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# Synthesis and characterization of a novel functionalized azanonaborane cluster for boron neutron capture therapy

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The reactivity of an azanonaborane cluster containing free amino groups $\{H_2N(CH_2)_4H_2NB_8H_{11}NH(CH_2)_4NH_2\}$ towards ketones and aldehydes is investigated. In a one step reaction, the reductive amination of some ketones and aldehydes (namely acetone, benzaldehyde, 3-hydroxybenzaldehyde, 4-hydroxybenzaldehyde, 4-nitrobenzaldehyde, 4-acetoxybenzaldehyde, and 4-acetamidobenzaldehyde) with an azanonaborane cluster in the presence of $H_3BNH_2(CH_2)_4NH_2$ gives monoalkylamino derivatives of the azanonaborane cluster $\{RHN(CH_2)_4H_2NB_8H_{11}NH(CH_2)_4NHR\}$ where (R = $(Me)_2CH$–, $C_6H_5CH_2$–, 3-$OHC_6H_4CH_2$–, 4-$OHC_6H_4CH_2$–, 4-$NO_2C_6H_4CH_2$–, 4-$MeOCOC_6H_4CH_2$–, or 4-$NH_2COC_6H_4CH_2$-). The functionalized derivatives of the $\{B_8N\}$ cluster can be used in boron neutron capture therapy for tumors (BNCT). Similarly, the reductive amination of 5-(4′-formylphenyl)-10,15,20-triphenylporphyrin with the $\{B_8N\}$ cluster gave a porphyrin bearing azanonaborane cluster, while a porphyrin dimer linked by an azanonaborane moiety was obtained following the same method, starting with a 2 : 1 molar ratio of porphyrin : $\{B_8N\}$ cluster. 5,10,15,20-Tetraformylphenylporphyrin gave the chance to increase the percentage of boron in the resulting boronated porphyrin, which is considered an important factor for a BNCT delivery agent. With these compounds, the cell toxicity using V79 cells was carried out to determine whether these compounds would have favorable biological properties.



## Introduction

Boron neutron capture therapy (BNCT) is a bimodal cancer treatment based on the selective accumulation of certain $^{10}B$ carriers in tumors. Subsequent irradiation with thermalized neutrons produces high linear energy transfer particles, $^4He^{2+}$ (α-particle) and $^7Li^{3+}$, that cause severe damage to tumor cells through ionization processes. Azanonaborane clusters $\{(R^1H_2N)B_8H_{11}NHR^2\}$ are readily prepared in good yields in easy steps from *nido*-$B_{10}H_{14}$, and the determination of their structures and unequivocal constitution have been reported.[1–3] These compounds have been shown to constitute a good entry into azacarbaborane[4] and azametalloborane chemistry.[5] The azanonaborane cluster also undergoes several other interesting reactions, such as: (i) a ligand-exchange reaction; (ii) N-deprotonation followed by subsequent N-alkylation; (iii) a halogenation reaction; (iv) hydrolytic decomposition to the new 5-vertex compound.[6–8] Recently, azanonaboranes containing free hydroxy groups[9] or free amino groups,[10] have been used as a new class of boron clusters and considered as a promising boron moiety in BNCT. The success of BNCT depends upon the selective uptake of boron within tumor cells.[11] Porphyrins appear to be particularly promising tumor-selective compounds because of their demonstrated tendency to accumulate in neoplastic tissue. Various boron clusters such as azanonaborane,[12] polyhedral *closo*- and *nido*-carborane and *closo*-dodecaborate anions containing porphyrin have been synthesized and designed as BNCT agents.[13–16] In order to develop the chemistry of azanonaboranes containing free amino groups such as (4-aminobutylamine)-*N*-aminobutyl)-azanonaborane(11) $\{H_2N(CH_2)_4H_2NB_8H_{11}NH(CH_2)_4NH_2\}$ (Fig. 1), we have become interested in the use of these compounds as starting substrates. Here we report the synthesis of new compounds containing an azanonaborane cluster by the reductive amination of some carbonyl compounds with (4-aminobutylamine)-*N*-aminobutyl)azanonaborane(11) $\{H_2N(CH_2)_4H_2NB_8H_{11}NH(CH_2)_4NH_2\}$ with the aim of obtaining azanonaboranes with enhanced biological significance.

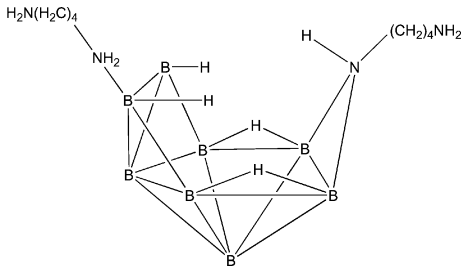


**Fig. 1** Structure of (4-aminobutylamine)-*N*-aminobutyl)azanonaborane(11) (*exo*-H atoms are omitted for clarity).

## Results and discussion

### Chemistry

The $B_8N$ cluster is an interesting starting material for the synthesis of a wide variety of boron-containing species. The eight boron atoms offer suitable boron content derivatives and have reasonable water solubility, which makes them good candidates for the selective delivery of boron for BNCT.[9,10] Functionalization of the azanonaboranes imparts a chemical reactivity which can assist their intracellular retention within tumors or provide the means to attach them to organic molecules. Azanonaborane clusters containing free amino groups $\{H_2N(CH_2)_4H_2NB_8H_{11}NH(CH_2)_4NH_2\}$ and $H_3BNH_2(CH_2)_4NH_2$ were prepared according to the literature.[10] Addition of carbonyl compounds (**1a–g**) to a mixture of $B_8N$ cluster with $H_3BNH_2(CH_2)_4NH_2$ in tetrahydrofuran (THF) at room temperature for 24 h (**1a**) or two days (**1b–g**), followed by chromatography, resulted in the isolation of monoalkylamino derivatives of the azanonaborane cluster $\{RHN(CH_2)_4H_2NB_8H_{11}NH(CH_2)_4NHR\}$ (**2a–g**) in good yields (Scheme 1). Additionally, the stability of the resulting clusters is higher than the previously reported $B_8N$ species[10] at room temperature. The monitoring of the reaction mixture by NMR spectroscopy showed no evidence of the presence of

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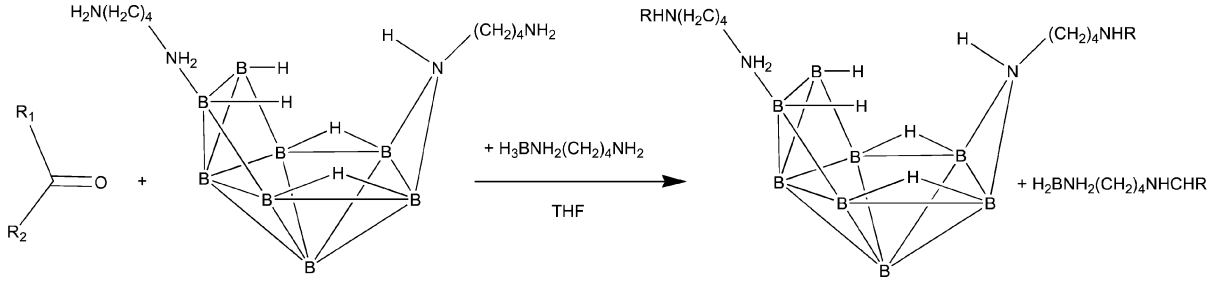



**1a** $R_1 = R_2 = CH_3$
**1b** $R_1$ =H, $R_2 = C_6H_5$
**1c** $R_1$ =H, $R_2$ = 3-$OHC_6H_5$
**1d** $R_1$ =H, $R_2$ = 4-$OHC_6H_5$
**1e** $R_1$ =H, $R_2$ = 4-$NO_2C_6H_5$
**1f** $R_1$ =H, $R_2$ = 4-$MeO_2CC_6H_5$
**1g** $R_1$ =H, $R_2$ = 4-$NH_2OCC_6H_5$

**2a** R = $(CH_3)_2CH$
**2b** R = $C_6H_5CH_2$
**2c** R = 3-$OHC_6H_4CH_2$
**2d** R = 4-$OHC_6H_4CH_2$
**2e** R = 4-$NO_2C_6H_4CH_2$
**2f** R = 4-$MeO_2CC_6H_4CH_2$
**2g** R = 4-$NH_2OCC_6H_4CH_2$

**Scheme 1**

$H_3BNH_2(CH_2)_4NH_2$ $\{\delta(^{11}B) = -19.97\}$ after 24 h for compound **2a** and two days for compounds **2b–g**. The appearance of a new broad band $\{\delta(^{11}B) = -24.65\}$ was attributed to the conversion of $H_3BNH_2(CH_2)_4NH_2$ to $H_2BNH_2(CH_2)_4NHCHR$ (Scheme 1). The constitution and purity of each of the compounds (**2a–g**) were established by NMR, mass spectroscopy and elemental analysis. Clean $^{11}B$ and $^{1}H$ NMR spectra were additional criteria of purity (Table 1). The $^{11}B$ NMR spectroscopic data of the series of compounds **2a–g** are very similar, although there are some variations in the proton shielding as the organic moiety changes (Table 1). $^{1}H$ NMR spectra of all compounds showed exchangeable singlets corresponding to the NH proton at $\delta$ = 5.8–6.2 ppm (Fig. 2). Also, $^{1}H$ and $^{13}C$ NMR spectra indicate the appearance of new signals corresponding to the organic moiety of each carbonyl compound. Mass spectra of all compounds showed fragments corresponding to the typical pattern of boron isotopes ($^{10}B$ and $^{11}B$).

In the case of compound **2a**, the $^{1}H$ NMR spectrum showed a heptet at 2.65–2.9 ppm corresponding to one CH proton and two doublets at 1.01–1.16 ppm corresponding to six $(CH_3)_2$ protons (Fig. 2). Moreover the ESI-MS spectrum showed the most intense molecular ion peak ($M^+$) at $m/z = 355$ corresponding to the $^{11}B$ isotope (see experimental section for details).

Computed $^{1}H$ and $^{13}C$ NMR chemical shifts, supported by 2D-$^{1}H$ $^{1}H$ cosy and 2D-$^{1}H$ $^{13}C$ cosy NMR spectra reflected the reductive amination of carbonyl compounds by the free amino groups in the $B_8N$ clusters. Additionally the assignments of $^{13}C$ NMR signals were based on DEPT experiments and chemical shift arguments (Fig. 3). The IR spectrum proved the absence of the free C=O group of carbonyl compounds. These observations suggested a mechanism for the formation of compounds **2a–g** in which the hydroborane $H_3BNH_2(CH_2)_4NH_2$ reduction of ketones and aldehydes most likely proceeds by an initial complexing of $H_3BNH_2(CH_2)_4NH_2$ with carbonyl oxygen[17]

**Table 1** 200 MHz ($^{11}B$, $^{1}H$) NMR data in $CDCl_3$ at 20 °C for **2a–g, 4a–b** and **6**

| 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 [$\delta(^{1}H)$] |
|---|---|---|---|---|---|---|---|---|---|---|
| **2a** | 1.62 [2.65] | −55.11 [−0.66] | −19.29 [1.18] | −34.16 [0.69] | −9.93 [2.64] | −9.93 [2.57] | −32.75 [0.69] | −31.41 [0.54] [−0.66] | [−2.17] [−2.021] | [−1.12] |
| **2b** | 1.54 [2.58] | −54.95 [−0.71] | −18.06 [1.28] | −34.31 [0.78] | −10.88 [2.54] | −10.88 [2.47] | −32.45 [0.78] | −30.95 [0.53] [−0.71] | [−2.05] [−2.12] | [−1.34] |
| **2c** | 1.71 [2.52] | −54.98 [−0.67] | −20.16 [1.32] | −33.95 [0.72] | −11.12 [2.56] | −11.14 [2.54] | −31.22 [0.72] | −30.67 [0.57] [−0.67] | [−2.32] [−2.29] | [−1.42] |
| **2d** | 1.66 [2.51] | −55.06 [−0.65] | −20.12 [1.21] | −34.06 [0.82] | −11.24 [2.62] | −11.24 [2.57] | −32.75 [0.82] | −30.59 [0.63] [−0.59] | [−2.39] [−2.39] | [−1.56] |
| **2e** | 1.72 [2.49] | −55.21 [−0.68] | −20.36 [1.28] | −34.14 [0.81] | −11.21 [2.58] | −11.21 [2.58] | −31.94 [0.81] | −30.06 [0.71] [−0.62] | [−2.17] [−2.17] | [−1.39] |
| **2f** | 1.79 [2.56] | −55.56 [−0.67] | −20.66 [1.29] | −34.16 [0.86] | −11.23 [2.52] | −11.23 [2.52] | −33.41 [0.86] | −30.26 [0.56] [−0.67] | [−2.23] [−2.025] | [−1.52] |
| **2g** | 1.64 [2.56] | −55.12 [−0.68] | −21.46 [1.18] | −34.05 [0.84] | −10.89 [2.58] | −10.89 [2.58] | −32.36 [0.84] | −30.76 [0.59] [−0.68] | [−2.22] [−1.99] | [−1.56] |
| **4a** | 1.59 [2.49] | −54.81 [−0.59] | −19.54 [1.23] | −32.76 [0.72] | −10.16 [2.53] | −10.11 [2.53] | −31.85 [0.72] | −30.25 [0.51] [−0.59] | [−1.99] [−1.89] | [−1.29] |
| **4b** | 1.76 [2.57] | −54.61 [−0.66] | −20.46 [1.19] | −33.23 [0.69] | −10.88 [2.53] | −10.89 [2.53] | −32.41 [0.69] | −30.52 [0.55] [−0.66] | [−2.14] [−2.03] | [−1.46] |
| **6** | 1.61 [2.51] | −54.72 [−0.65] | −19.36 [1.18] | −33.15 [0.66] | −10.78 [2.51] | −10.78 [2.51] | −31.87 [0.68] | −30.72 [0.57] [−0.65] | [−2.11] [−2.02] | [−1.56] |

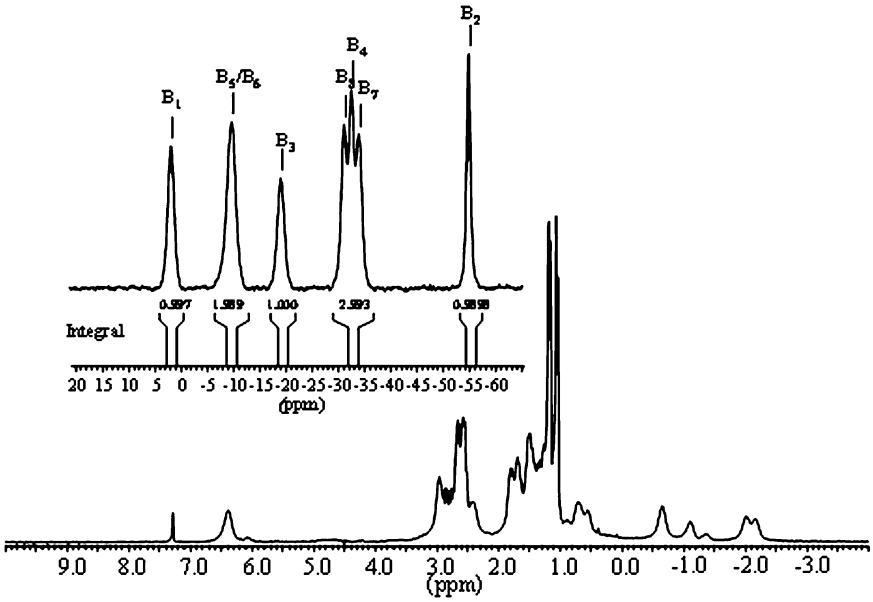



**Fig. 2** 200 MHz $^{11}B$ (top) and $^{11}B\{^1H\}$ (bottom) NMR spectra of compound **2a** in $CDCl_3$ at 20 °C.

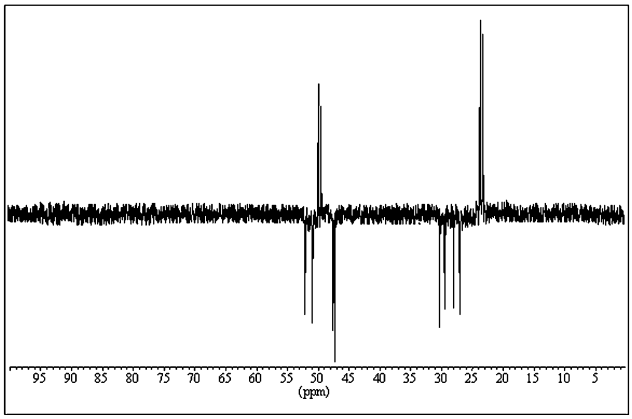



**Fig. 3** 200 MHz DEPT $^{13}C$ NMR spectrum of compound **2a** in $CDCl_3$ at 20 °C.

followed by an intramolecular hydride shift and removal of $H_2O$ to yield the required products.

Reaction of azanonaborane clusters with hydroxy acetone or isophthaloyl chloride under the same reaction conditions or at a lower temperature (0 °C) gave a white precipitate immediately. However, the monitoring of the reaction mixture by NMR spectroscopy showed that a progressive loss of the boron cluster occurred exclusively to give only boric acid. No reaction was found to occur upon treatment of the $B_8N$ cluster with acetylacetone.

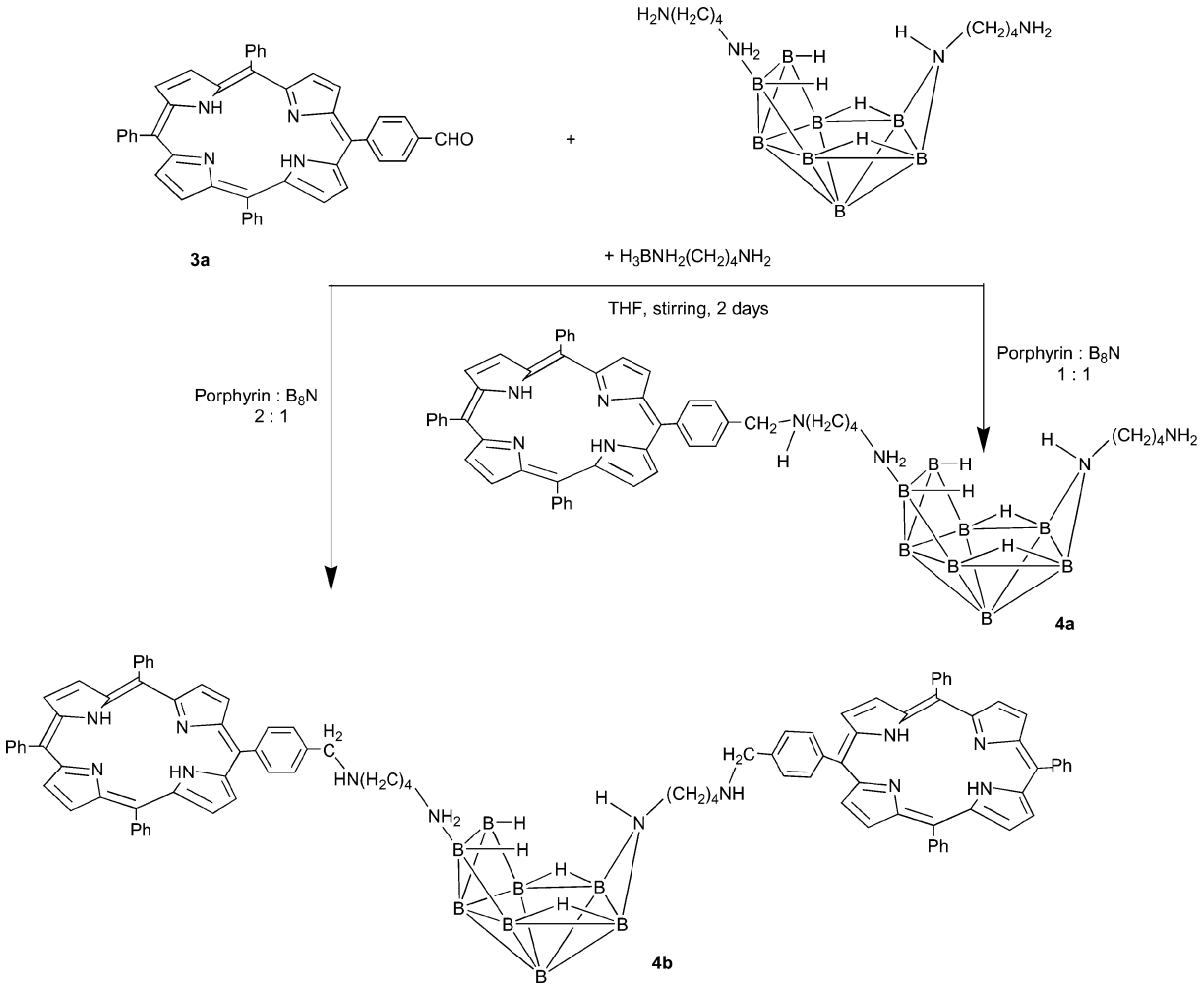
The observation that porphyrins have both selective uptake and persistence within tumors encouraged us to synthesize a series of pure compounds of porphyrins containing the $B_8N$ cluster that may be useful in cancer treatment. We have found that the reductive amination of 5-(4′-formylphenyl)-10,15,20-triphenylporphyrin (**3a**)[18] with a $B_8N$ cluster containing free amino groups in THF for two days followed by chromatography afforded both porphyrin monomer (**4a**) and dimer (**4b**) containing 9.5% and 5.6% boron by weight, respectively (Scheme 2). To achieve the incorporation of multiple azanonaborane cluster units, we started with 5,10,15,20-tetraformylphenylporphyrin **5**, affording boronated porphyrin bearing four azanonaborane clusters (**6**) in the *para*-position (Scheme 3). Compound **6** may be more useful as a boron carrier in BNCT than both compounds **4a** and **4b**, where it contains 20% boron by weight. The structures of the new compounds synthesized possess spectroscopic data in accord with the assigned structures **4a, 4b**, and **6**. To assess the potential of these boronated porphyrins in neutron capture therapy (NCT), we will consider achieving the water solubility of these porphyrins. Insertion of trivalent metal and addition of 1N HCl may be the route to our aim.

UV–visible spectra showed that the Soret band of the boronated porphyrins are red shifted nearly 3–5 nm comparing to the nonboronated starting porphyrins.

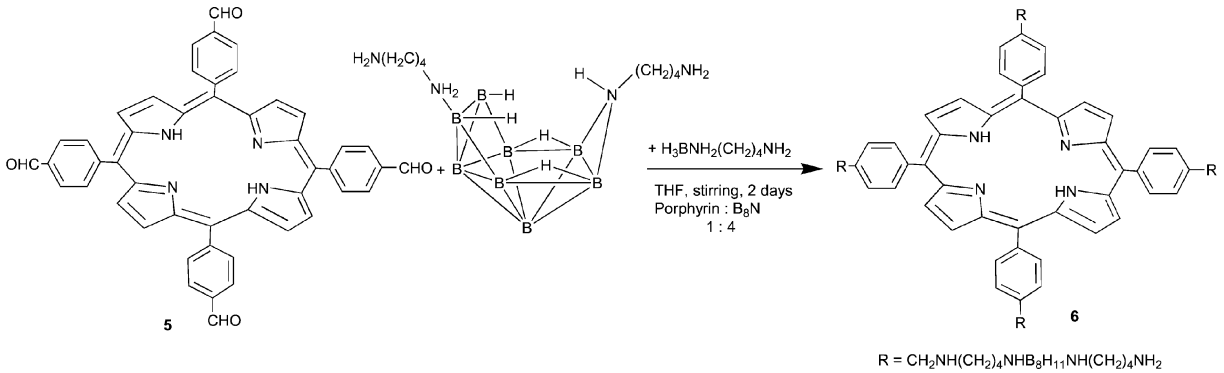
The vibrational frequency of the B–H band $\nu$(B–H) or the B–B band $\nu$(B–B) was not found to be sensitive to the connection of the carbonyl compounds with free amino groups. For compounds **2a–g, 4a–b** and **6**, $\nu$(B–H) lies in the range of 2521–2512 $cm^{-1}$, while $\nu$(B–B) varies from 1056 to 1048 $cm^{-1}$. Compared to the frequencies of $\{B_8N\}$[10] $\nu$(B–B) = 2529–2541 $cm^{-1}$, $\nu$(B–H) = 1065 to 1059 $cm^{-1}$, only slight differences were found, indicating that the intracluster bonding is not perturbed by substitution on the free amino groups.

## Biology

Polyamines such as putrescine, spermidine (SPD), and spermine (SPM) are important biochemical constituents that are essential for cell growth and differentiation[19,20] and their depletion has a growth inhibitory effect on tumors.[21] They accumulate in the tumor cells[22] and have transport systems which increase their uptake in malignant cells.[23] The potential of *N*-benzylpolyamines as vectors of boron for tumor targeting is evidenced by a recent *in vitro* study.[24] The *in vitro* toxicity test was carried out to determine whether these compounds were sufficiently nontoxic. *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 **2b** and **2e** at a concentration of 0.607 mM did not survive while **2d, 2f** and **6** were not toxic at this concentration, and **2a** already showed some toxicity. We conclude, in agreement with others, that the incorporation of a hydrophobic group into the chain of polyamines increases the compound's toxicity.[25] The survival ratio for **2c, 2f** and **2g** decreased when its concentrations in the medium increased from 0.607 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 (Fig. 4, Table 2). According to these results, a major limitation in the use of the present compounds appears to be their cellular toxicity, especially compounds such as **2a** or **2b**




**Scheme 2**




**Scheme 3**

and **2e** with hydrophobic groups. The *in vitro* toxicities of these compounds with $LD_{50}$ values below 607 μM were not measured at lower concentrations because the achievable concentration of boron would not be effective for BNCT.

## Conclusion

In conclusion, we have succeeded in synthesizing functionalized azanonaborane clusters *via* reductive amination of some aldehydes and ketones by a one step process. New types of compounds containing both porphyrin macrocycles and polyhedral azanonaborane units in one molecule have been prepared. These compounds might be suitable for use in BNCT. As compared with azanonaboranes containing free amino groups, results reported in this study have shown that their stability at room temperature is advantageous over previously reported azanonaboranes. The $B_8N$ clusters **2d, 2f** and **6** appear not to be toxic at suitable boron concentrations. These compounds might be useful as delivery agents for BNCT.

## Experimental

### Material and methods

The reagents, dry solvents THF and dichloromethane, were used as presented directly without further purification. All aldehydes and ketones (**1a–g**) were commercially available. 5-(4′-Formylphenyl)-10,15,20-triphenylporphyrin, 5,10,15,20-tetraformylphenylporphyrin, (4-aminobutylamine)-*N*-aminobutyl)azanonaborane(11) cluster $\{H_2N(CH_2)_4H_2NB_8H_{11}NH(CH_2)_4NH_2\}$ and $H_3BNH_2(CH_2)_4NH_2$ were prepared as

**Table 2** *In vitro* toxicity of $B_8N$ clusters by V79 Cells[a]

| $C_{media}$ | | Percentage of survival (%) | | | | | |
|---|---|---|---|---|---|---|---|
| (μg of B/mL) | (mM) | **2a** | **2b** | **2d** | **2e** | **2f** | **6** |
| 50 | 0.607 | <1 | <1 | 65.15 ± 5.3 | <1 | 59 ± 1.7 | 82 ± 0.87 |
| 75 | 0.868 | — | — | 41.45 ± 3.0 | — | 35.23 ± 1.3 | 60.2 ± 2.68 |
| 100 | 1.736 | — | — | 24.17 ± 3.2 | — | 18.13 ± 1.56 | 38.1 ± 0.85 |
| 150 | 2.343 | — | — | 17.25 ± 1.6 | — | 10.11 ± 0.93 | 22.2 ± 1.28 |
| 225 | 2.864 | — | — | 12.57 ± 1.2 | — | 7.23 ± 0.67 | 16.3 ± 0.45 |
| 300 | 3.472 | — | — | 6.72 ± 1.0 | — | 4 ± 0.87 | 7.0 ± 0.77 |

[a] V79 cells were incubated with boronated compounds for 16 h at compound concentrations corresponding to the boron amounts indicated. Cells were washed (PBS), trypsinized and seeded out for colony formation. After one week, colonies were washed, stained, washed again (ethanol) and counted.

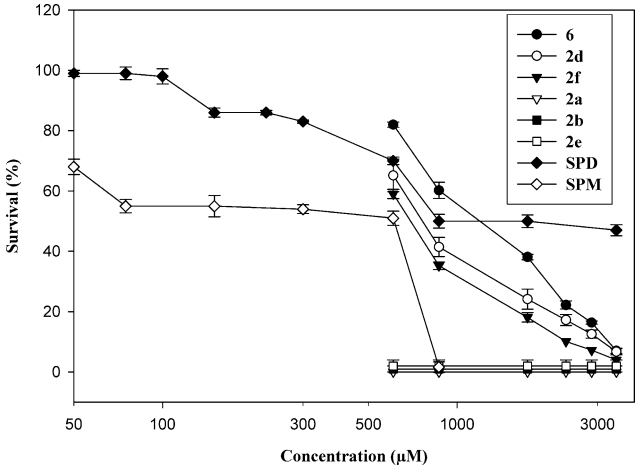



**Fig. 4** Percentage (± SD) of the *in vitro* survival cell with respect to the concentration of the $B_8N$ cluster compounds, SPD, and SPM. The data for SPD and SPM were taken from ref. 10.



described in the literature.[18,10] Plate chromatography was conducted on silica gel 60 (Fluka). Elemental analyses were performed by a Perkin-Elmer 2400 automatic elemental analyzer. All compounds gave elemental analysis within ±0.4%. UV–visible data were measured on a Varian Cary 50 bio instrument. The measurements for NMR ($^{11}$B, $^{1}$H and $^{13}$C) were carried out on a Bruker DPX 200 spectrometer. The chemical shifts $\delta$ are given in ppm relative to $\Delta E$ = 100 MHz for $\delta$ ($^{1}$H) (nominally $SiMe_4$), and $\Delta E$ = 32.083972 MHz for $\delta$ ($^{11}$B) (nominally $F_3BOEt_2$) in $CDCl_3$ or $CD_3CN$. IR ($cm^{-1}$) spectra were determined as a KBr disc on a Biorad FTS-7 spectrometer. Electron spray ionization (ESI) and direct chemical ionization (DCI, reactant gas $NH_3$) mass spectra were recorded on a Finnigan MAT 8222. Only the most intense molecular ion peak ($M^+$) corresponding to the $^{11}$B isotope was taken into consideration.

### General procedure for the synthesis of compounds 2a–g, 4a–b, and 6

Carbonyl compound (5 mmol) was added dropwise to a solution of azanonaborane cluster (1 mmol) and $H_3BNH_2(CH_2)_4NH_2$ (2 mmol) in 30 ml dry THF at room temperature. The reaction mixture was stirred for 24 h in the case of compound **2a** and two days in the case of compounds **2b–g, 4a–b**, and **6**. The solution was filtered off and all volatile components of the filtrate were removed under vacuum to give the desired compounds. The residue was chromatographed on silica gel using THF and $CH_2Cl_2$ (1 : 2) as an eluent to yield the desired product.

**2a.** Yield (0.5 g, 45%) as a colorless oil; $R_f$ = 0.3; (found: C, 47.33; H, 12.08; N, 15.71. $B_8H_{43}C_{14}N_4$ requires C, 47.53; H, 12.16; N, 15.84%); $\nu_{max}$(KBr disc)/$cm^{-1}$ 3238s ($NH_2$/NH), 2521s (BH), 1657w (δ, $NH_2$/NH), 1338s (BN), 2963m, 2898m, 1472m, 1442m, 1402m, 1157m, 1115s, 745m, (δ, γ, $CH_2$-groups); $\delta_H$(200 MHz; $CDCl_3$; $Me_4Si$) +6.35 (2H, bs, HN–), +3.93 (2H, bs, $H_2N$–$B_8$), +2.82 (6H, t, –$H_2$CHN), +2.75 (2H, hept, –HCNH), +2.61 (2H, m, $CH_2NH_2$–$B_8$), +1.38–1.61 (8H, m, –$CH_2CH_2$–, –$CH_2CH_2$–), +1.13 (6H, d, $(CH_3)_2$), +1.01 (6H, d, $(CH_3)_2$); $\delta_C$(200 MHz; $CDCl_3$; $Me_4Si$) 51.76 ($CH_2HNB_8$), 50.54 ($CH_2NH_2$–$B_8$), 49.59, 49.17 (2C, $CH_2NH$), 47.2, 46.95 (2C, HNCH), 29.93, 29.14, 27.66, 26.65 (4C, –$CH_2CH_2$–), 23.4, 22.56 (4C, $CH_3$); *m/z* (ESI) 355 ($M^+$, 10%).

**2b.** Yield: (0.58 g, 47%) as a colorless oil; $R_f$ = 0.32; (found: C, 58.95; H, 9.01; N, 12.37. $B_8H_{41}C_{22}N_4$ requires C, 59.0; H, 9.16; N, 12.51%); $\nu_{max}$(KBr disc)/$cm^{-1}$ 3239s ($NH_2$/NH), 2526s (BH), 1667w (δ, $NH_2$/NH), 1339s (BN), 2976m, 2895m, 1515m, 1467m, 1448m, 1166m, 1116s, 745m (δ, γ of $CH_2$-groups); $\delta_H$(200 MHz; $CD_3CN$; $Me_4Si$) +6.35 (2H, bs, HN–), +6.5–7.12 (10$H_{arom}$, m, CH), +3.86 (2H, bs, $H_2N$–$B_8$), +2.78 (2H, t, –$H_2$CHN–$B_8$), +2.71 (4H, m, –$H_2$CNH), +2.67 (2H, m, –$H_2CH_2N$–$B_8$), +1.4–1.65 (8H, m, –$CH_2CH_2$–, –$CH_2CH_2$–); $\delta_C$(200 MHz; $CD_3CN$; $Me_4Si$) 130.12, 130.02, 125.2, 117.1, 115.25, 115.12, 115.08 ($C_{arom}$), 51.63 ($CH_2HNB_8$), 50.34 ($H_2CH_2N$–$B_8$), 48.98, 49.4 (2C, $CH_2NH$), 30.02, 29.55, 28.23, 26.95 (4C, –$CH_2CH_2$–); *m/z* (DCI) 449 ($M^+$, 8%).

**2c.** Yield: (0.56 g, 38%) as a colorless solid; $R_f$ = 0.28; (found: C, 55.12; H, 8.05; N 11.56. $B_8H_{39}C_{22}N_4O_2$ requires C, 55.39; H, 8.16; N, 11.73%); $\nu_{max}$(KBr disc)/$cm^{-1}$ 3612s (OH), 3239s ($NH_2$/NH), 2522s (BH), 1661s (δ, $NH_2$/NH), 1336s (BN), 2973m, 2895m, 1513m, 1463m, 1446m, 1163m, 1115s, 742m (δ, γ of $CH_2$-groups); $\delta_H$(200 MHz; $CD_3CN$; $Me_4Si$) +6.35 (2H, bs, HN–), +6.97 (2H, s, OH), +6.7–7.19 (8$H_{arom}$, m, CH), +3.95 (2H, bs, $H_2N$–$B_8$), +2.88 (2H, t, –$H_2$CHN–$B_8$), +2.75 (4H, m, –$H_2$CNH), +2.71 (2H, m, –$H_2CH_2N$–$B_8$), +1.38–1.65 (8H, m, –$CH_2CH_2$–, –$CH_2CH_2$–); $\delta_C$(200 MHz; $CD_3CN$; $Me_4Si$) 156.8, 156.49, 130.41, 130.12, 125.83, 117.8, 115.54, 115.48, 115.43 ($C_{arom}$), 51.21 ($CH_2HNB_8$), 50.54 ($H_2CH_2N$–$B_8$), 48.98, 47.25 (2C, $CH_2NH$), 30.19, 29.24, 28.13, 27.25 (4C, –$CH_2CH_2$–); *m/z* (DCI) 479 ($M^+$, 12%).

**2d.** Yield: (0.64 g, 43%) as a colorless oil; $R_f$ = 0.31; (found: C, 55.09; H, 8.13; N, 11.43. $B_8H_{39}C_{22}N_4O_2$ requires C, 55.39; H, 8.16; N, 11.73%); $\nu_{max}$(KBr disc)/$cm^{-1}$ 3605s (OH), 3238s ($NH_2$/NH), 2521s (BH), 1665w (δ, $NH_2$/NH), 1335s (BN), 2976m, 2894m, 1512m, 1458m, 1438m, 1152m, 1113s, 745m (δ, γ of $CH_2$-groups); $\delta_H$(200 MHz; $CD_3CN$; $Me_4Si$) +6.35 (2H, bs, HN–), +6.95 (2H, s, OH), 6.5–7.12 (8$H_{arom}$, m, CH), +3.86 (2H, bs, $H_2N$–$B_8$), +2.78 (2H, t, –$H_2$CHN–$B_8$), +2.71 (4H, m, –$H_2$CNH), +2.67 (2H, m, –$H_2CH_2N$–$B_8$), +1.4–1.65 (8H, m, –$CH_2CH_2$–, –$CH_2CH_2$–); $\delta_C$(200 MHz; $CD_3CN$; $Me_4Si$) 156.23, 156.2, 130.12, 130.02, 125.2, 117.1, 115.25, 115.12, 115.08 ($C_{arom}$), 51.45 ($CH_2HNB_8$), 50.35 ($H_2CH_2N$–$B_8$), 48.89, 49.51 (2C, $CH_2NH$), 29.56, 28.95, 27.56, 26.53 (4C, –$CH_2CH_2$–); *m/z* (ESI) = 479 ($M^+$, 10%).

**2e.** Yield: (0.74 g, 45%) as a colorless oil; $R_f$ = 0.32; (found: C, 49.12; H, 6.87; N, 15.48. $B_8H_{37}C_{22}N_6O_4$ requires C, 49.3;

H, 6.91; N, 15.68%); $\nu_{max}$(KBr disc)/cm$^{-1}$ 3228s ($NH_2$/NH), 2527 (sBH), 1597w (δ, $NH_2$/NH), 1332s (BN), 2978m, 2895m, 1517m, 1468m, 1449m, 1167m, 1112s, 744m (δ, γ of $CH_2$-groups); $\delta_H$(200 MHz; $CD_3CN$; $Me_4Si$) +6.28 (2H, bs, HN–), +6.5–7.18 (8$H_{arom}$, m, CH), +3.95 (2H, bs, $H_2N$–$B_8$), +2.88 (2H, t, –$H_2$CHN–$B_8$), +2.76 (4H, m, –$H_2$CNH), +2.61 (2H, m, –$H_2CH_2N$–$B_8$), +1.4–1.65 (8H, m, –$CH_2CH_2$–, –$CH_2CH_2$–); $\delta_C$(200 MHz; $CD_3CN$; $Me_4Si$) 155.25, 130. 12, 130.02, 125.2, 117.1, 115.25, 115.12, 115.08 ($C_{arom}$), 52.05 ($CH_2HNB_8$), 50.86 ($H_2CH_2N$–$B_8$), 48.89, 49.41 (2C, $CH_2NH$), 29.69, 29.58, 27.95, 26.85 (4C, –$CH_2CH_2$–); *m*/*z* (DCI) 537 ($M^+$, 15%).

**2f.** Yield: (0.74 g, 42%) as a colorless solid; $R_f = 0.28$; (found: C, 55.23; H, 7.38; N, 11.12. $B_8H_{43}C_{26}N_4O_4$, requires C, 55.57; H, 7.65; N, 11.4%); $\nu_{max}$(KBr disc)/cm$^{-1}$ 3232s ($NH_2$/NH), 2523s (BH), 1310s (CO), 1615w (δ, $NH_2$/NH), 1336s (BN), 2984m, 2892m, 1511m, 1452m, 1437m, 1156m, 1115s, 745m (δ, γ of $CH_2$-groups); $\delta_H$(200 MHz; $CD_3CN$; $Me_4Si$) +6.26 (2H, bs, HN–), +6.56–7.12 (8$H_{arom}$, m, CH), +3.67 (2H, bs, $H_2N$–$B_8$), +2.68 (2H, t, –$H_2CHNB_8$), +2.65 (4H, m, –$H_2$CNH), +2.67 (2H, m, –$H_2CH_2NB_8$), 2.48 (6H, s, $CH_3OCO$), +1.4–1.66 (8H, m, –$CH_2CH_2$–, –$CH_2CH_2$–); $\delta_C$(200 MHz; $CD_3CN$; $Me_4Si$) 186.12, 186.15 (2C, CO), 176.23, 132.11, 131.25, 126.15, 117.25, 116.23, 115.12, 115.29 ($C_{arom}$), 52.21 ($CH_2HNB_8$), 51.56 ($H_2CH_2N$–$B_8$), 49.35, 48.58 (2C, $CH_2NH$), 31.59, 30.15, 27.46, 27.38 (4C, –$CH_2CH_2$–), 26.22, 26.13 (2C, $CH_3$); *m*/*z* (DCI) 563 ($M^+$, 10%).

**2g.** Yield: (0.73 g, 45%) as a colorless oil; $R_f = 0.25$; (found: C, 53.96; H, 7.56; N, 15.65. $B_8H_{41}C_{24}N_6O_2$ requires C, 54.19; H, 7.71; N, 15.8%); $\nu_{max}$(KBr disc)/cm$^{-1}$ 3270s (NHCO), 3232s ($NH_2$/NH), 2525s (BH), 1715s (CO), 1614w (δ, $NH_2$/NH), 1337s (BN), 2986m, 2895m, 1515m, 1456m, 1432m, 1155m, 1115s, 745m (δ, γ of $CH_2$-groups); $\delta_H$(200 MHz; $CD_3CN$; $Me_4Si$) +6.45 (2H, bs, HN–), +7.3 (2H, s, HNCO), 6.67–7.89 (8$H_{arom}$, m, CH), +4.01 (2H, bs, $H_2N$–$B_8$), +2.89 (2H, t, –$H_2$CHN–$B_8$), +2.76 (4H, m, $H_2$CNH), +2.65 (2H, m, –$H_2CH_2N$–$B_8$), +1.4–1.65 (8H, m, –$CH_2CH_2$–, –$CH_2CH_2$–); $\delta_C$(200 MHz; $CD_3CN$; $Me_4Si$) 198.12, 198.08, (2C, HNCO), 177.89, 135.12, 133.02, 128.2, 118.1, 117.25, 116.12, 115.85 ($C_{arom}$), 51.98 ($CH_2HNB_8$), 50.58 ($H_2CH_2N$–$B_8$), 49.58, 48.47 (2C, $CH_2NH$), 31. 95, 30.51, 28.63, 27.87 (4C, –$CH_2CH_2$–); *m*/*z* (DCI) 533 ($M^+$, 15%).

**4a.** Yield: (0.97 g, 37%) as a deep violet crystalline solid; $R_f = 0.28$; $\lambda_{max}$($CHCl_3$)/nm 420, 525, 564, 601 and 660 ($10^{-3}$ ε/dm$^3$ mol$^{-1}$cm$^{-1}$ 370.3, 16.8, 5.4, 5.2, and 2.6); $\delta_H$(200 MHz; $CDCl_3$; $Me_4Si$) −2.7 (2H, bs, HN–), +4.15 (2H, bs, $H_2N$–$B_8$), +4.2 (2H, s, $CH_2$), +7.05–8.25 (19H, m, $H_{arom}$), 8.68–8.92 (8H, m, β-pyrrole), +2.82 (2H, t, $H_2$CNH), +2.75 (6H, m, –$H_2CNH_2$), +1.4–1.62 (8H, m, –$CH_2CH_2$–, –$CH_2CH_2$–); $\delta_C$(200 MHz; $CDCl_3$; $Me_4Si$) 149.37, 148.82, 136.92, 135.8, 133.83, 130.82, 128.37, 125.32, 117.8, 115.03, 112.01, 108.75 ($C_{porphyrin}$), 51.36 ($CH_2HNB_8$), 50.25 ($CH_2NH_2$–$B_8$), 47.86 (C, $CH_2$), 49.19, 48.45 (2C, $CH_2NH$), 47.81, 46.19 (2C, HNCH), 30.18, 28.26, 27.13, 26.12 (4C, –$CH_2CH_2$–); *m*/*z* (ESI) = 902 ($M^+$, 30%), 630 (M–$B_8H_{11}NH(CH_2)_4NH_2$, 100).

**4b.** Yield: (0.22 g, 39%) as a deep violet crystalline solid; $R_f = 0.25$; $\lambda_{max}$($CHCl_3$)/nm 422, 525, 562, 600, and 660 ($10^{-3}$ ε/dm$^3$ mol$^{-1}$cm$^{-1}$ 370.1, 16.8, 5.2, 5.1, and 2.6); $\delta_H$(200 MHz; $CDCl_3$; $Me_4Si$) −2.72 (4H, bs, HN–), +4.1 (2H, bs, $H_2N$–$B_8$), +4.32 (4H, s, $CH_2$), +6.98–8.35 (38H, m, $H_{arom}$), 8.38–9.01 (16H, m, β-pyrrole), +2.92 (2H, t, $H_2$CNH), +2.75 (6H, m, –$H_2CNH_2$), +1.4–1.62 (8H, m, –$CH_2CH_2$–, –$CH_2CH_2$–); $\delta_C$(200 MHz; $CDCl_3$; $Me_4Si$) 149.8, 148.07, 139.22, 137.2, 136.78, 135.8, 135.05, 134.62, 133.72, 131.38, 130.71, 130.32, 129.55, 128.73, 128.3, 127.32, 125.62, 117.32, 116.01, 115.6, 111.43, 108.8 ($C_{porphyrin}$), 51.21 ($CH_2HNB_8$), 50.14 ($CH_2NH_2$–$B_8$), 49.46 (2C, $CH_2$), 49.18, 48.42 (2C, $CH_2NH$), 47.63, 46.12 (2CH, HNCH), 30.19, 29.26, 28.13, 26.95 (4C, –$CH_2CH_2$–); *m*/*z* (ESI) 1531 ($M^+$, 15%), 901 (M-630, 23), 630 (M-901, 100).

**6.** Yield: (0.35 g, 45%) as a deep violet crystalline solid; $R_f = 0.21$; $\lambda_{max}$($CHCl_3$)/nm 424, 526, 564, 600, and 661 ($10^{-3}$ ε/dm$^3$ mol$^{-1}$cm$^{-1}$ 369.9, 16.6, 5.1, 5.1, and 2.5); $\delta_C$(200 MHz; $CDCl_3$; $Me_4Si$) −2.71 (2H, bs, HN–), +4.2 (8H, bs, $H_2N$–$B_8$), +3.82 (8H, s, $CH_2$), +7.05–8.25 (19H, m, $H_{arom}$), 8.68–8.92 (8H, m, β-pyrrole), +2.84 (8H, t, $H_2CNH$), +2.75 (12H, m, –$H_2CNH_2$), +1.38–1.65 (16H, m, –$CH_2CH_2$–); $\delta_C$(200 MHz; $CDCl_3$; $Me_4Si$) 150.75, 149.28, 137.12, 136.14, 132.83, 130.82, 128.73, 126.23, 118.18, 115.03, 112.01, 108.75 ($C_{porphyrin}$), 51.36 ($CH_2HNB_8$), 50.25–48.17 (4$CH_2NH_2$–$B_8$), 53.19–51.05 (8C, $CH_2NH$), 48.1–42.15 (8C, HNCH), 32.15–25.16 (16C, –$CH_2CH_2$–); *m*/*z* (ESI) 1747 ($M^+$, 21%), 1446 (M-272, 18), 630 (M-1088, 100).

### Biological studies

All tests were repeated 2–3 times. For each compound, Petri dishes were seeded with V79 cells (Chinese hamster fibroblasts) in an 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 a medium containing varying concentrations of the boron compounds (the solubility of **6** was enhanced using 1N HCl) 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.[26] The medium was removed from the dishes. The cells were suspended by trypsinization, counted and seeded out into new dishes at different dilutions. The numbers of colonies formed after one week were compared to the numbers of colonies formed in the control without boron. The medium was removed, washed with PBS, dyed with GIEMSA for 10–15 minutes and washed again with ethanol.

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