The first synthesis of azanonaborane-containing sugars, possible boron carriers for neutron capture therapy

Mohamed E. El-Zaria,*[a] Afaf R. Genady[a] and Detlef Gabel[b]

Received (in Montpellier, France) 3rd October 2005, Accepted 14th February 2006
First published as an Advance Article on the web 3rd March 2006
DOI: 10.1039/b513959g

A novel class of boron containing sugars is described. They were synthesized in acceptable yields from readily available starting materials. Reaction of (4-aminobutylamine)-(*N*-aminobutyl)azanonaborane(11) [$H_2N(CH_2)_4H_2NB_8H_{11}NH(CH_2)_4NH_2$] with acetobromo-α-D-glucose or acetobromo-α-D-galactose in acetonitrile followed by hydrolysis with sodium methoxide afforded (4-α-glucopyranosylaminobutylamine)-(*N*-α-glucopyranosylaminobutyl)azanonaborane(11) or (4-α-galactopyranosylaminobutylamine)-(*N*-α-galactopyranosylaminobutyl)azanonaborane(11) in 65% and 63% yields, respectively. In a one step process, treatment of a B_8N cluster with D-ribose in methanol in the presence of a catalytic amount of glacial acetic acid gave (4-α-ribofuranosylaminobutylamine)-(*N*-α-ribofuranosylaminobutyl)azanonaborane(11) in 78% yield. The connection of monosaccharide units to the azanonaborane cluster enhances the stability and the water solubility of the resulting compounds, which are considered important factors for boron neutron capture therapy (BNCT) delivery agents. The *in vitro* toxicity test using B16 melanoma cells showed that azanonaborane clusters containing sugar are not toxic even at boron concentrations of 3 mM, whereas (4-aminobutylamine)-(*N*-aminobutyl)azanonaborane(11) has an LD_{50} of about 700 μM. Results obtained from these studies may provide a more definitive answer as to the potential usefulness of these compounds for BNCT.

Introduction

Treatment of cancer by boron neutron capture therapy (BNCT) requires the synthesis and evaluation of non-toxic agents that selectively target malignant cells in comparison to adjacent normal tissue and are retained intracellulary. When a therapeutic amount of ^{10}B (around 10 μg (g tissue)$^{-1}$) can be distributed throughout the target tumor, the two particles generated through the neutron capture reaction in boron ($^{10}B(n,\alpha)^7Li$) carry a high linear energy transfer if a sufficient number of very low energy thermal neutrons can be delivered.[1,2] Various boron containing carbohydrates have been synthesized and considered as potential agents for BNCT.[3–10] The first boronated sugar derivatives synthesized for BNCT were obtained by Maurer *et al.*[3] They contained the *o*-carborane moiety as boron carrier. The synthesis of further carborane containing sugars encompassed the reaction of lithium carborane with aldehydes.[4] One further sugar derivative synthesized is 3-*O*-carboranylmethyl glucose.[5] Its low water solubility required the use of DMSO as co-solvent. Uptake in cells was high resulting in a concentration of 65 μg (g cell mass)$^{-1}$ after 16 h, with a concentration in the medium of 6.8 μg g^{-1}. The stereoselective synthesis of thioglycosides of BSH ($B_{12}H_{11}SH^{2-}$) has been already successfully carried out with several sugars.[9] Recently, a BSH containing a glycoside of glucuronic acid has been prepared and is being considered as a potential prodrug for BNCT.[10] Nucleoside/metallocarborane conjugates and some simple metallocarborane derivatives substituted at boron-8 are characterized by low toxicity and high lipophilicity, an advantageous and preferred for potential boron delivering drugs.[11] The cellular uptake showed that some of these compounds gave boron concentrations within the range of 31 and 27 Pg/10^6 cells.

Carbohydrates are also important molecules for cell–cell interactions and cellular recognition. It has therefore been suggested to use these interactions also for BNCT. In view of the necessary accumulation of boron required for successful therapy, the receptor density on the cell surface might be too low for use in targeting boron for BNCT.

Compared with presently used ionic boron clusters,[1,2] azanonaborane [$RH_2NB_8H_{11}NHR$] is a neutral compound and exhibits remarkable chemical stability. *o*-Carborane requires the use of polyols or polyethers for solubilization in water.[12] In contrast, azanonaborane is reasonably soluble in water. For this purpose, a number of azanonaborane derivatives of biomolecules have been synthesized for use in BNCT of cancer.[13–18] Previous work focused on azanonaborane containing porphyrins,[15–17] free hydroxy groups,[13] as well as free amino groups.[14,18] The toxicity of the cluster *in vitro* and *in vivo* was found to be low, and governed mostly by its side chains.

Due to the increased metabolism of tumors, sugars might be taken up to a larger extent there than in surrounding tissue.[1,2,19] Sugars are potentially attractive compounds because

[a] *Department of Chemistry, Faculty of Science, University of Tanta, 31527 Tanta, Egypt. E-mail: MohamedEl-Zaria@web.de; Fax: +2-0040-3350804; Tel: +20-40-3297974*
[b] *Department of Chemistry, University of Bremen, D-28334 Bremen, Germany*

they either (1) may be taken up selectively into tumors due to the high metabolic rate of tumor cells *vs*. normal cells, *e.g*. through the Na-glucose cotransporter,[20] or (2) their nucleosides may be intracellularly converted to the corresponding nucleotides through phosphorylation by appropriate enzymes, or (3) they may potentially be incorporated into tumor DNA, thereby enhancing the cytotoxicity of the neutron capture reaction.[21] We have investigated the synthesis of *N*-glycosides of the sugars (D-glucose, D-galactose, and D-ribose) to (4-aminobutylamine) - (*N*- aminobutyl) azanonaborane (11) [$H_2N(CH_2)_4H_2NB_8H_{11}NH(CH_2)_4NH_2$] (Fig. 1). *In vitro* toxicity of these compounds has been studied and found to be low. They may therefore have potential as BNCT agents.

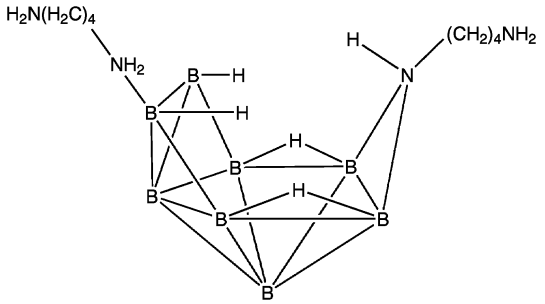
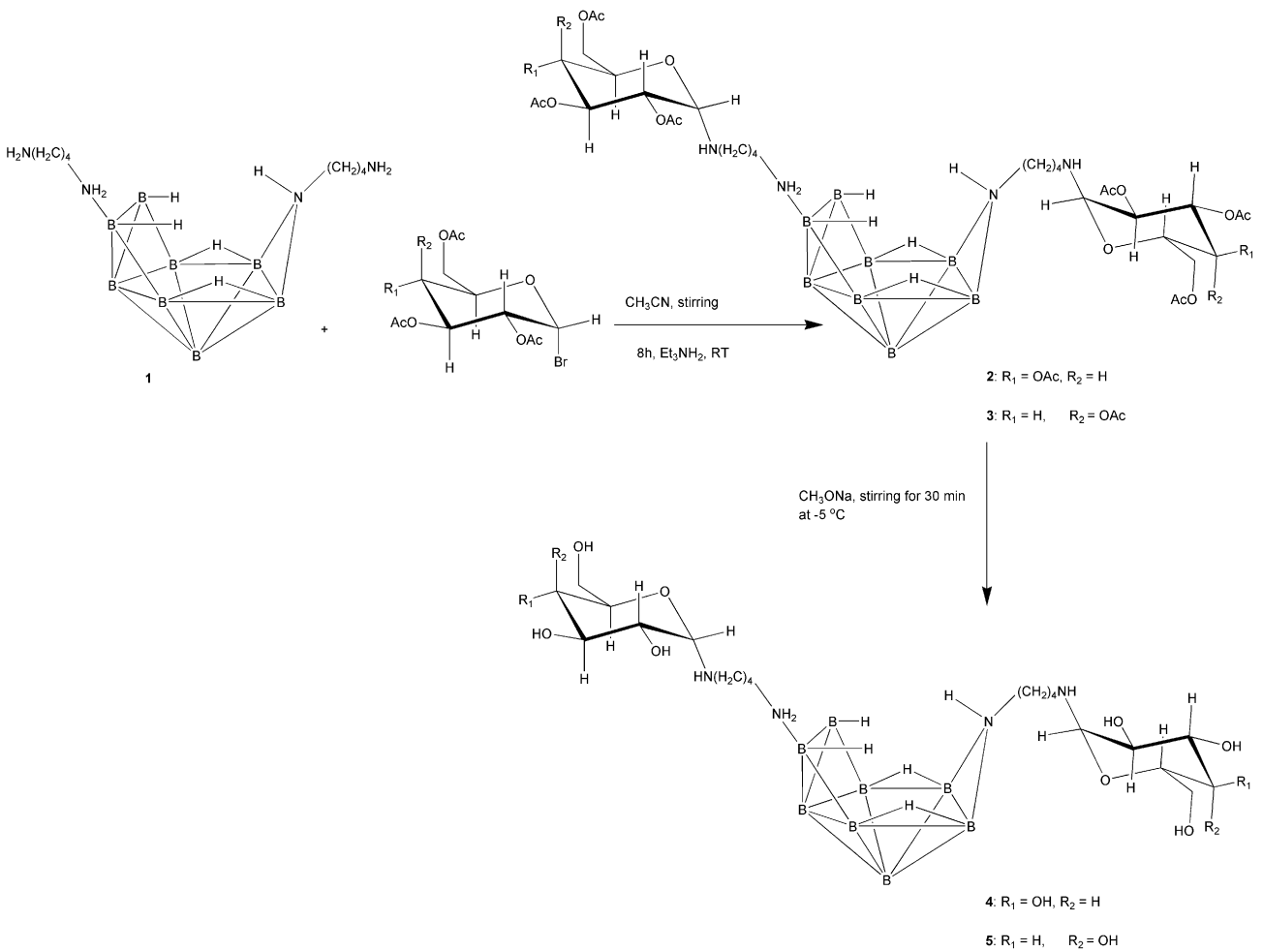


Fig. 1 Structure of (4-aminobutylamine)-(*N*-aminobutyl)azanonaborane(11) (*exo*-H atoms are omitted for clarity).

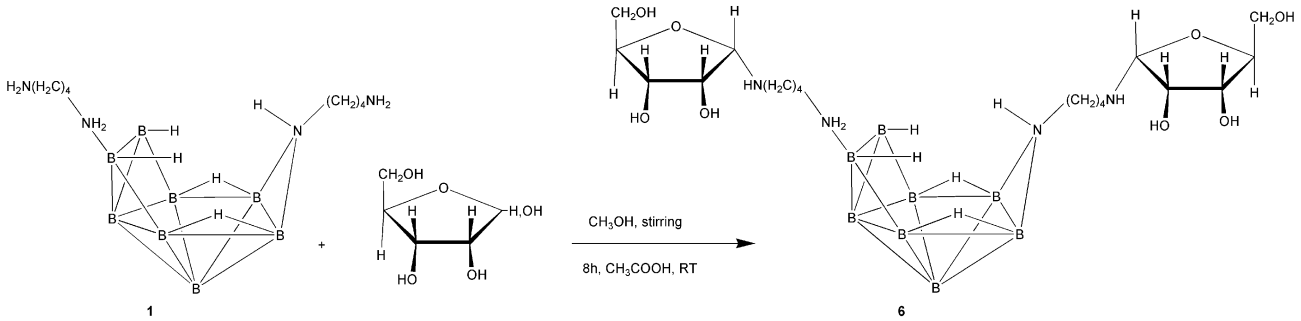
Results and discussion

Chemistry

(4-Aminobutylamine)-(*N*-aminobutyl)azanonaborane(11) [$H_2N(CH_2)_4H_2NB_8H_{11}NH(CH_2)_4NH_2$] **1**, easily prepared *via* a two step process starting from *nido*-$B_{10}H_{14}$ in reasonable yield according to previously described procedures,[14] was first reacted directly with D-glucose or D-galactose in methanol or ethanol, without obtaining the azanonaborane α-gluco- or α-galacto-pyranosyl-*N*-glycoside. The *N*-glycosides could be obtained by the reaction of the B_8N cluster **1** with acetobromo-α-D-glucose or acetobromo-α-D-galactose in CH_3CN in the presence of triethylamine at room temperature affording 4-(2,3,4,6-tetra-*O*-acetyl)-(α-D-glucopyranosylaminobutylamine)-(*N*-2, 3, 4, 6-tetra-*O*-acetyl-α-glucopyranosylaminobutyl)azanonaborane(11) **2** and 4-(2,3,4,6-tetra-*O*-acetyl)-(α-D-galactopyranosylaminobutylamine)-(*N*-2,3,4,6-tetra-*O*-acetyl-α-galactopyranosylaminobutyl)azanonaborane(11) **3** in 85% and 87% yields, respectively (Scheme 1).



Scheme 1



Scheme 2

Deacetylation of compounds **2** and **3** with freshly prepared CH_3ONa at −5 °C gave (4-α-glucopyranosylaminobutylamine)-(*N*-α-glucopyranosylaminobutyl)azanonaborane(11) **4** and (4-α-galactopyranosylaminobutylamine)-(*N*-α-galactopyranosylaminobutyl)azanonaborane(11) **5** in 65 and 73% yields, respectively (Scheme 1).

In contrast, D-ribose reacts directly with **1** in methanol at room temperature in the presence of one drop of glacial acetic as catalyst to afford the corresponding (4-α-ribofuransoylaminobutylamine)-(*N*-α-ribofuranosylaminobutyl)azanonaborane (11) **6** in 78% (Scheme 2).

The constitution and purity of each of the compounds (**2–6**) were established by IR, 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 **2–6** are very similar, although there are some variations in the proton shielding as the organic moiety changes (Table 1). Also, ^{1}H and ^{13}C NMR spectra indicate the appearance of new signals corresponding to the organic moiety of each sugar. Mass spectra of all compounds showed ions corresponding to the expected pattern of boron isotopes (^{10}B and ^{11}B).

NMR spectra of **2** and **3** showed the resonances of the anomeric protons at δ = 5.45 and 5.67 ppm, and of the anomeric carbons, at δ = 89.11 and 89.92 ppm, respectively. NMR spectra of compounds **4** and **5** showed the anomeric protons as broad signals at δ = 4.85 and 4.89 ppm, respectively, with the anomeric carbons at 89.8 and 89.52 ppm, respectively. The anomeric proton **6** resonates as a sharp signal at δ = 4.85 ppm; C-1′ resonates at δ = 87.95 ppm. These values are consistent withan α-configuration with a $^{4}C_1$(B) conformation.[22,23] The vibrational frequency of the B–H band ν(B–H) or the B–B band ν(B-B) was not found to be sensitive to the connection of the acetylated or free sugar moieties with free amino groups. For compounds **2–6**, ν(B–H) lies in the range of 2525–2538 cm^{-1}, and ν(B–B) from 1058 to 1047 cm^{-1}, comparable to the frequencies of $\{B_8N\}^{12}$ ν(B–H) = 2529–2541 cm^{-1}, ν(B–B) = 1065 to 1059 cm^{-1}, indicating that the intracluster bonding is not perturbed by substitution on the free amino groups. The IR spectra of compounds **4–6** showed absorption bands within the 2924–3932 cm^{-1} region characteristic for OH groups of carbohydrate moieties overlapped with NH. The ESI-MS spectra of compounds **4** or **5** and **6** showed the most intense molecular ion peak (M^+) at m/z = 598 and 538, respectively, corresponding to the ^{11}B isotope (see Experimental section for details).

The presence of the aminopyranosyl or aminofuranosyl residues increased the water solubility and stability of these compounds in comparison to azanonaboranes containing 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. The rate constant for all compounds was

Table 1 200 MHz (^{11}B, ^{1}H) NMR data in CD_3CN (**2** and **3**) and D_2O (**4–6**) 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 [$\delta(^{1}H)$]
2	1.76 [2.67]	−55.11 [−0.68]	−21.35 [1.22]	−33.32 [0.69]	−11.08 [2.64]	−11.08 [2.57]	−32.75 [0.71]	−31.76 [0.56] [−0.66]	[−2.18] [−2.021]	[−1.32]
3	1.71 [2.59]	−55.06 [−0.71]	−20.13 [1.26]	−34.31 [0.78]	−10.88 [2.54]	−10.88 [2.57]	−32.45 [0.78]	−30.95 [0.53] [−0.71]	[−2.09] [−2.12]	[−1.36]
4	1.69 [2.52]	−55.53 [−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]
5	1.66 [2.51]	−54. 93 [−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]
6	1.72 [2.49]	−55.53 [−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]

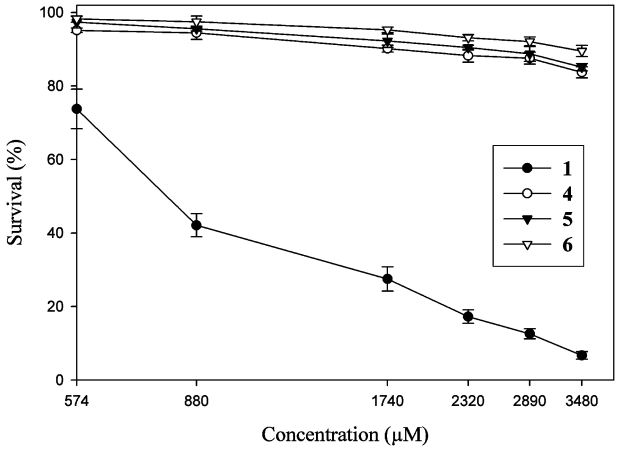


Fig. 2 Percentage (±SD) of *in vitro* cell survival with respect to the concentration of the B_8N cluster compounds **1**, **4**, **5** and **6**.

$k = 0.2\ day^{-1}$, corresponding to a half life of 14 days. It is possible to prepare a stock solution of all compounds with A concentration of 3.48×10^{-3} M (300 μg boron ml^{-1}).

Toxicity studies

Toxicity was measured in an *in vitro* test. 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*.[24] *In vitro* toxicity effects of B_8N clusters **1** and **4–6** against B16 melanoma cells are shown in Fig. 2. All compounds were tested up to a maximum concentration of boron (300 μg boron ml^{-1}). *In vitro* toxicity was evaluated by exposing B16 melanoma cells for 24 h to the test compounds, and comparing the number of surviving cells to the number of surviving cells not exposed to the test compounds. The *in vitro* toxicity of cluster **1** has an LD_{50} value of around 700 μM (Fig. 2), whereas B_8N cluster **1** containing porphyrins or aryl derivatives has LD_{50} values of around 650 and 880 μM, respectively.[16] Even at the higher levels of boron concentration, no toxicity from the drugs **4–6** was observed. The *in vitro* toxicities of these compounds with LD_{50} values below 574 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 the first azanonaborane cluster containing monosaccharides by two and one step processes. When compared with previously tested azanonaboranes, their lower *in vitro* toxicity, higher water solubility and higher stability at room temperature are advantageous over the previously reported azanonaboranes. The B_8N clusters **4–6** appear not to be toxic over a wide range of boron concentrations even at higher boron concentration (300 μg boron ml^{-1}, corresponding to a compound concentration of 3.48 mM). 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

Materials and methods

The reagents and dry solvents were used as presented directly without further purification. Acetobromo-α-D-glucose, acetobromo-α-D-galactose, and D-ribose were commercially available. The B_8N cluster **1** was prepared as described in the literature.[14] Plate chromatography was conducted on silica gel 60 F_{254} (Merck). Elemental analyses were performed by a Perkin-Elmer 2400 automatic elemental analyzer. Melting points were recorded using a Koeffler heated stage microscope and are uncorrected. The measurements for NMR (^{11}B, ^{1}H and ^{13}C) were carried out on a Bruker DPX 200 spectrometer. The chemical shifts δ are given in ppm relative to $\Xi = 200$ MHz for $\delta(^{1}H)$ (nominally $SiMe_4$), $\Xi = 50$ MHz for $\delta(^{13}C)$ (nominally $SiMe_4$), and $\Xi = 32.08$ MHz for $\delta(^{11}B)$ (nominally F_3BOEt_2) in D_2O or CD_3CN. IR (cm^{-1}) spectra were determined as KBr discs on a Biorad FTS-7 spectrometer. Electron spray ionization (ESI) mass spectra were recorded with a Bruker Esquire in CH_3OH. ^{11}B and boron-bonded ^{1}H data are found in Table 1.

Syntheses

General procedure for 4-(2,3,4,6-tetra-*O*-acetyl)-(α-D-glucopyranosylaminobutylamine)-(*N*-2, 3, 4,6-tetra-*O*-acetyl-α-glucopyranosylaminobutyl)azanonaborane(11) 2 and 4-(2,3,4,6-tetra-*O*-acetyl)-(α-D-galactopyranosylaminobutylamine)-(*N*-2, 3, 4, 6-tetra-*O*-acetyl-α-galactopyranosylaminobutyl)azanonaborane(11) 3. A solution of B_8N cluster **1** (1 mmol) was added to a solution of acetobromo-α-D-glycoside (2 mmol) in 20 ml anhydrous CH_3CN with one drop of triethylamine under a nitrogen atmosphere. The mixture was stirred for 8 h at room temperature. The solution was filtered off and all volatile components of the filtrate were removed under vacuum to give a yellow oil. The residue was chromatographed on silica gel using THF and CH_2Cl_2 (1 : 1) as eluent to yield the desired product. They were used without further purification.

2: Yield (0.35 g, 85%) as a colorless oil; $R_f = 0.65$; ν_{max}(KBr disc) cm^{-1} 3278s (NH_2/NH), 2525s (BH), 1752s (OAc), 1658w (δ, NH_2/NH), 1340s (BN), 2969m, 2889m, 1476m, 1452m, 1405m, 1151m, 1118s, 747m, (δ, γ, CH_2 groups); δ_H(200 MHz; CD_3CN; Me_4Si) +6.8 (2H, bs, HN–), +5.45 (2H, bs, H-1), +4.81–3.63 (10H, m, other glucose protons), +4.12 (2H, bs, H_2N–B_8), +3.11 (6H, t, –H_2CHN), +3.06 (2H, t, CH_2NH_2–B_8), +2.23 (24H, bs, CH_3CO), +1.38–1.55 (8H, m, –CH_2CH_2–, –CH_2CH_2–); δ_C(200 MHz; CD_3CN; Me_4Si) 170.3–169.07 (8C, –$COCH_3$), 89.11, 88.93 (2C, C-1), 73.15, 72.99 (2C, C-2), 70.78, 70.52 (2C, C-3), 70.21, 68.87 (2C, C-4), 67.23, 66.23 (2C, C-5), 61.79, 61.39 (2C, C-6), 51.58 (CH_2HNB_8), 50.15 (CH_2NH_2–B_8), 49.38, 49.27 (2C, CH_2NH), 29.93, 29.14, 27.66, 26.65 (4C, –CH_2CH_2–), 20.98–20.29 (8C, –$COCH_3$); *m*/*z* (ESI) 936 (M, 37%; M − 4, 34%; M − 5, 35%).

3: Yield (0.38 g, 87%) as a colorless oil; $R_f = 0.62$; ν_{max}(KBr disc) cm^{-1} 3276s (NH_2/NH), 2528s (BH), 1749s (OAc), 1661w (δ, NH_2/NH), 1343s (BN), 2972m, 2884m, 1471m, 1456m, 1410m, 1155m, 1121s, 745m, (δ, γ, CH_2 groups); δ_H(200 MHz; CD_3CN; Me_4Si) +6.84 (2H, bs, HN–), +5.67 (2H, bs, H-1),

+5.16–4.04 (10H, m, other glucose protons), +4.32 (2H, bs, H_2N–B_8), +3.21 (6H, t, –H_2CHN), +3.05 (2H, t, CH_2NH_2–B_8), +2.24 (24H, m, CH_3CO), +1.38–1.58 (8H, m, –CH_2CH_2–, –CH_2CH_2–); δ_C(200 MHz; CD_3CN; Me_4Si) 170.86–170.1 (8C, –$COCH_3$), 92.5, 89.92 (2C, C-1), 74.39, 72.82 (2C, C-2), 69.9, 69.06 (2C, C-3), 68.92, 68.49 (2C, C-4), 67.49, 65.86 (2C, C-5), 62.49, 61.03 (2C, C-6), 51.67 (CH_2HNB_8), 50.25 (CH_2NH_2–B_8), 49.12, 49.23 (2C, CH_2NH), 29.87, 29.65, 27.74, 26.36 (4C, –CH_2CH_2–), 20.88–20.31 (8C, –$COCH_3$); *m*/*z* (ESI) 936 (M, 46%; M − 2, 36%; M − 4, 21%).

General procedure for (4-α-glucopyranosylaminobutylamine)-(*N*-α-glucopyranosylaminobutyl)azanonaborane(11) 4 and (4-α-galactopyranosylaminobutylamine)-(*N*-α-galactopyranosylaminobutyl)azanonaborane(11) 5. Compound **2** or **3** was stirred in freshly prepared CH_3ONa (20 ml) at −5 °C. After 1 h, all volatile components were removed under vacuum to give a colorless oil. The residue was chromatographed on silica gel using THF as eluent to yield the desired product.

4: Yield: (0.23 g, 65%) as a colorless solid (mp 132.7–134.5 °C); (Found: C, 40.12; H, 8.89; N 9.23. $B_8H_{54}C_{20}N_4O_{10}$ requires C, 40.24; H, 9.05; N, 9.38%); ν_{max}(KBr disc) cm^{-1} 2924s (OH), 3239s (NH_2/NH), 2525s (BH), 1585s (δ, NH_2/NH), 1336s (BN), 2973m, 2895m, 1513m, 1463m, 1446m, 1163m, 1115s, 742m (δ, γ of CH_2 groups); δ_H(200 MHz; D_2O; Me_4Si) +6.15 (2H, bs, HN–), 4.85 (2H, bs, H-1, H-1′), 3.96 (2H, dd, H-6, H-6′), +3.85 (2H, bs, H_2N-B_8), 3.82 (2H, d, H-2, H-2′), 3.69 (2H, dd, H-6, H-6′), 3.58 (2H, dd, H-3, H-3′), 3.53 (2H, t, H-4, H-4′), 3.7–3.42 (2H, m, H-5, H-5′), +2.86 (2H, t, –H_2CHN–B_8), +2.75 (4H, m, –H_2CNH), +2.72 (2H, m, –H_2CH_2N–B_8), +1.43–1.65 (8H, m, –CH_2CH_2–, –CH_2CH_2–); δ_C(200 MHz; D_2O; Me_4Si), 89.52 (2C, C-1), 82.23 (2C, C-2), 76.23 (2C, C-3), 73.25 (2C, C-4), 68.99 (2C, C-5), 63.94 (2C, C-6), 51.05 (CH_2HNB_8), 50.14 (H_2CH_2N–B_8), 48.12, 47.25 (2C, CH_2NH), 30.2, 29.32, 28.25, 27.05 (4C, –CH_2CH_2–); *m*/*z* (ESI) 598 (M, 65%; M−2, 52%; M − 4, 43%).

5: Yield: (0.28 g, 73%) as a colorless solid (mp 135.0–136.5 °C); (Found: C, 40.03; H, 8.97; N 9.11. $B_8H_{54}C_{20}N_4O_{10}$ requires C, 40.24; H, 9.05; N, 9.38%); ν_{max}(KBr disc) cm^{-1} 2925s (OH), 3238s (NH_2/NH), 2527s (BH), 1605w (δ, NH_2/NH), 1335s (BN), 2976m, 2894m, 1512m, 1458m, 1438m, 1152m, 1113s, 745m (δ, γ of CH_2 groups); δ_H(200 MHz; D_2O; Me_4Si) +6.14 (2H, bs, HN–), 4.89 (2H, bs, H-1, H-1′), 3.99 (2H, dd, H-6, H-6′), 3.86 (2H, d, H-2, H-2′), +3.76 (2H, bs, H_2N–B_8), 3.74 (2H, dd, H-6, H-6′), 3.65 (2H, dd, H-3, H-3′), 3.57 (2H, t, H-4, H-4′), 3.72–3.41 (2H, m, H-5, H-5′), +2.73 (2H, t, –H_2CHN–B_8), +2.7 (4H, m, –H_2CNH), +2.64 (2H, m, –H_2CH_2N–B_8), +1.43–1.65 (8H, m, –CH_2CH_2–, –CH_2CH_2–); δ_C(200 MHz; D_2O; Me_4Si), 89.82 (2C, C-1), 82.56 (2C, C-2), 77.11 (2C, C-3), 73.41 (2C, C-4), 69.26 (2C, C-5), 63.64 (2C, C-6), 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) = 598 (M, 52%; M − 2, 46%, M − 4, 21%).

(4-α-Ribofuranosylaminobutylamine)-(*N*-α-ribofuranosylaminobutyl)azanonaborane(11) 6. A solution of B_8N cluster **1** (0.26 g, 1 mmol) was added to a solution of D-ribose (0.28 g, 2 mmol) in 20 ml anhydrous CH_3OH with one drop of glacial acetic acid under a nitrogen atmosphere. The mixture was stirred for 8 h at room temperature. All volatile components of the solution were removed under vacuum to give a colorless oil. The residue was chromatographed on silica gel using THF as eluent to yield the desired product **6**.

6: Yield: (0.42 g, 78%) as a colorless solid (mp 128.0–129.4 °C); (Found: C, 40.08; H, 9.11; N, 10.31. $B_8H_{50}C_{18}N_4O_8$ requires C, 40.26; H, 9.32; N, 10.43%); ν_{max}(KBr disc) cm^{-1} 2932s (OH), 3238s (NH_2/NH), 2538s (BH), 1597w (δ, NH_2/NH), 1332s (BN), 2978m, 2895m, 1517m, 1468m, 1449m, 1167m, 1112s, 744m (δ, γ of CH_2 groups); δ_H(200 MHz; D_2O; Me_4Si), +6.18 (2H, bs, HN–), 5.12 (2H, bs, H-1), 3.99 (2H, dd, H-6, H-6′), +3.95 (2H, bs, H_2N–B_8), 3.86 (2H, d, H-2, H-2′), 3.74 (2H, dd, H-6, H-6′), 3.65 (2H, dd, H-3, H-3′), 3.57 (2H, t, H-4, H-4′), 3.72–3.41 (2H, m, H-5, H-5′), +2.88 (2H, t, –H_2CHN–B_8), +2.76 (4H, m, –H_2CNH), +2.61 (2H, m, –H_2CH_2N–B_8), +1.45–1.65 (8H, m, –CH_2CH_2–, –CH_2CH_2–); δ_C(200 MHz; D_2O; Me_4Si), 87.95 (2C, C-1), 82.43 (2C, C-2), 75.12 (2C, C-3), 69.5 (2C, C-4), 64.14 (2C, C-5), 52.05 (CH_2HNB_8), 50.86 (H_2CH_2N–B_8), 48.29, 49.14 (2C, CH_2NH), 30.69, 29.98, 28.95, 27.85 (4C, –CH_2CH_2–); *m*/*z* (ESI) 538 (M, 83%; M − 2, 74%; M − 4, 59%).

Biological studies

All tests were repeated 2–3 times. For each compound Petri dishes were seeded with B16 melanoma cells grown in 9.69 g l^{-1} Eagle minimum essential medium (Biochrom KG) supplemented (EMEM) 10 ml l^{-1} penicillin–streptomycin (10 000 U–10 000 μg ml^{-1}, Biochrom KG), 2.2 g l^{-1} $NaHCO_3$ and 10% fetal calf serum (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 boron compounds and incubated for an additional 24 h at 37 °C. 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 phosphate-buffered saline (PBS), dyed with GIEMSA stain for 10–15 minutes and washed again with ethanol.

Acknowledgements

The financial support from the University of Bremen, Germany is gratefully acknowledged.

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