Carboranyl Bisglycosides for the Treatment of Cancer by Boron Neutron Capture Therapy

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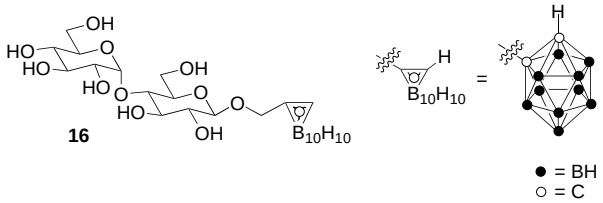
Boron neutron capture therapy is a special type of radiotherapy for the treatment of cancer by using boron compounds. Problems often arise from the low water solubility of these compounds, their unselective uptake into the cancer cells, and their toxicity. Here we describe the novel water-soluble ortho*-carboranyl bisglycosides **7** and **10** containing either lactose or glucose and the mixed bisglycosides **1** and **28** containing glucose, mannose, and galactose. The carboranyl bisglycosides show almost no toxicity toward bronchial carcinoma cells of line A549 up to a concentration of 0.50 mM. As anticipated, these compounds exhibit nearly no uptake into C6 glioma cells; they can therefore be used for a selective delivery into malignant cells by using conjugates of glycohydrolases and monoclonal antibodies which bind to tumor-associated antigens, since by enzymatic hydrolysis the bisglycosides are transformed into lipophilic compounds.*

KEYWORDS:

antitumor agents · boron neutron capture therapy · carboranes · glycosides · prodrugs

Introduction

Boron neutron capture therapy is under active investigation for the treatment of various types of cancer.[1, 2] The approach is based on the nuclear reaction of ^{10}B with thermal neutrons to produce high-energy $^{2}He^{2+}$ and $^{7}Li^{3+}$ particles. For a successful application of this method a sufficient amount of boron (20–30 μg per gram of tumor tissue) must be present in the malignant cells.[3] Substituted carboranes are highly suitable for delivering boron due to their high boron content and their stability in aqueous media. However, the use of most of these carboranes is somehow limited due to their low water solubility and their pronounced toxicity. We have recently shown that the water solubility of the highly lipophilic *ortho*-carborane unit can be greatly improved by the formation of glycosides such as carboranyl maltoside **16**.[4] Furthermore, these compounds are



16

taken up into the tumor tissue to a high extent.[5] This accumulation is probably caused by an interaction of the lipophilic carborane moiety with the phospholipid double layer of the membrane whilst the sugar moiety stays outside. We have also prepared novel fluorine-containing carboranes to allow a noninvasive determination of the boron compounds in the tumor tissue by ^{19}F NMR spectroscopy.[6]

However, a selective uptake of these novel carboranyl glycosides into tumor cells cannot be expected. We therefore designed a new type of carborane compounds that have sugar molecules on both sides of the lipophilic carborane unit. We anticipated that these compounds will not be incorporated into the cell membranes of neither normal nor malignant cells. However, using conjugates of glycohydrolases and monoclonal antibodies that bind to tumor-associated antigens would permit a selective cleavage of one or both sugar moieties to allow the remaining lipophilic carborane unit to either penetrate the cell membrane or to be incorporated into it.[7] We therefore prepared the *ortho*-carboranyl bisglycosides **7** and **10** containing either lactose or glucose, respectively, and the mixed bisglycosides **1**

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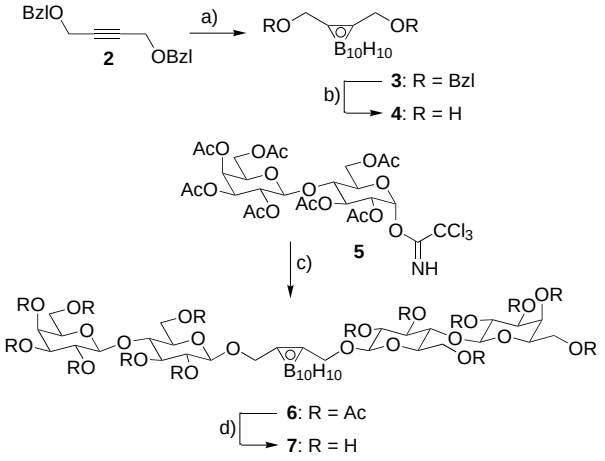
and **28** containing glucose as well as mannose and glucose as well as galactose, respectively. Thereafter we determined the cytotoxicity of the novel compounds, their accumulation in tumor cells, and—in combination with neutron irradiation—their lethal effect on tumor cells to test their suitability for cancer therapy by neutron capture.

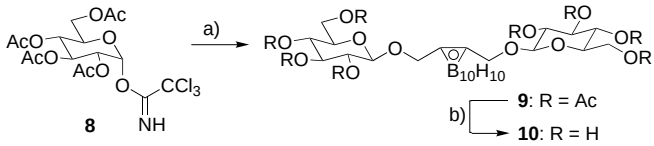
Results and Discussion

Syntheses

For the synthesis of the *ortho*-carboranyl bisglycosides **7** and **10**, decaborane(14) was heated in acetonitrile at reflux for 30 min to give the adduct $B_{10}H_{12}\cdot 2CH_3CN$,[8] which was then treated with the benzylated butynyl diol **2**[9] to give the carboranyl diol **3** in 48% yield; hydrogenolysis with palladium on charcoal as the catalyst led to the diol **4**[10] in 82% yield. Stereoselective reaction of **4** with lactose trichloroacetimidate (**5**)[11] in the presence of $BF_3\cdot Et_2O$ to give **6**, followed by solvolysis using sodium methoxide in methanol, afforded the desired carboranyl bislactoside **7** in 46% overall yield as a single diastereomer with the β configuration at the newly formed glycosidic bond (Scheme 1). Similarly, reaction of the carboranyl diol **4** with glucose trichloroacetimidate (**8**) and subsequent deprotection afforded carboranyl bisglucoside **10** in 54% overall yield (Scheme 2).

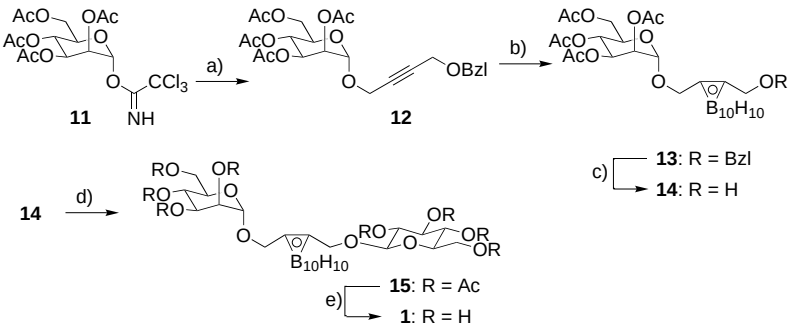


Scheme 1. *Synthesis of* ortho*-carboranyl bisglycoside **7**. a) $B_{10}H_{14}$, CH_3CN, reflux, then **2** in toluene, 48%; b) H_2 (3 bar), Pd/C, ethyl acetate, methanol, RT, 82%; c) **4**, $BF_3\cdot Et_2O$, dichloromethane, RT, 68%; d) NaOMe, MeOH, RT, 74%. Bzl = benzyl.*



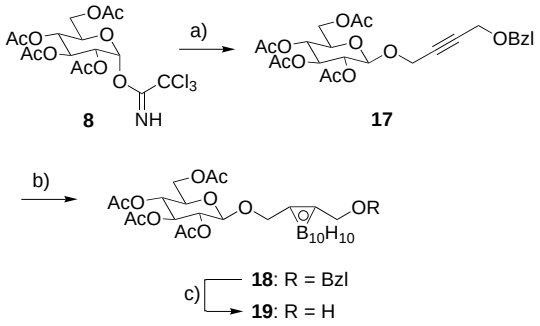
Scheme 2. *Synthesis of* ortho*-carboranyl bisglycoside **10**. a) **4**, $BF_3\cdot Et_2O$, dichloromethane, RT, 83%; b) NaOMe, MeOH, RT, 65%.*

For the synthesis of the unsymmetrical carboranyl bisglycoside **1**, the alkynyl mannoside **12** was prepared from mannose trichloroacetimidate (**11**) and 4-benzyloxy-2-butyn-1-ol[12] in 72% yield by using trimethylsilyl trifluoromethanesulfonate (TMSOTf) as promotor. Reaction of **12** with decaborane(14) gave the carboranyl mannoside **13**, which was then deprotected by hydrogenolysis with Pd on charcoal as the catalyst to afford the mannose carboranyl alcohol **14** in 37% overall yield. Reaction of **14** with glucose trichloroacetimidate (**8**) followed by solvolysis using sodium methoxide in methanol gave the desired unsymmetrical carboranyl bisglycoside **1** in 44% yield as a single diastereomer with the α configuration at the mannosidic and the β configuration at the glucosidic bond (Scheme 3).



Scheme 3. *Synthesis of the unsymmetrical carboranyl bisglycoside **1**. a) 4-Benzyloxy-2-butyn-1-ol, TMSOTf, dichloromethane, 0 °C, 72%; b) $B_{10}H_{14}$, CH_3CN, reflux, then **12** in toluene, reflux (**13** was not fully purified); c) H_2 (3 bar), Pd/C, ethyl acetate, methanol, RT, 37% over two steps; d) **8**, $BF_3\cdot Et_2O$, dichloromethane, RT, 68%; e) NaOMe, MeOH, RT, 66%.*

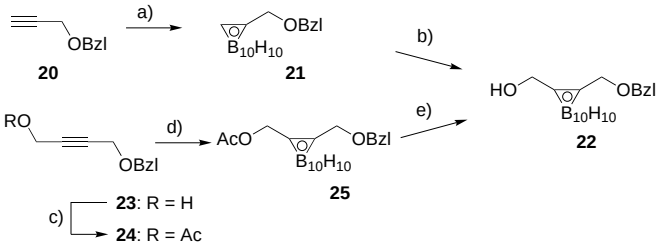
For the synthesis of the carboranyl bisglycoside **28** we first prepared the alkynyl glucoside **17** in 65% yield by reaction of 4-benzyloxy-2-butyn-1-ol with glucose trichloroacetimidate (**8**) using $BF_3\cdot Et_2O$ as promotor. Reaction of **17** with decaborane(14) gave **18**, which was then debenzylated by hydrogenolysis to afford the glucose carboranyl alcohol **19** in 33% yield over two steps (Scheme 4).



Scheme 4. *Synthesis of glucose carboranyl alcohol **1**. a) 4-Benzyloxy-2-butyn-1-ol, $BF_3\cdot Et_2O$, dichloromethane, RT, 69%; b) $B_{10}H_{14}$, CH_3CN, reflux, then **17** in toluene, reflux (**18** was not fully purified); c) H_2 (3 bar), Pd/C, ethyl acetate, methanol, RT, 33% over two steps.*

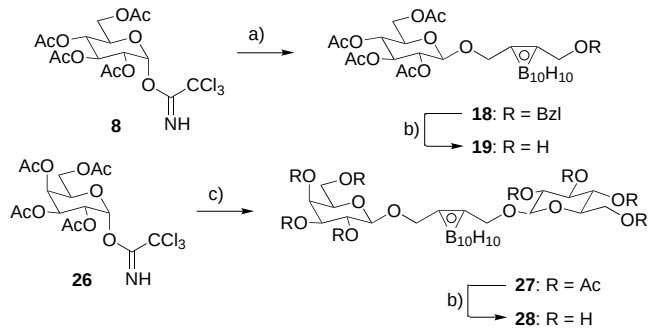
In the synthesis described above, the yield for the transformation of the alkynyl glucoside **17** into the glucose carboranyl alcohol **19** was only moderate. We therefore developed an access to **19** via the carboranyl alcohol **22**, which was prepared

in two different ways: First, alkyne **20** was transformed into the protected carboranyl alcohol **21** in 60% yield using decaborane(14); subsequent reaction with paraformaldehyde and tetrabutylammonium fluoride trihydrate (TBAF · 3 H_2O) in THF[13] gave the alcohol **22** in 87% yield. In the second approach, alkynol **23** was acetylated to give **24**, which was then treated with decaborane(14) and deprotected to yield the alcohol **22** in 44% overall yield (Scheme 5).



Scheme 5. *Synthesis of carboranyl alcohol **22**. a) $B_{10}H_{14}$, CH_3CN, reflux, then **20** in toluene, 60%; b) paraformaldehyde, TBAF · 3 H_2O, THF, RT, 87%; c) Ac_2O, DMAP, dichloromethane, RT, 89%; d) $B_{10}H_{14}$, CH_3CN, reflux, then **24** in toluene, 63%; e) NaOMe, MeOH, RT, 79%. DMAP = 4-(dimethylamino)pyridine.*

Glycosidation of the carboranyl alcohol **22** with glucose trichloroacetimidate (**8**) followed by hydrogenolysis of the benzyl group gave glucose carboranyl alcohol **19** in 50% yield over two steps. Subsequent reaction with galactose trichloroacetimidate (**26**) afforded bisglycoside **27** in 50% yield, which was then deprotected by solvolysis to lead to the desired carboranyl bisglycoside **28** in 89% yield (Scheme 6).



Scheme 6. *Synthesis of carboranyl bisglycoside **28**. a) **22**, $BF_3 \cdot Et_2O$, dichloromethane, RT (**18** was not fully purified); b) H_2 (3 bar), Pd/C, ethyl acetate, RT, 50% over two steps; c) **19**, $BF_3 \cdot Et_2O$, dichloromethane, RT, 50%; d) NaOMe, MeOH, RT, quant.*

Structure determination

The structures of the new compounds were mainly determined by ^1H and ^{13}C NMR spectroscopy. As is typical for carboranes, a broad signal for the protons attached to boron is found in all ^1H NMR spectra at $\delta = 0.5 - 3.5$. In addition, the IR spectra of these compounds displayed a strong B–H stretch signal at ca. 2590 cm^{-1}. For the protons at the anomeric centers of the β-glycosides, doublets with coupling constants of $J = 7.6 - 8.0$ Hz were observed. For example, the signal of the 1-H atom of the carboranyl bisglucoside **10** appears as a doublet at $\delta = 4.40$ with a coupling constant of $J = 7.6$ Hz, clearly indicating the β configuration. In contrast, for 1-H of the α-mannosides (e.g. **12**), a doublet with a small coupling constant $J = 1.6$ Hz at $\delta = 5.04$ is found. The diastereotopic protons of the sugar CH_2-O groups resonate as separated doublets with a large coupling constant of $J = 12.0 - 13.2$ Hz. For example, two doublets with a coupling of 12.4 Hz at $\delta = 4.28$ and $\delta = 4.49$ are found in the ^1H NMR spectrum of carboranyl bisglucoside **10**.

In vitro studies

Toxicities and enzymatic assay: The cytotoxicity of the carboranyl bisglycosides **1** and **10** was determined in cloning efficiency tests on human bronchial carcinoma cells of line A549.[14, 15] The incubation was performed in a serum-free medium (Ultra Culture) without the addition of fetal calf serum (FCS) or serum substitute (basal medium supplement, BMS) to prevent cleavage of the glucosidic bonds by glucohydrolase known to be present in the serum.[16] The carboranyl bisglucoside **10** as well as the mixed carboranyl bisglycoside of mannose and glucose **1** displayed almost no cytotoxicity up to a concentration of 0.50 mM. In contrast, hydroxymethylcarboranes are considerably cytotoxic with an ED_{50} value of 45 μM.[6] It was shown that the galactosyl moiety of carboranyl bisglycoside **28** was cleaved off with β-galactosidase (*Escherichia coli*) in aqueous solution at pH 7.3 and 37 °C.

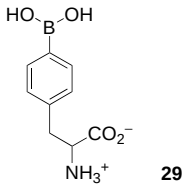
In vitro boron incorporation into B-16 melanoma cells: Our concept for a selective boron neutron capture therapy requires that the carboranyl bisglycosides are not incorporated into the cells. Boron incorporation into B-16 cells was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). The cells were cultured in Falcon dishes (150 mm ∅) until they had grown to fill the dishes ($4.0 - 5.0 \times 10^6$ cells per dish). The cells were incubated for 3 – 24 h with Eagle-MEM medium containing the compounds (boron concentration: 10.8 ppm). At 3, 12, and 24 h, the cells were washed three times with PBS(−) and processed for the determination of boron concentration by ICP-AES. The results are shown in Table 1. Both mixed bisglyco-

Table 1. *Boron incorporation into B-16 melanoma cells.[a]*

	Incubation time with the boron compound			
	3 h	12 h	24 h	24 [h][b]
mixed glycoside **1**	n.d.[c]	0.72 ± 0.082	n.d.[c]	1.0 ± 0.022
bisglucoside **10**	0.48 ± 0.050	0.69 ± 0.061	n.d.[c]	0.78 ± 0.052
maltoside **16**	6.1 ± 0.074	10 ± 0.58	20 ± 1.1	–
BPA (**39**)	1.4 ± 0.21	1.9 ± 0.060	3.1 ± 0.31	5.4 ± 0.080

[a] The cells were incubated for 3 – 24 h with Eagle-MEM medium containing the boron compounds (boron concentration: 10.8 ppm). The unit of the boron concentration is 10^{-6} g boron per 10^7 cells. Each value represents the mean ± SE of triplicate experiments. SE = standard error. [b] The boron concentration in the medium was 54 ppm. [c] n.d. = not determined. The boron concentrations were too low to be determined by ICP-AES.

side **1** and bisglucoside **10** exhibited lower uptake into B-16 cells in comparison with maltoside **1**. The boron concentrations in B-16 cells after administration of **10** and **1** were lower than 1.0 ppm, whereas with maltoside **16** 6.1 ppm at 3 h and 20.0 ppm at 24 h after administration were observed. When the cells were incubated in the medium containing a higher boron concentration (54.0 ppm) for 24 h, the boron uptakes into B-16 cells with mixed bisglycoside **1** and bisglucoside **10** were 1.0 ppm and 0.78 ppm, respectively. In comparison, with the amino acid 4-dihydroxyborylphenylalanine (BPA; **29**),[2, 17] which is used as a standard, an uptake of 3.1 ppm was determined.



29

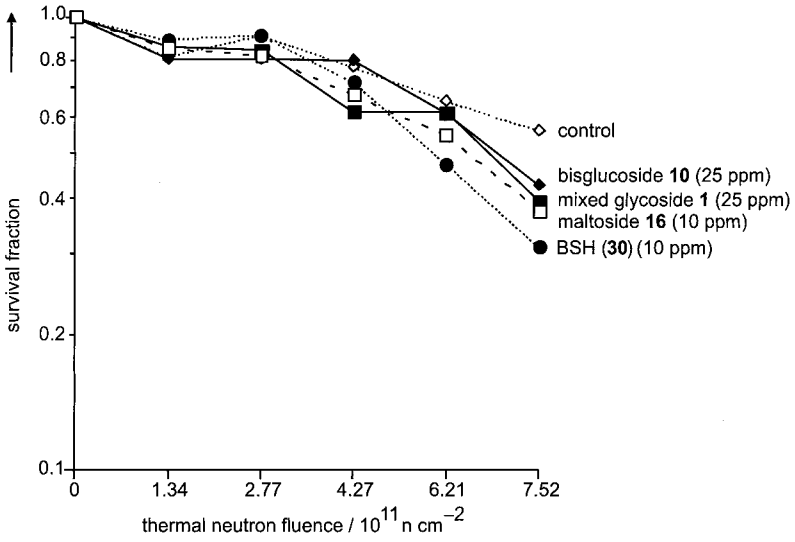


Figure 1. *Survival fraction of C6 glioma cells after exposure to boron-containing compounds and thermal neutron irradiation. No boron compound was applied in the control experiment. The concentrations indicated are based on the boron content.*

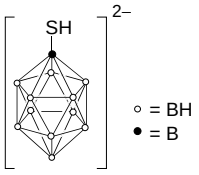
In vitro boron incorporation into C6 glioma cells: The C6 glioma cells were incubated with the medium containing various concentrations of the compounds (10, 25, and 40 ppm boron) for 6 h and the boron concentration in the cells was determined by ICP-AES. The results are summarized in Table 2. A high boron uptake (48×10^{-6} g boron per 10^7 cells) was observed in the case of carboranyl maltoside **16** (at 10 ppm in the medium), wheras the carboranyl bisglucoside **10** as well as the mixed bisglycoside **1** exhibited very low uptakes. Actually, a boron uptake of only 1.9 ppm was observed when the cells were incubated with the medium containing the mixed bisglycoside **1** (40 ppm of boron concentration in solution). In the case of bisglucoside **10**, the boron concentrations in the cells were too low to be determined by ICP-AES.

Table 2. *Boron incorporation into C6 cells.*[a]

	Concentration of the boron compound[b]		
	10 ppm	25 ppm	40 ppm
mixed glycoside **1**	n.d.[c]	0.40 ± 0.32	1.9 ± 0.66
bisglucoside **10**	n.d.[c]	n.d.[c]	n.d.[c]
maltoside **16**	48 ± 6.5	–	–

[a] The cells were incubated for 6 h with Eagle-MEM medium containing various concentrations of the boron compounds. The unit of the values is 10^{-6} g boron per 10^7 cells. Each value represents the mean $\pm$ SE of triplicate experiments. [b] Boron concentrations in the medium. [c] The boron concentrations were too low to be determined by ICP-AES.

In vitro survival study of C6 glioma cells after thermal neutron irradiation: Figure 1 shows the fraction of the cells that survived after the thermal neutron irradiation. A thermal neutron beam (thermal neutron fluence: 7.52×10^{11} n cm^{-2}) had some lethal effect on C6 glioma cells after they had been exposed to carboranyl bisglucoside **10**, mixed carboranyl bisglycoside **1**, and maltoside **16**, respectively. In comparison, the bisglycosides displayed a lower lethal effect toward the cells than the maltoside **16**. However, the boranyl thiol mercaptoundecahydrododecaborate (BSH; **30**),[2] which was applied in ^{10}B-enriched form, had the strongest lethal effect after exposure to the cancer cells and their subsequent treatment with the neutron beam.



30

Conclusions

For the treatment of cancer by using the neutron capture therapy, boron compounds were applied which accumulated in cells. However, a differentiation between malignant and normal cells has not been possible so far. In a new concept we developed novel carboranyl bisglycosides that do not penetrate the cell membranes, but could be selectively transformed at the surface of malignant cells into boron compounds, which can then be taken up by the cells. The transformation can be achieved by using a conjugate of an enzyme and a monoclonal antibody, which selectively binds to the tumor cell surface. The new compounds are highly water-soluble and show a low cytotoxicity in cell culture tests. In the in vitro studies, the boron incorporation with carboranyl bisglucoside **10** and mixed bisglycoside **1** into B-16 melanoma and C6 cells was very low in comparison to the application of maltoside **16** and BPA (**29**). Irradiation with a thermal neutron beam had only a slightly higher lethal effect on C6 glioma cells after exposure to the

boron compounds in comparison with the control experiment. The results clearly indicate that the bisglycosides are not taken up into the cells due to the two hydrophilic sugar moieties at both ends of the lipophilic carborane core. As shown in the enzymatic assay, these compounds together with an antibody–glucohydrolase conjugate could be used for the selective delivery of boron into malignant cells by the enzymatic formation of a lipophilic carborane moiety directly at the tumor cell surface, which can then penetrate the cell membrane.

Experimental Section

Synthesis of the carboranyl bisglycosides

General: ^{1}H NMR and ^{13}C NMR spectroscopy: Varian XL-500, XL-300 and VXR-200, Bruker AM-300; multiplicities were determined with an APT pulse sequence. Mass spectrometry: Varian MAT 311A. The prepared boron compounds contain the normal isotopic distribution of boron. A broad family of peaks is therefore detected together with the highest peaks, which correlate to ^{11}B as the main isotope being cited. IR spectroscopy: Bruker IFS 25. Elemental analyses were carried out in the analytical laboratory of the University of Göttingen.

All solvents were distilled prior to use. Reagents and materials were obtained from commercial suppliers and were used without further purification. All reactions were carried out under argon and monitored by TLC (Macherey–Nagel & Co., Polygram SIL G/UV$_{254}$). Products were isolated by column chromatography on silica gel (Merck).

1,2-Bis(benzyloxymethyl)-1,2-dicarba-*closo*-dodecaborane (3): Decaborane(14) (768 mg, 6.28 mmol, 1.2 equiv) in CH_3CN (10 mL) was heated at reflux for 30 min, then a solution of alkyne **2** (1.43 g, 5.37 mmol) in toluene (10 mL) was added and heating was continued for 39 h. For work-up methanol (1 mL) was added and the solution was heated for 10 min at reflux and then cooled to room temperature. Solvents were evaporated in vacuo and the residue was purified by gradient column chromatography (petroleum ether/ethyl acetate, 100:1 → 10:1) to afford **3** (990 mg, 2.57 mmol, 48%) as a white solid. R_f (petroleum ether) = 0.25; IR (KBr): $\tilde{\nu}$ = 3084, 3060, 3030, 3012 (C–H), 2952, 2918, 2868 (C–H), 2634, 2604, 2564 (B–H), 742, 698 (arom); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.89 (s, 4H, CH_2C_{CB}), 4.41 (s, 4H, CH_2Ph), 7.16–7.38 (m, 10H, Ph-H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 69.86 (CH_2C_{CB}), 73.28 (CH_2Ph), 76.37 (C_{CB}), 127.53, 128.07, 128.50, 136.63 (arom); elemental analysis (%): calcd for $C_{18}H_{28}B_{10}O_2$ (384.5): C 56.23, H 7.34; found: C 56.50, H 7.53.

1,2-Bis(hydroxymethyl)-1,2-dicarba-*closo*-dodecaborane (4): A mixture of borane **3** (1.23 g, 3.20 mmol) and Pd/C (348 mg, 10% Pd) in ethyl acetate (22 mL) and methanol (10 mL) was shaken in a hydrogen atmosphere (2.5 bar) at room temperature for 18 h. Filtration, evaporation of the solvents, and filtration through a short column of silica (petroleum ether/ethyl acetate, 2:1) gave **4** (537 mg, 2.63 mmol, 82%) as a white solid. IR (KBr): $\tilde{\nu}$ = 3360 (O–H), 2954, 2884 (C–H), 2590 (B–H); ^{1}H NMR (200 MHz, [D$_4$]methanol): δ = 4.10 (s, 4H, 2 × CH_2); ^{13}C NMR (50 MHz, [D$_4$]methanol): δ = 64.18 (CH_2), 81.78 (C_{CB}); MS (EI): *m/z* (%): 186 (100) $[M-H_2O]^+$ (calcd for $C_4H_{16}B_{10}O_2$: 204.3).

1,2-Bis(2a,3a,6a,2b,3b,4b,6b-hepta-*O*-acetyl-β-D-lactopyranosylmethyl)-1,2-dicarba-*closo*-dodecaborane (6): A solution of the carboranyl diol **4** (239 mg, 1.17 mmol) in dichloromethane (20 mL) was stirred at room temperature for 10 min over molecular sieves (3 Å), then a solution of lactose trichloroacetimidate (**5**) (2.58 g, 3.30 mmol, 2.8 equiv) in dichloromethane (5 mL) was added and the mixture was again stirred for 10 min. Subsequently, $BF_3 \cdot Et_2O$ (0.20 mL, 1.6 mmol) was added and stirring at room temperature was continued for 18.5 h. The reaction was quenched by addition of a 3:1 mixture of triethylamine and methanol (1 mL), then the organic layer was washed with water, brine, dried with sodium sulfate and concentrated in vacuo. Purification by gradient column chromatography (ethyl acetate/petroleum ether, 3.5:1 → 2:1) gave **6** (1.14 g, 0.79 mmol, 68%) as a white solid. R_f (petroleum ether/ethyl acetate, 1:2) = 0.43; $[\alpha]_D^{20} = -27.0$ (c = 0.5, chloroform); IR (KBr): $\tilde{\nu}$ = 2962, 2893 (C–H), 2589 (B–H), 1761 (C=O), 1371 ($OCOCH_3$), 1224 (C–O); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.95, 2.02, 2.03, 2.04, 2.06, 2.12, 2.13 (7s, 42H, 14 × CH_3), 3.61 (ddd, J = 10.1, 5.3, 2.1 Hz, 2H, 5a-H), 3.80 (t, J = 9.1 Hz, 2H, 4a-H), 3.86–3.89 (m, 2H, 5b-H), 3.94 (d, J = 12.8 Hz, 2H, C*H*(a)H(b)C_{CB}), 4.05 (dd, J = 11.2, 7.5 Hz, 2H, 6b-H(a)), 4.09 (dd, J = 8.9, 5.3 Hz, 2H, 6a-H(a)), 4.12 (dd, J = 8.9, 6.2 Hz, 2H, 6a-H(b)), 4.22 (d, J = 12.8 Hz, 2H, CH(a)*H*(b)C_{CB}), 4.45 (dd, J = 12.2, 1.9 Hz, 2H, 6b-H(b)), 4.47 (d, J = 8.0 Hz, 2H, 1a-H or 1b-H), 4.49 (d, J = 8.5 Hz, 2H, 1a-H or 1b-H), 4.87 (dd, J = 9.6, 7.8 Hz, 2H, 2a-H), 4.95 (dd, J = 10.3, 3.5 Hz, 2H, 3b-H), 5.08 (dd, J = 10.5, 7.9 Hz, 2H, 2b-H), 5.17 (t, J = 9.2 Hz, 2H, 3a-H), 5.33 (dd, J = 3.4, 0.9 Hz, 2H, 4b-H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 20.48, 20.58, 20.60, 20.66, 20.74, 20.85 (CH_3), 60.69, 61.74 (C-6a, C-6b), 68.60 (CH_2C_{CB}), 66.54, 69.04, 70.62, 70.88, 71.08, 72.24, 72.89, 75.82 (C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b), 75.70 (C_{CB}), 100.3, 100.9 (C-1a, C-1b), 169.0, 169.5, 169.6, 170.0, 170.1 170.3, 170.3 (OAc); MS (DCI): *m/z* (%): 1460 (100) $[M+NH_4+H]^+$, 1459 (98) $[M+NH_4]^+$; elemental analysis (%): calcd for $C_{56}H_{84}B_{10}O_{36}$ (1441): C 46.67, H 5.87; found: C 46.38, H 5.62.

1,2-Bis(β-D-lactopyranosylmethyl)-1,2-dicarba-*closo*-dodecaborane (7): A solution of carboranyl bislactoside **6** (585 mg, 0.41 mmol) in methanol (10 mL) was added to a solution of sodium methoxide (0.10 mL, 5.4 M). The mixture was stirred for 135 min at room temperature and the reaction was quenched by addition of Amberlyte IR-120 resin (H^+ form). The mixture was filtered, the solvent evaporated in vacuo, and the residue purified by column chromatography (methanol/chloroform, 1:1) to give **7** (256 mg, 0.30 mmol, 74%) as a slightly yellow foam. R_f (methanol/chloroform, 1:1) = 0.52; $[\alpha]_D^{20} = -27.0$ (c = 0.5, methanol); IR (KBr): $\tilde{\nu}$ = 3406 (O–H), 2924, 2890 (C–H), 2586 (B–H); ^{1}H NMR (500 MHz, [D$_4$]methanol): δ = 3.42 (ddd, J = 9.4, 4.4, 2.4 Hz, 2H, 5a-H), 3.47 (dd, J = 9.7, 3.3 Hz, 2H, 2b-H), 3.50 (t, J = 9.0 Hz, 2H), 3.52–3.55 (m, 2H), 3.57–3.62 (m, 4H), 3.69 (dd, J = 11.4, 4.5 Hz, 2H, 6a-H or 6b-H), 3.77 (dd, J = 12.4, 7.5 Hz, 2H, 6a-H or 6b-H), 3.80–3.84 (m, 4H, 6a-H or 6b-H, 4b-H), 3.90 (dd, J = 12.1, 2.3 Hz, 2H, 6a-H or 6b-H), 4.31 (d, J = 12.3 Hz, 2H, OCOC*H*(α)H(β)), 4.34 (d, J = 7.5 Hz, 2H, 1a-H or 1b-H), 4.45 (d, J = 8.0 Hz, 2H, 1a-H or 1b-H), 4.46 (d, J = 12.4 Hz, 2H, OCOCH(α)*H*(β)); ^{13}C NMR (200 MHz, [D$_4$]methanol): δ = 61.72, 62.46 (C-6a, C-6b), 69.94 (CH_2OCO), 70.21, 72.45, 74.31, 74.66, 76.38, 76.51, 76.97, 80.31 (C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b), 77.69 (C_{CB}), 103.5, 104.9 (C-1a, C-1b); MS (ESI): *m/z* (%): 877 (100) $[M+Na+H]^+$, 1728 (35) $[2M+Na-H]^+$ (calcd for $C_{28}H_{56}B_{10}O_{22}$: 852.9).

1,2-Bis(2a,3a,4a,6a-tetra-*O*-acetyl-β-D-glucopyranosylmethyl)-1,2-dicarba-*closo*-dodecaborane (9): A solution of glucose trichloroacetimidate (**8**) (3.22 g, 6.54 mmol, 4.0 equiv) and the carboranyl diol **4** (338 mg, 1.65 mmol) in dichloromethane (40 mL) was stirred for 1.5 h over molecular sieves (3 Å), then $BF_3 \cdot Et_2O$ (0.20 mL, 1.6 mmol) was added and stirring was continued for 15 h at room temperature. Work-up as described for **6** gave **9** (1.19 g, 1.37 mmol, 83%) as a white solid after purification by column chromatography (petroleum ether/ethyl acetate, 1:1). R_f (petroleum ether/ethyl acetate, 1:1) = 0.25; $[\alpha]_D^{20} = -41.4$ (c = 0.5, chloroform); IR (KBr): $\tilde{\nu}$ = 2964, 2898 (C–H), 2585 (B–H), 1759 (C=O), 1371 ($OCOCH_3$), 1234 (C=O); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 2.02, 2.03, 2.10, 2.11 (4s, 24H, 8 × CH_3), 3.70

(dd, $J = 10.1$, 4.5, 2.4 Hz, 2H, 5a-H), 3.99 (d, $J = 13.0$ Hz, 2H, C*H*(α)H(β)C_{CB}), 4.12 (dd, $J = 12.3$, 2.3 Hz, 2H, 6a-H(α)), 4.27 (dd, $J = 12.5$, 4.4 Hz, 2H, 6a-H(β)). 4.29 (d, $J = 12.9$ Hz, 2H, CH(α)*H*(β)C_{CB}), 4.52 (d, $J = 7.7$ Hz, 2H, 1a-H), 4.99 (dd, $J = 9.6$, 8.0 Hz, 2H, 2a-H), 5.06 (t, $J = 9.7$ Hz, 2H, 4a-H), 5.20 (t, $J = 9.5$ Hz, 2H, 3a-H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 20.54$, 20.63, 20.68 (CH_3), 61.48 (C-6a), 67.95, 70.75, 72.06, 72.28 (C-2a, C-3a, C-4a, C-5a), 68.85 (CH_2C_{CB}), 75.86 (C_{CB}), 100.8 (C-1a), 169.2, 169.3, 170.1, 170.5 (OAc); MS (DCI): *m/z* (%): 884 (100) $[M+NH_4+H]^+$; elemental analysis (%): calcd for $C_{32}H_{52}B_{10}O_{20}$ (864.9): C 44.44, H 6.06; found: C 44.37, H 5.83.

1,2-Bis(β-D-glucopyranosylmethyl)-1,2-dicarba-*closo*-dodecaborane (10): A solution of the carboranyl bisglucoside **9** (626 mg, 0.72 mmol) in methanol (20 mL) was treated with sodium methoxide (0.20 mL) for 150 min at room temperature as described for **7**. Twofold purification by column chromatography (ethyl acetate/methanol, 3:1; and dichloromethane/methanol, 5:2) gave the bisglucoside **10** (248 mg, 0.47 mmol, 65%) as a white foam. $[\alpha]_D^{20} = -20.2$ ($c = 0.5$, methanol); IR (KBr): $\tilde{\nu} = 3385$ (O–H), 2924, 2888 (C–H), 2588 (B–H); ^{1}H NMR (300 MHz, [D$_4$]methanol): $\delta = 3.18 - 3.36$ (m, 8H, 2a-H, 3a-H, 4a-H, 6a-H(α)), 3.61 – 3.67 (m, 2H, 5a-H), 3.84 – 3.88 (m, 2H, 6a-H(β)), 4.28 (d, $J = 12.4$ Hz, 2H, C*H*(a)H(b)C_{CB}), 4.40 (d, $J = 7.6$ Hz, 2H, 1a-H), 4.49 (d, $J = 12.4$ Hz, 2H, CH(a)*H*(b)C_{CB}); ^{13}C NMR (50 MHz, [D$_4$]methanol): $\delta = 62.65$ (C-6a), 69.95 ($C_{CB}CH_2$), 71.41, 74.77, 78.09 (C-2a, C-3a, C-4a, C-5a), 77.87 (C_{CB}), 103.8 (C-1a); MS (DCI): *m/z* (%): 547 (100) $[M+NH_4]^+$ (calcd for $C_{16}H_{36}B_{10}O_{12}$: 528.6).

4-Benzyloxy-2-butyn-1-yl 2a,3a,4a,6a-tetra-*O*-acetyl-α-D-mannopyranoside (12): TMSOTf (40 mL, 0.22 mmol) was added to an ice-cold solution of mannose trichloroacetimidate (**11**) (4.94 g, 10.0 mmol) and 4-benzyloxy-2-butyn-1-ol (1.94 g, 11.0 mmol, 1.1 equiv) in dichloromethane (60 mL) with stirring in the presence of molecular sieves (4 Å), and stirring was continued for 85 min. Work-up as described for **6** and purification by column chromatography (petroleum ether/ethyl acetate, 2:1) gave **12** (3.65 g, 7.21 mmol, 72%) as a yellow viscous oil. R_f (petroleum ether/ethyl acetate, 2:1) = 0.24; $[\alpha]_D^{20} = +58.8$ ($c = 1$, chloroform); IR (film): $\tilde{\nu} =$ 2932, 2860 (C–H), 1752 (C=O), 1372 ($OCOCH_3$), 1228 (C–O); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.00$, 2.04, 2.10, 2.16 (4s, 12H, $4 \times CH_3$), 4.02 (ddd, $J = 9.8$, 5.1, 2.5 Hz, 1H, 5a-H), 4.11 (dd, $J = 12.4$, 2.4 Hz, 1H, 6a-H(α)), 4.21 (t, $J = 1.7$ Hz, 2H, $CH_2C{\equiv}CCH_2OBzl$), 4.29 (dd, $J = 12.4$, 5.2 Hz, 1H, 6a-H(β)), 4.32 (dt, $J = 15.6$, 1.7 Hz, 1H, 1a-H(α)), 4.36 (dt, $J = 15.7$, 1.7 Hz, 1H, 1a-H(β)), 4.59 (s, 2H, CH_2Ph), 5.04 (d, $J = 1.6$ Hz, 1H, 1a-H), 5.29 (dd, $J = 3.3$, 1.8 Hz, 1H, 2a-H), 5.32 (t, $J = 10.2$ Hz, 1H, 4a-H), 5.36 (dd, $J = 10.1$, 3.2 Hz, 1H, 3a-H), 7.29 – 7.36 (m, 5H, Ph-H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 20.67$, 20.72, 20.86 ($4 \times CH_3$), 55.07, 57.26 ($2 \times CH_2C{\equiv}C$), 62.30 (C-6a), 65.99, 68.91, 69.34 (C-2a, C-3a, C-4a, C-5a), 71.77 (CH_2Ph), 80.61, 83.47 ($2 \times C{\equiv}C$), 96.00 (C-1a), 127.9, 128.0, 128.4, 137.2 (Ph-C), 169.6, 169.8, 169.9, 170.6 ($4 \times$ OAc); MS (DCI): *m/z* (%): 524.3 (100) $[M+NH_4]^+$ (calcd for $C_{25}H_{30}O_{11}$: 506.5).

2-Hydroxymethyl-1-(2a,3a,4a,6a-tetra-*O*-acetyl-α-D-mannopyranosylmethyl)-1,2-dicarba-*closo*-dodecaborane (14): Decaborane(14) (1.06 g, 8.67 mmol, 1.4 equiv) was heated in CH_3CN (20 mL) at reflux for 30 min, then a solution of alkynyl mannoside **12** (3.07 g, 6.06 mmol) in toluene (20 mL) was added and heating was continued for 15 h. Work-up as described for **3** and purification by column chromatography (petroleum ether/ethyl acetate, 2:1) afforded **13** (2.61 g; slightly impure according to TLC) which was directly used in the next step. R_f (petroleum ether/ethyl acetate, 2:1) = 0.35; ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.01$, 2.06, 2.08, 2.16 (4s, 12H, $4 \times CH_3$), 3.86 – 3.91 (m, 1H, 5a-H), 3.96 (d, $J = 12.1$ Hz, 1H, $C_{CB}CH(\alpha)H(\beta)$OBzl), 4.03 (s, 2H, CH_2Ph), 4.06 (dd, $J = 12.4$, 2.2 Hz, 1H, 6a-H(α)), 4.16 (d, $J = 12.1$ Hz, 1H, C_{CB}CH(α)*H*(β)OBzl), 4.21 (dd, $J = 12.2$, 5.4 Hz, 1H, 6a-H(β)), 4.55 (d, $J = 12.1$ Hz, 1H, OCOC*H*(α)H(β)), 4.61 (d, $J = 12.0$ Hz, 1H, OCOCH(α)*H*(β)), 4.68 (d, $J = 1.1$ Hz, 1H, 1a-H), 5.21 – 5.27 (m, 3H, 2a-H, 3a-H, 4a-H), 7.27 – 7.39 (m, 5H, Ph-H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 20.59$, 20.64, 20.76 ($4 \times CH_3$), 62.14 (C-6a), 65.44, 68.68, 68.78, 69.51 (C-2a, C-3a, C-4a, C-5a), 67.42 ($OCOCH_2$), 70.08 (CH_2OBzl), 73.46 ($PhCH_2$), 74.54, 76.32 ($2 \times C_{CB}$), 97.14 (C-1a), 127.8, 128.2, 128.6, 136.4 (Ph-C), 169.6, 169.8, 169.8, 170.4 ($4 \times$ OAc); MS (DCI): *m/z* (%): 643 (100) $[M+NH_4]^+$ (calcd for $C_{25}H_{40}B_{10}O_{11}$: 624.7).

A solution of **13** (2.12 g) in a mixture of methanol (10 mL) and ethyl acetate (10 mL) was hydrogenated by using palladium on carbon (10% Pd, 1.48 g) as the catalyst at 3 bar H_2 within 24 h at room temperature. The mixture was filtered and concentrated, and the residue was purified by gradient column chromatography (ethyl acetate/petroleum ether, 1.25:1 → 1.5:1) to afford **14** (980 mg, 1.83 mmol, 37% based on **12**) as a white foam. R_f (petroleum ether/ethyl acetate, 1:1) = 0.29; $[\alpha]_D^{20} = +40.7$ ($c = 1$, chloroform); IR (KBr): $\tilde{\nu} = 3002$, 2958, 2894 (C–H), 2584 (B–H), 1748 (C=O), 1372 ($OCOCH_3$), 1240, 1226 (C–O); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.02$, 2.08, 2.11, 2.17 (4s, 12H, $4 \times CH_3$), 2.70 – 2.82 (br s, 1H, OH, D_2O exchange), 3.97 (ddd, $J = 9.7$, 5.6, 2.6 Hz, 1H, 5a-H), 4.10 (d, $J = 11.6$ Hz, 1H, OCOC*H*(α)H(β)), 4.12 – 4.16 (m, 1H, 6a-H(α)), 4.20 (s, 2H, CH_2OH), 4.25 (dd, $J = 12.4$, 5.2 Hz, 1H, 6a-H(β)), 4.28 (d, $J = 12.0$ Hz, 1H, OCOCH(α)*H*(β)), 4.86 (s, 1H, 1a-H), 5.22 – 5.33 (m, 3H, 2a-H, 3a-H, 4a-H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 20.64$, 20.67, 20.78 ($4 \times CH_3$), 62.33 (C-6a), 64.04 (CH_2OH), 65.47, 68.72, 68.89, 69.67 (C-2a, C-3a, C-4a, C-5a), 67.66 ($OCOCH_2$), 74.88, 78.84 ($2 \times C_{CB}$), 97.20 (C-1a), 169.7, 170, 170.1, 170.7 ($4 \times$ OAc); MS (DCI): *m/z* (%): 595 (10) $[M+NH_4+acetyl]^+$, 552 (62) $[M+NH_4]^+$, 366 (100) $[C_{14}H_{19}O_{10}+NH_4-H]^+$; elemental analysis (%): calcd for $C_{18}H_{34}B_{10}O_{11}$ (534.6): C 40.44, H 6.41; found: C 40.47, H 6.69.

1-(2a,3a,4a,6a-Tetra-*O*-acetyl-α-D-glucopyranosylmethyl)-2-(2b,3b,4b,6b-tetra-*O*-acetyl-α-D-mannopyranosylmethyl)-1,2-dicarba-*closo*-dodecaborane (15): $BF_3 \cdot Et_2O$ (0.10 mL, 0.80 mmol) was added to a mixture of glucose trichloroacetimidate (**8**) (646 mg, 1.31 mmol, 1.6 equiv), mannose carboranyl alcohol **14** (452 mg, 0.85 mmol) and molecular sieves (4 Å) in dichloromethane (20 mL) with stirring at room temperature. After 145 min $BF_3 \cdot Et_2O$ (0.10 mL, 0.80 mmol) was added and stirring was continued for 4.5 h. Work-up as described for **6** and column chromatography (petroleum ether/ethyl acetate, 1:1) gave bisglucoside **15** (482 mg, 0.56 mmol, 66%) as a white foam. $[\alpha]_D^{20} = +14.8$ ($c = 0.5$, chloroform); IR (KBr): $\tilde{\nu} = 2961$, 2898 (C–H), 2590 (B–H), 1754 (C=O), 1371 ($OCOCH_3$), 1226 (C–O); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.00$, 2.01, 2.02, 2.08, 2.09, 2.10, 2.11, 2.16 (8s, 24H, $8 \times CH_3$), 3.72 (ddd, $J = 9.8$, 4.5, 2.3 Hz, 1H, 5a-H), 3.91 – 3.97 (m, 1H, 5b-H), 4.04 (d, $J = 12.0$ Hz, 2H, $2 \times CH(\alpha)H(\beta)C_{CB}$), 4.07 – 4.29 (m, 5H), 4.19 (d, $J = 12.1$ Hz, 1H, CH(α)*H*(β)C_{CB}), 4.40 (d, $J = 12.5$ Hz, 1H, CH(α)*H*(β)C_{CB}), 4.57 (d, $J = 7.9$ Hz, 1H, 1a-H), 4.85 (d, $J = 1.5$ Hz, 1H, 1b-H), 5.03 (dd, $J = 9.8$, 7.9 Hz, 1H, 2a-H), 5.10 (t, $J = 9.8$ Hz, 1H), 5.20 – 5.34 (m, 3H); ^{13}C NMR (200 MHz, $CDCl_3$): $\delta = 20.62$, 20.70, 20.79 (CH_3), 61.43, 61.55 (C-6a, C-6b), 62.28, 65.42, 67.38, 67.98, 68.76, 68.96, 69.66, 70.56, 72.15, 74.76, 74.85, 77.20, 96.85 (C-1b), 100.5 (C-1a), 169.2, 169.3, 169.7, 169.8, 169.9, 170.1, 170.4, 170.5 (OAc); MS (DCI): *m/z* (%): 884 $[M+NH_4+H]^+$ (calcd for $C_{32}H_{52}B_{10}O_{20}$: 864.9).

1-(β-D-Glucopyranosylmethyl)-2-(α-D-mannopyranosylmethyl)-1,2-dicarba-*closo*-dodecaborane (1): The carboranyl bisglycoside **15** (336 mg, 0.39 mmol) dissolved in methanol (10 mL) was deprotected within 50 min at room temperature by using a sodium methoxide solution (0.10 mL) as described for **7**. Purification by gradient column chromatography (ethyl acetate/methanol, 4:1 → 3.5:1) gave **1** (135 mg, 0.26 mmol, 66%) as a white foam. R_f (ethyl acetate/methanol, 4:1) = 0.12; $[\alpha]_D^{20} = +30.8$ ($c = 0.5$, methanol); IR (KBr): $\tilde{\nu} =$ 3386 (O–H), 2930, 2892 (C–H), 2588 (B–H); ^{1}H NMR (300 MHz, [D$_4$]methanol): $\delta = 3.25 - 3.27$ (m, 1H), 3.50 – 3.71 (m, 6H), 3.83 – 3.89 (m, 4H), 4.14 (d, $J = 12.4$ Hz, 1H, C*H*(α)H(β)C_{CB}), 4.15 (d, $J = 12.1$ Hz,

1H, C*H*(α)H(β)C_{CB}), 4.31 (d, J = 13.2 Hz, 1H, CH(α)*H*(β)C_{CB}), 4.32 (d, J = 8.0 Hz, 1H, 1a-H), 4.50 (d, J = 12.1 Hz, 1H, CH(α)*H*(β)C_{CB}), 4.58 – 4.60 (br signal, 1H, 1b-H); ^{13}C NMR (50 MHz, [D_4]methanol): δ = 62.48, 62.86 (C-6a, C-6b), 68.11, 68.31, 69.92, 71.22, 71.51, 72.38, 74.74, 75.53, 77.62, 77.97, 78.13, 101.6, 103.8 (C-1a, C-1b); MS (DCI): *m/z* (%): 546 (100) [M+NH_4]$^+$ (calcd for $C_{16}H_{36}B_{10}O_{12}$: 528.6).

4-Benzyloxy-2-butyn-1-yl 2a,3a,4a,6a-tetra-*O*-acetyl-β-D-glucopyranoside (17): Reaction of glucose trichloroacetimidate (**8**) (2.84 g, 5.76 mmol, 1.07 equiv) and 4-benzyloxy-2-butyn-1-ol (950 mg, 5.39 mmol) in dichloromethane (50 mL) for 165 min at room temperature over molecular sieves (3 Å) using $BF_3 \cdot Et_2O$ (0.30 mL, 2.4 mmol) as described for **6** gave, after purification by column chromatography (petroleum ether/ethyl acetate, 2:1), **17** (1.89 g, 3.73 mmol, 69%) as a slightly yellow viscous oil. $[\alpha]_D^{20} = -33.3$ (c = 1, chloroform); IR (film): $\tilde{\nu}$ = 3032, 2942, 2872 (C–H), 1756 (C=O), 1368 ($OCOCH_3$), 1225 (C–O); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.01, 2.03, 2.04, 2.08 (4s, 12H, 4 × CH_3), 3.71 (ddd, J = 10.0, 4.5, 2.5 Hz, 1H, 5-H), 4.14 (dd, J = 12.4, 2.3 Hz, 1H, 6a-H(α), 4.22 (t, J = 1.7 Hz, 2H, $CH_2C{\equiv}C$), 4.27 (dd, J = 12.4, 4.5 Hz, 1H, 6a-H(b)), 4.44 (t, J = 1.9 Hz, 2H, $CH_2C{\equiv}C$), 4.60 (s, 2H, CH_2Ph), 4.78 (d, J = 8.3 Hz, 1H, 1a-H), 5.02 (dd, J = 9.4, 7.9 Hz, 1H, 2a-H), 5.11 (t, J = 9.6 Hz, 1H, 4a-H), 5.25 (t, J = 9.4 Hz, 1H, 3a-H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 20.60, 20.69, 20.72 (4 × CH_3), 56.25, 57.28 (2 × $CH_2C{\equiv}C$), 61.74 (C-6a), 68.26, 71.02, 71.90, 72.80 (C-2a, C-3a, C-4a, C-5a), 71.73 (CH_2Ph), 80.92, 83.38 (2 × $C{\equiv}C$), 98.15 (C-1a), 128.0, 128.1, 128.5, 137.2 (4 × Ph-C), 169.4, 170.2, 170.6 (OAc); MS (DCI): *m/z* (%): 525 (100) [M+NH_4]$^+$; elemental analysis (%): calcd for $C_{25}H_{30}O_{11}$ (506.5): C 59.28, H 5.79; found: C 59.20, H 5.96.

1-Benzyloxymethyl-1,2-dicarba-*closo*-dodecaborane (21): Decaborane(14) (788 mg, 6.45 mmol, 1.25 equiv) and acetonitrile (20 mL) were heated at reflux for 30 min, then a solution of alkyne **20** (735 mg, 5.15 mmol) in toluene (20 mL) was added and the solution was heated at reflux for 20 h. Work-up as described for **3**, purification by column chromatography (petroleum ether/ethyl acetate, 10:1), and recrystallization from *n*-pentane gave **21** (810 mg, 3.06 mmol, 60%) as a white solid. R_f (petroleum ether/ethyl acetate, 10:1) = 0.64; IR (KBr): $\tilde{\nu}$ = 3083 (carborane C–H), 3060, 3039, 2891, 2867, 2848 (C–H), 2634, 2605, 2586, 2572, 2559 (B–H); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 3.88 (s, 2H, CH_2C_{CB}), 3.95 – 4.01 (br s, 1H, carborane C-H), 4.54 (s, 2H, CH_2Ph), 7.25 – 7.39 (m, 5H, Ph-H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 57.65 (carborane C-H), 70.79 (CH_2C_{CB}), 72.46 (carborane C), 73.70 (CH_2Ph), 127.7, 128.4, 128.7, 136.2 (4 × Ph-C); MS (EI): *m/z* (%): 264 (21) [M]$^+$, 92 (100) [Bzl+H]$^+$, 91 (91) [Bzl]$^+$; HR-MS: *m/z*: calcd. 266.2445; found 266.2444; elemental analysis (%): calcd for $C_{10}H_{20}B_{10}O$ (264.4): C 45.43, H 7.63; found: C 45.16, H 7.39.

4-Acetoxy-2-butyn-1-yl benzyl ether (24): To an ice-cold solution of 4-benzyloxy-2-butyn-1-ol (**23**) (3.09 g, 17.5 mmol) and Ac_2O (6.0 mL) in dichloromethane (100 mL) were added DMAP (115 mg, 0.94 mmol) and NEt_3 (10 mL) and the solution was stirred at room temperature for 18 h. The reaction was quenched by addition of aqueous HCl (1 M, 50 mL). Then the organic layer was separated and washed with aqueous HCl (1 M, 2 × 30 mL), sat. $NaHCO_3$ solution, and brine, and dried with sodium sulfate. Distillation (bp 138 °C, 0.1 mbar) gave **24** (3.42 g, 15.7 mmol, 89%) as a slightly yellow liquid. R_f (petroleum ether/ethyl acetate, 10:1) = 0.24; IR (film): $\tilde{\nu}$ = 2942, 2856 (C–H), 1748 (C=O), 1379 ($OCOCH_3$), 1224 (C–O), 741, 699 (Ph); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.11 (s, 3H, CH_3), 4.21 (t, J = 1.8 Hz, 2H, BzlOC$H_2C{\equiv}C$), 4.59 (s, 2H, CH_2Ph), 4.74 (t, J = 1.7 Hz, 2H, AcOC$H_2C{\equiv}C$), 7.25 – 7.40 (m, 5H, Ph-H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 20.67 (CH_3), 52.20, 57.22 (2 × $CH_2C{\equiv}C$), 71.63 (CH_2Ph), 80.45, 82.68 (2 × $C{\equiv}C$), 127.8, 128.0, 128.4, 137.1 (4 × Ph-C), 170.1 (OAc); MS (EI): *m/z* (%): 146 (37) [BzlOC$H_2C{\equiv}CCH_2$+H]$^+$, 107 (43) [BzlO]$^+$, 91 (92) [Bzl]$^+$, 43 (100) [Ac]$^+$; HR-MS for $C_{13}H_{14}O_3$ (218.3): calcd. 218.0943; found 218.0942.

1-Acetoxymethyl-2-benzyloxymethyl-1,2-dicarba-*closo*-dodecaborane (25): Decaborane(14) (991 mg, 8.11 mmol, 1.4 equiv) in acetonitrile (20 mL) was heated at reflux for 30 min, then alkyne **24** (1.26 g, 5.77 mmol) in toluene (20 mL) was added and the solution was heated at reflux for 13.5 h. Work-up as described for **3** and purification by column chromatography (petroleum ether/ethyl acetate, 10:1) gave carborane **25** (1.23 g, 3.66 mmol, 63%) as a white solid. R_f (petroleum ether/ethyl acetate 10:1) = 0.33; IR (KBr): $\tilde{\nu}$ = 3090, 3066, 3038, 2879 (C–H), 2598, 2574 (B–H), 1747 (C=O), 1363 ($OCOCH_3$), 1233 (C–O); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.04 (s, 3H, CH_3), 4.00 (s, 2H, BzlOCH_2C_{CB}), 4.56 (s, 2H, CH_2Ph or AcOCH_2C_{CB}), 4.62 (s, 2H, CH_2Ph or AcOCH_2C_{CB}), 7.22 – 7.40 (m, 5H, Ph-H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 20.40 (CH_3), 62.41, 70.09 (2 × CH_2C_{CB}), 73.48 (CH_2Ph), 74.60, 77.23 (2 × C_{CB}), 127.6, 128.2, 128.6, 136.3 (4 × Ph-C); MS (EI): *m/z* (%): 336 (7) [M]$^+$, 293 (8) [M – Ac]$^+$; elemental analysis (%): calcd for $C_{13}H_{24}B_{10}O_3$ (336.4): C 46.41, H 7.19; found: C 46.71, H 7.43.

1-Benzyloxymethyl-2-hydroxymethyl-1,2-dicarba-*closo*-dodecaborane (22): *Method A*: Deprotection of carborane **25**: A solution of carborane **25** (1.11 g, 3.29 mmol) in methanol (10 mL) was treated with a solution of sodium methoxide (0.10 mL, 5.4 M in methanol) for 60 min at room temperature; then the reaction was quenched by addition of Amberlyte IR-120 resin (H^+ form). After filtration and evaporation of the solvent in vacuo, the residue was purified by column chromatography (petroleum ether/ethyl acetate, 4:1) to afford the carboranyl alcohol **22** (764 mg, 2.60 mmol, 79%) as a white solid.

Method B: Reaction of carborane **21** with formaldehyde: A solution of carborane **21** (1.32 g, 4.99 mmol) in THF (7 mL) was added at room temperature to a solution of paraformaldehyde (374 mg, 12.5 mmol, 2.5 equiv) in THF (30 mL). Then a solution of tetrabutylammonium fluoride trihydrate (4.94 g, 15.7 mmol, 3.1 equiv) in THF (12 mL) was rapidly added by syringe and the mixture was stirred for 30 min. The reaction was quenched by addition of an aq sat. ammonium chloride solution (25 mL), water (100 mL), and diethyl ether (50 mL). The organic layer was separated, washed with water (100 mL), brine (50 mL), and dried with sodium sulfate. The solvents were evaporated and the residue was purified by flash chromatography (petroleum ether/ethyl acetate, 4:1) to afford carboranyl alcohol **22** (1.28 g, 4.35 mmol, 87%) as a white solid. R_f (petroleum ether/ethyl acetate, 6:1) = 0.15; IR (KBr): $\tilde{\nu}$ = 3089, 3067, 3034, 2878 (C–H), 2591 (B–H); ^{1}H NMR (200 MHz, $CDCl_3$): δ = 2.85 (t, J = 7.7 Hz, 1H, OH), 4.07 (s, 2H, BzlOCH_2C_{CB}), 4.07 (d, J = 7.6 Hz, CH_2OH), 4.59 (s, 2H, CH_2Ph), 7.25 – 7.42 (m, 5H, Ph-H); ^{13}C NMR (50 MHz, $CDCl_3$): δ = 64.18 (CH_2OH), 70.61 ($C_{CB}CH_2OCH_2$), 74.03 (CH_2Ph), 75.62, 78.85 (2 × C_{CB}), 127.9, 128.7, 128.8, 135.5 (4 × Ph-C); MS (EI): *m/z* (%): 294 (10) [M]$^+$, 107 (100) [BzlO]$^+$, 91 (64) [Bzl]$^+$; elemental analysis (%): calcd for $C_{11}H_{22}B_{10}O_2$ (294.4): C 44.88, H 7.53; found: C 44.72, H 7.24.

2-Hydroxymethyl-1-(2a,3a,4a,6a-tetra-*O*-acetyl-β-D-glucopyranosylmethyl)-1,2-dicarba-*closo*-dodecaborane (19): Glycosidation of carboranyl alcohol **22** and subsequent deprotection: Reaction of glucose trichloroacetimidate (**8**) (728 mg, 1.48 mmol, 1.3 equiv) and carboranyl alcohol **22** (339 mg, 1.15 mmol) in dichloromethane (15 mL) in the presence of molecular sieves (4 Å) within 18 h at room temperature using $BF_3 \cdot Et_2O$ (0.20 mL, 1.6 mmol) as promotor as described for **6** gave **18** as a white foam (528 mg) after purification by column chromatography (petroleum ether/ethyl acetate, 2:1). The compound contained traces of trichloroacetamide and was used without further purification in the next step. R_f (petroleum ether/ethyl acetate, 2:1) = 0.31; IR (KBr): $\tilde{\nu}$ = 3032, 2958, 2936, 2893 (C–H), 2639, 2590, 2574, 2559 (B–H), 1751 (C=O), 1364 ($OCOCH_3$), 1216 (C–O); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.02, 2.03, 2.05, 2.08 (4s, 12H, 4 × CH_3), 3.54 (ddd, J = 9.8, 4.6, 2.3 Hz, 1H, 5a-H), 3.94 (d, J = 11.6 Hz, 1H, C*H*(α)H(β)OBzl), 3.98 (d, J = 11.6 Hz, 1H, OCOC*H*(α)H(β)), 4.00 (d,

$J = 12.0$ Hz, 1H, CH(α)*H*(β)OBzl), 4.06 (dd, $J = 12.4$, 2.3 Hz, 1H, 6a-H(α)), 4.21 (dd, $J = 12.4$, 4.5 Hz, 1H, 6a-H(β)), 4.36 (d, $J = 12.8$ Hz, 1H, OCOCH(α)*H*(b)), 4.43 (d, $J = 7.9$ Hz, 1H, 1a-H), 4.53 (d, $J = 11.6$ Hz, 1H, C*H*(α)H(β)OBzl), 4.59 (d, $J = 11.7$ Hz, 1H, CH(α)*H*(β)OBzl), 4.99 (dd, $J =$ 9.4 Hz, 7.9 Hz, 1H, 2a-H), 5.05 (t, $J = 9.2$ Hz, 1H, 4a-H), 5.15 (t, $J =$ 9.4 Hz, 1H, 3a-H), 7.30 – 7.42 (m, 5H, Ph-H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 20.55$, 20.60, 20.64 (CH_3), 61.48 (C-6a), 67.97, 70.65, 71.93, 72.25 (C-2a, C-3a, C-4a, C-5a), 68.71, 70.05 ($2 \times CH_2C_{CB}$), 73.47 ($PhCH_2$), 75.19, 76.58 ($2 \times C_{CB}$), 100.4 (C-1a), 127.6, 128.3, 128.6, 136.5 ($4 \times$ Ph-C), 169.1, 169.3, 170.1, 170.4 ($4 \times$ OAc); MS (DCI): *m/z* (%): 644 (100) $[M+NH_4+H]^+$ (calcd for $C_{25}H_{40}B_{10}O_{11}$: 624.7).

A mixture of crude **18** and palladium on carbon (10%, 549 mg) in ethyl acetate (10 mL) was shaken in a hydrogen atmosphere (3 bar) for 18 h at room temperature. Work-up as described for **4** gave **19** as a white foam (309 mg, 0.58 mmol, 50% based on **22**) after purification by column chromatography (petroleum ether/ethyl acetate, 2:1). R_f (petroleum ether/ethyl acetate, 1:1) = 0.21; $[\alpha]_D^{20} = -34.0$ ($c = 0.9$, chloroform); IR (KBr): $\tilde{\nu} = 3493$ (O—H), 2955, 2945 (C—H), 2593 (B—H), 1756 (C=O), 1227 (C—O); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 2.02$, 2.04, 2.11, 2.11 (4s, 12H, $4 \times CH_3$), 2.90 – 3.06 (br s, 1H, OH), 3.71 (ddd, $J = 10.3$, 4.1, 2.8 Hz, 1H, 5a-H), 4.07 (d, $J = 12.1$ Hz, 1H, OCOC*H*(α)H(β)), 4.12 (s, 2H, C*H*$_2$OH), 4.19 (dd, $J = 12.5$, 3.0 Hz, 1H, 6a-H(α)), 4.24 (dd, $J = 12.5$, 4.2 Hz, 1H, 6a-H(β)), 4.44 (d, $J = 11.7$ Hz, 1H, OCOCH(α)*H*(β)), 4.54 (d, $J = 7.9$ Hz, 1H, 1a-H), 5.00 (dd, $J = 9.7$, 7.9 Hz, 1H, 2a-H), 5.08 (t, $J = 9.7$ Hz, 1H, 4a-H), 5.23 (t, $J = 9.4$ Hz, 1H, 3a-H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 20.56$, 20.65, 20.74 (CH_3), 61.39 (C-6a), 63.80 (CH_2OH), 68.06, 70.70, 71.99, 72.31 (C-2a, C-3a, C-4a, C-5a), 68.92 ($OCOCH_2$), 74.94, 79.00 ($2 \times C_{CB}$), 99.98 (C-1a), 169.4, 169.8, 170.1, 170.8 ($4 \times$ OAc); MS (DCI): *m/z* (%): 553 (100) $[M+NH_4]^+$; elemental analysis (%): calcd for $C_{18}H_{34}B_{10}O_{11}$ (534.6): C 40.44, H 6.41; found: C 40.71 H 6.36.[18] In a second approach, **19** was also prepared by reaction of alkynyl glucoside **17** with decaborane(14) followed by debenzylation as described for **14** in 33% overall yield.

1-(2a,3a,4a,6a-Tetra-*O*-acetyl-β-D-galactopyranosylmethyl)-2-(2b,3b,4b,6b-tetra-*O*-acetyl-β-D-glucopyranosylmethyl)-1,2-dicarba-*closo*-dodecaborane (27): Reaction of galactose trichloroacetimidate **26** (287 mg, 0.58 mmol, 1.3 equiv) and glucose carboranyl alcohol **19** (237 mg, 0.44 mmol) in dichloromethane (40 mL) over molecular sieves (4 Å) with $BF_3 \cdot Et_2O$ (0.10 mL, 0.80 mmol) as promotor for 21 h at room temperature as described for **6** gave the carboranyl bisglycoside **27** (190 mg, 0.22 mmol, 50%) as a white solid after purification by column chromatography (petroleum ether/ethyl acetate, 1:1). R_f (petroleum ether/ethyl acetate, 1:1) = 0.19; $[\alpha]_D^{20} = -39.4$ ($c = 0.9$, chloroform); IR (KBr): $\tilde{\nu} = 2963$, 2893 (C—H), 2588 (B—H), 1754 (C=O), 1371 ($OCOCH_3$), 1226 (C—O); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 2.00$, 2.02, 2.03, 2.07, 2.11, 2.12, 2.17 (7s, 24H, $8 \times CH_3$), 3.73 (ddd, $J = 10.0$, 4.5, 2.4 Hz, 1H, 5b-H), 3.94 (td, $J = 6.7$, 1.0 Hz, 1H, 5a-H), 4.02 (d, $J = 13.1$ Hz, 1H, OCOC*H*(α)H(β)C_{CB}), 4.03 (d, $J = 13.0$ Hz, 1H, OCOC*H*(α)H(β)C_{CB}), 4.12 – 4.16 (m, 3H, 6a-H(α), 6a-H(β), 6b-H(α)), 4.29 (dd, $J = 12.5$, 4.6 Hz, 1H, 6b-H(β)), 4.31 (d, $J =$ 13.0 Hz, 1H, OCOCH(α)*H*(β)C_{CB}), 4.33 (d, $J = 13.0$ Hz, 1H, OCOCH(α)*H*(β)C_{CB}), 4.51 (d, $J = 7.9$ Hz, 1H, 1a-H or 1b-H), 4.56 (d, $J =$ 7.9 Hz, 1H, 1a-H or 1b-H), 5.02 (dd, $J = 9.6$, 7.9 Hz, 1H, 2b-H), 5.04 (dd, $J = 10.5$, 3.5 Hz, 1H, 3a-H), 5.10 (t, $J = 9.7$ Hz, 1H, 4b-H), 5.20 (dd, $J = 10.6$, 7.7 Hz, 1H, 2a-H), 5.23 (t, $J = 9.5$ Hz, 1H, 3b-H), 5.40 (dd, $J =$ 3.4, 0.9 Hz, 1H, 4a-H); ^{13}C NMR (50 MHz, $CDCl_3$): $\delta = 20.56$, 20.63, 20.67, 20.72, 20.82 ($8 \times CH_3$), 60.92, 61.58 (C-6a, C-6b), 68.80, 68.93 ($2 \times CH_2C_{CB}$), 66.77, 68.03, 68.36, 70.46, 70.82, 71.06, 72.13, 72.35 (C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b), 76.13 ($2 \times C_{CB}$), 101.0, 101.4 (C-1a, C-1b), 169.2, 169.3, 169.4, 170.0, 170.2, 170.3, 170.6 ($8 \times$ OAc); MS (DCI): *m/z* (%): 884 (85) $[M+NH_4+H]^+$, 366 (100) $[C_{14}H_{19}O_{10}+NH_4+H]^+$; elemental analysis (%): calcd for $C_{32}H_{52}B_{10}O_{20}$ (864.9): C 44.44, H 6.06; found: C 44.71, H 5.93.

1-(β-D-Galactopyranosylmethyl)-2-(β-D-glucopyranosylmethyl)-1,2-dicarba-*closo*-dodecaborane (28): A solution of sodium methoxide (0.10 mL, 5.4 M) was added to carboranyl bisglycoside **27** (96 mg, 0.11 mmol) dissolved in methanol (10 mL) and the mixture was stirred for 60 min at room temperature. Work-up as described for **7** and washing of the resulting colorless film with diethyl ether gave carboranyl bisglycoside **28** as a white solid (59 mg, 99%), which was pure without further purification according to the NMR spectra. R_f (dichloromethane/methanol, 5:2) = 0.34; $[\alpha]_D^{20} = -8.0$ ($c = 0.5$, methanol); IR (KBr): $\tilde{\nu} = 3387$ (OH), 2924, 2892 (C—H), 2587 (B—H); ^{1}H NMR (500 MHz, [D$_4$]methanol): $\delta = 3.20$ (dd, $J = 9.1$, 7.9 Hz, 1H, 2b-H), 3.23 – 3.29 (m, 2H), 3.34 (t, $J = 8.7$ Hz, 1H), 3.44 (dd, $J = 9.6$, 3.3 Hz, 1H, 3a-H), 3.49 – 3.52 (m, 1H, 5a-H), 3.54 (dd, $J = 9.7$, 7.8 Hz, 1H, 2a-H), 3.64 (dd, $J = 11.9$, 5.5 Hz, 1H, 6a-H or 6b-H), 3.69 (dd, $J = 11.3$, 4.8 Hz, 1H, 6a-H or 6b-H), 3.76 (dd, $J = 11.4$, 7.2 Hz, 1H, 6a-H or 6b-H), 3.80 – 3.81 (m, 1H, 4a-H), 3.86 (dd, $J = 11.9$, 1.6 Hz, 1H, 6a-H or 6b-H), 4.28 (d, $J =$ 12.3 Hz, 1H, OCOCH(α)), 4.29 (d, $J = 12.4$ Hz, 1H, OCOCH(α)), 4.34 (d, $J = 7.8$ Hz, 1H, 1a-H or 1b-H), 4.41 (d, $J = 7.9$ Hz, 1H, 1a-H or 1b-H), 4.49 (d, $J = 12.4$ Hz, 2H, $2 \times$ OCOCH(β)); ^{13}C NMR (50 MHz, [D$_4$]methanol): $\delta = 62.36$, 62.44 (C-6a, C-6b), 69.67, 69.78 ($2 \times CH_2C_{CB}$), 70.10, 71.23, 72.01, 74.68, 76.71, 77.72 (C-2a, C-3a, C-4a, C-5a, C-2b, C-3b, C-4b, C-5b), 77.97 ($2 \times C_{CB}$), 103.6, 104.1 (C-1a, C-1b); MS (DCI): *m/z* (%): 546 (100) $[M+NH_4]^+$ (calcd for $C_{16}H_{36}B_{10}O_{12}$: 528.6).

Biological evaluation

Ezymatic assay: Mixed bisglycoside **28** (8.1 mg, 15.3 μmol) and β-galactosidase (from *E. coli*, Sigma G-2513, 2.3 U) in buffer (9.0 mL, pH 7.3) prepared from KH_2PO_4 (0.1 M) and NaOH (0.1 M) solutions were incubated at 37 °C. After 24 h the glycoside was cleaved with 80% conversion (TLC: dichloromethane/methanol, 2:1).

Cytotoxicity tests: Adherent cells of a human tumor cell line were seeded in triplicate in six multiwell plates at concentrations of 10^2, 10^3, 10^4, and 10^5 cells per cavity, and were incubated with freshly prepared solutions of the compound to be tested at various concentrations. After cultivation (12 d) at 37 °C and 7.5% CO_2 in air, the medium was removed; the clones were dried, stained with Löffler's methylene blue, and counted under a microscope. The relative clone-forming rate was determined according to the following formula: relative clone-forming rate [%] = (number of clones counted after exposure)/(number of clones counted in the control) $\times$ 100. Cells for the toxicity tests were cultivated at 37 °C and 7.5% CO_2 in air in DMEM (Biochrom) supplemented with L-glutamine (4 nM, Gibco), $NaHCO_3$ (44 nM, Biochrom), and 10% fetal calf serum (FCS; heat-inactivated for 30 min at 56 °C, Gibco). During exposure of the cells to the test substances, the medium did not contain any FCS to prevent enzymatic hydrolysis by glycohydrolases.[16]

Tumor cells for in vitro evaluation: B-16 melanoma and C6 rat glioma cell lines were used in the biological study. B-16 cells were maintained in Eagle's MEM (Nissui Pure Industries, Osaka, Japan) supplemented with 10% fetal bovine serum (JRH Biosciences) and 1% Antibiotics/Antimycotics solution (100X; Gibco BRL). C6 cells were maintained in Eagle's MEM supplemented with 10% fetal bovine serum (FBS; ICN Biochemicals Japan Co., Ltd.), gentamicin sulfate (50 mg L^{-1}), and amphotericin B (250 mg L^{-1}).

In vitro boron incorporation into B-16 melanoma cells: B-16 melanoma cells were cultured in Falcon 3025 dishes (150 mm ∅). When the cells had grown to fill up the dish, the cell number was counted ($4.0 - 5.0 \times 10^6$ cells per dish). Since all glassware and living organisms contain trace amounts of boron, one dish was used for a control experiment in which no synthesized compounds were added. The boron compounds, mixed glycoside **1** and bisglucoside **10** (1.0 and 5.0×10^{-4} M, 10.8 and 54 ppm boron, respectively), carboranyl maltoside **16** (1.0×10^{-4} M, 10.8 ppm boron) and BPA (**29**)

(1.0×10^{-3} M, 10.8 ppm boron) were added to the dishes. The cells were incubated for 3 – 24 h at 37 °C in 20 mL of the medium (Eagle-MEM, 10% FBS). The cells were washed three times with Ca- and Mg-free phosphate-buffered saline [PBS(−)], collected, chemically digested with 2 mL of 60% $HClO_4$/30% H_2O_2 (1:2) solution and then decomposed for 1 h at 75 °C. After filtration through a membrane filter (Millipore, 0.22 μm), the boron concentration was determined by ICP-AES (Shimadzu, ICPS-1000-III). The boron concentration of the control experiment was subtracted from the boron concentrations of the cells of each dish. Three replications of each experiment were carried out. The average boron concentration of each fraction is indicated in Table 1.

In vitro boron incorporation into C6 glioma cells: The cells in the cell culture dishes that were in the growth phase were washed three times with PBS(−) and were preincubated in the culture media containing various concentrations of the compounds (5, 10, and 15 ppm). After incubation for 6 h, the cells were washed four times in PBS(−) and the number of the viable cells was counted by the dye exclusion method as described above. The cells were centrifuged again and the supernatant was removed. Then the cells were chemically digested with a mixture of 0.15 mL of 70% $HClO_4$ and 0.3 mL of 30% H_2O_2 at 75 °C. After incubation for 1 h, the samples became colorless and were diluted with 2.0 mL of distilled water and filtered through a 0.45-μm filter. ICP-AES (VR1200, SEIKO Electric Co.) was used to measure boron concentrations in the samples. Finally, the cellular uptakes of boron were calculated based on the measured boron concentration of the digested samples and the number of the tumor cells present in the samples.

In vitro thermal neutron irradiation: C6 cells in growth phase were prepared in the cell culture dishes and reincubated in the culture media containing 25 ppm (based on the boron concentration) of carboranyl bisglucoside **10** and mixed glycoside **1**, and 10 ppm of maltoside **16** and BSH (**30**). After incubation for 6 h the cells were trypsinized and washed four times in PBS(−), and suspensions with a cell density of 2.0×10^4 cells mL^{-1} were prepared. One millilitre of the suspension in column-shaped teflon tubes was irradiated with thermal neutrons at the Kyoto University Research Reactor (KUR) at a neutron flux of 1.95×10^9 n cm^{-2} s^{-1}. After the thermal neutron exposure, a colony formation assay was performed in a routine manner to evaluate the survival of the cells.

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[1] a) R. F. Barth, A. H. Soloway, R. G. Fairchild, *Cancer* **1992**, *70*, 2995 – 3007; b) M. F. Hawthorne, *Angew. Chem.* **1993**, *105*, 997 – 1033; *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 950 – 984; c) C. Morin, *Tetrahedron* **1994**, *50*, 12521 – 12569; d) D. Gabel, *Chem. Unserer Zeit* **1997**, *31*, 235 – 240.

[2] A. H. Soloway, W. Tjarks, B. A. Barnum, F.-G. Rong, R. F. Barth, I. M. Codogni, J. G. Wilson, *Chem. Rev.* **1998**, *98*, 1515 – 1562.

[3] R. F. Barth, A. H. Soloway, *J. Neuro-Oncol.* **1997**, *33*, 3 – 7.

[4] Carboranyl glycosides: a) J. L. Maurer, A. J. Serino, M. F. Hawthorne, *Organometallics* **1988**, *7*, 2519 – 2524; b) C. Morin, M. Ramburrun in *Current Topics in the Chemistry of Boron* (Ed.: G. Kabalka), The Royal Society of Chemistry, Cambridge, **1994**, pp. 229 – 231; c) J. L. Maurer, F. Berchier, A. J. Serino, C. B. Knobler, M. F. Hawthorne, *J. Org. Chem.* **1990**, *50*, 838 – 843; d) V. Dahlhoff, J. Bruckmann, K. Angermund, C. Krüger, *Liebigs Ann. Chem.* **1993**, 831 – 835; e) W. Tjarks, A. K. M. Anisuzzaman, L. Liu, A. H. Soloway, R. F. Barth, D. J. Rerkins, D. M. Adams, *J. Med. Chem.* **1992**, *35*, 1628 – 1633; f) W. Tjarks, A. K. M. Anisuzzaman, A. H. Soloway, *Nucleosides Nucleotides* **1992**, *11*, 1765 – 1779; g) R. L. Sneath, Jr., J. E. Wright, A. H. Soloway, S. M. O'Keefe, A. S. Dey, W. D. Smolnycki, *J. Med. Chem.* **1976**, *19*, 1290 – 1294; h) G. B. Giovenzana, L. Lay, D. Monti, G. Palmisano, L. Panza, *Tetrahedron* **1999**, *55*, 14123 – 14136.

[5] L. F. Tietze, U. Bothe, *Chem. Eur. J.* **1998**, *4*, 1179 – 1183.

[6] L. F. Tietze, U. Bothe, I. Schuberth, *Chem. Eur. J.* **2000**, *6*, 836 – 842.

[7] L. N. Jungheim, T. A. Shepherd, *Chem. Rev.* **1994**, *94*, 1552 – 1566.

[8] R. Schaeffer, *J. Am. Chem. Soc.* **1957**, *79*, 1006 – 1007.

[9] A. Arai, I. Ichikizaki, *Bull. Chem. Soc. Jpn.* **1961**, *34*, 1571 – 1583.

[10] T. L. Heying, J. W. Ager, Jr., S. L. Clark, R. P. Alexander, S. Papetti, J. A. Reid, S. I. Trotz, *Inorg. Chem.* **1963**, *2*, 1097 – 1105.

[11] R. R. Schmidt, *Angew. Chem.* **1986**, *98*, 213 – 236; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212 – 235.

[12] C. P. Gorst-Allman, S. P. Steyn, *J. Chem. Soc. Perkin Trans. 1* **1987**, 163 – 168.

[13] H. Nakamura, K. Aoyagi, Y. Yamamoto, *J. Am. Chem. Soc.* **1998**, *120*, 1167 – 1171.

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[16] L. F. Tietze, R. Hannemann, W. Buhr, M. Lögers, P. Menningen, M. Lieb, D. Starck, T. Grote, A. Döring, I. Schuberth, *Angew. Chem.* **1996**, *108*, 2840 – 2841; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2674 – 2676.

[17] a) Y. Yamamoto, H. Nemoto, H. Nakamura, S. Iwamoto in *Current Topics in the Chemistry of Boron* (Ed.: G. Kabalka), The Royal Society of Chemistry, Cambridge, **1994**, pp. 159 – 154; b) H. Nemoto, J. Cai, N. Asao, S. Iwamoto, Y. Yamamoto, *J. Med. Chem.* **1995**, *38*, 1673 – 1678; c) M. Takagaki, K. Ono, Y. Oda, H. Kikuchi, H. Nemoto, S. Iwamoto, J. Cai, Y. Yamamoto, *Cancer Res.* **1996**, *56*, 2071 – 2020; d) Y. Yamamoto in *Advances in Neutron Capture Therapie Volume II, Chemistry and Biology* (Eds.: B. Larsson, J. Crawford, R. Weinreich), Elsevier, Amsterdam, **1997**, pp. 22 – 29; e) M. Takagaki, K. Ono, Y. Oda, H. Kikuchi, H Nemoto, S. Iwamoto, J. Cai, Y. Yamamoto in *Advances in Neutron Capture Therapie Volume II, Chemistry and Biology* (Eds.: B. Larsson, J. Crawford, R. Weinreich), Elsevier, Amsterdam, **1997**, pp. 243 – 248.

[18] Crystallographic data (excluding structure factors) for glucose carboranyl alcohol **19** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-154331. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223-336-033; e-mail: deposit@ccdc.cam.ac.uk). The auhors thank Dr. M. Noltemeyer and H.-G. Schmidt, Institut für Anorganische Chemie der Universität Göttingen (Germany), for the X-ray analyis of **19**.

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