Exploring the Biochemical Foundations of a Successful GLUT1-Targeting Strategy to BNCT: Chemical Synthesis and *in vitro* Evaluation of the Entire Positional Isomer Library of *ortho*-Carboranylmethyl Bearing Glucoconjugates

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ABSTRACT: Boron Neutron Capture Therapy (BNCT) is a non-invasive binary therapeutic modality applicable to the treatment of cancers. While BNCT offers a tumor-targeting selectivity that is difficult to match by other means, the last obstacles preventing the full harness of this potential come in the form of the suboptimal boron delivery strategies presently used in the clinics. To address these challenges, we have developed delivery agents that target the glucose transporter GLUT1. Here, we present the chemical synthesis of a number of *ortho*-carboranylmethyl substituted glucoconjugates and the biological assessment of all positional isomers. Altogether, the study provides protocols for the synthesis and structural characterization of such glucoconjugates and insights on their essential properties e.g. cytotoxicity, GLUT1-affinity, metabolism and boron delivery capacity. In addition to solidifying the biochemical foundations of a successful GLUT1-targeting approach to BNCT, we identify the most promising modification sites in D-glucose which are critical in order to further develop this strategy towards clinical use.

1. INTRODUCTION

Cancer is among the leading causes of death in modern society and thus constitutes a significant societal burden. Despite the continuous progress made in radio- and chemotherapies, there are still a vast number of patients with untreatable as well as recurring cancers. Head and neck cancers account for 10% of all cancers with 630 000 new cases diagnosed annually on a global scale.[1,2] While a combination of surgery and chemotherapy remains the primary method for treatment of these cancers, the conventional therapies face significant challenges as the percentage of inoperable and recurrent cancers in this category (20–40%) is exceptionally high.[2,3] In these cases, there is a clear need for novel and improved treatment options. Among the existing treatment possibilities available Boron Neutron Capture Therapy (BNCT) stands out as one of the most promising alternatives.[4]

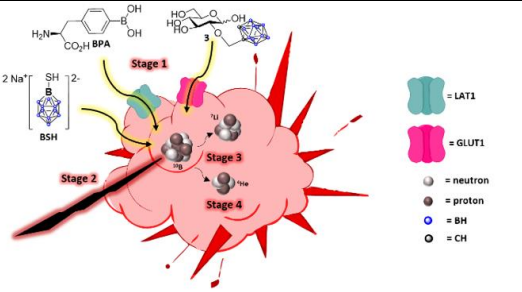


Figure 1. The principle of BNCT is showcased together with the different delivery strategies of boronophenylalanine (BPA), sodium borocaptate (BSH) and glucoconjugate **3**. **Stage 1**: Boron delivery agents enter the cancer cells. **Stage 2:** Irradiation by a precise neutron beam. **Stage 3:** ^{10}B captures a neutron and a fission reaction occurs. **Stage 4**: ^{4}He destroys the cancer cell.

BNCT is a combinatorial treatment featuring accumulation of ^{10}B-atoms in malignant tissue followed by irradiation with an external neutron beam in a narrow region (see Figure 1). In theory, an unrivalled selectivity can be achieved since the cross-section of ^{10}B is far greater than those of other elements present in a cellular environment, and the high energy α particles (^{4}He) generated in the fission reactions have a path length of 5–9 μm in tissue, *i.e.*, the diameter of a single cell.[5,6] Previously, nuclear reactors have been used as the sole neutron source in clinical BNCT. This has not only been suboptimal – it has been straightout inconvenient from the clinical practice standpoint, which is reflected in the fact that there is not a single phase 3 clinical study performed to date. To answer the needs of modern BNCT, in-hospital neutron sources have recently begun to emerge. The time to revisit the development of novel boron delivery agents is now at hand.[7,8]

While boron delivery agents have been developed for the better part of a century,[9] only three delivery agents are in actual clinical use, namely: boronophenylalanine (BPA),[10] sodium borocaptate (BSH)[11] and decahydrodecaborate (GB10).[12] None of these agents give good tumor to blood (T/B) or tumor to normal tissue (T/N) ratios which leads to the conclusion that new delivering strategies may be required in order to improve the overall treatment prospects of BNCT.[6,13,14,15] The development of delivery agents for BNCT is challenging and the requirements on the end products are numerous: sufficient aqueous solubility, minimal systemic toxicity, a minimum cellular uptake of 20–35 μg/g of the tumor, high tumor/normal tissue (T/N) and tumor/blood (T/B) ratios, and a detailed understanding on the behavior of the delivery agents in a biological setting.[9]

Our approach to target all of the current deficiencies of boron delivery agents is based on the potential embedded in glycoconjugates which target the glucose transporter GLUT1.[16,17] The biochemical foundation of our GLUT1-targeting strategy lies in the nature of the "Warburg effect",[18,19] *i.e.*, the increased expression of GLUT1 and uptake of D-glucose observed in head and neck cancers, analogous to most other tumors. This Warburg effect stems from the impaired and inefficient aerobic glucose metabolism observed in malignant cells where it is closely linked to the aberrant growth of tumors.[20] In contrast to the majority of previous studies reported on boron-glucoconjugates,[21,22] our emphasis is on critically assessing the biochemical foundations of this approach from a BNCT point-of-view. Recently, we reported promising results which indicated that an appropriately designed GLUT1-targeting strategy can outperform both the LAT1-targeting strategy of BPA and the passive transport strategies of BSH and GB10 (see Figure 1).[16]

Here, we significantly extend the initial exploratory studies by synthesizing and studying the cytotoxicity, GLUT1 affinity, and basis thereof, as well as cellular uptake displayed by the entire positional isomer library of *ortho*-carboranylmethyl substituted D-glucoconjugates in the relevant human oral adenosquamous carcinoma cell line CAL 27. In addition to these studies, we have addressed the metabolic fate of these glucoconjugates through an NMR-spectroscopic evaluation and ruled out the possibility for these substrates to enter the glycolysis or pentose phosphate metabolic pathways.[23] This study is central to laying the foundations of the GLUT1-targeting approach to BNCT, as the preclinical *in vitro* evaluations of the previously reported[16] and new glucoconjugates have been conducted under identical conditions thus allowing direct comparison of the results. The need for the study is evident as our results do not fully match those of Lippard et al.,[24] which implies that great care should be taken when interpreting or comparing results obtained with glucoconjugates bearing different substituents. Lastly, and most importantly, every single glucoconjugate synthesized displayed improved properties over the agents in current clinical use, thereby indicating that a correctly designed GLUT1-targeting approach to BNCT is a viable alternative to boron delivery agents used clinically at present.

2. EXPERIMENTAL SECTION

2.1 Synthesis and structural characterization

Reaction solvents were purified by the VAC vacuum solvent purification system prior to use when dry solvents were needed. All reactions containing moisture- or air-sensitive reagents were carried out under an argon atmosphere. All reagents were purchased from commercial sources. The NMR spectra were recorded with a Bruker Avance III NMR spectrometer operating at 500.13 MHz (^{1}H: 500.13 MHz, ^{13}C: 125.76 MHz, ^{11}B: 160.46 MHz). The probe temperature during the experiments was kept at 23 °C, unless otherwise stated. All products were characterized by utilization of the following 1D-techniques: ^{1}H, ^{13}C, ^{1}H-decoupled ^{11}B and 1D-TOCSY and the following 2D-techniques: Ed-HSQC, HMBC and COSY by using pulse sequences provided by the instrument manufacturer. Chemical shifts are expressed on the δ scale (in ppm) using TMS (tetramethylsilane), residual chloroform, methanol or 15% BF_3 in CDCl3 (^{11}B NMR) as internal standards. Coupling constants have been obtained through spectral simulations with the PERCH (PEak ReseaRCH) software, are given in Hz and provided only once, when first encountered. Coupling patterns are given as s (singlet), d (doublet), t (triplet) etc. HRMS were recorded using Bruker Micro Q-TOF with ESI (electrospray ionization) operated in positive mode. The purity of the compounds were determined to be > 95% in all cases. The substrate specific analytical data is provided below and representative ^{1}H, ^{13}C and ^{11}B NMR spectra are supplied in the supporting information. TLC was performed on aluminium sheets precoated with silica gel 60 F254 (Merck). Flash chromatography was carried out on silica gel 40. Spots were visualized by UV, followed by spraying the TLC plates with a solution of H_2SO_4:MeOH (1:5) and heating.

2.2 Experimental procedures

The general synthetic protocols applied to the synthesis of glucoconjugates **1**–**5** are described below. The synthetic routes leading to the new glucoconjugates **3** and **5** are presented in Scheme 1 and discussed in detail in the results and discussion section while the slightly modified synthetic routes employed to the synthesis of previously reported glucoconjugates **1**, **2**, and **4** are presented in Supporting Scheme 1 in the supporting information and not discussed at all. The protocols employed to the synthesis of **6** were discussed recently and have therefore been omitted from the present study.[16]

General procedure for glycosylation of glucose. To a solution of D-glucose (1.0 equiv.) in propargyl alcohol (5 ml/1 g of sugar), DOWEX 50 H^+-form was added (1 g/1 g of sugar) and the resulting mixture was refluxed for 17 h at 100 °C. The reaction mixture was brought to r.t., filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (DCM:MeOH 3:1).

General procedure for stereoselective acetylation of glucose. A suspension of NaOAc (0.5 g/1 g of sugar) in Ac_2O (7 ml/1 g of sugar) was gently heated to reflux followed by the addition of D-glucose (1.0 equiv.). After the addition was completed, the reaction mixture was heated at reflux for 30 min. The mixture was then brought to r.t., poured onto an ice-water bath and stirred for 2 h with external cooling. The precipitate was filtered, giving the crude product which was purified by recrystallization from ethanol.

General procedure for glycosylation of peracetylated glucose. To a solution of the peracetylated glucopyranose (1.0 equiv.) in dry DCM (9 ml/1 g of starting material), propargyl alcohol or BnOH (4 equiv.) and pre–activated 4 Å molecular sieves were added. The reaction mixture was cooled on an ice-bath and $BF_3{\cdot}OEt_2$ (6–8 equiv.) was added dropwise. The mixture was left in the ice-bath for 20 min. after which it was stirred for 17 h at r.t. The reaction mixture was then diluted with DCM (12 ml/1 g of starting material), poured into ice–water, stirred, and then the aqueous layer was decanted off. A satd. aq. solution of $NaHCO_3$ (12 ml/1 g of starting material) was added to the mixture, the aqueous layer was decanted off, and the organic layer was filtered through celite, washed with brine (12 ml/1 g of starting material), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc:Hexane 1:5).

General procedure for deacetylation. To an ice-water cooled solution of the corresponding glucopyranoside (1.0 equiv.) in MeOH:THF (5:1, 7 ml/1 g of starting material), NaOMe (1.0 equiv) was added until the pH ~ 10. The reaction mixture was stirred for 1–17 h at r.t. followed by the addition of Dowex 50 H^+-form until the pH was neutral. The reaction mixture was filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (DCM:MeOH 5:1).

General procedure for alkylation of free hydroxyl groups. To an ice-water cooled solution of the partially protected sugar (1.0 equiv.) in dry DMF (2 ml/100 mg of starting material), NaH (2.3 equiv./free OH group) was added portionwise. The mixture was brought to r.t. and the corresponding alkyl bromide was added (1.8 equiv./free OH group). The resulting mixture was stirred for 1.5 h, quenched with MeOH (0.4 ml/1 mmol of starting material), diluted with DCM (50 ml/1 g of starting material) and washed with a satd. aq. solution of $NaHCO_3$ (30 ml/1 g of starting material). The organic phase was separated, and the aqueous phase was extracted with DCM (3×30 ml/1 g of starting material). The combined organic phases were washed with brine (30 ml/1 g of starting material), dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc:Hexane 1:3).

General procedure for coupling with decaborane. Decaborane ($B_{10}H_{14}$,1.7 equiv.) and dry acetonitrile (5 ml/150 mg of starting material) were stirred under reflux for 1 h at 60 °C to form the $B_{10}H_{12}{\cdot}2CH_3CN$ adduct. Separately, the propargylated glucoconjugate (1.0 equiv.) was dissolved in dry toluene (5 ml/150 mg of starting material) and added to the reaction mixture which was left to reflux at 80 °C for 17 h. The mixture was quenched with dry MeOH (1.5 ml/150 mg of starting material), refluxed at 80 °C for another 30 min, brought to r.t. and concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc:Hexane 1:3).

General procedure for deprotection of benzyl groups. To a solution of the protected glucoside (1.0 equiv.) in dry EtOAc:MeOH (7:1, 1 ml/10–15 mg of starting material), Pd/C (10% Pd, 1.0 mass equiv.) was added. The reaction mixture was stirred in an autoclave under H_2-pressure (4 bar) for 4 h and filtered through celite. The celite was washed with EtOAc:MeOH (5:1, 1 ml/10–15 mg of starting material) and the filtrate concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc:MeOH 5:1).

General procedure for installation of 4,6-O-benzylidene acetals. To a solution of the corresponding deacetylated glucopyranoside (1 equiv.) in DMF (1 ml/40 mg starting material), PTSA (10 mol%) and benzaldehyde dimethyl acetal (1 equiv.) were added. The resulting mixture was stirred at 60 °C and 200 mbar for 2 h and concentrated. The crude product was purified by column chromatography (DCM:MeOH 5:1).

General procedure for selective ring-opening of the benzylidene acetal to give the 4-OH/6-OBn substrate. To a solution containing the corresponding 4,6-*O*-benzylidene acetal (1 equiv.) in DCM (6 ml/0.5 g of starting material), Et_3SiH (5 equiv.) was added, followed by dropwise addition of TFA (5 equiv.) at 0 °C. The resulting mixture was brought to r.t., stirred for 2 h, diluted with EtOAc (20 ml/0.5 g), washed with satd. aq. solution of $NaHCO_3$ (20 ml/0.5 g) and brine (20 ml/0.5 g). The organic phase was separated, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (EtOAc:Hexane 2:3).

General procedure for 1,2-orthoester formation. To a solution containing peracetylated glucose in DCM (12 ml/g of starting material), I_2 (1.4 equiv.) and Et_3SiH (1.4 equiv.) were added and the mixture was refluxed for 1 h. After 1 h, the mixture was brought to r.t. and 2,6-lutidine (4 equiv.), MeOH (6 equiv.) and TBAI (0.25 equiv.) were added. The mixture was refluxed for 3 h, then concentrated and dissolved in EtOAc. The resulting mixture was extracted with 5% aq. $Na_2S_2O_3$ (3×6 ml/g of starting material) and washed with EtOAc (4 ml/g of starting material). The organic phase was separated, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (EtOAc:Hexane 1:2).

General procedure for opening of the 1,2-orthoester. To a solution containing the 1,2-*orthoester* (1 equiv.) in acetone:H_2O 7:3 (15 ml/g of starting material), *p*-TsOH (0.4 equiv.) was added at 0 °C. The mixture was brought to r.t. and left to stir for 2 h after which it was neutralized with TEA and concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc:Hexane 1:2).

General procedure for formation of an imidate donor. To a solution containing the corresponding pyranose in DCM (20 ml/g of starting material), Cl_3CCN (10 equiv.) and DBU (0.25 equiv.) were added. The mixture was left to stir for 1 h at r.t. after which it was diluted with DCM (10 ml/g of starting material) and washed with brine (20 ml/g of starting material). The organic phase was separated, dried over Na_2SO_4, filtered and

concentrated. The crude product was purified by column chromatography (EtOAc:Hexane 2:3 containing 0.01 % TEA).

General procedure for glycosylation using TMSOTf. To a solution containing the corresponding acceptor (1 equiv.) in DCM (2.8 ml/100 mg of starting material), pre-activated 4 Å molecular sieves were added and the mixture was cooled to –20 °C. TMSOTf (2 equiv.) was added and the mixture was stirred for 10 min. The corresponding donor (1.4 equiv.) dissolved in DCM (2.8 ml/100 mg of starting material) was added dropwise to the solution. The reaction mixture was left to stir for 2 h at –20 °C after which it was brought to r.t., diluted with DCM (2 ml/g of starting material), washed with a satd. aq. solution of $NaHCO_3$ (2 ml/g of starting material) and brine (2 ml/g of starting material). The organic phase was separated, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by column chromatography (EtOAc:Hexane 1:4).

General procedure for installation of 1,2:4,6-di-O-isopropylidene acetals. To a solution containing D-glucose (1 equiv.) in acetone (50 ml/g of starting material), I_2 (0.2 equiv.) was added and the mixture was left to stir at r.t. for 3.5 h after which the mixture was quenched with 5% aq. $Na_2S_2O_3$ (8.6 ml/g of starting material). Acetone was removed under reduced pressure and the resulting mixture was extracted with DCM (3×9 ml/g of starting material). The organic phase was further washed with a satd. aq. solution of $NaHCO_3$ (9 ml/g of starting material), separated, dried over Na_2SO_4, filtered and concentrated. The crude product was purified by recrystallization from petroleum ether.

General procedure for 1,2:4,6-di-O-isopropylidene acetal removal. The corresponding protected sugar (1 equiv.) was dissolved in H_2O:TFA 1:1 (14 ml/g of starting material) and left to stir at r.t. for 21 h. The resulting mixture was concentrated under reduced pressure and the crude product was purified by column chromatography (DCM:MeOH 5:1) to give the desired product.

2.3 Substrate specific analytical data

In this section, the substrate specific analytical data featuring: synthetic protocol employed, reaction scale and yield, and NMR and MS characterization data is supplied for all intermediates and end products on the synthetic routes to **1**–**5**.

1,2,3,4,6-penta-O-acetyl-β-D-glucopyranoside. Synthesized from D-glucose (15.09 g, 84.0 mmol) and Ac_2O (113.40 g, 1.1 mol) according to the general procedure for stereoselective acetylation of glucose. This reaction gave the title compound as a white solid (18.52 g, 57%). TLC: R_f: 0.68 (EtOAc:Hexane 1:1).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 5.72 (d, 1H, $J_{1,2}$= 8.3 Hz, H-1), 5.25 (dd, 1H, $J_{3,2}$ = 9.6, $J_{3,4}$ = 9.4 Hz, H-3), 5.14 (dd, 1H, H-2), 5.13 (dd, 1H, $J_{4,5}$= 10.1 Hz, H-4), 4.29 (dd, 1H, $J_{6a,5}$ = 4.5, $J_{6a,6b}$= –12.5 Hz, H-6a), 4.12 (dd, 1H, $J_{6b,5}$ = 2.1 Hz, H-6b), 3.84 (ddd, 1H, H-5), 2.12 (s, 3H, 1-OCOC**H$_3$**), 2.09 (s, 3H, 6-OCOC**H$_3$**), 2.04 (s, 6H, 2-OCOC**H$_3$** and 4-OCOC**H$_3$**) and 2.02 (3-OCOC**H$_3$**) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 170.7 (6-O**C**OCH$_3$), 170.2 (3-O**C**OCH$_3$), 169.5 (4-O**C**OCH$_3$), 169.3 (2-O**C**OCH$_3$), 169.0 (1-O**C**OCH$_3$), 91.9 (C-1), 73.0 (C-3), 72.9 (C-5), 70.4 (C-2), 67.9 (C-4), 61.6 (C-6), 20.9 (1-OCO**C**H$_3$), 20.8 (6-OCO**C**H$_3$) and 20.7 (2-OCO**C**H$_3$, 3-OCO**C**H$_3$ and 4-OCO**C**H$_3$) ppm.

HRMS: *m/z* calcd. for $C_{16}H_{22}O_{11}Na$ $[M + Na]^+$ 413.1060; found 413.1030.

*Propargyl β-D-glucopyranoside (**15**).* Synthesized from 1,2,3,4,6-penta-*O*-acetyl-β-D-glucopyranoside (8.04 g, 21.0 mmol, 1.0 equiv.) according to the general procedure for glycosylation of peracetylated glucose to give propargyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside. This product was deprotected according to the general procedure for deacetylation to give the title compound as a white solid (2.35 g, yield over two steps 52%). TLC: R_f: 0.54 (DCM:MeOH 5:1).

^{1}H NMR (500.13 MHz, CD_3OD, 23 °C): δ = 4.46 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.44 (dd, 1H, $J_{CH2a,\ CH2b}$ = –15.6, $J_{CH2a,CH}$ = 2.4 Hz, 1-OC**H$_{2a}$**C≡CH), 4.41 (dd, 1H, $J_{CH2b,CH}$ = 2.4 Hz, 1-OC**H$_{2b}$**C≡CH), 3.87 (dd, 1H, $J_{6a,6b}$ = –12.0, $J_{5,6a}$ = 2.1 Hz, H-6a), 3.66 (dd, 1H, $J_{6b,5}$ = 5.7 Hz, H-6b), 3.36 (dd, 1H, $J_{3,2}$ = 9.3, $J_{3,4}$ = 8.9 Hz, H-3), 3.28 (dd, 1H, $J_{4,5}$ = 8.9 Hz, H-4), 3.27 (ddd, 1H, H-5), 3.20 (dd, 1H, H-2) and 2.86 (dd, 1H, 1-OCH$_2$C≡C**H**) ppm.

^{13}C NMR (125.76 MHz, CD_3OD, 23 °C): δ = 102.1 (C-1), 80.1 (1-OCH$_2$**C**≡CH), 78.1 (C-5), 78.0 (C-3), 76.2 (1-OCH$_2$C≡**C**H), 74.9 (C-2), 71.6 (C-4), 62.7 (C-6) and 56.5 (1-O**C**H$_2$C≡CH) ppm.

HRMS: *m/z* calcd. for $C_9H_{14}O_6Na$ $[M + Na]^+$ 241.0688; found 241.0720.

Propargyl 2,3,4,6-tetra-O-benzyl-β-D-glucopyranoside. Synthesized from **15** (0.81 g, 3.7 mmol), NaH (0.62 g, 26.0 mmol) and BnBr (4.176 g, 24.0 mmol) according to the general procedure for alkylation of free hydroxyl groups. The title compound was obtained as a white solid (1.78 g, 82%). TLC: R_f: 0.54 (EtOAc:Hexane 1:3).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 7.40–7.13 (m, 20H, arom. H), 4.97 and 4.69 (each d, each 1H, J = –10.8 Hz, 2-OC**H$_2$**Ph), 4.93 and 4.78 (each d, each 1H, J = –10.9 Hz, 3-OC**H$_2$**Ph), 4.82 and 4.53 (each d, each 1H, J = –10.8 Hz, 4-OC**H$_2$**Ph), 4.63 (d, 1H, $J_{1,2}$= 7.8 Hz, H-1), 4.61 and 4.54 (each d, each 1H, J = –12.2 Hz, 6-OC**H$_2$**Ph), 4.46 (dd, 1H, $J_{CH2a,CH2b}$ = –15.8, $J_{CH2a,CH}$ = –2.5 Hz, 1-OC**H$_{2a}$**C≡CH), 4.41 (dd, 1H, $J_{CH2b,CH}$ = –2.4 Hz, 1-OC**H$_{2b}$**C≡CH), 3.74 (dd, 1H, $J_{6a,6b}$ = –10.8, $J_{6a,5}$ = 1.8 Hz, H-6a), 3.69 (dd, 1H, $J_{6b,5}$ = 4.6 Hz, H-6b), 3.66 (dd, 1H, $J_{3,2}$ = 9.0, $J_{3,4}$ = 9.2 Hz, H-3), 3.62 (dd, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 3.49 (dd, 1H, H-2), 3.47 (ddd, 1H, H-5) and 2.46 (dd, 1H, 1-OCH$_2$C≡C**H**) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 138.7–127.7 (arom. C), 101.6 (C-1), 84.7 (C-3), 82.1 (C-2), 79.2 (1-OCH$_2$**C**≡CH), 77.8 (C-4), 75.9 (3-O**C**H$_2$Ph), 75.1 (4-O**C**H$_2$Ph and 1-OCH$_2$C≡**C**H), 75.0 (C-5), 74.9 (2-O**C**H$_2$Ph), 73.6 (6-O**C**H$_2$Ph), 68.9 (C-6) and 56.1 (1-O**C**H$_2$C≡CH) ppm.

HRMS: *m/z* calcd. for $C_{37}H_{38}O_6Na$ $[M + Na]^+$ 601.2566; found 601.2551.

*(O)-carboranylmethyl 2,3,4,6-tetra-O-benzyl-β-D-glucopyranoside (**16**).* Synthesized from propargyl 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranoside (0.83 g, 1.4 mmol) and $B_{10}H_{14}$ (0.33 g, 2.7 mmol) according to the general procedure for coupling with decaborane. The title compound was obtained as a white solid (0.64 g, 64%). TLC: R_f: 0.49 (EtOAc:Hexane 1:3).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 7.38–7.13 (m, 20H, arom. H), 4.89 and 4.81 (each d, each 1H, J = –11.0 Hz, 3-OC**H$_2$**Ph), 4.79 and 4.52 (each d, each 1H, J = –10.5 Hz, 4-OC**H$_2$**Ph), 4.77 and 4.76 (each d, each 1H, J = –11.5 Hz, 2-

OC**H₂**Ph), 4.56 and 4.50 (each d, each 1H, J = –12.1 Hz, 6-OC**H₂**Ph), 4.32 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.21 and 4.04 (each d, each 1H, J = –11.5 Hz, 1-OC**H₂**-carborane), 4.00 (br s, 1H, carborane-C**H**), 3.66 (dd, 1H, $J_{6a,6b}$ = –10.5, $J_{6a,5}$ = 1.9 Hz, H-6a), 3.63 (dd, 1H, $J_{6b,5}$ = 4.6 Hz, H-6b), 3.62 (dd, 1H, $J_{3,2}$ = 9.0, $J_{3,4}$ = 8.9 Hz, H-3), 3.60 (dd, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 3.42 (dd, 1H, H-2), 3.41 (ddd, 1H, H-5) and 3.00–1.45 (br m, 10H, carborane-B**H**) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 138.4–127.9 (arom. C), 103.2 (C-1), 84.7 (C-3), 81.7 (C-2), 77.6 (C-4), 75.8 (3-O**C**H₂Ph), 75.2 (2-O**C**H₂Ph and 4-O**C**H₂Ph), 75.0 (C-5), 73.7 (6-O**C**H₂Ph), 72.5 (carborane-**C**), 70.9 (1-O**C**H₂-carborane), 68.6 (C-6) and 58.2 (carborane-**C**H) ppm.

^{11}B NMR (160.46 MHz, $CDCl_3$, 23 °C): δ = –2.5, –4.4, –9.0, –11.6 and –12.9 ppm.

HRMS: *m/z* calcd. for $C_{37}H_{48}B_{10}O_6Na$ $[M + Na]^+$ 721.4279; found 721.4357.

*(O)-carboranylmethyl β-D-glucopyranoside (**1**).* Synthesized from **16** (0.10 g, 0.1 mmol) and 10% Pd/C (0.01 g, 0.1 mmol) according to the general procedure for deprotection of benzyl groups. This reaction gave the title compound as a white solid (0.04 g, 86%). TLC: R_f: 0.55 (EtOAc:MeOH 5:1).

^{1}H NMR (500.13 MHz, CD_3OD, 23 °C): δ = 4.78 (br s, 1H, carborane-C**H**), 4.35 and 4.12 (each d, each 1H, J = –11.5 Hz, 1-OC**H₂**-carborane), 4.26 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 3.86 (dd, 1H, $J_{6a,6b}$ = –11.8, $J_{6a,5}$ = 2.0 Hz, H-6a), 3.64 (dd, 1H, $J_{6b,5}$ = 5.7 Hz, H-6b), 3.32 (dd, 1H, $J_{3,2}$ = 9.2, $J_{3,4}$ = 8.9 Hz, H-3), 3.26 (dd, 1H, $J_{4,5}$ = 9.3 Hz, H-4), 3.26 (ddd, 1H, H-5), 3.18 (dd, 1H, H-2) and 2.98–1.46 (br m, 10H, carborane-B**H**) ppm.

^{13}C NMR (125.76 MHz, CD_3OD, 23 °C): δ = 104.5 (C-1), 78.2 (C-5), 77.8 (C-3), 74.8 (C-2), 74.6 (carborane-**C**), 71.6 (1-O**C**H₂-carborane), 71.4 (C-4), 62.6 (C-6) and 60.4 (carborane-**C**H) ppm.

^{11}B NMR (160.46 MHz, CD_3OD, 23 °C): δ = –2.3, –4.0, –8.5, –10.7 and –12.2 ppm.

HRMS: *m/z* calcd. for $C_9H_{24}B_{10}O_6Na$ $[M + Na]^+$ 361.2401; found 361.2381.

*Propargyl α-D-glucopyranoside (**17**).* Synthesized from D-glucose (0.50 g, 2.8 mmol), propargyl alcohol (2.41 g, 43.0 mmol) and DOWEX 50 H^+-form (0.50 g) according to the general procedure for glycosylation of glucose. This reaction gave the title compound as an off-white oil (0.21 g, 34%). TLC: R_f: 0.38 (DCM:MeOH 5:1).

^{1}H NMR (500.13 MHz, CD_3OD, 23 °C): δ = 5.00 (d, 1H, $J_{1,2}$ = 3.8 Hz, H-1), 4.31 (dd, 1H, $J_{CH2a,CH2b}$ = –15.8, $J_{CH2a,CH}$ = 2.5 Hz, 1-OC**H₂ₐ**C≡CH), 4.30 (dd, 1H, $J_{CH2b,CH}$ = 2.3 Hz, 1-OC**H₂ᵦ**C≡CH), 3.81 (dd, 1H, $J_{6a,6b}$ = –11.9, $J_{6a,5}$ = 2.3 Hz, H-6a), 3.68 (dd, 1H, $J_{6b,5}$ = 5.5 Hz, H-6b), 3.63 (dd, 1H, $J_{3,2}$ = 9.7, $J_{3,4}$ = 8.9 Hz, H-3), 3.57 (ddd, 1H, $J_{5,4}$ = 10.0 Hz, H-5), 3.42 (dd, 1H, H-2), 3.31 (dd, 1H, H-4) and 2.85 (dd, 1H, 1-OCH₂C≡C**H**) ppm.

^{13}C NMR (125.76 MHz, CD_3OD, 23 °C): δ = 98.6 (C-1), 80.1 (1-OCH₂**C**≡CH), 76.0 (1-OCH₂C≡**C**H), 75.0 (C-3), 74.1 (C-5), 73.3 (C-2), 71.7 (C-4), 62.5 (C-6) and 55.2 (1-O**C**H₂C≡CH) ppm.

HRMS: *m/z* calcd. for $C_9H_{14}O_6Na$ $[M + Na]^+$ 241.0688; found 241.0678.

*Propargyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranoside (**18**).* Synthesized from **17** (0.44 g, 2.0 mmol), NaH (0.34 g, 14.1 mmol) and BnBr (2.23 g, 13.0 mmol) according to the general procedure for alkylation of free hydroxyl groups. This reaction gave the title compound as a white solid (0.96 g, 82%). TLC: R_f: 0.41 (EtOAc:Hexane 1:3).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 7.40–7.11 (m, 20H, arom. H), 5.08 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.98 and 4.81 (each d, each 1H, J = –10.9 Hz, 3-OC**H₂**Ph), 4.83 and 4.47 (each d, each 1H, J = –10.8 Hz, 4-OC**H₂**Ph), 4.76 and 4.71 (each d, each 1H, J = –12.0 Hz, 2-OC**H₂**Ph), 4.60 and 4.47 (each d, each 1H, J = –12.4 Hz, 6-OC**H₂**Ph), 4.27 (dd, 1H, $J_{CH2a,CH}$ = –2.7, $J_{CH2a,CH2b}$ = –15.8 Hz, 1-OC**H₂ₐ**C≡CH), 4.27 (dd, 1H, $J_{CH2b,CH}$ = –2.2 Hz, 1-OC**H₂ᵦ**C≡CH), 3.98 (dd, 1H, $J_{3,2}$ = 9.7, $J_{3,4}$ = 9.1 Hz, H-3), 3.78 (ddd, 1H, $J_{5,4}$ = 10.0, $J_{5,6a}$ = 3.6, $J_{5,6b}$ = 2.0 Hz, H-5), 3.72 (dd, 1H, $J_{6a,6b}$ = –10.9 Hz, H-6a), 3.65 (dd, 1H, H-4), 3.63 (dd, 1H, H-6b), 3.61 (dd, 1H, H-2) and 2.43 (dd, 1H, 1-OCH₂C≡C**H**) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 138.9–128.7 (arom. C), 95.4 (C-1), 82.1 (C-3), 79.5 (C-2), 79.1 (1-OCH₂**C**≡CH), 77.6 (C-4), 75.9 (3-O**C**H₂Ph), 75.2 (4-O**C**H₂Ph), 74.9 (1-OCH₂C≡**C**H), 73.6 (6-O**C**H₂Ph), 73.1 (2-O**C**H₂Ph), 70.9 (C-5), 68.5 (C-6) and 54.5 (1-O**C**H₂C≡CH) ppm.

HRMS: *m/z* calcd. for $C_{37}H_{38}O_6Na$ $[M + Na]^+$ 601.2566; found 601.2581.

(O)-carboranylmethyl 2,3,4,6-tetra-O-benzyl-α-D-glucopyranoside. Synthesized from **18** (0.63 g, 1.1 mmol) and $B_{10}H_{14}$ (0.25 g, 2.1 mmol) according to the general procedure for coupling with decaborane. This reaction gave the title compound as a colorless oil (0.45 g, 59%). TLC: R_f: 0.44 (EtOAc:Hexane 1:3).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 7.36–7.12 (m, 20H, arom. H), 4.93 and 4.84 (each d, each 1H, J = –10.9 Hz, 3-OC**H₂**Ph), 4.81 and 4.47 (each d, each 1H, J = –10.7 Hz, 4-OC**H₂**Ph), 4.77 and 4.55 (each d, each 1H, J = –11.6 Hz, 2-OC**H₂**Ph), 4.67 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.56 and 4.47 (each d, each 1H, J = –12.1 Hz, 6-OC**H₂**Ph), 4.19 (br s, 1H, carborane-C**H**), 4.04 and 3.78 (each d, each 1H, J = –11.2 Hz, 1-OC**H₂**-carborane), 3.84 (dd, 1H, $J_{3,2}$ = 9.6, $J_{3,4}$ = 9.1 Hz, H-3), 3.67 (dd, 1H, $J_{6a,6b}$ = –10.7, $J_{6a,5}$ = 3.8 Hz, H-6a), 3.63 (ddd, 1H, $J_{5,4}$ = 9.9, $J_{5,6b}$ = 1.8 Hz, H-5), 3.61 (dd, 1H, H-4), 3.60 (dd, 1H, H-6b), 3.54 (dd, 1H, H-2) and 2.87–1.44 (br m, 10H, carborane-B**H**) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 138.6–127.9 (arom. C), 98.7 (C-1), 81.7 (C-3), 80.0 (C-2), 77.5 (C-4), 75.8 (3-O**C**H₂Ph), 75.4 (4-O**C**H₂Ph), 74.1 (2-O**C**H₂Ph), 73.7 (6-O**C**H₂Ph), 72.4 (carborane-**C**), 71.4 (C-5), 69.4 (1-O**C**H₂-carborane), 68.3 (C-6) and 57.9 (carborane-**C**H) ppm.

^{11}B NMR (160.46 MHz, $CDCl_3$, 23 °C): δ = –0.9, –3.2, –4.8, –8.8 and –13.2 ppm.

HRMS: *m/z* calcd. for $C_{37}H_{48}B_{10}O_6Na$ $[M + Na]^+$ 721.4279; found 721.4316.

*(O)-carboranylmethyl α-D-glucopyranoside (**2**).* Synthesized from (*O*)-carboranylmethyl 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranoside (0.30 g, 0.4 mmol) and 10% Pd/C (0.05 g, 0.4 mmol) according to the general procedure for removal of benzyl groups. This reaction gave the title compound as an off-white oil (0.12 g, 81%). TLC: R_f: 0.53 (EtOAc:MeOH 5:1).

^{1}H NMR (500.13 MHz, CD_3OD, 23 °C): δ = 4.84 (br s, 1H, carborane-C**H**), 4.80 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.22 and 3.98 (each d, each 1H, J = –11.2 Hz, 1-OC**H₂**-carborane), 3.81 (dd, 1H, $J_{6a,6b}$ = –11.9, $J_{6a,5}$ = 2.2 Hz, H-6a), 3.63 (dd, 1H, $J_{6b,5}$ = 6.1

Hz, H-6b), 3.59 (dd, 1H, $J_{3,2}$ = 9.8, $J_{3,4}$ = 8.9 Hz, H-3), 3.49 (ddd, 1H, $J_{4,5}$ = 10.0 Hz, H-5), 3.42 (dd, 1H, H-2), 3.25 (dd, 1H, H-4) and 3.04–1.42 (br m, 10H, carborane-B**H**) ppm.

^{13}C NMR (125.76 MHz, CD_3OD, 23 °C): δ = 100.6 (C-1), 74.8 (C-5), 74.7 (C-3), 74.5 (carborane-**C**), 73.3 (C-2), 71.6 (C-4), 69.9 (1-O**C**H_2-carborane), 62.6 (C-6) and 60.5 (carborane-**C**H) ppm.

^{11}B NMR (160.46 MHz, CD_3OD, 23 °C): δ = –2.4, –4.2, –8.5, –11.1 and –12.3 ppm.

HRMS: *m/z* calcd. for $C_9H_{24}B_{10}O_6Na$ $[M + Na]^+$ 361.2401; found 361.2395.

1,2-O-(1-methoxyethylidene)-3,4,6-tri-O-acetyl-α-D-glucopyranose. Synthesized from peracetylated D-glucopyranose(11.99 g, 30.7 mmol), I_2 (10.93 g, 43.1 mmol), Et_3SiH (5.01 g, 43.1 mmol), 2,6-lutidine (13.18 g, 0.123 mol), TBAI (2.84 g, 7.69 mmol) according to the general procedure for formation of 1,2-orthoester. This reaction gave the title compound as a colorless oil (10.1 g, 91%). TLC: R_f: 0.25 (EtOAc:Hexane 1:3).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 5.72 (d, 1H, $J_{1,2}$ = 5.2 Hz, H-1), 5.20 (dd, 1H, $J_{3,2}$ = 3.0, $J_{3,4}$ = 2.7 Hz, H-3), 4.90 (ddd, 1H, $J_{4,2}$ = –1.0, $J_{4,5}$ = 9.6 Hz, H-4), 4.32 (ddd, 1H, H-2), 4.21 (dd, 1H, $J_{6a,5}$ = 5.6, $J_{6a,6b}$ = –12.2 Hz, H-6a), 4.19 (dd, 1H, $J_{6b,5}$ = 2.6 Hz, H-6b), 3.95 (ddd, 1H, H-5), 3.29 (s, 3H, C(CH_3)OC**H$_3$**), 2.11 (s, 3H, 3-OCOC**H$_3$**), 2.10 (s, 3H, 6-OCOC**H$_3$**), 2.09 (4-OCOC**H$_3$**) and 2.71 (s, 3H, C(C**H$_3$**)OCH_3) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 170.8 (6-O**C**OCH_3), 169.8 (4-O**C**OCH_3), 169.3 (3-O**C**OCH_3), 121.7 (**C**(CH_3)OCH_3), 97.1 (C-1), 73.2 (C-2), 70.3 (C-3), 68.3 (C-4), 67.1 (C-5), 63.2 (C-6), 51.2 (C(CH_3)O**C**H_3), 20.9 (3-OCO**C**H_3, 4-OCO**C**H_3 and 6-OCO**C**H_3) and 20.2 (C(**C**H_3)OCH_3) ppm.

HRMS: *m/z* calcd. for $C_{15}H_{22}O_{10}Na$ $[M + Na]^+$ 385.1111; found 385.1121.

*1,2-O-(1-methoxyethylidene)-3,4,6-tri-O-benzyl-α-D-glucopyranose (**7**).* Synthesized over two steps, starting from 1,2-*O*-(1-methoxyethylidene)-3,4,6-tri-*O*-acetyl-α-D-glucopyranose and NaOMe according to the general procedure for deacetylation, and then a further reaction of 1,2-*O*-(1-methoxyethylidene)-α-D-glucopyranose (2.10 g, 6.89 mmol) withNaH (1.15 g, 48.01 mmol) and BnBr (7.30 g, 42.67 mmol) according to the general procedure for alkylation of free hydroxyl groups. This reaction gave the title compound as a colorless oil (2.28 g, 51%). TLC: R_f: 0.66 (EtOAc:Hexane 1:2).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 7.37–7.16 (m, 15H, arom. H), 5.78 (d, 1H, $J_{1,2}$ = 5.3 Hz, H-1), 4.71 and 4.60 (each d, each 1H, *J* = –11.9 Hz, 3-O**C**H_2Ph), 4.60 and 4.38 (each d, each 1H, *J* = –11.9 Hz, 4-O**C**H_2Ph), 4.57 and 4.50 (each d, each 1H, *J* = –11.9 Hz, 6-O**C**H_2Ph), 4.42 (ddd, 1H, $J_{2,3}$ = 3.6, $J_{2,4}$ = –0.8 Hz, H-2), 3.87 (dd, 1H, $J_{3,4}$ = 4.6 Hz, H-3), 3.79 (ddd, 1H, $J_{5,4}$ = 9.5, $J_{5,6a}$ = 2.2, $J_{5,6b}$ = 4.3 Hz, H-5), 3.71 (ddd, 1H, H-4), 3.66 (dd, 1H, $J_{6a,6b}$ = –10.8 Hz, H-6a), 3.64 (dd, 1H, H-6b), 3.28 (s, 3H, C(CH_3)OC**H$_3$**) and 1.65 (s, 3H, C(C**H$_3$**)OCH_3) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 138.2–127.7 (arom. C), 121.4 (**C**(CH_3)OCH_3), 97.9 (C-1), 78.9 (C-3), 76.0 (C-2), 75.0 (C-4), 73.5 (6-O**C**H_2Ph), 73.0 (4-O**C**H_2Ph), 72.0 (3-O**C**H_2Ph), 70.6 (C-5), 69.2 (C-6), 50.7 (C(CH_3)O**C**H_3) and 21.4 (C(**C**H_3)OCH_3) ppm.

HRMS: *m/z* calcd. for $C_{30}H_{34}O_7Na$ $[M + K]^+$ 545.1905; found 545.1883.

*2-O-acetyl-3,4,6-tri-O-benzyl-α-D-glucopyranose trichloroacetimidate (**8**).* Synthesized over two steps, starting from **7** and *p*-TsOH according to the general procedure for opening of 1,2-orthoester, and then a further reaction of 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranose (1.00 g, 2.03 mmol) with Cl_3CCN (2.93 g, 20.3 mmol) and DBU (0.12 g, 0.50 mmol) according to the general procedure for formation of an imidate donor. This reaction gave the title compound as a colorless oil (1.17 g, 91%). TLC: R_f: 0.51 (EtOAc:Hexane 1:4).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 8.56 (s, 1H, N**H**), 7.35–7.16 (m, 15H, arom. H), 6.52 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 5.07 (dd, 1H, $J_{2,3}$ = 10.0 Hz, H-2), 4.85 and 4.77 (each d, each 1H, *J* = –11.4 Hz, 3-O**C**H_2Ph), 4.83 and 4.57 (each d, each 1H, *J* = –10.6 Hz, 4-O**C**H_2Ph), 4.63 and 4.50 (each d, each 1H, *J* = –12.0 Hz, 6-O**C**H_2Ph), 4.09 (dd, 1H, $J_{3,4}$ = 9.3 Hz, H-3), 4.01 (ddd, $J_{5,4}$ = 10.1, $J_{5,6a}$ = 3.3, $J_{5,6b}$ = 1.9 Hz, H-5), 3.88 (dd, 1H, H-4), 3.81 (dd, 1H, $J_{6a,6b}$ = –11.1 Hz, H-6a), 3.70 (dd, 1H, H-6b) and 1.92 (s, 3H, 2-OCOC**H$_3$**) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 170.2 (2-O**C**OCH_3), 161.2 (imidate **C**NH), 138.4–127.9 (arom. C), 94.2 (C-1), 91.2 (imidate **C**Cl_3), 79.6 (C-3), 77.2 (C-4), 75.6 (4-O**C**H_2Ph), 75.5 (3-O**C**H_2Ph), 73.7 (6-O**C**H_2Ph), 73.6 (C-5), 72.5 (C-2), 68.0 (C-6) and 20.7 (2-OCO**C**H_3) ppm.

HRMS: *m/z* calcd. for $C_{31}H_{32}Cl_3NO_7Na$ $[M + Na]^+$ 658.1142; found 658.1487.

*Benzyl 2-O-acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranoside (**9**).* Synthesized from **8** (1.11 g, 1.75 mmol), BnOH (0.13 g, 1.25 mmol) and TMSOTf (0.56 g, 2.5 mmol) according to the general procedure for glycosylation using TMSOTf. The reaction gave the title compound as a white solid (0.70 g, 96%). TLC: R_f: 0.68 (EtOAc:Hexane 1:2).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 7.37–7.16 (m, 20H, arom. H), 5.08 (dd, 1H, $J_{1,2}$ = 7.9, $J_{2,3}$ = 9.5 Hz, H-2), 4.89 and 4.61 (each d, each 1H, *J* = –12.4 Hz, 1-O**C**H_2Ph), 4.79 and 4.56 (each d, each 1H, *J* = –10.8 Hz, 4-O**C**H_2Ph), 4.77 and 4.65 (each d, each 1H, *J* = –11.4 Hz, 3-O**C**H_2Ph), 4.64 and 4.57 (each d, each 1H, *J* = –12.2 Hz, 6-O**C**H_2Ph), 4.42 (d, 1H, H-1), 3.76 (dd, 1H, $J_{6a,5}$ = 1.9, $J_{6a,6b}$ = –10.9 Hz, H-6a), 3.72 (dd, 1H, $J_{6b,5}$ = 4.9 Hz, H-6b), 3.71 (dd, 1H, $J_{4,3}$ = 9.0, $J_{4,5}$ = 9.8 Hz, H-4), 3.64 (dd, 1H, H-3), 3.48 (ddd, 1H, H-5) and 1.93 (s, 3H, 2-OCOC**H$_3$**) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 169.6 (2-O**C**OCH_3), 138.3–127.7 (arom. C), 99.8 (C-1), 83.1 (C-3), 78.1 (C-4), 75.3 (C-5), 75.2 (4-O**C**H_2Ph and 3-O**C**H_2Ph), 73.7 (6-O**C**H_2Ph), 73.3 (C-2), 70.4 (1-O**C**H_2Ph), 68.9 (C-6) and 21.0 (2-OCO**C**H_3) ppm.

HRMS: *m/z* calcd. for $C_{36}H_{38}O_7Na$ $[M + Na]^+$ 605.2516; found 605.2524.

Benzyl 3,4,6-tri-O-benzyl-β-D-glucopyranoside. Synthesized from **9** (0.68 g, 1.17 mmol) and NaOMe (0.09 g, 1.75 mmol) according to the general procedure for deacetylation. This reaction gave the title compound as a white solid (0.57 g, 91%). TLC: R_f: 0.64 (EtOAc:Hexane 1:2).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 7.39–7.16 (m, 20H, arom. H), 4.95 and 4.63 (each d, each 1H, *J* = –11.7 Hz, 1-O**C**H_2Ph), 4.92 and 4.82 (each d, each 1H, *J* = –11.3 Hz, 3-O**C**H_2Ph), 4.84 and 4.55 (each d, each 1H, *J* = –10.8 Hz, 4-O**C**H_2Ph), 4.63 and 4.56 (each d, each 1H, *J* = –12.2 Hz, 6-O**C**H_2Ph), 4.36 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 3.76 (dd, 1H, $J_{6a,5}$ = 1.9, $J_{6a,6b}$ = –10.8 Hz, H-6a), 3.72 (dd, 1H, $J_{6b,5}$ = 4.8 Hz, H-6b), 3.63 (dd, 1H, $J_{4,3}$ = 8.9, $J_{4,5}$ = 9.8 Hz, H-4), 3.62 (ddd, 1H, $J_{2,2}$-

$_{OH}$ = 2.2, $J_{2,3}$ = 9.2 Hz, H-2), 3.58 (dd, 1H, H-3), 3.49 (ddd, 1H, H-5) and 2.30 (d, 1H, 2-OH) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 138.8–127.8 (arom. C), 101.8 (C-1), 84.7 (C-3), 77.7 (C-4), 75.4 (C-5), 75.3 (3-O**C**H_2Ph), 75.1 (4-O**C**H_2Ph), 74.9 (C-2), 73.7 (6-O**C**H_2Ph) 71.2 (1-O**C**H_2Ph) and 69.0 (C-6) ppm.

HRMS: *m/z* calcd. for $C_{34}H_{36}O_6Na$ $[M + Na]^+$ 563.2410; found 563.2405.

*Benzyl 3,4,6-tri-O-benzyl-2-O-propargyl-β-D-glucopyranoside (**10**).* Synthesized from Benzyl 3,4,6-tri-*O*-benzyl-β-D-glucopyranoside (0.54 g, 1.0 mmol), NaH (0.05 g, 1.9 mmol) and propargyl bromide (0.22 g, 1.5 mmol) according to the general procedure for alkylation of free hydroxyl groups. This reaction gave the title compound as a yellow oil (0.49 g, 85%). TLC: R_f: 0.72 (EtOAc:Hexane 1:2).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 7.41–7.15 (m, 20H, arom. H), 5.01 and 4.76 (each d, each 1H, *J* = –10.8 Hz, 3-O**C**H_2Ph), 4.95 and 4.65 (each d, each 1H, *J* = –11.9 Hz, 1-O**C**H_2Ph), 4.84 and 4.53 (each d, each 1H, *J* = –10.8 Hz, 4-O**C**H_2Ph), 4.62 and 4.55 (each d, each 1H, *J* = –12.2 Hz, 6-O**C**H_2Ph), 4.51 (dd, 1H, $J_{CH2a,CH}$ = 2.4, $J_{CHa,CH2b}$ = –15.3 Hz, 2-OC**H$_{2a}$**C≡CH), 4.45 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.43 (dd, 1H, $J_{CH2b,CH}$ = 2.4 Hz, 2-OC**H$_{2b}$**C≡CH), 3.75 (dd, 1H, $J_{6a,5}$ = 1.9, $J_{6a,6b}$ = –10.8 Hz, H-6a), 3.69 (dd, 1H, $J_{6b,5}$ = 4.9 Hz, H-6b), 3.60 (dd, 1H, $J_{3,2}$ = 9.1, $J_{3,4}$ = 8.7 Hz, H-3), 3.59 (dd, 1H, $J_{4,5}$ = 9.6 Hz, H-4), 3.50 (dd, 1H, H-2), 3.45 (ddd, 1H, H-5) and 2.44 (dd, 1H, 2-OCH_2C≡C**H**) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 138.7–127.7 (arom. C), 102.4 (C-1), 84.4 (C-3), 81.8 (C-2), 80.2 (2-OCH_2**C**≡CH), 77.8 (C-4), 75.9 (3-O**C**H_2Ph), 75.2 (4-O**C**H_2Ph), 75.1 (C-5), 74.4 (2-OCH_2C≡**C**H), 73.6 (6-O**C**H_2Ph), 71.2 (1-O**C**H_2Ph), 69.0 (C-6) and 59.8 (2-O**C**H_2C≡CH) ppm.

HRMS: *m/z* calcd. for $C_{37}H_{38}O_6Na$ $[M + Na]^+$ 601.2566; found 601.2569.

Benzyl 3,4,6-tri-O-benzyl-2-O-carboranylmethyl-β-D-glucopyranoside. Synthesized from **10** (0.47 g, 0.8 mmol) and $B_{10}H_{14}$ (0.10 g, 1.4 mmol) according to the general procedure for coupling with decaborane. This reaction gave the title compound as an off-white oil (0.29 g, 51%). TLC: R_f: 0.47 (EtOAc:Hexane 1:3).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 7.40–7.12 (m, 20H, arom. H), 4.90 and 4.55 (each d, each 1H, *J* = –11.5 Hz, 1-OC**H$_2$**Ph), 4.84 and 4.70 (each d, each 1H, *J* = –11.1 Hz, 3-OC**H$_2$**Ph), 4.77 and 4.55 (each d, each 1H, *J* = –10.8 Hz, 4-OC**H$_2$**Ph), 4.63 and 4.55 (each d, each 1H, *J* = –12.2 Hz, 6-OC**H$_2$**Ph), 4.35 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.19 and 4.01 (each d, each 1H, *J* = –10.8 Hz, 2-OC**H$_2$**-carborane), 3.73 (dd, 1H, $J_{6a,5}$ = 1.9, $J_{6a,6b}$ = –10.9 Hz, H-6a), 3.70 (dd, 1H, $J_{6b,5}$ = 4.5 Hz, H-6b), 3.7 (br s, 1H, carborane-C**H**), 3.62 (dd, 1H, $J_{4,3}$ = 9.0, $J_{4,5}$ = 9.8 Hz, H-4), 3.52 (dd, 1H, $J_{3,2}$ = 9.3 Hz, H-3), 3.41 (ddd, 1H, H-5), 3.22 (dd, 1H, H-2) and 2.86-1.36 (br m, 10H, carborane-B**H**) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 138.1–127.7 (arom. C), 101.3 (C-1), 84.2 (C-3), 82.6 (C-2), 78.2 (C-4), 75.8 (3-O**C**H_2Ph), 75.1 (C-5), 75.0 (4-O**C**H_2Ph), 73.7 (6-O**C**H_2Ph), 73.2 (2-O**C**H_2-carborane), 72.9 (carborane-**C**), 71.3 (1-O**C**H_2Ph), 68.6 (C-6) and 57.8 (carborane-**C**H) ppm.

^{11}B NMR (160.46 MHz, $CDCl_3$, 23 °C): δ = –3.07, –4.97, –9.36, –11.81 and –13.35 ppm.

HRMS: *m/z* calcd. for $C_{37}H_{48}B_{10}O_6K$ $[M + K]^+$ 737.4018; found 737.4057.

*2-O-carboranylmethyl-D-glucopyranose (**3**).* Synthesized from Benzyl 3,4,6-tri-*O*-benzyl-2-*O*-carboranylmethyl-β-D-glucopyranoside (0.11 g, 0.2 mmol) and 10% Pd/C (0.11 g, 1.0 mmol) according to the general procedure for hydrogenolysis. This reaction gave the title compound as a colorless oil (0.04 g, 77%, α:β 33:67). TLC: R_f: 0.51 (EtOAc:MeOH 5:1).

α anomer: ^{1}H NMR (500.13 MHz, CD_3OD, 23 °C): δ = 5.20 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.76 (br s, 1H, carborane-C**H**), 4.21 and 4.13 (each d, each 1H, *J* = –10.8 Hz, 2-OC**H$_2$**-carborane), 3.76 (dd, 1H, $J_{6a,6b}$ = –11.8, $J_{6a,5}$ = 2.4 Hz, H-6a), 3.73 (dd, 1H, $J_{3,2}$ = 9.7, $J_{3,4}$ = 9.0 Hz, H-3), 3.72 (ddd, 1H, $J_{5,4}$ = 10.0, $J_{5,6b}$ = 5.1 Hz, H-5), 3.68 (dd, 1H, H-6b), 3.29 (dd, 1H, H-4), 3.23 (dd, 1H, H-2) and 2.83–1.42 (br m, 10H, carborane-B**H**) ppm.

^{13}C NMR (125.76 MHz, CD_3OD, 23 °C): δ = 91.6 (C-1), 82.7 (C-2), 75.0 (carborane-**C**), 73.8 (C-3), 72.8 (C-5), 72.6 (2-O**C**H_2-carborane), 71.8 (C-4), 62.5 (C-6) and 60.1 (carborane-**C**H) ppm.

β anomer: ^{1}H NMR (500.13 MHz, CD_3OD, 23 °C): δ = 4.81 (br s, 1H, carborane-C**H**), 4.51 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.33 and 4.19 (each d, each 1H, *J* = –11.2 Hz, 2-OC**H$_2$**-carborane), 3.83 (dd, 1H, $J_{6a,5}$ = 2.1, $J_{6a,6b}$ = –11.9 Hz, H-6a), 3.63 (dd, 1H, $J_{6b,5}$ = 5.7 Hz, H-6b), 3.38 (dd, 1H, $J_{3,2}$ = 9.2, $J_{3,4}$ = 8.7 Hz, H-3), 3.72 (ddd, 1H, $J_{5,4}$ = 9.7 Hz, H-5), 3.25 (dd, 1H, H-4), 2.92 (dd, 1H, H-2) and 2.83-1.42 (br m, 10H, carborane-B**H**) ppm.

^{13}C NMR (125.76 MHz, CD_3OD, 23 °C): δ = 97.6 (C-1), 85.9 (C-2), 77.8 (C-3), 77.3 (C-4), 75.3 (carborane-**C**), 74.3 (2-O**C**H_2-carborane), 71.8 (C-5), 62.7 (C-6) and 59.9 (carborane-**C**H) ppm.

^{11}B NMR (160.46 MHz, CD_3OD, 23 °C): δ = –2.68, –4.40, –8.62, –10.93 and –12.50 ppm.

HRMS: *m/z* calcd. for $C_9H_{24}B_{10}O_6Na$ $[M + Na]^+$ 361.2401; found 361.2396.

*1,2:5,6-bis-O-(isopropylidene)-α-D-glucofuranose (**19**).* Synthesized from D-glucose (7.11 g, 39.5 mmol) and I_2 (2.00 g, 7.88 mmol) according to the general procedure of acetonide formation. This reaction gave the title compound as a white solid (4.55 g, 47%). TLC: R_f: 0.56 (EtOAc:Hexane 1:1).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 5.94 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 4.34 (ddd, 1H, $J_{5,4}$ = 7.8, $J_{5,6a}$ = 6.2, $J_{5,6b}$ = 5.3 Hz, H-5), 4.32 (ddd, $J_{3,2}$ = 0.7, $J_{3,4}$ = 2.8, $J_{3,3\text{-OH}}$ = 3.9 Hz, 1H, H-3), 4.17 (dd, 1H, $J_{6a,6b}$ = –8.7 Hz, H-6a), 4.07 (dd, 1H, H-4), 4.00 (dd, 1H, H-6b), 2.76 (d, 1H, 3-OH), 1.50 (s, 3H, 1,2-C**H$_3$**′), 1.45 (s, 3H, 5,6-C**H$_3$**′), 1.37 (s, 3H, 5,6-C**H$_3$**′′) and 1.32 (s, 3H, 1,2-C**H$_3$**′′) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 111.9 (1,2-^{q}C), 109.8 (5,6-^{q}C), 105.4 (C-1), 85.2 (C-2), 81.2 (C-4), 75.2 (C-3), 73.5 (C-5), 67.7 (C-6), 26.9 (1,2-**C**H_3′and 5,6-**C**H_3′), 26.3 (1,2-**C**H_3′′) and 25.2 (5,6-**C**H_3′′) ppm.

HRMS: *m/z* calcd. for $C_{12}H_{20}O_6Na$ $[M + Na]^+$ 283.1158; found 283.1164.

1,2:5,6-bis-O-(isopropylidene)-3-O-propargyl-α-D-glucofuranose. Synthesized from **19** (3.45 g, 13.3 mmol), NaH (0.61 g, 25.2 mmol) and propargyl bromide (2.37 g, 19.9 mmol) according to the general procedure for alkylation of free hydroxyl groups. This reaction gave the title compound as a yellow oil (3.55 g, 90%). TLC: R_f: 0.35 (EtOAc:Hexane 1:3).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 5.89 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.64 (dd, 1H, $J_{2,3}$ = 0.03 Hz, H-2), 4.30 (dd, 1H, $J_{CH2a,CH2b}$ = –15.9, $J_{CH2a,CH}$= –2.2 Hz, 3-OC**H$_{2a}$**C≡CH), 4.28 (dd, 1H, $J_{CH2b,CH}$= –2.4 Hz, 3-OC**H$_{2b}$**C≡CH), 4.28 (ddd, 1H, $J_{5,4}$ = 7.9, $J_{5,6a}$ = 6.5, $J_{5,6b}$ = 5.4 Hz, H-5), 4.14 (dd, 1H, $J_{4,3}$ = 2.7 Hz, H-4), 4.10 (dd, 1H, H-3), 4.09 (dd, 1H, $J_{6a,6b}$ = –8.8 Hz, H-6a), 4.00 (dd, 1H, H-6b), 2.49 (dd, 1H, 3-OCH$_2$C≡C**H**), 1.50 (s, 3H, 1,2-C**H$_3$**′), 1.43 (s, 3H, 5,6-C**H$_3$**′), 1.35 (s, 3H, 5,6-C**H$_3$**′′) and 1.32 (s, 3H, 1,2-C**H$_3$**′′) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 112.0 (1,2-^{q}C), 109.2 (5,6-^{q}C), 105.4 (C-1), 83.0 (C-2), 81.7 (C-3), 81.2 (C-4), 79.4 (3-OCH$_2$**C**≡CH), 75.0 (3-OCH$_2$C≡**C**H), 72.7 (C-5), 67.4 (C-4), 58.3 (3-O**C**H$_2$C≡CH), 27.0 (1,2-**C**H$_3$′and 5,6-**C**H$_3$′), 26.4 (1,2-**C**H$_3$′′) and 25.5 (5,6-**C**H$_3$′′) ppm.

HRMS: *m/z* calcd. for $C_{15}H_{22}O_6Na$ $[M + Na]^+$ 321.1314; found 321.1342.

*1,2:5,6-bis-O-(isopropylidene)-3-O-carboranylmethyl-α-D-glucofuranose (**20**).* Synthesized from 1,2:5,6-bis-*O*-(isopropylidene)-3-*O*-propargyl-α-D-glucofuranose (1.72 g, 5.77 mmol) and $B_{10}H_{14}$ (1.34 g, 11.0 mmol) according to the general procedure for coupling with decaborane. This reaction gave the title compound as a clear oil (1.08 g, 45%). TLC: R_f: 0.58 (EtOAc:Hexane 1:3).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 5.86 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.46 (dd, 1H, $J_{2,3}$ = 0.03 Hz, H-2), 4.28 (br s, 1H, carborane-C**H**), 4.15 (dd, 1H, $J_{6a,5}$ = 6.1, $J_{6a,6b}$ = –8.9 Hz, H-6a), 4.14 (ddd, 1H, $J_{5,4}$ = 9.0, $J_{5,6b}$ = 5.5 Hz, H-5), 4.12 and 4.04 (each d, each 1H, *J* = –10.8 Hz, 3-OC**H$_2$**-carborane), 4.01 (dd, 1H, $J_{4,3}$ = 3.2 Hz, H-4), 3.95 (dd, 1H, H-3), 2.94-1.55 (br m, 10H, carborane-B**H**), 1.48 (s, 3H, 1,2-C**H$_3$**′), 1.41 (s, 3H, 5,6-C**H$_3$**′), 1.34 (s, 3H, 1,2-C**H$_3$**′′) and 1.31 (s, 3H, 5,6-C**H$_3$**′′) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 112.3 (1,2-^{q}C), 109.6 (5,6-^{q}C), 105.3 (C-1), 84.2 (C-3), 82.4 (C-2), 81.3 (C-4), 72.4 (carborane-**C**), 72.2 (C-5), 71.6 (3-O**C**H$_2$-carborane), 68.1 (C-6), 57.8 (carborane-**C**H), 26.8 (1,2-**C**H$_3$′ and 5,6-**C**H$_3$′), 26.2 (1,2-**C**H$_3$′′) and 25.2 (5,6-**C**H$_3$′′) ppm.

^{11}B NMR (160.46 MHz, $CDCl_3$, 23 °C): δ = –3.11, –4.69, –8.77, –9.00, –11.13, –12.24, –13.08 and –13.41 ppm.

HRMS: *m/z* calcd. for $C_{15}H_{32}B_{10}O_6Na$ $[M + Na]^+$ 441.3027; found 441.3048.

*3-O-carboranylmethyl-D-glucopyranose (**4**).* Synthesized from **20** (0.40 g, 0.96 mmol) according to the general procedure for acetonide removal. This reaction gave the title compound as a white solid (0.29 g, 90%, α:β 30:70). TLC: R_f: 0.61 (DCM:MeOH 5:1).

α anomer: ^{1}H NMR (500.13 MHz, CD_3OD, 23 °C): δ = 5.05 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.84 (br s, 1H, carborane-C**H**), 4.30 and 4.29 (each d, each 1H, *J* = –10.8 Hz, 3-OC**H$_2$**-carborane), 3.75 (dd, 1H, $J_{6a,5}$ = 2.3, $J_{6a,6b}$ = –11.8 Hz, H-6a), 3.74 (ddd, 1H, $J_{5,4}$ = 9.9, $J_{5,6b}$ = 5.0 Hz, H-5), 3.68 (dd, 1H, H-6b), 3.48 (dd, 1H, $J_{3,2}$ = 9.5, $J_{3,4}$ = 9.0 Hz, H-3), 3.41 (dd, 1H, H-2), 3.38 (dd, 1H, H-4) and 3.05-1.42 (br m, 10H, carborane-B**H**) ppm.

^{13}C NMR (125.76 MHz, CD_3OD, 23 °C): δ = 94.0 (C-1), 85.6 (C-3), 75.6 (carborane-**C**), 74.8 (3-O**C**H$_2$-carborane), 73.6 (C-2), 72.9 (C-5), 71.2 (C-4), 62.4 (C-6) and 59.7 (carborane-**C**H) ppm.

β anomer: ^{1}H NMR (500.13 MHz, CD_3OD, 23 °C): δ = 4.84 (br s, 1H, carborane-C**H**), 4.44 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.32 and 4.29 (each d, each 1H, *J* = –11.0 Hz, 3-OC**H$_2$**-carborane), 3.82 (dd, 1H, $J_{6a,5}$ = 2.3, $J_{6a,6b}$ = –11.9 Hz, H-6a), 3.64 (dd, 1H, $J_{6b,5}$ = 5.7 Hz, H-6b), 3.36 (dd, 1H, $J_{4,3}$ = 8.7 Hz, $J_{4,5}$ = 9.8 Hz, H-4), 3.24 (ddd, 1H, H-5), 3.18 (dd, 1H, $J_{3,2}$ = 9.0 Hz, H-3), 3.17 (dd, 1H, H-2) and 3.05-1.42 (br m, 10H, carborane-B**H**) ppm.

^{13}C NMR (125.76 MHz, CD_3OD, 23 °C): δ = 98.1 (C-1), 88.2 (C-3), 77.7 (C-5), 76.1 (C-2), 75.5 (carborane-**C**), 74.7 (3-O**C**H$_2$-carborane), 71.2 (C-4), 62.5 (C-6) and 59.8 (carborane-**C**H) ppm.

^{11}B NMR (160.46 MHz, CD_3OD, 23 °C): δ = –2.87, –4.59, –8.83, –11.03, –11.90 and –12.65 ppm.

HRMS: *m/z* calcd. for $C_9H_{24}B_{10}O_6Na$ $[M + Na]^+$ 361.2401; found 361.2395.

*Benzyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**11**).* Synthesized from 1,2,3,4,6-penta-*O*-acetyl-D-glucopyranose (8.70 g, 22.3 mmol), BnOH (9.64 g, 89.2 mmol) and $BF_3{\cdot}OEt_2$ (17.92 g, 126.0 mmol) according to the general procedure for glycosylation of peracetylated glucose. This reaction gave the title compound as a white solid (4.17 g, 43%). TLC: R_f: 0.39 (EtOAc:Hexane 1:3).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 7.38–7.25 (m, 5H, arom. H), 5.17 (dd, 1H, $J_{3,2}$ = 9.7, $J_{3,4}$ = 9.4 Hz, H-3), 5.11 (dd, 1H, $J_{4,5}$ = 10.1 Hz, H-4), 5.07 (dd, 1H, $J_{2,1}$ = 8.0 Hz, H-2), 4.90 and 4.62 (each d, each 1H, *J* = –12.3 Hz, 1-OC**H$_2$**Ph), 4.55 (d, 1H, H-1), 4.27 (dd, 1H, $J_{6a,5}$ = 4.7, $J_{6a,6b}$ = –12.3 Hz, H-6a), 4.17 (dd, 1H, $J_{6b,5}$ = 2.4 Hz, H-6b), 3.67 (ddd, 1H, H-5), 2.11 (s, 3H, 6-OCOC**H$_3$**), 2.02 (s, 3H, 4-OCOC**H$_3$**), 2.00 (s, 3H, 2-OCOC**H$_3$**) and 1.99 (s, 3H, 3-OCOC**H$_3$**) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 170.8 (6-O**C**OCH$_3$), 170.4 (3-O**C**OCH$_3$), 169.5 (2-O**C**OCH$_3$), 169.4 (4-O**C**OCH$_3$), 136.8–127.9 (arom. C), 99.4 (C-1), 73.0 (C-3), 72.0 (C-5), 71.4 (C-2), 70.9 (1-O**C**H$_2$Ph), 68.5 (C-4), 62.1 (C-6), 20.9–20.8 (6-OCO**C**H$_3$ and 2-OCO**C**H$_3$) and 20.7 (3-OCO**C**H$_3$ and 4-OCO**C**H$_3$) ppm.

HRMS: *m/z* calcd. for $C_{21}H_{26}O_{10}Na$ $[M + Na]^+$ 461.1424; found 461.1398.

Benzyl β-D-glucopyranoside. Synthesized from **11** (4.94 g, 11.3 mmol) according to the general procedure for deacetylation. This reaction gave the title compound as a white solid (3.01 g, 99%). TLC: R_f: 0.46 (DCM:MeOH 7:1).

^{1}H NMR (500.13 MHz, CD_3OD, 23 °C): δ = 7.43–7.24 (m, 5H, arom. H), 4.92 and 4.66 (each d, each 1H, *J* = –11.8 Hz, 1-OC**H$_2$**Ph), 4.34 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 3.89 (dd, 1H, $J_{6a,5}$ = 2.6, $J_{6a,6b}$ = –11.9 Hz, H-6a), 3.68 (dd, 1H, $J_{6b,5}$ = 5.9 Hz, H-6b), 3.33 (dd, 1H, $J_{3,2}$ = 8.8, $J_{3,4}$ = 9.3 Hz, H-3), 3.29 (dd, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 3.25 (ddd, 1H, H-5) and 3.24 (dd, 1H, H-2) ppm.

^{13}C NMR (125.76 MHz, CD_3OD, 23 °C): δ = 139.1–128.7 (arom. C), 103.3 (C-1), 78.1 (C-3), 78.0 (C-5), 75.1 (C-2), 71.7 (C-5), 71.7 (1-O**C**H$_2$Ph) and 62.8 (C-6) ppm.

HRMS: *m/z* calcd. for $C_{13}H_{18}O_6Na$ $[M + Na]^+$ 293.1001; found 239.1009.

*Benzyl 4,6-O-benzylidene-β-D-glucopyranoside (**12**).* Synthesized from Benzyl β-D-glucopyranoside (2.98 g, 11.03 mmol), $C_6H_5CH(OCH_3)_2$ (2.68 g, 17.6 mmol) and *p*-TsOH (0.19 g, 1.10 mmol) according to the general procedure for installation of 4,6-*O*-benzylidene acetals. This reaction gave the title compound as a white solid (3.95 g, 85%). TLC: R_f: 0.66 (DCM:MeOH 10:1).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 7.51–7.30 (m, 10H, arom. H), 5.54 (s, 1H, C**H**Ph), 4.94 and 4.64 (each d, each

1H, J = –11.6 Hz, 1-OC**H**$_2$Ph), 4.51 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.37 (dd, 1H, $J_{6a,5}$ = 5.0, $J_{6a,6b}$ = –10.6 Hz, H-6a), 3.81 (dd, 1H, $J_{6b,5}$ = 10.0 Hz, H-6b), 3.81 (ddd, 1H, $J_{3,2}$ = 9.0, $J_{3,3\text{-OH}}$ = 2.3, $J_{3,4}$ = 9.3 Hz, H-3), 3.58 (dd, 1H, $J_{4,5}$ = 9.4 Hz, H-4), 3.56 (ddd, 1H, $J_{2,2\text{-OH}}$ = 2.6 Hz, H-2), 3.46 (ddd, 1H, H-5), 2.84 (d, 1H, 3-OH) and 2.67 (d, 1H, 2-OH) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 137.1–126.4 (arom. C), 102.2 (C-1), 102.1 (**C**HPh), 80.7 (C-3), 74.7 (C-4), 73.3 (C-2), 71.7 (1-O**C**H$_2$Ph), 68.8 (C-6) and 66.6 (C-5) ppm.

HRMS: *m/z* calcd. for $C_{20}H_{22}O_6Na$ $[M + Na]^+$ 381.1314; found 381.1307.

Benzyl 2,3-di-O-benzyl-4,6-O-benzylidene-β-D-glucopyranoside. Synthesized from **12** (1.00 g, 2.8 mmol), BnBr (1.58 g, 8.9 mmol) and NaH (0.25 g, 10.6 mmol) according to the general procedure for alkylation of free hydroxyl groups. This reaction gave the title compound as a white solid (1.21 g, 80%). TLC: R_f: 0.35 (EtOAc:Hexane 1:6).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 7.51–7.23 (m, 20H, arom. H), 5.58 (s, 1H, C**H**Ph), 4.95 and 4.68 (each d, each 1H, J = –11.6 Hz, 1-OC**H**$_2$Ph), 4.90 and 4.79 (each d, each 1H, J = –11.6 Hz, 3-OC**H**$_2$Ph), 4.90 and 4.77 (each d, each 1H, J = –11.6 Hz, 2-OC**H**$_2$Ph), 4.63 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1), 4.38 (dd, 1H, $J_{6a,5}$ = 5.0, $J_{6a,6b}$ = –10.5 Hz, H-6a), 3.82 (dd, 1H, $J_{6b,5}$ = 10.0 Hz, H-6b), 3.75 (dd, 1H, $J_{3,2}$ = 8.8, $J_{3,4}$ = 9.4 Hz, H-3), 3.71 (dd, 1H, $J_{4,5}$ = 9.4 Hz, H-4), 3.53 (dd, 1H, H-2) and 3.42 (ddd, 1H, H-5) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 138.6–126.1 (arom. C), 103.2 (C-1), 101.3 (**C**HPh), 82.3 (C-2), 81.7 (C-4), 81.1 (C-3), 75.6 (2-O**C**H$_2$Ph), 75.3 (3-O**C**H$_2$Ph), 71.7 (1-O**C**H$_2$Ph), 69.0 (C-6) and 66.2 (C-5) ppm.

HRMS: *m/z* calcd. for $C_{34}H_{34}O_6Na$ $[M + Na]^+$ 561.2253; found 561.2279.

*Benzyl 2,3,6-tri-O-benzyl-β-D-glucopyranoside (**13**).* Synthesized from Benzyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside (1.19 g, 2.2 mmol) according to the general procedure for selective ring–opening of the benzylidene acetal to give the 4-OH/6-OBn substrate. This reaction gave the title compound as a white solid (0.85 g, 72%). TLC: R_f: 0.61 (EtOAc:Hexane 1:2).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 7.39–7.25 (m, 20H, arom. H), 4.96 and 4.66 (each d, each 1H, J = –11.9 Hz, 1-OC**H**$_2$Ph), 4.96 and 4.72 (each d, each 1H, J = –10.9 Hz, 2-OC**H**$_2$Ph), 4.93 and 4.72 (each d, each 1H, J = –11.4 Hz, 3-OC**H**$_2$Ph), 4.62 and 4.59 (each d, each 1H, J = –12.1 Hz, 6-OC**H**$_2$Ph), 4.53 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 3.79 (dd, 1H, $J_{6a,5}$ = 3.7, $J_{6a,6b}$ = –10.4 Hz, H-6a), 3.73 (dd, 1H, $J_{6b,5}$ = 5.4 Hz, H-6b), 3.61 (ddd, 1H, $J_{4,3}$ = 8.9, $J_{4,4\text{-OH}}$ = 2.2, $J_{4,5}$ = 9.7 Hz, H-4), 3.49 (dd, 1H, $J_{2,3}$ = 9.2 Hz, H-2), 3.45 (dd, 1H, H-3), 3.45 (ddd, 1H, H-5) and 2.50 (d, 1H, 4-OH) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 138.7–127.8 (arom. C), 102.7 (C-1), 84.2 (C-3), 81.9 (C-2), 75.4 (3-O**C**H$_2$Ph), 74.9 (2-O**C**H$_2$Ph), 74.2 (C-5), 73.8 (6-O**C**H$_2$Ph), 71.7 (C-4), 71.3 (1-O**C**H$_2$Ph) and 70.4 (C-6) ppm.

HRMS: *m/z* calcd. for $C_{34}H_{36}O_6Na$ $[M + Na]^+$ 563.2410; found 563.2453.

*Benzyl 2,3,6-tri-O-benzyl-4-O-propargyl-β-D-glucopyranoside (**14**).* Synthesized from **13** (0.80 g, 1.49 mmol), NaH (0.07 g, 2.83 mmol) and propargyl bromide (0.39 g, 2.38 mmol) according to the general procedure for alkylation of free hydroxyl groups. This reaction gave the title compound as a white solid (0.79 g, 91%). TLC: R_f: 0.68 (EtOAc:Hexane 1:2).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 7.39–7.25 (m, 20H, arom. H), 4.97 and 4.66 (each d, each 1H, J = –11.9 Hz, 1-OC**H**$_2$Ph), 4.94 and 4.70 (each d, each 1H, J = –10.9 Hz, 2-OC**H**$_2$Ph), 4.89 and 4.77 (each d, each 1H, J = –10.8 Hz, 3-OC**H**$_2$Ph), 4.65 and 4.60 (each d, each 1H, J = –12.1 Hz, 6-OC**H**$_2$Ph), 4.49 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.37 (dd, 1H, $J_{CH2a,CH2b}$ = –15.3, $J_{CH2a,CH}$ = 2.4 Hz, 4-OC**H**$_{2a}$C≡CH), 4.26 (dd, 1H, $J_{CH2b,CH}$ = 2.4 Hz, 4-OC**H**$_{2b}$C≡CH), 3.85 (dd, 1H, $J_{6a,5}$ = 1.8, $J_{6a,6b}$ = –11.0 Hz, H-6a), 3.74 (dd, 1H, $J_{6b,5}$ = 5.2 Hz, H-6b), 3.60 (dd, 1H, $J_{3,2}$ = 9.2, $J_{3,4}$ = 9.0 Hz, H-3), 3.51 (dd, 1H, $J_{4,5}$ = 9.8 Hz, H-4), 3.48 (dd, 1H, H-2), 3.44 (ddd, 1H, H-5) and 2.40 (dd, 1H, 4-OCH$_2$C≡C**H**) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 138.5–127.7 (arom. C), 102.7 (C-1), 84.6 (C-3), 82.3 (C-2), 80.0 (4-OCH$_2$**C**≡CH), 77.5 (C-4), 75.9 (3-O**C**H$_2$Ph), 75.0 (2-O**C**H$_2$Ph), 74.7 (C-5), 74.6 (4-OCH$_2$C≡**C**H), 73.6 (6-O**C**H$_2$Ph), 71.3 (1-O**C**H$_2$Ph), 69.3 (C-6) and 60.0 (4-O**C**H$_2$C≡CH) ppm.

HRMS: *m/z* calcd. for $C_{37}H_{38}O_6Na$ $[M + Na]^+$ 601.2566; found 601.2551.

Benzyl 2,3,6-tri-O-benzyl-4-O-carboranylmethyl-β-D-glucopyranoside. Synthesized from **14** (0.75 g, 1.30 mmol) and $B_{10}H_{14}$ (0.30 g, 2.47 mmol) according to the general procedure for coupling with decaborane. This reaction gave the title compound as a clear oil (0.59 g, 65%). TLC: R_f: 0.59 (EtOAc:Hexane 1:3).

^{1}H NMR (500.13 MHz, $CDCl_3$, 23 °C): δ = 7.39–7.21 (m, 20H, arom. H), 4.96 and 4.68 (each d, each 1H, J = –11.0 Hz, 2-OC**H**$_2$Ph), 4.95 and 4.66 (each d, each 1H, J = –11.9 Hz, 1-OC**H**$_2$Ph), 4.93 and 4.57 (each d, each 1H, J = –11.0 Hz, 3-OC**H**$_2$Ph), 4.65 and 4.52 (each d, each 1H, J = –12.1 Hz, 6-OC**H**$_2$Ph), 4.46 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.15 and 3.80 (each d, each 1H, J = –10.2 Hz, 4-OC**H**$_2$-carborane), 3.66 (dd, 1H, $J_{6a,5}$ = 1.9, $J_{6a,6b}$ = –10.9 Hz, H-6a), 3.65 (dd, 1H, $J_{6b,5}$ = 3.9 Hz, H-6b), 3.52 (dd, 1H, $J_{3,2}$ = 9.1, $J_{3,4}$ = 9.0 Hz, H-3), 3.48 (dd, 1H, H-2), 3.45 (dd, 1H, $J_{4,5}$ = 9.7 Hz, H-4), 3.42 (br s, 1H, carborane-C**H**), 3.35 (ddd, 1H, H-5) and 2.78–1.44 (br m, 10H, carborane-B**H**) ppm.

^{13}C NMR (125.76 MHz, $CDCl_3$, 23 °C): δ = 138.2–127.9 (arom. C), 102.5 (C-1), 84.1 (C-3), 82.4 (C-2), 77.8 (C-4), 75.6 (3-O**C**H$_2$Ph), 74.8 (2-O**C**H$_2$Ph), 74.2 (C-5), 73.8 (6-O**C**H$_2$Ph), 73.3 (4-O**C**H$_2$-carborane), 72.5 (carborane-**C**), 71.3 (1-O**C**H$_2$Ph), 68.7 (C-6) and 58.1 (carborane-**C**H) ppm.

^{11}B NMR (160.46 MHz, $CDCl_3$, 23 °C): δ = –2.85, –4.83, –9.29, –11.78 and –13.34 ppm.

HRMS: *m/z* calcd. for $C_{37}H_{48}B_{10}O_6Na$ $[M + Na]^+$ 721.4279; found 721.4411.

*4-O-carboranylmethyl-D-glucopyranose (**5**).* Synthesized from Benzyl 2,3,6-tri-*O*-benzyl-4-*O*-carboranylmethyl-β-D-glucopyranoside (0.12 g, 0.18 mmol) and 10% Pd/C (0.12 g, 1.0 mmol) according to the general procedure for removal of benzyl groups. This reaction gave the title compound as a white solid (0.05 g, 77%, α:β 50:50). TLC: R_f: 0.47 (DCM:MeOH 5:1).

α anomer: ^{1}H NMR (500.13 MHz, CD_3OD, 23 °C): δ = 5.08 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 4.68 (br s, 1H, carborane-C**H**), 4.43 and 4.11 (each d, each 1H, , J = –10.6 Hz, 4-OC**H**$_2$-carborane), 3.79 (ddd, 1H, $J_{5,4}$ = 9.9, $J_{5,6a}$ = 2.1, $J_{5,6b}$ = 3.9 Hz, H-5), 3.78 (dd, 1H, $J_{3,2}$ = 9.5, $J_{3,4}$ = 9.0 Hz, H-3), 3.72 (dd, 1H, $J_{6a,6b}$ = –

12.0 Hz, H-6a), 3.68 (dd, 1H, H-6b), 3.32 (dd, 1H, H-2), 3.29 (dd, 1H, H-4) and 2.96–1.46 (br m, 10H, carborane-B**H**) ppm.

^{13}C NMR (125.76 MHz, CD_3OD, 23 °C): δ = 93.8 (C-1), 79.8 (C-4), 76.4 (C-2), 74.9 (C-3), 75.1 (carborane-**C**), 74.2 (4-O**C**H_2-carborane), 71.5 (C-5), 62.1 (C-6) and 60.5 (carborane-**C**H) ppm.

β anomer: ^{1}H NMR (500.13 MHz, CD_3OD, 23 °C): δ = 4.68 (br s, 1H, carborane-C**H**), 4.45 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.42 and 4.11 (each d, each 1H, J = –10.6 Hz, 4-OC**H**$_2$-carborane), 3.79 (dd, 1H, $J_{6a,5}$ = 1.8, $J_{6a,6b}$ = –12.0 Hz, H-6a), 3.66 (dd, 1H, $J_{6b,5}$ = 4.3 Hz, H-6b), 3.49 (dd, 1H, $J_{3,2}$ = 9.2, $J_{3,4}$ = 9.0 Hz, H-3), 3.32 (ddd, 1H, $J_{5,4}$ = 9.5 Hz, H-5), 3.30 (dd, 1H, H-4), 3.09 (dd, 1H, H-2) and 2.96–1.46 (br m, 10H, carborane-B**H**) ppm.

^{13}C NMR (125.76 MHz, CD_3OD, 23 °C): δ = 98.1 (C-1), 80.0 (C-4), 78.0 (C-3), 76.6 (C-2), 75.1 (carborane-**C**), 74.2 (4-O**C**H_2-carborane), 74.0 (C-5), 62.1 (C-6) and 60.5 (carborane-**C**H) ppm.

^{11}B NMR (160.46 MHz, CD_3OD, 23 °C): δ = –2.08, –3.96, –8.43, –10.61 and –12.09 ppm.

HRMS: *m/z* calcd. for $C_9H_{24}B_{10}O_6Na$ $[M + Na]^+$ 361.2401; found 361.2384.

2.4 Molecular modelling

The initial geometries of the ligands were optimized to a local minimum at DFT level, using the dispersion-corrected hybrid Tao-Perdew-Scuseria-Staroverov functional TPSSh-D3(BJ),[24-26] with the doubly polarized triple-zeta basis set def2-TZVPP.[28] The structures of the different ligands were aligned so that geometries would be as similar as possible. Partial atomic charges were computed using the restrained electrostatic potential (RESP) protocol.[29] For the RESP charge calculation, the molecule was divided into two parts, with one part consisting of the carborane and a linking carbon, and the other part comprising the sugar. Partial charges of hydrogens bonded to the same carbon were constrained to be equal. The geometry optimizations were performed with Turbomole 7.21,[30,31] and the RESP calculations with NWChem 6.8.[32]

Molecular docking studies were performed using AutoDock 4.2.6.[33,34] All rotatable bonds in the carborane part were set to nonrotatable (inactive). For docking, the number of torsional degrees of freedom for the carboranes was set to 8 (torsdof 8). The docking studies were performed using the XylE inward-open 4QIQ and outward-open 6N3I PDB structures. The XylE protein structures were mutated using PyMOL, changing Gln-415 to Asn-415. The most probable rotamer, that is, the one with least clashes with surrounding amino acids, as suggested by PyMOL was used. Each protein was prepared by removing the ligand and other superfluous small molecules (Zn for 4QIQ), adding hydrogens, merging them and then computing Gasteiger partial charges. For all proteins, a grid of size 46 x 56 x 60 was used, with a grid spacing value of 0.375. The grid center was in the middle of the protein cavity, for the grid box to cover the binding site. During docking, the protein was kept rigid and only ligand torsional angles changed. 2000 independent search runs, each with max 2.5 million energy evaluations and population size of 150 with max 27000 generations were performed using the default settings of the Lamarckian genetic algorithm (LGA), that is, a mutation rate of 0.02 and crossover rate of 0.8, with one top individual surviving to the next generation. Conformations were clustered (ranked) with a cluster RMS 2.0 Å.

Parameters for boron, missing from the standard distribution of Autodock, were added to the parameter file: R 2.285, R_{ii} 4.57, epsilon 0.179, vol 49.9744; other parameters were set to their corresponding carbon values. R and epsilon were taken from Couto et al.,[35] and were used to calculate R_{ii} and vol. The complete parameter definition was thus:

atom_par B 4.57 0.179 49.9744 –0.00143 0.0 0.0 0 –1 –1 0 # Boron for Carborane

For the binding pose analysis, clusters with less than 10 conformations, clusters at unphysical location, and clusters with positive binding energies were removed. We used CAVER Analyst "cavity computation" to recognize the binding sites and for estimating binding site volumes.[36]

2.5 Cytotoxicity

Human epithelial CAL 27 squamous carcinoma cell line (ATCC® CRL-2095™) was acquired from American Type Culture Collection (Manassas, VA, USA). TC-treated cell culturing flasks and 96-well plates were obtained from Corning (Corning, NY, USA). Dulbecco's Modified Eagle's Medium (DMEM), Dulbecco's phosphate buffer saline (10×DPBS), Hank's balanced salt solution (1×HBSS), fetal bovine serum (FBS), GlutaMax (100×), and Penicillin-Streptomycin (10,000 U/ml) were purchased from Gibco (Life Technologies, Carlsbad, CA, USA). CellTiter-Glo® luminescent cell viability kit was obtained from Promega Corporation (Madison, WI, USA). Pierce™ BCA Protein assay was purchased from Thermo Fisher Scientific (Waltham, MA, USA).

The commercial CellTiter-Glo® luminescent cell viability assay was used for the determination of the *in vitro* cytotoxicity of glucoconjugates (**1**–**5**) and borocaptate (BSH). The human epithelial CAL 27 squamous carcinoma cell line was used as a head and neck cancer cell model. Cells were seeded in 100 µl cell culture medium supplemented with 1×GlutaMax, 1% Penicillin-Streptomycin and 10% FBS at the density of 5,000 cells per well and allowed to adhere overnight on a 96-well plate. Once the cells had attached, the medium was removed and exchanged with 100 µl of complete cell culture medium containing either **1**, **2**, **3**, **4**, **5** or BSH at 5 µM, 25 µM, 50 µM, 125 µM, and 250 µM concentration. The cells were incubated for 6 and 24 h in a temperature- and humidity-controlled incubator (37 °C, 95% relative humidity and 5% CO_2). Fresh cell culture medium and 1% (v/v) Triton X-100 were used as negative and positive controls of cell viability, respectively. At the predetermined time points, the plates were equilibrated to r.t. for 30 min. The test solutions were discarded and the cells were washed with 100 µl of 1×DPBS twice. For the viability assay, 50 µl of 1×HBSS and CellTiter-Glo® cocktail were added to each well. The plates were immediately protected from light with aluminum foil and gently shaken on an orbital shaker for 2 min at r.t.. The ATP-generated luminescence was measured using a Varioskan LUX multimode microplate reader (Thermo Fisher Scientific, Waltham, MA, USA). The total protein content was quantified using the colorimetric bicinchoninic acid (BCA) protein assay. Each sample was used to normalize the cell viability results. The BCA assay procedure was carried out according to the manufacturer's protocol. Briefly, 25 µl of cell lysates from the Cell-TiterGlo® samples were pipetted to a 96-well clear bottom UV-transparent microplate. Then, 200 µl of working reagent were added to each well (1:8 ratio). The plates were wrapped with aluminum foil and mixed on an orbital shaker for 30 seconds before further incubation at 37 °C for 30 min. The absorbance was read at 562 nm and the protein content was calculated using a bovine serum albumin (BSA) standard curve (0–2000 µg/ml). All experiments were carried out in quadruplicate.

The statistical significance of mean cell viability was determined using unpaired Student's *t*-test and compared to the negative control (untreated cells).

2.6 GLUT1 protein quantification in CAL27 cells

CAL27 squamous cell carcinoma cells (ATCC® CRL-2095™) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). CAL27 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Gibco, ThermoFisher Scientific, Waltham, MA, USA) supplemented with 2 mM L-glutamine (ThermoFisher Scientific, Waltham, MA, USA), 10% heat-inactivated fetal bovine serum (Gibco, ThermoFisher Scientific, Waltham, MA, USA), penicillin (50 U/mL)- streptomycin (50 µg/mL) solution (ThermoFisher Scientific, Waltham, MA, USA) at 37 °C in a humidified incubator with 5% CO_2. The CAL27 cells were seeded onto 24 well plates at the density of 5×10^5 cells/wells. The cells were used in further studies two days after seeding, while the passage number of the cells was in the range of 8–20. The culture medium was removed and the cells were washed and pre-incubated at 37 °C for 10 min with pre-warmed HBSS without glucose (including 125 mM NaCl, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.3 mM KH_2PO_4, 1.3 mM $CaCl_2$ and 25 mM HEPES adjusted to pH 7.4) before the experiments.

The preparation of crude membrane fractions of CAL27 cells was accomplished in the following way. The cells were washed twice with ice-cold phosphate buffered saline (PBS), and 2 mL of PBS was added. The cells were scraped off the dishes and centrifuged at 250 × g, +4 °C, 10 min. The supernatant was removed, and the cell pellet was snap-frozen and stored at –80 °C before sample preparation. The Membrane Protein Extraction Kit (BioVision Incorporated, Milpitas, CA, USA) was used for extraction of crude membrane fractions from the cell pellet according to the manufacturer's protocol. The protein concentration was measured using a Bio-Rad Protein Assay (EnVision, Perkin Elmer, Inc., Waltham, MA, USA) and 50 µg of protein from each sample (n=3) were taken for further analysis.

These samples were further processed in order to prepare them for protein quantification studies. In more detail, a total of 50 µg of protein was dissolved in 7 M guanidine hydrochloride, 0.5 M Tris-HCl [pH 8.5] and 10 mM EDTA-Na [pH 8.0] in MilliQ water. The samples were then reduced by dithiothreitol (1:50, w/w) for 60 min at r.t., and alkylated by iodoacetamide (1:20, w/w) (Sigma-Aldrich, St. Louis, MO, USA) for 60 min at r.t. in the dark. The proteins were then precipitated with cold methanol and chloroform. The pellet (alkylated proteins) was resuspended in a 6 M urea solution and mixed for 10 minutes at r.t.. The alkylated proteins were dissolved completely by intermittent sonication (Branson 3510, Danbury, CT, USA) after diluting the samples with 0.1 M Tris-HCl to a final concentration of 1.2 M urea. The dissolved proteins were first digested with LysC (1/100, w/w) (Sigma-Aldrich, St. Louis, MO, USA) and 0.05% ProteaseMax (Promega Biotech AB, Nacka, Sweden) for 3 hours at r.t. and then, spiked with 10 µl (60 fmol) of the labelled JPT's peptides for absolute quantification (JPT Peptide Technologies GmbH, Berlin, Germany) (see the supporting information, table 4) and were further digested with (1/100, w/w) TPCK-Trypsin (Promega Biotech AB, Nacka, Sweden) for 18 hours at 37 °C. The tryptic digestion was stopped by adding 40 µl of 5% formic acid and the diluted samples were centrifuged at 18000 g × 5 min. at 4 °C. The supernatants were transferred to HPLC vials for quantification studies.

The absolute quantity of GLUT1 was determined by a LC-MS/MS-SRM setup. In more detail, the quantification of GLUT1 and the membrane marker Na^+K^+ATPase was based on three selected reaction monitoring (SRM) transitions of precursor and product ions from both the light and heavy peptide chains (see the supporting information, table 4 and figures 69–74), as previously described.[37] A total of 20 µl of the digested peptides (10 µg) were injected into an Agilent 1290 LC system coupled with an Agilent 6495 triple quadrupole mass spectrometer with an electrospray ionization source operated in the positive mode (Agilent Technologies, Santa Clara, CA, USA). Initially, the peptides were separated on a 2.1 × 250 mm, 2.7 µm column (Agilent Technologies, Santa Clara, CA, USA) and eluted by a gradient of 0.1% formic acid in water (A) and acetonitrile (B). A constant flow rate of 0.3 ml/min was utilized and the gradient was shifted in the following way: 2–7% B for 2 min, followed by 7-30% B for 48 minutes, 30-45% B for 3 min and 45–80% B for 2.5 min before re-equilibrating the column for 4.5 min. The data was acquired using the Agilent MassHunter Workstation Acquisition and processed using the Skyline software 20.1. The GLUT1 and Na^+K^+ATPase proteins were quantified based on the ratio between the light and heavy peptides.

2.7 GLUT1 affinity and cellular uptake studies

Determination of the GLUT1 affinity was conducted as described recently by our team.[16] In more detail, the CAL27 cells were cultured, seeded and pre-incubated as described above. The GLUT1 affinity of the glucoconjugates was studied through a *cis*-inhibition assay using a known radiolabeled GLUT1 substrate, [^{14}C]-D-glucose (PerkinElmer, Waltham, MA, USA). Briefly, the pre-incubated cells were further incubated at r.t. for 5 min with the glucoconjugates (0.1–1800 µM) containing 1.8 µM (0.1 mCi/ml) of [^{14}C]-D-glucose in HBSS (250 µL), with HBSS as a control. The reaction was quenched by the addition of ice-cold HBSS and the cells were washed twice with ice-cold HBSS, lysed with 250 µL of 0.1 M NaOH and the lysate was mixed with 1.0 mL of Emulsifier safe cocktail (PerkinElmer, Waltham, MA, USA). The radioactivity was measured by liquid scintillation counter (MicroBeta^2 counter, PerkinElmer, Waltham, MA, USA). The inhibition of [^{14}C]-D-glucose uptake in the presence of the boron cluster glucoconjugates was compared to the control (HBSS) and calculated as percentages (%) (see supporting information, Figure 75 and table 5).

The cellular uptake of the glucoconjugates was studied by adding the compounds in concentrations ranging from 10–400 µM (in pre-warmed HBSS buffer (250 µL)) onto the cell layer, followed by incubation at r.t. for 5 and 30 min, respectively. After incubation, the reaction was quenched with ice-cold HBSS, and the cells were washed and lysed as described previously. The lysate used in determination of cellular uptake was collected from 4 wells into Eppendorf tubes and centrifuged at 4 °C. 800 µL from each sample supernatant was collected and digested in 1.0 mL of conc. HNO_3 for 24 hrs. After sample digestion, Milli-Q water was then added in order to reach a total volume of 10 mL. The cellular uptake was then determined by analyzing the boron concentrations by inductively-coupled plasma mass-spectrometry (ICP-MS).

The NeXION 350D ICP-MS instrument (PerkinElmer Inc., Waltham, MA, USA) equipped with an ESI PrepFAST autosampler (Elemental Scientific, Omaha, NE, USA) was used.

A peristaltic pump and a nebulizer were used for sample injection. The radio frequency (RF) was used at a power of 1.6 kW during the operations and the nebulizer gas, auxiliary gas, and plasma gas flows were 0.90, 18, and 1.2 L min^{-1}, respectively. The sample uptake rate was 3.5 mL min^{-1} and the dwell time was set to 100 ms per AMU. To remove polyatomic interferences, a triple-quadrupole reaction system was used in the collision mode with kinetic energy discrimination (KED). Helium was used as the cell gas (3.7 mL min^{-1}). Yttrium-89 was used as an internal standard and mixed online with the samples to compensate for matrix effects and instrument drift. The boron concentration was determined against the certified multi-element calibration standard, TraceCERT Periodic Table Mix 1 (Sigma Aldrich) in acid conditions (6.7% HNO_3, TraceMetal™ grade, Fisher Chemical). The calibration range used for ^{11}B was 4–400 µg L^{-1}. The limit of detection (LOD) was 1.0 µg L^{-1}. Three replicates were analyzed from each concentration. The data was analyzed with the PerkinElmer Syngistix Data Analysis Software™.

2.8 NMR spectroscopic hexokinase studies

The activity of human hexokinase 1 on each of the glucoconjugates was studied by incubating each compound with the enzyme in an NMR tube at 30 °C and recording a time series of ^{1}H and ^{31}P NMR spectra overnight. The reaction mixture contained 2 mM glucoconjugate, 5 mM ATP and 5 mM $MgCl_2$ in 50 mM Tris-HCl buffer, pH 7.5, containing 10% of D_2O (EurisoTop). A 2 mM solution of D-glucose was used as a positive control. The experiments were performed on a 600 MHz Bruker Avance III NMR spectrometer equipped with a QCI H-P/C/N-D cryo probe and SampleJet automated sample changer. After the initial screening of both the ^{1}H and ^{31}P-NMR experiments, 5 µl (10 mU) of recombinant human hexokinase 1 (Abcam) was added to the NMR tube and recording of the time series of the spectra was initiated. The preheater block of the SampleJet was used as a sample incubator during the experiments. This enabled automated monitoring of several reactions in parallel.

3. RESULTS AND DISCUSSION

Synthesis and structural characterization of the positional isomer library. 33 chemical reactions (without accounting for method development) is required in order to access the entire library of positional isomers depicted in Figure 2. Due to our recent work on the synthesis of **6**,[16] and the α and β methyl glycosides thereof, we had already obtained the required knowledge in both boron cluster chemistry and carbohydrate chemistry to plan appropriate synthetic pathways.

From a design perspective, it is important to note that the chosen boron cluster contains ten boron nuclei per delivery agent in a charge-neutral way. While the carboranes exhibit three-dimensional aromaticity which could afford them some added stability,[38,39] we opted to use a neutral hydrophobic boron cluster in order to avoid undesirable interactions between charged boron clusters and amino acid residues which could have a detrimental effect on the transport process.[40–42] In addition, a glucoconjugate bearing a carboranylmethyl-substituent delivers ten times the amount of boron atoms per molecule compared to BPA, has a small degree of rotational freedom which may be important for passage through the transporter and retains a minimally intrusive nature from a steric point-of-view. The targeted glucoconjugates are thus a seemingly ideal solution for BNCT.

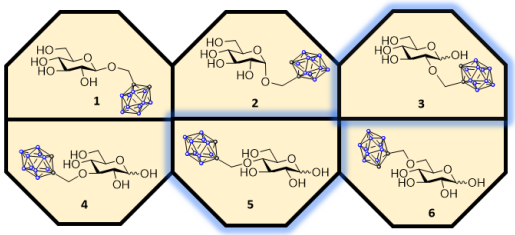
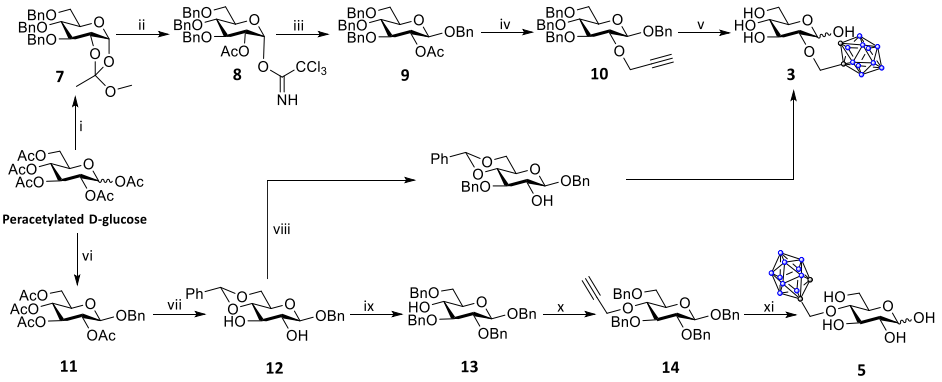


Figure 2. The molecular structures of all positional isomers of *ortho*-carboranylmethyl substituted D-glucose. All glucoconjugates were prepared in the present study. The ones for which synthetic protocols have not been previously reported in the literature are shaded with a blue glow (**3** and **5**). In the *ortho*-carboranylmethyl moiety, the blue dots represent boron atoms and the gray dots represent carbon atoms (hydrogen atoms omitted).

From a medicinal chemistry perspective, the importance of comparing the properties of the complete set of positional isomers under identical conditions cannot be sufficiently stressed: such studies are mandatory in order to identify the most promising modification sites. In the literature, the synthesis of **1**, **2** and **4** has been reported a few decades ago and the synthesis of **6** was recently described by our team.[16,43,44] While the previous work has left several questions unanswered regarding the potential of glucoconjugates as delivery agents for BNCT, the work has nevertheless been of importance in establishing functioning reaction protocols for the construction of boron-glucoconjugates. One observable limitation in some of the previous reports is the lack of ^{11}B NMR spectra as part of the reported structural characterization data. Here, we bridge this gap as all of the structures containing boron clusters were fully characterized by NMR-spectroscopy including ^{1}H-decoupled ^{11}B NMR. Focus will next be placed on the synthesis of the new glucoconjugates **3** and **5** which have not been reported previously in the literature. Both synthetic routes are depicted in Scheme 1.

For the synthesis of **3**, an 11-step synthetic route was originally devised starting from commercially available peracetylated D-glucose. From a synthetic perspective, it was important to consider the susceptibility of decaborane to nucleophiles,[45] *i.e.* protecting groups would be a necessity. The requirements for the protecting group strategy were twofold. First, in order to access boron cluster derivative **3**, a conjugation reaction between an alkyne and a decaborane-ACN complex was envisioned. This required the pre-installation of a propargyl group at the 2nd position. Second, *ortho*-carboranes are known to degrade under strongly basic conditions and therefore protecting groups requiring such deprotection conditions were avoided.[45]



Scheme 1. The synthetic routes and reaction conditions leading to **3** and **5**: i) 1) I_2, Et_3SiH, DCM, reflux, 1 h; 2) 2,6-lutidine, TBAI, MeOH, reflux, 3 h, 91% over two steps; 3) NaOMe, MeOH, RT, 1.5 h, quant.; 4) NaH, BnBr, DMF, 2 h, 51%; ii) 1) *p*-TsOH, acetone:H_2O 7:3, RT, 2 h, 88%; 2) Cl_3CCN, DBU, RT, 1 h, 91%; iii) BnOH, TMSOTf, DCM, –20 °C, 2 h, 96%; iv) 1) NaOMe, MeOH, THF, RT, 24 h, 91%; 2) NaH, propargyl bromide, DMF, RT, 2 h, 85%; v) 1) $B_{10}H_{14}$, ACN, 60 °C, 1 h; 2) **10**, toluene, 80 °C, 17 h, 51%; 3) H_2, 10% Pd/C, EtOAc:MeOH 7:1, 5 bar, 4 h, 77%; vi) BnOH, $BF_3{\cdot}OEt_2$, DCM, 0°C→RT, 56%; vii) 1) NaOMe, MeOH/THF, RT, 17 h, 99%; 2) $C_6H_5(OCH_3)_2$, *p*-TsOH, DMF, 60 °C, 0.2 bar, 2 h, 85%; viii) 1) Bu_2SnO, toluene; 120 °C, 3 h; 2) TBAB, CSF, BnBr, 120 °C, 80% (selectivity 2:1); ix) 1) NaH, BnBr, DMF, RT, 2.5 h, 80%; 2) Et_3SiH, TFA, DCM, RT, 3 h, 72%; x) NaH, propargyl bromide, DMF, RT, 2 h, 91%; xi) 1) $B_{10}H_{14}$, ACN, 60 °C, 1 h; 2) **14**, toluene, 80 °C, 20 h, 65%; 3) H_2, 10% Pd/C, EtOAc:MeOH 7:1, 5 bar, 4 h, 77%.

With these premises in mind, we started by converting peracetylated D-glucose into *ortho*-ester **7** via a four-step protocol. First, the anomeric acetate was displaced by an iodine with I_2 and Et_3SiH followed by conversion into an 1,2-*ortho*-ester species with 2,6-lutidine, TBAI and MeOH in a 91% yield (over two steps).[46]

The acetyl groups were next exchanged to benzyl groups through a two-step deacetylation[47]–benzylation[48] protocol with an overall yield of 51%. The selective ring-opening of the *ortho*-ester was performed with *p*-TsOH in acetone:H_2O 7:3 in order to obtain the 1-OH/2-OAc derivative. While the overall yield was 88%, the formation of an inseparable and unwanted 1-OAc/2-OH regioisomer was also noted in a 7:1 ratio in favor of the desired regioisomer. The mixture was converted to imidate donor **8** with DBU and Cl_3CCN in DCM in a 91% yield.[46,49] It should be noted that this yield would not be possible to obtain from the mixture as such and therefore, it is likely that acyl migration takes place under the basic conditions employed which increases the amount of the 1-OH/2-OAc isomer *in situ*.[50]

Following a previously reported robust TMSOTf promoted glycosylation protocol,[51,52] but instead employing BnOH as the acceptor, compound **9** could be isolated in excellent yield. In order to convert **9** into building block **10** which was required for the conjugation reaction with decaborane, a high-yielding two-step deacetylation-propargylation protocol was employed. At this stage, all hydroxyl groups were protected as benzyl ethers except the 2nd position which contained a propargyl-substituent. The benzyl ether has been found to be a suitable protecting group during the synthesis of boron cluster glucoconjugates of the chosen type.[16,53] The conjugation with decaborane ($B_{10}H_{14}$) was conducted in two stages, following the typical conditions reported in the literature.[45] Decaborane was first refluxed in dry ACN under inert conditions in order to form the reactive $B_{10}H_{12}{\cdot}2ACN$ species, which was then allowed to react with the alkyne functionality in **10** at elevated temperatures overnight. The reaction was quenched by the addition of dry MeOH before further work-up and purification. The moderate yield obtained after isolation of the end product is in the typically observed literature range.[45] In the last step, the benzyl protective groups were removed in a hydrogenolysis reaction, following our previously described protocol and in good yield. While this synthetic route to **3** is rather lengthy, a satisfactory overall yield of 11% could nevertheless be achieved.

In principle, both glucoconjugates **3** and **5** could be prepared through a shared synthetic route. This would shorten the overall synthetic routes significantly. Therefore, while shifting focus to the synthesis of **5**, we did address the possibility of using one of the intermediates for the generation of **3** as well. Starting from commercially available peracetylated D-glucose, we performed a $BF_3{\cdot}OEt_2$ promoted glycosylation with BnOH according to literature protocols, and, in similar yields.[54] Deacetylation under Zemplen conditions[47] followed by the formation of a 4,6-*O*-benzylidene acetal with *p*-TsOH gave **12** in an 84% yield over two steps.[55,56] In order to synthesize **3** by a shorter reaction route, we attempted a stereoselective benzylation of the 3-OH group of **12** through the use of a dibutylstannylene intermediate.[57,58] While the yield was high (80%) and the selectivity decent (2:1 in favor of the desired regioisomer), we were not able to separate the two regioisomers at this stage, or at any other stage for that matter, although we did complete the synthesis of **3** and **4** as a mixture through this route as well. Unfortunately, the lengthy route therefore seems to be required in order to access **3** in its pure form. Continuing on the synthesis of **5**, the hydroxyl groups in **12** were benzylated and the benzylidene acetal was selectively ring-opened with Et_3SiH and TFA[59] to give the 4-OH/6-OBn substrate in high yield. From this stage onwards, similar reaction conditions as described above were employed. In short, the free hydroxyl group was propargylated in excellent yield, followed by a conjugation reaction with the preformed $B_{10}H_{12}{\cdot}2ACN$ species and cleavage of the benzyl groups through hydrogenolysis. Altogether, glucoconjugate **5** was prepared in 8 synthetic steps with an overall yield of 12%.

On any given synthetic route, the results obtained are only reliable if the reported structural characterization data is accurate. In this work, high-resolution mass spectrometry (HRMS) and NMR spectroscopy were employed in verification of the structural features and purity of all products synthesized – with emphasis on the latter technique. NMR-spectroscopy is generally referred to as the number one tool for structural elucidation studies of organic molecules. Herein, we used a

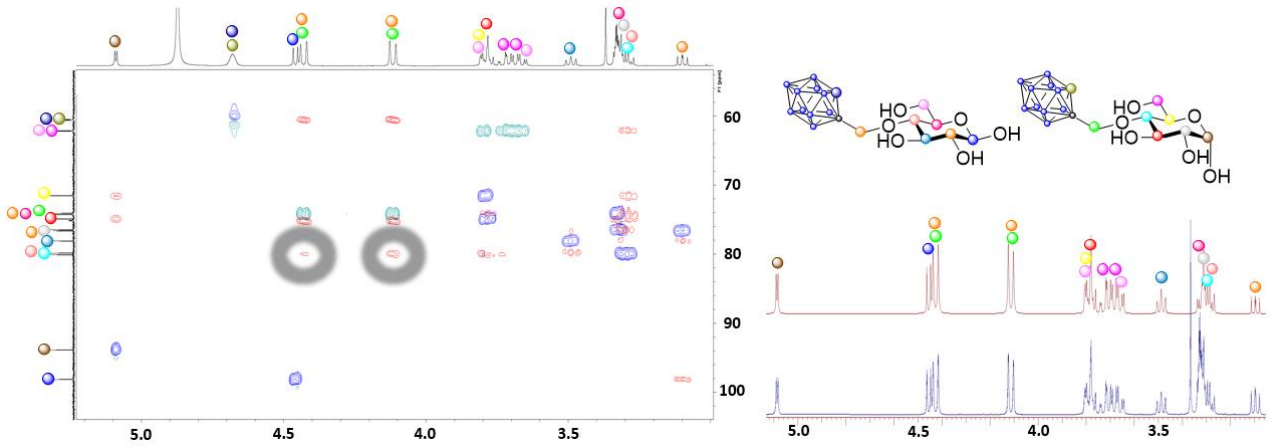


Figure 3. Left: An overlapping Ed-HSQC (CH/CH_3 in blue and CH_2 in green) and HMBC-spectrum (red) with the most important HMBC-correlations highlighted with grey circles. **Top right:** The molecular structures of the two anomers together with the colors used for visualization of the different signals in the NMR spectra. **Bottom right:** the 5.2–3.2 ppm region of the ^{1}H NMR spectrum highlighting the accuracy of the spectral simulations with the PERCH software (measured spectrum in blue, simulated spectrum in red). The 5.0–4.6 ppm region has been removed and the methanol and CD_3OD peaks have not been included in the simulation.

solid selection of 1D and 2D-NMR experiments (^{1}H, ^{13}C, 1H-decoupled ^{11}B, 1D-TOCSY, DQF-COSY, Ed-HSQC, HMBC) with further processing of the ^{1}H NMR spectra in the Peak ResearRCH (PERCH) software[60] in order to obtain accurate coupling constants (excerpts of representative spectra are displayed in Figure 3).

The PERCH software applies quantum mechanical optimization in the spectral simulations and the accurate coupling constants are required in order to verify the conformation of the glucoconjugates, which is important for understanding their interactions with GLUT1. Altogether, we were able to assign all signals (carboranylmethyl moiety, protective groups and carbohydrates) in the ^{1}H and ^{13}C NMR spectra of all products. While the characterization flow of anomeric mixtures was addressed in detail in our recent study,[16] and will not be repeated here, it is important to note that the carboranylmethyl-bearing glucoconjugates exist primarily in the $^4C_1$-chair conformation.

Finally, the purity of glucoconjugates **1–5** was assessed by absolute qNMR using maleic acid as an internal calibrant in accordance with the protocol reported by Pauli et al.[61] These experiments confirmed that the purity of all glucoconjugates evaluated in the *in vitro* studies exceeded 95%.

Investigating the interactions with the GLUT1 transporter. While a number of boron-glucoconjugates have been reported to date,[44,45] studies on the molecular level recognition and interaction with their intended transporters, the very essence of their biochemical functioning principle, are lacking. These foundations need to be addressed, not only from a functioning principle point-of-view but also from a medicinal chemistry and drug development perspective. Efforts in this direction have been reported for other classes of glucoconjugates recently.[24] However, a change in the substituents inflicts a change in the character of the molecules and may alter their behavior. Therefore, these aspects have become the focus of our GLUT1-targeting approach to the development of improved delivery agents for BNCT. GLUT1 is an interesting transporter that is well-suited for BNCT. Due to the "Warburg effect" a wide range of cancer cells overexpress GLUT1.[18,20] This stems from the inefficient glucose metabolism in cancer cells further coupled with a greater demand for energy consumption in cell proliferation. The head and neck cancers are no exception and GLUT1 has been reported to be responsible for the aberrant growth of the human CAL 27 carcinoma cell line, which is used as our model in the *in vitro* studies.[64,65] To gain insight on the GLUT1 expression level in the CAL 27 cells, we initially determined the GLUT1 amount by LC-MS/MS-SRM, and found it to be 4.7±0.97 fmol/µg of protein. For comparison, the GLUT1 expression in *e.g.* human embryonic kidney cells (HEK 293) was found to be significantly less pronounced, only 0.37±0.09 fmol/µg of protein. The expression of GLUT1 in the CAL 27 cancer cells is therefore 8–20 times higher than in the control cell line used. Even when these results were further normalized to Na^+/K^+-ATPase, the GLUT1 expression was still roughly 2.5-times higher in the CAL 27 cells. Although these factors indicate that there is significant potential in targeting GLUT1, this approach has previously been viewed as suboptimal from a BNCT-perspective due to open questions regarding the possibility of modified glucoconjugates to compete for the transporter with the high levels (6 mM) of D-glucose found in blood.[24,66] In our previous work,[16] we showed that glucoconjugate **6** has a significantly higher affinity towards the transporter thereby proving that such doubts were unfounded. In order to investigate if the 6th position was unique in this matter, or if this is a general feature of these types of glucoconjugates, we performed a similar *cis*-inhibition assay in the CAL 27 cell line with the entire positional isomer library in this work (the GLUT1-function and expression in the CAL 27 cell line had been validated). In short, the *cis*-inhibition assay was conducted as a competition assay between the individual glucoconjugates and radiolabeled [^{14}C]-D-glucose with a control experiment performed with D-glucose. This experimental setup is an accurate representation of the situation that the glucoconjugates would face in the intended application. The experimentally determined relative GLUT1 IC_{50}-values for glucoconjugates **1–5** were in the low µM-range: 84.6 µM for **1**, 107.6 µM for **2**, 122.6 µM for **3**, 86.2 µM for **4** and 32.07 µM for **5**. Compared to the > 1 mM IC_{50}-value displayed by D-glucose, the transporter-targeting capability of the glucoconjugates are at least 8–31 times

higher. From a practical point-of-view, the glucoconjugates are therefore expected to be able to compete for the transporter with the high D-glucose levels found in blood. While care should be taken in drawing conclusions based on affinity results alone, the experimental IC_{50}-values determined herein, and previously for **6** (44.0 μM), suggest that modification at positions 4 or 6 lead to the highest GLUT1-affinities.

In order to address the molecular level basis for the observed affinities, we used the molecular models constructed and validated previously.[16] These models are based on XylE, a D-xylose-proton symporter found in *E.coli* which is structurally similar to the GLUT1–4 proteins.[67] Most importantly, the required crystal structures for both the outward- (PDB ID 6N3I)[68] and inward-open (PDB ID 4QIQ)[69] conformations have been published thus resulting in the possibility for assessing the interactions of the glucoconjugates with the transporter on the outside and inside of the cell. Following virtual mutation of the Gln415 in XylE to Asn to match Asn411 in GLUT1, an identical carbohydrate binding site can be established.[70,71] Using the same model for the inward- and outward-open conformations is crucial, as it provides directly interconnectable information on the binding modes displayed by the glucoconjugates on the outside and inside of the cells. As a result, the use of a XylE-based model is warranted in our case. For GLUT1, only the inward-open crystal structure has been reported,[72] thus impeding comparison to the observed experimental affinities, where the outward-open structure is more relevant.

Through the use of our previously established protocols, we determined the binding energies of glucoconjugates **1**–**5** by molecular docking studies. It should be noted that molecules **3**–**6** exist as an anomeric mixture of α and β anomers (α:β 1:3 for **3**; 1:3 for **2**; 1:1 for **4** and 3:2 for **6**). While the properties of α and β anomers are indistinguishable in experimental studies, the individual anomers can be, and were, separately studied in the ligand-transporter modelling assay. In line with the observations made earlier for **6**,[16] and the experimental affinity values determined in the *cis*-inhibition assays, glucoconjugates **1**–**5** displayed significantly stronger binding affinities to both the outward- and inward-open conformation than the natural ligand D-glucose. In the outward-open conformation, the binding free energy difference was found to be ca. 5 kcal/mol in favor of the glucoconjugates compared to glucose. This corresponds to a remarkable binding affinity increase on the order of 10^3 (see supporting information for details). The energy differences between the individual glucoconjugates, on the other hand, were marginal.

We note that the computational values are by necessity and construction based on an approximate description of the binding process. Measured experimental values are naturally a more fitting representation of a true biological system. Both the experimental and computational studies are in line, thereby indicating that the affinity differences between the carboranylmethyl substituted glucoconjugates are minimal. These results are quite surprising and prompt a couple of questions. Is there no substantial difference between the modification site? How could such observations be explained?

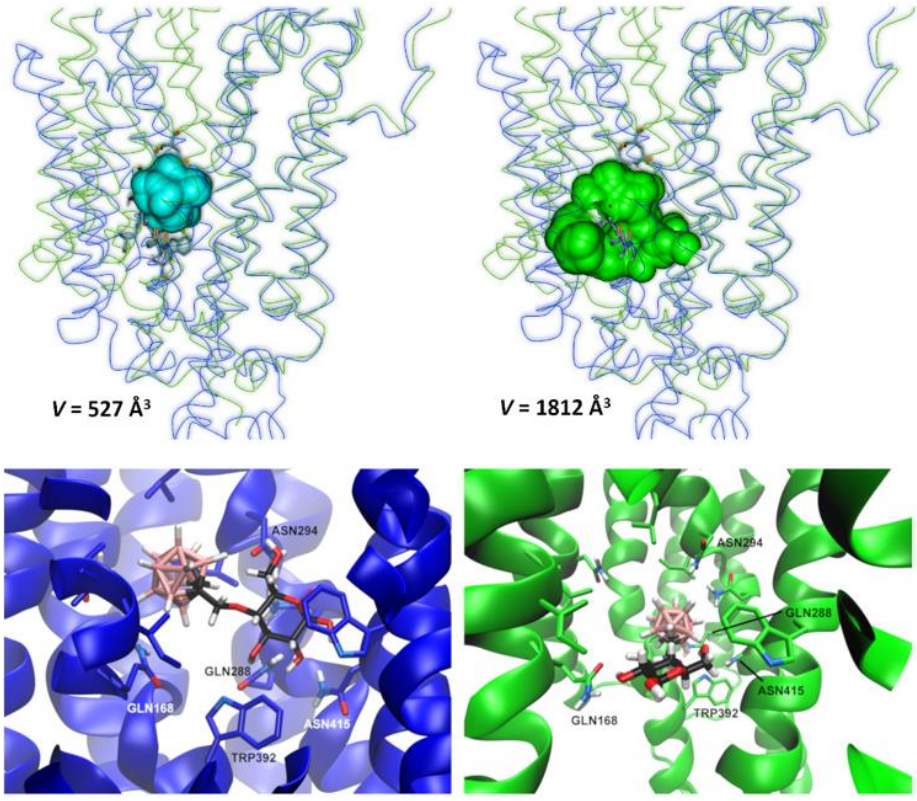


Figure 4. Top: the volumes of the binding pockets as recognized by CAVER Analyst[36] are highlighted; **top left)** the outward-open structure (cyan), **top right)** the inward-open structure (light green). Blue string = outward-open XylE, green string = inward-open XylE, natural ligand binding site amino acids as sticks, color scheme CAVER. **Bottom:** The β-anomer of **5** displayed in the binding site with the amino acids binding the natural ligand marked. The figures highlight the changes in the positions of especially GLN168 and TRP392 during the conformational shift from **bottom left)** the outward-open structure (blue) to **bottom right)** the inward-open structure (green).

We sought to address these questions by analyzing thoroughly the results obtained from the docking studies in both the outward- and inward-open conformations. In the outward-open conformation, two distinct binding poses can be identified for the glucoconjugates. In one, the carbohydrate part overlaps with the most favorable binding pose of free D-glucose, as identified by the docking study, while in the other, the glucoconjugates are slightly rotated. The amino acid residues involved in the recognition process are Gln168, Gln288, Asn294, Trp392 and Asn415.[67] In order to piece the puzzle together it was important to address the binding poses and amino acid residues participating in the glucoconjugate/protein complex also in the inward-open conformation and search for similarities/differences. In the inward-open conformation, the glucoconjugates display significantly lower binding affinities across the board. This may be beneficial from a functional point-of-view, as it implies that the glucoconjugates are less tightly bound to the transporter on the inside of the cell and thus more prone to be released – a seemingly ideal feature of a delivery agent. The reduced binding affinity to the inward-open structure could be due to the "wider nature" of the inward-open protein cavity; the binding site volume in the inward-open structure is three times larger than in the outward-open structure (see Figure 4 and the supporting information, tables 1–3). The wider binding site offers greater flexibility in terms of binding poses than the outward-open one.

The most noteworthy of the binding poses for the different glucoconjugates binding to the inward-open protein structure are the ones in which the hydrophobic boron cluster is located close to the amino acids identified to bind the natural ligand, *i.e.*, Asn415, Gln288, Trp392 (and Asn294) while the glucose-moiety points towards Gln168. The binding poses of the glucoconjugates in the outward-open structure are supportive of this inward-open ligand/transporter complex. The main amino acid residues involved in the glucoconjugate–binding are the same as in the outward-open conformation. In the eventual outward-open to inward-open conformational change process, the Gln168 amino acid is expected to move away as the entire *N*-domain moves away from the *C*-domain.[70] Therefore, the glucoconjugates would be expected to be capable of undergoing the necessary transformation from both binding poses in the outward-open state to the binding poses C–F in the inward-open state (data not shown). It is encouraging that the glucoconjugates interact with the transporter in a similar fashion in the top-candidate computational binding poses identified for both the outward- and inward-open structures. There is thus no need for the ligand to undergo any considerable conformational changes inside the narrow transporter channel, a process that would be expected to significantly hinder the cellular uptake.

Cytotoxicity and boron delivery capacity. With a concise view of the GLUT1–glucoconjugate interactions occurring on a molecular level obtained, we continued by addressing topics of interest from a translational medicine perspective. The most essential properties were deemed to be cytotoxicity, boron delivery capacity and metabolic fate. All of these studies were conducted *in vitro*. In this section, the focus will be on the cytotoxicity and boron delivery capacity with the metabolic fate addressed in the next section.

The cytotoxicity and cellular uptake studies were conducted in the human CAL 27 carcinoma cell line, the same cell line used in the affinity studies because it is a representative head and neck cancer cell line of clinical relevance and the GLUT1-function and expression had been validated.[64,65] In order for a boron delivery agent to be of potential interest, it should not impair the cellular viability of the cancer cells prior to BNCT irradiation.[73] In the cytotoxicity assays, the glucoconjugates **1**–**5** and BSH were incubated with the CAL 27 cells at concentrations of 5 μM, 25 μM, 50 μM, 125 μM and 250 μM for 6 h and 24 h. BSH served as a control of a delivery agent in clinical use and BPA was omitted because its IC_{50}-value has been reported to be in the low mM range.[10] Apart from this, the concentration range was selected based on the GLUT1-affinity results and the incubation times are representative of time frames used in clinical BNCT following intravenous administration of delivery agents. The results obtained from the cytotoxicity assays are summarized in Figure 5. The cell viability was quantified by a commercial CellTiter-Glo® assay and the IC_{50}-values were obtained from nonlinear regression fitting of the cell viability data. The IC_{50}-values were determined to be: 181 μM for **1**, 101 μM for **2**, 42 μM for **3**, 203 μM for **4**, 201 μM for **5** and 220 μM for BSH. The IC_{50}-value previously reported for **6** in the CAL 27 cell line was 215 μM.[16] It is important to note that the modest cytotoxicities displayed by the glucoconjugates, especially **4, 5** and **6**, would not hamper their use as delivery agents in BNCT. Based on cytotoxicity and affinity results; **4, 5** and **6** seemed to have the highest potential at this stage.

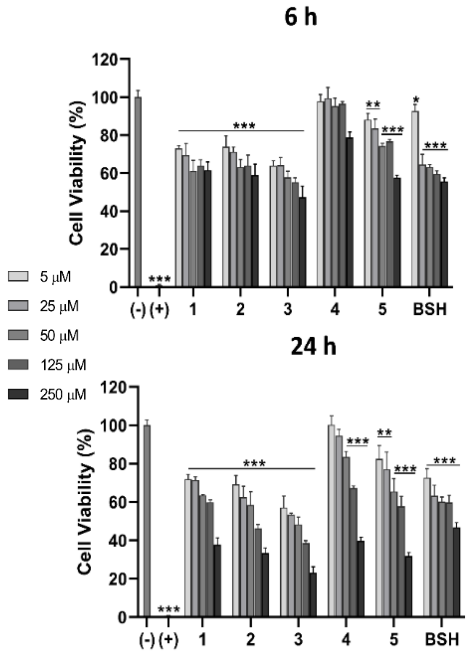


Figure 5. Cell cytotoxicity studies in the human CAL 27 cell line after incubation with cell culture medium (negative control), 1% Triton X-100 (positive control), glucoconjugates (**1**–**5**), and BSH at the concentrations of 5 μM, 25 μM, 50 μM, 125 μM, and 250 μM for 6 h and 24 h. Results are represented as the mean ± s.d. (n=4) in comparison with the negative control. The statistical significance was analyzed using an unpaired Student's *t*-test where the significance was set at *p<0.05, **p<0.01 and ***p<0.001.

The GLUT1-targeting approach differs from the LAT1-targeting approach of BPA and the passive transport mechanisms of BSH and GB10 (the delivery agents used in the clinics).[10–12,74] In cellular uptake studies, we therefore included both BSH and BPA in order to be able to compare the boron delivery capacity to the approaches and agents in clinical use. It should

be noted that while the term "cellular uptake" is used, the protocol employed does not differentiate between intracellular components and components trapped on the cell membrane. Regardless of if the compounds are trapped on the cell membrane or internalized – the boron nuclei are within the range required to exert a cell-killing effect since the α particles travel a distance of 5–9 μm in tissue.[73] Therefore, from a BNCT perspective, the results obtained are still equally valid.

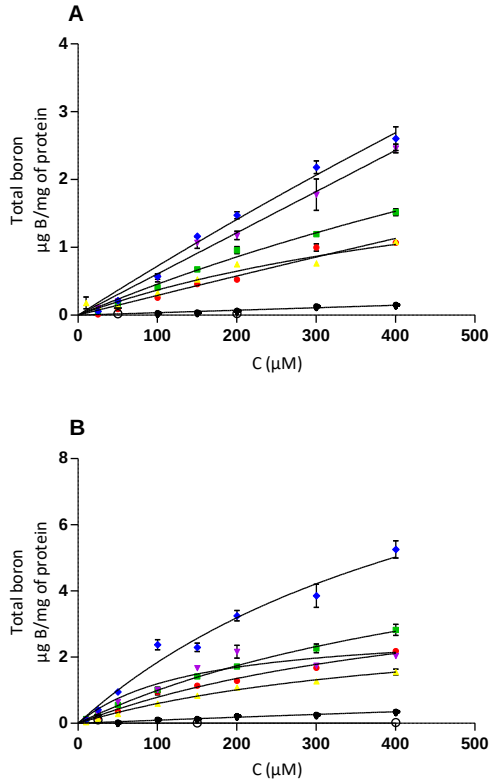


Figure 6. Cellular uptake results in the CAL27 cell line after incubation with glucoconjugates **1** (yellow), **2** (red), **3** (blue), **4** (green), **5** (purple), BPA (•) and BSH (○) in the 10–400 μM range for 5 min. (A; n=3) and 30 min. (B; n=3). The Michaelis–Menten kinetic parameters for glucoconjugates when available; at 5 min incubation time (A), glucoconjugate **1**: V_{max}= 2.654; K_m= 616.8: **3**: V_{max}= 28.65; K_m= 3859 and **4**: V_{max}= 2.208; K_m= 544.3. At 30 min incubation time (B), glucoconjugate **1**: V_{max}= 3.290; K_m= 450.8; **2**: V_{max}= 4.816; K_m= 511.2; **3**: V_{max}= 11.85; K_m= 543.0; **4**: V_{max}= 0.887; K_m= 111.4; **5**: V_{max}= 2.924; K_m= 145.0 and BPA: V_{max}= 3.625; K_m= 3737.

In the cellular uptake studies, we used our previously developed protocol.[16] Shortly, the boron content in the CAL 27 cell lysates was determined after incubation with the delivery agents and careful washing to remove unattached species from the matrix. A concentration range of 10–400 μM was selected based on the GLUT1-affinity studies and incubation times of 5 and 30 min. were selected based on the optimal performance of [^{14}C]-D-glucose under these conditions (see Supporting Information). Before ICP-MS analysis of the boron contents, the lysates from four wells were combined and digested. The results are summarized in Figure 6. Based on the results it is clear that the GLUT1-targeting approach bears a considerable potential since the boron delivery capacity of the glucoconjugates is significantly higher than those of the agents in current clinical use. While the alternate delivery strategy is the main cause for the observations, it should be noted that the glucoconjugates do contain ten times the amount of boron nuclei/delivery agent compared to BPA. Surprisingly, the boron delivery capacity of glucoconjugate **3** is considerably better than for the rest of the glucoconjugates. While this does not correlate with the affinity results obtained, a direct correlation is not expected as the affinity and uptake studies provide insights on two separate properties: the ability to compete for the transporter with the natural substrate and the ability to remain attached or internalized in the cells. The results obtained with glucoconjugates **1**–**5** in the present study are in line with those reported earlier by Lippard et al. with other types of glucoconjugates.[24] While modification at the second position was found to result in the best functioning molecule in the current work, and in the work by Lippard et. al., glucoconjugate **6,** which was the focus of our previous study,[16] displays improved overall qualities: higher GLUT1-affinity, lower cytotoxicity and higher boron delivery capacity. Therefore, while on a substrate-specific level glucoconjugate **6** remains the hit compound – the results obtained with the entire positional isomer library solidify the foundations of the GLUT1-targeting strategy and showcase the significant potential embedded in this approach.

Outruling potential metabolic pathways. The metabolic fate of glucoconjugates has remained an open question. Especially concerns regarding the possible incorporation of glucoconjugates into other biomolecules through metabolic routes has been raised. Reminiscent to the previous speculations regarding the competition with D-glucose for the transporters – scientific studies addressing this topic are lacking. The main metabolic pathways for D-glucose metabolism are the glycolysis route and the pentose phosphate pathway (PPP). In both pathways, the first step is the phosphorylation of the 6th position by the enzyme hexokinase.[75] While this position is blocked in glucoconjugate **6**, the possibility of entering these metabolic routes is in theory possible for glucoconjugates **1–5**. In order to determine if glucoconjugates **1–6** are substrates for hexokinase, we used an *in situ* NMR-spectroscopic approach to analyzing this important step. In this protocol, the individual glucoconjugates **1–6** and D-glucose (a positive control) were dissolved in a TRIS buffered aqueous solution (2 mM concentration of the glucoconjugates) containing an excess of ATP and Mg^{2+} ions. Recombinant human hexokinase 1 was added to the mixture and the NMR experiments were performed at 30 °C by recording ^{1}H and ^{31}P NMR spectra continuously. The ^{1}H and ^{31}P NMR spectra are well suited to follow the transformation as the natural abundance of these NMR-active nuclei is high, thereby leading to a sufficient sensitivity.[76] In a typical experiment, the chemical shift changes accompanied by the transformation of ATP to ADP and the eventual effect on the chemical shifts of the carbohydrate signals were carefully monitored. In our methodology validating experiment with D-glucose, we were able to observe the expected phosphorylation resulting in glucose-6-phosphate. The observed chemical shift changes in the ^{31}P NMR spectra were a decrease in the ATP signals appearing at –7.2, –11.3 and –22.3 ppm with a corresponding increase in the ADP signals appearing at –6.7 and –11.8 ppm. Changes in the ^{1}H chemical shifts of D-glucose were likewise noted (e.g. H-2 shifted from 3.19 ppm to 3.22 ppm) as a result of the phosphorylation.

With a functioning and validated protocol developed, we addressed the possibility of glucoconjugates **1–6** entering the glycolysis route or the PPP. None of the glucoconjugates were phosphorylated at the 6th position, or any other position for that matter, thus indicating that they are not substrates for human hexokinase and are thereby unlikely to enter these metabolic routes. Therefore, the previous concerns regarding the potential disruption of regular glucose metabolism by the glucoconjugates have been unfounded. From a BNCT perspective, the findings seem rather ideal. The glucoconjugates deliver significant boron contents to the cells but do not disturb the general glucose metabolism. Thus, they do not fulfil the energy requirements of the dividing cells and additional D-glucose uptake is probable. This may lead to further accumulation of glucoconjugates and explain in part the significant boron delivery capacity noted in the cellular uptake studies.

4. DISCUSSION

Understanding the biochemical foundations of any drug development or medicinal chemistry campaign provides a decisive advantage and the means for improving the selected approach, whichever it may be. In this study, we have concentrated on studying the biochemical foundations of a successful GLUT1-targeting approach to BNCT. Our approach represents an alternative boron delivery strategy to those presently in clinical use. The GLUT1-targeting approach has been long neglected due to a number of doubts concerning its suitability for BNCT. Here, we have addressed all of these, and shown that there is no need for concern. On the contrary, the glucoconjugates exhibit superior properties as measured by key indicators.

In more detail, we have synthesized the entire positional isomer library of *ortho*-carboranylmethyl bearing glucoconjugates and assessed their properties through a comprehensive *in vitro* evaluation featuring computational/experimental GLUT1-affinity, cytotoxicity, cellular uptake and metabolic fate studies. The glucoconjugates are (1) capable of competing for GLUT1 with the natural substrate, (2) display acceptable cytotoxicity, (3) have a significantly boosted boron delivery capacity compared to the delivery agents in clinical use, and (4) do not enter the common metabolic routes of D-glucose.

We have conducted all of the *in vitro* studies in the CAL 27 cancer cell line, a tumor type amenable to the treatment with BNCT and a solid model for head and neck cancers. Head and neck cancers have been targeted as they are suitable for BNCT and 20–40% of the cancers in this category are either inoperable or recurrent and require non-conventional cancer treatments. While still too early to draw definite conclusions regarding the potential of the glucoconjugates *in vivo*, our findings firmly support and lay the foundations for progressing to the *in vivo* stage. To this end, we are currently working on the synthesis of ^{10}B-enriched species, and *in vivo* biodistribution and tumor accumulation studies. The revitalization of BNCT is in full force.

SUPPORTING INFORMATION

Supporting information is available. Additional reaction routes, NMR spectra of all compounds prepared and additional details on molecular modelling and GLUT1-studies is supplied.

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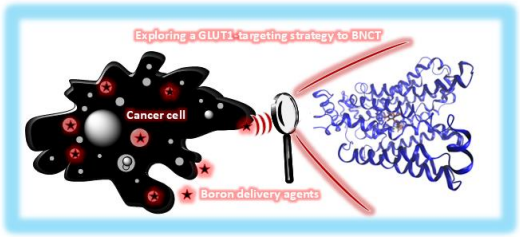
Exploring a GLUT1-targeting strategy to BNCT
Cancer cell
Boron delivery agents