Synthesis of New Boron-Rich Building Blocks for Boron Neutron Capture Therapy or Energy-Filtering Transmission Electron Microscopy

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The synthesis of a new ortho*-carborane derivative, tetracarboranylketone **4**, is reported here. Ketone **4** was prepared from a tetraalkynylated ketone by the addition of decaborane. The keto group was then easily modified to yield the glycosides **17**α and **18**β, which contain glucose or galactose, respectively, and the nucleotide **13b**. In addition to ketone **4**, which is acyclic, cyclic ketone **8** was also synthesised. X-ray diffraction analysis of compound **4** indicated the presence of two toluene guest molecules per molecule of the host compound. Furthermore, compound **4** displays a rather low cytotoxicity. These novel products can be used as building blocks to create a new class of biomolecules containing high-density carborane clusters. Such molecules may constitute powerful tools for applications like Boron Neutron Capture Therapy or Energy-Filtering Transmission Electron Microscopy.*

Introduction

BNCT and the requirement for high density B compounds

The design and synthesis of boron-rich compounds suitable for boron neutron capture therapy (BNCT) and also for electron-microscopy techniques like energy-filtering transmission electron microscopy (EFTEM) and electron spectroscopic imaging (ESI) have received increasing attention in recent years.[4,26] BNCT is a mode of radiotherapy that still holds great potential, even though the first clinical trials were conducted as early as the 1950s and early 1960s.[23] Basically, malignant tissues are injected with suitable boron compounds and irradiated with thermal neutrons; the ^{10}B atoms then transform into energetic ^{4}He and ^{7}Li atoms, which destroy the surrounding tissues, mainly by damaging the nucleus of each cell. Achieving an adequate amount of boron in malignant cells constitutes one of the major problems with this kind of therapy. The first compounds used for BNCT contained a single boron atom, but it was soon clear that a compound containing multiple boron atoms would have an advantage over compounds with a single atom. From compounds possessing comparable molar toxicity, higher boron concentrations could be administered with those having multiple boron atoms.[23] Hence, higher concentrations of boron within the tumour and a corresponding relatively lower cytotoxicity could be achieved with compounds containing multiple boron atoms.

Newly synthesised compounds for BNCT

Thus, the first carboranes were synthesised and they now represent a very promising tool for BNCT.[25,26,28] In the approach described here, we have performed the next step in the synthesis of molecules with high concentrations of boron by clustering several carboranes into a single compound. Thus, our product has the capacity to transport 40 boron atoms per molecule into cancer cells; this represents a fourfold increase relative to another recently synthesised carborane derivative.[27] Tetracarboranylketone **4** (see Scheme 1) is, amongst the carborane derivatives with 40 or more boron atoms (for example, see ref. [13]), the one with the highest content of boron with respect to the atomic mass (63%). Moreover, in contrast with other constructs, (for example, see ref. [11]), **4** can be bound to a biomolecule in a unique, specific position. Thus, glycosidic derivatives that can be used for BNCT can be easily obtained.

EFTEM as a visualisation technique in BNCT

A major issue in BNCT is the spatial mapping of boron in tissues, in order to evaluate the degree of segregation of boron atoms inside the cells. EFTEM is an important tool that can provide us with information in this regard. EFTEM is an electron-microscopy technique whereby inelastically scattered electrons are selected through an energy filter, in order to

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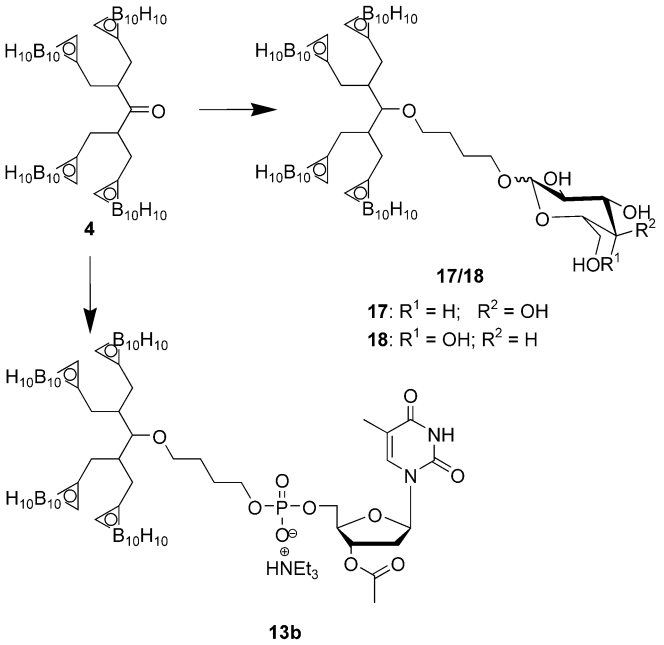


Scheme 1. *The versatile building block **4**, with four carboranyl moieties, can be used to label a biomolecule (for example, as in nucleotide **13b**), a technique that has potential applications for EFTEM. Alternatively, **4** can be conjugated to saccharides (for example, as in **17** and **18**) to form a product that is deliverable into malignant cells for BNCT applications.*

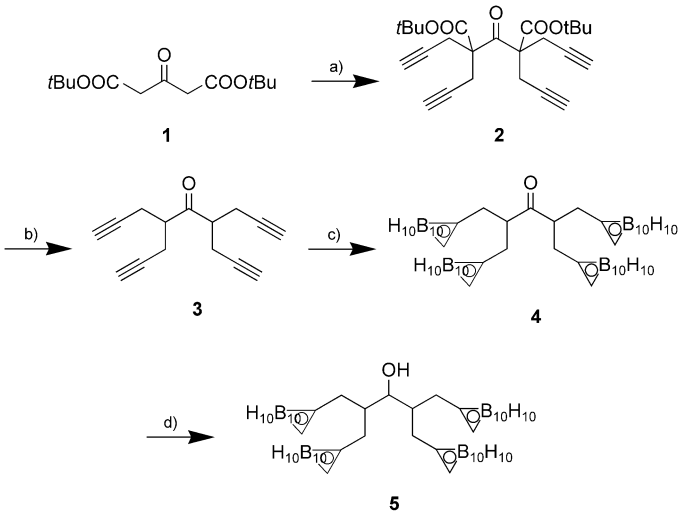
form a map of a particular element or elemental ratio. Previous studies[2,6,18] of boron imaging in cells or tissues have been carried out by using EFTEM. A spherical shape of the molecule carrying boron and the presence of a relatively high number of boron atoms in a small volume improves the detectability in EFTEM.[1] The compact structure of, for example, **4** and its nucleoside derivative **13b** (see Scheme 1), is therefore appropriate for EFTEM analysis. However, a boron load high enough to exceed the boron EFTEM detection limit has to be achieved. This limit is about 2000 ppm when boron is incorporated into a carbon matrix, as pointed out in ref. [30] and approximately 100 ppm when boron is incorporated into a biological material.[17] It was recently shown that even a single molecule of *ortho*-carborane, $(CH)_2(BH)_{10}$, can be visualised with high-resolution transmission electron microscopy.[16] Nonetheless, not much is known about the detection limit for biological probes, even though 80 incorporated boron atoms per macromolecule are expected to suffice for an EFTEM analysis.[19] The compact, ball-like tetracarboranylketone **4** (with a diameter of about 1.4 nm) was designed to achieve a highly localised concentration of boron in the centre of the molecule. This enables, in principle, the visualisation of a specific and spot-like signal by EFTEM, a development that represents a clear advantage of **4** in comparison to molecules used in former studies,[13,19] in which the coupling of boron atoms to the surface of macromolecules resulted in a distribution of those atoms over large areas, thereby reducing their potential to be detected as single labelled units.

Thus, the important novelty associated with tetracarboranylketone **4** lies in its versatility and potentially important applicability. It can be modified by simple synthetic steps in order to give, for example, a nucleoside derivative that could be useful for electron-microscopy studies or a glycoside derivative for BNCT-related purposes (Scheme 1). In addition, our approach may constitute an interesting strategy for the synthesis of molecules with multiple, covalently attached gold or other labels.

Results and Discussion

Syntheses

Herein we report the synthesis and analysis of the new *ortho*-carboranes **4** (acyclic), **8** (cyclic), **13b**, **17**α, **18**β and **22** (80 boron atoms). We have also estimated the cytotoxicity of compounds **4** and **5** on different cell lines. The first step of the synthesis consisted of binding four alkynylated side chains to a central ketone group (Scheme 2). The alkynylation of **1** was



Scheme 2. *Synthesis of the tetracarboranylalcohol **5**: a) Propargylbromide, NaH, DMF, RT, 61%; b) TFA, CH_2Cl_2, then distillation under high vacuum, 71%; c) $B_{10}H_{14}$ (5 equiv), CH_3CN/toluene, 80°C, 72 h, RT, 16%; d) $LiAlH_4$, RT, 84% yield. DMF = N,N-dimethylformamide, TFA = trifluoroacetic acid.*

performed in two steps with a 43% overall yield by using propargylbromide as an alkynylating agent.

The overall yield of the carboranylation of **3** was 16%. When it is taken into account that all four alkynyl moieties had to react with the decaborane unit, the yield relative to the formation of a single carborane cluster was 63%. **5** was easily accessible through reduction of **4** by lithium aluminium hydride, with a yield of 84%.

An alternative route for the synthesis of a tetracarboranylketone was to use 1,4-cyclohexanedione monoethyleneacetal **6** instead of 3′-oxoglutarate **1** (Scheme 3). The tetraalkyne derivative **7** was accessible in one step, with a yield of 44%. **7** was converted into the tetracarboranyl derivative **8** in one step with a 16% yield.

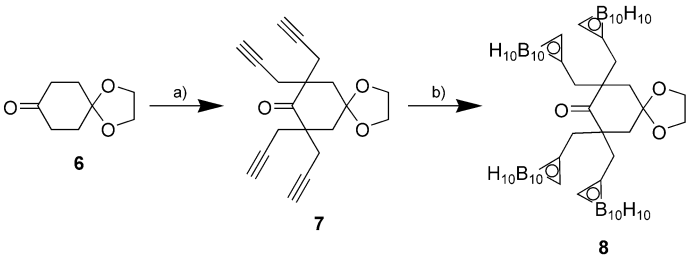
Unfortunately, the direct coupling of **5** to a biomolecule failed. This was probably due to low reactivity of the hydroxy

group in **5**, given the steric hindrance of the four carborane moieties. Therefore, we attached a small spacer molecule to **5**. This spacer had to be reactive enough to bind with the hydroxy group of **5**, but at the same time, it had to contain sufficient functionality to permit further reaction with a biomolecule. A good candidate was molecule **9**, which has recently been described in the literature,[10] even though its use as a spacer is, to our knowledge, new (Scheme 4).

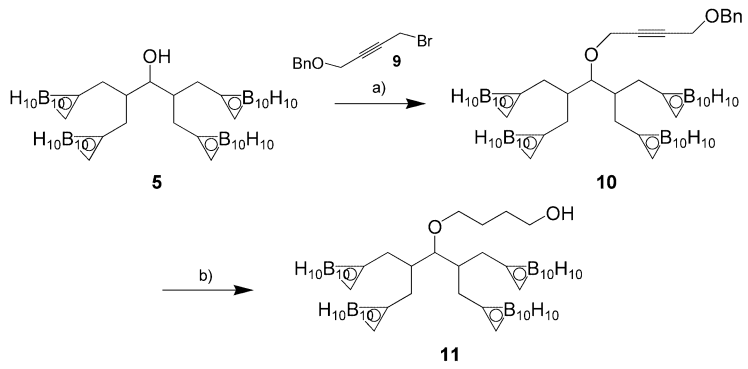
Spacer **9** has three useful fundamental features for our purpose. Firstly, the reactivity of the bromomethyl group is dramatically increased by the vicinity of the triple bond. This should ensure good yields in the etherification reaction with **5**. Secondly, the triple bond allows a rigid alignment of the molecule, thereby keeping the cumbersome benzyl groups out of the centre of reaction. Thirdly, the hydroxy function is protected with a benzyl group, which can be readily removed after etherification to leave a reactive hydroxy group. This could be used for successive binding of **9** to other boron-rich fragments or to a biomolecule. Thus, **10** was prepared in a single step with a 65% yield (Scheme 4).

Following hydrogenolytic cleavage of the benzyl group, the triple bond was hydrogenated and compound **11** was obtained. **11** was then converted into the phosphoramidite **12**, which reacted with the 5′ hydroxy group of a nucleoside, under the conditions applied for automated DNA synthesis[13] (Scheme 5).

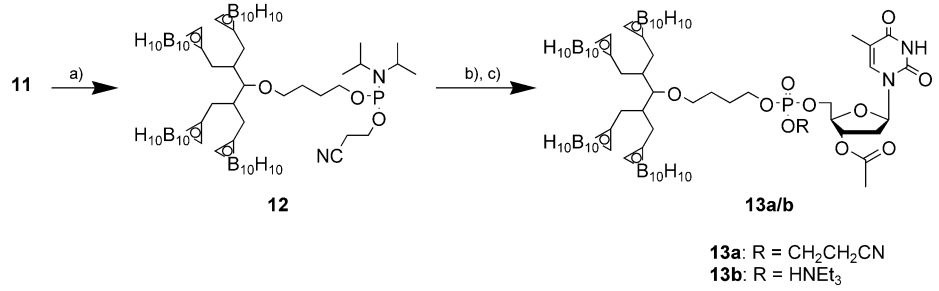
The strategy employed to couple a boron-rich alcohol like **5** to a saccharide was slightly different. In this case, the spacer used was 4-bromobut-2-ynol.[15] In the first step, imidate-activated glucose or galactose reacted with the spacer (Scheme 6). After separation of the resulting anomeric mixtures, the anomeric constitution of the carbohydrates **15**α or **16**β was determined. In the second step, **15**α and **16**β were conjugated to **5** to give, after deprotection, the glucosylated and galactosylated carboranes **17**α and **18**β, respectively (Scheme 6). In a very similar synthesis, the introduction of the bromobutynyl spacer into carborane **5** was successful (Scheme 7). As a consequence, **22** was accessible by treatment of **20** with a malonate. It has already been reported[22] that double alkylation of even base-sensitive structures such as carboranes is attainable with this ester by phase-transfer catalysis.



Scheme 3. *Synthesis of the cyclic tetracarboranylderivative **8**: a) Propargylbromide, toluene, aq. NaOH, phase-transfer catalysis with $BnEt_3NCl$, RT, 44%; b) $B_{10}H_{14}$, toluene, 80°C, 72 h, 16%. Bn = benzyl.*



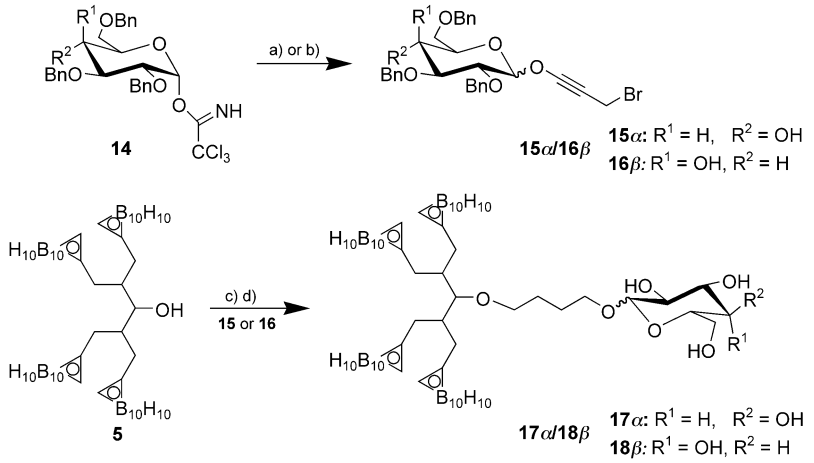
Scheme 4. *Synthesis of tetracarbonylalcohol **11**: a) Etherification of **5** to form **10** through phase-transfer catalysis, RT, 65%; b) Pd/C, EtOAc/H_2O, RT, 84%.*



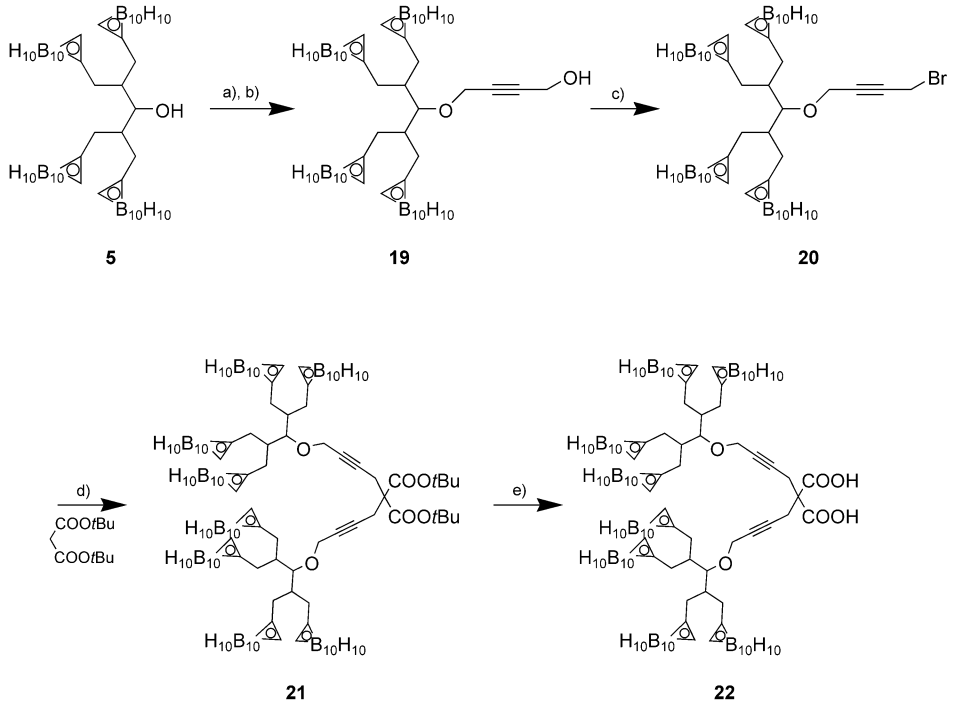
Scheme 5. *Coupling of boron-rich fragment **11** to a nucleoside: a) $Cl[(IC_3H_7)_2N]_2PO(CH_2)_2CN$ (2 equiv), Huenig base (3 equiv), CH_3CN, RT, 53%; b) coupling with 3-O′-acetylthymidine, with subsequent oxidation by cumol hydroperoxide in tetrazol/CH_3CN, RT, 20 min, 33%; c) deprotection with triethylamine, 20 min, RT, 74%.*

Structure determination

The structures of the boron-containing compounds were verified by ^1H, ^{11}B and ^{13}C NMR spectroscopy. In the ^1H NMR spectrum, a broad signal was visible at $\delta = 0.35$–3.35 ppm for the protons attached to boron, as expected for carborane compounds. In addition, the molecular composition of compound **4** was analysed with fast-atom-bombardment and high-resolution mass spectrometry. A solution of **4** in THF/toluene produced, after slow evaporation, transparent prisms which were analysed by X-ray diffraction. The crystal structure that was deduced from these analyses is represented in Figure 1. Moreover, diffraction data indicated two molecules of toluene per host molecule of tetracarboranylketone **4**.



Scheme 6. *Synthesis of the glycosylated carboranes* ***17***α *and* ***18***β*: a) Synthesis of* ***15***α*: **14** (R^1 = H, R^2 = OH), 4-bromobut-2-ynol, TMSOTf in CH_2Cl_2, −20 °C, 30 min, 41%; b) synthesis of* ***16***β*: 1.* ***14*** *(R^1 = OH, R^2 = H), 2-butyn-1,4-diol, TMSOTf, CH_3CN; 2. CBr_4, Ph_3P, CH_2Cl_2, −15 °C, 14% over two steps; c)* ***15***α *or* ***16***β*, aq. NaOH/toluene, $BnNEt_3Cl$; d) 20% Pd/C, H_2, acetone/water (10:1), RT,* ***17***α*: 7% over two steps,* ***18***β*: 30% over two steps. TMSOTf = trimethylsilyltrifluoromethane sulfonate.*



Scheme 7. *Synthesis of* ***22****, a carborane derivative with 80 boron atoms: a)* ***5****, 4-(2′-tetrahydropyranyloxy)-1-bromobut-2-yne[14] under phase-transfer conditions, toluene/aq. NaOH, RT; b) H^+ ionic exchange, MeOH, RT, 64% over two steps; c) PPh_3, CBr_4, CH_2Cl_2, RT, 83%; d) phase-transfer catalysis in toluene/aq. NaOH, $BnEt_3NCl$ as the phase-transfer catalyst, RT, 37%; e) TFA, RT, 1 h, 75%.*

A further X-ray determination was performed to characterise ketone **3** as a precursor of carborane **4**. The deduced molecular structure is shown in Figure 2. As with molecule **4**, the keto group of **3** was positioned upon a crystallographic twofold rotation axis.

Conclusion

In the present study, we have reported the synthesis of a new group of carboranes with a high percentage content of boron (up to 63%). All of the synthesised carborane derivatives were stable with respect to air and humidity; they could be stored at room temperature for several months without any detectable degradation. These molecules can be used as building blocks to create a new class of biomolecules that may be powerful tools in applications like BNCT or EFTEM. The ketone **4** has limited water solubility, but preliminary tests for alcohol **5** indicate a more pronounced solubility, due presumably to the hydroxy function. Compound **4** displayed a rather low cytotoxicity (see Experimental Section for further details). Further efforts should be directed at increasing the solubility of these compounds, since low water solubility is one of the major handicaps of carboranes.[12,29] Nevertheless, **4** represents a valid reactive handle for selective conjugation of high-density carborane clusters to molecules that recognise biological targets.

Experimental Section

General: ^1H, ^{13}C and ^{31}P NMR spectroscopy was carried out with a Bruker AM250 spectrometer in the Department of Spectroscopy of the German Cancer Research Center. ^{11}B NMR spectroscopy was carried out with a Bruker DRX200 spectrometer in the Inorganic Chemistry Institute of the University of Heidelberg, Germany. Electron-spray-ionisation mass spectra were collected with a Finnigan MAT TSQ 7000 in the Department of Spectroscopy of the German Cancer Research Center. Electron-impact (EI), chemical-ionisation (CI) and fast-atom-bombardment (FAB) mass spectra were recorded in the Institute of Organic Chemistry of the University of Heidelberg, Germany. Elemental analyses were carried out in the Microanalytic Laboratory of the Max-Planck Institute for Medical Research in Heidelberg, Germany. Reagents and materials were used without further purification and all the solvents were dried over

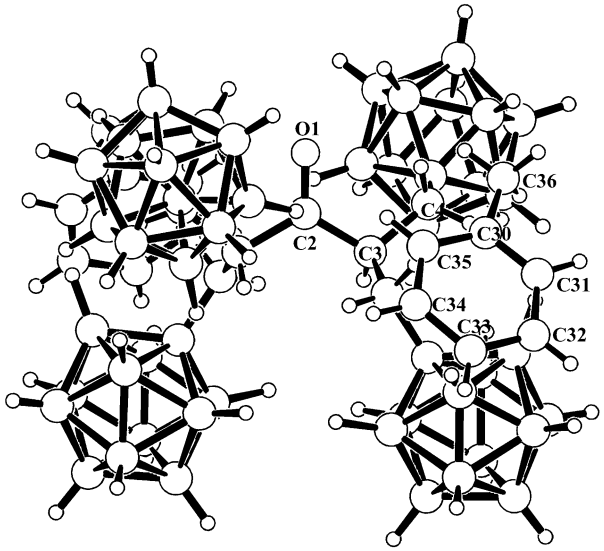


Figure 1. *Schematic SCHAKAL representation[14] illustrating compound* ***4****. Selective atoms are numbered. Likewise, a guest molecule of toluene (C31–C36) is shown.*

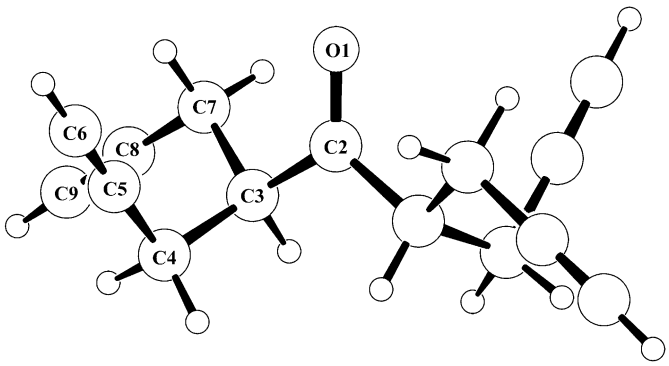


Figure 2. *Schematic SCHAKAL representation[14] illustrating compound* ***3****.*

molecular sieves before use. Most reactions were monitored by thin-layer chromatography (Polygram Sil G/UV$_{254}$, Macherey and Nagel; aluminium sheet 60 F254 neutral, type E, Merck). Products were isolated by column chromatography on silica gel (Macherey and Nagel) or aluminium oxide gel (Alox, activity III, Merck).

Di-*tert*-butyl-protected 3-oxo-2,2,4,4-tetra(prop-2′-ynyl)glutarate (2): 3-Oxoglutarate (252 mg, 0.976 mmol) **1** and propargylbromide (0.70 mL, 6.26 mmol, 80% solution in toluene) were dissolved in DMF (2 mL). NaH (182 mg, 4.17 mmol, 60% suspension in oil) was slowly added at 0 °C. The solution was stirred overnight at RT, then H_2O (4 mL) was added and the resulting mixture was extracted three times with diethyl ether. The combined organic layers were washed with H_2O, dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography on silica gel with petroleum ether/diethyl ether (19:1) to afford **2** (243 mg, 0.59 mmol, 61%): $R_f = 0.33$ (petroleum ether/diethyl ether, 9:1); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 2.07$ (t, $J = 2.7$ Hz, 4H, 4CH), 2.95–3.13 (dd, $J = 17.4$ Hz, 8H, 4CH_2), 3.75 (s, 12H, 6CH_3) ppm; ^{13}C NMR (62.90 MHz; $CDCl_3$): $\delta = 24.0$ (4C-1′), 27.7 (6CH_3), 62.5 (C-2, C-4), 72.2 (4C-2′), 79.0 (4C-3′), 84.1 (2CH), 167.5 (C-1, C-6), 196.7 (C-3) ppm; elemental analysis: calcd (%) for $C_{25}H_{30}O_5$ (410.5): C 73.15, H 7.37; found: C 73.35, H 7.35.

1,1,3,3-Tetra(prop-2′-ynyl)propan-2-one (3): **2** (213 mg, 0.519 mmol) was dissolved in a mixture of CH_2Cl_2/trifluoroacetic acid (1:1) and stirred overnight. The solvent then was removed under vacuum and the residue was heated to 150 °C at 0.4 mbar. With the start of gas production, a yellow solid was obtained. This solid was dissolved in CH_2Cl_2 and the solution was concentrated and purified by column chromatography (petroleum ether/diethyl ether, 9:1) to afford **3** (77.3 mg, 368 μmol, 71%): $R_f = 0.48$ (petroleum ether/diethyl ether, 4:1); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 2.04$ (t, $J = 2.7$ Hz, 4H, 4CH), 2.48–2.68 (ddd, $J = 6.9$, 17.3 Hz, 8H, 4CH_2), 3.13 (q, 2H, 2CH) ppm; ^{13}C NMR (62.90 MHz; $CDCl_3$): $\delta = 19.6$ (4C-1′), 48.8 (C-1, C-3), 70.9 (4C-2′), 80.6 (4C-3′), 209.4 (C-2) ppm; HRMS (FAB): calcd for $C_{15}H_{15}O$ (210.3): 211.112 $[M+H]^+$; found: 211.111.

1,1,3,3-Tetra-(1′,2′-dicarba-*closo*-dodecaboranylmethyl)propan-2-one (4): 3 (50.0 mg, 237 μmol), decaborane (149 mg, 1.24 mmol) and CH_3CN (50 μL) were mixed in toluene (1.0 mL) and heated for 72 h at 80 °C under an argon atmosphere. A mixture of HCl (37%) and methanol (5 mL, 1:1) was added dropwise. After heating under reflux conditions for 15 h, the mixture was allowed to cool down to RT. The organic phase was separated and purified by column chromatography on Alox (petroleum ether/diethyl ether, 4:1) to afford **4** (26.1 mg, 38 μmol, 16%) as a white powder: $R_f = 0.31$ (petroleum ether/ethyl acetate, 4:1); ^{1}H NMR (250.13 MHz; [D_8]THF): $\delta = 0.35$–3.35 (br m, 50H, 40BH, H-1, H-3, 4CH_2), 4.10 (s, 4H, 4H-2′) ppm; ^{13}C NMR (62.90 MHz; [D_8]THF): $\delta = 37.9$ (4CH_2), 48.6 (C-1, C-3), 64.7 (4C-2′), 73.9 (4C-1′), 204.3 (C-2) ppm; ^{11}B NMR (64.21 MHz; [D_8]THF): $\delta = -2.61$, −4.89, −9.46, −12.48 ppm; HRMS (FAB): calcd for $C_{15}H_{53}{}^{10}B_8{}^{11}B_{32}O$: 681.811 $[M-H]^-$; found: 681.810.

1,1,3,3-Tetra-(1′,2′-dicarba-*closo*-dodecaboranylmethyl)propan-2-ol (5): $LiAlH_4$ (18.1 mg, 0.476 mmol) was added to a solution of carborane **4** (361 mg, 0.529 mmol) in THF (2 mL) under an argon atmosphere. The mixture was left for 30 min at RT and 30 min under reflux conditions. After cooling, the reaction mixture was hydrolysed by dropwise addition of water (1 mL). Diethyl ether (5 mL) was added, followed by dropwise addition of H_2SO_4 (10%) with stirring at 0 °C, until the aqueous phase remained clear. The organic phase was separated and the aqueous phase was washed three times with diethyl ether. The combined organic layers were washed with water and a saturated solution of NaCl and then dried over Na_2SO_4. The solution was concentrated and the residue was purified by column chromatography on silica gel (petroleum ether/diethyl ether, 4:1) to afford **5** (303 mg, 442 μmol, 84%) as colourless crystals: $R_f = 0.088$ (petroleum ether/diethyl ether, 4:1); ^{1}H NMR (250.13 MHz; [D_8]THF): $\delta = 1.00$–3.50 (br m, 50H, 40BH, H-1, H-3, 8CH_2), 3.34 (m, 1H, H-2), 4.41, 4.47 (2s, 4H, 2H-2′, 2H-2″), 5.22 (d, 1H, OH) ppm; ^{13}C NMR (62.90 MHz, [D_8]THF): $\delta = 39.9$, 40.1 (CH_2, CH_2a), 42.1 (C-1, C-3), 63.6, 65.3 (2C-2′, 2C-2′a), 73.6 (C-2), 74.8, 75.7 (2C-1′, 2C-1′a) ppm; elemental analysis: calcd (%) for $C_{15}H_{56}B_{40}O$ (685.0): C 26.30, H 8.24; found: C 26.48, H 8.33.

2,2,6,6-Tetra-(prop-2′-ynyl)-1,4-cyclohexanedione monoethyleneacetal (7): NaOH (2.12 mg, 53.0 mmol) and benzyl triethylammonium chloride (819 mg, 3.61 mmol) in water (4 mL) were added to the solution of 1,4-cyclohexanedione monoethyleneacetal (**6**; 632 mg, 4.05 mmol) and propargylbromide (3.0 mL, 27 mmol, 80% solution in toluene). The reaction mixture, which was scarlet red, was vigorously stirred at 0 °C and turned brown after 15 min. After 44 h, water (15 mL) was added and the reaction mixture was allowed to cool to RT. It was then extracted three times with diethyl ether. The combined organic solution was washed with water,

dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography on silica gel (petroleum ether/diethyl ether, 9:1) to afford **7** (249 mg, 807 μmol, 44%) as a solid: $R_f = 0.32$ (petroleum ether/diethyl ether, 4:1); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 2.04$ (t, 4H, $J = 2.6$ Hz, 4H-3′), 2.37 (s, 4H, 2H-3, 2H-5), 2.52 (dd, 4H, $J = 16.8$ Hz, 4H-1′a), 2.73 (dd, 4H, 4H-1′b), 4.04 (s, 4H, CH_2CH_2) ppm; ^{13}C NMR (62.90 MHz; $CDCl_3$): $\delta =$ 26.9 (4C-1′), 39.1 (C-3, C-5), 49.5 (C-2), 64.3 (CH_2CH_2), 71.8 (4C-2′), 79.8 (4C-3′), 106.6 (C-4), 210.0 (C-1) ppm.

2,2,6,6-Tetra-(1′,2′-dicarba-*closo*-dodecaboranylmethyl)-1,4-cyclohexanedione monoethyleneacetal (8): The solution of cyclohexandione **7** (101 mg, 0.327 mmol), decaborane (235 mg, 1.73 mmol) and CH_3CN (165 mg) in toluene (1 mL) was stirred at 80 °C for 16 h under an argon atmosphere. The reaction mixture was heated for 4 h under reflux conditions and was then allowed to cool to 80 °C. To quench the excess of decaborane, a mixture of HCl (37%) and methanol (6 mL, 1:1) was added dropwise. The reaction mixture was heated for 16 h under an argon atmosphere, toluene (2 mL) was added and the mixture was allowed to cool to RT. The organic phase was separated and immediately purified by column chromatography on Alox (petroleum ether/diethyl ether, 4:1) to give **8** (41.0 mg, 50 μmol, 16%) as a solid: $R_f = 0.31$ (petroleum ether/diethyl ether, 4:1); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 0.90$–3.50 (br m, 40H, 40BH), 2.50 (s, 4H, 2H-3, 2H-5), 2.94, 3.05 (2d, 8H, $J =$ 15.9 Hz, 4CHa, 4CHb), 4.08 (s, 4H, CH_2CH_2) ppm; ^{13}C NMR (62.90 MHz; $CDCl_3$): $\delta = 37.9$, 43.6 (C-3, C-5, 4CH_2), 54.3 (C-2, C-5), 64.7 (4C-2′), 65.4 (CH_2CH_2), 73.0 (4C-1′), 106.5 (C-4), 208.4 (C-1) ppm.

(1,1,3,3-Tetra-(1′,2′-dicarba-*closo*-dodecaboranylmethyl)propan-2-yl)-(4″-benzyloxy-but-2″-ynyl) ether (10): Carborane **5** (162 mg, 0.236 mmol) was added to the solution of 4-benzyloxy-1-bromo-but-2-yne (**9**; 375 mg, 1.57 mmol) in toluene (1.0 mL). NaOH (432 mg, 10.8 mmol) and benzyl triethylammonium chloride (112 mg, 0.49 mmol) were dissolved in water (1 mL) and then added to the above reaction mixture. The mixture was stirred for 1 h while carborane **5** dissolved. Water (10 mL) and diethyl ether (10 mL) were added and the combined layers were stirred until the aqueous phase remained clear. After separation of the organic phase, the aqueous phase was extracted twice with diethyl ether. The combined organic layers were washed with water, dried over Na_2SO_4 and evaporated, then the residue was purified by column chromatography on silica gel (petroleum ether/diethyl ether, 4:1) to afford **10** (129 mg, 153 μmol, 65%) as a colourless powder: $R_f =$ 0.28 (petroleum ether/diethyl ether, 7:3); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 1.00$–3.50 (br m, 50H, 40BH, H-1, H-3, 4CH_2), 3.44, 3.69 (2br s, 4H, 2H-2′a, 2H-2′b), 3.49 (m, 1H, H-2), 4.23, 4.30 (2s, 4H, CH_2-1″, CH_2-4″), 7.31–7.39 (m, 5H, 5Ar-H) ppm; ^{13}C NMR (62.90 MHz; $CDCl_3$): $\delta = 38.9$, 40.3 (4CH_2), 41.8 (C-1, C-3), 57.6 (C-4″), 61.8, 62.8 (2C-2′a, 2C-2′b5a), 62.0 (C-1″), 72.6 (Bn-C), 72.6, 73.6 (2C-1′a, 2C-1′b), 80.5 (C-2), 80.5, 86.1 (C-2″, C-3″), 127.8, 128.4, 128.7, 136.8 (5Ar-C) ppm; HRMS (FAB): calcd (%) for $C_{26}H_{66}{}^{10}B_8{}^{11}B_{32}O_2$: 842.907 $[M]^-$; found: 842.907.

(1,1,3,3-Tetra-(1′,2′-dicarba-*closo*-dodecaboranylmethyl)propan-2-yl)-(4″-hydroxy-butyl) ether (11): The ether **10** (105 mg, 0.125 mmol) and 20% Pd/C (14.2 mg) in acetone/water (3.3. mL, 9:1) were stirred for 4 h at RT under an H_2 atmosphere. The catalyst was filtered and the solvent was evaporated. The residue was purified by column chromatography on silica gel (petroleum ether/diethyl ether, 1:1) to afford **11** (79 mg, 104 μmol, 84%) as a white powder: $R_f = 0.26$ (petroleum ether/diethyl ether, 1:1); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 1.00$–3.50 (br m, 55H, 40BH, H-1, H-3, 8CH_2, CH_2-2″, CH_2-3″, OH), 3.26 (m, 1H, H-2), 3.58, 3.68 (2t, 4H, CH_2-1″, CH_2-4″), 3.62, 3.75 (2br s, 4H, 2H-2′a, 2H-2′b) ppm; ^{13}C NMR (62.90 MHz, $CDCl_3$): $\delta = 26.9$, 28.6 (C-2″, C-3″), 38.4, 40.4 (4CH_2), 42.0 (C-1, C-3), 61.7, 62.8 (2C-2′a, 2C-2′b), 62.3 (C-4″), 72.7, 73.5 (2C-1′a, 2C-1′b), 76.0 (C-1″), 81.7 (C-2) ppm; elemental analysis: calcd (%) for $C_{19}H_{64}B_{40}O_2$ (757.1): C 30.14, H 8.52; found: C 29.80, H 8.11; HRMS (FAB): calcd (%) for $C_{19}H_{63}{}^{10}B_8{}^{11}B_{32}O_2$: 755.884 $[M-H]^-$; found: 755.889.

***β*-Cyanoethyloxy-*N*,*N*-diisopropyl-(4″-(1,1,3,3-tetra-(1′,2′-dicarba-*closo*-dodecaboranylmethyl)-propan-2-yloxy)but-1″-yloxy)phosphine (12)**: Chloro-*β*-cyanoethyloxy-*N*,*N*-diisopropylphosphine (18.2 mg, 77 μmol) was added to a stirred suspension of **11** (18.0 mg, 23.8 μmol) and *N*-ethyl-*N*,*N*-diisopropylamine (12 mg, 93 μmol) in CH_2Cl_2 (1 mL) under an argon atmosphere. After 2 h at RT the reaction mixture was diluted with ethyl acetate (5 mL) and quenched with an aqueous solution of $NaHCO_3$ (1 mL, 10%). The mixture was shaken vigorously and the organic phase was then separated, dried over Na_2SO_4 and evaporated. After purification by flash chromatography on silica gel (petroleum ether/diethyl ether, 1:1), **12** was obtained (12.1 mg, 12 μmol, 53%): $R_f = 0.26$ (petroleum ether/diethyl ether, 1:1); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 1.00$–3.50 (br m, 58H, 40BH, H-1, H-3, 4CH_2, CH_2-2″, CH_2-3″, 2NCH, CH_2CN), 1.17–1.26 (m, 12H, 4CH_3), 3.27 (m, 1H, H-2), 3.55–3.86 (m, 10H, 4H-2′, CH_2-1″, CH_2-4″, CH_2-O) ppm; ^{13}C NMR (62.90 MHz, $CDCl_3$): $\delta = 20.6$ (CH_2CN) 24.6, 24.7 (4CH_3), 29.7 (C-2″, C-3″), 38.3, 40.4 (4CH_2), 42.0 (C-1, C-3), 43.0, 43.1 (2NCH), 57.7 (CH_2O), 61.7, 62.8 (4C-2′), 63.1 (C-1″), 72.9, 73.7 (C-1′), 76.0 (C-4″), 81.8 (C-2), 118.0 (CN) ppm; ^{31}P NMR (101.26 MHz, $CDCl_3$): $\delta = 147.7$ (dt, $J_{P,1''} =$ 17.1 Hz, $J_{P,CH2\text{-}O} = 20.0$ Hz) ppm.

(3‴-*O*-Acetylthymidine-5‴-yl)-(4″-(1,1,3,3-tetra-(1′,2′-dicarba-*closo*-dodecaboranylmethyl)propan-2-yloxy)but-1″-yl)-(*β*-cyanoethyl)phosphate triester (13a): 3-*O*-Acetylthymidine (10.2 mg, 35.9 μmol) and 1*H*-tetrazol (3.5 mg, 48 mmol) were added to a stirred solution of **12** (12.0 mg, 12.4 μmol) in CH_3CN (0.15 mL) under an argon atmosphere. After 2 h at RT, a CH_2Cl_2/ethyl acetate mixture (5 mL, 1:1) was added. The organic phase was extracted with aqueous $NaHCO_3$ (10 mL, 10%) and H_2O (10 mL), dried over Na_2SO_4 and evaporated. The crude residue was dissolved in CH_3CN (0.20 mL) and oxidised with cumol hydroperoxide (25 mg, 80% in CH_3CN). After 5 min, 2-propanol (0.20 mL) was added to quench the reaction. The solvent was removed under vacuum and the residue was purified by column chromatography on silica gel (diethyl ether) to afford the triester **13a** (4.7 mg, 4 μmol, 33%) as a diastereomeric mixture: $R_f = 0.26/0.22$ (diethyl ether); ^{1}H NMR (250.13 MHz; $CDCl_3$, diastereomeric mixture): $\delta = 0.83$–3.50 (m, 64H, 40BH, 2H-2‴, CH_3-5‴, 2H-2″, 2H-3″, CH_2CN, CH_3CO, H-1, H-3, 4CH_2), 3.32 (m, 1H, H-2), 3.56–3.65, 3.72, 3.90, 4.11–4.16, 4.27–4.35 (m, 12H, H-6‴′, 2H-1″, CH_2OP, H-4‴, 2H-5‴, 4H-2′), 5.27–5.34 (m, 1H, H-3‴), 6.32 (m, 1H, H-1‴), 7.37 (m, 2H, 2H-4″), 8.24 (m, 1H, NH) ppm; ^{13}C NMR (62.90 MHz; $CDCl_3$, diastereomeric mixture): $\delta =$ 12.6 (CH_3-5‴′), 20.9 (CH_3CO), 22.7 (CH_2CN), 26.1, 29.7 (C-2″, C-3″), 36.9 (C-2‴), 38.1, 40.3 (4CH_2), 42.0 (C-1), 62.0, 62.8 (4C-2′), 62.3, 68.1, 74.9 (C-4″, C-1″, CH_2OP), 72.8, 73.6 (4C-1′), 73.7 (C-3‴), 81.6 (C-2), 85.0, 85.1 (C-1‴, C-4‴), 111.6 (C-5‴′), 116.2 (CN), 150.0 (C-2‴′), 163.1 (C-4‴′), 170.7 (CH_3CO) ppm, C-5‴ was not observed; ^{31}P NMR (101.26 MHz, $CDCl_3$): $\delta = -1.01$, -1.04 ppm; MS (ESI): calcd for $C_{34}H_{84}B_{40}N_3O_{10}P$: 1159.1 $[M+H]^+$; found: 1158.59.

(3‴-*O*-Acetylthymidin-5‴-yl)-(4″-(1,1,3,3-tetra-(1′,2′-dicarba-*closo*-dodecaboranylmethyl)propan-2-yloxy)but-1″-yl)phosphate diester (13b): The triester **13a** (4.2 mg, 3.6 μmol) was treated with triethylamine (0.5 mL) in CH_2Cl_2 (0.5 ml) for 20 min at RT. The solution was evaporated and purified on silica gel ($CHCl_3$/MeOH/triethylamine, 80:20:2) to give **13b** (3.2 mg, 2.9 μmol, 74%): $R_f = 0.11$

($CHCl_3$/MeOH, 9:1); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 1.00–3.50$ (br m, 62H, 40BH, CH_3-5‴, 2H-2″, 2H-3″, 2H-2″, H-1, H-3, 4CH_2), 1.34 (t, 9H, 3CH_3 (NEt_3)), 3.07 (q, 6H, 3CH_2 (NEt_3)), 3.30–4.22 (m, 9H, 2H-4″, 2H-1″, H-1‴, H-4‴, 2H-5‴, H-2), 5.34 (m, 1H, H-3‴), 6.84 (m, 1H, H-6‴), 7.72 (m, 1H, NH) ppm; ^{31}P NMR (101.26 MHz, $CDCl_3$): $\delta = -0.45$ ppm; HRMS (FAB): calcd for $C_{31}H_{80}{}^{10}B_{32}{}^{11}B_8N_2O_{10}P$: 1105.71 $[M-HNEt_3]^+$; found: 1102.940.

1-Bromo-4-(2′,3′,4′,6′-tetra-*O*-benzyl-α-D-glucopyranosyl)but-2-yne (15α) and 1-bromo-4-(2′,3′,4′,6′-tetra-*O*-benzyl-β-D-glucopyranosyl)but-2-yne (15β): 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl-trichloroacetimidate[20] (1.23 g, 1.80 mmol) and 1-bromobut-2-yn-4-ol[3] (373 mg, 2.50 mmol) were dissolved in CH_2Cl_2 (16 mL) and cooled to −30 °C under an argon atmosphere. TMSOTf (72 mg, 0.32 mmol) was added dropwise to this mixture and then the reaction mixture was stirred and allowed to warm up to RT overnight. The solution was washed with a saturated solution of $NaHCO_3$, the organic phase was separated and the aqueous phase was extracted twice with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and concentrated under vacuum. The residue was purified by column chromatography on silica gel (petroleum ether/diethyl ether, 17:3) to afford **15**α (501 mg, 744 μmol, 41%) and **15**β (392 mg, 582 μmol, 32%): **15**α: $R_f = 0.17$ (petroleum ether/diethyl ether, 17:3); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 3.57–3.80$, 3.94–4.03 (m, 6H, H-2′, H-3′, H-4′, H-5′, 2H-6′), 3.89 (t, 2H, $J_{1,4} = 1.9$ Hz, 2H-1), 4.30 (t, 2H, 2H-4), 4.43–5.00 (m, 8H, 4Ar-CH_2), 5.04 (d, 1H, $J_{1',2'} = 3.6$ Hz, H-1′), 7.11–7.39 (m, 20H, 20Ar-H) ppm; ^{13}C NMR (62.90 MHz; $CDCl_3$): $\delta = 14.0$ (C-1), 54.7 (C-4), 68.4 (C-6′), 70.7, 77.5, 79.3, 81.6 (C-2′, C-3′, C-4′, C-5′), 72.9, 73.4, 75.0, 75.7 (4Ar-CH_2), 81.5, 82.1 (C-2, C-2), 95.4 (C-1′), 127.5, 127.6, 127.6, 127.8, 127.9, 128.1, 128.3, 128.4, 137.8, 137.9, 138.2, 138.7 (20Ar-C) ppm; HRMS (FAB): calcd for $C_{38}H_{39}{}^{81}BrO_6Na$: 695.181 $[M+Na]^+$; found: 695.181; calcd for $C_{38}H_{39}{}^{79}BrO_6Na$: 693.182 $[M+Na]^+$; found: 693.182; **15**β: $R_f = 0.20$ (petroleum ether/diethyl ether, 17:3); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 3.43–3.76$ (m, 6H, H-2′, H-3′, H-4′, H-5′, 2H-6′), 3.89 (t, 2H, $J_{1,4} = 2.0$ Hz, H-1), 4.46–4.97 (m, 11H, H-1′, 2H-4, 4Ar-CH_2), 7.12–7.40 (m, 20H, 20Ar-H) ppm; ^{13}C NMR (62.90 MHz; $CDCl_3$): $\delta = 14.0$ (C-1), 56.3 (C-4), 68.8 (C-6′), 73.5, 74.7, 74.9, 75.6 (4Ar-CH_2), 74.9, 77.6, 82.0, 84.6 (C-2′, C-3′, C-4′, C-5′), 81.7, 82.0 (C-2, C-3), 101.6 (C-1′), 127.6, 127.6, 127.6, 127.7, 127.7, 127.8, 127.9, 128.2, 128.3, 138.1, 138.4, 138.6 (20Ar-C) ppm; HRMS (FAB): calcd for $C_{38}H_{39}{}^{79}BrO_6Na$: 693.183 $[M+Na]^+$; found: 693.181; calcd for $C_{38}H_{39}{}^{81}BrO_6Na$: 695.181 $[M+Na]^+$; found 695.183.

1-Bromo-4-(2′,3′,4′,6′-tetra-*O*-benzyl-β-D-galactopyranosyl)but-2-yne (16β): 2,3,4,6-Tetra-*O*-benzyl-α-D-galactopyranosyl-trichloracetimidate[20] (3.51 g, 5.12 mmol) and but-2-yne-1,4-diol (879 mg, 10.2 mmol) were suspended in CH_3CN (15 mL) and the reaction mixture was cooled to −15 °C. TMSOTf (184 mg, 0.82 mmol) was added dropwise. After 30 min, the reaction was quenched with solid $NaHCO_3$ (250 mg). The reaction mixture was allowed to warm to RT and was filtered. The filtrate was concentrated under vacuum and the residue was purified by column chromatography on silica gel (petroleum ether/diethyl ether, 7:3). This intermediate (307 mg) was dissolved in CH_2Cl_2 (3 mL) and CBr_4 (327 mg, 0.986 mmol) was added. Over 10 min triphenylphosphine (318 mg, 1.21 mmol) was added in small portions. The solution was stirred for 24 h and then concentrated under vacuum. The residue was extracted with diethyl ether and filtered. The ethereal extract was concentrated under vacuum and purified by column chromatography on silica gel (petroleum ether/diethyl ether, 8:2) to yield **16**β (56.0 mg, 80 μmol, 14%): $R_f = 0.09$ (petroleum ether/diethyl ether, 8:2); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 3.51–3.60$, 3.79–3.89 (2m, 8H, H-2′, H-3′, H-4′, H-5′, 2H-1, 2H-4), 4.40–4.79, 4.91–4.96 (2m, 11H, H-1′, 2H-6′, 4Ar-CH_2), 7.27–7.42 (m, 20H, 20Ar-H) ppm; ^{13}C NMR (62.90 MHz; $CDCl_3$): $\delta = 14.1$ (C-1), 56.0 (C-4), 68.7 (C-6′), 73.1, 73.5, 74.4, 75.0 (4Ar-CH_2), 73.4, 73.4, 79.2, 82.1 (C-2′, C-3′, C-4′, C-5′), 81.4, 82.4 (C-2, C-3), 101.7 (C-1′), 127.5, 127.7, 127.8, 128.1, 128. 2, 128.2, 128.3, 128.4, 137.8, 138.4, 138.5, 138.7 (20Ar-C) ppm; HRMS (FAB): calcd for $C_{38}H_{40}{}^{79}BrO_6$: 671.201 $[M+H]^+$; found: 671.193; calcd for $C_{38}H_{40}{}^{81}BrO_6$: 673.199 $[M+H]^+$; found: 673.199.

(1,1,3,3-Tetra-(1′,2′-dicarba-*closo*-dodecaboranylmethyl)-prop-2-yl)-(4″-α-D-glucopyranosyloxy-but-1″-yl) ether (17α): Carborane **5** (31.1 mg, 45 μmol) was added to a solution of glucoside **15**α (41.2 mg, 61 μmol) in toluene (0.5 mL). A solution of NaOH (108 mg, 2.7 mmol) and benzyl triethylammonium chloride (22.0 mg, 96.9 μmol) in water (0.3 mL) was added and the reaction mixture was stirred vigorously for 1 h. The reaction was quenched with water (2 mL) and diethyl ether (2 mL). After a further 30 min stirring, the organic phase was separated and the aqueous phase was extracted twice with diethyl ether. The combined organic layers were dried over Na_2SO_4 and evaporated under vacuum. The residue of the benzylated glucoside was purified by column chromatography on silica gel (petroleum ether/diethyl ether, 4:1). The product ($R_f = 0.39$ (petroleum ether/diethyl ether, 3:1) in acetone/water (10:1, 0.55 mL) was then treated with H_2 and catalytic amounts of Pd/C (2.6 mg, 20%) for 2 h at RT. The catalyst was filtered off and the solvent was evaporated under vacuum. The crude product was purified by column chromatography on silica gel ($CHCl_3$/MeOH, 9:1) to afford **17**α (3.1 mg, 3.4 μmol, 7.4%): $R_f = 0.21$ ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 0.83–3.30$ (m, 54H, 40BH, H-1, H-3, 4CH_2, 2H-2″, 2H-3″), 3.31–3.84 (m, 15H, H-2, 4H-2′, 2H-1″, 2H-4″, H-2‴, H-3‴, H-4‴, H-5‴, 2H-6‴), 4.84 (d, 1H, $J_{1''',2'''} = 2.6$ Hz, H-1‴) ppm; HRMS (FAB): calcd for $C_{25}H_{74}{}^{10}B_8{}^{11}B_{32}O_7$: 918.945 $[M]^-$; found: 918.943.

(1,1,3,3-Tetra-(1′,2′-dicarba-*closo*-dodecaboranylmethyl)prop-2-yl)-(4″-β-D-galactopyranosyloxy-but-1″-yl) ether (18β): **18**β was synthesised by the same method as described for **17**α but with galactoside **16**β (39.1 mg, 58 μmol), carborane **5** (18 mg, 26 μmol), NaOH (208 mg, 5.2 mmol) and benzyltriethylammonium chloride (13.2 mg, 58 μmol). **18**β (7.2 mg, 7.8 μmol, 30%): $R_f = 0.17$ ($CHCl_3$/MeOH, 9:1); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 0.83–3.25$ (m, 54H, 40BH, H-1, H-3, 4CH_2, 2H-2″, 2H-3″,), 3.30–4.08 (m, 15H, H-2, 4H-2′, 2H-1″, 2H-4″, H-2‴, H-3‴, H-4‴, H-5‴, 2H-6‴), 4.24 (d, 1H, $J_{1''',2'''} = 7.3$ Hz, H-1‴) ppm; ^{13}C NMR (62.90 MHz; $CDCl_3$): $\delta = 26.1$, 26.8 (C-2″, C-3″), 38.3, 40.3, 40.6 (4CH_2), 42.1, 42.1 (C-1, C-3), 62.0, 62.9, 63.0 (4C-2′), 62.7 (C-6‴), 69.4, 75.4 (C-1″, C-4″), 69.8, 71.7, 73.4, 73.9 (C-2‴, C-3‴, C-4‴, C-5‴), 81.5 (C-2), 103.1 (C-1‴) ppm; HRMS (FAB): calcd for $C_{25}H_{74}{}^{10}B_8{}^{11}B_{32}O_6Na$: 941.935 $[M+Na]^+$; found: 941.935.

(1,1,3,3-Tetra-(1′,2′-dicarba-*closo*-dodecaboranylmethyl)prop-2-yl)-(4″-hydroxy-but-1″-yl) ether (19): Carborane **5** (40 mg, 58 μmol) was added to a solution of 1-bromo-4-(2′-tetrahydropyranyloxy)-but-2-yne[15] (27.2 mg, 117 μmol) in toluene (0.40 mL). A solution of NaOH (110 mg, 2.7 mmol) and benzyltriethylammonium chloride (26.4 mg, 116 μmol) in water (0.3 mL) was added to the organic phase and the reaction mixture was vigorously stirred for 1 h. The reaction was quenched with water (4 mL) and diethyl ether (4 mL) and the reaction mixture was stirred until the turbidity disappeared. After separation of the two phases, the organic phase was extracted twice with diethyl ether. The combined organic layers were washed with water, dried over Na_2SO_4 and evaporated under vacuum. The residue was dissolved in methanol (1.5 mL) and stirred together with ion-exchange resin (82 mg, DOWEX 50WX8, H^+) for 16 h. The product was then filtered, evaporated under vacuum and purified by column chromatography on silica gel (petroleum

ether/diethyl ether, 7:3→3:2) to afford **19** (28 mg, 37 μmol, 64%) as a colourless foam: $R_f = 0.11$ (petroleum ether/diethyl ether, 7:3); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 1.00$–3.50 (br m, 50H, 40BH, H-1, H-3, 4CH_2), 3.45 (m, 1H, H-2), 3.68, 3.76 (2 br s, 4H, 4H-2′), 4.28–4.34 (m, 4H, 2H-1″, 2H-4″) ppm; ^{13}C NMR (62.90 MHz; $CDCl_3$): $\delta =$ 38.9, 40.4 (4CH_2), 41.8 (C-1, C-3), 61.8, 62.9 (4C-2′), 61.8 (C-1″), 72.6, 73.5 (4C-1′), 79.7, 88.3 (C-2″, C-3″), 80.9 (C-2) ppm; HRMS (FAB): calcd for $C_{19}H_{59}{}^{10}B_8{}^{11}B_{32}O_2$: 752.859 $[M]^-$; found: 752.860.

(1,1,3,3-Tetra-(1′,2′-dicarba-*closo*-dodecaboranylmethyl)prop-2-yl)-(4″-bromo-2″-butyn-1″-yl) ether (20): Triphenylphosphine (29.3 mg, 112 μmol) was added to a solution of carborane **19** (28.0 mg, 37.2 μmol) and CBr_4 (23.2 mg, 70.0 μmol) in CH_2Cl_2 (0.5 mL) and stirred for 1 h. The reaction mixture was concentrated under vacuum and purified by column chromatography on silica gel (petroleum ether/diethyl ether, 4:1→7:3) to afford **20** (25.1 mg, 30 μmol, 83%): $R_f = 0.28$ (petroleum ether/diethyl ether, 7:3); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 1.00$–3.50 (br m, 50H, 40BH, H-1, H-3, 4CH_2), 3.47 (m, 1H, H-2), 3.62, 3.70 (2 br s, 4H, 4H-2′), 3.95 (t, 2H, $J_{1'',4''} = 2.0$ Hz, 2H-4″), 4.32 (t, 2H, 2H-1″) ppm; ^{13}C NMR (62.90 MHz; $CDCl_3$): $\delta = 13.2$ (C-4″), 38.9, 40.5 (4CH_2), 41.9 (C-1, C-3), 61.9, 62.9 (4C-2′), 61.9 (C-1″), 72.5, 73.5 (4C-1′), 80.8 (C-2), 80.6, 85.1 (C-2″, C-3″); HRMS (FAB): calcd for $C_{19}H_{58}{}^{10}B_8{}^{11}B_{32}{}^{79}BrO$: 814.774 $[M-H]^-$; found: 814.771; calcd for $C_{19}H_{58}{}^{10}B_8{}^{11}B_{32}{}^{81}BrO$: 816.777 $[M-H]^-$; found: 816.774.

Di-*tert*-butyl-protected 2‴,2‴-bis-(4″-(1,1,3,3-tetra-(1′,2′-dicarba-*closo*-dodecaboranylmethyl)prop-2-yloxy)-2″-butyn-1″-yl) malonate (21): A solution of NaOH (93 mg, 2.3 mmol) and benzyltriethylammonium chloride (15.4 mg, 67.8 μmol) in water (0.5 mL) was added to a solution of carborane **20** (22.4 mg, 27.4 μmol) and di-*tert*-butylmalonate (2.75 mg, 12.7 μmol) in toluene (0.1 mL) and the reaction mixture was vigorously stirred for 1 h. The reaction was quenched with water (2 mL) and diethyl ether (2 mL). The organic phase was separated and the aqueous phase was washed twice with water. The combined organic layers were washed with water and dried over Na_2SO_4. After concentration under vacuum, the residue was purified by column chromatography on silica gel (petroleum ether/diethyl ether, 17:3) to afford **21** (8.2 mg, 4 μmol, 37%): $R_f = 0.19$ (petroleum ether/diethyl ether, 17:3); ^{1}H NMR (250.13 MHz; $CDCl_3$): $\delta = 1.00$–3.50 (br m, 122H, 80BH, 2H-1, 2H-3, 8CH_2, 4H-1″, 6CH_3), 3.35 (m, 2H, H-2), 3.66, 3.76 (2 br s, 8H, 8H-2′), 4.26 (m, 4H, 4H-4″) ppm; ^{13}C NMR (62.90 MHz; $CDCl_3$): $\delta = 23.4$ (2C-1″), 28.0 (6CH_3), 38.9, 40.3 (8CH_2), 41.9 (2C-1′), 56.3 (C-2‴) 61.6, 62.9 (8C-2′), 62.4 (2C-4″), 72.6, 73.6 (8C-1′), 78.0, 83.3, 84.2 (C-2″, C-3″, C-1‴, C-3‴), 81.0 (C-2) ppm; MS (FAB): calcd for $C_{49}H_{136}{}^{10}B_{16}{}^{11}B_{64}O_6$: 1686.8 $[M]^-$; found: 1686.6.

2‴,2‴-Bis-(4″-(1,1,3,3-tetra-(1′,2′-dicarba-*closo*-dodecaboranylmethyl)prop-2-yloxy)-2″-butyn-1″-yl) malonic acid (22): Malonate **21** (5.6 mg, 3.3 μmol) was dissolved in TFA (1.0 mL) and stirred for 1 h at RT. The solvent was removed by evaporation under vacuum to give **22** (4.2 mg, 3 μmol, 75%) as a highly viscous, colourless oil: $R_f = 0.21$ (petroleum ether/diethyl ether, 2:1); MS (ESI): calcd for $C_{41}H_{128}{}^{10}B_{16}{}^{11}B_{64}O_6$: 1582.0 $[M]^-$; found: 1582.1.

X-ray crystal structure analyses of molecules 3 and 4: The reflections of both compounds were collected with a Nonius CAD4 diffractometer by using $Mo_{K\alpha}$ radiation ($\lambda = 71.073$ pm, graphite monochromated). The stability of reflection intensity was controlled by monitoring three control reflections every 100 reflections.

Compound **3**: $C_{15}H_{14}O$, $M_r = 210.26$, $\rho = 1.13$ g cm^{-3}; colourless prism of dimensions 0.4×0.3×0.25 mm, monoclinic space group $P2/n$ with lattice parameters $a = 9.734(2)$, $b = 5.0136(7)$, $c =$ 12.774(4) Å, $\beta = 96.84(2)°$, $Z = 2$, unit cell volume $V = 619.0(2)$ Å^3, absorption coefficient $\mu = 0.07$ mm^{-1}. Data collection: intensity data of 1571 collected reflections in the range $2.5 \le 2\theta \le 27.9°$ were measured in the $\omega-2\theta$ scan mode (measuring temperature of 263 K); 1488 reflections were unique, of which 1246 were assigned to be observed; the data were Lorentz and polarisation corrected. Structure solution and refinement: direct methods (SHELXS-97[21]); refinement (against F^2, SHELXL-97[21]) with 102 parameters converging at $R1 = 0.037$ and $wR^2 = 0.099$ ($I > 2\sigma(I)$); max. and min. residual electron densities were 0.22 and −0.16 e Å^{-3}, respectively. All hydrogen atoms were refined isotropically.

Compound **4**: $C_{15}H_{54}B_{40}O{\cdot}C_7H_8$, $M_r = 774.6$, $\rho = 1.07$ g cm^{-3}; colourless prism of dimensions 0.5×0.4×0.4 mm, orthorhombic space group *Pbcn* with lattice parameters $a = 22.615(8)$, $b = 9.957(2)$, $c =$ 23.897(8) Å, $Z = 4$, unit cell volume $V = 5381(3)$ Å^3, absorption coefficient $\mu = 0.05$ mm^{-1}. Data collection: intensity data of 6474 collected reflections in the range $2.2 \le 2\theta \le 28.0°$ were measured in the $\omega-2\theta$ scan mode (measuring temperature of 293 K); 6474 reflections were unique, of which 2957 were assigned to be observed; the data were Lorentz and polarisation corrected. Structure solution and refinement: direct methods (SHELXS-97[21]); refinement (against F^2, SHELXL-97[21]) with 404 parameters converging at $R1 = 0.088$ and $wR2 = 0.247$ ($I > 2\sigma(I)$); max. and min. residual electron densities were 0.5 and −0.31 e Å^{-3}, respectively. All hydrogen atoms were refined isotropically except those of the toluene guest molecule, which were calculated. The toluene guest molecule was disordered at two positions with 50% multiplicity (in one of both disordered toluene positions, only the *xyz* coordinates were refined). In the carborane clusters we could not distinguish one carbon atom from the equivalent boron atoms. Therefore we refined all five equivalent positions in the cluster as boron atoms, each with a multiplicity of 120%.

CCDC-209845 (**3**) and CCDC-209846 (**4**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336033; or deposit@ccdc.cam.uk).

In vitro toxicity studies: Cytotoxicity tests on C6 rat glial cells[5] and A240286S human lung adenocarcinoma cells[8] were performed under flavin-protecting conditions.[9] The cell strains were cultivated, without antibiotics, in riboflavin-free[7] RPMI-1640 medium buffered with 3 mM $NaHCO_3$/9 mM 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) and supplemented with 10% fetal calf serum (Integro, Zaandam, Holland). The parameter measured was the IC_{50}, that is, the dose capable of inhibiting the cell growth by 50% after three days of incubation.[24] A 50 mM solution of **4** was prepared in tissue-culture-grade dimethylsulfoxide (DMSO, Sigma). Concentration gradients for ketone **4** were established, in quadruplicate, by serially diluting an initial mixture of **4** in RPMI-1640 full medium. The initial mixture resulted from the dilution of 1 volume of **4** (50 mM in DMSO) with 249 volumes of the medium. The six negative control wells per test and cell strain contained the highest DMSO concentration present in the test wells (0.2% *v/v*). Tests were performed in 96-well tissue-culture plates (Greiner, Frickenhausen, Germany) after collagen coating, with only the internal 10×6 well area used. For greater homogeneity of test results, the peripheral 36 wells of the plates were filled with phosphate-buffered saline. Each well was inoculated with either 0.9×10^4 freshly trypsinised C6 cells or 3×10^4 A240286S cells. The calculated final concentrations of **4** were in the range 1.56–50 μM. Test plates were incubated under 2.5% CO_2 at 36.5 °C in a humidified incubator for 72 h. After trypsinising the cells, cell numbers were read from cell-size histograms produced by a Casy1 cell analyser

by using Casystat software (Schärfe System, Reutlingen, Germany). Only C6 cells with diameters of 9–30 μm and A240286S cells with diameters of 11.5–30 μm were considered. The effect of **4** was expressed as a percentage of growth inhibition. The results are shown in Figure 3. The apparent IC_{50} of **4** in C6 and A240286S cells was 48 μM and 59 μM, respectively.

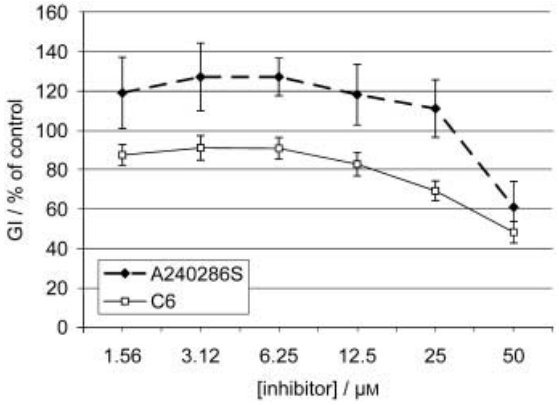


Figure 3. *Growth inhibition (GI) of human A240286S lung adenocarcinoma cells and rat C6 glial cells by ketone **4**, after exposure for 72 h. Symbols represent mean values from three independent experiments, each one performed in quadruplicate. The bars show the standard deviations.*

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