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# Synthesis and anticancer properties of dendritic glycoconjugates containing multiple *o*-carborane clusters†

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Boron-rich glycodendrimers containing three to six peripheral hydrophilic glucose moieties and three *ortho*-carborane clusters have been synthesized. Adding more number of glucose moieties compensated the hydrophobicity of *ortho*-carboranes and made the glycodendrimers completely water soluble. *In vitro* cytotoxicity of the synthesized glycodendrimers **16**, **18** and **20** has been evaluated against MCF-7 breast cancer cell lines and compared with that of the core compound **7** without any peripheral glucose moieties. The glycodendrimer **20** having nine glucose moieties has shown the highest cell death potential with an IC<sub>50</sub> value of 41.35 ng mL<sup>-1</sup> (0.01 μM) while lower cell death potential with an IC<sub>50</sub> value of 822.33 ng mL<sup>-1</sup> (0.8 μM) has been observed in the case of compound **7**. The cytotoxicity of glycodendrimer **20** was found to be 600 times higher than the cytotoxicity of the chemotherapeutic drug cisplatin (IC<sub>50</sub> 6 μM). The study of caspase-3 activity reveals that compounds **7**, **16**, **18** and **20** induce cell death in MCF-7 cell lines *via* apoptosis. The results indicate that the glycodendrimers containing multiple *o*-carborane clusters could be useful tumor specific anticancer agents.

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## Introduction

Dendrimer based drug delivery systems show enhanced cellular uptake because of their unique branching architecture and a large number of peripheral functional groups.<sup>1</sup> Macromolecular and dendrimer based drug delivery systems selectively carry and accumulate drugs inside tumors because of the leaky vasculature of tumor tissues in a method known to be the enhanced permeability and retention (EPR) effect.<sup>2–5</sup> Icosahedral carboranes possess remarkable anticancer properties. Incorporation of multiple carborane clusters into the dendritic platform makes it boron-rich for effective cancer treatment.<sup>6,7</sup> Dendritic molecules containing multiple *o*-carborane clusters showed higher cytotoxicity towards cancer cells than the commonly used anticancer agent cisplatin.<sup>8</sup> Glycoconjugates of carboranes have been synthesized for selective

boron delivery to cancer cells. Hydrophilic carrier compounds such as sugar moieties compensate for the hydrophobicity of carborane clusters and enhance their selectivity towards tumor cells.<sup>9–11</sup> Carbohydrate–protein interactions facilitate many biological processes and cellular adhesion at the molecular level. Due to the overexpression of lectins in tumor tissues polyglycosylated derivatives selectively target cancer cells.<sup>12</sup> Glycodendrimers have been used as selective delivery agents for cancer cells.<sup>13</sup> We reported dendritic glycoconjugates containing one *o*-carborane moiety and evaluated anticancer efficacy *in vitro* against two cancer cell lines (MCF-7 and A-431) and one normal skin epidermal cell line (HaCaT). The results of the study revealed that the synthesized compounds were less toxic towards HaCaT normal cell lines while more cytotoxic towards cancer cells. However, they showed lower anticancer activity when more sugar moieties were added to enhance water solubility.<sup>14</sup> These findings inspired us to synthesize dendritic molecules containing multiple *o*-carborane clusters with peripheral hydrophilic carbohydrate moieties. Herein, we report the preparation of a series of dendritic carboranyl glycoconjugates containing multiple *o*-carbore clusters in the core. The core has been appended to three, six and nine peripheral glucose moieties using Cu(I) catalyzed azide–alkyne click cycloaddition reactions.<sup>7,8,14</sup> The anticancer efficacy of the synthesized glycoconjugates has been investigated against MCF-7 breast carcinoma cells through *in vitro*

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cytotoxicity assay. Caspase-3 activity assay was carried out to explore the mode of cell death *i.e.* necrosis or apoptosis.

## Experimental

### General methods

All the chemicals used in this work were purchased from Sigma-Aldrich/Merck India Pvt. Ltd/Spectrochem India and were used without any further purification. 3-(4,5-Dimethylthiazol-2-yl)-2,5 di-phenyl-tetrazolium bromide (MTT), Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), antibiotic solution (penicillin-streptomycin), caspase assay buffer and caspase-3 colorimetric substrate were purchased from Sigma Aldrich and 96 wells plates were purchased from Tarsons, India. All experiments were carried out in oven-dried glassware under dry argon/nitrogen. Solvents were distilled under nitrogen from an appropriate drying agent. Reagents were purchased and were used without further purification. All compounds were purified by column chromatography on silica gel/activated neutral alumina (60–120 mesh, Spectrochem, India). The yield of the products refers to analytically pure samples.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Fourier Transform multi-nuclear NMR spectrometer at 400 MHz and 100 MHz respectively. Chemical shifts were reported relative to TMS ( $^1\text{H}$ :  $\delta = 0.00$  ppm),  $\text{CDCl}_3$  ( $^{13}\text{C}$ :  $\delta = 77.0$  ppm) and coupling constants are given in Hz. All  $^{13}\text{C}$  spectra are proton-decoupled.  $^{11}\text{B}$  NMR spectra are proton-decoupled and were recorded at 128 MHz relative to  $\text{BF}_3 \cdot \text{Et}_2\text{O}$ . Accurate mass measurements were performed for compounds using high resolution instruments (in the positive or negative mode) and a MALDI-TOF mass spectrometer.

### Cell culture

The MCF-7 cell line was obtained from the National Center for Cell Science, Pune, India, and was maintained in Dulbecco's minimal essential medium containing 10% fetal bovine serum, 1% L-glutamine, and 1% penicillin-streptomycin at 37 °C in a humidified incubator with 5%  $\text{CO}_2$ /95% air. MCF-7 cells at a concentration of  $20 \times 10^3$  cells in 200  $\mu\text{L}$  per well were seeded into 96-well culture plates and kept for 24 h. Then compounds **7**, **16**, **18** and **20** were added separately and kept for 48 h. After the incubation period, cell survival was monitored using MTT assay. The concentrations used were calibrated after a pilot experiment taking the test compounds on a log scale ( $1$ – $1000$  ng  $\text{mL}^{-1}$ ).

### Assay of cytotoxicity

After incubation for 48 h, the media were removed carefully from all wells, and the cells were washed in phosphate buffer saline (PBS) two times and used for MTT assay. Then, 100  $\mu\text{L}$  of 10% (v/v) MTT solution (5 mg  $\text{mL}^{-1}$  in PBS) in medium was added. After incubation for 3 h, the MTT solution was replaced with 100  $\mu\text{L}$  of 20% (w/v) sodium dodecyl sulfate solution and 0.6% (v/v) of 37% HCl in dimethyl sulfoxide, which acted as cell lysis buffer, breaking the cells and allowing the purple crystals to be released into the solution. The 96-well plates were then

shaken in a multi-microplate shaker to dissolve the MTT crystals, resulting in a homogeneous purple solution and the absorbance was measured at 570 nm using a plate reader. The darker the solution, the higher is the number of viable and metabolically active cells. The percentage of viable cells is calculated using the following formula:

$$\% \text{ Viable cell} = (\text{abs}_{\text{sample}} - \text{abs}_{\text{blank}} / \text{abs}_{\text{control}} - \text{abs}_{\text{blank}}) \times 100$$

### Measurement of apoptosis from caspase-3 activity

Caspase-3, which is an effector caspase in apoptosis, is the most studied mammalian caspase. It plays a central role in nuclear apoptosis which leads to cell death. The caspase-3 colorimetric assay is based on the hydrolysis of the peptide substrate acetyl-Asp-Glu-Val-Asp *p*-nitroanilide by caspase-3, resulting in the release of the *p*-nitroaniline (pNA) moiety, the concentration of which is calculated from the absorbance value at 405 nm. The caspase-3 activity was determined as per the reported procedure.<sup>15</sup> After replacing media with caspase assay buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 0.1% CHAPS, 1 mM EDTA, 10% glycerol and 10 mM DTT), cell lysates were incubated with a caspase-3 colorimetric substrate acetyl-Asp-Glu-Val-Asp-*p*-nitroanilide-7-amino-4-methylcoumarin. Reaction mixtures were incubated at 37 °C for 15 min to 1 hour, and the absorbance value was measured at 405 nm using an ELISA microplate reader.

### Live dead assay

To observe the morphological changes incurred upon MCF-7 cells after treatment with the compounds **7**, **16**, **18**, and **20**, the cells were seeded in culture dishes for 48 h at 37 °C. The cells were then stained with Hoechst 33342 and PI solution at room temperature for 10 min in the dark. The cellular morphology was observed under an inverted fluorescence microscope.

### Statistical analysis

All statistical analyses were performed using the SPSS Version 20.0 for Windows (significance was established at  $P < 0.05$ ). Data have been expressed as mean  $\pm$  SD and statistical significance was determined by one-way analysis of variance (ANOVA) combined with Duncan's multiple range tests. All experiments were performed in triplicate, unless otherwise indicated.

### Preparation and analytical data of compounds

**Compound 3.** To a solution of compound **1** (1.60 g, 12.11 mmol) in 30 mL of  $\text{Et}_3\text{N}$  and 10 mL of acetonitrile, *p*-bromo acetophenone (**2**) (1.68 g, 8.48 mmol) was added under an argon atmosphere. To the reaction mixture,  $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$  (85 mg, 0.12 mmol) was added followed by CuI (46 mg, 0.24 mmol) and refluxed at 90 °C for 8 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was cooled to room temperature and filtered using short silica pads and the solvent was removed under reduced pressure. The crude product was purified by silica gel chromatography using 10% ethyl acetate



in hexane. Pure product: 1.30 g. Yield: 61%. Colourless solid. MP: 137 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 7.91 (d, 2H,  $J = 8$  Hz, Ar-H), 7.57 (d, 2H,  $J = 8$  Hz, Ar-H), 7.48 (d, 2H,  $J = 8$  Hz, Ar-H), 6.88 (d, 2H,  $J = 8$  Hz, Ar-H), 3.82 (s, 3H,  $\text{OCH}_3$ -H), 2.59 (s, 3H,  $\text{COCH}_3$ -H);  $^{13}\text{C}$   $\{^1\text{H}\}$  NMR (100 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 197.4 (carbonyl-C), 160.1 (Ar-C), 135.9 (Ar-C), 133.3 (Ar-C), 131.5 (Ar-C), 128.6 (Ar-C), 128.3 (Ar-C), 114.7 (Ar-C), 114.2 (Ar-C), 93.0 (alkyne-C), 87.6 (alkyne-C), 55.4 ( $\text{OCH}_3$ -C), 26.6 ( $\text{COCH}_3$ -C); IR (KBr): 2954, 2033, 1672, 1509, 1274, 1078  $\text{cm}^{-1}$ ; ESI-MS: calcd for  $\text{C}_{17}\text{H}_{14}\text{O}_2$ : 250.29; found: 251.10  $[\text{M} + \text{H}]^+$ .

**Compound 4.** Decaborane (878 mg, 7.19 mmol, 1.2 equiv.) was refluxed at 95 °C in 5 mL of dry acetonitrile for 2 hours under an argon atmosphere. To this, compound 3 (1.5 g, 5.99 mmol, 1 equiv.) dissolved in 5 mL of dry toluene was added at room temperature and then refluxed at 95 °C for 17 hours. The progress of the reaction was monitored by TLC. Excess decaborane was quenched using 2 mL of methanol and then the resulting mixture was concentrated. The crude product obtained was purified by silica gel chromatography using 25% ethyl acetate in hexane. Pure product: 800 mg. Yield: 36%. Colourless solid. MP: 123 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 7.39 (d, 2H,  $J = 8$  Hz, Ar-H), 7.33 (d, 2H,  $J = 8$  Hz, Ar-H), 7.13 (d, 2H,  $J = 8$  Hz, Ar-H), 6.61 (d, 2H,  $J = 8$  Hz, Ar-H), 4.77 (s, 1H, OH-H), 3.69 (s, 3H,  $\text{OCH}_3$ -H), 1.91 (br s, 1H, tertiary CH-H), 1.38 (s, 3H,  $\text{CH}_3$ -H);  $^{13}\text{C}$   $\{^1\text{H}\}$  NMR (100 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 160.8 (Ar-C), 147.8 (Ar-C), 132.1 (Ar-C), 130.9 (Ar-C), 129.9 (Ar-C), 125.3 (Ar-C), 123.0 (Ar-C), 113.6 (Ar-C), 85.8 (cage-C), 85.3 (cage-C), 69.5 (tertiary CH-C), 55.3 ( $\text{OCH}_3$ -C), 25.2 ( $\text{CH}_3$ -C);  $^{11}\text{B}$   $\{^1\text{H}\}$  NMR: -2.82, -9.14, -10.82 ppm; IR (KBr): 3445, 3029, 2945, 2592 (B-H), 1608, 1516, 1274, 1078  $\text{cm}^{-1}$ ; ESI-MS: calcd for  $\text{C}_{17}\text{H}_{26}\text{B}_{10}\text{O}_2$ : 370.49; found: 370.48  $[\text{M}]^+$ .

**Compound 5.** Compound 4 (800 mg, 2.16 mmol) was added to a solution of PCC (1.39 g, 6.48 mmol) in 30 mL of dry dichloromethane at 0 °C and stirred at room temperature for 2 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was filtered over a short silica pad and concentrated. The crude product was purified by silica gel chromatography using 8% ethyl acetate in hexane. Pure product: 500 mg. Yield: 83%. Colourless solid. MP: 137 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 7.63 (d, 2H,  $J = 12$  Hz, Ar-H), 7.45 (d, 2H,  $J = 8$  Hz, Ar-H), 7.26 (d, 2H,  $J = 8$  Hz, Ar-H), 6.55 (d, 2H,  $J = 12$  Hz, Ar-H), 3.61 (s, 3H,  $\text{OCH}_3$ -H), 2.43 (s, 3H,  $\text{COCH}_3$ -H);  $^{13}\text{C}$   $\{^1\text{H}\}$  NMR (100 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 197.1 (Carbonyl-C), 161.1 (Ar-C), 137.9 (Ar-C), 135.2 (Ar-C), 132.1 (Ar-C), 131.0 (Ar-C), 128.1 (Ar-C), 122.6 (Ar-C), 113.8 (Ar-C), 86.0 (cage-C), 84.1 (cage-C), 55.3 ( $\text{OCH}_3$ -C), 26.7 ( $\text{CH}_3$ -C);  $^{11}\text{B}$   $\{^1\text{H}\}$  NMR: -3.28, -10.70, -11.82 ppm; IR (KBr): 3034, 2933, 2594 (B-H), 1656, 1523, 1259  $\text{cm}^{-1}$ ; ESI-MS: calcd for  $\text{C}_{17}\text{H}_{24}\text{B}_{10}\text{O}_2$ : 368.48 found: 369.47  $[\text{M} + \text{H}]^+$ .

**Compound 6.** To a solution of compound 5 (450 mg, 1.22 mmol) in a mixed solvent (18 mL ethanol and 6 mL toluene) system at 0 °C, 3 mL of  $\text{SiCl}_4$  was added carefully and stirred at room temperature for 15 hours. The progress of the reaction was monitored by TLC. Upon completion of reaction, it was quenched carefully by adding distilled water; the product was extracted using dichloromethane and concentrated

to get the crude product. The crude product was washed carefully with cold dichloromethane to get the pure product. Column chromatography was not required. Pure product: 350 mg. Yield: 27%. Colourless solid. MP: > 250 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 7.52 (s, 3H, Ar-H), 7.50 (d, 6H,  $J = 8$  Hz, Ar-H), 7.39 (d, 6H,  $J = 4$  Hz, Ar-H), 7.37 (d, 6H,  $J = 4$  Hz, Ar-H), 6.63 (d, 6H,  $J = 8$  Hz, Ar-H), 3.69 (s, 9H,  $\text{OCH}_3$ -H);  $^{13}\text{C}$  NMR  $\{^1\text{H}\}$  (100 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 160.9 (Ar-C), 142.0 (Ar-C), 140.8 (Ar-C), 132.2 (Ar-C), 131.2 (Ar-C), 130.5 (Ar-C), 127.0 (Ar-C), 125.5 (Ar-C), 123.1 (Ar-C), 113.6 (Ar-C), 85.9 (cage-C), 85.0 (cage-C), 55.3 ( $\text{OCH}_3$ -C);  $^{11}\text{B}$   $\{^1\text{H}\}$  NMR: -3.24, -4.21, -10.93, -11.75 ppm; IR (KBr): 3036, 2942, 2589 (B-H), 1612, 1523, 1246  $\text{cm}^{-1}$ ; ESI-MS: calc. for  $\text{C}_{51}\text{H}_{66}\text{B}_{30}\text{O}_3$ : 1056.78; found: 1057.92  $[\text{M} + \text{H}]^+$ .

**Compound 7.**  $\text{BBr}_3$  (2.8 mL, 2.8 mmol, 1 M in  $\text{CH}_2\text{Cl}_2$ , 10.5 equiv.) was added dropwise to a solution of compound 6 (280 mg, 0.266 mmol, 1 equiv.) in 30 mL of  $\text{CH}_2\text{Cl}_2$  at 0 °C and stirred at room temperature for 20 hours. The progress of the reaction was monitored by TLC. After completion, the reaction mixture was quenched with distilled water (20 mL) at 0 °C. The excess  $\text{CH}_2\text{Cl}_2$  was evaporated using a rotary evaporator. The crude product was then extracted using ethyl acetate (3  $\times$  20 mL), and combined organic layers were dried over anhydrous sodium sulfate and filtered. Removal of the solvent afforded the crude product, which was purified carefully by washing with cold dichloromethane. Pure product: 240 mg. Yield: 89%. Colourless solid. MP: > 250 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$  ppm): 7.45 (d, 6H,  $J = 8$  Hz, Ar-H), 7.35 (d, 6H,  $J = 8$  Hz, Ar-H), 7.11 (s, 3H, Ar-H), 7.06 (d, 6H,  $J = 8$  Hz, Ar-H), 6.50 (d, 6H,  $J = 8$  Hz, Ar-H);  $^{13}\text{C}$   $\{^1\text{H}\}$  NMR (100 MHz,  $\text{CD}_3\text{OD}$ ,  $\delta$  ppm): 159.4 (Ar-C), 141.7 (Ar-C), 139.3 (Ar-C), 132.2 (Ar-C), 131.1 (Ar-C), 130.2 (Ar-C), 126.6 (Ar-C), 124.5 (Ar-C), 121.4 (Ar-C), 115.0 (Ar-C), 86.8 (cage-C), 85.4 (cage-C);  $^{11}\text{B}$   $\{^1\text{H}\}$  NMR: -3.24, -4.21, -10.92, -11.75 ppm; IR (KBr): 3415, 3024, 2936, 2587 (B-H), 1607, 1518, 1256  $\text{cm}^{-1}$ ; ESI-MS: calcd for  $\text{C}_{48}\text{H}_{60}\text{B}_{30}\text{O}_3$ : 1009.31; found: 1010.77  $[\text{M} + \text{H}]^+$ .

**Compound 11.** To a solution of compound 7 (140 mg, 0.138 mmol, 1 equiv.) and compound 8 (113 mg, 0.496 mmol, 3.6 equiv.) in 30 mL of dry acetone under an argon atmosphere,  $\text{K}_2\text{CO}_3$  (171 mg, 1.242 mmol, 9 equiv.) was added and refluxed at 70 °C for 17 hours. The progress of the reaction was monitored by TLC. After completion of the reaction, the reaction mixture was filtered through a short silica pad and concentrated. The crude product was purified by silica gel chromatography using 25% ethyl acetate in hexane. Pure product: 145 mg. Yield: 73%. Colourless solid. MP: 151 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 7.55 (s, 3H, Ar-H), 7.50 (d, 6H,  $J = 8$  Hz, Ar-H), 7.40–7.36 (m, 12H, Ar-H), 7.23 (d, 6H,  $J = 8$  Hz, Ar-H), 6.91 (d, 6H,  $J = 8$  Hz, Ar-H), 6.69 (d, 6H,  $J = 8$  Hz, Ar-H), 4.84 (s, 6H,  $\text{OCH}_2$ -H), 4.62 (s, 6H,  $\text{OCH}_2$ -H), 2.16 (s, 3H, alkyne-H);  $^{13}\text{C}$   $\{^1\text{H}\}$  NMR (100 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 160.1 (Ar-C), 157.6 (Ar-C), 142.0 (Ar-C), 140.9 (Ar-C), 132.2 (Ar-C), 131.3 (Ar-C), 130.5 (Ar-C), 129.2 (Ar-C), 129.0 (Ar-C), 127.0 (Ar-C), 125.5 (Ar-C), 123.2 (Ar-C), 115.1 (Ar-C), 114.5 (Ar-C), 85.9 (cage-C), 85.0 (cage-C), 78.4 (alkyne-C), 75.7 (alkyne-C), 69.8 ( $\text{OCH}_2$ -C), 55.8 ( $\text{OCH}_2$ -C);  $^{11}\text{B}$   $\{^1\text{H}\}$  NMR: -3.84, -10.74 ppm; IR (KBr): 3207, 3026, 2924, 2591 (B-H), 2401, 2125, 1612, 1514, 1225  $\text{cm}^{-1}$ ; ESI-MS: calcd for  $\text{C}_{78}\text{H}_{84}\text{B}_{30}\text{O}_6$ : 1441.82; found: 1442.02  $[\text{M}]^+$ .



**Compound 12.** To a solution of compound **7** (170 mg, 0.168 mmol, 1 equiv.) and compound **9** (169 mg, 0.607 mmol, 3.6 equiv.) in 30 mL of dry acetone under an argon atmosphere,  $K_2CO_3$  (209 mg, 1.51 mmol, 9 equiv.) was added and refluxed at 70 °C for 17 hours. The progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was filtered through a short silica pad and concentrated. The crude product was purified by silica gel chromatography using 25% ethyl acetate in hexane. Pure product: 175 mg. Yield: 64%. Colourless solid. MP: 121 °C.  $^1H$  NMR (400 MHz,  $CDCl_3$ ,  $\delta$  ppm): 7.55 (s, 3H, Ar-H), 7.50 (d, 6H,  $J$  = 8 Hz, Ar-H), 7.40–7.36 (m, 12H, Ar-H), 6.68 (d, 6H,  $J$  = 8 Hz, Ar-H), 6.56–6.53 (m, 6H, Ar-H), 6.50–6.47 (m, 3H, Ar-H), 4.86 (s, 6H,  $OCH_2$ -H), 4.58–4.56 (m, 12H,  $OCH_2$ -H), 2.42–2.40 (m, 6H, alkyne-H);  $^{13}C$  { $^1H$ } NMR (100 MHz,  $CDCl_3$ ,  $\delta$  ppm): 159.9 (Ar-C), 158.9 (Ar-C), 142.0 (Ar-C), 140.8 (Ar-C), 138.7 (Ar-C), 132.2 (Ar-C), 131.3 (Ar-C), 130.5 (Ar-C), 127.0 (Ar-C), 125.5 (Ar-C), 123.4 (Ar-C), 114.6 (Ar-C), 101.7 (Ar-C), 85.8 (cage-C), 85.0 (cage-C), 78.2 (alkyne-C), 75.9 (alkyne-C), 69.6 ( $OCH_2$ -C), 55.9 ( $OCH_2$ -C);  $^{11}B$  { $^1H$ } NMR: –2.94, –10.75 ppm; IR (KBr): 3203, 3026, 2924, 2593 (B–H), 2402, 2123, 1606, 1514, 1218  $cm^{-1}$ ; ESI-MS: calcd for  $C_{87}H_{90}B_{30}O_9$ : 1606.52; found: 1606.89  $[M]^+$ .

**Compound 13.** To a solution of compound **7** (100 mg, 0.099 mmol, 1 equiv.) and compound **10** (118 mg, 0.357 mmol, 3.6 equiv.) in 30 mL of dry acetone under an argon atmosphere,  $K_2CO_3$  (123 mg, 0.891 mmol, 9 equiv.) was added and refluxed at 70 °C for 17 hours. The progress of the reaction was monitored by TLC. After completion of reaction, the reaction mixture was filtered through a short silica pad and concentrated. The crude product was purified by silica gel chromatography using 30% ethyl acetate in hexane. Pure product: 120 mg. Yield: 68%. Colourless solid. MP: 115 °C.  $^1H$  NMR (400 MHz,  $CDCl_3$ ,  $\delta$  ppm): 7.56 (s, 3H, Ar-H), 7.52 (d, 6H,  $J$  = 8 Hz, Ar-H), 7.42–7.38 (m, 12H, Ar-H), 6.71 (d, 12H,  $J$  = 8 Hz, Ar-H), 4.87 (s, 6H,  $OCH_2$ -H), 4.67–4.65 (m, 18H,  $OCH_2$ -H), 2.42 (t,  $J$  = 4 MHz, 3H, alkyne-H), 2.33 (t,  $J$  = 4 Hz, 6H, alkyne-H);  $^{13}C$  { $^1H$ } NMR (100 MHz,  $CDCl_3$ ,  $\delta$  ppm): 159.9 (Ar-C), 151.8 (Ar-C), 142.0 (Ar-C), 140.8 (Ar-C), 137.0 (Ar-C), 132.29 (Ar-C), 132.26 (Ar-C), 131.3 (Ar-C), 130.6 (Ar-C), 127.0 (Ar-C), 125.5 (Ar-C), 123.5 (Ar-C), 114.6 (Ar-C), 107.7 (Ar-C), 107.0 (Ar-C), 85.8 (cage-C), 85.0 (cage-C), 79.1 (alkyne-C), 78.3 (alkyne-C), 76.1 (alkyne-C), 75.4 (alkyne-C), 69.8 ( $OCH_2$ -C), 60.4 ( $OCH_2$ -C), 57.1 ( $OCH_2$ -C);  $^{11}B$  { $^1H$ } NMR: –3.67, –10.78 ppm; IR (KBr): 3201, 3018, 2931, 2592 (B–H), 2402, 2126, 1610, 1516, 1217  $cm^{-1}$ ; ESI-MS: calcd for  $C_{87}H_{90}B_{30}O_9$ : 1770.96; found: 1770.80  $[M]^+$ .

**Compound 15.** To a solution of alkynyl core **11** (150 mg, 0.104 mmol, 1 equiv.) and glucosyl azide **14** (159 mg, 0.374 mmol, 3.6 equiv.) in 5 mL of dry THF, 3 mL of distilled water was added. To this suspension, a solution of sodium ascorbate (19 mg, 0.093 mmol, 0.9 equiv.) and  $CuSO_4 \cdot 5H_2O$  (24 mg, 0.093 mmol, 0.9 equiv.) in 2 mL of water was added and the reaction mixture was stirred at room temperature for 24 hours. After completion of reaction, the crude product was extracted using ethyl acetate (3  $\times$  20 mL), dried over sodium sulfate and concentrated. The crude product was purified by

neutral alumina column chromatography using 2% methanol in ethyl acetate. Pure product: 192 mg. Yield: 68%. Colourless solid. MP: 95 °C.  $^1H$  NMR (400 MHz,  $CDCl_3$ ,  $\delta$  ppm): 7.67 (s, 3H, triazole-H), 7.55 (s, 3H, Ar-H), 7.50 (d, 6H,  $J$  = 8 Hz, Ar-H), 7.40–7.36 (t, 12H,  $J$  = 8 Hz, Ar-H), 7.24 (d, 6H,  $J$  = 8 Hz, Ar-H), 6.95 (d, 6H,  $J$  = 8 Hz, Ar-H), 6.69 (d, 6H,  $J$  = 8 Hz, Ar-H), 5.19–5.13 (3H (Glu-H); 6H ( $OCH_2$ -H)), 5.07–4.95 (m, 6H,  $OCH_2$ -H), 4.83 (s, 3H,  $OCH_2$ -H), 4.62–4.57 (m, 3H,  $OCH_2$ -H), 4.52–4.43 (m, 6H,  $OCH_2$ -H), 4.24–4.20 (m, 6H,  $OCH_2$ -H), 4.13–4.06 (m, 6H, Glu-H), 3.93–3.87 (m, 3H, Glu-H), 3.69–3.65 (m, 3H, Glu-H), 2.06–1.92 (4s, 36H, 12 $CH_3$ );  $^{13}C$  { $^1H$ } NMR (100 MHz,  $CDCl_3$ ,  $\delta$  ppm): 170.6, 170.2, 169.5, 169.4 (4  $\times$   $OAc$ -C), 160.2 (Ar-C), 158.3 (Ar-C), 143.7 (Ar-C), 142.0 (Ar-C), 140.9 (Ar-C), 132.4 (Ar-C), 132.2 (Ar-C), 131.3 (Ar-C), 131.0 (Ar-C), 130.5 (Ar-C), 129.3 (Ar-C), 128.9 (Ar-C), 128.6 (Ar-C), 127.0 (Ar-C), 125.5 (Ar-C), 124.3 (Ar-C), 123.2 (Ar-C), 114.9 (Ar-C), 114.5 (Ar-C), 100.5 (Glu-C), 85.9 (cage-C), 85.0 (cage-C), 72.5 ( $OCH_2$ -C), 72.0 (Glu-C), 71.8 (Glu-C), 71.0 ( $OCH_2$ -C), 69.8 (Glu-C), 68.2 (Glu-C), 67.8 (Glu-C), 62.9 ( $CH_2$ -C), 61.8 (glucose-C), 50.1 ( $CH_2$ -C), 27.8, 20.8, 20.6, 19.2 (4  $\times$   $CH_3$ -C);  $^{11}B$  { $^1H$ } NMR: –2.92, –10.78 ppm; IR (KBr): 3029, 2924, 2589 (B–H), 1751 (ester-CO), 1660, 1593, 1512, 1228, 1047  $cm^{-1}$ ; ESI-MS: calcd for  $C_{126}H_{155}B_{30}N_9O_{36}Na$ : 2723.32; found: 2723.76  $[M + Na]^+$ .

**Compound 16.** To the acetylated derivative **15** (100 mg, 0.037 mmol, 1 equiv.) dissolved in 10 mL of dry methanol, a solution of sodium methoxide (2 mg, 0.009 mmol, 1 equiv.) in 2 mL of dry methanol was added and stirred at room temperature for 45 minutes. After completion of the reaction monitored by TLC, the reaction mixture was neutralized by the addition of Amberlite IR-120 ( $H^+$ -form) ion exchange resin (pre-washed with methanol), filtered, and concentrated under reduced pressure to afford the desired hydroxylated product **16**. Pure product: 72 mg. Yield: 88%. Colourless solid. MP: 111 °C.  $^1H$  NMR (400 MHz,  $DMSO-d_6$ ,  $\delta$  ppm): 8.22 (s, 3H, triazole-H), 7.74 (s, 3H, Ar-H), 7.70–7.67 (m, 6H, Ar-H), 7.57 (d, 6H,  $J$  = 8 Hz, Ar-H), 7.46 (d, 6H,  $J$  = 8 Hz, Ar-H), 7.23 (d, 6H,  $J$  = 8 Hz, Ar-H), 6.94 (d, 6H,  $J$  = 8 Hz, Ar-H), 6.81 (d, 6H,  $J$  = 8 Hz, Ar-H), 5.03 (s, 9H,  $OCH_2$ -H), 4.93 (dd, 6H,  $J$  = 8 Hz,  $J$  = 8 Hz,  $OCH_2$ -H), 4.84 (s, 6H,  $OCH_2$ -H), 4.55–4.50 (m, 9H,  $OCH_2$ -H, Glu-H), 4.19 (d,  $J$  = 8 Hz, 3H, Glu-H), 4.07–4.02 (m, 3H, Glu-H), 3.97 (d,  $J$  = 12 Hz, 6H, Glu-H), 3.89–3.84 (m, 3H, Glu-H), 3.66–3.62 (m, 3H, Glu-H), 3.11–2.90 (m, 12H, Glu-H);  $^{13}C$  { $^1H$ } NMR (100 MHz,  $DMSO-d_6$ ,  $\delta$  ppm): 160.5 (Ar-C), 158.4 (Ar-C), 142.8 (Ar-C), 141.9 (Ar-C), 140.2 (Ar-C), 132.8 (Ar-C), 132.2 (Ar-C), 132.1 (Ar-C), 131.7 (Ar-C), 130.2 (Ar-C), 129.9 (Ar-C), 129.2 (Ar-C), 128.9 (Ar-C), 125.9 (Ar-C), 115.2 (Ar-C), 115.0 (Ar-C), 103.4 (Ar-C), 87.1 (cage-C), 86.3 (cage-C), 77.5 ( $CH_2$ -C), 77.1 ( $CH_2$ -C), 73.8 ( $OCH_2$ -C), 71.6 ( $OCH_2$ -C), 70.4 ( $OCH_2$ -C), 69.7 (Glu-C), 68.3 (Glu-C), 67.8 (Glu-C), 61.6 (Glu-C), 61.5 (Glu-C), 50.2 ( $CH_2$ -C);  $^{11}B$  { $^1H$ } NMR: –5.52, –13.13 ppm; IR (KBr): 3405, 2929, 2857, 2592 (B–H), 1627, 1607, 1512, 1387, 1247, 1065  $cm^{-1}$ ; ESI-MS: calcd for  $C_{102}H_{129}B_{30}N_9O_2 + Na$ : 2217.19; found: 2218.09  $[M + H + Na]^+$ .

**Compound 17.** To a solution of alkynyl core **12** (200 mg, 0.128 mmol, 1 equiv.) and glucosyl azide **14** (385 mg, 0.923 mmol, 7.2 equiv.) in 5 mL of dry THF, 3 mL of distilled water was added. To this suspension, sodium ascorbate (45 mg,





0.23 mmol, 1.8 equiv.) and  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (57 mg, 0.23 mmol, 1.8 equiv.) in 2 mL of water were added and stirred at room temperature for 24 hours. After completion of the reaction, the crude product was extracted using ethyl acetate ( $3 \times 20$  mL), dried over sodium sulfate and concentrated. The crude product was purified by neutral alumina column chromatography using 5% methanol in ethyl acetate. Pure product: 320 mg. Yield: 60%. Colourless solid. MP: 95 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 7.71–7.66 (m, 6H, triazole-H), 7.54–7.48 (m, 12H, Ar-H), 7.41–7.35 (m, 12H, Ar-H), 6.69 (d, 6H,  $J = 8$  Hz, Ar-H), 6.57 (dd, 6H,  $J = 4$  Hz, Ar-H), 5.17–4.94 (m, 30H,  $\text{OCH}_2$ -H), 4.84 (s, 6H,  $\text{OCH}_2$ -H), 4.61–4.55 (m, 6H,  $\text{OCH}_2$ -H), 4.50–4.45 (m, 6H, Glu-H), 4.24–4.19 (m, 12H, Glu-H), 4.12–4.05 (m, 12H, Glu- $\text{CH}_2$ ), 3.93–3.87 (m, 6H, Glu-H), 7.70–3.65 (m, 6H, Glu-H), 2.04, 1.99, 1.96, 1.90 (4s, 72H);  $^{13}\text{C}$   $\{^1\text{H}\}$  NMR (100 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 170.6, 170.1, 169.5, 169.4 ( $4 \times \text{OAc-C}$ ), 159.9 (Ar-C), 159.6 (Ar-C), 143.5 (Ar-C), 142.0 (Ar-C), 140.8 (Ar-C), 138.8 (Ar-C), 132.4 (Ar-C), 131.0 (Ar-C), 128.9 (Ar-C), 127.1 (Ar-C), 124.4 (Ar-C), 123.3 (Ar-C), 114.6 (Ar-C), 106.5 (Ar-C), 101.5 (Ar-C), 100.5 (Glu-C), 85.9 (cage-C), 85.1 (cage-C), 72.5 ( $\text{OCH}_2$ -C), 72.0 (Glu-C), 71.8 (Glu-C), 71.0 ( $\text{OCH}_2$ -C), 69.7 (Glu-C), 68.2 (Glu-C), 67.7 (Glu-C), 61.9 ( $\text{CH}_2$ -C), 61.8, 50.1 ( $\text{CH}_2$ -C), 27.7, 20.8, 20.6, 19.24 ( $4 \times \text{CH}_3$ -C);  $^{11}\text{B}$   $\{^1\text{H}\}$  NMR:  $-4.53$ ,  $-10.77$  ppm; IR (KBr): 3026, 2924, 2584 (B-H), 1757 (ester-CO), 1665, 1597, 1509, 1223, 1037  $\text{cm}^{-1}$ ; ESI-MS: calcd for  $\text{C}_{183}\text{H}_{288}\text{B}_{30}\text{N}_{18}\text{O}_{69} + 2\text{H}_2\text{O}$ : 4144.17; found: 4144.12  $[\text{M} + 2\text{H}_2\text{O}]^+$ .

**Compound 18.** To the acetylated derivative **17** (200 mg, 0.048 mmol, 1 equiv.) dissolved in 5 mL of dry methanol, a solution of sodium methoxide (5 mg, 0.097 mmol, 2 equiv.) in 2 mL of dry methanol was added and the reaction mixture was stirred at room temperature for 45 minutes. After completion of the reaction, the reaction mixture was neutralized by the addition of Amberlite IR-120 ( $\text{H}^+$ -form) ion exchange resin (pre-washed with methanol), filtered, and concentrated under reduced pressure to afford the desired hydroxylated product **18**. Pure product: 160 mg. Yield: 60%. Colourless solid. MP: 105 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{DMSO-d}_6$ ,  $\delta$  ppm): 8.21 (s, 6H, triazole-H), 7.76 (s, 6H, Ar-H), 7.71–7.67 (m, 12H, Ar-H), 7.65–7.46 (m, 6H, Ar-H), 6.83 (d, 3H,  $J = 8$  Hz, Ar-H), 6.61 (d, 9H,  $J = 8$  Hz, Ar-H), 5.02 (s, 18H,  $\text{OCH}_2$ ), 4.89 (s, 3H,  $\text{OCH}_2$ ), 4.53 (t,  $J = 4$  Hz, 18H,  $\text{OCH}_2$ ), 4.21 (d, 12H, Glu-H), 4.07–4.03 (m, 9H, Glu-H), 3.97 (d,  $J = 12$  Hz, 12H, Glu-H), 3.89–3.84 (m, 12H, Glu-H), 3.11–2.97 (m, 24H, Glu-H);  $^{13}\text{C}$   $\{^1\text{H}\}$  NMR (100 MHz,  $\text{DMSO-d}_6$ ,  $\delta$  ppm): 160.3 (Ar-C), 159.7 (Ar-C), 144.7 (Ar-C), 142.7 (Ar-C), 141.9 (Ar-C), 139.2 (Ar-C), 132.2 (Ar-C), 132.1 (Ar-C), 129.9 (Ar-C), 129.2 (Ar-C), 125.9 (Ar-C), 122.7 (Ar-C), 115.3 (Ar-C), 107.1 (Ar-C), 103.5 (Ar-C), 103.4 (Ar-C), 86.3 (cage-C), 77.4 ( $\text{CH}_2$ -C), 77.0 ( $\text{CH}_2$ -C), 73.8 ( $\text{OCH}_2$ -C), 71.6 ( $\text{OCH}_2$ -C), 70.6 ( $\text{OCH}_2$ -C), 67.9 (Glu-C), 67.8 (Glu-C), 61.6 (Glu-C), 61.5 (Glu-C), 50.2 ( $\text{CH}_2$ -C);  $^{11}\text{B}$   $\{^1\text{H}\}$  NMR:  $-5.63$ ,  $-13.24$  ppm; IR (KBr): 3417, 2937, 2842, 2593 (B-H), 1621, 1602, 1509 1379, 1244, 1045  $\text{cm}^{-1}$ ; ESI-MS: calcd for  $\text{C}_{135}\text{H}_{180}\text{B}_{30}\text{N}_{18}\text{O}_{45}$ : 3103.51; found: 3157.54  $[\text{M} + 3\text{H}_2\text{O}]^+$ .

**Compound 19.** To a solution of alkynyl core **13** (200 mg, 0.113 mmol, 1 equiv.) and glucosyl azide **14** (548 mg, 1.315 mmol, 11.6 equiv.) in 5 mL of dry THF, 3 mL of distilled water was added. To this suspension, sodium ascorbate (60 mg, 0.306 mmol, 2.7 equiv.) and  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  (76 mg, 0.306 mmol,

2.7 equiv.) in 2 mL of water were added and stirred at room temperature for 24 hours. After completion of the reaction monitored by TLC, the crude product was extracted using ethyl acetate ( $3 \times 20$  mL), dried over sodium sulfate and concentrated. The crude product was purified by neutral alumina column chromatography using 6% methanol in ethyl acetate. Pure product: 345 mg. Yield: 55%. Colourless solid. MP: 94 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 7.87–7.70 (m, 9H, triazole-H), 7.57 (s, 3H, Ar-H), 7.53–7.50 (m, 6H, Ar-H), 7.43, 7.41, 7.39 (t,  $J = 8$  Hz, 12H, Ar-H), 6.74 (t,  $J = 8$  Hz, 12H, Ar-H), 5.17–5.01 (m, 36H,  $\text{OCH}_2$ -H), 4.97–4.92 (m, 9H,  $\text{OCH}_2$ -H), 4.85 (s, 6H,  $\text{OCH}_2$ -H), 4.61–4.51 (m, 24H,  $\text{OCH}_2$ -H, Glu-H), 4.26–4.21 (m, 18H, Glu-H), 4.12–4.06 (m, 12H, Glu-H), 4.01–3.91 (m, 9H, Gluc-H), 3.72–3.68 (m, 9H, Gluc-H), 2.05, 1.99, 1.95, 1.88 (4s, 108H);  $^{13}\text{C}$   $\{^1\text{H}\}$  NMR (100 MHz,  $\text{CDCl}_3$ ,  $\delta$  ppm): 170.6, 170.1, 169.5, 167.7 ( $4 \times \text{OAc-C}$ ), 159.9 (Ar-C), 152.4 (Ar-C), 144.3 (Ar-C), 143.6 (Ar-C), 142.1 (Ar-C), 140.8 (Ar-C), 137.5 (Ar-C), 132.4 (Ar-C), 132.2 (Ar-C), 131.3 (Ar-C), 131.0 (Ar-C), 130.4 (Ar-C), 128.9 (Ar-C), 127.1 (Ar-C), 125.0 (Ar-C), 124.6 (Ar-C), 123.3 (Ar-C), 114.6 (Ar-C), 107.2 (Ar-C), 100.5 (Ar-C), 100.6 (Glu-C), 85.9 (cage-C), 85.2 (cage-C), 72.5 ( $\text{OCH}_2$ -C), 71.8 (Glu-C), 70.9 ( $\text{OCH}_2$ -C), 68.3 (Glu-C), 67.7 (Glu-C), 63.0 ( $\text{CH}_2$ -C), 61.8 (Glu-C), 60.4 (Glu-C), 50.0 ( $\text{CH}_2$ -C), 27.7, 20.8, 20.6, 19.2 ( $4 \times \text{CH}_3$ -C);  $^{11}\text{B}$   $\{^1\text{H}\}$  NMR:  $-5.34$ ,  $-10.75$  ppm; IR (KBr): 3021, 2928, 2586 (B-H), 1754 (ester-CO), 1659, 1591, 1512, 1207, 1021,  $\text{cm}^{-1}$ ; ESI-MS: calcd for  $\text{C}_{240}\text{H}_{303}\text{B}_{30}\text{N}_{27}\text{O}_{102}$ : 5521.42; found: 5520.58  $[\text{M-H}]^+$ .

**Compound 20.** To the acetylated derivative **19** (200 mg, 0.036 mmol, 1 equiv.) dissolved in 5 mL of dry methanol, a solution of sodium methoxide (6 mg, 0.018 mmol, 3 equiv.) in dry methanol was added and stirred at room temperature for 45 minutes. After completion of the reaction, the reaction mixture was neutralized by the addition of Amberlite IR-120 ( $\text{H}^+$ -form) ion exchange resin (pre-washed with methanol), filtered, and concentrated under reduced pressure to afford the desired hydroxylated product **20** as white foam. Pure product: 106 mg. Yield: 73%. Colourless solid. MP: 105 °C.  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ ,  $\delta$  ppm): 8.21–8.01 (m, 30H, triazole-H, Ar-H), 6.77 (br s, 12H, Ar-H), 4.78–4.63 (m, 54H,  $\text{OCH}_2$ -H), 4.41 (s, 12H, Glu-H), 4.20 (s, 12H, Glu-H), 4.07 (s, 15H, Glu-H), 3.88 (s, 18H, Glu-H), 3.68 (d, 12H,  $J = 12$  Hz, Glu-H), 3.58 (d, 24H,  $J = 8$  Hz, Glu-H), 3.46 (s, 12H, Glu-H);  $^{13}\text{C}$   $\{^1\text{H}\}$  NMR (100 MHz,  $\text{D}_2\text{O}$ ,  $\delta$  ppm): 160.2 (Ar-C), 151.7 (Ar-C), 143.3 (Ar-C), 142.7 (Ar-C), 138.0 (Ar-C), 135.8 (Ar-C), 132.7 (Ar-C), 132.3 (Ar-C), 128.0 (Ar-C), 125.3 (Ar-C), 125.1 (Ar-C), 120.5 (Ar-C), 110.1 (Ar-C), 107.4 (Ar-C), 102.4 (Ar-C), 87.4 (cage-C), 82.8 (cage-C), 75.9 ( $\text{CH}_2$ -C), 75.6 ( $\text{CH}_2$ -C), 73.0 ( $\text{OCH}_2$ -C), 72.9 ( $\text{OCH}_2$ -C), 69.5 ( $\text{OCH}_2$ -C), 68.4 (Glu-C), 67.8 (Glu-C), 62.6 (Glu-C), 60.7 (Glu-C), 50.2 ( $\text{CH}_2$ -C);  $^{11}\text{B}$   $\{^1\text{H}\}$  NMR:  $-6.23$ ,  $-14.52$  ppm; IR (KBr): 3425, 2954, 2834, 2589 (B-H), 1633, 1606, 1519, 1387, 1237, 1054  $\text{cm}^{-1}$ ; ESI-MS: calcd for  $\text{C}_{168}\text{H}_{231}\text{B}_{30}\text{N}_{27}\text{O}_{66} + 2\text{H}_2\text{O}$ : 4042.86; found: 4042.80  $[\text{M} + 2\text{H}_2\text{O}]^+$ .

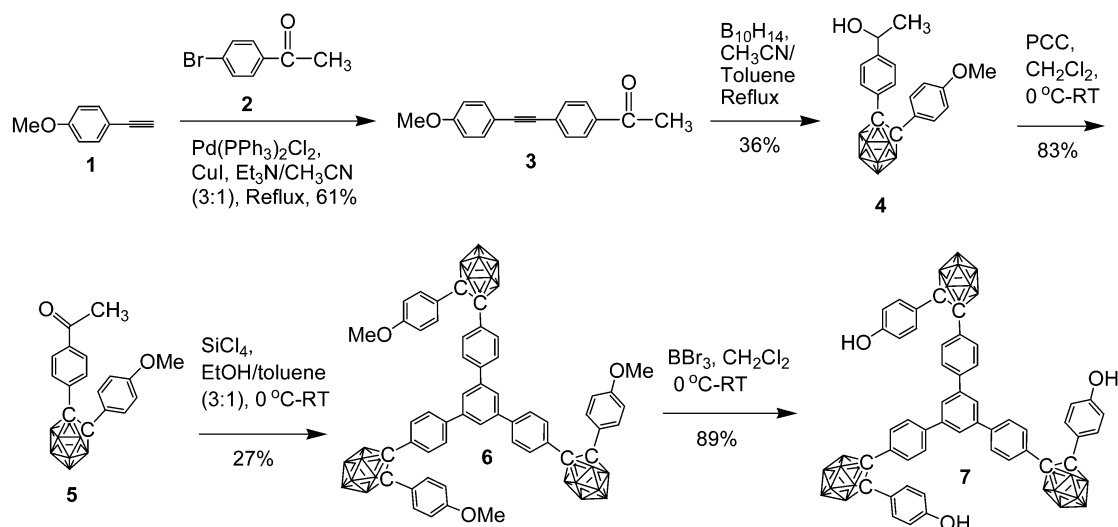
## Results and discussion

The dendritic carboranyl glycosides (**16**, **18** and **20**) were synthesized from compound **1** following a sequence of reaction

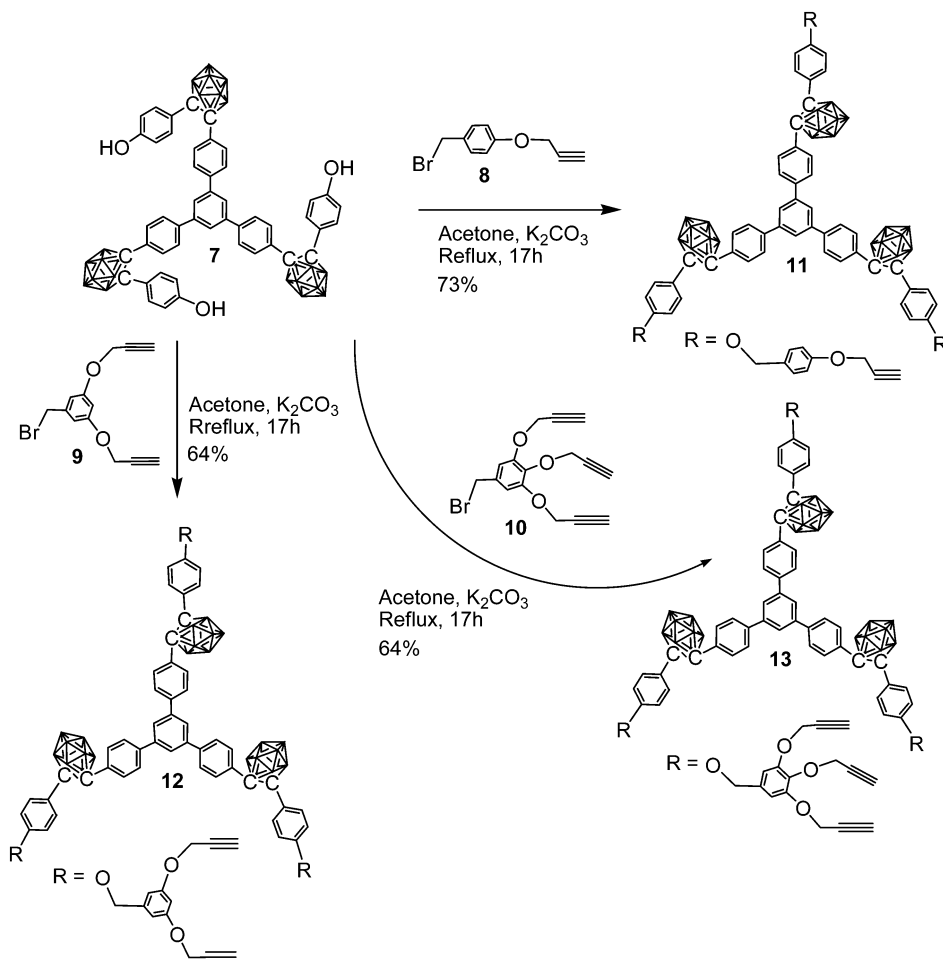


conditions. The synthetic method includes Sonogashira cross-coupling reaction, decaborane insertion and oxidation with PCC followed by a cyclotrimerization reaction mediated by

silicon tetrachloride to produce a symmetrical phenylene-carboranyl compound containing three *o*-carborane clusters. Finally, BBr<sub>3</sub> promoted demethylation reaction yielded the



Scheme 1 Synthesis of dendritic core **7** containing three *o*-carborane moieties.



Scheme 2 Synthesis of alkynyl core compounds **11**, **12** and **13**.



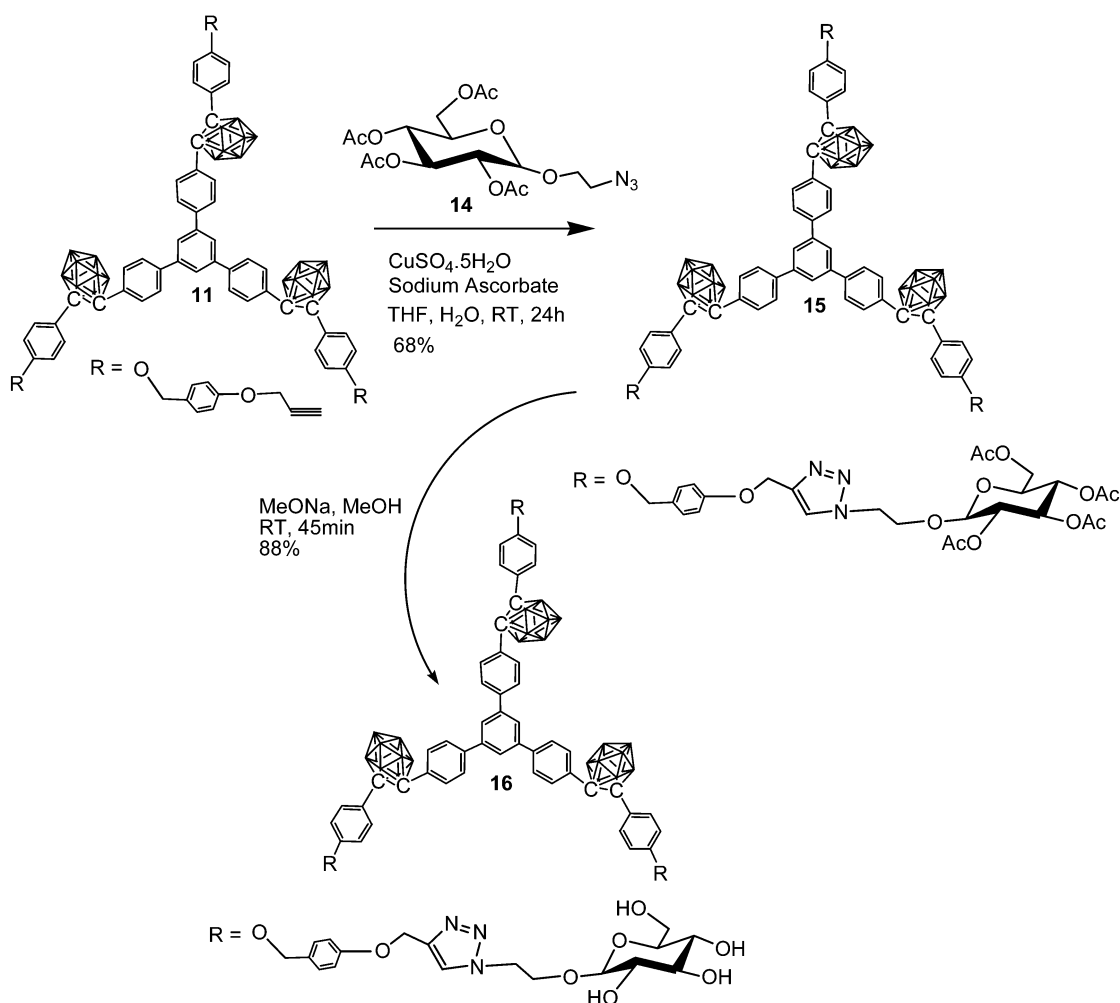
symmetrical hydroxylated compound **7** (Scheme 1). The core compounds **11**, **12** and **13** were prepared in good yield from nucleophilic substitution reaction of compound **7** with different alkyne dendrons in the presence of  $K_2CO_3$  in dry acetone. These core compounds further underwent a Cu(I) catalyzed azide-alkyne [3+2] cycloaddition reaction with glucosyl azide followed by Zemplén deacetylation reaction to furnish the desired boron rich dendritic glycoconjugates **16**, **18** and **20** in good to moderate yields.<sup>14,16–19</sup>

### Synthesis of the hydroxylated core **7**

Synthesis of the compound **7** was initiated from the Sonogashira cross-coupling reaction as illustrated in Scheme 1. Compound **1** was prepared as per the reported procedures.<sup>14</sup> The Sonogashira cross-coupling reaction of compound **1** with compound **2** (4-bromoacetophenone) produced the unsymmetrical alkyne **3** in 61% yield.<sup>16</sup> The alkynyl compound **3** was refluxed with decaborane to form compound **4**.<sup>17</sup> The  $^1H$  and  $^{13}C$  NMR data indicated the reduction of the sensitive carbonyl group of compound **3** to secondary alcohol. Thus, a pyridinium chlorochromate (PCC) mediated oxidation reaction of compound **4**

was necessary to obtain the required carbonyl compound **5**. Subsequently silicon tetrachloride mediated cyclotrimerization reaction of compound **5** provided the symmetrical phenylene core methoxy compound **6** containing three *o*-carborane clusters.<sup>18,19</sup> Disappearance of the carbonyl peak in the  $^{13}C$  NMR spectrum along with other NMR and mass spectral evidences confirmed the formation of compound **6**. The boron tribromide promoted demethylation of compound **6** led to the formation of the hydroxylated core **7** containing three hydroxyl groups and three *o*-carborane clusters in good yield.<sup>17</sup> The disappearance of the peak for  $-OCH_3-H$  at  $\delta$  3.69 ppm in  $^1H$  NMR and  $OCH_3-C$  at  $\delta$  55.3 ppm in  $^{13}C$  NMR along with mass spectral evidence confirmed the formation of compound **7** (see ESI†).

The alkynyl dendrons **8**, **9** and **10** used for the preparation of the alkynyl core compounds were synthesized from commercially available methyl-4-hydroxy benzoate, methyl-3,5-dihydroxy benzoate and methyl-3,4,5-trihydroxy benzoate respectively in accordance with the reported procedure (Scheme 2).<sup>20</sup> The dendritic alkynyl core compounds **11**, **12** and **13** were prepared from the hydroxylated compound **7** in quantitative yield as illustrated in Scheme 2.



Scheme 3 Synthesis of carboranyl dendrimer **16** containing three glucose moieties.



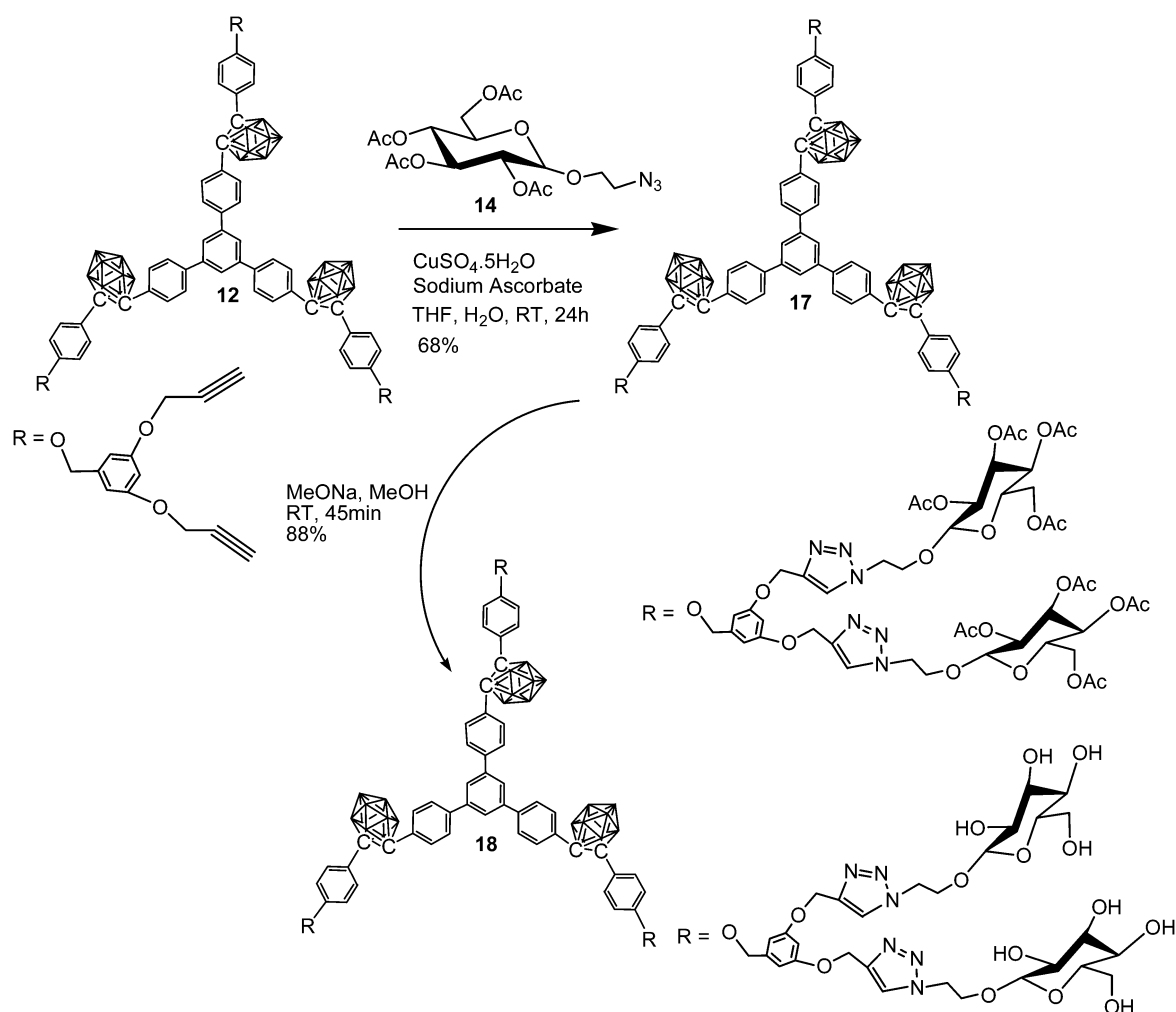
The hydroxylated compound **7** with compound **8** under reflux conditions in the presence of  $K_2CO_3$  in dry acetone produced alkynyl core **11** in 73% yield. Through a similar approach, the core compounds **12** and **13** were synthesized in good yields upon treatment of compound **7** with compounds **9** and **10**, respectively.<sup>8</sup> FT-IR analysis showed the presence of an alkyne C–C triple bond stretching band at  $2125\text{ cm}^{-1}$  and a terminal alkyne C–H stretching band at  $3200\text{ cm}^{-1}$  in all the three synthesized core compounds. The existence of the characteristic peaks for alkyne C–H protons in the range  $\delta\ 2.16\text{--}2.42\text{ ppm}$  in  $^1\text{H}$  NMR along with the mass spectral analysis confirmed the synthesis of the alkynyl core compounds (see ESI†).

### Synthesis of the glycodendrimers **16**, **18** and **20**

The glucosyl azide **14** was prepared from commercially available D-glucose in accordance with the literature procedure.<sup>21,22</sup> Preparation of the desired glycodendrimers was accomplished in two steps. Treatment of alkynyl cores with glucosyl azide using Huisgen-type Cu(I) catalyzed azide–alkyne “click” cycloaddition reaction yielded acetylated derivatives, which upon subsequent sodium methoxide promoted *trans*-acetylation

reactions in dry methanol yielded the desired final compounds.<sup>7,21,22</sup> At each step of the reactions, the formation of the anticipated products was confirmed by  $^1\text{H}$ ,  $^{13}\text{C}$  and  $^{11}\text{B}$  NMR data. The disappearance of alkyne C–H protons at around  $\delta\ 2.5\text{ ppm}$ , the appearance of four singlets for the acetyl protons around  $\delta\ 2\text{ ppm}$  in  $^1\text{H}$  NMR and peaks for cage-C and carbonyl-C in the appropriate regions in  $^{13}\text{C}$  NMR corroborate the formation of click reaction products. The complete disappearance of the peak for ester-H in  $^1\text{H}$  NMR and carbonyl-C in  $^{13}\text{C}$  NMR spectra respectively along with  $^{11}\text{B}$  NMR and FT-IR data confirmed the formation of the hydroxylated carborane–glucose conjugates. Formation of the desired compounds was further confirmed from their mass spectral analysis.

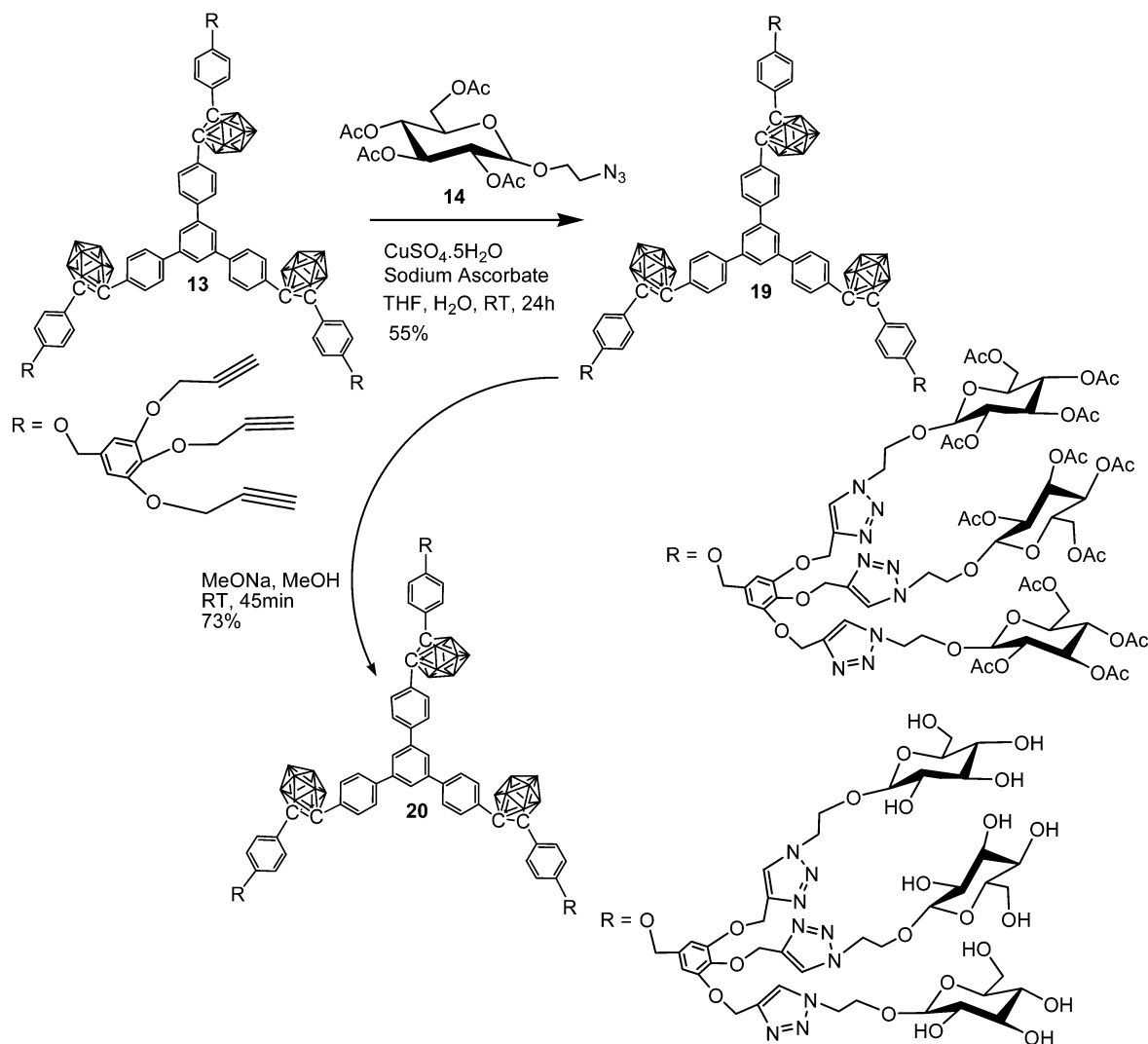
The reaction of the core **11** with the glucosyl azide **14** led to the formation of the acetylated derivative **15** in 68% yield, which upon deacetylation produced dendrimer **16** having three peripheral glucose moieties in 88% yield (Scheme 3). For the click cycloaddition product **15**  $^1\text{H}$  NMR shows the presence of a singlet for the triazole-H at  $\delta\ 7.67\text{ ppm}$  and four singlets for ester-H in the range  $\delta\ 2.06\text{--}1.92\text{ ppm}$ .  $^{13}\text{C}$  NMR shows four peaks for the ester carbons in the range  $\delta\ 170.6\text{--}169.4\text{ ppm}$  and



Scheme 4 Synthesis of carboranyl dendrimer **18** containing six glucose moieties.







Scheme 5 Synthesis of carboranyl dendrimer **20** containing nine glucose moieties.

two peaks for the cage-C at  $\delta$  85.9 ppm and 85.0 ppm respectively. FT-IR analysis shows a band at  $2589\text{ cm}^{-1}$  corresponding to B-H stretching of *o*-carborane clusters. The disappearance of the peaks for ester-H in  $^1\text{H}$  NMR and peaks for ester-C in  $^{13}\text{C}$  NMR along with mass spectral data confirms the formation of the fully hydroxylated product **16** containing three glucose moieties at the periphery.

The acetylated dendritic compound **17** was prepared in 60% yield upon reaction of core **12** with glucosyl azide **14**. A subsequent deacetylation reaction in the presence of sodium methoxide in dry methanol produced hydroxylated glycodendrimer **18** containing six peripheral glucose moieties in 80% yield (Scheme 4).  $^1\text{H}$  NMR for the click cycloaddition product **17** shows four singlets for ester-H in the range  $\delta$  2.04–1.90 ppm.  $^{13}\text{C}$  NMR shows four peaks for the ester carbons in the range  $\delta$  170.6–169.4 ppm and two peaks for the cage-C at  $\delta$  85.9 ppm and 85.1 ppm. FT-IR shows the presence of a B-H stretching band of *o*-carborane clusters at  $2584\text{ cm}^{-1}$  and an ester-CO stretching band at  $1757\text{ cm}^{-1}$ . The disappearance of the peaks

for ester-H in  $^1\text{H}$  NMR, peaks for ester-C in  $^{13}\text{C}$  NMR and mass spectral analysis confirmed the formation of hydroxylated dendrimer **18**.

Through a similar approach using click chemistry, the reaction of core **13** and glucosyl azide **14** produced acetylated glycodendrimer **19** in 55% yield. The *trans*-acetylation was carried out in the presence of sodium methoxide in dry methanol to obtain the desired dendrimer **20** in 73% yield containing nine peripheral glucose moieties (Scheme 5). The formation of acetylated dendrimer **19** was evident from the presence of four singlets for ester-H in the range  $\delta$  2.05 ppm–1.88 ppm.  $^{13}\text{C}$  NMR shows four peaks for the ester-C in the range  $\delta$  170.1 ppm–167.7 ppm and two peaks for the cage-C at  $\delta$  85.9 ppm and 85.2 ppm. FT-IR shows the presence of a B-H stretching band of *o*-carborane clusters at  $2586\text{ cm}^{-1}$  and an ester CO stretching band at  $1754\text{ cm}^{-1}$ . The formation of the hydroxylated water-soluble glycodendrimer **20** was evident from the absence of peaks for ester-H in  $^1\text{H}$  NMR and peaks for ester-Cs in  $^{13}\text{C}$  NMR in appropriate regions (see ESI†).



**Table 1** Water solubility and IC<sub>50</sub> values of the carboranyl glycoconjugates against MCF-7

Compound	Water solubility (mg mL <sup>-1</sup> )	Regression equation	IC <sub>50</sub> (ng mL <sup>-1</sup> )	IC <sub>50</sub> (μM)
7	—	$y = 97.3199 + -0.05955x$	823	0.8
16	—	$y = 92.9639 + -0.2233x$	216	0.1
18	20 ± 10	$y = 96.1886 + -0.4750x$	104	0.03
20	150 ± 10	$y = 97.9492 + -1.2318x$	41	0.01

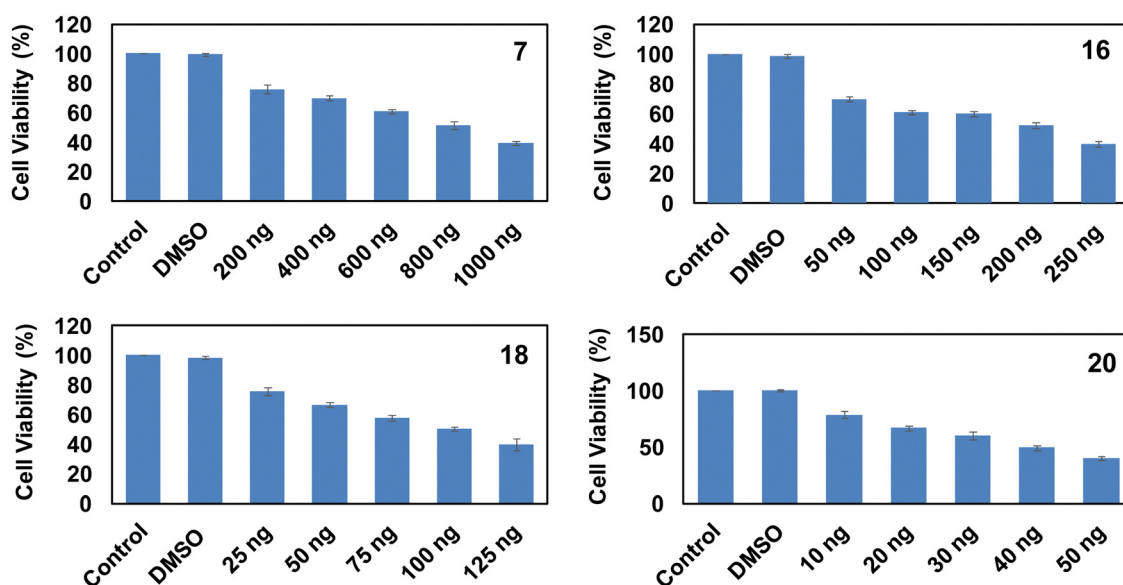
### Water solubility

The *o*-carboranyl core **7** with three hydroxyl groups and the glycodendrimer **16** containing three peripheral glucose moieties and twelve hydroxyl groups were completely insoluble in water. But the glycodendrimer **18** containing six glucose moieties and twenty-four hydroxyl groups at the periphery was sparingly water soluble up to 20 mg mL<sup>-1</sup>. With an increase in the number of peripheral glucose moieties to nine, the glycodendrimer **20** with thirty-six hydroxyl groups was found to be completely water soluble. The water solubility of **20** was enhanced to 150 mg mL<sup>-1</sup> (Table 1). There are only a few reports on the preparation of water soluble carboranyl glycoconjugates.<sup>11,14</sup> *m*- and *p*-carborane containing bis(glycophosphonates) with one and two galactosyl moieties showed very high water solubility, even higher than that of galactose.<sup>23</sup>

### In vitro cytotoxicity, live dead and apoptosis assay

*In vitro* cytotoxicity and caspase-3 activity studies of the synthesized glycodendrimers **16**, **18** and **20** were carried out against the MCF-7 breast cancer cell line to explore their ability to treat cancer as chemotherapeutic agents and promising boron neutron capture therapy (BNCT) drugs as well. MTT assay employs a colorimetric method for determining the percentage of viable cells based on mitochondrial dehydrogenase activity measurement at 595 nm. It is considered as an economic,

convenient and rapid move toward the detection of viable and dead cells. To test the cytotoxic effect of the glucose containing dendritic carboranyl glycoconjugates, a cell viability study was carried out with the conventional MTT reduction assay. The IC<sub>50</sub> value is a quantitative measure of the substance required to inhibit, *in vitro*, a given biological process or a biological component by 50%. Here, we measured the IC<sub>50</sub> values of the newly synthesized compounds **7**, **16**, **18** and **20** by evaluating the concentration of the compounds required to inhibit the survival of MCF-7 cancer cells by 50%. All the four compounds under study showed remarkable dose-dependent cytotoxicity toward the MCF-7 breast cancer cell line as shown in Fig. 1. The results of the *in vitro* cytotoxicity studies are shown in Table 1. Fig. 1 shows the % cell survivability of the cell line MCF-7 after treatment with the dendritic carboranyl compounds **7**, **16**, **18** and **20** containing zero, three, six and nine peripheral glucose moieties, respectively. The IC<sub>50</sub> value for the dendritic compound **20** containing nine glucose units at the periphery was found to be 41.35 ng mL<sup>-1</sup> (0.01 μM), whereas, it was found to be 822.53 ng mL<sup>-1</sup> (0.8 μM) for compound **7** without a glucose moiety at the periphery. The IC<sub>50</sub> value for the dendritic compound **16** containing three peripheral glucose units was found to be 215.65 ng mL<sup>-1</sup> (0.1 μM) and 104.47 ng mL<sup>-1</sup> (0.03 μM) in the case of dendritic compound **18** containing six glucose units at the periphery. These results indicate that the water soluble glycodendrimer **20** is most cytotoxic



**Fig. 1** *In vitro* cytotoxic effect of the carboranyl glycoconjugates at different concentrations (ng mL<sup>-1</sup>) on MCF-7 cells (breast cancer cell line) determined using MTT assay (data are expressed as mean ± SD, *n* = 10 with respect to control).



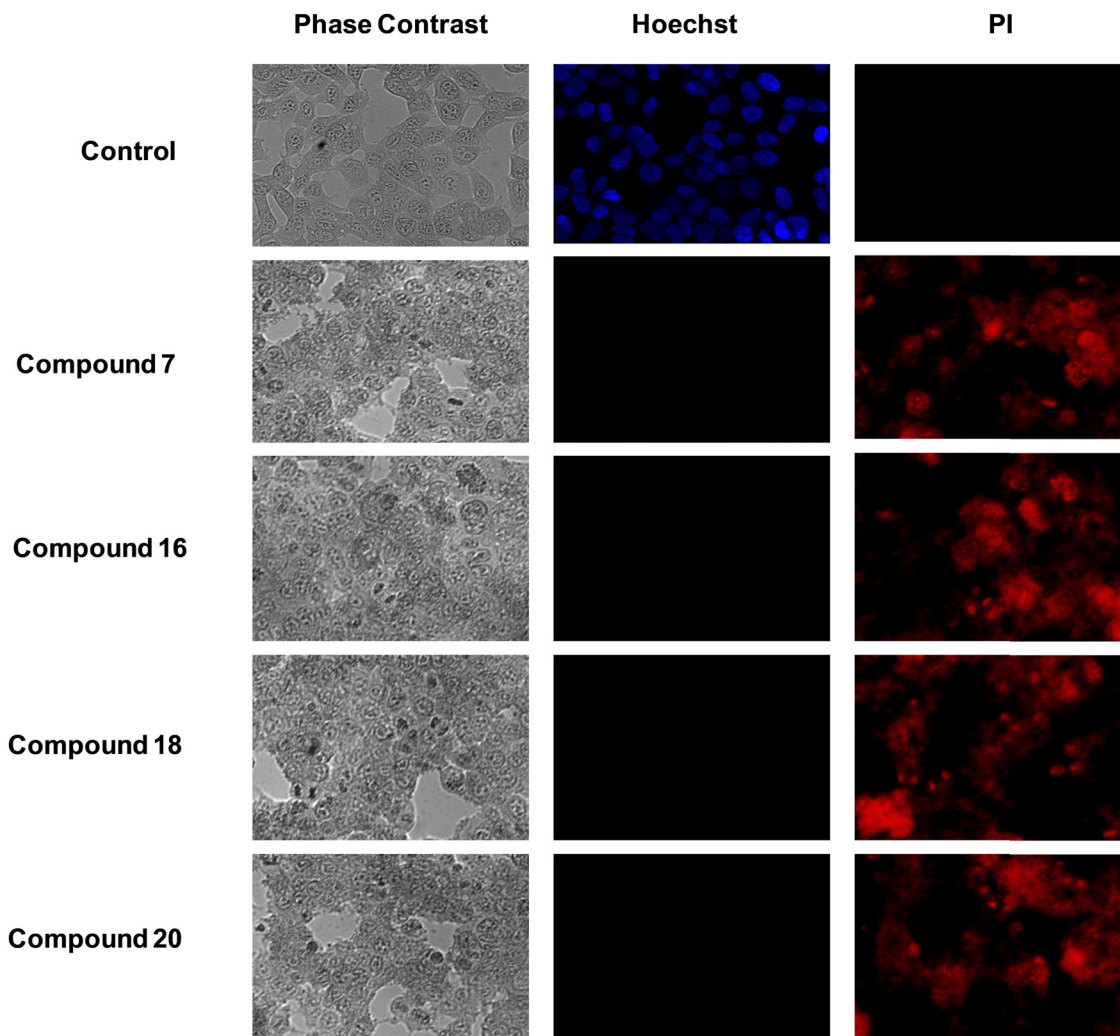


Fig. 2 Representative bright-field (phase contrast) and live/dead fluorescent images of MCF-7 cells treated with compound **7**, **16**, **18** and **20**. The cells were stained with Hoechst-PI to demonstrate the cellular viability after 48 h.

while the core compound **7** is least cytotoxic towards MCF-7. These results were compared with those of the commonly used cisplatin drug which has an  $IC_{50}$  value of  $6 \mu M$  towards the MCF-7 breast cancer cell line.<sup>24</sup> From the comparison of the  $IC_{50}$  value (Table 1), it was estimated that the water-soluble glycodendrimer **20** was 600-fold more cytotoxic than the chemotherapeutic drug cisplatin towards the MCF-7 breast cancer cell line. However, the core compound **7** was estimated to be more than 7-fold cytotoxic than cisplatin. Water insoluble glycodendrimer **16** and partially water soluble glycodendrimer **18** were found to be 60 and 200-fold more cytotoxic towards MCF-7 cancer cell lines than cisplatin, respectively. The cytotoxicity of the water-soluble carboranyl glycodendrimer **20** was comparable with that of the clinically used cancer drug paclitaxel. Its  $IC_{50}$  value towards the MCF-7 cancer cell line has been found to be between  $8-54 \text{ ng mL}^{-1}$  ( $0.009-0.06 \mu M$ ).<sup>25</sup> The cytotoxicity of the partially water-soluble carboranyl glycodendrimer **18** was comparable with that of the anticancer agent raloxifene ( $IC_{50}$   $0.025 \mu M$  towards MCF-7).<sup>26</sup> These preliminary

observations indicate that the dendritic molecules under study could be used as better anticancer therapeutics than cisplatin. The trend of cytotoxicity indicates that cytotoxicity increases with an increase in the number of peripheral glucose moieties and increasing water solubility. This result might be attributed to peripheral glucose moieties, which enhance the uptake of the carboranyl glycodendrimers into the cancer cells for the cell killing action.

Hoechst-propidium iodide (PI) staining assay is generally performed to detect apoptotic and necrotic cells by fluorescence microscopy.<sup>27</sup> Typically, the nuclei of healthy cells are spherical and the DNA is evenly distributed. However, the DNA becomes condensed during apoptosis but remains unchanged during necrosis. Thus, nuclear condensation can be used to distinguish between apoptotic and necrotic cells. Hoechst 33342 is a remarkable dye that binds to DNA, and is employed to observe nuclear condensation. For a normal cell, the nucleus is stained bright blue. The dead cells are characterized by red spots in propidium iodide staining. Usually, PI cannot pass



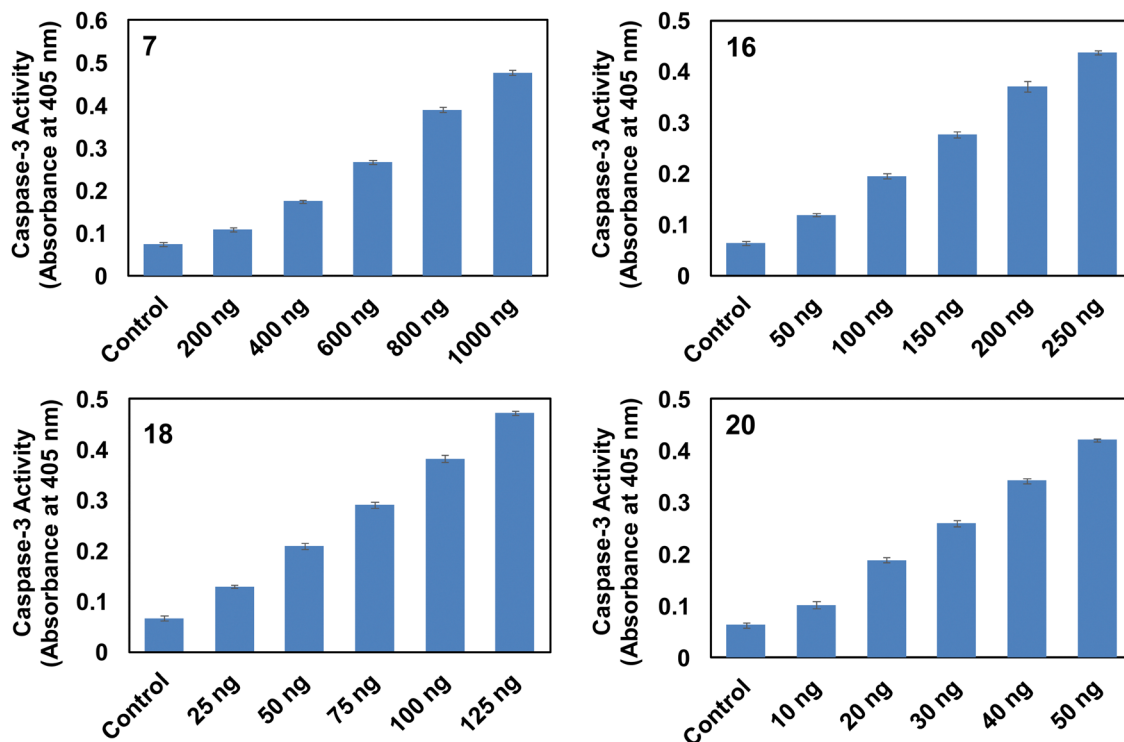


Fig. 3 Effect of different concentrations (in ng mL<sup>-1</sup>) of synthesized dendritic compounds **7**, **16**, **18** and **20** on caspase-3 activity in MCF-7 cancer cells.

through intact cell membranes but penetrates dead or damaged cells to bind with DNA and RNA by intercalating between the bases. In the present study, the cells stained with PI showed no fluorescence in the control indicating no dead cells in the range. On the other hand, compounds **7**, **16**, **18** and **20** displayed red fluorescence entirely in their vicinity, thereby validating cell death at their IC<sub>50</sub> value (Fig. 2).

After evaluating the IC<sub>50</sub> values of glycodendrimers against MCF-7 cancer cell lines, the mode of cell death was studied through caspase-3 activity assay (Fig. 3). Apoptosis or programmed cell death involves controlled dismantling of intracellular components while avoiding inflammation and damage to surrounding cells. This mode of cell death is coordinated by members of the caspase family of cysteine proteases and 14 different caspases are reported in mammalian cells. Out of different caspases, caspase-3 is essential in the execution phase of cell apoptosis.<sup>28</sup> It was found from caspase-3 activity assays (Fig. 3) that the non-glycoconjugated compound **7** and all the three dendritic glycoconjugated *o*-carboranyl dendrimers **16**, **18** and **20** induced caspase-3 activity in a dose-dependent manner, suggesting the induction of cell death *via* apoptosis. It was found that the water soluble carboranyl glycoside **20** containing nine peripheral glucose moieties showed the maximum induction in the caspase-3 activity at a lower concentration. In hormone responsive breast cancers, the steroid hormone estrogen plays a crucial role in its progression. It elicits its action by binding to the ligand binding domain of the estrogen receptor (ER, a protein), leading to its dimerization which in turn binds to DNA promoter elements, initiating gene transcription.<sup>29</sup> Through genome-wide

approaches, Bailey *et al.* reported that ER prevents p53-dependent apoptosis, promoting cell proliferation and can be treated with ER antagonists such as fulvestrant.<sup>29</sup> ER agonists or antagonists are compounds that can bind with it to either promote or inhibit estrogenic activity, respectively. Based on the inhibition of ER $\alpha$ , Endo *et al.* have shown that a carborane derivative has higher anti-estrogen response that is comparable to tamoxifen.<sup>30</sup> Therefore, it may be stated that *o*-carborane induces p53-dependent apoptosis by inhibiting ER as evidenced by induction of caspase-3 activity. Furthermore, the augmentation in the apoptosis process in glycoconjugated carboranes may be attributed to their enhanced bio-availability and enhanced cellular uptake.

## Conclusions

In summary, we have synthesized boron-rich glycodendrimers containing three *o*-carborane moieties. The glycodendrimer **16** contains three, **18** contains six and **20** possesses nine peripheral glucose moieties. Compound **20** contains thirty-six hydroxyl groups at the periphery and was found to be completely water soluble while partial water solubility was observed for dendrimers **18** with twenty-four hydroxy groups. The glycodendrimer **16** with twelve hydroxyl groups was not water soluble. The *in vitro* cytotoxicity test of dendritic compounds **7**, **16**, **18** and **20** against the MCF-7 breast cancer cell line revealed that all these compounds show stronger anticancer activity in comparison to the commonly used chemotherapeutic drug cisplatin. The water-soluble dendrimer **20** was found to





be 600-fold more cytotoxic than the chemotherapeutic drug cisplatin whereas **16** and **18** were more than 60 and 200-fold cytotoxic compared to cisplatin. The least cytotoxicity was observed for the core **7** without any glucose moieties. The high cytotoxicity of glycodendrimers **18** and **20** can be compared with that of clinically used anticancer agents paclitaxel and raloxifene. Caspase-3 activity assay suggested that the mode of cell death is *via* apoptosis. Preliminary experimental and comparison results suggest that the high boron content dendrimers containing three polyhedral *o*-carborane cages in a single molecule could act as effective anticancer agents for cancer therapy. The high boron content symmetrical dendrimer **20** might be able to incorporate a therapeutic dosage of boron atoms into malignant tissues at a lower concentration due to the enhanced permeability and retention (EPR) effect.

## Conflicts of interest

The authors declare no conflict of interest.

## Acknowledgements

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## Notes and references

- 1 A.-M. Caminade and C.-O. Turrin, *J. Mater. Chem. B*, 2014, **2**, 4055–4066.
- 2 S. H. Medina and M. E. El-Sayed, *Chem. Rev.*, 2009, **109**, 3141–3157.
- 3 M. R. Carvalho, R. L. Reis and J. M. Oliveira, *J. Mater. Chem. B*, 2020, **8**, 1128–1138.
- 4 H. Maeda, J. Wu, T. Sawa, Y. Matsumura and K. Hori, *J. Controlled Release*, 2000, **65**, 271–284.
- 5 J. Zhu and X. Shi, *J. Mater. Chem. B*, 2013, **1**, 4199–4211.
- 6 M. C. Parrott, E. B. Marchington, J. F. Valliant and A. Adronov, *J. Am. Chem. Soc.*, 2005, **127**, 12081–12089.
- 7 B. P. Dash, R. Satapathy, B. P. Bode, C. T. Reidl, J. W. Sawicki, A. J. Mason, J. A. Maguire and N. S. Hosmane, *Organometallics*, 2012, **31**, 2931–2935.
- 8 B. B. Jena, S. R. Jena, B. R. Swain, C. S. Mahanta, L. Samanta, B. P. Dash and R. Satapathy, *Appl. Organomet. Chem.*, 2020, **34**, e5754.
- 9 L. F. Tietze, U. Griesbach, I. Schuberth, U. Bothe, A. Marra and A. Dondoni, *Chem. – Eur. J.*, 2003, **9**, 1296–1302.
- 10 S. Stadlbauer, P. Wetzl and E. Hey-Hawkins, *Inorg. Chem.*, 2009, **48**, 5005–5010.
- 11 R. Satapathy, B. P. Dash, C. S. Mahanta, B. R. Swain, B. B. Jena and N. S. Hosmane, *J. Organomet. Chem.*, 2015, **798**, 13–23.
- 12 H. Lis and N. Sharon, *Chem. Rev.*, 1998, **98**, 637–674.
- 13 D. Goyard, P. I. Diriwari and N. Berthet, *RSC Med. Chem.*, 2022, **13**, 72–78.
- 14 B. R. Swain, C. S. Mahanta, B. B. Jena, S. K. Beriha, B. Nayak, R. Satapathy and B. P. Dash, *RSC Adv.*, 2020, **10**, 34764–34774.
- 15 J. O. Suberu, I. Romero-Canelon, N. Sullivan, A. A. Lapkin and G. C. Barker, *ChemMedChem*, 2014, **9**, 2791–2797.
- 16 B. P. Dash, R. Satapathy, J. A. Maguire and N. S. Hosmane, *Chem. Commun.*, 2009, 3267–3269.
- 17 B. P. Dash, R. Satapathy, E. R. Gaillard, K. M. Norton, J. A. Maguire, N. Chug and N. S. Hosmane, *Inorg. Chem.*, 2011, **50**, 5485–5493.
- 18 B. P. Dash, R. Satapathy, J. A. Maguire and N. S. Hosmane, *Org. Lett.*, 2008, **10**, 2247–2250.
- 19 B. P. Dash, R. Satapathy, J. A. Maguire, E. R. Gaillard and N. S. Hosmane, *J. Am. Chem. Soc.*, 2010, **132**, 6578–6587.
- 20 J. Camponovo, J. Ruiz, E. Cloutet and D. Astruc, *Chem. – Eur. J.*, 2009, **15**, 2990–3002.
- 21 Y. M. Chabre, P. P. Brisebois, L. Abbassi, S. C. Kerr, J. V. Marcotte, I. Fahy and R. Roy, *J. Org. Chem.*, 2011, **76**, 724–727.
- 22 N. P. Bizier, S. R. Atkins, L. C. Helland, S. F. Colvin, J. R. Twitchell and M. J. Cloninger, *Carbohydr. Res.*, 2008, **343**, 1814–1818.
- 23 S. Stadlbauer, P. Lonnecke, P. Wetzl and E. Hey-Hawkins, *Eur. J. Org. Chem.*, 2009, 6301–6310.
- 24 J. O. Suberu, I. Romero-Canelon, N. Sullivan, A. A. Lapkin and G. C. Barker, *ChemMedChem*, 2014, **9**, 2791–2797.
- 25 H. Onyüksel, E. Jeon and I. Rubinstein, *Cancer Lett.*, 2009, **274**, 327–330.
- 26 Y. Okamoto, X. Liu, N. Suzuki, K. Okamoto, M. Sekimoto, Y. R. S. Laxmi and S. Shibutani, *Int. J. Cancer*, 2008, **122**, 2142–2147.
- 27 I. Vermes, C. Haanen and C. Reutelingsperger, *J. Immunol. Methods*, 2000, **243**, 167–190.
- 28 L. M. Bomfim, F. A. de Araujo, R. B. Dias, C. Sales, C. A. G. Rocha, R. S. Correa, M. B. Soares, A. A. Batista and D. P. Bezerra, *Sci. Rep.*, 2019, **9**, 11483.
- 29 S. T. Bailey, H. Shin, T. Westerling, X. S. Liu and M. Brown, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 18060–18065.
- 30 Y. Endo, T. Yoshimi and C. Miyaura, *Pure Appl. Chem.*, 2003, **75**, 1197–1205.

