Boron neutron capture therapy (BNCT) has recently received considerable attention due to a highly selective therapy for treating cancer in clinical trial. BNCT is binary therapy based on the combination of two components: non-radioactive boron ($^{10}$B) atom and low energy thermal neutron. As the thermal neutron irradiates $^{10}$B atom, alpha ($\alpha$)-particle and lithium ion ($^7$Li) are generated as well as substantial sufficient energy to damage a cell is produced.

Since the $\alpha$-particle and $^7$Li ion traverse within confined distance (i.e. $\approx 5-10$ μm) corresponding diameter of tumor cell, BNCT can be utilized to selectively kill tumor cells but minimize irradiation damage on normal cells.

Major challenge in BNCT is to find a safe and selective boron delivery agent. BNCT agents for successful therapy have been required to accumulate high $^{10}$B concentration in tumor (i.e., 10-30 μg of $^{10}$B per gram of tumor) with high selectivity (i.e., tumor to normal cell ratio is 5:1) and with low toxicity. Currently successful BNCT agents are sodium borocaptate (BSH) and boronophenylalanine (BPA), which showed several advantages such as sufficient water solubility, and cell-uptake mechanism in the biological system. Therefore, demands of novel boron carrier in BNCT still remain.

Folate receptor (FR) has been an important therapeutic target for cancer treatment, because many cancer cells exhibit high levels of FR on their surface while FR expression in most normal cells is highly restricted. Also, folic acid is able to bind to FR with extremely high affinity ($K_d = 10^{-10}$ M) and transport a drug in cell via an endocytic process. Folate-conjugated drugs could be selectively delivered to highly FR-expressing cancer cell. Therefore, folic acid natural compound might be potentially a non-toxic and selective boron delivery agent in BNCT.

On the other hands, dicarba-closo-dodecaborane ($C_{2}B_{10}H_{12}$; commonly referred to as carborane; exists ortho, meta, and para isomers) has been widely considered for developing new BNCT agents, because use of carborane has benefits such as high boron content, stability to catabolism, hydrophobic interaction enhancement, and extraordinary chemical and thermal stability. Specifically, the volume of carborane has not only topologically similar with that of a phenyl ring rotating about its C1-C4 axis, but also the diameter of carborane is 5.25 Å in comparison with that of phenyl ring is 4.72 Å. Therefore, typical strategy has been to prepare carborane-conjugated biomolecules or to introduce carborane cluster in place of aryl group.

In the past, we have reported the synthesis and testing of new class of ortho-carborane-conjugated aminobenzoylglutamate as a BNCT agent. The carborane analog of folate showed several advantages such as sufficient water solubility for in vitro test, low toxicity towards melanoma cells (IC$_{50}$ 6.9 × 10$^{-4}$ M), and boron uptake of 0.37 μg B per 10$^{6}$ cells. Especially, the aminobenzoylglutamate moiety in the carborane seems to be very important natural carrier for water solubility, and cell-uptake mechanism in the biological system. Therefore, we hypothesized that the new class of folate compounds, introduced ortho-carborane in place of heteroaromatics, could be potentially promising BNCT agent to deliver on FR or internalize in cell nucleus as well as to minimize damage to normal cell for successful cancer therapy. On the other hands, a computational design quite recently showed that ortho-carborane could fit in the active site of human dihydrololate reductase (hDHFR).

In this paper, as our continuous work, we present the synthesis and biological data of ortho-carborane analogs from 4-aminobenzoic acid and 4-hydroxybenzoic acid as a potential boron neutron capture therapy agent.
synthetic and biological data of new class of ortho-carborane analog of folate sodium salt as a potential BNCT agent. The ortho-carboranes as shown in Figure 1 were synthesized from 4-aminobenzoic acid and 4-hydroxybenzoic acid. All carborane compounds were tested as mono- or disodium salt forms to confer water solubility.

Results and Discussion

The first carborane compounds (7a and 7b) have been synthesized as shown in Scheme 1 (also as shown in supplementary materials). The formylation reaction of 4-aminobenzoic acid in the presence of acetic anhydride and formic acid at room temperature (rt) gave 4-(N-formylamino)benzoic acid (2) in 92% yield. The in situ cesium salt of 2 easily transformed benzylated compound (3) in 88% yield. The N-alkylation reaction with propargyl bromide in acetone in the presence of tetra-n-butylammonium bromide under reflux for 12 h gave the propargyl product (4a) in 87% yield. Also, the same reaction of 3b with 4-pentynyl p-toluene-sulfonate (tosylate) led to 4b in 63% yield. The ortho-carboranes readily formed in 77% and 72% yields, respectively, by refluxing the solution of propargylation products and decaborane (B\textsubscript{10}H\textsubscript{14}) in toluene and acetonitrile cosolvent. Benzyl protecting groups of 5a and 5b were easily deprotected via catalytic transfer hydrogenation in the presence of 10% Pd/C and 4.5% formic acid in ethanol and the 6a and 6b were obtained in quantitative yields. The two acids were quantitatively transformed into sodium salts (7a and 7b) to increase the water solubility for in vitro toxicity and boron uptake tests.\textsuperscript{10,11} In the end, the ortho-carborane analogs of folate sodium salts (7a and 7b) were prepared from 4-aminobenzoic acid through 7 steps in 54% and 36% overall yields, respectively.

The second target compounds of ortho-carborane sodium salts (13a-c) were prepared from the 4-hydroxybenzoic acid (8) as shown in Scheme 2. The cesium salt of 4-hydroxybenzoic acid was treated with benzyl bromide in DMF at rt and the benzyl (4-hydroxy)benzoate (9) formed in 93% yield. The O-alkylation with propargyl bromide (n = 1), 4-pentynyl tosylate (n = 2) or 5-butylnyl tosylate (n = 3) gave the propargylated compounds (10a-c) in 96% (n = 1), 68% (n = 2), and 61% (n = 3) yields, respectively. The carboranization of the esters (10a-c) from decaborane in refluxing toluene and acetonitrile produced the ortho-carborane (11a-c) in 84% (n = 1), 68% (n = 2), and 72% (n = 3) yields, respectively. The carboranyl benzoic acids (12a-c) were quantitatively created via catalytic transfer hydrogenation in the presence of 10% Pd/C and 4.5% formic acid in ethanol. Also, the resulting three acids (12a-c) were quantitatively transformed into the sodium salts (13a-c) to confer the sufficient water solubility for the in vitro tests.\textsuperscript{12-14} In fact, the ortho-carborane sodium salts (13a-c) were prepared from 4-hydroxybenzoic acid through 6 steps in 68%, 39%,
and 37% overall yields, respectively.

Finally, the third target molecule of ortho-carborane analog of folate (16) was quantitatively prepared from 12a as shown in Scheme 3. The L-glutamic acid moiety was conjugated with the carborane compound (12a) through 2-ethoxy-l-ethoxy-carbonyl-l,2-dihydroxyquinoline (EEDQ)-mediated amide formation reaction. Benzyl group of 14 was selectively deprotected via catalytic transfer hydrogenation in the excellent yield. 15 was also transformed as the sodium salt before in vitro tests. 15 was also transformed as the sodium salt before in vitro tests. Eventually, the ortho-carborane analog of folate sodium salt (16) was synthesized from the carboranyl benzoic acid (12a) through 3 steps in 96% overall yield (from 4-hydroxybenzoic acid (8) through 8 steps in 66 % overall yield).

All ortho-carboranes as sodium salt (7a, 7b, 13a, 13b, 13c, 16) for BNCT were used for testing in vitro cytotoxicity in three kinds of tumor cells such as B-16 mouse melanoma cell line (i.e., FR positive malignant tumor), U 87 brain tumor cell, and A 431 epidermoid carcinoma cell line (i.e., epidermal growth factor receptor (EGFR) positive malignant tumor), as shown in Figure 2 (also as shown in supplementary materials Table 1). Especially, the cytotoxicity of sodium salt (n = 1) (16) was relatively lower than that of the other salts on B-16 and U 87, and that of sodium salt among them, 13a showed relatively low cytotoxicity on A 431. However, toxicities of elongated carboranes (n = 2 and 3) (7b and 13c) were relatively high. It might be the reason that the carborane salts are structurally associated with potent retinoid agonists and antagonist.

On the other hands, in comparison with BSH, all carboranes were efficiently accumulated into FR receptor positive tumor cell. Especially, 13a was stably incorporated into the cell after additional incubation as shown in Figure 3 (also as shown in supplementary materials Table 2). It was considered to be a good indication of the boron compound as a boron carrier for BNCT.

Conclusion

In summary, all six ortho-carborane analogs of folate were synthesized and characterized for developing a new BNCT agent. Ortho-carborane-conjugated benzoic acid sodium salts (7 and 13) were prepared from 4-aminobenzoic acid in 7 steps in 54 % (n = 1) and 36 % (n = 3) overall yields, respectively, and from 4-hydroxybenzoic acid in 6 steps in 68 % (n = 1), 39 % (n = 2), and 37 % (n = 3) overall yields, respectively. Furthermore, the ortho-carborane analog of folate sodium salt (16) was efficiently synthesized from the carboranyl acid (12a) in 96% overall yield (66% overall yield from 4-hydroxybenzoic acid, 9). In particular, the carboranes (n = 1) (7a, 13a and 16) have relatively low toxicity and high level of accumulation in FR positive cell. It could be potentially promising BNCT agent to deliver on FR target or internalize in cell nucleus for successful cancer therapy.

Acknowledgments. This work was supported by Korea University.
References


10. Characterization of 6a and 7a. (6a): $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 8.49 (s, 1H, formyl H), 8.23 (d, 2H, $J = 8.4$ Hz, aromatic H), 7.29 (d, 2H, $J = 8.4$ Hz, aromatic H), 4.57 (s, 2H, -NCH$_2$-), 4.13 (s, br, 1H, CH in carborane cage). Anal Calcd: C, 41.11; H, 5.96. Found: C, 41.17; H, 6.04. (7a): Anal Calcd: C, 38.48; H, 5.28. Found: C, 38.53; H, 5.29.

11. Characterization of 6b and 7b. (6b): $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 8.51 (s, 1H, formyl H), 8.25 (d, 2H, $J = 8.7$ Hz, aromatic H), 7.34 (d, 2H, $J = 8.7$ Hz, aromatic H), 4.53 (t, 2H, $J = 7.2$ Hz, -CH$_2$), 4.17 (s, br, 1H, CH in carborane cage), 4.05 (t, 2H, $J = 7.2$ Hz, -NCH$_2$-). Anal Calcd: C, 44.68; H, 6.63. Found: C, 44.51; H, 6.65. (7b): Anal Calcd: C, 42.04; H, 5.92. Found: C, 42.21; H, 5.99.

12. Characterization of 12a and 13a. (12a): $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 8.08 (d, 2H, $J = 9.3$ Hz, aromatic H), 6.91 (d, 2H, $J = 8.7$ Hz, aromatic H), 4.49 (s, 2H, -OCH$_2$-), 4.07 (s, br, 1H, CH in carborane cage). Anal Calcd: C, 40.80; H, 6.16. Found: C, 41.86; H, 6.33. (13a): Anal Calcd: C, 37.97; H, 5.42. Found: C, 38.05; H, 5.48.

13. Characterization of 12b and 13b. (12b): $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 8.06 (d, 2H, $J = 9.0$ Hz, aromatic H), 6.88 (d, 2H, $J = 9.0$ Hz, aromatic H), 4.14 (t, 2H, $J = 5.4$ Hz, -OCH$_2$-), 3.83 (s, br, 1H, CH in carborane cage), 2.77 (tt, 2H, $J = 5.4$ Hz, -CH$_2$-). Anal Calcd: C, 42.84; H, 6.54. Found: C, 42.89; H, 6.45. (13b): Anal Calcd: C, 39.99; H, 5.80. Found: C, 39.87; H, 5.84.

14. Characterization of 12c and 13c. (12c): $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 8.03 (d, 2H, $J = 8.7$ Hz, aromatic H), 6.89 (d, 2H, $J = 8.7$ Hz, aromatic H), 4.00 (t, 2H, $J = 6.0$ Hz, -OCH$_2$-), 3.62 (s, br, 1H, CH in carborane cage), 2.43 (tt, 2H, $J = 6.0$ Hz, -CH$_2$-). Anal Calcd: C, 44.70; H, 6.88. Found: C, 44.65; H, 6.89. (13c): Anal Calcd: C, 41.85; H, 6.15. Found: C, 41.77; H, 6.19.

15. Characterization of 15 and 16. (15): $^1$H NMR (CDCl$_3$, 300 MHz): $\delta$ 8.07 (d, 2H, $J = 8.7$ Hz, aromatic H), 6.88 (d, 2H, $J = 8.7$ Hz, aromatic H), 4.43 (s, 2H, -OCH$_2$-), 4.09-1.17 (m, 2H, CH in carborane cage, CH in glutamate), 1.96-2.49 (m, 4H, 2 × CH$_2$ in glutamate). Anal Calcd: C, 42.54; H, 5.95. Found: C, 42.68; H, 5.99. (16): Anal Calcd: C, 38.54; H, 4.96. Found: C, 38.65; H, 4.93.

Supplementary Materials

Synthesis and Biological Data of ortho-Carborane Analogs from 4-Aminobenzoic Acid and 4-Hydroxybenzoic Acid as a Potential Boron Neutron Capture Therapy Agent

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Experimental Section

All solvents and reagents were used as purchased without additional purification. Silica gel 60 (230-400 mesh, Merck) was used for column chromatography, and silica gel 60F254 plates (0.25 mm, Merck) were used for monitoring the reactions. 1H NMR spectra were recorded on a VARIAN at 300 MHz. Compounds 2 and 3 were synthesized following a method in reference 1 and 2.

Synthesis of 4-(Formyl-prop-2-ynyl-amino)benzoic Acid Benzyl Ester (4a). To a solution of 4-formylamino-benzoic acid benzyl ester (204 mg, 1.60 mmol) and propargyl bromide (380 mg, 3.20 mmol) in acetone (40 mL) were added potassium carbonate (885 mg, 6.40 mmol) and tetrabutylammonium bromide (515 mg, 1.60 mmol). The reaction mixture was heated at reflux for 12 h. After filtration, the solvent was evaporated in vacuo. Then the residue was purified by flash column chromatography using ethyl acetate/n-hexane (1/2) as the eluent to give the title compound 4a (87%) as a white solid. mp 74-79 °C; 1H NMR (CDCl3, 300 MHz): δ 8.57 (s, 1H, formyl H), 8.15 (d, 2H, J = 8.7 Hz, aromatic H), 7.47-7.34 (m, 7H, aromatic H), 5.38 (s, 2H, -CH2-Ph), 4.60 (d, 2H, J = 2.7 Hz, -NCH2-), 2.23 (t, 1H, J = 2.7 Hz, CH≡), 1.97 (t, J = 2.7 Hz, 1H, CH2=). Anal Calcd: C, 73.71; H, 5.15. Found: C, 73.72; H, 5.16.

Synthesis of 4-(Formyl-pent-2-ynyl-amino)benzoic Acid Benzyl Ester (4b). To a solution of 4-formylamino-benzoic acid benzyl ester (204 mg, 1.60 mmol) and 4-pentynyl p-toluenesulfonate (762 mg, 3.20 mmol) in acetone (40 mL) were added potassium carbonate (885 mg, 6.40 mmol) and tetrabutylammonium bromide (515 mg, 1.60 mmol). The reaction mixture was heated at reflux for 12 h. After filtration, the solvent was evaporated in vacuo. Then the residue was purified by flash column chromatography using ethyl acetate/n-hexane (1/2) as the eluent to give the title compound 4b (63%) as a white solid. mp 77-80 °C; 1H NMR (CDCl3, 300 MHz): δ 8.52 (s, 1H, formyl H), 8.13 (d, 2H, J = 8.7 Hz, aromatic H), 7.33-7.46 (m, 5H, aromatic H), 7.25 (d, 2H, J = 8.7 Hz, aromatic H), 5.37 (s, 2H, -CH2-Ph), 3.98 (t, 2H, J = 7.2 Hz, -NCH2-), 2.22 (td, 2H, J = 7.2 Hz, -CH2-), 1.97 (t, J = 2.7 Hz, 1H, CH2=), 1.76-1.86 (m, 2H, -CH2-). Anal Calcd: C, 74.75; H, 5.96. Found: C, 74.94; H, 5.91.

Synthesis of 4-[N,N-Formyl-(ortho-carboranylmethyl)-amino]benzoic Acid Benzyl Ester (5a). To a solution of compound 4a (234 mg, 0.80 mmol) in acetonitrile/toluene (1/3, 16 mL) was added decaborane (116 mg, 1.04 mmol). The reaction mixture was heated at reflux for 7 h. The solvent was evaporated in vacuo and the residue was purified by flash column chromatography using ethyl acetate/n-hexane (1/2) as the eluent to give the title compound 5a (72%) as a white solid. mp 114-117 °C; 1H NMR (CDCl3, 300 MHz): δ 8.49 (s, 1H, formyl H), 8.20 (d, 2H, J = 8.7 Hz, aromatic H), 7.47-7.34 (m, 7H, aromatic H), 5.38 (s, 2H, -CH2-Ph), 4.60 (d, 2H, J = 2.7 Hz, -NCH2-), 2.23 (t, 1H, J = 2.7 Hz, CH≡). Anal Calcd: C, 73.71; H, 5.15. Found: C, 73.72; H, 5.16.

Scheme 1
Synthesis of 4-[N,N-Formyl-(ortho-carboranylmethyl)-amino]benzoic Acid (6a). A solution of 4-hydroxybenzoic acid (6.138 g, 0.045 mmol) in methanol (90 mL) was added cesium carbonate (7.239 g, 0.023 mmol) and the solution was stirred at rt for 10 min. After concentrating the solution, it was azeotroped with toluene to remove remaining water. To the resulting solid, was added anhydrous DMF (60 mL) and benzyl bromide (6.3 mL, 0.053 mol), and then the reaction mixture was stirred at rt for 18 h. The reaction progress was monitored by TLC (ethyl acetate and hexane = 1:4). After filtering white solid, the filtrate was concentrated and purified on silica gel flash column chromatography using ethyl acetate/hexane (1:1) to give the benzyl 4-hydroxybenzoate (9) as a white solid in 93% yield (9.42 g). mp 79.3 °C; 1H NMR (CDCl₃, 300 MHz): δ 8.06 (s, 2H, -CH=Ph), 7.91 (s, 1H, aromatic H), 6.86 (d, 2H, J = 8.8 Hz), 7.33-7.46 (m, 5H), 7.99 (d, 2H, J = 8.8 Hz).

Synthesis of 4-(Prop-2-ynyloxy)benzoic Acid Benzyl Ester (10a). To a solution of 4-hydroxybenzoic acid benzyl ester (365 mg, 1.60 mmol) and propargyl bromide (380 mg, 3.20 mmol) in acetone (40 mL) was added potassium carbonate (885 mg, 6.40 mmol). The reaction mixture was heated at reflux for 12 h. After filtration, the solvent was evaporated in vacuo. Then the residue was purified by flash column chromatography using ethyl acetate/hexane (1:10) as the eluent to give the title compound 10a (96%) as a white solid. mp 52-56 °C; 1H NMR (CDCl₃, 300 MHz): δ 8.06 (d, 2H, J = 9.0 Hz, aromatic H), 7.35-7.46 (m, 5H, aromatic H), 6.99 (d, 2H, J = 9.0 Hz, aromatic H), 5.34 (s, 2H, -CH=Ph), 4.75 (d, 2H, J = 2.4 Hz, -OCH₂), 2.54 (t, 1H, J = 2.4 Hz, CH=). Anal Calcd: C, 76.68; H, 5.30. Found: C, 76.80; H, 5.32.

Synthesis of Sodium 4-[N,N-Formyl-(ortho-carboranylmethyl)-amino]benzoate (7a). To a solution of compound 6a (96 mg, 0.30 mmol) in methanol (6 mL) was added sodium bicarbonate (25 mg, 0.30 mmol). The reaction mixture was stirred at rt for 0.5 h and the solvent was evaporated in vacuo to give the title compound 7a (97%) as a white solid. Anal Calcd: C, 38.48; H, 5.28. Found: C, 38.53; H, 5.29.

Synthesis of Sodium 4-[N,N-Formyl-(ortho-carboranylprop-2-ynyloxy)-amino]benzoate (7b). To a solution of 6b (104 mg, 0.30 mmol) in methanol (5 mL) was added sodium bicarbonate (25 mg, 0.30 mmol). The reaction mixture was stirred at rt for 0.5 h and the solvent was evaporated in vacuo to give the title compound 7b (98%) as a white solid. Anal Calcd: C, 42.04; H, 5.92. Found: C, 42.21; H, 5.99.

Notes

Scheme 2
as the eluent to give the title compound 10b (68%) as a white solid. mp 51-55 °C; 1H NMR (CDCl3, 300 MHz): δ 8.03 (d, 2H, J = 9.0 Hz, aromatic H), 7.33-7.46 (m, 5H, aromatic H), 6.92 (d, 2H, J = 9.0 Hz, aromatic H), 5.34 (s, 2H, -CH2-Ph), 4.14 (t, 2H, J = 6.9 Hz, -OCH2-), 2.71 (td, 2H, J = 6.9 Hz, -CH2-), 2.06 (t, 1H, CH=). Anal Calcd: C, 77.12; H, 5.75. Found: C, 76.93; H, 5.65.

Synthesis of 4-(Pent-2-ynyl)benzoic Acid Benzy1 Ester (10c). To a solution of 4-hydroxybenzoic acid benzyl ester (365 mg, 1.60 mmol) and 4-pentynyl p-toluenesulfonate (762 mg, 3.20 mmol) in acetonitrile (40 mL) was added potassium carbonate (885 mg, 6.40 mmol). The reaction mixture was heated at reflux for 12 h. After filtration, the solvent was evaporated in vacuo and concentrate was purified by flash chromatography using ethyl acetate/hexane (1/10) as an eluent to give the title compound 10c (61%) as a white solid. mp 52-56 °C; 1H NMR (CDCl3, 300 MHz): δ 8.02 (d, 2H, J = 8.7 Hz, aromatic H), 7.33-7.46 (m, 5H, aromatic H), 6.91 (d, 2H, J = 8.7 Hz, aromatic H), 5.34 (s, 2H, -CH2-Ph), 4.12 (t, 2H, J = 6.3 Hz, -OCH2-), 2.42 (td, 2H, J = 6.3 Hz, -CH2-), 1.97-2.05 (m, 3H, -CH2-), CH=). Anal Calcd: C, 77.53; H, 6.16. Found: C, 77.34; H, 6.19.

Synthesis of 4-(Ortho-carboranylmethyloxy)benzoic Acid Benzyl Ester (11a). To a solution of compound 11a (213 mg, 0.80 mmol) in acetonitrile/toluene (1/3, 16 mL) was added decaborane (116 mg, 1.04 mmol). The reaction mixture was heated at reflux for 7 h, concentrated in vacuo and purified by flash column chromatography using ethyl acetate/n-hexane (1/10) as the eluent to give the title compound 11a (84%) as a white solid. mp 80-82 °C; 1H NMR (CDCl3, 300 MHz): δ 8.05 (d, 2H, J = 9.0 Hz, aromatic H), 7.46-7.39 (m, 5H, aromatic H), 6.87 (d, 2H, J = 9.0 Hz, aromatic H), 5.34 (s, 2H, -CH2-Ph), 4.46 (s, 2H, -OCH2-), 4.06 (s, br, 1H, CH in carborane cage). Anal Calcd: C, 53.11; H, 6.29. Found: C, 53.26; H, 6.27.

Synthesis of 4-(Ortho-carboranylpropyloxy)benzoic Acid Benzyl Ester (11b). To a solution of compound 10b (224 mg, 0.80 mmol) in acetonitrile/toluene (1/3, 16 mL) was added decaborane (116 mg, 1.04 mmol). The reaction mixture was heated at reflux for 7 h. The solvent was evaporated in vacuo and the residue was purified by flash column chromatography using ethyl acetate/n-hexane (1/10) as the eluent to give the title compound 11b (68%) as a white solid. mp 96-99 °C; 1H NMR (CDCl3, 300 MHz): δ 8.05 (d, 2H, J = 9.0 Hz, aromatic H), 7.37-7.46 (m, 5H, aromatic H), 6.88 (d, 2H, J = 9.0 Hz, aromatic H), 5.35 (s, 2H, -CH2-Ph), 4.12 (t, 2H, J = 6.0 Hz, -OCH2-), 3.80 (s, br, 1H, CH in carborane cage), 2.75 (t, 2H, J = 2.7 Hz, -CH2-). Anal Calcd: C, 54.25; H, 6.58. Found: C, 54.15; H, 6.60.

Synthesis of 4-(Ortho-carboranylpropyl)benzoic Acid Benzy1 Ester (11c). To a solution of compound 10c (235 mg, 0.80 mmol) in acetonitrile/toluene (1/3, 16 mL) was added decaborane (116 mg, 1.04 mmol). The reaction mixture was heated at reflux for 7 h, concentrated in vacuo and chromatographed on silica gel using ethyl acetate/n-hexane (1/10) as an eluent to give the title compound 11c (72%) as a white solid. mp 88-91 °C; 1H NMR (CDCl3, 300 MHz): δ 8.02 (d, 2H, J = 8.7 Hz, aromatic H), 7.34-7.46 (m, 5H, aromatic H), 6.86 (d, 2H, J = 8.7 Hz, aromatic H), 5.33 (s, 2H, -CH2-Ph), 3.98 (t, 2H, J = 6.0 Hz, -OCH2-), 3.62 (s, br, 1H, CH in carborane cage), 2.44 (t, 2H, J = 6.0 Hz, -CH2-), 1.97-2.04 (m, 2H, -CH2-). Anal Calcd: C, 55.32; H, 6.84. Found: C, 55.36; H, 6.92.

Synthesis of 4-(Ortho-carboranylethyl)benzoic Acid (12a). A solution of compound 11a (153 mg, 0.40 mmol) and 10% Pd/C (153 mg, 100 wt %) in 4.5% formic acid in methanol (10 mL) was stirred at rt for 3 h. The reaction mixture was filtered through celite bed and the filtrate was evaporated in vacuo to give the title compound 12a (99%) as a white solid. 1H NMR (CDCl3, 300 MHz): δ 8.08 (d, 2H, J = 9.3 Hz, aromatic H), 6.91 (d, 2H, J = 8.7 Hz, aromatic H), 4.49 (s, 2H, -OCH2-), 4.07 (s, br, 1H, CH in carborane cage). Anal Calcd: C, 40.80; H, 6.16. Found: C, 41.86; H, 6.33.

Synthesis of 4-(Ortho-carboranylpropyl)benzoic Acid (12b). To a solution of 11b (159 mg, 0.40 mmol) and 10% Pd/C (159 mg, 100 wt %) in 4.5% formic acid in methanol (10 mL) was stirred at rt for 3 h. The reaction mixture was filtered through celite bed and concentrated in vacuo to give the title compound 12b (99%) as a white solid. 1H NMR (CDCl3, 300 MHz): δ 8.06 (d, 2H, J = 9.0 Hz, aromatic H), 6.88 (d, 2H, J = 9.0 Hz, aromatic H), 4.14 (t, 2H, J = 5.4 Hz, -OCH2-), 3.83 (s, br, 1H, CH in carborane cage), 2.77 (t, 2H, J = 5.4 Hz, -CH2-). Anal Calcd: C, 42.84; H, 6.54. Found: C, 42.89; H, 6.45.

Synthesis of Sodium 4-(ortho-carboranylethyl)benzoate (13a). To a solution of compound 12a (88 mg, 0.30 mmol) in methanol (6 mL) was added sodium bicarbonate (25 mg, 0.30 mmol). The reaction mixture was stirred at rt for 0.5 h and concentrated in vacuo to give the title compound 13a (99%) as a white solid. Anal Calcd: C, 37.97; H, 5.42. Found: C, 38.05; H, 5.48.

Synthesis of Sodium 4-(ortho-carboranylethyl)benzoate (13b). To a solution of compound 12b (92 mg, 0.30 mol) in methanol (6 mL) was added sodium bicarbonate (25 mg, 0.30 mmol). The reaction mixture was stirred at rt for 0.5 h and concentrated in vacuo to give title compound 13b (99%) as a white solid. Anal Calcd: C, 39.99; H, 5.80. Found: C, 39.87; H, 5.84.

Synthesis of Sodium 4-(ortho-carboranylpropyl)benzoate (13c). To a solution of compound 12c (128 mg, 0.30 mmol) in methanol (5 mL) was added sodium bi-
carbonate (25 mg, 0.30 mmol). The reaction mixture was stirred at rt for 0.5 h and concentrated in vacuo to give title compound 13e (99%) as a white solid. Anal Calcd: C, 41.85; H, 6.15. Found: C, 41.77; H, 6.19.

Synthesis of 4-(Ortho-carboranylethoxy)benzoyl-di-benzyl Glutamate (14). To a solution of compound 12a (520 mg, 1.842 mmol) in chloroform (35 mL) was added dibenzyl L-glutamate (904 mg, 2.763 mmol). The reaction mixture was stirred at rt for 24 h. The organic solution was evaporated in vacuo and the residue was purified by flash column chromatography using ethyl acetate/n-hexane (1/10) as an eluent to give title compound 14 (76%). 1H NMR (CDCl3, 300 MHz): δ 8.08 (d, 2H, J = 8.4 Hz, aromatic H), 7.26-7.34 (m, 5H, benzyl aromatic H), 6.90 (d, 2H, J = 8.4 Hz, aromatic H), 5.17 (s, 2H, -CH2-Ph), 5.10 (s, 2H, -CH2-Ph), 4.45 (s, 2H, -OCH3), 4.05-4.16 (m, 2H, CH in carborane cage, CH in glutamate), 1.95-2.48 (m, 4H, 2 × CH2 in glutamate). Anal Calcd: C, 42.54; H, 5.95. Found: C, 42.68; H, 5.99.

Synthesis of Sodium-4-(ortho-carboranylethoxy)benzoyl glutamate (16). To a solution of compound 15 (127 mg, 0.30 mmol) in methanol (6 mL) was added sodium bicarbonate (25 mg, 0.30 mmol). The reaction mixture was stirred at rt for 0.5 h and concentrated in vacuo to give title compound 16 (99%) as a white solid. Anal Calcd: C, 38.54; H, 4.96. Found: C, 38.65; H, 4.93.

Biological Evaluation: in vitro Boron Incorporation into B-16 Melanoma Cells. Boron uptake by B-16 cells was determined by using the ICP-AES method. The cells were cultured in Falcon dishes (90 mm) until they were grown to ~3 × 10^5 cells/dish. The cells were incubated for 3 h with Eagle-MEM medium containing the compounds (boron concentration: 10.8 ppm). At 3 h, the cells were washed three times with PBS (−) and one was processed for the determination of boron concentration by ICP-AES. The other was again incubated for 3 h with boron compounds-free Eagle-MEM medium, and then processed for the determination of boron concentration by ICP-AES. Three replications of each experiment were carried out. The boron compounds exhibited higher uptake by B-16 cells in comparison with sodium borocaptate (BSH) after 3 h incubation and came out from the cells after 3 h of the compounds-free incubation.

Table 1. IC50 values (M)

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>B-16</th>
<th>I-87</th>
<th>A431</th>
</tr>
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<tbody>
<tr>
<td>Compounds</td>
<td>3h-incubation</td>
<td>Washed, then 3h-incubation</td>
<td></td>
</tr>
<tr>
<td>7a</td>
<td>1.5 × 10^-5</td>
<td>0.16 ± 0.006</td>
<td>0.099 ± 0.003</td>
</tr>
<tr>
<td>7b</td>
<td>1.5 × 10^-5</td>
<td>0.13 ± 0.045</td>
<td>0.10 ± 0.010</td>
</tr>
<tr>
<td>7c</td>
<td>1.1 × 10^-5</td>
<td>0.28 ± 0.03</td>
<td>0.20 ± 0.05</td>
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<tr>
<td>7d</td>
<td>7.0 × 10^-5</td>
<td>0.14 ± 0.01</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>7e</td>
<td>1.0 × 10^-5</td>
<td>0.29 ± 0.05</td>
<td>0.16 ± 0.04</td>
</tr>
<tr>
<td>7f</td>
<td>5.5 × 10^-6</td>
<td>0.28 ± 0.05</td>
<td>0.085 ± 0.02</td>
</tr>
<tr>
<td>BSH</td>
<td>0.15 ± 0.05</td>
<td>0.051 ± 0.011</td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Boron uptake by B-16 Cell (10^-5 gB/10^6 cells)

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Incubation time</th>
<th>Washed, then 3h-incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>7a</td>
<td>1.5 × 10^-5</td>
<td>0.16 ± 0.006</td>
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<tr>
<td>7b</td>
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<tr>
<td>7c</td>
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<td>7d</td>
<td>7.0 × 10^-5</td>
<td>0.14 ± 0.01</td>
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<tr>
<td>7e</td>
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<tr>
<td>7f</td>
<td>5.5 × 10^-6</td>
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</tr>
<tr>
<td>BSH</td>
<td>0.15 ± 0.05</td>
<td>0.051 ± 0.011</td>
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</tbody>
</table>

References