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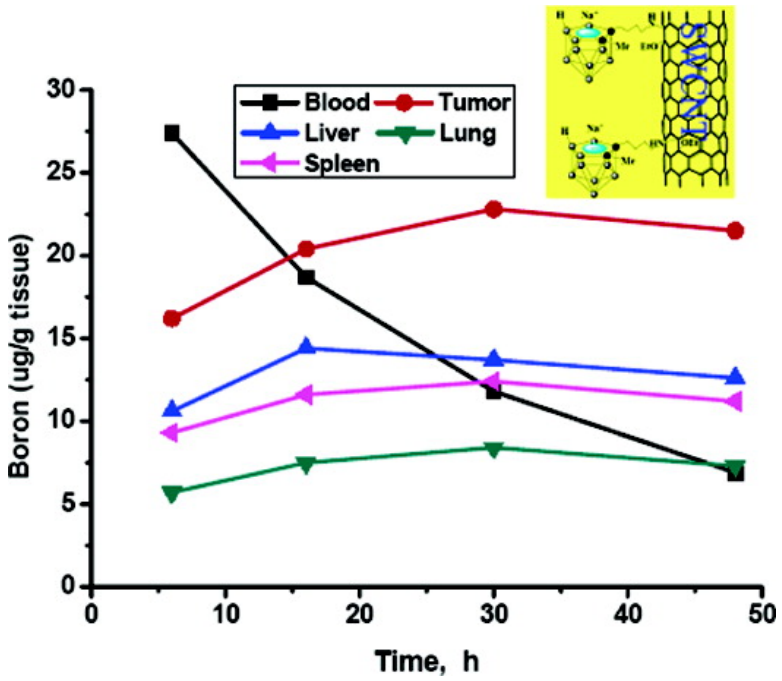

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Substituted Carborane-Appended Water-Soluble Single-Wall Carbon Nanotubes: New Approach to Boron Neutron Capture Therapy Drug Delivery

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Abstract: Substituted C_2B_{10} carborane cages have been successfully attached to the side walls of single-wall carbon nanotubes (SWCNTs) via nitrene cycloaddition. The decapitations of these C_2B_{10} carborane cages, with the appended SWCNTs intact, were accomplished by the reaction with sodium hydroxide in refluxing ethanol. During base reflux, the three-membered ring formed by the nitrene and SWCNT was opened to produce water-soluble SWCNTs in which the side walls are functionalized by both substituted *nido*-C_2B_9 carborane units and ethoxide moieties. All new compounds are characterized by EA, SEM, TEM, UV, NMR, and IR spectra and chemical analyses. Selected tissue distribution studies on one of these nanotubes, $\{([Na^+][1\text{-Me-}2\text{-}((CH_2)_4NH\text{-})\text{-}1,2\text{-}C_2B_9H_{10}][OEt])_n(SWCNT)\}$ (**Va**), show that the boron atoms are concentrated more in tumors cells than in blood and other organs, making it an attractive nanovehicle for the delivery of boron to tumor cells for an effective boron neutron capture therapy in the treatment of cancer.

Introduction

Carbon nanotubes (CNT) have attracted a great deal of attention since their discovery in 1991.[1] Not only are they interesting in their own right,[2] but methods have been developed that led to chemically modified CNTs having useful properties, such as solubility in polar and nonpolar solvents and moderate biocompatibility that make them potentially important nonomaterials.[2−8] Observations of enhanced water solubility of CNTs through side-wall derivation with biologically important moieties have been of special interest.[5,9−25] It has recently been reported that peptide-functionalized single-walled carbon nanotubes (SWCNTs) were able to cross cell membranes and concentrate

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in the cytoplasm of 3T6, 3T3 fibroblasts and phagocytic cells without showing obvious toxic effects.[9a] Similar results were obtained in HL60 cells, where it was found that functionalized SWCNTs can help transport large attached groups into cells without themselves exhibiting cell toxicity.[9b] These observations raised the question whether suitably derived SWCNTs would be useful boron delivery agents for use in boron neutron capture therapy (BNCT). Such substances could prove to be a useful addition to the group of tumor-targeting biomolecules, such as porphyrin substrates, epidermal growth factors, liposomes, and so forth, which have been investigated as possible BNCT drug delivery agents, with varying degrees of success.[26] It was this speculation that led us to synthesize and characterize water-soluble SWCNTs with appended monoanionic, substituted C_2B_9 carborane units and to study their boron tissue distributions. These results, reported herein, indicate that such modified SWCNTs could prove to be new boron delivery agents for effective BNCT in the treatment of cancer.

Experimental Section

Syntheses. All reactions were carried out under an argon atmosphere using standard Schlenk techniques. Diethyl ether and benzene were heated over sodium/benzophenone until a blue color was sustained, and distilled under nitrogen just before use. 1,2-Dichlorobenzene was dried over phosphorus pentoxide and distilled. *N*-Butyllithium (1.6 M in hexanes), 1-chloro-4-iodobutane, 1,4-diiodobutane, sodium azide, sodium iodide, and all other reagents (Aldrich), including organic solvents, were used as received. 1-Methyl- and 1-phenyl-*closo*-1,2-$C_2B_{10}H_{11}$ were obtained from Katchem Ltd. and used as received. Before use, the commercially available SWCNTs (Aldrich), characterized by a diameter of about 1 nm and a length in the range of 200−1000 nm, were refluxed with 6 M HCl for 1 day and then centrifuged, followed by repeated washing with deionized water until a pH of about 7 was reached, and then the residue dried in vacuo to ensure the removable of any metal catalysts present in SWCNTs. FT-IR spectra were measured using a BIO-RAD spectrophotometer with KBr pellets or organic solvent films. Scanning electron microscopy (SEM) images were obtained on a JSM-6700F field-emission microscope. The samples were previously sputter-coated with a homogeneous palladium layer for charge dissipation during the SEM imaging. Transmission electron microscopy (TEM) measurements were carried out on a JEOL Tecnai-G^2, FEI analyzer at 200 kV; the samples for TEM measurements were prepared by placing one drop of a diluted solution of the functionalized SWCNTs onto a copper grid coated with carbon followed by the solvent evaporation. Elemental analyses were measured on a EURO EA. UV−visible spectra were recorded on a UV-2550, Shimadzu UV−visible spectrophotometer. The samples for characterization by UV−visible spectrophotometry were dispersed in C_6H_6 with the help of an ultrasonic bath. ^{1}H, ^{13}C, and ^{11}B NMR spectra were recorded on a Bruker 400 analyzer at 400.13, 100.62, and 128.38 MHz, respectively. Inductively coupled plasma-optical emission spectroscopy (ICP-OES) measurements were made on a VISTA-MPX machine.

(1) Synthesis of IIa,b from 1-Chloro-4-iodobutane and *ortho*-Carborane. Synthesis of Intermediates Ia,b. A dry 250-mL three-necked round-bottom flask equipped with a magnetic stirring bar was charged with 1-methyl-1,2-$C_2B_{10}H_{11}$ (3.17 g, 20.00 mmol) and dissolved in 100 mL of a diethyl ether/benzene (v/v = 2/1) mixture. The solution was cooled to −78 °C, and then 12.6 mL (20.16 mmol) of the *n*-butyllithium solution was added dropwise with a syringe. The mixture was maintained at −78 °C for 30 min and then allowed slowly to warm to room temperature. The reaction mixture was stirred at that temperature for 4 h and cooled to 0 °C, and then 2.6 mL (20.60 mmol) of 1-chloro-4-iodobutane was added through a syringe. After addition, the reaction mixture was stirred for 30 min at 0 °C, slowly warmed to room temperature, stirred for an additional 2 h, and then refluxed for 4 h. At this point, the mixture was cooled to 0 °C and then quenched with deionized water. The organic phase was separated, and the aqueous layer was extracted with diethyl ether (3 × 20 mL). The extract was combined with the organic phase, and then the solvents were removed, leaving a pale yellow sticky residue. This residue was purified with thin-layer chromatography (SiO_2), developed with a *n*-pentane/ethyl acetate (v/v = 6/1) mixture, to give 5.10 g of (**Ia**) as a colorless sticky oil, which slowly solidified into wax during storage. Elemental Anal: Found for **Ia**: C, 29.50; H, 7.40, calcd for a 1-Me-2-$(CH_2)_4$Cl-1,2-$C_2B_{10}H_{10}$/1-Me-2-$(CH_2)_4$I-1,2-$C_2B_{10}H_{10}$ mixture in a 1/0.89 molar ratio, C, 29.52; H, 7.43. ^{1}H NMR ($CDCl_3$, relative to $SiMe_4$, ppm): δ 3.41 (t, 2H, $-CH_2Cl$), 3.05 (t, 2H, $-CH_2I$), 2.90−1.20 (m, br, 19H, CH_3-C_{cage}, $-(CH_2)_3-C_{cage}$, $B_{10}H_{10}$). ^{13}C NMR ($CDCl_3$, relative to $SiMe_4$, ppm): δ 77.67, 77.59, and 74.74 for C_{cage}, 44.08 ($-CH_2Cl$), 34.41, 33.96, 32.36, 31.66, 30.33, and 26.80 for $C_{cage}-(CH_2)_3-$, 23.14 and 23.09 for $C_{cage}-CH_3$, 5.34 ($-CH_2I$).

In a process similar to that described above for the synthesis of **Ia**, 4.41 g (20.00 mmol) of 1-Ph-*closo*-$C_2B_{10}H_{11}$, 12.60 mL (20.16 mmol) *n*-butyllithium, and 2.60 mL (20.61 mmol) of 1-chloro-4-iodobutane produced 6.18 g of **Ib**, which was purified as described above. Elemental Anal: Found for **Ib**: C, 41.58; H, 6.65, calcd based on a 1-Ph-2-$(CH_2)_4$Cl-1,2-$C_2B_{10}H_{10}$/1-Ph-2-$(CH_2)_4$I-1,2-$C_2B_{10}H_{10}$ molar ratio of 1/0.82, C, 41.62; H, 6.70. ^{1}H NMR ($CDCl_3$, relative to $SiMe_4$, ppm): δ 7.60−7.27 (m, 5H, $C_{cage}-C_6H_5$), 3.24 (t, 2H, $-CH_2Cl$), 2.88 (t, 2H, $-CH_2I$), 3.30−1.20 (m, br, 16H, $-(CH_2)_3-C_{cage}$, $B_{10}H_{10}$). ^{13}C NMR ($CDCl_3$, relative to $SiMe_4$, ppm): δ 130.87, 130.86, 130.52, and 129.12 for C_6H_5, 83.82, 82.06, and 82.01 for C_{cage}, 44.05 ($-CH_2Cl$), 34.22, 33.80, 32.37, 31.62, 30.26, and 26.71 for $C_{cage}-(CH_2)_3-$, 5.42 ($-CH_2I$).

Synthesis of IIa,b from NaI and Acetone. A 2.00 g sample of **Ia**, or 3.00 g sample of **Ib**, 6.50 g (43.15 mmol) of sodium iodide, and 120 mL of HPLC grade acetone were refluxed for 3 days and then cooled to room temperature. All volatiles were removed by pumping, and the residue was extracted with diethyl ether. The extract was then dried in vacuo to obtain a pale yellow sticky residue that was then purified by flash column chromatography (SiO_2, 2.5 × 30 cm, eluted with mixed solvents *n*-pentane/ethyl acetate in v/v = 5/1 ratio) to obtain 1-Me-2-$(CH_2)_4$I-1,2-$C_2B_{10}H_{10}$ (**IIa**) (2.30 g, 92% yield, based on chloride content in (**Ia**)) or 1-Ph-2-$(CH_2)_4$I-1,2-$C_2B_{10}H_{10}$ (**IIb**) (3.38 g, 95% yield based on chloride content in (**Ib**)) as colorless oils.

IIa: Elemental Anal: Calcd for $C_7H_{21}B_{10}I$: C, 24.71; H, 6.22. Found: C, 24.60; H, 6.20. ^{1}H NMR ($CDCl_3$, relative to $SiMe_4$, ppm): δ 3.12 (t, 2H, $-CH_2I$); 2.90−1.20 (m, br, 19H, CH_3-C_{cage}, $-(CH_2)_3-C_{cage}$, $B_{10}H_{10}$). ^{13}C NMR ($CDCl_3$, relative to $SiMe_4$, ppm): δ 76.63, 73.79 (C_{cage}); 32.98 ($-CH_2CH_2CH_2CH_2I$); 31.38 ($-CH_2CH_2CH_2CH_2I$); 29.36 ($-CH_2CH_2CH_2CH_2I$); 22.18 ($C_{cage}-CH_3$); 4.50 ($-CH_2CH_2-CH_2CH_2I$). ^{11}B NMR ($CDCl_3$, relative to $BF_3\cdot OEt_2$, ppm): δ −3.70 (1B, $^1J_{BH}$ = 161 Hz); −5.04 (1B, $^1J_{BH}$ = 171 Hz); −8.57 (2B, $^1J_{BH}$ = 90 Hz); −9.14 (2B, $^1J_{BH}$ = 108 Hz); −10.01 (4B, $^1J_{BH}$ = 151 Hz). IR (film on KBr, cm^{-1}) 3005 (s, s), 2983 (s, s), 2869 (m, s), 2587 (vs, s, ν_{BH}), 1955 (w, s), 1852 (w, s), 1452 (s, s), 1385 (m, s), 1296 (m, s), 1261 (m, s), 1221 (s,s), 1172 (s, s), 1022 (s, s), 947 (m, s), 789 (m, br), 729 (vs, s), 665 (w, s), 600 (w, s), 502 (m, s).

IIb: Elemental Anal: Calcd for $C_{12}H_{23}B_{10}I$: C, 35.83; H, 5.76. Found: C, 35.76; H, 5.70. ^{1}H NMR ($CDCl_3$, relative to $SiMe_4$, ppm): δ 7.60−7.29 (m, 5H, C_6H_5), 3.30−1.20 (m, br, 18H, $-(CH_2)_4-C_{cage}$, $B_{10}H_{10}$). ^{13}C NMR ($CDCl_3$, relative to $SiMe_4$, ppm): δ 130.06, 130.04, 129.72, 127.97 (C_6H_5); 82.66, 80.81 (C_{cage}); 32.71 ($-CH_2CH_2CH_2CH_2I$); 31.30 ($-CH_2CH_2CH_2CH_2I$); 29.13 ($-CH_2CH_2CH_2CH_2I$); 4.15 ($-CH_2-CH_2CH_2CH_2I$). ^{11}B NMR ($CDCl_3$, relative to $BF_3\cdot OEt_2$, ppm): δ −2.98

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(2B, $^1J_{BH}$ = 150 Hz); −9.10 (2B, $^1J_{BH}$ = 112 Hz); −9.72 (6B, $^1J_{BH}$ = 107 Hz). IR (film on KBr, cm^{-1}) 3437 (m, br), 2956 (w, s), 2587 (vs, s, ν_{BH}), 2253 (m, s), 1620 (m, br), 1493 (m, s), 1447 (s, s), 1221 (m, s), 1171 (m, s), 1071 (m, s), 999 (w, s), 918 (s, s), 751 (s, s), 716 (s, s), 694 (s, s), 651 (s, s), 498 (w, s).

(2) Synthesis of IIa,b from 1,4-Diiodobutane and *ortho*-Carborane. In a process similar to the preparation of **Ia**, 3.88 g of **IIa** was synthesized in 86% yield from 2.10 g (13.27 mmol) of 1-Me-*closo*-$C_2B_{10}H_{11}$, 8.70 mL (13.92 mmol) of *n*-BuLi (1.6 M in hexanes), and 1.86 mL (13.96 mmol) of 1,4-diiodobutane after purification by TLC as described above. In the same way, **IIb** was produced in 90% yield (2.47 g) from 1.50 g (6.81 mmol) of 1-Ph-*closo*-$C_2B_{10}H_{11}$, 4.38 mL (7.01 mmol) of *n*-BuLi (1.6 M in hexanes), and 0.93 mL (6.98 mmol) of 1,4-diiodobutane. The NMR spectra of **IIa** and **IIb** obtained by the two methods were identical.

Synthesis of IIIa,b. A 2.00-g (5.88 mmol) sample of **IIa**, or a 2.26-g (5.62 mmol) sample of **IIb**, was mixed with 3.90 g (59.39 mmol) of sodium azide and 120 mL of HPLC grade acetone and refluxed in the dark for 3 days in a 250-mL three-necked round-bottom flask equipped with a magnetic stirring bar. After cooling to room temperature, all solvents were removed by pumping, and the residue was extracted with diethyl ether. The diethyl ether was then removed from the extract to give the crude product that was later purified by TLC (SiO_2, developed with *n*-pentane/ethyl acetate in 5:1 ratio) to produce 1.31 g of 1-Me-2-$(CH_2)_4N_3$-1,2-$C_2B_{10}H_{10}$ (**IIIa**) (87% yield) or 1.59 g of 1-Ph-2-$(CH_2)_4N_3$-1,2-$C_2B_{10}H_{10}$ (**IIIb**) (89% yield) as colorless waxy solids.

IIIa: Elemental Anal: Calcd for $C_7H_{21}B_{10}N_3$: C, 32.92; H, 8.29; N, 16.46. Found: C, 32.88; H, 8.26; N, 16.40. ^{1}H NMR ($CDCl_3$, relative to $SiMe_4$, ppm): δ 3.25 (t, 2H, −CH_2N$_3$), 2.90−1.20 (m, br, 19H, −(CH_2)$_3$−C$_{cage}$, B$_{10}H_{10}$). ^{13}C NMR ($CDCl_3$, relative to $SiMe_4$, ppm): δ 79.01, 76.12 (*C$_{cage}$*); 52.22 (−$CH_2CH_2CH_2CH_2N_3$); 36.18 (−CH_2-$CH_2CH_2CH_2N_3$); 29.83 (−$CH_2CH_2CH_2CH_2N_3$); 28.21 (−$CH_2CH_2CH_2$-CH_2N_3); 24.49 (C$_{cage}$−*C*H$_3$). ^{11}B NMR ($CDCl_3$, relative to BF_3·OEt_2, ppm): δ −3.82 (1B, $^1J_{BH}$ = 167 Hz); −5.08 (1B, $^1J_{BH}$ = 157 Hz); −8.25 (2B, $^1J_{BH}$ = 94 Hz); −9.05 (2B, $^1J_{BH}$ = 112 Hz); −10.06 (4B, $^1J_{BH}$ = 149 Hz). IR (film on KBr, cm^{-1}) 3404 (vw, br), 2941 (s, s), 2872 (m, s), 2589 (vs, s, ν_{BH}), 2253 (w, s), 2098 (vs, s, $\nu_{N\equiv N}$), 1455 (s, s), 1383 (w, s), 1283 (s, s), 1256 (s, s), 1178 (w, s), 1026 (m, s), 923 (s, s), 749 (s, s), 650 (s, s), 556 (w, s).

IIIb: Elemental Anal: Calcd for $C_{12}H_{23}B_{10}N_3$: C, 45.40; H, 7.30; N, 13.24. Found: C, 45.29; H, 7.26; N, 13.20. ^{1}H NMR ($CDCl_3$, relative to $SiMe_4$, ppm): δ 7.60−7.30 (m, 5H, C_6H_5); 3.30−1.20 (m, br, 18H, −(CH_2)$_4$−C$_{cage}$, B$_{10}H_{10}$). ^{13}C NMR ($CDCl_3$, relative to $SiMe_4$, ppm): δ 131.06, 130.68, 130.57, 128.90 (*C*$_6$H$_5$); 83.57, 81.76 (*C$_{cage}$*); 50.59 (−CH_2N_3), 34.43 (−$CH_2CH_2CH_2CH_2N_3$); 28.11 (−$CH_2CH_2CH_2$-CH_2N_3); 26.52 (−$CH_2CH_2CH_2CH_2N_3$). ^{11}B NMR ($CDCl_3$, relative to BF_3·OEt_2, ppm): δ −3.48 (2B, $^1J_{BH}$ = 148 Hz); −9.50 (2B, $^1J_{BH}$ = 80 Hz); −10.19 (6B, $^1J_{BH}$ = 78 Hz). IR (film on KBr, cm^{-1}) 2938 (m, s), 2871 (m, s), 2587 (vs, s, ν_{BH}), 2254 (w, s), 2098 (vs, s, $\nu_{N\equiv N}$), 1493 (m, s), 1452 (s, s), 1350 (m, s), 1282 (s, s), 1189 (w, s), 1066 (m, s), 919 (s, s), 754 (s, s), 652 (s, s).

Synthesis of IVa,b. An 80.00-mg sample of pretreated SWCNTs was placed in a dry 100-mL three-necked round-bottom flask, equipped with a magnetic stirring bar and reflux condenser, suspended in 60 mL of 1,2-dichlorobenzene and irradiated in an ultra sonic bath for 30 min. At this point, 1.50 g (5.87 mmol) of **IIIa** was added, and the mixture was refluxed for 1 week. The solvent was removed, and the resulting residue was washed with *n*-hexane (5 × 30 mL) and then dried in high vacuum for 3 days, resulting in 93.30 mg of {(1-Me-2-[$(CH_2)_4N$═]-1,2-$C_2B_{10}H_{10})_n$(SWCNT)} (**IVa**). This yield gave a loading of 0.73 mmol of carborane per gram of SWCNTs or 9.67 × 10^2 carborane cages per SWCNT (based on the mass of a typical 1-μm-long and about 1-nm-diameter nanotube as 2.2 × 10^{-18}g). ^{1}H NMR (C_6D_6, relative to $SiMe_4$, ppm): δ 3.50−0.80 (m, br, 21H, −(CH_2)$_4$−C$_{cage}$, C*H*$_3$−C$_{cage}$, B$_{10}H_{10}$). ^{13}C NMR (C_6D_6, relative to $SiMe_4$, ppm): δ 66.66 (−$CH_2CH_2CH_2CH_2N$═); 37.81 (−$CH_2CH_2CH_2CH_2N$═); 29.42 (−$CH_2CH_2CH_2CH_2N$═); 27.90 (−$CH_2CH_2CH_2CH_2N$═); 22.76 (C$_{cage}$−*C*H$_3$) (Here, medium ^{13}C chemical shifts are used to describe broad peaks). ^{11}B NMR (C_6D_6, relative to BF_3·OEt_2, ppm): δ −5.01 (br); −9.99 (br); −10.45 (br). IR (KBr pellet, cm^{-1}) 3413 (w, br), 2940 (s, s), 2865 (m, s), 2582 (vs, s, ν_{BH}), 1704 (w, s), 1648 (m, br), 1558 (m, br), 1440 (s, s), 1363 (s, br), 1175 (m, s), 1020 (s, s), 946 (m, s), 728 (s, s), 678 (s, s), 501 (w, br).

In a procedure identical to the one described above for the preparation of **IVa**, 98.80-mg functionalized SWCNTs {(1-Ph-2-[$(CH_2)_4N$═]-1,2-$C_2B_{10}H_{10})_n$(SWCNT)} (**IVb**) were obtained from the reaction involving 1.83 g (5.76 mmol) of **IIIb**, 80.0 mg of pretreated SWCNTs, and 66 mL of 1,2-dichlorobenzene. The amount of carborane cage loading was calculated to be 0.81 mmol of carborane per gram of SWCNTs or 1.07 × 10^3 carborane cages per SWCNT. ^{1}H NMR (C_6D_6, relative to $SiMe_4$, ppm): δ 7.30−6.40 (m, br, 5H, C_6H_5); 4.10−0.80 (m, br, 18H, −(CH_2)$_4$−C$_{cage}$, B$_{10}H_{10}$). ^{13}C NMR (C_6D_6, relative to $SiMe_4$, ppm): δ 129.40, 129.26, 127.68, 127.28 (*C*$_6$H$_5$); 66.65 (−$CH_2CH_2CH_2CH_2N$═); 37.81 (−$CH_2CH_2CH_2CH_2N$═); 28.80 (−$CH_2CH_2CH_2CH_2N$═); 22.76 (−$CH_2CH_2CH_2CH_2N$═) (Here, medium ^{13}C chemical shifts are used to describe broad peaks). ^{11}B NMR (C_6D_6, relative to BF_3·OEt_2, ppm): δ −3.94 (br); −11.33 (br). IR (KBr pellet, cm^{-1}): 3439 (w, br), 3062 (w, s), 2929 (s, br), 2862 (m, s), 2575 (vs, s, ν_{BH}), 1648 (w, s), 1580 (m, br), 1494 (m, s), 1446 (s, s), 1367 (m, br), 1183 (w, br), 1109 (w, s), 1065 (m, s), 1032 (m, s), 1002 (m, s), 931 (w, s), 882 (w, s), 802 (w, s), 756 (m, s), 729 (s, s), 691 (s, s), 571 (w, br), 495 (w, s).

Synthesis of Va,b. A 2.00-g sample of sodium hydroxide was dissolved in 60 mL of 95% ethanol, and the resulting solution was added to 60.00 mg of **IVa** or 60.00 mg of **IVb** with constant stirring using an ultrasonic bath for 30 min. The resulting mixture was heated to reflux for 3 days and cooled to 0 °C, and the solution was then neutralized with aqueous HCl to a pH equal to about 5.0 to remove any unreacted NaOEt. Removal of all the volatiles under reduced pressure and washing with small amounts of cold water to remove sodium chloride produced a residue that was dried in vacuo for 3 days to yield 62.0 mg of {([Na^+][1-Me-2-($(CH_2)_4$NH-)-1,2-$C_2B_9H_{10}$][OEt])$_n$-(SWCNT)} (**Va**) or 62.3 mg of {([Na^+][1-Ph-2-($(CH_2)_4$NH-)-1,2-$C_2B_9H_{10}$][OEt])$_n$(SWCNT)} (**Vb**).

Va: ^{1}H NMR (DMSO-d_6, relative to $SiMe_4$, ppm): δ 3.22 (br, 2H, −OCH_2−); 2.62 (br, 2H, −CH_2−NH); 2.30 to −0.40 (m, br, 21H, −(CH_2)$_3$−C$_{cage}$, C*H*$_3$−C$_{cage}$, B$_9H_9$, −OCH$_2$C*H*$_3$); −2.50 to −3.30 (br, 1H, BH_{bridge}). ^{13}C NMR (DMSO-d_6, relative to $SiMe_4$, ppm): δ 60.81 (br), 56.00 (br) (*C$_{cage}$*); 55.98 (−O*C*H$_2$−); 35.02, 27.10, 21.83 (−$CH_2CH_2CH_2CH_2NH$−, CH$_3$−C$_{(cage)}$, 2C is covered by DMSO-d_6 peaks); 18.80 (−OCH$_2$*C*H$_3$). (Here, medium ^{13}C chemical shifts are used to describe broad peaks). ^{11}B NMR (DMSO-d_6, relative to BF_3·OEt_2, ppm): δ −11.40, −19.69, −35.63, −38.22 (br). IR (KBr pellet, cm^{-1}) 3577 (s, br), 3213 (s, s), 2930 (s, s), 2866 (s, s), 2514 (vs, s, ν_{BH}), 1610 (s, s), 1453 (s, br), 1196 (m, s), 1023 (m, br), 799 (w, br), 751 (w, br).

Vb: ^{1}H NMR (DMSO-d_6, relative to $SiMe_4$, ppm): δ 7.50−6.70 (m, br, 5H, C$_6H_5$); 3.46 (br, 2H, −OCH_2−); 2.60−0.30 (m, br, 20H, −(CH_2)$_4$−C$_{cage}$, B$_9H_9$, −OCH$_2$C*H*$_3$); −1.80 to −2.80 (br, 1H, BH_{bridge}). ^{13}C NMR (DMSO-d_6, relative to $SiMe_4$, ppm): δ 131.36, 127.13. 125.69 (*C*$_6$H$_5$); 67.10, 62.92 (*C$_{cage}$*); 55.99 (−O*C*H$_2$−); 35.08, 32.94, 27.20, and 26.82 (−$CH_2CH_2CH_2CH_2NH$−); 18.50 (−OCH$_2$*C*H$_3$). (Here, medium ^{13}C chemical shifts are used to describe broad peaks). ^{11}B NMR (DMSO-d_6, relative to BF_3·OEt_2, ppm): δ −10.90, −19.58, −35.79, −38.91 (br). IR (KBr pellet, cm^{-1}) 3582 (s, br), 3218 (s, br), 2933 (s, s), 2860 (m, s), 2515 (vs, s, ν_{BH}), 1600 (s, br), 1491 (s, s), 1443 (s, s), 1378 (w, s), 1180 (w, s), 1034 (m, s), 873 (w, s), 761 (m, s), 701 (s, s), 487 (w, s).

Tissue Distribution. The biodistributions of **Va** in both saline and dimethyl sulfoxide (DMSO) solvents were measured using six-week-old female BALB/c mice (provided by Shanghai Pharmaceutical Institute) in a method similar to that of the literature.[26] The mice were housed and treated humanely under standard conditions. EMT6 tumor

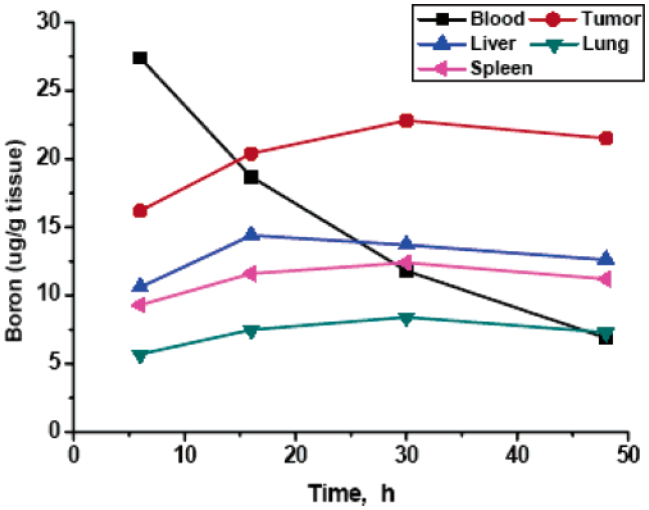

Figure 1. Boron tissue distributions of **Va** in saline.

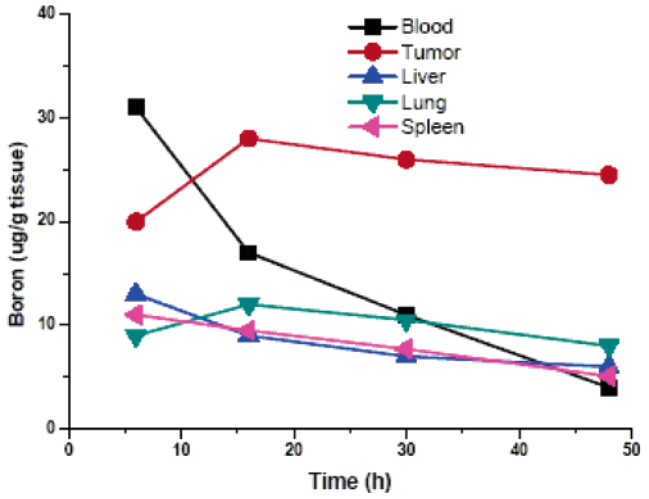

Figure 2. Boron tissue distributions of **Va** in DMSO.

cells, a mammary carcinoma,[27] were then transplanted into the right flank of the young female BALB/c mice of ~20-g body weight one week before testing. A 200 μL of a saline solution of **Va** at a concentration of 23 mg/mL, or 200 μL of a DMSO solution of **Va** at a concentration of 50 mg/mL, was slowly injected into the tail vein of the mice. For comparison, four tissues, tumor, blood, lung, liver, and spleen samples were collected and analyzed with ICP-OES. The mice were anesthetized (diethyl ether) and bled into heparinized syringes via cardiac puncture before surgery to collect blood in a method described in the literature.[28] The collected blood was then placed into tared cryogenic tubes and kept frozen at −70 °C. The mice were later sacrificed via cervical dislocation while anesthetized. The tumor and organs samples (liver, lung, and spleen) were collected, placed in tared cryogenic tubes, and kept frozen at −70 °C before being subjected to analysis with ICP-OES. The results are shown in Figures 1 and 2, for saline and DMSO, respectively. Each data point represents the average of five mice. For clarity, error bars are not shown in the graphical data; standard deviations were typically ~5−15% of the average values.

Results and Discussion

Syntheses and Spectra. The sequence of reactions leading to the side-wall functionalization of the SWCNTs with the substituted carborane units is shown in Scheme 1. The formation of the lithium salt of [1-R-*closo*-1,2-$C_2B_{10}H_{10}$]$^-$ (R = Me, Ph) followed literature procedures.[29] The monolithium compound was not isolated but was reacted, in situ, with 1-chloro-4-iodobutane to give a mixture of 1-R-2-$(CH_2)_4$Cl-1,2-$C_2B_{10}H_{10}$ and 1-R-2-$(CH_2)_4$I-1,2-$C_2B_{10}H_{10}$ in Cl/I molar ratios of 1:0.89 and 1:0.82 for R = Me and Ph, respectively. The approximately equimolar ratios of the two alkyl halides reflect the extremely high reactivity of the monolithium carborane. Because of this halide distribution, an extra step, that of converting the chloride to the iodide by refluxing with NaI, had to be introduced. Although this step was lengthy (3 days), the reaction did not adversely affect the overall yields of **IIa**,**b** (92−95%). Compounds **IIa**,**b** were also synthesized using 1,4-diiodobutane instead of 1-chloro-4-iodobutane. The methods are comparable, with 1,4-diiodobutane giving slightly lower yields of **IIa** and **IIb** (86 and 90%, respectively). However, because of the length of the former synthesis, the latter method would be preferable for standard syntheses of **IIa** and **IIb**. The subsequent conversion of the alkyl iodide to the corresponding azides (**IIIa**,**b**) proceeded in high yields (87−89%). The precursors **IIa**,**b** and **IIIa**,**b** were characterized by chemical analysis, infrared spectra, and NMR spectra. All data are consistent with the formulations shown in Scheme 1. The ^{13}C NMR spectra show the presence of the carborane cage carbons at δ 73−83 ppm, which is in the range of the reported C_{cage} resonances of other C_2B_{10} systems,[29] in addition to those of the C_{cage}-substituted moieties. The ^{11}B NMR spectra are also in accord with literature values.[29,30] In addition to showing infrared peaks at 2587 cm^{-1}, assigned to the B−H bond stretching, compounds **IIIa** and **IIIb** show strong absorptions at 2098 cm^{-1} due to the N═N stretching mode of vibrations (Figure 3). The attachment of the substituted carborane units to the SWCNTs was accomplished by the cycloaddition reaction of the nitrenes, **IIIa**,**b**, to the side walls of the SWCNTs, through thermally induced N_2 extrusion (see Scheme 1). This is a standard method for attaching groups to the side walls of SWCNTs.[2b,31] The loading of the carborane cage per gram of SWCNTs is 0.73 and 0.81 mmol for **IVa** and **IVb**, respectively. The absence of the N═N absorption bands in the IR spectra of **IV** confirm successful attachment of the carborane moiety to the SWCNTs. The ^{13}C NMR spectra of **IVa** and **IVb** show a shift of about 14.4 and 16.1 ppm in the resonances of the carbons α to the nitrogen atoms at δ = 66.66 and 66.65 ppm, respectively, which is in the range for carbons bonded to sp^3-hybridized nitrogen atoms. In addition, there is a significant broadening of the peaks in the ^{11}B NMR spectra, which has been observed for other groups on attachment to CNTs.[31] The possibility of a [3 + 2] cycloaddition of the azides to SWCNTs is excluded in our experiments by the long-term refluxing at high temperature. This was confirmed by the absence of −N═N−N− absorption in their IR spectra (Figure 3) and the presence of N_2 as a product. The carborane-functionalized SWCNTs were analyzed by SEM and TEM (see images in the Supporting Information). The functionalized SWCNTs are apparently bundled, which may partially be caused by aggregation of the

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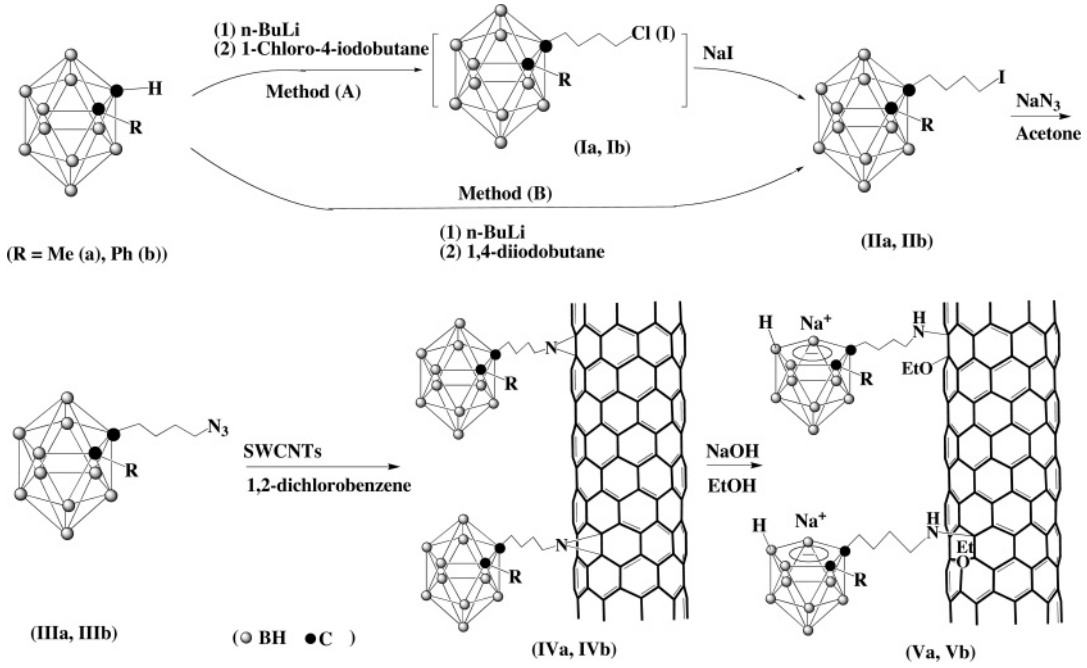
Scheme 1. Syntheses of Substituted Carborane-Appended SWCNTs



functionalized SWCNTs and sample preparation process. Although it was not possible to judge by TEM if the functional groups were covalently attached to the tubes, the solubility of the material in organic solvents, the NMR, IR, and ICP-OES data are decisive arguments in support of the carborane functionalization of the tubes.

The carborane cages attached to SWCNTs can be decapitated by refluxing for 3 days with alcoholic base to produce the water-soluble SWCNTs, **Va**,**b**. Compounds **Va**,**b** were found to be soluble in polar and moderately polar solvents such as DMSO, tetrahydrofuran, water, acetone, and dimethylformamide. For example, **Va** could be dissolved in water and DMSO to a concentration of 24 and 53 mg/mL, respectively. The NMR spectra of **Va**,**b** in DMSO-d_6 are clear enough to confirm that the three-membered ring formed by nitrene and SWCNTs has been opened by an ethoxide ion to give an ethoxo- and amido sidewall-functionalized SWCNTs. The ^{1}H NMR spectra of **Va**,**b** show the presence of the ethoxy groups and the broad B−H−B resonances at δ −2.50 to −3.30 ppm and −1.80 to −2.80 ppm for **Va** and **Vb**, respectively. All of these data are consistent with the successful syntheses of a hitherto unknown set of stable, water-soluble, carborane-appended SWCNTs, and thus this constitutes a new synthetic approach that can be extended to other systems of practical significance.

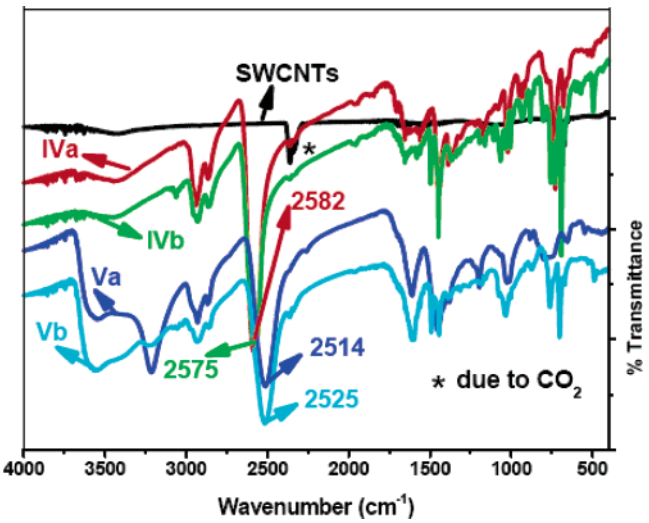


Figure 3. IR spectra of **IVa**, **IVb**, **Va**, **Vb**, and SWCNTs.

2. Biological Results. Tissue distribution studies were conducted as a function of time after tail injection of **Va**, dissolved in both saline and DMSO solvents, in mice using a literature method.[26] In brief, EMT6 tumor cells, a mammary carcinoma, were transplanted into the right flank of the young female BALB/c mice of ~20 g body weight one week before testing. Boron concentrations in blood and four tissues (tumor, lung, liver and spleen) were analyzed to gauge the relative advantage of SWCNTs delivery. Typical time course tissue distribution experiments examined tissue boron concentration at four time points over 48 h. The saline solution results, shown in Figure 1, demonstrate that maximum boron concentrations in the tumor (22.8 μg(boron/g(tissue))) were achieved after 30 h, then dropped very slowly until, after 48 h, the value was 21.5 μg(boron)/g(tissue), which is slightly lower than the desired value of 30 μg(boron)/g tumor for an effective BNCT. Interestingly, the boron concentration in blood drops rapidly and reaches a value of 6.9 μg(boron)/g(tissue) to give a tumor-to-blood ratio of 3.12, which is favorable for BNCT. The low boron concentration in the other tissues shows that there is a preferential uptake of **Va** by the tumor cells with a long retention time of over 48 h. This is the most important requirement of a successful BNCT drug. The same general results were found in DMSO solutions; there is enhanced boron uptake and retention by tumor cells of the carboranes attached to SWCNTs (see Figure 2). The main difference is that in DMSO the carborane is assimilated faster (a maximum concentration of 27.9 μg(boron)/g(tissue) in 16 h versus 22.8 μg(boron)/g(tissue) in 30 h in saline). The long-time (48 h) tumor-to-blood boron ratio in DMSO is 6.13, which is significantly greater than that

found in water. Given that the DMSO solution is more concentrated in **Va** than the saline solution (50 versus 23 mg/mL) the increased rate of boron uptake and retention is not suprising. In view of the fact that a number of studies show that unbound borane and carborane anions show no preferential absorption or retention in tumor cells,[26d,32] the use of SWCNTs as boron delivery vehicles shows promise. The actual mechanism of the accumulation of carborane-modified SWCNTs in tumors has not yet been determined. It could be the result of the increased and immature vasculature of the rapidly growing tumor cells. It has been shown that nonspecific hydrophobic bonding exists between nanotubes and proteins.[33] The nanotubes could nonspecifically associate with hydrophobic regions of the cell surfaces and then be internalized by endocytosis, and such a mechanism has been found in HL60 cells and in a number of other cell lines.[9c] The phenomenon of the enhanced permeability and retention effect (EPR), due to the increased vascular permeability and a decrease in the lymphatic drainage system in tumor cells, has been recognized as a general effect leading to the passive accumulation of macromolecular drugs in tumor cells.[34] It could well be that such an effect is operable in the accumulation of the **Va** in tumor cells. Whatever the mechanism, the data in Figures 1 and 2 clearly demonstrate the enhanced accumulation and retention of the carborane-attached SWCNTs in tumor tissue, compared to blood and to the other tissues tested.

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Conclusions

It has been shown that it is possible to link the substituted *nido*-carborane units to the side walls of SWCNTs. The resulting water-soluble nanotubes have been found to be tumor-specific and thus absorbed preferentially by EMT6 tumor cells. These results indicate that a further investigation of these functionalized SWCNTs as effective boron delivery agents for BNCT in cancer treatment is warranted. More complete biodistribution studies and cytotoxicity studies based on cell culture are needed. Such studies are currently underway in our laboratory.

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Supporting Information Available: NMR, IR, and UV−vis spectra of **I**−**V** and SEM, TEM of **IVa**. This material is available free of charge via the Internet at http://pubs.acs.org.

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