Abstract: Substituted C$_2$B$_{10}$ carborane cages have been successfully attached to the side walls of single-wall carbon nanotubes (SWCNTs) via nitrene cycloaddition. The decapitations of these C$_2$B$_{10}$ 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 \textit{nido}-C$_2$B$_{10}$ 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-Me-2-(CH$_2$)$_4$NH-]-1.2-C$_2$B$_{10}$H$_{10}$][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 nontoxic materials.\(^3-8\) Observations of enhanced water solubility of CNTs through side-wall derivatization 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...
in the cytoplasm of 3T6, 3T3 fibroblasts and phagocytic cells without showing obvious toxic effects.26 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.26 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$_3$B$_{10}$ carbanate 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 under nitrogen just before use. 1-Methyl- and 1-phenyl-sodium iodide, and all other reagents (Aldrich), including organic solvents, were used as received. 1-Methyl- and 1-phenyl-

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In a process similar to that described above for the synthesis of Ia, 4.41 g (20.00 mmol) of 1-Ph-closo-C$_2$B$_{10}$H$_{12}$, 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 Analysis: Found for Ib: C, 24.71; H, 6.22. 1 H NMR (CDCl$_3$, relative to SiMe$_4$, ppm): δ 7.60–7.70 (7H, C$_6$H$_5$), 3.30–3.40 (2H, CH$_2$-). 13 C NMR (CDCl$_3$, relative to SiMe$_4$, ppm): δ 129.12 (for C$_6$H$_5$), 120.60 (C$_2$H$_3$), 51.06 (C$_2$H). IR (film on KBr, cm$^{-1}$): 3050 (s), 2983 (s), 2869 (m), 1296 (w), 1261 (s), 1221 (s), 1172 (s), 1022 (s), 947 (s), 789 (br), 729 (vs), 665 (w), 600 (w), 502 (s).
(2B, JHut = 150 Hz); −9.10 (2B, JHut = 112 Hz); −9.72 (6B, JHut = 107 Hz). IR (film on KBr, cm−1) 3437 (m, br), 2956 (w, s), 2587 (vs, s, νPH), 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 ortha-Carborane was synthesized in 86% yield from 2.10 g (13.27 mmol) of 1-Me-Substituted Carborane-Appended Water-Soluble SWCNTs, 694 (s, s), 651 (s, s), 498 (w, s).

Carborane. A mixture was refluxed for 1 week. The solvent was removed, and the residue was extracted with diethylthyl ether. The diethyl ether was then removed from the extract to give the crude product that was later purified by TLC (SiO2, developed with n-pentane/ethyl acetate in 5:1 ratio) to produce 1.31 g of 1-Me-2-(CH2)5N3-1,2-C2B10H10 (IIa) (87% yield) or 1.59 g of 1-Ph-2-(CH2)9N3-1,2-C2B10H10 (IIb) (89% yield) as colorless waxy solids.

IIa: Elemental Anal: Calc'd for C12H23B10N3: C, 32.92; H, 4.82; N, 16.53. Found: C, 32.88; H, 8.26; N, 16.20. 1H NMR (CDCl3, relative to SiMe4, ppm): δ 3.25 (2H, −CH2N3), 2.90–1.20 (m, br, 19H, −(CH2)n−Cage, B2H10). 13C NMR (CDCl3, relative to SiMe4, ppm): δ 79.01, 76.12 (Cage), 52.22 (−CH2CH2CH2CH2N3); 36.18 (−CH2CH2CH2CH2N3); 29.83 (−CH2CH2CH2CH2N3); 28.21 (−CH2CH2CH2CH2N3); 24.49 (−CH2−Cage). 1B NMR (CDCl3, relative to BF3·OEt2, ppm): δ −3.82 (1B, JHut = 167 Hz); −5.08 (1B, JHut = 157 Hz); −8.25 (2B, JHut = 94 Hz); −9.05 (2B, JHut = 112 Hz); −10.6 (4B, JHut = 149 Hz). IR (film on KBr, cm−1) 3404 (vw, br), 2941 (s, s), 2872 (m, s), 2589 (vs, s, νPH), 2253 (w, s), 2098 (vs, s, νPH), 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).

IIb: Elemental Anal: Calc'd for C12H23B10N3: C, 32.92; H, 4.82; N, 16.53. Found: C, 32.88; H, 8.26; N, 16.20. 1H NMR (CDCl3, relative to SiMe4, ppm): δ 3.25 (2H, −CH2N3), 2.90–1.20 (m, br, 19H, −(CH2)n−Cage, B2H10). 13C NMR (CDCl3, relative to SiMe4, ppm): δ 131.06, 130.68, 130.57, 128.90 (C3H3); 83.57, 81.76 (C2); 50.59 (−CH2−Cage); 34.43 (−CH2CH2CH2CH2N3); 28.11 (−CH2CH2CH2CH2N3); 26.52 (−CH2CH2CH2N3). 1B NMR (CDCl3, relative to BF3·OEt2, ppm): δ −3.48 (2B, JHut = 148 Hz); −9.50 (2B, JHut = 80 Hz); −10.19 (6B, JHut = 78 Hz). IR (film on KBr, cm−1) 2938 (m, s), 2871 (m, s), 2587 (vs, s, νPH), 2254 (w, s), 2098 (vs, s, νPH), 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. 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-((CH2)2NH)-1,2-C2B10H10][OEt2]·(SWCNT) (Va) or 62.3 mg of [(Na+)1-Ph-2-((CH2)2NH)-1,2-C2B10H10][OEt2]·(SWCNT) (Vb).

V: 1H NMR (DMSO-d6, relative to SiMe4, ppm): δ 3.22 (2H, −OCH2−); 2.62 (2H, −CH2−N2−H2); 2.30 to −0.40 (m, br, 21H, −(CH2)n−Cage, CH−Cage, B2H10, −OCH2CH2−); −2.50 to −3.30 (1H, B2H2−Cage). 13C NMR (DMSO-d6, relative to SiMe4, ppm): δ 60.81 (br), 56.00 (br) (Cage); 55.98 (−OCH2−); 35.02, 27.10, 21.83 (−CH2CH2CH2CH2NH2−CH2−Cage). 2C is covered by DMSO-d6 peaks); 18.80 (−OCH2CH2−). (Here, medium 13C chemical shifts are used to describe broad peaks). 1B NMR (DMSO-d6, relative to BF3·OEt2, ppm): δ −11.40, −19.69, −35.63, −38.22 (br, IR (KBr pellet, cm−1) 3577 (br), 3213 (s, s), 2930 (s, s), 2866 (s, s), 2514 (vs, νPH), 1610 (s, s), 1453 (br, s), 1196 (m, s), 1023 (m, br), 799 (w, w), 751 (w, br).

Vb: 1H NMR (DMSO-d6, relative to SiMe4, ppm): δ 7.50−6.70 (m, br, 5H, C2H5); 3.46 (2H, −OCH2−); 2.60−0.30 (m, br, 20H, −(CH2)n−Cage, B2H10, −OCH2CH2−); 1.80 to −2.80 (1H, B2H2−Cage). 13C NMR (DMSO-d6, relative to SiMe4, ppm): δ 131.36, 127.13, 125.69 (C3H3); 67.10, 62.92 (Cage); 55.99 (−OCH2−); 35.02, 32.94, 27.20, and 26.82 (−CH2CH2CH2CH2NH−; 18.50 (−OCH2CH2−). (Here, medium 13C chemical shifts are used to describe broad peaks). 1B NMR (DMSO-d6, relative to BF3·OEt2, ppm): δ −10.90, −19.58, −35.79, −38.91 (br, IR (KBr pellet, cm−1) 3582 (br), 3218 (s, br), 2933 (m, s), 2680 (m, s), 2515 (vs, νPH), 1600 (s, s), 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. The mice were housed and treated humanely under standard conditions. EMT6 tumor
cells, a mammary carcinoma, 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 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-C2B10H10]− (R = Me, Ph) followed literature procedures. The monolithium compound was not isolated but was reacted, in situ, with 1-chloro-4-iodobutane to give a mixture of 1-R-2-(CH2)2Cl-1,2-C2B10H10 and 1-R-2-(CH2)2I-1,2-C2B10H10 in Cl/I molar ratios of 1.089 and 1.082 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-diodobutane instead of 1-chloro-4-iodobutane. The methods are comparable, with 1,4-diodobutane 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 (IIa,b) proceeded in high yields (87–99%). 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 13C 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 C2B10 systems, in addition to those of the C cage-substituted moieties. The 11B NMR spectra are also in accord with literature values. 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 N2 extrusion (see Scheme 1). This is a standard method for attaching groups to the side walls of SWCNTs. 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 13C 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 sp2-hybridized nitrogen atoms. In addition, there is a significant broadening of the peaks in the 11B NMR spectra, which has been observed for other groups on attachment to CNTs. 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=N absorption in their IR spectra (Figure 3) and the presence of N2 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

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 $\text{B-H-B}$ resonances at $\delta = -2.50$ 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.

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. In brief, EMT6 tumor cells, a mammary carcinoma, were transplanted into the right flank of the young female BALB/c mice of $20 \text{ g}$ 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 \mu g(boron)/g(tissue)) were achieved after 30 h, then dropped very slowly until, after 48 h, the value was $21.5 \mu g(boron)/g(tissue)$, which is slightly lower than the desired value of $30 \mu g(boron)/g$ tumor for an effective BNCT. Interestingly, the boron concentration in blood drops rapidly and reaches a value of $6.9 \mu g(boron)/g(tissue)$ 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 $27.9 \mu g(boron)/g(tissue)$ in 16 h versus $22.8 \mu g(boron)/g(tissue)$ 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

![Scheme 1. Syntheses of Substituted Carborane-Appended SWCNTs](image1)

![Figure 3. IR spectra of IVa, IVb, Va, Vb, and SWCNTs.](image2)
found in water. Given that the DMSO solution is more concentrated in $\text{Va}$ than the saline solution (50 versus 23 mg/mL) the increased rate of boron uptake and retention is not surprising. 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, 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. 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. 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. It could well be that such an effect is operable in the accumulation of the $\text{Va}$ 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.

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 $\text{I−V}$ and SEM, TEM of $\text{IVa}$. This material is available free of charge via the Internet at http://pubs.acs.org.

