Synthesis and in Vitro Evaluation of Boronated Uridine and Glucose Derivatives for Boron Neutron Capture Therapy

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Received October 16, 1991

The following boron-containing nucleoside and glucose derivatives have been synthesized as potential boron delivery agents for boron neutron capture therapy (BNCT): 2′-*O*-(*o*-carboran-1-ylmethyl)uridine (**4a**), 3′-*O*-(*o*-carboran-1-ylmethyl)uridine (**4b**), sodium 7-(uridin-2′-ylmethyl)dodecahydro-7,8-dicarba-*nido*-undecaborate (**5**), 5′-*O*-(*o*-carboran-1-ylmethyl)uridine (**9**), and 3′-*O*-(*o*-carboran-1-ylmethyl)-D-glucose (**13**). In vitro cellular uptake studies were performed with F98 rat glioma cells. Following 16 h incubation, cellular boron concentrations were determined by direct current plasma atomic emission spectroscopy (DCP-AES). Boron concentrations ranged from 65 to 103 μg/g of cells for the neutral closo structures compared with 1.5 μg/g of cells for the charged nido species. Cellular uptake of sodium mercaptoundecahydro-*closo*-dodecaborate (BSH), the compound currently being used in Japan for the treatment of malignant brain tumors by BNCT, was 2 μg/g of cells.

Boron neutron capture therapy (BNCT) of cancer is based on the nuclear reaction that occurs when boron-10 is irradiated with thermal neutrons to yield high-energy alpha particles and recoiling lithium-7 nuclei. In order for BNCT to succeed, it is necessary to have agents which possess tumor-targeting selectivity and can achieve boron-10 concentrations approaching 30 μg/g of tumor tissue.[1] Calculations have shown[2,3] that the therapeutic effectiveness will be 2–5 times greater if the compound is localized within the cell nucleus in contrast with the same boron-10 concentration uniformly distributed throughout the cell. This fact has been the rationale for the attempts to synthesize boron analogues of nucleic acid precursors. Such structures may achieve higher concentration differentials in rapidly proliferating malignant cells compared to the mitotically less active cells that make up most normal tissues. It needs to be mentioned that boron-containing nucleic acid analogues could accumulate as well in mitotically active normal hematopoitic, epidermal, and intestinal cells, which could cause significant problems during clinical BNCT.

Initial approaches concentrated on the synthesis of boron analogues of purine and pyrimidine bases.[4-11] However, many of these compounds were found to be too toxic or hydrolytically and biologically unstable, or they failed to become incorporated selectively into tumor cells or into nucleic acids. More recently, several boron-containing nucleosides and nucleotides have been synthesized with a borane,[12] cyanoborane,[13] dihydroxyboryl,[14,15] or carborane[16] group either linked to the phosphate[12] or the base[13-16] moiety. One of these compounds, 5-(dihydroxyboryl)-2′-deoxyuridine, is described as a competitive inhibitor in the phosphorylation of thymidine by thymidine kinase and is reported to replace thymidine to the extent of 5–15% in vitro.[17]

The first nucleoside containing the carborane cluster [2′-*O*-(*o*-carboran-1-ylmethyl)uridine] was synthesized in our laboratories.[18] The rationale for choosing a carborane cage as the boron moiety was a 10-fold increase in boron content compared with the above mentioned groups which contain just one boron atom. In addition, there is an increased lipophilicity due to the carborane moiety which could aid cellular penetration.

The premise for the synthesis of this carboranyl nucleoside was that such a structure would be preferentially

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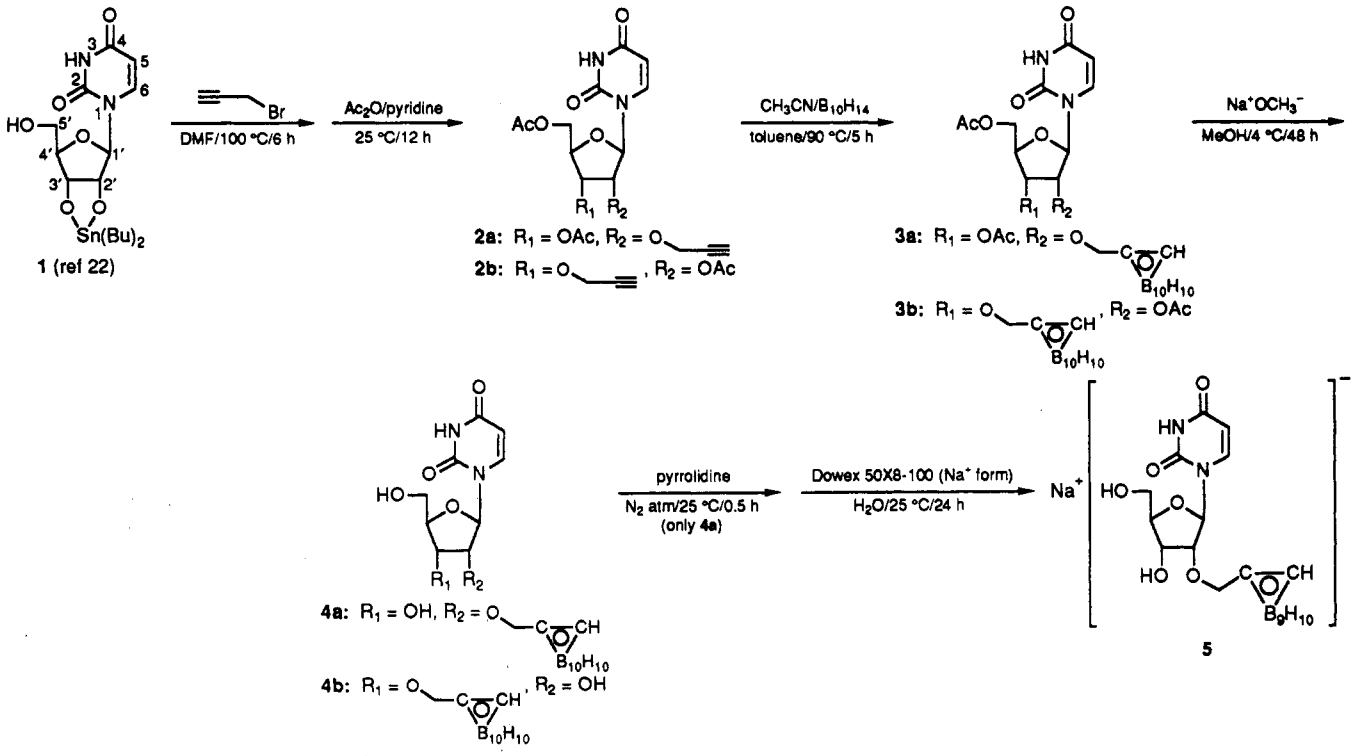
Scheme I



taken up by rapidly proliferating tumor cells through the action of the nucleoside transport system and become entrapped by the conversion to the corresponding 5′-monophosphate. Additional selectivity might be achieved if such a nucleotide were converted to the active precursors of the nucleic acids, namely the di- and triphosphates. Their incorporation into the tumor cell's nucleic acids could therefore arise by the action of appropriate polynucleotide ligases/polymerases. Initial cell culture studies have shown that this compound accumulates and persists in F98 glioma cells to a much greater extent than sodium mercaptoundecahydro-*closo*-dodecaborate ($Na_2B_{12}H_{11}SH$), the compound currently being used in Japan for the treatment of malignant brain tumors by BNCT.[19,20]

In order to correlate physicochemical properties with the biological parameters required for tumor cell penetration, incorporation, and persistence, we have now synthesized 3′-*O*-(*o*-carboran-1-ylmethyl)uridine, 5′-*O*-(*o*-carboran-1-ylmethyl)uridine, 3-*O*-(*o*-carboran-1-ylmethyl)-D-glucose, and the nido analogue of 2′-*O*-(*o*-carboran-1-ylmethyl)-uridine. It was our intention to obtain information of how the position of the carborane cage on the ribose portion, the presence of a negative charge, and the existence of the base moiety influenced the uptake of carboranyl nucleosides by conducting comparative in vitro uptake studies of these compounds with F98 glioma cells. In this report the synthesis of these compounds is described, and the results of the in vitro studies are presented.

Chemistry

C-Substituted *o*-carboranes are readily prepared from a bis-ligand decaborane derivative and the appropriate substituted acetylenes. However, acetylenes containing a free hydroxyl or amino group destroy the decaborane nucleus, failing to give any carborane products.[21]

The reaction of uridine derivatives containing a propynyl group attached to the oxygen of the 2′-, 3′-, or 5′-position with a bis(acetonitrile) decaborane complex results in the desired *o*-carborane-substituted uridines. This reaction only occurs when the hydroxyl functions of the ribose moiety are properly protected. The unprotected imide function on the pyrimidine does not cause significant degradation of the decaborane ligand.

The reaction sequences for the synthesis of *o*-carborane-containing uridines are shown in Schemes I and II. The dibutylstannylene function in 2′,3′-*O*-(dibutylstannylene)uridine (1)[22] (Scheme I) serves as an activating group for the 2′ and 3′ oxygen functions.[22] Thus, alkylation of 1 with propargyl bromide gives a mixture of 2′-*O*-(3-prop-1-ynyl)uridine and 3′-*O*-(3-prop-1-ynyl)uridine in a 3:2 ratio (as estimated by ^{1}H-NMR). The reaction was carried out in DMF at 100 °C over a period of 6 h, giving a total yield of 62% of the mixture after purification by column chromatography. Longer reaction times or higher temperatures increased the formation of side products

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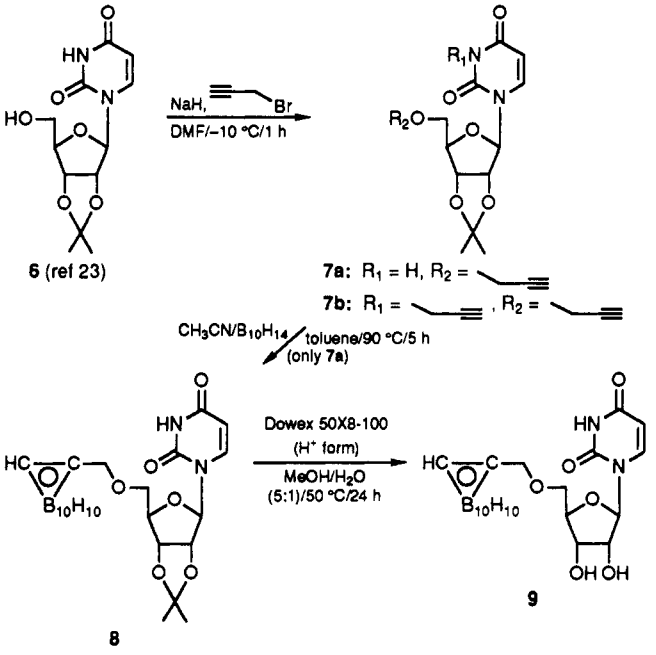
Scheme II

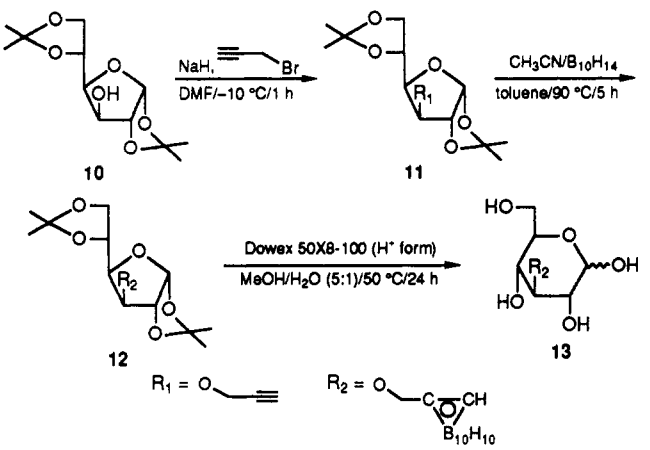


rather than improving the yield of the 2′- or 3′-O-alkylated products. Since 2′-*O*-(3-prop-1-ynyl)uridine and 3′-*O*-(3-prop-1-ynyl)uridine have the same R_f-values, they could not be separated either by column chromatography or crystallization. Acetylation resulted in a mixture of **2a** and **2b** (Scheme I) with a total yield of 81%; the molar ratio of both compounds was 61:39. The R_f-values for **2a** and **2b** were 0.45 and 0.37, respectively. The chromatographic separation and rigorous purification was difficult and time consuming because both compounds showed a tendency for tailing on silica gel with the solvent systems used.

The signals for the H-2′ and H-3′ protons in the ^{1}H-NMR spectrum (CD_3OD) of the mixture of 2′-*O*-(3-prop-1-ynyl)uridine and 3′-*O*-(3-prop-1-ynyl)uridine are part of a complex multiplet in the range from 4.0–4.5 ppm. The ^{1}H-NMR spectra of **2a** (R_f = 0.45) and **2b** (R_f = 0.37) display a doublet of a doublet for H-3′ at 5.22 ppm ($J_{2',3'}$ = 5.6 Hz, $J_{3',4'}$ = 4.0 Hz) and a doublet of a doublet for H-2′ at 5.29 ppm ($J_{1',2'}$ = 3.1 Hz, $J_{2',3'}$ = 5.4 Hz), respectively. The signals for the H-2′ proton of **2a** and the H-3′ proton of **2b** are at 4.49 ppm and in the region from 4.05–4.36 ppm, respectively, as a part of a complex multiplet. These significant downfield shifts of the H-3′ signal (**2a**) and the H-2′ signal (**2b**) from approximately 4.0–4.5 ppm to 5.22 ppm and 5.29 ppm, respectively, caused by O-acetylation at the 3′ or 2′ position, provide clear evidence that the compound with the R_f = 0.45 is in agreement with structure **2a**, and the compound with the R_f = 0.37 is in agreement with structure **2b**.

The reaction of 2′,3′-*O*-isopropylideneuridine (**6**)[23] (Scheme II) with a 1.5 molar excess of sodium hydride and propargyl bromide in DMF at −10 °C yielded a mixture of 43% **7a** (Scheme II), 24% **7b** (Scheme II), and 25% of unreacted **6**. The alkylation occurs predominantly at the 5′-O-position, but at the same time to a smaller extent at the 3-N-position. The use of equimolar amounts of reactants resulted in the formation of **7a** and **7b** in approximately the same molar ratio as for the reaction with a 1.5 molar excess, but with much reduced overall yield (29% of **7a**, 15% of **7b**, and 43% of **6**). Decrease of the reaction temperature to −20 °C had no significant influence on this ratio. Almost complete dialkylation could be achieved by using a 2 molar excess of sodium hydride and propargyl bromide. It is conceivable that a hindered, not as strong a base as sodium hydride can reduce the formation of **7b** and recovery of unreacted **6**. Experiments with such a base have not been carried out.

Scheme III



A comparison of the chemical shifts of the terminal alkyne protons and the methylene protons of the propynyl group of 2-(benzylthio)-4-*O*-(3-prop-1-ynyl)uracil[24] (2.51 and 4.95 ppm, respectively), 2-(benzylthio)-3-*N*-(3-prop-1-ynyl)uracil[24] (2.26 and 4.82 ppm, respectively), and the *N*-propynyl group of **7b** (2.16 and 4.69 ppm, respectively) indicates that in the dialkylated product, additional alkylation might have occurred at N-3 rather than O-4.

Compound **7b** appeared to be unstable even when it was stored under an inert-gas atmosphere at −20 °C over a period of 1–2 weeks. This is indicated by the formation of significant amounts of decomposition products, as confirmed by TLC. Since the compound **7b** was only a byproduct, no additional study was undertaken to confirm its structure or the characterization of its decomposition products. Compound **11** (Scheme III) was synthesized in 91% yield from 1,2:5,6-di-*O*-isopropylidene-D-glucose (**10**) (Scheme III) by analogy with **7a** and **7b**.

Compounds **3a** and **3b** (Scheme I) were synthesized by reacting the mixture of **2a** and **2b** with a bis(acetonitrile) decaborane complex in toluene. Both compounds were obtained in a ratio of 62/38 in 45% total yield. The difference in the R_f-values (0.59 for **3a** and 0.49 for **3b**) is comparable to the difference in the R_f-values of **2a** and **2b**. The chromatographic separation of the two boronated isomers is less difficult than the separation of their respective precursors because both compounds showed no tendency for tailing when appropriate solvent systems were used. Compounds **8** (Scheme II) and **12** (Scheme III) were synthesized by analogy with **3a** and **3b** in 70% and 77% yield, respectively.

It seems to us that the relatively low total yield for **3a** and **3b** is not due to a decomposition of decaborane resulting from the unprotected imide function of the base. If this were the case, we should obtain a yield in the same range for **8** as for **3a** and **3b**. It is more likely that the acetyl protective groups of the ribose portion have a limited stability under the reaction conditions required for the boronation which could result in an increased number of free hydroxyl functions, and subsequently an increase in the degradation of decaborane cages.

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The ^{1}H-NMR spectra ($CDCl_3$) of **2a**, **2b**, **7a**, **3a**, **3b**, and **8** display a doublet of a doublet for H-5 in the range of 5.6–5.9 ppm, caused through coupling with H-6 ($J_{5,6}$ = 8.1–8.2 Hz) and long range coupling with H-3 ($J_{3,5}$ = 2.0–2.3 Hz). These signals changed to doublets on deuteration of the NH proton by the addition of a drop of deuterated methanol to the $CDCl_3$ solutions.

Compounds **3a** and **3b** were almost quantitatively deacetylated by catalytic amounts of sodium methylate in methanol to yield the target compounds **4a** and **4b** which are not water soluble. Both products form stable diethyl ether adducts. However, the diethyl ether could be removed by heating the material for 1 hour at 100 °C under vacuum (0.5 mm).

The water-insoluble target compounds **9** (Scheme II) and **13** (Scheme III) were obtained in high yield by acidic hydrolysis of the isopropylidene protective groups of the corresponding precursors using strongly acidic ion-exchange resin (H^+ form). The removal of the *O*-isopropylidene groups from **12** resulted in the formation of **13** as an anomeric mixture. The product could not be characterized by mass spectroscopy (EI or FAB). This was expected for a glucose derivative such as **13** containing several hydroxyl functions.

The conversion of **4a** to the corresponding water-soluble nido compound **5** was achieved by stirring **4a** for 30 min at room temperature in pyrrolidine under N_2 atmosphere. The pyrrolidinium cation was replaced by a sodium cation by use of strongly acidic ion-exchange resin (Na^+ form). The sodium salt of **5** is soluble in such organic solvents as acetone, acetonitrile, ethanol, and methanol. It could be purified chromatographically on a silica gel column using dichloromethane/methanol 3:1 as a solvent system.

The sodium salt of **5** was analyzed by FAB-mass spectroscopy and elemental analysis. However, proton and carbon-13 NMR indicate the formation of two isomers in a 3:2 ratio. The formation of two isomeric forms of 7-phenyldodecahydro-7,8-dicarba-*nido*-undecaborate (−1), as the result of the basic degradation of 1-phenyl-*o*-carborane, has been reported by Hawthorne et al. in 1968.[25] The description of the separation and characterization of the two isomers of **5** will be the subject of a subsequent publication.

Biological Studies

The usefulness of these boronated nucleoside and glucose derivatives as potential boron delivery agents for BNCT will ultimately depend upon their in vivo tumor-localizing properties and their ability to selectively deliver the requisite amounts of boron to tumors. The first step in evaluating this potential is the in vitro uptake by tumor cells. Cellular uptake studies were carried out with a well-characterized rat glioma clone, designated F98,[26] and the results of these studies are summarized in Table I. The carboranyl uridines **4a**, **4b**, and **9** are rapidly incorporated into F98 glioma cells, and persist at high levels for as long as 48 h following cell culture in boron-free medium.[19,20]

This is in marked contrast with **5** (the nido analogue of **4a**), $Na_2B_{12}H_{11}SH$, and boric acid, where the values are somewhat lower than would even be expected if the compounds were freely diffusible and concentrated based upon cellular water content. Since **5** is an anionic structure, it may be incapable of penetrating the tumor cell membrane, and this may account for the fact that its cellular concentration is 2 orders of magnitude lower than its closo counterpart **4a**.

Table I. Cellular Uptake of Boron by F98 Glioma Cells after Incubation with Different Boron Compounds

boron compounds	boron in media at 0 h (μg of B/mL)	cellular uptake of boron at 16 h (μg of B/g cells)
4a	6.75	103.5 ± 2.9[a]
4b	6.75	96.2 ± 5.4
5	6.75	1.5 ± 0.2
9	6.75	83.8 ± 5.1
13	6.75	65.2 ± 2.1
boric acid	6.75	1.9 ± 0.1
BSH[b]	6.75	2.0 ± 0.6
BSH	67.5	9.8 ± 0.3

[a] Mean ± SD values for all compounds have been calculated on basis of three experiments. [b] Sodium mercaptoundecahydro-*closo*-dodecaborate.

The difference in the values of **4a** and **4b** is negligible. It seems to be of no importance for the cellular uptake of a carboranyl uridine if the carborane cage is linked to the 2′- or 3′-position of the ribose portion. The uptake of **13** was 32–37% less than that of **4b** and **4a**, and that of **9** was 13–19%. This might be due to the possibility that the pyrimidine portion and 5′-phosphorylation play a role in the uptake of a carboranyl uridine. The 5′-position in **9** is blocked by the carborane cage and can therefore not be phosphorylated. However, the cellular uptake of all closo structures is high (compared to BSH), which cannot be explained by the mentioned reasons.

The results from our preliminary biological studies are not adequate to make any statement regarding a possible mechanism of cellular uptake but do clearly demonstrate rapid incorporation for all *closo*-carborane-containing compounds. Further work is now underway to delineate the operative biochemical mechanism by which this occurs.

Conclusion

The compounds described here represent a novel class of boron-containing nucleoside and glucose derivatives that were synthesized in acceptable yields from readily available starting materials. The reactions as well as the workup procedures and the purifications for all products were readily feasible. Only the separation of the isomers **2a** and **2b** appeared to be a significant problem. Compounds **4a**, **4b**, **9**, and **13** show a high uptake in F98 glioma cells. Compound **5** does not accumulate in this cell line. The intracellular distribution of these compounds, the biochemical basis for their incorporation and persistence, and the in vivo uptake in brain tumor bearing rats are currently under investigation. Results obtained from these studies will provide a more definitive answer as to the potential usefulness of these compounds for clinical BNCT.

Experimental Section

FT-NMR spectra (proton and carbon-13) were obtained at The Ohio State University Chemical Instrument Center using a Bruker AM500 and at the College of Pharmacy of The Ohio State University using a Bruker AC250. Chemical shifts (δ) were reported in ppm downfield from an internal tetramethylsilane standard. Coupling constants (J) are reported in hertz. IR spectra were recorded on a RFX 40 FT-IR spectrometer (Laser Precision Corp.). The spectra were in accordance with the proposed structures. Melting points were determined on a Fisher-Johns melting point apparatus and are reported uncorrected. Mass spectra were obtained at The Ohio State University Chemical Instrument Center by use of a FG 70-250S mass spectrometer.

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Ionization was through fast atom bombardment (FAB) with XE using 3-NBA (3-nitrobenzyl alcohol) or MB (Magic Bullit, a mixture of dithiothreitol and dithioerythreitol in a ratio of 3:1 or 5:1) as matrix compounds. For all boron-containing compounds the mass of the most intense peak of the isotope pattern is indicated; the measured patterns agreed with the theoretical ones. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN; Atlantic Microlab, Inc., Norcross, GA; Analytische Laboratorien, Gummersbach, Germany. Precoated TLC plates with silica gel 60 F_{254} (0.25 mm layer thickness) and silica gel 60 (70–230 mesh ASTM) from E. Merck were used for TLC and column chromatography, respectively.

3′,5′-Di-*O*-acetyl-2′-*O*-(3-prop-1-ynyl)uridine (2a) and 2′,5′-Di-*O*-acetyl-3′-*O*-(3-prop-1-ynyl)uridine (2b). A solution of 15.5 g (32.6 mmol) of 2′,3′-*O*-(dibutylstannylene)uridine (1)[22] and 5.8 mL (65.2 mmol) of propargyl bromide in 200 mL of DMF was stirred at 100 °C for 6 h. Following evaporation of the solvent, the remaining residue was purified by column chromatography (dichloromethane/methanol 7:1), giving a mixture (5.7 g, 62% yield) consisting of roughly 60% 2′-*O*-(3-prop-1-ynyl)uridine and 40% 3′-*O*-(3-prop-1-ynyl)uridine (as judged by ^{1}H-NMR). The mixture was dissolved in 15 mL of acetic anhydride and 60 mL of pyridine and stirred at room temperature for 12 h. The solution was evaporated to dryness, and the remaining mixture of the acetylated isomers was separated by column chromatography (dichloromethane/acetone 8:2). **2a**: yield 3.7 g (50%) of a clear glass-like compound; R_f 0.45 (dichloromethane/acetone 8:2); MS (FAB$^+$, 3-NBA) 367 (M + H)$^+$; ^{1}H-NMR ($CDCl_3$) δ 2.14 (s, 3 H, Ac), 2.16 (s, 3 H, Ac), 2.44 (t, 1 H, $CH_2C{\equiv}CH$, $J_{1,3propynyl}$ = 2.3), 4.29–4.43 (m, 6 H, H_2-5′, $CH_2C{\equiv}CH$, H-4′, H-2′), 5.08 (t [dd], 1 H, H-3′, $J_{2',3'}$ = 5.4, $J_{3',4'}$ = 5.4), 5.94 (d, 1 H, H-1′, $J_{1',2'}$ = 4.6), 7.49 (d, 1 H, H-6), 8.76 (br s, 1 H, H-3); ^{1}H-NMR (CD_3OD) δ 5.22 (dd, 1 H, H-3′, $J_{2',3'}$ = 5.6, $J_{3',4'}$ = 4.0), 4.49 (t [dd], 1 H, H-2′, $J_{1',2'}$ = $J_{2',3'}$ = 5.6), 5.92 (d, 1 H, H-1′, $J_{1',2'}$ = 5.6). Anal. ($C_{16}H_{18}N_2O_8$) C, H, N. **2b**: yield 2.3 g (31%) of a clear glass-like compound; R_f 0.37 (dichloromethane/acetone 8:2); MS (FAB$^+$, 3-NBA) 367 (M + H)$^+$; ^{1}H-NMR ($CDCl_3$) δ 2.06 (s, 3 H, Ac), 2.10 (s, 3 H, Ac), 2.41 (t, 1 H, $CH_2C{\equiv}CH$, $J_{1,3propynyl}$ = 2.3), 4.05–4.34 (m, 6 H), H_2-5′, $CH_2C{\equiv}CH$, H-3′, H-4′), 5.29 (dd, 1 H, H-2′, $J_{1',2'}$ = 3.1, $J_{2',3'}$ = 5.4), 5.69 (dd, 1 H, H-5, $J_{5,6}$ = 8.1, $J_{3,5}$ = 2.1), 5.79 (d, 1 H, H-1′), 7.40 (d, 1 H, H-6), 8.55 (br s, 1 H, H-3). Anal. ($C_{16}H_{18}N_2O_8$) C, H, N.

3′,5′-Di-*O*-acetyl-2′-*O*-(*o*-carboran-1-ylmethyl)uridine (3a) and 2′,5′-Di-*O*-acetyl-3′-*O*-(*o*-carboran-1-ylmethyl)uridine (3b). A solution of 1.7 g (14 mmol) of decaborane[27] and 2.2 mL (41 mmol) of acetonitrile in 150 mL of dry toluene was heated under reflux for 1 h. The solution was cooled to room temperature, and 5 g (13.7 mmol) of a mixture of 61% 3′,5′-di-*O*-acetyl-2′-*O*-(3-prop-1-ynyl)uridine (**2a**) and 39% 2′,5′-di-*O*-acetyl-3′-*O*-(3-prop-1-ynyl)uridine (**2b**) was added. This reaction mixture was heated for 4–5 h at 90 °C. The progress of the reaction was checked by TLC (dichloromethane/acetone 8:2). Following evaporation of the toluene, the remaining residue was taken up in diethyl ether and filtered, and the filtrate was evaporated to dryness. Compounds **3a** and **3b** were separated by column chromatography (dichloromethane/acetone 8:2). **3a**: yield 1.85 g (28%) of a clear glass-like compound; R_f 0.59 (dichloromethane/acetone 8:2); MS (FAB$^+$, 3-NBA) 485 (M + H)$^+$; ^{1}H-NMR ($CDCl_3$) δ 1.3–3.0 (br m, 10 H, B-H), 2.10 (s, 3 H, Ac), 2.14 (s, 3 H, Ac), 3.90 (br s, 1 H, $C_{carborane}$-H), 3.98 (a) 4.19 (b) (AB q, 2 H, H_2-5′, $J_{5a',5b'}$ = 10.7), 4.28 (m, 4 H, H-2′, H-4′, $OCH_2C_{carborane}$), 4.83 (dd, 1 H, H-3′, $J_{2',3'}$ = 7.5, $J_{3',4'}$ = 5.3), 5.74 (d, 1 H, H-1′, $J_{1',2'}$ = 2.4), 5.75 (d, 1 H, H-5, $J_{5,6}$ = 8.2), 7.55 (d, 1 H, H-6), 9.05 (br s, 1 H, H-3). Anal.[28] ($C_{16}H_{28}N_2O_6B_{10}$) H, N, C: calcd 39.66, found 40.45, B: calcd 22.31, found 20.77. **3b**: yield 1.13 g (18%) of a clear glass-like compound; R_f 0.49 (dichloromethane/acetone 8:2); MS (FAB$^+$, MB, DMSO) 485 (M + H)$^+$; ^{1}H-NMR ($CDCl_3$) δ 1.3–3.0 (br m, 10 H, B-H), 2.12 (s, 3 H, Ac), 2.18 (s, 3 H, Ac), 3.86 (br s, 1 H, $C_{carborane}$-H), 3.89 (a) 4.02 (b) (AB q, 2 H, H_2-5′, $J_{5a',5b'}$ = 10.7), 4.10–4.38 (m, 4 H, H-3′, H-4′, $OCH_2C_{carborane}$), 5.38 (dd, 1 H, H-2′, $J_{1',2'}$ = 2.8, $J_{2',3'}$ = 5.4), 5.62 (d, 1 H, H-1′), 5.74 (dd, 1 H, H-5, $J_{5,6}$ = 8.1, $J_{3,5}$ = 2.0), 7.31 (d, 1 H, H-6), 8.73 (br s, 1 H, H-3). Anal. ($C_{16}H_{28}N_2O_8B_{10}$) C, H, N, B.

2′-*O*-(*o*-Carboran-1-ylmethyl)uridine (4a). A 1-g (2.07 mmol) quantity of 3′,5′-di-*O*-acetyl-2′-*O*-(*o*-carboran-1-ylmethyl)uridine (**3a**) was dissolved in 50 mL of dry methanol, and 3 drops of a 2% solution of sodium methylate in dry methanol were added at 0 °C. The reaction mixture was left in a refrigerator at 4 °C for 2 days. The progress of the reaction was checked by TLC (dichloromethane/methanol 7:1). Subsequently, 10 g of Dowex 50X8-100 ion-exchange resin (H^+ form) was added at 0 °C to neutralize the solution. The resin was filtered off, and the filtrate was evaporated to dryness. The remaining residue was purified by column chromatography (dichloromethane/methanol 7:1) and redissolved in 20 mL of diethyl ether. After 30 min, long needle-shaped crystals began to precipitate, and 100 mL of hexane was added to complete the precipitation. The crystals were filtered off and dried for 1 h at 100 °C under vacuum (0.5 mm). **4a**: yield 770 mg (93%) of white crystals; mp 136–137 °C; MS (FAB$^+$, 3-NBA) 401 (M + H)$^+$; ^{1}H-NMR (CD_3OD) δ 1.30–3.00 (br m, 10 H, B-H), 3.74 (a) 3.89 (b) (d of AB q, 2 H, H_2-5′, $J_{5a',5b'}$ = 12.46, $J_{4',5a'}$ = 2.83, $J_{4',5a'}$ = 2.36), 3.97–4.21 (m, 3 H, H-2′, H-3′, H-4′), 4.20 (a) 4.27 (b) (AB q, 2 H, $CH_2C_{carborane}$, $J_{a,b}$ = 11.3), 4.68 (br s, 1 H, $C_{carborane}$H), 5.67 (d, 1 H, H-5, $J_{5,6}$ = 8.1), 5.84 (d, 1 H, H-1′, $J_{1',2'}$ = 3.17), 8.05 (d, 1 H, H-6). Anal. ($C_{12}H_{24}N_2O_6B_{10}$) C, H, N, B.

3′-*O*-(*o*-Carboran-1-ylmethyl)uridine (4b). A 1-g (2.07 mmol) quantity of 2′,5′-di-*O*-acetyl-3′-*O*-(*o*-carboran-1-ylmethyl)uridine (**3b**) was reacted with catalytic amounts of sodium methylate as described for 3′,5′-di-*O*-acetyl-2′-*O*-(*o*-carboran-1-ylmethyl)uridine (**3a**). **4b**: purification by column chromatography (dichloromethane/methanol 7:1); yield 780 mg (94%) of white crystals; mp 202–204 °C; MS (FAB$^+$, MB) 401 (M + H)$^+$; ^{1}H-NMR (CD_3OD) δ 1.30–3.00 (br m, 10 H, B-H), 3.65 (a) 3.74 (b) (d of AB q, 2 H, H_2-5′, $J_{5a',5b'}$ = 12.2, $J_{4',5a'}$ = 2.92, $J_{4',5b'}$ = 3.12), 4.03 (a) 4.23 (b) (AB q, 2 H, $CH_2C_{carborane}$, $J_{a,b}$ = 11.1), 3.95–4.27 (m, 3 H, H-2′, H-3′, H-4′), 4.62 (br s, 1 H, $C_{carborane}$-H), 5.65 (d, 1 H, H-5, $J_{5,6}$ = 8.1), 5.83 (d, 1 H, H-1′, $J_{1',2'}$ = 5.85), 7.87 (d, 1 H, H-6). Anal. ($C_{12}H_{24}N_2O_6B_{10}$) C, H, N, B.

Sodium 7-(Uridin-2′-ylmethyl)dodecahydro-7,8-dicarba-*nido*-undecaborate (5). A 500-mg (1.25 mmol) quantity of 2′-*O*-(*o*-carboran-1-ylmethyl)uridine (**4a**) was dissolved in 10 mL of pyrrolidine and stirred for 30 min at room temperature under N_2 atmosphere. The pyrrolidine was evaporated, and the remaining residue was added to 80 g of Dowex 50X8-100 ion-exchange resin (Na^+ form) in 150 mL of double-distilled H_2O. After stirring for 24 h at room temperature, the resin was filtered off and the filtrate was evaporated to dryness. The resulting residue was purified by column chromatography (dichloromethane/methanol 3:1). **5**: yield 367 mg (71%) of white crystals; mp 290 °C dec; MS (FAB$^-$, 3-NBA) 390 [(M)$^-$ for the anion]. Anal. ($NaC_{12}H_{24}N_2O_6B_9$) C, H, N, B.

2′,3′-*O*-Isopropylidene-5′-*O*-(3-prop-1-ynyl)uridine (7a) and 2′,3′-*O*-Isopropylidene-3-*N*,5′-*O*-bis(3-prop-1-ynyl)-uridine (7b). A 2.1-g (52.5 mmol) quantity of sodium hydride (60% dispersion in mineral oil) was slowly added to an ice-cooled solution of 10 g (35.0 mmol) of 2′,3′-*O*-isopropylideneuridine (**6**)[23] in 70 mL of dry DMF. The mixture was stirred for 30 min at 0 °C until the hydrogen evolution had ceased. After cooling to −10 °C, 4.7 mL (52.5 mmol) of propargyl bromide was slowly added, and the solution was stirred for an additional 60 min at −10 °C. The reaction mixture was diluted with 700 mL of ethyl acetate and was washed three times with of 100 mL of 1 mM HCl. Subsequently, the organic layer was dried over anhydrous magnesium sulfate, filtered, and evaporated to dryness. Compounds **7a** and **7b** were obtained by separating the remaining residue on a silica gel column (dichloromethane/acetone 9:1). **7a**: yield 4.9 g (43%) of white crystals; R_f 0.14 (dichloromethane/acetone 9:1); mp 48–52 °C; MS (FAB$^+$, 3-NBA) 323 (M + H)$^+$; ^{1}H-NMR ($CDCl_3$) δ 1.36 (s, 3 H, isopropylidene), 1.58 (s, 3 H, isopropylidene), 2.48 (t, 1 H, $CH_2C{\equiv}CH$, $J_{1,3propynyl}$ = 2.4), 3.72 (a) 3.82 (b) (d of AB q, 2 H, H_2-5′, $J_{5a'5b'}$ = 10.4, $J_{4',5a'}$ = 3.8, $J_{4',5b'}$

(27) Decaborane ($B_{10}H_{14}$) was kindly provided by Callery Chemicals Co., Pittsburgh, PA. This highly toxic compound forms impact-sensitive mixtures with several materials. It is advisable to study the MSDS (Material Safety Data Sheet) for decaborane, which every company has to provide together with this product, before usage.

(28) Some microanalytical laboratories were unable to provide us with exact elemental analysis for the new boron compounds while others were.

= 2.8), 4.17 (a) 4.22 (b) (d of AB q, 2 H, $CH_2C{\equiv}CH$, $J_{1a,1b}$ = 15.8, $J_{1a,3}$ = 2.45, $J_{1b,3}$ = 2.35), 4.41 (m, 1 H, H-4'), 4.79 (dd, 1 H, H-2', $J_{1',2'}$ = 2.54, $J_{2',3'}$ = 6.2), 4.82 (dd, 1 H, H-3', $J_{3',4'}$ = 2.87), 5.71 (dd, 1 H, H-5, $J_{5,6}$ = 8.1, $J_{3,5}$ = 2.1), 5.90 (d, 1 H, H-1'), 7.56 (d, 1 H, H-6), 8.64 (br s, 1 H, H-3). Anal. ($C_{15}H_{18}N_2O_6$) C, H, N. **7b**: yield 3.0 g (24%) of a clear glass-like compound; R_f 0.62 (dichloromethane/acetone 9:1); MS (FAB$^+$, 3-NBA) 361 (M + H)$^+$; ^{1}H-NMR ($CDCl_3$) δ 1.36 (s, 3 H, isopropylidene), 1.59 (s, 3 H, isopropylidene), 2.16 (t, 1 H, $NCH_2C{\equiv}CH$, $J_{1,3propynyl}$ = 2.45), 2.48 (t, 1 H, $OCH_2C{\equiv}CH$, $J_{1,3propynyl}$ = 2.35), 3.72 (a) 3.83 (b) (d of AB q, 2 H, H_2-5', $J_{5a',5b'}$ = 10.4, $J_{4',5a'}$ = 3.84, $J_{4',5b'}$ = 2.7), 4.16 (a) 4.20 (b) (d of AB q, 2 H, $OCH_2C{\equiv}CH$, $J_{1a,1b}$ = 15.8, $J_{1a,3}$ = $J_{1b,3}$ = 2.35, 4.44 (m, 1 H, H-4'), 4.65 (a) 4.73 (b) (d of AB q, $NCH_2C{\equiv}CH$, $J_{1a,1b}$ = 16.5, $J_{1a,3}$ = 2.45, $J_{1b,3}$ = 2.46), 4.79 (d, 1 H, H-2', $J_{1',2'}$ = 2.5, $J_{2',3'}$ = 6.2), 4.81 (d, 1 H, H-3', $J_{3',4'}$ = 2.58), 5.78 (d, 1 H, H-5, $J_{5,6}$ = 8.1), 5.96 (d, 1 H, H-1'), 7.55 (d, 1 H, H-6). Anal. ($C_{15}H_{18}N_2O_6$) C, H, N.

5'-*O*-(*o*-Carboran-1-ylmethyl)-2',3'-*O*-isopropylideneuridine (8). A 4.4-g (13.7 mmol) quantity of 2',3'-*O*-isopropylidene-5'-*O*-(3-prop-1-ynyl)uridine (**7a**) was reacted with acetonitrile and decaborane as described for the mixture of 3',5'-di-*O*-acetyl-2'-*O*-(3-prop-1-ynyl)uridine (**2a**) and 2',5'-di-*O*-acetyl-3'-*O*-(3-prop-1-ynyl)uridine (**2b**). **8**: Purification by column chromatography (dichloromethane/acetone 8:2); yield 4.2 g (70%) of white crystals; mp 130–132 °C; MS (FAB$^+$, 3-NBA) 441 (M + H)$^+$; ^{1}H-NMR ($CDCl_3$) δ 1.36 (s, 3 H, isopropylidene), 1.56 (s, 3 H, isopropylidene), 1.3–3.0 (br m, 10 H, BH), 3.71 (a) 3.76 (b) (d of AB q, 2 H, H_2-5', $J_{5a',5b'}$ = 10.7, $J_{4',5a'}$ = 5.75, $J_{4',5b'}$ = 3.77), 3.93 (s, 2 H, $CH_2C_{carborane}$), 3.96 (br s, $C_{carborane}$-H), 4.19 (m, 1 H, H-4'), 4.79 (dd, 1 H, H-3', $J_{3',4'}$ = 4.7, $J_{2',3'}$ = 6.4), 4.99 (d, 1 H, H-2', $J_{1',2'}$ = 1.8), 5.57 (dd, 1 H, H-1'), 5.75 (dd, 1 H, H-5, $J_{5,6}$ = 8.1, $J_{3,5}$ = 2.15), 7.23 (d, 1 H, H-6), 8.87 (br s, H-3). Anal. ($C_{15}H_{28}N_2O_6B_{10}$) C, H, N, B.

5'-*O*-(*o*-Carboran-1-ylmethyl)uridine (9). A 1-g (2.27 mmol) quantity of 5'-*O*-(*o*-carboran-1-ylmethyl)-2',3'-*O*-isopropylideneuridine (**8**) and 30 g of Dowex 50X8-100 ion-exchange resin (H$^+$ form) were stirred in 150 mL methanol/water 5:1 at 50 °C for 24 h. The progress of the reaction was checked by TLC (dichloromethane/methanol 7:1). The resin was filtered off, and the filtrate was evaporated to dryness. The resulting residue was purified by column chromatography (dichloromethane/methanol 7:1) and dried for 1 h at 100 °C under vacuum (0.5 mm). **9**: yield 790 mg (87%) of white crystals; mp 145–147 °C; MS (FAB$^+$, 3-NBA) 401 (M + H)$^+$; ^{1}H-NMR (CD_3OD) δ 1.30–3.00 (br m, 10 H, B-H), 3.70 (a) 3.86 (b) (d of AB q, 1 H, H_2-5, $J_{5a',5b'}$ = 11.3, $J_{4',5a'}$ = 3.4, $J_{4',5b'}$ = 3.2), 4.08 (m, 5 H, H-2', H-3', H-4', $OCH_2C_{carborane}$), 4.58 (br s, 1 H, $C_{carborane}$-H), 5.70 (d, 1 H, H-5, $J_{5,6}$ = 8.1), 5.85 (d, 1 H, H-1', $J_{1',2'}$ = 3.5), 7.72 (d, 1 H, H-6). Anal. ($C_{12}H_{24}N_2O_6B_{10}$) C, H, N, B.

1,2:5,6-Di-*O*-isopropylidene-3-*O*-(3-prop-1-ynyl)-D-glucose (11). A 10-g (38.4 mmol) quantity of 1,2:5,6-di-*O*-isopropylidene-D-glucose (**10**) was reacted with equimolar amounts of sodium hydride and of propargyl bromide as described for 2',3'-*O*-isopropylideneuridine (**6**).[23] Diethyl ether and H_2O were used instead of ethyl acetate and 1 mM HCl for partition. **11**: Purification by column chromatography (dichloromethane/methanol 19:1); yield 11.3 g (91%) of a clear oil; MS (FAB$^+$, 3-NBA) 299 (M + H)$^+$; ^{1}H-NMR ($CDCl_3$) δ 1.28, 1.32, 1.39, 1.46 (4 s, 12 H, isopropylidene), 2.45 (t, 1 H, $CH_2C{\equiv}CH$, $J_{1,3propynyl}$ = 2.36), 3.95–4.26 (m, 7 H, H-3, H-4, H-5, H_2-6, $CH_2C{\equiv}CH$), 4.60 (d, 1 H, H-2, $J_{1,2}$ = 3.68, $J_{2,3}$ = 0), 5.85 (d, 1 H, H-1). Anal. ($C_{15}H_{22}O_6$) C, H.

3-*O*-(*o*-Carboran-1-ylmethyl)-1,2:5,6-di-*O*-isopropylidene-D-glucose (12). A 4.1-g (13.7 mmol) quantity of 1,2:5,6-di-*O*-isopropylidene-3-*O*-(3-prop-1-ynyl)-D-glucose (**11**) was reacted with decaborane and acetonitrile as described for 3',5'-di-*O*-acetyl-2'-*O*-(3-prop-1-ynyl)uridine (**2a**) and 2',5'-di-*O*-acetyl-3'-*O*-(3-prop-1-ynyl)uridine (**2b**). **12**: Purification by column chromatography (dichloromethane/acetone 8:2); yield 4.4 g (77%) of white crystals; mp 65–67 °C; MS (FAB$^+$, 3-NBA) 417 (M + H)$^+$; ^{1}H-NMR ($CDCl_3$) δ 1.30, 1.34, 1.40, 1.48 (4 s, 12 H, isopropylidene), 1.30–3.0 (br m, 10 H, BH), 3.92–4.16 (m, 7 H, H-3, H-4, H-5, H_2-6, $CH_2C_{carborane}$), 4.25 (br s, 1 H, $C_{carborane}$-H), 4.45 (d, 1 H, H-2, $J_{1,2}$ = 3.7, $J_{2,3}$ = 0), 5.85 (d, 1 H, H-1); ^{13}C-NMR ($CDCl_3$) δ 25.1, 26.1, 26.7 ($4CH_3$), 57.6 ($C_{carborane}$-H), 68.0 (C-6), 71.5 ($CH_2C_{carborane}$), 72.1 (C-5), 72.2 ($CH_2C_{carborane}$), 81.2 (C-4), 82.3 (C-3), 84.1 (C-2), 105.1 (C-1), 109.5, 112.2 ($2C(CH_3)_2$). Anal. ($C_{15}H_{32}O_6B_{10}$) C, H, B.

3-*O*-(*o*-Carboran-1-ylmethyl)-D-glucose (13). A 750-mg (2.27 mmol) quantity of 3-*O*-(*o*-carboran-1-ylmethyl)-1,2:5,6-di-*O*-isopropylidene-D-glucose (**12**) was reacted with Dowex 50X8-100 ion-exchange resin (H$^+$ form) as described for 2',3'-*O*-isopropylidene-5'-*O*-(*o*-carboran-1-ylmethyl)uridine (**8**). **13**: Purification by column chromatography (dichloromethane/methanol 7:1); yield 580 mg (95%) of white crystals; mp 177–179 °C; ^{1}H-NMR (CD_3OD) δ 1.30–3.0 (br m, 10 H, BH), 3.10–3.82 (m, 8 H, H-2, H-3, H-4, H-5, H_2-6, $CH_2C_{carborane}$), 4.57 (d, 0.5 H, H_β-1, $J_{1,2}$ = 7.52), 4.82 (br s, 1 H, $C_{carborane}$-H), 5.03 (d, 0.5 H, H_α-1, $J_{1,2}$ = 3.6). Anal. ($C_9H_{24}O_6B_{10}$) C, H, B.

Cell Uptake Studies. Cellular uptake studies of the boron compounds **4a**, **4b**, **5**, **9**, and **13** were carried out as follows: 2 × 10^6 F98 glioma cells[26] were seeded into 150 cm^2 culture flasks (Corning, Corning, NY) with 30 mL of Dulbecco's modified eagle's medium (DMEM), supplemented with 10% fetal bovine serum (FBS). The cells were incubated in an atmosphere containing a 5% CO_2/95% air mixture for 96 h at 37 °C. This approximated four doubling times, by which time the cell monolayers were semiconfluent. The medium was decanted and 30 mL of fresh DMEM + 10% FBS, containing 7.5 μL of a 0.25 M solution of either **4a**, **4b**, **5**, **9**, or **13** in DMSO were added to the culture flasks. Boric acid and sodium mercaptoundecahydro-*closo*-dodecaborate (BSH) were used as standards; 2.5 M (boric acid), 0.208 M (BSH), and 2.08 M (BSH) DMSO stock solutions were used in case of these compounds. The final boron concentrations of **4a**, **4b**, **5**, **9**, **13**, and boric acid in the cell cultures were 6.75 μg/mL. That of BSH was 6.75 and 67.5 μg/mL. The concentration of DMSO in all of these studies was 0.025%.

The insolubility of many of the nonionic boron compounds in aqueous solution necessitated the use of DMSO as a solubilizing agent. Alternatively, we used β-cyclodextrin as a solubilizing agent.[19,20] None of the nonionic boron compounds precipitated from the medium when either DMSO or β-cyclodextrin were used as solubilizing agents and the obtained cellular boron concentrations were comparable.

After incubating the cells with each compound for an additional 16 h, the medium was decanted and the cells were washed twice with approximately 40 mL of 0.01 M phosphate buffered saline (PBS, pH 7.4). No decrease in cellular boron uptake could be demonstrated when a 0.025% DMSO/PBS solution was used to wash the cells instead of PBS. The cells then were disaggregated by adding several milliliters of 0.25% trypsin and incubating them at 37 °C. Subsequently, the cells were washed twice with approximately 40 mL of PBS. After determining the total cell number for each preparation by counting them in a hemocytometer, boron concentrations were determined by means of direct current plasma-atomic emission spectroscopy (DCP-AES).[29] Values below 0.3–0.4 μg per sample were considered to be background. For computational purposes, 10^9 cells approximated 1 g.

Acknowledgment. The authors acknowledge Samuel A. McCalmont of Callery Chemical Co. (Pittsburgh, PA) for providing decaborane, and Dr. C. E. Cottrell for producing the ^{1}H-NMR and ^{13}C-NMR spectra on the Bruker AM500 funded by NIH Grant No. 1 S10 RR01458-01A1. This work was supported by Department of Energy Grant DE-FG02-90ER60972 and contract DE-AC02-76CH000616, National Cancer Institute Grants 1R 01 CA53896 and P-30 CA16058-11, and a grant from The Ohio State University Comprehensive Cancer Center.

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