Synthesis of 1-Amino-3-[2-(1,7-dicarba-*closo*-dodecaboran(12)-1-yl)ethyl]cyclobutanecarboxylic Acid: A Potential BNCT Agent

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The synthesis of an unnatural amino acid, 1-amino-3-[2-(1,7-dicarba-*closo*-dodecaboran(12)-1-yl)ethyl]cyclobutanecarboxylic acid, was achieved. This new potential BNCT agent was prepared via the monoalkylation of *m*-carborane with 4-bromobutene to produce 4-*m*-carboranyl-1-butene, which was then subjected to a 2 + 2 cycloaddition using dichloroketene. The resultant boronated cyclobutanone was reductively dechlorinated prior to the formation of the corresponding hydantoin, which was hydrolized to the title compound in excellent yield.

Introduction

Though the concept of boron neutron capture therapy (BNCT) was proposed by Locker some 60 years ago,[1] the successful application of BNCT to the treatment of cancer still presents a challenge in medical research. An early attempt to cure cancer using this protocol failed.[2−5] The failure was attributed to vascular damage caused by the nonselective uptake of the boronated agent resulting in a high boron concentration in the blood. The recent resurgence of interest in BNCT is due mainly to the encouraging results obtained in Japan[6] using sodium mercaptoundecahydrodecaborate ($Na_2B_{12}H_{11}SH$, BSH) and 4-dihydroxyborylphenylalanine (BPA). The Japanese data suggest that BNCT can play an important role in cancer therapy (especially in the treatment of glioblastoma multiforme where conventional chemotherapy and radiation therapy have had little success).

Boron neutron capture therapy (BNCT) is a binary therapy in which a substance containing boron-10 is preferentially deposited in the tumor prior to irradiation by slow (thermal) neutrons.[7] The interaction of a boron-10 atom with a thermal neutron produces an α-particle and a high energy lithium-7 ion. The linear energy transfer (LET) of these heavy charged particles (^{4}He and ^{7}Li) has a range of only 9 and 5 μm, respectively (approximately the size of one cell diameter), and thus are lethal to the cells in which they are generated. A prerequisite to successful BNCT is selective delivery of an adequate concentration of boron-10 to the tumor (10−30 μg ^{10}B/g of tumor). The quantity of boron in the tumor must exceed that in the surrounding tissue by a factor exceeding 3.[7−9] Various carrier molecules have been used to deliver boron to the tumor cells. These include carbohydrates,[10−12] amino acids,[13−16] nucleosides,[17,18] antisence agents,[19] porphyrins[20] antibodies,[21,22] and liposomes.[23] While these carrier molecules are under development, BSH and BPA are being used clinically for the treatment of brain tumors and malignant melanoma in human patients.[6,24,25]

A variety of boron-containing amino acid derivatives[13−16] have been examined as potential agents for BNCT. It is believed that amino acids are preferentially taken up by the fast growing tumor. In fact, the only drug (BPA) currently under investigation for clinical trials in the United States is an amino acid.[26] Carboranyl analogues of phenylalanine[15,16] have also been synthesized and are currently being evaluated as potential BNCT agents.

We wish to report the synthesis of an unnatural alicyclic, boron-containing α-amino acid, 1-amino-3-[2-(1,7-dicarba-*closo*-dodecaboran(12)-1-yl)ethyl]cyclobutanecarboxylic acid (**1**) (*m*-carboranyl-ACBC). 1-Aminocyclobutanecarboxylic acid (ACBC) is known to be preferentially retained in intracerebral tumors.[27,28] In addition, ACBC has been found to be nontoxic.[27] These

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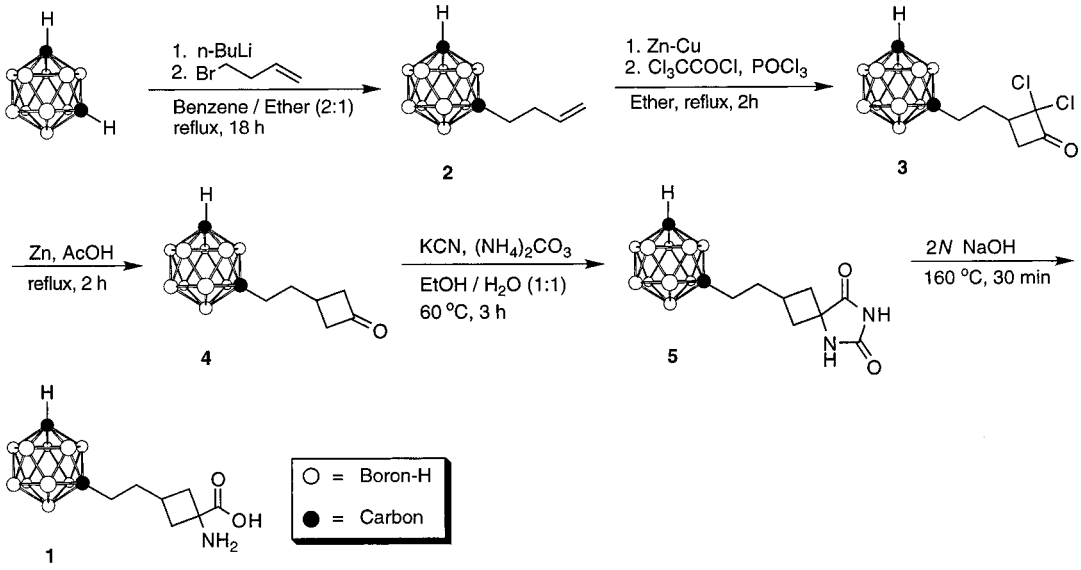
Scheme 1



properties make a boronated ACBC analogue a potential BNCT agent for the treatment of brain tumors. A carborane-substituted ACBC derivative was chosen as the target of this research because of the large number of boron atoms contained in the carborane and because of its chemical stability. The *o*-carborane cage is extremely stable under acidic conditions, but *nido* derivatives are generated upon reaction with primary or secondary amines.[29] This property has been advantageous in obtaining water-soluble caboranes[19,21,30] (*nido* carboranes are water soluble). Although some *nido*-carborane derivatives have been found to be nontoxic in *in vitro* studies,[31] the ionic nature of the cage has always been problematic in *in vivo* studies because they tend to react with proteins, resulting in nonspecific binding to biological materials.[32,33] In contrast to *o*-carborane, the *m*-carborane cage is stable under basic conditions.[29] Consequently, a *m*-carborane moiety was chosen for use in the preparation of a boronated ACBC derivative since it would not be expected to degrade under the reaction conditions that involve base and high temperature.[27]

Results and Discussion

The synthesis of 1-amino-3-[2-(1,7-dicarba-*closo*-dodecaboran(12)-1-yl)ethyl]cyclobutanecarboxylic acid (**1**) was accomplished in four steps (Scheme 1). The synthesis was initiated by the monoalkylation of *m*-carborane. When *m*-carborane was reacted with an equimolar quantity of butyllithium in a mixture of ether and benzene (1:2 ratio), followed by reaction with 4-bromobutene, 4-*m*-carboranyl-1-butene (**2**) was formed in high yield. This alkene was subjected to a 2 + 2 cycloaddition with dichloroketene that was generated *in situ* from the reaction of trichloroacetyl chloride with phosphorus oxychloride in the presence of a Zn−Cu couple.[34] The crude product obtained from the ketene addition was subjected to reductive dechlorination with Zn and acetic acid.[35] The resultant 3-[2-(1,7-dicarba-*closo*-dodecaboran(12)-1-yl)ethyl]cyclobutanone (**4**) was reacted with ammonium carbonate and potassium cyanide in a pressure tube.[36] The hydantoin **5** was formed in excellent yield. In the final step of the synthesis, the hydantoin was heated in the presence of sodium hydroxide to generate the desired amino acid **1** in high yield.

Conclusion

We report the first successful monoalkylation of *m*-carborane. It should be noted that only a few reports have appeared on the alkylation of *m*-carborane, but these syntheses generated mixtures of mono- and dialkylated products.[37] The synthesis of a 1-amino-3-[2-(1,7-dicarba-*closo*-dodeca-boran(12)-1-yl)ethyl]cyclobutanecarboxylic acid (**1**) was achieved, and the agent is being evaluated for potential use in BNCT.

Experimental Section

General Methods. All solvents were reagent grade and were distilled from appropriate drying agents under a nitrogen atmosphere prior to use. Diethyl ether was distilled from sodium benzophenone ketyl; benzene was distilled from calcium hydride and stored under nitrogen. *m*-Carborane was purchased from Dexsil Corporation (Hamden, CT) and purified by sublimation. All other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used as received.

Column chromatography was performed using silica gel (60 Å, 230−400 mesh) obtained from Baxter Co. (McGaw Park, IL). Reversed-phase column chromatography was performed utilizing octadecyl-functionalized silica gel obtained from Aldrich Chemical Co. (Milwaukee, WI). Analytical thin layer chromatography was performed on 250 μm silica plates obtained from Analtech Inc. (Newark, DE) and were visualized by phosphomolybdic acid, palladium chloride, and silver nitrate solutions.

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Melting points are uncorrected. Infrared spectra were obtained either neat or as Nujol mulls. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AC 250 at 250.13 and 62.89 Mz, respectively. ^{11}B NMR (^{1}H-decoupled) spectra were obtained on a Bruker AMX-400 at 128.38 Mz. Chemical shifts for ^{1}H and ^{13}C NMR spectra were referenced to $Si(CH_3)_4$ and measured with respect to the residual protons in the deuterated solvents. In the case of ^{11}B NMR, chemical shifts were measured with reference to external $BF_3{\cdot}OEt_2$. Resonances observed upfield of the reference ($BF_3{\cdot}OEt_2$) were assigned negative chemical shift value. Microanalyses were performed by Galbraith Laboratories Inc., Knoxville, TN. HR-FAB-MS (M + 1) were obtained on a ZAB-EQ instrument in a glycerol matrix.

Synthesis of 4-*m*-Carboranyl-1-butene (2). A three-necked round-bottomed flask equipped with an addition funnel, reflux condenser, and argon balloon was charged with *m*-carborane (35 mmol, 5.0 g) and a mixture of benzene (70 mL) and ether (35 mL). The reaction mixture was cooled to 0 °C, butyllithium (38 mmol, 24 mL of 1.6 M solution in hexane) was added via a syringe over a period of 10 min, and the mixture was allowed to stir at room temperature for 30 min. The reaction mixture was then cooled to 0 °C, and to it was added a mixture of 4-bromobutene (39 mmol, 3.9 mL) in a mixture of benzene (10 mL) and ether (5 mL). The reaction mixture was then refluxed at 90 °C in an oil bath for 18 h, cooled to room temperature, and quenched with water (2 mL). The solution was transferred to a separatory funnel and washed successively with water (2 × 20 mL) and brine (1 × 20 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure using a rotary evaporator. After drying at 50 °C at 0.2 mmHg, a colorless liquid (7.1 g) was obtained. The crude product was purified by column chromatography using silica gel (27 × 4 cm, hexane as eluent) to yield **2** as a colorless liquid (5.8 g, 84% yield): R_f = 0.76 (hexane, thin layer chromatography); ^{1}H NMR ($CDCl_3$) δ 5.67 (m, 1H), 5.00 (m, 2H), 2.91 (s, 1H), 2.03 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 136.13, 115.74, 75.32, 54.85, 36.06, 33.77; ^{11}B ($CDCl_3$) δ −1.81 (s, 1 B), −8.71 (s, 4 B), −11.32 (s, 3 B), 13.27 (s, 2 B); IR (neat) 3064, 2981, 2919, 2854, 2598, 1735, 1641, 1436, 1244, 1046, 994, 924, 908, 735 cm^{-1}; HR-FAB-MS (M + H; obtained in glycerol matrix) calcd for $C_6H_{19}B_{10}$ 198.241, found 198.243.

3-[2-(1,7-Dicarba-*closo*-dodecaboran(12)-1-yl)ethyl]cyclobutanone (4). The synthesis of this compound was achieved in two steps. 2,2-Dichloro-3-[2-(1,7-dicarba-*closo*-dodecaboran(12)-1-yl)ethyl]cyclobutanone (**3**) was synthesized from 4-*m*-carboranyl-1-butene (**2**), and then the halo ketone was treated with zinc and acetic acid to yield the desired compound **4**.

A 250 mL, three-necked, round-bottomed flask equipped with a reflux condenser and an argon balloon was charged with 4-*m*-carboranyl-1-butene (**2**) (29.1 mmol, 5.8 g) and diethyl ether (100 mL). Freshly prepared Zn−Cu couple (174 mmol, 22.5 g) was added followed by trichloroacetyl chloride (64 mmol, 7.2 mL) and phosphorus oxychloride (64 mmol, 5.9 mL). After the mixture was stirred at room temperature for 10 min, it was refluxed under an argon atmosphere. After 2 h at reflux, the reaction was cooled to room temperature and filtered through a pad of Celite. Additional ether (50 mL) was used to transfer the material. The solvent was removed under reduced pressure using a rotary evaporator, and the viscous 2,2-dichloro-3-[2-(1,7-dicarba-*closo*-dodecaboran(12)-1-yl)ethyl]cyclobutanone (**3**) was dried under high vacuum (0.2 mmHg at 50 °C).

The crude 2,2-dichloro-3-[2-(1,7-dicarba-*closo*-dodecaboran(12)-1-yl)ethyl]cyclobutanone (**3**) was dissolved in glacial acetic acid (50 mL) in a 100 mL round-bottomed flask, fitted with a reflux condensor, containing zinc dust (10 g, excess). The mixture was stirred at room temperature for 20 min, and then it was refluxed for 2 h. TLC indicated the complete diappearence of the starting material. The reaction was cooled to room temperature and filtered through a pad of Celite. Additional ethyl acetate (100 mL) was used to wash the Celite pad and transfer the material from the round-bottomed flask. The solvent was removed under reduced pressure using a rotary evaporator. The viscous mass was dissolved in ethyl acetate (100 mL) placed in a separatory funnel and then washed sequentially with water (2 × 20 mL), saturated sodium bicarbonate (2 × 20 mL), water (2 × 20 mL), and brine (1 × 20 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure using a rotary evaporator. The reaction yielded a viscous material (8.86 g) that was purified by column chromatography using silica gel (30 × 4 cm, 10% ethyl acetate in hexane) to yield **4** as a colorless oily product [3.1 g, 44% overall yield based on 4-*m*-carboranyl-1-butene (**2**)]: R_f = 0.56 (15% ethyl acetate in hexane, thin layer chromatography); ^{1}H NMR ($CDCl_3$) δ 3.16 (m, 2H), 2.93 (s, 1H), 2.64 (m, 2H), 2.24 (m, 1H), 1.96 (m, 2H), 1.68 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 206.7, 75.4, 54.9, 52.4, 36.3, 35.6, 23.4; ^{11}B ($CDCl_3$) δ −1.91 (s, 1 B), −8.66 (s, 4 B), −11.42 (s, 3 B), −13.22 (s, 2 B); IR (neat) 3060, 2946, 2929, 2868, 2596, 1784, 1452, 1385, 1104, 1062, 1008, 729 cm^{-1}. Anal. Calcd for $C_8H_{20}B_{10}O$: C, 39.98; H, 8.39; B, 44.98. Found: C, 40.44; H, 8.28; B, 43.63.

Hydantoin 5 of 3-[2-(1,7-Dicarba-*closo*-dodecaboran(12)-1-yl)ethyl]cyclobutanone (4). A 15 mL Ace pressure tube was charged with 3-[2-(1,7-dicarba-*closo*-dodecaboran(12)-1-yl)ethyl]cyclobutanone (**4**) (0.42 mmol, 0.10 g), aqueous ethanol (50%, 2 mL), potassium cyanide (0.46 mmol, 30 mg), and ammonium carbonate (1.0 mmol, 0.10 g). The reaction vessel was sealed and heated at 60 °C in an oil bath for 3 h. A white precipitate formed. The reaction vial was cooled to room temperature and the cap unscrewed carefully in a fume hood. The reaction mixture was quenched with aqueous acetic acid (2 mL of a 30% solution in water). The solvent was removed under reduced pressure using a rotatory evaporator and the resultant white solid taken up into ethyl acetate (50 mL) in a separatory funnel and washed sequentially with water (2 × 10 mL) and brine (1 × 10 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. A white solid was obtained (0.27 g), and this was purified by column chromatography using silica gel (20 × 4 cm, 10% methanol in methylene chloride) to yield **5** as a white solid (0.11 g, 84% yield): R_f = 0.65 (10% methanol in methylene chloride, thin layer chromatography); mp 332−334 °C (with decomposition); ^{1}H NMR (CD_3OD) δ 3.48 (s, 1H), 2.53 (m, 2H), 2.25 (m, 3H), 1.89 (m, 2H), 1.64 (m, 1H), 1.51 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 178.59, 155.82, 59.06, 57.54, 56.20, 37.73, 36.73, 36.48, 33.34, 25.46; ^{11}B (CD_3OD) δ −1.36 (s, 1B), −8.06 (s, 4B), −10.66 (s, 2 B), −11.56 (s, 1 B), −12.24 (s, 2 B); IR (Nujol) 3207, 3040, 2949, 2920, 2850, 2755, 2597, 1761, 1734, 1435, 1305, 1120, 778, 765, 727, 643 cm^{-1}. Anal. Calcd for $C_{10}H_{22}B_{10}O_2N_2$: C, 38.70; H, 7.14; N, 9.03; B, 34.83. Found: C, 38.31; H, 6.73; N, 8.70; B, 33.51.

1-Amino-3-[2-(1,7-dicarba-*closo*-dodecaboran(12)-1-yl)ethyl]cyclobutanecarboxylic Acid (1). The hydantoin of 3-[2-(1,7-dicarba-*closo*-dodecaboran(12)-1-yl)ethyl]cyclobutanone (**5**) (0.64 mmol, 0.20 g) was placed in a 15 mL Ace pressure tube along with a solution of sodium hydroxide (5 mL of 2 *N* NaOH). The reaction mixture was heated to 160 °C in an oil bath for 30 min. It was then cooled to room temperature and opened carefully. TLC indicated the disappearance of the starting hydantoin. The reaction mixture was acidified using concentrated hydrochloric acid, and the volatile solvents were removed under reduced pressure using a rotary evaporator. The white solid obtained was purified by column chromatography using octadecyl-functionalized silica gel (22 × 2 cm, 80% methanol in water). The product **1** was obtained as a white solid (0.15 g, 82% yield): R_f = 0.55 (in a mixture of butanol, water, and acetic acid in ratio of 10:1:0.5); mp compound turns brown (without melting) at 201 °C; ^{1}H NMR (CD_3OD) δ 3.49 (s, 1H), 2.63 (m, 2H), 2.40 (m, 3H), 1.89 (m, 2H), 1.61 (m, 2H); ^{13}C NMR (CD_3OD) δ 176.3, 77.5, 56.9, 38.4, 37.5, 36.8, 36.0, 35.2, 29.0; ^{11}B (CD_3OD) δ −1.45 (s, 1 B), −8.11 (s, 4 B), −10.70 (s, 2 B), −11.42 (s, 1 B), −12.29 (s, 2 B); IR (Nujol) 4000, 3434, 3060, 2947, 2909, 2849, 2598, 2294, 1719, 1677, 1566, 1457, 1377, 1269, 1233, 1183, 1139, 1072, 1009, 728 cm^{-1}. Anal. Calcd for $C_9H_{23}B_{10}O_2N$: C, 37.60; H, 8.07; N, 4.87; B, 38.32. Found: C, 36.54; H, 7.96; N, 5.24; B, 35.94. HR-FAB-MS (M + H; obtained in glycerol matrix) calcd for $C_9H_{24}B_{10}O_2N$: 286.282, found 286.284.

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