SYNTHESIS OF 1-AMINO-3-{2-[7-(6-DEOXY-α/β-D-GALACTOPYRANOS-6-YL)-1,7-DICARBA-*closo*-DODECABORAN(12)-1-YL]ETHYL}CYCLOBUTANECARBOXYLIC ACID HYDROCHLORIDE

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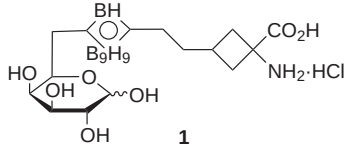
Dedicated to Professor Jaromír Plešek on the occasion of his 75th birthday.

1-Amino-3-{2-[7-(6-deoxy-α/β-D-galactopyranos-6-yl)-1,7-dicarba-*closo*-dodecaboran(12)-1-yl]ethyl}cyclobutanecarboxylic acid was synthesized as a potential new agent for boron neutron capture therapy. The key step in the synthesis is the alkylation of 3-{2-[1,7-dicarba-*closo*-dodecaboran(12)-1-yl]ethyl}cyclobutanone ethylene monothioketal with 1,2:3,4-di-*O*-isopropylidene-6-*O*-triflyl-α-D-galactopyranose which gave the precursor ketone that was then converted to the title amino acid *via* a Bücherer–Strecker synthesis followed by removal of isopropylidene groups in HCl. Preliminary toxicity data in A 435 cancer cells were obtained.
Keywords: Boron neutron capture therapy; Amino acids; Carbohydrates; Carboranes; BNCT.

In the last decade, there has been considerable interest in boron neutron capture therapy[1] (BNCT), a binary approach to the treatment of cancer in which a compound containing ^{10}B is selectively delivered to tumor tissues prior to irradiation by thermal neutrons[2]. The interaction of the boron-10 atom with a thermal neutron produces an α particle and a ^{7}Li ion. The linear energy transfer (LET) of these heavily charged particles has a range of approximately one cell diameter and is considered to be of sufficient energy to kill the cell. To minimize damage to normal tissue, the quantity of boron in the tumor (≈30 μg of ^{10}B per gram of tumor) must exceed that in the surrounding normal tissue by at least a factor of three[3,4]. The clinical success of BNCT depends on two factors: effective delivery of a sufficient quantity of boron to the targeted tumor site and a neutron flux sufficient to achieve the prerequisite nuclear reaction while minimizing damage to healthy tis-

sue. Early BNCT clinical trials were disappointing in that they failed to achieve either of these goals[5]. However, significant advances have been made in the design of nuclear reactors so that attention has now been focused on the design of selective tumor-seeking boron containing pharmaceuticals[6]. A variety of carrier molecules have been designed to deliver boron to tumor cells. These include carbohydrates[7], polyamines[8], amino acids[9], nucleosides[10], antisense agents[11], porphyrins[12], antibodies[13], and liposomes[14].

It is believed that amino acids are preferentially taken up by growing tumor cells. For this reason, boron-containing amino acid derivatives have been studied as potential BNCT agents. In fact, the only drug currently used in BNCT clinical trials in the United States is a boronated phenylalanine[15] (BPA). Recent positron emission tomography (PET) investigations carried out at the University of Tennessee on BNCT patients using both ^{18}F labeled BPA and ^{11}C labeled 1-aminocyclobutanecarboxylic acid (ACBC) revealed that cyclic amino acids localize in glioblastoma multiforme (GBM) and metastatic multiple melanoma (MM) tumors more avidly than BPA[16]. For this reason, we have focused our efforts on cyclic amino acids as the boron carrier. We reported the syntheses of a *m*-carborane containing ACBC derivative and a less liphophilic *nido* analogue[17]. The *nido* derivative provided good water solubility, but the ionic character of the cage has always been problematic in *in vivo* studies because it leads to non-specific protein binding[18]. In order to increase the water solubility of *m*-carboranyl-ACBC, we decided to synthesize the hydrochloride of 1-amino-3-{2-[7-(6-deoxy-α/β-D-galactopyranos-6-yl)-1,7-dicarba-*closo*-dodecaboran(12)-1-yl]ethyl}cyclobutanecarboxylic acid (**1**) as a potential BNCT agent.



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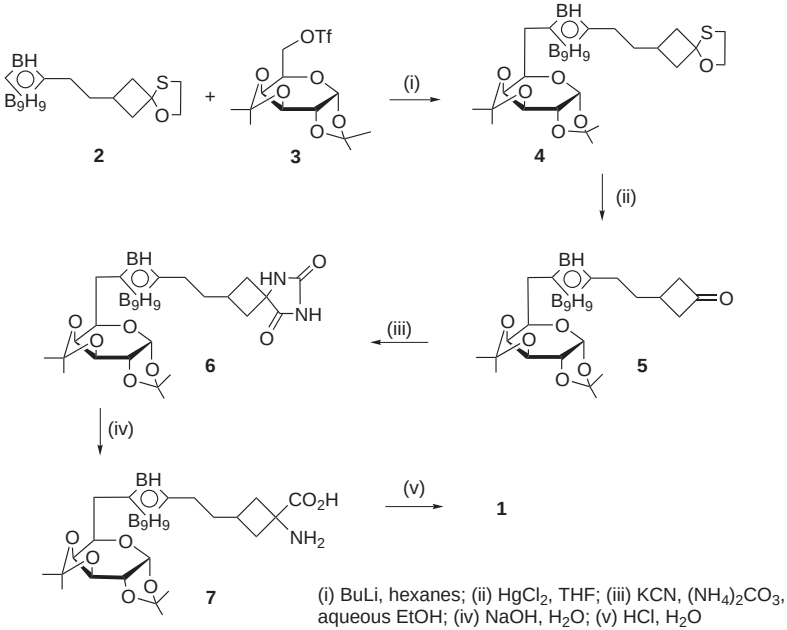
The key synthetic step in the formation of **1** is the preparation of the protected derivative of cyclobutanone **4** (Scheme 1). Ketone **4** was prepared by coupling the lithium salt of cyclobutanone monothioketal[19,20] **2** with the triflate of 1,2:3,4-di-*o*-isopropylidene-D-galactopyranose[21,22] (**3**). Monothioketal **2** was obtained by refluxing the *m*-carboranyl substituted cyclobutenone[19] with 2-mercaptoethanol.

The monothioketal group was removed from **4** by the action of mercuric chloride to generate **5**. Ketone **5** was allowed to react with ammonium car-

bonate and potassium cyanide in an Ace pressure tube to form the corresponding hydantoin in good yield. The hydantoin was then hydrolyzed with sodium hydroxide at 160 °C followed by acidic removal of the two isopropylidene groups to give the title compound which was found to readily dissolve in water (3.3 mg/ml).



SCHEME 1

A preliminary cell toxicity study was performed using A 435 breast cancer cells. The cells were placed in Leibovitz's (L-15) minimal essential media containing various concentrations of the boronated amino acid (0.1, 0.5, 1.0 mg/ml). The resulting colonies were then stirred and counted. Kill/survival ratios were calculated by dividing the number of colonies in the samples containing boron by the number of colonies in control samples. A ratio of 1.0 represents non-toxic behavior. Compound **1** exhibited essentially no toxicity (kill/survival ratio = 1.03 at 1.0 mg/ml).

EXPERIMENTAL

Diethyl ether was distilled from sodium benzophenone ketyl and benzene was distilled from calcium hydride and stored under nitrogen. *Meta*-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)). Analytical thin layer chromatography was performed on 250 μm silica plates obtained from Analtech Inc. (Newark (DE)) and were visualized by phosphomolybdic acid and silver nitrate solution.

Melting points are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded at 250.13 and 62.89 MHz, respectively (Bruker AC250 NMR spectrometer). Chemical shifts are given in ppm (δ-scale) and coupling constants (*J*) in Hz. ^{1}H resonances for the hydrogens of the carborane cages were extensively broadened due to the large quadropole moment of the boron nuclei and are not reported. Chemical shifts for ^{1}H and ^{13}C NMR were referenced to tetramethylsilane and measured with respect to the residual protons in the deuterated solvents. NMR data are reported only for the major diastereomeric product. Microanalyses were performed by Atlantic Microlab Inc., Atlanta (GA).

3-{2-[1,7-Dicarba-*closo*-dodecaboran(12)-1-yl]ethyl}cyclobutanone Ethylene Monothioketal (**2**)

A mixture of 3-{2-[1,7-dicarba-*closo*-dodecaboran(12)-1-yl]ethyl}cyclobutanone (0.488 g, 2.03 mmol), 2-mercaptoethanol (0.17 ml, 2.4 mmol), 4-toluenesulfonic acid monohydrate (20 mg, 0.10 mmol) and benzene (80 ml) was placed in a flask equipped with a water-separator and refluxed for 4 h. The reaction mixture was then neutralized with 0.1 M aqueous NaOH, washed with brine to neutral pH, and dried over anhydrous $MgSO_4$. The solvent was removed and the residue purified by column chromatography using silica gel and 10% ethyl acetate in hexane to give a colorless solid (0.58 g, 94%); m.p. 48–50 °C. ^{1}H NMR ($CDCl_3$): 4.01 (m, 2 H); 3.06 (m, 2 H); 2.90 (s, 1 H); 2.53 (m, 2 H); 2.20–1.75 (m, 5 H); 1.52 (m, 2 H). ^{13}C NMR ($CDCl_3$): 91.0, 76.0, 69.0, 54.8, 44.6, 36.4, 34.8, 33.7, 26.0. For $C_{10}H_{24}B_{10}OS$ (300.5) calculated: 39.97% C, 8.05% H; found: 40.02% C, 8.14% H.

3-{2-[7-(6-Deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)-1,7-dicarba-*closo*-dodecaboran(12)-1-yl]ethyl}cyclobutanone Ethylene Monothioketal (**4**)

To a stirred solution of compound **2** (0.536 g, 1.78 mmol) in THF (20 ml) was added (drop-wise) BuLi (1.17 ml, 1.60 mol/l in hexanes). The mixture was stirred at room temperature for 0.5 h and then a solution of 1,2:3,4-di-*O*-isopropylidene-6-*O*-trifyl-α-D-galactopyranose (**3**; 0.733 g, 1.87 mmol in 5 ml of THF) was added. The resulting orange solution was stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue dissolved in Et_2O (60 ml). The organic layer was washed with brine and dried over anhydrous $MgSO_4$. The filtrate was concentrated under reduced pressure and the residue purified by column chromatography using silica gel and 10% ethyl acetate in hexane to give a colorless oil (0.786 g, 81%). ^{1}H NMR ($CDCl_3$): 5.35 (d, 1 H, J = 5.1); 4.57 (dd, 1 H, J = 2.1, 7.8); 4.28 (dd, 1 H, J = 2.3, 5.0); 4.02 (m, 3 H); 3.75 (d, 1 H, J = 7.8); 3.04 (m, 2 H); 2.52 (m, 2 H); 2.31 (dd, 1 H, J = 2.9, 8.1); 2.18–1.70 (m, 6 H); 1.59 (s, 3 H); 1.52 (m, 2 H); 1.42 (s, 3 H); 1.34 (s, 3 H); 1.30 (s, 3 H). ^{13}C NMR ($CDCl_3$): 109.2, 108.6, 96.6, 90.0, 75.9, 73.1, 70.9, 70.0, 69.4, 67.0, 44.6, 38.0, 36.5, 34.9, 33.7, 26.0, 25.9, 25.0, 24.4. For $C_{22}H_{42}B_{10}O_6S$ (542.7) calculated: 48.69% C, 7.80% H; found: 49.10% C, 7.92% H.

3-{2-[7-(6-Deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)-1,7-dicarba-*closo*-dodecaboran(12)-1-yl]ethyl}cyclobutanone (**5**)

To a stirred solution of compound **4** (0.27 g, 0.50 mmol) and $HgCl_2$ (150 mg, 0.55 mmol) in THF (40 ml) was added 0.1 M aqueous NaOH dropwise (0.55 mmol, 55 ml) over a period of 30 min. The reaction was monitored by thin layer chromatography. After completion of the reaction, the solvent was removed under reduced pressure and the product extracted into ether (50 ml). The ether solution was dried over anhydrous $MgSO_4$ and concentrated to yield the crude product. After purification by column chromatography (silica gel and 15% ethyl acetate in hexane), viscous oil (0.21 g, 89%) was obtained. ^{1}H NMR ($CDCl_3$): 5.52 (d, 1 H, *J* = 5.1); 4.57 (dd, 1 H, *J* = 2.2, 7.9); 4.28 (dd, 1 H, *J* = 2.4, 5.2); 4.03 (dd, 1 H, *J* = 1.8, 7.9); 3.76 (dd, 1 H, *J* = 1.8, 8.7); 3.12 (m, 2 H); 2.62 (m, 2 H); 2.40–2.10 (m, 3 H); 1.93 (m, 2 H); 1.67 (m, 2 H); 1.59 (s, 3 H); 1.42 (s, 3 H); 1.34 (s, 3 H); 1.30 (s, 3 H). ^{13}C NMR ($CDCl_3$): 206.8, 109.3, 108.6, 96.5, 75.3, 73.3, 73.1, 70.8, 70.0, 67.0, 66.4, 52.4, 36.0, 36.2, 35.7, 25.9, 24.3, 23.5. For $C_{20}H_{38}B_{10}O_6$ (482.6) calculated: 49.77% C, 7.94% H; found: 50.10% C, 8.03% H.

Hydantoin of 3-{2-[7-(6-Deoxy-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranos-6-yl)-1,7-dicarba-*closo*-dodecaboran(12)-1-yl]ethyl}cyclobutanone (**6**)

A mixture of compound **5** (0.330 g, 0.684 mmol), potassium cyanide (0.067 g, 1.3 mmol) and ammonium carbonate (0.165 g, 1.72 mmol) in EtOH–H_2O (1 : 1, 8 ml) was placed in a 35 ml Ace pressure tube and sealed. The tube was heated in an oil bath at 70 °C for 12 h with stirring. After cooling, the tube was carefully opened in a fume hood, and the contents were transferred to a flask and evaporated to dryness. The residue was dissolved in Et_2O (50 ml), washed with brine, and dried over anhydrous $MgSO_4$. Evaporation of the solvent and column chromatography (silica gel, 10% MeOH in CH_2Cl_2) furnished a crystalline solid (0.35 g, 93%); m.p. 174–175 °C. ^{1}H NMR ($CDCl_3$): 9.28 (s, 1 H); 7.07 (s, 1 H); 5.54 (d, 1 H, *J* = 5.0); 4.58 (dd, 1 H, *J* = 2.2, 7.9); 4.29 (dd, 1 H, *J* = 2.3, 7.1); 4.04 (d, 1 H, *J* = 7.9); 3.76 (d, 1 H, *J* = 8.3); 2.64 (m, 2 H); 2.40–2.08 (m, 5 H); 1.84 (m, 2 H); 1.60 (s, 3 H); 1.52 (m, 2 H); 1.42 (s, 3 H); 1.35 (s, 3 H); 1.31 (s, 3 H). ^{13}C NMR ($CDCl_3$): 177.9, 157.1, 109.2, 108.6, 96.4, 75.6, 73.2, 73.0, 70.8, 69.9, 67.0, 58.8, 38.7, 37.9, 37.0, 34.2, 26.4, 25.8, 24.9, 24.3. For $C_{22}H_{40}B_{10}N_2O_7$ (552.7) calculated: 47.81% C, 7.29% H; found: 47.78% C, 7.25% H.

1-Amino-3-{2-[7-(6-deoxy-1,2:3,4-di-*O*-isopropylidene-(-D-galactopyranos-6-yl)-1,7-dicarba-*closo*-dodecaboran(12)-1-yl]ethyl}cyclobutanecarboxylic Acid (**7**)

A 35 ml Ace pressure tube containing compound **6** (0.315 g, 0.570 mmol), a stirring bar and 2 M aqueous NaOH (5 ml) was sealed and immersed in an oil bath, heated to 160 °C for 1 h. On cooling, the tube was opened carefully and the contents dissolved in a mixture of Et_2O (50 ml) and water (50 ml). The layers were separated and the ether layer was washed with water. The combined aqueous solution was neutralized with 2 M aqueous HCl (pH 7), some white solid precipitated. The mixture was extracted with CH_2Cl_2 (3 × 30 ml) and the combined organic layer was washed with distilled water (2 × 20 ml). Removal of the solvent under pressure afforded a solid (0.29 g, 97%); m.p. 202–204 °C (decomp.). ^{1}H NMR (CD_3OD): 5.48 (d, 1 H, *J* = 5.0); 4.59 (dd, 1 H, *J* = 2.2, 7.9); 4.32 (dd, 1 H, *J* = 2.36, 5.1); 4.06 (d, 1 H, *J* = 7.9); 3.78 (d, 1 H, *J* = 8.3); 2.60–2.05 (m, 7 H); 1.84 (m, 2 H); 1.56 (s, 3 H); 1.50 (m, 2 H); 1.37 (s, 3 H); 1.34 (s, 3 H); 1.30 (s, 3 H). ^{13}C NMR ($CDCl_3$): 176.6, 110.2, 109.8, 98.0, 77.6,

75.0, 74.4, 72.2, 71.4, 68.9, 55.8, 39.7, 37.7, 36.1, 35.4, 29.1, 26.3, 25.1, 24.3. For $C_{21}H_{41}B_{10}NO_7$ (527.7) calculated: 47.80% C, 7.83% H, 2.65% N; found: 47.56% C, 7.72% H, 2.53% N.

1-Amino-3-{2-[7-(6-deoxy-α/β-D-galactopyranos-6-yl)-1,7-dicarba-*closo*-dodecaboran(12)-1-yl]ethyl}cyclobutanecarboxylic Acid Hydrochloride (**1**)

To a solution of compound **7** (0.136 g, 0.258 mmol) in THF (15 ml) at 0 °C was added 4 M aqueous HCl (15 ml). The resulting mixture was stirred at room temperature for 20 h. All volatile components were removed under vacuum to afford a white powder (0.12 g, 95%). ^{1}H NMR (CD_3OD): 4.35 (d, 1 H, J = 5.8); 4.00–3.40 (m, 4 H); 2.66 (m, 2 H); 2.42 (m, 3 H); 2.20–1.75 (m, 4 H); 1.61 (m, 2 H). ^{13}C NMR (CD_3OD): 176.6, 98.2, 77.6, 75.3, 75.0, 74.8, 73.6, 72.9, 68.9, 55.8, 39.7, 37.7, 36.1, 35.4, 29.1. For $C_{15}H_{34}B_{10}ClNO_7$ (484.0) calculated: 37.22% C, 7.08% H, 2.89% N; found: 36.97% C, 7.05% H, 2.67% N.

Cell Toxicity Studies

A preliminary cell toxicity study was performed using a standard colony-forming assay with A 435 breast cancer cells obtained from American Cell Type Culture Collection (Rockville (MD)). The standard colony forming assay has been described elsewhere[23,24]. The cells were maintained in Leibovitz's (L-15) minimal essential growth media supplemented with 10% fetal bovine serum. The cells were incubated at 37 °C and 95% humidity in 35 ml flasks containing 10 ml of L-15 minimal essential media containing varying concentrations of the boronated amino acid (0.1, 0.5, 1.0 mg/ml) for a period of 24 h. 2 000 boron-exposed cells were then counted using a hemocytometer and plated in 35 mm Petri dishes containing 3 ml of non-boronated L-15 growth media. The cells were incubated for 7 days to allow for colony formation. The resulting colonies were then washed in isotonic saline solution, fixed with absolute methanol, and stained with crystal violet. The stained colonies contained within a 1 cm^2 area were then counted under a light microscope. Kill/survival ratios were calculated by dividing the number of colonies in the samples containing boron by the number of colonies in control samples. A ratio of 1.0 represents non-toxic behavior. Compound **1** exhibited essentially no toxicity (kill/survival ratio = 1.03 at 1.0 mg/ml).

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