Synthesis and Characterization of a Boronated Metallophthalocyanine for Boron Neutron Capture Therapy

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Synthesis of the first fully characterized, water-soluble boronated phthalocyanine is reported. Reaction of 4-nitrophthalonitrile with dimethyl malonate in the presence of base yielded dimethyl (3,4-dicyanophenyl)malonate which was converted into dimethyl (3,4-dicyanophenyl)propargylmalonate by sequential treatment with potassium hydroxide and propargyl bromide. Formation of the *o*-carborane cage was accomplished by reaction of the alkyne with decaborane in acetonitrile at reflux. High-temperature solid state condensation of the resulting *o*-carboranylphthalonitrile with cobalt(II) chloride followed by ester deprotection and cation exchange provided the water-soluble *closo*-carbonylphthalocyanine product. The product contains 40 boron atoms (27% boron by weight) and may be useful as a tumor-seeking boron delivery agent for boron neutron capture therapy of cancer.

Introduction

Boron neutron capture therapy (BNCT) is a binary method for cancer therapy which allows selective tumor irradiation. The principle of BNCT is based on the interaction of a nonradioactive ^{10}B nucleus with low-energy (thermal) neutrons to generate cytotoxic particles, ^{4}He and ^{7}Li, of ∼2.3 MeV kinetic energy. These fission fragments have mean free paths approximately equivalent to the average cell diameter, thus confining their considerable radiation damage to the immediate vicinity of cells in which they are located.[1] It has been calculated that a minimum cellular ^{10}B concentration of between 10 and 30 μg per gram of tumor is necessary for clinically effective BNCT.[1] The challenge for chemists, therefore, is to synthesize ^{10}B-containing compounds capable of specific localization and retention in tumor cells at or above these levels while simultaneously clearing normal, nontarget tissues and blood. Certain phthalocyanines (Pc's) have been proven to localize and persist in various solid tumors, and metallophthalocyanines have been used, *in vitro* and *in vivo*, as photosensitizers for photodynamic therapy (PDT).[2,3] Thus, the use of a metallophthalocyanine to deliver ^{10}B atoms to a tumor might be a viable strategy for meeting this challenge. Phthalocyanines usually exhibit notorious insolubility in both water and organic solvents that precludes adequate purification and characterization, so the key issue is to synthesize a boronated phthalocyanine having both lipophilic and hydrophilic substituents capable of enhancing the solubility of boronated phthalocyanines in therapeutic solutions. The first water-soluble boronated phthalocyanine, bearing only one *closo*-carborane cage, was synthesized by Soloway *et al*.[4] However, no detailed information on its purification and characterization was reported.

Experimental Section

Dimethyl (3,4-dicyanophenyl)malonate, **2**, and its potassium salt, **3**, were synthesized according to literature procedures.[5] 4-Nitrophthalonitrile was purchased from Tokyo Chemical Inc. (TCI). Decaborane was purchased from Dexsil Corp. and was sublimed prior to use. All other reagents and chemicals were purchased from Aldrich, and all reactions were carried out under high-purity argon unless otherwise indicated. High-resolution mass spectra (HRMS) were recorded on a VG-70SE mass spectrometer in EI mode for molecules less than 900 amu. Liquid secondary ion mass spectra (LSIMS) were recorded in a nitrobenzyl alcohol matrix on a VG-70SE mass spectrometer for molecules of more than 1000 amu. Infrared (IR) spectra were recorded on a Nicolet Impact 400 infrared spectrophotometer using KBr disks. Nuclear magnetic resonance (NMR) spectra for proton and carbon were recorded on a GE 300 NMR spectrometer with TMS as an internal standard. Ultraviolet−visible spectra (UV−vis) were recorded on a HP8452A diode array spectrophotometer. Melting points (mp) were measured by a Thomas Hoover capillary melting point apparatus and are uncorrected. Chromatography was performed with silica gel (Merck grade 60) from Aldrich. Microanalyses were performed by the Microanalysis Laboratory of College of Chemistry, University of California, Berkeley, CA.

Dimethyl (3,4-Dicyanophenyl)propargylmalonate, 4. To a suspension of compound **3** (3 g, 0.01 mol) in acetone (70 mL) was added propargyl bromide (1.8 g, 80% in toluene, 0.012 mol). The suspension was stirred and heated to reflux under argon for 20 h. The potassium bromide precipitate was filtered off and acetone was removed *in vacuo* to give an oily residue. Chromatography of the oily residue using hexane/THF (6:1) as the eluant generated 2.4 g (83%) of dimethyl (3,4-dicyanophenyl)propargylmalonate **4** (mp: 90−91 °C). HRMS for $C_{16}H_{12}N_2O_4$ (M^+): calcd, 296.0797; found, 296.0789 (Δ = 0.8 ppm). ^1H NMR ($CDCl_3$-d_1): δ 8.02 (d, J = 1.5 Hz, H), 7.89, 7.87 (2dd, J = 1.5 Hz, J = 8.4 Hz, H), 7.81 (d, J = 8.4 Hz, H), 3.84 (s, 6H), 3.23 (d, J = 2.4 Hz, 2H), 2.09 (t, J = 2.4 Hz, H). ^{13}C NMR ($CDCl_3$-d_1): δ 168.1, 141.2, 138.1, 133.9, 133.2, 133.1, 115.7, 115.2, 115.0, 114.9, 73.5, 61.6, 53.8, 25.7. IR (KBr disk): 2240 cm^{-1} (CN), 1733 cm^{-1} (C═O).

Dimethyl (3,4-dicyanophenyl)(*o*-carboranylmethyl)malonate, 6. To a two-necked round-bottomed flask charged with $B_{10}H_{14}$, **5** (0.82 g, 0.0067 mol), was added 50 mL of anhydrous acetonitrile by syringe. The solution was heated to reflux under argon for 1 h and then allowed to cool to room temperature. Compound **4** (2.0 g, 0.0067 mol) was added, and the resulting solution was futher refluxed under argon for 40 h or until TLC analysis indicated that no compoud **4** could be observed in the solution. Acetonitrile was evaporated *in vacuo* to give a waxy white solid. The solid was dissolved in a small volume of methanol and then adsorbed on a small portion silica gel and loaded onto a silica gel column which was eluted with hexane and THF (4:1). The first fraction was collected to give 1.4 g of the desired product **6**

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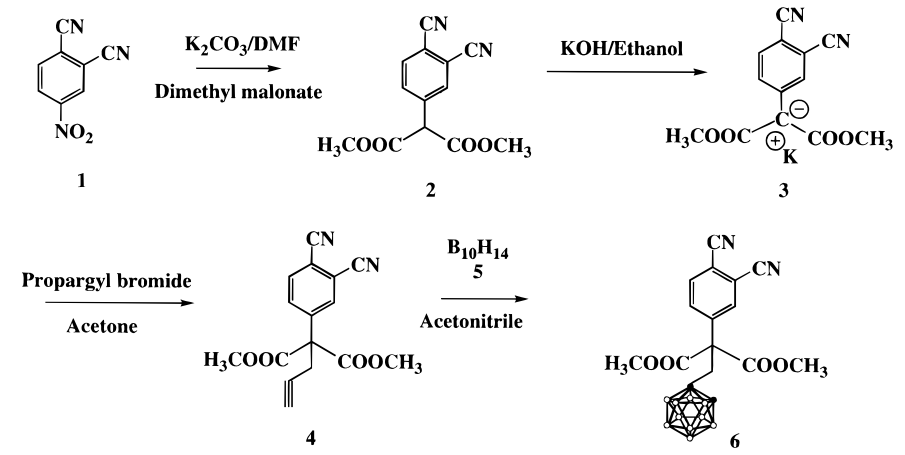
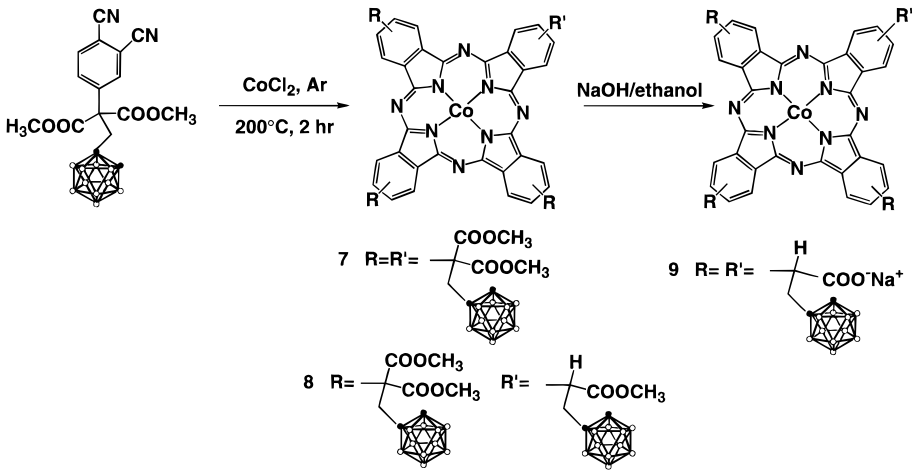
Scheme 1



Scheme 2



(50% yield) as a light yellow solid (mp: 184−185 °C). HRMS for $C_{16}H_{22}B_{10}N_2O_4$ (M^+): calcd, 414.2582; found, 414.2564 ($\Delta = 1.8$ ppm). ^{1}H NMR ($CDCl_3$-d_1): δ 8.16 (dd, $J = 1.5$ Hz, H), 7.99, 7.96 (2dd, $J = 1.5$ Hz, $J = 8.4$ Hz, H), 7.83 (d, $J = 8.4$ Hz, H), 3.82 (s, 6H), 3.41 (s, 2H), 2.0 (br, 10H), 1.3 (s, H). ^{13}C NMR ($CDCl_3$-d_1): δ 167.4, 140.5, 133.5, 133.4, 132.9, 116.3, 115.9, 114.8, 114.6, 70.5, 60.9, 54.1, 43.5, 26.4. IR (KBr disk): 2629 and 2582 cm^{-1} (BH), 2232 cm^{-1} (CN), 1748 cm^{-1} (C═O).

(2,9,17,23-Tetrakis(1,1-(dimethoxycarbonyl)-2-(*o*-carboranyl)ethyl)phthalocyanato)cobalt(II), 7. A mixture of compound **6** (300 mg, 0.17 mmol) and anhydrous $CoCl_2$ (98 mg, 0.77 mmol) was pulverized and transferred to a 20 mL single-necked flask. The mixture was heated to 200 °C under argon on an oil bath in about 40 min and kept at 200 °C for 2 h. The resulting dark blue solid was chromatographed and eluted with a solution of toluene and ethyl acetate (100:3) to give 84 mg of the desired product **7** (27% yield) as a dark blue solid. MS: *m*/*z* 1717 (MH^+, 94%). IR (KBr disk): 2587 cm^{-1} (BH), 1739 cm^{-1} (CO). UV−vis (CH_2Cl_2): λ (nm) (log ϵ) 266 (3.9), 328 (3.9), 602 (3.6), 630 (3.6), 670 (4.3). Anal. Calcd for $C_{64}H_{88}B_{40}N_8O_{16}Co$: C, 44.78; H, 5.16; N, 6.53. Found: C, 44.80; H, 5.32; N, 6.42.

(2,9,17-Tris(1,1-(dimethoxycarbonyl)-2-(*o*-carboranyl)ethyl)-23-(1-methoxycarbonyl)-2-((*o*-carboranyl)ethyl)phthalocyanato)cobalt-(II), 8. A mixture of compound **6** (300 mg, 0.17 mmol) and anhydrous $CoCl_2$ (93 mg, 0.73 mmol) was ground to a fine powder and then transferred to a 20 mL single-necked flask. The mixture was quickly heated to 210 °C and kept at 210−220 °C for 8 h under argon. A dark blue solid was formed. The solid was chromatographed using a solution of toluene and dichloromethane (100:3) as the eluant to give 80 mg of the desired product **8** (20% yield) as a blue solid. MS: *m*/*z* 1659 (MH^+, 60%). IR (KBr disk): 2589 cm^{-1} (BH), 1739 cm^{-1} (CO). UV−vis (CH_2Cl_2): λ (nm) (log ϵ) 206 (4.0), 328 (3.9), 604 (3.6), 668 (4.3). Anal. Calcd for $C_{62}H_{86}B_{40}N_8O_{14}Co$: C, 44.89; H, 5.22; N, 6.75. Found: C, 44.91; H, 5.12; N, 6.81.

Sodium Salt of (2,9,17,23-Tetrakis(1-carboxyl-2-(*o*-carbonyl)-ethyl)phthalocyanato)cobalt(II), 9. To a 25 mL single-necked flask charged with anhydrous ethanol (10 mL) was added cut sodium pieces (53 mg, 2.3 mmol) with stirring under argon. After the sodium was completely dissolved, compound **7** (80 mg, 0.05 mmol) was added under argon. The resulting blue solution was stirred at ambient temperature under argon for 4 days. The solution was concentrated *in vacuo* and the residue dissolved in water. A trace amount of undissolved particles was filtered off. The water layer was acidified with 0.1 M aqueous HCl solution. The precipitated deep blue product was filtered off and dissolved in a solution of acetone/water (8:2), passed through an ion exchange column packed with Dowex 50WX2-400 ion exchange resin in Na^+ form, and eluted with water. Acetone was evaporated by rotary evaporator and water was lyophilized to produce the sodium salt **9** in 60% yield as a blue solid. UV−vis (H_2O): λ (nm) (log ϵ) 636 (5.4), 290 (5.6), 192 (5.8). Anal. Calcd for $C_{52}H_{68}B_{40}N_8O_8CoNa_4 \cdot 6H_2O$: C, 38.45; H, 4.96; N, 6.90. Found: C, 38.49; H, 4.89; N, 6.75.

Results and Discussion

In this paper we report the synthesis and characterization of the first boronated metallophthalocyanine in which four *closo*-carborane cages are covalently linked to the periphery of the phthalocyanine ring. Water-solubility is obtained by hydrolyzing eight methyl ester groups followed by ion exchange to the sodium salt. Schemes 1 and 2 present the synthetic procedure used to prepare this water-soluble boronated metallophthalocyanine.

Substituted phthalocyanines are generally prepared either by solid state condensation of a substituted phthalonitrile in the presence of a metal template or by derivatization of a preformed

phthalocyanine. Two strategies are then obvious for the preparation of a boronated phthalocyanine: (1) attach the boron at an early stage by placing the boron moiety on the phthalonitrile or (2) add the boron group to a reactive functional group on a suitable preformed phthalocyanine derivative. A further consideration in choosing from these two strategies is the need to achieve a modicum of aqueous solubility in the final product. The second strategy is attractive in that, in principle, the essential boron group(s) can be added in a high-yield step at the culmination of the route, an important consideration when ^{10}B reagents are to be used. However, the solubility requirement led us to adopt the former approach since it appeared possible to incorporate a protected solubilizing group, the methyl esters, into the phthalonitrile and then deprotect in a later step.

Synthesis of dimethyl malonate, **2**, from commercially available nitrophthalonitrile, **1**, was readily accomplished by literature techniques.[5] The yield of **2** was comparable to the reported yield of 40%. Conversion of **2** to the desired propargylphthalonitrile, **4**, was readily carried out in 83% yield by conversion to the potassium malonate salt, **3**, followed by reaction with propargyl bromide. Addition of the boron was made at this stage by condensation of decaborane ($B_{10}H_{14}$), **5**, with the ethynyl group of **4**. It was found that *in situ* conversion of **5** to its bis(acetonitrile) adduct of decaborane followed by reaction with the alkyne resulted in higher product yields and shorter reaction times than reacting the preformed bis(acetonitrile) adduct with the alkyne in either THF or toluene. Carborane phthalonitrile **6** was isolated in 50% yield after chromatography, a typical yield for this type of condensation.

Solid state condensation of boronated phthalonitrile **6** to boronated phthalocyanine **7** was carried out by heating a pulverized mixture of **6** and cobalt(II) chloride at 200 °C under an argon atmosphere for 2 h. The relatively low yield of **7** (27%), while typical of this type of reaction, illustrates again the potential advantages of adding the boron at a later stage. When **6** and cobalt(II) chloride were quickly heated to 210 °C and kept at this temperature for 8 h, a different phthalocyanine was produced whose elemental composition and mass spectrum corresponded to partially decarboxylated phthalocyanine **8**.

Boronated metallophthalocyanines **7** and **8** were fully characterized by their spectroscopic data and elemental analyses. The liquid secondary ion mass spectra (LSIMS) of **7** and **8** in a nitrobenzyl alcohol (NBA) matrix show molecular ion clusters (MH^+) at nominal *m*/*z* 1717 and 1659 corresponding to the formulas $C_{64}H_{88}B_{40}N_8O_{16}Co$ and $C_{62}H_{86}B_{40}N_8O_{14}Co$, respectively. The clusters of the theoretical molecular ions for these formulae are almost identical to ion clusters observed at *m*/*z* 1717 and 1659, respectively. The infrared spectra of **7** and **8** exhibit strong B−H absorption bands at 2587 and 2590 cm^{-1}, respectively, and a strong carbonyl absorption band at 1739 cm^{-1}. The UV−vis spectra of **7** and **8** in dichloromethane show absorption maxima at 670 and 668 nm, respectively. Both compounds presumably exist as a complex set of positional isomers since the dysymmetric phthalonitrile starting material **6** can condense in either of two orientations. However, no isomeric separation was observed on thin layer, column, or HPLC chromatography.

Attempts to hydrolyze the ester groups of compound **7** under either mild or extreme acidic conditions failed. Hydrolysis under basic conditions (sodium ethoxide in absolute ethanol), however, was successful and was accompanied by decarboxylation to give the tetraacid upon acidification. This compound was not isolated for characterization, but was immediately converted to the highly water-soluble sodium salt **9** by cationic exchange. Compound **8** was hydrolyzed under the same conditions to give compound **9** after ion exchange. Elemental analysis of compound **9** illustrated that partial decarboxylation occurs under these conditions. Decarboxylation of carborane esters under similar conditions has been reported by Zakharkin *et al*. who reported that overnight room temperature treatment of ethyl 3-(2-phenylcarboranyl)propionate with potassium hydroxide in absolute ethanol produced 1-ethyl-2-phenylcarborane.[6] We have also previously observed decarboxylation of diethyl 2,2-bis(carboranylmethyl)malonate to 2,2-bis(carboranylmethyl)acetic acid with sodium ethoxide in absolute ethanol.[7] No degradation of the carborane cages of **7** or **8** to the *nido* open cage form was observed under these conditions as evidenced by the infrared spectrum of compound **9** which exhibited a B−H absorption band at 2587 cm^{-1} identical to the starting material **7**. Opening of the *closo*-carborane cage generally shifts this absorption to the vicinity of 2450 cm^{-1}. ^{11}B NMR spectra of **7** and **9** were substantially broadened by the presence of the paramagnetic Co(II) and were not useful in establishing the nature of the cage in **9**.

Conclusions

Compound **9** is the first fully characterized boronated phthalocyanine to be reported and contains four *closo* carborane cages covalently linked to the phthalocyanine periphery. Substantial aqueous solubility is obtained through the presence of four carboxylate residues as their Na^+ salts. This boronated phthalocyanine may be useful as a tumor-selective sensitizer for boron neutron capture therapy using thermal neutrons to activate ^{10}B through the $^{10}B(n,\alpha)^7Li$ reaction, or in photodynamic therapy by activation of the nitrogen macrocycle with light. Studies evaluating the *in vitro* and *in vivo* localizing ability of **9** are currently being carried out and will be reported in an appropriate venue in the near future. The synthesis of other boronated phthalocyanines in which the boron cages are added at a later stage to preformed derivatized phthalocyanines is currently being explored and will also be reported in the near future.

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