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Closomers of High Boron Content: Synthesis, Characterization, and Potential Application as Unimolecular Nanoparticle Delivery Vehicles for Boron Neutron Capture Therapy

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Unique nanosized closomers of high boron content that may exhibit potential as boron neutron capture therapy target species have been synthesized. The design of these boron-rich nanospheres is based in part on previous work involving dodeca(carboranyl)-substituted closomers [Thomas, J.; Hawthorne, M. F. *Chem. Commun.* **2001**, 1884–1885]. Coupling of *ortho*-carborane moieties through ester and ether linkages to the rigid [*closo*-$B_{12}(OH)_{12}]^{2-}$ scaffold resulted in the development of a 12(12)-closomer–ester derivative, dodeca[6-(1,2-dicarba-*closo*-dodecaboran-1-yl)hexanoate]-*closo*-dodecaborate (2–), **6**, and 12(12)-closomer–ether derivatives, dodeca[6-(2-methy1-1,2-dicarba-*nido*-dodecaboran-1-yl)hexyl]-*closo*-dodecaborane (14–), **14**, and dodeca[6-(7,8-dicarba-*nido*-dodecaboran-7-yl)hexyl]-*closo*-dodecaborane (14–), **15**. These closomers were investigated by UV–visible spectroscopy and cyclic voltammetry. Additionally, a deboronation method employing NaCN as the nucleophilic reagent was utilized to obtain sodium salts of the ether-linked *nido*-closomer polyanions, which were purified using a newly developed size-exclusion high pressure liquid chromatography method.

Introduction

Among the challenges precluding the widespread use of boron neutron capture therapy (BNCT) has been the difficulty in achieving selective delivery of large quantities of boron nuclei to malignant cancer cells. Effective therapy and tumor destruction upon irradiation with thermalized neutrons requires approximately 30 μg ^{10}B per gram of tumor tissue or 10^9 ^{10}B atoms per tumor cell.[2] To realize these high concentrations, much research has focused on liposomes as boron delivery vehicles because[3−9] of their ability to encapsulate and transport large quantities of boron-rich solute molecules, while their diminutive size (30−150 nm) enables these small vesicles to pass through the porous immature vasculature of rapidly growing tumor tissue,[10] conferring a certain degree of tumor specificity.

The concept of using liposomes as delivery vehicles was first proposed in 1974.[11,12] Initially intended as a means to augment antibody responses in vivo by transporting antigens, this technique has evolved over the past 30 years and, today, is effectively used to transfer an ever-expanding variety of drugs[13,14] and vaccines.[15] Additionally, the liposome system

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has proven to be a successful model for the transport of large quantities of boron for BNCT,[6,16,17] beginning with the first water-soluble polyhedral borane, $Na_3[B_{20}H_{17}NH_3]$ (TAC), which was encapsulated in the hydrophilic core of small unilamellar phospholipid vesicles. Biodistribution studies[3,4] showed that it was possible to deliver therapeutic doses of ^{10}B atoms to tumor cells upon the administration of relatively low injected doses of these loaded liposomes. BALB/c mice bearing EMT6 tumors indicated significant tumor boron uptake from the blood over the course of the 48-h experiment, to a maximum of 32 μg of boron per gram of tumor 30 h post-injection.[3,4]

In an effort to improve the effectiveness of such systems for BNCT, boron-rich compounds have also been modified for use as addenda to the liposome—for incorporation directly into the bilayer during its construction.[4,5,18] By the inclusion of the amphiphilic K[*nido*-7-$CH_3(CH_2)_{15}$-7,8-$C_2B_9H_{11}$] into a cholesterol/distearoylphosphatidylcholine membrane (0.6:1:1) encapsulating a 200 mM TAC solution, significantly enhanced tumor boron concentrations of 48 μg B/g of tumor were realized[4,5] upon the injection of a dose equivalent to 18 mg B/kg of body weight.

Considering these past achievements, liposomes represent a field of research in need of more vigorous investigation. Despite the obvious potential of such systems with regard to the continued advancement of BNCT, liposomes currently represent a relatively inefficient method of boron delivery. Regardless of the technique for boron integration (encapsulation in the hydrophilic core or incorporation into the lipid bilayer), total boron content accounts for approximately 5% of the whole liposome mass,[16] while the remainder is composed predominantly of the therapeutically inactive phospholipid and cholesterol building blocks.[1] Accordingly, boron-rich nanoparticles based on the attachment of boron clusters to a closomer framework,[19] a rigid near-spherical core of 12 boron atoms, provides an attractive alternative to liposomal boron delivery. In addition, such species may, in principle, be targeted to bioreceptors.

Nanosized closomer derivatives, ester and ether conjugates of the perhydroxylated [*closo*-$B_{12}(OH)_{12}]^{2-}$ scaffold, contain up to 40% boron by weight and are extremely small in size (approximately 3 nm), which should confer some tumor selectivity and lead to good permeation of solid tumors, similar to that seen in liposome-based systems.[20] Their potential role in BNCT is further enhanced by their amphiphilic nature, ensuring solubility and interaction with both hydrophilic and lipophilic systems, a spherical profile due to the icosahedral core, tunability through branching and functionalization, and increased water solubility that is characteristic of the corresponding anionic *nido*-carborane. In 2001, Thomas and Hawthorne described the synthesis of dodeca[7-(2-methyl-1,2-dicarba-*closo*-dodecaboran-1-yl)heptanoate]-*closo*-dodecaborate (2−) and its nido analogue,[1] boron-rich nanospheres containing 132 and 120 boron atoms, respectively. The obvious potential applications of such species, including BNCT, provided the impetus for the design of three additional ester- and ether-linked closomers, **6**, **14**, and **15**. Additionally, a novel mild deboronation method is presented for *ortho*-carboranes together with the animal toxicity data, indicating that the closomer-based boron-rich nanoparticles are well-tolerated in therapeutic doses by BALB/c mice.

Results and Discussion

The rigid icosahedral scaffold that is the [*closo*-$B_{12}(OH)_{12}]^{2-}$ anion (tetrabutylammonium (TBA) salt) precursor was used here to build 12(12)-closomer nanoparticles consisting of 12 *ortho*-carborane cages linked through ester and ether functions to the central boron core. The synthesis of these perfunctionalized salts required coupling of 12 activated radial-arm precursor molecules with the polyfunctional core.[21] The resulting highly boron-enriched closomer was very compact in size (approximately 3 nm). Conversion of the pendant *closo*-carborane moieties to the corresponding anionic nido form resulted in the synthesis of amphiphilic species with increased water solubility.

Carboxylate Closomers. To maintain the near-spherical nature of the [*closo*-$B_{12}(OH)_{12}]^{2-}$ core, 12-fold conjugation was desired. In 2001, the successful syntheses of the first dodecaborane ester and ether derivatives were reported.[22,23] Since that time, this process has been adapted to achieve a variety of perfunctionalized closomers,[24,25] including dodeca[7-(2-methyl-1,2-dicarba-*closo*-dodecaboran-1-yl)heptanoate]-*closo*-dodecaborate (2−) and its nido analogue,[1] upon reaction of the [*closo*-$B_{12}(OH)_{12}]^{2-}$ anion with an excess of the activated radial-arm-precursor species.

Boron-rich carboxylate ester closomers were constructed from the perhydroxylated scaffold together with the carborane-containing carboxylic acid moiety, **2**. Though this species was originally synthesized by Feakes et al. in their preparation of boron-containing cholesterol derivatives,[18] two new strategies for its synthesis are presented here. To create the six-carbon linker that is crucial for the accommodation of 12-carborane cages around a single $(TBA)_2$[*closo*-$B_{12}(OH)_{12}$] closomer, methodologies involving octynoic acid (method 1, Scheme 1) and 1,6-dibromohexane (method 2, Scheme 1) are presented.

Preparation of the precursor, **5**, via octynoic acid followed the literature method utilizing 6-bromohexanoic acid.[26] After

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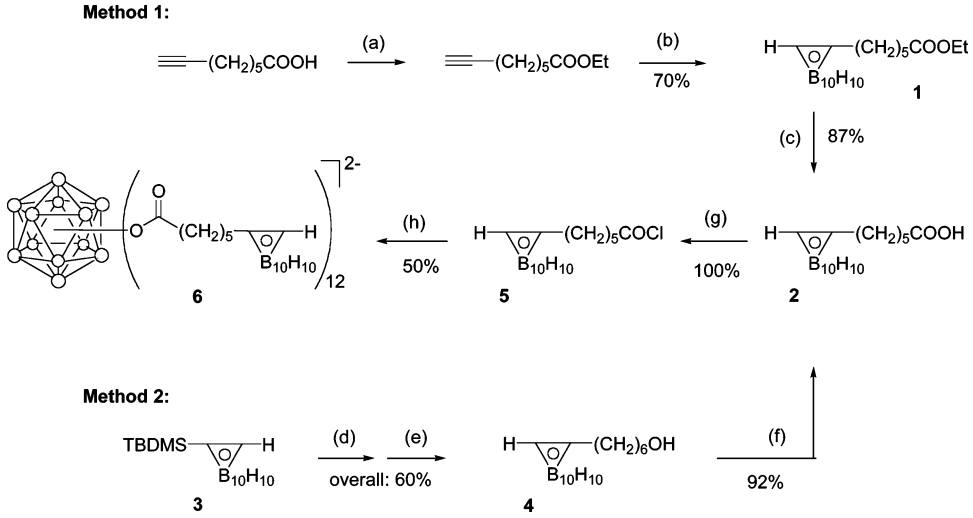
Scheme 1. Synthesis of *closo*-**6** from Acid Chloride **5**[a]



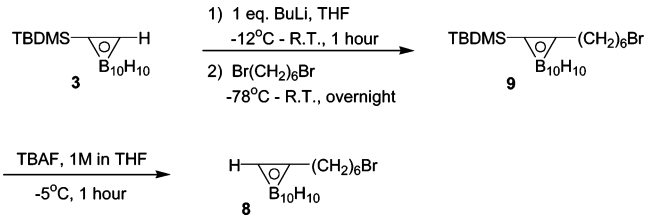
[a] Reagents and conditions: (a) EtOH, *p*-toluenesulfonic acid, benzene, reflux; (b; 1) $B_{10}H_{14}$, CH_3CN, toluene, reflux, overnight; (2) ethyl octyonate, reflux 72 h; (c) H_2SO_4, 1,4-dioxane, reflux, overnight; (d; 1) 1 equiv BuLi, 1 h; (2) 1,6-dibromohexane, −78 °C reflux, overnight; (e) wet NMP, 110 °C, overnight; (f) H_5IO_6/CrO_3, CH_3CN; (g) $SOCl_2$, CH_2Cl_2, reflux, overnight; (h) $(TBA)_2B_{12}(OH)_{12}$, *i*-Pr_2NEt, CH_2Cl_2, reflux.

the protection of the acid, the ethyl ester was reacted with $B_{10}H_{12}(NCCH_3)_2$, prepared by refluxing decaborane overnight in CH_3CN,[27,28] to yield ethyl-6-(1,2-dicarba-*closo*-dodecaboran-1-yl)hexanoate, **1**. Deprotection with sulfuric acid afforded 6-(1,2-dicarba-*closo*-dodecaboran-1-yl)hexanoic acid, **2**, in a 61% overall yield.

In an effort to avoid the inconvenient protection and deprotection steps outlined above, an alternative method was successfully attempted to create the desired carboxylic acid, **2**. The starting material, 1-(*tert*-butyldimethylsilyl)-1,2-dicarba-*closo*-dodecarborane, **3**, easily synthesized from 1,2-dicarba-*closo*-dodecarborane,[29] was first treated with 1 equiv of *n*-butyllithium. To the lithiated carbanion was added a large excess of 1,6-dibromohexane, which served to decrease the likelihood that carborane dimers would be formed. After the removal of the excess starting material, the crude bromo product was directly treated with wet *N*-methylpyrrolidine (NMP) and heated to 110 °C overnight. In addition to converting the bromo functionality to hydroxyl, NMP also removed the *tert*-butyldimethylsilyl (TBDMS) protecting group.[30] Purification of the crude product with silica-gel column chromatography afforded 6-(1,2-dicarba-*closo*-dodecaboran-1-yl)hexan-1-ol, **4**, as a colorless oil. The oxidation of **4** with H_5IO_6/CrO_3 solution in wet acetonitrile[31] afforded **2** in a 59% overall yield.

Initial attempts to create the percarborane closomer **6** via the conjugation of bis-tetrabutylammonium [*closo*-$B_{12}(OH)_{12}$] and **2** with carbonyldiimidazole met with little success. Though varied reaction conditions were explored, including the use of refluxing acetonitrile, 1,2-dichloroethane, and dichloromethane, no product could be isolated. Alternatively, carboxylic acid **2** was converted to the more reactive acid chloride **5** for the reaction with $(TBA)_2$[*closo*-$B_{12}(OH)_{12}$] in the presence of diisopropylethylamine in CH_2Cl_2. This afforded the desired ester-linked *closo*-closomer **6**, which was confirmed by ^1H, $^{13}C\{^1H\}$, ^{11}B, and $^{11}B\{^1H\}$ NMR spectroscopy and high-resolution mass spectrometry (HRMS) [electrospray ionization (ESI)].

Scheme 2. Synthesis of 6-(1,2-*closo*-Dodecarboran-1-yl)hexyl Bromide



Increased water solubility of carborane derivatives, a requirement for their use in vivo, can be achieved by the removal of one BH vertex to afford the corresponding *nido*-anion derivative. Attempts to achieve deboronation of radial-arm moieties of ester closomer **6** met with little success and instead resulted in the transesterification. Additional details on the methods employed are described below.

Ether-Linked Closomers. Earlier work[32] described the synthesis of 1-(6-bromo)hexyl-2-methyl-1,2-dicarba-*closo*-dodecarborane, **7**, which was used here in addition to the novel 1-(6-bromo)hexyl-1,2-dicarba-*closo*-dodecarborane, **8**, to produce boron-rich nanoparticles for evaluation related to BNCT.

The synthesis of ligand **8**, 1-(6-bromo)hexyl-1,2-dicarba-*closo*-dodecarborane, is shown in Scheme 2. *closo*-1-(*tert*-Butyldimethylsilyl)-1,2-carborane, **3**,[33] reacted with 1 equiv of *n*-butyllithium to form the lithiated intermediate, to which a large excess of 1,6-dibromohexane was added in situ. Tetrabutylammonium fluoride (TBAF) was used to remove

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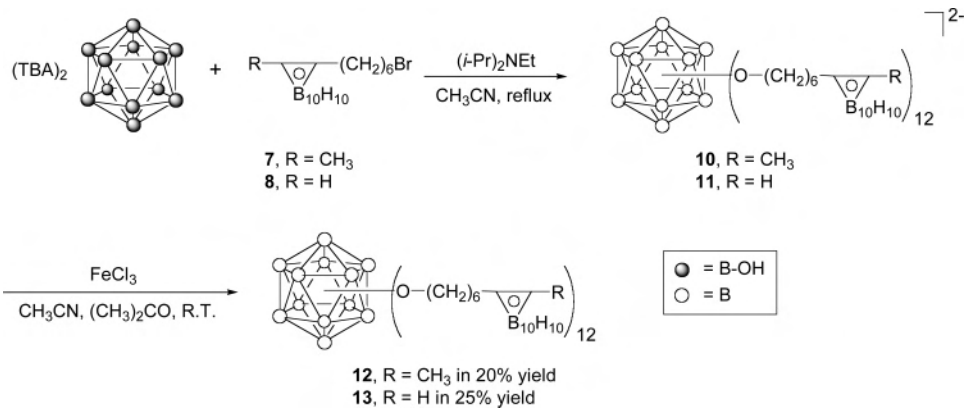
Scheme 3. Synthesis of Ether Closomers



the TBDMS protecting group from the crude product, **9**, to give 6-(1,2-dicarba-*closo*-dodecaboran-1-yl)bromohexane, **8**, in a 75% overall yield.

Conjugation of radial-arm precursors **7** and **8** to the closomer core (Scheme 3) produced ether-linked boron-rich closomers, monodisperse nanoparticles. To achieve the symmetrical fully substituted product (**10** or **11**), a large excess of the bromides, **7** or **8**, was reacted with the $(TBA)_2$-[*closo*-$B_{12}(OH)_{12}$] core in the presence of diisopropylethylamine in acetonitrile. After reacting for 14 days at the reflux temperature, the dianions, **10** and **11**, were obtained to which $FeCl_3$ was directly added to achieve the oxidation of the crude products to the neutral hypercloso derivatives. Upon completion of the oxidation reaction, the excess unreacted bromide precursors were recovered by column chromatography to afford the pure products, **12** and **13**, as dark yellow solids in 20 and 25% yields, respectively. The hypercloso derivatives, **12** and **13**, were characterized by ^1H, $^{13}C\{^1H\}$, ^{11}B, and $^{11}B\{^1H\}$ NMR spectroscopy, as well asHRMS [matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF)] to confirm the identity of the 12(12) *closo*-closomer. Efforts to obtain a single crystal of **13** for X-ray diffraction studies were unsuccessful.

Closomers Incorporating *nido*-Carboranes. Traditionally, *nido*-carboranes are obtained from *ortho*- or *meta*-carborane derivatives by degradation with strong bases or fluoride ions.[34−36] Accordingly, the ether-based nido analogues of **12** and **13** were prepared via the partial degradation of the pendant *ortho*-carboranes using CsF in a mixture of tetrahydrofuran (THF) and ethanol at the reflux temperature.[34] The mixed solvent system was used to accommodate the differing solubility properties of the closomer and CsF. After the normal workup of the reaction mixture, the white solid obtained from the organic layer is the cesium salt of the fully reduced (−14) *nido*-closomers, which turned pink upon exposure to air as a result of the air oxidation of the central boron core from the 2− to the monoanionic state.

For the purposes of bioevaluation (and eventual use in vivo), the water solubility of these compounds is of prime importance. To facilitate this, ion-exchange chromatography of the water-soluble closomer species is usually possible. However, the highly charged nido derivatives of Cs_2-**12** and Cs_2-**13** have extremely poor water solubility and tend to stick tightly on the column. Other degradation reagents, such as KF and literature-reported amines such as piperidine,[37,38] were also utilized under varying conditions, but no satisfactory nido-compound production was observed. This difficulty in obtaining water-soluble *nido*-closomers via ion exchange prompted a search for a new degradation reagent to directly produce water-soluble sodium salts of the closomers.

As strong bases, sodium hydroxide and sodium ethoxide have been used to degrade carboranes to their nido analogues,[39] both methods resulting in the sodium salt of these nido compounds. The extremely harsh conditions necessary to carry out such reactions preclude their use with base-sensitive species and result in the degradation of both *ortho*- and *meta*-carboranes. Alternatively, it was hypothesized that NaCN might also be used as an effective deboronating reagent because it is a nucleophilic ion that forms tight bonds with boron.[40] Previously, NaCN was effectively used to deboronate *arachno*-4,5-$C_2B_7H_{13}$ to give the *hypo*-$C_2B_6H_{13}^-$ anion.[41] As a basic salt (HCN, pK_a = 9.21), deboronation conditions with NaCN are significantly milder than the more common technique involving NaOH and ethanol and can provide the sodium salt of the degraded carboranes directly with no need for an ion exchange. To test this hypothesis, attempts to deboronate a model compound, *ortho*-carborane, were carried out under a variety of conditions and monitored by NMR. In each case, the reaction was carried out using 2 equiv of 0.1 M NaCN in either ethanol or methanol. After 60 h, no deboronated product was observed for the reactions carried out at room temperature, but a greater than 90% yield of the desired product was achieved after only 20 h at elevated reflux temperatures.

To test the stability of closomer−ether and closomer−ester linkages under these newly developed conditions, model

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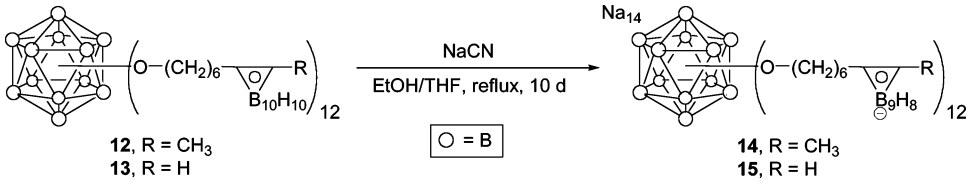
Scheme 4. Deboronation To Achieve *nido*-Closomers



compounds of each were deboronated with NaCN in refluxing ethanol. No cleavage of the ether linkage was observed, but the ester-linked compound was less stable and did undergo transesterification.

When the NaCN method was used, *nido*-closomers of the ether-linked **12** and **13** were obtained as sodium salts in a mixed solvent system of THF and ethanol (Scheme 4). Upon deboronation, *nido*-**15** was obtained as a white solid that showed superior solubility in water over its closo precursor. Though less water soluble, the sodium salt of *nido*-**14** was also obtained and proved soluble in ethanol and methanol. Upon exposure to air, solutions of the colorless compounds **14** and **15** turned purple as a result of the single-electron oxidation of their closomer cores. The identity of the nido compounds was proven by ^{1}H, ^{13}C{^{1}H}, ^{11}B, and ^{11}B{^{1}H} spectroscopy, as well as ESI-HRMS.

As a result of problems associated with transesterification under basic conditions, the NaCN method of deboronation was not applicable to the ester-linked closomer, **6**. Instead, degradation to the nido derivative was attempted in the presence of CsF and ethanol. The characteristic nido peaks were observed by ^{11}B NMR, but the cesium salt was, again, not water soluble. This extreme water insolubility precluded ion exchange to the sodium salt. While additional efforts are underway to further increase the water solubility of boron-rich ester closomers, currently no improved strategies are available, preventing the use of this nanoparticle in vivo.

Animal Toxicity and Biodistribution Studies. As a result of their similarity in terms of size and boron content, the results of animal toxicity studies of **14** and **15** are likely to be very similar and repetitious. Because the deboronation of **12** to give Na_{14}-**14** resulted in a nanoparticle with enhanced hydrophilicity that was obtained much more readily than **15**, likely a result of problems associated with the added steric bulk of the methylated carbon on each of its 12 radial arms, only the animal toxicity and biodistribution data for **14** is reported.

Size-exclusion high pressure liquid chromatography (HPLC) was employed to ensure the purity of **14** for animal toxicity studies. Size-exclusion chromatography was chosen because the functionalized closomers, with a mass of approximately 3000 Da, are significantly larger than any of the starting materials. Upon isolation of the product and removal of all solvents under vacuum, the clean salt was obtained as a pink solid.

Normal BALB/c mice were used in 48-h toxicity studies of the *nido*-closomer **14**. Five animals were each injected with 10 mg/kg of **14**; an additional two mice received a dose of 20 mg/kg, approximately 30% of which was administered subcutaneously to one of the remaining mice. The results indicated that a therapeutic dose (10 mg B/kg of body weight) of the *nido*-closomer **14** was well-tolerated by the mice and not toxic.

Murine biodistribution studies of *nido*-closomer **14** were conducted at Washington State University, Pullman, using BALB/c mice (16−20 g) bearing EMT6 tumors (125−350 mg at the time of sacrifice). Observation of the mice upon injection of the boronated compound with a dose equivalent to 10 mg B/kg of body weight continued for 48 h, at which time the animals were sacrificed for tissue analysis. Boron uptake by tumor tissue reached a maximum at 24 h post-injection, achieving a maximum tumor boron concentration of 10.5 μg B/g of tumor, with a tumor-to-blood ratio of 9.4. Other tissues, including the skin and brain, showed only minimal boron uptake (0.93 and 0.27 μg B/g of tissue, respectively), while the liver and kidneys had significantly higher boron uptake, as is expected for these organs. The complete graphical representation of the biodistribution data can be found in Supporting Information.

Electronic Spectra of Ether Closomers. The electronic spectra of the yellow, neutral, 24-electon species, **12** and **13**, were measured in solutions of methylene chloride (1.24×10^{-5} and 1.31×10^{-5} M, respectively). As a result of their structural similarity, both **12** and **13** have nearly identical electronic spectra that are dominated by a broad absorption centered at 459 nm (ϵ = 21 900 and 27 500 L mol^{-1} cm^{-1}, respectively). This absorption is attributed to the 24-electron *hypercloso* core. Furthermore, the absence of the 25-electron *hypercloso* radical anion is evident by the lack of absorbance at 540 nm.

Cyclic Voltammetry Studies of the Ether Closomers. Theoretical studies of ether closomers using density functional theory were recently published.[42] In this work, McKee offers further evidence in support of experimental observations that closomers functionalized through ether linkages readily undergo one-electron oxidations upon exposure to oxidants, including oxygen. To further quantify this observation, the redox chemistry of ether-closomers **12** and **13** was studied by cyclic voltammetry. As expected, cyclic voltammograms (0.10 M [TBA] PF_6, Ag/AgCl, CH_2Cl_2) of **12** and **13** are very similar. Two quasi-reversible one-electron processes were observed in each case, which are attributed to the dianion/radical (−2/−1) and radical/neutral (−1/0) redox couples. Half-wave potentials for closomer **12** were −1.22 and −0.47 V versus ferrocene for the (−2/−1) and (−1/0) couples, respectively. Similarly, closomer **13** exhibited half-wave potentials of −1.31 and −0.54 V for the same redox couples. These values are comparable to other ether-

(42) McKee, M. L. *Inorg. Chem.* **2002**, *41*, 1299−1305.

linked closomers. Each redox couple exhibits anodic-to-cathodic peak separations much larger than the Nernst value of 59 mV for a one-electron process and, therefore, each may be classified as quasi reversible.

Conclusion

Three novel boron-rich closomers, each with an isosahedral dodecaborane core and 12 pendant *ortho*-carborane moieties, have been synthesized and characterized. The ester closomer proved unsuitable for use in vivo because of extreme water insolubility, a result of the fact that conversion to the nido analogue was not possible as a result of problems associated with transesterification. The electronic spectra for each closomer were dominated by an absorbance at 459 nm, corresponding to the neutral *hypercloso* core. The ether closomers **12** and **13** exhibited interesting redox propoerties and exist in three oxidation states: a 26-electron dianion, a 25-electron radical anion, and a 24-electron neutral species. Electrochemical studies demonstrated two one-electron, quasi-reversible redox couples with half-wave potentials of approximately −1.25 and −0.5 V.

A novel deboronation method involving sodium cyanide for the selective degradation of *ortho*-carborane cages has been demonstrated. Previously, NaOH and KOH have been successfully used for this purpose but provided no selectivity in the deboronation of *ortho*- over *meta*-carboranes. When this technique was used, the sodium salt of *nido*-closomer **14** was obtained and exhibited increased water solubility over other *nido*-closomer salts, an important factor for the successful application of these naonparticles as boron delivery vehicles for BNCT. Promising animal toxicity data was obtained, indicating that ether closomer **14** caused no ill effects in healthy BALB/c mice 48 h after the injection of a therapeutic dose of 10 mg B/kg of body weight. Murine biodistribution studies of **14** indicated that a maximum tumor boron concentration of 10.5 μg B/g of tumor was achieved 24 h post-injection, with a tumor-to-blood ratio of 9.4.

Experimental Section

Column chromatography was performed on silica gel 60 (Geduran, EMD). NMR spectra were recorded on a Bruker ARX 500-MHz spectrometer. Mass spectroscopic analyses were performed at UCLA Pasarow Mass Spectrometry Laboratory: electron impact (EI) analyses were measured on a Micromass GCT; fast atom bombardment (FAB) analyses were measured on a VG ZAB-SE; ESI analyses were measured on an IonSpec 7.0T Ultima Fourier transform mass spectrometer; and MALDI-TOF analyses were measured on an Applied Biosystems DE-STR. UV−visible spectra were recorded on a HP 845x UV−visible spectrophotometer. HPLC was completed using a HP 1100 with a size exclusion by Tosoh Bioscience (G 2000 SW_{XL}, 7.8-mm i.d., δ = 30 cm, purchased from the sales office at 156 Keystone Dr., Montgomeryville, PA 18936).

All reactions were carried out under an argon atmosphere. Reagents were reagent grade and used without further purification unless otherwise stated. Solvents for spectroscopic measurements were spectroscopy or HPLC grade. THF was freshly distilled from sodium benzophenone ketyl under nitrogen; CH_2Cl_2, CH_3CN, and Et_2iPrN were freshly distilled from CaH_2.

All closomer syntheses employed the perhydroxylated icosahedral derivative of the parent [*closo*-$B_{12}(OH)_{12}]^{2-}$ at the core, with activated *ortho-closo*-carborane units as pendant moieties. The icosahedral [*closo*-$B_{12}(OH)_{12}]^{2-}$ scaffold was synthesized according to the literature.[21]

Ethyl-6-(1,2-dicarba-*closo*-dodecaboran-1-yl)hexanoate, 1. To a stirred solution of decaborane (1.0 g, 8.0 mmol) in toluene (50 mL) was added freshly distilled acetontrile (5 mL). The mixture was refluxed overnight and cooled to room temperature for the addition of ethyl octynoate (1.25 g, 7.43 mmol), which was followed with stirring for 72 h. Upon completion, the solvent was removed in vacuo to give a brown residue that was extracted with ether and washed with water. The organic layer was dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo to give the crude product that was purified by silica-gel column chromatography (petroleum ether/diethyl ether) to afford a colorless oil (1.5 g, 70%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.10 (q, 2H, J = 7.1 Hz, −CH_2O−), 3.57 (s, 1H, −CH−), 2.26 (t, 2H, J = 7.4 Hz, CH_2-CO_2−), 2.18 (m, 2H, CC$H_2$$CH_2$−), 1.59 (m, 2H, $CCH_2CH_2CH_2CH_2$), 1.46 (m, 2H, $CCH_2CH_2CH_2CH_2$), 1.29 [m, 2H, $C(CH_2)_3CH_2$−], 1.24 (t, 3H, J = 7.1 Hz, OCH_2CH_3). ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.29 (C═O), 75.18 (br), 61.01 (br), 60.28, 37.77, 33.84, 28.81, 28.28, 24.26, 14.17. ^{11}B NMR (160 MHz, $CDCl_3$): δ −2.11 (d, 2B), −5.56 (d, 2B), −9.08 (d, 2B), −11.78 (m, 4B). HRMS (EI, M^+, 100%, *m*/*z*): calcd for $C_{10}H_{26}B_{10}O_2$, 286.2941; found, 286.2920.

6-(1,2-Dicarba-*closo*-dodecaboran-1-yl)hexanol, 4. Under a nitrogen atmosphere, BuLi (1.2 M, 45 mL) was added into a THF (160 mL) solution of **3** (12.7 g, 49.1 mmol) at 0 °C. The reaction mixture was stirred for 30 min before warming to room temperature with stirring for 5 h. For the addition of 1,6-dibromohexane (24 mL, 155 mmol), the reaction mixture was cooled to 0 °C before warming to reflux for 72 h. Upon completion of the reaction, the solvent was removed in vacuo and the excess 1,6-dibromohexane was recovered by vacuum distillation. To the crude oily residue (20 g) was added a wet NMP solution (25 mL, v/v, 15% H_2O) with stirring at 110 °C overnight. The mixture was extracted with diethyl ether and washed with water, saturated $NaHCO_3$, and brine before drying over anhydrous magnesium sulfate. After filtration, the filtrate was concentrated in vacuo and the crude product was purified by silica-gel column chromatography (hexane/diethyl ether) to afford a colorless oil (7.2 g, 60%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.62 (t, 2H, J = 6.5 Hz, −CH_2O−), 3.56 (s, 1H, −CH−), 2.19 (m, 2H, CC$H_2$$CH_2$−), 1.52 (m, 2H, $CCH_2CH_2CH_2CH_2$), 1.45 (m, 2H, $CCH_2CH_2CH_2CH_2$), 1.35 [m, 2H, $C(CH_2)_3CH_2$−], 1.28 (m, 2H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 75.45 (br), 62.76, 61.09 (br), 38.08, 32.47, 29.24, 28.77, 25.39. ^{11}B NMR (160 MHz, $CDCl_3$): δ −2.34 (d, 2B), −5.83 (d, 2B), −9.31 (d, 2B), −12.14 (m, 4B). HRMS (FAB−, M^-, 100%, *m*/*z*): calcd for $C_8H_{24}B_{10}O$, 244.2836; found, 244.2834.

6-(1,2-Dicarba-*closo*-dodecaboran-1-yl)hexanoic Acid, 2. Method 1. Into a round-bottom flask charged with ethyl-6-(1,2-dicarba-*closo*-dodecaboran-1-yl)hexonate (1.4 g, 4.9 mmol) were added 1,4-dioxane (20 mL) and sulfuric acid (6 M, 7.5 mL). The mixture was heated to reflux overnight. The organic layer was separated, and the remaining water layer was extracted with diethyl ether. The combined organic layers were dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated in vacuo, and the residue was purified by silica-gel column chromatography (methylene chloride followed by diethyl ether) to afford a white solid (1.12 g, 87%).

Method 2. Into a round-bottom flask charged with 6-(1,2-dicarba-*closo*-dodecaboran-1-yl)hexanol (5.2 g, 21 mmol) in wet MeCN (90 mL, 0.75 vol % water) was added H_5IO_6/CrO_3 solution (80

mL) dropwise at 0 °C. The mixture was stirred for 1 h and then brought to room temperature with stirring for 1 h. The MeCN was removed in vacuo, and the residue was extracted with diethyl ether. The organic phase was washed with water and brine and then dried over anhydrous magnesium sulfate. After filtration, the filtrate was concentrated under vacuum to afford the pure product (4.6 g, 92%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.55 (s, 1H, $-CH-$), 2.35 (t, 2H, $J = 7.4$ Hz, CH_2CO_2-), 2.20 (m, 2H, CCH_2CH_2-), 1.62 (m, 2H, $CCH_2CH_2CH_2$), 1.48 (m, 2H, $CCH_2CH_2CH_2$), 1.32 [m, 2H, $C(CH_2)_3CH_2-$]. ^{13}C NMR (125 MHz, $CDCl_3$): δ 179.59 (C=O), 75.07 (br), 61.03 (br), 37.80, 33.62, 28.82, 28.25, 24.00. ^{11}B NMR (160 MHz, $CDCl_3$): δ −2.15 (d, 2B), −5.59 (d, 2B), −9.09 (d, 2B), −12.26 (m, 4B). HRMS (FAB−, M^-, 100%, *m*/*z*): calcd for $C_8H_{22}B_{10}O_2$, 258.2629; found, 258.2631.

6-(1,2-Dicarba-*closo*-dodecaboran-1-yl)hexanoic Acid Chloride, 5. Under a nitrogen atmosphere, to a solution of **2** (4.2 g, 16.3 mmol) in dichloromethane (50 mL) was added thionyl chloride (12 mL, 164.5 mmol). The reaction mixture was stirred at room temperature overnight before the solvent and all excess reagents were removed in vacuo to afford the pure product. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.55 (s, 1H, $-CH-$), 2.89 (t, 2H, $J = 7.3$ Hz, CH_2CO_2-), 2.20 (m, 2H, CCH_2CH_2-), 1.70 (m, 2H, $CCH_2CH_2-CH_2$), 1.49 (m, 2H, $CCH_2CH_2CH_2$), 1.33 [m, 2H, $C(CH_2)_3CH_2-$]. ^{13}C NMR (125 MHz, $CDCl_3$): δ 173.66 (C=O), 75.07 (br), 61.22 (br), 46.79, 37.87, 28.86, 27.81, 24.64. ^{11}B NMR (160 MHz, $CDCl_3$): δ −2.79 (d, 2B), −6.25 (d, 2B), −9.78 (d, 2B), −12.68 (m, 4B).

Dodeca[6-(1,2-dicarba-*closo*-dodecaboran-1-yl)hexanoate]-*closo*-dodecaborate (2−), 6. To a solution of $(TBA)_2[B_{12}(OH)_{12}]$ (140 mg, 0.17 mmol) in dry dichloromethane (5 mL) was added **5** (4.5 g, 16.3 mmol) in methylene chloride (20 mL) and diisopropylethylamine (0.43 mL, 2.47 mmol). The mixture was heated to reflux for 21 days. The deep brown solution was concentrated in vacuo to reveal a residue that was purified by silica-gel chromatography. A diethyl ether eluent was used initially to remove most of the impurities, and then acetone elution was used to afford the product as an off-white solid that was further purified by an ether and dichloromethane wash to give the pure product (315 mg, 50%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.68 (s, 12H, $-CH$), 3.11 (t, 16H, NCH_2CH_2-), 2.20 (m), 1.58 (m), 1.45 (m), 1.31 (m), 1.05 ($-CH_3$, TBA). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.07 (*C*OO), 75.70 (br, *C*B), 61.46 (br, *C*B), 59.09 (NCH_2, TBA), 37.93, 35.84, 29.28, 28.56, 25.07, 23.87 (CH_2, TBA), 19.72 (CH_2, TBA), 13.57 (CH_3, TBA). ^{11}B NMR (160 MHz, $CDCl_3$): δ −2.91 (24B), −6.43 (24B), −9.84 (24B), −12.03 (48B), −17.99 (12B, *B*−O). HRMS (ESI, M^{2-}, 100%, *m*/*z*): calcd for $C_{96}H_{252}B_{132}O_{24}$, 1609.088; found, 1609.116.

6-(1,2-Dicarba-*closo*-dodecaboran-1-yl)bromohexane, 8. Under a nitrogen atmosphere, BuLi (1.4 M, 22.4 mL) was added into a THF (130 mL) solution of 1-TBDMS-1,2-carborane (8.07 g, 30.95 mmol) at 0 °C with stirring before warming to room temperature for an additional 5 h. For addition of the 1,6-dibromohexane (17 mL, 110 mmol), the mixture was cooled to −78 °C, and then the mixture was brought to reflux for 72 h. At the conclusion of this time, TBAF (1 M, 50 mL) was added at 0 °C with stirring. To quench the reaction, water and diethyl ether were added, and the mixture was extracted; the organic layer was dried over anhydrous $MgSO_4$. After filtration, the filtrate was concentrated in vacuo; vacuum distillation of the crude product to remove excess 1,6-dibromohexane resulted in a crude residue that was purified by silica-gel column chromatography (hexane/methylene chloride) to afford a colorless oil (7.21 g, 74%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.57 (s, 1H, $-CH-$), 3.39 (t, 2H, $J = 6.7$ Hz, $-CH_2Br-$), 2.20 (m, 2H, CCH_2CH_2-), 1.83 (m, 2H, $CCH_2CH_2CH_2$), 1.44 (m, 4H, CH_2), 1.29 (m, 2H, CH_2). ^{13}C NMR (125 MHz, $CDCl_3$): δ 75.34 (br), 61.09 (br), 37.93, 33.67, 32.40, 29.04, 28.04, 27.62. ^{11}B NMR (160 MHz, $CDCl_3$): δ −2.43 (d, 2B, $J_{H-B} = 146$ Hz), −5.89 (d, 2B, $J_{H-B} = 134$ Hz), −9.36 (d, 2B, $J_{H-B} = 150$ Hz), −12.15 (m, 4B). HRMS (EI, M^+, *m*/*z*): calcd for $C_8H_{23}B_{10}Br$, 307.1964; found, 307.1976.

Dodeca[6-(2-methyl-1,2-dicarba-*closo*-dodecaboran-1-yl)hexyl]-*closo*-dodecaborane (2−), 12. To a solution of $(TBA)_2[B_{12}(OH)_{12}]$ (0.41 g, 0.5 mmol) and 1-(6-bromo)hexyl-2-methyl-1,2-dicarba-*closo*-dodecarborane (11.6 g, 36 mmol) in acetonitrile (90 mL) was added diisopropylethylamine (2.3 mL, 13.2 mmol). The mixture was heated to reflux for 21 days, after which the purple solution was concentrated in vacuo. The residue was dissolved in acetone to which excess ferric chloride was added to achieve oxidation overnight of the closomer dianion. Upon completion of the oxidation reaction, the mixture was concentrated and extracted with dichloromethane and washed with a dilute HCl solution and water. The organic layer was dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated in vacuo, and the residue was purified by column chromatography (hexane/dichloromethane) to afford a dark yellow solid that was further washed with ethanol to reveal the pure product. (330 mg, 20%). Mp 227−229 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.03 (t, 12 × 2H, $J = 6.1$ Hz, $-OCH_2-CH_2-$), 2.16 (t, 12 × 2H, CCH_2CH_2-), 2.00 (s, 12 × 3H, $-CH_3-$), 1.56 [m, 12 × 4H, $-(CH_2)_2-$], 1.37 [m, 12 × 4H, $-(CH_2)_2-$]. ^{13}C NMR (100 MHz, $CDCl_3$): δ 78.12 ($-OCH_2-$), 75.04 (br, *C*B), 70.27 (br, *C*B), 35.35, 32.11, 29.96, 29.20, 26.16, 23.25. ^{11}B NMR (160 MHz, $CDCl_3$): δ 42.62 (s, 12B), −4.94 (24B), −9.73 (96B). HRMS (MALDI-TOF, M^-, 100%, *m*/*z*): calcd for $C_{108}H_{300}B_{132}O_{12}$, 3218.614; found, 3218.612.

Dodeca[6-(1,2-dicarba-*closo*-dodecaboran-1-yl)hexyl]-*closo*-dodecaborane (2−), 13. Diisopropylethylamine (0.82 mL, 4.71 mmol) was added to a solution of $(TBA)_2[B_{12}(OH)_{12}]$ (319 mg, 0.39 mmol) and 1-(6-bromo)hexyl-1,2-dicarba-*closo*-dodecarborane, **8** (7 g, 22.7 mmol), in acetonitrile (40 mL). The mixture was heated to reflux for 21 days, after which the purple solution was concentrated in vacuo. The residue was dissolved in methylene chloride, to which Celite and sea sand were added. After drying in vacuo, a Soxhlet apparatus was employed for 48 h to remove the excess **8**. The Celite and sea sand residue was washed with acetone to obtain the crude product. To this purple acetone solution was added excess ferric chloride (520 mg, 3.2 mmol) at room temperature for 4 h for the oxidation of the closomer dianion. The reaction mixture was concentrated, extracted with diethyl ether (300 mL), and washed with water and brine. The organic layer was dried over anhydrous magnesium sulfate and filtered. The filtrate was concentrated, and the residue was purified by column chromatography (hexane/dichloromethane) to afford the dark yellow solid (310 mg, 25%). Mp 233−235 °C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.99 (t, 12 × 2H, $J = 5.8$ Hz, $-OCH_2CH_2-$), 3.57 (s, 12H, $-CH-$), 2.20 (t, 12 × 2H, CCH_2CH_2-), 1.50 [m, 12 × 4H, $-(CH_2)_2-$], 1.32 [m, 12 × 4H, $-(CH_2)_2-$]. ^{13}C NMR (100 MHz, $CDCl_3$): δ 75.19 (br, *C*B), 70.12 ($-OCH_2-$), 61.61 (br, *C*B), 38.10, 31.98, 29.48, 28.80, 25.94. ^{11}B NMR (160 MHz, $CDCl_3$): δ 42.50 (s, 12B), −2.04 (d, 12B), −5.17 (s, 12B), −8.90 (24B), −11.54 (72B). HRMS (MALDI-ICR, M^-, *m*/*z*): calcd for $C_{96}H_{276}B_{132}O_{12}$, 3050.426; found, 3050.427.

Dodeca[6-(7,8-dicarba-*nido*-dodecaboran-7-yl)hexyl]-*closo*-dodecaborane (14−), 15. To a solution of **13** (50 mg, 0.016 mmol) in THF (2.5 mL) was added NaCN in ethanol (approximately 0.1 M, 6 mL). The mixture was heated to reflux for 10 days. The light brown reaction mixture was filtered and dried in vacuo. After the

addition of water, the purple solution was dialyzed (with tubing having a molecular weight cutoff of 1000 Da) and concentrated. The compound was purified by size-exclusion HPLC (10 mM ammonium acetate/acetonitrile) to afford the product as a pink solid. ^{1}H NMR (500 MHz, CD_3OD): δ 3.95 (br, 12 × 2H, $-OCH_2CH_2-$), 1.72 (s, 12H, $-CH-$), 1.61 (br, 12 × 2H, CCH_2CH_2-), 1.46 (m, 12 × 4H, $-CH_2-$), 1.29 [m, 12 × 4H, $-(CH_2)_2-$], −3 (s, br). ^{13}C NMR (100 MHz, CD_3OD): δ 68.37 (br, *C*B), 61.88 (br, *C*B), 42.54, 40.88, 33.96, 32.66, 31.38, 27.54. ^{11}B NMR (160 MHz): δ −12.3 (24B), −14.3 (12B), −18.2 (s, 4 × 12B), −21.6 (12B), −33.6 (d, 12B), −37.9 (d, 12B). HRMS (ESI, 100%, *m*/*z*): calcd for the ether-linked nido closomer $C_{96}H_{276}B_{120}O_{12}Na_{14}$, 3243.160, 787.790 ($M^{4-}$), 625.632 ($M^{5-}$); found, 787.787 ($M^{4-}$), 625.633 ($M^{5-}$).

Dodeca[6-(7,8-dicarba-*nido*-dodecaboran-7-yl)hexyl]-*closo*-dodecaborane Animal Toxicity and Biodistribution Studies. Size-exclusion HPLC was employed to ensure the purity of **14** for animal toxicity studies. Size-exclusion chromatography was chosen because the functionalized closomers, with a mass of approximately 3000 Da, are significantly larger than any of the starting materials. A Tosoh Bioscience G 2000 SW_{XL} silica-based size-exclusion column (7.8 mm i.d. × 30 cm) was used with a 50:50 mixture of ammonium acetate and acetonitrile as the eluent. With a retention time of 13 min, **14** was easily separated from the unknown impurity that eluted at 23 min. Upon isolation of the product and removal of all solvents under vacuum, the clean salt was obtained as a pink solid.

A solution of **14** (7.8 mg dissolved in 3 mL of isotonic phosphate-buffered saline) was sterilized by filtration through a 0.22-μm Millipore membrane. Normal BALB/c mice (six males and one female, each 18−20 g) were used in 48-h toxicity studies of the *nido*-closomer **14**. Four males and one female were each injected with 10 mg/kg of **14** (167 μL). The remaining two males each received a dose of 20 mg/kg, approximately 30% of which was administered subcutaneously to one of the remaining mice. Though one subject appeared slightly in shock 14 h post-injection, all were fine 22, 38, and 48 h later. All mice were euthanized 48 h post-injection, and the blood was collected for boron analysis. The results indicate that a therapeutic dose (10 mg B/kg of body weight) of *nido*-closomer **14** was well-tolerated by the mice and not toxic.

Murine biodistribution studies of *nido*-closomer **14** were conducted at Washington State University, Pullman, using BALB/c mice (16−20 g) bearing EMT6 tumors (125−350 mg at the time of sacrifice). Observation of the mice upon injection of the boronated compound with a dose equivalent to 10 mg B/kg of body weight continued for 48 h, at which time the animals were sacrificed for tissue analysis by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Boron uptake by tumor tissue reached a maximum at 24 h post-injection, achieving a maximum tumor boron concentration of 10.5 μg B/g of tumor, with a tumor-to-blood ratio of 9.4. Other tissues, including the skin and brain, showed only minimal boron uptake (0.93 and 0.27 μg B/g of tissue, respectively), while the liver and kidneys had significantly higher boron uptake, as is expected for these organs. The complete graphical representation of the biodistribution data can be found in Supporting Information.

Animal experiments were done in accordance with the guidelines of the Animal Welfare Act and in compliance with protocols approved by the Vestar, Inc., Institutional Animal Care and Use Committee.

Cyclic Voltammetry of Ether Closomers. Electrochemical measurements were carried out using a potentiostat/galvanostat model 263A (Princeton Applied Research) interfaced to a Hewlett-Packard Pavillion desktop running PowerCV (Princeton Applied Research). Platinum wires were used as working and counting electrodes, while saturated Ag/AgCl was used as a reference in a three-electrode setup. Measurements were conducted with a scan rate of 100 mV/s in CH_2Cl_2 with 0.1 M $[TBA]PF_6$ and were referenced to an internal ferrocene standard.

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Supporting Information Available: Graphical biodistribution data from the animal toxicity studies, full experimental details, and characterization data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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