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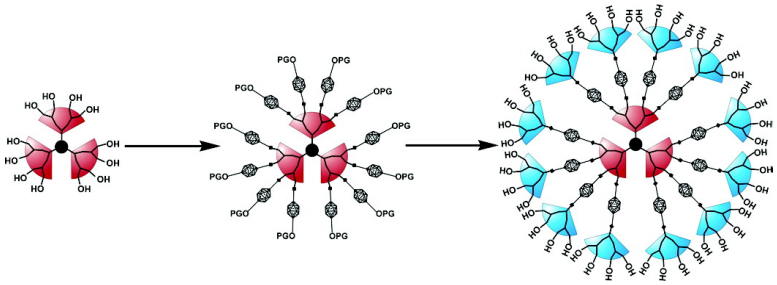

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Synthesis and Properties of Carborane-Functionalized Aliphatic Polyester Dendrimers

Matthew C. Parrott,[†] Erin B. Marchington,[†] John F. Valliant,[†,‡] and Alex Adronov*[,§,†]

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Abstract: The incorporation of multiple p-carborane cages within an aliphatic polyester dendrimer was accomplished through the preparation of a bifunctional carborane synthon. A p-carborane derivative having an acid and a protected alcohol functionality was found to efficiently couple to peripheral hydroxyl groups of low-generation dendrimers under standard esterification conditions. Deprotection of carborane hydroxyl groups allowed for further dendronization through a divergent approach using the highly reactive anhydride of benzylidene-protected 2,2-bis(hydroxymethyl)propanoic acid. This approach was used to prepare fourth- and fifth-generation dendrimers that contain 4, 8, and 16 carborane cages within their interior. Upon peripheral deprotection to liberate a polyhydroxylated dendrimer exterior, these structures exhibited aqueous solubility as long as a minimum of eight hydroxyl groups per carborane were present. Several of the water-soluble structures were found to exhibit a lower critical solution temperature. Additionally, irradiation of these materials with thermal neutrons resulted in emission of gamma radiation that is indicative of boron neutron capture events occurring within the carborane-containing dendrimers.

Introduction

Boron-10 delivery to biological tissues has been the subject of longstanding research because of the potential of boron neutron capture therapy (BNCT) in the treatment of diseases such as cancer.[1,2] BNCT is a binary method for radiation therapy that involves the irradiation of ^{10}B nuclei with thermal neutrons. Upon neutron capture, the ^{10}B nucleus undergoes fission resulting in the localized emission of high linear energy transfer (LET) particles (^{4}He and ^{7}Li)[3] having a penetration range of less than 10 μm in biological tissues, which amounts to approximately one cell diameter.[4] Therefore, if high concentrations of ^{10}B are achieved in tumor cells relative to surrounding healthy tissues, neutron irradiation should result in selective tumor annihilation. Considering the high neutron capture cross section of ^{10}B relative to other light elements,[2] BNCT is theoretically an ideal method for targeted delivery of radiation doses capable of selective tissue destruction. However, the principal obstacle to mainstream application of BNCT for cancer treatment has been the selective delivery of adequate boron concentrations, requiring a minimum of 10^9 ^{10}B atoms per cell within the target tissues.[5] To address this issue, polyhedral borane clusters, such as closo-$[B_{10}H_{10}]^{2-}$, closo-$[B_{12}H_{12}]^{2-}$, and the isoelectronic icosahedral family of carboranes, closo-$C_2B_{10}H_{12}$, have attracted significant attention because of the high boron content within each molecular cage (Figure 1).[1] Carboranes are particularly attractive because of their high stability, charge neutrality, and the relative ease with which they can be chemically modified.[6] For these reasons, the conjugation of carboranes to small biologically relevant molecules, such as nucleic acids, amino acids, sugars, and lipids, has been extensively investigated.[2] However, many of these studies have been plagued by the modification of biomolecule structure resulting from introduction of the carborane, which causes a loss of function or receptor recognition of the hybrid compounds.[5] More recently, the need for high cellular ^{10}B concentrations has prompted the conjugation of multiple carboranes with biological and synthetic macromolecules capable of specifically targeting cancer cells. For example, direct conjugation of monoclonal antibodies with carboranes and carborane-functionalized polylysine resulted in heterostructures bearing greater than 1300 boron atoms.[7] Again, the success of this approach was limited because of decreased in-vivo antigen

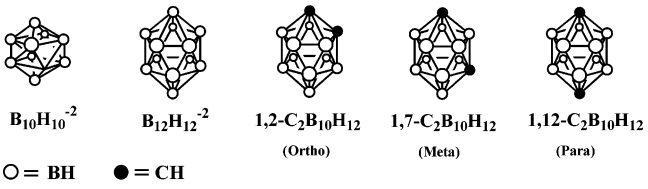

Figure 1. Structures of some common polyhedral boranes.

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specificity and decreased water solubility, resulting in greatly diminished tumor-localizing capability.[7] Additional promising methods have also been reported, including boron-rich oligomeric phosphate diesters[8] and carborane-loaded unilamellar liposomes.[9]

Over the past two decades, the use of synthetic macromolecules as drug-delivery agents has gained increasing momentum. The idea of using water-soluble polymers to mimic transport proteins was first introduced by Ringsdorf[10,11] and Kopecek[12,13] and has led to clinical trials of several polymer therapeutic agents for cancer chemotherapy.[14] Polymer-based drug delivery agents exhibit improved solubility and increased vascular circulation time because of a decreased rate of renal filtration, a process that is abated by increasing the molecular size of the delivery system.[15,16] This prolonged circulation time enables macromolecular drug delivery systems to passively target tumor tissues as a result of increased permeability of tumor vasculature to macromolecules and the limited lymphatic drainage away from a tumor.[17] Combined, these two factors allow the selective accumulation of macromolecules in tumor tissue, a phenomenon known as the enhanced permeation and retention (EPR) effect.[18−20]

Within the area of polymer therapeutics, dendritic macromolecules exhibit several distinct advantages over their linear counterparts. These include their precisely controlled architecture, monodispersity, and the ability to incorporate specific functional groups at the periphery or the interior of the molecule.[21−25] Dendrimers can therefore serve as highly versatile drug delivery vehicles, allowing for control over solubility, molecular weight, multiplicity of therapeutic agents, and potentially the incorporation of active targeting moieties.[17,26] In light of these advantages, several research groups have already investigated the incorporation of carboranes within a dendritic polymer architecture. Perhaps the first example was Yamamoto's ortho-carborane coupled to a cascade-type tetraol that exhibited enhanced water solubility over the non-dendron-functionalized starting material.[27,28] Following this, other groups investigated the coupling of multiple carborane cages to the peripheral groups of various dendrimers, including PAMAM,[29] poly(propylene imine),[30] carbosilane,[31] polylysine,[32] metallodendrimers,[33] and the dendrimer-like closomers.[34] Of these, only the PAMAM and polylysine structures exhibited some degree of aqueous solubility, though neither one proved to be an ideal boron delivery agent. PAMAM dendrimers are cytotoxic because of their polycationic nature,[35] and the polylysine scaffold, while clearly biocompatible, exhibited diminished aqueous solubility upon carborane introduction, requiring aqueous−organic solvent mixtures for bioconjugation reactions.[32] A more successful approach to producing water-soluble carborane-functionalized dendrimers, reported by Newkome and coworkers, involved the reaction of alkyne moieties with decaborane to form ortho-carborane cages within the interior of cascade macromolecules.[36] Aqueous solubility over a wide pH range was provided by peripheral sulfate groups, resulting in a unimolecular micelle-type structure. However, the biocompatibility and biodegradability of this hydrocarbon-based dendrimer has not been reported.

On the basis of these studies, it is clear that internal dendrimer functionalization is advantageous, allowing peripheral hydrophilic groups to impart aqueous solubility and to effectively mask the presence of hydrophobic carborane cages within the macromolecule. Additionally, the dendrimer scaffold must be chosen such that it imparts the required solubility features while also maintaining biocompatibility. Recently, Fréchet and coworkers developed an efficient divergent synthesis of aliphatic polyester dendrimers based on 2,2-bis(hydroxymethyl)propanoic acid (bis-MPA),[37] originally prepared by Ihre et al. in a convergent manner.[38,39] These structures were found to be promising as drug delivery agents, as they are biocompatible, nonimmunogenic, nontoxic, water-soluble, and well-tolerated in vivo.[26,40] We have therefore undertaken the development of similar aliphatic polyester dendrimers that incorporate an easily controllable number of carboranes within the interior of the dendrimer structure. Critical to this approach was the development of a bifunctional carborane synthon that matches the dual functionality of the bis-MPA monomer, allowing it to be inserted within the dendrimer synthesis at any generation using traditional carbodiimide esterification reactions. This flexibility in the position of carborane insertion provides control over the boron concentration within a specific dendrimer target compound.

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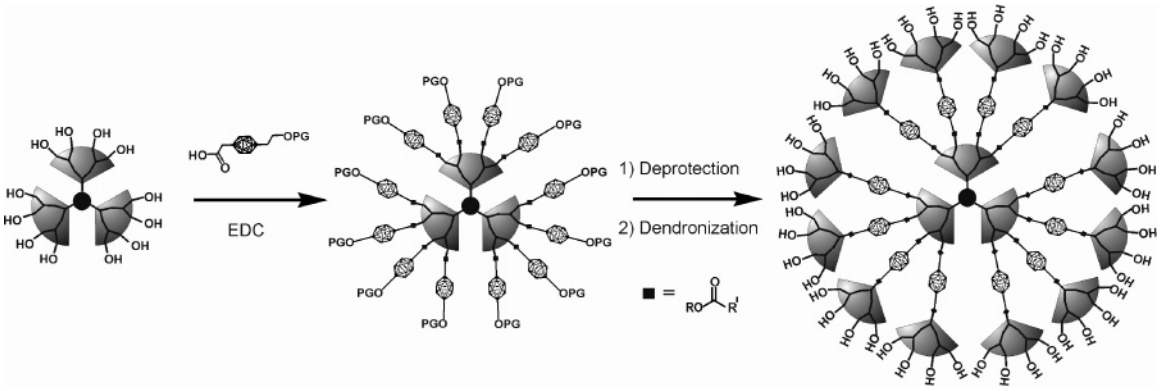
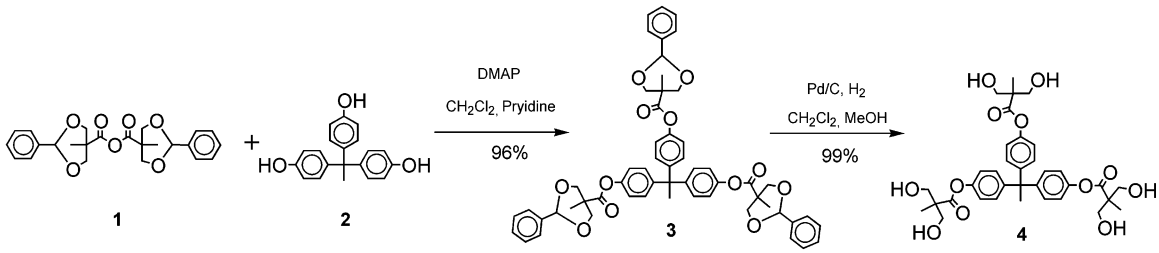


Figure 2. Schematic describing the strategy for incorporation of a carborane synthon into the polyester dendrimer.

Scheme 1



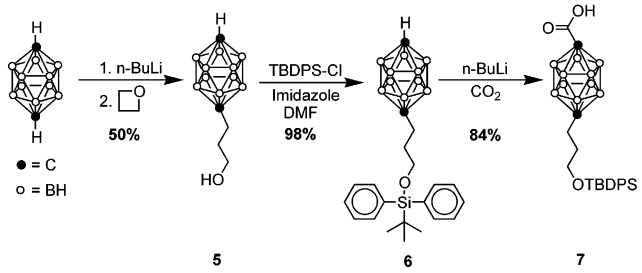
Here, we report the synthesis and properties of this novel class of carborane-functionalized dendrimers.

Results and Discussion

Dendrimer Synthesis. The general procedure for the divergent synthesis of aliphatic poly(ester) dendrimers, utilizing readily available and inexpensive 2,2-bis(hydroxymethyl) propionic acid (bisMPA) as the monomer, involves coupling of a highly reactive bisMPA anhydride with nucleophiles such as alcohols or amines.[37] The bisMPA anhydride (**1**) was prepared in two steps, according to literature procedures.[37] This anhydride was reacted with the 1,1,1-tris(hydroxyphenyl)ethane core using a catalytic amount 4-(dimethylamino)pyridine (DMAP) in 96% yield (Scheme 1). We have found that using a 3:2 mixture of CH_2Cl_2 and pyridine and a 2-fold excess of **1** relative to each alcohol functionality are optimal conditions for all generations. The excess anhydride **1** was quenched with water, and the pure first generation dendrimer was obtained after extraction and washing with $NaHSO_4$ (1 M), Na_2CO_3 (10% w/v), and brine. The benzylidene-protecting groups of **3** were quantitatively removed by hydrogenolysis using a catalytic amount of 10% (w/w) Pd/C and H_2 to produce **4**. Iteration of these steps allowed the production of a series of hydroxy-terminated dendrimer generations, from G-1 to G-4.

The general strategy for the incorporation of carborane cages into the polyester dendrimer synthesis involved the preparation of a bifunctional carborane bearing a carboxylic acid and a protected alcohol. The carboxylic acid of such a bifunctional structure could be coupled to the peripheral alcohols of the deprotected polyester dendrimer at any generation, and the protected alcohols of the resulting product would subsequently be deprotected to regenerate peripheral alcohol functionalities (Figure 2). The new array of peripheral alcohols would then be reacted with the bisMPA anhydride **1** again to produce higher generation dendrimers. By doing this, the modified carborane cage acts as a spacer between generations and can be inserted at any stage of the dendrimer synthesis.

Scheme 2



Preparation of the bifunctional carborane cage was accomplished by utilizing the relative acidity of the proton on the carbon vertexes ($pK_a = 26.8$).[41] A simple deprotonation of para-carborane with one equivalent of *n*-butyllithium (*n*-BuLi) was performed in dry THF (Scheme 2), leading to the formation of a statistical mixture of three species, including a monoanion, a dianion, and the starting material. The anions generated from this reaction were treated with 1 equiv of trimethylene oxide resulting in a hydroxypropyl group coupled directly to the cage.[42] The reaction was quenched with HCl (1 M), and the product was purified by column chromatography in dichloromethane giving **5** in 50% yield. The unreacted starting material (25%) could be recovered and reused, while the diol byproduct (25%) was a useful synthon in other reactions (vide infra). The resulting alcohol (**5**) was subsequently protected using *tert*-butyldiphenylsilyl chloride (TBDPS−Cl) to form **6** in 98% yield. Compound **6** was deprotonated with *n*-BuLi and the resulting anion was quenched with CO_2 to produce the desired carboxylate functionality. Acidic workup, followed by column chromatography in 9:1, DCM:methanol resulted in acid **7** (84% yield).

To investigate the esterification chemistry between acid **7** and the eventual hydroxyl-functionalized dendrimer, a model study

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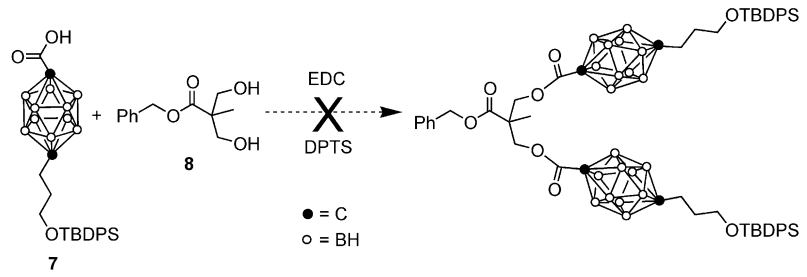
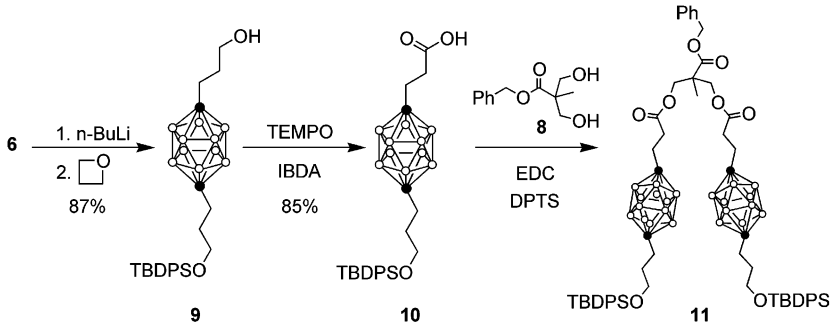
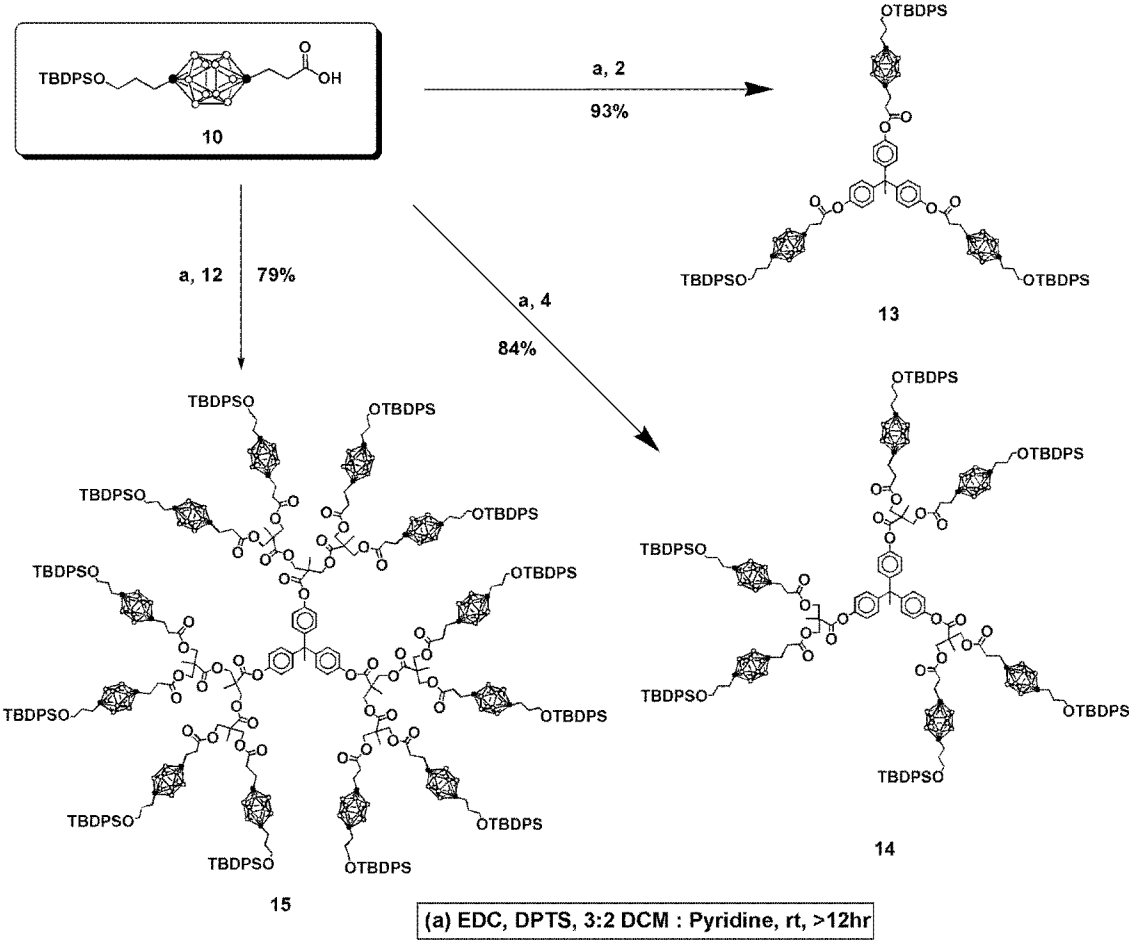
Scheme 3



Scheme 4

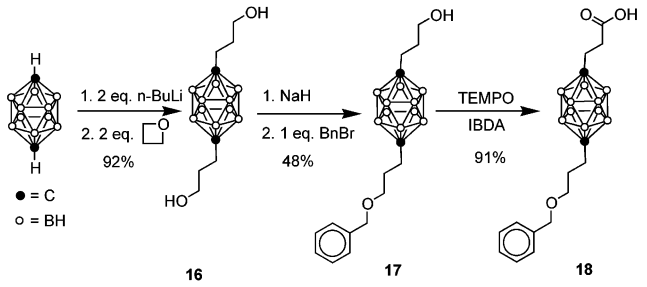


Scheme 5



was performed using benzylated bisMPA (**8**) as a dendrimer mimic (Scheme 3). Compound **8** was prepared in a single quantitative step by reacting bisMPA with benzyl bromide in the presence of DMAP.[39] Unfortunately, all attempts to couple **7** and **8** using carbodiimide chemistry were unsuccessful, resulting only in the isolation of starting material. The apparent lack of reactivity is likely due to steric hindrance of the proximal acid group caused by the bulky carborane cage. To reduce this deactivating effect, it was necessary to introduce a spacer between the carborane cage and the acid functionality.

Scheme 6

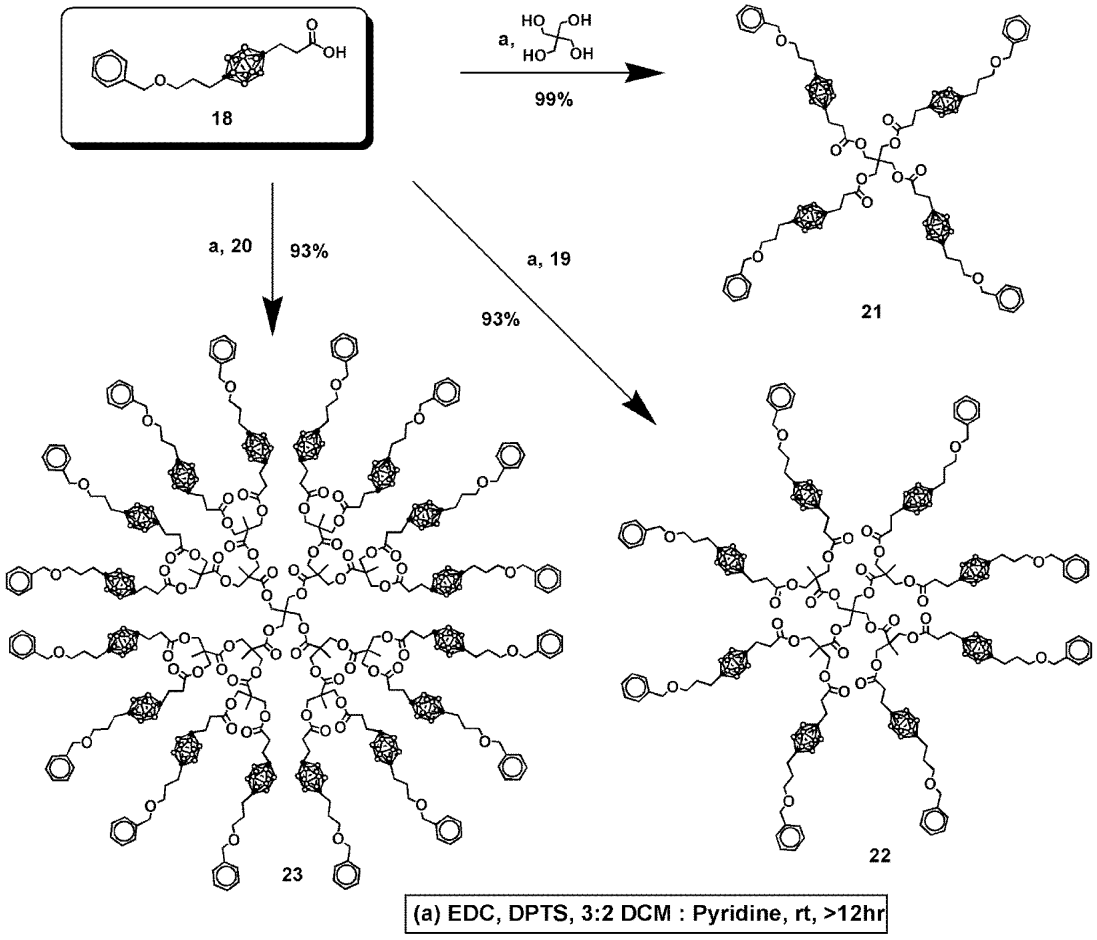


The original synthesis of the bifunctional carborane was easily modified by deprotonating the protected alcohol **6** using *n*-butylithium and reacting with a second equivalent of trimethylene oxide (Scheme 4). After an acidic workup, compound **9** was purified by column chromatography using 100% DCM as the eluent and was isolated in 87% yield. Alcohol **9** was then oxidized using iodobenzene diacetate (IBDA) and TEMPO in dichloromethane under ambient conditions.[43] This nitroxyl radical mediated oxidation was chosen for its mild and selective oxidation of primary alcohols, allowing us to avoid the more aggressive chromium(VI) oxides that deprotect the TBDPS group.[44] Compound **10** was precipitated from hexanes and was isolated in 85% yield. The reactivity of acid **10** was tested by coupling to **8** using EDC and DPTS.[45] This reaction produced **11** in quantitative yield, indicating that the spacer between the carborane and the acid group was indeed required to impart the necessary acid reactivity in the carbodiimide mediated esterification. The bifunctional carborane **10** was subsequently used as a synthon in the preparation of carborane-functionalized dendrimers.

The EDC/DPTS couplings between **10** and the 1,1,1-tris-(hydroxyphenyl)ethane core **2**, the first-generation bisMPA dendrimer **4**, and the second-generation bisMPA dendrimer **12** were carried out in 93%, 84%, and 79% yield, respectively (Scheme 5). In each case, a small excess of the carborane acid **10** was used (1.25 equiv per alcohol) to ensure complete functionalization. Compounds **13**−**15** were easily purified by column chromatography using various mixtures of hexanes and ethyl acetate as the eluent. These structures were fully characterized by ^{1}H NMR, ^{13}C NMR, and MALDI-TOF MS to ensure that complete functionalization of all peripheral alcohols on the dendritic precursors was obtained. In each case, lower mass structures corresponding to incompletely carborane-functionalized dendrimers were not observed, indicating that the coupling of **10** to the dendrimer periphery is a highly efficient process.

To add dendrimer generations at the periphery of these molecules, it was necessary to remove the TBDPS protecting groups, followed by coupling with anhydride **2**. Deprotection of the TBDPS groups was attempted under standard conditions using tetrabutylammonium fluoride (TBAF) in THF. However, both thin-layer chromatography and ^{1}H NMR indicated extensive degradation of the dendrimers during the course of this deprotection reaction. It was postulated that the alkoxides generated from removal of the TBDPS groups attacked the various esters in the dendrimer backbone, especially the

Scheme 7



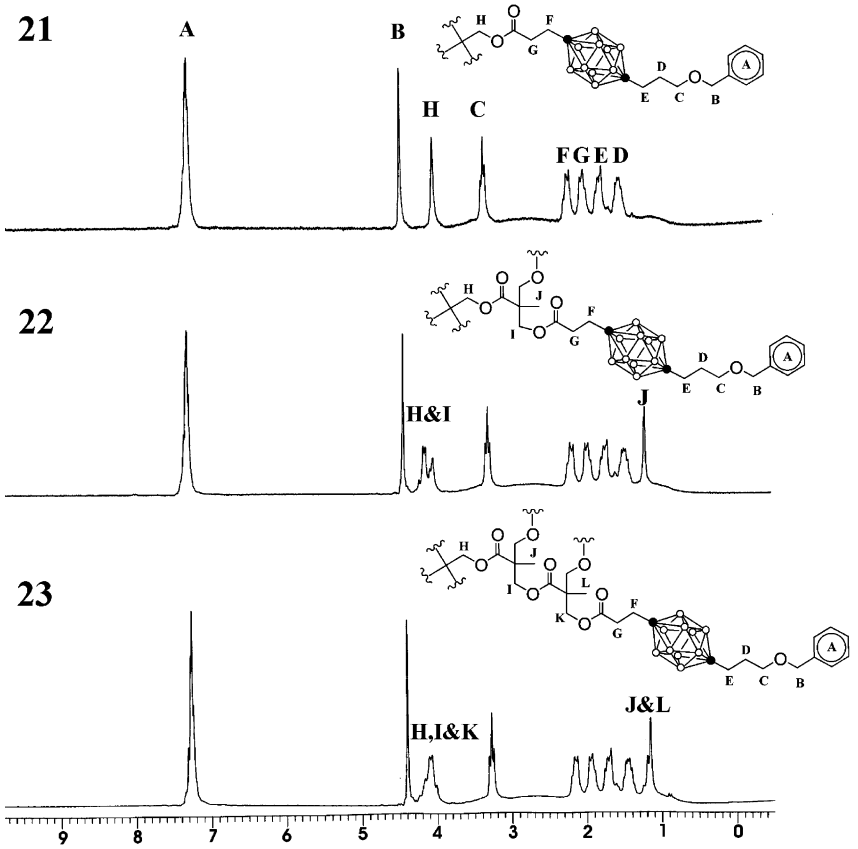


Figure 3. ^{1}H NMR spectra of **21**−**23**.

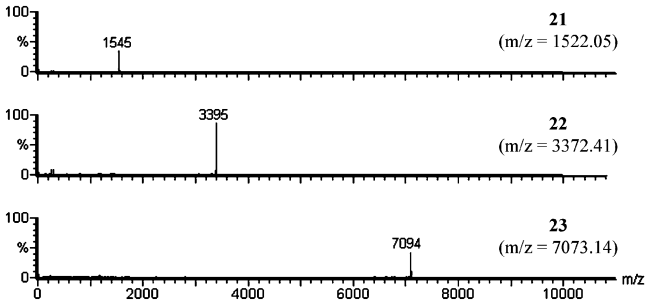


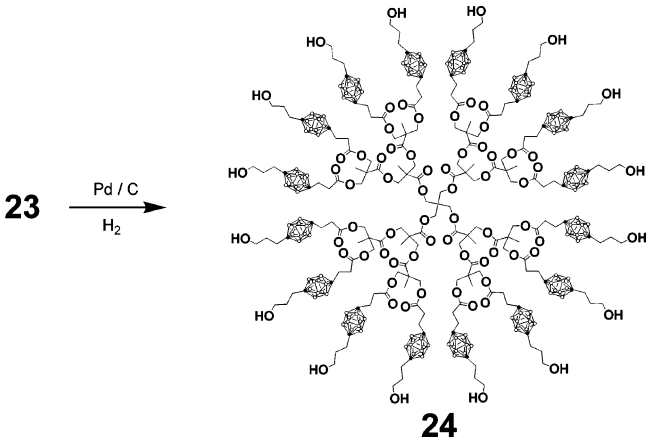
Figure 4. MALDI-TOF MS of **21**−**23** with calculated *m*/*z* values in parentheses.

relatively labile phenolic ester linkages between the core and the dendrons.

To avoid the TBAF deprotection and the ensuing degradation of the dendrimer, we decided to utilize a benzyl ether group to monoprotect the carborane diol **16** (Scheme 6). The benzyl ether was chosen for its stability to slightly acidic and basic media and for its mild and efficient deprotection conditions. Considering that benzyl ethers are quantitatively removed by hydrogenolysis with a catalytic amount of 10% (w/w) Pd/C and H_2,[44] identical to the conditions used to remove the benzylidene-protecting groups of the dendrimer, this deprotection is convenient and highly compatible with the dendrimer synthesis.

Starting with para-carborane, deprotonation of both carbon vertices using two equivalents of *n*-butyllithium (*n*-BuLi) was followed by ring opening of trimethylene oxide to produce diol **16** in 92% yield after acidic workup and crystallization from $CHCl_3$. This diol was monoprotected using benzyl bromide under basic conditions to produce **17** in 48% yield. The remaining free alcohol of **17** was subsequently oxidized using TEMPO and IBDA to yield acid **18** in 91% yield.

Scheme 8



As an additional precaution in the dendrimer synthesis, it was decided to substitute the 1,1,1-tris(hydroxyphenyl) ethane core with Pentaerythritol. This modification not only eliminates the weak phenolic ester linkages between the core and the dendrons but also increases the number of dendrons, and therefore the number of carboranes, within each dendrimer. The G-1 and G-2 protected dendrimers (**19** and **20**, respectively) having Pentaerythritol cores were easily prepared in high yields using the

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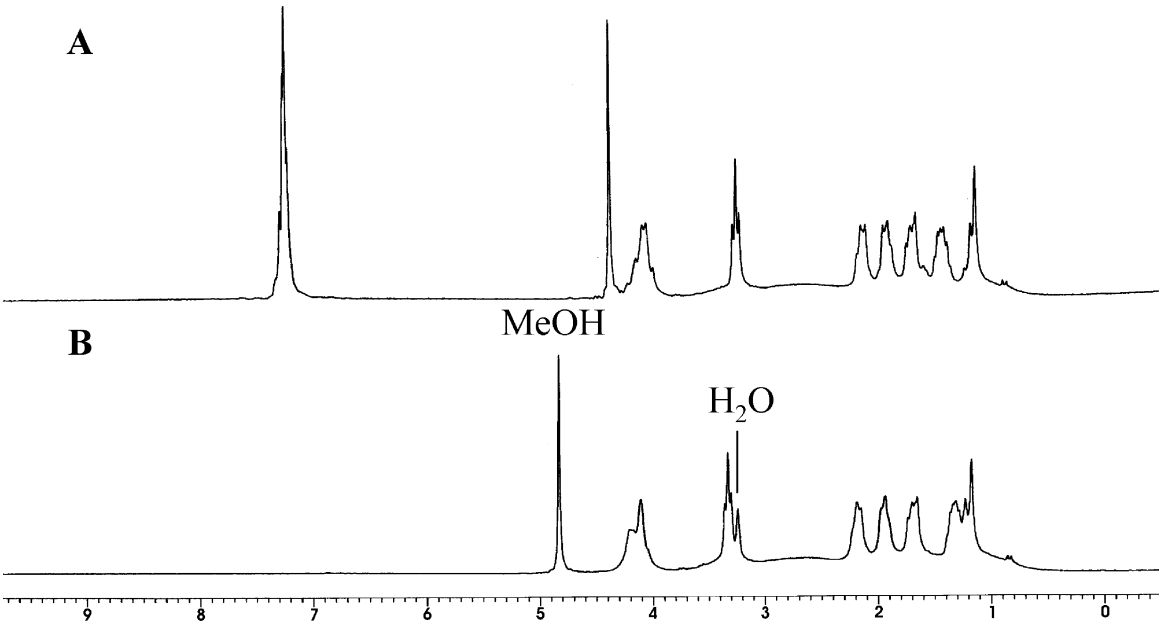

Figure 5. ^{1}H NMR before (A) and after (B) benzyl ether deprotection of **23**. Solvents used for spectra A and B were $CDCl_3$ and MeOD, respectively.

aforementioned procedures. As depicted in Scheme 7, carbodiimde coupling reactions were carried out on Pentaerythritol, the G-1 bisMPA dendrimer (**19**), and the G-2 bisMPA dendrimer (**20**) in 99%, 93%, and 93% yield, respectively. In each of these reactions, a small excess of the carborane acid **18** (1.25 equiv per alcohol) was required for complete functionalization. Compounds **21**−**23** were easily purified by column chromatography.

The ^{1}H NMR spectra for dendrimers **21**−**23** exhibit all of the expected resonances attributed to the branched polyester core, the carborane linker, and the peripheral-protecting groups (Figure 3). The broad carborane B−H resonances observable in these spectra prevent accurate integration of signals in the range of 0.8−3.8 ppm. However, the appearance of methyl and methylene signals at 1.1−1.3 ppm and 4.0−4.3 ppm (J, L and I, K, respectively) provides a clear indication of dendrimer growth (Figure 3).

MALDI-TOF MS provided the critical evidence for successful preparation of compounds **21**−**23** (Figure 4). The mass of the observed molecular ion clearly corresponded to the Na^+ adducts of each respective dendrimer, with no observable lower molecular weight fragments or incompletely functionalized materials. Again, this illustrates the efficiency of the esterification between the carborane acid **18** and the multitude of hydroxyl groups at the dendrimer periphery.

Deprotection of the peripheral benzyl ether groups was accomplished quantitatively by hydrogenolysis using a catalytic amount of 10% (w/w) Pd/C and H_2 (Scheme 8). The reaction conditions were essentially identical to those of the benzylidene deprotections discussed above. The success of this reaction could easily be confirmed by ^{1}H NMR because of complete disappearance of the aromatic signals at 7.2−7.4 ppm as well as by the benzylic proton signals at 4.4 ppm. As an example, the ^{1}H NMR spectra of **23** and **24** are depicted in Figure 5. It can clearly be seen that the strong aromatic signals at 7.3 ppm and the methylene protons at 4.4 ppm completely disappear, while the signals due to the dendrimer backbone remain unchanged. This result was significant as it confirmed that the deprotection reaction occurred without any degradation of the dendritic backbone, unlike the analogous reaction with TBDPS protecting groups.

Successful deprotection of the benzyl ether groups resulted in the regeneration of peripheral alcohols on the dendrimer, allowing for further divergent growth. These peripheral functionalities were reacted with the bisMPA anhydride **1** to further dendronize each molecule, allowing the carborane cages to be internalized within higher generation dendrimers. Scheme 9 depicts the deprotection and dendronization of **21** to the third-generation deprotected structure. Similar reaction sequences were used to produce hydroxyl-terminated dendrimers of varying generation containing 8 and 16 carborane cages. The structures of the largest members of these two dendrimer families are given in Figure 6. To name each of these molecules, we refer to the number of carborane cages, the overall dendrimer generation number, and the total number of peripheral functional groups. Thus, a structure containing eight carboranes attached to a first-generation core and G-4 dendrons coupled to each of the carboranes would be named 8-[G-5]-OH_{128}, as depicted in Figure 6.

Aqueous Solubility. Considering the potential therapeutic applications of carborane-containing compounds, it was important to evaluate the aqueous solubility of each of these structures. Specifically, we were interested in determining the minimum number of peripheral alcohol groups required per carborane to impart water solubility at a level of 1 mg/mL or higher. Table 1 summarizes these data and clearly indicates that an alcohol:carborane ratio of 4:1 or lower is not sufficient for aqueous solubility. A ratio of 8:1 was also not sufficient for complete water solubility in the structures containing four and eight carboranes but did impart solubility to the fifth-generation dendrimer containing 16 carboranes. Finally, an alcohol:carborane ratio of 16:1 allowed rapid dissolution of the structures containing four and eight carboranes to concentrations in excess of 5 mg/mL. It is expected that a cooperative effect between the hyrophilicity of the multiple hydroxyl groups and the overall globular shape of the dendrimers beyond generation four must dictate overall solubility.

Scheme 9

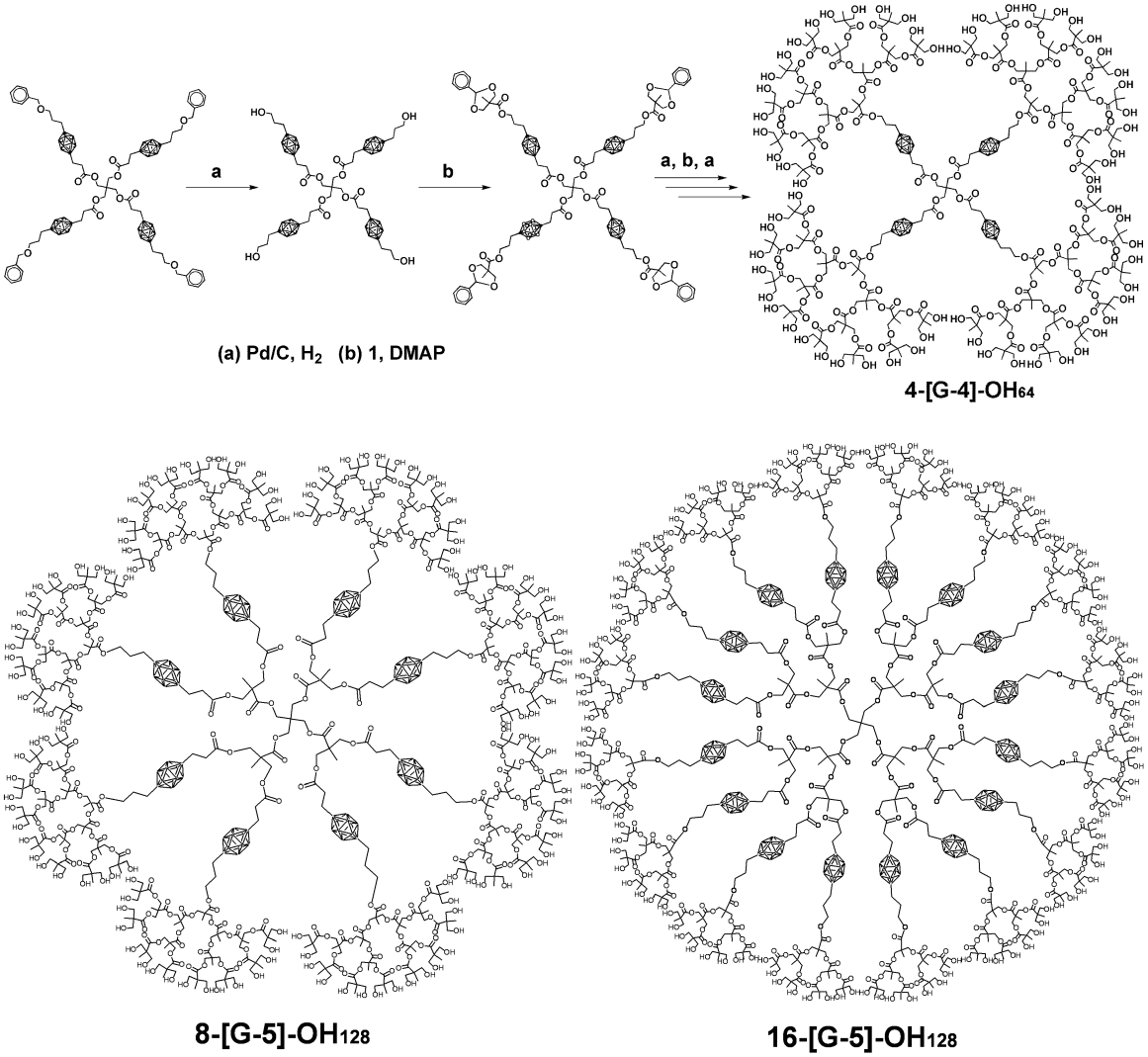

Figure 6. Structures of largest synthesized dendrimers containing 8 and 16 carborane cages.

Table 1. Aqueous Solubility of Carborane Functionalized Dendrimers

	# of OHs per carborane			
# of carboranes	2	4	8	16
4	no	no	<1 mg/mL[a,b]	8 mg/mL
8	no	no	<1 mg/mL[a,b]	6 mg/mL
16	no	no	1 mg/mL[b]	

[a] Exhibits LCST. [b] Completely soluble in 50/50 (v/v) MeOH/H_2O.

Interestingly, during the course of these measurements, we found that heating the aqueous suspensions of 4-[G-3]-OH_{32} and 8-[G-4]-OH_{64} did not improve solubility. In fact, these molecules precipitated from solution at elevated temperatures, indicating that they exhibit a lower critical solution temperature (LCST). Quantitative measurements of this behavior were made by % transmittance measurements as a function of temperature (Figure 7) and indicated an onset of precipitation at 52 °C and 83 °C for 4-[G-3]-OH_{32} and 8-[G-4]-OH_{64}, respectively. Dendrimer 16-[G-5]-OH_{128}, which was soluble at room temperature, was also found to precipitate at a temperature of 63 °C, although its solubility transition is more gradual than for the other two structures (Figure 7). However, the more water-soluble structures containing a 16:1 ratio of alcohols to carborane exhibited no LCST up to the boiling point of water (Table 1). LCST behavior within dendrimers has previously been reported by Kono and co-workers, but their PAMAM dendrimers were peripherally functionalized with isobutyramide groups that are known to impart LCST behavior in poly(*N*-vinylisobutyramide).[46] In our

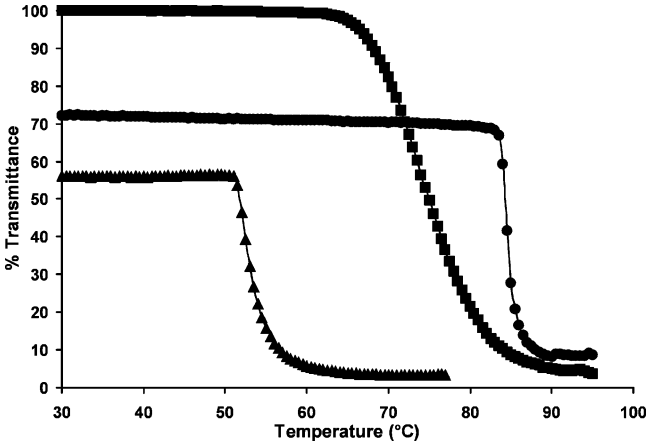

Figure 7. Plots of % transmittance as a function of temperature, indicating the LCST behavior of carborane-functionalized dendrimers 4-[G-3]-OH_{32} (▲), 8-[G-4]-OH_{64} (●), and 16-[G-5]-OH_{128} (■).

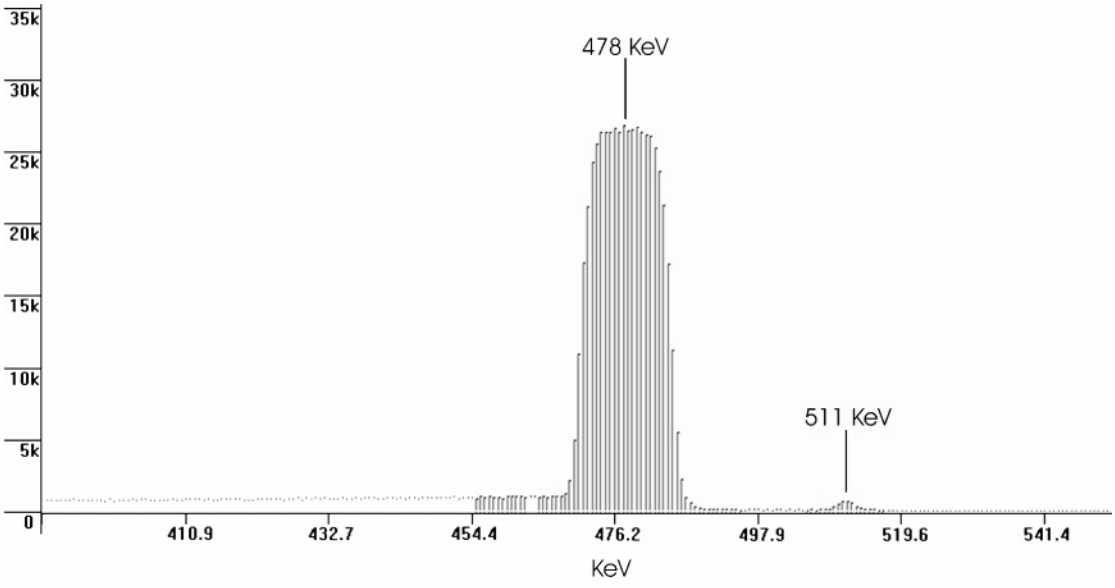

Figure 8. Gamma energy profile resulting from neutron capture events occurring within a 10 mg/mL solution of 16-[G-4]-OH_{64}.

case, the hydrophobic carborane functionality is encapsulated within the interior of the dendrimer, yet surprisingly imparts LCST behavior to an otherwise fully water-soluble structure. A detailed investigation into the LCST behavior of these dendrimers will be reported elsewhere.

Neutron Activation. To examine neutron activation of the dendrimer-encapsulated carborane cages, 10 mg/mL solutions of the hydroxy-terminated dendrimers 4-[G-3]-OH_{32}, 8-[G-4]-OH_{64}, and 16-[G-4]-OH_{64} in THF were placed in a thermal neutron beam at the Prompt Gamma Facility of the McMaster Nuclear Reactor. The energy profile of the emitted gamma radiation is given in Figure 8, clearly showing the characteristic gamma radiation signature at an energy of 480 keV, corresponding to boron neutron capture events. The small signal observed at 511 keV is due to gamma emission caused by annihilation of the positron byproduct from the boron neutron capture event. Irradiation was conducted for a period of 6 h at a flux of 10^7 neutrons/cm^2•s, during which no changes in signal intensity or energy were observed from the dendrimer samples. Additionally, NMR analysis of the dendrimer samples before and after neutron activation indicated no degradation during the course of these experiments. However, the detection of sample degradation because of neutron capture events at this neutron flux would not be expected, as the annihilation of only ~0.01% of the sample's ^{10}B atoms occurs during the 6-h experiment.

Conclusion

We have shown that a bifunctional carborane derivative bearing an acid group and a benzyl ether protected alcohol serves as a highly effective synthon for the incorporation of carborane cages within an aliphatic polyester dendrimer. The insertion of 4, 8, or 16 carboranes was accomplished in high yield using a previously reported divergent synthesis. It was subsequently possible to further dendronize the macromolecular periphery to install a controllable number of hydroxyl functionalities that imparted aqueous solubility to the final structures. We found that a minimum of eight alcohols was required to achieve water solubility. Additionally, it was found that all structures having an alcohol-to-carborane ratio of 8:1 exhibited a lower critical solution temperature, which varied with the total number of carboranes in the structure. Neutron activation experiments indicated that neutron capture was occurring within the synthesized dendrimers. This family of dendrimers has proven to be extremely versatile, allowing complete control over location and number of carborane moieties, as well as overall solubility. These structures should therefore serve as potential BNCT agents, and their investigation for this purpose will be investigated soon.

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Supporting Information Available: Full experimental details and characterization for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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