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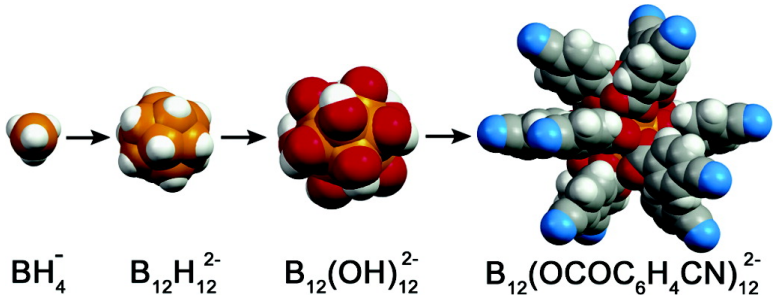

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Organic Syntheses on an Icosahedral Borane Surface: Closomer Structures with Twelvefold Functionality

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Abstract: The syntheses of a series of novel ester-linked derivatives of the icosahedral [*closo*-$B_{12}(OH)_{12}]^{2-}$ boron cluster (closomer esters) are described using several synthetic methods. The reaction of bis-(tetrabutylammonium)-*closo*-dodecahydroxy-dodecaborate, **[$NBu_4]_2$1**, with carboxylic acid chlorides and anhydrides, vinyl esters with a $Y_5(OiPr)_{13}O$ catalyst and 1,1′-carbonyldiimidazole-activated carboxylic acids yields the corresponding dianionic dodeca-ester closomers. The method using 1,1′-carbonyldiimidazole-activated carboxylic acids may be employed as a general synthetic strategy. The use of elevated reaction temperatures, achievable under pressure, to expedite syntheses is described. An attractive methodology using immobilized scavenger reagents for the expeditious purification of the closomer esters was employed. The developed methodology is compatible with a variety of peripheral functional groups attached to the termini of densely packed, carboxylate ester-linked radial arms bonded to the icosahedral borane surface. A closomer ester having twelve terminal amino groups was prepared, and without isolation, fully acetylated in good yield.

Introduction

The exquisite geometries of the two best-known polyhedral borane dianions (*closo*-$B_nH_n^{2-}$, $n = 10$ and 12) coupled with their availability, structurally robust nature, variable shape, resistance to thermal decomposition, transparency to ultraviolet radiation (>210 nm), invisibility to enzymes, and the well-understood chemical reactivity of their individual vertexes make these two polyhedral boranes especially attractive as molecular construction modules. The latter reactions, in principle, allow the synthesis of stoichiometrically and stereochemically discrete derivatives having as many as *n* molecular attachments to their polyhedral surfaces. These substituents may be identical or differ from each other in a predetermined manner. Alkyl and aryl derivatives and permethylated species of this sort based upon the [*closo*-$B_{12}H_{12}]^{2-}$ ion have been previously reported by this laboratory.[1−4] Following this, the scope of this chemistry was extended by the discovery of the BH hydroxylation reaction, with which all BH vertexes present in [*closo*-$B_{12}H_{12}]^{2-}$, [*closo*-$CB_{11}H_{12}]^-$, and *closo*-1,12-$C_2B_{10}H_{12}$ were converted to stable BOH vertexes by reaction with 30% hydrogen peroxide.[5−9] The icosahedral [*closo*-$B_{12}(OH)_{12}]^{2-}$ ion, **1**, was obtained in high yield. Previously reported studies[10] had described acid-catalyzed hydroxylation of [*closo*-$B_{12}H_{12}]^{2-}$ to give the monosubstituted [*closo*-$B_{12}H_{11}OH]^{2-}$ and the disubstituted [*closo*-$B_{12}H_{10}(OH)_2]^{2-}$ derivatives in low yields. We reported[11] stepwise hydroxylation of [*closo*-$B_{12}H_{12}]^{2-}$ with hot H_2SO_4 to produce substituted species having 1−4 BOH vertexes. A discrete stereoisomer of the di-, tri-, and tetrahydroxy species was obtained. Recently, Semioshkin reported[12] synthesis of disubstituted derivatives of [*closo*-$B_{12}H_{12}]^{2-}$ containing hydroxy and arylated carbonyl functions by reacting [*closo*-$B_{12}H_{12}]^{2-}$ with aroyl chlorides.

The hydroxyl functions of **1** were slowly converted to carboxylic acid esters[13,14] and alkyl or aralkyl ethers[15] by conventional reactions. The structures of the ether derivatives were initially described elsewhere[15] while this paper is specifically devoted to the chemistry of the carboxylate ester derivatives.[13,14]

Structures obtained by the attachment of twelve molecular passenger entities to an icosahedral borane surface has been differentiated from dendrimer structures and the resulting

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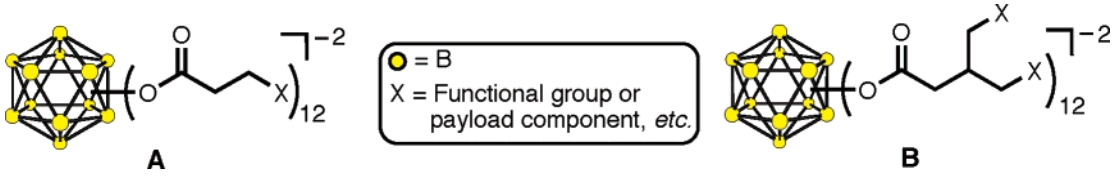

Figure 1. Generic twelvefold closomers equipped with oligomeric, **A**, and dendritic, **B**, radial arms.

derivatives have been designated as "closomers"[13] due to their obvious relationship to their supporting $[closo\text{-}B_nH_n]^{2-}$ ions. Another distinguishing feature of closomers is their potential to form compact, rigid, and densely populated surface-bonded arrays. Figure 1 describes two types of possible radial arm substituents that may be present in a closomer ester structure. Functionally, closomers may be viewed as molecular collector structures.

Closomer Derivatives as Molecular Collector Structures. The key component of molecular collector structures is the central collector species, which may take a variety of forms ranging from large polydispersed, chemically functionalized polymers to relatively small, monodispersed, and precisely functionalized molecules. The size and molecular weight of molecular collector structures can, in principle, range from small to large and span the 1−100 nm scale. Large and/or polydispersed collector molecules are not relevant to the structure or functional capabilities of the monodispersed closomer collector species. However, this relevance is encountered with other types of collectors such as the PANAM dendrimers,[16−29] C_{60} derivatives,[30−32] silesquioxanes,[33] and polyoxymetalate anions.[34,35]

The physical characteristics associated with dendritic structures, such as globular shape and a rigid outer shell, do not become pronounced until higher molecular weights are reached. Dendrimers of lower generation number and of functionality comparable to that of closomers are flexible and greatly differ from the compact, rigid, and densely packed structures of the latter. Generally speaking, the mass of the dendrimer superstructure comprises a much greater percentage of the total dendrimer structure than that associated with a simple closomer derivative of identical functionality. In addition, the radial arms of the closomer structure extend from a surface of high sphericity, may be designed for rigidity or flexibility, are in close proximity to one another and capable of interaction with their nearest neighbor radial arms, if desired. While dendrimer binding sites have been differentiated[16] for the purpose of cell-targeted drug delivery, this is not a common practice.

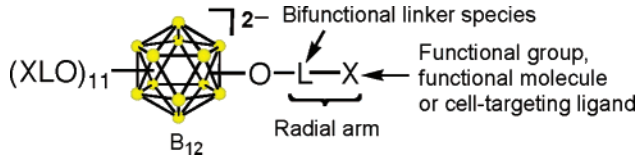

Figure 2. Generalized closomer construction components.

Figure 2 illustrates the various components used in closomer construction. The 12-fold functionality and the rigid, near-spherical icosahedral shape are unique to chemistry, save for the chemically quite different C_{60} cluster. Applications of these features may eventually involve the development of chemical strategies making possible the synthesis of closomer clusters in which discrete subsets of the twelve vertexes become the anchoring sites for a predetermined function, such as tumor cell targeting moieties,[36,37] gadolinium chelators as MRI contrast agents,[38] plasma membrane penetrators,[39] radionuclide chelators for diagnosis[40−42] or therapy,[43] fluorophores,[44] chemotherapeutics,[45] targeting,[46] and therapeutic[47] peptides, carbohydrates and glycobiologics,[48] synthetic antigens,[49] RNA and DNA segments,[50] immunoproteins,[51,52] etc. Ideally, the resulting diagnostics, therapeutics, nanodevices, or a combination of these, would have the ability to carry multiple copies of selected payload species or a diverse multicomponent and specifically tailored payload to targeted sites. Chemical differentiation of closomer vertexes must be achieved to construct more complex species having diversified and multifunctional payloads. The

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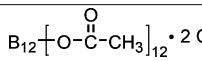
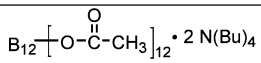
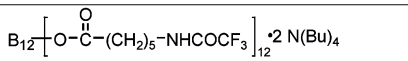
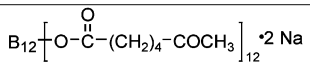
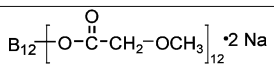
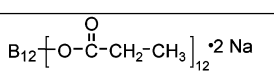
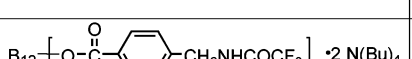
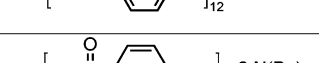
Table 1. Methods, Conditions and Yields for the Synthesis for Carboxylate Ester Closomers

Entry	Closomer Ester	Structure	Method /Reagents	Reaction time (days) and Temp (°C).	Solvent	Yield (%)
1	Cs_2 **2a**		1/Acetic anhydride	6 d; reflux	Neat	58
2	$[NBu_4]_2$-**2a**		4/Vinyl acetate	48 h; reflux	CH_2Cl_2	82
3	$[NBu_4]_2$-**2b**		5/CDI/ N-Trifluoroacetyl-6-aminocaproic acid	14 d; reflux	CH_2ClCH_2Cl	59 & 68[a]
4	Na_2-**2c**		5/CDI/6-Oxoheptanoic acid	14 d; reflux	CH_2ClCH_2Cl	53
5	Na_2-**2d**		5/CDI/ Methoxyacetic acid 2/Methoxyacetic anhydride	14 d; reflux 40 h; 150 °C	CH_2ClCH_2Cl CH_3CN	33 47
6	Na_2-**2e**		5/CDI/ Methoxyacetic acid 2/Propionic anhydride	14 d; reflux 40 h; 150 °C	CH_2ClCH_2Cl CH_3CN	86 54
7	$[NBu_4]_2$-**3b**		5/ CDI/4-(trifluoroacetylaminomethyl) benzoic acid	14 d; reflux	CH_2ClCH_2Cl	50
8	Na_2-**3c**		5/CDI/*p*-chlorobenzoic acid	14 d; reflux	CH_2ClCH_2Cl	12
9	$[NBu_4]_2$-**3d**		3/4-Cyanobenzoyl chloride 5/CDI/4-Cyanobenzoic acid	10 d; reflux 14 d; reflux	CH_3CN CH_2ClCH_2Cl	36 56 &67[a]

[a] = Alternate workup. See experimental procedure.

differentiation and stereochemical control of substitution at discrete sets of closomer vertexes is one major thrust of ongoing research.

Results and Discussion

This paper extends the original report[13] and further describes the synthesis, purification, and characterization of carboxylate ester-linked closomers containing a variety of carboxylic acid components and the stepwise transacylation of the trifluoroacetylated amide, [*closo*-B_{12}(4-$OCOC_6H_4CH_2NHCOCF_3$)$_{12}$]$^{2-}$ to form the corresponding dodeca-acetyl derivative with retention of the closomer structure.

Characteristics of Closomer Carboxylate Ester Syntheses. Regardless of the method, the esterification of **1** presents an unusual set of requirements due to the large number of available BOH vertexes per molecule of **1** and their compacted disposition on the icosahedral surface. As a consequence, it is to be expected that steric encumbrance of the remaining unreacted BOH vertexes becomes more pronounced as the esterification reaction proceeds. Thus, reaction times extending for several days are required for total reaction of all BOH vertexes, even with high concentrations of esterification reagents present in great excess (5−10 equiv. of acylation reagent per BOH group) at reaction temperatures of 60−100 °C. The elevation of reaction temperatures, attained by carrying out reactions under pressure, proved successful in certain cases, and the reaction time was greatly reduced under such conditions. The course of the esterification reactions was followed by observation of the ^{11}B NMR spectrum of crude reaction mixtures. Intermediate stages of esterification were characterized by an array of resonances centered near −16 ppm. Complete reaction was characterized by equivalent BOCOR vertexes and a singlet near −16 ppm.

Specific synthetic approaches to the total esterification of **1** were based upon (1) the uncatalyzed reaction of **1** with carboxylic acid anhydrides (2) the use of elevated reaction temperatures (150 °C) attained in a pressure vessel under an argon atmosphere to accelerate the reaction of **1** with carboxylic acid anhydrides in acetonitrile containing pyridine (3) the reaction of **1** with acyl and aroyl chlorides in the presence of tertiary amines (4) the transesterification reaction of **1** with vinyl acetate catalyzed by $Y_5(OiPr)_{13}O$ in methylene chloride solution at 40 °C and (5) the reaction of **1** with carboxylic acids in the presence of 1,1′-carbonyldiimidazole using 1,2-dichloroethane as solvent at the reflux temperature. Each of these methods will be discussed below. The extravagant use of excess reagents in these reactions required the development of product purification methods which remove these large quantities of starting material. At the same time, these procedures must purify the closomer product while accommodating its ionic properties. Extensive use has been made of reactive resins, ion-exchange and flash chromatography procedures. Table 1 lists the closomer carboxylate ester derivatives prepared in this study and their methods of synthesis.

Acylation of 1 Using Carboxylic Acid Anhydrides or Aroyl Chlorides (Methods 1−3). The closomer dodecaacetate, **$Cs_2$2a**, is one of the simplest derivatives of its type and its synthesis serves to demonstrate method 1 (Table 1); the uncatalyzed reaction of **$Cs_2$1** with acetic anhydride at 100 °C for up to 6 days duration. The structure of **$Cs_2$2a**, determined by X-ray

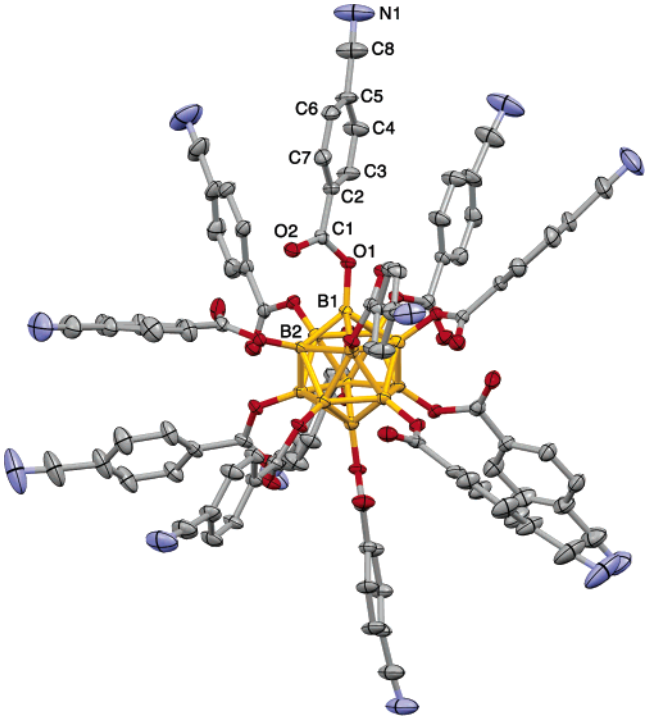


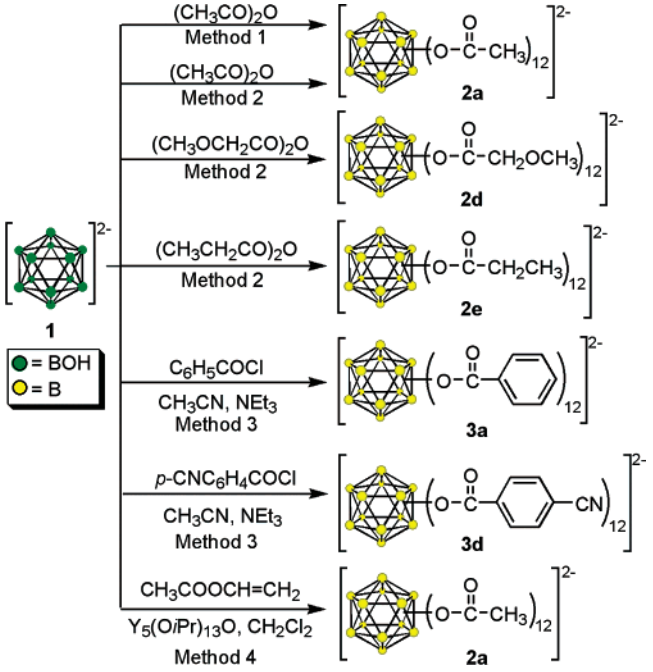
Figure 3. ORTEP depiction of the solid-state molecular structure of **3d**. Hydrogen atoms are omitted for clarity. Ellipsoids are drawn with 30% probabilities. Selected bond lengths (Å), Angles (°): B1−O1, 1.450(5); B1−B2, 1.810(6); C1−O1, 1.327(4); C1−O2, 1.197(4); C1−C2, 1.509(5); C5−C8, 1.450(6); C8−N1, 1.140(5); O1−B1−B2, 116.8(3); B1−O1−C1, 124.8(3); O2−C1−C2, 121.9(4); N1−C8−C5, 179.0(7).

diffraction, was previously reported along with its NMR spectra and physical properties.[13] Further work describing the acid−base-catalyzed hydrolysis and attempted enzymatic cleavage of **$Na_2$2a** is presented below.

The principal disadvantage of Method 1 is the long reaction time, even in the absence of added solvent. Pressurization of such reaction mixtures and the addition of a tertiary amine catalyst would allow the use of a reaction solvent and provide a means to increase the reaction temperature to well over 100 °C. These modifications (Method 2) provided a reaction temperature of 150 °C and reaction times were reduced to 40 h. A small autoclave pressurized with argon served as the reaction vessel. Both methoxyacetic and propionic acid anhydrides were successfully employed in the presence of pyridine and CH_3CN solvent to give **2d** (47%) and **2e** (54%), respectively. The progress of these reactions was monitored using ^{11}B NMR, as described above.

The use of acyl chlorides in lengthy esterification reactions with **[NBu_4]$_2$1** in the presence of triethylamine (Method 3) were generally plagued by self-condensation reactions of the acid chloride giving intractable byproducts. Benzoyl and 4-cyanobenzoyl chlorides provided satisfactory reactions in the presence of triethylamine and acetonitrile at the reflux temperature. Reaction times of several days were required for only moderate yields of closomer product. Single-crystal X-ray diffraction studies were carried out with salts of the benzoate (**3a**)[13] and the 4-cyanobenzoate (**3d**) closomer esters. The benzoate structure was previously reported while that of the 4-cyanobenzoate closomer, **3d** is presented here (Figure 3) along with representative bond lengths, which are unremarkable. In these structures, it is apparent that the 4-position of the attached phenyl groups

Scheme 1. Synthesis of Closomer Esters Using Methods **1** through **4**



is exposed and sterically available for further chemistry if reactive functional groups are attached. The twelve cyano groups present in the 4-cyanobenzoate are incipient functional groups if they can be reduced to the corresponding aminomethyl substituents or hydrolyzed to carboxyl groups without cleavage of the closomer-ester linkages.

Yttrium-Catalyzed Transesterification of 1 with Vinyl Acetate (Method 4). In all of the esterification procedures described above, a large excess of acid chloride or anhydride is required to complete the esterification of **1** often with reaction times of 6−10 days. Transesterification procedures are alternatives to direct esterification of BOH vertexes and an especially attractive route has recently been described. This method is the transesterification of vinyl carboxylates catalyzed by yttrium isopropoxide, $Y_5(OiPr)_{13}O$.[53] The formation of acetaldehyde provides the reaction driving-force and ensures irreversibility. The catalyzed reaction of **[NBu_4]$_2$1** with vinyl acetate was explored in CH_2Cl_2 solution at 40 °C. The product, **[NBu_4]$_2$2a**, was obtained in a high state of purity in 82% yield. The total reaction time was 48 h. This method may be easily extended to other esters since vinyl carboxylates are easily prepared by transvinylation procedures.[54] Catalyst efficiency is lost during the course of these reactions with **1**. This may be due to the chelation of the yttrium center by neighboring BOH vertexes. However, the high yield of product indicates that such reactions are quite effective. Scheme 1 illustrates methods 1 to 4 used for closomer ester synthesis.

Synthesis of Closomer Esters Using 1,1′-Carbonyldiimidazole-Activated Carboxylic Acids and Solid-Supported Purification Strategy (Method 5). The use of a wide variety of often complex dodecaester derivatives as closomer collector molecules for biomedical or material science applications is complicated by the frequent unavailability of starting materials suitable for synthesis methods 1−4. To address these issues,

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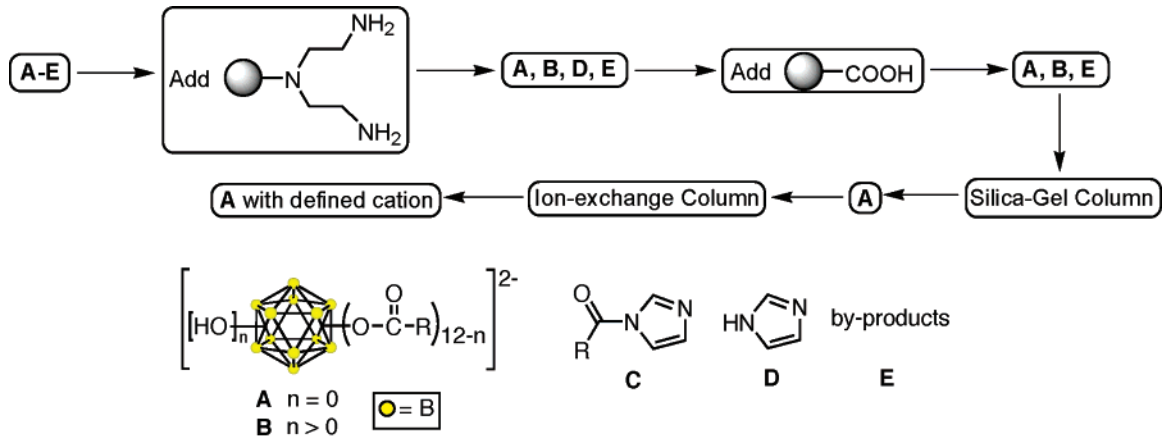

Figure 4. General procedure for purification of closomer esters using solid-supported reagents in method 5.

milder reactions of various 1,1′-carbonyldiimidazole (CDI)-activated carboxylic acid derivatives with **[NBu$_4$]$_2$1** were examined (method 5). In addition, these reactions were coupled to a solid-phase purification strategy using immobilized scavenger resins.

Activation of carboxylic acids with CDI to give reactive imidazolides is a simple procedure widely used in organic synthesis.[55,56] The use of the CDI method for the conversion of 4-cyanobenzoic acid to **[NBu$_4$]$_2$3d** was chosen as an example for detailed investigation. The imidazolide of 4-cyanobenzoic acid was produced by reacting the acid with CDI in 1,2-dichloroethane for 2 h at room temperature. The addition of **1** to the activated acid followed by heating for 14 days at the reflux temperature provided the fully substituted dodecaester **3d** in 67% yield following the reactive resin workup procedure described below (Figure 4). To achieve complete esterification of **1**, a large excess of the activated acid is necessary and 10 mole equiv. of activated acid per hydroxy function were used with reaction times of 10−14 days. Only with these extended reaction times were totally esterified closomers formed in moderate yield. The need for these conditions can be rationalized by noting the decreased reactivity of the boron-bound hydroxy functions relative to the reactivity of normal alcohols and the increasing steric requirements of the closomer core as reaction proceeds. During this and all subsequent closomer ester syntheses involving CDI as the coupling reagent, a boron-free solid separated upon prolonged heating at the reflux temperature. This side-product presumably arises from the decomposition of the activated imidazolide during the extended reaction periods. Perusal of the literature reveals no information regarding the fate of activated imidazolide solutions when heated for very long periods of time. Indeed, the majority of reactions which involve CDI are conducted at room temperature. Consequently, a control experiment was performed in which 4-cyanobenzoic acid was reacted with CDI in 1,2-dichloroethane as before, followed by heating the mixture to the reflux temperature for 14 days, during which time a solid separated. The rate of imidazolide decomposition was determined by measuring the amount of imidazolide present at two time points by quenching aliquots of the reaction mixture with isopropylamine. After refluxing for 7 and 14 days, the original imidazolide had decomposed 25% and 41%, respectively. Since the complete esterification of **1** requires 14 days and 10 mole equivalents of CDI-activated acid are used per hydroxyl group, a large excess of unreacted activated acid is present in the reaction mixture at the completion of the reaction. The removal of this material from the reaction mixtures is achieved as described below (Figure 4).

Scheme 2 depicts the synthesis of a variety of closomer esters bearing different functionalities on their icosahedral surfaces, from **1** and CDI activated acids.

Figure 4 summarizes the solid supported reagents and the purification methodology employed in CDI-activated closomer ester syntheses. This simple workup procedure could be applied to all closomer ester derivatives prepared with Method 5.

When the reaction is complete (Method 5), as indicated by ^{11}B NMR spectra, the heterogeneous reaction mixture contains the desired product, **A**, a trace amount of incompletely esterified closomer, **B**, as well as unreacted imidazolide, **C**, imidazole, **D**, and boron-free byproducts, **E**, formed by the thermal decomposition of the imidazolide. The esterification reactions of **1**, utilize 120 mol equiv. of carboxylic acid and CDI per mol equiv. of **1**. The use of such large amounts of acid and coupling reagent is necessary to drive the esterification of **1** to completion in a reasonable time. Numerous attempts to separate and purify **A** using chromatographic methods were unsuccessful.

Examination of the reaction products presented in Figure 4 reveals that the unreacted imidazolide **C** is an ideal candidate for separation using an immobilized primary amine resin[57−59] such as the commercially available StratoSpheres PL-DETA (diethylenetriamine) resin. The esterification reaction mixture was filtered and the filtrate was stirred with the amine resin for 5 h. After removal of the resin by filtration, only trace amounts of unreacted imidazolide were present. In the reaction mixtures containing **3b** and **3c**, the desired product is found in the solid, which separated during the reaction. This material was dissolved in DMF prior to amine resin purification. Next, an immobilized carboxylic acid resin (Amberlite IRC-50) was added to the filtrate and the suspension was stirred for 2 h to remove the free imidazole, **D**, as a salt, leaving only the boron cluster species (**A** and **B**) and the boron-free byproducts, **E**, in the solution. Subsequent filtration of the remaining mixture over

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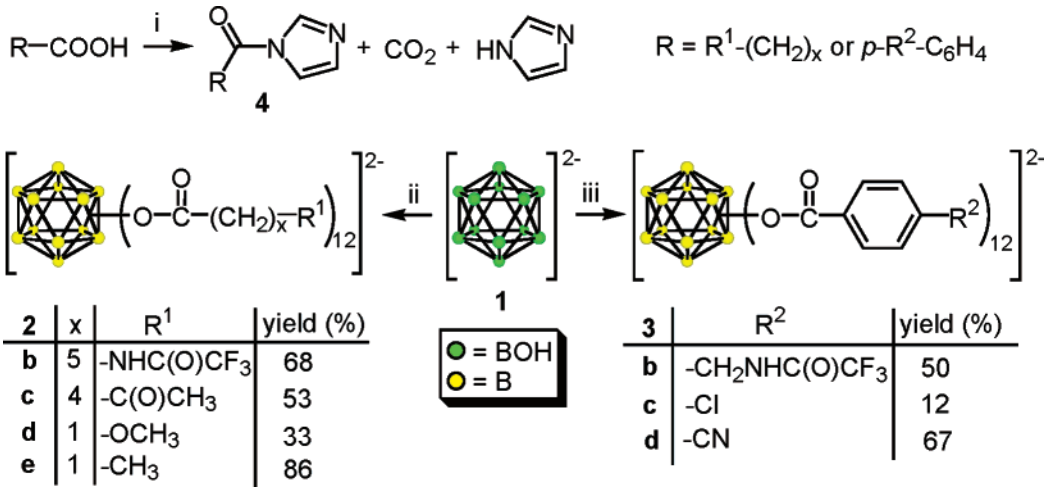
Scheme 2. Dodecaesterification of 1 Using Various Carboxylic Acids Activated by CDI.



2	x	R^1	yield (%)
b	5	-NHC(O)CF_3	68
c	4	-C(O)CH_3	53
d	1	-OCH_3	33
e	1	-CH_3	86

3	R^2	yield (%)
b	-CH_2NHC(O)CF_3	50
c	-Cl	12
d	-CN	67

(i) carbonyldiimidazole, 1,2-dichloroethane; (ii) **4** with R = $R^1-(CH_2)_x$, 1,2-dichloroethane, 14d reflux; (iii) **4** with R = *p*-$R^2-C_6H_4$, 1,2-dichloroethane, 14d reflux.

silica gel using a gradient of eluents (CH_3CN:CH_2Cl_2 30:70 and MeOH) resulted in pure closomer ester **A**. These esters were obtained as sodium and/or potassium salts, depending upon the alkali metals present in the silica gel used in the last filtration step. To obtain salts of the closomer esters with a specific cation, the alkali salt mixtures were subjected to a cation-exchange with the desired cation.

The workup procedure outlined in Figure 4 results in rapid purification of closomer esters using two scavenger resins followed by flash chromatography with silica gel and ion-exchange. In all cases, the closomer esters were obtained in very high purity suitable for analytical characterization.

Even though the use of acetonitrile provides better solubility of reactants and *N*,*N*-dimethylformamide enabled higher reaction temperatures, the reactions conducted in these solvents gave only incompletely esterified products. Excellent results were obtained when the esterification reactions were carried out in 1,2-dichloroethane at the reflux temperature (14 days).

The reaction of **1** with 4-(N-trifluoroacetylaminomethyl)-benzoic acid and CDI provided **3b** in 50% yield. When other amino protection groups were used, such as *tert*-butoxycarbonyl or 9-fluorenylmethoxycarbonyl, the esterification of **1** did not proceed to completion, presumably due to steric hindrance associated with the bulky protection groups. In **3b** the methylene group present between the amino group and the phenyl ring may significantly decrease the steric compression of the closomer ester radial arms. The fact that the corresponding closomer ester, *closo*-dodeca-[4-(trifluoroacetylamino)benzoyl-oxy]-dodecaborate, was only formed in 16% yield compared with a 50% yield for **3b**, supports this steric argument. When **1** was reacted with N-trifluoroacetyl-6-aminocaproic acid and CDI, the closomer ester, **2b**, was obtained in 68% yield. In **2b**, five methylene groups of each linker arm provide greater spatial separation of the peripheral trifluoroacetamido groups. When the linker arm chain length was reduced to four methylene groups or less, the esterification reaction did not proceed to completion.

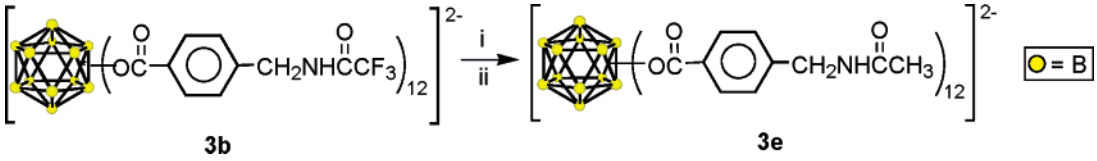
The reaction of **1** with 6-oxoheptanoic acid and CDI provided the closomer ester **2c** in 53% yield. The twelve carbonyl groups present in **2c** represent another type of peripheral functional groups attached to closomer esters. In addition to closomer esters having peripheral amino (see below) and carbonyl groups, the dodecaester **2d** with peripheral ether groups was prepared in 33% yield from methoxyacetic acid. The highest yield of a closomer ester was obtained with propionic acid, and the corresponding dodecapropionate, **2e**, was obtained in 86% yield. The lowest yield of all CDI-mediated esterification reactions was that of 4-chlorobenzoic acid, in which case after a reaction time of fourteen days, *closo*-dodeca-[4-chlorobenzoyloxy]-dodecaborate, **3c**, was isolated in only 12% yield.

Deacylation of a Twelvefold Peripheral Carboxamide and the in Situ Acetylation of the Dodecaamino Intermediate. The only known molecular collector systems carrying multiple amino functions in close proximity to one another are the commercially available starburst polyamidoamine dendrimers;[16] they continue to be investigated in a variety of applications in medicine, biotechnology and material science.[17] The icosahedral closomer esters **2b** and **3b** carry radial arms terminated with acylated primary amino groups. The presence of twelve amino groups about an icosahedral core provides an opportunity for the development of new dendritic structures and conveniently reactive functionalized molecular collectors. Amide **3b** was chosen for further study.

The hydrolysis of the twelve base-labile trifluoroacetamide groups present in **3b** without simultaneous cleavage of the internal carboxylate ester functions of the cluster was accomplished and generated the corresponding closomer with twelve terminal primary amino groups available for further conjugation reactions. Hydrolytic stability studies as a function of pH proved (see below) that the ester functions on the closomer surface are stable to weak bases. Stirring **3b** in 0.5 M NH_4OH in 50 vol % aqueous methanol for 3 days at room temperature liberated the corresponding free amine, which was subsequently transformed to the corresponding acetylated derivative **3e**. The yield of the overall reaction was 60% (Scheme 3).

For the first time, attachment of twelve equivalent primary amino groups to a small central collection species was accomplished. With twelve primary amino groups available for reaction, this closomer derivative has unique potential for a variety of applications. Detailed studies using closomers as dodecafunctional reactants are ongoing.

Scheme 3. Deprotection of **3b** and Acetylation of the Amine Product.



3b **3e**

(i) 0.5 M NH_4OH in 50 vol % CH_3OH/H_2O, r.t., 3 days, evaporated to dryness; (ii) acetic anhydride, NEt_3, DMF, r.t. 24 h.

Closomer Integrity Toward Possible Hydrolytic and Enzymatic Cleavage Reactions. Many biomedical applications suggested for closomer carboxylate esters require excellent hydrolytic stability across a range of pH and in the presence of esterases as well as minimal toxicity in biological systems. Dicesium dodecaacetate closomer **2a** was selected as a typical closomer ester for both pH-dependent and enzymatic hydrolysis studies.

To investigate the chemical stability with respect to acid- and base-catalyzed ester hydrolysis, a hydrolytic stability study was performed over a range of pH using conventional buffer systems prepared with D_2O and monitored using ^1H NMR spectroscopy at room temperature. The ratios of boron-bound acetate to free acetate ion were measured quantitatively by integrating the corresponding signals in the ^1H NMR spectra as a function of time. The loss of symmetry of the closomer resonance due to potential cleavage of the set of 12 acetate substituents was also qualitatively observed in the ^{11}B NMR spectra. Cleavage of acetate groups took place between pH 1 and 3 and between pH 12 and 14. No observable cleavage of the ester functions was observed between pH 4 to 10 over a period of 10 days at room temperature. Thus, closomer carboxylate esters are sufficiently stable to hydrolysis to allow manipulation in weak aqueous acid and base or by rapid manipulation at low temperatures outside this pH 4−11 range.

The enzymatic stability of the dodecaacetate closomer **2a** was examined by dissolving $\mathbf{Cs_2}$**2a** in a neutral PBS buffer solution and exposing it to fresh calf serum. At room temperature and at 37 °C, no ester cleavage was observed as determined by ^{11}B NMR spectroscopy over a period of 4 days. This result indicates that the carboxylic ester functions in **2a** are stable toward esterase enzymes present in the calf serum. This is an important observation since enzymatic closomer ester radial arm cleavage can probably be eliminated as a mechanism for structural degradation in vivo. The tight packing of the closomer ester groups about the closomer core may sterically inhibit the approach of enzyme.

Biodistribution of Disodium Closomer Dodecaacetate in Mice Bearing EMT-6 Tumors. The novel structures of the closomer carboxylate esters will undoubtedly suggest species of this type as viable candidates for further derivatization leading to pharmacophores, diagnostics and molecular device constructs for use in vivo. Furthermore, the closomer esters are of interest as candidate neutron target compounds for use in boron neutron capture therapy.[14] Consequently, a biodistribution experiment was conducted which would also indicate acute toxicity issues associated with these new materials and also identify any propensity for them to accumulate in BALB/C mouse tissues including EMT-6 murine carcinoma xenografts. The very water-soluble $\mathbf{Na_2}$**2a** was selected for this study.

Thirty-two EMT-6 tumor-bearing BALB/C mice (right dorsal hip area) were each injected with 0.20 mL of a PBS buffer solution of $\mathbf{Na_2}$**2a** giving an injected dose of 11 mg boron/kg body weight. All mice survived until they were euthanized for analysis at specific time intervals. Tissue samples were digested and analyzed for boron using ICP-AES. The tumor and organs of interest did not accumulate appreciable boron during the 48h course of the experiment. Only kidney reached a steady boron content of 2−3 ppm while blood, brain, liver, skin and tumor contained less than 1 ppm of boron throughout the course of the experiment, the facile excretion of $\mathbf{Na_2}$**2a** is in accord with its hydrophilicity. No physiological response was noted in accord with rapid clearance and enhanced hydrophilicity. Closomer esters having a collection of amphiphilic radial arms would be expected to provide a more complex response in biodistribution experiments. However, it is clear that the simple closomer core dianion is unremarkable with regard to systemic toxicity and organ accumulation. The synthesis of boron-enriched closomers for potential use in BNCT has been reported elsewhere.[14]

Conclusion

The synthesis and reactions of closomer carboxylate esters have been extended to (1) reveal new synthesis routes to these monodisperse globular multifunctional species (2) develop facile purification procedures which utilize convenient solid-supported scavenger reagents (3) the in situ preparation and subsequent reaction of a closomer species having twelve peripheral primary amino groups suitable for further studies (4) the demonstration that closomer carboxylate esters are generally stable to hydrolysis between pH 4 and 11 at room temperature and that these same species are inert to enzymatic hydrolysis catalyzed by the enzymatic components of fresh calf serum (5) a biodistribution study with tumor bearing BALB/C mice (EMT-6 murine carcinoma xenografts) gave little evidence of preferential uptake of $\mathbf{Na_2}$**2a** by blood, internal organs or tumor and no evidence of toxicity issues at injected doses of 11 mg B/kg body weight.

Further work will extend the utility of reactive functional groups carried by radial arms of closomer esters in order to address new applications.

Experimental Section

General. All reactions were carried out under N_2 or Ar using Schlenk techniques. Chemicals were purchased from commercial suppliers and used without further purification. All solvents were dried and distilled before use. The StratoSphere (PL-DETA) resin was purchased from Aldrich; the acidic resin (Amberlite IRC-50) was purchased from Fluka. The silica gel was from Sorbent Technologies (60 Å, 32−63 μm). *N*-trifluoroacetyl-6-aminocaproic acid,[60] 4-cyanobenzoyl chloride,[61] and 4-(trifluoroacetylaminomethyl)-benzoic acid[62] were prepared according to published methods.

The ^{11}B NMR spectra were obtained with a Bruker ARX500 spectrometer at 160 MHz and externally referenced to $BF_3{\cdot}Et_2O$;

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resonances upfield of the reference are designated as negative. The ^{1}H and ^{13}C NMR spectra were recorded at 500 and 126 MHz with Bruker ARX500 and AVANCE500 spectrometers. The IR spectra were acquired with a Nicolet Nexus 470 FT-IR instrument. The high-resolution mass spectra were obtained via MALDI and Electrospray (ESI) methods.

Synthesis Method 1. Acid Anhydride Method. Bis(cesium)-*closo*-dodecaacetoxy-dodecaborate ($Cs_2$2a). A suspension of the cesium salt of **1** (2.00 g, 3.57 mmol) in acetic anhydride (50 mL) was heated to reflux for 6 d under argon with vigorous stirring. The dark-brown reaction suspension was cooled to room temperature and filtered. The volume of the filtrate was reduced to about 10 mL in a vacuum and cooled to −30 °C. After 2 d, the cesium salt of **2** precipitated and was obtained as a tan solid (2.29 g, 58%). ^{1}H NMR (D_2O, 500 MHz) δ 1.9 (s, CH_3CO−); ^{13}C NMR (D_2O, 126 MHz) δ 22.0 (*C*H_3CO−), 174.0 (CO); ^{11}B {^{1}H} NMR (D_2O 160, MHz) δ −16. MS (Electrospray) *m*/*z* calcd for $C_{24}H_{37}O_{24}B_{12}^{1-}$ (M+H): 839.27 Found: 839.2 (M+H), 419.1 (M^{2-}).

Synthesis Method 2. Acid Anhydride Method at Elevated Temperatures. Disodium-*closo*-dodeca-(2-methoxyacetoxy)-dodecaborate ($Na_2$2d). Into a 100 mL round-bottom flask were added methoxyacetic acid (20 mL) and acetic anhydride (20 mL) and the reaction mixture was heated with stirring for 2 h. The acetic acid and acetic anhydride were removed by distillation, and the methoxyacetic anhydride was collected by fractional distillation under reduced pressure. A solution containing this methoxyacetic anhydride (3 mL), bis-(tetrabutylammonium)salt of **1** (100 mg, 0.120 mmol), and pyridine (2 mL) in acetonitrile (40 mL) was heated in an autoclave at 150 °C and 1500 psi for 40 h. After the completion of the reaction as indicated by an appearance of a singlet in ^{11}B NMR, the solvent was removed under reduced pressure and the resulting residue was passed through a small column of silica gel (10% MeOH in CH_2Cl_2). The resulting mixed salts of **2d** were cation-exchanged to disodium salt **2d** (71 mg, 47%). ^{1}H NMR (acetone-d_6, 500 MHz) δ 3.36 (s, CH_3O-, 36 H), 3.84 (s, −CH_2-, 24 H). ^{13}C {^{1}H} NMR (acetone-d_6, 126 MHz) δ 59.0 (*C*H_3O-), 71.4 (-*C*H_2-), 169.6 (C═O). ^{11}B {^{1}H} NMR (acetone, 126 MHz) δ −16.6. HRMS (MALDI): *m*/*z* calcd for $C_{36}H_{60}O_{36}B_{12}Na^{1-}$ (M+Na): 1221.3968. Found: 1221.4004 (3 ppm).

Disodium-*closo*-dodecapropionoyloxy-dodecaborate ($Na_2$2e). A 100-mL autoclave reaction vessel was charged with bis(tetrabutylammonium) salt of **1** (100 mg, 0.120 mmol), propionic anhydride (2 mL), pyridine (2 mL) and acetonitrile (40 mL). The pressure was increased to 1500 psi, and the vessel was heated to 150 °C. The reaction was monitored by ^{11}B NMR and found to be complete after 40 h. The solvent was removed under reduced pressure and the residue was passed through a silica gel column using 10% acetone in CH_2Cl_2 followed by 100% acetone as the eluents. A mixed salt of compound was obtained in the acetone fraction which was converted to disodium salt of **2e** (69 mg, 54%) after passing through cation-exchange resin.

Synthesis Method 3. Acid Chloride Method. Bis(tetrabutylammonium)-*closo*-dodeca-(4-cyanobenzoyloxy)-dodecaborate [(NBu_4)$_2$3d]. To a solution of bis(tetrabutylammonium) salt of **1** (50 mg, 0.061 mmol) and 4-cyanobenzoyl chloride (1.21 g, 7.31 mmol) in CH_3CN (30 mL), triethylamine (5 mL) was added and the reaction mixture was refluxed for 10 d. The course of the reaction was monitored by ^{11}B NMR. After the completion of the reaction, the solvent was removed under reduced pressure and the remaining solid was dissolved in CH_2Cl_2 (100 mL) and washed with water (3 × 50 mL). The organic layer was dried with anhydrous $MgSO_4$ and the solvent removed under vacuum. Column chromatography on silica gel with THF/CH_3CN (1:1) yielded a mixture of the (NBu_4)$_2$ and (NBu_4)(Na) salts of **3d** (102 mg, mixed cations). Ion exchange in CH_3CN/H_2O (1: 1) to the (NBu_4)$_2$ salt and recrystallization from 2-propanol/CH_3CN gave 49 mg of pure (NBu_4)$_2$**3d** in 36% yield. ^{1}H NMR (CD_3CN, 500 MHz) δ 1.02 (t, ^{3}J = 7.3 Hz, 24 H CH_3CH$_2$−), 1.44 (m, CH$_3$CH_2-, 16 H), 1.62 (m, −CH_2CH$_2$N−, 16 H), 3.08 (m, −CH_2N−, 16 H), 7.47 (d, ^{3}J = 8.3 Hz, Ar−H, 24 H), 8.08 (d, ^{3}J = 8.4 Hz, Ar−H, 24 H); ^{13}C {^{1}H} NMR (CD_3CN, 126 MHz) δ 13.6 (*C*H_3CH$_2$−), 20.0 (CH$_3$*C*H_2−), 24.1 (−*C*H_2CH$_2$N−), 59.4 (−*C*H_2N−), 114.8 (−CN), 118.9, 130.9, 131.8, 138.3, 163.7 (C═O); ^{11}B {^{1}H} NMR ($CHCl_3$, 160, MHz) δ −16.3. IR(KBr) ν 2960, 2230, 1718, 1316, 722 cm^{-1}. HRMS (MALDI), *m*/*z* calcd. for $C_{96}H_{48}O_{24}N_{12}B_{12}Na^{1-}$ (M+Na): 1906.4018, Found: 1906.4080 (3 ppm).

Synthesis Method 4. Catalyzed Vinyl Carboxylate Transesterification Method. Bis(tetrabutylammonium)-*closo*-dodecaacetoxy-dodecaborate [(NBu_4)$_2$2a]. To a solution of bistetrabutylammonium salt of **1** (165 mg, 0.202 mmol) in CH_2Cl_2 was added Y_5(O*i*Pr)$_{13}$O (120 mg, 0.0977 mmol) and vinyl acetate (5 mL, excess). The reaction mixture was heated to 40 °C for 24 h, at which time, another 120 mg of Y_5(O*i*Pr)$_{13}$O was added and continued the heating for another 24 h. The completion of the reaction was confirmed by the appearance of a singlet in the ^{11}B NMR spectrum. The mixture was filtered and solvents were removed under vacuum. Washing the remaining residue with diethyl ether and subsequent purification by column chromatography on silica gel (CH_3CN/MeOH, 7:3) yielded bis(tetrabutylammonium)-salt of **2a** (218 mg, 82%) as a colorless solid. ^{1}H NMR (CD_3OD, 500 MHz) δ 0.91 (t, ^{3}J = 7.4 Hz, C*H*$_3$CH$_2$−, 24 H), 1.38 (m, CH$_3$C*H*$_2$−, 16 H), 1.56 (m, −C*H*$_2$CH$_2$N−, 16 H), 1.83 (s, C*H*$_3$CO−, 36 H) 3.14 (m, − C*H*$_2$N−, 16 H); ^{13}C {^{1}H} NMR (CD_3OD, 126 MHz) δ 14.1 (*C*H_3CH$_2$−), 20.8 (CH$_3$*C*H_2−), 22.8 (*C*H_3CO−), 24.9 (−*C*H_2CH$_2$N−), 59.6 (−*C*H_2N−), 178.3 (*C*H_3CO−). ^{11}B {^{1}H} NMR (CH_3OH, 160 MHz) δ −15.6. MS (MALDI-TOF), *m*/*z* calcd for $C_{36}H_{64}O_{24}N_1B_{12}NH(Bu)_3^{1-}$ (M+NH(Bu)$_3$): 1025.9 Found: 1026.

Synthesis Method 5. Carbonyldiimidazole Method (CDI method). General Procedure. Synthetic Procedure. The carboxylic acid (7.35 mmol) and 1,1′-carbonyldiimidazole (1.19 g, 7.35 mmol) were added to 1,2-dichloroethane (25 mL) and stirred for 2 h at room temperature. Bis(tetrabutylammonium)-*closo*-dodecahydroxy-dodecaborate, **[NBu_4]$_2$1**, (50 mg, 0.061 mmol) in 1,2-dichloroethane (5 mL) was added and the mixture was heated to reflux for 14 d. The completion of the reaction was confirmed by formation of singlet in ^{11}B NMR.

Procedure for Purification Using Solid-Supported Reagents. When the reaction was complete, some insoluble materials were usually present in the reaction mixture and only one fraction (the solution or the separated solid) contained the product. The presence of the product was determined by checking the ^{11}B NMR of both the solution and the insoluble materials (in deuterated DMF). When the product was in the solution, the dichloroethane solution was transferred and stirred with 1.20 g of diethylenetriamine basic resin (StratoSpheres PL-DETA) for 5 h. After filtration, the solution was stirred with 1.00 g of acidic resin (Amberlite IRC-50 resin) for 2 h.

When the product was located in the solid residue, the dichloroethane solution was decanted, the residue was dissolved in distilled DMF (15 mL) and the resin wash steps were repeated. The product was obtained after 3-step flash chromatography on silica gel with $CH_3CN/CHCl_3$(3:7), CH_3CN and MeOH, respectively. Ion exchange to the desired cation was carried out using a Dowex 50X8-200 cation-exchange resin with CH_3CN/H_2O (1:1).

Bis(tetrabutylammonium)-*closo*-dodeca-[6-(2,2,2-trifluoroacetylamino)-hexanoyloxy]-dodecaborate [(NBu_4)$_2$2b]. N-Trifluoroacetyl-6-aminocaproic acid (1.67 g, 7.35 mmol) and 1,1′-carbonyldiimidazole (1.20 g, 7.35 mmol) were stirred in 1,2-dichloroethane (20 mL) for 2 h at room temperature. Bis(tetrabutylammonium) dodecahydroxy-dodecaborate **[NBu_4]$_2$1** (50 mg, 0.061 mmol) in 1,2-dichloroethane (5 mL) was added and mixture was heated to reflux for 14 d. After the reaction was complete, ^{11}B NMR of the precipitate showed the presence of the desired product. Using the solid-support purification method and subsequent column chromatography on silica gel (CH_3CN) and ion exchange gave pure tetrabutylammonium salt of **2b** (120 mg, 59%). Alternative purification was also accomplished as follows: The reaction mixture was evaporated to dryness and the resulting residue was treated with MeOH/acetone (1:1). The mixture was centrifuged

and the supernatant was decanted. The solvent was removed in a vacuum and the residue was dissolved in EtOAc (50 mL) and washed with H_2O (3 × 50 mL). The organics were removed under reduced pressure and the resulting residue was purified by flash chromatography (silica gel/MeOH) followed by ion exchange in CH_3CN/H_2O (1:1) to give the tetrabutylammonium salt of **2b** (138 mg, 68%). ^{1}H NMR (acetone-d_6, 500 MHz) δ 0.98 (t, ^3J = 7.4 Hz, CH_3CH$_2$−, 24 H), 1.43 (m, CH_2, 40 H), 1.61 (m, CH_2, 48 H), 1.83 (m, −CH_2CH$_2$N−, 16 H), 2.21 (m, − CH_2COO−, 24 H), 3.32 (m, −CH_2NH−, 24 H), 3.45 (m, −CH_2N−, 16 H), 8.77 (m, −NH−, 10 H); ^{13}C {^{1}H} NMR (acetone-d_6, 126 MHz) δ 13.8 (*C*H$_3$CH$_2$-), 20.4 (CH$_3$*C*H$_2$−), 24.4 (−*C*H$_2$CH$_2$N−), 25.5 (CH_2), 27.2 (CH_2), 29.1 (CH_2), 36.9 (CH_2), 40.6 (CH_2), 59.4 (−*C*H$_2$N−), 117.2 (q, $^1J_{C,F}$ = 287 Hz, *C*F$_3$−), 157.6 (q, $^3J_{C,F}$ = 36 Hz CF$_3$*C*═O), 172.2 (−*C*OO−); ^{11}B{^{1}H} NMR (acetone, 126 MHz) δ −17.1. IR (KBr) υ 2944 (CH), 1710 (C═O), 1211 (C−O) cm^{-1}. HRMS (MALDI): *m*/*z* calcd for $C_{96}H_{132}O_{36}N_{12}B_{12}F_{36}Na^{1-}$ (M+Na): 2866.9409. Found: 2867.0237 (29 ppm).

Disodium *closo*-dodeca-(5-acetylpentanoyloxy)-dodecaborate ($Na_2$2c). To a solution of 6-oxoheptanoic acid (1.06 g, 7.35 mmol) in 1,2-dichloroethane (20 mL), 1,1′-carbonyldiimidazole (1.20 g, 7.35 mmol) was added and the mixture was stirred for 2 h at room temperature. Bis(tetrabutylammonium) *closo*-dodecahydroxy-dodecaborate, $(NBu_4)_2$**1**, (50 mg, 0.061 mmol) in 1,2-dichloroethane (5 mL) was then added and the reaction mixture was heated to reflux for 14 d. The desired product was present in the dichloroethane solution as indicated by ^{11}B NMR. The resin workup procedure followed by silica gel flash chromatography (MeOH) of the crude product gave mixed salt of **2c** which was cation-exchanged to the disodium salt of **2c** (62 mg, 53%). ^{1}H NMR (CD_3CN, 500 MHz) δ 1.47 (m, CH_2, 48 H), 2.05−2.14 (m, CH_3 + CH_2, 60 H), 2.40 (m, −CH_2COO−, 24 H); ^{13}C {^{1}H} NMR (acetone-d_6, 126 MHz) δ 24.1 (CH_2), 25.4 (CH_3), 29.8 (CH_2), 36.3 (CH_2), 43.7 (CH_2), 172.3 (−CH$_2$*C*OO−), 208.3 (CH$_3$*C*═O). ^{11}B{^{1}H} NMR (acetone, 126 MHz) δ −17.2. HRMS (ESI): *m*/*z* calcd for $C_{84}H_{132}O_{36}B_{12}Na^{1-}$ (M+Na) 1870.9609. Found: 1871.0076 (25 ppm).

Disodium *closo*-dodeca-(2-methoxyacetoxy)-dodecaborate ($Na_2$2d). Methoxyacetic acid (0.560 mL, 7.35 mmol) and 1,1′-carbonyldiimidazole (1.20 g, 7.35 mmol) were stirred in 1,2-dichloroethane (20 mL) for 2 h at room temperature. Bis(tetrabutylammonium) *closo*-dodecahydroxy-dodecaborate, $(NBu_4)_2$**1**, (50 mg, 0.061 mmol) in 1,2-dichloroethane (5 mL) was then added and the mixture was heated to reflux for 14 d. The desired closomer product was present in the solution phase, and the respective solid support workup procedure was employed. Flash chromatography on silica gel (MeOH) of the crude product gave mixed salts of **2d**, which were cation-exchanged to the disodium salt of **2d** (25 mg, 33%).

Disodium-*closo*-dodecapropionyloxy-dodecaborate ($Na_2$2e). Propionic acid (0.550 mL, 7.35 mmol) and 1,1′-carbonyldiimidazole (1.20 g, 7.35 mmol) were added to 1,2-dichloroethane (20 mL) and stirred for 2 h at room temperature. Bis(tetrabutylammonium)-*closo*-dodecahydroxy-dodecaborate, **[NBu$_4$]$_2$1**, (50 mg, 0.061 mmol) in 1,2-dichloroethane (5 mL) was then added and the mixture was heated to reflux for 14 d. The desired product was present in the solution phase, and the respective solid support work up procedure was employed. Flash chromatography ($CH_3CN/CHCl_3$) of the crude product gave mixed salts of **2e**, which were cation-exchanged to the disodium salt of **2e** (55 mg, 86%). ^{1}H NMR (CD_3OD, 500 MHz) δ 1.08 (t, ^3J = 7.6 Hz, CH_3-, 36 H), 2.27 (m, ^3J = 7.5 Hz, −CH_2−, 24 H); ^{13}C {^{1}H} NMR (CD_3OD, 126 MHz) δ 10.0 (*C*H$_3$), 30.4 (*C*H$_2$) 175.7 (CO); ^{11}B {^{1}H} NMR (CD_3OD, 160 MHz) δ −17.9. IR (KBr): υ 2964 (CH), 1706 (C═O), 1240 (C−O) cm^{-1}. HRMS (MALDI): *m*/*z* calcd for $C_{36}H_{61}O_{36}B_{12}Na^{1-}$ (M+Na): 1029.4577. Found 1029.4597 (2 ppm).

Bis(tetrabutylammonium)-*closo*-dodeca-{[4-(2,2,2-trifluoroacetylamino)methyl]benzoyloxy}-dodecaborate [(NBu_4)$_2$3b]. A mixture of 4-(trifluoroacetylaminomethyl)benzoic acid (1.82 g, 7.35 mmol) and 1,1′-carbonyldiimidazole (1.20 g, 7.35 mmol) in 1,2-dichloroethane (20 mL) was stirred for 2 h at room temperature followed by the addition of **[NBu$_4$]$_2$1**(50 mg, 0.061 mmol). The reaction mixture was heated to reflux for 14 d. After the completion of the reaction, ^{11}B NMR of the reaction mixture showed the presence of desired product in the separated solid. The 1,2-dichloroethane solution phase, which did not contain the product, was discarded. The residue was treated with MeOH/acetone (1:1) and the mixture was centrifuged. The supernatant liquid, which contained the product, was collected. The solvent was removed under reduced pressure and the residue was again treated with CH_2Cl_2. The separated crude product was further purified by flash chromatography on silica gel (CH_3CN), followed by cation exchange [CH_3CN/H_2O (1:1)] to provide the pure bis(tetrabutylammonium) salt of **3b** (74 mg, 50%). ^{1}H NMR (acetone-d_6, 500 MHz) δ 0.94 (t, ^3J = 7.4 Hz, CH_3CH$_2$−, 24 H), 1.38 (m, CH$_3$CH_2−, 16 H), 1.76 (m, −CH_2CH$_2$N−, 16 H), 3.37 (m, −CH_2N−, 16 H), 4.50 (m, Ar−CH_2−, 24 H), 7.08 (m, Ar−H, 24 H), 8.06 (m, Ar−H, 24 H), 8.98 (s, −NH−,12 H); ^{13}C {^{1}H} NMR (acetone-d_6, 126 MHz) δ 13.8 (*C*H$_3$CH$_2$−), 20.3 (CH$_3$*C*H$_2$-), 24.3 (−*C*H$_2$CH$_2$N−), 43.9 (Ar−*C*H$_2$−), 59.2 (−*C*H$_2$N−), 117.2 (q, $^1J_{C,F}$ = 287 Hz, *C*F$_3$−), 127.4 (Ar−C), 131.7 (Ar−C), 136.0 (Ar−C), 140.0 (Ar−C), 157.6 (q, $^3J_{C,F}$ = 36 Hz, CF$_3$*C*═O), 164.6 (Ar−*C*═O). ^{11}B{^{1}H} NMR (acetone, 126 MHz) δ −14.4. IR (KBr) υ 2967 (CH), 1710 (C═O), 1321 (C−O) cm^{-1}. HRMS (MALDI), *m*/*z* calcd for $C_{120}H_{84}N_{12}O_{36}F_{36}B_{12}Na^{1-}$ (M+Na): 3106.5663. Found: 3106.4824 (27 ppm).

Disodium *closo*-dodeca-(4-chlorobenzoyloxy)-dodecaborate ($Na_2$3c). A mixture of *p*-chlorobenzoic acid (1.15 g, 7.35 mmol) and 1,1′-carbonyldiimidazole (1.20 g, 7.35 mmol) was stirred in 1,2-dichloroethane (20 mL) for 2 h at room temperature. **[NBu$_4$]$_2$1** (50 mg, 0.061 mmol) in 1,2-dichloroethane (5 mL) was then added and the reaction mixture was heated to reflux for 14 d. The desired product was present in the solution phase, and the respective solid support work up procedure was employed. The purification of the crude product via flash chromatography on silica gel (MeOH) and subsequent cation exchange gave the disodium salt of **3c** (15 mg, 12%). ^{1}H NMR (acetone-d_6, 500 MHz) δ 7.53 (d, ^3J = 8.5 Hz, Ar−H, 24 H), 8.03 (d, ^3J = 8.5 Hz, Ar−H, 24 H). ^{13}C {^{1}H} NMR (acetone-d_6, 126 MHz) δ 129.6, 130.3, 132.2, 139.5, 166.9 (C═O). ^{11}B {^{1}H} NMR (acetone, 126 MHz) δ −15.3. HRMS (ESI): *m*/*z* calcd for $C_{84}H_{48}O_{24}Cl_{12}B_{12}^{2-}$ (M^{2-}): 997.9970. Found 998.0081 (11 ppm).

Bis(tetrabutylammonium)-*closo*-dodeca-(4-cyanobenzoyloxy)-dodecaborate [(NBu_4)$_2$3d]. To a solution of 4-cyanobenzoic acid (1.07 g, 7.35 mmol) in 1,2-dichloroethane (20 mL), 1,1′-carbonyldiimidazole (1.20 g, 7.35 mmol) was added and the mixture was stirred at room temperature for 2 h. A solution of bis(tetrabutylammonium)salt of **1** (50 mg, 0.061 mmol) in 1,2-dichloroethane (5 mL) was then added and the reaction mixture was heated to reflux for 14 d. ^{11}B NMR of the solution and the separated solid indicated that the product is in the solution phase. The mixture was filtered and the solution phase was subjected to resin workup as described in Method 5 general procedure. The title compound, **3d** was isolated as a bis(tetrabutylammonium) salt (81 mg, 56%). Apart from the resin workup procedure, an alternative workup method was also applied as follows: The solvent was removed under vacuum and the residue was extracted with CH_2Cl_2 and H_2O. The organic layer was collected and the solvent evaporated under vacuum. The residue was fractionally recrystallized from CH_3CN to remove the imidazole, which has a poor solubility in CH_3CN. The mother liquor was evaporated to dryness and the residue was recrystallized from 2-propanol/CH_3CN to yield bis(tetrabutylammonium) salt of **3d** (97 mg, 67%) as a pale-white solid. ^{1}H NMR (CD_2Cl_2, 500 MHz) δ 1.02 (t, ^3J = 7.3 Hz, 24 H CH_3CH$_2$−), 1.44 (m, CH$_3$CH_2−, 16 H), 1.62 (m, −CH_2CH$_2$N−, 16 H), 3.08 (m, −CH_2N−, 16 H), 7.47 (d, ^3J = 8.3 Hz, Ar−H, 24 H), 8.08 (d, ^3J = 8.4 Hz, Ar−H, 24 H); ^{13}C {^{1}H} NMR (CD_2Cl_2, 126 MHz) δ 13.6 (*C*H$_3$CH$_2$−), 20.0 (CH$_3$*C*H$_2$−), 24.1 (−*C*H$_2$CH$_2$N−), 59.4 (−*C*H$_2$N−), 114.8 (−CN), 118.9 (Ar−C), 130.9 (Ar−C), 131.8 (Ar−C), 138.3 (Ar−C), 163.7 (C═O); ^{11}B {^{1}H} NMR ($CHCl_3$, 160, MHz) δ −16.3. IR(KBr) υ 2960 (CH), 2230 (CN),

1718 (C═O), 1316 (C−O) cm^{-1}. HRMS (MALDI), *m*/*z* calcd. for $C_{96}H_{48}O_{24}N_{12}B_{12}Na^{1-}$ (M+Na): 1906.4018, Found: 1906.4080 (3 ppm).

Bis(tetrabutylammonium)-*closo*-dodeca-{[4-(acetylamino)-methyl]-benzoyloxy}-dodecaborate [$(NBu_4)_2$3e]. A mixture of $(NBu_4)_2$**3b** (100 mg, 0.0321 mmol), 0.5 M NH_4OH (50 mL) and MeOH (50 mL) was stirred for 3 d at room temperature. The complete cleavage of the trifluoroacetyl protection group was monitored by ^{19}F NMR. After completion of the reaction, the solvent was removed under reduced pressure and the residue was dried under vacuum and then dissolved in a mixture of distilled DMF (10 mL), triethylamine (2.0 mL), and acetic anhydride (2.0 mL). The mixture was stirred at room temperature for 24 h. The solvent was removed by distillation under reduced pressure. The residue was then washed with water and dissolved in MeOH/H_2O (1:1). This solution was ion-exchanged to give bis-(tetrabutylammounium) salt of **3e** (48 mg, 58%). ^1H NMR (acetone-d_6, 500 MHz) δ 1.00 (t, ^3J = 7.3 Hz, CH_3CH$_2$−, 24 H), 1.38 (m, CH$_3$CH_2−, 16 H), 1.63 (m, −CH_2CH$_2$N−, 16 H), 1.96 (m, CH_3CO−, 36 H) 3.20 (m, −CH_2N−, 16 H), 4.31 (m, Ar−CH_2−, 24 H), 7.02 (m, Ar−H, 24 H), 7.96 (m, Ar−H, 24 H); ^{13}C {^1H} NMR (acetone-d_6, 126 MHz) δ 13.9 (*C*H$_3$CH$_2$−), 20.7 (CH$_3$*C*H$_2$−), 22.7 (*C*H$_3$CO−), 24.8 (−*C*H$_2$CH$_2$N−), 44.0 (Ar−*C*H$_2$−), 59.5 (−*C*H$_2$N−), 127.6 (Ar−C), 131.7 (Ar−C), 134.6 (Ar−C), 142.9 (Ar−C), 167.1 (Ar−*C*═O), 173.0 (*C*H$_3$CO−). ^{11}B {^1H} NMR (CH$_3$OH, 160 MHz) δ −15.3. HRMS (ESI), *m*/*z* calcd for $C_{120}H_{120}N_{12}O_{36}F_{36}B_{12}^{2-}$ (M^{2-}): 1217.9587. Found: 1217.9523 (5 ppm).

X-ray Crystallography of Bis(tetraphenylarsonium)-*closo*-dodeca-(4-cyanobenzoyloxy)-dodecaborate [$(Ph_4As)_2$3d]. X-ray data of **3d**: $C_{144}H_{88}As_2B_{12}N_{12}O_{24.80}$; FW = 2662.62; orthorhombic space group *Fddd*; *a* = 20.066(4) Å, *b* = 40.221(7) Å, *c* = 67.924(13) Å; α = 90°; β = 90°; γ = 90°; *V* = 54818(18) Å^3; *Z* = 16; ρ_{calcd} = 1.290 mg mm^{-3}; μ = 0.562 mm^{-1}; *F*(000) = 21766. A colorless crystal (plate, 0.20 × 0.07 × 0.02 mm^3), obtained from an acetonitrile solution, was used for data collection at *T* = 100 °K ($2\theta_{max}$ = 28.36°) giving 90131 unique reflections. The structure was solved by direct methods. The final discrepancy indices were *R* = 0.0615 and R_w = 0.1264 for 16844 independent reflections with $I > 2\sigma(I)$ (goodness-of-fit on F^2 = 0.994). The maximum and minimum values on a final difference electron density map were 0.596 and −0.752 eÅ^{-3}. All nonhydrogen atoms were included with anisotropic displacement parameters. All hydrogen atoms were kept in calculated positions. A summary of the crystallographic data and details of the structure determination is provided in Supporting Information. Data were collected on a Bruker Smart 1000 CCD diffractometer (Mo K_α, λ = 0.71073 Å, $\phi+\omega$-scans). Data were corrected for Lorentz and polarization effects. The structure was solved by direct methods and refined by full-matrix least-squares methods based on F^2 (Bruker-SHELXTL package).

Hydrolytic Stability Study of $Cs_2$2a, at Variable pH. A series of buffer solutions in D_2O (0.4 mL of commercial buffer solution was dried and redissolved in 0.4 mL D_2O) of various molarities (0.05 M in the case of pH 2, 3, 4, 5, 6, 7, 8, 10, 11, 12, 13, and 0.1 M in the case of pH 9), aqueous NaOH (2 M, pH 14), and aqueous HCl (1 M, pH 1) were prepared and a solution of **$Cs_2$2** (0.15 M) in D_2O (0.3 mL) was added to each. These solutions were kept at 30 °C for up to 240 h. The ratio of boron bound acetate to cleaved acetate was measured quantitatively by integrating the corresponding signals in the ^1H NMR spectra (500 MHz, δ 1.85 for BOCOC*H*$_3$; 1.95 for C*H*$_3$COOH and 1.76 for C*H*$_3$COO−, respectively) and converting the values into percent of esters cleaved. Cleavage of acetate groups took place between pH 1 and 3 and between pH 11 and 14. No cleavage of the ester functions was observed between pH 4 to 10 over a period of 240 h at room temperature.

Enzymatic Stability Study of $Cs_2$2a. A 1.0 mM solution of Cs_2**2** in neutral PBS buffer (1 mL) was added to calf's serum (1 mL) and the ^{11}B NMR of this mixture was monitored for 2 d at room temperature followed by 2 d at 37 °C. During this period of time the symmetry of the singlet for the anion in the ^{11}B NMR spectrum remained unchanged indicating that no ester hydrolysis had occurred. Previous experiments have shown that if ester hydrolysis takes place the singlet signal in the ^{11}B NMR spectrum becomes unsymmetrical with shoulders present on the side, reflecting the reduced symmetry of the boron cage. If complete ester hydrolysis had occurred, then the formed $Cs_2B_{12}(OH)_{12}$ would precipitate from the buffer solution.

Biodistribution Study of $Na_2$2a. In this study, thirty-two BALB/c tumor-bearing mice were used. They were implanted with EMT-6 tumor cells earlier along the right dorsal hip area. The mice were injected with 0.2 mL of **$Na_2$2a** through the lateral tail vein. The data collection times were 6, 16, 30, and 48 h postinjection. Blood and tissues were collected for boron analysis and histopathology. The summary of the results are as follows: **6-h postinjection**; 7 mice were in this group. The injections and tumors were good. In 3 mice, the liver and kidneys were dark red; in one mouse, the liver and kidneys were very dark red; and in two mice, the liver and kidneys were less pronounced red. **16-h postinjection**: 7 mice in this group. All the injections and tumors were good. In 3 mice the liver and kidney were dark red. **30-h postinjection**: 7 mice in this group. All the injections and tumors were good. In three mice, the liver and kidneys were dark red; and in one mouse the kidneys were dark red. **48-h postinjection**: 7 mice in this group. The injections and tumors were good, except the liver of one mouse was dark red. In summary the **$Na_2$2a** did not show any bioactivity save for apparent thrombocytopenia of the liver and kidneys.

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Supporting Information Available: Tables listing atomic coordinates, temperature factors, bond lengths and angles, and torsion angles and details of the refinement of the X-ray crystallographic data for compound **3d**; Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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