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Synthesis of [*closo*-$B_{12}(OH)_{11}NH_3$]$^-$: A New Heterobifunctional Dodecaborane Scaffold for Drug Delivery Applications

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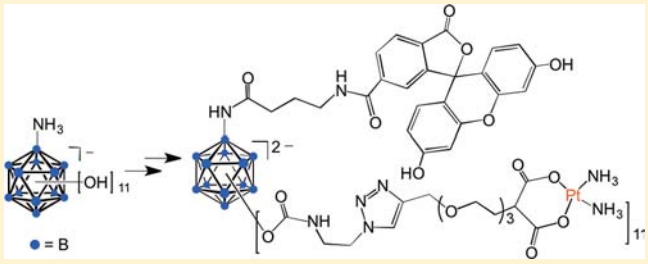
Supporting Information

ABSTRACT: Effective utilization of [*closo*-$B_{12}H_{12}$]$^{2-}$ derivatives in targeted drug delivery applications depends upon an efficient strategy to differentiate at least one of the 12 vertices on the B_{12}^{2-} core. Precursor molecules must also be able to withstand the initial harsh hydrogen peroxide treatment necessary for hydroxylation of the B−H vertices. We report here a method for preparation of the ammonio derivative [*closo*-$B_{12}(OH)_{11}NH_3$]$^-$ and also demonstrate its utility in construction of a targeted drug delivery scaffold. Treatment of the precursor [*closo*-$B_{12}H_{11}NH_3$]$^-$ with hydrogen peroxide gives the corresponding nitro derivative [*closo*-$B_{12}(OH)_{11}NO_2$]$^{2-}$ in good yield. The nitro group is easily reduced with hydrogen over a Raney nickel catalyst to produce [*closo*-$B_{12}(OH)_{11}NH_3$]$^-$. The 11 hydroxyl groups can then be readily converted to carbonates or carbamates. As a proof-of-principle of its utility as a drug delivery system, we used the resulting vertex-differentiated ammonio derivative to construct a platinated pro-drug possessing 11 copies of a carboplatin analogue conjugated to the B_{12}^{2-} core via carbamate linkage and a fluorescein molecule attached at the remaining vertex by an amide linkage. In vitro cytotoxicity assays demonstrated that activity of an untagged analog was similar to carboplatin against platinum-sensitive A459 cells and higher than carboplatin against platinum-resistant SK-OV-3 cells. Further fluorescence microscopy revealed that the fluorescein-tagged pro-drug localizes to the nuclei of A459 cells.



INTRODUCTION

Targeted drug delivery systems employing nanomolecular assemblies such as liposomes, polymeric and metallic nanoparticles, and dendrimers have recently garnered much attention for their potential usefulness in the detection and treatment of disease.[1] Such targeted nanocarrier drug delivery systems exhibit prolonged circulation within the blood as well as preferential biodistribution to targeted organs, helping reduce toxic effects within healthy tissues.[2] Dramatic clinical responses can therefore be achieved with reduced drug-associated adverse side effects.

The ability of the icosahedral borane dianion [*closo*-$B_{12}H_{12}$]$^{2-}$ and its dodecahydroxyl derivative [*closo*-$B_{12}(OH)_{12}$]$^{2-}$ to form functionalized 12-fold derivatives known as closomers[3,4] makes them attractive as molecular drug delivery scaffolds for a variety of biologically active agents. Closomers have recently been used to prepare a nontargeted high-performance MRI contrast agent in which polyethylene glycol linkers were used to anchor 12 Gd^{3+} chelates to the B_{12}^{2-} core.[5] This agent exhibited excellent persistence within tumor tissue due to the enhanced permeability and retention (EPR) effect.

Production of a targeted drug delivery agent requires the presence of heterofunctionalized linker arms on the same scaffold to permit dual attachment of payload molecules and the targeting moiety. A targeted version of the Gd^{3+}-chelated closomer has been successfully produced in which differentiation of a single B−H vertex in the B_{12}^{2-} cage was achieved by monoetherification of a single verb−OH vertex of [*closo*-$B_{12}(OH)_{12}$]$^{2-}$ and functionalization of the remaining 11 vertices to ester or carbonate groups.[6,7] This differentiation of a single vertex permitted attachment of an integrin-targeting moiety. The resulting targeted Gd^{3+}-chelated closomer was found to accumulate to higher levels in mice bearing human PC-3 prostate cancer xenografts than the corresponding nontargeted compound.[7]

Although the monoether derivative of [*closo*-$B_{12}(OH)_{12}$]$^{2-}$ is useful in construction of targeted delivery systems, its synthesis and purification are tedious—yields are only 35% after multiple purifications by size-exclusion chromatography.[6] This drawback prompted us to investigate a more efficient and convenient method for generating vertex-differentiated closomers. The first requirement was identifying a [*closo*-$B_{12}H_{11}(X)_1$]$^{2-}$ derivative capable of withstanding the harsh initial hydrogen peroxide treatment necessary to convert [*closo*-$B_{12}H_{12}$]$^{2-}$ to [*closo*-$B_{12}(OH)_{12}$]$^{2-}$.

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Of several alternatives examined, one of the most promising candidates was the monosubstituted derivative $[closo\text{-}B_{12}H_{11}NH_3]^-$, whose synthesis was first reported in 1967.[8] Synthesis of $[closo\text{-}B_{12}H_{11}NH_3]^-$ is achieved by reaction of $[closo\text{-}B_{12}H_{12}]^{2-}$ with NH_2OSO_3H, hydroxylamine-*O*-sulfonic acid. Under the conditions necessary for hydroxylation of the B−H vertices, the amino group of $[closo\text{-}B_{12}H_{11}NH_3]^-$ survives and is oxidized to form the nitro derivative $[closo\text{-}B_{12}(OH)_{11}NO_2]^{2-}$. The nitro group is easily reduced back to the amino form to permit further derivatization.

This paper describes for the first time the synthesis of $[closo\text{-}B_{12}(OH)_{11}NH_3]^-$ and also reports its use in the preparation of platinum-carrying prodrug to demonstrate its utility in producing vertex-differentiated scaffolds for drug delivery applications.

Platinum-based drugs are used as first-line chemotherapy agents for a wide variety of cancers such as small-cell lung cancer (SCLC), ovarian, testicular, and bladder cancer.[9] These drugs work by intercalating into DNA of tumor cells leading to inhibition of DNA replication and RNA translation.[10] Typically, platinum intracellular uptake is facilitated by transport proteins such as copper transport proteins (CTP), the absence or deficiency of which results in reduced intracellular uptake of carboplatin or other platinum based drugs.[11] Some of these shortcomings can be mitigated by the use of macromolecular delivery systems via dendrimers, gold nanoparticles, and other inorganic nanosystems.[12]

The platinated prodrug reported in this paper possesses carboplatin analogues attached to 11 of the B_{12}^{2-} vertices via carbamate linkages and a fluorescein molecule attached to the remaining vertex via an amide linkage (Figure 1). Subsequent

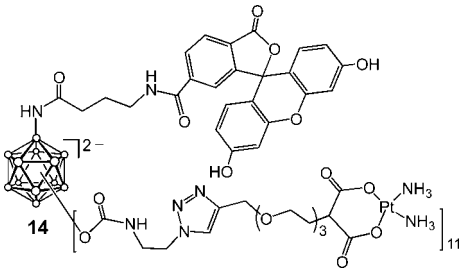


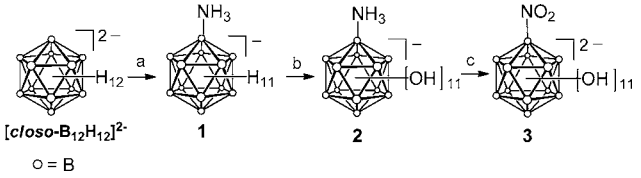
Figure 1. Chemically differentiated closomer carrying eleven platinum prodrugs.

cytotoxicity and intracellular localization studies with platinated closomer and fluorescein conjugated platinated closomer, respectively, are presented in this paper. In vitro cytotoxicity studies demonstrated that activity of the platinum-complexed closomer was equivalent to carboplatin against A459 cells, but it is almost twice that of carboplatin against platinum-resistant SK-OV-3 cells. Fluorescence microscopy of A459 cancer cells following incubation with the fluorescein-linked platinated closomer confirmed localization of closomers within cell nuclei.

RESULTS AND DISCUSSION

Chemistry. Synthesis of the vertex-differentiated closomer was initiated by the reaction of $[closo\text{-}B_{12}H_{12}]^{2-}$ with NH_2OSO_3H to give the $[closo\text{-}B_{12}H_{11}NH_3]^-$ ion (**1**) in 60% yield, which was isolated as a cesium salt (Scheme 1). The remaining 11 boron vertices of compound **1** were hydroxylated by refluxing with 30% hydrogen peroxide using a previously

Scheme 1. Synthesis of the Vertex-Differentiated B_{12}^{2-} Scaffold[a]



[a]Reagents and conditions: (a) NH_2OSO_3H, 80 °C; (b) H_2O_2, reflux; (c) H_2O_2, CsOH pH 8−10, reflux.

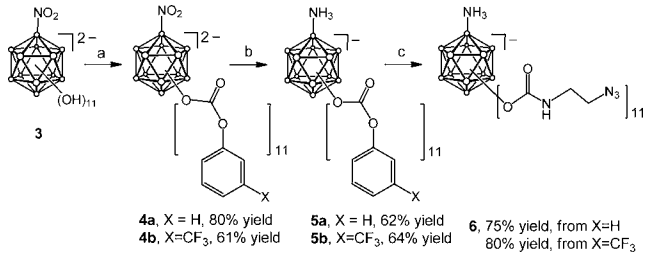
described procedure[3] to give $Cs[closo\text{-}B_{12}(OH)_{11}NH_3]$ (**2**) in 73% yield.

Functionalization of the 11 remaining hydroxyl groups required conversion of compound **2** to an organically soluble salt; however, the extreme aqueous insolubility of the cesium salt of compound **2** precluded this conversion. We observed that during the reaction of compound **1** with hydrogen peroxide the amino group oxidized to produce the nitro derivative $Cs_2[closo\text{-}B_{12}(OH)_{11}NO_2]$ (**3**) that was highly water-soluble. Therefore, the reaction was modified and optimized to isolate nitro derivative **3** instead of the amino derivative **2** by adding cesium hydroxide and adjusting the pH to 8 during the reaction (see the Experimental Section). This permitted ion exchange using Dowex 50 × 8 resin to produce the tetrabutylammonium salt of derivative **3**, which is soluble in organic solvents.

The structure of the derivative **3**, the $[closo\text{-}B_{12}(OH)_{11}NO_2]^{2-}$ anion, was confirmed by single-crystal X-ray diffraction analysis of its tetraphenylphosphonium salt, $[PPh_4]_2[closo\text{-}B_{12}(OH)_{11}NO_2]$ (see the Supporting Information for details).

The hydroxylated nitro derivative **3** was used as the starting material for the synthesis of a closomer-based platinum drug delivery system. As shown in Scheme 2, the hydroxyl groups

Scheme 2. Synthesis of the Amino B_{12}^{2-} Closomer[a]



[a]Reagents and conditions: (a) phenyl chloroformate, pyridine, CH_3CN, 80 °C, 24 h; (b) Raney nickel, 70 bar H_2, MeOH, 40 °C, 24 h; (c) 2-azidoethanamine, CH_3CN, 40 °C, 72 h.

first were converted to phenyl carbonates using our recently reported procedure.[4f] The reaction of $[Bu_4N]_2[closo\text{-}B_{12}(OH)_{11}NO_2]$ with phenyl chloroformate gave the corresponding carbonate **4a** in 80% yield; likewise, its reaction with *m*-CF_3-phenyl chloroformate gave the carbonate **4b** in in 61% yield. The choice of phenyl and *m*-CF_3-phenyl groups was based on their stability toward Raney nickel-catalyzed hydrogenation, which was used for reduction of the nitro group to recover the ammonio form of the closomer. Compounds **4a** and **4b** were stirred at 40 °C at 70 bar of hydrogen for 24 h to give the corresponding amino compounds **5a** and **5b** in 62%

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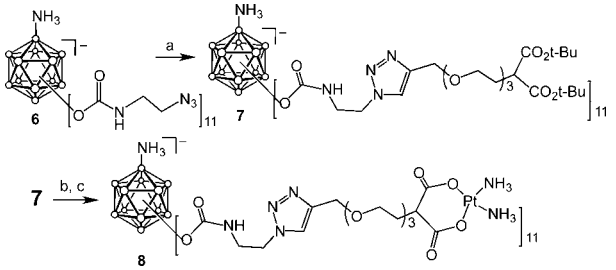
and 64% yield, respectively. ^{11}B NMR of the reaction mixture showed an upfield shift from −21 ppm to −27 ppm, corresponding to the B−NH_3 vertex. The Raney nickel process was found to be superior to other catalysts such as Pd/C or Pt for this conversion.

We have previously reported that carbamate closomers can be prepared by reaction of aryl carbonates with primary amines.[4f] Using this method, the 11 carbonates of compounds **5a** and **5b** were readily converted to carbamate groups by reaction with a 100-fold excess of 2-azidoethanamine. Carbamate **6** was obtained in 75% yield from **5a** at 40 °C and in 80% yield from **5b** at rt. Compound **6** was purified by size-exclusion chromatography on Lipophilic Sephadex LH-20 resin and characterized by NMR and MS analysis.

Next, a click chemistry approach[13] was used to attach alkyne-terminated hydrophilic PEG linkers containing a *t*-Bu-protected dicarboxylate side chain at their distal ends to the 11 azido groups of **6** (Scheme 3). The reaction was conducted using catalytic amounts of $CuSO_4 \times 5H_2O$ and sodium ascorbate to produce the 11-fold triazole-linked closomer **7** in 48% yield.

Scheme 3. Synthesis of the Pt-Complexed Closomer 8[a]



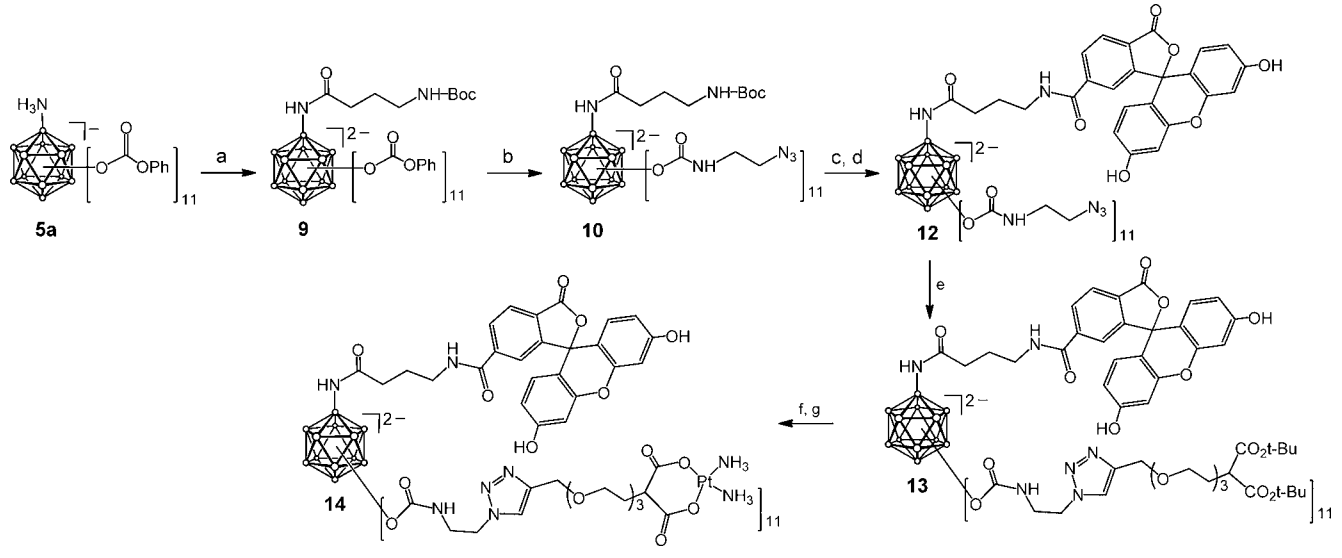
[a]Reagents and conditions: (a) alkyne linker, $CuSO_4 \cdot 5H_2O$, sodium ascorbate, TBTA, MeOH/CH_2Cl_2, rt, 72 h, 48%; (b) TFA, rt, 16 h, 97%; (c) 1 M NaOH, pH 7, $[Pt(NH_3)_2(H_2O)_2](NO_3)_2$, H_2O, rt, 16 h, 34%.

After deprotection of the dicarboxylate ligands of **7** using TFA and titration of the reaction mixture to pH 7 using 1 M NaOH, a 36-fold excess of aqueous *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ was added. Following dialysis to remove excess uncomplexed Pt, the solution was lyophilized and closomer-platinum complex **8** was obtained as white powder in 34% yield.

^{195}Pt NMR spectroscopy[1d] confirmed the formation of platinum complexes, showing a substantial −142 ppm chemical shift from δ = −1580 ppm for the starting material *cis*-$[Pt(NH_3)_2(H2O)_2](NO_3)_2$ to δ = −1722 ppm for closomer **8**. A platinum-release study of closomer **8** in PBS buffer indicated that the complex is stable at physiological pH and temperature (see the Supporting Information for details).

To permit observation of in vitro uptake of platinum-closomer complexes by tumor cells using fluorescence microscopy, reactions were devised to attach a fluorescein molecule to the closomer via linkage at the B(1) amino group. Further derivatization at this location requires activation of the ammonio group, which is challenging due to its strong basic character. Treatment with strong bases such as NaOH or KOH is usually necessary,[14] but this approach was not feasible given the delicate nature of the carbonate and carbamate functionalities present on the other 11 vertices. Sodium hydride, NaOMe, *N*,*N*-diisopropylethylamine, and DBU were tried. Of these, only DBU, which is a strong organic base, was effective. Thus, the deprotonation of the ammonio group of the phenyl carbonate salt **5a** was accomplished with DBU in situ, and a subsequent reaction with *N*-hydroxysuccinate-activated 6-amino-Boc butyric acid gave the conjugated carbonate closomer **9** in 86% yield (Scheme 4). In order to prevent intramolecular carbonate formation, an excess (6 equiv) of amino butyric acid was used. Employing the same method used for the synthesis of closomer **6** (Scheme 2, reaction c), closomer **9** was reacted with 2-aminoethyl azide (Scheme 4, reaction b) to give the azido carbamate closomer **10** in 76% yield.

Scheme 4. Synthesis of the Fluorescein Conjugated Closomer 14[a]



[a]Reagents and conditions: (a) ^{t}Boc-GABA-NHS ester, DBU, CH_3CN, rt, 24 h, 86%; (b) 2-azidoethanamine, CH_3CN, 40 °C, 72 h, 76%; (c) 80% TFA, 40 min, 98%; (d) 5(6)-carboxyfluorescein succinimidyl ester, Et_3N, CH_2Cl_2, 66%; (e) $CuSO_4 \times 5H_2O$, sodium ascorbate, TBTA, MeOH/CH_2Cl_2, rt, 72 h, 52%; (f) TFA, rt, 16h, 97%; (g) 1 M NaOH, pH 7, $[Pt(NH_3)_2(H_2O)_2](NO_3)_2$, H_2O, rt, 16 h, 46%.

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Boc deprotection of the primary amine in azido carbamate closomer **10** was achieved using 80% TFA in CH_2Cl_2[15] to give closomer **11** in quantitative yield (Scheme 4). Following washing with diethyl ether to remove excess TFA, **11** was reacted with commercially available 5(6)-carboxyfluorescein *N*-hydroxysuccinimide ester to give fluorescein-labeled closomer **12** in 66% yield.[16] The click reaction protocol used for synthesis of closomer **7** was similarly employed for linkage of PEGylated ligands to the 11 azido groups in closomer **12** to give closomer **13** in 52% yield (Scheme 4). Closomer **13** was purified by Sephadex LH-20 column chromatography, and its structure was confirmed by ^{1}H NMR and MS analysis.

The *t*-Bu protective groups of closomer **13** were cleaved with TFA, and the solution was brought to neutral pH by titration with 1 M NaOH. Platinum complexation at the 11 vertices was achieved using 36-fold excess *cis*-$[Pt(NH_3)_2(H_2O)_2](NO_3)_2$ in water. The solution was dialyzed to remove excess free platinum complex and then lyophilized. Fluorescein-conjugated, platinum-complexed closomer **14** was isolated in 46% yield. The structure was confirmed by NMR and MS analysis, and the purity was confirmed by reversed-phase HPLC analysis (see the Supporting Information text for details).

In Vitro Studies To Assess Cytotoxicity and Uptake of Platinum-Complexed Closomers. The structural similarities of closomers **8** and **14** with dendritic macromolecules[12] suggested they may be passively transported across cell membranes via an endosomal pathway to permit subsequent delivery of platinum into the cells.[17]

Since these 11 platinum prodrugs on closomer are structurally similar to carboplatin, we compared in vitro cytotoxicity of closomer **8** with carboplatin.

In vitro experiments were conducted in Pt-susceptible lung cancer cell line A549 and the Pt-resistant ovarian cancer line SK-0 V-3, which has reduced copper transporter-1 expression.[11a] Cells were incubated for 72 h with either closomer **8** or carboplatin at varying concentrations (1−100 μM). The concentration of closomer **8** was adjusted relative to carboplatin so that cells received equivalent amounts of platinum.

MTT assays following platinum treatment showed that cytotoxic activity of closomer **8** against A549 cells was equivalent to carboplatin (Figure 2). The IC_{50} value for closomer **8** was 2.96 μM, which was comparable to the IC50 of 3.2 μM obtained for carboplatin (Figure 4).

On the other hand, closomer **8** inhibited the proliferation of SK-OV-3 cells significantly higher than carboplatin (Figure 3). However, the IC_{50} values were higher compared to A549 cells line (22.5 μM for closomer **8** and 41.03 μM for carboplatin as shown in Figure 4), suggesting that these compounds share a similar pathway of cellular uptake, but closomer **8** is able to break the platinum resistance more efficiently than carboplatin and hence shows significantly higher inhibitory effects on SK-OV-3 proliferation (Figure 4).

Fluorescence microscopic intracellular localization studies of fluorescent closomer **14** were conducted by incubating A549 cells with 100 μM of closomer **14** for 3 h. Analysis of cells under fluorescent microscope revealed that closomer **14** colocalized with a nuclear staining dye (DAPI) in A549 cells, confirming nuclear localization of closomer **14** in these cells (Figure 5a−c).

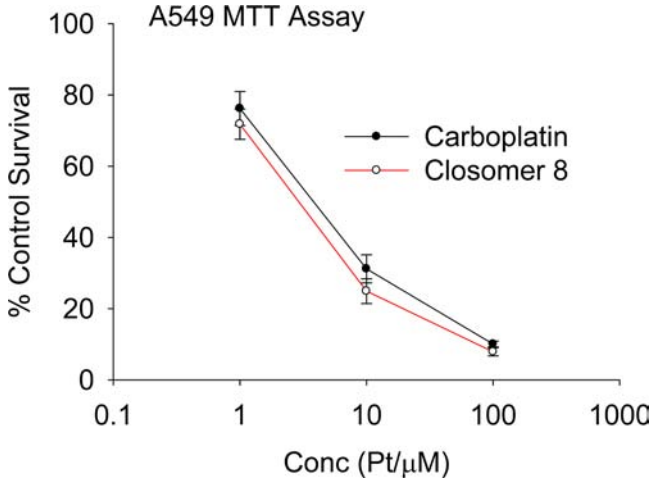

Figure 2. MTT assay of A549 cells treated with closomer **8** or carboplatin with equivalent platinum concentration. A549 cells were cultured overnight in 96-well plates at 37 °C followed by treatment with different doses of closomer **8** and carboplatin (1−100 μM) for 72 h. Graphs were plotted as % control survival. SDM, closomer **8** vs carboplatin (1−100 μM) $P > 0.05$.

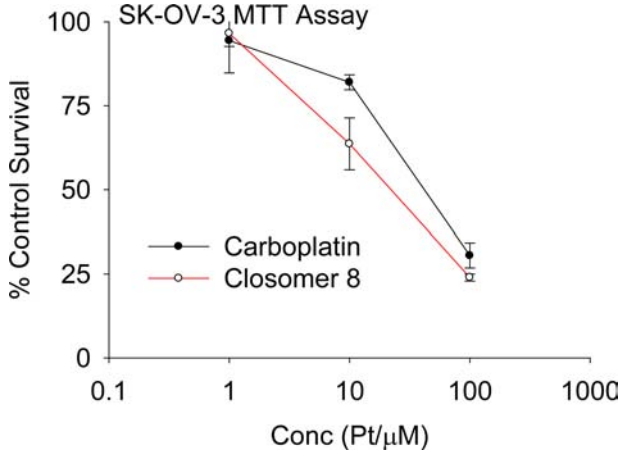

Figure 3. MTT assay of SK-OV-3 cells at equivalent platinum concentration. SK-OV-3 cells were cultured overnight in 96-well plates at 37 °C and treated with different doses of closomer **8** and carboplatin (1−100 μM) for 72 h. Graphs were plotted as % control survival. SDM, carboplatin vs closomer **8** 1 μM $P = 0.577$, carboplatin vs closomer **8** 10 μM $P = 0.003$, carboplatin vs closomer **8** 100 μM $P = 0.01$.

CONCLUSIONS

This study reports the synthesis and utility of a unique vertex differentiated icosahedral B_{12}^{2-} scaffold, [*closo*-$B_{12}(OH)_{11}NH_3]^-$ that can be used for nanomolecular drug delivery applications. Production of [*closo*-$B_{12}(OH)_{11}NH_3]^-$ is achieved by reduction of [*closo*-$B_{12}(OH)_{11}NO_2]^{2-}$ using a Raney nickel catalyst. Attachment of various moieties to the backbone can confer targeting and diagnostic properties to the closomer. Attachment of azido functionalities at 11 B−OH vertices permits reaction with alkyne terminated linkers by click chemistry to attach desired therapeutic moieties. The amino group at the remaining vertex can be used to conjugate a targeting peptide or antibody or a tracer moiety. To demonstrate the utility of this heterobifunctionalized closomer as a starting platform for preparation of cancer therapeutic agents, we used it to generate a platinated pro-drug carrying 11 carboplatin analogues. This closomer possesses in vitro toxicity against both A459, a platinum-sensitive line, and against the platinum-resistant ovarian cancer cell line SK-OV-3.

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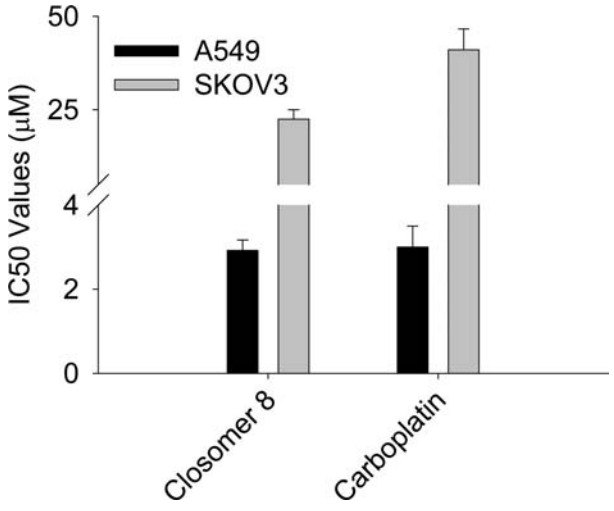

Figure 4. IC50 values for A549 and SK-OV-3 cells obtained by treatment of cells with closomer **8** and carboplatin.

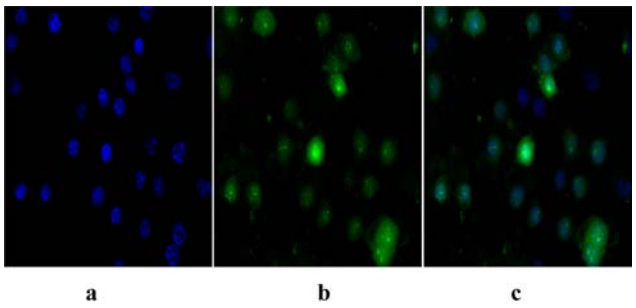

Figure 5. Intracellular localization of closomer **14** in A549 cells by fluorescence microscopy: (a) blue, DAPI-nuclear stain; (b) green, closomer **14**; (c) light blue (teal), merged images. A549 cells were cultured overnight in eight-well chamber slides at 37 °C followed by incubation with closomer **14** (100 μM) for 3 h.

EXPERIMENTAL SECTION

General Considerations. Standard Schlenk-line techniques were employed for all manipulations of air- and moisture-sensitive compounds. All solvents were purchased from VWR and used as received. $Cs_2[closo\text{-}B_{12}H_{12}]$ was purchased from BASF SE, and carboplatin was purchased from Strem (Newburyport, MA). Lipophilic Sephadex LH-20 was obtained from GE Healthcare. ^{1}H, ^{13}C, and ^{11}B NMR and ^{195}Pt spectra were recorded at rt on Bruker Avance 400 or 500 MHz spectrometers. The ^{11}B spectra were externally referenced to $BF_3{\cdot}Et_20$ while ^{1}H and ^{13}C spectra were referenced to residual solvent peaks. The ^{195}Pt spectra were externally referenced to Na_2PtCl_6. Mass spectrometry was conducted at a Mariner Biospectrometry Workstation. The high resolution mass spectrometry analysis was performed using an Applied Biosystems Mariner ESI-TOF. IR spectra were recorded on a Nicolet Nexus 470 FT-IR spectrometer using KBr pellets. Platinum concentrations of samples were measured by inductively coupled plasma optical emission spectroscopy (ICP-OES) using a PekinElmer OptimaTM 7000 DV instrument.

Synthesis of Closomers and Linkers. *Cs[closo-$B_{12}H_{11}NH_3$] (1).* A suspension of $Cs_2[closo\text{-}B_{12}H_{12}]$ (25.0 g, 0.061 mol) in water (150 mL) was heated to reflux. After all starting material was dissolved, an aqueous solution (20 mL) of hydroxylamine-*O*-sulfonic acid (13.9 g, 0.123 mol) was added to the reaction over a 1-h period. The reaction was stirred at reflux for another 2 h. The solution was concentrated to 30 mL and cooled to 4 °C overnight, with the crude product precipitating out as white crystals. NMR analysis revealed that the crude product contained unreacted starting material $Cs_2[closo\text{-}B_{12}H_{12}]$ as well as the diamino borane $closo\text{-}B_{12}H_{10}(NH_3)_2$ as a side product. The diamino borane was removed by methanol washes (40 mL × 3). Following the methanol washes, the mixture was stirred in acetonitrile (200 mL × 2) and filtered. $Cs_2[closo\text{-}B_{12}H_{12}]$ (5 g, 20%) was recovered as an insoluble solid. The desired complex $Cs[closo\text{-}B_{12}H_{11}NH_3]$ (10.0 g, 60%) was obtained from the filtrate as a white solid. ^{1}H NMR (400 MHz, CD_3CN): δ_H 4.70 (br, 3H), 0.48−1.63 (m, 11H). $^{11}B\{^{1}H\}$ NMR (161 MHz, D_2O): δ_B −6.36, −15.47, −16.35, −19.68. IR (cm^{-1}) ν = 3200−3300 (2 bands, N-H), 2500 (B-H), 1400 (N-H). MS (TIS): *m/z* calcd for $[B_{12}H_{14}N]^-$ (M^-) 158.2310, found 158.2341.

Cs[closo-$B_{12}(OH)_{11}NH_3$] (2). $Cs[closo\text{-}B_{12}H_{11}NH_3]$, **1** (8.0 g, 27.5 mmol), was dissolved in 100 mL of 30% H_2O_2, and the reaction was slowly heated to reflux with vigorous stirring. ^{11}B NMR was conducted daily to monitor the reaction progress. A solution of 30% H_2O_2 (30 mL) was added to the reaction every other day until the reaction became cloudy on the fourth or fifth day. After this point, the volume of H_2O_2 added to the reaction was increased to 60−80 mL every other day until the ^{11}B NMR signals beyond −32 ppm disappeared. The reaction required about 400 mL of 30% H_2O_2 total and was complete in 10 days. After completion, the mixture was cooled to rt. Crude product was separated by filtration and washed with hot water (50 mL × 4, 50 °C) to give compound **2** as a white solid (9.4 g, 73%). $^{11}B\{^{1}H\}$ NMR (161 MHz, D_2O, ppm): δ_B −14.78, −17.19, −17.66, −29.41. IR (cm^{-1}) ν = 2710−3700 (O-H and N-H), 1400 (N-H), 990−1310 (O-H and B-O). MS (TIS): *m/z* calcd for $[B_{12}H_{14}NO_{11}]^-$ (M^-) 334.1752, found 334.1562.

[Bu_4N]$_2$[closo-$B_{12}(OH)_{11}NO_2$] (3). Method 1. To compound $Cs[closo\text{-}B_{12}(OH)_{11}NH_3]$, **2** (1.80 g, 3.86 mmol), were added 30% H_2O_2 (20 mL) and 50% CsOH (30 drops. The suspension was slowly heated to reflux with vigorous stirring. The pH and H_2O_2 content of the reaction mixture were assessed daily, and reaction progress was also monitored daily using ^{11}B NMR. Additinal 50% CsOH was added dropwise as necessary to maintain the pH between 8 and 10. When H_2O_2 levels were found to have declined or reaction progress was observed to slow, additional 30% H_2O_2 was added to the reaction (20−40 mL per addition). Monitoring and addition of CsOH and H_2O_2 continued until the solution became completely clear and the ^{11}B NMR spectrum indicated that no compound **2** remained. Typically, reactions were complete by 7 d and required a total of 240 mL of 30% H_2O_2 (20 mL/day × 3 days and 40 mL/day × 4 days).

After completion of the reaction, the crude mixture was checked for H_2O_2 using KI/starch test paper. Any remaining H_2O_2 was destroyed by heating at reflux for an additional 2 days. Crude $Cs_2[closo\text{-}B_{12}(OH)_{11}NO_2]$ was precipitated as a yellow solid by adding the reaction mixture to 400 mL of MeOH. This yellow solid was separated and dissolved in hot water (50 °C, 150 mL). The solution was filtered, and the filtrate was concentrated and cation-exchanged[4] to the tetrabutylammonium salt of $[Bu_4N]_2[closo\text{-}B_{12}(OH)_{11}NO_2]$ (2.5 g, 78%).

Method 2. To compound $Cs[closo\text{-}B_{12}H_{11}NH_3]$, **1** (1.12g, 3.85 mmol), was added 30% H_2O_2 (40 mL), and the reaction mixture was slowly heated to reflux with vigorous stirring. On the third day, a precipitate started to form, and a 50% CsOH solution was added dropwise until the pH of the reaction mixture was 8. The pH was maintained between 8 and 10 with 50% CsOH, and H_2O_2 was added daily (20 mL/day × 2 days followed by 30 mL/day × 5 days) until the solution became completely clear. At this point, the crude mixture was checked for H_2O_2 using KI/starch test paper. Any remaining H_2O_2 was destroyed by heating the mixture at reflux for an additional 2 d. The crude product was precipitated and purified as described in method 1. The $Cs_2[closo\text{-}B_{12}(OH)_{11}NO_2]$ was then cation-exchanged to the $[Bu_4N]_2[closo\text{-}B_{12}(OH)_{11}NO_2]$ (2.3g, 70%). ^{1}H NMR (500 MHz, D_2O): δ_H 3.20−3.23 (m, 16H), 1.67 (m, 16H), 1.36−1.40 (m, 16H), 0.96 (t, 24H). $^{11}B\{^{1}H\}$ NMR (161 MHz, D_2O): δ_B −12.49, −16.71, −22.24. MS (TIS): *m/z* calcd for $[B_{12}H_{11}NO_{13}]^{2-}$ (M^{2-}) 181.5705, found 181.5754; $[B_{12}C_{16}H_{47}NO_{13}]^-$ ($[M + Bu_4N]^-$) 605.4274, found 605.3857.

[Bu_4N]$_2$[closo-$B_{12}(OCO_2C_6H_5)_{11}NO_2$] (4a). Under argon protection, a solution of phenyl chloroformate (0.96 g, 6.18 mmol) in 15 mL of anhydrous CH_3CN was added to a solution anhydrous pyridine (0.49 g, 6.20 mmol) and $[Bu_4N]_2[closo\text{-}B_{12}(OH)_{11}NO_2]$ (0.094 g, 0.11

mmol) in 15 mL of anhydrous CH_3CN. The resulting suspension was heated to reflux and stirred overnight. After completion of the reaction, the crude mixture was cooled to rt and the solvent removed. The residue was subjected to size-exclusion chromatography over Sephadex LH-20. All boron-containing portions were collected and concentrated. This crude product was then purified by silica gel column chromatography. Compound **4a** (0.19 g, 80%) was isolated by elution with a gradient solvent system of CH_2Cl_2/CH_3CN mixtures (v/v 99/1 to 5/1). ^{1}H NMR (500 MHz, CD_2Cl_2): δ_H 7.25−7.05 (m, 55H), 3.06−3.02 (m, 16H), 1.43−1.41 (m, 16H), 1.30−1.24 (m, 16H), 0.79 (t, 24H). $^{11}B\{^1H\}$ NMR (161 MHz, CD_2Cl_2): δ_B −14.9 (1B), −16.9 (10B), −21.6 (1B). MS (TIS): m/z calcd for $[B_{12}C_{77}H_{55}NO_{35}]^{2-}$ (M^{2-}) 842.1888, found 842.1487.

*$[Bu_4N]_2$[closo-$B_{12}(OCO_2C_6H_4CF_3)_{11}NO_2$] (**4b**).* Under argon protection, a solution of *m*-trifluoromethyl phenyl chloroformate (1.60 g, 7.13 mmol) in CH_3CN (20 mL) was added to a solution of anhydrous pyridine (0.56 g, 7.08 mmol) and $[Bu_4N]_2$[*closo*-$B_{12}(OH)_{11}NO_2$], **3**, (0.105 g, 0.12 mmol) in CH_3CN (20 mL). The mixture was stirred at rt overnight under argon. The solvent was removed by rotary evaporation, and the residue was purified in a manner identical to compound **4a** to give compound **4b** (220 mg, 61%) as a colorless oil. ^{1}H NMR (400 MHz, CD_2Cl_2): δ_H 7.24−7.43 (m, 44H), 3.00−3.04 (m, 16H), 1.45−1.52 (m, 16H), 1.24−1.33 (m, 16H), 0.83 (t, 24H). $^{11}B\{^1H\}$ NMR (128 MHz, CD_2Cl_2): δ_B −14.50 (1B), −16.92 (10B), −21.94 (1B). MS (TIS): m/z calcd for $[B_{12}C_{88}H_{44}F_{33}NO_{35}]^{2-}$ (M^{2-}) 1216.1189, found 1216.0248.

*$[Bu_4N]_2$[closo-$B_{12}(OCO_2C_6H_5)_{11}NH_3$] (**5a**).* Compound **4a** (0.084 g, 0.039 mmol) was dissolved in CH_2Cl_2 (1 mL) and diluted with CH_3OH (50 mL) in a 300-mL reaction vessel. Raney nickel catalyst (0.15 mL) was added to the solution, and the mixture was pressurized with H_2 to 70 bar and the temperature increased to 40 °C. The reaction was allowed to proceed under these conditions for 24 h. Assay of the crude product by mass spectrometry indicated the complete absence of compound **4a** at 24 h. The reaction was stopped, and the solvents were removed in vacuo. The product was purified by silica gel column chromatography using CH_2Cl_2/CH_3CN (v/v: 50/1) as the eluent. All boron-containing fractions were combined and dried to give compound **5a** as a white solid (0.045 g, 62%). ^{1}H NMR (400 MHz, CD_2Cl_2): δ_H 7.31−6.64 (m, 55H), 6.91 (br, 1H), 6.78 (br, 1H), 6.64 (br, 1H), 3.08−3.04 (m, 8H), 1.57−1.50 (m, 8H), 1.37−1.31 (m, 8H), 0.96 (t, 12H). $^{13}C\{H\}$ NMR (100 MHz, CD_2Cl_2): δ_C 152.2, 152.0, 128.9, 125.3, 121.9, 58.1, 23.6, 19.3, 13.3. $^{11}B\{^1H\}$ NMR (128 MHz, CD_2Cl_2): δ_B −16.6 (1B), −27.3 (11B) MS (TIS): m/z calcd for $[B_{12}C_{77}H_{58}NO_{33}]^-$ (M^-) 1654.4080, found 1654.1667.

*$[Bu_4N]_2$[closo-$B_{12}(OCO_2C_6H_4CF_3)_{11}NH_3$] (**5b**).* Compound **4b** (0.12 g, 0.041 mmol) was dissolved in CH_2Cl_2 (1 mL) and diluted with CH_3OH (50 mL) in a 300-mL reaction vessel. Raney nickel catalyst (0.15 mL) was added to the solution, and the reaction mixture was pressurized with H_2 to 70 bar and maintained at 40 °C overnight. After removal of the solvent, the crude product was purified by silica gel column chromatography using CH_2Cl_2/ CH_3CN (v/v: 50/1) as the eluent. All boron-containing fractions were combined and dried to give compound **5b** as a pale yellow oil (0.07 g, 64%). ^{1}H NMR (500 MHz, CD_2Cl_2): δ_H 7.25−7.49 (m, 44H), 6.80 (br, 1H), 6.70 (br, 1H), 6.60 (br, 1H), 3.03−3.06 (m, 8H), 1.53−1.60 (m, 8H), 1.37−1.42 (m, 8H), 1.00 (t, 12H). $^{11}B\{^1H\}$ NMR (128 MHz, CD_2Cl_2): δ_B −16.52 (11B), −27.29 (1B) MS (TIS): m/z calcd for $[B_{12}C_{88}H_{47}F_{33}NO_{33}]^-$ (M^-) 2402.2736, found 2402.1551.

*$[Bu_4N]$[closo-$B_{12}(O_2CNHCH_2CH_2N_3)_{11}NH_3$] (**6**).* Closomer **5a** (0.417 g, 0.158 mmol) or closomer **5b** (0.3 g, 0.158 mmol) was combined with acetonitrile (1 mL) and 2-azidoethylamine in a Schlenk tube. The mixture was stirred for 3 d at either 40 °C for **5a** or rt for **5b**. After completion of the reaction, volatiles were removed by rotary evaporation. Purification by lipophilic Sephadex LH-20 column chromatography using methanol as the eluent yielded 0.214 g (75%) of **6** starting from **5a** and 0.228 g (80%) starting from **5b**. ^{1}H NMR (500 MHz, CD_2Cl_2): δ_H 7.34 (br, 1H), 7.19 (br, 1H), 7.06 (br, 1H), 5.01 (s, 11H), 3.46−3.23 (m, br, 52H), 1.68−1.66 (m, 8H), 1.46−1.41 (m, 8H), 1.05 (t, 12H). $^{13}C\{H\}$ NMR (100 MHz, CD_2Cl_2): δ_C 156.8, 59.6, 51.6, 41.5, 24.7, 20.4, 14.4. ^{11}B NMR (161 MHz, CD_2Cl_2): δ_B −16.7 (11B), −27.5 (1B). MS (TIS): m/z calcd for $[B_{12}O_{22}C_{33}N_{45}H_{58}]^-$ (M^-) 1567.5956, found 1567.4906

Di-tert-butyl 2-(2-(2-(2-(Prop-2-yn-1-yloxy)ethoxy)ethoxy)ethyl)-malonate Linker. NaH (0.38 g, 16 mmol) was suspended in dry THF (100 mL), and dry di-*tert*-butyl malonate (4.4 mL, 20 mmol) was added to the reaction mixture with stirring until gas evolution ceased. A solution of 3-(2-(2-(2-iodoethoxy)ethoxy)ethoxy)prop-1-yne[18] (2.98 g, 10 mmol) was added dropwise to the reaction mixture, followed by stirring overnight. The THF was removed by evaporation, and the product was redissolved in CH_2Cl_2 and washed with 5% aqueous acetic acid. The organic fractions were combined, dried over $MgSO_4$, and concentrated. The final product was purified by silica gel column chromatography (using 95/5 = hexane/ethyl acetate (v/v) as the eluent). The purified product was isolated as a colorless oil (3.09 g, 80%). ^{1}H NMR (500 MHz, $CDCl_3$: δ_H 4.11 (d, 2H), 3.63−3.48 (m, 4H), 3.53 (m, 4H), 3.27 (t, 3H), 2.43 (t, 1H), 2.07 (q, 2H), 1.44 (s, 18H). $^{13}C\{H\}$ NMR (125 MHz, CD_2Cl_2): δ_C 168.6, 81.1, 79.5, 77.4, 77.1, 76.9, 74.5, 70.4, 70.3, 70.1, 68.9, 68.4, 58.2, 50.7, 28.6, 27.8. MS (TIS): m/z calcd for $[C_{20}H_{34}O_7Na]^+$ (M + Na^+) 409.2175, found 409.1574.

*Closomer **7**.* To a solution 0.123 g (0.062 mmol) of **6** and 0.957 g (2.48 mmol) di-*tert*-butyl 2-(2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)-ethoxy)ethyl)malonate in CH_2Cl_2 (5 mL) was added a solution of $CuSO_4{\cdot}H_2O$ (0.015 g, (0.062 mmol), sodium ascorbate (0.037 g, 0.186 mmol), and TBTA [tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)-methyl]amine] (0.065 g, 0.124 mmol) in methanol (5 mL). The mixture was vigorously stirred at rt for 3 d under argon. After completion, the reaction mixture was filtered through a Celite pad. The filtrate was concentrated and subjected to Sephadex LH-20 size-exclusion column chromatography with methanol as the eluent obtain the pure product 7 as a white solid (0.173 g, 48%). ^{1}H NMR (500 MHz, CD_2Cl_2): δ_H 8.20 (s, br, 11H), 4.60 (s, br, 11H), 3.69−3.33 (m, 176H), 3.32 (s, 11H), 2.00 (s, 22H), 1.47 (s, 198H). $^{13}C\{H\}$ NMR (125 MHz, CD_2Cl_2): δ_C 168.5, 156.2, 144.4, 124.1, 81.1, 70.4, 70.3, 70.1, 68.4, 66.7, 64.7, 50.8, 49.6, 41.6,35.1, 28.7, 27.6. ^{11}B NMR (161 MHz, CD_2Cl_2): δ_B −17.4 (11B), −26.6 (1B). MS (TIS): m/z calcd for $[C_{253}H_{432}B_{12}N_{45}O_{99}]^-$ (M^-) 5817.1409, found 5817.5415; $[C_{253}H_{432}B_{12}N_{45}O_{99}K]^{2-}$ (M + K)$^{2-}$ 2927.0381, found 2927.6770.

*Closomer **8**.* Compound 7 (0.150 g, 0.025 mmol) was dissolved in 80% TFA in CH_2Cl_2 and stirred for 16 h at rt. The solvent was removed under reduced pressure, and the reaction product was washed with Et_2O and dissolved in water. The pH of the solution was adjusted to pH 7 by titration with 1 M NaOH. Under an argon atmosphere, the solution was combined with an in situ prepared aqueous solution of $[Pt(NH_3)_2H_2O_2)](NO_3)_2$ (36-fold excess, 0.9 mmol) and stirred vigorously overnight in darkness. The reaction mixture was then dialyzed in deionized water for 2 d using 1000 MWCO (MWCO = molecular weight cutoff) Spectra/Por dialysis membrane tubing. The product **8** was obtained as an off-white solid after lyophilization (0.060 g, 34%). ^{1}H NMR (500 MHz, D_2O): δ_H 8.38 (s, br, 11H), 4.73 (s,br, 11H), 3.66 (br, 176H), 2.45 (s, 11H), 1.91 (s, br, 11H), 1.41 (s, 11H). $^{13}C\{H\}$ NMR (125 MHz, D_2O): δ_C 179.4, 177.1, 156.8, 145.3, 127.7, 69.6, 68.5, 63.1, 52.0, 40.9, 29.6. ^{11}B NMR (161 MHz, D_2O): δ_B −17.7 (11B), −24.7 (1B). ^{195}Pt (107 MHz, D_2O) δ_{Pt}: −1722. MS (ES): m/z calcd for $[C_{165}H_{300}B_{12}N_{67}O_{99}Pt_{11}Na]$ 1419.7455 $(M)^{-4}$, 1774.9337 $(M)^{-5}$, 1015.8307 $(M)^{+7}$, found 1419.8097 $(M)^{-5}$, 1774.4103 $(M)^{-4}$, 1015.4509 $(M)^{+7}$. ICP-OES analysis for Pt calcd 11, found 10.8.

*Closomer **9**.* Compound **5a** (0.379 g, 0.2 mmol) was combined with DBU (0.364 2.4 mmol) and *N*-Boc-γ-aminobutyric succinimide (0.36 g, 1.2 mmol) in 5 mL of acetonitrile. The mixture was stirred at rt for 24 h. After completion of the reaction, volatiles were removed by rotary evaporation. Lipophilic Sephadex LH-20 size-exclusion column chromatography with methanol as the eluent gave purified closomer **9** as a white solid (0.358 g, 86%). ^{1}H NMR (500 MHz, CD_2Cl_2): δ_H 7.39−7.07 (m, 55H), 3.29−3.18 (m, 8H), 3.06 (m, 4H), 2.48 (m, 2H), 1.76 (m, 8H), 1.74−1.55 (m, 8H), 1.39 (s, 9H), 0.97 (t, 9H). $^{13}C\{H\}$ NMR (125 MHz, CD_2Cl_2): δ_C 178.8, 166.7, 153.1, 152.7, 130.0, 126.4, 122.5, 55.2, 54.8, 48.9, 47.6, 40.1, 39.0, 33.6, 33.3, 29.1, 26.7, 24.1, 19.6, 13.1. ^{11}B NMR (161 MHz, CD_2Cl_2): δ_B −16.7 (11B), −23.5

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(1B). MS (TIS): m/z calcd for $[B_{12}O_{36}C_{86}N_2H_{72}]^{2-}$ (M^{2-}) 919.2527, found 919.7914

Closomer 10. Closomer **9** (0.492 g, 0.237 mmol) was combined with 2-azidoethylamine 2.04 g (0.024 mol) and 1 mL of acetonitrile in a Schlenk tube. The mixture was stirred at 40 °C for 3 d. After completion of the reaction, volatiles were removed by rotary evaporation. Sephadex LH-20 size-exclusion column chromatography with methanol as the eluent was used to purify the product. The boron-containing fractions were combined and dried to give **10** as a yellow solid (0.357 g, 76%). ^{1}H NMR (500 MHz, CD_2Cl_2): δ_H 3.43 (br, 22H), 3.24 (m, 22H), 3.09 (m, 8H), 2.43 (br, 2H), 1.81 (br, 2H), 1.61−1.59 (m, 8H), 1.44 (s, 9H), 1.37−1.35 (m, 8H), 0.98 (s, 12H). ^{13}C{H} NMR (125 MHz, CD_3CN): δ_C 178.6, 166.8, 156.9, 59.1, 54.8, 51.4, 49.2, 41.2, 39.3, 38.8, 32.9, 29.4, 28.6, 27.0, 24.4, 24.1, 20.1, 13.2. ^{11}B NMR (161 MHz, CD_2Cl_2) δ_B: −17.3 (11B), −26.6 (1B). MS (TIS): m/z calcd for $[B_{12}O_{25}C_{42}N_{46}H_{72}]^{2-}$ (M^{2-}) 874.8501, found 874.7748

Closomer 11. Under an argon atmosphere, **10** (0.070 g, 0.035 mmol) was dissolved in anhydrous dichloromethane (2 mL), and TFA (8 mL) was added to the solution. The reaction mixture was stirred for 40 min at ambient temperature. The solution was concentrated under reduced pressure and washed with Et_2O to give **11** as a white powder, which was used without further purification.

Closomer 12. Under an argon atmosphere, to a solution of **7** (0.066 g, 0.035 mmol) in anhydrous CH_2Cl_2 (5 mL) was added Et_3N (0.017g, 0.175 mmol). The solution was cooled to 0 °C, and 5(6)-carboxyfluorescein *N*-hydroxysuccinimide (5(6)-FAM SE) (0.066 g, 0.14 mmol) in CH_2Cl_2 (1 mL) was added. After stirring for 24 h at rt, CH_2Cl_2 was removed under reduced pressure. Lipophilic Sephadex LH-20 size-exclusion column chromotography with methanol as the eluent was used to obtain purified **12**, a yellow powder (0.052 g, 66%). ^{1}H NMR (500 MHz, CD_2Cl_2): δ_H 8.51−7.94 (m, 3H), 7.18 (m, 2H), 6.41 (m, 4H), 4.53 (s, 11H), 3.28 (br, 22H), 3.20 (br, 22H), 2.11 (d, 2H), 1.77 (d, 2H), 1.47 (m, 4H), 1.25 (q, 4H), 0.86 (t, 6H). ^{13}C{H} NMR (125 MHz, CD_3OD): δ_C 174.1, 168.3, 165.3, 164.5, 154.8, 152.8, 152.1, 128.2, 127.6, 127.2, 124.9, 123.8, 123.0 113.5, 112.4, 109.7, 108.9, 56.5, 52.4, 48.9, 44.6, 38.7, 36.4, 34.0, 33.8, 30.80, 27.0, 24.5, 23.8, 22.7, 22.0, 21.83, 17.7, 17.5, 11.0, 6.25. ^{11}B NMR (161 MHz, CD_2Cl_2): δ_B −17.4 (11B), −25.2 (1B). MS (TIS): m/z calcd for $[C_{58}H_{74}B_{12}N_{46}O_{29}]^-$ (M^-) 2010.6846, found 2011.1591; $[B_{12}C_{74}H_{125}N_{46}O_{39}]^{2-}$ (M^{2-}) 1005.3423, found 1005.1149.

Closomer 13. To solution 0.076 g (0.037 mmol) of **12** and 0.582 g (1.48 mmol) coupling reagent in 5 mL CH_2Cl_2 (5 mL) was added $CuSO_4{\cdot}H_2O$ (0.035g, 0.037 mmol), of sodium ascorbate (0.029 g, 0.148 mmol), and of TBTA (0.039 g, 0.074 mmol) in methanol (5 mL). The mixture was vigorously stirred at rt for 3 d under argon. After completion, the reaction mixture was filtered through a Celite pad. The filtrate was concentrated, and size-exclusion column chromatography using Sephadex LH-20 with methanol as the eluent was used to obtain the pure product **13** as a yellow solid (0.120 g, 52%). ^{1}H NMR (500 MHz, CD_2Cl_2): δ_H 8.14 (m, br, 14H), 7.35 (m, 6H), 4.57 (s, br, 11H), 3.63−3.49 (m, 176H), 3.35 (m, 11H), 2.57 (m, 2H), 1.47 (s, 198H). ^{11}B NMR (161 MHz, CD_2Cl_2): δ_B −17.4 (11B), −24.9 (1B). MS (ES): m/z calcd for $[C_{278}H_{448}B_{12}N_{46}O_{106}Na]^{2-}$ (M + Na)$^{2-}$ 3140.0974, found 3140.0496

Closomer 14. Closomer **13** (0.100 g, 0.016 mmol) was dissolved in 80% TFA in CH_2Cl_2. The reaction proceeded 16 h at rt, and the solvent was removed under reduced pressure. The product was washed with Et_2O and dissolved in water. The pH of the solution was adjusted to 7 by titration with 1 M NaOH.

Under an argon atmosphere, this pH 7 solution was mixed with an in situ prepared aqueous solution of $[Pt(NH_3)_2H_2O_2)](NO_3)_2$ (36 fold excess, 0.576 mmol) and stirred vigorously overnight in darkness. The product was purified by dialysis using 1000 MWCO Spectra/Por dialysis membrane tubing and lyophilized to dryness to afford **14** as a yellow powder (0.055 g, 46%). ^{1}H NMR (500 MHz, D_2O): δ_H 8.86 (m, 3H), 8.35 (s, br, 11H), 7.46 (m, 4H), 6.98 (m, 2H), 4.59 (s, br, 11H), 3.67 (m, br, 176H), 3.16 (m, 4H), 2.49 (s, br, 11H), 2.03 (br, 11H), 1.61 (br, 11H), 1.32 (m, 4H), 1.24 (m, 4H), 0.91 (t, 6H). ^{11}B NMR (161 MHz, D_2O): δ_B −17.4 (11B), −24.8 (1B). MS (ES): m/z calcd for $[C_{190}H_{316}B_{12}N_{68}O_{106}Pt_{11}]^-$ (M^-) 7519.86, found 7519.69. ICP-OES analysis for Pt calcd 11, found 10.86.

Cell Culture and Assay for Platinum Cytotoxicity. A549 (ATCC, Manassas, VA) and SK-OV-3 (a kind gift from Dr. Susan Deutscher) cells were maintained in DMEM + 10% FBS, 5% CO2 and humidified atmosphere. When the flasks reached 90% confluence, cells were harvested using TrypLE (Gibco, Grand Island, NY) according to the manufacturer's protocol. Briefly, cells were rinsed using 1XPBS to remove FBS and incubated with TrypLE for 10 min at 37 °C. Cells were then centrifuged at 323*g* for 8 min in a Fisher Scientific Accuspin 3R centrifuge (Pittsburgh, PA). Cell were resuspended in DMEM + 10% FBS and counted in an automatic cell counter (Invitrogen, Grand Island, NY). For cytotoxicity experiments, cells were plated in 96-well plates at a density of 10000 cells/well. Cells in triplicates were treated for 72 h with different doses of closomer **8** or carboplatin (1−100 μM). Platinum concentrations were determined by ICP, and the amount of closomer **8** was adjusted such that carboplatin and closomer **8** treated cells received equivalent doses of platinum at any given treatment concentration. Cytotoxicity was assessed by MTT assay using a commercial kit (Promega, Madison, WI). Following platinum treatment, MTT reagent was added directly to the culture plates, and the reaction was terminated after 4 h using the stop solution provided in the kit. Absorbance of dissolved formazan product was quantitated at 570 nm using a microplate reader. Percent survival was determined as the ratio of the absorbance of treated cells to untreated cells. Each experiment was repeated three times, and Figures 2 and 3 show the average of three experiments.

Intracellular Localization of Closomer 14 in A549 Cells. A459 cells were cultured and harvested as described above. Cells were seeded into an 8-well chamber slide (Corning, Tewksbury MA) at a density of 100000 cells/well and allowed to adhere overnight at 37 °C. Cells were incubated with closomer **14** for 3 h and washed with 1X PBS to remove free closomer. Cells were then mounted with DAPI-containing mounting solution and observed under the Olympus 1 × 70 fluorescence wide-field microscope equipped with an ORCA digital camera (Center Valley, PA). Images were taken at 40X (oil) magnification.

Statistical Analysis. Student t test (Sigma plot) was used for statistical analysis of results. A P value <0.05 was recorded as significant. All P values were at 95% confidence. Graphs were plotted using standard deviation and mean (SDM).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Single-crystal X-ray data for **3**, HPLC chromatograms for **8** and **14**, absorption and fluorescence spectra for **14**, and platinum-release studies in PBS for **8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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