Pergamon

Tetrahedron 55 (1999) 11177–11186

TETRAHEDRON

Synthesis of Boron-Containing Cholesterol Derivatives for Incorporation into Unilamellar Liposomes and Evaluation as Potential Agents for BNCT

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Received 4 June 1999; accepted 20 July 1999

Abstract: Four carborane-containing derivatives of cholesterol were prepared for incorporation into the bilayer of unilamellar liposomes and evaluation as potential agents for boron neutron capture therapy. The derivatives enable the evaluation of the linker moiety and the type of carborane head group on the bilayer stability and ultimate *in vivo* tumor specificity.

Key words: Carborane, Boron and Compounds, Esters, Ethers

INTRODUCTION

Boron neutron capture therapy (BNCT) is a binary cancer therapy which requires the site-specific delivery of large quantities of the boron-10 isotope to the tumor (>15 ug boron/g tumor).[1] After deposition of the boron-10 nuclei, the area is irradiated with thermal neutrons. The interaction of the thermal neutrons with the boron-10 nuclei results in the formation of two highly energetic species, a lithium ion and an alpha particle. The linear energy transfer (LET) of these particles dissipates within approximately 10 μm in tissue, the approximate diameter of a single cell. Therefore, irradiation of sufficient quantities of the boron-10 isotope in the tumor cells should result in the destruction of the tumor cells while normal neighboring cells remain essentially unaffected. Although phase I clinical trials are currently underway in both the United States and Europe, the continued development of BNCT is limited, in part, due to the scarcity of boron-containing compounds which will accumulate naturally in the tumor or, alternatively, a tumor selective delivery modality which is capable of transporting boron-containing compounds which have no inherent tumor specificity.

Unilamellar liposomes of a specific size and composition have demonstrated the ability to deliver therapeutic concentrations of boron selectively to the tumor in small animal experiments.[2,3] The selectivity of the liposomes has been attributed to the small size of the liposomes (< 70 nm) and their ability to seep through the immature vasculature characteristic of rapidly proliferating tumor masses.[4] The composition of the liposome bilayer used in the small animal experiments,[2,3] a 1:1 mole mixture of cholesterol and distearoylphosphatidylcholine (DSPC), provides the enhanced serum stability required for delivery.[5,6] Previous investigations have concentrated on the delivery of water-soluble polyhedral borane anions which have been encapsulated in the aqueous core of the liposomes. Although the liposome formulations have exhibited a high degree of tumor uptake at low injected doses relative to other BNCT studies,[7-12] the tumor boron concentrations required for application in BNCT have been achieved as a result of the encapsulation of hypertonic solutions of the polyhedral borane anions (generally ranging from 750-900 mOsM). Higher

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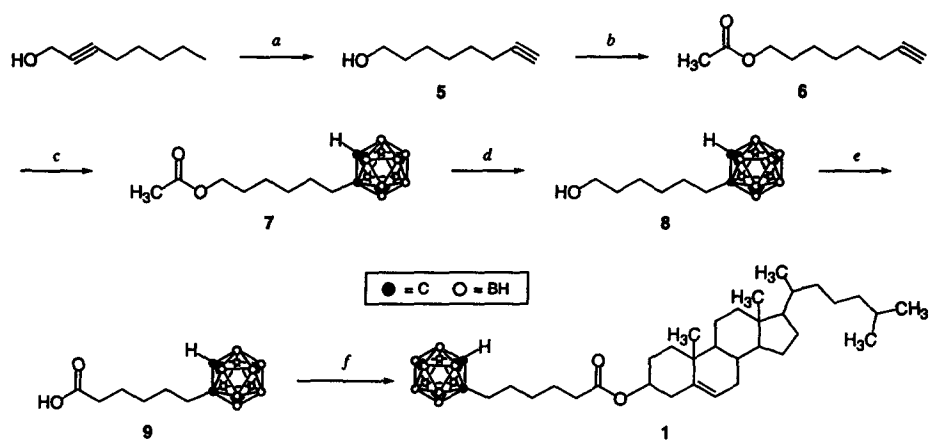
0040-4020/99/$ - see front matter
PII: S0040-4020(99)00640-7

concentrations of the water-soluble polyhedral borane anions cannot be encapsulated within the liposomes without deleterious affects to the stability of the liposome formulation. Additionally, the utilization of encapsulated polyhedral borane anions for BNCT is impeded by the low incorporation efficiencies (≈3%) obtained in the manufacture of unilamellar liposomes.[13] This limitation has particular significance when boron-10 enriched compounds are ultimately required.

Incorporation of lipophilic boron-containing compounds into the liposome bilayer of formulations which encapsulate hydrophilic boron-containing compounds would result in an increased injected dose while maintaining the integrity of the bilayer. The potassium salt of [*nido*-7-$CH_3(CH_2)_{15}$-7,8-$C_2B_9H_{11}$]$^-$, a *nido*-carborane compound characterized by a polar *nido*-carborane head group and a long lipophilic alkyl chain, has been successfully incorporated into the bilayer of liposomes for investigation as a potential agent in BNCT.[14] In addition to the desired dose enhancement, the resulting liposomes demonstrated enhanced tumor specificity, a property believed to result from the negative charge which is imparted on the liposome bilayer by the charged head group.[15,16] In an effort to maintain the basic constituents of the standard liposome bilayer, a series of boron-containing cholesterol derivatives have been synthesized for investigation in small animal experiments. The series of compounds produced enables the determination of the affect of the neutral *closo*- head group as compared to the polar *nido*- head group as well as the affect of the linker moiety on the stability and biodistribution of the resulting liposome formulations.

RESULTS AND DISCUSSION

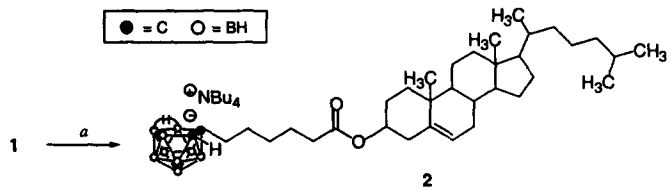
Four boron-containing cholesterol derivatives, suitable for incorporation into unilamellar liposomes and subsequent evaluation as potential agents for BNCT, have been prepared. The synthesis of cholesteryl-6-(1,2-dicarba-*closo*-dodecaboran(12)-1-yl)hexanoate (**1**) was accomplished in six steps (Scheme 1). The



Reagents and conditions: a) NaH/diaminopropane, 50 °C, overnight, *b)* acetyl chloride/triethylamine/diethyl ether, room temperature, overnight, *c)* $B_{10}H_{14}$/acetonitrile/toluene, reflux, overnight, *d)* *p*-toluenesulfonic acid/EtOH, reflux, two days, *e)* $CrO_3/H_2O/H_2SO_4$/acetone, room temperature, overnight, *f)* cholesterol/DCC/DMAP/diethyl ether, room temperature, four days.

Scheme 1.

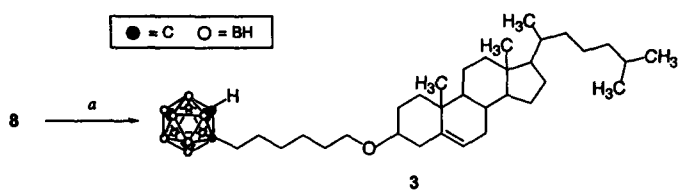
synthesis was initiated by the formation of the terminal alkyne (**5**) through a base catalyzed isomerization.[17] The terminal alcohol was protected by an acetate moiety, to form 1-(7-octynyl)ethanoate (**6**), prior to the formation of the *ortho*-carborane. Failure to protect the terminal alcohol may result in intramolecular alkoxide ion mediated cage degradation.[18] Treatment of decaborane with acetonitrile in toluene, followed by the addition of the protected terminal alkyne (**6**), produced 1-(6-(1,2-dicarba-*closo*-dodecaboran(12)-1-yl))hexylethanoate (**7**) in moderate yield (40%). Deprotection of the alcohol substituent yielded the free alcohol, 6- (1,2-dicarba-*closo*-dodecaboran(12)-1-yl)hexan-1-ol (**8**), which was then oxidized using the Jones' reagent to form 6-(1,2-dicarba-*closo*-dodecaboran(12)-1-yl)hexanoic acid (**9**). Coupling of the carboranyl acid to the cholesterol was achieved using N,N'-dicyclohexylcarbodiimide (DCC) as the coupling reagent and dimethylaminopyridine (DMAP) as a nucleophilic catalyst. Due to the base sensitivity of the ester linkage, the standard KOH/ethanol degradation of the *closo*-carborane substituent,[19] to form the *nido*-carborane moiety, could not be utilized. Anhydrous tetrabutylammonium fluoride in tetrahydrofuran (THF) was employed for the conversion[20] (Scheme 2) which produced the tetrabutylammonium salt of cholesteryl-6-(1,2-dicarba-*nido*-dodecaboran(12)-1-yl)hexanoate (**2**).



Reagents and conditions: a) tetrabutylammonium fluoride/THF, room temperature, three days.

Scheme 2.

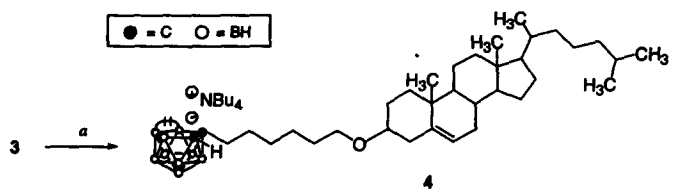
The synthesis of 1-(6-(1,2-dicarba-*closo*-dodecaboran(12)-1-yl)hexoxy cholesterol (**3**) was achieved by coupling the tosylate of the alcohol, 6-(1,2-dicarba-*closo*-dodecaboran(12)-1-yl)hexan-1-ol (**8**), to the cholesteryl anion (Scheme 3). Although the standard KOH/EtOH degradation pathway could be used in the



Reagents and conditions: a: i) TsCl/pyridine, 0 °C, overnight, *ii)* NaH/cholesterol, THF, reflux, overnight.

Scheme 3.

presence of the stable ether linkage, the milder tetrabutylammonium fluoride degradation (Scheme 4) was used to obtain the tetrabutylammonium salt of the product, 1-(6-(1,2-dicarba-*nido*-dodecaboran(12)-1-yl)hexoxy cholesterol (**4**).



Reagents and conditions: a) tetrabutylammonium fluoride/THF, room temperature, overnight.

Scheme 4.

The resulting compounds provide an opportunity to evaluate the influence of the ester linkage and the ether linkage as well as the influence of the neutral *closo*-carborane head group and the negatively charged *nido*-carborane head group on liposome stability and *in vivo* tumor selectivity. Additionally, the compounds, which have a six carbon tether between the carborane and the cholesterol moieties, have been prepared using reactions which are amenable to chain length alteration if desired. The new agents will be incorporated into unilamellar liposomes and evaluated in murine biodistribution experiments.

EXPERIMENTAL SECTION

General Methods.

Decaborane, $B_{10}H_{14}$, was obtained from Alfa Aesar (Ward Hill, MA) and sublimed[21] prior to use. **CAUTION:** ***Decaborane is a highly toxic, impact sensitive compound which forms explosive mixtures, especially with halogenated materials. A careful examination of the MSDS is recommended before usage.*** 2-Octyn-1-ol was obtained from Lancaster (Windham, NH) and used without further purification. The remaining reagents and chemicals were obtained from Aldrich Chemical Co. (Milwaukee, WI) and used without further purification. All solvents were reagent grade and were distilled from the appropriate drying agents under an argon atmosphere prior to use. Reactions were carried out under an inert atmosphere (argon or nitrogen) using anhydrous conditions.

Melting points were measured on a Mel-Temp II apparatus and are uncorrected. The ^{1}H, ^{13}C, and ^{11}B Fourier transform NMR spectra were obtained with a Varian INOVA instrument operating at 400 MHz, 100 Hertz, and 128 MHz, respectively. Proton and carbon chemical shifts were referenced to residual solvent protons; J values are given in Hertz. Proton integrations in the 0.50-2.50 ppm region of the spectrum are estimated in the boron-containing precursor compounds based on theoretical values or, in the case of the cholesterol derivatives, are not provided for multiplets in the region due to the complexity of the cholesterol spectrum in combination with the underlying boron-hydrogen baseline signal. Boron chemical shifts were externally referenced to $BF_3 \bullet Et_2O$ in C_6D_6; peaks upfield of the reference are designated as negative. The Fourier transform IR spectra were obtained using a Perkin-Elmer 1300 instrument. Microanalyses were performed by Quantitative Technologies, Inc. (Whitehouse, NJ).

7-Octyn-1-ol (5).

NaH (60% dispersion, 127 g, 3.17 mol) was transferred to a 2000-mL 2-neck flask equipped with a stir bar and argon inlet. The mineral oil was removed from the NaH by a hexane wash (1 L) which was decanted from the settled solid. The remaining hexane was removed under vacuum. The flask was cooled in an ice bath and 1,3-diaminopropane (1000 g, 13.5 mol) was added and the mixture stirred. The argon inlet was replaced by an oil bubbler and the flask was allowed to warm to room temperature. Hydrogen gas evolution was evident. The flask was heated overnight at 70 °C in an oil bath. After the heat was removed, the dark brown solution was allowed to cool to room temperature. 2-Octyn-1-ol (50 g, 0.40 mol) was added slowly to the stirred reaction mixture. The deep red solution was heated overnight at 50 °C in an oil bath. After heating the dark solution was allowed to cool to room temperature and then placed in an ice bath. The reaction was quenched with 600 mL of distilled water. The quenched solution was transferred to a 2000-mL separatory funnel and the mixture extracted with diethyl ether (5 x 300 mL). The combined ether layers were extracted with 1 M HCl (7 x 300 mL) and brine (2 x 300 mL). The ether solution was dried over anhydrous magnesium sulfate, filtered, and the solvent removed under reduced pressure by rotary evaporation. The yellow residue was purified by vacuum distillation to produce the clear liquid product in 39% yield. bp 61-62 °C (0.35 mmHg); ^{1}H NMR ($CDCl_3$) δ 3.51 (dt, 2H, J = 6.8, 1.2 Hz), 2.84 (bs, 1H), 2.08-2.12 (m, 2H), 1.88 (t, 1H, J = 2.8 Hz), 1.42-1.51 (m, 4H), 1.25-1.38 (m, 4H); ^{13}C ($CDCl_3$) δ 84.4, 68.1, 62.3, 32.3, 28.3, 28.2, 25.1, 18.1; IR (neat) 3316, 3300, 2934, 2860, 2116, 1055 cm^{-1}. Anal. Calcd. for $C_8H_{14}O$: C, 76.12; H, 11.20. Found: C, 75.73; H, 10.84.

1-(7-Octynyl)ethanoate (6).

A 2-L 3-neck flask equipped with an overhead stirrer and pressure equalized dropping funnel (PED) was charged with **5** (18.2 g, 0.144 mol). Freshly distilled diethyl ether (450 mL) and triethylamine (28.0 mL, 0.201 mol) were added to the stirred mixture. Acetyl chloride, CH_3COCl (18.0 mL, 0.253 mol), was added dropwise to the solution. The triethylammonium chloride immediately precipitated as a white solid. The solution was allowed to stir overnight at room temperature, after which, the reaction was quenched with 1 M NaOH (200 mL). The clear yellow solution was transferred to a 1-L separatory funnel and the water layer was removed. The diethyl ether layer was extracted with 1 M NaOH (4 x 200 mL), distilled H_2O (2 x 200 mL), 3 M HCl (3 x 200 mL), distilled H_2O (2 x 200 mL), brine (2 x 200 mL), and distilled H_2O (2 x 200 mL). The ether solution was collected, dried over anhydrous magnesium sulfate, filtered, and the solvent removed under reduced pressure by rotary evaporation. The remaining yellow residue was eluted through a silica gel plug and then purified by vacuum distillation to yield the colorless liquid product in 70% yield. bp 78-79 °C (0.83 mmHg); ^{1}H NMR ($CDCl_3$) δ 3.96 (t, 2H, J = 6.8 Hz), 2.10 (dt, 2H, J = 6.8, 2.8 Hz), 1.95 (s, 3H), 1.87 (t, 1H, J = 2.4 Hz), 1.54 (p, 2H, J = 6.8 Hz), 1.44 (p, 2H, J = 7.2 Hz), 1.24-1.38 (m, 4H); ^{13}C ($CDCl_3$) δ 170.8, 84.1, 68.1, 64.2, 28.3, 28.2, 28.1, 25.2, 20.7, 18.1; IR (neat) 3295, 2936, 2860, 2116, 1735, 1240, 1036 cm^{-1}. Anal. Calcd. for $C_{10}H_{16}O_2$: C, 71.38; H, 9.60. Found: C, 71.41; H, 9.43.

1-(6-(1,2-dicarba-closo-dodecaboran(12)-1-yl))hexylethanoate (7).

A 300-mL 3-neck flask equipped with a stir bar, argon inlet, condenser, and PED was charged with 150 mL of freshly distilled toluene. Natural abundance decaborane ($B_{10}H_{14}$, 10.0 g, 81.8 mmol) and freshly distilled acetonitrile (40 mL, 0.76 mol) were transferred to the flask. The solution was allowed to reflux overnight. To the bright yellow refluxing solution, **6** (13.7 g, 81.4 mmol) was added dropwise. The deep red-orange solution was allowed to reflux overnight. The reaction mixture was cooled to room temperature and transferred to a 250-mL separatory funnel. The mixture was extracted with 1 M NaOH (4 x 100 mL). The organic layer was collected, dried over anhydrous magnesium sulfate, filtered, and the solvent removed under reduced pressure by rotary evaporation. The remaining viscous red-orange liquid was extracted with 100-mL increments of boiling heptane until the liquid became an unworkable solid. The decanted heptane solutions were combined and the heptane removed under reduced pressure by rotary evaporation. The viscous dark yellow liquid was dissolved in chloroform and eluted through a silica gel plug. The remaining viscous yellow residue was purified by vacuum distillation to produce a colorless liquid product in 40% yield. bp 190 °C (1.1 mmHg); ^{1}H NMR ($CDCl_3$) δ 4.01 (t, 2H, J = 6.8 Hz), 3.58 (bs, 1H), 2.15-2.20 (m, 2H), 2.02 (s, 3H), 1.58 (p, 2H, J = 7.2 Hz), 1.40-1.50 (m, 2H), 1.23-1.35 (m, 4H); ^{13}C ($CDCl_3$) δ 171.0, 75.2, 64.1, 61.0, 37.9, 29.0, 28.4, 28.3, 25.4, 20.9; ^{11}B($CDCl_3$) δ –2.8 (d, 1B), –6.2 (d, 1B), –9.7 (d, 2B), –11.7 (d, 2B), –12.4 (d, 2B), –13.3 (d, 2B); IR (neat) 3059, 2936, 2861, 2580, 1736, 1241, 1035, 722 cm^{-1}. Although the elemental analysis of the compound was performed, the results were unsatisfactory for the percent carbon composition. Anal. Calcd. for $C_{10}H_{26}O_2B_{10}$: C, 41.92; H, 9.17. Found: C, 43.57; H, 9.46.

6-(1,2-dicarba-closo-dodecaboran(12)-1-yl)hexan-1-ol (8).

p-Toluene sulfonic acid monohydrate (0.50 g, 2.6 mmol) was transferred to a 250-mL Schlenk flask equipped with a stir bar and argon inlet. Absolute ethanol (50 mL) and **7** (9.1 g, 32 mmol) were transferred to the flask and a condenser was connected. The clear reaction mixture was allowed to reflux for 48 hours. The flask was allowed to cool to room temperature, the solution transferred to a round bottom flask and the solvent removed under reduced pressure by rotary evaporation. The clear viscous liquid was dissolved in chloroform (100 mL) and transferred to a separatory funnel. The solution was extracted with saturated $NaHCO_3$ (3 x 100 mL). The organic layer was collected, dried over anhydrous magnesium sulfate, filtered, and the solvent removed under reduced pressure by rotary evaporation to produce a clear viscous liquid product in 90% yield. ^{1}H NMR ($CDCl_3$) δ 3.59 (bs, 1H), 3.56 (t, 2H, J = 6.4 Hz), 2.16-2.20 (m, 2H), 1.52 (p, 2H, J = 6.8 Hz), 1.41-1.49 (m, 2H), 1.24-1.37 (m, 4H); ^{13}C ($CDCl_3$) δ 75.3, 62.5, 61.0, 37.9, 32.3, 29.1, 28.6, 25.2; ^{11}B($CDCl_3$) δ -2.7 (d, 1B), -6.2 (d, 1B), -9.7 (d, 2B), -11.7 (d, 2B), -12.4 (d, 2B), -13.3 (d, 2B); IR (neat) 3613, 3355, 2936, 2860, 2571, 1055, 722 cm^{-1}. Although the elemental analysis of the compound was performed, the results were unsatisfactory for the percent carbon composition. Anal. Calcd. for $C_8H_{24}OB_{10}$: C, 39.31; H, 9.92. Found: C, 40.64; H, 10.21.

6-(1,2-dicarba-closo-dodecaboran(12)-1-yl)hexanoic acid (9).

The Jones reagent was prepared using the following procedure: A 250-mL Schlenk flask equipped with a stir

bar was charged with CrO_3 (16.9 g, 0.169 mol) and distilled water (12.5 mL). The solution was cooled in an ice bath and concentrated sulfuric acid (14.4 mL) was added slowly to the flask. A precipitate immediately formed which dissolved after the addition of 30.0 mL of distilled water. While cool, acetone (50.0 mL) was added to the solution. After the preparation of the Jones reagent, a PED was attached to the flask. The PED was charged with a solution of **8** (6.1 g, 25 mmol) dissolved in acetone (30.0 mL). The solution was added dropwise to the cooled Jones reagent. After complete addition, the Schlenk flask was removed from the ice bath and the reaction was allowed to stir overnight at room temperature. The dark solution was transferred to a 500-mL round bottom flask and the solvent was removed at reduced pressure by rotary evaporation. The dark residue was transferred to a separatory funnel and extracted with diethyl ether (3 x 150 mL). The combined ether layers were extracted with brine (3 x 150 mL) and then dried over anhydrous magnesium sulfate, filtered, and the solvent removed at reduced pressure by rotary evaporation. The remaining solid was recrystallized from boiling hexane. The crystalline precipitate was filtered and dried under vacuum to yield the product in 56% yield. mp 107 °C; ^{1}H NMR ($CDCl_3$) δ 10.98 (bs, 1H), 3.57 (bs, 1H), 2.33 (t, 2H, J = 7.2 Hz), 2.16-2.20 (m, 2H), 1.60 (p, 2H, J = 7.6 Hz), 1.43-1.50 (m, 2H), 1.28-1.43 (m, 2H); ^{13}C ($CDCl_3$) δ 179.8, 75.2, 61.0, 37.8, 33.6, 28.8, 28.2, 24.0; ^{11}B($CDCl_3$) δ -2.6 (d, 1B), -6.1 (d, 1B), -9.6 (d, 2B), -11.7 (d, 2B), -12.4 (d, 2B), -13.4 (d, 2B); IR (KBr) 3071, 2943, 2867, 2610, 1704, 1232, 943, 723 cm^{-1}. Anal. Calcd. for $C_8H_{22}O_2B_{10}$: C, 37.18; H, 8.60. Found: C, 37.32; H, 8.97.

Cholesteryl-6-(1,2-dicarba-closo-dodecaboran(12)-1-yl)hexanoate (1).

A 250-mL Schlenk flask equipped with a stopper and stir bar was charged with **9** (1.99 g, 7.70 mmol), cholesterol (3.29 g, 8.51 mmol), dicyclohexylcarbodiimide (DCC, 1.76 g, 8.53 mmol), and 4-dimethylaminopyridine (DMAP, 0.12 g, 0.98 mmol). Freshly distilled diethyl ether (160 mL) was cannulated into the reaction vessel. A white precipitate of dicyclohexylurea formed immediately upon the addition of the ether. The reaction mixture was allowed to stir at room temperature for four days. The precipitate was filtered from the solution and the remaining solution was extracted with HCl (5%, 3 x 200 mL), saturated $NaHCO_3$ (1 x 200 mL), and brine (1 x 200 mL). The ether solution was collected and dried over anhydrous magnesium sulfate, filtered, and the solvent removed under reduced pressure by rotary evaporation. The product was isolated in 37% yield from the white foamy residue by filtration of the product from boiling 95% ethanol. mp 133-134 °C; ^{1}H NMR ($CDCl_3$) δ 5.37-5.38 (m, 1H), 4.56-4.63 (m, 1H), 3.56 (bs, 1H), 2.24-2.31 (m), 2.17-2.22 (m), 1.95-2.03 (m), 1.78-1.88 (m), 0.94-1.64 (m), 1.02 (s, 3H), 0.91 (d, 3H, J = 6.4 Hz), 0.87 (d, 6H, J = 6.4 Hz), 0.68 (s, 3H); ^{13}C ($CDCl_3$) δ 172.7, 139.6, 122.7, 73.4, 61.0, 56.7, 56.1, 50.0, 42.3, 39.7, 39.5, 38.1, 37.9, 37.0, 36.6, 36.2, 35.8, 34.2, 31.9, 31.8, 28.9, 28.3, 28.2, 28.0, 27.8, 24.4, 24.3, 23.8, 22.8, 22.5, 21.0, 19.3, 18.7, 11.8; ^{11}B($CDCl_3$) δ -2.6 (d, 1B), -6.0 (d, 1B), -9.5 (d, 2B), -11.7 (d, 2B), -12.4 (d, 2B), -13.3 (d, 2B); IR (nujol) 3047, 2921, 2852, 2595, 1717, 1268, 1007, 722 cm^{-1}. Anal. Calcd. for $C_{35}H_{66}O_2B_{10}$: C, 67.03; H, 10.63; B, 17.24. Found: C, 67.29; H, 10.79; B, 17.03.

Cholesteryl-6-(1,2-dicarba-nido-dodecaboran(12)-1-yl)hexanoate tetrabutylammonium salt (2).

Tetrabutylammonium fluoride (TBAF, 95%, 1.39 g, 5.05 mmol) and 5.0 mL of freshly distilled THF were

transferred to a small round bottom flask and dried over calcium hydride for three days. The mixture was filtered and the clear solution transferred to a round bottom flask equipped with a stir bar. A solution of **1** (0.56 g, 0.89 mmol) in 2.5 mL of freshly distilled THF was added to the flask and the mixture allowed to stir at room temperature for three days. Reaction progress was monitored by ^{11}B NMR spectroscopy. The reaction solvent was removed *in vacuo* and the oily residue dissolved in chloroform (20 mL). The solution was extracted with distilled water (2 x 10 mL). The organic layer was dried over anhydrous magnesium sulfate, filtered, and the solvent removed *in vacuo*. The pure white product was isolated in 49% yield from the pale yellow residue by filtration of the product from boiling 95% ethanol. mp 151 °C; ^1H NMR ($CDCl_3$) δ 5.35-5.36 (m, 1H), 4.54-4.62 (m, 1H), 3.15-3.19 (m, 8H), 2.20-2.26 (m), 2.28-2.31 (m), 0.94-2.02 (m), 1.01 (t, 12H, J = 7.2 Hz), 0.90 (d, 3H, J = 6.4 Hz), 0.86 (d, 6H, J = 6.4 Hz), 0.67 (s, 3H); ^{13}C ($CDCl_3$) δ 173.4, 139.8, 122.5, 73.6, 58.9 (4C), 56.7, 56.1, 50.0, 42.3, 39.7, 39.5, 39.2, 38.1, 37.0, 36.6, 36.2, 35.8, 34.7, 31.9, 31.8, 30.8, 29.3, 28.2, 28.0, 27.8, 25.1, 24.2, 24.0 (4C), 23.8, 22.8, 22.5, 21.0, 19.7 (4C), 19.3, 18.7, 13.6 (4C), 11.8; $^{11}B(CDCl_3)$ δ -11.5 (d, 2B), -14.0 (d, 1B), -18.2 (d, 3B), -22.1 (d, 1B), -33.7 (d, 1B), -37.6 (d, 1B); IR (nujol) 3204, 2511, 1734, 1172, 1026, 737 cm^{-1}. Anal. Calcd. for $C_{51}H_{102}O_2B_9N \bullet H_2O$: C, 69.85; H, 11.98; N, 1.63. Found: C, 69.53; H, 11.63; N, 1.62. The presence of water was confirmed by FT-IR spectroscopy.

1-(6-(1,2-dicarba-closo-dodecaboran(12)-1-yl)hexoxy cholesterol (3).

The tosylate of **8** was prepared using the following procedure: A 100-mL round bottom flask was charged with **8** (2.5 g, 10 mmol) and pyridine (30 mL). The flask was stoppered and cooled to 0 °C in the freezer. ρ-Toluenesulfonyl chloride (2.2 g, 12 mmol) was added to the cooled solution and placed back in the freezer overnight. Pyridinium chloride precipitated from the clear yellow solution. The mixture was poured into ice-cold distilled water (200 mL). The crystals dissolved and a white oily substance formed. The aqueous solution was transferred to a 500-mL separatory funnel and extracted with chloroform (3 x 150 mL). The chloroform layers were collected, dried over anhydrous magnesium sulfate, filtered, and the solvent removed at reduced pressure by rotary evaporation. The residue (3.8 g, 93%) was a yellow viscous liquid and was used without further purification. A 500-mL 3-neck flask equipped with a water condenser, argon inlet, stir bar and stopper, was charged with cholesterol (4.4 g, 11 mmol) and freshly distilled THF (80 mL). NaH (3.6 g, 90 mmol) was added to the solution. Hydrogen gas evolution was immediately apparent. The solution was allowed to stir at room temperature overnight. A PED was attached to the flask. The tosylate of **8** (3.8 g, 9.5 mmol) was dissolved in THF (60 mL), transferred to the PED, and added dropwise to the solution. The PED was rinsed with THF (60 mL), the PED removed, and replaced by a stopper. The solution was allowed to reflux overnight. The light brown reaction solution was quenched with distilled water. Ethyl acetate (100 mL) was added to the mixture and the mixture was transferred to a 500-mL separatory funnel. The mixture was extracted with distilled water (3 x 150 mL), 1 M NaOH (3 x 150 mL) and brine (2 x 150 mL). The organic layer was collected, dried over anhydrous magnesium sulfate, filtered, and the solvent removed at reduced pressure by rotary evaporation . The dark red-brown solid was purified by column chromatography on silica gel using hexane:dichloromethane (1:1.5); R_f = 0.79. The fractions were concentrated to dryness and the resulting yellow solid was recrystallized from absolute ethanol at room temperature to yield a white solid

(3) in 11% yield. mp 130-131 °C; ^{1}H NMR ($CDCl_3$) δ 5.33-5.34 (m, 1H), 3.55 (bs, 1H), 3.43 (t, 2H, J = 6.4 Hz), 3.06-3.15 (m, 1H), 2.33-2.35 (m), 2.17-2.21 (m), 1.96-2.03 (m), 1.84-1.87 (m), 1.44-1.58 (m), 1.26-1.33 (m), 1.02-1.22 (m), 1.00 (s, 3H), 0.91 (d, 3H, J = 6.8 Hz), 0.86 (d, 6H, J = 6.4 Hz), 0.68 (s, 3H); ^{13}C ($CDCl_3$) δ 141.0, 121.5, 79.0, 75.3, 67.7, 60.9, 56.8, 56.2, 50.2, 42.3, 39.8, 39.5, 39.2, 38.0, 37.3, 36.9, 36.2, 35.8, 32.0, 31.9, 29.9, 29.1, 28.8, 28.5, 28.2, 28.0, 25.8, 24.3, 23.8, 22.8, 22.6, 21.1, 19.4, 18.7, 11.9; ^{11}B($CDCl_3$) δ -2.7 (d, 1B), -6.2 (d, 1B), -9.6 (d, 2B), -11.7 (d, 2B), -12.5 (d, 2B), -13.4 (d, 2B); IR (nujol) 2590, 1097 cm^{-1}. Anal. Calcd. for $C_{35}H_{68}OB_{10}$: C, 68.56; H, 11.20. Found: C, 68.11; H, 11.26.

1-(6-(1,2-dicarba-nido-dodecaboran(12)-1-yl)hexoxy cholesterol, tetrabutylammonium salt (4).
Tetrabutylammonium fluoride (TBAF, 95%, 1.3 g, 4.7 mmol) and 5.0 mL of freshly distilled THF were transferred to a small round bottom flask and dried over calcium hydride for three days. The mixture was filtered and 0.9 mL of the yellow-brown clear solution transferred to a round bottom flask equipped with a stir bar. A solution of **3** (0.108 g, 0.175 mmol) in 0.5 mL of freshly distilled THF was added to the flask and the mixture allowed to stir at room temperature overnight. Evolution of hydrogen gas was apparent. The reaction solvent was removed *in vacuo*. The pure white product was isolated in 35% yield from the pale yellow residue by recrystallization from boiling 95% ethanol. mp 167-169 °C; ^{1}H NMR ($CDCl_3$) δ 5.30-5.31 (m, 1H), 3.39 (t, 2H, J = 7.2 Hz), 3.06-3.17 (m, 9H), 2.30-2.34 (m), 2.01-2.18 (m), 0.95-1.99 (m), 0.99 (t, 12H, J = 7.6 Hz), 0.88 (d, 3H, J = 6.4 Hz), 0.83 (d, 6H, J = 6.8 Hz), 0.64 (s, 3H); ^{13}C ($CDCl_3$) δ 141.1, 121.3, 78.8, 68.1, 58.9 (4C), 56.7, 56.1, 50.2, 42.3, 39.8, 39.5, 39.4, 39.2, 37.3, 36.9, 36.2, 35.7, 32.0, 31.9, 31.2, 30.2, 29.7, 28.5, 28.2, 28.0, 26.2, 24.3, 24.0 (4C), 23.8, 22.8, 22.5, 21.0, 19.7 (4C), 19.3, 18.7, 13.6 (4C), 11.8; ^{11}B($CDCl_3$) δ -11.5 (d, 2B), -14.0 (d, 1B), -18.2 (d, 3B), -22.4 (d, 1B), -33.7 (d, 1B), -37.6 (d, 1B); IR (thin film) 2931, 2516, 1472, 1102 cm^{-1}. Anal. Calcd. for $C_{51}H_{104}OB_9N$: C, 72.50; H, 12.43; N, 1.66. Found: C, 71.97; H, 12.47; N, 1.63.

ACKNOWLEDGMENTS

This research was supported by awards from the Robert A. Welch Foundation (AI1292), Research Corporation (CC3913), and Southwest Texas State University.

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