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Preparation and In Vivo Evaluation of Radioiodinated *closo*-Decaborate(2-) Derivatives to Identify Structural Components That Provide Low Retention in Tissues

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Abstract

Introduction—In vivo deastatination of ^{211}At -labeled biomolecules can severely limit their use in endoradiotherapy. Our studies have shown that the use of *closo*-decaborate(2-) moiety for ^{211}At -labeling of biomolecules provides high in vivo stability towards deastatination. However, data from those studies have also been suggestive that some astatinated *closo*-decaborate(2-) catabolites may be retained in tissues. In this study, we investigated the in vivo distributions of several structurally simple *closo*-decaborate(2-) derivatives to gain information on the effects of functional groups if catabolites are released into the blood system from the carrier biomolecule.

Methods—Thirteen *closo*-decaborate(2-) derivatives were synthesized and radioiodinated for evaluation. Tissue concentrations of the radioiodinated compounds were obtained in groups of 5 mice at 1 and 4 h post injection (pi). Dual label (^{125}I and ^{131}I) experiments permitted evaluation of 2 compounds in each set of mice.

Results—All of the target compounds were readily synthesized. Radioiodination reactions were conducted with chloramine-T and $\text{Na}[^{125/131}\text{I}]\text{I}$ in water to give high yields (75-96%) of the desired compounds. Biodistribution data at 1 and 4 h pi (representing catabolites released into the blood system) showed small differences in tissue concentrations for some compounds, but large differences for others. The results indicate that formal (overall) charge on the compounds could not be used as a predictor of tissue localization or retention. However, derivatives containing carboxylate groups generally had lower tissue concentrations. Acid cleavable hydrazone functionalities appeared to be the best candidates for further study.

Conclusions—Further studies incorporating hydrazone functionalities into pendant groups for biomolecule radiohalogenation are warranted.

Keywords

closo-decaborate(2-); radioiodination; radiolabeling; tissue retention

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1. Introduction

Biomolecules labeled with the α -particle emitting radionuclide [^{211}At]astatine are of interest as potential therapeutic radiopharmaceuticals [1-6]. Due to an inherent instability to deastatination, biomolecules can not be directly labeled with ^{211}At . Thus, coupling ^{211}At with biomolecules requires either conjugation of a pendant group that has been prelabeled with ^{211}At or a pendant group that has a high reactivity with electrophilic ^{211}At . A number of research groups have developed aryl pendant groups with radiohalogen-reactive organometallic functionalities for conjugation with biomolecules [7-9]. Although high radiochemical yields can be obtained with ^{211}At -labeling of biomolecules conjugated with aryl pendant groups, in vivo deastatination is often a major problem [10]. To circumvent this problem, our research group has been evaluating the use of boron cage moieties as radiohalogen-reactive moieties in pendant groups for biomolecule labeling [11-14]. Of the boron cage moieties evaluated, *closo*-decaborate(2-) conjugates provide highest labeling yields with minimal alteration of the biomolecule's properties. Most importantly, use of the *closo*-decaborate(2-) moiety in conjugated pendant groups provides an ^{211}At label that is stable towards in vivo deastatination [15].

While pendant groups containing a *closo*-decaborate(2-) moiety have favorable labeling and in vivo stability properties, it appears from some of our in vivo studies that catabolites of this dianionic boron cage may be retained in tissues longer than similar conjugates containing aryl pendant groups. Such retention (or residualization) has been noted previously with biomolecules labeled with radiometals [16-18]. It is not known why the tissue retention occurs, but it may be due in part to the dianionic character of the *closo*-decaborate(2-) moiety. Although the *closo*-decaborate(2-) moiety has been used in Boron Neutron Capture Therapy (BNCT) studies [19,20], very little is known about its in vivo properties when conjugated with biomolecules.

In this investigation, fundamental studies were conducted to evaluate the tissue distributions in mice of radioiodinated *closo*-decaborate(2-) derivatives containing different functional groups. While such data does not provide direct evidence of how these molecules might be retained or released from tissues when bioconjugates localize there, they can provide important information regarding the distribution of catabolites released into the blood stream. Relatively simple derivatives were prepared with the forethought that they might be incorporated into more complex pendant groups in a manner that they could be released in vivo after metabolism of the biomolecule. Literature reports have shown that design of radiolabeled biomolecule conjugates, such that they can be metabolized to release a predefined fragment, can greatly alter their retention in tissues such as kidney [21] and liver [22]. As the dianionic nature of the *closo*-decaborate(2-) moiety might affect the tissue retention, one of the questions addressed in the investigation was whether the (formal) ionic charge on the molecule had an influence on its retention in tissues. Varying formal charges were obtained by coupling functional groups that would be ionized under physiological conditions. Another question addressed is whether cleavable functional groups, such as esters (cleaved by esterases) or hydrazones (cleaved by acid pH in lysosomes), could be used to decrease tissue concentrations of radiolabeled *closo*-decaborate(2-) catabolites. Thus, the studies included *closo*-decaborate(2-) derivatives that contained amines, carboxylates, esters, ketones and hydrazones. Thirteen structurally simple derivatives of *closo*-decaborate(2-) (Figure 1) were prepared, radioiodinated and their biodistributions were evaluated in mice. The results of the investigation are described herein.

2. Materials and methods

2.1. General

Most of the reagents employed in the studies were obtained from Sigma-Aldrich (Milwaukee, WI) and were used without further purification. Previously reported syntheses were used to prepare the *closo*-decaborate(2-) derivatives, [Et₃NH]B₁₀H₉-CO-trioxadiazine, **1a** [15]; [Et₃NH][B₁₀H₉-CO], **14** [23]; [Et₃NH]₂[B₁₀H₁₀], **15** [23]. Reactions to prepare *closo*-decaborate(2-) derivatives **1a** – **13a** were followed by HPLC analyses. Solvents for HPLC analysis were obtained as HPLC grade and were filtered (0.2 μm) prior to use.

2.2. Radioactivity

All radioactive materials were handled according to approved protocols at the University of Washington. Na[¹²⁵I]I and Na[¹³¹I]I were purchased from Perkin-Elmer Life and Analytical Sciences, Inc. (Waltham, MA) as a high concentration solution in 0.1 N NaOH. Radioiodinations were conducted within a charcoal-filtered Plexiglas enclosure (Biodex Medical Systems Inc., Shirley, N.Y.) housed in a radiochemical fume hood. The radioiodination reactions were carried out in vials capped with Teflon-coated septa vented through a 10 mL charcoal-filled syringe.

Measurement of ¹²⁵I and ¹³¹I was accomplished on a Capintec CRC-15R Radioisotope Calibrator using the manufacturer's settings for those radionuclides. In the experiment evaluating an admixture of ¹³¹I with ¹²⁵I, the ¹²⁵I counts were compensated for spillover from the ¹³¹I window (15%). Tissue samples were counted in a Wallac 1480 gamma counter with the following window settings: channels 35-102 and 165-185 for ¹²⁵I and ¹³¹I.

2.3. Chromatography

HPLC separations of the non-radioactive compounds were obtained using a system that contained a Hewlett-Packard quaternary 1050 gradient pump, a variable wavelength UV detector (254 nm), and an ELSD 2000 evaporative light-scattering detector (Alltech, Deerfield, IL). Analysis of the HPLC data was conducted on Hewlett-Packard HPLC ChemStation software. Reversed-phase HPLC chromatography was carried out on an Alltech Altima C-18 column (5 μm, 250 × 4.5 mm) using a gradient solvent system at a flow rate of 1 mL/min. The gradient began with 0.05 M, pH 5.5, aqueous Et₃NHOAc and was increased linearly to 100% MeOH over a 15 min period. Following this, the elution continued with 100% MeOH for an additional 5 min. The retention time (t_R) of each iodinated compound is provided with the experimental procedure.

Products were purified from crude reaction mixtures using a Biotage SP Flash Purification System (Charlottesville, VA) on a reversed-phase C18 FLASH 25+M column or 40+M column. The purification used a gradient mixture composed of MeOH and 0.05 M Et₃NHOAc (TEAA). The gradient started with 100% 0.05 M TEAA, with MeOH being increased linearly to 100% over the next 20 min. Fractions were collected based on UV detection at 215 or 254 nm. Fractions containing pure products were determined by analytical HPLC and were combined, solvent evaporated, and isolated to provide the yields listed.

The product mixtures from radioiodination reactions were analyzed by radio-HPLC using a C-18 column (Altima C-18, 5 mm, 250 × 4.5 mm; Alltech, Deerfield, IL) eluting at a flow rate of 1 mL/min using the same gradient conditions as described for non-radioactive iodination products. It should be noted that, for radiolabeled compounds **12** and **13**, the pH of the Et₃NHOAc elution buffer was brought to 7.2 due to concerns for cleaving the hydrazone moiety during isolation. The HPLC equipment used in the analyses consisted of a Hewlett-Packard quaternary 1050 gradient pump, Waters 601 UV detector, and a Beckman model 170

radioisotope detector. Analysis of the HPLC data was conducted on Hewlett-Packard HPLC ChemStation software. Isolation of radiolabeled compounds was accomplished by collection of part (when broad) or the entire radioactive peak. The retention times (t_R) of the radioiodinated and astatinated compounds are provided in the experimental procedures.

2.4. Spectral Analyses

^1H NMR, ^{11}B NMR and mass spectral analyses were obtained on *closo*-decaborate(2-) derivatives. The data obtained is provided with the experimental procedures. Spectral data for compounds previously reported are not listed, but were consistent with the reported data. ^1H and ^{11}B NMR spectra were obtained on a Bruker AV-500 (500 MHz ^1H and 160.4 MHz for ^{11}B). ^1H NMR data are referenced to tetramethylsilane as an internal standard (δ), and ^{11}B NMR data are referenced to $\text{BF}_3\cdot\text{OEt}_2$ as an external standard. Mass spectral data were obtained on either a Waters Micromass Quattro Micro API Tandem Quadrupole Mass Spectrometer, (QHQ) MS/MS, with a liquid chromatograph inlet system and atmospheric pressure chemical ionization or electrospray ionization for low resolution mass spectra (LRMS), or a Bruker APEX Qe 47e Fourier transform (Ion Cyclotron Resonance) Mass Spectrometer, [FT(ICR)]MS, using an infusion inlet system and electrospray ionization for high resolution mass spectra (HRMS).

2.5. Syntheses

2.5.1 General procedure for the preparation of 2a - 6a— $[\text{Et}_3\text{NH}]\text{B}_{10}\text{H}_9\text{-CO}$, **14**, (1 eq) was added to a solution of the amine-containing compound, (5 eq), Et_3N (3 eq) and anhydrous DMF (10 mL) at room temperature. The resulting solution was then stirred at room temperature for 16 to 72 h (following the reaction progress by HPLC). After reaction completion, the volatile materials were removed on a rotary evaporator under vacuum. The crude product was dissolved in $\text{CH}_3\text{CN}/\text{MeOH}/\text{H}_2\text{O}$ (1/1/1) and purified via Biotage (C18 FLASH 25+M column). Yields of the adducts **2a** – **6a** ranged from 37% to 55%.

$[\text{Et}_3\text{NH}]_2\text{B}_{10}\text{H}_9\text{-CONHCH}_2\text{CH}_2\text{CH}_2\text{N(Me)CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$, 2a: Yield 92 mg (46%). ^1H NMR ($\text{DMSO}-d_6$, 500 MHz): δ -0.33-1.31 (m, 9H), 1.18 (t, J = 7.1 Hz, 18H), 1.52-1.59 (m, 2H), 1.69-1.75 (m, 2H), 2.12 (s, 3H), 2.29 (t, J = 7.1 Hz, 4H), 2.36 (t, J = 6.9 Hz, 2H), 2.83 (t, J = 6.9 Hz, 2H), 3.11 (q, J = 7.1 Hz, 12H). ^{11}B NMR ($\text{DMSO}-d_6$, 160.4 MHz): δ -0.44 (1B), -4.70 (1B), -7.25 (1B), -14.94 (1B), -17.22 (1B), -23.07 (3B), -26.44 (2B). LRMS (ES^-) $\text{C}_8\text{H}_{27}\text{B}_{10}\text{N}_3\text{O}$ (M) $^-$ calc 291.3. Found 291.2. HPLC: t_R = 2.9 min.

$[\text{Et}_3\text{NH}]_2\text{B}_{10}\text{H}_9\text{-CONH-CH}_2\text{CH}_2\text{NHCH}_2\text{CH}_2\text{CH}_2\text{NH-CH}_2\text{CH}_2\text{NH}_2$, 3a: Yield 76 mg (37%). ^1H NMR ($\text{DMSO}-d_6$, 500 MHz): δ -0.56-1.33 (m, 9H), 1.18 (t, J = 7.0 Hz, 18H), 1.53-1.66 (m, 2H), 1.97-2.03 (m, 5H), 2.45 (t, J = 5.9 Hz, 2H), 2.51 (t, J = 6.0 Hz, 2H), 2.53-2.71 (m, 8H), 3.12 (q, J = 7.0 Hz, 12H). ^{11}B NMR ($\text{DMSO}-d_6$, 160.4 MHz): δ -0.17 (2B), -3.46 (2B), -6.23 (1B), -14.95 (1B), -22.53 (1B), -25.97 (3B). LRMS (ES^-) $\text{C}_8\text{H}_{28}\text{B}_{10}\text{N}_4\text{O}$ (M) $^-$ calc 306.3. Found 306.2. HPLC: t_R = 2.6 min.

$[\text{Et}_3\text{NH}]_2\text{B}_{10}\text{H}_9\text{-CONHNH}_2$, 4a: Yield 61 mg (40%). ^1H NMR (D_2O , 500 MHz): δ -0.63-1.58 (m, 9H), 1.16 (t, J = 7.3 Hz, 18H), 3.08 (q, J = 7.3 Hz, 12H). ^{11}B NMR (D_2O , 160.4 MHz): δ 0.14 (1B), -3.69 (2B), -6.75 (1B), -15.09 (1B), -23.55 (3B), -28.77 (2B). LRMS (ES^-) $\text{CH}_{12}\text{B}_{10}\text{N}_2\text{O}$ (M) $^-$ calc 178.2. Found 178.1. HPLC: t_R = 2.9 min.

$[\text{Et}_3\text{NH}]\text{B}_{10}\text{H}_9\text{-CONHCH}_2\text{CO}_2\text{H}$, 5a: Yield 73 mg (43%). ^1H NMR (CD_3OD , 500 MHz): δ -0.45-1.47 (m, 9H), 1.15 (t, J = 7.0 Hz, 18H), 3.03 (q, J = 7.0 Hz, 12H), 3.53 (s, 2H). ^{11}B NMR (CD_3OD , 160.4 MHz): δ 6.19 (1B), 0.49 (1B), -1.39 (1B), -3.62 (1B), -5.96 (1B),

–15.32 (1B), –21.36 (1B), –22.73 (1B), –25.70 (2B). LRMS (ES[–]) C₃H₁₃B₁₀NO₃ (M) calc 221.2. Found 221.3. HPLC: t_R = 2.9 min.

[Et₃NH]₂B₁₀H₉-CONHCH₂CO₂Me, 6a: Yield 97 mg (55%). ¹H NMR (CD₃OD, 500 MHz): δ –0.50–1.45 (m, 9H), 1.12 (t, J = 7.1 Hz, 18H), 3.03 (q, J = 7.1 Hz, 12H), 3.65 (s, 3H), 3.69 (s, 2H). ¹¹B NMR (CD₃OD, 160.4 MHz): δ 5.60 (1B), 0.28 (1B), –1.45 (1B), –3.66 (1B), –5.81 (1B), –15.48 (1B), –21.14 (1B), –22.90 (1B), –25.79 (2B). LRMS (ES[–]) C₄H₁₅B₁₀NO₃ (M)[–] calc 235.2. Found 235.0. HPLC: t_R = 3.1 min.

[Et₃NH]₂B₁₀H₉-CH₂OH, 7a: (This compound was prepared using a method similar to that previously reported [24]) LiBH₄ (4 mL, 2 M solution in THF) was added to a solution of [Et₃NH]B₁₀H₉-CO, **14** (200 mg, 0.808 mmol) and anhydrous acetonitrile (15 mL) at room temperature. The resulting solution was heated to reflux for 1 h. After the solution was cooled to room temperature, 2 mL of MeOH was added and stirred for 1 h. The volatile materials were removed by rotary evaporator under vacuum. The crude product was dissolved in MeOH/water (1/1) and purified via Biotage (C18 FLASH 25+M column) to yield 151 mg (53%) of a colorless tacky solid. ¹H NMR (CD₃OD, 500 MHz): δ –0.36–1.73 (m, 9H), 1.13 (t, J = 7.1 Hz, 18H), 3.08 (q, J = 7.1 Hz, 12H), 3.35 (s, 2H). ¹¹B NMR (CD₃OD, 160.4 MHz): δ 4.00 (1B), 0.98 (1B), –1.78 (1B), –12.90 (1B), –19.49 (1B), –20.06 (1B), –20.62 (1B), –25.71 (1B), –26.28 (1B), –29.86 (1B). LRMS (ES[–]) CH₁₂B₁₀O (M)[–] calc 150.2. Found 150.2. HPLC: t_R = 2.9 min.

[Et₃NH]₂B₁₀H₉-COPh, 8a: A solution of [Et₃NH]₂B₁₀H₁₀, **15** (2.0 g, 6.20 mmol), benzoyl chloride (2.61 g, 18.6 mmol) and anhydrous CH₃CN (20 mL) was stirred at room temperature for 16 h. After that time, the volatile materials were removed by rotary evaporator under vacuum. The crude product was dissolved in MeOH/H₂O (1/1) and purified via Biotage (C18 FLASH 40+M column) to yield 1.7 g (64%) of a colorless oil. ¹H NMR (CD₃OD, 500 MHz): δ –0.19–1.06 (m, 9H), 1.27 (t, J = 7.3 Hz, 18H), 3.17 (q, J = 7.3 Hz, 12H), 7.24 (t, J = 7.6 Hz, 2H), 7.32 (t, J = 7.3 Hz, 1H), 8.00 (d, J = 8.1 Hz, 2H). ¹¹B NMR (CD₃OD, 160.4 MHz): δ 1.65 (1B), 0.74 (1B), –19.03 (1B), –26.50 (7B). HRMS (ES[–]) C₇H₁₅B₁₀O (M+H)[–] calc 225.2053. Found 225.2042. HPLC: t_R = 7.6 min.

[Et₃NH]₂B₁₀H₉-COPhOMe, 9a: A solution of [Et₃NH]₂B₁₀H₁₀, **15** (1.0 g, 6.20 mmol), 4-methoxybenzoyl chloride (3.17 g, 18.60 mmol) and anhydrous CH₃CN (25 mL) was stirred at room temperature for 2 h (turned to dark-brown color). The solution was triturated with 20% Et₂O/hexanes (150 mL). The residue was dissolved in CH₃CN (10 mL) and the trituration procedure was repeated. The crude product was dissolved in 5 mL of MeOH/CH₃CN (1/1) and purified via Biotage (C18 FLASH 40+M column) to yield 1.6 g (57%) of a light yellow solid, mp 138–140°C. ¹H NMR (CD₃CN, 500 MHz): δ –0.09–1.38 (m, 9H), 1.23 (t, J = 7.3 Hz, 18H), 3.14 (q, J = 7.3 Hz, 12H), 3.84 (s, 3H), 6.89 (d, J = 9.0 Hz, 2H), 8.17 (d, J = 9.0 Hz, 2H). ¹¹B NMR (CD₃CN, 160.4 MHz): δ 6.13 (2B), –17.86 (1B), –20.75 (2B), –23.30 (1B), –24.95 (2B), –26.87 (2B). HRMS (ES[–]) C₈H₁₇B₁₀O₂ (M+H)[–] calc 255.2165. Found 255.2160. HPLC: t_R = 9.8 min.

[Et₃NH]₂B₁₀H₉-COPhCO₂H, 10a: A solution of [Et₃NH]₂B₁₀H₁₀, **15** (2.0 g, 6.20 mmol), terephthaloyl chloride (6.29 g, 31.0 mmol) and anhydrous CH₃CN (25 mL) was stirred at room temperature for 1 h (turned to dark-brown color). The solution was triturated with 20% EtOAc/hexanes (150 mL). The remained residue was dissolved in CH₃CN (20 mL) and triturated with EtOAc (150 mL) again. The remained residue was dissolved in a solution of CH₃CN/H₂O/NEt₃ (8/1/1, 25 mL) and stirred at room temperature overnight. The crude solution was purified via Biotage (C18 FLASH 40+M column) to yield 1.1 g (48%) of a light-yellow tacky

solid. ¹H NMR (CD₃CN, 500 MHz): δ –0.34–1.48 (m, 9H), 1.22 (t, J = 7.3 Hz, 18H), 3.09 (q,

$J = 7.3$ Hz, 12H), 7.78 (d, $J = 8.4$ Hz, 2H), 7.98 (d, $J = 8.4$ Hz, 2H). ^{11}B NMR (CD_3CN , 160.4 MHz): δ 3.08 (1B), 2.20 (1B), -14.36 (1B), -18.21 (1B), -24.77 (2B), -25.48 (3B), -26.59 (1B). HRMS (ES^-) $\text{C}_8\text{H}_{15}\text{B}_{10}\text{O}_3$ ($\text{M}+\text{H}$) $^-$ calc 269.1957. Found 269.1961. HPLC: $t_R = 6.8$ min.

[Et₃NH]₂B₁₀H₉-C(Ph)=NNHPhCO₂H, 11a: A solution of [Et₃NH]₂B₁₀H₉-COPh, **8a** (200 mg, 0.469 mmol), H₂OC-Ph-NHNH₂, **20** (107 mg, 0.703 mmol) and MeOH (10 mL) was stirred at room temperature for 2 h. The light-yellow crude product solution was filtered, and purified via Biotage (C18 FLASH 25+M column) to yield 186 mg (71%) of a light-yellow

tacky solid. ^1H NMR (CD_3OD , δ 5 MHz): δ -0.12-1.40 (m, 9H), 1.26 (t, $J = 7.3$ Hz, 18H), 3.15 (q, $J = 7.3$ Hz, 12H), 6.72 (d, $J = 8.9$ Hz, 1H), 6.83 (d, $J = 8.9$ Hz, 1H), 7.13-7.18 (m, 2H), 7.26 (t, $J = 7.6$ Hz, 1H), 7.59 (d, $J = 7.0$ Hz, 1H), 7.73 (d, $J = 7.0$ Hz, 1H), 7.78 (d, $J = 8.8$ Hz, 1H), 7.86 (d, $J = 8.8$ Hz, 1H). ^{11}B NMR (CD_3OD , 160.4 MHz): δ 4.10 (1B), 1.35 (1B), -19.38 (1B), -22.49 (1B), -25.63 (6B). HRMS (ES^-) $\text{C}_{14}\text{H}_{21}\text{B}_{10}\text{N}_2\text{O}_2$ ($\text{M}+\text{H}$) $^-$ calc 359.2534. Found 359.2517. HPLC: $t_R = 8.5$ min.

[Et₃NH]₂B₁₀H₉-C(Ph-CO₂H)=NNHPhCO₂H, 12a: This compound was prepared by reaction of **10a** with **20**, under the same reaction and purification conditions described for

11a, to yield 176 mg (69%) yield of a light-yellow tacky solid. ^1H NMR (CD_3OD , δ 5 MHz): δ -0.07-1.04 (m, 9H), 1.25 (t, $J = 7.3$ Hz, 18H), 3.14 (q, $J = 7.3$ Hz, 12H), 6.85 (d, $J = 8.5$ Hz, 2H), 7.75 (d, $J = 8.0$ Hz, 2H), 7.78 (d, $J = 8.5$ Hz, 2H), 7.82 (d, $J = 8.0$ Hz, 2H). ^{11}B NMR (CD_3OD , 160.4 MHz): δ 1.11 (1B), 0.25 (1B), -22.86 (1B), -25.65 (7B). HRMS (ES^-) $\text{C}_{15}\text{H}_{21}\text{B}_{10}\text{N}_2\text{O}_4$ ($\text{M}+\text{H}$) $^-$ calc 403.2437. Found 403.2437. HPLC: $t_R = 9.3$ min.

[Et₃NH]₂B₁₀H₉-C(Ph-CO₂H)=NNHCOPhCO₂H, 13a: This compound was prepared by reaction of **10a** with **19**, under the same reaction and purification conditions described for

11a, to yield 161 mg (60%) yield of a light-yellow tacky solid. ^1H NMR (CD_3OD , δ 5 MHz): δ -0.09-1.02 (m, 9H), 1.24 (t, $J = 7.3$ Hz, 18H), 3.11 (q, $J = 7.3$ Hz, 12H), 7.68 (d, $J = 8.4$ Hz, 2H), 7.81 (d, $J = 8.4$ Hz, 2H), 7.94 (d, $J = 8.4$ Hz, 2H), 8.00 (d, $J = 8.4$ Hz, 2H). ^{11}B NMR (CD_3OD , 160.4 MHz): δ 1.62 (1B), 1.19 (1B), 0.80 (1B), -22.54 (1B), -26.20 (6B). HRMS (ES^-) $\text{C}_{16}\text{H}_{21}\text{B}_{10}\text{N}_2\text{O}_5$ ($\text{M}+\text{H}$) $^-$ calc 431.2387. Found 431.2401. HPLC: $t_R = 8.6$ min.

2.5.2. General procedure for preparing iodinated HPLC standards, 1b-13b

Method A: A 8.27 μmol quantity (203 μL of 10 mg/mL in deionized H₂O) of chloramine-T was added to a solution containing 17 μmol of the *closo*-decaborate(2-) derivative (**1a** – **7a**, **11a**, **12a** or **13a**), 8.27 μmol of NaI (12.4 μL , 100 mg/mL in deionized H₂O) and 0.8 mL of a 5% HOAc in 1:1 MeOH/H₂O solution. That mixture was stirred at room temperature for 1 min, then 8.27 μmol Na₂S₂O₅ (157 μL , 10 mg/mL in deionized H₂O) was added and the solution was stirred at room temperature for an additional 1 min. A small quantity of iodinated product was isolated from an analytical HPLC column for mass spectral analysis, and to be used as a HPLC retention time standard for the radioiodination reactions.

Method B: A 14 μmol quantity (188 μL , 10 mg/mL in MeOH) of *N*-chlorosuccinimide was added to a solution containing 28 μmol of the *closo*-decaborate(2-) derivative (**8a**, **9a** or **-10a**), 14 μmol of NaI (21 μL of 100 mg/mL in deionized H₂O), and 5% HOAc/MeOH (0.5 mL). The reaction solution was stirred at room temperature for 1 min, then 14 μmol Na₂S₂O₅ (134 μL , 20 mg/mL in deionized H₂O) was added and the resultant solution was stirred at room temperature for 1 min. A small quantity of iodinated product was isolated from an analytical HPLC column for mass spectral analysis, and to be used as a HPLC retention time standard for the radioiodination reactions.

[Et₃NH]₂B₁₀H₈I-CONH-CH₂CH₂CH₂(OCH₂CH₂)₃CH₂NH₂, 1b: This compound was prepared using synthetic Method A. Repeated attempts to obtain mass spectral data were unsuccessful. HPLC: t_R = 3.2 min.

[Et₃NH]₂B₁₀H₈I-CONHCH₂CH₂CH₂N(Me)CH₂CH₂CH₂NH₂, 2b: This compound was prepared using synthetic Method A. Repeated attempts to obtain mass spectral data were unsuccessful. HPLC: t_R = 3.2 min.

[Et₃NH]₂B₁₀H₈I-CONHCH₂CH₂NHCH₂CH₂CH₂NHCH₂CH₂NH₂, 3b: This compound was prepared using synthetic Method A. LRMS (ES⁻) C₈H₂₇B₁₀IN₄O (M)⁻ calc 432.2. Found 432.5. HPLC: t_R = 3.1 min.

[Et₃NH]₂B₁₀H₈I-CONHNH₂, 4b: This compound was prepared using synthetic Method A. LRMS (ES⁻) CH₁₁B₁₀IN₂NaO (M+Na)⁻ calc 327.1. Found 327.1. HPLC: t_R = 3.2 min.

[Et₃NH]₂B₁₀H₈I-CONHCH₂CO₂H, 5b: This compound was prepared using synthetic Method A. LRMS (ES⁻) C₃H₁₂B₁₀INO₃ (M)⁻ calc 347.1. Found 347.3. HPLC: t_R = 3.3 min.

[Et₃NH]₂B₁₀H₈I-CONHCH₂CO₂Me, 6b: This compound was prepared using synthetic Method A. LRMS (ES⁻) C₄H₁₄B₁₀INO₃ (M)⁻ calc 361.1. Found 361.1. HPLC: t_R = 3.6 min.

[Et₃NH]₂B₁₀H₈I-CH₂OH, 7b: This compound was prepared using synthetic Method A. LRMS (ES⁻) CH₁₁B₁₀IO (M)⁻ calc 276.1. Found 276.0. HPLC: t_R = 3.2 min.

[Et₃NH]₂B₁₀H₈I-COPh, 8b: This compound was prepared using synthetic Method A. LRMS (ES⁻) C₇H₁₃B₁₀IO (M)⁻ calc 350.1. Found 350.1. HPLC: t_R = 9.0 min.

[Et₃NH]₂B₁₀H₈I-COPhOMe, 9b: This compound was prepared using synthetic Method A. HRMS (ES⁻) C₈H₁₆B₁₀IO₂ (M+H)⁻ calc 381.1131. Found 381.1143. HPLC: t_R = 10.9 min.

[Et₃NH]₂B₁₀H₈I-COPhCO₂H, 10b: This compound was prepared using synthetic Method B. HRMS (ES⁻) C₈H₁₄B₁₀IO₃ (M+H)⁻ calc 395.0924. Found 395.0939. HPLC: t_R = 8.3 min.

[Et₃NH]₂B₁₀H₈I-C(Ph)=NNHPhCO₂H, 11b: This compound was prepared using synthetic Method B. HRMS (ES⁻) C₁₄H₂₀B₁₀IN₂O₂ (M+H)⁻ calc 485.1505. Found 485.1503. HPLC: t_R = 10.2 min.

[Et₃NH]₂B₁₀H₈I-C(PhCO₂H)=NNHPhCO₂H, 12b: This compound was prepared using synthetic Method A. HRMS (ES⁻) C₁₅H₂₀B₁₀IN₂O₄ (M+H)⁻ calc 529.1404. Found 529.1438. HPLC: t_R = 10.4 min.

[Et₃NH]₂B₁₀H₈I-C(PhCO₂H)=NNHCOPhCO₂H, 13b: This compound was prepared using synthetic Method A. HRMS (ES⁻) C₁₆H₂₀B₁₀IN₂O₅ (M+H)⁻ calc 557.1353. Found 557.1345. HPLC: t_R = 9.6 min.

2.5.3. General procedure for preparing radioiodinated derivatives, [¹²⁵/¹³¹I]1b-^{[125/131]13b}—To 100 μL of a 1 mg/mL solution of the compound in a 1:1 mixture of MeOH / 5% HOAc in H₂O was added 2-4 μL of Na¹³¹I or Na¹²⁵I in 0.1 N NaOH, followed by 20 μL of a 1 mg/mL solution of chloramine T in water. It should be noted that the radioiodination reactions with compounds **12** and **13** did not contain the 5% HOAc due to concerns that the hydrazone might be cleaved under the isolation conditions. After 1-5 min at room temperature, the reaction was quenched with 20 μL of a 1 mg/mL solution of sodium metabisulfite. The

radioiodinated compound was purified by collection from reversed-phase HPLC effluent, reduced to dryness and dissolved in PBS for injection.

The integrated radio-HPLC and isolated radiochemical yields* (in parentheses) for labeled *closo*-decaborate(2-) derivatives **1b** – **13b** were as follows*: [¹²⁵I]**1b**: 83% (84%); [¹²⁵I]**2b**: 95% (84%); [¹³¹I]**3b**: 95% (78%); [¹²⁵I]**4b**: 95% (96%); [¹³¹I]**5b**: 90% (80%); [¹²⁵I]**6b**: 95% (84%); [¹³¹I]**7b**: 75% (61%); [¹²⁵I]**8b**: 95% (80%); [¹²⁵I]**9b**: 90% (90%); [¹³¹I]**10b**: 70% (44%); [¹³¹I]**11b**: 95% (94%); [¹²⁵I]**12b**: 96% (98%); [¹³¹I]**13b**: 95% (87%). *Note that the isolated yield may not include all of a broad radio-HPLC peak, so it may be lower than the HPLC yield.

2.6. Biodistribution Studies

Biodistribution studies were conducted under a protocol approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Washington. Male nude mice, obtained from Simonson Laboratories (Gilroy, CA), were housed for 1 week in the isolator facility prior to beginning the study. Dual label experiments were conducted to minimize the number of mice required to obtain the information. In each experiment, the radioactive peaks corresponding to the desired products were isolated from the HPLC to provide high specific activity radioiodinated compounds. The isolated compounds (¹²⁵I- and ¹³¹I-labeled) were mixed to prepare an admixture containing predetermined μ Ci quantities of each radioiodinated compound. The admixture was diluted with phosphate buffered saline (PBS) to prepare a solution for injection of ~100 μ L. This quantity was injected into each of 10 athymic mice via the lateral tail vein. The actual amount of injectate each animal received was determined by weighing the administering syringe before and after injection. Groups of 5 mice were sacrificed by cervical dislocation at 1 and 4 h post injection. The tissues were excised, blotted free of blood, weighed, and counted. Blood weight was estimated to be 6% of the total body weight [25]. Calculation of percent injected dose (%ID) and percent injected dose per gram (%ID/g) in the tissues was accomplished counting $4 \times 1 \mu$ L of the injectate as standards for ¹²⁵I and ¹³¹I counts.

3. Results

3.1. Syntheses of **1a** – **13a**

Syntheses of the *closo*-decaborate(2-) derivatives was accomplished using three general approaches as shown in Figure 2, A, B and C. Reaction of carbonylated *closo*-decaborate(2-), [Et₃NH]B₁₀H₉-CO, **14**, with compounds having primary amines in anhydrous DMF at room temperature for 16-72 h provided the target adducts **2a**–**6a** in moderate yields (37-55%), as depicted in Fig. 2, A. Higher yields were obtained when the reacting amino compound was used as a solvent (e.g. 68% for **1a**; 93% for **2a**; 98% for **3a**; 81% for **4a**), but workup and purification was much more difficult using that method. Preparation of the hydroxymethyl derivative **7a** was accomplished in 53% yield by reaction of LiBH₄ with **14** in anhydrous acetonitrile at reflux for 1 h (Fig. 2, B). The reaction conditions were adapted from a procedure previously reported for a perchlorinated *closo*-decaborate(2-) derivative [26]. The procedures for preparation of the phenacyl derivatives **8a** - **10a** and their hydrazone adducts **11a** - **13a** are shown in Fig. 2, C. The phenacyl derivatives were prepared in 48-64% yield by reaction of the corresponding benzoyl chloride, **16**, **17**, or **18**, with the triethylammonium salt of *closo*-decaborate(2-), **15**, in anhydrous acetonitrile at room temperature for 1 – 16 h. A previously reported method for reaction of benzoyl chloride, **16**, employed the protio salt of B₁₀H₁₀²⁻ in water and dimethoxyethane provided **8a** in 35% yield [27]. In that same paper, the semicarbazone derivative of **8a** was prepared. In these studies, formation of the hydrazone derivatives **11a** - **13a** was found to be relatively fast (within 2 h) at room temperature, providing yields of 60-71%

when the hydrazide, **19** or **20**, was reacted with the phenacyl-*closo*-decaborate(2-) derivative **8a** or **10a**.

3.2. Radioiodination Reactions to prepare [¹²⁵I]-**1b**-[¹²⁵I]-**13b**

Iodination reactions were initially conducted on **1a**-**13a** to prepare **1b**-**13b** for HPLC standards. Iodination was achieved by reaction of the *closo*-decaborate(2-) with either chloramine-T (ChT) and NaI in MeOH/H₂O (Method A) or N-chlorosuccinimide (NCS) and NaI in MeOH/5% HOAc at room temperature for 1 min (Method B). The reactions employed 0.5 equivalents of oxidizing agent (ChT or NCS) and NaI so that 50% of the product was present with 50% of the starting *closo*-decaborate(2-) derivative (by ELSD detection). Iodination reactions gave a single product for most of the *closo*-decaborate(2-) derivatives, but two product peaks were observed with iodination of **9a**, **11a**, **12a** and **13a**. These may be regioisomeric iodinated derivatives, as mass spectral data for both iodinated peaks of **9b** indicated they had the same mass. The iodinated compounds were isolated from the analytical HPLC since only small quantities were required as standards. Identity of the isolated derivative was confirmed by obtaining mass spectral data. In those examples where there were two iodinated products, the major peak was isolated and identified by mass spectral analyses.

Radioiodination reactions were conducted on **1a** - **13a** to obtain either ¹²⁵I- or ¹³¹I-labeled *closo*-decaborate(2-) derivatives, [¹²⁵I]-**1b**-[¹³¹I]-**13b** (Figure 1). The two different radionuclides were used for labeling so that dual-label experiments could be conducted to minimize the number of mice required in the investigation. Since the radionuclides were obtained in aqueous base, the reactions were conducted in a mixture containing a small amount of HOAc in aqueous solution. However, concerns that hydrazone functionalities in **12** and **13** would be cleaved by HOAc led to its removal from the reaction and HPLC solvent when preparing those compounds. Radioiodination reactions employed Na[¹²⁵I] or Na[¹³¹I] and chloramine-T for 30 seconds at room temperature. In the radioiodination reactions a single broad peak or two (non-separated) radioactive peaks were seen. Based on the assumption that the radioactive peaks come from iodination at two different boron atoms in the cage or from the two possible geometric hydrazone isomers, both of the closely eluting peaks were isolated. High yields (70-96%) were obtained by radio-HPLC, but isolated yields were sometimes lower (44-98% yield) due to collecting portions of the radioactive peak(s) in some reactions (see experimental).

3.3. In vivo evaluations

Experiments were conducted in athymic mice to obtain biodistribution data for the 13 *closo*-decaborate(2-) compounds. In the experiments, 10 mice were injected with the pair of ¹²⁵I-/¹³¹I-labeled *closo*-decaborate(2-) derivatives and 5 mice were sacrificed at 1 and 4 h post injection. The 1 and 4 h time points were chosen as later time points had very low counts in most tissues, and the early time points were adequate to determine whether the radiolabel was being retained in a tissue. Selected tissue distribution data for the 13 radioiodinated *closo*-decaborate(2-) derivatives are provided in Table 1, and are plotted in Figure 3, panels A-I. Figure 3 is provided to more readily compare the concentrations of the 13 compounds in tissues. The tissue concentration data are plotted as “stacked” bar graphs, with the 1 h data being represented by the grey (bottom) portion of the bar and the 4 h data being represented as the black (top) portion of the bar. This form of presentation of data was chosen so that the concentrations in blood and tissues could be readily compared, and the decreases in tissue concentration with time (i.e. grey vs. black portions) could be easily assessed. It should be noted that, in most biodistribution experiments (except for compounds **6**, **7** & **8**), higher concentrations of the radioactivity were obtained in the urine than was present in the liver or intestines at the 1 h time point, but animal-to-animal variation in voiding provided urine data that was highly variable. Indeed, low urine volumes can have very high %ID/g values whereas

higher urine volumes can be more dilute resulting in lower %ID/g values, and in some cases, no urine is obtained which perturbed the average values significantly. Therefore, the urine concentration data are not included in this report. Interestingly, the concentrations of **12** and **13** in liver and intestine at the 1 hour time point seem to indicate that these compounds are excreted primarily through the hepatobiliary system.

The biodistribution studies were conducted to help identify which functional groups in the *closo*-decaborate(2-) derivatives had the lowest tissue concentrations and/or were retained the least in tissues. Of the derivatives that contain terminal (free) amine functional groups, [¹²⁵I] **1b**, [¹²⁵I]**2b**, [¹³¹I]**3b** and [¹²⁵I]**4b**, the diamino- and triamino-derivatives [¹²⁵I]**2b** and [¹³¹I]**3b** had the lowest concentrations in blood, muscle, lung and spleen. However, [¹²⁵I]**2b** and [¹³¹I]**3b** had relatively high kidney concentrations at 1 h pi, and [¹²⁵I]**2b** had higher liver and intestine concentrations than the other amine-containing derivatives at both time points. The glycine and glycine methyl ester-containing derivatives, [¹²⁵I]**5b** and [¹³¹I]**6b** had relatively low concentrations in most tissues, with the exception of the ester [¹³¹I]**6b**, which had relatively high concentrations and retention in liver and intestine. The hydroxymethyl derivative, [¹³¹I]**7b**, was unusual as it had relatively high concentrations in blood, lung, liver, intestine, and spleen. It also had particularly high concentrations in neck (containing thyroid) and stomach. The reason for the high concentrations in tissues is not understood, but it is unlikely to be due to an impurity or free radionuclide as the radioiodinated compounds were isolated from HPLC effluent much later than where free radioiodine elutes (solvent front). The phenacyl derivatives [¹²⁵I]**8b**, [¹²⁵I]**9b** and [¹³¹I]**10b** had different tissue concentrations and retention, presumably due to the substituent *para* to the ketone. Particularly noteworthy was the fact that the unsubstituted phenacyl derivative **8b** appears to be retained longer (black portion of bars in Figure 3 graphs) than the other two derivatives in all tissues. This may be due to the higher lipophilicity of the unsubstituted aryl ring. The derivative that was retained the least and had the lowest concentrations in most tissues is the *para*-carboxylate derivative [¹³¹I]**10b**. The final set of compounds studied were those containing a hydrazone functionality ([¹³¹I]**11b**, [¹²⁵I]**12b** and [¹³¹I]**13b**). The phenacyl-hydrazone derivative [¹²⁵I]**12b** had the lowest concentrations of the compounds studied in many tissues, with the exceptions of liver ([¹²⁵I]**5b** lower), intestines, neck and stomach. In contrast, hydrazone [¹³¹I]**13b** had high concentrations in a majority of tissues.

4. Discussion

The *closo*-decaborate(2-) moiety provides high reactivity with ²¹¹At and has a high stability towards deastatination in vivo, even when incorporated into rapidly metabolized biomolecules. This has led us to investigate incorporation of that moiety into a number of pendant groups for biomolecule labeling. Conjugation of pendant groups containing the *closo*-decaborate(2-) moiety with monoclonal antibodies (MAb) and their Fab' fragments has allowed direct ²¹¹At labeling of those proteins in high overall radiochemical yields (60-80%) without affecting their tumor- or hematopoietic cell-targeting ability [11,28]. However, results obtained in radioiodination studies appear to indicate that the radiolabel may be retained in some tissues (e.g. liver), potentially due to catabolites containing the *closo*-decaborate(2-) moiety. We felt that the way to circumvent this potential problem was to design pendant groups that contain a releasable *closo*-decaborate(2-) derivative. Investigators have shown that concentrations of radionuclides in kidney and liver can be decreased by incorporating a cleavable linker between the protein and the labeling moiety. For example, esters and diesters have been successfully used to decrease kidney concentrations [29-32]. It is postulated that esterases cleave the linker to provide a catabolite that is more readily cleared from the tissues. Other chemical structures that can be enzymatically cleaved in lysosomes, such as disulfides and peptides have also been studied [33-35]. Although not extensively explored for radioactive materials, the use of linkers containing acid cleavable functional groups can conceivably provide a mechanism of release

of radioactivity from kidney and liver. There are many examples in the literature of acid cleavable groups, such as diortho esters or hydrazones that have been used for release of drugs from bioconjugates [36-38].

Our goal in this investigation was to compare tissue concentrations of 13 different *closo*-decaborate(2-) derivatives to aid in the design of biomolecule pendant groups containing this radiohalogen-reactive moiety, such that retention of radioactivity in non-target tissues from catabolites released into the blood system could be minimized. It is recognized the results obtained from iv injection of the *closo*-decaborate(2-) derivatives can not provide direct evidence for the catabolite distributions when the same *closo*-decaborate(2-) derivatives are conjugated with biomolecules, as the biomolecules will have different catabolic pathways and different pharmacokinetics. However, the in vivo model used does provide valuable data regarding clearance of potential catabolites once they enter the blood stream. The tissue concentrations at 1 h pi are reflective of the initial uptake of the radiolabeled compound, and the differences seen between the 1 h and 4 h data reflect the clearance of catabolites. Importantly, the 4 hour data provide information on how well the derivatives and their catabolites were processed by the metabolically active organs; kidney, liver and intestines.

The terminal amine containing derivatives **1b**, **2b** and **3b** were evaluated as potential catabolites that may be released from a carbamate functionality through enzymatic-assisted cleavage, such as previously described for the "Ardec" reagent [39]. The *closo*-decaborate(2-) derivatives **1b**, **2b**, and **3b** contain varying numbers of amines such that their ionic charges (under physiological conditions) are different. The tissue concentrations of these compounds did not vary much, with the only appreciable difference being in the liver (e.g. 4h data: 3.75 ± 0.34 % ID/g for [^{125}I]**2b** vs. 0.42 ± 0.06 & 0.48 ± 0.17 % ID/g for [^{125}I]**1b** & [^{131}I]**3b** respt.). Compound **2b** has a net neutral formal charge, which may have contributed to its retention in the liver.

The *closo*-decaborate(2-) derivatives **5b**, **6b** and **7b** were prepared to evaluate the potential for ester containing *closo*-decaborate(2-) derivatives. The glycine adduct **5b** is similar in structure to *meta*-iodohippuric acid, which is readily excreted through the kidneys [40]. Interestingly, **5b** had the lowest concentration in the liver of the compounds studied, presumably due to its propensity to be rapidly cleared through the kidneys. The corresponding methyl ester, **6b**, was evaluated to demonstrate cleavage of the ester under the conditions studied. Interestingly, compound **6b** had relatively low concentrations in most tissues, except for the liver. The liver concentration of [^{131}I]**6b** was quite high at 1h (10.74 ± 2.63 % ID/g), and the 4 h data (7.75 ± 2.89 % ID/g) indicated that the ester had a slow clearance from that organ. The hydroxymethylene derivative **7b** was also evaluated, as that simple *closo*-decaborate(2-) could potentially be released in vivo from an ester derivative. However, its high concentrations in most tissues makes it unattractive for inclusion into a pendant group. The high neck (thyroid) and stomach concentrations with radioiodinated **7b** would appear to indicate that free radioiodine was present, but the fact that the concentration in those tissues decreases, rather than increases, at the 4 h time point may indicate that the radioactivity was not present as free radioiodine.

The acylhydrazine **4b** and ketones **8b**, **9b** and **10b** were evaluated as derivatives that might be released from an acid cleavable hydrazone. Lysosomes have a pH around 4 [41], so hydrazones that are cleaved at that pH (and 37°C) might be used to release a catabolite that is not retained in the tissues. Our ultimate goal is to use the *closo*-decaborate(2-) derivatives with ^{211}At ($t_{1/2} = 7.21$ h) and the optimal reagent for that will have minimal tissue concentrations at all time points. Therefore, the acyl hydrazine derivative **4a** does not appear to be a good candidate for preparing an optimal cleavable hydrazone derivative, since [^{125}I]**4b** had higher concentrations in blood, lung and kidney than most of the other derivatives tested at 1 h post injection. Of the phenacyl derivatives [^{125}I]**8b**, [^{125}I]**9b** and [^{131}I]**10b**, **9b** had the highest concentrations in

most tissues at 1 h pi, but **8b** had the highest concentrations at 4 h pi. Reaction of **8** or **10** with a phenylhydrazine or a phenacylhydrazine derivative provided the hydrazone derivatives, [^{131}I]**11b**, [^{125}I]**12b** and [^{131}I]**13b**. It was anticipated that the bis-aryl hydrazones would have higher concentrations in tissues than most of the other compounds tested, unless the hydrazone was cleaved. It should be noted that the structures of **11b** and **12b** are the same, except that the releasable ketone moiety in **12b** has a *p*-carboxyphenyl moiety (i.e. release of **10b**) and **11b** has a phenyl moiety (i.e. release of **8b**). It should also be noted that the structures of **12b** and **13b** are the same, except **13b** is a phenacylhydrazine adduct whereas **12b** is a phenylhydrazine adduct. The tissue distributions of compounds [^{131}I]**11b** and [^{125}I]**12b** are quite similar except [^{125}I]**12b** is slightly lower in several tissues and higher in the intestines, indicating that a significant portion of **12b** was excreted rapidly into the intestine through the hepatobiliary system. The lower tissue concentrations are most likely due to the extra carboxylate in **12b**. Dramatic differences are seen when comparing tissue concentrations of [^{125}I]**12b** and [^{131}I]**13b**. Hydrazone [^{131}I]**13b** had much higher concentrations than [^{125}I]**12b** in most tissues, with the exception of the intestines. However, it should be noted that the intestines are difficult to obtain relevant concentrations due to the animal-to-animal variation in radioactivity transit. Indeed, for [^{125}I]**12b** the animal-to-animal variation in concentrations in intestines was high, where at 4 h pi the 5 animals had 38.8 %ID/g, 27.1%, 2.5%, 0.81%ID/g and 0.26%ID/g. The large differences in tissue concentrations between hydrazones **12b** and **13b** at the 1 h time point may be due to differences in their structural nature. Derivative **12** is a phenylhydrazone and **13** is a phenacylhydrazone. Large differences in acid cleavage of different types of hydrazones have been reported [42,43]. While we cannot determine if hydrolysis of the hydrazones has occurred based on the data obtained, one interpretation might be that the phenylhydrazone **12b** was cleaved whereas the phenacylhydrazone **13b** was not. A more plausible explanation for the results is that phenylhydrazone **12b** was not cleaved, but rather cleared more rapidly than **13b** through the hepatobiliary route.

5. Conclusion

Pendant groups containing the *closo*-decaborate(2-) moiety are currently the only ones that provide conjugates which are stable to in vivo deastatination, but their use may be limited in some cases due to long retention of the catabolites in kidney and/or liver. Other studies have shown that clearance from kidney and liver can be greatly improved by using cleavable linker molecules to release catabolites from tissues. The studies described herein were designed to help identify structural features in *closo*-decaborate(2-) derivatives that would minimize tissue localization and retention if they were released from lysosomes. The data from the studies indicate that terminal amine-containing catabolites would not be the most optimal. Additionally, there appears to be no correlation of the formal charge on the compounds with the lowest tissue concentrations. The data suggests that ester derivatives of **5** would be worth studying, but there is some concern about cleavage of the esters formed. It appears from the data that the most promising functional group to incorporate into pendant groups for conjugation to biomolecules, such as antibodies and their fragments, is a hydrazone. Additional studies into use of hydrazones to minimize the concentrations of radiolabeled catabolites from biomolecule conjugates containing the *closo*-decaborate(2-) moiety are underway.

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Fig. 1.

Chemical structures of monosubstituted *closo*-decaborate(2-) derivatives. In the *closo*-decaborate(2-) structures, circles represent B-H or B atoms.

A. Modification of $B_{10}H_9-CO(1-)$ with amino compounds

- 1a: R = $-CH_2CH_2CH_2(OCH_2CH_2)_3CH_2NH_2$
 2a: R = $-CH_2CH_2CH_2N(Me)CH_2CH_2CH_2NH_2$
 3a: R = $-CH_2CH_2NHCH_2CH_2NHCH_2CH_2NH_2$
 4a: R = $-NH_2$
 5a: R = $-CH_2CO_2H$
 6a: R = $-CH_2CO_2Me$

B. Modification of $B_{10}H_9-CO(1-)$ by reduction

C. Preparation of benzoyl derivatives and modification with phenyl hydrazine (formation of hydrazone)

D. Radioiodinations of $B_{10}H_9-R$ Derivatives

Fig. 2.

Synthetic steps for preparation of the *closo*-decaborate(2-) derivatives, **1a-13a**, and their iodinated or radioiodinated products, **1b-13b**.

Fig. 3.

Concentrations (%ID/g) of the radioiodinated *closo*-decaborate derivatives (**1b-13b**) in blood and selected tissues of nude mice. The numbers on the x-axis indicate the compound number. Bar graphs are a composite (stacked) of the 1 h and 4 h data. The grey portion of each bar is the average %ID/g for 5 mice at 1 h post injection (pi) and the black portion of the bar is the average %ID/g for 5 mice at 4 h pi. Note that the concentration ranges differ for each tissue graph (y-axis; A-I), so that the differences in the concentrations (%ID/g) between the compounds in a specific tissue can be more readily seen.

Table 1
Tissue concentrations of radioiodinated *closo-decaborate*(2-) derivatives in mice at 1 hour and 4 hours post injection *

Cmpd#	Blood	Muscle	Lung	Kidney	Spleen	Liver	Intestine	Neck	Stomach
(1h data)									
1	2.69 ± 0.94	0.35 ± 0.08	1.60 ± 0.37	3.18 ± 0.92	0.70 ± 0.19	4.38 ± 0.34	1.06 ± 0.70	0.79 ± 0.25	0.98 ± 0.25
2	1.31 ± 0.29	0.24 ± 0.07	1.05 ± 0.27	4.51 ± 1.49	0.45 ± 0.11	7.86 ± 1.53	1.24 ± 1.52	1.03 ± 0.23	0.70 ± 0.13
3	1.68 ± 0.41	0.26 ± 0.08	1.26 ± 0.34	4.89 ± 2.07	0.56 ± 0.18	3.69 ± 0.96	1.20 ± 1.80	0.74 ± 0.20	0.60 ± 0.12
4	3.51 ± 1.09	0.51 ± 0.12	2.39 ± 0.34	5.86 ± 1.30	1.13 ± 0.21	3.70 ± 0.85	0.52 ± 0.12	2.56 ± 2.23	1.62 ± 0.48
5	1.29 ± 0.80	0.28 ± 0.10	1.16 ± 0.41	3.24 ± 1.42	0.49 ± 0.20	1.79 ± 0.75	0.35 ± 0.19	0.61 ± 0.23	0.47 ± 0.22
6	1.01 ± 0.25	0.16 ± 0.04	0.67 ± 0.16	2.10 ± 0.45	0.29 ± 0.07	10.74 ± 2.63	0.60 ± 0.66	0.44 ± 0.09	0.28 ± 0.07
7	2.70 ± 1.45	0.28 ± 0.16	2.37 ± 1.13	1.75 ± 0.42	2.04 ± 0.62	11.64 ± 3.89	6.39 ± 6.20	2.01 ± 0.42	7.57 ± 8.28
8	1.37 ± 0.16	0.13 ± 0.02	0.57 ± 0.09	1.72 ± 0.23	0.25 ± 0.04	3.90 ± 0.34	1.37 ± 0.51	0.62 ± 0.33	1.00 ± 0.68
9	3.82 ± 1.01	0.33 ± 0.03	1.44 ± 0.35	2.38 ± 0.60	0.45 ± 0.08	4.16 ± 0.43	0.45 ± 0.10	0.95 ± 0.49	0.61 ± 0.41
10	1.75 ± 0.44	0.23 ± 0.05	1.22 ± 0.40	2.27 ± 0.80	0.35 ± 0.06	6.37 ± 1.15	0.28 ± 0.07	0.51 ± 0.25	0.62 ± 0.41
11	0.74 ± 0.29	0.10 ± 0.03	0.34 ± 0.09	1.38 ± 0.33	0.18 ± 0.07	5.99 ± 1.45	4.69 ± 2.05	0.31 ± 0.27	3.36 ± 3.31
12	0.48 ± 0.20	0.08 ± 0.03	0.53 ± 0.17	1.53 ± 0.32	0.15 ± 0.06	3.64 ± 2.02	50.58 ± 27.45	1.18 ± 1.19	2.36 ± 1.70
13	2.86 ± 1.35	0.56 ± 0.17	3.94 ± 1.30	6.61 ± 1.14	1.33 ± 0.52	23.19 ± 6.63	33.75 ± 6.30	3.99 ± 0.36	5.91 ± 4.75

(4h data)									
1	0.17 ± 0.02	0.02 ± 0.00	0.14 ± 0.02	0.24 ± 0.01	0.05 ± 0.01	0.42 ± 0.06	0.34 ± 0.08	0.06 ± 0.02	0.15 ± 0.05
2	0.28 ± 0.05	0.03 ± 0.01	0.20 ± 0.02	1.06 ± 0.16	0.10 ± 0.02	3.75 ± 0.34	3.18 ± 1.56	0.52 ± 0.14	0.57 ± 0.20
3	0.20 ± 0.03	0.02 ± 0.01	0.14 ± 0.04	0.24 ± 0.04	0.06 ± 0.02	0.48 ± 0.17	0.52 ± 0.29	0.16 ± 0.05	0.30 ± 0.17
4	0.26 ± 0.12	0.02 ± 0.04	0.17 ± 0.07	0.17 ± 0.08	0.06 ± 0.02	0.49 ± 0.19	0.27 ± 0.2	0.26 ± 0.16	0.20 ± 0.05
5	0.17 ± 0.09	0.01 ± 0.04	0.13 ± 0.07	0.20 ± 0.08	0.04 ± 0.02	0.40 ± 0.16	0.83 ± 1.34	0.07 ± 0.04	0.06 ± 0.03
6	0.24 ± 0.10	0.05 ± 0.03	0.31 ± 0.14	0.57 ± 0.23	0.09 ± 0.04	7.75 ± 2.89	12.14 ± 19.33	0.28 ± 0.12	0.30 ± 0.14
7	0.77 ± 0.08	0.10 ± 0.01	1.33 ± 0.46	1.19 ± 0.09	1.64 ± 0.33	6.24 ± 2.17	23.60 ± 14.10	2.14 ± 0.27	1.86 ± 0.32
8	1.41 ± 0.29	0.15 ± 0.03	0.64 ± 0.02	4.02 ± 0.36	0.26 ± 0.06	3.78 ± 1.05	4.59 ± 1.79	0.57 ± 0.14	1.50 ± 1.21
9	0.70 ± 0.05	0.16 ± 0.09	0.38 ± 0.02	0.60 ± 0.16	0.15 ± 0.04	1.65 ± 0.33	3.32 ± 1.88	0.19 ± 0.02	0.26 ± 0.13
10	0.06 ± 0.01	0.03 ± 0.04	0.11 ± 0.01	0.24 ± 0.04	0.04 ± 0.02	0.44 ± 0.22	4.89 ± 4.07	0.04 ± 0.01	0.15 ± 0.09
11	0.40 ± 0.05	0.03 ± 0.01	0.16 ± 0.17	0.28 ± 0.03	0.05 ± 0.03	0.69 ± 0.06	13.61 ± 6.39	0.08 ± 0.02	2.70 ± 2.27
12	0.32 ± 0.04	0.01 ± 0.04	0.26 ± 0.03	0.22 ± 0.03	0.06 ± 0.02	0.20 ± 0.01	10.58 ± 18.81	0.29 ± 0.12	0.60 ± 0.36

(4h data)									
13	0.25 ± 0.04	0.05 ± 0.01	0.61 ± 0.07	1.55 ± 0.15	0.17 ± 0.05	1.26 ± 0.18	6.58 ± 7.08	0.16 ± 0.02	0.29 ± 0.11

* Values are average % injected dose / gram ± standard deviation for groups of 5 mice at 1 hour (top table) and 4 hour (bottom table).