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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 five mice at 1 and 4 h postinjection (pi). Dual-label (^{125}I and ^{131}I) experiments permitted evaluation of two compounds in each set of mice.

Results: All of the target compounds were readily synthesized. Radioiodination reactions were conducted with chloramine-T and Na[$^{125/131}$I]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

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 cannot be directly labeled with ^{211}At. Thus, coupling ^{211}At with biomolecules requires conjugation of either 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 the highest labeling yields with minimal alteration of the biomolecule's properties. Most importantly, use of the *closo*-decaborate(2-) moiety in

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conjugated pendant groups provides an ^{211}At label that is stable towards in vivo deastatination [11].

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 [15–17]. 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 studies [18,19], 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 [20] and liver [21]. 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-) (Fig. 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, USA) and were used without further purification. Previously reported syntheses were used to prepare the *closo*-decaborate(2-) derivatives, $[Et_3NH]B_{10}H_9$-CO-trioxadiamine (**1a**) [11]; $[Et_3NH][B_{10}H_9$-CO] (**14**) [22]; $[Et_3NH]_2[B_{10}H_{10}]$ (**15**) [22]. Reactions to prepare *closo*-decaborate(2-) derivatives **1a**–**13a** were followed by HPLC analyses. Solvents for

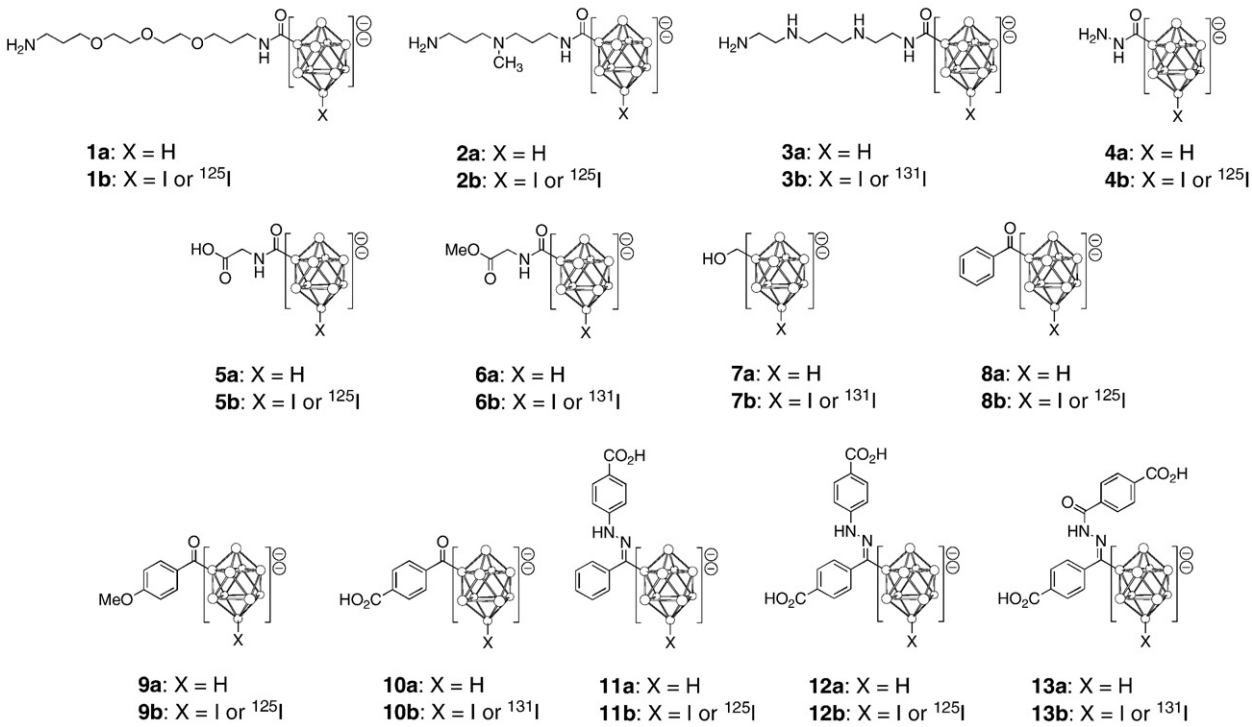

Fig. 1. Chemical structures of monosubstituted *closo*-decaborate(2-) derivatives. In the *closo*-decaborate(2-) structures, circles represent B-H or B atoms.