POLYHEDRAL BORON COMPOUNDS AS POTENTIAL LINKERS FOR ATTACHMENT OF RADIOHALOGENS TO TARGETING PROTEINS AND PEPTIDES. A REVIEW

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Dedicated to Professor Jaromír Plešek on the occasion of his 75th birthday.

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Polyhedral boron clusters (PBC) are three-dimensional inorganic aromatic systems. Some of them can easily be halogenated, and the halogen–boron bond in such systems is very strong. We consider the use of PBC as linkers for attachment of radioactive halogen isotopes to tumor-targeting proteins and peptides. In this review the major preconditions for such applications, such as biological considerations, knowledge concerning coupling chemistry and radiolabeling of PBC, are described. A review with 90 references.

Keywords: Tumour targeting; ^{123}I; ^{125}I; ^{76}Br; ^{211}At; Polyhedral boron clusters; Prosthetic groups; Boranes; Carboranes; Isotope labelling.

1. INTRODUCTION

There is a growing interest in labeling polyhedral boron compounds (PBC), such as carboranes and borate anions (Fig. 1), with halogens for biomedical application[1]. Initially, such labeling was performed with the aim to study

pharmacokinetics of boron compounds for boron neutron capture therapy (BNCT)[2,3]. In successful cases, radiolabeling would yield detailed information about boron pharmacokinetics, which is helpful in the refinement of patient treatment protocols, for example by providing information about the required dosage of tumor-seeking boron conjugates, and optimal treatment time. Without a radioactive label on the boron moiety, however, the measurement of the pharmacokinetics of boron-containing compounds is limited to the possibility of obtaining biopsy samples, and to accurately measuring their boron content[3]. In 1994, Wilbur *et al.*[3] already suggested that *nido*-carborate derivatives could be used as linkers for the attachment of radioiodine atoms to biologically active molecules.

Previously, it was proposed to use polyhedral boron clusters as prosthetic groups for iodination and astatination of small molecules. We believe that the most interesting application of such compounds is in the halogenation of proteins and peptides. To support this idea, we will review the state of the art of this field.

2. TUMOR-TARGETING PROTEINS – A PROMISING WAY OF INCREASING SPECIFICITY OF TUMOR THERAPY

Despite impressive progress in the treatment of tumors using surgery and external radiotherapy, cancer remains a highly lethal disease. Small tumor cell clusters and single cells can easily evade detection and remain un-

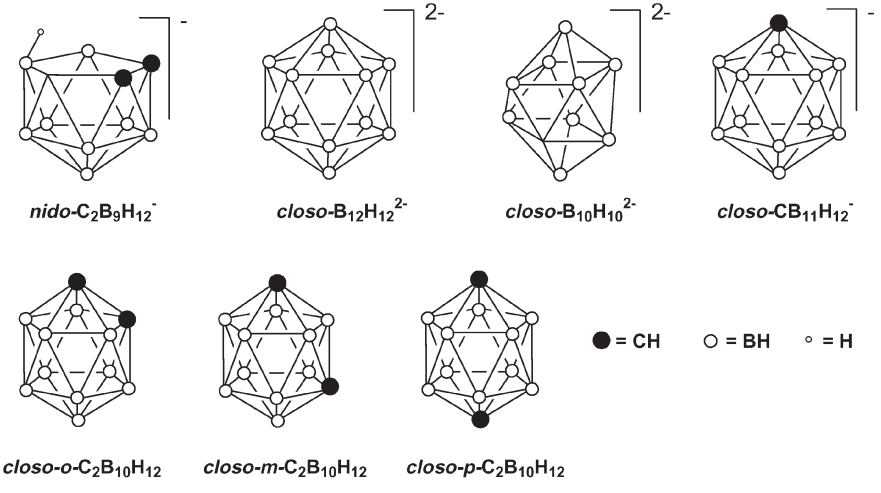

FIG. 1
Some polyhedral boron compounds of interest for labeling with radiohalogens

treated. Chemotherapy can help in this respect, but its applicability is limited due to lack of selectivity. In principle, the search for guerrilla cells can be successful with application of targeted delivery of cytotoxic substances. The cytotoxic substance (toxin, drug, or radionuclide) is conjugated to a tumor-seeking molecule, such as an antibody or signaling peptide. The use of radioactive nuclides for targeting tumor cells has apparent advantages: firstly, radioisotopes are not subject to multidrug resistance; and secondly, radiation emitted by a radionuclide delivered to a cancer cell can kill a neighboring cancer cell, even if the latter does not express an antigen (crossfire effect)[4]. Application of radioactive nuclides conjugated to tumor-seeking molecules is called "targeted radionuclide therapy". The targeting substances used in radionuclide diagnostics and therapy are of protein nature. They may be monoclonal antibodies and their fragments[5], or regulatory peptides and their analogs[6]. At present, a vast majority of nuclides used, or considered for use, in targeted radionuclide therapy decay with the emission of β-particles. Auger electron or α-particle-emitting nuclides are also under evaluation for targeting radionuclide therapy.

The same tumor-seeking substance may be used also for detection and characterization of tumors. Currently, the most efficient radiopharmaceutical is [^{18}F]-2-deoxy-2-fluoro-D-glucose ([^{18}F]FDG)[7]. This compound possesses an excellent sensitivity and is therefore useful in tumor diagnostics. However, its specificity is far from ideal, because it accumulates not only in tumors, but also in infection and inflammation foci. With the growing application of [^{18}F]FDG in oncology, there is an alarming increase in reports of false positive results. This may result in a percentage of patients with potentially curable tumors not receiving curative treatment[5]. By contrast, targeting molecules, which may be less sensitive than [^{18}F]FDG, have an exquisite specificity and can be used to discriminate between true tumors and nontumorous diseases.

In radioimmunodiagnostics, γ- or positron-emitting nuclides are required. Generally speaking, in radionuclide targeting, there is an advantage in using several nuclides with the same, or very similar, chemical properties, but with different physical properties. In this case, the same targeting molecule labeled by the same or a similar method can be used for initial detection of a tumor (*e.g.*, using a γ-emitting nuclide), for quantification of pharmacokinetics and for patient-specific dosimetry using a positron-emitting nuclide, and for therapy using a β^-- or α-emitter. From this point of view, radiohalogens are attractive. They share many chemical properties, and possesses a variety of half-lives and decay modes (Table I), which enables

optimization of half-life and emitted radiation, depending on the biomedical problem to be solved.

3. DEHALOGENATION – HISTORY AND ATTEMPTS TO AVOID IT

Historically, the first nuclide used for labeling of antibodies was ^{131}I. This nuclide is still the most widely used in targeting therapy, mainly due to availability and low cost[8]. An important aspect in the establishment of iodine isotopes as a label for proteins was their chemical properties, which enable a relatively direct simple iodination of tyrosine residues of proteins (Fig. 2) with a high yield.

Later, labeling of antibodies with radiometals using a chelating technique was introduced. The early comparative studies of the pharmacokinetics demonstrated better tumor localization of antibodies labeled with ^{111}In than with their radioiodinated counterparts[9,10]. Initially, a poor accumulation of radioiodine in tumors was ascribed to the action of ubiquitous dehalogenases[9]. It was noted that iodotyrosine, a chemical form of radioiodine label in proteins, has a structural similarity of thyroid hormones

TABLE I
Half-lines and decay properties of selected radiohalogens

Nuclide	Half-life	Mode of decay	Possible applications
^{18}F	1.8 h	β^+	PET[a]
^{75}Br	1.6 h	β^+	PET[a]
^{76}Br	16 h	β^+	PET[a]
^{77}Br	57 h	EC[b]	SPECT[c]
^{80m}Br	4.4 h	IT[d]/Auger	therapy
^{82}Br	35.3 h	β^-	therapy
^{123}I	13.2 h	EC	SPECT[c]
^{124}I	4.2 days	β^+	PET[a]
^{125}I	60 days	EC[b]	*in vitro*/therapy
^{131}I	8 days	β^-	therapy
^{211}At	7.2 h	α	therapy

[a] Positron emission tomography; [b] electron capture; [c] single photon emission tomography; [d] isomeric transition.

(Fig. 3), and therefore may be a substrate for deiodinases[11]. A number of studies were devoted to the search for nonphenolic linkers for radioiodine[12–18]. The most interesting results were obtained using derivatives of 3- and 4-iodobenzoic acid.

When such prosthetic groups were used for coupling of radioiodine to antibodies, a significant reduction in iodine uptake in the thyroid and stomach was observed, as was some elevated accumulation in tumors[19]. These results were interpreted as a confirmation of the role of dehalogenases in the decreased uptake of direct radioiodine label in tumors. However, the results of

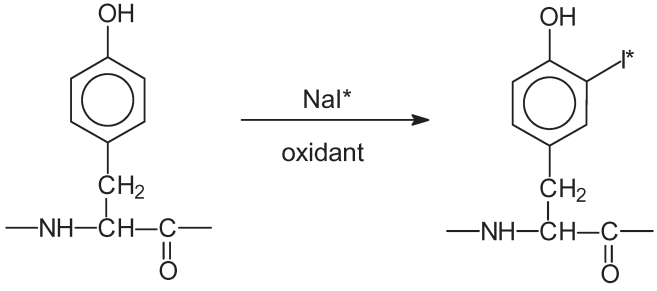

FIG. 2
Direct radioiodination of proteins. Radioactive iodide is *in situ* oxidized and attacks the activated phenolic ring of tyrosine

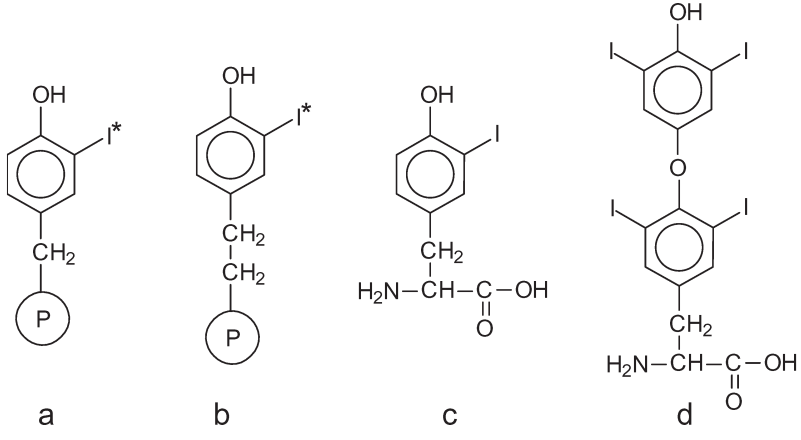

FIG. 3
Structural similarity between sites of iodination of a protein using direct oxidative iodination (a), the Bolton–Hunter method (b), and the thyroid hormones monoiodotyrosine (c) and thyroxine (d)

later investigations contradicted this theory. The results of a series of *in vitro* studies demonstrated that radioiodine leaks from cells in the form of iodotyrosine[20–23]. It was concluded that peptidases, and not deiodinases, are responsible for loss of radioiodine from cancer cells. A direct *in vitro* comparison of cellular retention of monoclonal antibodies or peptides labeled either directly, with the label on tyrosine, or indirectly using derivatives of iodobenzoic acid did not reveal sufficient improvement of retention of the radioactivity in the latter case[24,25]. On the other hand, the use of the Bolton–Hunter reagent (Fig. 3), which also has a tyrosine-like moiety, led to a reduction in thyroid and stomach uptake[26,27]. So-called "residualizing iodine labels", which contain iodinated tyramine (structurally similar to thyroid hormones) linked to the bulky saccharide part, demonstrated remarkable cellular retention[28,29].

The results of these observations may be generalized in the following way: when a tumor-seeking protein or peptide binds to a cellular structure, it is internalized. If the target structure has a receptor nature, it may be rapidly internalized in a clathrin-dependent pathway[30]. Alternatively, clathrin-independent endocytosis may take place, which is slower than clathrin- dependent endocytosis[30,31]. After internalization, the labeled protein ends up in the lysosome, where enzymatic proteolysis occurs (Fig. 4). If the label is attached to a lipophilic product of degradation, such as tyrosine, it could dissolve through the bilipid membrane of the lysosome and leave this compartment. Later, it can penetrate the cellular membrane in the same way, and escape into extracellular space. Its further fate depends on the chemical structure of the catabolite. It appears that iodotyrosine undergoes deiodination, while derivatives of iodobenzoic acid are rapidly excreted *via* the kidney. If the radiocatabolite cannot penetrate the cellular membrane, it will be trapped intracellularly until its excretion by exocytosis. Since exocytosis is relatively slow in comparison with diffusion, the cellular retention of the label is improved. We can conclude that improvement of intracellular retention and, consequently, improvement of tumor accumulation of the radiolabel should be sought by molecular design, which places the radiolabel on a structure that cannot penetrate the cellular membrane.

Textbook knowledge[32] informs us that the cellular membrane is permeable to small, uncharged polar molecules (*e.g.*, water and glycerol) and hydrophobic molecules (*e.g.*, O_2 or N_2). Large uncharged polar molecules and ions cannot penetrate the cellular membrane (Fig. 5). Conceivably, the electric charge of metal chelates is the reason for their good tumor accumulation.

Evidently, bulky hydrophilic molecules and ionic compounds are the most promising candidates for radionuclide carriers with improved cellular retention.

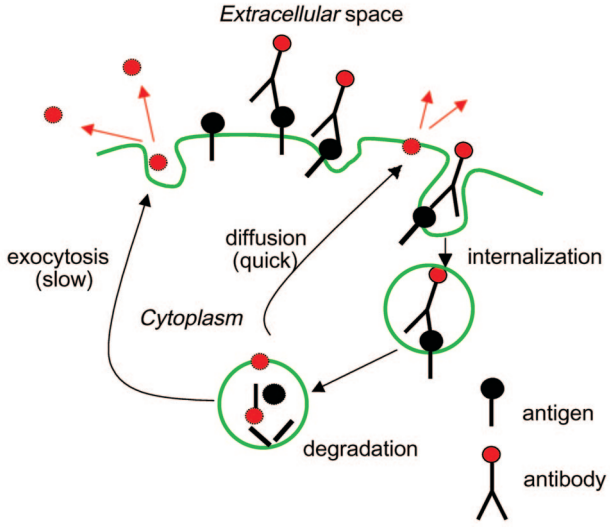

FIG. 4
Cellular processing of an antibody–antigen complex when targeting cancer cells. The antibody–antigen complex is internalized and degraded in lysosomes. If the labeled fragment is lipophilic, it diffuses quickly through the cellular membranes out of the cell. If the labeled degradation product can not diffuse through the lipid membranes, it remains inside the cell and probably undergoes relatively slow exocytosis

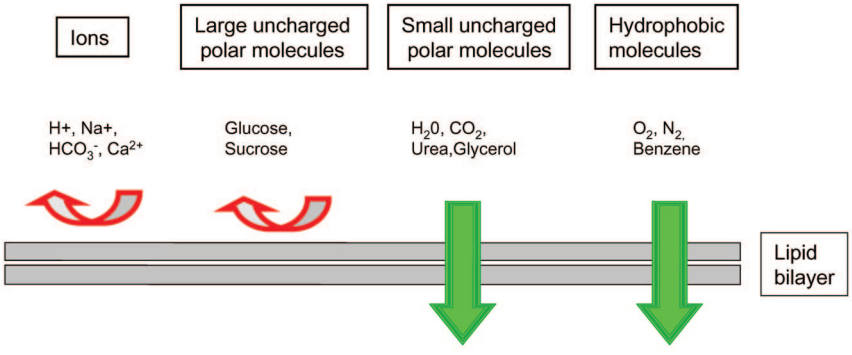

FIG. 5
Permeability of cellular membranes for various compounds

Research in these areas continues. The saccharide-based residualizing labels can be considered as an example of successful use of bulky hydrophilic groups for improvement of retention[28,29]. The most widely used compounds are conjugates of tyramine-cellobiose and dilactitol-tyramine (DLT) (Fig. 6). Results of *in vitro* studies demonstrate that this type of label provides as good a retention as do radiometal chelates[23,33]. A similar approach has been used in our laboratories by coupling of dextran to a targeting protein, with consequent conjugation of tyrosine and subsequent labeling in the saccharide part. Again, an improvement of cellular retention was obtained[34,35].

Despite the impressive results obtained, these methods have considerable restraints. The conjugation chemistry is complicated, and the low specific radioactivity of the conjugates produced limits for successful application of dilactitol-tyramine and tyramine-cellobiose labels[23].

Alternative approaches include the use of linkers, which are charged at lysosomal pH. Thus, radiolabeled *N*-succinimidyl 5-halopyridine-3-carboxylates have been used for labeling antibodies with radioiodine[16,17,36] and astatine[37]. An improvement in retention was observed *in vitro*[36], but the results of *in vivo* studies were controversial. In some cases, an improved tumor localization was observed[38], while in another study, the use of this method did not give improved tumor targeting[39].

Recently, use of *N*-succinimidyl 4-(guanidinomethyl)-3-iodobenzoate has been proposed for the same purpose. The use of this prosthetic group in the labeling of L8A4 provided a three- to four-fold improvement of radioactivity retention in comparison with either the Iodogen or the [^{125}I]SIPC-labeling methods[40]. The labeling chemistry is relatively simple and rapid. Somewhat troubling is the fact that the linker has a positive charge since it has been demonstrated that positively charged molecules are taken up pref-

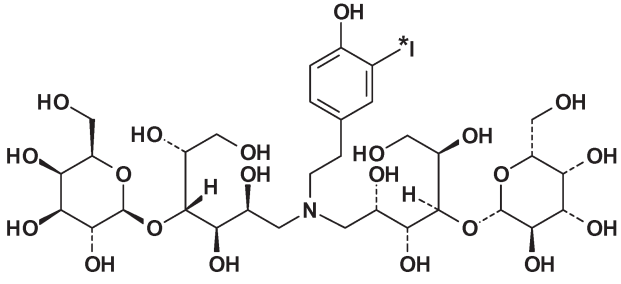

FIG. 6
Structure of radioiodinated dilactitol-tyramine (*I-DLT)

erentially in the kidneys[41–43]. It is possible that the use of positively charged prosthetic groups may lead to elevated kidney uptake and, consequently, to an increased dose burden for this radiosensitive organ. As an alternative, we propose to use PBCs as the prosthetic group.

The following features of these compounds may be important for such an application:

- high strength of halogen–boron bonds (higher than in their halogen–carbon counterparts)[44];
- most conceivably, the absence, due to the very exogenous nature of such compounds, of an enzymatic system for cleavage of the halogen–boron bond;
- also, some PBCs have a negative charge, which may improve intracellular retention of bound radiohalogens without elevated uptake in kidneys.

Though these features are attractive, they are not sufficient for the proposed application. A candidate prosthetic group for labeling of proteins should meet a number of indispensable requirements:

- possibility to synthesize derivatives, which can be conjugated to proteins;
- efficient methods for labeling the prosthetic group with radiohalogens;
- high stability of the construct in the blood circulation;
- rapid urinary excretion of radiocatabolites when they leave the tumor cell; in other words, no accumulation of catabolites in any specific organ.

Below, we will review the state of the art with regard to these requirements.

4. DERIVATIVES OF POLYHEDRAL BORON COMPOUNDS FOR ATTACHMENT TO TARGETING PROTEINS

The PBCs of interest in this context are mainly those shown in Fig. 1. To be realistic prosthetic groups, PBCs should be bifunctional, not only offering a site for binding of the radiohalogen, but also being linked to some functional group, which enables their coupling with tumor-seeking molecules. Fortunately, the problem of targeted delivery of boron into tumors has for several years been investigated for BNCT. Radionuclide targeting can in many cases utilize compounds which have been developed for use as boron carriers in BNCT. A few examples are given below.

The chemistry most thoroughly investigated is that of the carboranes (1,2-, 1,7-, and 1,12-*closo*-$C_2B_{12}H_{12}$; Fig. 1). These compounds contain within the boron cage two carbon atoms, which can be used for further functionalization. One example is the coupling with the tumor-seeking

peptide epidermal growth factor (EGF). The amino group of the diol-amino-carborane 2-{[2-(3-aminopropyl)-1,2-dicarba-*closo*-dodecacarboran(12)-1-yl]methoxy}propane-1,2-diol, designated as DAC-1 (Fig. 7) was treated with Traut's reagent to produce iminothiolated protein, which was then linked to EGF pretreated with *N*-[(3-maleimidobenzoyl)oxy]sulfosuccinimide[45].

Another interesting approach is the preparation of carboranyl amino acids (Fig. 7) and their subsequent incorporation into peptides. Work on the synthesis and evaluation of biological properties of polyhedral borane-containing amino acids including biological work on peptides containing *o*-carboranylalanine (*o*-Car) has recently been reviewed[46–50]. Alternatively, polycarboranyl amino acids can be synthesized and coupled with monoclonal antibodies[51–53].

Yet another example is the coupling of a radioiodine-labeled 4-isothiocyanatophenyl derivative of dodecahydro-7,8-dicarba-*nido*-undecaborate(–) with an antibody for a study of the pharmacokinetics of this boronated protein (Fig. 8)[2]. The same compound has also been used for astatination of streptavidin for two-step tumor targeting[54].

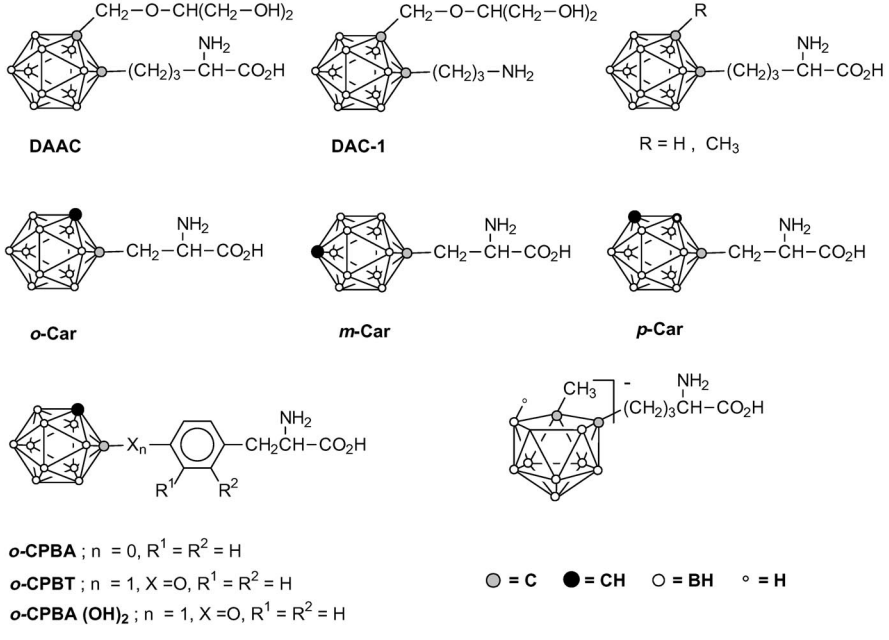

FIG. 7

Some carborane derivatives which can be used for incorporation of boron clusters into or attachment to proteins and peptides

Absence of carbon atoms in *closo*-dodecaborate ($B_{12}H_{12}^{2-}$) and *closo*-decaborate ($B_{10}H_{10}^{2-}$) complicates the synthesis of derivatives which could be used for coupling with tumor-seeking peptides and proteins. However, intensive research in this area has enabled the production of some compounds of interest. For instance, Alam *et al.*[55] report direct coupling of sulfanylundecahydro-*closo*-dodecaborate(2–) anion (BSH, Fig. 9) with antibodies. Between nine and thirteen molecules of the anion could be linked to one molecule of the antibody without losing immunoreactivity. Although the number of boron atoms in this case was not sufficient for BNCT, it would be more than sufficient for carrying radiohalogens.

Nagasawa and Narisada[56] describe the synthesis of carboxylic acid derivative of BSH derivative, which contains a carboxy group. An active

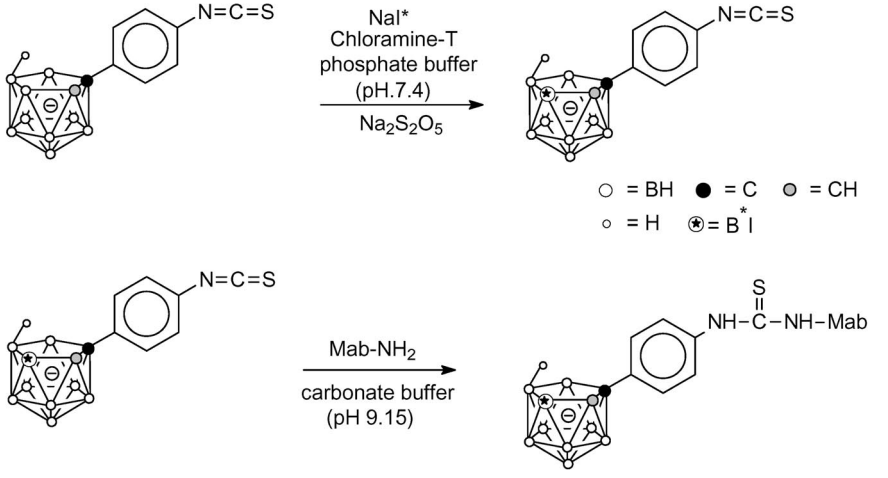

FIG. 8

Indirect labeling of an antibody using a *nido*-carborate derivative (regio- and stereoisomers are not shown for simplicity). Mab is a monoclonal antibody

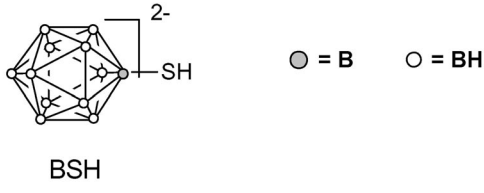

FIG. 9

Structure of the sulfanylundecahydro-*closo*-dodecaborate(2–) anion (BSH)

N-succinimidyl ester was prepared, which was then used for coupling with peptides and antibodies (Fig. 10).

A BSH derivative of dextran has also been attached to EGF [57]. The conjugate contained a high number of boron cages.

A number of *closo*-dodecaborate derivatives suitable for conjugation to proteins have recently been synthesized. In this regard, methods for synthesis of a number of oxonium derivatives of $[B_{12}H_{12}]^{2-}$ have been reported (Sivaev *et al.* 2000)[58]. The series includes oxonium derivatives containing amino and carboxyl functional groups, as well as an amino acid derived from *closo*-dodecaborate (Fig. 11).

These compounds may be built-in by various methods, either into synthetic peptides or coupled together with large tumor-seeking proteins including monoclonal antibodies. Another series of the reagents, which were produced by the same research group, includes monosubstituted amines of *closo*-dodecaborate obtained by reduction of the corresponding Schiff bases[59]. Examples of such compounds include the carboxy, amino, and isothiocyanate (DABI) derivatives shown in Fig. 12. In a pilot study by Tolmachev *et al.*[60], the isothiocyanate DABI [(4-isothiocyanatobenzoyl)amino]undecahydro-*closo*-dodecaborate was radioiodinated and coupled with an albumin.

In conclusion, it could be stated that methods for synthesis of bifunctional derivatives of PBCs exist, and that these derivatives can be utilized for radiohalogenation of tumor-targeting proteins and peptides.

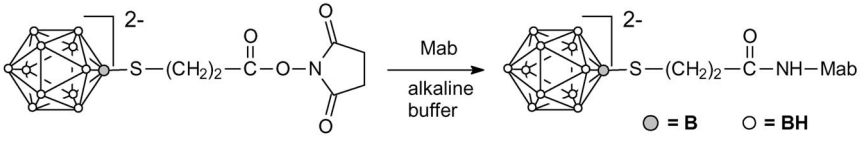

FIG. 10
Coupling of a sulfanylundecahydro-*closo*-dodecaborate derivative to an antibody (Mab)

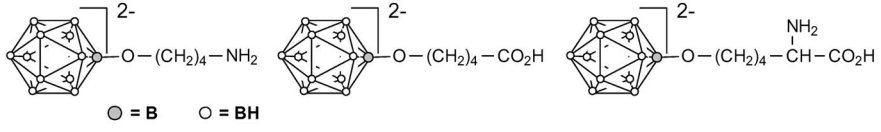

FIG. 11
Oxonium derivatives of *closo*-dodecaborate, which might be used for coupling of *closo*-dodecaborate to proteins

5. LABELING OF POLYHEDRAL BORON ANIONS

Detailed knowledge of the labeling chemistry of bifunctional compounds is another very important precondition for their use as a prosthetic group. Among PBCs, the labeling chemistry of *nido*-carborate ($C_2B_9H_{12}^-$; Fig. 1) is the one most thoroughly studied.

Nonradioactive iodination of the *nido*-carborate cage was first described 1965 by Olsen and Hawthorne[61] and, as has been mentioned previously, a *nido*-carborate derivative was the first radiohalogenated compound used for indirect radiohalogenation of proteins. Oxidative radioiodination using Chloramine T as an oxidant was described by Mizusawa *et al.* (Fig. 8)[2]. However, this compound was not used for radioimmunodetection but for pharmacokinetic studies of boronated antibodies for use in BNCT [62].

Later, the radioiodination chemistry of *nido*-carborates was studied in detail by Wilbur and coworkers[63]. It was found that radioiodination can easily be accomplished by using *N*-chlorosuccinimide as the oxidant. Under these conditions, the reactivity of *nido*-carborate was found to be 50 times higher than that of tyrosine.

Initial radioiodination attempts were directed at labeling small molecules, such as the diastereomeric mixture of *nido*-carborate-substituted 2-nitroimidazoles (Fig. 13) for potential use in BNCT and in scintigraphy for detection of tumor hypoxia[3], or for preparation of biotin conjugates for two-step targeting[64].

nido-Carborate derivatives were also suggested as a pendant group for direct iodination of proteins, which lack tyrosine residues or in which the tyrosine residues are buried inside the protein structure[65]. It was noted that

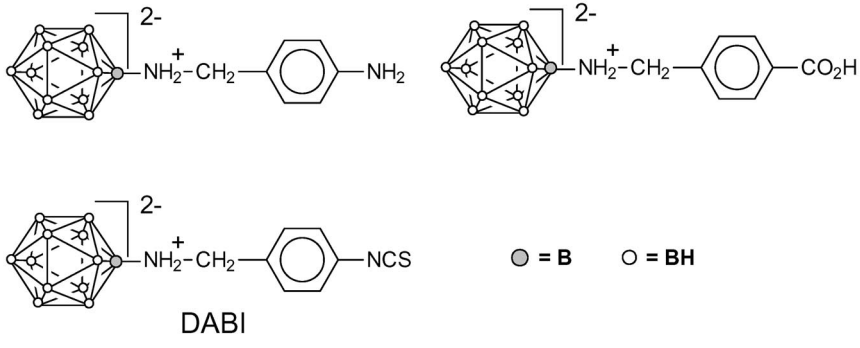

FIG. 12
Amino derivatives of *closo*-dodecaborate, which might be used for coupling of *closo*-dodecaborate to proteins

the radioiodination of carbon-based pendant groups is complicated if a thiourea bond is involved in the conjugation, whereas *nido*-carborate derivatives can easily be iodinated[64].

In our laboratories, a *nido*-carborate derivative has been coupled to dextran and was investigated as a potential residualizing label for peptides[66]. In Tolmachev *et al.*[66], dextran was oxidized with metaperiodate, and an amino derivative of *nido*-carborate (7-(3-aminopropyl)-7,8-dicarba-*nido*-undecaborate(–), ANC) was coupled to dextran using reductive amination (Fig. 14).

This conjugate was radioiodinated using Iodogen in a high yield (69–85%). A comparative study of *in vitro* stability has been performed demonstrating that ANC–dextran conjugate remains stable in rat liver homogenate for at least 24 h, while albumin is rapidly degraded under these conditions. The high reactivity of *nido*-carborate gives the possibility of directing the radioiodine label predominantly on the residualizing part of the protein–dextran conjugate, when the one-step labeling procedure is used.

Of special interest is the use of *nido*-carborate as pendant groups for attachment of the α-emitting radionuclide ^{211}At to tumor-seeking biomolecules. Due to the character of the emitted radiation, ^{211}At has a high potential for eradicating tumors[67]. The problem is that direct oxidative astatine labeling of proteins gives a very weak astatine–protein bond, which prohibits its application in targeted radionuclide therapy[67,68]. This problem

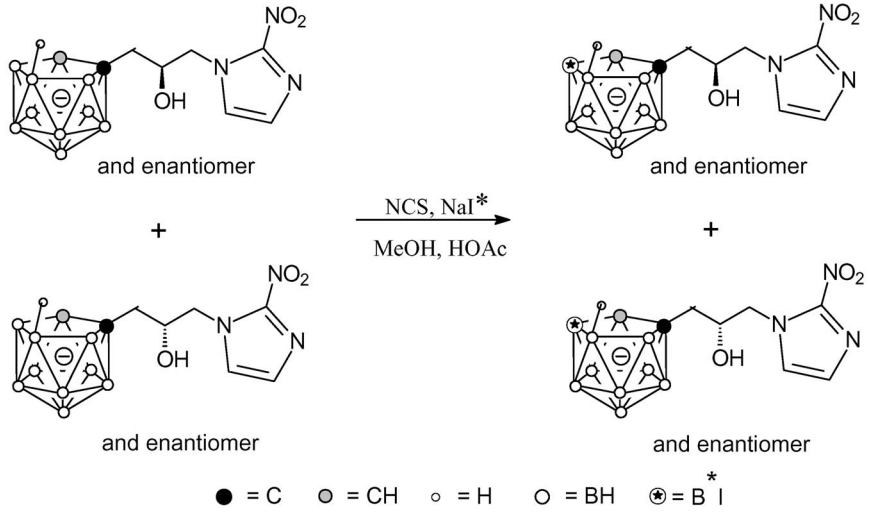

FIG. 13
Labeling of a *nido*-1,2-carboranyl derivative of 2-nitroimidazole for BNCT and hypoxia imaging

may be circumvented by astatine labeling of *N*-succinimidyl 3-(tributylstannyl)benzoate[15] or *N*-succinimidyl 5-(tributylstannyl)pyridine-3-carboxylate[37], followed by conjugation to the targeting protein. However, all *in vivo* distribution studies performed so far indicate a release of astatine from the targeting conjugate[69]. It may be that the use of PBCs would provide a more stable attachment of astatine to the prosthetic group than to aromatic prosthetic groups. Wilbur and coworkers, concentrating on the use of *nido*-carborate derivatives for astatination of biotin[70,71], found that *nido*-carborate can be directly labeled in almost quantitative yield, while the astatodestannylation reaction on arylstannanes gave a yield of only 50%.

In our laboratories, the *nido*-carborate derivative ANC (Fig. 14) was used for direct astatination of the tumor-seeking protein EGF [72,73]. Using two different conjugation methods, glutaraldehyde crosslinking (Fig. 15) and treatment with Traut's reagent and subsequent linking of ANC with *N*-[(3-maleimidobenzoyl)oxy]sulfosuccinimide (Fig. 16), ANC was attached to a peptide. The conjugates were astatinated using Chloramine T in high yields. The average labeling yield (70.5 ± 2.2%) using the glutaraldehyde conjugate was higher than that obtained by indirect labeling using *N*-succinimidyl astatobenzoate (44.1 ± 3.6%). *In vitro* stability tests indicated that the introduced label was as stable as was EGF labeled with

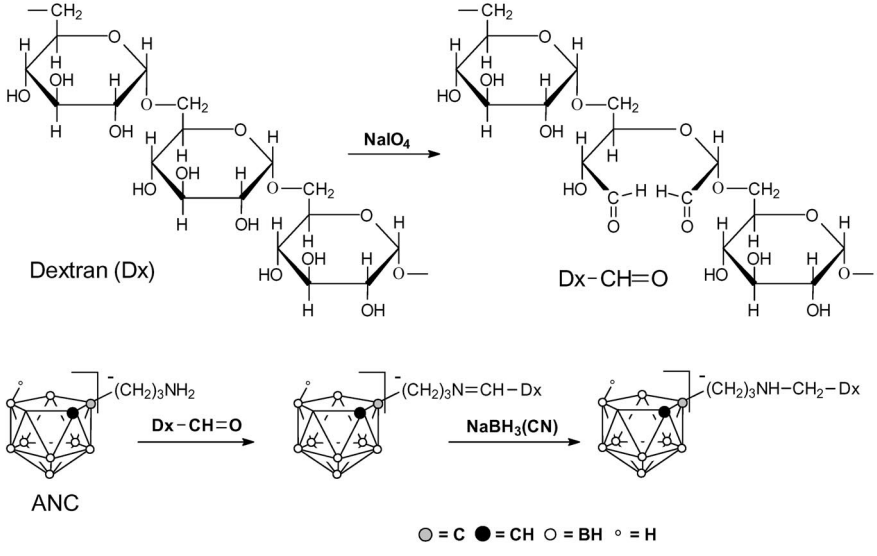

FIG. 14
Coupling of the amino *nido*-carborate ANC to dextran for radioiodination

astatobenzoate. It should be emphasized that the use of ANC in this case provided not only the higher yield and simpler labeling procedure, but also a potential for better intracellular retention of labeled catabolites.

Up to the present time, the labeling chemistry of carboranes has mainly concentrated on *nido*-carborates. It should be noted that other classes of carboranes (*e.g.* the three *closo*-$C_2B_{10}H_{12}$ compounds and the $CB_{11}H_{12}^-$ anion; Fig. 1) can also be halogenated[74–77], which opens the way for their radiohalogenation. However, physicochemical properties of carboranes should be taken into account for a sound choice of prosthetic group. For example, the *closo*-dicarboranes are fairly lipophilic. For this reason, a label attached to protein *via closo*-dicarboranes would most likely rapidly diffuse away from the tumor cell after degradation of the targeting protein. Although a derivative of 1,12-dicarba-*closo*-dicarborane has been radioiodinated[78], we believe that this kind of labeling can be performed in the study of

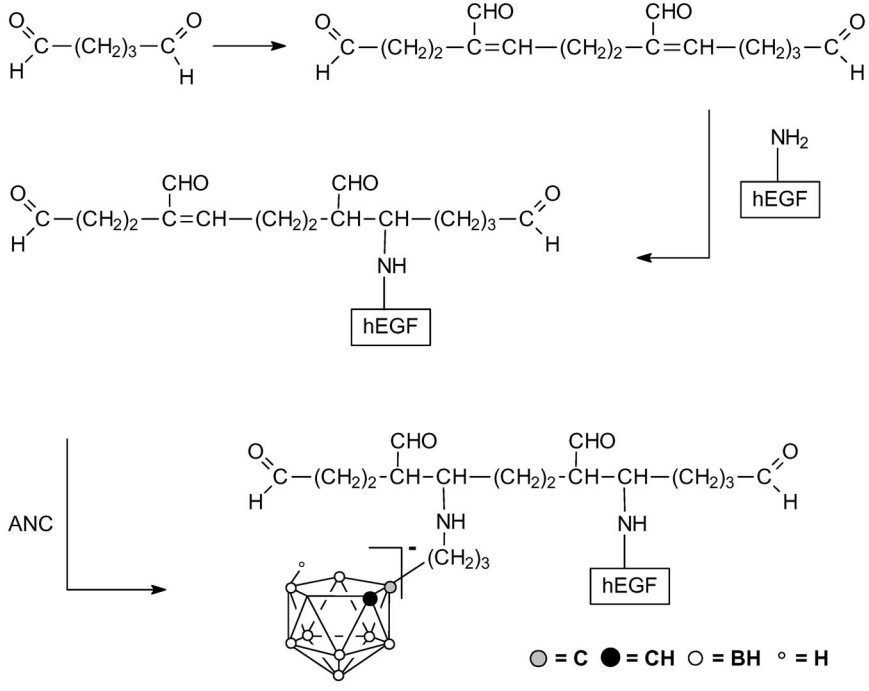

FIG. 15
Sketch showing the conjugation of hEGF (human EGF) and ANC (Fig. 14) using glutaraldehyde crosslinking. Glutaraldehyde is polymerized during the conjugation, which allows conjugation of hEGF and ANC

pharmacokinetics of boron-rich substances in BNCT, but not in tumor imaging or therapy.

On the other hand, the *closo*-monocarborane $CB_{11}H_{12}^-$ is negatively charged and may provide some advantages in intracellular retention of radiohalogens. Recently, the feasibility of radioiodination of *closo*-monocarborane has been established[79] by use of an amino derivative of $CB_{11}H_{12}^-$. In principle, such derivatives could be converted into the corresponding isothiocyanate, which has a potential for coupling with tumor-seeking proteins.

The progress in the derivatization of the *closo*-dodecaborate $B_{12}H_{12}^{2-}$ (Fig. 1), which opens the possibility of its conjugation to tumor-targeting substances, prompted our interest in its use as a pendant group for radio-

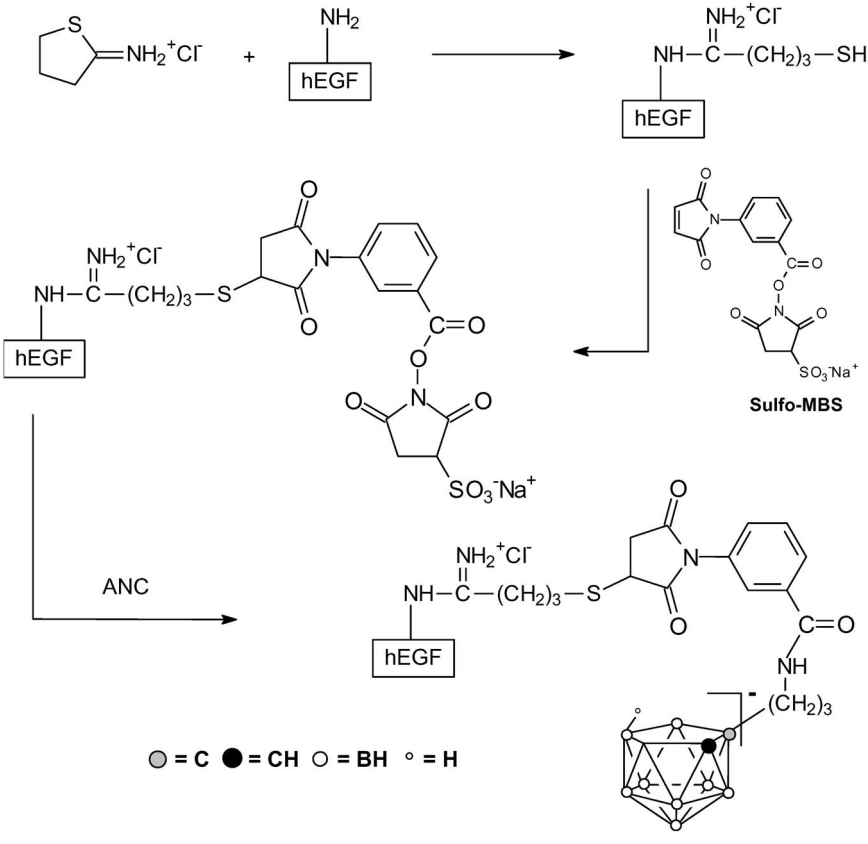

FIG. 16
Conjugation of hEGF (human EGF) and ANC (Fig. 14) using Traut's reagent and Sulfo-MBS. hEGF is thiolated with Traut's reagent and is then coupled to Sulfo-MBS in order to introduce a good leaving group that finally allows conjugation of ANC

halogenation, and the feasibility of radiohalogenation of $B_{12}H_{12}^{2-}$ was recently demonstrated[80,81]. It was found that *closo*-dodecaborate, as well as a conjugate formed by reacting allylated dextran with sulfanyl-*closo*-dodecaborate, could be radioiodinated in a high yield (*ca* 90%) using either Chloramine T or Iodogen (Fig. 17). An interesting finding was that at pH 5, the radioiodine label is directed to *closo*-dodecaborate, even if the labeling was carried out in the presence of tyrosine. This creates a potential for single-step labeling of tyrosine-containing conjugate into the residualizing part.

Recently, the factors determining the efficiency of the radioiodination chemistry of $B_{12}H_{12}^{2-}$ in aqueous solutions using Chloramine T have been studied[82]. The reaction was rapid (>90% yield in 30 s) and efficient in a wide range of pH (4–7.4). As little as 1 nmol of *closo*-dodecaborate can therefore be iodinated with a high yield.

DABI, a derivative of *closo*-dodecaborate, [(4-isothiocyanatobenzyl)amino]-undecahydro-*closo*-dodecaborate, has recently been radioiodinated with a yield of 93–95%, and coupled to a model protein bovine serum albumin, with an overall yield of 45–64%, depending on the protein-pendant group ratio (Fig. 18)[83,84]. Labeling of another *closo*-dodecaborate isocyanate derivative has recently also been reported[85].

Bromine isotopes show a variety of decay schemes and may be utilized in different areas of diagnostics and therapy. The decay properties of ^{76}Br make this compound an interesting candidate for use in diagnostics, whose nuclear properties make it interesting for radioimmuno-positron emission tomagraphy (PET)[86]. However, this nuclide not only shares the problem of poor intracellular retention with the other halogens, but also shows an un-

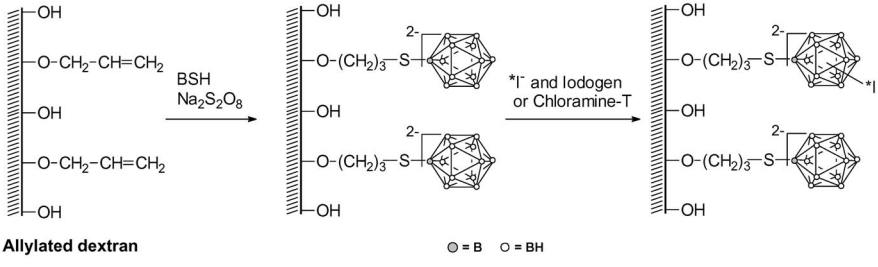

FIG. 17
Conjugation of BSH (Fig. 9) to allylated dextran and subsequent radioiodination of the dodecahydro-*closo*-dodecaborate(2–) cage in the conjugate

favorable distribution of radiocatabolites. The main catabolite, the radiobromide anion, is not excreted but remains distributed in the extracellular space, decreasing the contrast of the image. We have previously demonstrated that radioiodinated $B_{12}H_{12}^{2-}$ is very rapidly excreted *via* the kidneys[79]. One can expect that the same would be the case with its radiobrominated analog. The use of *closo*-dodecaborate as a pendant group would solve not only the problem of improving cellular retention, but also that of excretion of radiocatabolites after exocytosis.

As an initial step in the development of such a label, we studied the chemistry of *closo*-dodecaborate bromination in aqueous media[87,88]. Radiobromination of *closo*-dodecaborate was slower than radioiodination and took at least 3 min for completion. This may due to the fact that it is more difficult to oxidize bromine than iodine. On the other hand, the labeling yield was high, >90% in the pH range 2.5–7.0 and slightly less at pH 7.4. The encouraging results of this study have prompted continued research in this direction.

The use of *closo*-dodecaborate for coupling astatine to tumor-seeking proteins has also been proposed[89] and the influence of the major reaction parameters on the labeling efficiency was studied. A labeling yield of 75% was obtained; however, ^{211}At was found to be a capricious label. For example, the radiolysis of the solvent resulted in formation of oxidizing products, which provoked labeling even without the addition of oxidant. This phenomenon was later confirmed by astatine-induced iodination of *closo*-dodecaborate[90].

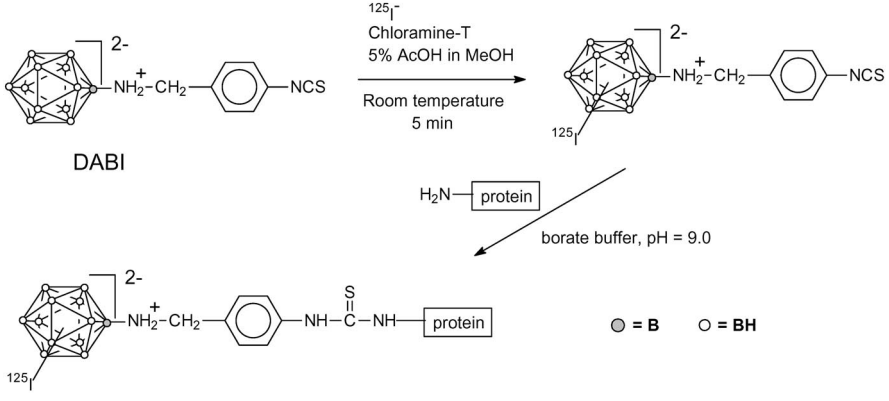

FIG. 18
Radioiodination of DABI and subsequent coupling to a model protein bovine serum albumin

6. PHARMACOKINETIC PROPERTIES OF RADIOHALOGENATED POLYHEDRAL BORON COMPOUNDS

Although a number of studies have been published on the labeling chemistry of PBCs, biological data are insufficient. However, the data in Tolmachev *et al.*[78] are encouraging. The results of this group's study of a *closo*-dodecaborate-derived BSH–dextran conjugate (Fig. 17) indicate stable intracellular retention after trapping by hepatocytes. Injection of both iodinated *closo*-dodecaborate-dextran conjugate and iodinated *closo*-dodecaborate in rats caused very little accumulation of radioiodine in the thyroid, which demonstrates high stability of such labels in the blood circulation. Iodinated *closo*-dodecaborate was very rapidly excreted *via* the kidney. If iodo-*closo*-dodecaborate is the major radiocatabolite of targeted proteins, such behavior would improve their imaging and therapeutic properties. It should be noted that more biological studies are required for validation of PBCs as linkers for radiohalogens.

7. CONCLUSIONS

The chemical and physicochemical properties of PBCs render these compounds promising candidates as pendant groups for linking of radiohalogens to tumor-targeting proteins and peptides. Methods for conjugation of PBCs to targeting proteins are available mainly as a result of developing the chemistry for BNCT. The radiohalogenation chemistry of many PBCs is being investigated. However, careful comparative biological characterization of targeting proteins labeled using PBCs is necessary before such compounds can be introduced into clinical practice.

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