Synthesis of Organic Compounds for Nuclide Therapy

Derivatives of Carboranes, 9-Aminoacridine and Anthracyclines

BY

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Abstract

This thesis addresses the synthesis of organic compounds, some of them are derivatives of compounds with DNA binding properties, for potential use in targeted nuclide therapy. The compounds synthesized therefore also need to contain potent nuclides. Here the nuclides considered are the radionuclide $^{121}$I, and the stable isotope $^{10}$B, which becomes radioactive upon neutron activation. $^{121}$I is an Auger-electron emitter, which emits particles that can travel only about 1-2 µm through human tissue and hence has to be delivered to the cancer cell nucleus to cause DNA damage. Neutron activated $^{10}$B emits highly cell killing α-particles and $^{1}$Li$^{+}$ ions, the application of which in Boron Neutron Capture Therapy (BNCT) has proven very promising.

The thesis can be divided into three parts:

i) A nido-carborate, 7-(3'-ammoniopropyl)-7,8-dicarba-nido-decaborate(-I), has been synthesized and radioiodinated for use as a pendant group for attachment of $^{121}$I to tumor-seeking macromolecules. Radiolabeling was achieved in greater than 95% yield.

ii) Both enantiomers of m-carboranylalanine, a carborane analogue of phenylalanine, have been prepared in high enantiomeric excess, and are of potential interest in BNCT. The synthesis involved amination of the N-acyl derivative formed from [3-(1,7-dicarba-closo-dodecarborene(12)-1-yl)]-2-propanoic acid and Oppolzer’s camphor sultam.

iii) Derivatives of the DNA intercalating compounds 9-aminoacridine, daunorubicin and doxorubicin have been synthesized and labeled with $^{121}$I. The 9-aminoacridines were synthesized with a variety of functional groups such as carboxyl, amino and hydroxyl. The anthracyclines daunorubicin and doxorubicin are efficient chemotherapeutic agents; the synthesis routes of ester, amide and amine derivatives of these compounds are presented.

The Chloramine T method was used for the radioiodinations, and the radioiodination precursors of both the acidine and the anthracycline derivatives, were made to contain either a trimethylstannyl group or a phenolic substituent. In the former case the trimethylstannyl group was replaced by $^{121}$I, and in the latter case, the compounds were radiolabeled directly at the α-position to the phenolic hydroxyl group. Both methods gave high radiolabeling yields.

Keywords: 9-Aminoacidin, Carborane, Daunorubicin, Doxorubicin, BNCT, Iodine-125

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“Our imagination is the only limit to what we can hope to have in the future.”

Kettering, C.
List of Papers

This thesis is based on the following papers, referred to in the text by their Roman numerals I-V


II Enantioselective synthesis of m-carboranylalanine a boron-rich analogue of phenylalanine. Charlotta Naeslund, Senait Ghirmai, Stefan Sjöberg. *Submitted*


V Synthesis and radioiodination of some daunorubicin and doxorubicin derivatives. Senait Ghirmai, Eskender Mume, Vladimir Tolmachev, Stefan Sjöberg. *Submitted*
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### Abbreviations

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<th>Abbreviation</th>
<th>Definition</th>
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</thead>
<tbody>
<tr>
<td>AcOH</td>
<td>Acetic acid</td>
</tr>
<tr>
<td>BNCT</td>
<td>Boron neutron capture therapy</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-Butyloxycarbonyl</td>
</tr>
<tr>
<td>BPA</td>
<td>p-Boronophenylalanine</td>
</tr>
<tr>
<td>BSH</td>
<td>Mercapto-undecahydro-closo-dodecaborate(-2)</td>
</tr>
<tr>
<td>BuLi</td>
<td>Butyl lithium</td>
</tr>
<tr>
<td>CAT</td>
<td>N-chloro-p-toluenesulfonamide, Chloramine-T</td>
</tr>
<tr>
<td>DCC</td>
<td>Dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>EC</td>
<td>Electron capture</td>
</tr>
<tr>
<td>EGF</td>
<td>Epidermal growth factor</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionization</td>
</tr>
<tr>
<td>HPLC</td>
<td>High pressure liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>High resolution mass spectroscopy</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NOE</td>
<td>Nuclear Overhauser effect</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>( R_f )</td>
<td>Fraction of retention</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>( t\text{-BDMSCl} )</td>
<td>\textit{tert}-Butyldimethylsilylchloride</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin layer chromatography</td>
</tr>
</tbody>
</table>
Populärvetenskaplig sammanfattning på svenska

Många typer av elakartade tumörer kan botas med konventionella metoder såsom kirurgi, bestrålning med yttre strålkälla eller behandling med cellgifter. Dessa metoder har emellertid begränsad framgång vid behandling av vissa tumörtyper med spridda tumörceller.

Målsökande radionuklidterapi är en behandlingsform som tilldrar sig allt större intresse i detta sammanhang. Här kan man utnyttja förhållandenat tumörceller kan ha en större förmåga att binda vissa antikroppar och peptider som därmed kan fungera som "målsökare". Till målsökaren kan man koppla en bärarmolekyl märkt med radioaktiva nuklider, eller stabila nuklider som görs radioaktiva genom att utsättas för lågenergetiska neutroner. Med hjälp av målsökaren kan nuklider transportereras till tumörceller och där avge sin strålning lokalt med celldöd som följd och med förhoppningsvis minimal skada av friska celler. Detta kan leda till att tumörer som tidigare enbart kunnat behandlas med smärtstillande ambition kanske kan komma att behandlas med ambitionen att bota.

För att forskning inom detta område skall kunna leda till kliniska tillämpningar krävs samarbete mellan olika ämnesområden. Denna avhandling är ett exempel på kemistens bidrag till sådan forskning.

I avhandlingen beskrives framställning av bärarmolekyler för två nuklider, dels I-125 som är en radioaktiv isotop av grundämnet jod, dels B-10 som är en aktiverbar isotop av grundämnet bor.

Kemiska föreningar innehållande nukliden B-10 har potentiell användning vid "bor-neutroninfängningsterapi" (Boron Neutron Capture Therapy, BNCT). Denna nuklid sönderfaller efter neutronaktivering till alfapartiklar och litiumjoner. Dessa avger sin
energi inom ett avstånd på circa en celldiameter och dödar de målsökta cellerna. Den andra nukliden, I-125 utsänder vid sitt radioaktiva sönderfall sk Augerelektroter, dessa har mycket kort räckvid men kan, om sönderfallet sker nära cellens DNA, orsaka celldöd.

Avhandlingen kan delas in i tre avsnitt:

i) Framställning och I-125-märkning av en organisk förening, ett sk nido-karborat, som kan bindas till antikroppar eller andra tumörsökande molekyler.

ii) Framställning av aminosyran m-karboranylalanin, med potentiell användning inom BNCT.

iii) Framställning av substanser som är härledda från föreningarna 9-aminoakridin, daunorubicin och doxorubicin med känd DNA-bindande förmåga. De framställda substanserna har mäkts med I-125 med avsikten att användas vid målsökande radionuklidterapi.
1. Introduction

1.1. Nuclide therapy

Most types of malignant tumors are treated using conventional treatment methodologies, such as surgery, external radiotherapy, and chemotherapy. However, these methods have limited success in the treatment of disseminated adenocarcinomas (e.g. breast cancer) and also of certain forms of locally spread brain tumors, such as glioblastomas. New therapeutic modalities are therefore worth considering in order to achieve better results.

Nuclide therapy is one approach that has recently gained considerable attention. It is based on the use of cytotoxic nuclear radiation for eradication of malignant cells. This radiation can be generated by radioactive nuclides or by stable nuclides, for example $^{10}$B and $^{157}$Gd, after neutron activation. If such nuclides could selectively be delivered to malignant cells, they would destroy these cells, while causing a minimum of damage to healthy tissues.

This thesis describes the synthesis of carborane-containing molecules for boron neutron capture therapy (BNCT), synthesis and radiolabeling of carboranes where the boron cage is used as a prosthetic group for the radionuclide; and synthesis and radiohalogenation of derivatives of the DNA intercalators, acridine and antracycline, for potential use in targeted nuclide therapy of cancer.
1.1.1. Radionuclides

Every radionuclide has a distinct set of nuclide properties such as half-life and emitted radiation (see Table 1.1) and different types are selected depending on the nature of the clinical problem. The physical half-life of the radionuclide has to be adjusted to the biological half-life of the labeled tumor seeking compound. When choosing a radionuclide for therapy, it is important that the emission characteristics of the radionuclide match the size and location of the particular tumor. High-energy beta emitters, such as $^{90}$Y can be used for treatment of large tumors. Other nuclides, like $^{131}$I, have lower beta energy and consequently shorter range and are more efficient for smaller tumors or even for single cells. Auger electron emitters, such as $^{125}$I and $^{123}$I, emit particles that can travel only about 1–2 µm and hence have to be inside the cancer cell nucleus to cause DNA damage. Alpha particle emitters are somewhere in between: the particles they emit travel in the range of 50–70 µm and locally deposit a large amount of energy.2

1.1.1.1. Radiohalogens

Radiohalogens have a wide range of half-lives and radiation emissions which make them attractive for various purposes (Table 1.1). This group of nuclides is particularly useful because their chemistry is well understood (except in the case of astatine). They form stable covalent bonds with carbon atoms in biomolecules, and they give high specific radiolabeling activity compared to most other types of radionuclides.3

The beta emitter, $^{131}$I is one of the most commonly used radionuclide for therapy. Positron emitting radiohalogens, such as $^{18}$F, $^{76}$Br, and $^{124}$I, can be used for diagnostics using positron emission tomography; and offer the advantages of allowing the assessment of patient radiation dosimetry to tissues and monitoring changes in tumor volume through PET scans.2,3
Astatine-211 is a radiohalogen that may potentially be of use in some therapeutic applications. It has a relatively short half-life (7.2 hours) and decays by α-particle emission, which is extremely cytotoxic. Thus, it could be an effective therapeutic agent if delivered specifically to the target cell.

Table 1.1. Data on decay characteristics of some radiohalogens

<table>
<thead>
<tr>
<th>Radionuclide</th>
<th>Half-life</th>
<th>Mode of decay</th>
</tr>
</thead>
<tbody>
<tr>
<td>¹⁸F</td>
<td>1.8 h</td>
<td>β⁺</td>
</tr>
<tr>
<td>⁷⁶Br</td>
<td>16 h</td>
<td>β⁺, EC</td>
</tr>
<tr>
<td>⁷⁷Br</td>
<td>56 h</td>
<td>EC</td>
</tr>
<tr>
<td>¹²³I</td>
<td>13.2 h</td>
<td>EC</td>
</tr>
<tr>
<td>¹²⁴I</td>
<td>4 d</td>
<td>EC, β⁺</td>
</tr>
<tr>
<td>¹²⁵I</td>
<td>60 d</td>
<td>EC</td>
</tr>
<tr>
<td>¹³¹I</td>
<td>8 d</td>
<td>β⁻</td>
</tr>
<tr>
<td>²¹¹At</td>
<td>7.2 h</td>
<td>α</td>
</tr>
</tbody>
</table>

Auger electron emitters are effective at killing cells if they can be incorporated into the cell nucleus. ¹²⁵I (half-life 60 days) and ⁷⁷Br (half-life 56 hours) decay by electron capture with the following emission of Auger electrons. Most of the compounds discussed in this thesis are labeled with ¹²⁵I. Its long half-life makes it interesting for in vitro applications, the fact that it also emits low-energy photons makes it convenient to work with, and its cost and availability are reasonable. ¹²³I (half-life, 13.2 hours) is also an Auger electron emitter. It is an attractive candidate for therapeutic use because of its short half-life; moreover, its γ-emission is easily imaged.⁴

1.1.2. Boron neutron capture therapy

BNCT is a binary therapy of cancer in which patients are first given a non-toxic ¹⁰B-enriched substance that becomes selectively localized in the tumor cells. The substance is then irradiated with low-energy neutrons which are “captured” by the ¹⁰B atoms. The nuclear reaction between ¹⁰B and the
neutrons produces an excited form of $^{11}$B, which instantaneously disintegrates into two highly cytotoxic particles, namely He and Li ions (Scheme 1.1). The range of these ions in tissue is approximately 9 and 5 µm, respectively, similar to the cell diameter.

$$^{10}\text{B} + ^1\text{n} \rightarrow ^{11}\text{B}^*$$

$$^{4}\text{He}^{2+} + ^7\text{Li}^{3+} + 2.79 \text{MeV (6%)}$$

$$^{4}\text{He}^{2+} + ^7\text{Li}^{3+} + 0.48 \text{MeV} + 2.31 \text{MeV (94%)}$$

Scheme 1.1. The boron neutron capture reaction

The ability of an atom to capture a neutron is related to the effective cross-sectional area of its atomic nucleus. Naturally occurring boron contains 80% $^{11}$B and 20% $^{10}$B. The extremely small cross section of $^{11}$B (0.0042 barn, 1 barn = $10^{-24}$ cm$^2$) renders it not interesting for boron neutron capture reactions. However, the cross-section of $^{10}$B is rather large (3837 barn) compared to those of elements found in the tissue. Thus, compounds prepared for BNCT are enriched with boron-10. Despite the fact that $^{14}$N and $^1$H have low cross-sectional areas (Table 1.2), these two elements contribute a significant radiation dose to the body during neutron capture therapy, due to their high concentration in living tissue. Consequently, a high concentration of $^{10}$B in the tumor cells is essential for the treatment dose to exceed the background radiation level.
Table 1.2. Thermal neutron capture cross-section values of tissue elements together with their weight percentages in tissue

<table>
<thead>
<tr>
<th>Nuclide</th>
<th>Weight (% in tissue)</th>
<th>Nuclear cross section (in barn)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>10.00</td>
<td>0.33</td>
</tr>
<tr>
<td>$^{12}$C</td>
<td>18.0</td>
<td>$3.4 \times 10^{-3}$</td>
</tr>
<tr>
<td>$^{14}$N</td>
<td>3.0</td>
<td>1.82</td>
</tr>
<tr>
<td>$^{16}$O</td>
<td>65.0</td>
<td>$1.8 \times 10^{-4}$</td>
</tr>
<tr>
<td>$^{23}$Na</td>
<td>0.11</td>
<td>0.43</td>
</tr>
<tr>
<td>$^{31}$P</td>
<td>1.16</td>
<td>0.18</td>
</tr>
<tr>
<td>$^{32}$S</td>
<td>0.2</td>
<td>0.53</td>
</tr>
<tr>
<td>$^{35}$Cl</td>
<td>0.16</td>
<td>43</td>
</tr>
</tbody>
</table>

The neutron exposure is limited by the tolerance of the normal tissue to the radiation dose produced by the tissue elements. A neutron fluency of $10^{12}$–$10^{13}$ neutron/cm² is the upper limit for normal tissues. The minimum $^{10}$B concentration necessary for an effective boron neutron capture therapy has been calculated to be between 10 and 30 µg of $^{10}$B per gram of tumor, depending on the cellular location of the targeted boron-10. The concentration needed for therapy is significantly less if the $^{10}$B nuclei are localized within the tumor cell nucleus rather than in the cytoplasm. Additionally, there must be sufficient tumor specificity to minimize the radiation dose to surrounding normal structures.

1.1.2.1. Background: clinical trials of BNCT

In 1935, not long after the discovery of neutrons, Taylor\textsuperscript{11} described the capture of thermal neutrons by $^{10}$B nuclei followed by their disintegration into $^4$He$^{2+}$ ($\alpha$-particles) and $^7$Li$^{3+}$. In 1936, Locher\textsuperscript{12} proposed the potential medical application of neutrons and boron neutron capture in cancer treatment. However, since there were no suitable boron compounds or controllable neutron sources, no clinical trials could be conducted until the
controllable neutron sources, no clinical trials could be conducted until the 1950s.

Initial BNCT clinical trials were carried out on patients with glioblastoma multiforme using $^{10}\text{B}$ enriched borate by Far and Sweet\textsuperscript{13} at Brookhaven National Laboratory in 1954. These trials failed, however, to prolong survival, likely due to the poor penetration of the neutron beam and low selectivity of the boronated compounds. Thus, there was a need for new boron-containing compounds, and polyhedral borane anions and carboranes have since been synthesized. The most promising of these compounds was the disodium salt of [B$_{12}$H$_{11}$SH]$^{2-}$ (BSH) (Figure 1.1) prepared and evaluated by Soloway.\textsuperscript{14}

![Figure 1.1. Structures of $p$-boronophenylalanine (BPA) and mercapto-undecahydro-closo-dodecaborate (BSH)](image)

Clinical trials of BNCT using BSH on patients with glioblastoma multiforme were first conducted by Hatanaka in Japan in 1968. The trials produced promising results,\textsuperscript{15,16} and BSH is currently in clinical trials both in Japan and Europe.

Another interesting compound is $p$-boronophenylalanine, BPA (Figure 1.1), which was first synthesized by Snyder et al.\textsuperscript{17} BPA has been shown to accumulate in melanoma cells with a higher preference for the L-isomer.\textsuperscript{18} L-$p$-boronophenylalanine is currently being used as a $^{10}\text{B}$ carrier in clinical BNCT trials in the US,\textsuperscript{19} Finland,\textsuperscript{20} and Sweden\textsuperscript{21} for treatment of the malignant brain tumor, glioblastoma multiforme. It is, however, clear from the experience with BPA that better boron carriers are required.
1.2. Targeting principle

Specific delivery of cytotoxic substances to tumor cells with minimal damage to normal tissue cells is an important requisite in nuclide therapy of cancer. One approach of obtaining specificity is tumor targeting. Ideally, the tumor targets would be substances that exist only in the tumor cells. This, however, is unlikely to be achieved since tumor cells are similar in many ways to normal cells. Thus, some cell surface molecules that are over-expressed in tumor cells compared to normal cells are instead used as targets. Targeting agents should selectively and with high affinity bind to the tumor targets. Monoclonal antibodies and natural ligands to receptors overexpressed in tumors (e.g. epidermal growth factor, EGF) are a few examples of such substances.

Several studies have reported that there is an overexpression of EGF receptors in tumor cells in the brain, bladder, breast, and lung compared to normal cells. These EGF receptors in tumors can be used to attain high selectivity when radionuclides or stable nuclides (for neutron activation) are conjugated with EGF. Monoclonal antibodies which target tumor sites by virtue of their tumor antigen specificity have also been demonstrated as being an effective drug delivery systems.

Specific drug delivery to tumors using liposomes is also an attractive research area at present. Liposomes are artificial microscopic spherical phospholipid vesicles surrounding an aqueous compartment. They are designed in such a way that they encapsulate both lipophilic and hydrophilic compounds. It has been reported that the liposomes selectively accumulate at tumor sites, making them potentially useful in transporting drugs to tumor cells. It is not yet clear as to why liposomes are selectively picked up by tumor cells. It is, however, believed that liposomes are of the right size to leak out of the blood vessels and capillaries that growing cancer cells induce.
The selectivity can be increased by conjugating the liposomes to site-directing agents, such as EGF or antibodies. Since the liposome wall is very similar to the cell membrane in composition, liposomes are expected to be biocompatible and biodegradable. Liposomes are stabilized to increase the length of time they circulate in the blood. Liposomes that are sterically stabilized with a polyethylene glycol (PEG) derivative are commonly employed. The efficacy of most of the compounds described in this thesis are or will be investigated using this targeting system.

![Figure 1.2. Schematic representation of a liposome. (by Göran Carlsson, Institute of Physical Chemistry, Uppsala University)](image)

1.2.1. Two-step targeting

Specific targeting of tumor cell DNA is one of the most challenging tasks in cancer treatment. Attaching a targeting agent such as EGF to liposomes significantly increases the tumor specificity. DNA damage using radio-nuclides is ascertained when the nuclides are inside the cell nucleus. This is particularly important for Auger electron emitters because of short range of the emitted particles. A “two-step targeting” has been developed to ensure specific delivery of the toxic substance to the nucleus of the cell. The substances employed for this technique comprise a toxic agent and a DNA
intercalator moiety. In the first step the carrier, for example, a liposome loaded with the drug, finds the tumor and is internalized. After internalization, the carrier is degraded and the drug is released into the cytoplasm. In the second step, the drug, with the help of its DNA-interacting part, finds its way to the nucleus of the tumor cell. This technique is illustrated in Figure 1.3.10

![Figure 1.3. The concept of two-step targeting. Step 1: the drug-loaded, stabilized liposome conjugated to EGF finds the tumor via its receptor affinity and is internalized. Step 2: the drug interacts with the DNA and causes double-strand DNA breaks.](image)

1.3. Carboranes and their reactivities

Molecules containing polyhedral boranes and carboranes are of particular interest in BNCT, which requires high $^{10}$B concentration. The three isomers ($o$-, $m$-, and $p$-) of dicarba-closo-dodecaboranes are the most extensively explored in the carborane family. The $o$-carborane (Figure 1.4) was first synthesized by the reaction of acetylene with complexes prepared from decaborane ($\text{B}_{10}\text{H}_{14}$) and Lewis base, such as acetonitriles, alkylamines, and alkylsulfides.36
Closo-carboranes are stable against strong acids, oxidizing agents, and reducing agents and are also thermally stable up to a temperature of 400°C. Under inert conditions, however, o-carborane rearranges to m-carborane at around 470°C and m-carborane isomerizes to p-carborane at 700°C. This isomerization of o-carborane is depicted in Figure 1.5.

The carboranes contain two C-H groups whose protons are fairly acidic. This allows a wide range of substitution reactions to occur at the carbon vertices of the carboranes. The C-H group is readily converted to a nucleophilic center, most commonly to CLi, using a strong base; this is subsequently followed by a reaction with an electrophilic organic reagent to introduce the desired functionality. Electrophilic halogenation, on the other hand, takes place at the boron atoms of the cage, usually occurring at the boron atoms farthest from the carbon atoms.37

The water solubility of a compound is important in biological applications. Thus, the highly lipophilic closo-carboranes have been derivatized so
as to increase their water solubility,\textsuperscript{38,39} or have been converted to their corresponding negatively charged nido-carborate forms. Basic degradation of an $o$-carborane to a nido-carborate was first reported in 1964 by Wieboeck and Hawthorne,\textsuperscript{40} who reported on the degradation stoichiometry of $o$-carborane by methanolic potassium hydroxide at 40°C (eq 1.1).

\begin{equation}
\text{o-C}_2\text{H}_{12}\text{B}_{10} + \text{CH}_3\text{O}^- + 2\text{CH}_3\text{OH} \xrightarrow{40\degree\text{C}} \text{C}_2\text{B}_9\text{H}_{12}^- + \text{H}_2 + \text{B(OCH}_3)_3
\end{equation}

It is known that one of the boron atoms nearest to the carbon atoms is removed during the nido formation. This is because those boron atoms are the most electropositive atoms in the icosahedral structure and hence are prone to attack by a base.

Amino substituted derivatives of carboranes can be used in BNCT by coupling them to targeting macromolecules such as antibodies and EGF-dextran conjugates.\textsuperscript{41} Radiohalogenation of the corresponding nido-analogue with $^{123}$I, $^{125}$I, or $^{211}$At could also be of interest for radionuclide therapy.\textsuperscript{42,43}
2. Ammonio derivatives of nido-carborates (I)

2.1. Introduction

Base degradation of the closo-carboranes to provide the corresponding nido-analogues has been known of for a long time. The mechanism of the degradation reaction was first studied with C-phenyl-o-carborane as a substrate by Hawthorne et al.\(^{40a}\) The authors have shown that either the boron atom at positions 3 or 6, the most electropositive boron atoms in the icosahedron, is removed in the degradation reaction, making the two carbon atoms near neighbors on the open pentagonal face of the carborate. The rate of degradation of this compound in aqueous ethanolic potassium hydroxide at 75°C was observed to be first order with respect to both carborane and hydroxide ion concentration. Degradation of \(m\)-carborane can be accomplished under similar conditions to give \([7,9-C_3B_9H_{12}]^-\) at a slower rate,\(^{40b,44}\) while \(p\)-carborane was found to be stable to ethanolic KOH and could only be degraded after promotion with crown ether\(^{45}\) or using more harsh conditions.\(^{46}\)

Boron cages are known to be fairly reactive towards electrophilic halogenation. Labeling closo-dodecaborate \([B_{12}H_{12}]^{2-}\) derivatives with bromine, iodine, and astatine has been demonstrated to be very rapid under mild labeling conditions.\(^{47,48}\) It has also been shown that the labeled products are stable \textit{in vivo}. Chloramine-T, iodogen, and \(N\)-chlorosuccinimide are the most commonly used oxidants in radiohalogenation reactions.
Radiolabeled polyhedral anions have been synthesized for a variety of applications. One of the serious problems with radiohalogens in medical applications is dehalogenation; a rapid release of radioactivity from the cells after the intracellular processing of the labeled compound. This problem could be circumvented by using boron cage anions as a prosthetic group for the radiohalogens. The boron-halogen bond, for example the B-I bond (bond dissociation energy = 381 ± 21 kJ/mol), is substantially stronger than the carbon–halogen bond, for example the C-I bond (bond dissociation energy = 209 ± 21 kJ/mol). Its relative stability is thus expected to be maintained in vivo and the boron-iodine derivative is expected to be less susceptible to the in vivo dehalogenation commonly encountered with carbon-iodine biomolecules.

Radiolabeled derivatives of boron cages could be used for the in vivo imaging of boron compounds, which enables the direct detection of boron when studying the biodistribution and pharmacokinetics of BNCT. The boron cage derivatives labeled with γ-emitting halogens are also of interest in determining boron concentrations in tumors in relation to BNCT. The use of polyhedral boron anions for radiohalogenation has recently been reviewed by Tolmachev and Sjöberg.

The radioiodination of nido-carborate was initially reported by Hawthorne and co-workers using the chloramine-T method. The authors labeled a nido-carborate derivative of [7-(4′-C₆H₄NCS)-nido-7,8-C₂B₉H₁₁]⁻ and investigated its use as a carrier for radioiodine in biodistribution studies.

Synthesis of the racemic 7-(3′-ammoniopropyl)-7,8-dicarba-nido-undecaborate(-1) and its corresponding hydrochloride, as well as iodination and radioiodination with ¹²⁵I for use of the boron cage as a suitable carrier of radioiodine in radio-diagnostics and radiotherapy, are presented in this chapter. It has been shown that the racemic 7-(3′-ammoniopropyl)-7,8-dicarba-nido-undecaborate(-1) could be conjugated to human epidermal growth factor and subsequently labeled with ²¹¹At in a high yield.
2.2. Synthesis and radiolabeling of 7-(3´-ammonio-propyl)-7,8-dicarba-nido-undecaborate(-1)

Synthesis

The *nido*-carborate, 7-(3´-ammoniopropyl)-7,8-dicarba-*nido*-undecaborate(-1) was synthesized using a slightly modified form of the method presented by Malmquist et al.\textsuperscript{54} (Scheme 2.1).

![Scheme 2.1](image)

Scheme 2.1. i) BuLi, t-BDMSCl; ii) 0°C, BuLi, oxetane, THF, reflux; iii) CBr\textsubscript{4}, (C\textsubscript{6}H\textsubscript{5})\textsubscript{3}P, CH\textsubscript{2}Cl\textsubscript{2}, 0°C; iv) Bu\textsubscript{4}NHSO\textsubscript{4}, NaOH, HN(Boc)\textsubscript{2}, CH\textsubscript{2}Cl\textsubscript{2}; v) Bu\textsubscript{4}NF, THF; vi) HCl, ether; vii) NaOH, MeOH, CO\textsubscript{2}.
The tert-butyldimethylsilyl protected bromo derivative, 2.3, was previously prepared in our laboratory from the corresponding alcohol.\textsuperscript{55} The di-Boc protected amine, 2.4, was prepared by phase transfer catalysis using di-tert-butyldimethylsilyl iminodicarboxylate (HN(Boc)\textsubscript{2}) and a mixture of Bu\textsubscript{4}NHSO\textsubscript{4} and NaOH in dichloromethane. The reaction afforded a 66\% yield after purification by chromatography. The protecting group, tert-butyldimethylsilyl, was removed by using a tetra-n-butylammonium fluoride solution in THF at a low temperature (~ –35°C) to give compound 2.5. Compound 2.6 was obtained by removing the Boc groups with hydrogen chloride gas in dry diethyl ether, giving a very high (97\%) yield.

A racemic mixture of the nido-carborate, 2.7, was achieved by refluxing the free base of 2.6 in ethanolic sodium hydroxide. Work-up required precipitation of the excess NaOH with CO\textsubscript{2} gas, filtration, and chromatographic purification of the final product. A sample of rac-2.7 was converted to the corresponding nido-carborate hydrochloride with dilute hydrochloric acid.

![Figure 2.1](https://example.com/figure2_1.png)

Figure 2.1. Numbering of the nido-cage

The structures of the nido compounds were analyzed by 1D and 2D NMR spectroscopy experiments (see Figure 2.1 for the numbering of the compound). The \textsuperscript{11}B NMR spectra (Figure 2.2) of 2.7 and 2.7·HCl show the usual large coupling constants to the exo-protons for both compounds and an additional smaller coupling of B-10 to the bridging H\textsubscript{endo} in the 2.7·HCl spectrum. The coupling of the B-10 to the H\textsubscript{endo} was, however, almost invisible in the nido-carborate, 2.7 spectrum, since it undergoes fast deuterium exchange
of the acidic H$_{endo}$ with the solvent. This was verified by the decrease of the H$_{endo}$ peak integral in the $^1$H NMR spectrum with time. The doublet of the signal in the typical region of the $^1$H$_{endo}$ signal was changed to a singlet in the $^1$H-$^1$B-10$_{endo}$ spectrum (Figure 2.3), proving coupling of B-10 to the H$_{endo}$. Assignment of the other boron atoms was carried out starting from B-10 because of its known chemical shift, and the fact that B-10 couples to boron atoms B-5, B-6, B-9, and B-11; B-1 couples to B-2 and B-6; and H-8 couples to H-3, H-4, and H-9.

![Figure 2.2. $^{11}$B-NMR-spectra (128.3 MHz) for compounds 2.7 (top) and 2.7-HCl (bottom) with coupling to $^1$H.](image)

![Figure 2.3. NMR spectra (CD$_3$OD solution) of 2.7 (with partial deuterium exchange of the the endo-proton): left, $^{11}$B NMR-spectra (128.3 MHz), right, $^1$H NMR spectra (400 MHz)](image)

Iodination of the nido compound, 2.7, was obtained by the electrophilic substitution of a developing I$^-$, which was generated in situ by oxidation of
NaI using \(N\)-chlorosuccinimide (Scheme 2.2). The resulting product was analyzed by HPLC and electrospray ionization (ESI) mass spectrometry. Both methods confirmed that monochloro-\(nido\), diiodo-\(nido\) and chloro-iodo-\(nido\) were formed in small amounts in addition to the desired mono-substituted product, 2.8. Compounds 2.8 were formed with the predominance of one of the regioisomeric racemic pairs, as can be seen from both the NMR spectrum (2:1 molar ratio) and HPLC analyses. Attempts to obtain total separation of the two regioisomeric racemic pairs by preparative HPLC were unsuccessful. Even though total recovery of the two regioisomers was impossible, small pure fractions of each isomer were collected and analyzed by MS and NMR spectroscopy. It is not established which of the two isolated isomers is which (2.8a or 2.8b), but it is suggested that the major compound is 2.8a due to less steric demands for its formation.

\[
\begin{align*}
\text{rac-2.7 (ANC)} & \xrightarrow{\text{I}} \text{rac-2.8a} \\
\text{rac-2.8b} & \xrightarrow{\text{I}} \text{rac-2.8a} \\
\end{align*}
\]

Scheme 2.2. The regioisomeric racemic pairs of 2.8 formed from iodination reaction of compound 2.7
Radioiodination of 7-(3′-ammoniopropyl)-7,8-dicarba-nido-undecaborate(-1)

Radiolabeling of 7-(3′-ammoniopropyl)-7,8-dicarba-nido-undecaborate(-1) was achieved using Na\[^{125}\text{I}\] and chloramine-T as an oxidizing agent. The “cold” iodinated compound 2.8 and non-radioactive sodium iodide were used as references during the thin layer chromatography separation. Radio-TLC diagrams of the radioiodinated rac-2.7 and the blank experiment are shown in Figures 2.4.

The same TLC system was used for the radioiodination reaction so as to be able to distinguish between unreacted Na\[^{125}\text{I}\] and the radioiodinated nido-carborate compound. Peak 1, with \( R_f = 0.2 \) (see Figure 2.4, left), appears at the same position in the system as does the non-radioactive sodium iodide. Peak 2, with \( R_f = 0.5 \), was co-eluted with the non-radioactive authentic sample of the compound 2.8. This peak is associated with the presence of compound 2.7 and an oxidant in the reaction mixture. It has never appeared in the blank experiments (Figure 2.4, right), in which neither CAT nor compound 2.7 was added. Therefore, we assume that this peak represents the radioiodinated product 2.8. According to radio-TLC analysis using a
phosphor imager, more than 80% radiolabeling efficiency was achieved. Unfortunately, the regioisomers of the labeled compound, 2.8, could not be separated in any of the radio-TLC systems investigated.
3. Carborane-containing amino acids (II)

3.1. Introduction

Boronated amino acids are of potential interest in the BNCT field.\textsuperscript{56} It is believed that such compounds would concentrate in rapidly growing cancer cells. Boron-containing amino acids that could potentially be used in BNCT may either be delivered \textit{per se}, or as a part of tumor-seeking small peptides.

The amino acid, L-\(p\)-boronophenylalanine (BPA), is currently in clinical use. As mentioned previously, it has been reported that BPA accumulates in melanoma and glioblastoma tumor cells in much higher concentrations than in normal tissue. Tumors, \textit{in vivo}, have been shown to take up the L-form of BPA in preference to the D-form.\textsuperscript{57}

L-\(p\)-boronophenylalanine is administered as a fructose complex due to its low water solubility. Therefore, several derivatives of BPA have been synthesized to increase its water solubility. Yamamoto attempted to increase the water solubility of BPA by introducing more hydroxyl functional groups, synthesizing some hydroxy amide derivatives of BPA. These are mono-ol (BPA(OH)), diol (BPA(OH)\(_2\)), and tetrol (BPA(OH)\(_4\)). Water solubility did increase with the increasing number of hydroxyl groups, but the cytotoxicity was found to decrease.\textsuperscript{58}

High boron concentration is a requisite of BNCT. Carboranyl amino acids contain ten boron atoms; thus a higher concentration of \(^{10}\)B could be delivered with carboranyl amino acids than with BPA, even at a lower concentration in the tumor cells.
Other applications, besides BNCT, have been described where use has been made of the carborane cage as hydrophobic pharmacophores\textsuperscript{59-61} in biologically active molecules. For example, \textit{o-}carboranylalanine has been used to replace phenylalanine and tyrosine in a number of biological active peptides. This work has recently been reviewed by Wyzlic et al.\textsuperscript{62}

The \textit{o-}carboranylalanine was first synthesized as a racemate by Zakharkin et al\textsuperscript{63} and by Brattsev et al\textsuperscript{64} in the late 1960s. The first stereoselective synthesis of \textit{o-}carboranylalanine was reported in 1976 by Schwyzer et al.\textsuperscript{65} Since then, many other groups have reported various methods of stereoselective synthesis of \textit{o-}carboranylalanine which offer improved enantiomeric purity.\textsuperscript{66} The next section describes the enantioselective synthesis of \textit{m-}carboranylalanine.

3.2. Synthesis of \textit{m-}carboranylalanine

The enantioselective synthesis of \textit{m-}carboranylalanine, \textbf{3.8} (carboranyl-amino acid) was achieved using the Oppolzer sultam methodology.\textsuperscript{67} The \textit{m-}carborane was protected using \textit{t-}BDMScI and directly alkylated with trimethylene oxide. Removal of the silyl-group using tetrabutylammonium fluoride afforded a 57% yield of the mono-substituted alcohol \textbf{3.3} from \textit{m-}carborane. A small amount of a di-substituted alcohol and unreacted \textit{m-}carborane were also isolated. The alcohol \textbf{3.3} was oxidized to its corresponding carboxylic acid, \textbf{3.4}, using Jones reagent in 77% yield (Scheme 3.1).
The Oppolzer’s sultam was used to introduce chirality in the target molecule. The m-carboranyl propanoic acid was coupled with the (S)-sultam, 3.2. The reaction was performed using bicyclohexylcarbodiimide (DCC) as an acylating agent and 4-dimethylaminopyridine (DMAP), giving a 90% yield of the compound (S)-3.5 (Scheme 3.2).

Hydroxyamination of the N-acylsultam was achieved by deprotonation with sodium hexamethyldisilazide at –78°C, followed by slow addition of the bright blue compound, 1-chloro-1-nitroso-cyclohexane 3.1, which after work-up, gave 79% yield of the diastereomerically pure hydroxyaminosultam, (S,S)-3.6. The N/O hydrogenolysis of the hydroxyaminosultam with zinc dust in AcOH/HCl at 4°C provided 82% yield of N-(α-aminoacyl)-sultam, (S,S)-3.7.

Hydrolysis of the amide group with LiOH/THF produced, after neutralization with HCl, 72% yield of the hydrochloride salt of (S)-m-carboranylalanine, 3.8. The sultam auxiliary was recovered upon extraction. The (R)-form of the amino acid was prepared by using the (R)-form of the sultam.

The enantiomeric purity of the two enantiomers was determined by HPLC separation of the diastereomeric derivatives, (S,S)-3.9 and (R,S)-3.9, respectively. Both (S,S)-3.9 and (R,S)-3.9 were prepared by reaction with Marfey reagent, N-α-(2,4-dinitro-5-flourophenyl)-(S)-alanine amide (Scheme 3.3). The enantiomeric excesses of both (S) and (R) were found to be greater than 98%.
Scheme 3.2. i) (S)-3.2, DCC, DMAP; ii) NaN(TMS)₂, 3.1; iii) Zn, HOAc/HCl; iv) LiOH, HCl

Scheme 3.3. i) N-α-(2,4-dinitro-5-flourophenyl)-(S)-alanine amide, NaHCO₃
4. Synthesis and radiolabeling of 9-aminoacridines (III, IV)

This chapter describes the syntheses and radioiodination of a series of 9-aminoacridine derivatives (9-aminoacridine is a well-known DNA intercalator) with the objective of using them as potential agents in targeted nuclide therapy. All the compounds presented in this and the next chapter will be investigated by applying the technique of active loading to liposomes. Their efficiency will be evaluated in light of the two-step targeting principle discussed in section 1.2.1.

4.1. DNA intercalation

The concept of DNA intercalation was initially explained by Lerman\textsuperscript{70} in 1961. It can generally be defined as the insertion of a flat polyaromatic chromophore between the base pairs of the double-helical DNA, driven by stacking and charge transfer interactions between the aromatic systems of the ligand and the DNA bases, causing unwinding of the deoxyribose-phosphate backbone.

Many compounds, such as acridine, anthracycline, phenanthridine, and naphthalimide derivatives, bind to DNA by intercalation. Studies with a series of DNA intercalating drugs have shown correlation between antitumor activity and the tightness of DNA binding.\textsuperscript{71,72} Part of this thesis deals with 9-aminoacridine and daunorubicin/doxorubicin DNA intercalators.
4.1.1. 9-Aminoacridine derivatives

A number of 9-aminoacridine derivatives have been synthesized for various purposes, taking advantage of their high DNA intercalative nature.\textsuperscript{73-76} Intercalation of the 9-amino substituted acridines has been studied by X-ray crystallography. It has been shown that the 9-amino group lies in the minor groove and that positions 4 and 5 on the acridine ring are oriented towards the major groove.\textsuperscript{77}

![Figure 4.1. Structure of 9-aminoacridine](image)

Comparative studies of some acridine derivatives have demonstrated that the DNA binding affinity of such compounds is remarkably diminished in the absence of the amino group at the C-9 position.\textsuperscript{78} Additionally, substitution at position 2 and the presence of bulky groups at position 3 are known to reduce the DNA binding.\textsuperscript{79} Protonation on the N-10 of the acridine moiety increases the DNA binding ability.\textsuperscript{80}

4.2. Synthesis of benzamide substituted aminoacridines

Synthesis of the stannylated compounds, \textbf{4.6} and \textbf{4.7}, was conducted in three steps, as outlined in Scheme 4.1. The starting 9-phenoxyacridine was readily available from acridone via the chloroacridine derivative.\textsuperscript{81} The chloroacridine was found to be unstable and easily convertible to 9(10\textit{H})-acridone with exposure to air. The 9-phenoxyacridine is stable to air and can be stored at room temperature for a prolonged time. High yields of the diamines, \textbf{4.2}\textsuperscript{82}
and 4.3, were obtained via the nucleophilic substitution reaction of 9-phenoxyacridine and the corresponding alkyldiamines. Excess of the diamines was required in these reactions to avoid disubstitution.

Several methods for coupling the amine to carboxylic acid were attempted, and the best was found to be via the acid chlorides. The acid chloride was reacted with the diamines, 4.2 and 4.3, under hydrous conditions to produce good yields of the iodo derivatives, 4.4 and 4.5, respectively. The stannylated compounds, 4.6 and 4.7 were then obtained from their respective iodo-derivatives by a Stille-type cross coupling reaction. Two isomers of these compounds were isolated. This issue is discussed in some detail in section 4.2.1.
In preparing the stannylated compounds, 4.6 and 4.7 (PPh₃)₄Pd or (PPh₃)₂PdCl₂ was employed as a catalyst. Azizian et al.⁸³ suggested a possible catalytic cycle involving the oxidative addition of the halide and the ditin compounds to Pd(II)-complexes and the reductive elimination of the stannylated product and trialkyltinhalide from the resulting Pd(IV)-complexes (Figure 4.2).

Later studies, however, do not entirely support the mechanism proposed in Figure 4.2. The Stille cycle seems to be a generally accepted model (Figure 4.3). This model clearly suggests that excess hexamethylditin is necessary in order to avoid the formation of homocouplings.

Figure 4.2. Possible catalytic cycle suggested by Azizian et al.⁸³ R= aryl, X= halide

Figure 4.3. Stille-type catalytic cycle

4.2.1. Tautomerism in acridine systems

Two tautomeric forms of 9-substituted acridines can potentially exist, as shown in Scheme 4.2. It has been possible to show the existence of the two tautomers of the 9-mercaptoacridine 4.12, in DMSO.⁸⁴ The authors found the thiono form to be dominant, accounting for 85% of the total of the two tautomers.
tautomers. However, when the 9-substituent is oxygen, the non-aromatic keto structure, 9(10H)-acridone, 4.11, is the only form observed.84-85

![Scheme 4.2. Tautomerism in 9-substituted acridines](image)

Tautomerism in 9-aminoacridine, 4.14, and its derivatives has been studied in more detail than the corresponding oxy- or thio-derivatives. The potential existence of amino- and imino-tautomers of 9-aminoacridines has been accounted for by means of calculations and spectroscopic investigations.86,87 Rak et al88 have demonstrated the existence of the two tautomers in equilibrium at room temperature. According to their calculations, the amino form, 4.14, was found to be thermodynamically more stable. The difference in energy between the amino and imino compounds was determined to be always positive from the theoretical calculations.

We have been able to observe isomerism in two of the 9-aminoacridine derivatives, compound 4.6 and 4.7, displayed in Scheme 4.1. Two isomers could clearly be observed in the stannylated compounds. The amount of the isomers depends on the type of substituent and the work-up procedures. The dependence on the type of substituent has been reported by Meszko et al.89 The authors have studied the effect on the tautomeric ratio of bulky substituents at the 9-position. Chromatography and NMR spectroscopy analysis did not reveal the existence of isomerism in the diaminoacridines, 4.2 and 4.3, or in the iodobenzamides, 4.4 and 4.5, which were purified in the presence of HCl. Compounds 4.4 and 4.5 were found to be monoprotonated according to
elemental analysis. It is known from the literature that single protonation of both tautomers of 9-aminoacridine leads to the same ion.\textsuperscript{90,91} In other words, the imino-form, which coexists with the amino-form in the free-base species, converts to the amino-form upon protonation giving an acridine ring protonated ion.

The stannylated compounds, \textit{4.6} and \textit{4.7}, are not stable to acids, they were purified in a slightly basic media in their free amine forms. In this case, two isomers were observed by TLC and separated by chromatography. High resolution mass spectroscopy showed that the two separated compounds indeed have the same mass. However, a slight difference in the $^1$H NMR of the two compounds could be seen, the main difference being the chemical shift of the amino NH proton. Further more, when a CDCl$_3$ solution of the minor product of compound \textit{4.7} was kept for 2 days at low temperature the $^1$H NMR spectrum of this compound was slightly changed and some NOE effect of the 9-NH proton to the protons at positions 1(8) and 4(5) could be observed indicating a fast equilibrium was established. It however, was not possible to show clearly whether these isomers were tautomers using the data at hand.

\textbf{4.3. Synthesis of tyrosine/tyramine substituted acridines}

The tyrosine and tyramine derivatives of 9-aminoacridine derivatives (compounds \textit{4.16-4.19}) were synthesized as shown in Scheme 4.3.
Scheme 4.3. i) L-tyrosine, phenol, 100°C, 98% yield; ii) 3-iodotyrosine, phenol, 100°C, 93% yield; iii) tyramine, phenol, 100°C, 95% yield; iv) 3-iodotyramine, phenol, 100°C, 95% yield; and v) Na[^125I], chloramine-T, MeOH, Na$_2$S$_2$O$_5$, NaI.

High yields of the four compounds could be easily obtained from the reaction of 9-phenoxyacridine with the amino functional group of the respective compounds in one hour reaction time.
4.4. Carboxylic acid and amine functionalized acridines

Synthesis of the carboxylic acid derivatives of acridines 4.31-4.33 was carried out as depicted in Schemes 4.4 and 4.5. The amine precursors, 4.27 and 4.28, for the substitution reaction with 9-phenoxyacridine could be achieved in straightforward, three-step reactions. Commercially available 3-(4-hydroxyphenyl)propionic acid, 4.22, was esterified and subsequently reduced with lithium aluminum hydride to its corresponding hydroxyl derivative, 4.23. The preparation of the iodo-derivative, 4.24, was performed using molecular iodine and Ag₂SO₄ in dichloromethane. The phenoxypropa-nols, 4.23 and 4.24, were alkylated with N-(3-bromopropyl)phthalimide to afford good yields of the phthalimide derivatives, 4.25 and 4.26, respectively. The amines were then liberated by the reaction of the phthalimides, 4.25 and 4.26, with hydrazine monohydrate. (Scheme 4.4).

The amines, 4.27 and 4.28, were reacted with 9-phenoxyacridine in phenol to provide high yields of the acridine derivatives, 4.29 and 4.30, respectively. The alcohols were then oxidized to their corresponding carboxylic acid analogues using Jones reagents. The iodo derivative, 4.32, was reacted
with hexamethylditin using tetrakis-triphenylphosphine palladium as a catalyst to give a good yield of the stannylated compound, 4.33 (Scheme 4.5).

Scheme 4.5. i) 9-Phenoxyacridine, molten phenol, 80°C; ii) CrO₃, H₂SO₄, acetone, 0–10°C; iii) Sn₂Me₆, Pd(PPh₃)₄, DMF; and iv) chloramine-T, Na[I¹²5], 3% acetic acid/MeOH, Na₂S₂O₅, NaI.

The amine derivatives 4.45-4.47 were synthesized as displayed in Schemes 4.6 and 4.7. The amines 4.41 and 4.42 were prepared using a process similar to that used for compounds 4.27 and 4.28 starting from Boc-protected tyramine, 4.35 and iodotyramine, 4.36 (Scheme 4.6). Compounds 4.41 and 4.42 were reacted with 9-phenoxycridine to give the Boc-protected acridines 4.43 and 4.44 respectively. Deprotection of the Boc group under acidic conditions afforded the acridine amines 4.45 and 4.46. The stannylated precursor, 4.47 was obtained from the iodo compound 4.46 by palladium(II) catalized coupling reaction.
Scheme 4.6. i) (Boc)$_2$O, 10% Et$_3$N/MeOH, 54°C, aq. HCl; ii) $N$-(3-bromopropyl)phthalimide, K$_2$CO$_3$, acetone, reflux; and iii) N$_2$H$_4$·H$_2$O, MeOH, reflux.

Scheme 4.7. i) 9-phenoxyacridine, phenol, 80°C; ii) dryHCl/EtOAc, RT; iii) Sn$_2$Me$_6$, (PPh$_3$)$_2$PdCl$_2$, 1,4-dioxane, 85°C; and iv) Chloramine-T, Na$_{125}$I
4.5. Radiohalogenation of acridine derivatives

The precursor compounds used for radiohalogenation, in this thesis contain either an organotin group or a phenolic substituent. Organotin precursors are widely used for radiohalogenation due to the fact that they give high yields and regiospecificity compared to many other organometallic intermediates. The weak carbon-tin bond is easily cleaved by all halogen electrophiles. Hence, stannylated precursors have been employed for electrophilic reactions with radiofluorine, radiobromine, radioiodine, and astatine. The precursors with a phenolic substituent can directly be radiohalogenated at the \( o \)-position to the hydroxyl group.

Radioiodination of the acridine benzamides

The stannylated benzamides, 4.6 and 4.7 (Scheme 4.1), were radiolabeled with \(^{125}\text{I}\) using chloramine-T as an oxidant. The stannylated precursor, 4.7, was employed as a model substance for optimizing the various reaction conditions. Parameters such as concentration of substrate, oxidant, and reaction time were investigated (Figure 4.4). The optimal reaction conditions provided as high as 92% radiolabeling yield.

The radioiodination yield, which is dependent on the reaction time, reached a plateau after 5 minutes (Figure 4.4a). This is rather slow compared to the radioiodination of proteins with chloramine-T (CAT), in which high yields could be obtained as rapidly as 30 seconds after addition of CAT. It is, however, as fast or faster than the radioiodination of other organotin intermediates.

The radiolabeling yield dramatically decreased as the amount of substrate was reduced (see Figure 4.4b). The best yields were achieved when at least 0.06 \( \mu \text{mol} \) of substrate was used, an amount higher than that commonly required for the direct iodination of proteins. For example, as little as 0.001 \( \mu \text{mol} \) of EGF can be labeled efficiently with iodine.
molecule of EGF has five tyrosine residues that can be iodinated, the amount of substrate (0.06 µmol) needed for the successful labeling of acridines is ten times larger. However, this is at least ten times less substrate than is usually employed for tin derivatives of N-succinimidyl benzoate.98–99

Figure 4.4. Dependence of radiochemical yields of radioiodination of 4.7 on various reaction conditions. Experiments were performed in duplicate. Error bars were calculated as maximal errors according to the formula, \( E_{\text{max}} = \frac{(Y_{\text{max}} - Y_{\text{min}})}{2} \). Total volume of reaction mixture was 70 µl. Vessels were vortexed during reaction. Reaction was quenched with 20 µl sodium metabisulfite (3.3 mg/ml MeOH). Sodium iodide (5 µl, 10 mg/ml MeOH) was added as carrier before analysis. a) 4.7 = 35 µg, CAT = 5 µg. b) CAT = 5 µg, time = 5 min.; c) 4.7 = 35 µg, time = 5 min.

The influence of the amount of oxidant, CAT, on labeling yields is shown in Figure 4.4c. The best yields were achieved using 1–5 µg of CAT, which corresponds to a 1:1 molar ratio of CAT to acridine. It should be noted that EGF could be labeled in a 110:1 CAT to EGF ratio; further increase in the amount of CAT decreased the labeling yield and increased the amount of byproduct.
Radioiodination of the acridine-carboxylic acid and amine

Radiolabeling the acridine carboxylic acid, 4.33 (Scheme 4.5), and the amine, 4.47 (Scheme 4.7), with $^{125}$I was performed using a protocol similar to that of the chloramine-T method used for acridine amide. The optimized reaction conditions afforded 70% yield of the radioiodinated product, 4.34, and 96% yield of the compound, 4.48, according to the radio-TLC analysis. Besides the reaction parameter previously mentioned, acidification of the media was particularly found to enhance the reaction yields of these compounds.

Radioiodination of acridine-tyrosine and tyramine

The 9-aminoacridine derivatives of tyrosine 4.16 and tyramine 4.18 were radioiodinated using a method similar to that used for the stannylated acridines. These compounds have an activated phenyl ring, which enables them to react directly with electrophilic radiohalogens at the $o$-position without first having to make the organotin precursors. This reduces the synthetic route by one step, which is advantageous in terms of cost and overall yield.

Optimal reaction conditions provided up to 96% and 68% yields of the tyrosine derivative 4.20 and the tyramine derivative 4.21 respectively.
5. Daunorubicin/doxorubicin derivatives (V)

Daunorubicin (5.1) and doxorubicin (5.2) (Figure 5.1) are two clinically interesting compounds in the anthracycline family. Structurally, they comprise a planar anthraquinone attached to a daunosamine sugar. Daunorubicin (daunomycin) was the first antibiotic of this class to show activity against acute leukaemia in humans. The replacement of one hydrogen atom in the daunorubicin acetyl side chain with a hydroxyl results in doxorubicin (also known as adriamycin). Doxorubicin exhibits antitumor activity against solid tumors, such as breast and lung cancers, and is generally known to be more potent than daunorubicin.

Figure 5.1. Structures of daunorubicin (5.1) and doxorubicin (5.2)

High cardiotoxicity and multi drug resistance are two serious problems limiting the clinical efficacy of these agents.\textsuperscript{100} Several derivatives of these compounds have therefore been developed to improve their clinical effi-
ciency. The most obvious positions for derivatization are at C-13, C-14, and N-3’ of the daunosamine sugar.

The mechanism of the antibiotic action of these molecules is related to their ability to intercalate between adjacent base pairs causing topoisomerase II inhibition. This leads to the production of hydroxyl free radicals that are toxic to both normal and tumor cells. One approach that potentially improves the therapeutic ability of these molecules is to target them specifically to the tumor cells. Delivering the drugs by loading them to liposomes would be expected to reduce their toxicity to healthy cells dramatically.

Daunorubicin and doxorubicin (Figure 5.1) are known for their effective intercalation to DNA. The mechanism for this intercalation is not precisely understood; however, the major binding force is believed to be the intercalation of the planar chromophore, stabilized by an external electrostatic binding of the amino sugar. Evidence for the latter mode of binding is the reduced effectiveness of the N-acetylated derivative of daunorubicin as a DNA-binding agent. 109 N-(trifluoroacetyl)adriamycin-14-valerate (AD 32) is a typical example of DNA non-binding analogue of doxorubicin.

This chapter describes the synthesis and radioiodination of esters, amides and amines derived from daunorubicin and doxorubicin. The compounds will be evaluated with regard to DNA binding and their possibility to be delivered to cancer cells using the two step targeting principle. The precursor compounds are labeled with 125I (an Auger emitting nuclide). Interestingly, compounds 5.19 and 5.22 (see Scheme 5.4 for the structures) could be loaded to stabilized liposomes and have been shown to be taken up by permeabilized cell lines in a preliminary experiment. Further biological experiments with these compounds are still underway and thus cannot be discussed in this thesis.
5.2. Synthesis of daunorubicin and doxorubicin derivatives

*Ester derivatives*

The ester derivatives of doxorubicin, 5.3-5.5, were synthesized via the 14-bromodaunorubicin as displayed in Scheme 5.1. The bromination of daunorubicin with molecular bromine in the presence of trimethylorthoformate, as the procedure described in the literature, gave 14-bromo-13-dimethylacetal of daunorubicin. This compound, upon being stirred in acetone followed by precipitation from diethyl ether, provided a high yield of the 14-bromodaunorubicin, 5.13. Since the doxorubicin/daunorubicin derivatives are acid sensitive, their hydrochloride salts were obtained by treating them with dry HCl in diethylether at low (-20°C to -15°C) temperatures.

\[
\text{Scheme 5.1. } i) \text{ CH(OMe)}_3, \text{MeOH/Dioxane (1:2), RT; b. Br}_2/\text{CHCl}_3, 30^\circ \text{C, c. } \text{Acetone, RT; ii) 3-iodobenzoic acid, K}_2\text{CO}_3, \text{RT; and iii) compound 5.12, K}_2\text{CO}_3, \text{RT; iv) Na}^{[125]}\text{I}, 3\% \text{AcOH/MeOH, CAT, Na}_2\text{S}_2\text{O}_3, \text{NaI} \]

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Amide derivatives

The synthetic route of amides, 5.6-5.8, is outlined in Scheme 5.2. The compound 4-iodobenzoic acid was converted to its corresponding stannylated analogue. The 4-iodobenzoic acid and 4-trimethylstannylbenzoic acid were activated by treatment with di-(N-succinimidyl)carbonate in pyridine\textsuperscript{112} to give the corresponding N-succinimidyl-derivatives, 5.14 and 5.15, respectively. Compounds 5.14 and 5.15 were reacted with daunorubicin hydrochloride in the presence of excess triethylamine in DMF to afford good yields of the daunorubicin derivatives, 5.6 and 5.7, respectively.

![Scheme 5.2.](image)

Scheme 5.2. i) a. For 5.6: Et\textsubscript{3}N, compound 5.14, RT, 1.5 h; b. for 5.7: Et\textsubscript{3}N, compound 5.15, RT, 1.5 h.; and ii) Na\textsuperscript{125I}, 1% AcOH/MeOH, CAT, Na\textsubscript{2}S\textsubscript{2}O\textsubscript{5}, NaI

The synthesis of the amides, 5.9-5.11, is described in Scheme 5.3. N-succinimidy1-5-bromonicotinic acid, 5.16, was prepared in 69% yield using the same method as was used for compounds 5.14 and 5.15. It was then converted to its trimethylstannyl analogue, 5.17 by palladium(II) catalyzed reaction. Reaction of the stannylated product, 5.17, with daunorubicin hydrochloride, followed by chromatographic purification afforded a 72% yield of the amide, 5.9. The tin compound, 5.9, was iodinated using NaI, with chloramine-T as an oxidizing agent, giving a good yield of the amide 5.10.
Scheme 5.3. i) Compound 5.17, triethylamine, RT, 1.5 h; ii) a. for 5.10, NaI, chloramine-T, Na$_2$S$_2$O$_5$, RT, 13 min, and b. for 5.11, Na$[^{125}$I], 1% AcOH/MeOH, CAT, Na$_2$S$_2$O$_5$, NaI; iii) Pd(PPh$_3$)$_2$Cl$_2$, Sn$_2$Me$_6$, 1,4-dioxane.

**Amine derivatives**

The amines, 5.18-5.23, were synthesized as outlined in Scheme 5.4. The amine, 5.19, was synthesized by the reductive alkylation of daunorubicin with 4-hydroxybenzaldehyde using sodium cyanoborohydride. The reaction required excess reagents for total consumption of the starting daunorubicin and was completed after 3 days. The reaction produced two major products. Isolation and purification of the two compounds by chromatography afforded 22% yield of compound 5.19 and 75% yield of compound 5.22 which was produced from reduction of the carbonyl group at position 13 by sodium cyanoborohydride. Similarly, the iodinated analogues 5.18 and 5.21 were synthesized using 4-hydroxy-3-iodobenzaldehyde (5.24) and provided 5.21 as a major product.
Scheme 5.4. i) Aldehyde 5.24, MeCN:H₂O (2:1), 1M solution NaBH₄CN/THF, RT; and ii) 4-hydroxybenzaldehyde, MeCN:H₂O (2:1), 1M solution NaBH₄CN/THF, RT; iii) Na[¹²⁵I], MeOH, CAT, Na₂S₂O₅, NaI; iv) I₂, Ag₂SO₄, CH₂Cl₂, RT.

5.3. Radiolabeling

Radioiodination of compounds 5.4, 5.7, 5.9, 5.19, and 5.22 with ¹²⁵I was performed using the chloramine-T method. The influence of certain reaction conditions: reaction time, concentration of substrate and oxidant were inves-
tigated. The acidity of the reaction media was also taken into account. The radioiodination yields of the labeled compounds obtained using the optimized conditions are shown in Table 5.1.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Radioiodinated compounds</th>
<th>Time (min)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.5</td>
<td>5</td>
<td>85 ± 0.2</td>
</tr>
<tr>
<td>2</td>
<td>5.8</td>
<td>5</td>
<td>95 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>5.11</td>
<td>5</td>
<td>91 ± 0.8</td>
</tr>
<tr>
<td>4</td>
<td>5.20</td>
<td>5</td>
<td>53 ± 1.2</td>
</tr>
<tr>
<td>5</td>
<td>5.23</td>
<td>5</td>
<td>72 ± 1.6</td>
</tr>
</tbody>
</table>

A representative radio-TLC diagram of $^{125}$I iodide of the model compound, 5.7, and a typical blank experiment are displayed in Figures 5.2.

Figure 5.2. a) Typical TLC chromatogram of compound 5.8 using the general labeling procedure. Peak 1 corresponds to $^{[125]}$I stabilized with carrier NaI ($R_f = 0.3$); peak 2 has the same retention factor ($R_f = 0.8$) as the iodinated "cold" reference, 5.6, and thus corresponds to the radiolabeled compound 5.8. b) Typical radiochromatogram of a blank experiment.

All radio-TLC analyses were performed using NaI as a carrier added after labeling to stabilize the radioiodide from oxidation by air during elution of the TLC plates. The retention factors of the labeled compounds were in agreement with the corresponding "cold" references obtained under the same chromatographic systems.
The optimization of the reaction conditions (Figures 5.3-5.5) is exemplified with the amine 5.22. The optimal conditions afforded high labeling yields of the stannylated precursors. The influence of reaction time on the radiochemical yield is shown in Figure 5.3. The labeling yield is strongly dependent on the reaction time, reaching its maximal level after four minutes of incubation. Further increase in reaction time had no effect on the labeling yield.

![Graph showing the influence of reaction time on radioiodination yield. Other conditions were: amount of substrate (5.22), 40 µg; amount of CAT, 80 µg; and total volume, 55 µl. Data presented represent average values from two experiments.](image)

The effect of the oxidant was examined by running the reaction at different concentrations of CAT (Figure 5.4). The optimal value was found to be 80 µg (0.35 µmol) CAT. Decreasing the concentration to less than 40 µg led to a sharp drop in labeling yield, while increasing it above 80 µg had no effect.
The dependence of the radiochemical yield on the amount of substrate was examined by varying the concentration in the 1–4 mg/ml range. This dependence is illustrated in Figure 5.5. The best yields were achieved for reaction mixtures containing at least 80 µg of the substrate.

The labeling of the stannylated precursor was further enhanced by acidification using 1% acetic acid in a methanol solution. However, subjecting the non-stannylated precursors to this condition did not improve the radiochemical yield.
6. Conclusion

The nido-carborate, 7-(3’-ammoniopropyl)-7,8-dicarba-nido-undecaborate (-1) was synthesized by base degradation of the aminopropyl-o-carborane analogue. The racemate nido-carborate was iodinated to give two pairs of regioisomers. Radiolabeling of the racemate with $^{125}$I was achieved in a high yield. The regioisomers of the radioiodinated nido-carborate could however, not be separated in any of the radio-TLC diagrams. The radiohalogenated nido-carborate was prepared for use of the boron cage as a prosthetic group for attachment of the radiohalogen to tumor targeting molecules.

The enantiomers of $m$-carboranylalanine have been synthesized in high enantiomeric excess.

A series of derivatives of daunorubicin, doxorubicin, and 9-aminoacridine of potential use in targeted nuclide therapy were synthesized in good yields. The precursor compounds were labeled with $^{125}$I using the chloramine-T method. These compounds contained either a trimethylstannyl group or a phenolic substituent. The trimethylstannyl group could regioselectively be substituted with $^{125}$I in high yields and short reaction time. The compounds with phenolic substituents could be radiolabeled in good yields directly at the $o$-position to the hydroxyl group. The efficiency with which the stannylated precursors could be labeled was improved by acidifying the reaction media.
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A doctoral dissertation from the Faculty of Science and Technology, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology. (Prior to October, 1993, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology”.)