CENTRAL NERVOUS SYSTEM TARGETED METALLOCARBORANE COMPLEXES
SYNTHESIS, CHARACTERIZATION AND EVALUATION OF CENTRAL NERVOUS SYSTEM TARGETED METALLOCARBORANE COMPLEXES

By

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ABSTRACT

A series of new methodologies to link a neurotransmitter receptor targeting vector (WAY) to carborane ligands and preparation of the corresponding metallocarboranes (M = Re, $^{99m}$Tc) as a new class of organometallic central nervous system (CNS) imaging probes is described. Alkyl- and amido-linked WAY-carborane conjugates (5, 6 and 16) were prepared and the corresponding metallocarboranes (M = Re (12, 13, 22a, 22b), $^{99m}$Tc (14a, 15, 23)) were synthesized in yields ranging from 10 to 95 % using microwave heating. During the course of this work, the first observed 3,1,2 versus 2,1,8 rhenacarborane isomerization process was discovered where isomerization and complexation occurred simultaneously and the relief of steric strain was found to be the driving force. An X-ray structure of 12 confirmed that the cluster had the 2,1,8 cage configuration. Re-carborane complexes 22a and 22b had similar carbon-carbon cage configuration (2,1,8) where the driving force behind isomerization in these cases was attributed to electronic effects.

The lipophilicities of $^{99m}$Tc-carborane complexes 14a, 15 and 23 were measured and were within the ideal range to cross the BBB (log P = 2.4-2.6). In vitro binding data of 12, 13 and 22b showed that complex 22b has high affinity for certain $\alpha$-adrenergic receptors ($K_i = 17-39$ nM) resulting in the first organometallic complex to effectively bind to this class of receptors. SPECT images of 14a in Sprague-Dawley rats showed no brain uptake, while quantitative biodistribution studies indicated modest, non-negligible brain uptake in the hypothalamus region (0.36 ± 0.08 %ID/g, 2 min p.i.). Complex 23 did
not show any brain uptake or accumulation in the heart or skeletal muscle where α-adrenergic receptors reside.

The charged nature of the \([M(CO)\text{3}(C_{2}B_{9}H_{10}R)]^−\) complexes (M = Re, \(^{99m}\text{Tc}\)), which may be limiting brain uptake, was addressed where the neutral \([M(CO)\text{2}(NO)(C_{2}B_{9}H_{10}R)]\) analogues (30, 34 and 37) were prepared. Reactivity differences between Re and \(^{99m}\text{Tc}\) were noted during nitrosation conditions where the initial products from the reaction led to nitration of the phenyl group in addition to nitrosation of the metal core. For the \(^{99m}\text{Tc}\)-complexes, direct production of the desired complexes (40 and 41) was observed where complex 38 was the only compound to show nitration of the phenyl ring at the tracer level. The fluorescence properties of these compounds was also measured where the quantum yield of 29 was low (\(\Phi_f = 0.015\); \(\tau_f = 1.57 \text{ ns}\); \(\lambda_{\text{em}} = 429 \text{ nm}\), \(\lambda_{\text{ex}} = 369 \text{ nm}\)).

Low yields and multistep syntheses associated with the preparation of substituted carborane ligands led to the development of a carborane-alkyne platform suitable for use in Cu(I)-catalyzed cycloaddition (“click”) chemistry. A series of alkyne-carborane ligands (53-55) were developed and conjugated to a WAY-azide (46). The metallocarborane WAY-complexes (M = Re (69-71), \(^{99m}\text{Tc}\) (72-74)) were generated in yields ranging from 45 to 71 %. These studies produced a series of alkynes that can be used to form anionic, neutral and cationic metallocarborane complexes.
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<table>
<thead>
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<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha$</td>
<td>alpha decay or particle</td>
</tr>
<tr>
<td>%ID/g</td>
<td>percent injected dose per gram of tissue</td>
</tr>
<tr>
<td>Ac</td>
<td>acyl group</td>
</tr>
<tr>
<td>$\beta$ (or $\beta^-$)</td>
<td>beta decay or emission</td>
</tr>
<tr>
<td>$\beta^+$</td>
<td>positron</td>
</tr>
<tr>
<td>BBB</td>
<td>blood-brain barrier</td>
</tr>
<tr>
<td>BNCT</td>
<td>boron neutron capture therapy</td>
</tr>
<tr>
<td>Bq</td>
<td>Becquerel</td>
</tr>
<tr>
<td>Ci</td>
<td>Curie</td>
</tr>
<tr>
<td>CNS</td>
<td>central nervous system</td>
</tr>
<tr>
<td>Cp</td>
<td>cyclopentadienyl</td>
</tr>
<tr>
<td>CT</td>
<td>computerized X-ray tomography</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulfoxide</td>
</tr>
<tr>
<td>DSD</td>
<td>diamond-square-diamond</td>
</tr>
<tr>
<td>ESA</td>
<td>effective specific activity</td>
</tr>
<tr>
<td>eV</td>
<td>electron volts</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>gamma ray</td>
</tr>
<tr>
<td>HOEt</td>
<td>$N$-hydroxybenzotriazole</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>IR</td>
<td>infrared</td>
</tr>
<tr>
<td>LC-MS</td>
<td>liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>MI</td>
<td>molecular imaging</td>
</tr>
<tr>
<td>MRI</td>
<td>magnetic resonance imaging</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry or mass spectrum</td>
</tr>
<tr>
<td>n</td>
<td>neutrino</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>nOe</td>
<td>nuclear Overhauser enhancement</td>
</tr>
<tr>
<td>p.i.</td>
<td>post injection</td>
</tr>
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<td>PET</td>
<td>positron emission tomography</td>
</tr>
<tr>
<td>SA</td>
<td>specific activity</td>
</tr>
<tr>
<td>SAAC</td>
<td>single amino acid chelate</td>
</tr>
<tr>
<td>SPE</td>
<td>solid phase extraction</td>
</tr>
<tr>
<td>SPECT</td>
<td>single photon emission computed tomography</td>
</tr>
<tr>
<td>TLC</td>
<td>thin layer chromatography</td>
</tr>
<tr>
<td>tR</td>
<td>retention time</td>
</tr>
<tr>
<td>US</td>
<td>ultrasound</td>
</tr>
<tr>
<td>UV</td>
<td>ultraviolet</td>
</tr>
<tr>
<td>WAY</td>
<td>1-(2-methoxyphenyl)piperazine</td>
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DECLARATION OF ACADEMIC ACHIEVEMENT

The author synthesized and characterized all the compounds listed in the experimental sections of this document. Samples submitted for analysis or conducted at other facilities are acknowledged in the experimental sections.

Published manuscripts:


Manuscripts in preparation:


Louie, A.S., Eleftheriou, N, Brennan, J.D., Valliant, J.F. Synthesis, characterization and X-ray crystal structure of dicarbonyl nitrosyl metallocarborane (M = Re, 99mTc) complexes targeted at CNS receptors. *Organometallics* 2011. (Chapter 4)

LIST OF COMPOUNDS

1

2

3

4

L = Lewis base

5

6

7

8

9

10

11

12

13

14a

14b

15
57 n = 1, R' = H
58 n = 1, R' = TMS
59 n = 1, R' = TMS
60 n = 3, R' = H
61 n = 3, R' = TMS
51, M = Re
52, M = ⁹⁹ᵐTc
53
54
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68
Chapter 1 - Introduction

1.1. Thesis overview

Molecular imaging (MI) agents can be used to detect changes to specific biochemical systems in vivo non-invasively. The discovery, evaluation and translation of viable MI agents can be used to facilitate the early detection, characterization and diagnosis of diseases including cancer and Alzheimer’s disease. The clinical benefits of MI probes derived from radiolabelled compounds (molecularly targeted radiopharmaceuticals) relies upon the development of compound that bind selectively to specific targets in vivo where changes in concentration or function of the target can be monitored as the disease of interest develops and progresses. To create viable probes, it is necessary to develop new prosthetic groups that form robust linkages with both targeting vectors and isotope of interest. This thesis describes the preparation of targeted organometallic carborane-based technetium-99m agents as a unique class of MI probes. During the course of this work, which focused on central nervous system (CNS) targets, novel chemistry of carboranes was discovered and investigated that can be exploited to prepare a new generation of MI agents.

1.2. Diagnostic imaging and radiopharmaceuticals

Historically, in vivo imaging visualizes gross anatomy and changes in structural abnormalities associated with disease or treatment effects. Imaging exploits the interaction of various forms of energy with tissues to non-invasively visualize inside the body. More recently, in vivo imaging of specific molecules and targets (e.g. proteins or
receptors), defined above, is an expanding field because of the advent of sensitive imaging technologies and the development of targeted imaging probes. MI is defined as the observation or measurement of biological functions at the molecular level in healthy and diseased living animals through some process non-invasively. MI can be used for early detection, characterization, and “real time” monitoring of disease as well as investigating the efficacy of drugs. Examples of clinically used MI modalities include single photon emission computed tomography (SPECT), positron emission tomography (PET), magnetic resonance imaging (MRI) and ultrasound (US).

Traditional radiological imaging techniques, such as X-ray, are based on interactions between the electromagnetic waves and the atoms constructing the human body; they do not provide any information regarding specific biochemical processes or targets. Nuclear medicine imaging and MRI are based on molecule-molecule and molecule-magnetic field interactions, respectively, thus, they provide molecular information. Nuclear medicine techniques (SPECT and PET) dominate MI research and clinical use because of their high sensitivity where it is possible to image traces at physiological concentrations. New technologies, such as dual-modality systems, are emerging where the strength of one imaging mode is paired with another to gain greater sensitivity and resolution (e.g. PET-MRI).

In diagnostic nuclear medicine, the imaging agents are known as radiotracers or radiopharmaceuticals. These are compounds that have been labelled with a radioisotope (or radionuclide) prior to injection into a subject (Figure 1.1). Radiopharmaceuticals can be prepared where the radionuclide is an integral structural component of the agent or
where it is simply linked to a targeting vector. The distribution of the radiotracer is monitored by external camera (SPECT or PET cameras) which visualizes the fate of the radiotracer creating an image that is related to the interaction of the agent and its target \textit{in vivo}.\textsuperscript{4, 5} Radioactive imaging is governed by the tracer principle. According to the tracer principle, the agent must (i) behave or interact with the system to be probed in a known and reproducible fashion; (ii) not alter or perturb the system in any measurable fashion; (iii) have a measurable concentration.\textsuperscript{6-9} The type of scanner used for detection of the tracer depends on the radioisotope selected to make the radiopharmaceutical. PET scanners generally offer superior sensitivity, high resolution and utilize shorter-lived radioisotopes, while SPECT offers the advantages of generally longer-lived isotopes and lower direct cost.\textsuperscript{10}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure1.png}
\caption{Graphical representation of a targeted molecular imaging probe or radiopharmaceutical.}
\end{figure}

A radionuclide can decay via three main processes: alpha (\(\alpha\)), beta (\(\beta\)) and gamma (\(\gamma\)) decay modes. In \(\alpha\) decay, the radionuclide emits a heavy, charged particle (\(\alpha\) particle or helium ion) and the resulting nuclide will be reduced by a mass number of four and an atomic number of two. An example of \(\alpha\) decay where radium-226 decays to radon-222 releasing an alpha particle (eq. 1.1).
β-Decay can be sub-classified into β⁻ emission, β⁺ (positron) emission and electron capture where a neutron or a proton in an atom is converted to a proton or a neutron, respectively (eq. 1.2). Consequently, the mass number remains unchanged but the atomic number is decreased by one. A well known example of β⁺ emission is the decay of the PET radionuclide fluorine-18 to oxygen-18 where a proton inside the nucleus is converted into a neutron releasing a pair of particles: a positron (β⁺) and a neutrino (ν). A positron is an electron with a unit-positive charge, the same mass as an electron and interacts with matter in a similar manner. A neutrino is a particle with no rest mass and no electric charge; it exists so that the law of conservation of energy is not violated.

γ-Decay involves the emission of energy, from an excited nuclear state to a lower energy or ground state, by either a high energy photon (γ-ray) or via internal conversion. Higher energy nuclear states are generally short-lived with the exception of metastable states denoted by the letter ‘m’ next to the atomic mass number (e.g. technetium-99m). γ-Decay is depicted as a vertical arrow in a decay scheme from one state to another (vide infra, Figure 1.2).

Some considerations associated with the development of radiopharmaceuticals include the cost of production of the isotope, its availability and the energy of emission.
A pure gamma ($\gamma$) emitter would be ideal (i.e. there is minimal or no $\alpha$ or $\beta$ emission) to reduce radiation dose to patients. Additionally, the half-life of the isotope should be appropriate to prepare the agent, to transport it to the patient and collect the images and be short enough so that it is eliminated from the body as quickly as possible.\textsuperscript{7,11} An effective agent also needs to have high specificity and selectivity.\textsuperscript{a} Examples of radionuclides used for PET and SPECT imaging include: $^{11}$C, $^{18}$F, $^{123/124}$I, $^{64}$Cu, $^{51}$Cr, $^{67/68}$Ga, $^{99m}$Tc, $^{111}$In and $^{201}$Tl.\textsuperscript{3,12}

1.3. Technetium-99m

The most widely used radionuclide for diagnostic imaging is technetium-99m ($^{99m}$Tc). $^{99m}$Tc has a $\gamma$ emission of 140 keV, which is within the desired range (30 and 300 keV) for commercial gamma cameras.\textsuperscript{13,14} With an energy below 30 keV, the $\gamma$ rays are absorbed by tissue and not detected. In contrast, an energy above 300 keV is not detected effectively with commonly available scintillators. $^{99m}$Tc is produced from the decay of $^{99}$Mo where approximately 87% of the total $^{99}$Mo decay is to $^{99m}$Tc and the remaining 13% decays to ground state $^{99}$Tc (Figure 1.2).\textsuperscript{13} Technetium-99m is conveniently available from a $^{99}$Mo/$^{99m}$Tc commercial generator and the $\gamma$ emission of technetium-99m allows the injection of more than 1.11 GBq (30 mCi)\textsuperscript{b} to patients.

\textsuperscript{a} High specificity and selectivity indicates the high probability of uptake in the target region(s) and a high target-to-background (non-target) ratio in order to obtain a resolved image, respectively.

\textsuperscript{b} Système International (SI) unit of radioactivity is the Becquerel (Bq) where Curie (Ci) is the older system and is still widely used.
without imposing excessive radiation exposure. An additional advantage of working with $^{99m}$Tc is its half-life of six hours which allows for preparation and purification of the $^{99m}$Tc-radiopharmaceuticals at a central pharmacy, distribution to hospitals, administration to patients, accumulation in target tissues and collection of images.$^{10}$ An account of the history of the production of technetium and the development of $^{99m}$Tc-based radiopharmaceuticals can be found in several publications.$^{10, 14-22}$

![Figure 1.2. Decay scheme of $^{99}$Mo to $^{99m}$Tc. $^{99}$Mo decays via $\beta^-$ emission to excited states of technetium with the probability of decay (a) 0.3 %, (b) 17 %, (c) <1 %, (d) 82 %; left to right arrows indicate $\beta^-$ decay while vertical arrows are isomeric transitions ($\gamma$ or internal conversion). Energy levels are not shown to scale.$^{13}$](image)

Technetium is a transition metal with no stable isotope. It cannot be readily incorporated into a compound by direct substitution of a native atom in the same fashion as carbon-11 or radiohalogens. Rhenium, being in the same group (group 7), is commonly used as a non-radioactive surrogate. Analogous complexes are prepared where Re is used in place of $^{99m}$Tc and the non-active product characterized by standard spectroscopic methods such as nuclear magnetic resonance (NMR) spectroscopy, infrared (IR) spectroscopy and mass spectrometry (MS). Characterization of radioactive
compounds is performed using high performance liquid chromatography (HPLC) where an ultraviolet (UV)-detector is connected in series with a radio-detector. In the case of $^{99m}$Tc, the analogous Re complex is co-injected with the $^{99m}$Tc-species to confirm the nature of the radioactive complex by matching HPLC retention times.

Technetium can form compounds in a wide variety of oxidation states ranging between -1 and +7 which allows for diversity in the development of radiopharmaceuticals. The most well studied oxidation states are Tc(I) and Tc(V) which have been shown to form complexes that are compatible with the aqueous reaction media used in radiopharmaceutical development. As shown in Figure 1.3, the types of Tc(V) complexes that have been developed include those with Tc(V)-oxo, Tc(V)-nitrido, Tc(V)-imido and Tc(V)-hydrazido cores. The Tc(V) core generally adopts a square-pyramidal geometry with the oxo, nitrido, imido or hydrazido group in the apical position. The core can be stabilized through donating ligand groups ($\sigma$ and $\pi$) having combinations of oxygen, nitrogen, sulfur and phosphorous atoms. Although much progress has been made in developing Tc(V) chelates, various drawbacks preventing routine clinical use have been noted including the formation of isomers (syn and anti), instability under physiological conditions and challenges in preparing the ligand and biomolecule conjugates. Recently, with the recent discovery of a convenient means to prepare $[^{99m}\text{Tc(CO)}_3(\text{H}_2\text{O})_3]^+$ in water, focus has shifted to the preparation of $^{99m}$Tc(I) agents. The chemistry of the $[^{99m}\text{Tc(CO)}_3]^+$ core is discussed further in section 1.6.
Figure 1.3. Tc(V) complexes with (a) oxo-, (b) nitrido-, (c) imido-, (d) hydrazido-cores (charges omitted as it depends on the nature of the R groups).

1.4. Technetium-99m radiopharmaceuticals

There are two main types of technetium radiopharmaceuticals: technetium-essential and technetium-tagged complexes. Technetium-essential is a radiopharmaceutical where the radiometal is a fundamental part of the molecule and thus the metal has direct and significant effect on the distribution of the agent. In the absence of the metal, the molecule would not localize to the site of interest. Examples of approved small molecule $^{99m}$Tc-essential complexes are listed in Table 1.1 and the structures are shown in Figure 1.4.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Trade name</th>
<th>Function</th>
</tr>
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<tbody>
<tr>
<td>$^{99m}$Tc-d, l-HMPAO</td>
<td>Ceretec</td>
<td>cerebral perfusion imaging for evaluation of a stroke</td>
</tr>
<tr>
<td>$^{99m}$Tc-ECD</td>
<td>Neurolite</td>
<td>cerebral perfusion imaging for evaluation of a stroke</td>
</tr>
<tr>
<td>$^{99m}$Tc-MAG$_3$</td>
<td>Technescan</td>
<td>renal function imaging</td>
</tr>
<tr>
<td>$^{99m}$Tc-sestamibi</td>
<td>Cardiolite</td>
<td>myocardial perfusion imaging</td>
</tr>
<tr>
<td>$^{99m}$Tc-tetrofosmin</td>
<td>Myoview</td>
<td>myocardial perfusion imaging</td>
</tr>
</tbody>
</table>

Table 1.1. $^{99m}$Tc-Essential complexes used clinically for diagnostic imaging.
Figure 1.4. Structures of $^{99m}$Tc-essential complexes: (a) $^{99m}$Tc-$d$,l-HMPAO (Ceretec), (b) $^{99m}$Tc-ECD (Neurolite), (c) $^{99m}$Tc-MAG$_3$ (Technescan), (d) $^{99m}$Tc-sestamibi (Cardiolite), (e) $^{99m}$Tc-tetrofosmin (Myoview).

The majority of successful technetium radiopharmaceuticals (i.e. $^{99m}$Tc-essential) are based on coordination complexes containing no targeting vector. Two important advances that have led to the widespread usage of $^{99m}$Tc-essential compounds include: (i) the development of a generator system leading to a routine and reliable source of technetium-99m and (ii) the development of single vial kits (containing reducing agents and ligand to be labelled) for the production of $^{99m}$Tc radiopharmaceuticals in high yields and purities in one step from $[{^{99m}}$TcO$_4]$.$^{24}$ There are numerous $^{99m}$Tc kits for producing radiopharmaceuticals to examine the brain, kidney, heart, bone, liver, lung and for labelling red blood cells.$^7$ $[{^{99m}}$TcO$_4]$ itself is used to examine the thyroid and Meckel’s diverticula.$^7,25$
Current research in the advancement of radiopharmaceuticals has shifted from the production of agents that are based on perfusion to looking for technetium agents that can target a specific biochemical process, site or receptor associated with disease. Known as technetium-tagged radiotracers, the technetium metal is basically a “passenger” in the molecule where the targeting moiety, such as an antibody, peptide or small molecule, directs the distribution of the tracer (vide supra, Figure 1.1). In this case, technetium is either coordinated to the biomolecule directly or via a chelate. The former has the metal bound to amino or thiol donors or it is orientated to mimic a specific geometry to retain specific binding. For instance, the replacement of a steroid ring with a metal chelate was used to generate a system with comparable size and shape to the steroid shown in Figure 1.5a. The commonly used strategy employs a $^{99m}$Tc-chelate that is conjugated to a targeting moiety as shown in Figure 1.5b. Bifunctional chelates have a specific collection and arrangement of donor atoms for binding to the radiometal and a linker group through which a targeting vector can be attached. An example is the N$_2$S$_2$ chelate system linked to a tropane molecule known as TRODAT (Figure 1.5b). Incorporation of a technetium complex can have a significant impact on the specificity of a targeting vector, consequently, the chelate should be sufficiently versatile that its properties and site of derivatization can be easily varied.
Figure 1.5. Bifunctional chelate complexes: (a) $^{99m}$Tc-chelate steroid mimic,\textsuperscript{26} (b) $^{99m}$Tc-chelate system linked to a targeting vector: $^{99m}$Tc-TRODAT.\textsuperscript{27,28}

There are a limited number of examples of targeted agents that are used clinically or that are being evaluated for clinical use. Examples of peptide-based agents include AcuTec (Figure 1.6a) which has been used to image thrombus and P829 which is FDA approved radiopharmaceutical for somatostatin receptor tumor imaging (Figure 1.6b).\textsuperscript{11} Another example of a $^{99m}$Tc(V) targeted radiopharmaceutical for neuroimaging is TRODAT (Figure 1.5b). This agent targets the dopamine transporter and is used to assess Parkinson’s disease.\textsuperscript{27,28} While proven successful in some trials, its use clinically has been relatively modest.
Figure 1.6. Targeted $^{99m}$Tc-radiopharmaceutical agents: (a) AcuTec, (b) P829.

1.5. CNS imaging probes

There is a need for non-invasive methods to better assess neurological diseases and disorders involving the central nervous system including Parkinson’s disease, schizophrenia, Alzheimer’s disease, epilepsy and addiction. PET and SPECT can provide a non-invasive means of studying changes in concentration and function of
neurotransmitter receptors and transporters associated with disease onset and
progression. Although the development of PET radiotracers for *in vivo* imaging of
specific targets in the CNS began more than 25 years ago, only a handful of established
PET agents exist for the imaging of CNS targets. A selection of effective PET
radiotracers for the study of binding sites and proteins in human brain and CNS system is
shown in Table 1.2.\textsuperscript{3,30-33}

<table>
<thead>
<tr>
<th>Neurotransmitter system/target protein</th>
<th>Radiotracer</th>
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<td>DAT</td>
<td>[^{[1]}\text{C}] \text{DASB}</td>
</tr>
<tr>
<td></td>
<td>[^{[1]}\text{C}] \text{methylphenidate}</td>
</tr>
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<td></td>
<td>[^{[1]}\text{F}] \text{CFT}</td>
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<td>[^{[1]}\text{F}] \text{fallypride}</td>
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<td>[^{[1]}\text{C}] \text{carfentanil}</td>
</tr>
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<td>[^{[1]}\text{C}] \text{WAY100635}</td>
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<td>5-HT	extsubscript{2A}</td>
<td>[^{[1]}\text{C}] \text{WAY100635}</td>
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<td>[^{[1]}\text{C}] \text{McN5652}</td>
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**Table 1.2.** PET radiotracers for studying binding sites and proteins in the brain.\textsuperscript{3,30-33}

The serotonin receptor mediates a wide variety of physiological responses in both
the peripheral and central nervous systems.\textsuperscript{34-36} The receptors are activated by serotonin
(5-hydroxytryptamine or 5-HT, Figure 1.7a) and are divided into many sub-classes where the serotonin 5-HT$_{1A}$ receptor is the most widely studied subtype because of their implications in various physiological functions (e.g. sleep and thermoregulation) and pathophysiological processes (e.g. migraine and depression). Serotonergic signaling appears to play a key role in the generation and modulation of various cognitive and behavioural functions. Disruptions in serotonergic systems have been linked to mental disorders such as schizophrenia, migraines, depression, eating disorders and obsessive compulsive disorder.

Arylpiperazines have high affinity for the 5-HT$_{1A}$ receptor. The lead compound known as WAY100635 (1) (Figure 1.7b) is a potent and selective 5-HT$_{1A}$ receptor antagonist (IC$_{50}$ = 2 nM). WAY-type molecules have been labelled with positron emitters $^{11}$C and $^{18}$F as a way to study the role of this receptor in various diseases and several major neuropsychiatric disorders such as depression, eating disorders and anxiety. The majority of agents used to examine 5-HT$_{1A}$ are PET...
tracers including $[^{11}C]WAY100635^{54,55}$ (Figure 1.7c) and $p-[^{18}F]MPPI^{56-58}$ (Figure 1.7d). Advances in SPECT research resulted in the discovery of $p-[^{123}I]-MPPI^{59}$ (Figure 1.7e). Although promising, PET compounds, particularly $^{11}C$ compounds, are generally limited to those facilities with a cyclotron and the high cost of $^{123}I$ hinders commercialization of viable agents which is driving the need to develop $^{99mTc}$-based agents that can target 5-HT$_{1A}$.

Progress in receptor pharmacology with PET radiotracers has led to the design of numerous technetium-99m candidate complexes. Fragments of WAY100635 (I), (i.e. arylpiperazine), have been combined with various bifunctional chelates consisting of a ligand that binds to technetium-99m and a linker that joins to the targeting vector.$^{60-65}$ For example, Johannsen et al. prepared a WAY-based complex using an N$_2$S$_2$ chelate to bind to $^{99mTc}$ (Figure 1.7f).$^{44,66}$ Other similar derivatives of arylpiperazine linked to other technetium(V) chelates (N$_x$S$_y$ or N$_x$P$_y$, x = 1-4, y = 4-x) have also been developed.$^{40,62,67,68}$ Although their IC$_{50}$ and K$_i$ values appear promising, these agents have low or negligible brain uptake likely due to the large size and/or high polarity of the chelate which can hinder compounds crossing the blood-brain barrier (BBB) (Table 1.3).$^{66}$ To date, no $^{99mTc}$ labelled compound has been developed which can image the 5-HT$_{1A}$ receptor in vivo. The failure is due to insufficient brain uptake and the inability of compounds to achieve high receptor binding affinity and selectivity.
<table>
<thead>
<tr>
<th>Compound</th>
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| ![Compound](image1.png)  
M = Re, IC$_{50}$ (nM): 5HT$_{1A}$ = 0.24, $\alpha_1 = 0.05$  
M = $^{99m}$Tc brain uptake 0.22 %ID at 5 min p.i. | 40 | ![Compound](image2.png)  
Brain uptake of 0.53 %ID/g at 5 min p.i. | 60 |
| ![Compound](image3.png)  
M = Re, IC$_{50}$ (nM): 5HT$_{1A}$ = 0.29, $\alpha_1 < 1$  
M = $^{99m}$Tc brain uptake 0.3-0.5 %ID/organ at 5 min p.i. | 62 | ![Compound](image4.png)  
M = Re, IC$_{50}$ (nM): 5HT$_{1A}$ = 0.58-103  
M = $^{99m}$Tc brain uptake 0.24-1.31 %ID at 2 min p.i. | 61 |
| ![Compound](image5.png)  
$^{99m}$Tc, Ki (nM): 5HT$_{1A}$ = 0.29  
$^{99m}$Tc brain uptake 0.14 %ID/g at 2 min p.i. | 67 | ![Compound](image6.png)  
$^{99m}$Tc, IC$_{50}$ (nM): 5HT$_{1A}$ = 1.29, $\alpha_1 =$8.12  
$^{99m}$Tc brain uptake 0.56 %ID/organ at 2.5 min p.i. | 44 |
| ![Compound](image7.png)  
SPECT image of rabbit showed trace amounts in the brain | 68 | ![Compound](image8.png)  
M = Re, IC$_{50}$ (nM): 5HT$_{1A}$ = 6-31  
M = $^{99m}$Tc brain uptake 0.49-1.15 %ID/organ at 1 min p.i. | 64 |
| ![Compound](image9.png)  
IC$_{50}$ (nM): 5HT$_{1A}$ = 20-1100 | 63 | ![Compound](image10.png)  
$^{99m}$Tc brain uptake 0.66 %ID/g at 2 min p.i. | 65 |

**Table 1.3.** Structures and key biological data on reported $^{99m}$Tc WAY-complexes.
For the majority of CNS targets there are no suitable radiotracers. Challenges include finding agents having high \textit{in vivo} affinity, specific binding, metabolic stability, and optimal pharmacokinetics. In the case of technetium-99m, there has been only one moderately successful agent developed to date (i.e. $^{99m}$Tc-TRODAT) which is largely due to difficulties in circumventing these challenges. It has been determined that technetium complexes that have low molecular weight (<600 Da), are neutral and lipophilic ($\log P = 0.5$-2.5)$^c$ can passively cross the blood-brain barrier. Small lipophilic organic drugs easily enter the brain, penetrating the lipid membranes by passive diffusion (in absence of chemical energy or active transporters). Polar or larger molecules use other routes such as transporters, facilitated diffusion or active transport. Very lipophilic agents may become trapped in the inner hydrophobic membrane after penetrating the hydrophilic outer region occupied by phospholipid polar head groups at the surface of the membrane.

One of the major obstacles for imaging neurotransmitter receptors is that the ability of an agent to cross blood-brain barrier. The inability to design coordination complexes of technetium-99m that can cross the BBB and that retain high affinity for targets in the CNS has motivated the search for new technetium synthons from which to develop effective molecular imaging probes.

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$^c$ Lipophilicity or $\log P$ is the partition coefficient between $n$-octanol and water.
1.6. Organometallic radiopharmaceuticals

Despite the preparation and screening of large numbers of coordination complexes of technetium-99m, only a handful of compounds have become successful clinical agents. To address this issue, the development of technetium-99m agents has shifted to looking at different classes of metal complexes. One rapidly emerging area is the use of organometallic complexes of technetium which has a demonstrated history of success. Technetium-sestamibi (Cardiolite, vide supra Figure 1.3d), for example, was the first clinically approved organometallic compound and is widely used as a myocardial perfusion agent.\(^69\)

Organometallic complexes are generally more compact than coordination complexes and they can be more stable in vivo than select chelate complexes. Furthermore, research in bioorganometallic chemistry has shown that the replacement of an organic portion of a targeting vector with an organometallic bioisostere can lead to an increase in selectivity and activity over the parent alone. For example, ferroquine or SRR97193 (Figure 1.8a) is an antimalarial drug that has completed phase I clinical trials and is currently in phase IIb trials.\(^70\) The ferrocene derivative of fluoroquinolone ciprofloxacin (Figure 1.8b) was found to be up to 100-fold more active as an antimalaria agent than the native compound.\(^70\) With respect to CNS, Gmeiner and co-workers observed sub-nanomolar K\(\text{i}\) value (i.e. high binding) for the dopamine D\(_4\) (0.52 nM) and serotonin 5-HT\(_{1A}\) (0.50 nM) receptors for a ferrocenylcarboxamide derivative (2) of a WAY-type vector shown in Figure 1.8c.\(^71\) This result in particular suggests that organometallic complex of technetium which exhibits high affinity to the CNS could be
developed.

![Chemical structures of (a) ferroquine, (b) ferrocenic ciprofloxacin and (c) ferrocenylcarboxamide-WAY (2).]

**Figure 1.8.** Chemical structures of (a) ferroquine, (b) ferrocenic ciprofloxacin and (c) ferrocenylcarboxamide-WAY (2).

As mentioned earlier, a common problem encountered in the development of metal-based neuroimaging agents is the poor diffusion of the complex across the blood-brain barrier. Technetium-chelates linked to WAY are limited by their large size and high polarity; some complexes have also been shown to be unstable *in vivo*. There is a need to develop technetium-99m complexes that are compact, stable and easily modified to facilitate uptake and selective binding to the targets of interest.

As an alternative to bulky chelate systems, organometallic complexes of Re(I) and 99mTc(I) have been examined as potential radiopharmaceutical agents. The first 99mTc organometallic cyclopentadienyl (Cp) complex, [Cp₉⁹mTc(CO)₃], was prepared by Wenzel in 1992 using the double ligand transfer methodology (Scheme 1.1). The Cp ligand and the carbonyl groups, from ferrocene and [Mn(CO)₅Br] respectively, were transferred simultaneously to the 99mTc metal in the preparation of [Cp₉⁹mTc(CO)₃]. The major disadvantages of this method that have not been overcome include the use of high reaction temperatures (160 °C), the use of organic solvents and the incorporation of a targeting vector after the formation of the [Cp₉⁹mTc(CO)₃] complexes. Incorporation of the radioisotope in the early synthetic steps means handling a radioactive substance in
further chemical transformations. The addition of the radioisotope in the last step would minimize radioactive exposure during subsequent synthetic steps.

![Scheme 1.1](image)

**Scheme 1.1.** Preparation of [Cp$^{99m}$Tc(CO)$_3$] via double ligand transfer.

Advances in organometallic chemistry of $^{99m}$Tc(I) can be attributed to the facile preparation of the tricarbonyltechnetium(I) core, $[^{99m}$Tc(CO)$_3]^+$. Alberto and co-workers prepared a $^{fac}$-$[^{99m}$Tc(CO)$_3]^+$ complex in the form of $[^{99m}$TcCl$_3$(CO)$_3]$$_2^-$ under 1 atm of gaseous carbon monoxide in the presence of BH$_3$-THF in organic solvents.$^{73-75}$ In the presence of water, substitution of the [Cl]$^-$ ions by water molecules forms the organometallic aqua ion $[^{99m}$Tc(CO)$_3$(H$_2$O)$_3]^+$ (3).$^{75}$ Later, the same group reported the preparation of 3 directly from $[^{99m}$TcO$_4]$ in saline under 1 atm of CO$_{(g)}$.$^{76}$ In the reaction, a small amount of NaBH$_4$ was used as a reducing agent where the authors found that NaBH$_4$ was an excellent reducing agent and worked synergistically with CO$_{(g)}$ to form $^{99m}$Tc(I).

One of the limitations of this procedure is the use of gaseous CO making it unsuitable for use in commercial radiopharmaceutical kits. Instead, boranocarbonate, [K]$_2$[BH$_3$CO$_2$], was developed as a solid, air-stable source of CO that also acts as a reducing agent replacing both NaBH$_4$ and CO$_{(g)}$. The advent of this reagent led to the commercialization of an instant kit (IsoLink by Mallinckrodt Medical) in which $[^{99m}$Tc(CO)$_3$(H$_2$O)$_3]^+$ is formed after adding $[^{99m}$TcO$_4]$ (Scheme 1.2). The availability of
the boranocarbonate has accelerated the research associated with the development of $^{99m}$Tc(I) radiopharmaceuticals and provided a means to arising products into the clinic.

Scheme 1.2. Preparation of $[^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$ (3) from $[^{99m}$TcO$_4$]$^-$.  

A wide range of mono-, bi- and tridentate chelates have been combined with $[^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$ where the aquo ligands were easily exchanged for chelate donor atoms affording stable complexes.$^{14, 18, 19, 69, 77-79}$ As an alternative to simple chelate complexes, [Cp$^{99m}$Tc(CO)$_3$] was also prepared in aqueous media. Initial coordination of cyclopentadienyl ligand and the related fulvene derivatives occurred in very low yields. When Cp was modified by the addition of an acyl group (Ac) to stabilize the cyclopentadienyl anion, the [AcCpM(CO)$_3$]$^+$ (M = Re, $^{99m}$Tc) complexes were prepared in very high yields.$^{80}$ The cyclopentadienyl WAY-based complex (Figure 1.9), [CpRe(CO)$_3$], demonstrated remarkable in vitro affinity for the serotonin 5-HT$_{1A}$ receptor (IC$_{50}$ = 6 nM).$^{66, 81}$
Although the cyclopentadienyl ligand can form stable metal complexes, they must have an acyl group adjacent to the ring which limits their synthetic versatility. As a potential alternative, *nido*-carboranes (C$_2$B$_9$H$_{12}$), which are isolobal to Cp, can be used to generate organometallic complexes that are low molecular weight and that have the means to be linked to targeting vectors using a number of different strategies (*vide infra*). Previous work in the Valliant group has shown that metallocarborane complexes are compact, lipophilic, and can be prepared rapidly in a microwave reactor in aqueous media thus making them versatile synthons for developing targeted radiopharmaceuticals.$^{82-84}$

**1.7. Carboranes and metallocarboranes**

Dicarba-*closo*-dodecaboranes (C$_2$H$_{10}$H$_{12}$), or carboranes, are polyhedral clusters of boron and carbon atoms. They are considered derivatives of boron hydrides in which one or more B or BH units have been replaced by isoelectronic carbon atoms. For the icosahedral cluster C$_2$B$_{10}$H$_{12}$, the carbon atoms in the cage participate in delocalized bonding yielding thermodynamically stable compounds. Carboranes with the carbon...
atoms adjacent to each other (in the 1,2-position) are known as ortho-carboranes. The meta- (1,7) and para- (1,12)-isomers can be prepared by thermal rearrangement (400 – 700 °C) of ortho-carborane (Scheme 1.3). 85

Scheme 1.3. Thermal rearrangement of ortho-carborane to meta- and para-carborane.

Carboranes are attractive systems for medicinal chemistry due to their high boron content, compact size, lipophilicity, stability and synthetic versatility. Carboranes have been studied for Boron Neutron Capture Therapy (BNCT) which is a binary treatment method for ablating tumours. The boron atoms in the BNCT agent capture thermal neutrons producing an energetically excited boron-11 nucleus which undergoes fission to generate two daughter nuclei (lithium-7 and helium-4). The fission products travel through the tissue causing irreversible cellular damage to the surrounding cells. The nuclides travel approximately 9-10 μm, which is on the order of a typical human cell. 86 The result is localized cytotoxicity inducing apoptosis (cell death). Various small-molecule carboranes have shown good tumor uptake and relatively low toxicity including glycophosphonate derivatives. 87, 88 In addition to BNCT, the role of carboranes has been extended into drug discovery, molecular imaging, and targeted radionuclide therapy. 89, 90 Examples of drug classes where the carborane is an isostere include estrogen receptor agonists 91-94 and antagonists, 95 androgen receptor antagonists, 96 retinoid agonists and
antagonists, and non-steroid anti-inflammatory drugs (NSAIDs). Additionally, other medicinal applications of carboranes or metallocarboranes include the identification of HIV protease inhibitors, anticoagulants and anti-tumor agents.

The preparation of ortho-carborane of the type C₂B₁₀H₁₂ involves an alkyne insertion reaction on decaborane (B₁₀H₁₄). Decaborane is initially activated with a Lewis base such as acetonitrile (CH₃CN), alkylamine (R₃N) or alkylsulfide (R₂S) to generate an adduct of the form B₁₀H₁₂L₂ (L = Lewis base). The alkyne inserts into the open face of the borane, displacing the Lewis base and the two bridging hydrogen atoms, leading to the closure of the carborane cage, RR'C₂B₁₀H₁₀ as shown in Scheme 1.4.

Scheme 1.4. Preparation of substituted carborane via alkyne insertion reaction from a decaborane adduct (4).

Carboranes can be functionalized at one or both of the carbon atoms and at select boron atoms. The various modes of functionalization provide flexibility which is advantageous for the design of radiopharmaceutical agents. Carbon substituted derivatives of ortho-carborane are generally prepared by two methods: the first approach involves the alkyne insertion reaction of a substituted acetylene with decaborane derivative, B₁₀H₁₂L₂ (4) shown previously in Scheme 1.4. The second approach involves reacting the weakly acidic CH group (pKₐ = 22.0) of commercially available ortho-carborane with an alkyl lithium reagent to form the mono-lithio-o-carborane which then
can be treated with a variety of electrophiles including carbon dioxide, halogens and alkyl halides to yield functionalized carboranes; shown in Scheme 1.5.

**Scheme 1.5. Preparation of substituted carborane derivative from \(o\)-carborane.**

The carbon and boron vertices exhibit orthogonal reactivity. As previously described, the weakly acidic carbon protons can be removed by strong non-nucleophilic bases while the hydrogen atoms on the boron vertices can be removed by strong Lewis acids. The carbon atoms act as nucleophiles in substitution reactions while the boron atoms generally act as electrophiles in substitution reactions; the latter resembling Friedel-Crafts reactions. The boron atoms can also be functionalized with organic groups (e.g. aryl, heteroaryl, alkynyl) using metal-catalysts. For example, select boron atoms can be iodinated under electrophilic conditions and subsequent palladium-catalyzed coupling reactions with Grignard reagents leads to boron-carbon bond formation.\(^{107-110}\)

The reactivity differences between the two atoms can be utilized to prepare a wide range of carbon and/or boron derivatives without the need to use protecting groups.

Although *closo*-carboranes are stable clusters, in the presence of a base (e.g. \(\text{OH}^-, \text{HNR}_2\))\(^{111-114}\) one of the boron vertices from *closo*-carborane is removed yielding the 7,8-dicarbaundecaborane(-1) ion, also known as *nido*-carborane (\(\text{C}_2\text{B}_9\text{H}_{12}^-\)) (Scheme 1.6). More recently, weaker bases such as dimethylsulfoxide (DMSO)\(^{115,116}\) and fluoride\(^{117,118}\) have been used to deboronate under milder reaction conditions. Regardless of the base
employed, the reaction occurs at the most electropositive boron atoms which are the two boron atoms adjacent to both cage carbon atoms (Scheme 1.6). There is an equal probability of removal of either of these boron atoms resulting in the formation of a racemic mixture for mono-carbon substituted or disubstituted clusters containing two difference carbon-substituents.85

![Scheme 1.6. Preparation of nido-carborane, (C_{2}B_{9}H_{12}^-), from closo-carborane (arrows indicate the most electropositive boron atoms).](image)

*nido*-Carborane can be isolated or generated *in situ* and reacted further with metal cores to generate organometallic complexes (Scheme 1.7). Further removal of the bridging hydrogen atom in the *nido*-carborane forms the dicarbollide dianion, [C_{2}B_{9}H_{11}]^{2-}. The open pentagonal face which contains two carbon and three boron atoms can form complexes with a variety of metals in an η^5^-fashion. Like in the case of the cyclopentadienide ligand, the dicarbollide dianion has p-like orbitals oriented towards the metal centre. The two species are considered to be isolobal (Figure 1.10).119
Hawthorne and co-workers were the first to recognize the similar bonding nature and prepared a carborane analogue of ferrocene. The X-ray crystal structure shows comparable bond distances between the iron metal centre and the atoms of the bonding face of the dicarbollide and those for the corresponding Cp complex. The iron dicarbollide complexes possess greater thermal stability and better resistance towards acids than the cyclopentadienyl analogs (i.e. ferrocene). This stability is attributed to the p orbitals of the dicarbollide which are directed towards the metal atom increasing overlap with the bonding orbitals and thus increasing the strength of the metal-ligand bond (Figure 1.10).

The first tricarbonyl-metallocarborane complexes contained Mn and Re. Since then, many carbonyl complexes have been prepared including Cr, Fe, Co, W and
In addition to carbonyl complexes, various other transition metals have been coordinated to the dicarbollide ion including metals from groups 4, 6, 8, 9, 10 and 11 such as Zr, Hf, Cr, Fe, Ru, Co, Rh, Ir, Ni, Pd and Cu. Metallocarborane complexes are not limited to transition metals as numerous main group metal complexes have been synthesized and characterized including Be, Al, Ga, and Sn; lanthanoid metals can also be coordinated to carboranes.

The first radiometal carborane derivative, prepared by Hawthorne and co-workers, is referred to as the Venus flytrap. The $^{57}$Co metallacarboranophane, a pyrazole bridged bis-dicarbollyl ligand (Figure 1.11a), was conjugated to an antibody and evaluated as a targeted radiopharmaceutical. Cobalt-57 has limited use as a radioisotope for diagnostic imaging due to its long half-life ($t_{1/2} = 271.8$ d), therefore the development of metallocarboranes for imaging has focused on technetium-99m and radiohalogens.

*nido*-Carborane derivatives can also be radiolabelled with iodine-125/131 (Figure 1.11b) and astatine-211 (Figure 1.11c). Iodination of carboranes generates a B-I bond that is stronger than an aryl C-I bond making them less susceptible to *in vivo* deiodination. The Valliant group investigated targeted radiohalogenated carboranes including a series of a carbohydrate derivatives and a carborane mimic of phenylalanine. Other applications of radioiodinated carboranes include using the *nido*-carborane as a bifunctional linker to conjugate to human epidermal growth factor, dextran, proteins such as bovine serum albumin, and cancer markers (biotin as an example). Additionally, radioiodinated carboranes for BNCT and hypoxia imaging of cancer and radioiodination of *closo*-decaborate(2-) ($RB_{10}H_{9}^{2-}$).
derivatives have also been developed.\textsuperscript{146}

![Diagram of radiolabelled carboranes](image)

**Figure 1.11.** Radiolabelled carboranes: (a) $^{57}$Co Venus flytrap complex, (b) radioiodinated *nido*-carborane, (c) astatinated *nido*-carborane.

Previous work in the Valliant group showed that it is possible to form rhenium(I) and technetium(I) complexes from the $[\text{M(CO)}_3]^+$ core ($\text{M} = \text{Re, }^{99m}\text{Tc}$).\textsuperscript{147} A method was developed to synthesize the metallocarborane from *nido*-carborane in aqueous media (Scheme 1.8) making these complexes accessible for the development of radiotracers.\textsuperscript{84} A second, and more rapid, method to prepare metallocarborane complexes in high yields was developed which utilized microwave-assisted heating.\textsuperscript{82} Following the development of this basic method to generate simple metallocarborane complexes, the focus of the research turned to the preparation of targeted metallocarborane complexes. Only two targeted $^{99m}\text{Tc}$-carborane systems have been reported thus far: an estrogen receptor probe\textsuperscript{148} and a carbohydrate derivative\textsuperscript{137} neither of which generated high affinity for the target of interest.
Scheme 1.8. Preparation of metallocarborane complexes (M = Re, $^{99m}$Tc) in aqueous media.

In terms of numbering carboranes and metallocarboranes (Figure 1.12), for a closo-carborane structure (C$_2$B$_{10}$H$_{12}$), one of the carbon atoms is placed at the apex and is numbered C1; the remaining numbering proceeds by the first five-membered ring of atoms, then the second ring and finally the opposite apex so that the other carbon atoms is given the lowest possible number. For the nido-carborane, the numbering starts at the apex (BH unit) and is labelled in the same order as the closo-carborane in which the carbons atoms of the nido-o-carborane are labelled C7 and C8. For the metallocarboranes, one of the carbon atoms is placed at the apex and the same order applies where the next highest priority atom is labeled as 2 which is either the other carbon atom or the metal. If the carbon atoms are in the ortho position, the carbon atoms would be 1,2 and the metal would be the third priority atom. When designating the complex name, the numbers are based on atom priority. For example, the complex {[Na][3,3,3-(CO)$_3$-1-R-3,1,2-closo-ReC$_2$B$_9$H$_{10}$]} has the metal atom in position 3 with three CO units attached and carbon atoms are designated C1 and C2 where C1 is substituted. Alternatively, if the cage has undergone isomerization (e.g. 1,2 to 1,7) where C2 is located where B11 was in the ortho-orientated metal complex, the complex would be re-number as {[Na][2,2,2-(CO)$_3$-8-R-2,1,8-closo-ReC$_2$B$_9$H$_{10}$]}. The metal is M2 and
the carbon atoms are in C1 and C8.119

![Carborane structures](image)

**Figure 1.12.** Numbering schematic for closo and nido carboranes and the associated 3,1,2 and 2,1,8 metal complexes. Bolded atoms are those in the foreground. Hydrogen atoms are omitted for clarity.

### 1.8. Scope and summary of research

Carboranes derivatives, more specifically Re and $^{99m}$Tc metallocarboranes, have the potential features needed to develop organometallic complexes capable of targeting CNS receptors. The approach to develop these novel agents is defined by the following key steps: (i) identifying a suitable targeting vector and derivatizing with the ligand(s) of interest, (ii) coordinating the metal with the ligand system, (iii) characterizing the metal (M = Re, $^{99m}$Tc) complex structures and identifying the properties and (iv) evaluating the biological aspects of the agent. After these steps, additional features may need to be optimized for the specific target of interest. For instance, targeting a receptor in the brain will likely require optimization so that the agent has the ability to cross the blood-brain barrier.

The focus of this thesis is the development of the methodology to link a known CNS targeting vector to metallocarboranes (M = Re, $^{99m}$Tc) as a means to create the first member of a new class of organometallic CNS imaging probes. The synthesis,
characterization and evaluation (lipophilicity and in vitro binding affinity) of metallocarborane complexes containing the vector and simple alkyl spacer groups is described in chapter 2. To further enhance the biological affinity of the complex, an alternative amide-linked derivative was prepared and the corresponding technetium-99m agent generated and examined (chapter 3). To facilitate the agents crossing the BBB, the charged nature of the \([M(CO)_3(C_2B_9H_{10}R)]^+\) (M = Re, \(^{99m}\)Tc) complex was addressed by the preparation of the neutral \([M(CO)_2(NO)(C_2B_9H_{10}R)]\) analogues (chapter 4). The multistep synthesis and low yields associated with the general preparation of WAY-substituted closo-carborane ligands led to the development of a carborane-alkyne platform using copper-catalyzed cycloaddition reaction/"click" chemistry (chapter 5).

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130. Schubert, D. M.; Bandman, M. A.; Rees, W. S., Jr.; Knobler, C. B.; Lu, P.; Nam,
W.; Hawthorne, M. F. Synthesis of group 13 element metallacarboranes and

1204-1206.

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Chapter 2 – Synthesis, Radiolabelling and Evaluation of Alkyl-linked Metallocarborane-WAY Complexes (M = Re, $^{99m}$Tc)

2.1. Overview

A variety of WAY-based (e.g. arylpiperazine) technetium-99m radiopharmaceuticals have been prepared including $^{99m}$Tc(V)-chelate and organometallic cyclopentadienyl (WAYCp$^{99m}$Tc(CO)$_3$) complexes. However, these complexes have modest to low binding to specific neurotransmitter receptors and/or negligible brain uptake which is typical of coordination complexes of $^{99m}$Tc. While, the [CpM(CO)$_3$] complexes show promise, the need to have an acyl group adjacent to the ring of the $\eta^5$ ligand complicates optimization. The objective of this research was to attempt to overcome limitations of existing compounds by developing a novel class of organometallic technetium-99m complexes that are synthetically versatile and designed to target neurotransmitter receptors.

Metallocarborane complexes can potentially address two of the issues associated with classical $^{99m}$Tc-based brain agents: they have the appropriate lipophilicity and size and are more compact than typical technetium-chelate systems. The aim was to develop the methods needed to conjugate a CNS targeting vector to carboranes and to then form complexes with technetium as a means to generate a new class of CNS imaging agents. The initial focus of this research was to develop a methodology to link the arylpiperazine unit of WAY100635 to the carborane ligand system (5 and 6) via a simple alkyl linker and subsequently prepare the corresponding metallocarborane WAY-complexes (M = Re (12 and 13), $^{99m}$Tc (14 and 15)) (Figure 2.1). The metallocarborane complexes were
evaluated for their lipophilicities, binding affinities to neurotransmitter receptors and ability to cross the blood-brain barrier (BBB).

Figure 2.1. Structures of the WAY-carborane ligands and metallocarborane WAY-complexes.

2.2. Synthesis and characterization of closo-carborane WAY conjugates 5 and 6

The preparation of the first generation of metallocarborane-WAY (M = Re, $^{99m}$Tc) complexes initially involved the synthesis of closo-WAY-carborane conjugates 5 and 6 from their alkynyl precursors 7 and 8. The binding affinity of a compound or complex can vary depending on spacer length and type between the targeting vector and the chelate or ligand system.¹⁻³ To test the impact of the spacer group, alkyl linkages consisting of one and three CH$_2$ units were initially incorporated between the carborane cage and the arylpiperazine.

The alkynyl precursors (7 and 8) were isolated from the reaction of 1-(2-methoxyphenyl)piperazine with the corresponding alkynyl halide (propargyl bromide and chloropentyne) in good yields following silica gel column chromatography (7: 92 % and 8: 74 %; Scheme 2.1a).⁴ Compound 7 was prepared following a literature procedure and the isolated compound matched the reported characterization data. The $^1$H NMR of
compound 8 shows resonances between 6.85-6.98 ppm for the aromatic protons and a shift at 1.95 ppm for the alkyne proton. The IR spectrum of 8 displays a peak at 3295 cm$^{-1}$ confirming the presence of the alkyne group while the mass spectrum shows a peak at 259.1812.

Scheme 2.1. Preparation of: (a) alkynyl halide precursors (7 and 8), (b) closo-carborane derivatives 5 and 6 via alkyne insertion reaction.

Carboranes 5 and 6 were prepared following the well-established alkyne insertion reaction where the Lewis base-adduct of decaborane 4 ($B_{10}H_{12}L_2$, $L=CH_3CN$) was treated with 7 or 8 (Scheme 2.1b). The isolated yields of carboranes 5 and 6 were low (< 5 %), even when considering that carborane reactions are typically between 6-75 %.$^5,^6$

Sneddon and co-workers reported the use of ionic liquids as non-coordinating solvents for the metal-catalyzed reactions of decaborane (Scheme 2.2). In addition to obtaining a hydroboration product (6-RC$_2$H$_4$B$_{10}$H$_{13}$) in high yield in the absence of a metal catalyst, functionalized carboranes were prepared in high yields via the alkyne
insertion reaction with decaborane and an ionic liquid.\textsuperscript{5} As described in section 1.7, the preparation of substituted carborane via alkyne insertion reaction is from treating decaborane adduct 4 with an alkyne which releases hydrogen gas as the by-product (Scheme 2.3). The rate determining process for the formation of functionalized carboranes is the insertion of the alkyne and 4. The presence of the ionic liquid, which is characterized as a strongly coordinating base, is postulated to assist with removing the bridging hydrogen atoms from the decaborane derivative 4 so that once the alkyne has inserted into 4, the cage closure could occur driving the reaction to completion.

\[ \text{B}_{10}\text{H}_{14} + \text{H}_2\text{C} = \text{C}(\text{H})\text{R} \xrightarrow{[\text{BMIM}][\text{BF}_4]} \text{6-RC}_2\text{H}_4\text{B}_{10}\text{H}_{13} \]

**Scheme 2.2.** Hydroboration reaction between decaborane and an olefin in the presence of an ionic liquid and in the absence of a metal catalyst.\textsuperscript{5}

![Scheme 2.2. Hydroboration reaction between decaborane and an olefin in the presence of an ionic liquid and in the absence of a metal catalyst.](image)

**Scheme 2.3.** Alkyne insertion reaction between decaborane 4 and substituted alkyne.

Adding catalytic amount of ionic liquid (1-butyl-3-methylimidazolium chloride, [BMIM][Cl]) to the reaction mixture containing the decaborane-adduct 4 and the alkynyl precursors 7 or 8 in boiling toluene, an increase in the yield occurred (5: 35 % and 6: 13 %; Scheme 2.1a). Although the use of an ionic liquid increased the yield of derivatized carborane compounds, the product mixture was more complex than the
traditional insertion reaction (i.e. without the ionic liquid) and occasionally required multiple purifications via silica gel chromatography.

To address the modest yields and repeated purifications needed to prepare 5 and 6 using the ionic liquid catalyzed insertion reaction, an alternative synthetic strategy was investigated. Rather than preparing 7 and 8 and converting them to the respective substituted carboranes via insertion (Scheme 2.1), derivatized carboranes bearing a reactive halo-alkyl unit (Cl, Br, and I) were prepared (Scheme 2.4) and reacted with 1-(2-methoxyphenyl)piperazine in a substitution reaction (Scheme 2.5 and Scheme 2.6). The series of halo-alkyl carboranes (Br: 9, Cl: 10 or I: 11) were prepared via a classic alkyne insertion; reacting decaborane adduct 4 and either propargyl bromide or chloropentyne in acetonitrile. The yields of the halocarboranes 9, 10 and 11 were 59, 7 and 96 %, respectively, and the characterization data is consistent with literature data.

![Scheme 2.4. Preparation of closo-carboranes 9, 10 and 11.](image-url)
The $^1$H NMR spectrum of 9 shows two overlapping resonances at approximately 4 ppm which can be assigned to the methylene group and the unsubstituted carborane cage CH unit. The $^{11}$B{$^1$H} NMR spectrum of 9 contains multiple resonances between -2.9 to -13.4 ppm in the expected pattern of a closo-carborane derivative. The iodo-carborane derivative 11 was generated from the chloro-carborane 10 by a Finkelstein reaction with sodium iodide (96 % yield). Conversion to the iodide can be readily monitored using $^1$H NMR: the methylene group at 3.51 ppm for 10 shifts to 3.13 ppm for 11. Additionally, changes in the chemical shifts in the $^{13}$C{$^1$H} NMR spectra for each derivative are observed: chloro 10 (32.0-74.3 ppm) and iodo 11 (3.6-61.7 ppm). High resolution mass spectra support the identities of carboranes 10 and 11 with their respective isotope patterns and m/z values.

Synthesis of carborane 5 was achieved via a substitution reaction of closo-
carborane 9 with 1-(2-methoxyphenyl)piperazine heated at reflux to yield the product in 25 % yield (Scheme 2.5). No significant increase in yield was observed for the preparation of 5 using the halo-alkyl carborane route, however, the substitution reaction produced fewer by-products decreasing the need for repeated purification which was required for the insertion reaction that employed an ionic liquid.

Reactivity of the chloro 10 and iodo 11 derivatives with 1-(2-methoxyphenyl)piperazine as well as a one-pot reaction was compared for the preparation of 6. The one-pot in situ generation of 11 from 10 in the presence of 1-(2-methoxyphenyl)piperazine led to a 25 % yield of 6. Chloro-carborane 10 was reacted with 1-(2-methoxyphenyl)piperazine to produce 6 in 15 % yield. The two step synthesis where the chloro-carborane 10 was converted to 11, followed by the isolation of the iodo-carborane 11 and subsequent reaction with the arylpiperazine generated compound 6 with the highest yield (57 %) (Scheme 2.6). The yield and purification of 6 from the substitution reaction is significantly superior to the alkyne insertion reaction using an ionic liquid (13 %) even with the increase in the number of synthetic steps. The iodo-carborane 11 is a better leaving group than the chloro carborane compound 10 which is likely the reason for the increase in yield.

Characterization of compounds 5 and 6 prepared using the improved synthetic method (i.e. substitution) matched with the data obtained using the alkyne insertion route, validating this alternative pathway. The 1H NMR spectrum of 5 contains resonances at 6.86-7.02, 4.02 and 3.85 ppm confirming the presence of the ortho-substituted aromatic ring, CH proton of the carborane cage and arylmethoxy group, respectively. The
$^{11}$B{$_1^1$H} NMR spectrum of 5 contains resonances between -4.1 and -14.3 ppm which are characteristic of a mono-substituted closo-carborane species (Figure 2.2). A broad peak at 2576 cm$^{-1}$ in the infrared spectrum is attributed to the BH units of the carborane. The isotopic pattern in the mass spectrum of 5 with a m/z 350.3109 further supports the identity of the isolated compound.

**Figure 2.2.** $^{11}$B{$_1^1$H} NMR (160 MHz, CDCl$_3$) spectrum of 5.

Compound 6 has similar chemical resonances in the $^1$H NMR spectrum as 5 with the exception of the additional signals between 1.70 and 2.37 ppm due to the addition of the methylene groups in the spacer group. Six resonances between -2.7 and -13.5 ppm in the $^{11}$B{$_1^1$H} NMR spectrum of 6 are also consistent with a closo-type cluster. The IR spectrum of 6 contains a BH stretch at 2589 cm$^{-1}$ and the mass spectrum shows a C$_2$B$_{10}$ isotopic pattern with a molecular ion peak at m/z = 378.3452 (Figure 2.3).
X-ray quality single crystals of \textit{closo}-carboranes 5 and 6 were obtained by slow evaporation of dichloromethane (Table 2.1). Carborane 5 crystallized with two independent molecules in the unit cell (Figure 2.4). The C-C cage bond lengths in 5 span 1.62 and 1.65 Å while the B-B bond distances range from 1.75 to 1.81 Å. The crystal packing of 5 displayed an alternating head-to-tail, tail-to-head pattern with the carborane cage in a zigzag-type arrangement along the b axis (Figure 2.5). Compound 6 (Figure 2.6) has a similar C-C cage bond length (1.65 Å) and average B-B bond distances (1.76 Å) as 5. Both these structures have comparable bond lengths to similar substituted \textit{closo}-carboranes reported in the literature (C-C = 1.65 Å and B-B ranged from 1.76-1.78 Å).\textsuperscript{7} The crystal packing of 6 was different than 5 and showed no unique features.
Figure 2.4. X-ray crystal structure of 5 (showing 50 % thermal probability ellipsoids; hydrogen atoms and solvent molecules omitted for clarity).

Figure 2.5. Crystal packing of 5 viewed along the b axis.
Figure 2.6. X-ray crystal structure of 6 (showing 30% thermal probability ellipsoids; hydrogen atoms omitted for clarity).

Table 2.1. X-ray crystal data for 5 and 6.

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¹ R = \[ \frac{\sum |F_o| - |F_c|}{\sum |F_o|} \] for reflections with I > 2.00σ(I)

ᵇ R_w = \[ \sqrt{\frac{\sum w(F_o^2 - F_c^2)^2}{\sum w(F_o^2)^2}} \] for all reflections
2.3. Synthesis and characterization of alkyl-linked WAY-metallocarborane complexes (M = Re (12 and 13), $^{99m}$Tc (14 and 15))

2.3.1. Preparation of rhenacarborane complexes 12 and 13

Removal of a BH vertex from the closo-carborane generates a more reactive nido-carborane cage species which is necessary prior to reaction with the metal centre. Preparation of the nido-carborane derivative can be accomplished by heating the closo-carborane in a basic solution or in the presence of a suitable nucleophile (e.g. hydroxide, amine, fluoride).\(^8\)\(^-\)\(^{13}\) The nido-carborane can subsequently be reacted with a metal centre such as $[\text{M(CO)}_3]^+$ (M = Re, $^{99m}$Tc).

Previously our group reported the synthesis of metallocarborane complexes (M = Re, $^{99m}$Tc) in water making the target compounds suitable for use as radiopharmaceuticals (Scheme 2.7).\(^{14}\) Following this standard method, closo-carborane 5 was converted to its nido-carborane analogue in refluxing ethanolic sodium hydroxide. nido-Carborane 5 was isolated and subsequently heated to reflux in aqueous ethanol with $[\text{Re(CO)}_3(\text{H}_2\text{O})_3][\text{Br}]$ in the presence of sodium fluoride to prepare the metal complex 12. The addition of fluoride to the reaction was used to prevent premature decomposition of the metal(I) complex over the long reaction time.\(^{14-16}\) After extensive heating (7 days), no complex was detected by thin layer chromatography (TLC) or mass spectrometry. Microwave-assisted heating, which is becoming an increasingly popular method to accelerate chemical reactions in drug discovery, was explored as a means to increase the yield.\(^{17}\) Our group investigated using microwave heating for the preparation of rhenacarborane complexes using a conventional microwave and a Parr microwave vessel.
The reaction between *nido*-5 and [Re(CO)$_3$(H$_2$O)$_3$][Br] in the microwave reactor under pressure led to the generation of the desired complex 12, however, the reproducibility of each reaction was poor due to the inability to set or monitor the temperature and pressure in the vessel.

**Scheme 2.7.** Preparation of metallocarboranes (M = Re, $^{99m}$Tc) in aqueous media.$^{14}$

The use of an automated microwave reactor (Biotage) and microwave compatible reaction vials led to a rapid and efficient method for preparing metallocarborane complexes (M = Re, $^{99m}$Tc). With this method, the yield improved and the synthesis became reproducible compared to conventional heating. The automated microwave reactor allows for the selection of the desired reaction temperature, reaction time and monitoring of pressure of the reaction. Using the microwave reactor, the *nido*-carborane analogues (*nido*-5 and *nido*-6) were prepared *in situ* from the reaction between *closo*-carborane (5 and 6) and sodium fluoride (Scheme 2.8). Subsequently, multiple additions of [Re(CO)$_3$(H$_2$O)$_3$][Br] was added to the reaction vial to generate the respective rhenacarborane complexes 12 and 13 in one pot. High, reproducible yields of the desired products were obtained using either silica gel or reverse phase column chromatography (12: 82 % and 13: 95 %). With the rapid reaction times (< 15 min) associated for metal
complexation using microwave heating, the addition of a second equivalent of fluoride to the reaction solution to stabilize the metal(I) centre was no longer be necessary for preparing rhenacarborane complexes.

Scheme 2.8. Preparation of rhenacarborane complexes 12 and 13 using microwave heating.

The rhenium carborane complex 12 has a broad resonance at 1.88 ppm in the $^1$H NMR spectrum consistent with the carborane CH which was at 4.02 ppm in the closo-carborane derivative 5. Upon complexation of the $[\text{Re(CO)}_3]^+$ core to the carborane cage, an AB spin system at 3.79 and 3.53 ppm appeared due to the diastereotopic protons of the spacer between the metallocarborane and the WAY molecule. The $^{13}$C$\{^1$H$\}$ NMR spectrum contains a resonance at 197.8 ppm due to the CO units of the $[\text{Re(CO)}_3]^+$-core; the presence of the CO units was confirmed in the IR spectrum with $\nu$(CO) stretches at 2005 and 1907 cm$^{-1}$. A single peak arising from the carbonyl groups in the $^{13}$C$\{^1$H$\}$ NMR implies that the Re(CO)$_3$ unit is in constant motion relative to the carborane cage,$^{18}$ other rhenacarborane carbonyl complexes have also observed a single resonance for the
carbonyl units.\textsuperscript{7,16,18,19} The $^{11}$B\textsuperscript{1}H NMR spectrum of 12 shows resonances between -5.8 and -20.3 ppm (Figure 2.7) which are characteristic of rhenacarborane complexes, while the original \textit{closo}-carborane compound 5 exhibited resonances between -4.1 and -14.3 ppm. The mass spectrum has a B\textsubscript{9}Re isotopic distribution and a m/z of 609.2366 which is consistent with the molecular ion of 12 (Figure 2.8).

\textbf{Figure 2.7.} $^{11}$B\textsuperscript{1}H NMR (160 MHz, CD\textsubscript{2}Cl\textsubscript{2}) spectrum of 12.
Single quality crystals of compound 12 were obtained by slow evaporation from a chloroform solution and analyzed by X-ray crystallography. Upon refinement of the data, the expected 3,1,2 isomer of the rhenium-carborane, where the two cage carbon atoms are adjacent to one another in the C₂B₃ bonding face, was not observed (Figure 2.9a). Instead, the carbon atom bearing the arylpiperazine group migrated out of the five-membered ring bound to the metal atom generating the 2,1,8-configuration of the rhenacarborane complex 12 (Figure 2.9b). The isomerization can be envisioned as a 120° rotation of the C-B-B triangle (Figure 2.9c). At the time the compound was isolated, this was the first observed and characterized rhenacarborane to undergo 3,1,2 to 2,1,8 isomerization. Isomerization of this nature for other sterically hindered metal-carborane (e.g. Pt, Ni, Rh, Ir) complexes has been reported. An example of this type of
isomerization includes the preparation of a phenyl substituted platinum carborane complex using microwave heating in which the carbon atoms in the carborane cage of the product migrated to a 1,7 configuration from the 1,2 position (Scheme 2.9).\textsuperscript{23}

Figure 2.9. (a) The expected 3,1,2-Re carborane complex; (b) the observed 2,1,8-Re carborane complex 12; (c) pictorial illustration of isomerization: 120° rotation of C-B-B triangle in the carborane cage.

Scheme 2.9. Pt-carborane complex showing isomerization (1,2 to 1,7) using microwave heating producing a similar isomerization product as 12.

In the X-ray crystal structure of 12 (Figure 2.10, Table 2.2), one of the piperazine nitrogen atoms is protonated which results in no residual counterion (Na\textsuperscript{+}) in the structure. Protonation of a nitrogen atom in the piperazine has been cited in literature and is supported by the slightly longer N-C bond within the ring.\textsuperscript{24} The average Re-B bond distance in the metallocarborane is 2.31 Å which is similar to the Re-C\textsubscript{cage} distance of
2.32 Å. Other closely related rhenium-carborane complexes that have been structurally characterized have similar average Re-B and Re-C<sub>cage</sub> bond distances (ca. 2.32 Å).<sup>15</sup> The metal-carbon bond distance of 12 is comparable to the average bond length (2.31 Å) observed for the analogous cyclopentadienyl-WAY rhenium complex (CpRe(CO)<sub>3</sub>WAY) reported by Alberto and co-workers.<sup>25</sup>

**Figure 2.10.** X-ray structure of 12 (showing 50 % thermal probability ellipsoids; hydrogen atoms and solvent molecule were omitted for clarity).
Table 2.2. X-ray data for 12 containing one molecule of CH₂Cl₂ in the lattice.

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² R = [Σ|F_o| – |F_c|]/[Σ|F_o|] for reflections with I > 2.00σ(I)
ᵇ R_w = \{[Σw(F_o² – F_c²)²]/[Σw(F_o²)²]\}¹/² for all reflections

To confirm that the bulk material of 12 exists as the 2,1,8-isomer, a nuclear Overhauser enhancement (nOe) experiment was performed. Irradiation of the carborane CH proton did not cause enhancement of the proton resonances in the substituent, indicating that the carborane CH and the pharmacophore units are not in close proximity thus corroborating the X-ray data. A variable temperature experiment in the microwave reactor was undertaken to further understand the observed isomerization. A series of reactions was conducted over a temperature range of 100-200 °C at a fixed time of 10 min for each reaction. The products were then analyzed by ¹H NMR spectroscopy, and the results indicated that complex formation begins at temperatures greater than 150 °C.
Results showed no evidence for either isomerization of the free nido-ligand 5 prior to complexation or the initial formation of the 3,1,2-rhenium carborane complex followed by isomerization to the 2,1,8-configuration. It can therefore be concluded that isomerization of the carborane cage and the coordination of the rhenium metal core occur simultaneously.

The results observed for complex 12 led to further probing of the steric and electronic effects associated with rhenacarborane cage isomerizations. Carborane ligands substituted with a bulky group such as a benzene ring observed isomerization upon complexation with [Re(CO)3]+ (Scheme 2.10a). When a spacer (such as CH2 unit) was introduced between the phenyl group and the cage, the steric strain is alleviated and the resulting rhenium complex retains its original configuration (with the carbons in the 1,2 orientation) (Scheme 2.10b). This 3,1,2-benzyl complex was subjected to prolonged heating in the microwave reactor but carborane cage isomerization was not observed. Based on these studies, the isomerization of complex 12 is most likely attributed to the close proximity of the substituent to the metal centre and the steric bulk of the arylpiperazine unit is the driving force for the carborane cage isomerization. Similar to complex 12, the phenyl (Scheme 2.10a) and phenol substituted carboranes displayed simultaneous coordination with [Re(CO)3]+ and isomerization.
Scheme 2.10. Reaction between [Re(CO)$_3$(H$_2$O)$_3$]$^+$ and (a) a phenyl-substituted carborane generating the isomerized complex; (b) a benzyl-substituted carborane retained its cage configuration in the product.

Compound 6 was converted to its corresponding *nido*-6 *in situ* with sodium fluoride and reacted with [Re(CO)$_3$(H$_2$O)$_3$]$^+$ generating complex 13 (Scheme 2.11). The $^1$H NMR spectrum of 13 contains resonances at 1.91 and 1.75 ppm which are attributed to the additional CH$_2$ units and the unsubstituted carborane proton, respectively. The $^{13}$C{$^1$H} NMR spectrum has a resonance at 200.4 ppm which is consistent with the coordination of the [Re(CO)$_3$]$^+$ core and the tripodal arrangement of the CO units is confirmed in the infrared spectrum of 13 showing CO frequencies at 1995 and 1897 cm$^{-1}$. The $^{11}$B{$^1$H} NMR spectrum shows resonances between -5.2 and -19.7 ppm which are characteristic of rhenacarborane complexes and the peaks are spread over a similar range as found for 12. The mass spectrum contains a molecular ion peak at m/z = 637.2679 with an isotopic pattern consistent with a B$_3$Re complex supporting the formation of the
desired complex.


Cage configuration of rhenacarborane 13 was determined from a $^1$H nOe experiment: irradiation of the broad cage CH proton resonance (at 1.75 ppm) caused enhancements of the proton resonances of the piperazine ring in the pharmacophore, indicating that the unsubstituted carborane CH is in close proximity to the arylpiperazine substituent and that the ortho- or 1,2 carbon-carbon cage configuration was retained (Figure 2.11). The steric strain which drove the isomerization of 12 is alleviated by the introduction of the longer linker between the carborane cage and the substituent and the results of complexation are similar to the benzyl-carborane derivative seen in Scheme 2.10b. Repeated attempts to grow X-ray quality crystals of 13 were not successful.
Figure 2.11. $^1$H nOe spectrum of complex 13 where the carborane-CH was irradiated (arrow) leading to enhancements of the proton resonances in the piperazine substituent (circled) indicating the retention of the 3,1,2-configuration.

The movement of the CH unit in the polyhedron to sites more distant from each other decreases the electrostatic repulsion of the carbon atoms. Thus, the isomerized 2,1,8 rhenacarborane 12 (Figure 2.9b) is less polar than the original 3,1,2 complex 12 (Figure 2.9a). The lipophilicity of the analogous $^{99m}$Tc complexes support the slight polarity differences of these complexes (section 2.4.2, vide infra). The less polar complex (2,1,8) seems to favour crystallization for these derivatives over the rhenacarborane complex which retains the 1,2 carbon configuration.

The precise mechanism of carborane isomerization or rearrangement is not known but two widely disputed pathways are diamond-square-diamond (DSD) and triangular face rotation (Figure 2.12 and Figure 2.13, respectively). These routes are considered a visual schematic of the isomerization rather than a true reaction mechanism. Isomerization from $o$-carborane to $m$-carborane has been proposed by both DSD and
triangular face rotation rearrangements. For the carbon units to move from the 1,2 to the 1,7 orientation of the 2,1,8-rhenacarborane complexes, it is suspected that a mixture of DSD and triangular face rotation could be proposed via pseudocloso intermediate polyhedral connectivity.\textsuperscript{21}

Figure 2.12. Schematic representation of the diamond-square-diamond isomerization where the icosahedron (diamond: highlighted in blue) opens to a cuboctahedron (square: highlighted in blue) then a new bond forms (dotted line) to regenerate another icosahedron/diamond (highlighted in red and blue; the structure has rotated). Some solid spheres representing BH vertices were removed and bonds in the background were shaded gray for clarity.

Figure 2.13. Schematic representation of triangular face rotation isomerization (top vertex removed for clarity): the closo structure adopts a pseudocloso structure with the absence of a bond between the carbon atoms (highlighted in red); the B-B-C triangle (highlighted in blue) subsequently undergoes a 120° rotation leading to the isomerized product reforming the closo configuration. Some solid spheres representing BH vertices were removed and bonds in the background were shaded gray for clarity.

2.3.2. Preparation of $^{99m}$Tc-carborane complexes 14a, 14b and 15

The characterized rhenium complexes 12 and 13 serve as the HPLC reference
standards for confirming the generation of the analogous $^{99\text{m}}\text{Tc}$-carborane complexes (14a and 15). Following a previously published procedure, $^{14}$ nido-5 and sodium fluoride were combined with $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ (3) in a sealed vial and the mixture was heated (Scheme 2.12). Preparation of 3 from $[^{99\text{m}}\text{TcO}_4]^-$ required approximately 30 min of reaction time prior to the addition of a solution of nido-5 and sodium fluoride. The reaction mixture was then heated at 95 °C for 2 hours in a heating block to yield complex 14a in a 45 % yield according to radio-HPLC. Reducing reaction times is desired for working with radioactive compounds to minimize losses by radioactive decay. With the successful use of microwave heating for preparing rhenium carborane complexes, the analogous synthesis of the technetium analogues was explored.

It should be noted that there is a distinct concentration difference between rhenium reactions and technetium reactions. Reaction with rhenium take place using macroscopic quantities of precursor (mol to $10^{-3}$ mol) while technetium reactions occur at trace levels ($10^{-9}$ to $10^{-12}$ mol). Fortunately, the reactions between carborane ligands and $[^{99\text{m}}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ were found to be equally effective as the rhenium complexation which lead to the publication of the first paper describing synthesis of $^{99\text{m}}\text{Tc}$-carborane complexes.
complexes using microwave-assisted heating.\textsuperscript{16}

The microwave reaction was applied to the synthesis of $14a$. A series of reactions was conducted between $[^{99m}{\text{Tc}}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ with nido-$5$ at temperatures ranging between 100-195 °C at a fixed time (5 min) and the crude reaction mixtures were analyzed by HPLC. The $^{99m}\text{Tc}$-complex was detected by radio-HPLC and its retention time was compared to the retention time of the rhenium complex $12$ (observed by UV-HPLC). A slight difference is observed between the retention time of the UV-detector and the radio-detector due to the serial configuration of the two detectors.

The experimental results indicated that at temperatures greater than 190 °C, only one complex was synthesized $14a$ ($t_R = 15.7$ min) where the peak in the radio-HPLC correlates with the rhenacarborane $12$ ($t_R = 15.0$ min) in the UV chromatogram. For reaction temperatures between 140 and 180 °C, two $^{99m}\text{Tc}$ complexes were observed: $14a$ and a species with a shorter retention time ($t_R = 14.9$ min); at temperatures <140 °C, only the $t_R = 14.9$ min species ($14b$) was present. These observations suggest the formation of the 3,1,2-$^{99m}\text{Tc}$-carborane complex $14b$ at lower temperatures followed by conversion to the 2,1,8 isomer $14a$ as the temperature is increased (Scheme 2.13).

\begin{equation}
\text{Scheme 2.13. Complexation and cage isomerization observed from the reaction between nido-5 and } [^{99m}\text{Tc}(\text{CO})_3]^+.
\end{equation}
These results were confirmed by the conversion of 14b ($t_R = 14.9$ min) to the thermodynamically stable isomer 14a by heating 14b to 195 °C for 5 min. The resulting species had a retention time of $t_R = 15.7$ min which is the same as 14a and matches the rhenium standard 12. Figure 2.14 shows the chromatograms of the conversion of 14b to 14a. The top set of radio- and UV-chromatograms shows the radioactive product 14b ($t_R = 14.9$ min) co-injected with complex 12 ($t_R = 15.0$ min) (Figure 2.14a). As mentioned previously, the HPLC detectors are set up in series with the UV-detector before the radio-detector where 14b eluted before the rhenium standard 12 indicating that 14b is not the analogous $^{99m}$Tc complex of 12. Figure 2.14b shows the product mixture after 14b was heated further. The chromatogram shows a peak with a retention time of 15.7 min which is the same as 14a as well as some degradation or oxidation products of the complex ($t_R < 12$ min) arising from the additional microwave heating. The retention times of 14a and 14b are quite similar so to confirm that they are not the same complex, 14b and the product mixture after heating were co-injected with 12 (Figure 2.14c). The chromatogram shows complexes 14a and 14b are distinct peaks where 14a correlates to that for 12. The optimal reaction condition for the preparation of 14a is 195 °C for 10 min while 120 °C for 10 min is most favourable for 14b. Both 14a and 14b were isolated by solid phase extraction (SPE) and HPLC where the decay corrected radiochemical yields of 14a and 14b were 36 and 21 %, respectively.
Figure 2.14. HPLC chromatograms (top: γ; bottom: UV) of (a) isolated 3,1,2-\(^{99m}\)Tc carborane complex 14b co-injected with 2,1,8-Re carborane 12; (b) reaction mixture containing 14b following heating in the microwave generating 2,1,8-\(^{99m}\)Tc carborane complex 14a; (c) co-injection of \(^{99m}\)Tc complexes (14b and 14a) and Re carborane 12.
The WAY-carborane conjugate 14b was the first observed and isolated 3,1,2-$^{99m}$Tc complex prepared using microwave heating. The temperature threshold for complexation and isomerization for $^{99m}$Tc do not match those seen for Re. Recall that complex 8 showed simultaneous complexation and cage isomerization with Re where the presence of the 3,1,2 isomer was not observed at lower reaction temperatures. We suspect the difference in isomerization behaviour between Re and $^{99m}$Tc can be attributed to the different reduction-oxidation potentials of the two metals. Other carborane derivatives (e.g. pyridine-substituted) have since been reported to have similar complexation trends as 14b. Complexation between $[^{99m}$Tc(CO)$_3]^+$ and the pyridyl-carborane compound generated the 3,1,2-$^{99m}$Tc pyridyl complex at slightly lower complexation temperatures (\(< 100 \, ^{\circ}C\) than 14b (Scheme 2.14). At a higher reaction temperature (\(> 160 \, ^{\circ}C\)) a mixture of 3,1,2- and 2,1,8-$^{99m}$Tc pyridyl complexes was observed. For rhenium complexation, only small amounts of the 3,1,2-Re pyridyl complex was observed at temperatures \(< 120 \, ^{\circ}C\) where reaction temperatures around 140 \(^{\circ}C\) generated more than 50 \% of the mixture consisting of the 2,1,8-Re complex (Scheme 2.14). Similar to the experimental results for 12 and 14b, different complexation products were found between the pyridyl-carborane derivative and [Re(CO)$_3$(H$_2$O)$_3$]$^+$ and $[^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$ at different temperatures.
Scheme 2.14. Reaction between \([\text{M(CO)}_3]^+\) (\(\text{M} = \text{Re, } ^{99m}\text{Tc}\)) and a pyridyl-carborane derivative to give metallocarborane (\(\text{M} = \text{Re, } ^{99m}\text{Tc}\)) complexes at different temperatures.  

The \(^{99m}\text{Tc}\)-carborane complex 15 was prepared via microwave heating using a procedure similar to 14a; analysis of the reaction mixture by HPLC showed a good retention time correlation of 15 with the rhenium complex 13 suggesting successful preparation of the technetium-99m analogue. Following solid phase extraction (SPE) and HPLC purification, the decay corrected radiochemical yield of 15 was determined to be 19 %. The low yield was attributed to the non-specific binding to the HPLC column associated with HPLC purification. The conversion of complexes 14a and 15 were 60 and 50 %, respectively, based on the analyses of the crude reaction mixtures by radio-HPLC.

Complexes 14a and 15 were re-suspended in 5-10 % ethanol/saline solutions and examined for stability. No decomposition was observed by radio-HPLC analysis over 6 hours using different HPLC conditions (mobile phase and pH). Examination of a 5-10 % ethanol/saline solution of the complex for stability was selected because this
solution/formulation can be used for *in vivo* testing of the complex.

When producing technetium-99m radiopharmaceuticals using traditional instant kit methods, the presence of a large excess of unlabelled material can be problematic. If the free ligand has affinity for the target similar to that for the radiolabelled compound it can saturate the target, consequently blocking the $^{99m}Tc$ complex from docking and potentially producing undesirable side effects.\(^{31}\) This is especially important for targets that are expressed in relatively low concentrations (i.e. low $B_{\text{max}}$).\(^{32}\) Specific activity (SA) and effective specific activity (ESA) are measurements to identify the purity of a complex. Specific activity is defined as the ratio of radioactivity divided by the mass of the sum of all radioactive and stable isotopes of the element involved (eq. 2.1) and is usually expressed in units of Bq/mol.\(^{33}\) Effective specific activity is the radioactivity per unit mass of total sample where the mass of the total sample is the sum of the mass of radioactive and stable isotope sample as well as any residual unlabelled compound (eq. 2.2).

\[
\text{SA} = \frac{\text{Radioactivity (MBq)}}{\text{Amount of radioactive + stable isotopes of the element (ng or mmol)}} \quad (\text{eq. 2.1})
\]

\[
\text{ESA} = \frac{\text{Radioactivity (MBq)}}{\text{Mass of total sample (mg or mmol)}} \quad (\text{eq 2.2})
\]

Mass of total sample = mass of radioactive/stable isotopes + mass of unlabelled ligand

Following HPLC isolation of complexes 14a and 15, the specific activity and effective specific activity of each complex was determined using analytical HPLC by
measuring the amount of $^{99}$Tc (ground state) in the sample and/or unlabelled ligand in the sample. The measured specific activities and effective specific activities of 14a and 15 ranged from 6.9-82.0 $\times$ 10$^4$ MBq/mmol and 1.9-5.5 $\times$ 10$^4$ MBq/mmol, respectively (Table 2.3). The values of SA and ESA for the $^{99m}$Tc-carborane complexes are orders of magnitude lower than those found for the preparation of standard $^{99m}$Tc-chelate systems using solid phase labelling (4.1 $\times$ 10$^8$ MBq/mmol)$^{34}$ or solid phase purification (3.7-4.6 $\times$ 10$^8$ MBq/mmol).$^{35}$ Another $^{99m}$Tc-chelate with a similar targeting vector ($^{99m}$Tc-DADT-WAY) has an estimated effective specific activity of greater than 4.4 $\times$ 10$^7$ MBq/mmol (1180 mCi/μmol) following solid phase purification$^{36}$ while a diamine $^{99m}$Tc-chelate reported a specific activity in the range of 3.0 x 10$^7$ MBq/mmol (800 mCi/μmol).$^{37}$

Although the specific activities of the $^{99m}$Tc carborane complexes are lower than standard $^{99m}$Tc-chelate complexes, we have been able to remove excess nido-ligand using HPLC purification to prepare pure complexes which can be used in further in vivo studies.

<table>
<thead>
<tr>
<th></th>
<th>Specific activity ($\times$ 10$^4$ MBq/mmol)</th>
<th>Effective Specific Activity ($\times$ 10$^4$ MBq/mmol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14a</td>
<td>6.9</td>
<td>5.5</td>
</tr>
<tr>
<td>15</td>
<td>82.0</td>
<td>1.9</td>
</tr>
</tbody>
</table>

Table 2.3. Measured specific activity and effective specific activity values for 14a and 15.
2.3.3. Optimizing the metal (M = Re, $^{99m}$Tc) complexation reaction with carboranes

The complexation of the WAY-carboranes with $[\text{M(CO)}_3(\text{H}_2\text{O})_3]^+$ (M = Re, $^{99m}$Tc) requires high temperatures (> 180 °C). A high reaction temperature for complexation is not amenable for thermally sensitive biovectors. Consequently, we attempted to optimize the metal complexation reaction by examining an alternative microwave reactor. Complexation reactions were run in parallel between nido-6 and $[\text{M(CO)}_3(\text{H}_2\text{O})_3]^+$ (M = Re, $^{99m}$Tc) comparing two microwave reactors: the standard microwave (Biotage) versus a microwave reactor (CEM) fitted with a cooling cavity insert. The cavity contains a cooling liquid which circulates to keep the reaction cavity and the reaction mixture at a temperature no higher than a set value. The heat generated by microwave irradiation of the reaction mixture is therefore minimized. The purpose of this study was to determine if the energy from microwave irradiation is sufficient to drive the complexation at ambient temperature or if high temperature and pressure is required for complexation. Similar concentrations/quantities and work-up procedures were followed for both microwave reactors where the programmed reaction conditions were the only variables. The reaction mixtures were analyzed by radio- and UV-HPLC.

For the Biotage system, reaction between nido-6 and $[\text{Re(CO)}_3(\text{H}_2\text{O})_3]^+$ used the optimal conditions described previously (Scheme 2.11): 195 °C for 10 min (maximum power 90 W). For the CEM system, the reaction conditions were 30 °C for 10 min (maximum power 200 W). The power outputs were automatically set depending on the program and temperature entered for the reaction. As previously described, the
rhenacarborane (13) could be prepared in high yield using the Biotage microwave. On the other hand, the reaction was unsuccessful in the CEM system showing only unreacted nido-6 in the UV-HPLC chromatogram. Complexation at the tracer level was attempted between nido-6 and \([^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+\) in each of the two microwave systems. The maximum temperature for the Biotage was 195 °C for 10 min with an average power of 60 W. The CEM system was set to 20 °C for 10 min and a maximum power output of 300 W where maximum temperature of 35 °C was measured. Isolation of the product and the removal of technetium salts using a C18 solid phase extraction cartridge were accomplished before radio-HPLC analysis. The SPE fractions were measured for radioactivity in the dose calibrator from the CEM reaction and no radioactive product was detected. The radio-HPLC chromatogram confirmed the absence of \(^{99m}\text{Tc}\)-carborane 15 as no gamma signal was seen while the UV signal indicated unreacted nido-6. The \(^{99m}\text{Tc}\) carborane complex 15 was synthesized using the Biotage microwave system and confirmed by co-injection with its corresponding rhenium standard (13).

Although the CEM microwave reactor was able to reach a higher power output while keeping the reaction vessel temperature below 40 °C, microwave irradiation alone was not able to drive the complexation of WAY-based carborane ligand with either \([\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3]^+\) or \([^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+\). High temperatures appear to be required for the preparation of these metallocarborane (M = Re, \(^{99m}\text{Tc}\)) complexes which may limit their use for temperature sensitive targeting vectors. Nonetheless, the use of microwave-assisted heating decreased the reaction time from greater than 2 h using conventional heating to less than 15 min to produce \(^{99m}\text{Tc}\)-carborane derivatives.
2.4. Biological evaluation of metallocarborane complexes

2.4.1. *In vitro* binding affinities of rhenium carborane complexes 12 and 13 to neurotransmitter receptors and transporters

Following the development of a methodology to prepare arylpiperazine-metallocarboranes (M = Re (12 and 13), $^{99m}$Tc (14a and 15)), assessments of the binding affinities to neurotransmitter receptors and transporters, lipophilicities and brain uptake were determined. The rhenium complexes 12 and 13 were sent to the National Institute of Mental Health’s Psychoactive Drug Screening Program (University of North Carolina at Chapel Hill) to assess the affinity and selectivity of the metallocarboranes 12 and 13 for several CNS targets in an *in vitro* binding assay. The screens were run in duplicate and in parallel with the 5-HT$_{1A}$ receptor antagonist WAY100635 as a positive blind control. The control compound showed affinities that are consistent with literature values for the 5-HT$_{1A}$ binding.38, 39 The complexes were examined for binding against a series of receptors and transporters: serotonin (5-HT$_{1A}$, 5-HT$_{1B}$, 5-HT$_{1D}$, 5-HT$_{1E}$, 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$, 5-HT$_{3}$, 5-HT$_{5A}$, 5-HT$_{6}$, 5-HT$_{7}$), adrenergic ($\alpha_{1A}$, $\alpha_{1B}$, $\alpha_{1D}$, $\alpha_{2A}$, $\alpha_{2B}$, $\alpha_{2C}$, $\beta_{1}$, $\beta_{2}$, and $\beta_{3}$), and sigma ($\sigma_{1}$ and $\sigma_{2}$) receptors and monoamine transporters (DAT, NET and SERT). The summary of the binding affinities can be found in Table 2.4.

Complexes 12 and 13 showed either less than 50 % specific binding in the primary assay or greater than 1000 nM binding for: serotoninergic receptors (5-HT$_{1B}$, 5-
HT{sub 1D}, 5-HT{sub 1E}, 5-HT{sub 2A}, 5-HT{sub 2C}, 5-HT{sub 3}, 5-HT{sub 5A}, 5-HT{sub 6}, 5-HT{sub 7}), adrenergic receptors (α{sub 2A}, α{sub 2B}, β{sub 1}, and β{sub 2}), sigma (σ{sub 1} and σ{sub 2}) receptors, DAT, NET and SERT transporters.

Complex 12 showed weak affinity for the 5-HT{sub 1A} receptor (K_{i} = 834 nM) and modest binding to the α{sub 1D} adrenergic receptor (K_{i} = 270 nM), while complex 13 showed higher affinity for both 5-HT{sub 1A} (118 nM) and 5-HT{sub 2B} (211 nM) serotonergic receptors than complex 12. Modest to poor binding of 12 and 13 were observed for the α-adrenergic receptors ranging from 115 to 575 nM while both complexes (12 and 13) showed binding greater than 900 nM for the β-adrenergic receptors. Although neither complex had high or selective binding to any one class of the neurotransmitter receptors screened, one common trend was observed. The extension of the linker length between the targeting WAY portion and the carborane unit, (12 = one CH{sub 2} unit and 13 = three CH{sub 2} units), improved binding affinities to the serotonin 5-HT{sub 1A} and 5-HT{sub 2B} receptors as well as the α{sub 1A}, α{sub 1B}, α{sub 1D}, α{sub 2C} and β{sub 3} adrenergic receptors. These results assisted in designing subsequent WAY-carborane ligands having increased affinity and selectivity to these classes of neurotransmitter receptors. The preparation of these second generation compounds is described in chapter 3.
**Table 2.4.** Binding assay results of rhenacarborane complexes 12 and 13 to serotonin, adrenergic and sigma receptors as well as dopamine, serotonin and norepinephrine transporters.

<table>
<thead>
<tr>
<th>Compound</th>
<th>5-HT₁₆</th>
<th>5-HT₁₇</th>
<th>5-HT₁₈</th>
<th>5-HT₂₆</th>
<th>5-HT₂₇</th>
<th>5-HT₂₈</th>
<th>5-HT₃</th>
<th>5-HT₃₆</th>
<th>5-HT₆</th>
<th>5-HT₇</th>
<th>σ₁</th>
<th>σ₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>834 ± 54</td>
<td>*</td>
<td>2113</td>
<td>4807</td>
<td>*</td>
<td>1165</td>
<td>*</td>
<td>*</td>
<td>1714</td>
<td>5085</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>13</td>
<td>118 ± 8</td>
<td>*</td>
<td>3937</td>
<td>*</td>
<td>*</td>
<td>211 ± 16</td>
<td>*</td>
<td>*</td>
<td>6993</td>
<td>1080</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>WAY100635</td>
<td>0.5 ± 0.1</td>
<td>*</td>
<td>661 ± 82</td>
<td>*</td>
<td>2117</td>
<td>302 ± 11</td>
<td>*</td>
<td>*</td>
<td>2345</td>
<td>*</td>
<td>248 ± 23</td>
<td>*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Compound</th>
<th>α₁₆</th>
<th>α₁₇</th>
<th>α₁₈</th>
<th>α₂₆</th>
<th>α₂₇</th>
<th>α₂₈</th>
<th>β₁</th>
<th>β₂</th>
<th>β₃</th>
<th>DAT</th>
<th>NET</th>
<th>SERT</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>1166</td>
<td>*</td>
<td>270 ± 15</td>
<td>*</td>
<td>575 ± 25</td>
<td>*</td>
<td></td>
<td>*</td>
<td>1185</td>
<td>*</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>13</td>
<td>115 ± 6</td>
<td>122 ± 7</td>
<td>174 ± 8</td>
<td>*</td>
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<td></td>
<td>*</td>
<td>960 ± 85</td>
<td>*</td>
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</tr>
<tr>
<td>WAY100635</td>
<td>62 ± 4</td>
<td>162 ± 9</td>
<td>36 ± 1</td>
<td>*</td>
<td>562 ± 28</td>
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<td></td>
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</tr>
</tbody>
</table>

*a* errors not recorded for values >1000 nM

* indicates <50 % binding in primary assay
2.4.2. Lipophilicity measurements of $^{99mTc}$-complexes 14a, 14b and 15

Although rhenium complexes 12 and 13 did not show high or selective binding to a particular neurotransmitter receptor, this does not preclude the ability of these compact and lipophilic carborane complexes to cross the blood-brain barrier. Lipophilicity (log P) measurements are a fundamental physico-chemical parameter and one basis of structure-activity relationships in medicinal chemistry. Lipophilicity is used to predict the ability to deliver the radiotracer to the desired organ (e.g. cross the BBB) and to anticipate the rate of clearance from non-target tissues. In order to gauge the ability of the $^{99mTc}$-carborane complexes 14 and 15 to cross the BBB, the lipophilicity values were measured.

Technetium-99m complexes that are both neutral and lipophilic have been found to cross the BBB by passive diffusion. In order for an agent to diffuse through the BBB, a technetium complex should partition between $n$-octanol and water to yield log P value between 0.5 and 2.5, where $P = [\text{compound}]$ in $n$-octanol$/[\text{compound}]$ in water. It should be noted that in the literature, log $P_{7.4}$ or log D is often referred to as simply log P. In general, the distinct term listed is used to describe the method employed for the partition analysis. Log D refers to the distribution coefficient of a compound between $n$-octanol and a buffer at pH 7.4 to represent physiological pH. The main difference between log P and log D is that log P refers to the partitioning of one species, most commonly the neutral/uncharged molecule, whereas log D includes the partitioning obtained by measuring all species present in solution (protonated and deprotonated) and therefore accounts for solubility affects associated with hydrogen bonding and ionization.
Radiotracers show optimal passive brain entry \textit{in vivo} with moderate lipophilicity and log D values in the approximate range of 2.0-3.5.\textsuperscript{43}

The most widely accepted means of determining lipophilicity, for radioactive materials and the method used in this work, is the shake-flask method.\textsuperscript{42} The $^{99m}$Tc-carborane complex ($14a$, $14b$ or $15$), in a 5-10 \% ethanol/saline solution, was added to an equal ratio of $n$-octanol and phosphate buffer solution (pH 7.4). Following equilibration, the partitioning of each of the $^{99m}$Tc-carborane complexes was measured in each layer using a gamma counting instrument (Figure 2.15). Complexes $14a$ and $14b$ contain the same biovector and linker but different carbon-carbon cage orientation which is expected to affect the lipophilicity value. The $^{99m}$Tc-carborane isomer $14a$, which has a log P value of 2.4, has the carbon atoms apart leading to a less polar complex, thus causing a mild increase in lipophilicity compared to $14b$ (log P = 2.26). Although the cage orientations of $14a$ and $14b$ are different, the log P values were found to be almost the same.

![Figure 2.15. Log P values for $^{99m}$Tc-carborane complexes $14a$, $14b$ and $15$.](image)

The longer linker found in complex $15$ caused a slight increase in the lipophilicity (log P = 2.6) due to the presence of more lipophilic groups (i.e. methylene units). It has been shown that log P increases approximately 0.5 units with every additional methylene
group (n = 1 to n = 7). In contrast, other experiments have shown that the log P values for technetium compounds that differ by one methylene group change by +0.1 or decrease by -0.08. It appears that the impact of the alkyl chain length on lipophilicity depends on each class of compound being evaluated. Nonetheless, the lipophilicity values for $^{99m}$Tc-WAY complexes $14a$, $14b$ and $15$ falls within the ideal range (log D = 2.0-3.5) in which compounds can passively diffuse through the BBB.

2.4.3. SPECT imaging study of the $^{99m}$Tc-carborane complexes $14a$ and $15$

While the in vitro binding data of rhenium complexes $12$ and $13$ did not indicate specific binding to a class of neurotransmitter receptors, the lipophilicity values of the $^{99m}$Tc-carborane complexes ($14a$, $14b$ and $15$) indicate that these complexes may potentially cross the BBB. In vivo SPECT imaging studies on the $^{99m}$Tc-WAY complexes $14a$ and $15$ were performed on healthy rats to determine whether these complexes have any brain uptake. Following the injection of approximately 111 MBq, a planar dynamic scan was obtained for 1 h followed by a CT scan with the camera positioned over the subject’s head. The scan monitored the location of the radiotracer through the subject’s body by acquiring a series of planar/stationary images over time. Each image is a result of summed data over a short interval where the sequence of the projections over a longer period would generate an animation of the tracer’s distribution. The images indicated no noticeable brain uptake for complex $14a$ or $15$. Complex $14a$ showed distribution of the activity primarily in the abdomen (liver, kidneys, etc.) which was only partially in the field of view. Unexpectedly, some lung uptake was also
observed (Figure 2.16). To confirm that the uptake is specific and significant, a blocking study will need to be performed where the rhenium complex 12 would be administered before the $^{99m}$Tc analogue. If the $^{99m}$Tc-complex is targeting a specific receptor, the receptor would be saturated or partially saturated by the pre-treatment with rhenium complex showing a decrease in the specific binding to that site. The $^{99m}$Tc uptake to that region would therefore be considered significant and not attributed to non-specific uptake of a lipophilic compound. Complex 15 showed primary uptake in the abdomen area and no observed uptake in lung as seen in complex 14a. The low amount of radioactivity used in the first study was the suspected reason for the absence of detectable brain uptake. A second study with 14a was performed to test this hypothesis where an increased amount of activity (ten times the previous scan) was injected into the subject; this resulted in an increase in facial uptake, but no observable brain uptake.

![Image](image.png)

**Figure 2.16.** Summed-dynamic scintigraphic images (over 1 h) of $^{99m}$Tc-carborane 14a administrated to a healthy Copenhagen rat.

The lack of brain uptake may be due to two main factors. First, the use of
anaesthesia has shown to affect the uptake of compounds into the brain.\textsuperscript{47} The \textit{in vivo} imaging study requires restricted body motion during the scan, thus the subjects are under sedation. Anaesthesia has been shown to reduce neural activity and metabolism, physiologic neural functions which can affect the distribution of the agent being analyzed.\textsuperscript{47-49} Mizugaki \textit{et al.} examined brain uptake of a carbon-11 agent using two different forms of anaesthesia (intravenous and inhaled) and found dissimilar uptake of the agent.\textsuperscript{50} They concluded that the effect of anaesthesia is a problem in pharmacokinetic studies with experimental animals. Another group found a reduction in neuronal function during anaesthesia which caused decreased regional brain perfusion of [\textsuperscript{99m}Tc]HMPAO.\textsuperscript{51} Even for functional MRI studies, caution is warranted when interpreting the data in anaesthetized animals because the effects are not fully known.\textsuperscript{52} The second issue may be associated with the charge of the \textsuperscript{99m}Tc-carborane complexes which may present these complexes from crossing the BBB. Metallocarborane are unlike charged organic compounds since they possess diffuse anionic charge which should allow the agents to cross the BBB. To test this hypothesis, a method to produce neutral analogues was developed and is described in chapter 4.

\textbf{2.4.4. Biodistribution of \textsuperscript{99m}Tc-carborane complex 14a}

Reliable quantitation of organ uptake associated with the \textsuperscript{99m}Tc compounds in rodents using \textit{in vivo} SPECT images is difficult especially when the field of view is centered over the head and is limited to top half the subject’s body. In order to quantify the amount of activity taken up by various regions of the body, a biodistribution study
was undertaken. The lack of uptake observed during the SPECT study may have been affected by the use of anaesthesia to sedate the subject during the experiment (vide supra). Due to the unknown effects of anaesthesia on the uptake of these complexes, the biodistribution study was determined using non-anaesthetized healthy Sprague-Dawley rats at four time points (2, 15, 60 and 120 min) in triplicate. The organs evaluated included: heart, lung, liver, kidneys, stomach, small intestine, caecum, large intestine, pancreas, spleen, eyes and brain. The areas of the brain were sectioned into cerebellum, cortex, hypothalamus, hippocampus, and the mid/hind area. Additionally, samples of blood, skeletal muscle, bone, skin and adipose were collected. The main focus of this study was to evaluate the distribution of the $^{99m}$Tc WAY-carborane complex and determine if any brain uptake occurred. Because the pathway of excretion was not considered (the bladder, urine and subject’s bedding were not measured), the total amount of activity given to the subject and the amount of activity accounted for at the end of the study did not match.

The biodistribution data of $14\text{a}$ in healthy Sprague-Dawley rats are shown in Figure 2.17 in terms of percent injected dose per gram of tissue (%ID/g). The complex $14\text{a}$ showed significant liver accumulation over 120 min (from 0.09 to 0.45 %ID/g) indicating this is the path of excretion and that $14\text{a}$ is lipophilic. The measured lipophilicity (section 2.4.2) of this complex supports these observations. Spleen uptake was also high with a maximum at 15 min post injection (p.i.) (0.18 ± 0.03 %ID/g) followed by a gradual decrease through the rest of the study. Lung uptake was approximately constant and ranged from 0.06-0.1 %ID/g through the study. Brain uptake
in the mid/hind, hippocampus, cortex, and cerebellum were low; only a small difference in the distribution was observed where a maximum of 0.06 ± 0.009 %ID/g at 60 min p.i was observed in the hippocampus. However, the hypothalamus region showed a maximum uptake of 0.36 ± 0.08 %ID/g, 2 min p.i. with a washout rate parallel to the accumulation rate of the liver. After 120 min p.i., 0.08 ± 0.05 %ID/g remained in the hypothalamus. Lack of uptake in the hippocampus, a region of high serotonin 5-HT_{1A} receptors, indicates that this tracer is not targeting the 5-HT_{1A} receptor which is consistent with the \textit{in vitro} data. Brain uptake for complex \textbf{14a} in the hypothalamus region is comparable to reported brain uptake seen for 5-HT_{1A} targeted iodo-radioligands (0.22-0.71 %ID/g ≤ 5 min p.i.),\textsuperscript{53, 54} and also comparable to the values of other \textsuperscript{99m}Tc WAY-based radiotracers with bifunctional chelate systems (0.24-1.31 %ID/g ≤ 5 min p.i.).\textsuperscript{55-59} The uptake in the hypothalamus cannot be directly correlated to the modest binding of the \(\alpha\)-adrenergic receptors observed in the \textit{in vitro} assay because \(\alpha\)-adrenergic receptors are not primarily located in this region of the brain.
Figure 2.17. Biodistribution of $^{99m}$Tc-carborane complex 14a in healthy Sprague-Dawley rats (n = 3).
The biodistribution study was repeated at two time points (2 and 15 min) in triplicate. In this second study with non-anaesthetized subjects, the hypothalamus region of the brain still showed the greatest uptake compared to the other brain regions. However, the overall uptake for each of the brain regions was significantly lower than the first study. The relative uptake of all other organs had a similar pattern to the first experiment, however, again the absolute values were generally lower. In order to compare the two sets of measurements, a t-test (assuming equal variances) was preformed using Microsoft Excel’s data analysis. The t-test compared each region in the two studies at the 2 and 15 min time points and is used to determine the statistical similarities or differences with the probability level at 95 % confidence. Uptake in each region at the 2 min time point is statistically similar for the two studies. On the other hand, the cerebellum, hypothalamus and mid/hind brain regions at the 15 min time point has t_stat values of 3.02, 4.46, 4.48 which is greater than the t_table value (2.77) indicating that the uptake in these brain regions are not the statistically the same. Each of the other regions in the subject’s body was examined at the 15 min time point and found to be statistically similar at 95 % confidence. In these studies, the use a standard or control substance was neglected which would have assisted in normalizing biological variances associated with the subject’s in the two experiments. The studies of complex 14a did show brain uptake but region specific uptake was not conclusive.

As noted above, the effects of anaesthesia on brain uptake have been reported, and we were interested in investigating the effects anaesthesia on the distribution of the $^{99m}$Tc WAY-carborane compound 14a. For this study, the subjects were under
anaesthesia (isoflurane) during the injection and at select time points (15 and 60 min) and the subjects were awake otherwise. The t-test (95 % confidence) analysis was used to compare uptake in each region between the non-anaesthetized and anaesthetized experiments. The abdominal organs showed no statistical difference between both studies at the 15 and 60 min time points. However, statistical differences between anaesthetized and non-anaesthetized subjects were observed in the hippocampus, hypothalamus, mid/hind brain, skin and eyes at either 15 min and/or 60 min time point. The use of anaesthesia does not appear to affect the distribution of compound 14a in organs that are not part of the central nervous system. However, regions of the brain and eyes, which are a part of the CNS, shows statistical differences between the two studies suggesting that our study supports the literature on the effects of anaesthesia on the uptake of brain agents.

From the biodistribution studies, three conclusions can be drawn: (i) clearance of the 99mTc-carborane complex occurs via the liver, supporting the lipophilic nature of these complexes, (ii) modest brain uptake is observed in the hypothalamus region of the brain, which regulates hormone levels in the case where no anaesthesia was used and (iii) CNS binding and brain distribution was impacted by the use of anaesthesia. Given the low affinity of the compounds for key targets, a decision was made to focus on identifying high affinity compounds.

2.5. Summary

Alkyl-linked WAY-carborane ligands 5 and 6 were synthesized using an alkyne
insertion reaction catalyzed by an ionic liquid in modest yields (5: 35 % and 6: 13 %). A new synthesis of 5 and 6 was developed, employing halocarboranes 9 and 11, which resulted in comparable or higher yields (5: 25 % and 6: 57 %) and fewer by-products. The corresponding rhenium complexes 12 and 13 were generated in good yields from \textit{in situ} production of the \textit{nido}-5 and \textit{nido}-6 in a microwave reactor. At the same time, the first observed and fully characterized rhenacarborane cage isomerization of complex 12 was found. Exploration of the isomerization led to the conclusion that the relief of steric strain is the driving force for the isomerization; furthermore, the isomerization and \([\text{Re(CO)}_3]^+\) complexation of 12 occurred simultaneously.

Complexation of carborane ligands 5 and 6 with \([^{99}\text{Tc(CO)}_3(\text{H}_2\text{O})_3]^+\) yielded complexes 14a, 14b and 15. The reactivity difference between rhenium and technetium-99m was demonstrated with the isolation of the 3,1,2-\(^{99}\text{Tc}\) isomer, 14b. Lipophilicity was measured for WAY-based \(^{99}\text{Tc}\)-carborane complexes 14a, 14b and 15 (log P = 2.26-2.6) where the lipophilicity values are within the ideal range to cross the BBB. \textit{In vitro} binding affinities of 12 and 13 indicated no high or selective binding to the series of neurotransmitter receptors or monoamine transporters screened. However, it was observed that by increasing the length of the linker (from one CH\(_2\) unit to three) improved the binding of complex 13 for most targets. \textit{In vivo} SPECT images of 14a and 15 showed no brain uptake however, biodistribution studies of 14a on non-anaesthetized subjects indicated modest, non-negligible brain uptake in the hypothalamus region (0.36 ± 0.08 %ID/g, 2 min p.i.). The preparation and evaluation of WAY-metallocarborane complexes (M = Re (12 and 13), \(^{99}\text{Tc} \ (14 \text{ and } 15)\)) has provided clues into the design of
second generation compounds having enhanced affinity and brain uptake.

2.6. Experimental

2.6.1. Reagents and general procedures

Unless otherwise stated, all chemicals and reagents were purchased and used as received from Sigma-Aldrich without further purification. Decaborane and \( o \)-carborane were obtained and used as received from Katchem Ltd. and 1-butyl-3-methylimidazolium chloride ([BMIM][Cl]) was purchased from Strem Chemicals. Compounds prepared according to literature procedures were 1-(2-methoxyphenyl)-4-(prop-2-ynyl)-piperazine (7), \( ^4 [\text{Re(CO)}_3(\text{H}_2\text{O})_3][\text{Br}], \) \( ^{60} \) and \( ^{99m}\text{Tc(CO)}_3(\text{H}_2\text{O})_3]^+ \) (3). \( ^{35} \)

Reactions were performed under an inert atmosphere unless otherwise stated. All solvents were obtained from Caledon, and either dried by a Pure-Solv Solvent Purification System (Innovative Technology Inc.) or dried over calcium hydride (acetonitrile and toluene), sodium/benzophenone (tetrahydrofuran) or sodium iodide (acetone) and distilled prior to use. Deuterated solvents for NMR samples were purchased from Cambridge Isotope Laboratories. Technetium-99m complexes were prepared from pertechnetate \( ^{99m}\text{TcO}_4^- \) which was obtained from a \( ^{99}\text{Mo}/^{99m}\text{Tc} \) generator (Lantheus Medical Imaging) in saline (0.9 % NaCl).

Reactions were monitored using Alugram Sil G/UV254 thin-layer chromatography (TLC) plates. Carborane-containing species were visualized with 0.2 % \( \text{PdCl}_2 \) in hydrochloric acid (3.0 M), which upon heating gave dark brown spots. Silica Gel 60PF254 containing gypsum (EM Science) was used for making preparative TLC
plates and plates for the Chromatotron Model 7924T (Harrison Research). Column chromatography was accomplished with Silica Gel 60 (EMD Chemical Inc.) or Ultra Pure Silica Gel 60 (Silicycle). Automated normal and reverse-phase (C18) silica gel chromatography were performed on a Biotage SP1 purification system operated at ambient temperature using solvent gradients as specified. Solid phase extraction cartridges (C18) obtained from Waters were used following pretreatment with water (10 mL), methanol or ethanol (10 mL), a second wash with water (10 mL) and HCl (10 mM, 10 mL).

2.6.2. Instrumentation

Nuclear magnetic resonance spectra ($^1$H, $^{13}$C{^1}H, $^{11}$B{^1}H) were recorded on either a Bruker 500 or 600 MHz spectrometer at ambient temperature. The chemical resonances ($\delta$) are reported in parts per million (ppm), with the $^1$H NMR shifts referenced to the residual proton signal of the deuterated solvent and the $^{13}$C shifts referenced to the carbon signal of the solvent. The chemical shifts ($\delta$) for $^{11}$B{^1}H NMR spectra are reported relative to an external reference of BF$_3$·Et$_2$O. Infrared spectra were acquired on a Bio-Rad FTS-40, Nicolet 510 or 6700 Fourier Transform IR spectrometer at ambient temperature. Samples were run on a KBr plate or as KBr pellets. A Gallenkamp melting point apparatus was used to determine melting points. Reactions involving microwave heating were performed on a Biotage Initiator 60 or Initiator 8 microwave reactor using crimp-sealed vials. Mass spectra were obtained from the McMaster Regional Centre for Mass Spectrometry on a Micromass Quattro Ultima (LC-ESI/APCI triple quadrupole)
mass spectrometer for electrospray ionization (ES) mass spectrometry, a Micromass GC-EI/CI time of flight mass spectrometer instrument for chemical ionization (CI) and electron ionization (EI) spectra and a Micromass Global Ultima (MALDI/CapLC-ESI quadrupole time of flight) mass spectrometer for high resolution mass spectra. A Capintec dose calibrator was used to determine the activity of samples containing technetium-99m (> 300 kBq).

High performance liquid chromatography was performed on a Varian ProStar model 230 or 230I solvent delivery system fitted with a Varian ProStar model 230 or 335 PDA detector and an in-line radioactivity detector (IN/US γ-RAM). The wavelength for detection was set at 250 or 254 nm and the dwell time in the gamma detector was 0.5 s with a 10 μL loop. A Varian Nucleosil C18 100-5 (250 x 4.6 mm) column was used with a sample volume of either 100 μL or 500 μL. The following solvent gradients were employed: method A (solvent A = acetonitrile (0.1 % formic acid), solvent B = 20 mM ammonium acetate, 1 mL/min) 0-15 min 65-100 % A, 15-20 min 100 % A; method B (solvent A = aqueous tetraethylammonium phosphate pH 2.0-2.5, solvent B = methanol, 1 mL/min) 0-3 min 100 % A, 3-6 min 100-75 % A, 6-9 min 75-67% A, 9-20 min 67-0 % A, 20-22 min 0 % A, 22-25 min 0-100 % A, 25-30 min 100 % A; method C (solvent A = acetonitrile, solvent B = water, 1 mL/min) 70:30 A:B isocratic for 15 min; method D (solvent A = acetonitrile (0.1 % formic acid), solvent B = water (0.1 % formic acid), 1 mL/min) 70:30 A:B isocratic for 15 min; method E (solvent A = acetonitrile (0.1 % formic acid), solvent B = 20 mM ammonium acetate, 1 mL/min) 70:30 A:B isocratic for 15 min; method F (solvent A = acetonitrile, solvent B = 0.1 M ammonium formate, 1
mL/min) 70:30 A:B isocratic for 15 min; method G (solvent A = methanol solvent B = water, 1 mL/min) 70:30 A:B isocratic for 15 min.

2.6.3. Synthetic procedures

1-[C_{12}H_{17}N_{2}O]-closo-C_{2}B_{10}H_{11} (5). Method A: A solution of compound 7 (0.33 g, 1.4 mmol) in toluene (1.5 mL) was added to a suspension of 1-butyl-3-methylimidazolium chloride (0.050 g, 0.29 mmol) and decaborane (0.071 g, 0.58 mmol) in toluene (1 mL). The heterogeneous mixture was heated at reflux (110 °C) for 2 h. The mixture was concentrated to a viscous yellow liquid using a rotary evaporator. The product was purified by preparative TLC and an eluent of 100 % dichloromethane. The product was obtained following extraction of the silica with 10 % methanol/dichloromethane and evaporation under reduced pressure to yield a white solid (0.07 g, 35 %).

Method B: Diisopropylethylamine (1.0 mL, 5.7 mmol) was added to a stirred solution of compound 9 (1.0 g, 4.2 mmol) and 1-(2-methoxyphenyl)piperazine (1.0 g, 5.2 mmol) in tetrahydrofuran (30 mL). The reaction mixture was heated at reflux (66 °C) for 24 h. Upon cooling, the resultant suspension was filtered and the solvent was removed under reduced pressure. The product was purified by flash silica gel chromatography employing a gradient of 75 to 100 % dichloromethane/hexanes giving an off-white solid (0.37 g, 25 %).

TLC R_f (100 % dichloromethane) = 0.79; m.p. = 133-135 °C; ^1H NMR (500 MHz, CDCl_3) δ: 7.02 (m, 1 H, Ar-H), 6.92 (m, 2 H, Ar-H), 6.86 (m, 1 H, Ar-H), 4.02 (s,
1 H, CH), 3.85 (s, 3 H, OCH₃), 3.12 (s, 6 H, NCH₂, CH₂), 2.80 (s, 4 H, NCH₂); ¹³C{¹H} NMR (125 MHz, CDCl₃) δ: 152.2, 140.8, 123.2, 120.9, 118.2, 111.3, 74.9, 62.1, 58.2, 55.4, 54.9, 50.7; ¹¹B{¹H} NMR (160 MHz, CDCl₃) δ: -4.1, -6.5, -10.1, -12.5, -13.6, -14.3; IR (KBr, cm⁻¹): ν 2576, 1500; HRMS (CI⁺) m/z for C₁₄H₂₈N₂OB₁₀: calculated: 350.3132, observed: 350.3109 [M⁺].

1-[C₁₄H₂₁N₂O]-closo-C₂B₁₀H₁₁ (6). Method A: A solution of decaborane (0.082 g, 0.67 mmol) in toluene (5 mL) was added to a suspension of 1-butyl-3-methylimidazolium chloride (0.041 g, 0.24 mmol) in toluene (5 mL). A solution of compound 8 (0.23 g, 0.89 mmol) in toluene (5 mL) was added to the reaction mixture. The heterogeneous mixture was heated at reflux (110 °C) for 7 days, then concentrated to a viscous yellow liquid using a rotary evaporator. The product was purified using by automated silica gel chromatography (12+M) employing a gradient of 0-2 % methanol/dichloromethane giving a beige solid (0.033 g, 13 %).

Method B: Diisopropylethylamine (0.10 mL, 0.57 mmol) and 1-(2-methoxyphenyl)piperazine (1.5 g, 7.8 mmol) were combined in tetrahydrofuran (3 mL) and added to a stirred solution of compound 11 (0.11 g, 0.35 mmol) in tetrahydrofuran (5 mL). The reaction mixture was stirred for 24 h at 22 °C. The solvent was removed from the resulting suspension under reduced pressure to yield a white solid. Dichloromethane (30 mL) was added to dissolve the residue. The solution was transferred into a separatory funnel and washed with water (3 x 20 mL); the aqueous fractions were combined and the product was extracted with dichloromethane (2 x 25 mL). The combined organic fractions were dried over anhydrous sodium sulfate, filtered and concentrated under

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reduced pressure. The product was purified by automated silica gel chromatography employing a gradient of 0 to 2% methanol/dichloromethane giving a beige solid (0.076 g, 57%).

TLC Rf (1% methanol/dichloromethane) = 0.12; m.p. = 138 °C; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 7.00 (m, 1 H, Ar-H), 6.93 (m, 2 H, Ar-H), 6.86 (m, 1 H, Ar-H), 3.85 (s, 3 H, OCH$_3$), 3.60 (s, 1 H, CH), 3.08 (s, 4 H, NCH$_2$), 2.61 (s, 4 H, NCH$_2$), 2.37 (m, 2 H, CH$_2$), 2.28 (m, 2H, CH$_2$), 1.70 (m, 2 H, CH$_2$); $^{13}$C{$^1$H} NMR (125 MHz, CDCl$_3$) $\delta$: 152.4, 141.4, 123.1, 121.2, 118.3, 111.5, 75.4, 61.5, 57.2, 55.5, 53.5, 50.7, 36.1, 26.7; $^{11}$B{$^1$H} NMR (160 MHz, CDCl$_3$) $\delta$: -2.7, -6.2, -9.7, -11.9, -12.5, -13.5; IR (KBr, cm$^{-1}$): ν 2589, 1501; HRMS (Cl+) m/z for C$_{16}$H$_{32}$B$_{10}$N$_2$O: calculated: 378.3445, observed 378.3452 [M+].

C$_{16}$H$_{22}$N$_2$O (8). The synthesis was adapted from a procedure by Khatuya et al.$^4$ Sodium iodide (1.2 g, 8.0 mmol) was added to a solution of 5-chloro-1-pentyne (0.83 mL, 7.8 mmol) in acetonitrile (20 mL) and the resulting mixture was stirred at 25 °C for 1 h. A solution of 1-(2-methoxyphenyl)piperazine (1.0 g, 5.2 mmol) in acetonitrile (15 mL) was added dropwise to the reaction mixture followed by a single addition of potassium carbonate (9.4 g, 68 mmol). The reaction was heated at reflux (82 °C) for 48 h. The reaction was cooled to room temperature and distilled water (50 mL) was added. The product was extracted with ethyl acetate (3 x 30 mL) in a separatory funnel; the combined organic fractions were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The product was purified by either by flash or automated (40+M) silica column chromatography using a gradient of 6-50% ethyl
acetate/hexanes. The product was isolated as a pale yellow solid (1.0 g, 74 %). TLC $R_f$ (50 % ethyl acetate/hexanes) = 0.38; m.p. = 62 °C; $^1$H NMR (600 MHz, CDCl$_3$) $\delta$: 6.98 (m, 1 H, Ar-H), 6.92 (m, 2 H, Ar-H), 6.85 (m, 1 H, Ar-H), 3.85 (s, 3 H, OCH$_3$), 3.09 (s, 4 H, NCH$_2$), 2.64 (s, 4 H, NCH$_2$), 2.50 (t, 2 H, CH$_2$, $J = 4.0$ Hz), 2.25 (td, 2 H, CH$_2$, $J = 7.0$ Hz, $J = 2.3$ Hz), 1.95 (t, 1 H, CH, $J = 2.6$ Hz), 1.75 (m, 2 H, CH$_2$); $^{13}$C {$^1$H} NMR (125 MHz, CDCl$_3$) $\delta$: 152.3, 141.5, 122.9, 121.1, 118.3, 111.3, 84.3, 68.5, 57.5, 55.4, 53.5, 50.7, 25.9, 16.5; IR (KBr, cm$^{-1}$): $\nu$ 3295, 2149, 1501; HRMS (CI+) m/z for C$_{16}$H$_{22}$N$_2$O: calculated: 259.1810, observed: 259.1812 [M+H]$^+$. 

1-[$\text{CH}_2\text{Br}$]-closo-$\text{C}_2\text{B}_{10}\text{H}_{11}$ (9). Decaborane (2.7 g, 22.1 mmol) was dissolved in acetonitrile (30 mL) and heated for 1 h at reflux (82 °C). To the resultant light yellow solution, propargyl bromide (80 % wt in toluene, 3.7 mL, 33.2 mmol) was added dropwise with stirring. The reaction mixture was heated at reflux for 5 days. The solvent was removed under reduced pressure leaving an orange oil. The residue was dissolved in diethyl ether (30 mL), transferred into a separatory funnel and washed with aqueous sodium hydroxide (0.1 M, 3 x 25 mL). The organic fraction was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure to give a viscous oil. Silica gel (5 g) was added to the yellow residue and stirred with hexanes (3 x 100 mL) for 1 h. The suspension was filtered and the filtrate was concentrated under reduced pressure to yield compound 9 as a white solid (3.1 g, 59 %). TLC $R_f$ (100 % hexanes) = 0.44; m.p. = 32 °C; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 4.00 (s, 1 H, CH), 3.96 (s, 2 H, CH$_2$); $^{13}$C {$^1$H} NMR (125 MHz, CDCl$_3$) $\delta$: 71.4, 61.1, 32.3; $^{11}$B {$^1$H} NMR (160 MHz, CDCl$_3$) $\delta$: -2.9, -5.5, -9.2, -11.0, -12.9, -13.4; IR (KBr, cm$^{-1}$): $\nu$ 3067, 2594; HRMS (EI) m/z for
C$_3$H$_{13}$B$_{10}$Br: calculated: 238.1131, observed 238.1125 [M+].

1-[C$_3$H$_6$Cl]-closo-C$_2$B$_{10}$H$_{11}$ (10). Decaborane (2.1 g, 17.2 mmol) was dissolved in acetonitrile (25 mL) and heated for 1 h at reflux (82 °C). To the resultant light yellow solution, chloropentyne (2.5 mL, 23.6 mmol) was added with stirring. The reaction mixture was heated at reflux for 5 d. The solvent was removed using a rotary evaporator leaving an orange oil. The residue was dissolved in diethyl ether (50 mL), transferred into a separatory funnel and washed with water (3 x 30 mL). The organic fraction was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. Silica gel (5 g) was added to the orange oil and stirred with hexanes (3 x 100 mL) for 1 h. The suspension was filtered and the filtrate was concentrated under reduced pressure to yield 10 as a white solid (0.25 g, 7 %). TLC R$_f$ (50 % diethyl ether/hexanes) = 0.62; m.p. = 50 °C; $^1$H NMR (500 MHz, CDCl$_3$) δ: 3.59 (s, 1 H, CH), 3.51 (t, 2 H, CH$_2$, $J$ = 6.1 Hz), 2.39 (m, 2 H CH$_2$), 1.97 (m, 2 H, CH$_2$); $^{13}$C{$_1$H} NMR (125 MHz, CDCl$_3$) δ: 74.3, 61.8, 43.4, 35.7, 32.0; $^{11}$B{$_1$H} NMR (160 MHz, CDCl$_3$) δ: -1.8, -5.2, -8.8, -11.3, -11.7, -12.6; IR (KBr, cm$^{-1}$): ν 3058, 2574; HRMS (EI+) m/z for C$_5$H$_{17}$B$_{10}$Cl: calculated: 222.1949, observed 222.1950 [M+].

1-[C$_3$H$_6$I]-closo-C$_2$B$_{10}$H$_{11}$ (11). Compound 10 (0.25 g, 1.1 mmol) was dissolved in acetone (10 mL) and combined with a solution of sodium iodide (0.27 g, 1.8 mmol) in acetone (10 mL); the reaction mixture was heated at reflux (56 °C) for 3 d. After cooling to room temperature, the heterogeneous mixture was filtered and the precipitate was washed with diethyl ether (25 mL). The filtrate was concentrated and the residue was re-dissolved in diethyl ether (50 mL), transferred into a separatory funnel and washed with
10 % sodium thiosulfate (3 x 25 mL). The organic fraction was dried over anhydrous sodium sulfate, filtered and the solvent removed under reduced pressure to yield \( \text{11} \) as a pale yellow solid (0.34 g, 96 %). TLC \( R_f \) (100 % petroleum ether) = 0.22; m.p. = 53 °C; \(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \): 3.58 (s, 1 H, CH), 3.13 (t, 2 H, CH\(_2\), \( J = 6.5 \) Hz), 2.34 (m, 2 H CH\(_2\)), 1.98 (m, 2 H, CH\(_2\)); \(^{13}\)C\(^{1}\)H NMR (125 MHz, CDCl\(_3\)) \( \delta \): 61.7, 39.1, 32.5, 31.0, 3.6; \(^{11}\)B\(^{1}\)H NMR (160 MHz, CDCl\(_3\)) \( \delta \): -2.7, -6.1, -9.7, -12.1, -12.5, -13.5; IR (KBr, cm\(^{-1}\)): \( \nu \) 3058, 2600, 1174, 721; HRMS (EI) m/z for C\(_5\)H\(_{17}\)B\(_{10}\)I: calculated: 314.1306, observed 314.1314 [M+].

**General preparation of rhenacarborane complexes 12 and 13**

The desired \textit{nido}-carborane was generated by heating the corresponding \textit{closo}-carborane derivatives (5 or 6) with sodium fluoride (7 eq.) in aqueous ethanol (10-15 %) in a microwave reactor (195 °C, 10-15 min). Complexation was accomplished by heating the [Re(CO)\(_3\)(H\(_2\)O)\(_3\)][Br] with the \textit{nido}-carborane in a microwave reactor at 180-195 °C for 10 min. Two subsequent additions of [Re(CO)\(_3\)(H\(_2\)O)\(_3\)][Br] (1.0 and 0.5 eq.) were performed where each addition was accompanied by heating the mixture at 180-195 °C for 10 min.

\[ \{[\text{Na}[2,2,2-(\text{CO})_3-8-(\text{C}_{12}\text{H}_{17}\text{N}_2\text{O})-2,1,8-\text{closo}-\text{ReC}_2\text{B}_9\text{H}_{10}]} \} \] (12). The procedure employed 0.093 g (0.26 mmol) of compound 5. Following the final heating, the mixture was cooled to room temperature and diluted with water (10 mL). The product was then extracted with dichloromethane (3 x 10 mL) in a separatory funnel. The organic fractions were combined, dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The product was isolated as an ivory solid (0.13 g, 82 %) by either flash or automated
(12+M) silica gel chromatography and a gradient of 12-100 % ethyl acetate/hexanes or using a reverse phase automated purification system and a gradient of 20-100 % acetonitrile/water. TLC \( R_f \) (50 % ethyl acetate/hexanes) = 0.24; m.p. = 110 °C; \(^1\)H NMR (600 MHz, CD\(_2\)Cl\(_2\)) \( \delta \): 7.12 (m, 1 H, Ar-H), 6.93 (m, 3 H, Ar-H), 3.84 (s, 3 H, OCH\(_3\)), 3.79 (AB, 1 H, CH\(_2\), \( J = -13.7 \) Hz), 3.61 (m, 4 H, NCH\(_2\)), 3.53 (AB, 1 H, CH\(_2\), \( J = -13.7 \) Hz), 3.37 (m, 2 H, NCH\(_2\)), 3.12 (m, 2 H, NCH\(_2\)), 1.88 (s, 1 H, CH); \(^{13}\)C\(\{^1\)H\} NMR (150 MHz, CD\(_2\)Cl\(_2\)) \( \delta \): 197.8, 153.1, 138.1, 125.7, 121.5, 119.8, 112.1, 66.8, 57.0, 56.9, 55.8, 48.4, 48.4, 44.1, 28.8; \(^{11}\)B\(\{^1\)H\} NMR (160 MHz, CD\(_2\)Cl\(_2\)) \( \delta \): -5.8, -8.0, -11.4, -18.5, -20.3; IR (KBr, cm\(^{-1}\)): \( \nu \) 2539, 2005, 1907, 1502; HRMS (ES-) m/z for C\(_{17}\)H\(_{27}\)N\(_2\)O\(_4\)B\(_9\)Re: calculated: 609.2353, observed 609.2366 [M+]. UV-HPLC (method A): \( t_R \) = 15.0 min; method B: \( t_R \) = 22.6 min.

\{[Na][3,3,3-(CO)\(_3\)-1-(C\(_{14}\)H\(_{21}\)N\(_2\)O)-3,1,2-closo-ReC\(_2\)B\(_9\)H\(_{10}\)]\} (13). The procedure employed 0.032 g (0.086 mmol) of compound 6. Following the final heating, the mixture was cooled to room temperature, diluted with water (10 mL) and extracted with dichloromethane (3 x 15 mL). The organic fractions were combined, dried over anhydrous sodium sulfate, filtered and evaporated to dryness. The product was isolated as an ivory solid (0.052 g, 95 %) by either flash or automated (12+M) silica gel chromatography and a gradient of 12-100 % ethyl acetate/hexanes or using a reverse phase automated purification system and a gradient of 20-100 % acetonitrile/water. TLC \( R_f \) (5 % methanol/dichloromethane) = 0.52; m.p. = 193 °C; \(^1\)H NMR (600 MHz, (CD\(_3\))\(_2\)CO) \( \delta \): 7.04 (m, 1 H, Ar-H), 6.99 (m, 2 H, Ar-H), 6.91 (m, 1 H, Ar-H), 3.86 (s, 3 H, OCH\(_3\)), 3.38 (m, 4 H, NCH\(_2\)), 2.81 (s, 4 H, NCH\(_2\)), 1.91 (m, 6 H, CH\(_2\)), 1.75 (s, 1 H, 101
CH); $^{13}$C{H} NMR (125 MHz, (CD$_3$)$_2$CO) $\delta$: 200.4, 153.5, 140.4, 124.8, 121.9, 119.5, 113.0, 79.2, 58.0, 55.9, 54.2, 53.2, 48.5, 37.7, 25.4; $^{11}$B{H} NMR (160 MHz, (CD$_3$)$_2$CO) $\delta$: -5.2, -7.8, -9.8, -11.2, -12.1, -18.0, -19.7; IR (KBr, cm$^{-1}$): $\nu$ 2542, 1995, 1897, 1500; HRMS (ES-) m/z for C$_{19}$H$_{31}$N$_2$O$_4$B$_9$Re: calculated: 637.2697, observed 637.2679 [M$^+$.]

UV-HPLC (method A): $t_R = 14.8$ min; (method B): $t_R = 23.4$ min.

**General preparation of $^{99m}$Tc-carborane complexes**

The *nido*-carboranes were generated by heating the *closo*-carborane derivatives 5 or 6 with sodium fluoride (7 eq.) in aqueous ethanol (10-15 %) in a microwave reactor at 195 °C for 10 min. To a solution of the ligand (5: 6 mM, 6: 5 mM), [$^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$ (370-1850 MBq, 1 mL) was added to the reaction vessel. The mixture was heated in the microwave reactor (14a and 15: 195 °C for 10 min or 14b: 120 °C for 10 min). The crude reaction mixture was passed through a C18 solid phase extraction cartridge where residual [$^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$ was eluted with HCl (10 mM) and the product was eluted with acetonitrile. The excess ligand was removed by HPLC (method A).

{[Na][2,2,2-(CO)$_3$-8-(C$_{12}$H$_{17}$N$_2$O)-2,1,8-closo-$^{99m}$TcC$_2$B$_9$H$_{10}$]} (14a). Decay corrected radiochemical yield: 36 %. Radio-HPLC (method A): $t_R = 15.7$ min; (method B): $t_R = 22.9$ min.

{[Na][3,3,3-(CO)$_3$-1-(C$_{12}$H$_{17}$N$_2$O)-3,1,2-closo-$^{99m}$TcC$_2$B$_9$H$_{10}$]} (14b). Decay corrected radiochemical yield: 21 %. Radio-HPLC (method A): $t_R = 14.9$ min.

{[Na][3,3,3-(CO)$_3$-1-(C$_{14}$H$_{21}$N$_2$O)-3,1,2-closo-$^{99m}$TcC$_2$B$_9$H$_{10}$]} (15). Decay corrected radiochemical yield: 19 %. Radio-HPLC (method A): $t_R = 15.2$ min.; (method B): $t_R = 23.1$ min.
2.6.4. Analyses

**Stability study.** The $^{99m}$Tc complexes $14a$ and $15$ were dissolved in ethanol (95 %, 0.5 mL) and transferred to a vial containing saline (0.9 % NaCl, 4.5 mL). Analytical HPLC of the samples were performed every 30 min using a Varian Nucleosil C18 column (methods C-G). No decomposition was observed by radio-HPLC over 6 h under several different analytical HPLC conditions (mobile phase and pH).

**Specific activity measurements.** Calibration curves were prepared for the external standard (anisole), $nido$-$5$, $nido$-$6$, $12$ and $13$ using the Agilent 1100 system (Quad Pump module G1311A, Degasser G1322A, ALS G1313A, Colcom G1316A) and an in-line radioactivity detector (BioScan) with the Varian Nucleosil C18 100-5 (250 x 4.6 mm) column. The wavelength for detection was set at 254 nm. The method used: solvents A = acetonitrile (0.1 % formic acid), solvent B = 20 mM ammonium acetate; gradient: 0-15 min, 65-100 % A, 15-20 min 100 %. For the external standard (anisole) a flow rate at 0.5 mL/min was used; for all other compounds, a flow rate of 1 mL/min was used. The concentration of the external standard ranged from 16.4–41 000 ng over nine calibration points done in triplicate. The calibration curves for the $nido$-$5$ (5.8–1160 ng) and rhenium complex ($12$) (5.3–1050 ng) were obtained over seven calibration points using an external standard (800 ng) done in triplicate. The calibration curves for the $nido$-$6$ (5.7–1140 ng) and rhenium complex ($13$) (5.5–1100 ng) were obtained over seven calibration points using an external standard (800 ng) done in triplicate. Limits of quantification (LOQ) and limits of detection (LOD) were determined based upon linear regression analysis of the data points which were measured in triplicate. LOQ: $nido$-$5$ =
19.5 ng, nido-6 = 94.4 ng, complex 12 = 9.5 ng, complex 13 = 129.6 ng; LOD: nido-5 = 6.9 ng, nido-6 = 24.8 ng, complex 12 = 6.1 ng, complex 13 = 32.1 ng. The specific activities and effective specific activities of 14a and 15 were obtained using samples containing 41.9 and 110.3 MBq, respectively.

**Lipophilicity.** The lipophilicities of compounds 14a and 15 were determined by the partition between n-octanol and phosphate buffer (0.02 M, pH 7.4) following a previously published method.40 Approximately 222 kBq (6 μCi, 200 μL) of the technetium complex (5-10 % ethanol/saline) was added to an equal ratio of n-octanol (10 mL) and phosphate buffer (10 mL) in a separatory funnel. The mixture was shaken for 3 min at ambient temperature; after resting the mixture for 5 min, the octanol (2 mL) phase was distributed into crimp-sealed vials. Additional phosphate buffer (2 mL) was added to each. The samples were vortexed for 10 min followed by centrifugation for 10 min. An aliquot (approximately 0.1 mL) was removed from each phase and placed into pre-weighed tubes. The remaining phosphate buffer was removed and replaced with a new aliquot of buffer (~2 mL). The vials were vortexed and centrifuged where a total of three repeats were obtained. The activity of each aliquot was measured using a gamma counter (Perkin Elmer 1470 Wizard) and the partition coefficient was determined by calculating the ratio of activities (n-octanol/buffer) and its logarithm was determined to express lipophilicity.

2.6.5. *In vitro* binding assay

Kᵢ determination was conducted by the National Institute of Mental Health’s
Psychoactive Drug Screening Program, Contract No. NO1MH32004. For experimental details please refer to the PDSP website http://pdsp.med.unc.edu/. Complexes 12 and 13 were assayed against the serotonin, the $\alpha$- and $\beta$-adrenergic, DAT, NET, SERT and the sigma receptors. WAY100635 was prepared by Vasdev and co-workers (University of Toronto) following literature procedures.\textsuperscript{61, 62}

2.6.6. \textit{In vivo} SPECT imaging

\textit{In vivo} SPECT imaging was performed at the McMaster Centre for Pre-clinical and Translation Imaging Facilities (Hamilton Health Sciences and McMaster University). Animal acquisitions were performed on a Gamma Medica Ideas X-SPECT (North Ridge, CA) preclinical small animal imaging system. CT acquisitions consisted of 1024 projections acquired over 360 deg with a 70 Kvp, 205 $\mu$A cone beam X-ray. Cobra Exxim (Feldkemp filtered backprojection cone beam reconstruction software) was used to reconstruct the image at a voxel size of 0.155 microns and a matrix size of 5123. The SPECT images were acquired using the dual head system fitted with a low energy high resolution collimator, a 20 % energy window was set over the 140 Kvp peak. Gamma Medica FLEX-SPECT software was used to reconstruct the data at a 1.15437 mm voxel size and 803 matrix size. Fusion of the SPECT and CT data was completed using in-house software. Male Copenhagen rats (250-300 g) or male white Sprague-Dawley rats (250-300 g) (Charles River) were positioned with the head in the centre of field of view, thus, only a portion of the abdomen was also in the field of view. The subjects were under anaesthesia (isoflurane) for the duration of the scan. The $^{99m}$Tc complexes 14a and
in a solution of 5-10% ethanol/saline were injected via tail vein. The SPECT/CT images of 14a were acquired using 10x2 s/frame for 32 frames at time of injection, and 2x20 s/frame for 32 frames at 30 and 45 min post injection or 3x30 s/frame for 32 frames at 0, 30 and 45 min post injection. The 2-D planar images of 15 were acquired using 30 s/frame for 120 frames.

2.6.7. Biodistribution studies

The animal studies in male white Sprague-Dawley rats (250-300 g) were carried out in accordance with the McMaster Animal Research Ethics Board. 370-555 kBq (10-15 μCi, 0.3 mL) of 14a in a solution of < 10% ethanol/saline (300 μL) was injected into the tail vein. After injection, the rats were sacrificed either by exsanguination under anaesthesia (isoflurane) or decapitation at select time points 2, 15, 60 and 120 min. Various organs and regions were isolated for weighing and counting. The results were presented in terms of the percentage of injected dose per gram of tissue (%ID/g).

2.6.8. X-ray crystallography

A colourless flat needle crystal of 5 was mounted on a glass fibre and placed in a cold stream of liquid nitrogen (77 K). Data was collected at 100 K on a Bruker APEX2 diffractometer equipped with CCD area detector using a graphite monochromator MoKα radiation and phi and omega scans. The SMART suite was used for data collection and SAINT was used for data reduction; data was scaled using SADABS with both face-indexed absorption correction as well as a correction using redundant data. Data was
collected in omega- and phi-scans and integrated as two component twin using the program SAINT. An empirical absorption correction using the program TWINABS was applied. The structure was then solved by direct methods and refined by full matrix least-squares refinement on \( F^2 \) using the Bruker SHELXTL program library. The non-hydrogen atoms were refined anisotropically and the hydrogen atoms were placed in calculated position and refined as riding on their constituent atoms. In the final stages of refinement, \( R = 5.53\% \) and \( wR^2 = 7.77\% \) were reported.

A colourless block crystal of 6 was mounted on a glass fibre. Data was collected at 295 K on a Bruker D8 diffractometer equipped with CCD area detector using a fine focused parallel mirrors, CuK\( \alpha \) radiation and phi and omega scans. The SMART suite was used for data collection and SAINT was used for data reduction; data was scaled using SADABS with both face-indexed absorption correction as well as a correction using redundant data. The structure was then solved by direct methods and refined by full matrix least-squares refinement on \( F^2 \) using the Bruker SHELXTL program library. The non-hydrogen atoms were refined anisotropically with no disorder. The hydrogen atoms were located from the difference map and allowed to refine independently of their constituent atoms. In the final stages of refinement, \( R = 4.45\% \) and \( wR^2 = 12.11\% \) were reported.

A colourless parallel-piped crystal of 12 was mounted on a MiTeGen polymer mount and placed in a cold stream of liquid nitrogen (77 K). Data was collected at 100 K on a Bruker APEX2 diffractometer equipped with CCD area detector using a graphite monochromator MoK\( \alpha \) radiation and phi and omega scans. The APEX2 suite was used
for data collection and SAINT was used for data reduction; data was scaled using SADABS with both face-indexed absorption correction as well as a correction using redundant data. The structure was then solved by direct methods and refined by full matrix least-squares refinement on F² using the Bruker SHELXTL program library. The non-hydrogen atoms were refined anisotropically. The hydrogen atoms were generated with the exception of H1 on C1 which was located. N1 is protonated, as evidenced by the slightly longer N-C bond within the ring. This has been previously cited in the literature. Protonation at N1 ensures that there is no residual counterion in the structure. One molecule of CH₂Cl₂ was located and refined. In the final stages of refinement, R = 5.12 % and wR² = 12.06 % were reported. CCDC # 791174.

2.7. References


21. Safronov, A. V.; Dolgushin, F. M.; Petrovskii, P. V.; Chizhevsky, I. T. Low-temperature "$1,2 \rightarrow 1,7$" isomerization of sterically crowded icosaheiral closo-((2,3,8-η$^3$):(5,6-η$^2$)-norbornadien-2-yl)rhodacarborane via the formation of a
pseudocloso intermediate. Moleculare structures of \([3,3-((2,3,8-\eta^3):(5,6-\eta^2)-C_3H_7CH_2)-1,2-(4'-MeC_6H_4)\_2-3,1,2-pseudocloso-RhC_2B_9H_9]\) and \(1,2 \rightarrow 1,7\) isomerized products. *Organometallics 2005*, 24, 2964-2970.


Chapter 3 – Synthesis, Radiolabelling and Evaluation of Second Generation WAY-derived Metallocarborane Complexes (M = Re, $^{99m}$Tc)

3.1. Overview

Modification of spacer length and type (i.e. functional group) between the radiometal and a WAY-type pharmacophore has dramatic effects on binding affinity and selectivity towards specific receptor subtypes as well as the overall biodistribution of the complex. For example, the replacement of a methylene group in a linker with an ether-oxygen spacer in a technetium(V) oxo-complex increased brain uptake from 0.5 %ID/organ to 2.1 %ID/organ 2 min post-injection in rats.

In the previous chapter, we observed varying in vitro binding affinities in the rhenium complexes 12 and 13 which contain different spacer lengths (Figure 3.1). Neither of these complexes showed high or selective binding to any of the neurotransmitter receptors or transporters examined, however, it was observed that the longer spacer in 13 resulted in better binding. In order to enhance the binding affinity, the preparation of an alternative linkage between the arylpiperazine moiety and the carborane cage was explored.

Figure 3.1. Structures of rhenacarborane complexes 12 and 13.

The introduction of an amide functional group as a linker was considered based
on the subnanomolar binding displayed in the ferrocene derivative 2 to the serotonin 5-HT$_{1A}$ receptor (Figure 3.2).² Computations have shown that the diameter of benzene (4.7-5 Å) is similar that of o-carborane (5.3-5.7 Å) thus, the space occupied by the three dimensional sweep of a phenyl group is similar to o-carborane.³⁻⁵ The molecular volume of o-carborane has been calculated from the optimized structure and from density values and range between 148-255 Å$^3$.⁶ The addition of [Re(CO)$_3$]$^+$ in place of a BH vertex on a carborane has shown to increase the molecular volume by 59-70 Å$^3$.⁷,⁸ The molecular volume of ferrocene (Cp$_2$Fe) is 192 Å$^3$,⁹ while the ferrocene derivative, [(C$_5$Me$_5$)$_2$Fe] or [(Cp*)$_2$Fe] has a molecular volume of 303 Å$^3$ and the metallocarborane sandwich complex, [(C$_2$B$_9$H$_{11}$)$_2$Fe] has as molecular between 277-287 Å$^3$.¹⁰ The size of a rhenacarborane is only slightly larger than the ferrocene complex which suggests that the carborane analogue of 2 could exhibit similar potent binding. As a result, the chemistry for preparing the amido-butyl linked carborane 16 was developed, and the cluster was complexed with [M(CO)$_3$(H$_2$O)$_3$]$^+$ (M = Re, $^{99m}$Tc) to generate the corresponding rhenium 22b and technetium 23 complexes (Figure 3.2). Subsequently, the *in vitro* binding affinity of 22b was determined, the lipophilicity and the ability of 23 to cross the BBB were evaluated where parts of this work along with the alkyl-linked complexes (chapter 2) was published in the Journal of Medicinal Chemistry.¹¹
Figure 3.2. Structures of compound 2, 16, 22b and 23.

3.2. Preparation of amido-linked carborane-WAY conjugate 16

Carborane 16 was synthesized following the convergent route outlined in Scheme 3.1. The mono-carboxylic acid carborane 17 was prepared via mono-lithiation of o-carborane in diethyl ether followed by carbonylation with dry ice (CO₂) (Scheme 3.1).¹² Quantitative conversion of 17 to the acid chloride carborane 18 was obtained using thionyl chloride (SOCl₂) in the presence of catalytic amounts of N,N-dimethylformamide (DMF). In the absence of DMF, 18 was not generated. Kahl and co-workers used phosphorus pentachloride (PCl₅) to prepare 18; however, we found removal of excess reagent and by-products (SO₂, HCl) using thionyl chloride was more convenient and could be done using vacuum techniques allowing for high yielding and effectively one
pot coupling with the amine 19.

Scheme 3.1. Preparation of sodium salt of 16.

The nitrile derivative 20 was obtained by combining 1-(2-methoxyphenyl)piperazine with 4-bromobutyronitrile. Following the reduction of 20 with lithium aluminum hydride the corresponding amine 19 was isolated (Scheme 3.1).
3.1). The synthesis of the target carborane 16 was achieved through coupling of the acid chloride 18 and the amine 19 where the resulting product mixture contained both the closo- and nido-carborane species (Scheme 3.1). In the presence of amines, closo-carborane species can undergo deboronation to generate the corresponding nido-carborane derivative.\(^{14}\) Deboration is advantageous because this process needs to occur prior to complexation with the \([\text{M(CO)}_3\]^+\) unit (\(\text{M} = \text{Re, } ^{99m}\text{Tc}\)). The presence of excess 19 and diisopropylethylamine in the reaction led to \textit{in situ} loss of a BH vertex yielding product 16 as the diisopropylethlammonium salt. Conversion of this mixture to solely nido-carborane 16 (17 % yield) as the sodium salt was accomplished using sodium hydroxide in ethanol.

The IR spectrum of the nitrile derivative 20 shows a band at 2245 cm\(^{-1}\) while after treatment with LiAlH\(_4\) the CN band of 19 was absent and an NH infrared band at 3353 cm\(^{-1}\) appeared indicating successful conversion. The NMR spectra of 19 and 20 are consistent with those reported in the literature prepared using different reagents.\(^{13}\) The sodium salt of carborane compound 16 shows \(^1\text{H}\) NMR resonances between 6.91-7.04 ppm, 3.86 ppm and 2.53 ppm indicative of the aromatic protons, the methoxy group and the carborane proton, respectively. The \(^{13}\text{C}\{^1\text{H}\}\) NMR spectrum of 16 contains a resonance at 173.9 ppm which is attributed to the amide carbonyl group. The \(^{11}\text{B}\{^1\text{H}\}\) NMR spectrum contains nine resonances ranging from -9.6 to -36.1 ppm where the two resonances at -32.8 and -36.1 ppm are characteristic of nido-carborane compounds (Figure 3.3). These two resonances are attributed to the BH unit at the apex (B1) of the compound and the vertex (B10) in the B\(_3\)C\(_2\) open face directly across from the two
carbon atoms which shares the fluxional bridging proton. The chemical shifts are consistent with similar nido-carborane compounds reported in the literature. The low solubility of 16 made characterization challenging because the borosilicate in the NMR tube produces a broad baseline resonance in the spectrum. The infrared spectrum of 16 contains absorbance bands at 3419, 2523 and 1593 cm\(^{-1}\) characteristic of the NH stretch, BH stretch and carbonyl group of an amide, respectively. The mass spectrum contains an isotopic pattern consistent with a C\(_2\)B\(_9\) compound and the peak at the expected m/z (424.3593).

Figure 3.3. \(^{11}\)B\(\{^1\text{H}\}\) NMR (160 MHz, (CD\(_3\))\(_2\)CO) spectrum of 16.

The X-ray crystal structure of the nido-carborane ligand 16 confirms that the two carbon atoms are in the 1,2 position (Figure 3.4, Table 3.1). The C-C bond length in the carborane cage is 1.67 \(\text{Å}\) which is similar to the closo-carborane 6 (1.65 \(\text{Å}\)) and is slightly longer than typical nido-carborane derivatives (1.55 \(\text{Å}\)). The average B-B cage bond length of 16 is 1.77 \(\text{Å}\) which falls within the typical B-B bond length range (1.71-1.86 \(\text{Å}\))
of other nido-carborane analogues.\textsuperscript{18-22} The sodium counterion is not present in the crystal lattice of 16 because one of the piperazine nitrogen atoms (N2) is protonated, generating a zwitterionic complex. Also, disordered solvent molecules (methanol, dichloromethane and water) were located in the crystal lattice of 16.

\textbf{Figure 3.4.} X-ray crystal structure of 16 (showing 30\% thermal probability ellipsoids; hydrogen atoms omitted for clarity).
Table 3.1. X-ray data for 16.

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<th>parameter</th>
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<td>empirical formula</td>
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^a R = [Σ|F_o| - |F_c|]/[Σ|F_o|] for reflections with I > 2.00σ(I)
^b R_w = {[Σw(F_o^2 - F_c^2)^2]/[Σw(F_o^2)^2]}^{1/2} for all reflections

Direct coupling between the carboxylic acid-derivatized carborane 17 and the arylpiperazine amine 19 was attempted to improve the overall yield. Despite using a combination of coupling reagents, additives and solvents, preparation of 16 via these methods was not successful (Table 3.2). Attempts to prepare carborane active ester derivatives of N-hydroxysuccinimidyl or tetrafluorophenyl resulted in a complex mixture of products. The electron withdrawing nature and bulk of the carborane cage hinders the ability of the carboxylic acid group to interact with the coupling agent in order to generate an amide bond. There was also no success in forming carborane-amide linkage directly off the cage using traditional solid-phase peptide synthesis coupling agents such
as HATU, HBTU and TBTU.

**Table 3.2.** Reagents used in attempts to prepare 21.

<table>
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<th>Coupling agent</th>
<th>Additive</th>
<th>Solvent</th>
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<td>EDC</td>
<td>HOBt</td>
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<tr>
<td>HATU</td>
<td>HOAt</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>HBTU</td>
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<td>Ethyl acetate</td>
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</tr>
</tbody>
</table>

a EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; HATU = N,N,N',N'-tetramethyl-O-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate; HBTU = N,N,N',N'-tetramethyl-O-(1H-benzotriazol-1-yl)uronium hexafluorophosphate; TBTU = N,N,N',N'-tetramethyl-O-(benzotriazol-1-yl)uronium tetrafluoroborate; DCC = dicyclohexylcarbodiimide

b HOBt = N-hydroxybenzotriazole; HOAt = N-hydroxy-7-azabenzotriazole

As a test reaction, an attempt was made to prepare the carboxylic acid substituted metallocarborane 21 by heating 17 with [Re(CO)₃(H₂O)₃]⁺ (Scheme 3.2). Unfortunately, the high temperature and pressure required for metal complexation in the microwave reactor led to decarboxylation yielding o-carborane.

**Scheme 3.2.** Attempted reaction between 17 and [Re(CO)₃(H₂O)₃][Br] to prepare 21. Decarboxylation was the dominant process yielding o-carborane.
3.3. Complexation and characterization of metallocarborane complexes (M = Re (22), $^{99m}$Tc (23))

Compound 16 was combined with [Re(CO)$_3$(H$_2$O)$_3$]$^+$ in a microwave reactor to prepare complex 22. Following the work-up, thin layer chromatography indicated the formation of two products. Using preparative TLC, each product was isolated and the mass spectral analysis showed each to have the same m/z and isotopic signature. Separation of these two complexes was initially attempted using reverse phase C18 automated purification, however, this was not successful. Separation was accomplished using silica gel column chromatography. Based on NMR spectroscopy and X-ray crystallographic data, the products were identified as 22a and 22b. The products were present in a ratio of 1:3 (22a:22b) based on the integration of the NH protons in the $^1$H NMR spectrum of the crude reaction mixture (Figure 3.5).

![Figure 3.5. $^1$H NMR (600 MHz, CD$_3$CN) spectrum of the reaction mixture containing complexes 22a and 22b and spectrum expansion (dotted region).]
The $^1$H NMR spectrum of the minor component 22a shows resonances at 6.12 and 2.61 ppm for the amide proton and the unsubstituted carborane CH proton, respectively. In the $^1$H NMR spectrum of the major complex 22b, the amide proton is found downfield (~ 6.6 ppm) while the carborane proton resonance is at 1.86 ppm. Prior to complexation, the unsubstituted carborane CH of nido-ligand 16 displayed a signal in the NMR spectrum at 2.53 ppm. Upon complexation of the [Re(CO)$_3$]$^+$ unit to the C$_2$B$_3$ bonding face of the carborane cage, the carborane CH proton shifts to a lower frequency (upfield). The $^1$H NMR spectrum of the major product 22b displays this expected movement in the carborane CH signal; on the other hand, the minor isomer 22a only shows a small resonance shift suggesting that the unsubstituted carborane proton is distant to the metal core.

The $^{13}$C{$^1$H} NMR spectrum of 22a has a resonance at 172.6 ppm for the amide carbonyl and the $^{11}$B{$^1$H} NMR spectrum contains six signals between -6.9 and -19.6 ppm characteristic of rhenacarborane-type complexes (Figure 3.6). The $^{13}$C{$^1$H} NMR spectrum of 22b shows the amide carbonyl resonance at 168.5 ppm and the $^{11}$B{$^1$H} NMR spectrum has seven chemical resonances between -5.8 and -19.9 ppm (Figure 3.7). The differences in the $^{11}$B{$^1$H} NMR spectra resonances of 22a and 22b suggests dissimilar orientation of the carbon atoms in the cage structure. The infrared spectrum of 22b shows strong bands at 2002 and 1917 cm$^{-1}$ characteristic of the [Re(CO)$_3$]$^+$ core and the band at 1652 cm$^{-1}$ is attributed to the carbonyl group of the amide. Complex 22a has similar IR bands at 2003 and 1895 cm$^{-1}$ from the [Re(CO)$_3$]$^+$ core.
**Figure 3.6.** $^{11}$B$^{1}$H NMR (160 MHz, CD$_3$CN) spectrum of 22a.

**Figure 3.7.** $^{11}$B$^{1}$H NMR (160 MHz, CD$_3$CN) spectrum of 22b.

The nOe spectrum of the major complex 22b was collected and showed no enhancement of any of the resonances in the complex following irradiation of the unsubstituted carborane proton (at $\delta$ 1.86 ppm) indicating that cage isomerization has occurred and the metallocarborane complex was the 2,1,8 isomer. Unlike complex 12 from chapter 2, the steric bulk of the arylpiperazine group is not likely driving the isomerization of the carborane cage. Carborane 16 with the amido-butyl linkage is
similar to compound 6 which has a propyl spacer between the carborane and arylpiperazine. When compound 6 was coordinated with [Re(CO)₃]⁺ to yield 13, no cage isomerization was observed presumably due to the lower steric strain associated with the extended linkage. Based on work done in our group on substituent effects on carborane cage isomerization of rhenacarborane complexes,²³ the electron withdrawing nature of the amide directly attached to the carborane cage is the driving factor for isomerization. For instance, a mono-substituted rhenacarborane containing a CH₂-pyridyl group undergoes isomerization at room temperature following methylation or protonation of the nitrogen atom in the pyridine group (Scheme 3.3).²³ These results suggest that positively charged or electron withdrawing substituents (such as the amide group) may lower the isomerization energy barrier. The proposed pathways for isomerization were discussed in chapter 2.

Scheme 3.3. Room temperature isomerization upon protonation or methylation of the pyridyl-substituted rhenacarborane complex.

Previously, our group reported observing additional signals in the ¹H NMR spectrum of an estrogen-based rhenacarborane complex and speculated that these
resonances were due to the presence of a second rhenacarbaborane isomer. However, conclusive evidence for this isomer and its configuration had not been achieved. The crystal structures described here offered definitive confirmation of the 2,1,8 isomer. X-ray quality crystal structures of $22a$ and $22b$ were obtained from slow evaporation from a solution of 10% methanol/dichloromethane (Figure 3.8 and Figure 3.9, Table 3.3). The configuration of both $22a$ and $22b$ consists of a 1,7 C-C cage orientation such that the $\text{[Re(CO)$_3$]}^+$ core is bonded through a CB$_4$ face. In the minor component, $22a$, the unsubstituted CH unit migrated away from the metal core (Figure 3.8). Complex $22b$ has the carbon atom bonded to the arylpiperazine group moved away from the metal bonding face showing a similar orientation as complex $12$ (Figure 3.9).
**Figure 3.8.** X-ray crystal structure of 22a (showing 50 % thermal probability ellipsoids; hydrogen atoms and solvent molecules were omitted for clarity).

**Figure 3.9.** X-ray crystal structure of 22b (showing 50 % thermal probability ellipsoids; hydrogen atoms omitted for clarity).
Table 3.3. X-ray crystal data for 22a and 22b.

<table>
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<tr>
<th>parameter</th>
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<th>22b</th>
</tr>
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</tr>
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<td>9.6837(5)</td>
</tr>
<tr>
<td>b, Å</td>
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<td>12.8205(7)</td>
</tr>
<tr>
<td>c, Å</td>
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<td>23.6507(13)</td>
</tr>
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<sup>a</sup> R = [Σ|F<sub>o</sub>| - |F<sub>c</sub>|]/[Σ|F<sub>o</sub>|] for reflections with I > 2.00σ(I)

<sup>b</sup> R<sub>w</sub> = {Σ[w(F<sub>o</sub><sup>2</sup> - F<sub>c</sub><sup>2</sup>)<sup>2</sup>]/Σ[w(F<sub>o</sub><sup>2</sup>)]<sup>1/2</sup> for all reflections

The bond lengths between the Re metal and the cage carbon atom of 22a and 22b are 2.32 Å and 2.33 Å, respectively. The bond distance is comparable to other 2,1,8-rhenacarborane complexes (2.35 Å).<sup>8</sup> The Re-B bond lengths range from 2.29-2.36 Å and 2.28-2.34 Å for complexes 22a and 22b, respectively, which is similar to Re-B bond lengths (2.27-2.35 Å) of a phenol substituted rhenium carborane complex with an analogous cage orientation of the substituents.<sup>8</sup> The average B-B bond length for complexes 22a and 22b is 1.78 Å and it is similar to complex 12 (1.78 Å) and other mono-substituted 2,1,8-rhenacarboranes (1.80 Å).<sup>23</sup> One of the piperazine nitrogen atoms (N2) is protonated in both 22a and 22b which accounts for the absence of a counterion in
the crystal lattice. Complex 22a also contains two dichloromethane solvent molecules in the lattice.

The characterized rhenium complexes 22a and 22b were used as HPLC reference standards for the radiolabelling of compound 16 with $^{99m}$Tc. The corresponding $^{99m}$Tc-complex 23 was prepared by reacting compound 16 and $[^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$ in a microwave reactor (Scheme 3.4). With this new linkage (an amide directly bonded to the carborane cage versus a simple alkyl linker), the optimal temperature for achieving a high yield of 23 was determined. A suspension of compound 16 in 10 % aqueous ethanol solution and $[^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$ was heated at various temperatures between 100 and 195 °C for 10 min. The highest conversion (63 %) was observed at 195 °C which was the maximum attainable temperature under these reaction conditions. The optimal reaction condition for generating 23 is the same as that reported for the preparation of $^{99m}$Tc-carborane complexes 14b and 15 described in chapter 2 where extending the reaction time did not increase the yield. The $^{99m}$Tc-carborane complex 23 showed a 63 % conversion based on radio-HPLC, however, following HPLC purification, the decay corrected isolated yield was 14 %. The low yield is attributed to the non-specific binding to the HPLC column associated with the purification process as seen earlier with the other derivatives (14a, 14b and 15). The radio-HPLC retention time of 23 is 14.0 min which correlates with rhenacarborane complex 22b in the UV-HPLC (t$_R$ =13.7 min). Complex 22b was the major complex isolated from the reaction between 16 and [Re(CO)$_3$(H$_2$O)$_3$]$^+$ and has the 2,1,8-configuration with the arylpiperazine group migrated away from the metal core.
Scheme 3.4. Preparation of 23 from 16 and $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$.

The nido-carborane salt 16 was combined with the $^{99m}\text{Tc}$ precursor in a suspension in 10 % aqueous ethanol due to the low solubility of this ligand. Following heating in the microwave reactor with $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$, the reaction mixture was transparent however, upon cooling a precipitate formed. To investigate whether the reaction yield would increase if a homogeneous solution of 16 was generated before the addition of $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$, a series of different aqueous-organic mixtures were used. When compound 16 was dissolved in either aqueous acetonitrile or dimethylsulfoxide, the solutions were transparent and colourless. Following complexation with $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$, the reaction mixtures were analyzed by HPLC and showed none of the desired product in the radio-chromatograms. Acetonitrile and DMSO are donor solvent ligands which can exchange with the labile water ligands of the $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+$ generating a new and potentially less reactive technetium complex, $[^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_{3-n}(\text{L})_n]^+$ (L = CH$_3$CN or DMSO, n = 1-3). Consequently, if all three water molecules are replaced by the less labile solvent molecules before complexation, the coordination of the $\eta^2$-carborane compound would be hindered and no product would be observed. Research by Liu and co-workers found that the three water molecules of
[\textsuperscript{99m}Tc(CO)\textsubscript{3}(H\textsubscript{2}O)\textsubscript{3}]\textsuperscript{+} could be fully replaced by acetonitrile ligands during HPLC analysis using acetonitrile as an elution solvent\textsuperscript{24}. The use of acetonitrile and DMSO increased the solubility the carborane ligand 16 but also interfered with the complexation reaction with [\textsuperscript{99m}Tc(CO)\textsubscript{3}(H\textsubscript{2}O)\textsubscript{3}]\textsuperscript{+} to generate the metallocarborane complex 23.

A solution of \textsuperscript{99m}Tc-complex 23 (in 5-10 % ethanol/saline) was examined for stability using radio-HPLC. Complex 23 showed no degradation of the product using different mobile phases and pH for up to 6 hours. The specific activity and effective specific activity of 23 were measured by analytical HPLC to be

\[ 91.3 \times 10^4 \text{ MBq/mmol} \text{ and } 18.5 \times 10^4 \text{ MBq/mmol}, \text{ respectively.} \]

The specific activity of 23 is similar to 15 \((82.0 \times 10^4 \text{ MBq/mmol})\) and the effective specific activity of 23 is higher than both 14a \((5.5 \times 10^4 \text{ MBq/mmol})\) and 15 \((1.9 \times 10^4 \text{ MBq/mmol})\). However, the SA and ESA are approximately three orders of magnitude lower than those reported for standard \textsuperscript{99m}Tc-chelate complexes \((3.7-4.6 \times 10^8 \text{ MBq/mmol})\).\textsuperscript{25} This is a result of the modest amount of activity used to label the ligand. The SA and ESA of 23 may not be as high as \textsuperscript{99m}Tc-chelates but it does not limit the use of this complex for further biological testing.

The lipophilicity of 23 was measured using \textit{n}-octanol/phosphate buffer (pH 7.4) shake-flask method and found to be \( \log P = 2.4 \pm 0.2 \). Complex 23 contains a mix of polar (amide group) and less polar components (the 1,7 orientation in the carborane cage and the butyl linkage) producing a lipophilicity value similar to complex 14a \((\log P = 2.4)\). Complex 23 falls within the ideal range \((2.0-3.5)\)\textsuperscript{26} in which compounds can passively diffuse through the BBB indicating it has potential for brain uptake.
3.4. Biological evaluation of metallocarborane complexes (M = Re (22b), $^{99m}$Tc (23))

3.4.1. *In vitro* binding assay for 22b

The predominant rhenacarborane isomer 22b was examined for its *in vitro* binding affinity toward a family of serotonin receptors, a series of α and β adrenergic receptors, sigma receptors and monoamine transporters in parallel with complexes 12 and 13 (chapter 2). The summary of the binding affinities of complexes 12, 13 and 22b can be found in Table 3.4. For complex 22b, less than 50% binding was observed for the serotonin receptors (5-HT$_1A$, 5-HT$_1B$, 5-HT$_1E$, 5-HT$_2A$, 5-HT$_2C$ and 5-HT$_3$), adrenoceptors ($\beta_1$ and $\beta_2$) and the monoamine transporters (DAT, NET, SERT) and greater than $\mu$M affinity was found for the serotonin (5-HT$_1D$ and 5-HT$_5A$) and β$_3$-adrenergic receptors. Complex 22b showed some affinity for two serotonin receptors 5-HT$_2B$ ($K_i = 128$ nM) and 5-HT$_7$ ($K_i = 40$ nM). Interestingly, complex 22b has a high affinity towards $\alpha_{1A}$, $\alpha_{1D}$ and $\alpha_{2C}$ adrenergic receptors with $K_i = 17$, 21 and 39 nM, respectively, but a greater than 100 nM affinity for the $\alpha_{1B}$ receptor. These results are promising but not surprising since many arylpiperazine derivatives are recognized as potential lead compounds to target the serotonin and the α-adrenergic receptors.$^{27-29}$ The degree of homology between the receptors (approximately 45%) leads to this poor differentiation.$^{30}$
Table 3.4. Binding assay of rhenacarborane complexes 12, 13 and 22b to serotonin, adrenergic and sigma receptors as well as dopamine, serotonin and norepinephrine transporters.

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<th>5-HT_{2B}</th>
<th>5-HT_{2C}</th>
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<td>2113</td>
<td>4807</td>
<td>*</td>
<td>1165</td>
<td>*</td>
<td>*</td>
<td>1714</td>
<td>5085</td>
<td>*</td>
<td>*</td>
<td>3630</td>
</tr>
<tr>
<td>13</td>
<td>118 ± 8</td>
<td>*</td>
<td>3937</td>
<td>*</td>
<td>*</td>
<td>211 ± 16</td>
<td>*</td>
<td>*</td>
<td>6993</td>
<td>1080</td>
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<td>*</td>
<td>4073</td>
<td>*</td>
<td>*</td>
<td>128 ± 7</td>
<td>*</td>
<td>*</td>
<td>3924</td>
<td>692 ± 77</td>
<td>40 ± 5</td>
<td>*</td>
<td>201 ± 9</td>
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<table>
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<td>*</td>
<td>1751</td>
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* errors not recorded for values >1000 nM
* indicates <50 % binding in primary assay
There are a number of PET agents identified for imaging adrenergic receptors which are derived primarily from carbon-11 and fluorine-18.\textsuperscript{31-41} Only a few WAY-based $^{99m}$Tc-chelate complexes have demonstrated good binding to the serotonin 5-HT$_{1A}$ and the $\alpha_1$-adrenergic receptors, however, these complexes showed no selectivity for either receptor. For example, the technetium(V) oxo-complex linked to a WAY derivative (phenol piperazine) (Figure 3.10a) has affinity for both serotonin 5-HT$_{1A}$ ($IC_{50} = 1.29$ nM) and the $\alpha_1$-adrenergic ($IC_{50} = 8.1$ nM) receptors\textsuperscript{42} while another oxo-based complex (M = Re, $^{99}$Tc) with a “3+1” mixed chelate linked to the WAY (methoxyphenylpiperazine) molecule (Figure 3.10b) has equally high affinities for the 5-HT$_{1A}$ receptor and the $\alpha_1$-adrenergic receptor.\textsuperscript{43} Another research group observed subnanomolar affinities for both the 5-HT$_{1A}$ and $\alpha_1$-adrenergic receptors for a series of Re(III) complexes with a nitrile linkage to the WAY molecule (Figure 3.10c).\textsuperscript{44} Saidi \textit{et al.} reported a cytectrene organometallic $^{99m}$Tc cyclopentadienyl-complex (Figure 3.10d) that showed high brain uptake and the pattern of distribution of the agent in autoradiographic studies indicated binding to both 5-HT$_{1A}$ and $\alpha_1$-adrenergic receptors.\textsuperscript{45} To the best of our knowledge, no other $^{99m}$Tc-organometallic complex has been reported to selectively target the $\alpha$-adrenergic receptors.
Figure 3.10. (a)-(c) WAY-based $^{99m}$Tc complexes and (d) a cyctectene organometallic $^{99m}$Tc complex that have high affinity for the serotonin and the $\alpha_1$-adrenergic receptors.

$\alpha$-Adrenergic receptors are of particular interest for molecular imaging applications because of their involvement in cognitive function such as attention and memory, and motor activity. Studies indicate that the involvement of $\alpha$-adrenergic receptors in pain and Alzheimer’s disease is through abnormalities in neurotransmission. The exact role of the adrenergic receptor system in normal and disease physiology is difficult to study because of a lack of a suitable molecular imaging probe. The identification of complex 22b as a potential lead for imaging $\alpha$-adrenergic receptors would assist in locating and understanding the function of this type of receptor.
3.4.2. SPECT imaging study and biodistribution of $^{99m}$Tc-carborane complex 23

In light of the promising results from the in vitro binding data observed for 22b, further biological testing was examined. A scintigraphic study of the $^{99m}$Tc-carborane analogue 23 was performed following administration (~ 100 MBq) to healthy Sprague-Dawley rats by tail vein injection. A planar dynamic scan was acquired for 1 h followed by a CT scan with the camera positioned over the subject’s head. No noticeable brain uptake for complex 23 was observed and the complex showed primarily uptake in the abdomen. Unlike complex 14a (chapter 2), no uptake in the lung was observed.

In order to quantify the distribution of the $^{99m}$Tc-carborane complex 23, a biodistribution study was performed using healthy Sprague-Dawley rats without anaesthesia. In the biodistribution study, the organs analyzed include: heart, lungs, liver, kidneys, muscle, stomach, intestine (small and large), caecum, pancreas, spleen, bladder, adrenal glands and eyes. Uptake in the bone, skin, adipose, urine and blood were also measured. The brain was sectioned into the cerebellum, cortex, hypothalamus, hippocampus and mid/hind region. Four time points were selected and studied: 2, 15, 60 and 120 min post injection. Minimal brain uptake was observed (0.005-0.05 %ID/g) with the exception of one region (Figure 3.11). The highest uptake of 23 in the brain was in the hypothalamus (0.16 %ID/g, 15 min p.i.) which is the same region of increased brain uptake found for complex 14a. This brain uptake is less than that seen for 5-HT$_{1A}$ targeted iodo-radioligands (0.22-0.71 %ID/g ≤ 5 min p.i.),$^{50,51}$ and other $^{99m}$Tc WAY-based radiotracers with bifunctional chelate systems (0.24-1.31 %ID/g ≤ 5 min p.i.).$^{42-44,52,53}$
Figure 3.11. Biodistribution results of $^{99m}$Tc-carborane complex 23 in healthy rats (n = 3).
Lung and stomach showed modest but similar uptake as the highest region in the brain. Complex 23 showed clearance via the liver with increasing activity observed over the 120 min which supports the lipophilic nature of carborane complexes and is comparable to the trend displayed in the biodistribution study in complex 14a. The *in vitro* binding assay indicated binding of the rhenium complex 22b to α-adrenergic receptors which are involved in contraction and growth of smooth and cardiac muscle as well as regulation of arterial blood pressure, however, minor uptake of 23 was observed for the heart and skeletal muscle: 0.05 %ID/g (2 min p.i.) and 0.03 %ID/g (15 min p.i.), respectively.54

The technetium(V) oxo complexes (*vide supra*, Figure 3.10a and Figure 3.10b) that showed high affinities to both serotonin and α-adrenergic receptors also displayed minimal heart uptake (0.6-0.9 %ID/organ, 5 min p.i.) in their respective biodistribution studies.42, 43 On the other hand, the 99mTc(III) nitrile linked-WAY complex (Figure 3.10c), which has subnanomolar binding to both receptors, observed heart uptake that ranged between 1.3 and 3.2 ID/g, 5 min p.i. depending on the length of the linker between the chelate and arylpiperazine group.44 The authors performed a preliminary SPECT study on a monkey but observed negligible brain uptake. The correlations between *in vitro* binding results and *in vivo* uptake are not always consistent. Anaesthesia is generally used to sedate the subject during the SPECT or scintigraphic imaging and may, as described previously, affect the uptake of the complex in the brain (*vide supra*, section 2.4.3).

While identifying a high affinity metallocarborane for the α-class of adrenergic
receptors is a major step, the carborane complex carries a negative charge which may limit the ability of the complex to cross membrane barriers. This was supported by the minimal uptake observed in the biodistribution study. The development of neutral complexes is required to address this issue (chapter 4) will be accomplished by taking advantage of the versatility associated with the chemistry of metallocarboranes.\textsuperscript{5,55}

3.5. Summary

A new amido-butyl nido-carborane derivative 16 was prepared and characterized by NMR and IR spectroscopies and X-ray crystallography. The corresponding rhenium (22a and 22b) and technetium (23) complexes were produced by reacting $[\text{M(CO)}_3(\text{H}_2\text{O})_3]^+$ ($\text{M} = \text{Re, } {99\text{mTc}}$) with 16. The rhenium complexation reaction resulted in the formation of two isomers (22a and 22b). Both complexes have the carbon-carbon cage orientation in the 1,7 position and the isomerization was attributed to electronic effects. The major rhenacarborane product 22b showed high binding affinity to $\alpha$-adrenergic receptors resulting in the first organometallic complex that has high and selective binding to this class of neurotransmitter receptor. The lipophilicity of $^{99\text{mTc}}$-carborane complex 23 ($\log P = 2.4$) indicated that it may cross the BBB. SPECT imaging and biodistribution studies of 23 in healthy rats, unfortunately, did not show any significant brain uptake or accumulation in heart or skeletal muscle where $\alpha$-adrenergic receptors reside. The chemistry developed for the preparation of the metallocarborane-WAY complex is a platform for making neutral analogues to facilitate crossing of the blood brain barrier and as the basis for identifying higher affinity analogues.
3.6. Experimental

3.6.1. Reagents and general procedures

Unless otherwise stated, all chemicals and reagents were purchased and used as received from Sigma-Aldrich without further purification. Decaborane and \( o \)-carborane were obtained and used as received from Katchem Ltd. Compounds prepared according to literature procedures were 1,2-dicarba-\textit{closo}-dodecaborane-1-carboxylic acid (17),\(^{12}\) \([\text{Re}(\text{CO})_3(\text{H}_2\text{O})_3][\text{Br}]\),\(^{56}\) and \([^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+\) (3).\(^{25}\)

Reactions were performed under an inert atmosphere unless otherwise stated. All solvents were obtained from Caledon, and either dried by a Pure-Solv Solvent Purification System (Innovative Technology Inc.) or dried over calcium hydride (acetonitrile and dichloromethane), sodium/benzophenone (diethyl ether) and distilled prior to use. Deuterated solvents for NMR samples were purchased from Cambridge Isotope Laboratories. Technetium-99m complexes were prepared from pertechnetate \([^{99m}\text{TcO}_4]^-\) which was obtained from a \(^{99}\text{Mo}/^{99m}\text{Tc}\) generator (Lantheus Medical Imaging) in saline (0.9 % NaCl).

Reactions were monitored using Alugram Sil G/UV254 thin-layer chromatography plates. Carborane-containing species were visualized with 0.2 % PdCl\(_2\) in hydrochloric acid (3.0 M), which upon heating gave dark brown spots. Amines were visualized with ninhydrin spray. Silica Gel 60PF\(_{254}\) containing gypsum (EM Science) was used for making preparative TLC plates. Column chromatography was accomplished with Silica Gel 60 (EMD Chemical Inc.) or Ultra Pure Silica Gel 60
(Silicycle). Solid phase extraction cartridges (C18) obtained from Waters were used following pretreatment with water (10 mL), methanol or ethanol (10 mL), a second wash with water (10 mL) and HCl (10 mM, 10 mL).

3.6.2. Instrumentation

Nuclear magnetic resonance spectra ($^1$H, $^{13}$C{${}^1$H}, $^{11}$B{${}^1$H}) were recorded on either a Bruker 500 or 600 MHz spectrometer at ambient temperature. The chemical resonances ($\delta$) are reported in parts per million (ppm), with the $^1$H NMR shifts referenced to the residual proton signal of the deuterated solvent and the $^{13}$C shifts referenced to the carbon signal of the solvent. The chemical shifts ($\delta$) for $^{11}$B{${}^1$H} NMR spectra are reported relative to an external reference of BF$_3$·Et$_2$O. Infrared spectra were acquired on a Bio-Rad FTS-40, Nicolet 510 or 6700 Fourier Transform IR spectrometer at ambient temperature. Samples were run on a KBr plate or as KBr pellets. A Gallenkamp melting point apparatus was used to determine melting points. Reactions involving microwave heating were performed on a Biotage Initiator 60 or Initiator 8 microwave reactor using crimp-sealed vials. Mass spectra were obtained from the McMaster Regional Centre for Mass Spectrometry on a Micromass Quattro Ultima (LC-ESI/APCI triple quadrupole) mass spectrometer for electrospray ionization (ES) mass spectrometry; a Micromass GC-EI/CI time of flight mass spectrometer instrument for chemical ionization (CI); and a Micromass Global Ultima (MALDI/CapLC-ESI quadrupole time of flight) mass spectrometer for high resolution mass spectra. A Capintec dose calibrator was used to determine the activity of samples containing technetium-99m (> 300 kBq).
High performance liquid chromatography was performed on either a Varian ProStar model 230 or 230I solvent delivery system fitted with a Varian ProStar model 230 or 335 PDA detector and an in-line radioactivity detector (IN/US γ-RAM, 0.5 s with a 10 μL loop) or on the Agilent 1100 system (Quad Pump module G1311A, Degasser G1322A, ALS G1313A, Colcom G1316A) interfaced to a BioScan radioactivity detector (APIFC2-00507). The wavelength for detection was set at 250 or 254 nm. A Varian Nucleosil C18 100-5 (250 x 4.6 mm) column was used with a sample volume of either 100 μL or 500 μL. The following solvent gradients were employed: method A (solvent A = acetonitrile (0.1 % formic acid), solvent B = 20 mM ammonium acetate, 1 mL/min) 0-15 min, 65-100 % A, 15-20 min 100 % A; method B (solvent A = aqueous tetraethylammonium phosphate pH 2.0-2.5, solvent B = methanol, 1 mL/min) 0-3 min 100 % A, 3-6 min 100-75 % A, 6-9 min 75-67% A, 9-20 min 67-0 % A, 20-22 min 0 % A, 22-25 min 0-100 % A, 25-30 min 100 % A; method C (solvent A = acetonitrile, solvent B = water, 1 mL/min) 70:30 A:B isocratic for 15 min; method D (solvent A = acetonitrile (0.1 % formic acid), solvent B = water (0.1 % formic acid), 1 mL/min) 70:30 A:B isocratic for 15 min; method E (solvent A = acetonitrile (0.1 % formic acid), solvent B = 20 mM ammonium acetate, 1 mL/min) 70:30 A:B isocratic for 15 min; method F (solvent A = acetonitrile, solvent B = 0.1 M ammonium formate, 1 mL/min) 70:30 A:B isocratic for 15 min; method G (solvent A = methanol solvent B = water, 1 mL/min) 70:30 A:B isocratic for 15 min.
3.6.3. Synthetic procedures

\{[\text{Na}\{1-(\text{C}_{16}\text{H}_{24}\text{N}_{3}\text{O}_{2}\text{-nido-C}_{2}\text{B}_{9}\text{H}_{11}})\}] (16)\}. To a solution of 1,2-dicarba-closo-dodecaborane-1-carboxylic acid (17) (1.0 g, 5.3 mmol) in dichloromethane (50 mL), thionyl chloride (1.2 mL, 16 mmol) and N,N-dimethylformamide (0.30 mL, 3.9 mmol) were added. The solution was heated at reflux for 3.5 h. The solvent was removed under reduced pressure to yield 18 (1.1 g, 5.3 mmol). Compound 18 was immediately dissolved in dichloromethane (30 mL) and cooled to 0 °C. Diisopropylethylamine (1.8 mL, 10 mmol) and compound 19 (2.8 g, 11 mmol) were dissolved in dichloromethane (35 mL) and added to the reaction vessel. The reaction was allowed to warm to room temperature and stirred overnight. The reaction was washed with hydrochloric acid (0.01 M, 3 x 30 mL) and brine (30 mL). The organic fraction was dried over sodium sulfate, filtered and concentrated under reduced pressure. The reaction mixture was purified by silica gel column chromatography using a gradient of 0-10 % methanol/ethyl acetate. The product was stirred an ethanolic solution of sodium hydroxide (5 eq.) overnight, and then concentrated under reduced pressure. The residue was redissolved in ethyl acetate and washed with hydrochloric acid (0.1 M), brine and water. The organic fraction was concentrated and lyophilized to yield 16 as a white solid. Yield: 0.4 g, 17 %. TLC Rf (10 % methanol/dichloromethane) = 0.53; mp = >230 °C; \(^1\)H NMR (500 MHz, (CD\(_3\))\(_2\)CO) \(\delta\): 7.04 (m, 1 H, Ar-H), 6.98 (m, 2 H, Ar-H), 6.91 (m, 1 H, Ar-H), 6.65 (s, 1 H, NH), 3.86 (s, 3 H, OCH\(_3\)), 3.45 (s, 6 H, NCH\(_2\)), 3.27 (m, 4 H, NCH\(_2\), CH\(_2\)), 2.53 (s, 1 H, CH), 1.89 (m, 2 H, CH\(_2\)), 1.79 (m, 2 H, CH\(_2\)), 1.64 (m, 4 H, CH\(_2\)); \(^{13}\)C \({}^1\)H NMR (125 MHz,
(CD$_3$)$_2$CO) $\delta$: 173.9, 152.7, 139.6, 123.9, 121.0, 118.8, 112.0, 56.3, 54.9, 54.0, 52.9, 47.7, 40.6, 37.1, 27.1, 20.0; $^{11}$B{${}^1$H} NMR (160 MHz, (CD$_3$)$_2$CO) $\delta$: -9.6, -10.6, -14.6, -17.1, -17.7, -21.4, -23.0, -32.8, -36.1; IR (KBr, cm$^{-1}$): $\nu$ 3419, 2916, 2523, 1593; HRMS (ES+) m/z for C$_{18}$H$_{35}$B$_9$N$_3$O$_2$: calculated: 424.3567, observed: 424.3593 [M$^+$].

C$_{15}$H$_{25}$N$_3$O (19). The synthesis was adapted from a procedure by Chu and co-workers. To a solution of compound 20 (4.1 g, 15.8 mmol) in dry diethyl ether (150 mL) at 0 °C, 25 mL lithium aluminum hydride (1.0 M Et$_2$O) was added dropwise with stirring. The reaction mixture was stirred at 0 °C for 10 min, then stirred at room temperature for 2 h and finally heated at reflux for 2 h. The mixture was cooled to 0 °C and saturated aqueous NaHCO$_3$ (50 mL) was added slowly. The reaction mixture was stirred for 10 min at 0 °C and 10 min at room temperature. The mixture was filtered through Celite and the filtrate was added to a separatory funnel. The layers were separated and the aqueous fraction was further extracted with dichloromethane (3 x 20 mL). The combined organic fractions were dried with anhydrous Na$_2$SO$_4$, filtered and the solvent was removed to yield a yellow oil of compound 19 (3.8 g, 91 %). Characterization data matched the data in the literature. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 6.91 (m, 4 H, Ar-H), 3.82 (s, 3 H, OCH$_3$), 3.07 (s, 4 H, NCH$_2$), 2.70 (s, 1 H, NH$_2$), 2.62 (s, 4 H, NCH$_2$), 2.39 (t, 2 H, CH$_2$, $J = 7.5$ Hz), 1.55 (m, 2 H, CH$_2$), 1.47 (s, 2 H, CH$_2$); HRMS (ES+) m/z for C$_{15}$H$_{25}$N$_3$O: calculated: 264.2076, observed 264.2065 [M$^+$.]

C$_{15}$H$_{21}$N$_3$O (20). The synthesis was adapted from a procedure by Chu and co-workers. To a solution of 1-(2-methoxyphenyl)piperazine (5.1 g, 26.5 mmol) in acetonitrile (50 mL), sodium carbonate (5.6 g, 52.8 mmol) and bromobutyronitrile (2.0
mL, 20.1 mmol) were added with stirring. The reaction mixture was heated to reflux (82 °C) for 24 hours. When the mixture was cooled, anhydrous Na2SO4 was added. The mixture was filtered and the solvent removed under reduced pressure to yield a yellow oil. The product 20 was purified using silica gel column chromatography using a gradient of 75-100 % ethyl acetate/hexanes (4.6 g, 88 %). Characterization data matched the data in the literature.13 1H NMR (500 MHz, CDCl3) δ: 6.93 (m, 4 H, Ar-H), 3.86 (s, 3 H, OCH3), 3.08 (s, 4 H, NCH2), 2.64 (m, 4 H, NCH2), 2.52 (t, 2 H, CH2, J = 6.8 Hz), 2.45 (t, 2 H, CH2, J = 7.1 Hz), 1.86 (m, 2 H, CH2, J = 7.0 Hz); HRMS (CI+) for C15H21N3O: calculated: 259.1685, observed 259.1675 [M+].

{[Na][2,2,2-(CO)3-8-(C16H24N3O2)-2,1,8-closo-ReC2B9H10]} (22).

Complexation was accomplished by heating [Re(CO)3(H2O)3][Br] (1.0 eq.) and 16 (0.30 g, 0.067 mmol) in aqueous ethanol (10-15 %) in a microwave reactor at 180-195 °C for 10 min. Two subsequent additions of [Re(CO)3(H2O)3][Br] (1.0 and 0.5 eq.) were performed where each addition was accompanied by heating the mixture at 180-195 °C for 10 min. Following the final heating, the solvent was evaporated and the mixture was suspended in acetonitrile (15 mL) and filtered. The complexes 22a and 22b were isolated as white solids following purification by silica gel column chromatography or preparative TLC using a gradient of 10 % methanol/dichloromethane.

{[Na][2,2,2-(CO)3-1-(C16H24N3O2)-2,1,8-closo-ReC2B9H10]} (22a). Yield of minor isomer was not measured. Rf (10 % methanol/dichloromethane) = 0.76. 1H NMR (600 MHz, CD3CN) δ: 7.05 (m, 1 H, Ar-H), 6.94 (m, 3 H, Ar-H), 6.12 (s, 1 H, NH), 3.83 (s, 3 H, OCH3), 3.11 (m, 7 H, NCH2, CH2, CH2), 3.00 (m, 5 H, NCH2, CH2), 2.61 (s, 1 H,
CH), 1.69 (m, 2 H, CH2), 1.49 (m, 2 H, CH2); $^{13}$C{$^1$H} NMR (150 MHz, CD$_3$CN) δ: 199.8, 172.6, 153.4, 140.5, 125.0, 122.0, 119.4, 113.0, 57.3, 56.1, 53.9, 48.4, 38.3, 37.3, 27.8, 21.2; $^{11}$B{$^1$H} NMR (160 MHz, CD$_3$CN) δ: -6.9, -10.6, -13.1, -17.5, -18.8, -19.6; IR (neat, cm$^{-1}$): ν 3426, 2917, 2849, 2560, 2003, 1895, 1627; HRMS (ES-) m/z for C$_{21}$H$_{34}$B$_9$N$_3$O$_5$Re: calculated: 694.2894, observed 694.2951 [M+].

$\{[\text{Na}][2,2,2-(\text{CO})_3-1-(\text{C}_{16}\text{H}_{24}\text{N}_3\text{O}_2)-2,1,8\text{-closo-ReC}_2\text{B}_9\text{H}_{10}]\}$ (22b). Yield: 0.046 g, 10 %. TLC R$_f$ (5 % methanol/dichloromethane) = 0.31; m.p. = >230 °C; $^1$H NMR (600 MHz, CD$_3$CN) δ: 7.03 (m, 1 H, Ar-H), 6.94 (m, 3 H, Ar-H), 6.53 (s, 1 H, NH), 3.83 (s, 3H, OCH$_3$), 3.22 (m, 4 H, NCH$_2$), 3.12 (m, 6 H, NCH$_2$, CH$_2$), 2.89 (m, 2 H, CH$_2$), 1.86 (s, 1 H, CH), 1.58 (m, 2 H, CH$_2$), 1.47 (m, 2 H, CH$_2$); $^{13}$C{$^1$H} NMR (125 MHz, CD$_3$CN) δ: 199.7, 168.5, 153.4, 141.1, 124.5, 122.0, 119.4, 112.9, 57.7, 56.1, 53.8, 49.2, 39.6, 28.6, 27.3, 22.2; $^{11}$B{$^1$H} NMR (160 MHz, CD$_3$CN) δ: -5.8, -7.7, -8.9, -12.2, -18.8, -19.9; IR (KBr, cm$^{-1}$): ν 2517, 2002, 1917, 1896, 1652; HRMS (ES-) m/z for C$_{21}$H$_{35}$N$_3$O$_5$B$_9$Re: calculated: 695.2972, observed 695.2999 [M+H]. UV-HPLC (method A): t$_R$ = 13.7 min.

$\{[\text{Na}][2,2,2-(\text{CO})_3-8-(\text{C}_{16}\text{H}_{24}\text{N}_3\text{O}_2)-2,1,8\text{-closo-}^{99m}\text{TcC}_2\text{B}_9\text{H}_{10}]\}$ (23). To a solution of the nido-carborane 16 ligand (aqueous ethanol 10 %, 4 mM), [$^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$ (370-1850 MBq, 1 mL) was added to the reaction vessel. The mixture was heated in the microwave reactor at 195 °C for 10 min. The crude reaction mixture was passed through a C18 solid phase extraction cartridge where residual [$^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^+$ was eluted with HCl (10 mM) and the product was eluted with acetonitrile. The excess ligand was removed by HPLC (method A). Decay corrected
radiochemical yield: 14 %. Radio-HPLC (method A): $t_R = 14.0 \text{ min}$.

3.6.4. Analyses

**Stability study.** The $^{99m}$Tc-carborane complex 23 was dissolved in ethanol (95 %, 0.5 mL) and transferred to a vial containing saline (0.9 % NaCl, 4.5 mL). Analytical HPLC of the sample was performed every 30 min using a Varian Nucleosil C18 column (methods C-G). No decomposition was observed by radio-HPLC over 6 h under several different analytical HPLC conditions (mobile phase and pH).

**Specific activity measurements.** Calibration curves for the external standard (anisole), the *nido*-compound 16 and the Re complex 22 were obtained using the Agilent 1100 system with the Varian Nucleosil C18 column. The wavelength for detection was set at 254 nm. Method A was used with a flow rate at 0.5 mL/min for the external standard (anisole) and a flow rate of 1 mL/min for all other compounds. The concentration of the external standard ranged from 16.4–41 000 ng over nine calibration points. The calibration curves for the *nido*-species (16) (5.2–1040 ng) and the rhenium complex (22b) (5.4–1070 ng) were obtained over seven calibration points containing the external standard (800 ng). LOQ: *nido*-16 = 56.6 ng, complex 22b = 23.8 ng; LOD: *nido*-16 = 17.4 ng, complex 22b = 10.0 ng. The specific activity and effective specific activity of 23 was obtained using a sample containing 86.8 MBq.

**Lipophilicity.** The lipophilicity of compound 23 was determined by the partition between *n*-octanol and phosphate buffer (0.02 M, pH 7.4) following a previously published method.57 Approximately 222 kBq (6 μCi, 200 μL) of the technetium complex
(5-10 % ethanol/saline) was added to an equal ratio of n-octanol (10 mL) and phosphate buffer (10 mL) in a separatory funnel. The mixture was shaken for 3 min at ambient temperature; after resting the mixture for 5 min, the octanol (2 mL) phase was distributed into crimp-sealed vials. Additional phosphate buffer (2 mL) was added to each. The samples were vortexed for 10 min followed by centrifugation for 10 min. An aliquot (approximately 0.1 mL) was removed from each phase and placed into pre-weighed tubes. The remaining phosphate buffer was removed and replaced with a new aliquot of buffer (~2 mL). The vials were vortexed and centrifuged where a total of three repeats were obtained. The activity of each aliquot was measured using a gamma counter (Perkin Elmer 1470 Wizard) and the partition coefficient was determined by calculating the ratio of activities (n-octanol/buffer) and its logarithm was determined to express lipophilicity.

3.6.5. In vitro binding assay

Kᵢ determination was conducted by the National Institute of Mental Health’s Psychoactive Drug Screening Program, Contract No. NO1MH32004. For experimental details refer to the PDSP website http://pdsp.med.unc.edu/. Compound 22b was assayed against the serotonin, the α- and β-adrenergic, DAT, NET, SERT and sigma receptors.

3.6.6. In vivo SPECT imaging

In vivo SPECT imaging was performed at the McMaster Centre for Pre-clinical and Translation Imaging Facilities (Hamilton Health Sciences and McMaster University).
Animal acquisitions were performed on a Gamma Medica Ideas X-SPECT (North Ridge, CA) preclinical small animal imaging system. CT acquisitions consisted of 1024 projections acquired over 360 deg with a 70 Kvp, 205 μA cone beam X-ray. Cobra Exxim (Feldkemp filtered backprojection cone beam reconstruction software) was used to reconstruct the image at a voxel size of 0.155 microns and a matrix size of 5123. The SPECT images were acquired using the dual head system fitted with a low energy high resolution collimator, a 20 % energy window was set over the 140 Kvp peak. Gamma Medica FLEX-SPECT software was used to reconstruct the data at a 1.15437 mm voxel size and 803 matrix size. Fusion of the SPECT and CT data was completed using in-house software. Male white Sprague-Dawley rats (250-300 g) (Charles River) were positioned with the head in the centre of field of view, thus, only a portion of the abdomen was also in the field of view. The $^{99m}$Tc compound 23 in a solution of 5-10% ethanol/saline was injected via tail vein. The subject was anaesthetized (isoflurane) for the duration of the scan. SPECT/CT images were acquired using 3x30 s/frame for 32 frames at 0, 30 and 45 min post injection.

3.6.7. Biodistribution study

The animal studies in male white Sprague-Dawley rats (250-300 g) were carried out in accordance with the McMaster Animal Research Ethics Board. 370-555 kBq (10-15 μCi, 0.3 mL) of 23 in a solution of < 10 % ethanol/saline (300 μL) was injected into the tail vein. After injection, the rats were sacrificed either by exsanguination under anaesthesia (isoflurane) or decapitation at select time points 2, 15, 60 and 120 min.
Various organs and regions were isolated for weighing and counting. The results were presented in terms of the percentage of injected dose per gram of tissue (%ID/g).

3.6.8. X-ray crystallography

A colourless chip crystal of 16 was mounted on a MiTeGen polymer mount and placed in a cold stream of liquid nitrogen (77 K). Data was collected at 100 K on a Bruker APEX2 diffractometer equipped with CCD area detector using a graphite monochromator MoKα radiation and phi and omega scans. The SMART suite was used for data collection and SAINT was used for data reduction; data was scaled using SADABS with both face-indexed absorption correction as well as a correction using redundant data. The structure was then solved by direct methods and refined by full matrix least-squares refinement on $F^2$ using the Bruker SHELXTL program library. Non-hydrogen atoms were refined anisotropically and the hydrogen atoms of the carborane cage were either located in the difference map or generated and refined as riding on their constituent atoms. N2 is protonated, as evidenced by the slightly longer N-C bond within the ring. This has been previously cited in the literature. Protonation at N2 ensures that there is no residual counterion in the structure. There were disordered solvent molecules (MeOH, CH2Cl2 and H2O) in the lattice. In the final stages of refinement, $R = 9.97\%$ and $wR^2 = 28.25\%$ were reported.

A colourless plate crystal of 22a was fixed on a MiTeGen polymer mount and placed in a cold stream of liquid nitrogen (77 K). Data was collected at 100 K on a Bruker APEX2 diffractometer equipped with CCD area detector using a graphite
monochromator MoKα radiation and phi and omega scans. The APEX2 suite was used for data collection and SAINT was used for data reduction; data was scaled using SADABS with both face-indexed absorption correction as well as a correction using redundant data.\textsuperscript{58-60} The structure was then solved by direct methods and refined by full matrix least-squares refinement on $F^2$ using the Bruker SHELXTL program library.\textsuperscript{61} Non-hydrogen atoms were refined anisotropically, and hydrogen atoms were located in the difference map and then fixed to their constituent atoms. Two disordered molecules of CH$_2$Cl$_2$ were removed using SQUEEZE.\textsuperscript{63} Protonation of one of the nitrogen atoms in the piperazine ring meant that there was no residual counterion in the complex. In the final stages of refinement, $R = 3.55\%$ and $wR^2 = 6.61\%$ were reported.

A colourless rod-shaped crystal of 22b was mounted on a MiTeGen polymer mount and placed in a cold stream of liquid nitrogen (77 K). Data was collected at 100 K on a Bruker APEX2 diffractometer equipped with CCD area detector using a graphite monochromator MoKα radiation and phi and omega scans. The APEX2 suite was used for data collection and SAINT was used for data reduction; data was scaled using SADABS with both face-indexed absorption correction and a correction using redundant data.\textsuperscript{58-60} The correct space group (P21/c) was chosen by examination of reciprocal space with the program MAX3D;\textsuperscript{64} this indicated a c-glide that otherwise was not detected by APEX2. The structure was then solved by direct methods and refined by full matrix least-squares refinement on $F^2$ using the Bruker SHELXTL program library.\textsuperscript{61} Non-hydrogen atoms were refined anisotropically, and hydrogen atoms were located from the difference map. The hydrogen atoms that were well-behaved were allowed to refine,
others were fixed at calculated positions and were refined as riding on the atom to which they were bonded. Protonation of one of the nitrogen atoms in the piperazine ring meant that there was no residual counterion in the complex. In the final stages of refinement, $R = 3.89\%$ and $wR^2 = 6.83\%$. CCDC No. 795365.

3.7. References


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Chapter 4 – Preparation of Neutral [M(CO)₂(NO)] WAY-Metallocarborane Complexes (M = Re, ⁹⁹ᵐTc)

4.1. Overview

In chapters 2 and 3, the preparation of WAY-based tricarbonyl metallocarborane complexes [M(CO)₃RC₂B₉H₁₀]⁻ (M = Re, ⁹⁹ᵐTc) was reported using a microwave reactor. The in vitro binding data of the rhenacarborane complexes 12, 13 and 22b showed that complex 22b had high and selective binding to the α₁-adrenergic receptors (Figure 4.1). Unfortunately, the scintigraphic images and biodistribution studies indicated minimal brain uptake. The anionic charge on the tricarbonyl metal complex may be one factor that inhibits the ability for the complex to cross the BBB. This chapter outlines an approach to charge compensate the metal core [M(CO)₃] (M = Re, ⁹⁹ᵐTc) where one CO unit is replaced with a [NO]⁺ to render the carborane complex neutral. Herein, the synthesis is described and the work includes the characterization of the rhenium and technetium-99m carborane-WAY complexes in the form of [M(CO)₂(NO)RC₂B₉H₁₀] (M = Re (30, 34, 37), ⁹⁹ᵐTc (38, 40, 41)) (Figure 4.1).

Figure 4.1. Structures of metallocarborane complexes.
4.2. Introduction

The ligand $[\text{NO}]^+$ is isoelectronic with $[\text{CO}]$ but is a stronger $\pi$-acceptor and when complexed with metals, in place of CO, it changes the overall complex charge by $+1$. Transition metal-NO complexes are well known in organometallic chemistry$^1$ and there are various methods of incorporating an $[\text{NO}]^+$ unit into metallocarboranes including the rhenacarborane complexes reported here.$^2$ One option is to generate the $[\text{Re(CO)}_2(\text{NO})]^2+$ core as described by Rattat $et$ $al.$ (Scheme 4.1),$^2$ then complex it with the ligand. This method has been used successfully for chelates such as picolinic acid, however, it has not been successful in making neutral carborane complexes.$^2$ An alternative method reported by Stone and co-workers involved directly converting the $[\text{Re(CO)}_3]$-carborane complex 24 to the $[\text{Re(CO)}_2(\text{NO})]^2+$ analogue 25 in high yields by treatment of the precursor with $[\text{NO}][\text{BF}_4]$ in THF at $-80 \, ^\circ\text{C}$ (Scheme 4.2).$^5$ As opposed to reactions conducted in organic solvents, our group reported an aqueous nitrosation method for transforming $[\text{M(CO)}_3(\text{RC}_2\text{B}_9\text{H}_{10})]$ to $[\text{M(CO)}_2(\text{NO})(\text{RC}_2\text{B}_9\text{H}_{10})]$ ($\text{M} = \text{Re, } ^{99m}\text{Tc}$) using sulfuric acid and sodium nitrite making this methodology suitable for radiopharmaceutical development (Scheme 4.3).$^6$ One benefit of substituting the CO unit with an $[\text{NO}]^+$ is that the resultant nitrosated rhenium complex becomes luminescent. In addition to nuclear imaging, the Re complex could be used in parallel for $in$ $vitro$ screening of new compounds that are isostructural to the $^{99m}\text{Tc}$ complex using fluorescence microscopy. The photophysical, electrochemical and spectroelectrochemical properties and emission trends of $[\text{Re(CO)}_2(\text{NO})(\text{RC}_2\text{B}_9\text{H}_{10})]$ complexes for other classes of derivatives ($R = \text{H}$ (25) and $\eta^5$-$\text{C}_3\text{H}_5$) have been reported...
by Fischer and Kunkely et al.\textsuperscript{7,8}

Scheme 4.1. Preparation of $[\text{Re(CO)}_2(\text{NO})]$ core then coordination to a picolinic acid ligand.\textsuperscript{2}

Scheme 4.2. Preparation of $[\text{Re(CO)}_2(\text{NO})(\text{C}_2\text{B}_{9}\text{H}_{11})]$ \textsuperscript{25} using $[\text{NO}]\text{[BF}_4\text{]}$.\textsuperscript{5}

Scheme 4.3. Preparation of $[\text{M(CO)}_2(\text{NO})(\text{RC}_2\text{B}_{9}\text{H}_{10})]$ from $[\text{M(CO)}_3(\text{RC}_2\text{B}_{9}\text{H}_{10})]$\textsuperscript{6}.

The luminescent behaviour of \textsuperscript{25} (methyltetrahydrofuran, 77 K) showed a $\lambda_{\text{max}}$ in the blue region of the visible spectrum while the $[\text{Re(CO)}_3\text{C}_2\text{B}_{9}\text{H}_{11}]$ carborane analogue \textsuperscript{24} afforded no observable emission.\textsuperscript{7} Excitation of \textsuperscript{25} (methyltetrahydrofuran, 77 K) at 285 nm led to the corresponding emission at 435 nm while luminescence was
undetectable in any solvent at room temperature. A solid microcrystalline sample of 25 showed a strong and broad emission at 298 K ($\lambda_{\text{ex}} < 300$ nm, $\lambda_{\text{em}} \approx 400$ nm). An absorption was similarly noted for the cyclopentadienyl analog $[\text{CpRe(CO)}_2(\text{NO})]^+$. Complex 25 is the first Re(I) metalallocarbonane complex that displays visible light emission. Prior to this report, a weak, stereogenerated luminescence was reported for a nickel carborane sandwich complex by Hawthorne and a blue photoluminescence was observed from a nido-carborane-diphosphine anion and its corresponding gold complex which does not occur with the parent closo-carborane compound. Fischer et al. found that iodination of 24 to yield 26 led to similar emission characteristic as 25 (Scheme 4.4).

![Scheme 4.4. Preparation of 26 via iodination of 24.](image)

Earlier studies on the electronic spectra of 25 displayed bands at 348 nm and a shoulder at 428 nm. Jellis and co-workers examined the photolysis of 25 in solutions and noted the nitrosyl ligand dynamics at low-temperatures. The authors observed a new isonitrosyl isomer following visible light photolysis and a $\eta^2$-NO linkage following UV photolysis in Nujol; a CO-loss product was determined following photolysis in a solution of methyltetrahydrofuran.
Despite the ability for many metallocarboranes to generate substituted complexes, complex 25 demonstrated some considerable resistance to binding other ligands either thermally or chemically. Banks and co-workers overcame this challenge to introduce a bipyridyl ligand bearing a pendant alkyl bromine group 27 (Figure 4.2) that would be used to tether to pharmacophores and/or radioactive labels. Complex 27 displayed absorption in the UV-vis spectrum at $\lambda_{\text{max}} = 663$ nm and was subsequently radiolabel with $^{131}$I to achieve compound 28 (Figure 4.2). Studies on mice showed rapid brain uptake (brain/serum ratio of 295 $\mu$l/g, 2 min after subcutaneous injection) and equilibrium with steady state of 0.1 % of the injected dose being taken up per gram of brain tissue (41.8 $\mu$l/g). These levels are comparable to other compounds that cross the BBB and exert effect within the CNS. The ability of the nitrosated rhenacarborane complex to cross the BBB suggests that the analogous $^{99m}$Tc complex could be used for imaging CNS receptors.

Figure 4.2. Structures of 27 and 28.
4.3. Synthesis and characterization of nitrosated rhenacarboranes 30, 34 and 37

The tricarbonyl rhenacarborane complexes, \([\text{Re}(\text{CO})_3\text{RC}_2\text{B}_9\text{H}_{10}]^-\), were converted to the dicarbonyl nitrosyl rhenacarborane complexes \([\text{Re}(\text{CO})_2(\text{NO})\text{RC}_2\text{B}_9\text{H}_{10}]^-\) in aqueous solvents.\(^6\) The rhenium complex 12 was reacted with sodium nitrite and sulfuric acid in aqueous acetonitrile to yield a single product 29 as a bright yellow solid (Scheme 4.5). The IR spectrum shows a strong \(\nu(N=O)\) stretching absorption at 1777 cm\(^{-1}\) along with two \(\nu(C=O)\) absorptions at 2083 and 2025 cm\(^{-1}\) which are consistent with similar \([\text{Re}(\text{CO})_2(\text{NO})]\)-carborane complexes.\(^5,\,6\) The \(^1\)H NMR spectrum displays aromatic proton resonances at 7.85 (d of d), 7.69 (d) and 6.87(d) ppm (each with an integration value of one) suggesting that an ortho-substituted phenyl ring is no longer present. The mass spectrum had a peak at \(m/z = 657.2347\) [M+H] which is 45 mass units higher than the expected [M+] ion. This data suggested that under these reaction conditions, in addition to nitrosation of the metal centre, a nitro group also added to generate 29 rather than 30 where there was no evidence for the product desired (Scheme 4.5). An estrogen-based (i.e. phenol substituted) dicarbonyl nitrosyl rhenacarborane complex has been prepared previously where a low yield was obtained and attributed to the production of a major side-product which was suspected to be the nitrated aromatic ring derivative.\(^{13}\)
Scheme 4.5. Nitrosation of 12 yielding 29 with a nitrated phenyl ring.

The reaction between sodium nitrite (NaNO₂) and sulfuric acid (H₂SO₄) generates nitrous acid, HNO₂ (Figure 4.3), which is the *in situ* [NO]⁺ source. A solution of nitrous acid disproportionates at room temperature to generate nitric acid, HNO₃, which could nitrate an activated phenyl ring (Scheme 4.6).¹⁴ The aryl group is activated towards electrophilic substitution therefore the isolation of 29 was not unexpected.

\[
\begin{align*}
\text{NO}_2^- & \xrightarrow{\text{H}^+} \text{HNO}_2 & \xrightarrow{\text{H}^+} \text{H}_2\text{O} + \text{NO}^+ \\
3 \text{HNO}_2 & \iff \text{H}_3\text{O}^+ + \text{NO}_3^- + 2 \text{NO}
\end{align*}
\]

**Figure 4.3.** Mechanisms for the generation of [NO]⁺ and HNO₃.
X-ray quality single crystals of \(29\) were obtained by slow evaporation of a solution of methanol/dichloromethane and confirmed that the phenyl group was nitrated in addition to nitrosation of the metal core (Figure 4.4 and Table 4.1). The bond length between the Re metal and the cage carbon atom is 2.32 Å, which is similar to that found in complexes \(12, 22a\) and \(22b\) (2.32-2.33 Å) and to other 2,1,8-rhencarborane complexes reported (2.35 Å).\(^{13}\) The average Re-B bond length is 2.31 Å which is comparable to the average Re-B bond lengths of \(12\) (2.31 Å), \(22a\) (2.32 Å) and \(22b\) (2.31 Å). The Re-L (L = C-O, N-O) units are disordered and each ligand (L) was refined to contain two-thirds CO and one-third NO. The Re-C-O and Re-N-O angles are nearly linear with angles between 173-175°.
Figure 4.4. X-ray crystal structure of 29 (showing 50 % thermal probability ellipsoids; hydrogen atoms omitted for clarity).
Table 4.1. X-ray data for 29.

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<tr>
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^a R = [Σ|F_o| - |F_c|]/[Σ|F_o|] for reflections with I > 2.00σ(I)
^b R_w = {[Σw(F_o^2 - F_c^2)^2]/[Σw(F_o^2)^2]}^{1/2} for all reflections

In attempts to decrease the production of 29 in the reaction, the presence of oxygen in the reaction mixture was minimized. Even with this precaution, only minor amounts of the preferred complex 30 was found to be present after short reaction times. However, as the reaction proceeded the yield of 29 increased, even under dilute and cold (0 °C) reaction conditions. A weaker acid, hydrochloric acid, was used in an attempt to minimize the amount of nitric acid formation but this did not decrease the formation of 29. Alternatively, the addition of a sacrificial competitor, an aryl moiety which could preferentially mop up any residual nitrating agent was used. An activated dimethoxybenzene was selected and three equivalents (relative to the carborane) was
added to 12 and the mixture was treated with sodium nitrite (one equivalent) and either sulfuric acid or hydrochloric acid as the acid sources. The sacrificial scavenger did not prevent nitration on the aromatic ring of complex 12 (Scheme 4.7).

\[
\text{Scheme 4.7. Nitrosation of complex 12 and a sacrificial scavenger, } m\text{-dimethoxybenzene.}
\]

In light of these challenges, alternative nitrosating agents were investigated. Diazald, \(N\)-methyl-\(N\)-nitroso-\(p\)-toluenesulfonamide, is reported to nitrosate \([\text{M(CO)}_3]\) centres to form \([\text{M(CO)}_2(\text{NO})]\) complexes in coordination and organometallic (cyclopentadienyl) complexes. Successful preparation of substituted \([\text{M(CO)}_2(\text{NO})]\) complexes of Nb, Ta, Cr, Mo and W have been reported using Diazald. A model system, 1-(2-methoxyphenyl)-4-(pent-4-ynyl)piperazine (8), was initially used to determine if nitration would occur on the aromatic ring using Diazald (Scheme 4.8). After 30 hours, at ambient temperatures and open to atmosphere, the nitration product was not observed. When the same reaction conditions were applied to the analogous \([\text{Re(CO)}_3]\)-complex 13 (Scheme 4.9), nitration of the aromatic ring was absent but so was nitrosation of the metal centre. Only the starting material was recovered. The reaction mixture was heated to 60 °C but neither nitrosation nor nitration was observed, consequently this route was abandoned.
Scheme 4.8. Treatment of 8 with Diazald as the nitrosating agent.

Scheme 4.9. Reacting 13 with Diazald showed no nitration of the phenyl ring or nitrosation of the metal core.

It was apparent that the reactivity of the aromatic group and the metal centre were comparable. Consequently, an alternative approach was needed to prepare the target compounds. One approach was to convert the nitro functional group to a hydrogen atom to generate complex 30. The retrosynthetic analysis in Scheme 4.10 shows the pathway to achieve the ortho-substituted phenyl derivative 30 from the nitrated derivative 29. The first attempt involved the reduction of the nitro group using sodium sulfide but this was unsuccessful; however, employing hydrated tin(II) chloride in refluxing ethanol produced the amine complex 31 (Scheme 4.11). The product 31 was isolated following extraction and used in the next step without further purification. Thin layer chromatography of the isolated product showed a single spot that was positive by palladium and ninhydrin stains indicating a carborane compound containing an amine.
The $^1$H NMR spectrum of 31 shows a similar pattern in the aromatic region as 29 but the signals are at a lower resonance frequency due to the amine group. The high resolution mass spectrum has a peak at m/z = 627.2609 supporting the formation of the amine derivative.

Scheme 4.10. Retrosynthetic analysis to prepare complex 30 from 29.

Deamination was attempted using two methods: $t$BuONO$^{21}$ and a mixture of CH$_3$COOH, NaNO$_2$, NaHSO$_3$$^{22}$ (Scheme 4.11) but neither were successful. Deamination was ultimately achieved using hypophosphorous acid and sodium nitrite (Scheme 4.11).$^{23}$ The $^1$H NMR of the complex 30 (4 % yield), following purification by silica gel column chromatography, has resonances centered at 6.91 ppm as a multiplet and integration of four which is characteristic of the ortho-substituted product. The $^{13}$C{$^1$H} NMR spectrum of 30 shows two resonances at ~189 ppm (the [Re(CO)$_3$]-complex 12 contained only one resonance at ~198 ppm) attributed to the CO units. The high resolution mass
spectrum supports the formation of \(30\) with the characteristic isotope pattern of \(B_9\)Re and a peak at \(m/z = 612.2488 \ [M+H]\).

Following a similar methodology, rhenium complex \(13\) was reacted with sodium nitrite and sulfuric acid to yield \(32\) as a yellow product (Scheme 4.12). The nitrated aromatic ring of complex \(32\) was treated with tin(II) chloride to give the amine complex \(33\) (92 % yield) which was subsequently transformed to the complex \(34\) using hypophosphorous acid and sodium nitrite (Scheme 4.12). Complex \(32\) was isolated in 96 % yield and shows signals in the \(^1H\) NMR spectrum at 7.85 (d of d), 7.70 (d) and 6.88 (d) ppm which are attributed to a 1,2,4-trisubstituted phenyl ring. The \(^{13}C\{^1H\}\) NMR spectrum of \(32\) shows two resonances at 188.9 and 188.4 ppm attributed to the carbonyl groups in the [Re(CO)\(_2\)(NO)] core (Figure 4.5). Although \(32\) has a 3,1,2 configuration, the resonances of the CO units in the NMR spectra do not show any distinguishing feature associated with the carborane cage configuration compared to 2,1,8 carborane cage orientation. The conversion of \(32\) to \(33\) shifted the aromatic protons resonances in the \(^1H\) NMR spectrum to 6.68 (d), 6.28 (d) and 6.14 (d of d) ppm and has a similar splitting pattern as \(32\) (Figure 4.6a). An absorbance at 3441 cm\(^{-1}\) in the IR spectrum supports the presence of the amine group in \(33\). The \(^{11}B\{^1H\}\) spectrum of \(33\) has six resonance between 0.7 and -15.8 ppm which are similar to those reported for other [Re(CO)\(_2\)(NO)] carborane complexes.\(^5\), \(^6\)

Figure 4.5. $^{13}\text{C}^\text{1{H}}$ (DEPTq) NMR (125 MHz, CDCl$_3$) spectrum of 32.
Figure 4.6. (a) $^1$H NMR (600 MHz, CD$_3$CN) spectrum of 33 and spectrum expansion (dotted region), (b) $^1$H NMR (600 MHz, CD$_3$CN) spectrum of 34 and spectrum expansion (dotted region).

Deamination of 33 using hypophosphorous acid and sodium nitrite gave complex 34 (29 % yield). The $^1$H NMR spectrum of 34 shows resonances at 6.97 and 6.90 ppm with integration values equaling one and three that can be attributed to the ortho-substituted phenyl group (Figure 4.6b). The original [Re(CO)$_3$]$^+$ complex 13 displayed three resonances at 7.04 (1H), 6.99 (2H) and 6.91 (1H) for the aromatic ring. The
\(^{11}\)B\(^{1}\)H\} NMR spectrum of 34 contains six resonances between 0.0 and -16.3 ppm while the original complex 13 had seven resonances between -5.2 and -19.7 ppm.

Compound 22b was nitrosated with sodium nitrite and sulfuric acid which resulted in the formation of 35 in 40 % yield following purification by silica gel column chromatography (Scheme 4.13). Reduction of the aromatic nitro group to the amine 36 was achieved by heating 35 at 70 °C with SnCl\(_2\cdot2\)H\(_2\)O in ethanol for 16 h. Deamination of 36 was accomplished with hypophosphorous acid and sodium nitrite to yield the yellow product 37 (38 % yield). The \(^1\)H NMR spectrum of 35 contains resonances at 7.82 (d of d), 7.70 (d) and 6.94 (d) ppm attributed to the 1,2,4-trisubstituted phenyl ring. The IR spectrum shows peaks at 2088, 2031 and 2001 cm\(^{-1}\) which are consistent with a \([\text{Re(CO)}_2(\text{NO})]^{2+}\) core; (complex 22b showed two strong IR absorbance peaks at 2002 and 1917 cm\(^{-1}\)). The \(^{11}\)B\(^{1}\)H\} NMR spectrum of 35 displays six resonances between -0.95 and -16.7 ppm (Figure 4.7). Following the reduction of the aromatic nitro group to the amine, the \(^1\)H NMR spectrum of complex 36 displayed resonances at 6.69 (d), 6.29 (d) and 6.15 (d of d) ppm which supports the presence of a tri-substituted phenyl group. The \(^{11}\)B\(^{1}\)H\} NMR spectrum shows nine resonances between 0.3 and -19.7 ppm which are consistent with a substituted \([\text{Re(CO)}_2(\text{NO})\text{RC}_2\text{B}_9\text{H}_{10}]\) complex. The mass spectrum displays a distinct isotope distribution for a B\(_9\)Re complex and a peak at m/z = 712.3128 corresponding to [M+H]. Following deamination, the resonances in the aromatic region of the \(^1\)H NMR spectrum of 37 integrate to one and three for the peaks at 6.96 and 6.90 ppm, respectively and the high resolution mass spectrum shows a m/z of 695.3055 (M+H) which is consistent with the formation of 37. The \(^{13}\)C\(^{1}\)H\} NMR spectrum of 37
displays two resonances at 189.1 and 188.5 ppm attributed to the carbonyl groups attached to the metal centre and a resonance at 164.4 ppm from the amide carbonyl while originally the [Re(CO)₃] complex 22b had resonances at 199.7 and 168.5 ppm for the carbonyl of the rhenium core and the carbonyl of the amide, respectively.

Scheme 4.13. Preparation of complex 37.
The neutral rhenium complexes 30, 34 and 37 were prepared successfully via functional group interconversion. Although additional steps were needed to prepare these nitrosated complexes (30, 34 and 37) from the [Re(CO)₃]-carborane complexes 12, 13 and 22b, the nitro (29, 32 and 35) and amino (33 and 36) derivatives are supplementary rhenacarborane complexes which can also be examined for their binding affinity to CNS receptors. It is unlikely, however, that the nitrated derivatives will be potent ligands as researchers have found that alteration to substituent groups and/or the positions of the substituents in the aromatic ring result in minor changes to the binding affinity to a specific receptor.²⁴-²⁷

4.4. Synthesis of nitrosated ⁹⁹ᵐTc-carborane complexes 38, 40 and 41

With the neutral rhenium complexes 30, 34 and 37 prepared, attempts were made to prepare the corresponding ⁹⁹ᵐTc-carborane complexes. The concentration of the metal
in technetium reactions (10^{-6} to 10^{-9} M) are orders of magnitude smaller than the corresponding reactions involving rhenium (1 to 10^{-3} M). As a result, the \([^{99m}Tc(CO)_3RC_2B_9H_{10}]^-\) complexes (14a, 15 and 23), prepared using the methods described in chapters 2 and 3, were reacted with an excess sulfuric acid and sodium nitrite. It was assumed that the resulting \([^{99m}Tc(CO)_2(NO)RC_2B_9H_{10}]^-\) complex would have both the metal core nitrosated and the aromatic ring nitrated as the main product particularly when the excess of nitrosating agent is considered.

The \([^{99m}Tc(CO)_3RC_2B_9H_{10}]^-\) complex 14a (from chapter 2) was treated with sulfuric acid (0.2 M) and sodium nitrite and stirred for 5 min at room temperature. The reaction mixture was quenched with dilute acid and loaded onto a solid phase extraction cartridge. The cartridge was flushed with water to remove excess [NO]+ and the product was eluted from the cartridge using acetonitrile. The primary product was identified as 38 where the retention time of the radio-HPLC correlated with the UV-HPLC of 29, the nitrosated/nitrated complex (Scheme 4.14). The reaction time was reduced (30 s) in an attempt to minimize the amount of nitration on the aromatic ring and found that it was possible to form complex 39 in 29 % yield where approximately 7 % was the nitrated product 38 while the remainder was the unreacted complex 14a. The products were confirmed by comparing the retention times in the radio-HPLC chromatogram with the retention times of the rhenium complexes 29 and 30 in the UV-HPLC chromatograms.
Scheme 4.14. Combining 14a with sulfuric acid and sodium nitrite to generate the \([^{99m}\text{Tc}(\text{CO})_2(\text{NO})\text{RC}_2\text{B}_9\text{H}_{10}]\) complexes.

The \([^{99m}\text{Tc}(\text{CO})_3]-\text{complex 15 (chapter 2)}\) with the longer spacer was treated with sulfuric acid and sodium nitrite and stirred for 5 min yielding only one product: the nitrosyl \(^{99m}\text{Tc}\)-product \(38\). There was no indication of nitration of the phenyl ring even when the reaction time was extended to 15 min (Scheme 4.15a). The retention time of \(38\) in the radio-HPLC chromatogram matched the retention time of the rhenium complex \(34\) in the UV-HPLC chromatogram (Figure 4.8). Complex 23 was nitrosated with sulfuric acid and sodium nitrite and the main product was determined to be \(41\) (Scheme 4.15b). The retention time of \(41\) (\(t_R = 13.6\) min) in the radio-HPLC correlated to the retention time of complex \(37\) (\(t_R = 13.0\) min) in the UV-HPLC indicating no nitration of the phenyl ring has occurred in the reaction. In summary, the \(^{99m}\text{Tc}\) complexes 15 and 23, unlike
14a, when reacted with excess nitrosating agent did not lead to nitration of the activated phenyl ring. Furthermore, there are clear differences with the preparation of the metal analogues. The main difference associated with the nitrosation reaction is concentration of the metal in the reaction. Technetium reactions are orders of magnitude more dilute than the corresponding reactions involving rhenium (vide supra). Properties associated with the metals (i.e. reduction-oxidation potential) can also contribute to the reactivity and the products formed (vide infra).

Scheme 4.15. Preparation of $^{99m}$Tc-carborane complexes 40 and 41.
Figure 4.8. HPLC chromatograms (γ and UV) of the isolated $^{99m}\text{Tc}$ carborane complex 40 co-injected with Re complex 34; residual nido-6 was present in the reaction.

The neutral $[^{99m}\text{Tc}(\text{CO})_2(\text{NO})]$-carborane complexes 38, 40 and 41 exist in one of two carborane cage configurations (3,1,2 or 2,1,8), however, there is no correlation between the cage configuration and nitration of the phenyl ring. Both $^{99m}\text{Tc}$-carborane complexes 38 and 41 are in a 2,1,8 configuration while complex 40 is in the 3,1,2. Complex 38 was the only derivative of the series that showed nitration of the aromatic ring suggesting that cage orientation is not the factor driving nitration. The type of substitutents attached to the carborane cage has been shown to affect complexation reaction conditions, isomerization of the carborane cage unit and stability of the resulting complex.6, 28-30 However, all three derivatives have the same methoxyphenylpiperazine unit and the phenyl ring is activated by the same functional groups present. The complexes do differ by the linkage length and type between the cage and this substituent. Consequently, the additional activation of the phenyl ring may be associated with the
proximity of the carborane cage which leads to nitration of the phenyl ring of only complex 38. An additional explanation is that the close proximity of the substituent in 14a hinders the reaction at the metal centre leading to preferential reaction at the aromatic ring.

4.5. Evaluation of dicarbonyl nitrosyl metallocarborane complexes 38, 40 and 41

Prior to measuring the lipophilicity or conducting any biological evaluations on the $^{99m}$Tc-carborane analogues 38, 40 and 41, the stabilities of these complexes were evaluated. Literature reports indicate that some substituted $[^{99m}$Tc(CO)$_2$(NO)]$^{2+}$ carborane complexes are unstable in aqueous media. From these studies, it was concluded that both steric bulk of the attached substituent and electronic properties of the substituents influence stability. The authors attempted to further investigate whether hydrolysis was occurring at the metal core or whether the decomposition was due to radiolysis, however, no conclusion was drawn.

The $[^{99m}$Tc(CO)$_2$(NO)]-complexes (38, 40 and 41) showed no degradation in acetonitrile over 6 hours as determined by radio-HPLC. When acetonitrile was exchanged for ethanol or DMSO, degradation of all three complexes was observed (< 30 min). Ethanol and DMSO were selected because small amounts of these solvents in aqueous media can be used in conjunction with biological testing. The nucleophilic nature of ethanol and DMSO are likely responsible for the degradation of the $[^{99m}$Tc(CO)$_2$(NO)] core. The identities of the decomposition products were not determined because of the tracer level concentration which is at the detection limit of
conventional mass spectrometry. To identify the decomposition products, an attempt was made to prepare non-radioactive standards by testing the stability of rhenium complex \( ^{\text{34}} \text{Re} \) in ethanol. Monitoring \( ^{\text{34}} \text{Re} \) in ethanol by UV and LC-MS, after 24 h, no significant degradation was observed. The total ion mass chromatogram did however display some minor decomposition products in the spectrum, nonetheless, the amounts were too low to compare with the technetium-99m products. Due to the lack of stability of \(^{99m}\text{Te} \) complexes (38, 40 and 41), these complexes are not suitable for testing in vivo.

During the preparation of the neutral \([\text{M(CO)}_2(\text{NO})]^-\)-complexes (\( \text{M} = \text{Re} (30, 34, 37), ^{99m}\text{Tc} (38, 40, 41) \)), it was found that different products resulted from the reaction between the \([\text{M(CO)}_3]^+\)-complexes (\( \text{M} = \text{Re}, ^{99m}\text{Tc} \)) and the nitrosating agents (i.e. sulfuric acid and sodium nitrite). Moreover, the stability of the neutral \([\text{M(CO)}_2(\text{NO})]^-\)-complexes (\( \text{M} = \text{Re}, ^{99m}\text{Tc} \)) in nucleophilic solvents varied depending on the nature of the metal (Re vs. \(^{99m}\text{Tc} \)). One contributing factor that can account for these differences between rhenium and technetium is the different reduction-oxidation potential of each of the two metals (\( E_{\text{M(II)/M(I)}} = -1.54 \) (Re) and -1.29 (Tc)).\(^{31} \) It is more difficult to reduce rhenium than the analogous technetium complex ranging from 190 to 320 mV.\(^{31, 32} \) However, a direct correlation between reactivity and stability and the metal’s redox potential has not been repeated. Our group has observed differences associated with rhenium and technetium in regards to metallicarbon complex stability\(^6 \) and cage isomerization\(^{30} \) while other researchers have reported differences for lipophilicity values and receptor binding data between the non-radioactive rhenium derivatives and their technetium counterparts for the same “3+1” chelate complexes.\(^{33, 34} \)
4.6. Fluorescence measurements of complex 29

SPECT imaging, using a gamma-emitting radiopharmaceutical, can show the distribution of an agent within the body, however, nuclear imaging (PET/SPECT) cannot visualize agents at a cellular or subcellular level. On the other hand, a widely employed technique for visualizing biological processes is fluorescence microscopy which examines the distribution of fluorescent probes \textit{in vitro} and is able to accurately depict processes at a subcellular level at high resolution (resolution range = 15 to 20 nm). Pairing these two powerful techniques would provide information about the overall distribution of an agent and specific data regarding its function and distribution at the cellular level giving greater insight to the uptake and mechanistic properties of the agent.

Functionalized carborane complexes of the type \([M(CO)\textsubscript{2}(NO)]^{2+}\), using the congener metal pair rhenium and technetium-99m, have the potential to correlate optical (M = Re) and scintigraphic (M = \(^{99m}\text{Tc}\)) imaging linking \textit{in vivo} data to \textit{in vitro} data.

The electronic spectra and luminescence behaviour of \([\text{Re(CO)}\textsubscript{2}(NO)\textsubscript{2}B\textsubscript{9}H\textsubscript{10}]\) complex has been reported in the literature. The UV/Vis spectrum of 29 was measured and showed a maximum absorbance at 369 nm (\(\lambda_{\text{max}}\)) with a molar extinction coefficient of 1406 M\(^{-1}\)cm\(^{-1}\). Fluorescence excitation and emission peaks for complex 29 were at 369 nm and 429 nm, respectively. The \(\lambda_{\text{max}}\) of 29 is similar to a dibenzyl substituted 2,1,8-[Re(CO)\textsubscript{2}(NO)] complex 42 (348 nm) where an emission was observed at 411 nm when this complex was excited at 348 nm. The \([\text{CpRe(CO)}\textsubscript{2}(NO)]^{+}\) 43 (Figure 4.9) showed two long-wavelength absorptions at \(\lambda_{\text{max}} = 380\) and 330 nm. In the solid state, complex 43 displayed a luminescence at \(\lambda_{\text{max}} = 570\) nm, however, in solution
the complex was not emissive but photoreactive.

The cyclopentadienyl complex 43 (Figure 4.9) was reported to be photoreactive which is not surprising since various nitrosyl metal complexes can undergo a photorelease of neutral [NO] generating the product where the metal oxidized by one unit. Complex 43 was apparently can not be oxidized to the stable Re(II) derivative. The authors found that in the presence of strong nucleophiles, such as triphenylphosphine (PPh₃), an associative substitution takes place yielding the [CpRe(CO)(PPh₃)(NO)]⁺ complex. The [(η⁵-C₅Me₅)⁹⁹Tc(CO)₂(NO)]⁺ complex was prepared from treating [(η⁵-C₅Me₅)⁹⁹Tc(CO)₃] with [NO][PF₆] by Ensing and co-workers, however further exploration of this complex was not reported.

![Figure 4.9. Structures of compound 29, 42 and 43.](image)

The quantum yield of 29 was low and determined to be Φᵢ = 0.015 and the average fluorescence lifetime was measured to be τᵢ = 1.57 ns. These data indicate that the organometallic complex 29 has inherently weak fluorescence which is consistent with similar carborane complexes such as 42 and 44 (Figure 4.10). The observed lifetime is short with respect to other luminescent Re complexes suggesting that the fluorescence is due to cage-centered electronic transitions and not metal-to-ligand charge transfer (τᵢ
The determination of the quantum yield and fluorescence lifetime for 43 was attempted by Armstrong et al., however, the extremely weak fluorescence led to unreliable data. Instead, the authors measured the unsubstituted 2,1,8-[Re(CO)2(NO)] complex (44) which showed a low quantum yield (0.002) and an average fluorescence lifetime of 8.1 ns. The versatility of carboranes provides a means to alter the electronic nature of the appended group by tuning the quantum efficiency or other key fluorescence properties in this class of molecules.

Figure 4.10. Structures of compound 44.

The quantum yield of 29 is comparable to the single amino acid chelate (SAAC) complex 45 (0.003-0.015) that has been used to image cells in vitro using fluorescence microscopy (Figure 4.11). The complex 45 was integrated within a peptide sequence (fMLF) which targets the formyl peptide receptor of neutrophils and the fluorescence microscopy image of human leukocytes incubated with this complex showed cellular uptake (Figure 4.11). Consequently, despite the poor stability of the 99mTc complexes, the [Re(CO)2(NO)]-carborane complexes are viable as in vitro imaging probes worthy of further exploration.
Figure 4.11. SAAC complex 45 (left); fluorescence microscopy image of human leukocytes incubated with SAAC-fMLF complex (right).

4.7. Summary

Neutral metallocarborane complexes in the form \([M(CO)_{2}(NO)RC_{2}B_{9}H_{10}]\) (M = Re, \(^{99m}\)Tc) were prepared. The nitrosation conditions to convert \([\text{Re}(CO)_{3}RC_{2}B_{9}H_{10}]^{-}\) to \([\text{Re}(CO)_{2}(NO)RC_{2}B_{9}H_{10}]^{-}\) for complexes 12, 13 and 22b led to nitration of the activated phenyl group in addition to nitrosation of the metal core. Functional group interconversion was employed to generate the amino derivatives which following deamination led to the desired complexes (30, 34 and 37). Nitrosation of the \([^{99m}\text{Tc}(CO)_{3}RC_{2}B_{9}H_{10}]^{-}\) complexes 14a, 15 and 23 with sulfuric acid and sodium nitrite led to the production of 38, 40 and 41 as the primary products of the reactions. Of these, complex 38 was the only compound in which nitration occurred at the phenyl ring. The \(^{99m}\)Tc complexes 38, 40 and 41 were stable in acetonitrile but decomposed in ethanol and
DMSO. Complex 29 displays an emission at 429 nm when excited at 369 nm. The quantum yield was low ($\Phi_f = 0.015$) and the average fluorescence lifetime was measured as $\tau_f = 1.57$ ns.

4.8. Experimental

4.8.1. Reagents and general procedures

Unless otherwise stated, all chemicals and reagents were purchased and used as received from Sigma-Aldrich without further purification. Compounds 12, 13 and 22b were prepared according to literature procedures. Deuterated solvents for NMR samples were purchased from Cambridge Isotope Laboratories.

Reactions were monitored using Alugram Sil G/UV$_{254}$ thin-layer chromatography plates. Carborane-containing species were visualized with 0.2 % PdCl$_2$ in hydrochloric acid (3.0 M) which upon heating gave dark brown spots. Amines were visualized with a ninhydrin spray which upon heating gave a coloured (purple-pink) spot. Silica Gel preparative TLC plates (AnalTech) were used and column chromatography was preformed using Ultra Pure Silica Gel 60 (Silicycle). Automated normal and reverse-phase (C18) silica gel chromatography were performed on a Biotage SP1 purification system operated at ambient temperature using the specified solvent gradients and column sizes.

4.8.2. Instrumentation

Nuclear magnetic resonance spectra ($^1$H, $^{11}$B/$^1$H) were recorded on either a
Bruker 500 or 600 MHz spectrometer at ambient temperature. The $^{13}$C{$^{1}$H} NMR data were acquired using a uniform driven equilibrium Fourier transform (uDEFT) approach according to Piotto et al. (except using a 60 kHz composite smoothed Chirp) on a Bruker 600 MHz spectrometer at ambient temperature. The chemical resonances (δ) are reported in parts per million (ppm), with the $^{1}$H NMR shifts referenced to the residual proton signal found in the deuterated solvent and the $^{13}$C shifts referenced to the carbon signal of solvent. The chemical shifts (δ) for $^{11}$B{$^{1}$H} NMR spectra were reported relative to an external reference of BF$_3$·Et$_2$O. Infrared spectra were acquired on a Nicolet 510 Fourier Transform IR spectrometer at ambient temperature. Samples were run neat on a KBr plate. Reactions involving microwave heating were performed on a Biotage Initiator 60 or Initiator 8 microwave reactor using crimp-sealed Emrys vials. Mass spectrometry analyses were obtained from the McMaster Regional Centre for Mass Spectrometry utilizing a Micromass Quattro Ultima (LC-ESI/APCI triple quadrupole) mass spectrometer for electrospray ionization (ES) mass spectrometry; a Micromass GC-EI/CI time of flight mass spectrometer instrument for chemical ionization (CI) and electron ionization (EI) spectra; and a Micromass Global Ultima (MALDI/CapLC-ESI quadrupole time of flight) mass spectrometer for high resolution mass spectra.

High performance liquid chromatography was performed on the Agilent 1100 system (Quad Pump module G1311A, Degasser G1322A, ALS G1313A, Colcom G1316A) interfaced to a BioScan radioactivity detector (APIFC2-00507). An Agilent Zorbax C18 (5μ, 250 x 4.6 mm) column was used with a flow rate of 1.0 mL/min. The following solvent gradients were employed (solvent A = acetonitrile with 0.1 % formic
acid, solvent B = 20 mM ammonium acetate): Method A: 0-15 min, 40-100 % A, 15-17 min 100 % A, 17-20 min, 100-40 % A. Method B: 0-15 min, 40-100 % A, 15-22 min 100 % A, 22-25 min, 100-40 % A.

4.8.3. Optical measurements

The absorption characteristics of 29 (3.0 mM in acetonitrile) were examined using a wavelength range of 200-800 nm on a Cary 300 UV/Vis instrument. The fluorescence emission spectra were measured at 21°C using a Jobin Yvon-SPEX Fluorolog-3 model 212 T-format spectrofluorometer (ISA, Edison, NJ) with excitation at 369 nm. The emission and excitation wavelength bandwidths were 3 nm, and integration times were 1.0 s. Spectra were corrected for solvent contributions. The Raman intensities of the sample and the blank were matched to account for inner filter effects due to the large amount of analyte required to obtain usable emission spectra at room temperature. The quantum yield was determined using quinine bisulfate in 0.1 M H$_2$SO$_4$ as the standard reference. All solutions had optical densities of less than 0.5 to minimize variation due to the inner filter effect. Fluorescence spectra were integrated using the Fluorolog-3 software and corrected for solvent contributions.

The fluorescence lifetime was assessed using time-correlated single-photon counting (TCSPC) on an IBH 5000U instrument (HORIBA Jobin Yvon, Edison, NJ). A pulsed 370 nm light-emitting diode operating at 1.0 MHz with 1.5 ns pulse duration was used for excitation. The fluorescence data were collected without excitation polarizers in place and the emission polarizer set the magic angle to 54.7°; the excitation and emission
monochromators were set to 370 and 429 nm, respectively, with a long pass filter (400 nm) in place; and the excitation and emission bandpasses were 12 and 32 nm, respectively. Decay data were collected into 1024 channels and corrected with an instrument response function. The decay data was fit to a biexponential decay model with $\chi^2 = 1.25$. The lifetime components and their percent contribution were $\tau_1 = 1.18 \pm 0.01$ ns (81%) and $\tau_2 = 3.18 \pm 0.05$ ns (19%), with an average lifetime of $<\tau> = 1.57$ ns. Reporting the decay data fit to a three-exponent decay model did not improve $\chi^2$ and increased the standard deviations of the fit. The optical density was less than 0.5 in order to avoid inner-filter effects.

4.8.4. Synthetic procedures

[2,2-(CO)$_2$-2-(NO)-8-C$_{12}$H$_{16}$N$_3$O$_3$-2,18-closo-ReC$_2$B$_9$H$_{10}$] (29). To a solution of 12 (0.38 g, 0.60 mmol) in acetonitrile (15 mL), H$_2$SO$_4$(aq) (2 M, 1.27 mL, 2.54 mmol) and NaNO$_2$(aq) (1.65 M, 0.048 g, 0.70 mmol) was added and stirred at room temperature overnight. The solution was transferred to a separatory funnel containing NaOH(aq) (0.1 M, 30 mL). The mixture was extracted with diethyl ether (50 mL) and the organic fraction was washed with water (40 mL). The organic layer was dried over sodium sulfate, filtered and concentrated. The crude reaction mixture was purified using a reverse phase (C18) Biotage automated purification system and a gradient of 25-100 % acetonitrile:water. Yield 0.070 g, 18 %. R$_f$ (dichloromethane) = 0.83. $^1$H (500 MHz, CDCl$_3$) $\delta$: 7.85 (dd, 1H, $J = 8.8$, 2.5 Hz, Ar-H), 7.69 (d, 1H, $J = 2.4$ Hz, Ar-H), 6.87 (d, 1H, $J = 8.9$ Hz, Ar-H), 3.93 (s, 3H, OCH$_3$), 3.22 (s, 4H, NCH$_2$), 2.81 (d, 2H, $J = 3.7$ Hz,
CH$_2$), 2.72 (m, 4H, NCH$_2$), 2.57 (s, 1H, CH); $^{13}$C{$_1^1$H} (125 MHz, CDCl$_3$) $\delta$: 188.9, 188.4, 151.4, 147.6, 142.2, 117.9, 116.9, 106.7, 66.1, 65.8, 56.1, 54.2, 50.1, 42.0;

$^{11}$B{$_1^1$H} (160 MHz, CDCl$_3$) $\delta$: 0.7, -4.2, -5.3, -7.5, -10.8, -11.9, -16.2; IR (KBr, cm$^{-1}$): $\nu$ 2833, 2567, 2083, 2025, 1777; HRMS (ES$^+$) for C$_{16}$H$_{27}$B$_9$N$_4$O$_6$Re: calculated 657.2326, observed 657.2347 [M+H]. HPLC (method B): $t_R$ = 19.3 min.

[2,2-(CO)$_2$-2-(NO)-8-C$_{12}$H$_{17}$N$_2$O-2,18-closo-ReC$_2$B$_9$H$_{10}$] (30). Complex 29 (0.049 g, 0.075 mmol) and SnCl$_2$·2H$_2$O (0.12 g, 0.53 mmol) in ethanol (1.5 mL) were heated at 70 °C for 17 h with stirring. Following cooling the reaction to room temperature, NaOH$_{(aq)}$ (1 M, 10 mL) was added to render the mixture basic (pH ~10) and the mixture was transferred to a separatory funnel. The solution was extracted with acetonitrile (3 x 20 mL) and the combined organic fractions were washed with brine (20 mL). The organic fraction was dried over sodium sulfate, filtered and concentrated under vacuum. The isolated compound and used without further purification. The compound 31 was positive by palladium and ninhydrin strains indicating a carborane species containing an amine group. $R_f$ (10 % methanol/dichloromethane) = 0.01. HRMS (ES$^+$) for C$_{16}$H$_{27}$B$_9$N$_4$O$_6$Re: calculated 627.2584, observed 627.2609 [M+H].

Hypophosphorous acid (16 $\mu$L, 50 wt. % in water) was added to the residue followed by water (1 mL) and the mixture sonicated to create a suspension. The reaction mixture was cooled to 0 °C and an aqueous solution of NaNO$_2$ (1.7 mg, 0.17 M, 0.025 mmol) was added dropwise. The reaction was stirred on ice for 1 h and then was allowed to stand overnight at room temperature. The reaction was adjusted to pH = 10 using NaOH$_{(aq)}$ (1 M, 5 mL) and the mixture transferred into a separatory funnel and extracted
with acetonitrile (3 x 5 mL) and the combined organic fractions were washed with brine (20 mL). The organic fraction was dried over sodium sulfate, filtered and concentrated to dryness. The product 30 was isolated as a yellow solid following silica gel column chromatography using dichloromethane as the eluent. Yield 2 mg, 4 %. Rf (dichloromethane) = 0.71. \(^{1}H\) (600 MHz, CD\(_{3}\)CN) \(\delta\): 6.91 (m, 4H, Ar-H), 3.79 (s, 3H, OCH\(_{3}\)), 2.97 (s, 4H, NCH\(_{2}\)), 2.81 (m, 2H, CH\(_{2}\)), 2.73 (s, 1H, CH), 2.63 (s, 4H, NCH\(_{2}\)); \(^{13}C\{^{1}H\} (150 MHz, CD\(_{3}\)CN) \(\delta\): 189.9, 189.4, 153.5, 142.7, 123.6, 122.0, 119.2, 112.8, 66.5, 55.9, 55.4, 51.4, 43.6; \(^{11}B\{^{1}H\} (192 MHz, CD\(_{3}\)CN) \(\delta\): 0.7, -4.1, -6.9, -10.1, -12.3, -15.7; IR (KBr, cm\(^{-1}\)): v 2917, 2820, 2572, 2082, 2023, 1772; HRMS (ES\(^{+}\) for C\(_{16}\)H\(_{28}\)B\(_{9}\)N\(_{3}\)O\(_{4}\)Re: calculated 612.2475, observed 612.2488 [M+H].

HPLC (method B): \(t_R = 19.7\) min.

\[3,3-(CO)\_2-3-(NO)-1-C_{14}H_{20}N_{3}O_{3}-3,1,2-closo-ReC_{2}B_{9}H_{10}\] (32). To a solution of 13 (0.12 g, 0.18 mmol) in 3:2 v/v acetonitrile:water (10 mL), H\(_{2}\)SO\(_{4}\)\(_{aq}\) (2 M, 0.32 mL, 0.64 mmol) and NaNO\(_{2}\) (0.035 g, 0.51 mmol) was added and the reaction was stirred at room temperature. After 15 h, an additional amount of H\(_{2}\)SO\(_{4}\)\(_{aq}\) (2M, 2 eq.) and NaNO\(_{2}\) (2 eq.) was added and the mixture was stirred for another 6 h. The reaction mixture was transferred to a separatory funnel containing NaOH\(_{aq}\) (0.1 M, 15 mL) and the mixture was extracted with dichloromethane (3 x 25 mL). The combined organic fractions were dried over sodium sulfate, filtered and concentrated under reduced pressure. The product 32 was isolated as a yellow solid following silica gel column chromatography using 5 % methanol/dichloromethane as the eluent. Yield 0.12 g, 96 %. Rf (5 % methanol:dichloromethane) = 0.89. \(^{1}H\) (500 MHz, CDCl\(_{3}\)) \(\delta\): 7.85 (dd, 1H, \(J = 8.8, 2.5\)
Hz, Ar-H), 7.70 (d, 1H, $J = 2.5$ Hz, Ar-H), 6.88 (d, 1H, $J = 8.8$ Hz, Ar-H), 3.94 (s, 3H, OCH$_3$), 3.24 (s, 4H, NCH$_2$), 2.59 (m, 5H, NCH$_2$, CH), 2.31 (t, 2H, $J = 7.3$ Hz, CH$_2$), 2.02 (m, 2H, CH$_2$), 1.61 (m, 2H, CH$_2$); $^{13}$C{$^{1}$H} (125 MHz, CDCl$_3$) $\delta$: 188.9, 188.4, 151.4, 147.5, 142.2, 118.0, 116.8, 106.7, 57.8, 56.1, 53.1, 50.1, 42.1, 38.1, 29.9, 27.2; $^{11}$B{$^{1}$H} (160 MHz, CDCl$_3$) $\delta$: 0.8, -4.7, -7.5, -10.8, -11.8, -16.3; IR (KBr, cm$^{-1}$): ν 2938, 2819, 2574, 2082, 2024, 1773; HRMS (ES+) for C$_{18}$H$_{31}$B$_9$N$_4$O$_6$Re: calculated 685.2639, observed 685.2627 [M+H].  HPLC (method A): $t_R = 16.6$ min.

[3,3-(CO)$_2$-3-(NO)-1-C$_{14}$H$_{22}$N$_3$O-3,1,2-closo-ReC$_2$B$_9$H$_{10}$] (33). Compound 32 (0.11 g, 0.16 mmol) and SnCl$_2$·2H$_2$O (0.35 g, 1.6 mmol) were suspended in ethanol (2 mL). The reaction was heated at 70 °C for 22 h. After the reaction cooled to room temperature, sodium hydroxide (1 M, 10 mL) was added and the mixture was transferred into a separatory funnel and extracted with dichloromethane (2 x 25 mL) and acetonitrile (3 x 50 mL). The combined organic fractions were washed with brine (25 mL) and then dried over sodium sulfate, filtered and concentrated to dryness. Compound 33 was isolated following purification using silica gel column chromatography using 5 % methanol/dichloromethane as the eluent. Yield = 97 mg, 92 %.  $R_f$ (5 % methanol/dichloromethane) = 0.66.  $^1$H (600 MHz, CD$_3$CN) $\delta$: 6.68 (d, 1H, $J = 8.3$ Hz, Ar-H), 6.28 (d, 1H, $J = 2.4$ Hz, Ar-H), 6.14 (dd, 1H, $J = 8.3$, 2.5 Hz, Ar-H), 3.72 (s, 3H, OCH$_3$), 2.83 (s, 4H, NCH$_2$), 2.72 (s, 1H, CH), 2.43 (s, 4H, NCH$_2$), 2.24 (t, 2H, $J = 6.9$ Hz, CH$_2$), 2.02 (m, 2H, CH$_2$), 1.54 (m, 2H, CH$_2$); $^{13}$C{$^{1}$H} (150 MHz, CD$_3$CN) $\delta$: 189.9, 189.3, 154.6, 145.2, 133.8, 120.3, 107.1, 100.9, 58.2, 55.7, 54.4, 52.1, 43.7, 38.7, 27.9;
$^{11}$B {$^1$H} (160 MHz, CD$_3$CN) δ: 0.7, -4.1, -7.0, -10.0, -12.2, -15.8; IR (KBr, cm$^{-1}$): ν 3441, 2918, 2849, 2571, 2082, 2026, 1777; HRMS (ES$^+$) for C$_{18}$H$_{33}$B$_9$N$_4$O$_4$Re:
calculated 655.2897, observed 655.2924 [M+H].

[3,3-(CO)$_2$-3-(NO)-1-C$_{14}$H$_{21}$N$_2$O-3,1,2-closo-ReC$_2$B$_9$H$_{10}$] (34).

Hypophosphorous acid (38 µL, 50 wt. % in water) was added to complex 33 (0.032 g, 0.049 mmol) followed by the addition of water (1 mL). The mixture was sonicated to create a suspension. The reaction was cooled to 0 °C and an aqueous solution of NaNO$_2$
(3.4 mg, 0.43 M, 0.049 mmol) was added dropwise. The reaction was stirred on ice for 1 h and then was allowed to stand overnight at room temperature. NaOH(aq) (1 M, 10 mL) was added to make the pH = 10. The mixture was transferred into a separatory funnel and extracted with dichloromethane (3 x 20 mL) and acetonitrile (3 x 20 mL). The combined organic fractions were dried over sodium sulfate, filtered and concentrated to dryness. The product 34 was purified using a preparative TLC plate purification and an eluent of 5 % methanol/dichloromethane; the yellow product was isolated following extraction of the silica with a 10 % methanol/dichloromethane solution. Yield 9 mg, 29 %.

R$_f$ (5 % methanol/dichloromethane) = 0.75. $^1$H (600 MHz, CD$_3$CN) δ: 6.97 (m, 1H, Ar-H), 6.90 (m, 3H, Ar-H), 3.80 (s, 3H, OCH$_3$), 3.04 (s, 4H, NCH$_2$), 2.73 (s, 1H, CH), 2.63 (s, 4H, NCH$_2$), 2.03 (m, 4H, CH$_2$, CH$_2$), 1.62 (m, 2H, CH$_2$); $^{13}$C {$^1$H} (150 MHz, CD$_3$CN) δ: 189.8, 189.3, 153.4, 142 (observed using HSQC), 123.9, 122.0, 119.2, 112.8, 57.8, 56.0, 54.0, 50.4, 43.7, 38.3, 27.0; $^{11}$B {$^1$H} (160 MHz, CD$_3$CN) δ: 0.0, -4.7, -7.6, -10.5, -12.7, -16.3; IR (KBr, cm$^{-1}$): ν 2919, 2849, 2558, 2085, 2026, 1999, 1780, 1732; HRMS (Cl$^+$) for C$_{18}$H$_{31}$B$_9$N$_3$O$_4$Re: calculated 637.2769, observed 637.2839 [M+].
HPLC (method A): $t_R = 15.9$ min.

$[2,2-(CO)2-2-(NO)-8-C_{16}H_{23}N_4O_4-2,18-closo-ReC_2B_9H_{10}]$ (35). To a solution of $22b$ (0.012 g, 0.017 mmol) in acetonitrile (2 mL), H$_2$SO$_4$(aq) (2 M, 42 $\mu$L, 0.084 mmol), NaNO$_2$ (0.0042 g, 0.061 mmol) and water (0.20 mL) were added. The reaction was stirred vigorously for 27 h at room temperature at which point NaOH(aq) (1 M, 5 mL) was added and the mixture transferred to a separatory funnel and extracted with acetonitrile (2 x 5 mL). The organic fractions were combined and washed with brine (10 mL) and dried over sodium sulfate, filtered and concentrated to dryness. The product 35, as a yellow solid, was isolated following silica gel column chromatography using 5 % methanol/dichloromethane as the eluent. Yield: 5 mg, 40 %. $R_f$ (5 % methanol/dichloromethane) = 0.68. $^1$H (600 MHz, CD$_3$CN) $\delta$: 7.82 (dd, 1H, $J = 8.8, 2.5$ Hz, Ar-H), 7.70 (d, 1H, $J = 2.5$ Hz, Ar-H), 6.94 (d, 1H, $J = 8.9$ Hz, Ar-H), 6.55 (s, 1H, NH), 3.91 (s, 3H, OCH$_3$), 3.22 (s, 4H, NCH$_2$), 3.12 (m, 2H, CH$_2$), 2.73 (s, 1H, CH), 2.52 (s, 4H, NCH$_2$), 2.34 (t, 2H, $J = 6.8$ Hz, CH$_2$), 1.44 (m, 4H, CH$_2$, CH$_2$); $^{13}$C{$^1$H} (150 MHz, CD$_3$CN) $\delta$: 189.1, 188.5, 164.4, 152.2, 148.7, 118.5, 117.7, 107.6, 58.5, 56.6, 53.9, 50.6, 42.7, 41.0, 27.7, 24.5; $^{11}$B{$^1$H} (192 MHz, CD$_3$CN) $\delta$: -0.95, -6.2, -8.2, -11.9, -13.5, -16.7; IR (KBr, cm$^{-1}$): v 2917, 2576, 2088, 2031, 2001, 1906, 1783; HRMS (ES+) for C$_{20}$H$_{34}$B$_9$N$_5$O$_7$Re: calculated 742.2853, observed 742.2884 [M+H]. HPLC (method A): $t_R = 12.0$ min.

$[2,2-(CO)2-2-(NO)-8-C_{16}H_{25}N_4O_2-2,18-closo-ReC_2B_9H_{10}]$ (36). Complex 35 (0.005 g, 0.007 mmol) and SnCl$_2$·2H$_2$O (0.019 g, 0.084 mmol) in ethanol (95 %, 0.5 mL) were heated at 70 °C for 16 h. Upon cooling, NaOH(aq) (1 M, 5 mL) was added and the
reaction was transferred to a separatory funnel and extracted with acetonitrile (2 x 5 mL). The combined organic fractions were washed with brine (10 mL), dried over sodium sulfate, filtered and concentrated to dryness. The product 36 was purified by silica gel column chromatography using 10 % methanol/dichloromethane. Yield: 0.004 g, 83 %.

Rf (10 % methanol/dichloromethane) = 0.46. $^1$H (600 MHz, CD$_3$CN) δ: 6.69 (d, 1H, J = 8.3 Hz, Ar-H), 6.56 (s, 1H, NH), 6.29 (d, 1H, J = 2.4 Hz, Ar-H), 6.15 (dd, 1H, J = 8.3, 2.5 Hz, Ar-H), 3.73 (s, 3H, OCH$_3$), 3.12 (m, 2H, CH$_2$), 2.87 (s, 4H, NCH$_2$), 2.73 (s, 1H, CH), 2.51 (s, 4H, NCH$_2$), 2.35 (t, 2H, J = 6.6 Hz, CH$_2$), 1.44 (m, 4H, CH$_2$, CH$_2$); $^{13}$C{$^1$H} (150 MHz, CD$_3$CN) δ: 189.1, 188.5, 164.4, 154.6, 145.3, 133.7, 120.3, 107.1 100.9, 65.4, 58.6, 55.8, 55.3, 54.4, 52.0, 49.9, 42.7, 41.0, 27.7, 24.4; $^{11}$B{$^1$H} (160 MHz, CD$_3$CN) δ: 0.3, -5.2, -7.1, -8.6, -10.7, -12.5, -15.6, -18.5, -19.7; IR (KBr, cm$^{-1}$): ν 3428, 3360, 2917, 2849, 2564, 2088, 2032, 1784, 1669; HRMS (ES+) for C$_{20}$H$_{36}$B$_9$N$_5$O$_5$Re:
calculated 712.3112, observed 712.3128 [M+H].

$[2,2-(CO)_{2}-2-(NO)-8-C_{16}H_{24}N_{3}O_{2}-2,18-closo-ReC_{2}B_{9}H_{10}]$ (37).

Hypophosphorous acid (24 μL, 50 wt. % in water) was added to 36 (0.008 g, 0.01 mmol) followed by the addition of water (1 mL). The mixture was sonicated to create a suspension. The reaction was cooled to 0 °C and an aqueous solution of NaNO$_2$ (0.97 mg, 0.070 M, 0.014 mmol) was added dropwise. The reaction was stirred on ice for 30 min and then was allowed to stand overnight at room temperature. NaOH$_{(aq)}$ (1 M, 5 mL) was added to the reaction and the mixture was transferred to a separatory funnel and extracted with acetonitrile (2 x 5 mL). The combined organic fractions were washed with brine and dried over sodium sulfate, filtered and concentrated to dryness. The product 37
was isolated following silica gel column chromatography using 5 %
methanol/dichloromethane. Yield 3 mg, 38 %. R_f (5 % methanol/dichloromethane) =
0.33. ^1H (600 MHz, CD$_3$CN) δ: 6.96 (m, 1H, Ar-H), 6.90 (m, 3H, Ar-H), 6.56 (s, 1H,
NH), 3.80 (s, 3H, OCH$_3$), 3.12 (m, 2H, CH$_2$), 3.01 (s, 4H, NCH$_2$), 2.73 (s, 1H, CH), 2.55
(s, 4H, NCH$_2$), 2.37 (t, 2H, J = 6.7 Hz, CH$_2$), 1.45 (m, 4H, CH$_2$, CH$_2$); ^13C{^1H} (150
MHz, CD$_3$CN) δ: 189.1, 188.5, 164.4, 153.5, 142.7, 123.6, 122.0, 119.1, 112.8, 58.6,
56.0, 54.3, 51.3, 42.7, 41.0, 27.7, 24.5; ^11B{^1H} (160 MHz, CD$_3$CN) δ: -0.2, -5.4, -7.3,
-10.9, -12.6, -15.8; IR (KBr, cm$^{-1}$): ν 3434, 2917, 2849, 2576, 2088, 2030, 2001, 1783;
HRMS (ES+) for C$_{20}$H$_{35}$B$_9$N$_4$O$_5$Re: calculated 695.3062, observed 695.3055 [M+H].
HPLC (method A): t$_R$ = 13.3 min.

4.8.5. Radiolabelling with $^{99m}$Tc

The technetium-$^{99m}$Tc complexes 14a, 15 and 23 were prepared and isolated
following solid phase extraction and the methods described in chapters 2 and 3.$^{29}$
Pertechnetate [{$^{99m}$TcO$_4$}] was obtained from a $^{99}$Mo-$^{99m}$Tc generator (Lantheus Medical
Imaging) in saline (0.9 % NaCl). Solid phase extraction cartridges (C18; Waters) were
used following pre-treatment with water (10 mL), methanol (10 mL), a second wash with
water (10 mL) and HCl (10 mM, 10 mL). A Capintec dose calibrator was used to
determine the activity of samples containing technetium-$^{99m}$ (> 300 kBq).

$[2,2-(CO)_2-2-(NO)-8-C_{12}H_{17}N_2O-2,18-closo-^{99m}$Tc$C_2B_9H_{10}]$ (38). A typical
reaction involved the addition of sulfuric acid (2 M, 0.10 mL) and sodium nitrite (2.8 mg,
0.041 mmol) to an aliquot of 14a (803 MBq (21.7 mCi), in acetonitrile (0.80 mL)). The
reaction was stirred at room temperature for 2-5 min then the resultant yellow solution was diluted with HCl (10 mM, 2 mL) and loaded onto a C18 SPE cartridge. The excess salts were eluted with water (5 mL) and 38 was eluted with acetonitrile (5 mL). Isolated yield = 80 %; HPLC (method B): \( t_R = 19.4 \) min.

\[
[3,3-(\text{CO})_2-3-(\text{NO})-1-C_{14}H_{21}N_2O-3,1,2\text{-}\text{closo-}^{99}\text{mTcC}_2\text{B}_9\text{H}_{10}] \quad (40).
\]

A typical reaction for preparing 35 involved the addition of sulfuric acid (2 M, 0.10 mL) and sodium nitrite (3.2 mg, 0.046 mmol) to an aliquot of complex 15 (10 MBq (0.273 mCi), in acetonitrile (0.50 mL)). The reaction was stirred for 1-2 min then the resultant yellow solution was diluted with water (2 mL) and loaded onto a C18 SPE cartridge. The excess salt was eluted with water (5 mL) and the product was eluted with acetonitrile (5 mL). Isolated yield = 78 %; HPLC (method A): \( t_R = 16.2 \) min.

\[
[2,2-(\text{CO})_2-2-(\text{NO})-8-C_{16}H_{24}N_3O_2-2,18\text{-}\text{closo-}^{99}\text{mTcC}_2\text{B}_9\text{H}_{10}] \quad (41).
\]

In a typical reaction, sulfuric acid (2 M, 0.10 mL) and sodium nitrite (3.5 mg, 0.051 mmol) were added to an aliquot of complex 23 (154 MBq (4.15 mCi), in acetonitrile (0.50 mL)). The reaction was stirred for 5 min then the resultant yellow solution was diluted with HCl (10 mM, 2 mL) and loaded onto a C18 SPE cartridge. The excess salt was eluted with water (5 mL) and the product was eluted with acetonitrile (5 mL). Isolated yield = 81 %; HPLC (method A): \( t_R = 13.6 \) min.

4.8.6. X-ray crystallography

A rod-shaped orange crystal of 29 was fixed on a MiTeGen polymer mount and placed in a cold stream of liquid nitrogen (77 K). Data was collected at 100 K on a
Bruker APEX2 diffractometer equipped with CCD area detector using a graphite monochromator MoKα radiation and phi and omega scans. The SMART suite was used for data collection and SAINT was used for data reduction; data was scaled using SADABS with both face-indexed absorption correction as well as a correction using redundant data. The structure was then solved by direct methods and refined by full matrix least-squares refinement on F² using the Bruker SHELXTL program library. Non-hydrogen atoms were refined anisotropically and the hydrogen atoms were located and then refined isotropically as their thermal parameters remained stable throughout refinement. There was a three-fold disorder of the CO/NO ligand of the metal core. The disorder was modeled by forcing two carbon atoms to be located over three positions around the rhenium atom. In the final stages of refinement, R = 2.26 % and wR² = 4.84 % were reported.

4.9. References

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Chapter 5 – Metallocarborane Complexes (M = Re, $^{99m}$Tc) for the Development of Targeted Imaging Agents via the Copper(I)-Catalyzed Cycloaddition (“Click”) Reaction

5.1. Overview

Low yields and multistep syntheses typically associated with linking targeting vectors to carboranes\(^1\),\(^2\) create the need to develop alternative and more efficient synthetic strategies for the preparation of metallocarborane-based imaging agents. For instance, alkyne-insertion reactions to form carboranes cannot be performed in the presence of nucleophilic species (e.g. alcohols, acids, amines) because these polar groups degrade the decaborane adduct (4) leading to poor or negligible yields.\(^3\),\(^4\) During the preparation of a $^{99m}$Tc-carborane complex that mimics estradiol, the alcohol groups in the diphenol compound needed to be protected before generating the carborane conjugate and later deprotected to yield the desired compound.\(^1\) An additional issue is that carborane are hindered which reduces the yield of coupling reactions with functional group linked immediately adjacent to the cage.

In order to make the carborane ligand system more accessible in terms of bioconjugation, a series of alkyne-carborane ligands (53-55, Figure 5.1) was developed and uses copper(I)-catalyzed cycloaddition reaction (“click” chemistry) to link to a biomolecule. In this chapter, a proof of principle study was completed, using an azide based on the WAY100635 molecule (1) and a series of established and new carborane-derived alkynes, in order to prepare metallocarborane WAY-complexes (M = Re (69-71), $^{99m}$Tc (72-74)) (Figure 5.1).
5.2. Copper-catalyzed cycloaddition (“click”) reactions

Sharpless et al. coined the term “click” for reactions leading to highly selective and high yielding carbon-heteroatom bond formation.\(^5\) One of the most well known “click” reactions, shown in Scheme 5.1, is the Cu(I)-catalyzed (3+2) cycloaddition where an alkyne reacts with an azide to form a stable 1,4-disubstituted 1,2,3-triazole.\(^6\) The transformation is efficient, regioselective and has minimal side products. The synthesis can be accomplished without protection of other functional groups, is not impeded by steric crowding, can be performed in aqueous media and does not require the exclusion of atmospheric oxygen; these properties are ideal for the synthesis of radiotracers containing...
biomolecules.\textsuperscript{5-7} Furthermore, the nitrogen atoms in the 1,2,3-triazole provide weak hydrogen bonding sites which improve the solubility of appended substituents in water.

\[ \text{Scheme 5.1. Cu(I)-catalyzed (3+2) cycloaddition between an alkyne and an azide forming 1,4-disubstituted 1,2,3-triazole.} \]

The advantages of Cu(I)-catalyzed (3+2) cycloaddition reactions listed earlier have been exploited for the preparation of radiopharmaceuticals. The applications of which have been recently reviewed.\textsuperscript{8, 9} One approach for technetium-99m involved using the nitrogen donor in the triazole as a site to bind radiometals.\textsuperscript{7, 10} Mindt \textit{et al.} used the triazole as an integral part of a metal chelating system which also contained a linkage to a biomolecule (Scheme 5.2a).\textsuperscript{7} The one-pot procedure represents an improvement for the preparation of metal-based conjugates by creating a convergent synthetic method. Additional work by Mindt \textit{et al.} generated a series of imaging probes using the cycloaddition reaction via one set of reagents.\textsuperscript{11} Folic acid was selected as the biomolecule and various chelate systems, probes or precursors containing an alkyne were prepared. The cycloaddition linked the two systems together for the preparation of SPECT, PET, MRI or near-infrared fluorescence imaging probes (Scheme 5.2b).\textsuperscript{11}
Scheme 5.2. Examples of Cu(I)-catalyzed (3+2) cycloaddition reactions: (a) the preparation of a 99mTc-triazole chelate system,7 (b) the preparation of a series of folic acid derivatives,11 (c) flutamide linked to a 99mTc dipyridyl chelate.12

Although the triazole ring formed following the cycloaddition reaction can be used as a coordination site to the metal centre,7 it can also be used as a linker between a radionuclide prosthetic group and the targeting vector. Moore et al. prepared a [Tc(CO)3]+ flutamide complex to target prostate cancer,12 in which the radiometal was bound with a dipyridylamine chelate and the triazole was used solely as a linker to the targeting vector (Scheme 5.2c). Additionally, Seridi et al. recently reported a [M(CO)3Cl]-pyridyltriazole-based complex (M = Re, 99mTc) containing an arylpiperazine...
pharmacophore used to target CNS receptors where the product showed a lipophilicity value within range needed to cross the BBB (Figure 5.2). Copper-catalyzed cycloaddition reactions of this nature can be used to link a wide variety of targeted agents or used as pharmacokinetic modifiers. This chemistry can be used as a more general platform for creating novel carborane-based radiopharmaceuticals that is superior to existing bioconjugation methods.

Figure 5.2. [M(CO)3Cl]-pyridyltriazole-based complex (M = Re, 99mTc) containing an arylpiperazine pharmacophore.

5.3. Preparation of bis(pyridyl) triazole 50 and metal chelate complexes (M = Re (51), 99mTc (52))

While there are numerous examples of using Cu(I)-catalyzed cycloaddition reaction in organic chemistry, only a few of these involve polyhedral boranes, carboranes and metallocarboranes. “Click” chemistry was used to prepare polyhedral borane [closo-B_{12}H_{12}]^{2-} derivatives containing various functional substituents (hydroxyl, halides, amino and aryl) and carborane compounds linked to amino acids and adenosine. Hyaluronan carborane-conjugates were synthesized to target the CD44 antigen in cancer and for potential use in BNCT therapy while Djeda et al. prepared a carborane dendrimer
containing 810 boron atoms for BNCT applications.\textsuperscript{17} Nucleoside-boron cluster conjugates and metallocarborane complexes (M = Fe, Co) based on pyrimidine and purine were generated using “click” chemistry for boron carriers for BNCT and boron containing antivirals.\textsuperscript{18-20}

Applying standard organic reactions to carborane-containing compounds typically results in yields that are generally lower than those reported for non-carborane compounds. An example is the attempted preparation of compound 16 (chapter 3) via direct coupling between the carboxylic acid group on the carborane and an amine; following standard procedures used in solution and solid phase peptide synthesis resulted in negligible yields. To become familiar with “click” chemistry and to establish a point of reference for the carborane “click” reactions, a known chelate was used to develop model reactions. The use of a coordination complex was designed to eliminate the complexities that can occur with carborane (i.e. redox chemistry). A family of bifunctional chelators, known as the single amino acid chelates (SAACs), was developed in our group (Figure 5.3).\textsuperscript{21-25} This class of chelates is derived from lysine and can contain pyridine, imidazole, carboxylate, thiophene, thiazole and quinoline donor groups. The tridentate bis(pyridyl)amino coordinates with \([^{99m}\text{Tc(CO)}_3(\text{H}_2\text{O})_3]^+\) rapidly in high yields to form inert \(d^6\) low spin octahedral metal complexes (Scheme 5.3).\textsuperscript{26}
Figure 5.3. Single amino acid chelate derivatives.

Scheme 5.3. Preparation of $^{99m}$Tc(CO)$_3$-bis(pyridyl)amino chelate complex using microwave heating.

The bis(pyridyl)amino compound was selected and used to develop the “click” chemistry with the WAY azide compound because of the flexibility of this ligand where it is capable of conjugating with a targeting vector through an active ester unit and ability to be purified easily using a copper resin/C18 solid phase extraction protocol. The arylpiperazine azide compound (46) was selected as a proof of concept for the copper(I)-catalyzed cycloaddition reaction for both the chelate and carborane ligand reactions. The azide (46) was prepared in 93 % yield from the chloro compound (47, 53 % yield) by an $S_N2$ reaction (Scheme 5.4). The NMR spectra of 46 and 47 are similar, with the expected differences found in the resonances of the alkyl linker (46): $^1$H δ 3.39, 2.67 ppm; $^{13}$C{$^1$H} δ 57.3, 48.3 ppm; (47): $^1$H δ 3.62, 2.80 ppm; $^{13}$C{$^1$H} δ 59.9, 40.9 ppm).
presence of an infrared band at 2099 cm\(^{-1}\) in the IR spectrum of 46 is characteristic of an azide (N\(_3\)) functional group.

![Scheme 5.4. Preparation of arylpiperazine azide 46.](image)

The bis(pyridyl)amino chelate\(^{23}\) (48) was coupled to propargylamine using EDC and HOBt to couple an alkyne to the chelate yielding 49 (42 \%) (Scheme 5.5). The \(^1\)H NMR spectrum of 49 shows resonances between 8.49 and 7.11 ppm which are consistent with the presence of the pyridyl rings in the compound. Spectroscopic data for 49 support the presence of the amide can be found in the \(^{13}\)C\textsubscript{\textsuperscript{1}H}\ NMR (173 ppm, CO) and infrared (\(\nu = 3296 \text{ cm}^{-1}\) (NH) and 1654 cm\(^{-1}\) (CO)) spectra.

![Scheme 5.5. Synthesis of 49.](image)

To prepare the triazole derivative 50, the optimal copper catalyst, solvent and reaction time were evaluated in a series of microreactions analyzed by liquid chromatography-mass spectrometry (LC-MS). The copper catalysts evaluated include copper(II) sulfate,\(^{19,28}\) copper(II) acetate\(^{29}\) and copper(I) iodide.\(^{30}\) The solvents examined
were aqueous $t$-butanol and methanol; the additives such as sodium ascorbate (reducing agent) and diisopropylethylamine (DIPEA, base) were also assessed. The reactions were performed at room temperature in order to determine the optimal reaction time while also considering thermally sensitive biomolecules that may be used in the future.

The majority of the reactions explored produced the desired compounds in low yields or as complex mixtures of products (Table 5.1). The copper catalyst that proved to be most efficient for coupling 49 and 46 was copper(II) acetate. The triazole 50 was isolated in 18 % yield following silica gel column chromatography (Scheme 5.6). The low yield could be attributed to the potential coordination of the copper to donor atoms. Ethylenediaminetetraacetic acid disodium salt was used to extract the copper from the coordination site. Compared to the $^1$H NMR spectrum of 49, an additional set of resonances are present for 50 at 6.99-6.84 and 3.84 ppm which are attributed to the substituted phenyl and methoxy moieties, respectively. The high resolution mass spectrum supports the identity of the compound as 50 ($m/z = 598.3607$ [M$^+$]).

**Table 5.1.** Reagents used in attempts to prepare 50.

<table>
<thead>
<tr>
<th>Copper catalyst</th>
<th>Additive</th>
<th>Solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>$Cu^{II}SO_4$</td>
<td>sodium ascorbate</td>
<td>$t$-butanol$_{(aq)}$</td>
</tr>
<tr>
<td>$Cu^{II}(OAc)_2$</td>
<td>DIPEA</td>
<td>methanol</td>
</tr>
<tr>
<td>$Cu^I$I</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**Scheme 5.6. Synthesis of 50.**

Complexation between 50 and [M(CO)$_3$]$^+$ (M = Re, $^{99m}$Tc) was accomplished using microwave-assisted heating (Scheme 5.7). Isolation of the [Re(CO)$_3$]$^+$ complex 51 was obtained in 15 % yield after purification using silica gel column chromatography. The complexation yield of 51 is lower than that reported in the literature for similar compounds, which could be due to the potential coordination of [Re(CO)$_3$]$^+$ to the triazole and piperazine moieties which may lead to various chelate complexes, consequently, lowering the yield. The proton signals in the $^1$H NMR spectrum of 51 associated with the methylene groups adjacent to the pyridine rings are split into two sets of doublets with geminal coupling values ($J$ = -16.5 Hz) (Figure 5.4) which is consistent with analogous complexes reported in the literature. The $^{13}$C{$_^1$H} NMR spectrum of 51 displays two shifts at 197.2 and 196.4 ppm attributed to the CO groups. The mass spectrum of 51 contains a peak with a molecular ion at 868.2997.
Scheme 5.7. Synthesis of 51 and 52.

Figure 5.4. $^1$H NMR (600 MHz, CD$_3$OD) spectrum of 51 and expansion centered about the diastereotopic CH$_2$ protons (dotted region).

Compound 50 and $[^{99m}\text{Tc(CO)}_3(\text{H}_2\text{O})_3]^+$ were combined and heated in a microwave reactor at 120 °C for 5 min (Scheme 5.7) where the corresponding $^{99m}\text{Tc}$-
derivative 52 was generated in 91 % yield. The product peak shows good HPLC retention time correlation with the rhenium analogue (51) (Figure 5.5). The preparation of 51 and 52 has established a new “click” methodology for preparing the Re(I) and $^{99m}$Tc(I) bis(pyridyl)amino-triazole complexes and a similar synthetic strategy can now be employed for the generation of the metallocarborane-triazole (M = Re, $^{99m}$Tc) complexes.

**Figure 5.5.** Chromatograms of the crude reaction mixture: (a) $\gamma$-chromatogram of $^{99m}$Tc-carborane 52, (b) UV-chromatogram, (c) UV-chromatogram co-injected with Re complex 51.
5.4. Synthesis of carborane-alkyne derivatives 53-55

As demonstrated in chapters 2 and 3, the spacer length and type between the WAY targeting vector and radiometal leads to different binding affinities and biodistribution profiles. In this chapter, a series of derivatives (53-55) were prepared which contain different linkages between the carborane and alkyne. The linkers are designed to allow for flexibility in the choice of ligands to optimize affinity distribution, pharmacokinetics and/or lipophilicity of the complex. The first carborane-alkyne compound (53) contains an methylene spacer and was prepared by reacting mono-lithiated o-carborane (56) with trimethylsilyl propargyl bromine to generate a protected alkyne-carborane derivative (57) (Scheme 5.8). In the published procedure, acetic acid and tetrabutylammonium fluoride were used to remove the protecting group and yield the closo-carborane alkyne derivative. Our interest involves the preparation of the metallocarborane complex thus isolation of the closo-carborane species was not necessary. A one pot deprotection and deboronation was achieved by combining 57 with sodium fluoride in methanol using conventional heating followed by the addition of an equivalent amount of water and heating in a microwave reactor to afford the nido-carborane species 53 as the sodium salt in 95 % yield following purification by reverse phase (C18) silica chromatography (Scheme 5.8).

**Scheme 5.8.** Synthesis of 53.
Diastereotopic protons associated with the CH$_2$ group in 53 are evident at 2.29 and 2.52 ppm in the $^1$H NMR spectrum (Figure 5.6). The $^1$H NMR spectrum also displays resonances at 2.15 and 1.79 ppm corresponding to the alkyne proton and the unsubstituted proton of the carborane cage, respectively. The $^{11}$B{$^1$H} NMR spectrum of 56 shows two characteristic resonances at -33.3 and -37.0 ppm which are a distinctive feature of nido-carborane compounds. The IR spectrum displays a distinctive broad peak at 2519 cm$^{-1}$ corresponding to the BH absorption. The mass spectrum of 53 contains a peak at 173.1951 and a distinctive B$_9$ pattern supporting the formation of the desired compound.

![NMR Spectrum](image)

**Figure 5.6.** $^1$H NMR (500 MHz, CD$_3$OD) spectrum of 53 and expansion centered about the characteristic resonances (dotted region).
Attempts were made to prepare methyl- and propyl-linked alkyne \textit{closo}-
carboranes (58-61) prior to running the “click” reaction. Mono-lithiated \textit{o}-
carborane (56) was combined with 5-chloropent-1-yne, 5-iodopent-1-yne and prop-2-ynyl-4-
methylbenzenesulfonate (Scheme 5.9); all three reactions recovered only the unreacted
starting materials upon work-up. Presumably, the basic reaction conditions appear to
induce deprotonation of the alkynyl proton which prevents substitution by the carborane
anion. A similar observation was noted by Di Meo \textit{et al.} where polymerization of the
alkyne was found.\textsuperscript{16} In order to avoid this result, the acidic protons of 5-chloropent-1-
yne, 5-iodopent-1-yne or prop-2-ynyl-4-methylbenzenesulfonate were protected using
trimethylsilylchloride. Unfortunately, reaction of the protected 5-halopent-1-yne and
prop-2-ynyl-4-methylbenzenesulfonate compounds with mono-lithiated \textit{o}-
carborane (56) failed to generate the desired products (59 and 61) despite using different solvents and
reaction temperatures (Scheme 5.9). Another route was explored where 3-iodopropyl-
\textit{o}-
carborane (11) was reacted directly with sodium acetylide suspension (18 wt. % in
xylene) but this reaction also failed to produce the alkyne-carborane 60 (Scheme 5.10).

\begin{equation}
\text{Scheme 5.9. Attempted preparation of the alkyne-derivatized carboranes 58-61.}
\end{equation}
Scheme 5.10. Attempted synthesis of the alkyne-carborane 60 by reacting 11 with sodium acetylide.

As an alternative strategy, a heteroatom was used to link the carborane and alkyne. The synthesis of carborane 54 was prepared by combining 11 and N-methyl propargylamine in the presence of diisopropylethylamine (Scheme 5.11). The isolated yield of 54 was 82 % following purification using silica gel column chromatography. The \(^1\)H NMR spectrum of 54 has resonances which correspond to the closo-carborane CH (3.61 ppm), the CH\(_2\) alkyl chain (3.34, 2.46, 2.29, 1.67 ppm), the alkyne proton (2.26 ppm) and the overlapping methyl group (2.31 ppm). The six resonances in the \(^{11}\)B\{\(^1\)H\} NMR spectrum between -2.7 and -13.5 ppm are characteristic of a closo-carborane species. The mass spectrum of 54 displays a distinctive B\(_{10}\) pattern with a molecular mass peak at 256.2844.

Scheme 5.11. Synthesis of 54 via a S\(_N\)2-type reaction.

The ligand 54 is versatile in that the amine group in the linker provides a site for methylation which would lead to the preparation of a cationic carborane. Following complexation with the \([\text{M(CO)}_3]^+\) core (M = Re, \(^{99m}\)Tc), the resulting product,
[M(CO)_3RC_2B_9H_10] will be neutral overall which is desired for compounds designed to cross membrane barriers.\textsuperscript{32} Compound 55, as the triflate salt, was prepared by reacting 54 with methyl triflate at room temperature in 93\% yield (Scheme 5.12). Compound 55 shows a similar NMR spectrum as 54 except that the resonance at 3.07 ppm has an integration of six rather than three protons. The mass spectrum of 55 has a peak at m/z = 268.3059 and an isotopic distribution characteristic of a carborane derivative.

![Scheme 5.12. Synthesis of 55.](image)

5.5. Attempted preparation of rhenacarborane-alkyne derivatives 62-64

An attempt was made to prepare the metallocarborane alkyne-complexes \(62-64\) (Figure 5.7) that could be “clicked” to various functionalized azides. In the previous chapters, the preparation of rhenacarborane complexes in a solution of aqueous ethanol using microwave heating was reported. Following these conditions, the carborane-alkyne ligands \(53-55\) were treated with the rhenium(I) salt, \([\text{Re(CO)}_3(\text{H}_2\text{O})_3]^+\), in attempts to prepare complexes \(62-64\).
The reaction between 53 and [Re(CO)₃(H₂O)₃]⁺ did not result in the formation of the desired product 62; instead the reaction conditions promoted acid-catalyzed hydration of the alkyne yielding the methyl ketone by-product 65 (Scheme 5.13). No literature precedent has been published showing alkyne hydration with rhenium as a catalyst, however, other transition metals such as ruthenium, palladium, copper and gold do catalyze such reactions.³³ Additionally, high temperatures (270-300 °C) in a microwave reaction have been reported to result in the hydration of phenylacetylene in the absence of a catalyst.³⁴

Scheme 5.13. Hydration of 53 producing the methyl ketone by-product 65.

The [Re(CO)₃(H₂O)₃]⁺ complex has a pKa of 7.5;³⁵ however, when the complex was dissolved in water (0.01 M), a pH value of approximately 1.5 to 2 was measured. In order to minimize the alkyne hydration by-product formation 65, a phosphate buffered saline solution (pH 7.4) was used as the solvent. The resulting pH of the dissolution of...
[Re(CO)$_3$(H$_2$O)$_3$]$^+$ in the buffer was between 6.0 and 6.4. Reactions involving
[Re(CO)$_3$(H$_2$O)$_3$]$^+$ cannot be basic as this leads to rhenium cluster formation at elevated
temperatures. Alternatively, organic bases (piperidine, pyridine or
diisopropylethylamine) were employed as the reaction solvent wherein the amine could
deprotonate of the bridging hydrogen generating the diicarbollide carborane species, thus
enhancing complexation with the [Re(CO)$_3$]$^+$ core. A variable temperature study (100-
180 °C, 5 min) for the reaction of 53 and [Re(CO)$_3$(H$_2$O)$_3$]$^+$ using these
solvents/reagents, indicated that none of these conditions successfully produced the
metallocarborane complex 62. The $^{11}$B{H} NMR spectrum of the reaction mixture
showed a dozen resonances between -5 and -23 ppm indicating a complex product
mixture. Compound 62 could not be detected in this mixture using mass spectrometry.

Amines have been shown to readily deboronate closo-carboranes to nido-
carboranes, thus there was the possibility that the tertiary nitrogen present in the
linker of carborane 54 could induce deboronation (Figure 5.8). Moreover, the presence
of the nitrogen atom in the compound may also assist with the deprotonation of the
bridging hydrogen prior to complexation with [Re(CO)$_3$]$^+$ thus permitting a lower
reaction temperature, which would be advantageous for thermally sensitive biomolecules
conjugated to the carborane unit. Heating carborane 54 in water both conventionally and
using a microwave reactor did not produce the nido-carborane derivative indicating
amine was not sufficiently basic (pK$_a$ ~ 10)$^{38}$ or the carborane compound may be too
bulky to induce deboronation. Conversion of 54 to the nido-species was accomplished by
extensive heating (24 h) in the presence of sodium hydroxide. Combining nido-54 and
[Re(CO)₃(H₂O)₃]⁺ in aqueous ethanol in a microwave reactor (100-195 °C) produced small quantities of the desired complex 63 and the nido-ligand remained even at the highest temperatures (Figure 5.8).

![Structures of 54 and 63.](image)

**Figure 5.8.** Structures of 54 and 63.

The initial attempts to prepare the nido-carborane derivative of 55 using sodium hydroxide were also unsuccessful, despite the fact it was successful for the mono-methylated carborane 54. This may be due to a competing Hoffman-type elimination reaction. Using the milder organic base piperidine, the nido-carborane species was formed and isolated (Scheme 5.14). The optimal reaction temperature between nido-carborane 55 and [Re(CO)₃(H₂O)₃]⁺ was explored and the resulting reaction yielded a mixture of products including the mono-methylated derivative 57.

![Scheme 5.14. Preparation of nido-55 using piperidine.](image)

**Scheme 5.14.** Preparation of nido-55 using piperidine.

As a result of the lack of success in the coordination of 53-55 with [Re(CO)₃]⁺ to generate metallocarborane 62-64, a decision was made to perform the “click” reaction
first, to generate the triazole-carborane compounds 66-68, followed by metal coordination. An advantage of incorporating the metal in the last step, especially $^{99m}$Tc, would be to minimize excess radiation exposure during preparation.

5.6. Preparation of triazole-carborane derivatives 66-68

The three alkyne-carborane compounds described previously possess vastly different electrostatics charges: anionic 53, neutral 54 and cationic 55. It was expected that these different analogues may require varying conditions for the cycloaddition reaction to occur. The optimal copper catalyst, solvent and reaction time for coupling the carborane ligands 53-55 and azide 46 were examined in a series of microreactions, in a similar manner as the bis(pyridyl) chelate 49 discussed above. Alkyl-carborane 53 and amino-carborane 54 were combined with 46 to yield triazole-carboranes 66 and 67 using copper(II) sulfate and sodium ascorbate in aqueous $t$-butanol (Scheme 5.15). For the ammonium analogue 55, the same copper catalyst was found to be optimal but solvent was changed to methanol to increase the solubility of the ligand. The yields of 67 and 68 were 76 % and 65 %, respectively, however, compound 66 was isolated with only 3 % yield; the proximity of the alkyne to the carborane cage likely hindered the cycloaddition reaction. Similar low or negligible yields have been observed in the preparation of other carborane derivatives in which the reactivity of a pendant functional group is hindered by the bulky cage. For example, Mollard et al. attempted to perform a reaction at a terminal carboxylic acid group in a tricarboranyl compound but found they needed to insert a linker to relieve the steric congestion for the reaction to occur. The extended spacer
between the cage unit and alkyne in 54 and 55 alleviates the steric bulk producing higher yields for triazole 67 and 68. Although carborane ligands 53-55 have different linkages and electrostatic charges, a similar set of reaction conditions that works for all three compounds was identified.

![Scheme 5.15. Preparation of the carborane triazole compounds 66-68.](image)

The $^1$H NMR spectrum of compound 66 shows characteristic resonances at 7.78, 6.98-6.88, 3.81 and 1.79 ppm which are attributed to the triazole proton, the aromatic protons, the methoxy protons and the carborane CH proton, respectively. The $^{11}$B{$^1$H} spectrum of 66 has eight resonances between -10.4 and -37.0 ppm which are consistent with a nido-carborane. For compound 67, the resonance at 7.58 ppm in the $^1$H NMR spectrum is from the triazole proton and the signal at 3.56 ppm is assigned to the carborane CH group and is consistent with a closo-carborane compound (Figure 5.9).

The mass spectrum of 67 shows a distinctive $B_{10}$ isotope pattern with a m/z at 517.4451. The $^{13}$C{$^1$H} NMR spectrum of 67 displays a resonance at 141.1 ppm which is attributed
to the quaternary triazole carbon and the overlapping peaks at 123 ppm corresponding to the triazole CH and aromatic CH (Figure 5.10). Compound 68 contains a distinct resonance at 8.57 ppm which is attributed to the triazole proton in the $^1$H NMR spectrum while the resonance at 4.76 ppm is due to the unsubstituted carborane proton. The $^{11}$B{$^{1}$H} NMR spectrum of 68 shows five resonances between -2.4 and -12.6 ppm which are consistent with a closo-carborane species.

Figure 5.9. $^1$H NMR (500 MHz, CDCl$_3$) spectrum of 67.
5.7. Preparation of metallocarborane complexes (M = Re (69-71), $^{99m}$Tc (72-74))

The nido-carborane triazole 66 was combined with [Re(CO)$_3$(H$_2$O)$_3$]$^+$ in aqueous ethanol but failed to produce 69. When aqueous acetonitrile was used as the solvent and one equivalent of base (diisopropylethylamine) was introduced to the reaction, the product 69 was isolated following column chromatography in 69 % yield (Scheme 5.16). The rhenium complex 69 was isolated as the diisopropylethylammonium salt where the resonances at 8.24, 3.83, 3.26 and 1.46 ppm in the $^1$H NMR spectrum can be attributed to the counter ion. The $^{11}$B{$^1$H} NMR spectrum of 69 contains six resonances between -5.5 and -19.9 ppm which are characteristic of rhenacarborane complexes (Figure 5.11). The IR spectrum has two strong $\nu$(CO) peaks at 1995 and 1890 cm$^{-1}$ consistent with the

Figure 5.10. $^{13}$C{$^1$H} (DEPTq) NMR (125 MHz, CDCl$_3$) spectrum of 67.

The nido-carborane triazole 66 was combined with [Re(CO)$_3$(H$_2$O)$_3$]$^+$ in aqueous ethanol but failed to produce 69. When aqueous acetonitrile was used as the solvent and one equivalent of base (diisopropylethylamine) was introduced to the reaction, the product 69 was isolated following column chromatography in 69 % yield (Scheme 5.16). The rhenium complex 69 was isolated as the diisopropylethylammonium salt where the resonances at 8.24, 3.83, 3.26 and 1.46 ppm in the $^1$H NMR spectrum can be attributed to the counter ion. The $^{11}$B{$^1$H} NMR spectrum of 69 contains six resonances between -5.5 and -19.9 ppm which are characteristic of rhenacarborane complexes (Figure 5.11). The IR spectrum has two strong $\nu$(CO) peaks at 1995 and 1890 cm$^{-1}$ consistent with the
bound tricarbonyl units in \([\text{Re(CO)}_3]^+\).

Scheme 5.16. Synthesis of 69 from 66 using microwave heating.

![Scheme 5.16](image)

Figure 5.11. \(^{11}\text{B}\{^1\text{H}\}\) NMR (160 MHz, CD₃CD) spectrum of 69.

The \textit{nido}-carboranes analogues of 67 and 68 were prepared \textit{in situ} from their \textit{closo}-analogues using previously established procedures (i.e. sodium fluoride in aqueous ethanol), prior to the addition of the \([\text{Re(CO)}_3(\text{H}_2\text{O})_3]^+\). This route was unsuccessful in the case of 67 and 68 so new conditions were explored. Diisopropylethylamine was used for \textit{in situ} deboronation of \textit{closo}-amino-triazole (67) and \textit{closo}-dimethylamino-triazole (68) in acetonitrile (Scheme 5.17). Complexation with \([\text{Re(CO)}_3]^+\) was then achieved in a solution of aqueous acetonitrile in the presence of approximately one equivalent of diisopropylethylamine using microwave heating (180-190 °C). Following purification,
rhenacarborane complexes 70 (as the diisopropylethylammonium salt) and 71 (as the zwitterion) were isolated with yields of 45 and 71 \%, respectively.

Scheme 5.17. Synthesis of rhenacarborane complexes 70 and 71.

The \(^{11}\text{B}\{1\text{H}\}\) spectrum of 71 has resonances between -5.5 to -19.8 ppm and the mass spectrum supports the formation of 71 with a peak at \(m/z = 791.3980\ [\text{M+H}]\) displaying a distinct B\(_9\)Re isotopic pattern. The \(^{11}\text{B}\{1\text{H}\}\) NMR spectrum of rhenacarborane 70 shows six resonances between -5.4 and -19.9 ppm which are consistent with a rhenacarborane complex. Somewhat surprisingly, the \(^{13}\text{C}\{1\text{H}\}\) NMR spectrum of 70 displays three CO resonances (one CO resonance was expected) suggesting this compound exists as multiple complexes upon standing in solution. Both hydrogen donors and acceptors are present within this compound which could lead to both intramolecular and intermolecular interactions or isomerization of the carborane cage may have occurred. Two sets of resonances for both the triazole (149.5, 148.9 ppm) and CH (125.1, 124.6 ppm) carbon atoms were identified in the \(^{11}\text{C}\{1\text{H}\}\) NMR spectrum of 70 with the assistance of 2-D NMR data. Based on these NMR data, we have speculated the complexes could be a mixture of 70\(a\), 70\(b\) 70\(c\) or 70\(d\) (Figure 5.12).
Isomerization of the carborane cage has been seen for previous complexes in this thesis and similar intramolecular hydrogen bonding between the triazole-CH and a donor atom within the molecule has been seen in other triazole compounds. Crystallization of this complex was attempted without success.

Figure 5.12. Proposed structural complexes of compound 70 in solution.

When the same reaction conditions used for rhenium complexation were used to prepare the technetium complexes 72-74, the reaction afforded negligible yields of the
desired compounds. It appears that diisopropylethylamine may interfere with the complexation between the nido-carborane derivatives and the $[^{99m}\text{Tc(CO)}_3]^{+}$ core. The nido-carborane derivative 66 was reacted directly with $[^{99m}\text{Tc(CO)}_3(\text{H}_2\text{O})_3]^{+}$ in aqueous acetonitrile (with no addition of diisopropylethylamine) to yield 72. The technetium-99m product 72 was isolated from residual $^{99m}\text{Tc}$ salts following solid phase extraction in 63 % decay corrected yield. The retention time of complex 72 correlates with the rhenium complex 69 (Figure 5.13).

![Figure 5.13. HPLC chromatograms ($\gamma$ and UV) of the isolated $^{99m}\text{Tc}$ carborane complex 72 co-injected with Re complex 69; residual nido-66 is present in the reaction mixture.](image)

The nido-carborane analogues of 67 and 68 were prepared in situ using a previously reported procedure for deboronation utilizing sodium fluoride as the base. $[^{99m}\text{Tc(CO)}_3(\text{H}_2\text{O})_3]^{+}$ was subsequently added and the reaction mixture heated (Scheme 5.18). The isolated yields of 73 and 74, following purification via a solid phase
extraction, were 49 % and 53 %, respectively. The $^{99m}$Tc-carborane complexes (73 and 74) had the expected retention time correlation with the corresponding rhenacarborane complexes 70 and 71 in the HPLC chromatograms (Figure 5.14).

Scheme 5.18. Reaction between 67 or 68 and $[^{99m}$Tc(CO)$_3]^+$ to yield 73 and 74, respectively.

Figure 5.14. HPLC chromatograms ($\gamma$ and UV) of the isolated $^{99m}$Tc carborane complex 74 co-injected with Re complex 71; residual nido-68 and $[^{99m}$TcO$_4]^-$ are present in the reaction.
The syntheses of the metal derivatives of the carborane triazole ligands 66-68 demonstrated that the methods used for preparing rhenacarboranes does not necessarily translate to the same reaction conditions for the radiolabelling with $^{99m}$Tc. In this thesis, differences associated with isomerization of metallocarborane ($\text{M} = \text{Re}, \text{99mTc}$) complexes (chapters 2 and 3) was observed as well as the stabilities of metallocarboranes of the type $[\text{M(CO)}_2(\text{NO})]^2+$ ($\text{M} = \text{Re}, \text{99mTc}$) (chapter 4). In addition to metallocarborane complexes, variations between the metal congeners ($\text{M} = \text{Re}, \text{99mTc}$) have been noted by other research groups for other chelate complexes. For example, the lipophilicity and receptor binding data are not the same for analogous rhenium and technetium (3+1) chelate complexes.43, 44 These differences can be attributed to two main factors: (i) the concentration difference between the metal and reagents in the reactions where the technetium reactions ($10^{-6}$ to $10^{-9}$) are orders of magnitude smaller than the corresponding rhenium reactions (1 to $10^{-3}$ M); (ii) rhenium and technetium-99m have a notable difference is their reduction-oxidation potential (vide supra). The results of this work emphasize that while rhenium can be used as a non-radioactive standard for confirming the preparation of the technetium analogue, the properties, stabilities and reactivity of rhenium complexes cannot be assumed to be identical to the technetium-99m complexes.

The synthetic strategy used to prepare the $^{99m}$Tc bis(pyridyl) chelate 50 by “click” chemistry was applied to the generation of the $^{99m}$Tc-carborane triazole analogues. With the exception of 66 (3 %) in which the short linkage between the alkyne and carborane hindered the cycloaddition reaction, the yields of the carborane-triazole compounds 67 (76 %) and 68 (65 %) were reasonable and superior to most carborane-biomolecule
conjugation reactions. The corresponding rhenacarborane-triazole complexes 69-71 were produced in yields of 45-71 % and the 99mTc carborane complexes were generated in yields of 49-63 %.

5.8. Summary

The carborane-alkyne (53-55) derivatives were generated in good yields (82-95 %). Although the carborane compounds (53-55) have distinctive electrostatic charges and unique linkages between the carborane cage and alkyne, a general reaction condition could be used to react the carborane ligands with a WAY-based azide (46) to generate the carborane triazole ligands (66-68). This methodology should therefore be useful in preparing other carborane-biomolecule conjugates. With this series of carborane-triazole ligands, it was found that different reaction conditions were required to complex rhenium or technetium-99m to the ligands (66-68). The metallocarborane complexes (M = Re, 99mTc) were isolated using microwave heating in under 15 min in good yields (45-71 %). The carborane ligands were compared to an organic chelate and indicated that the carborane ligand system can benefit from copper(I)-catalyzed cycloaddition reaction to directly conjugate a targeting vector to the carborane system for the preparation of targeted 99mTc radiopharmaceuticals.

5.9. Experimental

5.9.1. Reagents and general procedures

Unless otherwise stated, all reagents were purchased and used as received from
Sigma-Aldrich without further purification. 1,2-Dicarba-closo-dodecaborane (ortho-carborane) was purchased and used as received from Katchem Ltd. Compound 11 was prepared in chapter 2, 57\(^{16}\) and [Re(CO)\(_3\)(H\(_2\)O)\(_3\)][Br]\(^{45}\) were prepared according to literature procedures. All solvents, except for \(t\)-butanol (Fisher Scientific), were obtained from Caledon and either used as received or dried via a Pure-Solv Solvent Purification System (Innovative Technology Inc.) prior to use. Deuterated solvents for NMR samples were purchased from Cambridge Isotope Laboratories.

Reactions were monitored using Alugram Sil G/UV\(_{254}\) thin-layer chromatography plates using a UV lamp or an appropriate stain or spray to visualize the compound. Carborane-containing species were visualized with 0.2 % PdCl\(_2\) in hydrochloric acid (3.0 M) which, upon heating, gave dark brown spots. Phosphomolybdic acid was used as a general visualizing stain and amines were visualized with ninhydrin spray. Silica gel column chromatography was accomplished with Ultra Pure Silica Gel 60 (Silicycle) and preparative plates from AnalTech were used. Automated normal and reverse-phase (C18) silica gel chromatography were performed on a Biotage SP1 purification system operated at ambient temperature using solvent gradients as specified.

5.9.2. Instrumentation

Nuclear magnetic resonance spectra (\(^1\)H, \(^{13}\)C\(_{\{1\}H}\), \(^{11}\)B) were recorded on either Bruker 500 or 600 MHz spectrometers at ambient temperature. The \(^{13}\)C\(_{\{1\}H}\) DEPT experiment where the quaternary carbon atoms were obtained using pulsed field gradients (31%:31%:31%). The chemical resonances (\(\delta\)) are reported in parts per million (ppm),
with $^1$H NMR shifts referenced to the residual proton signal of the deuterated solvent and
the $^{13}$C{$_1$H$_1$} shifts referenced to the carbon signal of the solvent. The chemical shifts ($\delta$)
for $^{11}$B{$_1$H$_1$} NMR spectra are reported relative to an external reference of BF$_3$·Et$_2$O.
Infrared spectra were acquired on a Nicolet 510 Fourier Transform IR spectrometer at
ambient temperature and the samples were run on a KBr plate. Reactions involving
microwave heating were performed on a Biotage Initiator 60 microwave reactor using
crimp-sealed Emrys vials. Mass spectrometry analyses were obtained from the
McMaster Regional Centre for Mass Spectrometry utilizing a Micromass Quattro Ultima
(LC-ESI/APCI triple quadrupole) mass spectrometer for electrospray ionization (ES)
mass spectrometry; and a Micromass Global Ultima (MALDI/CapLC-ESI quadrupole
time of flight) mass spectrometer for high resolution mass spectra.

High performance liquid chromatography was performed on an Agilent 1100
system (Quad Pump module G1311A, Degasser G1322A, ALS G1313A, Colcom
G1316A) interfaced with a BioScan radioactivity detector (APIFC2-00507). The
wavelength for detection was set at 254 nm. Agilent Zorbax C8 100-5 (250 x 4.6 mm)
analytical column was used with a flow rate of 1 mL/min. The following solvent
gradients were employed (solvent A = acetonitrile with 0.1 % formic acid, solvent B = 20
mM ammonium acetate): Method A 0-15 min, 40-100 % A; 15-20 min, 100 % A.
Method B 0-20 min, 25-75 % A; 20-23 min, 75-100 % A; 23-25 min, 100-25 % A.

5.9.3. Synthetic procedures

[C$_{13}$H$_{19}$N$_5$O] (46). The synthesis of 46 was adapted from a procedure reported by
Zhang et al. Sodium azide (2.2 g, 33.8 mmol) was added to a solution of 47 (1.1 g, 4.3 mmol) in anhydrous N,N-dimethylformamide (25 mL) and heated at 60 °C for 18 h under an inert atmosphere. The mixture was transferred to a separatory funnel containing ethyl acetate (75 mL) and washed with water (4 x 100 mL) and brine (100 mL). The organic fraction was dried over sodium sulfate, filtered and concentrated to a yellow-orange oil. Yield: 1.05 g, 93 %. Rf (50 % ethyl acetate/hexanes) = 0.44. 1H NMR (500 MHz, CDCl₃) δ: 6.99 (m, 1H, Ar-H), 6.94, (m, 2H, Ar-H), 6.85 (m, 1H, Ar-H), 3.86 (s, 3H, OCH₃), 3.39 (t, 2H, J = 6.1 Hz, CH₂), 3.11 (s, 4H, NCH₂), 2.71 (s, 4H, NCH₂), 2.67 (t, 2H, J = 6.1 Hz, CH₂); 13C{1H} NMR (125 MHz, CDCl₃) δ: 152.3, 141.3, 123.0, 121.1, 118.3, 111.3, 57.3, 55.4, 53.5, 50.6, 48.3; IR (KBr, cm⁻¹): ν 2940, 2817, 2099, 1500; HRMS (ES+) m/z for C₁₃H₂₀N₅O: calculated: 262.1668, observed: 262.1652 [M+H][C₁₃H₁₉ClN₂O] (47). Synthesis adapted from a procedure reported by Chen et al. 1-(2-methoxyphenyl)piperazine (1.7 g, 8.8 mmol) and potassium carbonate (3.1 g, 22.4 mmol) were suspended in anhydrous N,N-dimethylformamide (40 mL) under an inert atmosphere. 1-Bromo-2-chloroethane (1.1 mL, 13.2 mmol) was added dropwise and the mixture was stirred at room temperature for 24 h. Water (80 mL) was added to quench the reaction and the mixture was transferred to a separatory funnel and extracted with ethyl acetate (3 x 80 mL). The combined organic fractions were washed with brine (3 x 80 mL). The organic fraction was dried over sodium sulfate, filtered and the solvent was evaporated under reduced pressure. The product was isolated following silica gel column chromatography using 50 % ethyl acetate/hexanes. Yield: 1.2 g, 53 %. Rf (50 % ethyl acetate:hexanes) = 0.46. 1H NMR (500 MHz, CDCl₃) δ: 6.99 (m, 1H, Ar-H), 6.93
(m, 2H, Ar-H), 6.85 (m, 1H, Ar-H), 3.85 (s, 3H, OCH₃), 3.62 (t, 2H, J = 7.1 Hz, CH₂), 3.01 (s, 4H, NCH₂), 2.80 (t, 2H, J = 7.1 Hz, CH₂), 2.72 (s, 4H, NCH₂); $^{13}$C{$^{1}$H} NMR (125 MHz, CDCl₃) δ: 152.3, 141.2, 123.0, 121.0, 118.2, 111.2, 59.9, 55.4, 53.4, 50.5, 40.9; IR (KBr, cm⁻¹): ν 2941, 2820, 1500; HRMS (ES+) m/z for C₁₃H₂₀N₂OCl: calculated: 255.1264, observed: 255.1244 [M+H].

$[^{[C_{20}H_{24}N_{4}O]}]$ (49). $^{N,N}$-Bis-(2-pyridyl) aminovaleric acid 48 was synthesized based on a related literature procedure, substituting 2-pyridinecarboxaldehyde in place of 2-quinolinecarboxaldehyde.²³ To a solution of $^{N,N}$-bis-(2-pyridyl) aminovaleric acid 48 (1.01 g, 3.37 mmol) in $^{N,N}$-dimethylformamide (35 mL), 1-hydroxybenzotriazole hydrate (0.50 g, 3.70 mmol), $^{N}$-(3-dimethylaminopropyl)-$^{N'}$-ethylcarbodiimide hydrochloride (0.71 g, 3.70 mmol) and diisopropylethylamine (2.0 mL, 11.5 mmol) were added. After stirring the reaction at room temperature for 4 h, propargylamine (0.26 mL, 4.06 mmol) was added and the reaction stirred a further 18 h. The mixture was transferred to a separatory funnel containing ethyl acetate (100 mL) and washed with water (5 x 100 mL). The organic fraction was dried over sodium sulfate, filtered and concentrated to dryness under vacuum. Pure product (49) was obtained as yellow oil following silica gel column chromatography using a gradient of 0-5 % methanol/chloroform. Yield: 0.48 g, 42 %. $R_f$ (10 % methanol/chloroform) = 0.35. $^{1}$H NMR (500 MHz, CDCl₃) δ: 8.49 (d, 2H, J = 4.2 Hz, Py-H), 7.61 (dt, 2H, J = 7.6, 1.7 Hz, Py-H), 7.44 (d, 2H, J = 7.8 Hz, Py-H), 7.11 (m, 2H, Py-H), 7.09 (s, 1H, NH), 4.00 (m, 2H, CH₂), 3.74 (s, 4H, CH₂), 2.50 (t, 2H, J = 6.8 Hz, CH₂), 2.14 (m, 3H, CH₂, CH), 1.58 (m, 4H, CH₂); $^{13}$C{$^{1}$H} NMR (125 MHz, CDCl₃) δ: 173.1, 159.7, 148.9, 136.6, 123.3, 122.1, 80 (assigned using HMBC), 238
71.2, 60.1, 53.3, 35.6, 29.0, 25.3, 23.5; IR (KBr, cm$^{-1}$): $\nu$ 3296, 3051, 2934, 1654;
HRMS (ES+) $m/z$ for C$_{20}$H$_{25}$N$_4$O: calculated: 337.2028, observed: 337.2040 [M+H].

[C$_{33}$H$_{43}$N$_9$O$_2$] (50). Compound 49 (0.096 g, 0.29 mmol), compound 46 (0.077 g, 0.29 mmol), sodium ascorbate (0.029 g, 0.15 mmol), copper (II) acetate monohydrate (0.069 g, 0.35 mmol) were combined in a solution of methanol/water (2:1 v/v, 2 mL) and stirred at room temperature for 15 hours. A solution of ethylenediaminetetraacetic acid disodium salt (0.1 M, 10 mL) was added and the reaction mixture was stirred for an additional 30 min. Sodium hydroxide solution (1 M) was added to the reaction mixture until the pH was greater than ~10. The mixture was added to a separatory funnel and extracted into dichloromethane (3 x 20 mL). The combined organic fractions were dried over sodium sulfate, filtered and concentrated to dryness under vacuum. The product 53 was isolated as an orange solid (0.031 g, 18 %) following silica gel column purification using 10 % methanol/dichloromethane. $R_f$ (10 % methanol/dichloromethane) = 0.22. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$: 8.49 (dd, 2H, $J$ = 4.8, 0.7 Hz, Py-H), 7.67 (s, 1H, CH), 7.62 (dt, 2H, $J$ = 7.6, 1.7 Hz, Py-H), 7.46 (d, 2H, $J$ = 7.8 Hz, Py-H), 7.13 (m, 2H, Py-H), 6.99 (m, 1H, Ar-H), 6.91 (m, 2H, Ar-H), 6.84 (d, 1H, $J$ = 7.7 Hz, Ar-H), 4.47 (d, 2H, $J$ = 5.6 Hz, CH$_2$), 4.45 (t, 2H, $J$ = 6.5 Hz, CH$_2$), 3.84 (s, 3H, OCH$_3$), 3.76 (s, 4H, CH$_2$), 3.05 (s, 4H, NCH$_2$), 2.86 (t, 2H, $J$ = 6.5 Hz, CH$_2$), 2.68 (s, 4H, NCH$_2$), 2.53 (t, 2H, $J$ = 6.9 Hz, CH$_2$), 2.14 (t, 2H, $J$ = 6.9 Hz, CH$_2$), 1.58 (m, 4H, CH$_2$); $^{13}$C($^1$H) NMR (125 MHz, CDCl$_3$) $\delta$: 173.3, 152.3, 149.1, 144.9, 141.2, 136.6, 123.3, 123.2, 122.9, 122.1, 121.1, 118.4, 111.3, 60.2, 57.7, 55.5, 53.5 (2C), 50.6, 47.7, 36.0, 35.0, 25.8, 23.5; IR (KBr, cm$^{-1}$): $\nu$ 3284, 3057, 2938, 2819, 1665, 1500; HRMS (ES+) $m/z$ for C$_{33}$H$_{44}$N$_9$O$_2$: 239
calculated: 598.3618, observed: 598.3607 [M+H].

\[\text{[C}_{36}\text{H}_{43}\text{N}_{9}\text{O}_{5}\text{Re}[\text{Br}]\ (51)\right.\]

Compound 50 (0.030 g, 0.050 mmol) and [Re(CO)\textsubscript{3}(H\textsubscript{2}O)\textsubscript{3}][\text{Br}] (0.031 g, 0.077 mmol) were combined in an Emrys vial and aqueous acetonitrile (50 %, 2 mL) was added. The crimp sealed vial was heated in a microwave reactor for 5 min at 120 °C. The solvent was removed under reduced pressure and the product was isolated as an off-white solid following silica gel column purification using 10 % methanol/dichloromethane. Yield: 0.007 g, 15 %. R\textsubscript{f} (10 % methanol/dichloromethane) = 0.28. \textsuperscript{1}H NMR (600 MHz, CD\textsubscript{3}OD) \(\delta\): 8.85 (d, 2H, \(J\ = 5.1\) Hz, Py-H), 7.98 (s, 1H, CH), 7.94 (dt, 2H, \(J\ = 7.8, 1.5\) Hz, Py-H), 7.58 (d, 2H, \(J\ = 7.9\) Hz, Py-H), 7.37 (t, 2H, \(J\ = 6.4\) Hz, Py-H), 6.95 (m, 4H, Ar-H), 4.91 (AB, 1H, \(J\ = -16.5\) Hz, CH\textsubscript{2}), 4.82 (AB, 1H, \(J\ = -16.5\) Hz, CH\textsubscript{2}), 4.56 (t, 2H, \(J\ = 6.4\) Hz, CH\textsubscript{2}), 4.48 (s, 2H, CH\textsubscript{2}), 3.84 (s, 3H, OCH\textsubscript{3}), 3.82 (m, 2H, CH\textsubscript{2}), 3.02 (s, 4H, NCH\textsubscript{2}), 2.90 (t, 2H, \(J\ = 6.4\) Hz, CH\textsubscript{2}), 2.69 (s, 4H, NCH\textsubscript{2}), 2.39 (t, 2H, \(J\ = 7.1\) Hz, CH\textsubscript{2}), 1.98 (m, 2H, CH\textsubscript{2}), 1.75 (m, 2H, CH\textsubscript{2}); \textsuperscript{13}C\{\textsuperscript{1}H\} NMR (150 MHz, MeOD) \(\delta\): 197.2, 196.4, 175.4, 162.2, 153.9, 153.1, 146.0, 142.3, 141.6, 126.9, 124.8, 124.7, 124.6, 122.2, 119.4, 112.9, 71.5, 68.8, 58.5, 56.0, 54.2, 51.8, 36.2, 35.6, 25.5, 23.8; IR (KBr, cm\textsuperscript{-1}): \(\nu\) 3253, 2937, 2828, 2028, 1914, 1662; HRMS (ES+) \(m/z\) for C\textsubscript{36}H\textsubscript{43}N\textsubscript{9}O\textsubscript{5}Re: calculated: 868.2945, observed: 868.2997 [M+]. UV-HPLC method B \(t\textsubscript{R}\) = 11.2 min

\{[\text{Na}[\text{1-C}_{3}\text{H}_{3}-\text{nido-C}_{2}\text{B}_{9}\text{H}_{9}]]\ (53)\}. Sodium fluoride (0.25 g, 6.0 mmol) was added to a solution of 57 (0.25 g, 0.98 mmol) in methanol (2 mL) in an Emrys microwave vial. The unsealed mixture was heated with stirring at 50 °C for 1 h. Water (2 mL) was added to the mixture and the vial was crimp sealed. The mixture was heated for 15 min
at 185 °C in a microwave reactor. The solvent was removed under reduced pressure and the residue was re-dissolved in methanol (10 mL). The mixture was filtered and the filtrate was concentrated to dryness. The product 53 was isolated following purification on reverse phase silica (12+M) using a gradient of 0-100 % acetonitrile/water to yield a reddish oil. Yield: 0.18 g, 95 %. Rf (10 % methanol/dichloromethane) = 0.33. 1H NMR (500 MHz, CD3OD) δ: 2.52 (AB, 1H, J = -17.8, 2.7 Hz, CH2), 2.29 (AB, 1H, J = -19.0 Hz, CH2), 2.15 (t, 1H, J = 2.9 Hz, CH), 1.79 (s, br, 1H, carborane-H); 11C{1H} NMR (125 MHz, CD3OD) δ: 84.6, 69.2, 56.7(br), 46.7(br), 28.6; 11B{1H} NMR (160 MHz, CD3OD) δ: -10.5, -10.9, -13.7, -17.2, -18.4, -22.2, -33.3, -37.0; IR (KBr, cm⁻¹): ν 3285, 2906, 2519; HRMS (ES-) m/z for C5H14B9: calculated: 173.1933, observed: 173.1951 [M+].

[1-(N-CH3)C6H9N-closo-C2B10H11] (54). N-Methyl propargyl amine (1.6 mL, 19.0 mmol) and diisopropylethylamine (2.5 mL, 14.4 mmol) were added to a solution of 11 (3.0 g, 9.6 mmol) in anhydrous tetrahydrofuran (70 mL) under an inert atmosphere. The reaction mixture was heated at reflux for 23 h and then the solvent was evaporated under reduced pressure. The residue was dissolved in dichloromethane (75 mL) and washed with HCl (0.01 M, 3 x 75 mL) in a separatory funnel. The organic fraction was dried over sodium sulfate, filtered, and concentrated under vacuum. The crude product was purified by silica gel column chromatography with a gradient of 0-5 % methanol/dichloromethane and recovered as an orange oil. Yield: 2.0 g, 82 %. Rf (5 % methanol/dichloromethane) = 0.79. 1H NMR (500 MHz, CDCl3) δ: 3.61 (s, br, 1H, carborane-CH), 3.34 (d, 2H, J = 2.2 Hz, CH2), 2.46 (t, 2H, J = 6.7 Hz, CH2), 2.31 (s, 3H, carborane-CH), 3.44 (s, 3H, N-CH3), 2.34 (s, 3H, N-CH3).
CH₃), 2.29 (m, 2H, CH₂), 2.26 (t, 1H, J = 2.2 Hz, alkyne-CH), 1.67 (m, 2H, CH₂);

¹¹C{¹H} NMR (125 MHz, CDCl₃) δ: 77.5, 75.2, 74.1, 61.6, 54.2, 45.7, 41.5, 35.9, 27.0;

¹¹B{¹H} NMR (160 MHz, CDCl₃) δ: -2.7, -6.3, -9.7, -12.0, -12.5, -13.5; IR (KBr, cm⁻¹):
ν 3304, 3062, 2944, 2802, 2592; HRMS (ES+) m/z for C₉H₂₄B₁₀N: calculated: 256.2839, observed: 256.2844 [M+H].

{[1-(N-(CH₃)₂)C₆H₉N-closo-C₂B₁₀H₁₁][CF₃SO₃]} (55). Methyl triflate (0.33 mL, 2.9 mmol) was added dropwise to a solution of 54 (0.58 g, 2.3 mmol) in anhydrous dichloromethane (40 mL) under an inert atmosphere. The reaction was stirred at room temperature for 48 h, then diluted with dichloromethane (15 mL). The mixture was transferred to a separatory funnel and washed with distilled water (3 x 25 mL). The organic fraction was dried over sodium sulfate, filtered and concentrated to dryness. The product 55 was obtained as an off white-solid following purification by silica gel column chromatography using a gradient of 0-10 % methanol/dichloromethane. Yield: 0.89 g, 93 %.
Rᵣ (10 % methanol/dichloromethane) = 0.29. ¹H NMR (600 MHz, CD₃CN) δ: 4.30 (s, br, 1H, carborane-CH), 4.13 (d, 2H, J = 2.5 Hz, CH₂), 3.26 (m, 2H, CH₂), 3.21 (t, 1H, J = 2.5 Hz, alkyne-H), 3.07 (s, 6H, CH₃), 2.30 (m, 2H, CH₂), 1.90 (m, 2H, CH₂); ¹¹C{¹H} NMR (150 MHz, CD₃CN) δ: 82.8, 75.7, 71.7, 63.5, 55.6, 51.5, 34.3, 23.5, ¹¹B{¹H} NMR (192 MHz, CD₃CN) δ: -2.6, -5.6, -9.3, -11.2, -11.6, -12.7. IR (KBr, cm⁻¹): ν 3302, 3050, 2982, 2591; HRMS (ES+) m/z for C₁₀H₂₆B₁₀N: calculated: 268.3074, observed: 268.3059 [M+].

{[Na][1-C₁₆H₂₂N₅O-nido-C₂B₉H₉]} (66). Aqueous copper (II) sulfate (0.1 M, 0.44 mL, 0.044 mmol) was added to a suspension of 53 (0.15 g, 0.78 mmol), compound
46 (0.27 g, 1.03 mmol) and sodium ascorbate (0.018 g, 0.091 mmol) in aqueous t-butanol (50 %, 6 mL). After stirring for 2.5 h at room temperature, ice-water (20 mL) was added to the reaction. The reaction mixture was filtered under vacuum where a precipitate was collected. The solid was washed with water (40 mL). The filter flask was switched to a clean flask and acetonitrile (40 mL) was added to dissolve the precipitate. The acetonitrile filtrate was concentrated to dryness under reduced pressure and the crude residue was purified by silica gel column chromatography (10 % methanol/dichloromethane). Yield: 0.012 g, 3 %. Rf (10 % methanol/dichloromethane) = 0.85. $^1$H NMR (500 MHz, CD$_3$CN) δ: 7.78 (s, 1H, CH), 6.88-6.98 (m, 4H, Ar-H), 4.53 (t, 2H, $^J$ = 5.9 Hz, CH$_2$), 3.81 (s, 3H, OCH$_3$), 3.12-3.03 (m, 6H, NCH$_2$, CH$_2$), 2.82 (s, 6H, NCH$_2$, CH$_2$) 1.79 (s, br, 1H, carborane-H); $^{13}$C $^1$H NMR (150 MHz, CD$_3$CN) δ: 153.4, 149.6, 142 (assigned using HBMC), 123.9, 123.5, 121.9, 119.4, 112.8, 58.0, 56.0, 54.1, 50.6, 47.4, 46 (assigned using HSQC), 36.3; $^{11}$B $^1$H NMR (160 MHz, CD$_3$CN) δ: -10.4, -11.0, -13.6, -16.4, -19.0, -21.9, -33.4, -37.0; IR (KBr, cm$^{-1}$): ν 2917, 2835, 2521, 1499; HRMS (ES-) m/z for C$_{18}$H$_{33}$B$_9$N$_5$O: calculated: 434.3523, observed: 434.3521 [M+]; UV-HPLC method A $t_R$ = 8.6 min.

[1-C$_{20}$H$_{31}$N$_6$O$^{closo}$-C$_2$B$_{10}$H$_{11}$] (67). Aqueous copper (II) sulfate (0.1 M, 0.49 mL, 0.049 mmol) was added to a solution of 54 (0.24 g, 0.95 mmol), compound 46 (0.29 g, 1.11 mmol) and sodium ascorbate (0.022 g, 0.011 mmol) in aqueous t-butanol (50 %, 4 mL). The reaction was stirred at room temperature for 1 h then ice-water (20 mL) was added. The mixture was transferred into a separatory funnel and the product was extracted with chloroform (3 x 20 mL). The combined organic fractions were dried over
sodium sulfate, filtered and concentrated to dryness. The product 67 was isolated following silica gel column chromatography using a gradient of 0-5 % methanol/dichloromethane. Yield: 0.37 g, 76 %. R<sub>f</sub> (10 % methanol/dichloromethane) = 0.49. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ: 7.58 (s, 1H, CH), 7.01 (m, 1H, Ar-H), 6.92 (m, 2H, Ar-H), 6.86 (m, 1H, Ar-H), 4.49 (t, 2H, J = 6.3 Hz, CH<sub>2</sub>), 3.86 (s, 3H, OCH<sub>3</sub>), 3.65 (s, 2H, CH<sub>2</sub>), 3.56 (s, br, 1H, carborane-H), 3.07 (s, 4H, NCH<sub>2</sub>), 2.89 (t, 2H, J = 6.3 Hz, CH<sub>2</sub>), 2.70 (m, 4H, NCH<sub>2</sub>), 2.35 (t, 2H, J = 6.8 Hz, CH<sub>2</sub>), 2.23 (m, 5H, NCH<sub>3</sub>, CH<sub>2</sub>), 1.65 (m, 2H, CH<sub>2</sub>); <sup>13</sup>C{<sup>1</sup>H} NMR (125 MHz, CDCl<sub>3</sub>) δ: 152.4, 144.4, 141.1, 123.3, 123.2, 121.1, 118.2, 111.4, 75.4, 61.5, 57.7, 55.5, 55.4, 53.5, 52.4, 50.8, 47.8, 42.1, 35.8, 27.3; <sup>11</sup>B{<sup>1</sup>H} NMR (160 MHz, CDCl<sub>3</sub>) δ: -2.8, -6.3, -9.7, -11.9, -12.6, -13.5; IR (KBr, cm<sup>-1</sup>): ν 3047, 2942, 2819, 2584; HRMS (ES+) m/z for C<sub>22</sub>H<sub>43</sub>B<sub>10</sub>N<sub>6</sub>O: calculated: 517.4429, observed: 517.4451 [M+H].

{{[1-C<sub>21</sub>H<sub>34</sub>N<sub>6</sub>O-closo-C<sub>2</sub>B<sub>10</sub>H<sub>11</sub>][CF<sub>3</sub>SO<sub>3</sub>]] (68). Aqueous copper (II) sulfate (0.1 M, 0.24 mL, 0.024 mmol) was added to a solution of 55 (0.20 g, 0.48 mmol), compound 46 (0.15 g, 0.57 mmol) and sodium ascorbate (0.013 g, 0.066 mmol) in methanol (3 mL). After stirring the reaction at room temperature for 1 h, ice-water (10 mL) was added. The mixture was transferred into a separatory funnel and extracted with dichloromethane (3 x 10 mL). The combined organic fractions were dried over sodium sulfate, filtered and concentrated to dryness. The product 68 was isolated following silica gel column chromatography using a gradient of 5-10 % methanol/dichloromethane. Yield: 0.21 g, 65 %. R<sub>f</sub> (10 % methanol/dichloromethane) = 0.13. <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) δ: 8.57 (s, 1H, CH), 7.21 (m, 1H, Ar-H), 7.16 (m, 2H, Ar-H), 7.10 (m, 1H, 244
Ar-H), 4.85 (t, 2H, J = 6.1 Hz, CH₂), 4.76 (s, 1H, carborane-H), 4.06 (s, 3H, OCH₃), 3.55 (s, 6H, NCH₃), 3.51 (s, 2H, CH₂), 3.45 (m, 2H, CH₂), 3.23 (s, 4H, NCH₂), 3.15 (t, 2H, J = 6.1 Hz, CH₂), 2.92 (s, 4H, NCH₂), 2.55 (m, 2H, CH₂), 2.30 (m, 2H, CH₂); ¹³C{¹H} NMR (125 MHz, CD₃OD) δ: 151.0, 139.4, 133.4, 127.1, 121.8, 120.2 (CF₃SO₃⁻), 119.4, 117.7 (CF₃SO₃⁻), 116.6, 110.0, 72.8, 60.7 (2C), 56.7, 55.4, 53.1, 49.0, 48.2, 46 (assigned using HSQC), 32.1, 21.0; ¹¹B{¹H} NMR (160 MHz, CDCl₃) δ: -2.4, -5.6, -9.2, -11.2, -12.6; IR (KBr, cm⁻¹): ν 3045, 2942, 2822, 2590, 1499; HRMS (ES⁺) m/z for C₂₃H₄₅B₁₀N₆O: calculated: 531.4585, observed: 531.4587 [M⁺].

{[C₈H₂₀N][3,3,3-(CO)₃-1-C₁₆H₂₂N₅O-3,1,2-closo-ReC₂B₉H₁₀]} (69). Compound 66 (0.011 g, 0.024 mmol) was dissolved in acetonitrile (0.25 mL) in an Emrys vial. Diisopropylethylamine (DIPEA, 7 μL, 0.040 mmol), solid [Re(CO)₃(H₂O)₃][Br] (0.018 g, 0.045 mmol) and water (0.25 mL) were added to create a suspension. The crimp sealed vial was heated in the microwave reactor at 160 °C for 10 min. The solvent was removed from the reaction mixture under reduced pressure. The product 69 was isolated following purification by silica gel column chromatography (10 % methanol/dichloromethane).

Yield: 0.014 g, 69 %. Rf (10 % methanol/dichloromethane) = 0.32. ¹H NMR (500 MHz, CDCl₃) δ: 8.24 (s, br, 1H, NH-DIPEA), 7.67 (s, 1H, CH), 7.00 (m, 1H, Ar-H), 6.93 (m, 2H, Ar-H), 6.85 (m, 1H, Ar-H), 4.46 (t, 2H, J = 5.7 Hz, CH₂), 3.83 (m, 4H, OCH₃, CH-DIPEA), 3.26 (s, 4H, CH₂, CH₂-DIPEA), 3.09 (s, 4H, NCH₂), 2.91 (s, 2H, CH₂), 2.74 (s, 4H, NCH₂), 1.75 (s, br, 1H, carborane-H) 1.46 (m, 15 H, CH₃-DIPEA); ¹³C{¹H} (125 MHz, CDCl₃) δ: 199.5, 152.3, 145.2, 140.9, 124.8, 123.4, 121.3, 118.6, 111.4, 57.6, 55.6, 55.3, 53.5, 51.9, 50.6, 48.2, 43.3, 36.5, 29.3, 19.2, 17.9, 12.5; ¹¹B{¹H} NMR (160 MHz, CDCl₃) δ: 32.1, 21.0;
MeOD) $\delta$: -5.5, -7.9, -10.3, -11.6, -18.4, -19.9. IR (KBr, cm$^{-1}$): ν 2995, 2823, 2549, 1995, 1890; HRMS (ES-) m/z for C$_{21}$H$_{32}$B$_9$N$_5$O$_4$Re: calculated: 702.2910, observed: 702.2874 [M$^+$. UV-HPLC method A $t_R = 10.9$ min.

$\{[C_8H_{20}N][3,3,3-(CO)_{3}-1-C_{20}H_{31}N_{6}O_{3,1,2-closo-ReC_2B_9H_{10}}]\}$ (70). Compound 67 (0.037 g, 0.072 mmol) and diisopropylethylamine (0.025 mL, 0.14 mmol) were suspended in aqueous acetonitrile (70 %, 0.7 mL) solution in an Emrys vial. The crimp sealed vial was heated in a microwave reactor for 5 min at 180 °C. To the resulting solution, solid [Re(CO)$_3$(H$_2$O)$_3$][Br] (0.031 g, 0.077 mmol) was added and the vial was re-sealed and heated in the microwave reactor for 5 min at 180 °C. Two subsequent additions of [Re(CO)$_3$(H$_2$O)$_3$][Br] (0.030-0.032 g, 0.077 mmol) and diisopropylethylamine (0.012 mL, 0.069 mmol) were made, each followed by heating for a further 10 min at 190 °C. The reaction mixture was concentrated to dryness under reduced pressure. The residue was suspended in chloroform (10 mL) and washed with water (3 x 10 mL) in a separatory funnel. The organic fraction was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (5-10 % methanol/dichloromethane) to yield 70 (0.027 g, 45 %). $R_f$ (10 % methanol/dichloromethane) = 0.44. $^1$H NMR (500 MHz, CD$_3$CN) $\delta$: 8.04 (m, 1H, CH), 6.95 (m, 1H, Ar-H), 6.90 (m, 3H, Ar-H), 4.56 (m, 2H, CH2), 4.41 (m, 2H, CH$_2$), 3.83 (m, 2H, CH$_2$), 3.80 (s, 3H, OCH$_3$), 3.78 (m, 2H, CH$_2$), 3.67 (m, 2H, CH-DIPEA), 3.15 (m, 2H, CH$_2$-DIPEA), 3.12 (s, 2H, CH$_2$), 2.99 (s, 4H, NCH$_2$), 2.89 (s, 5H, CH$_2$, NCH$_3$), 2.63 (s, 4H, NCH$_2$), 1.85 (s, br, 1H, carborane-H), 1.32 (m, 15H, CH$_3$-DIPEA); $^{13}$C ($^1$H) NMR (125 MHz, CD$_3$CN) $\delta$: 200.61, 198.89, 198.50, 246
195.76, 192.59, 192.29, 153.40, 149.49, 149.45, 148.86, 142.52, 125.10, 124.59, 123.65,
121.99, 121.94, 119.14, 119.12, 112.78, 67.37, 67.30, 63.31, 57.42, 57.37, 57.22, 57.19,
56.98, 56.06, 55.94, 53.92, 52.61, 51.36, 51.32, 49.84, 49.80, 49.73, 44.05, 38.42, 38.40,
37.97, 37.93, 29.77, 27.46, 27.43, 25.37, 18.73, 17.36, 12.98; ¹¹B{¹H} NMR (160 MHz,
CD₃CN) δ: -5.4, -7.9, -10.1, -11.6, -18.3, -19.9; IR (KBr, cm⁻¹): ν 2944, 2821, 2545,
2025, 1995, 1888; HRMS (ES⁺) m/z for C₂₅H₄₁B₉N₆O₄Re: calculated 775.3584,
observed: 775.3638 [M⁺]. UV-HPLC method A tᵣ = 11.9 min

[3,3,3-(CO)₃-1-C₂₁H₃₄N₆O-3,1,2-closo-ReC₂B₉H₁₀] (71). To a solution of 68
(0.035 g, 0.052 mmol) in acetonitrile:water (2:1 v/v, 0.8 mL) in an Emrys vial,
diisopropylethylamine (9.0 μL, 0.052 mmol) was added. The vial was sealed and heated
in a microwave reactor for 10 min at 180 °C. Diisopropylethylamine (9.0 μL, 0.052
mmol) and solid [Re(CO)₃(H₂O)₃][Br] (0.023 g, 0.057 mmol) were added to the vial
which was re-sealed and subsequently heated for a further 10 min at 180 °C. A second
addition of diisopropylethylamine (9.0 μL, 0.052 mmol) and [Re(CO)₃(H₂O)₃][Br] (0.023
g, 0.057 mmol) was performed and the reaction mixture was heated at 190 °C for 10
additional minutes. The mixture concentrated to dryness under vacuum and purified by
silica gel column chromatography using acetonitrile as the eluent. Yield 0.029 g, 71 %.
Rᵣ (100 % acetonitrile) = 0.57. ¹H NMR (500 MHz, CD₃CN) δ: 8.31 (s, 1H, CH), 6.95
(m, 1H, Ar-H), 6.89 (m, 3H, Ar-H), 4.53 (t, 2H, J = 5.9 Hz, CH₂), 4.45 (s, 2H, CH₂), 3.80
(s, 3H, OCH₃), 3.02 (m, 6H, CH₂, NCH₂), 2.94 (s, 6H, CH₃), 2.84 (t, 2H, J = 5.9 Hz,
CH₂), 2.62 (s, 4H, NCH₂), 1.88 (m, 4H, CH₂), 1.84 (s, br, 1H carborane-H), 1.32 (m, 2H,
CH₂), ¹³C{¹H} NMR (125 MHz, CDCl₃) δ: 200.5, 153.4, 142.5, 135.6, 129.3, 123.7,
122.0, 119.1, 112.8, 64.4, 59.6, 57.9, 56.0, 53.9, 53.6, 51.4, 51.2, 48.5, 37.3, 29.8, 23.6; 

$^{11}$B{\textsuperscript{1}H} NMR (160 MHz, CDCl$_3$) δ: -5.5, -8.0, -10.1, -11.6, -18.3, -19.8; IR (KBr, cm$^{-1}$): ν 2943, 2822, 2537, 1994, 1884; HRMS (ES+) m/z for C$_{26}$H$_{45}$B$_9$N$_6$O$_4$Re:

- calculated: 791.3897, observed: 791.3980 [M+H]. UV-HPLC method A $t_R = 12.1$ min

5.10. Radiochemistry

Pertechnetate [$^{99m}$TcO$_4$]$^{-}$ was obtained from a $^{99}$Mo/$^{99m}$Tc generator (Lantheus Medical Imaging) in saline (0.9% NaCl). Compound [$^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^{+}$ was prepared according to the literature procedure. Reactions involving microwave heating were performed on a Biotage Initiator 8 microwave reactor using crimp-sealed Emrys vials. Solutions of acetonitrile and aqueous acetonitrile (50%, 2:1 v/v) were de-oxygenated before used. Solid phase extraction cartridges (C18) obtained from Waters were used following pre-treatment with water (10 mL), methanol or ethanol (10 mL) and a second wash with water (10 mL) and 10 mM HCl solution (10 mL). A Capintec dose calibrator was used to determine the activity of samples containing technetium-99m.

$[\text{C}_{36}\text{H}_{43}\text{N}_{9}\text{O}_{5}\text{^{99m}}\text{Tc}]$ (52). In a typical reaction, [$^{99m}$Tc(CO)$_3$(H$_2$O)$_3$]$^{+}$ (257-374 MBq, 0.5 mL) was added to a solution of 50 (0.5 mg, 0.8 μmol) in acetonitrile (0.5 mL) in an Emrys vial. The crimp sealed vial was heated in a microwave reactor for 5 min at 120 °C. Radiochemical yield (based on radio-HPLC analysis): 91%. Radio-HPLC method B $t_R = 11.8$ min.

$\{[\text{Na}][3,3,3-(\text{CO})_{3}\text{-1-C}_{16}\text{H}_{22}\text{N}_{5}\text{O}_{3,1,2-\text{closo}}\text{-99mTcC}_{2}\text{B}_{9}\text{H}_{10}]\}$ (72). In a typical experiment, a solution of 66 (0.6 mg, 1.3 μmol) in aqueous acetonitrile (50%, 0.5 mL)
and \([^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+\) (103–203 MBq, 0.5 mL) were combined in an Emrys vial. The crimp sealed vial was heated in a microwave reactor for 5 min at 180 °C. The reaction mixture was loaded onto a solid phase extraction cartridge which was washed with HCl (10 mM, 7 mL) and 10 mM HCl:acetonitrile (1:1, 2 mL). The product (72) was then eluted in acetonitrile (8 mL). Decay corrected radiochemical yield: 63 %. Radio-HPLC method A \(t_R = 11.3\) min.

\{[\text{Na}][3,3,3-(\text{CO})_3-1-\text{C}_{20}\text{H}_{31}\text{N}_6\text{O}-3,1,2-\text{closo-}^{99m}\text{TcC}_2\text{B}_9\text{H}_{10}]\} (73). In a typical reaction, compound 67 (0.7 mg, 1.4 μmol) and sodium fluoride (0.5 mg, 11.9 μmol) were combined in an Emrys vial and aqueous acetonitrile (50 %, 0.5 mL) was added. The crimp sealed vial was heated in a microwave reactor (180 °C, 5 min) to generate the nido-carborane derivative of 67 in situ. \([^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+\) (233–430 MBq, 0.5 mL) was added to the reaction vial and the mixture was heated in a microwave reactor for 10 min at 180 °C. Following the reaction, the mixture was loaded onto a solid phase extraction cartridge and washed with HCl (10 mM, 7 mL) and 10 mM HCl:acetonitrile (50 %, 2 mL). The product (73) was eluted in acetonitrile (8 mL). Decay corrected radiochemical yield: 49 %. Radio-HPLC method A \(t_R = 12.1\) min.

\[3,3,3-(\text{CO})_3-1-\text{C}_{21}\text{H}_{34}\text{N}_6\text{O}-3,1,2-\text{closo-}^{99m}\text{TcC}_2\text{B}_9\text{H}_{10}\] (74). In a typical experiment, compound 68 (2.4 mg, 3.5 μmol) and sodium fluoride (2.2 mg, 52.4 μmol) were combined in an Emrys vial and aqueous acetonitrile (2:1 v/v, 0.5 mL) was added. The crimp sealed vial was heated in a microwave reactor (180 °C, 5 min) to generate the nido-carborane derivative of 68 in situ. \([^{99m}\text{Tc}(\text{CO})_3(\text{H}_2\text{O})_3]^+\) (339–392 MBq, 0.5 mL) was added to the reaction vial and the mixture was heated in a microwave reactor for 10
min at 180 °C. The crude reaction mixture was passed through a C18 solid phase extraction cartridge where residual 99mTc salts were eluted with HCl (10 mM) and 10 mM HCl:acetonitrile (50 %, 2 mL). The product was eluted in acetonitrile (8 mL). Decay corrected radiochemical yield: 53 %. Radio-HPLC method A $t_R = 12.7$ min

5.11. References


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Chapter 6 – Future Directions

There are a series of experiments that are warranted based on the data arising from the research described in this thesis. These experiments were not completed owing to time and lack of resources needed to perform assays in-house. Nonetheless, the completion of the following studies could yield important information to further support the hypotheses stated in this thesis. There are some short term goals that should be completed and there are also three promising areas of research emerging from this work are described below.

6.1. Short term projects

Three rhenacarborane complexes 12, 13 and 22b (Figure 6.1) were evaluated for their binding affinities towards CNS receptors. Complex 22b was found to have high and selective binding to the α-adrenergic receptors. The synthetic strategy employed in preparing the rhenacarborane complex 22b generated an additional rhenacarborane derivative that has not yet been evaluated. The minor isomer 22a (prepared from the reaction between 16 and [Re(CO)3(H2O)3]+; chapter 3) is a candidate for evaluation of its receptor binding affinity towards CNS receptors. For compounds to have high binding to a specific receptor, the compound needs to fit into the binding pocket and/or have specific association with groups in the binding region. The variation in binding affinity may provide further information regarding the binding pocket itself since complexes 22a and 22b have the same targeting vector in the complex but the relative position of the substituents on the carborane cage is different.
Figure 6.1. Structures of rhenium complexes 12, 13, 22a and 22b

The preparation of the neutral complexes 30, 34 and 37 in the form \([\text{Re(CO)}_2(\text{NO})\text{RC}_2\text{B}_9\text{H}_{10}]\) (chapter 4) led to a synthetic strategy that involved the initial generation of nitro- and amino-substituted derivatives. The substituents in the phenyl group and position of the substituents in the ring have been shown to some affect the binding affinities to neurotransmitter receptors and transporters.\(^1\)-\(^4\) Evaluation of the affinity of complexes 29, 32, 33, 35 and 36 towards CNS receptors is also warranted where these additional complexes may show potent and selective affinity to specific receptors leading to identification of a potential \(^{99m}\text{Tc}\)-carborane imaging agent (Figure 6.2).
In chapter 5, “click” chemistry was used to conjugate the arylpiperazine biomolecule to the carborane ligand and a series of metallocarborane WAY-complexes (M = Re, $^{99m}$Tc) was prepared. Measuring the log P values of the $^{99m}$Tc complexes 72-74 would assist in determining the lipophilicity associated with each linkage (Figure 6.3). Estimates of lipophilicity have been determined using one of three reported methods: (1) computational models based on summation of molecular fragments, (2) instrumental techniques (e.g. UV-Vis spectroscopy, gas chromatography or HPLC) and (3) partition measurements between water or buffer and $n$-octanol. The change in log P associated with 53 and 54 could be estimated using a computer program; for instance, there is an increase in the calculated log P (from CambridgeSoft ChemDraw program) of approximately +0.4 to +0.5 units due to the increase in the CH$_2$ units from 53 to 54 (Figure 6.3). Unfortunately, programs do not accurately recognize electrostatic charges, polyhedral carborane clusters or metal atoms thus, an accurate calculated value for the carborane complexes can not be determined while instrumental techniques are tedious and has it inherent limitations. Physically measuring the lipophilicity of $^{99m}$Tc
complexes 72-74 using the shake-flask method remains the gold standard and is the only viable option. The log P values would assist in the selection of the desired linkage when preparing targeted carborane complexes.

Figure 6.3. Structures of 53, 54, 72-74.

6.2. Long term projects

6.2.1. Investigation of the stability of $[^{99m}\text{Tc} (\text{CO})_2 (\text{NO})]^{-}$-carborane complexes

In chapter 4, the neutral $[^{99m}\text{Tc} (\text{CO})_2 (\text{NO})]^{-}$-carborane complexes (38, 40 and 41) were found to decompose in the presence of nucleophilic solvents such as ethanol and DMSO (Figure 6.4). These solvents were selected for use because, unlike many other organic solvents, the presence of small amounts of ethanol and DMSO is permitted in biological studies. Mono- and di-substituted $[^{99m}\text{Tc} (\text{CO})_2 (\text{NO})]^{-}$-carborane complexes (i.e. phenyl-, benzyl- and dibenzyl-substituted) have been reported in literature and have been observed to decompose under aqueous conditions. The stabilities of these
complexes were not evaluated in ethanol or DMSO since these complexes were not intended to undergo biological evaluations. On the other hand, Alberto and co-workers were able to prepare the chelate complexes of the form \([M(CO)2(NO)]\) (\(M = \text{Re, } ^{99m}\text{Tc}\)), where the syntheses were achieved in the presence of ethanol and no observed degradation was noted for the \(^{99m}\text{Tc}\) complex. While research into the stability and reactivity of the \([\text{Re}(CO)2(NO)]\) complex has been extensive in comparison to the \([^{99m}\text{Tc}(CO)2(NO)]\), a greater number of \(^{99m}\text{Tc}\) complexes need to be prepared and examined in order to gain a greater understanding associated with their reactivity and stability. There are significant differences associated with rhenium and technetium-99m complexes both in literature and in this thesis. There is a need to further understand the stability of these neutral carborane-based \(^{99m}\text{Tc}\) complexes to prevent degradation and expand the research associated with these carborane-based metal complexes. These results would also impact other research groups working with nitrosated Tc-based complexes. Experiments that determine whether or not steric and/or electronic effects are associated with degradation from nucleophilic solvents should be completed by varying the structure around the carborane core. LC-MS analysis could be used to identify by-products and provide information regarding the process of degradation.
6.2.2. Experimental optimization and change of lipophilicity of metallocarboranes for radioimaging applications

This thesis shows that the carborane-based $^{99m}$Tc complexes described have lipophilicity values that are in the correct range to cross the BBB but the anionic charge may prevent crossing the membrane. A compound’s charge and lipophilicity could be optimized using the following methods including: (1) addition of a charge compensation group to the unsubstituted carbon atom or boron atom, (2) addition of a polar group to the unsubstituted carbon atom or (3) introduction of a diethylene glycol spacer to a boron atom. These options would be investigated and their relative merits compared to one another to help identify the best mode of altering lipophilicity. The chemistry accomplished in this proposed research would lead to the development of some general methods for improving the lipophilicity of other carborane complexes for MI applications.

Alternative modes of charge compensation to generate neutral $^{99m}$Tc carborane complexes include the addition of a methylated pyridyl group ($75^{8-10}$ to the unsubstituted

Figure 6.4. Structures of 38, 40 and 41.
carbon atom or a dialkylsulfidium \((76)^{11,12}\) or trialkylammonium \((77)^{12}\) groups to a BH unit rendering the complex neutral overall (Figure 6.5). The addition of these groups will add steric bulk to the complex which may also affect the binding associated with the resulting complex depending on the target receptor site.

**Figure 6.5.** Structures of charge compensated metallocarborane complexes \(75-77\).

Carboranes are lipophilic compounds and this was observed with the measured log \(P = 2.4-2.6\) for complexes \(14a, 15\) and \(23\). These anionic complexes, \([{^{99m}\text{Tc}(\text{CO})_3\text{RC}_2\text{B}_9\text{H}_{10}]^-}\), are within the ideal range to cross the BBB (log \(P = 2-3.5\)).\(^{13}\) A neutral complex made by replacing a CO unit with a \([\text{NO}]^+\) in the \([\text{Re}(\text{CO})_3]^-\)-core increased the log \(P\) value by \(+0.68\) for a phenol rhenacarborane compound.\(^{14}\) Addition of these lipophilic and charge compensation group by nitrosation would likely increase the log \(P\) to a value that is higher than the ideal range to cross membrane barriers. A complex that is too lipophilic could bind non-specifically to proteins or get trapped in membrane layers leading to a poorly resolved image. In order to counterbalance the increase in lipophilicity associated with charge compensation using the \([\text{NO}]^+\), considerations need to be made with the introduction of a polar group which would subsequently keep log \(P\) within the desired range. One method would be to add polar
functional groups such as carboxylic acid (78),\textsuperscript{15, 16} alcohol (79),\textsuperscript{17, 18} amide (80)\textsuperscript{16} or ester (81)\textsuperscript{19} linkages to the unsubstituted carbon atom where the preparation of these carborane compounds have been reported in literature (Figure 6.6). The introduction of the targeting biomolecule could be either introduced before or after the addition of these four groups. Lipophilicity values provide a guide to whether a compound or complex has the ability to cross the BBB.

\begin{center}
\includegraphics[width=\textwidth]{carborane_compounds.png}
\end{center}

**Figure 6.6.** Structures of carborane compounds 78-81.

An alternative to substitution at the carbon atom would be to incorporate a polar linker between the carborane cage and targeting vector which is a viable approach for dicarbon-substituted carboranes. However, as demonstrated in this thesis, modification to the linkage between the targeting vector and carborane cluster can have a dramatic impact on the binding affinity of the complex. Another option would be to attach a polar group or linkage to a boron atom away from the targeting vector which would minimize the binding affects but change the overall polarity of the complex. A method to prepare this type of linkage would include a ring opening of cyclic oxonium derivatives with hydroxide (Scheme 6.1). This type of reaction has been reported for the oxonium derivatives of closo-dodecaborate\textsuperscript{20, 21} and cobalt bis(dicarbollide).\textsuperscript{22, 23}
Many cobaltacarborane complexes have been used for metal extraction\textsuperscript{24} and as boron neutron capture therapy (BNCT) agents.\textsuperscript{25} A common method of derivatizing these complexes at a boron atom is to react the cobaltacarborane with 1,4-dioxane in the presence of BF\textsubscript{3}·Et\textsubscript{2}O\textsuperscript{24-26} to generate an oxonium substituted complex (Scheme 6.2). The synthesis of cyclic oxonium derivatives of other metallocarborane as well as monocarborane anions [CB\textsubscript{11}H\textsubscript{12}]\textsuperscript{-} and [1-CB\textsubscript{9}H\textsubscript{10}]\textsuperscript{-} have been reported.\textsuperscript{20} A ring-opening reaction occurs in the presence of a variety of nucleophilic (Nu) reagents such as fluoride, chloride, and hydroxide anions, imide, cyanide and amines, phenolate and pyrrolyl salts.\textsuperscript{20,22,25} The resulting product is a diethylene glycol spacer between the cluster and the nucleophile. If an oxonium group was introduced to Re(I) and \textsuperscript{99m}Tc(I) metallocarborane complexes and reacted with a tertiary amine, the resulting complex would be a zwitterionic and this methodology could be another alternative method of charge compensation disubstituted metallocarboranes (Scheme 6.3).
Scheme 6.2 Preparation of cobaltacarborane complexes containing a diethylene glycol linkage.

Scheme 6.3 Proposed synthesis to introduce a polar linkage and charge compensating modality into disubstituted metallocarborane complexes.

6.2.3. Development of “click” methodology for the incorporation of sensitive targeting vectors into radiometal imaging probes

In chapter 5, the use of “click” chemistry to conjugate a targeting vector to the carborane ligand was developed. To further investigate the strength of using “click” chemistry in the preparation of targeted $^{99m}$Tc probes, other targeting compound containing various functional groups and/or thermally sensitive biovectors should be explored. For example, the preparation of metallocarborane complexes ($M = \text{Re}, \ 99m\text{Tc}$) conjugated to a carbohydrate molecule$^{27}$ and a phenol derivative$^{14}$ have been reported. The synthetic procedures required the protection of nucleophilic group(s) that could
degrade the borane compound during the alkyne insertion reaction, followed by removal of these same protecting group(s) to prepare the desired carborane conjugate (Scheme 6.4).

Scheme 6.4 Synthetic procedures for the preparation of (a) carbohydrate, (b) phenol-substituted metallocarborane complexes.

The application of “click” chemistry could be used to improve the synthesis of these metallocarborane complexes without the need for the extra protection and deprotection synthetic steps. The azide derivative of these two biomolecules could be reacted with one of the carborane-alkyne (53, 54 or 55) in a single step where the triazole ring would be used a linker (Scheme 6.5). Following, the preparation of the corresponding metallocarborane could be accomplished.
Scheme 6.5 Carborane “click” chemistry for the preparation of carbohydrate and estradiol analogues without the need for extra protection and deprotection steps.

The use of carborane “click” chemistry could extend to other target compounds containing a greater variety of functional groups such as the peptide bombesin which has affinity for tumors and a flutamide derivative which targets overexpressed receptors in the early stages of prostate cancer. The advantage of using the diagnostic radioisotope technetium-99m is that it can be paired with the therapeutic radioisotope rhenium-186/188 for the generation of analogous therapeutic agent. One major disadvantage of preparing metallocarboranes is the high temperatures required for complexation with the metal to the carborane conjugate. This issue must be addressed for carboranes to be used as ligands for the preparation of molecular imaging probes and targeted therapeutics.

6.3. References


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