THE PREPARATION OF METALLOCARBORANE AND IODINATED CARBORANE AMINO ACID ANALOGUES FOR MOLECULAR IMAGING AND THERAPY

THE PREPARATION OF METALLOCARBORANE AND IODINATED CARBORANE AMINO ACID ANALOGUES FOR MOLECULAR IMAGING AND THERAPY

By

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Abstract

This thesis demonstrates the versatility of the carborane cage by using a known carborane containing analogue of the amino acid phenylalanine, carborananylalanine (Car **5**) as a novel platform for radiolabelling targeting vectors with both ^{99m}Tc and ^{125}I. Initial racemic synthesis of Car was undertaken based on a literature procedure. Optimization of the procedure yielded all key intermediates leading to *closo*-carboranylalanine which was obtained in 67% yield. A new method for cage degradation of Car **5** involving microwave heating in water to give the desired *nido*-Car **9** was developed such that the product could be isolated in quantitative yield where the only additional product was boric acid. The removal of boric acid was non-trivial but was ultimately achieved through conversion to the more volatile borate ester.

The synthesis of Re-Car **12** was achieved by microwave heating *nido*-Car **9** with $[Re(CO)_3(H_2O)_3]Br$ at 180 °C for 15 min in a microwave. The reaction produced multiple carborane products including the desired product **12**, an amino acid rhenium chelate complex **15**, and a di-rhenium complex **14**. Conditions were altered to maximize the amount of the desired compound which was separated from impurities through semi-preparative HPLC albeit in low yield (3%). Analysis of **12** by ^{1}H nOe NMR experiments revealed that cage isomerization had occurred under the employed conditions resulting in the formation of the 2, 1, 8-cage isomer of **12**.

The iodination of *nido*-Car was found to take place in high yield at room temperature with a reaction time of less than 10 minutes. When the stoichiometry was kept to a 1:1 ratio between **9** and I_2 no other side products were observed and purification by semi-preparative HPLC gave pure I-Car **16** in 48% yield.

It was found that the radiolabelling of *nido*-Car with ^{99m}Tc proceeded with very few side products as compared to the cold rhenium standard (Re-Car **12**). Variations of the labelling conditions (time, temperature and pH) resulted in a 45% percent conversion to the desired ^{99m}Tc-Car **13**. The HPLC retention time of the ^{99m}Tc product correlated to the Re-Car **12** reference standard.

Radiolabelling of *nido*-Car with ^{125}I using Iodogen® as an oxidant was fast, efficient and high yielding. It was found that under the standard labelling conditions, *nido*-Car would give high conversion (>95%) to ^{125}I-Car **18** in less than 10 minutes at room temperature. Not only was **9** highly reactive towards radioiodination, but the resulting product was found to be remarkably stable with no signs of degradation up to two weeks.

A dilution study to examine the reactivity of **9** towards radioiodination was performed. At concentrations of 4.5 mM and 2.3 mM the conversion of **9** to ^{125}I-Car **18** occurred in >95% conversion and 70% conversion occurred at a ligand concentration of 1.1 mM. A head-to-head experiment using equimolar amounts of tyrosine to *nido*-Car was also performed and found that ^{125}I-Car was formed prefentially with an average percent conversion of 93%.

This work demonstrates that a versatile radiolabelling platform using carboranes can be developed. Because the ligand is a non-natural amino acid it can serve for preparing bioconjugates and targeted molecular imaging and therapy agents.

Acknowledgements

There are so many people I need to thank for supporting me over the long journey that was my Masters. First and foremost I would like to thank Dr. John Valliant. Thank you so much for providing me with so many wonderful opportunities, for the guidance and motivation (e.g. taking on that boric acid bet) and for inspiring me to go after what I want. I would also like to thank my other committee members Dr. David Emslie and Dr. Paul Harrison for their useful opinions on the project, and the many facility staff for their reliable and prompt work with samples. I would also like to acknowledge Laura Harrington and Alicia Jeanveau for their hard work keeping the Valliant group in order, and helping me out with my work as well.

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List of Abbreviations

ACN	Acetonitrile
β^+	Positron
β^-	Beta (negative) particle
BNCT	Boron neutron capture therapy
BPA	p-Boronophenylalanine
br	Broad signal (NMR spectroscopy)
Car	Carboranylanine
Chloramine-T	*N*-chloro-*p*-toluenesulfonamide, sodium salt
cm^{-1}	Wavenumbers (IR)
COSY	Correlation spectroscopy (NMR)
Cp	Cyclopentadiene
d	Doublet (NMR)
dd	Doublet of doublets (NMR)
D	Deuterium (in NMR solvent)
DOPA	3,4-Dihydroxy-6-phenylalanine
DTPA	Diethylene triamine pentaacetic acid
E_γ	Gamma ray energy
E_β	Beta particle energy
ESI$^-$	Negative ion electrospray mass spectroscopy
EtOH	Ethanol
eV	Electron Volt
FDOPA	3,4-Dihydroxy-6-[^{18}F]fluoro-$_L$-phenylalanine
γ	Gamma ray
HMBC	Heteronuclear Multiple Bond Correlation (NMR)
HMPAO	Hexamethylpropyleneamine oxime
HPLC	High Performance Liquid Chromatography
hr	Hours
HRMS	High Resolution Mass Spectroscopy
HSQC	Heteronuclear Single Quantum Coherence (NMR)
Hz	Hertz
IMT	Iodo-alpha-methyl-$_L$-tyrosine
Iodogen®	1, 3, 4, 6-tetrachloro-3α, 6α-diphenylglycouril
IR	Infrared spectroscopy
J	Coupling constant (NMR)
LAT1	$_L$-type amino acid transporter
LCMS	[High Performance] Liquid chromotagraphy-mass spectroscopy

m	Multiplet (NMR)
MDP	Methylene diphosphonate
MeOH	Methanol
MET	Methionine
MIBG	Meta-iodobenzylguanidine
min	Minutes
MRI	Magnetic Resonance Imaging
NMR	Nuclear Magnetic Resonance Spectroscopy
o	*Ortho*
ov	Overlapping (peaks in NMR)
PET	Positron Emission Tomography
ppm	Parts per million (chemical shift)
q	Quartet
RT	Room Temperature
s	Singlet (NMR)
SAAC	Single amino acid chelate
SPECT	Single Photon Emission Computed Tomography
t	Triplet (NMR)
$t_{1/2}$	Half-life
TLC	Thin Layer Chromatography
t_r	Retention time (HPLC)
VFC	Venus Flytrap Cluster

Chapter 1

Introduction and Objectives

1.1 Molecular Imaging

Molecular imaging (MI) is a multidisciplinary science that can be broadly described as the characterization and measurement of biological processes at the cellular and molecular levels.[1] Cassidy and Radda[2] elaborated, indicating the term "molecular imaging" can be used to describe numerous events including the imaging of molecules which are present in the living system, the use of reporter agents that monitor specific cellular processes or molecular targets and the application of natural or labelled substrates to follow specific pathways. MI techniques utilize nearly the entire electromagnetic spectrum including ultrasonic, gamma ray and X-ray frequencies. The *in vivo* MI techniques which are playing a key role in the field today are: optical imaging, magnetic resonance imaging (MRI), ultrasound and nuclear imaging methods, which include positron emission tomography (PET) and single photon emission computed tomography (SPECT)[2]; these modalities rely on the development of targeted imaging agents to create a means to image specific biological targets/processes.

1.2 Radioimaging and Radiotherapy

In the case of molecular radioimaging, compounds containing radioactive elements are administered to the subject under study and the emitted radiation is observed by a series of external detectors. The two primary types of *in vivo* imaging techniques are PET and SPECT. PET scanners detect the radiation given off when positron emitting isotopes decay (i.e 511 keV photons), while SPECT systems are designed to detect lower energy gamma rays given off by specific radionuclides.

Radioimaging is attractive in that it has exquisite sensitivity since only minute quantities of radiolabelled material are required to generate high signal to noise ratios. The small amount of material is the basis of the tracer principle, where a minute quantity of radioactive substance (a radiopharmaceutical) is enough to obtain good images, but not so much to interfere with the biological system itself.[3,4]

Some radioisotopes can also be used for therapeutic applications. A number of targeted radiotherapeutic agents using β^-, x-ray and α-emitting isotopes have been reported.[5,6,7] At present there are only a handful of clinically approved compounds for radiation therapy and these are based on the β^- isotopes ^{90}Y and ^{131}I. Ibritumomab tiuxetan, sold under the name Zevalin™ and tositumomab (typically sold as Bexxar™) are both radiolabelled monoclonal antibodies. These drugs are both used for the treatment of non-Hodgkin lymphoma. Zevalin can be labelled with In-111 for imaging purposes prior to cancer treatment with the Y-90 labelled derivative.[8] Another common

radiotherapy drug is [^{131}I]MIBG (meta-iodobenzylguanidine) which is used in the treatment of neuroendocrine tumours.[9] Na[^{131}]I is in fact the most widely used therapeutic agent where it is employed for thyroid ablation in hyperthyroid disorders and treatment of thyroid cancer.[10]

1.3 Isotopes for SPECT

1.3.1 Technetium-99m

^{99m}Tc is the most widely used radionuclide in diagnostic medicine.[11] It is available from a molybdenum-99/technetium-99m (^{99}Mo/^{99m}Tc) generator at a very modest cost. The ^{99}Mo is generally produced in low specific activity by neutron irradiation of Mo, or in high specific activity by chemical separation of neutron irradiated ^{235}U which produces ^{99}Mo as a fission product. The ^{99}Mo, as MoO_4^{2-}, is loaded onto a generator where it strongly adheres to alumina. ^{99}Mo decays through β^- emission (E_β max = 1.23 MeV) giving ^{99m}Tc which can be easily eluted with a dilute saline solution as sodium ^{99m}Tc-pertechnetate (Na[$^{99m}TcO_4$]).[12] ^{99m}Tc has a half-life of 6.02 hours and a useful γ-energy (140 keV) for imaging, as gamma rays of this energy can be very easily detected with the thallium-doped sodium iodide crystals present in scintillation cameras.[13] The ^{99m}Tc decay product (^{99}Tc) has a half life of 2.1 x 10^5 years and undergoes β^- emission (E_β max = 294 keV) to ^{99}Ru which is stable.

The pertechnetate salt eluted from the ^{99}Mo generator contains technetium in the +7 oxidation state, which does not easily form complexes with chelates or other donor ligands. This form is usually reduced to a lower oxidation state to facilitate labelling of molecules. Depending on the coordinating ligand(s) present, a range of oxidation states and coordination numbers can occur for technetium including Tc (I) to Tc (VI) and 4-7 respectively. Amongst all radionuclides ^{99m}Tc has the most diverse coordination and organometallic chemistry.[13]

Many prominent radiopharmaceuticals have been developed using ^{99m}Tc including Sestamibi (Cardiolite™ Figure 1.1), which is an isonitrile based myocardial perfusion imaging agent,[14] and tetrofosmin (Myoview™) which is a (1,2-bis[bis(2-ethoxyethyl)phosphino]ethane) ^{99m}Tc complex also used for myocardial perfusion imaging.[13] ^{99m}Tc based brain imaging agents have played an important role to fill the need for early diagnosis of brain injuries. One example of a FDA approved technetium based brain perfusion agent is a Tc(V) complex of hexamethylpropyleneamine oxime (HMPAO or Ceretec™, Figure 1.1). This agent was shown to have good *in vivo* brain uptake and retention in cerebral tissue.[13]

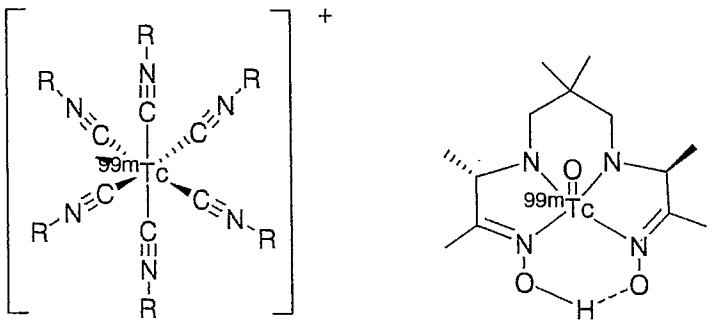
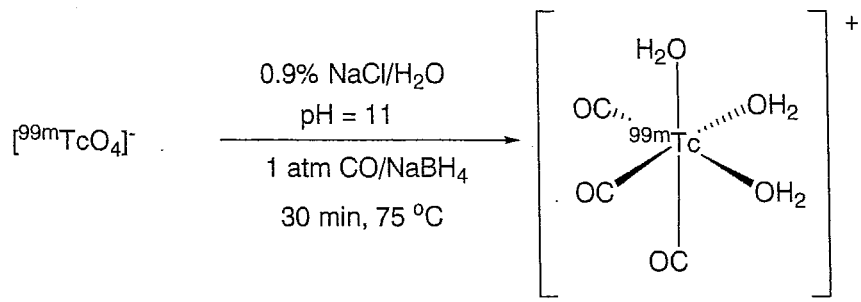


Figure 1.1: Structures of ^{99m}Tc-$(MIBI)_6^+$ and ^{99m}Tc-HMPAO

^{99m}Tc-methylene diphosphonate (MDP) is the most commonly used imaging agent for diagnosis of osetomyelitis and is highly accurate (90%) but has relatively low specificity (33%) because it accumulates at areas of high bone-mineral turnover, which is not necessarily indicative of infection.[15] After a positive ^{99m}Tc-MDP bone scan, patients are often given a second bone scan with ^{99m}Tc-HMPAO labelled white blood cells, as labelled leukocytes do not accumulate in areas of increased bone turnover this scan helps eliminate false positive ^{99m}Tc-MDP bone scans.[15] ^{99m}Tc-HMPAO labelled leukocytes have also been successfully used in conjunction with ^{99m}Tc-MDP to improve the specificity of ^{99m}Tc-MDP (from 33% to 78%) for the diagnosis of osetomyelitis.[16]

Many of the above mentioned technetium derivatives contain the metal as Tc(I). This oxidation state is attractive for preparing radiopharmaceuticals because it can form octahedral complexes which can exist with a low-spin d^6 electronic configuration. These complexes are therefore inert and will not decompose prematurely *in vivo*.

An increasing number of technetium compounds have been developed based on the $^{99m}Tc(CO)_3(H_2O)_3^+$ core which is water and air stable and was first developed by Alberto et al. in 1998 (Scheme 1.1).[17] The reported synthesis gave high yields (>95%) of $^{99m}Tc(CO)_3(H_2O)_3^+$ and involved direct reduction of pertechnetate in a saline solution using sodium borohydride under one atmosphere CO for carbonylation. They later altered the conditions to use potassium boranocarbonate $[K_2(BH_3CO_2)]$ which acts as a source of carbon monoxide (eliminating the need for gaseous carbon monoxide) and a reducing agent.[18]



Scheme 1.1: Synthesis of the trisaquotricarbonyl technetium-99m (I) species

This $^{99m}Tc(CO)_3(H_2O)_3^+$ core and its cold rhenium analogue are extremely useful synthetic precursors because the three water ligands can be readily exchanged with other ligands (k_{ex} = 0.49 s^{-1} for $^{99m}Tc(CO)_3(H_2O)_3^+$, k_{ex} = 0.0054 s^{-1} for $Re(CO)_3(H_2O)_3^+$ at 298K).[19] Biologically relevant bidentate (e.g. histamine) and tridentate systems (e.g. histidine) technetium compounds have been synthesized using the $^{99m}Tc(CO)_3(H_2O)_3^+$ core. The ligands were selected to match the criteria needed for use in radiopharmacy, specifically to have the potential of being bifunctionalized (to be further linked to a biomolecule) and exhibit fast and efficient complex formation. *In vivo* studies found that the tridentate ligands gave superior and faster clearance from all organs and tissues compared to the bidentate ligands which had high retention in the kidneys, liver and blood pool.[20]

1.3.2 Isotopes of Iodine

Iodine is second to technetium in terms of importance to nuclear medicine. There are a number of radioactive isotopes of iodine that can be used for MI and therapy applications. One of the most established forms of radionuclide therapy involves the treatment of thyroid carcinoma and thyrotoxicosis with sodium iodide-131 ($E_{\beta max}$= 610 keV, $t_{1/2}$= 8 d).[21] Another therapeutic isotope of iodine that is in widespread use is ^{125}I. ^{125}I has a half life of 60 days and gamma emission energy of 35 keV and is the main radionuclide used in brachytherapy.[22] Brachytherapy involves seed capsules containing radioactive isotopes (such as ^{125}I, ^{192}Ir and ^{103}Pd) which are inserted directly into a patient

for a localized radiation therapy.[13] ^{125}I is also being explored as a nuclide for Auger electron therapy.[23]

Other isotopes of iodine which are gaining notoriety are ^{123}I, which has high specific activity (235 Ci/mmol) and a long half-life (13 hrs). ^{123}I is often used in diagnostic radiopharmaceuticals as it emits gamma ray energy of 159 keV, which is similar to ^{99m}Tc and can penetrate tissue effectively without excessive radiation dose to the patient. ^{124}I is a long-lived PET isotope ($t_{1/2}$ = 4.2 d) that is produced through proton or deuteron reaction on enriched ^{124}Te and is widely used for antibody labelling.[13]

Iodination reactions can be done using a variety of labelling methods. ^{124}I and ^{123}I for instance have both been used to label meta-iodobenzylguanidine (MIBG) through halogen exchange where the cold iodinated version has the halogen displaced for the radioactive isotope.[24] This approach has certain drawbacks, that the non-radioactive starting material and the labelled product share the same physical and chemical properties, making separation impossible and resulting in low effective specific activity products.[25] Another form of radioiodination involves initial oxidation of iodide to form an oxidized iodine species (typically I-Cl or $I\text{-}OH_2^+$) which then reacts with the substrate.[26,13] One issue that arose with direct labelling was degradation of proteins or peptides which were designed to act as targeting vectors under the typical labelling conditions (i.e. acidic solution with hydrogen peroxide, potassium iodate etc.). Various oxidants including Chloramine-T and Iodogen have been developed to allow for labelling

of sensitive compounds. Oxidative labelling methods are discussed further in Section 1.7.

1.4 Targeted Radiopharmaceuticals

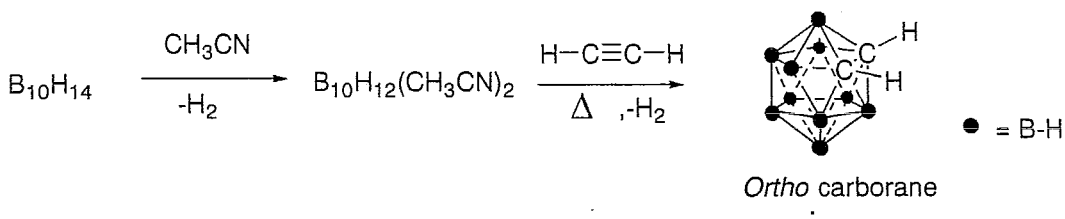
The use of radiolabelled probes is an essential component of molecular radioimaging as they are designed to achieve high target-to-non-target ratios. One way to maximize target specificity is to create tracers that have a high affinity for specific cell surface proteins.[27] A protein binding ligand, which is referred to as the targeting vector, serves as a transport vehicle for the radionuclide. The extent to which the compound accumulates at the target is dependent on several factors including the affinity for receptors, the B_{max} value of the protein and pharmacokinetics.[28]

One other point that needs to be considered when designing a radiopharmaceutical is that metabolism can also have a significant impact on the viability of a tracer. It is crucial that the radionuclide not be cleaved prematurely *in vivo* otherwise, non-specific binding will confound visualization of specific uptake while in the case of therapy, healthy tissue can receive enhanced and unwanted doses of radiation. The chemical link between radionuclide and biomolecule must therefore be robust and stable *in vivo* at least until the agent reaches the target or is cleared from the body. There are a limited number of ligands/complexes that meet these criteria and that can be prepared under mild conditions for ^{99m}Tc and iodine. There is a need to develop new synthons which form

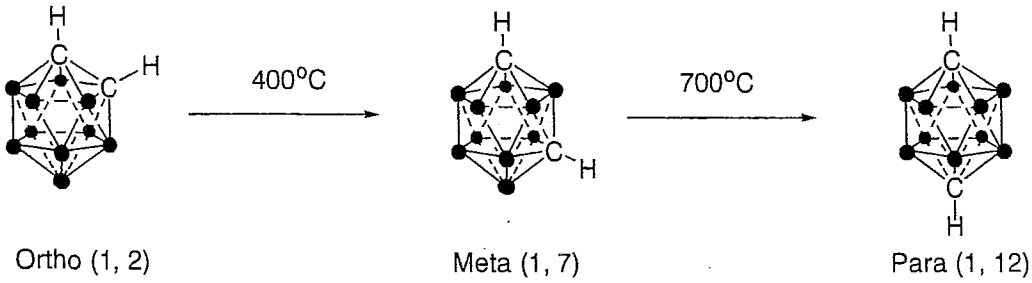
robust ^{99m}Tc and/or iodine derivatives and that can be used in conjunction with modern drug discovery methods to prepare novel MI and therapy agents.

1.5 Carboranes and Metallacarboranes

Carboranes are polyhedral clusters of boron and carbon atoms, of which 1,2-dicarba-*closo*-dodecaborane (*ortho*-carborane) is the best known example (Scheme 1.3). O*rtho*-carboranes can be readily prepared by reaction of alkynes with decaborane in the presence of a Lewis base. The alkyne insertion reaction (Scheme 1.2) can occur with both mono and di-substituted alkynes, affording a large number of substituted *ortho*-carboranes in one synthetic step. *Ortho*-carboranes are remarkably robust and are stable in the presence of strong acids, oxidizing agents and alcohols as well as being thermally stable at temperatures up to 400 °C. Under an inert atmosphere, *ortho*-carborane will isomerize to *meta*-carborane (1,7-dicarba-*closo*-dodecaborane) between 400-500 °C, which will further isomerize to *para*-carborane (1,12-dicarba-*closo*-dodecaborane) at temperatures between 600-700 °C (Scheme 1.3).[29]



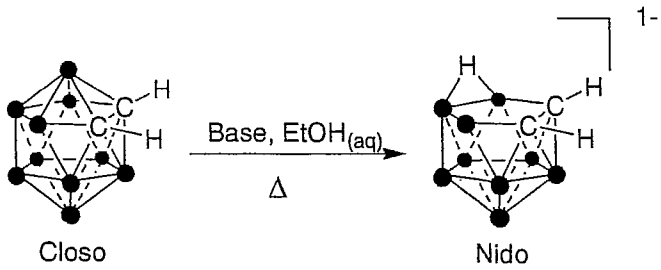
Scheme 1.2: Alkyne insertion reaction to form *ortho*-carborane



Scheme 1.3: *Ortho, meta* and *para* isomers of *closo*-$C_2B_{10}H_{12}$

Ortho-carboranes can be readily functionalized either prior to the alkyne insertion (substituted alkyne) or after removal of the weakly acidic (pKa ~ 23.3) C-H hydrogen atom. In the latter case *n*-butyl lithium creates a nucleophilic species which can be used to react with a variety of electrophiles including alkyl chlorides, carbonyl containing molecules and epoxides.[30,29]

Closo-carboranes are converted to the anionic *nido*-cluster form by removal of a boron vertex under basic conditions (Scheme 1.4). Further treatment with base removes the bridging hydrogen in *nido*-carborane and results in the formation of the dicarbolloide dianion which is formally isolobal to cyclopentadienide.[31] This dicarbollide dianion is capable of binding metals in a η^5 fashion, and numerous stable metallocarborane complexes have been reported[32] including ferracarboranes, cobaltacarboranes, aluminacarboranes and gallacarboranes (Figure 1.2).[32, 33]



Scheme 1.4: Formation of a *nido*-carborane

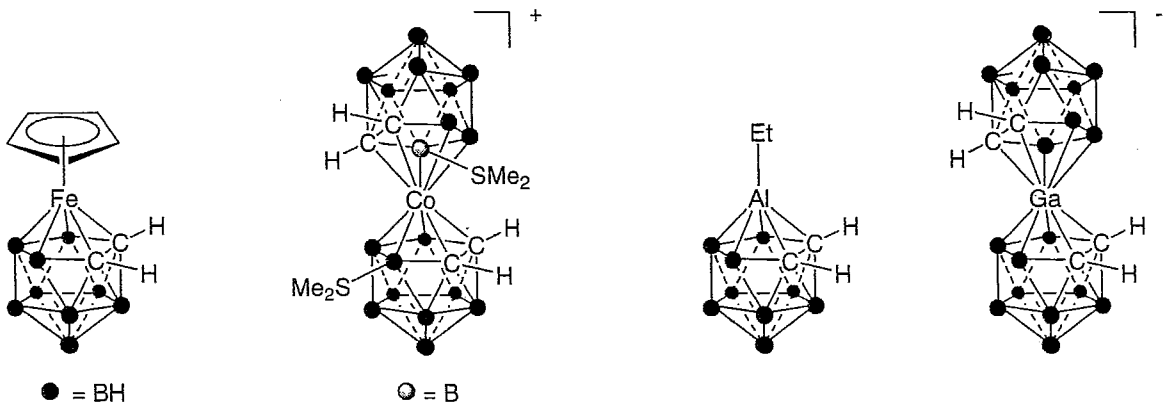


Figure 1.2: Various examples of metallocarboranes

1.6 Carboranes as Radiometal Carriers

Metallocarboranes are attractive synthons for developing molecular imaging agents because they are compact, easy to derivatize and can be produced in high effective specific activity. Hawthorne and co-workers developed a pyrazole based bridging carborane ligand called the "Venus flytrap cluster" which was coordinated to both the

non-radioactive cobalt (^{59}Co), and ^{57}Co (gamma emitting isotope) for use for radioimaging (Figure 1.3).[34]

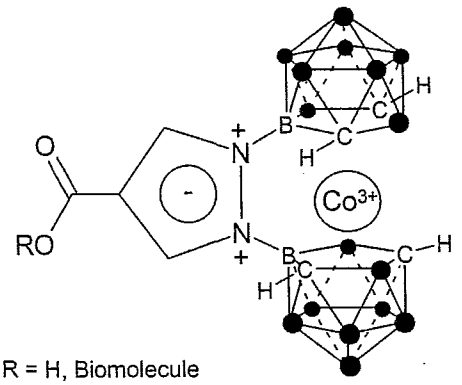
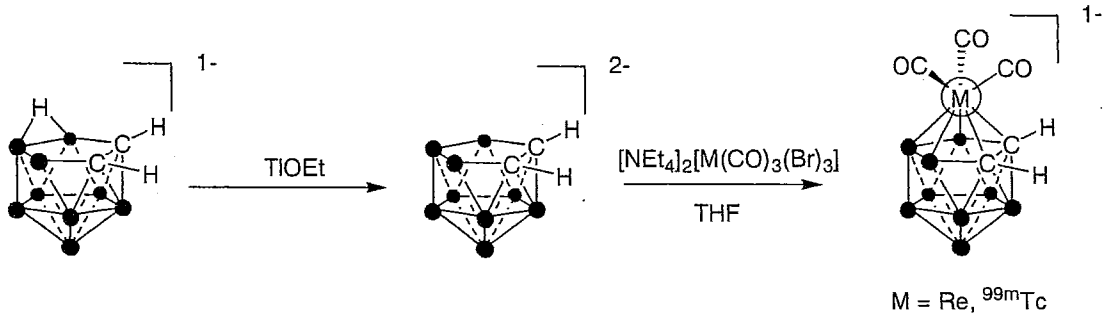


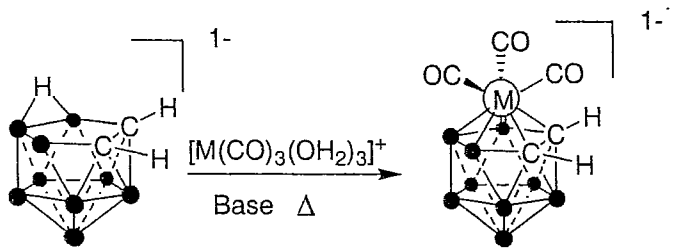
Figure 1.3: Venus fly trap cluster cobalt complex

Carborane complexes of technetium were first reported by the Valliant group in 2002. Both $^{99m}Tc/Re$ complexes have the general formula $[RR'C_2B_9H_9M(CO)_3]^-$ (M = Tc, Re) and were prepared initially in modest yield by treatment of *nido*-carborane with base to yield the dicarballoide dianion which was further reacted with $[NEt_4]_2[M(CO)_3(Br)_3]$ to yield the metallacarborane (Scheme 1.5). [35] The rhenium complexes are important as they are non-radioactive, can be prepared in macroscopic quantities, fully characterized and then used as reference standards for the ^{99m}Tc analogues. Furthermore, radioactive rhenium complexes can be used for therapy as there are two radioactive isotopes of rhenium that are commercially available: rhenium-188,

which has a half life of 16.9 h and an $E_{\beta max}$ = 2.1 MeV and rhenium-186 which has a half life of 3.7 d and a $E_{\beta max}$ = 1.1 MeV.



Scheme 1.5: Initial synthesis of $[C_2B_{10}H_{11}M(CO)_3]^-$ (M = Tc, Re)



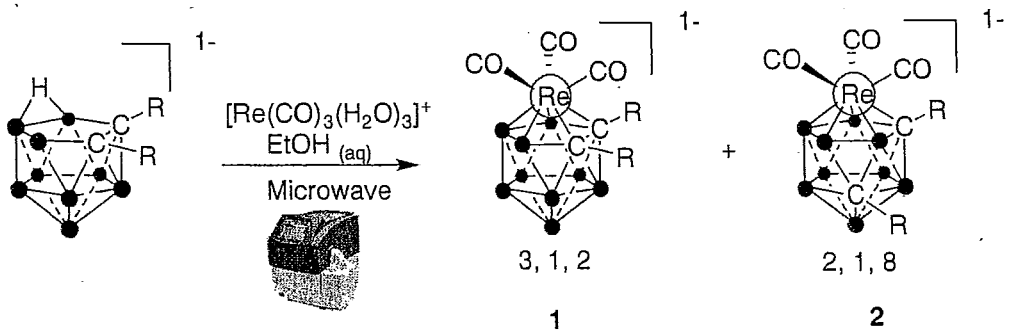
Scheme 1.6: Metallocarborane formation from a *nido*-carborane

M = Re, ^{99m}Tc

The continued investigation of ^{99m}Tc and Re carborane complexes led to their synthesis using the precursor $[M(CO)_3(H_2O)_3]^+$ which is available at both the macroscopic and tracer levels (M = $^{185/187}$Re, $^{186/188}$Re, ^{99m}Tc).[36] The metallacarborane complexes are

produced by heating the *nido*-carborane ligand with $[M(CO)_3(OH_2)_3]^+$ (Scheme 1.6) and can be generated in water.[37] This conversion was initially conducted using conventional heating, and in 2006 was accomplished in a microwave reactor with temperatures ranging from 100-200 °C.[38]

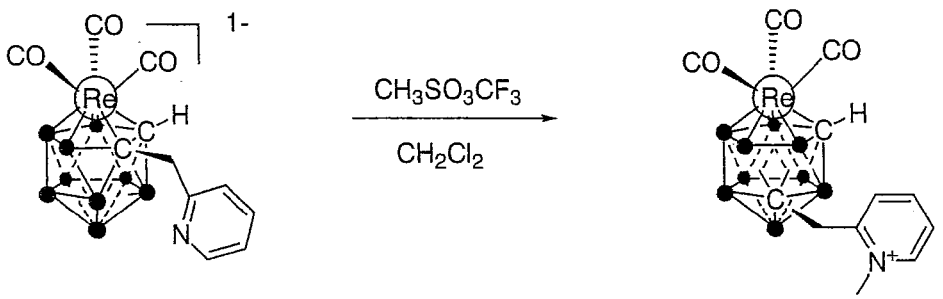
One other feature of the reaction used to make the ^{99m}Tc/Re carborane complexes is that heating in the microwave can bring about cage isomerizations.[39] Rhenacarboranes have been found to isomerize between the 3, 1, 2 and the 2, 1, 8 isomers depending on the nature of the substituents and reaction conditions (Scheme 1.7).



Scheme 1.7: Rhenacarborane cage isomerization

It was found that unsubstituted rhenacarboranes isomerized from the 3, 1, 2 to 2, 1, 8 isomer at ~170 °C. Though the mechanism of the isomerization is not entirely clear, NMR studies suggest the cage isomerization does not occur during formation of the *nido* species, but seems to happen simultaneously with metal complexation.[40] Factors that

affect whether or not isomerization occurs were both steric and electronic. It was found that bulky substituents bound to the cage C-atoms favored the formation of the 2, 1, 8 isomer, and isomerization is more likely to occur when strongly electron withdrawing substituents are attached to the cage C-atoms. In fact, upon methylation of a benzyl pyridine substituent, which does not isomerize upon complexation, the cage isomerized at room temperature (Scheme 1.8).[39]

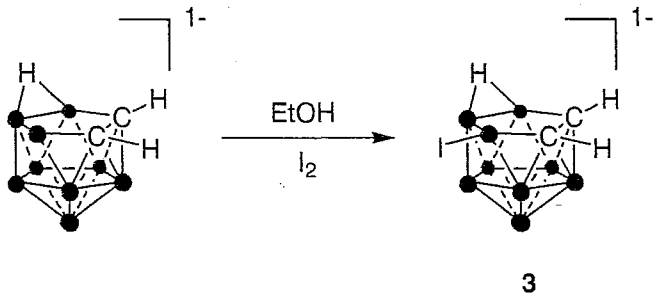


Scheme 1.8 Room temperature isomerization to give 2,1,8 rhenacarborane cage isomer

1.7 Halogenated Carboranes

Hawthorne *et al.* reported that *nido*-carboranes can be readily labelled with iodine (Scheme 1.9) through an electrophilic substitution of a boron atom adjacent to the carbons on the open face of the *nido*-carborane. The reaction of the potassium salt of *nido*-carborane with elemental iodine in absolute ethanol gave the monoiodinated product in 75% yield. [41] In order to iodinate more sensitive starting materials, the same authors later investigated using Chloramine-T and sodium iodide to iodinate 7-(4-C_6H_4NCS)-

nido-7,8-$C_2B_9H_{11}^-$ which was subsequently conjugated to an amino group of a protein.[42] This method was later employed using radioactive iodine and the resulting compound investigated as a carrier for radioiodine in *in vivo* studies. The iodination reactions proceeded with good yields in a short amount of time, but a significant amount of non-specific binding (likely due to impurities) was a problem with these studies.[43] Another study utilized both Chloramine-T and Iodogen as oxidants for iodination of dextran coupled 3-aminoproyl-*nido*-carborane derivatives and performed *in vitro* studies. Radioiodination with ^{125}I was found to proceed in yields between 69-85% and the label was stable in rat liver homogenates.[44]



3

Scheme 1.9: Iodination of *nido*-carborane

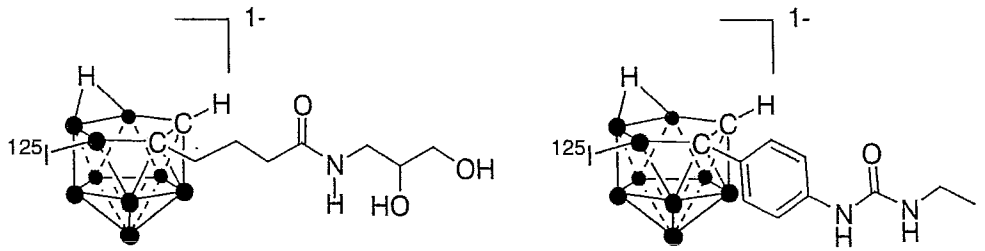


Figure 1.4: Examples of successfully radioiodinated *nido*-carboranes

A benefit of forming the iodinated carborane species is that the B-I bonds (bond dissociation energy = 361 kJ/mol) are more stable and robust *in vivo* compared to aryl iodides (C-I bond dissociation energy = 253.1 ± 35.6 kJ/mol) which often undergo premature deiodination *in vivo*.[45,46] Some examples of successfully radioiodinated *nido*-carboranes involve C- substituted clusters with amides,[47] carbohydrates[48] and benzamidyl groups (Figure 1.4).[49]

Another halogen which has been used for the labelling of carboranes is astatine. ^{211}At has a half life of 7.2 hours and decays through alpha particle emission which is useful for radiotherapy. Although astatine-211 is an attractive isotope for radiotherapy, there have been problems finding targeting vectors that can form stable bonds to the heaviest halogen, which tend to undergo premature deastatination *in vivo*. In an attempt to address this issue, astatination of *nido*-carboranes has been investigated. [49,50] Direct comparison of halogenated (^{125}I/^{211}At) carborane compounds to astatinated benzamide derivatives was conducted by Wilbur *et al.* Radioastatination was achieved using a variety of different *nido*-carboranes in yields ranging between 39 and 86% (unoptimized)

which were similar to the yields obtained for radioiodinations of the various analogues. *In vivo* screening studies showed ^{211}At-labelled benzamides undergo rapid deastatination while the ^{211}At-*nido*-carborane derivatives seemed to be slightly more stable though the difference was not significant enough to be stated indisputably. The most promising results were those involving a bridging di-carborane ligand (bis-(*nido*-carboranylmethyl)benzene) which was referred to as a Venus flytrap type complex (VFC) (Figure 1.5). These radiohalogenated *nido*-carboranes did not release ^{211}At *in vivo* which is strong evidence that if linked to the appropriate targeting vector, they could serve as robust radiopharmaceuticals.[49]

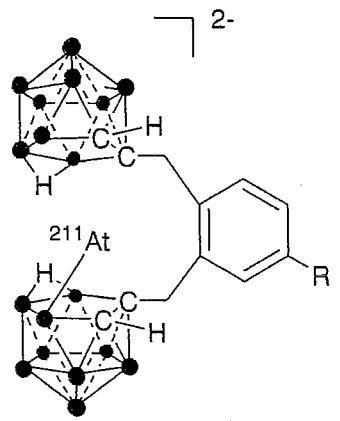
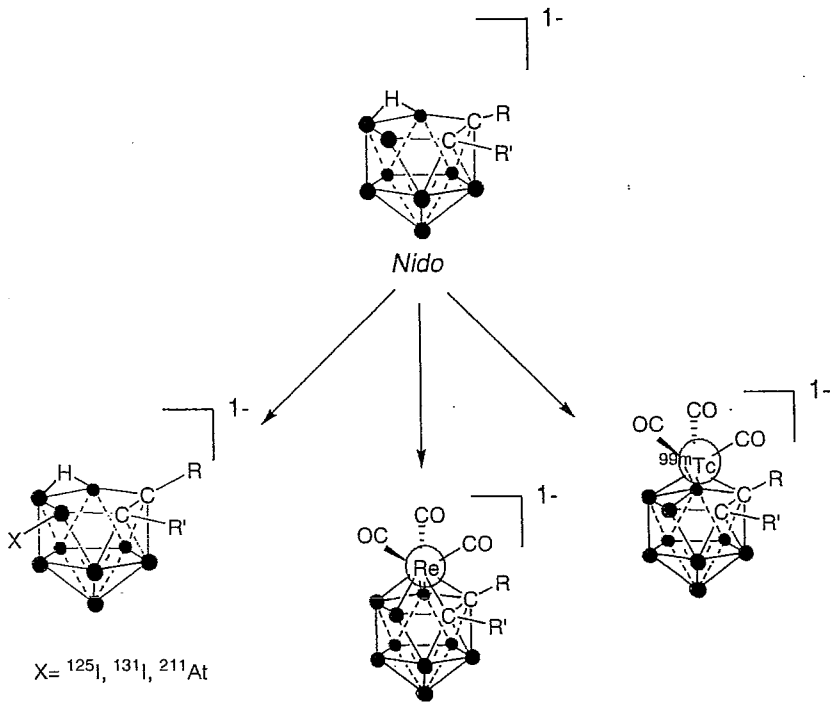


Figure 1.5: Astatine labelled Venus fly trap carborane complex where R = H or benzyl coupled diethylene triamine pentaacetic acid (DTPA)

Carboranes are one of very few synthons that can be labelled with both technetium and radiohalogens. This unique feature makes carboranes highly versatile in

the sense that when incorporated into targeting vectors they can be tagged with the radioisotope that best matches with the pharmacokinetics of the vector and the intended application (e.g quantitative imaging, dynamic imaging, therapy) (Scheme 1.10). The key to exploiting this is to develop new ways to incorporate carboranes into targeting vectors and to evaluate ways to label the synthons with both ^{99m}Tc and radiohalogens of interest. One attractive strategy involves carborane based amino acid analogues.



Scheme 1.10: The various radiolabelled derivatives of the $C_2B_9H_{10}$ cluster that have been reported showing the versatility of the *nido*-carborane ligand

1.8 Amino acids for SPECT/PET and Amino Acid Transporters

Amino acid transport is upregulated in many tumour types including brain, prostate, colon and breast.[51] This feature has lead researchers to label amino acids in order to use SPECT and PET to detect and characterize tumours. The most common radionuclides used are carbon-11 ($t_{1/2}$ = 20.3 min) and fluorine-18 ($t_{1/2}$ = 12.3 h) for PET and iodine-123 ($t_{1/2}$ = 13 h) for SPECT. Some examples of natural amino acids which have been labelled with ^{11}C are $_L$-[^{11}C]methionine (MET), and $_L$-[1-^{11}C]tyrosine. Of the natural labelled amino acids, [^{11}C]MET is the most well established radiopharmaceutical for imaging brain and extracranial tumours.[51]

3-[^{123}I]iodo-alpha-methyl-$_L$-tyrosine (IMT, Figure 1.6) is a non-natural aromatic amino acid that has been prepared in moderate to high radiochemical yield (60-80%) and has been primarily used for imaging brain tumours.[51] [^{123}I]IMT is quite metabolically stable resulting in very little loss of iodine *in vivo* and is a substrate for the $_L$-type amino acid transporter (LAT1) which transports large neutral amino acids in a Na^+ independent manner. Peak uptake of [^{123}I]IMT in a patient occurs ~15 minutes after injection with about a 2:1 tumour to brain ratio, which is equivalent to [^{11}C]MET.

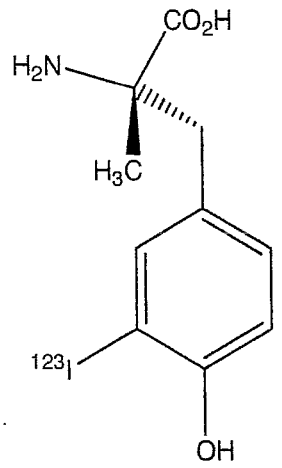


Figure 1.6: 3-[^{123}I]Iodo-alpha-methyl-$_{L}$-tyrosine

Another aromatic amino acid analogue is 3,4-dihydroxy-6-[^{18}F]fluoro-$_{L}$-phenylalanine (FDOPA), a fluorinated version of naturally occurring $_{L}$-3,4-dihydroxy-6-phenylalanine ($_{L}$-DOPA). DOPA plays an important role in the biosynthesis of dopamine, consequently [^{18}F]FDOPA has been used in the investigation of the dopaminergic system of the brain. This use of [^{18}F]FDOPA has immediate application in Parkinson disease research as it is believed that Parkinson disease is caused by insufficient formation and usage of dopamine in the dopaminergic system. [^{18}F]FDOPA also has practical purposes for PET imaging of primary brain tumours where it has been shown to have high uptake as a result of amino acid transport systems. A comparison between [^{18}F]FDOPA and [^{11}C]MET for imaging of supratentorial brain lesions showed similar spatial distribution and standardized uptake values for tumour to non-tumour ratios (2.05 ± 0.91 for [^{11}C]MET and 2.04 ± 0.53 for [^{18}F]FDOPA).[52] Another study

found that [^{18}F]FDOPA had 96% sensitivity and 86% specificity for detecting brain tumours.[53] These results indicate that non-natural amino acids can be highly effective as targeting vectors for radiopharmaceuticals.

The development of targeted radioimaging agents often involves the labelling of small biomolecules (i.e. amino acids or peptides) to afford site specificity. One major obstacle that has hindered the development of targeted agents is that upon the introduction of the radioisotope (especially with radiometals), the derivatization process has an adverse affect on the molecular recognition of the vector or it enhances non-specific binding.[54] To overcome these issues groups have recently tried to develop amino acid based probes for systems capable of binding a wide range of substrates and their derivatives.

The LAT1 system is an important route by which living cells take up aromatic or branched amino acids from extracellular fluids. The expression of LAT1 is restricted to specific tissues such as brain, placenta, and testis LAT1 and is also highly expressed in malignant tumours. An important attribute to the LAT1 system is its broad substrate selectivity, which implies that not only natural amino acids will be taken up by the system, but also that amino acid related compounds have a high probability of uptake.[55]

With this in mind, Alberto *et al.* successfully demonstrated the first examples of small molecule-metal complex conjugates that were actively internalized into cells by LAT1 (Figure 1.7). The LAT transport system works through a 1:1 exchange of amino

acids. To determine the uptake of the Re-amino acid derivatives, the efflux of radiolabelled amino acids were measured where the results showed selective uptake of the amino acid analogue. It was found that for the structure shown in Figure 1.7, n=4 had the highest affinity for the receptor giving a K_i = 308 μM, and n=5 had the lowest affinity (K_i = 5300 μM).[54]

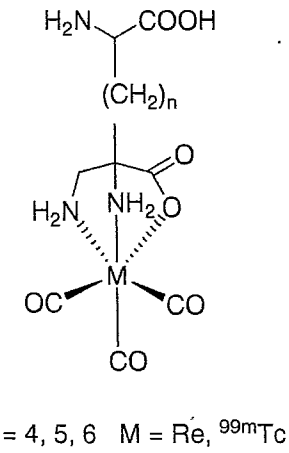


Figure 1.7: Re/^{99m}Tc complex developed by Alberto *et al.* as a probe for the LAT1 system

1.9 Peptides as Targeting Vectors

Metal binding amino acid analogues are also being used to develop peptide–targeted radiopharmaceuticals. Low molecular weight peptides, generally less than 40 amino acids are attractive targeting vectors because sequences that bind to one specific protein can be identified through phage display or other related screening methods.[21,56]

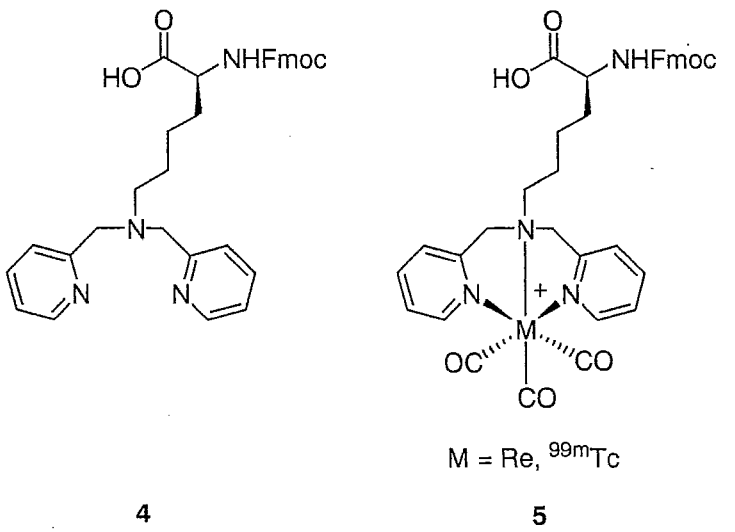
Furthermore, they generally exhibit sought after properties for radiopharmaceuticals such as fast clearance from non-target tissue, rapid tissue penetration and low antigenicity.[21] Another important feature is that peptides can generally be readily produced in large quantities at a reasonable cost thus rendering them as potential candidates for clinical applications.[21]

An example of a peptide-based molecular imaging agent is the ^{99m}Tc-labelled somatostatin analogue Depreotide. Somatostatin, a 14 amino acid long peptide hormone, is naturally present in the hypothalamus, pancreas, brain stem and gastrointestinal tract. Depreotide has shown promising results and appears to differentiate between malignant and benign lung lesions.[57] Somatostatin has also been labelled with ^{111}In to generate an agent (Ocreoscan^{TM}) which is approved for clinical use.

1.10 The Single Amino Acid Chelate (SAAC)

Our research group has developed a means to prepare peptide-based radiotracers using non-natural amino acids that chelate Tc(I). The single amino acid chelates (SAAC, Figure 1.8) which are derived from lysine, have a tridentate chelating group at the ε-amine. The ligand **4** can be incorporated into small peptides using standard solid-phase synthesis methods. Fmoc-protected SAAC and its Re complex can be incorporated into peptides as if they were natural amino acids. The corresponding ^{99m}Tc complexes can be prepared in high yield from $[^{99m}Tc(CO)_3(OH_2)_3]^+$.[58,59] An example of one such peptide is

shown in Figure 1.9, which was successfully labelled with ^{99m}Tc in 90% yield with greater than 98% radiochemical purity.[59]



4 5

Figure 1.8: The SAAC ligand **4** and its Re(I)/^{99m}Tc(I) complex **5**

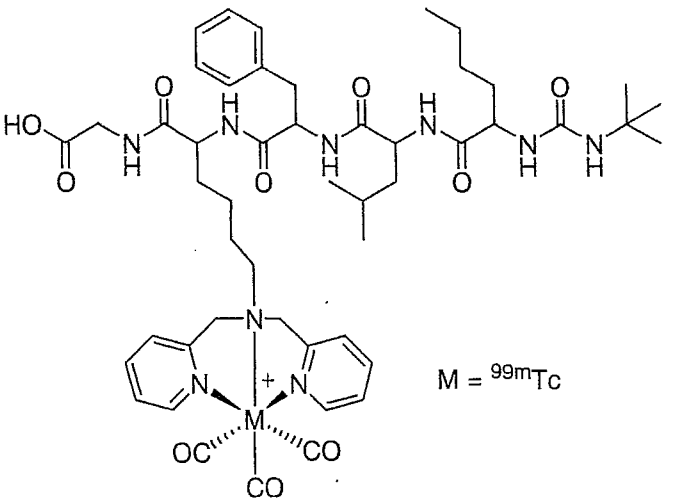


Figure 1.9: SAAC containing tert-butyl urea derivative peptide labelled with ^{99m}Tc [59]

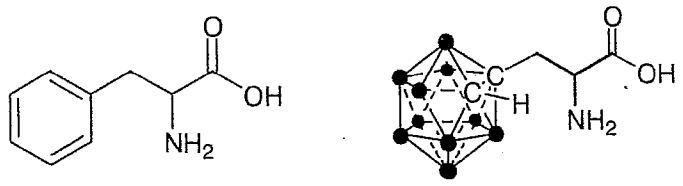
1.11 SAAC System Limitations

Although the SAAC amino acid system has proven effective at incorporation into peptides as if it were a natural amino acid, there are limitations to its usefulness. SAAC can be linked to other amino acids, but the resulting peptide may have different properties compared to that containing a lysine amino acid instead of the non-natural derivative. One main reason for this is the significant difference in size from lysine to SAAC, and an even more substantial difference with the metal chelate. Another issue which impedes the effectiveness of the SAAC system is the ligand's selectivity for chelation against competing groups in the peptides such as disulfide bridges or other types of donating ligands present in the peptide backbone. Additionally, peptides are generally thermally sensitive which is problematic because labelling of SAAC with technetium-99m often requires elevated temperature.[60]

Because of both the advantages and limitations of the SAAC system, there is a demand for novel non-natural amino acid ligand systems that can be used in radiolabelling experiments. The aim is to identify the next generation of ligands that are compact, easily labelled and that can be incorporated into peptides.

1.12 Carboranylalanine

Carboranylalanine (Car) is an analogue of the amino acid phenylalanine where a carborane is present in place of the phenyl ring (Figure 1.10). Car was first reported in two independent publications by Brattsev/Stanko and Zakharkin as a racemic mixture.[61,62] Since then, multiple synthetic routes have been developed to make carboranylalanine, both in the racemic form, and stereoselectively.[63,64,65,66] The carborane cage is known to occupy roughly the same volume as a benzene ring rotating around its C1-C4 axis.[67] This leads one to believe that Car, being considered as a more lipophilic version of phenylalanine, should be recognized by transporters and receptors which bind phenylalanine.



Phenylalanine *o*-Carboranylalanine

Figure 1.10: Structures of phenylalanine and *o*-carboranylalanine

Investigations using Car include an *in vitro* analysis of Car for antimicrobial activity. It was found that the antibacterial effect of Car against some plant pathogenic bacteria was comparable to the widely used agricultural antibiotic, streptomycin. This same study also found that in general Car appeared to be more toxic to Gram positive than to Gram negative bacteria.[68] Other investigations with Car include probing the aromatic recognition site of chymotrypsin.[69] It was found that although slightly larger, Car was easily accommodated by the recognition site of chymotrypsin. This result lead Leukart *et al.* to further examine Car as a replacement for amino acids in polypeptide hormones.[70] They substituted Car in position 4 of enkephalin (commonly shown to be a critical site for biological activity and opiate receptor affinity) to give H·Tyr-Gly-Gly-Car-Leu-OH. The non-natural peptide was investigated for its ability to displace [^{3}H]naloxone from rat brain opiate receptor preparations. [Car^4, Leu^5]-enkephalin bound better than [Leu^5]-enkephalin. It was concluded that the site for recognition involves a phenyl recognition 'pocket' which is capable of accommodating the carborane icosahedron, similar to the recognition site in chymotrypsin.

As Car contains a carborane cage, there also has been a great deal of interest in using this non-natural amino acid for boron neutron capture therapy (BNCT). BNCT is an experimental therapy where a boron containing drug is taken up selectively in the target cells (typically cancer). The compound on its own is supposed to be non-toxic on administration, but once sufficient time has passed to allow accumulation in the target tissue, and excess to be cleared from the patient, the target area is subjected to a thermal, or epithermal, beam of neutrons. When these neutrons are absorbed by the ^{10}B nucleus, the result is a fission reaction which produces an alpha particle, a lithium nucleus and a gamma ray with a total energy of the decay event of ~2.8 MeV. These species cause irreparable damage to DNA. As the majority of human tissue components are composed of atoms with a low boron capture cross-section (C, H, N, O etc), the exposure of neutrons is very selective with minimum dose to healthy tissue so long as the BNCT agent has high boron content. BNCT agents should also be highly selective *in vivo* to maximize the concentration of boron in the target to further avoid delivering unwanted doses to healthy tissue. Carboranes are very well suited for this application as they contain numerous boron atoms in a compact cage. Carboranylalanine, for instance, has 46.8% boron by weight compared to another commonly used BNCT agent p-boronophenylalanine (BPA) which is 5.2% boron by weight.[71]

One study in the application of Car for BNCT involved measuring uptake of both D and L enantiomers of Car in V-79 Chinese hamster lung cells. Both enantiomers were incubated at concentrations of 100-200 μg/mL with the cells and were found to be

entirely non-cytotoxic. Both D and L-Car were taken up and retained in V-79 cells and subjected to repeated washing in boron free medium with no resultant loss in uptake. It was found that when subjected to thermal neutron irradiation, both enantiomers were effective as neutron sensitizers with dose enhancement factors between 1.4 ± 0.63 (D-Car at 100 μg/mL) to 2.9 ± 0.09 (L-Car at 200 μg/mL).[72] These results suggest that not only would Car be an effective BNCT agent but could also be used as a radionuclide delivery vector.

1.13 Objectives

The objective of this thesis was to synthesize a complement to the single amino acid chelate (SAAC) system using carboranes. This would allow both technetium and iodine-based molecular imaging agents to be produced using a single core ligand. The first step towards this goal was the synthesis and characterization of a carborane analogue of phenylalanine (carboranylalanine). The second step would be to identify a cage degradation method to produce the *nido*-carboranylalanine which is needed to effectively label the cluster with technetium and iodine. The third phase involves the preparation of the non-radioactive standards and optimization of the radiolabelling methods for both of the isotopes of interest.

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Chapter 2

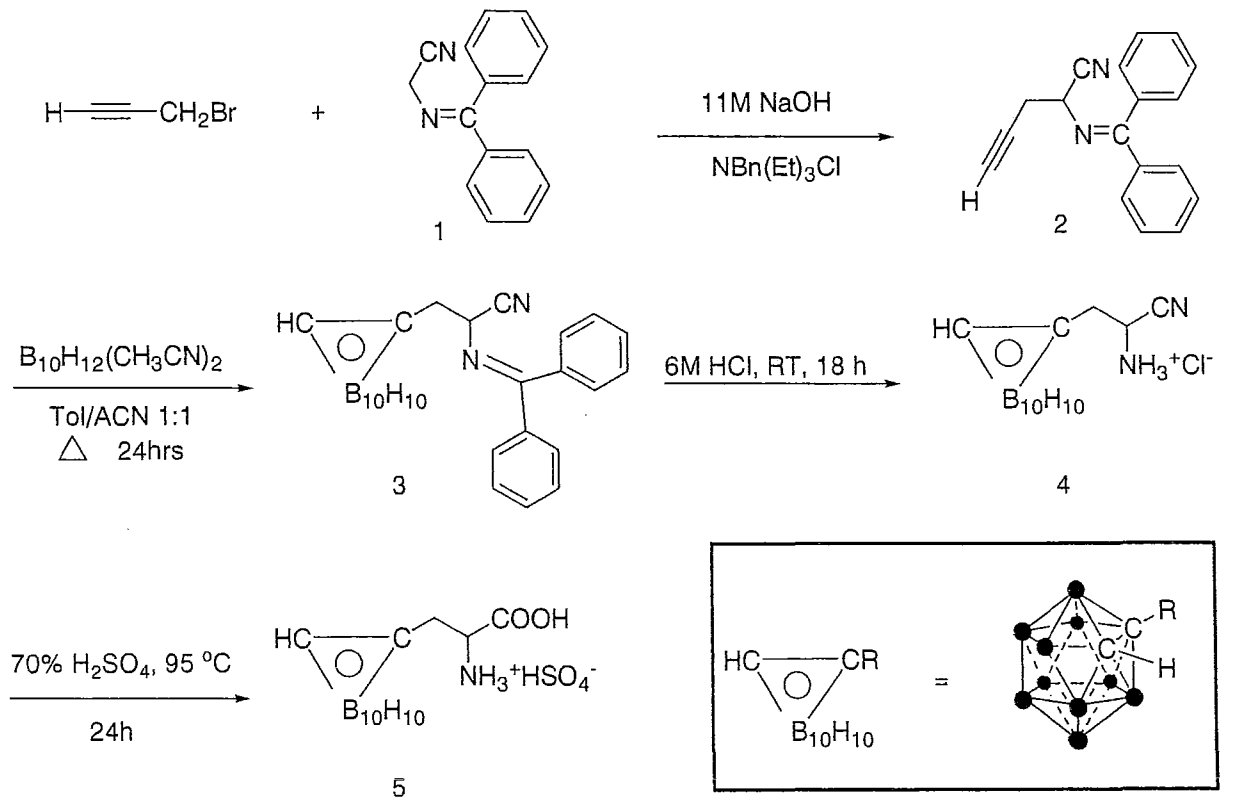
Synthesis of Carboranylalanine (Car) and Derivatives: *Nido*-Car, Re-Car and I-Car

2.1 Synthesis of Carboranylalanine

As discussed in Section 1.13, carboranylalanine (Car) was reported in two independent publications by Brattsev/Stanko and Zakharkin as a racemic mixture[1,2] and has been previously incorporated into biologically active peptides.[3] Since then, multiple synthetic routes have been developed to make carboranylalanine, both in the racemic form, and stereoselectively.[4,5,6,7] The approach taken for this thesis was to synthesize Car as a racemic mixture to determine its potential as a radiotracer ligand. Future work for the project could involve the more involved and lower yielding enantio-selective synthesis once the suitability of Car as a ligand for technetium and iodine has been established.

Although numerous synthetic methods have been published, the multi-step synthesis of carboranylalanine was attempted following the procedure reported by Wyzlic et al.[8] The synthesis started with a phase-transfer alkylation of commercially available [(diphenylmethylene)amino]acetonitrile **1** (Scheme 2.1).[8] This method was selected as the starting materials are all commercially available, the number of synthetic steps were

minimal and reportedly high yielding. The literature reported the use of anhydrous potassium carbonate as a suitable base to abstract a methylene proton on **1** to promote alkylation (Scheme 2.1). It was found that under these conditions, only small quantities of product **2** were obtained. The method was altered to include the use of 11M NaOH and benzyltriethylammonium chloride as a phase transfer catalyst.[9] It was found that isolated yields increased to 56 % however it was necessary to monitor reactions carefully to prevent any significant formation of the disubstituted alkylation product. Characterization data matched those previously reported in the literature.[8]

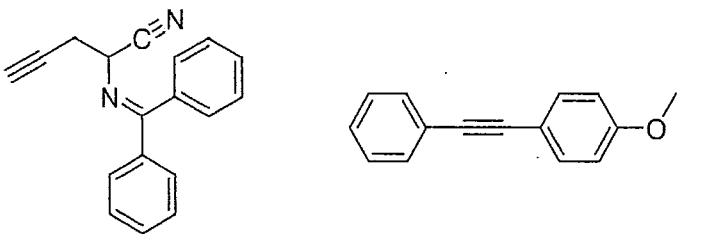


Scheme 2.1: Synthesis of carboranylalanine

The next step in the synthesis involved an alkyne insertion reaction to form the carborane **3**. To do this, decaborane was first converted to $B_{10}H_{12}(CH_3CN)_2$ **6**.[10] The decaborane-acetonitrile complex can be formed *in situ* or isolated and combined with the alkyne in a separate step. Both of these methods were employed for this synthesis of **3** where the maximum yield of 25% was achieved by heating the decaborane-acetonitrile complex with the alkyne in dry acetonitrile and toluene. Reaction of 2-[(diphenylmethylene)amino]pent-4-ynenitrile **2** and $B_{10}H_{12}(CH_3CN)_2$ in ionic liquids, which have been reported to increase insertion yields,[11] was performed. However it was found that there was no significant increase in the amount of the carborane isolated.

The isolated yield of 25% achieved for **3**, may appear low but can be considered a moderate yield for alkyne insertion reactions. Under conventional heating, alkyne insertion reactions often take up to 24 hours with terminal alkyne yields between 6-75%.[11] In an attempt to improve general alkyne insertion reactions, microwave heating was investigated. Microwave heating, in certain cases, can lead to increased yields and reduced reaction time.[12] Caution was taken when these investigations were initiated due to the fact that hydrogen gas is released when the alkyne is inserted into the decaborane-acetonitrile complex. Another mole of hydrogen gas is released in the formation of the decaborane-acetonitrile complex, so to minimize pressure buildup in the microwave vial **6** was made and isolated prior to microwave heating. As well, larger microwave vials than necessary were used to help accommodate the increase in pressure. Two alkynes were selected for this investigation (Figure 2.1). The first being the terminal alkyne used

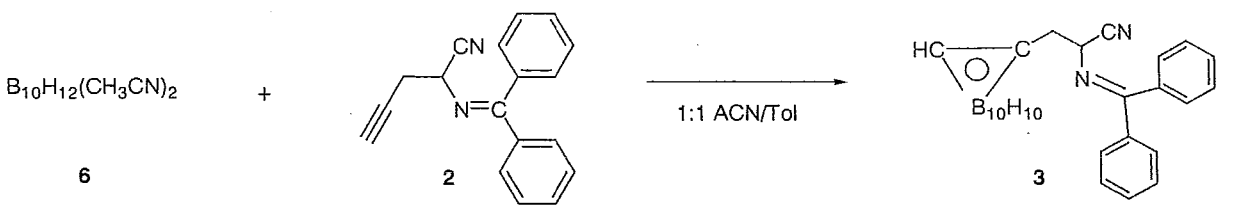
in the synthesis of carboranylalanine, and an internal alkyne **7** which are generally less reactive towards carborane formation due to steric bulk.



2 **7**

Figure 2.1: Alkynes used for microwave-assisted alkyne insertion

The alkyne insertion reactions were carried out successfully at temperatures ranging from 160 to 180 °C in 1:1 toluene/acetonitrile using the alkynes shown in Figure 2.1. Reactions were monitored by HPLC where the data for the alkyne insertion reactions are presented in Table 2.1 and Table 2.2. The percent conversion was calculated using the areas of the resulting HPLC chromatogram peaks.

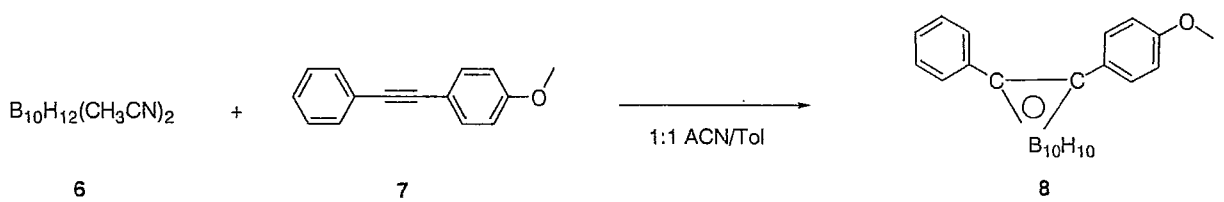


6 **2** **3**

Scheme 2.2: Microwave assisted alkyne insertion reaction of **2**

Reaction Time (min)	*Reaction Temperature (°C)*	*% Conversion to 3*
5	160	43
5	170	62
5	180	55
10	160	68
15	160	63

Table 2.1: Microwave assisted alkyne insertion reaction results for Scheme 2.2



6 7 8

Scheme 2.3: Microwave assisted alkyne insertion reaction of **7**

Reaction Time (min)	*Reaction Temperature (°C)*	*% Conversion to 8*
10	160	16
10	170	15
10	180	15
15	170	16
20	170	18

Table 2.2: Microwave assisted alkyne insertion reaction results for Scheme 2.3

It was found that for the insertion of alkyne **2**, the carboranylalanine precursor, the highest percent conversion (68%) to the carborane product **3** occurred at 160°C for 10 minutes. This percent conversion indicates that pending isolation procedures, the microwave-assisted alkyne insertion reactions give a higher conversion to the desired carborane than conventional methods, where the highest reported yield for this carborane is 36% with a reaction time of at least 3 hours.[8] Microwave-assisted alkyne insertion reactions for **7** to give carborane **8** (Scheme 2.3) were found to require higher temperatures and gave lower percent conversion compared to **3**. This is not surprising due to the sterically crowded nature of the alkyne which has two phenyl groups directly bound to the triple bond. Further work is required to explore the utility of the method using various alkynes, but preliminary data indicates that microwave assisted alkyne insertion reactions are worth investigating as a way to increase yields and reduce reaction times.

Continuing with the synthesis of Car, and with compound **3** in hand, a two-step hydrolysis reaction was necessary to form the final product. Imine hydrolysis was performed by treating **3** with 5M HCl to give the hydrochloride salt **4**. The side product of the reaction was benzophenone, which was separated from the desired product through column chromatography (chloroform/methanol, 10:1, v/v). Upon isolation of the amino containing product (41% yield), the nitrile was converted to the acid (**5**) by hydrolysis using 75% H_2SO_4. After being heated for 24 h at 95°C, the impurities were removed by

filtering the crude reaction mixture while still hot, then allowing the product to precipitate out while cooling the filtrate in an ice bath. The desired product **5** was isolated in good yield (67%) as a white solid.

Characterization of *closo*-carboranylalanine **5** was initially carried out by multi-nuclear NMR spectroscopy. Figure 2.2 shows the ^{11}B NMR spectrum of **5**. There are 10 unique boron environments that exist for carboranylalanine, but only 5 signals are resolved in the ^{11}B NMR spectrum. This is not surprising because the chemical and magnetic environments of the boron atoms are similar. Those peaks which are resolved are quite broad due to the large quadrupole moment of the ^{11}B nucleus.[13] This ^{11}B NMR spectrum also indicates that the carborane is in fact *closo* with no peaks appearing below -20 ppm which are characteristic of the deboranated cluster.[13] The ^{13}C NMR spectrum (Figure 2.3) shows 5 unique carbon signals as expected for *closo-o*-carboranylalanine including the carboxylic acid carbon signal at 169.9 ppm.

The ^{1}H NMR (Figure 2.4) spectrum of **5** exhibited all the expected peaks including the reported ABX spin system associated with the methylene protons appearing at 2.73 ppm, 3.13 ppm and the single X hydrogen at 4.16 ppm. The two multiplets are separated by 0.4 ppm, and resolve well to show an estimated J_{AB} coupling of 16.2 Hz and J_{AX} and J_{BX} coupling of 4.4 and 6.6 Hz respectively. This spectrum also exhibits the expected broad overlapping signals between 1.0 and 3.3 ppm corresponding to the B-H signals of the carborane cage. To aid in the interpretation of the NMR spectra, 2D experiments were conducted, including HMBC (Figure 2.6) and HSQC (Figure 2.5).

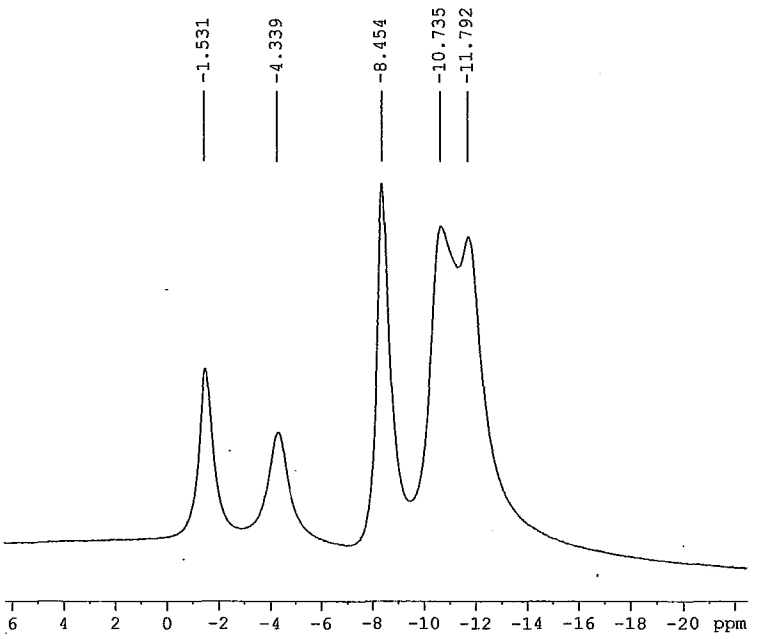


Figure 2.2: $^{11}B\{^{1}H\}$ NMR (160 MHz, CD_3OD) spectrum of *closo*-carboranylalanine **5**

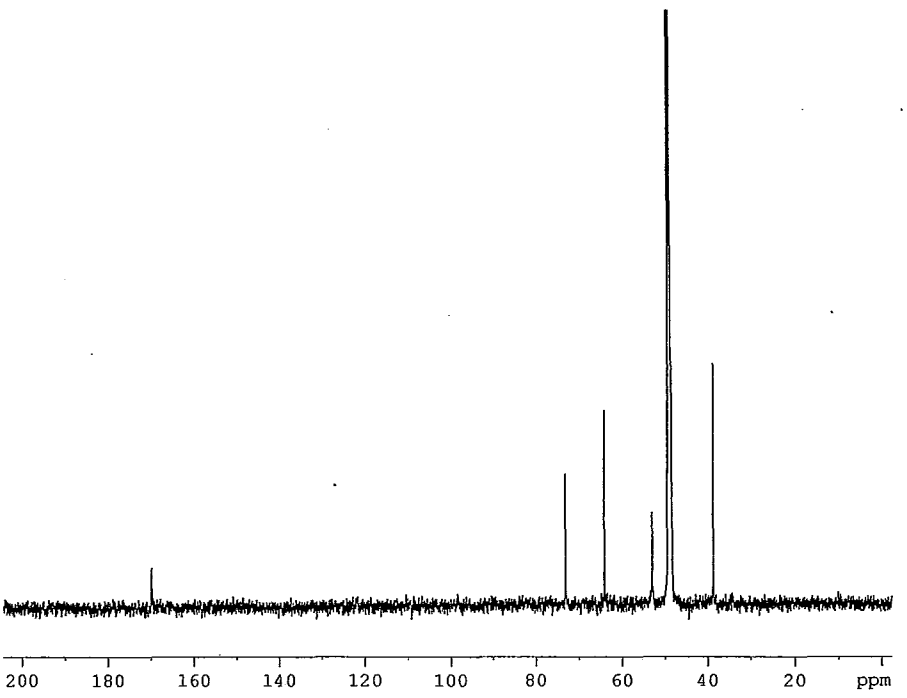


Figure 2.3: ^{13}C NMR spectrum (126 MHz, CD_3OD) of *closo*-carboranylalanine **5**

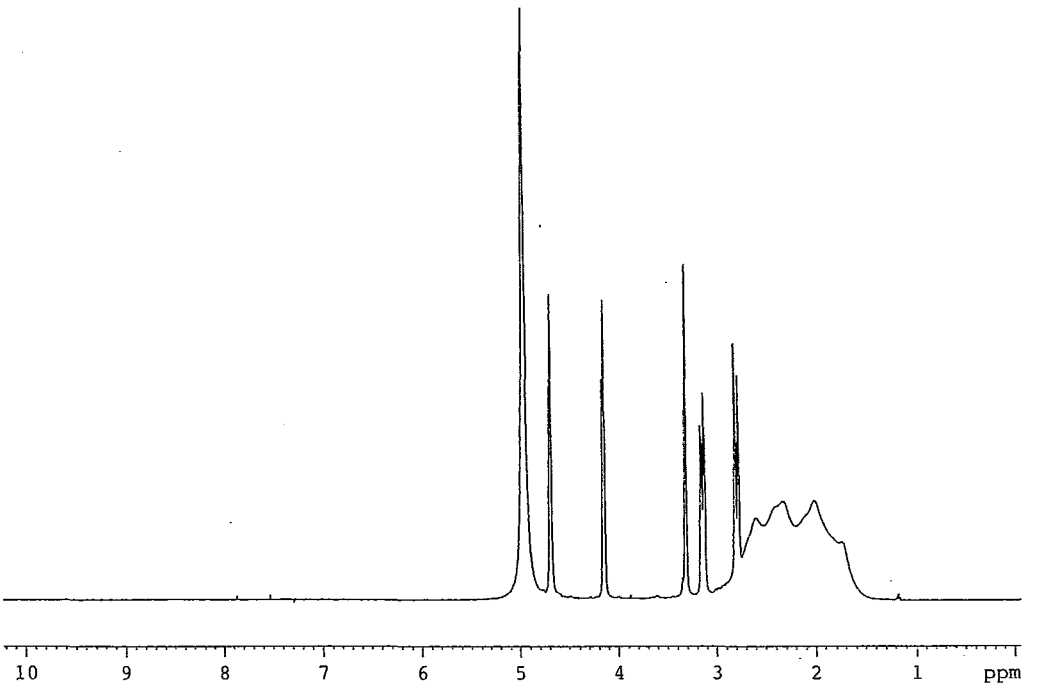


Figure 2.4: ^{1}H NMR spectrum (500 MHz, CD_3OD) of *closo*-carboranylalanine **5**

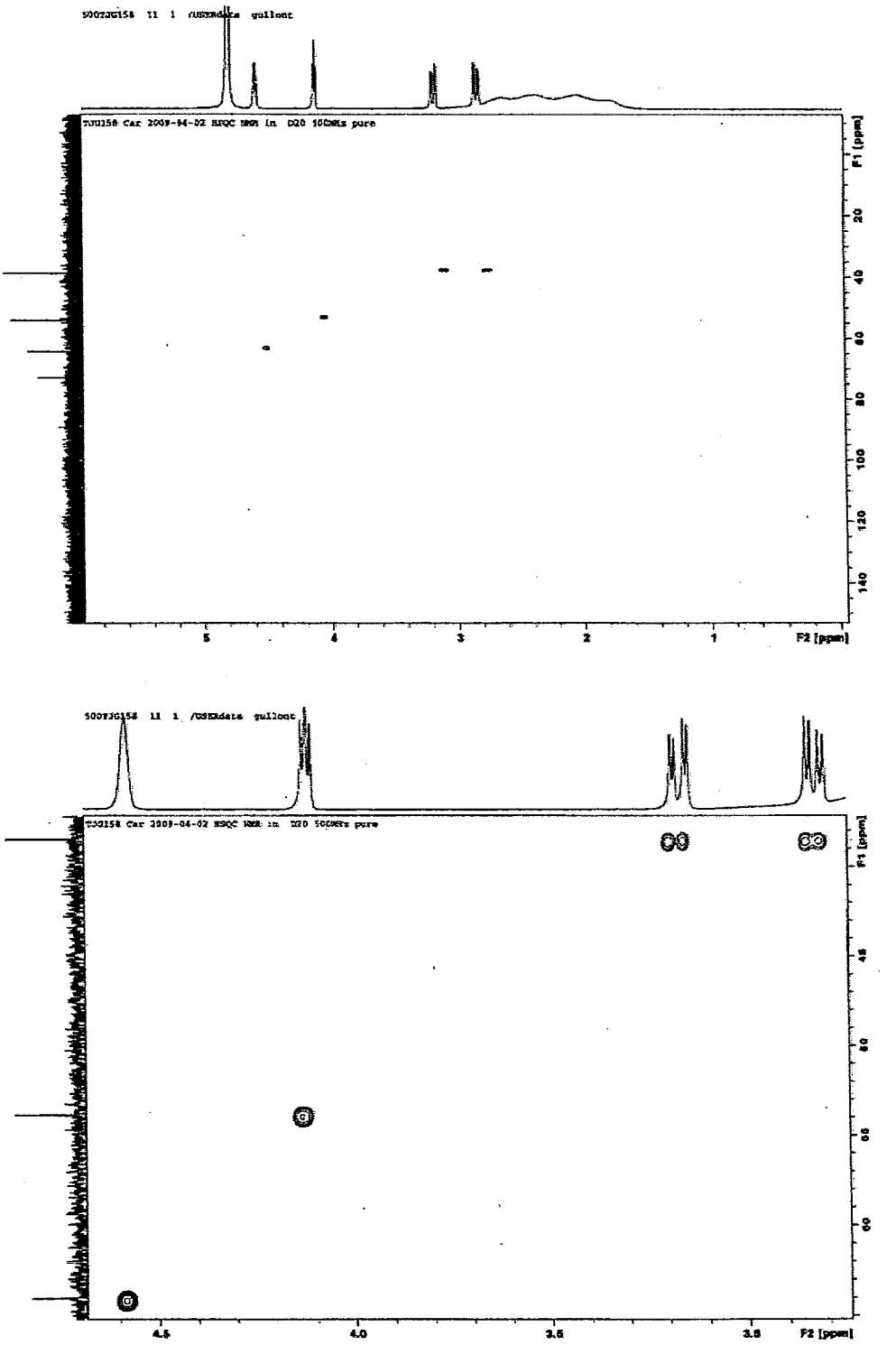


Figure 2.5: HSQC spectra (500 MHz, D_2O) of Carboranylalanine **5**

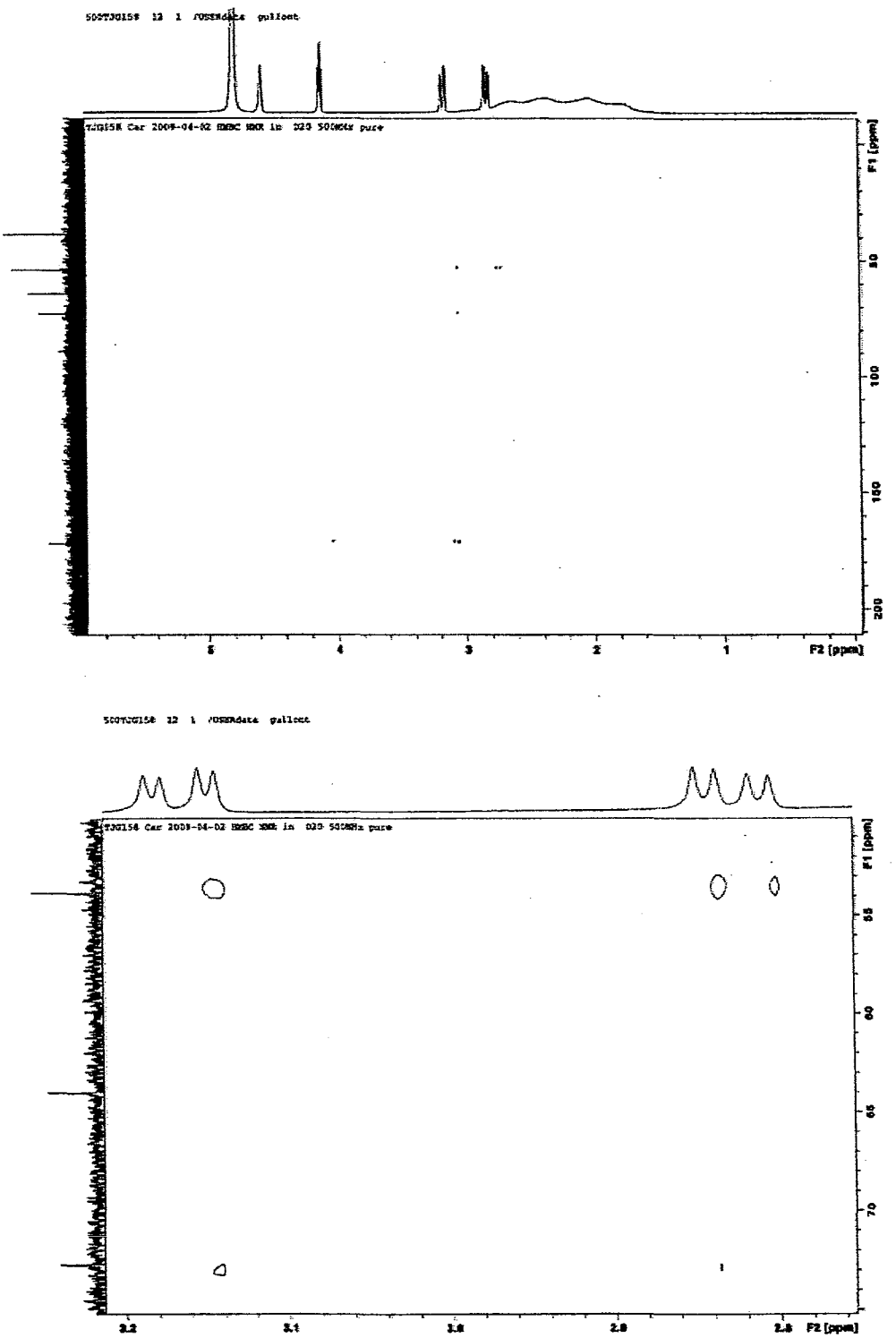


Figure 2.6: HMBC spectra (500 MHz, D_2O) of carboranylalanine **5**

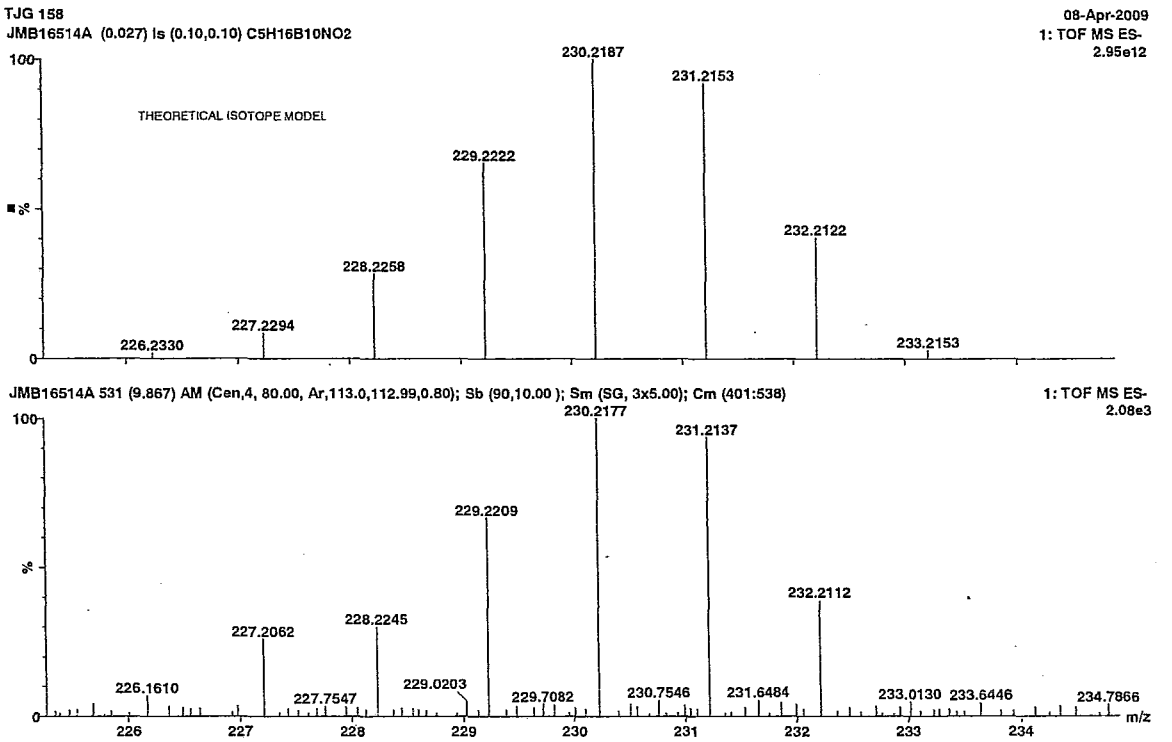
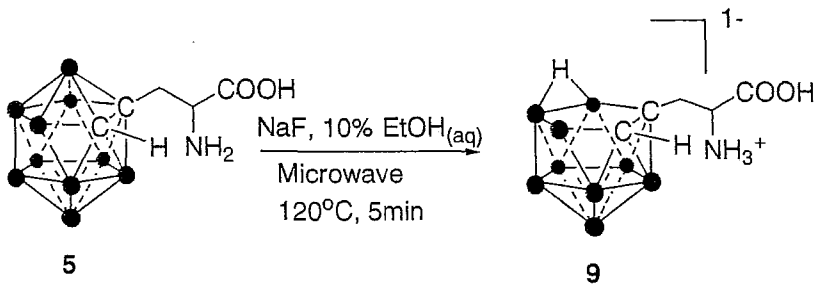


Figure 2.7: High resolution negative ion electrospray mass spectrum of carboranylalanine **5**

Figure 2.7 shows the high resolution negative electrospray mass spectrum of carboranylalanine (bottom) where it can be seen that the m/z isotope distributions match well with the isotope model (top).

2.2 *Synthesis of Nido-Carboranylalanine (Nido-Car)*

With the desired carboranylalanine ligand (**5**) in hand, cage degradation to form the *nido* complex (**9**, Scheme 2.4) was examined. Previous findings showed that the cage degradation occurs following first order kinetics with respect to *o*-carboranylalanine under slightly acidic conditions in water and methanol.[14] After 3 days of stirring at room temperature in a citric acid buffer solution (pH= 5), ^{11}B NMR analysis revealed only a small amount of conversion to the *nido* ligand (< 20%). To achieve a greater degree of conversion, microwave-assisted synthesis was investigated.[15] *Closo*-carboranylalanine was combined with sodium fluoride (7 eq.) in aqueous ethanol and the mixture heated for 5 minutes at 120°C. ^{11}B NMR of the crude reaction mixture (Figure 2.8) showed that only trace amounts of the *closo*-carborane remained in solution along with other boron side products.



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Scheme 2.4: Synthesis of *nido*-carboranylalanine (*nido*-Car) **9**

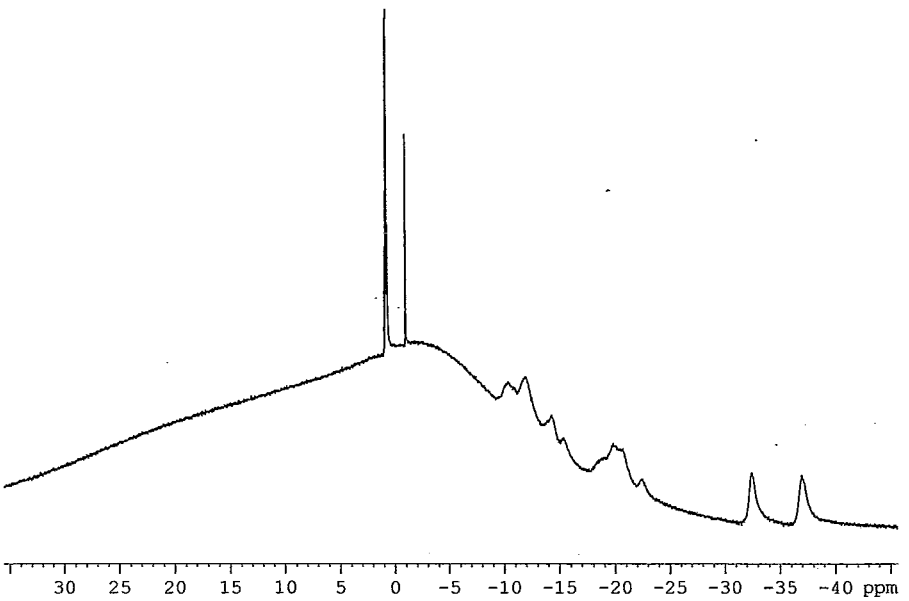


Figure 2.8: $^{11}B\{^1H\}$ NMR spectrum (160 MHz, D_2O) of the crude reaction mixture containing *nido*-carboranylalanine **9**

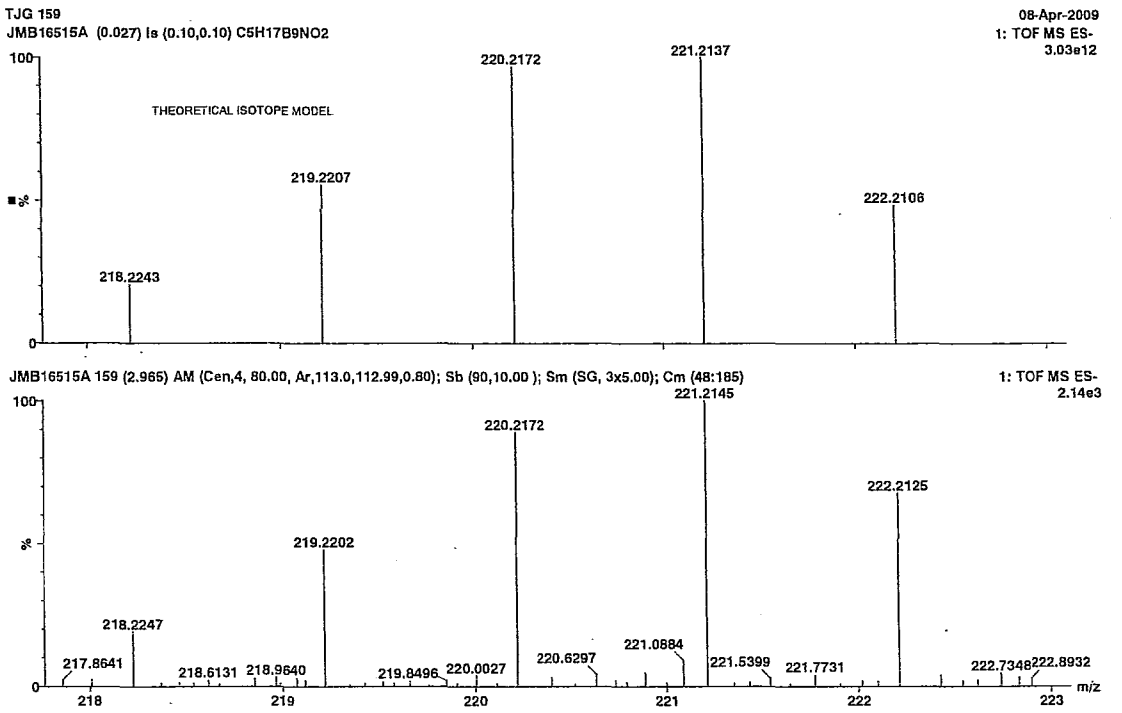
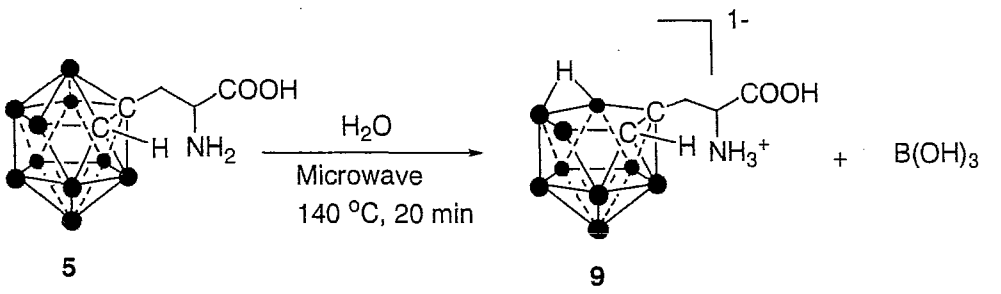


Figure 2.9: High resolution negative ion electrospray mass spectrum of *nido*-carboranylalanine **9** (bottom), and theoretical isotope model (top).

Later, it was found that the presence of NaF in the reaction mixture was not needed as the conversion from *closo* **5** to *nido* **9** could occur in water under microwave heating. The only side product from this reaction was boric acid which could be seen as a sharp signal in the ^{11}B NMR at 20 ppm (Scheme 2.5).[16]



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Scheme 2.5: Synthesis of *nido*-carboranylalanine (*nido*-Car) **9** in water

Issues arose when it came to isolation of *nido*-carboranylalanine from the boric acid side product. Many efforts were made to remove the boric acid including: silica gel column chromatography, preparative TLC, solid-phase extraction using both reversed phase silica and ion exchange resins. It appeared as though the boric acid had very similar properties to the product, and could not be separated through conventional chromatographic methods. Even semi-preparative HPLC was not able to remove the boric acid; a semi-preparative HPLC fraction of *nido*-Car which was shown to be pure by analytical HPLC, still contained residual boric acid according to the $^{11}B\{^1H\}$ NMR. To verify that the peak at 20 ppm corresponds to boric acid, a sample of *nido*-Car was spiked with boric acid and the $^{11}B\ \{^1H\}$ NMR recorded. It can be seen from Figure 2.11 that the boric acid peak clearly overlaps with the peak at 20 ppm present in the sample of *nido*-Car confirming that boric acid was in fact still present.

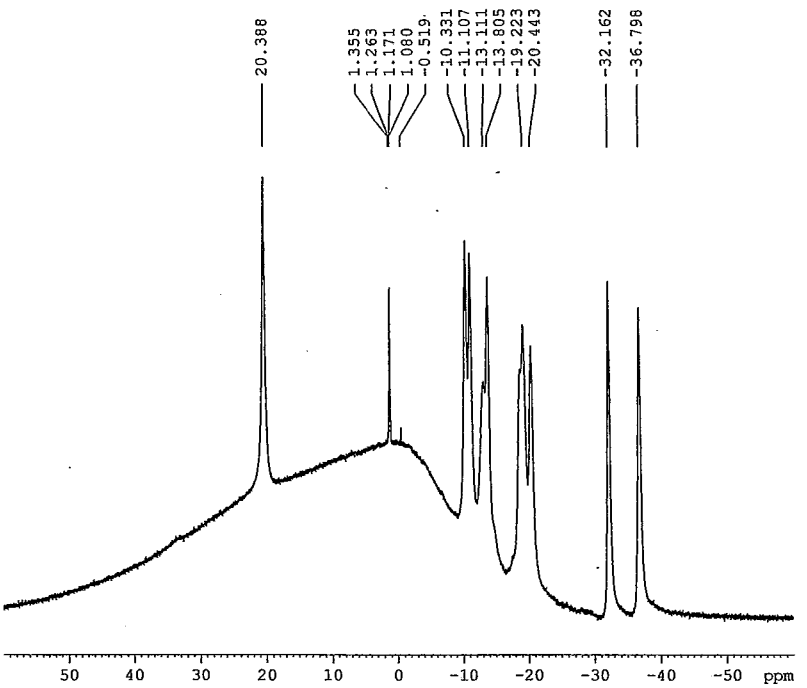


Figure 2.10: $^{11}B\{^{1}H\}$ NMR spectrum (160 MHz, D_2O) of *nido*-Car **9** containing boric acid

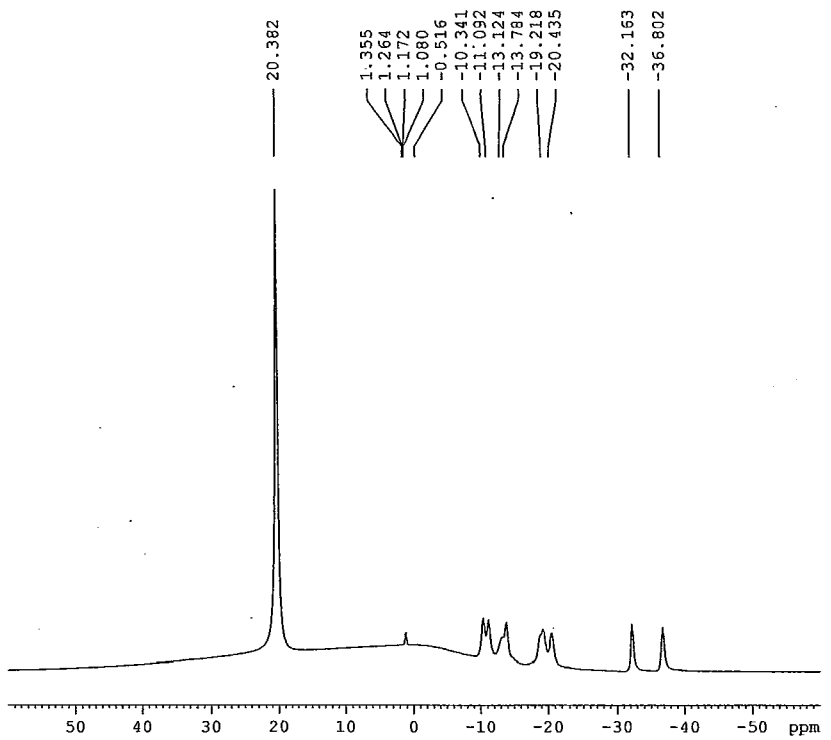


Figure 2.11: $^{11}B\{^{1}H\}$ NMR spectrum (160 MHz, D_2O) *nido*-Car **9** spiked with boric acid

It was hypothesized that perhaps there was a hydrogen-bonding interaction occurring between the amino acid portion of carboranylalanine and the boric acid. Other reports of such interactions include the supramolecular hydrogen-bonded structure of a 1:2 adduct of melamine with boric acid where boric acid forms hydrogen bonds with the nitrogen donor sites of melamine (Figure 2.12).[17] As well, 1:1 and 1:2 complexes of boric acid with polyhydroxy compounds such as chromotropic acid have been studied extensively by Shao *et al.*[18]

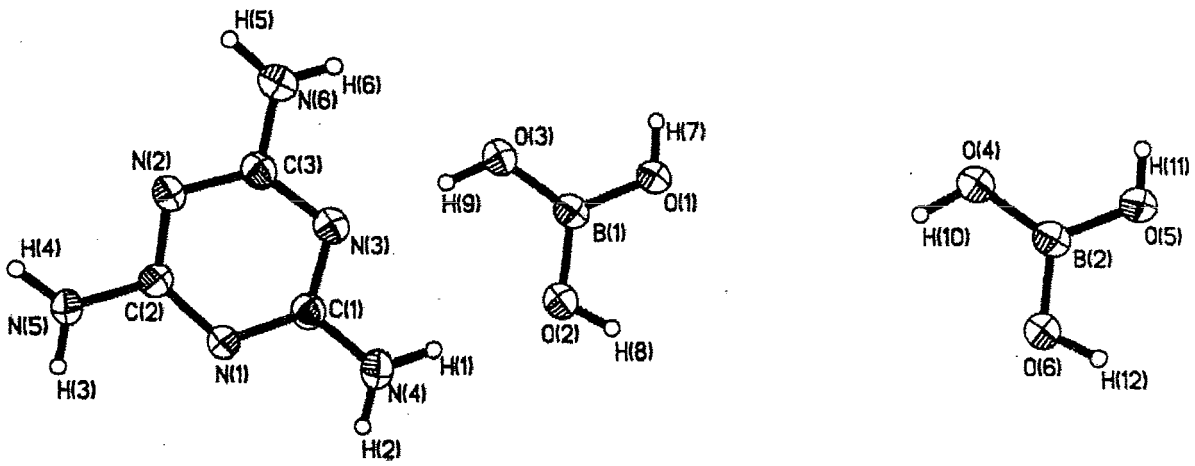
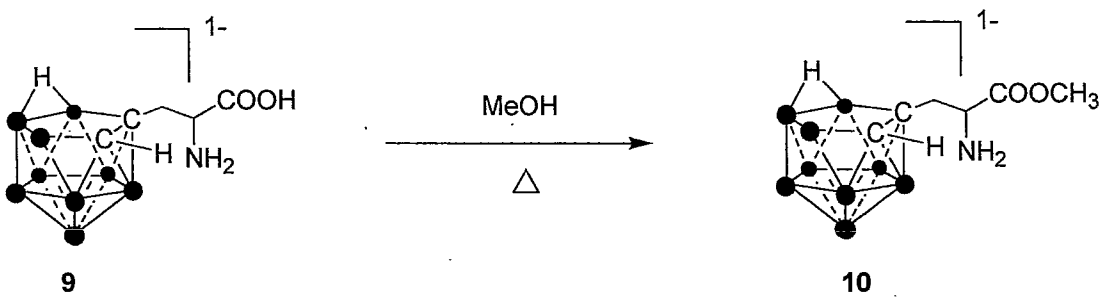


Figure 2.12: Molecular structure of a 2:1 boric acid: melamine complex. The thermal ellipsoids are given at 50% probability[17]

To facilitate the removal of boric acid, one possible approach was to convert it into a species that was more easily removed. Conversion of the boric acid to the more volatile trimethyl borate through repetitive treatment with methanol and acetic acid[19] (4 times) at room temperature was attempted followed by the removal of all volatile components under vacuum (using a V10 evaporator system). This strategy ultimately lead to the isolation of the *nido*-car with no boric acid (Figure 2.13). It was determined necessary to carry out the removal of the volatile species as close to room temperature as possible because if the mixture was heated on a rotatory evaporator instead of the V10, conversion to the corresponding methyl ester **10** occurred (Scheme 2.6).



9 10

Scheme 2.6: Formation of *nido*-Car methyl ester **10**

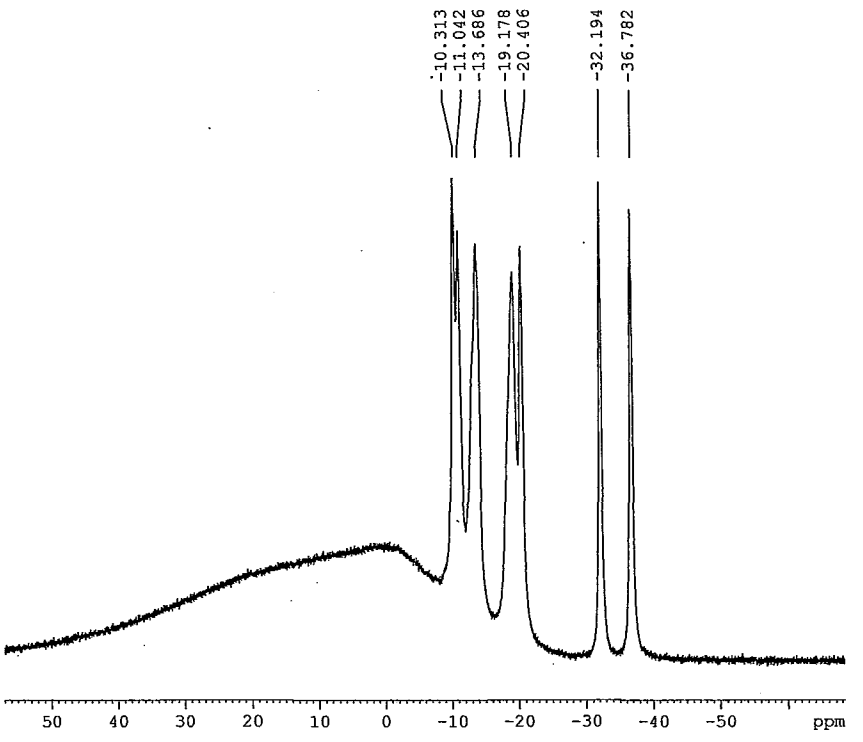


Figure 2.13: ^{11}B {^{1}H} NMR spectrum (160 MHz, D_2O) of pure *nido*-carboranylalanine **9**. Note the absence of the boric acid peak at 20 ppm

Figure 2.13 shows the ^{11}B $\{^1H\}$ NMR of the purified product with no boric acid present. *Nido*-Car displays the expected signals for the cage borons bearing the bridging hydrogen at -32 and -37 ppm. Two dimensional NMR spectra were obtained to aid in the assignment of the ^1H and ^{13}C NMR spectra of **9** (Figure 2.14 and Figure 2.15). It is important to point out that during cage degradation of the *closo* species, there are two possible sites of deboronation (Figure 2.16), where either of the two boron atoms connected to both carbon atoms of the cage can be removed. This results in the formation of four isomers, two of which can be distinguished by ^1H NMR. The peaks which clearly demonstrate the two products are the broad C-H's of the carborane cage which show up at 2.03 and 2.00 ppm. The ^{13}C NMR shows two broad signals at 46.2 and 57.8 ppm which were assigned using a HSQC spectrum (Figure 2.14). It can be observed in the HSQC that both C-H carborane peaks in the ^1H NMR are shown as being attached to the signal at 46.2 ppm, indicating that although these peaks resolve in the ^1H NMR spectrum, they overlap to show a single broad signal in the ^{13}C NMR spectrum. The broad ^{13}C signal at 57.8 ppm is assigned as the functionalized carborane carbon as no ^1H NMR signals are shown to be attached to it in the HSQC spectrum.

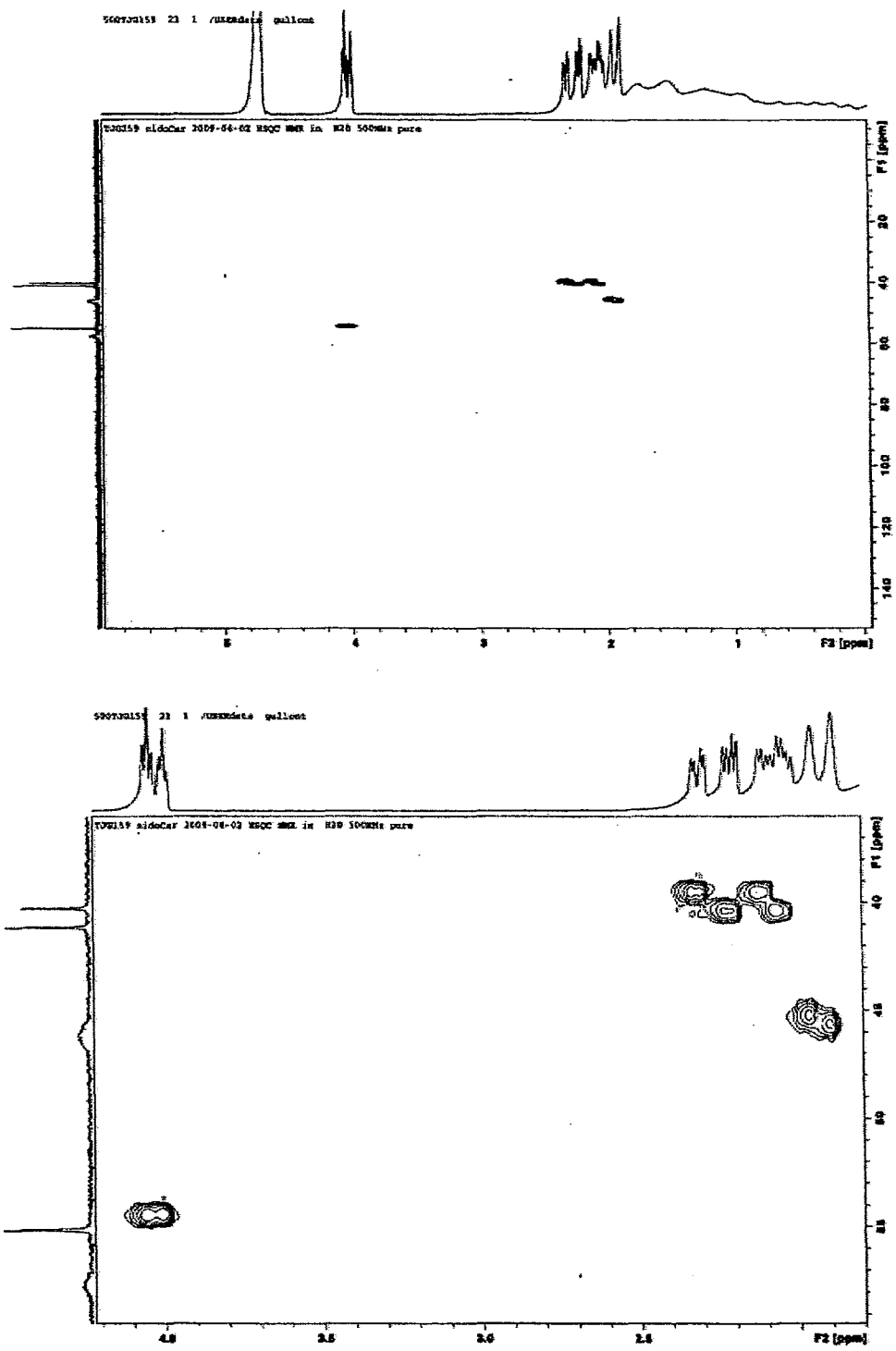


Figure 2.14: HSQC spectra (500 MHz, D_2O) of *nido*-carboranylalanine **9** (top), and expansion of the same spectrum (bottom)

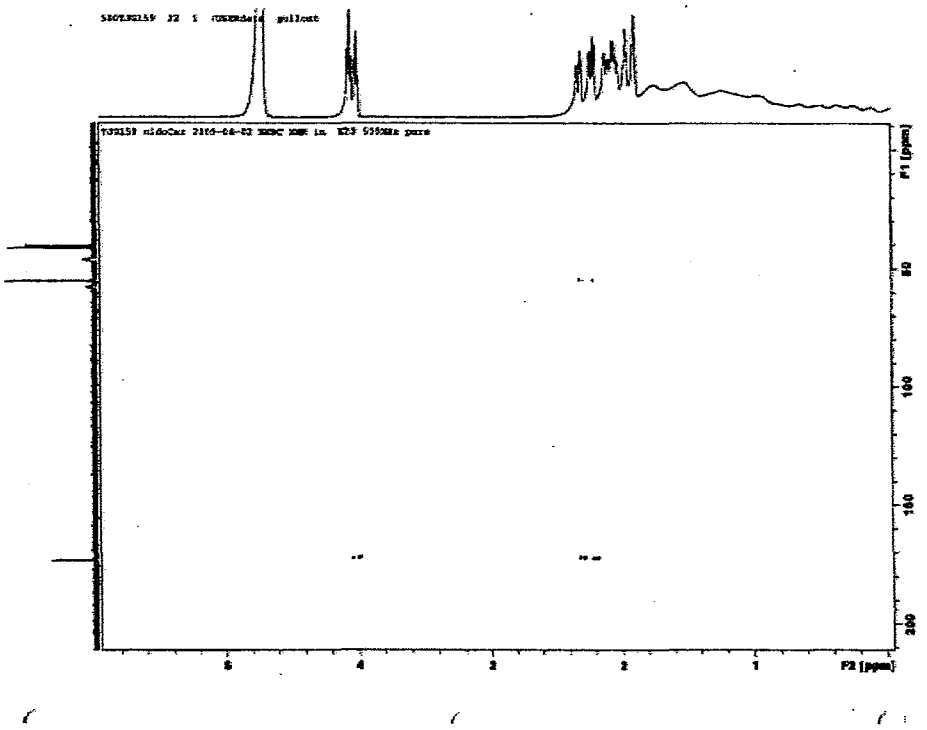


Figure 2.15: HMBC spectrum (500 MHz, D_2O) of *nido*-carboranylalanine **9**

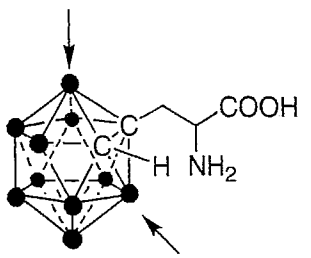
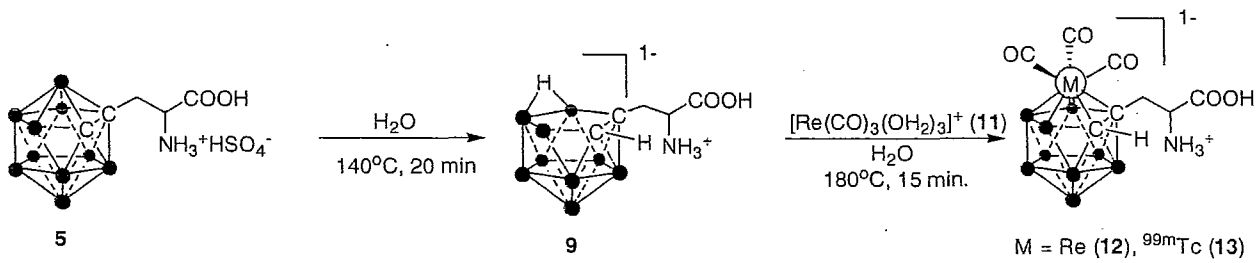


Figure 2.16: *Closo*-Car **5** with possible sites of deboronation (leading to *nido*-Car **9**) indicated with arrows

2.3 Synthesis of Re-Carboranylalanine

Following the isolation of **9**, the next step was to determine the optimal conditions for the formation of the $Re(CO)_3^+$ complex. This was first investigated by conversion of *closo*-Car **5** to *nido*-Car **9** in the microwave, followed by the direct addition of $[Re(CO)_3(H_2O)_3]Br$ **11** to the vial which was re-sealed and subjected to further microwave heating (Scheme 2.7). The mass spectrum of the crude reaction mixture showed an interesting result (Figure 2.18). Not only was Re-Car **12** formed during this reaction (based on the expected m/z ratio), but another rhenium containing compound was present. Based on the m/z value it appeared that a second $Re(CO)_3^+$ core had added to *nido*-Car, presumably through chelation with the amino acid functionality (Figure 2.17).



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Scheme 2.7: Synthesis of Re-Car **12**

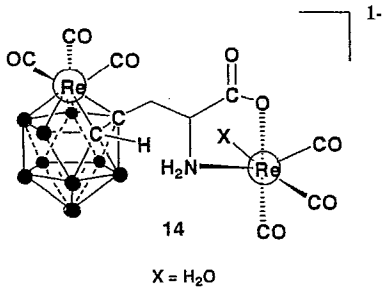


Figure 2.17: Structure of di-Re Car **14**

The dirhenium species **14** was not the desired product, so attempts were made to minimize its formation by varying reaction time, temperature, stochiometry and pH. The optimal conditions were heating at 180 °C for 15 minutes with 1.2 equivalents of $[Re(CO)_3(H_2O)_3]Br$ **11**. If a larger excess of **11** was used, the amount of di-Re **14** product formed was significant. Another option investigated to avoid the second rhenium addition, was to run the reaction under acidic conditions in order to protonate the amine, theoretically decreasing the chance of chelation with the amino acid. It was found that dropping the pH had no significant effect on the proportional amount of di-Re Car to the desired product and if the pH was dropped very low (>1) none of the desired product formed.

The analytical HPLC chromatogram of the crude reaction mixture from the reaction of *nido*-Car and **11** at 160 °C for 15 minutes with no adjustment to the pH can be seen in Figure 2.19. Clearly a mixture of products was produced during this reaction, although the mass spectrum of the reaction mixture indicated a limited number of

carborane or rhenacarborane products which have a very distinctive isotope pattern due to the naturally abundant isotopes present in the product (^{10}B, ^{11}B, ^{185}Re and ^{187}Re).

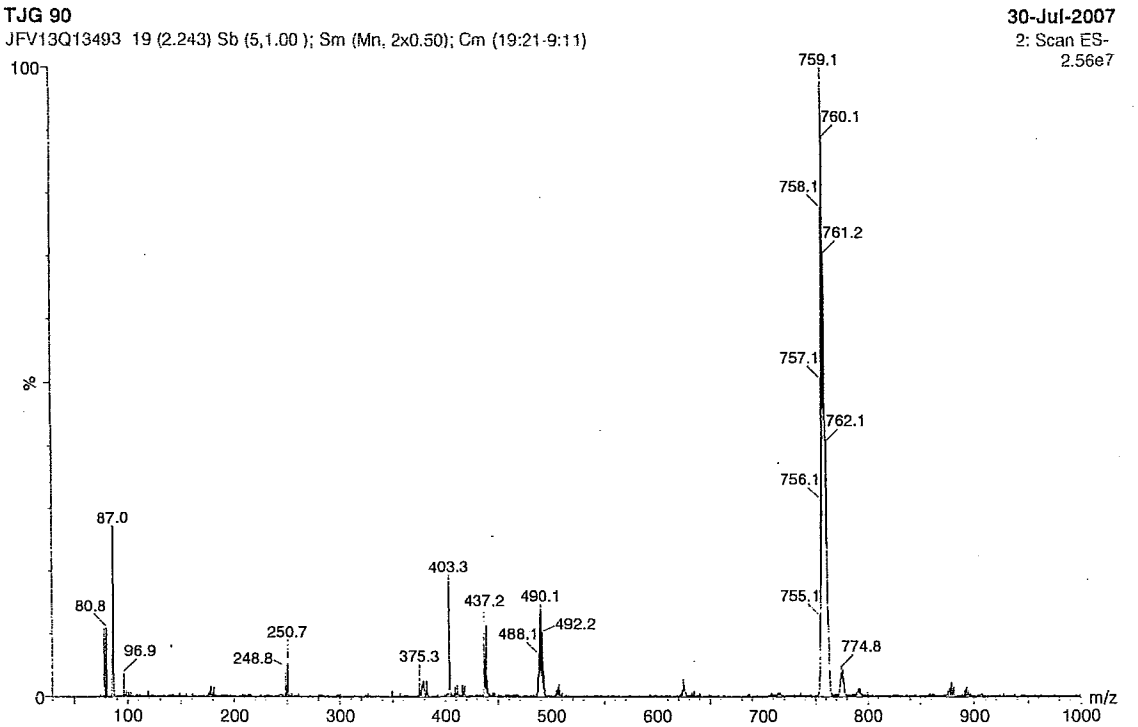


Figure 2.18: Negative ion electrospray mass spectrum of the crude reaction mixture shown in Scheme 2.7 containing Re-Car **12** (m/z = 490.1) and di-Re Car **14** (m/z = 759.1)

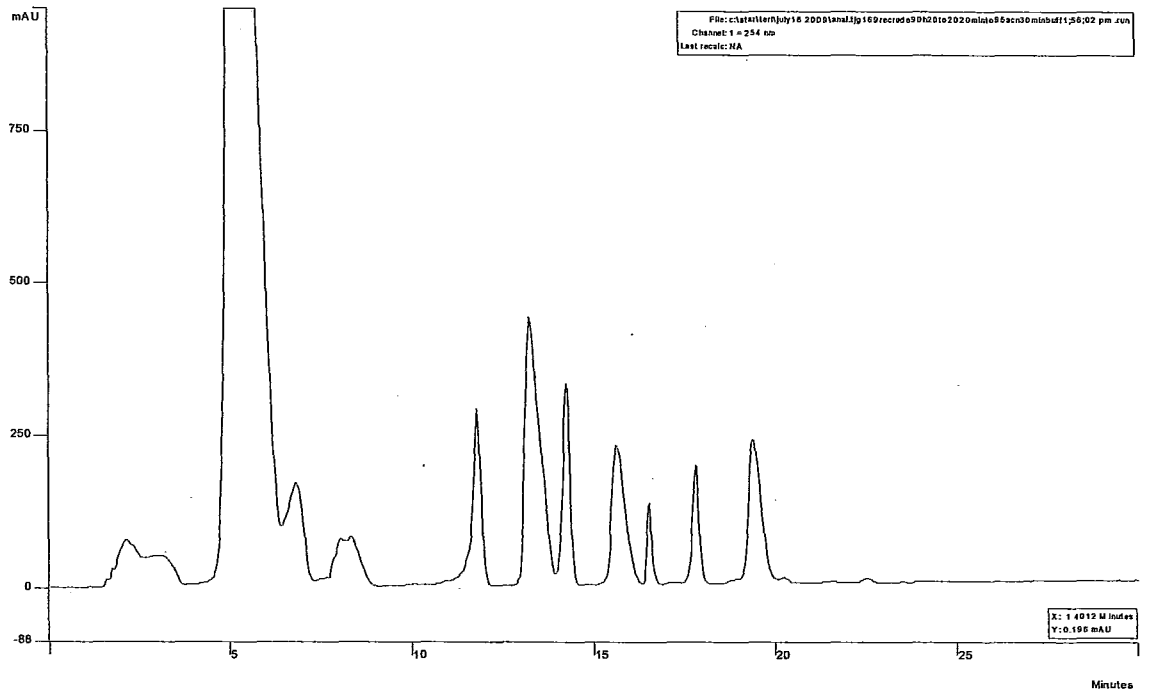


Figure 2.19: Analytical HPLC chromatogram (λ = 254 nm) of the crude reaction mixture of *nido*-Car **9** with $[Re(CO)_3(H_2O)_3]Br$ **11** (1:1.2 eq) at 160 °C for 15 min

Although the HPLC looks complex, there are only three peaks containing carborane derivatives (disregarding residual *nido*-Car which shows up at 8.5 min). LCMS of the crude reaction mixture (Figure 2.20) showed that there were two peaks corresponding to the expected 490 m/z for Re-Car (12.8 min, 15.4 min), and another at 19.6 min which matches with the di-Re complex **14**. Another value in the LCMS for the peak at 19.6 minutes at 800.2 m/z likely corresponds to an adduct of the dirhenium species where the X ligand has been exchanged for a higher molecular weight molecule. Using semi-preparative HPLC, the three carborane peaks were isolated (Figure 2.21),

which eluted at 15 min, 17 min and 22 min under the conditions used to separate the products. The other peaks present in the HPLC chromatogram are speculated to be salts formed during the reaction including numerous Re clusters.[20] It is important to note that chemistry at the macroscale does not necessarily reflect that at the tracer level. Although the formation and isolation of Re-Car led to quite low yields (~3%), at the tracer level it was found (Chapter 3) that the number of side products was significantly reduced.

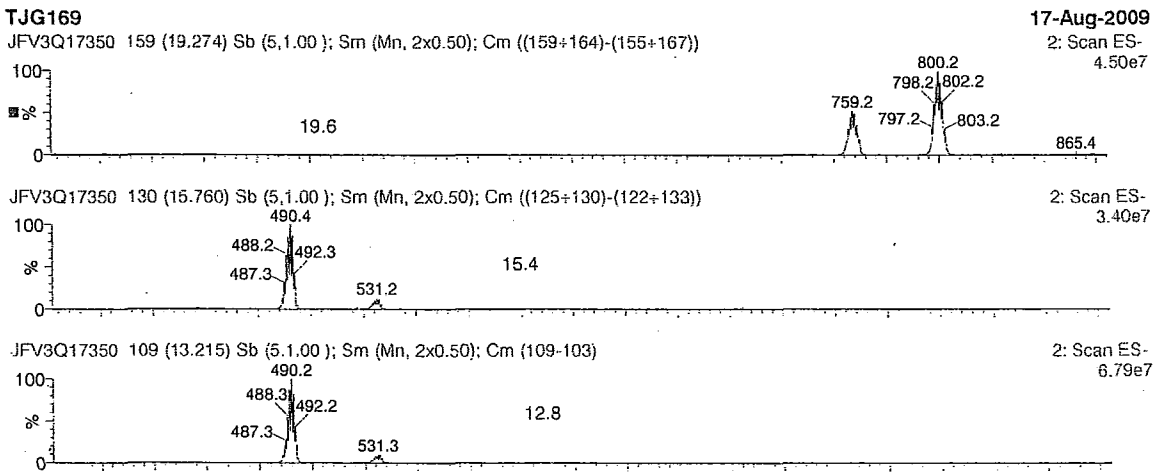


Figure 2.20: Negative ion electrospray LCMS spectrum for Re-Car reaction mixture showing the m/z values for the three peaks present in the LCMS at 19.6 min (top), 15.4 min (middle) and 12.8 min (bottom).

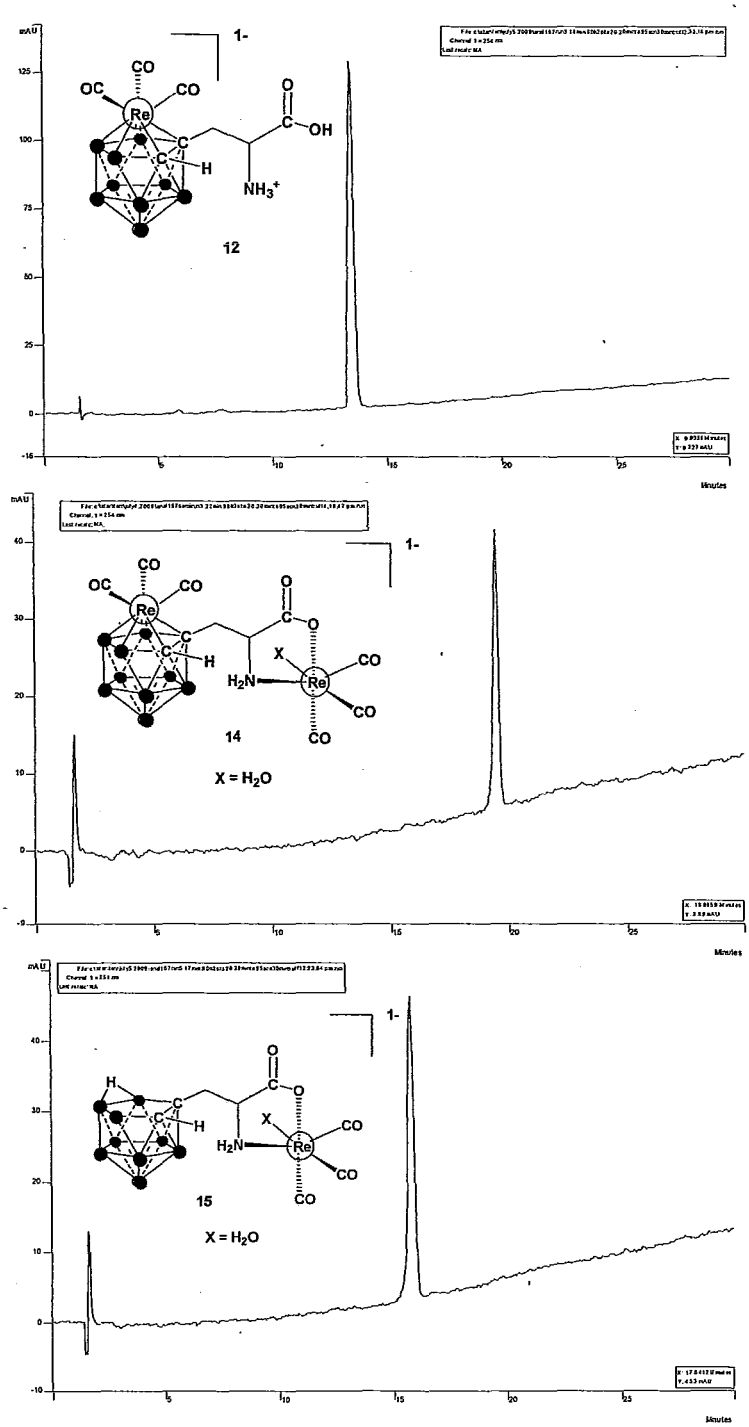


Figure 2.21: Analytical HPLC chromatograms of pure Re-Car products. Desired Re-Car metallocarborane **12** (top), di-Re Car **14** (middle) and Re-chelate carborane **15** (bottom)

It was found that the peak at 17 minutes (**15**) would change HPLC retention time depending on how the sample was handled. Samples were left in the buffer solution (due to isolation by semi-preparative HPLC) which consisted of an ammonium acetate/H_2O; formic acid/ACN mixture, and kept cold in a freezer. From this sample HPLC chromatograms would show a single peak at 17 min. If however, the solvent was removed on a rotatory evaporator with heating, then redissolved in the same buffer solution and immediately injected, two peaks of different retention times (14 and 17 min) were present in the HPLC (Figure 2.22). If this sample was left at room temperature and reinjected, the peak at 14 minutes reverts back to the one at 17 minutes.

The ^{11}B $\{^1H\}$ NMR spectrum of the peak at 17 minutes (Figure 2.23) resembles that of a *nido* species due to the presence of two peaks around -30 ppm. The proposed structure for this compound **15** is seen in Figure 2.24. If this were the case, then the bridging hydrogen would still be present, and another ligand would also be bound to the rhenium core (X). The concentration of potential X ligands changes when the buffer solvent is removed on the rotary evaporator. This change in concentration would likely lead to a switching of X ligands (water, acetate or formic acid) in the complex **15** and could explain the change in retention time as well as the switch back once the original concentration is restored.

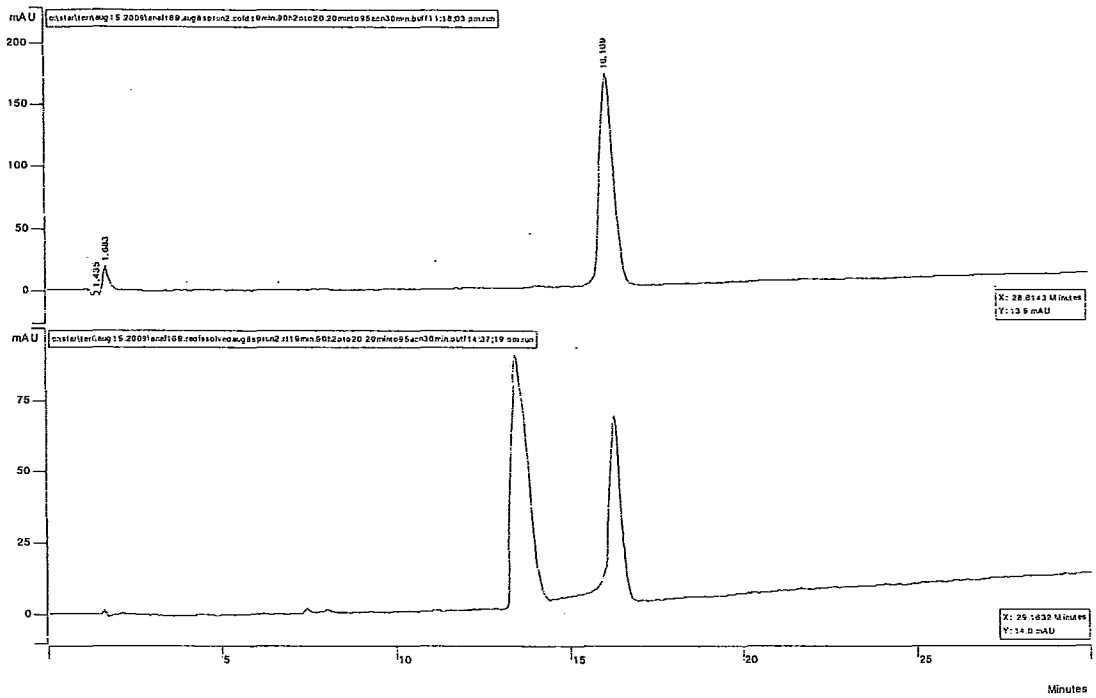


Figure 2.22: HPLC chromatograms of peak at 17 min at r.t. (top) and after rotary evaporation (bottom)

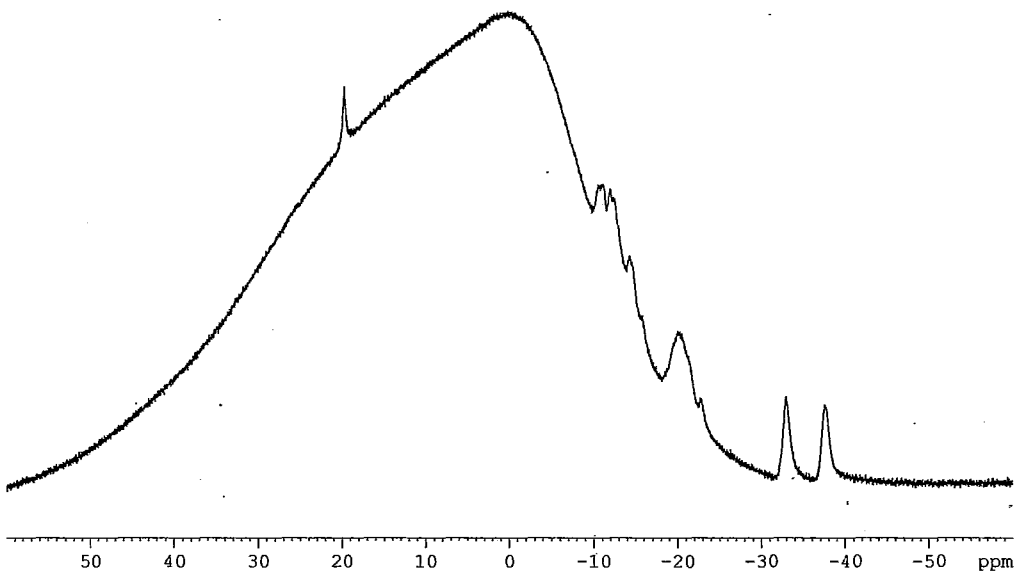
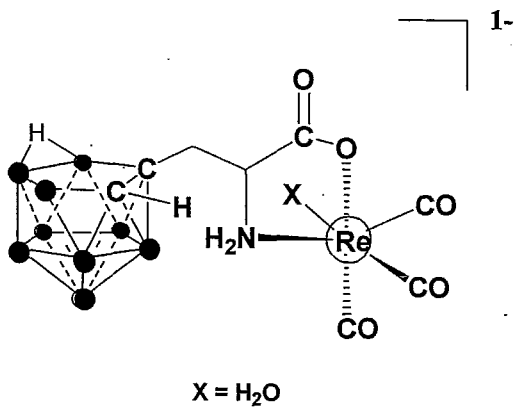


Figure 2.23: $^{11}B\{^{1}H\}$ NMR spectrum (160 MHz, D_2O) of Re-Car chelate **15** HPLC peak at ~17 minutes at room temperature



X = H_2O

Figure 2.24: Re(Car) chelation product **15**

The peak at 17 minutes was established as not being the desired metallocarborane product, so the peak at 14 minutes became the primary focus. To check the stability of this peak compared to the peak at 17 minutes, the same experiment was performed by

leaving the sample in buffer, checking the HPLC chromatogram of a cold sample, one at room temperature and another after having the solvent removed on the rotatory evaporator and redissolved in the buffer. No change was found in the HPLC traces for this peak. The $^{11}B\{^{1}H\}$ NMR spectrum shows no sign of the bridging hydrogen, but resembles the typical metallocarborane spectrum with peaks between -25 and 0 ppm (Figure 2.25).[21] The high resolution ESMS was also consistent with the desired product (Figure 2.26).

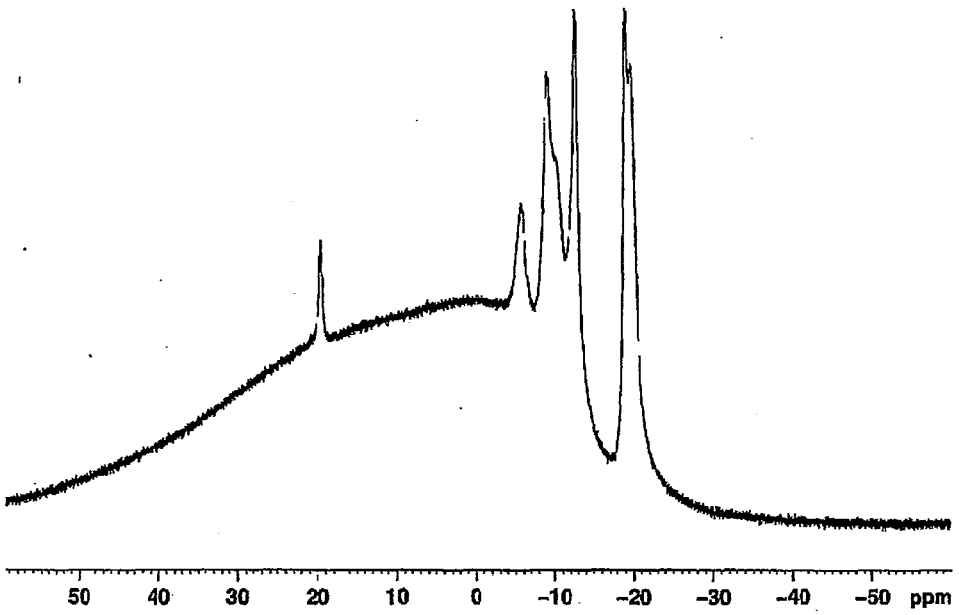


Figure 2.25: $^{11}B\{^{1}H\}$ NMR spectrum (160 MHz, D_2O) of Re-Car **12** which gives rise to the HPLC peak at 14 minutes

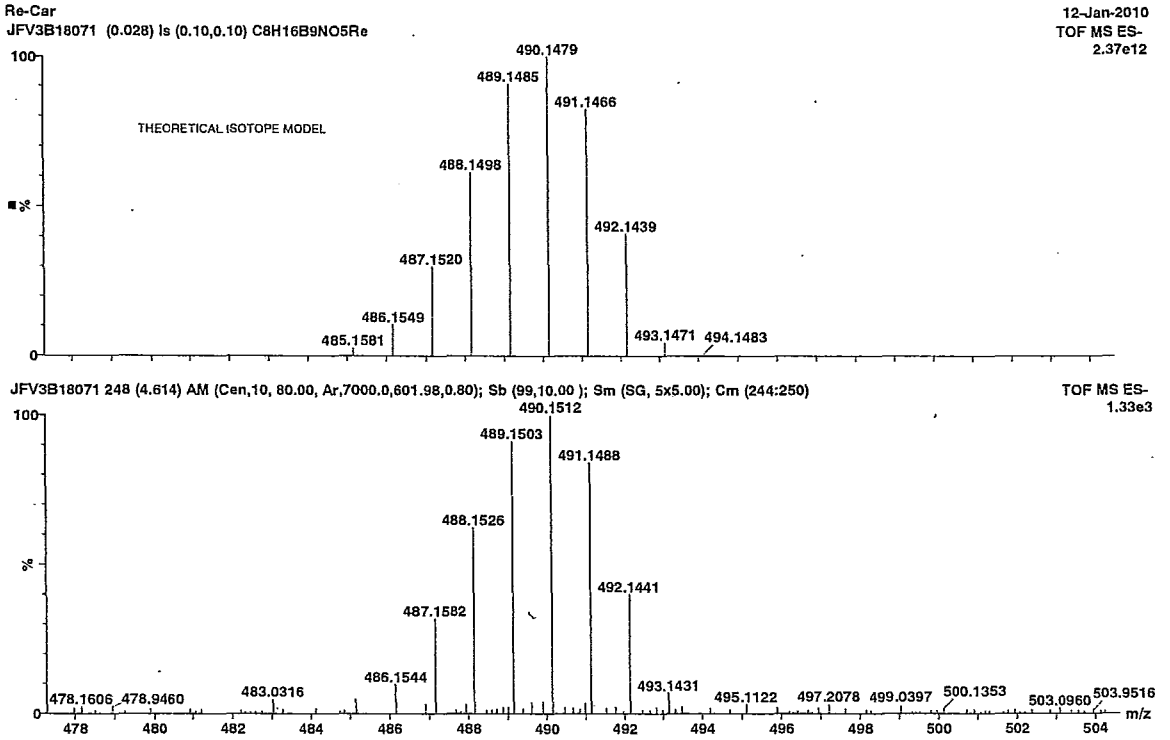


Figure 2.26: High resoluation negative ion electrospray mass spectrum of Re-Car **12** (bottom) with isotope model (top)

The desired product was isolated through semi-preparative HPLC with some difficulty as the separation between the desired peak and an impurity was less than a minute. Numerous HPLC fractions were combined and the buffers were removed through use of a C18 SepPak® cartridge. The SepPak® was first activated with ethanol, acetonitrile and then flushed with water prior to loading the sample. The sample was loaded in water, and the SepPak® was washed with a substantial amount of water without any breakthrough of the sample. The desired product could then be eluted with 100% acetonitrile and **12** isolated following rotary evaporation.

Multi-NMR spectra were obtained on the Re-Car product **12** on the 700 MHz NMR spectrometer to maximize resolution and sensitivity. There are in fact a total of four isomers because the parent molecule (Car **5**) was prepared as a racemic mixture, however two sets of peaks were identified, which would be expected due to the two diastereomers formed during cage degradation (Figure 2.27).

The ^{1}H NMR spectrum was very similar to that of the *nido*-Car ligand **9** although it is interesting to note that the two C-H protons from the carborane overlap to give a single peak at 2.07 ppm. This is unlike in the proton NMR spectrum for *nido*-Car where the two isomers gave two separate peaks at 2.00 and 2.03 ppm. The ABX spin-systems for the two diastereomers resolved in the ^{1}H NMR spectrum for Re-Car which allowed for their assignments through the aid of two-dimensional NMR (HSQC and HMBC). The differences in chemical shift between **9** and **12** were quite small, but the resolution of the peaks was improved. When the rhenium tricarbonyl-carborane complex is formed this could restrict rotation of the functional group that is pendant to the cage which would help distinguish the different environments of the ABX spin system. The coupling constants for the system were extremely similar in the two sets of diastereomers, with J_{ab} = 16.2 Hz for both. The coupling constants for the BX splitting were both 10.2 Hz compared to the AX coupling constants which were 1.8 and 1.7 Hz.

The ^{13}C NMR spectrum is shown in Figure 2.29 where the carbonyl carbon signal is at 173 ppm. This is the only carbon signal that did not resolve for the two

diastereomers (excluding the C=O ligands of the rhenium). The other four carbon environments resolved for the different isomers but in all cases by less than 0.4 ppm.

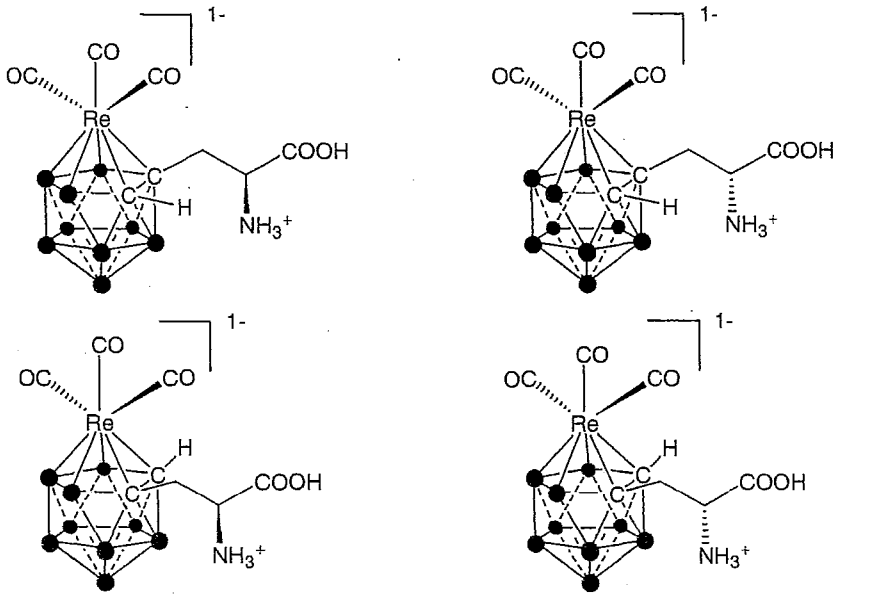


Figure 2.27: Structural isomers of Re-Car **12**

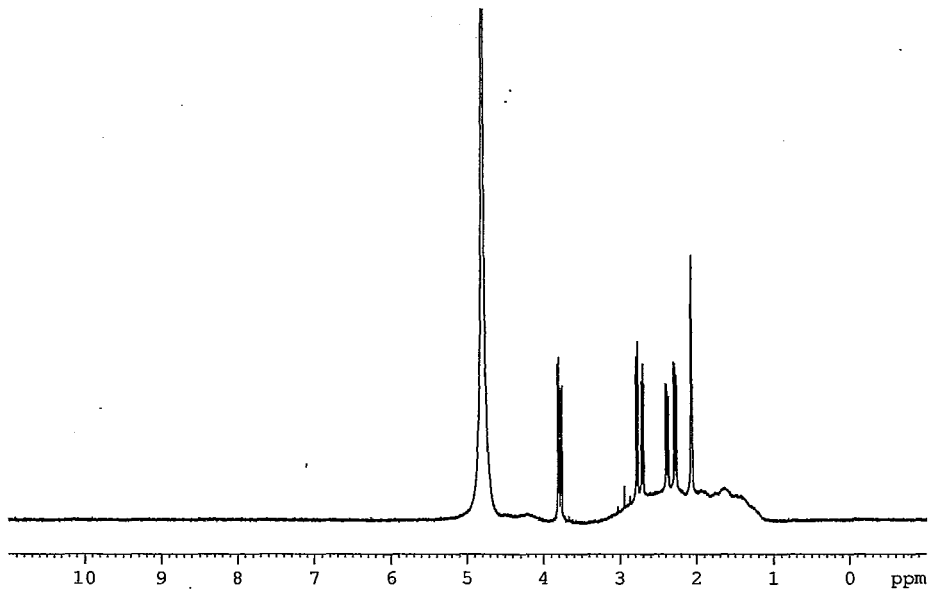


Figure 2.28: ^{1}H NMR spectrum (700 MHz, D_2O) of Re-Car **12**

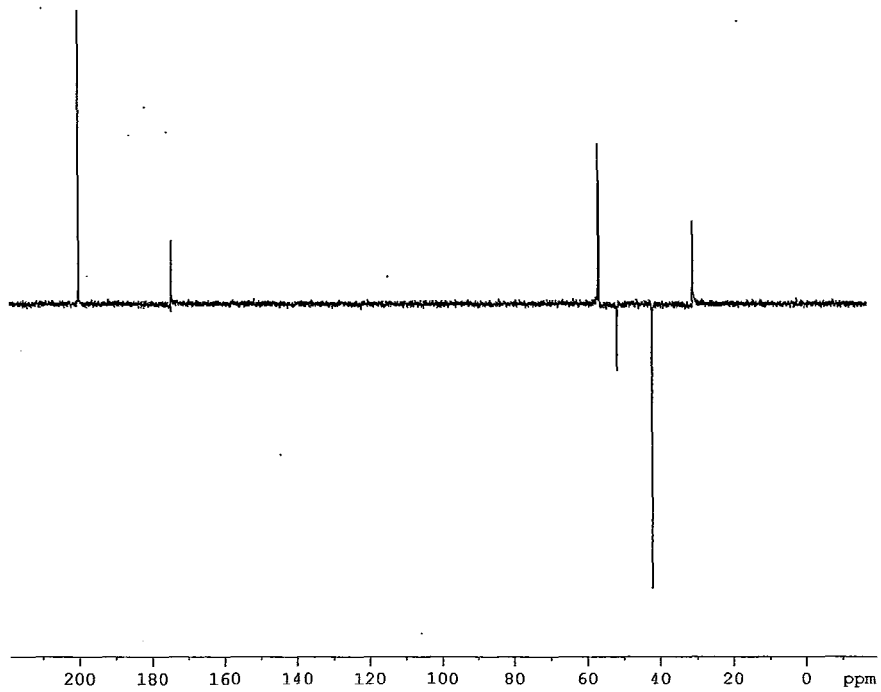


Figure 2.29: Jmod spin sort ^{13}C NMR spectrum (176 MHz, D_2O) of Re-Car **12**

Nuclear Overhauser effect (nOe) spectra were obtained to determine whether or not the cage of the carborane had actually isomerized from the 3, 1, 2 isomer to the 2, 1, 8 isomer.[22] The nOe revealed no enhancement in the C-H of the carborane signal when the methylene protons were irradiated (Figure 2.30). This is a clear indication that the methylenes are not in close proximity to the carborane C-H, while enhancement of the C-H of the ABX spin system was observed. These findings lead to a new representation of Re-Car, where the substituted carbon on the bonding face has rotated away from the rhenium yielding the 2,1,8-isomer (Figure 2.31).

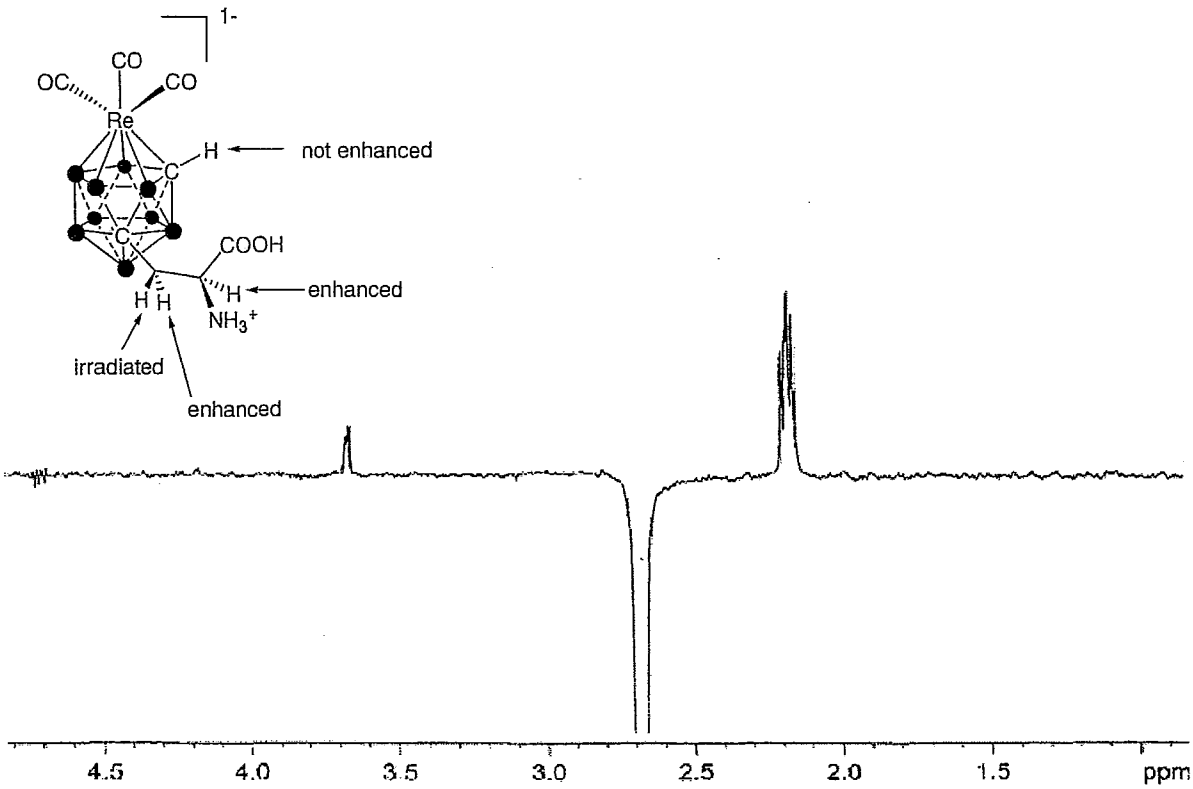


Figure 2.30: **1D Selective nOe spectrum of Re-Car** 12**, irradiation at 2.69 ppm**

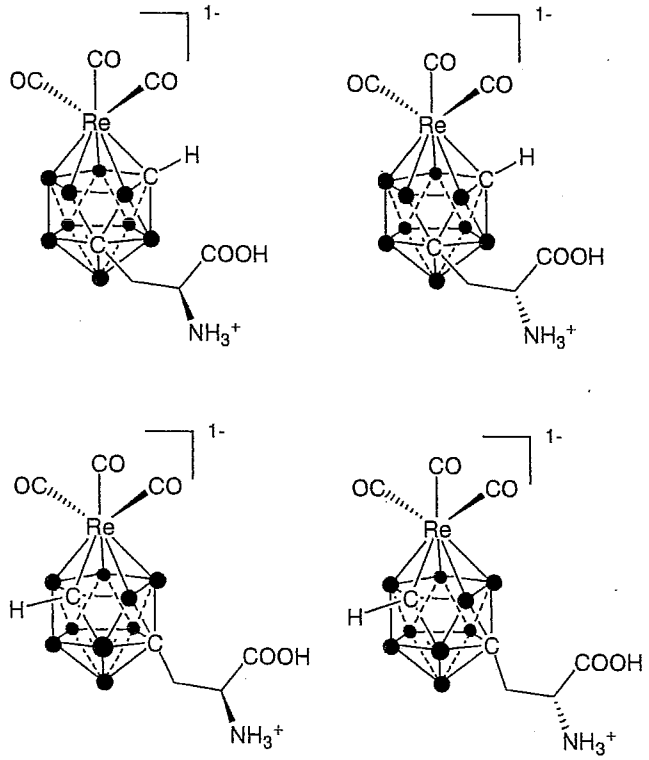
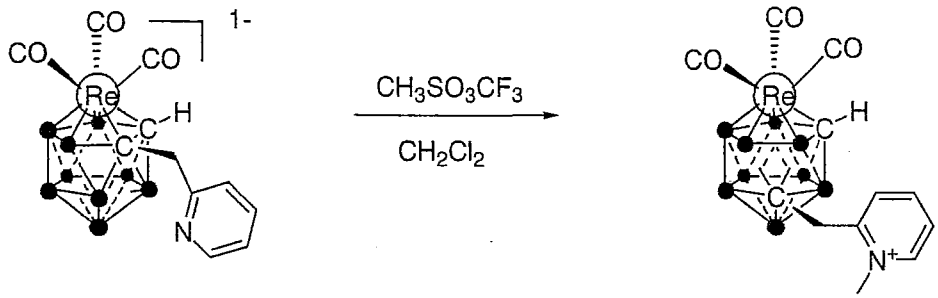


Figure 2.31: 2, 1, 8 isomers of Re-Car **12**

This was the expected result when comparing the structure of **12** to other rhenacarboranes examined by Armstrong and Valliant for cage isomerization.[22] Cage isomerization was shown to occur with carboranes which have bulky groups (i.e. phenyl ring) without a spacer to relieve the steric strain. Although the substituted carbon in Re-Car is not overly bulky, the more likely cause of the cage isomerization in this case is an electronic effect. Rhenacarboranes were shown to isomerize from the 3, 1, 2 isomer to the 2, 1, 8 isomer when the cage substituents are strongly electron withdrawing. One example of the isomerization is shown in Scheme 2.8 where the methylation of the benzyl pyridine substituent resulted in room temperature cage isomerization.[22] This example is

similar to the structure of Re-Car because the reactions took place under acidic medium meaning the amine is likely protonated, making the substituent highly electron withdrawing.



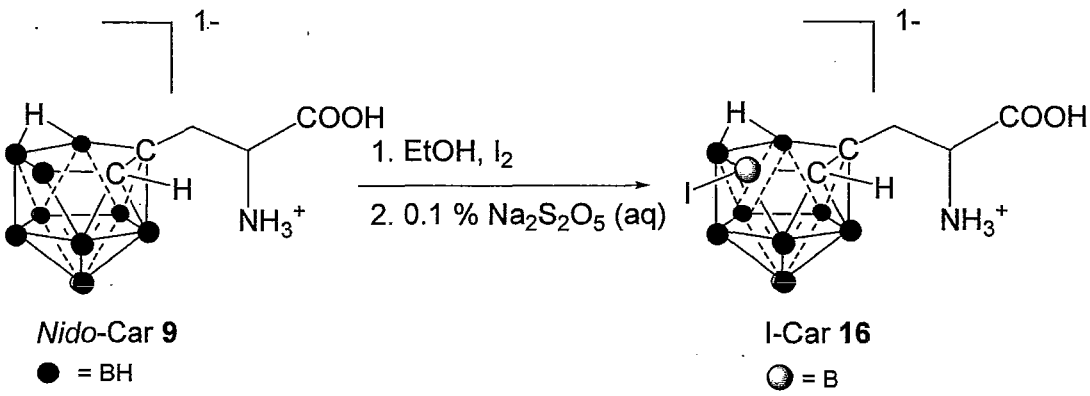
Scheme 2.8: Room temperature isomerization to give 2,1,8 rhenacarborane cage isomer

In summary the rhenium complex of carboranylalanine was isolated by semi-preparative HPLC in low yield with sufficient product obtained to allow for full characterization and use as a reference standard for the technetium work (Chapter 3). The competing products were a di-Re species **14** and $Re(CO)_3^+$ complex **15** which both resulted from chelation of the rhenium core by the amino acid functionality. The resulting rhenium complex undergoes salt and/or ligand exchange reactions when the nature and concentration of a buffer solution are varied.

2.4 Synthesis of I-Carboranylalanine (I-Car)

Nido-carboranes can be labelled at room temperature with iodine, a reaction which was initially reported by Olsen and Hawthorne in 1965.[23] The iodination of *nido*-carboranylalanine was examined using similar conditions to those initially reported in 1965. *Nido*-car was dissolved in absolute ethanol and a solution of I_2 in absolute ethanol was added dropwise while the reaction mixture was stirred at room temperature (

Scheme **2.9**). The reaction mixture was left to stir for an additional 15 minutes where upon iodine decolouration was evident and used to monitor the progress of the reaction. The reaction was then quenched with a 0.1 % solution of sodium metabisulfite in water to ensure consumption of any unreacted I_2 which could lead to the di-iodinated product (m/z 472). This latter product was observed in the mass spectrum (Figure 2.32) when a 2:1 molar ratio of iodine to *nido*-Car was used. If 1:1 stoichiometry was used and the reaction quenched prior to workup no di-iodinated product was observed.



Scheme 2.9: Synthesis of I-Car **16**

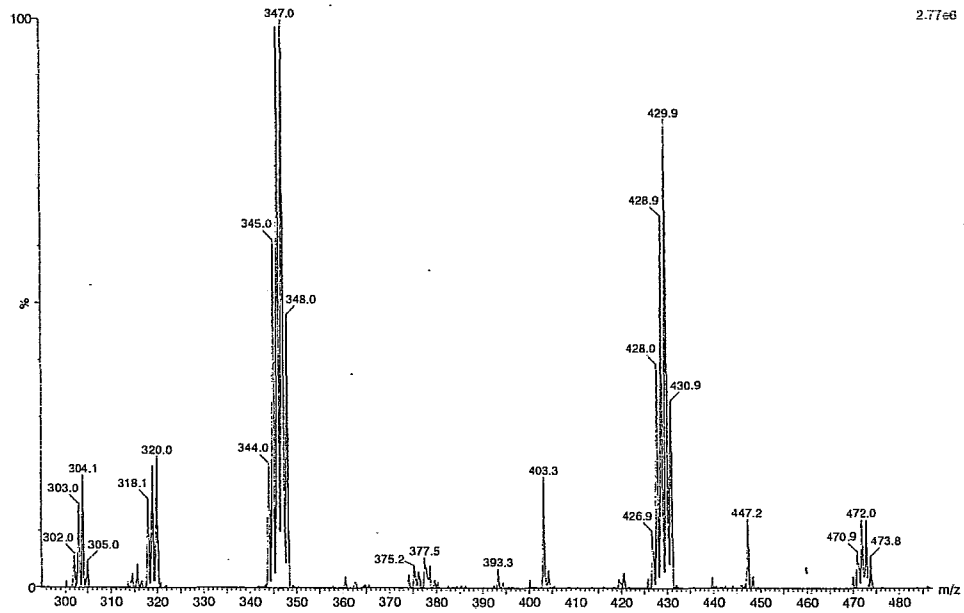


Figure 2.32: Electrospray mass spectrum containing di-iodinated carboranylalanine

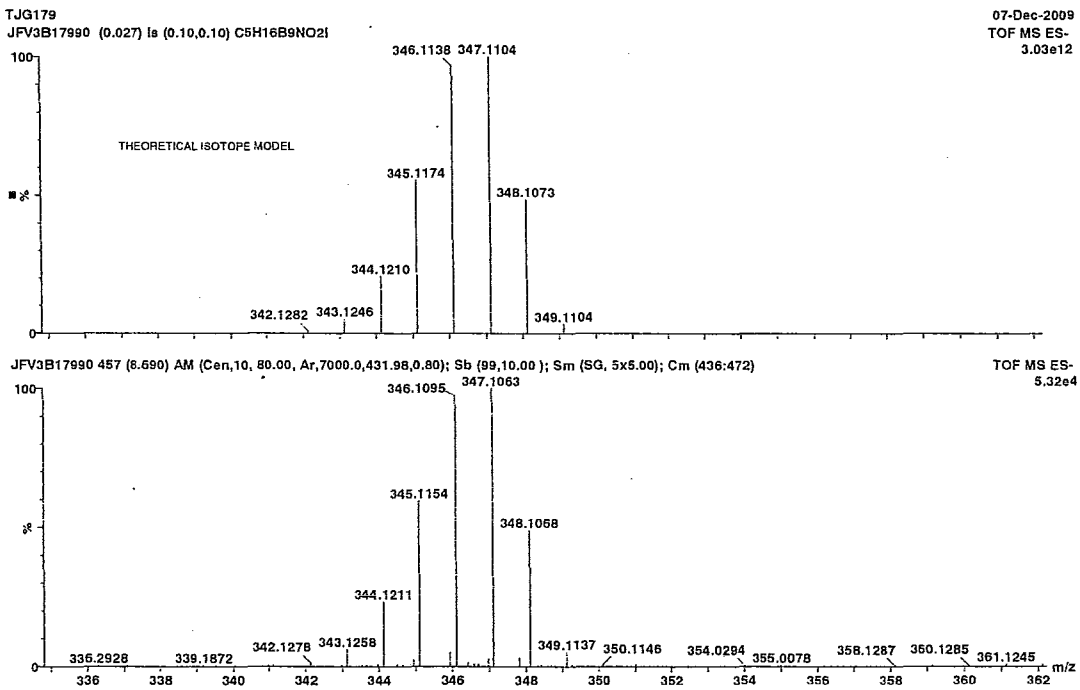


Figure 2.33: High resolution electrospray mass spectrum of I-Car **16** (bottom) with isotope model (top)

The analytical HPLC chromatogram of the reaction mixture of *nido*-Car **9** with one equivalent of I_2 stirred at room temperature for 5 minutes can be seen in Figure 2.34 with a significant separation of *nido* **9** starting material (14 min) and iodinated product **16** (17 min). The reaction proceeds almost quantitatively and the product can be easily isolated through semi-preparative HPLC methods to give pure **16** (Figure 2.35).

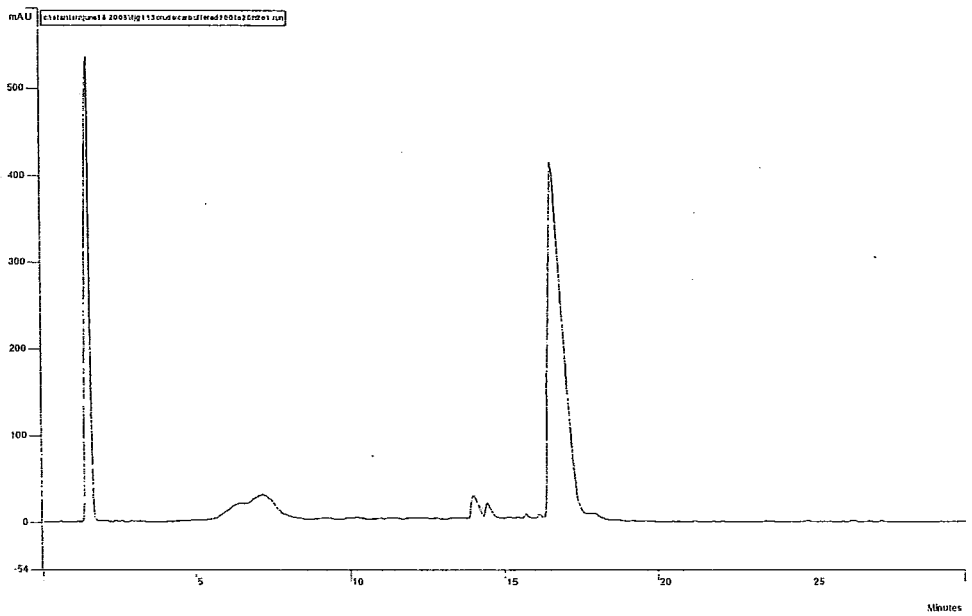


Figure 2.34: Analytical HPLC chromatogram of the reaction mixture from the combination of **9** and I_2 (1:1) to give I-Car **16**. (H_2O:ACN) 95:5 to 20:80 from 0-20 min., to 5:95 20-30 min.

The buffer system used during purification of **16** was 0.1 % formic acid in H_2O and 0.2 mM ammonium acetate in ACN and these buffers were found to be difficult to remove from **16**. Repeated lyophilization and addition of water were not able to remove

all of the buffer. As well, repeated evaporation on the V10 evaporator system which is generally more effective than freeze drying was also not successful. Instead, a C-18 solid phase extraction cartridge was used for purification following activation with EtOH, ACN and water prior to loading of the sample. It was found that once the sample was loaded, the buffers and I-Car could be separated using a water wash as I-Car had, as expected, a greater retention to the reverse-phase cartridge.

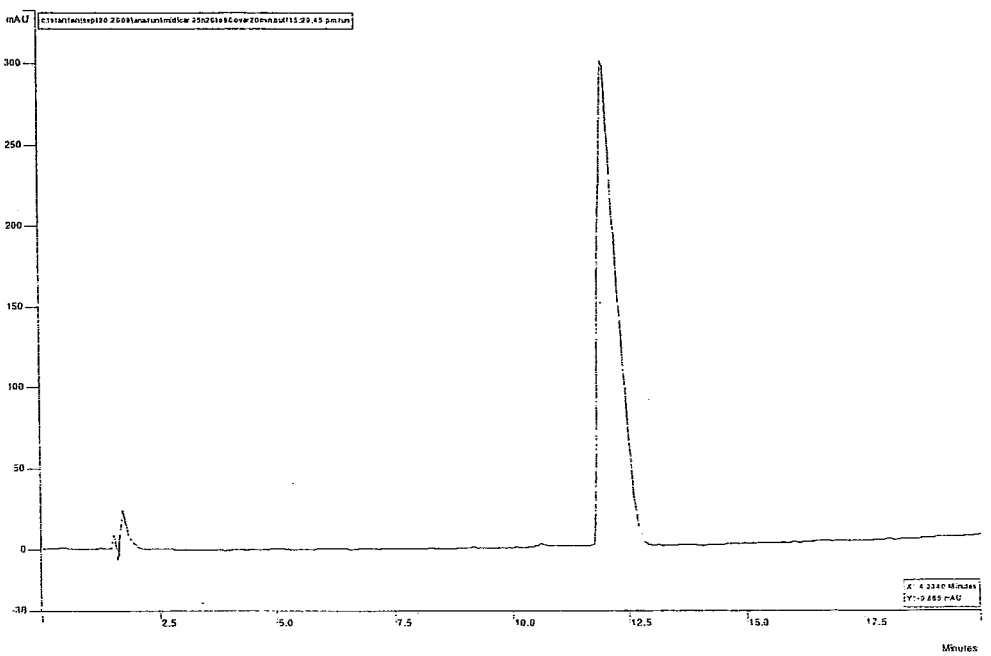


Figure 2.35: Analytical HPLC chromatogram of pure I-Carboranylalanine **16.** (H_2O:ACN) 95:5 to 20:80 from 0-20 min., to 5:95 20-30 min.

During the iodination reaction, it is important to recognize that a mixture of products are formed. Initially, carboranylalanine is synthesized as a racemic mixture.

These two enantiomers are subjected to cage degradation which can occur through removal of one of two borons directly bound to the two carbons in the carborane cage which leads to a mixture of four isomers. During iodination, substitution occurs at either of the two boron atoms bound to the carbon atoms in the open face of the *nido*-carborane. Figure 2.36 shows all the possible products which result during the iodination of *nido*-Car. Comparing these eight structures, it can be concluded that four sets of enantiomers (1+8, 2+7, 3+6, 4+5) are formed. The mixture of the corresponding diastereomers explains the broad nature of the HPLC peak.

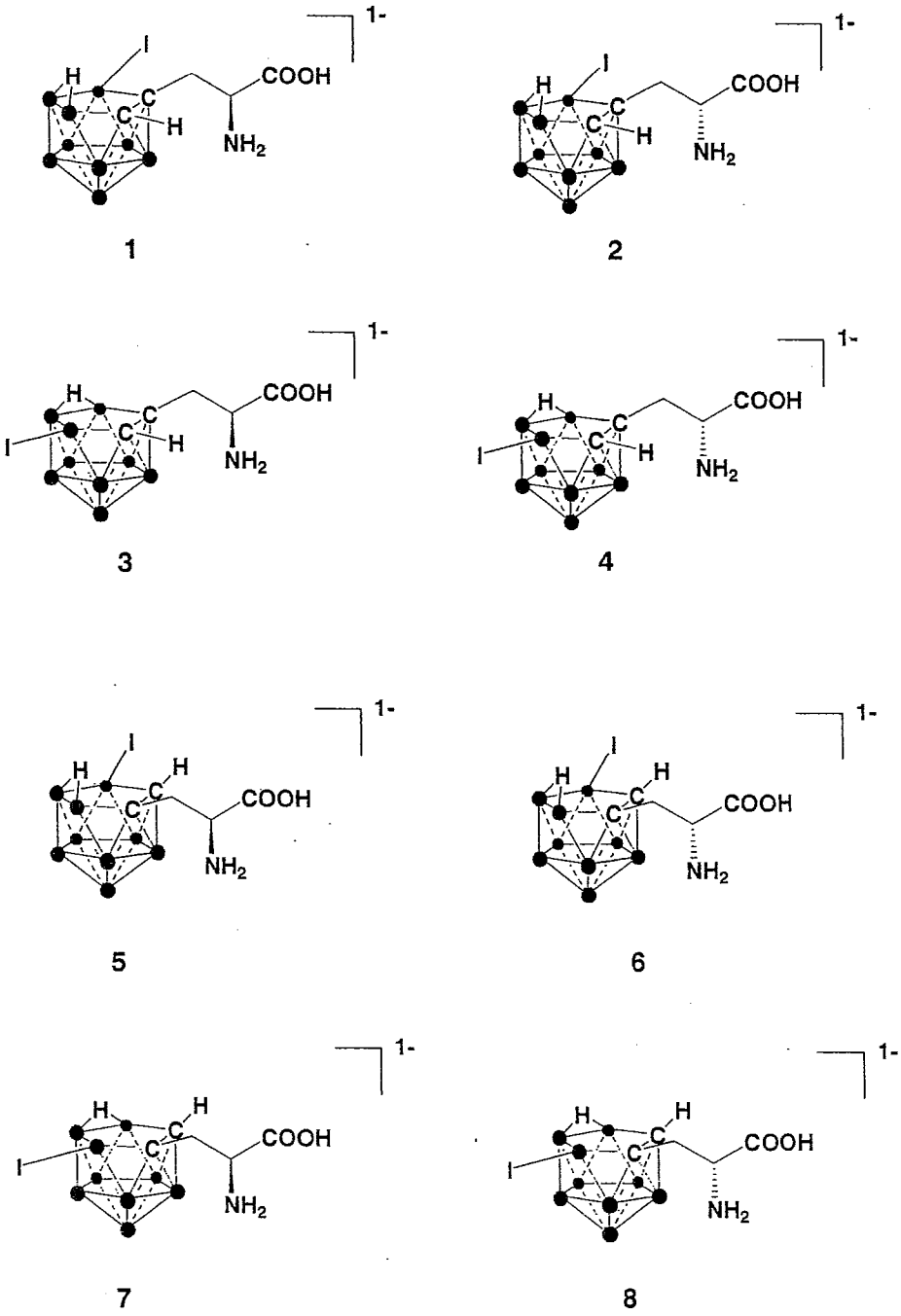


Figure 2.36: Structural isomers of I-Car **16**

Purified I-Car was fully characterized using NMR spectroscopy at 700 MHz. The ^{1}H NMR spectrum clearly shows multiple sets of peaks, where solvent suppression and Gaussian multiplication processing helped enhance the resolution of the various peaks, although complete resolution was not possible (Figure 2.39). To aid in the peak assignments, 2D NMR (COSY, HMBC and HSQC) spectra were obtained. The ^{1}H NMR signals at 4.2 and 3.8 ppm correspond to the CH proton on carbon *2* (Figure 2.37) which couples to the two hydrogens on the adjacent C-*3*. The two hydrogens on C-*3* are diasterotopic, leading to the signal of the proton on C-*2* appearing as a doublet of doublets. As there are four sets of enantiomers in total, four sets of peaks are present for each proton. The C-*2* proton is a clear example of this, where the signals at 4.2 ppm represent 2 sets of peaks (8 peaks resolved) and the signals at 3.8 ppm are the other 2 sets (overlapping).

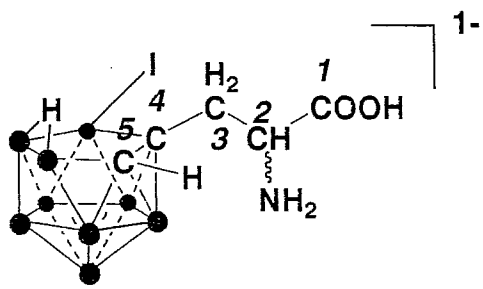


Figure 2.37: Numbering system for I-Car **16**

Although the isomers did not seem to separate on the HPLC, during the removal of the buffers by solid phase extraction whereby small fractions of product were collected it was found that two of the diastereomers did retain slightly more than the others. The

isomers where the iodine atom is located adjacent to the substituted carbon (1, 2, 7 and 8) would likely have slightly different retention compared to those where the iodine atom is next to the carborane C-H (3-6) in Figure 2.36. This separation was observed when examining the fractions by ^{1}H NMR. In the ^{1}H NMR spectra of these fractions (Figure 2.38), the peaks at 4.2 and 3.8 ppm (which are the X protons of the ABX spin system) are present in different amounts depending on the fraction eluted from the SepPak®.

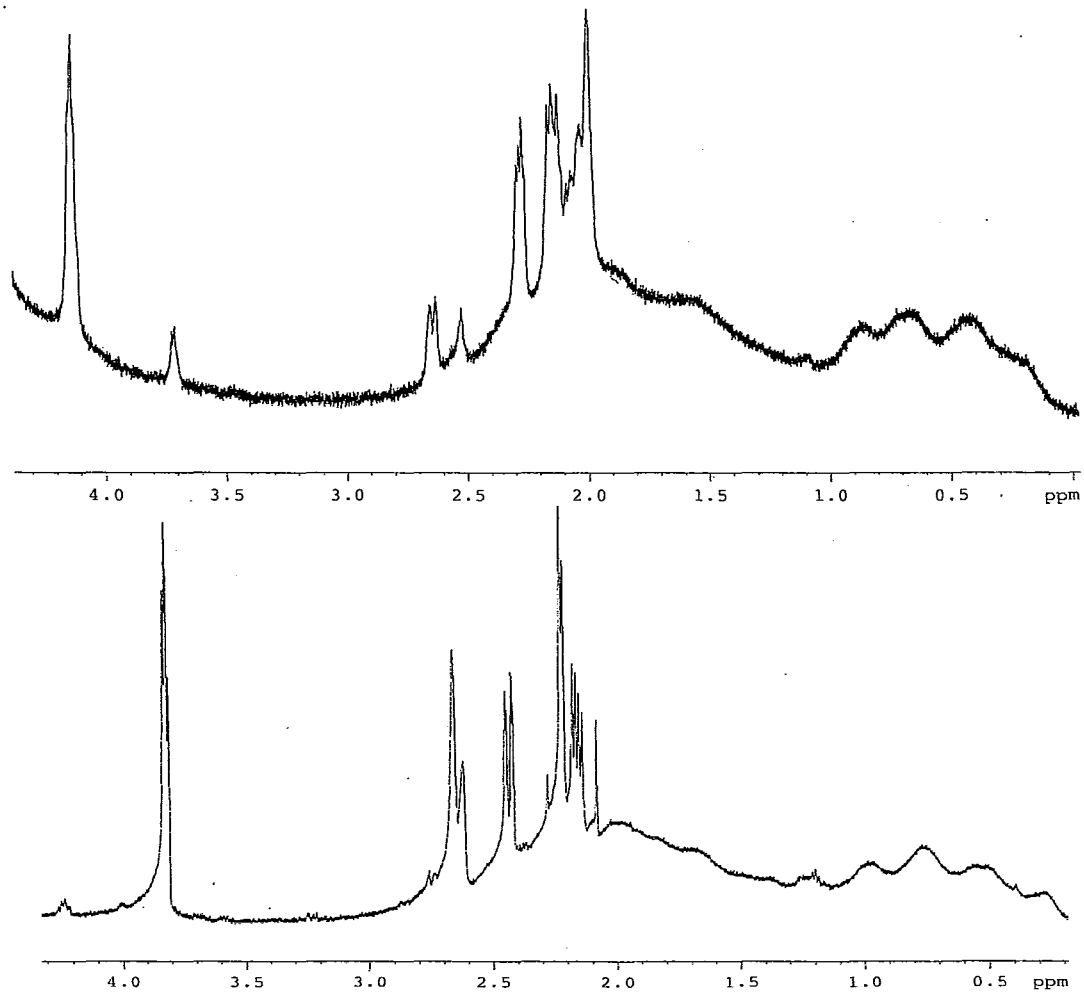


Figure 2.38: ^{1}H NMR spectrum (600 MHz, D_2O) of I-Car solid phase extraction fractions. First fraction (v = 1 mL) (top) and second fraction (v = 1 mL) (bottom)

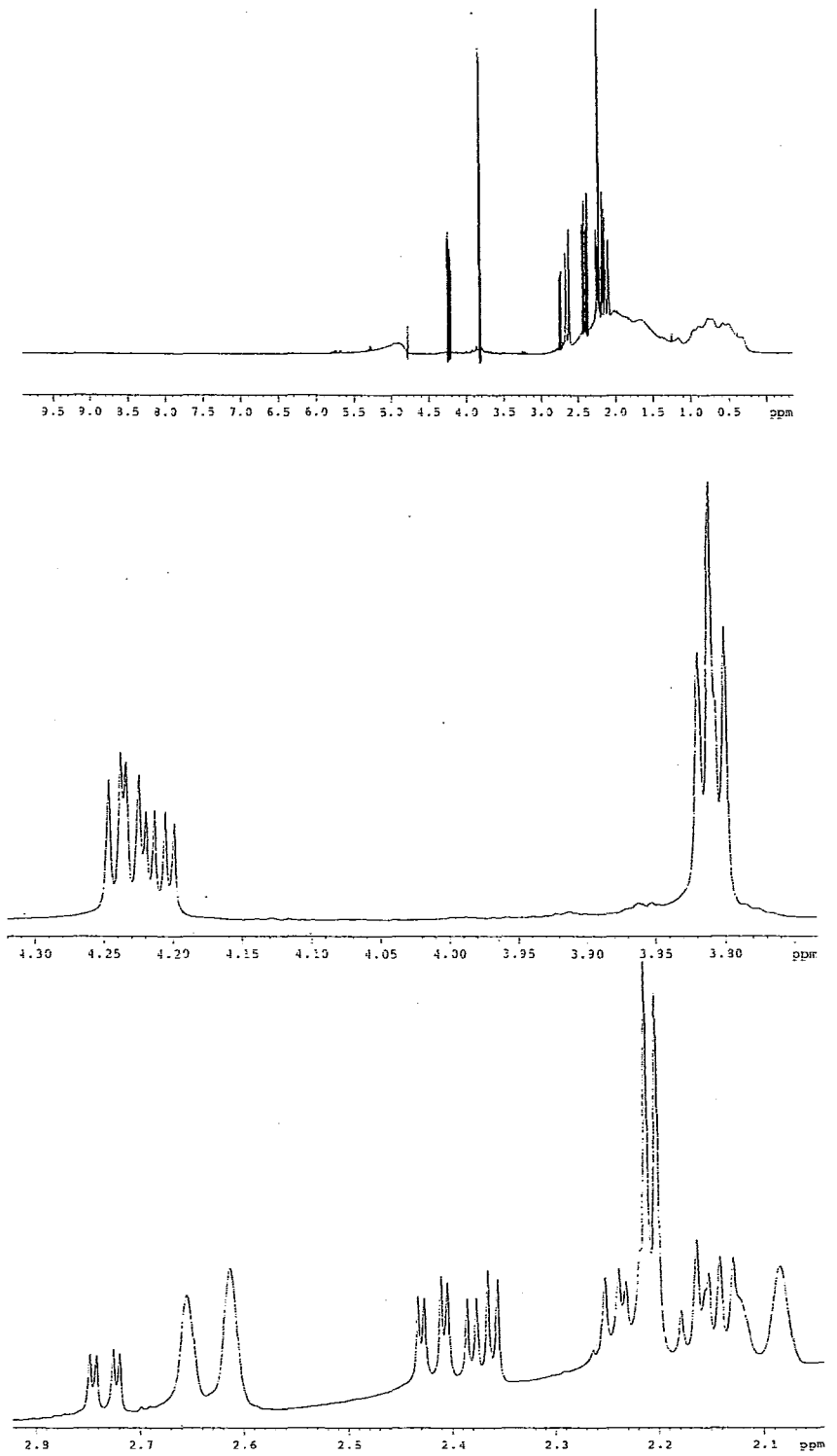


Figure 2.39: ^{1}H NMR spectra (700 MHz, D_2O) of I-Car 16 including the full spectrum (top) and two expansions

The COSY for **16** is shown in Figure 2.40. This spectrum aided in the assignment of the ABX spin systems of the isomers. The COSY spectrum confirmed that the peaks at 3.8 and 4.2 ppm are actually part of different spin systems as they correlate to different peaks between 2-2.8 ppm. The C-**5** hydrogen atoms on the carborane are present at 2.67, 2.63, 2.14 and 2.10 ppm. These were identified by the broad nature of the peaks, and that there is no correlation between these signals and any other protons in the COSY spectrum.

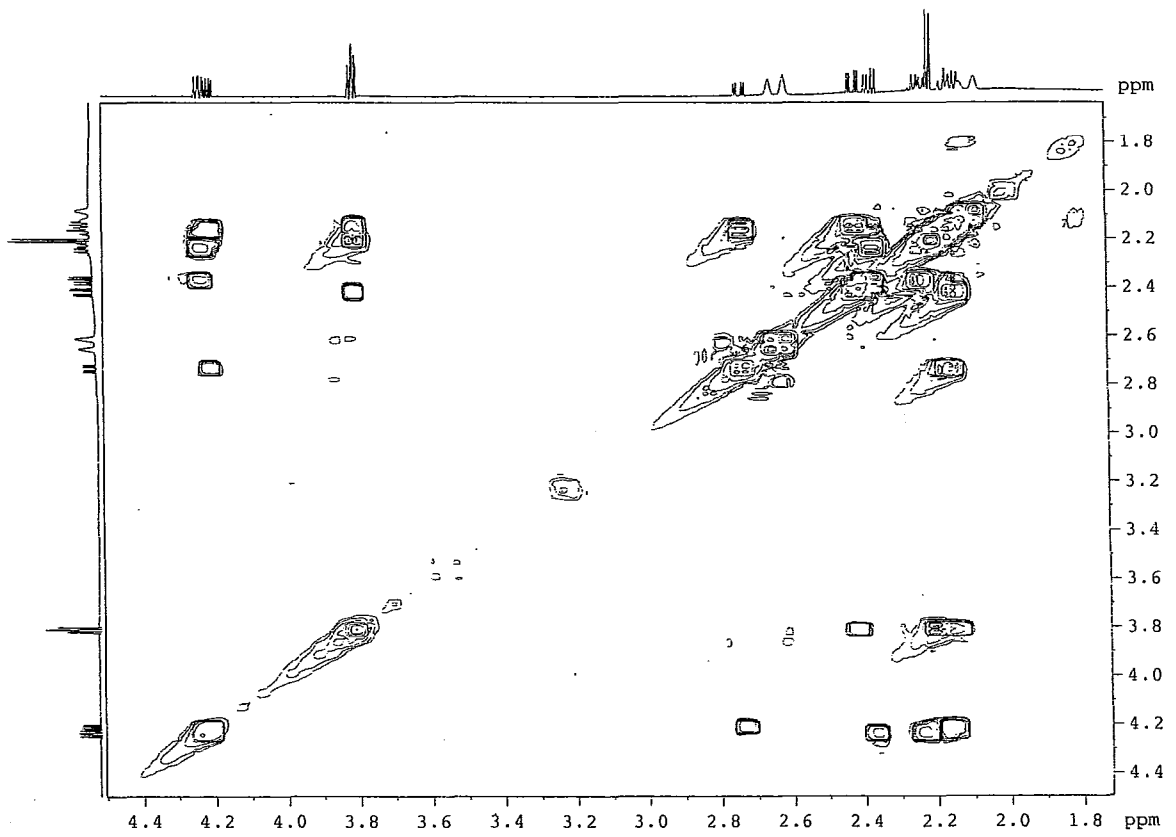


Figure 2.40: COSY ^{1}H NMR spectrum (700 MHz, D_2O) of I-Car **16**

The ^{13}C NMR (Figure 2.41) further confirms that numerous isomers are present. In a single isomer there would be five expected signals, but as there are four sets of diastereomers, a maximum of 20 peaks could be observed (although not all resolve). A clear example of the isomer signals is the carbon peaks for C-***1*** of the carboxylic acid which appears at ~ 173 ppm. The expansion of the spectrum shows that the four different diastereomeric carboxylic acid carbon peaks are resolved for this chemical environment. The carborane carbon atoms (38-62 ppm) are difficult to distinguish in the ^{13}C NMR since they show as weak and broad signals (Figure 2.41). HSQC revealed that two of the C-***5*** signals appear at approximately 56.3 and 56.8 ppm which correlate to the broad signals in the ^{1}H spectrum at 2.67 and 2.63 ppm. The two other C-***5*** signals are at approximately 39.2 and 39.7 ppm and correlate to the ^{1}H signals at 2.10 and 2.14 ppm. These signals would be extremely hard to identify without the 2D NMR experiments as they overlap with the C-***2*** and C-***3*** signals.

The ^{11}B $\{^{1}H\}$ NMR spectrum shows peaks from -40 to 0 ppm with no sign of residual *nido*-Car or boric acid (Figure 2.43). High resolution electrospray mass spectrometry of the purified compound (molecular formula $C_5H_{16}B_9NO_2I$) gave a calculated mass of 348.1063 and found 348.1068 giving a difference of 1.4 ppm.

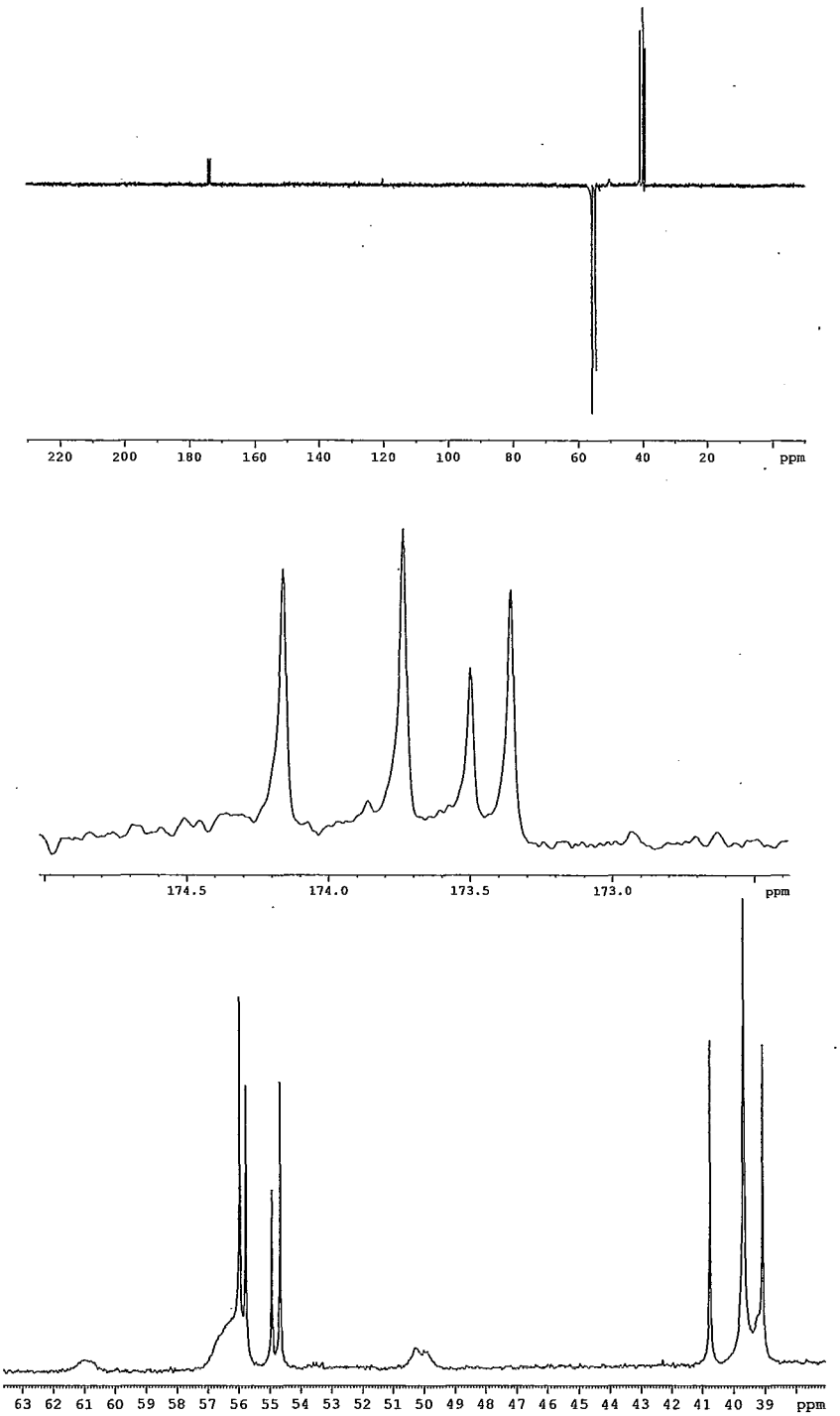


Figure 2.41: ^{13}C NMR spectra (176 MHz, D_2O) of I-Car 16 including the full spectrum (top) and two expansions

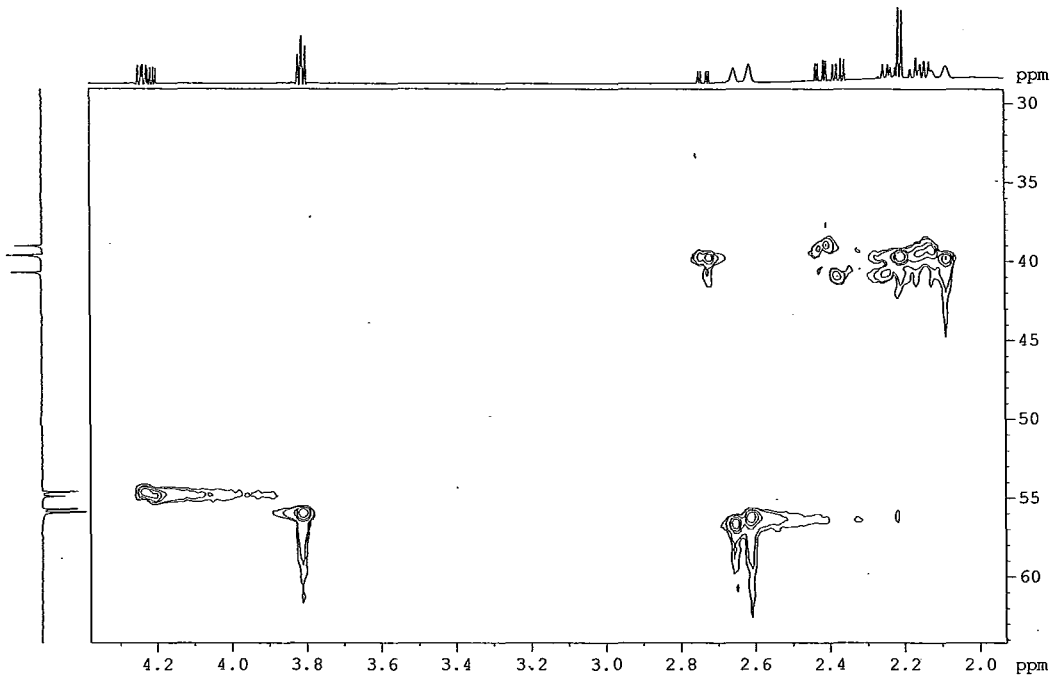


Figure 2.42: HSQC spectrum (700 MHz, D_2O) of I-Car **16**

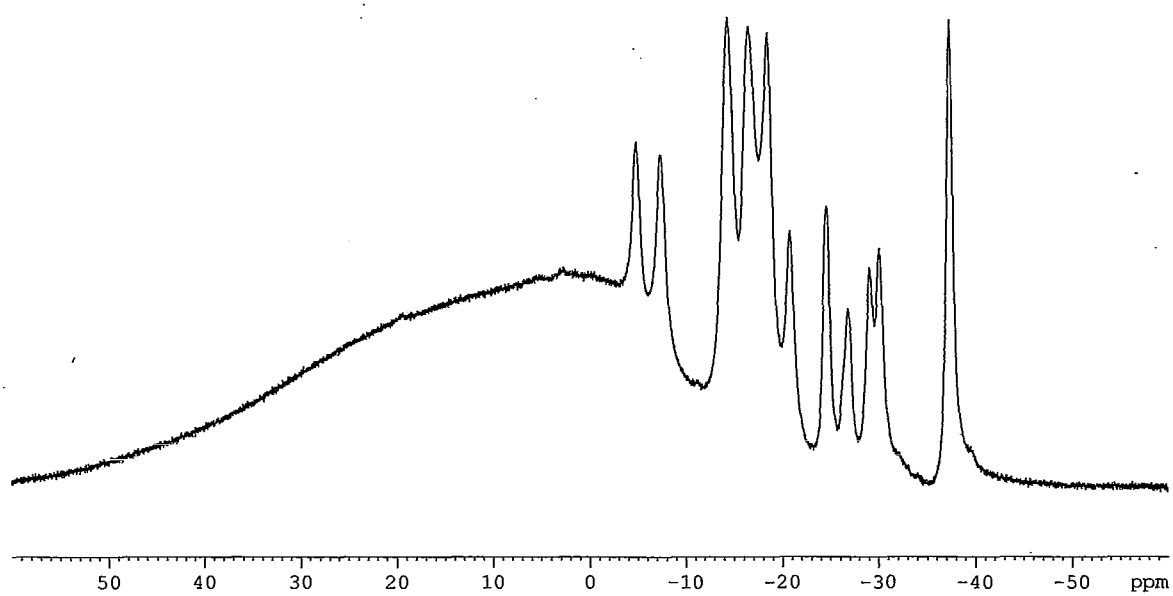


Figure 2.43: $^{11}B\{^1H\}$ NMR spectrum (160 MHz, D_2O) ICar **16**

2.5 Summary and Conclusions

This chapter focused on the synthesis of carboranylalanine (Car **5**) and the cage degradation of this species to form *nido*-Car **9** followed by the synthesis of Re-Car **12** and I-Car **16**. Although the synthesis of Car was known, it was found that some changes to the original procedure[8] resulted in increased yields and easier purification. The self-degradation of Car to form *nido*-Car had been investigated from a kinetic standpoint[14] previously, but the work in this chapter outlines a simple synthesis using microwave heating in water. The only side product from the formation of *nido*-Car in this manner was boric acid. A unique method was employed to allow for the quantitative and fast removal of boric acid through conversion to the more volatile borate methyl ester.

Once *nido*-Car was successfully isolated, complexation to the rhenium tri-carbonyl core was undertaken. It was found that under the standard conditions employed by the Valliant group to form rhenacarboranes, a mixture of products was obtained including the desired Re-Car **12**, a chelation product **15** and a dirhenium species **14**. Re-Car was found to be more thermodynamically stable than **15**, so under slightly higher temperatures the amount of **15** was minimal, but the dirhenium species **14** was found to form regardless of change in temperature, time and pH of the reaction. The isolation of **12** was achieved in low yield through semi-preparative HPLC.

Nido-Car also serves as a precursor for iodination reactions to give I-Car **16**. The iodination reactions were found to take place in high yield at room temperature with reaction times of less than 15 minutes. When the stoichiometry was kept to a 1:1 ratio

between **9** and I_2 no other side products were observed and purification by semi-preparative HPLC gave pure **16** in high yields. As Car was synthesized as a mixture of enantiomers, cage degradation can occur in two different sites and iodination of the carborane cage can also occur at two sites, there is a mixture of eight different isomers present in I-Car **16**. These isomers show as one peak in the HPLC chromatograms, and did not fully resolve in the NMR spectra. The main goal of this work involves the incorporation of *nido*-Car into a larger targeting moiety such as a peptide, so the subtle differences between the isomers would not likely affect the binding affinity of the full targeting vector therefore further investigation of these compounds is warranted.

2.6 Experimental Section

2.6.1 Material and Instruments

Decaborane and NaF were purchased from Katchem Ltd and EM Science respectively. Other reagents were purchased from Sigma-Aldrich Inc. $[Re(CO)_3(H_2O)_3]Br$, **11** was prepared following literature procedures[24] and solvents were purchased from Caledon, dried over calcium hydride (CH_2Cl_2, CH_3CN, and toluene) and were freshly distilled prior to use.

NMR spectra were recorded on Bruker Avance 200, 600, 500 or 700 spectrometers with chemical shifts reported in ppm relative to the residual proton signal of the deuterated solvent (^1H NMR) or the carbon signal of the solvent (^{13}C NMR). Boron-11 NMR spectra were referenced externally to $BF_3{\cdot}Et_2O$. Infrared spectra were acquired using a Nicolet 6700 FT-IR spectrometer. All spectra were recorded at 22 °C. A Biotage Initiator Sixty Microwave Reactor was employed for reactions requiring microwave heating. Thin layer chromatograms (Merck F254 silica gel on aluminum plates) were visualized using 0.1% $PdCl_2$ in 3 M HCl (aq), UV light and/or ninhydrin. Purification of all products was carried out either by flash chromatography using Ultrapure Silica Gel from Silicycle (70-230 mesh), or by using a Biotage SP1 autopurification system unless otherwise indicated. Melting points were measured uncorrected on a Gallenkamp melting point apparatus. Low-resolution mass spectra were obtained on a Waters/Micromass Quattro Ultima spectrometer using electrospray (ES) ionization. High-resolution mass spectra were obtained on a Waters/Micromass Q-ToF

Ultima Global spectrometer. HPLC experiments were performed on a Varian Prostar Model 230 instrument, fitted with a Varian Prostar model 330 PDA detector. The wavelength for detection was set at λ = 254 nm, and the dwell time in the gamma detector was 0.5 s. The analytical column used was a Phenomenex Gemini (L x ID = 100 x 4.6 mm) analytical column (5 μm C18). The semi-preparative column used was a Phenomenex Gemini (L x ID = 250 x 10 mm) analytical column (5 μm C18). Elution protocols Method 1: Solvent A) H_2O + 20mM ammonium acetate, solvent B) CH_3CN + 0.1% formic acid. Gradient: 0-20 min, 90:10 A/B to 20:80 A/B; 20-30 min, 20:80 A/B to 100 B. The flow rate was set at 1 mL/min (analytical) and 4 mL/min (semi-preparative).

2.6.2 Experimental Data

Synthesis of 2-[(diphenylmethylene)amino]pent-4-ynenitrile 2

To a solution of 2.0 g (9.07 mmol) of [(diphenylmethylene)amino]acetonitrile in dichloromethane (40 mL) were added propargyl bromide (3.17 mL, 16.3 mmol), NaOH (15 mL of 11 M solution) and benzyltriethylammonium chloride (0.207 g, 0.91 mmol). The two-phase mixture was stirred vigorously at room temperature until reaction was complete according to TLC (~1.5 h). The mixture was diluted with water (15 mL) and extracted with dichloromethane (3 x 40 mL). The organic phases were combined and dried ($MgSO_4$), filtered and concentrated leaving a yellow oil. The desired product was isolated using column chromatography (hexanes/ethyl acetate, 10:1, v/v) as pale yellow crystals (1.3 g, 56%). TLC R_f 0.32 (hexanes/ethyl acetate, 6:1, v/v). ^{1}H NMR (200.13

MHz, $CDCl_3$) δ 7.20-7.68 (m, 10H, aromatic), 4.41 (t, 1H, *H*CCH_2, J= 7.2), 2.85 (m, 2H, CH_2), 2.11 (t, 1H, *H*C≡C, J= 2.6). ^{13}C NMR (125.77 MHz, $CDCl_3$) δ 174.35 (N=*C*Ph_2), 138.27 (aromatic), 134.94, 131.46, 129.61, 129.23, 129.12, 128.29, 127.57, 118.31 (CH), 78.29 (HC≡*C*), 72.16 (HC≡C), 52.27 (*C*HCH_2), 25.45 (*C*H_2).

Synthesis of $B_{10}H_{12}(CH_3CN)_2$ ***6***[25]

A solution of decaborane (2.108 g, 17.25 mmol) in acetonitrile (30 mL) was heated at reflux for 5 hours. The heterogeneous mixture was cooled to r. t. and filtered, yielding the desired product as a white solid (3.16 g, 91%) m.p. 205-210 °C (decomposition).

Synthesis of 3-(o-carboran-1-yl)-2-[diphenylmethylene)amino]propionitrile ***3***

To a solution of $B_{10}H_{12}(CH_3CN)_2$ (1.53 g, 7.56 mmol) in toluene (25 mL) and acetonitrile (25 mL) was added 2-[(diphenylmethylene)amino]pent-4-ynenitrile (1.3 g, 5.03 mmol) under argon. The solution was heated at reflux for 24 h, upon which it was cooled to room temperature and the solvent removed by rotary evaporation. The desired product was isolated by column chromatography (hexanes/ethyl acetate, 10:1, v/v) as light yellow crystals (0.480 g, 25%). TLC R_f 0.19 (hexanes/ethyl acetate, 10:1, v/v). ^{1}H NMR (200.13 MHz, $CDCl_3$): δ 7.22-7.64 (m, 10H, aromatic), 4.35 (t, 1H, C*H*, J = 6.5), 3.83 (br s, 1H, C*H* of carborane), 2.94 (d, 2H, CH_2, J=6.2), 0.8-3.5 (br m, 10H, BH). ^{11}B NMR {^{1}H} (160.46 MHz, $CDCl_3$): δ -0.87, -3.91, -8.49, -10.49, -11.85. ^{13}C NMR (125.77

MHz, $CDCl_3$): δ 175.60 (N=*C*Ph_2), 137.71 (aromatic), 134.53, 131.91, 130.01, 129.33, 129.09, 128.48, 127.00, 117.81 (*C*N), 70.74 (*C* of carborane), 60.16 (H*C* of carborane), 51.60 (HCCH_2), 42.29 (*C*H_2).

Synthesis of 3-(o-carboran-1-yl)2-aminopropionitrile ***4***

3-(*o*-Carboran-1-yl)-2-[diphenylmethylene)amino]propionitrile (0.186 g, 0.493 mmol) was dissolved in benzene (10 mL) and stirred with 5 M HCl (12 mL) for 18 h at room temperature. The mixture was diluted with water (4 mL) and the organic layer was removed. The aqueous layer was extracted with ether (3 x 30 mL), the organic layers combined, dried ($MgSO_4$), filtered and the solvent removed by rotary evaporation. The desired product was isolated by column chromatography (chloroform/methanol, 10:1, v/v) as a white powder (42 mg, 41%). TLC R_f 0.50 (chloroform/methanol, 10:1, v/v). MS (ESI$^-$) *m/z* 212.2 $[M-H]^-$. ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 4.24 (br s, 1H, C*H* of carborane), 3.81-3.88 (m, 1H, C*H*), 2.54-2.80 (m, 2H, CH_2), 0.6-3.8 (br m, 10H, BH). ^{11}B NMR (160.46 MHz, $CDCl_3$): δ -0.67, -1.60, -3.57, -4.50, -8.32, -9.35, -10.10, -11.48, -12.56. ^{13}C NMR (125.77 MHz, $CDCl_3$): δ 120.29, 70.85, 60.07, 42.42, 41.89.

Synthesis of closo-o-carboranylalanine ***5***

3-(*o*-Carboran-1-yl)2-aminopropionitrile (137.1 mg, 0.646mmol) was heated at 95 °C in 75% H_2SO_4 for 24 h. The mixture was cooled in an ice bath and the resulting off-white

precipitate collected by vacuum filtration. The powder was dissolved in hot water and any insoluble impurities removed by filtering the hot solution. The solution was cooled in an ice bath and the product was isolated as a white solid (100 mg, 67% yield). TLC R_f 0.64 (n-BuOH/CH_3COOH/H_2O 4:1:1, v/v). HRMS (ESI$^-$) *m/z* for $C_5H_{18}B_{10}O_2N$: calcd 230.2187, observed 230.2196 [M^+]. ^1H NMR (200.13 MHz, CH_3OH-d_4): δ 4.70 (br s, 1H, C*H* of carborane), 4.16 (dd, 1H, C*H*), 3.13 (dd, 1H, CH_2, J_{AB}= 16.2, J_{BX}= 6.6 Hz), 2.73 (dd, 1H , CH_2, J_{AB} = 16.2, J_{AX} = 4.4 Hz), 1.0-3.3 (br m, 10H, BH). ^{11}B NMR {^1H} (160.46 MHz, CH_3OH-d_4): δ -1.53, -4.34, -8.45, -10.74, -11.79. ^{13}C NMR (125.77 MHz, CH_3OH-d_4): δ 169.92 (*C*OOH), 73.24 (*C* of carborane), 64.20 (*C*H of carborane), 53.16 (*C*H_2), 38.79 (*C*H_2). IR (KBr) ν: 2987, 2587, 1744, 1574, 1493, 1205 cm^{-1}.

Synthesis of nido-carboranylalanine ***9***

Closo-o-carboranylalanine (85 mg) was weighed out in a 2-5 mL microwave vial. Distilled water (4 mL) was added, the vial sealed and heated in the microwave for 20 minutes at 140 °C. The solvent was removed by rotary evaporation resulting in a white powder. The product was dissolved in MeOH (2 mL) and acetic acid (2 mL), shaken by hand for 2 minutes, and the solvent removed on the V10 evaporator system. This procedure was repeated 3 additional times. Distilled water (4 mL) was then added to the vial, shaken by hand for 2 mintues and the solvent removed on the V10 evaporator system. This was repeated 3 additional times. The compound was further purified through semi-preparative HPLC using Method 1 (36 mg, 45% yield). HRMS (ESI$^-$) *m/z*

for $C_5H_{17}B_9O_2N$: calcd 221.2137, observed 221.2145 [M^+]. ^1H NMR (700.17 MHz, D_2O): δ 3.87-3.92 (ov m, 2H, C*H*NH_2), 2.08-2.37 (ov m, 4H, CH_2), 2.03 (br s, C*H* carborane), 2.00 (br s, C*H* carborane), -0.2-2.4 (br m, BH). ^{11}B NMR (160.46 MHz, D_2O): δ -10.30, -11.01, -13.67, -19.16, -20.33, -32.15, -36.74. ^{13}C NMR (125.77 MHz, D_2O): δ 173.27 (*C*OOH), 173.07 (*C*OOH), 57.75 (*C* carborane), 55.14 (*C*H), 55.09 (*C*H), 46.17 (*C*H carborane), 41.14 (*C*H_2), 40.26 (*C*H_2). HPLC (Method 1): t_r = 11.68, 11.92. IR (KBr) ν: 3198, 2921, 2527, 1729, 1581, 1475, 1400, 1123 cm^{-1}.

Synthesis of Rhena-carboranylalanine ***12***

Closo-o-carboranylalanine (26.7 mg) was weighed out in a 2-5 mL microwave vial. Distilled water (3.5 mL) was added to the vial which was then heated in the microwave for 20 minutes at 160 °C. $[Re(CO)_3(H_2O)_3]Br$ (65.5 mg, 1.2 eq) was then added to the microwave vial which was then heated for an additional 15 minutes at 180 °C. The solvent was removed under vacuum. The off-white product was isolated using semi-preparative HPLC (Method 1). Multiple runs were necessary to obtain pure product (1.71 mg, 3% yield). Two diastereomers are present in the final product, for characterization the two ABX spin systems are assigned either abx or a*b*x*. ^1H NMR (700.17 MHz, D_2O): δ 3.80 (ov dd, 1H, C*Hx*), 3.76 (ov dd, 1H, C*Hx**), 2.78 (ov dd, 1H, C*Ha*Hb, J_{ax}= 1.7 Hz), 2.71 (ov dd, 1H, C*Ha**Hb*, J_{a*x*} = 1.8 Hz), 2.38 (dd, 1H, CHa**Hb**, J_{a*b*} = 16.2 Hz, J_{b*x*} = 10.2 Hz), 2.28 (dd, 1H, CHa*Hb*, J_{ab} = 16.2, Hz, J_{bx} = 10.2 Hz), 2.07 (br ov, C*H* of carborane), 1.1-3.0 (br m, BH). ^{11}B NMR (160.46 MHz, D_2O): δ -5.70, -9.30, -12.76,

-19.14, -19.71. ^{13}C (176.07 MHz, D_2O): δ 198.62 (*C*=O), 173.20 (*C*OOH), 55.44 (*C*H),55.09 (*C**H),50.36 (*C* of carborane), 50.28 (*C** of carborane), 40.70 (CH_2), 40.60 (C^*H_2), 29.67 (*C*H of carborane), 29.51 (*C*H of carborane). HRMS (ESI^-) *m/z* for $C_8H_{16}B_9O5NRe$: calcd 492.1424, observed 492.1441 [M^+]. HPLC (Method 1) t_r = 12.8 min. IR (KBr) ν: 3596, 3263, 2540, 2002, 1888, 1628, 1401 cm^{-1}.

Synthesis of Iodo-carboranylalanine ***16***

Nido-carboranylalanine (41.3 mg) was dissolved in ethanol (4 mL), and a solution of iodine (23.6 mg) in ethanol (3 mL) was slowly added over 5 minutes while stirring the mixture at room temperature. The solution was left to stir for an additional 5 minutes at which time 3 mL of a 0.1% solution of $Na_2S_2O_5$ in water was added. The solvent was then removed, and the product isolated through semi-preparative HPLC (Method 1) (31 mg, 48% yield). HRMS (ESI^-) *m/z* for $C_5H_{16}B_9O_2NI$: calcd 348.1063, observed 348.1068 [M^+]. HPLC: t_r = 9.2, 9.8. ^1H (700.17 MHz, D_2O): δ 4.22-4.26 (ov m, C*H*), 3.81-3.83 (ov m, C*H*), 2.74-2.76 (ov m, CH_2), 2.67 (br, C*H* of carborane), 2.63 (br, C*H* of carborane), 2.4-2.45 (ov m, CH_2), 2.15-2.30 (ov m, CH_2), 2.14 (br, CH of carborane), 2.10 (br, C*H* of carborane), 0.3-2.2 (br m, B*H*). ^{11}B NMR (160.46 MHz, D_2O): δ -4.90, -7.40, -14.44, -16.62, -18.56, -20.78, -24.58, -26.81, -29.09, -30.10, -37.48. ^{13}C (176.07 MHz, D_2O): δ 174.16 (*C*OOH), 173.73 (*C*OOH), 173.50 (*C*OOH), 173.36 (*C*OOH), 60.94 (*C*H carborane), 56.29 (*C*H carborane), 55.97 (*C*H), 55.77 (*C*H), 54.94 (*C*H), 54.67 (*C*H), 50.30 (*C* carborane), 49.92 (*C* carborane), 40.75 (CH_2), 39.70 (CH_2), 39.66 (CH_2),

carborane), 49.92 (*C* carborane), 40.75 (CH_2), 39.70 (CH_2), 39.66 (CH_2), 39.08 (CH_2). HPLC (Method 1) t_r = 17.2 min. IR (KBr) ν: 3444, 3228, 2537, 1627, 1406, 1338 cm^{-1}.

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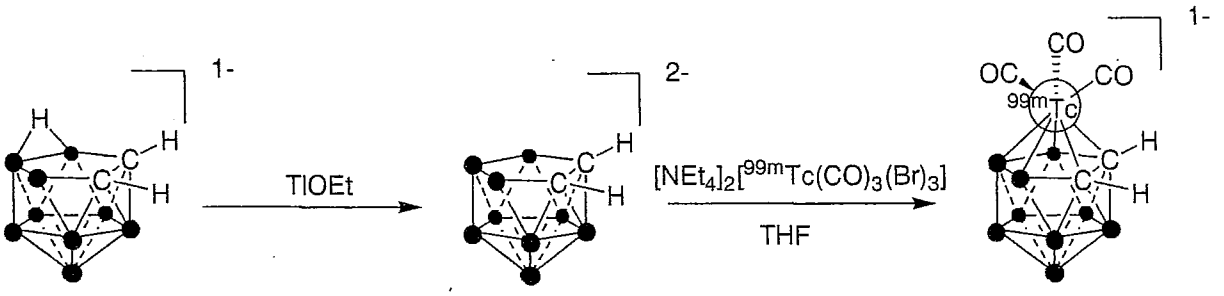
Chapter 3

Synthesis of ^{99m}Tc-Car and ^{125}I-Car

3.1 Synthesis of ^{99m}Tc-Car

Nido-carborane complexes with ^{99m}Tc were first developed by the Valliant group in 2002.[1] The initial conditions are shown in Scheme 3.1 and involved the formation of the dicarbollide dianion through removal of the bridging hydrogen in the *nido*-carborane using thallium ethoxide followed by addition of $[NEt_4]_2[^{99m}Tc(CO)_3(Br)_3]$ with gentle reflux overnight to give the metallocarborane in 80% isolated yield. The standard synthesis method for formation of these complexes has been modified to use another precursor developed by Alberto et al., $[M(OH_2)_3(CO)_3]^+$ where M= ^{99m}Tc/Re, which contains highly labile water ligands.[2] The choice of solvent is also relevant if these compounds were to be applied to biological systems, with water being the ideal selection. It was found that the initial use of $[^{99m}Tc(OH_2)_3(CO)_3]^+$ in water led to low yields (<10%) likely due to degradation of the precursor under the strongly basic conditions, but the use of a weaker base (KF) and heating for 3 hours in water gave the desired ^{99m}Tc-carborane derivative with 80% yield following purification with a C18 SepPak® cartridge.[3] Three hours is a significant reaction time for ^{99m}Tc reactions as it has a half life of only six hours, meaning half of the activity would be lost before the isolation of the product is even attempted. To this end, microwave heating was investigated to reduce reaction

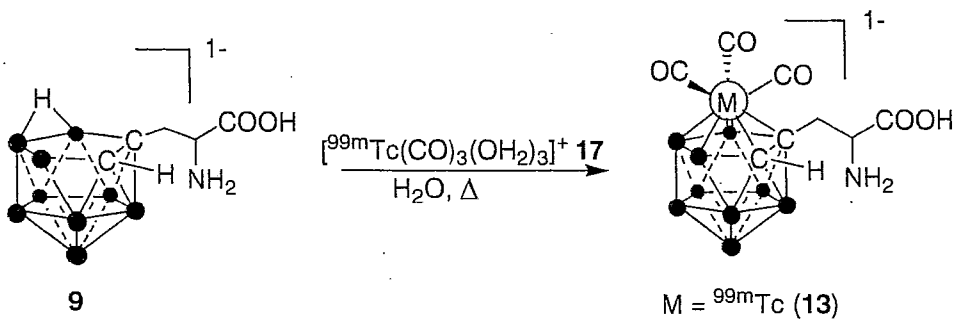
times for the radiolabelling of carboranes.[4] It was found that for both the rhenium and technetium analogues reaction times were significantly reduced (hours to minutes) without sacrificing high yields.



Scheme 3.1: Initial synthesis of $[C_2B_{10}H_{11}{}^{99m}Tc(CO)_3]^-$

The latest methods for the preparation of ^{99m}Tc carborane complexes were implemented for the labelling of *nido*-Car using the cold rhenium standard (Section 2.3) for characterization by HPLC. The $[^{99m}Tc(CO)_3(H_2O)_3]^+$ **17** core was prepared through the combination of $K_2[BH_3CO_2]$, $Na_2B_4O_7 10H_2O$, Na/K Tartrate, Na_2CO_3 with $Na[^{99m}TcO_4^-]$ in saline as per standard methods.[5] The non-radioactive starting materials were sealed in a 2-5 mL Biotage microwave vial and purged with argon. Pertechnetate was added and the mixture microwave heated at 120 °C for 3 minutes. 500 μL of the $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and 500 μL of a 1 mg/mL solution of *nido*-Car **9** were combined in a reaction vial (0.5-2 mL) purged with argon and heated in a microwave (Scheme 3.2). Under these standard conditions, the pH of the reaction is about 11. As all of the non-

radioactive rhenium chemistry was performed under acidic conditions, a parallel experiment at the tracer level was examined where the pH of the technium-99m core was dropped to about 6 using a 1M HCl solution. Using these slightly acidic conditions, it will ensure that the carboxylic acid and the amine remain protonated which would significantly decrease the chances of the chelation product forming at the tracer level as was seen at the macroscale (Figure 2.24, Section 2.3).



9

M = ^{99m}Tc (**13**)

Scheme 3.2: Preparation of ^{99m}Tc-Car **13**

Although numerous conditions were varied during the radiolabelling (time, temperature and pH) only a maximum of two product peaks were observed at the tracer level. This is a very significant difference compared to the crude reactions involving rhenium where a mixture of products formed which included the desired Re-Car **12**, a dirhenium complex **14** and a rhenium chelation product **15** (Figure 3.1).

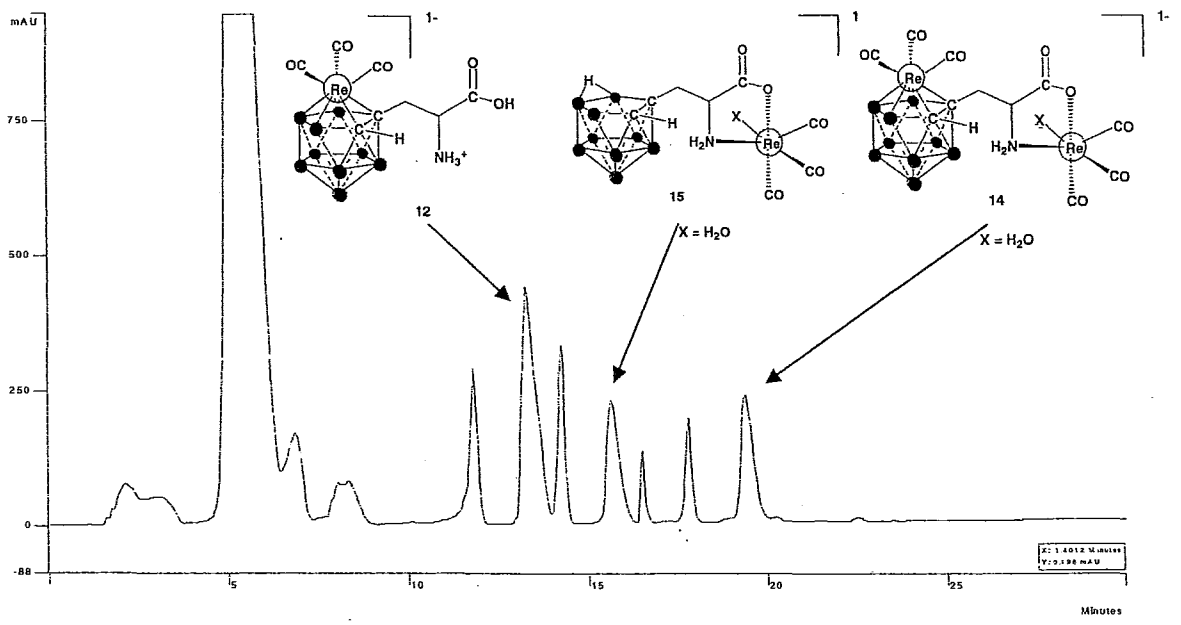


Figure 3.1: Analytical HPLC chromatogram of the crude reaction mixture of *nido*-Car **9** with $[Re(CO)_3(H_2O)_3]Br$ **11** (1:1.2 eq) at 160 °C for 15 min to give a mixture of compounds including three carboranes products (**12**, **15** and **14**)

For the technetium reactions carried out at pH 6 with heating for 5 minutes at 180 °C, two major product peaks in the HPLC at R_t = 15 and 17 minutes are present (Figure 3.2). If the reaction was heated at lower temperature (160 °C) for 15 minutes, the two peaks were still present, but the relative amount of each changed (Figure 3.3). At the lower temperature, the product that eluted later (17 min) became the major product suggesting that it is the kinetic product and the peak at 15 minutes is the thermodynamic product.

When the pH was not adjusted, and remained at 11, there was less overall conversion to product (increased pertechnetate or $^{99m}Tc(CO)_3^+$) but better separation between peaks, and a slightly cleaner baseline (Figure 3.5). It is also interesting to note

that under the higher pH, the one main product peak corresponded with the more thermodynamically favored product from the corresponding reactions at pH 6.

The peak at 17 minutes did not match with any of the rhenium standards, while the peak at 15 minutes was a good match to the metallocarborane Re-Car species **12** as can be seen from Figure 3.6.

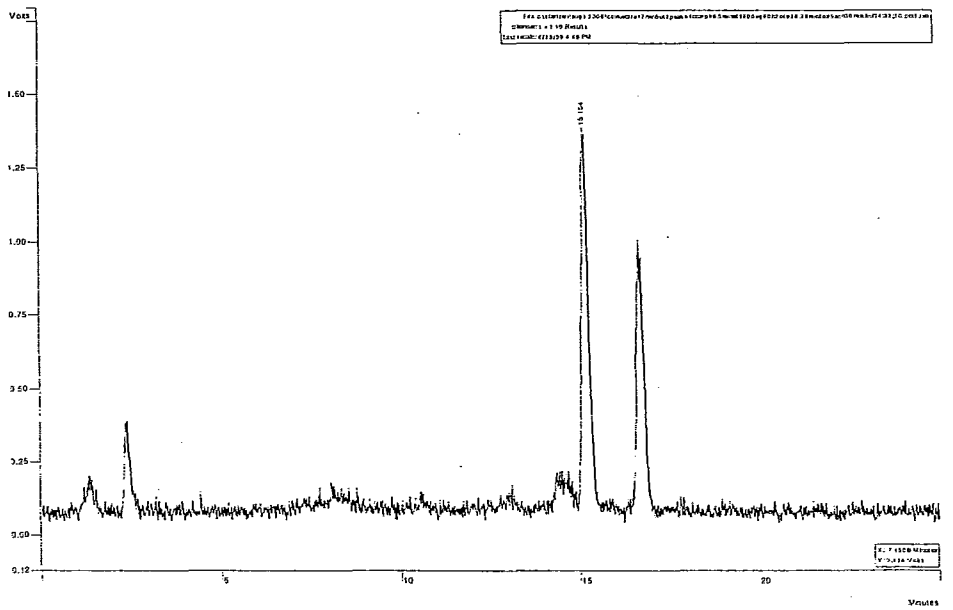


Figure 3.2: HPLC chromatogram of crude reaction mixture of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ **17** with *nido*-Car **9** at pH 6 with microwave heating at 180 °C for 5 minutes

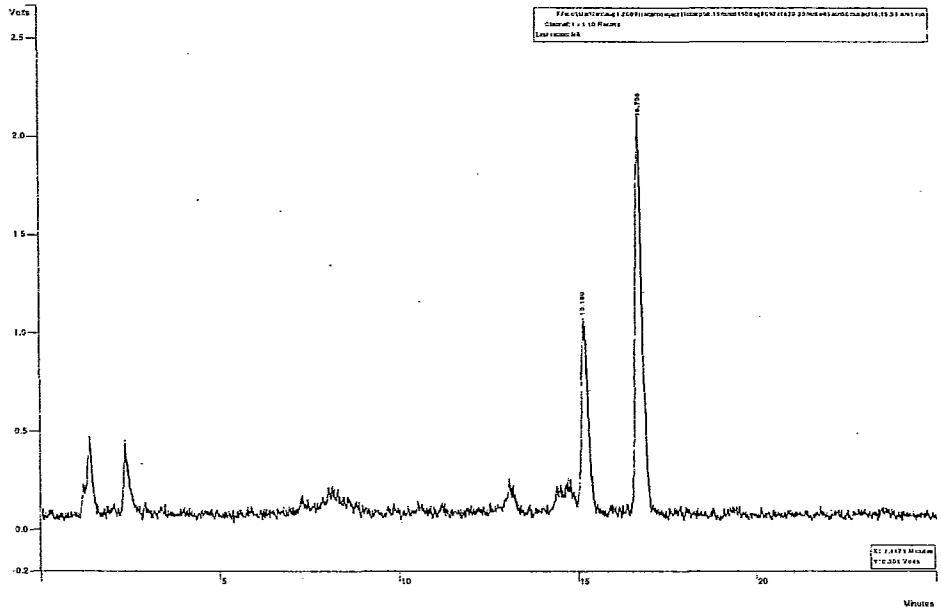


Figure 3.3: HPLC chromatogram of crude reaction mixture of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ **17** with *nido*-Car **9** at pH 6 with microwave heating at 160 °C for 15 minutes

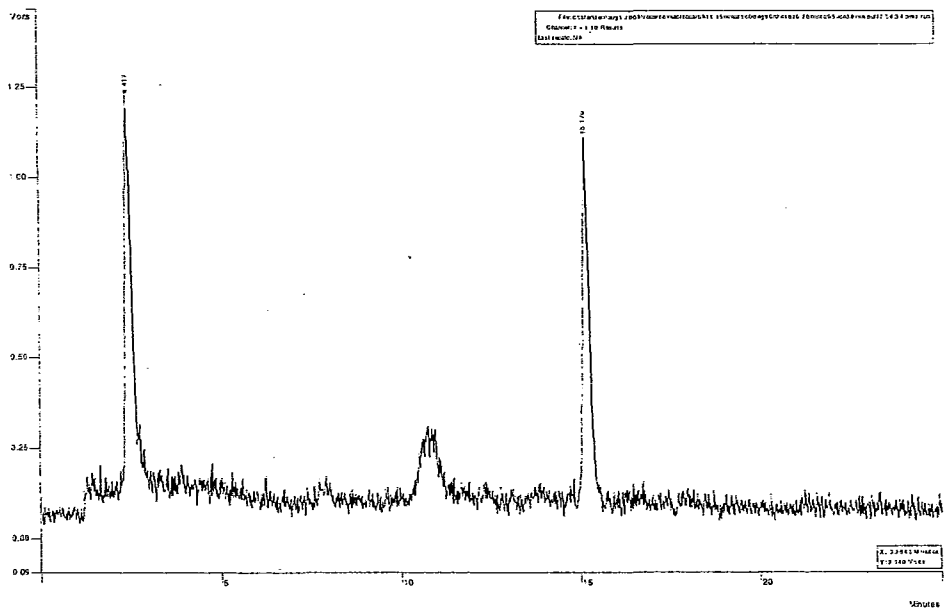


Figure 3.4: HPLC chromatogram of crude reaction mixture of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ **17** with *nido*-Car **9** at pH 11 with microwave heating at 160 °C for 15 minutes

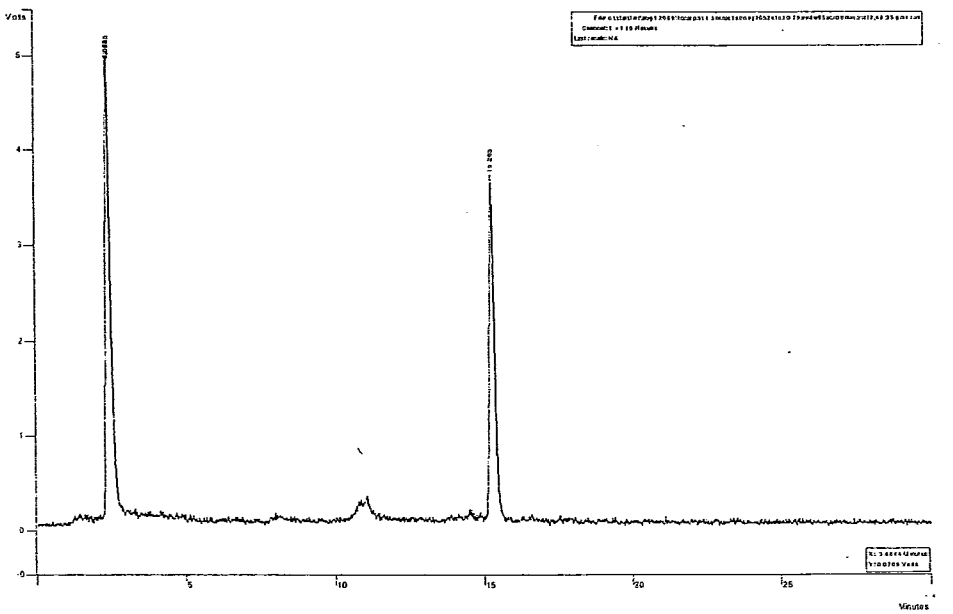


Figure 3.5: HPLC chromatogram of crude reaction mixture of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ **17** with *nido*-Car **9** at pH 11 with microwave heating at 180 °C for 5 minutes

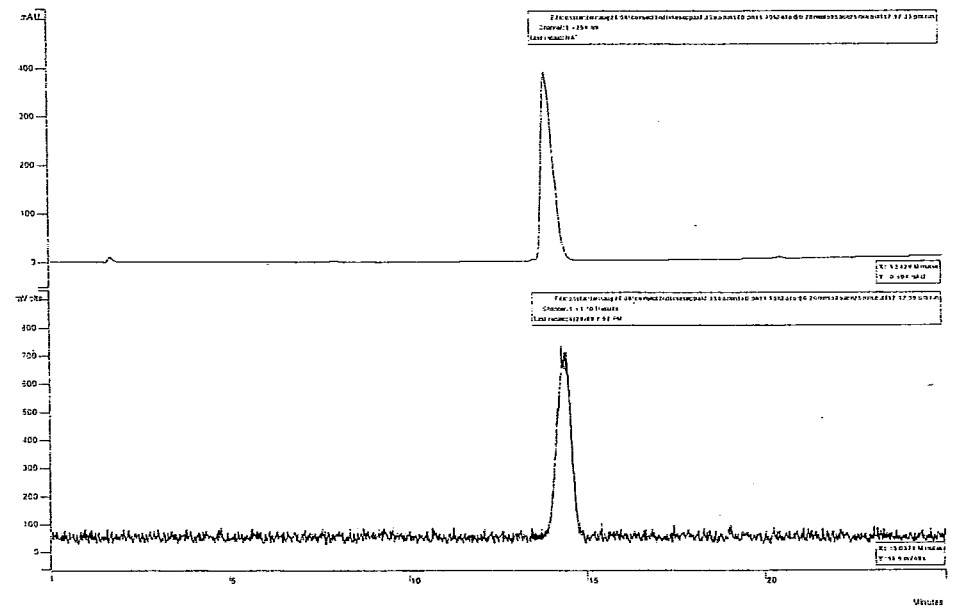


Figure 3.6: HPLC chromatograms of Re-Car **12** standard (UV, top) and isolated ^{99m}Tc-Car **13** (gamma, bottom) showing good correlation of retention times

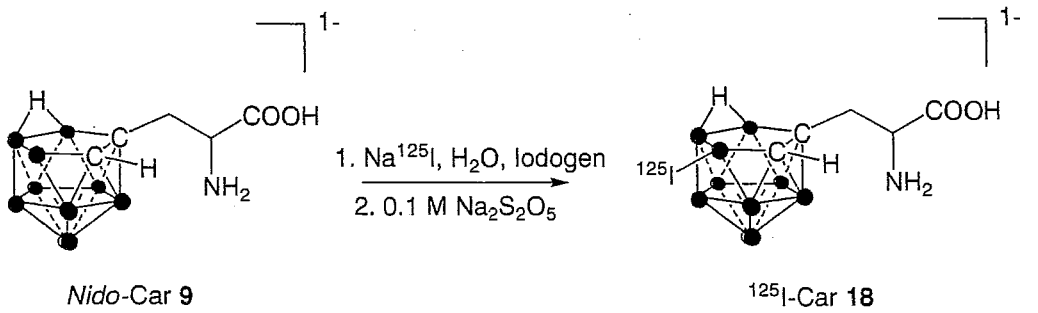
No significant difference was observed in product formation between reactions at pH 11 carried out at 160 °C for 15 minutes (Figure 3.4) or 180 °C for 5 minutes (Figure 3.5). The reaction conditions which gave the highest percent converion (~45%) to the desired product **13** were at pH 6 with microwave heating at 180 °C for 5 minutes. The product was isolated using a C18 SepPak® cartridge to give a clean chromatogram as can be seen in Figure 3.6 in 10% isolated radiochemical yield (decay corrected) in greater than 99% radiochemical purity.

It is interesting to note the difference between the chemistry at the macroscale compared to the technetium work at the tracer level. Re-Car was difficult to isolate due to the mixture of products which overlapped during HPLC elution. In contrast ^{99m}Tc-Car could be easily separated from the few side products using a C18 SepPak® rather than semi-preparative HPLC (as was used for Re-Car isolation) due to the differences in retention between impurities. This is an important point as radiochemistry must be accomplished as quickly as possible to prevent loss of activity, and one step purification such as was implemented with the C18 SepPak® catridge compared to the time-consuming isolation using semi-preparative HPLC, is highly desirable.

3.2 Synthesis of ^{125}I-Car

Radioiodination of *nido*-Car **9** was performed by first coating the walls of an eppendorf tube with 20 μL of a 1 mg/mL solution of Iodogen® and drying by rotary

evaporation. To the oxidant coated tube, 100 μL of a 1 mg/mL solution of *nido*-Car **9** (in H_2O) was added, along with 5-10 μL of $Na^{125}I$ solution in a mildly basic solution (pH 8-11) of concentration of 10 mCi/mL (Scheme 3.3). The activity of the reaction was measured and the reaction quenched with a 0.1 M solution of sodium metabisulfite after 10 min.



Nido-Car **9** ^{125}I-Car **18**

Scheme 3.3: Radioiodination of *nido*-Car using $Na^{125}I$ and Iodogen as the oxidant to form ^{125}I-Car **18**

The radiolabelling of *nido*-Car was found to be remarkably fast and efficient. Figure 3.7 shows a crude reaction mixture of ^{125}I-Car **18**. The typical conversion to the desired product was greater than 95%. It can be seen from Figure 3.8 that there is excellent correlation between the radioiodinated product and the non-radioactive standard. Not only were these reactions high yielding with no side products but it was found the product showed no signs of degradation when left in solution for two weeks (Figure 3.9).

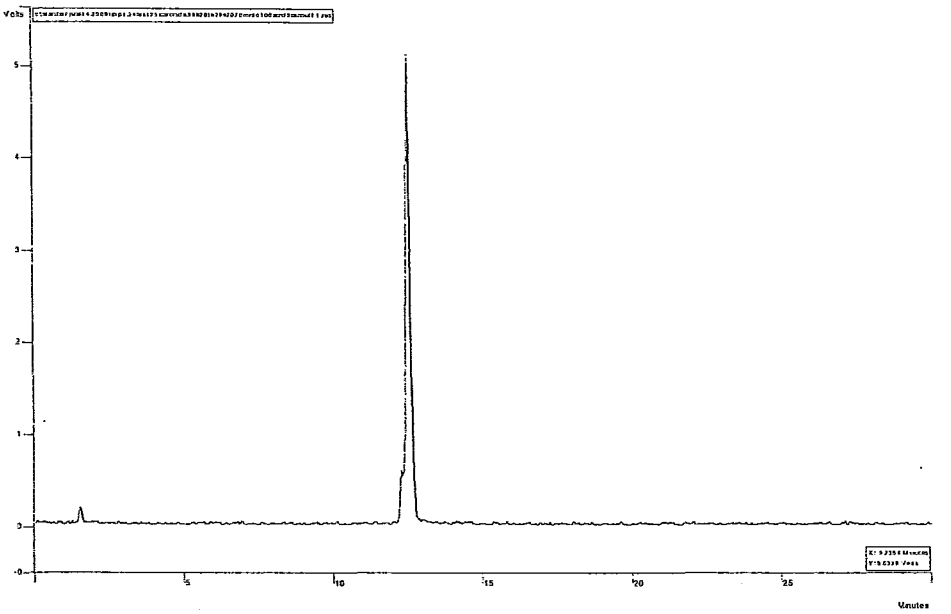


Figure 3.7: Gamma HPLC chromatogram of crude ^{125}I-Car **18** reaction mixture

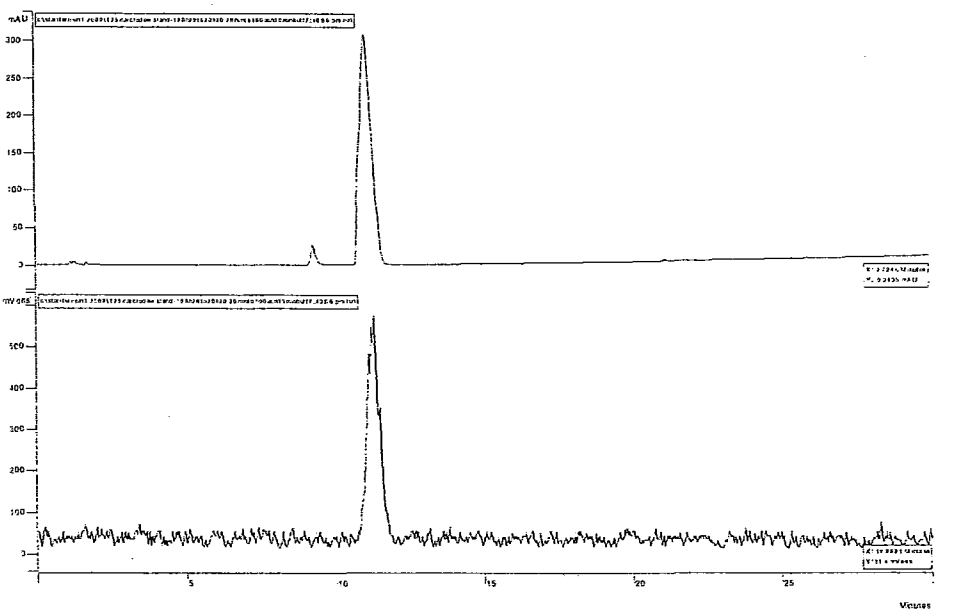


Figure 3.8: Comparison between non-radioactive standard I-Car **16** (UV trace, top) and ^{125}I-Car **18** (gamma trace, bottom)

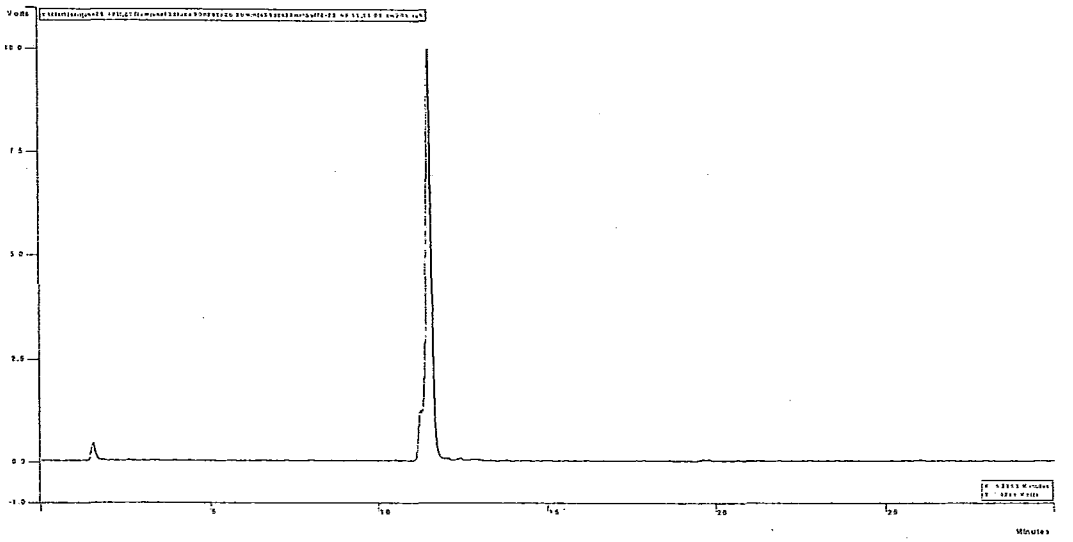


Figure 3.9: HPLC chromatogram of ^{125}I-Car **18** after 2 weeks sitting in solution (4.1 μCi/100 μL)

Further investigation into the efficiency of the radioiodination of *nido*-Car involved a dilution study where the concentration of *nido*-Car was decreased to see if iodination could still take place in order to increase the effective specific activity for the samples, and simplify purification.

3.2.1 Effect of Ligand Concentration

The dilution study involved using the same amount of radioiodine (10 μL of a 10 mCi/mL solution of $Na^{125}I$) and making serial dilutions to the stock solution of *nido*-Car (1 mg/mL, 500 μg/mL, 250 μg/mL, 100 μg/mL, 10 μg/mL and 1 μg/mL) of which 100

μL of the solutions were used and doing the reactions in triplicate for consistency. It was found that at 1 mg/mL and 500 μg/mL (using 100 μL), the average conversion to ^{125}I-Car (based on HPLC area calculations) was greater than 95%. At 250 μg/mL, the average conversion was 70% with the main impurity likely being iodide salts which elute at the solvent front (Figure 3.10).

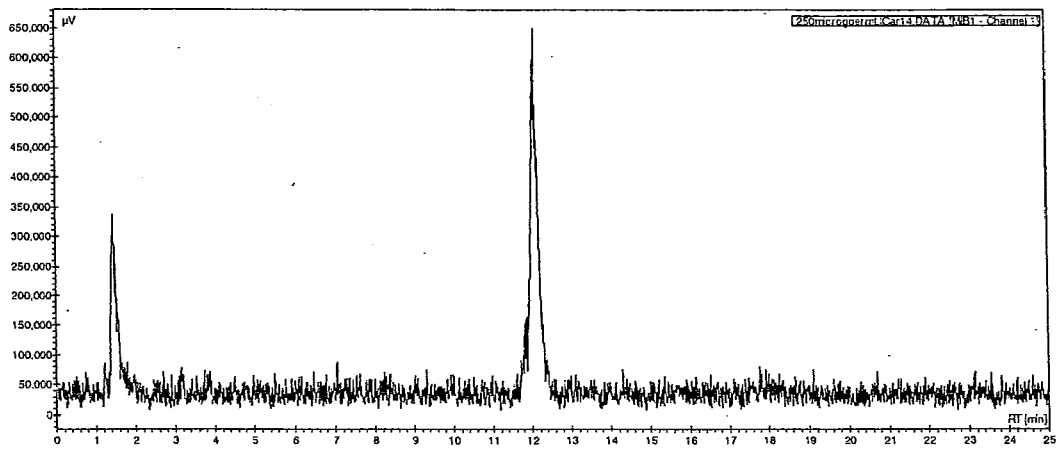


Figure 3.10: γ-HPLC chromatogram of the crude reaction mixture of *nido*-Car (250 μg/mL solution) with Na^{125}I with 78% conversion to the desired product **18**

Below starting material concentrations of 250 μg/mL, the percent conversion dropped signifcantly (100 μg/mL = 19%, 10 μg/mL = 18% and 1 μg/mL = <5%) which is not surprising as the concentration of 1 μg/mL contains a nanomolar concentration (4.5 x 10^{-9} M) of *nido*-Car. When these reactions can efficiently label at such low concentrations, the need for purification becomes virtually obsolete as the effective specific activity will be basically the same as the specific activity. It was found that at a

concentration of 100 μg/mL any nonradioactive impurities were below the detection limit of the UV detector. The acheivment of 70% conversion at concentrations of 250 μg/mL (1.1×10^{-7} M) is very impressive as direct radiolabelling reactions are typically done at concentrations of 10^{-4} M to 10^{-5} M.

3.2.2 Labelling Car in the Presence of Tyrosine

The carborane ligand and ^{125}I-Car were shown to be highly reactive and very stable respectively, but for radiopharmaceutical development, it is also important that iodine will selectively react with the desired vector when in the presence of competing functional groups. As the *nido*-Car ligand will ultimately be incorporated into peptides and radiolabelled it is important to determine if the group can be labelled selectively. The obvious competing amino acid is tyrosine which is activated towards electrophilic species and in proteins tyrosine is predominately labelled relative to other amino acids.[6]

To determine the selectivity, head-to-head experiments of *nido*-Car to tyrosine were performed in equimolar amounts. These experiments were undertaken by adding equimolar amounts of both tyrosine (in a H_2O/EtOH solution) and *nido*-Car in a volume of 100 μL each to a Iodogen® coated eppendorf tube, followed by the addition of ~170 μCi of ^{125}I. This mixture was quenched with a 0.1 M solution of sodium metabisulfite after 10 min. The resulting chromatograms (e.g. Figure 3.11) showed high conversion to ^{125}I-Car with an average conversion (after three trials) of 93%. Tyrosine was labelled without the presence of *nido*-Car to confirm that the labelling would take place under the

same conditions, and the labelling was successful with >90% conversion. These experiments show very promising results where future work for this project could involve the incorporation of Car into a targeting peptide to be used for radioiodination.

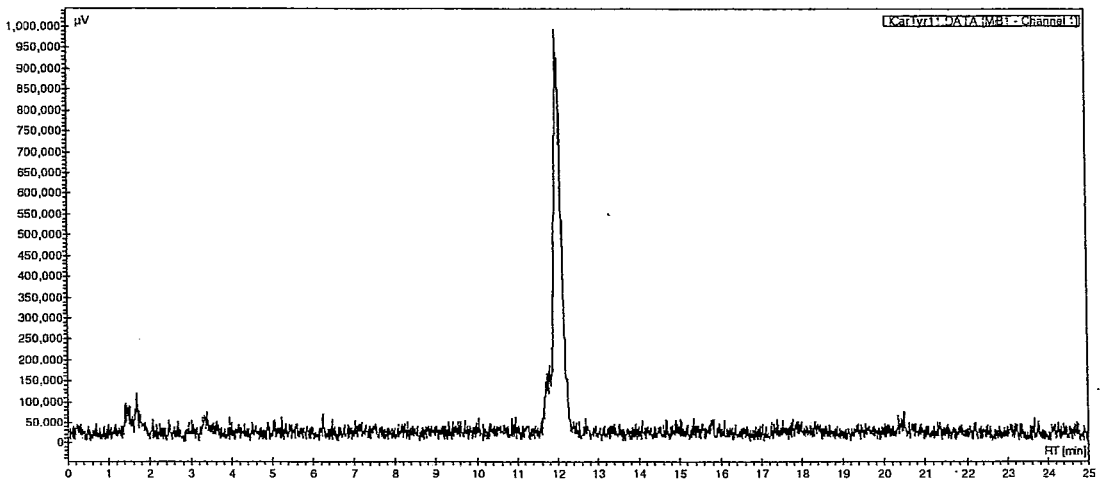


Figure 3.11: γ-HPLC chromatogram of a equilmolar reaction of tyrosine to *nido*-Car **9** with ^{125}I showing high conversion to ^{125}I-Car **18** (96%)

3.2.3 Summary and Conclusions

The focus of this chapter was the radiolabelling of *nido*-Car **9** with both ^{99m}Tc and ^{125}I. It was found that the radiolabelling of *nido*-Car with ^{99m}Tc proceeded with very few side products as compared to the cold rhenium standard (Re-Car **12**). This is not an unexpected result as the chemistry at the tracer level is significantly different due to the huge excess of ligand to metal, making it highly unlikely that two ^{99m}Tc(CO)$_3$ cores could chelate to one *nido*-Car as was the case with the rhenium analogue (formation of di-

rhenium complex **14**). Variations of the labelling conditions (time, temperature and pH) resulted in a 45% percent conversion to the desired ^{99m}Tc-Car **13**.

Much like the cold analogue (I-Car **16**), the radiolabelling of *nido*-Car with ^{125}I was a fast, efficient and high yielding reaction. It was found that under the standard labelling conditions, *nido*-Car would give quantitative conversion to ^{125}I-Car **18** in less than 10 minutes at room temperature. Not only was **9** highly reactive to radioiodination, but the resulting product was found to be remarkably stable with no signs of degradation up to two weeks.

A dilution study was performed to examine the reactivity of **9** at unusually low concentrations for labelling. It was found that >95% conversion occurred at concentrations of 4.5 mM and 2.3 mM and 70% conversion occurred at 1.1 mM. These results are highly encouraging as at reduced concentrations, the effective specific activity is significantly increased and purification of the radioactive product could theoretically not be necessary.

A head-to-head experiment using equimolar amounts of tyrosine to *nido*-Car found that ^{125}I-Car was formed prefentially with an average percent conversion of 93% indicating that the incorporation of *nido*-Car into peptides is plausible as it should be labelled preferentially.

3.3 Experimental Section

3.3.1 Material and Instruments

HPLC experiments were performed on a Varian Prostar Model 230 instrument, fitted with a Varian Prostar model 330 PDA detector and an IN/US γ-RAM gamma detector. The wavelength for detection was set at λ = 254 nm, and the dwell time in the gamma detector was 0.5 s with a 10 μL loop. The analytical column used was a Phenomenex Gemini (L x ID = 100 x 4.6 mm) analytical column (5 μm C18). Elution protocols Method 1: Solvent A) H_2O + 20mM ammonium acetate, solvent B) CH_3CN + 0.1% formic acid. Gradient elution, 0-20 min, 90:10 A/B to 20:80 A/B; 20-30 min, 20:80 A/B to 100 B. The flow rate for all methods was set at 1 mL/min (analytical).

3.3.2 Experimental Data

*Synthesis of 99m-Tc carboranylalanine **13** at pH 11*

500 µL of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ **17** (5.46 mCi) and 500 µL of a 1 mg/mL solution of *nido*-Car **9** were combined in a reaction vial (0.5-2 mL) purged with argon. The vial was heated at 180 °C in a microwave for 5 minutes. The reaction mixture was examined by analytical HPLC (Method 1) by first diluting a small amount of the sample (~1 µL) with 500 µL of water. Purification of **16** was accomplished by first activating a C18 SepPak® cartridge with 10 mL of EtOH, 10 mL of ACN and 10 mL of 10 mM HCl. The sample was loaded and washed with 10 mM HCl to remove $[^{99m}Tc(CO)_3(H_2O)_3]^+$ and

$[^{99m}TcO]^-$, followed by elution with a gradient from 4:1 10 mM HCl/CH_3CN to 100% ACN collecting 1 mL fractions. The fractions containing activity were examined by analytical HPLC for purity (263 μCi, 10% decay corrected yield). HPLC (Method 1): t_r = = 15.2 min.

*Synthesis of 99m-Tc carboranylalanine **13** at pH 6*

500 μL of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ **17** (1.48 mCi) and 500 μL of a 1 mg/mL solution of *nido*-Car **9** in H_2O were combined in a reaction vial (0.5-2 mL) purged with argon. A solution of 0.1 M HCl was added dropwise until a pH of 6 was reached, then the vial was heated at 195 °C in a microwave for 5 minutes. The reaction mixture was examined by analytical HPLC (Method 1) by first diluting a small amount of the sample (~1 μL) with 500 μL of water. A 82% conversion to the desired product was observed by HPLC. HPLC (Method 1): t_r = 15.2 min.

*Synthesis of ^{125}I carboranylalanine **18***

A 1 mg/mL solution of Iodogen® was prepared in chloroform and 20 μL of the solution was added to an eppendorf tube. The chloroform was removed from the eppendorf tube using a rotary evaporator leaving a film of Iodogen®. To this tube, 100 μL of a 1 mg/mL solution of *nido*-Car **9** was added, followed by 10 μL of a 10 mCi/mL solution of $Na^{125}I$ in sodium hydroxide (pH 8-11). The reaction was left for 5-10 minutes, followed by the

addition of 20 μL of 0.1 M $Na_2S_2O_5$. The reaction was analyzed by HPLC by first diluting a small amount of the sample (10 μL) with 500 μL of water. HPLC (Method 1): t_r = 11.2 min.

*Synthesis of ^{125}I carboranylalanine **18** in the Presence of Tyrosine*

A 1 mg/mL solution of Iodogen® was prepared in chloroform and 20 μL of the solution was added to an eppendorf tube. The chloroform was removed from the eppendorf tube using a rotary evaporator leaving a film of Iodogen®. To this tube, 100 μL of a 1 mg/mL solution of *nido*-Car **9** and 100 μL of a 0.82 mg/mL solution of L-tyrosine were added followed by 20 μL of a 10 mCi/mL solution of $Na^{125}I$ in sodium hydroxide (pH 8-11). The reaction was left for 5-10 minutes, followed by the addition of 50 μL of 0.1 M $Na_2S_2O_5$. The reaction was analyzed by HPLC by first diluting a small amount of the sample (10 μL) with 500 μL of water. Percent conversion to I-Car (averaged of 3 experiments) = 93.2% by HPLC (Method 1): t_r = 11.2 min.

3.4 References

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Chapter 4

Conclusions and Future Work

4.1 Conclusions

The work presented in this thesis describes the synthesis of carboranylalanine (Car **5**), a known carborane containing analogue of the amino acid phenylalanine, the cage degradation of Car to form *nido*-Car **9** using microwave heating followed by the synthesis of Re-Car **12** and I-Car **16** to act as reference standards for the radiolabelling of **9** with both ^{99m}Tc and ^{125}I. This work clearly demonstrates the versatility of the carborane cage as a novel platform for radiolabelling using multiple isotopes.

The racemic synthesis of Car was achieved following a publication by Wyzlic et al.[1] Although the synthesis of Car was known, it was found that some changes to the original procedure resulted in increased yields. Car had previously been shown to self-degrade forming *nido*-Car **9**[2] but it was found that this process did not give quantitative conversion, so an alternative route was established using microwave heating in water. It was found that basic conditions were not necessary for cage degradation, likely due to the presence of the amine in Car which would aid in the conversion. The only side product from the formation of *nido*-Car in this manner was boric acid. A new and facile method was developed to remove the boric acid through conversion to the more volatile borate ester.

Once *nido*-Car **9** was successfully isolated, complexation to the rhenium tri-carbonyl core was examined. It was found that under the standard conditions for

rhenacarborane formation, a mixture of products was obtained including the desired Re-Car **12**, a chelation product **15** and a dirhenium species **14**. Re-Car could be separated from the mixture in low yield by isolation using semi-preparative HPLC.

Nido-Car also serves as a platform for iodination reactions to give I-Car **16**. The iodination reactions were found to take place in high yield at room temperature with reaction times of less than 15 minutes. When the stoichiometry was kept to a 1:1 ratio between **9** and I_2 no other side products were observed and purification by semi-preparative HPLC (from any residual **9**) gave pure **16** in 48% yield.

With the cold rhenium and iodine standards in hand, the radiolabelling of *nido*-Car **9** with both ^{99m}Tc and ^{125}I was examined. It was found that the radiolabelling of *nido*-Car with ^{99m}Tc proceeded with very few side products as compared to the cold rhenium standard (Re-Car **12**). Variations of the labelling conditions (time, temperature and pH) resulted in 45% percent conversion to the desired ^{99m}Tc-Car **13**. Although some side products were obtained during the formation of **13** it should be noted that isolation could still be achieved using a C18 SepPak® which was simpler than using semi-preparative HPLC.

The radiolabelling of *nido*-Car with ^{125}I was a fast, efficient and high yielding reaction. It was found that under the standard labelling conditions, *nido*-Car gave quantitative conversion to ^{125}I-Car **18** in less than 10 minutes at room temperature in the presence of Iodogen® as the oxidant. The resulting product was found to be extremely stable with no signs of degradation up to two weeks in solution.

A dilution study of the nido-Car found that >95% conversion to ^{125}I-Car occurred at concentrations of 4.5 mM and 2.3 mM and 70% conversion occurred at 1.1 mM. These are significantly lower concentrations compared to other direct radiolabelling procedures.[3] These results are encouraging because at these concentrations, the effective specific activity is high enough that it may not be necessary to remove unlabelled ligand even for targeted probes.

To ensure that *nido*-Car can be labelled sectively when incorporated into a peptide a head-to-head experiment using equimolar amounts of tyrosine to *nido*-Car was performed. It was found that ^{125}I-Car formed preferentially with greater than 93% forming in competitive experiments. Under the same reaction conditions but without *nido*-Car present, tyrosine was converted to ^{125}I-tyrosine quantitatively.

4.2 Future Work

Nido-Car is a highly versatile ligand capable of forming both metallocarboranes and iodinated products. One immediate project for the future work should be the testing of the synthesized products for their uptake in tumour cell lines. In Section 1.8 amino acid transporter systems, particularly LAT1, were discussed. The LAT1 transporter system has been shown to actively internalize large non-natural amino acids including Re/^{99m}Tc containing amino acid analogues developed by Alberto *et al.*[4] Given the range of amino acids capable of being accepted by the LAT1 system, it is highly probable that *nido*-Car labelled with various isotopes would be recognized as well. As amino acid

transport is upregulated in numerous tumour types, the radiolabelled *nido*-Car derivatives, if successfully recognized by the transporter, could be used for tumour imaging (e.g. SPECT or PET) and therapy.

Another potential application of the chemistry described in this thesis would be the incorporation of *nido*-Car into peptide-based targeting vectors for a range of biological targets. One issue which would need to be addressed would be the high labelling temperatures necessary for the metallocarborane complexation as peptides often degrade at elevated temperatures. Of the results described in this work, the better option for labelling of *nido*-Car in peptides would be with halogens. Radioiodination using ^{125}I was rapid and selective to the carborane in the presence of competing sites (tyrosine) indicating that if present in a peptide, *nido*-Car should be the solitary iodination site. As there are numerous isotopes of iodine, the relevance of this work is greatly varied depending on the desired application.

The synthesis of Re-Car was found to give multiple side products as a result of the amino acid functionality chelating to the rhenium tricarbonyl core. One way to potentially improve on the results obtained in this work would be to protect the amino acid functionality of *nido*-Car. The feasibility of this was examined by Armstrong and the initial results presented at the Society of Nuclear Medicine conference in Toronto, ON in 2009.[5] The synthesis of a protected Car derivative was successfully undertaken from commercially available Fmoc-D-propargyl-Gly-OH as a simplified synthesis to

protected Car. Further work on this project would be necessary to examine the effects of a protecting group with regards to metallocarborane formation.

4.3 References

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