CARBORANE AND METALLOCARBORANE CARBOHYDRATE CONJUGATES

THE SYNTHESIS AND CHARACTERIZATION

OF

CARBORANE AND METALLOCARBORANE - CARBOHYDRATE CONJUGATES

By

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Abstract

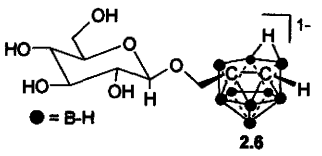
This thesis describes the synthesis and characterization of a series of carborane and metallocarborane-carbohydrate conjugates as model systems for developing a novel class of radiopharmaceuticals. The role of the carborane group is to provide a site for binding radioactive elements while the carbohydrate moieties are present either as a targeting vector, or as a means by which to increase the hydrophilicity of the overall complex. In this research, the versatility of carboranes was demonstrated since it was shown that carbohydrate-*nido*-carborane derivatives could be labeled with both metals (Re/^{99m}Tc) and halogens (^{125}I/^{127}I).

The initial synthetic target was compound **2.6**, a simple *nido*-carboranyl glycoside of glucose. The syntheses of this model ligand and its Re-metallocarborane (**2.1**, **3.4**) and iodinated (**2.13**) derivatives were carried out in order to determine the optimal methods and conditions for synthesis and purification of bifunctional ligands and the corresponding radioactive analogues. Microwave irradiation was found to greatly enhance the synthesis of Re and ^{99m}Tc-metallocarborane complexes which were isolated in 31% and 58% yield respectively. Analysis of the Re complexes by ^{1}H nOe NMR spectroscopy revealed that rearrangement of the carborane cage from the expected 3,1,2-ReC_2B_9 isomer to the 2,1,8- isomer occurred under the synthetic conditions employed.

Iodination and radioiodination of model compound **2.6** was carried out using Na[^{127}I] or Na[^{125}I] in the presence of Chloramine-T or Iodogen as oxidants at room temperature. Reactions were complete in 5 min and the products isolated in 21% and 29% yield for ^{127}I and ^{125}I, respectively.

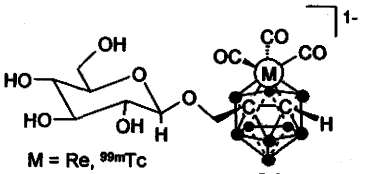
Building on these results, bifunctional compounds **4.3** and **4.12** were prepared. Using microwave heating, these compounds were labeled with ^{99m}Tc in 62% and 44% yield, respectively. Compounds **4.3** and **4.12** contained a benzoic acid functionality through which conjugation to targeting vectors could be accomplished. To demonstrate this, benzamides **4.14** and **4.16** were synthesized using an active ester approach. The products were isolated in 41% and 35% yield and subsequently labeled with ^{125}I using the methods developed for the model system. Compounds [^{125}I]-**4.23** and [^{125}I]-**4.24** were obtained in 73% and 92% yield, respectively. The stability of these [^{125}I]-labeled compounds was excellent, showing less than 1% degradation after 24 hours in solution. In order to assess the effect of the carbohydrate moiety upon lipophilicity, the log P of the radiolabeled benzamides was measured and found to be 1.53±0.01 for [^{125}I]-**4.23** and 0.82±0.04 for [^{125}I]-**4.24**. This result confirmed the increase in hydrophilicity associated with the presence of the carbohydrate moiety.

Progress was also made towards preparing a glucose-*nido*-carborane conjugate (**5.9**) whose Re and Tc complexes were pursued as metallocarborane analogues of the clinical PET tracer [^{18}F]FDG. The key precursor was made in good overall yield and the product fully characterized. Future work should focus on preparing the radiolabeled analogues.



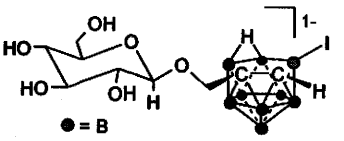
● = B-H

2.6



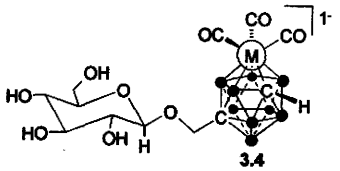
M = Re, ^{99m}Tc

2.1

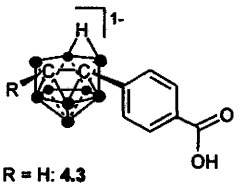


● = B

2.13

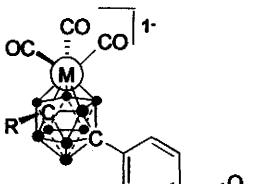


3.4



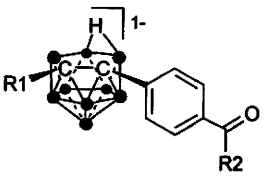
R = H: **4.3**

R = CH_2-β-D-glucose: **4.12**



R = H: **4.2**

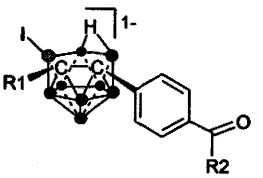
R = CH_2-β-D-glucose: **4.1**



R1 = CH_2-β-D-glucose, R2 = OH: **4.12**

R1 = H, R2 = $NH(CH_2)_2N(C_2H_5)_2$: **4.14**

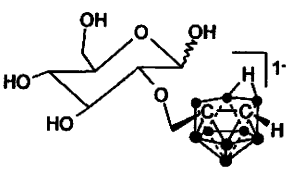
R1 = CH_2-β-D-glucose, R2 = $NH(CH_2)_2N(C_2H_5)_2$: **4.16**



R1 = CH_2-β-D-glucose, R2 = OH: **4.22**

R1 = H, R2 = $NH(CH_2)_2N(C_2H_5)_2$: **4.23**

R1 = CH_2-β-D-glucose, R2 = $NH(CH_2)_2N(C_2H_5)_2$: **4.24**



5.9

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Finally, I thank my family and friends for their constant love and support throughout this endeavour.

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Glossary

Ac_2O	Acetic anhydride
β^+	Positron
β^-	Beta (negative) particle
BNCT	Boron neutron capture therapy
br	Broad signal (IR or NMR spectroscopy)
CEA	Carcinoembryonic antigen
Chloramine-T	*N*-chloro-*p*-toluenesulfonamide, sodium salt
CI-MS	Chemical Ionization Mass Spectrometry
cm^{-1}	Wavenumbers (IR)
COSY	Correlation spectroscopy (NMR)
Cp	Cyclopentadiene
d.	Days (Isotope half life or reaction duration)
d	Doublet (NMR)
dd	Doublet of doublets (NMR)
ddd	Doublet of doublets of doublets (NMR)
D	Deuterium (in NMR solvent)
DBU	1,8-diaza-bicyclo-[5.4.0.]-undec-7-ene
DCM	Dichloromethane
DIC	Diisopropylcarbodiimide
DLT	Double ligand transfer reaction
DMF	Dimethylformamide
DTPA	Diethylenetriaminepentaacetic acid (chelate)
E_γ	Gamma ray energy
E_β	Beta particle energy
EDC·HCl	*N*-(3-dimethylaminopropyl)-*N'*-ethylcarbodiimide hydrochloride
ECDG	Ethylenedicysteinedeoxyglucose
EI-MS	Electron Ionization Mass Spectrometry
ESMS	Electrospray Ionization Mass Spectrometry
EtOH	Ethanol
eV	Electron volt
FDG	[^{18}F]-2-Fluoro-2-deoxyglucose
γ	Gamma ray
GluT	Glucose transporter
HMBC	Heteronuclear Multiple Bond Correlation (NMR)
HPLC	High Performance Liquid Chromatography
hr	Hours
HRMS	High Resolution Mass Spectrometry

HSQC	Heteronuclear Single Quantum Coherence (NMR)
Hz	Hertz
I	Nuclear spin
IDA	Iminodiacetic acid (chelate)
Iodogen®	1,3,4,6-tetrachloro-3α,6α-diphenylglycouril
IR	Infrared spectroscopy
J	Coupling constant (NMR)
LC-MS	[High Performance] Liquid chromatography-mass spectrometry
m	Multiplet (NMR), also medium absorption (IR)
MeOH	Methanol
MIBI	Methoxyisobutylisonitrile
min	Minutes
MW	Microwave (irradiation)
M.W.	Molecular Weight
NMR	Nuclear Magnetic Resonance spectroscopy
o	*Ortho* (1,2-dicarba-*closo* or 7,8-dicarba-*nido* carborane)
PBF	Phosphate-buffered sodium fluoride solution
PBS	Phosphate-buffered saline solution
PET	Positron Emission Tomography
ϕ	Benzene or aryl group
ppm	Parts per million (chemical shift)
q	Quartet (NMR)
RT	Room temperature
s	Singlet (NMR), also strong absorption (IR)
SPE	Solid Phase Extraction
SPECT	Single Photon Emission Computed Tomography
t	Triplet (NMR)
$t_{½}$	Half - life
TBAF	Tetrabutylammonium fluoride
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS-OTf	Trimethylsilyl trifluoromethanesulfonate
t_R	Retention time (HPLC)
TsOH	*Para*-toluenesulfonic acid
VFC	Venus Flytrap Cluster
vide infra	See below
vide supra	See above
w	Weak absorption (IR)

Chapter 1

Introduction and Objectives

1.1 Radiotracers and Radioimaging

Radioisotopes and radiopharmaceuticals are used in medicine to assess the function of organs and for determining the state of a particular disease. Radioimaging is based upon the *tracer principle*,[1] which refers to the fact that the amount of the radioactive substance (the radiopharmaceutical) introduced into the patient is sufficient that adequate imaging data may be acquired, but not so large that the imaging agent interferes with the biological processes being observed.[2] Radioimaging is accomplished by two main methods: Single Photon Emission Computed Tomography (SPECT) and Positron Emission Tomography (PET).

SPECT involves the detection of gamma (γ) photons emitted from the appropriate radionuclide by a gamma camera. To obtain tomographic data, between one and three gamma cameras that rotate around the patient detect photons at various positions. In doing so, images are taken as slices along a particular direction, and are subsequently reconstructed to give three-dimensional images of the distribution of the radionuclide within the patient. In each camera, incident gamma rays first pass through a collimator, which is a device made of a dense substance (typically lead), with one or several holes through which the photons must pass in order to strike the detector. Since the photons from gamma ray emitting nuclei are emitted in random directions, the collimator is necessary to block stray photons and detect only those within the field of view of the

camera. Gamma rays passing through the collimator strike a scintillating device typically consisting of thallium-doped sodium iodide crystals (NaI(Tl)). Gamma photons interact with the NaI(Tl) detector to produce flashes of light which are subsequently converted into an electrical signal by means of a photomultiplier tube and amplifiers. A pulse height analyzer is used to select only electrical pulses resulting from a particular gamma ray energy (i.e. the E_γ of the radionuclide being used). The resulting data are collected by a computer, where image reconstruction is accomplished using mathematical algorithms.[3,4]

Positron Emission Tomography is accomplished by detecting the coincident 511 keV gamma rays resulting from positron-electron annihilation. A β^+ particle emitted from the nucleus eventually collides with an electron and annihilation occurs, resulting in the two photons, which travel 180° to each other. PET scanners consist of ring-shaped detectors that count only those photons coming from the subject that result in a coincidence event. Image reconstruction is accomplished in the same manner as for SPECT. Because only coincident photons are used to create images, use of a collimator is not required. This results in higher sensitivity versus SPECT. SPECT, however, is the more widely employed technique because single photon emitting radionuclides are generally more available and less expensive than positron emitters.[3,5]

1.2 Technetium Radiopharmaceuticals

Technetium-99m (^{99m}Tc) is the most widely used radioisotope in medical imaging due to its low cost, availability and its nearly ideal nuclear properties ($t_{½}$ = 6.02 hr., E_γ = 140 keV). Approximately 85% of current radioimaging procedures involve ^{99m}Tc,[6] which

is readily available from a ^{99}Mo / ^{99m}Tc generator, in which molybdenum-99 molybdate (MoO_4^{2-}), adhered to a column of alumina, decays via β^- emission to give technetium-99m. (Figure 1.1).[7] The ^{99m}Tc is eluted from the generator as sodium ^{99m}Tc-pertechnetate ($Na[^{99m}TcO_4]$) with a dilute saline solution. Pertechnetate ($^{99m}TcO_4^-$) is the starting material for all technetium radiopharmaceuticals.[6,7]

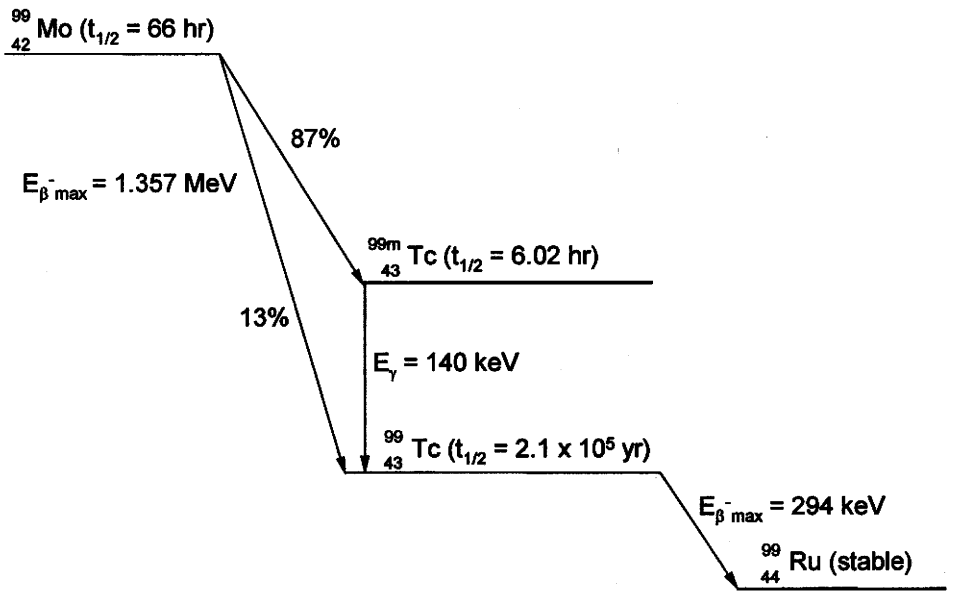


Figure 1.1: A simplified nuclear decay scheme for ^{99}Mo / ^{99m}Tc.

Technetium-based radiopharmaceuticals are divided into two main classes: technetium-essential compounds and technetium-tagged radiopharmaceuticals. In technetium-essential compounds, the metal atom is an integral structural component of the radiopharmaceutical, and the observed biodistribution depends on the size, charge and hydrophobicity / hydrophilicity of the complex as a whole.[6] Some examples of Tc-

essential radiopharmaceuticals are shown in Figure 1.2. Most technetium-essential radiopharmaceuticals are perfusion agents, which are used for the study of blood flow to and from various structures such as the heart, brain, and kidneys.[6-10]

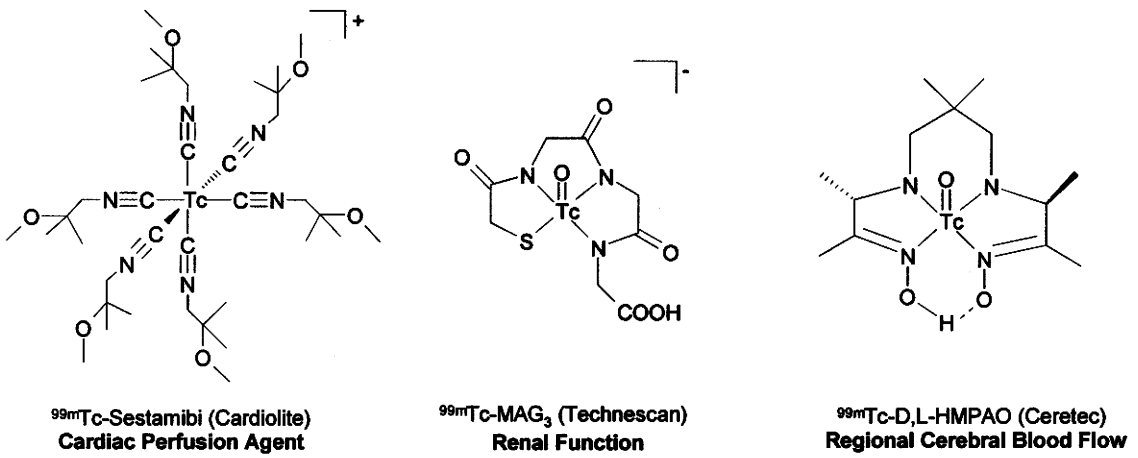


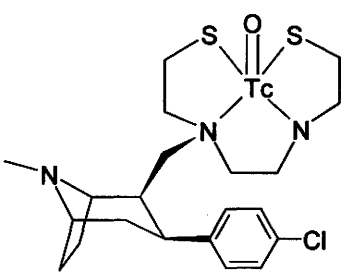
Figure 1.2: Examples of Tc-essential radiopharmaceuticals.

Technetium - tagged radiopharmacueticals are those in which the radiometal is attached to a targeting vector, such as a peptide, hormone, or antibody. In so doing, the isotope is ostensibly delivered to a site that binds the biomolecule, thereby affording a way of imaging specific biological targets. Examples of Tc-tagged compounds are given in Figure 1.3.[6,11-14] The technetium atom in these complexes is generally attached to the biomolecule through a bifunctional chelate. A bifunctional chelate is a ligand which binds Tc while also possessing an additional functional group for bioconjugation.

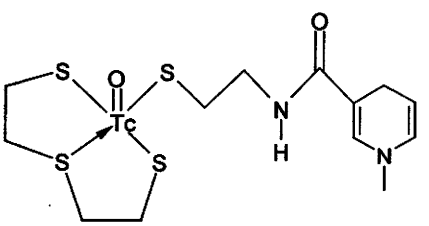
Chelate complexes of Tc(V), which typically contain a Tc(V)-oxo core, $[Tc{=}O]^{3+}$, have been one of the most actively investigated classes of bifunctional chelates for

designing targeted radiopharmaceuticals.[15] Tc(V) ligands that have been studied include N_2S_2, N_3S_1, and S_4 chelates, and those that form (3+1) type complexes. These ligands typically form square pyramidal complexes with Tc(V) (Figure 1.3).[6,16]

One of the disadvantages of Tc(V) chelates is their large size, which can influence the ability of a targeting agent to bind to the site for which it has specificity. Another disadvantage is that the chelates are often susceptible to catabolism by enzymes *in vivo*.[17] Consequently, there is an active search for complexes of technetium that are smaller in size, have more versatile bioconjugate chemistry (*vide infra*), and that possess superior resistance to catabolism.



[^{99m}Tc]-TRODAT-1
Dopamine Transporter
(N_2S_2)

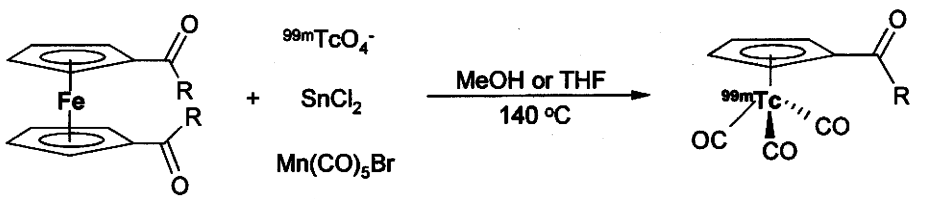


[^{99m}Tc]-3+1-dihydropyridine analogue
Hypoxia
(3+1)

Figure 1.3: Examples Tc-tagged radiotracers.

1.3 Organometallic Ligands for Technetium

The use of organometallic complexes of Tc(I) to prepare Tc-tagged radiopharmaceuticals is attractive because of their small size and expected high stability.[18] The first organometallic complexes produced at the tracer level were cyclopentadienyl complexes of ^{99m}Tc. $Cp^{99m}Tc(CO)_3$ was synthesized by reacting an acyl-substituted ferrocene with bromopentacarbonyl manganese (I), $SnCl_2$, and ^{99m}Tc-pertechnetate (Scheme 1.1).[19] This reaction is referred to as the Double Ligand Transfer reaction (DLT) because the cyclopentadienyl ligand bound to iron and the carbonyl ligands on manganese are both transferred to technetium.[15,19]



Scheme 1.1: Wenzel's synthesis of a $Cp^{99m}Tc(CO)_3$ complex.

A number of problems are associated with the DLT methodology. Firstly, the major product of the reaction was the manganese tricarbonyl derivative, rather than that of technetium. This might be expected, owing to the large excess of the Mn complex present in the reaction, compared to the trace amounts of ^{99m}Tc typically used during radiolabelling (10^{-7} - 10^{-8} M).[20] This problem is compounded by the fact that the manganese and technetium cyclopentadienyl complexes are not readily separable. This situation is not suitable for the preparation of radiopharmaceuticals, where a pure product

is essential, not only from the viewpoint of quality control, but in the case of receptor-targeted tracers, the excess manganese complex would likely displace the Tc complexes from the target receptors, thus limiting the effectiveness of the radiopharmaceutical.[15]

Another drawback of the double ligand transfer reaction is that the transformations are carried out in organic solvents (THF or methanol), whereas an aqueous synthesis is necessary for routine medical applications. Furthermore, high temperatures (140 °C) are required to conduct the reaction, which precludes the use of thermally sensitive targeting molecules such as peptides.[21]

Katzenellenbogen and co-workers have worked to improve the double ligand transfer reaction. They prepared substituted cyclopentadienyltricarbonyl rhenium species by reaction of perrhenate with ferrocene, $Cr(CO)_6$ and $CrCl_3$.[15] They have employed these methods for the preparation of cyclopentadienyltricarbonyl rhenium - tagged proteins and peptides[15] and tagged phenyltropanes for targeting the dopamine transporter.[22] They have also used this methodology to prepare $Cp^{99m}Tc(CO)_3$ conjugates of Octreotide. Octreotide is a short, cyclic peptide that binds somatostatin receptors, which are often overexpressed on cancer cells. Katzenellenbogen *et al.* have found that their ^{99m}Tc complexes compare favourably in terms of stability and biological activity with that of OctreoScan®; an approved radiopharmaceutical in which Octreotide is labelled with ^{111}In ($t_{½}$ = 67 hr, E_γ = 173 keV, 89%, E_γ = 247 keV, 94%). In this complex, the radiometal is bound to the peptide by a diethylenetriaminepentaacetic acid (DTPA) chelate.[23]

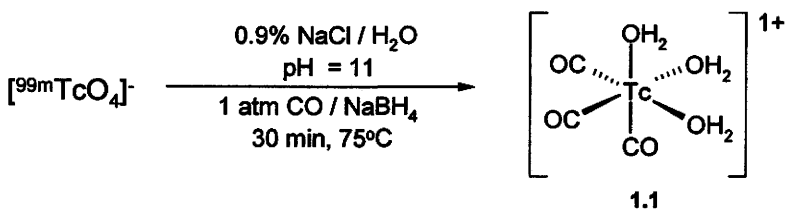
The main drawbacks of the modified double ligand transfer method are that the reaction still requires high temperatures (160 °C), organic solvents (methanol), and the targeting agent can only be added after the Cp complex is formed (indirect labelling). It is advantageous to perform the labelling step after the organometallic ligand is attached to the targeting agent because the radiolabelled complex can be isolated in a shorter period of time, thus minimizing both loss of activity and radiation exposure to the handler.

1.4 The $Tc(CO)_3^+$ Core

In the cyclopentadienyltricarbonyl technetium complexes prepared by Wenzel, Katzenellenbogen and co-workers, the oxidation state of the metal was Tc(I), as opposed to the Tc(V) state found in most chelate complexes. Tc(I) is an attractive oxidation state because octahedral complexes can exist with a low -spin d^6 electronic configuration. As a result, the rate of ligand substitution, or catabolism, at the metal centre in these Tc(I) complexes should be much slower than that in Tc(V) complexes.[18,24,25]

An example of Tc(I) - based organometallic radiopharmaceuticals are the Tc(I)-isonitrile complexes prepared by Davison and co-workers.[6,8,26,27] The most notable example is $[^{99m}Tc(MIBI)_6]^+$ (Cardiolite, Figure 1.2), which is used clinically as a tracer of myocardial perfusion.[6,8] The Tc(I)-isonitriles were prepared from $^{99m}TcO_4^-$ by reduction with $Na_2S_2O_4$, followed by reaction with an excess of isonitrile ligands.[26,27] Unfortunately, the Tc(I) isonitrile complexes are inert and therefore not suitable starting materials for preparing other organometallic radiopharmaceuticals. A Tc(I) core where a certain degree of ligand substitution is possible is needed for such a purpose.

Alberto and co-workers recently prepared a trisaquotricarbonyltechnetium(I) species (**1.1**) from pertechnetate under mild conditions in aqueous media (Scheme 1.2).[24,28] In their synthesis, sodium [^{99m}Tc]-pertechnetate was reduced and carbonylated under one atmosphere of carbon monoxide in saline solution at pH = 11, in the presence of sodium borohydride. They have since modified their synthesis to involve the use of potassium boranocarbonate ($K_2(BH_3CO_2)$), which acts both as a reductant and an *in situ* source of carbon monoxide.[18,29,30]



1.1

Scheme 1.2: Synthesis of the $[Tc(CO)_3(H_2O)_3]^+$ species.

Compound **1.1**, and its non-radioactive analogue of rhenium are useful synthetic precursors because the three water molecules can be displaced by a variety of ligands.[31-34] The majority of compounds that have been prepared from the $M(CO)_3^+$ core are simple coordination complexes. For the purposes of preparing targeted radiopharmaceuticals, a wide range of bidentate and tridentate bifunctional chelate complexes have been reported.[21,25,35-48] Some recent examples (Fig. 1.4) include $^{99m}Tc(CO)_3$ complexes of histidine, picolinic acid, pyridine-Schiff-base bidentate ligands, as well as, iminodiacetic acid, N-2-picolylamineacetic acid, various amine, thioether, phosphine, and N,N-bis(2-pyridylmethyl)-amino tridentate ligands. Studies that investigated the formation and

stability of these compounds indicated that formation of tridentate complexes could be done at ligand concentrations as low as 10^{-6}M, and that the tridentate complexes were generally more stable, as they resulted in a closed coordination sphere about the metal centre. In contrast, in the complexes involving bidentate ligands, one coordination site remained occupied by a labile water or chloride ligand, which allowed for exchange with other ligands, including plasma proteins.[39,43,46,47]

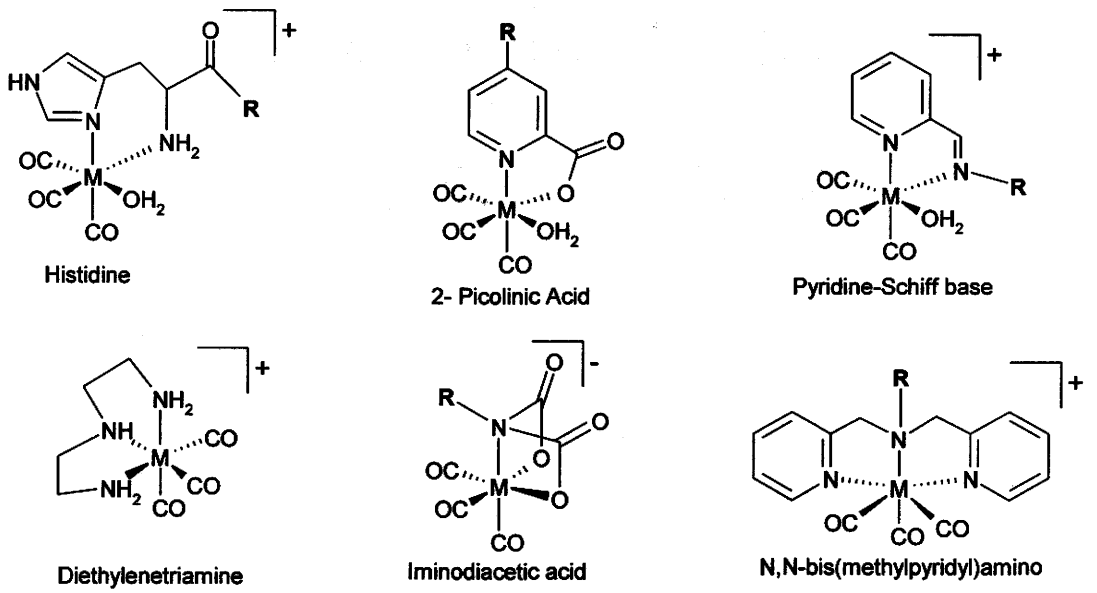


Figure 1.4: Examples of bidentate and tridentate ligands for the $M(CO)_3^+$ core.[43,47]

As an alternative to simple chelate complexes, the $M(CO)_3^+$ core affords the opportunity to prepare more traditional organometallic complexes of technetium (including $CpTc(CO)_3$) in water. Attempts to prepare $CpM(CO)_3$ complexes (M = Re, Tc) from $[NEt_4]_2[Re(CO)_3Br_3]$ or $[Tc(CO)_3(H_2O)_3]^+$ in aqueous media, however, are

complicated by the fact that cyclopentadiene is not soluble in water, and its conjugate base, the cyclopentadienide ion is difficult to prepare in water.

To circumvent these problems, Alberto and co-workers used acyl-substituted cyclopentadienes to prepare $RCpM(CO)_3$ complexes in water (R = acyl). The presence of the carbonyl group increased the acidity of Cp by five orders of magnitude, which allowed for the preparation of the metal complexes in good yield (Figure. 1.5). Alberto and colleagues have used this approach to prepare carbonyl-substituted cyclopentadienyltricarbonyl technetium(I) complexes bound to derivatives of 1-(2-methoxyphenyl)-piperazine for targeting of serotonergic receptors (Scheme 1.3).[18,49]

The presence of the carbonyl group on cyclopentadiene is disadvantageous in the sense that it can act as a competing ligand for the $Tc(CO)_3^+$ core.[18] Additionally, the cyclopentadienide-containing ligands can dimerize at elevated temperatures.[49]

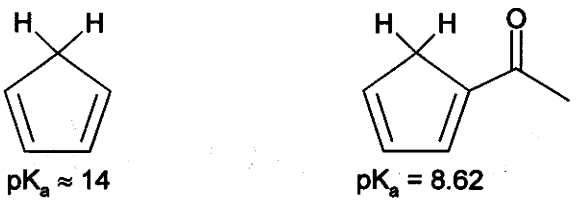
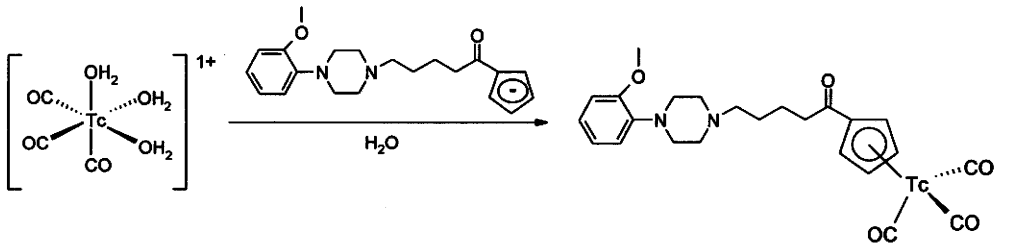


Figure 1.5: Comparison of pKa of cyclopentadiene to acetyl cyclopentadiene.



Scheme 1.3: Aqueous synthesis of a Cp-Tc complex for targeting of serotonergic receptors.[18]

1.5 Carboranes and Metallocarboranes

Carboranes are clusters of boron, carbon and hydrogen, which can form sandwich type complexes with a variety of metals.[50-55] Of particular interest are the ligands derived from dicarba-*closo*-dodecaboranes (Figure 1.6), which are a class of boron hydride clusters containing two carbon atoms in the structural framework. The first of these twelve - vertex species to be prepared was 1,2-dicarba-*closo*-dodecaborane (1,2-$C_2B_{10}H_{12}$), which was synthesized by insertion of acetylene into decaborane(14) ($B_{10}H_{14}$) in the presence of CH_3CN as a weak Lewis base.[56-59] This product is commonly referred to simply as "*ortho*-carborane". Thermal rearrangement occurs upon heating of *ortho*-carborane to produce the 1,7- (*meta*) and 1,12- (*para*) isomers.[60,61]

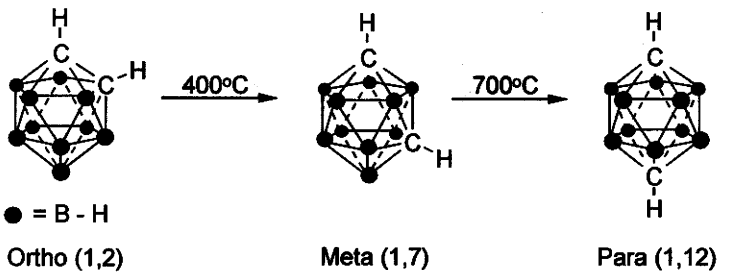
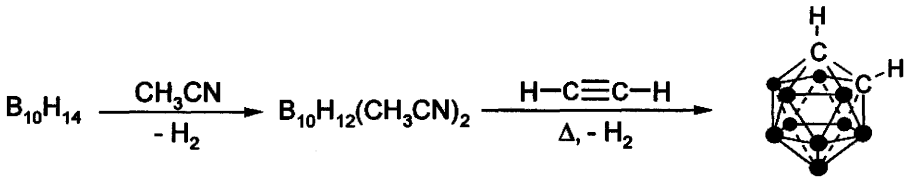


Figure 1.6: *Ortho*, *meta* and *para* isomers of *closo*-$C_2B_{10}H_{12}$.

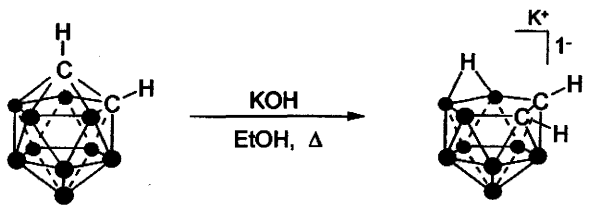
The preparation of *ortho*-carborane involves reacting an alkyne with a Lewis acid-base adduct of decaborane(14). Common Lewis bases include nitriles or sulfides (Scheme 1.4).[56-61] The alkyne insertion reaction can be accomplished using a variety of different alkynes, making it possible to prepare a large number of unique carbon-substituted *ortho*-carborane derivatives in a single step. This includes both mono and disubstituted alkynes. Carboranes are also synthetically versatile in that the C-H hydrogen atoms can be removed with *n*-butyllithium, allowing the resulting anion to be used as a nucleophile. Functionalization of the cluster may then be accomplished by the addition of a variety of electrophilic species, including alkyl halides, chlorosilanes, and α,β-unsaturated ketones.[61,62] Alternatively, carbon atom substitution with aryl and heteroaryl groups can be accomplished via copper(I) derivatives, in a manner similar to many transition metal-catalyzed reactions.[63]

Further synthetic versatility of carboranes comes from the reactivity of their boron atoms. The boron atoms of the carborane are considered chemically orthogonal to the carbon atoms in that the boron atoms tend to participate in electrophilic substitution reactions, such as those which resemble the Friedel-Crafts reaction,[61] whereas the carbon atoms participate in nucleophilic substitution reactions. The B-atoms can also be functionalized with organic groups (aryl heteroaryl, alkynyl) via metal-catalyzed reactions involving activation of a B-I in an iodine-substituted carborane with a Pd(0) catalyst, followed by reaction with an active organometallic compound, such as a Grignard or an organozinc compound.[64]



Scheme 1.4: Decaborane-alkyne insertion reaction to form *ortho*-carborane.

Reaction of *ortho*-carborane with strong Lewis bases results in removal of one boron atom from the cage, yielding the 7,8-dicarba-*nido*-undecaborate anion $[C_2B_9H_{12}]^-$. This reaction was first observed by Hawthorne and co-workers using alcoholic solutions of potassium hydroxide (Scheme 1.5).[65,66] The degradation of the carborane cage using piperidine[67] and, more recently, fluoride[68,69] has been reported. These methods allow degradation of the carborane to be carried out in the presence of functional groups that are sensitive to strongly basic reaction conditions.



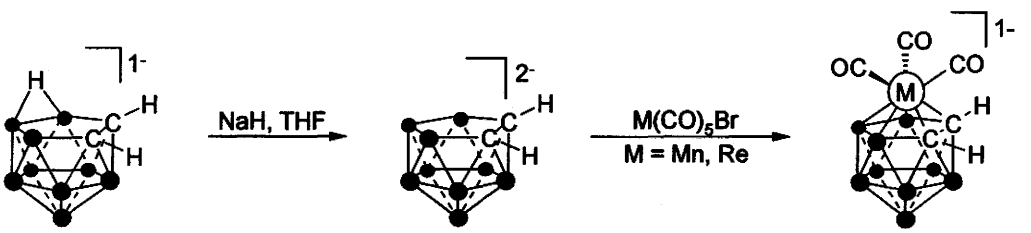
Scheme 1.5: Degradation of *closo-ortho*-carborane to the *nido* form.

The B3 and B6 boron atoms of the *closo-ortho*-carborane cage are more electrophilic than the other boron atoms, as they both have two carbon atoms as nearest neighbours. As a result, these two atoms are more susceptible to attack by nucleophilic bases. These atoms are removed from the cage with equal probability, which, in the case

of mono-carbon substituted, or hetero di-carbon substituted carboranes, results in the formation of racemic mixtures of *nido*-carboranes.[65,66,70]

Nido-ortho carboranes contain an "extra" hydrogen atom on the open face of the cluster. The exact location of this hydrogen atom in the *nido*-carborane cage remains a controversy. It has often been assumed to occupy a B-H-B bridging position on the open face of the cage. Some studies have indicated that this atom occupies an *endo* - position, localized on one of the boron atoms of the open face, as a BH_2 unit.[71] Others have suggested a fluxional environment or asymmetrically bridging position for this atom.[72-74]

The "extra" hydrogen atom of *nido-ortho*-carborane is weakly acidic (pK_a = 13.5)[75] and may be removed by base to give a doubly charged $[C_2B_9H_{11}]^{2-}$ species, commonly referred to as the "dicarbollide dianion".[51,66] The dicarbollide dianion has an open, approximately pentagonal face which may bind metal centres in a manner analogous to cyclopentadienide.[50,51] In fact, dicarbollide dianion is considered isolobal to Cp^-.[52] Using reagents of the type $[M(CO)_5Br]$ (M = Mn, Re), metal tricarbonyl-dicarbollyl complexes were among the first metallocarboranes ever reported (Scheme 1.6).[50] Since then, a wide variety of metallocarborane complexes have been prepared and characterized, involving numerous transition metals and main group elements.[51,53,54,76-80]



Scheme 1.6: Synthesis of π-dicarbollyl $M(CO)_3$ complexes.

1.6 Carboranes in Radiopharmaceutical Chemistry

One of the advantages of working with carborane ligands is that unlike un-derivatized Cp^-, they are amenable to forming metal complexes in water. The first example of the preparation of metallocarboranes in water was by Hawthorne and co-workers who prepared bis(dicarbollide) complexes of iron, cobalt and nickel using hot aqueous sodium hydroxide to effect removal of the *nido*-carborane "bridging" hydrogen atom.[51,53,76,81] Since this discovery, several groups have investigated the use of carboranes as carriers of radiometals.

Hawthorne and co-workers prepared a bis-dicarbollyl ligand for ^{57}Co (t = 271 d, E_γ = 122 keV), which is referred to as the "venus flytrap cluster" (VFC, Figure 1.7).[82,83] The ligand was prepared by reacting 4-carbomethoxypyrazole anion with the 11-vertex carborane *closo*-1,8-$C_2B_9H_{11}$ to form a B-N bridged bis(*nido*-7,9-$C_2B_9H_{11}$) species, containing the *nido-meta*-carboane anion.[84] Reaction with aqueous base both removed the carborane bridging hydrogens, and hydrolyzed the ester substituent on the pyrazole ring to a carboxylic acid, through which functionalization to a biomolecule could be accomplished. The labelling reaction was done using Co^{3+} to form the flytrap cluster.[82,83] The VFC-Co^{3+} complex exhibited high stability, characteristic of other *commo*-bis-dicarbollides (bis-dicarbollyl metallocarboranes sharing the metal ion as a common vertex) prepared at the macroscopic scale.[51]

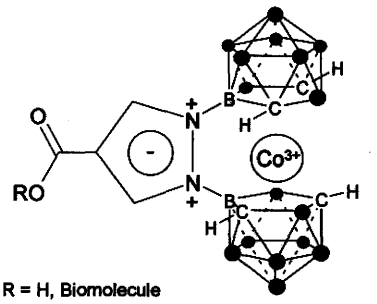
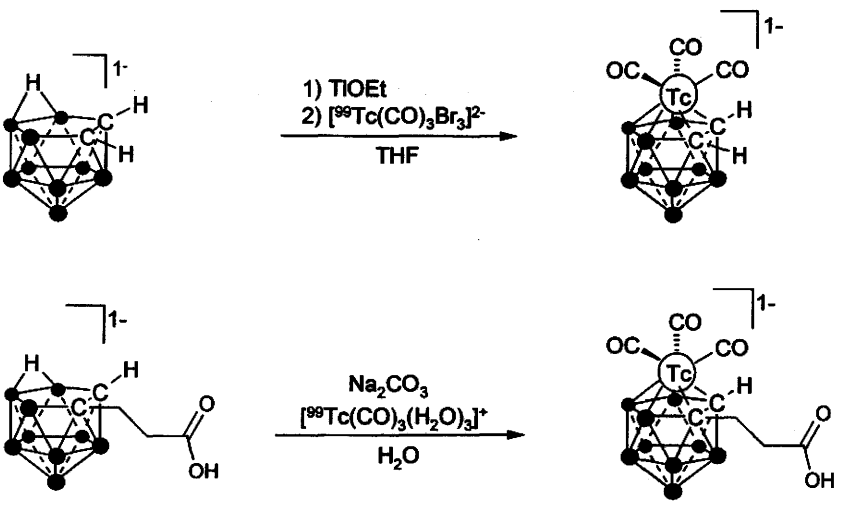


Figure 1.7: Cobalt(III)-bis-dicarbollyl "venus flytrap" cluster.

The venus flytrap cluster was subsequently conjugated to the anti-carcinoembryonic antigen (CEA) monocolonal antibody T84.66 and evaluated as a targeted radiopharmaceutical. The antibody was found to fully retain its immunological activity when bound to the VFC. The biodistribution of the T84.66-VFC conjugate was evaluated in mice, and showed both excellent tumour uptake and low accumulation in normal tissues. The VFC conjugate also compared favourably in terms of stability and biodistribution with a ^{111}In-DTPA conjugate of T84.[85,86] Unfortunately, ^{57}Co is of limited use in nuclear medicine due to its long half-life and less than ideal nuclear properties. It is therefore of interest to prepare carborane complexes of Tc, and in particular ^{99m}Tc, the isotope which, as mentioned previously, is in widespread use in nuclear medicine.

Our group has recently prepared and characterized the first metallocarborane complex of technetium.[87] The synthesis involved the use of the $Tc(I)(CO)_3^+$ core and has been found to be amenable to both organic and aqueous reaction media (Scheme 1.7). In the case of reactions in organic solvents, the syntheses were performed using TlOEt as the base to remove the bridging hydrogen atom. As this reagent is not suitable for an application in nuclear medicine, alternative bases were investigated. For reactions in

aqueous solution, Na_2CO_3 was used to successfully prepare the desired products. The Re(I) and Tc(I) complexes formed were evaluated for stability under biological conditions by way of cysteine and histidine challenge experiments. The metallocarboranes proved to be exceptionally stable, as no decomposition was detected under the reported conditions.[87]



Scheme 1.7: Synthesis of Tc-Metallocarboranes in organic (top) and aqueous (bottom) media.[87]

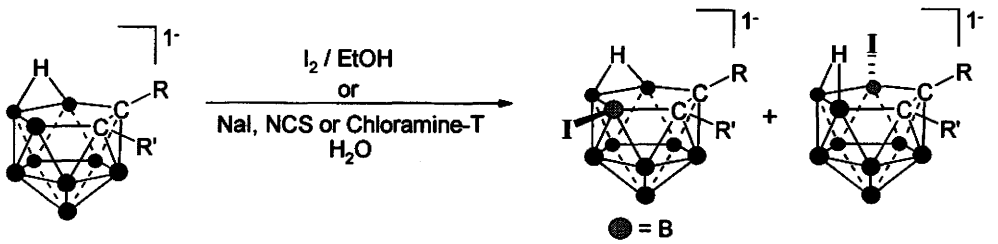
In addition to binding metals, the *nido*-carborane moiety can also be labeled with radioiodine. *Nido*-carboranes react with iodide in the presence of an oxidant to yield singly-iodinated *nido*-carboranes in which the halogen is bound to the carborane cage via a B-I bond (Scheme 1.8).[88,89] This is potentially useful for the synthesis of radiopharmaceuticals based on iodine isotopes, as radioiodinated *nido*-carborane derivatives are prepared in a manner analogous to that for radiolabelled aryl iodides; that is, electrophilic iodination, using similar reaction conditions (i.e., *in situ* oxidation of I^-).[90]

Iodinated *nido*-carborane derivatives are considered advantageous in that the B-I bond formed is expected to be stronger, and thus, less susceptible to *in vivo* de-iodination than aryl C-I bonds.[91,92] Additionally, the iodination of *nido*-carboranes has been reported to be much more rapid than that of tyrosine.[93-95] The use of a carborane prosthetic group is therefore seen as a potential advantage for the direct radiohalogen labeling of peptide or protein-based radiopharmaceuticals, in both the ease of incorporation of the label and the subsequent stability of the complex.

Hawthorne and co-workers have prepared carborane-phenylisothiocyanate,[89,96,97] amino acid,[98] and phosphate diester[99,100] derivatives labelled with ^{125}I and have conjugated these to tumor-localizing antibodies.[89,100] Wilbur and co-workers have prepared a radioiodinated *nido*-carborane derivative of 2-nitroimidazole as a tracer of hypoxic tumor tissues.[93,101] Furthermore, they have prepared radioiodinated *nido*-carborane derivatives as prosthetic groups for direct protein labeling,[95] derivatives bearing a biotin moiety for selective binding to antibody-streptavidin conjugates in a tumor "pre-targeting" methodology,[102] as well as *nido*-carborane derivatives for radiolabelling with not only radioactive isotopes of iodine, but also a congener, astatine-211, which is of interest for radionuclide therapy due to its α-particle emission.[94,103] Tolmachev and co-workers have also used *nido*-carborane derivatives as pendant groups for direct labelling of proteins (e.g. epidermal growth factor) with radioiodine isotopes and astatine-211.[92,104]

With the ability to bind not only metals and radiometals, but also radiohalogens, carboranes are intriguing and potentially valuable ligands for radiopharmaceutical preparations. Firstly, carboranes can be incorporated into a variety of organic molecules

through its versatile carbon and boron atom substitution chemistry. Secondly, these products can be labelled with with both ^{99m}Tc or radiohalogens. This means that a single carborane derivative can be labelled in such a manner to match the isotope with the desired application.



Scheme 1.8: Formation of iodinated *nido*-carboranes.

1.7 Carbohydrate - Based Radiopharmaceuticals and FDG

With the ability to prepare Tc and Re metallocarboranes in hand, the next step was to develop new synthetic methods for preparing metallocarborane - biomolecule conjugates for targeting specific receptors and/or diseases. Due to their key role in many biological processes, carbohydrates are attractive targeting moieties for radiopharmaceuticals and for pharmaceuticals in general, (e.g. targeting of lectins,[105,106] glucose transporters,[107] or hexokinase[108,109]). A clinically-used radiopharmaceutical that takes advantage of the importance of carbohydrates in diseases like cancer[110] is [^{18}F]-2-fluoro-2-deoxy-glucose, commonly referred to as FDG (Figure 1.8). FDG is used clinically as a tracer for glucose metabolism associated with various conditions, including

cancer. Imaging of the tumour uptake of FDG is accomplished using Positron Emission Tomography (PET), and is the largest single application of PET.[111-116]

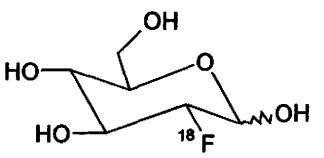


Figure 1.8: Structure of [^{18}F]-FDG.

Carbohydrates can also be used to influence the pharmacokinetics of a bioactive species. The bioactivity and hydrophilicity of carbohydrates can be exploited in order to give more favourable *in vivo* properties to drugs or radiotracers. For example, numerous glycopeptide enkephalin analogues have been investigated as analgesics. Several of these have been shown to be more potent than morphine. This is believed to be a result of increased bioavalability due to their greater hydrophilicity and ability to penetrate the blood-brain-barrier via the glucose transporter GLUT-1.[117-122] Also, the effects of glycosylation on the bioactivity of various other clinically relevant species, such as AZT (anti-HIV/AIDS),[123,124] geldanamycin (anti-cancer),[125] vasopressin (anti-diuretic)[126] and renin inhibitors (hypertension)[127] have been investigated. The conjugation of the carbohydrate in these examples has, in general increased the bioavailability of these agents, presumably due to an increase in hydrophilicity from the sugar. In radiopharmaceutical research, Wester and co-workers have prepared and radiolabelled several series of peptide-carbohydrate conjugates for the imaging of tumour angiogenisis or somatostatin receptors overexpressed by cancer cells. They found that glycosylation of

these peptides resulted in increased hydrophilicity, resulting in reduced liver uptake, increased renal excretion and increased tumour uptake, versus the non-glycosylated analogues.[128-135] Since carboranes are generally quite lipophilic, the preparation of radiolabelled carborane-carbohydrate conjugates is expected to offset this limitation.[106,136,137]

The synthesis of carborane-carbohydrate conjugates is practical, as many examples of *closo*-carboranyl-carbohydrate derivatives have been prepared during research for boron neutron capture therapy (BNCT),[60,106,136-146] a method under investigation for cancer therapy. Therefore, the synthesis of *nido*- and subsequently metallocarborane-carbohydrate conjugates should also be feasible.

1.8 Objectives

The objective of this thesis was to develop an efficient synthesis of carborane and metallocarborane derivatives of glucose and other simple sugars. The initial focus was to prepare the *nido*-carborane ligands derived at the C-1 position of glucose and galactose, and then investigate their coordination chemistry with rhenium. These compounds would not be expected to possess biological activity. These commpounds also serve as excellent model systems for studying the impact of the sugar on lipophilicity and reactivity of carboranes towards complex formation with the $M(CO)_3^+$ core. The synthesis of an advanced C-1 analogue is described in Chapter 4, and that of a C-2 (i.e. FDG-like) functionalized carborane-glucose derivative is described in Chapter 5.

1.9 References

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Chapter 2

C-1 Glucose and Galactose Carborane and Metallocarborane Derivatives

The initial targets for Re metallocarborane - carbohydrate conjugates were the glycosides **2.1**, **2.2**, and **2.3** (Figure 2.1). The position of functionalization on the carbohydrate ring for these compounds was the anomeric (C-1) carbon. This site was selected because it is synthetically the most feasible in that the carbohydrate-carborane linkage can be achieved through the formation of a simple glycosidic bond.[1,2] These compounds serve as test substrates for exploring and optimizing the complexation reactions with Re and ^{99m}Tc, the results of which can then be applied to the synthesis of other, more advanced carbohydrate derivatives, and for evaluating the impact of the carbohydrate on the lipophilicity of the metallocarborane core. An application of this idea has been reported recently in which D-glucose and D-galactose have been used to enhance the water solubility of potential ^{18}F-labelled radiopharmaceuticals based on the RGD (arginine-glycine-aspartic acid) peptide sequence.[3,4]

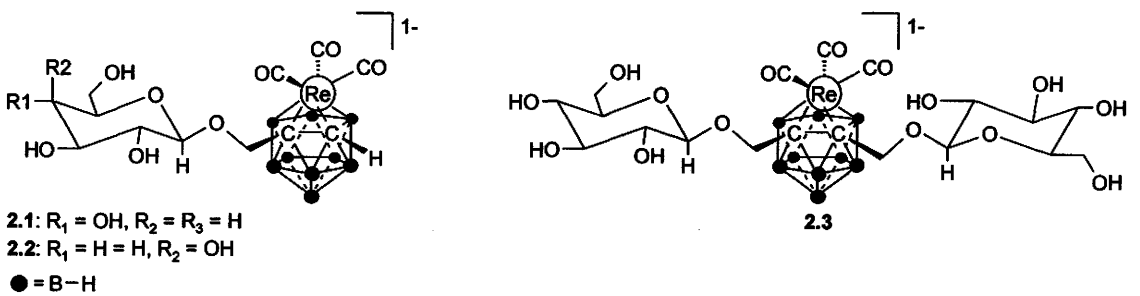


Figure 2.1: Initial synthetic targets based on glucose and galactose.

2.1 Synthesis of a Re(CO)$_3$-metallocarboranyl glycoside of D-Glucose

The synthesis of **2.1** began by following published methods for the preparation of precursors **2.4** and **2.5**. Carbohydrates functionalized with a *closo*-carborane at the C-1 position were reported by Panza *et al.*[5] as delivery vehicles for boron neutron capture therapy (BNCT), which is an experimental binary cancer treatment strategy. The corresponding *nido*-carborane and metallocarborane derivatives have not been reported. The retrosynthesis illustrates how the synthesis of **2.1** can be approached through precursors **2.4**, **2.5**, and **2.6** (Figure. 2.2). The Re complex was to be prepared from the *nido*-carborane **2.6**, which can in turn prepared by the deboronation of the *closo*-carboranyl glycoside **2.5**. This *closo*-carborane derivative could be synthesized via decaborane insertion with alkyne **2.4**, which could be prepared by glycosylation of β-D-glucose pentaacetate with propargyl alcohol.

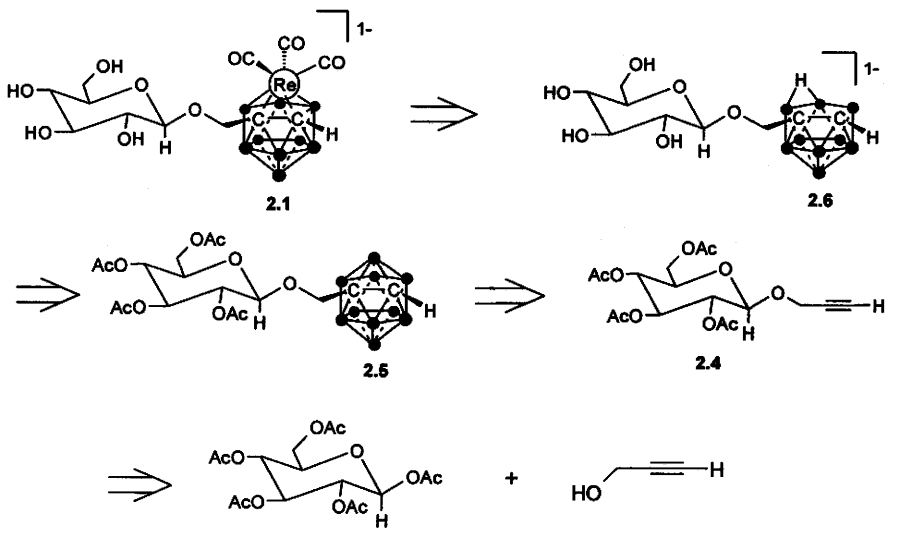
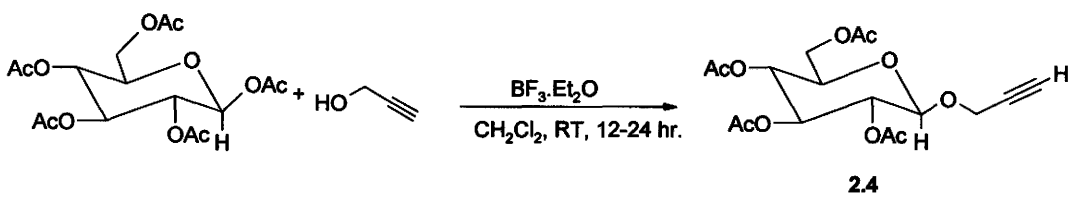


Figure 2.2: Retrosynthetic approach to target **2.1**.

The first product in the synthesis of **2.1** was 2-propynyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside **2.4**. This compound was prepared using a modification of the method reported by Mereyala *et al.* (Scheme 2.1).[6] The synthesis involved reacting β-D-glucose pentaacetate and propargyl alcohol in the presence of boron trifluoride diethyl etherate. The desired product was obtained in 71% yield after column chromatography. The modified version of the synthesis involved the use of a full two mole equivalents of propargyl alcohol versus 1.2 equivalents in the literature method. In addition, the reaction was allowed to proceed for a period of 12 to 24 hours instead of two hours as was reported.[6] This modification was deemed necessary after initial attempts to prepare **2.4** failed to consume the starting carbohydrate material, β-D-glucose pentaacetate. Attempts to purify these initial products by silica gel column chromatography were not successful in separating product from reactant, since the two compounds were virtually indistinguishable in terms of their retention on silica gel. Thus, by increasing the proportion of propargyl alcohol in the mixture, and allowing the reaction to proceed for a longer period of time, the carbohydrate starting material could be completely consumed. The infrared, ^{1}H NMR and $^{13}C\{^{1}H\}$ NMR spectra of the product were in agreement with reported data.[6]

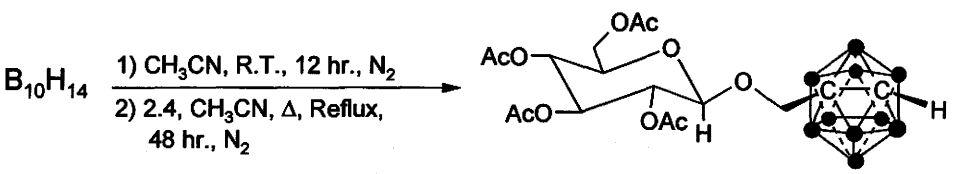


2.4

Scheme 2.1: Synthesis of 2-propynyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (**2.4**).

Compound **2.5** was prepared according to Scheme 2.2. The formation of the *o*-carborane unit occurred via insertion of the triple bond of **2.4** into the boron cage, in a similar fashion to that described by Panza *et al.*[5] Decaborane(14), $B_{10}H_{14}$, was stirred in dry acetonitrile to form the Lewis acid-base adduct $B_{10}H_{12}(CH_3CN)_2$ to which the alkyne **2.4** was added.[7-12] The resulting solution was stirred under reflux for 48 hours. Compound **2.5** was purified by silica gel column chromatography, and recrystallization from ethyl acetate and hexanes. The product, a white solid, was isolated in 63% yield.

Infrared spectroscopy (Fig. 2.3) clearly showed a B-H stretch at 2588 cm^{-1}, in the range characteristic of *closo-ortho*-carboranes.[13] Also appearing in the IR spectra was the C=O stretch of the acetate protecting groups at 1757 cm^{-1}. Electrospray ionization mass spectrometry (ESMS) showed the target mass ($[M+NH_4]^+$; m/z = 523) with a ^{10}B / ^{11}B isotopic distribution pattern consistent with that for a carborane cage containing ten boron atoms.[14] The ^1H and $^{13}C\{^1H\}$ NMR spectra were consistent with those previously reported for compound **2.5**.[5]



2.5

Scheme 2.2: Synthesis of compound **2.5**.

The $^{11}B\{^{1}H\}$ NMR spectrum (Figure 2.4) of **2.5** showed the expected pattern for a monosubstituted *closo-ortho*-carborane cage.[5,15,16] Two signals, nearly overlapping at -2.25 and -3.94 ppm, corresponded to two boron atoms. A further single peak at -8.48 ppm, arose from another two boron atoms. Finally, a large, overlapping signal centered around -12.58 ppm, corresponded to six boron atoms.

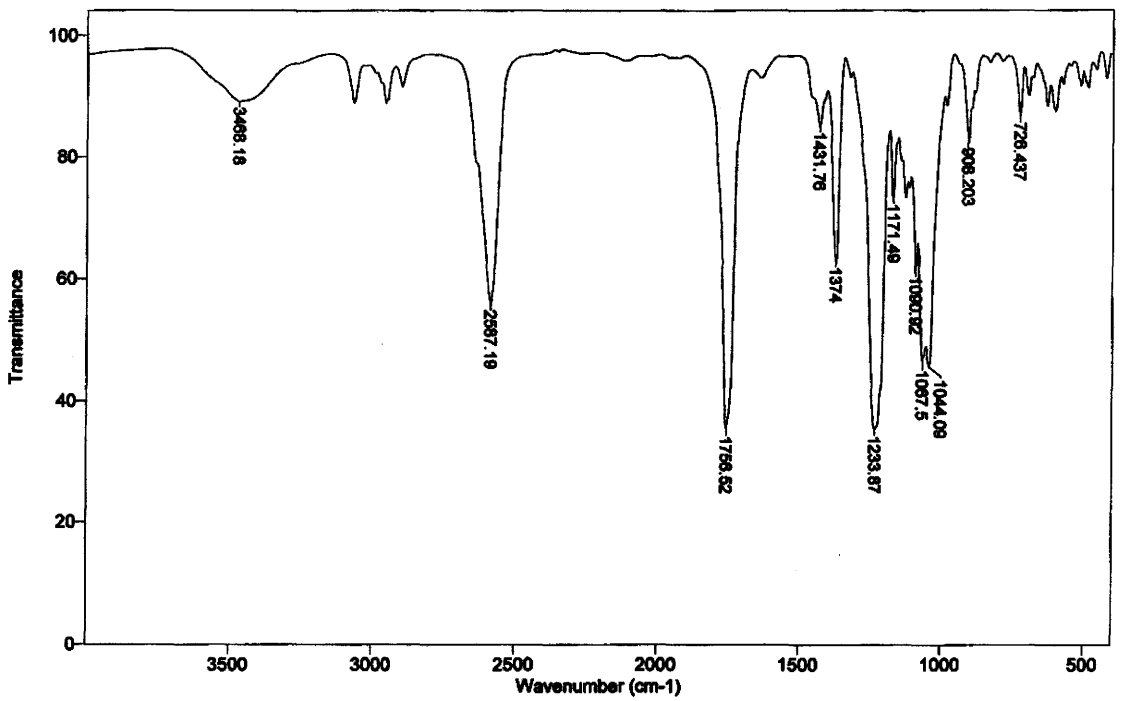


Figure 2.3: FT-IR spectrum of **2.5** (KBr Pellet).

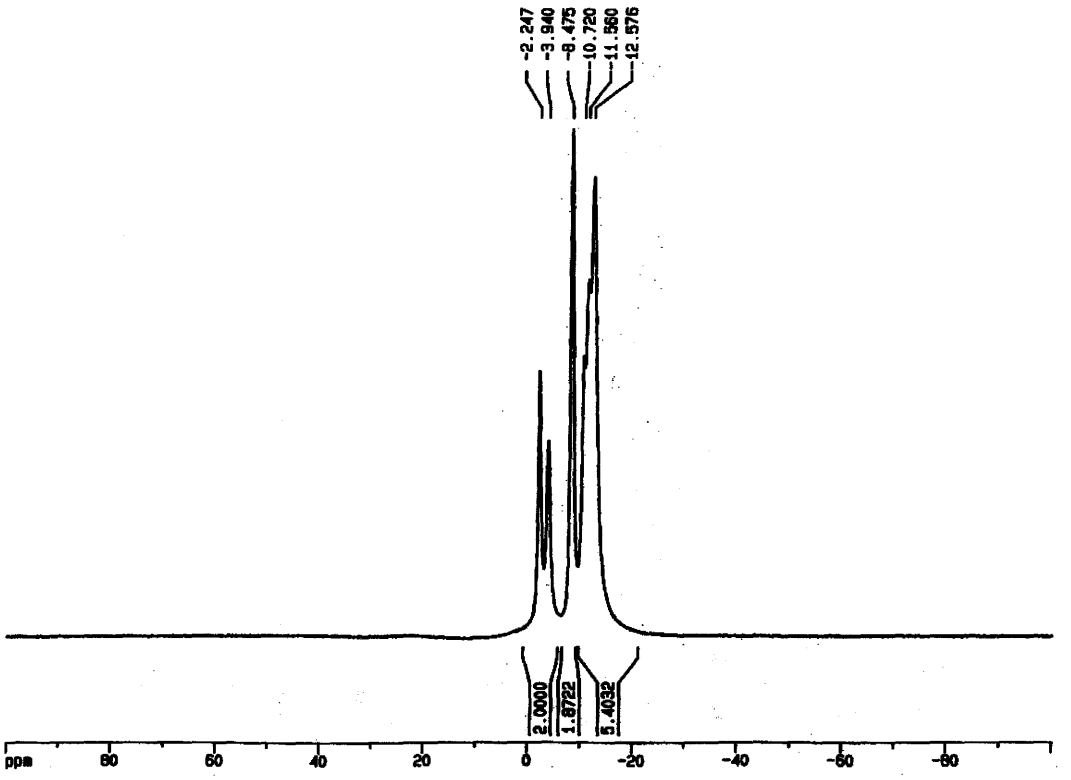


Figure 2.4: $^{11}B\{^1H\}$ NMR spectrum of **2.5** (160 MHz, $CDCl_3$).

Degradation of the *closo*-carborane cage in compound **2.5** to the *nido* form was initially attempted using the method of Hawthorne *et al.*[17,18] Compound **2.5** was treated with KOH in ethanol, and the mixture was heated to reflux for 12 hours. This method was also expected to result in the simultaneous deprotection of the acetate esters on C-2, 3, 4 and 6.[5] The intended target was compound **2.6a**, a more water-soluble precursor to metallocarboranyl glycoside **2.1**, which has not been previously reported and characterized. The reaction proceeded as illustrated in Scheme 2.3 (steps 1 and 2), where an excess of KOH (20 equivalents) was used for the conversion. Upon completion of the reaction, residual KOH was precipitated from the ethanolic solution by conversion to

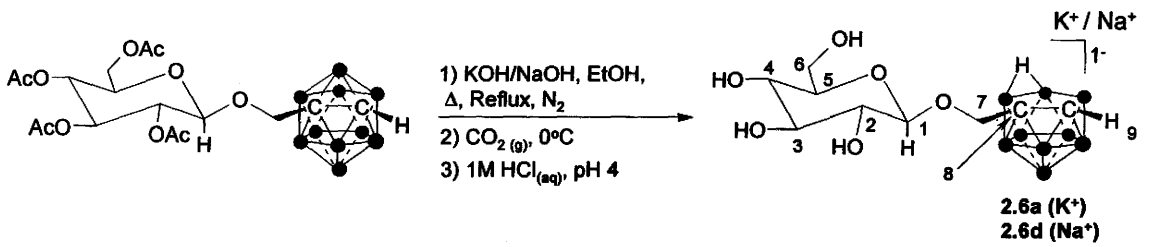
K_2CO_3 by passing $CO_{2\,(g)}$ through the solution, which was cooled to 0 °C. The resulting precipitate was removed by filtration on a pad of Celite.

FT-IR spectra of the crude reaction product showed a B-H stretch at approximately 2525 cm^{-1}, which is characteristic of *nido-ortho*-carboranes.[13,18] An intense absorption in the carbonyl stretching region of the IR spectrum was also observed, which could suggest the presence of partially deprotected products. This possibility was discounted by electrospray mass spectrometry, which clearly indicated the target mass (m/z = 326, B_9 isotopic distribution), and no signals corresponding to the target bearing extra acetate groups. The carbonyl absorption was thus attributed to potassium acetate and / or ethyl acetate, which are by-products of the deacetylation reactions.

To eliminate these unwanted products, the crude product was dissolved in water, and the resulting basic solution (pH >11) was adjusted to pH = 4 with 1M HCl. This was expected to result in conversion of potassium acetate to acetic acid and potassium chloride. The *nido*-carboranyl glucose derivative could then be extracted into methanol, acetone or tetrahydrofuran; solvents in which the solid potassium chloride has poor solubility. Further purification of the product was effected by silica gel column chromatography, where the desired product, **2.6a**, was obtained as a glassy solid in 83% yield.

In addition to compound **2.6a**, the potassium salt of the *nido*-carboranyl glycoside derivative of β-D-glucose, three other variants of this compound were produced. Compound **2.6b**, in which the acetate protecting groups remained intact, was prepared as

an organic-soluble *nido*-carboranyl glycoside, and was intended for reactions to produce **2.1** under anhydrous conditions. Compound **2.6c**, the tetraethylammonium salt, was prepared from **2.6a** by cation exchange. Finally, the sodium salt, **2.6d** was prepared in a manner analogous to that for **2.6a**, except that NaOH, rather than KOH was used to cleave the sugar protecting groups and degrade the carborane cage. Following silica gel column chromatography, compound **2.6d** was obtained in 63% yield. Since **2.6d** was used as the major synthetic precursor in the work described in Chapter 3, details of its characterization will be described here.



Scheme 2.3: Conversion of tetraacetyl-*closo*-carboranyl glucose **2.5** to deprotected *nido*-carboranyl glycosides **2.6a/d**. The numbering system used for assigning NMR spectra is also indicated.

The infrared spectrum of compound **2.6d** (Figure 2.5) showed the absorptions expected for the deprotected *nido*-carboranyl glucose derivative. A broad peak centered at 3409 cm^{-1} was observed due to the O-H stretching of the glucose hydroxyl groups. The B-H stretch characteristic of *nido*-carboranes appeared at 2526 cm^{-1}.[13,18] The electrospray mass spectrum (Figure 2.6) indicated the target anion mass of m/z = 326.4.

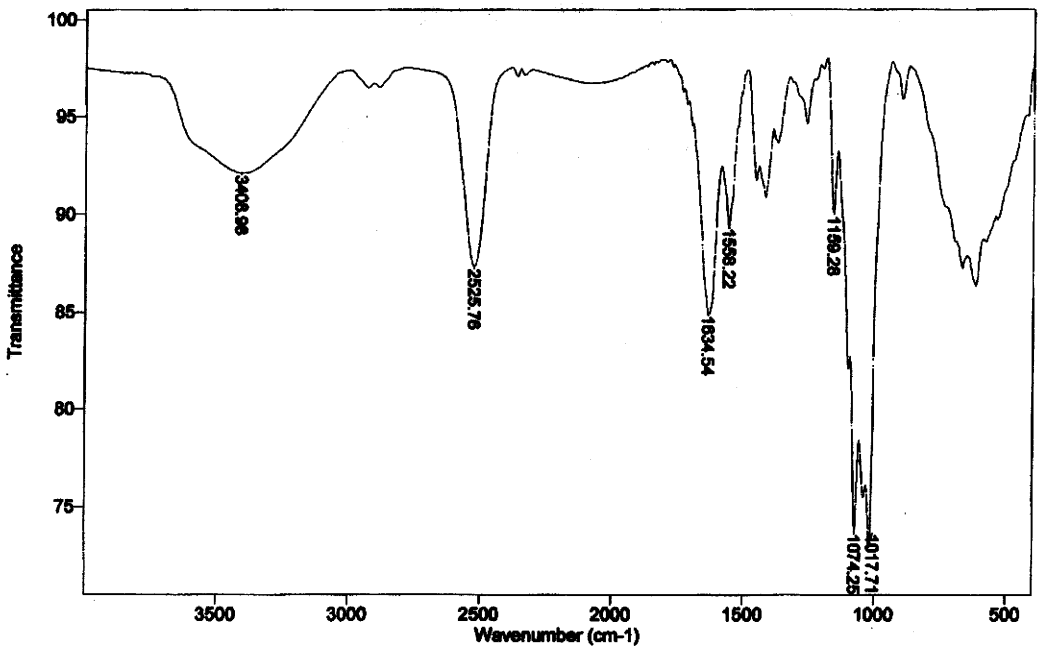


Figure 2.5: FT-IR spectrum of **2.6d** (KBr Pellet).

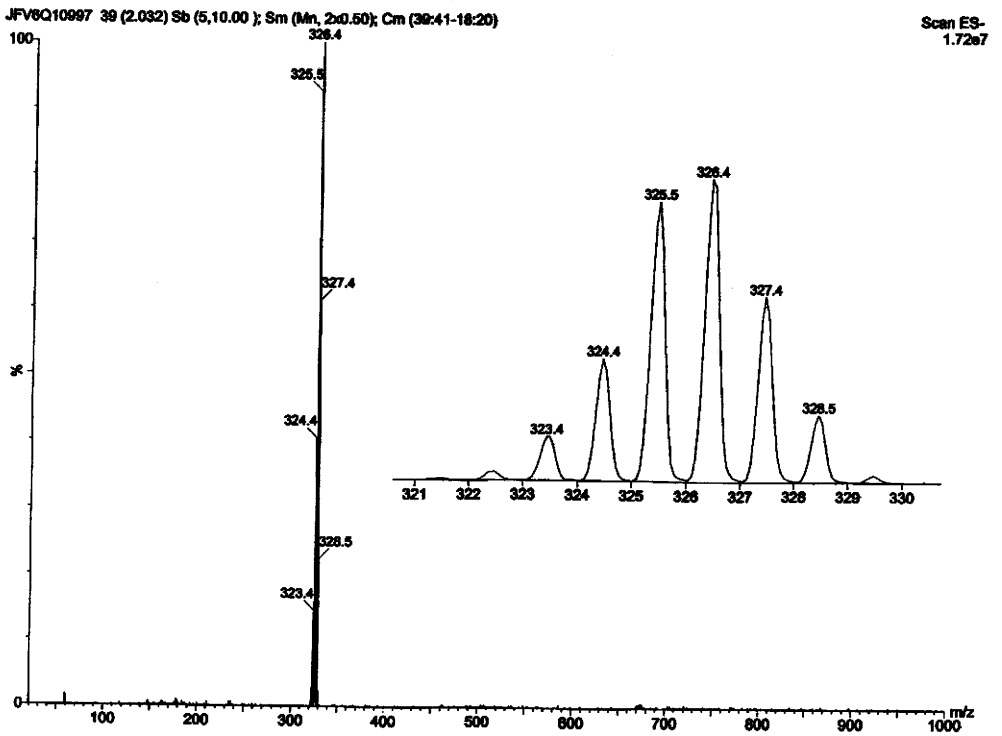


Figure 2.6: Negative ion electrospray mass spectra of **2.6d**.

During the cage degradation reaction, either one of the B3 or B6 atoms of the C_2B_{10} cage can be removed with equal probability.[18] Degradation of a mono-substituted *closo-ortho*-carborane, results in the formation of two enantiomeric *nido*-carborane anions. These would normally be indistinguishable from each other by spectroscopic means. However, since β-D-glucose contains stereogenic centres in close proximity to the carborane cage (Figure 2.7), pairs of signals in the ^1H, $^{13}C\{^1H\}$ and $^{11}B\{^1H\}$ NMR spectra associated with the diastereomeric products were observed.[19]

The ^1H and $^{13}C\{^1H\}$ NMR spectra of all variants of compound **2.6** proved to be more complex than the two precursors as a result of the formation of two diastereomeric products during the degradation / deprotection reaction. Many of the carbohydrate signals were overlapping, with other signals from **2.6d**, and also with the solvent signal(s) (CD_3OD). In order to correctly assign signals, ^1H-^1H COSY, ^1H-^{13}C HSQC and ^1H-^{13}C HMBC spectra were acquired. In some cases with this compound (and with later *nido*-carboranyl glycosides - *vide infra*), overlapping proton NMR signals made definite assignment of ^{13}C signals difficult. It was noted that the sequence of carbohydrate chemical shifts was similar to that reported for glycoside derivatives of β-D-glucose, as indicated in a compilation of carbohydrate NMR data by Bock and Thøgersen.[20] Therefore, assignments of the $^{13}C\{^1H\}$ NMR spectra could be made by reference to this data in cases where there was ambiguity in the two-dimensional ^1H-^{13}C NMR spectra of the actual compounds.

A general observation regarding the ^1H NMR spectrum of **2.6d** (Figure 2.8) was that all of the signals appeared at lower frequencies than the corresponding signals in **2.4**

or **2.5**. This was attributed to the loss of the electron - withdrawing ester protecting groups, as well as the generation of the *nido*-carborane cage, which is less electron withdrawing than the electron-deficient *closo* cluster. The spectrum illustrated the diastereomeric nature of the product (*vide infra*) by the presence of two distinct anomeric doublets ($^3J_{1,2} = {}^3J_{1^*,2^*} = 7.8$ Hz) at 4.46 and 4.36 ppm. Two pairs of doublets (3.97 and 3.60 ppm, $^2J_{7a,7b} = -11.0$ Hz) and (3,82 and 3.72 ppm, $^2J_{7a^*,7b^*} = -10.7$ Hz), arose from the methylene protons of the C-1 substituent group. The doublets corresponding to H-7a and H-7a* were overlapping with the signals arising from the H-6a/6a* and H-6b/6b* protons, respectively. Protons H-3/3* and H-4/4* appeared as an overlapping signal at 3.46 ppm. Protons H-5/5* and H-2/2* each gave complex signals at 3.36 and 3.01 ppm, respectively. The broad singlet corresponding to the carborane cage terminal C-H was observed at 2.07 ppm. The broad peak between 0.30 and 2.50 ppm is attributed to the carborane B-H protons. The broad signal observed at -2.63 ppm, arising from the "extra" hydrogen atom, is due to the fluxional briding hydrogen atom located between the three boron atoms of the open C_2B_3 face of the *nido-o*-carborane cage.[21-23]

The $^{13}C\{^1H\}$ NMR spectrum of **2.6d** (Figure 2.9) also indicated a mixture of diastereomers. Two anomeric carbon signals were observed at 103.26 and 102.85, as were pairs of signals corresponding to the C-3 (77.99 and 77.89 ppm) and C-5 (77.74 and 77.62) carbons of the diastereomeric pair. The C-7, C-2, C-4 and C-6 carbon atoms of the two diastereomeric forms of **2.6d** each gave single signals at 78.35, 75.05, 71.51 and 62.48 ppm, respectively. The C-8 and C-9 carbon atoms belonging to the carborane cage were more difficult to observe by $^{13}C\{^1H\}$ NMR. However, broadened signals with

reduced intensity were observed in the baseline of the spectrum at 45.85 and 47.80 ppm, which from the HSQC spectra were assigned the cage terminal (C-9/9*) carbon atoms. A further pair of weak, broadened signals were observed at 59.02 and 43.75 ppm, and were assigned the substituted cage carbon atoms (C-8/8*). The exact origin of these weak signals is unclear; whether it was a result of quadrupolar effects from the boron nuclei, relaxation effects, or the fluxional nature of "extra" hydrogen atom on the open face of the *nido*-carborane cage.[15,19,22]

A significant change was observed in the $^{11}B\{^{1}H\}$ NMR spectrum of **2.6d** (Figure 2.10) versus that of **2.5**. With the removal of one boron atom from the mono-substituted carborane cage, each boron atom becomes chemically and magnetically unique, resulting in the more complicated spectrum (nine distinct signals are expected, in the case of a mono-substituted *ortho*-carborane derivative,[18] some of which were overlapping in the case of compounds **2.6**). The signals of the spectrum were shifted to lower frequency relative to the *closo* compound due to the distribution of the negative charge over the cage.[18] Two signals in particular - those appearing at -32.92 and -37.49 ppm - were taken as diagnostic of the *nido*-carborane cluster.[15] The observed pattern of the signals was in reasonable agreement with that reported for mono-substituted *nido*-carborane anions, particularly those bearing chiral pendant groups.[15,19,24]

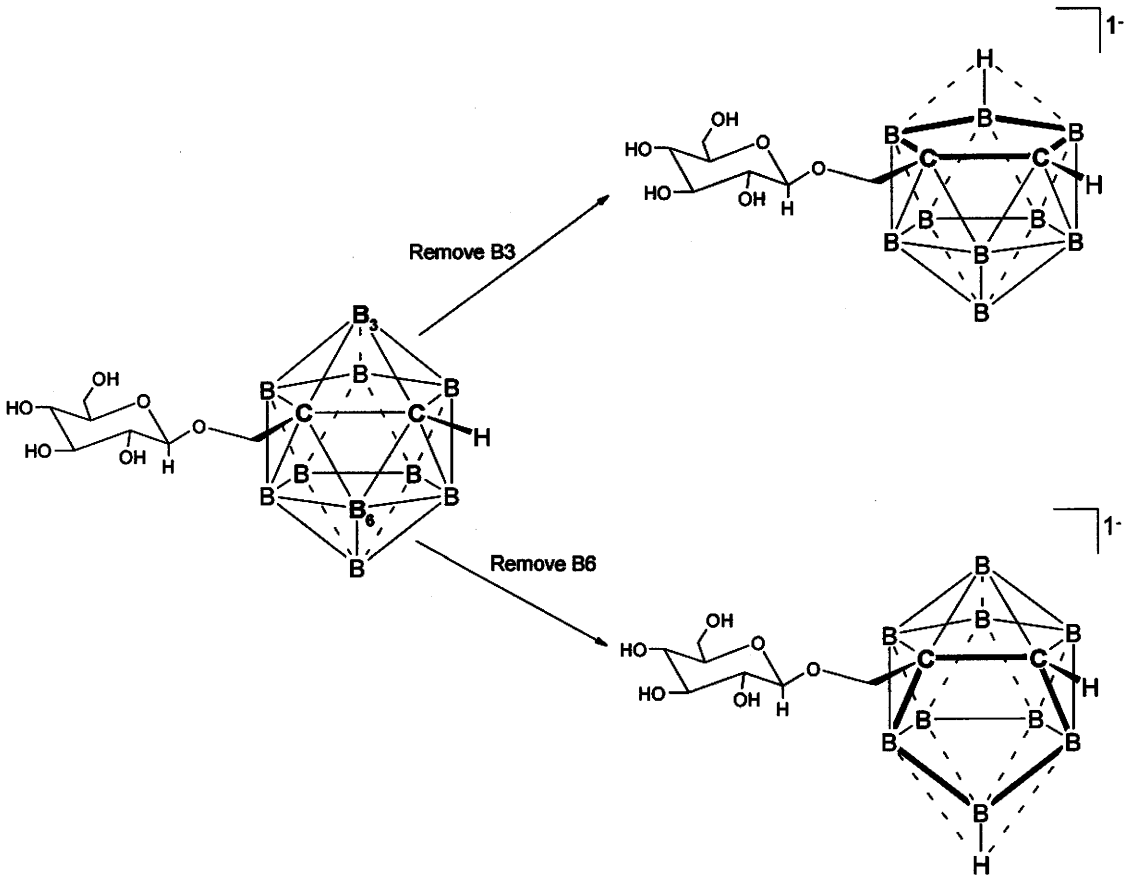


Figure 2.7: Diagram showing the *closo-* to *nido-* conversion in the case of *o*-carboranes substituted by a pendant group bearing chiral centres (β-D-glucose). Terminal B-H bonds have been omitted for clarity [15]

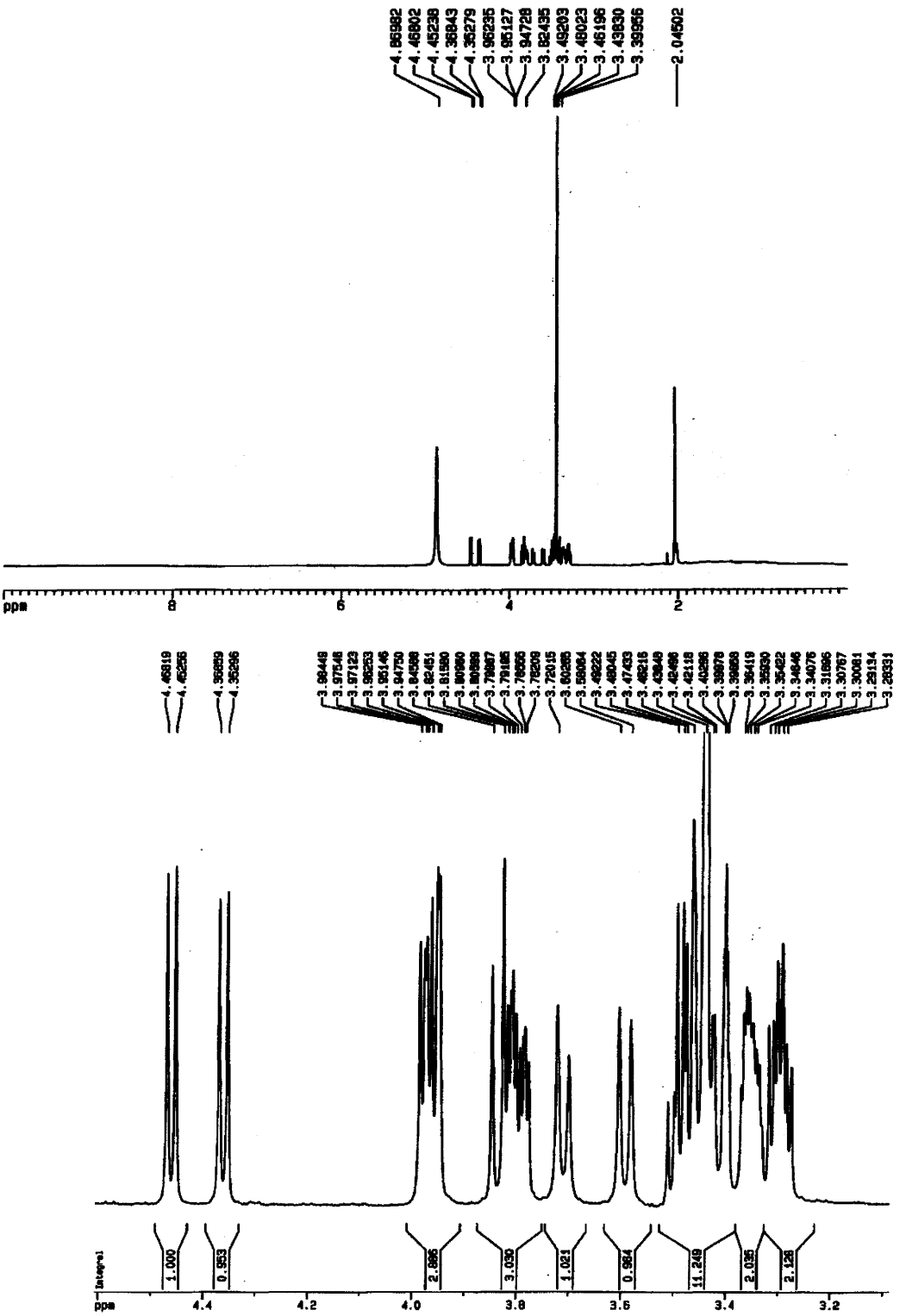


Figure 2.8: The ^{1}H NMR spectrum and an expansion of the region 3.1 to 4.6 ppm of **2.6d** (500 MHz, CD_3OD).

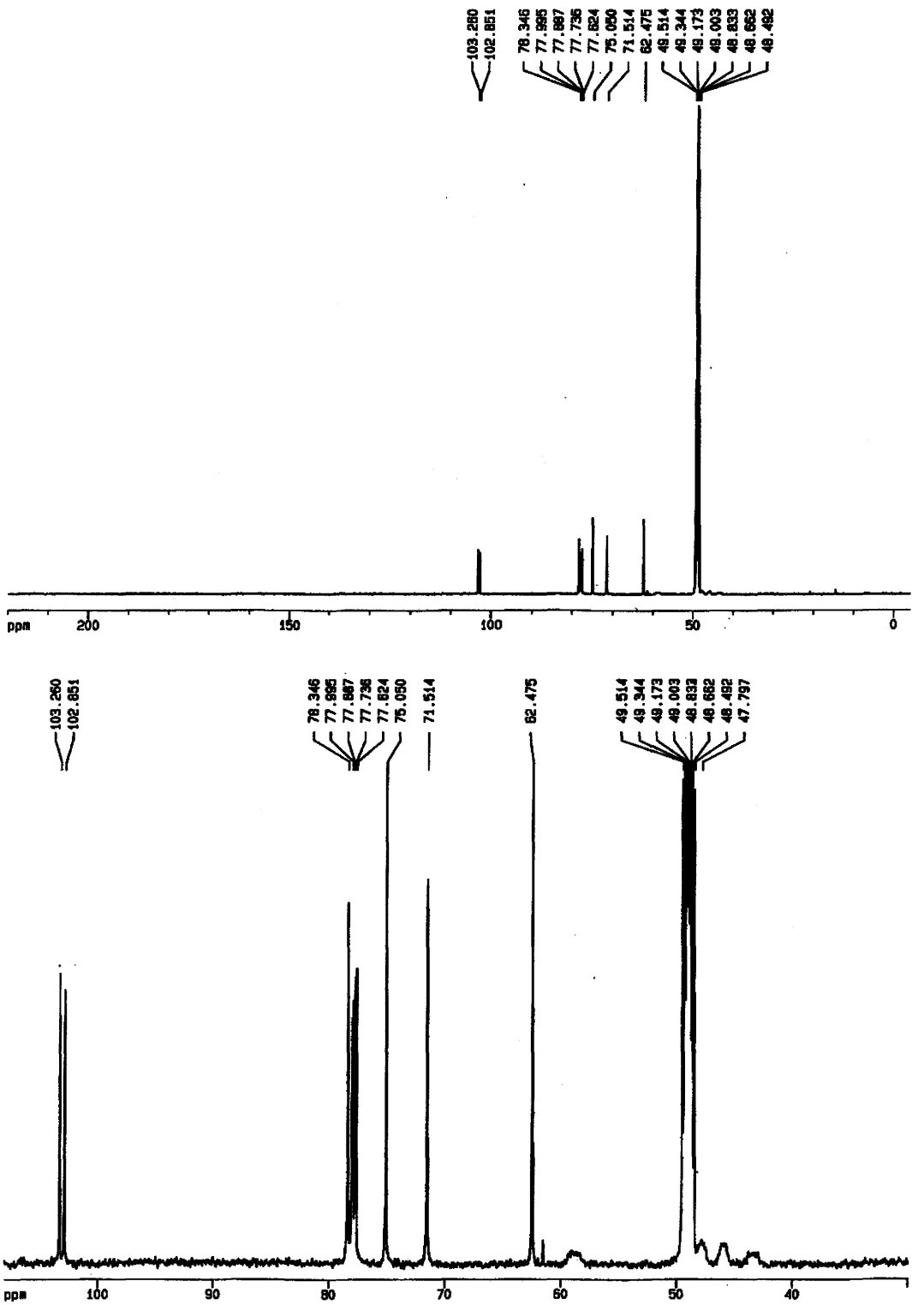


Figure 2.9: The $^{13}C\{^{1}H\}$ NMR spectrum and associated expaantion of the region between 30 and 110 ppm of **2.6d** (125 MHz, CD_3OD).

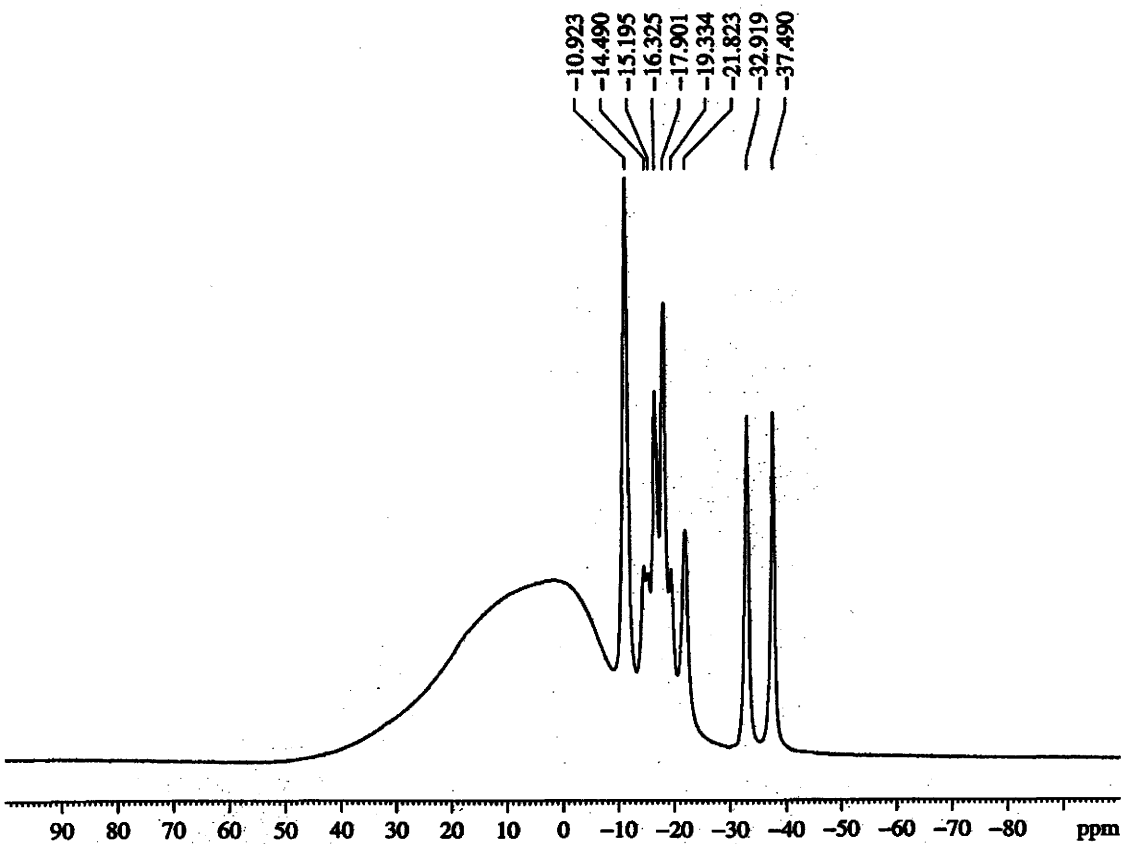


Figure 2.10: The $^{11}B\{^{1}H\}$ NMR spectrum of **2.6d** (192 MHz, CD_3OD).

Another synthetic challenge was to prepare a *nido*-carboranyl analogue of the tetra-acetyl protected glucose, which would afford the opportunity to carry out metal complexation reactions without having to be concerned about competition from the free hydroxyl groups of the glucose moiety. This target was the tetra-acetate-protected nido-carboranyl glycoside **2.6b**. In order to prepare the desired target, it was necessary to use a cage degradation protocol that would not result in the removal of the acetate protecting groups. Two such strategies were investigated; the use of piperidine[25] and fluoride

ion.[26,27] Reactions in which the degradation was attempted using piperidine were unsuccessful in preparing **2.6b**, thus the reaction was attempted using cesium fluoride.[26] The electrospray mass spectrum of the crude product showed a distribution of species corresponding to the *nido*-carboranyl glycoside, bearing zero, one, two, three, or four acetate protecting groups. The use of CsF was thus abandoned, and the use of tetrabutylammonium fluoride hydrate[27] was used successfully to prepared compound **2.6b**. Compound **2.5** and TBAF·xH$_2$O were combined and dissolved in tetrahydrofuran (Scheme 2.4), and stirred open to the air at room temperature for approximately twelve hours. At this time, TLC indicated that all of the starting material had been consumed. The desired product was obtained in 37% yield following silica gel column chromatography.

The IR spectrum of the product featured the expected *nido*-carborane B-H stretch at 2526 cm^{-1}, as well as the expected carbonyl absorption at 1756 cm^{-1} from the acetate protecting groups. The negative ion electrospray mass spectrum indicated the mass of the target anion, m/z = 494, with the B_9 isotopic distribution, while the positive ion spectrum showed the tetrabutylammonium cation mass m/z = 242. The ^{1}H and ^{13}C{^{1}H} NMR spectra were complicated by the presence of diastereomeric products of the cage degradation reaction. The ^{1}H spectrum of **2.6b** bore some resemblance to that of **2.5**, in that the signals corresponding to protons 2, 3, and 4 appeared grouped together at higher frequency than that for H-1. The broad signal corresponding to the carborane cage terminal C-H (H-9) appeared at 1.96 ppm, and the broad signal attributed to the bridging hydrogen of the open face of the carborane cage was observed at -2.65 ppm.

Scheme 2.4 Synthesis of compound **2.6b**.

Compound **2.6c**, tetraethylammonium 7-methyl-(β-D-glucopyranosyloxy)-7,8-dicarba-*nido*-undecaborate(-1) was also prepared (Figure 2.11). In the synthesis of **2.1**, the use of compound **2.6c** would serve to maintain a single cation throughout the remainder of the synthesis of **2.1**, since a mixture of such counter ions lead to difficulty in isolating a single final product (*vide infra*). *Nido*-carboranyl glycoside **2.6c** was isolated when **2.6a**, the potassium salt, was dissolved in water, tetraethylammonium bromide added, and the mixture cooled to 0 °C. This resulted in the formation of a white precipitate, which upon analysis was found to consist of the desired product. The infrared spectrum of **2.6c** indicated the expected O-H stretch at 3417 cm^{-1}, and the *nido* B-H stretch at 2526 cm^{-1}. The electrospray mass spectrum differed only in the presence of the tetraethylammonium ion (m/z = 130) in the positive ion spectrum.

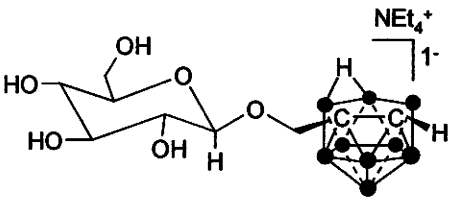
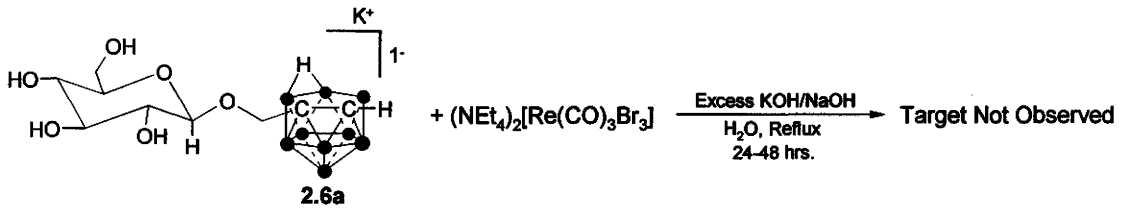


Figure 2.11: Structure of compound **2.6c**.

The ^{1}H and $^{11}B\{^{1}H\}$ NMR spectra of **2.6c** were virtually identical to those for **2.6a/d**, except for those signals in the proton spectrum arising from the tetraethylammonium cation, a quartet at 3.46 ppm, and triplet at 1.39 ppm. The $^{13}C\{^{1}H\}$ NMR spectrum of **2.6c** was also very similar to that for **2.6a/d**. The signals corresponding to the C-7 carbon in **2.6c** appeared as a pair of signals at 77.96 and 77.85 ppm, whereas in **2.6a** and **2.6d**, they were overlapping in a single peak. The tetraethylammonium signals appeared at 53.12 (methylene) and 7.76 (methyl) ppm.

Previous research in our laboratory established the synthesis of $M(CO)_3$-metallocarborane derivatives (M = Re, ^{99}Tc) in both aqueous and organic solutions.[28] Using a similar approach, the preparation of **2.1**, was likewise attempted in both aqueous and organic (THF) media. For the initial attempts at aqueous synthesis of **2.1** (Scheme 2.5), compound **2.6a**, the potassium (or sodium) salt of the *nido-ortho*-carboranyl glucose derivative was reacted with an excess of aqueous potassium (or sodium) hydroxide. This was expected to generate *in situ* the doubly charged "dicarbollide dianion".[28,29] To this solution was then added the reagent $[NEt_4]_2[Re(CO)_3Br_3]$, which was prepared using the method described by Alberto and co-workers.[30] The resulting reaction solutions were allowed to stir at reflux for periods of 24 to 48 hours. Negative ion electrospray mass

spectrometry was performed on the crude products in order to determine whether the target anion mass (m/z = 595) was present in the mixture. The mass spectra were instead dominated by the anion **2.6** (m/z = 326) and by several species with masses centered on m/z = 590, 605, 619, and 633, and at m/z = 878 (Figure 2.12), which were attributed to the formation of clusters of μ-hydroxo - bridged units of the $Re(CO)_3^+$ core.[30-32]



Scheme 2.5: Initial method for the preparation of **2.1** in water.

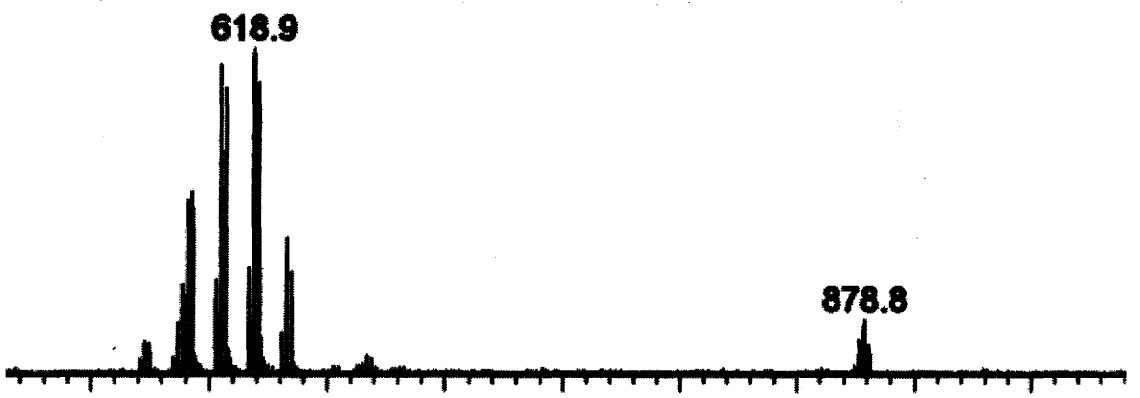


Figure 2.12: Rhenium cluster species in electrospray mass spectrum.

When $[NEt_4]_2[Re(CO)_3Br_3]$ is dissolved in water, it forms the hydrolysis product, $[Re(CO)_3(H_2O)_3]^+$. Alberto and co-workers showed that the hydrolysis product forms clusters when it is dissolved in basic aqueous solution. It is indefinitely stable when dissolved in acidic solutions.[30] Reaction of the tris-aquo species with alkali hydroxides resulted in the formation of three distinct cluster species, depending on the quantity of base added. Equimolar addition of base (NaOH) resulted in the formation of tetranuclear $[Re_4(CO)_{12}(\mu_3\text{-}OH)_4]^+$, whereas addition of 1.33 and greater than 1.5 equivalents of base resulted in the formation of trinuclear $[Re_3(CO)_9(\mu_2\text{-}OH)_3(\mu_3\text{-}OH)]^-$ and dinuclear $[Re_2(CO)_6(\mu_2\text{-}OH)_3]^-$ complexes, respectively (Figure 2.13). The molecular mass 878, corresponding to the trinuclear species was routinely observed in mass spectra of samples taken during attempts to prepare **2.1**. The masses of the other two known species, however, were not typically observed. The mass for the tetranuclear species (m/z = 1149) was observed in mass spectral and LC-MS data for later successful attempts to prepare **2.1**.[31-33]

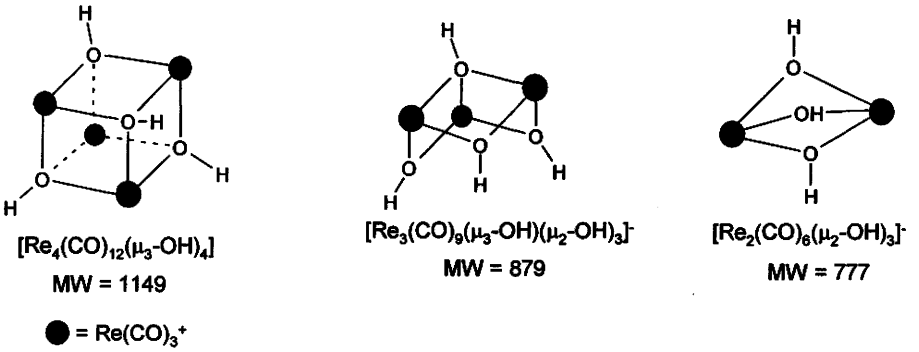
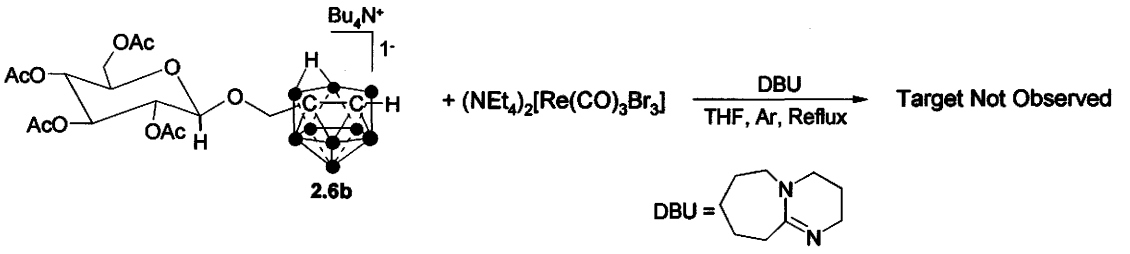


Figure 2.13: Rhenium cluster species investigated by Alberto *et al.*

Since the formation of the rhenium clusters takes place readily in the presence of water, attempts were made to prepare **2.1** or its tetra-acetate protected analogue from **2.6b** in THF solution using different bases. The initial attempt was made using thallium ethoxide, following a method previously reported by researchers in our group.[28] This reagent was employed to remove the bridging hydrogen atom of **2.6b**, and to precipitate the di-thallium salt of the resulting dicarbollide dianion species. Unfortunately, in the case of compound **2.6b**, the target mass was not observed, whereas the formation of the same rhenium clusters with m/z = 590, 605, 619, and 633 were again observed, along with the ligand mass of **2.6b** (m/z = 494).

In light of these results, the complexation reaction was attempted using the non-nucleophilic base 1,8-diaza-bicyclo-[5.4.0]-undec-7-ene (DBU, Scheme 2.6), with the belief that the nucleophilic bases used previously may have interfered with the reaction, resulting in the formation of rhenium cluster species. Therefore, the use of a so-called "bulky base" might promote the removal of the bridging hydrogen without interference with either the ligand or the rhenium reagent. The mass spectra of the crude reaction mixtures were dominated by the *nido*-ligand mass (m/z = 494), perrhenate (ReO_4^-, m/z = 250), and bromide (m/z = 78, 80). Again, no target mass was observed.

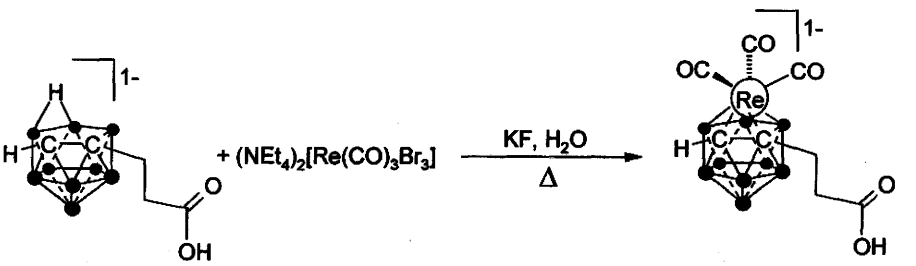


Scheme 2.6: Attempted synthesis of **2.1** in THF using DBU as base.

An attempt was made to generate the di-thallium dicarbollide salt, derived from **2.6a** in aqueous solution, with the anticipation that it could be collected by precipitation. To achieve this, **2.6a** was again stirred with excess KOH or NaOH in water at reflux. An aqueous solution of $TlNO_3$ was added dropwise to the solution as it was cooled in an ice bath. As expected, a yellow precipitate was formed and collected by filtration. The precipitate was re-dissolved in boiling water and reacted with $[NEt_4]_2[Re(CO)_3Br_3]$. Again, the mass spectrum of the crude product mixture was dominated by the ligand mass and rhenium cluster species. This was attributed to the basic nature of the solution formed from dissolution of the dithallum salt of **2.6a**. To remedy this in a subsequent reaction, the di-thallium species was again dissolved in boiling water, and the resulting basic solution was adjusted to a neutral pH by dropwise addition of 1M $HCl_{(aq)}$. The reagent $[NEt_4]_2[Re(CO)_3Br_3]$ was added and the reaction stirred at reflux for approximately 24 hours. Electrospray mass spectra of the resulting crude solution this time indicated two species: the ligand mass (**2.6a**, m/z = 326) and the target anion mass for **2.1** (m/z = 595), with the expected isotopic distribution for the B_9Re cluster. The

product solution was analyzed by HPLC and by LC-MS. Semi-preparative HPLC was used in an attempt to isolate the desired product, but the quantity obtained was insufficient for further analysis by NMR.

Following these attempts, our group discovered that fluoride ion can be used to prepare Re metallocarboranes in aqueous solution (Scheme 2.7).[34] Fluoride ion is a weak base and a poor nucleophile in aqueous solution that does not interact with the $M(CO)_3^+$ core.[35] As a result, reactions with fluoride do not promote the formation of rhenium clusters nearly to the extent seen with hydroxide.



Scheme 2.7: Synthesis of Re-metallocarboranes in the presence of aqueous KF.

Following our group's reported fluoride method, compound **2.6a** was reacted with $[NEt_4]_2[Re(CO)_3Br_3]$ in a 1.0 M solution of KF. The product mass spectrum showed the presence of the ligand mass and the target mass, plus some rhenium cluster species which appeared at m/z = 590 and 633, and at 878. Reduction of the concentration of KF to 0.1 M in subsequent reactions appeared to eliminate these undesired products. Attempts to isolate and purify sufficient quantities of **2.1** for NMR analysis were unsuccessful. Analysis by LC-MS of those solutions that had reacted for 24 - 48 hours indicated

formation of small quantities of the desired product, relative to the ligand **2.6a**. The reaction was therefore allowed to proceed longer, with monitoring by analytical HPLC (Figure 2.14). After a period of seven days, the peak corresponding to the starting material had diminished almost completely, and the peak corresponding to **2.1** had increased accordingly.

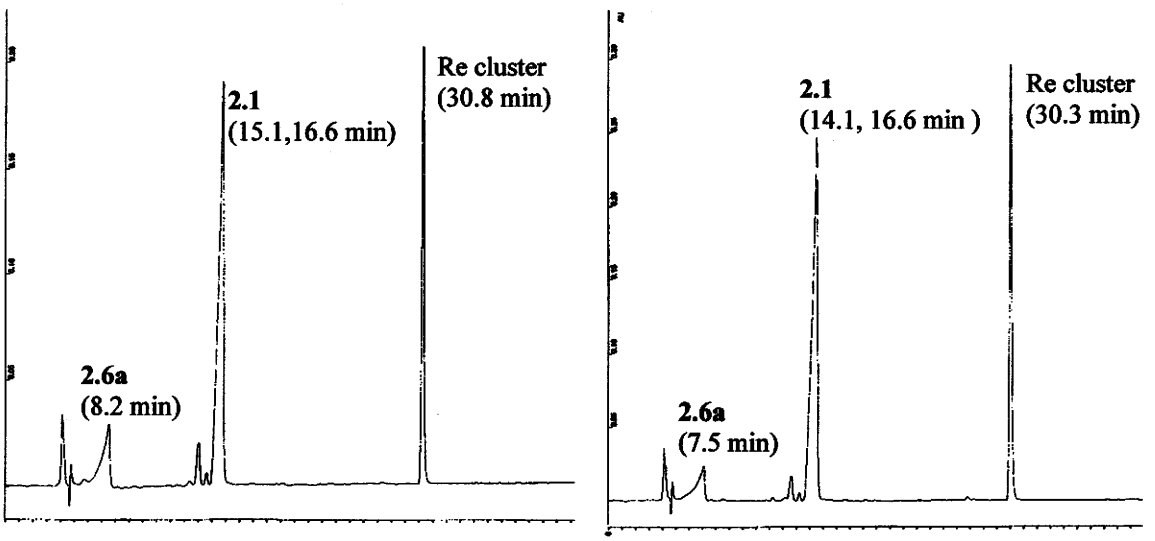
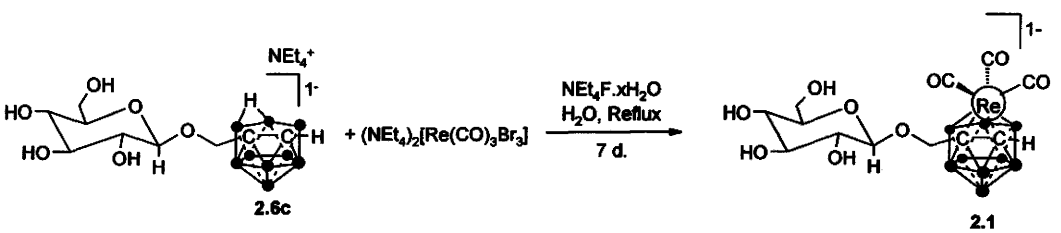


Figure 2.14: HPLC traces for synthesis of **2.1** using KF: Left: HPLC at 2 days; Right: HPLC after 3 days. Column: Varian Dynamax Microsorb 4.6x250mm C_{18}, Elution method A (see experimental section).

Analysis of the KF reaction by HPLC and LC-MS suggested that the product formed as a mixture of K^+ and NEt_4^+ salts, since the two HPLC peaks with t_R = 15.1 and 16.6 minutes corresponded to the target anion mass m/z = 595. To simplify purification, the NEt_4^+ salt, **2.6c**, was used as the ligand and tetraethylammonium fluoride as the base

in order to maintain a single cation in the reaction (Scheme 2.8). The reaction was allowed to proceed for seven days at reflux. The crude reaction solution was analyzed by HPLC (Figure 2.15) and LC-MS (Figure 2.16). The peak at t_R = 16.41 min corresponded to the target mass m/z = 595. Unlike the products of the KF reaction, this was a well-resolved and well-defined peak. Semi-preparative HPLC was employed to isolate pure **2.1** as the teteraethylammonium salt in 16% yield. The low yield of the target was attributed to loss of material during the purification step, as the analytical HPLC of the reaction mixture suggested a higher yield of compound **2.1** than that obtained.



Scheme 2.8: Preparation of **2.1** from **2.6c** with tetraethylammonium fluoride.

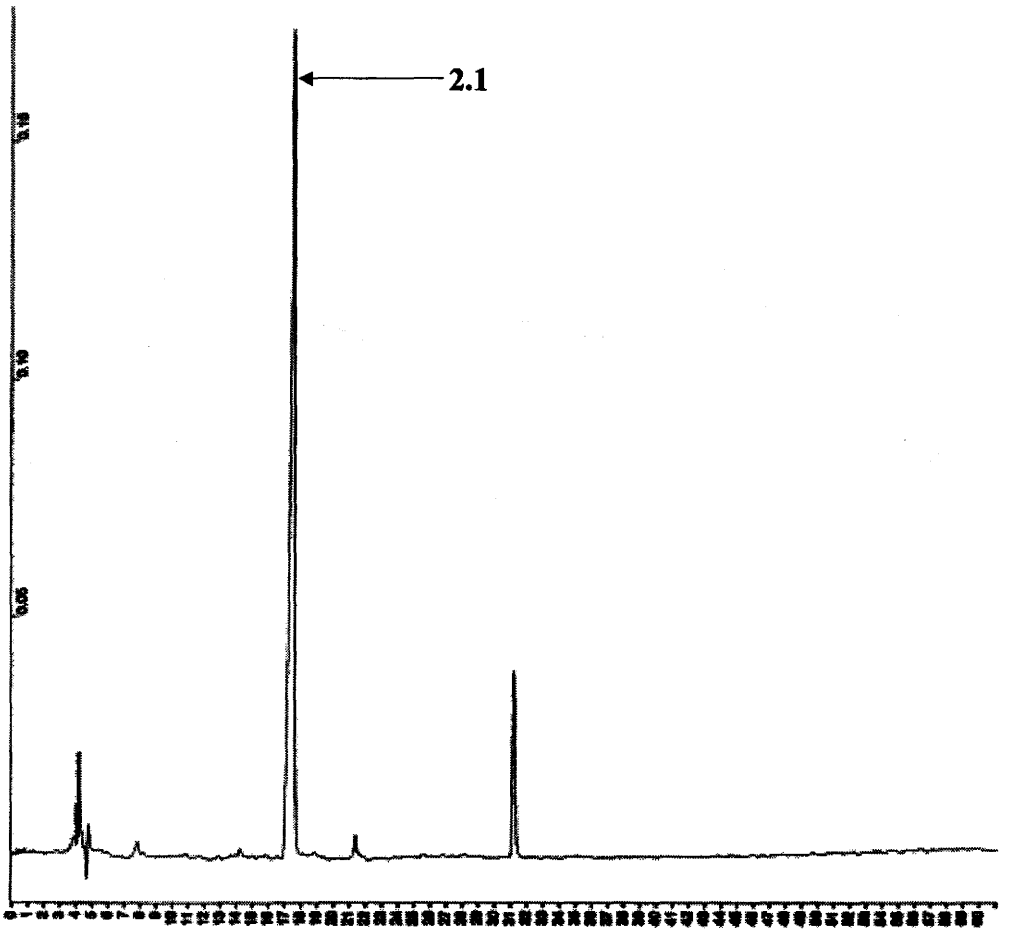


Figure 2.15: HPLC trace of 7-day reaction of **2.6c** with NEt_4F and $(NEt_4)_2[Re(CO)_3Br_3]$. Column: Varian Dynamax Microsorb 4.6x250mmC_{18}. Elution Method **A**.

The FT-IR spectrum (Figure 2.17) of **2.1** featured the characteristic O-H stretch at 3425 cm^{-1} and B-H stretch at 2537 cm^{-1}. A pair of C≡O stretches was observed at 1999 and 1898 cm^{-1}, which were characteristic of the Re carbonyl ligands in a complex having local C_{3v} symmetry.[36] The electrospray ionization mass spectrum (Figure 2.18) of the purified product gave the target mass m/z = 595.3, with an isotopic distribution

characteristic of a ReB_9 cluster in the negative ion mode, and the tetraethyammonium cation mass m/z = 130.2 in the positive ion spectrum.

The ^1H and $^{13}C\{^1H\}$ NMR spectra (Figures 2.19 and 2.20), interestingly, appeared to indicate the formation of unequal amounts of the two expected diastereomers of **2.1**. As there is no basis for diastereoselectivity in the reaction, it is likely that during the HPLC purification, one diastereomer was collected preferentially over the other. In the ^1H NMR spectrum, the anomeric doublets at 4.28 and 4.19 ppm appeared with integration ratios of approximately 10:1 in favour of the lower frequency doublet. Protons H-2, 3 and 4 appeared as pseudo triplets at 3.11, 3.28 and 3.22 ppm, respectively. The metallocarborane cage C-H proton, H-9 was observed as a broad singlet at 1.81 ppm. Also appearing in the proton spectrum was the characteristic pair of expected teteraethylammonium cation signals at 3.16 (quartet) and 1.21 ppm (triplet).

The $^{13}C\{^1H\}$ NMR spectrum of **2.1** featured a C≡O carbon signal at 200.39 ppm, which is characteristic of the presence of the $Re(CO)_3^+$ core.[37] The signal corresponding to the anomeric carbon, C-1 appeared at 103.68 ppm, while the signals arising from the remaining carbohydrate ring carbons C-2, 3, 4, 5, and 6 were assigned to the peaks at 74.74, 77.43, 71.45, 77.19 and 62.72 ppm, respectively. The methylene spacer carbon, C-7 was assigned to the signal at 75.82 ppm. The tetraethyammonium carbon atoms were assigned the signals at 53.06 (methylene) and 7.67 ppm (methyl). The low-intensity signal just below 30 ppm was attributed to the carborane terminal carbon C-9, based on the HSQC spectrum, while the C-8 carbon was not observed, possibly due to T_1 relaxation associated with quaternary (or higher-coordinate, in the case of carboranes)

carbon atoms.[38] Combined with this effect, the small quantity of sample available for analysis may also have hindered the observation of the C-8 signal.

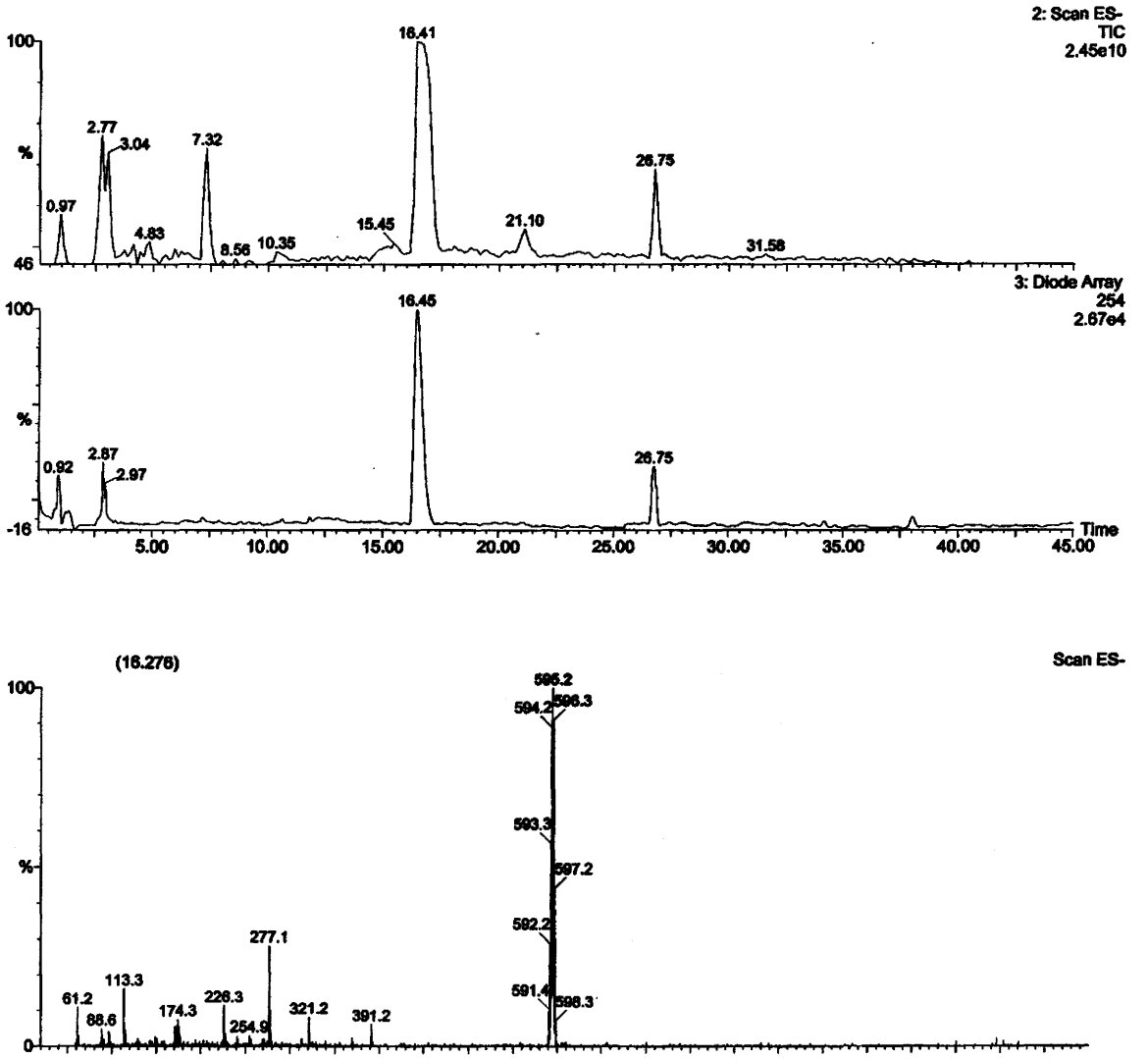


Figure 2.16: LC-MS analysis of crude tetraethylammonium salt of **2.1**. Top: Total ion chromatogram (ESMS) for **2.1**. Middle: UV trace of **2.1**. Bottom: Negative ion ESMS for **2.1**.

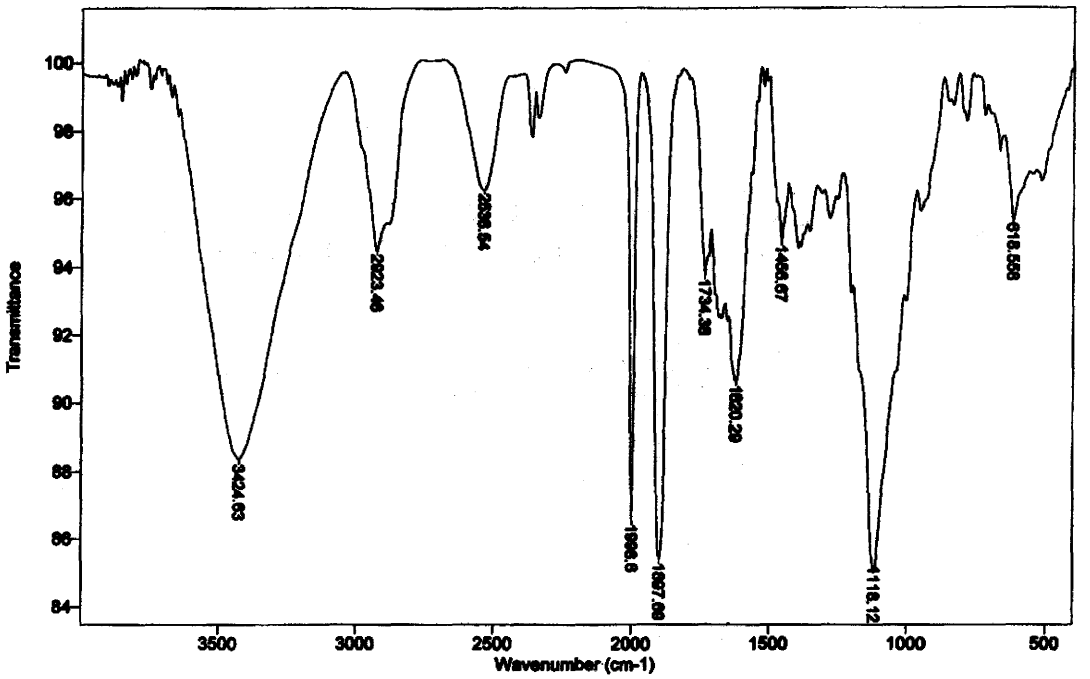


Figure 2.17: FT-IR spectrum of **2.1** (KBr Pellet).

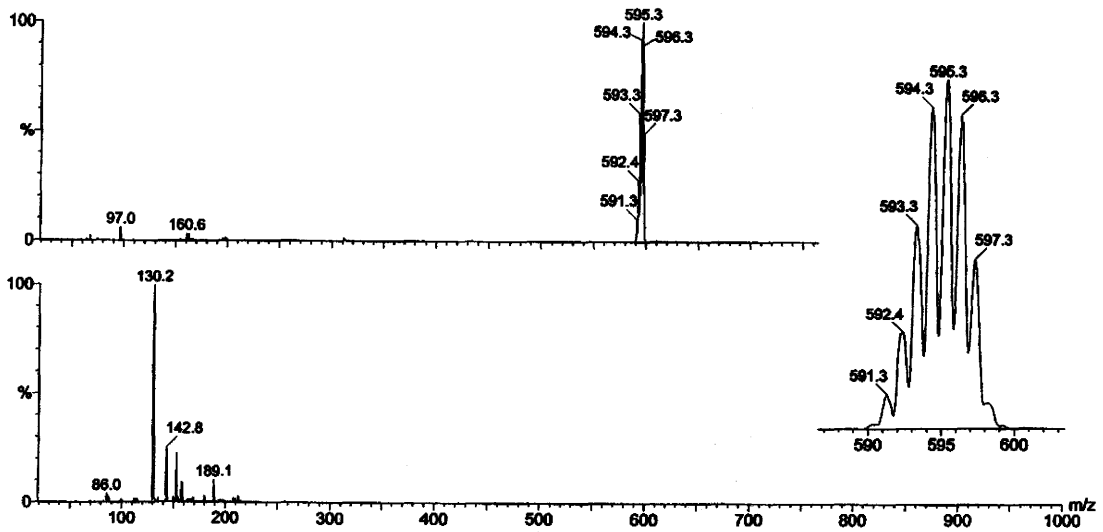


Figure 2.18: Positive and negative ion electrospray mass spectra of **2.1**.

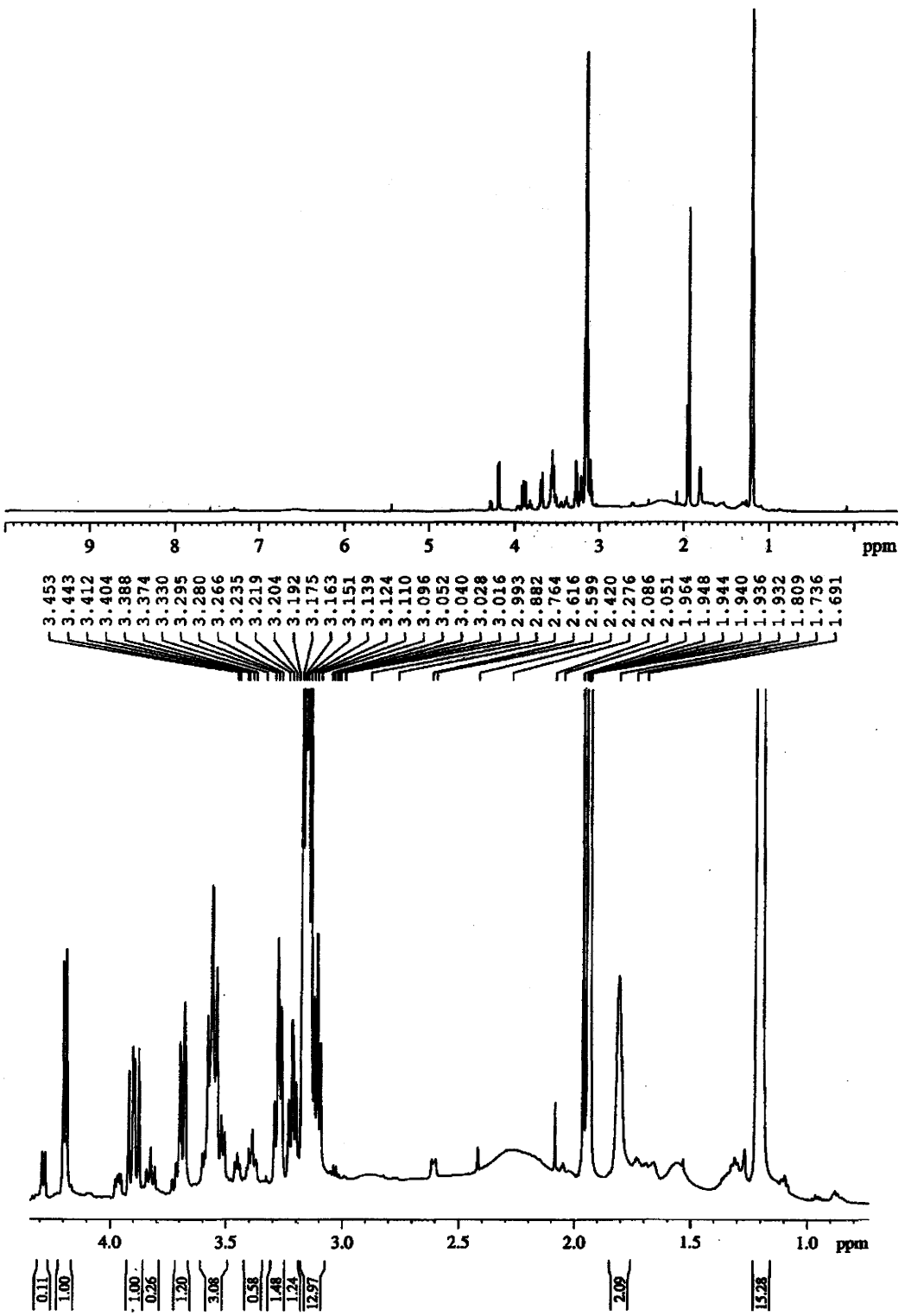


Figure 2.19: The ^{1}H NMR spectrum and expansion between 0.70 and 4.40 ppm of NEt_4-**2.1**. (600 MHz, CD_3CN).

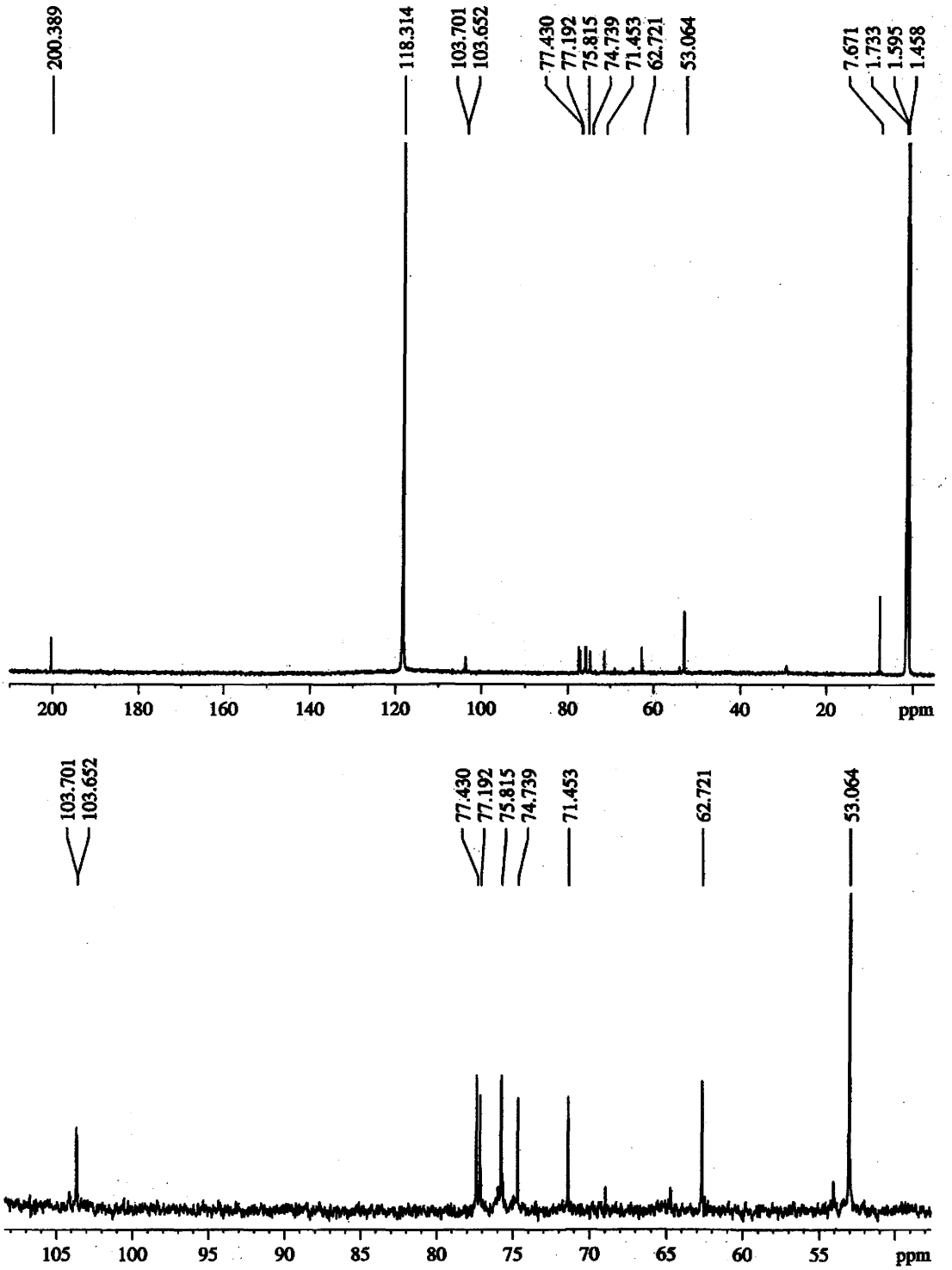


Figure 2.20: The $^{13}C\{^{1}H\}$ NMR spectrum and expansion between 47 and 109 ppm of **2.1** (150 MHz, CD_3CN).

The $^{11}B\{^1H\}$ NMR spectrum of **2.1** (Figure 2.21) was expected to give nine signals, corresponding to nine chemically distinct boron atoms, as was expected for **2.1**. Signals appeared at -5.82, -7.65, -8.78, -11.62, -18.35, -19.55, and -20.13 ppm, with the peaks at -8.78 and -11.62 ppm each appearing to consist of two overlapping signals. The signals in the $^{11}B\{^1H\}$ spectrum for **2.1** were shifted to higher frequency versus those in the *nido*-precursors.

The long reaction time needed to achieve reasonable yields of **2.1** could be the result of the interaction of the rhenium tricarbonyl core with the glucose hydroxyl groups. This argument remains conjecture at present because attempts to detect such a Re-glucose complex by ESMS were unsuccessful. Hydroxyl groups are known to bind the $Re(CO)_3^+$ core, as evidenced by the formation of the rhenium clusters discussed above.[31-33] It is reasonable to expect that the products of the rhenium core and the glucose hydroxyl groups would form at a rate that is faster than the formation of the metallocarborane. With sufficient heating and time, however, the kinetic product should eventually convert to the more thermodynamically stable metallocarborane.

Such a long reaction time would be unsuitable for labeling with ^{99m}Tc, whose half life is 6 hours. It is known, however, that the rate of reactions with the $Tc(CO)_3^+$ core are much faster than those involving $Re(CO)_3^+$, which is based on the trend of decreasing reactivity of transition metals upon descending a group in the periodic table.[39] In addition, during a radiolabelling experiment, a large excess (10,000 fold) of ligand over radiometal reagent is used, which generally drives the reactions to completion in a more reasonable time frame.

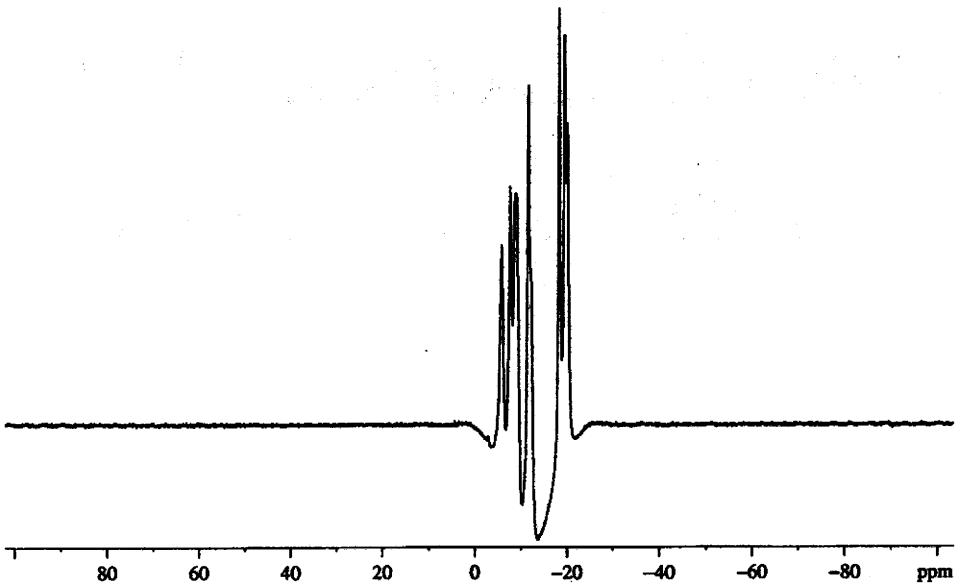


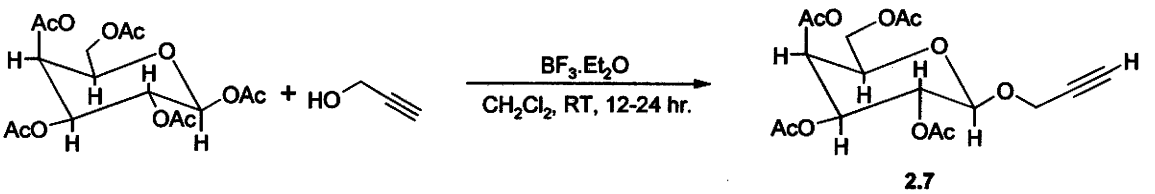
Figure 2.21: $^{11}B\{^{1}H\}$ NMR spectrum of **2.1** (192 MHz, CD_3CN).

2.2 Synthesis of a $Re(CO)_3$-metallocarboranyl glycoside of D-galactose

The synthesis of the metallocarboranyl derivative of β-D-galactose, compound **2.2**, was pursued in concert with the metallocarboranyl glycoside of glucose described above. The synthesis of target **2.2** was approached in a manner analogous to that of the glucose derivative, compound **2.1**. The same synthetic strategy was followed, except that the starting material was β-D-galactose pentaacetate.

Propargyl galactoside **2.7** was prepared by reaction of β-D-galactose pentaacetate with propargyl alcohol in dichloromethane in the presence of boron trifluoride diethyl etherate (Scheme 2.9).[6] The desired product was obtained in 65% yield. The FT-IR

spectrum of compound **2.7** gave the expected acetylenic C-H stretch at 3267 cm^{-1}, a weak C≡C stretch at 2124 cm^{-1}, and a strong carbonyl C=O stretch at 1751 cm^{-1}. The electrospray mass spectrum showed a peak at m/z = 404.4, corresponding to the ammonium ion adduct of **2.7**, $[M+NH_4]^+$. The ^1H and $^{13}C\{^1H\}$ NMR spectra of **2.7** were in agreement with those reported in the literature.[6]



Scheme 2.9: Synthesis of **2.7**.

Closo-carboranyl galactoside **2.8** was synthesized by the decaborane - alkyne insertion reaction as was described above for compound **2.5**. The *closo*-carboranyl derivative of β-D-galactose was obtained in 40% yield. The infrared spectrum gave the expected absorptions for the target compound. The *closo* carborane B-H stretch was observed at 2595 cm^{-1}. The acetate ester carbonyl C=O absorption appeared at 1739 cm^{-1}. Electrospray mass spectrometry gave a peak with the B_{10} isotopic distribution at m/z = 523.5, corresponding to the $[M+NH_4]^+$ ion.

The ^{1}H NMR spectrum of **2.8** showed a similar appearance to that of the alkyne precursor, **2.7**. A doublet at 5.38 ppm was observed for the H-4 proton, with a coupling constant of 3.4 Hz, coupling to H-3. This interaction was indicative of the stereochemical relationships between these protons in D-galactose.[40-42]. A broad signal corresponding to the carborane terminal hydrogen atom, H-9, was observed to be overlapping with one of the H-7 methylene spacer signals at 3.94 ppm . Like the *closo*-carboranyl glucose **2.5**, the ^{1}H NMR spectrum for compound **2.8** featured a distorted baseline in the region between 1.5 and 2.8 ppm, arising from the carborane B-H protons.

The $^{13}C\{^{1}H\}$ NMR spectrum for **2.8** was also similar to that of its alkyne precursor. The carborane carbon signals were observed at 71.51 and 58.11 ppm, for the quaternary (C-8) and terminal (C-9) carbon atoms, respectively.

Degradation of the carborane cage of *closo*-carboranyl glycoside **2.8** was accomplished as with the related glucose-based compound, **2.5**, by reaction with ethanolic KOH solution at reflux. Silica gel column chromatography gave target **2.9** in 80% yield. The infrared spectrum displayed the characteristic broad O-H stretching absorption at 3526 cm^{-1}, and the *nido*-carboranyl B-H stretch at 2526 cm^{-1}. The electrospray mass spectrum gave the target anion mass m/z = 326 with the expected B_9 isotopic distribution. The $^{11}B\{^{1}H\}$ NMR spectrum of **2.9** appeared identical to that for the singly substituted glucose *nido*-carboranyl derivatives.

The ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of **2.9** (Figures 2.22 and 2.23, respectively) indicated the expected formation of diastereomers as a consequence of the cage degradation reaction. The anomeric proton signals appeared as two distinct doublets at

4.31 and 4.22 ppm. One of the four expected H-7 protons gave a doublet at 3.90 ppm with a coupling constant of -10.6 Hz. A multiplet signal at 3.87 ppm was attributed to the equatorial H-4 proton. Another multiplet signal, at 3.75 ppm contained the H-6 signals in addition to another H-7 signal, all overlapping. A further isolated doublet, corresponding to an H-7 proton was observed at 3.63 ppm. The overlapping multiplet signal at 3.51 ppm integrated to seven protons, and was attributed to two H-2 protons, two H-3 protons, two H-5 protons and one H-7 proton. The carborane terminal C-H proton gave a broad signal at 1.94 ppm. The distorted baseline arising from carborane B-H protons was observed between 2.4 and -0.4 ppm. A broad hump was observed at -2.70 ppm and attributed to the bridging hydrogen of the *nido*-carborane cage.

The $^{13}C\{^{1}H\}$ NMR spectrum of compound **2.9** appeared very similar to that of its glucose-based counterpart, compound **2.6a**. Two anomeric carbon signals were observed at 103.97 and 103.58 ppm. A single overlapping signal at 78.38 ppm corresponded to the methylene spacer carbons, C-7. The C-5 and C-3 carbons each gave two distinct signals at 76.33 and 76.19 ppm, and at 74.96 and 74.85 ppm, respectively. Carbon atoms C-2, 4, and 6 gave single signals at 72.60, 70.40, and 62.23 ppm, respectively. As was the case for **2.6**, broadened, low-intensity signals were observed in the baseline of the spectrum at 53.58, 47.73, and 45.94 ppm. On the basis of HSQC/HMBC correlations, these signals were assigned the C-8 and C-9/9* carbon atoms, respectively.

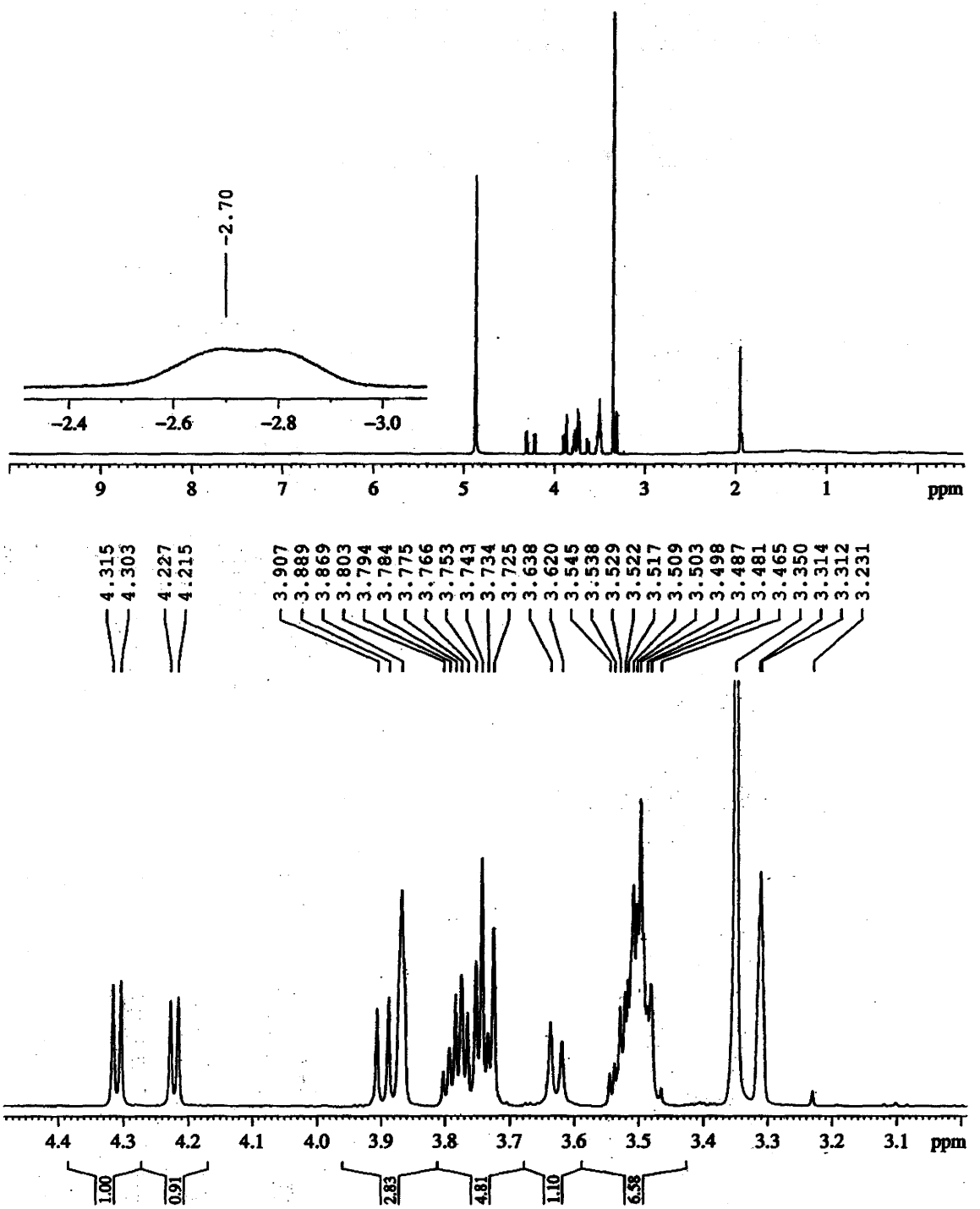


Figure 2.22: The ^{1}H NMR spectrum and expansion between 3.0 and 4.5 ppm of **2.9** (600 MHZ, CD_3OD).

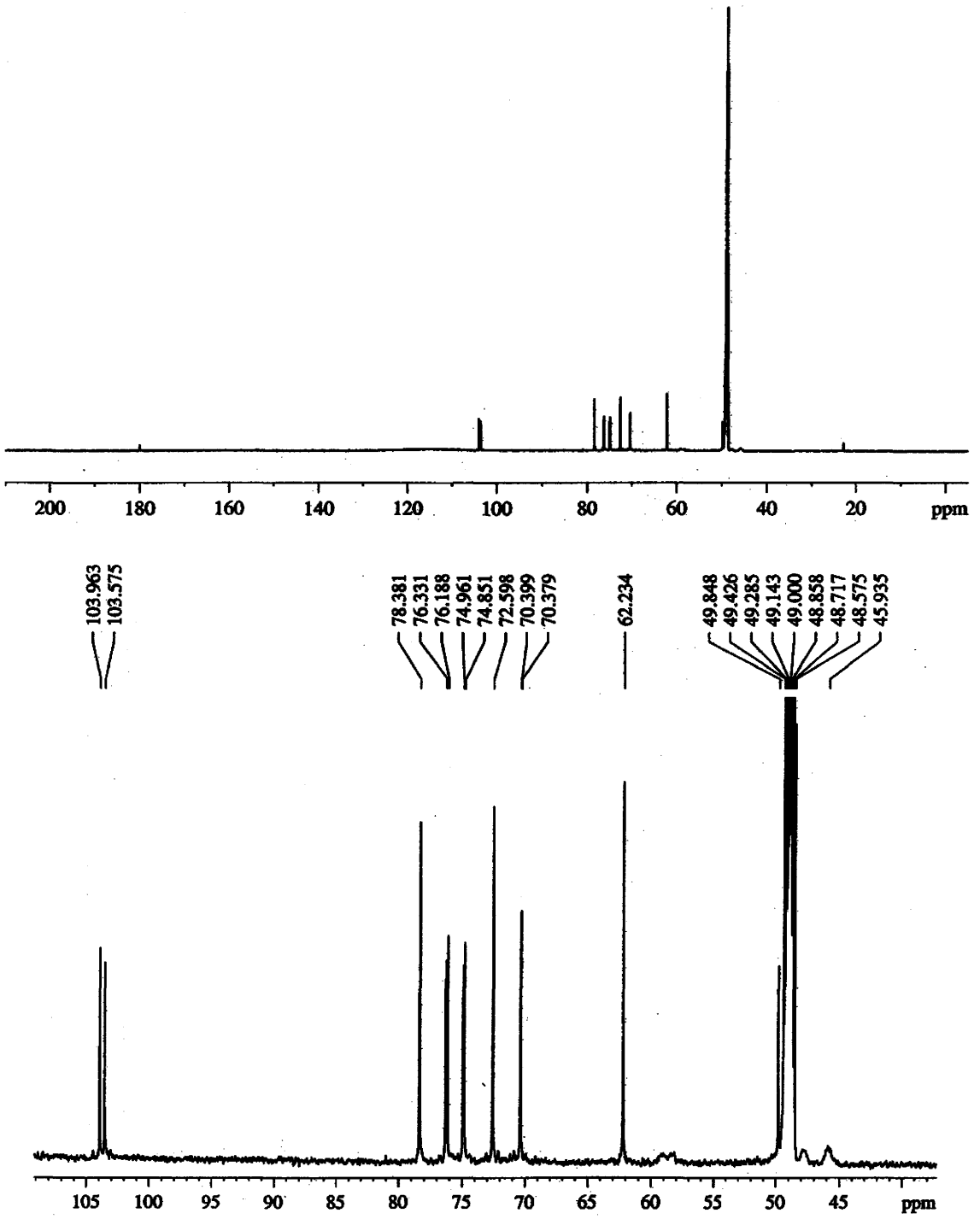


Figure 2.23: The $^{13}C\{^{1}H\}$ NMR spectrum and expansion between 37 and 110 ppm of **2.9** (150 MHz, CD_3OD).

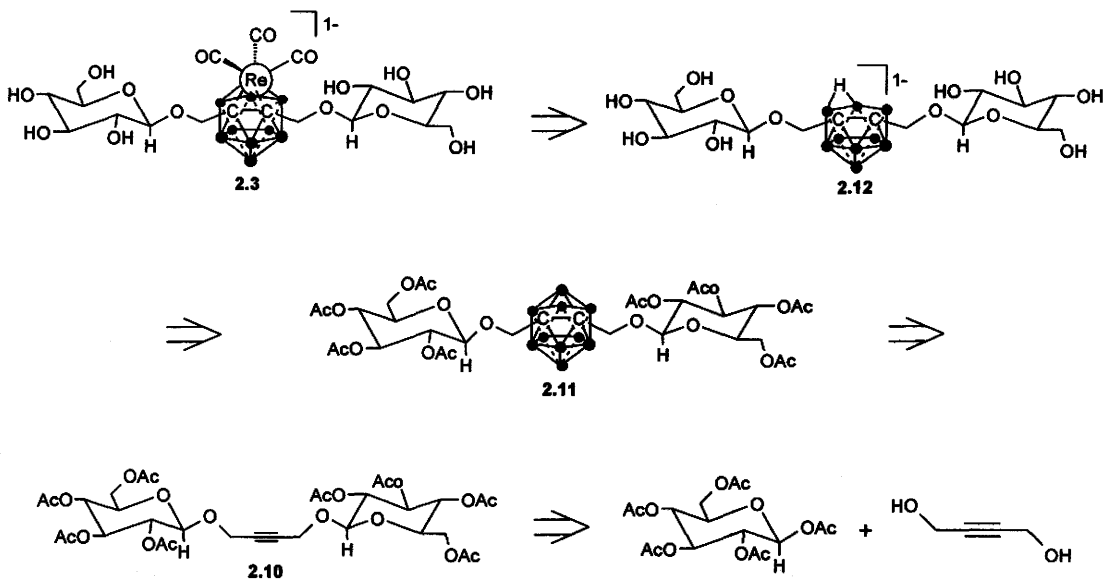
Attempts were made to isolate the tetraethylammonium salt of **2.9** by addition of 2.0 M NEt_4Br to an aqueous solution of $[K^+]$ **2.9** that had been cooled in ice. Although a white solid was formed, as in the analogous reaction to prepare **2.6c**, this solid readily re-dissolved upon heating to room temperature, complicating the isolation process. Centrifugation of the resulting cloudy solution was attempted in order to separate the desired product, but this was not successful. Extraction of the residue with THF yielded only a small quantity of the desired product.

Synthesis of target **2.2** was accomplished by combining **2.9** (K^+ salt) with NEt_4F and $[NEt_4]_2[Re(CO)_3Br_3]$ in water and stirring at reflux for seven days, as was done in the successful preparation of **2.1**. After the above-mentioned time period, ESMS analysis of the crude product indicated the presence of the target mass (m/z = 595). The analytical HPLC trace was very similar in appearance to those of crude mixtures of **2.1** in which the carborane starting material was **2.6a**, with two peaks appearing to correspond to the potassium and tetraethylammonium salts of target compound. These peaks occurred at t_R = 16.9 and 18.1 min, with the second peak having a much larger area. Semi-preparative HPLC was used to isolate this second peak. The product was obtained in very low yield (4%). This low yield was attributed to both poor conversion of **2.9** to the product, and to loss of material during purification. Because of this disappointing yield, alternative methods of preparing metallocarboranyl glycosides were investigated, and are presented in Chapter 3.

2.3 Toward the synthesis of a $Re(CO)_3$-metallocarboranyl bis-glycoside of D-glucose

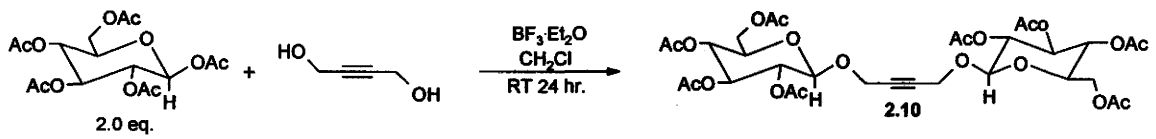
The synthesis of compound **2.3** was undertaken in order to demonstrate that two carbohydrates could be linked to a single metallocarborane cage. This is an important target because the bis-glycoside metallocarborane derivative based on β-D-glucose can be expected to possess even greater hydrophilicity than compound **2.1**, the singly-substituted metallocarboranyl glucose derivative. Furthermore, multiple carbohydrate targeting agents could potentially facilitate enhanced relative binding affinities due to multi-valent binding effects.[43-49]

Like the synthesis of compound **2.1**, the synthesis of **2.3** was planned following the preparation of known derivatives **2.10** and **2.11**.[5,50,51] A retrosynthetic scheme is presented in Scheme 2.10 and shows the strategy used for the preparation of **2.3**.



Scheme 2.10: Retrosynthetic approach to **2.3**.

Compound **2.10**, 1,4-bis-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyloxy)-2-butyne, was prepared in a similar fashion to compound **2.4**. Reaction of two mole equivalents of β-D-glucose pentaacetate with 2-butyne-1,4-diol in dry dichloromethane in the presence of boron trifluoride diethyl etherate (Scheme 2.11) gave the desired compound in 38% yield following workup with sodium bicarbonate and silica gel column chromatography.



Scheme 2.11: Synthesis of compound **2.10**.

The product was analyzed by FT-IR spectroscopy, which showed a strong carbonyl absorption at 1755 cm^{-1}, but little else outside the fingerprint region. Chemical ionization mass spectrometry gave the target mass, m/z = 747. The yield and melting point of **2.10** compared favourably with reported data, and the ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra were consistent with the literature reports.[5,50]

Compound **2.11** was also prepared in a manner similar to that reported in the literature.[5] First, the adduct $B_{10}H_{12}(CH_3CN)_2$ was prepared from the reaction of CH_3CN with $B_{10}H_{14}$ overnight at room temperature. Stirring at reflux for forty-eight hours followed addition of alkyne **2.10**. Purification of the resulting reaction mixture by column chromatography and recrystallization from ethyl acetate and hexanes afforded the desired product **2.11** in 62% yield. The FT-IR spectrum of **2.11** contained the expected B-H

stretch at 2588 cm^{-1}, and the acetate C=O stretch at 1757 cm^{-1}. Positive ion electrospray mass spectrometry gave two masses containing the B_{10} isotopic distribution: m/z = 883.9, corresponding to the ammonium ion adduct $[M+NH_4]^+$, and m/z = 888.8, corresponding to the sodium ion adduct $[M+Na]^+$. The ^1H and $^{13}C\{^1H\}$ NMR spectra were consistent with the reported data.[5,51] The $^{11}B\{^1H\}$ NMR spectrum (Figure 2.24) contained two signals, at -3.96 and -11.72 ppm, with an integration ratio of 1:4. This pattern is consistent with a symmetrically substituted *closo- ortho*-carborane.[5]

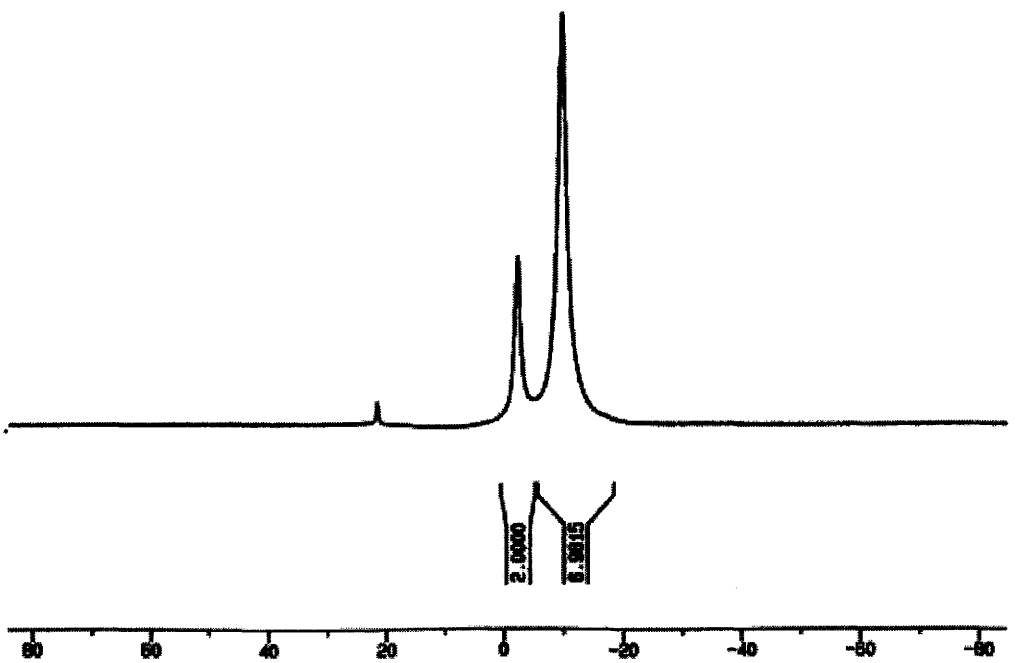


Figure 2.24: $^{11}B\{^1H\}$ NMR spectrum of **2.11** (160 MHz, $CDCl_3$).

The synthesis of the deprotected *nido*-carboranyl derivative of **2.11**, sodium 7,8-bis(β-D-glucopyranosyloxymethyl)-7,8-dicarba-*nido*-undecaborate(-1), **2.12**, was performed in the same manner as was for compound **2.6d**. *Closo*-carborane **2.11** was stirred overnight stirring in an ethanolic solution of NaOH at 60 °C. Following the conversion of the excess sodium hydroxide to insoluble sodium carbonate, the reaction mixture was neutralized with 1M HCl. It was found that the resulting crude product could be isolated by column chromatography on a short plug of silica, whereas a longer column would result in strong retention of the deprotected bis-sugar, and subsequent loss of much of the desired compound. Following this isolation step, target **2.12** was obtained in 52% yield. The infrared spectrum of **2.12** showed the expected broad O-H absorption at 3359 cm^{-1}, as well as the characteristic *nido*-carborane B-H stretch at 2512 cm^{-1}. The electrospray mass spectrum indicated the target anion mass with a peak at m/z = 518.5.

The ^{1}H NMR spectrum (Figure 2.25) of **2.12** was similar in appearance to those of the mono-substituted *nido*-carboranyl glycosides described above. The appearance of the product as a mixture of diastereomers arising from the cage degradation reaction was also observed in the proton (and carbon) NMR spectra of this compound as well. Two doublets, arising from the anomeric protons H-1/1* appeared at 4.65 and 4.38 ppm, both of which contained the expected coupling constant of 7.8 Hz. The methylene spacer protons H-7a and its diastereomeric counterpart, H-7a* appeared as an overlapped signal at 4.17 ppm. The protons H-7b and 7b*, as well as the glucose C-6 protons H-6a and 6a* gave rise to an overlapping signal at 3.91 ppm. The protons H-6b and 6b* gave a signal at 3.78 ppm. The protons H-3/3* and H-4/4* gave rise to an overlapping signal at 3.46 ppm,

while H-2/2* and H-5/5* also gave an overlapping signal at 3.35 ppm. Similarly to **2.6d**, the carborane B-H gave a wavy and broad baseline signal between -0.30 and 2.60 ppm, and the "fluxional" carborane proton gave a broad signal at -2.48 ppm.

The $^{13}C\{^{1}H\}$ NMR spectrum of **2.12** (Figure 2.26) showed the anomeric carbon signals as a just-resolved pair at 104.11 and 104.04 ppm. The C-3 and C-5 carbons, and their diastereomeric counterparts gave a trio of overlapping signals at 77.92, 77.84, and 77.76 ppm. Due to the overlap of both the carbon and proton signals corresponding to the C-3 and C-5 protons and carbons, it was not possible to give a definitive assignment to these signals. Similarly, the signals arising from the methylene spacer group and the C-2 carbons gave four signals, one at 76.13 ppm, which could be assigned to a spacer group, either C-7 or its diastereomeric partner C-7*, and a further three at 75.06, 74.93 and 74.84 ppm, which could not be assigned separately. The C-4/4* carbon atoms gave the signals at 71.51 and 71.46 ppm. The signals at 62.58 and 62.53 were assigned the C-6/6* carbon atoms. Finally, broad signals were observed at 59.97 and 58.84 ppm, and were attributed to the carborane carbon atoms C-8/8*.

In the $^{11}B\{^{1}H\}$ NMR spectrum of **2.12** (Figure 2.27), only four signals, appearing at -9.91, -16.95, -33.84 and -37.75 ppm were observed, whereas more would be expected due to the lack of symmetry present when a *nido*-carborane is substituted with chiral groups.[19] It is believed that the observed signals between -9.91 and -16.95 actually consisted of multiple overlapping signals, and this is substantiated by the fact that these signals are unusually broad.

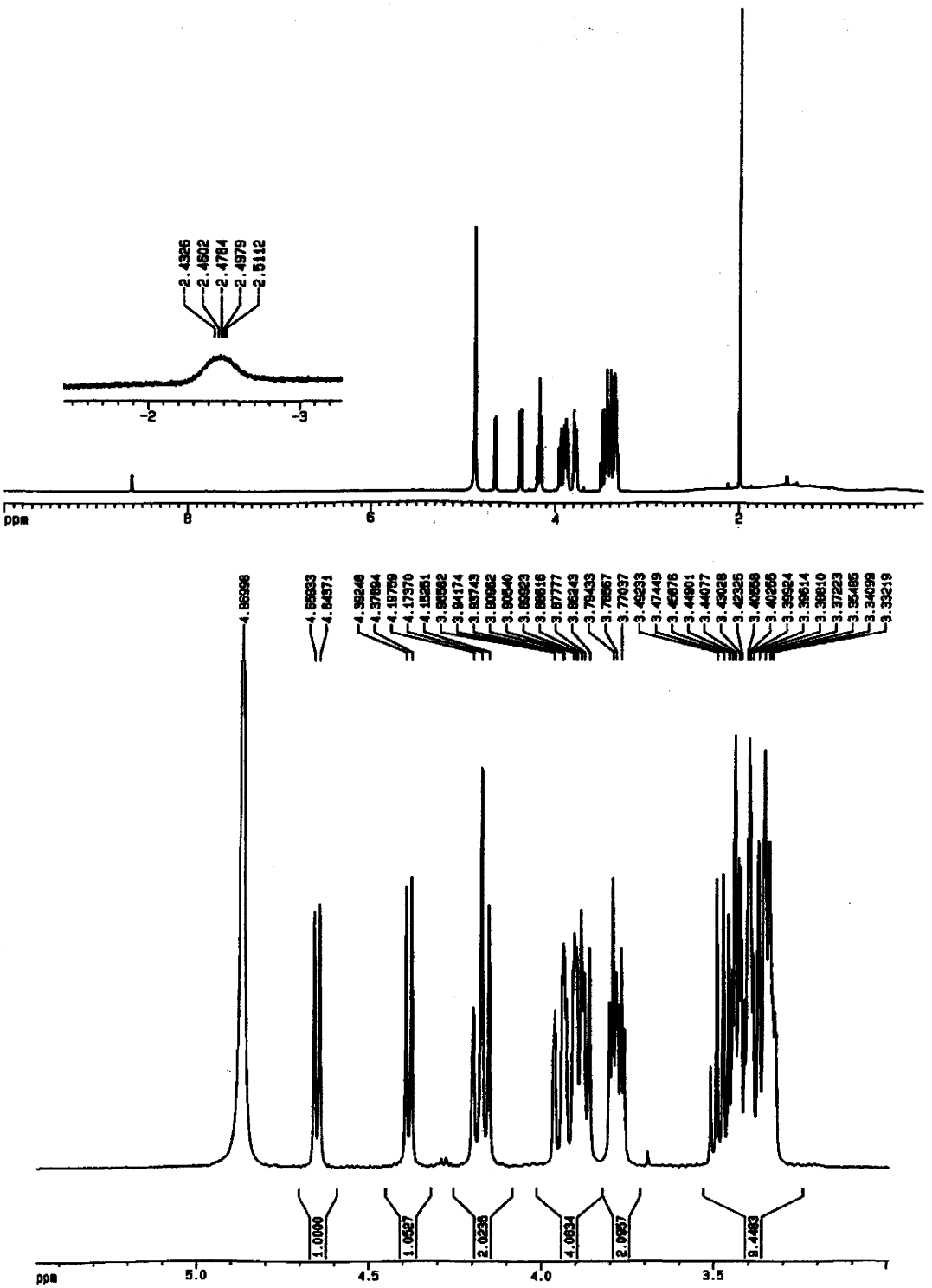


Figure 2.25: The ^{1}H NMR spectrum and expansion between 3.0 and 5.5 ppm of **2.12** (500 MHz, CD_3OD).

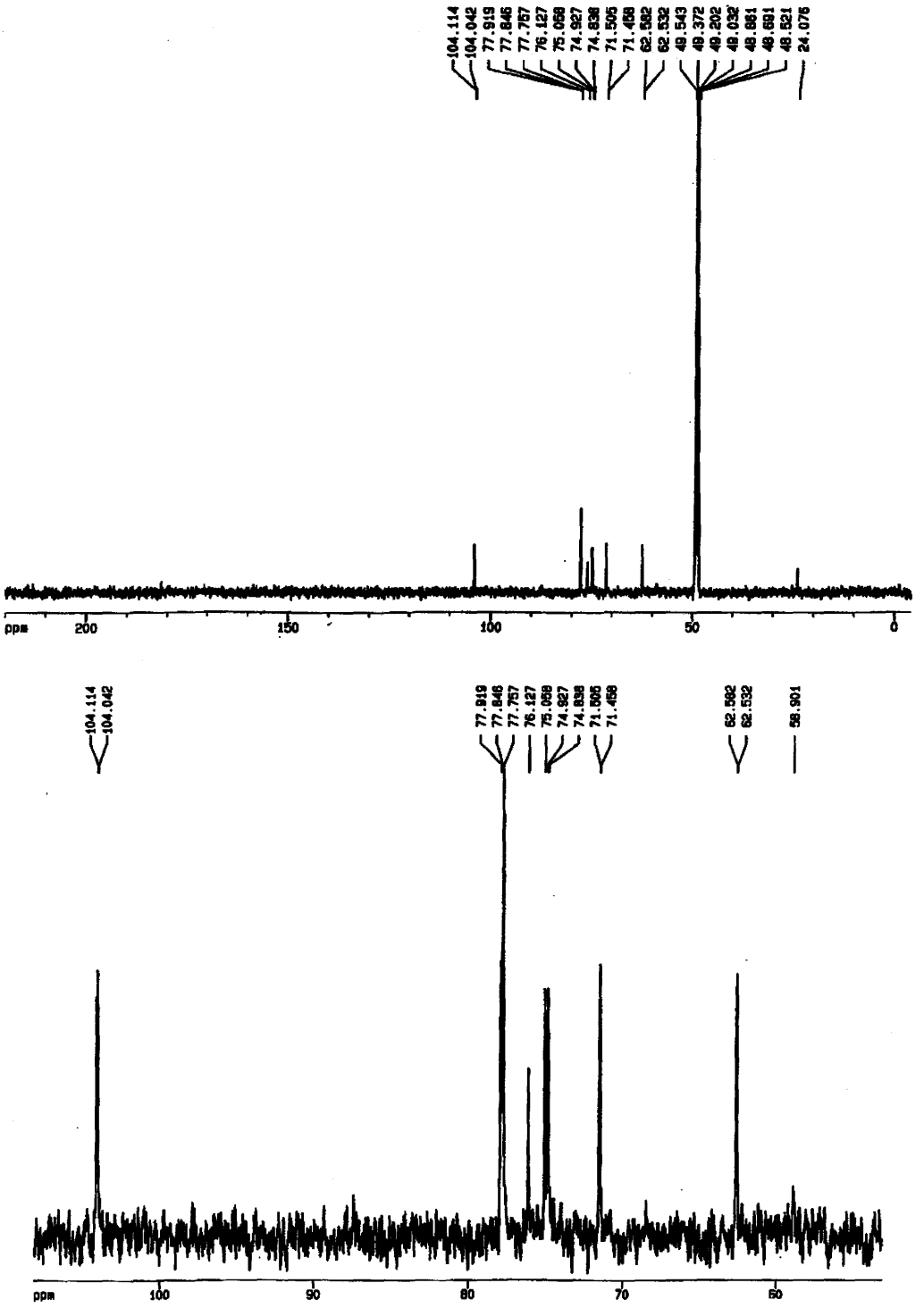


Figure 2.26: The $^{13}C\{^{1}H\}$ NMR spectrum and expansion between 50 and 110 ppm of **2.12** (126 MHz, CD_3OD).

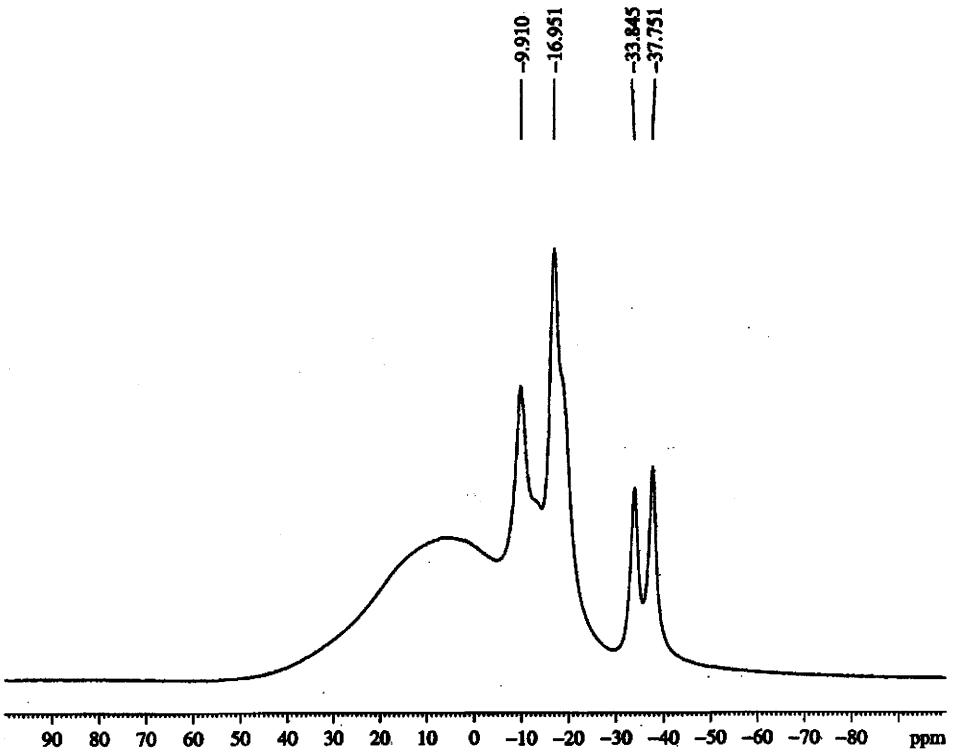
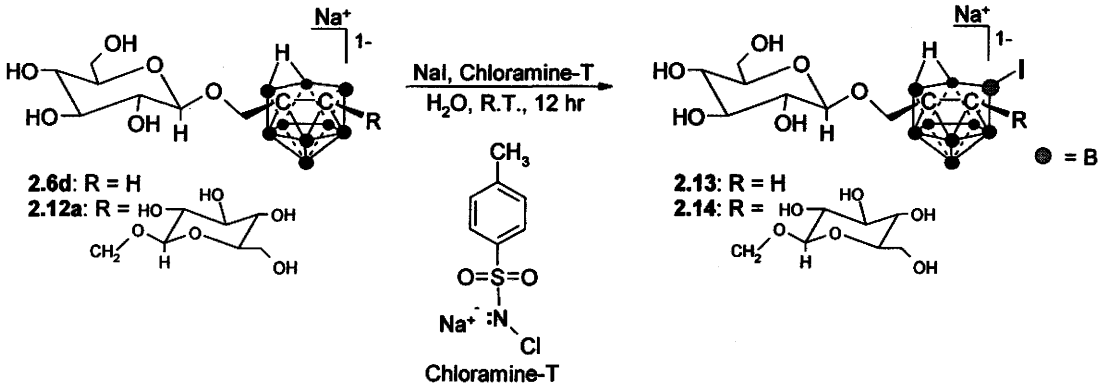


Figure 2.27: $^{11}B\{^{1}H\}$ NMR spectrum of **2.12** (192 MHz, CD_3OD).

Using the aqueous fluoride approach, the synthesis of **2.3** was attempted in a manner analogous to the syntheses of **2.1** and **2.2**. Although electrospray mass spectrometry indicated the presence of the target anion mass (m/z = 787), HPLC analysis indicated that very little of the desired compound was present in the reaction mixture. Optimization of this reaction will be discussed in Chapter 3.

2.4 Iodinated nido-carboranyl glycosides

Since *nido*-carboranes can also be used to bind halogens and radiohalogens, in addition to metals, the synthesis of iodinated analogues of **2.6** and **2.12** was investigated. Compounds **2.13** and **2.14**, the iodinated analogues of **2.6d** and **2.12a**, respectively, were prepared following similar methods to those described by Wilbur and co-workers (Scheme 2.12).[52,53]



Scheme 2.12: Synthesis of iodinated *nido*-carboranyl glycosides **2.13** and **2.14**. Only one regioisomer of **2.13** and **2.14** shown.

The *nido*-carboranyl glycoside **2.6d** was combined with a slight excess of NaI and dissolved in distilled water. To the resulting solution was added slowly a ten-fold molar excess of the oxidizing agent Chloramine-T. With stirring, the iodine colour faded, and had dissipated completely within a few minutes. Analysis of the crude mixture by electrospray mass spectrometry gave a mass peak at m/z = 452, containing an isotope

distribution characteristic of a B_9 cluster, and corresponding to the expected mass of the target compound. Additionally, HPLC analysis on a reversed-phase (C_{18}) column indicated a peak with a longer retention time than that of the starting material. Since the retention of the iodination product on normal-phase silica TLC plates appeared identical to that of the starting material, compound **2.13** was isolated in 21% yield using semipreparative HPLC. Infrared spectroscopy on **2.13** showed the expected O-H and B-H stretches at 3417 and 2540 cm^{-1}, respectively.

The ^{1}H and $^{13}C\{^{1}H\}$ NMR spectral data for **2.13** are listed in Table 2.1. Like the starting compound, **2.6d**, the NMR spectra (Figures 2.28 and 2.29) of **2.13** indicated the presence of diastereomers. For example, two distinct anomeric proton signals were observed at 4.40 and 4.36 ppm, and two anomeric carbon signals were observed at 103.89 and 102.13 ppm. Notable differences between the ^{1}H NMR spectra for **2.13** and **2.6d** were as follows: the carborane terminal proton signal for **2.13** appeared at 2.54 ppm, a shift of 0.52 ppm to higher frequency versus that in **2.6d**. The baseline signal for the B-H protons was also shifted slightly to higher frequency, ranging between 0.10 and 2.60 ppm, whereas in precursor **2.6d**, this signal had spanned as low as -0.30 ppm. Finally, a slight difference was observed between the "bridging" proton signals, with that for compound **2.13** appearing at -2.73 ppm, representing a shift of 0.10 ppm to lower frequency versus the same signal for compound **2.6d**.

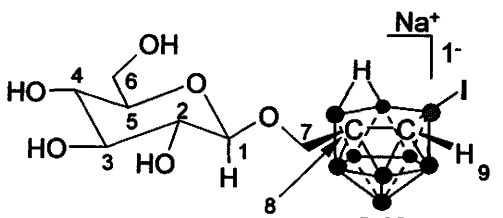
The $^{11}B\{^{1}H\}$ NMR spectrum of **2.13** (Figure 2.30), as was expected, showed a considerable change, relative to that of the precursor, **2.6d**. A total of seven resolved signals were observed at -6.20, -15.78, -18.08, -21.39, -24.71, -29.56 and -37.31 ppm.

Since nine signals are expected in total, due to the lack of symmetry present, it is reasonable to conclude that the partially overlapped signals at -15.78 and -18.08 ppm were, by coincidence, also overlapped with the signals from the remaining boron atoms.

For the purpose of comparison of this ^{11}B NMR spectrum with that of a previously characterized iodo-*nido*-carborane derivative, the 7-(*p*-isothiocyanatophenyl)-9- *iodo*-7,8-dicarba-*nido*-undecarborate(-1) species, prepared by Hawthorne and co-workers, will be considered.[24] The structure of this previously reported compound is shown in Figure 2.31. Except for the fact that the *nido*-carborane moiety bears an achiral substitutent, one would expect this compound to be similar, in terms of the chemical environments of the boron atoms, to compound **2.13**. Indeed, the ^{11}B NMR data for this compound are in good agreement with those obtained for **2.13**. Both the pattern of the signals and the values of the chemical shifts are similar, and are in agreement with the expected appearance of the spectrum predicted by Hermanek and co-workers.[15,24]

Iodination of a monosubstituted carborane is expected to give unequal amounts of the two possible products (structural isomers). In unsubstituted *nido-o*-carborane, the iodine atom can attach to either one of the two boron atoms adjacent the carbons with equal probability, and the products are indistinguishable. However, in mono-substituted *nido*-carborane derivatives, one might expect a larger atom such as iodine to attach preferentially to the boron vertex adjacent the unsubstituted carbon atom, in order to minimize steric interactions between the iodine and the pendant group.[54] The ratio of these two species of **2.13** is 40:1, according to the ^{1}H NMR spectrum. ESMS and HPLC experiments were run to verify that the signals from the minor isomer do not correspond

to unreacted starting material. However, since the anion **2.6** could not be observed in either the HPLC chromatogram, or the mass spectrum of HPLC-purified **2.13**, it is reasonable to conclude that no starting material remained. Rather, the observation of these signals may indicate the presence of a minor structural isomer of **2.13**, in addition to the diastereomers present as a result of the cage degradation reaction.



2.13

Proton	Chemical Shift	Carbon	Chemical Shift
H-1	4.40	C-1*	103.89
H-1*	4.36	C-1	102.13
H-7a,6a,6a*	3.95	C-7	78.37
H-6b,6b*7a*,7b*	3.78	C-3,3*	77.90
H-7b	3.68	C-5,5*	77.64, 77.42
H-3,3*,4,4*,5/5*	3.43	C-7*	77.03
H-5/5*	3.33	C-2,2*	75.11, 75.02
H-2,2*	3.28	C-4,4*	71.35
H-9/9*	2.54	C-6,6*	62.49, 62.29
B-H	0.10 - 2.60	C-9,9*	57.17, 56.28
B-H-B	-2.73	C-8,8*	53.42

Table 2.1 ^{1}H and ^{13}C NMR assignments for compound **2.13**.

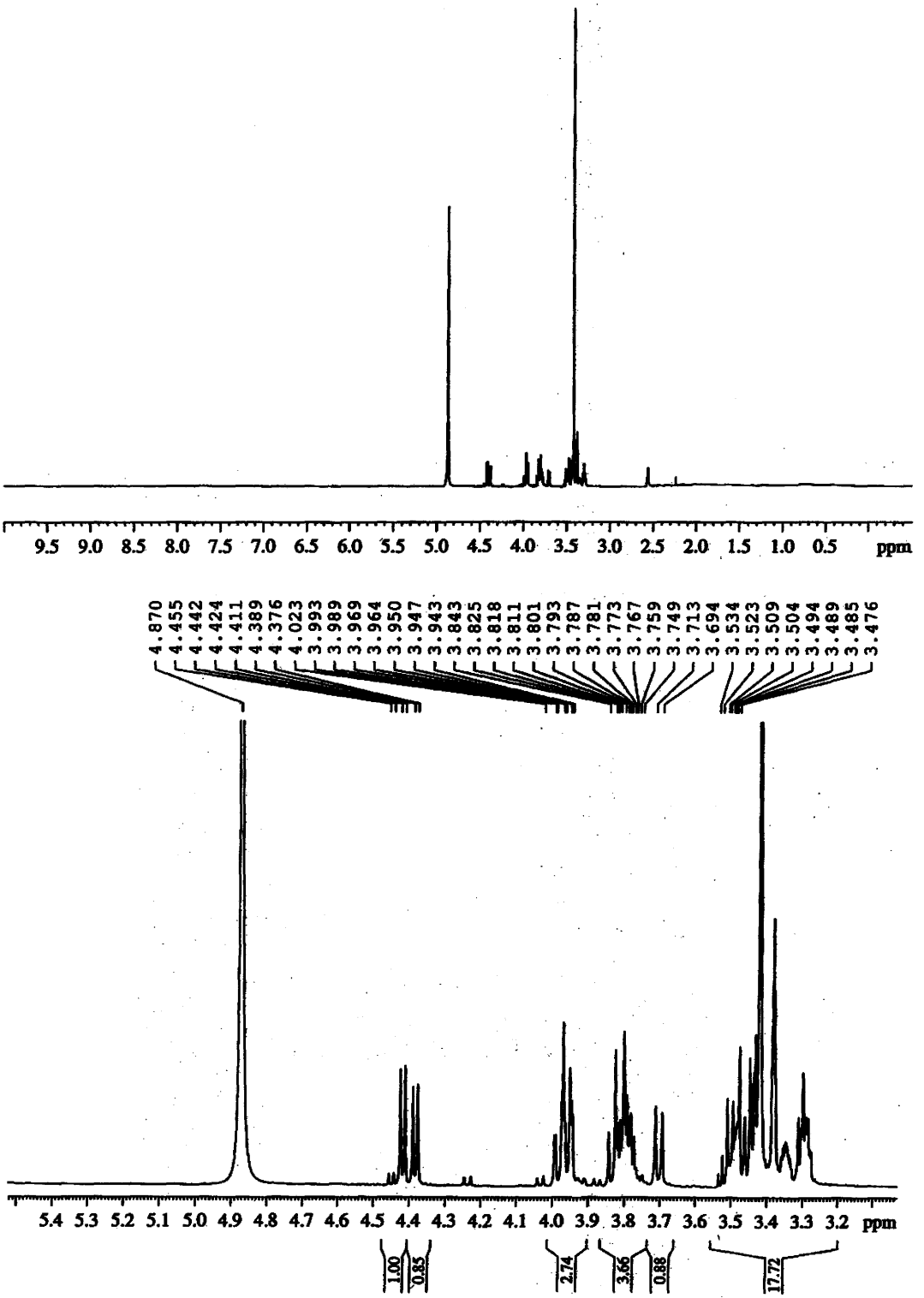


Figure 2.28: The ^{1}H NMR spectrum and expansion between 3.0 and 5.5 ppm of **2.13** (600 MHz, CD_3OD).

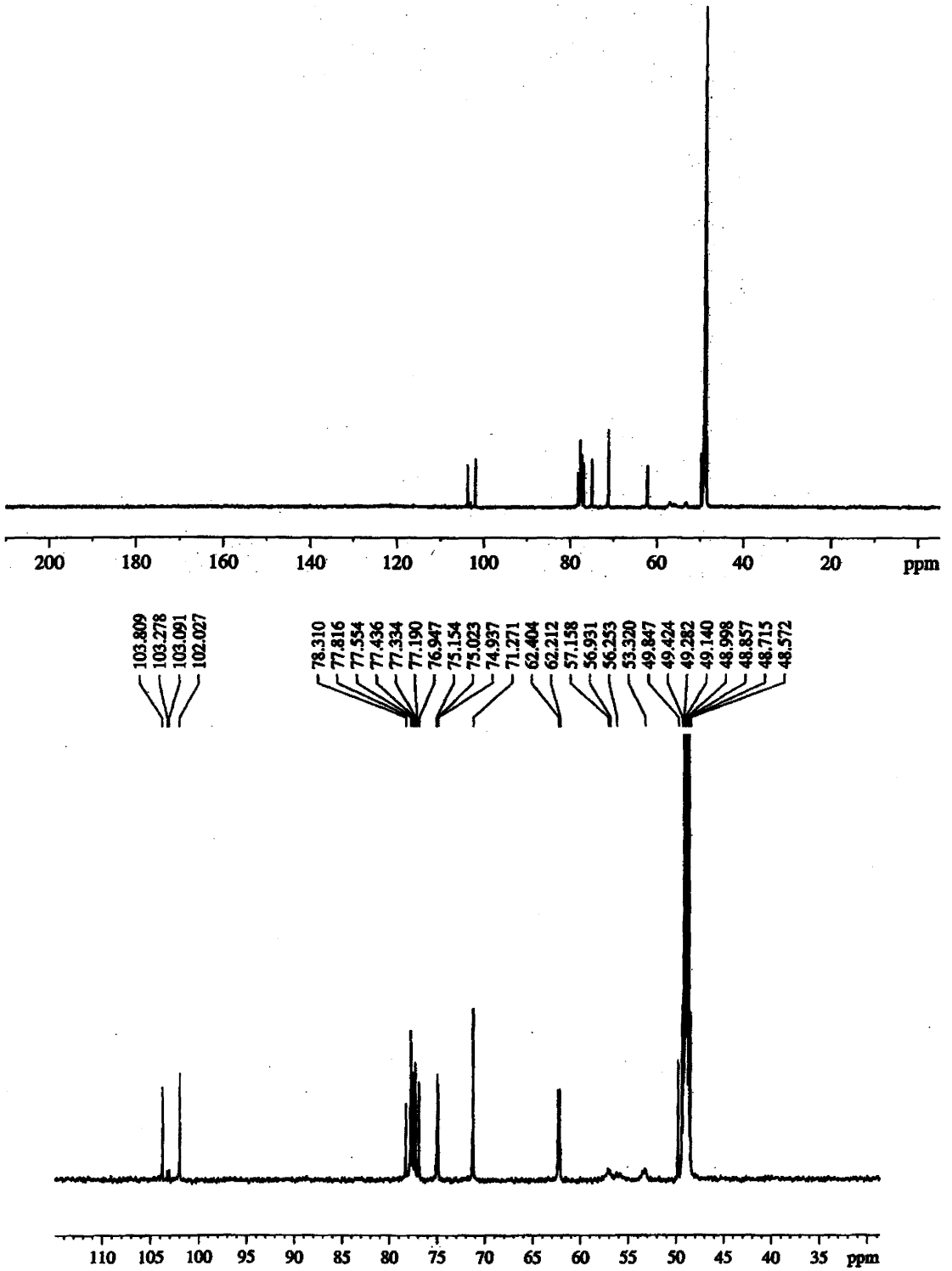


Figure 2.29: The $^{13}C\{^{1}H\}$ NMR spectrum and expansion between 29 and 115 ppm of **2.13** (151 MHz, CD_3OD).

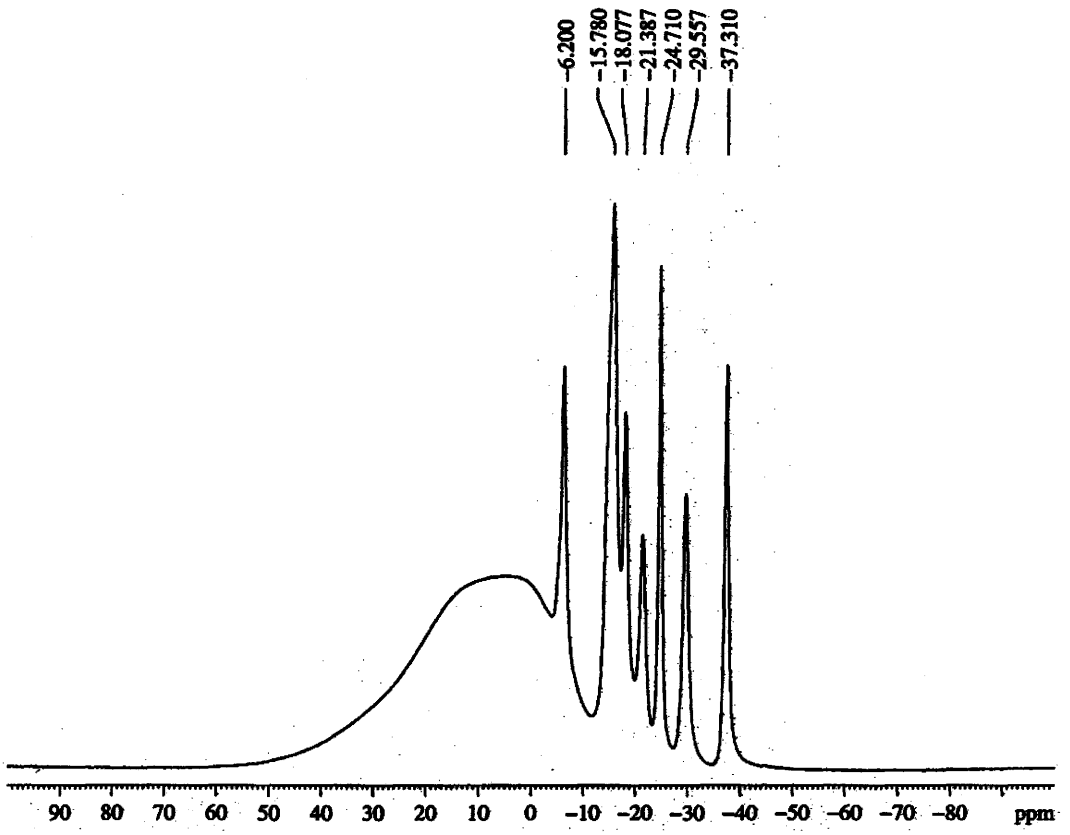


Figure 2.30: $^{11}B\{^1H\}$ NMR spectrum of **2.13** (192 MHz, CD_3OD).

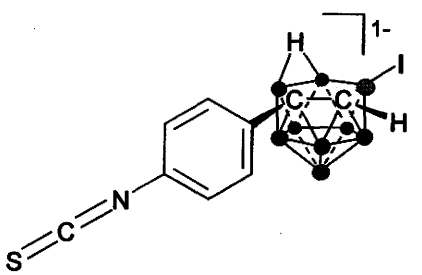


Figure 2.31 [*nido*-7-(*p*-C_6H_4NCS)-9-I-7,8-$C_2B_9H_{11}$]$^-$ species prepared by Hawthorne *et al.*[24]

In the proton-coupled ^{11}B NMR spectrum, only the signal arising from the boron atom bound to iodine should show no splitting due to coupling to a proton.[55,56] In the case of compound **2.13**, (Figure 2.32), however, the proton-coupled spectra showed only two signals which clearly appeared as doublets, while the remaining signals were broad singlets, which was likely due to the diastereomeric nature of the compound. Therefore, with the spectra available for the characterization of **2.13** alone, it is not possible to determine conclusively the site of iodination, relative to the site of carbon substitution.

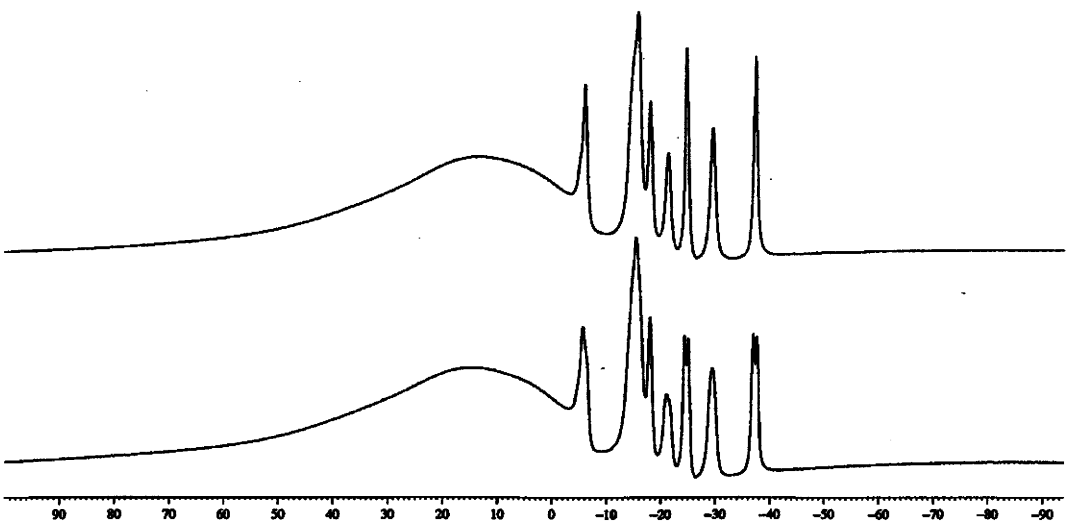


Figure 2.32: Stacked plot comparison of $^{11}B\{^{1}H\}$ (top) and ^{11}B (bottom) NMR spectra of **2.13**.

2.5: Radiolabelling with ^{125}I

The radiolabelling of compound **2.6** with ^{125}I was investigated following the successful synthesis of the non-radioactive standard **2.13**. Although Chloramine-T was the oxidant used for the synthesis of non-radioactive **2.13**, further non-radioactive iodination experiments were conducted to compare the use of Chloramine-T with that of another oxidant, Iodogen®, in their respective abilities to yield iodinated-*nido*-carboranyl glycoside derivatives. A short series of experiments revealed that reaction with Iodogen® resulted in greater yields in a shorter time period than did Chloramine-T. As a result, this oxidant was the one initially employed for radiolabelling reactions. An additional benefit of the use of Iodogen® is that it is insoluble in water and is introduced to the iodination reactions as a film on the inside surfaces of the reaction vessels, and therefore remains in the solid phase so as to simplify purification procedures.

Synthesis of compound [^{125}I]-**2.13** was successfully accomplished by adding 100μg of the *nido*-carborane precursor **2.6d** (as 100μL of a 1mg/mL solution in 5%HOAc/H_2O) to a vial filmed with Iodogen. After addition of 5.5 MBq of Na[^{125}I], the reaction was allowed to proceed at room temperature for 5 minutes, followed by quenching with sodium metabisulfite solution. HPLC analysis of the crude reaction mixture indicated a major radioactive product whose retention time corresponded to that of the desired target by reference to the standard **2.13** (Fig. 2.33). This major product accounted for >90% of the total radioactivity, as estimated by integration of the γ-HPLC traces. The use of semipreparative HPLC permitted the isolation of [^{125}I]-**2.13** from precursor **2.6d** in 29% radiochemical yield. This low yield can be attributed to loss of

material during transfer, and "cutting" of the product γ-HPLC peak to exclude the leading and trailing edges. The radiochemical purity of [^{125}I]-**2.13** was 99%.

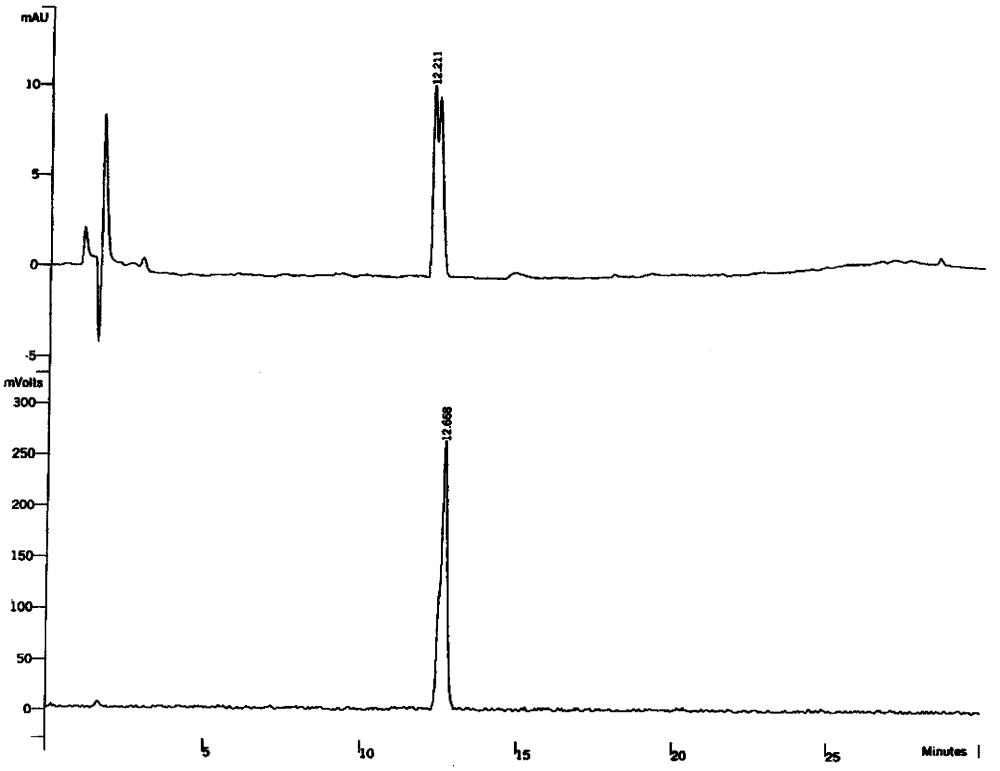


Figure. 2.33: HPLC chromatograms for synthesis of [^{125}I]-**2.13**. Top: UV trace of **2.13**. Bottom: γ-trace of [^{125}I]-**2.13**.

2.6 Summary and Conclusions

A new synthetic method was devised that yielded two novel metallocarboranyl glycoside derivatives. The two main products, **2.1** and **2.2**, were fully characterized and, despite their low yields, they represent the first examples of metallocarborane carbohydrate conjugates, and should be suitable standards for radiolabelling with ^{99m}Tc. The successful use of the fluoride-mediated complexation reaction also supports moving

to the next step, which is to prepare ^{99m}Tc-carborane-carbohydrate complexes, and to embark upon the synthesis of more advanced, biologically relevant derivatives.

Progress was also made towards the synthesis of a metallocarboranyl bis-glycoside as a model of a more hydrophilic analogue, which could also use multivalent effects to enhance binding to receptor targets. Although the synthesis of the metal complex was not immediately successful under conventional reaction conditions, the preparation of the *nido*-carboranyl bis-glycoside **2.12a** will allow further exploration of synthetic strategies, which is the focus of Chapter 3.

In addition to the synthesis of metallocarboranyl glycoside derivatives, two iodinated *nido*-carborane derivatives, **2.13** and **2.14** were prepared and characterized. A radioactive analogue, [^{125}I]-**2.13** was prepared readily under mild conditions. These compounds are intended to serve as a demonstration of the versatility of the carborane moiety, not only in the variety of organic groups with which it can be derived, but also in that it can bind different classes of radioisotopes.

2.7 Experimental Section

All reagents used were purchased from Aldrich, except for decaborane(14), which was purchased from Katchem, Czech Rep. $[NEt_4]_2[Re(CO)_3Br_3]$ was prepared by the method of Alberto and co-workers.[30] Solvents were purchased from Caledon. Dichloromethane and acetonitrile were dried over calcium hydride and distilled prior to use. Propargyl alcohol (bp = 114 °C) was purified by distillation, with contaminant water being removed as an azeotrope with benzene. NMR spectroscopy was performed on

either a Bruker Avance DRX-500 (^{1}H frequency = 500.13 MHz, ^{13}C = 125.77 MHz, ^{11}B = 160.46 MHz) or Avance AV-600 (^{1}H = 600.13 MHz, ^{13}C = 150.90 MHz, ^{11}B = 192.55 MHz) spectrometer. Proton and carbon assignments were made with the assistance of COSY, HSQC and HMBC spectra. The residual absorption of the NMR solvent relative to tetramethylsilane was taken as the internal reference for ^{1}H and ^{13}C spectra. ^{11}B spectra were referenced using a $BF_3{\cdot}Et_2O$ external standard and processed using backward linear prediction in order to eliminate the broad background signal arising from the borosilicate glass NMR tubes. In addition, $^{11}B\{^1H\}$ NMR spectra of the ultimate metallocarborane complexes were acquired in quartz NMR tubes. Infrared spectra were recorded on a BioRad FTS-40 FT-IR instrument. Mass spectra were obtained using a Micromass Quattro-LC Triple Quadrupole mass spectrometer. High-performance liquid chromatography was performed with a Varian ProStar HPLC system. Compounds were detected by UV asbsorbance at 254nm. Radioactive (^{125}I) compounds were detected with a IN/US γ-RAM Model 3 detector incorporated into the HPLC system. Elution conditions: Method **A**: 80:20 H_2O:CH_3CN to 54:46 H_2O:CH_3CN, 0-20 min, 54:46 H_2O:CH_3CN to 100% CH_3CN, 20-25 min, 100% CH_3CN 25-30 min; Column: Varian Dynamax Microsorb C_{18} 4.6x250mm or Varian Nucleosil C_{18} 4.6x250mm; flow rate = 1.0 mL/min (Analytical) or Varian Dynamax C_{18} 10x250mm; flow rate = 4.7 mL/min (Semipreparative). Method **B**: 80:20 H_2O:CH_3CN to 20:80 H_2O:CH_3CN, 0-20 min, 20:80 H_2O:CH_3CN to 100% CH_3CN, 20-25 min, 100% CH_3CN 25-30 min. Solvents contained 0.05% TFA. Column: Varian Dynamax C_{18} 10x250mm; flow rate = 4.7 mL/min. Method **C**: 80:20 H_2O:CH_3CN to 20:80 H_2O:CH_3CN, 0-20 min, 20:80 H_2O:CH_3CN to 100%

CH_3CN, 20-25 min, 100% CH_3CN 25-30 min. Solvents contained 0.05% TFA. Column: Phenomenex Gemini C_{18} 4.6x100mm; flow rate = 1.0 mL/min. Method **D**: 80:20 H_2O:CH_3CN to 75:25 H_2O:CH_3CN, 0 to 10 min; 75:25 H_2O:CH_3CN to 100% CH_3CN 10 to 20 min. Column: Varian Dynamax C_8 10x250mm; flow rate = 4.7 mL/min. Thin layer chromatograms (Merck F_{254} silica gel on aluminum plates) were visualized using 0.1% $PdCl_2$ in 3M HCl.

2-Propynyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (2.4):

β-D-Glucose pentaacetate (10.0 g, 0.026 mol) and 4Å molecular sieves were added to a flame-dried 2-neck round-bottom flask equipped with a gas inlet (to dry N_2 line) and a rubber septum. The solid glucose pentaacetate was dissolved in dry dichloromethane (60mL), and freshly distilled propargyl alcohol (3.0 mL, 0.052 mol) added through the septum via syringe. The flask was then cooled to 0°C in an ice bath, and boron trifluoride diethyl etherate (6.4 mL, 0.051 mol) was added through the septum with a syringe. The reaction vessel was removed from the ice bath and allowed to stir at room temperature overnight (approximately 12 hours) under an atmosphere of dry nitrogen. The reaction mixture was filtered through a pad of celite, and the reaction was quenched by the addition of a saturated solution of $NaHCO_3$. The resulting mixture was stirred for approximately 30 minutes, by which time CO_2 evolution had ceased. The mixture was shaken in a separatory funnel, in which the organic and aqueous layers were separated, and the aqueous layer was washed with two 50 mL portions of dichloromethane. The combined organic extracts were dried over Na_2SO_4, and the

solvent was removed under reduced pressure, yielding a yellow-brown oil, which eventually crystallized to an off-white solid. The crude solid was purified by silica gel column chromatography (1:1 ethyl acetate : hexanes). Yield: 71% (7.1 g). ^{1}H NMR ($CDCl_3$): δ 5.24 (dd, 1H, $^3J_{2,3}$ = 9.5 Hz, $^3J_{3,4}$ = 9.5 Hz, H-3), 5.10 (dd, 1H, $^3J_{3,4}$ = 9.5 Hz, $^3J_{4,5}$ = 10.0 Hz, H-4), 5.01 (dd, 1H, $^3J_{1,2}$ = 8.0 Hz, $^3J_{2,3}$ = 9.5 Hz, H-2), 4.78 (d, 1H, $^3J_{1,2}$ = 8.0 Hz, H-1), 4.37 (d, 2H, $^4J_{7,9}$ = 2.4 Hz, H-7), 4.27 (dd, 1H, $^2J_{6a,6b}$ = -12.3 Hz, $^3J_{5,6a}$ = 4.7 Hz, H-6a), 4.15 (dd, 1H, $^2J_{6a,6b}$ = -12.3 Hz, $^3J_{5,6b}$ = 2.3 Hz, H-6b), 3.73 (ddd, 1H, $^3J_{4,5}$ = 10.0 Hz, $^3J_{5,6a}$ = 4.7 Hz, $^3J_{5,6b}$ = 2.3 Hz, H-5) 2.48 (t, 1H, $^4J_{7,9}$ = 2.4 Hz, H-9), 2.09, 2.06, 2.03, 2.01 (4s,12H OAc CH_3). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.54, 170.15, 169.32 (OAc C=O), 98.14 (C-1), 78.09 (C-8), 75.42 (C-9), 72.77 (C-3), 71.94 (C-5), 70.99 (C-2), 68.36 (C-4), 61.78 (C-6), 55.88 (C-7), 20.63, 20.60, 20.52 (OAc CH_3). IR (KBr): ν 3277 (alkyne C-H), 2121 (C≡C), 1741 (OAc C=O) cm^{-1}. MS (ESI): m/z = 404.1 $[M\text{-}NH_4]^+$, 408.1 $[M\text{-}Na]^+$, 425.1 $[M\text{-}K]^+$. MP: 114-116 °C (lit. 114-116°C).[50] TLC (1:1 Hexanes: Ethyl Acetate): R_f = 0.53.

1,2-dicarba-*closo*-dodecaboranyl-1-methyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (2.5):

Decaborane(14) (1.0 g, 8.1 mmol) was placed in a flame-dried 2-neck round bottom flask equipped with a magnetic stir bar, a reflux condenser, and a rubber septum. The solid was dried under vacuum for 1-2 hours. The flask was then placed under an atmosphere of dry nitrogen, and acetonitrile (50 mL) was added through the septum via a syringe. The resulting solution was allowed to stir at room temperature overnight

(approx. 12 hours) to form the $B_{10}H_{12}(CH_3CN)_2$ adduct. To this solution was added 2-propynyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (2.0 g, 5.19 mmol), and the reaction mixture heated to reflux under N_2 atmosphere for 48 hours, during which time the solution changed from colourless to dark yellow. This solution was filtered and concentrated under reduced pressure, yielding a dark yellow solid. The solid was purified by silica gel column chromatography (200g, 400mL silica gel, elution with 10% v/v ethyl acetate / hexanes to 30% ethyl acetate / hexanes in increments of 10% ethyl acetate, followed by 30%-50% EtOAc/ hexanes in increments of 5% EtOAc. Two column volumes were used for 10, 30-50% and one column volume for 20%). This resulted in a slightly yellow, glassy solid, which was shown to be impure by TLC. Recrystallization from ethyl acetate / hexanes yielded a light, white solid. Yield: 63% (1.7 g). ^{1}H NMR ($CDCl_3$): δ 5.20 (dd, 1H, $^3J_{2,3}$ = 9.6 Hz, $^3J_{3,4}$ = 9.5 Hz, H-3), 5.05 (dd, 1H, $^3J_{3,4}$ = 9.5 Hz, $^3J_{4,5}$ = 10.0 Hz, H-4,), 4.98 (dd, 1H, $^3J_{1,2}$ = 7.9 Hz, $^3J_{2,3}$ = 9.6 Hz, H-2), 4.49 (d, 1H, $^3J_{1,2}$ = 7.9 Hz, H-1), 4.25 (d, 1H, H-7a, $^2J_{7a,7b}$ = -11.0 Hz), 4.23 (dd, 1H, $^2J_{6a,6b}$ = -12.4 Hz, $^3J_{5,6a}$ = 4.7 Hz, H-6a), 4.14 (dd, 1H, $^2J_{6a,6b}$ = -12.4 Hz, $^3J_{5,6b}$ = 2.4 Hz, H-6b), 3.96 (d, 1H, H-7b), 3.93 (s, br, 1H, H-9), 2.69 (ddd, 1H, $^3J_{4,5}$ = 10.0 Hz, $^3J_{5,6a}$ = 4.7 Hz, $^3J_{5,6b}$ = 2.4 Hz, H-5), 2.09, 2.06, 2.03, 2.01 (4s, 12H, OAc CH_3), 2.8 - 1.5 ppm (br m, carborane B-H). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.41, 169.98, 169.26 (4C, OAc C=O), 100.56 (C-1), 72.23 (C-3), 72.08 (C-5), 71.52 (C-8), 70.78 (C-2), 70.27 (C-7), 68.09 (C-4), 61.56 (C-6), 58.08 (C-9), 20.62, 20.53, 20.48 (4C, OAc CH_3).

^{11}B NMR ($CDCl_3$): δ -4.10 (2B) -9.50 (2B), -11.73, -13.64 (6B). IR (KBr): ν 2587 (B-H), 1757 (OAc C=O) cm^{-1}. MS (ESI): m/z: 523.7 $[M+NH_4]^+$ with B_{10} isotopic distribution. MP: 182-184 °C. TLC (1:1 hexanes : ethyl ecetate): $R_f = 0.57$.

Potassium [7-methyl-(β-D-glucopyranosyloxy)-7, 8-dicarba-*nido*-undecaborate] (2.6a):

1,2-dicarba-*closo*-dodecaboran-1-yl-methyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (0.51 g, 1.0 mmol) and KOH (1.2 g, 22 mmol) were placed in a 100mL round bottom flask equipped with a reflux condenser and placed under N_2 atmosphere. Absolute ethanol (20 mL) was added and the solids dissolved upon heating. The resulting solution was stirred at reflux overnight (12 hrs). The reaction was cooled to room temperature and the excess KOH was precipitated as K_2CO_3 by passing a stream of CO_2 gas through the solution. The solid was removed by vacuum filtration of the mixture through a glass frit and the residue washed with cold ethanol (20 mL). The solution was then concentrated under reduced pressure, yielding a white solid. The solid was dissolved in distilled water, and the pH (initially ~ 10) was adjusted to ~ 4 by dropwise addition of 1M HCl and frequent monitoring with pH paper. The solution was again concentrated to a white solid by rotary evaporation. Crude TLC analysis indicated three Pd-active spots, with the target spot in between a fast-running spot and a spot adhering to the baseline. Removal of these impurities was accomplished by silica gel column chromatography (50% → 70% acetone in CH_2Cl_2). The resulting slightly yellow oil was mixed with diethyl ether and treated to rotary evaporation, yielding an off - white foam. This product

was then dried *in vacuo* to remove residual solvents. Yield: 83% (0.3 g). ^{1}H NMR (500 MHz, CD_3OD): δ 4.51 (d, H-1, $^{3}J_{1,2}$ = 7.8 Hz), 4.41 (d, 1H, $^{3}J_{1^*,2^*}$ = 7.8 Hz, H-1*), 4.01 (d, 1H, $^{2}J_{7a,7b}$ = - 10.9 Hz, H-7a), 4.01 (2dd, H-6a,6a*), 3.88 (d, 1H, $^{2}J_{7a',7b'}$ = -10.7 Hz, H-7a*), 3.86 (2dd, H-6b,6b*), 3.77 (d, H-7b*), 3.64 (d, H-7b), 3.54 - 3.48 (m, H-3,3*, H-4,4*), 3.40 (m, H-5,5*), 3.34 (m, H-2,2*), 2.07 (br s, H-9,9*), 2.14 - -0.30 (br, m, B-H), -2.5 (br, B-H-B). ^{13}C{^{1}H} NMR (126 MHz, CD_3OD) δ 103.41, 103.01 (C-1,1*), 78.46 (C-7,7*), 77.12, 77.01 (C-3,3*), 77.84, 77.74 (C-5,5*), 75.16 (C-2,2*), 71.55 (C-4,4*), 62.51 (C-6,6*). ^{11}B NMR (160 MHz CD_3OD): δ -10.83 (2B), -16.72 (4B), -21.87 (1B), -33.02 (1B), -37.50 (1B). IR (KBr): ν 3429 (br, O-H), 2526 (B-H), cm^{-1}. MS (ESI): m/z: 326.3 [M]$^{-}$ (B_9 isotopic distribution). HRMS (ESI): Calculated for $C_9H_{24}B_9O_6$: 326.2455. Observed: 326.2452. TLC (25% CH_3OH / CH_2Cl_2): R_f = 0.38.

Tetrabutylammonium [7-methyl-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyloxy)-7,8-dicarba-*nido*-undecaborate] (2.6b):

1,2-Dicarba-*closo*-dodecaboran-1-yl-methyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (0.68 g, 1.34 mmol) and tetrabutylammonium fluoride hydrate (1.80 g, 6.87 mmol) were combined in a round bottom flask and dissolved in THF (5 mL). The reaction was allowed to stir overnight (approx. 12 hours), in the open air, until TLC indicated disappearance of the starting carborane derivative. The target compound, corresponding to the fastest-running TLC spot, was isolated via silica gel column chromatography (5% CH_3OH / CH_2Cl_2). The solvent was removed under reduced pressure to give an off - white, gummy solid. Yield: 37 % (0.37 g). ^{1}H NMR (500 MHz,

$CDCl_3$): δ 5.25 (m, 2H, H-3), 5.04 (m, 2H, H-4), 4.96 (m, 2H, H-2), 4.93 (d, 1H, H-1, $^3J_{1,2}$ = 8.1 Hz), 4.53 (d, 1H, $^3J_{1,2}$ = 8.0 Hz ,H-1), 4.28 (dd, 1H, $^2J_{6a,6b}$ = -12.2 Hz, $^3J_{5,6a}$ = 4.6 Hz, H-6a), 4.20 (dd, 1H, $^2J_{6a,6b}$ = Hz, $^3J_{5,6a}$ = 5.2 Hz, 6a), 4.16 (dd, 1H, $^3J_{5,6b}$ = 1.9 Hz), 4.11 (dd, 1H, $^3J_{5,6b}$ = 2.0 Hz, H-6b), 3.89 (d, 1H, $^2J_{7a,7b}$ = - 11.8 Hz, H-7a), 3.83 (d, 1H, $^2J_{7a,7b}$ = -11.6 Hz, H-7a), 3.72 (m, 2H, H-5), 3.62 (d, 1H, H-7b), 3.56 (d, 1H, H-7b), 3.16 (m, 16H, $NCH_2CH_2CH_2CH_3$), 2.15, 2.11, 2.06, 2.04, 2.01, 2.00, 1.99 (8s, 32H, OAc CH_3), 1.96 (br s, 2H, H-9), 1.63 (m, 16H, $NCH_2CH_2CH_2CH_3$), 1.45 (m, 16 H, $NCH_2CH_2CH_2CH_3$), 1.02 (t, 24H, $NCH_2CH_2CH_2CH_3$), 2.5 - -0.3 (br m, B-H), -2.68 (br, B-H-B). $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$): δ 170.75, 170.70, 170.23, 169.87,169.54 (OAc *C*=O), 99.05, 98.84 (C-1), 76.34, 76.02 (C-7), 73.24, 73.18 (C-3), 71.76, 71.48 (C-5), 71.30, 71.24 (C-2), 68.78, 68.65 (C-4), 62.09, 61.94 (C-6), 58.92 ($NCH_2CH_2CH_2CH_3$), 23.93 ($NCH_2CH_2CH_2CH_3$), 20.95, 20.87, 20.73, 20.67, 20.61 (OAc CH_3), 19.66 ($NCH_2CH_2CH_2CH_3$), 13.60 ($NCH_2CH_2CH_2CH_3$). IR (KBr): ν 2525.92 (B-H), 1756 (C=O), cm^{-1}. MS (ESI) m/z = 494.2 with B_9 isotopic distribution $[M]^-$, 242.2 $[Bu_4N]^+$ TLC (5% CH_3OH / CH_2Cl_2): R_f = 0.39.

Tetraethylammonium [7-methyl-(β-D-glucopyransyloxy)-7,8-dicarba-*nido*-undecaborate] (2.6c):

Potassium 7-methyl-(β-D-glucopyranosyloxy)-7,8-dicarba-*nido*-undecaborate(-1) (0.103 g, 0.282 mmol) was dissolved in 0.8-1.0 mL of distilled deionized water and placed in a water / ice bath at 0 °C. Tetraethylammonium bromide solution (2.0 M; 141 μL, 0.282 mmol) was added with an Eppendorf pipette. During the next few minutes, a

white precipitate was observed to form, first observed as small droplets, until the reaction vessel contained a congealed white mass. The solid was collected by vacuum filtration on a Hirsch funnel, and dried using a lyophilizer, yielding a white solid (0.075 g, 0.165 mmol, 58%). ^{1}H NMR ($(CD_3)_2CO$): δ 4.35 (d, 1H, $^{3}J_{1,2}$ = 7.8 Hz, H-1), 4.25 (d, 1H, $^{3}J_{1^*,2^*}$ = 7.8 Hz, H-1*), 3.84 (d, 1H, $^{2}J_{7a,7b}$ = -10.9 Hz, H-7a), 3.81 (m, 2H, H-6a, 6a*), 3.70 (d, 1H, $^{2}J_{7a^*,7b^*}$ = -10.7 Hz, H-7a*), 3.71 (m, 2H, H-6b, 6b*), 3.59 (d, 1H, $^{2}J_{7a,7b}$ = -10.9 Hz, H-7b), 3.49 - 3.40 (m, 3H, H-3,3*, H-7b*), 3.46 (q, 8H, $(CH_3CH_2)_4N^+$), 3.40 (m, 2H, H-4,4*), 3.26 (m, 2H, H-5,5*), 3.20 (m, 2H, H-2,2*), 1.90 (br s, 2H, H-9,9*), 1.39 (t, 12H, $(CH_3CH_2)_4N^+$). ^{13}C{^{1}H} NMR ($(CD_3)_2CO$): δ 103.07 (C-1), 102.58 (C-1*), 77.96, 77.85 (C-7,7*), 77.43, 77.37 (C-3,3*), 77.28, 77.20 (C-5,5*), 74.85 (C-2,2*), 71.61 (C-4,4*), 62.70 (C-6,6*), 53.12 ($(CH_3CH_2)_4N^+$), 7.76 ($(CH_3CH_2)_4N^+$). ^{11}B NMR ($(CD_3)_2CO$): -8.86 (2B), -15.99 (4B), -21.01 (1B), -31.52 (1B), -35.85 (1B). IR (KBr): ν 3417 (s, br, O-H), 2526 (s, B-H) cm^{-1}. ESI-MS m/z = 326.3 [M]$^-$, 130.9 [NEt$_4$]$^+$. HRMS(ESI) Calculated for $C_9H_{24}B_9O_6$: 326.2455. Calculated: 326.2462. HPLC (80:20 H_2O : AcN → 100 % AcN, t = 60 min, 100 % → t = 70 min): t_R = 7.8 min. TLC (25% CH_3OH / CH_2Cl_2): R_f = 0.54.

Sodium [7-methyl-(β-D-glucopyranosyloxy)-7, 8-dicarba-*nido*-undecaborate] (2.6d): The product was prepared in a manner similar to that for **2.6a**. Isolation was by silica gel column chromatography (1:3 MeOH:CH_2Cl_2). Quantities used: Compound **2.5**: (2.02 g, 4.02 mmol), NaOH: (3.20g, 80 mmol). Yield: 0.88g (63%). ^{1}H NMR (CD_3OD): δ 4.46, 4.36 (2d, 2H, $^{3}J_{1,2}$ = $^{3}J_{1^*,2^*}$ = 7.8 Hz, H-1,1*), 3.97 (m, 3H, H-7a,6a,6a*), 3.82 (m, 3H, H-7a*,6b,6b*), 3.72 (d, 1H, $^{2}J_{7a^*,7b^*}$ = -10.7 Hz, H-7b*), 3.60 (d, 1H, $^{2}J_{7a,7b}$ = -11.0 Hz, H-

7b), 3.46 (m, 4H, H-3,3*,4,4*), 3.36 (m, 2H, H-5,5*), 3.01 (m, 2H, H-2,2*), 2.02 (br s, 2H, H-9,9*), -0.30 - 2.50 (br m, B-H), -2.63 (B-H-B). $^{13}C\{^{1}H\}$ NMR (CD_3OD): δ 103.26, 102.85 (C-1,1*), 78.35 (C-7,7*), 77.99, 77.89, (C-3,3*), 77.74, 77.62 (C-5,5*), 75.05 (C-2,2*), 71.51 (C-4,4*), 62.48 (C-6,6*), 59.02, 43.75 (C-8,8*), 47.80, 45.85 (C-9,9*). $^{11}B\{^{1}H\}$ NMR (CD_3OD): δ -10.92, -14.49, -15.20, -16.32, -17.90, -19.33, -21.82, -32.92, -37.49. IR (KBr): ν 3409 (O-H), 2526 (B-H) cm^{-1}. TLC(1:3 CH_3OH:CH_2Cl_2): R_f = 0.34. ESI-MS: m/z = 326.4 [M⁻]. HRMS (ESI): Calculated for $C_9H_{24}B_9O_6$: 326.2455. Observed: 326.2430.

Tetraethylammonium [1-(methyl-β-D-glucopyranosyloxy)-3,3,3-tricarbonyl-3-rhenium-1,2-dicarba-*closo*-dodecaborate] (2.1):

Tetraethylammonium [7-methyl-(β-D-glucopyranosyloxy)-7,8-dicarba-*nido*-undecaborate] (0.21 g, 0.46 mmol), NEt_4F (0.35 g, 2.32 mmol), and $[NEt_4]_2[Re(CO)_3Br_3]$ (0.432 g, 5.61 mmol) were combined in a round bottom flask and dissolved in distilled water (10 mL). The vessel was fitted with a condenser and stirred at reflux for seven days. Analytical HPLC indicated disappearance of the starting material, and LC-MS indicated that the major peak in the chromatogram corresponded to the target mass. Semi-preparative HPLC (Method A) was used to isolate the product. Yield: 45 mg (of 276 mg crude, 16%). ^{1}H NMR (CD_3CN): δ 4.19 (d, 1H, $^{3}J_{1,2}$ = 7.6 Hz, H-1), 3.90 (2d, 2H, $^{2}J_{7a,7b}$ = $^{2}J_{7a*,7b*}$ = -10.8 Hz, H-7a,7a*), 3.69 (dd, 1H, $^{2}J_{6a,6b}$ = -11.5 Hz, H-6a), 3.56 (m, 2H, H-6b, 7b), 3.28 (t, 1H, H-3), 3.22 (t, 1H, H-4), 3.16 (q, m, NCH_2CH_3, H-5), 3.11 (m, 1H, H-2), 1.81 (br s, 1H, H-9), 1.21 (t, NCH_2CH_3). $^{13}C\{^{1}H\}$ NMR (CD_3CN): δ 200.39 (C≡O),

103.68 (C-1), 77.43 (C-3), 77.19 (C-5), 75.82 (C-7), 74.74 (C-4), 62.72 (C-6), 53.06 (NCH_2CH_3), 7.67 (NCH_2CH_3). $^{11}B\{^1H\}$ NMR (CD_3CN): δ -5.82, -7.65, -8.78, -11.62, -18.35, -19.55, -20.13. IR (KBr): ν 3425 (broad, O-H), 2537 (B-H), 1999, 1898 (C≡O in local C_{3V} symmetry) cm^{-1}. ES-MS (negative ion mode): m/z = 595, *nido*-B_9 isotopic distribution. HRMS (ESI): Calculated for $C_{12}H_{23}B_9O_9Re$: 595.1794. Observed: 595.1785.

2-Propynyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (2.7):

The reaction and work-up was performed in a manner analogous to that for the synthesis of 2-propynyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside, except that β-D-galactose-pentaacetate was used as the starting compound (2.7 g, 6.96mmol). Propargyl alcohol, (0.8 mL 14.1mmol) and $BF_3{\cdot}Et_2O$ (1.8 mL 14.3mmol) were used in this reaction. The product obtained from the column chromatography was a colourless oil which formed a white solid upon standing in air for a sufficient period of time. Spreading the oil in a thin layer over a watch glass assisted in drying the product. Yield: 65% (1.8 g). ^1H NMR ($CDCl_3$): δ 5.38 (d,1H, $^3J_{3,4}$= 3.4 Hz, H-4), 5.20 (dd, 1H, $^3J_{1,2}$= 8.0 Hz, $^3J_{2,3}$ = 10.4 Hz, H-2), 5.04 (dd, 1H, $^3J_{2,3}$ = 10.4 Hz, $^3J_{3,4}$= 3.4 Hz, H-3), 4.72 (d, 1H, $^3J_{1,2}$= 8.0 Hz, H-1), 4.37 (d, 2H, $^4J_{7,9}$ = 2.1 Hz, H-7), 4.17 (dd, 2H, $^2J_{6a,6b}$ = -11.3 Hz, $^3J_{5,6a}$ = 6.5 Hz, H-6a), 4.11 (dd, 2H, $^2J_{6a,6b}$ = -11.3 Hz, $^3J_{5,6b}$ = 6.9 Hz, H-6b), 3.92 (m,1H, H-5), 2.46 (m, 1H, $^4J_{7,9}$ = 2.1 Hz, H-9), 2.13, 2.06, 2.04, 1.97 (4s, 12H, OAc CH_3). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.49, 170.33, 170.22, 169.66 (4C, OAc C=O), 98.74 (C-1), 78.30 (C-8), 75.49 (C-9), 70.95, 70.91 (C-3, C-5), 68.58 (C-2), 67.07 (C-4), 61.29 (C-6), 56.00 (C-7), 20.90, 20.76, 20.68, 20.32 (4C, OAc CH_3). IR (KBr): ν 3267 (alkyne C-H),

2124 (w, C≡C), 1751.11 (s, OAc C=O) cm^{-1}. ESI-MS: m/z = 404.4 $[M+NH_4]^+$. MP: 74-77 °C (lit. 55-57°C).[6] TLC (1:1 Hexanes:Ethyl Acetate): R_f = 0.52.

1,2-dicarba-*closo*-dodecaboranyl-1-methyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside (2.8):

The product was synthesized in a manner analogous to that for the glucose derivative. Reagent quantities: $B_{10}H_{14}$: 1.0 g (7.9 mmol), CH_3CN: 50 mL, 2-propynyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoside: 2.0 g (5.12 mmol). Yield: 40% (1.0 g). ^1H NMR ($CDCl_3$): δ 5.38 (d, 1H, H-4, $^3J_{3,4}$ = 3.4 Hz), 5.14 (dd, 1H, H-2, $^3J_{1,2}$ = 7.9 Hz, $^3J_{2,3}$ = 10.5 Hz), 5.00 (dd, 1H, $^3J_{2,3}$ = 10.5 Hz, $^3J_{3,4}$ = 3.4 Hz, H-3), 4.44 (d, 1H, $^3J_{1,2}$ = 7.9 Hz, H-1), 4.26, (d, 1H, H-7a, $^2J_{7a,7b}$ = -10.8 Hz), 4.12 (m, 2H, H-6a,b), 3.94 (d, 1H, $^2J_{7a,7b}$ = -10.8 Hz, H-7b) 3.94, (br s, 1H, H-9), 3.89 (m, 1H, H-5), 2.15, 2.08, 2.04, 1.98 (4s, 12H, OAc CH_3). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 170.23, 169.98, 169.88, 169.55 (OAc C=O), 101.11 (C-1), 71.51 (C-8), 71.20 (C-5), 70.26, 70.17 (C-3, 7), 68.30 (C-2), 66.72 (C-4), 61.08 (C-6), 58.10 (C-9), 20.65, 20.57, 20.55, 20.46 (OAc CH_3). IR (KBr): 2595, 2577 (B-H), 1749 (OAc C=O) cm^{-1}. MP: 143.5-145.5 °C. ESI-MS: 523.5 $[M+NH_4]^+$. HRMS: Calculated for $C_{17}H_{32}B_{10}O_{10}NH_4$: 522.3352. Observed: 522.3347. TLC (1:1 Hexanes : Ethyl Acetate):R_f = 0.56.

Potassium [7-methyl-(β-D-galactopyranosyloxy)-7,8-dicarba-*nido*-undecaborate] (2.9):

The product was prepared in the same manner as for the glucose derivative. Reagent quantities: 1,2-dicarba-*closo*-dodecaboran-1-ylmethyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranoisde: 2.04 g (4.04 mmol), KOH: 4.4 g (78 mmol). Yield: 80 % (1.17 g). ^{1}H NMR (CD_3OD): δ 4.31,4.22 (2d, 2H, $^{3}J_{1,2} = {}^{3}J_{1^*,2^*} = 7.2$ Hz, H-1,1*), 3.90 (d, 1H, $^{2}J_{7a,7b} = -10.6$ Hz H-7), 3.87 (m, 2H, H-4,4*), 3.75 (m, 4H, H-6a,6a*,6b,6b*), 3.63 (d, 1H, H-7), 3.51 (m, 7H, H-2,2*,3,3*,5,5*,7), 1.94 (br s, $C_{cage}H$, H-9,9*), 2.4 - -0.4 (br m, B-H), -2.70 (br, BHB). ^{13}C{^{1}H} NMR (CD_3OD): δ 103.97, 103.58 (C-1,1*), 78.38 (C-7,7*), 76.33, 76.19 (C-5,5*), 74.96, 74.85 (C-3,3*), 72.60 (C-2,2*), 70.40 (C-4,4*), 62.23 (C-6,6*), 53.83 (C-8,8*), 47.73, 45.94 (C-9,9*). ^{11}B NMR (CD_3OD): δ -10.80, -14.42, -15.13, -16.39, -17.93, -19.37, -21.88, -32.98, -37.47. IR (KBr): ν 3525.75 (broad, O-H), 2525.61 (*nido* B-H) cm^{-1}. ESI-MS: 326.4 [M$^-$]. HRMS (ESI): Calculated for $C_9H_{24}B_9O_6$: 326.2455. Observed: 326.2433. TLC (25% CH_3OH /CH_2Cl_2): $R_f = 0.50$.

Tetraethylammonium [1-(methyl-β-D-galactopyranosyloxy)-3,3,3-tricarbonyl-3-rhenium-1,2-dicarba-*closo*-dodecaborate] (2.2):

Potassium [7-methyl(β-D-galactopranosyloxy)-7,8-dicarba-*nido*-undecaborate] (0.19 g,0.52 mmol) was dissolved in distilled water with tetraethylammonium fluoride (0.39 g, 2.6 mmol) and $[NEt_4]_2[Re(CO)_3Br_3]$ (0.48 g, 0.62 mmol) in a round bottom flask fitted with a reflux condenser. The resulting solution was heated to the reflux temperature for seven days. The solution was cooled to room temperature, frozen and lyophilized to a

light yellow solid (0.85 g). The desired product was isolated by semi-preparative HPLC to give 20 mg out of 495 mg injected (4% yield). ^{1}H NMR (CD_3OD): δ 4.16 (2d, 2H, $^{3}J_{1,2}$ = 7.6 Hz, H-1,1*), 3.97 (d, 2H, $^{2}J_{7a,7b}$ = -10.6 Hz, H-7a,7a*), 3.80 (d, 2H, $^{3}J_{3,4}$ = 2.7 Hz, H-4,4*), 3.69, m, 4H, H-6a,6a*,6b,6b*), 3.60 (2d, 2H, H-7b,7b*), 3.52 (m, 2H, H-2,2*), 3.44 (m, 4H, H-3,3*,5,5*), 3.29 (q, $(CH_3CH_2)_4N$), 1.64 (br s, 2H, $CH_2C_{cage}C_{cage}H$,H-9,9*), 1,29 (t, $(CH_3CH_2)_4N$), 3.10 - 0.80 (br m, B-H).^{13}C{^{1}H} NMR (CD_3OD): δ 200.49 (C≡O), 104.97, 104.93 (C-1,1*), 76.60 (C-7,7* C-5,5*), 74.85 (C-3,3*), 72.69 (C-2,2*), 70.33 (C-4,4*), 62.39 (C-6,6*), 53.91 ($CH_2C_{cage}C_{cage}H$, C-8,8*), 53.28 ($(CH_3CH_2)_4N$), 28.65 ($CH_2C_{cage}C_{cage}H$, C-9,9*), 7.60 ($(CH_3CH_2)_4N$). ^{11}B{^{1}H} NMR (CD_3OD): δ -5.72 (1B), -7.61 (1B), -8.64 (2B), -11.56 (2B), -18.29 (1B), -19.47 (1B), -20.16 ppm (1B). FT-IR (KBr Pellet): ν 3427 (br, O-H), 2544 (B-H), 2001, 1899 (C≡O) cm^{-1}. ESI MS: m/z = 595 [M]$^{-}$. HRMS (ESI): Calculated for $C_{12}H_{23}B_9O_9Re$: 595.1794. Observed: 595.1791.

1,4-Bis-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyloxy)-2-butyne (2.10):

β-D-glucose pentaacetate (5.5 g, 14mmol) and 2-butyne-1,4-diol (0.6 g, 7.09 mmol) were placed in a flame-dried 100 mL 2-neck round bottom flask under N_2 atmosphere. The solids were dissolved with 40 mL of dry CH_2Cl_2 and cooled in an ice bath. Boron trifluoride diethyl etherate (3.5 mL, 28 mmol) was added through a septum via syringe. The reaction allowed to warm to room temperature and stirred for 12 - 24 hours. The reaction was quenched by the addition of a saturated aqueous solution of $NaHCO_3$. The aqueous and organic layers were shaken in a separatory funnel and

separated. The aqueous layer was washed twice with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4, and the solid was subsequently removed under reduced pressure, yielding a gummy, yellowish-white solid. The solid was purified by silica gel column chromatography (60/40 ethyl acetate / hexanes), yielding a colourless syrup which was crystallized to a white solid with ethyl acetate / hexanes. Yield: 38% (2.0 g). ^{1}H NMR ($CDCl_3$): δ 5.26 (dd, 2H, $^3J_{2,3}$ = 9.4 Hz, $^3J_{3,4}$ = 9.5, H-3), 5.12 (dd, 2H, $^3J_{3,4}$ = 9.5, $^3J_{4,5}$ = 10.0 Hz, H-4), 5.01 (dd, 2H, $^3J_{1,2}$ = 7.9 Hz, $^3J_{2,3}$ = 9.4 Hz, H-2), 4.73 (d, 2H, $^3J_{1,2}$ = 7.9 Hz, H-1), 4.45 , 4.40 (2d, 4H, $^2J_{7a,7b}$ = -14.7 Hz, H-7a, 7b), 4.29 (dd, 2H, $^2J_{6a,6b}$ = -12.3 Hz, $^3J_{5,6a}$ = 4.4 Hz, H-6a), 4.17 (dd, 2H, $^2J_{6a,6b}$ = -12.3 Hz, $^3J_{5,6b}$ = 2.4 Hz, H-6b), 3.76 (ddd, 2H, $^3J_{4,5}$ = 10.0 Hz, $^3J_{5,6a}$ = 4.4 Hz, $^3J_{5,6b}$ = 2.4 Hz, H-5), 2.10, 2.08, 2.03, 2.01 (4s, 24H, OAc CH_3). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 170.53, 170.14, 169.32 (OAc *C*=O), 98.34 (C-1), 81.75 (C-8), 72.71 (C-3), 71.93 (C-5), 71.12 (C-2), 68.32 (C-4), 61.78 (C-6), 56.11 (C-7), 20.64, 20.52 (OAc *C*H_3). IR (KBr): ν 1755 (C=O) cm^{-1}. CI-MS: m/z = 747 $[M]^+$. TLC (1:1 Hexanes/Ethyl Acetate): R_f = 0.13. MP: 119-122°C (lit. 119-122°C).[50]

1,2-Bis(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyloxymethyl)-1,2-dicarba-*closo*-dodecaborane (2.11):

Decaborane(14) (0.25 g, 2.0 mmol) was dried under vacuum for approximately 2 hours in a 2-neck round bottom flask. The system was then placed under N_2 atmosphere, and dry CH_3CN (13.4 mL) added through a septum via syringe. The solution was stirred overnight at room temperature. Compound **2.10** (1.0 g, 1.4 mmol) was added and the solution was heated to reflux with stirring for 48 hours, forming a dark yellow solution.

The solution was filtered and concentrated to dryness under reduced pressure, yielding a light yellow solid. The crude product was purified by silica gel column chromatography (50/50 ethyl acetate / hexanes → 65/35 EtOAc / hexanes in 5% increments of EtOAc). The resulting product was a glassy, yellowish solid. TLC analysis showed three Pd - active spots, with the slowest - running corresponding to the desired product. The impure solid was recrystallized from ethyl acetate and hexanes, yielding a white solid. Yield: 62% (0.74 g). ^{1}H NMR ($CDCl_3$): δ 5.22 (dd, 2H , $^{3}J_{2,3}$ = 9.5 Hz, $^{3}J_{3,4}$ = 9.5 Hz, H-3), 5.08 (dd, 2H, $^{3}J_{3,4}$ = 9.5 Hz, $^{3}J_{4,5}$ = 10.0 Hz, H-4), 5.01 (dd, 2H, $^{3}J_{1,2}$ = 7.9 Hz, $^{3}J_{2,3}$ = 9.5 Hz, H-2), 4.55 (d, 2H, $^{3}J_{1,2}$ = 7.9 Hz, H-1), 4.32 (d, 2H, $^{2}J_{7a,7b}$ = -13.0 Hz, H-7a), 4.28 (dd, 2H, $^{2}J_{6a,6b}$ = -12.4 Hz, $^{3}J_{5,6a}$ = 4.4 Hz, H-6a), 4.15 (dd, 2H, $^{2}J_{6a,6b}$ = -12.4 Hz, $^{3}J_{5,6b}$ = 2.3 Hz, H-6b), 4.02 (d, 2H, $^{2}J_{7a,7b}$ = -13.0 Hz, H-7b), 3.72 (ddd, 2H, $^{3}J_{4,5}$ = 10.0 Hz, $^{3}J_{5,6a}$ = 4.4 Hz, $^{3}J_{5,6b}$ = 2.3 Hz, H-5), 2.10, 2.10, 2.03, 2.01 (4s, 24H, OAc CH_3). $^{13}C\{^{1}H\}$ NMR ($CDCl_3$): δ 170.50, 170.09, 169.32, 169.21 (OAc C=O), 100.80 (C-1), 75.90 (C-8), 72.40 (C-3), 72.19 (C-5), 70.88 (C-2), 68.87 (C-7), 68.14 (C-4), 61.61 (C-6), 20.65, 20.62, 20.51 (OAc CH_3). ^{11}B NMR ($CDCl_3$): δ -3.96 (2B), -11.72 (8B). IR (KBr): ν 2588 (B-H), 1757 cm^{-1} (OAc C=O) cm^{-1}. MS (ESI): m/z: 883.9 $[M+NH_4]^+$, 888.8 $[M+Na]^+$ (with B_{10} isotopic distribution). TLC (1:1 Hexanes/Ethyl Acetate): R_f = 0.16. MP: 176-179 °C.

Sodium [7,8-bis(β-D-glucopyranosyloxymethyl)-7,8-dicarba-*nido*-undecaborate] (2.12a):

Compound **2.11** (0.50 g, 0.58 mmol) and NaOH (0.46 g, 12 mmol) were placed in a round-bottom flask and dissolved in 50 mL of absolute ethanol. The flask was fitted

with a condenser, and the solution heated to approximately 60°C for 12 hours. Following the heating period, the flask was cooled to room temperature, and $CO_{2\,(g)}$ passed through the solution, precipitating the excess NaOH as Na_2CO_3. The solid was removed by filtration over a pad of Celite, and the resulting solution concentrated to dryness by rotary evaporation, giving a white solid. The crude solid was dissolved in distilled water and the pH of the solution adjusted to 6 by dropwise addition of 1M HCl. The neutralized solution was concentrated to dryness by rotary evaporation. The resulting solid was washed with small portions of methanol, and the combined extracts were concentrated, and passed through a short plug of silica gel. The target compound was eluted with 30% methanol in dichloromethane, giving 0.16 g, (52%). ^{1}H NMR (CD_3OD) δ 4.65, 4.38 (2d, 2H, $^3J_{1,2} = {}^3J_{1^*,2^*} = 7.8$ Hz, H-1,1*), 4.17 (2d, 2H, H-7a,7a*), 3.91 (m, 4H, H-6a,6a*,7b,7b*), 3.78 (m, 2H, H-6b,6b*), 3.43 (m, 4H, 3,3*,4,4*), 3.35 (m, 4H, H-2,2*,5,5*), -0.30 - 2.60 (br, m, 18H, B-H), -2.48 (br s, B-H-B). $^{13}C\{^1H\}$ NMR (CD_3OD) δ 104.11, 104.04 (C-1,1*), 77.92, 77.85, 77.76 (C-3,3*,5,5*), 76.13, 75.06, 74.93, 74.84 (C-7,7*,2,2*), 71.51, 71.45 (C-4,4*), 62.58, 62.53 (C-6,6*). $^{11}B\{^1H\}$ NMR (CD_3OD): δ -9.91, -16.95, -33.84, -37.75. IR (KBr): ν = 3359 (O-H), 2512 (B-H) cm^{-1}. TLC (1:3 CH_3OH:CH_2Cl_2): R_f = 0.11. ESI-MS: m/z = 518.5 [M$^-$]. HRMS(ESI) Calculated for $C_{16}H_{36}B_9O_{12}$: 518.3094. Observed: 518.3120.

Sodium [(7-methyl-β-D-glucopyranosyloxy)-iodo-7,8-dicarba-*nido*-undecaborate] (2.13):

Compound **2.6d** (0.093 g, 0.27 mmol) and NaI (0.048 g, 0.32 mmol) were dissoved in 5 mL of distilled H_2O in a 25 mL round bottom flask. Chloramine-T (0.13 g, 0.57 mmol) was added, and the resulting solution stirred for 24 hours at room temperature. After quenching with 0.1M $Na_2S_2O_5$, the solution was concentrated to dryness by rotary evaporation, dissolved in 20 mL of a 80:20 H_2O:Acetonitrile mixture, and the target compound isolated by semipreparative HPLC (Dynamax C_{18} 10x250mm column, elution method **D**). Yield: 0.027 g (21 %). ^{1}H NMR (600 MHz, CD_3OD): δ 4.42 (d, 1H, $^3J_{1,2}$ = 7.8 Hz, H-1), 4.38 (d, 1H, $^3J_{1^*,2^*}$ = 7.8 Hz, H-1*), 3.98 (m, 3H, H-6a,6a*,7a), 3.83 (d, 1H, $^2J_{7a^*,7b^*}$ = -10.9 Hz, H-7a*), 3.80 (m, 3H, H-6b,6b*,7b*), 3.70 (d, 1H, $^2J_{7a,7b}$ = -11.4 Hz, H-7b), 3.48 (m, 5H, H-3,3*,4,4*, 5 or 5*), 3.36 (m, 1H, H-5 or 5*), 3.29 (m, 2H, H-2,2*), 2.56 (br s, 2H, H-9,9*), 0.10 - 2.60 (br m, B-H), -2.74 (br s, B-H-B). ^{13}C{^{1}H} NMR (151 MHz, CD_3OD): δ 103.81 (C-1*), 102.03 (C-1), 78.31 (C-7*), 77.82 (C-3,3*), 77.55, 77.44 (C-5/5*), 76.95 (C-7*), 75.02, 74.94 (C-2,2*), 71.27 (C-4,4*), 62.40, 62.21 (C-6,6*), 56.25 (C-9,9*), 53.32 (C-8,8*). ^{11}B{^{1}H} NMR (192 MHz, CD_3OD): δ -6.20, -15.78, -18.08, -21.39, -24.71, -29.56, -37.31. IR (KBr): ν 3417 (O-H), 2540 (B-H) cm^{-1}. HPLC (Elution method **C**): t_R = 12.2 min. ESI-MS: m/z = 452.2 [M]$^-$. HRMS (ESI$^-$): Calculated for $C_9H_{23}B_9O_6I$: 452.1421. Observed: 452.1438.

Sodium [7,8-bis(methyl-β-D-glucopyranosyloxy)-iodo-7,8-dicarba-*nido*-undecaborate] (2.14):

Compound **2.12** (0.045 g, 0.08 mmol) and NaI (0.020 g, 0.13 mmol) were dissolved in 5 mL of distilled H_2O in a 25 mL round bottom flask. Chloramine-T (0.052 g, 0.23 mmol) was added, and the resulting solution stirred at room temperature for 24 hours. The solution was concentrated to dryness on a rotary evaporator, re-dissolved in 80:20 H_2O:Acetonitrile (20 mL), and the product isolated by semipreparative HPLC (Dynamax C_8 column, elution method **D**). Yield: 0.010g (18 %). ^{1}H NMR (600 MHz, CD_3OD): δ 4.44 (m, 4H, 3xH-1, H-7a), 4.35 (m, 2H, H-1, H-7a), 4.23 (d, 1H, H-7a), 4.07 (d, 1H, H-7a), 4.01 (3d, 3H, H-7b), 3.90 (m, 5H, H-7b, 4xH-6a), 3.72 (m, 4H, H-6b), 3.36 (m, 16H, 4xH-2, 4xH3, 4xH4, 4xH-5), 0.10 - 2.70 (br m, B-H), -2.65 (br s, B-H-B). ^{13}C{^{1}H} NMR (151 MHz, CD_3OD): δ 105.02, 104.46, 104.32, 104.17 (4xC-1), 78.28, 78.18, 78.02 (4xC-3, 4xC-5), 75.51, 75.43, 75.23, 75.17, 75.06 (4xC-7, 4xC-2), 71.93, 71.77, 71.71 (4xC-4), 62.90 (4xC-6). ^{11}B{^{1}H} NMR (192 MHz, CD_3OD): δ -4.45, -15.20, -19.43, -24.73,-29.57, -36.82. IR (KBr): ν 3422 (O-H), 2545 (B-H) cm^{-1}. ESI-MS: m/z = 644.2 [M]$^-$. HRMS (ESI$^-$): Calculated for $C_{16}H_{35}B_9O_{12}I$: 644.2061. Observed: 644.2050.

Radiolabelling procedures: [^{125}I]-2.13:

To a 2 mL reaction vial filmed with 20 μg of Iodogen® was added 100 μL of a solution containing **1** (1.0 mg/mL in 5% aqueous acetic acid). To this was added Na[^{125}I] (0.74 - 5.6 MBq, 20-150 μCi) in 0.1N NaOH. The vials were stirred for five minutes before

addition of 10 μL 0.1M $Na_2S_2O_5$. The product was isolated via semi-preparative HPLC (Method **B**, Dynamax 10x250mm C_{18} column at 4.7mL/min). Yield: 29%: HPLC t_R 12.7 (Method **C**).

2.8 References

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Chapter 3

Microwave - Assisted Synthesis of Metallocarboranes

3.1 Introduction to Microwave - Assisted Syntheses

Due to the difficulties encountered while synthesizing the metallocarboranyl glycosides **2.1** and **2.2**, such as the long reaction times required with conventional reflux heating, and low yields following HPLC purification of incomplete reaction mixtures, alternative strategies were sought to improve the syntheses of these targets. Most notably, microwave-assisted heating was investigated as a means of shortening reaction times and permitting higher yields of the target compounds to be obtained, with reactions driven to completion in order to avoid time-consuming HPLC purifications.

The use of microwave heating in synthesis has gained a great deal of interest in recent years. It has been applied successfully to a variety of reactions in organic synthesis.[1-3] In addition, a number of examples of the synthesis of organometallic[4,5] complexes have been reported to be enhanced by microwave irradiation. For example, several groups have reported significant rate enhancement of the synthesis of metal carbonyl complexes,[4-6] organometallic di-olefin, arene and cyclopentadienyl complexes,[7] and thiolate complexes.[8] Also, Mingos and co-workers have reported the microwave-assisted synthesis of metallocarborane derivatives.[9]

The use of microwave heating has also been applied to the synthesis of radiopharmaceutical compounds. For example, Hung and co-workers have used microwave irradiation to effect rapid preparation of ^{99m}Tc-based perfusion agents such as

^{99m}Tc-Sestamibi, ^{99m}Tc-teboroxime, and ^{99m}Tc-bicisate.[10-12] More recently, Oh *et al.* have shown that microwave heating enhanced the synthesis of ^{99m}Tc-ciprofloxacin, a tracer of bacterial infection.[13] In addition, Park and co-workers have reported the microwave-assisted synthesis of ^{188}Re complexes of mercaptoacetyltriglycine (MAG_3) and 2,9-dimethyl-4,7-diazadecane-2,9-dithiol (TDD) which would serve as bifunctional chelate complexes in potential therapeutic radiopharamaceuticals.[14]

Microwave heating, in contrast to traditional heating methods, involves transfer of microwave energy to molecules via dipole rotation or ionic conduction. This direct transfer of energy (as opposed to conduction) allows for a more efficient heating of the reaction medium.[1,15] Also, in sealed vessels, higher temperatures and pressures can be achieved, which assist in driving a reaction to completion in a shorter amount of time, relative to reflux heating.[1] With a well-established precedent and growing interest in organic, organometallic and radiopharmaceutical synthesis, the use of microwave heating may afford an opportunity to prepare functionalized metallocarboranes in better yields with reduced reaction times.

3.2 Initial Attempts to Prepare Metallocarboranyl Glycosides via Microwave Irradiation

In addition to microwave heating, alternative reagents were employed in order to further assist in optimizing the synthesis of the model metallocarboranyl glycosides. The rhenium reagent $[Re(CO)_3(H_2O)_3]Br$ was prepared by the method of Zubieta *et al*,[16] replacing the compound $(NEt_4)_2[Re(CO)_3Br_3]$ that was used previously. The use of the trisaquo compound eliminated any issues involving mixed counter ions, thus the more

water-soluble carboranyl glycoside **2.6d**, the sodium salt of *nido*-carboranyl glucose ligand **2.6**, was used for all further syntheses of the mono-glucose-substituted metallocarboranyl glycoside. Aqueous solutions of sodium fluoride were subsequently used as the reaction medium.

Initial synthetic attempts were conducted using a domestic microwave oven (SANYO EM-S250, 900W). Reactions were performed in an open Erlenmeyer flask. HPLC analysis of the product mixtures generated by this method indicated a more rapid conversion of the ligand to the desired compound. However, heating in the open vessel resulted in bumping and rapid evaporation of the solutions. The rapid loss of the reaction medium resulted in degradation of the carbohydrate-based compounds, thus it appeared that successful syntheses would require an efficient containment the reaction medium.

Subsequent reactions were attempted in screw-cap Teflon vials. Since these vessels were not airtight, the same problems arose as in the case of reactions in open flasks. Glass pressure tubes (Aldrich) equipped with O-ring seals were also used in attempted syntheses. However, under the pressures that developed during microwave irradiation, the seals failed within 40 seconds at the maximum power output of the oven.

The next type of vessel to be tested for use in the synthesis of the metallocarboranyl glycoside targets was a Parr Microwave Acid Digestion Bomb (Parr Instruments). This device consisted of a Teflon reaction cup with a volume of 45 mL. This reaction cup was equipped with an O-ring seal, which was held inside a microwave-transparent polymer casing that hand-tightened to provide a seal capable of withstanding internal pressures up to 1200 psi (Figure 3.1). Although this vessel was initially

successful in synthesizing **2.1** (Na^+ salt) in good yield, (77% following silica gel column chromatography), subsequent attempts to prepare more advanced derivatives such as **2.3** were less successful, owing to a number of difficulties. The main difficulty in preparing the metallocarborane-carbohydrate derivatives using the Parr reactor was the lack of control over temperature and pressure within the vessel. Thus, failure of the Teflon seals was observed, and the final use of the Parr bomb resulted in its destruction. Mingos and co-workers have previously reported attempts to synthesize organometallic compounds in the Parr Microwave Acid Digestion Bomb, and similarly found this vessel unsuitable for their purposes.[17] It became apparent that a research-grade microwave reactor was required which would allow for monitoring and control of temperature and pressure within the reaction vessel.

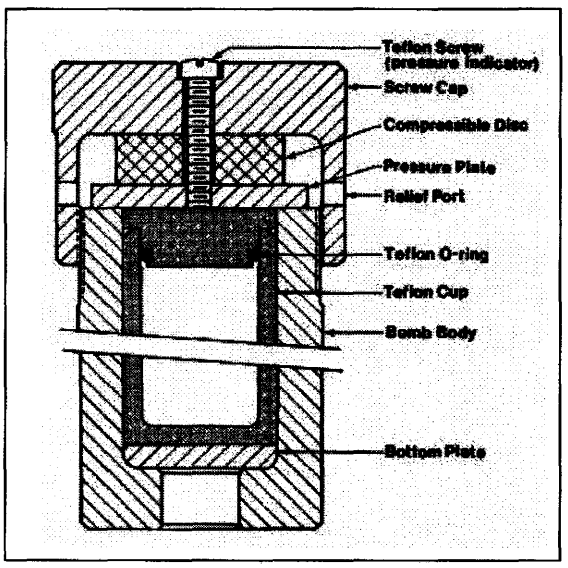
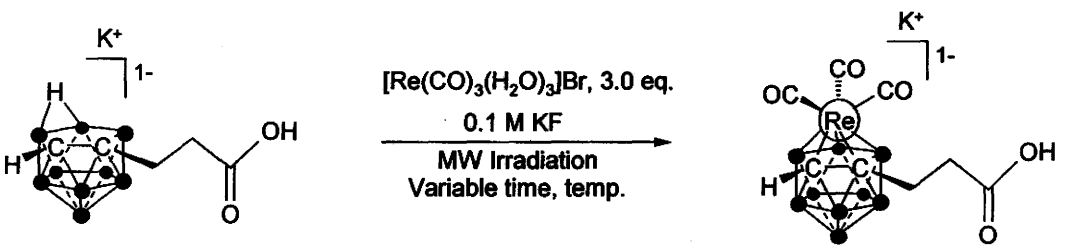


Figure 3.1: Schematic diagram of the Parr Microwave Acid Digestion Bomb.[18]

3.3 Systematic Investigation Using the Biotage Initiator Sixty Reactor

The reactor chosen for further study was the Biotage Initiator microwave. This instrument is capable of applying a maximum of 300W of power, and has a maximum operating temperature of 250 °C. Reactions are performed inside glass vials equipped with a magnetic stirring vane, and sealed with an aluminum cap and rubber septum which are crimped onto the vial. The Biotage Initiator Sixty instrument is equipped with a robot arm and test tube racks capable of holding up to sixty vials. This equipment allowed for sequential, automated performance of multiple reactions.

A generic carborane ligand was initially chosen to study the fluoride ion-mediated metallocarborane-forming reaction under microwave irradiation. This ligand was the carboxylic acid-derived *ortho*-carborane **3.1**, prepared by the method of Valliant and co-workers.[19-21] The metallation reaction (Scheme. 3.1) was evaluated with respect to time and temperature.



3.1 **3.2**

Scheme 3.1: Microwave-assisted synthesis of compound **3.2**.

Two series of reactions were conducted, in which the time and temperature were varied, respectively. In each microwave reaction vial (solution volumes permitted were 2-

5 mL) were placed 5-10 mg of ligand **3.1**, three equivalents of $[Re(CO)_3(H_2O)_3]Br$, and 2.2 mL of 0.1 M aqueous KF. Variable time reactions were set to run at 180°C, and variable temperature reactions were fixed at five minutes' duration. These parameters were programmed into the Initiator instrument, and the reactions run accordingly. Crude reaction solutions were analyzed by HPLC. The desired product was identified by the HPLC peak whose UV absorbance contained a local maximum at approximately 275 nm, which was found to be diagnostic of compounds containing the $Re(CO)_3^+$ core. This peak was collected and analyzed by negative ion electrospray mass spectrometry. The mass peak m/z = 475 confirmed the target ion. The approximate percent yield was calculated based on the ratio of the product peak area to that of the *nido*-carborane starting material. The results are summarized in Figures 3.2 and 3.3.

From the plots, it can be seen that the reactions were most strongly influenced by temperature. It became apparent that reactions run at 200°C resulted in the most rapid conversion to the product. A further series of reactions was run in which the temperatures were varied from 100 to 200°C, keeping the reaction time constant at 5 minutes, and running the reactions in 0.5 M KF solution. The progress of reactions was monitored by ^1H NMR spectroscopy. From the stacked plot of ^1H NMR spectra (Figure 3.4) for this series, it can be seen that reactions at temperatures below 150°C did not result in significant conversion to the desired product within the 5 minute reaction time, whereas reaction at 200°C resulted in nearly complete conversion in as little as 5 minutes.

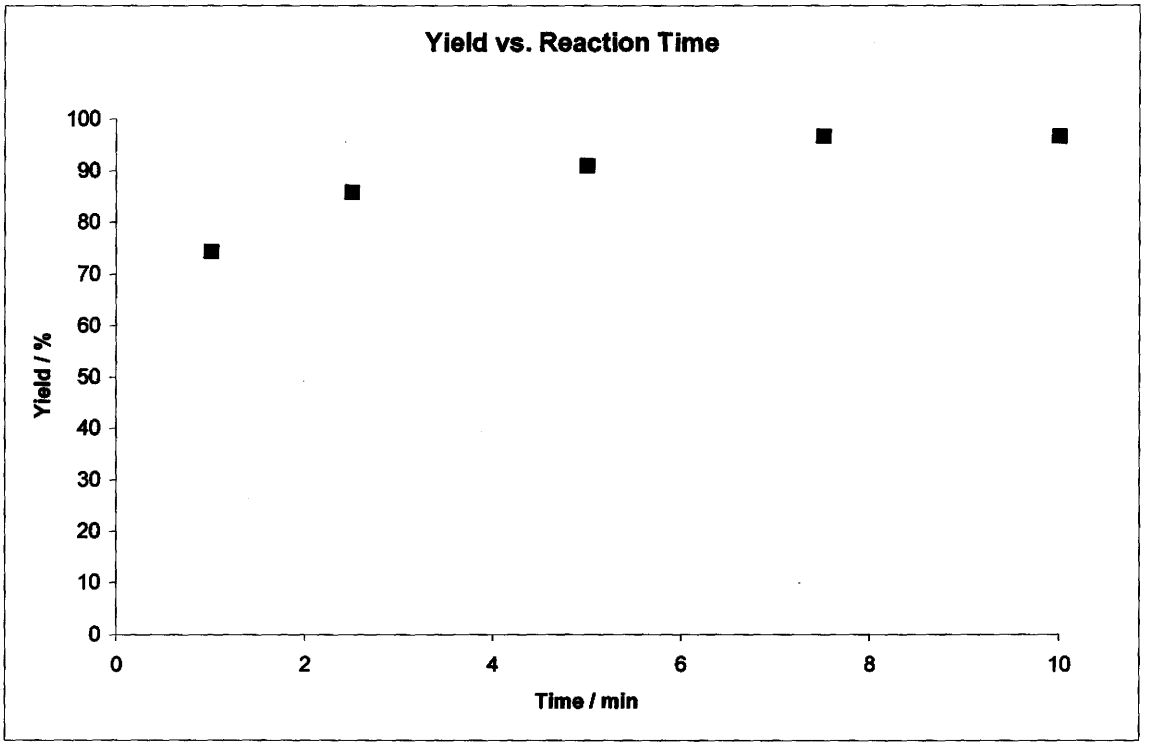


Figure 3.2: Plot of estimated yield vs. reaction time for initial MW investigation.

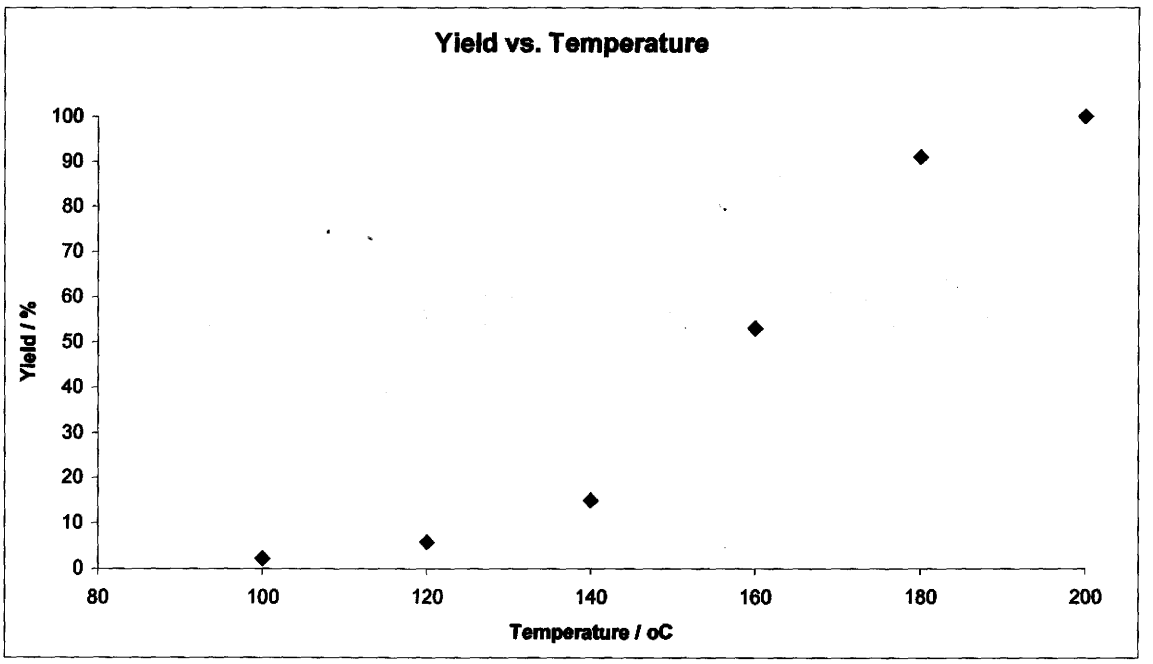


Figure 3.3: Plot of estimated yield vs. reaction temperature for initial MW investigation.

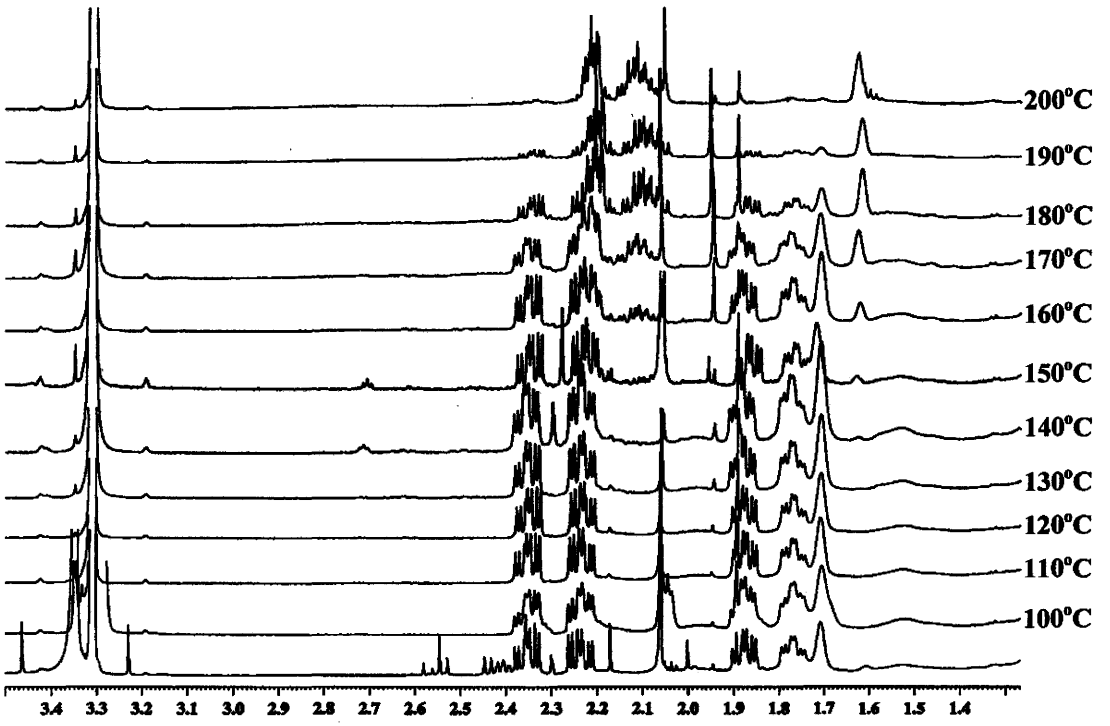
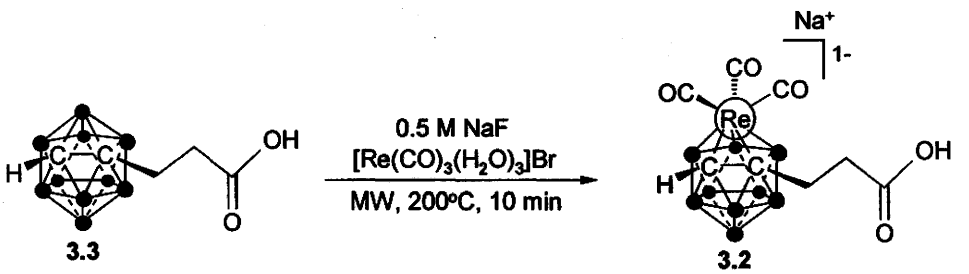


Figure 3.4: Stacked ^{1}H NMR spectra of MW temperature series for conversion of **3.1** (bottom) to **3.2**.

To simplify purification, reactions were driven to completion by multiple additions of the rhenium reagent $[Re(CO)_3(H_2O)_3]Br$ and subsequent microwave irradiations until the *nido*-carborane ligand could no longer be observed in the NMR spectra. It is worth noting that for this purpose, it was preferable to use $^{11}B\{^{1}H\}$ NMR spectroscopy, since two low-frequency signals near -33 and -38 ppm are both diagnostic of *nido-ortho*-carboranes,[22,23] and far-removed from the signals corresponding to the product rhenium complex.

Additionally, during the course of our group's research, it was discovered that some rhenacarborane complexes could be prepared directly from the *closo*-carborane precursor.[21] In the case of target **3.2**, it was possible to synthesis this compound directly from *closo*-carborane **3.3** (Scheme 3.2) by the methods described above, even though **3.3** has poor water solubility at room temperature. Using this protocol, compound **3.2** was obtained in 93% yield. Characterization of **3.2** was consistent with that reported previously.[19]



Scheme 3.2: MW-assisted synthesis of **3.2** directly from *closo*-carborane precursor.

3.4 Application of the Biotage Initiator Sixty to the Synthesis of Metallocarboranyl Glycosides

With optimal conditions determined for the synthesis of simple $Re(CO)_3$-metallocarborane derivatives using the Biotage Initiator Sixty microwave, the synthesis of the target metallocarboranyl glycosides **2.1** and **2.3**, subsequently renamed **3.4** and **3.5**, respectively (Figure 3.5) was undertaken.

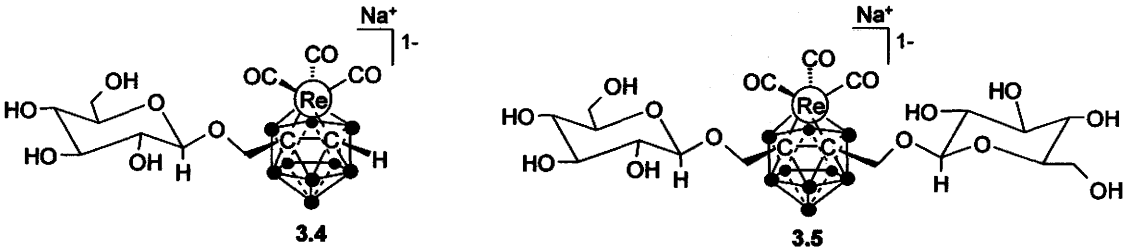
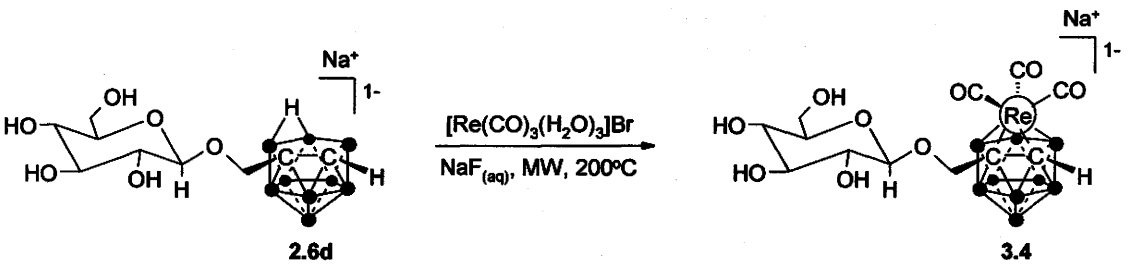
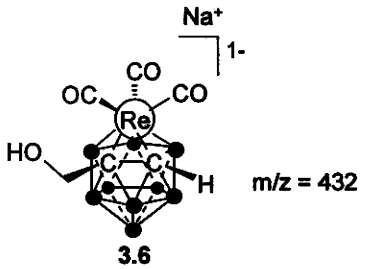


Figure 3.5: Metallocarboranyl glycoside targets.

The synthesis of metallocarboranyl glycoside **3.4** is illustrated in Scheme 3.3. The *nido*-carboranyl glycoside **2.6d** was used as the precursor in this synthesis. Initial attempts to prepare **3.4** were undertaken by combining **2.6d** with three equivalents of $[Re(CO)_3(H_2O)_3]Br$ in approximately 2.2mL of 0.5M NaF in a 5mL microwave reaction vial. The vials were sealed and heated for 15 minutes at 200°C. Analysis by electrospray mass spectrometry indicated the presence of the target anion at m/z = 595. Additionally, a second mass peak was observed at m/z = 432. This mass peak, as did the target peak, contained the isotope distribution corresponding to the B_9Re cluster. The anion mass of 432 appeared to correspond to loss of glucose from the overall complex (Figure 3.6).



Scheme 3.3: Initial MW-assisted synthesis of **3.4**.



3.6

Figure 3.6: Suspected degradation product of synthesis of compound **3.4**.

The formation of **3.6** was believed to be the result of either the high temperature of the reaction (200°C), or the Brønsted acidity of the aquo ligands in the reagent $[Re(CO)_3(H_2O)_3]Br$, which are known to be weakly acidic (pKa =7.5).[24-26] To explore the cause of the degradation, **2.6d** was combined with an excess (3 mole equivalents) of $[Re(CO)_3(H_2O)_3]Br$, and heated at high temperature. Without F^- present, minimal complexation should take place. Consequently, the acidity of the Re(I) starting material would become apparent.[27] In a parallel experiment, **2.6d** was heated with sodium fluoride, without any rhenium reagent to see if simply the high reaction temperature is responsible for the observed degradation. Each reaction vial was heated for 5 minutes at 200°C. Samples of the two reactions were analyzed by electrospray mass spectrometry (Figure 3.7).

The product solution for the reaction containing no fluoride was discoloured, appearing brown, suggesting decomposition of the glucose moiety, which was further evidenced by the odour of burned sugar upon opening the vial. The negative-ion electrospray mass spectrum for the product of the "no F^-" reaction did not readily indicate the presence of the target anion at m/z = 595. Rather, a number of lower mass peaks, in

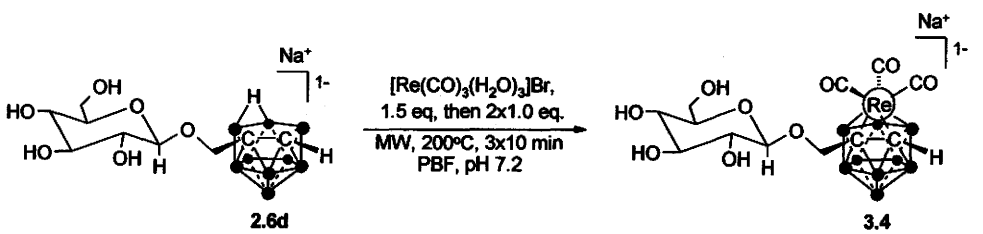
particular, the familiar byproduct peak at m/z = 432 were present. Additionally, the HPLC chromatogram showed the presence of multiple species, those being either rhenium clusters or metallocarborane derivatives resulting from the loss of the glucose group.

In contrast to this result, the product solution for the "F^- only" reaction was not obviously discoloured. The HPLC trace indicated a single major species, and the negative-ion electrospray mass spectrum gave the mass peak at m/z = 326, corresponding to the *nido*-carboranyl glycoside anion **2.6**, as the dominant species. No other peaks were observed containing the boron isotope distribution for the B_9 cluster.

Based on these results, it s is reasonable to conclude that the excess rhenium compound contributed to the degradation, and also that the presence of fluoride is required in order to prevent excessive degradation of the product. These experiments suggested that buffering the reaction mixtures should reduce the extent of the unwanted degradation of **3.4**.

The effect of pH on the degree of degradation of **3.4** was subsequently examined. Four buffer solutions, with pH values of 7.3 (phosphate), 8.5, 9.7, and 10.9 (carbonate/bicarbonate), were prepared and used as reaction media for the synthesis of target **3.4**. Aliquots of the crude product mixtures were analyzed by HPLC and electrospray mass spectrometry. These results showed that a reaction medium with pH close to 7 is optimal for the synthesis of compound **3.4**, since lower pH is expected to result in compound degradation and higher pH is conducive to excess rhenium cluster formation.

Following this discovery, the synthesis of compound **3.4** (Scheme 3.4) was revised to incorporate an appropriately buffered reaction medium. Jaouen and co-workers have recently labeled biomolecues with maleimide-functionalized $CpM(CO)_3$ complexes (M = Mo, W), for which they have used a phosphate buffered saline solution with a reported pH of 7.4 in the ultimate labeling step.[28] For the purposes of synthesizing **3.4**, the formulation of this solution was modified, where the NaCl / KCl was replaced with sodium fluoride. The overall fluoride concentration was maintained at 0.5M. This buffer, hereafter referred to as PBF ("phosphate-buffered fluoride"), consisted of 0.003M NaH_2PO_4 and 0.01 M Na_2HPO_4 in deionized water. The pH of this and subsequent solutions were typically around 7.2. To further assist in minimizing the degradation of **3.4** during microwave irradiation, the initial amount of $[Re(CO)_3(H_2O)_3]Br$ in the reactions was reduced from three equivalents to 1.5 equivalents. The reaction time was reduced from 15 minutes to 10 minutes per irradiation. Additional aliquots of $[Re(CO)_3(H_2O)_3]Br$ were still added (usually an additional 1 eq) to the reaction mixture to ensure complete consumption of the starting material. Analysis of reactions using these new conditions by HPLC and mass spectrometry indicated a lesser extent of degradation and rhenium cluster formation than those run under non-buffered conditions. Compound **3.4** was obtained in 31% yield following silica gel column chromatography.



2.6d 3.4

Scheme 3.4: Modified synthesis of **3.4** incorporating a buffered medium.

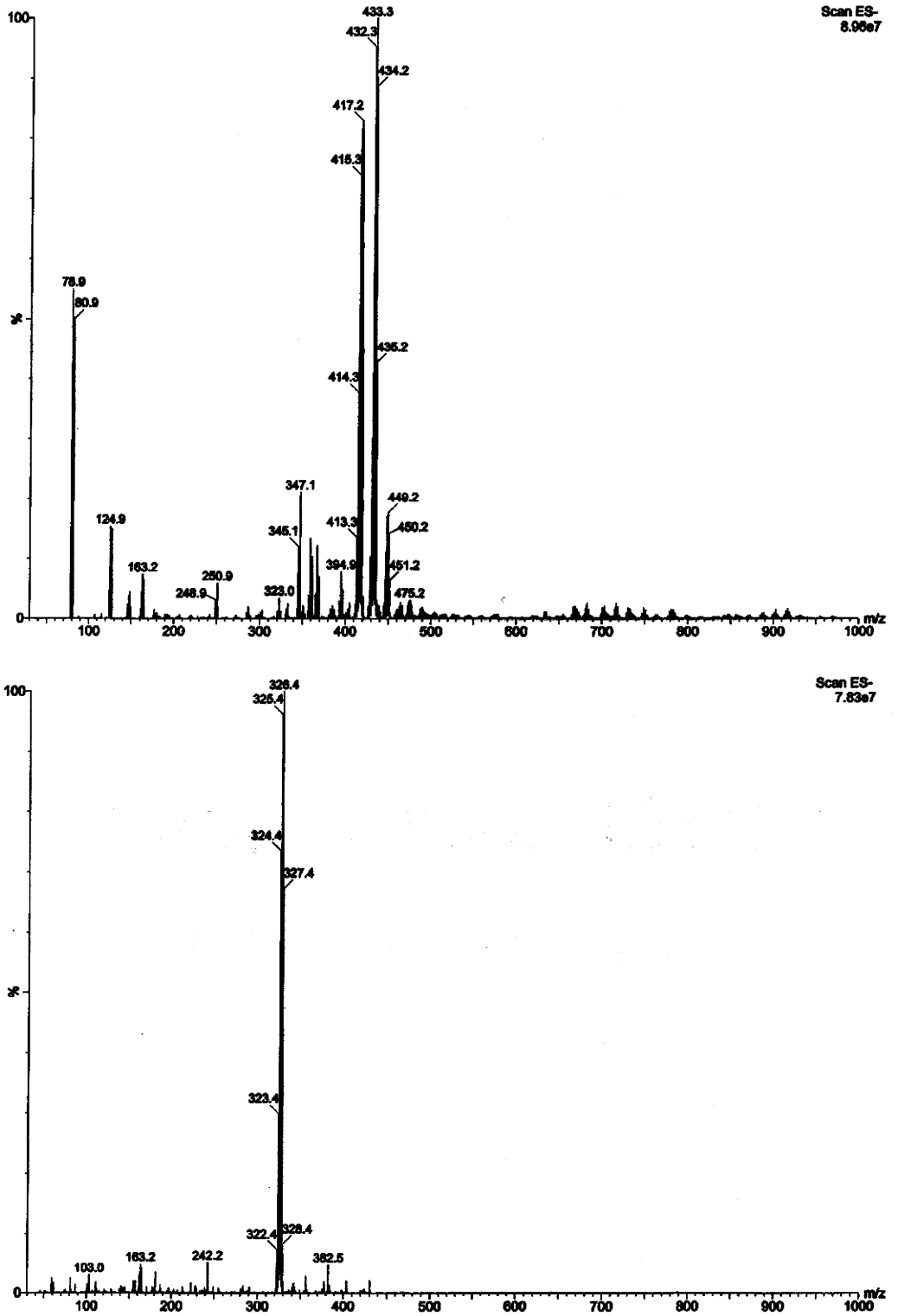


Figure 3.7: Electrospray mass spectra of MW irradiation test reactions. Top: Vial A; **2.6d** with $[Re(CO)_3(H_2O)_3]Br$, no NaF. Bottom: Vial B; **2.6d** with NaF, no $[Re(CO)_3(H_2O)_3]Br$.

3.5: Carborane Cage Isomerization in Microwave-Assisted Reactions

During the course of our group's investigation into the microwave assisted syntheses of various Re(I) metallocarboranyl targets, it was discovered through NMR spectroscopic and X-ray crystallographic studies of the products that for certain metallocarboranes, one of the carbon atoms of the carborane cage migrates out of the metal bonding face of the cluster to the lower belt of cage atoms (Figure 3.8).[29,30]

The carborane cage isomerization of *ortho* (1,2) to *meta* (1,7) to *para* (1,12) at elevated temperatures is well known for dicarba-*closo*-dodecaborane,[31-33] and it has been reported that the presence of bulky substituents on the carbon atoms of substituted *ortho*-carborane derivatives resulted in a decrease in the temperature required to effect the *ortho*- to *meta*- isomerization.[34] Several groups have reported the migration of the carbon atoms away from the *ortho*-configuration in various metallocarborane derivatives as well.[9,35-44] The carborane cage isomerization described as a 120° rotation of the triangular face containing the migrating carbon atom to transform the "*ortho*" 3,1,2 MC_2B_9 cage configuration into the 2,1,8-MC_2B_9 isomer.[30,45] It has been established that the separation of the carbon atoms in carborane clusters is associated with their increased thermodynamic stability.[46,47] Furthermore, the isomerization of carborane and metallocarborane complexes bearing bulky groups as carbon atom substituents or as ligands on the metal centers is rationalized in part by a lowering of the energy barrier to isomerization by relief of steric crowding about the carborane cage.[30,35-37,40,41] It has also been shown, in the case of other examples of $Re(CO)_3$-metallocarborane derivatives

prepared by our group, that electronic factors, as well as steric ones appear to affect the likelihood of isomerization, and the temperatures at which it proceeds.[29,30]

Figure 3.8: Illustration of 3,1,2 and 2,1,8-MC_2B_9 cage isomers. [38]

Unfortunately the metallocarboranyl glycosides studied in this research, along with **3.2**, could not be obtained in crystalline form. Consequently it was not possible to directly determine the configuration of the metallocarborane cluster. Therefore, it was necessary to use other means by which the nature of the metallocarborane cages could be determined, namely, NMR spectroscopy.

The $^{11}B\{^1H\}$ NMR spectrum of **3.2** (Figure 3.9) shows an apparent 1:2:1:2:1:2 ratio of signal intensities. This pattern was consistent with other Re-metallocarboranes reported by our group to contain the 3,1,2 cage configuration.[30] By contrast, the $^{11}B\{^1H\}$ NMR spectrum of **3.4** (Figure 3.10) shows a somewhat different pattern for the signals in this region, which may be evidence for the existence of **3.4** as a different cage isomer. However, due to the apparent overlap of signals in these spectra, another method to probe the environment of the metallocarborane cage would be preferable.

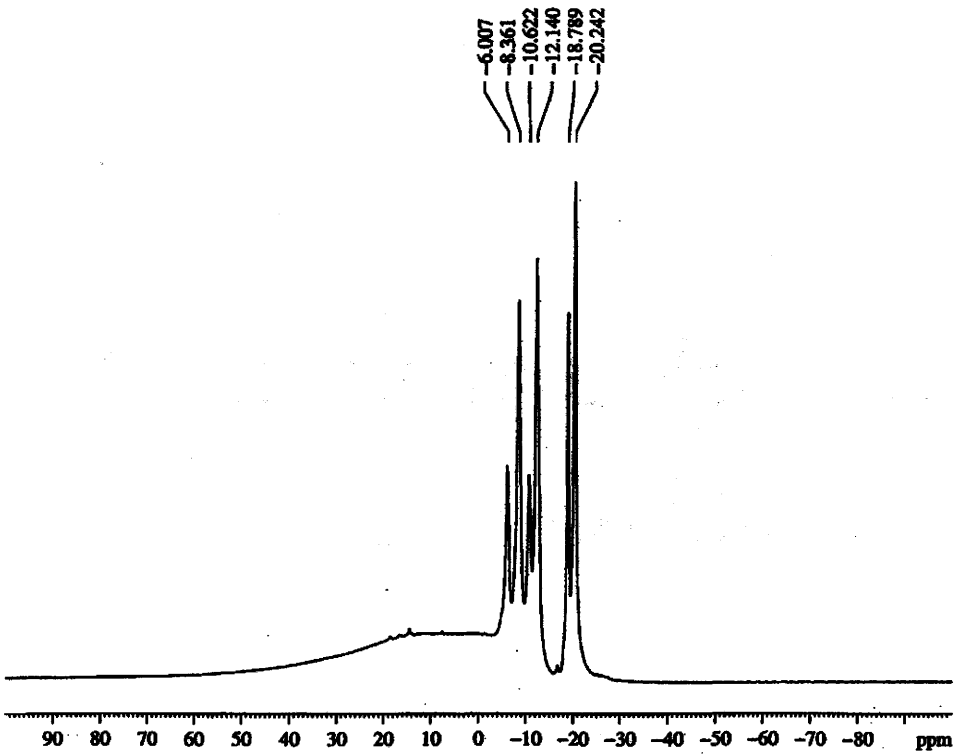


Figure 3.9: $^{11}B\{^1H\}$ NMR spectrum of **3.2** (192 MHz, CD_3OD).

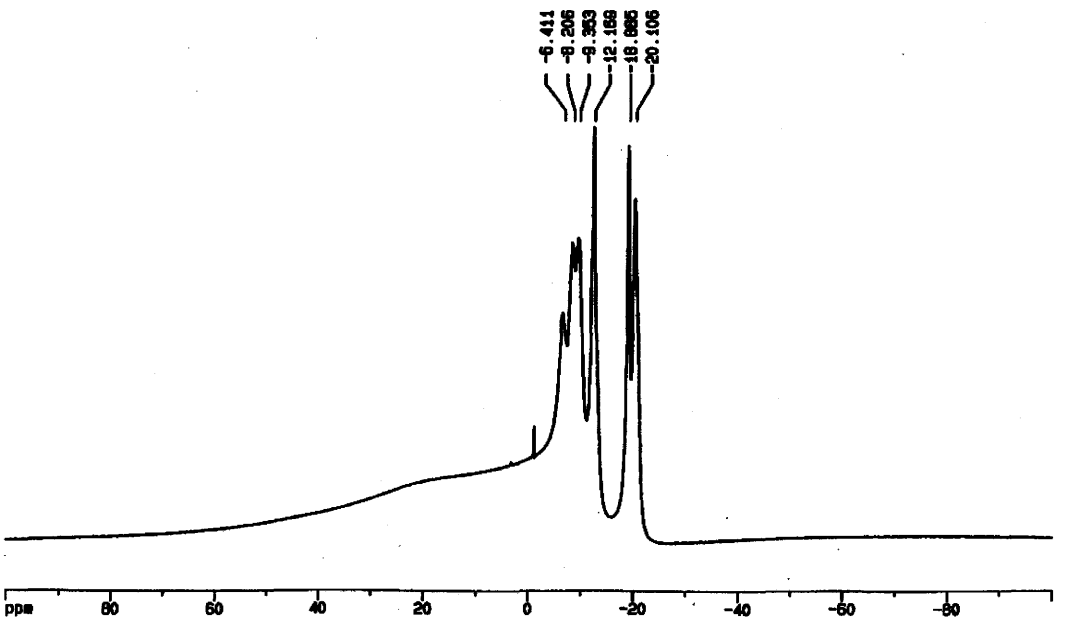


Figure 3.10: $^{11}B\{^1H\}$ NMR spectrum of **3.4** (160 MHz, CD_3OD).

The other NMR method that was employed as a means by which to indicate carborane cage isomerization was the selective nOe experiment. Since the nuclear Overhauser effect is a through-space interaction, it can give information on the relative proximity of the various protons within a molecule.[48] Due to the different distances between carbon atoms in the two metallocarborane cage isomers encountered in this research, the use of nOe spectroscopy might give information as to the nature of metallocarborane species such as **3.4**. Specifically, nOe enhancements from 2,1,8 isomers were expected to be less than those in the 3,1,2 isomers, or absent completely due to the greater distances in the "isomerized" cages. Indeed, upon irradiation of a proton on a group bound to the carborane terminal C-H proton or, for di-substituted derivatives, the proton nearest the carborane cage, we have observed such enhancements for a proton or protons on substituent groups for 3,1,2-metallocarboranes, whereas for 2,1,8-isomers, such enhancements were absent.[29,30] Therefore ^{1}H nOe spectroscopy should be suitable for determining the nature of the metallocarborane cages for compounds **3.4** and **3.5**, whose characterization is discussed in the next section.

*3.6 Characterization of Compounds **3.4** and **3.5***

The FT-IR spectrum of compound **3.4** was consistent with that expected, and was virtually identical to that obtained for compound **2.1**, which was prepared via conventional heating. The electrospray ionization mass spectrum of **3.4** gave the target anion mass peak at m/z = 595, with the expected isotope distribution pattern for the ReB_9

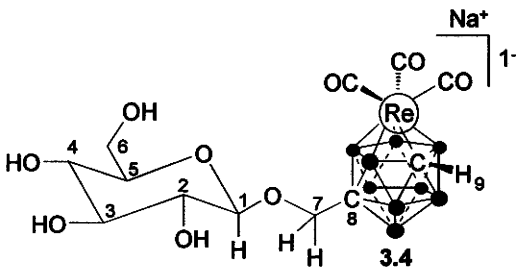
cluster. The ^{1}H and ^{13}C NMR spectral data for compound **3.4** are summarized in Table 3.1.

As was noted for compound **2.1**, the ^{1}H NMR spectrum (Figure 3.11) of **3.4** gave doubled signals as an indication of a diastereomeric product. The anomeric proton signals (H-1 and H-1*) appeared as an overlapping pair of doublets at 4.25 ppm. A second overlapping pair of doublets appeared at 4.00 ppm, corresponding to pendant group methylene protons H-7a and H-7a*. Pairs of overlapping doublets of doublets were observed at 3.86 and 3.69 ppm, arising from the glucose methylene protons H-6a and H-6a* and from H-6b and H-6b*, respectively. Another pair of doublets corresponding to H-7b and H-7b* were overlapping at 3.64 ppm. The overlapping signal at 3.35 ppm arose from protons H-3, H-3*, H-4 and H-4*, while a further overlapping signal at 3.24 ppm corresponded to H-2, H-2*, H-5 and H-5*. The broad signal at 1.67 ppm arose due to the carborane terminal protons H-9 and H-9*. Like all the previous carborane and metallocarborane derivative examined, the broad baseline signal in the region 0.90 to 3.10 ppm arose from the carborane B-H protons.

The $^{13}C\{^{1}H\}$ NMR spectrum (Figure 3.12) of **3.4** was also similar to that of **2.1**. The signal at 200.43 ppm arose from the carbonyl ligands bound to the metal core. Separate anomeric carbon signals were observed at 104.31 and 104.28 ppm, corresponding to C-1 and C-1*, although a precise assignment of these resonances to the individual diastereomers was not possible. The signal at 77.85 ppm was an overlapping resonance arising from C-3, C-5, and their respective diastereomeric counterparts. The pendant methylene carbon signal appeared at 76.66 ppm. The remaining carbohydrate

signals, C-2/2*, C-4/4* and C-6/6*, gave the signals at 75.17, 71.42, and 62.53 ppm. The substituted carborane carbon atom, C-8/8*, gave the signal at 53.77, while the terminal atom, C-9/9* gave the signal at 29.60 ppm.

The $^{11}B\{^1H\}$ NMR spectrum (Fig 3.10, *vide supra*) of **3.4** gave signals at -6.41, -8.21, -9.38, -12.17, -18.89, and -20.10 ppm.



Proton	δ (ppm)	Carbon	δ (ppm)
H-1, 1*	4.25	C≡O	δ 200.43
H-7a, 7a*	4.00	C-1, 1*	104.31, 104.28
H-6a, 6a*	3.86	C-3, 3*, 5, 5*	77.85
H-6b, 6b*	3.69	C-7, 7*	76.66
H-7b, 7b*	3.64	C-2, 2*	75.17
H-3, 3*, 4, 4*	3.35	C-4, 4*	71.42
H-2, 2*, 5, 5*	3.24	C-6, 6*	62.53
H-9, 9*	1.67	C-8, 8*	53.77
B-H	3.10 - 0.90	C-9, 9*	29.60

Table 3.1: The ^1H and ^{13}C NMR spectral assignments for **3.4**.
* Denotes signals arising due to diastereomers

Figures 3.13 and 3.14 show the 1D selective nOe spectra of compounds **2.6d** and **3.4** overlaid with the ^1H NMR spectrum. The *nido*-carborane precursor was subjected to nOe analysis for the purposes of comparison, since these precursors to **3.4** and **3.5** are expected to contain the "*ortho*" configuration of the carbon atoms. If nOe enhancements are observed for the *nido*-carborane ligands, the nature of the metallocarborane cages in

3.4 and **3.5** should determine whether similar enhancements are observed. Indeed, upon irradiation of the *nido*-carborane terminal C-H proton (2.02 ppm) in **2.6d**, enhancement of the signals corresponding to both the methylene "spacer" protons between the glucose and carborane moieties was observed. By contrast, irradiation of the carborane CH frequency (1.67 ppm), in **3.4**, the nOe spectrum shows no enhancements of either the signals arising from the methylene "spacer" protons adjacent the carborane cage, or the anomeric proton signals. By analogy to results obtained for other examples, this lack of signal enhancement would indicate that compound **3.4** exists as the 2,1,8-ReC_2B_9 cage isomer.

Since metallocarboranyl glycoside **2.1** (NEt_4^+ salt) was prepared previously via conventional reflux heating, it was of interest to determine whether microwave-assisted heating was solely responsible for the carborane cage isomerization that was observed for compound **3.4**. The 1D selective nOe spectrum of **2.1** (Figure 3.15) clearly shows no enhancement of the anomeric or pendant methylene "spacer" signals upon irradiation of the terminal carborane C-H. This implies that compound **2.1** also contains the 2,1,8 configuration of the metallocarborane cage. Rather than suggesting that microwave heating alone is the cause of the observed isomerization, it is necessary to recall that the synthesis of **2.1** required seven days at reflux to reach completion. It is likely that the length of heating, in the case of this compound, was a contributing factor, as well as the relief of possible steric interactions between the glucose pendant group and the $Re(CO)_3$ core, which would be identical to that which could occur in the case of compound **3.4**.

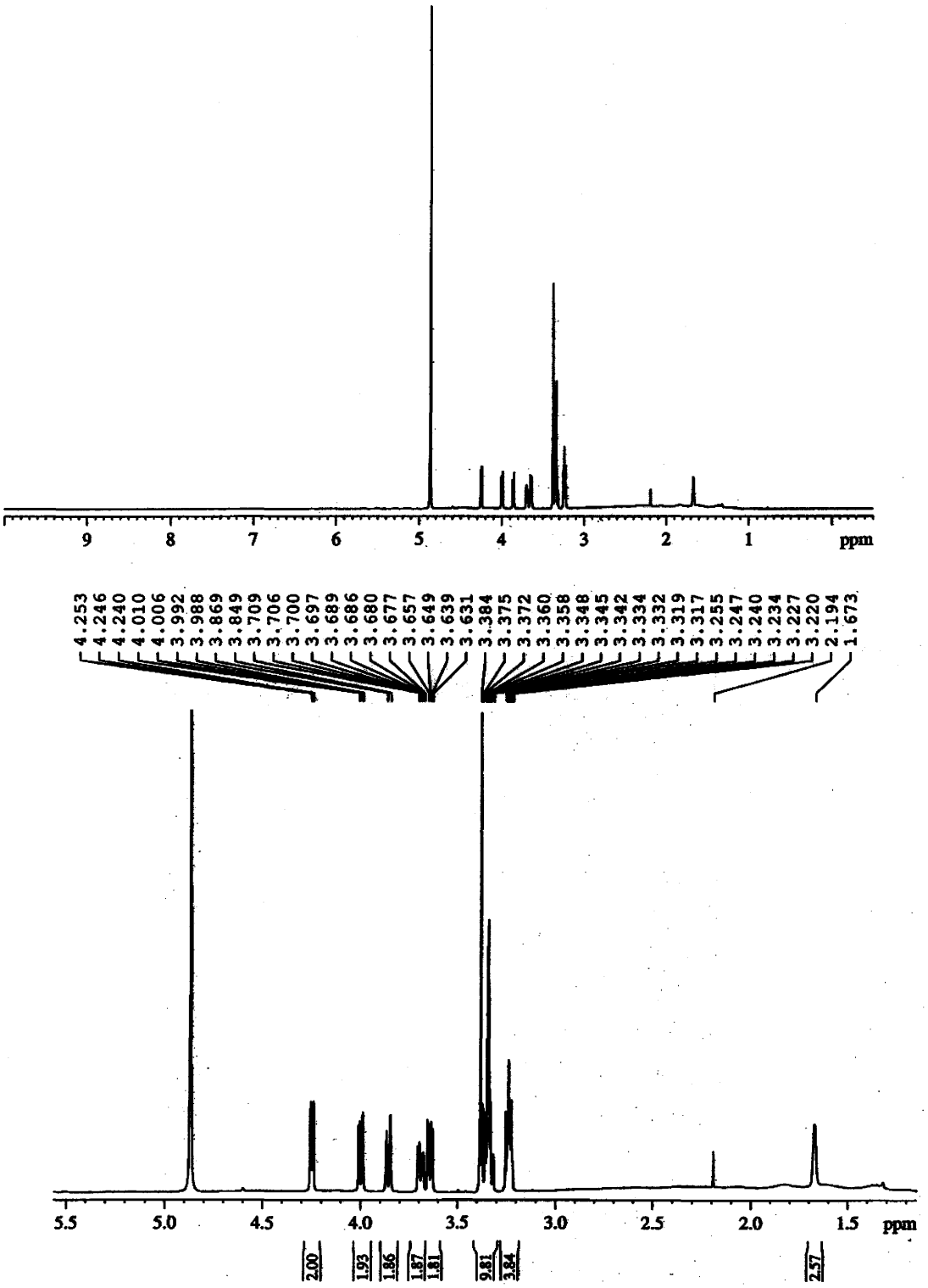


Figure 3.11: The ^{1}H NMR spectrum and expansion between 1.0 and 5.5 ppm of **3.4** (600 MHz, CD_3OD).

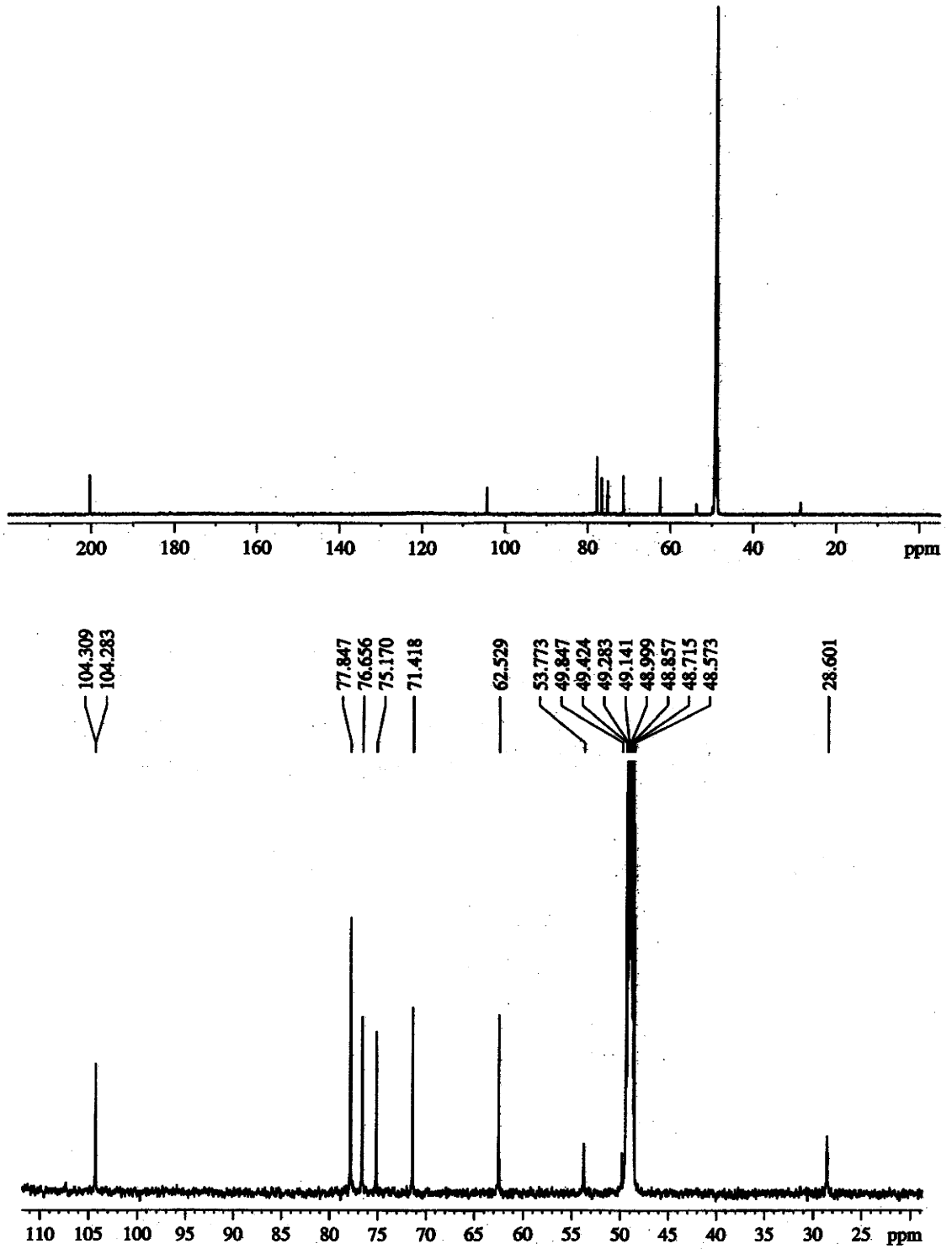


Figure 3.12: The $^{13}C\{^{1}H\}$ NMR spectrum and expansion between 20 and 110 ppm of **3.4** (150 MHz, CD_3OD).

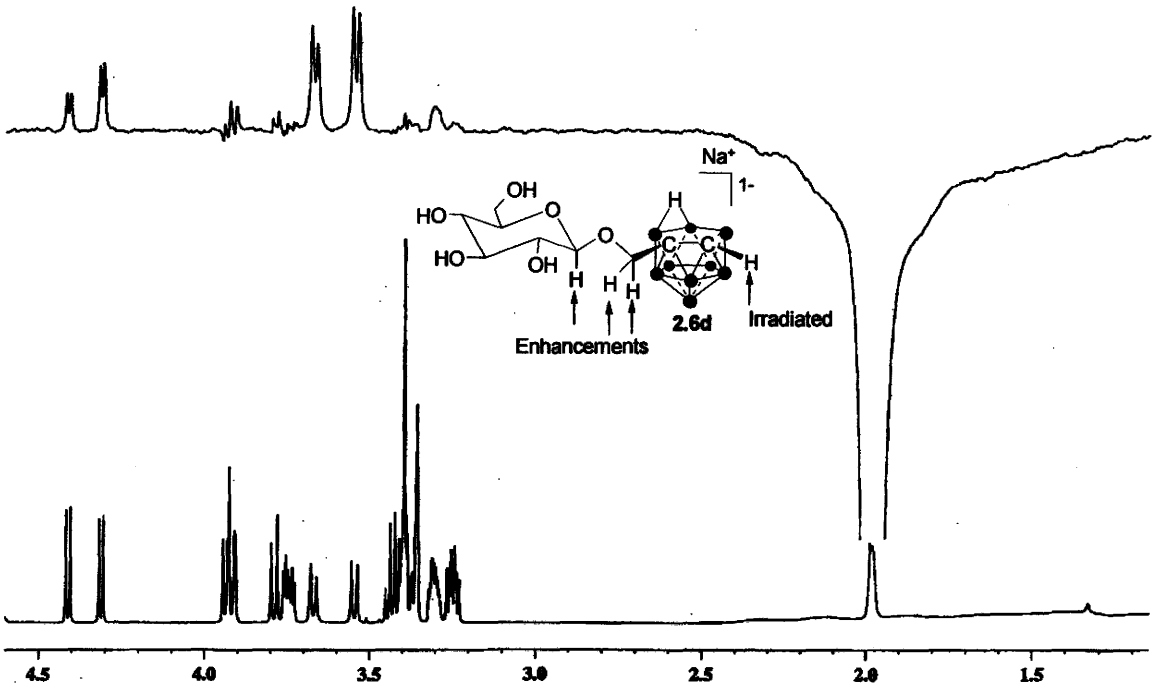


Figure 3.13: 1D Selective nOe spectrum of ligand **2.6d**; ^{1}H NMR spectrum below (600 MHz, CD_3OD).

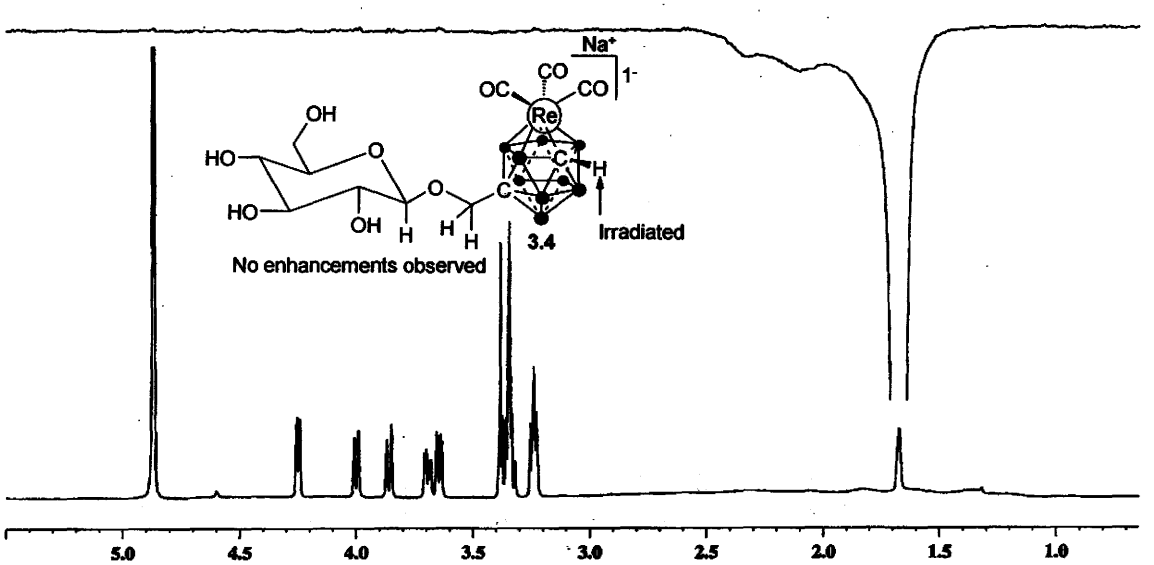


Figure 3.14: 1D Selective nOe spectrum of **3.4**; ^{1}H NMR spectrum below (600 MHz, CD_3OD).

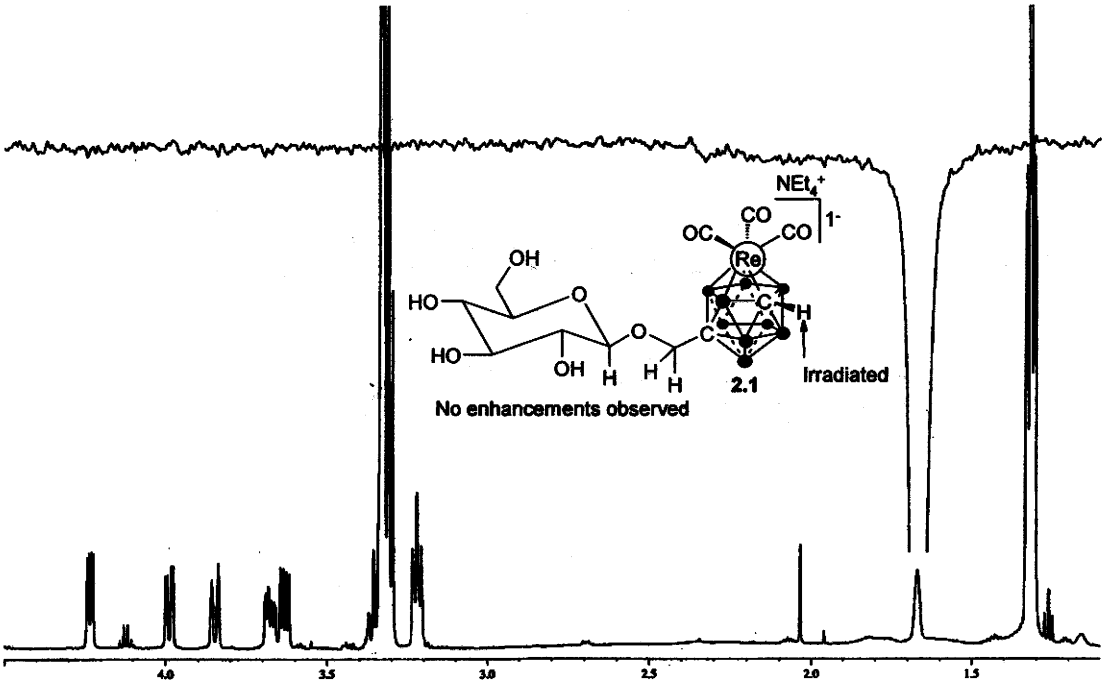


Figure 3.15: 1D selective nOe spectrum of **2.1**; ^{1}H NMR spectrum below (600 MHz, CD_3OD).

Following its isolation by HPLC, compound **3.5** was characterized by NMR and IR spectroscopy, and by electrospray mass spectrometry. The IR spectrum gave a broad O-H stretch at 3410 cm^{-1}, and the diagnostic B-H stretch at 2550 cm^{-1}. The C≡O stretching absorptions appeared at 2002 and 1907 cm^{-1}.

The ^{1}H and ^{13}C NMR spectral data for compound **3.5** are listed in Table. 3.2. As was mentioned in the discussion of the elucidation of the configuration of the metallocarborane cage, the ^{1}H NMR spectrum (Figure 3.16) of **3.5** contains anomeric and pendant methylene "spacer" signals in such a pattern as to indicate two distinct carbohydrate environments. This hypothesis is supported by the nOe data, which is suggestive of a 2,1,8-ReC_2 configuration in the metallocarborane cluster, which would

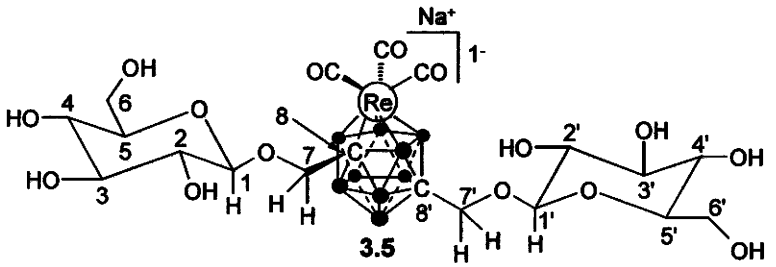
necessitate two chemically distinct glucose environments; one bound to the carbon atom on the metal-bonding face of the cluster, the other bound to the carbon atom which has migrated away from the metal bonding face.

The two highest-frequency signals in the ^{1}H NMR spectrum of **3.5** appeared at 4.25 and 4.12 ppm and were assigned the labels H-1 and H-1′, respectively. These signals each integrated to two protons. The signal at 4.25 ppm appeared as a partially overlapping pair of doublets, while the signal at 4.12 ppm was a single doublet. Both of these signals contained coupling constants of 7.7 Hz, and are characteristically anomeric proton signals.[49] These signals have arisen due to the two distinct glucose environments present in the structure. The overlapping nature of the signal at 4.25 ppm (H-1) is due to the presence of the diastereomers that resulted from the cage degradation that formed the *nido*-carboranyl precursor **2.12**. That this "doubling" is visible in this signal may be a result of a more hindered environment around the metal-binding face of the carborane cluster. By contrast, the second carbohydrate group likely experiences greater rotational freedom, thus the signal arising from H-1′ is that of the average between two diastereomeric counterparts. The signal at 3.98 ppm was an overlapping pair of doublets corresponding to one of the pendant methylene "spacer" protons. HSQC and HMBC spectra confirmed that the protons giving this signal were on the methylene carbon atom proximal to the metal binding face of the cluster, and were therefore labeled H-7a/7a*. The multiplet signal at 3.84 ppm was attributed to one of the glucose C-6 protons from both carbohydrate environments, and their respective diastereomeric counterparts. Next was a doublet at 3.76 ppm, which was attributed to one of the H-7a′ methylene protons.

The diastereomeric counterpart to this signal was buried under the overlapping signal from the second glucose C-6 proton from both environments, which appeared at 3.68 ppm. The remaining methylene signals, H-7b′ and H-7b′* gave a doublets at 3.45 ppm, the second of which was buried under an overlapping signal arising from H-3, H-3′, H-4, and H-4′ at 3.34 ppm. Overlapping signals were also observed at 3.23 and 3.19 ppm, arising from H-2 and H-2′, and from H-5 and H-5′, respectively. The carborane B-H protons gave the typical wavy baseline signal in the range 0.80 to 3.00 ppm.

The assignment of the $^{13}C\{^1H\}$ NMR spectrum (Figure 3.17) of **3.5** was relatively straightforward. Differentiation between carbon atoms in the separate glucose environments was possible only in the case of the signals arising from the carborane carbon atoms and the pendant methylene carbon atoms. Overlap of signals precluded this differentiation for the carbohydrate carbon atoms. The carbonyl carbon of the $Re(CO)_3$ core gave the usual signal at 200.90 ppm. A group of signals appeared at 104.36, 104.16, and 104.05 ppm, and were attributed to the anomeric carbon signals. The signal at 78.66 ppm was assigned the methylene carbon atom C-7′. The signals at 77.89 and 77.71 ppm were attributed to C-5/5′, and to C-3/3′, respectively. The signal at 76.69 was assigned methylene carbon atom C-7, in the metal-bonding face of the carborane cage. The signals at 75.25, 71.48, and 62.60 ppm were assigned C-2/2′, C-4/4′, and C-6/6′, respectively. The low-intensity signal at 58.01 ppm was assigned to C-8′, the "migratory" carborane carbon atom. The carbon atom in the metal-bonding face of the cluster gave a signal at 54.42 ppm. The intensity of this signal is barely above the baseline in the 1D $^{13}C\{^1H\}$

NMR spectrum, and it was identified through HMBC (Figure 3.18) correlations to the H-7a and H-7b signals.



3.5

Proton	δ (ppm)	Carbon	δ (ppm)
H-1, 1*	4.25	C≡O	200.90
H-1′, 1′*	4.12	C-1, 1*, 1′, 1′*	104.36, 104.16, 104.05
H-7a, 7a*	3.98	C-7′, 7′*	78.66
H-6a, 6a*, 6a′, 6a′*	3.84	C-5, 5*, 5′, 5′*	77.89
H-7a′/7a′*	3.76	C-3, 3*, 3′, 3′*	77.71
H-7a′/7a′*, H-6b, 6b*, 6b′, 6b′*	3.68	C-7, 7*	76.69
H-7b, 7b*	3.65	C-2, 2*, 2′, 2′*	75.25
H-7b′/7b′*	3.45	C-4, 4*, 4′, 4′*	71.48
H-7b′/7b′*, H-3,3*,3′,3′*,4,4*,4′,4′*	3.34	C-6, 6*, 6′, 6′*	62.60
H-2, 2*, 2′, 2′*	3.23	C-8′, 8′*	58.01
H-5, 5*, 5′, 5′*	3.19	C-8, 8*	54.42
B-H	0.80 - 3.00		

′ Denotes signals arising from separate glucose environment
* Denotes signals arising from diastereomers

Table 3.2: ^{1}H and ^{13}C NMR spectral assignments for **3.5**.

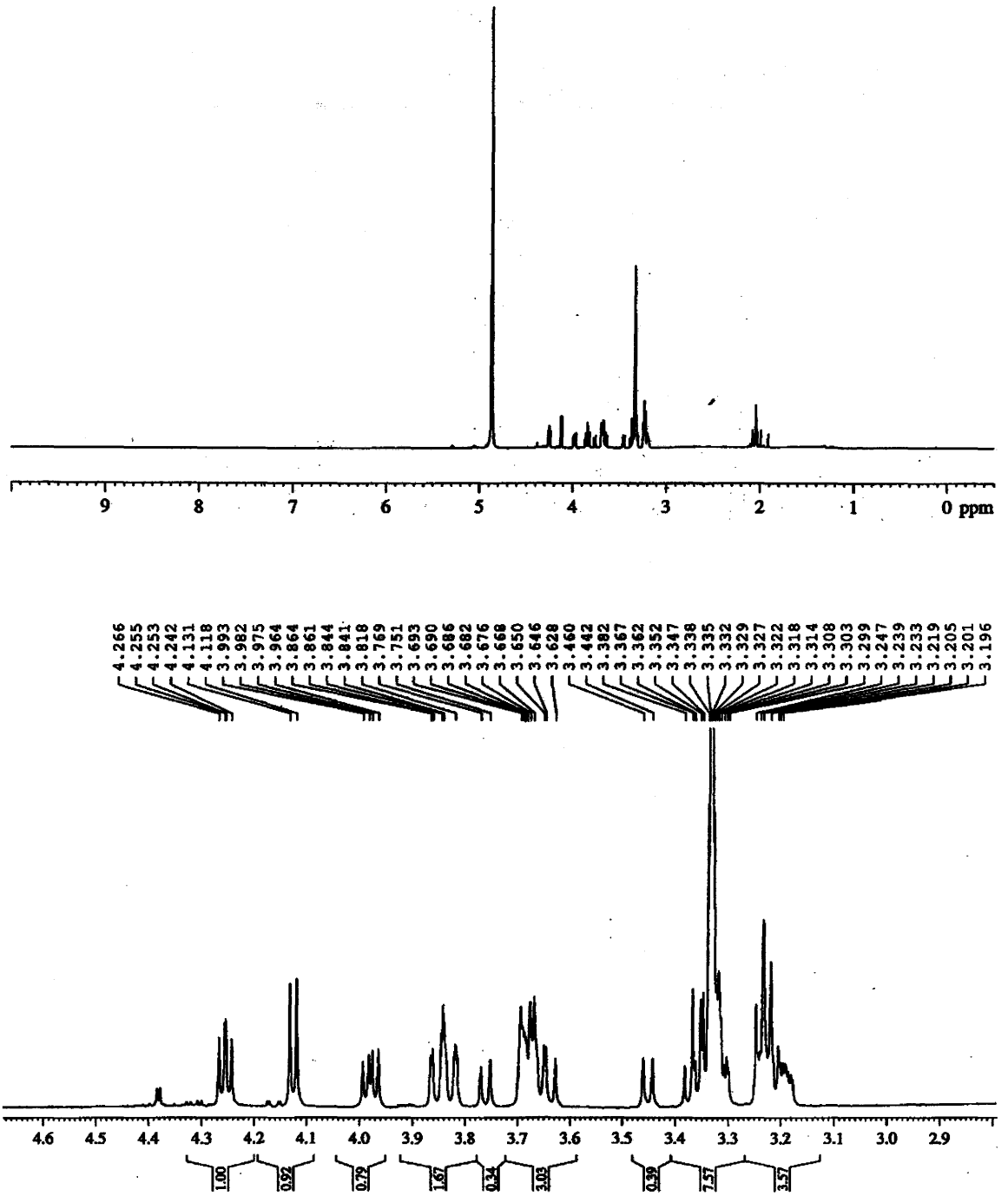


Figure 3.16: The ^{1}H NMR spectrum and exansion between 2.8a nd 4.7 ppm of **3.5** (600 MHz, CD_3OD).

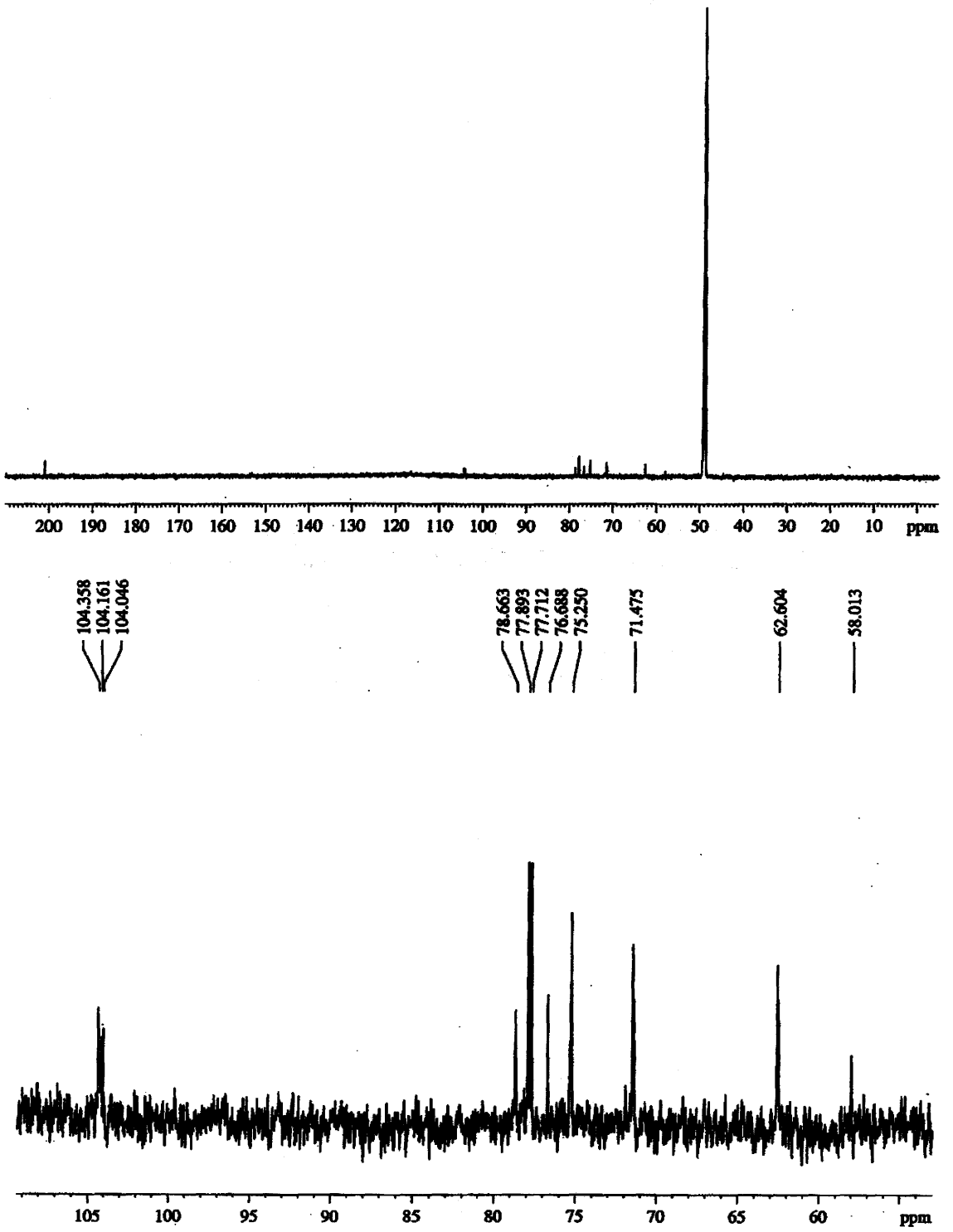


Figure 3.17: The $^{13}C\{^{1}H\}$ NMR spectrum and expansion between 52 and 110 ppm of **3.5** (150 MHz, CD_3OD).

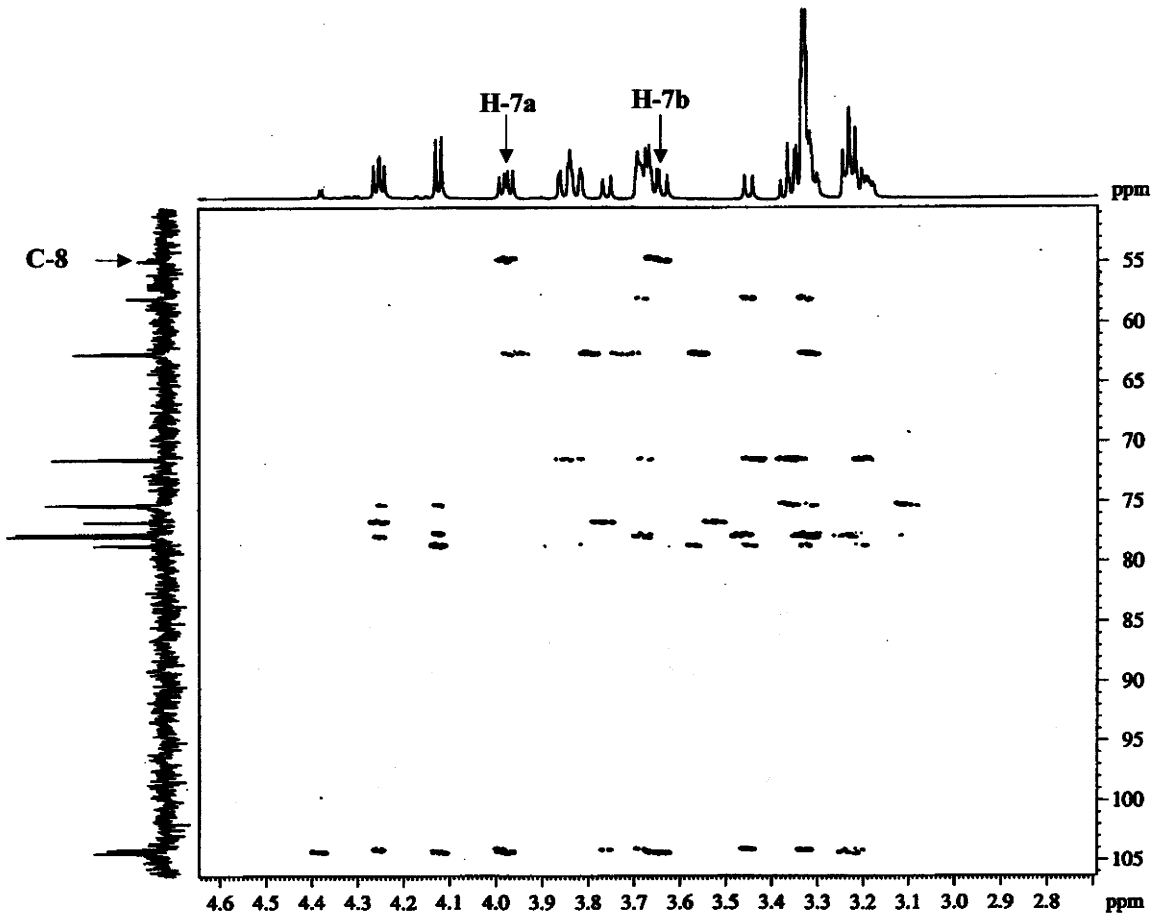


Figure 3.18: ^{1}H-^{13}C HMBC spectrum of **3.5** (CD_3OD).

The case of determining the nature of the metallocarborane cage in **3.5** is somewhat more complex than for **3.4**. The ^{1}H NMR spectrum of **3.5** indicated a total of eight methylene "spacer" signals, and two anomeric proton signals, which would be accounted for by the presence of two distinct glucose-pendant environments, in addition to the two diastereomeric forms of the overall metallocarborane complex, which was the result of the formation of the *nido*-carborane cage in the presence of the chiral sugar

pendant groups.[22,50,51] It is interesting to note that one of the two anomeric signals appears as a doublet, while the other appears as two overlapping doublets, the "extra" signal a result of the diastereomeric nature of the complex. The presence of two distinct carbohydrate environments is also supported by the COSY, HSQC and HMBC spectra of compound **3.5**. These environments are believed to arise from the isomerization of the carborane cage to the 2,1,8 configuration.

To confirm this hypothesis, ^{1}H nOe spectra of **3.5** (Figure 3.20) and its *nido*-carborane precursor **2.12** (Figure 3.19), were acquired. Since these compounds did not contain a free carborane C-H proton, nOe experiments involved irradiation the protons closest to the carborane cage, those being the methylene protons of the pendant glucose units. The results for **2.12** showed that the cage carbon atoms were adjacent to each other, as expected for this *nido-ortho*-carborane derivative. Notably, irradiation of one set of methylene protons (4.17 ppm) resulted in enhancement of the other methylene protons (3.91 ppm) as identified through two-dimensional NMR techniques. Additionally, some enhancement was also seen of the anomeric proton signals at 4.65 and 4.38 ppm.

Irradiation of one of the methylene signals of **3.5** (at 3.98 ppm) resulted in enhancement of the signal arising from this proton's coupling partner, and the signal from the anomeric proton (pair of doublets at 4.25 ppm), that must be nearest the irradiated methylene protons (connectivity confirmed by HMBC). No enhancements of the signals arising from the remaining methylene "spacer" signals, nor the second anomeric signal were observed, suggesting that the "second" glucose environment is removed from the metal-bonding face of the metallocarborane, as in the 2,1,8 isomer.

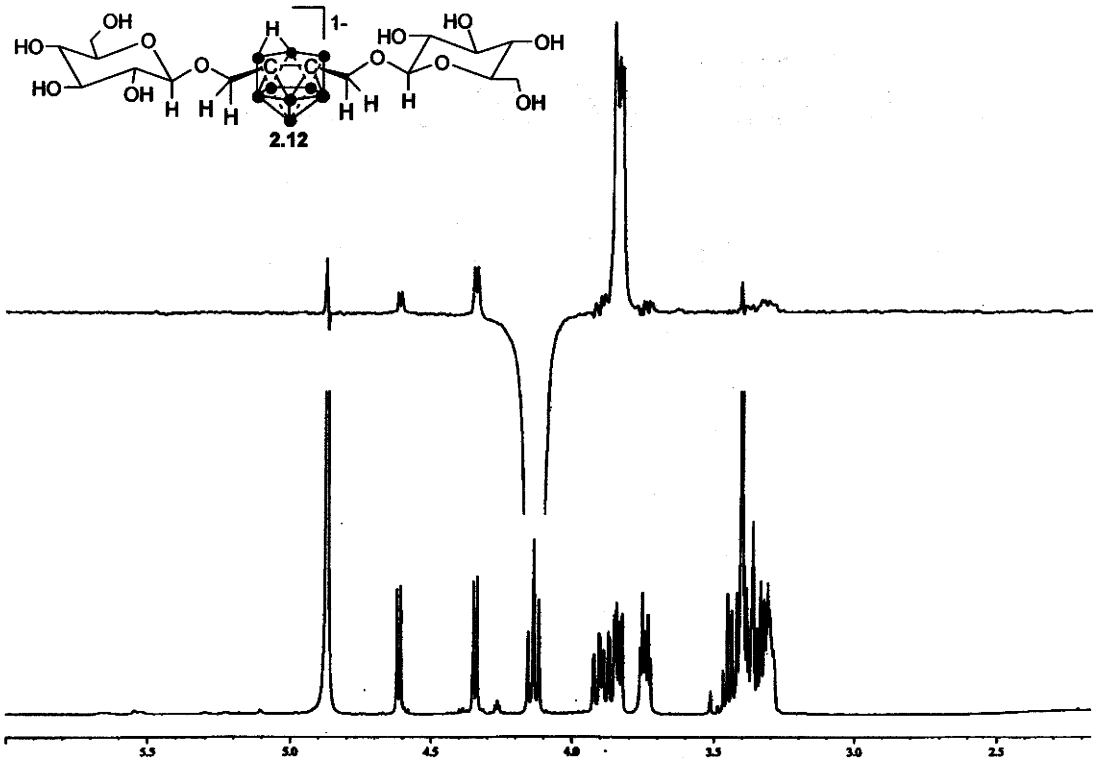


Figure 3.19: 1D selective nOe spectrum of ligand **2.12**; ^{1}H NMR spectrum below (600 MHz, CD_3OD).

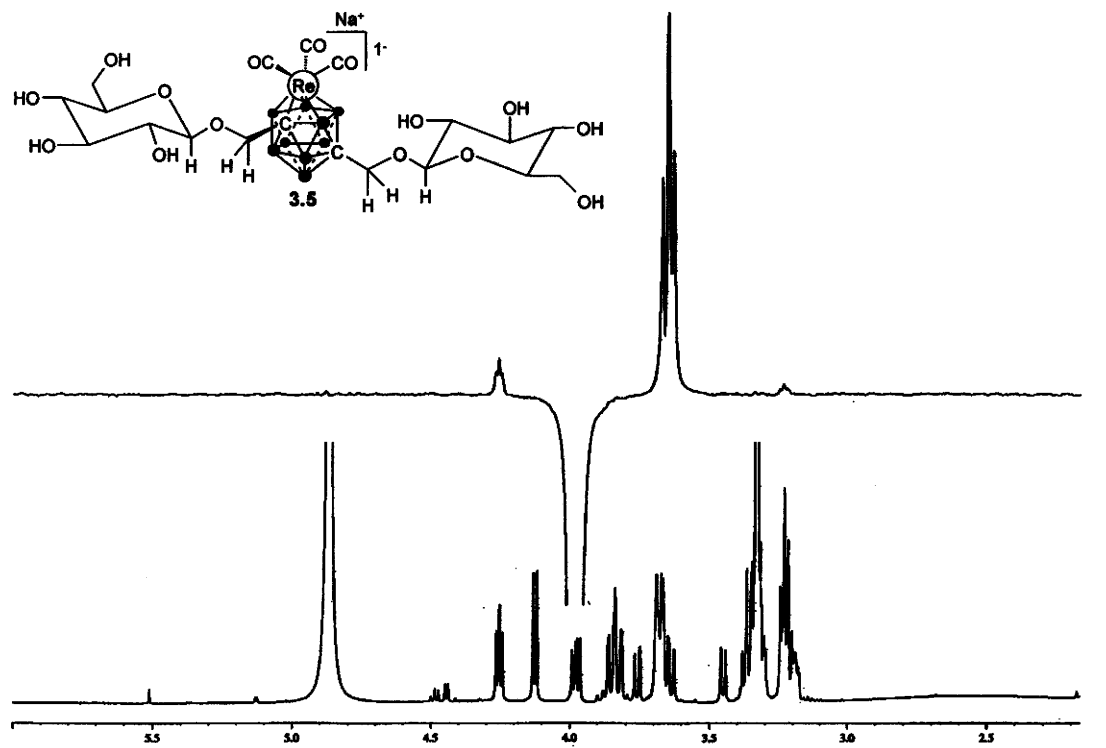
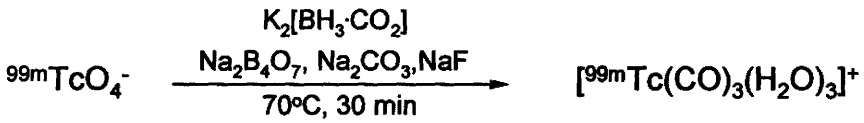


Figure 3.20: 1D selective nOe spectrum of **3.5**; ^{1}H NMR spectrum below (600 MHz, CD_3OD).

3.7 Radiolabelling With ^{99m}Tc: Synthesis of ^{99m}Tc-3.4

Following the successful preparation of compounds **3.4**, which are the standards to which radioactive products are compared for characterization, the synthesis of the ^{99m}Tc-radiolabelled analogue, namely **^{99m}Tc-3.4**, was investigated. The reagent $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared following methods developed in our group (Scheme 3.5),[20,52] which is a modification of the synthesis of this species first reported by Alberto and co-workers.[53]

Initial attempts to prepare **^{99m}Tc-3.4** were done by conventional heating. Aliquots of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ were added to vials containing ligand **2.6d** in 0.5M NaF. The vials were heated at 95°C for 2-3 hours. HPLC analysis indicated an isolable major radioactive product, with pertechnetate and other undetermined radioactive species as minor products. Following isolation, comparison of the product obtained with the non-radioactive standard (Figure 3.21), **3.4**, revealed that the major radioactive species did not have the same retention time as the standard. The isolated radioactive product eluted from the HPLC column approximately two minutes prior to standard **3.4**. Several repetitions of this experiment gave consistent results of this nature. In order to correlate properly with the standard, the observed products (γ-peak) should elute approximately 0.3 minutes *after* the standard (UV peak), as the physical layout of our HPLC system has the UV detector ahead of the γ-detector, connected such that the time delay between the two is approximately 0.3 minutes at a flow rate of 1.0 mL per minute.



Scheme 3.5: Preparation of $[^{99m}Tc(CO)_3(H_2O)_3]^+$.

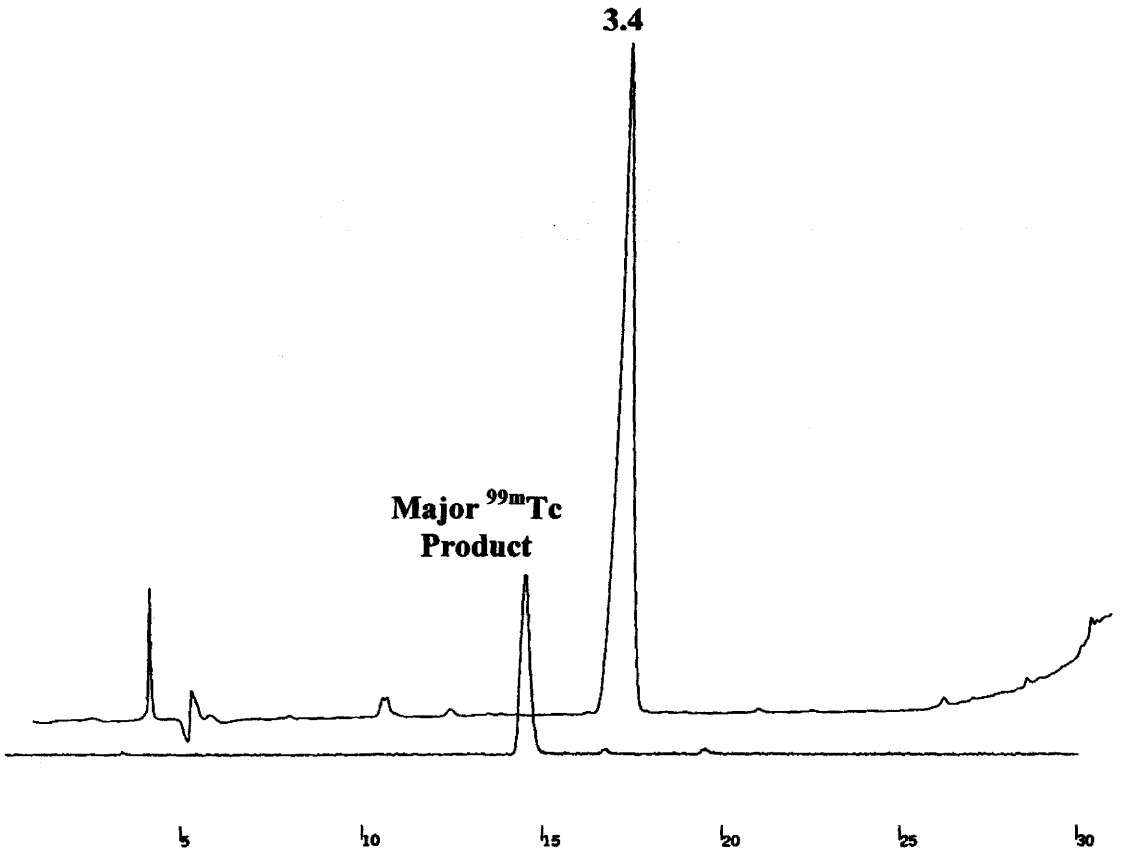


Figure 3.21: HPLC Chromatogram for ^{99m}Tc-radiolabelling experiment with conventional heating. Top: UV trace. Bottom: γ-trace.

Radiolabelling reactions were subsequently performed using microwave irradiation. Two series of four microwave reactions were conducted to explore key labelling parameters. In the first series, the ligand **2.6d** was dissolved in PBF, as was the case in the optimized preparation of the "cold" rhenium standard. In the second series, ligand **2.6d** was dissolved in 0.5M NaF. To these ligand solutions were added aliquots of $[^{99m}Tc(CO)_3(H_2O_3)]^+$, containing approximately 1 mCi (37 MBq) of activity. Each reaction in the respective series was subsequently irradiated over a different time interval, ranging between 5 and 20 minutes. Aliquots of the completed reactions were taken and analyzed by HPLC. In all cases, only two radioactive species were detected by HPLC following MW irradiation: residual $^{99m}TcO_4^-$, and a major radioactive species eluting at approximately 20 minutes. Integration of the peaks in the γ-HPLC trace suggested radiochemical yields of 80-95% for the reactions (Table 3.3). A typical example is shown in Figure 3.22, while Figure 3.23 shows stacked plots illustrating the similarity of all reactions in both series.

One reaction was selected and the major product isolated in 70% radiochemical yield (decay-corrected), according to a solid-phase extraction (SPE) procedure developed by our group.[52,54] Comparison of this isolated product with **3.4** is illustrated in Figure 3.24. Direct comparison with the non-radioactive standard **3.4** using a different HPLC elution method (Method **B** - *vide infra*, Experimental section) was done since this method was developed for the analysis compound **3.4**, and is more amenable to such a comparison. Under this method, co-injection of the isolated product with standard **3.4**

showed that the two compounds correlated well with each other. Thus it can be concluded that the major product of these reactions was the desired target, ^{99m}Tc-**3.4**.

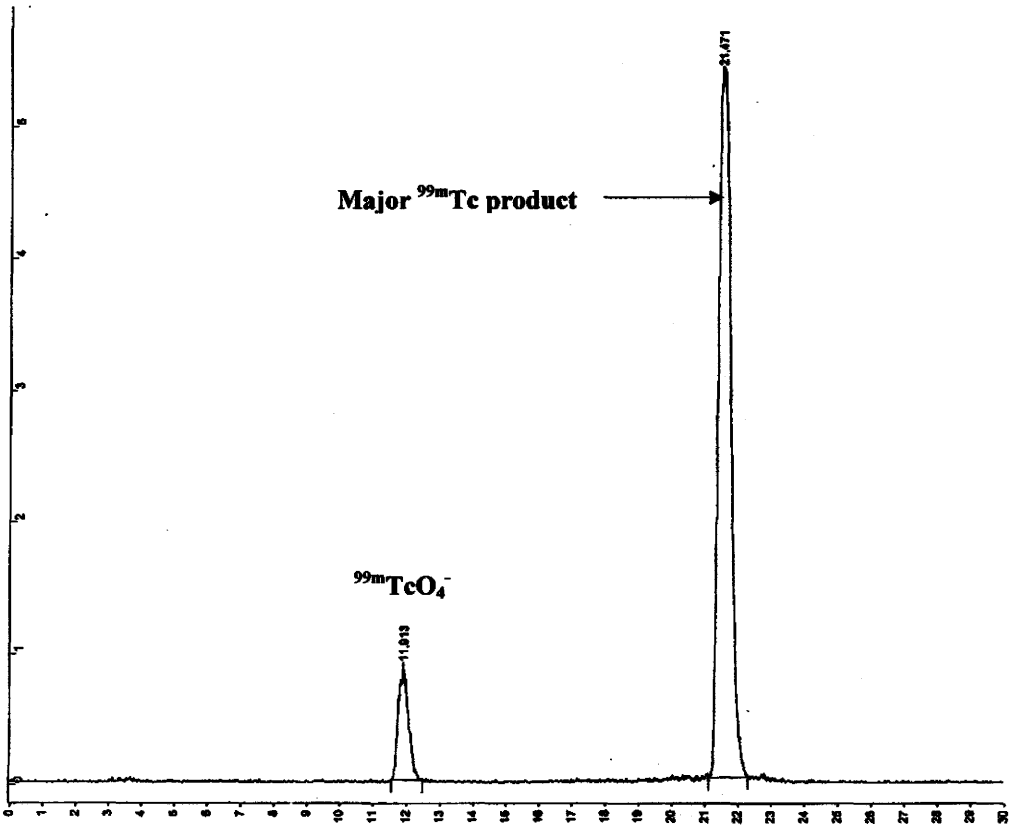


Figure 3.22: γ-HPLC chromatogram of crude reaction to prepare ^{99m}Tc-**3.4**. Elution Method A.

The results of this experiment showed that there was no apparent improvement in the radiochemical yield in reactions in which the ligand was dissolved in PBF versus 0.5M sodium fluoride. That is, the degradation of the carbohydrate moiety that plagued the synthesis of **3.4** was not observed on the tracer level. Therefore, for simplicity, the use of the buffered medium was discontinued in subsequent reactions.

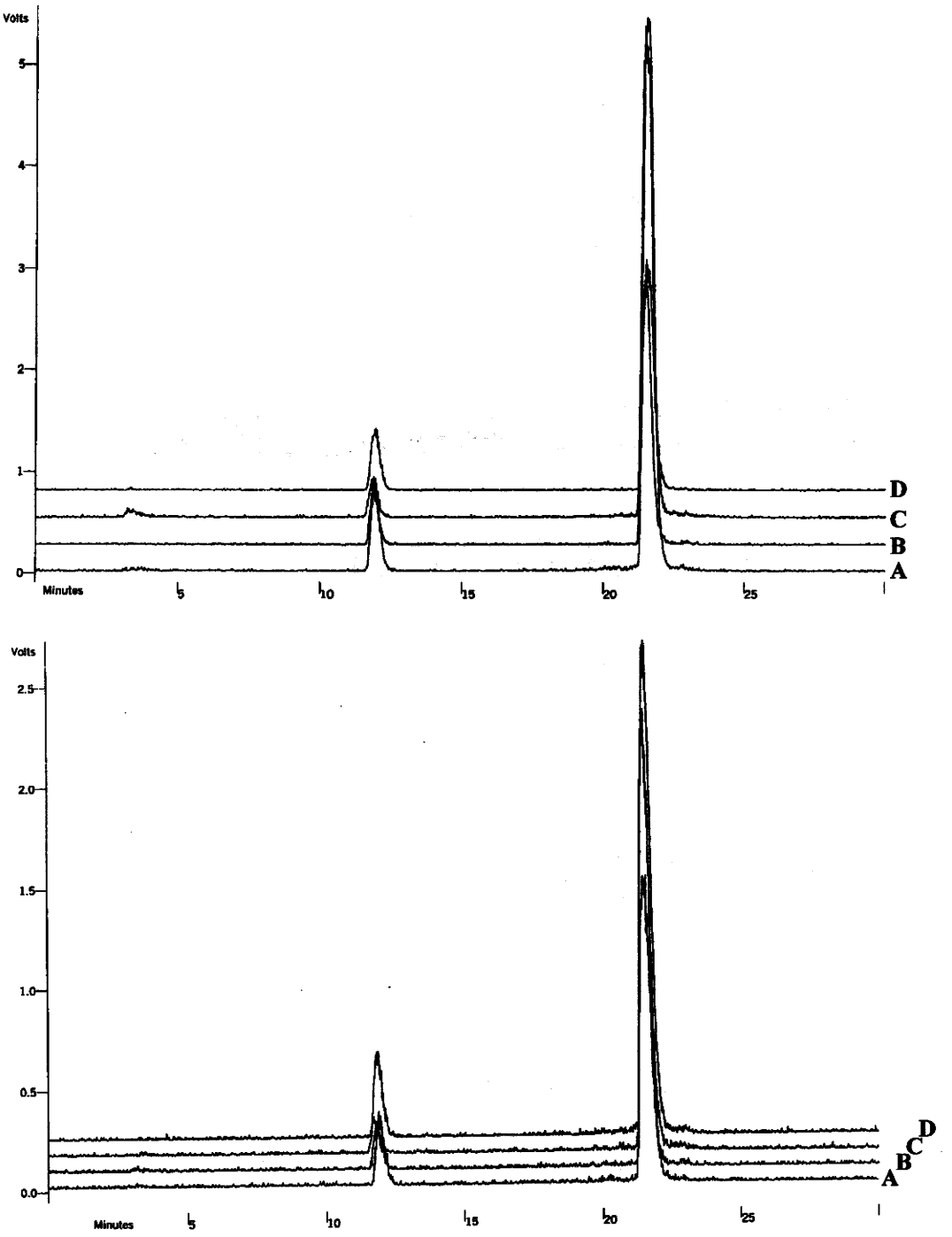


Figure 3.23: γ-HPLC traces for synthesis of [^{99m}Tc]-**3.4**:
Top: Series 1 (No buffer); Bottom: Series 2 (Buffered medium).
A: 5min; **B**: 10 min; **C**: 15 min; **D**: 20 min irradiation. Elution method **A**.

Vial #	Buffered Y/N	Irradiation Time (min)	Estimated RCY (%)
1a	N	5	89
1b	N	10	85
1c	N	15	95
1c	N	20	82
2a	Y	5	89
2b	Y	10	90
2c	Y	15	94
2d	Y	20	88

Table 3.3: Reaction Media, Times, and Yields for MW-assisted synthesis of ^{99m}Tc-3.4.

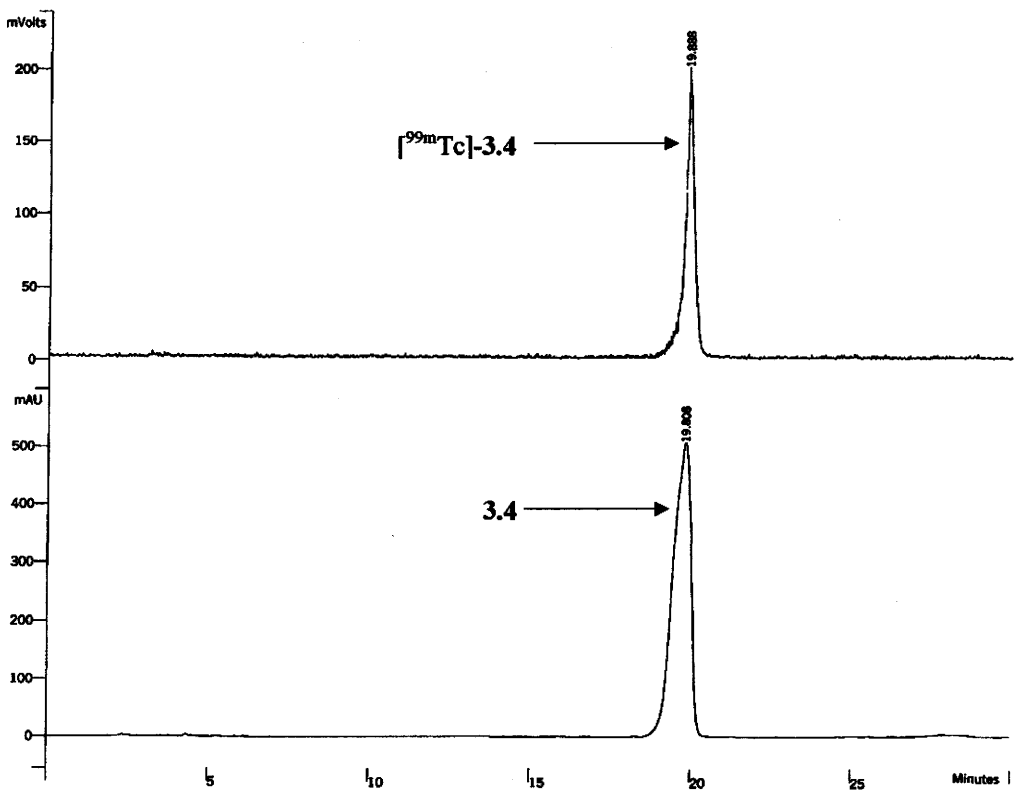


Figure 3.24: HPLC traces of isolated ^{99m}Tc-3.4 and standard 3.4. Top: γ-trace. Bottom: UV-trace. (Varian Nucleosil 4.6x250mm C_{18} Column, elution method B).

Another aspect of optimization was to probe the effect of ligand concentration on the yield of **^{99m}Tc-3.4**. In the above reactions, the concentration of ligand **2.6d** was 0.01M, which had been shown to be an optimal concentration for the synthesis of other ^{99m}Tc-metallocarborane derivatives.[20,52] However, for the synthesis of radiolabelled compounds in high specific activity, and, preferably through the use of a radiolabelling kit, it is desirable to use the lowest possible concentration of ligand. For example, concentrations as low as 10^{-6} M have been sufficient for binding the $^{99m}Tc(CO)_3^+$ core by some tridentate ligands.[55]

With this in mind, four ligand solutions were prepared with concentrations as follows: 10^{-2}M, 10^{-3}M, 10^{-4}M, and 10^{-5}M. Aliquots of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ (~37 MBq) were added to vials containing the ligand solutions. The vials were heated at 200°C for 15 minutes, after which aliquots were taken and analyzed by HPLC. The results are summarized in Table 3.4, and are illustrated in Figure 3.25. As can be seen from these results, the yield of **^{99m}Tc-3.4** decreased markedly with the decrease in concentration of **2.6d**. Thus, it appears that the optimal ligand concentration for this system is 10^{-2}M.

Vial #	[L]	Estimated RCY (%)
1	10^{-2} M	91
2	10^{-3} M	39
3	10^{-4} M	6
4	10^{-5} M	<1

Table 3.4: Yield versus concentration of ligand **2.6d** in synthesis of **^{99m}Tc-3.4**.

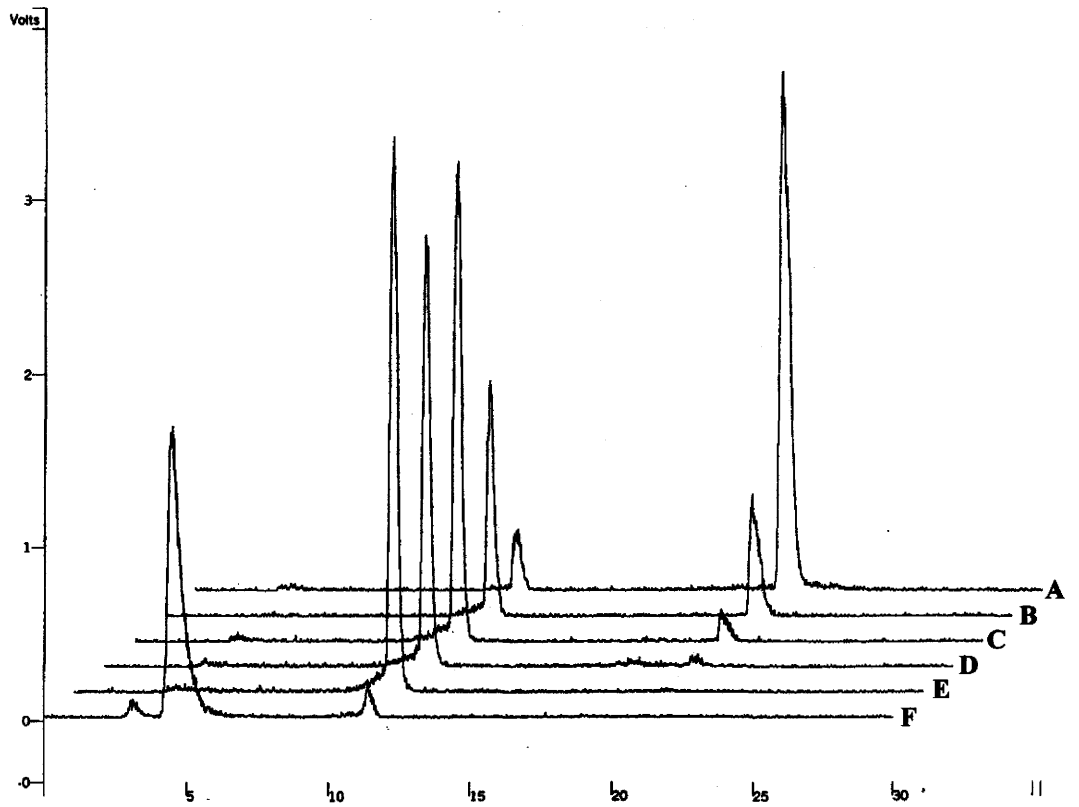


Figure 3.25: Stacked γ-HPLC traces illustrating change in yield of ^{99m}Tc-3.4 with ligand concentration: **A**: $[L^-] = 10^{-2}$M; **B**: $[L^-] = 10^{-3}$M; **C**: $[L^-] = 10^{-4}$M; **D**: $[L^-] = 10^{-5}$M; **E**: $^{99m}TcO_4^-$; **F**: $[^{99m}Tc(CO)_3(H_2O)_3]^+$ reaction aliquot. (Elution Method A).

Following the elucidation of the optimal radiolabelling conditions for this system, the synthesis of ^{99m}**Tc-3.4** was repeated in order to obtain a calculated average radiochemical yield over several experiments. Thus five repeat syntheses were performed, and ^{99m}**Tc-3.4** was isolated with an average radiochemical yield of 58%, corrected for decay. The last of these experiments was done using 11.4 mCi (422 MBq) of $[^{99m}Tc(CO)_3(H_2O)_3]^+$ as an example of a radiosynthesis with an activity level nearer

that which could be clinically useful. At this scale of reaction, sufficient activity remained for an *in vitro* stability assay, in the form of cysteine and histidine challenge experiments. Since the amino acids L-cysteine and L-histidine are ubiquitous in the human body, and since they also contain sulfur and nitrogen atoms, respectively, which can potentially bind the radiometal, the outcome of incubation of a radiolabeled compound in the presence of excess quantities of these amino acids can give an estimate of the stability of potential radiotracers under physiological conditions. To one sample was added 10μL of a 10^{-2}M solution of L-cysteine, and to another was added 10μL of a 10^{-2} M solution of L-histidine. The resulting solutions were incubated for 18 hours at 37°C before aliquots were analyzed by γ-HPLC. The results are illustrated in Figure 3.26. As can be seen from the HPLC traces, the majority of the radioactivity remained as the target compound, 75% based on HPLC peak integration for the cysteine challenge experiment, and 79% for the histidine challenge. The remainder of the activity, in both cases, occurred as pertechnetate. These results indicate that ^{99m}**Tc-3.4** possesses good, but not infinite stability under the challenge conditions employed.

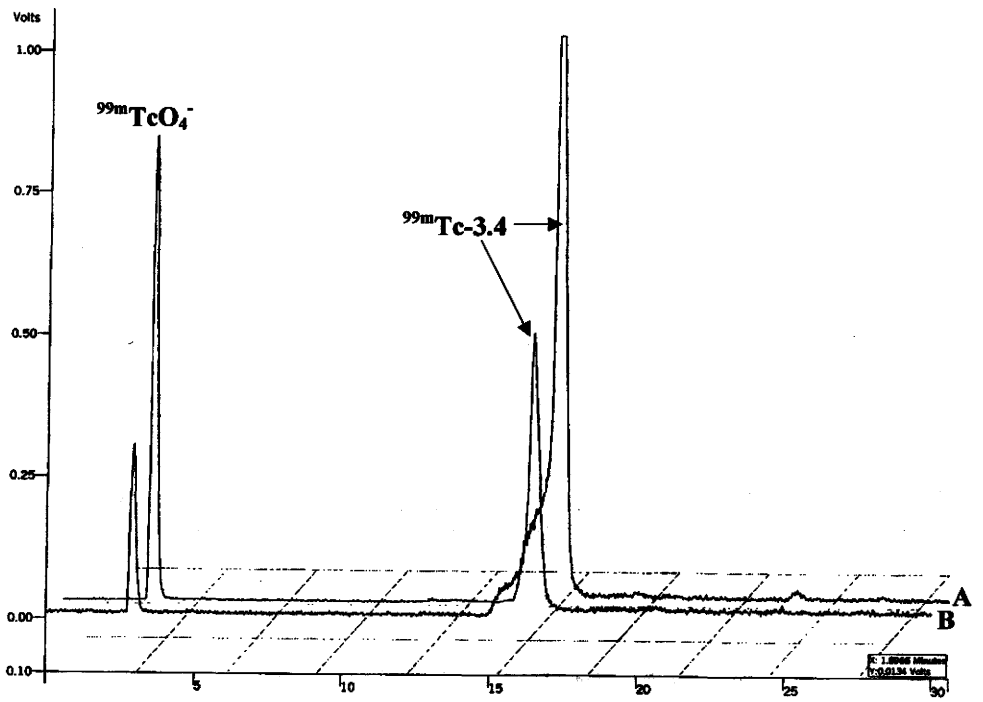


Figure 3.26: γ-HPLC of *in vitro* assays after 18 hrs with ^{99m}Tc-**3.4**. **A**: L-Cys challenge; **B**: L-His challenge. (Elution method **B**).

3.8 Summary and Conclusions

The focus of this chapter was to investigate the use of microwave irradiation in the synthesis of metallocarboranyl glycoside targets **3.4** and **3.5**. Optimal conditions for the synthesis of Re(I) metallocarborane derivatives were determined using precursors **3.1** and **3.3** as test substrates, and compound **3.2** as the example target. The Biotage Initiator Sixty microwave reactor was employed for these evaluations, and was determined to be an ideal platform for the synthesis of Re(I) metallocarboranes in aqueous media. It was determined that reactions at 200°C resulted in the most rapid and high-yielding

conversions to the desired products and that buffering the reaction was essential for efficient complex formation.

During the investigation into the microwave-assisted reactions, it was determined that isomerization of the metallocarborane cages was occurring. The observed isomerization was characterized by the migration of one of the carbon atoms away from the metal-binding face of the carborane cluster. In the absence of molecular structures from x-ray crystallography, NMR spectroscopy was found to be a valuable tool for elucidating the nature of the meallocarborane cage environments. Chiefly, 1D selective nOe experiments were able to indicate that isomerization had occurred by the lack of enhancement of signals for certain protons, which, in the *closo-* or *nido-ortho*-carborane precursors did give enhancements, thereby indicating that the same protons in the metal complexes were further apart, spatially, and thus, isomerization had occurred.

Finally, successful radiolabelling with ^{99m}Tc was accomplished using microwave-assisted heating. The optimal conditions for this synthesis involved a 15-minute irradiation at 200°C, with the ligand **2.6d** present at a concentration of 10^{-2}M. Compound ^{99m}Tc-**3.4** was prepared and isolated in 58% radiochemical yield. This complex exhibited good stability towards L-cysteine and L-histidine challenge conditions.

With the methods established in this chapter to synthesize and characterize metallocarboranyl glycosides such as the model compound **3.4**, and its radioactive analogue ^{99m}Tc-**3.4**, it is now possible to investigate more advanced compounds, including a bifunctionalized carboranyl glycoside, and a C-2 functionalized glucose derivative, which is proposed as an analogue of FDG.

3.9 Experimental Section

High-performance liquid chromatography was performed with a Varian ProStar HPLC system. Compounds were detected by UV asbsorbance at 254nm. Radioactive (^{99m}Tc) compounds were detected with a IN/US γ-RAM Model 3 detector incorporated into the HPLC system. HPLC Elution method **A**:[52] 100% A, 0 to 3 min; 100% A to 75:25 A:B , 3-6 min; 75:25 A:B to 67:33 A:B 6 to 9 min; 67:33 A:B to 100% B, 9 to 20 min; 100% B, 20 to 22 min; 100% B to 100% A, 22 to 25 min; 100% A, 25-30 min. Column: Varian C_{18} Nucleosil 4.6x250mm. Flow rate: 1.0 mL/min. Solvent A = triethylammonium phosphate (TEAP) buffer, pH 2-2.5. Solvent B = Methanol.

Elution method **B**: 80:20 A:B to 54:46 A:B, 0 to 20 min; 54:46 A:B to 100% B, 20 to 25 min; 100% B, 25 to 30 min. Column: Varian C_{18} Nucleosil 4.6x250mm. Flow rate: 1.0 mL/min. Solvent A = Water. Solvent B = Acetonitrile.

Elution method **C**: 80:20 A:B to 75:25 A:B, 0 to 10 min; 75:25 A:B to 100% B 10 to 20 min. Column: Varian C_8 Dynamax 10x250mm. Flow rate: 4.7 mL/min. Solvent A = Water. Solvent B = Acetonitrile.

Sodium [*rac*-1-(n-propanoic acid)-3,3,3-tricarbonyl-3-rhenium-1,2-dicarba-*closo*-dodecaborate] (3.2):

1,2-Dicarba-*closo*-dodecaboran-1-yl propanoic acid **3.3** (25mg, 0.12 mmol) and $[Re(CO)_3(H_2O)_3]Br$ (76 mg, 0.19 mmol) were dissolved in 0.5 M NaF (2.4 mL) in a sealed Emrys vial (5 mL) containing a magnetic stir bar. The mixture was then heated at 200 °C for 15 min. in a microwave reactor; a second portion of $[Re(CO)_3(H_2O)_3]Br$ (63

mg, 0.16 mmol) was added, and the reaction mixture was heated for an additional 15 min. at 200 °C. The solvent was then removed by rotary evaporation, giving a brown gum. The crude product was isolated using an automated purification system and a gradient of 2 to 20 % MeOH in CH_2Cl_2, extracted with acetone to remove excess salts, then dried *in vacuo*, yielding an amber oil (54 mg, 93%). ^{1}H NMR (CD_3OD): δ = 2.33 (m, 2 H, CH_2COOH), 2.07 (m, 2 H, CH_2C_{cage}), 1.62 (br s, 1 H, $C_{cage}H$). $^{13}C\{^1H\}$ NMR (CD_3OD): δ = 200.61, 179.33, 53.74, 36.85, 36.24, 29.03. $^{11}B\{^1H\}$ NMR (CD_3OD): δ = -6.01, -8.36, -10.62, -12.14, -18.79, -20.24. FTIR (KBr): ν 3629, 2543, 2003, 1910, 1715, 1613 cm^{-1}. TLC (CH_2Cl_2:CH_3OH; 6:1): R_f = 0.50. HRMS (ESI^-) Calculated for $C_8H_{15}B_9O_5Re$: 475.1370. Observed: 475.1385 [M^-].

Sodium [8-methyl(β-D-glucopyranosyloxy)-2,2,2-tricarbonyl-2-rhenium-1,8-dicarba-*closo*-dodecaborate] (3.4):

Compound **2.6d** (0.066 g, 0.19mmol) and $[Re(CO)_3(H_2O)_3]Br$ (0.17 g, 0.42 mmol) were placed in an Emrys microwave reaction vial (2-5mL) and dissolved in a phosphate-buffered solution (pH 7.3) containing 0.5M NaF (2.2mL). An aluminum cap containing a Teflon septum was attached to the vial and sealed by crimping. The reaction vessel was heated in the Biotage Initiator Sixty reactor for 10 minutes at 200°C, after which the vial was un-capped and a further 0.12g (mmol) of the rhenium reagent added. The vial was sealed again and irradiated for a further 10 minutes at 200°C, resulting in a clear yellow solution. The aqueous solution was diluted with acetonitrile and concentrated to dryness by rotary evaporation. The residue was extracted into methanol,

and the target compound isolated by silica gel column chromatography (10-15% MeOH/CH_2Cl_2). Further purification was accomplished using a C_{18} SepPak® cartridge (Waters), giving the target compound in 31% yield (0.037 g). ^{1}H NMR (CD_3OD) δ 4.25 (2d, 2H, $^3J_{1,2}$ = 7.8 Hz, H-1,1*), 4.00 (2d, 2H, $^2J_{7a,7b}$ = -10.7 Hz, H-7a,7a*), 3.86 (2dd, 2H, $^2J_{6a,6b}$ = -11.9 Hz, H-6a, 6a*), 3.69 (2dd, 2H, H-6b,6b*), 3.64 (2d, 2H, H-7b,7b*), 3.35 (m, 4H, H-3,3*,4,4*), 3.24 (m, 4H, H-2,2*,5,5*), 1.67 (br s, 2H, H-9,9*), 3.10 - 0.90 (br m, B-H). ^{13}C{^{1}H} NMR (CD_3OD) δ 200.43 (C≡O), 104.31, 104.28 (C-1,1*), 77.85 (C-3,3*,5,5*), 76.66 (C-7,7*), 75.17 (C-2,2*), 71.42 (C-4,4*), 62.53 (C-6,6*), 53.77 (C-8,8*), 29.60 (C-9,9*). ^{11}B{^{1}H} NMR (CD_3OD): δ -6.41, -8.21, -9.38, -12.17, -18.89, -20.10. IR (KBr): ν 3433 (O-H), 2539 (B-H), 2002, 1904 (C≡O) cm^{-1}. TLC (1:4 MeOH:CH_2Cl_2): R_f = 0.45. ESI-MS: m/z = 595.3 [M$^-$]. HRMS(ESI): Calculated for $C_{12}H_{23}B_9O_9Re$: 595.1794. Observed: 595.1776. HPLC (Elution method **B**): t_R = 19.8 min.

Sodium [1,8-Bis-(methyl-β-D-Glucopyranosyloxy)-2,2,2-tricarbonyl-2-rhenium-1,-8-dicarba-*closo*-dodecaborate] (3.5):

Nido-carborane ligand **2.12** (0.068 g, 0.13 mmol), and $[Re(CO)_3(H_2O)_3]Br$ (0.23 g, 0.57 mmol) were dissolved in NaF (10mL) and placed in the Parr microwave vessel. The vessel was assembled and heated in the SANYO microwave oven for 1.5 minutes at the 80% power setting. The resulting solution was concentrated to dryness by rotary evaporation. Semi-preparative HPLC (Elution method **C**) gave the product in 14% yield (0.014 g). ^{1}H NMR (600 MHz, CD_3OD): δ 4.25 (2d, 2H, $^3J_{1,2} = {}^3J_{1*,2*}$ = 7.7 Hz, H-1,1*), 4.12 (d, 2H, $^3J_{1',2'}$ / $^3J_{1'*,2'*}$ = 7.7 Hz, H-1',1'*), 3.98 (2d, 2H, $^2J_{7a,7b} = {}^2J_{7a*,7b*}$ = -10.7 Hz,

H-7a,7a*), 3.84 (m, 4H, H-6a,6a*,6a′,6a′*), 3.76 (d, 1H, $^{2}J_{7a',7b'}$ = -10.6 Hz, H-7a′ or 7a′*), 3.68 (m, 5H, H-7a′ or 7a′*, H-6b,6b*,6b′,6b′*), 3.65 (2d, 2H, H-7b,7b*), 3.45 (d, 1H, H-7b′ or 7b′*), 3.34 (m, 9H, H-7b′ or 7b′*, H-3,3*,3′,3′*,4,4*,4′,4′*), 3.23 (m, 4H, H-2,2*,2′,2′*), 3.19 (m, 4H, H-5,5*,5′,5′*), 0.80 - 3.00 (br m, B-H). $^{13}C\{^{1}H\}$ NMR (151 MHz, CD_3OD): δ 200.90 (C≡O), 104.36, 104.16, 104.05 (C-1,1*,1′,1′*), 78.66 (C-7′,7′*), 77.89 (C-5,5*,5′,5′*), 77,71 (C-3,3*,3′,3′*), 76.69 (C-7,7*), 75.25 (C-2,2*,2′,2′*), 71.48 (C-4, 4*,4′,4′*), 62.60 (C-6,6*,6′,6′*), 58.01 (C-8′8′*), 54.42 (C-8,8*). $^{11}B\{^{1}H\}$ NMR (160 MHz, CD_3OD): δ -5.40, -8.91, -12.01, -18.21. IR (KBr): ν 3410 (O-H), 2550 (B-H), 2002, 1907 (C_{3v} C≡O) cm^{-1}. ESI-MS: m/z = 787.2 $[M]^-$. HRMS (ESI): Calculated for $C_{19}H_{35}B_9O_{15}Re$: 787.2432. Observed: 787.2421.

^{99m}Tc radiolabelling: [^{99m}Tc]-3.4:

To a sealed 2 mL Emrys microwave vial containing **2.6d** (0.01M) in 0.5 mL de-gassed aqueous sodium fluoride was added a 350 μL aliquot of the solution containing $[^{99m}Tc(CO)_3(H_2O)_3]^+$ (422 MBq, prepared according to reported methods.[20,52,53]). The vial was placed in a Biotage Initiator Sixty microwave reactor and heated for 15 minutes at 200°C. The product solution was taken up in a syringe and loaded onto a C_{18} Environmental SepPak Plus™ cartridge (Waters). Conditioning: i)EtOH, 10 mL; ii) CH_3CN, 10 mL; iii) 1:1 CH_3CN:0.1M HCl, 10 mL; iv) 0.1M HCl, 10 mL. Elution: i) 0.1M HCl, 7 mL; ii) 4:1 0.1M HCl:CH_3CN, 4 mL; iii) 1:1 0.1M HCl:CH_3CN, 4 mL; iv) 1:4 0.1M HCl:CH_3CN, 4 mL; v) CH_3CN, 5 mL. Compound [^{99m}Tc]-**3.4** was obtained in 58% radiochemical yield, corrected for decay (n = 6). HPLC (Method **B**): t_R = 19.9 min.

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Chapter 4

Synthesis and Bioconjugation of Funcionalized Metallocarborane Glycosides

Following the successful preparation of metallocarboranyl glycoside **3.4**, the subsequent objective was to prepare a derivative that could be readily conjugated to a targeting vector. To this end, the synthesis of an "advanced" metallocarboranyl glycoside **4.1** (Figure 4.1) was investigated as a prosthetic group that can be linked to a range of biomolecules, including peptides, proteins or antibodies. As illustrated in Figure 4.1, this derivative contains three distinct components: i) the metallocarborane to bind a radiometal or radiohalogen, ii) the carbohydrate, which in this class of compounds would be present to mask the hydrophobic carborane, and iii) the carboxylic acid, through which conjugation to biologically-active molecules could occur. It has been shown that by adding glucose units (glycosides) to radiolabelled peptides has improved pharmacokinetics (e.g. clearance from non-target tissue, renal excretion) versus those without the carbohydrate appendage.[1-4] Unfortunately, the synthesis of these types of tracers is difficult and there is no general strategy for glycosylating and radiolabelling simultaneously.

In order to have the capacity to comapre the impact of the glucose group on bioconjugation chemistry and eventually the biology, the non-glycosylated analogue, **4.2** was prepared in addition to the target glycoside.

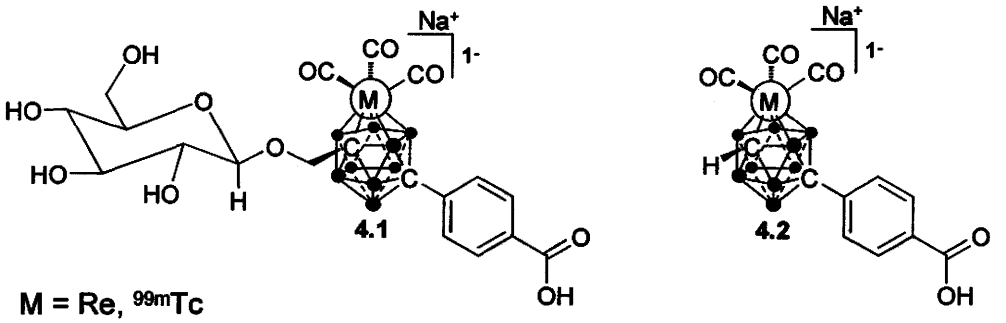


Figure 4.1: Benzoic acid-derived metallocarborane targets. Proposed bifunctional glycoside **4.1** contains the acid functionality for the purposes of bioconjuation, and the sugar to mask the hydrophobic nature of the carborane. The non-glycosylated analogue, **4.2**, can be used to assess the effect of the carbohydrate on labelling, bioconjugation chemistry and biology.

4.1 Synthesis of Non-Glycosylated Metallocarboranyl Benzoic Acid Derivative ***4.2***

A retrosynthetic scheme is presented in Figure 4.2, and shows how the ultimate target metallocarboranyl-benzoic acid **4.2** was approached. A microwave-assisted metal insertion reaction yielded **4.2** from the *nido*-carborane precursor **4.3**. Compound **4.3** was prepared from the *closo*-carborane derivative **4.4** by a base-mediated cage degradation/saponification procedure similar to that used to prepare the *nido*-carboranyl glycoside ligands discussed in previous chapters. The *closo* carborane derivative **4.4** was prepared from alkyne **4.5** by the decaborane-alkyne insertion reaction used in the syntheses of all of the *closo*-carborane derivatives prepared thus far. Alkyne **4.5** was obtained from the trimethylsilyl-protected alkyne **4.6**, which was prepared from readily available starting materials via a Sonogashira cross-coupling reaction.[5-7] The precursor methyl 4-iodobenzoate was obtained in gram quantitites using literature methods,[6] while trimethylsilylacetylene is available commercially.

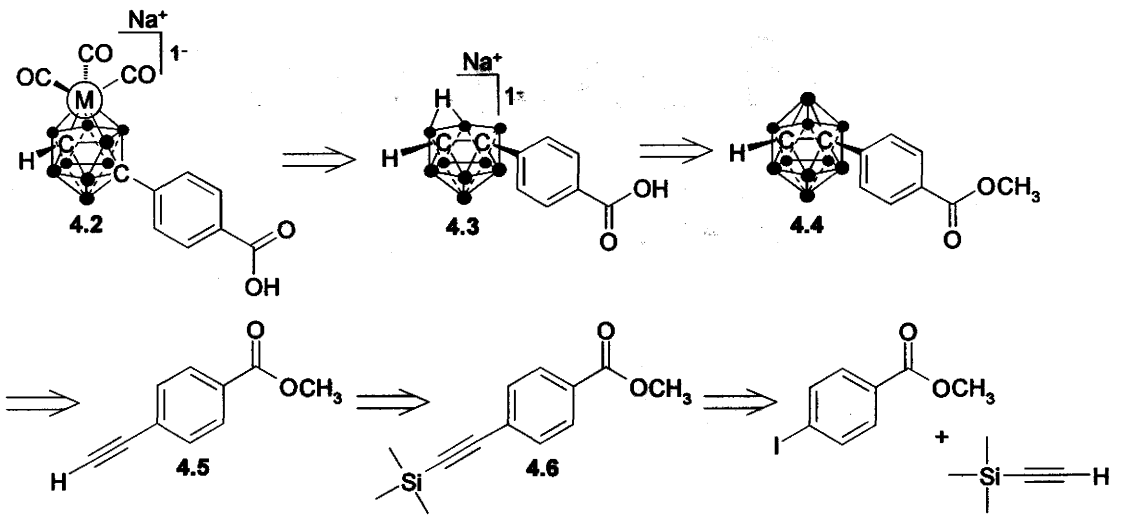
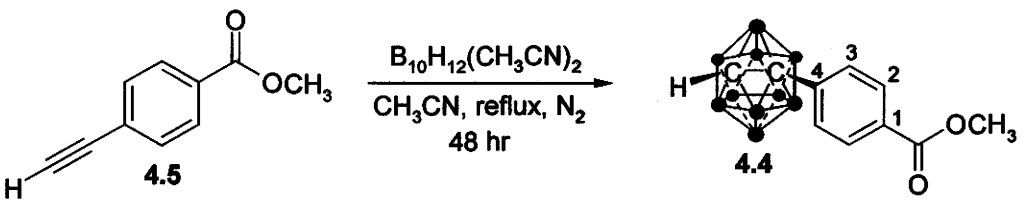


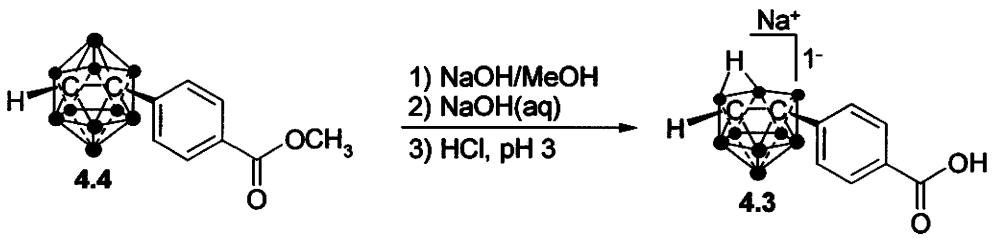
Figure 4.2: Retrosynthetic approach to non-glycosylated metallocarborane **4.2**.

The syntheses of compounds **4.5** and **4.6** followed those in the previous report of these compounds by Li and co-workers, and the products obtained were in agreement with this published account.[6] Compound **4.4** was prepared (Scheme 4.1) in a manner similar to the previous synthesis of *closo*-carboranyl glycosides.[8] Decaborane(14) was stirred for approximately 12 hours prior to the addition of compound **4.5**. The resulting solution was stirred at reflux for 48 hours. Silica gel column chromatography isolated the desired product in 56% yield. Compound **4.4** was characterized by ^{1}H, $^{13}C\{^{1}H\}$, and $^{11}B\{^{1}H\}$ NMR spectroscopy, IR spectroscopy, and electrospray ionization mass spectrometry, which were in agreement with those data reported previously for this known compound.[9]



Scheme 4.1: Synthesis of compound **4.4** and illustration of numbering scheme used for NMR analysis.

With compound **4.4** in hand, the synthesis of the *nido*-carboranyl benzoic acid ligand **4.3** (Scheme 4.2) was undertaken. The *closo*-carborane starting material was combined with ten equivalents of sodium hydroxide and stirred in methanol at reflux overnight to effect the cage degradation. At this point, electrospray mass spectrometry indicated the presence of the target anion (m/z = 253). However, a mass peak corresponding to the *nido*-carborane-methyl ester was also observed at m/z = 268. To ensure complete hydrolysis of the ester, the methanol was removed, and the product residue was re-dissolved in water, to which was added a further equivalent of aqueous sodium hydroxide. After a few hours of stirring at room temperature, the pH of the solution was adjusted to approximately 3. Removal of the solvent gave the desired compound in 67% yield.



Scheme 4.2: Synthesis of **4.3**.

The infrared spectrum of the product gave the expected carboxylic acid O-H stretching signal at 3576 cm^{-1}, the *nido*-carborane B-H stretch at 2526 cm^{-1}, and the carboxylic acid C=O stretch at 1606 cm^{-1}. Electrospray mass spectrometry for **4.3** gave the target anion mass at m/z = 254.0.

The ^{1}H NMR spectrum of **4.3** (Figure 4.3) showed the changes that were expected to occur upon degradation of the cage and saponification of the benzoate methyl ester. A slight upfield shift of the aryl proton signals was observed, with H-2 and H-3 giving rise to doublets at 7.78 and 7.25 ppm, respectively. The carborane cage terminal proton appeared at 2.27 ppm, a shift to lower frequency by 1.77 ppm versus the *closo*-carborane precursor. As in all of the previous *nido*-carborane derivative prepared during the course of this research the B-H proton signal shifted to appear in the range 2.60 to -0.30 ppm. Finally, the "bridging" proton gave rise to a broad signal centered at -2.42 ppm.

The $^{13}C\{^{1}H\}$ NMR spectrum of **4.3** (Figure 4.4) was also indicative of the desired compound. A low-intensity signal was observed at 170.98 ppm, corresponding to the carboxylic acid C=O carbon atom. The signal at 151.90 ppm corresponded to one of the aryl ring *ipso* carbon atoms. HMBC correlation to the H-2 proton (proximal to the carboxyl group) confirmed the assignment of this carbon signal to C-1, the carbon attached to the carboxylic acid functional group. The signal at 129.58 ppm corresponded to C-2, the aryl carbon atom nearest the carboxyl group. The signal at 126.66 ppm appeared to consist of two overlapping signals, which were corresponding to the C-3 and C-4 carbon atoms. The carborane carbon signals, appeared broadened and with reduced intensity, as was the case for the other *nido*-carborane derivatives prepared and

characterized previously. The substituted carborane carbon atom gave rise to the signal at 62.19 ppm, while the terminal carborane carbon gave the signal at 45.13 ppm. The $^{11}B\{^1H\}$ spectrum of **4.3** (Figure 4.5) gave the expected pattern for a mono-substituted *nido*-carborane derivative. A total of nine signals were observed at -7.85, -9.49, -12.87, -15.68, -17.27, -19.06, -22.32, -32.03, and -34.93 ppm, with a 1:1:1:1:1:1:1:1:1 integration ratio.[10]

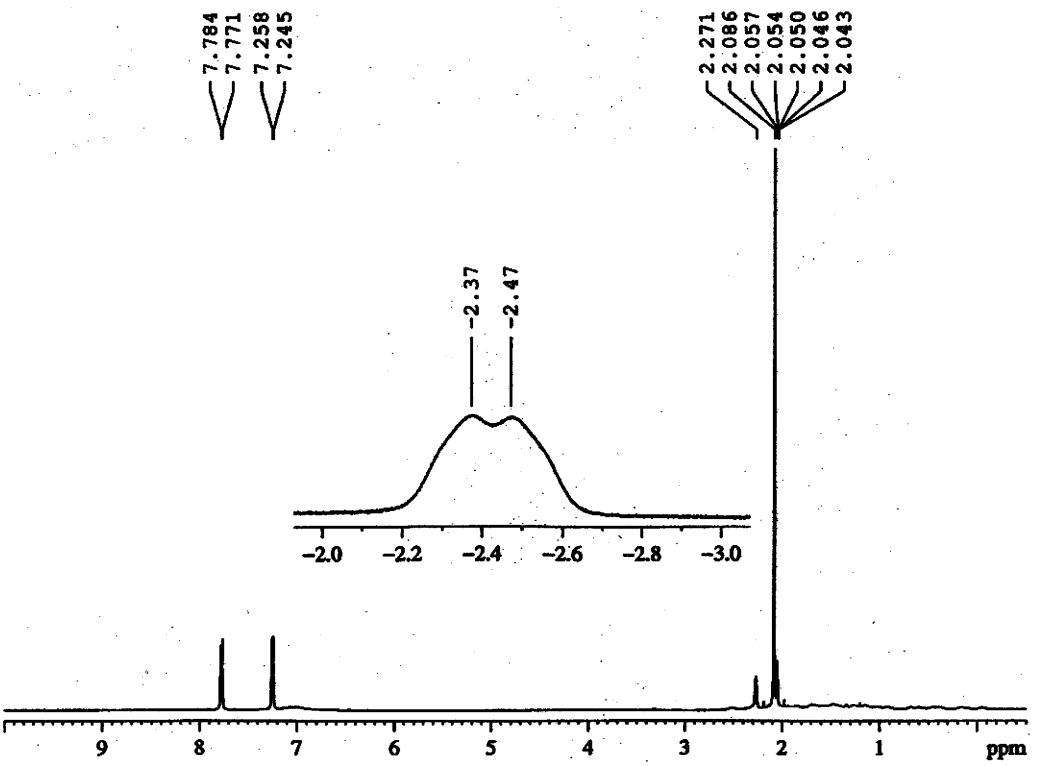


Figure 4.3: The ^1H NMR spectrum of **4.3** (600 MHz, acetone-d_6).

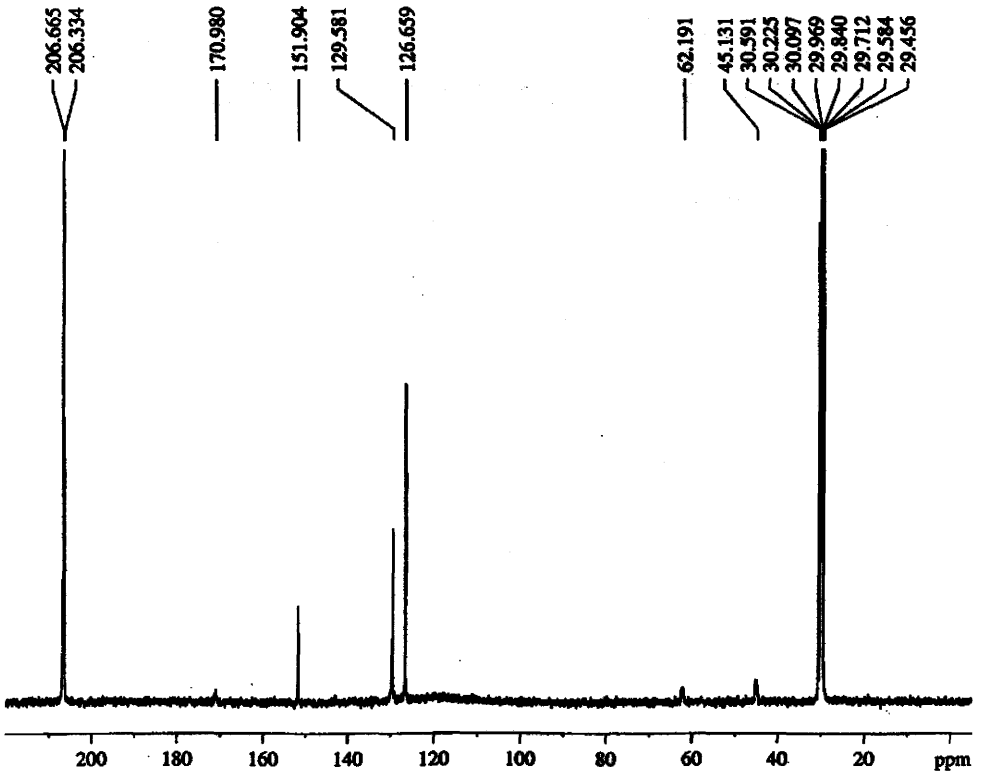


Figure 4.4: The $^{13}C\{^{1}H\}$ NMR spectrum of **4.3** (151 MHz, acetone-d_6).

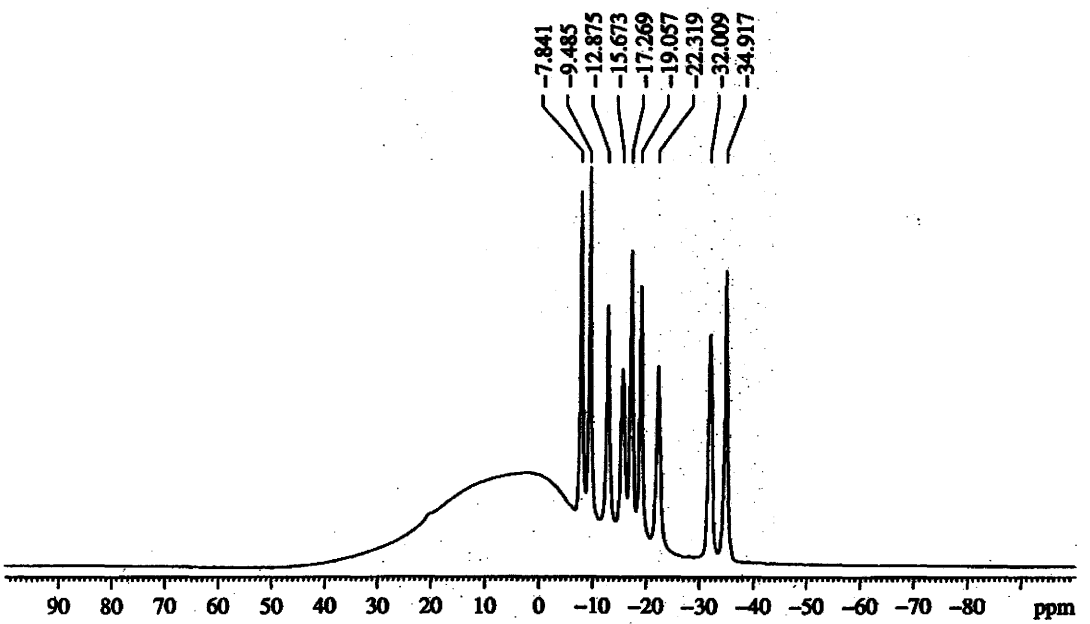
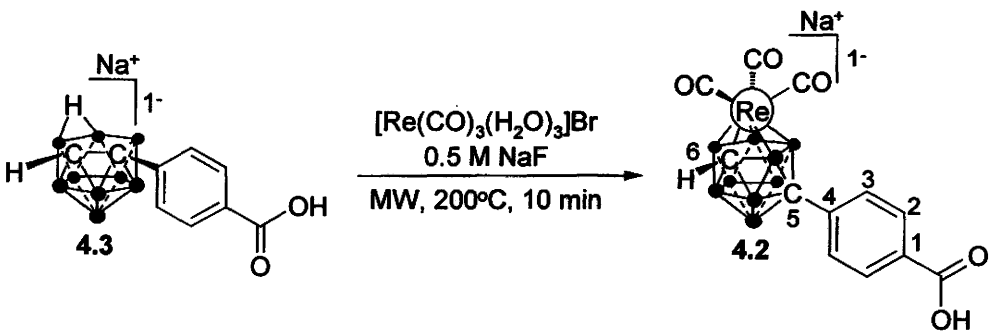


Figure 4.5: The $^{11}B\{^{1}H\}$ NMR spectrum of **4.3** (192 MHz, acetone).

Synthesis of the target rhenium complex **4.2** (Scheme 4.3) was affected in a manner similar to that used for the microwave-assisted reactions described previously. The ligand **4.3** was dissolved in 0.5 M NaF and combined in a microwave vial with 1.5 equivalents of $[Re(CO)_3(H_2O)_3]Br$. The resulting solution was heated to 200°C for ten minutes in the microwave reactor. Following this irradiation, a further quantity of the rhenium reagent was added, and the vial heated for another ten minutes at 200°C in order to ensure complete conversion of the precursor. The target compound was isolated in 44% yield following silica gel column chromatography and an additional reversed-phase purification on a C_{18} silica cartridge using the Biotage SP4 automated purification system.



Scheme 4.3: Microwave-assisted synthesis of **4.2**.

The infrared spectrum of compound **4.2** gave the absorptions expected from the structure. The carboxylic acid O-H stretch appeared at 3427 cm^{-1}. The rhenacarborane B-H stretch was observed at 2551 cm^{-1}. The C≡O stretch of the carbonyl ligands on the rhenium metal center gave the expected pair of signals at 2008 and 1890 cm^{-1}. Finally,

the benzoic acid C=O stretch appeared at 1605 cm^{-1}. Electrospray mass spectrometry indicated the target anion mass at m/z = 523.2.

The ^{1}H NMR spectrum (Figure 4.6) of **4.2** gave the expected pair of doublets at 7.82 and 7.45 ppm, integrating to two protons each, corresponding to the protons on the aryl ring. The carborane CH proton gave a broad singlet at 1.90 ppm. The carborane B-H protons gave a broad baseline signal between 1.80 and 3.80 ppm. A second pair of aromatic signals were observed and correspond to the 2,1,8 isomer in which the CH group migrates out of the bonding face of the carborane, as opposed to the aryl group. In order to relieve the most steric bulk about the $Re(CO)_3$ core, the substituted carbon atom is expected to be the most likely to undergo migration. However, it is possible, although less likely, that the unsubstituted carbon could migrate instead. This would result in the formation of a pair of 2,1,8-rhenacarborane isomers of **4.2**, as depicted in Figure 4.7. Integration of the signals in the ^{1}H NMR spectrum of **4.2** indicates that during the synthesis, approximately 5% of the minor isomer is formed. The formation of a mixture of "carbon atom" isomers of 2,1,8-rhenacarborane derivatives has been shown in other reported examples of metallocarboranes.[11]

The $^{13}C\{^{1}H\}$ NMR spectrum (Figure 4.8) of compound **4.2** was consistent with the desired product. The carbonyl ligands on the metal core gave the signal at 200.22 ppm. The benzoic acid carbonyl carbon atom gave a barely-visible signal just above 175 ppm. The aromatic ring gave signals at 133.78 and 129.99 for the carbonyl carbon-bonding (C-1) and carborane carbon-bonding (C-4) carbon atoms, respectively. The signals at 128.75 and 124.70 ppm, corresponded to the C-2, and C-3 carbon atoms,

respectively. The signals at 56.83 and 28.96 ppm arose from the substituted (C-5) and unsubstituted (C-6) carborane carbons, respectively. The $^{11}B\{^1H\}$ NMR spectrum (Figure 4.9) of compound **4.2** gave a pattern of signals that was very similar to that of compound **3.4**, which is consistent with a 2,1,8-isomeric form of the ReC_2B_9 cluster.

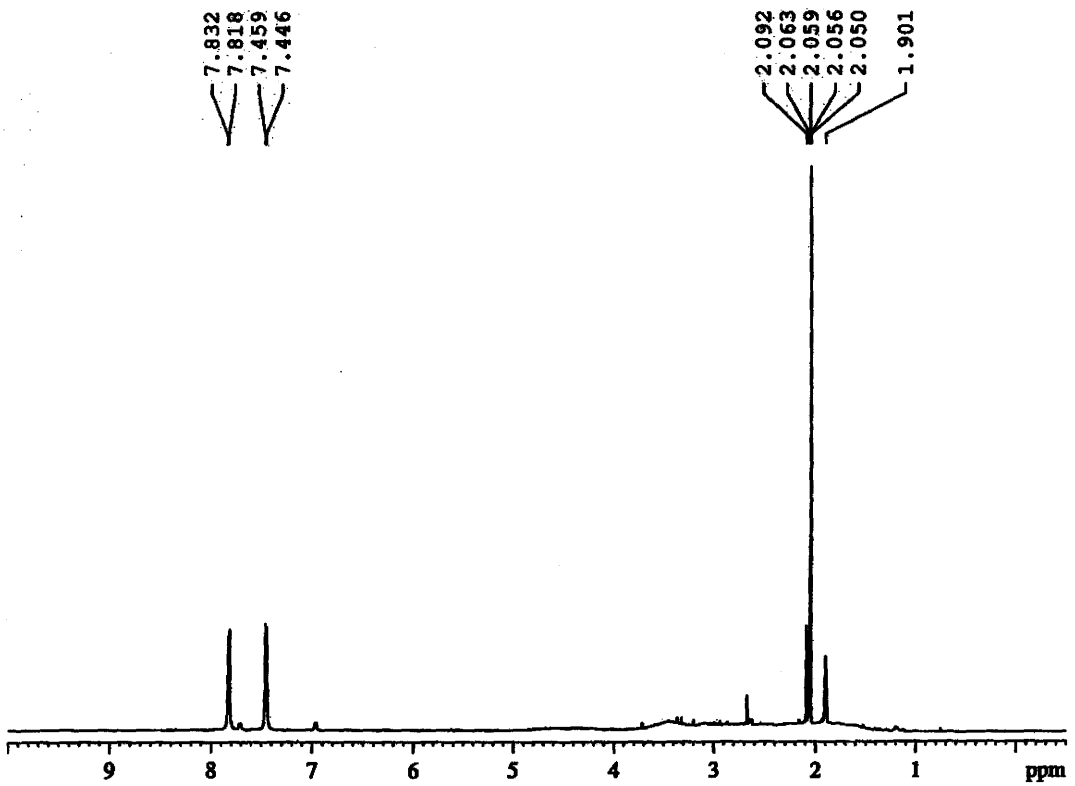


Figure 4.6: The ^1H NMR spectrum of **4.2** (600 MHz, acetone-d_6).

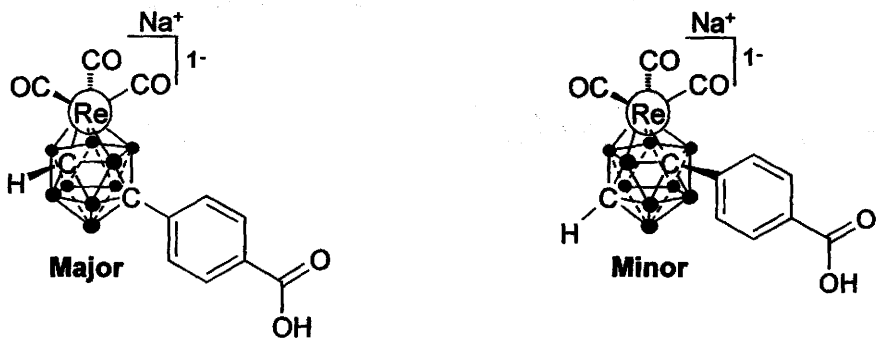


Figure 4.7: Structures of the possible "carbon atom isomers" of **4.2**.

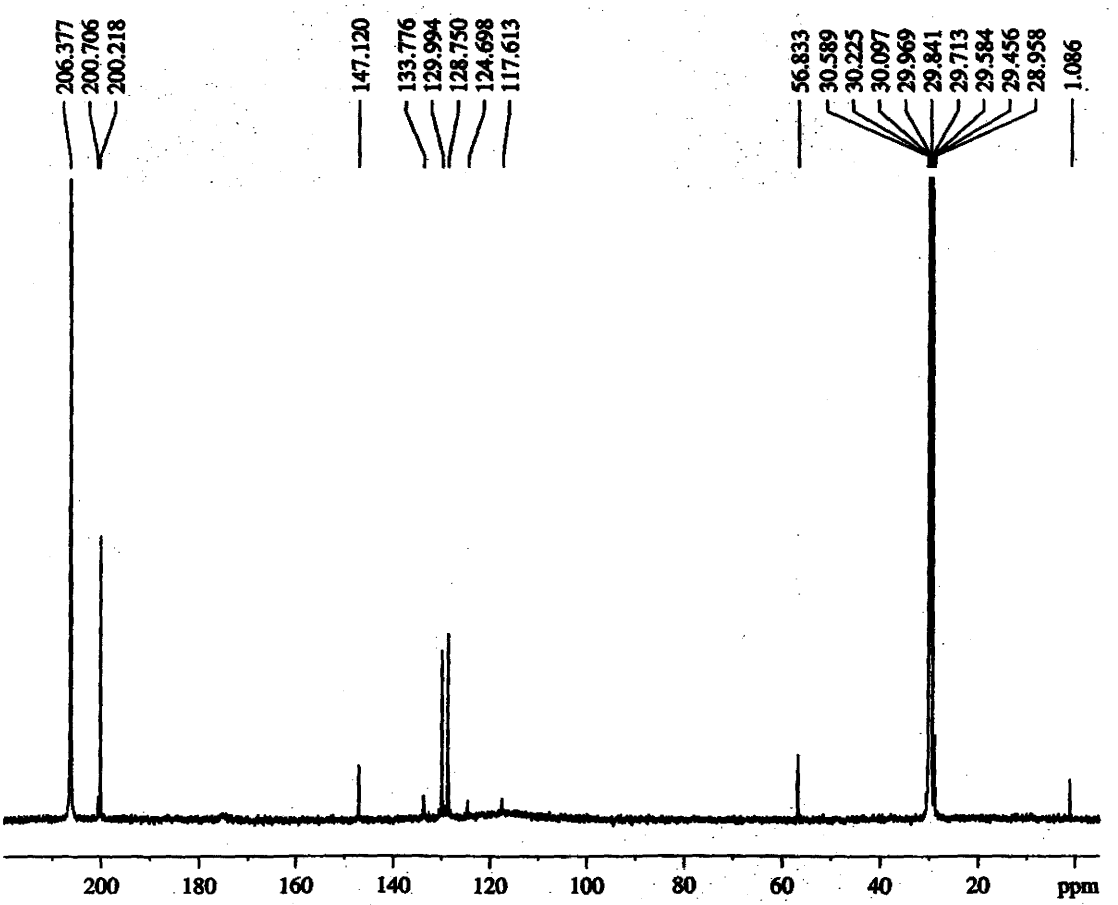


Figure 4.8: The $^{13}C\{^{1}H\}$ NMR spectrum of **4.2** (150 MHz, acetone-d_6).

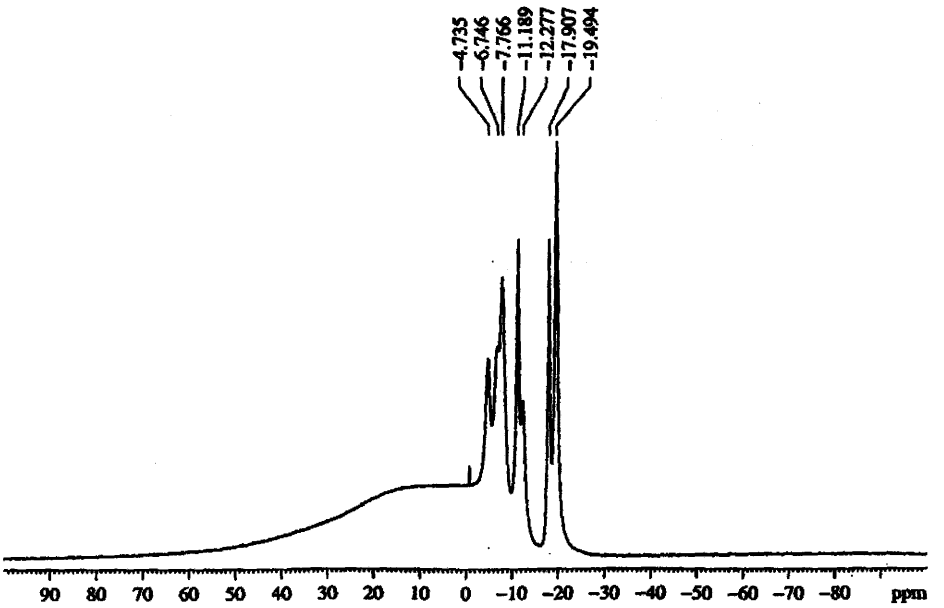
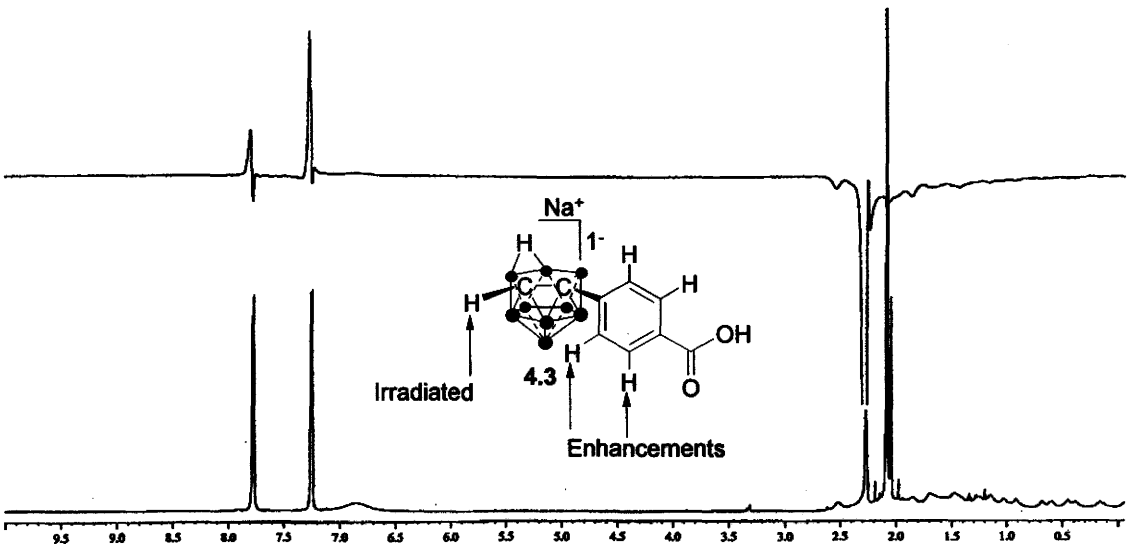


Figure 4.9: The $^{11}B\{^1H\}$ NMR spectrum of **4.2** (192 MHz, acetone-d_6).

To verify that isomerization of the cage had in fact occurred, an nOe experiment was conducted in a fashion similar to that for other mono-substituted rhenacarborane derivatives. Irradiation at the carborane CH signal frequency gave a spectrum (Figure 4.10) in which very slight enhancements of the aryl proton signals were observed. This result is in contrast to that for the *nido*-carborane ligand, in which more marked enhancements of the aryl proton signals were observed. Thus, it may be concluded, based on both the $^{11}B\{^1H\}$ NMR and selective nOe spectra, that isomerization did occur during the formation of compound **4.2**.



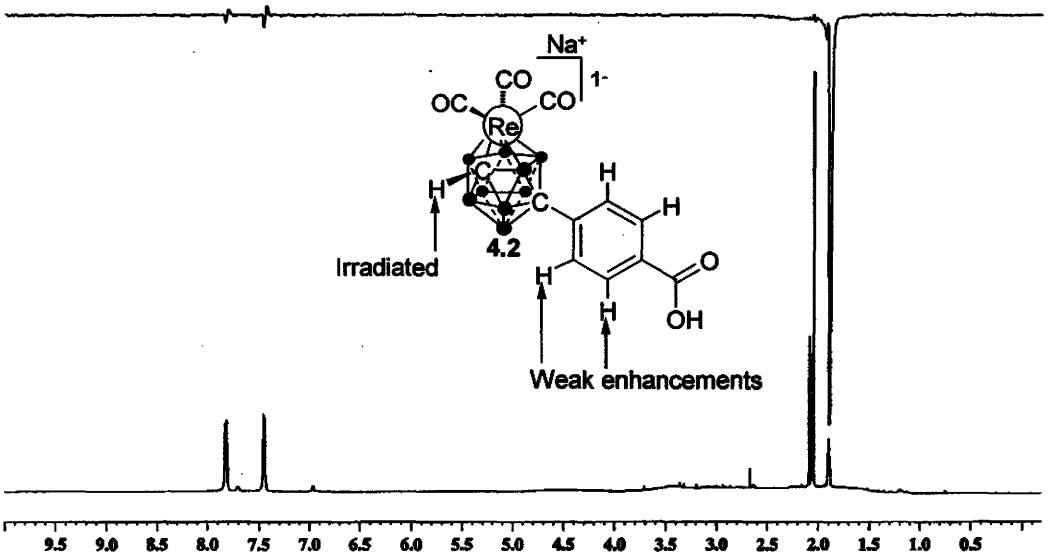
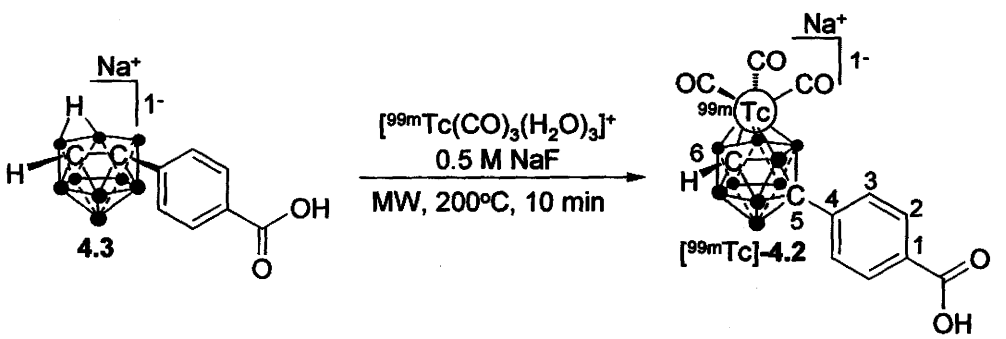


Figure 4.10: Selective 1D ^{1}H nOe spectra of **4.3** (top), and **4.2** (bottom) (600 MHz, acetone-d_6).

4.2 Synthesis of [^{99m}Tc]-4.2

The synthesis of [^{99m}Tc]-**4.2** was successfully conducted according to the methods used to prepare [^{99m}Tc]-**3.4**. The trisaquo species, [$^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared from Na[$^{99m}TcO_4$] and added to microwave vials containing compound **4.3** (Scheme 4.4). The vials were heated to 200°C for 10 minutes in the Biotage Initiator Sixty microwave reactor, and the product solutions analyzed by HPLC. Analysis of the crude reaction mixtures indicated the presence of a major radioactive product, with a minor amount of pertechnetate. Integration of the γ-chromatogram peaks suggested that the major product accounted for 95% of the radioactivity. The major product was isolated by solid-phase extraction using a C_{18} SepPak cartridge. Co-injection of this material with **4.2**, the non-radioactive standard resulted in matching peaks in the UV and γ-HPLC traces (Figure 4.11), thus confirming that the major radioactive product was [^{99m}Tc]-**4.2**. The product was obtained in 62% radiochemical yield (decay-corrected, n=7). The two peaks in the chromatogram correspond to the major and minor isomers of [^{99m}Tc]-**4.2**.



Scheme 4.4: Microwave-assisted synthesis of [^{99m}Tc]-**4.2**.

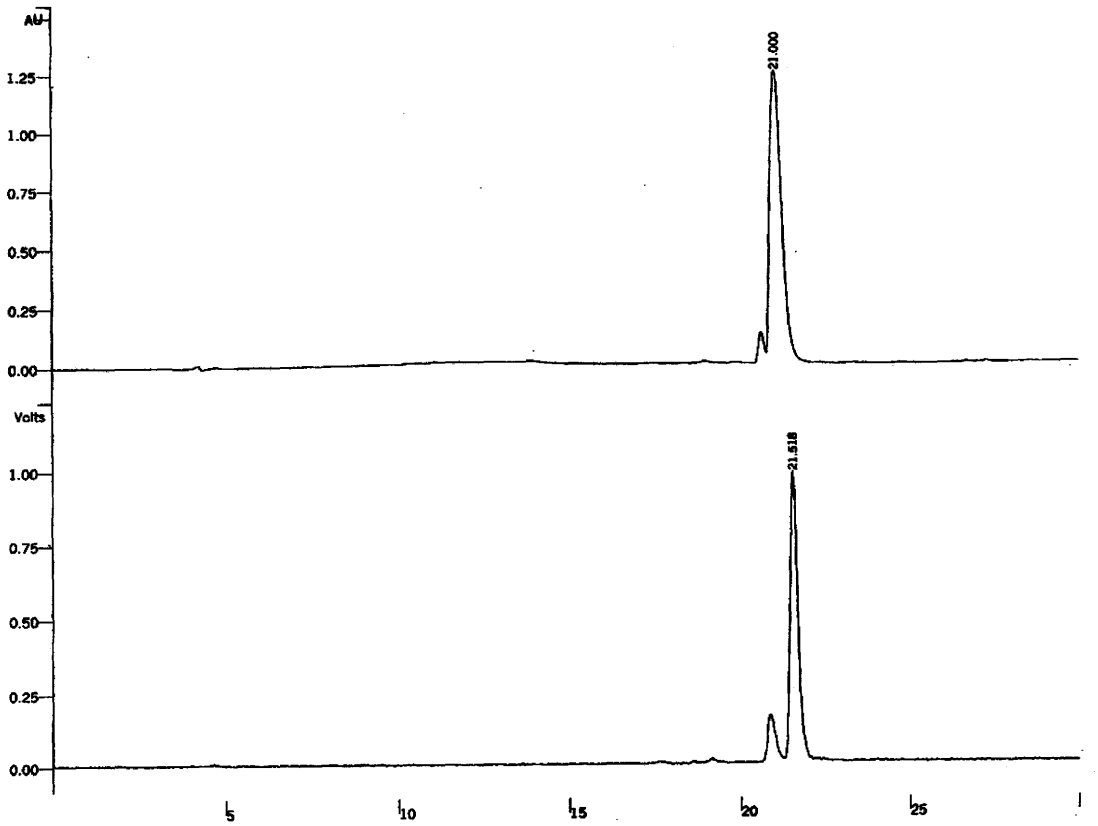


Figure 4.11: HPLC trace for synthesis of ^{99m}Tc-**4.2**. Top: UV-trace of standard **4.2**. Bottom: γ-trace of isolated [^{99m}Tc]-**4.2**.

In vitro stability studies were performed for [^{99m}Tc]-**4.2** by way of cysteine and histidine challenge experiments. Separate aliquots of isolated [^{99m}Tc]-**4.2** were incubated in 1.5 mL phosphate buffer at pH 7.4 at 37°C following addition of 10μL of either 10mM L-cysteine or 10mM L-histidine. Good stability was observed over 4 hours, with 95% of the complex remaining intact. The stability over 24 hours was 57% and 41% in the presence of L-cysteine and L-histidine, respectively, which was surprising given the known stability of other metallocarboranes.

*4.3 Synthesis of a Bioconjugatable Metallocarboranyl Glycoside - **4.1***

The target metallocarborane **4.1** was approached through the retrosynthetic scheme shown below (Figure 4.12), where compound **2.4** conveniently serves as a key precursor. The metallocarborane **4.1** was prepared from the *nido*-carboranyl glycoside derivative **4.12**, using microwave irradiation, as was the case for the Re and ^{99m}Tc complexes **3.4** and **4.2**. The *nido*-carborane derivative **4.12** was prepared in the familiar fashion, that is alcoholic hydroxide deboronation/ester hydrolysis, from the protected *closo*-carborane derivative **4.11**. Compound **4.11** was prepared from alkyne **4.10** by the typical decaborane-alkyne insertion, while **4.10** was prepared from **2.4** through a Sonogashira cross-coupling reaction with methyl 4-iodobenzoate.[5]

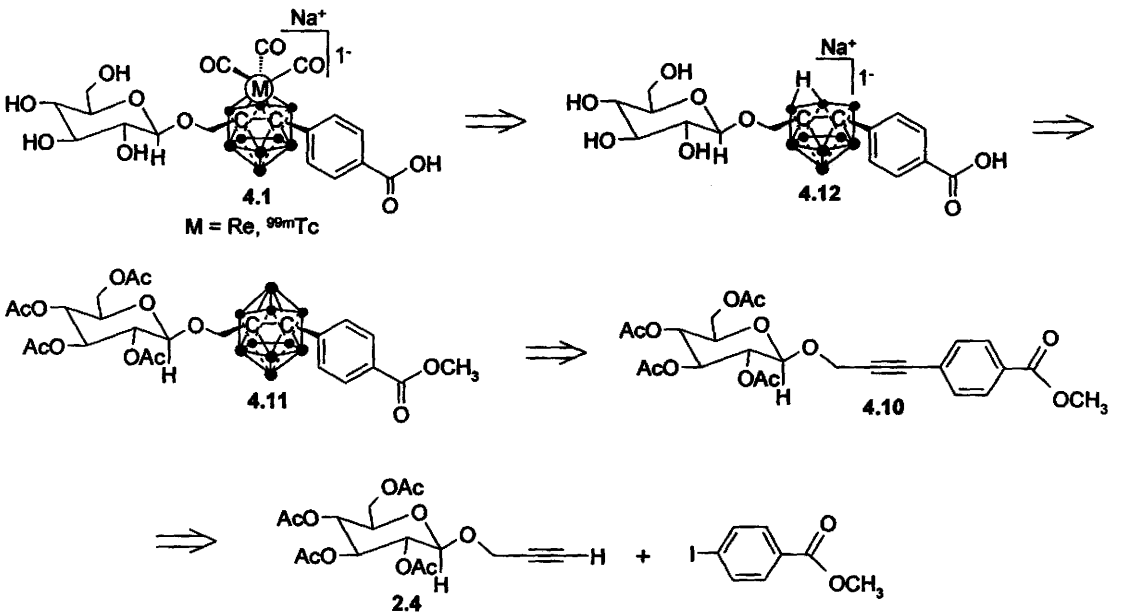
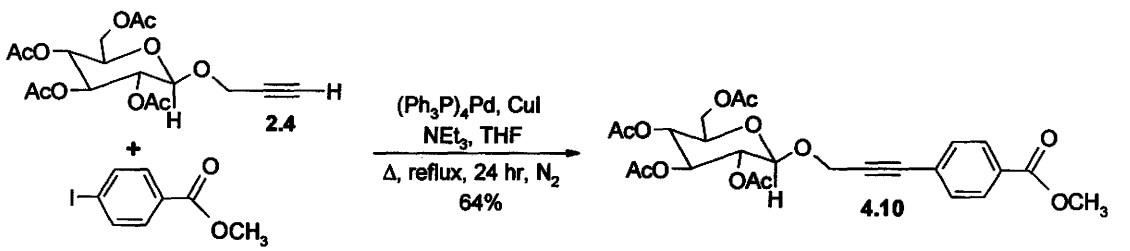


Figure 4.12: Retrosynthetic approach to compound **4.1**.

The synthesis of compound **4.10** (Scheme 4.5) was approached from compound **2.4**, which was used in the synthesis of compounds **2.1** and **3.4**, and could be easily prepared in multigram quantities. The issue of how to efficiently functionalize compound **2.4** with a carboxylic acid functionality was resolved by considering the Sonogashira reaction, in which aryl iodides may be coupled with alkynes in the presence of palladium and copper catalyts.[5] Roy and co-workers have fused this technology with carbohydrate chemistry, and have prepared several examples of alkyne-derived, acetyl-protected carbohydrate derivatives using this type of chemistry.[12-14] Methyl 4-iodo-benzoate was selected to introduce the acid group in order to minimize steric interferences (relative to the *ortho*- and *meta*-isomers) during the later decaborane-alkyne insertion and carborane metallation reactions. Additionally, the methyl ester could be conveniently hydrolyzed to give the desired carboxylic acid functionality as part of the process of forming the *nido*-carborane derivative (*vide infra*).



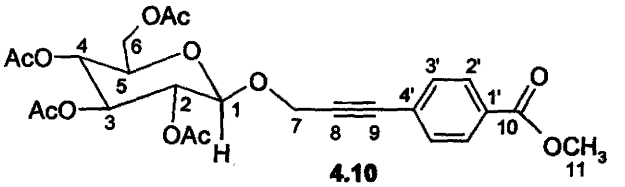
Scheme 4.5: Synthesis of compound **4.10**.

To prepare alkyne **4.10**, the aryl iodide was dissolved in a 1:1 v:v solution of dry THF and triethylamine, along with 5-10 mol% each of copper(I) iodide and

tetrakis(triphenylphosphine) palladium(0). To this solution was added slowly a solution of compound **2.4** in dry THF. Compound **2.4** was added in a dropwise manner to minimize homo-coupling of the alkyne, which had been reported as a potential byproduct of this type of reaction by Roy *et al.*[14,15] The resulting solution was stirred at reflux for 24 hours, and the desired product, **4.10** obtained in 64% yield following silica gel column chromatography and recrystallization from ethyl acetate and hexanes. The product was characterized by ^{1}H and $^{13}C\{^{1}H\}$ NMR and IR spectroscopy and by electrospray mass spectrometry. The NMR data are summarized and tabulated in Table 4.1.

The infrared spectrum of **4.10** gave the expected C=O stretching bands at 1758 and 1722 cm^{-1}, indicative of the acetate and benzoate groups, respectively. The electrospray mass spectrum gave a peak at m/z = 538, corresponding to the ammonium ion adduct, $[M+NH_4]^+$. The ^{1}H NMR spectrum of **4.10** was consistent with the desired product, and similar to that of **2.4**, in terms of the signals arising from the carbohydrate protons. The methylene "spacer" protons, between the glucose moiety and the alkyne, gave rise to the signal at 4.61 ppm. This signal appeared as appeared as a singlet, since, unlike the case of **2.4**, there was no acetylenic proton with which coupling could occur. The methoxy group protons appeared as a singlet at 3.93 ppm. To prove that the Sonogashira reaction had coupled the propargyl glycoside to the benzoate ester, the connection between the sugar and the benzene ring was established using ^{1}H-^{13}C HMBC spectra. Notably, the aryl proton, H-3′ (7.50 ppm), which was determined to be that proton closest to the benzene ring's point of attachment to the alkyne, gave a correlation

to the alkyne carbons C-8 and C-9, thus showing that the sugar and the aryl ring were connected to each other through the alkyne.



4.10

Proton	Chemical Shift	Carbon	Chemical Shift
H-2′ (Aryl)	8.00	OAc C=O (C-6)	170.53
H-3′ (Aryl)	7.50	OAc C=O (C-3)	170.16
H-3	5.27	OAc C=O (C-2, 4)	169.30
H-4	5.12	C-10 (C=O)	166.30
H-2	5.04	C-3′	131.60
H-1	4.84	C-1′	130.11
H-7	4.61	C-2′	129.51
H-6a	4.29	C-4′	126.79
H-6b	4.17	C-1	98.50
H-11 (OCH_3)	3.93	C-8, 9	86.40, 86.28
H-5	3.77	C-3	72.80
OAc CH_3 (C-6)	2.08	C-5	72.01
OAc CH_3 (C-2)	2.04	C-2	71.17
OAc CH_3 (C-4)	2.03	C-4	68.37
OAc CH_3 (C-3)	2.01	C-6	61.83
		C-7	56.71
		C-11 (OCH_3)	52.20
		OAc CH_3	20.63, 20.52

Table 4.1: Numbering scheme and table of ^{1}H and ^{13}C NMR data for **4.10**.

The synthesis of the *closo*-carboranyl glycoside **4.11** (Scheme 4.6) was accomplished following the familiar method of alkyne insertion with decaborane(14) in acetonitrile.[16,17] The product was obtained in 60% yield following purification by silica

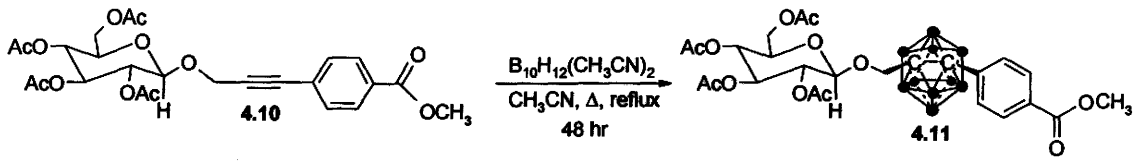
gel column chromatography and recrystallization, and was characterized by ^{1}H, $^{13}C\{^{1}H\}$ and $^{11}B\{^{1}H\}$ NMR spectroscopy, IR spectroscopy and electrospray mass spectrometry.

The IR spectrum of **4.11** gave the expected *closo*-carborane B-H stretching absorption at 2587 cm^{-1}, as well as the C=O stretches at 1758 and 1730 cm^{-1}. The electrospray mass spectrum gave a mass peak at m/z = 657.8, containing the expected isotope pattern for a B_{10} cluster, which corresponded to the $[M+NH_4]^+$ ion.

The ^{1}H NMR spectrum of **4.11** was similar to that of the alkyne precursor. A notable change was observed for the signals due to the methylene "spacer" protons, H-7, which appeared as two separate doublets at 3.83 and 3.69 ppm with a geminal coupling of -12.6 Hz. These signals were shifted by nearly 1 ppm to lower frequency versus the singlet observed for **4.10**. Additionally, a broad, "hilly" baseline was observed between 1.70 and 3.20 ppm, which was attributed to the carborane cage B-H protons.

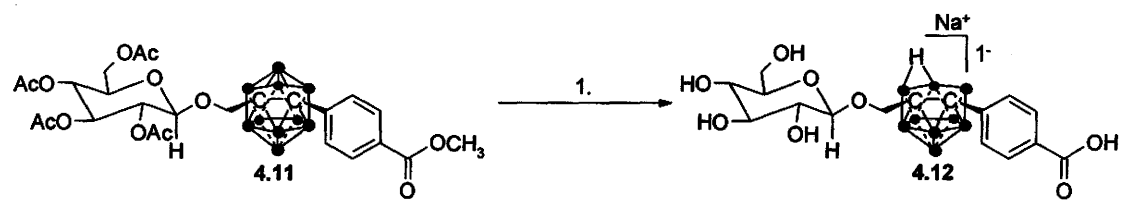
The $^{13}C\{^{1}H\}$ NMR spectrum of **4.11** was also little different with respect to that of compound **4.10**. The most notable change that occurred was in the signal arising from the C-7 methylene carbon atom, which appeared at 67.86 ppm. This represented shift to higher frequency by approximately 11 ppm, versus the corresponding signal in **4.10**.

The $^{11}B\{^{1}H\}$ NMR spectrum of **4.11** closely resembled that of compound **2.11**, the *closo*-carboranyl bis-glycoside, in that only two separate resonances were observed These signals appeared at -3.73 and -10.96 ppm, with a 1:4 ratio of their peak areas, respectively.



Scheme 4.6: Synthesis of compound **4.11**.

Compound **4.11** was converted to the deprotected *nido*-carboranyl glycoside **4.12** using sodium hydroxide in alcohol (Scheme 4.7).[18,19] Compound **4.11** was combined with 10 equivalents of sodium hydroxide in methanol, and the solution heated to 50°C for 12 hours. The resulting mixture was concentrated to dryness, re-dissolved in water, to which was added 1 equivalent of NaOH (from 1M NaOH). This step was conducted in order to ensure hydrolysis of the benzoate ester. The solution was stirred for 3 hours at room temperature, before the solution was adjusted to pH 3 with 1M HCl. The solution was concentrated to dryness, and the desired product obtained in 76% yield following silica gel column chromatography.



Scheme 4.7: Synthesis of compound **4.12**. Conditions: 1. a) NaOH (10 eq)/CH_3OH, 50°C, 12 hr. b) 1M NaOH (1 eq), RT, 3 hr. c) 1M HCl, pH 3.

The conversion of **4.11** to **4.12** was illustrated in the infrared spectrum by the appearance of a broad absorption at 3433 cm^{-1}, which corresponded to the O-H stretch of the hydroxyl groups of the glucose moiety, and of the carboxylate group. The *nido*-carborane B-H stretch appeared at 2529 cm^{-1}, and the benzoic acid C=O stretch appeared at 1592 cm^{-1}. The electrospray mass spectrum gave a peak at m/z = 446.4, containing the expected isotope pattern for the B_9 cluster, which corresponded to the target anion mass.

The ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra (Figure 4.13, 4.14) were complicated by the presence of diastereomers, as was the case for all of the *nido*-carboranyl carbohydrate derivatives investigated thus far. With the assistance of ^{1}H-^{1}H COSY, ^{1}H-^{13}C HSQC and ^{1}H-^{13}C HMBC spectra, many of the signals could be assigned (Table 4.2) to the particular diastereomers. In addition, a minor, but noticeable and consistent difference in the intensities of the signals for the two diastereomers (the lower-intensity signals of the second diastereomer were denoted with an asterisk (*)) was of use in assigning some of the signals in compound **4.12**. These differences likely arose during fractionation of the product during purification.

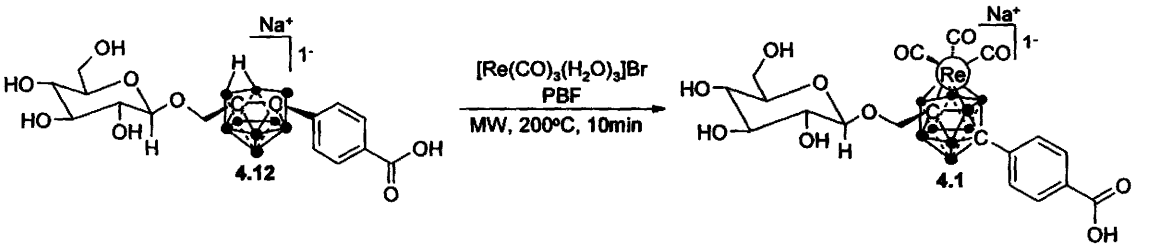
The aryl protons of compound **4.12** gave the signals at 7.76, 7.74, 7.43 and 7.38 ppm, appearing as distinct doublets (^{3}J = 8.4 Hz), corresponding to H-2′*, H-2′, H-3′*, and H-3′, respectively. The anomeric proton, H-1 gave a doublet ($^{3}J_{1,2}$ = 7.7 Hz) at 3.95 ppm. The methylene proton, H-7a*, gave a doublet ($^{2}J_{7a^*,7b^*}$ = -10.9 Hz) at 3.81 ppm. Following were three distinct doublets of doublets at 3.71 ($^{2}J_{6a^*6b^*}$ = -12.1 Hz, $^{3}J_{5^*6a^*}$ = 2.4 Hz), 3.66 ($^{2}J_{6a,6b}$ = -11.9 Hz, $^{3}J_{5,6a}$ = 2.5 Hz), and 3.60 ppm ($^{3}J_{5^*,6b^*}$ = 5.1 Hz), corresponding to H-6a*, H-6a, and H-6b*, respectively. The H-6b resonance appeared in

an overlapping signal at ppm, which also contained the resonances corresponding to the H-1* and H-7a protons. A doublet ($^2J_{7a,7b}$ = -11.1 Hz) corresponding to H-7b appeared at 3.44 ppm. An overlapping signal centered at 3.24 ppm corresponded to the protons H-3, H-3*, H-4 and H-4*. Doublets of doublets, which had collapsed into pseudo-triplets, appeared at 3.18 and 3.10 ppm, corresponding to H-2 and H-2*, respectively. Similarly, two ddd signals corresponding to H-5 and its diastereomeric counterpart, H-5* appeared at 3.09 and 2.94 ppm, respectively. The doublet at 2.92 ppm arose from H-7b*. The carborane B-H protons gave a broad baseline signal between -0.30 and 2.50 ppm, while the *nido*-carborane "bridging" proton gave a broad signal at -2.20 ppm.

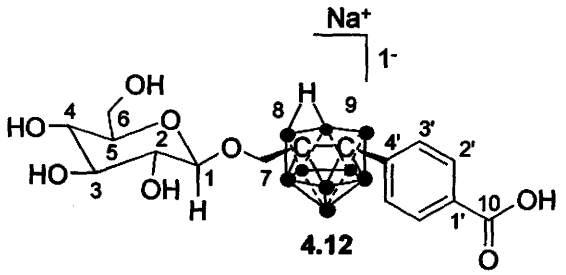
The benzoic acid carbonyl carbon (C-10) resonance appeared at 174.18 ppm. The aryl carbon resonances appeared at 147.09, 147.01, 133.52, 132.70, 132.63, 129.19 and 128.98 ppm, corresponding to C-1′, C-1′*, C-4′/4′*, C-3′*, C-3′, C-2′*, and C-2′, respectively. The anomeric carbon signals appeared at 103.89 and 103.52 ppm, corresponding to C-1* and C-1, respectively. The cluster of signals at 77.66, 77.49 and 77.38 were attributed to the C-3, C-3*, C-5 and C-5* carbon atoms, although definite assignments could not be made due to their close proximities. The signals at 75.74, 75.25, 75.02 and 74.80 ppm corresponded to C-7*, C-2, C-2*, and C-7, respectively. The carborane cage carbon atoms gave broad, reduced-intensity signals at 66.55 (C-9*), 66.13 (C-9), 63.55 (C-8) and 62.92 ppm (C-8*). Finally, the signals for the glucose C-6 and C-6* carbon atoms appeared at 62.53 and 62.34 ppm, respectively.

The $^{11}B\{^1H\}$ NMR spectrum of **4.12** (Figure 4.15) gave six distinguishable signals at -8.53, -9.98, -14.13, -16.89, -33.12 and -36.69 ppm. Although nine total signals are expected for a substituted nido-carborane, overlapping signals are not unexpected.

The synthesis of the Re complex **4.1** (Scheme 4.8) was approached in a manner similar to that described in Chapter 3 for the synthesis of Re-metallocarboranyl glycosides. The *nido*-carborane ligand, **4.12** was combined with $[Re(CO)_3(H_2O)_3]Br$ in a 5mL microwave vial, dissolved in PBF (pH ~7.2) containing 0.5M NaF, and heated to 200°C for 10 minutes. Since ^{11}B NMR indicated that a small amount of **4.12** remained after the initial irradiation, further quantities of the rhenium reagent were added, and the vial re-sealed and irradiated, in order to complete the conversion to the desired product. Compound **4.1** was isolated in 26% yield by silica gel column chromatography. The product was characterized by ^1H, $^{13}C\{^1H\}$ NMR and IR spectroscopy and by electrospray mass spectrometry. The ^1H and ^{13}C NMR data are summarized in Table 4.3.



Scheme 4.8: Synthesis of compound **4.1**.



4.12

Proton	Chemical Shift	Carbon	Chemical Shift
H-2'*	7.76	C-10 (C=O)	174.18
H-2'	7.74	C-1'	147.09
H-3'*	7.43	C-1'*	147.01
H-3'	7.38	C-4',4'*	133.52
H-1	3.95	C-3'*	132.70
H-7a*	3.81	C-3'	132.63
H-6a*	3.71	C-2'*	129.19
H-6a	3.66	C-2'	128.98
H-6b*	3.60	C-1*	103.89
H-1*, H-7a, H-6b	3.52	C-1	103.52
H-7b	3.44	C-3, 3*, 5, 5*	77.66, 77.49, 77.38
H-3, 3*, 4, 4*	3.24	C-7*	75.74
H-2*	3.18	C-2	75.25
H-2	3.10	C-2*	75.02
H-5	3.02	C-7	74.80
H-5*	2.94	C-4	71.54
H-7b*	2.92	C-4*	71.45
B-H	2.50 to -0.30	C-9*	66.55
B-H-B	-2.20	C-9	66.13
		C-8	63.28
		C-8*	62.92
		C-6	62.53
		C-6*	62.34

Table 4.2: Numbering scheme and ^{1}H and ^{13}C NMR data for **4.12**.

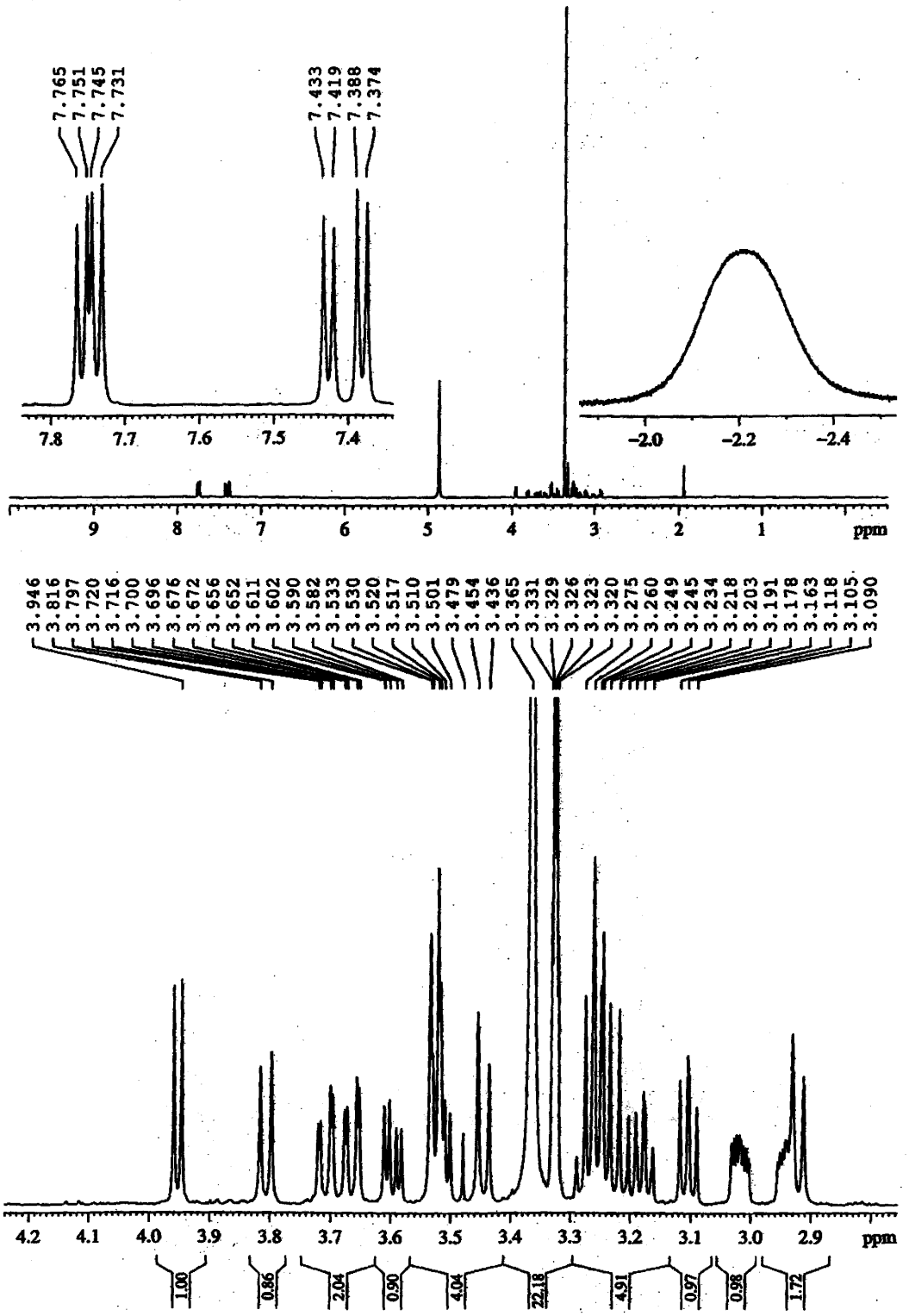


Figure 4.13: The ^{1}H NMR spectrum and expansion between 2.8 and 4.2 ppm of **4.12** (600 MHz, CD_3OD).

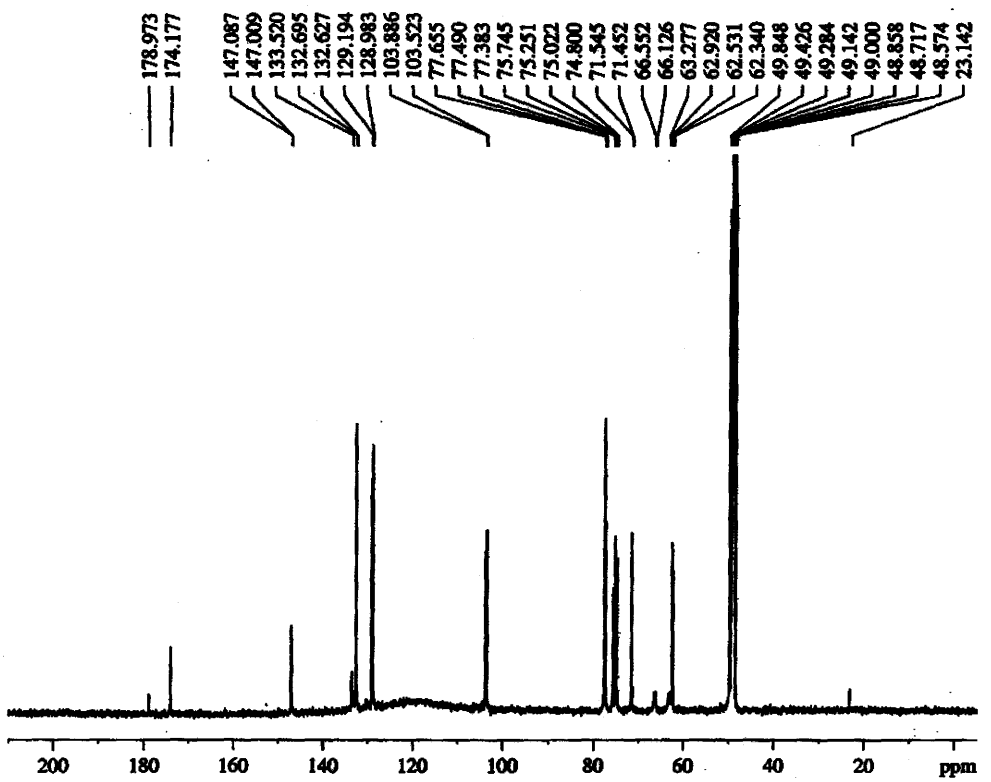


Figure 4.14: The $^{13}C\{^{1}H\}$ NMR spectrum of **4.12** (600 MHz, CD_3OD).

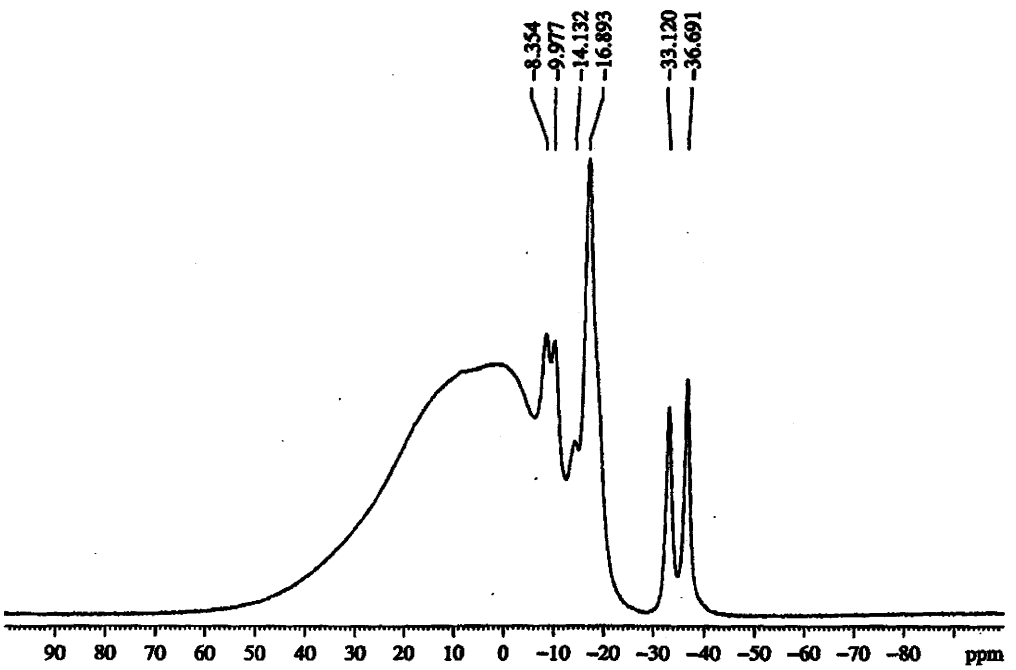


Figure 4.15: The $^{11}B\{^{1}H\}$ NMR spectrum of **4.12** (192 MHz, CD_3OD).

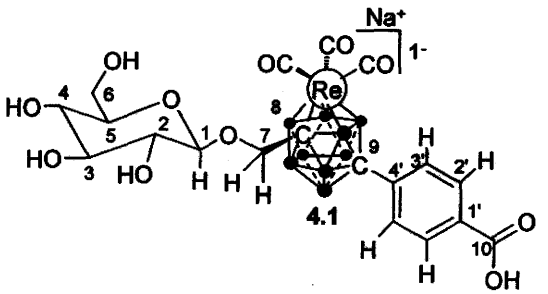
The infrared spectrum of **4.1** gave the expected broad O-H stretching absorption at 3440 cm^{-1} and the carborane B-H stretch at 2538 cm^{-1}. The C≡O stretching absorptions appeared at 2004 and 1909 cm^{-1}, and the carboxylic acid C=O stretch was observed at 1603 cm^{-1}. The electrospray mass spectrum gave the target anion mass peak at m/z = 715.4, with the expected isotope distribution for the B_9Re cluster. The assignment of the ^1H and $^{13}C\{^1H\}$ NMR spectra are listed in Table 4.3.

In the ^1H NMR spectrum of **4.1** (Figure 4.16), the aryl protons appeared as simple doublets. By contrast, the aryl protons in the ^1H NMR spectrum of *nido*-carborane **4.12** appeared as two doublets each, due to the diastereomeric nature of the product. However, doubling of the carbohydrate signals in **4.1** was observed, which would indicate the product remained as a mixture of diastereomers. Similarly, in the $^{13}C\{^1H\}$ NMR spectrum (Figure 4.17) no doubling of aryl group signals was observed, while doubling was observed for certain carbohydrate group signals, specifically for the C-1 and C-7 carbon atoms. In light of this, the data for **4.1** would suggest that the aryl ring of this metallocarboranyl glycoside exists in an environment with greater rotational freedom. This situation can be rationalized by the rearrangement of the carborane cage to the 2,1,8 configuration, that is, with the carbon bearing the aryl group migrating out of the metal bonding face of the cluster.

Comparison of the ^1H-^{13}C HMBC spectra of **4.12** (Figure 4.18) and **4.1** (Figure 4.19) can also give evidence for isomerization as described above and in Chapter 3. In the spectrum of the *nido*-carboarane precursor, correlations were observed between the signals arising from the methylene protons (H-7) of the glycoside moiety and those from

both carborane carbon atoms C-8 and C-9. However, in the HMBC spectrum for compound **4.1**, correlations were observed only between the H-7 and C-8 signals. This would again imply that the aryl-binding carbon atom, C-9, has migrated to a more remote location within the carborane cage.

The nOe spectra of compound **4.1** and **4.12** were acquired (Figure 4.20) and compared. For the *nido*-carborane precursor, **4.12**, irradiation of the aryl proton proximal to the carborane cage resulted in enhancements of the signals arising from both the methylene carbons linking the sugar to the carborane, as well as the anomeric carbon signals. However, irradiation of the same proton signal in the spectrum of **4.1** resulted in no such enhancements, suggesting that the aryl ring and carbohydrate were further apart in the rhenium complex, as would be the case in the 2,1,8 metallocarborane isomer.



Proton	Chemical Shift	Carbon	Chemical Shift
H-2′	7.65	C≡O	200.43
H-3′	7.10	C-10	170.32
H-1, 1*	4.30	C-1′	154.59
H-7a, 7a*	4.10	C-2′	129.61
H-6a	3.88	C-4′	127.33
H-7b, 7b*, 6b	3.73	C-3′	125.81
H-3, 4	3.37	C-1, 1*	104.41, 104.34
H-2, 5	3.26	C-3, 5	77.99, 77.94
B-H	3.20 to 1.20	C-7, 7*	76.79, 76.75
		C-2	75.23
		C-4	71.48
		C-6	62.62
		C-9	56.08
		C-8	54.74

Table 4.3: ^{1}H and ^{13}C NMR assignments for compound **4.1**.

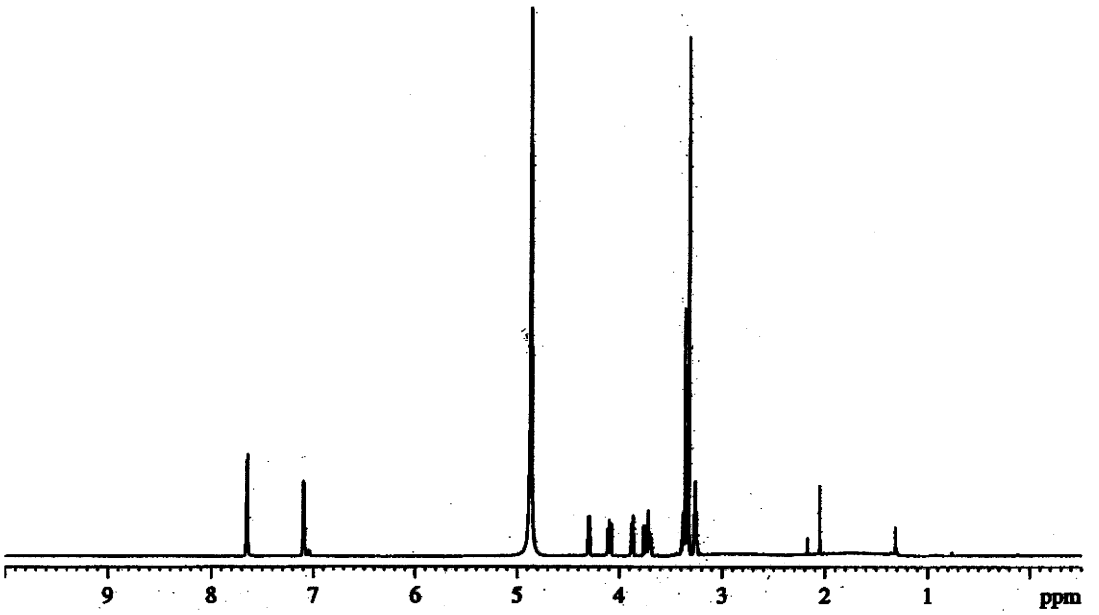


Figure 4.16: ^{1}H NMR spectrum of **4.1** (600 MHz, CD_3OD).

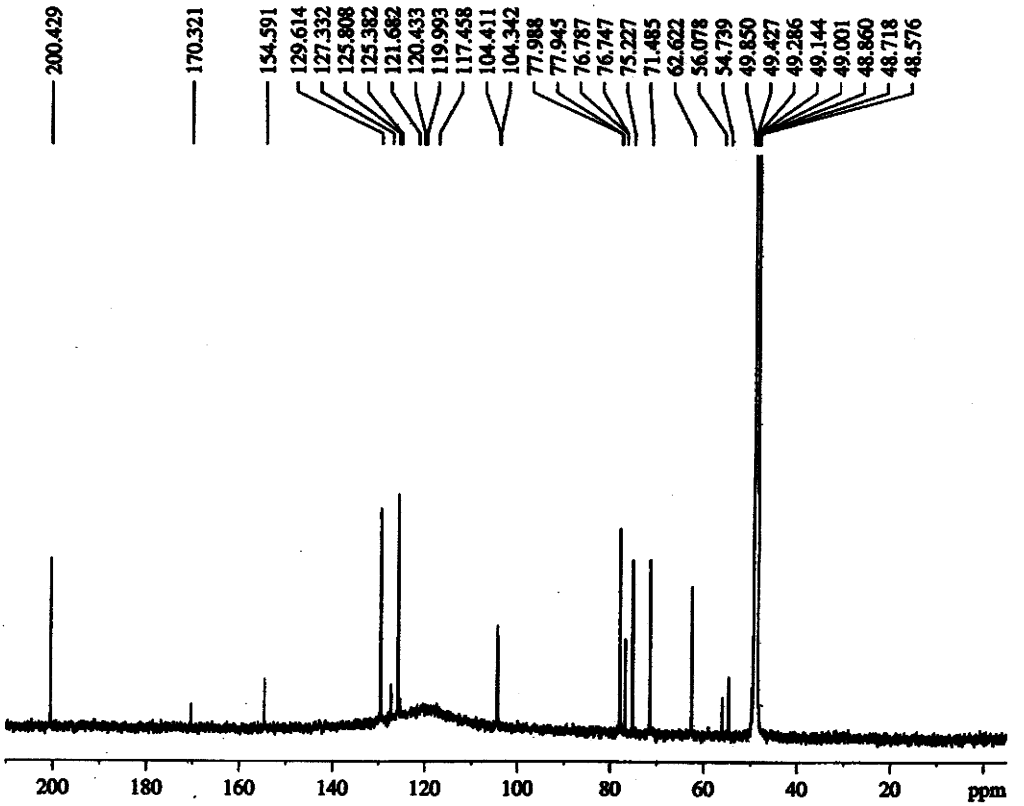


Figure 4.17: The $^{13}C\{^{1}H\}$ NMR spectrum of **4.1** (600 MHz, CD_3OD).

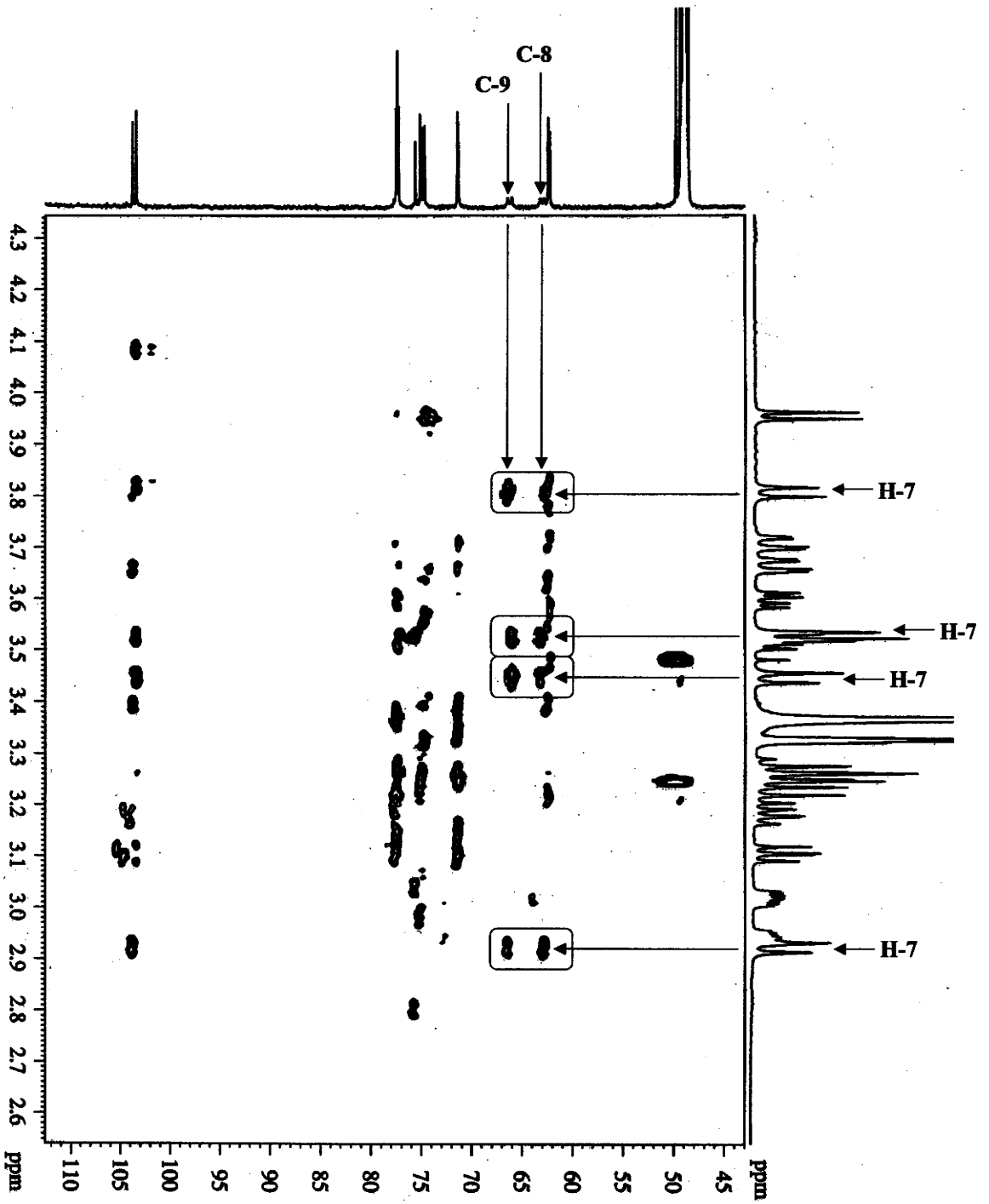


Figure 4.18: The ^{1}H-^{13}C HMBC spectrum of **4.12** (CD_3OD).

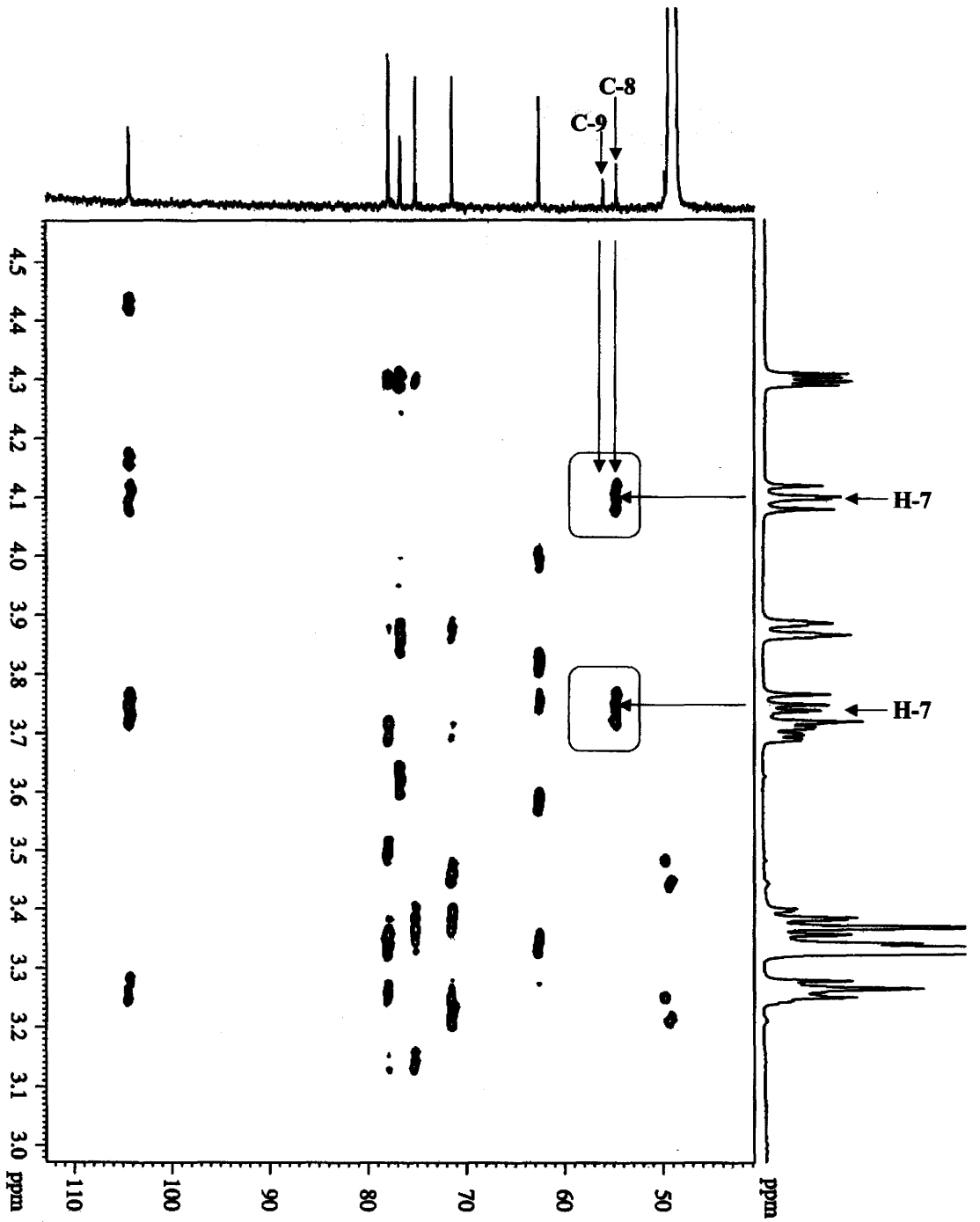


Figure 4.19: The ^{1}H-^{13}C HMBC spectrum of **4.1**.

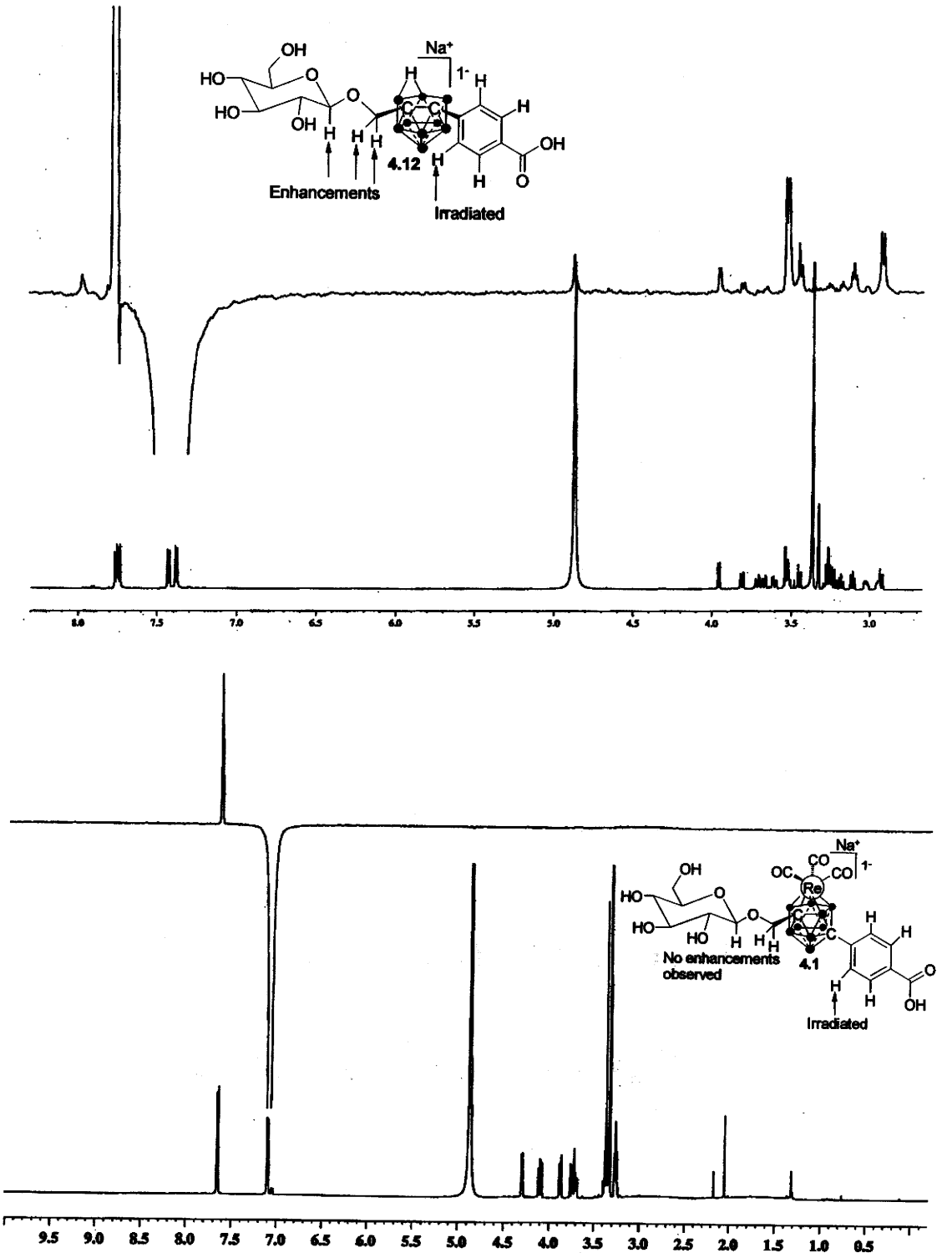
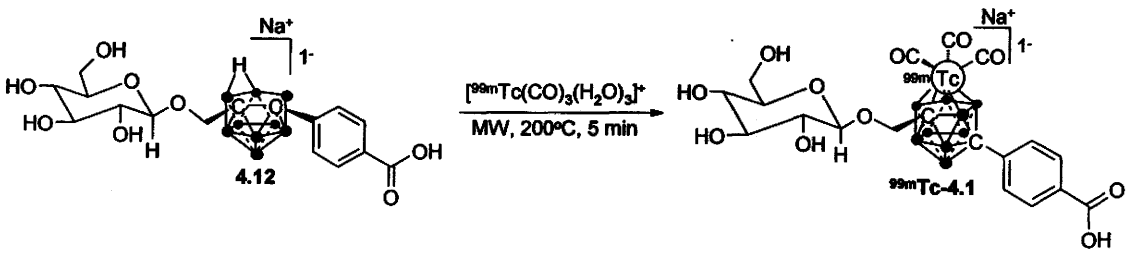


Figure 4.20: ^{1}H NMR and nOe spectra for **4.12** (top) and **4.1** (bottom) (600 MHz, CD_3OD).

*4.4 Synthesis and Isolation of [^{99m}Tc]-**4.1***

Following the microwave-assisted methodology used to successfully prepare [^{99m}Tc]-**3.4**, and [^{99m}Tc]-**4.2**, the synthesis of the ^{99m}Tc complex of **4.1** was attempted in a similar manner (Scheme 4.9). $[^{99m}Tc(CO)_3(H_2O)_3]^+$ was added to a 10mM solution of the ligand **4.12** and the mixture heated to 200°C for five minutes. An aliquot of the crude reaction was analyzed by HPLC (TEAP method). Integration of the peaks in the resulting γ-chromatogram (Figure 4.21) indicated 66% conversion to a major radioactive product, with 24% as minor radioactive products, and 10% as pertechnetate ($^{99m}TcO_4^-$). Separation of the major radioactive species was attempted by the reversed-phase SPE protocol that was used to successfully isolate [^{99m}Tc]-**3.4**. The activity in resulting fractions was counted and those containing the most activity were analyzed by HPLC (H_2O/ACN method), with co-injection of the rhenium standard. The resulting chromatogram (Figure 4.22) showed that the major radioactive species matched with the standard, and thus was identified as [^{99m}Tc]-**4.1**. The γ-trace also showed that the SPE protocol was successful in separating [^{99m}Tc]-**4.1** from all other radioactive species (Figure 4.23).



Scheme 4.9: Synthesis of ^{99m}Tc-**4.1**.

Although the SPE method isolated the desired product from other radioactive species, it was not able to remove unlabelled **4.12**. To address this issue, purification using an automated chromatographic purification instrument, the Biotage SP4, was attempted. This instrument consists of an automated solvent pumping unit, a UV detector, and an automated fraction collector. Columns are purchased as cartridges, with an accompanying "samplet" upon which the compound to be purified is adsorbed, and are assembled into a hand-tightened "compression module" apparatus. Both normal or reversed-phase cartridges are available, making this a convenient tool to purify radiolabelled compounds.

Crude product mixtures of [^{99m}Tc]-**4.1** were loaded onto samplets and attached to the main column, a C_{18} cartridge (column volume = 12 mL), which was pre-equilibrated with water containing 0.05% TFA. The gradient elution was as follows: 100% H_2O (0.05%TFA), 3.0 CV, 100% H_2O to 100% ACN (0.05% TFA), 12.0 CV, 100% ACN, 3.0 CV. In total, 72 fractions (3mL) were collected. The UV trace indicated a peak, corresponding to *nido*-carborane precursor **4.12**, eluting between fractions 38 and 41. In the case of ^{99m}Tc-**4.1**, it is not possible to observe this compound by UV absorbance, thus eluate fractions were obtained in the "Collect All" mode; that is, all solvent that passed through the column was collected and subsequently counted for activity. The greatest amount of activity appeared in fractions 44 and 45. HPLC co-injection of these fraction with the Re standard **4.1** confirmed the major product as [^{99m}Tc]-**4.1** in the absence of ligand **4.12**. Compound [^{99m}Tc]-**4.1** was thus obtained in **44%** decay corrected radiochemical yield (n = 3).

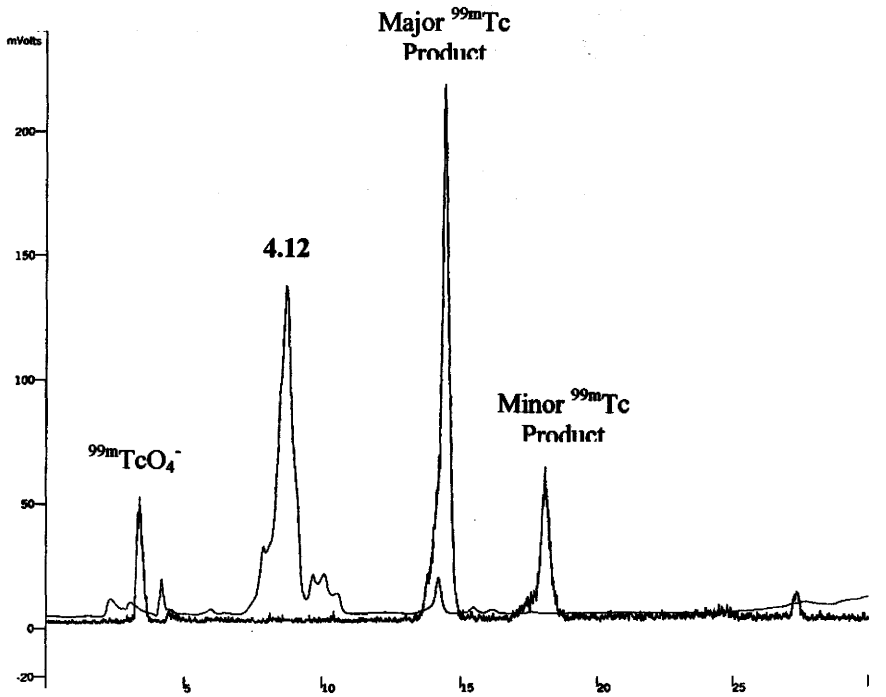


Figure 4.21: HPLC Chromatogram of crude ^{99m}Tc-**4.1** product mixture. Top: UV, Bottom: γ-trace.

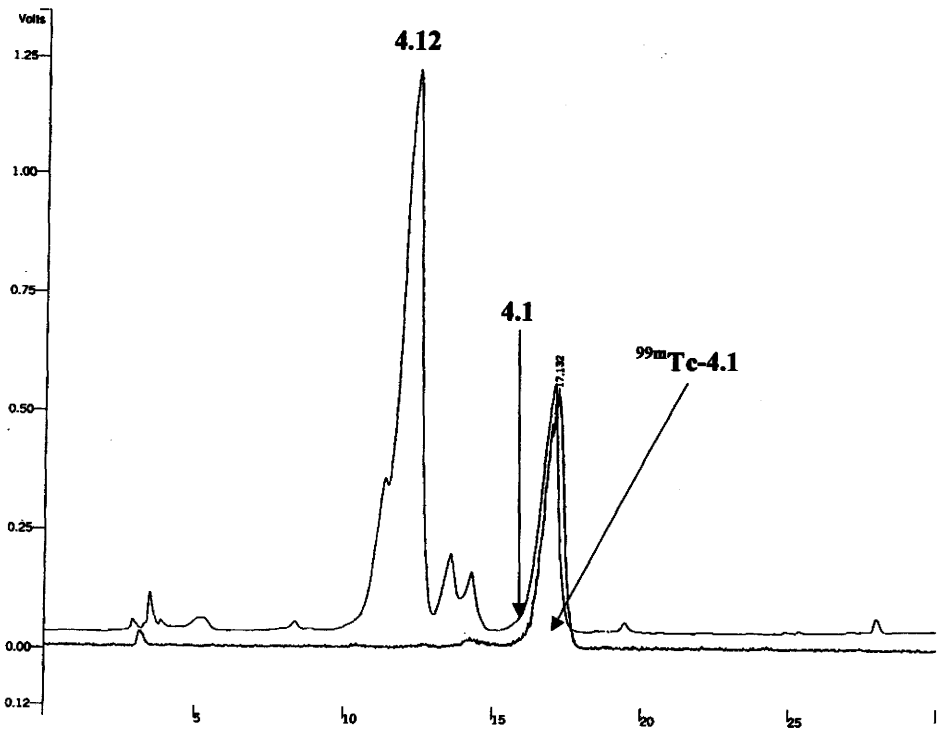


Figure 4.22: ^{99m}Tc-**4.1** after solid-phase extraction. Top: UV. Bottom: γ-trace.

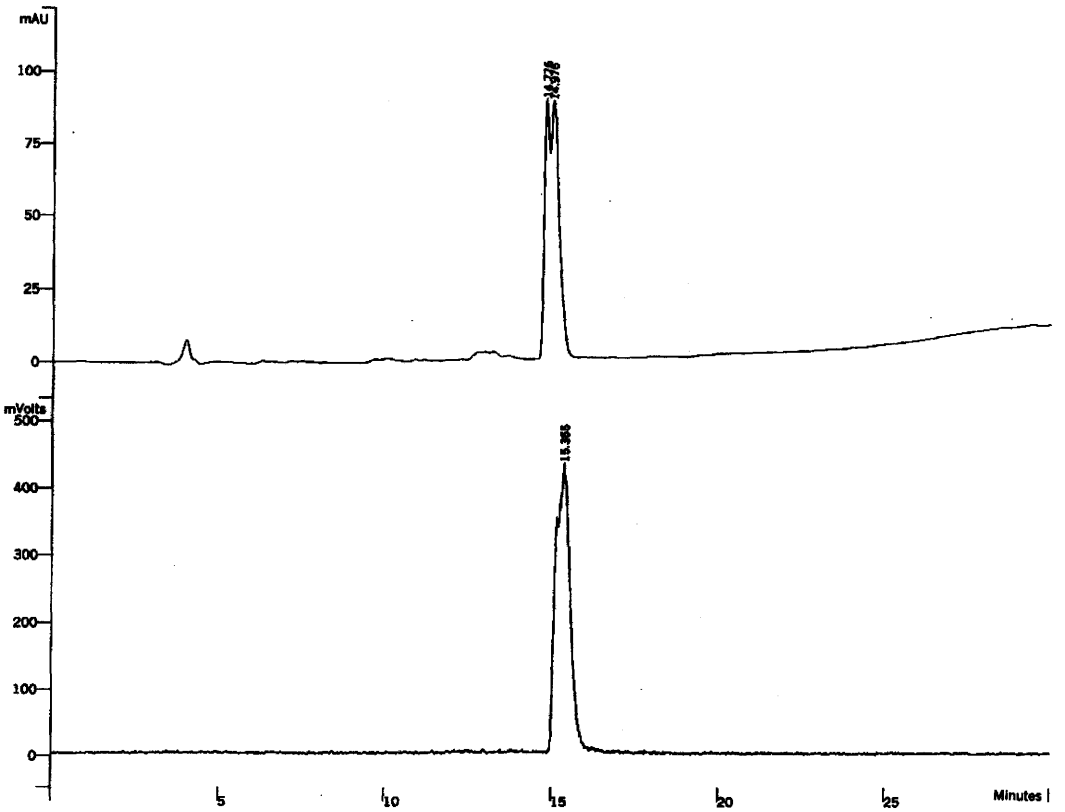


Figure 4.23: HPLC chromatograms for synthesis of [^{99m}Tc]-**4.1**. Top: UV trace of non-radioactive **4.1**. Bottom: γ-HPLC trace of [^{99m}Tc]-**4.1**.

4.5 Attempts to Prepare Model Conjugates of 4.1 and 4.2

Following the successful synthesis of Re complexes **4.1** and **4.2**, and their ^{99m}Tc radiolabelled analogues, the preparation of model conjugates of these compounds was investigated. The initial plan was to prepare active esters (Figure 4.24) through which all subsequent conjugates could potentially be prepared. The target conjugates were simple benzamides, as several benzamide compounds have been prepared and evaluated as tracers of malignant, melanotic melanoma.[20-25]

The synthesis of N-hydroxysuccinimide (NHS) and 2,3,5,6-tetrafluorophenyl (TFP) activated ester derivatives of **4.1** and **4.2** were attempted using carbodiimide

coupling reagents, including DCC, DIC, and EDC·HCl. However, none of these were successful, as isolation of the products in reasonable yields from starting materials and/or byproducts by various methods could not be achieved. In addition, the use of EDC·HCl complicated the reaction as the urea byproduct, protonated at its tertiary amine, appeared to undergo salt formation with the negatively charged metallocarboranes. This was undesirable, as an organic cation would alter the hydrophilic properties of the derivatives, of which the sodium salts were preferred. Perhaps the most significant complicating factor was the difficulty in preparing reasonable quantities of metallocarboranyl glycoside **4.1**, due to its tendency to degrade under microwave irradiation, and the difficulty in isolation of the product by chromatographic methods. In light of these difficulties, focus shifted to the iodinated analogues of *nido*-carboranes **4.3** and **4.12**, which could be more readily prepared and isolated.

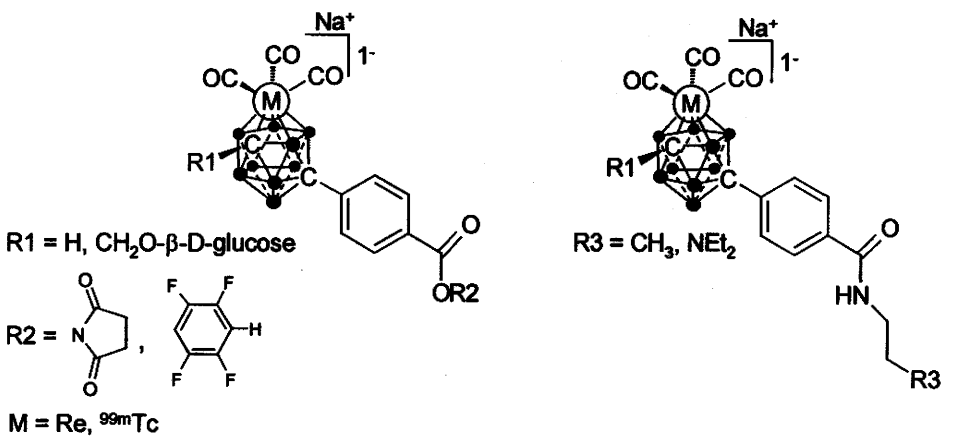


Figure 4.24: Proposed activated ester and benzamide derivatives of **4.1** and **4.2**.

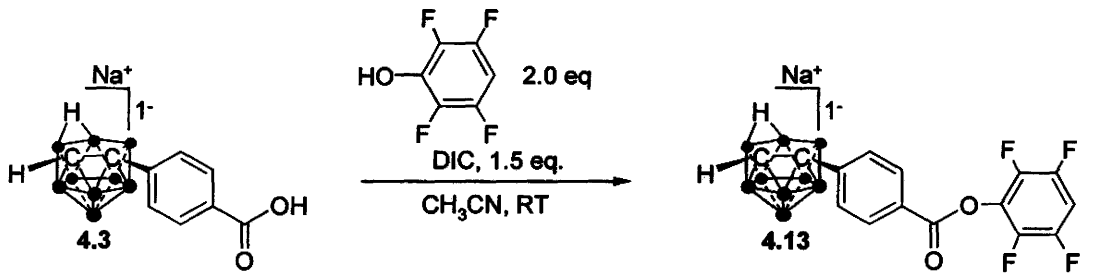
*4.6 Synthesis of nido-carborane-benzamide derivatives of **4.3** and **4.12** as Iodination Precursors*

In Chapter 2, the iodinated *nido*-carboranyl glycoside **2.13** was prepared as a demonstration of the versatility of the carborane moiety. Additionally, the conditions required to prepare the non-radioactive standard, as well as the ^{125}I-radiolabelled targets were extremely mild compared to those required for preparation of the rhenium and technetium-99m metallocarboranes as discussed above and in Chapter 3. Iodination and radioiodination reactions were run at room temperature, with ^{125}I labeling being completed in a matter of minutes. With these mild labeling conditions in mind, analogous radioiodination was pursued for model conjugates of *nido*-carborane ligands **4.9** and **4.12**.

The synthesis of an iodinated benzamide derivative of *nido*-carboranyl benzoic acid compound **4.3** was begun by pursuing an activated ester derivative. Through such an intermediate, both a simple benzamide derivative could be prepared for the purposes of this investigation, as well as other conjugates, such as those of peptides, etc. Since much difficulty had been encountered previously with the synthesis of NHS activated esters, a 2,3,5,6- tetrafluorophenyl (TFP) benzoate derivative of **4.3** was investigated. Scheme 4.10 illustrates the synthetic route used to prepare this activated ester, designated **4.13**. Briefly, compound **4.3** was combined with 2 equivalents 2,3,5,6-tetrafluorophenol in dry acetonitrile, to which was added 1.5 equivalents of DIC. Following overnight reaction at room temperature, TLC analysis indicated consumption of the starting material. The precipitated urea byproduct was removed by filtration, and the crude reaction product separated via preparative TLC. The desired product, **4.13**, was thus obtained in 74%

yield. Confirmation of the target was obtained from ^{1}H, $^{13}C\{^{1}H\}$ and $^{11}B\{^{1}H\}$ NMR spectroscopy, IR spectroscopy, and electrospray mass spectrometry.

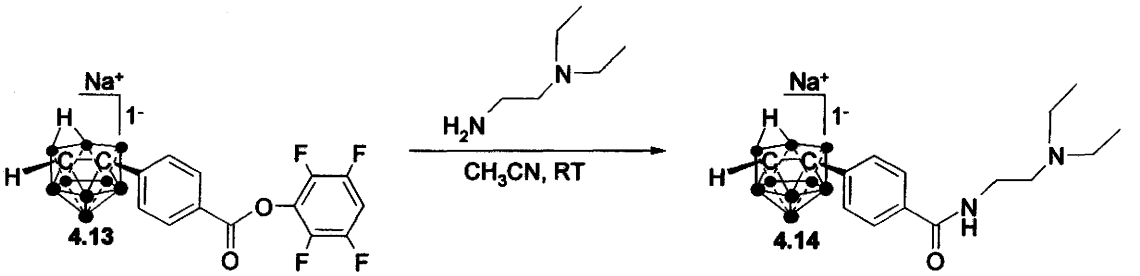
Inclusion of the TFP group in the structure of **4.13** was confirmed by the presence of a multiplet signal at 7.56 ppm in the ^{1}H NMR spectrum of **4.13**, which arose due to multiple proton-fluorine couplings. Additionally, the $^{13}C\{^{1}H\}$ NMR spectrum gave a series of multiplet signals in the aromatic region, which were attributed to the carbon atoms of the TFP group. These multiplets arose due to ^{13}C-^{19}F coupling. No significant change in the $^{11}B\{^{1}H\}$ NMR spectrum was observed for **4.13**, relative to precursor **4.3**. Finally, the negative-ion electrospray mass spectrum of the product gave a mass peak at m/z = 402.1, corresponding to the molecular ion of **4.13**.



Scheme 4.10: Synthesis of activated ester **4.13**.

Conversion of **4.13** to the *nido*-carboranyl N,N-diethyl(aminoethyl) benzamide target **4.14** (Scheme 4.11) was accomplished by stirring a solution of **4.13** in dry acetonitrile with 1.05 equivalents of N,N-diethylethylenediamine at room temperature overnight. Compound **4.14** was obtained in 41% yield following isolation by preparative

TLC. The product was characterized by ^{1}H, $^{13}C\{^{1}H\}$, and $^{11}B\{^{1}H\}$ NMR spectroscopy, IR spectroscopy, electrospray mass spectrometry, and HPLC.



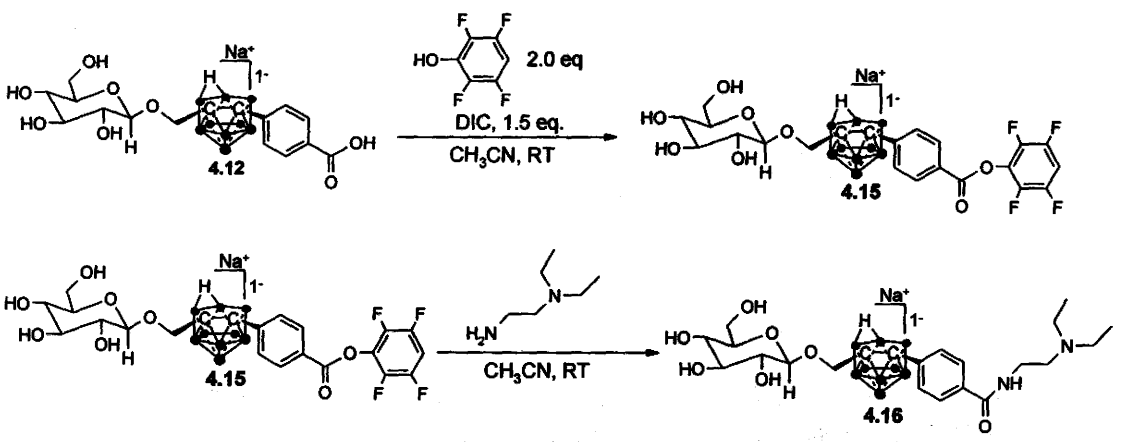
Scheme 4.11: Synthesis of model benzamide **4.14**.

The ^{1}H NMR spectrum of compound **4.14** gave signals which confirmed the presence of the N,N-diethyl(aminoethyl)benzamide structural unit. Notably, a broad signal at 8.25 ppm indicated the proton on the amide nitrogen atom, as well as multiplet signals at 3.78 and 3.39 ppm, corresponding to the methylene groups of the ethylenediamine structural element. Finally a quartet at 3.31 ppm and a triplet at 1.33 ppm corresponded to the terminal ethyl groups. Signals corresponding to the carbon atoms of the benzamide moiety were observed in the $^{13}C\{^{1}H\}$ NMR spectrum and assigned with the assistance of ^{1}H-^{13}C HSQC and HMBC spectra. The $^{11}B\{^{1}H\}$ NMR spectrum of **4.14** was essentially unchanged relative to those of precursors **4.3** and **4.13**, with nine signals being observed from nine unique boron nuclei in the mono-substituted *nido*-carborane structure.

The infrared spectrum of **4.14** contained the expected *nido*-carborane stretching absorption at 2527 cm^{-1}, as well as an amide N-H signal at 3407 cm^{-1} and the amide C=O

stretch at 1640 cm^{-1}. The electrospray mass spectrum of **4.14** gave a signal corresponding to the target anion mass at m/z = 352.4, containing the expected isotope distribution for the B_9 cluster.

With methods established for the synthesis of *nido*-carboranyl-benzamide derivatives, the synthesis of a glycoside analogue was undertaken. Initial attempts to prepare a glycose-*nido*-carboranyl-benzamide conjugate were made in a manner analogous to that for the synthesis of compound **4.14**, namely, the synthesis of the 2,3,5,6-tetrafluorophenyl activated ester derivative (**4.15**) of *nido*-carboranyl glycoside **4.12**, followed by reaction with the amine to afford the target benzamide **4.16** (Scheme 4.12). This route was deemed practical initially, as carbodiimide coupling reactions have been reported in which carbohydrate derivatives bearing no protecting groups are present.[26,27]



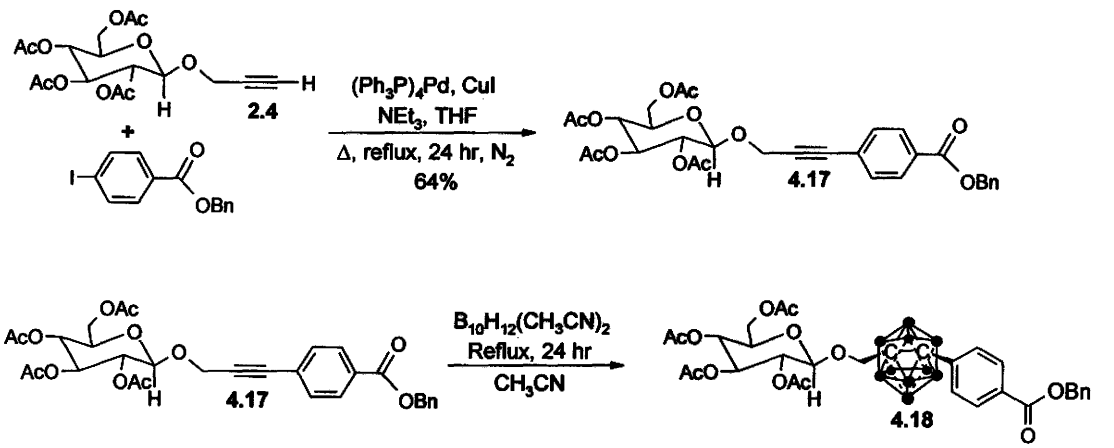
Scheme 4.12: Initial synthetic route to target benzamide **4.16**.

Following this method, the activated ester **4.15** was extremely difficult to isolate in high purity, and the overall yield was poor. Additionally, the benzamide target **4.16** could not be obtained in good yield with acceptable purity via reaction of **4.15** and subsequent purification via preparative TLC. Therefore, an alternative approach to **4.16** was explored.

The modified route involved retaining the acetate protecting groups on the carbohydrate during active ester formation. To do this required preparation of the benzyl ester protected alkyne derivative, **4.17**. Following conversion to the *closo*-carborane (**4.18**), the benzyl ester was selectively removed in the presence of the acetate esters, giving the free benzoic acid derivative **4.19**, and permitting subsequent formation of an active ester derivative (**4.20**) that was simple to isolate. From compound **4.20**, the synthesis of benzamide **4.21** was straightforward, and subsequent NaOH/EtOH degradation and saponification gave the *nido*-carborane labelling precursor **4.16**.

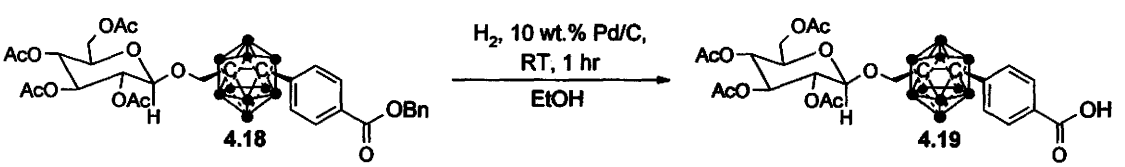
The synthesis of compounds **4.17** and **4.18** (Scheme 4.13) were performed in a manner analogous to that for their methyl ester counterparts, **4.10** and **4.11**, the only difference being the use of benzyl-4-iodobenzoate in the synthesis of alkyne **4.17**. Yields were also similar, with alkyne **4.17** being obtained in 65% yield, and *closo*-carborane **4.18** in 50% yield. The ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of these two compounds were very similar to those of their respectve methyl ester analogues, the major difference being the presence of extra signals in the aromatic regions of these compounds, thus denoting the benzyl ester group. Additionally, the $^{11}B\{^{1}H\}$ NMR spectrum of **4.18** was virtually indistinguishable from that of the methyl ester analogue, **4.11**.

Electrospray mass spectrometry was also used to characterize **4.17** and **4.18**. Alkyne **4.17** gave a positive ion signal at m/z = 614.0, and a negative ion signal at m/z= 655.3, corresponding to $[M+NH_4]^+$ and $[M+CH_3COO]^-$ adducts, respectively. The *closo*-carboranyl glycoside **4.18** gave a negative ion signal at m/z = 774.5, corrsponding to $[M+CH_3COO]^-$, which also contained the expected B_{10} isotope distribution.



Scheme 4.13: Synthesis of compounds **4.17** and **4.18**.

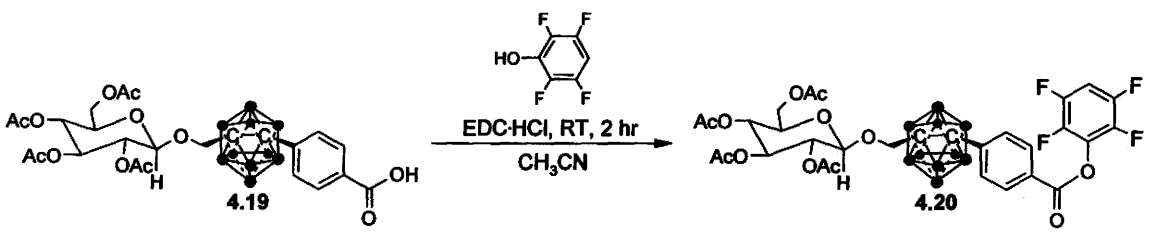
Conversion of compound **4.18** to the free benzoic acid form was accomplished via catalytic hydrogenolysis (Scheme 4.14). This reaction was facile and high-yielding. The target **4.19** was obtained in 98% after reacting 1 hour room temperature.



Scheme 4.14: Synthesis of compound **4.19**.

Except for the disappearance of the benzyl group signals and a broad acid proton signal at 10.39 ppm, the ^{1}H, $^{13}C\{^{1}H\}$ and $^{11}B\{^{1}H\}$ NMR spectra of **4.19** remained similar to those of its precursors. Meanwhile, the infrared spectrum of **4.19** was little changed versus that for **4.18**, except for the broad O-H stretching signal centered around 3245 cm^{-1}. The electrospray mass spectrum gave a signal at m/z = 624.4, with the appropriate isotope distribution, corresponding to the $[M-H]^-$ species.

With the peracetylated *closo*-carboranyl glycoside **4.19** in hand, the synthesis of the active ester was attempted. Compound **4.20**, the tetrafluorophenyl ester of **4.19**, was prepared using EDC as the coupling agent (Scheme 4.15). Analysis by TLC indicated complete reaction after two hours at room temperature in acetonitrile. After liquid-liquid extraction, the product could be crystallized by dissolving in the minimum volume of diethyl ether, followed by addition of a small quantity of hexanes. This crystallization procedure furnished **4.20** in 70% yield, and in sufficient purity to continue the synthesis toward the benzamide target compound.



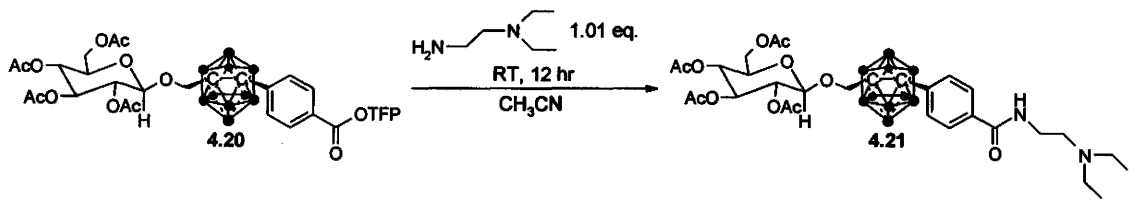
Scheme 4.15: Synthesis of compound **4.20**.

As was the case with the non-glycosylated derivative **4.13**, the most distinguishable feature of the ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of compound **4.20** were the signals corresponding to the tetrafluorophenyl group. Otherwise, these spectra, and the $^{11}B\{^{1}H\}$ NMR spectrum, resembled those of the immediate precursors. The molecular weight of **4.20** was confirmed via electrospray mass spectrometry. A positive ion appeared at m/z = 791.2, corresponding to $[M+NH_4]^+$, while negative ions were observed at m/z = 832.4 and 886.4, corresponding to $[M+CH_3COO]^-$ and $[M+CF_3COO]^-$ adducts, respectively. As expected, the B_{10} isotope distribution was observed for all of these signals.

Compound **4.20** was readily converted to the N,N-diethyl(aminoethyl) benzamide derivative by reaction with 1.01 eq.of N,N-diethylethylenediamine for 12 hours at room temperature in dry acetonitrile (Scheme 4.16). Following isolation by silica gel column chromatography, compound **4.21** was obtained in 86% yield.

The signals corresponding to the aryl and carbohydrate groups in the ^{1}H NMR spectrum of **4.21** were not significantly changed versus those of the precursors to this material. The remaining signals could be attributed to the benzamide structural element. The amide N-H proton gave a broad signal at 7.08 ppm. The multiplet signals at 3.49 and 2.67 ppm arose from the methylene protons of the ethylenediamine moiety, and the quartet at 2.59 ppm and the triplet at 1.05 ppm corresponded to the terminal ethyl group methylene and methyl group protons, respectively. Two-dimensional heteronuclear correlation experiments were used to assign the ^{13}C NMR signals to the corresponding atoms of the benzamide fragment.

Acquisition of the $^{11}B\{^1H\}$ NMR spectrum of **4.21** revealed no obvious changes versus the carborane-containing precursors to this compound. In particular, no signals corresponding to the *nido*-carborane fragment were observed. This was a concern as *ortho*-carborane cage degradation has been reported previously to occur in the presence of some amines.[28] This result indicated that the *closo*-carborane was stable in the presence of the quantity of N,N-diethylethylenediamine required to affect the transformation from **4.20** to **4.21**.

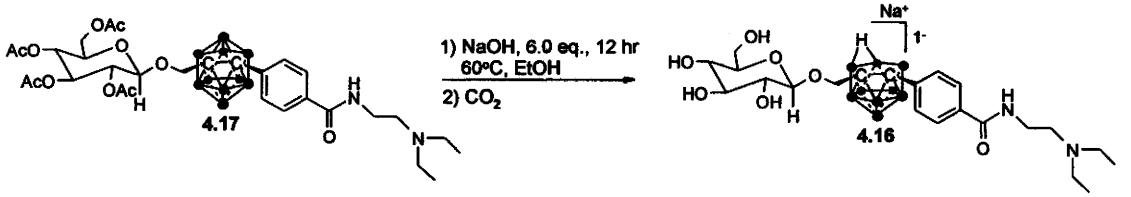


Scheme 4.16: Synthesis of compound **4.21**.

The infrared spectrum of **4.21** indicated stretching vibrations consistent with the amide group. For example an N-H stretch was observed at 3406 cm^{-1}, and an amide C=O stretch was observed at 1652cm^{-1}. Electrospray mass spectrometry gave signals consistent with those expected for compound **4.21**. A positive ion signal was observed at m/z = 724.3, corresponding to the [M+H]+ adduct, and a negative ion signal was observed at m/z = 781.6, corresponding to the $[M+CH_3COO]^-$ adduct. Both signals contained isotope distributions characteristic of the B_{10} cluster.

Compound **4.21** was converted to the labelling precursor, **4.16** by the familiar carborane cage degradation/acetate ester saponification reaction employed for all

previous *nido*-carboranyl glycosides prepared in this investigation (Scheme 4.17). Briefly, compound **4.17**, dissolved in ethanol was reacted with six equivalents of NaOH at elevated temperature (60°C) overnight. After cooling, the excess NaOH was removed by precipitation as Na_2CO_3 by bubbling CO_2 through the solution, followed by filtration. The solution containing the product was concentrated and subsequently purified by silica gel column chromatography, giving the desired product in 35% yield.



Scheme 4.17: Synthesis of compound **4.16**.

The ^{1}H NMR spectrum of **4.16** (Figure 4.25) was indicative of the desired benzamidyl-*nido*-carboranyl-glycoside structure. As was observed in the *nido*-carboranyl glycoside compound prepared previously (e.g. **2.6, 2.12, 4.12**), a doubling of signals was observed due to the formation of diastereomers upon carborane cage degradation in the presence of the carbohydrate.[29] Notable features of this spectrum include the two distinct amide N-H proton signals at 7.89 and 7.84 ppm, the triplet at 1.26 ppm, corresponding to the terminal methyl group protons of the N,N-diethyl(aminoethyl) fragment (the remaining signals from this fragment were overlapping with signals from the glucose moiety), the broad B-H signal, shifted to lower frequency relative to that in the *closo*-carborane based precursors, and the broad, "bridging" proton signal at -2.20 ppm.

The $^{13}C\{^{1}H\}$ NMR spectrum of **4.16** also featured a doubling of signals. Assignment of the signals was made with the assistance of ^{1}H-^{13}C HSQC and HMBC experiments. Aside from the signals corresponding to the benzamide fragment, notable features included the broad, reduced-intensity signals corresponding to the *nido*-carborane carbon atoms. These signals appeared at 67.23 ppm (glucose-CH_2-C_{cage}-C_{cage}-aryl, C-8), and 61.58 and 61.28 ppm (glucose-CH_2-C_{cage}-C_{cage}-aryl, C-9).

The $^{11}B\{^{1}H\}$ NMR spectrum of **4.16** (Figure 4.26) was also indicative of the presence of the *nido*-carborane cluster. Of the nine signals expected from the asymmetrically-substituted structure,[19] seven were observed, due to overlap. The spectrum of **4.16** gave signals at -7.13, -10.00, -14.82, -18.02, -19.74, -32.92, and -36.42 ppm, the two lowest-frequency signals being characteristic of the *nido-o*-carborane fragment.[10]

The presence of the *nido*-carborane moiety was also indicated in the infrared spectrum of **4.16** by the characteristic B-H stretch at 2326 cm^{-1}. Additionally, the presence of free hydroxyl groups on the glucose portion of the molecule was indicated by the broad O-H stretching signal at 3431 cm^{-1}. Finally, the amide C=O stretch was observed at 1641 cm^{-1}. The electrospray mass spectrum of **4.16** gave the target anion mass at m/z = 544.3, with the characteristic B_9 isotope distribution.

With compounds **4.14** and **4.16** prepared and characterized, and the previously-described **4.12** in hand, it was then possible to investigate the synthesis of their non-radioactive iodo-*nido*-carborane derivatives as standards for eventual radiolabelling with ^{125}I.

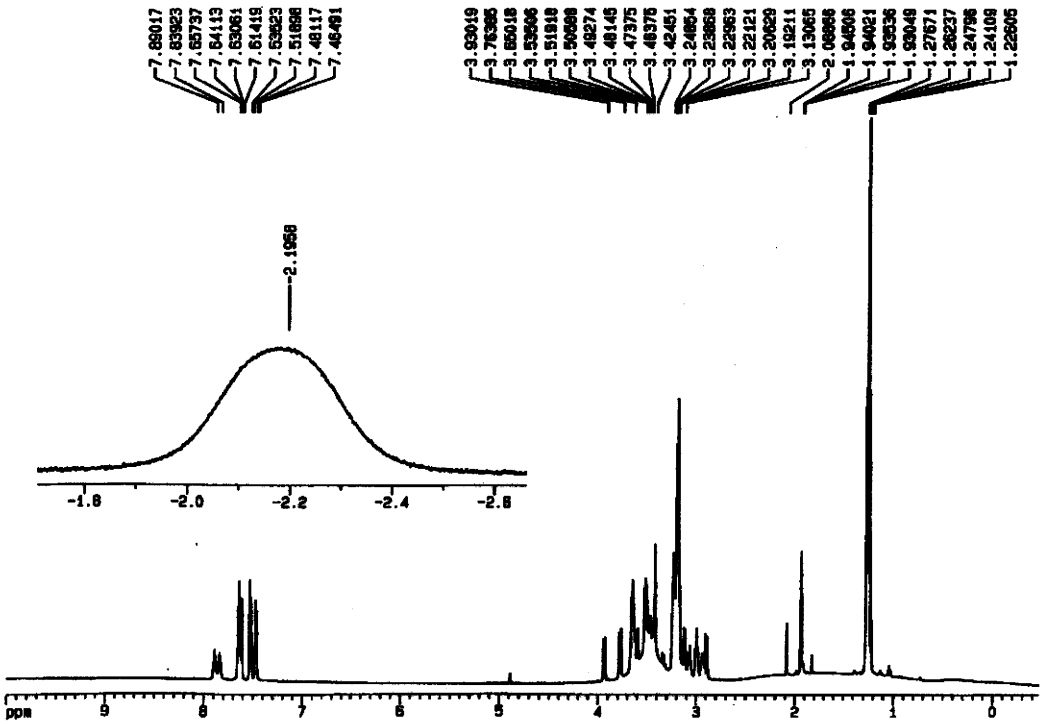


Figure 4.25: The ^{1}H NMR spectrum of compound **4.16** (500 MHz, CD_3CN).

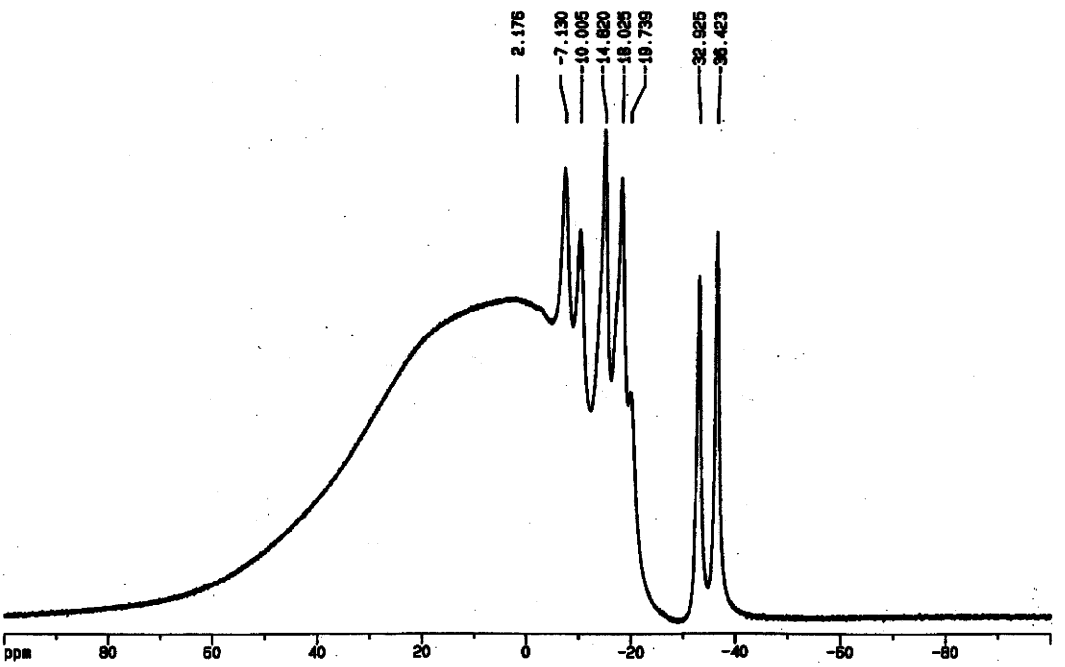
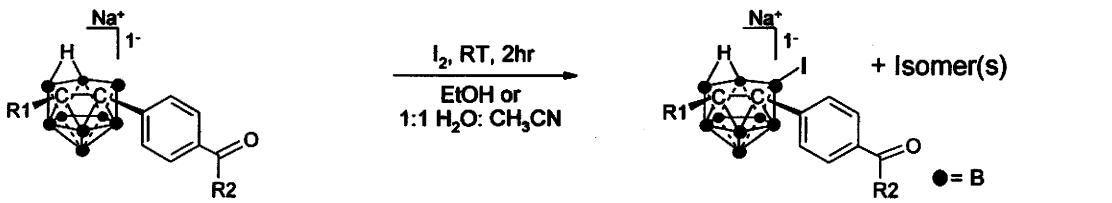


Figure 4.26: The $^{11}B\{^{1}H\}$ NMR spectrum of compound **4.16**.

4.7 Synthesis of iodinated derivatives of ***4.12, 4.14, and 4.16***

Due to the lengthy and tedious nature of the HPLC methods required to isolate compound **2.13** following its synthesis via reaction of **2.6d** with NaI in the presence of Chloramine-T, an alternative approach was employed in the syntheses of subsequent iodo-*nido*-carboranes **4.22, 4.23,** and **4.24**. This approach (Scheme 4.18) involved reaction of the *nido*-carborane precursor with molecular iodine, as per the original method of *nido*-carborane iodination.[30] This would be expected to simplify the isolation of the targets, if complete consumption of the starting materials could be effected.



4.12: R1 = CH_2-β-D-glucose, R2 = OH
4.14: R1 = H, R2 = $NH(CH_2)_2N(C_2H_5)_2$
4.16: R1 = CH_2-β-D-glucose, R2 = $NH(CH_2)_2N(C_2H_5)_2$

4.22: R1 = CH_2-β-D-glucose, R2 = OH
4.23: R1 = H, R2 = $NH(CH_2)_2N(C_2H_5)_2$
4.24: R1 = CH_2-β-D-glucose, R2 = $NH(CH_2)_2N(C_2H_5)_2$

Scheme 4.18: Synthesis of iodinated *nido*-carborane derivatives using I_2.

Compound **4.22** was prepared by reaction of precursor **4.12** with I_2 in ethanol solution. Analysis by HPLC and electrospray mass spectrometry indicated complete consumption of the starting material after two hours at room temperature, as evidenced by the absence of the HPLC and MS signals corresponding to **4.12**. The reaction was quenched with sodium metabisulfite ($Na_2S_2O_5$) to prevent formation of the di-iodinated product, which is possible if a sufficient quantity of I_2 is present and/or the reaction is allowed to proceed for long periods of time.[30,31] The target was isolated by solid-phase

extraction using a C_{18} SepPak^{TM} cartridge (Waters). After loading the crude product onto the pre-conditioned cartridge, and washing with water to remove salts (e.g. NaI, $Na_2S_2O_5$), the product was eluted with a 1:1 mixture of water and acetonitrile, and was obtained in 32% yield.

The HPLC trace of **4.22** (Figure 4.27) gave a series of four peaks, which, under LC-MS analysis all corresponded to the target anion mass of m/z = 572 for **4.22**. The appearance of four HPLC peaks corresponding to **4.22** can be rationalized by the possibility of regioisomers resulting from iodine substitution onto either of the boron atoms adjacent to carbon in the open face of the *nido*-carborane cage. Previous researchers have noted the appearance of the two possible regioisomers upon iodination of asymmetrical *nido*-carborane derivatives.[32,33] In the case of iodination of compound **4.12**, the structure of the precursor consists not only of an asymmetrically-substituted *nido*-carborane, but also exists as a pair of diastereomers due to the fixed stereochemistry of the carbohydrate moiety.[19,29] Therefore, iodination of **4.12** would result in two regioisomers for each diastereomer, thus generating a total of four species for product **4.22**.

The ^{1}H NMR (Figure 4.28) and $^{13}C\{^{1}H\}$ NMR spectra of **4.22**, were consistent with multiple isomers. In the latter (Figure4.29), four distinct anomeric carbon signals were evident. The $^{11}B\{^{1}H\}$ NMR spectrum of **4.22** (Figure 4.30) was indicative of the iodinated carborane cage, with a signal appearing at -29.27 ppm. This signal had apparently shifted to higher frequency relative to the corresponding signal (-33.11 ppm) in the $^{11}B\{^{1}H\}$ NMR spectrum of precursor **4.12** (Figure 4.16).[10] Due to the presence of

multiple isomers of **4.22**, many of the ^{1}H and ^{13}C NMR signals were overlapping with one another. Although ^{1}H-^{13}C HSQC and HBMC permitted the assignment of many signals, complete and unique assignment of all signals was not possible. The ^{1}H and ^{13}C NMR data for compound **4.22** are tabulated in Table 4.4.

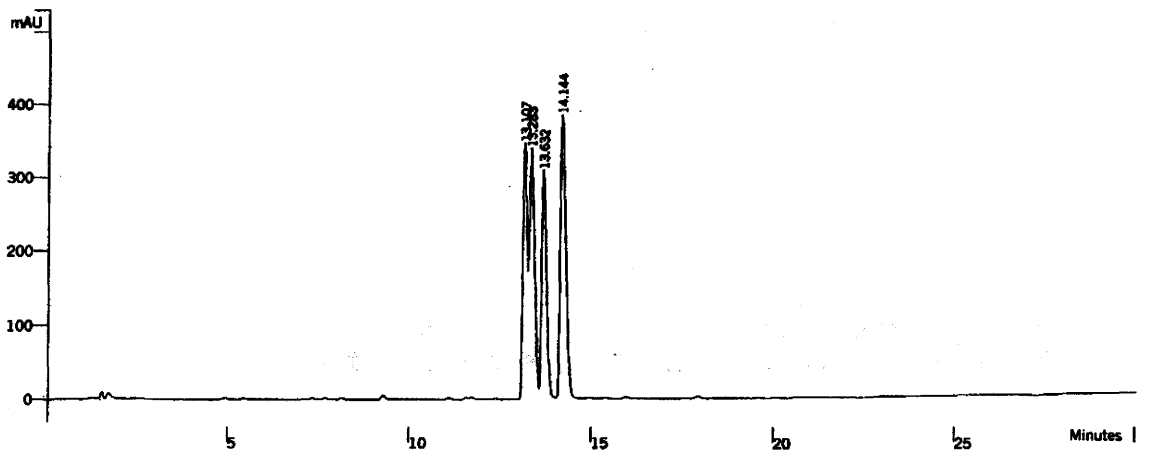


Figure 4.27: HPLC trace of compound **4.22** (Elution method **B**).

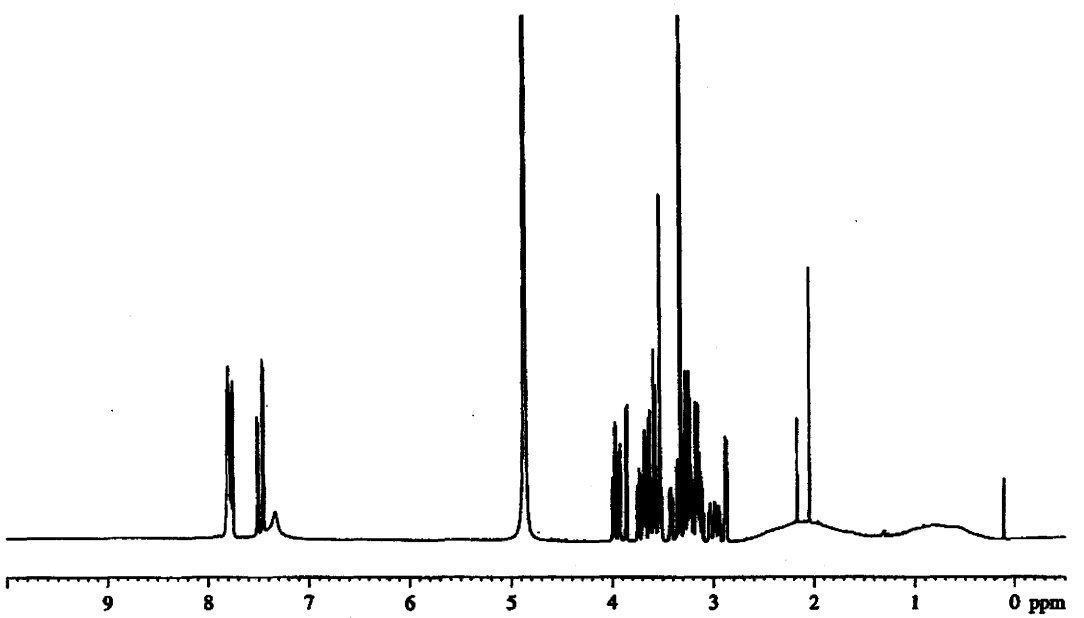


Figure 4.28: ^{1}H NMR spectrum of **4.22** (600 MHz, CD_3OD).

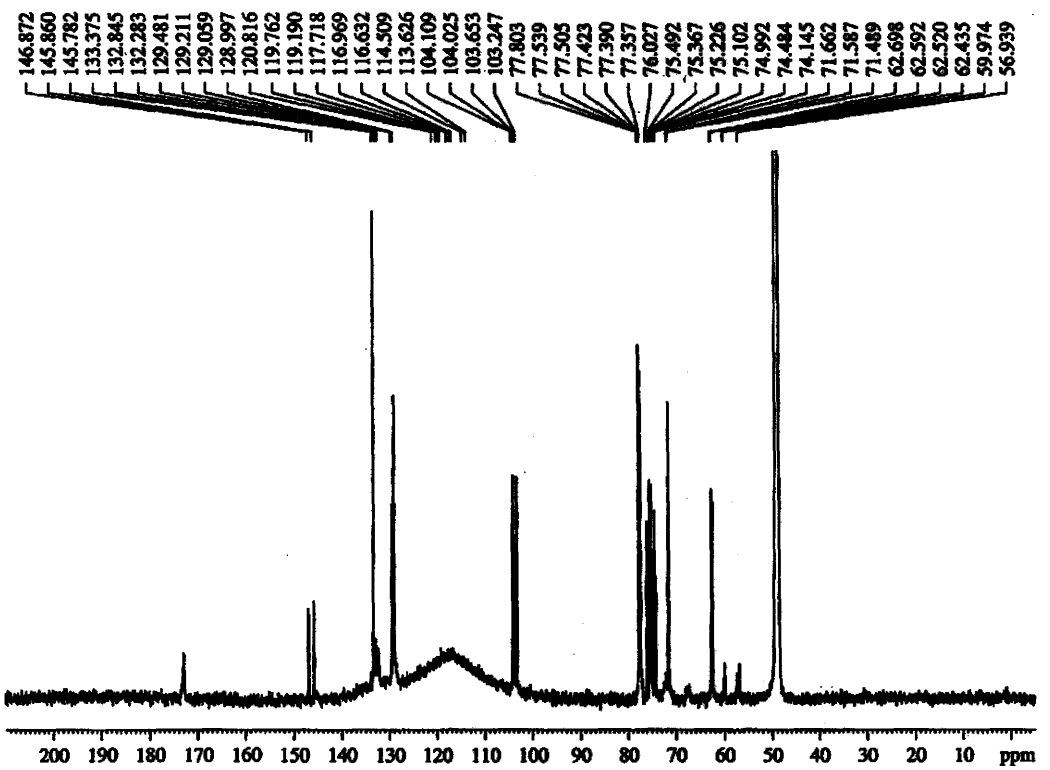


Figure 4.29: The $^{13}C\{^{1}H\}$ NMR spectrum of **4.22** (150 MHz, CD_3OD).

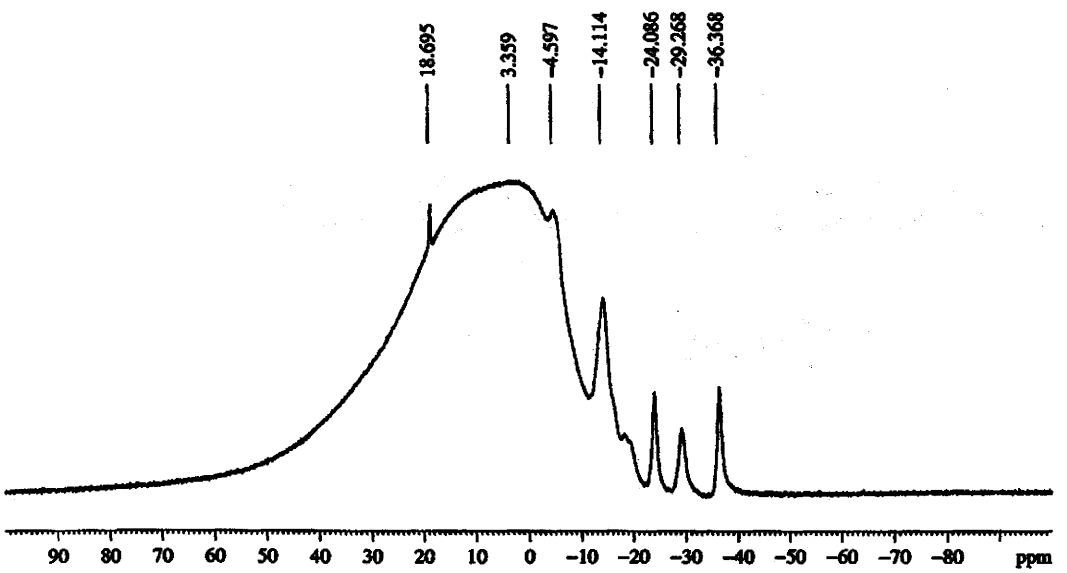
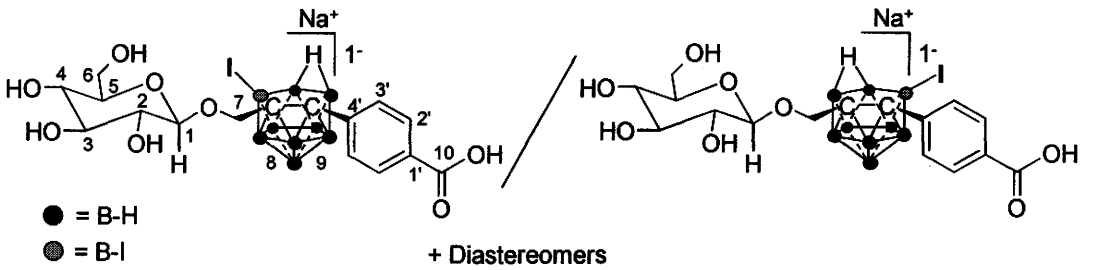


Figure 4.30: $^{11}B\{^{1}H\}$ NMR spectrum of **4.22** (192 MHz, CD_3OD).



Proton	Chemical Shift	Carbon	Chemical Shift
H-2′	7.81 - 7.75	C-10	172.99
H-3′	7.51, 7.46	C-1′	146.98, 146.87, 145.86, 145.78
H-7	3.99	C-3′	133.38
H-1	3.97	C-4′	132.84, 132.28
H-7	3.93	C-2′	129.48, 129.21, 129.06, 129.00
H-1	3.86	C-1	104.11, 104.02, 103.65, 103.25
H-6$^{(*)}$	3.74	C-3, C-5	77.80, 77.54, 77.50, 77.42, 77.39, 77.36
H-7,6,6$^{(+)}$	3.70 - 3.66	C-7	76.03
H-1	3.63	C-7, C-2	75.49, 75.37, 75.23
H-6$^{(*)}$,6$^{(x)}$,7,6$^{(+)}$,6	3.62 - 3.52	C-7	75.10, 74.99
H-6$^{(x)}$	3.41	C-8/9	72.25
H-1	3.36	C-4	71.66, 71.59, 71.49
H-2,3,4,7	3.30 - 3.09	C-8/9	67.63
H-5	3.03	C-6	62.70, 62.59, 62.52, 62.44
H-5$^{(*)}$	2.99	C-8/9	59.97
H-5$^{(x)}$	2.95	C-8/9	57.45, 56.94
H-5$^{(+)}$	2.88	Symbols (*),(+),(x) denote signals assigned to separate isomers	
H-7	2.87		
B-H	2.80 - 0.10		
B-H-B	-2.30, -2.44		

Table 4.4: NMR data and numbering scheme for compound **4.22**.

The iodinated-*nido*-carboranyl benzamides **4.23** and **4.24** were prepared from *nido*-carboranyl benzamdides **4.14** and **4.16**, respectively. Analysis of the crude reactions by ESI-MS indicated exclusive formation of the mono-iodinated products, with no residual starting material, nor any di-iodinated products being observed. Compounds **4.23** and **4.24** were isolated via reversed-phase SPE as was compound **4.22**. Yields were poor for both compounds (less than 20%), which is believed to be a result of the hydrophobic compounds adhering readily to the stationary phase.

The electrospray mass spectrum of compound **4.23** gave the target anion mass at m/z =478.1, while that of compound **4.24** gave the target anion signal at m/z = 670.1. In the case of both benzamides, multiple HPLC peaks were observed, indicating a mixture of two (**4.23**, Figure 4.31) or four isomers (**4.24**, Figure 4.32) for these compounds.

Due to the formation of multiple isomers upon iodination, the use of compounds **4.22-4.24** for a clinical application could be questioned. However, **4.22** is intended for use as a prosthetic group to a larger targeting vector, such as a peptide or antibody, and as such, the presence of that group as multiple isomers would not be expected to influence the binding behaviour of the larger moiety. In the case of the benzamides, changing the position of the iodine lablel is expected to have little impact on target affinity, according to the recent literature. For example, benzamides have been prepared and investigated with the label in various positions (*ortho*, *meta*, *para*) about the aryl ring.[21,25,34]

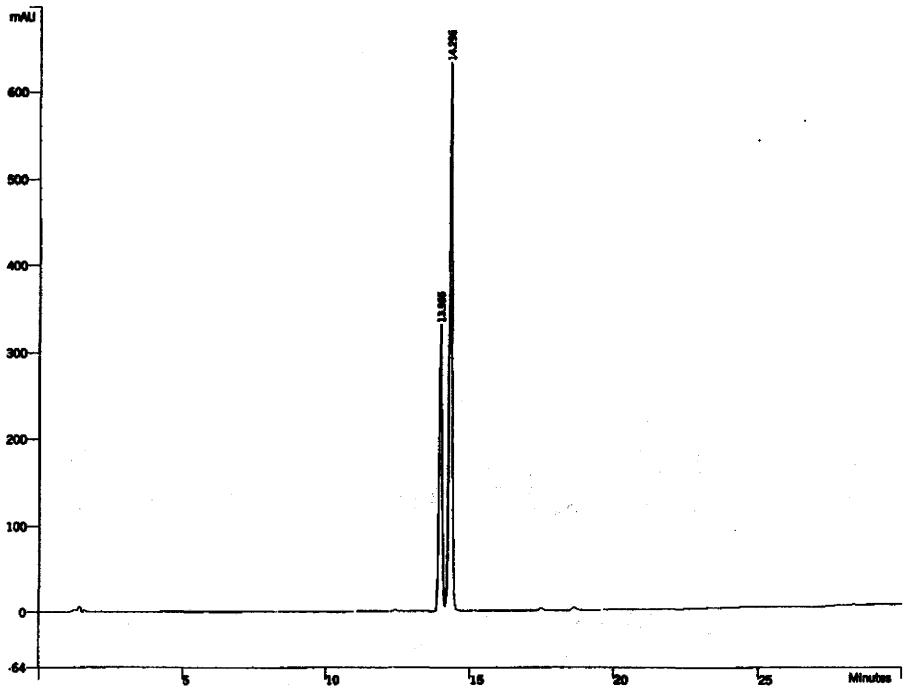


Figure 4.31: HPLC trace of compound **4.23** (Elution method **B**).

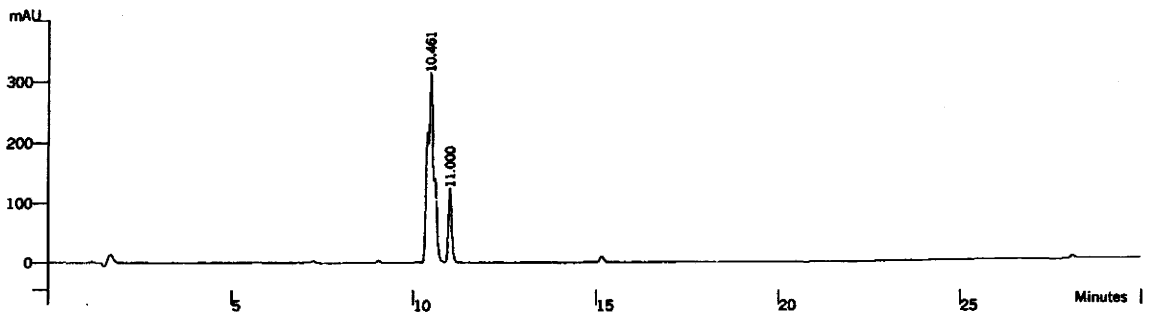


Figure 4.32: HPLC trace of compound **4.24** (Elution method **B**).

4.8 Radiolabelling with ^{125}I, log P Calculations and Comparison

With methods established in Chapter 2 for radioiodination using the simple glycoside model, **2.6**, the radiolabelling of the more advanced derivatives **4.22**, **4.23**, and **4.24** was undertaken. Compound [^{125}I]-**4.22** was prepared and isolated via HPLC in 50% radiochemical yield in a manner similar to that for [^{125}I]-**2.13**. The radiochromatogram for [^{125}I]-**4.22** gave a series of four peaks correlating with the non-radioactive standard **4.22**. The relative intensities of these peaks were reasonably consistent with those of the standard (Figure 4.33). The slight discrepancy observed may be due to different rates of formation of the various isomers of **4.22** at the tracer level concentrations employed (10^{-10} M). Additionally, the manual collection of HPLC eluate may have resulted in the loss of some material from the earlier and later features, resulting in a slight distortion of the distribution of isomeric species observed in the final product.

The synthesis of benzamides [^{125}I]-**4.23** and [^{125}I]-**4.24** were initially explored by reacting using conditions similar to those described above for the model derivatives of **2.6** and **4.12**. For initial experiments, the non-glycosylated precursor **4.23** was reacted with Na[^{125}I] in the presence of Iodogen®, with the only difference in the case of this reaction being that the reaction medium consisted of a 1:1 mixture of acetonitrile and water containing 5% acetic acid, as the organic solvent was required to dissolve the starting material, which was not soluble in aqueous solution, nor in methanol or ethanol.

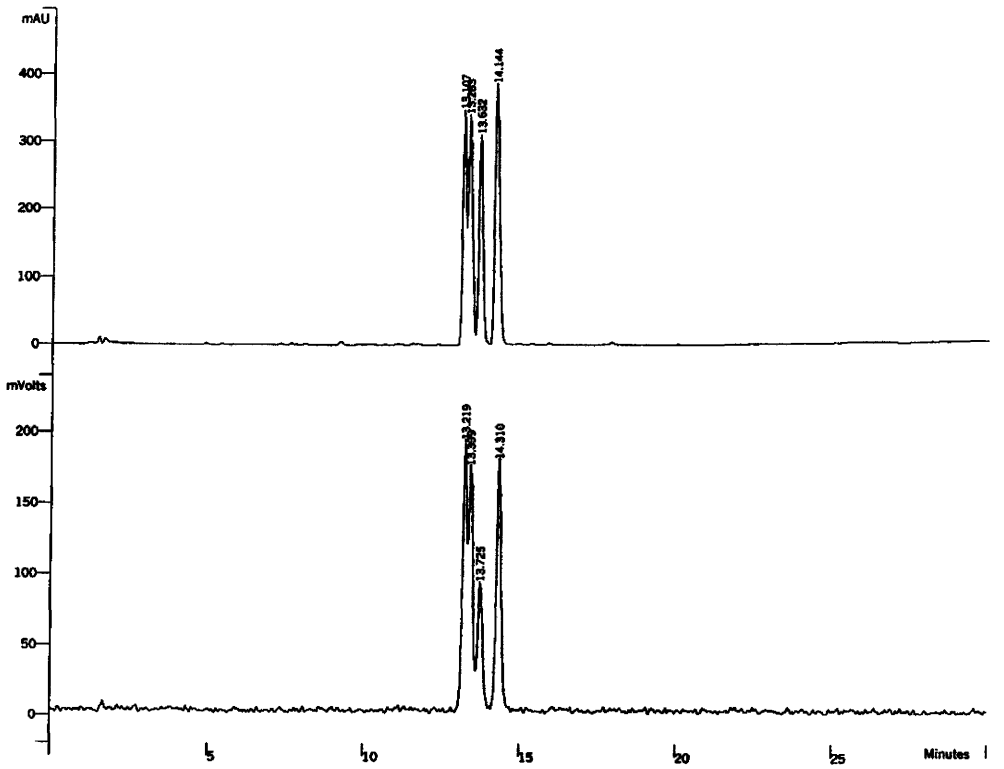


Figure 4.33: HLPC analysis for ^{125}I radiolabelling of **4.12**. Top: UV trace of non-radioactive standard **4.22**. Bottom: γ-HPLC trace of **[^{125}I]-4.22**.

This initial radiolabelling reaction gave unexpected results, with no radioactive peak observed to correspond to the target. Following this unsuccessful reaction, a non-radioactive experiment was also performed with "cold" sodium iodide under the same conditions. Analysis of the product mixture by HPLC and LC/MS revealed what appeared to be the formation of a degradation product. From the mass species obtained, this product appeared to be the result of N-dealkylation of the iodinated *nido*-carboranyl benzamide. Degradation of tertiary amines such as that present in the N,N-diethyl(aminoethyl) benzamide structural element has been reported to occur in the

presence of strong oxidants under acidic conditions.[35] Further non-radioactive experiments were conducted which showed that the extent of degradation was less when Chloramine-T was used as the oxidant. Therefore, this oxidant was used in subsequent preparations involving the benzamide derivatives.

Using a new procedure, which involved adding 20μg of Chloramine-T (as 20μL of a 1mg/mL solution in water), rather than Iodogen®, to the radiolabelling reactions, the synthesis of compound [^{125}I]-**4.23** was attempted again. Under these conditions, the γ-HPLC traces showed two peaks that matched with those corresponding to the non-radioactive standard. Incorporation of activity as the desired target was estimated to be >95% from peak integration of the analytical traces. Compound [^{125}I]-**4.23** was obtained in 73% radiochemical yield following isolation by semipreparative HPLC (Figure 4.34). Glycoside compound [^{125}I]-**4.24** was prepared and isolated in 92% yield in a likewise manner (Figure 4.35). In the case of these radiolabelled compounds, the intensities of the HPLC peaks matched well with those of the standards.

HPLC analysis of the isolated products showed that they were obtained in >99% radiochemical purity, and that their respective precursors had been removed. Stability of the radioiodinated compounds was excellent, with 98% of [^{125}I]-**4.23** remaining intact after 24 hours, and >99% of [^{125}I]-**4.24** remaining intact after 72 hours (Figure 4.36).

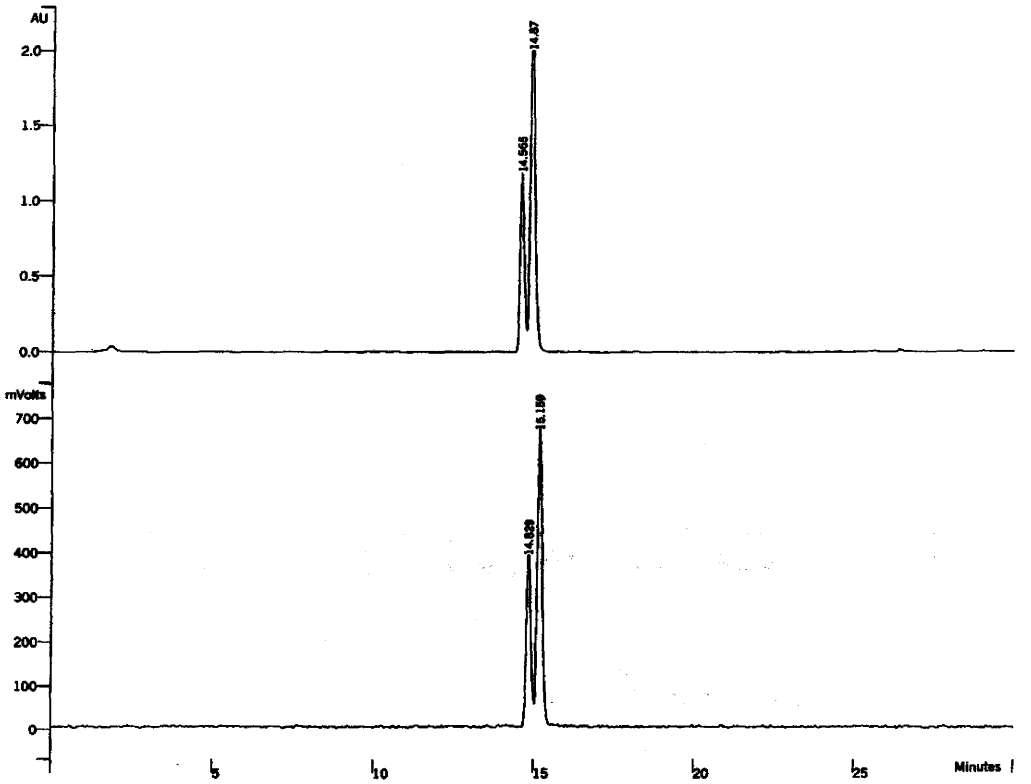


Figure 4.34: HPLC analysis for ^{125}I radiolabelling of **4.14**. Top: UV trace of non-radioactive standard **4.23**. Bottom: γ-HPLC trace of [^{125}I]-**4.23**.

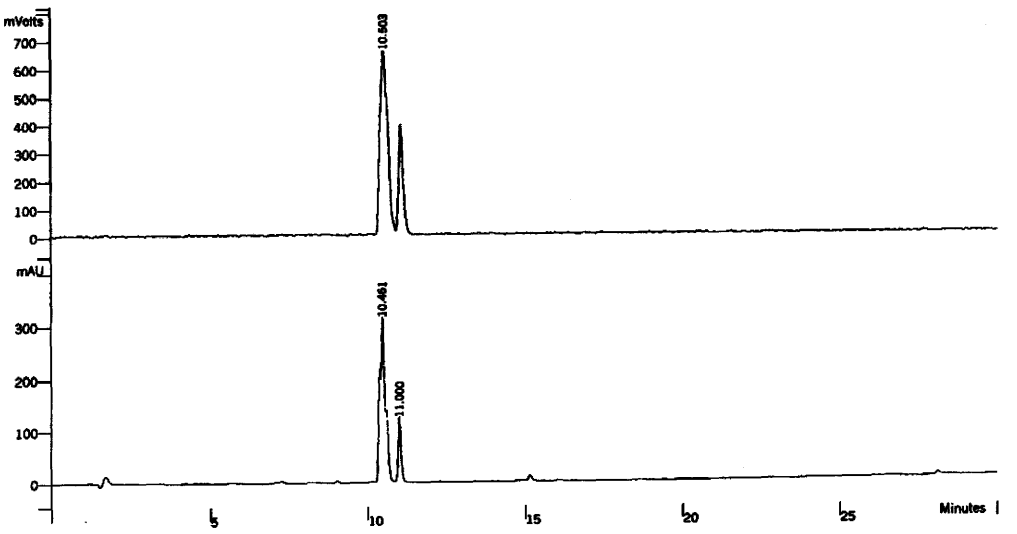


Figure 4.35: HPLC analysis for ^{125}I-radiolabelling of **4.16**. Top: γ-HPLC trace of [^{125}I]-**4.24**. Bottom: UV trace of non-radioactive standard **4.24**.

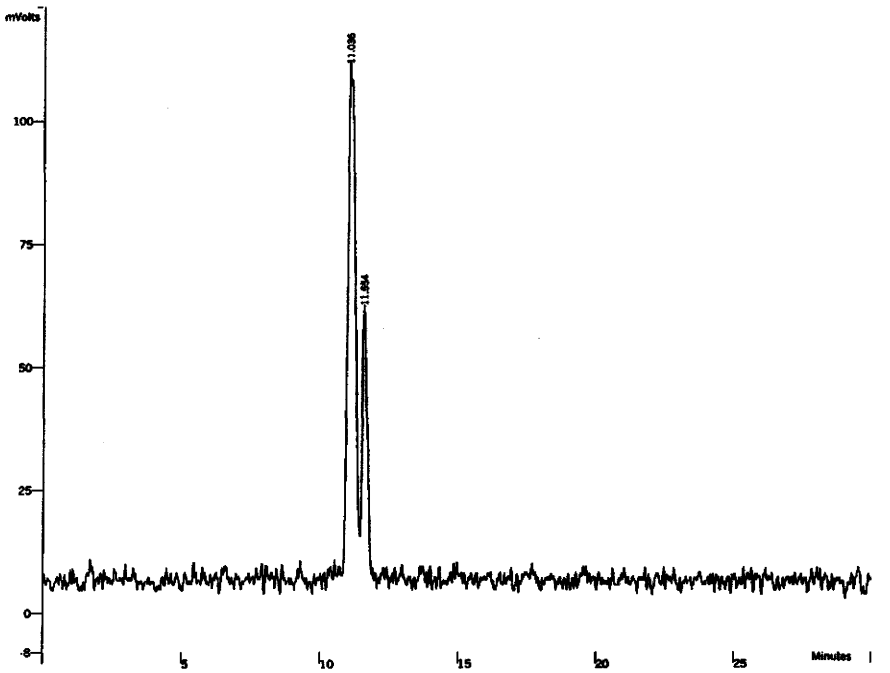


Figure 4.36: γ-HPLC trace of [^{125}I]-**4.24** illustrating radiochemical stability after 72 hours at room temperature.

For the purposes of rapid isolation, it was also possible to use solid phase extraction on C_{18} SepPak^{TM} cartridges (Waters). Although the tracers were obtained in excellent radiochemical purity (>99%), the specific activity was low, as the SPE method did not separate the radioiodinated benzamdes from their precursors. This however, was not considred important for certain experiments such as calculating log P by activity counting, or melanoma cell uptake, which has been reported not to affect the uptake of benzamide derivatives by melanotic melanoma cells.[23]

With the synthesis and isolation of benzamides [^{125}I]-**4.23** and [^{125}I]-**4.24** accomplished, the measurement of their relative lipophilicities was undertaken. The

lipophilicities of these tracers were estimated as log P by standard activity counting methods.[36] Following isolation by HPLC or SPE, the eluates containing the products were evaporated by a stream of air, followed by re-dissolution in phosphate-buffered saline (pH 7.4). As was observed by Wilbur and co-workers, the radioiodinated *nido*-carborane derivatives had a tendency to adhere to glass vessels. They subsequently chose to use plastic containers in which to evaporate and store their tracers.[37] In the case of compounds [^{125}I]-**4.23** and [^{125}I]-**4.24**, these products adhered to both plastic and glass vessels. It was found that silanization of glass vials with Sigmacote^{TM} (Aldrich) remedied this problem sufficiently that the majority of the product was removed by redissolving in buffer, and only a small amount remained adhered to the vessel following initial evaporation of the HPLC solvent. The solutions of the compounds were shaken with a mixture of PBS and 1-octanol for 10 minutes in a vortex mixer, then centrifuged at 3000 rpm. Aliquots of the two layers were taken, and counted for ^{125}I activity. From the ratios of counts for each layer, the log P values calculated for compound [^{125}I]-**4.23** was 1.53±0.01,and that for compound [^{125}I]-**4.24** was 0.82±0.04. For comparison, the reported log P value for IBZA is 1.34.[22] These results confirm an increase in hydrophilicity obtained by inclusion of a glucose moiety in the molecule. The difference of 0.71 log units between the two compounds appears to be in reasonable agreement with results obtained for glycopeptides by Haubner and co-workers,[4] who noted a difference of ~0.5 log units between short peptide sequences and their glycosylated analogues.

4.9 Summary and Conclusions

A functionalized metallocarboranyl glycoside (**4.1**) containing a benzoic acid functionality for bioconjugation was prepared. The metallation of the ligand was carried out at the macroscopic scale and tracer level using microwave irradiation.

In addition to glycoside **4.1** a non-glycosylated metallocarboranyl benzoic acid derivative, **4.2**, was prepared and radiolabelled with ^{99m}Tc in a manner analogous to that for **4.1**. The purpose of producing this compound was to compare the hydrophilic properties of the two derivatives, in order to observe the effect of including the glucose moiety on the overall complex in targeted derivatives.

However, difficulties with the synthesis and isolation of the initial Re complexes (i.e. due to degradation of glycosides during microwave heating), and difficulties with the synthesis and purification of active ester and benzamide model conjugates led to the abandonment of Re/^{99m}Tc chemistry, and a focus on the synthesis of iodinated and radioiodinated analogues.

To this end, a pair of iodinated *nido*-carboranyl N,N-diethyl(aminoethyl) benzamides were prepared as model conjugates of **4.9** and **4.12**. Subsequently, the ^{125}I-labelled analogues were produced in good radiochemical yield, and they displayed excellent stability. The log P of each radiolabelled benzamide compound was measured by activity counting methods, and as expected, the log P value for the glycoside compound (0.82±0.04) was lower than that of its non-glycosylated analogue (1.53±0.01), indicating greater hydrophilicity associated with the presence of the carbohydrate.

4.10 Experimental Section

HPLC Elution conditions: **Method A**: 80:20 A:B to 20:80 A:B; 0-20 min, 20:80 A:B to 100% B; 20-25 min, 100% B; 25-30 min (A = H_2O containing 0.05% TFA, B = CH_3CN containing 0.05% TFA), Varian C_{18} Nucleosil 4.6x250mm column, flow rate = 1.0 mL/min. **Method B:** 80:20 A:B to 20:80 A:B; 0-20 min, 20:80 A:B to 100% B; 20-25 min, 100% B; 25-30 min (A = H_2O containing 0.05% TFA, B = CH_3CN containing 0.05% TFA), Phenomenex Gemini C_{18} 4.6x100mm column, flow rate = 1.0 mL/min. **Method C:** 80:20 A:B to 20:80 A:B; 0-20 min, 20:80 A:B to 100% B; 20-25 min, 100% B; 25-30 min (A = H_2O containing 0.05% TFA, B = CH_3CN containing 0.05% TFA), Varian C_{18} Dynamax 10x250mm semipreparative column, flow rate = 4.7 mL/min.

4′-(sodium [7,8-dicarba-*nido*-undecaboranyl])-7-benzoic acid (4.3):

Methyl 4-[1,2-dicarba-*closo*-dodecaboranyl] benzoate (1.1 g, 4.0 mmol) and sodium hydroxide (0.86 g, 21 mmol) were combined in a round-bottom flask, and dissolved in methanol (75mL). The flask was heated overnight (approximately 12 hours) in an oil bath at 65°C. The reaction was cooled to room temperature, and the solvent removed by rotary evaporation. The solid residue was re-dissolved in distilled water (10 mL). The resulting solution was stirred at room temperature for four hours, whereupon the addition of 1.0 M HCl (14.6 mL) adjusted the solution to pH 3. The solution was diluted with acetonitrile and concentrated to dryness on a rotary evaporator, giving a white solid. The target compound was isolated via silica gel column chromatography (5-25% CH_3OH/CH_2Cl_2), giving a thick, light yellow syrup. Yield: 1.02 g (93%). ^1H NMR

(Acetone-d_6): δ 7.78 (d, 2H, $^3J_{2,3}$ = 8.1 Hz, H-2), 7.25 (d, 2H, H-3), 2.27 (br s, C_{cage}-*H*), -0.30 - 2.60 (br m, B-H), -2.42 (br, B-H-B). $^{13}C\{^1H\}$ NMR (Acetone-d_6): δ 170.98 (C=O), 151.90 (C-1), 129.58 (C-2), 126.66 (C-3, C-4), 62.19 (C_{cage}-C_{cage}-H), 45.13 (C_{cage}-H). $^{11}B\{^1H\}$ NMR (192 MHz, Acetone): δ -7.85 (1B), -9.49 (1B), -12.87 (1B), -15.68 (1B), -17.27 (1B), -19.06 (1B), -22.32 (1B) -32.03 (1B), -34.93 (1B). IR (KBr): ν 3576 (O-H), 2526 (B-H), 1606 (C=O). TLC (1:9 CH_3OH:CH_2Cl_2 + 0.1% CH_3COOH): R_f = 0.15. HPLC: t_R = 17.4 min (Method A). ESI-MS: m/z = 254.3 $[M]^-$. HRMS (ESI) Calculated for $C_9H_{16}B_9O_2$: 254.2031. Observed: 254.2025.

4′-(sodium [2,2,2-tricarbonyl-2-rhenium-1,8-dicarba-*closo*-undecaboranyl])-8-benzoic acid (4.2):

Compound **4.3** (0.10 g, 0.36 mmol) was combined with $[Re(CO)_3(H_2O)_3]Br$ (0.22 g, 0.55 mmol) in a 20mL Emrys microwave vial, and dissolved in 12mL of 0.5M aqueous NaF solution. The vial was capped and sealed, and heated to 200°C for 10 minutes in the microwave reactor. To the resulting solution was added a further 0.15 g (mmol) of the rhenium reagent. The vial was re-capped and subjected to a second 10-minute irradiation at 200°C. The product solution was diluted with acetonitrile and concentrated to dryness on a rotary evaporator. This residue was extracted with acetone and treated via silica gel column chromatography (gradient 1:9 CH_3OH:CH_2Cl_2 to 1:4 CH_3OH:CH_2Cl_2). The fractions containing the desired product were collected and found by TLC to be impure despite the chromatography. Final purification was accomplished using a Biotage SP4 system with a reversed-phase C_{18} cartridge(12mL column; Elution: 100% H_2O, 6CV;

100% H_2O to 100% CH_3CN, 18 CV; 100% CH_3CN, 6 CV), yielding 0.087g (44%). ^1H NMR (600 MHz, Acetone-d_6): δ 7.82 (d, 2H, $^3J_{2,3}$ = 7.9 Hz, H-2), 7.45 (d, 2H, $^3J_{2,3}$ = 7.9 Hz, H-3), 1.90 (br s, H-6), 1.0 - 3.7 (br, B-H). $^{13}C\{^1H\}$ NMR (150 MHz, Acetone-d_6): δ 200.22 (C≡O), 147.12 (*ipso*-*C*-COOH, C-1), 133.78 (*ipso*-*C*-C_{cage}, C-4), 129.99 (C-2), 128.75 (C-3), 56.83 (C_{cage} C-5), 28.96 (C_{cage}-H, C-6). $^{11}B\{^1H\}$ NMR (192 MHz, Acetone-d_6): δ -4.74, -6.75, -7.77, -11.19, -12.28, -17.91, -19.49. IR (KBr): ν 3427 (O-H), 2551 (B-H), 2008, 1890 (C≡O), 1605 (C=O) cm^{-1}. TLC (1:9 CH_3OH : CH_2Cl_2+0.1%CH_3COOH): R_f = 0.18. HPLC (Method **B**): t_R = 21.0 min. ESI-MS: m/z = 532.2 [M^-]. HRMS (ESI): Calculated for $C_{12}H_{15}B_9O_5Re$: 523.1371. Observed: 523.1387.

Methyl 4′-(2-propynyl-2″,3″,4″,6″-tetra-O-acetyl-β-D-glucopyranosyloxy)-3-benzoate (4.10):

Methyl 4-iodo-benzoate (2.53 g, 9.6 mmol) was placed in a 2-neck round-bottom flask (equipped with a condenser and magnetic stir bar) under an atomosphere of nitrogen, and dissolved in 50 mL of dry tetrahydrofuran. To this was added $Pd(PPh_3)_4$ (560 mg), CuI (111 mg) and triethylamine (50 mL). A solution of 2-propynyl-2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside (3.42 g, 8.8 mmol) in dry THF (40 mL) was added dropwise to the reaction vessel. The reaction mixture was then heated, and stirred at reflux for 12 hours, during which time the colour of the solution changed from colourless to light yellow, to dark brown with a white suspended solid. At this time, TLC indicated consumption of the starting materials. The solution was filtered through a pad of Celite,

concentrated on a rotary evaporator to a dark orange-brown oil, and subsequently re-dissolved in 100 mL of dichloromethane and placed in a separatory funnel. The organic solution was shaken twice with 250 mL of 0.1 M HCl, followed by 250 mL of brine. The organic layer was then dried over anhydrous Na_2SO_4, filtered, and concentrated to a dark orange-brown oil on the rotary evaporator. Silica gel column chromatography (10% - 50% ethyl acetate / hexanes) isolated the target compound as an orange oil that could be crystallized to an off-white solid with diethyl ethyl acetate and hexanes. The target compound was obtained in 64% yield (2.93 g). ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 8.00 (d, 2H, $^3J_{2',3'}$ = 8.3 Hz, H-2′), 7.50 (d, 2H, $^3J_{2',3'}$ = 8.3 Hz, H-3′), 5.27 (dd, 1H, $^3J_{2,3}$ = 9.4 Hz, $^3J_{3,4}$ = 9.4 Hz, H-3), 5.12 (dd, 1H, $^3J_{3,4}$ = 9.4 Hz, $^3J_{4,5}$ = 10.0 Hz, H-4), 5.04 (dd, 1H, $^3J_{1,2}$ = 8.0 Hz, $^3J_{2,3}$ = 9.4 Hz, H-2), 4.84 (d, 1H, $^3J_{1,2}$ = 8.0 Hz, H-1), 4.61 (s, 2H, H-7), 4.29 (dd, 1H, $^2J_{6a,6b}$ = -12.3 Hz, $^3J_{5,6a}$ = 4.6 Hz, H-6a), 4.17 (dd, 1H, $^2J_{6a,6b}$ = -12.3 Hz, $^3J_{5,6b}$ = 2.3 Hz, H-6b), 3.93 (s, 3H, benzoate OCH_3 H-11), 3.77 (ddd, 1H, $^3J_{4,5}$ = 10.0 Hz, $^3J_{5,6a}$ = 4.6 Hz, $^3J_{5,6b}$ = 2.3 Hz, H-5), 2.08, 2.04, 2.03, 2.01 (4s, 12H, OAc CH_3). ^{13}C{^{1}H} NMR (125.77 $CDCl_3$): δ 170.53, 170.16, 169.30 (OAc C=O), 166.30 (Benzoate C=O), 131.60 (C-3′), 130.11 (C-1′), 129.51 (C-2′), 126.79 (C-4′), 98.50 (C-1), 86.40, 86.28 (C-8,9), 72.80 (C-3), 72.01 (C-5), 71.17 (C-2), 68.37 (C-4), 61.83 (C-6), 56.71 (C-7), 52.20 (benzoate OCH_3 C-11), 20.63, 20.52 (OAc CH_3). IR (KBr): ν 1758, 1722 cm^{-1}(C=O). ESI-MS: m/z = 538.6 $[M+NH_4]^+$. HRMS (ESI): Calculated for $C_{25}H_{28}O_{12}NH_4$: 538.1925. Observed: 538.1915. MP: 80-83 °C. TLC (2:1 hexanes:ethyl acetate): R_f = 0.24.

Methyl 4′-(1-methyl-(2″,3″,4″,6″-tetra-O-acetyl-β-D-glucopyranosyl)-1,2-dicarba-*closo*-dodecaboranyl)-2-benzoate (4.11):

Decaborane (14), $B_{10}H_{14}$ (1.35 g, 11.0 mmol), was dried under vacuum for two hours in a flame-dried round-bottom flask, which, after the drying period was placed under an atmosphere of nitrogen. Dry acetonitrile (50 mL) was added to the flask via syringe through a septum, and the resulting solution stirred at room temperature for 12 hours. Compound **4.10** (2.57 g, 4.94 mmol) was added, and the solution heated to reflux and stirred for a further 48 hours. The solution was cooled to room temperature and concentrated on a rotary evaporator, yielding an orange-brown oil. Column chromatography (10% - 50% ethyl acetate/hexanes) gave a light, slightly yellow solid. Recrystallization from ethyl ether and hexanes gave the desired compound as a light, white solid in 60% yield (1.89 g). ^{1}H NMR ($CDCl_3$): δ 8.03 (d, 2H, $^3J_{2',3'}$ = 8.5 Hz, H-3′), 7.70 (d, 2H, $^3J_{2',3'}$ = 8.5 Hz, H-2′), 5.13 (dd, 1H, $^3J_{2,3}$ = 9.5 Hz, $^3J_{3,4}$ = 9.5 Hz, H-3), 4.99 (dd, 1H, $^3J_{3,4}$ = 9.5 Hz, $^3J_{4,5}$ = 9.9 Hz, H-4), 4.95 (dd, 1H, $^3J_{2,3}$ = 9.5 Hz, $^3J_{1,2}$ = 7.8 Hz, H-2), 4.31 (d, 1H, $^3J_{1,2}$ = 7.8 Hz, H-1), 4.11 (dd, 1H, $^2J_{6a,6b}$ = - 12.4 Hz, $^3J_{5,6a}$ = 4.7 Hz, H-6a), 3.95 (dd, 1H, $^2J_{6a,6b}$ = - 12.4 Hz, $^3J_{5,6b}$ = 2.3 Hz, H-6b), 3.94 (s, 3H, benzoate OC*H*$_3$ H-11), 3.83 (d, 1H, $^2J_{7a,7b}$ = -12.6 Hz, H-7a), 3.69 (d, 1H, $^2J_{7a,7b}$ = -12.6 Hz, H-7b), 3.52 (ddd, 1H, $^3J_{4,5}$ = 9.9 Hz, $^3J_{5,6a}$ = 4.7 Hz, $^3J_{5,6b}$ = 2.3 Hz, H-5), 2.12, 2.03, 2.01, 1.99 (4s, 12H, OAc C*H*$_3$) 1.70 - 3.20 (br m, B-H). ^{13}C{^{1}H} NMR ($CDCl_3$): δ 170.33, 170.06, 169.22, 169.03 (OAc *C*=O), 165.73 (benzoate *C*=O C-10), 134.28 (C-1′), 132.38 (C-4′), 131.16 (C-3′), 129.85 (C-2′), 100.18 (C-1), 81.33 (C-9), 79.36 (C-8), 72.30 (C-3), 71.96 (C-5), 70.71 (C-2), 68.10 (C-4), 67.89 (C-7), 61.51 (C-6), 52.48 (benzoate O*C*H$_3$ C-11),

20.60, 20.53 (OAc CH_3). $^{11}B\{^1H\}$ NMR ($CDCl_3$): δ -3.73 (1B), -10.96 (4B). IR (KBr): ν 2587 (B-H), 1758, 1730 (C=O) cm^{-1}. ESI-MS: m/z = 657.8 $[M+NH_4]^+$. HRMS(ESI): Calculated for $C_{25}H_{38}B_{10}O_{12}NH_4$: 657.3696. Observed: 657.3715. TLC (2:1 hexanes : ethyl acetate): R_f = 0.27. MP: 167-170 °C.

4′-(sodium [7-methyl-(β-D-glucopyranosyloxy)-7,8-dicarba-*nido*-undeaboranyl])-8-benzoic acid (4.12):

Compound **4.11** (1.6 g, 2.5 mmol) and NaOH (1.0 g, 26 mmol) were dissolved in methanol (40 mL), and stirred with mild heating (~50°C) for approximately 12 hours. At this time, TLC indicated complete consumption of the starting material. The methanol was removed by rotary evaporation, and the resulting white solid re-dissolved in 10 mL of distilled water, to which was added 2.5 mL of 1.0 M NaOH (2.5 mmol). The resulting solution was stirred at room temperature for three hours, at which time ESI-MS indicated formation of the desired product (m/z = 222.6 $[M-H]^{2-}$, 445.3 $[M]^-$). The solution was treated with 1M HCl until a pH of 3 was reached. The solution was dried by rotary evaporation, yielding a white solid. The crude product was dissolved in a minimum amount of methanol and cooled in a freezer for 2 hours. The resulting precipitate was removed on a pad of Celite on top of a fine glass frit. The filtrate was subsequently concentrated to an off-white solid rotary evaporation. This crude product was treated to silica gel column chromatography (1:4 MeOH:CH_2Cl_2 to 2:3 MeOH:CH_2Cl_2). The fractions containing the desired product were concentrated by rotary evaporation, re-dissolved in a minimum quantity of methanol, and allowed to stand in a freezer

overnight. The resulting precipitate was removed as described above. The solvent was removed by rotary evaporation, yielding a white solid (0.88 g, 1.9 mmol, 76%). ^{1}H NMR (CD_3OD): δ 7.76 (d, 2H, $^{3}J_{2'^*,3'^*}$ = 8.4 Hz, H-2'*), 7.74 (d, 2H, $^{3}J_{2',3'}$ = 8.4 Hz, H-2'), 7.43 (d, 2H, H-3'*), 7.38 (d, 2H, H-3'), 3.95 (d, 1H, $^{3}J_{1,2}$ = 7.7 Hz), 3.81 (d, 1H, $^{2}J_{7a^*,7b^*}$ = -10.9 Hz, H-7a*), 3.71 (dd, 1H, $^{2}J_{6a^*,6b^*}$ = -12.1 Hz, $^{3}J_{5^*,6a^*}$ = 2.4 Hz, H-6a*), 3.66 (dd, 1H, $^{2}J_{6a,6b}$ = -11.9 Hz, $^{3}J_{5,6a}$ = 2.5 Hz, H-6a), 3.60 (dd, 1H, $^{3}J_{5^*6b^*}$ = 5.1 Hz, H-6b*), 3.52 (m, 3H, H-1*, H-7a, H-6b), 3.44 (d, 1H, $^{2}J_{7a,7b}$ = -11.1 Hz, H-7b), 3.24 (m, 4H, H-3,3*,4,4*), 3.18 (dd, $^{3}J_{1^*,2^*}$ = 7.7 Hz, H-2*), 3.10 (dd, 1H, H-2), 3.02 (ddd, $^{3}J_{4,5}$ = 9.3 Hz, $^{3}J_{5,6b}$ = 5.4 Hz, H-5), 2.94 (ddd, 1H, H-5*), 2.92 (d, 1H, H-7b*), 2.50 to -0.30 (br m, 18 H, B-H), -2.20 (br s, 2H, B-H-B). ^{13}C{^{1}H} NMR (CD_3OD): δ 174.18 (C=O, C-10), 147.09 (C-1'), 147.01 (C-1'*), 133.52 (C-4',4'*), 132.70 (C-3'*), 132.63 (C-3'), 129.19 (C-2'*), 128.98 (C-2'), 103.89 (C-1*), 103.52 (C-1), 77.66, 77.49, 77.38 (C-3,3*,5,5*), 75.74 (C-7*), 75.25 (C-2), 75.02 (C-2*), 74,80 (C-7), 71.54 (C-4), 71.45 (C-4*), 66.55 (C-9*), 66.13 (C-9), 63.28 (C-8), 62.92 (C-8*), 62.53 (C-6), 62.34 (C-6*). ^{11}B{^{1}H} NMR (CD_3OD): δ -8.25, -10.03, -13.95, -16.81, -33.11, -36.64. IR (KBr): ν 3433 (O-H), 2530 (B-H), 1592 (C=O) cm^{-1}. TLC (1:3 CH_3OH:CH_2Cl_2 + 0.1%HOAc): R_f = 0.38. HPLC: t_R = 12.6 min (Method **A**); t_R = 11.5min, 11.7 min (Method **B**). ESI-MS: m/z = 446.4 [M]$^-$. HRMS (ESI): Calculated for $C_{16}H_{28}B_9O_8$: 446.2671. Observed: 446.2681.

4′-(sodium [1-methyl-(β-D-glucopyranosyloxy)-2,2,2-tricarbonyl-2-rhenium-1,8-dicarba-*closo*-dodecaboranyl])-8-benzoic acid (4.1):

Compound **4.12** (0.078 g, 0.17 mmol) and $[Re(CO)_3(H_2O)_3]Br$ (0.14 g, 0.34 mmol) were dissolved in 0.25 M NaF (2.2 mL), and placed in a 5 mL Biotage microwave reaction vial. The vial was fitted with a magnetic stir bar, metal cap and septum, and then heated with microwave irradiation in the Initiator for 10 minutes at a temperature of 200°C. The resulting solution had discoloured slightly to light yellow following irradiation. The vial was uncapped, and a further 0.079 g (0.2 mmol) of $[Re(CO)_3(H_2O)_3]Br$ was added. The vial was re-sealed and irradiated a further 10 minutes at 200 °C. After this heating interval, ^{11}B NMR still indicated the presence of the nido-carboranyl ligand, so a third amount of $[Re(CO)_3(H_2O)_3]Br$ (0.080 g, 0.2 mmol) was added to the reaction, and the vial was again heated to 200 °C for 10 minutes. The product mixture was transferred to a round-bottom flask, diluted with acetonitrile (10 mL), and concentrated to dryness on a rotary evaporator. The crude reaction residue was dissolved in a minimum amount of methanol and subjected to silica gel column chromatography (gradient elution, 10% to 30% CH_3OH in CH_2Cl_2), giving the target compound as a light brown oil. Yield: 0.032 g (26 %). ^1H NMR (CD_3OD): δ 7.65 (d, 4H, $^3J_{2',3'}$ = 8.4 Hz, H-2′), 7.10 (d, 4H, H-3′), 4.30 (2d, 2H, $^3J_{1,2}$ = 7.7 Hz, H-1,1*), 4.10 (2d, 2H, $^2J_{7a,7b}$ = -10.8 Hz, H-7a,7a*), 3.88 (2dd, 2H, H-6a,6a*), 3.73 (m, 4H, H-7b,7b*, 6b,6b*), 3.37 (m, 4H, H-3,3*,4,4*), 3.26 (m, 4H, H-2,2*,5,5*), 1.20 - 3.20 (br m, B-H).$^{13}C\{^1H\}$ NMR (CD_3OD): δ 200.43 (C≡O), 170.32 (C=O, C-10), 154.59 (C-1′), 129.61 (C-2′), 127.33 (C-4′), 125.81 (C-3′), 104.41, 104.34 (C-1,1*), 77.99, 77.94 (C-3,3*,5,5*),

76.79, 76.75 (C-7,7*), 75.23 (C-2,2*), 71.48 (C-4,4*), 62.62 (C-6,6*), 56.08 (C-9), 54.74 (C-8). IR(KBr): ν 3440 (O-H), 2538 (B-H), 2004, 1909 (C≡O), 1603 (C=O) cm^{-1}. TLC (1:3 CH_3OH:CH_2Cl_2+0.1% HOAc): R_f = 0.51. HPLC (Method A): t_R = 16.8 min. ESI-MS: m/z = 715.4 $[M]^-$. HRMS (ESI): Calculated for $C_{19}H_{27}B_9O_{11}Re$: 715.2009. Observed: 715.1964.

2″,3″,5″,6″-tetrafluorophenyl-4′-(sodium[(7,8-dicarba-*nido*-undecaboranyl)])-7-benzoate (4.13):

Compound **4.3** (0.11g, 0.41mmol) and 2,3,5,6-tetrafluorophenol (0.15g, 0.90mmol) were dissolved in 1mL of dry acetonitrile. To this solution was added diisopropylcarbodiimide (100μL, 0.65mmol), and the reaction stirred overnight at room temperature, during which time a white precipitate formed that was attributed to the urea byproduct of DIC. Following the reaction period, the precipitate was removed by filtration, and the filtrate solution concentrated by rotary evaporation. The target compound was isolated in 74% yield by preparative TLC (15% CH_3OH/CH_2Cl_2), and used without futher purification. ^1H NMR (600.13 MHz, Acetone-d_6): δ 7.97 (d, 2H, H-9), 7.56 (m, 1H, H-1), 7.45 (d, 2H, H-10), 2.36 (br s, 1H, C_{cage}-H, H-13), -0.10-2.80 (br, B-H), -2.46 (br, B-H-B). $^{13}C\{^1H\}$ NMR (150.90 MHz, Acetone-d_6): δ 163.38 (C=O, C-7), 155.92 (Aryl *ipso*, C-8), 147.91-140.90 (TFP C-F , C-2, 3, 5, 6), 130.81 (TFP *ipso*, C-4), 130.49 (C-9), 127.53 (C-10), 123.18 (benzoate *ipso*, C-11), 104.50 (TFP C-H, C-1), 62.32 (C_{cage}, C-12), 44.23 (C_{cage}-H, C-13). $^{11}B\{^1H\}$ NMR (160.46 MHz, Acetone-d_6): δ -7.46, -9.20, -12.62, -15.44, -16.99, -18.97, -22.24, -31.79, -34.63. IR (neat, on KBr disc):

ν 2530 (B-H), 1751 (C=O). TLC (1:9 CH_3OH:CH_2Cl_2): R_f = 0.30. ESI-MS: m/z = 402.1 $[M]^-$. HRMS (ESI): Calculated for $C_{15}H_{16}B_9O_2F_4$: 402.1971. Observed: 402.1985.

N,N-diethyl(aminoethyl)-4′-(sodium [7,8-dicarba-*nido*-undecaboranyl])-7-benzamide] (4.14):

Compound **4.13** (0.13g, 0.30mmol) was dissolved in 1mL dry acetonitrile, followed by addition of N,N-diethylenthylenediamine (45.5μL, 0.32mmol). The reaction mixture was stirred overnight at room temperature, whereupon TLC indicated consumption of the starting material. The crude material was isolated via preparative TLC (20% CH_3OH/CH_2Cl_2+1%NEt_3). The target bands were cut from the plate, extracted with acetone, filtered, concentrated by rotary evaporation, and dried under vacuum, giving 0.0294g (26%). ^{1}H NMR (500.13 MHz, Acetone-d_6): δ 8.25 (br, 1H, (amide N-H, H-12), 7.64 (d, 2H, $^3J_{2,3}$ = 8.5 Hz, H-2), 7.29 (d, 2H, H-3), 3.78 (m, 2H, $^3J_{8,9}$ = 5.4 Hz, H-8), 3.39 (m, 2H, H-9), 3.31 (q, 4H, $^3J_{10,11}$ = 7.2 Hz, H-10), 2.27 (br s, C_{cage}H, H-6), 1.33 (t, 6H, H-11), -0.30 - 2.80 (br, B-H). ^{13}C{^{1}H} NMR (125.77 MHz, Acetone-d_6): δ 170.54 (C=O, C-7), 152.30 (C-1), 129.87 (C-4), 127.32 (C-2), 127.13 (C-3), 62.32 ($C_{cage}C_{cage}$H, C-5), 55.41 (C-9), 49.02 (C-10), 44.87 ($C_{cage}C_{cage}$H, C-6), 37.77 (C-8), 10.20 (C-11). ^{11}B{^{1}H} NMR (160.46 MHz, Acetone-d_6): δ -7.68, -9.38, -12.76, -15.65, -16.99, -18.99, -22.13, -31.88, -34.85. IR (KBr pellet): ν 3407 (N-H), 2527 (B-H), 1640 (C=O). TLC (20% CH_3OH/CH_2Cl_2+1%NEt_3): R_f = 0.50. HPLC: t_R = 13.3 min (Method **B**). ESI-MS: m/z = 352.4 $[M]^-$. HRMS (ESI) Calculated for $C_{15}H_{30}B_9ON_2$: 352.3242. Observed: 352.3251.

Benzyl 4′-(2-propynyl-2″,3″,4″,6″-tetra-O-acetyl-β-D-glucopyranosyl) benzoate (4.17):

Compound **2.4** (2.01g, 5.20mmol), benzyl 4-iodobenzoate (1.94g, 5.73mmol), tetrakis(triphenylphosphine) palladium(0) (0.321g, 0.28mmol) and copper(I) iodide (0.053g, 0.28mmol) were combined in a round-bottom flask and dissolved in 100mL of a 1:1 solution of dry THF and freshly distilled triethylamine. The resulting mixture was heated to reflux and allowed to stir overnight (~12hours), during which time a white precipitate was formed, and the solution became amber-brown in colour. The solid was removed by filtration, and the filtrate concentrated by rotary evaporation giving an amber-brown oil. The crude material was dissolved in chloroform (50mL) and extracted thrice with 50mL of 0.01M HCl, followed by washing three times with 50mL of distilled water. The organic layer was dried over Na_2SO_4 before filtering through a pad of silica gel, washing with 100mL $CHCl_3$, followed by 300mL of ethyl acetate. The filtrate was concentrated by rotary evaporation. The resulting oil was re-dissolved in the minimum amount of ethyl acetated and the target compound isolated via silica gel column chromatography (10-15-20-25-30-40% ethyl acetate/hexanes). The fractions containing the product were concentrated to an amber oil by rotary evaporation. This oil was crystallized from ether/hexanes to give an off-white solid (2.02g, 65%). ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 8.04 (d, 2H, $^3J_{2',3'}$ = 8.4 Hz, H-2′), 7.49 (d, 2H, $^3J_{2',3'}$ = 8.4 Hz, H-3′), 7.39 (m, 5H, Aryl H), 5.37 (s, 2H, H-11), 5.26 (dd, 1H, $^3J_{2,3}$ = 9.5 Hz, $^3J_{3,4}$ = 9.8 Hz, H-3), 5.12 (dd, 1H, $^3J_{3,4}$ = 9.8 Hz, $^3J_{4,5}$ = 10.0 Hz, H-4), 5.04 (dd, 1H, $^3J_{2,3}$ = 9.5 Hz, $^3J_{1,2}$ = 8.0 Hz, H-2), 4.83 (d, 1H, $^3J_{1,2}$ = 8.0 Hz, H-1), 4.61 (s, 2H, H-7), 4.29 (dd, 1H, $^2J_{6a,6b}$ = -

12.3 Hz, $^{3}J_{5,6a}$ = 4.7 Hz, H-6a), 4.17 (dd, 1H, $^{2}J_{6a,6b}$ = -12.3 Hz, $^{3}J_{5,6b}$ = 2.4 Hz, H-6b), 3.76 (ddd, 1H, $^{3}J_{4,5}$ = 10.0 Hz, $^{3}J_{5,6a}$ = 4.7 Hz, $^{3}J_{5,6b}$ = 2.4 Hz, H-5), 2.08, 2.04, 2.02, 2.01 (4xs, 12H, OAc CH_3). $^{13}C\{^{1}H\}$ NMR (125.77 MHz, $CDCl_3$) δ 170.59, 170.22, 169.35 (OAc C=O), 165.68 (C=O, C-10), 135.83 (OBn *ipso* C), 131.64 (C-3′), 130.11 (C-1′), 129.67 (C-2′), 128.63, 128.35, 128.23 (OBn aryl C), 126.92 (C-4′), 98.53 (C-1), 86.50, 86.29 (C-8, C-9), 72.82 (C-3), 72.02 (C-5), 71.17 (C-2), 68.37 (C-4), 66.95 (C-11), 61.84 (C-6), 56.75 (C-7), 20.67, 20.55 (OAc CH_3). IR (KBr pellet): ν 1757, 1725 (ester C=O). TLC (1:2 ethyl acetate:hexanes): R_f = 0.19. ESI-MS: m/z = 655.3 $[M+CH_3COO]^-$. HRMS (ESI): Calculated for $C_{31}H_{32}O_{12}+CH_3COO$: 655.2027. Observed: 655.2026.

Benzyl 4′-(1-methyl-(2″,3″,4″,6″-tetra-O-acetyl-β-D-glucopyranosyl)-1,2-dicarba-*closo*-dodecaboranyl)-2-benzoate (4.18):

Decaborane(14) (0.49g, 4.0mmol) was dried under vacuum for two hours prior to addition of 20mL dry acetonitrile. The resulting solution was stirred at room temperature overnight. Alkyne **4.17** (0.79g, 1.3mmol) was added, and the solution heated to reflux for 24 hours, giving a deep yellow solution from which a precipitate formed on cooling. The precipitate was removed by gravity filtration, and the filtrate concentrated by rotary evaporation, giving a foamy, yellow solid. The solid was re-dissolved in a minimum quantity of ethyl acetate, and purified by silica gel column chromatograph (1:2 ethyl acetate: hexanes). The fractions containing the desired product were concentrated by rotary evaporation, giving a light yellow oil which crystallized slowly at room temperature. The column product was shown to be impure by TLC, and was re-

crystallized from ethyl acetate and hexanes, giving a white, crystalline solid. (0.48g, 50%). ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 8.06 (d, 2H, $^{3}J_{2',3'}$ = 8.4 Hz, H-2′), 7.69 (d, 2H, $^{3}J_{2',3'}$ = 8.4 Hz, H-3′), 7.39 (m, 5H, aryl H), 5.38 (s, 2H, H-11), 5.12 (dd, 1H, $^{3}J_{2,3}$ = 9.5 Hz, $^{3}J_{3,4}$ = 9.6 Hz, H-3), 4.99 (dd, 1H, $^{3}J_{3,4}$ = 9.6 Hz, $^{3}J_{4,5}$ = 10.0 Hz, H-4), 4.95 (dd, 1H, $^{3}J_{1,2}$ = 7.8 Hz, $^{3}J_{2,3}$ = 9.5 Hz, H-2), 4.31 (d, 1H, $^{3}J_{1,2}$ = 7.8 Hz, H-1), 4.10 (dd, 1H, $^{2}J_{6a,6b}$ = -12.3 Hz, $^{3}J_{5,6a}$ = 4.7 Hz, H-6a), 3.96 (dd, 1H, $^{2}J_{6a,6b}$ = -12.3 Hz, $^{3}J_{5,6b}$ = 2.4 Hz, H-6b), 3.82 (d, 1H, $^{2}J_{7a,7b}$ = -12.7 Hz, H-7a), 3.69 (d, 1H, $^{2}J_{7a,7b}$ = -12.7 Hz, H-7b), 3.52 (ddd, 1H, $^{3}J_{4,5}$ = 10.0 Hz, $^{3}J_{5,6a}$ = 4.7 Hz, $^{3}J_{5,6b}$ = 2.4 Hz, H-5), 2.10, 2.00, 1.99 (4 x s, 12H, OAc CH_3), 1.70-3.40 (br, B-H). $^{13}C\{^{1}H\}$ NMR (125.77 MHz, $CDCl_3$): δ 170.35, 170.07, 169.23 (OAc C=O), 165.09 (OBn C=O, C-10), 135.59 (OBn *ipso* aryl C) 134.40 (C-1′), 132.43 (C-4′), 131.19 (C-3′), 130.00 (C-2′) 128.68, 128.49, 128.34 (5 x OBn aryl C), 100.26 (C-1), 81.35 (C-9), 79.43 (C-8), 72.33 (C-3), 71.98 (C-5), 70.73 (C-2), 68.12 (C-4), 67.92, 67.24 (C-7, C-11), 61.52 (C-6), 20.60, 20.53, 20.49 (OAc CH_3). $^{11}B\{^{1}H\}$ NMR (160.46 MHz, $CDCl_3$): δ -2.95, -10.30. IR (KBr Pellet): n 2594 (B-H), 1758, 1726 (OAc, OBn C=O). TLC (1:1 ethyl acetate:hexanes): R_f = 0.67. ESI-MS: m/z = 714.4 $[M-H]^-$. HRMS (ESI): Calculated for $C_{31}H_{41}B_{10}O_{12}$: 714.3586. Observed: 714.3588.

4′-(1-methyl-(2″,3″,4″,6″-tetra-O-acetyl-β-D-glucopyranosyl)-1,2-dicarba-*closo*-dodecaboranyl)-2-benzoic acid (4.19):

Compound **4.18** (0.55g, 0.77mmol) was dissolved in 100mL of absolute ethanol in a round-bottom flask. To this solution was added slowly 0.11g of 10 wt.% (Pd/C). The flask was purged twice with hydrogen from a balloon, using a long, stainless steel needle

to bubble the gas through the solution. The reaction was stirred for 1 hour at room temperature under an atmosphere of hydrogen, at which time TLC indicated complete disappearance of the starting material. The product mixture was filtered over a pad of Celite and concentrated to dryness by rotary evaporation. The solid residue so obtained was re-dissolved in DCM and filtered through a plug of glass wool to remove the last residue of catalyst, and concentrated to dryness by rotary evaporation, giving a white, foamy solid (0.47g, 98%). ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 10.39 (br s, 1H, COO*H* H-10), 8.09 (d, 2H, $^{3}J_{2',3'}$ = 8.4 Hz, H-2′), 7.74 (d, 2H, $^{3}J_{2',3'}$ = 8.4 Hz, H-3′), 5.14 (dd, 1H, $^{3}J_{2,3}$ = 9.5 Hz, $^{3}J_{3,4}$ = 9.7 Hz, H-3), 5.00 (t, 1H, $^{3}J_{3,4}$ = 9.7 Hz, $^{3}J_{4,5}$ = 9.9 Hz, H-4), 4.96 (dd, 1H, $^{3}J_{1,2}$ = 7.8 Hz, $^{3}J_{2,3}$ = 9.5 Hz, H-2), 4.34 (d, 1H, $^{3}J_{1,2}$ = 7.8 Hz, H-1) 4.11 (dd, 1H, $^{2}J_{6a,6b}$ = -12.3 Hz, $^{3}J_{5,6a}$ = 4.6 Hz, H-6a), 3.95 (dd, 1H, $^{2}J_{6a,6b}$ = -12.3 Hz, $^{3}J_{5,6b}$ = 2.4 Hz, H-6b), 3.82 (d, 1H, $^{2}J_{7a,7b}$ = -12.6, H-7a), 3.73 (d, 1H, H-7b), 3.54 (ddd, 1H, $^{3}J_{4,5}$ = 9.9 Hz, $^{3}J_{5,6a}$ = 4.6 Hz, $^{3}J_{5,6b}$ = 2.4 Hz, H-5), 2.12, 2.04, 2.01, 1.99 (4s, 12H, OAc CH_3), 1.80-3.30 (br, B-H). $^{13}C\{^{1}H\}$ NMR (125.77 MHz, $CDCl_3$): δ 170.65, 170.13, 169.31, 169.12 (OAc C=O), 169.93 (COOH C=O, C-10), 134.99 (C-1′), 134.84 (C-4′), 131.29 (C-3′), 130.40 (C-2′), 100.13 (C-1), 81.17 (C-9), 79.33 (C-8), 72.30 (C-3), 71.94 (C-5), 70.71 (C-2), 68.14 (C-4), 67.88 (C-7), 61.65 (C-6), 20.60, 20.51, 20.48 (OAc CH_3). $^{11}B\{^{1}H\}$ NMR (160.46 MHz, $CDCl_3$): δ -2.80, -10.40. IR (KBr pellet): ν 3245 (O-H), 2593 (B-H), 1758 (C=O). TLC (ethyl acetate+0.1% HOAc): R_f = 0.85. ESI-MS: m/z = 624.4 $[M-H]^-$. HRMS (ESI): Calculated for $C_{24}H_{35}B_{10}O_{12}$: 624.3113. Observed: 624.3114.

2‴,3‴,5‴,6‴-tetrafluorophenyl-4′-(1-methyl-(2″,3″,4″,6″-tetra-O-acetyl-β-D-glucopyranosyl)-1,2-dicarba-*closo*-dodecaboranyl)-2-benzoate (4.20):

Compound **4.19** (0.22g, 0.35mmol) and tetrafluophenol (0.12g, 0.75mmol) were dissolved in 2mL dry acetonitrile, followed by EDC·HCl (0.082g, 0.43mmol). The reaction was allowed to stir at room temperature for two hours until TLC indicated consumption of starting material. The solution was concentrated to dryness, then re-dissolved in 4mL CH_2Cl_2 and added to a separatory funnel. The dichloromethane solution was extracted three times with 0.01M HCl (4mL), and washed thrice with distilled water (4mL). The organic layer was dried over anhydrous Na_2SO_4, filtered, and concentrated to a light yellow oil by rotary evaporation. The oil was re-dissolved in a minimum volume of ether, cooled in an ice bath, with addition of hexanes to induce crystallization, yielding a white solid (0.19g, 70%). ^{1}H NMR (500.13 MHz, $CDCl_3$): δ 8.22 (d, 2H, $^3J_{2',3'}$ = 8.6 Hz, H-2′), 7.82 (d, 2H, $^3J_{2',3'}$ = 8.6 Hz, H-3′), 7.07 (m, 1H, TFP H-11), 5.15 (dd, 1H, $^3J_{2,3}$ = 9.6 Hz, $^3J_{3,4}$ = 9.5 Hz, H-3), 5.01 (dd, 1H, $^3J_{3,4}$ = 9.5 Hz, $^3J_{4,5}$ = 10.0 Hz, H-4), 4.97 (dd, 1H, $^3J_{1,2}$ = 7.8 Hz, $^3J_{2,3}$ = 9.6 Hz, H-2), 4.36 (d, 1H, $^3J_{1,2}$ = 7.8 Hz, H-1), 4.15 (dd, 1H, $^2J_{6a,6b}$ = -12.4 Hz, $^3J_{5,6a}$ = 4.6 Hz, H-6a), 4.00 (dd, 1H, $^2J_{6a,6b}$ = -12.4 Hz, $^3J_{5,6b}$ = 2.4 Hz, H-6b), 3.86 (d, 1H, $^2J_{7a,7b}$ = -12.8 Hz, H-7a), 3.77 (d, 1H, $^2J_{7a,7b}$ = -12.8 Hz, H-7b), 3.56 (ddd, 1H, $^3J_{4,5}$ = 10.0 Hz, $^3J_{5,6b}$ = 2.4 Hz, $^3J_{5,6a}$ = 4.6 Hz, H-5), 2.12, 2.02, 2.01, 2.00 (4xs, 12H, OAc CH_3), 1.70 - 3.30 (br, B-H). ^{13}C{^{1}H} NMR (125.77 MHz, $CDCl_3$): δ 170.28, 170.03, 169.22, 169.04 (OAc C=O), 161.37 (TFP ester C=O, C-10), 147. 08, 145.12 (2t, TFP C-F), 141.69, 139.68 (2d, TFP C-F), 136.04 (C-1′), 131.61 (C-3′), 130.90 (C-2′), 129.55 (TFP C-F), 129.36 (C-4′), 103.62 (t, TFP C-H, C-11), 100.25 (C-1), 80.67 (C-9),

79.50 (C-8), 72.28 (C-3), 72.04 (C-5), 70.73 (C-2), 68.08 (C-4), 67.98 (C-7), 61.43 (C-6), 20.57, 20.48, 20.43 (OAc CH_3). $^{11}B\{^1H\}$ NMR (192.55 MHz, $CDCl_3$): δ -2.79, -10.27. IR (KBr Pellet): ν 2596 (B-H), 1758 (C=O). TLC (1:2 ethyl acetate:hexanes): R_f = 0.31. ESI-MS: m/z = 886.4 $[M+TFA]^-$. HRMS (ESI): Calculated for $C_{30}H_{36}B_{10}O_{12}F_4$+$CF_3COO$: 886.2982. Observed: 886.2977.

N,N-diethyl(aminoethyl)-4′-(1,2-dicarba-*closo*-dodecaboranyl-2-methyl-2″,3″,4″,6″-tetra-O-acetyl-β-D-glucopyranosyl)-1-benzamide (4.21):

Compound **4.20** (0.25g, 0.32mmol) was dissolved in 2.5mL dry acetonitrile. To this solution was added 47μL (0.33mmol) of N,N-diethylethylenediamine via Eppendorf pipette. The reaction was stirred overnight at room temperature, whereupon TLC indicated consumption of the starting material. The solvent was removed by rotary evaporation, and the resulting off-white foam re-dissolved in a minimum volume of DCM. The target was isolated via silica gel column chromatography (1:4 Acetone:DCM containing 1 vol.% triethylamine). The target fractions were combined and concentrated by rotary evaporation yielding a colourless oil which formed a white foam upon drying under vacuum (0.20g, 86%). ^1H NMR (600.13 MHz, $CDCl_3$): δ 7.77 (d, 2H, $^3J_{2',3'}$ = 8.4Hz, H-2′), 7.68 (d, 2H, $^3J_{2',3'}$ = 8.4Hz, H-3′), 7.08 (br, 1H, N-H, H-15), 5.13 (dd, 1H, $^3J_{2,3}$ = 9.5 Hz, $^3J_{3,4}$ = 9.4 Hz, H-3), 5.01 (dd, 1H, $^3J_{3,4}$ = 9.4 Hz, $^3J_{4,5}$ = 10.0 Hz, H-4), 4.96 (dd, 1H, $^3J_{2,3}$ = 9.5 Hz, $^3J_{1,2}$ = 7.8 Hz, H-2), 4.32 (d, 1H, $^3J_{1,2}$ = 7.8 Hz, H-1), 4.13 (dd, 1H, $^2J_{6a,6b}$ = -12.4 Hz, $^3J_{5,6a}$ = 4.6 Hz, H-6a), 3.99 (dd, 1H, $^2J_{6a,6b}$ = -12.4 Hz, $^3J_{5,6b}$ = 2.3 Hz, H-6b), 3.83 (d, 1H, $^2J_{7a,7b}$ = -12.8 Hz, H-7a), 3.68 (d, 1H, $^2J_{7a,7b}$ = -12.8 Hz, H-7b),

3.54 (ddd, 1H, H-5), 3.49 (m, 2H, $^3J_{11,15}$ = 5.3 Hz, H-11), 2.67 (t, 2H, $^3J_{11,12}$ = 5.8 Hz, H-12), 2.59 (q, 4H, $^3J_{13,14}$ = 7.1 Hz, H-13), 2.13, 2.03, 2.01, 2.00 (4s, 12H, OAc CH_3), 1.05 (t, 6H, H-14), 1.60 - 3.10 (br, B-H). $^{13}C\{^1H\}$ NMR (150.90 MHz, $CDCl_3$): δ 170.49, 170.12, 169.29, 169.13 (OAc C=O), 165.79 (Amide C=O, C-10), 137.10 (C-4′), 132.70 (C-1′), 131.31 (C-3′), 127.41 (C-2′), 100.39 (C-1), 81.67 (C-9), 79.47 (C-8), 72.34 (C-3), 71.91 (C-5), 70.74 (C-2), 68.05 (C-4, C-7), 61.49 (C-6), 51.13 (C-12), 46.71 (C-13), 37.30 (C-11), 20.67, 20.63, 20.55, 20.52 (OAc CH_3), 11.90 (C-14). $^{11}B\{^1H\}$ NMR (160.46 MHz, $CDCl_3$): δ -2.03, -9.37. IR (KBr Pellet): ν 3406 (N-H), 2592 (B-H), 1758 (OAc C=O), 1652 (amide C=O). TLC (1:2 Acetone:CH_2Cl_2+1%NEt_3): R_f = 0.70. ESI-MS: m/z = 724.3 $[M+H]^+$, 782.6 $[M+CH_3COO]^-$. HRMS (ESI): Calculated for $C_{30}H_{50}B_{10}O_{11}N_2$+$CH_3COO$: 782.4542. Observed: 782.4537.

N,N-diethyl(aminoethyl)-4′-(sodium [(7-methyl-(β-D-glucopyranosyloxy)-7,8-dicarba-*nido*-dodecaboranyl])-8-benzamide (4.16):

Compound **4.21** (0.19g, 0.26mmol) was dissolved in 5mL of absolute ethanol. To this solution was added 775μL (0.062g, 1.6mmol) of a 80mg/mL solution of NaOH in 17% H_2O in ethanol, and the reaction stirred overnight with heating in an oil bath at 60°C, whereupon TLC indicated consumption of the starting material. The reaction was cooled to room temperature, and bubbled with CO_2 to precipitate excess NaOH as Na_2CO_3. The solid was removed by filtration, washed with ethanol, and the filtrate concentrated to a colourless oil by rotary evaporation. Re-dissolution in methanol, filtration through a plug of glass wool and rotary evaporation gave a white solid. The

crude material was dissolved in a minimum volume of methanol and the target compound isolated via silica gel column chromatography (gradient 10-20% CH_3OH:CH_2Cl_2 containing 1% (vol.) NEt_3, increment :5% CH_3OH), giving 0.052g (35%). ^1H NMR (500.13 MHz, CD_3CN): δ 7.89, 7.84 (2xt, br, 2H, H-15 (amide N-H), 7.65, 7.62 (2xd, 4H, H-2′), 7.53, 7.47 (2xd, 4H, H-3′), 3.94 (d, 1H, $^3J_{1,2}$ = 7.7 Hz, H-1), 3.77 (d, 1H, $^2J_{7a,7b}$ = -10.9 Hz, H-7a), 3.65 (m, 4H, H-11), 3.61 (dd, 1H, H-6a), 3.54-3.40 (m, 6H, H-1, H-6a, 2xH-6b,2xH-7), 3.25-3.14 (m, 16H, 2xH-3, 2xH-4, H-12, H-13), 3.07 (m, 1H, H-2), 3.00 (m, 2H, H-2, H-5), 2.94 (m, 1H, H-5), 2.90 (d, 1H, H-7b), 1.26 (t, 12H, $^3J_{13,14}$ = 7.2 Hz, H-14), -0.40 - 2.60 (br, B-H), -2.20 (br, B-H-B). $^{13}C\{^1H\}$ NMR (125.77 MHz, CD_3CN) δ 171.29 (C=O, C-10), 147.87, 147.75 (C-1′), 133.01 (C-3′), 131.26, 131.20 (C-4′), 127.34, 127.05 (C-2′), 103.12, 102.93 (C-1), 77.43, 77.38 (C-3), 76.86, 76.55 (C-5), 74.91, 74.84, 73.96 (C-2, C-7), 71.58, 71.29 (C-4), 67.23 (C-9), 62.73, 62.58 (C-6), 61.58, 61.28 (C-8), 55.42, 55.36 (C-12), 49.19, 49.04 (C-13), 37.42, 37.26 (C-11), 9.62, 9.55 (C-14). $^{11}B\{^1H\}$ NMR (160.46 MHz, CD_3CN): δ -7.13, -10.00, -14.82, -18.02, -19.74, -32.92, -36.42. IR (KBr pellet): ν 3431 (O-H), 2526 (B-H), 1641 (C=O). TLC (15%CH_3OH / CH_2Cl_2+1%NEt_3): R_f = 0.55. HPLC: t_R = 8.87min (Method **B**). ESI-MS: m/z = 544.3 $[M]^-$. HRMS (ESI): Calculated for $C_{22}H_{42}B_9O_7N_2$: 544.3879. Observed: 544.3882.

4′-(sodium [7-methyl-(β-D-glycopyranosyl)-iodo-7,8-dicarba-*nido*-undecaboranyl])-8-benzoic acid (4.22):

To a solution of compound **4.12** (37.5mg, 80.2μmol) in ethanol (500μL) was added gradually over 1 hour 2mL of I_2 in EtOH (21mg, 82.7μmol). After addition of the

iodine solution was complete, the reaction was stirred at room temperature for a further hour, followed by addition of 1.7mL of 0.1M $Na_2S_2O_5$ to quench the reaction. The resulting mixture was concentrated to dryness by rotary evaporation. The resulting residue was re-dissolved in 0.5 mL H_2O and loaded onto a pre-conditioned C_{18} SepPak® cartridge (Waters), which was subsequently eluted with 7mL H_2O, 3mL 1:1 H_2O:CH_3CN, and 5mL CH_3CN for a total of 15 1mL fractions. Fractions 10 and 11 contained a single, UV and Pd-active product, which was confirmed by mass spectrometry to be isolated **4.22**. The fractions containing the target were combined, concentrated by rotary evaporation, and lyophilized giving a light white solid. (15mg, 32%). ^{1}H NMR (600.13 MHz, CD_3OD): δ 7.81-7.75 (m, 8H, H-2′), 7.51 (d, 2H, ^3J = 8.3 Hz, H-3′), 7.46 (d, 3H, ^3J = 8.3 Hz, H-3′), 3.99 (d, 1H, ^2J = -11.0 Hz, H-7), 3.97 (d, 1H, ^3J = 7.7 Hz, H-1), 3.93 (d, 1H, ^2J = -10.9 Hz, H-7), 3.86 (d, 1H, ^3J = 7.6 Hz, H-1), 3.74 (dd, 1H, ^2J = -12.0 Hz, ^3J = 2.4 Hz, H-6$^{(*)}$), 3.70-3.66 (m, 3H, H-7, H-6, H-6$^{(+)}$), 3.63 (d, 1H, ^3J = 7.6 Hz, H-1), 3.62 - 3.52 (m, 7H, 3xH-7, H-6, H-6$^{(*)}$, H-6$^{(x)}$, H-6$^{(+)}$), 3.41 (dd, 1H, ^2J = -11.8 Hz, ^3J = 5.6 Hz, H-6$^{(x)}$), 3.36 (d, 1H, ^3J = 7.5 Hz, H-1), 3.30 - 3.09 (m, 13H, H-7, 4xH-2, 4xH-3, 4xH-4), 3.03 (ddd, 1H, H-5), 2.99 (ddd, 1H, H-5$^{(*)}$), 2.95 (ddd, 1H, H-5$^{(x)}$), 2.88 (ddd, 1H, H-5$^{(+)}$), 2.87 (d, 1H, ^2J = -10.8 Hz, H-7), 2.80 - 0.10 (br, B-H), -2.30, -2.44 (br, B-H-B). $^{13}C\{^1H\}$ NMR (150.90 MHz, CD_3OD) δ 172.99 (C=O, C-10), 146.98, 146.87, 145.86, 145.78 (C-1′), 133.38 (C-3′), 132.48, 132.28 (C-4′), 129.48, 129.21, 129.06, 129.00 (C-2′), 104.11, 104.02, 103.65, 103.25 (C-1), 77.80, 77.54, 77.50, 77.42, 77.39, 77.36 (C-3, C-5), 76.03 (C-7), 75.49, 75.37, 75.23 (C-2, C-7), 75.10, 74.99 (C-7), 71.25 (C8/9), 71.66, 71.59, 71.49 (C-4), 67.63 (C-8/9), 62.70, 62.59, 62.52, 62.44 (C-6), 59.97 (C-8/9), 57.45, 56.94

(C-8/9). $^{11}B\{^1H\}$ NMR (192.55 MHz, CD_3OD): δ -4.60, -14.11, -24.09, -29.27,-36.37. IR (KBr): ν 3433 (O-H), 2542 (B-H), 1607 (C=O). ESI-MS: m/z = 572.1 $[M]^-$. HRMS (ESI): Calculated for $C_{16}H_{27}B_9O_8I$: 572.1638. Observed: 572.1639. HPLC: t_R = 12.34, 12.50, 12.84, 13.36 (Method **B**).

N,N-diethyl(aminoethyl)-4′-(sodium [iodo-7,8-dicarba-*nido*-undecaboranyl])-7-benzamide (4.23):

Compound **4.14** (4.0 mg, 11 μmol) was dissolved in 500μL of a 1:1 mixture of water and acetonitrile. To this solution was added 366μL of a 0.0295M solution of I_2 in ethanol (10.8μmol) gradually over 1 hour. After all the iodine solution had been added, the reaction was allowed to stir a further hour at room temperature before quenching with 216μL of 0.1M $Na_2S_2O_5$, and concentration to dryness by rotary evaporation. The crude material was re-dissolved in 200μL of 1:1 water:acetonitrile, which was loaded onto a pre-conditioned C_{18} SepPak® cartridge. The SPE cartridge was eluted first with 5mL of H_2O, then 5mL CH_3CN, with a total of 10 1mL fractions being collected. TLC indicated a UV and Pd-active spot eluting in fractions 7 and 8 (CH_3CN). The collected fractions were concentrated by rotary evaporation and lyophilized giving a white film. Yield: 1mg (19%). ESI-MS: m/z = 478.1 $[M]^-$. HRMS (ESI): Calculated for $C_{15}H_{29}B_9ON_2I$: 478.2209. Observed: 478.2208.HPLC: t_R = 13.96, 14.30 (Method **B**).

N,N-diethyl(aminoethyl)-4′-(sodium [7-methyl-(β-D-glucopyranosyloxy)-iodo-7,8-dicarba-*nido*-undecaboranyl])-8-benzamide (4.24):

Compound **4.16** (9mg, 15.9μmol) was dissolved in 1200μL of a 5:1 mixture of ethanol and acetonitrile. To this was added dropwise 544μL of a 0.0295M solution of I_2 (16.1μmol) in ethanol. The colour of the iodine dissipated rapidly initially, but persisted after all of the solution had been added. The reaction was stirred at room temperature for two hours, at which time 322μL of a 0.1M solution of $Na_2S_2O_5$ was added to quench the reaction by conversion of the molecular iodine to sodium iodide. The mixture was concentrated to dryness by rotary evaporation, giving a clear, colourless residue which was re-dissolved in 400μL of a 3:1 water:acetonitrile mixture, loaded onto a C_{18} SepPak® cartridge and eluted with (i) 6mL H_2O, (ii) 3mL CH_3CN, collecting 1mL fractions in test tubes. TLC analysis of the fractions showed all carborane-containing material eluted in the first acetonitrile fraction. This material was concentrated to remove the acetonitrile, then lyophilized, giving a light, white solid. Yield: 2mg (18%). $^{11}B\{^1H\}$ NMR (192 MHz, CD_3CN): δ -4.99, -14.22, -23.89, -29.19, -36.32. ESI-MS: m/z = 670.1 [M$^-$]. HRMS: Calculated for $C_{22}H_{41}B_9O_7N_2I$: 670.2849. Observed: 670.2830. HPLC (Method **B**): t_R = 10.5 min, 11.0 min.

Radiolabelling with ^{99m}Tc:

Synthesis as described for compound [^{99m}Tc]-**3.4**. Compound [^{99m}Tc]-**4.1** was isolated using a Biotage SP4 chromatographic purification instrument in reversed-phase mode. Reaction solutions were loaded onto C_{18} silica samplets, and subsequently

mounted onto 12+M C_{18} cartridges. Elution was as follows: 100% A, 3.0 CV; 100% A to 100% B, 12.0 CV; 100% B, 3.0 CV. Solvent A = H_2O+0.1%TFA. Solvent B = CH_3CN+0.1%TFA. 1 CV = 12mL. Fractions containing the major fraction of activity were collected and analyzed; those found to contain the radiochemically pure product were combined and counted for total activity. Isolation of [^{99m}Tc]-**4.2** was accomplished via C_{18} SepPak^{TM} (Waters) using the elution method described for [^{99m}Tc]-**3.4**. [^{99m}Tc]-**4.1**: Yield: 44% (corrected for decay, n= 3). HPLC: t_R = 15.4 min (Method A). [^{99m}Tc]-**4.2**: Yield: 62% (corrected for decay, n = 6). HPLC: t_R = 12.5 min (Method A).

Radiolabelling - [^{125}I]-4.22:

To a 2 mL reaction vial filmed with 20 μg of Iodogen® was added 100 μL of a solution containing **4.12** (1.0 mg/mL in 5% aqueous acetic acid). To this was added Na[^{125}I] (0.92 - 3.9 MBq, 25-105 μCi) in 0.1N NaOH. The vials were stirred for five minutes before addition of 10 μL 0.1M $Na_2S_2O_5$.Isolation was by HPLC (Elution method **C**) or solid phase extraction on a C_{18} SepPak^{TM} cartridge. Conditioning: i)EtOH, 10 mL; ii) CH_3CN, 10 mL; iii) 1:1 CH_3CN:H_2O, 10 mL; iv) H_2O, 10 mL. Elution: i) 7 mL H_2O; ii) 3 mL 1:1 H_2O:CH_3CN; iii) 5 mL CH_3CN. [^{125}I]-**4.22**: 50%RCY; t_R = 13.2, 13.4, 13.7, 14.3 min (Elution method **B**).

Radiolabelling - [^{125}I]-4.23, [^{125}I]-4.24:

To a 2 mL reaction vial filmed with 20 μg of Iodogen® was added 100 μL of a solution containing **4.14** or **4.16** (1.0 mg/mL in 1:1 CH_3CN:5% aqueous acetic acid). To this was

added Na[^{125}I] (0.85 - 17.9 MBq, 23-484 μCi) in 0.1N NaOH. The vials were stirred for five minutes before addition of 10 μL 0.1M $Na_2S_2O_5$. The products were isolated by HPLC (Method **C**) or by solid phase extraction on C_{18} SepPak^{TM} cartridges. Conditioning: i)EtOH, 10 mL; ii) CH_3CN, 10 mL; iii) 1:1 CH_3CN:H_2O, 10 mL; iv) H_2O, 10 mL. Elution: i) 5mL H_2O; ii) 3 mL 3:1 H_2O:CH_3CN, iii) 2 mL 1:1 H_2O:CH_3CN, iv) 5 mL 1:3 H_2O:CH_3CN, v) 5 mL CH_3CN.

[^{125}I]-**4.23**: Yield: 73%. HPLC: t_R = 14.8, 15.2 min (Method **B**).

[^{125}I]-**4.24**: Yield: 92% . HPLC: t_R = 10.5, 11.1 min (Method **B**).

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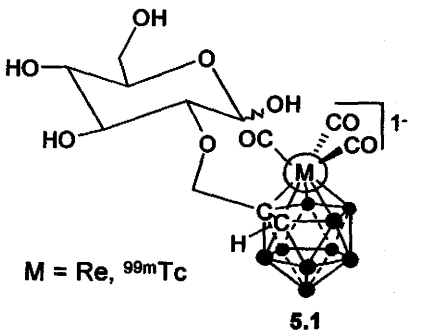
Chapter 5

Towards a C-2 Functionalized Metallocarborane Derivative of Glucose as a Mimic of 2-Fluoro-2-Deoxy-D-Glucose

Building on the synthesis of the carborane and metallocarborane-carbohydrate derivatives described in Chapters 2-4,the next phase was to explore the feasibility of having a site of functionalization other than the anomeric (C-1) carbon. In order to produce a glucose derivative that can interact with hexokinase in a manner analogous to FDG, it is necessary to functionalize at the C-2 position of the carbohydrate ring.[1-4] The proposed derivative **5.1** (Figure 5.1), carrying a metallocarborane at C-2 is particularly attractive, as its technetium complex could be investigated as an analogue of the PET imaging radiopharmaceutical [^{18}F]-2-fluoro-2-deoxy-glucose (FDG), which is widely used clinically to image tumours.[5,6] It would also be of interest to prepare a derivative incorporating ^{186}Re or ^{188}Re, which are attractive radionuclides for preparing targeted radiotherapy agents, a feature that is not available for fluorine-based agents.



5.1

Figure 5.1: Proposed C-2 functionalized metallocarboranyl glucose target.

5.1 Synthesis of a C-2 Functionalized Glucose Metallocarborane

The retrosynthetic approach to **5.1** was based in part on the work of Dumas and co-workers[4] (Figure 5.2). The ultimate target can be prepared from *nido*-carboranyl glucose derivative, **5.9**, by the methods previously established for the synthesis of **2.1**. The *nido*-carborane derivative was prepared from a *closo*-carborane derivative such as **5.8**, which in turn was prepared by rearrangement of the carbohydrate ring of **5.7**. Simple cleavage of the benzyl ether protecting groups generated **5.7** from **5.6**. Decaborane-alkyne insertion yield the *closo*-carborane derivative **5.6** from alkyne **5.5**, which was prepared from alkylation of the tri-benzyl-protected glucofuranose **5.4**. This C-2 free alcohol species was prepared by alcoholysis of the tri-benzylated, 1,2:isopropylidene acetal-protected glucofuranose **5.3**. Compound **5.3** was synthesized by benzylation of the free alcohols of 1,2-O-isopropylidene-α-D-glucofuranose, which is commercially available.

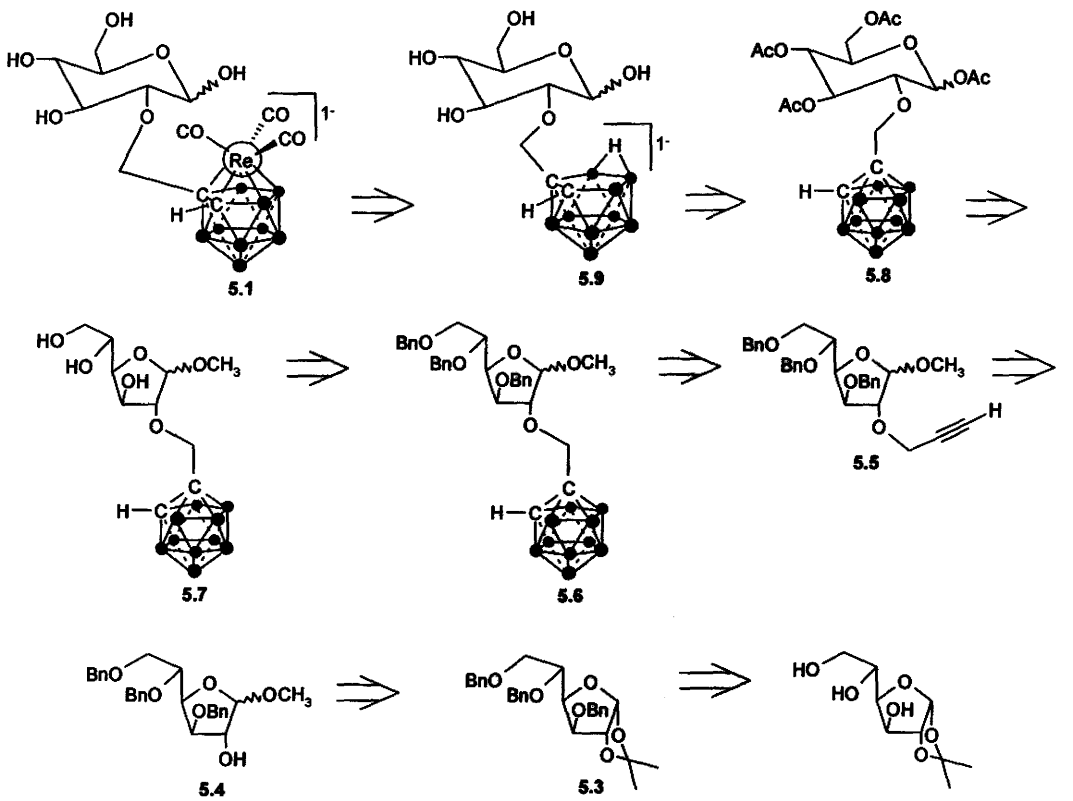
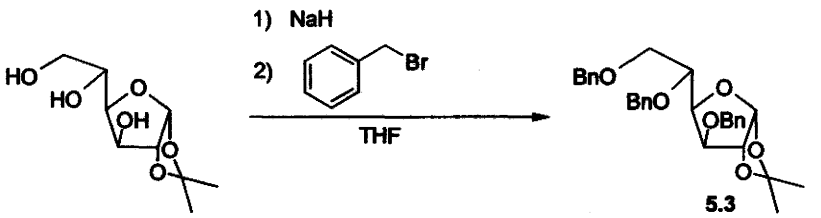


Figure 5.2: Retrosynthetic approach to **5.1**.

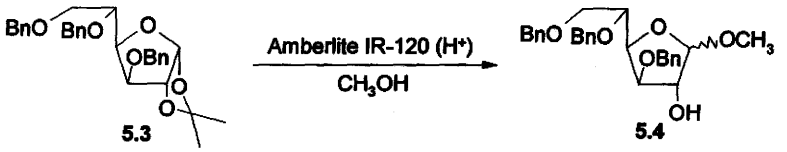
Compound **5.3** was prepared in a manner similar to that described Lee *et al.*[7] The starting material, 1,2-O-isopropylidene-α-D-glucofuranose was reacted with benzyl bromide at room temperature in the presence of sodium hydride. The synthesis was modified from the literature method in that it used tetrahydrofuran as the solvent instead of DMF, as its lower boiling point would simplify the synthesis and purification steps. Excess quantities of sodium or potassium hydride and benzyl bromide were also used to ensure deprotonation and subsequent benzylation of the C-3, C-5, and C-6 alcohols (Scheme 5.1). Excess hydride was destroyed by alcoholic and aqueous workup, and the organic material was extracted into dichloromethane following evaporation of the

original solvent. The target was obtained in 71% yield following purification by silica gel column chromatography. The ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of the product were consistent with reported data.[7]



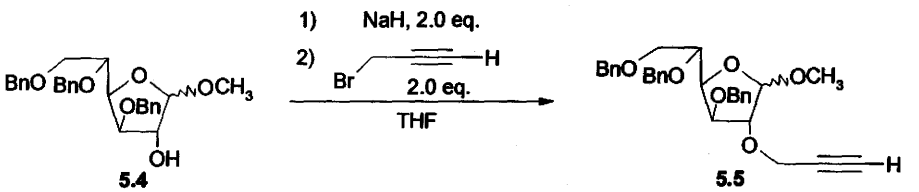
Scheme 5.1: Synthesis of precursor **5.3**.

In order to make the glucose C-2 position available for derivatization, compound **5.3** was converted to **5.4** by acid-catalyzed alcoholysis (Scheme 5.2),[7] which resulted in the introduction of the methoxy group on the anomeric carbon of the glucose ring. The IR spectrum gave a broad absorption at 3495 cm^{-1}; consistent with the presence of a free alcohol. The product was purified by silica gel column chromatography and was obtained in 86% yield. The α and β anomers of **5.4** were formed in approximately equal quantities as evidenced by NMR integration. Although the two anomers were separable by chromatography, such a separation was not deemed necessary, since the final target **5.1** was expected to consist of an equilibrium mixture of the α and β anomers of glucose. As was the case with its precursor, the characterization data for **5.4** were consistent with the literature.[7]



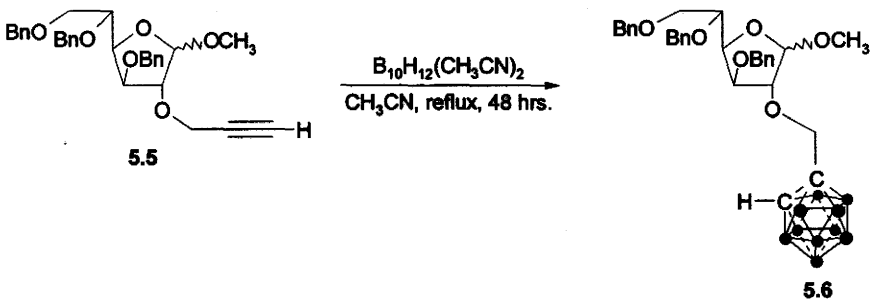
Scheme 5.2: Alcoholysis of **5.3** to **5.4**.

Compound **5.5** was prepared by deprotonation of **5.4** with sodium hydride, and subsequent alkylation with propargyl bromide (Scheme 5.3). Initially, the reaction was performed in dichloromethane. This was the solvent of choice for Dumas *et al.* in their alkylation reactions at this position in which the electrophiles were N-substituted amino alkyl bromide derivatives.[4] Attempts to prepare **5.5** in DCM required long reaction times (five days or more) and resulted in low yields. Changing the solvent to THF resulted in complete overnight consumption of the starting material. Silica gel column chromatography isolated the product as a mixture of anomers in 94% yield. The IR spectrum indicated the presence of an alkyne terminal C-H stretch at 3288 cm^{-1}, and an alkyne C≡C stretch at 2119 cm^{-1}. The positive ion electrospray mass spectrum showed a peak at m/z = 503.4, which corresponded to $[M+H]^+$. The characterization of compound **5.5** was in agreement with that which has been reported recently.[8]



Scheme 5.3: Synthesis of **5.5**.

Compound **5.5** was subjected to a decaborane-alkyne insertion reaction to produce compound **5.6** (Scheme 5.4). The product was obtained in 33% yield, following two rounds of silica gel column chromatographic purification. The IR spectrum of the product contained the expected *closo-ortho*-carborane B-H stretch at 2595 cm^{-1}. The mass spectrum gave the target m/z value of 620, with a B_{10}-isotope distribution. As was the case with **5.5**, the ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of compound **5.6** were assigned with the assistance of the two-dimensional NMR techniques indicated above. The assignments of the signals are summarized in Table 5.1.

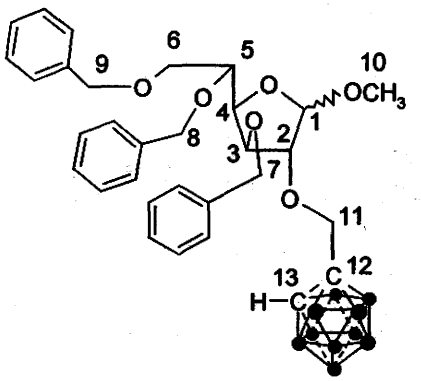


Scheme 5.4: Synthesis of *closo*-carborane **5.6**.

The ^{1}H NMR spectrum of **5.6** (Figure 5.3) was, in general, similar in appearance to that of **5.5**, in that it was complicated by overlapping signals due to the presence of both α and β anomers. There were, however some notable differences. In the ^{1}H NMR, a number of signals of both anomers were shifted to slightly lower frequency (by 0.1 to 0.2 ppm). For example, proton H-1α appeared at 4.89 ppm, and H-1β at 4.70 ppm. Following

insertion of the alkyne into the carborane cage, protons H-11 gave two pairs of doublets, one pair for each glucofuranose anomer. The H-11α doublets appeared at 3.88 and 3.75 ppm, while those for H-11β appeared at 3.58 and 3.53 ppm. These protons are upfield from the signals arising from H-11 in **5.5**, which were between 4.06 and 4.23 ppm. As a result of the shift, the H-11 resonances were no longer overlapped with other resonances (notably, H-2α, 3α, 5α, and 5β), which was not the case for the alkyne precursor. This change allowed for a more complete assignment of the ^{1}H and ^{13}C spectra of **5.6**. Two doublets of doublets of doublets were observed at 4.01 and 3.97 ppm, and were assigned the H-5β and H-5α protons, respectively. The 5β signal gave coupling constants of 9.1 Hz, 2.0 Hz, and 4.6 Hz, corresponding to the $^{3}J_{4\beta,5\beta}$, $^{3}J_{5\beta,6a\beta}$, and $^{3}J_{5\beta,6b\beta}$ interactions, respectively. The 5α signal contained coupling constants of 7.7 Hz, 2.2 Hz, and 5.6 Hz, corresponding to the $^{3}J_{4\alpha,5\alpha}$, $^{3}J_{5\alpha,6a\alpha}$, and $^{3}J_{5\alpha,6b\alpha}$ interactions, respectively. The next signal observed was a broad singlet at 3.93 ppm, indicative of a carborane terminal proton, H-13. The second H-13 signal was buried amongst several other resonances at approximately 3.70 ppm. Due to the close spacing of the C-13 signals in the ^{13}C spectrum and the lack of other multiple bond correlations involving H-13 and C-13, an unambiguous assignment of these two protons to specific anomers was not possible. The pseudo triplet at 3.81 ppm was assigned to proton H-2α. The H-2β signal was part of the overlapping group of signals at 3.68 ppm. Both of these protons had shifted substantially to lower frequency with respect to their counterparts in the ^{1}H NMR spectrum of **5.5**. A "hilly", uneven baseline was observed in the range 2.7 - 1.6 ppm, which was attributed to the carborane B-H protons.

Many of the signals in the $^{13}C\{^{1}H\}$ NMR spectrum of **5.6** (Figure 5.4) remained unchanged compared to that of the precursor. The only significant changes occurred with signals from those carbon atoms involved in the conversion of the alkyne of **5.5** to the *closo*-carborane in compound **5.6**. Carbon atoms 2α and 2β appeared at 84.29 and 87.82 ppm, respectively, while 11α and 11β appeared at 71.30 and 70.48 ppm, respectively. These two pairs of carbon signals shifted to higher frequency from their counterparts in **5.5**. In contrast, the carbons directly involved in the insertion reaction, C-12 and C-13, shifted to lower frequency. C-12α and C-12β appeared at 72.24 and 71.70 ppm, respectively, while the C-13 carbon atoms gave rise to the signals at 57.46 and 57.36. Because of the proximity of these signals to one another, definitive assignment to individual anomers was not possible.



5.6

Proton	Chemical Shift	Carbon	Chemical Shift
Ar-H	7.36-7.24	Ar-*C*-CH_2	138.62 - 137.34
H-1α	4.89	Ar-*C*-H	128.56 - 127.50
H-7, 8, 9	4.81-4.42	C-1β	107.42
H-1β	4.71	C-1α	101.57
H-4α, β	4.25	C-2β	87.82
H-3α	4.08	C-2α	84.29
H-5β	4.01	C-3α	81.65
H-5α	3.97	C-3β	79.74
H-13α / β	3.93	C-4β	79.64
H-11aα, 3β	3.88	C-4α	76.79
H-6aα, 6aβ	3.84	C-5β	76.50
H-2α	3.81	C-5α	76.29
H-11bα	3.75	C-7, 8, 9	73.39 - 72.24
H-2β, 6bα, 6bβ, 13α/β	3.70-3.66	C-12α	72.24
H-11aβ	3.58	C-12β	71.70
H-11bβ	3.53	C-11α	71.30
H-10α	3.36	C-6α	70.66
H-10β	3.35	C-11β	70.48
B-H	2.55 - 1.85	C-6β	69.77
		C-13 α/β	57.46, 57.36
		C-10β,10α	55.98, 55.64

Table 5.1: Assignment of ^{1}H and ^{13}C NMR spectra of **5.6**.

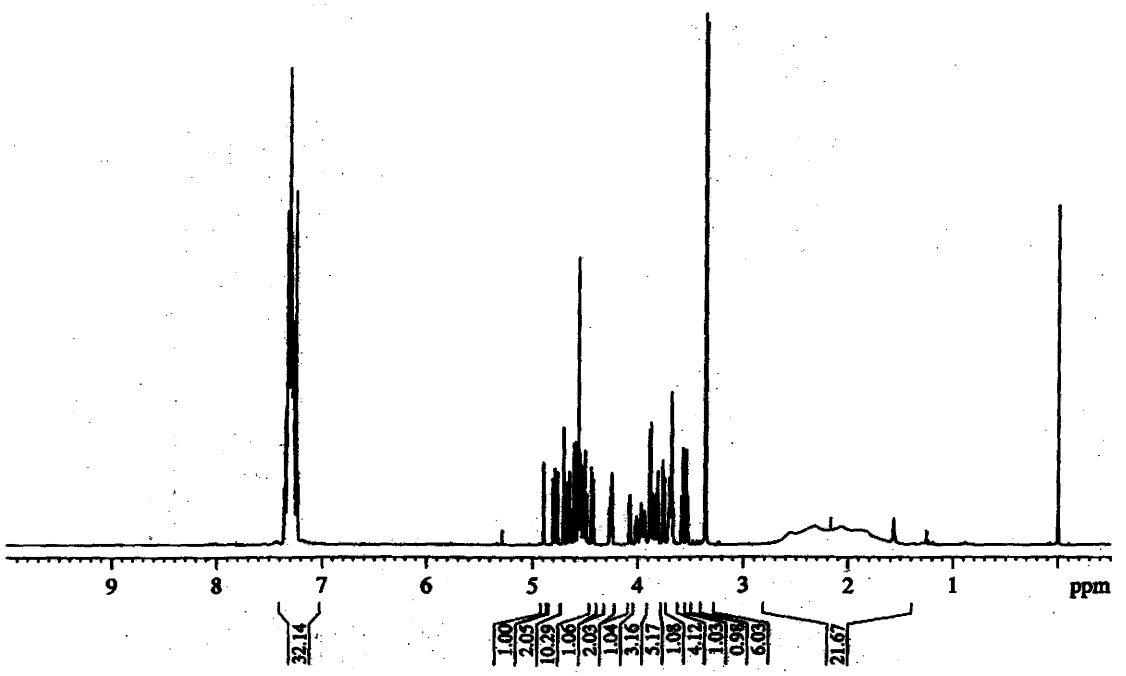


Figure 5.3: ^{1}H NMR spectrum of **5.6** (600 MHz, $CDCl_3$).

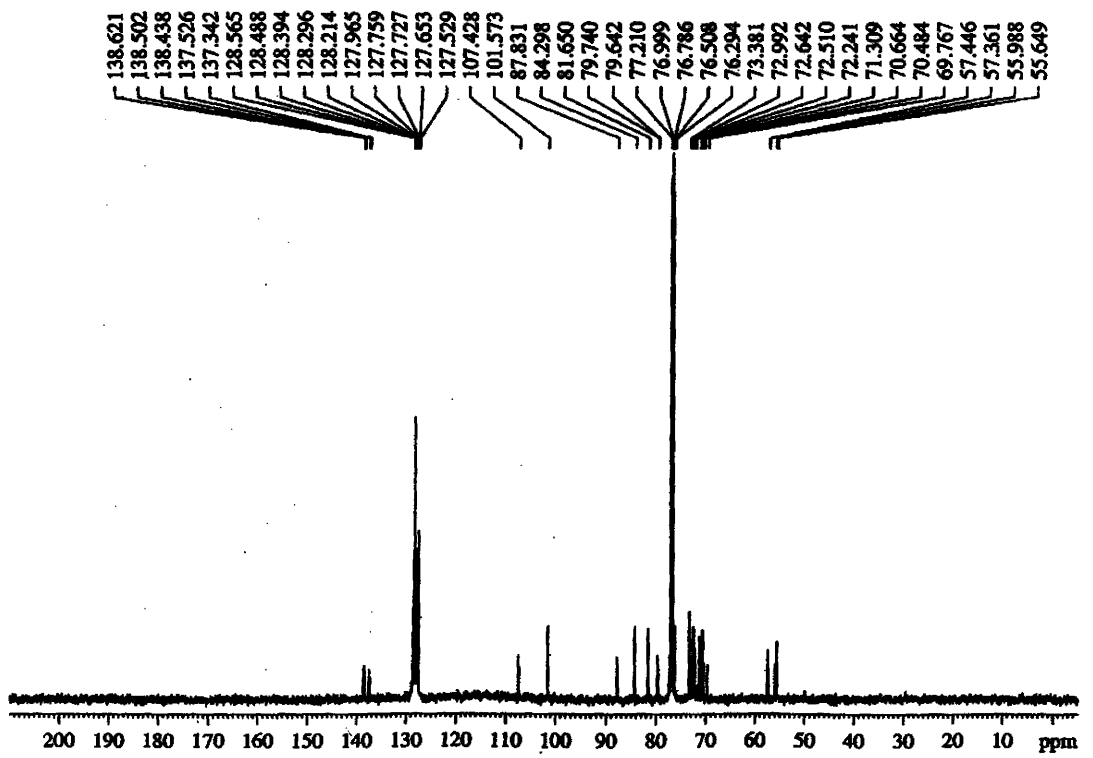


Figure 5.4: The $^{13}C\{^{1}H\}$ NMR spectrum of **5.6** (150 MHz, $CDCl_3$).

The $^{11}B\{^1H\}$ NMR spectrum of **5.6** (Figure 5.5) gave the expected pattern of signals for a singly substituted, *closo-ortho* carborane.[9-11] Signals appeared at -3.50 and -5.24 ppm, (two boron atoms), and at -9.64, -12.32, and -13.63 ppm (total of eight boron atoms).

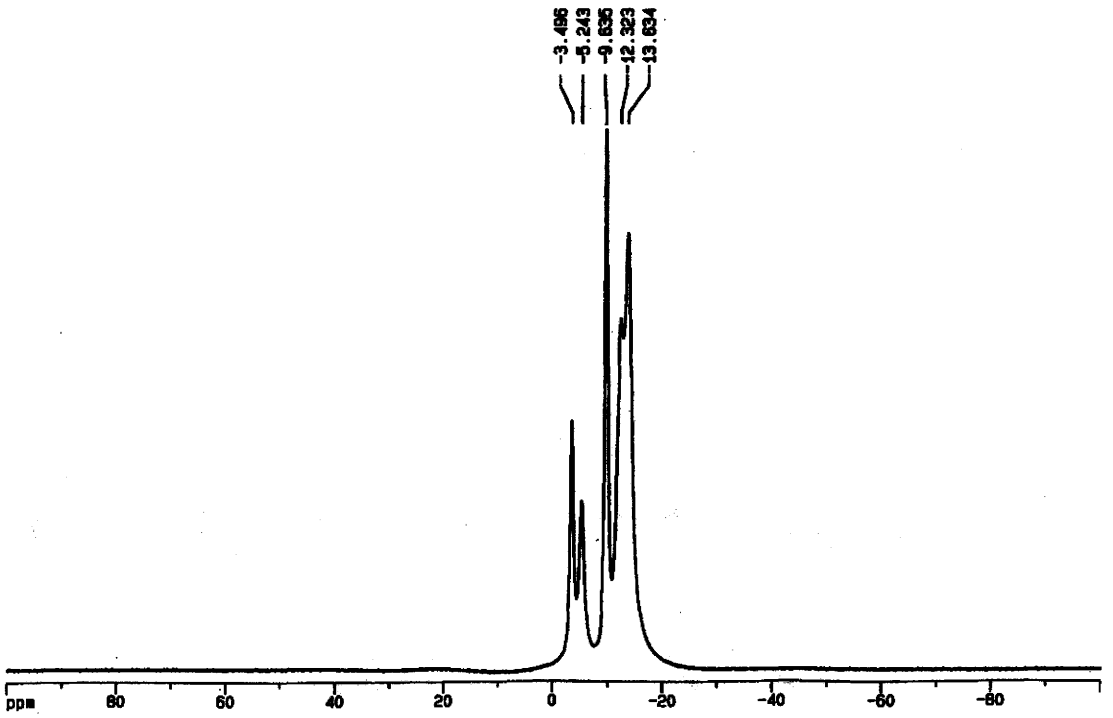
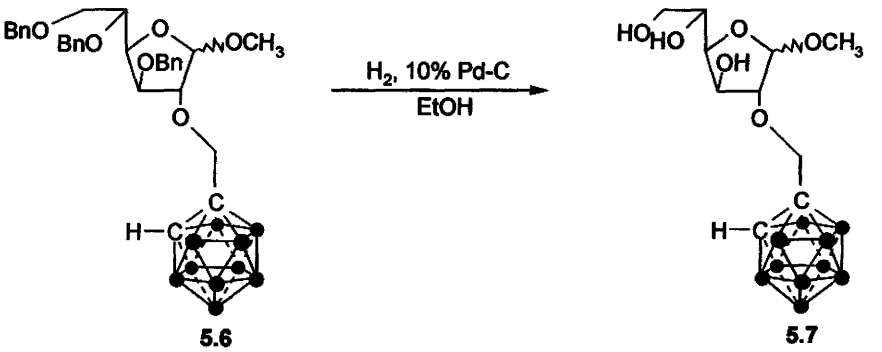


Figure 5.5: $^{11}B\{^1H\}$ NMR spectrum of **5.6** (160 MHz, $CDCl_3$).

The benzyl ether protecting groups of **5.6** were cleaved by catalytic hydrogenolysis to give 1-O-methyl-2-O-methyl-(1,2-dicarba-*closo*-dodecaboranyl)-α,β-D-glucofuranose **5.7** (Scheme 5.5). The product was obtained in 83% yield following silica gel column chromatography. The IR spectrum contained a broad absorption at 3423 cm^{-1}, confirming the presence of free hydroxyl groups in the product. In addition, the B-

H stretch was observed at 2592 cm^{-1}. Electrospray ionization mass spectrometry exhibited the target mass (m/z = 349.5). This mass also contained the expected isotopic distribution for the B_{10}-containing carborane cluster. The $^{11}B\{^1H\}$ NMR spectrum again gave the expected pattern of signals for a monosubstituted *closo-ortho* carborane cage. Resonances appeared at -3.78, (two boron atoms), -9.17 (two boron atoms), -11.32 and -12.88 ppm (six boron atoms).



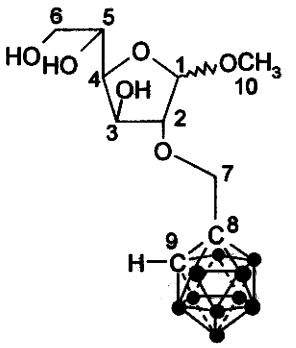
Scheme 5.5: Hydrogenolysis of the benzyl ethers of **5.6** to form **5.7**.

The loss of the benzyl ethers was further confirmed by the absence of any aromatic signals in the ^1H NMR spectrum. In addition, the signals arising from the benzylic methylene group protons were also absent from the proton spectrum of **5.7**. The majority of the spectrum of **5.7**, as would be expected, did not appear to be significantly changed from that of **5.6**. One of the obvious changes was in the broad signal appearing at 4.58 ppm, arising from the carborane terminal proton, H-9, of both anomers. This observation is in agreement with the work reported by Tietze and Bothe, in which the chemical shifts of the carborane terminal protons for a variety of carboranyl glycosides

were shifted to higher frequency upon deprotection, appearing in the range 4.38 to 4.80 ppm.[12]

As was the case with the ^{1}H NMR spectrum, the $^{13}C\{^{1}H\}$ NMR spectrum was characterized by the disappearance of the aromatic and benzylic carbon signals. The signals for C-3, 5, and 6 experienced shifts of approximately 5 ppm to lower frequency, with signals appearing at 75.87, 74.53, 71.92, 71.61, 65.04, and 64.56 ppm. These signals corresponded to C-3α, 3β, 5α, 5β, 6β, and 6α, respectively. The resonances for the remainder of the carbon atoms of **5.7** were shifted to slightly higher (between 1 and 3 ppm) frequency versus the tri-benzyl precursor.

The next reaction in the synthesis of **5.1** was to be the simultaneous deprotection of the C-1 hydroxyl group and rearrangement of **5.7**, in which the glucose ring was to convert from the furanose (five-membered ring) form to the pyranose (six-membered ring) form, giving **5.8**. Dumas and co-workers reported a similar reaction in which treatment of a glucofuranose derivative with acetic anhydride in the presence of trimethylsilyl trifluoromethanesulfonate (TMS-OTf) resulted in a simultaneous rearrangement of the carbohydrate ring and conversion of the free alcohols and the anomeric methoxy substituent into acetate esters. In the present study, such a reaction would result in compound **5.8** (Figure 5.2).[4,13,14]

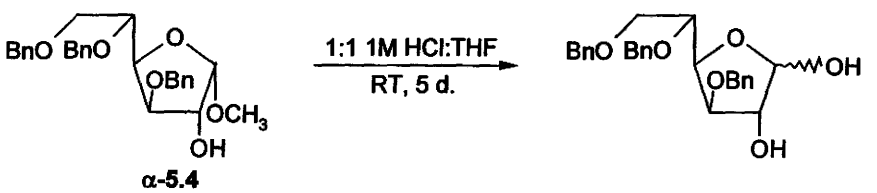


5.7

Proton	Chemical Shift	Carbon	Chemical Shift
H-1α	4.97	C-1β	108.45
H-1β	4.82	C-1α	103.04
H-9α/β	4.58	C-2β	90.65
H-3α	4.31	C-2α	87.56
H-3β	4.21	C-4β	82.43
H-7aα	4.10	C-4α	78.82
H-7aβ, 7bβ	4.07	C-3α	75.87
H-7bα	4.04	C-12α	74.72
H-4α/β	4.01	C-12β, C-3β	74.53
H-5β	3.91	C-7α	72.63
H-2α	3.86	C-7β	72.02
H-2β, 5α	3.86	C-5α	71.92
H-6aβ	3.80	C-5β	71.61
H-6aα	3.74	C-6β	65.04
H-6bβ	3.64	C-6α	64.56
H-6bα	3.59	C-9α/β	61.04, 60.30
H-10α	3.39	C-10α/β	55.87, 55.81
H-10β	3.35	C-3β	74.53
B-H	2.60 - 1.50	C-3β	74.53

Table 5.2: Assignment of ^{1}H and ^{13}C NMR spectra of **5.7**.

Attempts to prepare **5.8** by the reaction described above resulted in consumption of the starting material within 45 minutes. Unfortunately, the reaction resulted in an inseparable, intractable mixture. As a result, an alternative means of cleaving the C-1 methoxy acetal were investigated. These included acidic hydrolysis using aqueous HCl, an acidic ion exchange resin, and *p*-toluenesulfonic acid (TsOH). Using compound α-**5.4** as a test substrate, and THF as a co-solvent (neither **5.4** nor **5.7** are water soluble), it was found that neither of the latter two methods resulted in any consumption of the starting material. The method in which the substrate was dissolved in a 1:1 (v : v) mixture of THF and 1M HCl (Scheme 5.6), did show a disappearance of the starting material. It was found that if the solution was heated to reflux, the reaction resulted in an intractable product. If the reaction was stirred at room temperature, a new product appeared, according to TLC. This reaction solution was neutralized with 1M NaOH, and an aliquot submitted for ESMS. A mass m/z = 473.4 was observed in the positive ion spectrum, corresponding to the sodium ion adduct of the target compound (MW[target] = 450.53).



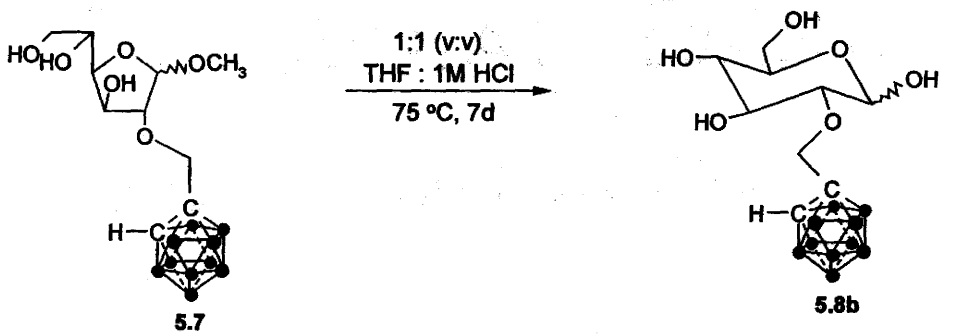
α-**5.4**

Scheme 5.6: Test reaction for hydrolysis of α-**5.4**.

The product of the hydrolysis at C-1 of compound **5.4** is expected to be a glucofuranose ring, since carbon C-5 (whose oxygen atom bonds to C-1 in the pyranose

form of glucose) is protected as a benzyl ether in the present form.[7] However, if the same reaction were to be conducted upon **5.7**, where C-5 bears a free alcohol, it is likely that at least some of the pyranose form would be made in the mixed aqueous / THF solution used in the transformation. This prediction is based on the tendency of glucose to adopt the pyranose form in aqueous solution.[15,16] This hydrolysis reaction, applied to compound **5.7** would potentially yield compound **5.8b**, with four free hydroxyl groups, rather than through the tetra-acetate **5.8**, which was proposed as an intermediate in Figure 5.2.

Hydrolysis of the methoxy substituent of **5.7** (Scheme 5.7) was attempted in a manner similar to that described above for α-**5.4**. It was found that heating to approximately 75 °C resulted in faster conversion. Over a period of seven days, the TLC spot corresponding to **5.7** was replaced by one with a lower R_f, as would be expected for a compound bearing an extra hydroxyl group. The reaction solution was neutralized by cautious addition of aqueous $NaHCO_3$, and the product was purified by silica gel column chromatography, giving the desired compound in 73% yield. ESMS gave the mass m/z = 371.2, corresponding to $[M+Cl]^-$. The IR spectrum of the product gave a broad O-H stretch at 3386 cm^{-1} and the *closo*-carborane B-H stretch at 2589 cm^{-1}. The $^{11}B\{^1H\}$ spectrum showed no change in the pattern of signals versus compound **5.7**, indicating that the *closo*-carborane cage had remained intact during the hydrolysis and subsequent basic work-up. Analysis of **5.8b** by ^1H and $^{13}C\{^1H\}$ NMR spectroscopy completed the characterization, the one-dimensional spectra being assigned with the assistance of the ^1H-^1H COSY, ^1H-^{13}C HSQC and HMBC techniques. Proton and carbon chemical shift assignments are given in Table 5.3.



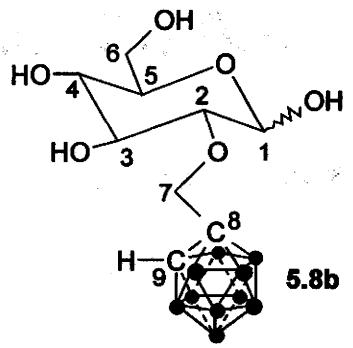
Scheme 5.7: Hydrolysis of **5.7** to **5.8b**.

Since **5.8b** is a key target, the ^{1}H (Figure 5.6) and $^{13}C\{^{1}H\}$ (Figure 5.7) NMR spectra are presented in detail. The proton and carbon spectra indicated the presence of both the α and β anomers of the glucose derivative, with signal integrations indicating a 36:64 (α : β) ratio, very close to the reported equilibrium distribution for D-glucose in aqueous solution (38:62 α:β).[15,16] All NMR spectra of **5.8b** were acquired in deuterated acetone, which permitted the observation of most hydroxyl protons. Due to this extra coupling between ring protons 1, 3 and 4 and their associated hydroxyl group protons, the signals corresponding to these ring atoms were further complicated. As a result, it was difficult to identify by inspection the characteristic anomeric doublets which serve as a good starting point for assigning the structure (with the assistance of the ^{1}H-^{1}H COSY spectrum). Signals in the 1-D spectrum which could be easily identified were those arising from the 2-O-methyl-(1, 2-dicarba-*closo*-carboranyl) pendant group. These signals included the characteristic broad singlets at 5.01 and 4.86 ppm, arising from the carborane terminal protons H-9β and H-9α, respectively. Of particular importance in

assigning the proton spectrum were the four doublets arising from the H-7 protons. These signals were easily identified as they contain only the coupling to their respective geminal partners. These signals appeared at 4.39 and 4.26 ppm (H-7aβ and H-7bβ) and at 4.31 and 4.23 ppm (H-7aα and H-7bα). The ^{1}H-^{13}C HMBC spectrum was used to identify carbon atoms C-2α and C-2β as a result of the three-bond correlation between the H-7 protons and C-2. Subsequently, the ^{1}H-^{13}C HSQC spectrum identified protons H-2α and H-2β as doublets of doublets at 3.30 (partially overlapped with another signal) and 3.00 ppm, respectively. The coupling constants within these two signals identified compound **5.8b** as a mixture of the α and β pyranose forms of the C-2 functionalized closo-carboranyl glucose derivative. The first of these two signals, which was later attributed to H-2α, contained coupling constants of 3.5 Hz and 9.6 Hz, corresponding to the $^{3}J_{1\alpha,2\alpha}$ and $^{3}J_{2\alpha,3\alpha}$ interactions, respectively. These values illustrate the cis-diaxial relationship between protons H-1α and H-2α and also the trans-diaxial relationship between H-2α and H-3α in a system based on α-D-glucopyranose.[17-19] In contrast, the coupling constants in α-**5.6**, a compound based on a five-membered glucofuranose ring, the corresponding coupling constants are $^{3}J_{1,2}$ = 4.0 Hz and $^{3}J_{2,3}$ = 3.3 Hz. The second signal contained coupling constants of 7.8 Hz and 9.2 Hz, which could only have arisen from two trans-diaxial interactions. These coupling constants were assigned to the $^{3}J_{1\beta,2\beta}$ and $^{3}J_{2\beta,3\beta}$ interactions, respectively. Consequently, this doublet of doublets was assigned to H-2β. With the H-2 proton signals established, COSY correlations to the anomeric signals could then be used to identify these protons, and correlations to the H-3 signals

would allow for the assignment of the remainder of the spectrum. Proton H-1α gave a pseudo-triplet at 5.27 ppm, while H-1β gave an almost fully resolved doublet of doublets at 4.59 ppm. Proton H-3α appeared as a doublet of doublets of doublets at 3.83 ppm. This signal contained the coupling constant $^{3}J_{3\alpha,4\alpha}$ = 9.2 Hz, which was taken as further evidence for a glucopyranose ring. Proton H-6aβ appeared as a complex multiplet signal at 3.78 ppm, which was ovelapping with the H-5α signal at 3.73 ppm. The protons H-6aα and H-6bβ ovelapped together at 3.64 ppm, while proton H-6bα appeared at 3.49, partially ovelapping a signal from the β-anomer. Completing the H-6a-6b-5 ABX systems was the signal for H-5β, which appeared at 3.25 ppm. The coupling constants within the systems for the α and β anomers were comparable to those found in the precursors to compound **2.1**, based on the D-glucopyranose ring. The signal for H-3β appeared as a multiplet (ddd), which was overlapping with the H-6bα signal. Protons H-4α and H-4β appeared at 3.34 and 3.31 ppm, respectively, as parts of an overlappping multiplet signal, which also contained part of H-2α. Yet another piece of evidence for the glucopyranose structure was found in the correlation of these H-4 signals to hydroxyl protons, since the C-4 carbon bears a free hydroxyl group in the pyranose form. In contrast, the oxygen attached to C-4 bonds to the anomeric carbon in the furanose structure, whereas C-5 bears a free alcohol. Proton H-5β appeared as a collapsed doublet of doublets of doublets at 3.25 ppm. Completing the proton spectrum of **5.8b** was the broad B-H signal, which was observed in the region between 1.60 and 2.70 ppm.

The $^{13}C\{^{1}H\}$ NMR spectrum contained eighteen signals, as was expected for a mixture of two isomers, each containing nine carbon atoms. The intensities of the α-anomer signals were approximately half those of their β-anomer counterparts. This disparity in signal height allowed for a complete assignment of the ^{13}C spectrum from the HSQC spectrum, after, as was mentioned above, the C-2 signals were identified with the HMBC data. The β-anomer signals appeared at 97.30, 85.87, 77.24, 77.05, 75.45, 73.72, 71.81, 62.68, and 59.72 ppm, corresponding to C-1β, 2β, 5β, 3β, 8β, 7β, 4β, 6β, and 9β, respectively. The α-anomer signals appeared at 91.36, 82.54, 75.24, 73.57, 72.40, 72.11, 71.90, 62.76, and 59.96 ppm, corresponding to C-1α, 2α, 8α, 3α, 5α, 4α, 7α, 6α, and 9α, respectively.



Proton	Chemical Shift	Carbon	Chemical Shift
O-$H_1\beta$	6.10	C-1β	97.30
O-$H_1\alpha$	5.52	C-1α	91.36
H-1α	5.27	C-2β	85.87
H-9β	5.01	C-2α	82.54
H-9α	4.86	C-5β	77.24
O-$H_3\beta$	4.64	C-3β	77.05
H-1β	4.59	C-8β	75.45
O-$H_3\alpha$	4.47	C-8α	75.24
H-7aβ	4.39	C-7β	73.72
O-$H_4\beta$	4.36	C-3α	73.57
H-7aα	4.31	C-5α	72.40
O-H4α	4.27	C-4α	72.11
H-7bβ	4.26	C-7α	71.90
H-7bα	4.23	C-4β	71.81
H-3α	3.83	C-6α	62.76
H-6aβ	3.78	C-6β	62.68
H-5α	3.73	C-9α	59.96
H-6aα, H-6bβ	3.64	C-9β	59.72
H-6bα	3.49		
H-3β	3.47		
H-4α	3.34		
H-4β	3.31		
H-2α	3.30		
H-5β	3.25		
H-2β	3.00		
B-H	2.7 - 1.6		

Table 5.3: Assignment of ^{1}H and ^{13}C NMR spectra of **5.8b**.

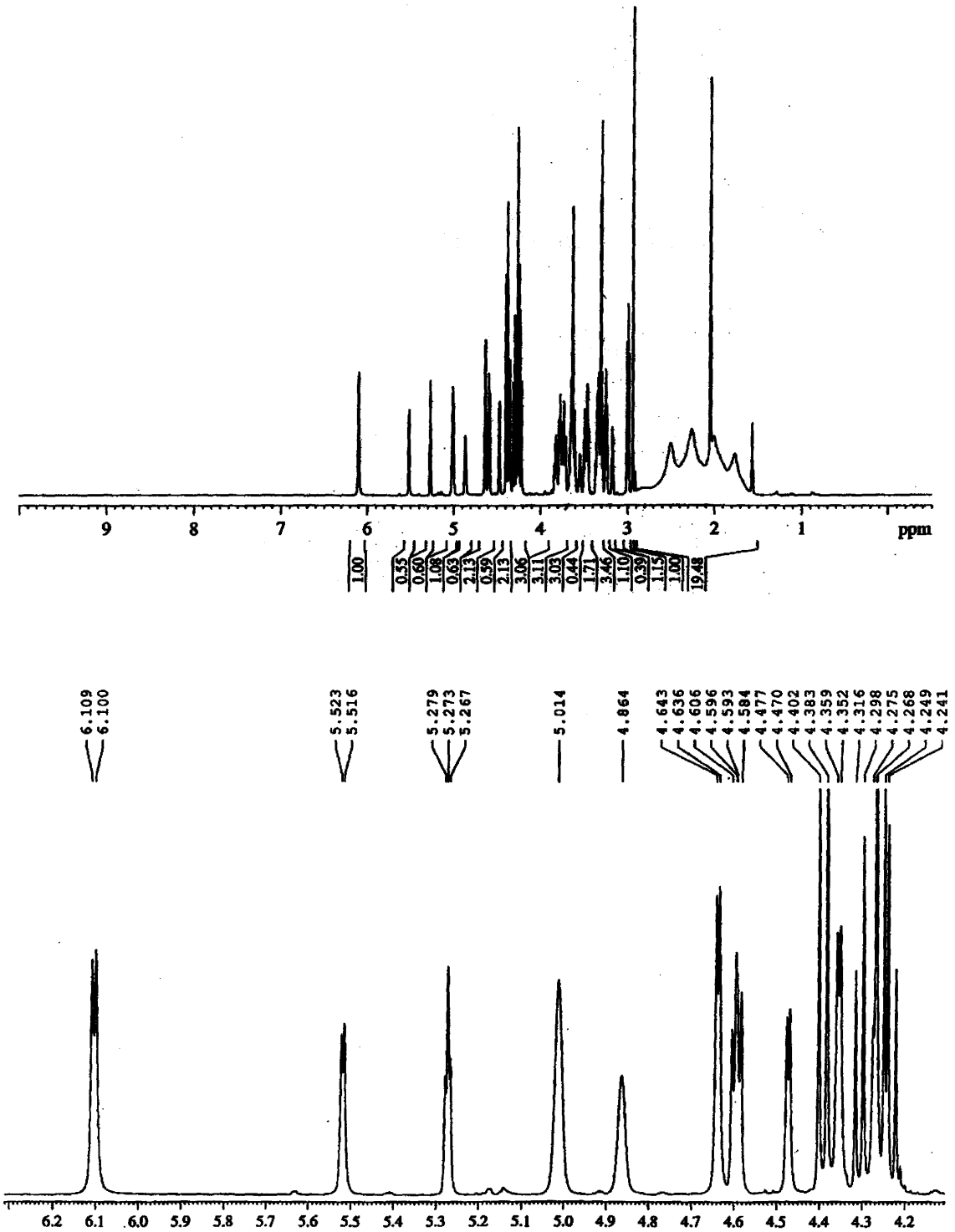


Figure 5.6: ^{1}H NMR spectrum and expansion between 4.1 and 6.2 ppm of **5.8b** (600 MHz, Acetone-d_6).

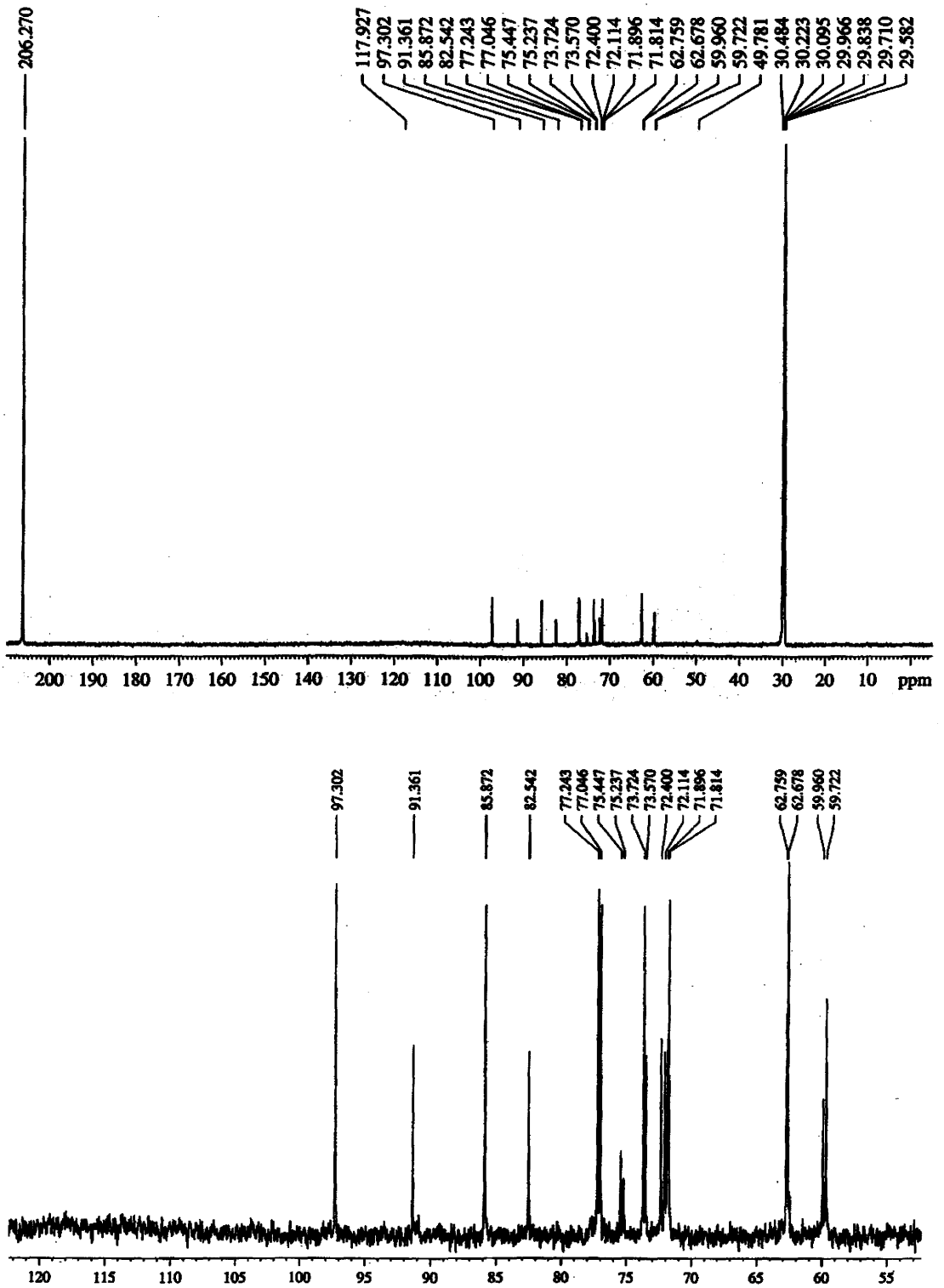


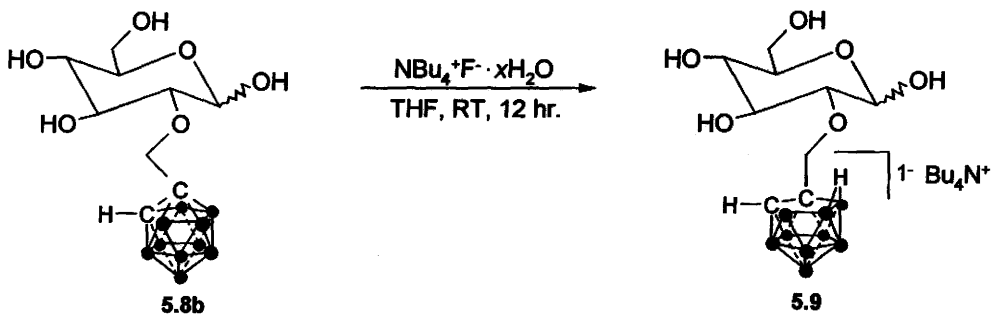
Figure 5.7: The $^{13}C\{^{1}H\}$ NMR spectrum and expansion between 52 and 123 ppm of **5.8b** (150 MHz, Acetone-d_6).

Degradation of the *closo*-carborane cage of **5.8b** to give *nido*-carborane derivative **5.9** was first attempted using the original method of Hawthorne *et al.*[20] Compound **5.8b** was dissolved in ethanol containing five equivalents of KOH, and heated to reflux. A change from a clear, colourless solution to a light brown one was observed within an hour. The reaction was analyzed by TLC, which gave a complex series of palladium-active spots, the most prominent of which was significantly faster running than that of the starting material. This result was in contrast to the expectation that a slower running spot would result from the formation of an ionic compound. Acidification of the reaction mixture with 1M HCl, as described in Chapter 2 for the synthesis of the *nido*-carboranyl glycosides **2.6** and **2.9**, did not affect the TLC analysis of the reaction mixture. In addition, electrospray mass spectra of the products obtained did not indicate the presence of the target anion. It is believed that the harsh reaction conditions may have resulted in the degradation of the starting material, rather than formation of the desired compound. In light of this, an alternative strategy was explored.

Conversion of **5.8b** to **5.9** was attempted using tetrabutylammonium fluoride hydrate,[21] as was done for the preparation of **2.6b**. The starting material and five equivalents of TBAF·xH$_2$O were dissolved in wet THF, and stirred in air at room temperature (Scheme 5.8). Analysis by TLC indicated complete consumption of the starting material after approximately twelve hours. The TLC chromatogram consisted of a streak from the baseline to approximately half the length of the plate, and appeared to consist of more than one distinct spot. This may be the result of the presence of a total of four diastereomers as the reaction product; that is, two carbohydrate anomers, α and β,

can undergo removal of B3 or B6 from the carborane cage of each isomer of **5.8b**, resulting in a total of four compounds. The desired product was isolated in 65 % yield following silica gel column chromatography.

The IR spectrum of the product gave the O-H stretch at 3384 cm^{-1}, and the *nido*-carborane B-H stretch at 2525 cm^{-1}. The product was analyzed by ESMS and gave the mass of the target anion, m/z = 326.6. The $^{11}B\{^1H\}$ NMR spectrum of compound **5.9** (Figure 5.11) resembled those of the asymmetrically substituted *nido*-carborane derivatives **2.6** and **2.9**. Signals were observed at -9.87, -10.41, -14.63, -16.69, -18.43, -22.00, -32.58, and -36.97 ppm. The signal at -16.69 ppm appeared to consist of two overlapping peaks, thus all nine of the expected boron resonances were accounted for. Three extra peaks, each with very low intensity relative to the main set of signals, were observed at -12.65, -20.30, and -35.80 ppm. The presence of these peaks was likely due to the fact that the compound can contain a mixture of up to four diastereomers.



Scheme 5.8: Conversion of **5.8b** to **5.9**.

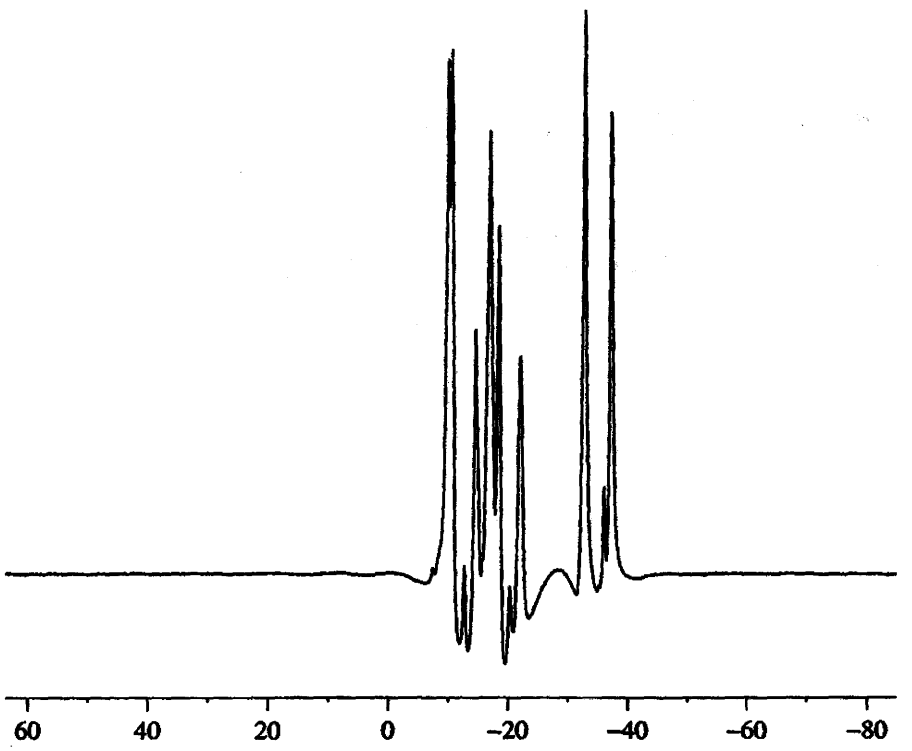


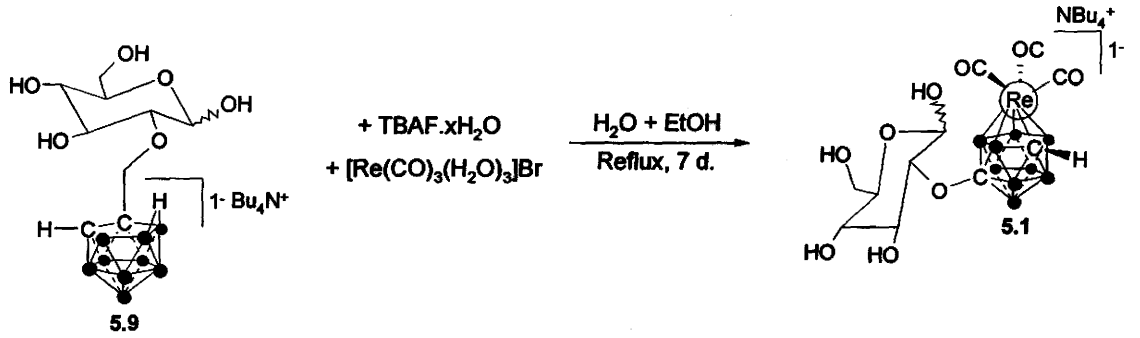
Figure 5.8: $^{11}B\{^1H\}$ NMR spectrum of **5.9** (192 MHz, CD_3CN).

The ^1H NMR spectrum of compound **5.9**, apart from the large degree of signal overlap, appeared to be little changed from the *closo*-carborane precursor, **5.8b**, in terms of the signals arising from the carbohydrate portion of the molecule. Two pairs of overlapping doublets appeared at 5.20 and 4.44 ppm, arising from the anomeric protons of the pairs of α and β anomer diastereomers, respectively. The carborane terminal protons, H-9 gave a broad signal at 1.94 ppm, which, coincidentally, overlapped with the residual proton solvent signal of CD_3CN. The "hilly" baseline signals arising from the carborane B-H protons had shifted to the range 2.3 to -0.3 ppm. Finally, the "bridging"

hydrogen of the *nido*-carborane gave a broad signal at -2.60 ppm. The most important feature of the $^{13}C\{^1H\}$ NMR spectrum of **5.9** was the appearance of the signal arising from C-9, the carborane terminal carbon atom (46.71 ppm). As was the case with the *nido*-carboranyl glycosides **2.6**, **2.9**, and **2.12**, this signal appeared broadened and with reduced intensity. In contrast to the two *nido*-carboranyl glycosides mentioned above, the signal from C-8, the substituted carborane carbon atom, was not observed in the $^{13}C\{^1H\}$ NMR spectrum of **5.9**, likely a result of overlap with other signals.

The final C-2 glucose target, **5.1** was approached through a procedure (Scheme 5.9) that was modified slightly from the one used to prepare compounds **2.1** and **2.2**. Instead of using $(NEt_4)_2[Re(CO)_3Br_3]$ as the active rhenium species, an alternative reagent was used. The compound $[Re(CO)_3(H_2O)_3]Br$ was prepared by heating $[Re(CO)_5Br]$ in water, at reflux for several days.[22] Compound **5.9**, TBAF·xH_2O, and $[Re(CO)_3(H_2O)_3]Br$ were dissolved in water and heated to reflux over a period of seven days. The electrospray mass spectrum of the crude reaction mixture was dominated by the ligand anion mass, but clearly showed the target mass m/z = 595. Rhenium clusters with m/z = 879 and m/z = 1149 were also observed. The product was obtained in less than 10% yield following purification by semipreparative HPLC. The low yield was attributed to the loss of material during HPLC purification. Analysis of the crude HPLC spectra suggested that the reaction proceeds in approximately 50% yield. The synthesis of **5.1** via microwave irradiation was attempted, in light its use in the synthesis of metallocarboranes **3.4**, **3.5**, **4.1** and **4.2**. Unfortunately, this resulted in complete degradation of the product.

IR spectroscopy gave the expected features for the $Re(CO)_3$-metallocarboranyl target. A broad, O-H stretch was observed at 3348 cm^{-1}, the metallocarborane B-H stretch appeared at 2544 cm^{-1}, and the C≡O stretches at 1997 and 1893 cm^{-1} were indicative of the C_{3v} local symmetry of the carbonyl ligands. The $^{11}B\{^1H\}$ NMR spectrum of **5.1** (Figure 5.9) was virtually identical in appearance to those of metallocarborane derivatives **2.1** and **3.4**. Signals were observed at -5.85, -7.93, -9.14, -11.67, -18.41, -19.62 and -20.24 ppm. Due to this similarity, and given the results of the analyses of compounds **2.1** and **3.4**, it is speculated that compound **5.1** consisted of the 2,1,8 metallocarborane isomer.



Scheme 5.9: Synthesis and proposed structure of **5.1**.

The ^1H NMR spectrum of compound **5.1** changed little from that of the ligand **5.9**. However, signals arising from the diastereomers, which formed upon degradation of the carborane cage, were overlapped to a greater extent in the spectrum of compound **5.1**. The signal arising from the carborane terminal proton, H-9, shifted to 1.80 ppm. This value was comparable to that observed in the ^1H NMR spectra of compounds **2.1** and **2.2**.

The baseline signal arising from the carborane B-H protons shifted to the range 3.00 to 0.80 ppm, which was also observed for the above-mentioned metallocarboranyl glycosides. Finally, no broad signal was observed around -2.60 ppm, indicating that the "bridging" hydrogen atom had been removed during formation of compound **5.1**.

The $^{13}C\{^1H\}$ NMR spectrum of compound **5.1** featured the characteristic resonance at 200.51 ppm, arising from the carbonyl carbons of the $Re(CO)_3^+$ core. Apart from this signal and the two signals arising from the C-1 carbons of the α and β anomers, few signals in the $^{13}C\{^1H\}$ NMR spectrum could be assigned conclusively, due to a lack of observable proton - carbon correlations in the HSQC and HMBC spectra.

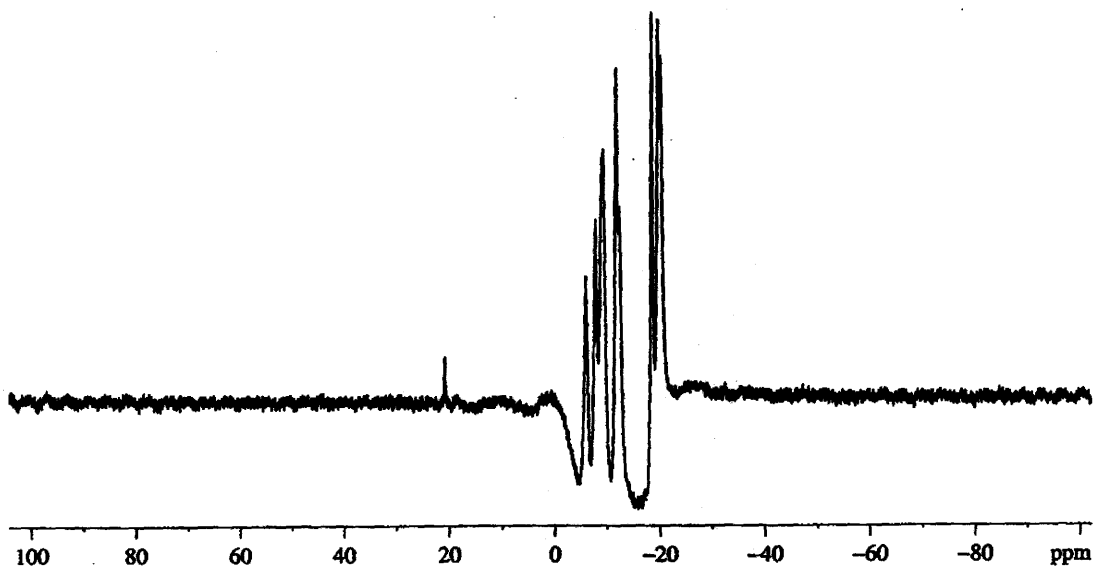


Figure 5.9: $^{11}B\{^1H\}$ NMR spectrum of **5.1** (192 MHz, CD_3CN).

5.2 Summary and Conclusions

A series of carborane conjugates and one metallocarborane conjugate of glucose were prepared in which the site of functionalization was the C-2 carbon atom of the sugar. Compound **5.1**, the Re(I) metallocarborane, was prepared, albeit in low yield, using the methods developed for the synthesis of metallocarboranyl glycosides **2.1** and **2.2**. Owing to the low yields obtained via conventional heating, the synthesis of **5.1** was attempted using microwave-assisted heating, as was the case for the successful preparations of metallocarboranyl glycosides **3.4** and **4.1**. However, complete degradation of the carbohydrate was observed under these conditions, suggesting that the high temperature and pressure attained under microwave irradiation was too harsh for this more sensitive compound, and that labeling of this derivative with Re and ^{99m}Tc is not practical. Alternatively, the iodination/radioiodination strategy used for the synthesis of **2.13** could possibly be applied to compound **5.9**, since the reaction conditions are milder than those required for metallation with rhenium and technetium. If successful, this might provide another means to a novel analogue of FDG based on isotopes of radioiodine.

5.3 Experimental Section

3,5,6-tri-O-benzyl-1,2-O-isopropylidene-α-D-glucofuranose (5.3):

Sodium hydride (1.4 g, 60 wt. % in oil, 34 mmol) was suspended in dry THF (20 mL) in a 3-neck round bottom flask under argon, and cooled in an ice bath. 1,2-O-isopropylidene-α-D-glucofuranose (1.1 g, 5.0 mmol), dissolved in dry THF (90 mL), was

added slowly, resulting in a cloudy, light yellow mixture. Benzyl bromide (4.0 mL, 30 mmol) was subsequently added dropwise, and the solution was allowed to warm to room temperature, and was stirred over 24 hours, at which time TLC indicated consumption of the starting material. The excess NaH was destroyed by slow addition of isopropanol, followed by methanol and water. This resulted in a clear yellow solution containing a suspended white solid. The solid was removed by filtration, and the solution concentrated by rotary evaporation, producing a yellow syrup. The crude product was purified by silica gel column chromatography (1:7 ethyl acetate : L.B. petroleum ether) to give a light yellow oil. (Yield: 2.3 g, 94%). ^{1}H NMR (CD_2Cl_2): δ 7.30 - 7.26 (m, 15 H, ϕ-H), 5.90 (d, 1H, $^{3}J_{1,2}$ = 3.8 Hz, H-1), 4.82, 4.52 (2d, 2H, $^{2}J_{8a,8b}$ = -11.4 Hz, H-8), 4.67, 4.52 (2d, 2H, $^{2}J_{7a,7b}$ = -11.4 Hz, H-7), 4.65 (d, 1H, $^{3}J_{1,2}$ = 3.8 Hz, H-2) 4.60 (s, 2H, H-9), 4.30 (dd, 1H, $^{3}J_{3,4}$ = 3.0 Hz, $^{3}J_{4,5}$ = 9.2 Hz, H-4), 4.12 (d, $^{3}J_{3,4}$ = 3.0 Hz, H-3), 4.03 (ddd, 1H, $^{3}J_{4,5}$ = 9.2 Hz, $^{3}J_{5,6a}$ = 1.9 Hz, $^{3}J_{5,6b}$ = 5.5 Hz, H-5), 3.93 (dd, 1H, $^{2}J_{6a,6b}$ = -10.7 Hz, $^{3}J_{5,6a}$ = 1.9 Hz, H-6a), 3.70 (dd, 1H, $^{2}J_{6a,6b}$ = -10.7 Hz, $^{3}J_{5,6b}$ = 5.5 Hz, H-6b), 1.49 (s, 3H, Acetal CH_3), 1.33 (s, 3H, Acetal CH_3). $^{13}C\{^{1}H\}$ NMR (CD_2Cl_2): δ 139.54,139.34, 138.37 (*ipso* ϕ-C), 128.92 - 127.90 (ϕ-C-H), 112.18 (C-10), 82.41, 82.35 (C-2,3), 79.45 (C-4), 76.25 (C-5), 73.87 (C-9), 72.92 (C-8), 72.48 (C-7), 71.48 (C-6), 27.10, 26.62 (2 x Acetal CH_3). IR (Neat, between KBr disks): ν 3090, 3065, 3032 (aromatic C-H), 1605, 1497 (aromatic C=C) cm^{-1}. ESI-MS: m/z = 491.6 $[M+H]^+$, 513.6 $[M+Na]^+$, 529.5 $[M+K]^+$. TLC (1:7 ethyl acetate : petroleum ether): R_f = 0.71.

Methyl-3,5,6-tri-O-benzyl-α, β-D-glucofuranose (5.4):

Compound **5.3** (1.77 g, 3.60 mmol) was dissolved in methanol (100 mL) in a round bottom flask equipped with stir bar and a condenser. To this solution was added Amberlite IR-120 (H^+) resin (30 mL), and the reaction mixture heated to reflux under nitrogen for 24 hours. The Amberlite resin was removed by filtration, and the filtrate concentrated to a light amber oil by rotary evaporation. The crude product was dissolved in 1:1 ethyl acetate : petroleum ether and the product isolated as a colourless oil by silica gel column chromatography (2:3 ethyl acetate : petroleum ether) (Yield 1.43 g, 86 %). ^{1}H NMR ($CDCl_3$): δ 7.36 - 7.24 (30H, *ϕ*-H), 5.02 (d, 1H, $^3J_{1\alpha,2\alpha}$ = 4.5 Hz, H-1α), 4.79 - 4.49 (m, 12H, H-7, 8, 9), 4.78 (s, 1H, H-1β), 4.39 (dd, 1H, $^3J_{3\beta,4\beta}$ = 4.9 Hz, $^3J_{4\beta,5\beta}$ = 8.9 Hz, H-4β), 4.30 (dd, 1H, $^3J_{3\alpha,4\alpha}$ = 4.4 Hz, $^3J_{4\alpha,5\alpha}$ = 8.3 Hz, H-4α), 4.24 (m, 1H, H-2α), 4.18 (m, 1H, 2β), 4.05 (m, 2H, H-3α, H-5β), 4.03 (m, 1H, H-5α), 3.96 (d, 1H, H-3β), 3.89 (dd, 1H, $^2J_{6a\beta,6b\beta}$ = -10.5 Hz, $^3J_{5\beta,6a\beta}$ = 1.7 Hz, H-6aβ), 3.86 (dd, 1H, $^2J_{6a\alpha,6b\alpha}$ = -10.7 Hz, $^3J_{5\alpha,6a\alpha}$ = 1.8 Hz, H-6aα), 3.72 (dd, 1H, $^3J_{5\beta,6b\beta}$ = 5.4 Hz, H-6bβ), 3.69, (dd, 1H, $^3J_{5\alpha,6b\alpha}$ = 5.9 Hz, H-6bα), 3.46 (s, 3H, OCH_3 H-10α), 3.36 (s, 3H, H-10β), 2.87 (br, 1H, C-OH, H-11α). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 138.84, 138.58, 137.88 (*ipso ϕ-C*), 128.32 - 127.32 (*ϕ-C*-H), 109.95 (C-1β), 102.10 (C-1α), 83.80 (C-3α), 82.86 (C-3β), 79.91(C-4β), 78.47 (C-2β), 77.75 (C-4α), 76.60 (C-5β), 76.10 (C-5α), 75.97 (C-2α), 73.39 - 71.62 (C-7, 8, 9), 71.20 (C-6α), 70.69 (C-6β), 55.88 (C-10β), 55.77 (C-10α). IR (KBr Pellet): ν 3495 (br, O-H) cm^{-1}. ESI- MS: 465.6 $[M+H]^+$, 487.6 $[M+Na]^+$, 503.5 $[M+K]^+$. TLC (2: 3: ethyl acetate : petroleum ether): $R_f(\alpha)$ = 0.76, $R_f(\beta)$ = 0.66.

3,5,6-tri-O-benzyl-1-O-methyl-2-O-(2′-propynyl)-α,β-D-glucofuranoside (5.5):

Sodium hydride (1.5 g, 60 wt. % in oil, 37 mmol) was weighed into a flame dried, 3-neck round bottom flask under argon atmosphere, and suspended in 60 mL freshly distilled dry THF. The reaction vessel was placed in an ice bath, and compound **5.4** (3.9 g, 8.3 mmol), dissolved in 5 mL dry THF, added via syringe through a septum. Propargyl bromide (2.0 mL, 80 wt. % in toluene, 18 mmol) was subsequently added dropwise via syringe, resulting in a light yellow solution. The reaction, which was allowed to warm to room temperature overnight, formed a dark orange-brown mixture. The excess NaH was destroyed by the cautious addition of isopropanol, followed by methanol and water. The resulting mixture was concentrated on a rotary evaporator. The resulting residue was dissolved in CH_2Cl_2, and shaken with water in a separatory funnel. The DCM layer was removed, and the remaining aqueous layer was washed twice more with CH_2Cl_2, the organic extracts being collected. The DCM extracts were washed with H_2O, and dried over Na_2SO_4. After filtration of the solid, the filtrate was concentrated to an orange-brown oil by rotary evaporation. Silica gel column chromatography (1 : 4 ethyl acetate : hexanes) was used to isolate the product as an orange oil (3.9 g, 94%). ^{1}H NMR ($CDCl_3$): δ 7.41 - 7.19 (m, 30H, ϕ-H), 5.02 (d, 1H, $^3J_{1\alpha,2\alpha}$ = 4.1 Hz, H-1α), 4.87 (s, 1H, H-1β), 4.79 - 4.50 (m, 12H, H-7, 8, 9), 4.31 (m, 2H, H-4α, 4β), 4.23 (m, 4H, H-2α, 3α, 11), 4.06 (m, 6H, H-2β, 3β, 5α, 5β, 11), 3.88 (m, 2H, H-6aα, 6aβ), 3.71 (m, 2H, H-6bα,6bβ), 3.41 (s, 3H, H-10α), 3.38 (s, 3H, H-10β), 2.45, 2.44 (2t, 2H, $^4J_{11,13}$ = 2.3 Hz, H-13α,13β). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 138.89 - 137.72 (ϕ-*C*-CH_2-O), 128.75 - 127.28 (ϕ-*C*-C-H), 108.33 (C-1β), 101.33 (C-1α), 84.90 (C-2β), 82.72 (C-2α), 81.61 (C-3α), 80.03, 79.91

(C-3β, 4β), 79.20 (C-12α), 79.03 (C-12β), 76.79 (C-4α), 76.51 (C-5α), 76.45 (C-5β), 75.28 (C-13α), 75.12 (C-13β), 73.30 - 72.02 (C-7, 8, 9), 71.20 (C-6α), 70.67 (C-6β), 57.64 (C-11α), 56.98 (C-11β), 55.95 (C-10β), 55.41 (C-10α). IR (neat, between KBr disks): ν 3288 (m, C≡C-H), 2119 (w, C≡C) cm^{-1}. ESI-MS: m/z = 503.4 $[M+H]^+$. HRMS (ESI): Calculated for $C_{31}H_{34}O_6H$ = 503.2434. Observed = 503.2428. TLC (1:4 ethyl acetate : hexanes): $R_f(\alpha)$ = 0.40, $R_f(\beta)$ = 0.33.

3′,5′,6′-tri-O-benzyl-1′-O-methyl-2′-O-methyl-(1,2-dicarba-*closo*-dodecaboranyl)-α,β-D-glucofuranoside (5.6):

Decaborane(14) (0.82 g, 6.72 mmol) was dried under vacuum for approximately two hours. The reaction vessel was placed under nitrogen atmosphere and freshly distilled acetonitrile (40 mL) was added via syringe through a septum. The resulting solution was stirred overnight (approximately 12 hours) at room temperature. Compound **5.5**, (2.14 g, 4.26 mmol), was dissolved in dry acetonitrile and added to the reaction solution via syringe through a septum. The septa were replaced by ground - glass stoppers, and the solution heated to reflux for 48 hours, which eventually formed a deep yellow coloured solution. The solution was filtered and concentrated under reduced pressure, yielding a yellow solid. The solid was dissolved in ethyl acetate and the product purified by silica gel column chromatography (10% → 20% ethyl acetate / hexanes). A second silica gel column (30% hexanes / CH_2Cl_2) was used to further purify the product, which was isolated as a clear, slightly yellow oil (0.879 g, 1.42 mmol, 33%). ^1H NMR ($CDCl_3$): δ 7.36 - 7.24 (m, 30 H, ϕ-H), 4.89 (d, 1H, $^3J_{1\alpha,2\alpha}$ = 4.0 Hz, H-1α), 4.81 - 4.42

(m, 12H, H-7,8,9), 4.71 (br s, 1H, H-1β), 4.25 (m, 2H, H-4 α,β), 4.08 (dd, 1H, $^3J_{2\alpha,3\alpha}$ = 3.3 Hz, $^3J_{3\alpha,4\alpha}$ = 5.2 Hz, H-3α), 4.01 (ddd, 1H, $^3J_{4\beta,5\beta}$ = 9.1 Hz, $^3J_{5\beta,6a\beta}$ = 2.0 Hz, $^3J_{5\beta,6b\beta}$ = 4.6 Hz, H-5β), 3.97 (ddd, 1H, $^3J_{4\alpha,5\alpha}$ = 7.7 Hz, $^3J_{5\alpha,6a\alpha}$ = 2.2 Hz, $^3J_{5\alpha,6b\alpha}$ = 5.6 Hz, H-5α), 3.93 (br s, 1H, H-13 α, 13β), 3.88 (d, 1H, $^2J_{11a\alpha,11b\alpha}$ = -10.3 Hz, H-11aα), 3.88 (m, 1H, H-3β), 3.84 (m, 2H, H-6aα, 6aβ), 3.81 (m, 1H, H-2α), 3.75 (d, 1H, $^2J_{11a\alpha,11b\alpha}$ = -10.3 Hz, H-11bα), 3.70 - 3.66 (m, 4H, H-2β, 6bα, 6bβ, 13 α/β), 3.58 (d, 1H, $^2J_{11a\beta,11b\beta}$ = -10.4 Hz, H-11aβ), 3.53 (d, 1H, $^2J_{11a\beta,11b\beta}$ = -10.4 Hz, H-11bβ), 3.36 (s, 3H, OCH_3 H-10α), 3.35 (s, 3H, OCH_3 H-10β), 2.55 - 1.85 (br m, 20H, BH). $^{13}C\{^1H\}$ NMR ($CDCl_3$): δ 138.62 - 137.34 (*ipso* ϕ-C), 128.56 - 127-50 (ϕ-C-H), 107.42 (C-1β), 101.57 (C-1α), 87.82 (C-2β), 84.29 (C-2α), 81.65 (C-3α), 79.74 (C-3β), 79.64 (C-4β), 76.79 (O/L with $CDCl_3$; C-4α), 76.50 (C-5β), 76.29 (C-5α), 73.39 - 72.24 (C-7,8,9), 71.30 (C-11α), 70.66 (C-6α), 70.48 (C-11β), 69.77 (C-6β), 57.46, 57.36 (C-13 α/β), 55.98 (OCH_3 C-10β), 55.64 (OCH_3 C-10α). $^{11}B\{^1H\}$ NMR ($CDCl_3$): δ -3.50, -5.24 (2B), -9.64 (2B), -12.32, -13.63 (6B). IR (KBr): ν 2591 (B-H), cm^{-1}. EI-MS: m/z = 620.2, $[M]^+$. HRMS: Calculated for $C_{31}H_{44}B_{10}O_6$: 622.4068. Observed: 622.4059. TLC (1:4 ethyl acetate : hexanes): R_f = 0.40.

1′-O-methyl-2′-O-methyl-(1,2-dicarba-*closo*-dodecaboranyl)- α,β-D-glucofuranoside (5.7):

10% Pd/C (65 mg) was added to a solution of compound **5.6** (0.59 g, 0.95 mmol) in absolute ethanol (25 mL). The reaction vessel was fitted with a rubber septum and

flushed three times with hydrogen from a balloon. The reaction was allowed to stir overnight (12 hours) under an atmosphere of hydrogen, whereupon TLC indicated complete consumption of the starting material. The solution was filtered through a pad of Celite, which in turn was washed with ethanol (20 mL). The filtrate solution was concentrated to a colourless oil by rotary evaporation. The crude product was purified by silica gel column chromatography (10% methanol / CH_2Cl_2) to afford the product as a colourless oil (0.28 g, 83%). ^{1}H NMR (CD_3OD): δ 4.97 (d, 1H, $^3J_{1\alpha,2\alpha}$ = 4.0 Hz, H-1α), 4.82 (br s, 1H, H-1β), 4.58 (br s, 2H, H-9 α, 9β), 4.31 (m, 1H, H-3α), 4.21 (m, 1H, H-3β), 4.10 (d, 1H, $^2J_{7a\alpha,7b\alpha}$ = -10.9 Hz, H-7aα), 4.07 (m, 2H, H-7aβ, 7bβ), 4.04 (d, 1H, $^2J_{7a\alpha,7b\alpha}$ = -10.9 Hz, H-7bα), 4.01 (m, 2H, H-4α, 4β), 3.91 (m, 1H, H-5β), 3.86 (m, 1H, H-2α), 3.86 (m, 2H, H-2β, 5α), 3.80 (dd, 1H, $^2J6_{a\beta,6b\beta}$ = -11.5 Hz, $^3J_{5,6a\beta}$ = 2.9 Hz, H-6aβ), 3.74 (dd, 1H, $^2J_{6a\alpha,6b\alpha}$ = -11.4 Hz, $^3J_{5,6a\alpha}$ = 3.4 Hz, H-6aα), 3.64 (dd, 1H, $^2J6_{a\beta,6b\beta}$ = -11.5 Hz, $^3J_{5,6b\beta}$ = 5.7 Hz, H-6bβ), 3.59 (dd, 1H, $^2J_{6a\alpha,6b\alpha}$ = -11.4 Hz, $^3J_{5,6b\alpha}$ = 6.1 Hz, H-6bα), 3.39, 3.35 (2s, 6H, OCH_3 H-10 α, H-10β), 1.49 - 1.80 (br m, B-H). $^{13}C\{^1H\}$ NMR (CD_3OD): δ 108.45 (C-1β), 103.04 (C-1α), 90.65 (C-2β), 87.56 (C-2α), 82.43 (C-4β), 78.82 (C-4α), 75.87 (C-3α), 74.53 (C-3β), 72.63 (C-7α), 72.02 (C-7β), 71.92 (C-5α), 71.61 (C-5β), 65.04 (C-6β), 64.56 (C-6α), 61.04, 60.30 (C-9 α, C-9β), 55.87, 55.81 (C-10 α, C-10β). $^{11}B\{^1H\}$ NMR (CD_3OD): δ -2.84, -4.72 (2B), -9.17 (2B), -11.32, -12.88 (6B). IR (Neat, between KBr disks): ν 3423 (s, br, O-H), 2591 (s, B-H). ESI-MS: m/z = 349.5, B_{10} isotopic distribution $[M]^-$. TLC (1:9 CH_3OH : CH_2Cl_2): 0.46. HRMS (ESI): Calculated for $C_{10}H_{26}B_{10}O_6Cl$: 386.2401 $[M+Cl]^-$. Observed: 386.2396.

2′-O-methyl-(1,2-dicarba-*closo*-dodecaboranyl)-α,β-D-glucopyranose (5.8b):

Compound **5.7** (0.67 g, 1.9 mmol) was placed in a round bottom flask and dissolved in a 1:1 (v:v) solution of THF and 1M HCl (20 mL total). The flask was fitted with a condenser and heated in an oil bath at 75 °C. The reaction was stirred in air for seven days, at which time TLC indicated nearly complete consumption of the starting material. The solution was cooled, and aqueous $NaHCO_3$ was added cautiously until the pH was in the range 7 - 8. The solvent was removed by rotary evaporation and the product purified by silica gel column chromatography, giving a colourless oil. Yield: 0.47g, 73%. ^{1}H NMR ($(CD_3)_2$=O): δ 6.10 (d, 1H, $^3J_{1\beta,OH1\beta}$ = 5.6 Hz, O-H$_1$β), 5.52, (d, 1H, $^3J_{1\alpha,OH1\alpha}$ = 3.9 Hz, O-H$_1$α), 5.27 (dd, 1H, $^3J_{1\alpha,OH1\alpha}$ = 3.9 Hz, $^3J_{1\alpha,2\alpha}$ = 3.5 Hz, H-1α), 5.01 (br s, 1H, H-9β), 4.86 (br s, 1H, H-9α), 4.64 (d, 1H, $^3J_{3\beta,OH3\beta}$ = 4.4 Hz, O-H$_3$β), 4.59 (dd, 1H, $^3J_{1\beta,OH1\beta}$ = 5.6 Hz, $^3J_{1\beta,2\beta}$ = 7.8 Hz, H-1β), 4.47 (d, 1H, $^3J_{3\alpha,OH3\alpha}$ = 4.2 Hz, O-H$_3$α), 4.39 (d, 1H, $^2J_{7a\beta,7b\beta}$ = -11.4 Hz, H-7aβ), 4.36 (d, 1H, $^3J_{4\beta,OH4\beta}$ = 4.5 Hz, O-H$_4$β), 4.31 (d, 1H, $^2J_{7a\alpha,7b\alpha}$ = -11.0 Hz, H-7aα), 4.27 (d, 1H, $^3J_{4\alpha,OH4\alpha}$ = 4.4 Hz, O-H$_4$α), 4.26 (d, 1H, $^2J_{7a\beta,7b\beta}$ = -11.4 Hz, H-7bβ), 4.23 (d, 1H, $^2J_{7a\alpha,7b\alpha}$ = -11.0 Hz, H-7bα), 3.83 (ddd, $^3J_{2\alpha,3\alpha}$ = 9.6 Hz, $^3J_{3\alpha,4\alpha}$ = 9.2 Hz, H-3α) 3.78 (m, $^2J_{6a\beta,6b\beta}$ = -11.6 Hz, $^3J_{5\beta,6a\beta}$ = 1.8 Hz, H-6aβ), 3.73 (m, $^3J_{4\alpha,5\alpha}$ = 9.4 Hz, $^3J_{5\alpha,6a\alpha}$ = 2.8 Hz, $^3J_{5\alpha,6b\alpha}$ = 6.0 Hz, H-5α), 3.64 (m, H-6aα, H-6bβ), 3.49-3.47 (m, 2H, H-3β, H-6bα), 3.34-3.30 (m, 3H, H-2α, H-4α, H-4β), 3.25 (m, 1H, H-5β), 3.00 (dd, 1H, $^3J_{1\beta,2\beta}$ = 7.8 Hz, $^3J_{2\beta,3\beta}$ = 9.2 Hz, H-2β), 2.70 - 1.6 (br m, B-H). ^{13}C{^{1}H} NMR ($(CD_3)_2$=O): δ 97.30 (C-1β), 91.36 (C-1α), 85.87 (C-2β), 82.54 (C-2α),

77.24 (C-5β), 77.05 (C-3β), 75.45 (C-8β), 75.24 (C-8α), 73.72 (C-7β), 73.57 (C-3α), 72.40 (C-5α), 72.11 (C-4α), 71.90 (C-7α), 71.81 (C-4β), 62.76 (C-6α), 62.68 (C-6β), 59.96 (C-9α), 59.72 (C-9β). $^{11}B\{^1H\}$ NMR ($(CD_3)_2$=O): δ -3.03, -4.68 (2B), -8.82 (2B), -11.20, -12.68 (6B). IR (neat, between KBr disks): ν 3386 (s, br, O-H), 2589 (s, *closo*-B-H). ESI-MS: m/z = 371.4 $[M+Cl]^-$ HRMS Calculated for $C_9H_{24}B_{10}O_6Cl$: 372.2244. Observed: 372.2247. TLC (1:9 CH_3OH : CH_2Cl_2): R_f = 0.32.

2'-O-methyl-(tetrabutylammonium [7,8-dicarba-*nido*-undecaboranyl])-α,β-D-glucopyranose (5.9):

Compound **5.8b** (0.27 g, 0.82 mmol) and tetrabutylammonium fluoride hydrate (1.09 g, 4.2 mmol) were combined in a round bottom flask and dissolved in THF (15 mL). The reaction was stirred, open to the atmosphere, for 24 hours, at which time TLC indicated complete consumption of the starting material. The reaction solution was concentrated to a dark yellow-brown oil by rotary evaporation, and the product isolated as a colourless oil following silica gel column chromatography (0.302 g, 65%). ^1H NMR (CD_3CN): δ 5.21 (2d, O/L, H-1α,1*α), 4.46 (2d, H-1β,1*β), 4.07, (d, H-7aβ), 3.85 (d, H-7a*β), 3.60 (m, H-6a,6a*,6b,6b*α/β, H-7bβ, 7b*β, H-7aα,7a*α,7bα,7b*α, H-3α,3*α, H-5α,5*α), 3.32 (m, H-3β,3*β), 3.25 (m, H-4β,4*β), 3.21 (m, H-5β,5*β), 3.16 (m, H-2α,2*α), 3.08 (t, $CH_3CH_2CH_2CH_2N$), 2.88, 2.83 (2dd, H-2β, 2*β), 1.96 (br s, H-9), 2.40 - -0.30 (br m, B-H), 1.59 (m, $CH_3CH_2CH_2CH_2N$), 1.35 (m, $CH_3CH_2CH_2CH_2N$), 0.96 (t, $CH_3CH_2CH_2CH_2N$), -2.60 (br s, B-H-B). $^{13}C\{^1H\}$ NMR (CD_3CN): δ 97.93. 97.83 (C-

1β,1*β), 91.49, 91.34 (C-1α,1*α), 83.62, 82.95 (C-2β, 2*β), 80.58, 80.39, 80.32, 80.07 (C-2α, 2*α, 7α,7*α),78.77, 78.38, 77.26, 77.13, 77.02, 76.97 (C-3β, 3*β, 5β, 5*β), 73.63, 72.35, 71.89, 71.78 (C-4β, 4*β) 71.64, 71.53 (C-7β,7*β), 64.67, 63.01, 59.32 (C-6α,6β), 59.32 ($CH_3CH_2CH_2CH_2N$), 46.71 (C-9), 24.29 ($CH_3CH_2CH_2CH_2N$), 20.31 ($CH_3CH_2CH_2CH_2N$), 13.80 ($CH_3CH_2CH_2CH_2N$). $^{11}B\{^1H\}$ NMR (CD_3CN): δ -9.87, -10.41, -14.63, -16.69, -18.43, -22.00, -32.58, -36.97. FT-IR (Neat, between KBr disks): ν 3384 (br, O-H), 2525 (B-H) cm^{-1}. ESI-MS: m/z = 326.5 $[M]^-$, 242.4 $[NBu_4]^+$. HRMS (ESI): Calculated for $C_9H_{24}B_9O_6$: 326.2455. Observed: 326.2464.

2′-O-methyl-(tetrabutylammonium [3,3,3-tricarbonyl-3-rhenium-1,2-dicarba-*closo*-dodecaboranyl])-α,β-D-glucopyranose (5.1):

Compound **5.9** (0.16 g. 0.29 mmol), tetrabutylammonium fluoride hydrate (0.41 g, 1.6 mmol), and $[Re(CO)_3(H_2O)_3]Br$ (0.15 g, 0.38 mmol) were combined in a pressure tube. The reagents were dissolved in water (15 mL), to which was added ethanol (5 mL) in order to improve the solubility of ligand **5.9**. The pressure tube was equipped with a magnetic stir bar, sealed, and heated in a sand bath (T = 150 °C) for seven days. The reaction mixture was concentrated to a brown oil by rotary evaporation. Silica gel column chromatography (1:9 CH_3OH : CH_2Cl_2) separated a mixture of **5.9** and **5.1** from the crude product. Semipreparative HPLC was used to isolate the target compound as a yellow oil (8 mg, 4%). ^1H NMR (CD_3CN): δ 5.14 (2d, H-1α), 4.46 (2d, H-1β), 3.91 (d, H-7), 3.65 (m, H-7, H-6, H-5α, H-4α, H-3α), 3.34 (m, H-3β), 3.23 (m, H-4β), 3.19 (m, H-5β), 3.08 (m, H-2α, $CH_3CH_2CH_2CH_2N$), 3.00 - 0.80 (br m, B-H), 2.76 (m, H-2β), 1.80 (br s, H-9),

1.59 (m, $CH_3CH_2CH_2CH_2N$), 1.35 (m, $CH_3CH_2CH_2CH_2N$), 0.96 ($CH_3CH_2CH_2CH_2N$). $^{13}C\{^1H\}$ NMR (CD_3CN): δ 200.51 (C≡O), 97.56 (C-1β), 91.55 (C-1α), 84.81, 82.19, 78.83, 77.58, 77.14, 77.07, 73.89, 73.34, 72.47, 71.72, 71.46, 62.69 (C-6), 59.32 ($CH_3CH_2CH_2CH_2N$), 29.24, 24.30 ($CH_3CH_2CH_2CH_2N$), 20.32 ($CH_3CH_2CH_2CH_2N$), 13.78 ($CH_3CH_2CH_2CH_2N$). $^{11}B\{^1H\}$ NMR: δ -5.85 (1B), -7.93 (1B), -9.14 (2B), -11.68 (2B), -18.41 (1B), -19.62 (1B), -20.24 (1B) ppm. FT-IR (Neat, between KBr disks): ν 3348 (br, O-H), 2544 (B-H), 1997, 1893 (s, C≡O) cm^{-1}. ESI-MS: m/z = 595.6 $[M]^-$, 242.5 $[NBu_4]^+$. HRMS (ESI): Calculated for $C_{12}H_{23}B_9O_9Re$: 595.1794. Observed: 595.1823.

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Chapter 6

Conclusions and Future Work

6.1 Conclusions

The synthesis and characterization of novel carborane and metallocarborane-carbohydrate conjugates was achieved where the products represent new platforms from which to prepare novel radiopharmaceuticals, particularly those based on ^{99m}Tc. The compounds initially prepared were simple glycoside derivatives of glucose and galactose, which served as model systems to develop the synthetic methods needed to prepare more advanced compounds. The advanced compounds included a glucose derivative in which the site of attachment of the metallocarborane pendant group was the C-2 position, rather than the anomeric position in the initial model species. This compound (**5.1**) was pursued as a potential ^{99m}Tc-based analogue of [^{18}F]FDG. Another advanced derivative was the disubstituted carboranyl glycoside (**4.1**) in which glucose and benzoic acid moieties were attached to the carbon atoms of the carborane. In this compound, the benzoic acid group was intended to serve as a linker site to a targeting vector such as a peptide, protein, or antibody while the glucose moiety was intended to enhance the overall hydrophilicity of the conjugate. The synthesis of the non-glycosylated, metallocarborane-benzoic acid derivative **4.2** was undertaken in order to permit the comparison of hydrophilic properties of any model conjugates produced from **4.1** and **4.2**.

With novel *nido*-carborane-based precursors in hand, synthesis of the non-radioactive rhenium-metallocarborane-carbohydrate derivatives was investigated.

Difficulties in achieving rapid, high-yielding syntheses of these materials, which would be required for routine radiopharmaceutical preparations, led to the development of alternate methods involving microwave heating. Microwave irradiation of reaction mixtures resulted in shorter reaction times and higher yields, and permitted synthesis of the first examples of ^{99m}Tc-carborane-carbohydrate conjugates. The characterization of products obtained through microwave irradiation (**3.4, 3.5, 4.1, 4.2**) revealed that isomerization of the carborane cages occurred, resulting in the carbon atoms moving futher apart and forming the 2,1,8-MC_2B_9 cage isomers, rather than the 3,1,2 "ortho" configuration reported previously for several Re and ^{99m}Tc-metallocarborane derivatives prepared by our group via conventional reflux heating.[1-3] Although initially unexpected, this isomerization was rationalized with reference to numerous examples of carborane and metallocarborane cage isomerization reported in the literature.[4-13]

Although the metallocarborane derivatives listed above could be prepared via microwave heating and subsequently characterized, a serious drawback was encountered with the microwave-based method, namely the degradation of the glucose-bearing compounds *in situ*. The observed degradation was characterized as loss of the carbohydrate from the metallocarborane group via hydrolysis. This could be controlled to a certain degree by conducting reactions in a buffered medium, but significant degradation was still observed. Indeed, with compounds **3.5** and **5.1**, degradation was extensive, and this precluded their further development. *In situ* degradation of compound **4.1**, coupled with difficulties in preparing active ester derivatives, also precluded the

development of model conjugates based on this metallocarborane. Therefore, an alternative application was explored, that being carborane iodination and radioiodination.

Iodination was initially undertaken with ligand **2.6** as a model system, as had been done with the metallocarborane chemistry. Following the successful synthesis and isolation of **2.13** and [^{125}I]-**2.13**, the synthesis of iodinated *nido*-carborane conjugates of **4.3** and **4.12** was completed. Chosen as model conjugates were benzamides **4.14** and **4.16**, which were subsequently iodinated to yield **4.23** and **4.24** as standards for characterization. Using standard radioiodination procedures,[14,15] [^{125}I]-**4.23** and [^{125}I]-**4.24** were prepared and subsequently isolated in good radiochemical yields and purity. Log P experiments confirmed an increase in hydrophilicity with the presence of the glucose moiety in [^{125}I]-**4.24**.

In conclusion, several *nido*-carborane-, iodo-*nido*-carborane- and metallocarborane-carbohydrate conjugates have been prepared and characterized as models for novel radiopharmaceuticals. This research has expanded the aqueous-based metallocarborane chemistry developed by our group to include the first examples of metallocarborane-carbohydrate derivatives, and has also introduced the use of microwave irradiation to the aqueous synthesis of Re and ^{99m}Tc metallocarborane derivatives. A bifunctional carboranyl glycoside derivative was also prepared. This compound gives the potential for conjugation to molecules (or molecular fragments) that could serve as targeting vectors through its benzoic acid functionality, while enhancing the hydrophilicity of the final complex, relative to a non-glycosylated analogue.

In a more general capacity, this reasearch has shown that a single *nido*-carborane-based precursor can be labelled with a choice of either a metal or iodine radioisotope. For example, the same ligands (**2.6, 4.12**) used to prepare ^{99m}Tc-metallocarborane complexes were used to prepare [^{125}I]-labelled iodo-*nido*-carborane derivatives. Therefore, depending on the availability of isotopes and/or the sensitivity of the biologically active group to labelling conditions, one such carborane-based ligand could potentially be used to suit numerous applications. Such a choice is not available to traditional metal chelates, or to aryl derivatives that serve as precursors to radioiodination.

6.2 Future Work

Several avenues of research could be explored to further advance the carborane and metallocarborane chemistry described in this thesis. Since a major limitation arose in the metallation chemistry, from the degradation of glycoside compounds under microwave irradiation, it might be possible to investigate the synthesis of compounds that are more stable to these forcing conditions. Such an alternative approach might be to prepare metallocarboranyl-*C*-glycoside derivatives, rather than the *O*-glycosides discussed in this research. *C*-glycosides are carbohydrate derivatives in which a pendant group is attached to the anomeric carbon of the carbohydrate through a carbon atom, rather than oxygen, eliminating the relatively sensitive acetal linkage associated with *O*-glycosides. Instead, *C*-glycosides are known to be more stable to enzymatic (e.g. by glycohydrolases) and chemical hydrolysis.[16,17] Since the degradation observed with the synthetic targets discussed in Chapters 3-5 was believed to be a result of hydrolysis of the

glycoside bonds in those species, the synthesis of *C*-glycoside analogues might furnish more robust platforms for preparing carbohydrate-derived metallocarboranes. This would be of particular interest in the case of a potentially bioconjugatable species, such as a *C*-glycoside analogue of **4.1**, the synthesis of which was complicated by, and that of subsequent model conjugates was precluded by *in situ* degradation.

The synthesis of a number of *closo*-carboranyl *C*-glycosides has been reported by Tietze and co-workers as potential agents for BNCT.[17] Since these compounds are accessible, as were the alkynyl and *closo*-carboranyl *O*-glycoside precursors to compounds **3.4**, **3.5**, and **4.1**, it should be practical to prepare the analogous nido-carboranyl and metallocarboranyl *C*-glycosides. In this case, one might expect the *C*-glycosides to be more robust to the conditions (e.g. MW irradiation at 200°C) for preparing the metal complexes. This should permit higher yields of the Re complexes, and allow for further developments, such as easier access to conjugates of a metallocarborane-carbohydrate analogue of **4.1**.

The strategy of preparing more robust derivatives, potentially capable of withstanding the rather harsh microwave heating conditions used to prepare Re and ^{99m}Tc metallocarboranes, might be suitable for preparing a conjugatable prosthetic group for the indirect labelling of, for example, peptides, proteins, or antibodies. However, it would also be advantageous to prepare a bioconjugate which can be labelled directly, as this route, in general, minimizes radiation exposure to the handler, and is more amenable to radiopharmaceutical kit formulations that are currently preferred in a clinical setting. This is at present more practical for iodination, since the conditions required for those

syntheses are milder, occurring rapidly at room temperature, whereas microwave irradiation at 200°C is incompatible with sensitive molecules such as peptides, proteins, and as this research has shown, carbohydrates. However, since radioiodine isotopes are typically prepared in nuclear reactors or accelerators,[18] these are less available and more expensive than ^{99m}Tc, which is available at low cost from a generator. Therefore, it is of interest to investigate the possiblity of modifying the carborane in order to lower the temperature required to affect metal complexation. One such modification could be one which increases the acidity of the *nido*-carborane "bridging" hydrogen atom, thus making the dicarbollide dianion more accessible, since it has typically been through this species that most metallocarborane derivatives have been approached.[19] To this end, Teixidor and co-workers have reported that while the pK_a (calculated) of the bridging proton in *nido*-carborane (charge = -1) is 13.5, the pK_a of neutral *nido*-carborane species is substantially lower (e.g. pK_a for 7,8-$C_2B_9H_{13}$ = -4.6; pK_a for 9-$SMe_2C_2B_9H_{11}$= 6.7).[20] Therefore, it may be possible to prepare "charge-compensated" *nido*-carborane ligands such that i) the metallation reaction is facilitated and ii) the resulting metal complexes are neutral, and therefore, may have the additional ability to cross otherwise impenetrable cell membranes or the blood-brain barrier.[21]

A number of examples of these charge compensated *nido*-carboranes and subsequent metallocarborane complexes have been reported. Typically, the charge-compensation arises from B-substitution by Lewis base ligands such as dialkyl sulfides,[22-28] amines or pyridine.[29-31] One such metal complex contains the $[Mn(CO)_3]^+$ unit.[25] Furthermore, the addition of the dialkly sulfide ligand in this case was accomplished in

aqueous solution. Therefore, it may be possible to prepare analogous Re and ^{99m}Tc complexes in aqueous media.

Another avenue of future research would be to investigate the synthesis of bioconjugates of compound **4.1** or **4.22**, or related analogues (e.g. *C*-glycoside). The synthesis of conjugates of many peptide-, protein- or antibody-based targeting vectors known to bind specific receptors (e.g. on cancer cells) *in vivo* could be investigated. If successful conjugates can be prepared, then radiolabelling and subsequent investigation of biological properties such as *in vitro* uptake by cells, or biodistribution in small animal models can be undertaken in order to determine their potential for future clinical use. Through the synthesis of the model benzamide **4.24**, it was shown that a *nido*-carborane-carbohydrate can be conjugated to an amine-bearing entity, and that the presence of the sugar increased the hydrophilicity of the complex. Furthermore, analogous conjugates of **4.2** or **4.23** could be prepared so that, as was done in this investigation, the respective properties (lipophilicity, receptor uptake, biodistribution) of the conjugates can be determined and compared.

Finally, if suitable bioconjugates can be prepared and radiolabelled, and whose properties are suitable for imaging their biological targets, the synthesis of analogues based on therapeutic radionuclides could be investigated. Since there are radioactive isotopes of rhenium (^{186}Re, ^{188}Re) that have properties amenable to targeted radionuclide therapy, the similar chemistry of rhenium and technetium give the possibility of "matched pairs" of radiopharmaceuticals, one based on ^{99m}Tc for imaging, and the other, based on radioactive rhenium, for therapy. An advantage of ^{188}Re, in particular, is that it

can be obtained from a generator. Similarly, with radioiodine isotopes, there also exists the possibility of preparing compounds with a radiohalogen label for specific application. For example, as was shown in this investigation, ^{125}I is appropriate for basic development, and *in vitro* studies of promising compounds. Meanwhile, ^{123}I and ^{131}I have useful properties for imaging and therapy, respectively.

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