THE TOTAL SYNTHESIS OF BIOTINYLATED BORON COMPOUNDS AND 1,3,2 BENZODIAZABOROLES DERIVATIVES AND THEIR APPLICATION AS POTENTIAL BNCT DELIVERY

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By

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DEDICATION

This dissertation is dedicated to my late mom, I. K. Dlamini, she encouraged me to study MSc but the Lord took her before she could see the fruits of her motivation and your love

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ABSTRACT

In this study, boron neutrons capture therapy (BNCT) principles which have been reported as the best treatment of glioblastoma multiforme tumors thus far were explored. The aim of this study was to synthesizing boron containing compounds which have a potential as boron delivery agents (BDA) with enhanced tumor selectivity. The tumor identification moiety chosen was biotin (a vitamin H) which is essential for every cell's metabolism. The mechanism in which biotin accumulates in the tumor cell is through the receptors which are over-expressed in the tumor cells. Therefore the possibility that biotinylated boron compounds could be deposited more on tumor cells than to healthy cells is high.

The synthetic approach involved the synthesis the boronic acids, 4-anilineboronic acid and 4-(hydromethyl) phenylboronic acid followed by their conjugation with *D*-biotin. These two boronic acids were chosen because their reactive grouping (OH, NH_2) could bind to the carboxylic acid reactive part of *D*-biotin via the formation of an ester linkage. Of these two boronic acids, 4-(hydromethyl) phenylboronic acid was successfully synthesised and biotinylated at 62% yield to form a new boron compound.

In this project it proposed that the addition of a fluorescent marker on this biotinylated compounds could potentially improve their imaging properties. The luminescence properties and stability of 1,3,2-benzodiazaboroles derivatives have been reported in literature. In this project, 1,3,2-benzodiazaboroles derivative of biotinylated boronic acid (already synthesised) was in 72% when reacted with *o*-phenylenediamine. It is anticipated that this compounds could have better tumor solubility, water solubility, less toxicity (brought about by the attached biotin) and enhanced luminescent properties (as reported for all 1,3,2-benzodiazaboroles derivatives). However, the biological testing on these compounds was not performed in this study.

LIST OF ABBREVIATIONS

ABNS	accelerator based neutron source
MIT	Massachusetts Institute of Technology
BSH	Sodium borocaptate
BPA	p-boronophenylalanine
GBM	Glioblastoma multiforme
LET	Linear energy transfer
BBB	brain-blood-barrier
ACBC	1-amino-cyclobutanecarboxylic acid
ABCPC	1-amino-3-boronocyclopentane-carboxylic acid
LBL	lactose binding lectin
TK1	thymidines kinase 1
MNP's	magnetic nanoparticles
PAMAM	Polyamidioamine
EGFR	epidermal growth receptor
PET	Positron Emission Tomography
SPECT	Single Photon Emission Computer Tomography
BNL	Brookhaven National Laboratory
BMRR	Brookhaven Medical Research Reactor
ESI	Electro spray ionisation
g	gram(s)
GC	gas chromatography
h	hour(s)

HOMO	highest occupied molecular orbitals
LUMO	lowest unoccupied molecular orbital
TLC	thin layer chromatography
M^+	parent molecular ion
NMR	nuclear magnetic resonance
Hz	hertz
COSY	correlation spectroscopy
HMBC	heteronuclear multiple bond correlation
δ	chemical shift in part per million downfield from tetramethylsilane
t	triplet
m	multiplet
d	doublet
s	singlet
DEPT	distortionless enhancement bipolarization transfer
J	coupling constant
ppm	part per million
IR	infra-red
mol	moles
THF	tetrahydrofuran
WHO	World health Organisation

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TABLE OF CONTENTS

Introduction

1.1 Boron neutron capture therapy principles

Boron neutron capture therapy (BNCT) is a hypothetical form of radiotherapy which is believed to have great potential in cancer treatment than conventional radiotherapies.[1] In BNCT, a stable non-radioactive nuclide ^{10}B captures a neutron when irradiated with low-energy neutron source to become ^{11}B which disintegrate into alpha particle and lithium-7 nuclei as shown in **Equation 1**.[2]

$$^{10}B + n_{th} \longrightarrow [^{11}B] \longrightarrow {}^{4}He + {}^{7}Li + 2.31MeV \qquad \text{(Equation 1)}$$

The alpha and lithium particles have a high linear energy transfer (LET) of 1.47MeV and 0.84 MeV respectively and a total of 2.31MeV.[3] The combined energy is responsible for causing DNA damage in the tumour cells by replacing the average LET within the cell.[4] Apart from the high LET, these nuclei have a path length of 5-10μm which is approximately one cell diameter.[5] Therefore, during BNCT experiment that a ^{10}B containing tumour cell is totally destroyed while normal neighbouring cells are spared.[6]

BNCT is a binary form of radiotherapy meaning it involves two distinct aspects; drug delivery and activation. To attain any success in BNCT, the right concentration (20-35 μg/kg) of boron 10 must have accumulated in the tumour cell. The boron concentration in tumour cells must be 3-folds greater than that deposited on healthy cells (approximately 5mg) to spare them from any damage when irradiated with a neutron.[7] The challenge in BNCT studies has been finding drugs which selectively deposit boron-10 to tumour cells at the right concentration.[8]

Boron-10 success in neutron capture therapy has been attributed to its non-radioactive which is bought about its existence with 20% of natural occurring boron.[6] The chemical stability of boron makes incorporation to a number of compounds possible for better drugs development.[3] The resultant particles (lithium and alpha) on neutron capture for boron have high LET and their combined path length is approximately one cell diameter for total cell disruption.[9]

However, boron-10 is not the only nuclide capable of capturing a neutron when bombarded with slow neutrons, other non-toxic elements including lithium (917 barns), gadolinium (248 000

barns) and gold (566). Nitrogen and hydrogen can also capture neutrons producing protons and gamma rays respectively. The advantage of boron over other nuclides is that it has a large cross-section for neutron capture (3800 barns).[10] It should be noted that gadolinium, ^{157}Gd, has the greatest cross section (248 000>>3800 barns) hence could capture most neutrons than boron itself. However the difficulty in the imaging of Gd using MRI limits its usage in capture therapy therefore boron is used ideally in neutron capture experiments.[7, 11]

The other factor that influences BNCT principles is the availability of adequate flux of neutrons targeting the tumour cells. The nature of the neutron source, tumour allocation and the degree of radiation tolerance of the surrounding tissues influences the degree of neutron delivery and capture on tumour cells.[12] The challenge in BNCT is to find the accurate balance on the two factors, boron concentration and good neutron source.[7]

1.2 History of BNCT and present status

It was four years after the discovery of a neutron by J Chadwick in 1932 at Cambridge University's Cavendish Laboratory that Dr Gordon Locher presented the concepts of BNCT. Locher pointed out that non-toxic compounds of boron, lithium, gadolinium or gold were capable of killing cancer cells when irradiated with a neutron. The superiority of Boron-10 amongst these elements was further outlined based on its large cross section. In 1938, Kruger and Goldhaber gave the exact energies of the particles that resulted from ^{11}B disintegration as 0.8MeV for ^{7}Li and 1.47MeV for the alpha particle with combined path length of 5-10μm approximately one cell diameter. About the 1940's, all BNCT experiments were only carried *in vitro* on mouse tumors.[3]

The first BNCT clinical trials were performed in 1951 at the Brookhaven National Laboratory (BNL) on 10 patients with the therapeutically persistent malignant, glioblastoma multiforme (GBM). The patients were treated with borax and irradiated with thermal neutron beams at Massachusetts Institute of Technology by Sweet.[13] All 10 patients treated died of serious side effects such as radiodermatoses of the scalp and ulcers.[5] The failure of these experiments were attributed to the borax lack of specificity on tumor–healthy cells.[9] Borax was deposited on the

cerebral capillaries not the desired glioblastoma tumours which led to healthy cell disruption during neutron irradiation. In 1952, Brownell, Javid and Sweet separately reported that the neutron irradiation contributed to the failure of borax clinical trials.[14] Taking into consideration the neutron source influence as stated by Sweet, Farr *et al.*, proposed that not enough thermal neutron reached the tumor due to scattering on irradiation[15] Further reports quantified the depth of penetration of the reactor-based thermal neutron to be limited to 3-4 cm.[16] The scattered neutron intensity was higher than what surrounding cells could handle which was the reason for the scalp damage. Efforts to avoid the scalp damage through surgical insertion of elastic bandages before drug administration could not prevent scalp damage and subsequently patient's death.[3] It was for these reasons that clinical trials were abandoned in the United States of America in 1954.

In 1958, Soloway, Sweet and Brownell carried clinical studies at the Massachusetts General Hospital using less toxic and more boronated compounds.[17] The two compounds tested were p-carboxyphenylboronic acid and sodium perhydrodecaborate ($Na_2B_{10}H_{10}$). High solubility, chemical stability and biological inertness have been observed in Sodium perhydrodecaborate of all the boron compounds evaluated about that time.[3, 9, 16] About 18 patients were treated at Massachusetts Institute of Technology (MIT) of which p-carboxyphenylboronic acid was administered to 16 patients and the other two patients took the $Na_2B_{10}H_{10}$ and thermal neutrons were used in those experiments. Of the 18 patients who died, 14 showed cerebral death while brain necrosis on 9 and tumor recurrent was seen on two patients. The survival period of the treated patients was between 10 days up to $11\frac{1}{2}$ months.[3] The BNCT clinical trials were for the second time discontinued in the United States in 1961 as they were unsuccessful.[18]

Dr Hiroshi Hatanaka, previously with Sweet's group in the USA started his BNCT studies in Japan. In 1968 he performed clinical experiments using sodium borocaptate (BSH) $Na_2B_{12}H_{11}SH$ synthesized by Soloway in 1967.[5, 11] Hatanaka reported the first success of BNCT on patients with malignant glioma. By 1986, a 5 year survival rate of grade 3-4 malignant glioma was calculated to be 58% when BNCT was used exclusively for tumours within 6cm from brain surface.[19] These results sparked interest worldwide and from 1986-1993, 120 patients have been treated by Hatanaka with six surviving for more than 10 years.[19]

Mishima *et al.,* in 1987 reported the success of clinical trials performed using a single boronated compound (p-boronophenylalanine (BPA)) as a boron carrier.[3, 5, 19] This boron compound which had been first synthesized by Sweet in 1972 contained an amino acid as a tumor targeting moiety. BPA enhanced boron delivery to the tumor cells using the amino acids affiliation with over-expressed plasma in tumor cells as opposed to healthy cells. BSH and BPA demonstrated favourable tumor-to-blood and tumor-to-normal tissue boron ratios hence they are the only acceptable boron delivery agents.[20]

The success of clinical trials on BPA and BSH at the Brookhaven National Laboratory (BNL) and Massachusetts Institute of Technology (MIT) was achieved using reactor based epithermal neutrons for tumors closer to the surface.[2] These results were of much improvement from the thermal neutrons that were previously used in earlier. The epithermal neutrons beams were of higher energy than the neutron sources previously used, boasting with better penetration of up to 10 cm from the surface.[21] From 1994-1999 at BNL about 53 patients were successfully treated using the epithermal beam in the United States.[22] The application of epithermal beams in BNCT research would spread to other countries like Argentina, across Europe and in Australia.

The epithermal beams were irradiated from nuclear reactors which were expensive to install and maintain thus inaccessible for more clinical trials.[7] The accelerator based neutron source (ABNS) have been reported United Kingdom at the University of Birmingham in 1994 as an alternative to the neutron reactors in epithermal beams production.[23] The use of accelerators was reported to have cheaper installation (could easily mounted in any hospital) and maintenance cost when compared to advantages over neutron rectors.

The use of accelerator based neutron sources is viewed by BNCT researchers as the best approach of neutron delivery. Therefore, research is currently focussed in the development of boron compounds which are non-toxic, highly selective on tumor cells, could deliver about 25-35 µg/Kg of boron into the tumor. A lot of such compounds have been synthesized and only few of them have been clinically proven because of the cost implications and political reasons.[24]

1.3 Early applications of BNCT principles on brain tumors

In the early 1930's, an outbreak of a vicious type of brain cancer was reported in some parts of the world including the United States of America.[25] This type of cancer was called glioblastoma multiforme (GBM) because their multiform tumors were located resultant from the glial cells within the brain.[26] GBM which is graded class IV astrocytoma by the World Health Organisation (WHO), is the most common and aggressive tumor of all brain tumors.[27] According to the WHO, glioblastoma multiforme accounts for about 60-65% of all primary brain tumors.[26, 28] Globally, malignant tumors were responsible for about 7.6 million annual deaths in 2008, a number that could rise to 22 million in 2030.[29] The mortality of the GBM remains high as to date the recovery rate is 0% while the survival period depends on the method of therapy used.[30]

Radiotherapy, chemotherapy, surgery and their combinations were first used on GBM patients.[31] Surgical procedures alone have been reported to have a 2-year survival rate at 0%.[32] However, an improved of the survival period from 3-4 to 7-12 months was reported when surgery and radiotherapy were jointly used in GBM patients.[33] Chemotherapy has also been used as an alternative or in addition to surgery and radiotherapy.[34] A number of chemotherapeutic agents have been developed for glioblastoma multiforme; however the survival rate observed was of small deviation from other forms of therapy.[35] The major limitation of chemotherapy was that the chemotherapic agents could not transverse through the highly selective blood-brain-barrier (BBB).[36] Recently, agents which have the potential to cross through the BBB have been synthesized. However, the main challenge surrounding their use most chemotherapeutic agent has been that, if taken in large doses, the excess could be lethal to neighbouring normal cells.[37]

The poor progress of conventional methods in the treatment of brain cancers has encouraged the use BNCT applications over the last two decades.[27] The frequent mutation of receptors and genes alterations makes the manipulation of radiation in radiotherapy and chemotherapic agents developments cumbersome.[38] BNCT principles provided a more effective alternative because of it binary nature, thus possessed more tumor selectivity and less toxicity to neighbouring healthy cells.[39] Early BNCT clinical trials on GBM treatment gave reported a survival rate of that is superior that conventional method (5-10 year survival of about 58 %).[19] Different boron compounds have been investigated for their boron delivery capabilities on GBM tumors.

BNCT principles have also been evaluated on other kinds of tumors other than GBM. Recent studies have been conducted on tumors which include head and neck, lungs, hepatic and most recently liver metastases.[40]

1.4 BNCT advantages over Conventional methods

The measure of success in cancer treatment is based on the number of tumor cells killed while preserving the neighbouring health cells.[1] Conventional methods consist of chemotherapy, radiotherapy, surgery and scarcely used immunotherapy and gene therapy. Patients are treated using various combinations of these methods depending on the type and location of the tumor.[12] These methods fails the core of cancer treatment in that health cells are most vulnerable to damage because of the toxicity of the agents used in chemotherapy. Some chemotherapeutic agents find it difficult to transverse through the biological barriers and may break down without reaching their target tumors or attack healthy cells.[41]

BNCT hold a higher tumor selectivity when compared to the conventional methods which is largely due to its binary nature. The boron accumulation to the tumor can be maximised independently before radiation altercations to improve selectivity.[25, 30] In BNCT, only cells with about 20-35µg/kg of boron 10 can be killed when irradiated with low energy neutrons and the particles have a combined path length of 5-10 µm which is one cell equivalent. The one cell restriction minimise cytotoxicity in BNCT. Drugs used in BNCT can transverse through biological barriers like the brain-blood-barrier (BBB) into the brain tumors. The drugs are usually administered intravenously to the specific tumor site.[2, 41] Renal clearance of boron compounds is high hence lowering toxicity to healthy cells.

Apart from the development of a good boron delivery agent, the neutron source could be manipulated to maximise therapeutic effect of BNCT model of cancer treatment. Separate studies have been conducted from 1967 in an effort to develop neutron sources that deposit adequate neutron flux, ideally for tumors deep inside.[23] Even though in early clinical trials, expensively assembled neutron reactors were used to produce thermal neutrons, the recent

development of accelerator based neutron sources have afforded a cheap, deep penetrating alternative in neutron production.

1.5 Boron Delivery Agents

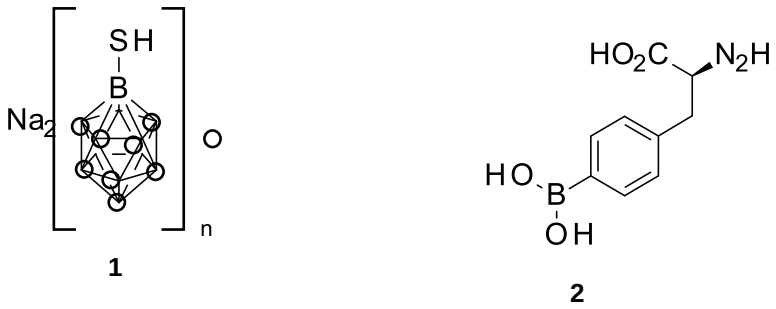
Boron delivery agents (BDA) are compounds that contain at least one boron-10 and are capable of attaching on tumor cells for effective cell disruption on neutron capture. An ideal BDA must have low toxicity to normal cells, high tumor uptake to low in normal cells. The tumor to normal tissue ration should be greater than 3.[25] The BDA must deliver the required boron concentration to the tumor cell (approximately 20-35 µg/Kg). The boron compound's water solubility, chemical stability and lipophilicity must be investigated for it to be considered a BDA.[5] The ability for boron compound to transverse through the biological barriers such as brain-blood-barrier (BBB) with dissociating must be taken into consideration.[1, 31] The rate of which the BDA clears from the blood and in normal cells after the BNCT experiment have been performed is considered in BDA development.

The development of boron delivery agents (BDA) also known as boron carries began in 1951 with the first generation being borax.[24] The lack of clinical success of the first generation led to the development of the second generation compounds (BSH and BPA). The second generation compound's clinical success was the turning point of BNCT research. However, there have some limitations such as water solubility and tumor selectivity that hindered their progress in boron delivery. Further improvements of the limitations of the second generation compounds led to the development of the third generation BDA.[16] The approach development of the third generation BDA is influenced by the quest to achieve 'ideality' of the boron carrier for effective BNCT experiments. Examples of the different generation of the boron delivery agents are given in the coming sections.

1.5.1 The first and second generations of BDA

The first generation of boron compounds investigated for BNCT was borax in 1951 followed by p-carboxyphenylboronic acid and Sodium perhydrodecaborate ($Na_2B_{10}H_{10}$) in 1958.[3] All clinical trials on these compounds were unsuccessful as a 0% survival rate was reported. The failures of the clinical trials of these compounds were attributed toxicity they possessed on neighbouring healthy cells. Severe side effects such as scalp death were reported when these compounds were clinically proven for BNCT.[3] The lack of success of the first generation of BDA encouraged more research on other compounds which were later known as the second generation BDA.

Boron containing compounds such as sodium borocaptate (BSH) **(1)** and boronophenylalanine (BPA) **(2) (Figure 1)** were reported to make up the second generation of boron delivery agents. These compounds showed better boron delivery of boron content to the tumor cells with minimal side effects.



1 **2**

Figure 1

Earlier BNCT clinical trials conducted on BSH and BPA were reported to be a success. [31] The first success of clinical trials on BSH was reported by Hatanaka in 1968. A 5-10 years survival rate at 58% brain tumors was reported.[19] BSH was also reported to readily transverse though the BBB better than other compound synthesised because of their interaction with the protein plasma through a sulfhydryl group.[40] The chemical stability and high boron content was reported for BSH which accounts for the success obtain during clinical trials.[6]

The limitations of BSH reported were its toxicity to neighbouring normal cells when taken in high doses and its low water solubility. Several reports suggested that BSH may have compromised tumor selectivity. This was because of the ionic nature of dianionic dodecaboratethio ($[^{10}B_{12}H_{11}]^{2-}$ -S-) that could easy bind to other organic compounds.[3] The high boron content of the boron cluster of BSH together with the ease to bind to organic compounds

could mean that the boron delivery may extend to the surrounding normal cells.[42] Boron deposition on neighbouring cells at high concentration could results to cell death on neutron irradiation. Another limitation of BSH was that it could readily oxidise to form untreatable toxic substances when in blood circulation for prolonged periods.

To counter some of the BSH limitations, studies were conducted led to development of BSH derivatives. The derivative's development involved the addition on group on the sulfhydryl grouping while the boron cluster is retained. Kusaka *et al.*, reported the addition of L-amino acids to dianionic dodecarboratethio($[^{10}B_{12}H_{11}]^{2-}$ -S-) unit from BSH to improve tumor selectivity.[43] Amino acids are known to be taken more readily by tumor cells because of their increased metabolism. On that basis, incorporating them to the boron cluster could result in a compound that is tumor selective and could deliver enough boron content. Examples of compounds that belong to this class are (*R*)-2-Amino-3-(dodecaboranylthio)pro-panoic acid disodium salt (**3)** and (*S*)-2-amino(dodecarbonylthio)pentanoic acid disodium salt (**4) (Figure 3)**.[43]

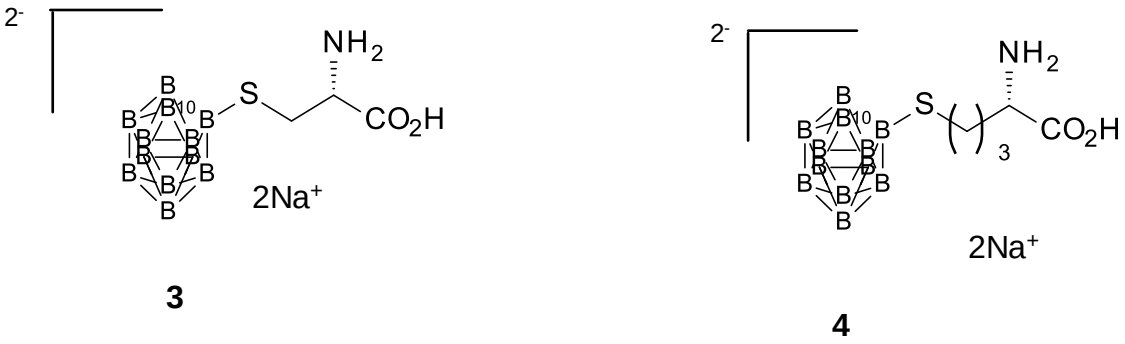
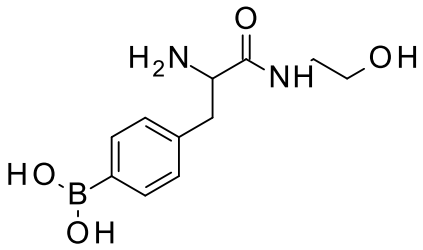


Figure 2

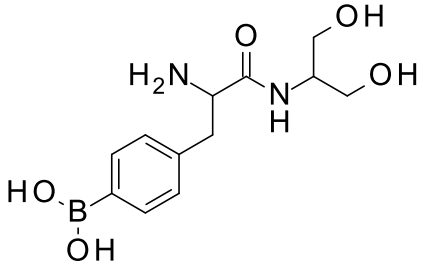
In 1987, Mishima *et al.*, started clinical trials on patients with malignant melanoma using BPA.[44] The rationale on using phenylalanine, an amino acid, as a boron transporter was that the membranes of the malignant cells was more permeable when compared to normal cells.[3] BPA was reported to deposit adequate amounts of boron concentration for BNCT studies with low toxicity.[45] Several studies conducted demonstrated that BPA has favourable tumor-blood ratio and tumor-to normal tissue boron ratios. [26] The success of the clinical trials on BPA have been reported by different groups could reaffirm it as acceptable boron delivery agent.

The inability of BPA to cross the BBB to deliver ^{10}B into brain tumors when taken in large doses have been outlined amongst its limitations. The poor water solubility ($16mgL^{-1}$) of BPA in large doses has been documented as the reason behind the difficulty to pass through the biological barriers.[46] BPA has also been reported to lack metabolic stability hence it could be renal cleared out of the cell.[1] This property would mean neutron irradiation must follow shortly after drug induction which may be lethal to normal cells that have the ability to hold on BPA much longer.

Several strategies have been employed to improve BPA deficiencies. These include complexion of BPA with cis-diol sugars such as glucose and fructose to improve water solubility.[47] Fructose-BPA has been the most used BPA derivative for *in vivo* and *in vitro* BNCT experiments.[48] Nemoto *et al.*, have also reported the synthesis of hydroxylforms-BPA conjugates to improve water solubility. BPA(OH)**(5)** and $BPA(OH)_2$ **(6)** in **Figure 3** were synthesised and their water solubility and cellular uptake were confirmed to be better to that of BPA was reported by Nemoto *et al.*[49] Capuani *et al.* have reported that preloading with L-DOPA would improve BPA uptake on the tumor.[50]



5: BPA (OH)



6: $BPA(OH)_2$

Figure 3

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Other BPA modifications reported for better boron delivery in the brain tumor have been through the simultaneous administration with BBB disruptors. Mannitol have been reported as the ideal BBB disruptor that promotes the passage of BPA into the brain cells.[51]

The success of the second generation compounds and their entire derivatives as boron delivery compounds was the turning point of BNCT concept on brain tumors.[52] However, none of these compounds could meet all the requirements of a "perfect" boron delivery agent discussed earlier. There need to develop boron delivery agents that could near perfection led to the synthesis of the third generation boron delivery agents.

1.5.2 Third Generation BDA's

The imperfection of both the first and secondary generations of boron delivery agents led to the synthesis of thousands of compounds which are known as the third generation of boron carriers.[16] These compounds were synthesized to achieve ideal properties with the key focus being enhanced tumor to normal cell selectivity, un-matched water solubility, high lipophilicity and availability to transverse through blood barriers like brain-blood-barrier. The biology of the tumor cells; as in what they absorb or feed on, unique receptors they may have, have been considered in boron carrier development. Compounds known to possess a similar interaction that tumor cells have with biological compounds have been boronated for BNCT studies.[6]

Boron delivery agents are classified based upon their molecular weight; as low molecular weight compounds and high molecular weight compounds. There is no structural relationship between compounds on either of the two classes. The low molecular weight compounds include boronated amino acids, carbohydrates, porphyrins, peptides, phosphates, phosphonates, phenylureas, nicotinamides, thioureas, lipids precursors and nucleic acid derivatives.[25] Amongst the high molecule weight compounds, monoclonal antibodies (MoAbs), liposomes and other tumor targeting nanoparticles have been studied for their boron delivery agents potential.[6, 53] The limitation in boron carrier development has been *in vivo* studies, while most have been confirmed *in vitro* to possess ideal BDA properties. Most third generation BDA have been clinically proven on human trials leaving BPA and BSH the only compounds used to treat BNCT patients.

1.5.2.1 Amino Acids derivatives as BDA

Amino groups proved to be effective as tumor seeking agents for they have more affinity for tumor cells compared to normal cells.[54] The over-expressed plasma of the tumor cells allows large amounts of amino acid to get into the cell to sustain its metabolism.[6] The use of amino acids derivatives as boron delivery agents began in 1987 when BPA was first used by Hatanaka. BPA have an amino group phenylalanine in its structure. BPA has been clinically proven that it can deposit the required concentration, >2.5mg, of boron-10 to tumor cells for BNCT experiments. However issues related to water solubility of BPA, resulted in the synthesis of a number of much improved BPA derivatives and synthesis of new compounds.

Naturally occurring amino acids such as glycine, aspartic acid, tyrosine, serine, cysteine, alanine and methionine have been boronated in the development of boron delivery agents .[6, 16] Of all the boron compounds conjugated with natural amino acids, tyrosine analogues have been well documented in BNCT studies.[55] Extensive research focus has been on unnatural amino acids mainly because of their metabolic stability compared to natural ones.[16] Adding to the stability deficiencies, boronated-natural amino acids were restricted to one boron compound hence they were taken in large doses during clinical trials. Attempts to attain higher boron delivery in the tumor and metabolic stability while improving water solubility led to the evaluation of carborane-containing amino acids.[53-54, 56]

The first compound studied was carboranylalanine (CBA) which is had aniline attached to a cluster of 10 boron atoms.[57] This compound showed some improved boron delivery capabilities at low dosage and higher lipophilicity when compared to BPA. However CBA possessed excessive lipophilicity than necessary thus causing retention in the blood. This limitation was countered by the use of dipeptides on lipophilic groups of the compound. Carboranylalanine have been reported to possess fungi exhibiting potential.[55] Carborane analogues are subject to research as boron delivery agents. Boronated unnatural amino acid- 1-aminocycloalkanecarboxylic acids are currently explored as boron delivery agents. These amino acids were found to be non-toxic, high boron concentration and could cross the brain-blood-barrier better compared to BPA.[58] Furthermore these compounds showed an astonishing tumor to blood ratio of 8 and tumor to normal brain ratio of 21 after 2 hours of administering.

The boronated derivatives of 1-amino-cyclobutanecarboxylic acid (ACBC) and 1-amino-3-boronocyclopentane-carboxylic acid (ABCPC) are the most studied of all cyclic amino acids.[59]

The *nido* and the *meta*-carborane derivatives have been reported. The boronated nido-carboranes have been reported as being ineffective as boron delivery agents because of the hydrophobic nature of the *closo* part of their structure. To counter this hydrophobicity issue, *meta*-carboranes analogues have been the focus in BNCT research. Example of such compounds is ACBC conjugated with polyol group **(7) (Figure 4**) to attain acceptable water solubility.[55]

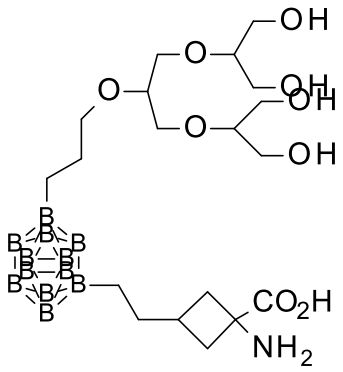

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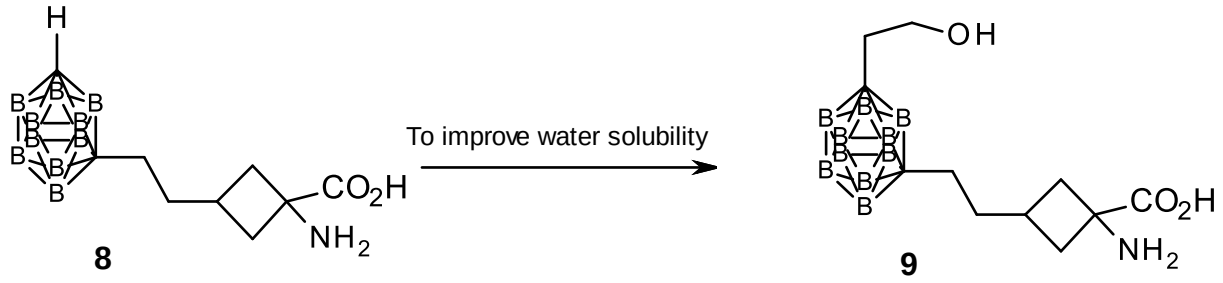
Figure 4

Srivastava *et al.*, reported the synthesis of 1-amino-3[2-(1, 7-dicarba-closo-dodecaboran (12)-1-yl)ethyl]cyclobutanecarboxylic acid **(8) (Scheme 1**), a ACBC derivative that could be attached in intracerebral tumors. *In vivo* studies on nido-carboranes failed because of the non-specific binding to biological materials these compounds have as a result of the ionic cage reaction with amino acids. To improve the metabolic stability for *in vivo* studies, Srivastava, synthesized non-toxic1-amino-3[2-(7-(2-hyrdoxyehyl)-1,7-dicarba-closo-dodecaboran(12)-1-yl)ethyl]cyclobutanecarboxylic acid **(9)**.[53]



Scheme 1

Kabalka *et al*., reported there was an unnatural amino acid which together with it derivatives when attached to boron-10 could selectivity attached to tumor better than BPA and BSH. This compound belongs to the 1-aminocycloalkanecarboxylic acids which were reported to transverse through the BBB.[60] The compound was 1-amino-3-boronocyclopentane-carboxylic acid (ABCPC).[54, 59] The cis and trans isomers of ABCPC were tested for their tumor selectivity capabilities. The *cis*-ABCPC ,as a mixture of its *L* and *D* enantiomers **(10,11) (Figure 5)** was reported to have tumor to blood ration of 8 and tumor to normal brain ratio of 21 on melanoma infected rats.[59] Furthermore, *cis*-ABCPC like BPA delivers 70% of the pool of boron in loosely bound form to the nucleus and cytoplasm of human glioblastoma cells.[56] The advantage of having free ion is that they can be eliminated off the cells easily when BNCT experiment has been completed. ABCPC was found to possess both improve water solubility and ease to pass through the BBB when compared with BPA.

10 cis-*L*-ABCPC

11 cis-*D*-ABCPC

Figure 5

The most sought-out in unnatural amino acids- BNCT research has been the synthesis of ABCPC and its derivatives and testing in both *in vivo* and *in vitro* for melanoma. Within the first hour of treatment with ABCPC, boron delivery was reported to be as double that of BPA when tested on T98G human glioblastoma cells.[54] The *in vitro* experiments reported thus far were conducted on B-16 melanoma cell line although a number of other cell lines could still be used.[61] There have been no reported experiments carried out on humans due to economical and ethical reasons. An increase in the number of tests on animal models until dosage is optimised could pave way for more clinical trials.

1.5.2.2 Carbohydrate derivatives

The use of carbohydrates in boron delivery agent's development began shortly after it was reported that BPA, a clinically acceptable drug for BNCT, had poor water solubility. Fructose, a carbohydrate was conjugated with the L-BPA's boronic acid moiety to improve it water solubility and its tumor identification capabilities without altering its toxicity.[62] Tumor specificity of carbohydrates attached boron carriers is the same as with amino acids, in that tumor cells take more of carbohydrates for metabolic purposes than normal cells.[63] Uptake of boronated carbohydrates is through the carbohydrate-specific receptor proteins on the surface of tumor cells.

Carbohydrate derivatives of BSH are reported to have superior tumor selectivity than BSH itself. Other classes of carbohydrates being researched besides fructose include glucose, maltose, ribose and lactose.[6] Boron compounds containing these classes have been synthesized and some have been tested both *in vivo* and *in vitro*. Carboranes-carbohydrates conjugates have been reported as having potential in BNCT. Carboranes are known to deliver the high boron content in the tumor even though they are hydrophobic[64]. Carbohydrates have been reported to be capable to improve the water solubility of carborane in the same way fructose does to BPA.[65] Therefore in the synthesis of carborane-carbohydrate compounds the balance between lipophilicity and hydrophobicity must be achieved, a good compound must be amphiphilic.[66]

Synthesis of carboranyl-glucose derivatives have also been reported as an alternative for BNCT experiments. Carborane-glucose conjugates can easily be labelled with ^{18}F or radiometals on the

C1 position making them useful in radiopharmaceutical development.[64] Glycosides have poor water solubility and forms mixtures of enantiomers. Hence, to effectively bind to carborane, they should first be attached to an alkynyl group. Tietze and Bothe synthesized *ortho*-carborane glycoside of monosaccharides and observed poor water solubility across the monosaccharides studied. Those findings narrowed synthesis of carborane-carbohydrate conjugates to disaccharides and most recently polysaccharides like lactose and maltose.[67]

Of all carbohydrates, lactose has been the most exploited in BNCT research. The existence of the lactose binding lectin (LBL) in the tumor cell surface has directed researchers to synthesizing lactose derivatives to act as inhibitors for metastatic growth[68]. It was reported for that lactose-carboranes are retained in the melanoma cells better than both BPA and BSH[66]. Bonechi at al reported [1,2-dicarba-closo-dodecaboran(12)-1-yl-methyl](β-D-galacto-pyranosyl)-(1→ 4)-β-D-glucopryanoside molecule (LCOB) **(Figure 6)** as a potential boron delivery agent for BNCT. This molecule showed favourable amphiphilic character and thermal stability making it ideal for BNCT.[69]

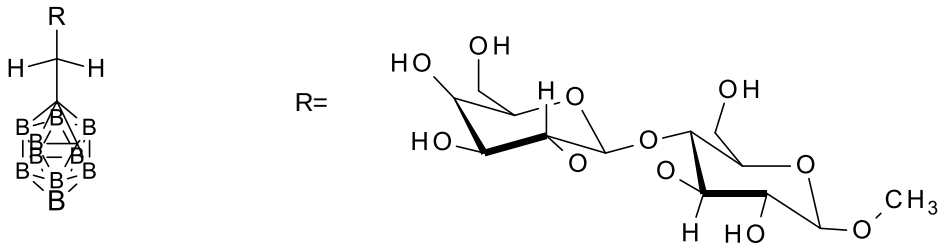


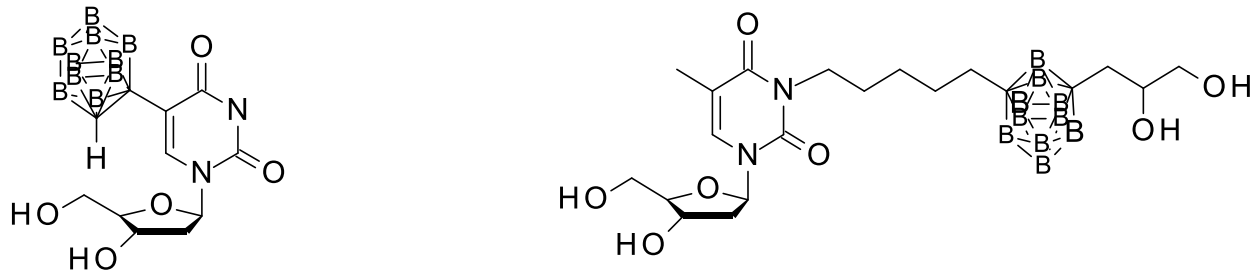
Figure 6

1.5.2.3 DNA precursors

Boron compounds containing precursors that have the affinity to bind to DNA in the cells were evaluated for their potential as boron delivery agents for BNCT.[70] The biochemical precursors attached to boron containing compounds include purines, pyrimidines and nucleosides among a host of others still under research. Purine and pyrimidine boron conjugates were first to be synthesized and evaluated. It was reported that those conjugates were unstable in aqueous media.

Further improvements gave stable and bulky compounds which could not mimic the natural occurring purines and pyrimidines hence their boron conjugates were not ideal for BNCT. Boron nucleosides conjugates were then evaluated and screened for BNCT shortly after the limitations of purines and pyrimidines were reported.[71] Biologically, tumor cells are known to have an increased uptake of nucleic acids mainly due to the rapid cell division. Taking that concept into consideration, incorporations to boron compounds for BNCT purposes seems to be realistic hypothesis.[9, 71]

Several single and multiple boronated compounds containing different kinds of nucleosides have been studied for BDA potential. The most successful of all boronated nucleoside has been carboranyl thymidine conjugates. These conjugates could convert to corresponding phosphate, 5'-monophosphate, as soon as they reach the cytosolic thymidines kinase 1(TK1) a receptor that is more on tumor cells than on healthy cell. Carboranyl thymidines expected high tumor specificity was however compromised by their bulkiness and high lipophilicity that is known with all carboranes. The use of *closo*-carboranes in-favour of nido or mera carboranes together with the attachment of hydroxyl groups would improve the water solubility and subsequently improve interaction with TK1. To further improve tumor selectivity most carboranyl-thymidines have a sugar moiety attached to it. Example of such compound that has been studied for BNCT is 3-(dihydropropyl-carboranyl-pentyl) thymidine (N5-2OH) and β-5-O-carboranyl-2'-deoxyindole (D-CDU) **(12)**.[6, 9] High rates of phosphorylation which meant increased uptake by the TK1 together with low toxicity were observed on N5- 2OH**(13) (Figure 7)**. Biological evaluation both *in vivo* and *in vitro* favours N5-2OH derivatives as the future of all boronated nucleoside for BNCT studies.



12 13

Figure 7

1.5.2.4 Monoclonal antibodies

Monoclonal antibodies (mAbs) have been used as cancer targeting and therapeutic reagents because they possess antigen-binding specificity. The radioimmunoconjugation of MoAbs and radioactive isotopes enhance tumor targeting capability and also improves imaging functionalities.[72] The use of boronated monoclonal antibodies (mAbs) as potential boron delivery agents for BNCT has been investigated by a number of researchers. The mAbs tumor specificity was confirmed in vitro but the immunoreactivity was lost in vitro.[13] These could be attributed to the high molecular weight of these compounds and their rapid clearance in the blood plasma hence it could not reach tumors across blood barriers. It is for these reasons that boronated monoclonal antibodies alone have shown little success in BNCT studies.[31] Recent research on mAbs for BNCT have involved the attachment of other tumor targeting groups with improved stability in vitro whilst depositing enough boron concentration.[73] Dendrimers, peptides and biotin are some of the groups that have been incorporated to monoclonal antibodies. Pressman and Keighley in 1948 reported the use of labelled biotinylated antibodies in cancer treatment on rat's kidney tumors.[74]

1.5.2.5 Nanoparticles

The challenge in brain cancer treatment has always been the development of drugs that are capable of penetrating the highly selective blood-brain-barrier. The barrier is structurally made of layers of endothelial cells with restricted permeability on polar compounds, of which most

drugs are polar.[75] Boron containing drugs synthesized have to be highly lipophilic and small sized to pass cross the BBB to the tumor. Nanoparticles research in drug delivery was motivated by their small size (between 10 and 1000nm) and their ability to utilise the tight junctions between the selective endothelial cells by opening them. Nanoparticles are therefore coated on boron compounds or chemically bonded to deliver sufficient boron concentration to the tumor cells. Research on nanoparticles containing BDA has resulted in the synthesis of a number of compounds some of which have been biologically tested both *in vivo* and *in vitro*.[76]

The oldest generation of the boronated nanoparticles has been the metallic nanoparticles.[16] These compounds were less success which could be attributed to the imbalance between tumor identification and sufficient boron delivery.[77] It was obvious that direct incorporation of any metallic nanoparticle to any boron molecule was insufficient for boron delivery. Factors like the identity of the metal in the nano group, the nature of boron compounds, the lipophilicity and water solubility of the two had to be taken into consideration. Ciani *et al.*, reported that carborane derivatized polymers which were both fairly lipophilic and water soluble could be conjugated to gold nanoparticles to enhance boron delivery to the tumor. The rationale for using carborane derivative of poly (ethylene oxide)-b-poly (caprolactone) diblock-copolymer (PEO-b-PCL) was that polymerized carborane had improved water solubility and tumor selectivity with non-toxic end products.[78] Gold has also been reported by Mandal *et al.*, to have great capabilities of forming good boronated compounds because of their biological inertness.[79] A number of metallic nanoparticles have been studied which include cobalt, iron and chromium.[80]

Boronated nanoparticle researchers have recently outlined the usage of magnetic nanoparticles (MNP's) to have a potential in boron transportation to the tumor.[9] Magnetic nanoparticles superiority as a catalyst, high stability and good tumor selectivity, have encourage its functionality as a drug carrier.[81] Carborane analogues enriched with magnetic nanoparticles used in the presence of starch, demonstrated prolonged boron accumulation on the tumor (of 51.4 µg / g).[76] The accurate mechanism on how magnetic nanoparticles accumulate in the tumor is still under investigation.[82] Further development of BDA with nanoparticles have led to peptides and proteins being incorporated into these compounds to improve tumor selectivity.[31]

1.5.2.6 Liposomes as BDA

Liposomes ability to selectively attach to tumor cells in large quantities has resulted to it being used as a vehicle for drug delivery. Tumor endothelial cells have increased permeability to liposomes when compared to normal cells because of their large pores and lymphatic drainage in the tumor cell is impaired hence liposomal clearance relatively slow.[5] These two factors make liposomes good drug delivery vehicles for ^{10}B when attached to boron compounds which sparked a number of studies for BNCT. Liposomes as boron delivery agent's studies started in the early 1990's using two approaches; encapsulation of boron compounds into liposomes and the incorporation of boron conjugated lipids into liposomal layer **(Figure 8)**.

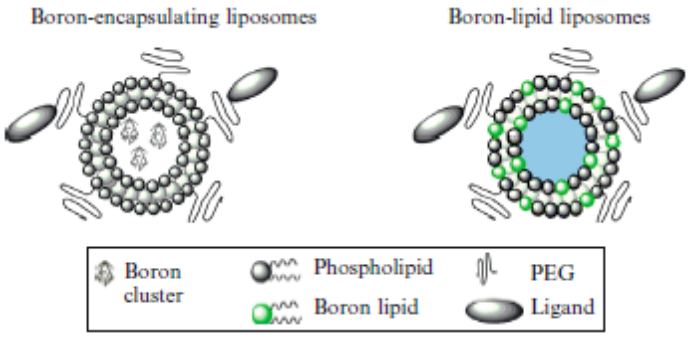


Figure 8: Showing boron encapsulating liposomes and boron-lipid liposomes for BNCT[83]

In the encapsulation method, boronated compounds are taken into the vacant inner part of the liposomes, once there the liposomes release it inside the tumor cell. In this approach, boron compounds used must have affinity for tumors, size should be maintained to nano scale, lipophilicity and water solubility remain an important factors.[31] Carboranes have been the most used boron compounds after the early usage of BSH among other borane anions. The advantage of carboranes is that they have high boron content and have metabolic stability than anionic compounds. However carboranes are hydrophilic while they are lipophilic, to compensate that hydrophlicity, groups with high water solubility are attached to the carboranes. Boron compounds permeability into the liposomal moiety is important as much as the liposome tumor affinity. The liposome structure could be attached to groups that do not only improve their tumor selectivity but prolong tumor-cleavage time. Amongst the groups that have been reported are; PEG and receptor targeting folate.[83]

Recently, boron-lipid conjugates have been synthesized to fit within the liposome bilayer so that when the liposomes are taken in by the tumor cells they deposit the boron compound. The first generation of boron-lipid conjugate reported was synthesized from *nido*-carboranes attached to water soluble lipids to improve the hydrophlicity which carboranes are known to possess. A number of boron- lipid conjugates are currently under investigation both *in vivo* and *in vitro*. However, their development is restricted to choosing the right carborane, lipid group and size maintain at nano scale. Nakamura reported the synthesis of closo-dodecarate conjugates of cholesterols as having potential as boron delivery agent. [5]

1.5.2.7 Receptor based drug delivery

To understand this form of drug delivery it is important to review the general cell metabolism, the role of receptors, population of receptors on tumor cells when compare to normal cells and lastly study how drugs are delivered into the receptors.

1.5.2.7.1 Over-expression of receptors

Cells are a basic building block of all living things. In humans, cells have a wide variation in size, functionality and structure but collectively they give 'life'. Human cells have broad nutritional requirement for their growth and survival.[84] Nutrients needed for cell metabolism are either synthesised without the body as main or by-product of a process within the body or they are administered orally through food-enriched with them and rarely intraperitoneally.[85] Deficiency of the essential nutrients for cell metabolism could result in the cell malfunction and eventually cell death subsequently human mortality.

Neoplasm, also known as a tumor, are cells that possess abnormally high growth rate when compare to other cells of the same structure and functionality. Neoplasm can be classified as benign, in situ and malignant neoplasm.[86] These tumors differ by their originality, extent of multiplication and physical recognition. For example, benign have slow growth and normal cell resemblance while malignant cells multiply aggressively.[87] Of all types of neoplasms, only malignant can be classified as cancer for they form a tumor that invade and destroy the host cell.[87]

Tumorous cells have a great appetite for nutrients to sustain their over-division when compared to normal cells. To subsidize their appetite, tumor cells have over-expressed receptors in their cell membrane to attract the nutrients they desire. Some of the nutrients that are readily taken into the tumor cells in large amounts include amino acids, carbohydrates, nucleic acids and vitamins. The target receptor sites include cancer cell structure, tumor extracellular matrix and tumor endothelial surface receptors.[88] The vitamins that a favoured by tumor cells include Vitamin 12, folate and biotin.[89]

1.5.2.7.2 Receptor targeting mechanism

The mechanisms of action that drugs are delivered into the tumor cells *via* their overexpressed receptors remain unknown.[90] Several studies have been conducted to determine how drugs administered through any form of therapy gets into the cells do. [91] Knowing the mechanism could help develop new drugs based on that mechanism in cases that the tumor shows some resistance existing drug. The toxicity could be due to the mechanism in which drugs used selectively chooses the tumor cell over the healthy cell in these conventional methods. For example chemotherapic agents that get into the cells through liposomal pathway have a great potential of being deposited in the healthy cells that are rich in liposomes.[92]

1.5.2.7.3 Biotin conjugates in tumor targeting

Biotin is a vitamin H, essential for the cell gluconeogenesis, fatty acids biosynthesis, amino acid catabolism and other metabolic pathways.[93] Humans cannot synthesize biotin and therefore it must be administered orally and taken into the cells via intestinal absorption. Food rich with biotin include the egg yolk, vegetables and the liver. Biotin deficiency results in the loss of hair colour, dry scalp, fatigue, mouth cracks, dry eyes, loss of appetite, and depression.[94] Biotin deficiency is very rare in humans because there is a large pool of foods that can are rich in biotin.[95] However, a person taking raw egg white is more likely to have these symptoms because egg white contains high avidin content which strongly bind to biotin and could limit biotin supply to the cells.[72, 94, 96]

Structurally biotin is made of a bicyclic ring and a reactive carboxylic group that can bind to the target group by different mechanism that may include esterification **(Figure 9)**. [72] These two reactively different ends are joined by most notable valeric acid side chain made of CH_2's and carboxylic group. There valeric system is not involved in avidin binding therefore making it chemical biotinylation possible without compromising its affinity to avidin or streptavidin.[97] A spacer groups could be added to the valeric acid side chain to improve biotin reactivity and enhance accessibility of biotinylated molecules into avidin or streptavidin.

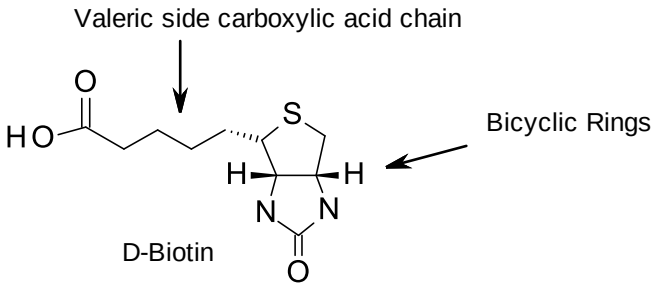


Figure 9

The biotin binding capability to avidin could be improved by the addition of a highly reactive longer spacer groups on the valeric acid chain. NHS is one group that has been added to the valeric acid chain not to lengthen the spacer arm but to improve biotin binding capabilities to a host of other molecules to be biotinylated **(Figure 10)**.[72]

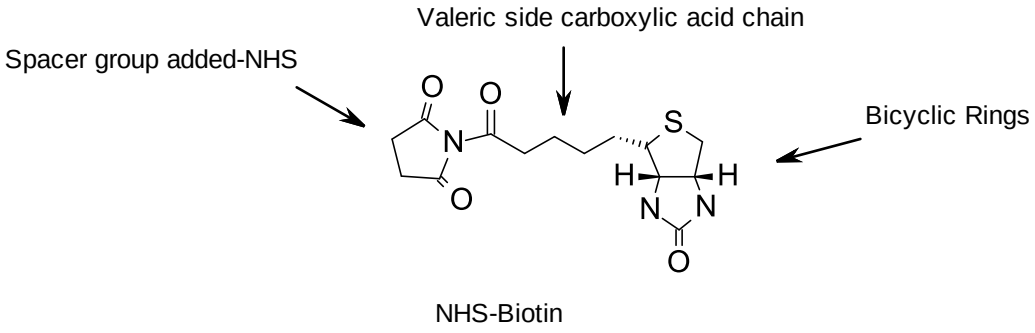


Figure 10

1.5.2.7.3.1 Synthesis of biotinylated therapeutic agents

Biotin's ease to chemically bind to a host of potential drugs without losing it reactivity together with existence of the over-expressed biotin receptors in tumor cells, have encouraged the usage of biotin as receptor targeting moiety for many anti-cancer drugs.[98] The physical structures of biotin receptor remain unknown but it is believed to be numerically superior when compared to other receptors such as vitaminB_{12}.[99] Other properties that make biotin ideal in anti-cancer drug conjugation are that; biotin does not have the water solubility problem that some drugs possess and it is less toxic to normal cells. [100]

Two routes have been taken in the development of biotinylated drugs; one has been the biotin conjugation to clinically proven anti-cancer drug to improve both the drug tumor selectivity and imagining. The other approach has been the synthesis of new biotinylated compounds and tested for their anti-cancer potentials. Most studies involved the conjugation of biotin to clinically proven tumor exhibiting drug. Example of such studies is the biotinylation of paclitaxel, a clinically anti-cancer drug for ovarian, breast and head and neck tumors.[101]

The limitation of biotin-drugs conjugates is that they are restricted to small doses since only one molecule can be attached per drug moiety which can either be a ligand, antibody or a peptide. Biotinylated conjugates are also vulnerable to renal clearance to the kidney because of the high water solubility and not so strong bond on its receptor site.[102] To overcome this limitations, multiple biotinylated compounds have been understudy in several research centres.[72, 103]

1.5.2.7.3.2 Pre-targeting technique of biotinylated molecules

In the late 1980's, Goodwin *et al.,* proposed the pre-targeting concept as a potential drug targeting strategy with less rapid clearance and high tumor-to-non-tumor accumulation ratio. Pre-targeting involves the induction of a targeting body to the tumor site before that therapeutic agent that has affinity to the targeting body is taken. The motivation for the pre-targeting techniques was the finding that the half-life of the mAb in circulation is usually 2-3 days while the optimal concentration of mAbs on the tumor was about 24 hours.[73] In pre-targeting an unlabelled

antibody and the label are administered separately. Paganelli *et al.*, reported the usage of avidin-biotin system as a pre-targeting label that can precede the therapeutic agent.[104]

Avidin is a tetrameric glycoprotein that is of high content in the egg white with molecular mass of 66 000 Da. Avidin is more of a protein nature with a isoelectric point pI= 10. On it structure avidin has four biotin binding sites; each having a strong biotin affinity **(Figure 11)**.[85, 97] Biotin-containing molecules are more biospecific to avidin conjugates even under harsh pH and temperatures conditions.[72] However avidin is not exclusively bio-specific to biotin, other negatively charged molecules are also capable of binding to the positively charged protein part of its structure.[105] This non-specificity hinders biotin binding and could limit antibodies deposition into the cell.

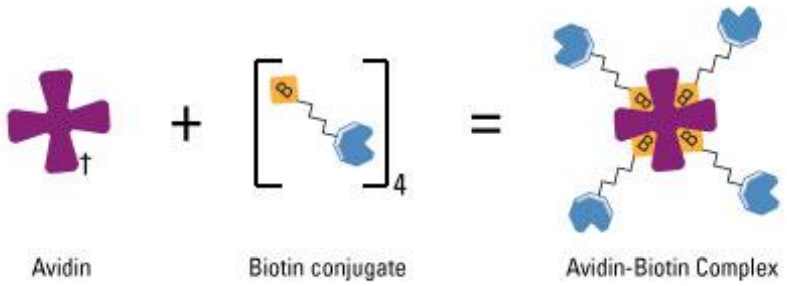


Figure 11-Schematic diagram showing biotin interactions with avidin while attached to an antibody.[97]

Streptavidin is an avidin, look alike, a tetrameric protein, with four biotin binding sites, similar affinity constant with biotin as avidin. Streptavidin unlike avidin is of bacterial origin, from the bacterium, *Streptomyces avidini*.[72, 106] The difference in origin between avidin and streptavidin slightly influences some of their characteristics. Streptavidin for example, have slightly lower pI (≈5-6) hence background interactions are minimal due to better bio specificity than that observed with avidin. Streptavidin lack the glycoprotein moiety in its structure thus binding to carbohydrate receptors impossible.[72, 100] Streptavidin has a higher half-life when compared to avidin hence it can bind longer before it is eventually excreted. Both avidin and streptavidin are used separately or simultaneously to target biotin containing molecules once they are injected into the body.[105, 107]

Paganelli *et al.*, proposed that avidin- biotin pre-targeting could be carried-out in steps of 1 to 5.[104] One step is the more direct deposition of anti-cancer drugs to the tumor where biotinylated monoclonal antibodies with therapeutic agent are injected (could not be classified as pre-targeting). The two- step pre-targeting was first reported by Paganelli group in 1988 who conducted clinical trials on ovarian cancer patients. The 15 patients were injected intraperitoneally with biotinylated antifolate receptor mAbs followed by ^{111}In-labeled streptavidin with 2-5 days interval. In vivo studies showed the increase in tumor uptake after 2-48 hours. This result encouraged more studies on pre-targeting particular in the quest to reduce the time between the induction of target body and biotinylated therapeutic agent.[73, 104]

The three step approach in pre-targeting which shows much promise on tumors such as gliomas was also proposed by Paganelli. This method involves the injection of biotinylated mAbs into the target tumors, followed by the injection of avidin or streptavidin to remove excess mAbs in circulation and lastly in induction of the biotinylated therapeutic agent.[100] Clinical studies conducted on 48 patients with high-grade gliomas showed tumor degradation extending over a year when treated with anti-tenascin A and yttrium-90 labelled biotin.[91]

It was noted after this studies that the half-life of avidin was short when compared to streptavidin therefore avidin was proposed to be used more as a clearing agent than a targeting agent. The second step of the 3-step method was then modified by addition of both avidin and streptavidin sequentially with avidin acting as a clearing agent for circulating antibody. Streptavidin lower non-specific binding profile and higher half-life it showed better pre-targeting characteristics. This sequential addition of both avidin and streptavidin in the 3- step form the basis of the 4 step method. [73, 100, 108]

The five-step protocol came about as a solution to problems that excess, unbound streptavidin caused while in circulation. Streptavidin low clearance became a disadvantage when an excess of it remained in circulation before the administration of the biotinylated therapeutic agent.[109] The possibility of the labeled biotin to bind to the streptavidin in circulation without reaching the tumor became high thus was compromising the effectiveness of the targeting concept. Biotinylated albumin was added after addition of streptavidin to clears the unbound freely circulating streptavidin before the addition of the biotinylated therapeutic agent.[108] The pre-targeting protocols are summarised in Table 1.

Table 1: Pre-targeting protocols

Method	Steps (administration protocol)	Explanation
1-Step	Biotinylated mAb with therapeutic agent	Direct accumulation to the tumor(no pre-targeting)
2-Step	Biotinylated mAb	Accumulation on the tumor
	(Strept)Avidin labelled with therapeutic agent	Streptavidin or avidin binds to the biotinylated mAb
3-Step	Biotinylated mAb	Accumulation to the tumor
	Streptavidin or avidin	Binds to biotinylated mAb
	Biotinylated therapeutic agent	Binds to Streptavidin or avidin
4-Step	Biotinylated mAb	Accumulation on the tumor
	Avidin	Clears and clears non bond biotinylated mAb that could still be in circulation
	Streptavidin	Binds to biotinylated mAb accumulated on the tumor
	Biotinylated therapeutic agent	Binds to streptavidin sites available
5-Step	Biotinylated mAb	Accumulation on the tumor
	Avidin	Clears non-bound biotinylated mAb that could still be in circulation
	Streptavidin	Binds to biotinylated mAb accumulated on the tumor
	Biotinylated albumin	Clears non-bound streptavidin in circulation
	Biotinylated therapeutic agent	Binds to streptavidin sites available

1.5.2.7.3. 3 Biotinylated receptor targeting compounds for BNCT

Receptor targeting concept has been widely exploited in drug development for a variety of cancer cells. A number of chemotherapic agents have been synthesized based on their receptor binding capabilities and could be extended to BNCT studies as well.[31] Boron compounds with receptor-targeting groups can selectively deposit ^{10}B to tumor cells with over-expressed receptors. Biotinylated molecules when conjugated with boron compounds could possible result in boron delivery agents with improved tumor selectivity, water solubility (biotin is highly water soluble) and less toxic. Multistep pre-targeting with avidin and streptavidin could be advantageous in depositing increased boron content into the tumor with better attachment for ^{10}B delivery.

However the boron concentration deposited into the tumor cannot be influenced only extent avidin-biotin interaction but also the boron content of the boron compound attached. There have no reports on boronated-biotinylated compounds that have been synthesized and evaluated in vivo or in vitro for BNCT studies. In this project such compounds were to be synthesised and detailed description would be in discussion section.

1.5.2.8 Other boron delivery agents

Some low molecular weight groups which when enriched with boron could possess tumor specific properties are under development. The groups attached include, porphyrins, benzimidozoles,[5] oxodium derivatives, polyamine, phenathridium derivatives, antibactetrial protein avidin,[9] purine, pyrimidine, nicotinamides, azululenes, dequalinim and retinoic acids.[6] Other boronated dendrimers such as polyamidioamine(PAMAM) have berrn reported to deliver great content of boron to tumor cells is the epidermal growth receptor (EGFR) and its mutant isoform EGFRvIII.[90] The boron delivery potentials of a majority of boronated compounds of these groups have not been evaluated extensively both *in vivo* and *in vitro*.[20]

1.5.3 Imaging of BNCT target agents

Most failures of BNCT clinical experiments were attributed to the insufficient ^{10}B content on the target tumor and the distribution of the boron *in vivo* studies could not be determined.[110] Advances has been made to improve the tumor selectivity of boron delivery agents by the addition of different tumor targeting moiety on boron containing compounds as discussed earlier.[4, 18] The imaging improvements on BDA are equally important to tumor selectivity advances. It is believed that if a ^{10}B compound could be monitored from intake, transportation through blood barriers and until it reaches the tumor, toxicity of neighbouring cells could be minimised. The binary nature of BNCT clearly supports this believe because irradiation of neutrons could be performed when the imaged drugs have reached the target tumor.

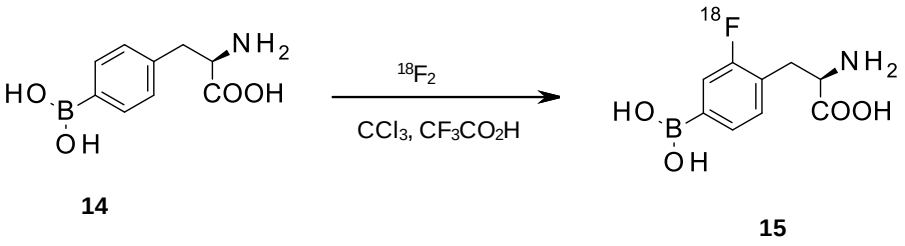
Imaging studies on boron delivery agents for BNCT experiments are currently conducted across the world. However most studies have been conducted on clinically proven BDA such as BPA and its derivatives. This is because BPA analogues satisfy most of the requirements for a good boron delivery. [111]

1.5.3.1 Imaging for BPA

Various studies have shown that BPA was capable of accumulating on various tumors in large quantities relative to normal cells. The exact mechanism have not been established but passive diffusion is the most possible way in which the amino acid of the BPA can get into the tumor cells of increased metabolism.[112] The ratio of boron distribution on the tumor relative to the normal could not be established during trials on BPA before neutron irradiation. The early development to imaging of BPA involved the use of magnetic resonance imaging methods. However MRI method's dependence on ^{11}B that on ^{10}B meant that the existing dosage had to be multiplied to achieve any imaging. Doubling the BPA dosage would in turn increase the boron concentration on normal cells (>5μg/kg) thus causing toxicity of neutron irradiation. The use of MRI on boron 10 compounds was unsuccessful.[110]

Recent developments of imaging methods have seen the use of positron-emitting radionuclides that could be monitored using Positron Emission Tomography (PET) and Single Photon

Emission Computer Tomography (SPECT).[113] BPA and its analogues have been radiolabelled with Fluoride-18 for PET studies to determine the tumor to normal cells boron ratio. The advantage of PET imaging of ^{18}F over other radionuclide is that structurally the compound is not modified.[114] Vähätalo *et al*., reported the synthesis of BPA could be fluorinated to a radioactive ^{18}F-BPA **(14)** by direct bombardment of BPA **(15)** with trifluoroacetic acid-freon-11 **(Scheme 2)**.[115]



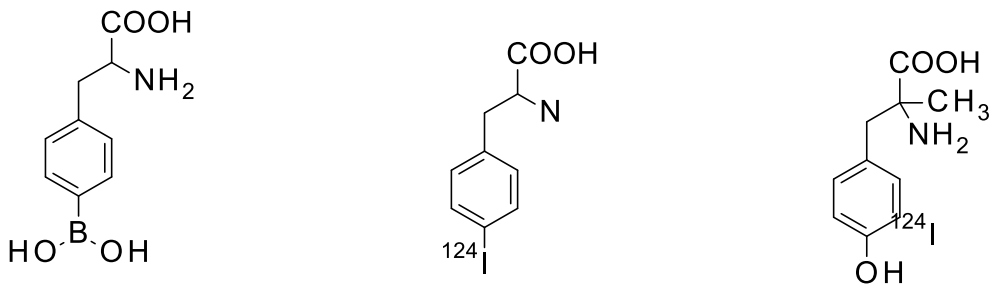
14 **15**

Scheme 2

In a compressive study to determine the difference in tumor accumulation between labelled BPA and unlabelled BPA, Chandra *et al.*, in 2002, reported that there was no significant difference in boron content delivered by ^{18}F-BPA and unlabelled BPA using SIMS-based imaging technique of iron microscopy.[112, 116] The success of ^{18}F-BPA labelling encouraged the development of radioactive labelled BPA and other newly developed boron delivery agents.

1.5.3.2 Other radio tracers for BPA

Earlier studies on radio tracers for BNCT have been restricted to the use of ^{18}F on clinically proven drug BPA as discussed above. However, current research has stretched to using other radio tracers that could be imaged by Positron Emission Tomography (PET) on BPA and other boron delivery compounds.[117] Weinreich *et al.*, have reported the synthesis of iodine BPA derivatives by direct electrophilic labelling with 1,3,4,6-tetrachloro-3a,6a-diphenyl-glycoluril (Iodogen ^{TM}) on the most vulnerable dihydroxylboronic acid part of BPA at good yields of 45-65 and 95% purity to give products in **Figure 12**.[118] Vahätälo *et al.*, confirmed tumor deposition of ^{124}I-BPA by PET imaging before it reaches its radioactive decay at 132 hours.[119]



16 **17** **18**

Figure 12

Figure 12: Showing p-Boronophenylalanine **(16)** and its iodo labelled analogues p-[124I]Iodophenylalanine **(17)** and DL-3[124I]Iodo α-methyltyrosine **(18)**.[118]

The difficulty in iodinating BPA has been outlined by both Weinreich and Vähätalo. The production of ^{124}I was through nuclear reactions which could be the reason why clinical trials have not been conducted on ^{124}I BPA. [119] On the other hand, ^{18}F-BPA ease to label by Vahätälo procedure of direct electrophilic labelling without any major structural modification have seen it being extensively studied. Aihara *et al.* reported the first success of clinical trial using PET-^{18}F-BPA imaging on tumors other than GBM (head and neck tumors).[48, 111]

1.5.3.3 Limitations of ^{18}F radio tracer for BPA

Imahori *et al.* first reported that ^{18}F BPA have been can bind to the malignant tumor cells via the over-expressed L-amino receptors with their amino moiety in the structure in the same way as BPA.[120] However, Bergman *et al.*, reported that the lipophilicity of BPA could be affected by the presence of the flourine radiolabel hence compromising the tumor identifying moiety of BPA. Radiotracers are relatively known to undergo decay after a certain period of time to form fragments which are to be removed by the body transport system. The ^{18}F decay is called defluorination and it happens after 110 minutes after administration.[121] In a review, Pike in 2009, had reported that defluorination of radiotracers produces ions that accumulates to the skull causing inaccurate quantification when PET imaging is conducted.[117]

1.6 Neutron Sources

The other aspect of the binary nature of BNCT is the radiotherapy- the supply of neutrons to the boron 10 attached or taken in by the tumor. Therefore finding the right neutron source is as vital as the synthesis of an ideal boron carrier.[11] The neutron source whose beam delivers high neutron flux enough to irradiate ^{10}B to ^{11}B without causing damage to neighbouring normal cells is ideal for BNCT. Economic considerations have also influenced the development of neutron sources in that sources of low capital cost and maintenance are favourable. Neutron beam characteristics including their composition and rate at which neutrons are released are important in choosing the neutron source.[12] Ideally, neutrons that reach the tumor at minimum velocity possible are capable of spending time needed to irradiate ^{10}B. A neutron beam, free of unwanted rays like gamma rays and photons which are lethal to normal cells and can penetrate tumor deep inside can perfect BNCT principles.[5, 12]

1.6.1 Nuclear reactor as neutron source

The use of neutrons for BNCT was first reported in 1939 by Gordon Locher but if was only around 1951 that the first clinical trials were performed in Brookhaven National Laboratory(BNL) on patients with gliomas. Thermal neutron beams for nuclear receptors were used to supply neutrons to patients injected with borax and later other boron agents were tested. About that time to early 1990's similar studies started at Massachusetts Institute of Technology (MIT), Brookhaven Medical Research Reactor (BMRR) and Japan on a wide range of boron carriers.[11] The experiments showed promise on tumors located about 4cm deep from the surface but failed for tumors deeper than that. Other limitations on the use of neutron beams for clinical trials were that, nuclear reactors were not collimated to protect health cells and they came at a high installation and maintenance costs with security clearance required. In general, there few nuclear reactors available for research and are located away from health centres hence in-accessible to a large number of tumor patients.[12, 122]

It was until 1994 that clinical trials using a better neutron beam with superior tumor penetration capability when compared to thermal neutrons was reported in the United States of America at BNL and MIT.[16, 22] The epithermal neutron beams were reported to reach tumors approximately 10cm deeper with neutrons of energies of 0.5 keV<En<10 keV or faster (En>0.5). Different types of nuclear reactors have been used as sources of epithermal neutrons across the world. BMRR, Finnish Research reactor and High Flux Reactor (HFR) have been reported to deliver epithermal neutrons. In 1997, epithermal neutrons beams released by the High Flux Reactor were reported in Petten, Netherlands. However it was discovered that a few alterations on the incident epithermal beam had to be done to improve cytotoxicity. These alterations include, beam moderation, filtration, neutrons collimation and purification.[123]

1.6.2 Accelerator based neutron source

Epithermal neutron beams just like thermal neutron beams were sourced from nuclear reactors so they shared the same limitation of high installation cost and inaccessibility to patients. Therefore there was a need for cheap, safe neutron source before researchers at the University of Birmingham reported accelerators as an alternative neutron source.[24] The advantages of accelerator based neutrons source (ABNS) over reactors has been their ease of installation in hospitals, could be turned off when neutron field is no longer required, is considered safe hence no licences required.[7]

The studies on the development of ABNS is influenced their design which include accelerator hardware, neutron producing target, cooling system and type of moderator.[23] The target that was reported to capable of producing low-energy neutrons when bombarded with 2.5MeV protons was lithium.[6, 10, 122] Lithium targets came with notable disadvantages of having a low melting point (180ºC) and low thermal conductivity thus bulk of the heat would be held in the target. Moreover, lithium's high reactivity when exposed to air could make lithium not an ideal neutron producing target.[124]

Recently, targets with much higher melting point than lithium which were capable of producing neutrons were reported. Carbon and Beryllium have been investigated as lithium alternatives;

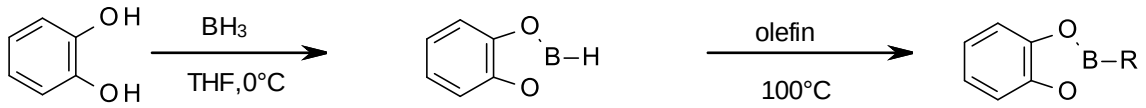
however insufficient number of neutrons was produced. It was proposed that if the proton energy could be increased to 4 MeV while the beryllium target is used then the neutron production improve. While this hypothesis was proved to be true, the basis of developing a cheap and less complex alternative to reactors was distorted.

Randers-Pehrson *et al.*, reported the use of lithium-beryllium hybrid target as ideal for neutron production. They proposed that if a thin beryllium layer were to be bombarded with protons of 4MeV and followed by cooling with liquid lithium would not only produce adequate neutrons but was cost effective as well.[124] To slow down the neutrons for effective capture by ^{10}B, a moderator made of heavy or light water (D_2O and H_2O) is used to scatter excessive radiation.[1] Aluminium and its halide, AlF_3, have been used as moderators for ABNS while the best moderator reported has been Fluental$^{@}$ which is a mixture of Al, AlF_3 and LiF.[122]

Clinical trials on accelerator-based BNCT that have been performed around the world, has been on linear accelerators for they are already available in most hospitals. Linear accelerators could be manipulated to produce beams as per patient needs through the Monte Carlo calculation.[2] Another class of accelerators that have been investigated for neutron production for BNCT has been the recirculation type, namely the cyclotrons. In the cyclotrons there is a continuous circulation of one particle making it compact and cost effective in terms of maintenance However cyclotrons produce beams inadequate for accelerator-based BNCT. Studies on how to improve effectiveness of cyclotrons are currently underway in Japan and in the United States.[23]

1.7 Previous work done by the group

The chemistry heterosubstituted boranes have been reported in literature for their potentials in organic synthesis. The most notable research area in which these compounds have been studied extensively is in hydroboration. The use of heterosubstituted boranes as hydroborating agents was first proposed by Brown *et al.,* in 1971.[125] This group reported the preparation of catecholborane **(20)** by the hydrolysis of alkaneboronic acids **(19)**, as a possible hydroboration agent for olefins **(21)** that could give better yields when compared to earlier generation of non-cyclic agents**(Scheme 3)**.[126]



19 **20** **21**

Scheme 3

The limitation of catecholborane reported by Gupta *et al*., was that it only hydrogenated alkenes and alkynes at a temperature range of 70-100 ºC which resulted to a possibility of the formation of proportionating products.[127] In 1975 Kono *et al.,* reported the use of metal catalyst for hydroboration that could be performed at lower temperatures. Manning and Noth investigated the concept of hydroboration using Wilkinson catalyst ($Rh(PPh_3)_3Cl$).[128] Several hydroboration of olefins studies have been performed using this catalysts and other newly synthesised metal catalysts. The research however has not been restricted to finding a new catalyst but other researchers have focused in the development of catecholborane derivatives with had better stability.[129]

Hadebe *et al.,* reported the synthesis of nitrogen and sulphur analogues of catecholborane **(20)**, 1,3,2-benzodiazaborolane **(22)** and 1,3,2-benzodithiaborolane **(23)** which had better stability when compared to catecholborane at elevated temperature.[128] The nitrogen analogues of catecholborane, 1,3,2-benzodiazaborolane, was reported as the most stable of all the 3 compounds for there were no disproportionation observed by Hadebe *et al.,* when it was subjected to hydroboration experiment with 1-octene. The stability was measured based on the

extent of disproportionation of the three compounds when reacted with octane under temperatures ranging from 100 to 150 ºC over a certain period of time as shown in **Figure 13**.

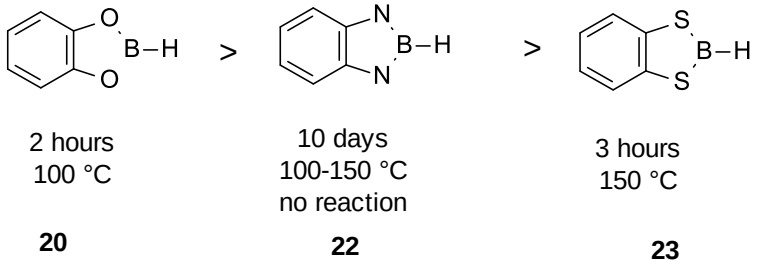
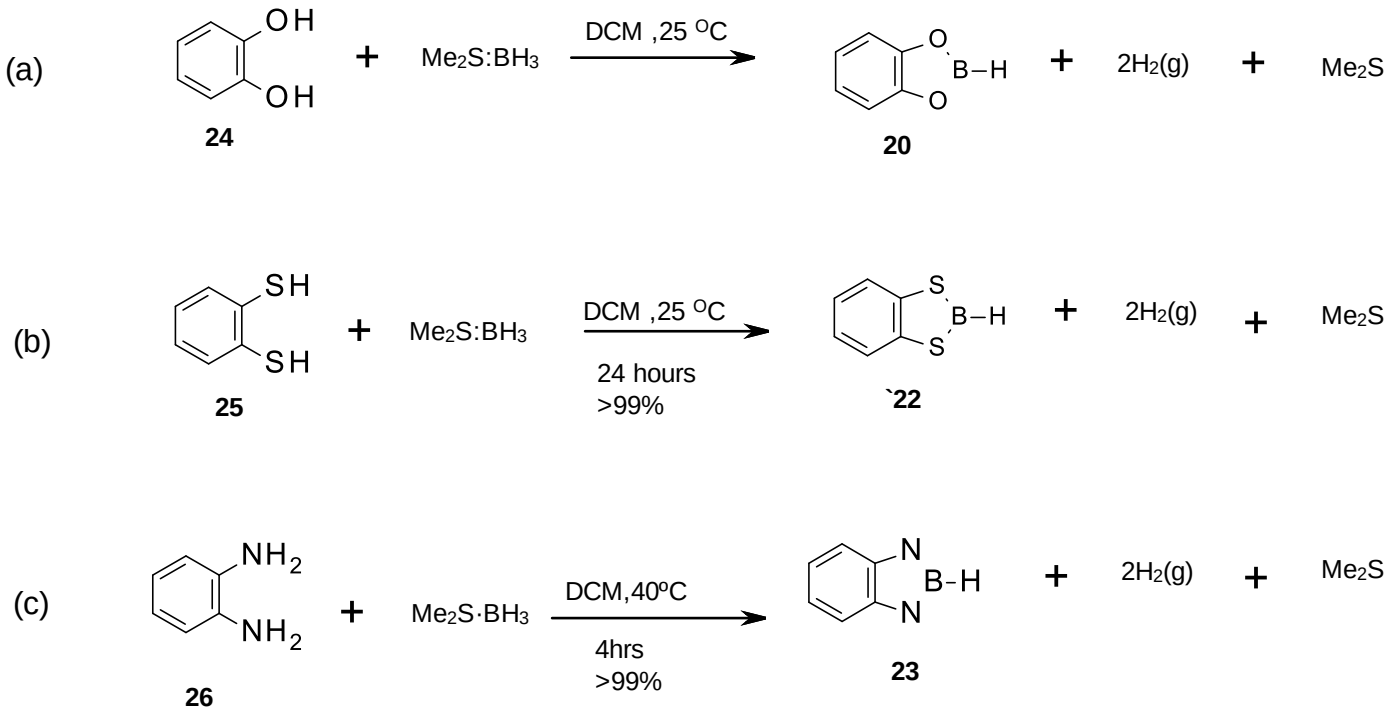


Figure 13

Hadebe *et al.,* reported disproportionation products of the catecholborane [tri(catecholato)diboron,H_3B/PPH_3 and boric acids]that have previously been reported by Westcott *et al*. The un-catalysed reaction between 1,3,2-benzodiazaborolane with 1-octene was a non-started after 10 days yet when catalysed by Rhodium catalysed [RhCl $(PPh_3)_3$], a 70% conversion was reported by Hadebe *et al.,* with no disproportionation products observed using GC-MS spectroscopic means and ^{11}B NMR. The stability (absence of disproportionation product) of 1,3,2-benzodiazaborolane was attributed to the electrophilicity of the boron atom because back bonding of π-electrons between the vacant $2p_z$ of the boron atom and the nitrogen atoms when compared to the oxygen analogues.[128]

Hadebe and Robinson report the synthesis of 1,3,2-benzodiazaborolane **(22)** and 1,3,2-benzodithiaborolane**(23)** using a procedure that had earlier been proposed for catecholborane preparation **(20) (Scheme 3)** by Brown *et al.*[125, 128] This scheme involved the reaction of dimethylsulfide borane complex($Me_2S.BH_3$) with 1,2-diaminobenzene and products were afforded at high yields of up to 99%.[130]



Scheme 4

Scheme 4 (a) Synthesis of catecholborane (b) Synthesis of 1,3,2-benzodithiaborolane (c) Synthesis of 1,3,2-benzodiazaborolane.

Encouraged by these results, the Robinson group has been involved in the preparation of a wide range of heterocyclic hydroborating agents and further evaluating their activity on different types of mechanisms.[131] Robinson and Hadebe have synthesised a number of 1,3,2- diazaboralane analogues by adding of different groups on their π-spacer end (**Figure 14)**.[130, 132]

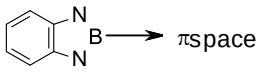
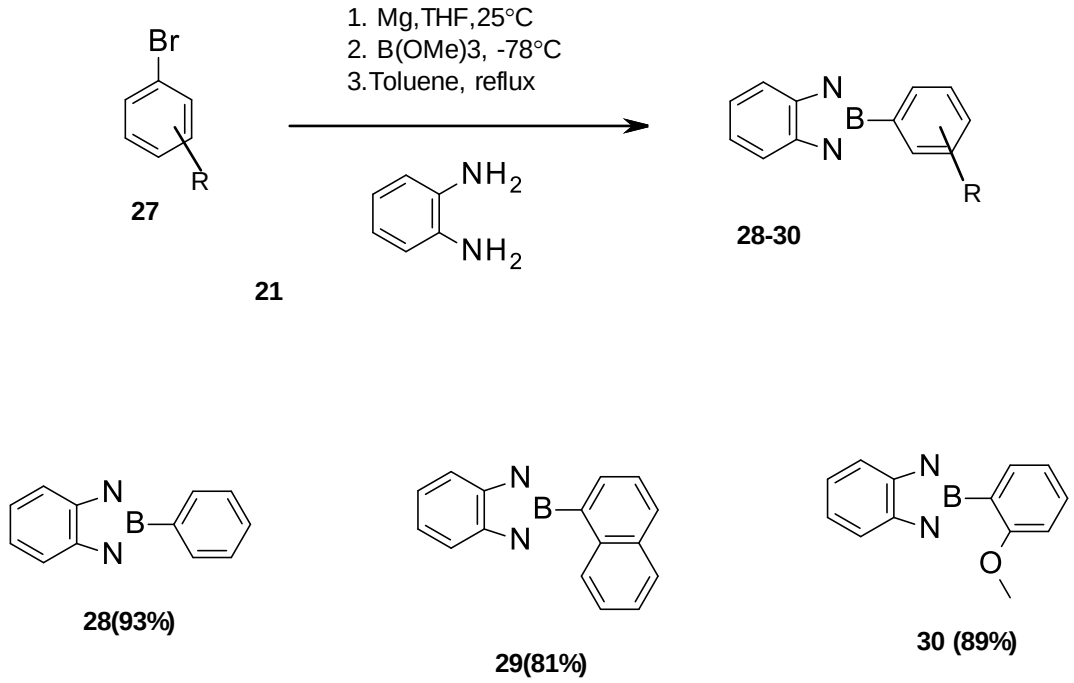


Figure 14

Sithebe *et al.*, a member of our group, recently created a library of 1,3,2- diazaboralane analogues. The 1,3,2-diazaboralane analogues were synthesized *via* the boronation of bromobenzene derivatives to a boronic acid followed by addition of *o*-phenylenediamine group

(Scheme 5). Cross-coupling of this compounds (**22-24**) to a variety of bromobenzene in the presence of a palladium catalyst was reported on yields of 67-85 percent.[133]



Scheme 5

Currently, a number of organoboron compounds are being synthesised by different members of the group for hydroboration of olefins, cyclic alkene and Suzuki-Miyaura cross coupling reactions.[130] The reported stability of 1,3,2-benzadiaboroles by Hadebe *et al.*, has sparked interest from other groups in different parts of the world. This so found interest has led to the preparation of a wide range of the 1,3,2-benzadiaboroles analogues from different organic synthetic protocols.[131]

1.7.1 Luminescence properties of 1,3,2-benzadiaboroles

Molecular three coordinate organoboron compounds were first reported by William and co-workers as having luminescent properties, a research that was taken further by Weber *et al*.[134] The chemistry of 1,3,2-benzadiaboroles, an organoboron compound, has widely been investigated in recent years as an alternative to radio tracers which not only have a short-life but could be cytotoxic in medicinal applications.[135]

The luminescence capabilities of 1,3,2-benzadiaboroles have been attributed by Weber *et al.*, together with a host of research groups to the extended π-donating system of the three coordinate 1,3,2-benzadiaboroles with benzene, biphenyl, flourene, thiophene and dithiophene.[132] It has been reported that the boron centre has a vacant $2p_z$-orbital so it can act as an acceptor of π-orbitals from dimethylamino groups and also can accept from the attached π-orbitals of aromatic groups such as benzene.[136] The π-back-donation results in an electron jump from the LUMO to the HOMO and when the stability of the boron is achieved, the electron are de-activated to the LUMO , they fluorescent.[137]

Weber *et al.*, reported that the luminescence properties of the π-complexes of 1,3,2 diazoborolyl compound were not solvent dependent. A blue luminescence was observed in most compounds on different solvents. Robinson's group has not extensively studied the florescence properties of most of the 1,3,2 diazoborolane analogues they have synthesized.

It has been proposed by this group that because of the remarkable finding on both the stability and fluorescence properties of 2-benzo-1,3,2-diazobenzoborales, they could be vital in the synthesis of cancer drugs. In this study, it is proposed that if a clinically proven or newly developed anti-cancer drug were to be added 2-benzo-1,3,2-diazobenzoborales moiety both its physiological stability and luminescence properties could be improved.

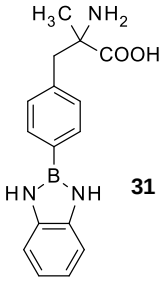
The understanding is that if therapeutic agents targeting tumor could florescence, then tracing that drug from administration to when it transverse through body barriers to the tumor location would be possible. As discussed earlier in BNCT context, boron delivery agents have been labelled with radio traces to improve their imaging properties. The limitations that come with labelling using radio traces particularly for clinically proven drug such as BPA have also been discussed. The limitations of radio traces include their short- half-life and release of toxic substances during deactivation. In this study, it is proposed that if tumor targeting boronic acids could be converted to be 1,3,2 benzodiazaboroles derivatives both stability and fluorescent properties could be enhanced. In the following sections, this proposition will be investigated experimentally.

1.8 Project Objectives

The BNCT principles require good delivery of the boron-10 atom into the tumor in the right concentration before it can be irradiated with a neutron beam to form ^{11}B integrates (alpha and lithium nuclei) that cause total cell total death.[59] While progress in the research on the development of a cheap, good neutron source has been made, the development of an 'ideal' boron delivery agent is still very much under research.[138] Researches have so far been unsuccessful in their quest to design drugs that meets all the requirements of 'drug ideality' (discussed earlier 1.4) hence there is still a need to develop drug that could at least meet most of those requirements.[20]

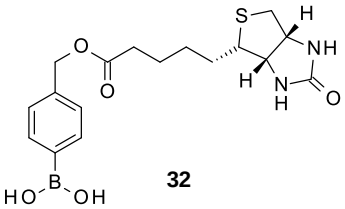
The aims of this project were:

- To Synthesize 2-(4-(1H-benzo[*d*][1,3,2]diazaborol-2-2(3H)-yl)benyzyl)-2-aminopropanoic acid **(31)** , an α-methyl BPA attached with fluorescent 1,3,2-benzodiazaborolane grouping.
- Synthesise a boron 11 containing compounds that contained a receptor targeting biotin. **Those biotinylated boron compounds were (Figure 15)**:



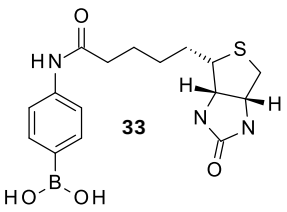
31

2-(4-(1H-benzo[*d*][1,3,2]diazaborol-2-2(3H)-yl)benyzyl)-2-aminopropanoic acid **(31)**



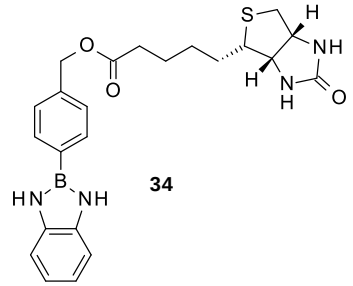
32

4-((5-((3a*R*,6*S*,6a*S*-hexahydro-2-oxo-1*H*-thieno[3,4-*d*] imidazole-6-yl)pentanoyloxymethy)phenylboronic acid **(32)**



33

4-((5-((3a*R*,6*S*,6a*S*)-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-6-yl)pentanamido) phenylboronic acid **(33)**



34

4-(1*H*,benzo[*d*][1,3,2] diazaborol-2(3*H*)-yl)benzyl 5-((3a*R*,6*S*,6a*S*)-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-6-yl) pentanoate **(34)**

Figure 15

- Optimise the method or synthesis routes and try using boron-10 instead of boron-11 for BNCT studies.
- Perform biological evaluation of compounds for their tumor targeting capabilities *in vivo and in vitro.*

2. Discussion

2.1 Preface

The focus of this project was to synthesise boron compounds that could be used as boron delivery agents for boron neutron capture therapy (BNCT). In this section, the choices of the compounds to be synthesised will be justified. To elucidate the findings from the laboratory investigations, different synthetic strategies reported in literature were considered. The findings from the lab are to be reported in this section together with their justification.

The boron compounds that were to be synthesised as previously shown in **Figure 15**:

- 2-(4-(1H-benzo[*d*][1,3,2]diazaborol-2-2(3H)-yl)benyzyl)-2-aminopropanoic acid **(31)**
- 4-((5-((3a*R*,6*S*,6a*S*-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-6-yl)pentanoyloxymethy)phenylboronic acid **(32)**
- 4-((5-((3a*R*,6*S*,6a*S*)-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-6-yl)pentanamido)phenylboronic acid **(33)**
- 4-(1*H*,benzo[*d*][1,3,2] diazaborol-2(3*H*)-yl)benzyl 5-((3a*R*,6*S*,6a*S*)-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-6-yl)pentanoate **(34)**

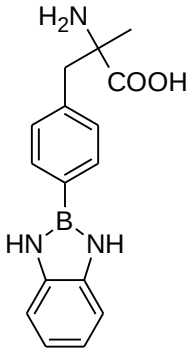
2.1 Synthesis of BPA analogues with fluorescence organoboron moiety,1,3,2-benzodiazoboroles

The failures of many early BNCT studies were attributed to the lack of imaging of boron delivery agents.[110] A number of imaging developments have been made on clinically proven drugs e.g. boronophenylalanine (BPA) and it analogues as discussed in the introduction (Section 1.5.3). Radio tracers such as fluorine-18 and iodo-124 have been attached to BPA and its analogues for imaging purposes in BNCT studies.[120] The clinically proven radio-labelled BPA is the fluorine labelled BPA, ^{18}F BPA has been used in experiments together with fructose to improve its water solubility.[119] Other limitations of existing radio traces include short half-life, reduced lipophilicity, and excretion of toxic materials on deactivation.[117]

The limitations of imaging using radio-labelling of BPA have underlined the need to develop other compounds that possess luminescence properties. William and co-workers first reported that organoboron compounds, 1,3,2-benzodiazoboroles derivatives possess fluorescent properties and could be perfect for imaging.[132] The main advantage of these compounds over existing radio traces was their stability (longer half-life). The stability on these compounds was reported by Hadebe *et al.,* as mentioned in the introduction **Section 1.7**.[136] The long half-life of the fluorescent 1,3,2-benzodiazoboroles compounds with tumor targeting moiety can be imaged as it transverse blood barriers into the tumor cells without dissociating.

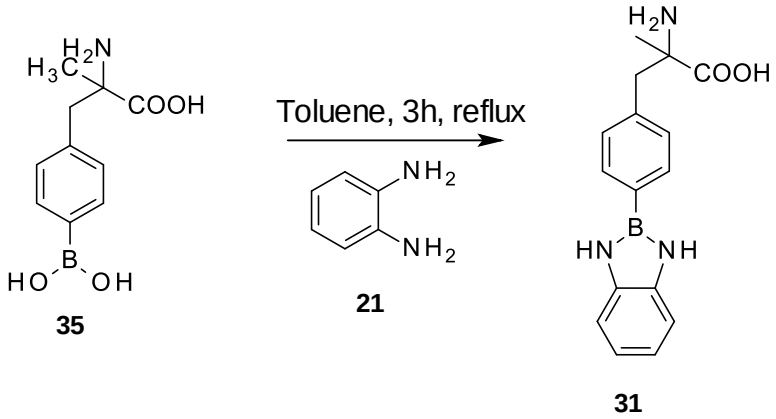
In this study it is proposed that if BPA, a clinically proven BNCT drug that attaches to the tumor,[59] could be attached to a fluorescence organoboron moiety-1,3,2-benzodiazoboroles, the limitations in imaging discussed above for ^{18}F-BPA could possibly be eliminated. Furthermore, Hubner *et al.,* reports that amino acids with quaternary–carbon were more robust in glioblastoma multiforme tumor localisation when compared to BPA.[139] Taking this two properties into consideration, it is proposed an addition of a fluorescence organoboron grouping (1,3,2-benzodiazoboroles) to α-methyl-BPA can afford a boron delivery agent with better imaging properties.

2.4.1 Synthesis of 2-(4-(1H-benzo[*d*][1,3,2]diazaborol-2-2(3H)-yl)benyzyl)-2-aminopropanoic acid (31)



31

There has not been any reports on the total synthesis of 2-(4-(1H-benzo[*d*][1,3,2]diazaborol-2-2(3H)-yl)benyzyl)-2-aminopropanoic acid **(31)** in literature. The thinking was that the synthesis of **31** was possible over two steps. The first step being the preparation of 2-amino-2-methyl-3-(4-dihydroboxybortlphenyl) propionic acid (α-methyl BPA) **(35)** was to be prepared, followed by nucleophilic substitution of *o*-phenylenediamine on the boronic acid part of α-methyl BPA **(Scheme 6)**. The second step was modelled from what was proposed by Slabber *et al.*, a member of our group in converting a series of boronic acids to 1,3,2-diazaboroles derivatives.[140]

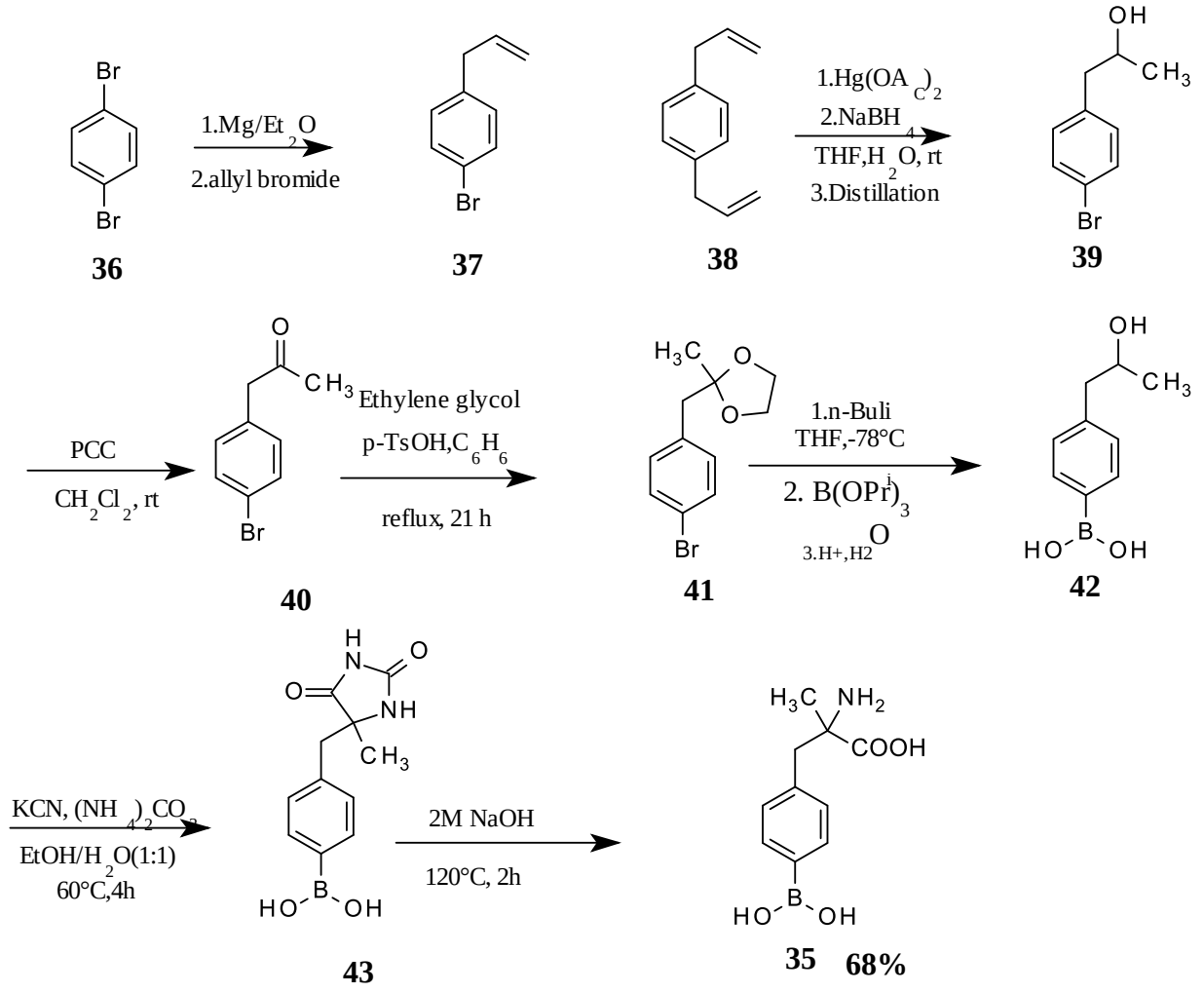


35 **21** **31**

Scheme 6

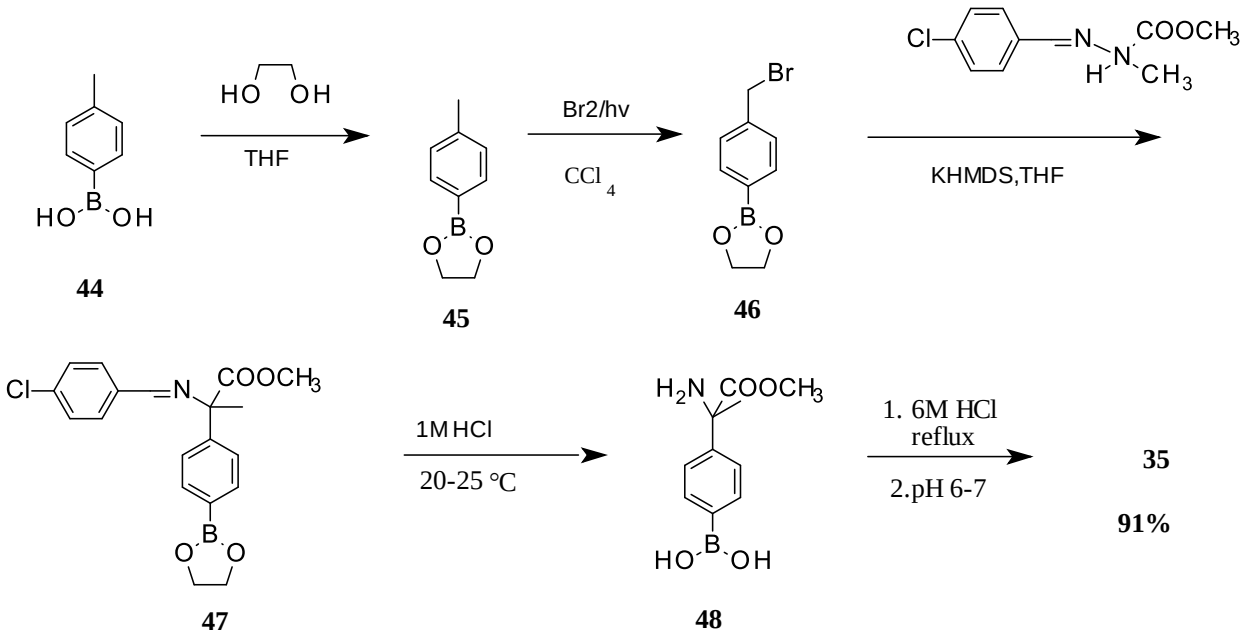
2.4.1.1 Synthesis of 2-amino-2-methyl-3-(4-dihydroboxybortlphenyl) propionic acid-(α-methyl BPA (35)

Zaidlewicz *et al.,* reported the synthetic route for preparing α-methyl BPA at a yield of 68%.[141] The synthesis began with the alkylation of 1,4 dibromobenzene **(36)** using allylbromide *via* a formation of a Grignard reagent to afford 4-bromoallylbenzene in mixture of 84:14 **(37-38)**. The mixture would then be hydrolysed using mercury acetate with sodium borohydride to afford **(39)** which was oxidised using pyridium chlorochromate (PCC) to give **(40)**. Ketone protection using ethylene glycol gave product **(41)** which was boronated with triisopropylborate to give product **(42**). The amide grouping was then introduced to afford hydantoin **(43)** which was hydrolysed to afford the desired product **(35)**.[142]



Scheme 7

Zaidlewicz *et al.*, reported another route for the synthesise α-methyl BPA. This synthesis begins with the protection of *p*-tolyboronic acid **(44)** to from an ester **(45)** which would be brominated to give product **(46)**. The brominated boronic ester **(46)** is then reacted with the carbanion by deprotonation of *D,L* aniline and potassium bis(trimethylsilyl)amide (KHMDS) to afford **(47)** which produces the amine **(48)** on de-protection with a strong acid. Further deprotection of **48** with 6 M HCl under reflux would afford the desired product **35** at 91 % yield **(Scheme 8)**.[142]

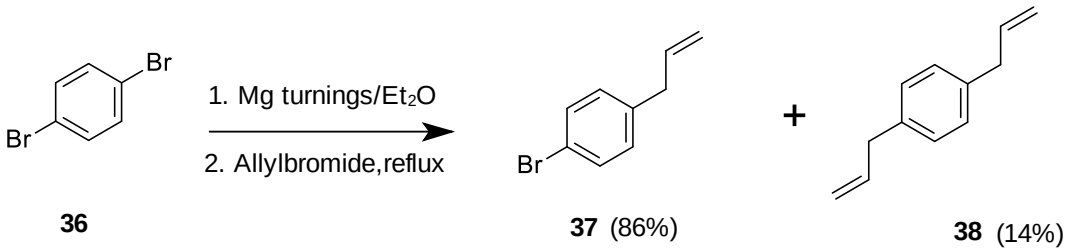


Scheme 8

The synthesis route on scheme **7** was chosen because the chemicals were commercially available.

2.4.1.1.2 Synthesis of 1-ally-4-bromobenzene (37)

Zaidlewicz *et al.,* reported that synthesis of 1-ally-4-bromobenzene **(37)** could be achieved by reacting 1,4 dibromobenzene with allybromide *via* the formation of a Grignard reagent **(Scheme 9)**. The formation of the side product 1,4-diallylbenzene **(38)** was also reported to be at 14 % ratio to the desired product. [142] The formation of the di-nucleophilic addition of the ally group could be attributed to the addition of excess allylbromide (more than 1 equivalent) and performing the reaction under high temperatures.

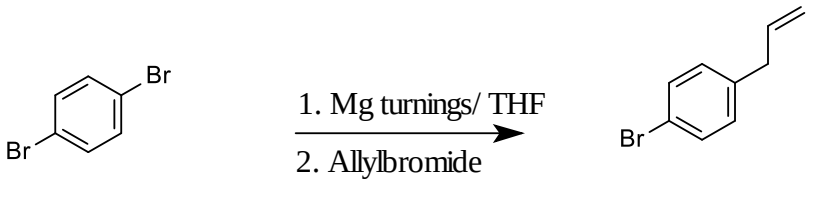


36 **37** (86%) **38** (14%)

Scheme 9

Taber *et al.,* have reported the preparation of 1-ally-4-bromobenzene **(37)** from the reaction of 1,4 dibromobenzene with allylbromide *via* the formation of a Grignard reagent at a 99% yield.[143]

The procedure by Taber *et al.,* was followed and the desired product **(37)** was achieved as a yellow oil with a 75% yield. The product was confirmed using spectroscopic techniques (^{1}H NMR, ^{13}C NMR and COSY). However, the desired product was in a mixture with the starting material **(Scheme 10)**. Efforts to separate the starting material and the product using chromatography were unsuccessful since the two compounds were very similar structurally and thus could be eluted about the same time, a similar problem to that encountered by Zaidlewcz *et al.*[141]



36 **37**

Scheme 10

The ^{1}H NMR spectrum showed integral ratios of the desired product **(37)** as reported by Taber *et al.,* and Zaidlewcz *et al.*, The formation of the allyl group was confirmed by the presence of a multiplet (at H-2′) at δ 5.80-5.91 ppm that integrated for 1 proton and the singlet H-3′a at δ 5.02 ppm together with the doublet H-3′b at δ 4.98 ppm integrated to 1 proton each. The COSY showed the expected coupling between the doublet (H-3.5) at δ 7.34 (with J = 8.4) ppm and a doublet (H-2,6) at δ 6.98 ppm (with J = 8.1) for two protons. The peak at δ 7.34 also coupled

with the doublet at δ 7.49 ppm (with J = 8.3) which gave an indication that the product was a mixture with the starting material. The ratio of the desired product to the residual starting material was predicted using the integral ratios of 2:3. The complete ^{1}H NMR and COSY assignments are showed in **Figure 16** and **Figure 20** below.

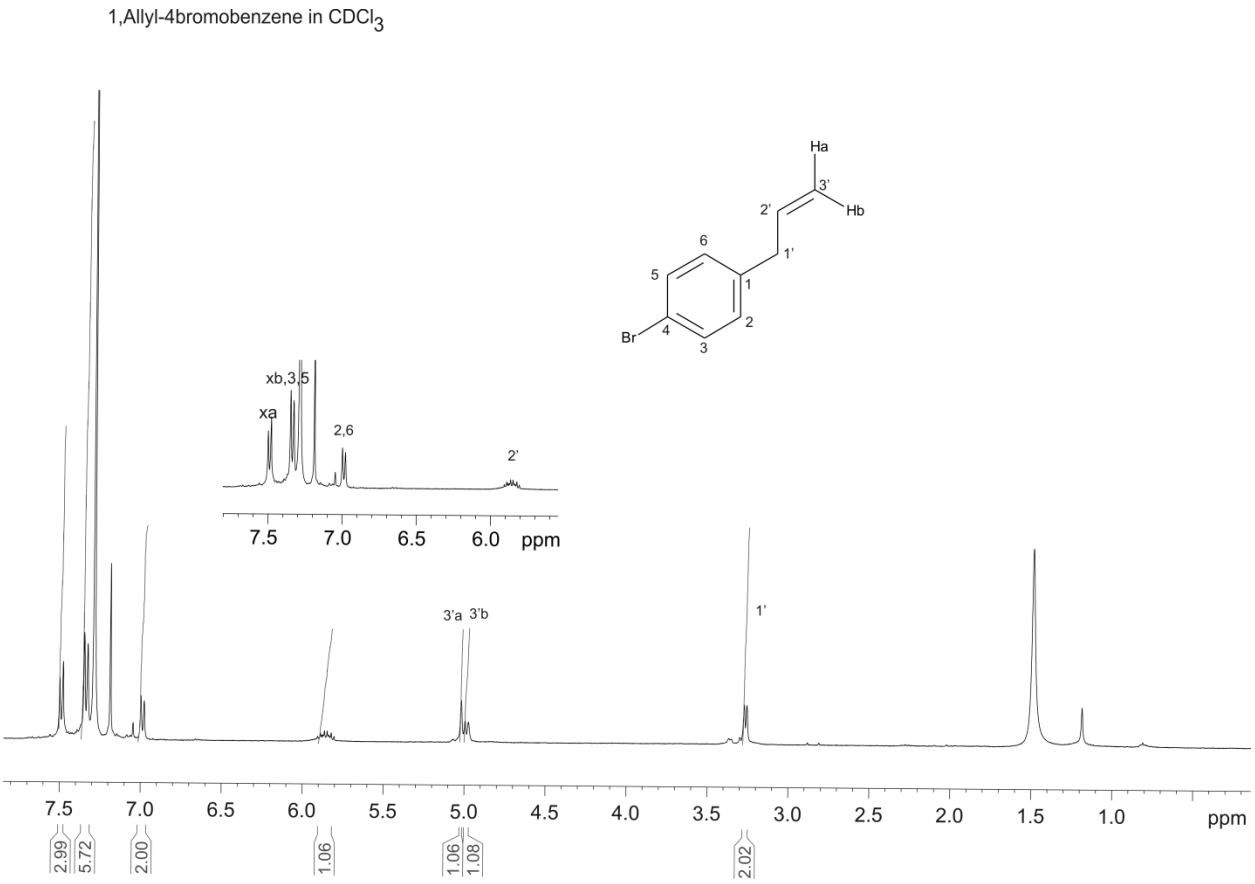


Figure 16: ^{1}H NMR spectrum of compound 37

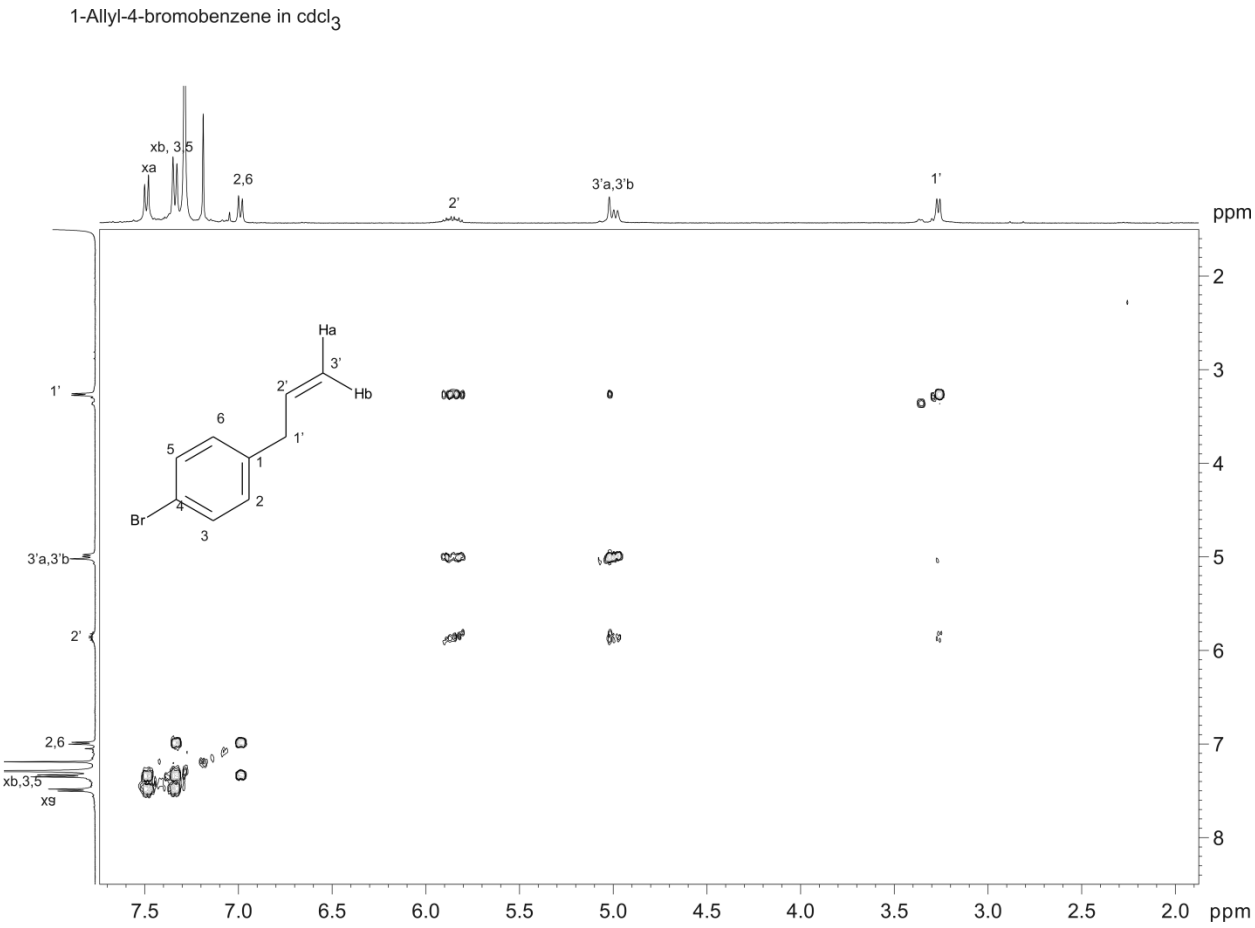


Figure 17: COSY NMR spectrum of compound 37

Note: xa, xb peaks belong to the residual starting material.

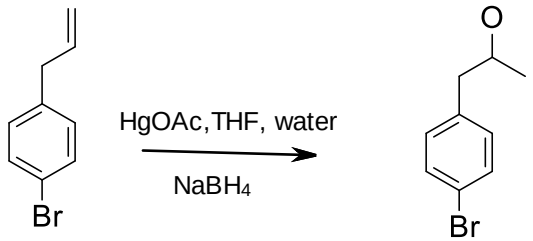
The ^{13}C NMR spectrum was in good agreement with the structure of the product. However peaks for the C-Br (C-4) could not be observed on the ^{13}C NMR spectrum. The absence of the C-4 could be explained by the quadrupolar relaxation mechanism. This mechanism operates on nuclei that have a spin that is greater than $\frac{1}{2}$ since electric quadrupole moment is more dominant compared to the dipole-dipole interaction that occur in nuclei with spin of $\frac{1}{2}$. The effects of the quadrupole moment is that the charge distribution is given a spheroid shape making interaction among the nucleus and surrounding electrons more possible.[144] The extent of the nucleus-electron interaction is reported as quadrupole coupling constant, Q. The value of Q is directly proportional to the relaxation time, T_1. Therefore the shorter the value of Q, the shorter the

relaxation time hence the sharp peaks are observed on both the ^{13}C or ^{1}H spectrum which is the case for all nuclei with a spin= $\frac{1}{2}$. On the other hand for nuclei with a large Q (mostly with spin > $\frac{1}{2}$) have very fast relaxation times which leads to very broad peaks that can hardly be observed in carbon and proton NMR spectrum. ^{79}Br has a nucleus spin of $\frac{3}{2}$ and it undergoes rapid quadrupolar relaxation therefore couplings between carbons cannot be observed in ^{13}C NMR spectrum.

The sharp single absorption peak at 998.6 cm^{-1} on the infrared spectrum indicates the presence of the C-H on an alkene of an allyl group. The assignment of all spectroscopic data was in correspondence with that expected for 1-ally-4-bromobenzene.

2.4.1.1.2 Synthesis of 1-(4bromophenyl) propan-2-ol (39)

The synthesis of 1-(4bromophenyl) propan-2-ol was reported by Zaidlewicz *et al.,* to be achievable by the oxymercuration reaction on 1-ally-4-bromobenzene **(37).** A 99 % yield of compound **39** was reported in the literature in using mercury acetate, sodium borohydride and water with THF as the solvent **(Scheme 11)**.



37 **39**

Scheme 11

This reaction as reported by Zaidlewicz *et al.,* was attempted with the use of other reports from literature. The ^{1}H NMR spectrum confirmed the presence of 1-(4 bromophenyl) propan-2-ol (**39)** with peaks in agreement with those by Zaidlewicz *et al.* However the product was in very low yields of about 10 % in a mixture with the starting materials, the separation of which was difficult. Numerous attempts that included optimising the reaction condition and taking the crude to the next step (preparation of **40**) were unsuccessful. Efforts to prepare 1-(4-Bromophenyl)

propon-2-one **(40)** from the mixture produced a mixture of unknown compounds. With all the problems obtained in this experiment and lack of any alternative, it was decided that a new approach was to be developed.

2.5 Synthesis of Biotinylated boronic acid compounds for BNCT

2.5.1 Preface

The use of biotin, a vitamin H, as a tumor targeting moiety has been widely reported in literature.[104] The conjugation of any compound with biotin is known as biotinylation. A number of biotinylated antibodies have been reported for the treatment of different kinds of tumors.[94] The role of biotin when this conjugation is done is to improve the drug selectivity on the tumor cells while sparing normal cells. The mechanism in which this selectivity occurs is reported to be through the over expressed biotin receptors on tumor cells when compared to normal cells hence biotinylated compounds favour binding on tumor cells.[106] The biotinylation of known drugs for cancer treatment have been explored on different types of cancer therapies most notable on chemotherapic drugs as discussed in the introduction section 2.5.2.7.3.

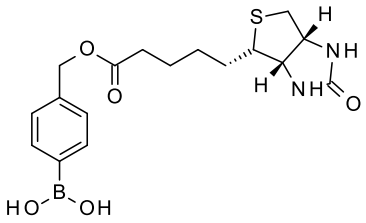
On the other hand the physiological usefulness of boronic acids was first reported by Lorand and co-workers in 1959.[145] Boronic acids versatility to react with substituents such as 1,2 and 1,3-diols including saccharides was attributed to their biological application.[146] Fang *et al.,* following work done by Yoon *et al.,* reported that when boronic acid compounds bind to saccharides a fluorescence intensity change was experienced.[145] The fluorescent properties of these boronic acids make them important in drug imaging on tumor cells. Furthermore, Jennifer *et al.,* reported the stability of boronic acids in aqueous media being brought by its ease to convert from a neutral trigonal form to an anionic tetrahedral form when in contact with water hence these compounds are water soluble. [147]

Boronic acid derivatives have been studied for decades for their potential as boron carriers for BNCT.[145] The first clinical success was reported in 1987 when boronophenylalanine (BPA) was first synthesized by Mashima *et al.,* after the failure on borax which was attributed to it toxicity to neighbouring cells, a problem not reported on boronic acids.[5, 9, 80] The chemical stability that

boronic acids possess has encouraged researchers to synthesis more analogues in development of boron delivery agents.[148]

This work was aimed at the synthesizing boronic acid compounds that would be conjugated to biotin as tumor targeting moiety. These compounds are referred to as biotinylated boron acid compounds. It is proposed that if arylboronic acid could be biotinylated (conjugated to biotin), this would could not only target selectively tumor cells but could improve the water solubility via the water affinity of biotin.[149] Pre-targeting with avidin or streptavidin which have strong affinity to biotinylated compounds would be expected to bring success of *in vivo* experiments as discussed in the introduction.[73]

The biotinylated boron compounds that were to be synthesised in this project are shown in **Figure 18**.



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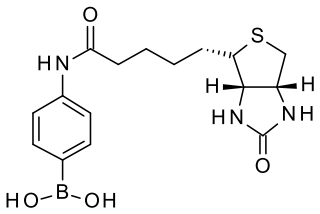

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Figure 18

- 4-((5-((3a*R*,6*S*,6a*S*-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-6-yl)pentanoyloxymethy)phenylboronic acid **(32)**
- 4-((5-((3a*R*,6*S*,6a*S*)-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-6-yl)pentanamido)phenylboronic acid **(33)**

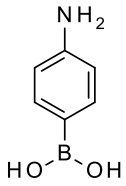
The total synthesis of this biotinylated boron compound has not been reported extensively in literature. Therefore, in this study, the proposed route could be to first synthesise the boronic acid compounds subsequently followed by the conjugation of biotin by chemical means.

2.5.2 Synthesis of boronic acids that could be biotinylated

The choice of the boronic acids to be biotinylated was based on the mechanism in which they can be conjugated to *D*-biotin by chemical means. The structural constituent of the compound to be biotinylated (nature of its most active group) influences the biotin conjugation mechanism. The identity of the spacer part of the biotin would also direct the mechanism of conjugation. ***D***-biotin for example has a carboxylic acid group on its spacer end of which conjugating can take place without any loss of biological activity.[150] It is therefore important to choose the compound to be biotinylated taking into considerations all reactions possible for the carbonyl part of biotin.

The conjugation mechanism that was chosen in this study to bind biotin to boronic acids was *via* the formation of an ester linkage. The reason behind this choice was that *D*-biotin with a carboxylic end was to be used as to biotinylate the boronic acid compounds. Therefore it was essential that the boronic acids have either a reactive alcohol or amine end.

In this study 4-anilineboronic acid **(49)** and 4-(hydromethyl)phenyl boronic acid **(50)** were chosen as the boronic acids to be synthesised that could form an ester or amide linkage to biotin **(Figure 19).**



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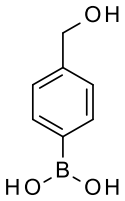

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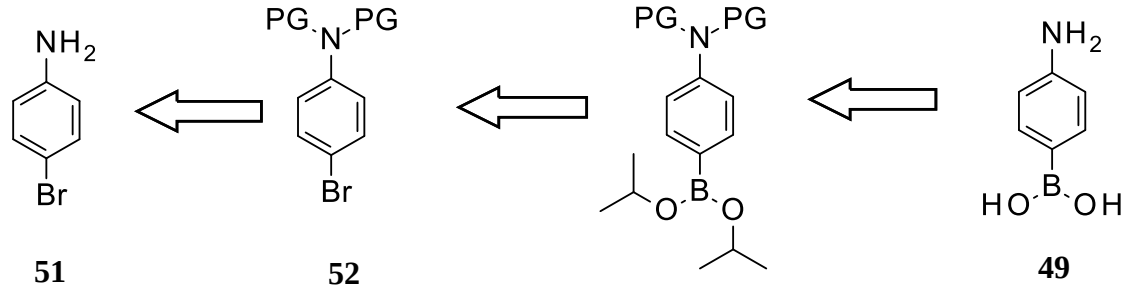
Figure 19

2.5.2.1 Proposed synthesis route of 4-anilineboronic acid (49)

The two synthetic pathways for the preparation of 4-anilineboronic acid **(49)** possible could be achieved *via* the boronic acid addition to an aniline containing compound or by aniline addition to a boronated compound. In this study, the boronation of an aniline containing group was

chosen because the boronating agents were available. Boronic acid addition procedures reported were straight forward when compared to amine addition.

was to be added thus an attack on the before the addition of the boronic acid on Br on the *para*-position to give 4-anilineboronic acid **(49) (Scheme 12)**.



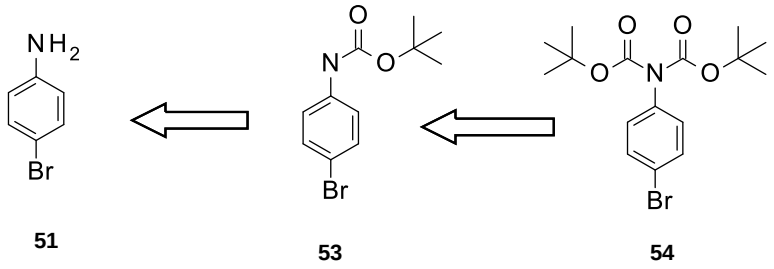
51 **52** **49**

Scheme 12

2.5.2.1.1 Amine protection of 4-bromo aniline

The protection of amino group is a common practice in organic synthesis. A number of protecting grouping have been reported in literature.[151] These protecting groups are either structurally aromatic or linear and they differ in terms of size, stability and de-protection protocols. Bruekelman *et al.,* reported the an aromatic protection of 4-bromoaniline using 2,4, dione to afford an n-substituted 2,5 dimethylpyrrole compound, 1-(4-bromophenyl)-2,5-dimethyl-pyrrole.[152] Gross *et al.,* had also reported aryl triazene as another form of aromatic protecting group for amines.[153]

The first attempt to protect 4-bromoaniline was using a non-aromatic protecting group di-tetra-butyldicarbonate (Boc_2O). The use of Boc_2O to protect amides in mainly both organic and inorganic chemistry has been widely reported.[154] However, the Boc protection of both NH's of the amines have not been reported in literature. Mun *et al.,* and Lammin *et al.,* both reported the protection of 4-bromoaniline using Boc can afford a single equivalent amine protected product, tert-Butyl 4-bromophenyl carbamate **(53) (Scheme 13)**.[155]



51 **53** **54**

Scheme 13

In this study it was proposed that the modifications of the reaction conditions for the synthesis of compound **53** could give the desired all amine protected compound **54.** The modification approaches proposed involved increasing the reaction time, addition excess of di-tetra-butyldicarbonate, performing the reaction under heat and using a more powerful base. The sequential addition of Boc on isolated compound **53** was chosen because of the availability of reports in the literature. The first step was to repeat the procedures that have been proposed by Mun *et al.,* and Lammin *et al.,* The procedure that gave the highest percentage yield was then considered for further modifications.

One procedure where the highest percentage yield of tert-butyl 4-bromophenyl carbamate **(53)** was obtained involved reacting of the two equivalence of Boc_2O with 4-bromoaniline in the presence of three equivalence of sodium hydrogen carbonate as a base at 0 ºC. The product was achieved as colourless needles at 95% yield. The product was characterised as tert-butyl 4-bromophenyl carbamate **(53)** using spectroscopic techniques (^1H, ^{13}C NMR, GC-MS and IR)**.**

The ^1H NMR spectrum showed a singlet at 7.42 ppm integrated for four protons of the aromatic ring, another singlet at 1.47 ppm integrated for nine protons of the Boc protection instead of 18 in di-boc protected amine as shown in **Figure 20**. The un-protected N-H proton was observed at 9.47 ppm. The spectroscopic data was in good agreement with that reported in literature by Lammin *et al.*[154] The absorption peak at 3372.8 cm^{-1} on the IR spectrum gave an indication of the presence of the unprotected amine whereas the singlets at 1672 cm^{-1} (C=O for amide stretch) and 2980.8 cm^{-1} (alkyl C-H) would mean the Boc grouping was attached on the other side. The ^{13}C NMR spectrum was in agreement with that reported by Mun *et al.* [155]The products purity was

confirmed using the GC-MS spectroscopy where a peak at retention times (16.0 minutes) the molecular mass of 271 which was consistent with what was expected for $C_{11}H_{14}BrNO_2$.

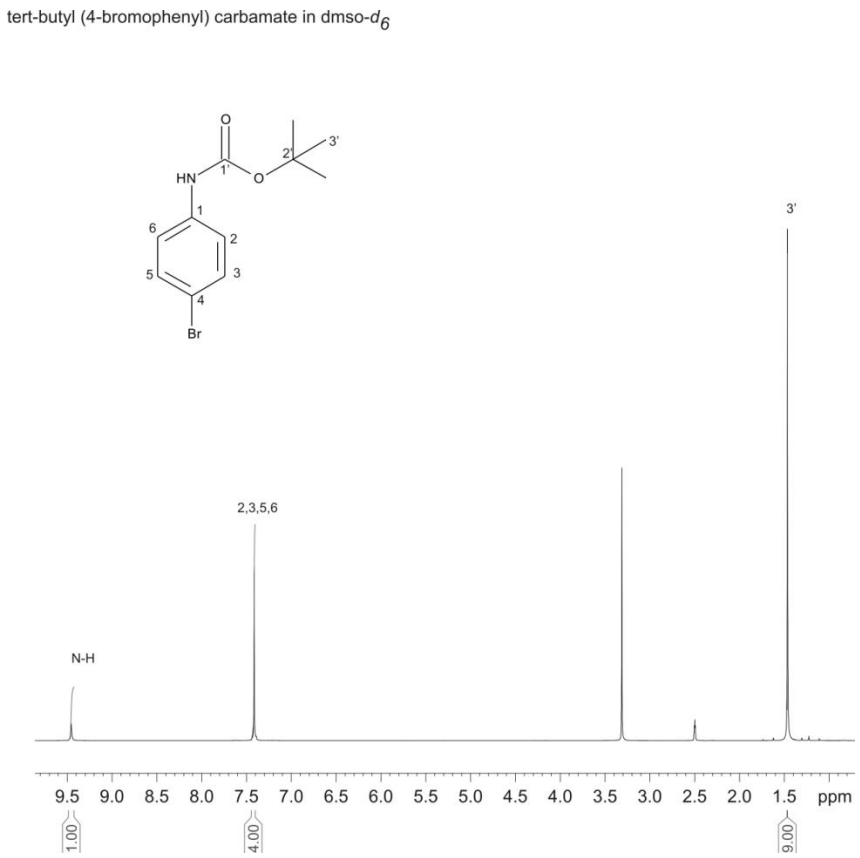


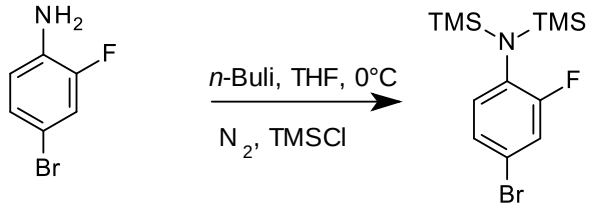
Figure 20: ^{1}H NMR spectrum of product 53

With clean product **53** isolated, the next step was to further protect the remaining NH. Repetition of the same procedure discussed above on **53** did not give product **54**. The use of stronger bases such as potassium carbonate and sodium hydride together with excess of Boc_2O on synthesised product **53** did not give the desired product **54**. All the starting materials were recovered under this modification even when the reaction was performed on elevated temperatures (0 ºC to 110 ºC). The reason for the unsuccessful amine protection could be that the lone pairs on the nitrogen are not available as they are delocalised onto the carbonyl group.

The Boc protected amines have been reported to be unaffected by catalytic hydrogenation and can be easily deprotonated by trifluoacetic (TFA) and HCl.[151] However, other reports suggested that they can easily be attacked by strong bases such as butyllithium (*n*-buli).[152] Boc_2O structural constituents of the carbonyl group (C=O) is thought to bring about the vulnerability of protected

amines in strong bases. As can be seen **Scheme 12**, the next step was the boronic acid addition to the bromine most commonly using butyllithium hence the nucleophilic attack on the Boc carbonyl was not possible. To address this problem, an additional step which involves the protection of the carbonyl using ethylene glycol was needed. This additional step was going to bring some complexity to the synthetic pathway and in turn lowering the overall percentage yield. Therefore it can be concluded that Boc protection was not a suitable protecting method for this synthetic pathway proposed in **Scheme 12**.

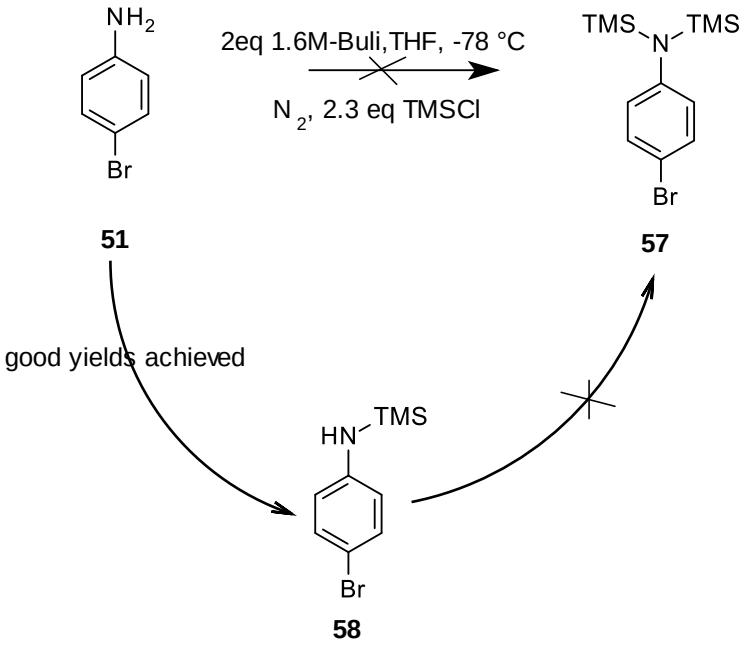
A protecting group that was inert to strong bases had to be investigated. Protecting groups such as trimethylsilane (TMS) which does not have a carbonyl grouping was a realistic possibility. The stability of trimethylsilane protected amines in the presence of a base such as *n*-buli was reported by Bruekelman *et al.*[152] Das *et al.* reported the preparation of trimethylsilyl protected 4-bromo-aniline fluorine substituted derivative, 4-bromo-2-floroaniline **(56)** from 4-bromo-2-fluoro aniline **(55).** The product was isolated as a colourless liquid at 47% yield when reaction was run for 2.5 hours at 0ºC **(Scheme 14)**



55 **56**

Scheme 14

Sudhakar *et al.,* reported the formation of (n- (4-bromophenyl)-1,1,1-trimethyl-n-(trimethylsilyl)-silanamine) **(57)** with 99 % yield when protecting 4-bromoaniline **(51)** stirred with 2 equivalence of 1.6M Buli at -78ºC followed by the addition of 2.3 equivalence of trimethylsilylchloride (TMSCl) **(Scheme 15)**.[156]



Scheme 15

Following the procedure reported by Sudhakar *et al.*, the desired product **57** was not obtained. Repeated efforts with increased reaction times, excess addition of TMSCl gave a product which was characterized by spectroscopic techniques as 4-bromo-N-(trimethylsiyl) benzanamine (**58).**

^{1}H NMR spectrum **(Figure 24)** showed a singlet at -0.06 ppm integrated for 9 protons which were assigned for CH_3's of the TMS. If product **57** had been formed a singlet about the same chemical shift would have integrated to 18 protons. A singlet at 5.40 ppm which integrated to one proton was assigned to the unprotected NH. The doublets of the aromatic rings were observed at 6.57 and 7.13 ppm with a *J*- value of 8.75 Hz.

4-bromo-*N*-(trimethysilyl)benzenamine in dmso-d_6

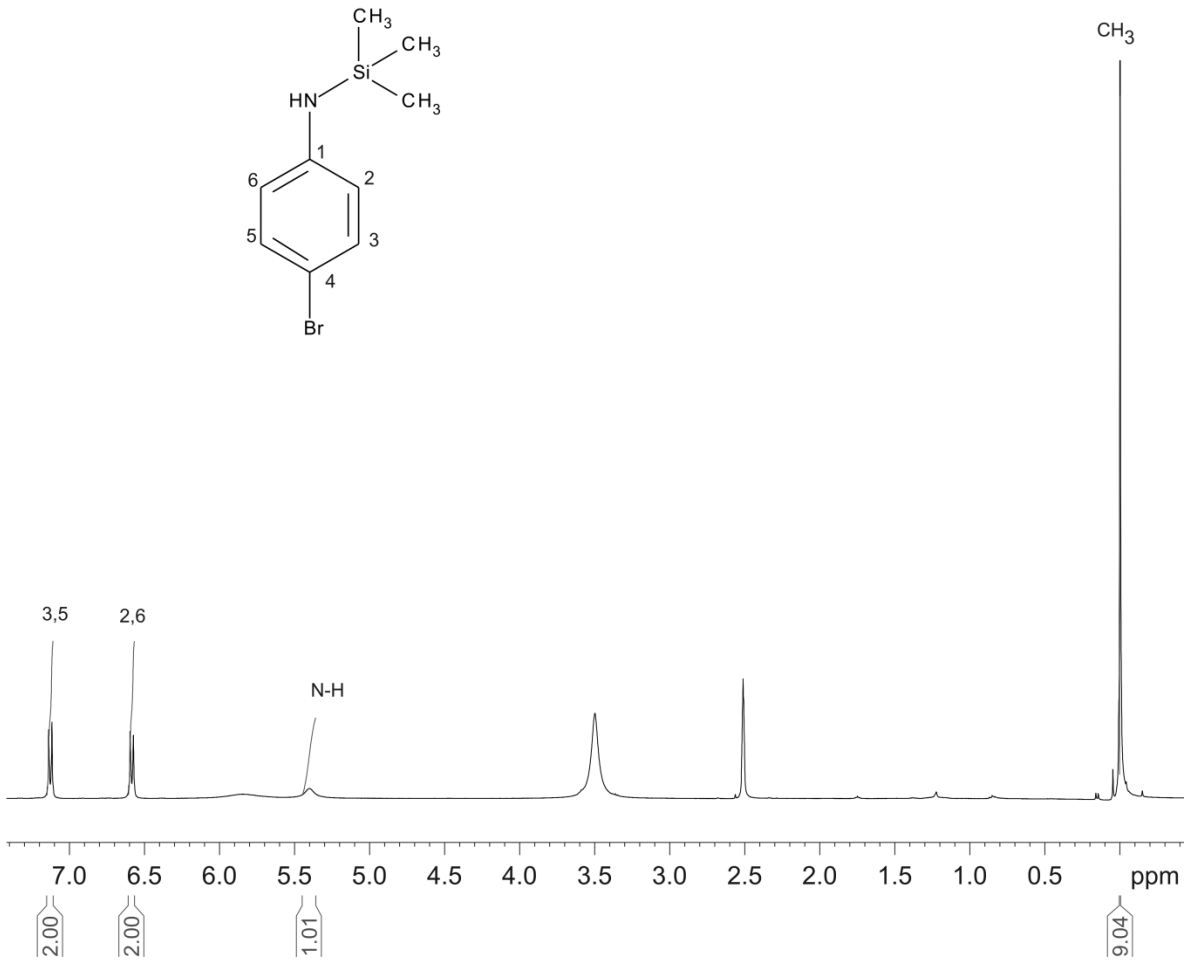


Figure 21: ^{1}H NMR spectrum of compound 58

The ^{13}C NMR spectrum would confirm the purity of the product, the peak at δ 2.3 ppm was assigned for the CH_3's of the TMS. The absorption singlet at 1230 cm^{-1} in the IR spectrum was correctly assigned to Si-CH_3 bond stretch while the broad band at 3383 cm^{-1} was assigned for the unprotected N-H.

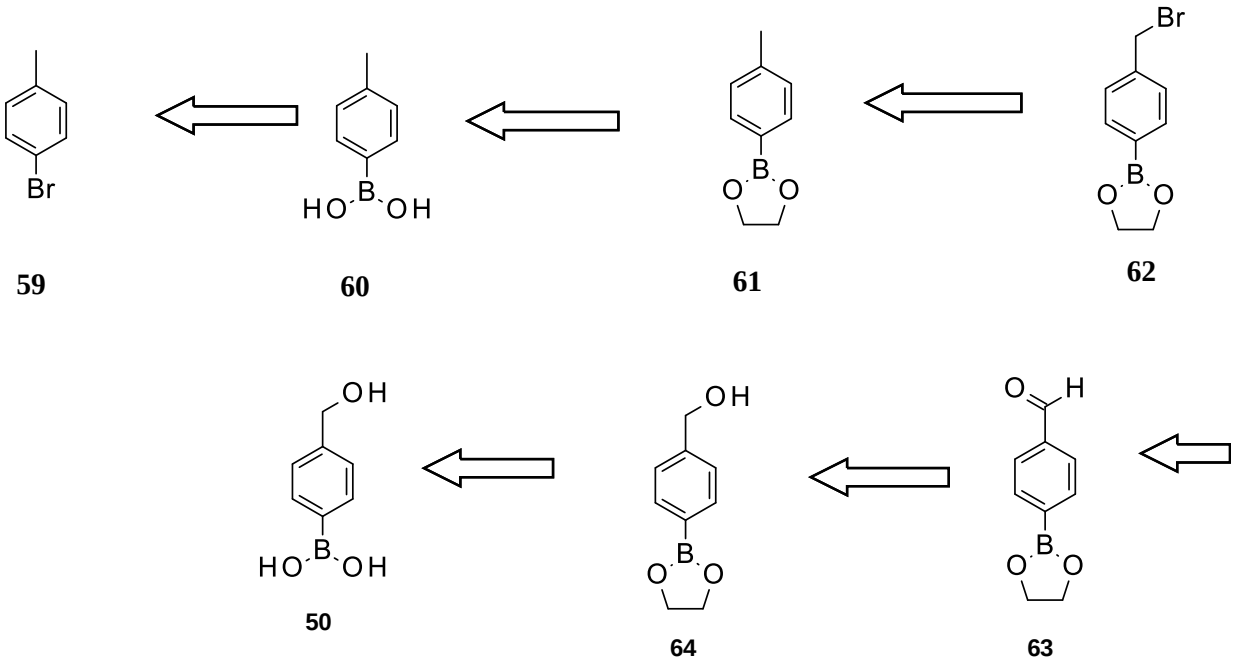
In spite of the success that Sudhakar *et al.,* reported with supporting NMR data, efforts to get TMS double protection of amine were unsuccessful for repeatedly partially protected products

were obtained. The reasons of this mono-protection when TMS is used could be attributed to the weakness of the Si-N bond. This bond interaction would mean even if the reaction had gone to completion TMS would fall off during the workout. The one equivalent protection is favoured for the H-N-Si bond is much stronger than the Si-N-Si.[157] Literature reports have supported this reasoning which therefore left question as to how Sudhakar *et al.*, managed to successfully protect amines using TMS. Literature reports gave another reasoning of TMS being a high donating system of π-electro systems and could stabilize on aromatic substituents hence this stability could make the substitution on the second nitrogen difficult.[158]

Other protection alternatives such as aromatic protecting groups were not explored because of time limitations. The attention was turned to the synthesis of boronic acid, 4-(hydromethyl) phenyl boronic acid **(50)**, which has an alcohol available to form an ester with carboxylic acid of the biotin.

2.5.2.2 Synthesis of 4-(hydromethyl) phenyl boronic acid (50)

The proposed synthesis route for the preparation of 4-(hydromethyl)phenyl boronic acid **(50)** begins with the boronation of *p*-bromotoluene **(59)** to form *p*-tolyboronic acid **(60)** which will be converted to a methyl boronic ester **(61)**. Bromination of the ester using NBS/ will provide **(62)** would then be followed by it oxidation to form an aldehyde **(63)** which would be reduced to form an alcohol. The alcohol-boronic ester **(64)** would then be di-protected using a strong acid to give the desired product **(50) (Scheme 16)**.



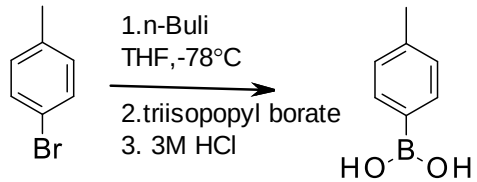
59 **60** **61** **62** **50** **64** **63**

Scheme 16

In the sections that follow, each step of the proposed synthesis route on **Scheme 16** will be discussed. The literature findings on the synthesis of each step will be reviewed and procedure used will be discussed followed by the results obtained.

2.5.2.2.1 Synthesis of *p*-tolyboronic acid (60)

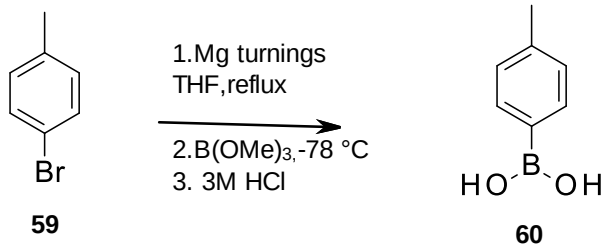
Two possible routes for the synthesis of *p*-tolyboronic acid **(60)** from 4-bromotoluene have been reported in literature. One of the methods is the boronation *via* direct lithium exchange using *n*-butylithium at -78 ºC followed by a boronating agent either trimethylborate or triisopropyl borate before the deprotection using a strong acid hydrochloric acid (HCl) **(Scheme 17)**.



59 **60**

Scheme 17

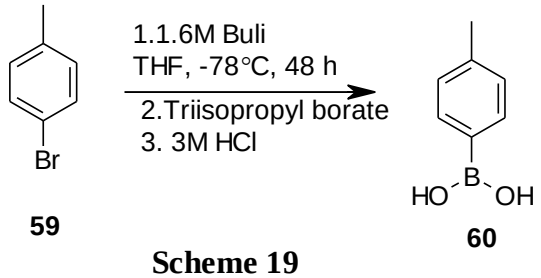
Zhu *et al.,* and Wong *et al.,* reported that compound **(60)** could also be synthesised through the formation of a Grignard reagent by reacting magnesium and 4-bromotoluene followed by dropwise addition of boronating agent (trimethylborate) before the deprotection using concentrated HCl **(Scheme 18)**. [159]



59 **60**

Scheme 18

Using the first route model *via* lithium exchange which uses triisopropyl borate as a boronation agent, *p*-tolyboronic acid was successfully isolated in good yield of 71% as a white solid with a purity of 98% when the reaction was run for total time of 48 hours **(Scheme 19)**. The reaction was monitored using ^{1}H NMR spectroscopy because the product could not move up the silica gel coated TLC plate. This baseline attachment was observed with all the boronic acids synthesised in this study. Therefore separation of products from a crude mixture using silica gel fitted chromatographic columns was avoided.



59 **60**

Scheme 19

The product was confirmed using spectroscopic techniques such as ^{1}H, ^{11}B and ^{13}C NMR spectroscopy. The peak at 7.90 ppm which integrated for two protons in the ^{1}H NMR spectrum confirmed the presence of the protons of the hydroxyl groups of the boronic acid and the four aromatic protons were represented as two doublets at 7.15 ppm and 7.66 ppm (J = 7.8 Hz) each integrating for two protons grouped based upon the similarities of their environments. The peak at 2.31 ppm was correctly assigned to the CH_3 because it integrated for three protons as shown in **Figure 22**.

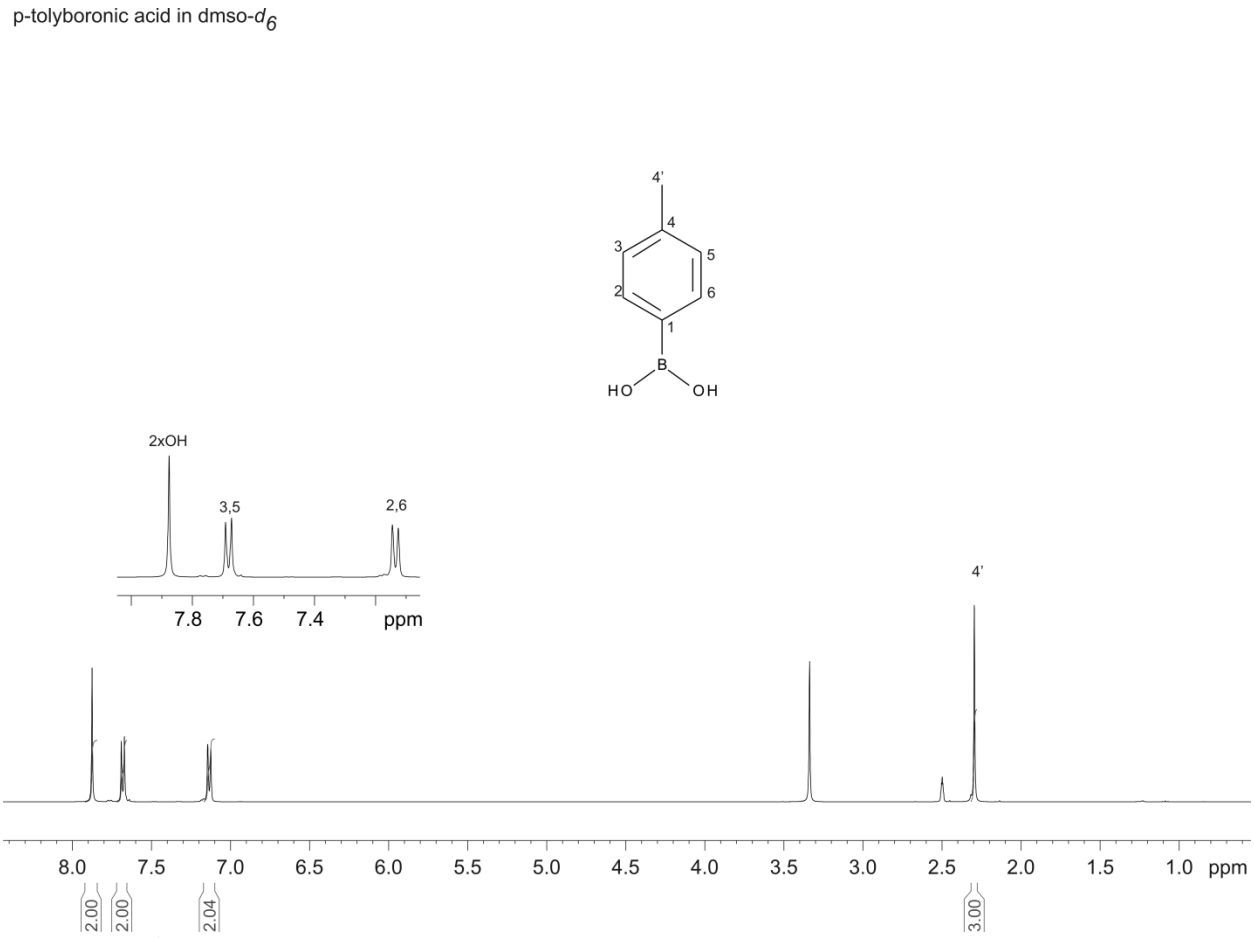


Figure 22: ^{1}H NMR spectrum for compound 60

The carbon peak for the CH_3 in the ^{13}C NMR spectrum was observed at 21.6 ppm while the two carbons with the aromatic protons had peaks at 128.4 ppm (C-3,5) and 134.6 ppm (C-2,6). The peak for the quaternary carbon (C-4) was assigned a peak at 139.4 ppm. The carbon peak for C-1 was not observed in the ^{13}C NMR spectrum. The nuclear spin ^{11}B nucleus has been quantified to be $\frac{3}{2}$ which means it has a very large value of Q and the relaxation time is very fast.[160] The peak broadening could be attributed to the ease of ^{11}B into coupling with any nuclei attached to them. It is because of this quadrupolar broadening that C-B peak of compound **(60)** cannot be observed on the ^{13}C NMR spectrum **(Figure 23)**. The absence of the C-B peak was also observed in all boron containing compounds synthesized in this work.

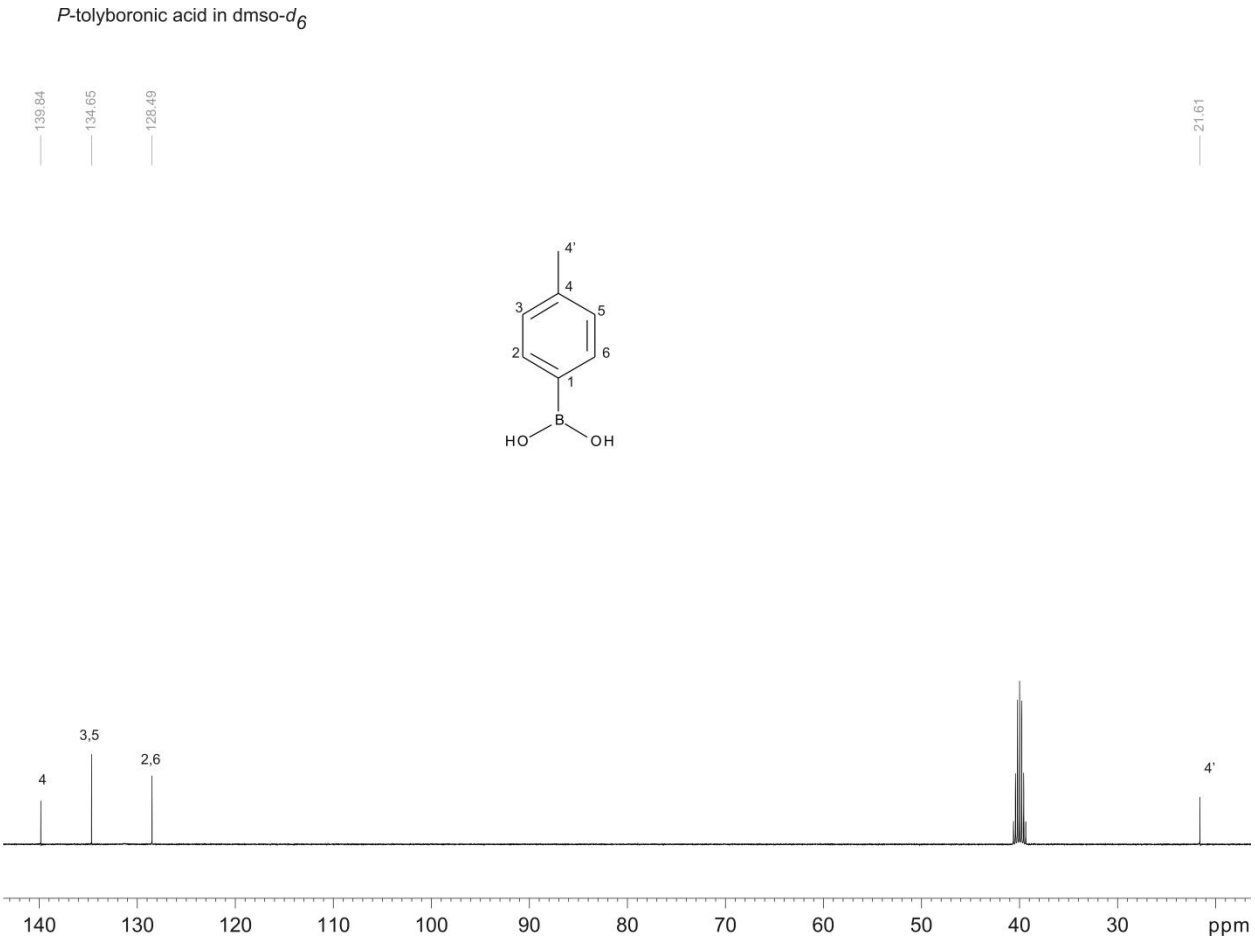


Figure 23: ^{13}C NMR spectrum for compound 60

The peak at δ 29.1 ppm on the ^{11}B NMR spectrum **(Figure 24)** was in agreement with one reported in literature for a boronic acid [$(B(OH)_2$].

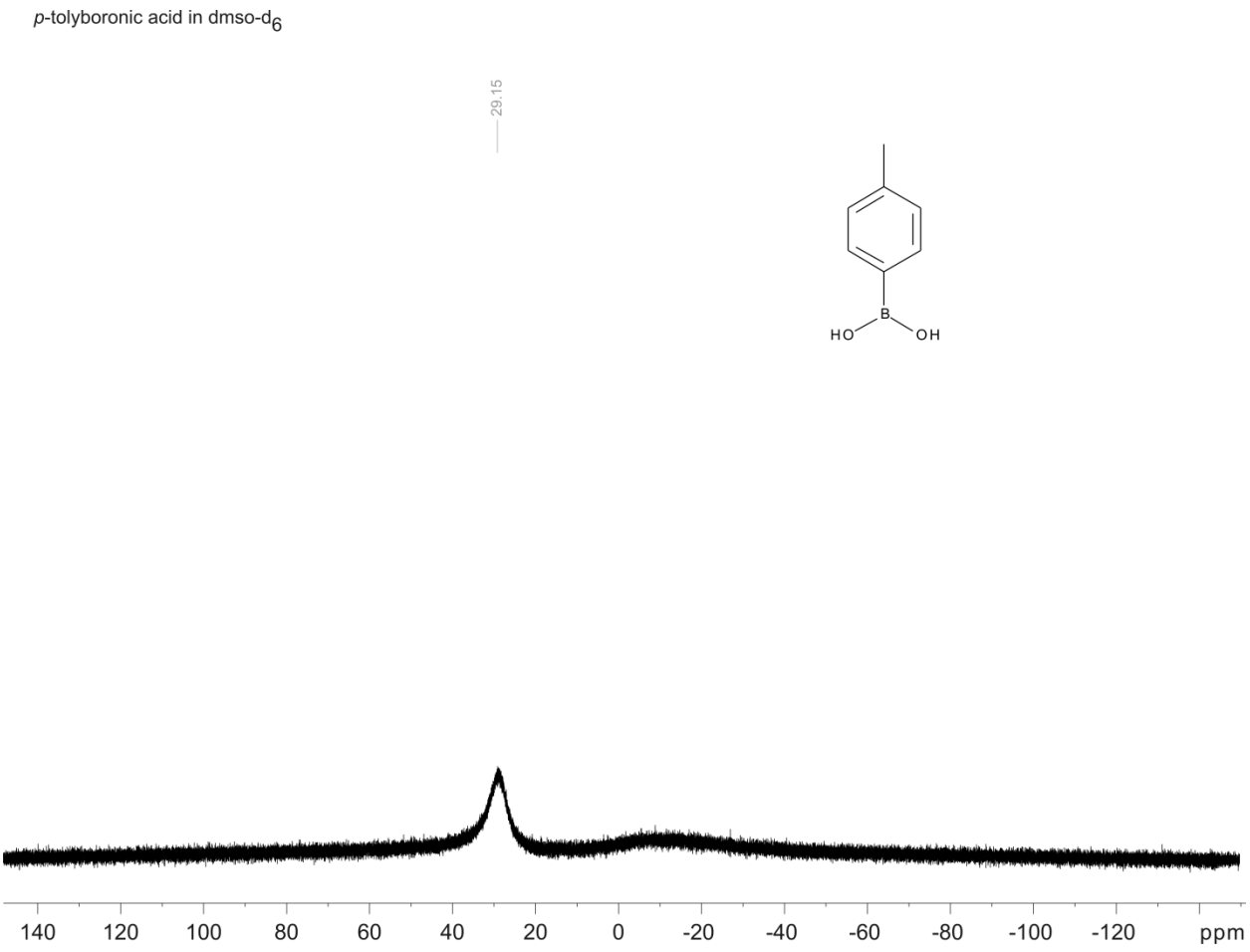


Figure 24: ^{11}B NMR spectrum of compound 60

The broad IR peak at absorption 3243.5 cm^{-1} represents the hydroxyl groups while the peak at 1341.1 cm^{-1} was assigned to B-O based on literature findings.[161] The TOF-MS spectroscopy was also used to confirm the purity of the product. However, on the spectrum obtained **(Figure 28)**, the peak corresponding to the desired product was not observed. The TOF-MS instrument was used ahead of the GC-MS instrument. The reason of this choice was because boronic acids are too polar to pass through the silica gel column of the GC-MS. On the other hand in the TOF-MS instrument, compounds do not go through a silica gel column. However the accurate mass of the *p*-tolyboronic acid and subsequently for all free boronic acids could not be obtained. Fragmentation when the boronic acid were ionised is suspected to be the reason for unfamiliar

masses obtained on the TOF-MS spectrum. Most of those peaks could not be assigned to any section of these compounds making it difficult to determine the fragmentation pattern. The accurate masses of boronic acids synthesised in this study were not reported and such information is missing in most reports in the literature where boronic acids have been synthesised.

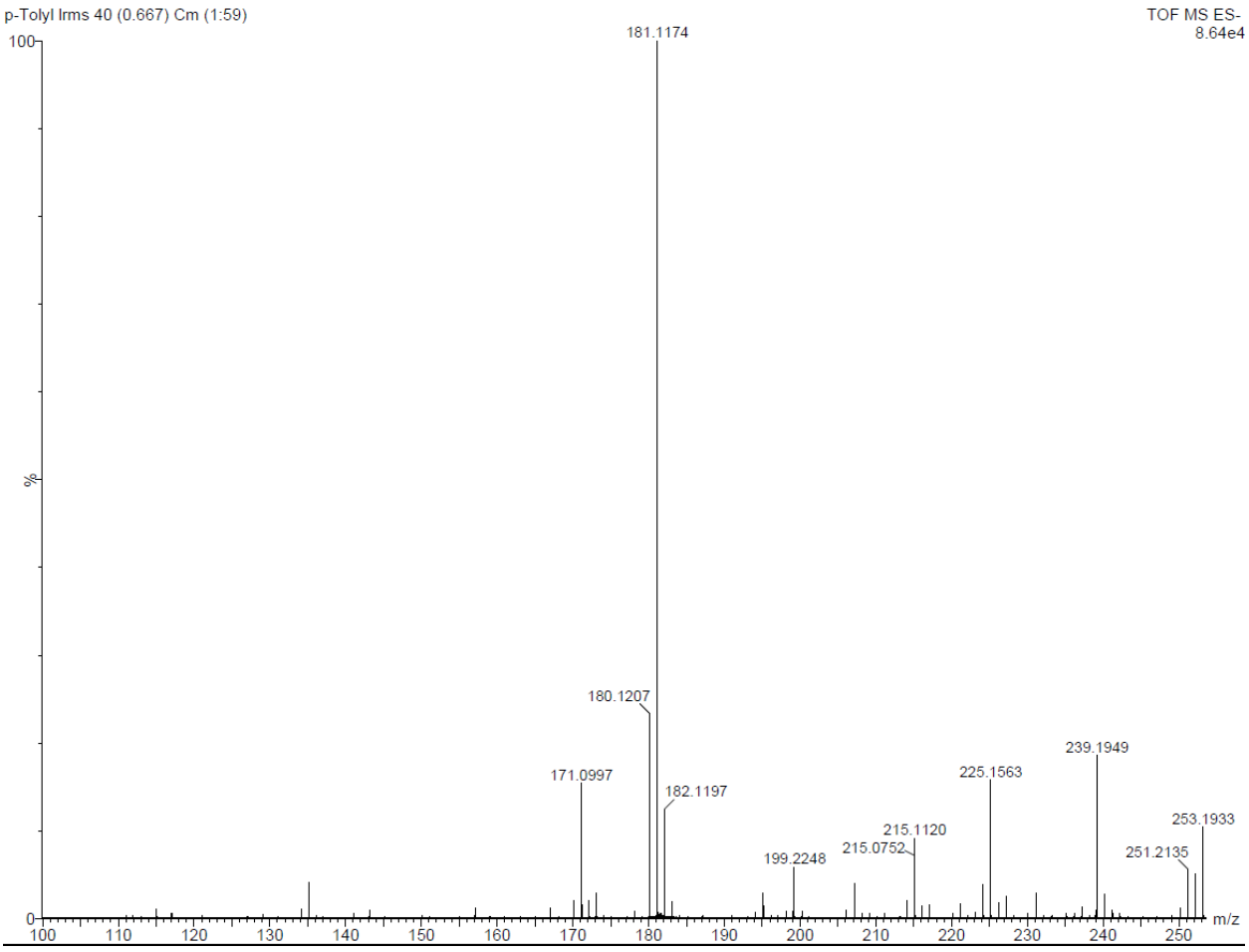


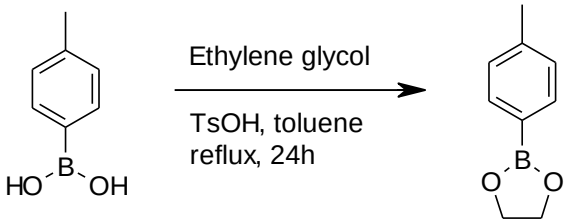
Figure 25: Mass spectrum of *p*-tolyboronic acid

2.5.2.2.2 Synthesis of 2-(4-tolyl)[1,3,2]dioxaborolane (61)

With the *p*-tolyboronic acid (**60)** synthesised, 2-(4-toly)[1,3,2]dioxaborolane was prepared from reacting with a diol, ethylene glycol to protect the hydroxyl group of the boronic acid moiety.

Hydroxyl protection using diols is mostly used in organic to form corresponding esters which have a pKa that is 3 units lower than the parent boronic acid thus increasing reactivity while maintaining stability.[162] If the hydroxyl groups were not protected, bromination would have favoured the more reactive $B(OH)_2$ as opposed to the methyl of the *p*-tolyboronic acid.

The boronic ester was achieved with a 90% percentage yield under Dean-Stark apparatus by reacting *p*-tolyboronic acid with 2 equivalence of ethylene glycol in dry toluene for 24 hours as described by Gomez-Blance *et al.***(Scheme 20)**.[163]



60 **61**

Scheme 20

The product and purity was confirmed using ^{1}H, ^{11}B and ^{13}C NMR spectroscopy. The peaks in the aromatic region in the ^{1}H NMR spectrum were kept as doublets with a slight up-field shift to those of the starting material, the peak at 8.20 ppm belonging to the hydroxyl groups of the boronic acids vanished. A new singlet at 4.37 ppm that integrated to four protons was observed and they were assigned to the two CH_2's of the diol ester a results that was in agreement with literature **(Figure 25)**.

2-(4-toly[1,3,2]dioxaborolane in $CDCl_3$

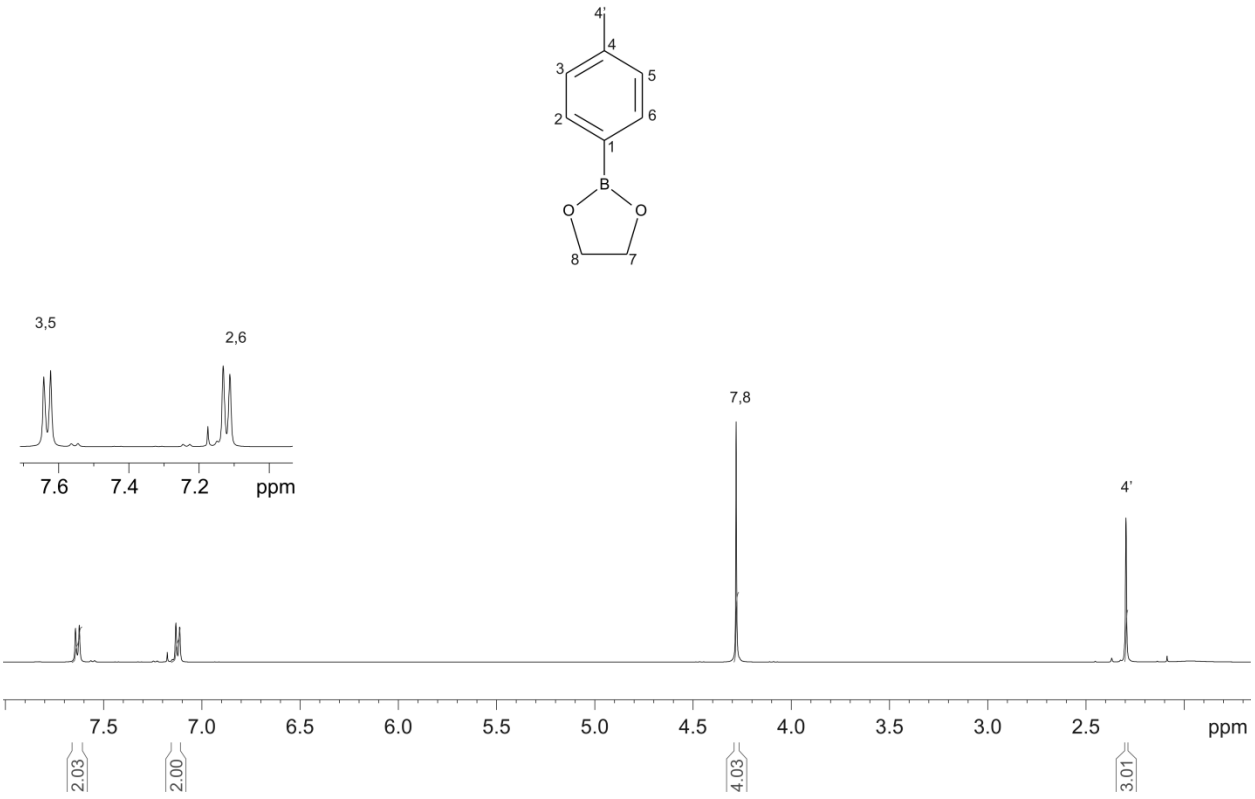


Figure 25: ^{1}H NMR spectrum for compound 61

A down-field chemical shift of the boron peak from δ 29.1 ppm of the starting material to δ 31.5 ppm was observed from ^{11}B NMR spectrum which was consistent with literature findings for boronic esters peaks as shown **Figure 30**. The carbons of the two methylene groups of the protection were observed at chemical shift 66.0 ppm which is about the range for (C-O). The broad singlet absorption peak in the IR spectrum assigned in the starting material for the two (OH) at 3243.5 cm^{-1} disappeared and two singlets at absorptions 2982.7 and 2911.9 cm^{-1} were

assigned to the two C-H stretch of the newly formed acetal grouping.

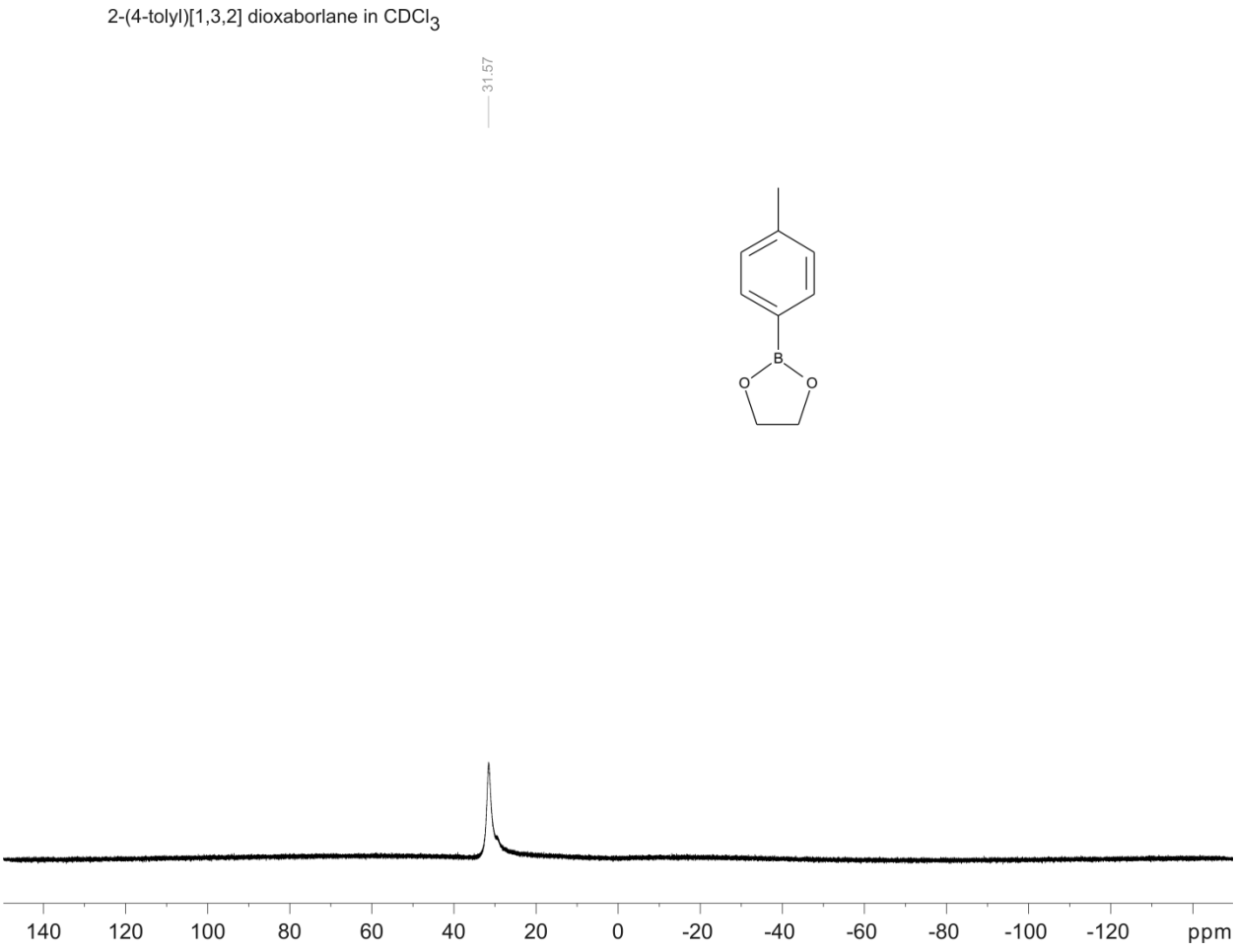


Figure 27: ^{11}B NMR of compound 61

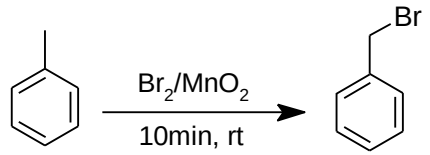
2.5.2.2.3 Synthesis of 2-(4-(bromomethyl)phenyl)1-3-2-dioxaborolane (62)

Bromination of alkanes, specifically alkyl benzenes have been performed over the last few decades using a wide range of brominating agents which differ in their mechanisms in which they brominate and the reactivity.[164] Brominating agents for direct bromination include Br_2,[165] n-Bromosuccinimide (NBS) and HBr which goes by radical formation mechanism as proposed by independent studies by Bloomfield and Goldfinger in good yields.[166] However there are a wide range of side- chain brominating agents which are used in conjunction with a catalyst have been

reported to be capable of brominating an alkane such include $CBrCl_3$, potassium bromide, BrF[167], Cu(II)Br, sodium bromate and bromotrimethylsilane.[168]

In this study, Br_2 and NBS were given first preference because high yields of brominated compounds have been reported in literature while lower yields were reported for the side-chain brominating agents.[169] Traynham *et al.*, reported the similarities of critical steps Br_2 and NBS radical mechanism specifically bromine-transfer and hydrogen abstraction.[170] In comparison, Br_2 reactivity has been reported to be higher than NBS simple because of the ease of Br_2 to form radicals even small amounts of radical initiator are used.[171]

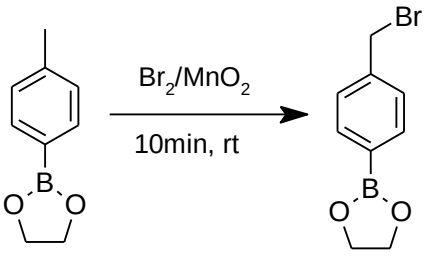
Jiang *et al.*, reported method of brominating using Br_2 alkanes that had product yields of 99 %. In this study, toluene **(65)** was brominated using bromide (Br_2) in the presence of peroxide, manganese oxide (MnO_2) as a radiation initiator. The reaction was carried out at room temperature for 10 minutes in CH_2Cl_2 **(Scheme 21)**.[172] The success of this procedure on the formation of benzyl bromide **(66)** from toluene was also achieved by Mathew *et al.*, who reported a 70% product yield when the reaction was performed over ten minutes [173]



65 **66**

Scheme 21

The procedure by Jiang *et al.*, was adopted to brominate 2-(4-tolyl)[1,3,2]dioxaborolane **(61) (Scheme 22).** The reaction was performed both on the bench and under microwave conditions from 10 to 30 minutes.

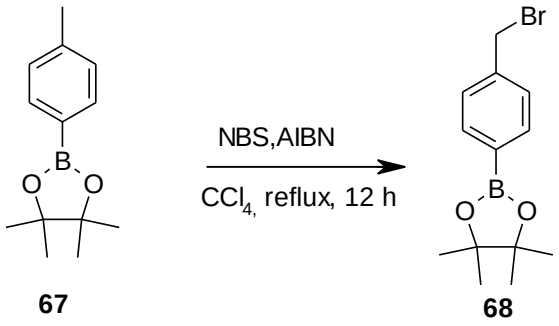


61 **62**

Scheme 22

The ^{I}H NMR spectrum of samples taken during the course of the experiements showed the presence of fragments whose identity could not be ascertained. This result could not change even when the reaction time was increased to 30 minutes. It was supposed that the Br_2 was too robust for the multi-functional grouped starting material with a rather "weaker" ester protection. This proposition was confirmed by that the fragmentation of the starting material was observed on ^{1}H NMR soon after the addition of the Br_2 on product **61**. The limitations of the use of Br_2 have been reported by Podgorsek *et al.*, and include being corrosive, toxic to weak bonds and hard to handle which was in agreement with our findings.[174] Baba *et al.*, reported NBS as the most mild, less-corrosive method of bromination alkanes when compared to Br_2 thus it was employed in the following section.

Das *et al.*, reported the radical bromination of 4,4,5,5-tetramethyl-2-*p*-tolyl-1,3,2-dioxaborolane **(67)** using NBS in the presence of catalytic amounts of a radical initiator AIBN (azobisisobutyronitrile) to form 2-(4-(bromomethyl)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane**(68)** using CCl_4 (carbon tetrachloride) as a solvent under 12 hour reflux. [175]

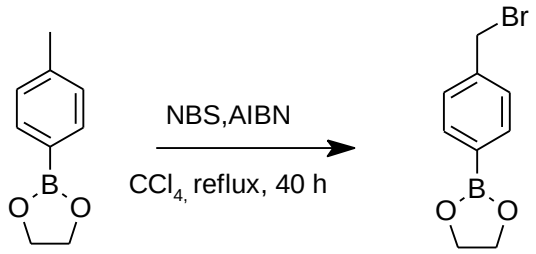


67 **68**

Scheme 23

Using Das *et al.*, method to synthesis 2-(4-bromomethyl)-1,3,2-dioxaborolane **(62)** from 2-(4-tolyl)[1,3,2]dioxaborolane **(61)**, the brominated ester was in a crude with starting material results that were correlated to those of Zaidlewicz *et al.***(Scheme23)**. [142] Efforts to separate this mixture using chromatographic means were unsuccessful and increasing the reaction time up to 40 hours proved slightly improved the bromination to 63% product yield when calculated based on the major product. Carbon tetrachloride have been reported as the best solvent for the bromination using NBS, however due to its high cost, other cheap solvents like chloroform ($CHCl_3$) and methylene chloride (CH_2Cl_2) were also tried. The conversion rate when solvents other than CCl_4

were used lowered to as little as 50% yields for $CHCl_3$ and 40% for CH_2Cl_2 even if the reaction times were increased to as long as 72 h.



61 **62**

Scheme 24

The desired product was confirmed to be in a mixture with the starting material using ^{1}H NMR spectroscopy. The doublets on the aromatic region at δ ppm: 7.42. 7.81, 7.72 and 7.22 ppm would confirm the product to be in a mixture. The coupling between each pair of doublets (a pair of 7.43-7.81 (*J* = 7.93 Hz) and 7.22-7.72 *(J* = 8.03 Hz) was observed in the COSY spectrum. A singlet at δ 4.51 ppm that integrated to 2 protons was assigned to the CH_2 (H-4´) which was expected upon the bromination of the starting material. A downfield shift for the methylene's of the acetal (H-7,8) from that of the starting was observed. All the peaks outside the aromatic region that corresponded to those reported for the starting material **(61)**. It was therefore concluded that the other compound was 2-(4-tolyl)[1,3,2]dioxaborolane **(61)**. The product abundance relative to the starting material was estimated to be 1.1:1 using the integral ratios of the two compounds. The fully assigned ^{1}H NMR spectrum is shown in **Figure 28** below.

2-(4-bromomethyl)phenyl) 1,3,2- dioxaborolane in $CDCl_3$

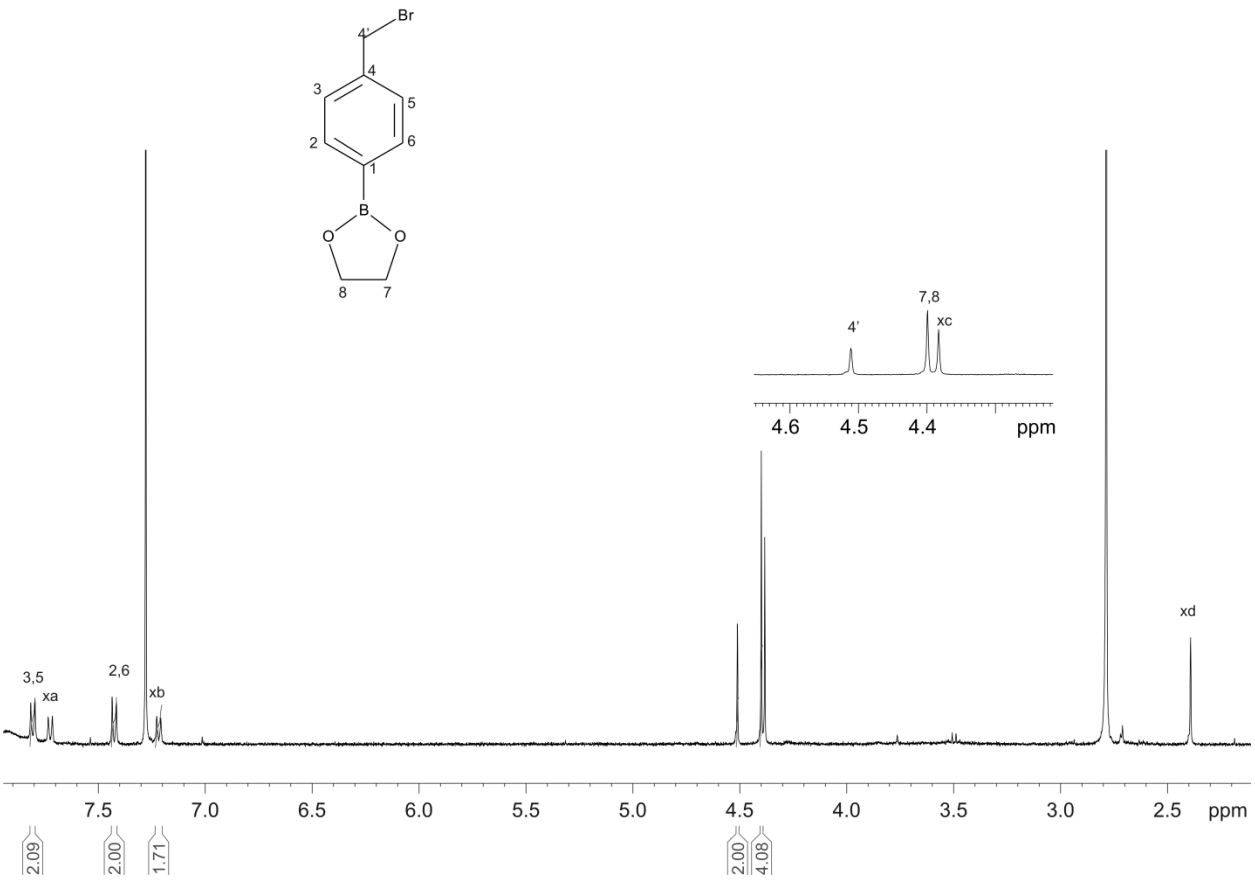


Figure 28: ^{1}H NMR spectrum of compound 62

Note: Peaks marked xa, xb, xc, xd are those that belong to the residual starting material

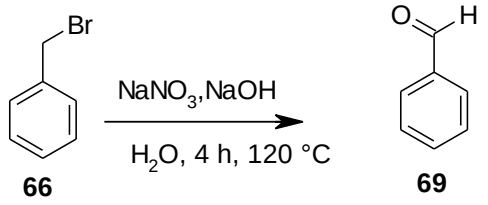
The ^{11}B NMR spectroscopy only showed two peaks at 31.9 and about 30.4 which confirmed the presence of boronic esters. The peak at δ 31.0 ppm in the ^{13}C NMR spectrum was correctly assigned to the CH_2 attached to the Br (C-4′). This assignment would confirm that bromination had occurred since the C-Br peak could not be observed in the spectrum as discussed earlier. The carbon peaks of the rest of the compound together with those of the starting material were as expected. The absorption single peak at 667.0 cm^{-1} in the IR spectrum could possibly indicate the presence of a C-Br stretch. Efforts to separate this mixture using chromatographic techniques were unsuccessful because they had very close r_f values on the TLC plate. Using a highly

sensitive grace chromatography could not make any difference so it was decided that the mixture be oxidised in the next step.

2.5.2.2.4 Synthesis of 4-(1,3,2-dioxaborolan-2-yl)benzaldehyde (63)

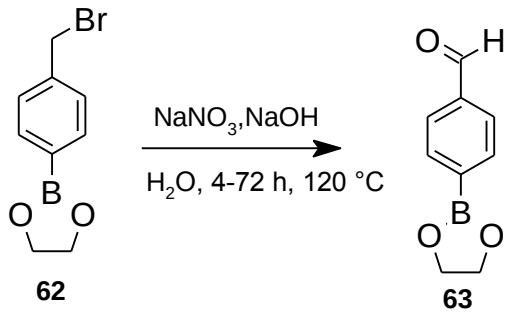
The transformation of an organic halide to a corresponding carbonyl compounds have been well documented in organic chemistry. Oxidising agents that have been reported include those that could be considered as mild such as $NaIO_4$-DMF[176], Kornblum's oxidation with $KHCO_3$-DMSO[177] and $NaNO_3$-NaOH [178] to the most harsh such as the hydrogen peroxide under different reagent pairing such as H_2O_2-EtOH[179], TMA-H_2O_2[180] and H_2O_2-H_2O.[181] However, the oxidation of 2-(4-bromomethyl)-1,3,2-dioxaborolane **(62)** has not been reported in literature. It is for this reason that reactions done were modelled from procedures reported for benzyl bromide.

Kulanglappar *et al.*, reported a mild oxidation of benzyl bromide **(66)** and its derivatives using sodium nitrate as an oxidant at 120 ºC under aqueous conditions to give **69** (**Scheme 25)**. A percentage yield of 91% was achieved when the reaction was performed for 4 hours and for the *ortho*- and *para*- substituted benzyl bromide derivatives the reaction time was increased to up to 24 hours for an electron donating group NO_2 attached.[178]



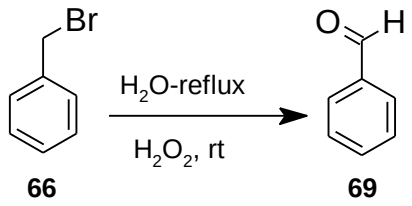
Scheme 25

Attempts to oxidise 2-(4-bromomethyl)-1,3,2-dioxaborolane **(61)** using the procedure reported by Kulanglappar *et al.,* proved unsuccessful since the oxidation could not start off even when reaction time was increased up to 3 days **(Scheme 26)**. The starting material was observed even when the reaction was performed in high temperatures hence the change of the oxidant was proposed.



Scheme 26

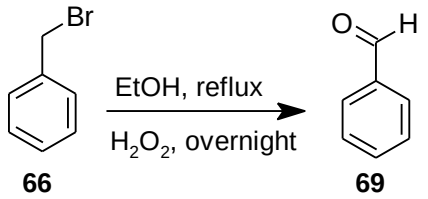
Patil *et al.,* reported the conversion of various benzyl bromides of different electron-donating and electron-withdrawing capabilities to corresponding benzaldehydes. Benzyl bromides were refluxed in water using hydrogen peroxide to afford good yields (79-85%). This procedure was reported by Patil *et al.,* to proceed regardless of the identity of the substituent,R. **(Scheme 27)**[181]



Scheme 27

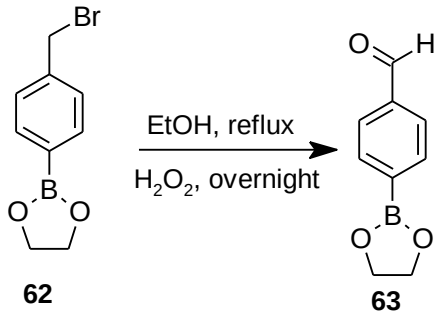
Solubility problems were encountered when the procedure proposed by Patil *et al.,* was tried on 2-(4-bromomethyl)-1,3,2-dioxaborolane **(56)** therefore a better solvent other than water in which the starting material could dissolve was to be used.

Tang *et al.,* reported ethanol as the best solvent of which the conversion could afford better yields and take shorter periods of time when compared to other solvents such as methylene chloride and THF. Benzyl bromide was oxidised to a benzaldehyde using hydrogen peroxide (as the oxidant) on ethanol (as a solvent) under reflux for 3 hours. Good percentage yields in a range of 76-94% were reported for most starting materials with an electron donating group in its substitutes while strong withdrawing nitro group at *para*-position had a poor percentage yield of 20 **(Scheme 28)**. [179]



Scheme 28

The solvent ethanol together with H_2O_2 as an oxidant was used to oxidise 2-(4-bromomethyl)-1,3,2-dioxaborolane **(56)** with the view of synthesising 4-(1,3,2-dioxaborolan-2-yl)benzaldehyde **(57)** as reported by Tang *et al.***(Scheme 29)**



Scheme 29

Several experiments were performed based on **Scheme 29** with the reaction times varying from 3 hours to 3 days and the reaction was monitored using TLC and ^{1}H NMR at 2 hour intervals until it was stopped when there was no change in the spectra. During the first 3 hours the reaction had not started and there was a spot corresponding to the starting material on the TLC. The reaction only started after 5 hours and the TLC showed four spots with one corresponding to the starting material while the identity of the three compounds was not known at this stage; it was only suspected that one could be the product. The reaction was allowed to reflux until the spots of the same R_f with the starting material had vanished and then it was stopped after 3 days.

The ^{1}H NMR spectrum of the crude confirmed the absence of the desired product but unknown species. This result was consistent on a number of experiments even when variation on the amount of the oxidant and reaction times was done. Isolation of the compounds from the crude was performed using column chromatography and they were identified using spectroscopic means such as ^{1}H and ^{11}B NMR spectroscopy.

One fraction was confirmed to be the benzyl bromide derivative with an unknown substituent on the *para*-position. The two doublets between δ 7.76-7.87 ppm (J = 8.6 Hz) confirmed the *para*-substitution and a triplet at 4.39 ppm which integrated for two protons on $CDCl_3$. These results corresponded with the peaks of a commercially available benzyl bromide even though a singlet was expected for the benzyl CH_2 as seen in **Figure 32**. Solvent influence could be attributed with the change formation of the singlet instead of a doublet. There was no peak on the ^{11}B NMR which justified that this fraction had no boron hence confirming it as benzyl bromide derivative.

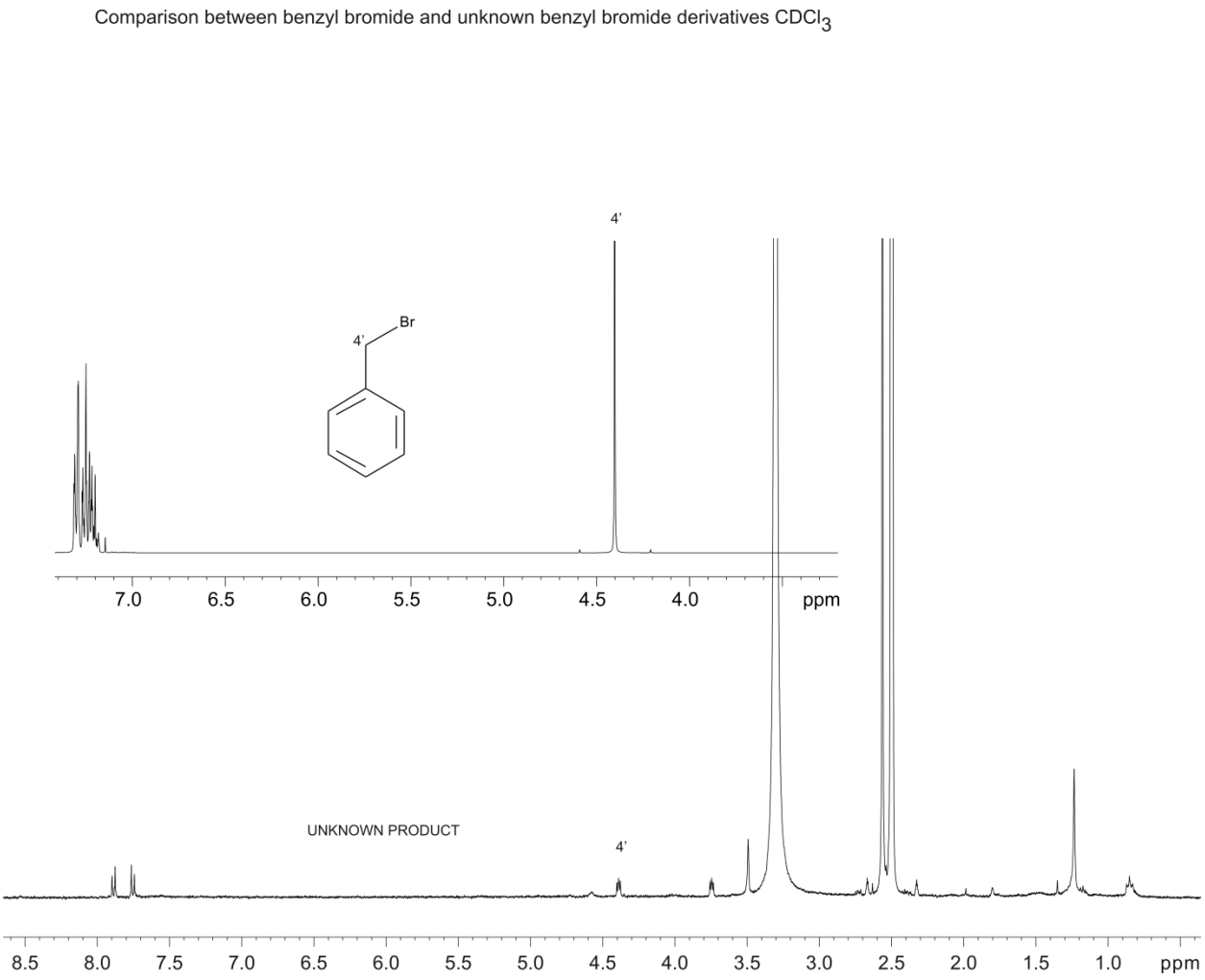


Figure 29: ^{1}H NMR spectrum of one fraction when compared to commercially available benzyl bromide

The second fraction isolated confirmed by ^{1}H and ^{11}B NMR spectroscopy as a non-cyclic compound judging by the absence of peaks about the aromatic region. The peak on the proton

NMR that intergraded for 3 protons was a singlet at δ 2.00 ppm as shown in **Figure 33**. The ^{11}B NMR spectrum of the same fraction had a peak at 22.0 ppm. This spectroscopic data when interpreted confirmed that this fraction was boric acid and this conclusion is supported in literature.

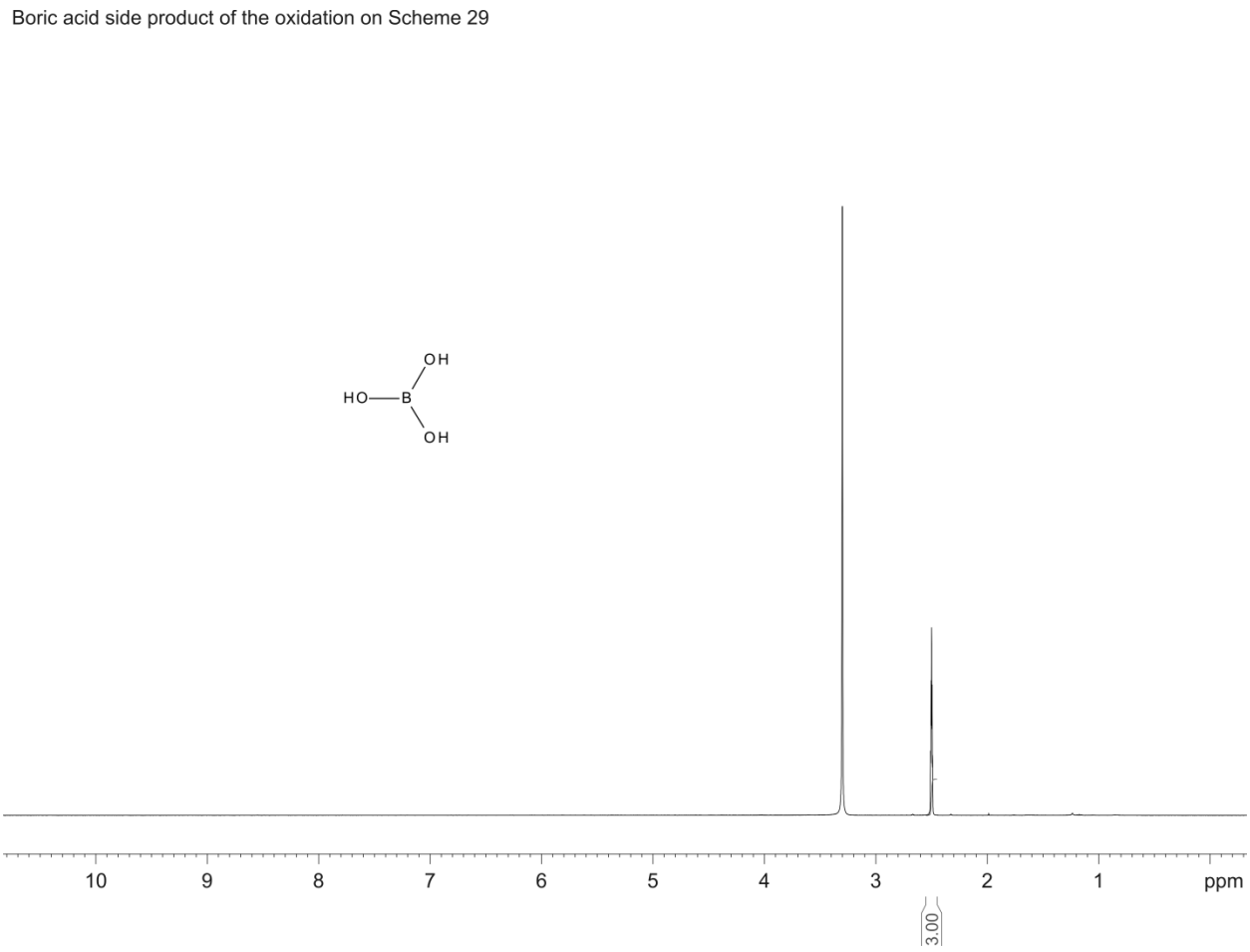


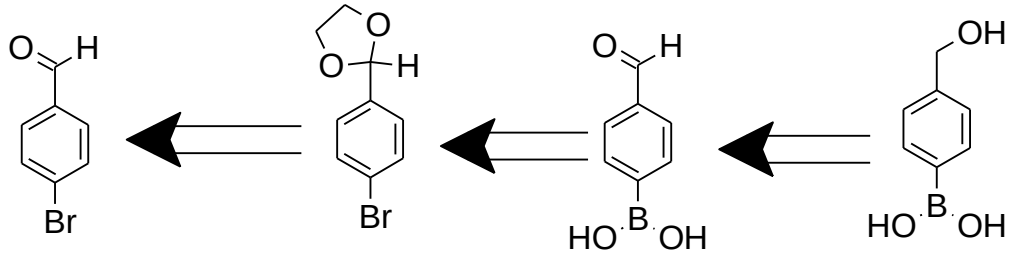
Figure 30: ^{I}H NMR spectrum of the second fraction identified as boric acid

The oxidation step proved to be the most challenging in this synthesis route and it hindered the progress to synthesis of the biotinylated compound **(50)**. The failures of this step could be attributed to the harsh nature of the oxidising agent hydrogen peroxide which fragmented the starting material. However, mild conditions have failed to kick-off the oxidation of **(56)** for unknown reasons but the boronate ester could possibly be acting as a strong electron-

withdrawing group thus making de-bromination difficult. It was decided that a new synthesis route which could avoid an oxidation step was to be evaluated.

2.5.2.3 Second proposed route for the synthesis of 4-(hydromethyl) phenyl boronic acid (50)

With the problems already encountered in the previous method due to the oxidation step, the thinking was that the second synthesis route would start with an already oxidised starting material. A starting material containing the aldehyde group was proposed as an ideal way to start the synthesis route and the available aldehyde in the laboratory was 4-bromobenzaldehyde **(70)**. The aldehyde was protected using ethylene glycol to form an acetal **(71)** which would be boronated on the bromine side by lithiation to form a boronic acid, 4-formyl phenyl boronic acid **(72)**. The boronated aldehyde would then be reduced to form an alcohol to give a 4-(hydromethyl) phenyl boronic acid **(50) (Scheme 30)**.



70 **71** **72** **50**

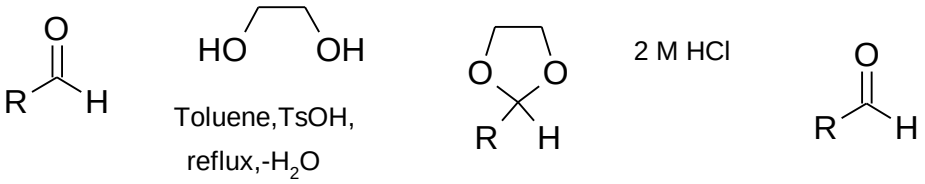
Scheme 30

2.5.2.3.1 Synthesis of 2-(4-bromophenyl)-1,3-dioxolane (71)

The preparation of 2-(4-bromophenyl)-1,3-dioxolane **(71)** from 4-bromobenzaldehyde **(70)** is commonly practised in organic synthesis which is aimed at improving the stability of the aldehyde for further additions on the *para*-position.[182] Aldehydes instability is brought about by the high electronegativity of the oxygen group than the carbon hence the oxygen has greater

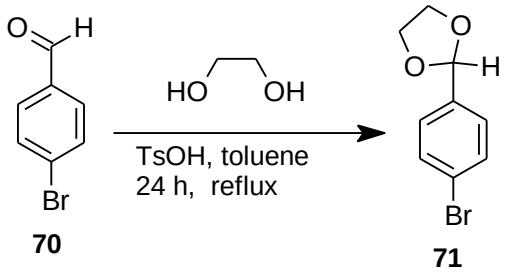
share of the double bond electrons. The carbon become partially positively charged thus making it vulnerable to any nucleophilic attack.

The standard procedure protection of aldehydes involves the use of *p*-toluenesulfonic acid in reflux with solvents either benzene or toluene under Dean-Stark apparatus to allow water removal. The products that results from protection of aldehydes in this way are called acetals. The advantage of acetal protection method is that it could easily be de-protected by hydrolysis means using a strong acid at room temperature to give back the aldehyde (**Scheme 31)**.[183]



Scheme 31

Hons *et al.,* obtained 2-(4-bromophenyl)-1,3-dioxilane **(71)** as a colourless oil by reacting of 4-bromobenzaldehyde **(70)** with 2 equivalence of ethylene glycol in the presence of catalytic amount of *p*-toluenesulfonic acid using anhydrous toluene as a solvent. A yield of 95% was reported when the reagents were refluxed under Dean and Stark apparatus for 24 h under nitrogen **(Scheme 31)**.[184] This procedure has generally been reported in a number of studies in literature in excellent yields from 85-99%. [182, 185]



Scheme 32

Following the procedure as reported by Hons *et al.,* 2-(4-bromophenyl)-1,3-dioxilane**(71)** was obtained as a white oil in a yield of 68% yield and its presence was confirmed using ^{1}H, ^{13}C

NMR spectroscopy. An up-field shift of the proton peak from δ 10.0 to 5.78 ppm was observed. Multiplets at δ 4.03 and δ 4.12 ppm that each integrated to 2 protons were assigned for protons (H2′ and H3′) as shown in **Figure 34**. The chemical shifts and the integral ratios of the product's ^{1}H NMR spectrum were consistent with results reported in by Cousin *et al*.[186] The ^{13}C NMR spectrum confirmed that C1′ was attached with the oxygen of was assigned a peak at 60 ppm. The DEPT 135 spectrum gave indication that C1′ was attached to one proton which was expected. The two carbons, C2′ and C3′, were correctly assigned peaks at 14.3 and 20.7 ppm in the ^{13}C NMR spectrum. The two broad bands at *v* 2889 cm^{-1} and 2988 cm^{-1} confirmed the presence of the C-H stretch at diol carbons (C2′ and C3′).

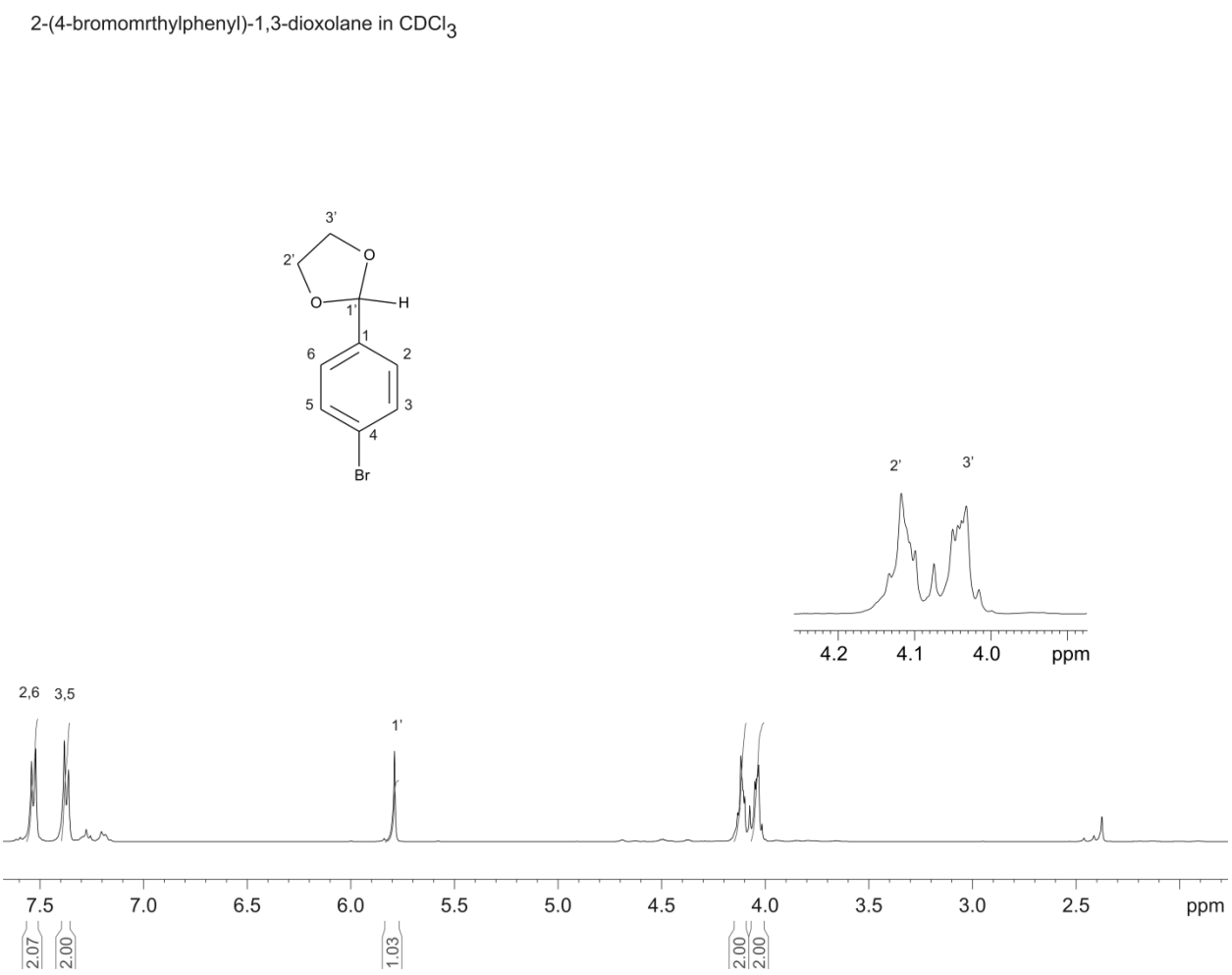
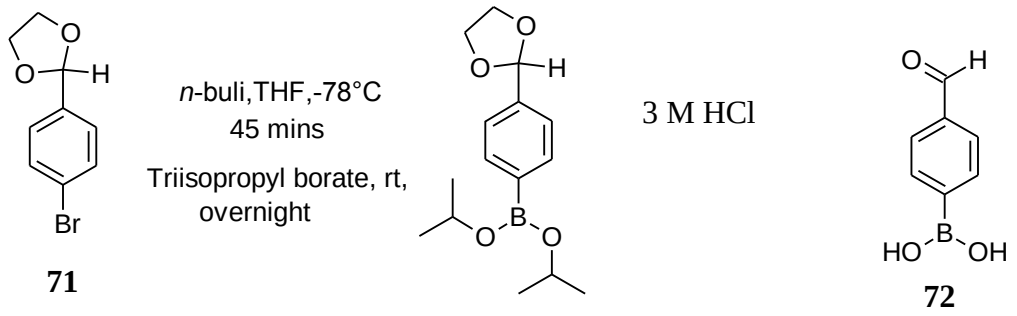


Figure 32: ^{1}H NMR spectrum for compound 71

2.5.2.3.2 Synthesis of 4-formyl phenyl boronic acid (72)

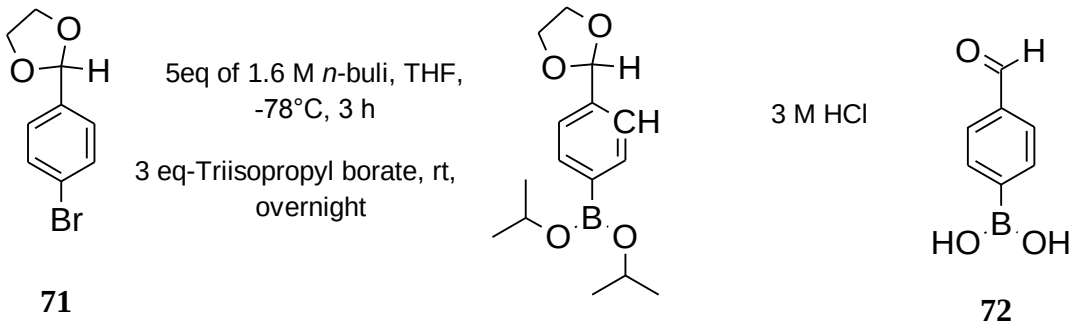
The standard procedure for the preparation of boronic acids from cyclic halide was discussed earlier in this section. Metal-exchange with butylithium is favoured because it does not involve any heating which is needed in Grignard formation to avoid de-protection of the acetal and subsequently nucleophilic attack on the carbonyl carbon instead of the bromine.

Cousin *et al.,* [186]followed by Wu *et al.,* [187]reported that 4-formyl phenyl boronic acid **(72)** could be obtained when the bromine end of 2-(4-bromophenyl)-1,3-dioxilane **(71)** is lithiated with *n*-butyllithium in anhydrous THF at temperatures below -78ºC after which 2 equivalence of the boronation agent either triisopropyl borate or trimethyl borate is added drop wise and stirred over-night at room temperature **(Scheme 33)**. The second part of this procedure would be the de-protection step using a strong acid HCl to afford the desired product.[186-187]



Scheme 33

The reaction under the condition reported Cousin *et al.,* could not yield the desired product for unknown reasons. The ^{1}H NMR spectrum was of that of the starting material **71**. The success in synthesizing compound **72** was obtained when a few method modifications of the procedure on **Scheme 33** were implemented. The modifications include performing the reaction at lower temperatures of -84ºC, adding five equivalence of 1.6 M *n*-buli, 3 equivalence of triisopropyl borate and letting the boronated mixture to stir for 24 hours. The product **72** was obtained as a yellow solid in good yields of 75% when **Scheme 34** was followed.



71 **72**

Scheme 34

The ^{1}H, ^{13}C, COSY NMR spectroscopy and infrared spectroscopy were used to confirm the presence of the product. On the ^{1}H NMR spectrum, singlet aldehyde peak H-4´ was observed at δ 10.03 ppm and another singlet that integrated to two protons that belonged to the hydroxyl groups of the boronic acids was observed at 8.20 ppm. A slight up-field shift of the aromatic doublets peaks (to 7.98 and 7.84 ppm) was observed. A complete peaks assignment and integral ratios of the desired compound is shown in **Figure 36**. The ^{13}C NMR spectrum confirmed the presence the aldehyde peak at 193.94 ppm. The ^{11}B NMR spectrum confirmed the presence of the boronic acid in the product with a broad peak at 27.6 ppm. The strong absorption band at *v* 1337 cm $^{-1}$ (correctly assigned for B-O) in the infrared spectrum could indicate that the product was a boronic acid. The spectroscopic interpretation were in agreements with what was reported by Cousin *et al.*[186]

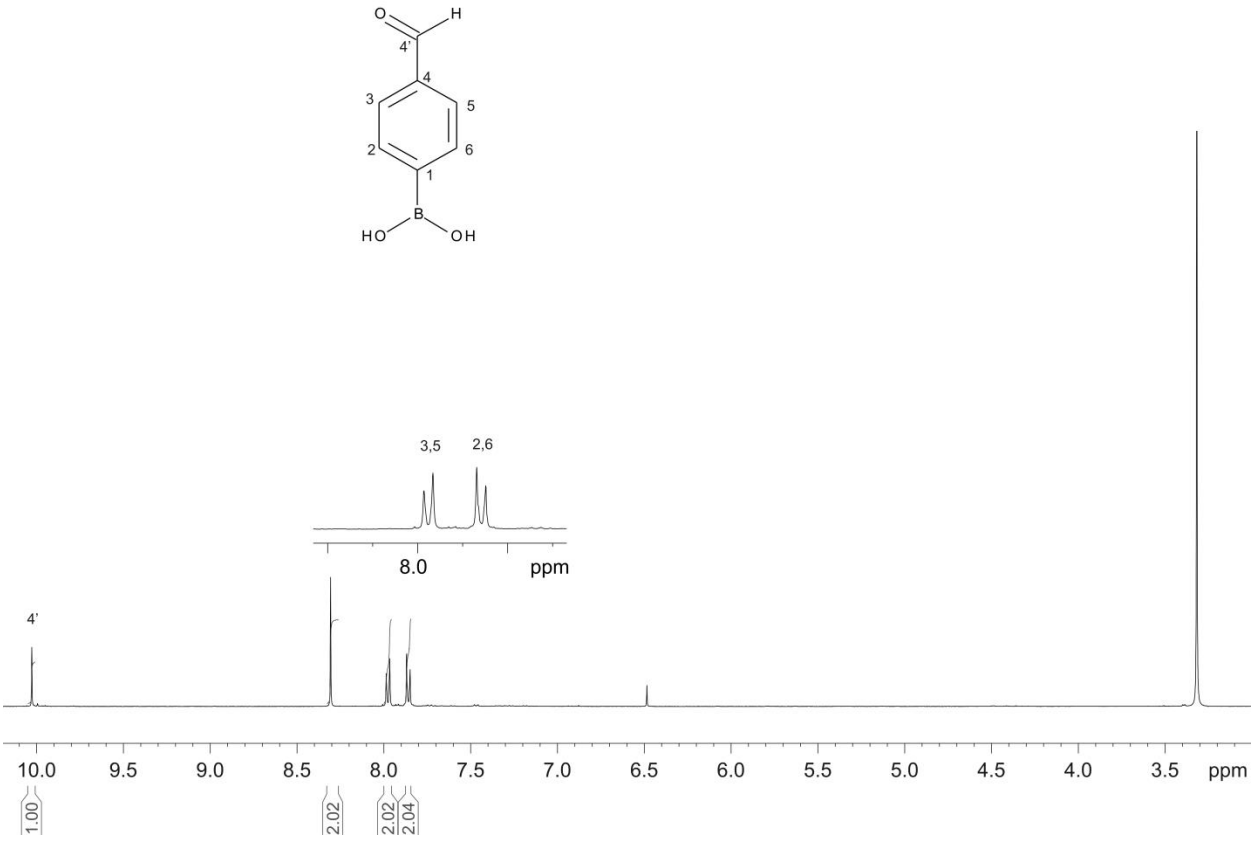
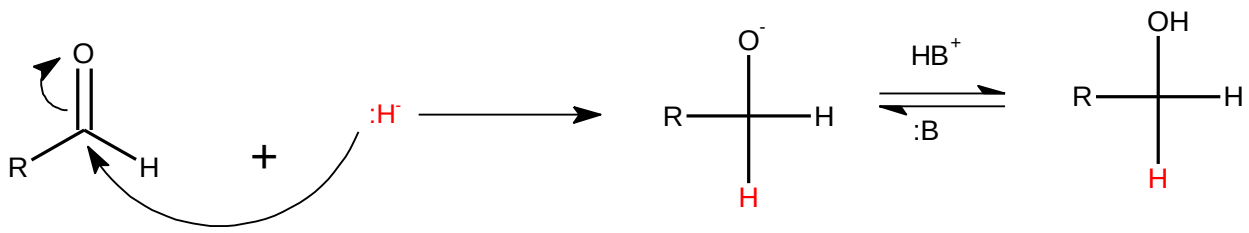


Figure 33: ^{1}H NMR spectrum of compound 72

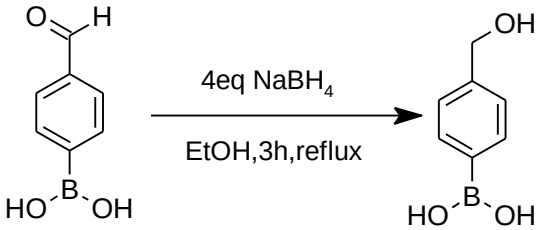
2.5.2.3.3 Synthesis of 4-(hydromethyl)phenyl boronic acid(50)

The conversion of an aldehyde to form a primary alcohol has been well documented in literature. This conversion could be achieved *via* the addition of a hydride ion to the aldehyde to form an alkoxide ion which is followed by protonation by an acid to produce an alcohol (**Scheme 35).**[183]



Scheme 35

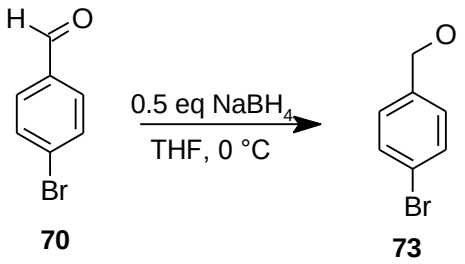
On basis of this mechanism, 4-(hydromethyl)phenyl boronic acid **(50)** formation was expected from reacting 4-formyl phenyl boronic acid **(72)** with sodium borohydride as the source of hydride ion. Zhang *et al.*, reported the synthesis and isolated 4-(hydromethyl)phenyl boronic acid **(44)** as a white solid when 4 equivalence of $NaBH_4$ was added to the aldehyde before refluxing with EtOH for 3hours, a procedure previously proposed by Platter *et al.* **(Scheme 36)**. [188]



72 **50**

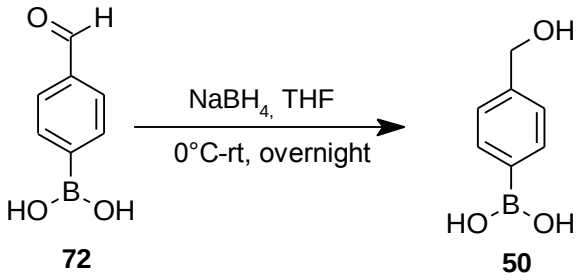
Scheme 36

The procedure reported by Zhang *et al.*, scheme was avoided because it was performed at reflux temperature which from previous experience on working with boronic esters, working on high temperature increases possibilities of compound decomposition. Therefore, reactions performed at room temperature were given first preference. In a separate study, Zhang *et al.*, reported that the reduction of aldehyde to a corresponding alcohol could be achieved by using sodium borohydride as a reducing agent and THF as a solvent. Percentage yield of 95% was reported when 0.5 equivalence of sodium borohydride was added to 4-bromobenzaldeyhde **(70)** in THF later quenched with water to afford 4-bromobenzyl alcohol **(73) (Scheme37)**.[189]



Scheme 37

Using Zhang *et al.* procedure, 4-(hydromethyl)phenyl boronic acid **(50)** was achieved as a white solid in 95% yield when 3.5 equivalence of sodium borohydride was added to 4-formyl phenyl boronic acid **(72)** and the mixture was stirred overnight at room temperature.[188, 190]



Scheme 38

The ^{1}H, ^{13}C, COSY and ^{11}B NMR spectroscopy was used to confirm the formation of the pure product 4-(hydromethyl)phenyl boronic acid **(72)**. The clear indication that the reduction was complete was the disappearance of the aldehyde peak at 10.2 ppm. Two new peaks were observed; a triplet at δ 5.10 ppm which integrated to one proton assigned for the (OH). A doublet at δ 4.48 ppm which integrated for 2 protons was assigned for H-4′ as shown in **Figure 37**. The coupling between the two peaks (at δ 5.10 and δ 4.48) was confirmed using COSY NMR spectrum as shown by a rectangular box in **Figure 38**. An up-field chemical shift of doublets in the aromatic region together with the singlets of the hydroxyl group was observed when ^{1}H NMR spectrum of product was compared with that of starting material **(72)**. The absence of the aldehyde peak (δ 194.3 ppm) in the ^{13}C NMR spectrum confirmed that reduction had gone to completion. Furthermore, in the ^{13}C NMR spectrum a peak at δ 63.3 ppm was assigned correctly for the C-O. All peaks assignment and integrals were in agreement with literature.[191]

4-(hydromethyl)phenyl boronic acid in dmso-d_6

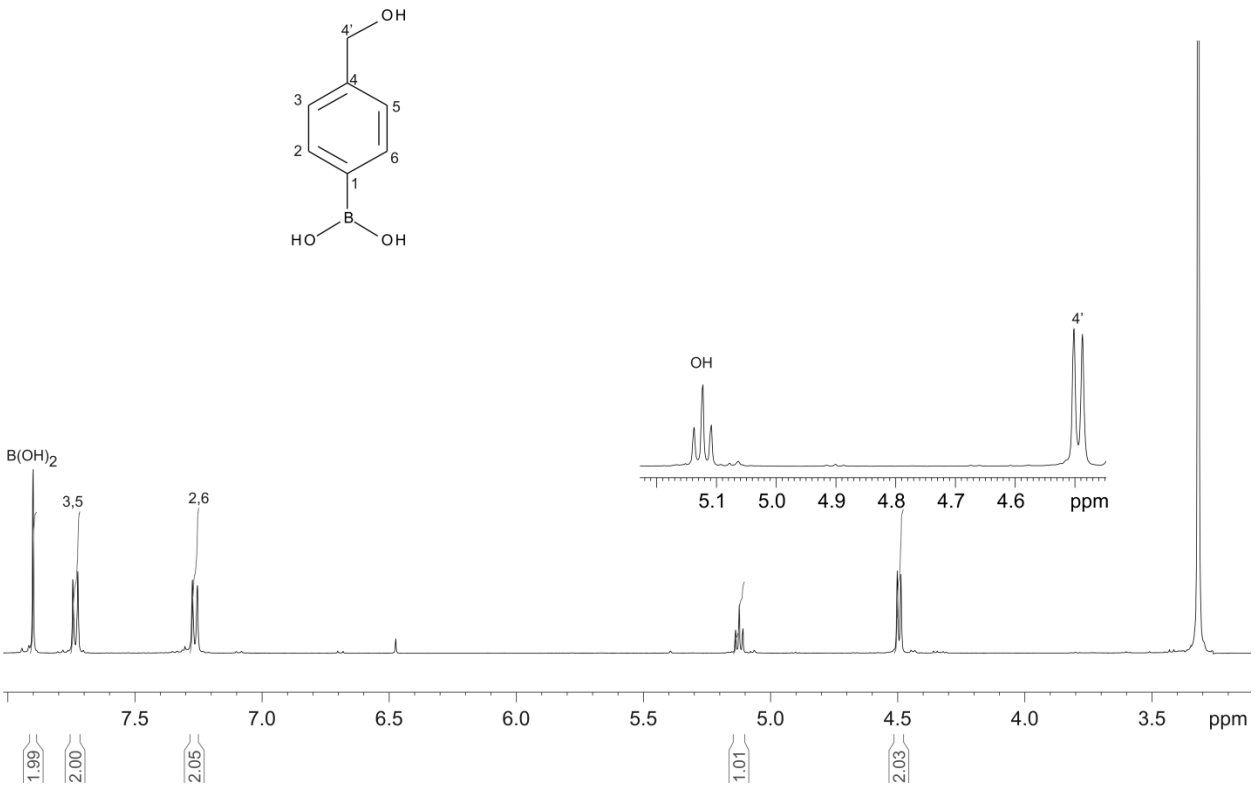


Figure 34: ^{1}H NMR spectrum for compound 50

4-(hydromethyl)phenyl boronic acid in DMSO-d_6

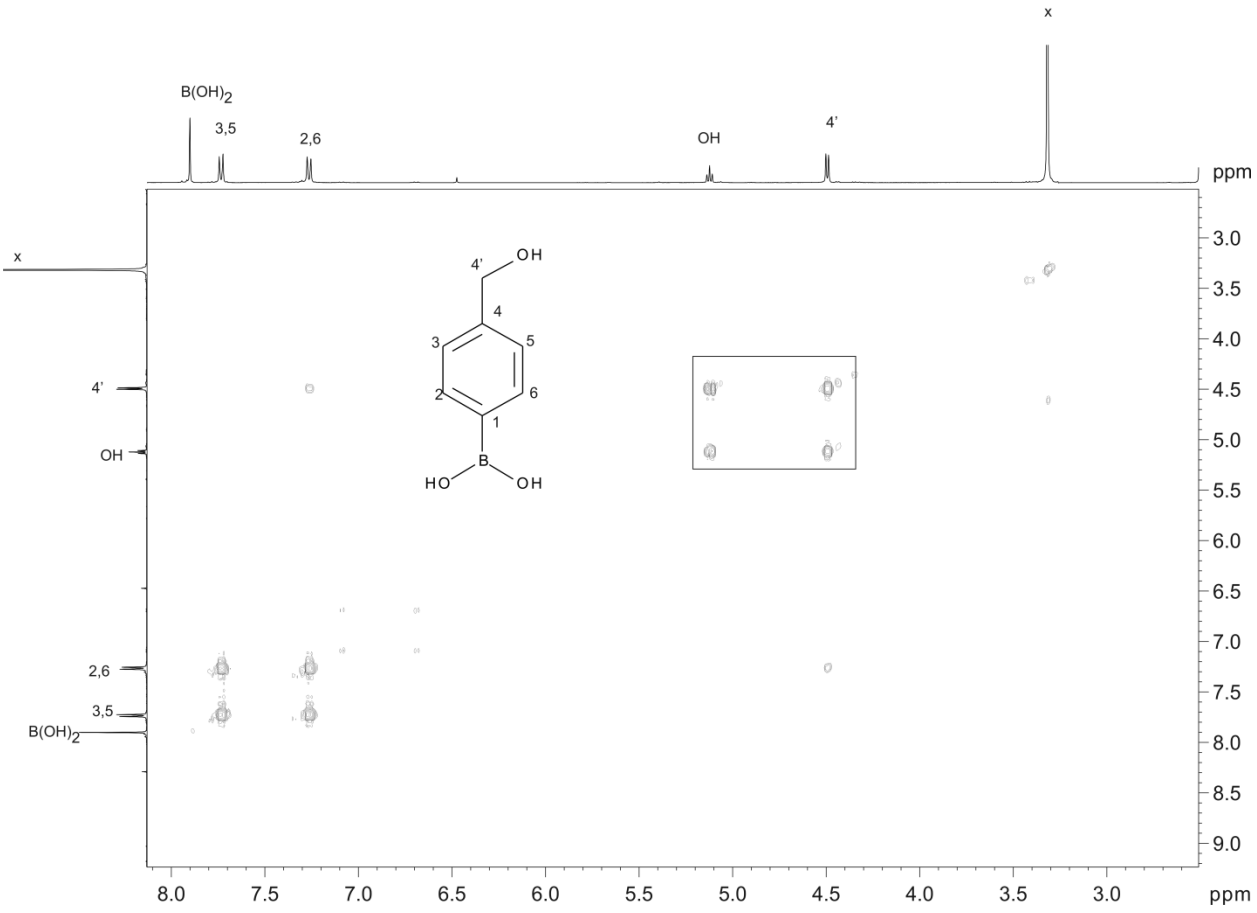


Figure 35: COSY spectrum for compound 50

The boron peak at 27.9 ppm in the ^{11}B NMR spectrum confirmed boron existence as a boronic acid. The reduction was further confirmed by infrared spectrum by the absence of the strong band (at ν 1663 cm^{-1}) that was assigned for the aldehyde C=O stretch on the starting material. A broad absorption band observed at ν 2921 cm^{-1} was correctly assigned to the C-H stretch on C-4′. The combined spectroscopic data analysis confirmed that the desired product was achieved.

The successful preparation of 4-(hydromethyl)phenyl boronic acid **(50)** meant that the aim of synthesizing a boronic acid that could be biotinylated was achieved. As stated earlier (**Section 2.5.2**) when the choice to synthesize this compounds was justified, the mechanism of conjugating to biotin was through an ester linkage. In the subsequent sections the different

strategies possible for the addition of biotin on compound (**72)** will be investigated and results will be discussed.

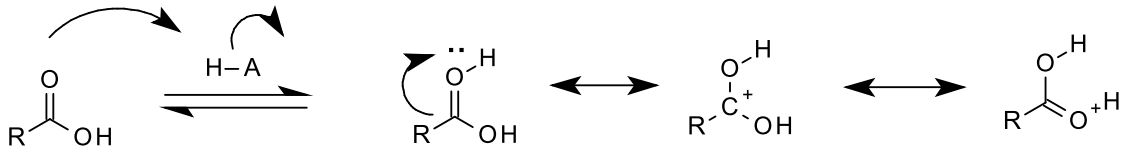
2.5.3 Synthesis of biotinylated boronic acids via the formation of an ester linkage

As it have been discussed earlier, the most active part in the *D*-biotin is the carbonyl on the spacer so the most possible conjugation with the alcohol part of the boronic acid could be *via* the esterification.[72]

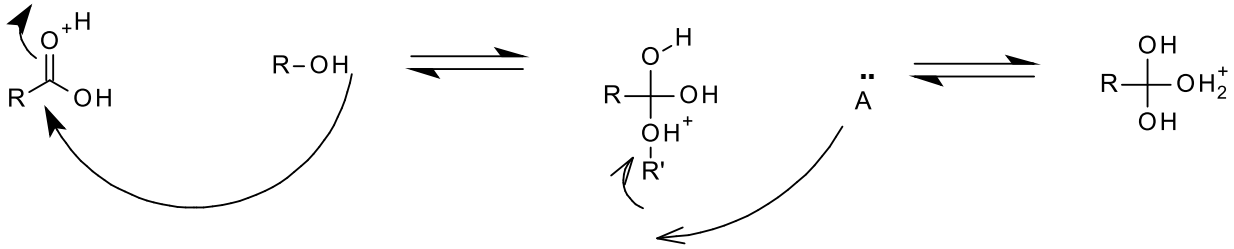
There have been a number of esterification mechanisms that have been reported in literature such as Fischer esterification, nucleophilic acyl substitution and Steiglich esterification. These esterification techniques differ by the manner in which they are catalysed. Catalyst properties such as its identity, its mode of action work and rate of activity influence the choice of the esterification mechanism. Catalyst functionality could either be of the alcohol or the carboxylic acid and the activity could be robust or mild. The two esters that could be formed are alcohol and amine esters with the latter formation being faster than of (C-O) formation.[183]

The Fischer esterification which was first described by Emil Fischer and Arthur Speier in 1895 is an acid catalyst whose mechanism involves a protonation of the carbonyl oxygen to increase electrophilic character of the carbonyl group.[192] The addition of the proton is facilitated by the addition of the acid such as sulphuric acid and *p*-TsOH. A nucleophile attack of the alcohol oxygen occurs on the electrophilic carbonyl by the 1.2 addition to form an intermediate with hydroxyl groups. Proton transfer then follows to give out water and a protonated ester. Water is eliminated followed by deprotonating to give the desired ester (**Scheme 39)**.[183]

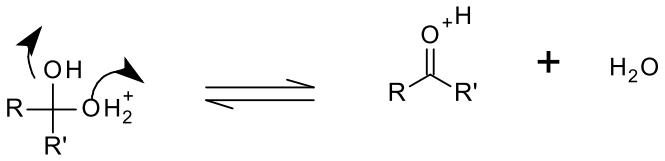
Protonation of carboxylic acid



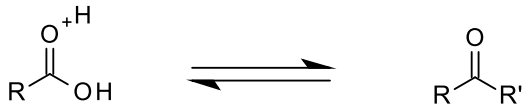
Nucleophilic addition of the alcohol



Elimination of the weaker base- water



Deprotonation to give an Ester

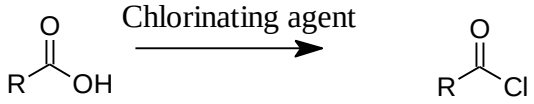


Scheme 39

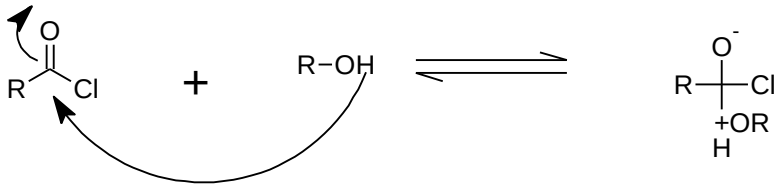
Esterification of carboxylic acids with an alcohol can also be done *via* the formation of acyl halide. A halide replaces the hydroxyl group that makes up the carboxylic grouping to form acyl

halide. To achieve this halide addition, a number of halogenating agents have been reported in the literature. These halogenating agents include thionyl chlorides, phosphorus pentachlorides, oxayl chloride and thionyl bromide.[193] Acyl halides (mostly bromides or chlorides) undergo nucleophilic acyl substitution reaction where it is attacked by a nucleophile to form a tetrahedral intermediate which result in the expulsion of the halide group. If that nucleophile is negatively charged hydroxyl containing compound, these mechanism is as discussed and the resultant compound will be an ester. On the other hand for a neutral nucleophile, the tetrahedral intermediate formed is of high acidity thus when reacted with a strong base a proton is eliminated.[194] The proton removal results in a deprotonated tetrahedral intermediate of which the weaker base, chloride ion is eliminated to give an ester **(Scheme 40)**.[191]

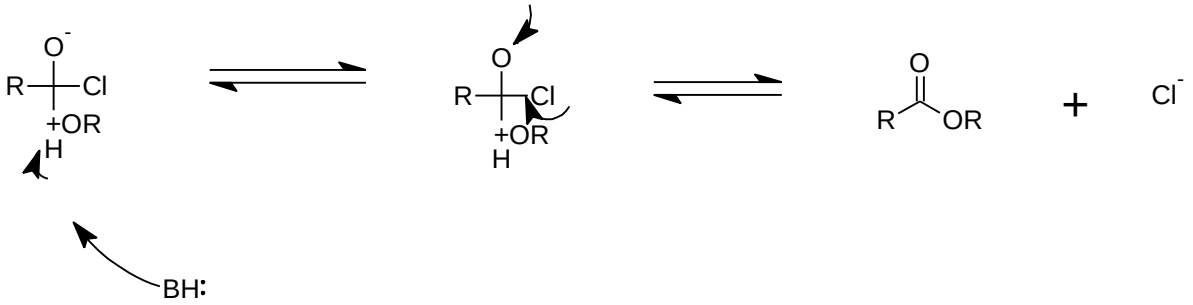
Acyl-halide formation



Nucleophilic attack by the alcohol



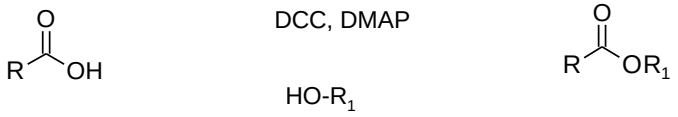
Deprotonation and Elimination of weak base



Scheme 40

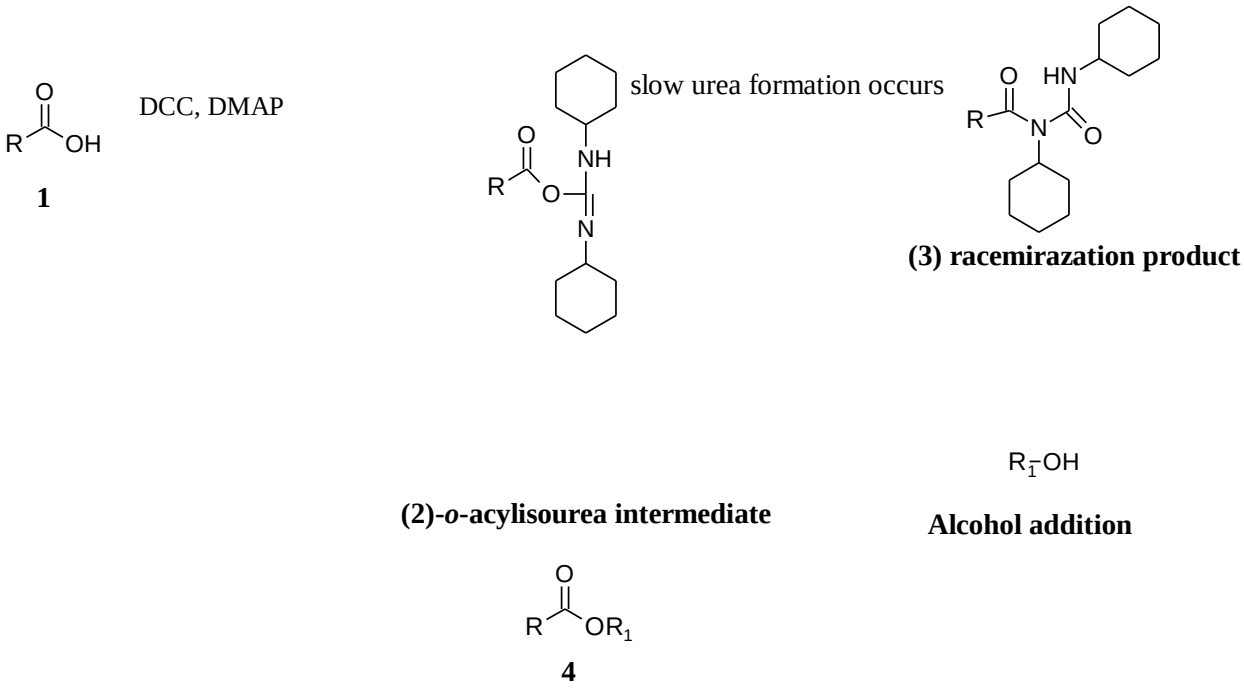
Steiglich esterification has been reported as a mild esterification that is ideal for compounds that fragments in the presence of acid making Fischer esterification.[195] This type of esterification which was first proposed by Steiglich and Höfle in 1969, begins with DCC (dicyclohexylcarbodiimide) reacting with the carboxylic acid to form an *o*-acylisourea intermediate **(2)** which then reacts with the alcohol to form an ester **(4)**. [196] An unavoidable formation of a side product **(3)** due to racemization have been reported as when only DCC

lowering the ester product yield **(Scheme 38)**.[197] The formation of *o*-acylisourea is usually catalysed by DMAP **(**dimethylaminopyridine) an activator of the hydroxyl groups. However usage of DMAP cannot help avoid the formation of the urea side product.[198]



General scheme

Mechanism

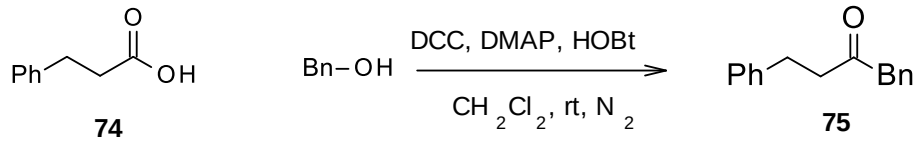


Scheme 41

Thompson *et al.,* reported that carboxylic activation was not limited to DCC or water soluble carbodiimide,1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) but other compounds such as 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMTMM) were

capable of activating carboxylic acids.[199] DMTMM was reported to have a better water solubility and higher carboxylic conversion yields when compared to carbodiimides.[200]

Sheikh *et al.,* studied the esterification of 3-phenylpropionic acid with benzyl alcohol using DCC. The formation of DCC abduct was observed as theoretically accepted but it was achieved in very low yields. The formation of a stable acyl urea, a side product, was attributed to the low yields (34%) of the desired product. The increase of the product yield to 68% was observed when catalytic amounts of DMAP were added. However, urea formation could not be completely eliminated by the addition of DMAP. Sheikh *et al.,* investigated the use of 1-hydroxybenzotriazole (HOBt) as a racemization suppressor reported the increase in product ester yield DCC-DMAP-HOBt-alcohol system has been produce ester linkages with minimal acyl urea formation (**Scheme 42).**[201]



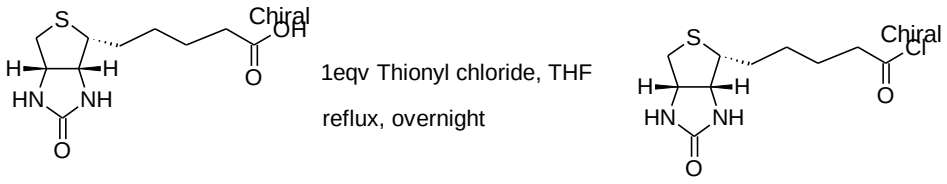
Scheme 42

Joullie *et al.,* reported that other benzotriazole derivatives such as 1-hydroxy-7-azabenzotriazole (HOAt) and hydroxy-6-chlorobenzotriazole (Cl-HOBt) to be have a better coupling capability when compared to HOBt hence reducing racemization.[202]

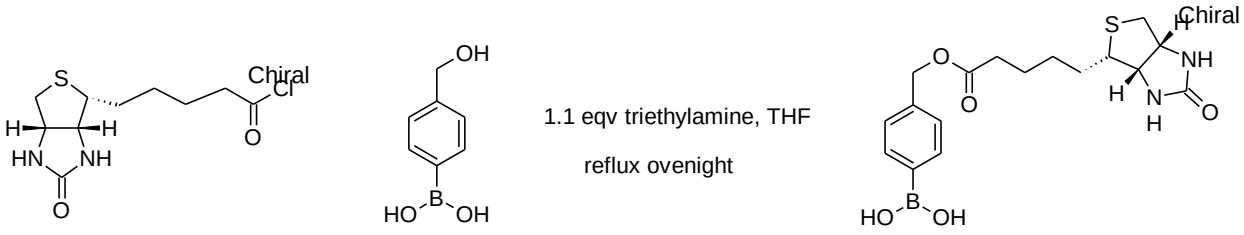
2.5.3.1 Proposed esterification protocols for the biotinylation of 4-(hydromethyl)phenyl boronic acid(50)

The biotinylation of 4-(hydromethyl) phenyl boronic acid **(50)** *via* the formation of an ester linkage was chosen as a possible route to make the desired compound **32.** In trying to achieve this, first esterification mechanism *via* the formation of acyl chloride was proposed. The chlorinating agent chosen was thionyl chloride. The biotin was first reacted with one equivalent thionyl chloride under reflux overnight after which the volatiles were removed before the addition of 4-(hydromethyl)phenyl boronic acid **(50)** with a base triethylamine to give **(32) (Scheme 43)**.

Step 1: Biotin activation(carbonxylic OH chlorination) to form acyl chloride



Step 2 : Biotinchloride derivative reacted with an alcohol to form an ester



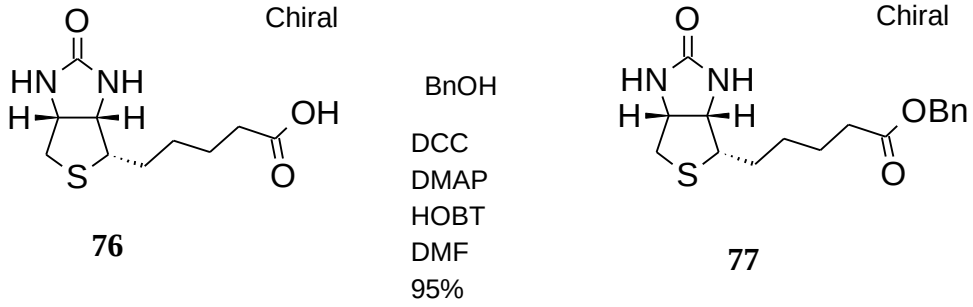
50 **34**

Scheme 43

The ^{1}H NMR spectrum showed the presence of unknown fragments which neither belonged to the biotin part or alcohol. The fragmentation was suspected to be as a result of the robust activity of the thionyl chloride on the multi-grouped, biotin. Fragments formation would persist even when the reaction was performed at room temperature. This esterification protocol was

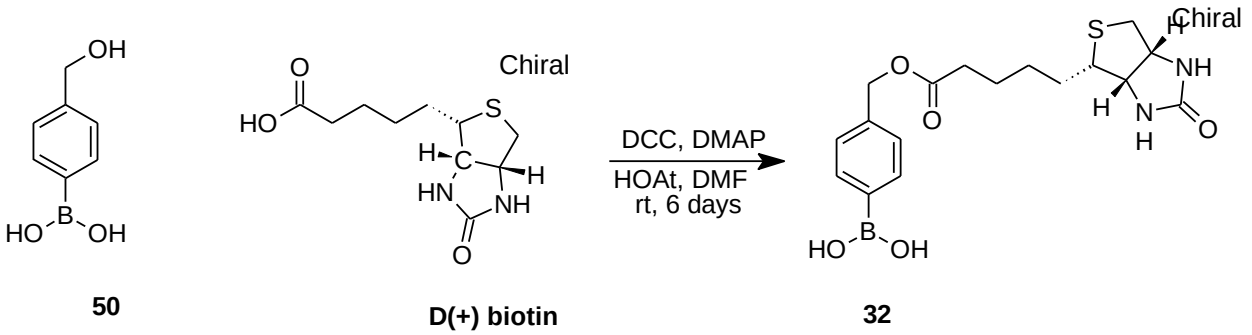
abandoned and a milder one such as Steiglich esterification was proposed as an alternative method.

The addition of biotin (biotinylation) to alcohols using Steiglich esterification mechanism has been reported in literature as the chemically favourable esterification protocol. Hansen *et al.*, reported a 80% yield when biotinylating (E)-vinyl iodide *via* an ester linkage using DCC/DMAP system[150] while Johnson reported an improved yield of 95% when biotinylating benzyl alcohol using DMAP/DCC in the presence of catalytic amounts of HOBt.[203]



Scheme 44

Taking into consideration the separate reports by Joullie *et al.* and Al-Warhi *et al.* that 1-hydroxy-7-azabenzotriazole (HOAt) had facilitated the coupling process better and gave better yields when compared with that HOBt, HOAt was chosen in this study.[204] Johnson's scheme was retained with only benzyl alcohol being replaced by 4-(hydromethyl)phenyl boronic acid **(50)**. The proposed scheme was for the synthesis of **(32)**.



Scheme 45

Using **Scheme 45**, compound **32** was achieved as a white powder at 62% yield. The product was confirmed using ^{1}H, ^{13}C, ^{11}B NMR spectroscopy together with IR and 2D NMR spectroscopy such as COSY, HMBC. The ^{1}H NMR spectrum showed the disappearance of the triple that corresponded to the OH of the starting material which was at δ 5.12 ppm indicated that esterification had occurred. The conversion of the doublet of the CH_2 at δ 4.49 ppm of the starting material to a singlet δ 5.09 ppm (C-4′) which integrated for two protons was observed which was expected if an ester bond have been formed. The peaks and the integral ratios that belonged to the *D*-biotin were as observed in corresponding chemical shifts with commercial available biotin in the same solvent. The boronic acid's aromatic protons were also observed in the expected chemical shifts. **Figure 36** shows the assignment of peaks in the ^{1}H NMR spectrum of the product. The COSY spectrum further confirmed that the singlet C-4′ (highlighted in **Figure 37**) was not coupled to any proton which was expected if an ester linkage between the boronic acid and biotin had formed.

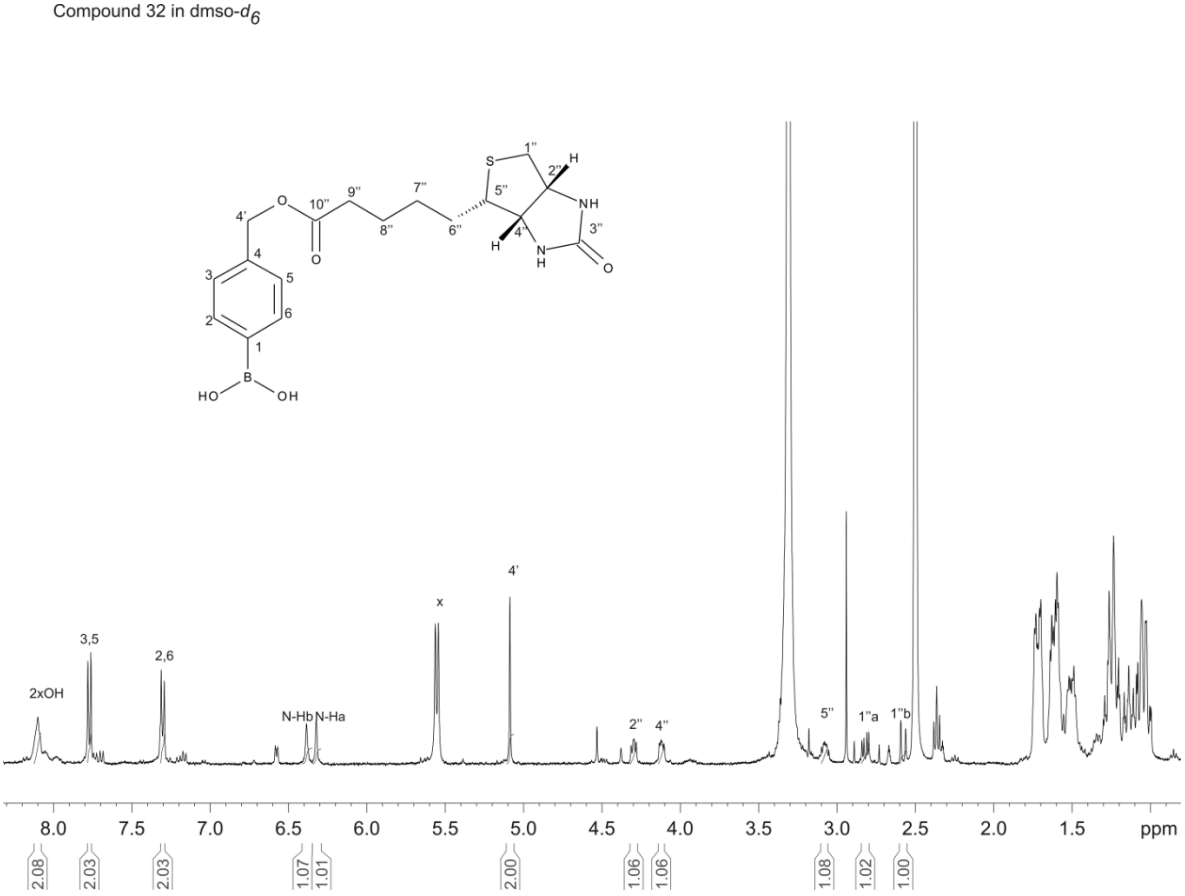


Figure 36: ^{1}H NMR spectrum for compound 32

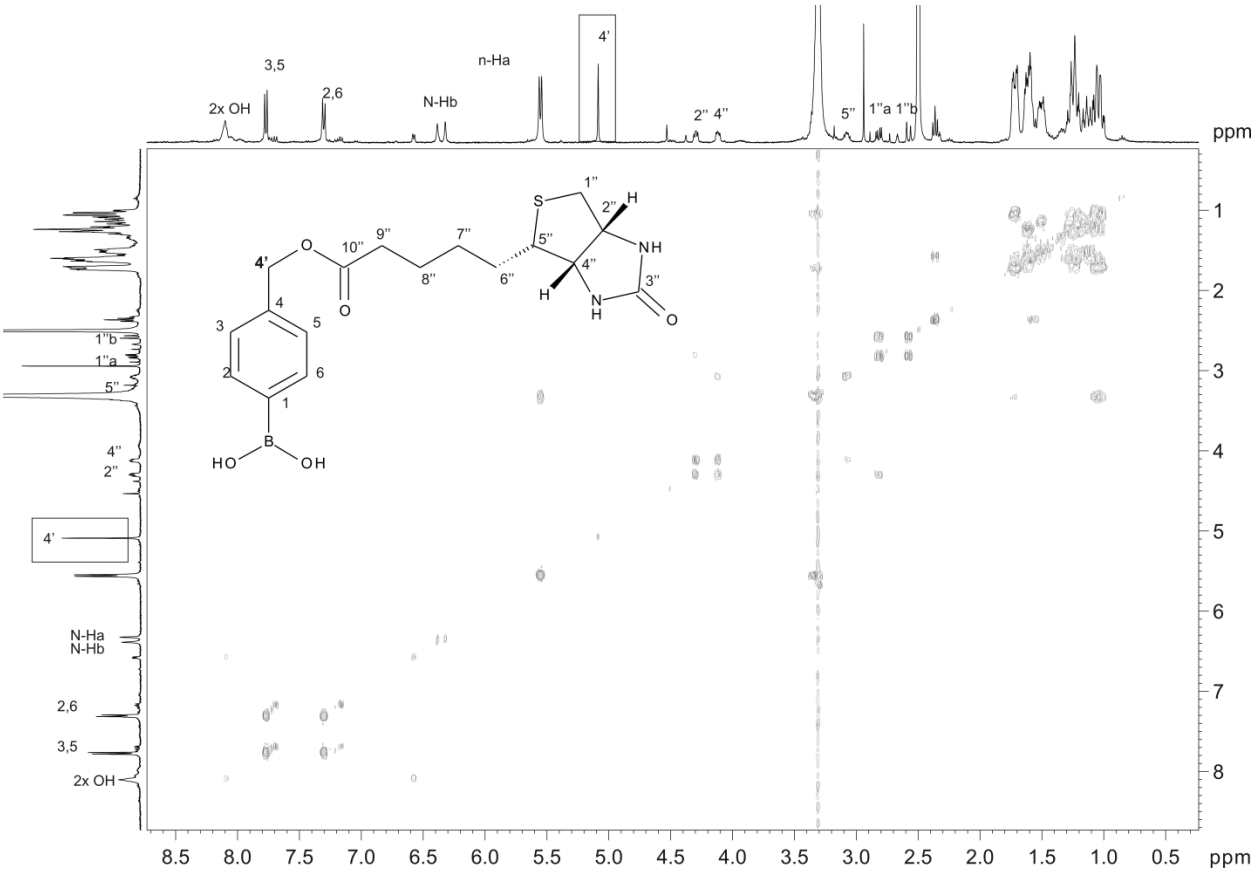


Figure 37: COSY NMR Spectrum for compound 32 showing that C-4´ is not coupled to any proton

The ^{13}C NMR spectrum confirmed the presence of the ester with a peak at δ 173 ppm which it long range coupling with protons of C-4´ was observed in the HMBC spectrum. The assignments of the carbons in the ^{13}C NMR spectrum are shown in **Figure 38**. The expected long–range coupling between the ester-carbon (C-10´´) with protons of C-4´ has been highlighted in the HMBC spectrum in **Figure 39**.

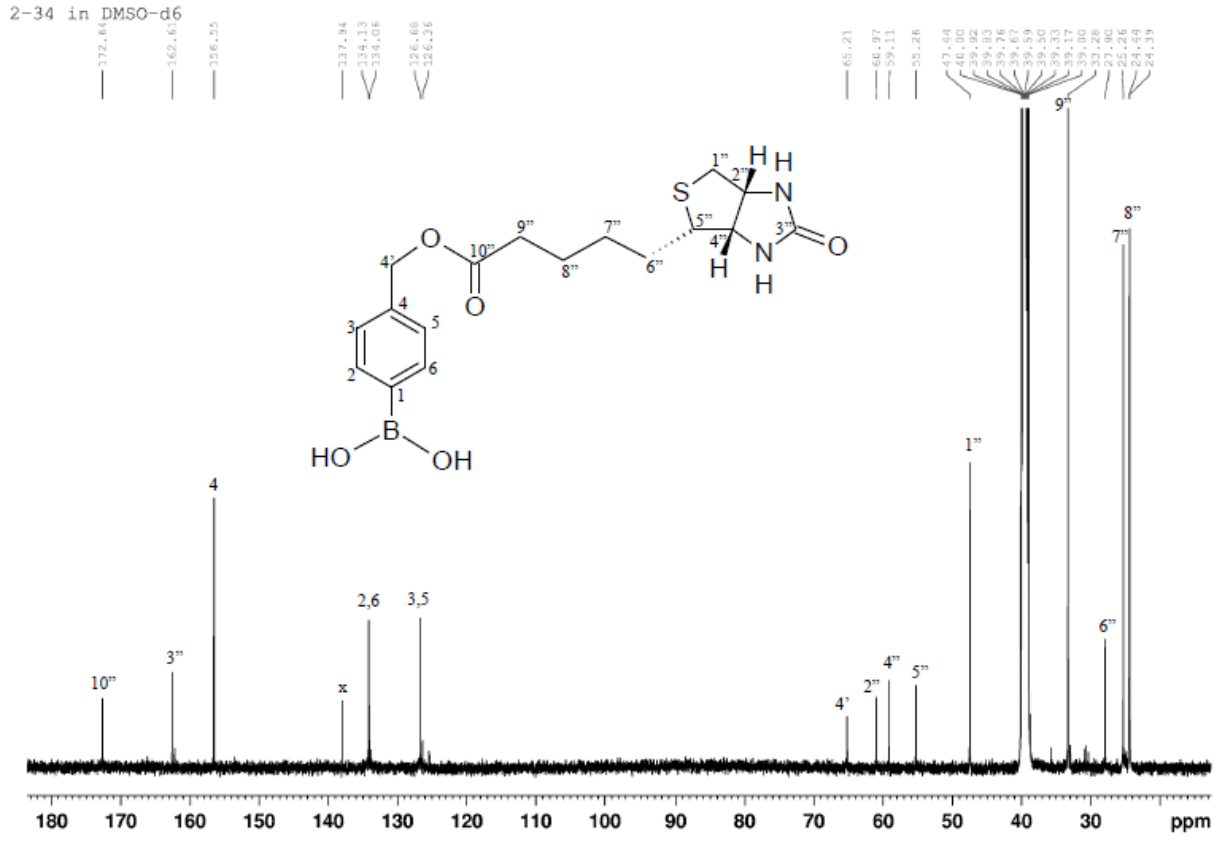


Figure 38: ^{13}C NMR spectrum for compound 32 with the ester carbon highlighted.

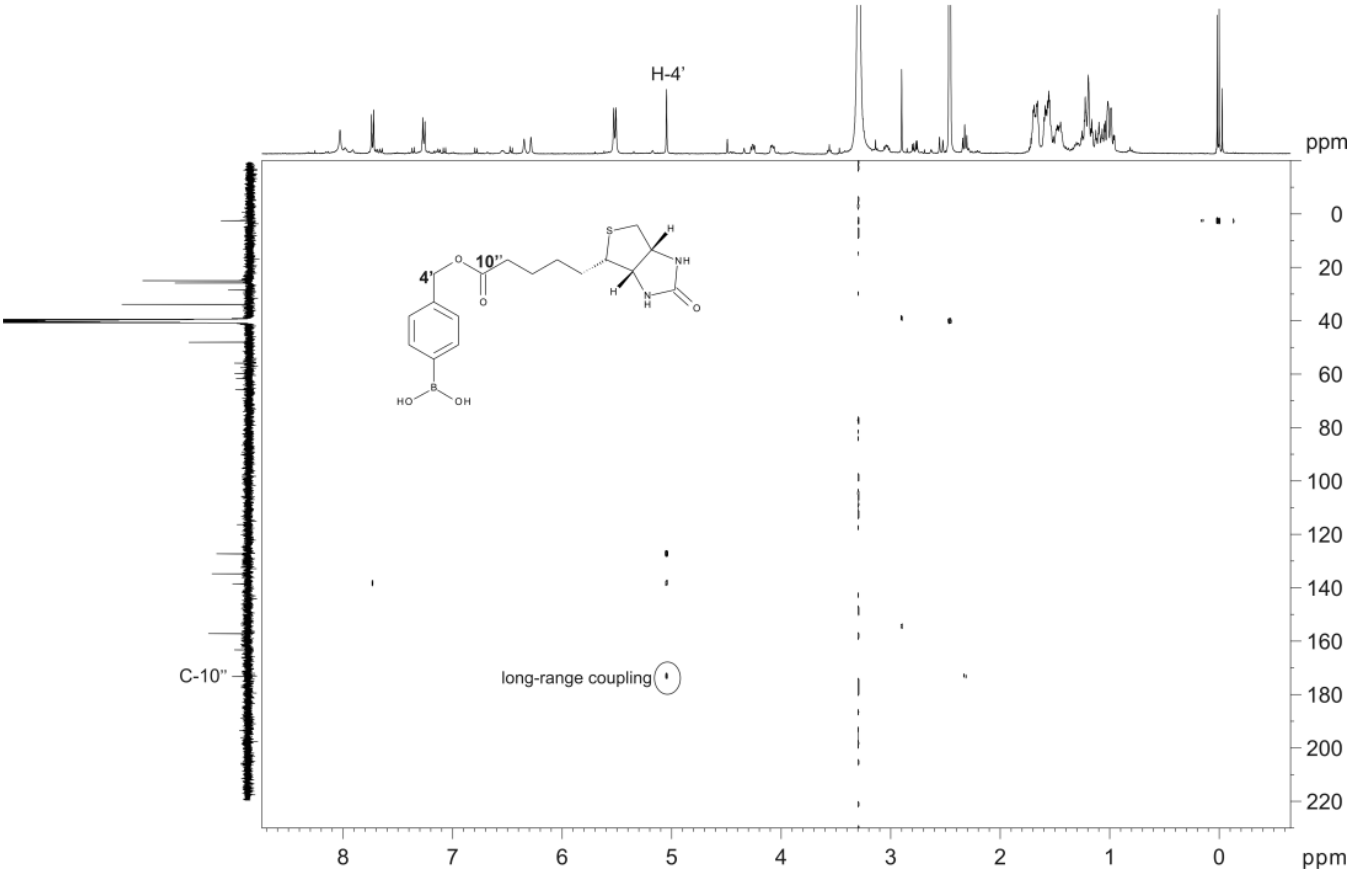


Figure 39: HMBC spectrum showing long-range coupling between protons 4′ and the ester carbon.

All the biotin peaks were retained and the ^{11}B peak was not observed which could be attributed to the solvent influence. To indicate the possibility of the desires product using infrared spectrum, the possible absorptions for the carbons making up the ester linkage were considered (C-4′ and C-10′′). A strong band 1687cm^{-1} was assigned for C=O of the ester carbon (C-10′′) while its C-O stretch was assigned 1088 cm^{-1}. It should be noted that the absorption range for C=O esters that has been reported is 1690-1750 cm^{-1} to be 1700cm^{-1}. The small in wavelength shift in this compound could be attributed to the factors such its bulkiness and complexity. To further confirm the presence of the ester linkage, a strong band at 1045 cm^{-1} was assigned to C′4-O stretch. This assignment was also correct for the linkage of the two carbons that make the ester (C′$_4$- O- C′′$_{10}$) as shown in **Figure 40.**

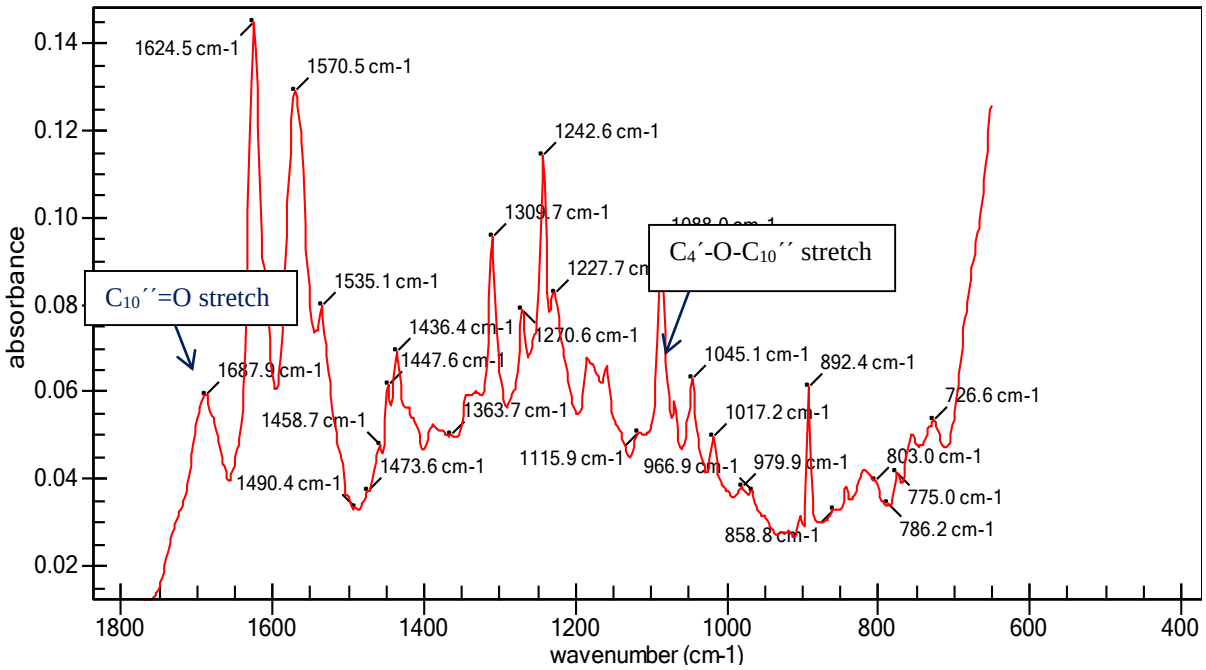


Figure 40: The Infrared spectrum of compound 32

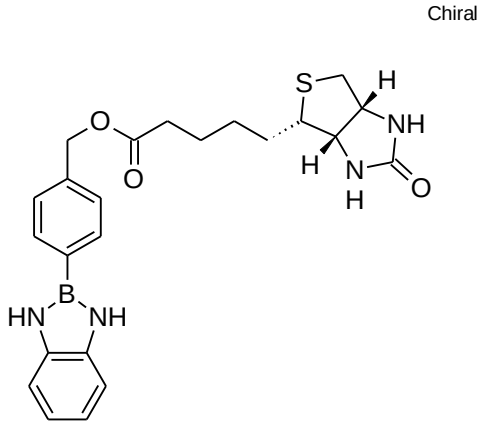
The combined spectroscopic data analysis confirmed the presence of the **compound 32** which was one the major product in this study**.**

2.5.4 Synthesis of biotinylated 1,3,2-benzodiazaboroles derivatives

Is it believed that the presence of biotin in compound **32** could improve both its tumor selectivity and water solubility. While a fluorescence intensity during the binding of boronic acids with saccharides have been reported by Yoon *et al.*, there have been no other reports suggesting that boronic acids can be traced after binding to saccharides.[145] Therefore, tracing these compounds as it transverse through the numerous selective blood barriers to its target tumor would still be a problem.

In this study, it is proposed that attaching the 1,3,2-benzodiazoboroles to replace the boronic part of the biotinylated compound would give that compound the fluorescence property. The 1,3,2-benzodiazaboroles compound were reported to have luminescence properties.

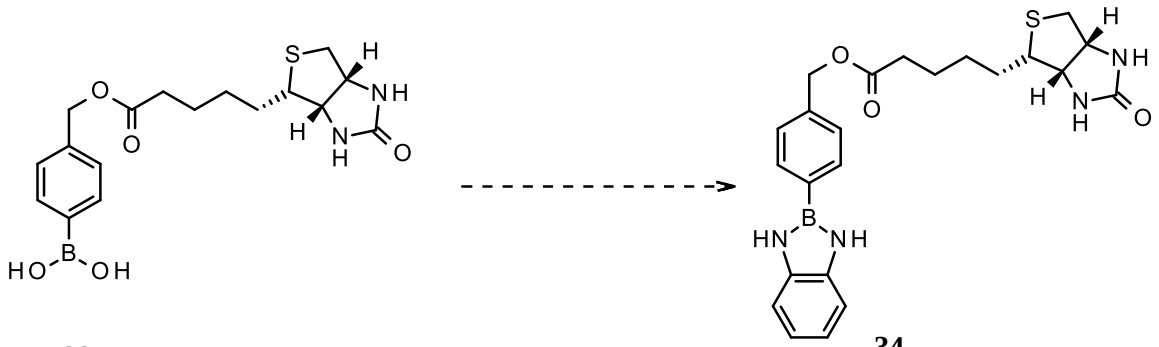
The compound that would both be biotinylated while having the 1,3,2-benzodiazoboroles chosen in this study was 4-(1*H*,benzo[*d*][1,3,2] diazaborol-2(3*H*)-yl)benzyl 5-((3a*R*,6*S*,6a*S*)-hexahydro 2-oxo-1*H*-thieno[3,4-*d*]imidazole-6-yl)pentanoate **(34)**



34

2.5.4.1 Proposed Synthesis of Biotinylated 1,3,2-benzodiaboroles derivatives (34)

The preparation of 4-(1*H*,benzo[*d*][1,3,2] diazaborol-2(3*H*)-yl)benzyl 5-((3a*R*,6*S*,6a*S*)-hexahydro 2-oxo-1*H*-thieno[3,4-*d*]imidazole-6-yl)pentanoate **(34)** began with the synthesis of biotinylated boronic acid 4-((5-((3a*R*,6*S*,6a*S*-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-6-yl)pentanoyloxymethy)phenylboronic acid **(32)** as discussed in the previous section **(Scheme 46).**



32 **34**

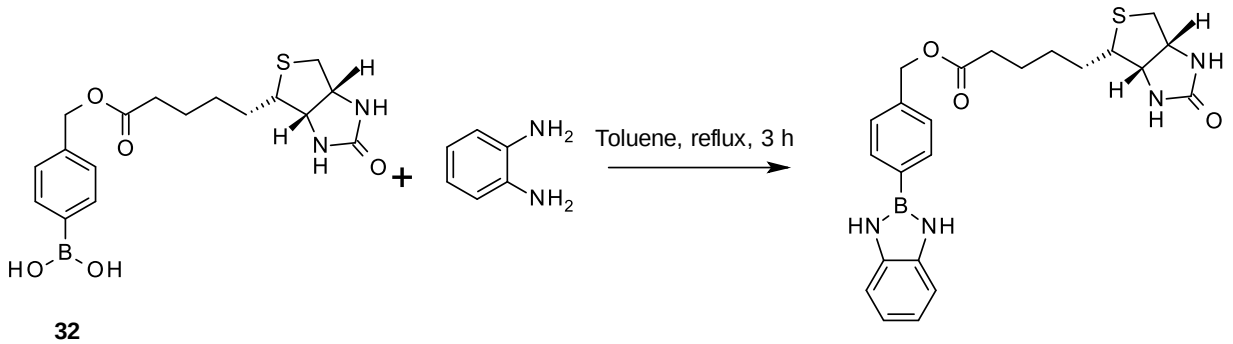
Scheme 46

The preparation of 4-(1*H*,benzo[*d*][1,3,2] diazaborol-2(3*H*)-yl)benzyl 5-((3a*R*,6*S*,6a*S*)-hexahydro 2-oxo-1*H*-thieno[3,4-*d*]imidazole-6-yl)pentanoate **(34)** have been not been reported in literature. However, Slabber *et al.*, have reported that the synthesis of 1,3,2-benzodiazaboroles derivatives from boronic acids could be achieved by reacting with *o*-phenylenediamine subsequently dehydrate the boronic acids (**Scheme 47)**.

R=ph, *p*-PhCl, *p*-PhOMe, *p*-PhMe, *p*-PhSMe, $CH_2CH(CH_3)(CH_3)$

Scheme 47

Taking into consideration this methodology proposed by Slabber *et al*., it was proposed that the conversion of boronic acid part of our compounds to 1,3,2-benzodiazaborales can be possible.



32 **34**

Scheme 48

The product was obtained at 72% yield and was characterised to be 4-(1*H*,benzo[*d*][1,3,2] diazaborol-2(3*H*)-yl)benzyl 5-((3a*R*,6*S*,6a*S*)-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-6-yl)pentanoate **(34)** using spectroscopic techniques such as ^{1}H, ^{13}C, ^{11}B NMR and IR. The ^{1}H NMR spectrum showed the absence of the peak at δ 8.10 ppm which was previously assigned for hydroxyl groups that make up the boronic acid in **(32)**. The protons that make the benzene ring of the attached *o*-phenylenediamine were assigned multiplets at δ 6.79- 6.82 ppm-(H-6′, 9′) and δ 7.03- 7.06 ppm (H- 7′,8′). The *o*-phenylenediamine N-H peaks (B) were observed as a singlet δ 9.10 ppm. The assignment in the aromatic region together with the integral ratios were consistent with those reported by Slabber *et al.,* for 1,3,2-benzodiaboroles derivatives.[140] Outside the aromatic region all peaks were observed as expected with a slight down-field shift from those of the starting material **(Compound 32)**. The protons assignment and integrals of the desired product in the ^{1}H NMR is shown on **Figure 41** below. The amides protons were labelled A and B to distinguish those from biotin and those from *o*-phenylenediamine.

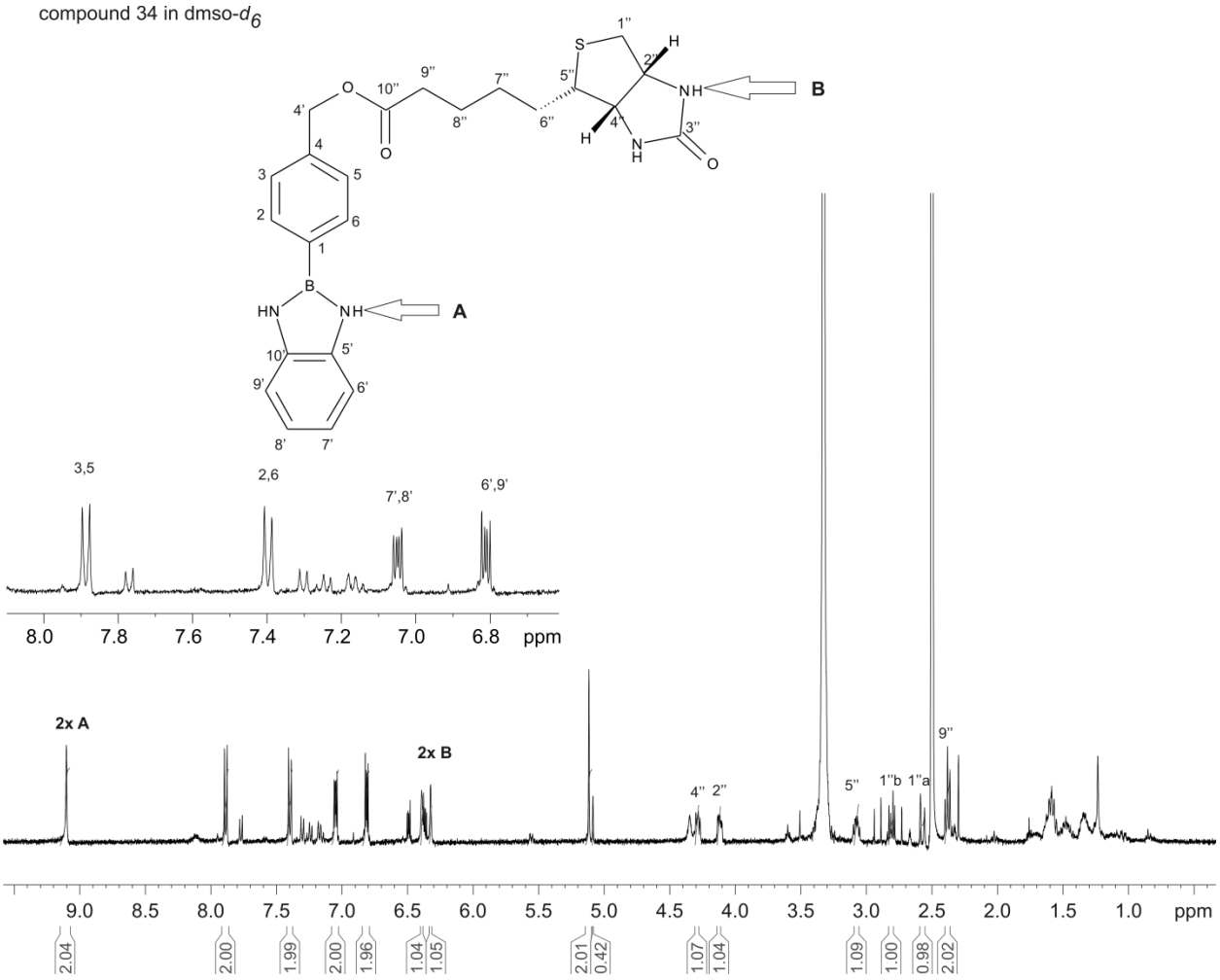


Figure 41: ^{1}H NMR spectrum of compound 34

The ^{1}H NMR spectrum also gave indication that the some of the side product in the product. Two peaks on close proximity (δ5.08 ppm and 5.11 ppm) were observed in a region where only one H-4′ singlet was supposed to be observed. The identity of side product to be the starting material was confirmed when ^{1}H NMR spectrum of the desired product was plotted in comparison with that of the starting material. All the peaks of the starting material matched those observed in the ^{1}H NMR of the product. A clear match was with a singlet at δ 5.08 ppm corresponded with the H-4′ reported for **compound 32**. The starting material ratio of abundance in the product was estimated using integral ratios to be 1:5. The comparison of the product to the starting material is shown in **Figure 42**.

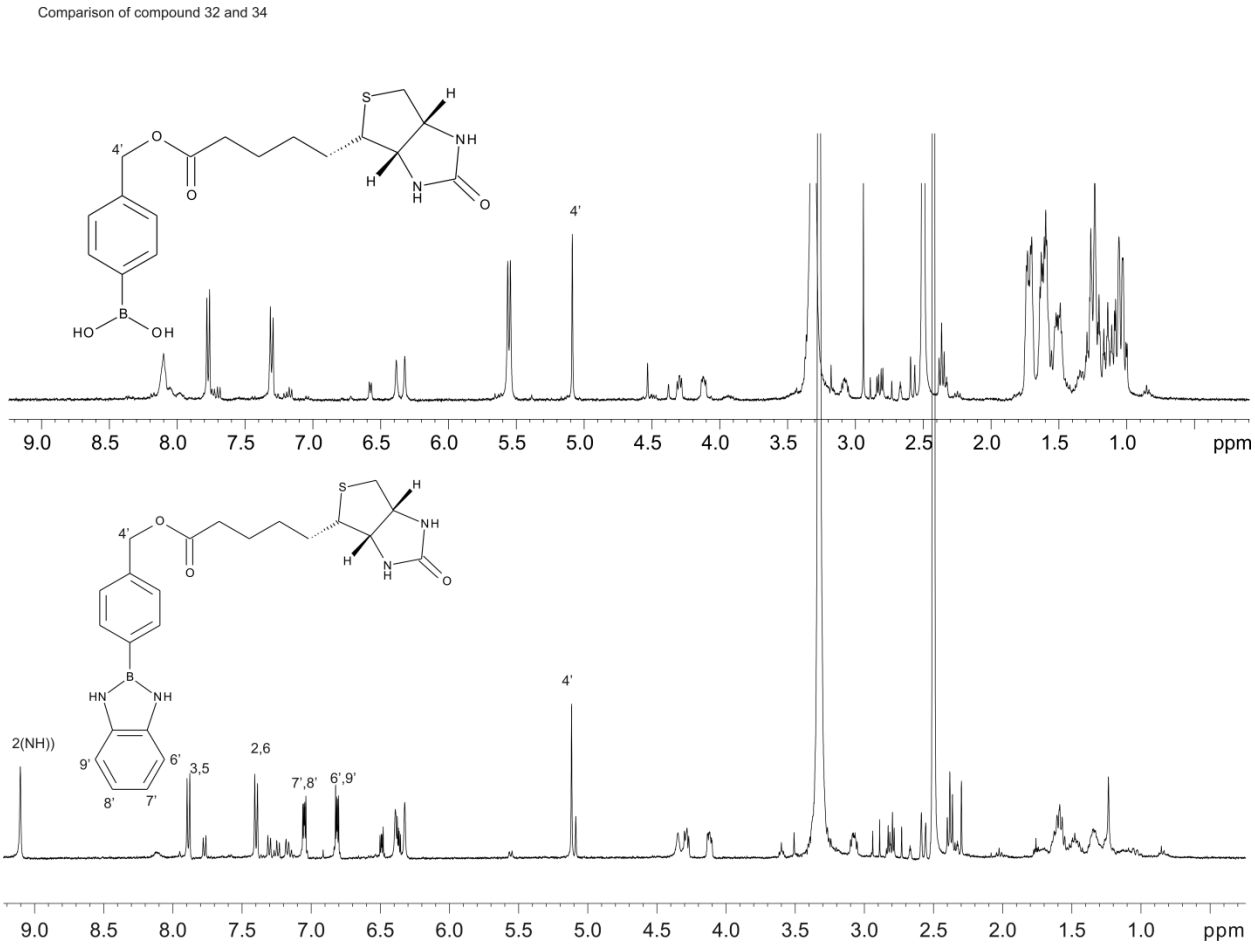


Figure 42: The comparison of the 1H NMR spectrum of compound 32 and 34 to show the new peaks due to the formation of the 1,3,2 benzodiazaboroles moiety.

Efforts to isolate the product from the crude using flash chromatography were unsuccessful. The difficulties of separating boronic acids using silica-gel loaded chromatographic means have been outlined earlier in this study. The desired product could then be flashed down the pass the silica gel. The ^{1}H NMR spectrum of the combined fractions showed the existence of the only two doublets in the aromatic region and some peaks that corresponded to the biotin were not observed.

The peaks that *o*-phenylenediamine brings to the product were given special attention in the ^{13} C NMR spectrum assignments. For the protonated carbons of aromatic ring in *o*-phenylenediamine, a peak at δ 109.1 ppm was assigned for C-7′, 8′ and the other peak δ 111.3 ppm was assigned C-6′,9′. The carbons directly bonded to the nitrogen (C5′, 10′) were observed in more down-field

at δ 134.1 ppm than the C-N (C- 2´´, 4´´) reported for biotin. A slight up-field shift for the peaks that makes up the aromatic ring from the boronic was observed. Carbons-C-3,5 previously observed at δ127.2 ppm in the starting material now observed at δ125.6 ppm for that were expected identical from those assigned for the starting material. This assignments was in agreement with what was reported by Slabber *et al.*, for 1,3,2-benzodiazaboroles.[140]

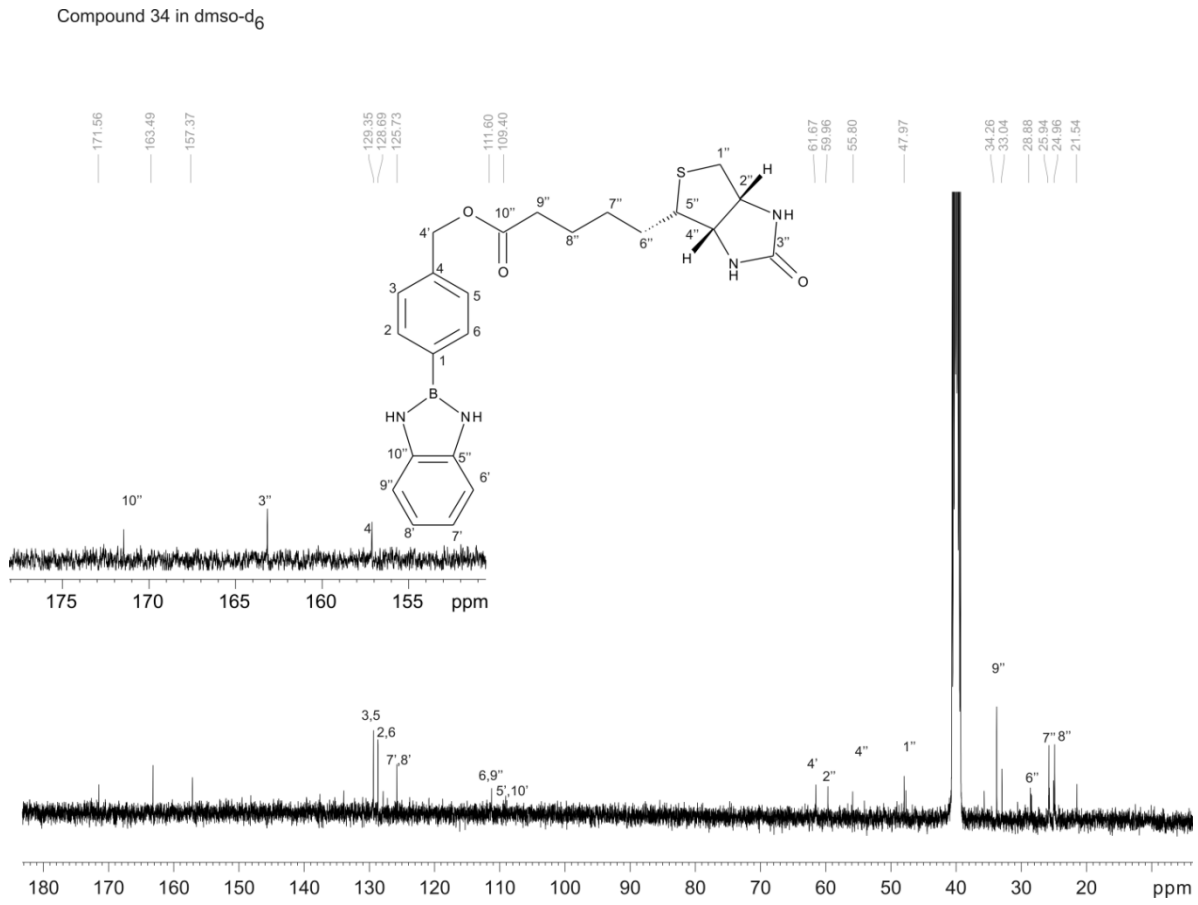


Figure 43: ^{13}C NMR spectrum of compound 34

2.6 Conclusion

The first objective of this study was transform clinically proven BPA derivative (α-methyl BPA) 1,3,2-benzodiaboroles derivatives by reacting with *o*-phenylendiamine. The route proposed for the synthesis of α-methyl boronophenylalanine (BPA) with a fluorescence maker 1,3,2-benzodiaboroles marker could not be taken to completion because of purification problems in most crucial steps. The focus shifted to the biotin conjugation of boronic acids 4-anilineboronic acid and 4-(hydromethyl) phenylboronic acid which were to be biotinylated. The synthesis of 4-anilineboronic acid began with the protection of the amine in the 4-bromoaniline was not be achieved. Numerous attempts with known protection groups such as *tert*-butyloxycarbonyl and trimethylsilane would only gave a one equivalent protection. Other protection protocols were not explored due to time constrains.

The other boronic acid proposed, 4-(hydromethyl) phenylboronic acid, was successfully synthesised after numerous attempts with different synthetic routes. This compound was achieved as a pure yellow solid at combined yield of 79% over three steps. After full characterisation using spectroscopic techniques such as ^{1}H, ^{11}B, ^{13}C NMR, the compound was then conjugated to biotin through the formation of an ester linkage. The biotinylated boronic acid was achieved a white solid with a yield of 62%. The formation of the ester linkage was confirmed with the presence of expected peak at 173 ppm on ^{13}C NMR spectrum and the long range coupling of ester carbon to the methylene protons of the boronic acid on the HMBC spectrum. 1,3,2-benzodiazoboroles derivative was achieved at 72% by reacting with *o*-phenylendiamine already synthesised biotinylated boronic acid under reflux for 3h.

The biological evaluation of the the two biotinylated products for their water solubility, fluorescent , chemical stability and their tumor selectivity capabilities both *in vivo* and *in vitro* were mot perfomed due to time constrains.

2.7 Future work

The binary nature of BNCT allows independent manipulation of the boron delivery and neutron irradiation to increase therapeutic index of this form of treatment for deadly, incurable glioblastoma multiforme tumors (GBM). While significant ground has been made to develop cheap, highly effective accelerator based neutron source, the development of new boron delivery agents is currently other research.

A number of boron compounds which have a potential as boron delivery agents have been synthesised across the world but most have not been clinically proven. The lack of clinical trials has been attributed to the difficulty to trace these compounds *in vivo* during experiments. Literature reports have reported the use of radio tracers on boron compounds to counter the imaging limitations. However, the short–life of most of the radio tracers could mean it was possible that deactivation could occur before the drug reach the target or before neutron irradiation. In this project the synthesis of a 1,3,2-benzodiaboroles derivative 4-(1*H*,benzo[*d*][1,3,2] diazaborol-2(3*H*)-yl)benzyl 5-((3a*R*,6*S*,6a*S*)-hexahydro 2-oxo-1*H*-thieno[3,4-*d*]imidazole-6-yl) pentanoate was achieved in god yield. These kinds of compounds have been well documented to be having luminescent properties and to be stable under physiological conditions. It can therefore be a realistic prospect that all boronic acids with has boron delivery potentials be added the 1,3,2-benzodiaboroles moiety to improve their imaging stability.

The lack of adequate tumor selectivity of boron compound has been the reason of a lot of failed earlier clinical trials on humans. The approached currently used by researches in the development of new boron delivery agents has been to incorporate boron compound with tumor targeting moieties. The identity of these moieties has been influenced by the known metabolism of tumor cells which include what they feed on. Groups such amino acids, carbohydrates, liposomes, peptides, nanoparticles, DNA precursors and receptor targeting boron derivatives have been reported in different studies as carriers of boron to the tumor cells.

This study also underlines the realistic prospect of biotinylated boron compounds as boron delivery agents. This is supported by literature findings that biotinylated chemotherapeutic drugs are deposit favourably to the tumor cells when compared to the normal cells through their over-

expressed receptors. In this study, a new biotinylated boronic acid 4-(5-((3a*R*,6*S*,6a*S*-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-6 yl)pentanoyloxymethy)phenylboronic acid was successfully synthesised which could possibly be a kind of new novel of this kinds of compounds for BNCT studies. However, conducting biological evaluation of the tumor targeting capabilities of this compound both *in vivo* and *in vitro* could still be needed. This study could be making the "birth" of the research on biotinylated boronic acids for BNCT. The synthesis of 4-((5-((3a*R*,6*S*,6a*S*)-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-6-yl)pentanamido)phenylboronic acid using different synthetic routes than those tried in this work would form part of the future works.

3. Experimental Section

3.1 General Methodology

All glassware used in this study was washed using soap, rinsed with water followed with acetone and dried in the oven over night at standardised temperature of 140 ºC. Freshly dried disposable needles were used for all reagents addition. Rubber stoppers were used to cover reaction flasks if they were not charged with a reflux condenser.

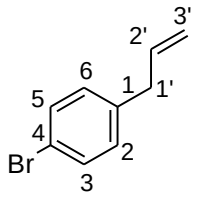
The ^{1}H NMR (400 MHz), ^{13}C NMR (100 MHz) and ^{11}B NMR (100 MHz) spectroscopic data were obtained using Brucer Avance III 400 MHz spectrometer equipped with a 5 mm BBO-Z or 5mm TBIz probe at 30 ºC. Standard size NMR tubes together with deuterated solvents (DMSO-$_{d6}$ and $CDCl_3$). All NMR spectra were reported in part per million referenced to TMS at 0. ^{11}B NMR spectra were referenced to BF_3:OEt_2.

The High-resolution mass spectra were obtained using the Water Acquits LCT premier (TOF) ultra-perfomance liquid chromatography-mass spectrometry. Low resolution mass spectra were recorded using a Thermo Finnigan trace GC, coupled with a Polaris Q Mass spectrometer.

Thin layer chromatography was performed using silica gel coated (60 F_{254}) plates obtained from Merck while for product isolation using chromatographic means, the Grace was used together with flash chromatography on SP Silica Gel 60 from Merck. Chemicals which include starting materials together with reagents were obtained from sigma Aldrich.

3.2 Synthesis of 2-amino-2-methyl-3-(4-dihydroboxybortlphenyl) propionic acid-(α-methyl BPA (31)

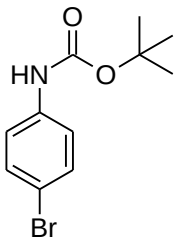
3.2.1 Synthesis of 1-ally-4-brombenzene (37)



1,4 Dibromobenzene (2.475 g, 10.5 mmol) dissolved in 5.2 mL dry THF was slowly added with stirring magnesium turnings (0.255 g, 10.5 mmol) under reflux. When the addition was complete, copper iodide (200 mg, 1.05 mmol) dissolved in 1 mL of THF was added using a syringe followed by the addition of 2 iodine granules. The mixture was refluxed under N_2 for 10 h until the magnesium turnings were completely used up. The reaction flask was cooled in the ice bath. Allylbromide (0.9 mL, 10.5 mmol) was added neat while keeping the flask at 0 ºC. The resultant reaction mixture was refluxed for 5 h, allowed to cool down to room temperature and quenched with NH_4Cl (10 mL). The reaction mixture was then diluted with H_2O (20 mL) then extracted with CH_2Cl_2 (3 × 20 mL). The combined extracts were dried over Na_2SO_4 and concentrated to give 75% of **37** as yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 3.26 (2H, d, J = 6.85 Hz, H3´a and H3´b), 4.95-5.03 (2H, m, H1´a and H1´b), 5.80-5.90 (1H, m, H2´), 7.34 (2H, d, J = 8.4 Hz, H2 and H6), 7.49 (2H, d, H3 and H5). ^{13}C NMR ($CDCl_3$, 128 MHz): δ_c 39.5 (C1´), 121.0 (C3 and C5), 121.9 (C2 and C6), 132.0 (C3´), 133.1 (C2´), 138.9 (C1). IR (neat) νcm^{-1}: 1583, 1466, 1380, 1065, 998, 847 and 670.

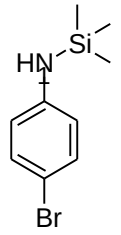
3.3 Synthesis of 4 aniline boronic acid

3.3.1 Synthesis of *tert*-Butyl 4-bromophenyl carbamate (53)



4-Bromoaniline (1 g, 5.8 mmol) together with di-*tert*-butyldicarbonate (2.54 g, 11.6 mmol) was dissolved in acetonitrile:H_2O (1:1). Shortly after, $NaHCO_3$ (1.46 g, 17.4 mmol) was separately dissolved in the same solvent system and the solution was cooled to 0 ºC. The solution prepared earlier was added to the chilled $NaHCO_3$ and the mixture was stirred while maintaining the temperature at 0 ºC for 3 h. The reaction was warmed to room temperature and further stirred overnight. The acetonitrile was removed under reduced pressure. The crude product was isolated by filtration from the white precipitate that formed when the flask attained room temperature after the rotary evaporator. The crude was then extracted with dichloromethane (3 × 15 mL) and the combined extracts were dried over Na_2CO_3. The solution was concentrated under reduced pressure to a colourless liquid that quickly recrystallized into 0.95 g (95%) of **53** as colourless needles. ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H 1.46 (9H s, H3′), 7.44 (4H, s, H2, H3, H5 and H6), 9.45 (1H, s, NH). ^{13}C NMR (DMSO-d_6, 128 MHz): δc 27.3 (C3′), 28.5 (C4), 80.0 (C2′), 120.7 (C3 and C5), 132.6 (C2 and C6), 139.3 (C1), 153.5 (C1′). IR (neat) νcm^{-1}: 3370, 2980, 1697, 1503, 1393, 1363, 1263 and 691. GCMS (EI): 57(100%); 171(68%); 271(53%, M^+).

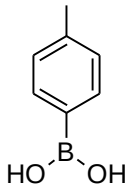
3.3.2 Synthesis of 4-bromo-N-(trimethylsiylbenzanamine (58)



4-Bromoaniline (2.0 g, 11.6 mmol) was dissolved in THF (20 mL) and the solution was stirred at -78 ºC (liquid N_2 + EtOAc) under N_2 atmosphere. 1.6 M Buli in hexane (14.5 mL, 23.2 mmol) was added dropwise. The mixture was stirred at this temperature for 4 h followed by the dropwise addition of trimethylsilylchloride (1.57 g, 14.5 mmol). The reaction flask was allowed to cool to room temperature and then stirred overnight. The resultant white precipitate was isolated by filtration and washed with minimum solvent. The thin layer chromatography was used to monitor the reaction at 6:4 (hexane: EtOAc). TLC plate would confirm the brown liquid as a starting material and further confirmation was done with ^{1}H NMR spectroscopy. Therefore the brown was stored as recovered starting material and further analysis was done on the white solid of mass 1.3 g (74%) of **58**. ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H -0.06 (9H, s, 3 × CH_3), 5.40 (1H, s, NH), 6.57 (2H, d, *J* = 8.7 Hz, H2 and H6), 7.13 (2H, d, *J* = 8.7 Hz, H3 and H5). ^{13}C NMR (DMSO-d6, 128 MHz): δc 2.3 (3 × CH_3), 116.5 (C3 and C5), 131.6 (C2 and C6). IR (neat) *v*cm^{-1}: 3383, 1628, 1250, 812 and 711.

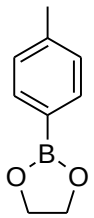
3.4 Synthesis of 4-(hydromethyl)phenyl boronic acid (50)

3.4.1 Synthesis of *p*-tolyboronic acids (60)



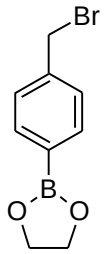
A 1.6 M solution of *n*-butyllithium in hexane (2.73 mL, 29.0 mmol) was added dropwise to a stirring solution of 4-bromotolouene (1 g, 5.8 mmol) in THF (20 mL) at -78 ºC under N_2 atmosphere. The mixture was stirred at this temperature for 4 h after which, triisopropyl borate (4.0 mL, 17.4 mmol) was added dropwise keeping the temperature at -78 ºC. The mixture was left to gradually warm to room temperature. The mixture was further stirred for 48 h at room temperature. The reaction flask was cooled to 0 ºC and 3 M HCl was added and the resultant mixture was stirred for 5 h. The crude product was extracted using ethyl acetate and the combined extracts were washed twice with brine and dried over $MgSO_4$. The crude product was concentrated under vacuum to give 0.71 g (71%) of **60** as a white solid. mp 251.5-256.6 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H 2.31 (3H, s, CH_3), 7.15 (2H, d, J = 7.81 Hz, H2 and H6), 7.66 (2H, d, J = 7.82 Hz, H3 and H5), 7.88 (2H, s, $B(OH)_2$). ^{11}B NMR (DMSO-d_6, 100 MHz): 28.8. ^{13}C NMR (DMSO-d_6, 128 MHz): δc 21.6 (CH_3), 128.4 (C3 and C5), 134.7 (C2 and C6), 139.8 (C4) and no peak for C1. IR (neat) $v$cm^{-1}: 3243.5, 1611, 1401, 1341, 732 and 681.

3.4.2 Synthesis of 2-(4-tolyl)[1,3,2]dioxaborolane (61)



Ethylene glycol (1.64 ml, 29 mmol) was added dropwise to a stirring solution of *p*-tolyboronic acid (2.0 g, 14.7 mmol), *p*-toluenesulfonic acid (catalytic amount, 1.4 mmol) in dry toluene under N_2. After the addition was complete, the reaction mixture was heated to reflux vigorously to remove water from the ethylene glycol. The mixture was refluxed for 25 h after which, the reaction was quenched with $NaHCO_3$ and the crude product was washed with water followed by brine and dried over $MgSO_4$. The crude was isolated without any purification to give 1.8 g (90%) of **61** as a white solid. mp 66-69 ºC; ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 2.39 (3H, s, H4´a, H4´b, H4´c), 4.37 (4H, s, H7a, H7b and H8a, H8b), 7.22 (2H, d, *J* = 7.47 Hz, H2 and H6), 7.73 (2H, d, *J* = 7.47 Hz, H3 and H5). ^{11}B ($CDCl_3$, 100 MHz): δ_B 31.6. ^{13}C NMR ($CDCl_3$, 128 MHz): δc 21.7 (C4´), 66.0 (C7 and C8), 128.6 (C3 and C5), 134.8 (C2 and C6), 141.6 (C4) and no peak for C1. IR (neat) *v*cm^{-1}: 2982, 2911, 1598, 1397, 1371, 1240,726 and 661.

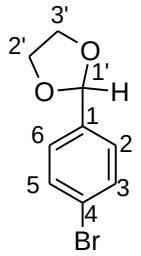
3.4.3 Synthesis of 2-(4-(bromomethyl)phenyl)1-3-2-dioxaborolane (62)



N-Bromosuccinimide (1,18 g, 6.6 mmol) and azoisisobutyronitrile (1.09 g, 6.6 mmol) was added to a solution of 2-(4-tolyl)[1,3,2]dioxaborolane (1.0 g, 6.6 mmol) in carbon tetrachloride. The mixture was refluxed at 150 ºC for 40 h. Solvents were evaporated under vacuum to obtain 0.63 g (63%) of 62 as yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ_H 4.40 (4H, s, H7a, H7b and H8a, H8b), 4.51 (2H, s, H4´), 7.42 (2H, d, J = 7.93 Hz, H2 and H6), 7.80 (2H, d, J = 7. 93 Hz, H3 and H5). ^{11}B NMR ($CDCl_3$, 400 MHz): δ_B 31.5. ^{13}C NMR ($CDCl_3$, 100 MHz): δc 31.0 (C4´), 65.9 (C7 and C8), 128.6 (C3 and C5), 134.8 (C 2 and C6), 141.6 (C4) and no peak for C1. IR (neat) νcm^{-1}: 2993, 2874, 1663, 1406, 1309, 696 and 667.

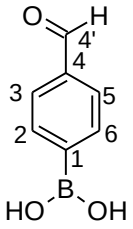
3.5 Second proposed synthesis of 4-(hydromethyl)phenyl boronic acid (50)

3.5.1 Synthesis of 2-(4-bromophenyl)-1,3-dioxilane (71)



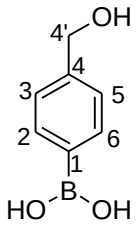
Ethylene glycol (0.34 g, 5.4 mmol) was added together with catalytic amount of *p*-toluenesulfonic acid to 4-bromonezaldehyde (0.50 g, 2.7 mmol) in dry toluene (30 mL). The mixture was refluxed under Dean-Stark condenser for 24 h. After the 24 h have elapsed the water collected on the Dean-Stark was discarded and the reaction mixture was quenched with ice-cold saturated $NaHCO_3$ and the organic layer was washed with saturated $NaHCO_3$ and brine. The organic solution was then dried over Na_2SO_4. From the isolated colourless oil white material crystallised out immediately. The product was isolated by flash chromatography using hexane: EtOAc (9:1) to give 0.34 g (68%) of **71** as colourless oil. ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H 3.94-3.97 (2H, m, H2′ or H3′), 4.01-4.04 (2H, m, H2′ or H3′), 5.78 (1H, s, H1′), 7.28 (2H, d, *J* = 8.34 Hz, H2 and H6), 7.54 (2H, d, *J* = 8.34 Hz, H3 and H5). ^{13}C NMR (DMSO-d_6, 128 MHz): δc 14.3 (C2′ or C3′), 20.7 (C2′ or C3′), 60.0 (C1′), 129.2 (C3 and C5), 131.1 (C2 and C6). MS (ESMS) m/z: 228.9 [M+] for the calculated 229.

3.5.2 Synthesis of 4-formyl phenyl boronic acid (72)



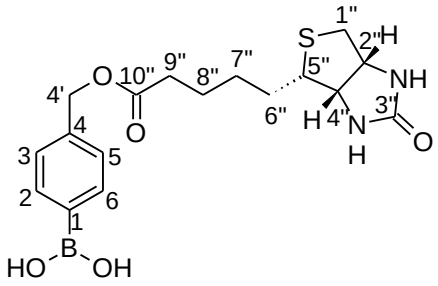
1.6 M *n*-Buli in hexane (0.54 g, 8.5 mmol) was added dropwise to a solution of 2-(4-bromophenyl)-1,3-dioxilane (0.40 g, 1.7 mmol) in THF (15 mL) under nitrogen at -78 ºC (maintained by liquid N_2 and EtOAc). The mixture was stirred at this temperature for 3 h after which, triisopropxylborate (0.96 g, 5.1 mmol) was added. The reaction mixture was allowed to gradually warm to room temperature, it was then stirred for 24 h. Freshly prepared 3 M HCl (2 mL) was added to the reaction flask and resultant mixture was stirred for 1 h. The reaction mixture was extracted with ethyl acetate and the combined extracts were washed with brine and dried over Na_2SO_4. The product was purified by flash chromatography (hexane: EtOAc 3:7) to obtain 0.30 g (75%) of **72** as a yellowish powder. mp 143-147 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H 7.87 (2H, d, *J* = 8.04 Hz, H2 and H6), 7.81 (2H, d, *J* = 8.04 Hz, H3 and H5), 8.31 (2H, s, $B(OH)_2$), 10.02 (1H, s, CHO). ^{11}B NMR (DMSO-d_6, 100 MHz): δ_B 27.9. ^{13}C NMR (DMSO-d_6, 100 MHz): δc 129.0 (C3 and C5), 135.3 (C2 and C6), 137.8 (C4), 193.8 (C4′). IR (neat) ν_{max} /cm^{-1}: 3195, 2842, 1663, 1503, 1337.

3.5.3 Synthesis of 4-(hydromethyl) phenyl boronic acid (50)



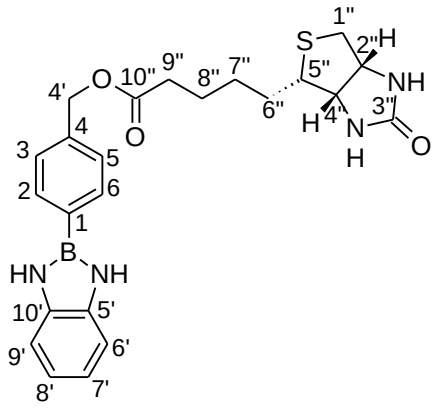
To a stirring solution of 4-formyl phenyl boronic acid (0.10 g, 0.067 mmol) in THF (20 mL), $NaBH_2$ (0.01 g, 0.23 mmol) was added slowly at 0 ºC. After the addition was complete, the mixture was stirred under N_2 atmosphere for 12 h (overnight) at room temperature. The reaction was quenched with 10 mL of water by dropwise addition. The organic solution was extracted with diethyl ether and dried over $NaSO_4$. Solvents were removed under reduced pressure to give colourless oil that solidified to 0.095 (95%) of **50** as a white powder. mp 243-249 ºC; ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H 4.49 (2H, d, J = 5.80 Hz, H4´a and H4´b), 5.14 (1H, t, OH), 7.25 (2H, d, J = 8.08 Hz, H2 and H6), 7.72 (2H, d, J,= 8.09 Hz, H3 and H5), 7.92 (2H, s, $B(OH)_2$). ^{11}B NMR (DMSO-d_6, 100 MHz): δ_B 28.8. ^{13}C NMR (DMSO-d_6, 100 MHz): δc 63.3 (C4´), 125.9 (C2 and C6), 134.4 (C3 and C5), 144.8 (C4), no peak observed for $CB(OH)_2$. IR (neat) νcm^{-1}: 3411, 2921, 1514 and 1335.

3.5.4 Synthesis of 4-(5-((3a*R*,6*S*,6a*S*-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-6 yl)pentanoyloxymethy)phenylboronic acid (32)



4-(Hydromethyl) phenyl boronic acid **50** (0.10 g, 0.65 mmol) was introduced in reaction flask followed by HOAt (0.007 g, 0.05 mmol), DMAP (0.005 g, 0.05 mmol) and biotin (0.132 g, 0.54 mmol). Dry DMF was added and the mixture was stirred under N_2 atmosphere until all reagents had dissolved. DCC (0.167 g, 0.81 mmol) was then added slowly. After the addition was complete, the mixture was stirred for 6 days at room temperature. The precipitate was then isolated by filtration and the DMF was removed under reduced pressure to give a yellowish solid. The yellow solid was washed with water while it was still on the flask and the filtered off and further dried under vacuum to remove excess water to give 0.062 g (62%) of **32** as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H 1.18 (2H, m, H7′′a and H7′′b), 1.47-1.65 (4H, m, H6′′a and H6′′b, H8′′a and H8′′b), 2.35 (2H, t, H9′′a and H9′′b), 2.57 (1H, d, H1′′a), 2.79-2.84 (1H, m, H1′′b), 3.05- 3.10 (1H, m, H5′′), 4.09- 4.13 (1H, m, H2′′), 4.26- 4.32 (1H, m, H4′′), 5.08 (2H, s, H4′a and H4′b), 6.32 (1H, s, N-Ha), 6.38 (1H, s, NHb), 7.29 (2H, d, *J* = 7.95 Hz, H2 and H6), 7.77 (2H, d, *J* = 7.96 Hz, H3 and H5), 8.10 (2H, s, $B(OH)_2$). ^{13}C NMR (DMSO-d_6, 128 MHz): δc 24.8 (C 8′′), 25.7 (C-7′′), 28.4 (C 6′′), 33.7 (C-9′′), 48.0 (C1′′), 55.8 (C5′′), 59.7 (C 4′′), 61.4 (C 2′′), 65.8 (C-4′), 127.2 (C3 and C5), 134.6 (C2 and C6), 157.1 (C4), 163.1 (C3′′), 173.1 (C10′′). IR (neat) *v*cm^{-1}: 3321, 2926, 2917, 2848, 1687, 1624, 1570, 1309, 1242, 1088, 1045, 892, 803 and 726.

3.5.5 Synthesis of 4-(1*H*,benzo[*d*][1,3,2] diazaborol-2(3*H*)-yl)benzyl 5-((3a*R*,6*S*,6a*S*)-hexahydro 2-oxo-1*H*-thieno[3,4-*d*]imidazole-6-yl)pentanoate (34)



o-Phenylendiamine (0.057 g, 0.5 mmol) was added to 4-(5-((3a*R*,6*S*,6a*S*-hexahydro-2-oxo-1*H*-thieno[3,4-*d*]imidazole-6ylpentanoyloxymethyl)phenylboronic acid (0.100 g, 0.2 mmol) dissolved in dry toluene (50 mL). The reaction flask was connected to a Dean-Stark condenser with a N_2 line. The mixture was refluxed for 6 h at 150 ºC. When the reaction was complete, the flask was allowed to cool to room temperature then on ice-cold water bath to aid its precipitation. The precipitate was isolated by filtration and the filtrate was dried over vacuum to give 0.072 g (72%) of **34** as a brown solid. ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H 1.18 (2H, m, H7´´a and H7´´b), 1.47-1.65 (4H, m, H6´´a and H6´´b,H8´´a and H8´´b), 2.35 (2H, t, H9´´a and H9´´b), 2.57 (1H, d, H-1a´´), 2.78- 2.83 (1H, m, H1b´´) 3.05- 3.08 (1H, m, H5´´), 4.11- 4.13 (1H, m, H2´´), 4.27-4.30 (1H, m, H4´´), 5.12 (2H, s, H4´a and H4´b), 6.32 (1H, s, NHa), 6.38 (1H, s, NHb), 6.79-6.82 (2H, m, H6´ and H9´), 7.03- 7.06 (2H, m, H7´ and H8´), 7.38 (2H, d, *J* = 8.12 Hz, H-2 and H6), 7.87 (2H, d, *J* = 8.12 Hz, H3 and H5), 9.10 (2H, s, 2×NH). ^{13}C NMR (DMSO-d_6, 128 MHz): δc 21.5(C-8"), 25.4 (C-7"), 28.5 (C-6"), 32.9 (C-9"), 47.9 (C-1"), 55.9 (C-5"), 59.5 (C-4"), 61.3 (C-2"), 109.1 (5", 10"), 111.3 (C-6",9"), 125.6 (C-7',8'),128.6 (C- 3,5), 129.4 (C-2,6), 157.2 (C- 4), 163.0 (C-3"), 171,7 (C-10"). IR (neat) *v*cm^{-1}:3303, 2932, 2850, 1695, 1639, 1665, 1537, 1432 1338, 1268, 1166, 868, 739.

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