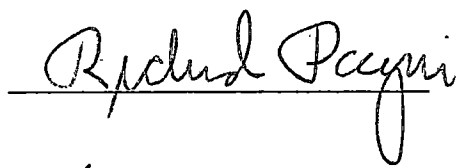
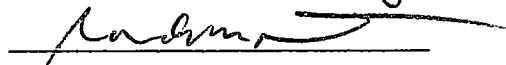


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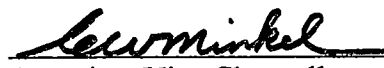
I am submitting herewith a thesis written by Robert R. Singhaus Jr. entitled "Syntheses of Boronated 1-Aminocyclobutanecarboxylic Acids; Sources of Potential Boron Neutron Capture Therapy Agents." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Chemistry.


Dr. George W. Kabalka, Major Professor

We have read this thesis
and recommend its acceptance:

Accepted for the Council:


Associate Vice Chancellor and
Dean of The Graduate School

**Syntheses of Boronated 1-Aminocyclobutanecarboxylic Acids; Sources
of Potential Boron Neutron Capture Therapy Agents**

A Thesis
Presented for the
Master of Science Degree
The University of Tennessee, Knoxville

Robert R. Singhaus Jr.
August 1999

Dedication

I dedicate this thesis to my youngest siblings, my sister Courtney Parrill (age 9) and my brother Jamie Parrill (age 7). You gave me something worth looking forward to when coming home. Whenever I feel sad I think of you and a smile comes to my face that I cannot hide. I love you both dearly. May all of your hopes and dreams come true.

Acknowledgments

I want to express my deepest appreciation and thanks to my research advisor, Dr. George W. Kabalka, for his guidance, encouragement and financial support. He has provided an exceptional working and learning environment for my graduate studies.

I would also like to express thanks to Dr. Rajiv Srivastava for providing me with assistance and teaching me the proper techniques for doing research and also as a person I can discuss chemistry with. I owe most of the skills and knowledge that I have acquired to him.

Also, I would like to thank the spirit of open-mindedness. As the fellow graduate students in Dr. Kabalka's group knows, I am an X-Phile. This TV show has shown me that opening your mind just a little bit, to all things, not just the paranormal, can be a wonderful thing. Likewise the people that I have met and become friends with (Max Hair, Shane Kelley, David Tejedor, George Pacer, Kathy Yang, Mike McGinnis) have introduced me to many things. For this I say thank you. These things have helped me to be even more open-minded, which has helped greatly in making me what I am today. Remember: I Want to Believe that The Truth is Out There so Trust No One and Deny Everything!

Abstract

The syntheses of several boronated 1-aminocyclobutanecarboxylic acids (ACBC) were achieved. These compounds were synthesized as potential Boron Neutron Capture Therapy (BNCT) Agents. The first derivative synthesized was *m*-carboranyl ACBC. This compound failed to show the desirable properties that a BNCT agent must have. This led the investigator to explore another group of compounds. The second set of derivatives synthesized was a series of 4-dihydroxyborylphenyl analogs of 1-aminocyclobutanecarboxylic acid. The number of carbon spacers (as methylene units) was varied between the cyclobutane ring and the aromatic ring to introduce different degrees of lipophilicity into the molecule. Four compounds with 0, 2, 3 and 7 methylene spacers were synthesized and are awaiting evaluation as Boron Neutron Capture Therapy Agents.

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List of Symbols and Abbreviations

Å	Angstrom
ACBC	1-Aminocyclobutanecarboxylic acid
AES	Atomic Emission Spectroscopy
$^{10}\text{B}(n,\alpha) ^7\text{Li}$	Boron Neutron Capture Reaction
BNCT	Boron Neutron Capture Therapy
BNL	Brookhaven National Laboratory
(Boc) ₂ O	di- <i>tert</i> -butyl dicarbonate
BPA	<i>p</i> -Boronophenylalanine
br s	broad singlet
BSH	Sodium Borocaptate ($\text{Na}_2\text{B}_{12}\text{H}_{11}\text{SH}$)
CBU-2'	2'-O-(<i>o</i> -carboran-1-ylmethyl) uridine
CDCl_3	chloroform- <i>d</i>
°C	degree Celsius
cm	centimeter
d	doublet
DCI	deuterium chloride
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
EELS	electron energy loss spectroscopy
eV	electron volt

g	gram
γ	gamma
Δ	heat
hr	hour
HR-FAB-MS	high resolution fast atom bombardment mass spectrometry
Hz	Hertz
IR	infrared spectroscopy
J	coupling constant
kg	kilogram
LDL	low-density lipoproteins
LET	linear energy transfer
M	molarity
<i>m</i> -	meta
m	multiplet
mA	milliamperes
MeV	mega electron volt
min	minutes
MIT	Massachusetts Institute of Technology
mg	milligram
MGH	Massachusetts General Hospital
mL	milliliter
mm	millimeters

mmHg	millimeters of Mercury
mmol	millimole
m.p.	melting point
MW	megawatt
MRI	Magnetic Resonance Imaging
N	Normality
NMR	Nuclear Magnetic Resonance
n/s/ μ g	neutrons per second per microgram
<i>o</i> -	ortho
PET	Positron Emission Tomography
<i>p</i> -	para
PTSA	<i>p</i> -toluenesulfonic acid
R _f	retardation factor
s	singlet
SIMS	secondary-ion mass spectroscopy
S _N 2	substitution by a nucleophile second order
SPECT	single photon emission computer tomography
t	triplet
<i>t</i> -	tertiary
t _{1/2}	half-life
THF	tetrahydrofuran
TLC	thin layer chromatography

UV

ultraviolet

μm

micrometer

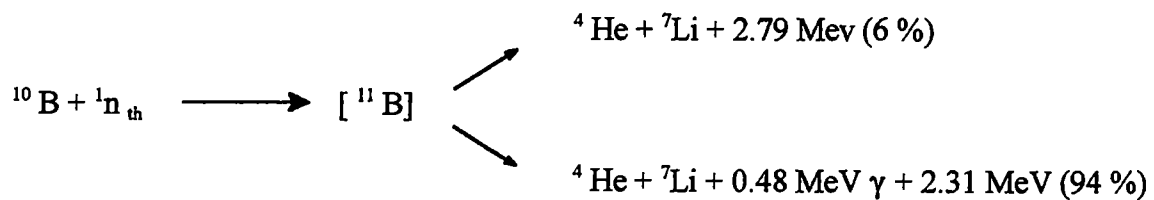
Chapter 1

Boron Neutron Capture Therapy

I. Introduction

Boron Neutron Capture Therapy (BNCT) was proposed by Locher in 1936 as a possible treatment for cancer.¹ BNCT is a binary therapeutic method based on the selective concentration of boron-10 (^{10}B) containing compounds in cancer cells, followed by the activation of the boron by low-energy thermal neutrons ($^1\text{n}_{\text{th}}$) resulting in cell death (*i.e.* a cytotoxic effect) due to the release of α particles and a lithium nuclei.

The cytotoxic agents are highly energetic helium ions and lithium nuclei (Eq. 1-1) with the range of approximately one cell diameter (6 - 10 μm).



Eq. 1-1 Boron Neutron Capture Reaction

There are a number of nuclides that have a high propensity for absorbing low-energy thermal neutrons. ^{10}B is the most attractive for the following reasons: (a) it is nonradioactive and readily available, comprising approximately 20 % of naturally occurring boron; (b) the particles emitted by the capture reaction are largely high LET (linear energy transfer); (c) their path lengths are approximately 1 cell diameter (10 - 14

μm), theoretically limiting the radiation effect to those tumor cells that have taken up a sufficient amount of ^{10}B and simultaneously sparing normal cells; and (d) the extensive chemistry of boron is such that it can be incorporated into a multitude of different chemical structures.

For BNCT to be successful, a sufficient quantity of ^{10}B must be localized within the tumor cell and enough thermal neutrons must be delivered and absorbed by the ^{10}B to sustain a lethal $^{10}\text{B}(\text{n},\alpha)^7\text{Li}$ reaction. These criteria are essential if BNCT is to be a useful modality for the treatment of various types of cancer. Among these aforementioned aspects, one must also take into consideration the neutron sources, the methods of analysis of the boron compounds and delivery of the boron containing compounds to the tumor cells. These will be discussed in the remainder of this chapter.

II. Early History of Boron Neutron Capture Therapy

The first observation of charged particles from slow-neutron irradiation of boron was made at Cambridge University on December 10, 1934.² The first radiobiological studies using the neutron- ^{10}B reaction were performed at the University of Illinois in 1938.³ Within the next few years, several articles described reduced viability of mouse tumor transplants after their exposure to boric acid and irradiation by slow neutrons in vitro³ and regression of mouse sarcomas infiltrated with a paste of boric acid powder in sesame oil after their irradiation by slow neutrons in vivo.⁴ It was realized that there would be clinical advantages to irradiation with neutrons of intermediate energies that could be slowed further ('thermalized') in the brain, where the slower neutrons would be

more likely to react with ^{10}B .⁵

Work on BNCT began at the Brookhaven National Laboratory Medical Department in the summer of 1950.⁶ In 1951 an irradiation facility with a 5 cm x 10 cm rectangular neutron port in its steel/lead/boron- shielded floor was built at the top of the Brookhaven Graphite Research Reactor, surrounded by concrete. The neutron port was at the apex of a 3.5 foot high conical air space in the reactor shielding beneath the floor of the facility, with bismuth plates used as extra gamma radiation shielding at the base of the cone. Preliminary experiments were performed at the Massachusetts General Hospital (MGH) in which nontoxic doses of borax (~ 70 mg/kg) were injected intravenously into volunteers with brain tumors.^{7,8}

The first patient in Brookhaven's initial 24-month, 10-patient study was irradiated at the Brookhaven Graphite Research Reactor on February 15, 1951, just six months after it was commissioned.⁹⁻¹⁴ All the patients had undergone a neurosurgical operation for partial removal of a malignant cerebral glioma and showed clinical evidence of tumor recurrence. Eight of these patients had received conventional radiation therapy for their brain tumors. These advanced malignant cerebral gliomas were irradiated by thermal neutrons, after an aqueous solution of 96 % ^{10}B -enriched borax was administered intravenously over a period of several minutes. There were no serious side-effects of BNCT in the first ten patients, although the large doses of borax infused before irradiation, ~200 mg/kg, were slightly toxic.¹⁵⁻¹⁷

A second series, comprising nine malignant glioma patients, was treated with a less toxic borate preparation, sodium pentaborate with D-glucose in the molar ratio

2:1,^{18,19} but at a higher ^{10}B dose than in the first series: 32-50 mg ^{10}B per kg body weight (median, 42 mg/kg) instead of 16-43 mg ^{10}B /kg (median, 26 mg/kg). A troublesome side effect of BNCT in the second series was intractable radiation dermatitis of the scalp, sometimes with ulceration. The median survival time after BNCT for the second series was 147 (range 93-337) days, which was longer than that for the first series (median 97, range 43-185 days).²⁰

In the third series of patients treated by BNCT at Brookhaven, the pentaborate (26-60 mg ^{10}B /kg, median 50 mg/kg) was delivered through a long warmed tube into the internal carotid artery of the tumor bearing hemisphere while the patient was positioned at the irradiation port. Survivals after BNCT in the nine patients of the third group (median, 96 days, range 29-158 days) were similar to those of 10 patients in the first group and to those of patients in the northeast USA with similar cerebral tumors treated only by conventional postoperative therapies during the 1950's. None of the nine developed severe radiation dermatitis, a fortunate outcome that sustained prospects for further clinical trials of BNCT at Brookhaven.

III. Neutron Sources

A. Nuclear Reactors

BNCT was conceptualized before a viable source of neutrons had been discovered. This restriction was lifted following the initial development of nuclear reactor technology in the 1940's. Farr, Sweet and their coworkers conducted the first clinical trials of BNCT with a thermal neutron beam in 1954.¹²

Nuclear reactors have been the exclusive source of neutrons for BNCT. Thermal neutron beams with energies in the range of 0.025 eV, epithermal beams with energies of 1-10,000 eV, or fast neutron beams with energies greater than 10,000 eV may be used. Reactor derived neutrons are produced in a fission reaction with a most probable energy of 0.75 MeV. However, the type of beam extracted from the reactor depends on the moderators and/or filters that are interfaced with the reactor core. Fast neutrons with high energy are obtained by extracting a beam of neutrons that has little or no moderation or filtration. These neutrons have been used for fast neutron therapy,²¹ in which the radiation dose is delivered when energetic neutrons collide with elements in tissues such as H and produce forward recoiling protons, which in turn produce biologic damage through ionizations. Because there is no radiation localized at the cellular level, as is possible with BNCT, there is little advantage for the use of such beams from reactors. Thermal neutrons, however, are produced if the fast neutrons, born in the fission reaction, are sufficiently slowed down or heavily moderated by scattering media such as heavy or light water (D_2O or H_2O) or graphite. These neutrons are attenuated rapidly in tissues, and it is difficult to obtain sufficient thermal neutrons at the depth required for BNCT without severely irradiating surface tissues.²² Thermal neutron beams have been used in Japan for treating brain tumors²³⁻²⁶ and superficial melanomas.²⁷⁻²⁸

Alternatively, an epithermal neutron beam (1-10,000 eV) may be produced by using a filter or moderator as a spectrum shifter to slow the fast neutrons in the intermediate or epithermal neutron energy range. By filtering out residual thermal neutrons with absorbers such as B, Li, or Cd, a relatively pure epithermal beam can be

produced.²⁹ Epithermal neutrons are relatively nondestructive, transferring little energy to H atoms by collisions and seldom being captured by H or N to produce gamma rays and protons. An epithermal beam, when used to irradiate tissues, provides better penetration than thermal neutrons, but still produces thermal neutrons at depth in tissue because of the moderating effects of the tissue itself. Such a beam of neutrons peaks the thermal neutron flux at a few centimeters beneath the tissue surface,³⁰ thus circumventing problems previously encountered with the poor penetrating properties of incident thermal beams. Increased penetration of epithermal neutrons with increasing neutron energy would appear to encourage the use of the highest energy epithermal (or low-energy fast neutrons) available. However, above ~ 10 keV, the energy transferred by collisions with H produces a significant dose compared with the $^{10}\text{B}(n,\alpha)^7\text{Li}$ reaction. If neutrons with energies less than 10 keV are used, this harmful dose is negligible. Thus, current efforts are directed toward the production of epithermal neutron beams, which will yield neutron fluences with energies in the 1-10,000 eV range.³¹ Before epithermal neutron beams can be used clinically, their radiobiologic effects must be carefully characterized in both non-tumor-bearing and tumor bearing animals.

B. Alternative Neutron Sources

A limitation of BNCT is that it is currently dependent on nuclear reactors as a source of neutrons. Given their high cost and the strong resistance to locating them in major metropolitan areas, there is a need to develop alternative neutron sources. Neutron sources such as Californium-252, $^7\text{Li}(p,n)^7\text{Be}$, and spallation neutron sources are all

being investigated as alternatives to nuclear reactors. While ultimately such sources may prove to be capable of providing a sufficiently high flux of neutrons for BNCT, they have not as yet been shown to have the required intensity and purity.³²⁻³⁹

1. Isotopic Sources

²⁵²Cf would be the most suitable of the various isotopic sources that have been considered and used for neutron production.³⁴ Neutron spectra and beam characteristics are similar to those of reactor fission sources with a mean energy of 1 MeV. Gram amounts of ²⁵²Cf, which emits 2.34×10^6 n/s/μg would be necessary for beams that could be used for therapy. The $t_{1/2}$ of 2.65 years would provide a simple and reliable source, which could be used in localities where no suitable nuclear reactor is available. Unfortunately, ²⁵²Cf is not readily available since it is a synthetic nuclide. ²⁵²Cf must be produced in large quantities to fulfill the neutron requirements for BNCT. This would be a very challenging and expensive task that would be difficult to overcome. These problems might make the use of ²⁵²Cf inaccessible for use in BNCT.

2. Low Energy Proton Accelerators

Epithermal neutrons for BNCT also can be produced in low energy proton accelerators³⁵⁻³⁷ by irradiating lithium targets. Proton beam currents in the range of 1-10 mA would be required, and additional neutron moderation with materials such as D₂O would be necessary in order to obtain suitable epithermal neutrons. Although the availability of such beams has not been demonstrated experimentally, mA beam currents are technically feasible and preliminary design studies have been carried out.³⁷

3. High Energy Proton (Spallation) Sources

Neutrons of various energies are produced by irradiating heavy elements such as copper, lead, and uranium with high energy protons. The spectra include neutrons with energies higher than those in fission spectra, but these can be moderated to produce epithermal beams suitable for BNCT.³⁸ Measurements carried out with 72-MeV neutrons on copper indicate that irradiation times in the order of hours will be required.³⁹

Although thermal neutron beams are used clinically in Japan, the consensus is that the increased penetration afforded by epithermal beams will be used in the proposed clinical trials in the United States and Europe.^{40,41} Work is in progress at a number of reactors to produce beams with a preponderance of neutrons in the region between 1 and 10,000 eV. These facilities are developed using heavy water, aluminum, alumina, and sulfur as moderators or filters. The epithermal beam at the Brookhaven Medical Research Reactor has been installed and tested and an evaluation of its physical and radiobiological parameters shows that its intensity and purity are adequate for clinical use.^{30,42}

IV. Methods of Analysis of Boron in BNCT

Analytical methods for boron required for BNCT research span a broad spectrum of procedures. They include the macroscopic and noninvasive estimation of ^{10}B concentrations in tumor and surrounding tissue with ^{10}B magnetic resonance imaging (MRI),⁴⁵⁻⁴⁸ and the microscopic visualization of ^{10}B localization in tumor and tissue sections with α -track autoradiography. Intracellular boron distribution and quantification

are subjects that have begun to yield important results. These will be discussed in the following pages.

A. Noninvasive Macroscopic Estimation of ^{10}B Concentrations In Vivo

Effective BNCT requires knowledge of the tumor's location, the concentrations of ^{10}B in the tumor, blood, and normal tissues, and the rate at which the ^{10}B -containing species is eliminated from these biological compartments. Noninvasive methods for both tumor visualization and the determination of ^{10}B concentrations are required. In principle, a chemically stable radiolabel having suitable characteristics for imaging and attached to a small fraction of the ^{10}B compound in question could be employed,⁴³ for use with single photon emission computer tomography (SPECT) and positron emission tomography (PET) to visualize the compound in both primary and secondary metastatic tumors.⁴⁴ However, each double-labeled ^{10}B delivery system introduces complications and time constraints.

Recent research has explored the potential capability of NMR-active ^{11}B and ^{10}B nuclei in MRI and in the determination of the boron content of tumors loaded with the appropriate isotope.⁴⁵ ^1H NMR imaging would be employed as an adjunctive modality. Continued development of this technique has achieved some success⁴⁵⁻⁴⁸ with the more responsive ^{11}B nucleus, and the ^{10}B nucleus may prove to be useful in the near future. It may eventually be possible not only to locate tumors with $^{10}\text{B}/^1\text{H}$ MRI methods, but also to visualize in a quantitative fashion the local ^{10}B concentrations in tumor and normal tissue.

B. Methods for the Macro and Micro Determination of Boron in Biological Samples

The need exists to determine accurately the concentration of boron present in biological research samples taken at biopsy and tissues obtained from animal biodistribution experiments. Such samples are often available in macroscopic quantities of 50 to 100 mg or more. Following the oxidative dissolution of weighed tissue samples, boron analysis of the aqueous sample is accomplished with the newly developed, inductively coupled or direct current plasma-atomic emission spectroscopy (ICP-AES⁴⁹ and DCP-AES,⁵⁰ respectively) methods. These AES methods determine boron in tissue samples at levels as low as 1 ppm. Extension of this method to microscopic tissue samples of 1 mg obtained by needle biopsy is feasible with a modified sample preparation procedure.

An alternative to ICP-AES and DCP-AES for the analysis of biological samples utilizes the quantification of the prompt γ -photons emitted during thermal neutron capture by the ^{10}B present in the sample,⁵¹⁻⁵³ and comparison of the result with calibration measurements. This method requires a source of thermal neutrons and lacks the precision at low ^{10}B concentrations characteristic of the plasma-AES methods.

C. The Visualization and Microdosimetry of Boron in Biological Specimens

There is a need for experimental methods capable of providing the spatial distribution of boron in tumor and surrounding tissue sections taken from experimental animals or humans at biopsy. The inhomogeneous structure of tumors makes such

studies vital to the understanding of the effectiveness of candidate boron-containing compounds in reaching individual tumor cells in the tumor structure. The α -track autoradiography method^{54,55} is ideally suited for this purpose. The procedure involves the placement of very thin (6-10 μm) tissue sections upon nitrocellulose sheets followed by exposure of the array to a thermal neutron source. The high linear energy transfer (LET) α and ${}^7\text{Li}$ products of the resulting ${}^{10}\text{B}(\text{n},\alpha){}^7\text{Li}$ reaction that are generated within the tissue sample sensitize the nitrocellulose for hydrolytic attack by strong aqueous base. Because of the limited track range of these particles and their high LET characteristics, individual neutron capture events are recorded and converted into visible tracks on the nitrocellulose film. Superposition of this track data upon that obtained from conventional optical representations of the same tissue section gives the relative density of ${}^{10}\text{B}$ nuclei with respect to the histology of the sample. By using calibration samples, this method can also be employed to obtain the ${}^{10}\text{B}$ concentration in tissue from track density data at ${}^{10}\text{B}$ concentrations as low as 1 μg per g sample.⁵⁶

D. Techniques for Imaging Boron on a Subcellular Scale

The design of new boron-containing drugs and the elucidation of their associated tumor localization mechanisms could best be served if the movement and relative concentration of the drug within the targeted tumor cells could be followed over time. The importance of such a capability to drug design derives from the fact that the most effective drugs will be those with the ability to enter tumor cells and bind with the cytoplasm, organelles, or even the nucleus. For example, if one were to synthesize a

candidate DNA-binding boron system, it would be important to confirm this proposed intracellular targeting by direct observation of boron within the cell nucleus. At present, methods for such subcellular imaging have demonstrated their usefulness in specific examples and await further development.

The first method referred to here is the secondary-ion mass spectroscopic (SIMS) microscopy method,^{57,58} which may be applied to the quantitative determination of boron in single, freeze-fractured and dried, boron-containing cells taken from culture. This method has been applied to the imaging of cells targeted by efficacious boron drugs; it has a spatial resolution of 1 μm .

The second method is electron energy loss spectroscopic (EELS) imaging,⁵⁹ which employs elemental mapping with an analytical electron microscope or an energy-filtering transmission electron microscope. Preliminary results for boron with EELS have been reported,⁶⁰ but as in the case of the SIMS method, further development is required before this method of drug evaluation becomes generally useful.

V. Development of Tumor Localizing Boron Compounds

Attempts to develop tumor-selective boron compounds for BNCT date back to studies carried out by Soloway, Sweet, and Brownell at the Massachusetts General Hospital (MGH) in the 1960s.⁶¹⁻⁶³ Among the many compounds that were screened in animals, two *p*-carboxybenzeneboronic acid and sodium decahydrodecaborate ($\text{Na}_2\text{B}_{10}\text{H}_{10}$), had favorable tumor-localizing properties. Both were nontoxic and gave tumor-to-brain boron concentration ratios of 5-8 to 1, which were maintained for several

hours.⁶² Subsequently, ^{10}B -enriched analogues were synthesized and evaluated in clinical trials at the MGH and Massachusetts Institute of Technology (MIT). Although these trials failed,^{64,65} the tumors were destroyed; however there was significant radiation damage to the vascular endothelium of normal blood vessels in all of patients due to the high blood levels of ^{10}B . It became clear that new boron compounds were needed that could attain both high tumor-to-normal-tissue and tumor-to-blood ratios. To identify such boron-containing compounds, a new method of evaluating them was developed using mice bearing a subcutaneously transplanted ependymoblastoma.⁶⁶ The boron compounds were administered over a period of time and blood levels were allowed to fall before various tissues were analyzed. From these studies, two sulfhydryl-containing boron anions, $\text{B}_{12}\text{H}_{11}\text{SH}^{2-}$ and $\text{B}_{10}\text{Cl}_8(\text{SH})_2^{2-}$, were identified.⁶⁷ As a result, $\text{Na}_2\text{B}_{12}\text{H}_{11}\text{SH}$ (sodium borocaptate or BSH) has been used since 1968 by Hatanaka and his colleagues in Japan to treat patients with brain tumors with BNCT.²³⁻²⁵ Despite the hiatus in BNCT clinical trials, which ended in the United States in 1961, research has continued on the synthesis of new boron compounds that would preferentially localize in neoplastic cells.

In addition to compounds selective to brain tumors, there has been special interest in developing agents that could be used to target malignant melanomas. It was reasoned that because the biosynthetic pathway for melanin requires phenylalanine as a precursor, ^{10}B -containing derivatives of this amino acid might preferentially be incorporated into melanoma cells.⁶⁸ Mishima et al., using *p*-boronophenylalanine (BPA), have carried out extensive in vitro and in vivo experiments with this compound^{69,70} and have initiated clinical studies in a group of patients with cutaneous melanomas.^{27,28} BPA is

metabolically unstable, however, and is degraded to boric acid, which can readily diffuse out of cells. Boron-containing amino acids that possess antineoplastic activity have been described by Spielvogel et al.,⁷¹ and these may also have potential use for BNCT. These boron compounds contain a single boron atom, and efforts are underway to synthesize and evaluate carborane-containing amino acids that may be more lipophilic, thereby permitting facile penetration of the tumor cell membrane and possessing greater resistance to metabolic degradation. The first such structure, carboranylalanine, previously had been synthesized,⁷² but the in vitro and in vivo tumor-localizing properties of this compound have not been reported.

The increased rate of mitosis of malignant cells has stimulated interest in the synthesis of boron-containing precursors of nucleic acids, which, if incorporated into DNA, would greatly enhance the destructive effects of α -particles and ^7Li nuclei.⁷³ Schinazi and Prusoff⁷⁴ synthesized the first boron-containing nucleoside, 5-borono-2'-deoxyuridine. Yamamoto⁷⁵ and Soloway⁷⁶ and their coworkers have since synthesized a group of carboranyluridines. The compounds that Soloway's group synthesized show high in vitro cellular uptake,⁷⁷ especially when compared with $\text{Na}_2\text{B}_{12}\text{H}_{11}\text{SH}$. One of them, 2'-O-(*o*-carboran-1-ylmethyl) uridine (CBU-2'), which has been studied intensively, also appears to have high in vivo uptake when injected intratumorally into brain tumor-bearing rats.⁷⁸ Uptake of this compound is independent of nucleoside transport systems and cell division,⁷⁹ and preliminary data suggest that it is not incorporated into RNA or DNA. The lipophilicity of CBU-2', in part, could account for its enhanced uptake, and efforts are under way to determine the biochemical mechanisms for its intracellular

uptake and retention. It will be important to correlate the chemical structure of these compounds with their tumor cell-localizing properties. Boron-containing nucleotides are also being synthesized, and these may prove to be better substrates for incorporation into nucleic acids. Masking of phosphate groups may be necessary for these compounds to penetrate the tumor cell membrane. A potential advantage of nucleic acid precursors for targeting, especially of brain tumors, is that the mitotic index of glioma cells is many times greater than that of surrounding normal glial cells, and much larger amounts of the compound might be incorporated into the rapidly proliferating tumor cell population.

In addition to the development of boron-containing biochemical precursors, other classes of low molecular weight boron compounds have been synthesized. These include phthalocyanines,⁸⁰ promazines,^{80,81} thiouracils,⁸² and porphyrins.^{83,84} Kahl et al. have synthesized a tetrakis-carborane carboxylate ester of 2,4-(α,β -dihydroxyethyl)-deuteroporphyrin IX (BOPP),⁸³ which shows high *in vitro* uptake and persistence. The boronated porphyrins, however, show significant tumor and normal tissue uptake,^{85,86} and the latter may prove to be a significant limitation for *in vivo* BNCT. Low-density lipoproteins (LDL) and liposomes also have the potential to selectively deliver ¹⁰B to tumor cells. Kahl and coworkers have synthesized the first boronated LDL in which carborane carboxylic acid esters of fatty alcohols were used to replace the cholesterol core.⁸⁷ CHO and V-79 Chinese hamster cells exposed to the boronated LDL and irradiated at the Brookhaven National Laboratory Medical Research Reactor showed steep reductions in percent survival compared with control subjects. If boronated LDL show high tumor and low normal tissue uptake *in vivo*, they may be excellent delivery

agents for BNCT. A related approach is to use the boron-containing liposomes as tumor-targeting agents.⁸⁸ Although in the early stages of development, these appear to have some potential for selectively delivering ^{10}B to tumor cells.

VI. Delivery of Boron-Containing Compounds to Tumor Cells

During the 1960s and early 1970s interest developed in the potential use of polyclonal antibodies directed against tumor-associated antigens for the delivery of drugs and radioisotopes to tumors.⁸⁹⁻⁹¹ In 1964 Soloway suggested that antibodies might be used for the selective targeting of ^{10}B to tumors.⁹² Hawthorne et al.⁹³ reported on the incorporation of the diazonium salt from 1-(4-aminophenyl)-1,2-dicarbo-*closo*-dodecaborane into antibodies directed against bovine serum albumin and polyclonal antibodies directed against human and mouse histocompatibility antigens.⁹³ It was claimed from *in vitro* experiments that these immunoconjugates were capable of delivering enough boron to human and murine lymphocytes to sustain a lethal $^{10}\text{B}(\text{n},\alpha)^7\text{Li}$ reaction, as evidenced by reduced viability following neutron irradiation. However, the immunoconjugates contained only 0.2 % natural boron by weight, which was equal to 6 atoms of ^{10}B /molecule of antibody. In retrospect, it appears that there must have been some other explanation for the reduced cell viability that was observed. Sneath et al.⁹⁴ showed that water-solubilizing groups had to be incorporated into protein-binding polyhedral boranes if protein-binding functional groups were linked to IgG molecules by means of the carbodiimide reaction without evidence of precipitation.⁹⁵

With the advent of hybridoma technology and the development of monoclonal

antibodies directed against a wide variety of tumor-associated antigens, new possibilities opened up for the targeting of ^{10}B . Studies by Barth et al. initially focused on the linkage of the polyhedral borane disodium mercaptoundecahydro-*closo*-dodecaborate ($\text{Na}_2\text{B}_{12}\text{H}_{11}\text{SH}$) to antibodies either by thiol disulfide exchange⁹⁶ or by means of the heterobifunctional reagent N-succinimidyl-3-(undecahydro-*closo*-dodecaboranyldithio)-propionate.⁹⁷ Using polyclonal antibody directed against human thymocytes (anti-thymocyte globulin) and 95 % ^{10}B -enriched $\text{B}_{12}\text{H}_{11}\text{SH}^2$, Barth et al. were able to incorporate approximately 140 ^{10}B atoms/molecule of antibody.⁹⁸ This was associated with a slight reduction in immunoreactivity. Utilizing N-succinimidyl-3-(undecahydro-*closo*-dodecaboranyldithio)propionate, they were able to incorporate as many as 1500 atoms of ^{10}B /molecule of antibody, but there was a 90 % reduction in immunoreactivity,⁹⁷ which was attributed to the large number of sites that were modified.

It has been estimated that ~35-50 μg of ^{10}B must be delivered per g of tumor in order to achieve a tumoricidal effect.⁹⁹⁻¹⁰¹ If this is extrapolated to the cellular level, then $\sim 10^9$ atoms of ^{10}B must be delivered per cell.¹⁰¹ If boronated antibody alone is to be used as the delivery system, then a very large number of boron atoms must be linked per molecule. The exact number would depend upon a number of parameters, including the antigen site density on the target cell and the affinity constant of the antibody molecule.¹⁰² Alam et al. have used a monoclonal antibody, designated 17-1A, which was produced against a human colorectal cancer-derived cell line to produce boron-containing immunoconjugates.¹⁰³ This antibody recognizes an epitope expressed with a density of 10^6 antigenic sites/cell and has an affinity constant of $1.05 \times 10^8 \text{ M}^{-1}$.

Assuming that all antigenic receptor sites could be saturated, this would require that each molecule of antibody must deliver a 1000 atoms of boron in order to attain the critical number. This value is set as the minimum requirement.

Mizusawa et al.¹⁰⁴ and Goldenberg et al.¹⁰⁵ conjugated antibodies directed against carcinoembryonic antigen with *p*-[1,2-dicarba-*closo*-[1-³H]dodecaboran(12)-2-yl] benzenediazonium ion. The resulting immunoconjugates were estimated to have 30-50 atoms of ¹⁰B/molecule of antibody. Although retention of immunoreactivity and selective *in vivo* localization were observed in hamsters carrying human colon cancer xenografts,¹⁰⁵ the small number of boron atoms per molecule of antibody would preclude the delivery of a sufficient amount of boron to sustain a lethal ¹⁰B(n,α)⁷Li reaction. In order to minimize the number of sites of an antibody molecule that would be modified during boronation, and to maximize the number of ¹⁰B atoms that could be linked to an antibody molecule, Alam et al. have synthesized water-soluble, boron-containing macromolecules which can be attached to one or two sites on the antibody molecule. A polyhedral borane isocyanate decaborate was linked to polylysine and the resulting macromolecule contained 21 to 28 % boron by weight and up to 2000 boron atoms/molecule of polymer.¹⁰⁶ This in turn has been linked to monoclonal antibodies 17-1A and IB16-6, which is directed against the B16 melanoma, utilizing two heterobifunctional reagents, N-succinimidyl-3-(2-pyridyldithio)propionate and *m*-maleimidobenzoyl N-hydroxysuccinimide ester.^{107,108} More than 1000 atoms of boron have been incorporated per antibody molecule by modifying only one site and the resulting immunoconjugates retained 40-90 % of the immunoreactivity of the native

antibody.¹⁰⁸ The *in vitro* cellular uptake of the boronated 17-1A was studied by means of electron energy loss spectroscopy utilizing a Zeiss 902 microscope. This instrument can detect elemental boron with a high degree of sensitivity and spatial resolution.¹⁰⁹ Preliminary observations suggest that there is intracellular uptake of boron by colorectal cancer cells exposed *in vitro* to the immunoconjugate.¹⁰⁸ These observations have important dosimetric implications, since intracellular uptake of ^{10}B would increase the selective therapeutic effect achieved as a result of $^{10}\text{B}(\text{n},\alpha)^7\text{Li}$ reaction.^{110,111} This is illustrated by Monte Carlo calculations for hamster V-79 cells, which show that ^{10}B located external to the cell will produce ~10 % of the dose delivered by a uniform distribution while cytoplasmic and nuclear locations would each deliver 2.5x the dose from a uniform distribution.¹¹⁰ If boronated antibodies are not internalized, this clearly would be a disadvantage.

In vivo distribution studies have shown that there was a marked reduction in the amount of boronated 17-1A localized in human colon cancer tumors that have been implanted subcutaneously into nude mice and a corresponding increase in the amount in the liver compared to the native antibody.¹⁰⁸ The problem of altered distribution is one that has been encountered with a variety of immunoconjugates including immunotoxins, radiolabeled antibodies, and drug-antibody conjugates, and methodology will have to be developed that produces less modification of the antibody molecule. New developments in hybridoma technology such as the production of recombinant antibodies that have two combining sites, one specific for the tumor-associated antigen and the other for the tumoricidal agent, potentially might have applicability to BNCT, if the boronated species

itself were immunogenic and reactive with one of the reactive sites on the hybrid molecule. Advances in the chemistry of immunoconjugation, especially the ability to increase the distance between the combining site for the tumor-associated antigen and the tumoricidal moiety, also might have applicability to developing better boronated immunoconjugates. The boronated polylysine Alam *et al.* have synthesized has a high negative charge, and this may have adversely affected its tumor-localizing properties and increased its clearance by the reticulendothelial system. The production of antibodies directed against more universally expressed tumor-associated antigens would reduce the problem of antigenic heterogeneity of tumor cells. The potential for using monoclonal antibodies for targeting tumoricidal agents including ^{10}B is great, and although significant problems exist, these theoretically are not insoluble.

One other category of macromolecular species that potentially may be useful for the delivery of ^{10}B is what may be termed "encapsulating complexes," such as liposomes, microspheres, and low density lipoproteins.¹¹² Theoretically large amounts of ^{10}B could be encapsulated, and if these encapsulating complexes could be targeted to the tumor by linkage to a monoclonal antibody using existing methodology or targeting an endogenously expressed cell surface receptor, they might be powerful delivery systems. Again, there may be preferential localization in the reticuloendothelial system, and methodology would have to be developed to minimize this and maximize tumor uptake.

It appears unlikely that antibodies alone will be able to deliver the required amount of boron to sustain a lethal $^{10}\text{B}(\text{n},\alpha)^7\text{Li}$ reaction and they will probably will be used in combination with other tumor-localizing boron compounds. Just as effective

cancer chemotherapy is based on using a combination of drugs, it is believed that successful BNCT will depend on a variety of ways to deliver the necessary amount of ^{10}B to cancer cells.

VII. Clinical Studies

Early clinical trials on the use of BNCT were carried out at BNL by Farr et al.^{113,114} and at MGH/MIT by Sweet et al.^{115,116} during the 1950s and early 1960s. At both centers, therapy focused on treating malignant gliomas, in particular, glioblastoma multiforme. The rationale stemmed from the fact that even when the tumor was surgically removed as completely as possible, even by such a radical procedure as hemispherectomy, glioblastomas invariably recurred, with a mean life expectancy of less than 6 months. Neoplastic cells in the healthy brain that escaped excision served as foci for regrowth and death. It was reasoned that if these residual glioblastoma cells could be destroyed by BNCT, prolongation of life or even a cure might be possible.¹¹⁶ Based on the optimistic assumption that the blood-brain barrier would effectively exclude boron from normal brain tissue, a series of patient irradiations were carried out at BNL. In these clinical trials, two inorganic boron-containing compounds, either borax ($\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$) or sodium pentaborate ($\text{Na}_2\text{B}_{10}\text{O}_{16} \cdot 10\text{H}_2\text{O}$), were given intravenously. These compounds, distributed widely throughout the body following the distribution of body water, and produced transient but mild and tolerable toxic effects.¹¹⁶ Initially, irradiations were carried out at the Brookhaven Graphite Research Reactor and then later at Brookhaven Medical Research Reactor. The latter reactor was designed and built

specifically for medical and biologic research. In particular, it was hoped that the newly constructed patient treatment port would facilitate irradiations and enhance clinical efficacy by reducing background radiation due to improvements in reactor design. Despite the initial enthusiasm, these clinical studies were a failure and the treated patients died without any evidence of prolonged survival time.¹¹³ Recent analysis of these results by Slatkin indicated that BNCT had arrested the growth of the metastases in at least one of these patients with metastatic carcinoma.⁶⁴ In all other cases, the lack of therapeutic efficacy was attributed to the poor penetration of thermal neutrons in tissue and the nonselectivity of the boron compounds, which resulted in boron concentrations in tumor that were only transiently higher than those in the healthy brain.

Currently, BNCT appears to offer the most promise for treating primary and metastatic brain tumors, specifically glioblastoma multiforme and malignant melanomas for which there currently is no effective therapy. The pioneering work of Hatanaka et al.²³⁻²⁵ has stimulated worldwide interest in using BNCT to treat brain tumors. In 1963, Hatanaka joined Sweet at the MGH in Boston and, over the course of several years, developed a strong interest in BNCT. On his return to Japan, Hatanaka initiated a clinical trial of BNCT for the treatment of brain tumors.²³ Between 1968 and 1992, he has treated more than 110 patients by a combination of surgery and BNCT using the following procedure.²⁴ As much of the tumor as possible is surgically excised. At varying times after surgery the patients are given an intra-arterial fusion of $\text{Na}_2\text{B}_{12}\text{H}_{11}\text{SH}$ (BSH), which has been shown to have favorable tumor-localizing properties.⁶⁷ This is followed by irradiation using a beam of thermal neutrons directed to the operative site in

the brain from which the tumor had been resected. The Musashi Institute of Technology reactor, which has been used for irradiating the overwhelming majority of these patients, has a low neutron flux ($1.5 \times 10^9 \text{ n.cm}^{-2} \text{ s}^{-1}$) that irradiation times of 3-5 hours have been required.²⁴ Furthermore, the depth of penetration is no more than 6 cm, limiting effective treatment to more superficial tumors. Fourteen hours after an infusion of approximately 40-60 mg $^{10}\text{B/kg}$, ^{10}B concentrations in the tumor ranged from 13-60 $\mu\text{g/gm}$ (mean, 26.3 $\mu\text{g/gm}$), mean blood concentration was 18.2 $\mu\text{g/ml}$, and tumor-to-blood ratios of 48 patients was 1.69. These results are noteworthy for several reasons. First, there was no evidence of radiation injury to the healthy brain, except for one patient who had received an exceptionally large dose of neutrons. Second, of 38 patients who had Grades 3 and 4 cerebral gliomas and 12 of whom had more superficial tumors located within 6 cm of the cortical surface, the mean survival time was 44 months (median, 25.6 months).²⁵ These include several long-term survivors, including a man who was treated in 1972 and is still alive and well with no evidence of tumor and a woman and a girl who appear to have been cured.¹¹⁷

As encouraging as Hatanaka's results are, a number of questions have been raised about them. These include the use of varying treatment combinations before the initiation of BNCT, varying time intervals between surgery and administration of the capture agent and irradiation, lack of randomization of patients, and most important, poor depth of penetration of the neutron beam. Nevertheless, Hatanaka's results have stimulated groups to initiate programs that eventually may lead to controlled clinical trials.

An interdisciplinary research has been assembled in Europe. Pharmacokinetic studies of $\text{Na}_2\text{B}_{12}\text{H}_{11}\text{SH}$ (BSH) currently are in progress. During the early 1990s, approximately 55 patients with malignant brain tumors of various types have been injected intravenously with BSH at doses ranging from 5-25 mg/kg body weight from 3-24 hours before surgical resection of their tumors.¹¹⁸ Considerable variability has been seen in tumor boron concentrations, and this has been attributed to the administration of steroids and mannose, which were given before surgical resection or biopsy of the glioblastomas to reduce the amount of cerebral edema. It has been decided that more accurate uptake data can be obtained in patients who have not received steroids or mannose, and future distribution studies will be carried out in such patients. The High Flux Reactor at the Joint Research Centre in Petten, The Netherlands, is being used for the clinical trial that Fankhauser is directing. This is a 45 MW water-cooled reactor, one beam port of which has been modified for patient irradiations. This beam has been characterized by Moss and his colleagues at Petten and is capable of delivering a fluence of up to $5 \times 10^8 \text{ n.cm}^{-2} \text{ s}^{-1}$ with energies in the range of a few eV to a few keV (i.e. high flux epithermal neutrons).¹¹⁹ It will be filtered through liquid argon/sulfur/aluminum to obtain an optimized beam that has minimum fast neutron and gamma photon contamination.

The second phase of study that Fankhauser is directing is to define the normal brain tolerance to the epithermal beam in patients that have glioblastomas.¹²⁰ Patients will be divided into three groups. Group I will consist of those having total resection of their gliomas, Group II will consist of those with partial resection or open biopsy, and

Group III will consist of those with unresectable tumor with stereotactic biopsy only. After complete or partial resection, patients will be given BSH, be reoperated on for biopsy of the tumor bed, and boron determinations will be carried out on the biopsied tissues. Patients in Group I who have had maximal surgery will then receive BNCT, after which they will be monitored closely by computerized axial tomography and magnetic resonance imaging. If there is clinical or radiologic evidence of recurrence or necrosis, they will be reoperated on to remove recurrent tumor and/or necrotic tissue. Patients in Group II and III who have unresectable tumors will be treated in the same way as those in Group I, except reoperation will be suboptimal and their life expectancy will be shorter. Additional treatment (chemotherapy or photon radiation) for those patients in Group I who have recurrence will be left up to the discretion of the local neurosurgeons and oncologists managing the cases. This will be a complex study with many variables; nevertheless, it represents an attempt to examine the efficacy of BNCT using BSH as the capture agent in a rigorous, although not randomized study.

Mishima et al. have taken the lead in the treatment of melanomas by BNCT.¹²¹ Therapeutic efficacy initially was established in an animal model using Duroc pigs,⁷⁰ which had spontaneously occurring melanomas. After this, clinical studies were initiated using ¹⁰B-boronophenylalanine (BPA) as the capturing agent.²⁷ The first patient was a 66-year-old man with melanoma metastatic to the occipital scalp who had a resected primary on his toe. The location of the scalp metastasis precluded surgical resection and the patient, therefore, was considered a candidate for BNCT. BPA at a dose of 100 mg/kg body weight was injected perilesionally around the cutaneous melanoma, followed

by a single neutron irradiation with $\sim 10^{13}$ n.cm⁻² at the Kyoto University Research Reactor Institute. Two months after irradiation, there was evidence of regression and eventually local cure with loss of pigmentation at the melanoma site.²⁷ Nevertheless, the patient died of metastatic disease.²⁸ By October 1992, 12 patients with cutaneous melanoma who were not candidates for surgery have been treated by BNCT.¹²⁰ One such patient was an 80-year-old man with a melanoma on the sole of his foot. Multiple doses of a ¹⁰B-BPA fructose complex, which is more soluble in water than ¹⁰B-BPA, were injected perilesionally around the melanoma. After allowing sufficient time for the BPA to clear from surrounding normal tissues, the foot was irradiated with a collimated beam of thermal neutrons. Within two weeks, the melanoma showed signs of regression. By 9 weeks, only a small pigmented spot remained. Two years later, there was no evidence of local recurrence. Additional patients have been treated, and more recently the ¹⁰B-BPA-fructose complex has been administered intravenously. The challenge that lies ahead is to extend this form of therapy to patients with melanoma who have disease in extracutaneous sites, which currently cannot be treated by any available form of therapy.

VIII. Conclusion

BNCT is perhaps one of the most complex cancer therapeutic modalities, and its ultimate success or failure is dependent on the delivery of an adequate concentration of the boron-containing capture agents and neutrons being delivered to the tumor. Efforts are under way to develop new boron delivery systems, and there are a number of promising leads. Amino acids comprise a large number of these delivery systems.

Therefore the next chapter will explore the various boron-containing amino acids that have been synthesized for use in BNCT.

Chapter 2

Chemistry of Boron Neutron Capture Therapy

I. Introduction

The greatest challenge to achieving clinical success using Boron Neutron Capture Therapy is the development of suitable boron-containing molecules that can deliver an appropriate dose of ^{10}B to tumor cells. These compounds must be able to target tumor cells but not healthy cells and also persist in the tumor cell sufficiently long so that the radiation delivered can effectively kill the tumor cells. Even though the concept of BNCT was introduced more than 60 years ago, the lack of appropriate compounds has limited development of this therapy. Even with the two compounds currently being used for BNCT, there is a lack of information as to how these compounds accumulate in the tumor. This knowledge would certainly be of use in the development of more effective tumor-targeting compounds. The focus of this chapter is on the chemical aspects of BNCT, in particular, the boron-containing amino acids.

II. Requirements for BNCT Agents

Initially, BNCT agents were limited to those that were commercially available or easily synthesized. Currently, the emphasis is on the development of boron-containing compounds that accumulate in the tumor cell. Three parameters that have essentially remained constant since the beginning of BNCT involve finding compounds that (1) achieve tumor concentrations in the range of 20 - 35 $\mu\text{g } ^{10}\text{B/g}$ of tumor; (2) exhibit a

tumor:normal tissue differential greater than 1 and preferably 3 - 5 and (3) have low toxicity so that the dose administered would be well tolerated. Another requirement is that the concentration differential should persist during the entire irradiation procedure. It was discovered in the early clinical trials that, if the blood boron concentration exceeded those in the tumor, irreparable damage to the blood vessel walls occurred.¹²² For this reason an additional requirement was made. The tumor:blood ratio must be at least greater than one (preferably higher) during the irradiation.

The design and development of drugs have become very sophisticated. No longer can compounds be synthesized just because they can be. There must be a biochemical/physiological basis for drug design, as well as an understanding of how they target the tumor cell. It is important that the boron portion of the compound not adversely affect tumor-targeting, solubility in the blood, transport through the vasculature, membrane permeability, and incorporation into tumor cells at a sufficient concentration. Also, there must not be a significant increase in the compound's toxicity. The problem with compound development is that it is difficult to predict the effect of the boron moiety on these parameters.

It also is important to gain knowledge about the location of the agent within the tumor cell. It has been presumed that the target of the high linear energy transfer (LET) particles is the nucleus. Thus, locating the boron-containing species near the nucleus would be highly advantageous.¹²³⁻¹²⁵ It has been postulated that the boron concentration could be decreased by a factor of 2-5 if the boron compound were to locate near the cell nucleus. However, as in chemotherapy, several agents are frequently used in conjunction

to impede tumor cell replication. It is possible that, in the future, a combination of boron compounds, each with the ability to target different tumor cell organelles (i.e. nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, etc.), may be used for BNCT.

III. Types of Boron Compounds

A. Boronic Acids and Compounds with One Boron Atom

The most common type of organic compound containing only one boron is a boronic acid, $\text{RB}(\text{OH})_2$. Most of the structures proposed for BNCT agents are those with an aromatic ring attached to the boron because these compounds are oxidatively stable, as well as hydrolytically stable. They have also been demonstrated to be metabolically inert.¹²⁶⁻¹²⁹

4-Dihydroxyborylphenylalanine (BPA) (Figure 2-1), a boronic acid, is one of only two compounds being used clinically for BNCT.¹³⁰⁻¹³⁴ The hydroxyboryl group ($\text{B}-\text{OH}$) can be viewed as an isostere of the carbonyl group. When this group and an amine are attached to a carbon, it is isosteric with an amide. Efforts have also been made to incorporate boronic acids into heterocycles to prepare analogs of purines and pyrimidines.¹³⁵⁻¹³⁸

Replacement of the hydroxyl group in the boronic acid with an organic species yields a borinic acid ($\text{R}_2\text{B}-\text{OH}$) and replacement of the hydroxyl group in the borinic acid yields a trialkylborane (R_3B). The problem with these compounds is that

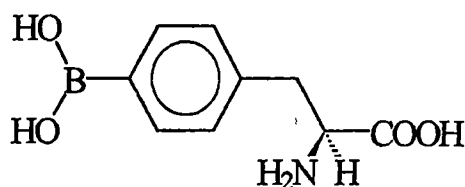


Figure 2-1 L-Configuration of 4-Boronophenylalanine (BPA)

replacement of the -OH groups of the boronic acids with organic groups decreases both the aqueous solubility and the oxidative stability.

B. Borane Zwitterions

Boranes (BH_3 , RBH_2 , R_2BH) are hydrolytically unstable. However, certain complexes of boranes are both hydrolytically and metabolically stable. These complexes are stabilized by electron-donating functional groups.^{139,140} These structures are zwitterionic with an anionic boron atom and a cationic nitrogen or phosphorus. These complexes enhance the water solubility when incorporated into compounds that have biochemical properties. Other Group V elements, such as phosphorus, have also been used in borane zwitterions. The structures of two borane zwitterions are presented in Figure 2-2.

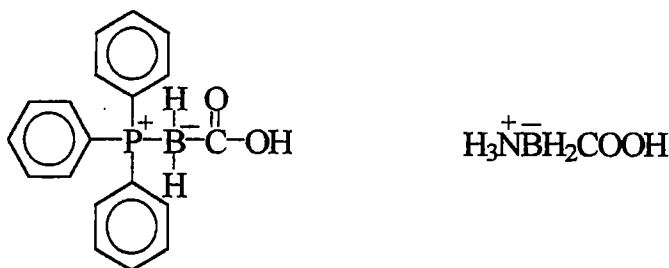


Figure 2-2 Examples of Borane Zwitterions

C. Polyhedral Borane Anions and Carboranes

The structures discussed previously contain only a single boron atom. Since BNCT is dependent upon a boron atom being located in a tumor cell, it is plausible that compounds of similar structure that contain multiple boron atoms could be used as agents in BNCT. If these structures possess similar properties, then higher doses of boron could be administered to the tumor cell. It is for this reason that much of the compound development for BNCT has been focused on multiple boron atom-containing compounds. Polyhedral borane anions and carboranes are attractive candidates. Both types of compounds contain multiple boron atoms. Two polyhedral borane anions, $B_{10}H_{10}^{2-}$ and $B_{12}H_{12}^{2-}$, were used in the early development of BNCT agents.¹⁴¹⁻¹⁵⁴ These borane anions are of interest because of their stability and the fact that functional groups can be incorporated into their structural framework. The sulfhydryl derivative, sodium mercaptoundecahydrododecaborate ($Na_2B_{12}H_{11}SH$, BSH) (Figure 2-3) is one of only two clinically approved agents. The polyhedral borane anions are charged species which can affect transport, especially the ability to cross lipophilic barriers. These structures can also bind to various plasma proteins and other biopolymers.

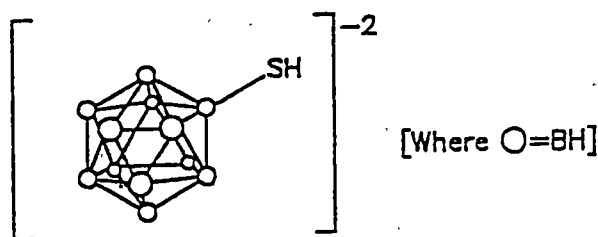
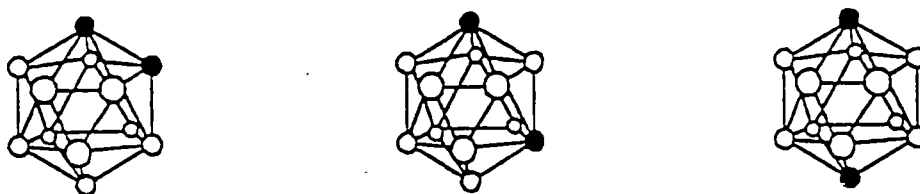


Figure 2-3 BSH

Carboranes, of the $C_2B_{10}H_{12}$ variety, are organic in nature and methods for their incorporation into many types of organic/biochemical structures have become very well known.¹⁵⁵⁻¹⁶⁵ *ortho*-Carboranes have been used more extensively than the corresponding *meta* and *para* isomers (Figure 2-4). Recently there has been a renewed interest in the *meta* and *para* isomers because of their stability towards base.¹⁶⁶⁻¹⁷¹ The *ortho*-carboranes are not stable towards base, in particular nitrogen containing bases. The carborane moiety is approximately the size of a phenyl group in three-dimensional space.¹⁷² Also, the carboranyl moiety is extremely hydrophobic and its lipophilicity is comparable to that of the adamantyl group.¹⁷³ On the surface, this may appear to be a desirable property, since lipophilic compounds have been known to cross the blood-brain-barrier with great ease. However, attachment of such a lipophilic moiety to a compound often results in structures that bind nonspecifically through hydrophobic bonds to biopolymers. Thus, high lipophilicity can make it difficult to achieve a high tumor:blood to tumor:normal tissue boron ratio. A balance between a compound's hydrophilic and lipophilic properties is needed if suitable differentials between blood and normal tissue and, ultimately, between normal tissue and tumor is to be attained. The *meta* and *para* isomers of the carboranes can be synthesized from the *ortho* derivative via high temperature rearrangement.¹⁵⁶ Unfortunately, the reactivities of the *meta* and *para* isomers are reduced when compared to the *ortho* isomer.



Where $\circ = \text{BH}$, $\bullet = \text{CH}$

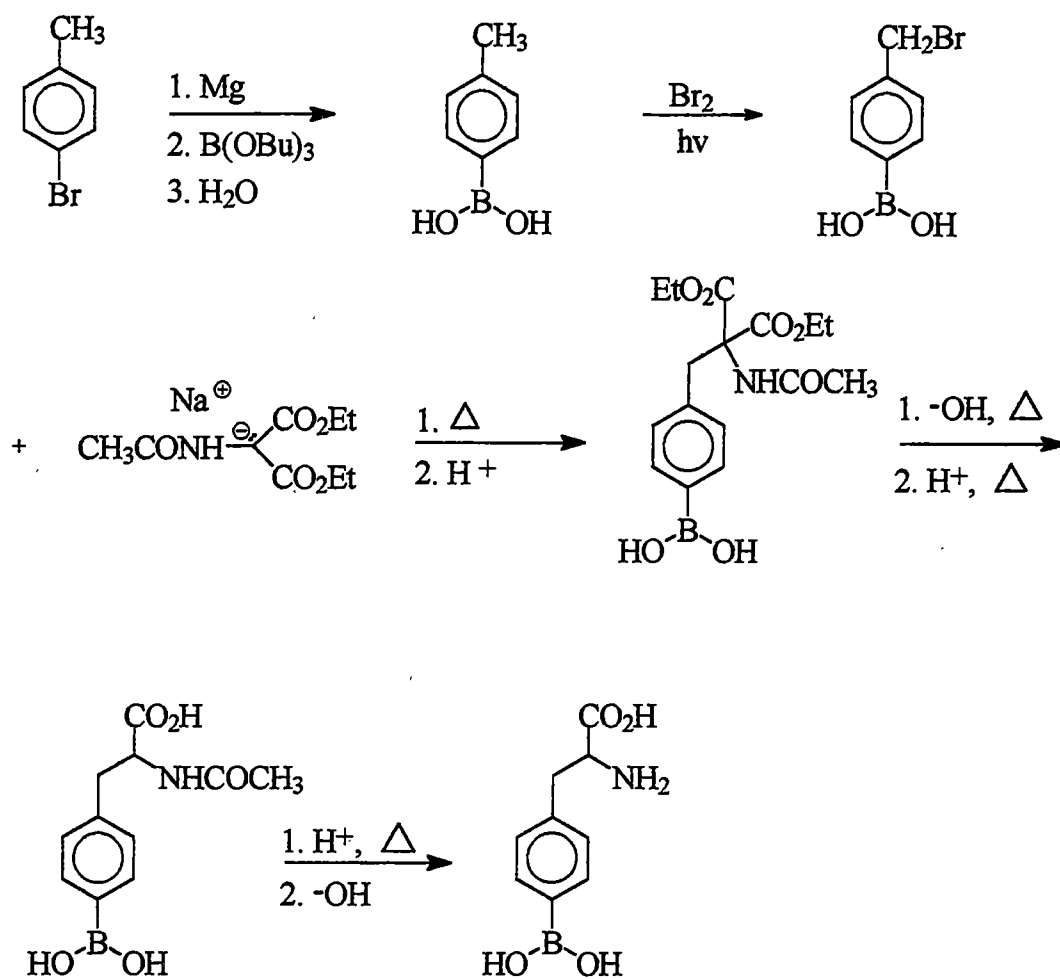
Figure 2-4 Structures of *o*-, *m*- and *p*-carborane

IV. Boron-Containing Amino Acids

A. Boronic Acid Derivatives

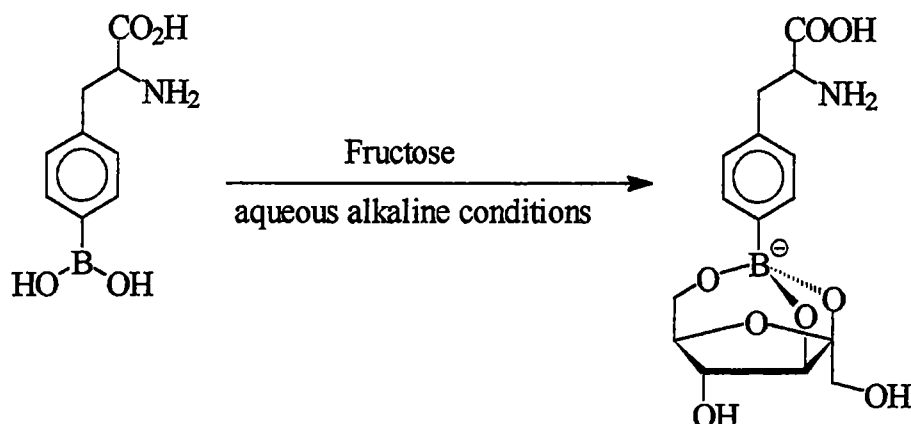
The main reason for the interest in boron-containing amino acids is the fact that 4-dihydroxyborylphenylalanine (BPA) (Figure 2-1) is one of the two clinically used BNCT agents. BPA has been shown to preferentially accumulate in melanoma cells, thus making it a good candidate for BNCT. BPA was first synthesized by Snyder¹⁷⁴ in 1958 (Eq 2-1). The premise was that BPA strongly resembled tyrosine and thus might be a suitable carrier of ^{10}B into melanoma cells.

Many syntheses of BPA have been reported.¹⁷⁵⁻¹⁷⁸ BPA has also been synthesized as its *ortho* and *meta* isomer,¹⁷⁹⁻¹⁸² but only the *para* isomer has been used in biological evaluation. Initially it was recognized that BPA had low aqueous solubility which could be problematic for intravenous delivery. To avoid this problem, researchers used the boronic acid's ability to complex with carbohydrates to increase the compound's aqueous solubility.¹⁸³⁻¹⁸⁶ Fructose is the carbohydrate of choice. The reaction of BPA with excess fructose converts the boronic acid into a water-soluble borate complex (Eq 2-2).¹⁸⁷ In



Eq 2-1 Snyder's BPA Synthesis

fact, it is the fructose derivative that is administered to patients prior to neutron irradiation.



Eq 2-2 Reaction of BPA with Fructose

Others have attempted to increase the water solubility by incorporating nonionic, hydrophilic groups into the BPA molecule.¹⁸⁸⁻¹⁹⁰ The carboxyl group in these molecules were converted to the mono-, di-, and tetrahydroxy amide derivatives. These unfortunately change the nature of the compound, possibly changing the effects these compounds might have as BNCT agents. Figure 2-5 shows the structures of these compounds. These compounds have demonstrated high aqueous solubility and some tumor uptake.

Boron compounds have one limitation in that the ability to study their *in vivo* kinetics is not possible. Unfortunately there exists no radioactive isotope of boron. Therefore, to overcome this problem, a fluorine-labeled BPA was synthesized. This compound is ¹⁸F-BPA which can be monitored by PET (Positron Emission Tomography) to determine the tumor targeting ability of BPA. The radiolabel was inserted into the 2-

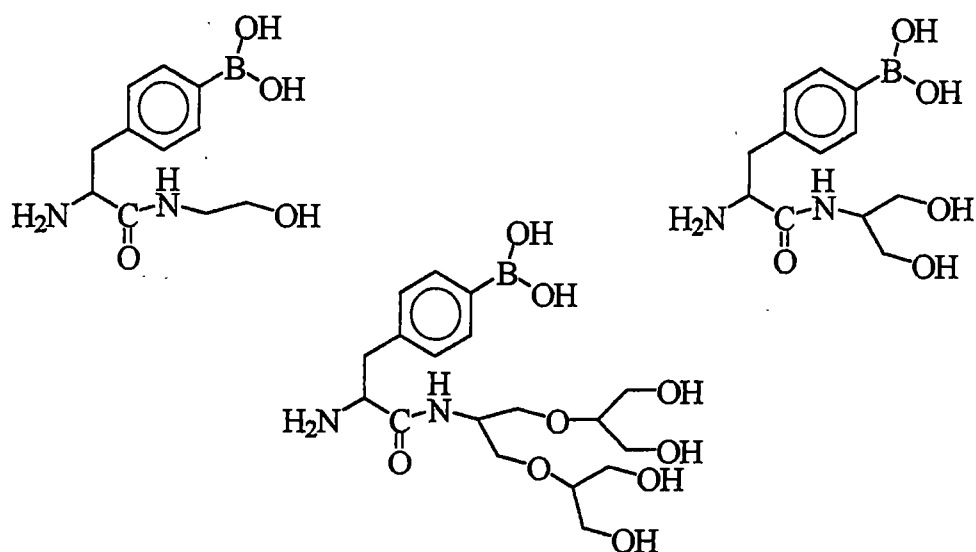


Figure 2-5 Hydroxyamide Derivatives of BPA

position on the aromatic ring¹⁹¹⁻¹⁹³ (Figure 2-6).

¹⁸F-BPA is analogous to BPA in that the uptake into tumor cells and retention is the same. PET studies of ¹⁸F-BPA in patients with brain tumors have demonstrated the ability of the compound to target cancerous masses.^{44a,194-196}

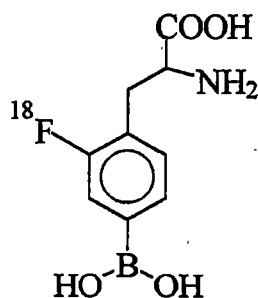


Figure 2-6 ¹⁸F-BPA

Experiments in tumor-bearing animals have shown the (L) isomer to be the more effective, supporting the assumption that the amino acid transport system may be active

for L-BPA (Figure 2-1) in achieving high tumor concentrations.¹⁹⁷⁻¹⁹⁹

Several other types of boronic acid amino acid derivatives have been synthesized. Examples include those that are similar to aspartic acid and cysteine (Figure 2-7).¹²⁸ Unfortunately, there have been few data reported on their use in BNCT.

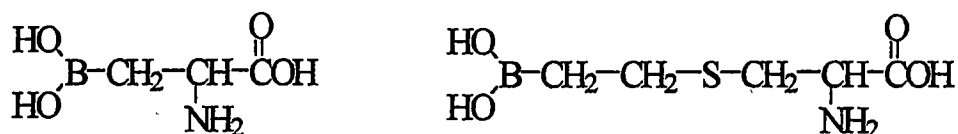


Figure 2-7 Boronic Acid Derivatives of Aspartic Acid and Cysteine

Some research has focused on the replacement of the carboxyl group with the dihydroxyboryl group.^{200,201} Figure 2-8 shows the structures of the proline,²⁰⁰ phenylalanine^{201b} and valine^{201c} analogs of these compounds. It was felt that these compounds would act as transition state inhibitors of enzymes, but little work has been done to show their usefulness as BNCT agents.

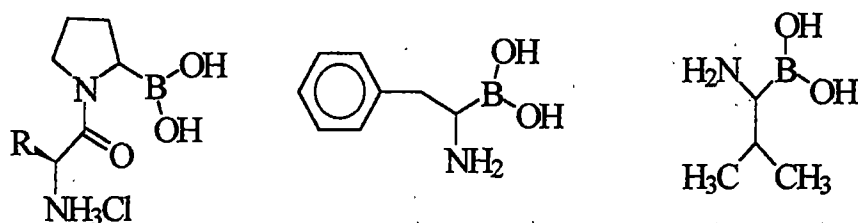


Figure 2-8 Amino Acids with Replacement of $-\text{COOH}$ with $-\text{B}(\text{OH})_2$

B. Zwitterionic Derivatives

Other types of boron-containing amino acids also exist. One class of compounds is the borane zwitterion. One example is $\text{H}_3\text{NBH}_2\text{COOH}$, which can be viewed as an

isostere of glycine.^{139,140} Figure 2-2 shows the structures of two borane zwitterionic species. These types of compounds have led to a development of several boron-containing amino acids as shown in Figure 2-9. Unlike other boron-containing amino acids, these compounds have undergone distribution studies to determine their efficacy as BNCT agents.

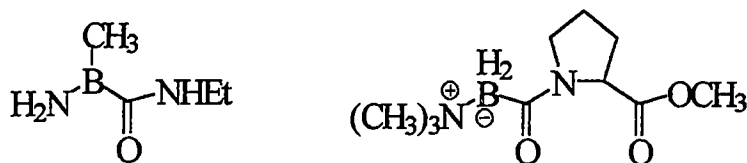


Figure 2-9 Boron-containing Amino Acid derivatives

C. Carboranyl Amino Acids

A new set of compounds was envisioned that contained a boron cluster, resulting in many boron atoms per molecule. These were sought after for the simple fact that less compound will be needed to concentrate into tumor cells prior to irradiation. With each molecule containing several boron atoms, it was perceived that the BNCT reaction would be more effective in killing tumor cells. The boron clusters that are the most common are the carboranes. Carboranes contain 10 boron atoms per molecule, as well as two carbons for manipulation in organic synthesis.

One of the first carborane amino acids described was *o*-carboranylalanine that was first synthesized by Zakharkin et al.²⁰² and Brattsev et al.²⁰³ as a racemic mixture. Unfortunately the yields were not very high, but more recent techniques have led to

higher yields.^{172,204-206} These syntheses yield both enantiomers of *o*-carboranylalanine. A stereoselective synthesis of the L-isomer ((S)-configuration) has also been reported.²⁰⁷ Recently a more useful stereoselective synthesis have been used to prepare both the D- and L-isomers.²⁰⁸⁻²¹³ Figure 2-10 shows the structures of these *o*-carboranylalanines.

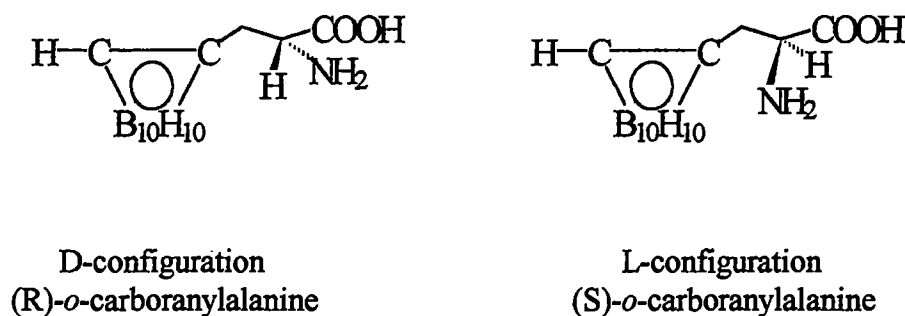


Figure 2-10 D- and L-*o*-Carboranylalanine

Since the carboranyl group is similar in size to a phenyl group, it was speculated that this compound would simulate BPA in its biochemical properties. Studies have demonstrated that *o*-carboranylalanine attained higher blood concentrations and lower tumor levels than BPA at similar time intervals and showed no tumor selectivity.²¹⁴⁻²¹⁸ These results clearly indicate that the carboranyl moiety affects the biodistribution. The carboranyl moiety is highly lipophilic and 112 times more hydrophobic than the indole side chain of tryptophan, which may explain the high blood levels through noncovalent interactions with blood lipids.¹⁷² These results are supported by studies on phenylalanine and tyrosine residues in which bioactive peptides are replaced with the *o*-carboranyl moiety.^{207,219,220} These analogues were biologically active and some showed prolonged

activity when compared to their phenylalanine counterparts. These results show the need to moderate the lipophilicity of the carboranyl group by placing functional groups on the molecule that will counteract the carboranyl moiety's lipophilicity.

Many other types of carborane-containing amino acids have been synthesized and evaluated.^{141,170,204-206,221-232} Two compounds are *p*-carboranylalanine and 2-methyl-*o*-carboranylalanine (Figure 2-11), which were synthesized by Sjöberg et al.¹⁷⁰ It has been demonstrated that *o*-carboranylalanine spontaneously self-degrades at a pH around the isoelectric point. This self-degradation is an intramolecular process in which the *o*-carborane cage is transformed into the corresponding *nido*-compound by action of both the carboxylate ion and the protonated amine function. This degradation could complicate the use of the free amino acid *in vivo*. For this reason *p*-carborane was used, since it is known to be more stable towards degradation to *nido*-compounds than *o*-carborane.²³³ The *p*-carboranylalanine was shown to indeed be stable towards degradation. However, 2-methyl-*o*-carboranylalanine degrades in a fashion similar to *o*-carboranylalanine.

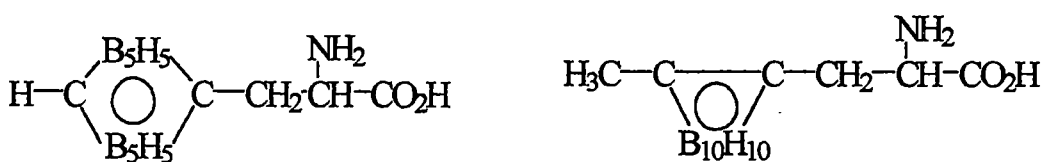


Figure 2-11 *p*-Carboranylalanine and 2-Methyl-*o*-carboranylalanine

Several carboranyl derivatives of BPA have been synthesized by Wyzlic et al.²³⁴ Two of these compounds, *p*-(*o*-carboran-1-yl)-phenylalanine (CBPA) and *O*-(*o*-carboran-1-ylmethyl)-tyrosine (CBT) are shown in Figure 2-12. These compounds are highly stable to acidic conditions and are expected to be transported into tumor cells by the same transport system that is operative for BPA. As with other carborane compounds these molecules are highly lipophilic and hydrophobic. The molecules are non-toxic up to several hundred $\mu\text{g/ml}$ solution.

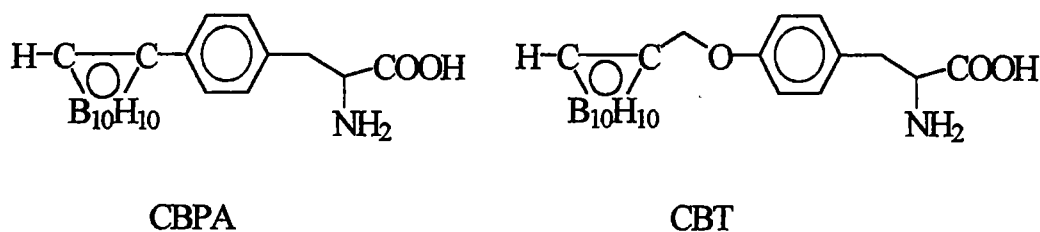


Figure 2-12 Carboranyl Derivatives of BPA

Another carboranyl derivative of BPA has been synthesized by Moore and his colleagues.²³⁰ The compound, 3,4-dihydroxy-5-carboranylphenylalanine (DOPA) (Figure 2-13), was synthesized because it might provide selective uptake of boron more efficiently than BPA while having greater aqueous solubility than the *closo*-carboranylphenylalanine. The procedure described for the synthesis of this compound could be used for the synthesis of other boronated hydroxylated aromatic amino acids for use in BNCT. Also, unlike BPA, this compound would not have to lose a boron atom to generate indolequinones, the suspected precursors of melanin that occur in significantly elevated amounts in melanomas.

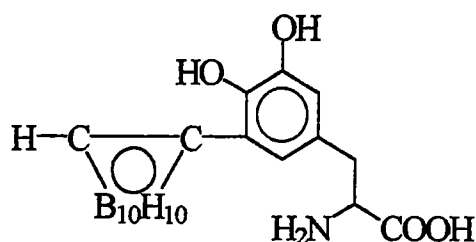


Figure 2-13 DOPA

Carboranyl tyrosine derivatives have also been made for use in BNCT. The compound O-(*o*-carboran-1-yl)methyl-3-hydroxytyrosine (CMHT) and its glycerol ester (CMHTG) (Figure 2-14) were synthesized by Hishiaki et al.²³¹ The water solubility of CMHT is 1.13 mmol/l at 20 °C, whereas the water solubility of the glycerol ester increased to 1.80 mmol/l. Unfortunately it is difficult to obtain CMHTG in a pure state, since CMHTG gradually decomposes at room temperature into glycerol and CMHT.

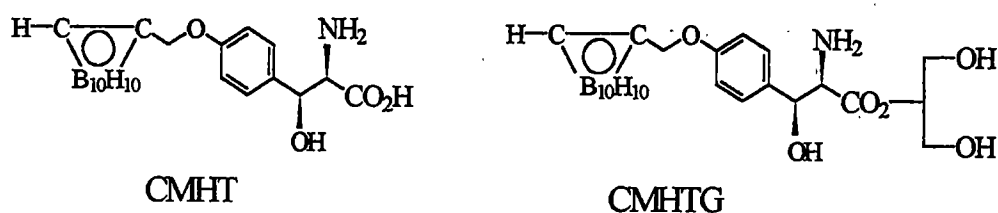
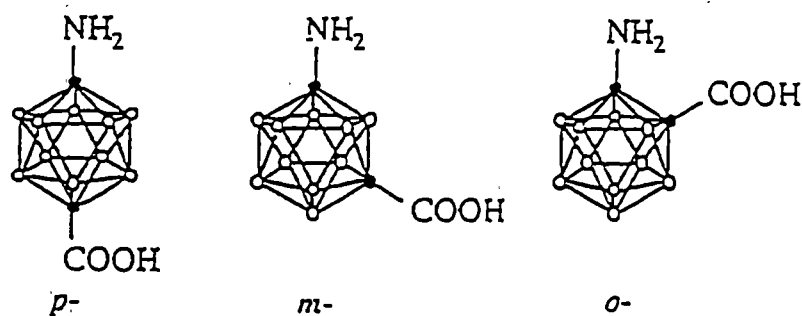


Figure 2-14 Carboranyl Tyrosine Derivatives

Carboranyl amino acids that have the amino and carboxyl groups on different carbons of the carborane cage have also been synthesized.^{171,235} These compounds contain *o*-, *m*-, and *p*-carborane cages (Figure 2-15). These compounds were synthesized because it was thought that carboranes bearing dissimilar polar functional groups on the

two carbons of the icosahedral cage might be water soluble and possible tumor localizers, and they could be even more important synthons for attachment to known tumor seekers such as porphyrins.



Where ● = carbon and ○ = boron-hydrogen

Figure 2-15 Carboranyl Amino Acids with -NH_2 and -COOH on Different Carbons

Another type of carboranyl amino acid has been prepared by Hawthorne and is based on the *nido*-carborane.²²⁷ He took advantage of the *o*-carborane's instability towards base to degrade it into the negatively charged *nido* derivative (Figure 2-16). These *nido* compounds show increased water solubility, but unfortunately are toxic. An alternative considered by Hawthorne is the attachment of polyols to these *nido* molecules,^{223,236} to further increase the water solubility. Unfortunately it has not been determined what the balance of lipophilic/hydrophilic properties of carboranyl amino acids must be to be useful in BNCT.

In addition to the carborane boron clusters, several polyhedral boron clusters of amino acids have been prepared. The decaborane analog of methionine was prepared by Jacobs et. al.¹⁴¹ DL-S-(10-Dimethylsulfidodecaborane)methionine (Figure 2-17)

was prepared as a potential tumor antibody-binding agent. This compound is a neutral

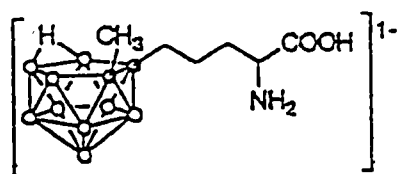


Figure 2-16 *nido*-Carboranyl Amino Acid

polyhedral borane with protein incorporating functionality provides the basis for several lines of research designed to synthesize water-soluble, boron-containing polypeptides. This amino acid will be used to synthesize low and medium weight polymers to attach to tumor antibodies. In this way, large numbers of boron atoms might be localized in the cell.

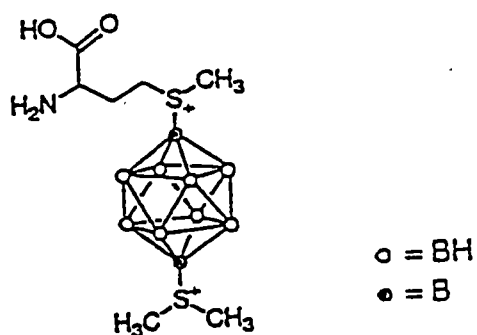


Figure 2-17 Polyhedral Borane Analog of Methionine

V. Conclusion

Clearly, amino acids play an important role as possible BNCT agents. The goal of this research was to synthesize amino acids that have better properties than those currently available. The model amino acid derivative chosen was 1-aminocyclobutane-carboxylic acid. The syntheses of boronated derivatives of this compound will be discussed in the remainder of this thesis.

Chapter 3

Syntheses of Boronated 1-Aminocyclobutanecarboxylic Acids

I. Introduction

Amino acids appear to be preferentially taken up by growing tumor cells. It is for this reason that boron-containing amino acids are being evaluated as BNCT agents. The focus of this research is on the synthesis of boronated amino acids. 1-Aminocyclobutanecarboxylic acid and its boronated derivatives, are the molecules of interest and their syntheses will be discussed in the remainder of this thesis.

II. 1-Aminocyclobutanecarboxylic acid

1-Aminocyclobutanecarboxylic acid (ACBC) (Figure 3-1) is an unnatural alicyclic amino acid that has a high affinity for a variety of tumors,²³⁷ including brain tumors.²³⁸ Only minimal amounts of ACBC are taken up by normal brain tissue, resulting in higher tumor-to-non tumor concentration ratios than seen for ¹⁸F-FDG (¹⁸F-Fluorodeoxyglucose) or ¹¹C/ ¹⁸F labeled natural amino acids.

ACBC has been used at the University of Tennessee Medical Center for clinical



Figure 3-1 1-Aminocyclobutanecarboxylic acid (ACBC)

PET oncology studies of brain tumors.²³⁹ PET oncology studies with appropriate radiotracers provide a unique and noninvasive method for studying biochemical and metabolic changes in cancer tissue. Therefore PET can be used to monitor the growth or regression of tumors. The C-11 analog of ACBC is used for the PET studies (Figure 3-2).

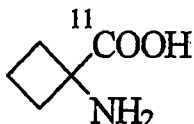


Figure 3-2 ¹¹C analog of ACBC

As a radiotracer, it has demonstrated great ability in identifying malignant tumors. In general, C-11 amino acids are rapidly transported into malignant cells and show high uptake, slow washout and rapid clearance from non-specific tissues and are excellent agents for the diagnosis and management of treatment of cancer using PET.²⁴⁰

The C-11 analog of ACBC has been shown to be as effective as, if not better than, F-18 BPA at localizing in tumors. This is illustrated in PET studies at the University of Tennessee Medical Center (Figure 3-3).

The synthesis of 1-[¹¹C]ACBC is straightforward. A modified Bucherer-Strecker synthesis is carried out at high temperature and pressure (Eq 3-1).²³⁹ The reaction involves the condensation of ¹¹C-labeled cyanide and cyclobutanone in an aqueous solution of ammonium carbonate and ammonium chloride to form a hydantoin. The hydantoin is then hydrolyzed by sodium hydroxide to the amino acid.

ACBC has been shown to be effective as a tumor specific agent. It would appear

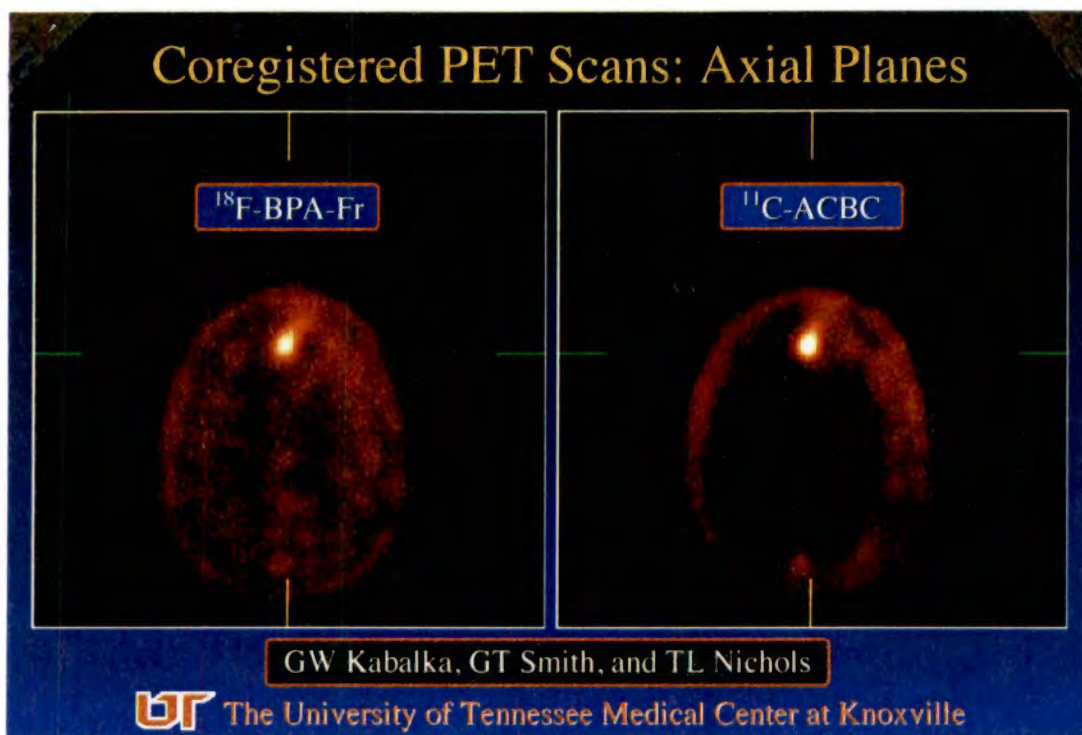
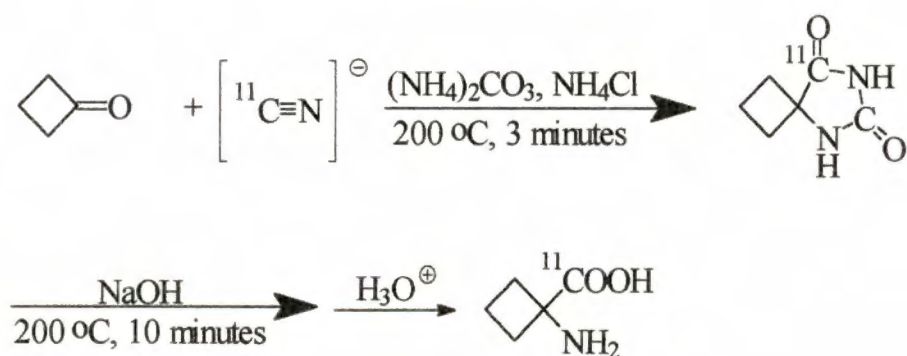


Figure 3-3 Comparison of ^{18}F -BPA and ^{11}C -ACBC by PET

that the synthesis of derivatives of ACBC that contain at least one boron atom would also be tumor specific. These compounds could then be used for BNCT. The syntheses of boronated derivatives of ACBC will be discussed in the following sections.



Eq 3-1 Synthesis of ^{11}C -labeled ACBC

III. *m*-Carboranyl ACBC

The first ACBC derivative that was synthesized was *m*-carboranyl ACBC (Figure 3-4).²⁴¹ The use of carboranes in BNCT is well documented. A carboranyl derivative was chosen first because the carborane molecule contains 10 boron atoms, which makes it an excellent candidate for BNCT. Lower *in vivo* concentrations of the compound would thus be required, which is a desirable property for BNCT agents. *m*-Carborane was chosen over *o*-carborane because of its stability towards base.

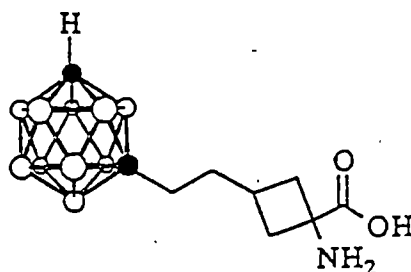
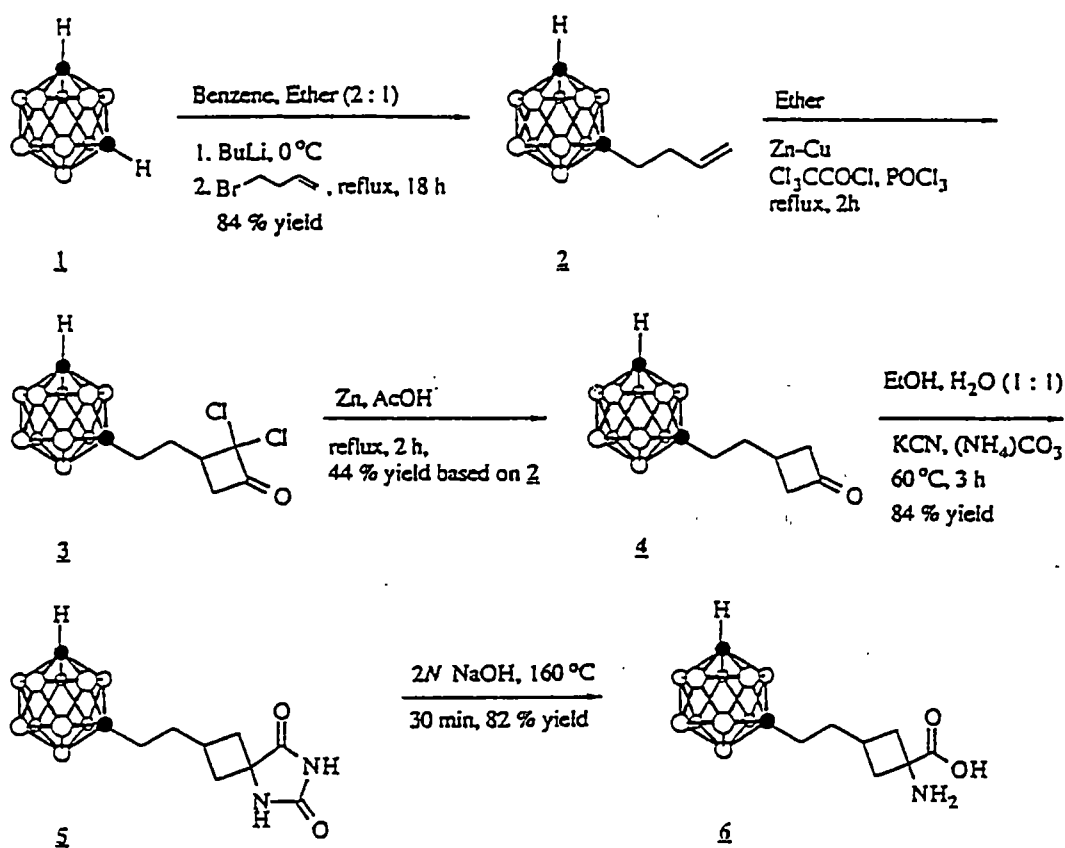


Figure 3-4 *m*-carboranyl ACBC

The synthesis of *m*-carboranyl ACBC is shown in Eq 3-2. The starting material for the synthesis is *meta*-carborane 1. *m*-Carborane is a cage molecule that consists of 12 atoms, 2 carbons and 10 boron atoms. All the atoms in the molecule are hexavalent and the C-H bonds are sufficiently acidic to be deprotonated by strong bases. The first step in the synthesis is the alkylation of the *m*-carborane cage.²⁴² *m*-Carborane 1 is allowed to react with a slight excess of butyllithium, which lithiates one of the C-H bonds creating a carbanion, which in turn attacks 4-bromo-1-butene in S_N2 fashion to yield alkenyl carborane 2. The precipitation of lithium bromide salts during the reaction indicates the product is being formed. It should be noted that, in this reaction, only monoalkylation of



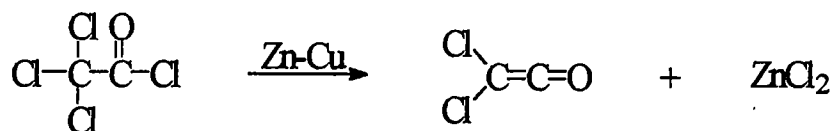
○ = Boron-H

● = Carbon

Eq 3-2 Synthesis of *m*-carboranyl ACBC

the *m*-carborane cage occurs.

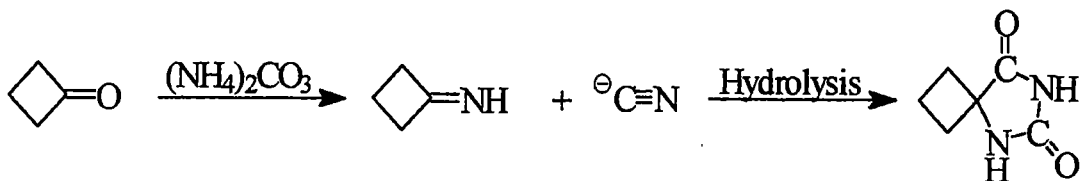
With the alkene in hand, synthesis of the four-membered ring was accomplished by allowing alkene **2** to react with zinc-copper couple,²⁴³ trichloroacetyl chloride and phosphorus oxychloride to yield the corresponding 2,2-dichlorocyclobutanone derivative **3**.²⁴³ This reaction proceeds through a [2 + 2] cycloaddition reaction of the alkene and dichloroketene.^{243,244} Dichloroketene is produced *in situ* by reaction of the zinc-copper couple and trichloroacetyl chloride (Eq 3-3). Dichloroketene is used for the cycloaddition reaction instead of ketene for several reasons: a) it is more stable than ketene, b) it is less likely to dimerize, and c) cycloaddition reactions with ketene usually requires a large excess of the alkene, which is not ideal for this synthesis.



Eq 3-3 Synthesis of dichloroketene

The crude 2,2-dichlorocyclobutanone **3** was then subjected to a reductive dechlorination with zinc dust and acetic acid to yield the desired cyclobutanone **4**.²⁴⁴ Cyclobutanone **4** was allowed to react with excess ammonium carbonate, potassium cyanide in an aqueous ethanol mixture in a pressure tube to yield the corresponding hydantoin **5**.²⁴⁵ This reaction proceeds through an imine intermediate that forms via the reaction of the cyclobutanone and the ammonium carbonate (Eq 3-4). This imine is then attacked by the nucleophilic cyanide to yield the hydantoin. The pressure tube is used

instead of a reflux apparatus in order to prevent the escape of the ammonia vapors that form during the reaction and to speed up the reaction.



Eq 3-4 Imine formation of the cyclobutanone then cyanide attack and hydrolysis

The last step of the synthesis involves hydrolysis of the hydantoin. This was accomplished by heating the hydantoin **5** in 2 M NaOH at 160 °C in a pressure tube to give the amino acid **6** in high yield.²⁴⁶ This reaction requires the use of pressure to enhance the reaction rate. This compound proved to be stable towards base, as expected, even at high temperatures.

Unfortunately *m*-carboranyl ACBC, **6**, was insoluble in aqueous solution due to the lipophilic carborane cage. Therefore this compound would be difficult to use as a BNCT agent. Polar derivatives of this compound are currently being synthesized in an effort to improve the water solubility. These derivatives are of the carbohydrate and polyethylene glycol variety. The hydrophilic groups will be attached to the other carbon of the carborane cage which can be functionalized as described earlier i.e. reaction with butyllithium. Since the *m*-carboranyl ACBC did not demonstrate the desired properties, attention was focused on small molecule ACBC derivatives, in hopes that they would show improved water solubility. These compounds are discussed in the following section.

IV. Small Molecule ACBC's

The compounds that are the focus of this section of the thesis are boronic acid derivatives of ACBC. Boronic acids were chosen due to their structural similarity to L-BPA, one of only two clinically approved BNCT agents (Figure 3-5). The similarities are in the phenylboronic acid portion of each molecule. These types of boronic acids are stable, thus making them useful for BNCT.

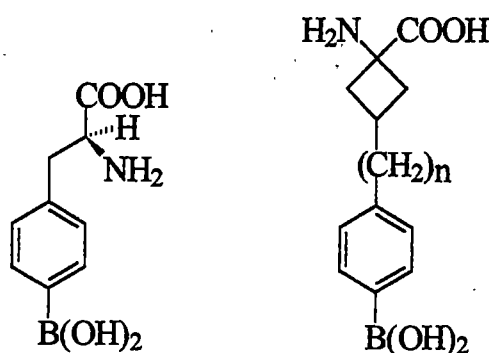
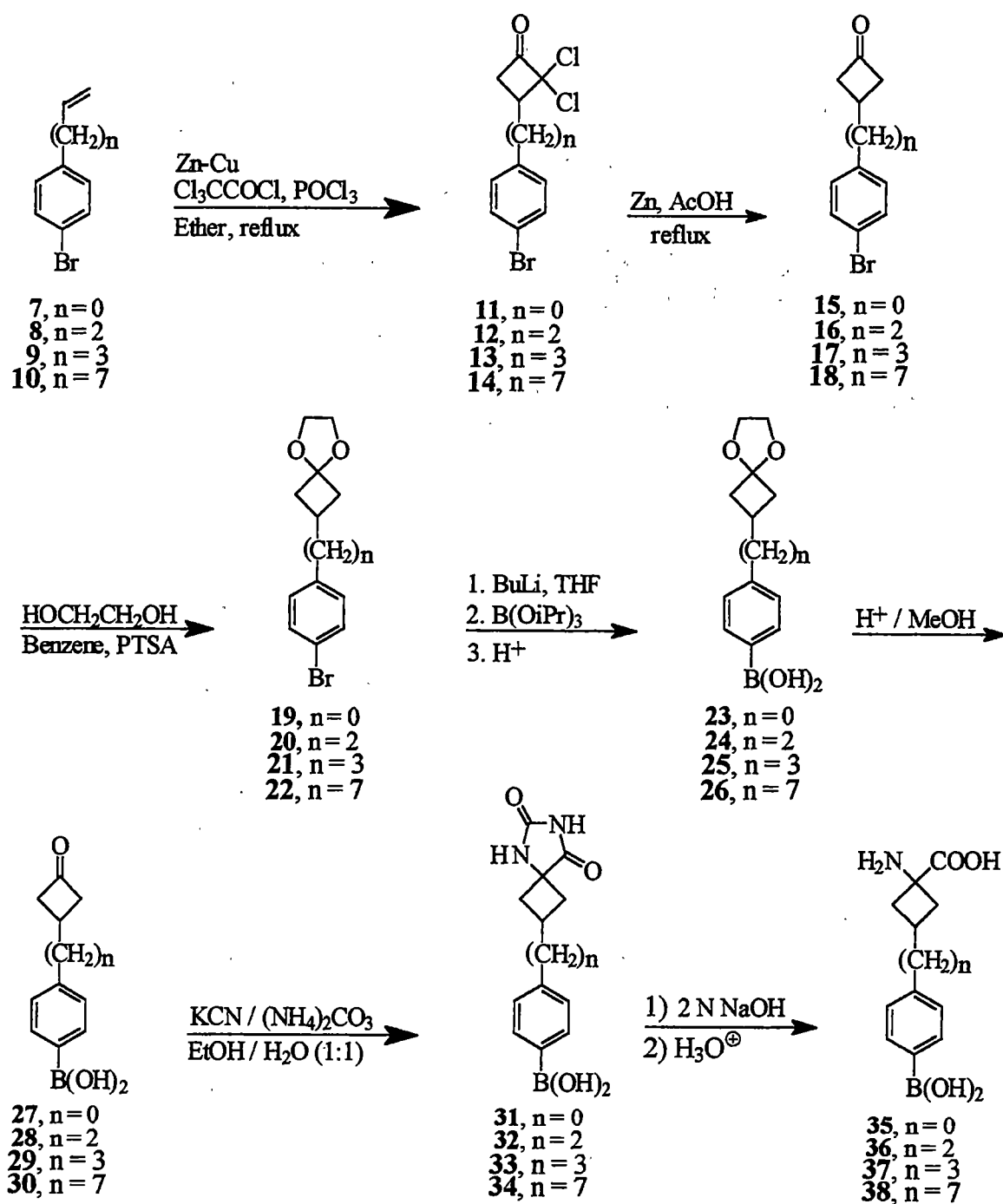


Figure 3-5 L-BPA and small molecule ACBC compounds

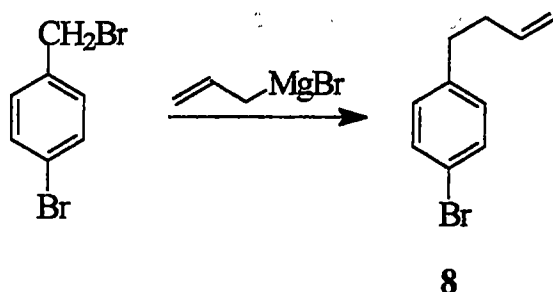
Our goal was to synthesize several derivatives where $n = 0, 1, 2, \dots, 7$ and then determine how these methylene spacers affect properties of the compounds, such as the lipophilicity. Another reason for synthesizing these compounds is that they can be complexed with fructose to increase the water solubility. Researchers have taken advantage of boronic acids ability to complex with carbohydrates to form the water soluble borates to increase the water solubility.¹⁸³⁻¹⁸⁶ BPA is administered to patients as the fructose complex.¹⁸⁷

The syntheses of these small molecule ACBCs is shown in Eq 3-5. The starting material shown for the syntheses is an alkene, which had to be synthesized. For $n = 0$, the starting material was *p*-bromostyrene which is commercially available. However, for



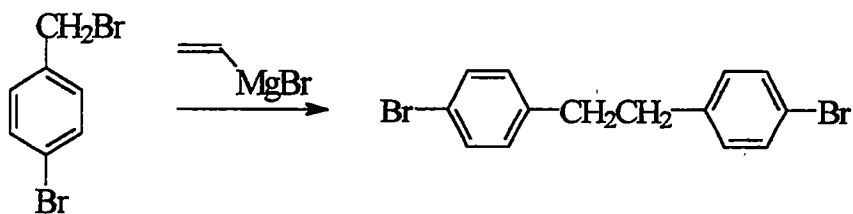
Eq 3-5 Syntheses of small molecule ACBCs

small molecule ACBC's with $n = 1, 2, \dots, 7$ the alkenes had to be synthesized. For the case of $n = 2$, the synthesis was straightforward. 4-Bromobenzyl bromide was stirred overnight at room temperature with excess allylmagnesium bromide and then refluxed for 1-2 hours to afford the corresponding alkene **8** in high yield (Eq 3-6).²⁴⁷ A cloudy appearance due to the MgBr_2 salts indicated that a reaction had occurred.



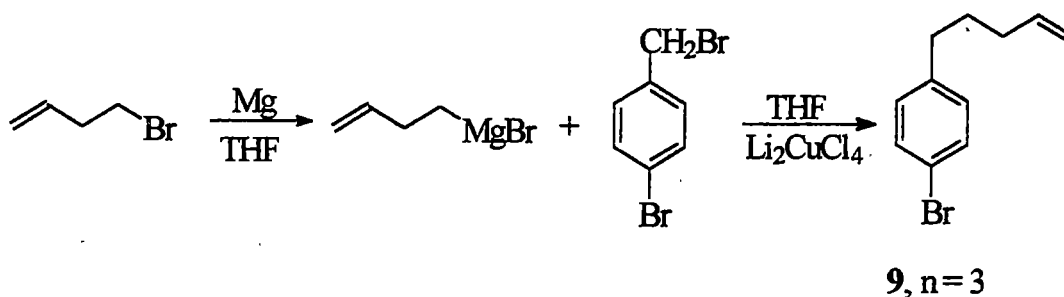
Eq 3-6 Reaction of 4-bromobenzyl bromide with allylmagnesium bromide

It was discovered that the addition of other alkenyl Grignard reagents, such as vinylmagnesium bromide, to 4-bromobenzyl bromide did not yield the desired alkenes; instead the dimerized product of 4-bromobenzyl bromide was isolated (Eq 3-7).



Eq 3-7 Reaction of 4-bromobenzyl bromide with vinylmagnesium bromide

An alternate synthesis of these alkenes was then developed using Li_2CuCl_4 (lithium tetrachlorocuprate) to catalyze the reaction.²⁴⁸ The synthesis of the alkene with $n = 3$ is outlined in Eq 3-8. In the first step, 4-bromo-1-butene was allowed to react with magnesium to yield the corresponding Grignard compound. This Grignard was then added to 4-bromobenzyl bromide in the presence of a catalytic amount of Li_2CuCl_4 (~ 5 mol%) to yield the desired alkene. This reaction was successfully used to synthesize the remaining alkenes ($n = 1, 4, \dots 7$).



Eq 3-8 Synthesis of alkene with $n = 3$ in the presence of Li_2CuCl_4

With the syntheses of the alkenes accomplished, the syntheses of the small molecule ACBCs was initiated. The first step in the synthesis was to construct the four membered ring. The alkenes **7**, **8**, **9** and **10** were subjected to a [2+2] cycloaddition with dichloroketene, which was generated *in situ* via the reaction of Zn-Cu couple and trichloroacetyl chloride, in the presence of phosphorus oxychloride, to yield the 2,2-dichlorocyclobutanones **11**, **12**, **13** and **14**.^{243,244} The crude 2,2-dichlorocyclobutanones were then reductively dechlorinated by zinc in refluxing acetic acid to afford the

cyclobutanones **15**, **16**, **17** and **18**.²⁴⁴

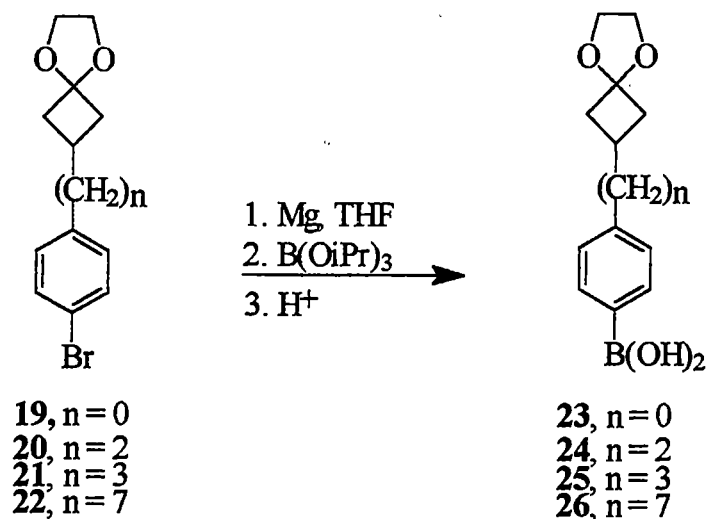
At this point, the boron atom was introduced into the molecule. It should be noted that the boron atom could not be introduced earlier. Control experiments have shown that the boron atom of *p*-tolylboronic acid is removed during the reductive dechlorination reaction to yield toluene. Since the boron is incorporated via a lithiation reaction, the presence of a carbonyl group would interfere with the lithiation reaction. In order to circumvent this problem the ketone had to first be protected as the ketal. This was accomplished by dissolving the cyclobutanones **15**, **16**, **17** and **18** in benzene and refluxing the solutions in the presence of a large excess of ethylene glycol and a catalytic amount of *p*-toluenesulfonic acid (PTSA) in a Dean-Stark trap to azeotropically remove the water.²⁴⁹ The solutions were refluxed overnight, to yield the ethylene ketals **19**, **20**, **21** and **22** in high yield. After protection, the lithiation reactions were successful. The ketals **19**, **20**, **21** and **22** were reacted with a slight excess of butyllithium in THF to form the lithiated species which were then added to triisopropyl borate to form the borate complexes that were then hydrolyzed with aqueous acid to yield the corresponding boronic acid / ketals **23**, **24**, **25** and **26**.²⁵⁰

The next step required removal of the carbonyl protecting group, so that the Bucherer-Strecker synthesis could be implemented. The boronic acid / ketals **23**, **24**, **25** and **26** were taken into an HCl / methanol mixture and stirred overnight at room temperature to remove the ethylene glycol protecting group and form the cyclobutanone boronic acids **27**, **28**, **29** and **30**.²⁵¹ The regeneration of the ketones permitted the syntheses of the amino acids.

Cyclobutanones **27**, **28**, **29** and **30** were each taken into an aqueous mixture of ethanol and allowed to react with a large excess of ammonium carbonate and an excess of potassium cyanide in a pressure tube for 3 hours at 80 °C, to yield the hydantoins **31**, **32**, **33** and **34**.²⁴⁵ These hydantoins were then subjected to a base hydrolysis using 2 N NaOH in a pressure tube at 160 °C for 30 minutes to afford the desired boronic acid amino acids **35**, **36**, **37** and **38**.²⁴⁶ Fortunately, during the hydrolyses of the hydantoins, the boron atoms are not cleaved from the molecule.

There are also alternative pathways for the syntheses of these compounds. These pathways differ from the one presented in that they all incorporate the boron atom into the molecule using alternate approaches. One alternative method involves a slight modification in the way in which boron is introduced at the cyclobutanone stage. The ethylene ketals **19**, **20**, **21** and **22** can be subjected to a Grignard reaction (Eq 3-9).²⁵² This reaction is analogous to the butyllithium reaction in that it creates a nucleophilic carbon that can attack the electron deficient boron atom creating a borate complex that is then hydrolyzed to the boronic acid. The ethylene ketals **19**, **20**, **21** and **22** are each allowed to react with a slight excess of magnesium turnings to form the corresponding Grignard reagent which is then added to triisopropyl borate and hydrolyzed in aqueous acid to yield the boronic acid ketals **23**, **24**, **25** and **26**. One disadvantage to the Grignard reaction is that it does not work well on a small scale, whereas the butyllithium reaction does. On a large scale, the butyllithium method would be difficult because the reaction temperature must be kept at -78 °C which is difficult in large reactions. Therefore the Grignard reaction would be more advantageous for large scale productions. The choice of two

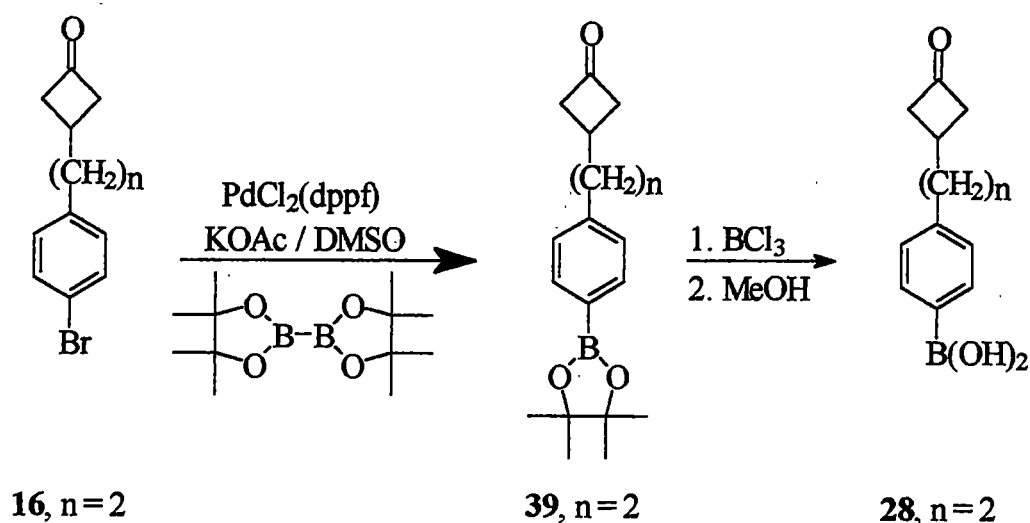
methods of incorporating the boron at the ketal stage allows for flexibility in the synthesis.



Eq 3-9 Introduction of boron by a grignard reaction

Another way to incorporate boron into the molecule is to use a Suzuki-type reaction. The bromophenylcyclobutanone **16** is dissolved in DMSO along with 3 equivalents of potassium acetate, 8 mol% of $\text{PdCl}_2(\text{dppf})$ [dppf = diphenylphosphinoferrocene], 1.1 equivalents of bis(pinacolato)diboron and heated at 80 °C for 2.5 hours to yield the desired boronic ester **39** (Eq 3-10).²⁵³ This boronic ester is then hydrolyzed to the boronic acid by treating it with boron trichloride and then quenching with methanol to afford the boronic acid ketone **28** (Eq 3-10).²⁵⁴ This sequence allows for the formation of the boronic acid ketone without having to first protect the ketone, as in the case of the Grignard or butyllithium reaction. This sequence requires two steps to form the boronic acid ketone as in both the Grignard and butyllithium reactions. One advantage of this reaction is that a boronic ester is formed initially instead of a boronic acid. The boronic

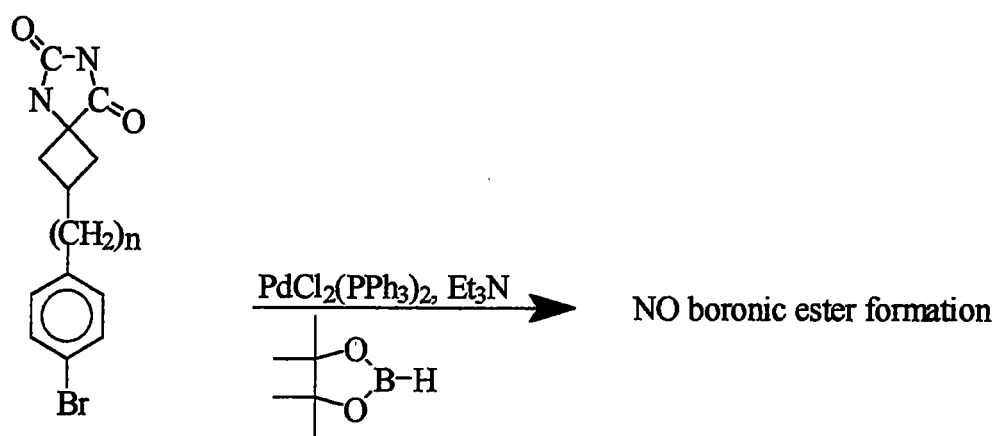
ester is more easily purified than a boronic acid. One serious drawback to this reaction is the cost. The diboron reagent is expensive, which would make it unacceptable for large scale syntheses. Also, the palladium catalyst is expensive, although the palladium is used in only catalytic amounts. From a cost standpoint the Grignard and butyllithium reactions are cost effective. Therefore, on a large scale, these reactions would be more advantageous.



Eq 3-10 Formation of boronic acid / ketone via a palladium reaction

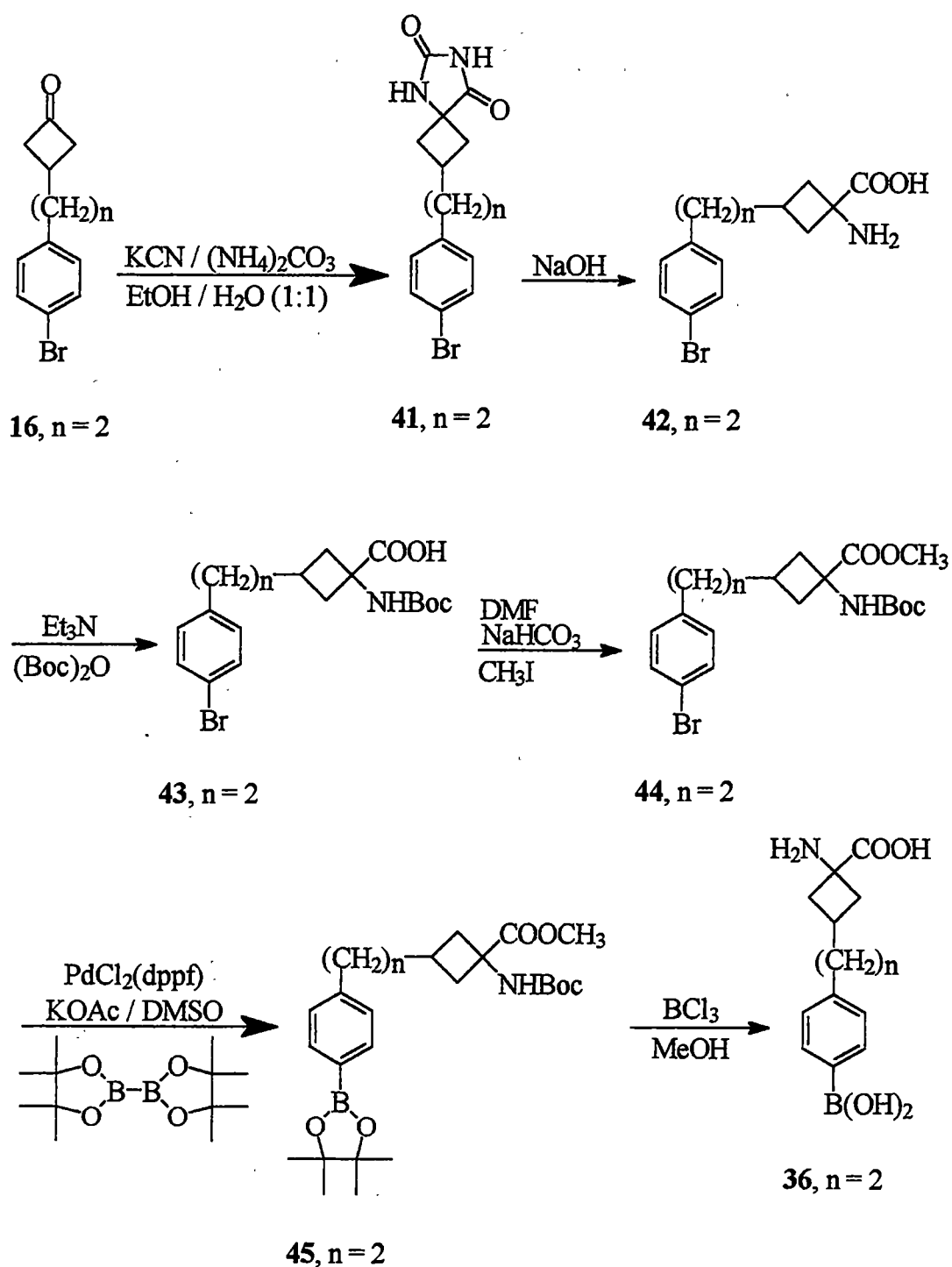
The boron atom can also be introduced into the molecule via the palladium reaction at a totally different stage in the synthesis. This synthesis involves the formation of the amino acid **42** from the ketone, with subsequent protection of both amino acid functional groups. The palladium/diboron reaction is then used at this stage. This synthesis is outlined in Eq 3-12. The first step in the synthesis is the preparation of the hydantoin of the ketone **16** by the method described earlier. Once the hydantoin is

formed, a variety of potential methods can be used to incorporate the boron atom into the molecule. One method involved the use of palladium chemistry along with pinacolborane (Eq 3-11)²⁵⁵ but it proved unsuccessful in our hands. Another attempt was made using the palladium/bis(pinacolato)diboron chemistry. This method also failed to introduce boron into the molecule. It was discovered in control experiments that a hydantoin interferes with the reaction.

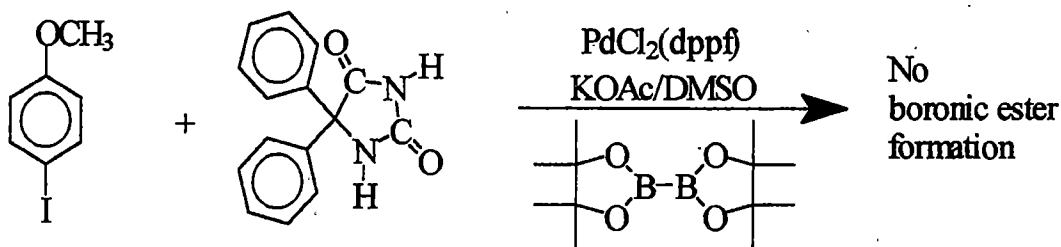


Eq 3-11 Reaction of hydantoin with pinacolborane and palladium catalyst

No boronic ester formation was observed when *p*-iodoanisole was subjected to the Suzuki conditions in the presence of 5,5-diphenylhydantoin (Eq 3-13). These results are attributed to the low solubility of hydantoins in organic solvents, such as DMSO, and also to the fact that the acidic protons on the hydantoin apparently interfere with the palladium catalyst.



Eq 3-12 Synthesis of product via palladium reaction on protected amino acid



Eq 3-13 Palladium reaction in the presence of a hydantoin

Hydantoin **41** was then hydrolyzed by base to the corresponding amino acid **42**. These amino acids had to be protected before the palladium reaction could be used. The first group to be protected was the amine. The amino acid **42** was taken into a 10 % Et_3N in MeOH and 2 equivalents of di-*tert*-butyl dicarbonate $[(\text{Boc})_2\text{O}]$ was added and the mixture heated at 50-60 °C for 1 hour to yield the corresponding Boc-protected amino acid **43**.²⁵⁶ The reaction mixtures were cloudy at the start of the reaction due to the insoluble nature of the amino acids. The amino acids dissolved in the solvent after about 30 minutes which indicated that N-carboxylation was complete. The next step in the synthesis required that the carboxylic acid be protected. The protecting group chosen was the methyl ester. The *N*-Boc amino acid compound **43** was taken into DMF and treated with excess NaHCO_3 , and a large excess of CH_3I and stirred at room temperature overnight to yield the protected amino acid **44**.²⁵⁷ The order in which these protection reactions take place proved to be important. If the carboxylic acid was protected first, the amine group might be methylated, which would be problematic since the methyl group could not easily be removed. For this reason, the amine group was protected first. Once

the amino acid was protected, the palladium reaction could be used. It is important to note that the protected amino acid **44** is much more soluble in organic solvents than the corresponding hydantoin. The protected amino acid was soluble in many solvents, including DMSO which was used for the palladium reaction. The protected amino acid **44** was taken into DMSO and treated with 3 equivalents of KOAc, 8 mol% of PdCl₂(dppf) and 1.1 equivalents of bis(pinacolato)diboron at 80 °C for 2.5 hours to yield the boronic ester **45**. The last step involved removal of the protecting groups to yield the desired amino acid. The protecting groups could be removed using boron trichloride at the same time the boronic ester was being hydrolyzed to the boronic acid. It is known that methyl esters are hydrolyzed by BCl₃.²⁵⁸ Also, the Boc-protecting group was removed by BBr₃.²⁵⁴ BCl₃ has been shown to hydrolyze boronic esters to the boronic acids (Eq 3-10). Therefore it appeared feasible to remove all the protecting groups in one step with BCl₃. The protected amino acid **45** was taken into CH₂Cl₂ and a large excess of BCl₃ in heptanes was added and the mixture stirred at -78 °C for one hour and then at room temperature overnight. The reaction was quenched with methanol to yield the desired amino acid **36**.

Several other attempts to remove the protecting groups were evaluated. One attempt involved removal of the pinacol ester at the same time the *t*-Boc was removed. Two routes were examined. First, the *t*-Boc group was removed by using 50 % trifluoroacetic acid in methylene chloride,²⁵⁹ but the boronic ester was not hydrolyzed to the boronic acid. The second method involved removing the *t*-Boc group with 3 M HCl in ethyl acetate.²⁶⁰ While this method successfully removed the *t*-Boc group, it also failed to hydrolyze the boronic ester to the boronic acid. These methods failed due to the stability

of boronic esters.

Each of the syntheses presented has advantages and disadvantages. For example, the Grignard or butyllithium reactions allow formation of the boronic acid two steps before final product formation. The problem is that boronic acids are sometimes difficult to work with. They are difficult to purify (by column chromatography) due to their high polarity. Also, it is difficult to dry these compounds since dehydration readily occurs resulting in formation of a reactive boroxine. As noted, the disadvantage of the palladium reaction is cost, even though the reactions are simpler to carry out than the Grignard and butyllithium reactions. One advantage that the palladium reaction offers is that the boronic acid is formed in the last step. Thus the problems that exist due to this functional group do not have to be dealt with until late in the sequence. The Grignard and butyllithium reactions are probably the best synthetic method since they are shorter and the cost is modest.

V. Conclusion

ACBC has shown to be as good as, if not better than, BPA as a tumor seeking agent based on PET studies at The University of Tennessee Medical Center. The usefulness of the boronated analogs of ACBC as potential BNCT agents is yet to be determined. Derivatives of ACBC, such as the ones described here, will require testing in order to determine their efficacy in treating cancer.

Experimental

General Methods:

All solvents used were reagent grade and were distilled from appropriate drying agents under a nitrogen atmosphere prior to use. Diethyl ether and THF were distilled from sodium benzophenone ketyl; benzene and DMSO were distilled from calcium hydride and stored under nitrogen. *m*-Carborane was purchased from Dexsil Corporation (Hamden, CT) and purified by sublimation. All other chemicals were purchased from Aldrich Chemical Co. (Milwaukee, WI) and used as received.

Column chromatography was performed using silica gel (60 Å⁰, 230-400 mesh) obtained from Baxter Co. (McGraw Park, IL). Reverse-phase column chromatography was performed utilizing octadecyl functionalized silica gel obtained from Aldrich Chemical Co. (Milwaukee, WI). Analytical thin layer chromatography was performed on 250 micron silica plates obtained from Analtech Inc. (Newark, DE) which were visualized by phosphomolybdic acid, palladium chloride, silver nitrate solutions and UV light.

Melting points were measured on a Mel-Temp apparatus and are uncorrected. Infrared spectra were obtained either neat or as Nujol mulls and were recorded on a Bio-Rad FTS-7 instrument. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker AC-250 at 250.13 and 62.89 MHz respectively. ¹¹B NMR spectra were obtained on a Bruker AMX-400 at 128.38 MHz. Chemical shifts for ¹H and ¹³C NMR were referenced to Si(CH₃)₄ and measured with respect to the residual protons in the deuterated solvents. In the case of ¹¹B NMR, chemical shifts were measured with reference to external

$\text{BF}_3 \cdot \text{OEt}_2$. Resonances observed upfield of the reference ($\text{BF}_3 \cdot \text{OEt}_2$) were assigned negative chemical shift values. Microanalyses were performed by Galbraith Laboratories Inc., Knoxville, TN. HR-FAB-MS ($M + 1$) were obtained on a ZAB-EQ instrument in a glycerol matrix.

Synthesis of 1-Amino-3-[2-(1,7-dicarboclosododecaboran(12)-1-yl)ethyl]cyclobutane-carboxylic Acid (*m*-Carboranyl ACBC) (6):

Synthesis of 4-*m*-carboranyl-1-butene (2):

A three-necked round-bottomed flask equipped with an addition funnel, reflux condenser and argon balloon was charged with *m*-carborane 1 (35 mmol, 5.0 g) and a mixture of benzene (70 mL) and ether (35 mL). The reaction mixture was cooled to 0 °C and butyllithium (38 mmol, 24 mL of 1.6 M solution in hexane) was added via a syringe over a period of 10 min, the reaction mixture was then allowed to stir at room temperature for 30 min. The reaction mixture was then cooled to 0 °C and to it was added a mixture of 4-bromo-1-butene (39 mmol, 3.9 mL) in a mixture of benzene (10 mL) and ether (5 mL). The reaction mixture was then refluxed at 90 °C in an oil bath for 18 hr, cooled to room temperature, and quenched with 2 mL of water. The solution was transferred to a separatory funnel and washed successively with water (2 x 20 mL) and brine (1 x 20 mL). The organic layer was dried over MgSO_4 , filtered and concentrated under reduced pressure using a rotoevaporator. After drying at 50 °C at 0.2 mm, 7.1 g of colorless liquid was obtained. The crude product was purified over 27 x 4.5 cm of silica

gel column with hexane as eluent to yield 5.8 g (84 %) of a colorless liquid **2** ; $R_f = 0.76$ (in hexane, thin layer chromatography); ^1H NMR (CDCl_3) 5.67 (m, 1H), 5.0 (m, 2H), 2.91 (s, 1H), 2.03 (m, 4H); ^{13}C NMR (CDCl_3) 136.13, 115.74, 75.32, 54.85, 36.06, 33.77; ^{11}B (CDCl_3) -1.81 (s, 1 B), -8.71 (s, 4 B), -11.32 (s, 4 B), 13.27 (s, 2 B); IR (neat) 3064, 2981, 2919, 2854, 2598, 1735, 1641, 1436, 1244, 1046, 994 924, 908, 735 cm^{-1} . HR-FAB-MS ($\text{M} + \text{H}$; obtained in glycerol matrix) calcd. for $\text{C}_6\text{H}_{19}\text{B}_{10}$: 198.241, Found: 198.243.

3-[2-(1,7-Dicarbacosododecaboran(12)-1-yl)ethyl]cyclobutanone (4):

The synthesis of this compound was achieved in two steps. In the first step, 2,2-dichloro-3-[2-(1,7-dicarbacosododecaboran(12)-1-yl)ethyl]cyclobutanone **3** was synthesized from 4-*m*-carboranyl-1-butene **2**. In the second step, the haloketone was treated directly with zinc and acetic acid to yield the desired compound.

A 250 mL three-necked round-bottomed flask equipped with a reflux condenser and an argon balloon was charged with 4-*m*-carboranyl-1-butene **2** (29.1 mmol, 5.77 g) and diethyl ether (100 mL). To this, freshly made Zn-Cu couple (174.3 mmol, 22.53 g) was added followed by trichloroacetyl chloride (64 mmol, 7.2 mL) and phosphorous oxychloride (64 mmol, 5.9 mL). After stirring the reaction mixture at room temperature for 10 min., it was refluxed for 2 hrs. under an argon atmosphere. After 2 hrs. at reflux, the reaction mixture was cooled to room temperature and filtered through a pad of Celite. An additional 50 mL of ether was used to transfer the material. The solvent was removed under reduced pressure using a rotatory evaporator and the viscous material containing

the 2,2-dichloro-3-[2-(1,7-dicarbaclosododecaboran(12)-1-yl)ethyl]cyclobutanone **3** was dried under high vacuum (0.2 mm at 50 °C).

The crude 2,2-dichloro-3-[2-(1,7-dicarbaclosododecaboran(12)-1-yl)ethyl]cyclobutanone **3** was dissolved in glacial acetic acid (50 mL) along with zinc dust (10 g, excess). The reaction mixture was stirred at room temperature for 20 min. After the initial reaction ceased, the mixture was refluxed for 2 hrs. TLC indicated the disappearance of the starting material. The reaction mixture was cooled to room temperature and filtered through a pad of Celite. Ethyl acetate (100 mL) was used to wash the Celite pad and transfer the material from the round bottom flask. The solvent was removed under reduced pressure using a rotoevaporator. The viscous mass was dissolved in ethyl acetate (100 mL) in a separatory funnel. It was washed sequentially with water (2 x 20 mL), saturated sodium bicarbonate (2 x 20 mL), water (2 x 20 mL), and brine (1 x 25 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure using a rotoevaporator. The reaction yielded 8.86 g of a viscous material which was purified by column chromatography using silica gel (30 x 4 cm, 10 % ethyl acetate in hexane) to yield 3.10 g (44 % overall yield based on 4-*m*-carboranyl-1-butene) of a colorless oily product, **4**: $R_f = 0.56$ (in 15 % ethyl acetate/hexane over TLC); ^1H NMR (CDCl_3) 3.16 (m, 2H), 2.93 (s, 1H), 2.64 (m, 2H), 2.24 (m, 1H), 1.96 (m, 2H), 1.68 (m, 2H); ^{13}C NMR (CDCl_3) 206.7, 75.4, 54.9, 52.4, 36.3, 35.6, 23.4; ^{11}B NMR (CDCl_3) -1.91 (s, 1 B), -8.66 (s, 4 B), -11.42 (s, 3 B), -13.22 (s, 2 B); IR (neat) 3060, 2946, 2929, 2868, 2596, 1784, 1452, 1385, 1104, 1062, 1008, 729 cm^{-1} . Anal. Calcd. For $\text{C}_8\text{H}_{20}\text{B}_{10}\text{O}$: C, 39.98; H, 8.39; B, 44.98. Found; C, 40.44; H,

8.28; B, 43.63.

Hydantoin of 3-[2-(1,7-dicarbacosododecaboran(12)-1-yl)ethyl]cyclobutanone (5):

A 15 mL Ace pressure tube was charged with 3-[2-(1,7- dicarbacosododecaboran(12)-1-yl)ethyl]cyclobutanone **4** (0.42 mmol, 0.10 g), aqueous ethanol (50 % , 2 mL), potassium cyanide (0.46 mmol, 0.03 g), and ammonium carbonate (1.0 mmol, 0.10 g). The reaction vessel was sealed and heated at 60 °C in an oil bath for 3 hrs. A white precipitate formed. The reaction vial was cooled to room temperature and the cap unscrewed carefully in fume hood. The reaction mixture was quenched with aqueous acetic acid (2 mL of 30 % acetic acid in water). The solvent was removed under reduced pressure using a rotatory evaporator and the resultant white solid taken up into ethyl acetate (50 mL) in a separatory funnel. It was washed sequentially with water (2 x 10 mL), and brine (1 x 10 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated under reduced pressure. A white solid was obtained (270 mg) and this was purified using column chromatography (silica gel, 10 % methanol in dichloromethane). Chromatographic purification yielded 0.11 g (84 % yield) of a white solid: R_f = 0.65 (in 10 % methanol/methylene chloride on TLC); mp; 332-334 °C (with decomposition); ^1H NMR (CD_3OD) 3.48 (s, 1 H), 2.53 (m, 2 H), 2.25 (m, 3 H), 1.89 (m, 2 H), 1.64 (m, 1 H), 1.51 (m, 1H); ^{13}C NMR ($\text{DMSO} - d_6$) 178.59, 155.82, 59.06, 57.54, 56.20, 37.73, 36.73, 36.48, 33.34, 25.46; ^{11}B NMR (CD_3OD) -1.36 (s, 1B), -8.06 (s, 4B), -10.66 (s, 2B), -11.56 (s, 1B), -12.24 (s, 2B); IR (nujol) 3207, 3040, 2949, 2920, 2850, 2755, 2597, 1761, 1734, 1435, 1305, 1120, 778, 765, 727, 643 cm^{-1} . Anal. Calcd. for

C₁₀H₂₂B₁₀O₂N₂: C, 38.7; H, 7.14; N, 9.03; B, 34.83. Found: C, 38.31; H, 6.73; N, 8.70; B, 33.51.

1-Amino-3-[2-(1,7-dicarbaclosododecaboran(12)-1-yl)ethyl]cyclobutanecarboxylic acid (6):

The hydantoin of 3-[2-(1,7-dicarbaclosododecaboran(12)-1-yl)ethyl]cyclobutanone **5** (0.64 mmol, 0.20 g) was placed in a 15 mL Ace pressure tube along with a solution of sodium hydroxide (5 mL of 2 N NaOH). The reaction mixture was heated to 160 °C in an oil bath for 30 min. It was then cooled to room temperature and opened carefully. TLC indicated the disappearance of the starting hydantoin. The reaction mixture was acidified using concentrated hydrochloric acid and volatile solvents removed under reduced pressure using a rotatory evaporator. The white solid obtained was purified using column chromatography (2 x 22 cm, octadecyl functionalized silica gel, 80 % methanol in water). The product (0.15 g, 82 % yield) was obtained as a white solid: R_f = 0.55 (in a mixture of butanol, water, and acetic acid in a ratio of 10:1:0.5); mp; compound turns brown at 201 °C; ¹H NMR (CD₃OD) 3.49 (s, 1H), 2.63 (m, 2H), 2.40 (m, 3H), 1.89 (m, 2H), 1.61 (m, 2H); ¹³C NMR (CD₃OD) 176.3, 77.5, 56.9, 38.4, 37.5, 36.8, 36.0, 35.2, 29.0; ¹¹B NMR (CD₃OD) -1.45 (s, 1B), -8.11 (s, 4B), -10.70 (s, 2B), -11.42 (s, 1B), -12.29 (s, 2B); IR (nujol) 4000, 3434, 3060, 2947, 2909, 2849, 2598, 2294, 1719, 1677, 1566, 1457, 1377, 1269, 1233, 1183, 1139, 1072, 1009, 728 cm⁻¹. Anal. Calcd. for C₉H₂₃B₁₀O₂N: C, 37.60; H, 8.07; N, 4.87; B, 38.32. Found: C, 36.54; H, 7.96; N, 5.24; B, 35.94. HR-FAB-MS (M + H; obtained in glycerol matrix) Calcd. for C₉H₂₄B₁₀O₂N:

286.282. Found: 286.284.

Small molecule ACBC's:

4-(4-bromophenyl)but-1-ene (8):

A 500 mL three-necked round-bottomed flask equipped with an addition funnel, reflux condenser and argon balloon was charged with 4-bromobenzyl bromide (80 mmol, 20 g) in 50 mL of anhydrous ether. To this, allylmagnesium bromide (96 mmol, 96 mL of 1 M solution in ether) was added via an addition funnel over a period of 30 min, and the reaction was allowed to stir at room temperature for 2 hours. After stirring at room temperature, the reaction mixture was refluxed in an oil bath for 3 hours. Progress of the reaction was monitored by TLC. After the starting material (aryl bromide) disappeared, the contents of the flask were cooled to room temperature and quenched by water (20 mL) and transferred to a separatory funnel. The aqueous layer was drained off and extracted twice with ether (2 x 25 mL). The combined organics were washed with water (2 x 20 mL), brine (1 x 20 mL), dried over magnesium sulfate and concentrated on rotatory evaporator. After drying at 50 °C at 1 mm Hg, a colorless liquid (16 g) was obtained. The crude product was purified by Kugelrohr distillation to yield **8** as a colorless liquid (14 g, 83% yield); R_f = 0.86 (hexane, thin layer chromatography); ^1H NMR (CDCl_3) 7.37 (d, J = 8.24 Hz, 2 H), 7.06 (d, J = 8.28 Hz, 2 H), 5.61 (m, 1 H), 5.06 (m, 2 H), 2.67 (t, J = 8.2 Hz, 2 H), 2.35 (m, 2 H). ^{13}C NMR (CDCl_3) 140.68, 137.52, 131.28, 130.16, 119.49, 115.24, 35.21, 34.69; IR (neat) 3067, 2975, 2928, 2856, 1639, 1486, 1436, 1402, 1072, 1011, 994, 913, 803. Anal. Calcd. for $\text{C}_{10}\text{H}_{11}\text{Br}$: C, 56.87; H,

5.21. Found: C, 57.07; H, 5.65.

5-(4-bromophenyl)pent-1-ene (9):

A 250 mL three-necked round-bottomed flask equipped with an addition funnel and an argon balloon was charged with 4-bromobenzyl bromide (44.4 mmol, 11.1 g) in 25 mL of anhydrous THF. To this was added butenylmagnesium bromide (37 mmol, 74 mL of 0.5 M solution in THF) via an addition funnel over a period of 20 minutes. During this reaction time the mixture was kept at -50 °C using a dry ice acetone bath. After the addition of the Grignard was over, a solution of lithium tetrachlorocuprate (0.18 mmol, 1.85 mL of 0.1 M solution in THF) was added while the reaction was still at -50 °C. The resulting solution was allowed to stir overnight at -50 °C after which the bath temperature was allowed to warm to room temperature. Next, the reaction mixture was quenched with saturated ammonium chloride (40 mL) and the solvent was removed on a rotary evaporator. The resultant material was taken into ether (100 mL) and washed with water (2 x 20 mL), brine (1 x 20 mL) and dried over magnesium sulfate and concentrated on rotary evaporator and dried at 50 °C at 1 mm Hg to obtain a colorless liquid (3.6 g). The crude reaction mixture was purified on a silica gel column, using hexane as the eluent, to obtain a colorless liquid (2.8 g, 33.4 % based on 4-bromo-1-butene); R_f = 0.87 (hexane, thin layer chromatography); ^1H NMR (CDCl_3) 7.36 (d, J = 8.25 Hz, 2 H), 7.02 (d, J = 8.35 Hz, 2 H), 5.78 (m, 1 H), 5.01 (m, 2 H), 2.54 (t, J = 7.75 Hz, 2 H), 2.05 (m, 2 H), 1.66 (m, 2 H); ^{13}C NMR (CDCl_3) 141.26, 138.23, 131.25, 130.16, 119.36, 114.89, 34.60, 33.08, 30.35.

9-(4-bromophenyl)non-1-ene (10):

This alkene was prepared similar to 9 by reacting 4-bromobenzyl bromide (28.8 mmol, 7.19 g) in THF (20 mL) and a solution of octenylmagnesium bromide (26 mmol, 52 mL of 0.5 M solution in THF) in the presence of lithium tetrachlorocuprate (0.29 mmol, 2.9 mL of 0.1 M solution in THF) to obtain a colorless liquid. The crude reaction mixture was purified on a silica gel column, using hexane as the eluent, to obtain a colorless liquid (3.09 g, 42 % based on 8-bromo-1-octene); R_f = 0.85 (hexane, thin layer chromatography); ^1H NMR (CDCl_3) 7.36 (d, J = 8.26 Hz, 2 H), 7.02 (d, J = 8.22 Hz, 2 H), 5.78 (m, 1 H), 4.96 (m, 2 H), 2.53 (t, J = 7.53 Hz, 2 H), 2.01 (m, 2 H), 1.56 (m, 2 H), 1.29 (m, 8 H); ^{13}C NMR (CDCl_3) 141.71, 139.04, 131.19, 130.09, 119.21, 114.14, 35.29, 33.73, 31.24, 29.26, 29.08, 28.99, 28.85; IR (neat) 3076, 2976, 2927, 2855, 1641, 1488, 1462, 1403, 1073, 1011, 995, 909, 827, 798, 723. Anal. Calcd. for $\text{C}_{15}\text{H}_{21}\text{Br}$: C, 64.06; H, 7.53. Found: C, 64.13; H, 7.73. HRMS: calcd. for $\text{C}_{15}\text{H}_{21}\text{Br}$: 280.083; Found: 280.085.

3-(4-bromophenyl)cyclobutanone (15):

The synthesis of this ketone was similar to that of compound 16. A solution of 4-bromostyrene 7 (136.6 mmol, 25 g) in ether (200 mL) was reacted with a mixture of trichloroacetyl chloride (204.9 mmol, 22.85 mL) and phosphorus oxychloride (204.9 mmol, 19.09 mL) in the presence of Zn-Cu couple (273.1 mmol, 17.86 g) to obtain 2,2-dichloro-3-(4-bromophenyl)cyclobutanone 11. The dichloroketone 11 was subjected to a reductive dechlorination with zinc dust (20 g, excess) and acetic acid (50 mL) to yield a light yellow liquid. The product was purified by Kugelrohr distillation to obtain 3-(4-

bromophenyl)cyclobutanone **15** as a colorless liquid (24 g, 78 % overall yield based on alkene **7**): R_f = 0.19 (5% ethyl acetate, thin layer chromatography); ^1H NMR (CDCl_3) 7.43 (d, J = 8.4 Hz, 2 H), 7.16 (d, J = 8.4 Hz, 2 H), 3.62 (m, 1 H), 3.44 (m, 2 H), 3.17 (m, 2 H). ^{13}C NMR (CDCl_3) 205.52, 142.32, 131.49, 128.07, 119.97, 54.29, 27.69; IR (neat) 2972, 2920, 1747, 1488, 1383, 1229, 1101, 1073, 1009, 818. HR-FAB-MS ($M + H$) calcd. for $\text{C}_{10}\text{H}_9\text{BrO}$: 223.984. Found: 223.982.

3-[2-(4-bromophenyl)ethyl]cyclobutanone (**16**):

The synthesis of this compound was achieved in two steps. First, 2,2-dichloro-3-[2-(4-bromophenyl)ethyl]cyclobutanone **12** was synthesized from alkene **8** by a 2 + 2 cycloaddition of dichloroketene and then the resultant dichloroketone **12** was subjected to reductive dechlorination with zinc dust and acetic acid to obtain cyclobutanone **16**.

A 500 mL three-necked round-bottomed flask equipped with an addition funnel, reflux condenser and argon balloon was charged with 4-(4-bromophenyl)but-1-ene **8** (47.0 mmol, 10 g), and diethyl ether (100 mL) along with freshly prepared Zn-Cu couple (125 mmol, 8.2 g). A solution of trichloroacetyl chloride (71.0 mmol, 7.9 mL) and phosphorus oxychloride (71.0 mmol, 6.6 mL) in ether (20 mL) was placed in the addition funnel. The contents of the addition funnel was added dropwise over a period of 30 minutes. After the addition was over, the reaction mixture was stirred at room temperature for 1 hour, then at reflux for 3 hours under argon atmosphere. After 3 hours of reflux, the reaction mixture was cooled to room temperature and filtered through a pad of Celite. Additional ether (2 x 20 mL) was used to transfer the material. The combined

organics were neutralized with saturated sodium bicarbonate and the product was extracted into ether (200 mL), then washed with water (3 x 20 mL), brine (2 x 20 mL), dried over magnesium sulfate and concentrated on rotary evaporator to obtain a light yellow liquid containing **12**.

The crude reaction mixture containing **12** was taken into glacial acetic acid (30 mL) along with zinc dust (7.7 g, excess). The reaction mixture was stirred at room temperature for 30 minutes and then heated at 120 °C in an oil bath for 2 hours. TLC indicated the disappearance of the starting material. The contents of the flask was cooled to room temperature and filtered through a pad of Celite. Additional ethyl acetate (2 x 20 mL) was used to wash the Celite pad and transfer the material from the flask. The solvent was removed on a rotary evaporator and the residue was dissolved into ethyl acetate (200 mL) and washed with water (2 x 20 mL), brine (1 x 20 mL), dried over magnesium sulfate and concentrated to obtain a light yellow thick liquid (14 g). The crude product was purified by column chromatography on silica gel (30 x 4 cm, 10% ethyl acetate in hexane) to yield **16** as a colorless liquid (10 g, 84% overall yield based on olefin **8**): R_f = 0.26 (10% ethyl acetate in hexane, thin layer chromatography); ^1H NMR (CDCl_3) 7.33 (d, J = 8.03 Hz, 2 H), 6.98 (d, J = 7.49 Hz, 2 H), 3.06 (m, 2 H), 2.58 (m, 4 H), 2.28 (m, 1 H), 1.79 (m, 2 H); ^{13}C NMR (CDCl_3) 207.59, 140.29, 131.38, 130.02, 119.62, 52.32, 37.64, 33.90, 23.25; IR (neat) 2963, 2932, 2855, 1785, 1787, 1487, 1452, 1403, 1384, 1269, 1097, 1071, 1010, 807. Anal. Calcd. for $\text{C}_{12}\text{H}_{13}\text{BrO}$: C, 56.92; H, 5.14. Found: C, 56.92; H, 5.92.

3-[3-(4-bromophenyl)propyl]cyclobutanone (17):

This ketone was prepared similar to 15. A solution of trichloroacetyl chloride (18.8 mmol, 2.08 mL) and phosphorus oxychloride (18.8 mmol, 1.7 mL) in ether (15 mL) was reacted with 5-(4-bromophenyl)pent-1-ene 9 (12.5 mmol, 2.82 g) in 25 mL of ether in the presence of Zn-Cu couple (25 mmol, 2.16 g) to obtain dichlorocyclobutanone 13. The dichloro ketone 13 was subjected to dechlorination in the presence of Zn (3 g, excess) and acetic acid (20 mL) to obtain a light yellow thick liquid that was purified by Kugelrohr distillation to obtain a colorless liquid (2.89 g, 86.5 % yield based on olefin 9). $R_f = 0.35$ (5 % ethyl acetate in hexane, thin layer chromatography); ^1H NMR (CDCl_3) 7.39 (d, $J = 7.75$ Hz, 2 H), 7.04 (d, $J = 7.75$ Hz, 2 H), 3.11 (m, 2 H), 2.64 (m, 4 H), 2.35 (m, 1 H), 1.61 (br s, 4 H); ^{13}C NMR (CDCl_3) 208.04, 140.93, 131.29, 130.01, 119.45, 52.39, 35.62, 34.95, 29.76, 23.66; IR (neat) 2931, 2856, 1788, 1496, 1457, 1400, 1383, 1204, 1107, 1071, 1009, 828, 800. Anal. Calcd. for $\text{C}_{13}\text{H}_{15}\text{BrO}$: C, 58.44; H, 5.66. Found: C, 58.65; H, 5.88.

3-[7-(4-bromophenyl)heptyl]cyclobutanone (18):

This ketone was prepared similar to 15. A solution of trichloroacetyl chloride (16.4 mmol, 1.82 mL) and phosphorus oxychloride (16.4 mmol, 1.52 mL) in ether 10 mL) was reacted with alkene 10 (10.9 mmol, 3.09 g) in ether (25 mL) in the presence of Zn-Cu couple (21.8 mmol, 1.43 g). The dichloroketone 14 (4.98 g, crude) thus obtained was reacted with Zn (1.5 g) in acetic acid (20 mL) to obtain a thick liquid that was purified over a silica gel column using 10 % ethyl acetate in hexane to obtain a colorless liquid

(2.59 g, 73 % yield based on alkene **10**). $R_f = 0.73$ (10 % ethyl acetate in hexane, thin layer chromatography); ^1H NMR (CDCl_3) 7.36 (d, $J = 8.3$ Hz, 2 H), 7.03 (d, $J = 8.24$ Hz, 2 H), 3.11 (m, 2 H), 2.59 (m, 4 H), 2.31 (m, 1 H), 1.56 (br s, 4 H), 1.29 (br s, 8 H); ^{13}C NMR (CDCl_3) 208.31, 141.54, 131.07, 130.01, 119.09, 52.34, 36.14, 35.14, 31.09, 29.18, 28.92, 28.07, 23.66; IR (neat) 2926, 2853, 1784, 1488, 1462, 1402, 1385, 1205, 1111, 1073, 1011, 829, 798. Anal. Calcd. for $\text{C}_{17}\text{H}_{23}\text{BrO}$: C, 63.16; H, 7.17. Found: C, 63.26; H, 7.22.

3-(4-bromophenyl)cyclobutanone ethylene ketal (19**):**

This compound was synthesized as described for ethylene ketal **20**. A solution of 3-(4-bromophenyl)cyclobutanone **15** (78.15 mmol, 17.59 g) in benzene (250 mL) with ethylene glycol (391 mmol, 21.8 mL) was refluxed in the presence of a catalytic amount of *p*-toluenesulfonic acid (3.907 mmol, 0.743 g) to obtain a light yellow liquid. The crude material was purified by Kugelrohr distillation to obtain **19** as a colorless liquid (19.28 g, 82.5 % yield): $R_f = 0.29$ (5% ethyl acetate in hexane, thin layer chromatography); ^1H NMR (CDCl_3) 7.38 (d, $J = 8.44$ Hz, 2 H), 7.11 (d, $J = 8.25$ Hz, 2 H), 3.89 (m, 4 H), 3.24 (m, 1 H), 2.71 (m, 2 H), 2.41 (m, 2 H). ^{13}C NMR (CDCl_3) 143.73, 131.29, 128.37, 119.69, 105.74, 64.23, 63.69, 43.10, 29.44; IR (nujol) 2948, 2840, 1487, 1461, 1377, 1297, 1165, 1072, 1011, 947, 886, 837. Anal. Calcd. for $\text{C}_{12}\text{H}_{13}\text{BrO}_2$: C, 53.53; H, 4.83; Br, 29.74. Found: C, 53.39; H, 4.99; Br, 29.84.

3-[2-(4-bromophenyl)ethyl]cyclobutanone ethylene ketal (20):

A 500 mL three-necked round-bottomed flask equipped with a Dean-Stark trap and reflux condenser was charged with ketone **16** (33 mmol, 8.3 g), ethylene glycol (83 mmol, 4.6 mL) and *p*-toluenesulfonic acid (3.3 mmol, 0.6 g) in benzene (180 mL). The reaction mixture was refluxed at 120 °C in an oil bath for 20 hours and the progress of the reaction was monitored by thin layer chromatography. After 20 hours of reflux, the contents of the flask was cooled to room temperature and to this 3 N NaOH (3 mL) was added to make the reaction mixture basic. The contents of the flask was then transferred to a separatory funnel and washed with water (3 x 20 mL), brine (1 x 20 mL), dried over magnesium sulfate and concentrated on a rotary evaporator to yield colorless thick oil (9.6 g). The product was purified by Kugelrohr distillation to obtain **20** as a colorless thick oil that solidified upon standing at room temperature (8.9 g, 91% yield): R_f = 0.26 (10% ethyl acetate in hexane, thin layer chromatography); ^1H NMR (CDCl_3) 7.30 (d, J = 825 Hz, 2 H), 6.95 (d, J = 8.2 Hz, 2 H), 3.77 (s, 4 H), 2.43 (t, J = 7.38 Hz, 2 H), 2.33 (m, 1 H), 1.90 (m, 4 H), 1.69 (m, 2 H). ^{13}C NMR (CDCl_3) 140.99, 131.19, 130.04, 119.30, 106.48, 63.89, 63.45, 40.92, 37.88, 33.42, 24.46; IR (neat) 2975, 2926, 2853, 1485, 1452, 1409, 1293, 1176, 1115, 1011, 946, 808. Anal. Calcd. for $\text{C}_{14}\text{H}_{17}\text{BrO}_2$: C, 56.57; H, 5.72. Found: C, 56.81; H, 5.90.

3-[3-(4-bromophenyl)propyl]cyclobutanone ethylene ketal (21):

This ketal was prepared similar to **20**. A solution of **17** (10.5 mmol, 2.8 g) and ethylene glycol (105 mmol, 5.85 mL) in benzene (50 mL) was refluxed in the presence of

p-toluenesulfonic acid (1.05 mmol, 0.199 g). Water was azeotropically removed by a Dean-Stark trap to obtain the desired compound. The product was purified by Kugelrohr distillation to obtain a colorless liquid (2.96 g, 90.95 % yield). R_f = 0.44 (5 % ethyl acetate in hexane, thin layer chromatography); ^1H NMR (CDCl_3) 7.34 (d, J = 8.23 Hz, 2 H), 6.99 (d, J = 8.19 Hz, 2 H), 3.83 (s, 4 H), 2.53 (t, J = 6.85 Hz, 2 H), 2.36 (m, 2 H), 1.98 (m, 1 H), 1.87 (m, 2 H), 1.45 (br s, 4 H); ^{13}C NMR (CDCl_3) 141.47, 131.28, 130.11, 119.34, 106.66, 63.96, 63.51, 41.05, 35.91, 35.14, 29.37, 24.91; IR (neat) 2976, 2928, 2855, 1486, 1408, 1292, 1176, 1117, 1069, 1012, 946, 859, 825, 792. Anal. Calcd. for $\text{C}_{15}\text{H}_{19}\text{BrO}_2$: C, 57.89; H, 6.15. Found: C, 58.02; H, 6.48.

3-[7-(4-bromophenyl)heptyl]cyclobutanone ethylene ketal (22):

This ketal was prepared similar to 20. The ketone 18 (7.23 mmol, 2.34 g), ethylene glycol (36.0 mmol, 2 mL), *p*-toluenesulfonic acid (0.723 mmol, 0.137 g) and benzene (35 mL) were used. The crude material was purified on a silica gel column to obtain 22 as a thick liquid (2.42 g, 90.9 % yield). R_f = 0.67 (10 % ethyl acetate in hexane, thin layer chromatography); ^1H NMR (CDCl_3) 7.36 (d, J = 8.29 Hz, 2 H), 7.02 (d, J = 8.27 Hz, 2 H), 3.86 (s, 4 H), 2.53 (t, J = 7.43 Hz, 2 H), 2.39 (m, 2 H), 1.94 (m, 3 H), 1.56 (m, 2 H), 1.42 (br s, 2 H), 1.28 (m, 8 H); ^{13}C NMR (CDCl_3) 141.65, 131.13, 130.04, 119.13, 106.66, 63.84, 63.37, 40.98, 36.37, 35.22, 31.18, 29.30, 29.03, 27.57, 24.97; IR (neat) 2976, 2926, 2853, 1487, 1463, 1409, 1346, 1294, 1178, 1071, 1012, 946, 798. Anal. Calcd. for $\text{C}_{19}\text{H}_{27}\text{BrO}_2$: C, 61.96; H, 7.66. Found: C, 62.23; H, 7.44.

3-(4-boronophenyl)cyclobutanone ethylene ketal (23):

This compound was synthesized similar to that of 3-[2-(4-boronophenyl)ethyl]-cyclobutanone ethylene ketal **24**. The aryl bromide **19** (14.87 mmol, 4.0 g), THF (80 mL), butyllithium (17.84 mmol, 11.15 mL of 1.6 M solution in hexane) and triisopropyl borate (17.84 mmol, 4.12 mL) was used. The compound was purified on a silica gel column (22 x 1.5 cm, 30 % ethyl acetate in hexane) to obtain **23** as a white solid (2.89 g, 83 % yield). R_f = 0.46 (50% ethyl acetate in hexane, thin layer chromatography); mp; 158-160 °C; ^1H NMR (CDCl_3) 8.17 (d, J = 7.81 Hz, 2 H), 7.40 (d, J = 7.81 Hz, 2 H), 3.96 (m, 4 H), 3.41 (m, 1 H), 2.78 (m, 2 H), 2.59 (m, 2 H). ^{13}C NMR (CDCl_3) 149.63, 135.83, 126.34, 106.01, 64.35, 63.78, 43.12, 30.34; IR (nujol) 3370, 2954, 2845, 1607, 1480, 1375, 1344, 1293, 1081, 1018, 949, 895, 849. Anal. Calcd. for $\text{C}_{12}\text{H}_{15}\text{BO}_4$: C, 61.58; H, 6.46; B, 4.62. Found: C, 63.40; H, 6.56; B, 4.62.

3-[2-(4-boronophenyl)ethyl]cyclobutanone ethylene ketal (24):

A 250 mL three-necked round-bottomed flask equipped with argon balloon was charged with aryl bromide **20** (6.7 mmol, 2.0 g) in anhydrous tetrahydrofuran (40 mL). The contents of the flask were cooled to -78 °C in a dry ice-acetone bath and butyllithium (8.0 mmol, 5.0 mL of 1.6 M solution in hexane) was added dropwise via a syringe. The resultant yellow solution was stirred at -78 °C for 30 minutes and triisopropyl borate (8.0 mmol, 1.9 mL) was added via a syringe. The reaction mixture was allowed to stir overnight (during this time the bath temperature was allowed to come to room temperature on its own). After overnight stirring, the contents of the flask was cooled to

0 °C and then quenched with dilute sulfuric acid (6 mL of 2 N H₂SO₄). The reaction mixture was stirred for an additional 30 minutes at 0 °C and then transferred to a separatory funnel. The product was extracted into ether (100 mL) and the organic layer was washed with water (2 x 20 mL), brine (1 x 20 mL), dried over magnesium sulfate and concentrated on rotary evaporator to obtain a colorless thick liquid (1.9 g). The compound was purified by column chromatography using silica gel (25 x 2 cm, 30% ethyl acetate in hexane) to obtain **24** as a white solid (1.5 g, 87% yield): R_f = 0.56 (50% ethyl acetate in hexane, thin layer chromatography); mp; 78-80 °C; ¹H NMR (CDCl₃) 8.06 (d, J = 7.5 Hz, 2 H), 7.23 (d, J = 7.25 Hz, 2 H), 3.81 (s, 4 H), 2.53 (m, 2 H), 2.36 (m, 1 H), 1.95 (m, 4 H), 1.74 (m, 2 H). ¹³C NMR (CDCl₃) 147.14, 135.74, 128.12, 106.66, 63.96, 63.50, 40.99, 37.96, 34.47, 24.71; IR (nujol) 3370, 2954, 2851, 1609, 1461, 1409, 1377, 1343, 1293, 1175, 1069, 786, 718. Anal. Calcd. for C₁₄H₁₉BO₄: C, 64.51; H, 7.31; B, 4.12. Found: C, 63.47; H, 7.08; B, 4.26.

3-[3-(4-boronophenyl)propyl]cyclobutanone ethylene ketal (25):

This compound was prepared similar to **24**. A solution of **21** (8.39 mmol, 2.6 g) in THF (50 mL) was treated with butyllithium (10.1 mmol, 6.29 mL of 1.6 M solution in hexane) followed by triisopropyl borate (10.1 mmol, 2.32 mL) to obtain the product. The compound was purified on a silica gel column (using 50 % ethyl acetate in hexane) to obtain **25** as a white solid (1.76 g, 76 % yield). R_f = 0.51 (50 % ethyl acetate in hexane, thin layer chromatography); mp; 128-130 °C; ¹H NMR (DMSO-d₆) 7.99 (s, 2 H, disappears by the addition of D₂O), 7.74 (d, J = 7.82 Hz, 2 H), 7.2 (d, J = 7.76 Hz, 2 H),

3.31 (s, 4 H), 2.03 (m, 2 H), 1.86 (m, 1 H), 1.45 (m, 4 H), 1.24 (m, 2 H); ^{13}C NMR (DMSO- d_6) 144.22, 134.21, 127.41, 105.88, 63.37, 62.91, 40.71, 35.56, 35.10, 29.01, 24.46; IR (nujol) 3203, 3239, 2925, 2855, 1609, 1460, 1409, 1375, 1347, 1298, 1178, 1068, 1020, 948, 860, 838, 722, 698. Anal. Calcd. for $\text{C}_{15}\text{H}_{21}\text{BO}_4$: C, 65.24; H, 7.67. Found: C, 69.45; H, 7.60. FAB-MS ($\text{M} + \text{H}$; obtained in glycerol matrix) calcd. for $\text{C}_{18}\text{H}_{26}\text{BO}_4$: 333.188, Found: 333.189.

3-[7-(4-boronophenyl)heptyl]cyclobutanone ethylene ketal (26):

This compound was prepared similar to 24. A solution of 22 (5.94 mmol, 2.19 g) in THF (50 mL) was treated with butyllithium (7.12 mmol, 4.46 mL of 1.6 M solution in hexane) followed by triisopropyl borate (7.12 mmol, 1.64 mL) to obtain the product. The product was purified on a silica gel column (50 % ethyl acetate in hexane) to obtain 26 as a white solid (1.58 g, 80 % yield). R_f = 0.61 (50 % ethyl acetate in hexane, thin layer chromatography); mp; 76-80 °C; ^1H NMR (DMSO- d_6) 7.91 (s, 2 H disappears by the addition of D_2O), 7.68 (d, J = 7.68 Hz, 2 H), 7.12 (d, J = 7.71 Hz, 2 H), 3.75 (s, 4 H), 2.53 (t, J = 7.29 Hz, 2 H), 2.28 (m, 2 H), 1.81 (m, 3 H), 1.53 (br s, 2 H), 1.34 (m, 2 H), 1.23 (br s, 8 H); ^{13}C NMR (DMSO- d_6) 144.33, 134.21, 127.39, 105.91, 63.37, 62.89, 40.74, 35.95, 35.26, 30.89, 28.89, 28.65, 27.22, 24.61; IR (nujol) 3550, 3382, 2953, 2924, 2853, 1611, 1463, 1406, 1378, 1342, 1297, 1181, 1161, 1139, 1091, 1025, 993, 848, 782. Anal. Calcd. for $\text{C}_{19}\text{H}_{29}\text{BO}_4$: C, 68.69; H, 8.8. Found: C, 69.00; H, 8.50. FAB-MS ($\text{M} + \text{H}$; obtained in glycerol matrix) calcd. for $\text{C}_{22}\text{H}_{34}\text{O}_5\text{B}$: 389.250. Found 389.253.

3-(4-boronophenyl)cyclobutanone (27):

This compound was synthesized similar to that of cyclobutanone **28**. The boronic acid ketal **23** (12.83 mmol, 3.0 g), methanol (30 mL) and concentrated HCl (0.5 mL) were used. The product was purified on a silica gel column (20 x 2 cm, 30 % ethyl acetate in hexane) to obtain **27** as a white solid (1.92 g, 79 % yield). R_f = 0.46 (50% ethyl acetate in hexane, thin layer chromatography); mp; 94-98 °C; ^1H NMR (CDCl_3) 7.63 (d, J = 7.29 Hz, 2 H), 7.20 (d, J = 7.52 Hz, 2 H), 3.56 (m, 1 H), 3.30 (m, 2 H), 3.08 (m, 2 H). ^{13}C NMR (CDCl_3) 208.81, 147.25, 135.29, 126.76, 55.19, 29.57; IR (nujol) 3358, 2941, 2855, 1768, 1601, 1461, 1375, 1339, 1149, 1094, 1084, 749. Anal. Calcd. For $\text{C}_{10}\text{H}_{11}\text{BO}_3$: C, 63.21; H, 5.84; B, 5.69. Found: C, 63.50; H, 6.05; B, 6.12.

3-[2-(4-boronophenyl)ethyl]cyclobutanone (28):

A 100 mL round bottomed flask was charged with boronic acid ketal **24** (6.9 mmol, 1.8 g) in methanol (30 mL) along with concentrated hydrochloric acid (0.5 mL). The contents of the flask was stirred at room temperature overnight. TLC indicated the disappearance of the starting ketal. The solvent was removed on a rotary evaporator and the residue was taken into ether (100 mL), washed with water (2 x 20 mL) and brine (1 x 20 mL). The organic layer was dried over magnesium sulfate and concentrated on rotary evaporator to yield a faint yellow viscous material (1.7 g). This crude material was purified on a silica gel column (20 x 2 cm, 30% ethyl acetate in hexane) to obtain **28** as a white solid (1.2 g, 78% yield): R_f = 0.56 (30% ethyl acetate in hexane, thin layer chromatography); mp; 93-95 °C; ^1H NMR (CDCl_3) 8.12 (d, J = 7.75 Hz, 2 H), 7.27 (d, J =

7.76 Hz, 2 H), 3.09 (m, 2 H), 2.69 (m, 4 H), 2.35 (m, 1 H), 1.93 (m, 2 H). ^{13}C NMR (CDCl_3) 208.07, 146.37, 135.86, 128.07, 52.43, 37.69, 34.91, 23.45; IR (nujol) 3340, 2957, 2852, 1761, 1608, 1460, 1407, 1376, 1347, 1278, 1167, 1042, 817, 720. Anal. Calcd. For $\text{C}_{12}\text{H}_{15}\text{BO}_3$: C, 66.10; H, 6.93; B, 4.96. Found: C, 65.95; H, 6.94; B, 5.12.

3-[3-(4-boronophenyl)propyl]cyclobutanone (29):

This compound was synthesized similar to compound 28. The ethylene ketal 25 (3.62 mmol, 1.0g), methanol (30 mL) and concentrated HCl (0.5 mL) were used. The product was purified on a silica gel column (using 50 % ethyl acetate in hexane) to obtain 29 as a white solid (0.69 g, 83 % yield). R_f = 0.52 (50 % ethyl acetate in hexane, thin layer chromatography); mp: 105-110 °C; ^1H NMR (DMSO-d_6) 8.1 (s, 2 H disappears by adding D_2O), 7.87 (d, J = 7.79 Hz, 2 H), 7.33 (d, J = 7.77 Hz, 2 H), 3.25 (m, 2 H), 2.75 (m, 4 H), 2.49 (m, 1 H), 1.72 (m, 4 H); ^{13}C NMR (DMSO-d_6) 208.19, 144.12, 134.23, 127.41, 51.99, 35.13, 35.01, 29.48, 23.15; IR (nujol) 3364, 2925, 2855, 1761, 1609, 1457, 1413, 1375, 1345, 1167, 1121, 1042, 1017, 799, 722. Anal. Calcd. for $\text{C}_{13}\text{H}_{17}\text{BO}_3$: C, 67.28; H, 7.38; B, 4.66. Found: C, 66.90; H, 7.90; B, 4.64.

3-[7-(4-boronophenyl)heptyl]cyclobutanone (30):

This compound was synthesized similar to compound 28. The ethylene ketal 26 (5.8 mmol, 1.94 g), methanol (20 mL) and concentrated HCl (0.5 mL) were used. The product was purified on silica gel column (50 % ethyl acetate in hexane) to obtain 30 as a white solid (1.26 g, 75 % yield). R_f = 0.57 (50 % ethyl acetate in hexane, thin layer

chromatography); mp; 70-76 °C; ^1H NMR (CDCl_3) 8.13 (d, $J = 7.34$ Hz, 2 H), 7.30 (d, $J = 7.51$ Hz, 2 H), 3.10 (m, 2 H), 2.64 (m, 4 H), 2.32 (m, 1 H), 1.66 (br s, 2 H), 1.55 (m, 2 H), 1.32 (br s, 8 H); ^{13}C NMR (CDCl_3) 208.71, 147.69, 135.63, 128.05, 52.44, 36.24, 31.16, 29.27, 29.15, 28.16, 23.78. IR (nujol) 3375, 3304, 2925, 2853, 1765, 1611, 1461, 1411, 1375, 1349, 1171, 1128, 1085, 1043, 1018, 831, 796, 724. Anal. Calcd. for $\text{C}_{17}\text{H}_{25}\text{BO}_3$: C, 70.85; H, 8.74; B, 3.75. Found: C, 72.50; H, 7.65; B, 4.03. FAB-MS ($M + H$; obtained in glycerol matrix) calcd. for $\text{C}_{20}\text{H}_{30}\text{BO}_4$: 345.224, Found: 345.223.

Hydantoin of 3-(4-boronophenyl)cyclobutanone (31):

This compound was synthesized similar to hydantoin 32. The cyclobutanone 27 (3.70 mmol, 0.700 g), aqueous ethanol (50 %, 12.0 mL), potassium cyanide (7.4 mmol, 0.48 g) and ammonium carbonate (18.5 mmol, 1.78 g) were used. The product was purified by recrystallization from water (containing 5 % ethanol) to yield 31 as a faint yellow solid (0.748 g, 77.67 % yield). $R_f = 0.65$ (10% methanol in methylene chloride, thin layer chromatography); mp; 248-250 °C; ^1H NMR ($\text{DMSO}-d_6$) 7.59 (d, $J = 8.9$ Hz, 2 H), 7.10 (d, $J = 7.6$ Hz, 2 H), 2.62 (m, 2 H), 2.22 (m, 2 H), benzylic protons (2 H) are covered up by H_2O of DMSO. ^{13}C NMR ($\text{DMSO}-d_6$) 178.87, 155.98, 146.24, 134.35, 125.65, 57.32, 30.79; IR (nujol) 3313, 2954, 2928, 2852, 1718, 1614, 1460, 1374, 1311, 1132, 1018, 768. Anal. Calcd. for $\text{C}_{12}\text{H}_{13}\text{BN}_2\text{O}_4$: C, 55.42; H, 5.04; N, 10.77. Found: C, 53.85; H, 5.06; N, 10.10. FAB-MS ($M + H$; obtained in glycerol matrix) calcd. for $\text{C}_{15}\text{H}_{17}\text{O}_5\text{BN}_2$: 317.1. Found 317.1.

Hydantoin of 3-[2-(4-boronophenyl)ethyl]cyclobutanone (32):

A 15 mL Ace pressure tube was charged with 3-[2-(4-boronophenyl)ethyl]-cyclobutanone **28** (1.1 mmol, 0.28 g), aqueous ethanol (50%, 7.0 mL), potassium cyanide (3.0 mmol, 0.19 g) and ammonium carbonate (5.0 mmol, 0.48 g). The reaction vessel was sealed and heated at 60 °C in an oil bath for 4 hours. A faint yellow precipitate formed. The reaction vessel was cooled to room temperature and the cap was carefully unscrewed in the fume hood. The reaction mixture was acidified by dilute hydrochloric acid. The solvent was removed on a rotary evaporator and the solid obtained was recrystallized by water (containing 5% ethanol) to yield **32** as a faint yellow solid (0.23 g, 62% yield); $R_f = 0.39$ (5% methanol in methylene chloride, thin layer chromatography); mp; 224-226 °C (with decomposition); ^1H NMR (DMSO- d_6) 7.68 (d, $J = 7.65$ Hz, 2 H), 7.12 (d, $J = 7.75$ Hz, 2 H), 2.45 (m, 4 H), 2.19 (m, 1 H), 1.86 (m, 2 H), 1.65 (m, 2 H). ^{13}C NMR (DMSO- d_6) 178.78, 155.89, 143.70, 134.18, 127.32, 57.76, 38.11, 32.74, 25.89; IR (nujol) 3296, 2954, 2851, 1738, 1713, 1607, 1460, 1375, 1327, 1133, 1022, 768. Anal. Calcd. for $\text{C}_{14}\text{H}_{17}\text{BN}_2\text{O}_4$: C, 58.36; H, 5.96; N, 9.72; B, 3.75. Found: C, 57.54; H, 6.15; N, 9.17; B, 1.49.

Hydantoin of 3-[3-(4-boronophenyl)propyl]cyclobutanone (33):

This compound was synthesized similar to hydantoin **32**. The cyclobutanone **29** (3.714 mmol, 0.862 g), aqueous ethanol (50 %, 12.0 mL) , potassium cyanide (7.4 mmol, 0.483 g) and ammonium carbonate (18.57 mmol, 1.784 g) were used. The product was purified on a silica gel column (10 % methanol in methylene chloride) to obtain **33** as a

white solid (0.744 g, 66.4 % yield). R_f = 0.46 (5 % methanol in methylene chloride, thin layer chromatography); mp; 220 -224 °C; ^1H NMR (DMSO- d_6) 7.89 (s, 2 H disappears by the addition of D_2O), 7.88 (d, J = 7.71 Hz, 2 H), 7.36 (d, J = 7.69 Hz, 2 H), 2.68 (m, 3 H), 2.50-2.29 (m, 3 H), 2.08 (m, 1 H), 1.62 (m, 4 H); ^{13}C NMR (DMSO- d_6) 178.99, 156.04, 144.16, 134.29, 127.48, 57.88, 38.28, 37.07, 35.14, 28.25, 26.32; IR (nujol) 3168, 3113, 3045, 2925, 2855, 1776, 1733, 1609, 1461, 1411, 1377, 1315, 1184, 1132, 1109, 1021, 804, 746, 723. Anal. Calcd. for $\text{C}_{15}\text{H}_{19}\text{BN}_2\text{O}_4$: C, 59.63; H, 6.34; N, 9.27; B, 3.58. Found: C, 59.67; H, 6.30; N, 8.67; B, 3.31.

Hydantoin of 3-[7-(4-boronophenyl)heptyl]cyclobutanone (34):

This compound was synthesized similar to hydantoin 32. The cyclobutanone 30 (3.67 mmol, 1.057 g), aqueous ethanol (50 %, 12.0 mL), potassium cyanide (3.67 mmol, 0.239 g) and ammonium carbonate (9.18 mmol, 0.882 g) were used. The product was purified on a silica gel column (10 % methanol in methylene chloride) to obtain 34 as a white solid (0.261 g, 40 % yield); R_f = 0.72 (10 % methanol in methylene chloride, thin layer chromatography); mp; 206-208 °C (with decomposition); ^1H NMR (DMSO- d_6 with 10 % D_2O) 8.13 (d, J = 7.71 Hz, 2 H), 7.59 (d, J = 7.77 Hz, 2 H), 2.99 (t, J = 6.5 Hz, 2 H), 2.88 (m, 2 H), 2.73-2.55 (m, 2 H), 2.32 (m, 1 H), 1.97 (br s, 2 H), 1.79 (m, 2 H), 1.67 (br s, 8 H); ^{13}C NMR (DMSO- d_6) 178.84, 155.92, 144.25, 134.16, 127.33, 57.76, 38.25, 37.02, 36.48, 35.22, 28.85, 28.62, 26.43, 26.31; IR (nujol) 3339, 3169, 3114, 3058, 2922, 2853, 1779, 1733, 1611, 1515, 1461, 1411, 1377, 1317, 1253, 1183, 1124, 1021, 808, 746, 723. Anal. Calcd. for $\text{C}_{19}\text{H}_{27}\text{BN}_2\text{O}_4$: C, 63.70; H, 7.60; B, 3.02; N, 7.82. Found: C,

65.88; H, 7.65; B, 2.92; N, 7.46. FAB-MS ($M + H$; obtained in glycerol matrix) calcd. for $C_{22}H_{32}BN_2O_5$: 415.241. Found: 415.241.

1-Amino-3-(4-boronophenyl)cyclobutanecarboxylic acid (35):

This compound was synthesized similar to amino acid 36. The hydantoin 31 (1.54 mmol, 0.40 g) and sodium hydroxide (4.0 mL of 2 *N* solution) were used. The product was purified on a reverse phase column (2 x 16 cm, using 50 % methanol in water with 2 % acetic acid) to obtain 35 as a white solid (0.188 g, 52 % yield). R_f = 0.57 (mixture of isopropanol, water and acetic acid in a volume ratio of 10:0.8:0.5); mp; compound turns brown (without melting) at 262-265 °C; ^1H NMR (DCI) 7.54 (d, J = 6.14 Hz, 2 H), 7.16 (d, J = 6.03 Hz, 2 H), 3.69 (m, 1 H), 2.85 (m, 2 H), 2.42 (m, 2 H). ^{13}C NMR (DCI) 174.91, 147.05, 134.89, 126.74, 54.46, 37.79, 32.85; IR (nujol) 3361, 2924, 2852, 1741, 1607, 1460, 1375, 1348, 1193, 844, 811, 744. HR-FAB-MS ($M + H$; obtained in glycerol matrix) Calcd. for $C_{14}H_{19}BNO_5$: 292.136, Found 292.136.

1-Amino-3-[2-(4-boronophenyl)ethyl]cyclobutanecarboxylic acid (36):

The hydantoin of 3-[2-(4-boronophenyl)ethyl]cyclobutanone 32 (0.63 mmol, 0.18 g) was placed in a 15 mL Ace pressure tube along with a solution of sodium hydroxide (4.0 mL of 2 *N* NaOH). The contents of the tube were sealed and then heated at 160 °C in an oil bath for 40 minutes. It was then cooled to room temperature and opened carefully. TLC indicated the disappearance of the starting hydantoin. The reaction mixture was neutralized by dilute hydrochloric acid, and the volatile solvents were removed under

reduced pressure using a rotary evaporator. The solid thus obtained was purified on a silica gel column (17 x 2 cm, using a mixture of isopropanol, water and acetic acid in a volume ratio of 2: 0.1: 0.1 mL) to obtain **36** as a white solid (0.18 g, 69% yield): $R_f = 0.43$ (mixture of isopropanol, water and acetic acid in a volume ratio of 2: 0.1: 0.1 mL); mp; compound turns brown (without melting) at 224-228 °C; ^1H NMR (DCl) 6.96 (d, $J = 7.63$ Hz, 2 H), 6.56 (d, $J = 7.77$ Hz, 2 H), 1.96 (m, 2 H), 1.84 (m, 2 H), 1.72 (m, 1 H), 1.38 (m, 2 H), 1.09 (m, 2 H); ^{13}C NMR (DCL) 174.77, 146.35, 134.72, 129.37, 129.04, 54.65, 38.43, 36.68, 33.22, 28.24; IR (nujol) 3404, 2958, 2852, 1741, 1608, 1530, 1461, 1375, 1207, 1176, 830, 721. HR-FAB-MS ($M + H$; obtained in glycerol matrix) calcd. for $\text{C}_{16}\text{H}_{23}\text{BNO}_5$: 320.167, Found 320.167.

1-Amino-3-[3-(4-boronophenyl)propyl]cyclobutanecarboxylic acid (37):

This compound was synthesized similar to amino acid **36**. The hydantoin **33** (0.662 mmol, 0.200 g) and sodium hydroxide (2 mL of 2 *N* solution) were used. The product was purified using a reverse phase column (2 x 16 cm, using 50 % methanol in water with 2 % acetic acid) to obtain **37** as a white solid (0.112 g, 61 % yield). $R_f = 0.46$ (mixture of acetonitrile, methanol and water in a volume ratio of 10: 2: 1.5 mL); mp; compound turns brown (without melting) at 228-232 °C; ^1H NMR (DMSO- d_6 with 5 % D_2O) 7.84 (d, $J = 7.5$ Hz, 2 H), 7.39 (d, $J = 7.5$ Hz, 2 H), 2.90-2.38 (m, 6 H), 2.22 (m, 1 H), 1.63 (br s, 4 H); ^{13}C NMR (CD_3OD) 176.31, 145.79, 134.93, 128.67, 57.24, 39.29, 38.02, 36.67, 29.53, 26.47; IR (nujol) 3352, 2922, 2855, 1720, 1610, 1459, 1376, 1253, 1187, 1118, 979, 828, 723. HR-FAB-MS ($M + H$; obtained in glycerol matrix) calcd. for

$C_{17}H_{25}BNO_5$: 334.183. Found: 334.182.

1-Amino-3-[7-(4-boronophenyl)heptyl]cyclobutanecarboxylic acid (38):

This compound was synthesized similar to amino acid 36. The hydantoin 34 (1.03 mmol, 0.369 g) and sodium hydroxide (7 mL of 3 N solution) were used. The product was purified on a reverse phase column (2 x 16 cm, using 50 % methanol in water with 2 % acetic acid) to obtain 38 as a white solid (0.288 g, 84 % yield). R_f = 0.61 (mixture of acetonitrile, methanol and water in a volume ratio of 10: 2: 0.5 mL, thin layer chromatography); mp; compound softens and turns brown (without clear melting) at 204-208 °C; ^1H NMR (CD_3OD with 5 % D_2O) 7.05 (d, J = 7.05 Hz, 2 H), 6.88 (d, J = 7.43 Hz, 2 H), 2.33 (m, 4 H), 2.06 (m, 2 H), 1.61 (br s, 1 H), 1.33 (br s, 2 H), 1.19 (br s, 2 H), 1.05 (br s, 8 H); ^{13}C NMR (CD_3OD) 177.36, 144.02, 134.16, 128.21, 55.93, 38.32, 36.93, 36.76, 32.51, 30.52, 30.25, 29.62, 27.87; IR (nujol) 3504, 3147, 2923, 2853, 1703, 1636, 1611, 1582, 1529, 1460, 1376, 1281, 1248, 1172, 1099, 989, 835, 723. HR-FAB-MS ($M + H$; obtained in glycerol matrix) calcd. for $C_{21}H_{33}BNO_5$: 390.246. Found: 390.245.

Experimental for Eq 3-10:

Pinacol ester of 3-[2-(4-boronophenyl)ethyl]cyclobutanone (39):

A 50 mL round-bottomed flask with a sidearm was equipped with a reflux condenser and a stir bar. It was then flame dried under nitrogen flow and allowed to cool under nitrogen flow. Next the apparatus was transferred to a nitrogen filled glove bag where it was charged with $PdCl_2(dppf)$ (0.08 mmol, 0.065 g), bis(pinacolato)diboron (1.1

mmol, 0.279 g). Next the glassware was removed from the glove bag and charged with dry potassium acetate (3.0 mmol, 0.294 g). Then dry DMSO (6 mL) was added to the mixture. Lastly, the 3-[2-(4-bromophenyl)ethyl]cyclobutanone **16** (1.0 mmol, 0.253 g) was added and the mixture was heated at 90 °C for 2 hours. When the reaction was over, the mixture was taken into 60 mL of ethyl acetate and washed with water (3 x 20 mL) and brine (1 x 20 mL). The organic layer was dried over magnesium sulfate, filtered and the solvent removed on a rotary evaporator. The crude material was purified using silica gel column chromatography (20 % ethyl acetate in hexanes) to yield **39** (0.234 g, 78 % yield) as a slight yellowish solid. R_f = 0.64 (20% ethyl acetate in hexanes, thin layer chromatography) ^1H NMR (CDCl_3) 7.74 (d, J = 7.79 Hz, 2 H), 7.19 (d, J = 7.78 Hz, 2 H), 3.11 (m, 2 H), 2.67 (m, 4 H), 2.37 (m, 1 H), 1.91 (m, 2 H), 1.33 (s, 12 H). ^{13}C NMR (CDCl_3) 207.86, 144.69, 134.89, 127.73, 63.56, 63.36, 52.32, 37.67, 34.67, 24.87, 24.78, 23.29. HR-FAB-MS ($M + H$); calcd. for $\text{C}_{18}\text{H}_{25}\text{BO}_3$; 301.198, Found: 301.199.

3-[2-(4-boronophenyl)ethyl]cyclobutanone (**28**):

A 50 mL round bottomed flask with a sidearm was flame dried under nitrogen flow. The flask was then allowed to cool under nitrogen flow. Next the boronic ester/ketone **39** (0.668 mmol, .200 g) was added under a nitrogen flow. Then 10 mL of anhydrous methylene chloride was added via syringe to the boronic ester/ketone **39**. Then the mixture was cooled to -78 °C in a dry ice/acetone bath. Then BCl_3 (12 mL of 1.0 M solution in hexanes) was added slowly via syringe. The mixture was allowed to stir at -78 °C for 1 hour, then at room temperature overnight. The reaction was again cooled to -78

°C and 10 mL of methanol was added carefully. The mixture was allowed to warm to room temperature. The solvent was removed on a rotary evaporator. The resultant material was taken into ethyl acetate and washed with water and brine. The solvent was removed again on rotary evaporator. The product was purified on a silica gel column (20 % ethyl acetate in hexanes to elute out the unreacted boronic ester, then with 100 % ethyl acetate to elute out the boronic acid) to yield **28** (0.058 g, 39.8 % yield) as a yellowish solid. R_f = 0.56 (30% ethyl acetate in hexane, thin layer chromatography); ^1H NMR (CDCl_3) 8.16 (d, J = 7.57 Hz, 2 H), 7.33 (d, J = 7.64 Hz, 2 H), 3.17 (m, 2 H), 2.73 (m, 4 H), 2.41 (m, 1 H), 1.98 (m, 2 H). ^{13}C NMR (CDCl_3) 208.04, 146.39, 135.69, 126.10, 52.46, 37.73, 34.94, 23.48.

Experimental for Eq 3-12:

Hydantoin of 3-[2-(4-bromophenyl)ethyl]cyclobutanone (41):

A 500 mL round bottomed flask was equipped with a stir bar and reflux condenser. Next 3-[2-(4-bromophenyl)ethyl]cyclobutanone **16** (65.5 mmol, 16.58 g) was placed in the flask. Next, 220 mL of 50% ethanol was added, then potassium cyanide (163.8 mmol, 10.66 g), and ammonium carbonate (327.5 mmol, 31.4 g) were added and the mixture was refluxed for 5 hours. Then an additional 5 g of ammonium carbonate was added and refluxed for another hour (this was repeated two more times). Next the mixture was transferred to a pressure tube vessel with an additional 8 g of ammonium carbonate and it was heated at 90 °C for 1 hour. The reaction was stopped and allowed to cool, then the cap was carefully unscrewed. The mixture was poured into a large beaker,

where it was diluted with 100 mL of water. This mixture was then placed in the cold room overnight, where a solid precipitated. The solid was filtered and washed with water. The solid was then taken into 200 mL of 95% ethanol and heated to boiling. The hot solution was filtered. A brown solid started to form on the filter paper. The material was allowed to air dry overnight. Meanwhile the filtrate had no crystals forming, so the solvent was removed yielding a brown solid that was washed with ethyl acetate. A white solid precipitated out of the solution. This solid was filtered to give a pure product. It should be noted that the brown solid that formed on the filter paper after heating in ethanol is also product, although not as pure as the white solid obtained later. The reaction yielded 5.54 g (26.2%). $R_f = 0.56$ (6 % methanol in methylene chloride, thin layer chromatography); mp 240-242 °C (with decomposition); ^1H NMR (CD_3OD) 7.33 (d, $J = 8.2$ Hz, 2 H), 7.00 (d, $J = 8.17$ Hz, 2 H), 2.52 (m, 4 H), 2.33 (m, 1 H), 1.87 (m, 2 H), 1.68 (m, 2 H); ^{13}C NMR (CD_3OD) 180.23, 157.72, 141.42, 131.76, 130.60, 119.70, 59.28, 39.08, 37.35, 33.23, 27.33. Anal. Calcd. for $\text{C}_{14}\text{H}_{15}\text{BrN}_2\text{O}_2$; C, 52.03; H, 4.68; N, 8.67. Found: C, 52.27; H, 4.78; N, 8.60.

1-Amino-3-[2-(4-bromophenyl)ethyl]cyclobutanecarboxylic acid (42):

A pressure tube was charged with the hydantoin of 3-[2-(4-bromophenyl)ethyl]-cyclobutanone **41** (1.55 mmol, 0.502 g) and sodium hydroxide (10 mL of 3 M solution) and heated at 200 °C for 1 hour. The reaction was allowed to cool and the cap was carefully unscrewed. Next the mixture was neutralized with 10 % HCl. Next the mixture was filtered and the solid material was heated in methanol and filtered again. The filtrate

was taken into a round-bottomed flask and the solvent was removed to yield a white solid (.210 g, 45.3 % yield). R_f = 0.83 (mixture of isopropyl alcohol, water and acetic acid in the ratio of 10: 0.5: 0.5 mL, thin layer chromatography); ^1H NMR (CD_3OD) 7.38 (d, J = 8.25 Hz, 2 H), 7.12 (d, J = 8.26 Hz, 2 H), 2.55 (m, 5 H), 1.84 (m 4 H); ^{13}C NMR ($\text{DMSO}-d_6$) 172.66, 141.74, 131.04, 130.60, 118.59, 53.55, 37.76, 34.93, 32.00, 27.68; ES+ ($M + \text{H}$) calcd. for $\text{C}_{13}\text{H}_{16}\text{NO}_2\text{Br}$: 298.2. Found: 298.8.

1-(*N*-(*t*-butoxycarbonyl)amino-3-[2-(4-bromophenyl)ethyl]cyclobutanecarboxylic acid (43):

A 50 mL round-bottomed flask with a reflux condenser was charged with 25 mL of 10 % triethyl amine in methanol, di-*t*-butyldicarbonate $[(\text{Boc})_2\text{O}]$ (8.8 mmol, 1.92 g) and 1-Amino-3-[2-(4-bromophenyl)ethyl]cyclobutanecarboxylic acid **42** (4.4 mmol, 1.303 g). The mixture was heated at 50 °C for 1 hour. The amino acid was insoluble initially, but after 30 minutes the solution cleared indicating that the reaction was complete. The solvent was removed on rotary evaporator. The resultant material was taken into methylene chloride (100 mL) and washed with 10 % HCl (2 x 20 mL), water (1 x 20 mL) and brine (1 x 20 mL). The organic layer was dried over magnesium sulfate, filtered and the solvent removed on a rotary evaporator. The product was purified on a silica gel column (using methylene chloride, methanol and acetic acid in a volume ratio of 18: 2: 0.5 mL) to yield **42** as a white solid (1.426 g, 81.4 % yield). R_f = 0.95 (methylene chloride, methanol and acetic acid in the ratio of 18: 2: 0.5 mL, thin layer chromatography); ^1H NMR (CDCl_3) 11.02, 7.37 (d, J = 8.22 Hz, 2 H), 7.02 (d, J = 8.22

Hz, 2 H), 2.73 (m, 1 H), 2.38 (m, 4 H), 1.87 (m, 4 H), 1.42 (s, 9 H); ^{13}C NMR (CDCl_3) 179.08, 155.18, 140.89, 131.31, 130.07, 119.43, 80.31, 54.74, 38.73, 37.74, 32.68, 28.24, 27.86. HR-FAB-MS ($\text{M} + \text{H}$) calcd. for $\text{C}_{14}\text{H}_{14}\text{NO}_3\text{Br}$: 323.016. Found: 323.015.

Methyl ester of 1-(*N*-(*t*-butoxycarbonyl)amino-3-[2-(4-bromophenyl)ethyl]-cyclobutanecarboxylic acid (44):

A 50 mL round-bottomed flask was charged with 1-(*N*-(*t*-butoxycarbonyl)amino-3-[2-(4-bromophenyl)ethyl]cyclobutanecarboxylic acid **43** (2.57 mmol, 1.02 g) and dissolved into DMF (35 mL). Next, sodium bicarbonate was added (18.7 mmol, 1.57 g) along with iodomethane (32.1 mmol, 2 mL). The mixture was allowed to stir in a water bath overnight. The mixture was taken into 125 mL of ethyl acetate and washed with water (3 x 25 mL) and brine (1 x 30 mL). The organic layer was dried over magnesium sulfate, filtered and the solvent removed on a rotary evaporator. The resultant material was purified on a silica gel column (20 % ethyl acetate as the eluent) to yield **44** as a viscous yellowish liquid (1.007 g, 95 % yield). R_f = 0.68 (20 % ethyl acetate in hexane, thin layer chromatography); ^1H NMR (CDCl_3) 7.36 (d, J = 7.73 Hz, 2 H), 7.01 (d, J = 8.20 Hz, 2 H), 3.74 (s, 3 H), 2.69 (m, 1 H), 2.39 (m, 4 H), 1.87 (m, 4 H), 1.42 (s, 9 H); ^{13}C NMR (CDCl_3) 174.60, 154.58, 140.81, 131.13, 129.92, 119.24, 79.64, 54.74, 52.26, 38.60, 37.73, 32.57, 28.14, 27.77. HR-FAB-MS ($\text{M} + \text{H}$) calcd. for $\text{C}_{19}\text{H}_{26}\text{NO}_4\text{Br}$: 411.105. Found: 411.104.

Pinacol boronic ester of methyl ester of 1-(*N*-(*t*-butoxycarbonyl)amino-3-[2-(4-bromophenyl)ethyl]cyclobutanecarboxylic acid (45):

This compound was prepared similar to 39. Yielded 0.682 g (63.9 %). ¹H NMR (CDCl₃) 7.72 (d, *J* = 7.87 Hz, 2 H), 7.16 (d, *J* = 7.80 Hz, 2 H), 3.75 (s, 3 H), 2.67 (m, 2 H), 2.39 (m, 4 H), 1.91 (m, 4 H), 1.42 (s, 9 H), 1.33 (s, 12 H); ¹³C NMR (CDCl₃) 174.71, 154.61, 145.41, 134.80, 127.74, 83.54, 79.79, 54.89, 52.35, 38.79, 37.87, 33.49, 32.68, 29.48, 24.80.

1-Amino-3-[2-(4-boronophenyl)ethyl]cyclobutanecarboxylic acid (36):

This compound was synthesized similar to compound 28. After neutralizing the reaction and removing the solvent the resultant material was washed with ether to remove any remaining pinacol. The resultant solid was dissolved in DMSO-*d*₆ and the ¹³C NMR was taken. The carbon signals that represent the methyl ester, the *t*-butoxycarbonyl and the pinacol boronic ester are not present indicating that the desired reaction had taken place. The only compound present is the amino acid/boronic acid.

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