The Chemistry of Neutron Capture Therapy

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I. Introduction

The term neutron capture therapy (NCT) has referred to the radiation generated from the capture reaction of thermal neutrons by various nuclides. This radiant energy has been used as a means of selectively destroying tissue and, for this reason, has been largely but not exclusively focused on the treatment of malignant tumors. The objective has been to destroy tumor cells and their processes without compromising nearby or contiguous normal tissue from which the tumor has arisen. In essence, NCT is a radiation therapy procedure whose goal is to eradicate tumor cells whose precise location may not be fully known so that such residual cancer cells do not become the foci for tumor recurrences. Current radiation procedures do not possess such selectivity, and their existing utility is based on the assumption that malignant cells will have greater radiation sensitivity than their normal counterparts. This assumption unfortunately is not always realized and various malignant cells may be highly resistant to both chemo- and radiotherapy. The question has been, how can these cells be eradicated without affecting normal cells? It is this search for the selective destruction of tumor cells that has led to the development of NCT.

This development arises from the fact that there are nuclides of certain elements with high propensities to absorb thermal or slow neutrons and that the radiation produced is comprised of what is termed high linear energy transfer (LET) particles. They have a short mean free path and because of their size and energy, they have the potential of having their radiant energy confined to the cell from which they arise and it is of lethal magnitude. One of the major challenges in the development of NCT has been a chemical one. Namely, can chemical structures of these thermal neutron absorbing nuclides be designed and synthesized that have the capacity for selectively targeting malignant cells and at a concentration level sufficient to deliver an effective radiation dosage? One of the limitations has been a lack of information regarding permeability differences between tumor and contiguous normal tissues for certain constituents as well as the different biochemical requirements that each possess. Even when such information is known, how can this knowledge be utilized in the area of compound development?

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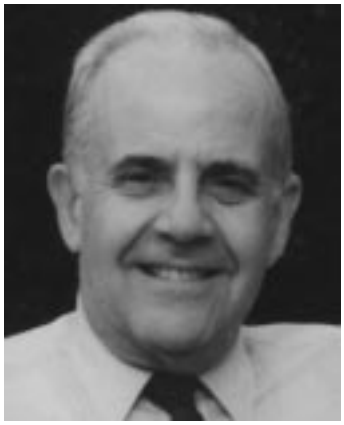

Albert H. Soloway completed his B.S. in chemistry at Worcester Polytechnic Institute in 1948 and his Ph.D. in organic chemistry in 1951. He was a Postdoctoral Fellow of the National Cancer Institute at Sloan-Kettering Institute for Cancer Research for two years, after which time he spent several years at Eastman Kodak Co. as a research chemist. In 1956, he joined the Department of Neurosurgery of the Massachusetts General Hospital and began working with Dr. William H. Sweet in the area of boron neutron capture therapy (BNCT). His full-time academic career began in 1966 as an Associate Professor at Northeastern University, and at the time of his departure in 1977, he was Professor of Medicinal Chemistry and Chemistry and Dean of the College of Pharmacy and Allied Health Professions. At The Ohio State University he was Dean of the College of Pharmacy from 1977 to 1988 and Professor of Medicinal Chemistry from 1977 to 1998. On April 1, 1998, he became Dean and Professor Emeritus and remains actively involved in the development of tumor-targeting compounds for BNCT.

Werner Tjarks studied chemistry at the University of Bremen in Germany where he received his M.S. in 1986 and his Dr. rer. nat. in 1989 under the direction of Professor Detlef Gabel. This was followed by a period as a Postdoctoral Fellow at The Ohio State University College of Pharmacy in Professor Albert H. Soloway's group and subsequently as a Research Associate in Professor Jörgen Carlsson's group at Uppsala University in Sweden. In 1997 he returned to The Ohio State University as a Research Scientist where he is currently responsible for compound development within the multidisciplinary NCT group.

Beverly A. Barnum obtained a B.S. in Chemistry from California State University at Fullerton under the supervision of Professor Greg Williams studying ferro-organometallic chemistry. She earned a Ph.D. degree in Inorganic Chemistry at the University of Pennsylvania in 1995 under the direction of Professor Larry G. Sneddon, investigating several aspects of "polyhedral borane chemistry". She was the recipient of a National Cancer Institute Oncology Training Fellowship in 1995 and 1996 at The Ohio State University and is currently a Postdoctoral Researcher in the College of Pharmacy. Her research interests have expanded into the challenging area of BNCT chemistry under the guidance of Professor Albert H. Soloway.

Feng-Guang Rong received his B.S. (chemistry) in 1982 at the East China Normal University and a M.S. in chemical engineering in 1985 at the East China University of Science and Technology (ECUST) with Professor Guang-Zhao Mu. He completed his Ph.D. in 1991 in a joint program sponsored by Tohoku University in Sendai, Japan, and ECUST with Professors Yoshinori Yamamoto and Fuying Liu. He was Associate Professor and Professor at Qingdao Institute of Chemistry and Technology and subsequently held research positions at The Ohio State University College of Pharmacy where he has been involved in boron neutron capture therapy with Professor Albert Soloway. In 1996, he joined the Chemical Abstracts Service as an Information Chemist.

The purpose of this review is to present the scientific basis for NCT, the background as well as the current status in its development and what we can anticipate in the future, especially with respect to tissue targeting of chemical compounds. The emphasis, as we shall see, has largely focused on boron compounds because of the unique nuclear attributes of the nonradioactive ^{10}B nuclide and the fact that stable boron compounds can be designed and synthesized with structures and chemical/physical properties analogous to those of their carbon counterparts. The challenge has been to use such knowledge in the creation of new agents that will not only target tumor cells but persist therein for a sufficient duration of time so that the radiation dose delivered will be effective and selective for malignant cells. While the concept of NCT was proposed more than 60 years ago, the limited involvement of synthetic medicinal chemists with a clear understanding of the necessary attributes that a compound must possess to be an effective NCT agent has been a major impediment in the development of this therapy. Even with those agents that are being used clinically, there is a clear paucity of information as to the biochemical pharmacologic mechanisms by which tumor accretion and persistence is attained. Such

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knowledge may certainly be beneficial in the development of more effective tumor-targeting compounds.

From this very brief introduction, one can appreciate the singular importance of chemistry, not only in the synthesis of new agents but also in probing the chemical mechanisms by which these compounds target neoplastic cells selectively. Such information may well be an important precondition to the full clinical development of NCT and in its widespread use as an effective and contributing modality in the treatment of various cancers. Presently, available treatments for many solid tumors remain inadequate in their therapeutic management and control and these limitations have been the stimulant for creating new therapies.

II. Limitations of Current Therapies of Cancer

The initiative for developing new methods for treating cancer has arisen from the failures with

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existing therapies. In the case of most solid tumors, surgical extirpation of the malignancy, especially if it is a solitary nodule and can be removed without life-threatening consequences, is the method of choice. However, there is always the inherent uncertainty as to whether all of the malignant cells have been removed, since residual ones can become the foci for tumor recurrences either at the site of the original tumor or at other locations. The clear difficulty in developing cancer therapies stems from the need to destroy such malignant cells selectively without compromising the normal cells from which they are derived as well as other cells that are essential for normal homeostasis. In contrast with bacterial and even viral infections where the host's own immune system is able to contribute to a useful therapeutic outcome, in the case of cancer this does not seem to be very effective and must be buttressed by external forms of therapy.

The problem with existing modalities has become very apparent in the treatment of malignant brain tumors.[1] In their surgical removal, there is great uncertainty as to whether all malignant cells have been removed. In contrast with most malignant tumors, those of the brain appear to possess little propensity to metastasize to other organs but are highly infiltrative of the brain itself.[2] Surgery alone does not meet the therapeutic objective, and for this reason, it has been used in combination with conventional radiotherapy and cytoreductive chemotherapy.

In the case of radiotherapy, the dose required to destroy the cancer cells is of such a magnitude that nearby normal brain cells likewise are severely compromised.[3] The key limitations in the use of radiation are normal tissue tolerance and the need to destroy tumor cells whose precise loci are unknown. Similarly, in the case of chemotherapy, the drug's effect is unfortunately not confined to tumor

cells, but also adversely affects normal brain cells.[4] Once again, the dose administered is limited by normal tissue tolerance. Additionally, reversing the toxic effects of the administered radiation and/or chemotherapy is not possible.

It is to obviate, or at least moderate, the toxic effect upon normal brain that the administered dose is fractionated, whether this be chemotherapy or radiotherapy. The difficulty arises from the fact that highly malignant tumors demonstrate increasing resistance to both conventional radiation and chemotherapy with exposure to these agents and in relationship to normal brain. To enhance the effect of these modalities upon tumor, they have been used in synergy; the objective being that the combination will be greater than the sum of their individual parts. Yet, the problem of achieving selective destruction of tumor cells without adversely affecting normal brain cells still remains an unachieved objective, and it is for this reason that new methods for the treatment of malignant brain tumors have been considered.

III. Rationale for the Development of Binary Systems for Cancer Treatment

It is this search for the specific and selective destruction of tumor cells that has led to the development of binary systems. Binary systems involve the use of two components for the treatment of cancer. Each component should be relatively innocuous to mammalian cells, but their combination generates a highly lethal cytocidal effect. If this combination can be largely restricted to tumor cells, then nearby normal cells will be spared and an important step can be made toward achieving a more selective form of treatment for those solid malignancies that are refractory to existing therapies. One of the advantages of a binary system is the potential that each component can be manipulated independently. Only when the timing is correct for achieving the maximal cytotoxic effect upon malignant cells, together with the maximal tolerated dose to contiguous normal cells, are the two components juxtaposed. If this approach is to succeed, it is essential that at least one of the components be confined rather specifically to tumor cells while the second component is exposed to all cells in a particular area. In this way, knowing a priori the precise location of the tumor cells is not necessary to achieve their destruction. There are several binary systems that are in various stages of development. These include the use of radiation sensitizers,[5,6] photon activation therapy,[7,8] photodynamic therapy,[9−11] gene therapy,[12,13] and neutron capture therapy (NCT).[14−45] The focus of this review is the chemistry of neutron capture therapy, the nuclides that have been considered, and their incorporation into structures designed to meet the therapeutic objective.

IV. Concept and Nuclides in Neutron Capture Therapy

The concept of NCT is based upon the observation that there are certain nuclides, both radioactive and nonradioactive, whose nuclei possess an unusual capacity for absorbing thermal or slow neutrons.[46] These neutrons have an energy of 0.025 eV, clearly below the threshold energy required to ionize tissue components. However, this neutron absorption by the atom's nucleus produces an activated nucleus that undergoes prompt fission, with the generation of lethal energetic particles. If this energy could be largely confined to malignant cells, then their selective destruction would be achieved without concomitant injury to contiguous normal cells and their supporting structures. A list of nuclides with high capture cross sections for thermal neutrons is displayed in Table 1.[47] These values are large by nuclear standards and in most instances are at least 2 orders of magnitude greater than those observed for the normal elemental composition of tissue (see Table 2).[47] However, cross section capture is only one attribute; the nature of the fission products is another. The latter determines whether these components are confined to the cell in which they originate or whether they produce radiation that is not retained cellularly. The latter result is self-defeating since the objective is to confine the radiation largely to malignant cells. Also, one can appreciate that radioactive nuclides *per se*, unless restricted to malignant cells, could deliver undesired radiation to various normal structures. Therefore, NCT has largely revolved around the use of nonradioactive nuclides.

Early in the development of NCT, radioactive ^{235}U was considered as a possible nuclide.[48−50] The fission particles generated by the capture reaction of thermal neutrons by ^{235}U are very large, ranging in mass numbers from 85−104 to 130−149. Because of their size and energy, the destructive radiation would be

Table 1. Capture Cross Section Values of Various Nuclides for Thermal Neutrons

nuclide	cross section capture value[a]	nuclide	cross section capture value[a]
^{6}Li	942	^{151}Eu	5800
^{10}B	3838	^{155}Gd	61000
^{22}Na[b]	32000	^{157}Gd	255000
^{58}Co[b]	1900	^{164}Dy	1800
^{113}Co	19800	^{184}Os	3000
^{126}I[b]	6000	^{199}Hg	2000
^{135}Xe[b]	2600000	^{230}Pa[b]	1500
^{148m}Pm[b]	10600	^{235}U[b]	580
^{149}Sm	42000	^{241}Pu[b]	1010

[a] Cross section capture values in barns (1 barn = 10^{-24} cm^2). [b] Radioactive.

Table 2. Thermal Neutron Capture Cross Section Values of Tissue Elements and Their Percentages

nuclide	weight (% in tissue)	neutron capture cross section[a]	nuclide	weight (% in tissue)	neutron capture cross section[a]
H	10.00	0.332	P	1.16	0.18
C	18.0	0.0034	S	0.20	0.53
N	3.0	1.82	Cl	0.16	32.68
O	65.0	1.8×10^{-4}	K	0.20	2.1
Na	0.11	0.43	Ca	2.01	0.4
Mg	0.04	0.053	Fe	0.01	2.57

[a] Neutron cross section values in barns (1 barn = 10^{-24} cm^2).

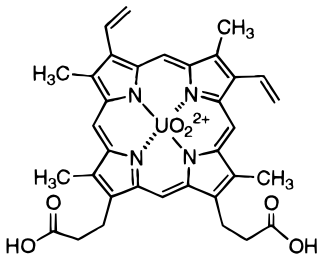


Figure 1. Uranyl protoporphyrin.

clearly confined to the cell in which the capture reaction occurs. However, in general, uranium compounds are toxic (i.e., uranyl protoporphyrin, Figure 1), and while ligands of uranium have been designed that are biologically stable (i.e., uranyl phthalocyanine tetrasulfonate), that in itself is not sufficient. The complexes must have the capability of selectively targeting tumor cells. These early studies focused on brain tumors, but there was a lack of appreciation of the biochemical requirements that must be met for uranium compounds to be useful as a clinical modality for NCT. Recently, there has been very little research involving ^{235}U compounds as potential NCT agents.

Another nuclide considered at that time was nonradioactive ^{6}Li.[51,52] The particles generated from its capture reaction are also large and are referred to as high linear energy transfer (LET) particles. Be-

$$^{6}_{3}\mathrm{Li} + {}^{1}_{0}\mathrm{n} \rightarrow [{}^{7}_{3}\mathrm{Li}] \rightarrow {}^{4}_{2}\mathrm{He} + {}^{3}_{1}\mathrm{H} + 4.3\ \mathrm{MeV} \quad (1)$$

cause of their size and energy, they are confined to a radius of approximately 9−10 μm, comparable to the dimensions of a single cell. If lithium-6 compounds could be synthesized possessing a strong predilection for neoplastic cells under in vivo conditions, then their selective destruction could be achieved. However, the major limitation in its use is that ^{6}Li is an alkali metal and under biological conditions its compounds would be readily broken down to lithium cations. Clinical studies were carried out with ionic lithium[53] but, as with ^{235}U, there has been little research involving this nuclide over the past three decades.

Nonradioactive boron-10, comprising approximately 20% of natural boron, is similar to ^{6}Li in that the fission products generated are largely high LET particles.[54−57] One clear advantage of boron-10, compared with the other nuclides shown in Table 1, is that not only are the fission products high LET but many different boron compounds can be synthesized having hydrolytically stable linkages between boron and other elements such as carbon, oxygen, and nitrogen. Also, its small atomic size permits its

$$^{10}_{5}\mathrm{B} + {}^{1}_{0}\mathrm{n} \rightarrow [{}^{11}_{5}\mathrm{B}] \begin{cases} \rightarrow {}^{4}_{2}\mathrm{He} + {}^{7}_{3}\mathrm{Li} + 2.79\ \mathrm{MeV}\ (6\%) \\ \rightarrow {}^{4}_{2}\mathrm{He} + {}^{7}_{3}\mathrm{Li} + \gamma 0.48\ \mathrm{MeV} + 2.31\ \mathrm{MeV}\ (94\%) \end{cases} \quad (2)$$

replacement of carbon in many organic structures, generating isosteres that offer the potential for simulating biologically those compounds from which they are derived. In addition to such covalent bonds with various heteroatoms, there are boron clusters

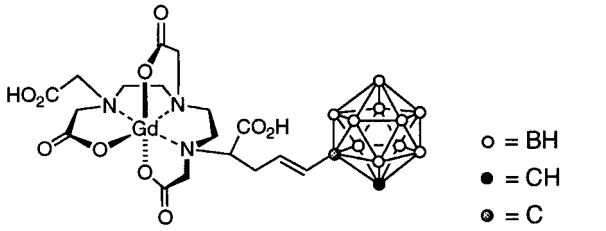


Figure 2. Proposed Gd-157 NCT agent.

that possess remarkable hydrolytic and metabolic stability. These include various polyhedral borane anions and carboranes. It is for this reason that in the area of compound development for NCT, much of the activity has focused upon the use of boron-10. Thus, the term boron neutron capture therapy (BNCT) is widely perceived as being synonymous with neutron capture therapy (NCT).

More recently, however, researchers have begun to explore the potential of using various gadolinium nuclides having high capture cross section values for thermal neutrons; the more prominent one of these, ^{157}Gd,[58−61] possesses a capture cross section value that is 66 times that observed for boron-10 (Table 1).[47] This advantage is counteracted in part by the nature of the radiation emitted; the products are γ rays and Auger and Coster-Kronig electrons.[62] The former are not confined to the cells from which they arise, and the latter, because of their low energies, must be closely associated with the tumor cell DNA in order to deliver a cytocidal effect to this highly sensitive cellular component. Some researchers have proposed using GdNCT in combination with BNCT,[63−68] but the need is to develop tumor targeting agents that are selective for specific subcellular organelles (Figure 2).

The potential for developing NCT rests upon the fact that the various normal elemental constituents of tissue have very low capture cross section values for thermal neutrons compared with boron-10 (3838 barns, see Table 2).[47] However, two of these, hydrogen and nitrogen, because of their high concentration in tissue can contribute to the radiation dose derived from the tissue exposed to the neutron beam. To

$$^{1}_{1}\mathrm{H} + {}^{1}_{0}\mathrm{n} \rightarrow [{}^{2}_{1}\mathrm{H}] \rightarrow {}^{2}_{1}\mathrm{H} + \gamma 2.23\ \mathrm{MeV} \quad (3)$$

$$^{14}_{7}\mathrm{N} + {}^{1}_{0}\mathrm{n} \rightarrow [{}^{15}_{7}\mathrm{N}] \rightarrow {}^{14}_{6}\mathrm{C} + {}^{1}_{1}\mathrm{p}\ 0.63\ \mathrm{MeV} \quad (4)$$

minimize their contribution as a percentage of the total radiation dose, it becomes essential that the boron-10 concentration in tumor approximates 20−35 μg/gram or 10^{9} ^{10}B atoms/cell.[57,69] Under such conditions, approximately 85% of the radiation dose arises from the ^{10}B capture reaction. It is for this reason that the cellular concentration of boron-10 be adequate, yet at the same time, there must be sufficient tumor specificity to minimize the radiation dose to surrounding normal structures. Thus, both requirements are important, and one without the other negates the advantage of BNCT as a potential clinical modality.

V. Types of Neutrons and Their Sources

The second component of this binary system is the thermal neutrons. A full examination of the types

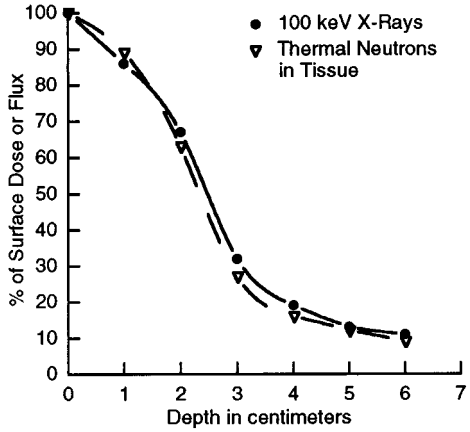


Figure 3. Depth dose reduction curves demonstrating the penetration of tissue by thermal neutrons vs X-rays.

of neutrons, their energies, and sources by which they are produced is not germane to this review. However, a brief examination may be desirable to acquaint readers of this review as to the nuclear requirements in the development of BNCT. Thermal neutrons, as stated, have an energy of approximately 0.025 eV that is well below the threshold value for the elastic scattering interaction between neutrons and hydrogen atoms. Such scattering, involving neutron energies in excess of 10 keV, produces recoil protons capable of ionizing tissue components.[70] In Figure 3 is shown the ability of thermal neutrons to penetrate tissue compared with "soft" or 100 keV X-rays. It is apparent that the two beams are very comparable in tissue penetrating properties and that within 2.5 cm (1 in.) of the tissue's surface, the incident flux is reduced by a factor of 50%.[71] The use of thermal neutrons has presented a major limitation in the treatment of more deep-seated tumors of the brain since they are readily dissipated when traversing tissue. More recently, the approach has been to develop beams with somewhat more energetic neutrons that become "thermalized" as they penetrate tissue. These beams are referred to as "epithermal" neutron beams, and their energies range from 0.5 to 10 keV.[72] These beams can deliver acceptable neutron fluences to tumors that may be 3−6 cm below the surface and has been the basis for the development of beams with suitable fluxes of epithermal neutrons.[73] Presently, the available beams are not monoenergetic nor do they possess a narrow energy spectrum. They contain thermal, epithermal, and fast neutrons, as well as gamma rays. The latter two will contribute to the radiation dose nonselectively, and for this reason, their contribution to the incident beam must be greatly minimized. The methods for developing epithermal beams using various filters and moderators are clearly beyond the scope of this review. However, beam development will become of increasing importance if BNCT demonstrates clinical potential in treating hitherto refractory malignancies, such as those of the CNS. Currently, nuclear reactors are the principal source for these neutrons. The reason is that they are capable of delivering fluences of the order of $10^{12}-10^{13}$ n/cm^2 and therby keep the radiation times of sufficiently short duration. Isotopic sources of neutrons, such as those derived from ^{252}Cf,[74] would have to be very large and, thus, have not been seriously considered. Alternatively, there is active ongoing research related to the development of accelerators capable of producing neutron beams with the appropriate energy spectrum and flux.[75−78] One of the primary reasons for such consideration is that these devices, in contrast with nuclear reactors, could be housed in medical centers where existing radiation procedures are performed and be part of such a facility. There are clinicians who feel that BNCT will only become a practical modality when neutron accelerators are developed.[79]

VI. The Basis for the Selection of Brain Tumors and Approaches in Brain Tumor Targeting

Two questions have been raised: why has the primary focus of BNCT been directed toward the treatment of malignant brain tumors and could such therapy be applied to other tumors as well? One of the main reasons that BNCT was directed toward treating brain tumors was the fact that these tumors have little history for metastasizing to other organ sites, and if tumor eradication could be achieved in the brain, then there might be a significant increase in disease-free life expectancy. Dr. William H. Sweet, a neurosurgeon, had a seminal role in taking this form of therapy from a conceptual hypothesis to a clinical trial more than 40 years ago.[55,80] While the results were unsuccessful,[81] the failure stemmed not from a flaw in the concept per se, but from the inadequacies in the boron compounds and in the neutron beams that were available. Subsequently, Dr. Hiroshi Hatanaka embarked upon the clinical use of BNCT with a compound that appeared to possess more desirable tumor targeting properties than compounds previously used.[82] His work over a 20 year span provided a significant impetus since he reported that several patients whom he treated were long-term survivors of malignant brain tumors.[83]

Not all of the clinical efforts focused on brain tumors. Dr. Yukata Mishima, a dermatologist, was interested in treating malignant melanoma. Using a boron-containing amino acid,[84] he demonstrated the effectiveness of this binary system in destroying isolated clusters of melanoma cells.[85]

These three clinicians have contributed significantly to the ongoing interest and continued development of BNCT. The focus of the new research activities has been directed toward the following: the design, synthesis, and evaluation of more selective tumor targeting agents; optimizing their delivery; improving neutron beam characteristics from reactors and accelerators.

Clinical trials are continuing in Japan,[85,86] two are now underway in the United States[87−90] with different agents and modified beams, a new trial has begun Europe,[91] and a second is under active consideration.[92] Only at their conclusion will it be possible to assess the potential of BNCT as a therapeutic modality. Although the approach is largely directed toward the treatment of patients with brain tumors, any improvement in life expectancy and their quality of life may spur efforts toward the treatment of other solid tumors that have become refractory to conven-

tional therapies. Among these may be hepatomas,[93] those of the head/neck and lung cancers, where local control of the malignancy is a current limitation of existing therapies. There is no present expectation that whole body neutron irradiation will be possible nor desirable, and thus, the treatment of widely metastasized and disseminated tumors by BNCT is not anticipated.

Developing agents that will effectively target malignant brain tumors has been a daunting task. The reason is that such compounds must not only be able to selectively target tumor cells in the presence of neurons and normal glial cells but at concentrations that will achieve a therapeutic effect. The design of BNCT agents for use in treating brain tumors was initially based on the increased permeability of the blood−brain barrier (BBB) in the tumor by contrast with normal brain.[94] This strategy failed to take into account that there may be isolated clusters of tumor cells that are protected by the normal BBB. Such a cluster could become the focus for tumor recurrence. To eradicate these, compounds must be able to cross the normal BBB and target islets of tumor cells therein. Thus, in addition to designing agents that possess a strong affinity for tumor cells, the mode of drug delivery that will enhance brain concentration has also become a major issue.[95−98] To increase the drug concentration to the brain, and thereby the tumor, arterial administration (i.e., internal carotid artery) has been applied.[95−98] Although elevated drug concentrations in tumors are achieved in contrast with intravenous injection, nonetheless, the BBB still severely restricts entrance of a variety of compounds into the brain, especially high molecular weight entities.[99,100] It is only more recently with techniques designed to temporarily disrupt the BBB (i.e., hyperosmotic mannitol and RMP 7), that the possibility now exists to expose both normal and malignant cells to compounds which heretofore would not enter the brain or least not at the necessary concentration levels.[95−98]

Also, there has been greater consideration toward using biochemical and metabolic differences between tumor and normal brain as the basis for achieving the selective targeting of gliomas. Knowledge relating to the biochemistry of brain tumors continues to evolve.[101,102] These include both qualitative and quantitative changes in cell membrane chemistry as reflected, among others, by alteration in antigenic determinants, and in differences in cellular receptors (i.e., growth factors, protein kinases, signal transducers) and ionic channels. As a consequence, there can be expected to be changes in the utilization of various biochemical building blocks that form the complex structural elements of the tumor cell. Changes have been observed in the various chemical compartments that make up the cell and include differences in the following: composition of polysaccharides as shown by altered glycogen levels; the lipid composition of phospholipids and sterols; the proteins and/or amino acids; the nucleic acids and/or their precursors. The key question is, how can this information based upon biochemical differences between tumor and normal tissue be translated into drug design? Can potential precursor compounds, containing ^{10}B or other neutron absorbers, be used that show elevated concentrations in tumor? Such questions apply not only to brain tumors but to all tumors.

VII. Compounds Used in Initial Clinical Studies of Brain Tumors

The requirements for compounds used clinically in the 1950s and early 1960s was that they should possess low toxicities, high tumor:brain boron ratios and persistence of such differentials with a tumor concentration in the range of 20−35 μg boron/g. Initially, localization studies using sodium borate (i.e., Borax and pentaborate) and boric acid and its derivatives were undertaken (Figure 4).[103−105] Their low toxicities, even in brain tumor patients, together with adequate tumor concentrations, prompted their use in a clinical trial. The results were clearly disappointing since patients succumbed from recurrent disease.[106] The failure was attributed to two major limitations: (1) the thermal neutron flux delivered to the area where the neoplasm had been surgically removed was inadequate due to the beam's traversing of the scalp and intact skull to reach the target area; and (2) the compounds used had tumor:brain differentials that were transient and reached unity within a relatively short period of time. The former limitation was met by reflecting scalp and bone flaps exposing the tumor bed directly to the neutron beam. The second limitation was addressed by searching for compounds that would show greater persistence in tumor in contrast with those levels in normal brain.

Of the compounds that were evaluated at that time, a series of aromatic boronic acids were screened (Table 3).[107] In attempting to correlate their physiochemical attributes with their distributive properties in tissue, it became apparent that those which were highly lipophilic achieved significant concentrations in the brain, demonstrated CNS toxicity, and achieved low tumor:brain ratios. On the other hand, there were a number of arylboronic acids which possessed low toxicities, very suitable tumor:brain

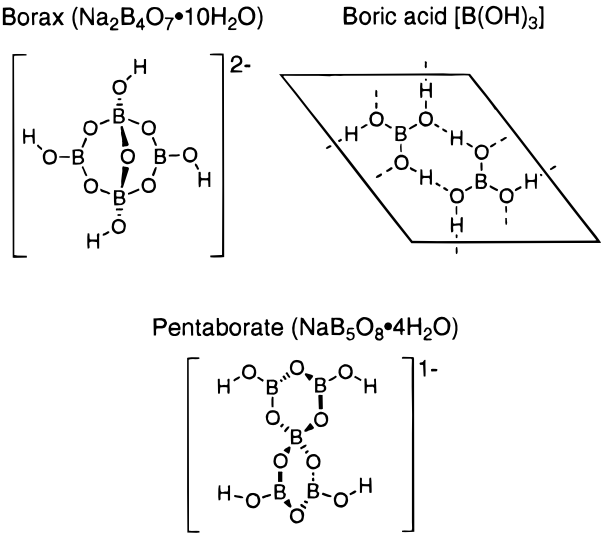


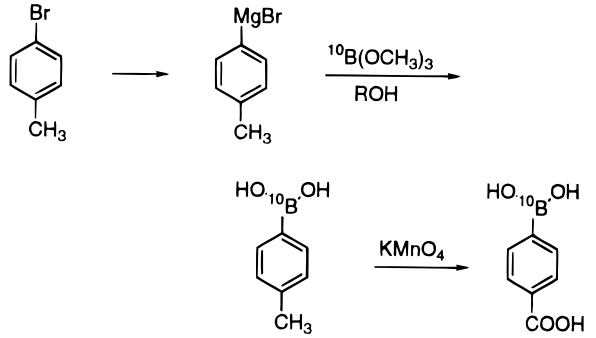
Figure 4. Sodium borates and boric acid.

Table 3. Various Substituted Benzeneboronic Acids, Their Aqueous/Benzene Partition Coefficients, and Their Average Tumor/Brain Boron Ratios in Mice

substituted benzeneboronic acid X-Ph-B(OH)$_2$	aqueous benzene partition coefficient	tumor/brain boron ratios[a]
4-Si(CH$_3$)$_3$	0.03	0.1[b]
3-CF$_3$	0.4	0.2[b]
4-OC$_2$H$_5$	1	0.6
4-CH$_3$	2	0.3
4-F	3	0.3
3-NHCOOC$_2$H$_5$	14	0.6
4-CHO	29	0.6
3-NO$_2$-4-COOH	51	2.5
4-COOH [PCPB]	67	5.7
4-B(OH)$_2$	>200	2.3
4-CH$_2$CH(NH$_3^+$)COO$^-$ [BPA]	>200	8.5
3-NH$_2$-4-COOH	>200	6.4
3-NH$_2$	>200	1.2
3-NHCONH$_2$	>200	7.5

[a] Values obtained within 15 min following intraperitoneal injection of a dose of 18−140 mg B/kg mouse. [Soloway, A. H.; Whitman, B.; Messer, J. R. *J. Med. Pharm. Chem.* 1962, *5*, 191.] [b] Animals died at low doses of the administered compound.

Scheme 1. Synthesis of Boron-10-Enriched PCPB, *p*-Carboxyphenylboronic Acid [A. H. Soloway, unpublished results]



concentration ratios and, importantly, these differentials persisted for much longer time periods than was observed with the borates.[108−110] The selection of *p*-carboxyphenylboronic acid (PCPB) as a BNCT agent was based upon these criteria plus the fact that it could be readily synthesized in ^{10}B form (Scheme 1). Interestingly enough, this compound was not transformed metabolically but was excreted in the urine unchanged. At the time that these arylboronic acids were being synthesized and evaluated both toxicologically and pharmacokinetically, 4-dihydroxyborylphenylalanine (BPA) was synthesized by Snyder and his associates[111] and evaluated biologically.[84] It showed properties analogous to other hydrophilic arylboronic acids, but it was unrecognized then that this compound gave significant tumor:blood ratios and thus possessed an important advantage over the other compounds. It remained for Mishima and his associates, two decades later, to show its potential for treating melanomas,[112,113] and subsequently, Coderre et al.[114,115] used it in treating malignant brain tumors by BNCT.

While these aromatic boronic acids were being screened, a major development was occurring in boron chemistry that would have very important

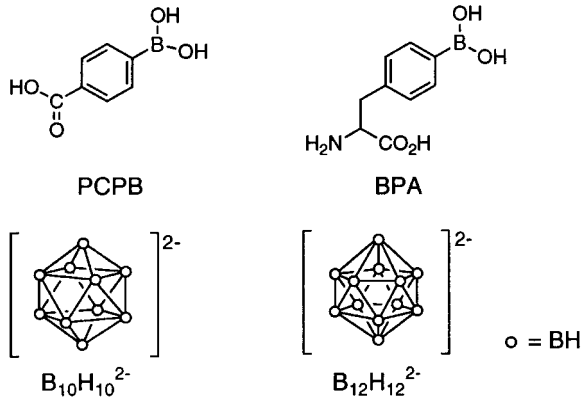


Figure 5. PCPB, *p*-carboxyphenylboronic acid; BPA, *p*-boronophenylalanine; and polyhedral borane anions: decahydrodecaborate,$B_{10}H_{10}^{2-}$, and dodecahydrododecaborate, $B_{12}H_{12}^{2-}$.

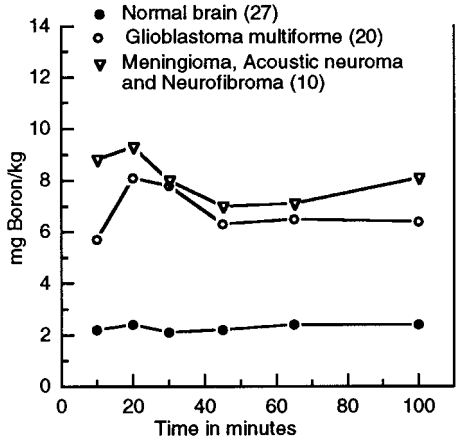
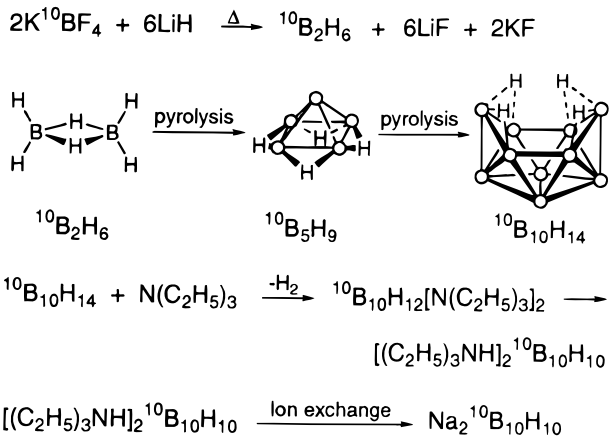


Figure 6. Boron concentrations in human brain and in brain tumors following intravenous administration of sodium decahydrodecaborate, $Na_2B_{10}H_{10}$, (number of samples).

significance for BNCT. Hawthorne and collaborators discovered the polyhedral borane anions, $B_{10}H_{10}^{2-}$ and $B_{12}H_{12}^{2-}$,[116−119] and demonstrated that, in contrast with classical boron hydrides, these cage structures had very remarkable chemical and hydrolytic stabilities (Figure 5). Their availability for biological study shortly after their discovery led to the consideration of sodium decahydrodecaborate ($Na_2B_{10}H_{10}$) as a potential BNCT agent.[109] The compound's toxicity in terms of mg of boron/kg was approximately 50% that of boric acid.[109] Comparably low toxicity values were also obtained with the $B_{12}H_{12}^{2-}$ anion. The $B_{10}H_{10}^{2-}$ anion was ultimately evaluated in patients, and as with PCPB, this compound was also excreted unchanged.[108] It was observed that administration of $Na_2B_{10}H_{10}$ via the internal carotid artery resulted in high boron concentrations in the brain tumor with concomitantly low levels in normal brain. Also the compound had low systemic toxicity. It appeared at that time that the ultimate BNCT agent had been found. Thus, PCPB and $Na_2B_{10}H_{10}$ were prepared as their ^{10}B-enriched analogues (Schemes 1 and 2) and used in a clinical trial.

The results of this clinical study, as with the borates, was a total failure. In the final instance, it became readily apparent that although tumor boron concentration may have been adequate and that an

Scheme 2. Synthesis of Boron-10-Enriched Decahydrodecaborane [A. J. Leffler and A. Gatti, unpublished results]



appropriate neutron fluence was delivered, radiation injury was the crucial contributing factor to the resultant morbidity and mortality. The neuropathological findings demonstrated severe radiation necrosis within the vasculature of the brain exposed to the neutron beam.[80] How could this severity be explained? In retrospect, the drug development approach was extraordinarily simplistic, viewing the brain as a monolithic entity and not one composed of discrete compartments and structures. The observed effects can be attributed to the fact that the blood concentrations of the agents used was greater than those found in the tumor, and as a consequence, the vascular endothelium, which is sensitive to high LET radiation, was compromised during the radiation procedure.

The results had a long-term negative impact on the further development of BNCT, especially from a clinical perspective in the United States. However, the failure can be directly attributed to one of chemistry and not to the concept of NCT. Simply achieving good tumor:brain differentials with an adequate tumor concentration that persisted during the radiation period was insufficient in and of itself. It was now appreciated that elevated boron levels in blood would pose life-threatening consequences by the radiation's effect on the small arterioles since the dose delivered to the vascular endothelium would be unacceptable. This important structure certainly must not be adversely affected by the impinging neutrons.

VIII. "Second Generation" Boron Compounds

The recognition that boron levels in the blood clearly must not exceed those in tumor as well as those in normal brain spurred an effort to uncover such structures. Key in this determination was a screening procedure in tumor-bearing animals that would allow blood boron levels to fall and to assess whether adequate tumor concentrations persisted. In essence, were there compounds whose ability to concentrate in tumor was independent of their kinetic parameters in blood and how could these be determined? Simplistically, compounds could be administered over a sequence of days followed by a hiatus

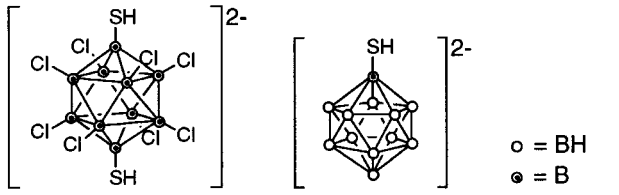


Figure 7. "Second generation" mercaptoboron compounds: 1,10-$B_{10}Cl_8(SH)_2^{2-}$ and $B_{12}H_{11}SH^{2-}$, mercaptoundecahydrododecaborate, BSH.

in drug administration. This would permit the boron levels in blood to fall before sacrificing the animals and measuring boron content in various tissues including tumor and brain. This was a departure from previous screening efforts and it was unclear whether there would be any compounds that possessed such a tumor targeting property.

In the process of such an evaluation, two compounds were uncovered which demonstrated this selective uptake by tumor cells. These were $Na_2B_{10}Cl_8(SH)_2$ and $Na_2B_{12}H_{11}SH$ (Figure 7).[82] These compounds were initially provided by E. I. DuPont Co. In view of the fact that the latter structure has a higher percentage of boron and a significantly lowered toxicity based upon its boron content, sodium mercaptoundecahydrododecaborate ($Na_2B_{12}H_{11}SH$, BSH) was selected for further biological evaluation. Interestingly enough, the original material provided by the DuPont researchers was not pure BSH, but undoubtedly contained at least some of the dimer, $Na_4B_{12}H_{11}SSB_{12}H_{11}$.[120] Subsequently, it was shown that this dimer produced appreciably higher concentrations in tumor but, at the same time, increased toxicity as shown by changes in liver enzymes.[121] This dimeric structure readily undergoes homolytic cleavage, especially under acidic as well as biological conditions, generating an interestingly stable free radical $B_{12}H_{11}S^{\bullet 2-}$, as shown by its ESR spectrum.[122,123] Whether this moiety is a factor in tumor localization remains to be determined.

The early method for the synthesis of BSH involved the reaction of the hydrated form of $(H_3O)_2B_{12}H_{12}$ at high temperature and pressure with liquid H_2S (Scheme 3).[124] Since this reaction was carried out initially in an iron vessel, some of the product was undoubtedly oxidized to the dimer. When the reaction was carried out in a glass-lined vessel, pure BSH was the only product obtained. Subsequently, scientists at the Shionogi Research Laboratories developed an alternate method for synthesizing the $B_{12}H_{11}SH^{2-}$ anion using *N*-methylthiopyrrolidone.[125] This eliminated the need for using liquid H_2S. Their method is the one that is currently being used for its preparation. More recently, Brattsev and Morris have developed a new method for the preparation of BSH from the thiourea derivative $B_{12}H_{11}SC(NH_2)_2^{1-}$ via electrochemical synthesis of the latter.[126]

The material initially provided by DuPont showed much more interesting biological properties, as shown in Table 4, than did the pure BSH. Tumor:blood boron ratios were appreciably higher with the impure BSH than was observed with the purified anion. With the latter, the ratios ranged from 1.0 to 1.3. In contrast, the former showed tumor:blood differentials of 5−6.[82]

Scheme 3. Summary of BSH ($B_{12}H_{11}SH^{2-}$) Synthesis and Reactions

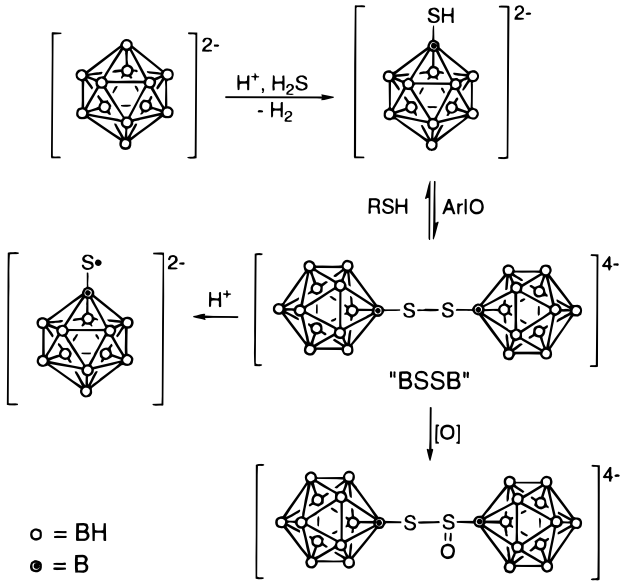


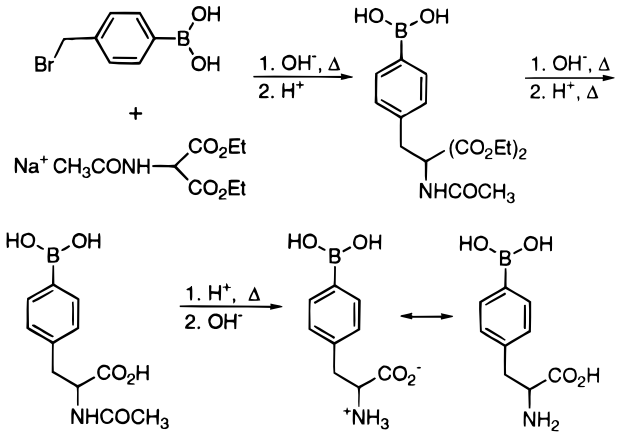
Table 4. In Vivo Distribution of Original Dupont BSH Sample

animal	tumor[a]	brain[a]	blood[a]	tumor/blood ratio
1	17.2	1.1	4.1	3.9
2	21.8	1.5	5.0	4.4
3	6.5	1.4	1.7	3.8
4	4.4	1.0	2.1	2.1
5	33.1	1.3	6.2	5.3
6	37.6	0.8	2.1	17.9
7	5.0	0.6	1.8	2.8
8	13.3	0.5	2.0	6.7
9	7.5	1.6	1.8	4.2
				av = 4.7 ± 5.0

[a] Tissue concentrations in μg B/g tissue. Dose: 35 μg B/g mouse/day. Total dose: 175 μg B/g mouse.

The basis by which tumor accretion is achieved with these sulfhydryl-containing polyhedral borane anions remains unknown now, nearly 30 years after its initial clinical introduction in Japan. Certainly, there is a great difference observed biologically between the anions, $B_{12}H_{12}^{2-}$ and $B_{10}H_{10}^{2-}$, and their mercapto-containing counterparts, The key question is, what is the precise chemical species that is responsible for tumor localization? Clearly, the charge of the boron cage alone cannot be the sole basis for the observed selectivity, nor is it apparent whether it is the boron entity itself or some complex involving a macromolecular species (i.e., a protein) in blood that is the responsible structure for achieving an elevated concentration in the tumor. In essence, the biochemical pharmacology of this compound remains to be delineated even though it has been used extensively in patients in Japan and has undergone clinical pharmacokinetic studies in Europe,[127] and such distributive studies have now begun in the United States.[128] Recently, a clinical trial has begun in Europe at the nuclear reactor in Petten, The Netherlands, using BSH.[91] Five patients have been treated, and the expectation is that 40 more will be treated in the coming months to determine both the safety and efficacy of BNCT with this agent.

Scheme 4. Synthesis of BPA by Snyder et al. (1958).



We do know that BSH is fairly readily oxidized to $B_{12}H_{11}SSB_{12}H_{11}^{4-}$ and $B_{12}H_{11}SS(O)B_{12}H_{11}^{4-}$, especially in aqueous solutions containing oxygen (see Scheme 3).[122,123] Thus, such transformations will most assuredly occur in blood but it remains to be determined whether such structures per se or their more proximately metabolized entities are the responsible species in tumor localization. What has been observed is that the tetravalently charged dimer is largely retained in the blood, probably through ionic binding and the relative biological effectiveness (RBE) value in animal irradiations is significantly lower than that for BSH.[129] It is the clinical utilization of this latter compound that has contributed in part to an ongoing interest in developing other BNCT agents that contain the polyhedral borane anions.

Another compound that may also be thought of as a "second generation" compound is 4-dihydroxyborylphenylalanine (BPA). Mishima spurred interest in this compound based upon his consideration for treating malignant melanoma with BNCT.[85] The fact that BPA is an amino acid and may be viewed as an analogue of phenylalanine or tyrosine was the driving force behind his interest. Since phenylalanine is the precursor of melanin, it was reasoned that a boron-containing analogue (Scheme 4) would mimic the natural constituent and achieve more selective uptake in those cells with an abnormally elevated melanin formation. However, unless the boron moiety is lost in the course of metabolism, BPA cannot be viewed as a precursor of melanin. Nevertheless, the fact that it is an aromatic amino acid may well be the basis by which it achieves elevated levels and persistence in melanomas.[130−132] These results prompted the subsequent evaluation of BPA in brain tumor-bearing animals, and once again, this compound appeared to meet the necessary criteria for becoming a useful BNCT agent.[114,115] While the levels in normal brain are higher than those observed for BSH, the levels in brain tumor are also significantly greater. These results have led to an ongoing clinical trial at the Brookhaven National Laboratory using BPA in brain tumor patients;[87] to date, 38 patients have been irradiated[133] with no evidence of radiation injury to normal brain.[134] Another important clinical trial of BNCT, using BPA, involves clinicians and researchers at Beth Israel Deaconess

Medical Center and Massachusetts Institute of Technology.[135] They have treated 17 patients: five with peripheral melanoma; one with melanoma metastatic to the brain; 11 with glioblastoma multiforme.[136] These studies are continuing but no untoward effects have been observed in these patients. These promising results with BPA have stimulated interest in the synthesis of other boron-containing amino acids that will be described below.

More recently in animal studies, BPA and BSH have been used in combination.[137,138] These agents have been administered, following blood−brain barrier disruption, via the internal carotid artery that provides the blood supply to the tumor. The results have shown measurable increases in tumor boron concentration and following neutron irradiation, 25% of the rats bearing the highly refractory F98 glioma implanted intracranially have been cured. This is the first example in which this particular, highly virulent tumor has been successfully treated by any regimen.

IX. Criteria for BNCT Agents

In the early years of BNCT (1940−1961), agents were considered that were either readily available commercially or easily synthesized. They were evaluated exclusively for the treatment of brain tumors. Since that time, other tumors have been considered and the design of new tumor-targeting entities has not focused solely on malignant brain tumors.

The current clinical use of chemical entities for targeting tumors has focused on two approaches, one in diagnosis and the second in therapy. An important question has been, can the background in drug development for either of these uses benefit the design of new BNCT agents? Radiopharmaceuticals and MRI compounds, as diagnostic agents, are based upon achieving a suitable concentration differential between tumor and surrounding normal structures. This is precisely the objective sought for BNCT compounds. However, there is one major difference with nuclear medicine drugs: these are either frequently used as carrier-free entities or are administered in very low concentrations. Their pharmacodynamics and pharmacokinetics, as well as the concentration differentials achieved, may be directly dependent upon the actual chemical amount that is administered. If the chemical level is of trace proportions, by comparison with an amount of 20−35 μg of boron-10/g that is required for BNCT compounds, it is probable that the development of BNCT drugs cannot be based upon the observations and research underpinnings of such diagnostic agents. The sensitivity of the MRI contrast agents depends, among other matters, upon the relaxation of the paramagnetic nuclides. The factors relative to their concentrations and measurements are quite different than are the requirements for BNCT compounds.

The chemotherapy of malignant tumors has not been based upon the actual chemical concentration of the anticancer agent in the tumor itself but is strictly related to a biological endpoint. Therefore, the design of a treatment regimen with such cytoreductive agents has been an empirical one and is not based upon achieving a predetermined concentration level of the compound in tumor. In some cases, it is not the compound itself but a metabolic product that may be the active entity. Its concentration level and rate of further metabolism in tumor cells may not be known. For BNCT, however, the basis for treatment is strictly rooted in the concentration that is attained within the tumor cells and it is for this reason that cancer chemotherapy likewise has not provided a useful basis for the development of new BNCT compounds.

The three most important parameters for compound development have essentially not changed since the early days of BNCT: (1) achieving tumor concentrations in the range of 20−35 μg ^{10}B/g; (2) a tumor:normal tissue differential greater than 1 and preferably 3−5; (3) sufficiently low toxicity so that the dose administered would be well tolerated first in animals and subsequently in patients. An added requirement was that the concentration differential would obviously persist during the entire neutron irradiation period. Although such parameters were readily achieved, it became apparent that they were insufficient in and of themselves. The initial clinical trials were an unmitigated failure, and in probing the reason for this lack of success, it became apparent that one of the reasons is that the brain, as any other organ, cannot be viewed as a single, monolithic entity. In a very simplistic way, organs are composed not only of various normal cells but also of blood and blood vessel walls. A conclusion of the pathological findings of the early clinical trials in the late 1950s and early 1960s was that the blood boron concentrations exceeded those in the tumor and produced irreparable damage to the blood vessel walls especially the small arterioles, as described above.[81] Destruction of normal brain cells occurred secondarily, and as a consequence of this radiation, injury to the vasculature of the brain resulted.

Therefore, an additional parameter was added, namely, that the tumor:blood ratio must be at least greater than one and preferably higher during the radiation procedure. Implicit in this requirement is that the boron compound or its metabolic products must concentrate and persist in tumor cells while blood values fall to appropriately low levels.[82] Therefore, the high LET radiation dose to the vascular endothelium must be below that value that will compromise the normal functioning of the sensitive blood vessel walls. Clearly, the approach in drug design and development has become a more sophisticated one. No longer are compounds merely synthesized simply because they can be prepared. There must be a biochemical/physiological basis to compound development as well as an understanding of how this influences tumor cell targeting.

In essence, in the process of compound design, Figure 8 may be viewed as the composite structure. It is important that the boron component not adversely effect the ability of the tumor-targeting entity to remain soluble in blood, be transported through the vasculartur, cross lipophilic membranes and become incorporated into the neoplastic cells at a sufficient concentration. The difficulty has been in

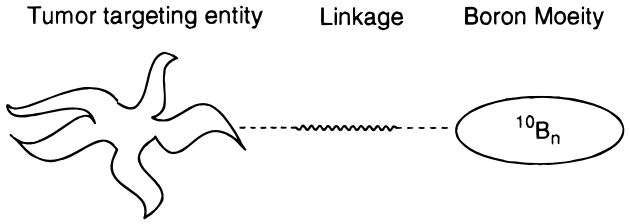


Figure 8. NCT compound design.

predetermining how the boron moiety will affect the tumor-targeting entity in its physiological, biochemical, and tumor-seeking properties. Additionally, it is essential that there not be a significant increase in the compound's toxicity. The key question has been how can these various objectives be attained in one agent?

From the standpoint of targeting tumor cells, what would be ideal site or sites within or on the cell? Since it has been presumed that the target of the high LET particles from the capture reaction is the tumor cell nucleus, locating the boron compound in close proximity to the nucleus would appear to be highly desirable.[139−141] It has been estimated that the cellular boron concentration required may be reduced by a factor of 2 to 5 if the boron compound were located in or near the nucleus. It is for this reason that in order to enhance the relative biological effectiveness (RBE) of the boron capture reaction that the tumor cell nucleus has been one of the sites that has been targeted. However, just as in cancer chemotherapy where several agents are frequently used in combination to deliver multiple noxious impediments to tumor cell replication, one can anticipate that there will ultimately be a combination of different boron compounds each with the capacity for targeting different receptors or tumor cell organelles (i.e., nucleus, mitochondria, endoplasmic reticulum, Golgi apparatus, etc.). It will be this combination of entities that must achieve 10^9 boron-10 atoms/cell.

X. Types of Boron Entities

Before considering specifically the basis for the different classes of boron compounds that have been synthesized as potential BNCT agents, the nature of the various boron moieties will be discussed. These may be broken down into those possessing a single boron atom and those having multiple boron atoms as boron clusters containing B−B linkages.

A. Boronic Acids and Related Analogues Containing Single Boron Atoms

The parent of these structures may be viewed as boric acid and dihydroxyboryl analogues have arisen by the replacement of a single hydroxyl group with an organic moiety. Certainly this organic entity markedly influences the biological properties of the compound. Many of the structures considered as potential BNCT agents have aromatic groups attached. The basis for this selection is the oxidative and hydrolytic stability of this aryl carbon−boron bond and the demonstration that certain of these linkages have been observed to be metabolically

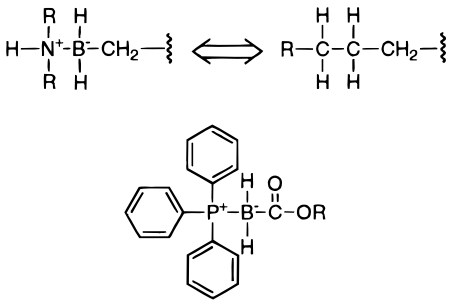


Figure 9. Borane zwitterions.

inert.[142−145] The basis for the continuing interest in such compounds resides in the fact that presently 4-dihydroxyborylphenylalanine (BPA) is one of the two compounds that is being used clinically in BNCT trials.[85,87−90] Related to these is the hydroxyboryl group (B−OH) itself which may be viewed as an isostere of the carbonyl function. When such a group is attached to nitrogen, it is isosteric with an amide. Efforts have been made to incorporate such an entity into heterocycles analogous to purines and pyrimidines.[146−149]

Replacement of a hydroxyl function with an organic species in the boronic acid yields a borinic acid and replacement of the last hydroxyl group produces a trialkyl or triaryl borane. A major limitation in the use of the latter such compounds would be their decreasing aqueous solubility. Not all of the efforts to incorporate boron into heterocycles have involved the insertion of the B−OH entity. Others have incorporated boron between two nitrogen atoms.[150−156] Unfortunately, such structures are hydrolytically unstable and therefore are not useful as tumor targeting compounds.

B. Borane Zwitterions

In general, boranes, as epitomized by BH_4^-, are hydrolytically unstable giving rise to boric acid. However, there is another type of borane structure which retains both hydrolytic and metabolic stability while increasing aqueous solubility. These are boranes that are stabilized by electron-donating functional groups such as occurs in the aminoboranes.[157,158] These may be functionally considered as isosteric with a carbon−carbon single bond. Such structures are zwitterionic with an anionic boron atom having four valence electrons and a cationic nitrogen. Such a complex may enhance the compound's water solubility, and this moiety has been incorporated into compounds that have biochemical antecedents. These will be presented in more detail, subsequently. Other group V elements have also been incorporated into related borane zwitterions. These include phosphorus as shown in Figure 9.[159]

C. Polyhedral Borane Anions and Carboranes

The chemical structures considered heretofore contain a single boron atom. All things being equal, there would be clear advantages for those compounds possessing multiple boron atoms. The advantage may be that if compounds possess comparable molar toxicity, higher boron concentrations could be admin-

istered with those having multiple boron atoms and thereby higher tumor concentrations may be achieved. The issue obviously is not attaining a specific molar concentration in neoplastic cells but the necessary boron level. It is for this reason that a great deal of effort in developing BNCT agents has focused on compounds containing multiple boron atoms. This was the precise reason that in the initial stages of compound evaluation, Borax and derivatives of pentaborate were considered. However, there was no significant advantage of these structures by comparison with boric acid. Nevertheless, the concept of developing agents with multiple boron atoms remains an attractive one with the proviso that these structures must be stable to hydrolytic and physiological conditions and possess low mammalian toxicity. This was the basis for synthesizing aryl diboronic acids[160] and boronic acids attached to a boron heterocycle.[149]

The polyhedral borane anions and many of the various carboranes appear to meet the requirement of possessing high boron percentages, and for this reason, there has been significant effort in the area of compound development directed toward the incorporation of such entities into organic structures. The two polyhedral borane anions, $B_{10}H_{10}^{2-}$ and $B_{12}H_{12}^{2-}$, and dimers of the former have been used in the development of BNCT agents.[161−174] Interest persists in such structures because of their inherent stability, their potential for incorporating functional groups and various organic moieties into these clusters, and the fact that the sulfhydryl derivative, sodium mercaptoundecahydrododecaborate ($Na_2B_{12}H_{11}SH$, BSH), is one of the two compounds that has been and is being used clinically. The polyhedral borane anions are charged species, and there has been concern that their incorporation could significantly influence a compound's transport, especially with respect to its ability to cross lipophilic barriers such as those in cell membranes or the BBB. Additionally, such structures may bind nonspecifically to various plasma proteins and other biopolymers. The question has been, how would such ionic charges influence tumor targeting?

On the other hand, the carboranes, especially those containing the $C_2B_{10}H_{12}$ nucleus, are very organic in nature, and the method for their incorporation into various organic/biochemical substrates has now become well developed.[65,175−184] Much of this effort has dealt with the use of the 1,2-$C_2B_{10}H_{12}$ (ortho), in contrast with the corresponding 1,7- (meta) and 1,12- (para) isomers (Figure 10). However, in recent years, there has been an increased interest in the latter two because of their enhanced stability toward degradation by various bases.[185−190] The ortho isomer can be readily synthesized from appropriately substituted acetylenes and their reaction with various nitrile and sulfide adducts of decaborane ($B_{10}H_{14}$) (Scheme 5).[175,176] Hydrophilic groups on the acetylenic compounds must be masked in order that the synthetic sequence will produce the desired *o*-carborane compound, otherwise they will degrade the decaborane complex. Yields are frequently in the 40−60% range. From a molecular standpoint, the carborane moiety approximates the three-dimensional sweep of the phenyl

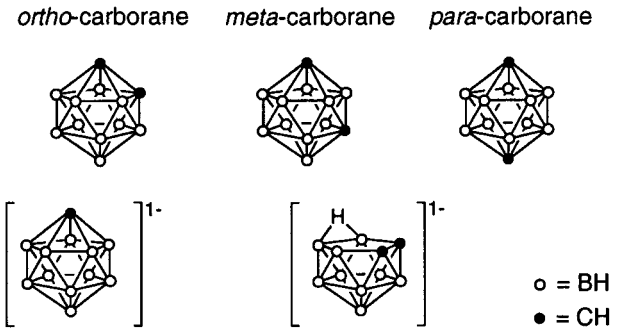
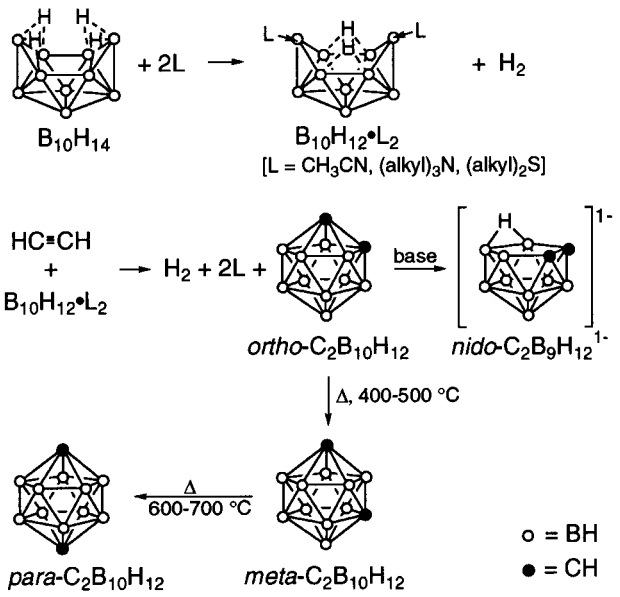


Figure 10. The dicarba-*closo*-dodecaboranes(12): 1,2-$C_2B_{10}H_{12}$ (ortho); 1,7-$C_2B_{10}H_{12}$ (meta); 1,12-$C_2B_{10}H_{12}$ (para). Carborane anions: *closo*-$CB_{11}H_{12}^{1-}$ and *nido*-$C_2B_9H_{12}^{1-}$.

Scheme 5. Preparation of Various Dicarbon Carboranes



group.[191] Interestingly enough, such carborane moieties are extremely hydrophobic and comparable to the adamantyl group in lipophilicity.[192] On the face of it, this would appear to be a desirable physiological property, since lipophilic compounds have been shown to cross the BBB with great facility. However, attachment of such a highly lipophilic entity to any compound could result in structures which bind nonspecifically through hydrophobic bonds to various biopolymers especially lipids in blood and other tissues. Thus, the objective of achieving high tumor:blood and tumor:normal tissue boron ratios could be compromised if the resulting compounds contain a very highly lipophilic component. There must be a suitable balance between a compound's hydrophilic and lipophilic properties if one is to attain suitable differentials between blood and normal tissue and ultimately between normal tissue and tumor. The meta and para isomers are derived from the ortho isomer via high temperature rearrangement of the latter (Scheme 5).[175] Thus, to succeed with functionalized analogues, the functional groups must be stable to the high temperatures that will be required. Alternatively, the meta and para isomers could be used as the starting materials for synthesizing the desired compounds; however, these compounds have reduced reactivity relative to the ortho isomer.

A third group of boron clusters used in BNCT chemistry is the negatively charged carboranes represented by the *closo*-$CB_{11}H_{12}^{1-}$ [193-197] and the *nido*-$C_2B_9H_{12}^{1-}$ [186,188,198-206] cages. The latter is derived through base degradation of an *o*-carborane nucleus. Their physicochemical properties resemble those of the aforementioned negatively charged polyhedral borane anions, and both have been considered as potential moieties for incorporation into tumor-targeting structures.

XI. "Third Generation" Boron Compounds

A. Evaluation Studies

The one factor that unifies the boron compounds in this category is that they have not reached the stage of being evaluated clinically. Many have been screened in cell culture: some using purified enzymes to determine their metabolism; others have been evaluated in rodents with a variety of subcutaneously transplanted animal and human tumors; and fewer still have been screened against these same tumors that are implanted intracranially. From the available data, how does one decide to eliminate or further evaluate a compound, since additional studies are both costly and time-consuming? The key question in compound development is assessing the usefulness of enzymatic, cell culture, and animal studies in determining the compound's clinical potential. This is not a trivial problem since the constant question is, how do these newer agents compare with those two compounds that are now being used clinically? The approach is not necessarily the replacement of existing agents, although that is possible, but to increase tumor specificity with additional compounds that may function by different biochemical and physiological mechanisms than those that apply to existing agents.

Tumor targeting is only one factor. A second one, of equal and crucial importance, is the issue of toxicity, since regardless of a compound's ability to achieve adequate and selective tumor concentration, if its toxicity is at an unacceptable level, then there is little need for any further evaluation. Again, the question is can the studies in cell culture and small animals be translated ultimately into humans? Clearly, it would not be advantageous to synthesize multigram amounts of each compound, especially if the targeted structure required a multistep synthetic sequence. The issue of toxicity is a recurrent theme with every new agent, and it may be anticipated that compounds with demonstrated selectivity for neoplastic cells may possess some inherent cellular toxicity. It is not an either/or condition but one of degrees. Thus, a certain level of toxicity must be expected but the real question is can that level be tolerated in order to attain the needed tumor cell boron concentration?

B. Classes of Boron Compound

The structures that may be classified as "third generation" compounds have, in general, a more biochemical and physiological basis for achieving tumor cell selectivity than the structures that have been previously designed and synthesized. Table 5 contains a listing of these generalized compounds.

Table 5. Classes of "Third Generation" Boron Compounds

1. Cellular Building Blocks
   a. Boron-Containing Nucleic Acid Precursors
   b. The Development of Boron-Containing Amino Acids and Related Peptides
   c. Lipids and Phospholipids
   d. Carbohydrates
2. Lipoproteins
3. Liposomes
4. Porphyrins and Phthalocyanines
5. DNA Binders
   a. Alkylating Agents
   b. Intercalators
   c. Groove Binders
   d. Polyamines
   e. Di- and Oligonucleotides Antisense Agents
6. Receptor/Antigen Binders
   a. Antibodies Monoclonal/Bispecific
   b. Growth Factors
   c. Hormones
7. Other Compounds
   a. Radiation Sensitizers
   b. Phosphates, Phosphonates and Phosphoramidates
   c. Cyclic Thiourea Derivatives
   d. Amines
   e. CNS Depressants
      i. Promazines
      ii. Hydantoins and Barbiturates
   f. Miscellaneous

1. Cellular Building Blocks

Compounds falling into this category have their origins in cancer chemotherapy.[207] Many that have been designed may be viewed as analogues of biochemical building blocks. The rationale for their synthesis is that, in contrast with normal cells, tumor cells are in various stages of cell division and therefore may have an elevated requirement for certain constituents necessary for cell replication. In cytoreductive chemotherapy, such fraudulent analogues have the capacity for competing with natural substrates and, in this manner, interfere with the replicative cellular process. The problem is that such inhibition cannot be confined solely to malignant cells since rapidly proliferating normal cells are similarly compromised. However, in the case of BNCT, the objective is to design boron-containing compounds that sufficiently emulate naturally occurring precursors needed for tumor cell replication. They should, at the same time, not adversely affect the cell's biochemical machinery. The objective is to use this biochemical metabolism to achieve selective boron incorporation, and it is the latter that provides the basis for specific cellular destruction. In essence, this may be viewed as a "Trojan horse" approach at the cellular level.

Clearly, the toxicity of such structures cannot be avoided; the question is not whether nontoxic analogues, which can be acted upon by biochemical systems, can be designed, but whether the level of toxicity will be tolerable. Synthesizing such biochemically active compounds certainly increases the compound's potential for being toxic. Thus, it re-

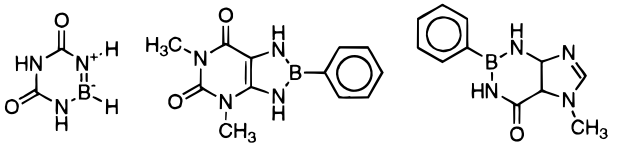


Figure 11. Hydrolytically unstable boron-containing pyrimidines and purines.

mains to be determined how successful this strategy of synthesizing boron-containing cellular building blocks will be in creating new BNCT agents.

a. Boron-Containing Nucleic Acid Precursors. Of all of the cellular building block analogues listed in Table 5, the most widespread chemical effort has been related to the design and synthesis of potential precursors of the nucleic acids. The rationale for their synthesis is readily understandable since the presumed target of the high LET radiation generated by the boron capture reaction is the tumor cell nucleus. Thus, synthesizing compounds with the capacity for competing biochemically with normal precursors of nucleic acids and becoming incorporated into cellular nucleic acids would seem to have merit. This approach appears to be especially pertinent since malignant cells have shorter doubling times than their normal counterparts and therefore require larger quantities of such biochemical substrates.

Initial efforts focused on the nucleic acid bases themselves, the purines and pyrimidines. Boron incorporation into such structures took many approaches. Some involved ring insertion in which the boron atom replaced carbon in such structures.[146−156] The more readily synthesizable compounds are those in which boron is flanked by two nitrogen atoms (Figure 11). However, such linkages are hydrolytically unstable in general, and when their stability is enhanced by placing bulky or aromatic groups on boron, the compounds cease to emulate biochemically, the normally occurring substrates.

Another approach was to attach the boron moiety directly on a pyrimidine nucleus. This led to the synthesis of 5-(dihydroxyboryl)uracil,[208,209] other dihydroxyboryl-substituted bases,[210−212] and carborane-containing pyrimidines (Figure 12).[213−217] Subsequently, this work led to the first boron-containing nucleoside, 5-(dihydroxyboryl)-2′-deoxyuridine (DBDU), prepared by Schinazi and Prusoff.[210] The rationale was clear; thymidine analogues, 5-bromodeoxyuridine (BUdR) and 5-iododeoxyuridine (IUdR), have K_m values[218] comparable with those of thymidine itself and their phosphorylation to the corresponding nucleotides has been used to measure the fraction of cells in S-phase in human brain tumors.[219] Interestingly enough, the labeling index with BUdR was greatest with the more highly malignant gliomas, but even so, the percent of cells that were labeled showed great variability. Biological studies with DBDU showed that this boron analogue of thymidine was nontoxic to Vero cells at a concentration level of 1.6 mM.[220−222] Although there was no chemical evidence for its phosphorylation or DNA incorporation, nevertheless in radiobiological studies using ^{10}B-enriched compound, it could be inferred that DBDU replaced thymidine to the

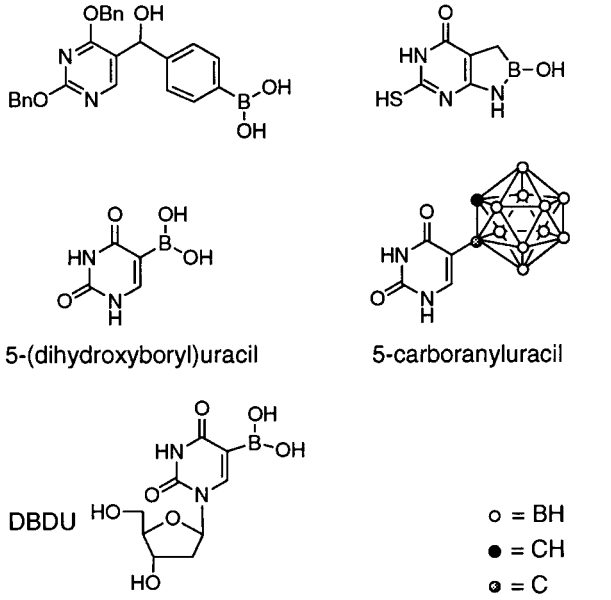


Figure 12. Boron-containing nucleic acid bases. Structure of 5-(dihydroxyboryl)-2′-deoxyuridine, DBDU.

extent of 5−15%.[220−222] Subsequent synthetic studies have focused almost exclusively on the development of boron-containing nucleosides, since these are further along in the metabolic chain than are the purines and pyrimidines and their intracellular conversion to the corresponding nucleotide could result in their intracellular entrapment. This is a key objective in the development of tumor-targeting agents, independent upon whether such structures are incorporated into DNA. In the case of certain nucleosides, there are a carrier-mediated process and other transport delivery systems operating independently of the compound's lipophilicity that permit such structures to penetrate the BBB and various cell membranes.[223] It is for this reason that such boron analogues have been considered as potential BNCT agents for targeting brain tumors.

Yamamoto et al. developed a new synthetic method for the preparation for such compounds, starting with 5-halogenated nucleoside derivatives (Figure 13).[211,212,224] The initial compounds contained the boronic acid moiety. A second, more recent approach, has focused once again on incorporating the hydroxyboryl group into pyrimidine analogues.[147] However, instead of flanking the boron moiety by two nitrogen atoms, an hydroxyboryl group was attached to both a carbon and a nitrogen atom (Figure 14). A key question was whether such a ring structure would possess hydrolytic stability. The synthesis of the benzoborauracils[146] and their corresponding nucleosides[225] demonstrated the stability of this new ring system, which may be viewed as being more closely analogous to the pyrimidine nucleus than any structure synthesized heretofore. However, it remains to be determined whether analogues without the aryl moiety can be synthesized, compounds that would be formally related to both deoxyuridine and thymidine, and if such structures remain intact under physiological conditions.

Another important class of boron-containing nucleosides are those which may be viewed as contain-

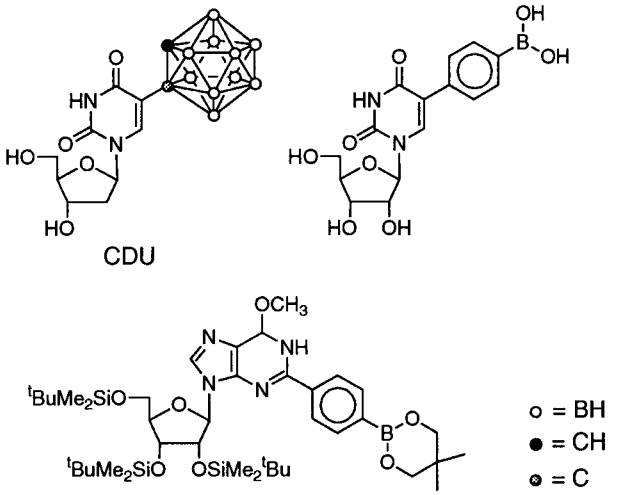


Figure 13. Boron-containing nucleosides and precursors developed by Yamamoto et al.

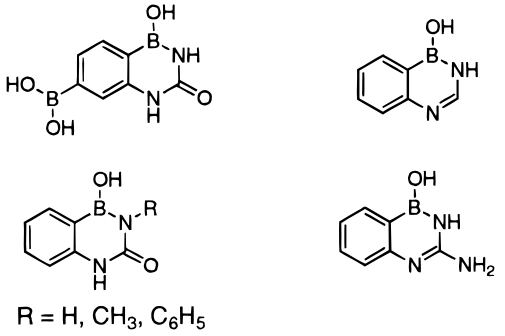


Figure 14. Hydroxyboryl isosteres of benzopyrimidines.

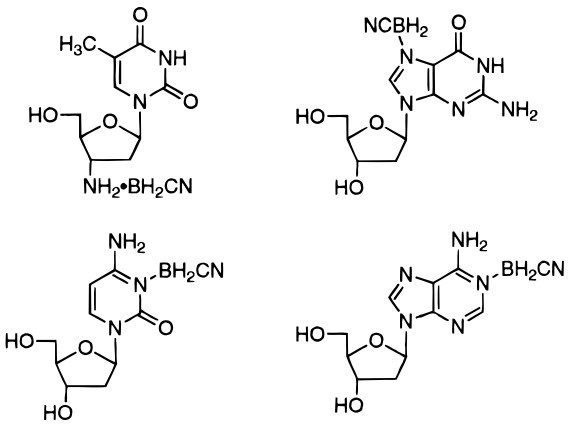


Figure 15. Nucleoside derivatives developed by Spielvogel et al.

ing borane zwitterions. Spielvogel and his associates have pioneered in their development.[226−233] In the case of the pyrimidines, they have attached the cyanoborane moiety to the N3 position of 2′-deoxycytidine and the 3′ position of an analogue thymidine. Such structures have been more fully elaborated in the case of the purine nucleosides where the same cyanoborane function has been attached at both the N1 and N7 positions. Representative structures of both the pyrimidine and purine nucleosides are shown in Figure 15.

All of these structures possess a single boron atom. Although this is not a limitation per se, since of greater importance is the compound's biochemical

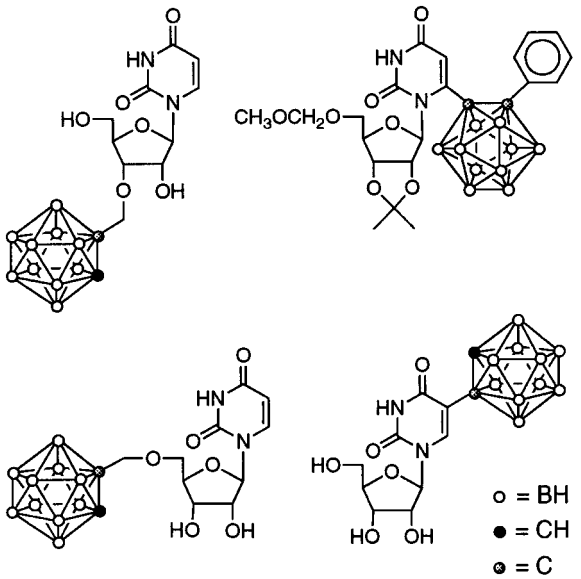


Figure 16. Carborane-containing nucleoside derivatives.

properties, nonetheless, the boron percentage in all of these compounds is relatively low. It is precisely for this reason that a number of investigators have incorporated stable boron clusters into nucleosides.[202,211−216,224,234−251] The insertion of a carboranyl group, having 10 boron atoms, could be advantageous since such nucleosides would have a 10-fold increase in the number of boron atoms and a correspondingly higher percentage of boron. Figure 16 shows some of the various structures that were initially synthesized with groups on the 2′, 3′, 5′, 5, and 6 positions. In some cases, the carbohydrate moiety is different than the 2′-deoxyribose group. Also, substituents have been attached to the C2 position of the *o*-carborane nucleus.

An important question in designing nucleosides is assessing their potential for becoming phosphorylated to their corresponding nucleotides. 5-Carboranyldeoxyuridine (CDU)[240] was the first carboranylpyrimidine nucleoside that was shown to be phosphorylated,[234] albeit by no means comparable with the rates for thymidine or deoxyuridine. Nevertheless, this was a very important step. One basis for predicting phosphorylation is to use the information obtained from affinity chromatography, where the interaction of kinases with immobilized nucleotides has been explored. In this context, the accessibility and binding of the nucleosides/nucleotides to the active sites of the kinases may be a useful predictor in determining the rate and extent of phosphorylation. Kinase binding to immobilized nucleotides was enhanced by the use of a series of linked spacer atoms, which both insulate and project the nucleotide from the matrix surface.[252] When this tether was approximately 10 Å, by interposing 6−8 methylenes or a comparable linker chain of atoms, there was a substantial increase in the strength of enzymatic binding. When this approach of insulating and projecting the bulky carboranyl group from the pyrimidine nucleoside was used,[253−262] an enhanced rate of nucleotide formation was observed in the action of human thymidine kinase (TK).[257−261,263] However, the rates of phosphorylation of the tethered

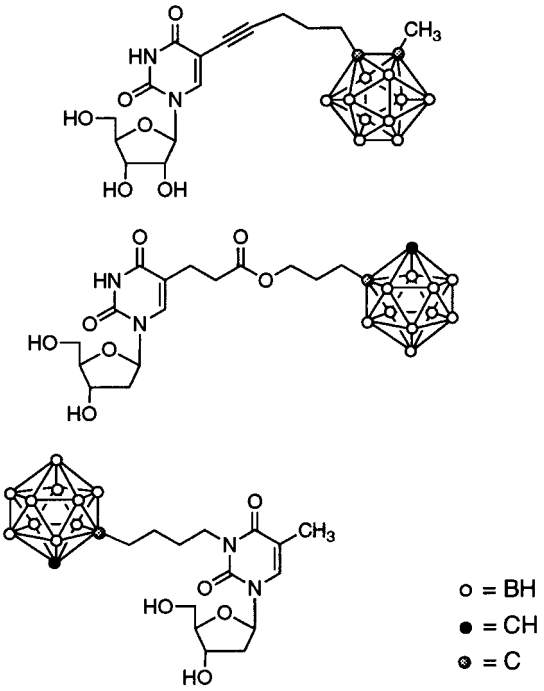


Figure 17. Tethered carboranyl pyrimidine nucleosides.

nucleosides are not readily predictable, and those factors that enhance reactivity by kinases need to be explored further. In Figure 17, the structures of some of these different tethered carboranyl pyrimidines are presented.

One of the key limitations with all of these carboranyl nucleosides is that their very significant lipophilicities severely compromise any assessment of their enzymatic reactivity or in vitro cellular evaluation. To ameliorate this problem and achieve a suitable hydrophilic/lipophilic balance, the *closo*-carboranes can be converted to their anionically charged nido counterparts.[43,202] A number of these have now been synthesized. Alternatively, ionic functions could be inserted into the molecule or nonionic hydrophilic moieties can be attached. In general, the *nido*-carborane structures have been reported to demonstrate increased toxicity, but this generalization remains to be proven with the newer compounds that have been synthesized. These compounds and those in which ionic groups have been inserted have the potential for nonspecific ionic binding to various biopolymers in the vascular system. It is for this reason that the attachment of nonionic hydrophilic functions to the carborane nucleus[213,214,259,261,263] or the ribose portion[239] of the nucleoside has been undertaken. The objective of this work has been to improve aqueous solubility without compromising TK binding or tumor targeting. In Figure 18 are examples of such structures. It has been shown that increased water solubility resulted, in certain instances, in a significant improvement in the rate of phosphorylation.[261] However, in the case of CDU, inserting the dihydroxypropyl group resulted in a decrease in this rate.[261] It remains to be determined whether these results can be translated into useful in vivo tumor-targeting and retentive agents.

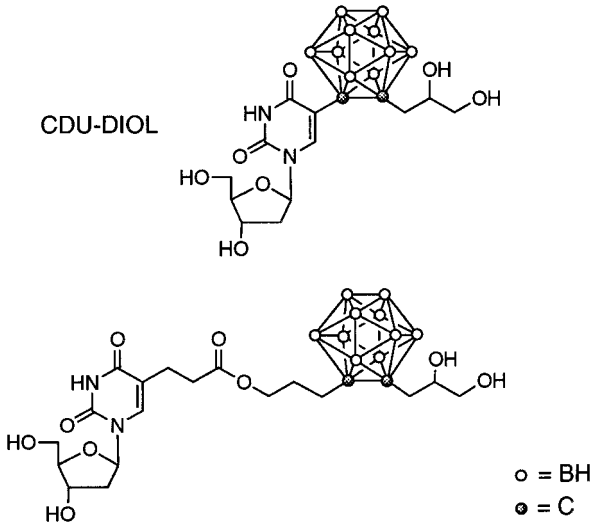


Figure 18. Hydrophilically enhanced carboranyl pyrimidine nucleosides.

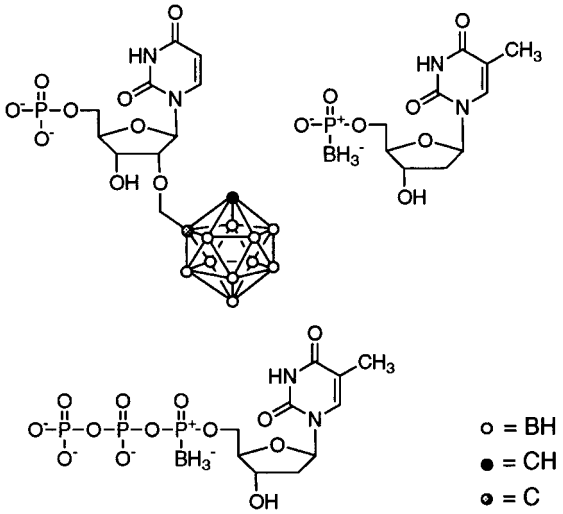


Figure 19. Boron-containing nucleotides.

This approach is essentially an empirical one. The use of computer drug modeling[264] of the structure and docking to the active site of TK may provide a more rational approach to drug design. However, there is uncertainty as to whether designing compounds that will meet biochemical objectives can result in superior agents for incorporation and retention by malignant cells under in vivo conditions. To date this has not been accomplished but it is clear that the development of boron-containing nucleosides will be continuing especially in view of the results that have been attained with cancer chemotherapy and in the development of new antiviral agents.

In addition to nucleosides, nucleotides have been prepared and biologically evaluated (Figure 19).[159,227,231,234,245,246,248,265−270] Although such ionic structures may be viewed as lacking an ability to cross the cell membrane, their penetrability will be based upon the overall lipophilic/hydrophilic balance and not the fact that they are nucleotides. Since some of these contain a carborane moiety, their lipophilic properties will be enhanced. More importantly, the enzymatic cleavage of such phosphates under in vivo conditions may make these compounds prodrugs.

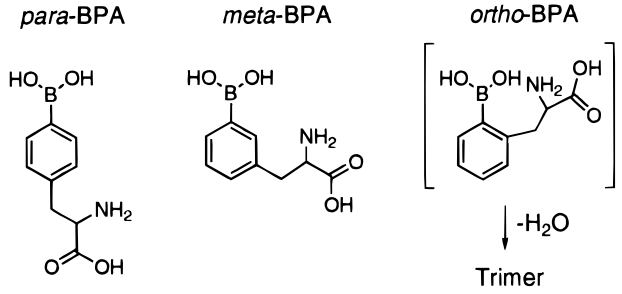


Figure 20. Dihydroxyboryl analogues of phenylalanine.

b. The Development of Boron-Containing Amino Acids and Related Peptides. The interest in the development of boron-containing amino acids and related peptides stem certainly in part from the fact that 4-dihyroxyborylphenylalanine (BPA) is one of the two clinically used BNCT agents. The view is that such cellular building blocks may be required to a greater extent in more rapidly proliferating cells (i.e., tumor cells) than in their normal counterparts. This requirement may lead to the synthesis of peptides and proteins derived from these amino acids and thereby their retention in the cell's matrixes. This, once again, would meet the objective for both tumor targeting and retention.

In addition to the para isomer of BPA,[111,271−274] the ortho and meta positional isomers have been synthesized and evaluated.[275−278] The ortho isomer exists as a cyclic internal anhydride.[278] In Figure 20, dihydroxyboryl analogues of phenylalanine that have been prepared are shown.

Only the para isomer of BPA has been subjected to extensive biological evaluation. Early on, it was recognized that the compound's low aqueous solubility was an impediment to its intravenous administration. To overcome this problem, researchers initially used the ability of the boronic acid moiety to complex with carbohydrates as a means of increasing the compound's aqueous solubility.[279−282] This has been achieved by using a fructose complex, which has been used in clinical studies. Others have attempted to increase water solubility significantly by the incorporation of nonionic, hydrophilic groups into BPA.[283−285] The latter would obviously change the biochemical nature of the amino acid. In Figure 21, structures are presented in which the carboxyl function has been converted to mono-, di-, and tetrahydroxy amide derivatives.

One of the limitations in evaluating the pharmacodynamics of any of these boron compounds is an inability to study their real-time in vivo kinetics. There are no useful boron radionuclides, and it is for this reason that ^{18}F−BPA was synthesized in which the radiolabel was inserted into the 2 position of the aromatic ring.[286−288] The utilization of this structure is predicated on the fluoro analogue of BPA being completely analogous to BPA itself in terms of its uptake and retention by tumors. Positron emission tomography of ^{18}F−BPA in patients with malignant brain tumors has demonstrated the targeting ability of BPA.[289−291]

An important question with BPA was which of the para isomers, the D and L analogues, was more useful in tumor targeting? The view was that the isomer

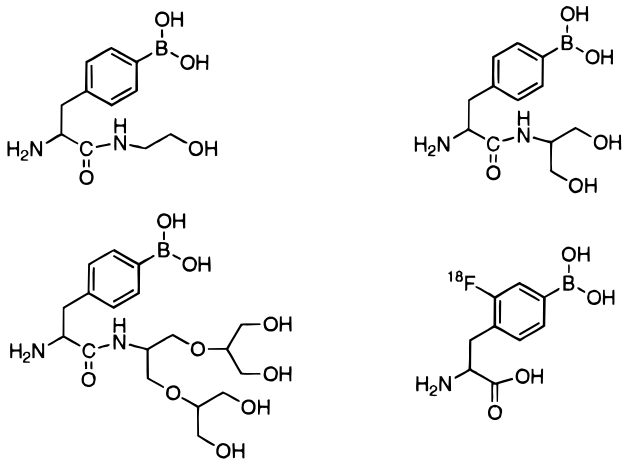


Figure 21. Hydrophilic derivatives of BPA, and a positron-emitting analogue of BPA (^{18}F−BPA).

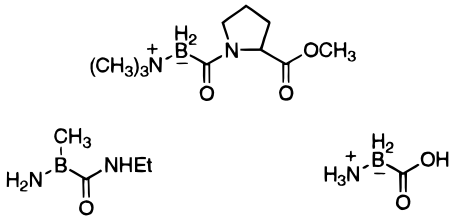


Figure 22. Boron-containing amino acid derivatives.

analogous to the naturally occurring amino acid (L) should be more effective. In vivo experiments in tumor-bearing animals and in vitro cell culture studies have shown that this is the case, supporting the assumption that the amino acid transport system may be operative for L-BPA in achieving elevated tumor concentrations.[130−132] A number of different dihydroxyboryl-containing amino acids have been synthesized. These include those related to aspartic acid and cysteine[144] but little has been reported on their potential as BNCT agents. Other research has focused on the replacement of the carboxyl group in the amino acid with a dihydroxyboryl group.[292,293] The rationale was that such compounds may act as transition-state inhibitors of enzymes but little has been done with respect to their possible use as BNCT compounds.

Not all of the syntheses of boron-containing amino acids have focused on those with the boronic acid moiety. Others have incorporated the borane zwitterionic group. This is epitomized by ammoniacarboxyborane, H_3NBH_2COOH, which may be viewed as an isostere of glycine.[157,158] This development has led to synthesis of a number of borane-containing amino acids shown in Figure 22, and distribution studies in tumor-bearing animals are described. Understandably, there has been interest in making analogues that contain boron clusters, and it was for this reason that one of the earlier compounds described was *o*-carboranylalanine (*o*-Car). It was first synthesized independently by Zakharkin and his associates[294] and Brattsev et al.[295] The compound was prepared as the racemic mixture. Subsequently, there have been other methods for its preparation in higher yield,[191,296−298] as well as the stereoselective synthesis of the L-isomer ((*S*)-configuration) initially by Schwyzer et al.[299] More recently, Kahl,[300−302]

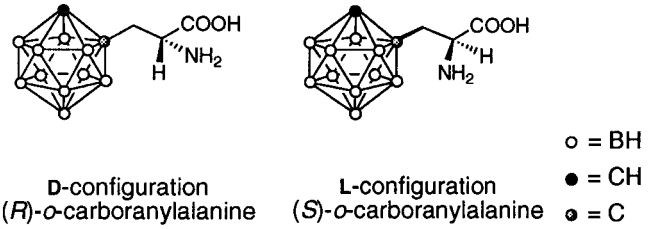


Figure 23. The L- and D-enantiomers of *o*-carboranylalanine.

Sjöberg[303,304] and Moroder[305] have independently developed more useful stereoselective syntheses and have prepared both the L and D forms (Figure 23). Since the carboranyl group approximates the phenyl group in its three-dimensional sweep, it was envisaged that this amino acid may simulate phenylalanine in its biochemical properties. Of special interest was a biological assessment of *o*-Car and its in vivo comparison with the clinically useful BPA. Improved synthetic procedures for *o*-Car as well as a greater understanding of its physiochemical properties were necessary. These provided the basis for the studies with tumor-bearing mice, demonstrating that *o*-Car attained higher blood concentrations and lower tumor levels than did BPA at comparable time intervals and that the former showed no evidence of tumor selectivity.[306−310] The fact that the carboranyl moiety is highly lipophilic and 112 times more hydrophobic than the indole side chain of tryptophan may account for its persistence in blood through noncovalent association with blood lipids.[191] These results are supported by previous studies in which phenylalanine (Phe) and tyrosine (Tyr) residues in various bioactive peptides and polypeptides are replaced with the *o*-Car moiety.[299,311,312] Such analogues were also biologically active and, in some instances, showed a prolongation of activity by comparison with their Phe counterparts. These results demonstrate the need to ameliorate the lipophilicity of the carboranyl group by the incorporation of functionalities that will balance the compound's lipophilic properties. At this time, a number of different carborane-containing amino acids have been synthesized and evaluated.[161,189,296−298,313−324] Not all of these are classical α-amino acids. A very interesting amino acid structure has been prepared by Kahl and Kasar[190,325] involving *o*-, *m*-, and *p*-carboranes in which the amino and carboxyl functions are located on the different carbon atoms of the carborane nucleus. Another category is described by Kabalka et al.[326−328] in which either an *o*-carborane or a *nido*-carborane nucleus is inserted into 1-aminocyclobutanecarboxylic acid. The low aqueous solubility of these *o*-carborane-containing amino acids is a key disadvantage once again in their biological evaluation. Hawthorne et al. used the degradation of the *o*-carborane moiety by base to produce the negatively charged *nido*-carborane species.[319] Such structures show improved water solubility. An alternate approach considered by him and others is to attach polyol structures to such compounds.[315,329] It has not been ascertained what is the appropriate balance between the lipophilic/hydrophilic properties that the amino acids must possess. Another very interesting carborane-containing amino acid was generated by the insertion of the carborane group into 3,4-dihydroxyphenylalanine (DOPA).[322] This compound, in contrast with BPA, would not have to lose the boron moiety in order to generate the indolequinones, the purported precursors of melanin that occur in significantly elevated amounts in melanomas. The structure is shown in Figure 24. In addition to carborane-containing amino acids, a polyhedral borane anion analogue of methionine has been described (Figure 25).[161]

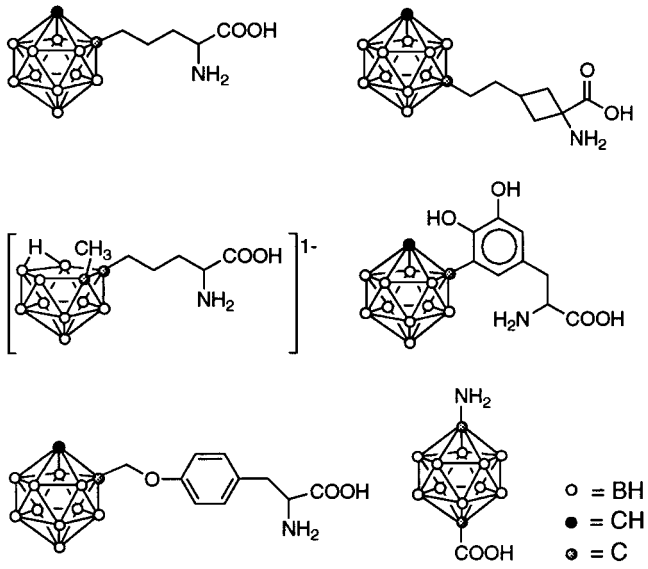


Figure 24. Carborane-containing amino acid analogues.

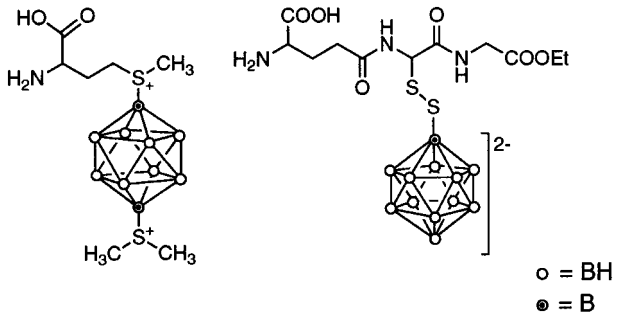


Figure 25. Boron-containing analogue of methionine, DL-*S*-(10-dimethylsulfidooctahydrodecaboranyl)methionine, and BSH-glutathione disulfide derivative.

The synthesis of these boron-containing amino acids stimulated interest in the development of boron-containing peptides. The rationale was based on the projected increased need by tumor cells for protein precursors and the feasibility that small peptides may cross cellular membranes and be utilized by tumor cells. The early boron-containing di- and tripeptides were those derived from the zwitterionic borane-containing amino acid analogues and their coupling to various amino acids.[157,330] Examples of these are shown in Figure 26. Interest in such compounds primarily arose from their hypocholesterolemic and triglyceridemic activity and not solely from their BNCT potential.

A second effort in the development of boron-containing peptides focused on the use of carboranylalanine by Schwyzer et al.[311,312,331−333] Work by Hawthorne et al., using *o*-carborane-containing amino acids of both the closo and nido analogues, was done

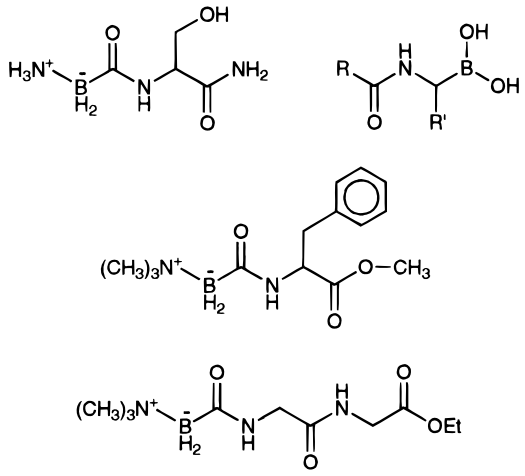


Figure 26. Boron-containing peptides.

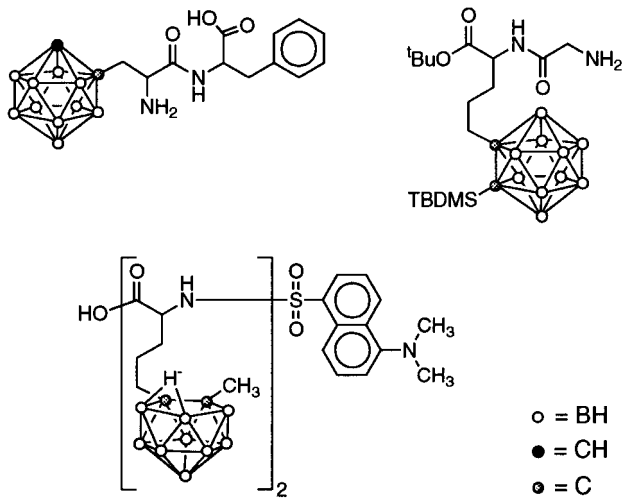
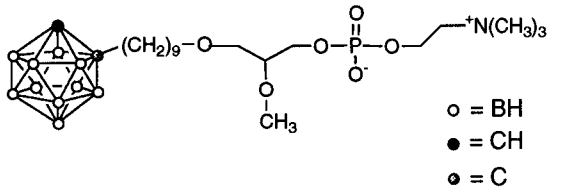


Figure 27. Carborane-containing peptides.

in conjunction with the development of boron-containing antibodies that will be discussed later in this review.[199,205,319] Others related to their use as tumor targeting agents and involved racemic mixtures as well as enantiomeric structures.[313,334] Representative compounds are shown in Figure 27.

There has been little advantage in developing peptides that could be formed from BPA together with other amino acids since these would possess a reduced boron percentage by comparison with BPA itself. However, they might provide very useful information as to the types of dipeptides that achieve tumor cell uptake and persistence. Nevertheless, the major emphasis in developing peptides has been with boron clusters, the carboranes, as well as the polyhedral borane anions. An example of the latter is the glutathione analogue, shown in Figure 25, whose synthesis[335] was driven by the improved tissue distribution characteristics of BSH and BSSB when used in concert with glutathione.

The preparation and evaluation of small boron-containing peptides is in an early stage of development by comparison with BPA and other boron-containing amino acids. It remains to be determined whether the rationale for their development, namely, the improved tumor to normal tissue and blood ratios, as well as enhanced tumor concentrations vis-à-vis



rac-1-(9-*o*-Carboranyl)nonyl-2-methyl-glycero-3-phosphocholine (B-Et-11-OMe)

Figure 28. Carborane-containing phospholipid.

the amino acids from which they are derived, can be achieved. In this area as well, developing more hydrophilic analogues of peptides and proteins has been viewed as an important objective.[336−338]

c. Lipids and Phospholipids. The observations that various natural and synthetic ether lipids possess a proclivity for and a persistence within a variety of both spontaneous and chemically induced tumors appears, based in part, on the absence of the enzyme O-alkylglycerol monooxidase in these neoplasms. Such accumulation, demonstrated by a radioiodinated phosphocholine analogue, led Lemmen and his collaborators to synthesize a carborane-containing phospholipid (Figure 28).[339] The results from both cell culture studies and in tumor-bearing animals were not encouraging.[340−344] The conclusion was that the trace level of compound used with the nonboronated analogues achieved subcellular concentrations by a different pathway than is obtained when larger amounts of the boronated phospholipid are used. Once again, this demonstrates that it is not possible to extrapolate from promising results in which trace amounts of a substance are used to those, such as BNCT, in which there is clearly a different order of magnitude of the amount that must be administered.

d. Carbohydrates. There are significant differences in the carbohydrate composition of the cell membrane surface of malignant cells by comparison with the normal cells from which they are derived.[102] Can such compositional changes in these various carbohydrate-containing compounds be used as the basis for the selective targeting of tumor cells? Even if the specific chemical differences were clearly elucidated, could these be used to achieve the needed differential concentrations between tumor and normal tissues? One approach, involving low molecular weight substances, could focus on providing the tumor with carbohydrate precursors of those glycoproteins, glycolipids, mucins, and nucleosides that have been shown to occur in elevated amounts in tumor vis-à-vis their normal counterparts. The incomplete biochemical knowledge, certainly from a quantitative standpoint and especially with respect to proximal precursors of structural carbohydrates, makes this a difficult task, and of course, what would be the impact of the boron moiety upon the utilization and incorporation of such carbohydrates? Another approach has been undertaken to design boron-containing carbohydrates based on the view that such structures may achieve elevated concentrations in tumors through the action of the glucose transport system.[345,346] The early boron cage compounds containing a carbohydrate moiety were those that were developed for binding to antibodies.[329,347−349] The

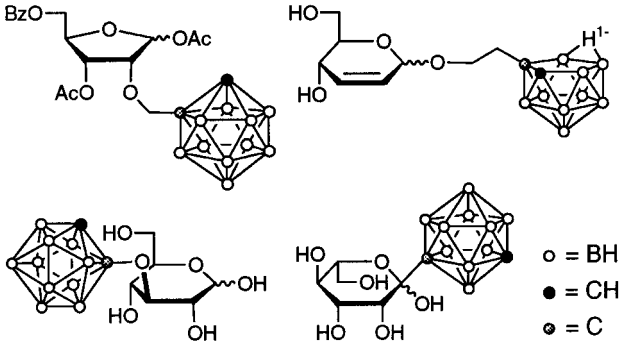


Figure 29. Carborane-containing analogues of carbohydrates.

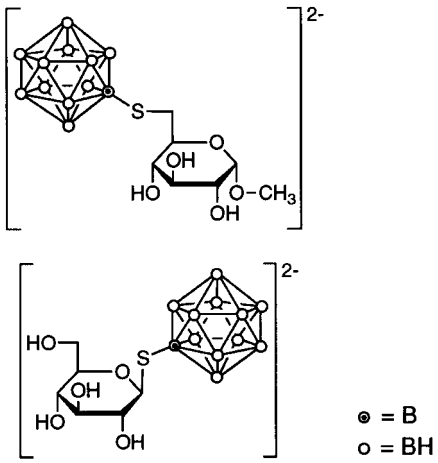


Figure 30. Thiododecaborate-containing sugars.

purpose of the carbohydrate functionality was solely to increase the compound's aqueous solubility. Subsequently, carborane-containing analogues of glucose, mannose, ribose, and gulose have been prepared (Figure 29).[202,247,248,350,351]

More recently, a series of carbohydrates have been synthesized in which the BSH moiety has been attached to the carbohydrate group.[345,346] Representative structures are shown in Figure 30. As may be expected, these compounds are very water soluble. There is no evidence that any of the above-mentioned compounds behave as precursors of structural carbohydrates or that they use the active transport system to achieve differential uptake in tumors.[352] However, their biodistribution evaluation is limited and much more remains to be accomplished.

This concludes the section on boron-containing cellular building blocks. By and large, these are low molecular weight compounds that may be used for all tumors. The compounds described in subsequent sections are both low and high molecular weight structures where the latter may be useful for targeting tumors other than those of the CNS. For those arising from the brain or metastatic to it, the compounds must possess the capability of penetrating the normal BBB and targeting tumor cells therein. In general, this restricts the usefulness of the macromolecular species unless techniques involving the disruption of the BBB are used.

2. Lipoproteins

One of the observed differences between tumor cells and their normal counterparts is the rate of metabolism of low-density lipoproteins (LDLs).[353] This difference is based upon the increased need that tumor cells possess for cholesterol in order to facilitate new membrane formation and thus could be viewed as a cellular building block. The overexpression of the LDL receptor on the tumor cell membrane is responsible for its LDL accretion. It was this observation that led Kahl to first propose using such receptor mediation as the basis for selectively delivering boron to tumor cells.[354,355] His idea was to extract cholesterol from the LDL particle and replace it with a boron species that would simulate cholesterol in its physiochemical properties and thereby deposit the boronated compound intracellularly. The initial compounds synthesized were esters of carborane carboxylic acid with various fatty acid alcohols (Figure 31).[356] Since the core of these particles is hydrophobic, other carborane-containing substances have also been used in place of these esters by virtue of the fact that the carborane moiety itself is highly lipophilic.[357] In vitro studies of these boron-containing LDLs demonstrated high cellular uptake.[8,358,359] After this work, Kallio and associates,[360,361] Smith and Moore,[362−364] and Tambunchong[365] have also explored the potential use of boron-containing LDLs as tumor delivery agents. This approach may offer good potential for tumors other than those of the CNS, provided their in vivo stability is satisfactory. For the latter, however, studies with radiolabeled LDL particles showed their inability to cross the BBB, compromising their utility in targeting those malignant cells that may indeed be protected by this barrier.[366,367]

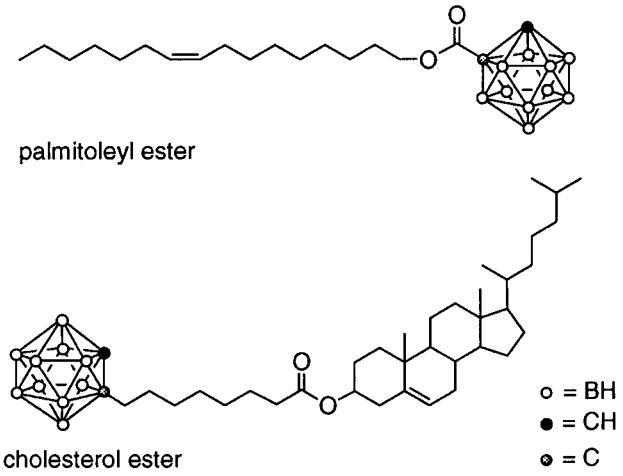


Figure 31. Boronated components for reconstitution with low-density lipoproteins (LDLs).

3. Liposomes

While LDLs are natural lipoproteins with a proclivity for those tumor cells in which the receptor for this vesicle is overexpressed, liposomes can be viewed as related synthetic vesicles (Figure 32). Hawthorne and his associates have conceived and mounted a major effort to develop liposomes as a boron delivery agent to tumors.[164,368−374] The concept was that since small unilamellar liposomes have been shown to penetrate the tumor cell membrane and localize intracellularly, incorporating boron compounds within

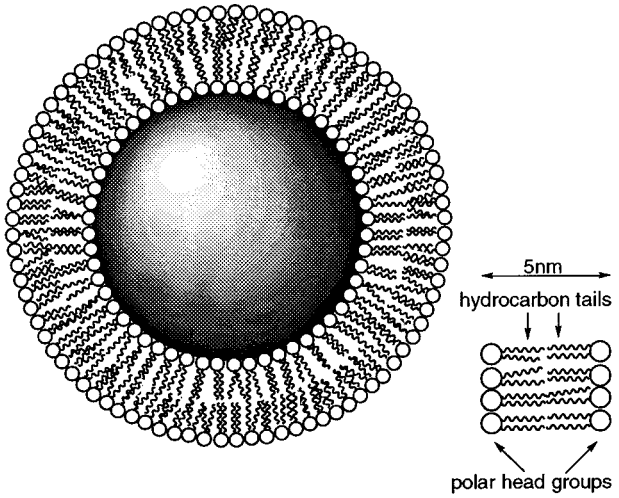


Figure 32. Diagram of unilamellar phospholipid vesicle, where the wall of the liposome is a lipid bilayer.

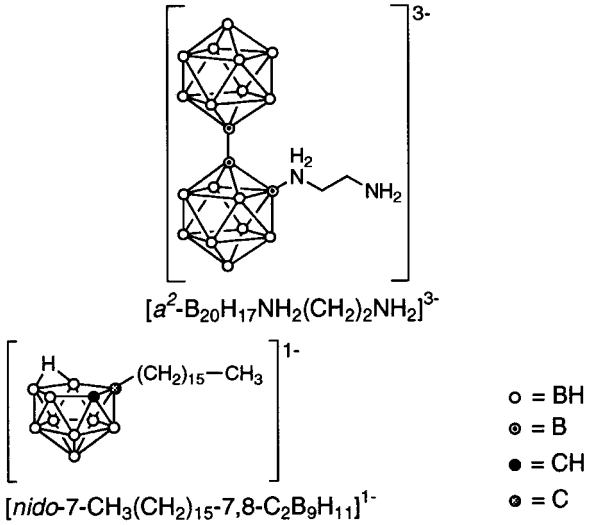


Figure 33. Boron compounds used for incorporation into liposomes.

such vesicles would provide a basis for achieving selectivity between tumor and normal cells. Initially, the boron compounds selected for incorporation into the aqueous core of the liposome were polyhedral borane anions such as $B_{10}H_{10}^{2-}$, $2\text{-}NH_3B_{10}H_9^{1-}$, and BSH.[368,369,374] Their rapid elimination from the tumor cell following deposition by the liposome prompted Hawthorne to use a boron species bearing an isocyanate group. This latter functionality has the capacity for becoming directly bound covalently to intracellular structures.[374] An alternate approach used derivatives of B_{20} polyhedral borane anions that can be oxidized intracellularly to electrophilic species. These could then be subjected to nucleophilic attack by intracellular biopolymers.[164,368−372,374] Several of the structures developed for this purpose are shown in Figure 33. The most effective of these compounds to date is $Na_3[\alpha^2\text{-}B_{20}H_{17}NH_2CH_2CH_2NH_3]$.[164] The in vivo results with mice, bearing subcutaneously transplanted tumors, are very impressive in terms of both tumor boron uptake and retention.[164]

This approach focused on the inclusion of boron compounds into the aqueous core of the liposome. A second approach involved the incorporation of lipophilic boron compounds, with the necessary physicochemical characteristics, into the membrane bilayer.[371,373,374] The species consists of a hydrophilic component attached to a lipophilic alkyl chain. This approach as well showed merit in subcutaneous tumors by achieving useful tumor:blood ratios when the aqueous core contained isotonic and especially hypertonic buffers. When both the aqueous core and the membrane bilayer contain appropriate boron compounds, useful tumor:blood boron values were achieved and the tumor boron concentrations persisted even after 48 h.[371,374] A key question is whether these liposomes can pass through the blood−brain barrier (BBB) and localize in intracranial tumors. Unilamellar liposomes with diameters as small as 60 nm are incapable of crossing the BBB.[375] Nevertheless, in studies with fluorescent-labeled boron-containing liposomes, there was qualitative evidence for their inclusion into tumor with concomitant exclusion from normal brain.[374] However, this result was based upon the fluorescent tag and not upon boron content. The studies involving the subcellular microdistribution of boron in such structures remain to be reported.

Liposomes have also been used by others to deliver boronated thiouracils[376] to melanoma cells and as a targeting vehicle for BSH[377,378] through intracerebral administration.[377] Immunoliposomes have been applied for the selective delivery of boronated antibodies directed against carcinoembryonic antigen.[379−381] An advantage of using liposomes is that the boron species itself does not need to possess tumor targeting properties. Once the compound is deposited either intracellularly within the tumor cell or interstitially within the tumor by the liposome and has properties that result in cell penetration and binding to subcellular organelles, persistence would be achieved. Selectivity, therefore, resides with the capability of the liposome to target and penetrate the tumor in contrast with its low retention by contiguous normal tissue and blood. This may well be the case for most solid tumors. With respect to brain tumors, the unique restrictive nature of the normal BBB limits penetrability of the CNS, especially for liposomes greater than 60 nm. However, in the total scheme of things for all tumors, liposomes may well be an important delivery system for BNCT agents.

4. Porphyrins and Phthalocyanines

The antecedents for using porphyrins for BNCT is rooted in the epic work of Doughtery[382] in treating solid tumors by photodynamic therapy (PDT), another binary system. The observation that porphyrins showed both selective uptake and persistence within tumors spawned interest in the development of boronated derivatives of both natural and synthetic analogues. Two groups have synthesized dihydroxyboryl-containing porphyrins[383,384] but the major focus has been in inserting boron cage structures into the porphyrin nucleus, since by doing this, a higher boron percentage may be achievable. Such boron-containing porphyrins were synthesized by Haushalter and Rudolph 20 years ago.[385−387] Their objective was not designing new BNCT agents but developing catalysts for reversible multielectron reductions. They pre-

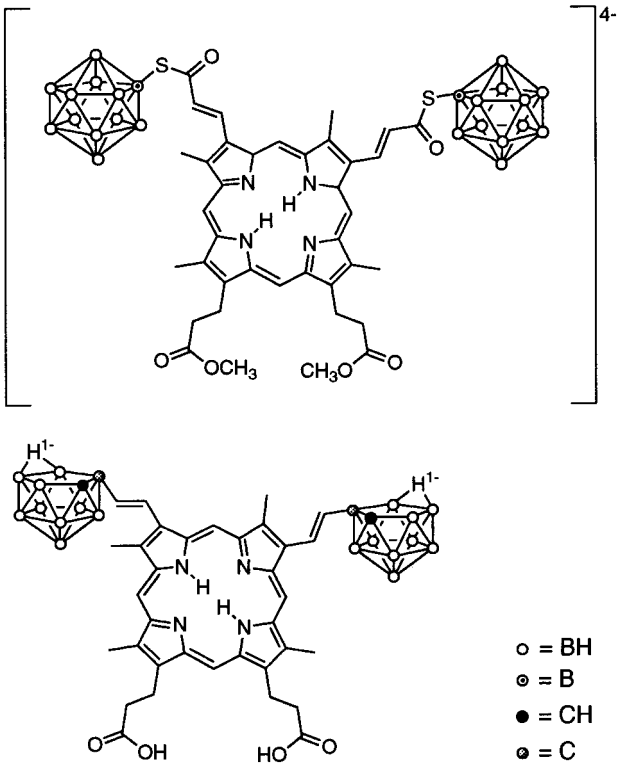


Figure 34. Boron-containing porphyrins by Gabel et al. and Miura et al.

pared tetracarboranylporphyrins having four *closo*- or *nido*-carborane moieties attached directly or via methylene or aromatic linkages to the porphyrin nucleus. Their work led to major efforts independently and jointly by Kahl,[388−404] Miura,[405−411] and Gabel[346,412−416] and their associates in the development of water-soluble, boron-containing porphyrins for BNCT (Figure 34). More recently, they have been joined by Phadke and Morgan.[417,418] The precise mechanism for the accretion of porphyrins by solid tumors is unclear, including the question of whether a receptor-mediated process is involved. Nevertheless, these structures, related to hematoporphyrin and tetraphenylporphyrin, may provide a basis for achieving the selective targeting and persistence, the twin requirements for any BNCT agent. An important question has been related to the toxicity of these compounds. Although most of the boron-containing porphyrins have photosensitizing properties, their toxicity is not dependent upon the individual parts of the molecule but upon the entire structure.

The compound that has been most widely studied biologically is the tetrakis-carboranecarboxylate ester of 2,4-bis-(α,β-dihydroxyethyl) deuterioporphyrin IX (BOPP) (Figure 35),[395] not only from the standpoint of BNCT but also as a potential sensitizer for PDT.[419,420] Its distributive studies in tumor-bearing rodents have appeared to very promising, and subcellular localization showed specific targeting of the mitochondria.[398,419,420] However, recent in vivo studies of BOPP in rats with intracerebral tumors have shown[421] conclusively that this compound is highly toxic at the levels required to obtain useful tumor concentrations. Even so, the tumor:brain concentrations attained are less than satisfactory, as are the

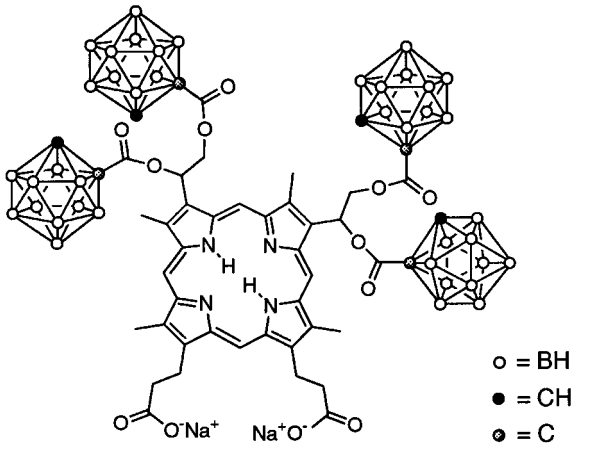


Figure 35. Boron-containing porphyrin (BOPP) by Kahl et al.

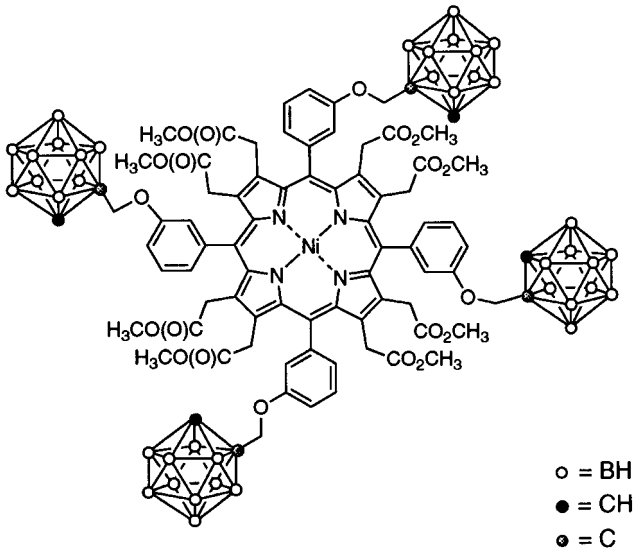


Figure 36. Structure of nickel tetracarboranylphenylporphyrin (Ni-TCP).

tumor:blood ratios. These data conflict with the earlier studies and make it highly unlikely that BOPP will become a clinically useful BNCT agent. Nevertheless, this conclusion does not rule out other boronated porphyrins.

Porphyrins have the possibility to complex with various metals[422] such as Ni, Cu, and Mn, and this provides an opportunity to determine the microdistribution of such boronated porphyrins using their stable chelates with radioactive metals. A more promising compound with significantly lowered toxicity is a nickel tetraphenylporphyrin that contains four carborane groups (Figure 36).[405,410,411] The boron content of this molecule is approximately 22%. In considering its possible use for treating brain tumors, biodistribution studies showed tumor:normal brain and tumor:blood boron differentials in mice in the range of 10 and 250, respectively, after 4 days. The tumor boron content was at the necessary concentration level for therapy, but it remains to be determined whether such studies using a subcutaneous mammary carcinoma can be replicated in animals with intracranial gliomas, since this tumor type was the one for which this compound was currently being proposed.

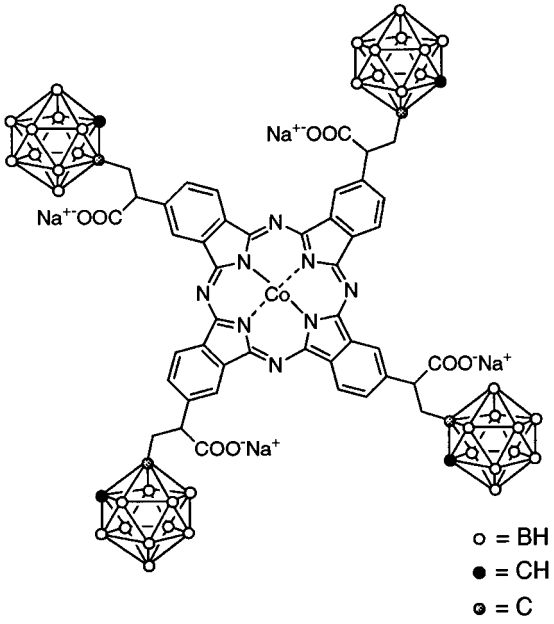


Figure 37. Boronated metallophthalocyanine by Kahl et al.

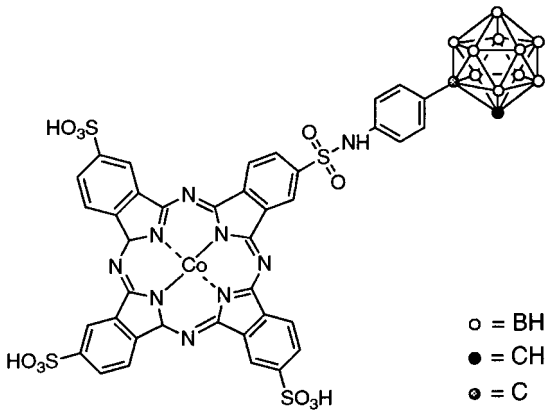


Figure 38. Boronated metallophthalocyanine by Soloway et al.

Research involving boron-containing phthalocyanines[423−425] are at a much more elementary stage of development both as chemical entities and certainly in terms of their biological evaluation. As with the porphyrins, phthalocyanines have the capability of forming extraordinarily stable metal conjugates (Figures 37 and 38) and, thus, provide the basis for their radiodetection. In general, phthalocyanines are much more stable chemically and biologically than the porphyrins; however, they, too, are highly photosensitizing in humans, even at trace levels. Few compounds in this category have been synthesized and fully characterized both chemically and biologically.

5. DNA Binders

The development of boron-containing pyrimidines, purines, nucleosides, and nucleotides is predicated on the basis that if a boron species could be incorporated into the tumor's DNA, the effect of the capture reaction would be enhanced by a factor of 2−5 over what would be the case if the boron compounds were evenly distributed in the cytoplasm.[139−141] Incorporating compounds into DNA

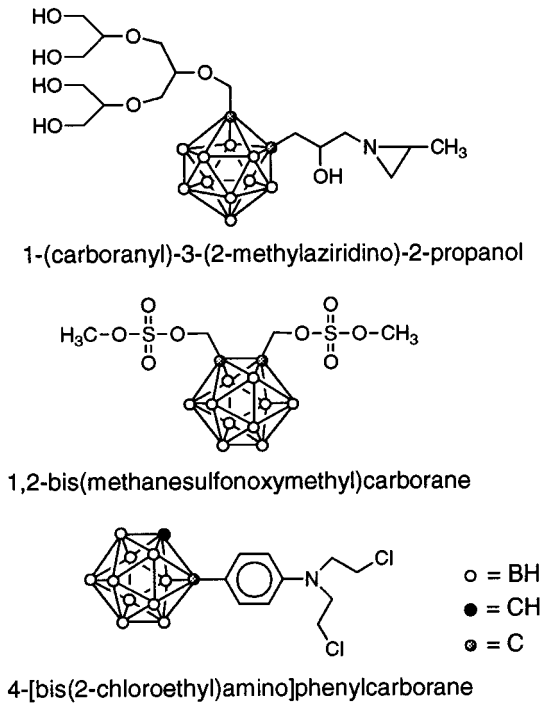


Figure 39. Boron-containing alkylating agents.

through metabolism is one approach to achieve this goal. An alternative is the development of compounds that could bind to the tumor cell's nucleus covalently or bind tightly to tumor DNA through noncovalent bonds. The rationale for their development is that the target of the high LET radiation generated by the capture reaction has been conceived as the nucleus of the tumor cell and its destruction would produce cell death. Therefore, positioning the neutron absorber in close juxtaposition to this cellular organelle has been viewed as being the ultimate location. This is the current dogma. However, the targeting and selective destruction of any specific subcellular organelle may achieve the same biological endpoint. Thus, the development of agents possessing a strong and selective proclivity for the tumor cell's mitochondria, endoplasmic reticulum, or endosomes, for example, may produce the same cytocidal objective.

a. Alkylating Agents. Since nitrogen mustards and other cancer chemotherapeutic alkylating agents are electrophiles that are covalently incorporated into tumor DNA and, as a result, interfere with tumor replication, the basis for the synthesis of such boron-containing analogues is understandable.[426,427] The following early structures were synthesized (Figure 39). Preliminary biological studies at that time were not very promising, and in retrospect, those results may be explainable. Bifunctional alkylating agents are DNA cross-linking compounds that render the DNA strands hydrolytically unstable. They produce a biological endpoint and not necessarily a concentration differential between tumor and contiguous normal tissue. Importantly as well, the actual boron concentration may not be at a level required for effective therapy by BNCT. More recently, compounds have been synthesized involving the insertion of the carboranyl group into aziridines.[239,428−431] One

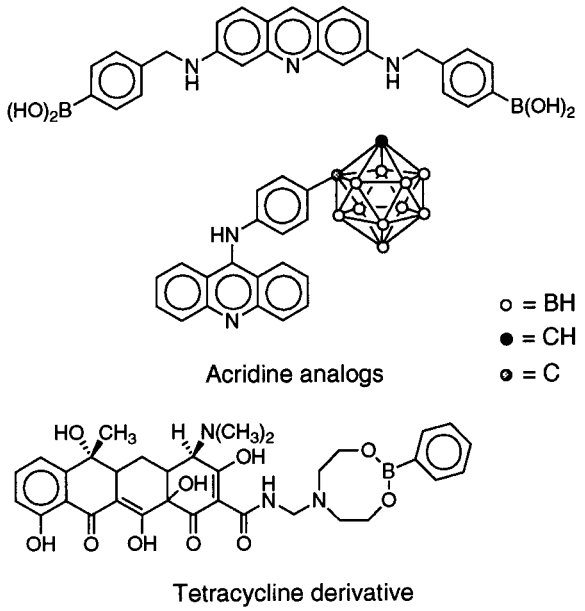


Figure 40. Boron-containing acridines and tetracyclines.

of these, 1-carboranyl-3-(2-methylaziridino)-2-propanol (Figure 39), showed significant growth inhibition toward certain tumor lines, and there was evidence for its selective incorporation.[429] However, it possessed poor aqueous solubility. The use of Yamamoto's cascade polyol technique with such aziridines greatly increased their aqueous solubility, reduced their cytotoxicity, and enhanced their uptake by cancer cells.[430] It remains to be determined whether such in vitro studies are replicated in vivo and what the potential of boronated aziridines as BNCT agents is.

b. Intercalators. Acridine dyes have been shown to stain the nuclei of many different cell types and therefore may have the potential of being boron delivery agents to tumor cells. They act as intercalating agents. The first boron-containing acridine dye was synthesized by Snyder and Konecky and contained two aryl dihydroxyboryl groups.[41] This compound was too toxic to be a useful BNCT agent but it led to the synthesis of other less toxic carborane-containing acridines.[432] These acridines are shown in Figure 40. Although these latter compounds were less toxic and achieved higher concentrations in tumor compared with normal brain and blood, these values were significantly lower than those found in the liver, kidney, and spleen. Knowing now the biological properties of the carborane nucleus, this result is not unexpected. Insertion of more hydrophilic groups into the carborane part of the molecule could conceivably improve its biodistribution.[433,434] Also, no acridines have been prepared in which the boron moiety is a polyhedral borane anion. Such structures may improve the hydrophilic/lipophilic balance for the acridine dyes.

In addition to acridines, there are other vital dyes used in histology that may not be intercalating agents but demonstrate selectivity for certain subcellular organelles. To these carrier molecules, boron moieties could be attached. It is essential that there be integrity of the boron linkage to this dye molecule. An Evan's Blue boron analog[435] was prepared but

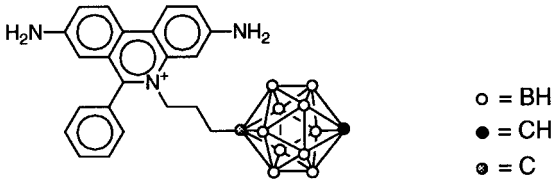


Figure 41. Boron-containing phenanthridine.

under in vivo conditions; separation of the dihydroxyboryl group from the dye moiety occurred.

The planar tetracyclic nucleus in the tetracyclines has the capacity for intercalation, and this was the basis in part for the synthesis of dihydroxyboryl analogues of the tetracyclines.[436−438] One of the compounds that was synthesized was evaluated both in tumor cell culture and in adenocarcinoma-bearing mice. The preliminary results indicated some potential, suggesting possible binding to ribosomes. However, this research was carried out more than 20 years ago and nothing further has been reported since that time.

More recent work has focused on the development on boron-containing phenanthridinium analogues.[16,188,434,439−441] The basic ring structure in these tricyclic planar compounds intercalates very readily into the DNA molecule, and this has been the basis for the synthesis of the boronated analogues. A representative structure is shown in Figure 41. The compounds contain *ortho*-, *nido*-, and *para*-carborane moieties. The latter was used because of the increased stability of that nucleus to basic conditions and the fact that the closo derivatives of the *o*-carboranes are slowly degraded to their nido structures in aqueous media.[439] In vitro studies with human glioma cells demonstrated binding to the cell nuclei but also to other cellular sites on and within viable tumor cells. This result may stem from the highly lipophilic nature of the carborane moiety, which may require the insertion of a hydrophilic functionality into the carborane nucleus in order to balance its lipophilicity.[16,440,442,443] In this way, one would hope to simulate more closely the physicochemical characteristics of ethidium and propidium, two established intercalators. In the past, polyols have been inserted onto the carborane nucleus in order to increase a compound's aqueous solubility, but it may be of equal importance in balancing the hydrophobic attributes of the various $C_2B_{10}H_{12}$ carboranes.

c. Groove Binders. Related to the intercalators are what have been termed DNA groove binders. Once again, the goal has been to develop compounds that will selectively target DNA and specifically tumor DNA. The initial focus was to use bibenzimidazole compounds related to Hoechst 33258, a compound that has been shown to target the minor groove of DNA with a great deal of specificity. Kelly, Martin, and their colleagues have led the way with this development. They and others have pursued both dihydroxyboryl[59,444−446] and carboranyl analogues[444,447,448] as well as the attachment of a gadolinium chelate to such a carrier.[59,445,449] The latter could provide the basis for GdNCT. The compounds that have been synthesized are shown in Figure 42.

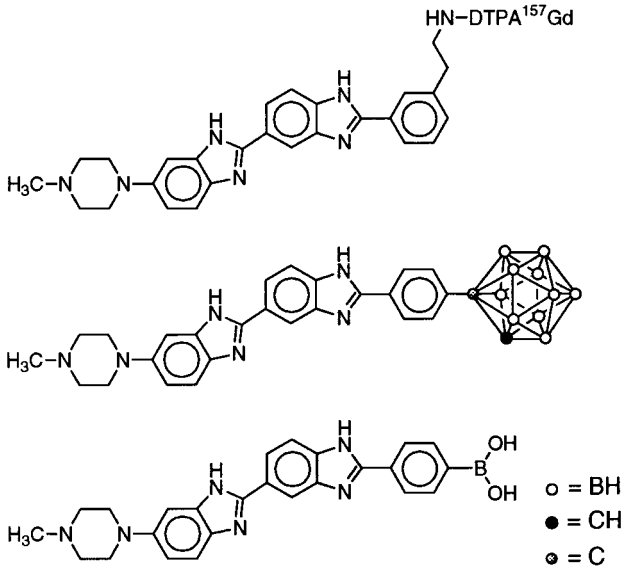


Figure 42. NCT DNA groove binders.

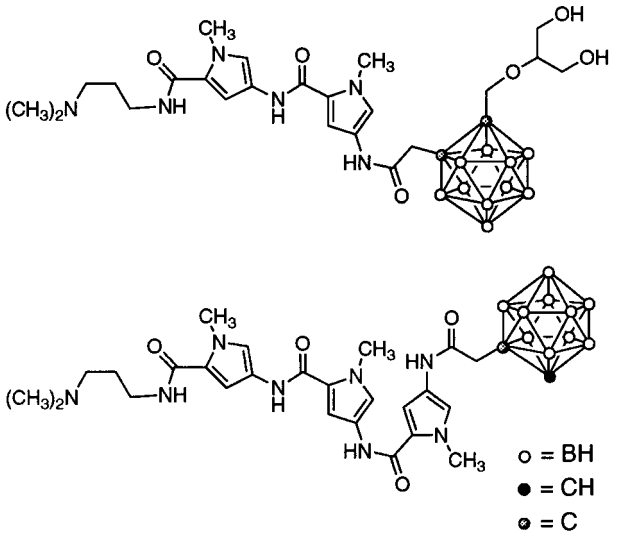


Figure 43. Carborane analogues of DNA binders netropsin and distamycin.

Use of this type of carrier has also been explored by others. Other types of compounds with a strong proclivity for DNA are netropsin and distamycin. These target DNA sites that are rich in adenine and thymine. Yamamoto and his associates[450,451] have succeeded not only in synthesizing various carboranyl analogues of these compounds but in inserting polyols into such compounds by methodology that was developed for the amino acids and nucleosides (Figure 43). One of these, an analogue of distamycin containing four hydroxyl groups, showed very strong binding to a DNA fragment. It will be very interesting to see the biodistribution of such an analogue, its toxicity, and especially the radiobiological effects both in vitro and in vivo.

d. Polyamines. Polyamines, such as putrescine, spermidine, and spermine, are important biochemical constituents that are essential for cell growth and differentiation. Of importance from the standpoint of BNCT, elevated concentrations of these substances are found in rapidly proliferating tumor cells whose polyamine transport system is upregulated. Furthermore, these structures, under physiological conditions, exist as cationic entities and bind very tightly to DNA via electrostatic interactions. Insertion of cytoreductive cancer chemotherapeutic agents into the polyamine scaffold generated more pharmacologically active compounds than the parent compound from which they are derived,[452] and this observation served as the basis for the synthesis of boron-containing polyamines.[262,433,453–456] Representative compounds related to spermidine and spermine are shown in Figure 44. DNA binding studies demonstrated that these structures simulated and competed with the naturally occurring polyamines.[456] In vitro cellular uptake studies showed extremely rapid depletion of compounds from the media, achieving cellular levels comparable to those with the clinically useful BSH and BPA but at media concentrations that were 100 to 1000-fold less.[456] At present, the major limitation observed with these boron-containing polyamines is both their cellular and in vivo toxicity.[456] Less toxic analogues have now been prepared,[457] but it remains to be determined what, if any, clinical utility these will have. However, even if the boronated polyamines themselves are limited by their in vivo toxicity, they might be encapsulated into liposomes in order to ameliorate this limitation. The liposomal content could then be delivered intracellularly or interstitially. In the latter instance, the boronated polyamines could be taken up directly by tumor cells or after the breakdown of the liposome, transported intracellularly by an upregulated system. In all instances, the boronated polyamines would bind to tumor DNA. In essence, the liposome is the targeting entity and the polyamine provides the basis for intracellular retention of boron through ionic binding to DNA.

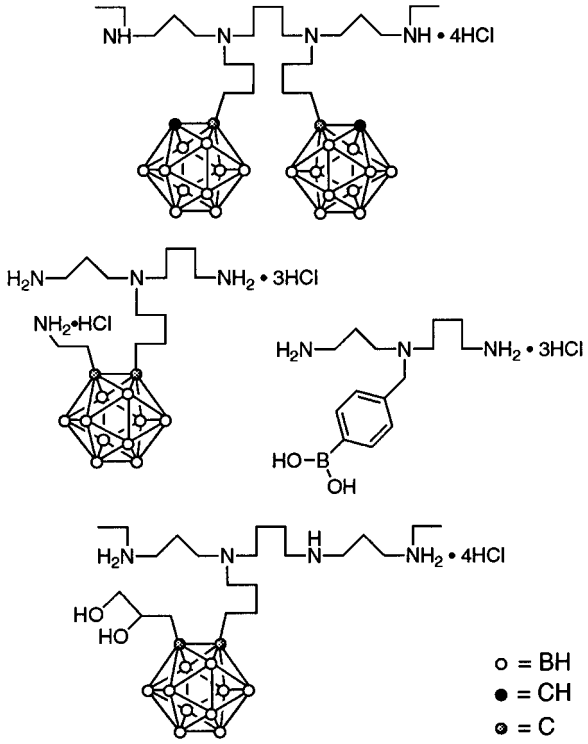


Figure 44. N-Substituted boronated polyamines.

e. Di- and Oligonucleotides: Antisense Agents. The development of oligodeoxynucleotides (oligos) as

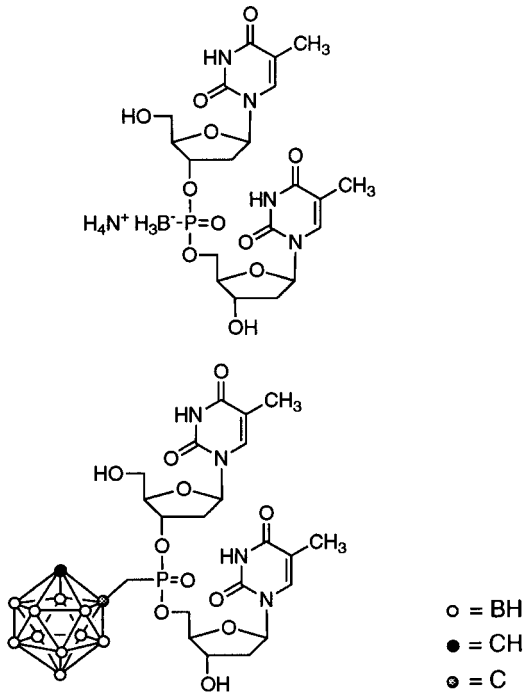
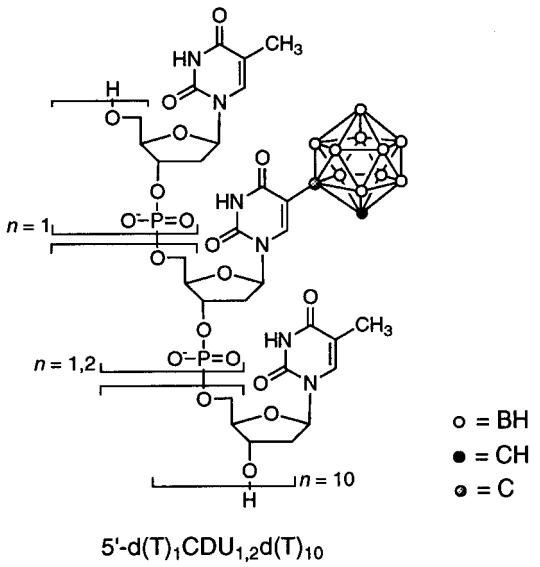


Figure 45. Boron-containing dinucleotide analogues.

potential boron delivery agents is predicated on the uniqueness of tumor oncogenes and their potential to form triple-stranded helical structures through hybridization with antisense boron-containing oligos. As with any oligos, it is essential that these compounds be stable under in vivo conditions, are able to cross tumor cell membrane, exhibit hybridization with tumor DNA, and demonstrate selectivity for tumor vis-à-vis normal cells that are contiguous with the tumor.

The development of antisense oligos provided the stimulus for the synthesis of boron-containing analogues. There are two general types of boronated oligos that have been described, involving (1) modification and incorporation of the boron moiety into the phosphodiester bond and (2) insertion of the boron component into the nucleoside portion of the molecule without any alteration of the phosphodiester linkage. The initial compounds that were prepared were reported by Spielvogel and his collaborators. They fall into the first category in which one of the nonbridging oxygen atoms of the phosphodiester backbone is replaced by the BH_3 moiety; the latter can be viewed as being isoelectronic with an oxygen atom.[226,269,458−460] Other structures have involved the attachment of the carboranyl moiety to this internucleotide linkage.[265,461,462] Representative structures are shown in Figure 45. Other oligos have been prepared in which the phosphodiester linkage is the native one and either the *o*-carborane cage or the cyanoborane group have been inserted into the nucleoside component (Figure 46).[463−467] In the case of carboranyl oligos, molecular modeling studies have shown that the bulkiness of the carborane nucleus can interfere with hybridization and thereby affect the stability of any triple helical structure expected in hybridization.[464] Biological studies with such boronated oligos show that they are substrates for



5′-d(T)$_1$CDU$_{1,2}$d(T)$_{10}$

Figure 46. Oligonucleotide derivative of 5-(o-carboranyl)-uracil (CDU).

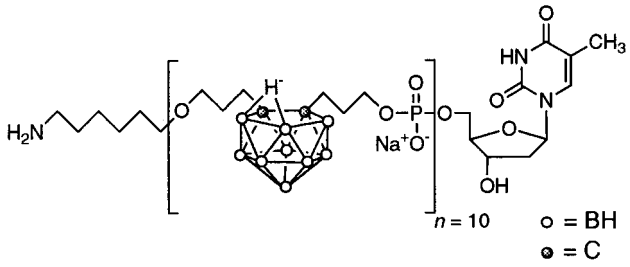


Figure 47. Carboranyloligophosphates by Kane and Hawthorne.

polynucleotide kinase and can act as primers for certain polymerases.

Although not strictly classical oligos that can bind through hybridization with DNA, the work of Kane, Hawthorne, and Gabel[186,204,206,468−472] in the development of novel carborane-containing oligomeric phosphodiesters is certainly related. These carboranyloligophosphates, containing up to 35% boron, can be assembled using automated synthesizer techniques. A representative structure is shown in Figure 47. The compounds reported are derived from *o*-carborane and possess the advantages that they are homogeneous, very hydrophilic, and lend themselves to functionalization. They can be converted quantitatively from the *closo*-carborane to the nido analogues. The developers of these compounds are evaluating their potential for use in liposomes, in immunological delivery systems and for their incorporation into ligands that can target receptors.

6. Receptors/Antigen Binders

The idea of using macromolecular species to deliver boron to tumor cells is not a new concept.[41] It stems from suggestions more than 30 years ago when only polyclonal antibodies were available for targeting tumor-associated antigens. The work of Milstein and Köhler[473] in developing monoclonal antibodies (Mabs) had not been conceived then and remained to be carried out (Figure 48). The biological, let alone the chemical, nature of receptors at that time still had

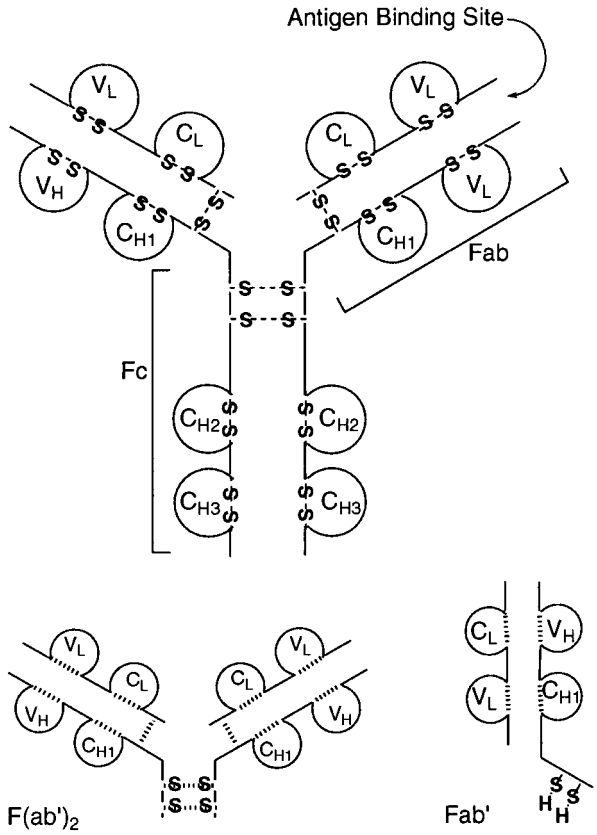


Figure 48. Schematic representation of IgG and its fragments. Antibodies are constructed from four polypeptide chains and are called "immunoglobulins" (Ig). IgG is a Y-shaped symmetric dimer composed of four subunits: two light chains (L) and two heavy chains (H), where each chain consists of two units, V and C, indicating their variable and constant regions. Partial digestion by proteases at different sites in the hinge region results in cleavage, forming fragments: e.g. Fab (fragment antigen binding) and Fc (Fragment crystallizable); or others like (Fab′) or $F(ab')_2$.

to be developed. Nevertheless, it was appreciated that incorporating boron compounds into proteins and other macromolecular species would ultimately become a necessary task, and early beginnings were made in the development of that methodology.[347,474−476] It was recognized that the attachment of boron to such biopolymers must take place where a significant amount of boron was incorporated since the ultimate boron loading of the tumor must be in the range of 20−35 μg ^{10}B/g. At the same time, it was appreciated that the attachment of the boron species to the macromolecule should be a covalent one since ionically bound structures may be readily exchanged under in vivo conditions with similar polymeric entities in the vascular system or in the tissues. Also, and most importantly, this linkage must occur without altering the binding site of the antibody molecule since this would eradicate its tumor specificity.

a. Antibodies: Monoclonal/Bispecific. The potential of using boron-containing antibodies as tumor-targeting entities was proposed before the actual synthetic work was undertaken.[41,474] Those early studies focused on the types of boron compounds that were to be used, the linkages that would be required, and the effect of such covalent attachment and its boron moiety upon the physiochemical properties of the protein conjugate that was formed. For this purpose, any protein molecule would provide useful information in the ultimate fabrication of a boronated antibody against a tumor-associated antigen. The technology acquired in research with antibodies has also been used in the development of boron-containing growth factors and other macromolecular species that are described below. The types of boron compounds that were considered useful for this purpose were the following: (1) polyhedral borane anions and their derivatives; (2) the stable carboranes. The basis once again was that the boronated biopolymer must contain an appropriate level of boron ($\sim 10^3$ boron atoms) in order to be a useful BNCT agent, and this in turn meant that the boron-binding structure must possess a very high boron percentage. Many of these initial small molecular structures that bind to proteins can be found in Hawthorne's review.[32] Each of these contain 9−12 boron atoms. It was estimated then that the number of boron atoms per protein molecule may have to be in the range of 200−1000,[69] and this translates into approximately 20−100 boron cages. The number of cages per se may not be critical, but implicit is that there must be 20−100 specific chemical reactions on the protein molecule in order to attain an adequate level of boron loading. There were two questions associated with this approach: (1) could that number of boron atoms be incorporated into any protein or macromolecular species without producing frank denaturation as shown by protein precipitation;[475−478] (2) could 20−100 discrete chemical reactions occur on a protein molecule without generating a massive alteration in its specificity for a tumor-associated antigen? Some of the initial protein-binding compounds were not particularly hydrophilic, and this property severely restricted the number of groups that could be incorporated. However, by inserting a water-solubilizing glucose moiety into the protein-binding boron cage compounds, the resulting immunoconjugates retained their water solubility.[347] It was possible to incorporate between one and two thousand boron atoms into a gamma globulin molecule without causing protein precipitation.[347] Other approaches to circumvent the solubility problem involved the use of negatively charged carboranes[198,479] or polyhedral borane anions,[480,481] as well as the insertion of different carbohydrate groups.[329,348,349]

Of much more serious concern, was the issue of altering 20−100 different functional groups on the protein molecule while retaining its conformational and targeting specificity. This seemed a highly unlikely possibility. This assumption was buttressed by the fact that the water-soluble compound $Cs_2B_{12}H_{11}SS(CH_2)_2COO[N(CO)_2(CH_2)_2]$ was capable of incorporating, on average, 1300 ^{10}B atoms per antibody molecule but this occurred with a concomitant 90% loss of its immunoreactivity.[482] The conclusion from this research was that inserting small molecular weight boron moieties into an antibody molecule was unproductive and that a possible preferable approach was to attach to one, or at best, to a handful of sites on the antibody molecule, a boron-containing oligo-

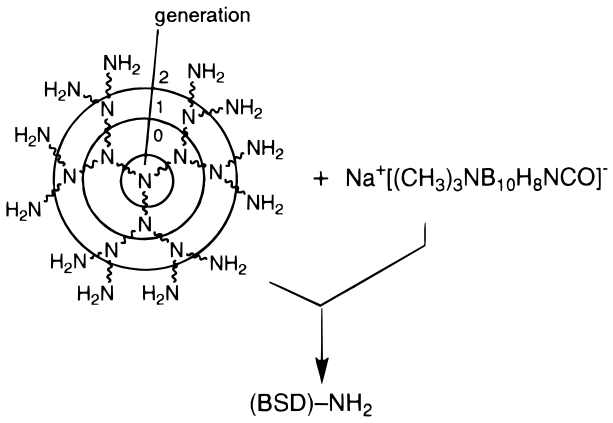


Figure 49. A "starburst" dendrimer (SD), consisting of repetitive polyamido amino groups (PAMAM) arranged in a starburst pattern. This second genaration starburst dendrimer has 12 reactive amino groups and reacts with $Na(CH_3)_3NB_{10}H_8NCO$ to form a boronated starburst dendrimer (BSD).

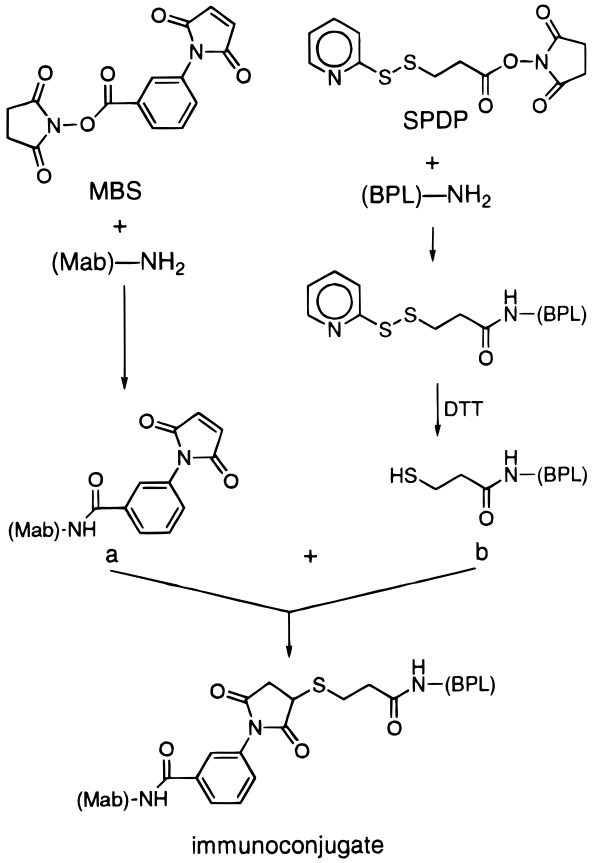
mer that would have 200−1000 boron atoms. The basis for such numbers arose from the calculations that multiple capture events must take place within tumor cells to ensure a lethal consequence following neutron irradiation. If one assumes an antigen site density on tumor cells of 10^6, then 10^3 ^{10}B atoms per antibody molecule would produce the estimated requirement of 10^9 boron atoms per cell (approximately 17−35 μg ^{10}B/g).[483] Thus, there emerged two requirements in order to prepare boronated Mabs: (1) synthesis of a boronated macromolecule containing 200−1000 boron atoms; (2) linkage technology to attach such structures to the Mabs.

The initial approaches involved the use of a preformed macromolecule containing a large number of functional groups to which the boron moiety could be covalently attached. These involved the use of polylysine,[163,484−490] polyornithine,[491] and dextran.[492−495] In the case of the commercially available polyamino acids, amine-binding boron compounds were used for attachment to the free amino functions in the polypeptide. In this way, boronated structures were prepared containing 24% boron by weight, with an average of 1500 boron atoms per antibody molecule. One of the limitations with such structures is that the polymer itself was not a discrete and homogeneous entity and that hetereogeneity was markedly increased following boronation since the number of boron groups attached to each polymeric molecule would vary. To ameliorate this situation, preformed homogeneous macromolecules were used. Such compounds are referred to as starburst dendrimers and a polyamido amino dendrimer was the species that was boronated (Figure 49).[496−498] Although the heterogeneity of the product was improved, boronated dendrimers understandably are not homogeneous structures since the number of boron ligands bound to each dendrimer molecule would be variable. However, there was improvement over the commercially available heterogeneous polylysine that was initially used. To develop homogeneous macromolecular boron-containing compounds, automated synthesizer techniques have now been employed. The first examples of such a homogeneous boron polymer were those derived from carboranyl amino acids. The number of such carborane-containing molecules that could be linked together was 10 but the process was very slow and precluded the preparation of longer chain peptides.[319] Also, these structures were very hydrophobic and in order to make them more water soluble, these *closo*-carboranyl polypeptides were converted to their corresponding nido analogues with the loss of one boron atom per cage.[199,205,319,499] Thus, a polypeptide was synthesized containing as many as 90 boron atoms.[205] This demonstrated the feasibility of using automated techniques for the synthesis of homogeneous boron polymers. A more suitable vehicle for preparing homogeneous boron macromolecules involves the use of oligomeric boron-containing phosphodiesters. Such structures have already been described in the section on oligonucleotides. These are water-soluble homogeneous polymers containing 35% boron by weight.[500]

A very important question is, how can any of these boron-containing oligomers be linked to an antibody? It is apparent that there must be a functional group on the boron macromolecule that has the capacity for becoming incorporated into a functionality on the antibody. Approaches for achieving such covalent attachment are described by Barth, Alam et al.,[163,483,485,497] Hawthorne and his collaborators,[199,200,499] and others.[501] Both homobifunctional and heterobifunctional agents have been used to achieve this objective. However, it is important to realize that in order to minimize heterogeneity in the resulting conjugate, ideally only one boron macromolecule should be attached to an antibody molecule. Such an attachment must occur under mild conditions to prevent any significant conformational changes in the antibody. One effective strategy for achieving this objective has been to insert a sulfhydryl group into the boronated macromolecule.[485] This is particularly effective when the polymer itself contains no sulfhydryl groups. A functionality then is inserted into the antibody molecule which can react readily and covalently with this sulfhydryl group. A good example is the maleimido group.[485] In Scheme 6, an example is shown of how this linkage technology is used to attach a boron macromolecule to an antibody. Thus, the incorporation of water-soluble boronated polymers into antibodies can be achieved without producing frank denaturation, and these conjugates do retain their aqueous solubility. A more critical question relates to the retention of their immunoreactivity. In vitro studies[163,485,496] have demonstrated that such bioconjugates possess significant immunoreactivity, comparable in many respects with the native antibody itself. Certainly this was an encouraging observation. The next and very crucial requirement relates to the conjugate's in vivo targeting.

One of the major problems observed with all of these immunoconjugates in vivo is that only a very small percentage of the total administered dose actually targets the tumor itself. By far, the major portion of the conjugate accumulates in the liver.[199,200,496,497,499] Studies with only the dendrimers themselves showed convincingly that with increasing molecular weight, there was a significantly greater

Scheme 6. Boronation of a Monoclonal Antibody (Mab) Utilizing Heterobifunctional Reagents. The Boronated Macromolecule (BPL) Was Formed from the Linkage of $Me_3NB_{10}H_8NCO^{1-}$ with Poly-DL-lysine (poly-DL)[a]



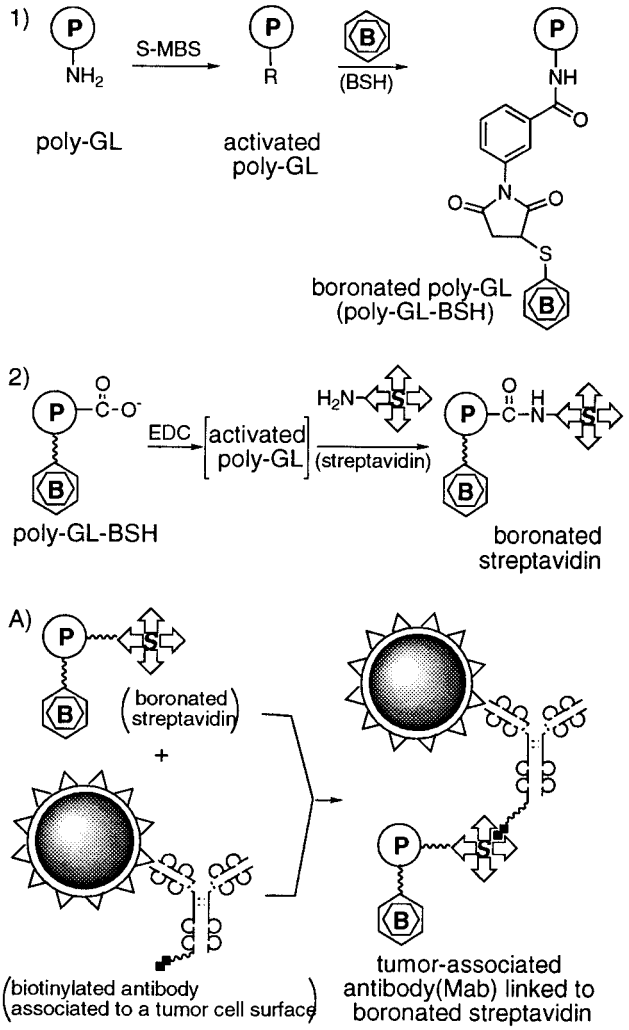
[a] BPL was attached to antibodies (Mab) utilizing *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) and *m*-maleimidobenzoyl *N*-hydroxysulfosuccinimide ester (MBS). Masked sulfhydryl groups were introduced into BPL using SPDP. Unmasking of the sulfhydryl group using dithiothreitol (DTT) results in (BPL)-SH. Maleimido groups were introduced into antibody molecules (Mabs) with MBS, yielding (Mab)-MB. Linkage of the boronated macromolecule (BPL)-SH to the antibody (Mab)-MB took place by the addition of a sulfhydryl group on BPL to the double bond on the maleimido group of (Mab)-MB. By using this method, incorporation of a large number of boron atoms per antibody molecule can be achieved without significantly modifying the in vitro immunoreactivity of the antibody for the tumor-associated antigen.

concentration attained in the liver than with any other tissue including the tumor.[496] Even with a water-soluble, homogeneous boron polymer that contains 35% boron by weight,[500] and assuming 1000 boron atoms, the polymer's molecular mass begins to approach 30 000 Da. Insertion of such a boron-containing compound into an antibody or its smaller immunoreactive fragments, $(Fab')_2$ and Fab′, could well be a significant factor in modifying their biodistribution.[329,485] The problem of using such entities as brain tumor targeting vehicles is even more seriously compromised, in view of the boronated antibody's need to traverse the BBB. To overcome this limitation, other targeting strategies for brain tumors have been considered, especially intratumoral injection.[502]

However, the in vivo problems encountered with these boronated Mabs dampened enthusiasm for their use and has led to two different approaches in macromolecular targeting. One involves the use of the streptavidin/biotin conjugate,[503−505] and the second focuses on the potential of bispecific antibodies. The streptavidin/biotin approach is based upon the extremely high binding constant ($K = 10^{15}$/mol) that these two molecular structures have for each other. Verro et al. and Sano have independently attempted to use this system for selective tumor targeting. In essence, a boronated streptavidin and a biotinylated Mab against a tumor-associated antigen have been prepared. This biotinylated structure has been shown to possess high affinity for tumor-associated antigens on tumor cell surfaces. This, then, becomes the target for the boronated streptavidin. One approach has used a commercially available poly-(D-glutamate D-lysine) (poly-GL) to which the BSH molecule was attached via a heterobifunctional agent.[503,504] This boronated poly-GL was then activated by a carbodiimide reagent and reacted with streptavidin. Another approach in synthesizing a boron-containing streptavidin has been to prepare a genetically engineered mutant possessing multiple cysteine residues that are lacking in the native streptavidin (Scheme 7).[505] These residues provide the basis via sulfhydryl-specific bifunctional reagents to incorporate more than 200 boron atoms using the BSH molecule as the boron species. These attempts using boronated streptavidin and biotinylated Mabs can be viewed as being related to the second approach, the potential use of bispecific antibodies.

The development of bispecific antibodies, generated from hybrid hybridomas or quadromas, as BNCT targeting agents is an attempt to circumvent the direct alteration of the antibody molecule with its resultant nonspecific extraction by the liver after systemic administration. Barth and his colleagues[506−509] and Hawthorne and his associates[510,511] independently have focused on the development of antibodies with two discrete combining sites: (1) the tumor-associated antigen; (2) a boron polymer. Such bispecific antibodies have now been prepared by both groups. They involve the use of different types of boron polymers. It has now been shown for the first time that the preparation of antibodies against different boron clusters can be accomplished. In one case, antibodies against polyhedral borane anions attached to a dendrimer molecule have been produced (Figure 50). This antibody recognized a variety of different types of polyhedral boranes anions but failed to react with the dihydroxyboryl group or the *o*-carborane group. In the second instance, antibodies against the *nido*-carboranyl group attached to a phosphodiester oligomer was achieved. This demonstrates the immunoreactivity of these boron clusters and the potential that such antibodies may offer for boron targeting of tumor cells. One can envisage that the bispecific antibody will pretarget tumor cells, as with the biotinylated Mab, by its ability to recognize tumor-associated antigens on the cell's surface, followed by intravenous administration and targeting of the boron-containing polymers to the antibody's boron combining site. This initial work has laid the groundwork for further developments in

Scheme 7. Macromolecular Boron Targeting Using the Streptavidin/Biotin Strategy[a]



[a] Boronated polymers can be linked to antibody molecules that are attached to tumor cells via a carrier component such as streptavidin, a common carrier protein. One method is to boronate streptavidin by coupling boronated polymers directly onto the carrier. A glutamate-lysine copolymer (poly-GL) is boronated with BSH using the heterobifunctional linker, sulfo-MBS (S-MBS), which is then coupled to streptavidin using 1-ethyl-3(3-dimethylaminopropyl) carbodiimide (EDC) (as shown in lines 1 and 2). An antibody against a tumor-associated antigen can be biotinylated permitting its pretargeting of the tumor cell membrane and allowing the boronated streptavidin to be linked to the tumor cell utilizing the high binding affinity of streptavidin for biotin (Reaction A).

the use of bispecific antibodies as boron delivery agents.

b. Growth Factors. The basis for the development of growth factors as boron carriers stems from the fact that these structures appear to play an important role in the malignant transformation of normal cells and that receptors for these growth factors may be overexpressed in tumor cells.[512] Furthermore, they are small polypeptides with significantly lower molecular masses than antibodies and range in mass from 6 to 25 kDa.[513] Therefore, boron conjugates derived from these growth factors would be expected to be intermediate in molecular weight between small organo- and inorganoboron compounds and antibodies. The hoped for result is that these structures would achieve more rapid and effective

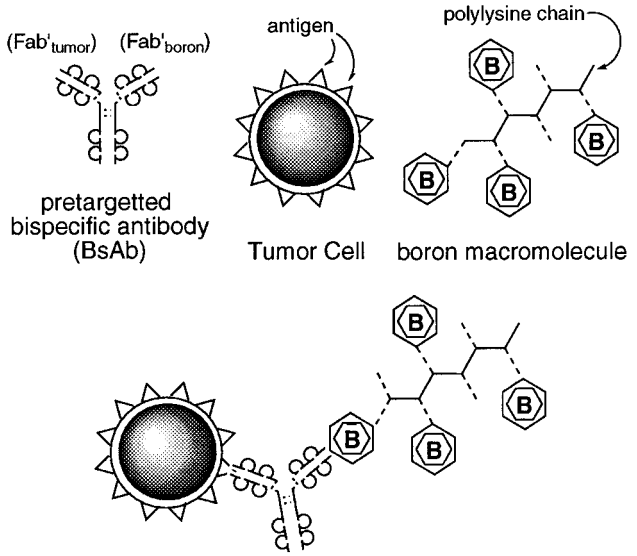


Figure 50. Schematic representation of a bispecific antibody (BsMab). This bispecific antibody (BsAb) has two different combining sites: one recognizes a tumor-associated cell surface antigen; the other recognizes a boron-containing macromolecule (such as a boron-rich polymer). Following administration, the antibody first localizes on the tumor cell surface by reacting with a tumor associated antigen. After localization, a boron-containing macromolecule is administered and reacts with the anti-boron combining site of the antibody molecule.

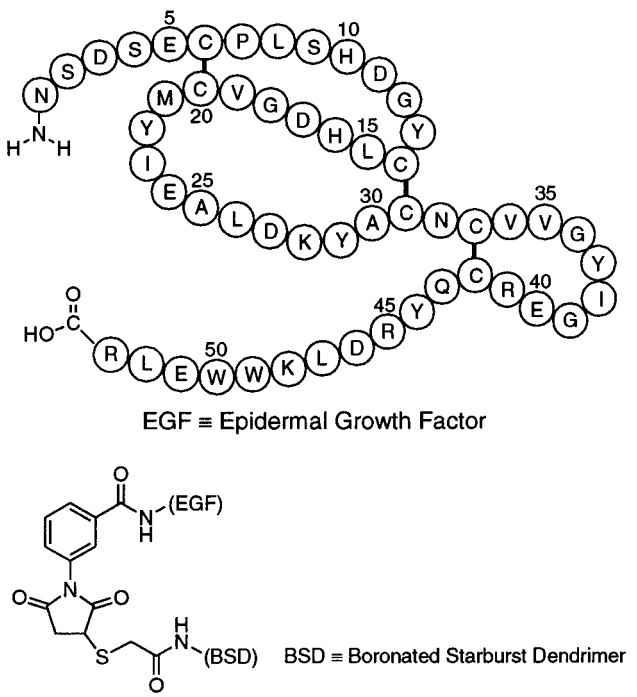


Figure 51. Schematic representations of the structure of human epidermal growth factor (EGF) and the boronated starburst dendrimer (BSD) conjugated to EGF.

tumor targeting than has been observed with monoclonal antibodies.

The growth factor which has attracted the greatest interest as a boron targeting agent is epidermal growth factor (EGF) (Figure 51). It is a 53 amino acid single chain polypeptide with a molecular weight of 6045 and is heat and acid stable. Its use as a boron carrier was first proposed by Carlsson et al.[304] The rationale is that there are a number of different types

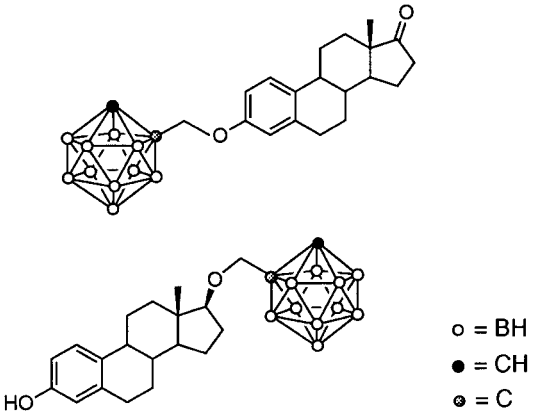


Figure 52. Boronated hormone analogues.

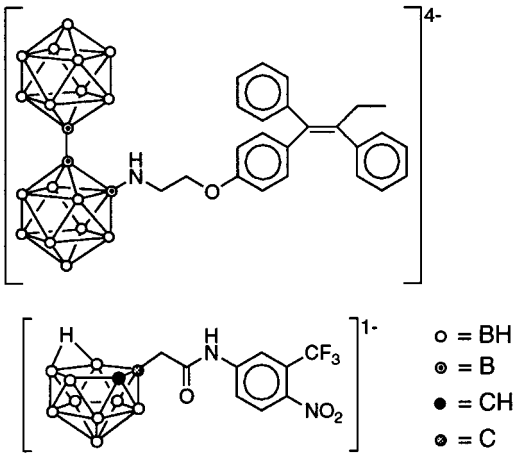


Figure 53. Boronated hormonal antagonists.

of tumors that demonstrate significant amplification of the EGF receptor (EGFR) on their surface, and this is especially true for the majority of high grade gliomas.[514]

In contrast, EGFR is low or undetectable in normal brain. Since EGF is a polypeptide, the methodology that has been developed for incorporating boron into antibodies has been applied to EGF. In one case, boronated dextran has been used as the boron moiety, and this has been conjugated to EGF.[324,515−519] In another, a boronated dendrimer was coupled to EGF via heterobifunctional reagents.[520−524] In vitro studies with these bioconjugates, using competitive binding assays, indicated their specificity for EGFR positive cells. However, as with the boronated antibodies, there was very significant extraction and retention by the liver following their intravenous administration with very low levels in intracranial tumors.[525] This result led Barth and his associates to use intratumoral delivery of boronated EGF.[525] Their data in glioma-bearing rats with EGFR positive tumors demonstrate that intracerebral administration may be the most effective way for delivering such conjugates or any other boronated macromolecules to brain tumors. A key question is whether such an intratumoral route of administration will be successful in targeting all tumor cells even those that may reside several centimeters from the main tumor mass? Such cells could be the basis for tumor recurrences.

c. Hormones. As with growth factors but clearly preceding them, there are tumors that are formed in various tissues and organs whose development and rate of proliferation is strongly hormone-dependent. Of significant importance, the steroid hormones have receptors that are localized in the cell's nucleus, the key target for the high LET radiation. In the case of breast cancer, approximately one-third of the tumors are responsive to endocrine therapy,[526] and it was this observation that stimulated Sweet to synthesize the first carboranyl derivative of estradiol (Figure 52).[527,528] The preparative method involved the synthesis of the acetylenic derivative of estradiol, protection of the alcoholic functions, and conversion of the carbon−carbon triple bond to the *o*-carborane using a decaborane complex. Subsequently, he and others have synthesized boron analogues of hormonal antagonists and other estrogenic and androgenic compounds.[393,529−533] One of the advantages of these boronated steroid hormones is their relatively low molecular weights, offering the potentiality for the rapid targeting of tumor cells in contrast with monoclonal antibodies and even growth factors. Key requirements for such compounds were that they be chemically stable under in vivo conditions, be generally nontoxic, and retain their hormonal activity. The objective was that these analogues might be useful in the selective targeting of hormone-sensitive malignancies for treatment by BNCT. Several of the estrogen-related compounds synthesized demonstrated the desired specificity for those malignant cells possessing elevated estrogenic receptor levels. However, the crucial limitation was that the receptor density observed was in the range of 10^4-10^5, and thus, the maximum number of ^{10}B atoms that could be achieved with these carborane-containing compounds would be 10^5-10^6 boron-10 atoms per cell. This number is at least 3 orders of magnitude lower than the amount required to produce a lethal effect on the tumor cell. This result emphasizes once again that though it is very desirable that the targeting compound possess a high degree of specificity for the tumor by comparison with those contiguous normal cells from which it is derived, nevertheless, it, in and of itself, is not sufficient. The absolute concentration must approximate that value which will generate a lethal effect within the tumor cell, namely, 10^9 boron-10 atoms per cell. Anything less than that concentration would be ineffective.

More recently, however, there has been renewed interest in the synthesis of boron-containing steroids by Schneiderova et al.[534] and others.[535,536] Also, Hawthorne and Groudine have focused on the coupling of a boron-containing moiety to a ligand possessing binding specificity for intracellular hormone receptors but lacking the ability to activate these receptors (Figure 53).[537] In essence, these can be viewed as hormonal antagonists. The glucocorticoid, mineralcorticoid, thyroid, estrogen, progesterone, androgen, and retinoic acid receptors are the ones that have been proposed for targeting. Related is the incorporation of the carborane moiety into the ovine corticotrophin releasing hormone.[538]

The low molecular weight compounds, especially those analogous to the steroids, may have intrinsic

advantages, especially with regard to the rapidity of tumor targeting. Nevertheless, important as that may be, it is apparent that adequate receptor site density is a sine qua non in the development of useful BNCT agents.

7. Other Compounds

In this category, there are a number of diverse chemical entities. In some cases, compounds were synthesized simply because the chemical methods were available for their preparation without there being any biological rationale for their development. Only subsequently were those compounds evaluated as tumor-targeting agents. In other instances, nonboronated analogues had been prepared initially, and the biological results have provided the impetus for the synthesis of their boron-containing counterparts. Thus, there may be no commonality either from a chemical or biological standpoint for the compounds that are described in this very broad category of agents.

a. Radiation Sensitizers. The development of boron-containing radiation sensitizers arose from the observation that various nitroimidazoles appear to be taken up selectively by poorly vascularized areas of tumor and retained therein by their reductive alkylation through the metabolic formation of electrophiles.[5,6] Since most solid tumors contain areas that are hypoxic and these, as a consequence of their hypoxia, are resistant to conventional gamma radiation, sensitizing such regions by use of bioreductive cytotoxic compounds may become an important means for eradicating these cells. Such quiescent but potentially viable cells can become the foci for tumor recurrences, and therefore, their selective destruction becomes critical for the success of any radiation procedure. The objective of Threadgill and Scobie, who first proposed the development of boronated analogues of these nitroimidazoles,[539−544] was that such structures would still retain the capacity that radiation sensitizers have for targeting hypoxic cells. Cells exposed to suitable levels of high LET radiation would not have radiation resistance.

The initial focus was to prepare *o*-carboranyl linked to 2-nitroimidazoles. The approach was to attach the *closo*-carborane through various linkers to the 1 position of the nitroimidazole molecule. Synthetic difficulties were encountered in the direct preparation of such boron analogues of misonidazole using acetylenic derivatives of 2-nitroimidazole and decaborane ligands.[539] It was presumed that the conditions needed for carborane formation were too harsh and that reduction of the nitroimidazole group occurred by action of decaborane itself. As a consequence, it was considered essential that the carborane component must be formed before it is inserted into the nitroimidazole. A mild 1,3-dipolar cycloaddition scheme was developed for synthesizing these carboranyl nitroimidazoles.[540] The hydrophobic nature of these carborane-containing nitroimidazoles and their low water solubility led to the tethering of the carborane through a series oxyethylene units (Figure 54).[541,543] Such polyether-linked carboranylnitroimidazoles possessed improved aqueous solubility and preliminary biological studies in tumor-bearing mice demonstrated tumor uptake and retention.[544]

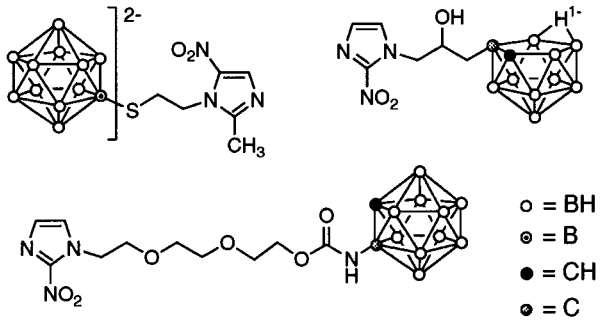


Figure 54. Boronated radiation sensitizers.

Wilbur et al.[185] used a carboranyl epoxide as the basis for also synthesizing boron-containing misonidazole congeners. To improve their aqueous solubility for biological testing, they converted the *closo*-carborane by strong base to the corresponding *nido*-carboranyl misonidazoles. The compounds that were prepared were less lipophilic than misonidazole itself but still possessed selective toxicity for hypoxic cells.[545] These structures could be readily radioiodinated,[546] and this would serve as a noninvasive method for measuring the boron concentration in tumor tissue. This information would be very useful in radiation treatment planning.

Another approach in the development of boron-containing radiation sensitizers is described by Swenson et al.[547] Their research involved a displacement reaction and the incorporation of the BSH moiety into 1-(2-bromoethyl)-2-methyl-5-nitroimidazole. The product from this reaction is a negatively charged species and in contrast with the carboranylmisonidazoles is not highly lipophilic. Representative structures of those boron-containing radiation sensitizers are shown in Figure 54.

b. Phosphates, Phosphonates, and Phosphoramidates. The early impetus for considering phosphorus-containing boron compounds as potential BNCT agents stems, certainly in part, from the observation that ^{32}P-phosphate concentrated and persisted to an extraordinarily high degree in a variety of different malignant brain tumors.[548] Such an active accretion of this anion by various CNS neoplasms with its apparent exclusion from normal brain, heightened expectations that promising boron-containing phosphorus compounds could be developed. However, it is clear that the tumor-localizing properties of trace levels of radioactive entities used for diagnosis cannot be extrapolated or fully serve as the basis for the development of BNCT agents. Nevertheless, phosphorus-containing boron compounds have been developed and screened. The earliest work reported was by Kacymarczyk and his collaborators.[549,550] In those instances, phosphorylation involved various substituted polyhedral borane anions of the B_{10}, B_{12}, and B_{20} cage structures. They demonstrated that different hydroxyl derivatives could be converted to phosphates and to pyrophosphates (Figure 55). The *o*-diphosphates appear to be very stable in aqueous solutions for a long period of time. Biodistribution studies in tumor-bearing ro-

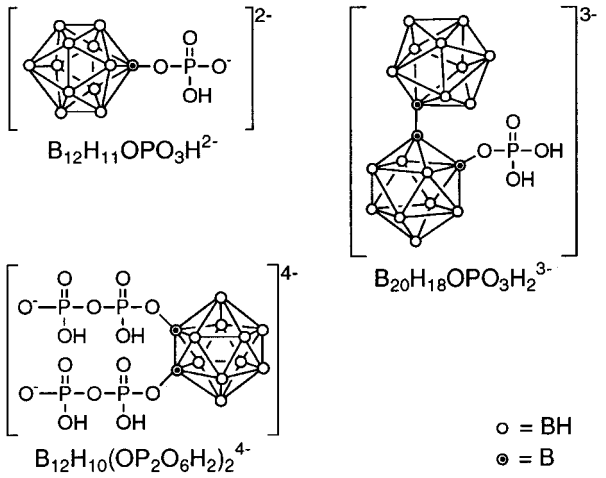


Figure 55. Phosphate esters of polyhedral boranes by Kaczmarczyk et al.

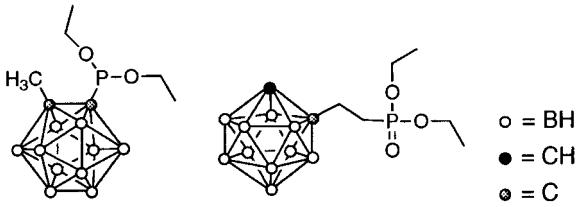


Figure 56. Phosphorus-containing carboranes.

dents showed a significant boron incorporation and persistence in the tumor by comparison with those levels observed in blood and normal brain at the same time intervals. These results are directly attributable to the free phosphate function and not to the borane anions since neither the parent hydroxyl compounds nor those in which the hydroxyl functions on the phosphate were masked demonstrated similarly tumor-targeting and tissue retentive properties.[550] The biochemical basis for the tumor's accumulation of these phosphates remains to be elucidated. Nothing has been done further with these particular phosphates until recently, but preliminary studies have shown that these compounds are highly toxic.[551]

The chemical development of phosphorus-containing carboranes (Figure 56) was carried out by both Zakharkin and Bregadze in the 1960s and 1970s.[552,553] It is only more recently that carboranylphosphonates have been proposed by Semioshkin and Lemmen et al. as potential BNCT agents based on the accumulation of nonboronated phosphonates by bone cells.[554] In the formation of carborane- and dodecaborane-containing oligomers, Schinazi, Hawthorne, Spielvogel, Gabel, and their collaborators independently have synthesized phosphorus-containing boron clusters.[186,203,204,206,265,461,468−472]

A more complete biological assessment of boron-containing phosphates and phosphonates as potential tumor-targeting BNCT agents still remains to be done. However, the interesting observations of Kaczmarczyk point to the potential that these compounds may have as BNCT agents and the need for further exploration.

c. Cyclic Thiourea Derivatives. The rationale for the development of boron-containing thiosemicarbazides, thioureas, and thiouracils is that their

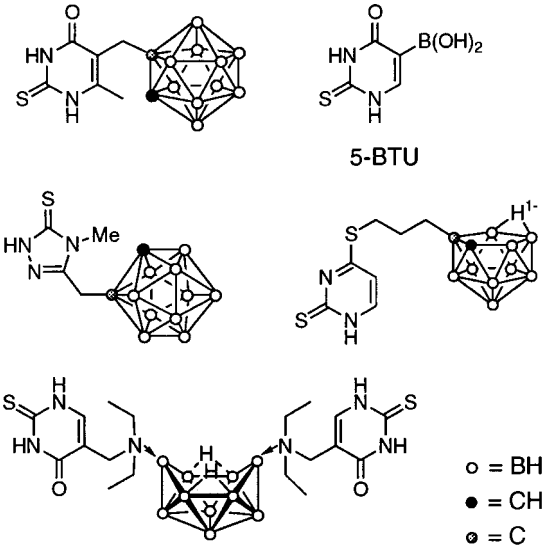


Figure 57. Boron-containing cyclic thioureas.

boron-free analogues have the capacity for becoming incorporated into the polymeric structure of melanin through their covalent bonding into melanin precursors based on the enol form of the thiourea moiety.[555,556] Thus, the boron analogues may have potential use in treating melanomas by BNCT. Roberto and Larsson described the preparation of a decaborane adduct of 5-(dimethylamino)methylthiouracil.[557−559] Subsequently, Tjarks and Gabel described the synthesis of carborane-containing cyclic thioureas both as closo and nido derivatives.[201] They also synthesized the first dihydroxyboryl-containing thiouracils.[560−562] Their biodistribution in tumor-bearing rodents demonstrated low toxicity as well as significant uptake and selectivity for malignant melanomas.[560,563] By comparison with BPA, 5-(dihydroxyboryl)-2-thiouracil (BTU) showed a different boron distributive pattern in the tumor.[560] This observation supports the possible desirability of using a cocktail of tumor-seeking boron compounds as a means of achieving a more uniform boron distribution within the tumor. Also, BTU had greater tumor persistence than did BPA.[560] Substantial synthetic work by Wilson has focused on the synthesis of *closo*-carboranylthiouracils.[564−568] Biodistribution studies in melanoma-bearing mice, especially with liposomal formulations of these derivatives, showed selective tumor uptake.[376] More recently, Gabel and his collaborators have synthesized *nido*-carboranyl and $B_{12}H_{11}$-substituted methimazoles.[20,346,569] No biological data for these compounds have been published as yet. Representative structures are shown in Figure 57. One final comment, thiouracils are strongly sequestered by the thyroid.[564] Therefore, boronated derivatives may offer the potential for treating various diseases of the thyroid.

d. Amines. Although there is no apparent biochemical reason for the synthesis of boron-containing amines themselves as tumor-targeting agents, they can serve as intermediates in the development of other compounds. Also, the use of liposomes as carrier entities did show that certain amines bind tightly to specific subcellular organelles.[164,374] Their synthesis in compounds containing the dihydroxy-

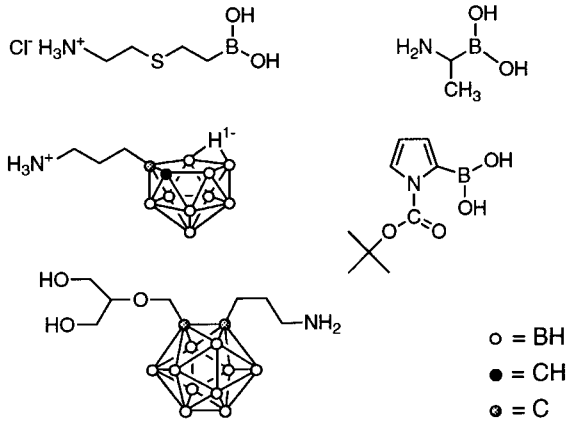


Figure 58. Boronated amines.

boryl group[144] and polyhedral borane anions[368,570−572] are well described. Their preparation in agents having an *o*-carboranyl function has been complicated by the fact that there is a chemical incompatibility between basic amines and the *closo*-carborane nucleus. The latter is readily degraded in the presence of strong amines to the corresponding nido structure with the loss of a boron atom and an opening of the cage structure. However, by masking the amine with various protective groups and isolating the amine as its hydrohalide salts, these two incompatible groups exist stably in the same molecule.[304,314,567,573−575] This afforded the opportunity of evaluating cationic carborane-containing compounds as tumor-targeting agents.[16,304,317,343,576] A correlation of this property with the physicochemical parameters of these boronated amines remains to be carried out. Representative structures are shown in Figure 58.

e. CNS Depressants: Promazines, Hydantoins, and Barbiturates. *i. Promazines.* The rationale for synthesizing boron-containing promazines stemmed from localization studies of chlorpromazine (CPZ) in melanoma-bearing mice.[577] The results demonstrated that concentrations of CPZ in tumor exceeded 100 μg/g with tumor:normal tissue ratios greater than 15 and having a biological half-life that approximated 10 days. If boron analogues containing a carborane nucleus had comparable biological properties, then the boron concentration in tumor would be at a clinically useful level. The first boron-containing promazines were described by Nakagawa and Aono[162] in which the boron moieties were attached to the aliphatic nitrogen of CPZ. The results with these compounds were not very promising owing in part to their low aqueous solubility and the probability that this aliphatic nitrogen, in protonated form, was involved in the binding to melanin and, thereby, to melanoma cells, via a charge-transfer complex with an indole nucleus.[578] The boron-containing quaternary ammonium compound may interfere with the compound's intracellular transit and in its binding to melanin. For this reason, carborane-containing promazines were synthesized in which the boron entity was incorporated into the aromatic rings of the phenothiazine nucleus.[424,425,579] Examples of such structures are shown in Figure 59. Acute toxicity of these compounds, with the exception of the *nido*-carborane analogue, was acceptable, and

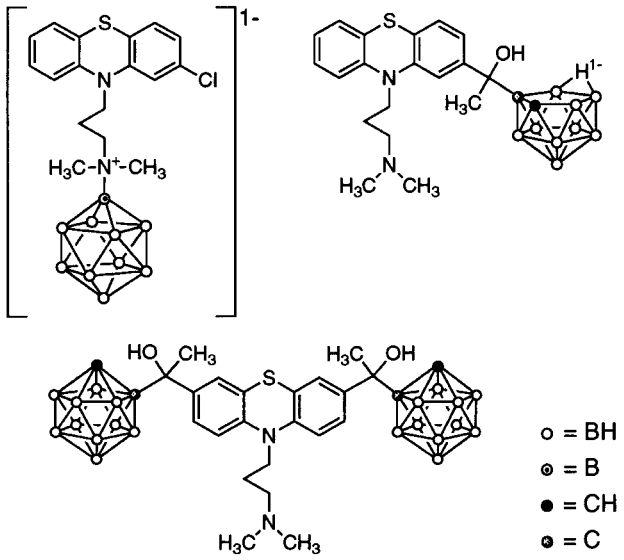


Figure 59. Boron-containing promazines.

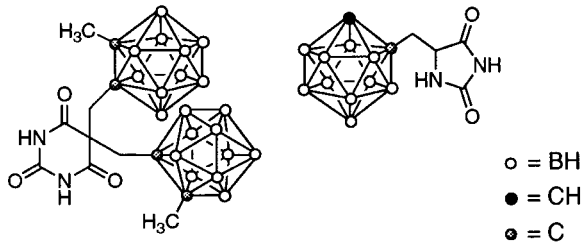


Figure 60. Boronated barbiturate and hydantoin.

the compounds were well tolerated in the same species of mice in which the B16 melanoma was transplanted. However, the localization studies with one of these under in vivo conditions produced disappointing concentration levels in tumor with appreciably larger amounts in the liver.[425] These results, together with the fact that the compounds were quite hydrophobic, had to be administered in DMSO, and produced significant sedation, indicate the need for more hydrophilic analogues that could be more readily solubilized in aqueous systems. Such syntheses have not been carried out to date.

ii. Hydantoins and Barbiturates. In 1970, the first carboranyl hydantoins and barbiturates were independently synthesized by Brattsev et al.[580] and Zakharkin et al.[294] The former prepared both boron-containing barbiturates and thiobarbiturates by the condensation of the appropriate barbituric acid with carboranyl aldehydes. The latter synthesized 5-(*o*-carboranylmethyl)hydantoin. More recently, Kazantsev and Kazantsev[581] reported on the preparation of other carboranyl thiohydantoins and Wyzlic, Tjarks et al. described the preparation of oxo- and thiohydantoins, from *o*-carboranylalanine (*o*-Car).[298]

Also, this latter group synthesized 5,5-bis[(2-methyl-*o*-carboran-1-yl)methyl]barbiturate.[298] In Figure 60 are representative structures in this category. The basis for the synthesis of those compounds described more recently is that certain CNS depressants have been shown not only to enter the brain rapidly but to achieve concentration ratios of the order of 4:1 between primary brain tumors and normal brain.[582] These compounds were initially screened as anticon-

vulsants, and the biodistribution study of representative compounds in tumor-bearing animals is planned.[298]

f. Miscellaneous. In this last category of compounds, there are structures which do not fall into any of the above classifications. Among these are a cyclic dihydroxyboryl ester-containing benzamides and nicotinamides.[583,584] They were designed on the basis of the fact that N-alkyl-substituted iodobenzamides have been used as radiopharmaceuticals.[585] Other compounds involved variously substituted *o*- and *m*-carboranes. These included benzonitriles,[586] bis(carboxyalkylcarbamoyl) derivatives,[587] phosphordiamidate nucleosides,[588] carboranes containing the azulene framework, [589,590] kojic acid, [591] isocyanates,[592] and carborane superclusters of dendrimers.[593] Others have involved the insertion of *nido*-7-$CB_{10}H_{12}^{2-}$ nucleus into various structures.[594,595] The biochemical/physiological basis by which any of these compounds would selectively target tumor cells is, in most instances, not clearly enunciated, justified, or even addressed. Thus, it will only be determined from biological studies whether any of the compounds in this category possess the requisite characteristics to be potentially useful BNCT agents.

XII. Boron Compound Detection and Analysis

A complete assessment of various methods for boron compound detection and analysis is beyond the scope of this review. However, a cursory examination is important since aspects in the synthesis and purification of the compounds relate to one's ability to detect them and to determine, at least in a qualitative way, the degree of compound purity (i.e., by thin-layer chromatography (TLC)[596,597] and by HPLC[598,599]). The common feature in all of these compounds is, of course, the boron atom and/or various boron-containing complexes. Do any of these boron linkages possess unique properties that will permit their facile detection? Also, what are the IR, NMR, and mass characteristics of different compounds and can these be used for compound detection and structure proof by spectrophotometric techniques? Finally, since the boron compounds are ultimately to be administered to mammalian systems, how can these compounds be measured in biological material and, importantly as well, are there methods for determining the subcellular location of these compounds? All of these issues are important in compound development.

It should be noted in the microdetermination of boron[600,601] and especially those involving boron cage structures that early work and, even currently, some results from certain commercial establishments show both low boron and carbon values. This may stem in part from inadequate mixing with a suitable oxidizing agent prior to combustion, and the resulting values arise from the possible formation of the refractory boron carbide.

Assessing compound purity by TLC, following a particular reaction sequence and prior to recrystallization or column chromatography, has become established procedure. With regard to the boron cage structures and those compounds containing boron hydrides, visualization by means of a $PdCl_2$ spray has become a very useful and rapid TLC method.[597] Detection is based upon the compound's ability to reduce the palladium salt to the corresponding metal as shown by the appearance of a black spot on the plate. In certain instances, it may be necessary to heat the plate to facilitate the reduction. Thus, the rapidity of the spot's appearance stems from the reducing effectiveness of the boron compound. Interestingly enough, the *nido*-carboranes are more rapid reducing agents than their closo counterparts and the former generate a spot that is more brownish in hue.

The infrared spectra of boron structures is a well-established method in compound identification. Of particular importance with respect to the boron hydrides is the very intense boron−hydrogen stretching vibration bands occurring at approximately 2470 cm^{-1} for the $B_{12}H_{12}^{2-}$ cage, 2520 cm^{-1} for *nido*-carboranes, and 2600 cm^{-1} for their closo counterparts. The development of fourier transform infrared (FTIR) instrumentation has enhanced the sensitivity and the rapidity of boron cluster measurement and has been used, as well, in the assay of polyhedral boranes in plasma.[602]

Proton NMR spectra of carborane- and polyhedral borane-substituted structures display a very broad "wave-like" signal for the terminal B−H protons ranging from $\delta = 3.00$ to -0.75 ppm. In the case of the *nido*-carboranes, a broad doublet can be observed having an unusual chemical shift at approximately $\delta = -2.5$ to -3.0 ppm. This is caused by the so-called "extra proton", or bridging proton, loosely bound to the atoms of the open pentagonal face of the boron cluster. Also, boron-11 and carbon-13 NMR are widely used in compound characterization. The former was used in demonstrating the metabolic inertness of the $B_{10}H_{10}^{2-}$ anion (Figure 61).[108]

Since naturally abundant boron consists of a mixture of 19.7% ^{10}B and 80.3% ^{11}B, the mass spectroscopy of those compounds containing carboranes or polyhedral borane clusters having 10 or more boron atoms results in very complex isotopic patterns for both molecular ions and fragments of these compounds (Figure 62).

The ability to measure boron compounds in biological fluids and tissues is of great importance in determining the potential utility of a compound and its comparison with other BNCT agents. The techniques that have been developed for measuring boron in biological materials have included the use of the following: prompt γ-ray analysis arising from the 478 keV γ-ray that occurs from the ^{10}B capture reaction;[603,604] FTIR, described above;[602] direct current plasma atomic emission spectroscopy (DCP-AES);[605] inductively coupled plasma atomic emission spectroscopy (ICP-AES);[606,607] inductively coupled plasma mass spectroscopy (ICP-MS).[360] These methods have permitted the measurement of boron levels in various tissues and fluids in the range of 0.002−1.0 μg of boron per sample.

Since the microdistribution of the boron compound has a direct impact on the radiation dose necessary for cellular destruction, efforts have been made using

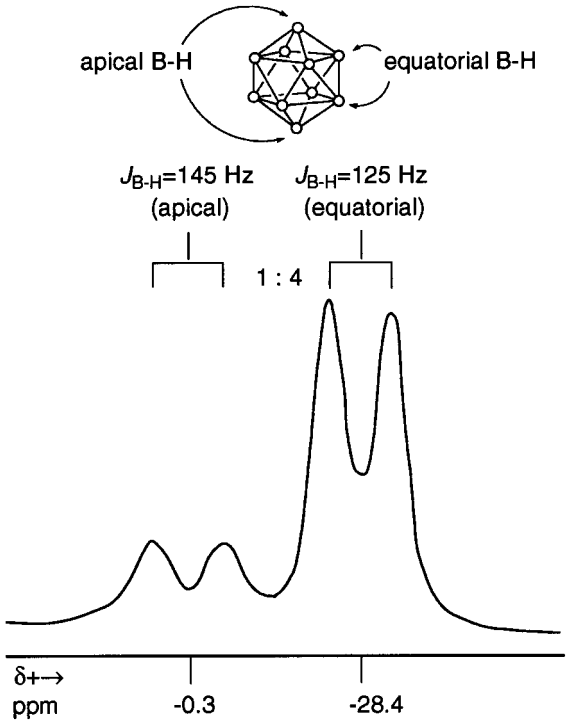


Figure 61. Proton-coupled boron-11 NMR of $B_{10}H_{10}^{2-}$, ex vivo, demonstrating the unchanged status of the compound post administration [adapted from: Sweet, W. H.; Soloway, A. H.; Wright, R. L. *J. Pharm. Exp. Therap.* **1962**, *137*, 263].

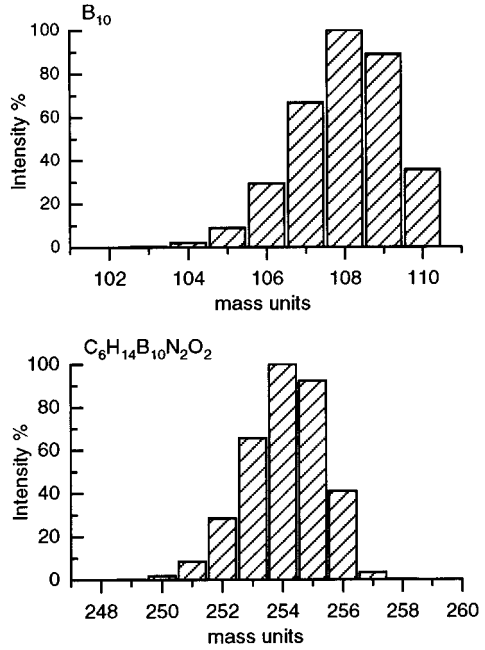


Figure 62. Computer-generated typical isotopic patterns for B_{10} and for 5-carboranyluracil (molecular formula = $C_6H_{14}B_{10}N_2O$).

alpha track autoradiography to pinpoint the precise subcellular location of the boron compound.[608−610] In addition to such studies, other techniques have been employed. These include the use of electron spectroscopic imaging (ESI) and electron energy loss spectroscopy (EELS) in combination with transmission electron microscopy[611] and ion microscopy based on secondary ion mass spectrometry (SIMS).[612−614] Morrison and his collaborators have pioneered the utilization of this latter methodology in examining the intracellular location of various BNCT agents. These approaches relate to the boron atom itself and not to the specific boron compound. Nevertheless, it affords an unique opportunity for comparing how the metabolic process affects the different agents since the objective, ultimately, is to determine the subcellular position of the boron atom. One can not only begin to estimate the radiation dose that will be delivered but also to determine whether metastatic or infiltrative clusters of tumor cells, separate from the main mass, have achieved adequate boron concentration for BNCT. In the cases of malignant brain tumors, this may be of crucial importance.

XIII. Optimizing Delivery of Compounds to Tumors

While compound development may be viewed as the major approach in optimizing delivery of a neutron absorber to a tumor, it is apparent that other strategies may be very important, especially in the targeting of malignant brain tumors in which the BBB is operative. The objective in all cases is to maximize the concentration in the tumor while concomitantly minimizing the levels in normal tissues and blood, in the path of the neutron beam. Currently in clinical trials, treatments of brain tumors by BNCT are using the intravenous route to deliver both BSH and BPA. Although convenient technically, this route certainly would be inappropriate for high molecular weight agents due to the BBB and even for many low molecular weight substances, depending upon their physiochemical properties. Better delivery strategies are needed. One approach with low molecular weight compounds is to administer the drug via the internal carotid artery that is supplying nutrients to the brain tumor. The rationale is that, in this way, the tumor may be exposed initially to higher concentrations of the compound and thereby hopefully attain elevated levels in this first pass through the arterial system. This approach is not an entirely new concept for BNCT and has been used previously by clinicians in earlier trials.

Optimizing drug delivery to brain tumors has been an ongoing concern at several centers[100,615−617] focusing on treatment by cytoreductive chemotherapy. Such approaches do have potential for BNCT as well. These strategies have been classified[618] as invasive, pharmacological and physiological methods. Among these are intratumoral injection, implantation of sustained release polymers,[616] convection-enhanced delivery, BBB disruption (using hyperosmotic mannitol or a nonapeptide bradykinin agonist, RMP-7), and transport through cationization.[619] The uniqueness of the BBB has made drug delivery to brain tumors a daunting task.

XIV. Potential Non-Cancer Uses of Boron Neutron Capture Therapy

The use of high LET radiation for nonmalignant conditions is only now in its infancy. However, the earliest consideration for using BNCT in situations other than for the treatment of cancer was considered over 30 years ago with its possible use for hypophy-

sectomy.[620] The transphenoidal injection into the pituitary gland of boron compounds that would remain sequestered therein would permit its selective destruction by thermal neutron irradiation. At that time, use of proton beams and the Bragg peak in order to ablate the pituitary gland was under active development. The clear advantage of using the proton beam generated in a cyclotron was the fact that this beam could be precisely focused in contrast with the beam of neutrons. However, while theoretically possible, nothing further was done with hypophysectomy by BNCT.

More recently, there has been interest in using the high LET particles from BNCT in treating rheumatoid arthritis by what is referred to as boron neutron capture synovectomy (BNCS). Yanch first proposed the use of BNCS as an alternative to the use of short-lived, low-energy β-emitting radionuclides for the radiation destruction of the inflamed synovium.[621,622] The advantage is that no radioactive material is administered and the high LET radiation will be generated in situ only in the affected neutron-irradiated joints. Since the high LET particles from BNCT have a very limited range of only $5-9\ \mu$m, the selective targeting of the inflamed cells lining the synovial membrane becomes of more crucial importance than occurs with the use of β-particle therapy. This is a very different use of BNCT, but it remains to be seen whether agents can be developed that will meet the more rigorous requirements that are necessary in order to achieve the desired objective.

Another possible use of BNCT could involve the treatment of hyperthyroidism as occurs in Graves' disease. Radioiodine has proven to be an excellent modality for destroying hypermetabolic thyroid tissue. One of its major limitations is that this treatment is generally confined to older people due to a high incidence of hypothyroidism several years following therapy. Various thiouracils have been developed that control hyperthyroidism and have been used in patients that are not candidates for radioiodine treatment.[623] Boron-containing thiouracils have been synthesized,[562] and these would be expected to target the thyroid gland in view of the fact that non-boron analogues completely block thyroid secretion. The question is would such boron compounds prove to be nontoxic, achieve suitable boron cellular concentrations, show persistence, and thereby offer advantage using BNCT over conventional treatment modalities?

These are merely a few of the possibilities where BNCT may be used in nonmalignant conditions. There may be other possibilities but it is incumbent that the concentration levels in the targeted tissue be adequate while tissue differentials (target:normal tissue) are appropriate. Since there are usually other means for treating nonmalignant conditions, the major thrust for BNCT has been devoted to the treatment of solid tumors for which existing therapies have failed.

XV. Future Directions

Before discussing specific directions in various areas, it is important to appreciate a major difference that binary radiotherapeutic systems present versus conventional radiation therapy. This difference involves the matter of dose escalation. While in conventional radiation therapy increasing the dose may simply involve increasing the duration that the tissue is exposed to the radiation, the matter is a much more complex one with respect to binary systems such as BNCT. To increase the dose for the latter, it is essential that the ^{10}B cellular content be elevated and that the percentage of tumor cells having the necessary boron content be increased. Increasing the neutron fluence per se may not be sufficient, and increasing the administered boron dose of any one particular agent may not necessarily produce an increase in the percentage of tumor cells that are adequately targeted. In view of the tumor's heterogeneity and variable cell cycle status, it may be highly desirable to use fractionated neutron and compound dosages and to give multiple agents in a cocktail mixture. Different compounds may achieve different concentrations in the pool of tumor cells. This latter matter will be discussed further under the area of compound development.

A. Types of Tumors

While it is always difficult to provide with any degree of certainty the future directions that may occur in any research endeavor, notwithstanding, the outline of these directions are taking shape now with respect to the types of tumors to be treated. It is probable that the focus of BNCT will be expanded from brain tumors[624] and melanomas to encompass other malignancies for which existing therapies have only marginally increased useful life expectancy. Among these may be small lung cell carcinoma, primary hepatoma, and head and neck tumors. However, other tumors, where the major need is achieving better local control prior to any evidence of metastasis, will be of increasing importance. It is necessary that efficacy must be demonstrated first with those tumors that are currently being treated by BNCT, and if that becomes established, there are many other solid tumors that will become candidates for therapy.

B. Compound Development

In the area of compound development, more sophisticated techniques will come to the fore in the area of design, including, of course, computer drug modeling. The more simplistic approach of attempting to balance a compound's lipophilic properties by the insertion of hydrophilic components will give way to a more comprehensive examination of the entire structure and a determination of whether certain elements in the structure bind to various blood components, cell membranes, connective tissues, and/or intracellular entities. Tumor-targeting will become less an art and more a science as we acquire a greater understanding of the biochemical and physiological differences between tumor cells and their normal counterparts and how to use these differences in compound design, synthesis, and targeting.

Implicit in these comments is a greater need to examine the biochemical pharmacological profile of

each agent, and specifically, what are the precise chemical species that are involved in tumor accretion? Is it the compound per se or a metabolic component? Is it a low molecular weight entity itself or is it a conjugate to some blood component that is transported to the tumor cell and sequestered as a complex? It is surprising that while both BSH and BPA are being used clinically, we still know little about the basis by which these agents achieve specific tumor cell selectivity. Knowing the mechanism for tumor accretion and persistence is very important in the design of new and possibly more effective agents.

It is clear that both BSH and BPA appear to act by different mechanisms in achieving concentration differentials between tumor, blood, and normal tissues. A more favorable result occurred treating murine brain tumors in vivo by combining these two agents than with either one alone. This observation lends support to the concept that a cocktail of different compounds will be used in the future in animal therapy as well as clinically. This situation parallels the case with cancer chemotherapy. Thus, we may anticipate that the administration of a mixture of compounds will be based upon a biodistributive pattern of the combination that will maximize both the tumor boron concentration as well as the differential to other structures in the path of the neutron beam.

Many of the recent compounds that have been synthesized have attempted to target the tumor cell's nucleus since it was viewed that this was the ultimate focus of the high LET radiation. However, the selective destruction of any key subcellular organelle within the tumor cell may produce cell death. Thus, targeting the cell's mitochondria, the endoplasmic reticulum, or the Golgi apparatus may be also be desirable, and it can be anticipated that as we learn more about the specific types of chemical structures which will bind selectively to such subcellular components and especially to those in malignant cells that more effective BNCT agents will be produced.

The outline for such developments are already underway, especially in the area of photodynamic therapy (PDT),[625] and these clearly have application to NCT. The use of certain dyes that have the potential to accumulate in particular subcellular organelles may lend themselves for use as BNCT agents. Porphyrins[419] and the cationic Rhodamine 123[626] have demonstrated their capacity to selectively target the mitochondria of tumor cells. Boronated analogues of the former have been described, and the formation of boron-containing cationic lipophilic dyes as mitochondrial agents would also seem highly appropriate. Another subcellular target is the lysosomes. Lysosomal localization is also an approach in compound development for PDT. Among the dyes that possess lysosomal targeting are Nile Blue[627] and lutetium texaphyrin.[628] Boron moieties could be inserted into such structures, and they may provide the basis for selectively destroying tumor lysosomes by BNCT. Interestingly enough, the texaphyrin dye, as with many photosensitizers following in vivo irradiation, appears to generate an apoptotic pattern that is considered to be an early event in the destruction of malignant cells.

Now, initially, the objective has been to deliver in the range of 10^9 boron atoms/tumor cell; this may be attained by any combination of compounds. However, if apoptosis is the basis for cell death,[629] then radiation that adversely affects multiple subcellular organelles (i.e., nucleus, mitochondria, and lysosomes) may achieve that effect at much lower dosages. Certainly, this must be demonstrated, but such a synergistic response cannot be ruled out a priori and needs to be explored.

In considering tumor targeting, we have not raised the issue as to whether the cells are growing under normal oxygen levels (oxic) or under hypoxic conditions and how this will affect a compound's tumor concentration. Some boron-containing hypoxic cell sensitizers have been synthesized and undergone preliminary evaluation, as described above. Even so, the results are very limited and there is no comparison of the pharmacodynamics of such analogues between oxic and hypoxic regions of the tumor.

Also, there is the important matter as to whether the cells are in a proliferating or nonproliferating mode and the affect that this will pose upon compound uptake and retention. The use of boronated nucleic acid precursors was designed with the view that such compounds may have the capability of targeting tumor cells in their S phase.[630] However, what about cells that are not undergoing cell division? What types of structures will meet that requirement?

Finally, there is the possibility of selectively targeting tumor blood vessels. This has been proposed as the basis by which, certainly in part, PDT is effective in destroying certain tumors. The concept is to damage those blood vessels supplying nutrients to the tumor and thereby adversely affect its growth and development. To accomplish this, the design and synthesis of certain small boron-containing peptides possessing a strong avidity for such vessels may be a basis for achieving this objective by BNCT.[631]

An overriding issue with all compounds is the matter of systemic toxicity. One cannot expect that any particular agent capable of selectively targeting tumor cells will be completely nontoxic to various normal tissues. The current objective is to achieve 20−35 μg boron-10/g tumor, and if this is to be attained with a single agent, then high doses may have to be administered. These could lead to significant systemic toxicity. Alternatively, if a cocktail or sequential series of drugs are given, the dosage of each may be such that a lower level of toxicity would occur. If there is no synergistic amplification, then the overall mixture may be less toxic and more readily tolerated.

There is growing concern as to what will be the next generation of NCT agents and what will be the process leading to their eventual use in clinical trials. It is essential that those compounds demonstrating promise as tumor-targeting agents must ultimately be screened in animals with lesions at sites simulating those observed in humans. All new compounds must be compared biologically against the two agents

that are being used in clinical BNCT trials, namely BSH and BPA. Any new chemical entity must be at least as good as these, in terms of tumor boron concentration and toxicity in order to merit further evaluation. Such tests may include extensive toxicological studies in larger animals as well as radiobiological assessments, using boron-10 enriched compounds. In essence, there should be specific steps in a compound's evaluation where there is a "go/no-go" decision; in other words, compound elimination is an important and integral part of the entire process, since for practical reasons not every compound that has been synthesized should undergo extensive evaluation. That should be reserved for only the more promising ones, and these ultimately may be used in combination with both BSH and BPA.

C. Methods of Delivery

Though not primarily within the scope of this review, the methods for the delivery of compounds will be more carefully scrutinized. These may be oral, intramuscular, intravenous, intraarterial, intrathecal, or intratumoral. Are the rates of administration important? It seems, at this juncture, that for the treatment of brain tumors, the use of agents that disrupt the normal BBB have succeeded in producing elevated concentrations of boron compounds in animal tumors with outstanding therapeutic results.[525] Their clinical use in the development of BNCT certainly needs to be evaluated much more fully and undoubtedly will be.

D. Neutron Sources

Another important matter, clearly outside the scope of this review, relates to the issue of neutron sources. Many radiation oncologists have expressed the view that only with the development of neutron accelerators, housed in a clinical facility, will neutron capture therapy as a new therapeutic modality for the treatment of cancer and other conditions be fully explored and hopefully realized. The use of nuclear reactors as the sole source for adequate fluxes of thermal and epithermal neutrons continues to be a significant impediment, since by and large these are not located at medical centers. As a result, it becomes difficult if not impossible to use all of the requisite clinical skills to deliver maximal radiation dosages to the tumor while ensuring that normal tissue tolerance is not exceeded. Efforts are now underway at universities, at national laboratories, and in industry to examine the feasibility of accelerator development and to determine the technical problems that must be overcome before construction of prototypical accelerators can be undertaken.

E. Measurement of Compound Levels in Tissue

Of importance in the development of any clinical radiation treatment plan, is the need to be able to measure tissue concentrations of the neutron absorber by noninvasive methods prior to the implementation of any radiation protocol. The poor nuclear magnetic imaging characteristics of the various boron nuclides, especially ^{10}B, has been a very serious limitation in measuring boron concentrations noninvasively.[632] Can the capture gamma ray generated by the nuclear reaction be a basis for the in vivo measurement of boron? An alternate approach would be to attach radioactive nuclides to the boron moiety with the expectation that such linkages are metabolically stable and that the labeled analogue behaves in pharmacodynamic and pharmacokinetic studies completely analogous to its nonlabeled counterpart. As a consequence, these would serve as the basis for measuring boron concentrations in tumor and normal tissues noninvasively. Such nuclides may be either γ- or positron-emitters. Work is already underway in the use of ^{18}F-labeled BPA for that specific purpose,[633] and one can anticipate that such efforts will continue. Alternatively, one could envisage the incorporation of stable nuclides that have more favorable MRI characteristics than ^{10}B. Such entities must be capable of being incorporated covalently into the boron component, this linkage must be stable under physiological conditions, and most importantly once again, the insertion of the MRI moiety must not perturb the biodistribution of the boron-containing compound. In other words, the boron compound with and without the MRI agent must behave in the same way from a biological standpoint. One potential nuclide is ^{19}F.

F. Nonmalignant Use of NCT

The use of the high LET radiation from the capture reaction for the treatment of nonmalignant conditions, such as in BNCS, has only just begun. In essence, it may be viewed as in situ α-particle radiation. For this to be an effective therapeutic procedure, there are very stringent requirements placed upon the targeting compound in view of the low tissue penetration of the high LET radiation. However, the intrinsic advantage of delivering such radiation selectively and without having to administer radioactive nuclides may make the chemical development of such targeting agents a worthwhile and attractive endeavor. We can expect that further research efforts along these lines may lead to additional clinical uses for NCT.

G. Other Nuclides for NCT

Much of the foregoing has focused on the use of ^{10}B in neutron capture therapy. Obviously this is not the only nuclide that can or should be considered. Fairly recently, researchers have begun to consider the use of gadolinium neutron capture therapy (GdNCT), since ^{157}Gd has a particularly large cross-section capture value of 255 000 barns for thermal neutrons. There are two major limitations in the development of GdNCT: (1) the radiation-emitted, Auger electrons have even weaker tissue penetrating properties than those derived from ^{10}B; (2) the atomic weight of this gadolinium nuclide is more than 15 times that of boron and 13 times that of carbon. Therefore, developing biochemical building block analogues incorporating ^{157}Gd cannot be readily envisaged. The key question in synthesizing gadolinium agents for NCT is whether these compounds will bind suf-

ficiently and closely to specific organelles so that the low-energy radiation produced will result in a tumoricidal or an apoptotic dose.

Just as a combination of agents derived from a specific neutron absorber may be the basis for NCT, similarly a mixture of compounds involving different neutron absorbers is certainly a possibility as well. Therefore, NCT may involve, for example, ^{10}B and ^{157}Gd compounds used in combination. Ultimately, what is important is determining the total radiation dose that will be delivered to tumor or other abnormal tissue and its surrounding normal tissues. How can the dose to the former be maximized and to the latter be minimized? Knowing the cellular and subcellular distribution of each neutron-absorbing entity becomes important in arriving at a reasonable approximation of the total estimated radiation dosage to the various tissues that are exposed.

XVI. Summary

Neutron Capture Therapy is a binary system for the treatment of cancer and other conditions where the in situ delivery of high LET radiation generated by the combination of thermal neutrons and a neutron absorber offers the opportunity for selective cellular destruction. The result is based upon biochemical and physiological differences that tumor/abnormal cells and adjacent tissues may have for a particular agent, arising from its targeting capabilities. One of the main limitations in the development of NCT has been a chemical one, namely, the synthesis of compounds with the capacity for achieving specific tissue targeting, at a concentration level necessary to meet the therapeutic objective and to do so with an acceptable degree of systemic toxicity.

Critical to the success of NCT is the need for the development of neutron absorbing compounds that possess the following attributes alone or in combination, that they: (1) demonstrate an ability to target tumor or other abnormal cells selectively in the presence of normal cells; (2) achieve appropriate concentrations in cellular, crucial subcellular organelles or receptors that will generate a cytocidal or apoptotic effect following neutron irradiation; (3) have abnormal/tumor:normal tissue concentration ratios, including those in blood, in excess of 3 or 4 to 1; (4) achieve compound persistence, especially during the course of neutron radiation which is essential for estimating the dose delivered to tumor/abnormal cells, normal tissue and the vascular system; and, importantly, (5) are sufficiently nontoxic systemically so that adequate tumor/abnormal concentrations can be attained. The key issues are can various neutron absorbing compounds be designed, synthesized, and evaluated that will demonstrate such properties and what criteria must be used in uncovering such agents?

Clearly, there is a need for greater involvement of synthetic chemists, who have a full understanding of the biochemical/physiological requirements that must be met and the approaches that need to be carried out to achieve the specific targeting of tumor or any other abnormal tissue whose selective destruction is desired.

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