University of Helsinki
Faculty of Science
Department of Chemistry
Laboratory of Radiochemistry
Finland

Studies of 4-dihydroxyborylphenylalanine and its radiolabelled analogues to implement clinical trials of boron neutron capture therapy in Finland

Jyrki Vähätalo

Academic Dissertation

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tomography (PET) studies with [^{18}F]FBPA in Finland and to perform L–BPA boron biodistribution clinical (phase I) trials with patients suffering from neurofibromatosis 2 (NF2).

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ABSTRACT

Boron compounds have been known for thousands of years starting with the Babylonians. Natural boron exists as 19.9% of ^{10}B isotope and 80.1% of ^{11}B isotope. The ^{10}B is used as a control for nuclear reactors, as a shield for neutron radiation, in instruments used for detecting neutrons and in ^{10}B containing pharmaceuticals as an emerging binary therapy called boron neutron capture therapy (BNCT).

Boron neutron capture therapy is an experimental combination of chemo- and radiotherapy: a ^{10}B containing pharmaceutical is administered to the patient, in whom it accumulates preferentially in to the neoplastic tissue. The tumour is then irradiated with neutrons. In the ensuing neutron capture reaction ^{10}B absorbs neutrons and self-destructs releasing powerful but very short-range alpha radiation and recoil lithium in the tumour. For the Finnish BNCT clinical trials an aromatic amino acid, 4-dihydroxyborylphenylalanine (BPA) was chosen to be the first boron containing pharmaceutical.

BPA synthesised via the asymmetric pathway by Malan and Morin was developed to be the boron containing pharmaceutical in the first series of Finnish BNCT clinical trials. The solubility of BPA was enhanced by complex formation with fructose. After completion of the development work BPA was administered to brain tumour patients in conjunction with clinical studies for development and testing of BNCT. We conclude that the synthesis development, complementary preclinical and clinical observations justify the safe use of BPA up to clinical phase III studies.

Radiotracers are radioactive nuclide containing chemical species that are used as markers to follow the course of a chemical reaction, physical process or to show the localisation of a substance. When used in *in vivo* studies radiotracers are referred to as radiopharmaceuticals. In our studies a direct electrophilic radioiodinating method using Iodogen as an oxidant gave reproducible amounts of radioiodinated phenylalanine instead of radioiodinated BPA.

Fluorine-18 is one of the most widely used clinical positron emitter. The radiofluorinated analogue of BPA, 4-dihydroxyboryl-2-[^{18}F]fluorophenylalanine ([^{18}F]FBPA), has been demonstrated to be a useful radiotracer in life sciences leading to PET patient studies for BNCT. In this work we have developed a concise procedure producing relatively high specific radioactivity [^{18}F]FBPA for clinical studies.

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications. In the text they are referred to their Roman numerals (**I-IV**):

I. Vähätalo J, Tuominen J, Kokkonen J, Kríž O, Karonen S-L and Kallio M. Trace impurities identified by high performance liquid chromatography/electrospray mass spectrometry in two different synthetic batches of 4-boronophenylalanine. *Rapid Commun Mass Spectrom* **1998**, *12*, 1118-1122. [1)]

II. Kulvik M*, Vähätalo J*, Buchar E, Färkkilä M, Järviluoma E, Jääskeläinen J, Kríž O, Laakso J, Rasilainen M, Ruokonen I and Kallio M. Clinical implementation of 4-dihydroxyborylphenylalanine synthesised by an asymmetric pathway. *Eur J Pharm Sci* **2003**, *18*, 155-163. [2)]

III. Vähätalo J, Kulvik M, Savolainen S and Karonen S-L. Radioiodination techniques for aromatic amino acids; possible tracers for BPA. *Frontiers in Neutron Capture Therapy* Hawthorne MF, Wiersema RJ and Shelly K (editors), Kluwer Academic/Plenum Publishers, New York, **2001**, 835-838. [3)]

IV. Vähätalo JK, Eskola O, Bergman J, Forsback S, Lehikoinen P, Jääskeläinen J and Solin O. Synthesis of 4-dihydroxyboryl-2-[^{18}F]fluorophenylalanine with relatively high-specific activity. *J Label Compd Radiopharm* **2002**, *45*, 697-704. [1)]

* Contributed equally to this work and should be regarded as the first author

CONTENTS

1. INTRODUCTION

1.1. Boron

Boron compounds have been known for thousands of years starting with the Babylonians. The element was isolated in 1808 by Sir Humphry Davy (1778-1829), Joseph-Louis Gay-Lussac (1778-1850) and Louis Jacques Thénard (1777-1857). In 1824 Jöns Jakob Berzelius (1779-1848) identified boron as an element. Name boron comes from the Arabic word *buraq* and the Persian word *burah* from borax ($Na_2B_4O_7 \bullet 10H_2O$), the principal ore of boron.

Extensive borax deposits are found in the Andes, in the Mojave Desert of California, USA, in Tibet and in Turkey. Pentahydrate species, tincalconite ($Na_2B_4O_7 \bullet 5H_2O$), is used in large quantities in the manufacturing of insulation fiberglass. Boric acid (**12**), $[B(OH)_3]$, is an important boron compound in textile products. Boron compounds are used in the manufacture of borosilicate glasses. Boron is an essential mineral for plants. For humans the World Health Organization (WHO) classifies boron as a trace element that is probably essential (WHO 1996). For example, there is experimental evidence to indicate that boron may be beneficial for optimal calcium metabolism (Hunt *et al.* 1997, Armstrong *et al.* 2000).

Boron is an electron deficient element, possessing a vacant *p*-orbital. Compounds of boron often behave as Lewis acids, bonding with electron rich species. Boron is similar to carbon with its capability to form stable covalently bonded molecular networks. Boron compounds are being investigated for a broad range of applications, such as constituents of antibiotics (Dunitz *et al.* 1971, Kohno *et al.* 1996) and as anticancer bioconjugates (Prusoff *et al.* 1993, Luo & Prestwich 1999, Murmu *et al.* 2002, Paterson *et al.* 2003).

Natural boron consists of 19.9% ^{10}B isotope and 80.1% ^{11}B isotope. 11 radioactive boron isotopes are known. The longest living radioactive boron isotope is ^{8}B with the half-life of 0.77 s. The ^{10}B isotope is used as a control for nuclear reactors, as a shield for neutron radiation, in instruments used for detecting neutrons and in ^{10}B-containing pharmaceuticals as an emerging binary therapy called boron neutron capture therapy (BNCT).

1.2. Boron neutron capture therapy

Boron neutron capture therapy is an experimental combination of chemo- and radiotherapy: a ^{10}B containing pharmaceutical is administered to the patient, in whom it accumulates preferentially in to the neoplastic tissue. The tumour is then irradiated with neutrons. In the ensuing neutron capture reaction ^{10}B absorbs neutrons and self-destructs releasing powerful but very short-range alpha radiation and recoil lithium in the tumour (Taylor & Goldhaber 1935, Locher 1936, Perks *et al.* 1988, Barth *et al.* 1990, Slatkin 1991, Barth *et al.* 1992, Carlsson *et al.* 1992, Sauerwein 1993, Savolainen & Kallio 1993, Barth & Soloway 1994, Flam 1994, Lundquist *et al.* 1994, Pignol & Chauvel 1995, Barth *et al.* 1996, Kallio *et al.* 1996, Burian *et al.* 1997, Sweet 1997, Barth *et al.* 1999, Diaz *et al.* 2000, Barth 2003). The alpha and ^{7}Li-particles released upon neutron capture by ^{10}B have a very short range (5–10 μm) and a high linear energy transfer (LET). Consequently, the lethal damage is restricted to the ^{10}B containing cell and cells in its immediate vicinity. The most important component is the dose resulting from the $^{10}B(n,\alpha)^{7}Li^*$ reaction, Figure 1. All other dose components (e.g. gamma contamination of the incident neutron beam, $^{14}N(n,p)^{14}C^*$ and $^{1}H(n,\gamma)^{2}H$ reactions or fast neutrons) involved with the neutron irradiation are non-selective (Seppälä *et al.* 1999).

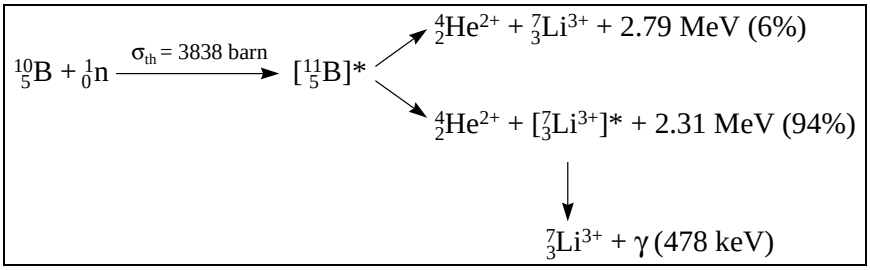

Figure 1. Boron neutron capture nuclear reaction, briefly $^{10}B(n,\alpha)^{7}Li^*$.

In early BNCT trials in the 1950's and early 1960's borax, boric acid, *p*-carboxyphenylboronic acid [$B(OH)_2PhCOOH$], sodium pentaborate (NaB_5O_8) and disodium decahydrodecaborate ($Na_2B_{10}H_{10}$) (**23**), were used as pharmaceuticals (Godwin *et al*. 1955, Asbury *et al*. 1972, Slatkin 1991). Currently, among various synthetic boron compounds (Hawthorne 1993, Morin 1994, Wyzlic *et al*. 1994, Lesnikowski & Schinazi 1995, Gabel 1996, Mehta & Lu 1996, Sjöberg *et al*. 1997, Soloway *et al*. 1998, Suominen 1998, Hawthorne & Lee 2003) only two compounds are used as pharmaceuticals: an aromatic amino acid, 4-dihydroxyborylphenylalanine [*p*-(2-carboxy-2-aminoethyl)-benzeneboronic acid, 4-boronophenylalanine, BPA] (**1**) and an inorganic salt; disodium mercaptoundecahydro-*closo*-dodecaborate (borocaptate sodium, BSH) (**2**), Figure 2.

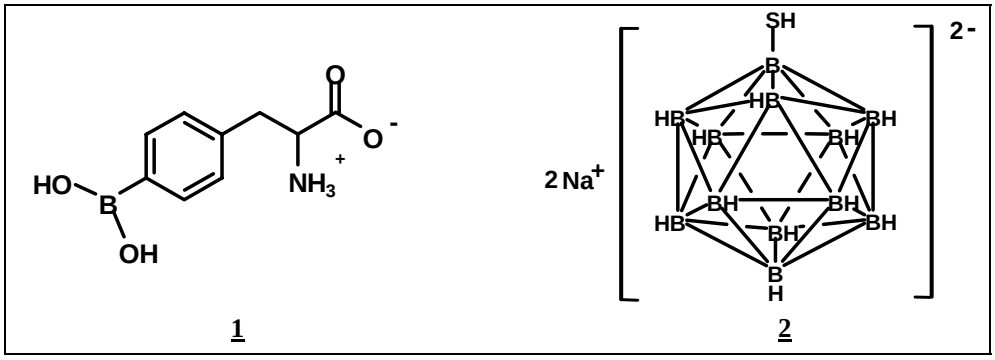

Figure 2. Structures of BPA **1** and BSH **2**.

1.3. 4-dihydroxyborylphenylalanine

BPA is a structural analogue of natural aromatic amino acids phenylalanine (Phe) (**11**) and tyrosine (Tyr) (**10**). The *para*- or 4-position hydrogen of Phe or the hydroxyl group of Tyr are substituted in BPA by the dihydroxyboryl group, $-B(OH)_2$. The first synthetic method for BPA affording racemic D, L– BPA was developed in the 1950's (Snyder *et al*. 1958). Natural amino acids belong to L–series, an *in vitro* experiment has also demonstrated that there is a preferential tissue uptake of L–BPA compared to D–BPA, Figure 3, (Coderre *et al*. 1987). Enantiomerically purified L–BPA can be obtained via enzymatic resolution of the D, L–BPA ethyl esters (Tong *et al*. 1971, Roberts *et al*. 1980). In the 1990's synthetic pathways yielding enantiomeric excess of L–BPA have been developed (Samsel 1992, Malan & Morin 1996, Nakao *et al*. 1996, Nakamura *et al*. 1998, Malan & Morin 1998).

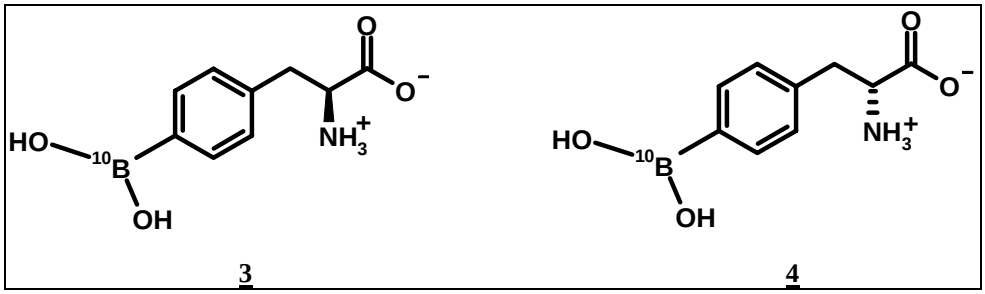

Figure 3. Enantiomers of BPA: L–BPA **3** and D–BPA **4**

At physiological pH value (7.4) BPA exists as *zwitterion* or inner salt that the net charge is zero. At physiological pH the solubility of *zwitterionic* BPA is only 1.6 g/l, which is too low for patient administration as an intravenous (i.v) infusion. Hydrochloric salt of BPA (BPA • HCl, pH 1.5 for 0.1 M water solution) has been used for perlesional clinical trials of malignant melanoma (Mishima *et al*. 1989a and 1989b). For a clinical study BPA has been administered orally as slurry in water or fruit juice (Coderre 1992). Boric acid forms an anionic complex with carbohydrates (Böeseken 1949), phenylboronic acids react with fructose to form an anionic complex (Torssell 1957) and in basic solution boric acid moiety of BPA takes an anionic sp^3 structure. Fructose was found to formulate the strongest and most stable complex with BPA of the *cis*-diol monosaccharides studied, Figure 4. The solubility of BPA as an anionic fructose complex (BPA–F) is about 100 g/l (Mori *et al*. 1989).

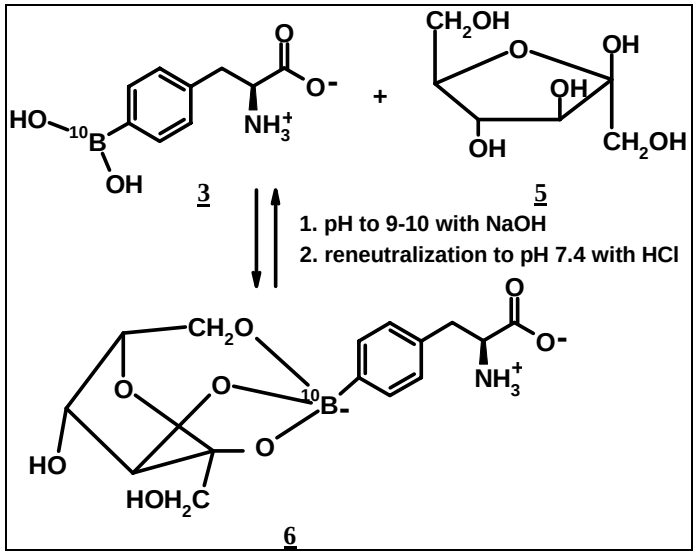

Figure 4. Formulation of anionic BPA–F for *in vivo* administration schematically: **5** β-furanose ring of fructose and **6** the plausible structure of BPA–F (Shull *et al*. 2000)

Melanoma cells accumulate actively aromatic amino acids for use as precursors in the synthesis of the pigment melanin. Melanogenesis starts with the oxidation of Tyr to 3,4-dihydroxyphenylalanine (DOPA) by tyrosinase, a key enzyme of melanin synthesis. It seems the L–BPA mimics L–Tyr in the early stage of melanogenesis and it accumulates in melanoma

tissue to a greater extent than in normal tissue providing sufficiently high boron concentrations for melanoma BNCT (Ichihashi *et al*. 1982, Coderre *et al*. 1987, Coderre *et al*. 1988, Belkhou *et al*. 1992, Packer *et al*. 1992, Tsuboi *et al*. 1998). The design of ^{10}B compounds for use in treating malignant brain tumours was initially based on the increased permeability of the blood-brain barrier (BBB) in the tumour by contrast with normal brain (Sweet *et al*. 1963). Similarly to all natural amino acids L–BPA can diffuse into cells. Active uptake of Tyr or Phe and leucine into brain is relatively effective compared to other amino acids (Oldendorf 1971). According to animal studies L–BPA seems to be taken into cells most similarly to Tyr with the L amino acid transport system (Wittig *et al*. 2000). Results of the evaluation of BPA in brain tumour-bearing animals have appeared to meet the necessary criteria for becoming a useful BNCT drug for gliomas (Soloway *et al*. 1961, Coderre *et al*. 1990, Coderre *et al*. 1992, Coderre *et al*. 1994, Matalka *et al*. 1994). Clinical trials in patients with melanomas and gliomas were considered to be warranted on the basis of the preclinical evaluation of L–BPA. Generally, in order to continue to clinical phases with a potential boron compound sufficiently low toxicity, ^{10}B concentration of 10-35 μg/g (parts per million, ppm) in tumour, and ^{10}B tumour to surrounding normal tissue ratio greater than 1, preferably more than three, should be demonstrated in preclinical phase.

1.4. Boron analysis in biological samples for boron neutron capture therapy

In theory, a single ^{10}B neutron capture reaction is capable to destroy a cancer cell. In practice, a concentration of 10-35 ppm ^{10}B, equivalent to 10^8-10^9 atoms of ^{10}B per cell, is required to destroy the cell (Fairchild and Bond 1985, Hawthorne 1993, Soloway *et al*. 1998). This required concentration range is due to the localization of the boron pharmaceutical at or inside the cell. Concentrations of approximately 10 ppm ^{10}B are required in the neighbourhood of the DNA and about 30-35 ppm is required for cytoplasmic positions or for extracelluary bound boron pharmaceutical (Probst 1999). Modern nuclear reactor based epithermal (0.5 eV-10 keV) neutron beams fulfil the requirements for effective BNCT with neutron fluxes of about 10^9 neutrons/cm^2 s (e.g. Perks *et al*. 1988, Moss 1990, Rogus *et al*. 1994, Liu *et al*. 1996, Burian *et al*. 1997, Moss *et al*. 1997, Auterinen *et al*. 2001, Kortesniemi 2002, Seppälä 2002).

Assessment of tumour ^{10}B levels is required for dosimetric modelling in BNCT. In treatment planning, the distribution of dose components: total absorbed gamma dose (D_g), dose from the boron neutron capture reaction (boron dose, D_B), absorbed dose from the nitrogen capture reaction (D_N) and absorbed fast neutron dose predominantly from recoil protons (D_{fast_n}) are computed in a geometric model of a patient's head (or body) (Seppälä 2002). The direct pharmacokinetic analysis of ^{10}B in a patient is impossible because the continuous measurement of the tissue boron concentrations *in vivo* is technically difficult. As surrogate for determining the *in vivo* tissue boron content, whole blood concentrations are used instead. Currently, it is assumed that each of the various regions of interest has an even average boron concentration. However, the observed mean glioma tissue to whole blood boron concentrations after L–BPA administration have varied from 1.4 (Elowitz *et al*. 1998) to 4 (Coderre *et al*. 1998). Variable boron concentrations in different tumour types (melanomas, brain tumours) and different parts of the same tumour have been reported (Mallesch *et al*. 1994, Elowitz *et al*. 1998, Coderre *et al*. 1998, Kulvik *et al*. manuscript in preparation). Nevertheless, the irradiation time for BNCT is adjusted on the basis of the preirradiation whole blood boron concentration, assuming a mean boron concentration ratio of 1:1 for blood to healthy tissue and 1:3.5 for blood to tumour tissue. This data is derived from preclinical (e.g. Coderre *et al*. 1990, Coderre *et al*. 1992, Coderre *et al*. 1994, Matalka *et al*. 1994) and clinical (Coderre 1992, Mallesch *et al*. 1994, Coderre *et al*. 1997, Elowitz *et al*. 1998)

biodistribution studies in patients with gliomas. Kinetic models for estimating whole blood ^{10}B time-concentration curves for L–BPA mediated BNCT have been created (Ryynänen *et al*. 2000, Kiger *et al*. 2001, Ryynänen *et al*. 2002, Kiger *et al*. 2003).

Numerous methods for preclinical and clinical trials boron analysis in BNCT have been investigated (Probst 1999). Boron analytical techniques used in biological samples for BNCT are inductively coupled plasma–atomic emission spectrometry (ICP–AES) (Tamat *et al*. 1987, Bauer *et al*. 1989, Johnson *et al*. 1992, Bauer *et al*. 1993, Laakso *et al*. 2001), direct current plasma–atomic emission spectrometry (DCP–AES) (Barth *et al*. 1991), inductively coupled plasma–mass spectrometry (ICP–MS) (Vanhoe *et al*. 1993, Nyomora *et al*. 1997) and prompt γ-ray activation analysis (Kobayashi & Kanda 1983, Matsumoto & Aizawa 1990, Raaijmakers *et al*. 1995).

When the Finnish BNCT project was approaching preclinical phase the most applicable methods for the on-line boron determination at the Finnish BNCT facility had to be chosen. Methods based on prompt γ-ray activation analysis, ICP-AES and ICP-MS were evaluated as the most suitable ones. The prompt γ-ray activation analysis based method was not technically feasible at the Finnish BNCT facility. ICP-AES was decided to be the principal method and ICP-MS was chosen to be the secondary method in reserve. A new ICP–AES was developed at the Finnish BNCT facility to determine the blood boron concentration during and after infusion of BPA (Laakso *et al*. 2001). The ICP-AES method uses protein removal with trichloroacetic acid before analysis was compared with the ICP-MS, which uses wet ashing as sample pre-treatment. The chosen ICP-AES method was found to be feasible and accurate for boron determination during clinical trials in BNCT (Laakso *et al*. 2001). The cross calibration of the ICP-MS and ICP-AES instruments was validated. Therefore, ICP-MS was found to be a secondary boron determination instrument in reserve for clinical trials at the Finnish BNCT facility. During the year 2003 a rapid method for the direct analysis of boron in whole blood by ICP-AES has been implemented at the Finnish BNCT facility based on the method developed originally by Bauer *et al*. (1993) (Auterinen *et al*. 2003).

1.5. Boron neutron capture therapy research and development in Finland

A research and development project to carry out clinical applications of BNCT was established in the early 1990's in Finland (Savolainen & Kallio 1993, Auterinen & Kallio 1994, Savolainen *et al*. 1997). The Finnish BNCT epithermal neutron beam in Otaniemi, Espoo uses the FiR1 reactor, which is a light-water moderated 250 kW Triga Mark II type nuclear reactor (Auterinen *et al*. 2001, URL: http://www.vtt.fi/pro/pro1/bnct/index.htm). Malignant gliomas were chosen as the first target of BNCT in Finland (Kallio *et al*. 1996, Joensuu *et al*. 2003). A multidisciplinary research and development team consisting of experts in administration, chemistry, engineering, medicine, pharmacy, physics, and veterinary sciences has been pursuing BNCT to bring it into clinical practice. There have been about 70 scientists developing the therapy. The basic preclinical research programs were successfully completed by 1998 (Aschan 1999, Kosunen 1999, Benczik 2000, Färkkilä *et al*. 2001, Laakso *et al*. 2001, Kortesniemi 2002, Ryynänen 2002, Seppälä 2002, **II**). In collaboration with Katchem Ltd, Czech Republic (URL: http://www.katchem.cz), the Finnish research group has improved the manufacturing process of L–BPA (**I**, **II**). Based on this work the BPA manufactured by Katchem was used in the first clinical phase I/II trials. The licensing procedure of the neutron beam and BNCT facility was completed in 1999. The first patient was treated in May 1999. At present, all ongoing clinical trials are sponsored by Boneca Corporation (URL: http://www.boneca.fi). The patient treatments are carried out in

collaboration with Technical Research Centre of Finland VTT, Helsinki University Central Hospital and Boneca Corporation. The clinical research is focused on phase I/II studies on safety and efficacy of L–BPA mediated BNCT in primary or recurrent gliomas as well as on recurrent inoperable head and neck carcinomas after previous conventional radiotherapy.

1.6. Preclincal and clinical trials of a boron pharmaceutical

Developing a pharmaceutical is a demanding, long, risky, and expensive project. Synthetic organic chemistry is crucial in the development of novel chemical entities. During early research and preclinical testing, molecules undergo laboratory investigation and animal model testing for pharmacology, efficacy and toxicity. Detailed regulations for pharmaceutical and medical device industry have been published including guidelines to current Good Manufacturing Practice (cGMP) (PIC/S 2002), Good Laboratory Practice (GLP) (OECD 1998) and Good Clinical Practice (GCP) (ICH 1996).

Currently, only three compounds have been evaluated to be used as modern clinical pharmaceuticals in BNCT: BPA, BSH and disodium decahydrodecaborate, currently known as GB-10 (**23**) (Hawthorne & Lee 2003). However, numerous potential boron compounds have been synthesised and many compounds have been tested preclinically (Hawthorne 1993, Morin 1994, Wyzlic *et al*. 1994, Lesnikowski & Schinazi 1995, Gabel 1996, Mehta & Lu 1996, Sjöberg *et al*. 1997, Soloway *et al*. 1998, Suominen 1998, Hawthorne & Lee 2003).

Generally, the clinical phase I consist of clinical pharmacology: pharmacokinetics [LAD(M)E: liberation, absorption, distribution, (metabolism and excretion) that can be combined as elimination] and when possible pharmacodynamics. Phase I trials include blood tests and biopsies to evaluate how the new compound is working physiologically. Small groups of patients are treated with a certain dose of a potential compound. During the trial the dose is usually increased by group in order to find the highest dose that does not cause unacceptable harmful side effects. Although the primary purpose of phase I trials is to find the safest dose of a new pharmaceutical, researchers can also evaluate if the new pharmaceutical benefits people. Phase I cancer trials usually have 15 to 30 participants. After a phase I trial is completed, researchers decide whether there are enough data to support further study with a phase II trial whether further research should be discontinued.

Boron biodistribution studies can be classified as phase I trials of BNCT. Boron biodistribution studies are also called ‘preludes’ for clinical BNCT trials: for example in a glioma boron biodistribution study a ^{10}B containing pharmaceutical is administrated to a patient prior to craniotomy for resection of glioma, blood samples are collected and biopsies of tumour and tissues are obtained for boron elemental assay (Sweet & Javid 1952, Sweet *et al*. 1963, Finkel *et al*. 1989, Coderre 1992, Hariz *et al*. 1994, Mallesch *et al*. 1994, Ceberg *et al*. 1995a, Stragliotto & Fankhauser 1995, Gabel *et al*. 1997, Kageji *et al*. 1997, Tagaki *et al*. 1997, Coderre *et al*. 1998, Elowitz *et al*. 1998, Horn *et al*. 1998). Patients participating in this kind of BNCT phase I trials are not irradiated with neutrons. In Finland L–BPA boron biodistribution studies of meningioma, Schwannoma and neurofibromatosis 2 (NF2) patients have been performed (Kulvik *et al*. manuscript in preparation, **II**). Boron biodistribution trials are traditionally performed to verify the basic requirements of boron pharmaceuticals prior to clinical trials with neutron irradiation.

Generally, phase II trials, also called clinical investigations, continue to test the safety of the new pharmaceutical, and begin to evaluate how well it works against a specific type of cancer.

Phase II cancer trials usually have less than 100 participants. When a phase II trial begins, it is not yet known if the pharmaceutical tested works against the specific cancer being studied. Unpredictable side effects can also occur in these trials.

Usually in BNCT phase I and II clinical trials are combined. The tumour is resected surgically before neutron irradiation. A L–BPA mediated BNCT glioma phase I/II trial has been completed in Brookhaven National Laboratory (BNL), USA. 53 patients participated to the phase I/II trial in BNL between September 1994 and May 1999. The safety of L–BPA mediated BNCT in patients with malignant gliomas was shown. In the trial the safe upper limit of modelled radiation dose that the central nervous system can tolerate was determined using the Brookhaven Medical Research Reactor (BMRR) neutron beam (Chanana *et al*. 1999, Diaz 2003). The analysis of 24 patients has reported of the L–BPA mediated BNCT trial (defined as *phase I* trial with neutron irradiation, not as combined *phase I/II* trial) in Harvard/Massachusetts Institute of Technology (MIT), USA, for intracranial tumours (gliomas and metastatic melanomas). Two melanoma patients have showed a complete radiographic response (Busse *et al*. 2003). In Studsvik, Sweden, a phase II L–BPA mediated BNCT trial has been started March 2001. The Swedish trial is based on the results from the phase I/II trial completed at BNL. No severe BNCT related acute toxicities were reported with the analysis of the first 17 glioma patients (Capala *et al*. 2003). In Finland, the analysis of the first 18 glioma patients revealed also that L–BPA mediated BNCT was generally well tolerated (Joensuu *et al*. 2003). In 2003 new phase I/II protocols for recurrent gliomas and recurrent inoperable head and neck carcinomas after previous conventional radiotherapy have been opened in Finland. In Japan combined phase I/II types L–BPA mediated BNCT trials with patients of gliomas and melanomas are going on (Fukuda *et al*. 1999, Takahashi *et al*. 2003, Imahori personal communication, July 2003). In Italy, the first human study of the L–BPA mediated BNCT to treat liver metastases has been reported (Pinelli *et al*. 2002).

Generally, phase III trials, also called formal therapeutic trials, focus on how a new treatment compares to standard, or the most widely accepted, treatment. In phase III trial, participants have an equal chance to be assigned to one of two or more groups (randomisation): one group gets the standard treatment and the other group gets the novel treatment tested. Phase III trials usually have hundreds to thousands of participants, in order to find out if there are true differences in the effectiveness of the treatment being tested. The researchers will inform the medical community and the public of the trial results. In most cases, a trial's results are first reported in peer-reviewed scientific journals. Phase IV trials, also called post-licensing studies, are used to further evaluate the long-term safety and effectiveness of a treatment.

L–BPA mediated BNCT is currently undergoing clinical phase I, II and combined phase I/II trials. As an experimental combination of chemo- and radiotherapy BNCT poses a number of unique problems. Therefore the implementation of clinical trials and the interpretation of the clinical results are challenging. It has been proposed that the BNCT community needs to standardize each aspect of the design, implementation, and reporting of clinical trials before proceeding into phase III clinical trials (Gupta *et al*. 2003).

1.7. Radiotracers

Radiotracers are radioactive nuclide containing chemical species that are used as markers to follow the course of a chemical reaction, physical process or to show the localisation of a substance. The activity of the radioisotope is monitored to follow the process under investigation. Radiotracers are referred to as radiopharmaceuticals when used in *in vivo*

studies. In the life sciences radiotracers have many applications, e.g. in evaluation of a radiopharmaceutical by tissue uptake distribution studies or by autoradiography in experimental animals, or in imaging techniques like Positron Emission Tomography (PET) and Single Photon Emission (Computed) Tomography (SPET or SPECT), Table 1.

Table 1. Some isotopes used in diagnostic radiopharmaceuticals

Isotope	Half-life	Radiation	Detection
^{11}C	20.4 min	β^+	PET
^{18}F	109.7 min	β^+	PET
^{75}Br	1.6 h	β^+	PET
^{99m}Tc	6.01 h	γ	SPET
^{123}I	13.2 h	γ	SPET

1.8. 4-dihydroxyboryl-2-[^{18}F]fluorophenylalanine

Fluorine-18 is one of the most widely used clinical positron emitters (e.g. Stöcklin & Pike 1993, Kilbourn 1990, Bergman 2001). Electrophilic fluorinating agents provide a rapid means of introducing ^{18}F into organic molecules through aromatic electrophilic substitution. The direct electrophilic radiofluorination of BPA was first reported by Ishiwata *et al.* (1991a). The radiochemical yield of 4-dihydroxyboryl-2-[^{18}F]fluoro–D,L–phenylalanine (4-borono-2-[^{18}F]fluoro–D,L–phenylalanine, D, L–[^{18}F]FBPA) was 25-35% corrected to end of bombardment (EOB), which in this case means to the end of the production of ^{18}F with cyclotron. The specific activity was 35-60 MBq/μmol at the end of synthesis (EOS). The overall synthesis time was about 80 min and the radiochemical purity over 99% determined by analytical high performance liquid chromatography (HPLC). Synthesis of [^{18}F]FBPA with fructose was reported by Reddy *et al.* (1995): BPA was radiofluorinated as described by Ishiwata *et al.* (1991a) and then treated with fructose. The fractions containing the fructose complex of [^{18}F]FBPA ([^{18}F]FBPA–F) were identified by reverse chiral thin layer chromatography and by HPLC.

The tissue distribution study of D, L–[^{18}F]FBPA in normal mice showed that the compound has potential as a tracer for pancreas imaging because of its rapid clearance from all other tissues (Ishiwata *et al.* 1991a). Brain uptake was found to be constant for 2 hours. The results in normal mice suggested also no incorporation of D, L–[^{18}F]FBPA into protein synthesis or very slow incorporation. Defluorination of the compound was anticipated from the constant radioactivity levels in bone including bone marrow. The radiation-absorbed dose to the bladder wall was found to be higher than any other organ but the dose was lower than for 6-[^{18}F]fluoro-DOPA (Ishiwata *et al.* 1991a). The potential of D, L–[^{18}F]FBPA, D–[^{18}F]FBPA and L–[^{18}F]FBPA for melanoma imaging by PET was studied using animal models (Ishiwata *et al.* 1992a, Ishiwata *et al.* 1992b): a high uptake of racemic or L–enantiomer was found in subcutaneous murine B16 melanoma or in Greene's melanoma No. 179 for the first 6 h after an injection of [^{18}F]FBPA. For D–enantiomer radioactivity levels in all tissues investigated were very low compared with the L–form (Ishiwata *et al.* 1992b). The tumour uptake and metabolism of D, L–[^{18}F]FBPA in mice bearing FM3A mammary carcinoma resulted in high FM3A-to tissue uptake ratios. The tracer was found to be stable for metabolic alteration (Ishiwata *et al.* 1991b). The cellular distribution of L–[^{18}F]FBPA and [6-^{3}H]thymidine ([^{3}H]Thd, a DNA precursor) in two murine B16 melanoma sublines and FM3A mammary

carcinoma using double-tracer microautoradiography showed that the L–[^{18}F]FBPA accumulation was primarily related to the activity of DNA synthesis and, secondarily, to the degree of pigmentation in melanocytes (Kubota *et al*. 1993).

Dynamic PET data of [^{18}F]FBPA incorporation into 33 cases of primary glioma has been represented as Gjedde-Patlak plots as defined by Patlak *et al*. (1983) (Imahori *et al*. 1998a). A three-compartment model using rate constants [K_1(ml/g/min, k_2 (min^{-1}) and k_3 (min^{-1})] based on the equation proposed by Huang *et al*. (1980) has been used for the pharmacokinetic analysis of [^{18}F]FBPA (Imahori *et al*. 1998a). Dynamic PET studies have revealed that [^{18}F]FBPA is selectively incorporated in the malignant tumour cells showing high radioactivity and tumour to normal tissue ratios that were greater than 1 in all patients, reaching the maximum value of 6 (Mishima *et al*. 1997, Imahori *et al*. 1998a, 1998b and 1998c). The rate constant K_1 value, thought to be a quantitative parameter of the amino acid transport process, differed significantly between the malignant tumour group (glioblastoma multiforme) and the benign tumour group (astrocytoma grade II) (Imahori *et al*. 1998a). Tumour tissue uptakes L–[^{18}F]FBPA, better than the racemic mixture of the radiofluorinated BPA analogue, D, L–[^{18}F]FBPA, (Imahori *et al*. 1998a). L–[^{18}F]FBPA was accumulated gradually after bolus injection reaching a constant level 42 min after injection and this constant was defined as the incorporation constant. The constant is thought to reflect the appropriate L–[^{18}F]FBPA accumulation in tumour tissue. Based on the incorporation constant, the methods for estimating tumour ^{10}B concentrations are devised (Imahori *et al*. 1988b and 1988c). The similarity of pharmacokinetics of L–[^{18}F]FBPA and L–BPA given as BPA–F was proposed to have been confirmed. PET studies using L–[^{18}F]FBPA are concluded to provide images of treatable brain tumours for BNCT and to permit the determination of local ^{10}B levels (Imahori *et al*. 1998b and 1998c). The kinetic constants of [^{18}F]FBPA metabolism as determined by PET can be significant in predicting the prognosis and indicating the feasibility of BNCT in patients with gliomas (Takahashi *et al*. 2003).

The distribution of [^{18}F]FBPA–F by PET has been found to be consistent with identified tumour by magnetic resonance imaging (MRI) in two patients with malignant gliomas (Kabalka *et al*. 1997). The [^{18}F]FBPA–F tumour to normal brain uptake ratio was 1.9 in the first patient and 3.1 in the second patient at 52 min after bolus injection of [^{18}F]FBPA–F (Kabalka *et al*. 1997). The observed difference in uptake kinetics between [^{18}F]FBPA–F and [^{18}F]FBPA was proposed to possibly be due to that [^{18}F]FBPA–F has kinetics closer to the most common PET tracer in oncology, 2-[^{18}F]fluoro-2-deoxy–D–glucose (2-FDG), than L–[^{18}F]FBPA as a free amino acid (Kabalka *et al*. 1997). The knowledge of the distribution of [^{18}F]FBPA–F by PET is concluded to be capable of providing *in vivo* [^{18}F]FBPA–F biodistribution data that may prove valuable for patient selection and BNCT treatment planning (Kabalka *et al*. 1997). The isodose contours derived from [^{18}F]FBPA-PET studies have been shown to correspond more closely to the observed BNCT clinical results than do the isodose contours generated by modelling calculations (Nichols *et al*. 2002). PET imaging with [^{18}F]FBPA can be used to identify potential tumours that may be amenable to BNCT (Kabalka *et al*. 2003)

2. AIMS OF THE PRESENT STUDY

The aim of this study was to

- evaluate L–BPA (papers **I** and **II**) in order to implement clinical phase I and phase I/II trials of BNCT in Finland.

- develop a radiolabelled analogue of L–BPA (papers **III** and **IV**) for clinical imaging studies in Finland.

3. METHODS

3.1. Evaluation of 4-dihydroxyboryl–L–phenylalanine (papers **I** and **II**)

When the Finnish BNCT project was approaching the first clinical phase I/II trial careful chemical analysis was made of the two different synthetic batches available of L–BPA. The first batch of L–BPA was purchased from Boron Biologicals (Raleigh, North Carolina, USA, URL: www.boronbiologicals.com). It was synthesised by the racemic Snyder pathway (Snyder *et al*. 1958). The another batch was a preliminary product from Katchem Ltd. (Prague, Czech Republic) synthesised by a pathway developed by Malan & Morin (1996). The latter pathway was evaluated as the most applicable to the first series of the Finnish BNCT clinical trials because the final product was enantiospecific affording only desired enantiomer of BPA, L–BPA.

Chemical characterisation of L–BPA synthesised by a pathway developed by Malan & Morin (1996) was verified by ^{1}H NMR, ^{13}C NMR, ^{10}B NMR, and IR spectrometry in Katchem Ltd. Melting points were determined in open capillary tubes and were uncorrected. Chemical purity of BPA batches was studied employing a reversed-phase (RP) isocratic HPLC both in Prague and in Helsinki. Elemental analyses were carried and specific rotation information was collected to investigate the enantiospecifity of the synthesised L–BPA in Katchem Ltd.

In order to verify that also BPA synthesised with the novel method is nontoxic, an animal study was carried out. The solubility of L–BPA was enhanced by complex formation with fructose (Yoshino *et al*. 1989). Careful attention was given to the pharmaceutical quality of the BPA–F preparations. Solutions for i.v. infusion of BPA–F were prepared at a concentration of 30 g/l (0.14 M), combining L–BPA with a 10% molar excess of fructose in sterile water. After completion of the development work L–BPA infusion solution was administered to brain tumour patients in conjunction with clinical studies for development and testing BNCT as a part of clinical phase I trial to develop novel indications for BNCT (Kulvik *et al*. manuscript in preparation, **II**). Appropriate notification of a clinical trial on medicinal products in human subjects (form 723) with appendices were presented to the Finnish National Agency for Medicines prior to initiation of the clinical studies with L–BPA.

3.2. Development of radiolabelled analogues of 4-dihydroxyboryl–L–phenylalanine (papers **III** and **IV**)

In the life sciences radiolabelled analogues of L–BPA have many applications, e.g. in exploring novel clinical applications for the $^{10}B(n,\alpha)^{7}Li^{*}$ reaction in tumour models *in vitro* or by autoradiography in experimental animals, or in cancer patients using imaging techniques like PET or SPET. In addition, uptake, metabolism, and pharmacokinetics of L–BPA prior to clinical BNCT studies can be noninvasibly estimated using PET or SPET techniques with radiolabelled analogue of L–BPA.

In order to improve available radiotracers for BNCT we wanted to test possibilities to label BPA directly with radioiodine. The dihydroxyboryl group in aromatic molecules is fragile and can be substituted by electrophiles (Kabalka *et al*. 1982, **I**). Therefore gentle chemical oxidants, Iodogen (1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril) (Fraker & Speck 1978), Figure 7a, and lactoperoxidase (Karonen 1981), were chosen. A direct Iodogen radioiodination technique for Tyr was also tested, Figure 7b.

A procedure to produce relatively high specific radioactivity (SA) L–[^{18}F]FBPA was developed, Figure 5. Electrophilic radiofluorine was produced using a post-target conversion of [^{18}F]F$^-$ to [^{18}F]F$_2$. Liquid chromatography with mass spectrometric detection is used to estimate the specific radioactivity of L–[^{18}F]FBPA and to verify the quality control for chemical identity of the target compound.

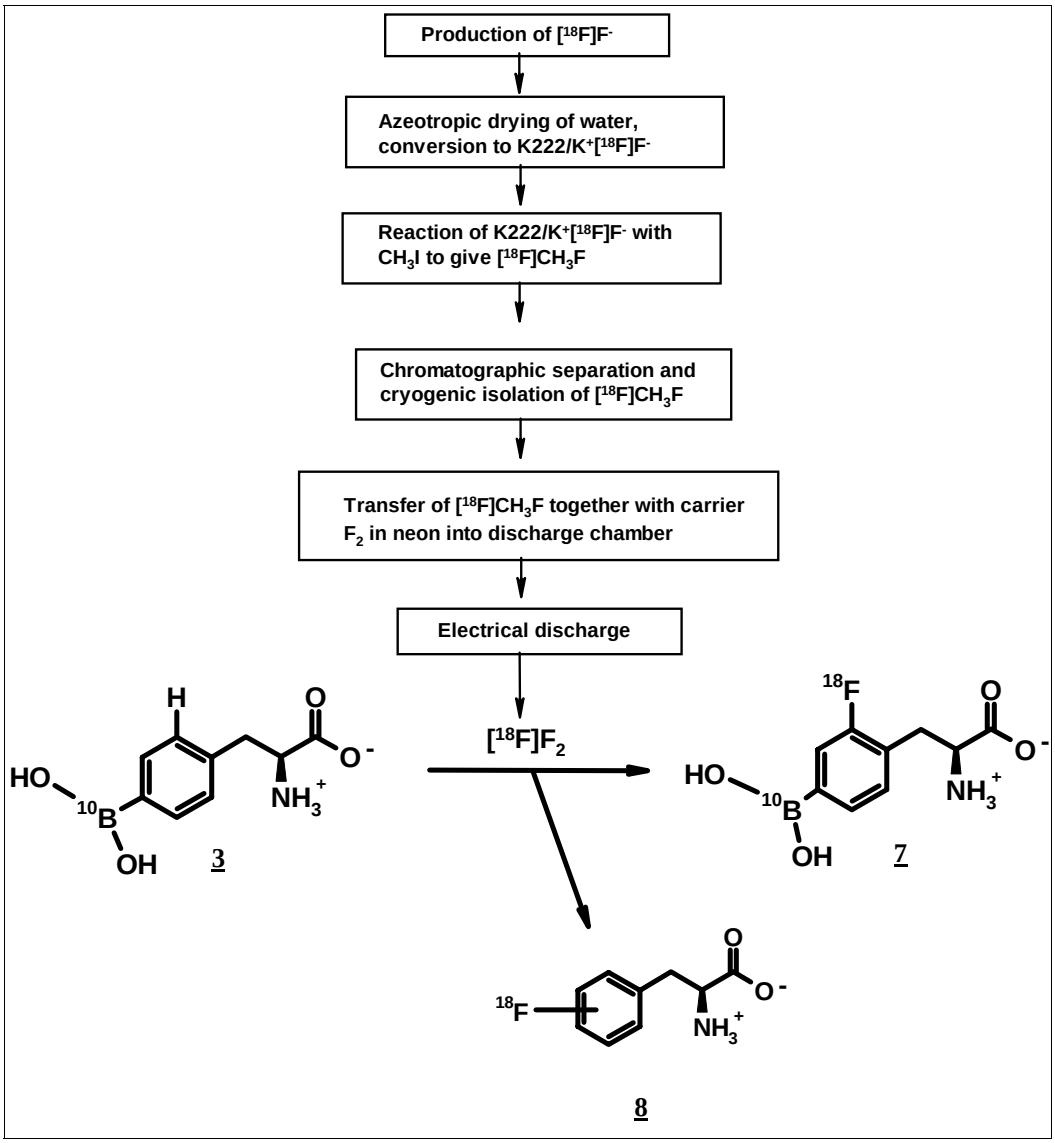

Figure 5. Flow-chart for the synthesis of electrophilic fluorine starting from nucleophilic fluorine (Bergman & Solin 1997, Bergman 2001) and following direct electrophilic radiofluorination of L–BPA schematically; the precursor: L–BPA **3**, the target compound: L–[^{18}F]FBPA **7**, and the principle by-products: 2,3,4-[^{18}F]fluorophenylalanines **8**.

4. RESULTS

4.1. Evaluation of 4-dihydroxyboryl–L–phenylalanine (papers **I** and **II**)

A new impurity during the development of L–BPA synthesis was identified by liquid chromatography with mass detection (LC-MS). The palladium catalysis cross-coupling reaction of phenylboronic acids with haloarens in the presence of bases yields corresponding biaryls (Miyaura *et al*. 1981). In the Malan & Morin (1996) pathway for synthesis of L–BPA, an unprotected 4-iodophenyl boric acid with a protected analogue can lead to a competitive cross-coupling reaction, causing the formation of 4'-dihydroxyborylbiphenylalanine (biBPA). The impurity biBPA could be avoided in later synthetic batches of L–BPA. In collaboration with Katchem Ltd, Czech Republic the Finnish research group improved the manufacturing process of L–BPA. Based on this work the BPA manufactured by Katchem was used in first clinical phase I/II trials.

Table 2. General requirements of ^{10}B enriched L–BPA for clinical trials

Requirements of final product	Methods of verification
Chemical characterisation	^{1}H NMR, ^{13}C NMR, ^{10}B NMR, IR spectrometry, melting point determination
Chemical purity (>98%)	RP HPLC
Enantiospecific (chiral) purity (~ 100%)	specific rotation determination, chiral HPLC
Enrichment factor of ^{10}B (>99%)	mass spectrometry
Impurities should be identified; if a new compound is identified with unknown toxicity	HPLC, LC/MS, NMR; synthesis of the detected impurity compound and toxicological evaluation
Residual solvents under limits as specified in the European Pharmacopoiea	gas chromatography
Pharmaceutical quality: no microbial contamination and bacterial endotoxins detected under limit as specified in the European Pharmacopoiea	sterility test, bacterial endotoxin test

The purity of the L–BPA batches used for clinical administration was verified by NMR and RP HPLC. The final product was 98.5–99.9% pure L–BPA with Phe (<1%) and to a lesser extent Tyr (<0.5%) as the analysed residual impurities. Potential trace impurities in the final product are boric acid and biBPA.

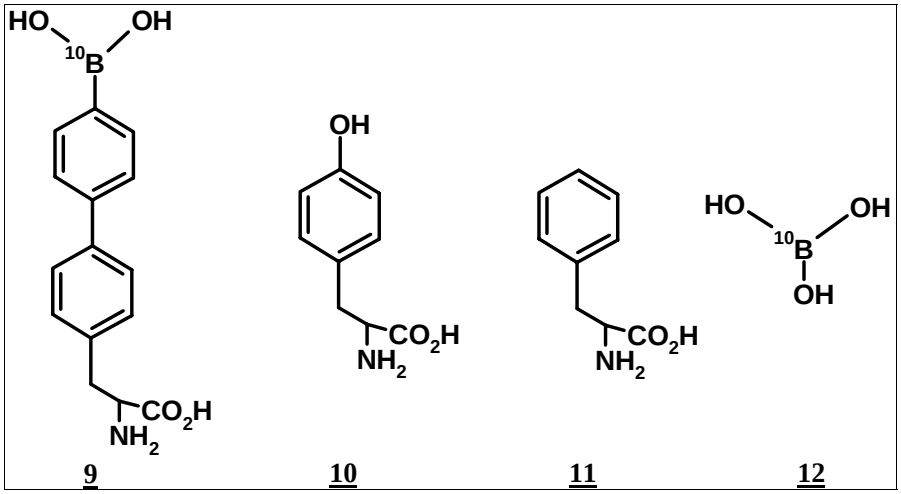

Figure 6. Impurities found: Tyr **10**, Phe **11**, and potential trace impurities anticipated: biBPA **9**, and boric acid **12**, in L–BPA batches used for clinical administration.

BPA has been reported to be nontoxic compound (LaHann *et al*. 1993). In accordance with earlier studies no adverse effects were observed in the acute toxicity of L–BPA studied in male albino Sprague–Dawley rats. The pH and osmolarity of the BPA–F solution are in the physiological range. An endotoxin test was carried out by the turbidimetric kinetic method for each synthesised batch of L–BPA to ensure that the batch was pyrogen free, i.e. contained bacterial endotoxins under the limit specified in the European Pharmacopoeia (3rd edition 2.6.14). The sterility tests of the L–BPA batches were carried out in the hospital pharmacy of HUCH, Helsinki according to the European Pharmacopoeia (3rd edition 2.6.1). No clinically significant adverse effects of L–BPA had been reported and we did not observer such either (**II**). The data were considered sufficient for starting L–BPA mediated clinical BNCT phase I trials (boron biodistribution studies) and phase I/II trials.

4.2. Development of radiolabelled analogues of 4-dihydroxyboryl–L–phenylalanine (papers **III** and **IV**)

A direct electrophilic radioiodinating method using Iodogen as an oxidant gave reproducible amounts of 4-[^{125}I]IPhe instead of radioiodinated BPA. A direct electrophilic Iodogen technique for radioiodination of Tyr gave an excellent radiochemical yield (>99%) 3-[^{125}I]ITyr. A formation of a corresponding di-iodo compound, 3,5-[^{125}I]di-iodotyrosine (3,5-[^{125}I]diTyr) was observed, but it was avoidable using suitable labelling conditions.

An alternative concise procedure to that reported by Ishiwata *et al*. (1991a), which produces relatively high SA L–[^{18}F]FBPA was developed. The amount of precursor could be reduced from 100 µmol to 4.8 µmol. On average, the radiochemical yield (as calculated from the initial amount of [^{18}F]F^{-}) of L–[^{18}F]FBPA) was 3.4%. The specific activity was 0.85-1.52 GBq/µmol at EOS. The overall synthesis time was about 50 min and the radiochemical purity over 98% determined by analytical HPLC.

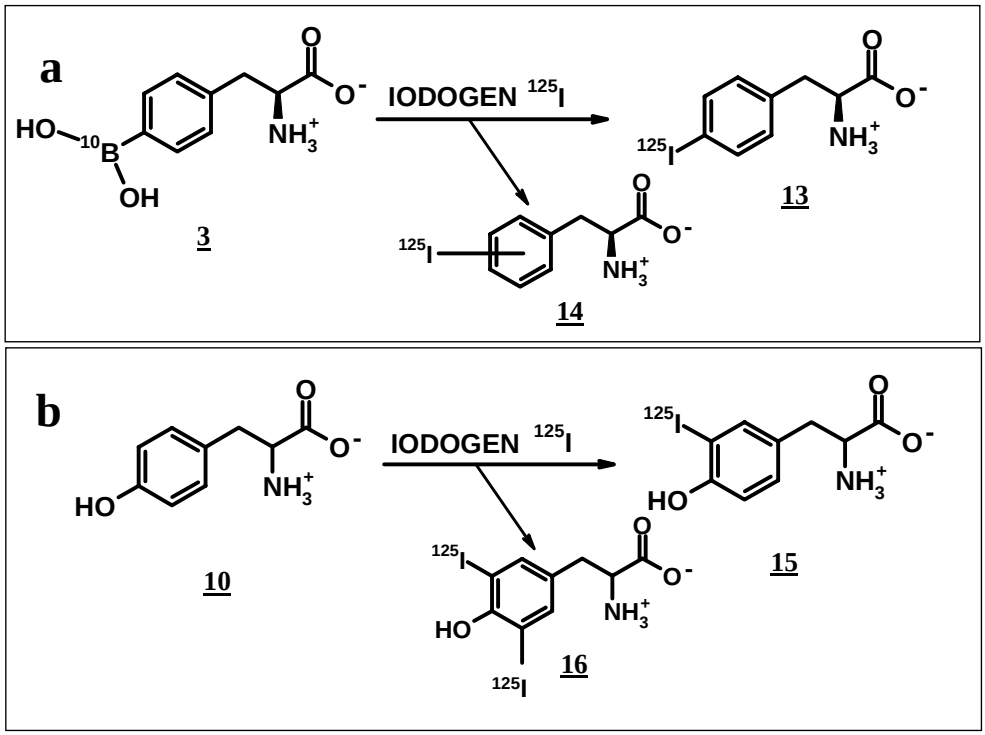

Figure 7. Radioiodination of L–BPA (**a**) **3** and Tyr **10** (**b**) using Iodogen as oxidant. Products are 4-IPhe **13**, 2,3-IPhes **14**, 3-ITyr **15** and 3,5-diITyr **16**.

5. DISCUSSION

5.1. Boron neutron capture therapy

Despite the success in the synthetic boron chemistry only few boron compounds have emerged in clinical BNCT trials. For example many structural BPA modifications have been synthesised, including α-methyl BPA (**17**) and 1-amino-3-(4-dihydroxyborylbenzyl) cyclobutanecarboxylic acid (**18**) (Zaidlewicz *et al*. 2004). Some BPA modifications have also been studied preclincally, e.g. 2- and 3-BPAs (**19** and **20**) (Hiratsuka *et al*. 2000) and 4-dihydroxyborylphenylalaninol (**21**) (Masunaga *et al*. 2001, Masunaga *et al*. 2003), Figure 8. The incorporation of ^{11}C-labelled 1-amino-1-[^{11}C]cyclobutanecarboxylic acid (1-[^{11}C]ACBC) [a structural analogue of 1-amino-3-(4-dihydroxyborylbenzyl)cyclobutanecarboxylic acid (**18**) but without the –B(OH)$_2$ group] into 20 cases of suspected recurrent brain tumours has been represented as Gjedde-Patlak plots showing high average tumour to contralateral gray matter ratio of 5.0 (Hübner *et al*. 1998). Boronated porphyrins are one of the most widely studied preclinical boron compounds (e.g. Gabel 1989, Kahl *et al*. 1990, Miura *et al*. 1990, Woodburn *et al*. 1993, Miura *et al*. 1998, Kreimann *et al*. 2003). One of the most biologically studied boronated porphyrin is the tetrakis-carboranecarboxylate ester of 2,4-bis-(α,β-dihydroxy-ethyl) deuterioporphyrin IX (BOPP) (**22**), Figure 9 (Fairchild *et al*. 1990, Hill *et al*. 1992, Huang *et al*. 1993, Ceberg *et al*. 1995b, Hill *et al*. 1995, Callahan *et al*. 1999, Zhou *et al*. 1999). BOPP has also been studied in a phase I clinical trials for photodynamic therapy (PDT)

(Rosenthal *et al*. 2001). Disodium decahydrodecaborate, currently known as GB-10, (**23**) Figure 8, a boron compound used in the early 1960's, is being re-evaluated for clinical glioma BNCT trials (Diaz *et al*. 2002, Hawthorne & Lee, 2003).

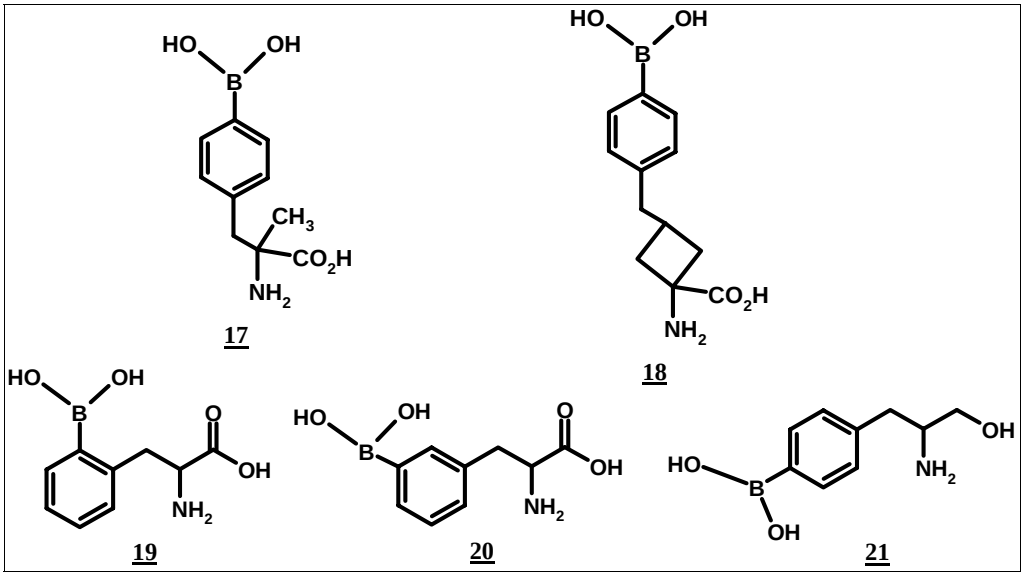

Figure 8. Synthesised structural modifications of BPA: α-methyl BPA (**17**), 1-amino-3-(4-dihydroxyborylbenzyl)cyclobutanecarboxylic acid (**18**), 2-BPA(**19**), 3-BPA (**20**) and 4-dihydroxyborylphenylalaninol (**21**).

By now, in Finland two potential boron containing pharmaceutical have been evaluated for BNCT in order to start clinical trials of patients with malignant gliomas. In the beginning of the 1990's boronated low-density lipoproteins (LDLs) were planned to used as ^{10}B containing pharmaceutical by the Finnish BNCT research group (Auterinen & Kallio 1994). Clinical uptake studies with ^{99m}Tc and ^{111}In labelled LDLs in malignant gliomas were performed (Kallio *et al*. 1993, Leppälä *et al*. 1995). However, in spite of chemical and preclinical studies on boronated LDLs, clinical patient studies could not be initiated due to technical difficulties: the B-100 protein component of LDLs binds to specific LDL receptor. *In vitro* processing in boronation denatures easily the fragile B-100 protein hampering the uptake of boronated LDLs to malignant glioma cells. (Ylä-Herttuala, unpublished results; personal communication, September 2003). Currently, in Finland, Karyon Ltd (URL: http://www.karyon.fi) is developing novel boron pharmaceuticals under the project entitled targeted boron neutron capture therapy (TBNCT). The research and development is focused on a potential lead compound K 1020 for malignant gliomas. Currently, K 1020 is in preclinical phase and it is planned to enter a clinical phase I trial in 2006 (Grayson 2003, Slätis personal communication, January 2004).

In 1996 L–BPA was chosen to be the first boron containing pharmaceutical in Finland because it was considered biochemically to be more attractive than the only available boron pharmaceutical, BSH, and because by the mid 1990s there were already reports of clinical experiences with BPA administered intravenously (Mallesch *et al*. 1994, Coderre *et al*. 1997). Clinical trials were planned to start with BPA synthesised by the Snyder (1958) pathway affording after enzymatic purification the L–enantiomer. In the mid 1990's there were serious

problems worldwide for obtaining L–BPA that fulfilled the chemical and pharmaceutical quality and purity requirements for *in vivo* administration. Fortunately, in 1996 Malan & Morin published a novel enantiospecific pathway affording L–BPA and Katchem Ltd. (Prague, Czech Republic) started to produce L–BPA synthesised by this pathway. After a period of intensive experimentation with synthesis of L–BPA in Katchem Ltd., analytical research & development of L–BPA and planning and preparing of clinical trials by the Finnish BNCT research group, the first patient boron biodistribution study (a phase I trial) of L–BPA was carried out in August 1998.

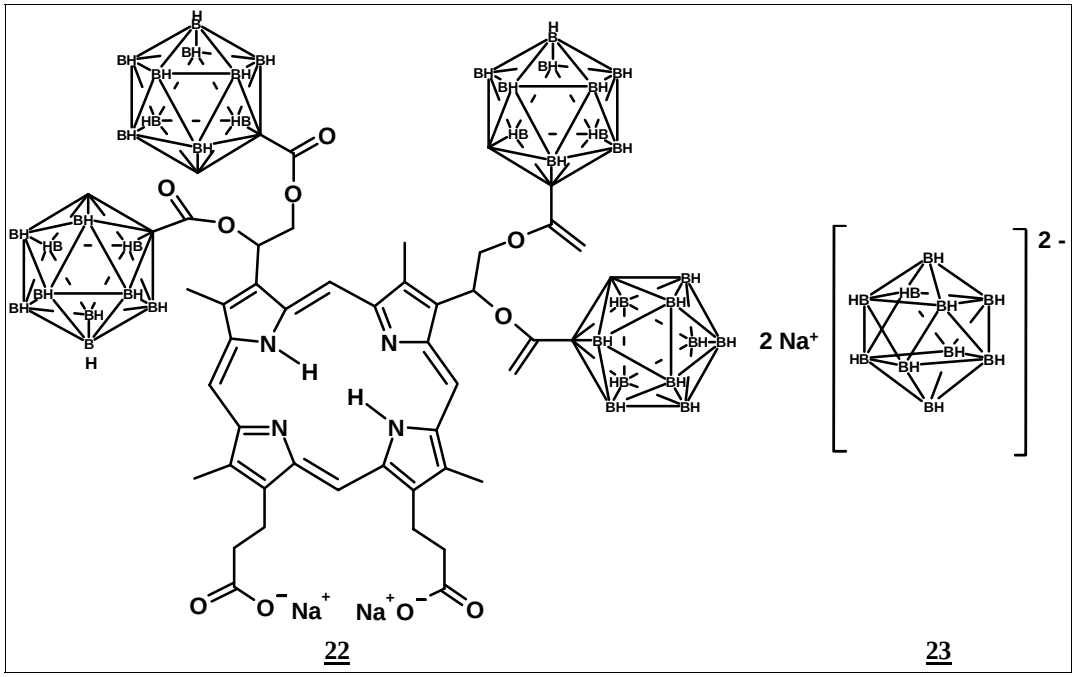

Figure 9. Structure of BOPP **22** and GB-10 **23**.

In order to model BNCT radiation doses the average estimated boron concentration of tissue is mainly derived from the preclinical (e.g. Coderre *et al*. 1987, Coderre *et al*. 1988, Coderre *et al*. 1992, Coderre *et al*. 1994, Matalka *et al*. 1994) and clinical (Coderre 1992, Mallesch *et al*. 1994, Coderre *et al*. 1997, Elowitz *et al*. 1998) glioma biodistribution studies. Metabolism of a boron pharmaceutical administered is usually neglected because the most important therapeutic factor in BNCT is the $^{10}B(n,\alpha)^{7}Li^{*}$ reaction, not the molecule containing the ^{10}B atom. Only few studies to investigate L–BPA metabolites have been reported (Svantesson *et al*. 2002). In order to develop clinical BNCT metabolites of L–BPA are not necessary to be fully elucidated especially if L–BPA is viewed as acting as an inactive prodrug, and ^{10}B as the active pharmaceutical affecting the viability of target cells through the ($^{4}He^{2+}$) particle and lithium ($^{7}Li^{3+}$) (**II**). However, for phase I trials in addition to boron analyses, HPLC analysis of L–BPA, for example according to a method developed by Di Pierro *et al*. (2000) in plasma, urine and tissues may afford additional useful information. Generally, clinical boron biodistribution studies can be considered as a solid part of phase I trials in order to study average boron concentration of malignant tissues of a novel promising boron containing pharmaceutical.

L–BPA mediated clinical trials are running at the Finnish BNCT facility (Joensuu *et al*. 2003). In Finland, the basic research & development research programs were successfully completed by 1998 in animal models (Benczik 2000), dosimetry (Aschan 1999, Kosunen 1999), dose planning (Seppälä 2002), kinetic mathematical modelling of ^{10}B (Ryynänen 2002) and technological development (Kortesniemi 2002). The quantification of BPA with phantoms has been evaluated using ^{1}H magnetic resonance spectrometry (MRS) and clinical studies are in progress (Heikkinen *et al*. 2003). Boron biodistribution studies of meningioma, Schwannoma and NF2 patients have been successfully completed (Kulvik *et al*. manuscript in preparation). [^{18}F]FBPA-PET studies of new tumour types including meningioma, Schwannoma and NF2 for BNCT are in progress (Minn *et al*. manuscript in preparation).

Worldwide BNCT research and development for brain tumours and melanoma are going on and some novel clinical applications for the $^{10}B(n,\alpha)^{7}Li^{*}$ reaction have been proposed, for example as treatment of liver (Pinelli *et al*. 2002), head and neck, prostate cancer and superficial sarcomas (Gupta *et al*. 2003), soft tissue sarcomas (Pignol *et al*. 1998), undifferentiated thyroid carcinoma (Dagrosa *et al*. 2003) and rheumatoid arthritis (Yanch *et al*. 1999). The methodology of BNCT is complicated because the irradiation conditions of both the neutrons and the boron compound must be considered. In BNCT the need for international and interdisciplinary co-operation is as pronounced today that it has been before (Kortesniemi 2002). A successful BNCT project toward clinical phase III trials requires fundamental knowledge of biology, chemistry, engineering, medicine, pharmacy and physics including robust administration and economics. Based on the data from Pharma Industry Finland (PIF) the estimated cost of developing a new pharmaceutical is 560 million €, and the time from concept to sale is on average 12-13 years. The risks in research are high; according to international statistics only one or two in 10 000 synthesised substances end up on the market. The risks in a prospective clinical BNCT development compared to the pharmaceutical development can be even higher because in BNCT both pharmaceutical and medical device developing areas must be considered.

Standardization of the design, implementation, and reporting of clinical trials as proposed by Gupta *et al*. (2003) will help to continue with clinical phase III trials in BNCT. However, accurately documented patient cases could simplify demonstrating the effectiveness (or ineffectiveness) of prospective BNCT protocols without performing 'traditional' phase III trials (randomisation). For example characterisation of a small but representative group of cancer patient cases with metabolic [^{18}F]FBPA PET and anatomical MRI or CT images before and after treatment could help to demonstrate the clinical response of the of L–BPA mediated BNCT without traditional phase III trial randomised comparison studies to standard treatments.

5.2. Radiolabelled analogues of 4-dihydroxyboryl– L–phenylalanine

The dihydroxyboryl group, $–B(OH)_2$, in aromatic molecules is electron withdrawing. $B(OH)_2$– deactivates strongly direct electrophilic radiolabelling and acts as a good leaving group. No radiolabelled BPA was observed using gentle oxidants and mild electrophilic radioiodination techniques, which we investigated. The hydroxyl group, –OH, is very different from the dihydroxyboryl group in direct electrophilic aromatic radiohalogenation: the OH– group is electron donating and activates the aromatic ring toward direct electrophilic labelling, resulting in efficient radioiodination techniques with excellent radiochemical yield, Figure 10. However, Weinreich *et al*. (1997) have reported of direct electrophilic radioiodination of BPA using Iodogen with a positron emitter ^{124}I leading to one labelled

radioiodinated product. The structure of the product was not identified. *In vivo* studies with ^{123}IBPA have been also reported (Lim *et al*. 1999). We suggest that the radioiodinated product on these studies was not radioiodinated BPA, but a radioiodinated phenylalanine. We have also reported some preliminary studies of radioiodinated BPA as a poster presentation in a nuclear medicine international congress (Vähätalo *et al*. 1997). After accurate evaluation we could not positively identify any amount of ^{125}IBPA either. Appropriate identification of the desired target compound is a crucial step before starting any further studies with hypothetic radiotracer of *IBPA (Suominen *et al*. 2001). Globally, radioiodinated BPA, 4-dihydroxyboryl-2-[*I]iodophenylalanine is a desired radiotracer for *in vivo* studies but unfortunately studies in order to produce *IBPA has so far been unsuccessful.

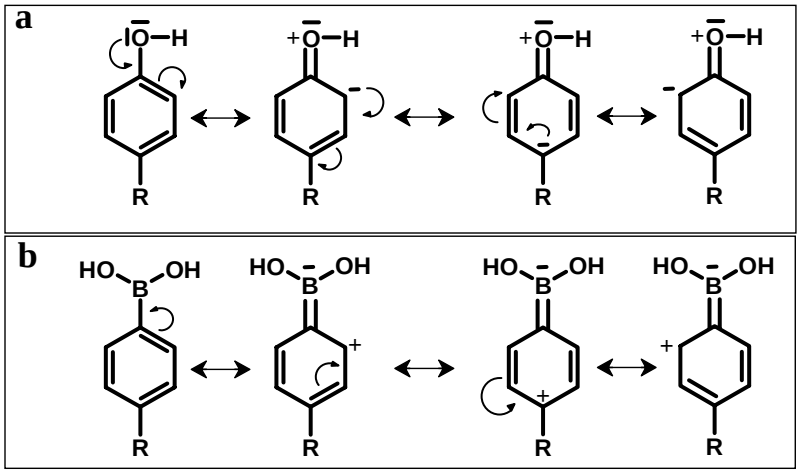

Figure 10. Resonance structures of Tyr (**a**) and (**b**) BPA, **R** = $–CH_2CH(NH_3^+)COO^-$.

The radiofluorinated analogue of BPA, [^{18}F]FBPA, has been demonstrated to be a useful radiotracer in life sciences leading to PET patient studies for L–BPA mediated BNCT in Japan, in USA and in Finland. The transport of L–BPA is supposed to be mediated by the L amino acid transport system (Wittig *et al*. 2000). The fluorine atom is usually substituted into a pharmaceutical in place of a hydrogen atom or a hydroxyl group; fluorine may affect the lipophilicity of the molecule (Bergman 2001). However, clinical experience with L–[^{18}F]FBPA has demonstrated that accumulation of ^{18}F-labelled BPA and L–BPA administered as BPA-F is analogous (Imahori *et al*. 1998a, 1998b and 1998c, Nichols *et al*. 2002, Takahashi *et al*. 2003). Therefore, L–[^{18}F]FBPA accumulation is supposed to be mediated by the same amino acid transport system than L–BPA, at least the L amino acid transport system is clearly shown to be the dominant mechanism affecting the L–[^{18}F]FBPA accumulation in malignant gliomas.

There is discrepancy in the use of the radiofluorinated analogue of BPA as free amino acid or complexed with fructose. Kabalka *et al*. (1997) have reported a patient PET study with the radiofluorinated analogue of BPA complexed with fructose. There is probably no need to use [^{18}F]FBPA complexed with fructose because the actual uptake mechanism to malignant cells is supposed to be mediated through the L amino acid transport system (Imahori *et al*. 1998a, 1998b and 1998c, **II**). In addition, the complex formulation of BPA–F is an equilibrium reaction, Figure 4. An *in vitro* study has shown that BPA–F dissociates and reaches equilibrium between the free molecules of BPA and the BPA–F complex in the diluted

condition present in plasma (Kakihana *et al*. 1993). The equilibrium kinetics of BPA–F and [^{18}F]FBPA–F has not been studied but one can assume that in diluted conditions of the radiolabelled compounds the equilibrium status should be toward the reactants, i.e. free [^{18}F]FBPA and free fructose, instead of complex [^{18}F]FBPA–F. Kabalka *et al*. (1997) have reported that a quality control of [^{18}F]FBPA–F for radiochemical identity was performed. In addition there are some variation is preparative HPLC conditions: Kabalka *et al*. (1997) have eluted [^{18}F]FBPA between 28 and 32 min – Imahori (personal communication, 1998) between 16 and 20 min corresponding to the original procedure by Ishiwata *et al* (1991a). Currently the radiochemical identity of [^{18}F]FBPA is recommended to be verified with a stable reference compound (Kabalka *et al*. 2000).

Direct electrophilic radiofluorination with [^{18}F]F_2 or [^{18}F]AcOF of BPA is an efficient and fast way to incorporate ^{18}F into BPA. Nucleophilic pathways to radiofluorinate BPA would be too arduous and complicated to realise, if possible at all. Direct electrophilic radiofluorination is simple to perform; most effort is applied to the separation and identification of the radiofluorinated products. However, selective labelling and better radiochemical yield, perhaps even successful radioiodination procedures are anticipated using precursors with appropriate leaving groups, such as trialkylstannyl derivatives of BPA, Figure 11. In addition to [^{18}F]FBPA studies in BNCT, ^{11}C-labelled BPA (**25**) studies are in progress (Studenov *et al*. 2001).

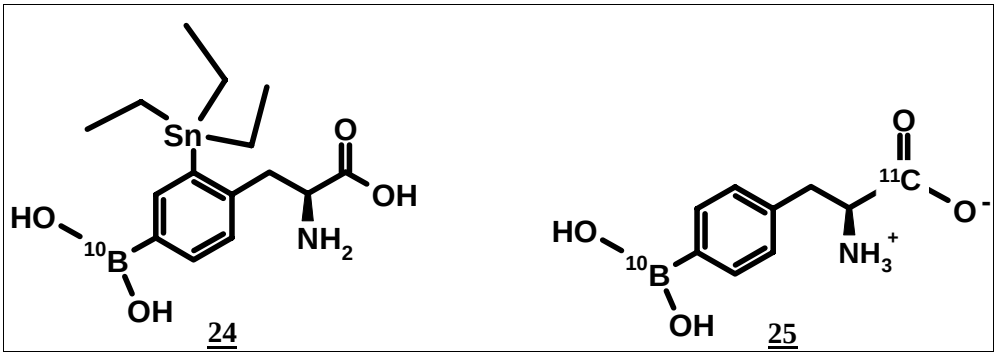

Figure 11. Structure of hypothetic molecule of triethylstannylated BPA (**24**) (under radiochemical labelling conditions dihydroxyboryl-, amino- and/or carboxyl-groups may have required to be protected) and ^{11}C labelled BPA (**25**) currently in preclinical evaluation (Studenov *et al*. 2001)

6. CONCLUSIONS

The conclusions of this thesis are:

- LC-MS was shown to be a novel analytical instrument to check the purity of BPA prior to clinical studies in BNCT (paper **I**),
- L–BPA analysis and synthesis development and complementary preclinical and clinical observations justify a safe use of BPA–F up to clinical phase III BNCT studies (paper **II**),
- BPA cannot be directly radioiodinated for tracer studies in BNCT (paper **III**),
- A concise procedure to produce relatively high SA L–[^{18}F]FBPA for clinical positron emission tomography studies in BNCT was developed (paper **IV**).

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