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Research Article

Synthesis of 4-dihydroxyboryl-2-[^{18}F] fluorophenylalanine with relatively high-specific activity

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Summary

An alternative procedure to produce 4-dihydroxyboryl-2-[^{18}F]fluorophenylalanine ([^{18}F]FBPA) for positron emission tomography studies in boron neutron capture therapy is described. Relatively high-specific activity electrophilic radiofluorine is 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 [^{18}F]FBPA and to verify the quality control for chemical identity of the target compound. [^{18}F]FBPA produced according to this method fulfilled the pharmaceutical quality requirements for an injection in patients. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: electrophilic fluorine; 4-dihydroxyborylphenylalanine; boron neutron capture therapy; specific radioactivity; LC–MS

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Introduction

Boron neutron capture therapy (BNCT) is an experimental binary radiotherapy.[1] ^{10}B enriched 4-dihydroxyborylphenylalanine (boronophenylalanine, BPA) is one of the most used drugs in BNCT clinical trials.[2–4] Positron emission tomography (PET) studies have been performed with a radiofluorinated analogue of BPA, 4-dihydroxyboryl-2-[^{18}F]fluorophenylalanine ([^{18}F]FBPA), in order to study the pharmacokinetics of BPA and to obtain information on ^{10}B distribution in patients with melanoma and gliomas.[5–8] PET studies with [^{18}F]FBPA have been based on the synthesis developed by Ishiwata and his colleagues reported in 1991.[9] The objective of our work was to develop an alternative procedure to produce relatively high-specific radioactivity (SA) [^{18}F]FBPA.[10,11] Semi-preparative HPLC conditions for separation of the product were developed such that the formulation for injection would include only sterile filtration of the HPLC fraction. We then identified the main by-products, and estimated the SA of the target compound by using both HPLC and liquid chromatography with mass spectrometric detection (LC–MS).

Results and Discussion

Nucleophilic displacement of activated nitro groups by [^{18}F]F$^-$ is an efficient no-carrier-added (NCA) route to radiofluorinated aromatic molecules.[12] This approach has been applied, for example, in the multistep synthesis of radiofluorinated phenylalanine (FPhe)[13] and DOPA.[14] However, nucleophilic approaches to [^{18}F]FBPA are not generally efficient because boron is a strong Lewis acid, and the dihydroxyboryl group is electron withdrawing which restrains the aromatic ring towards nucleophilic attacks.

It is suggested that [^{18}F]FBPA accumulation in tissues reflects several pathways involved in amino acid utilization including transport and metabolic processes, but slow, if any, incorporation in proteins.[5–8,15–18] Thus, as an amino acid transport tracer, there is no need to produce NCA or even high SA [^{18}F]FBPA for *in vivo* applications. However, high SA and the concomitant low amount of mass for the product make preparative chromatographic separation easier and the synthesis method more amendable to automation and routine production.

[^{18}F]FBPA has been synthesized for clinical PET studies via electrophilic fluorination with [^{18}F]F_2 produced by bombarding a mixture of 99.5% Ne and 0.5% F_2 gas and converted to acetyl hypofluorite (SA 130 MBq/μmol).[7,8] Generally, by using ^{18}O(p,n)^{18}F reaction from enriched oxygen gas targets for making electrophilic radiofluorine higher SA for the product can be achieved.[5]

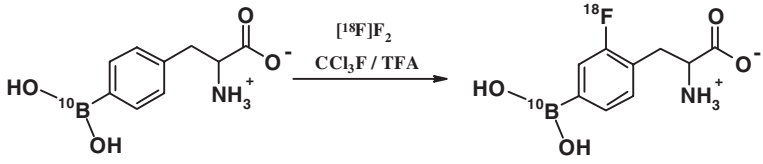
High SA [^{18}F]F_2 [up to 55 GBq/μmol (decay corrected to the end of cyclotron bombardment, EOB)] can be produced using a post-target conversion of [^{18}F]F^- to [^{18}F]F_2.[19] Utilizing this method and starting with about 6.8 GBq [^{18}F]F^- the SA of [^{18}F]F_2 was about 4 GBq/μmol (EOB), as calculated from the SA of the final product.

Electrophilic radiofluorination of BPA with carrier-added (CA) [^{18}F]F_2 is a relatively simple way to incorporate radiofluorine into BPA. Selective labelling and better radiochemical yields are anticipated using precursors with appropriate organometallic leaving groups, such as trialkylstannyl derivatives of BPA. Despite the lack of a specific organometallic precursor, the labelling method is applicable because molecular fluorine is a highly reactive chemical agent for direct aromatic electrophilic substitution. On the other hand, a large number of radioactive side products may be generated due to the high reactivity, which makes the chromatographic separation more challenging. In our work, using ethanolic saline as HPLC mobile phase, we managed to separate [^{18}F]FBPA from the radioactive side products: radiochemical purity exceeded 98% in six consequtive syntheses (Table 1).

In direct radiofluorination of BPA regioselectivity is achieved because electrophilic radiofluorine attacks primarily the aromatic carbon 2 (*ortho*-position) producing [^{18}F]FBPA or the aromatic carbon 4 (*para*-position) leading to undesired deboronation. Displacement of the dihydroxyboryl group itself has been succesfully exploited in aromatic radioiodination[20,21] and radiobromination[22] but attempts have failed

Table 1. Data for six consecutive radiosynthesis of [^{18}F]FBPA

Batch	$^{18}F^-$(GBq) EOB	[^{18}F]FBPA (MBq) EOS	SA (GBq/μmol) EOS
I	7.4	187	—
II	7.4	200	0.85
III	7.0	213	1.52
IV	6.3	162	1.22
V	6.3	114	1.30
VI	6.3	145	—



Scheme 1. Radiosynthesis of [^{18}F]FBPA

to produce desired radiofluorinated products via deboronation reactions.[23,24]

The direct radiofluorination of BPA was performed in a trifluoro acetic acid–freon-11 (TFA–CCl_3F)-mixture (Scheme 1). The amount of precursor could be reduced from 100[9] to 4.8 μmol by using relatively high SA electrophilic radiofluorine synthesized according to our method. On average, the radiochemical yield (as calculated from the initial amount of [^{18}F]F^-) was 3.4%. The mean value of radiochemical yield increases to 13%, when calculated from the estimated amount of [^{18}F]F_2 radioactivity. The major chemical impurity present after the process was the unreacted precursor BPA. [^{18}F]FBPA was efficiently and reproducibly separated from this and other side products with semi-preparative reversed phase (RP) HPLC (Figure 1). The main radiofluorinated by-products were [^{18}F]FPhes as characterized with non-radioactive reference compounds and HPLC. The summed yield of [^{18}F]FPhes was in average 1.1% (EOB) as calculated from the initial amount of [^{18}F]F^-. The synthesis time was 50 min, 30 min less than in the original procedure.[9] The radiochemical purity of [^{18}F]FBPA was unchanged in the final solution (0.9% NaCl, 1% ethanol, 0.01% acetic acid) up to 3 h after the end of synthesis (EOS).

The retention times of BPA ($R_t = 2.5$ min) and the FPhes ($R_t = 3.0-3.5$ min) were determined using RP C18 column and analytical HPLC: the target compound eluted slightly after BPA at 2.7 min, which is in accordance with the general claim of aromatic fluorine substitution making a compound more lipophilic and thus prolonging its retention time in RP system.

In addition to HPLC, FBPA mass signal with LC–MS was detected. We had already shown that trace amounts of BPA or its derivatives can be identified by LC–MS.[25] The mass concentration was estimated by using BPA as the reference material. Currently, a synthetic pathway for the stable reference compound is also available.[26] Strong ion concentration of a sample tends to disturb the mass spectrometric

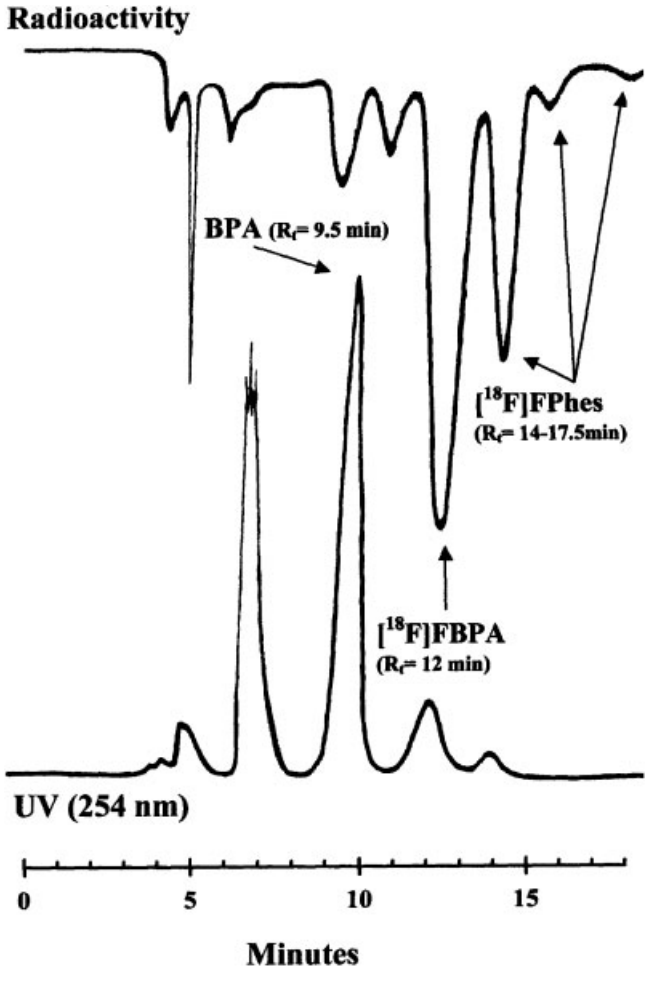


Figure 1. Chromatograms of the semi-preparative HPLC separation of [^{18}F]FBPA

detection: in our experiments, the final solution contained 0.9% NaCl which was found to suppress the FBPA mass signal. However, the correct mass signal of FBPA ($[M+H]^+$ 228.2) was detected. We used LC–MS to estimate the mass concentration of FBPA in the final formulated solution, the value of which was then used to calculate the SA of [^{18}F]FBPA. We assume the detected mass concentration to be underestimated due to the strong ion concentration, and therefore the calculated SA may be slightly overestimated. In our experiments the SA achieved was 0.9–1.5 GBq/μmol (EOS). For clinical PET studies, we will use higher amounts of initial activities increasing the SA of [^{18}F]FBPA

up to 3.7 GBq/μmol which is unambiguously higher SA than in the earlier studies.

Experimental

Production of eletrophilic radiofluorine

High SA $[^{18}F]F_2$ was produced using a post-target conversion system starting from $[^{18}F]F^-$.[19] Briefly, the $[^{18}F]F^-$ was produced with MGC-20 cyclotron by irradiating 700 μl 96% ^{18}O enriched H_2O in a non-pressurized silver target chamber with 17 MeV protons using 10 μA beam current. Radiofluorine labelled fluoromethane was synthesized from iodomethane and [^{18}F]fluoride. After preparative gas chromatographic purification, $[^{18}F]CH_3F$ was mixed with carrier F_2 (1.2 μmol) in a neon matrix. The constituents were atomized with an electronic discharge, after which rearrangement and ^{18}F for ^{19}F exchange took place. Finally, $[^{18}F]F_2$ was available for the radiolabelling experiments.

Synthesis of [^{18}F]FBPA

BPA of 1 mg (4.8 μmol) (Katchem Ltd., Prague, Czech Republic) was dissolved in 250 μl TFA and 350 μl CCl_3F. $[^{18}F]F_2$ was bubbled with neon as a sweep gas into this solution. TFA and CCl_3F were evaporated under neon flow, the residue was dissolved in 700 μl of 0.2 M acetate buffer (AcOH/NaOAc, pH 4.2) and injected to a semi-preparative HPLC column (Waters μBondapak C18, $7.8 \times 300\,mm^2$). The column was eluted with 0.9% NaCl containing 1% ethanol and 0.01% acetic acid with a flow rate of 3 ml/min. The separation was monitored with a UV-absorption detector (254 nm) connected in series with a NaI crystal for radioactivity detection. The retention time of [^{18}F]FBPA was 12 min. The fraction containing [^{18}F]FBPA was collected and formulated for injection by passing the fraction through a single-use sterile apyrogenic filter (0.22 μm) into a sterile lagenula. A sample of this fraction was collected for quality control tests.

Quality control (QC) of [^{18}F]FBPA

Chemical and radiochemical purity and stability tests of the final product were performed with analytical HPLC. Analyses were done with analytical HPLC column (Waters μBondapak C18, $3.9 \times 300\,mm^2$)

eluted with 0.1% AcOH/CH_3CN 99/1 (v/v) with a flow rate of 1.8 ml/min. The retention time of [^{18}F]FBPA was 2.7 min.

LC–MS analyses were performed with PE SCIEX API 150EX mass spectrometer (Perkin-Elmer) equipped with Turbo Ion Spray coupled with RP Supelcosil ABZ + Plus column ($4.6 \times 150\,mm^2$, 5 μm). A mixture of H_2O/MeOH/HCOOH 80/20/0.1 (v/v/v) was used as eluent with a flow rate of 0.2 ml/min. The amount of FBPA was determined using BPA as reference compound. Selected ion monitoring (SIM) was performed on m/z 209.2 and 228.2, corresponding to the protonated molecules $[M + H]^+$ of BPA and FBPA, respectively.

Tests of sterility (European Pharmacopoiea 3rd edition 2.6.1) and tests for bacterial endotoxins (Limulus Amebocyte Lysate assay, European Pharmacopoiea 3rd edition 2.6.14) were done to ensure the pharmaceutical quality of the final product from six consecutive [^{18}F]FBPA syntheses.

Conclusion

We have developed an alternative concise procedure to produce relatively high SA [^{18}F]FBPA for PET studies for BPA-based BNCT. [^{18}F]FBPA is stable in the final solution for at least 3 h after EOS and it was shown to fulfil pharmaceutical quality requirements. Based on this radiochemical study our clinical PET studies with [^{18}F]FBPA are warranted and are in progress.

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References

1. Diaz AZ, Coderre JA, Chanana AD, Ma R. *Ann Med* 2000; **32**: 81–85 and references cited therein.
2. Mishima Y, Honda C, Ichihashi M, *et al. Lancet* 1989; **ii**: 388–389.

3. Chanana AD, Capala J, Chadha M, *et al Neurosurgery* 1999; **44**: 1182–1193.
4. Joensuu H, Kankaanranta L, Seppälä T, *et al. J Neuro-Oncol*, in press.
5. Kabalka GW, Smith GT, Dyke JP, *et al. J Nucl Med* 1997; **38**: 1762–1767.
6. Mishima Y, Imahori Y, Honda C, Hiratsuka J, Ueda S, Ido T. *J Neuro-Oncol* 1997; **33**: 163–169.
7. Imahori Y, Ueda S, Ohmori Y, *et al. J Nucl Med* 1998; **39**: 325–333.
8. Imahori Y, Ueda S, Ohmori Y, *et al. Clin Cancer Res* 1998; **4**: 1825–1841.
9. Ishiwata K, Ido T, Meija AA, Ichihashi M, Mishima Y. *Appl Radiat Isot* 1991; **42**: 325–328.
10. Vähätalo JK, Karonen S-L, Kulvik M, Bergman J, Lehikoinen P, Solin O. *J Label Compd Radiopharm* 1999; **42**: S536–S538.
11. Eskola O, Vähätalo J, Lehikoinen P, Bergman J, Forsback S, Solin O. *J Label Compd Radiopharm* 2001; **44**: S849–S851.
12. Attinà M, Cacace F, Wolf AP. *J Label Compd Radiopharm* 1983; **20**: 501–514.
13. Lemaire C, Guillaume M, Christianes L, Palmer AJ, Cantineau R. *Appl Radiat Isot* 1987; **38**: 1033–1038.
14. Lemaire C, Guillaume M, Cantineau R, Christiaens L. *J Nucl Med* 1990; **31**: 1247–1251.
15. Ishiwata K, Ido T, Kawamura M, Kubota K, Ichihashi M, Mishima Y. *Nucl Med Biol* 1991; **18**: 745–751.
16. Ishiwata K, Ido T, Honda C, Kawamura M, Ichihashi M, Mishima Y. *Nucl Med Biol* 1992; **19**: 311–318.
17. Ishiwata K, Shiono M, Kubota K, *et al. Melanoma Res* 1992; **2**: 171–179.
18. Kubota R, Yamada S, Ishiwata K, Tada M, Ido T, Kubota K. *Br J Cancer* 1993; **67**: 701–705.
19. Bergman J, Solin O. *Nucl Med Biol* 1997; **24**: 677–683.
20. Kabalka GW, Sastry KAR, Muralidhar K. *J Label Compd Radiopharm* 1982; **19**: 795–799.
21. Vähätalo J, Kulvik M, Savolainen S, Karonen S-L. Radioiodination techniques for aromatic amino acids; possible tracers for BPA. In *Frontiers in Neutron Capture Therapy*, Hawthorne MF, Wiersema RJ, Shelly K (eds). Kluwer Academic/Plenum Publishers: New York, 2001; 835–838.
22. Kabalka GW, Sastry KAR, Pagni PG. *J Radioanal Chem* 1982; **74**: 315–321.
23. Kabalka GW. *Acc Chem Res* 1984; **17**: 215–221.
24. Adam MJ. *Appl Radiat Isot* 1986; **37**: 811–815.
25. Vähätalo J, Tuominen J, Kokkonen J, Kriz O, Karonen S-L, Kallio M. *Rapid Commun Mass Spectrom* 1998; **12**: 1118–1122.
26. Kabalka GW, Reddy NK, Wang L, Malladi RR. *Org Prep Proced Int* 2000; **32**: 290–293.

