JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2004; **47**: 181–190.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.811

Research Article

Novel probes for imaging amyloid-β: F-18 and C-11 labeling of 2-(4-aminostyryl)benzoxazole derivatives

Hiroshi Shimadzu*, Takahiro Suemoto, Masako Suzuki, Tsuyoshi Shiomitsu, Nobuyuki Okamura, Yukitsuka Kudo and Tohru Sawada
BF Research Institute, Inc. c/o National Cardiovascular Center 7-1, 5-Chome, Fujishirodai, Suita, Osaka 565-0873, Japan

Summary

2-(4-Methylaminostyryl)-6-(2-[^{18}F]fluoroethoxy)benzoxazole ([^{18}F]BF-168) was prepared and found to be a potential probe for imaging amyloid-β. The precursor, a 6-(2-tosyloxyethoxy)benzoxazole derivative, was fluorinated with [^{18}F]KF and Kryptofix 222 in acetonitrile, and the crude product purified by semi-preparative HPLC to give [^{18}F]BF-168. The radiochemical purity was $>95\%$ and the maximum specific activity was 106 TBq/mmol at the end of synthesis. The synthesis time was 110 min from the end of bombardment.

2-(4-[*N*-methyl-^{11}C]methylaminostyryl)-5-fluorobenzoxazole ([^{11}C]BF-145) was also prepared from 2-(4-aminostyryl)-5-fluorobenzoxazole, [^{11}C]MeI and 5 N NaOH in DMSO, and purified by semi-preparative HPLC. The radiochemical purity was $>95\%$ and the specific activity was 40–70 TBq/mmol at the end of synthesis. The synthesis time was 45 min from the end of bombardment. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: fluorine-18; 2-(4-methylaminostyryl)-6-(2-[^{18}F]fluoroethoxy)benzoxazole; BF-168; 2-(4-[*N*-methyl-^{11}C]methylaminostyryl)-5-fluorobenzoxazole; BF-145; amyloid-β; PET

Introduction

Assessing the deposition of amyloid-β (Aβ) in the living brain is important for the diagnosis of Alzheimer's disease (AD) at an early stage. Radioactive probes which have a high binding affinity to Aβ are required in non-invasive imaging techniques, such as positron emission tomography (PET) or single

*Correspondence to: H. Shimadzu, BF Research Institute, Inc. c/o National Cardiovascular Center 7-1, 5-Chome, Fujishirodai, Suita, Osaka 565-0873, Japan. E-mail: shimadzu@po.iijnet.or.jp

Contract/grant sponsor: Program for Promotion of Fundamental Studies in Health Sciences of the Organization for Pharmaceutical Safety and Research of Japan

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Received 29 September 2003
Revised 4 December 2003
Accepted 6 December 2003

photon emission computed tomography (SPECT), which are expected to facilitate the evaluation of AD. Many Aβ imaging probes, such as FDDNP,[1] BSB,[2] TZDM,[3] IMPY,[3] 6-OH-BTA-1,[4] and SB-13[5] have recently been reported.

Previously, we also reported the synthesis of (2-[^{18}F]fluoroethyl)ethylamino-6-diethylaminoacridine ([^{18}F]BF-108).[6] But this probe was not removed rapidly enough from the brain of mice. So, we further searched for compounds which specifically bind to Aβ(l-40) with a β-sheet structure using the fluorescence method that we adapted from LeVine's[7] and Wood's[8] methods, and found that some 2-(4-aminostyryl)benzoxazole derivatives bind to Aβ(1-40) stronger than thioflavin T, a positive control. Many of these derivatives also showed a high brain uptake (2–5% ID/g) at 2 min post injection in mice. Then, F-18 labeling was investigated in various ways, and resulted in the successful preparation of 2-(4-methylaminostyryl)-6-(2-[^{18}F]fluoroethoxy)benzoxazole ([^{18}F]BF-168). It was confirmed that this radioactive probe combines selectively with the injected Aβ peptide in the rat brain. Thus, we have chosen [^{18}F]BF-168 as a new candidate to image the Aβ peptide. C-11 labeling of 2-(4-aminostyryl)benzoxazole derivatives was also performed. 2-(4-[N-methyl-^{11}C]methylaminostyryl)-5-fluorobenzoxazole ([^{11}C]BF-145) was prepared from 2-(4-aminostyryl-5-fluorobenzoxazole and it was confirmed that [^{11}C]BF-145 also binds specifically to the injected Aβ peptide in the rat brain.

Results and discussion

Several 2-(4-aminostyryl)benzoxazole derivatives have been prepared and evaluated as precursors for F-18 radiolabeling. Firstly, 5- or 6-nitro-2-(4-dimethylaminostyryl)benzoxazole was heated with KF and Kryptofix 222 (K.222) in DMSO, without any reaction, only gradual decomposition of the starting material being noticed at temperatures over 160°C. No 5- or 6-fluoro-2-(4-dimethylaminostyryl)benzoxazole was formed. Secondly, the 5- or 6-fluoro-2-(4-dimethylaminostyryl)benzoxazole was heated with [^{18}F]KF (15 μA, 30 min irradiation) and K.222 in DMSO. A ^{19}F-^{18}F exchange reaction occurred to a small extent in the case of the 6-fluoro derivative, but only gave 3.4 MBq of radiolabeled product with a specific activity of ca. 370 MBq/mmol. These values showed that this reaction using the fluoro-derivative was not suitable for practical use. Thirdly, fluorodestannylation of 2-(4-dimethylaminostyryl)-5-tributylstannylbenzoxazole was attempted using fluorine- or acetyl hypofluorite-diluted neon gas in various solvents and at different temperatures. However, the required compound, 5-fluoro-2-(4-dimethylaminostyryl)-benzoxazole, was not obtained in either cold or hot experiments, while iododestannylation of the same compound proceeded readily to give 5-iodo-2-(4-dimethylaminostyryl)benzoxazole by the usual method. So, using the iodo-derivative obtained, an iodine–fluorine exchange reaction was attemp-

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ted by heating a mixture of the iodo-derivative, KF and K.222 in DMSO, but none of the required compound, 5-fluoro derivative, was obtained. Next, the fluorination of 2-(4-dimethylamino)benzoxazole was attempted in both cold and hot conditions using fluorine- or acetyl hypofluorite-diluted neon gas in various solvents and at different temperatures, but without success. Finally, 2-(4-dimethylaminostyryl)-6-(2-tosyloxyethoxy) benzoxazole (7) was chosen as a substrate for F-18 labeling of 2-(4-dimethylaminostyryl)-6-(2-fluoroethoxy)benzoxazole (8, BF-168).[9] The synthetic routes to the authentic 8 and the precursor 7 are shown in Figure 1. The non-radioactive fluorination was successfully carried out by reacting 7 with powdered potassium fluoride in the presence of K.222 in acetonitrile at 80°C for 20 min. The reaction mixture was concentrated to dryness and the residue was purified by column chromatography on silica gel to give 8 in 46% yield as an E/Z mixture. The authentic 8 (E isomer) also gave an E/Z mixture on standing at room temperature in solution. The same phenomenon was observed in the majority of 2-(aminostyryl)benzoxazole derivatives. As it has been reported that E and Z isomers are almost equal in their binding ability to Aβ,[10] the evaluation for Aβ recognition using an E/Z mixture is acceptable. Radiosynthesis was performed in a manner similar to that of the cold synthesis described above, except that [^{18}F]KF prepared *in situ* from F-18 fluoride and

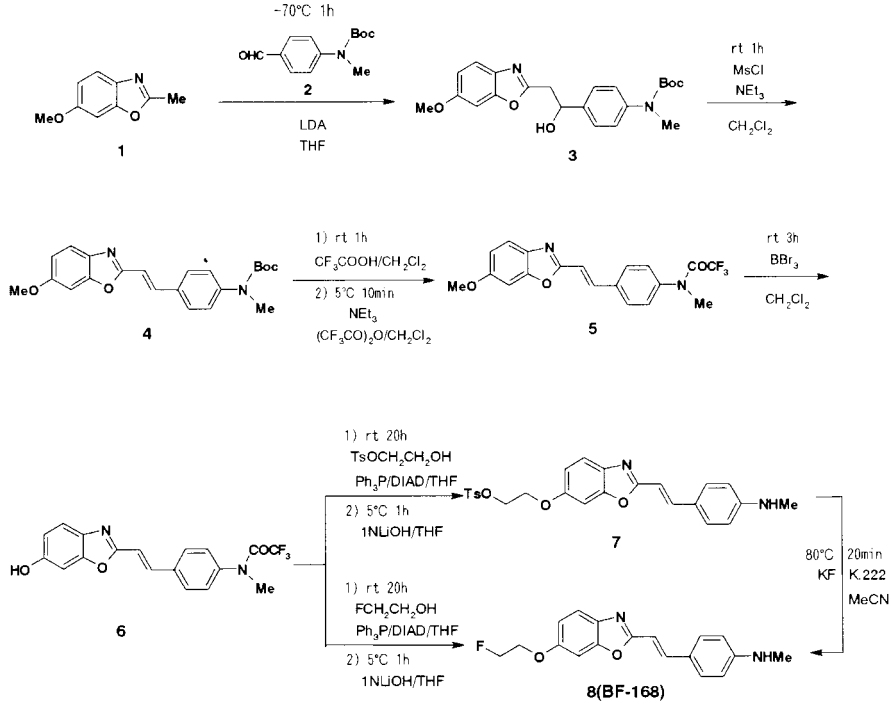

Figure 1. Synthetic route to BF-168

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potassium carbonate was used instead of potassium fluoride (Figure 2). The desired product was isolated by semi-preparative reversed-phase HPLC. The radioactive fraction containing [^{18}F]8 was collected, concentrated under reduced pressure and transferred to a product vial. The synthesis time was 110 min from the end of bombardment. The analytical HPLC showed two peaks corresponding to the *E* and *Z* isomers and that the radiochemical purity was >95% (Total % of *E* and *Z* isomers). The specific activity was a maximum of 106 TBq/mmol. Biodistribution of [^{18}F]8 was studied in Slc:ICR mice (male, 28–32 g, SLC). The mice were decapitated at specific times (2, 10, 30, 60, 120 and 180 min) after intravenous injection of 0.24–0.38 MBq of [^{18}F]8. The organs of interest were removed and weighed, and the radioactivity measured with an automatic γ-counter (Wizard 1480, Turku, Finland). The percentage injection dose per gram (% ID/g) was calculated. Each % ID/g value is an average $\pm$ standard deviation of three separate experiments. A high brain uptake (1.6% ID/g) at 30 min and low uptake (0.76%) at 180 min post injection were obtained as shown in Table 1, and a hot spot was clearly observed at 180 min post i.v. administration on the same site of the rat brain where Aβ(1-42) (and a buffer only on the opposite site) had been injected 6 days before[11] (Figure 3). These results suggest that [^{18}F]8 has the potential to be used as a PET radiotracer for diagnosing AD in its early stage.

In addition to F-18 labeling, we also attempted C-11 labeling and found that 2-(4-[*N*-methyl-^{11}C]methylaminostyryl)-5-fluorobenzoxazole ([^{11}C]15,

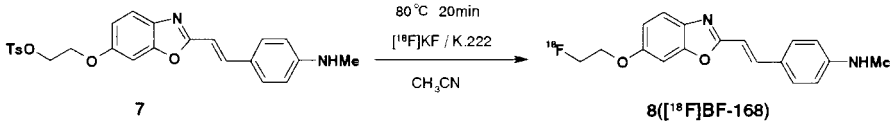

Figure 2. Synthesis of [^{18}F]BF-168

Table 1. Biodistribution of [^{18}F]BF-168 in mice after i.v. injection

Organ	2 min	10 min	30 min	60 min	120 min	180 min
Blood	2.6 ± 0.46	1.6 ± 0.17	1.4 ± 0.066	1.8 ± 0.35	1.3 ± 0.091	1.1 ± 0.16
Brain	3.9 ± 0.22	3.2 ± 0.35	1.6 ± 0.007	1.3 ± 0.040	0.93 ± 0.12	0.76 ± 0.13
Liver	8.7 ± 0.14	7.2 ± 0.74	5.7 ± 0.59	5.2 ± 0.083	4.4 ± 0.68	3.1 ± 0.39
Kidney	9.1 ± 0.34	3.8 ± 0.40	2.3 ± 0.051	2.0 ± 0.37	1.1 ± 0.15	0.86 ± 0.11
Heart	4.8 ± 0.090	2.2 ± 0.26	1.6 ± 0.062	1.4 ± 0.033	1.1 ± 0.11	0.94 ± 0.10
Lung	6.2 ± 0.51	2.9 ± 0.40	1.7 ± 0.081	1.5 ± 0.044	1.7 ± 0.94	1.4 ± 0.71
Bone	1.7 ± 0.40	2.7 ± 1.3	4.1 ± 2.0	4.0 ± 1.4	5.4 ± 1.2	6.6 ± 2.9
Muscle	1.9 ± 0.22	1.5 ± 0.38	1.2 ± 0.035	0.93 ± 0.19	0.76 ± 0.087	0.66 ± 0.10
Skin	1.2 ± 0.24	1.8 ± 0.071	1.7 ± 0.41	1.4 ± 0.16	1.1 ± 0.18	0.82 ± 0.060
Spleen	3.2 ± 0.13	1.8 ± 0.28	1.3 ± 0.11	1.1 ± 0.047	0.92 ± 0.15	0.71 ± 0.14
Intestine	3.3 ± 0.13	8.0 ± 1.1	11 ± 0.55	12 ± 2.3	12 ± 7.1	12 ± 2.3

All values show the %ID/g: mean of three mice $\pm$ standard deviation.

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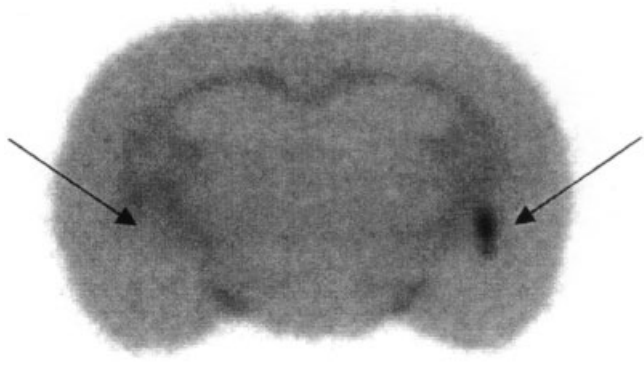

Figure 3. Autoradiographic image at 180 min post i.v. administration of 70 MBq of [^{18}F]BF-168 in rat brain slice injected with Aβ(1-42) dissolved in phosphate buffer (right arrow) and buffer only (left arrow)

[^{11}C]BF-145) was easily synthesized. Thus, [^{11}C]15 was prepared from a precursor, 2-(4-aminostyryl)-5-fluorobenzoxazole (12), with C-11-labeled methyl iodide (20 μA, 30 min irradiation) and 5 N NaOH in DMSO[12] at room temperature for 5 min, followed by purification of the product by semi-preparative HPLC. The synthesis time was 45 min from the end of bombardment. The radiochemical purity was $>95\%$ and the specific activity was 40–70 TBq/mmol at the end of synthesis. The [^{11}C]15 solution was injected into the same model rat with which [^{18}F]8 was used, and after 70 min, hot spots were observed on the same sites of the rat brain slice where Aβ(1-42) had been injected by autoradiography and were well correlated with the result of Aβ immunostaining with the 6F/3D monoclonal antibody using the same slice[13] (Figure 4). This result suggests that [^{11}C]15 also has the potential to be used as a probe for imaging Aβ.

Experimental

General method

The authentic compounds, 2-(4-methylaminostyryl)-6-(2-tosyloxyethoxy)benzoxazole (7) and 2-(4-methylaminostyryl)-6-(2-fluoroethoxy)benzoxazole (8, BF-168) were prepared from 2-methyl-6-methoxybenzoxazole (1) and 4-(*N*-butoxycarbonyl-*N*-methyl)aminobenzaldehyde (2) as shown in Figure 1, and 2-(4-aminostyryl)-5-fluorobenzoxazole (12) and 2-(4-methylaminostyryl)-5-fluorobenzoxazole (15, BF-145) prepared from 4-fluoro-2-aminophenol (9) as shown in Figure 5, both of which were supplied by Tanabe R&D Service Co., Ltd., Osaka City. All other chemicals used in this study were purchased from commercial suppliers. NMR spectra were recorded on a Varian

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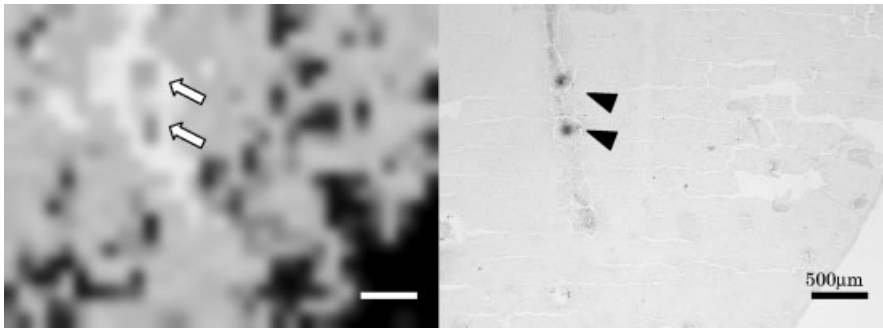

Figure 4. Autoradiographic image (left) at 70 min post i.v. administration of 90 MBq of [^{11}C]BF-145 in rat brain slice injected with Aβ(1-42) and immunostaining (right) with the 6F/3D monoclonal antibody using the same slice (magnified figure)

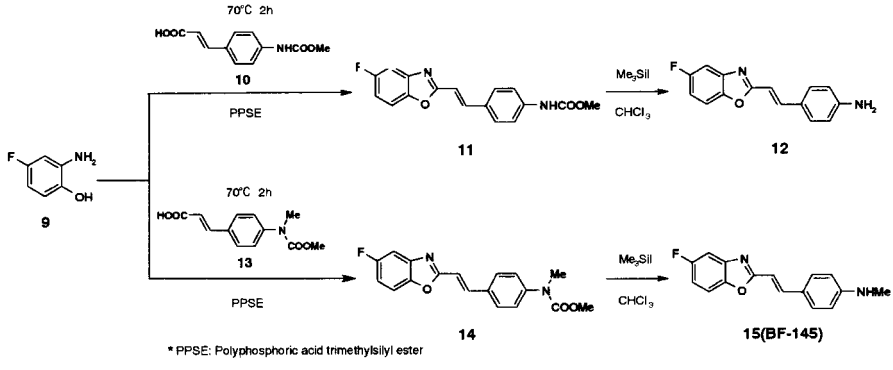

Figure 5. Synthetic route to BF-145

GEMINI-300 spectrometer. A JASCO PU-987 pump and a JASCO UV-970 UV/Vis detector were used for the semi-preparative HPLC, and a Shimadzu C-R4AX Chromatopac, a Shimadzu SPD-6A UV detector, a JASCO PU-980 pump and an Aloka RLC-700 radioanalyzer were used for the analytical HPLC. Autoradiography was performed by using a Fuji Film BAS-III imaging film and a Fuji Film BAS2000 bioimaging analyzer.

2-(4-Methylaminostyryl)-6-(2-tosyloxyethoxy)benzoxazole (7)

To a solution of 2-[4-(*N*-methyl-*N*-trifluoroacetyl)aminostyryl]-6-hydroxy benzoxazole (6) (400 mg, 1.1 mmol), triphenylphosphine (637 mg, 2.4 mmol) and 2-hydroxyethyl tosylate (525 mg, 2.4 mmol) in dry THF (10 ml), diisopropyl azodicarboxylate (DIAD, 0.48 ml, 2.4 mmol) was added. The reaction mixture was stirred for 20 h at room temperature and evaporated to dryness. The residue was purified by silica gel column chromatography (eluent:

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hexane/ethyl acetate 2:1) to give 601 mg (97%) of an intermediate, 2-[4-(*N*-methyl-*N*-trifluoroacetyl)aminostyryl]-6-(2-tosyloxyethoxy)benzoxazole, as lemon-yellow crystals which melted at 143–144°C. To a solution of the intermediate (400 mg, 0.71 mmol) in THF (5 ml), 1 N LiOH (1.43 ml) was added and stirred for 2 h under ice-water cooling. To the reaction solution, a saturated aqueous NH_4Cl solution and AcOEt were added. The organic phase was washed with saline solution, dried with anhydrous $MgSO_4$ and evaporated to dryness. The residue was recrystallized from a mixture of AcOEt and hexane to give 320 mg (96%) of 7 as an orange crystalline powder. m.p. 118–120°C. ^{1}H-NMR ($CDCl_3$) δ: 2.44 (3H, s) 2.89 (3H, s) 4.05(1H, br) 4.20 (2H, t, $J = 4.8$ Hz) 4.40 (2H, t, $J = 4.8$ Hz) 6.61 (2H, d, $J = 8.6$ Hz) 6.77 (1H, dd, $J = 8.7$, 2.3 Hz) 6.78(1H, d, $J = 16.2$ Hz) 6.91 (1H, d, $J = 2.3$ Hz) 7.33 (2H, d, $J = 8.5$ Hz) 7.43 (2H, d, $J = 8.5$ Hz) 7.49 (1H, d, $J = 8.7$ Hz) 7.62 (1H, d, $J = 16.2$ Hz) 7.82 (2H, d, $J = 8.6$ Hz); Elemental anal. Calculated for $C_{25}H_{24}N_2O_5S \cdot 1/10H_2O$: C, 64.39; H, 5.23; N, 6.01; S, 6.87. Found: C, 64.18; H, 5.12; N, 6.01; S, 6.86.

2-(4-Methylaminostyryl)-6-(2-fluoroethoxy)benzoxazole (8, BF-168)

Method A. The title compound 8 was prepared similarly to 7 from 6, using 2-fluoroethanol instead of 2-hydroxyethyl tosylate. An intermediate, 2-[4-(*N*-methyl-*N*-trifluoroacetyl)aminostyryl]-6-(2-fluoroethoxy)benzoxazole, was obtained in 90% yield as lemon-yellow crystals which melted at 124–125°C. Deprotection of the intermediate by LiOH gave 8 in 87% yield as an orange crystalline powder. m.p. 136–137°C. ^{1}H-NMR ($CDCl_3$) δ: 2.89 (3H, s) 4.06 (1H, br) 4.27 (2H, dt, $J = 27.8$, 4.2 Hz) 4.79 (2H, dt, $J = 47.4$, 4.2 Hz) 6.61 (2H, d, $J = 8.6$ Hz) 6.79 (1H, d, $J = 16.2$ Hz) 6.93 (1H, dd, $J = 8.8$, 2.3 Hz) 7.06 (1H, d, $J = 2.3$ Hz) 7.43 (2H, d, $J = 8.6$ Hz) 7.54 (1H, d, $J = 8.8$ Hz) 7.62 (1H, d, $J = 16.2$ Hz); Elemental anal. Calculated for $C_{18}H_{17}FN_2O_2$: C, 69.22; H, 5.49; N, 8.97; F, 6.08. Found: C, 68.95; H, 5.40; N, 8.99; F, 5.80.

Method B[14]. To a solution of 7 (10 mg) in acetonitrile (2 ml), powdered potassium fluoride (10 mg) and K.222 (20 mg) were added, and the mixture stirred at 80°C for 20 min. The reaction mixture was evaporated to dryness under reduced pressure, and the residue purified by silica gel column chromatography (eluent: chloroform only) to give 3.1 mg (46%) of 8 as a yellowish orange solid. However, the compound was obtained as an E/Z mixture and it was then shown that the authentic sample (*E*-isomer) of 8 prepared by method A was also converted to an identical E/Z mixture after being allowed to stand in solution, especially in polar solvents. The compounds were identified by means of NMR, HPLC and TLC. The retention times of the *E* and *Z* isomer were 6.1 and 6.6 min, respectively. ^{1}H-NMR ($CDCl_3$, $E/Z = 7/3$ mixture) δ: 2.89 (2.1H, s) 2.90 (0.9H, s) 4.04 (1H, br)

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4.20–4.24 (1H, m) 4.30–4.33 (1H, m) 4.69–4.73 (1H, m) 4.85–4.89 (1H, m) 6.25 (0.3H, d, $J = 13.2$ Hz) 6.61 (2H, d, $J = 8.7$ Hz) 6.80 (0.7H, d, $J = 16.5$ Hz) 6.83 (0.3H, d, $J = 12.9$ Hz) 6.92–6.98 (1H, m) 7.02 (0.7H, d, $J = 2.4$ Hz) 7.07 (0.3H, d, $J = 2.4$ Hz) 7.44 (1.4H, d, $J = 8.4$ Hz) 7.55 (1H, d, $J = 9.0$ Hz) 7.60 (0.3H, d, $J = 8.7$ Hz) 7.63 (0.7H, d, $J = 16.2$ Hz) 7.82 (0.6H, d, $J = 8.4$ Hz).

2-(4-Methylaminostyryl)-6-(2-[^{18}F]fluoroethoxy)benzoxazole ([^{18}F]8)

[^{18}F]Fluoride was prepared by the ^{18}O(p,n)^{18}F nuclear reaction (15 μA, 30 min irradiation) in a CYPRIS HM-18 cyclotron (Sumitomo Heavy Industries Ltd., Tokyo) on an enriched water (ca. 95% ^{18}O, ISOTEC) target which was adsorbed onto an anion exchange resin (AG1-X8, BIO-RAD) and was then eluted with 7.5 mM potassium carbonate (0.3 ml × 2) and a solution of K.222 (10 mg) in acetonitrile (l.5 ml) was added. The resulting solution was transferred to a glassy carbon vessel. Then, water and acetonitrile were removed in a stream of helium at 100°C under reduced pressure for 10 min. To the residue, acetonitrile (1.5 ml) was added and the resultant mixture was dried again in a stream of helium at 100–115°C under reduced pressure for 20 min. To the residue, a solution of precursor 7 (ca. 2 mg) in acetonitrile (1.5 ml) was added, and the mixture stirred at 80°C for 20 min. After cooling, CH_3CN/H_2O 60:40 (1.0 ml) was added and the mixture injected onto a semi-preparative HPLC column (YMC, ODS-AQ-323, 10 × 250 mm + SHISEIDO CAPCEL PAK C18 10 × 150 mm; eluent: CH_3CN/H_2O 60:40; flow rate: 4.0 ml/min). The eluate was monitored by both UV (254 nm) and radioactivity detectors, and the fraction containing the desired product eluted at 18–23 min was collected in a flask which contained 0.1 N HCl (0.5 ml), and concentrated under reduced pressure to a volume of ca. 5 ml. The resultant solution was transferred to a vial. The radioactivity of the product obtained was 120–950 MBq at the end of synthesis. The synthesis time was 110 min from the end of bombardment. HPLC analysis (Wakosil-II5C18 HG 4.6 × 150 mm; CH_3CN/0.01 M sodium phosphate buffer (pH6.5) 60:40; 2.0 ml/min: UV detector at 254 nm; radioactivity detector) showed that the radiochemical purity was >95% (*E*/*Z* total) and the maximum specific activity was 106 TBq/mmol at the end of synthesis.

2-(4-Methylaminostyryl)-5-fluorobenzoxazole (15, BF-145)

To a solution of 12 (2.7 mg) in DMSO (1.0 ml), 5 N NaOH (27 μl) and methyl iodide (2 μl) were added and the mixture stirred at room temperature for 5 min. HPLC analysis (conditions are as above) of the reaction mixture showed three peaks corresponding to the starting material (12, Rt: 4.8 min, 52%), required compound (15, Rt: 8.2 min, 15%) and *N*,*N*-dimethylated compound (Rt: 14.4 min, 19%) respectively. In this case, the *Z* isomers were

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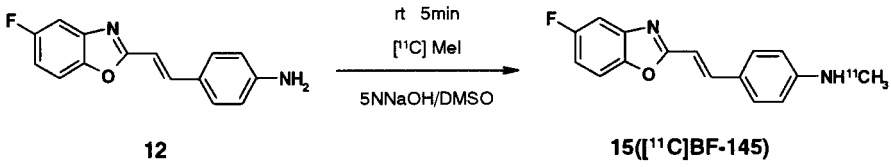

Figure 6. Synthesis of [^{11}C]BF-145

not detectable or had the same retention time as *E* isomers. The isolation of 15 was not attempted.

2-(4-[N-Methyl-^{11}C]methylaminostyryl)-5-fluorobenzoxazole([^{11}C]15, [^{11}C]BF-145)

In a reaction vessel were placed a solution of the precursor (12, ca. 1 mg) in DMSO (0.5 ml) and 5 N NaOH (10 µl). [^{11}C]Methyl iodide (prepared from [^{11}C]CO_2 (20 µA, 30 min irradiation), 0.1 M LAH/THF and 57%HI) were transferred into the solution via a stream of nitrogen (Figure 6). The radioactivity of the vessel was monitored, and when the numeric value of radioactivity was maximized, the flow rate of nitrogen was lowered and then kept at a low value (ca. 30 ml/min). After 5 min at room temperature, the reaction mixture was injected onto a semi-preparative HPLC column (YMC, ODS-AQ-323. 10 × 250 mm; eluent: CH_3CN/0.01 M sodium phosphate buffer (pH6.5) 60:40; flow rate: 6.0 ml/min). The eluate was monitored with both UV (254 nm) and radioactivity detectors, and the fraction containing the desired product, which eluted at ca. 15–16 min, was collected in a flask which contained EtOH (0.2 ml), and concentrated under reduced pressure. The resulting solution was transferred to a vial. The radioactivity of the product obtained was 198–362 MBq at the end of synthesis. HPLC analysis (the same conditions) showed that the radiochemical purity was > 95% and the specific activity was 40–70 TBq/mmol at the end of synthesis.

Acknowledgements

This study was supported by the Program for Promotion of Fundamental Studies in Health Sciences of the Organization for Pharmaceutical Safety and Research of Japan. The authors thank Mr. Norimasa Ejima of the Institute for Biofunctional Research Ltd. for operating the cyclotron and for his assistance in dealing with the automated synthesis apparatus.

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