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Research Article

A novel probe for imaging amyloid-β: Synthesis of F-18 labelled BF-108, an Acridine Orange analog

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

The synthesis of 3-(2-[^{18}F]fluoroethyl)ethylamino-6-diethylaminoacridine ([^{18}F]BF-108), a potential positron-labelled probe for imaging amyloid-β is described. The precursor tosylate derivative was fluorinated with [^{18}F]KF/Kryptofix 222 in acetonitrile, and the crude product was purified by semi-preparative HPLC to give the radiolabelled BF-108. The radiochemical purity was >95% and the maximum specific activity was 33.9 TBq/mmol at the end of the synthesis (EOS). The synthesis time was 130 min from the end of bombardment (EOB). Copyright © 2003 John Wiley & Sons, Ltd.

Key Words: ^{18}F; 3-(2-fluoroethyl)ethylamino-6-diethylaminoacridine; BF-108; amyloid-β; PET

Introduction

Assessing the deposition of amyloid-β 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 amyloid-β are required for non-invasive imaging techniques, such as positron emission

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

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tomography (PET) or single photon emission computed tomography (SPECT) which are expected to facilitate the evaluation of AD. Many amyloid-β imaging probes, such as FDDNP,[1] BSB,[2] TZDM[3] and 6-OH-BTA-1,[4] have recently been reported. We have also searched randomly for compounds which specifically bind to amyloid-β(1-40) with a β-sheet structure using the thioflavin T fluorescence method developed by LeVine[5] and Wood,[6] and found that 3,6-bis(diethylamino)acridine (BF-009) inhibits amyloid-β(1-40)-thioflavin T binding more than Congo Red, a positive control. Moreover, BF-009 has a partition coefficient (octanol-water) much larger than that of Congo Red. This finding suggests that BF-009 has an increased permeability across the blood brain barrier. To ascertain the validity of the design for the radiolabelling, 3-(2-fluoroethyl)ethylamino-6-diethylaminoacridine (BF-108), a fluorinated derivative of BF-009, was synthesized and it was confirmed that this compound has a binding activity and a partition coefficient as large as BF-009. Thus, we chose [^{18}F]BF-108 as a candidate for the radioactive probe for imaging the amyloid-β peptide.

Results and discussion

The synthetic route to the authentic BF-108 (**6**) is shown in Figure 1. The precursor tosylate (**7**) was prepared by reacting a 3-(2-hydroxyethyl)ethylamino derivative (**5**) with an excess amount of *p*-toluenesulfonic anhydride in pyridine.[7] As the tosylate (**7**) was found to be hygroscopic and unstable, it had to be stored in a freezer. It was converted to an unknown compound when left standing at room temperature. Furthermore, it is sensitive to basic reagents and reacts readily, for example, with potassium carbonate to give compound **5**. This property may cause a problem in the synthesis of radioactive BF-108, because, in the radiofluorination, an excess amount of potassium carbonate, which is used for detachment of the radioactive fluoride anion from the ion-exchange resin, is always present in the reaction mixture. So use of a minimum amount of potassium carbonate and an addition of KH_2PO_4 to neutralize the reaction solution were thought to be advantageous for obtaining the desired product. The non-radioactive fluorination was successfully carried out by reacting **7** with powdered potassium fluoride in the presence of Kryptofix 222 in acetonitrile at 80°C for 40 min. The reaction mixture was concentrated to dryness and the residue was purified by column chromatography on silica-gel to give

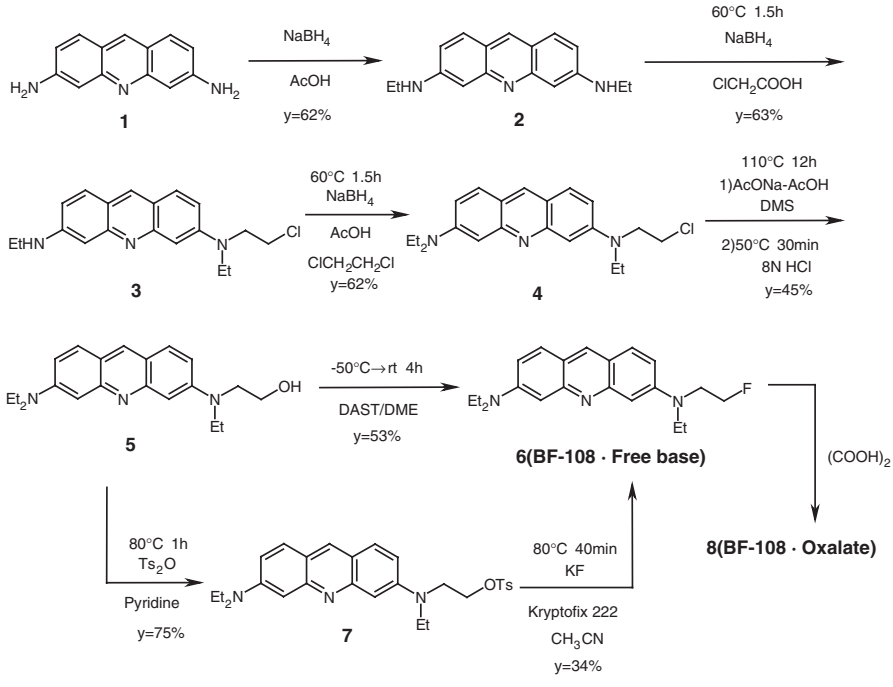
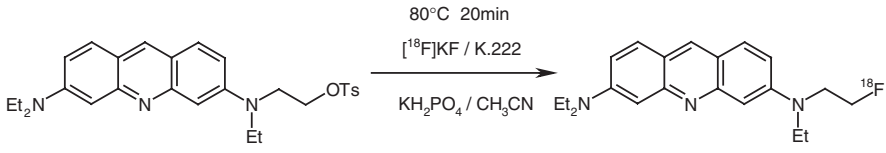


Figure 1. Synthetic routes to BF-108

6 in 34% yield. Compound **6** thus obtained was compared to an authentic sample of **6** prepared from **5** and (diethylamino)sulfur trifluoride (DAST). Their HPLC retention times and NMR spectra were identical.

The radiosynthesis was performed in a manner similar to that of the non-radiosynthesis described above except that KH_2PO_4 was further added (Figure 2). The desired product was isolated by semi-preparative reversed-phase HPLC (Figure 3). The radioactive fraction containing [^{18}F]**6** was collected, concentrated under reduced pressure and transferred to a product vial. The synthesis time was 130 min from EOB. The analytical HPLC showed that the radiochemical purity was $>95\%$ and the maximum specific activity was 33.9 TBq/mmol.

Biodistribution of [^{18}F]**6** was determined in Slc:ICR mice (male, 28–32 g, SLC). The mice were decapitated at specific times (2, 10, 30, 60 and 180 min) after intravenous injection of 0.3–0.4 MBq of [^{18}F]**6** in saline. The organs of interest were removed and weighed, and the amount of radioactivity was counted with an automatic γ-counter (Wizard 1480, Turku, Finland). The percentage injection dose per gram (%ID/g) was calculated by comparison of the tissue counts to the weights of tissues and standards. Each %ID/g value is an average



7 [^{18}F]**6**

Figure 2. Synthesis of [^{18}F]BF-108

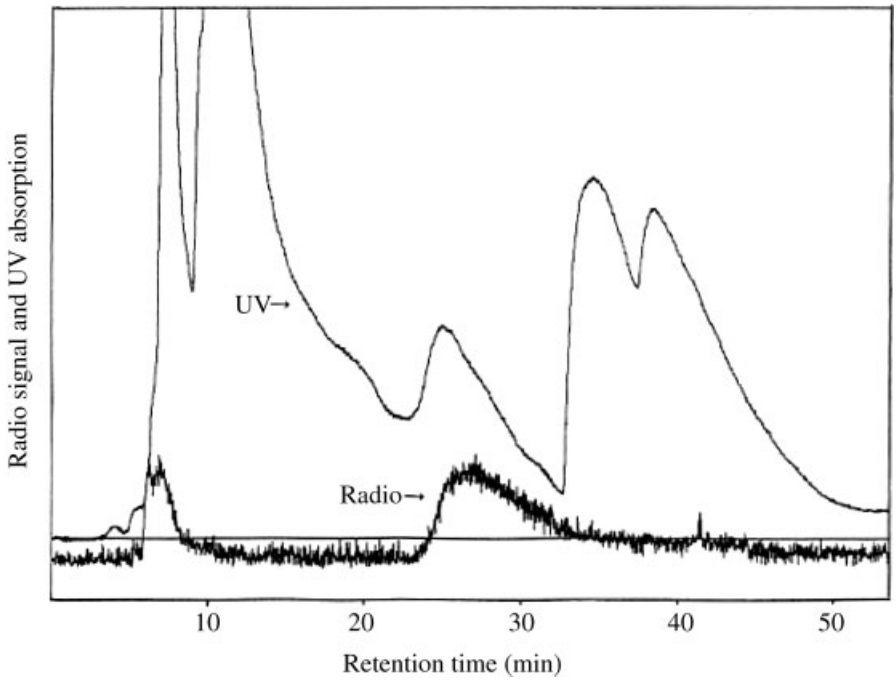


Figure 3. Semi-preparative HPLC separation of [^{18}F]BF-108

$\pm$ standard deviation (SD) of three or four separate experiments. A high brain uptake (1.53% ID/g) was obtained at 30 min post injection as shown in Figure 4.[8] This result suggests that [^{18}F]**6** has the potential to be used as a radiotracer for the early diagnosis of AD.

Experimental

General method

Compounds: 3-(2-hydroxyethyl)ethylamino-6-diethylaminoacridine (**5**) and 3-(2-fluoroethyl)ethylamino-6-diethylaminoacridine oxalate (**8,**

organ	2min	10min	30min	60min	120min	180min
brain	0.42 ± 0.09	1.13 ± 0.25	1.53 ± 0.36	1.39 ± 0.13	1.34 ± 0.31	1.04 ± 0.25
blood	2.89 ± 0.56	2.65 ± 0.68	2.57 ± 0.51	2.32 ± 0.27	1.91 ± 0.32	1.56 ± 0.43
liver	7.82 ± 1.51	6.28 ± 1.39	8.08 ± 7.74	2.99 ± 0.33	2.45 ± 0.43	1.75 ± 0.32
kidney	14.26 ± 2.84	14.54 ± 0.56	10.79 ± 3.01	6.42 ± 0.58	2.86 ± 0.20	3.81 ± 3.35
heart	8.87 ± 1.98	3.47 ± 0.28	2.60 ± 0.38	2.17 ± 0.31	1.89 ± 0.33	1.37 ± 0.24
lung	20.06 ± 1.93	13.89 ± 2.77	7.52 ± 0.76	5.36 ± 0.80	3.06 ± 0.63	2.61 ± 0.58
spleen	3.07 ± 0.62	3.91 ± 0.50	2.78 ± 0.31	2.02 ± 0.16	1.50 ± 0.27	1.10 ± 0.15
intestine	2.60 ± 0.84	5.32 ± 1.11	7.97 ± 2.16	9.51 ± 0.76	9.75 ± 1.60	4.38 ± 0.51
bone	1.84 ± 0.32	2.95 ± 0.58	4.31 ± 1.06	5.41 ± 1.77	8.00 ± 2.28	8.96 ± 0.82
muscle	2.65 ± 0.51	2.50 ± 0.38	1.82 ± 0.27	1.48 ± 0.16	1.27 ± 0.27	0.91 ± 0.18
skin	1.05 ± 0.15	2.11 ± 0.35	2.15 ± 0.39	1.83 ± 0.20	1.57 ± 0.28	1.12 ± 0.17

All values showing the %ID/g; mean of three or four mice ± standard deviation.

Figure 4. Biodistribution in mice after i.v. injection of [^{18}F]BF-108

BF-108 · oxalate) were prepared from 3,6-diaminoacridine as shown Figure 1 and also 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 GEMINI-300 spectrometer. A JASCO PU-987 pump and a JACSO 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.

*3-(2-p-Toluenesulfonyloxyethyl)ethylamino-6-diethylaminoacridine (**7**)*

Compound **5** (50 mg, 0.12 mmol) was dissolved in pyridine (25 ml). To this solution, *p*-toluenesulfonic anhydride (500 mg, 1.56 mmol) was added and the mixture was stirred at 80°C for 1 h. The reaction mixture was evaporated to dryness, and the residue was purified by silica gel column chromatography (eluent: chloroform/methanol 10:1(v/v)) to give **7** (55 mg,0.04 mmol) as a brown hygroscopic solid in 39% yield. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3 H, t, $J = 7.2$ Hz) 1.31 (6 H, t, $J = 7.2$ Hz) 2.35 (3 H, s) 3.57 (2 H, t, $J = 7.2$ Hz) 3.58 (4 H, q, $J = 7.2$ Hz) 3.83 (2 H, t, $J = 5.1$ Hz) 4.33 (2 H, t, $J = 4.8$ Hz) 6.95–7.05 (2 H, m) 7.24–7.28 (3 H, m) 7.35 (1 H, br.s) 7.64–7.71 (2 H, m) 7.73 (2 H, d, $J = 6.6$ Hz) 8.34 (1 H, s); Anal. Calculated for $C_{28}H_{33}N_3O_3S \cdot H_2O$: C, 65.99; H, 6.92; N, 8.24. Found: C, 66.05; H, 6.78; N, 8.03.

*3-(2-Fluoroethyl)ethylamino-6-diethylaminoacridine (**6**, BF-108)*

Method A.A solution of DAST (0.83 ml, 6.28 mmol) in 1,2-dimethoxyethane (20 ml) was added to a solution of **5** (1.06 g, 3.14 mmol) in 1,2-dimethoxyethane (40 ml) at –50°C under stirring. After 10 min, the cooling bath was removed and the reaction mixture was allowed to warm up to room temperature. After 4 h, to the resulting reaction mixture, chloroform and an aqueous potassium carbonate solution were added and the separated organic phase was evaporated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (eluent: chloroform/methanol/NH_4OH 100:10:1) to give 550 mg (53%) of **6** as a brown solid. The oxalate (**8**) was prepared by treating **6** with a solution of oxalic acid in a mixture of methanol and diethyl ether.

Free base (**6**); ^{1}H-NMR ($CDCl_3$) δ:1.32 (9 H, t, $J = 7.2$ Hz) 3.58 (4 H, q, $J = 7.2$ Hz) 3.68 (2 H, q, $J = 7.2$ Hz) 3.87 (2 H, td, $J = 4.5$, 25.8 Hz) 4.77 (2 H, td, $J = 4.8$, 47.4 Hz) 7.01 (1 H, dd, $J = 2.4$, 9.6 Hz) 7.04 (1 H, dd, $J = 2.4$, 8.1 Hz) 7.33 (1 H, br.s) 7.49 (1 H, br.s) 7.67 (1 H, d, $J = 9.6$ Hz) 7.68 (1 H, d, $J = 9.6$ Hz) 8.34 (1 H, s).

Oxalate (**8**); ^{1}H-NMR (DMSO-d_6) δ:1.23 (9 H, t, $J = 6.6$ Hz) 3.5–3.7 (6 H, m) 3.91 (2 H, td, $J = 4.5$, 25.8 Hz) 4.77 (2 H,td, $J = 4.5$, 47.1 Hz) 6.79 (1 H, s) 6.87 (1 H, s) 7.26 (1 H, d, $J = 9.3$ Hz) 7.30 (1 H, d, $J = 9.3$ Hz) 7.94 (2 H, d, $J = 9.3$ Hz) 8.83 (1 H, s) Elemental anal. Calculated for $C_{21}H_{26}N_3F \cdot C_2H_2O_4 \cdot 0.2H_2O$: C, 63.79; H, 6.61; N, 9.70; F 4.39. Found: C, 63.68; H, 6.33: N, 9.66; F 4.27; mp 203–205°C.

Method B.To a solution of **7** (6.0 mg) in acetonitrile (2 ml), powdered potassium carbonate (6 mg) and Kryptofix 222 (15 mg) were added, and the mixture was stirred at 80°C for 40 min. The reaction mixture was evaporated to dryness under reduced pressure, and the residue was purified by silica gel column chromatography (eluent: chloroform/methanol 10:1) to give 1.4 mg (34%) of **6** as a brown solid. The compounds obtained by Methods A and B were identified by means of NMR, HPLC and TLC.

*3-(2-[^{18}F]Fluoroethyl)ethylamino-6-diethylaminoacridine ([^{18}F]BF-108 · free base, [^{18}F]**6**)*

[^{18}F]fluoride prepared by the $^{18}O(p,n)^{18}F$ nuclear reaction (15 μA, 20 min) in a CYPRIS HM-18 cyclotron (Sumitomo Heavy Industries Ltd., Tokyo) on an enriched water (ca.95%^{18}O) target was adsorbed onto an anion exchange resin (AG1-X8, BIO-RAD) and was eluted with

7.5 mM potassium carbonate (0.3 ml × 2) and a solution of 20 mg of Kryptofix 222 in 3.0 ml of acetonitrile was added. The resulting solution was transferred to a glassy carbon vessel and 0.5 mg of potassium dihydrogen phosphate in 0.05 ml water was added. Then, water and acetonitrile were removed in a stream of helium at 115°C under reduced pressure. To the residue, a solution of the precursor **7** (ca.1 mg) in 1.0 ml of acetonitrile was added, and the mixture was stirred at 80°C for 20 min. After cooling, 1.0 ml of 0.01 M phosphate buffer was added and the mixture was injected onto a semi-preparative HPLC column (YMC, ODS-AQ-323,10 × 250 mm + SHISEIDO CAPCEL PAK C18 10 × 150; eluent: CH_3CN/0.01 M sodium phosphate buffer (pH6.5) 60:40; flow rate: 4.0 ml/min) The effluent was monitored with both UV (270 nm) and radioactivity detectors and the fraction containing the desired product at 25–30 min was collected and concentrated under reduced pressure to less than half of the initial volume, and transferred to a vial. The product activity was 200–850 MBq at EOS. The synthesis time was 130 min from EOB. HPLC analysis (Wakosil-II5C18 HG 4.6 × 150 mm; CH_3CN/0.01 M sodium phosphate buffer (pH6.5) 60:40; 1.0 ml/min: UV detector at 270 nm; radioactivity detector) showed that the radiochemical purity was > 95% and the maximum specific activity was 33.9 TBq/mmol at EOS.

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References

1. Barrio JR, Huang S-C, Cole G, Satyamurthy N, Petric A, Phelps ME, Small G. *J Label Compd Radiopharm* 1999; **42**: S194–S195.
2. Skovronsky DM, Zhang B, Kung M-P, Kung HF, Trojanowski JQ, Lee VM-Y. *Proc Natl Acad Sci USA* 2000; **97**: 7609–7614.

3. Zhung Z-P, Kung M-P, Hou C, Lee C-W, Trojanowski JQ, Lee VM-Y, Kung HF. *J Label Compd Radiopharm* 2001; **44**: S29–S32
4. Mathis C, Holt D, Wang Y, Huang G-F, Debnath M, Klunk W. *Neurobiol Aging* 2002; **23**: S349.
5. LeVine H. *Protein Sci* 1993; **2**: 404–410.
6. Wood SJ, Maleeff B, Hart T, Wetzel R. *J Mol Biol* 1996; **256**: 870–877.
7. Barrio JR, Petric A, Satyamurthy N, Small GW, Cole GM, Huang S-C. *WO* 00/10614 (PCT/US 99/18966).
8. Okamura N, Suemoto T, Suzuki M, Shiomitsu T, Shimadzu H, Kudo Y, Sawada T. *Neurobiol Aging* 2002; **23**: S352.

