SUPPLEMENTARY INFORMATION

Feasibility of Amylin Imaging in Pancreatic Islets with β-Amyloid Imaging Probes

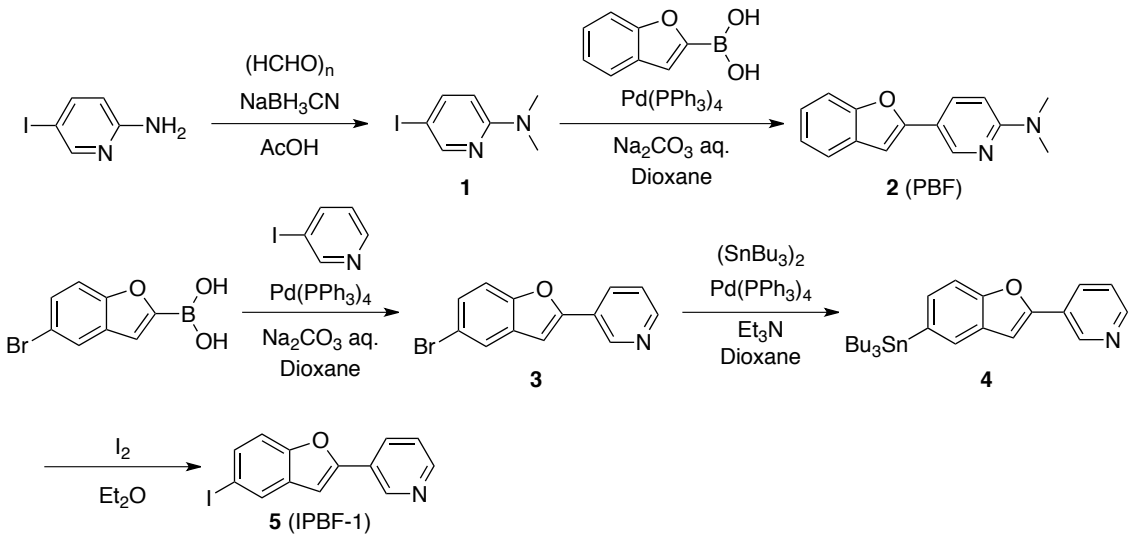
Masashi Yoshimura, Masahiro Ono, Hiroyuki Watanabe, Hiroyuki Kimura,

Hideo Saji

Department of Patho-Functional Bioanalysis, Graduate School of Pharmaceutical Sciences, Kyoto University, 46-29 Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan.

Chemistry

Novel benzofuran derivatives (IPBF-1 and PBF) were prepared according to the following scheme. All reagents were commercial products and used without further purification unless indicated otherwise. ^{1}H NMR spectra were recorded using a JEOL JNM-ECS400 or JEOL ECA-500 spectrometer, and chemical shifts are reported in δ (ppm) relative to TMS as an internal standard. Coupling constants are reported in Hertz. Multiplicity was defined as singlet (s), doublet (d), triplet (t), or multiplet (m). Mass spectra were obtained on a SHIMADZU LCMS-2010 EV. HPLC was performed with a Shimadzu system (LC-20AD pump with a SPD-20A UV detector, λ = 254 nm) using a Cosmosil C_{18} column (Nacalai Tesque, 5C_{18}-AR-II, 4.6×150 mm) and acetonitrile/water or acetonitrile/20 mM phosphate buffer (pH 7.0) as the mobile phase at a flow rate of 1.0 mL/min. All key compounds were proven by this method to show ≥97% purity.



5-Iodo-*N*,*N*-dimethylpyridine-2-amine (1).

To a solution of 2-amino-5-iodopyridine (1.1 g, 5 mmol) in acetic acid (20 mL) was added paraformaldehyde (1.5 g, 50 mmol) and $NaBH_3CN$ (942.6 mg, 15 mmol). The reaction mixture was stirred at room temperature for 24 h and then quenched with ice water and 2N NaOH aq. The solution was extracted with AcOEt (100 mL x 2). The organic layers were combined and dried over $MgSO_4$. Evaporation of the solvent afforded a residue, which was purified by silica gel chromatography (AcOEt / hexane = 1 / 5) to give 1.162 g of **1** (93.7%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.29 (d, J = 2.3 Hz, 1H), 7.61 (dd, J = 8.9, 2.3 Hz, 1H), 6.35 (d, J = 8.9, 1H), 3.05 (s, 6H). MS(ESI) m/z 249 [MH^+].

5-(Benzofuran-2-yl)-*N*,*N*-dimethylpyridin-2-amine (2).

To a solution of 2-benzofuranboronic acid (242.9 mg, 1.5 mmol) in 1,4-dioxane (8 mL) was added **1** (372.2 mg, 1.5 mmol), $Pd(PPh_3)_4$ (173.3 mg, 0.15 mmol), and Na_2CO_3 (2M in water, 1.5 mL). The reaction mixture was refluxed for 2 h. The solution was allowed to cool to room temperature and then extracted with AcOEt (60 mL x 2). The organic layers were combined and dried over $MgSO_4$. Evaporation of the solvent afforded a residue, which was purified by silica gel chromatography (AcOEt / hexane = 1 / 5 and $CHCl_3$ / MeOH = 40 / 1) to give 221 mg of **2** (61.8%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.69 (d, *J* = 2.3 Hz, 1H), 7.89 (dd, *J* = 8.9, 1.7 Hz, 1H), 7.53 (d, *J* = 7.4 Hz, 1H), 7.49 (d, *J* = 7.7 Hz, 1H), 7.23-7.20 (m, 2H), 6.82 (s, 1H), 6.58 (s, 1H), 3.16 (s, 6H). MS(ESI) m/z 239 [MH^+].

3-(6-Bromobenzofuran-2-yl)pyridine (3).

To a solution of 5-benzofuran-2-ylboronic acid (602.1 mg, 2.5 mmol) in 1,4-dioxane (12.5 mL) was added 3-iodopyridine (676.5 mg, 3.3 mmol), $Pd(PPh_3)_4$ (288.9 mg, 0.25 mmol), and Na_2CO_3 (2M in water, 2.5 mL). The reaction mixture was refluxed for 5 h.

The solution was allowed to cool to room temperature and then extracted with AcOEt (60 mL x 2). The organic layers were combined and dried over Na_2SO_4. Evaporation of the solvent afforded a residue, which was purified by silica gel chromatography (AcOEt / hexane = 3 / 1) to give 352.3 mg of **3** (51.4%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (d, *J* = 2.3 Hz, 1H), 8.61 (dd, *J* = 4.9, 1.7 Hz, 1H), 8.13-8.11 (m, 1H), 7.74 (s, 1H), 7.42-7.38 (m, 3H), 7.07 (s, 1H).

3-(6-(Tributylstannyl)benzofuran-2-yl)pyridine (4).

To a solution of **3** (352.3 mg, 1.29 mmol) in 1,4-dioxane (10 mL) was added bis(tributyltin) (1,491 mg, 2.57 mmol), $Pd(PPh_3)_4$ (149.1 mg, 0.129 mmol), and Et_3N (5 mL). The reaction mixture was stirred for 4 h at 100ºC. The solution was allowed to cool to room temperature and then filtered through celite. Evaporation of the solvent afforded a residue, which was purified by silica gel chromatography (AcOEt / hexane = 1 / 3) to give 325.9 mg of **4** (52.2%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (d, *J* = 1.5 Hz, 1H), 8.58 (dd, *J* = 4.6, 1.5 Hz, 1H), 8.14-8.11 (m, 1H), 7.70 (s, 1H), 7.53 (d, *J* = 7.8 Hz, 1H), 7.40-7.36 (m, 2H), 7.11 (s, 1H), 1.61-1.52 (m, 6H), 1.39-1.32 (m, 6H), 1.12-1.07

(m, 6H), 0.90 (t, J = 7.3 Hz, 9H).

3-(6-Iodobenzofuran-2-yl)pyridine (5).

To a solution of **4** (325.9 mg, 0.673 mmol) in dry diethylether (10 mL) was added I_2 (170.8 mg, 0.673 mmol). The reaction mixture was stirred for 5 min at room temperature. The solution was allowed to cool to room temperature and then quenched with sat $Na_2S_2O_3$ aq. The solution was extracted with AcOEt (60 mL x 2). The organic layers were combined and dried over Na_2SO_4. Evaporation of the solvent afforded a residue, which was purified by aminopropyl silica gel chromatography (AcOEt / hexane = 1 / 3) to give 165.1 mg of **5** (76.4%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.10 (s, 1H), 8.60 (d, J = 4.9 Hz, 1H), 8.12-8.11 (m, 1H), 7.95 (s, 1H), 7.60 (d, J = 8.9 Hz, 1H), 7.40 (dd, J = 7.7, 4.6 Hz, 1H), 7.33 (d, J = 8.6 Hz, 1H), 7.05 (s, 1H). MS(ESI) m/z 322 [MH^+].

Radiolabeling

The radioiodination of [^{125}I]IPBF was carried out according to ref. 1. The identity of [^{125}I]IPBF was verified by a comparison of the retention time with that of the nonradioactive compound (Figure S1).

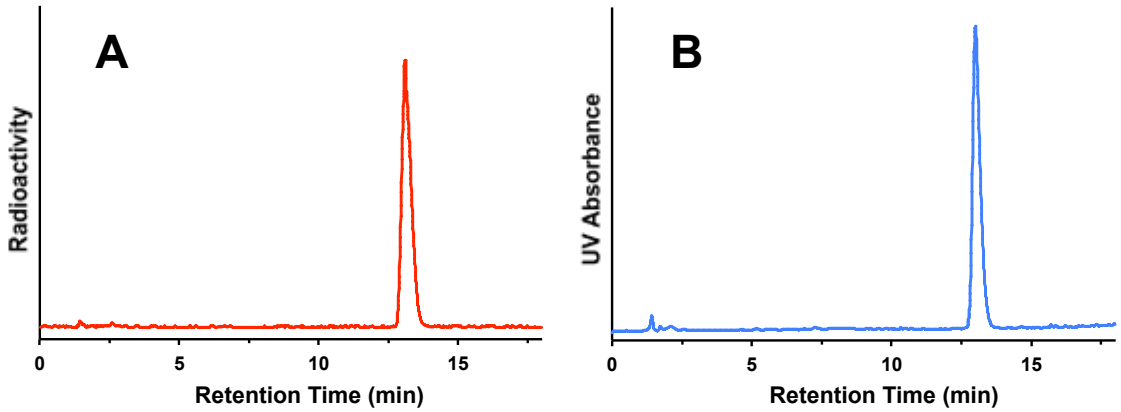


FIGURE S1. Representative HPLC profiles of [^{125}I]IPBF (A) and nonradioactive IPBF (B). HPLC conditions: Cosmosil C_{18} column (Nacalai Tesque, 5C_{18}-AR-II, 4.6×150 mm), MeCN/20 mM phosphate buffer (pH 7.0) = 80/20, 1.0 mL/min, UV absorbance at 254 nm.

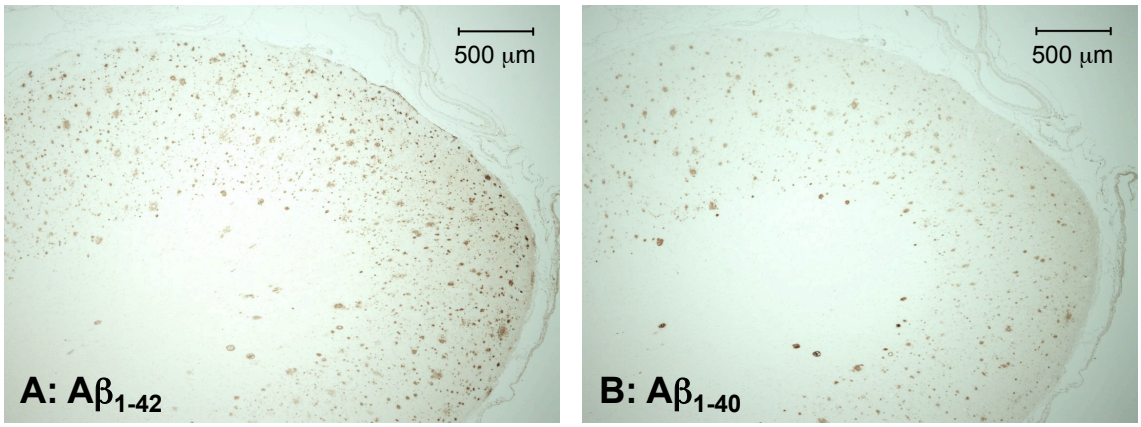


FIGURE S2. Immunohistochemical staining of AD brain sections with antibodies against $A\beta_{1-42}$ (A) and $A\beta_{1-40}$ (B).

Reference

1. Ono, M. *et al*. Development of novel ^{123}I-labeled pyridyl benzofuran derivatives for SPECT imaging of β-amyloid plaques in Alzheimer's disease. *PLoS One* **8**, e74104 (2013).