Supplementary Data

Preferred features of a fluorine-19 MRI probe for amyloid detection in the brain

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Supplementary Materials and Methods

Preparation of compounds 3 and 5

Materials

Fluorinated reagents, 3’,5’-Bis(trifluoromethyl)benzylamine, 2,2,2-trifluoroethoxy-ethanol and 1,1,1,3,3,3-hexafluoro-2-propanol were purchased from Wako Pure Chemical Industries, SynQuest, and MP Biomedicals, respectively. Monoprotected poly(ethylene glycol)s were prepared according to the method reported by Loiseau and colleagues in 2004 [1].

Synthesis of compound 3

General procedure A:

Synthesis of compound 2 (m = 2 – 6)

Diisopropylazodicarboxylate (1.9 M toluene solution) (1.5 equiv) was added to a cooled mixture (in an ice-bath) of 2-(4’-dimethylaminostyryl)-6-hydroxy-benzoxazole (1 equiv), monotosylated polyethylene glycol (1.5 equiv) and triphenylphosphine (1.5 equiv) in N,N-dimethylformamide. The mixture was stirred for 10 min and then warmed up to room temperature and stirred for 6 h. The mixture was diluted with ethyl acetate (20 parts) and the solution was washed three times with a small amount of water and once with brine. After drying the extract over $MgSO_4$, the organic solvents were evaporated under reduced pressure and the remaining viscous oil was purified by silica gel column chromatography.

General procedure B:

Synthesis of compound 3a

Potassium carbonate (6 equiv) was added to a mixture of the tosylate compound 2 (1 equiv) and 3’,5’-bis(trifluoromethyl)benzylamine(5 equiv) in N,N-dimethylformamide and the mixture heated at 90°C for 9 h. The mixture was diluted with ethyl acetate (20 parts) and the extract was washed three times with a small amount of water and once with brine. After drying over $MgSO_4$, the organic solvent was removed under vacuum. The residue was purified by silica gel column chromatography.

General procedure C:

Synthesis of compounds 3b, 3c, and 3d

A solution of appropriate alcohol (1.5 equiv) in tetrahydrofuran (THF) was added to a cooled suspension of sodium hydride (60% oil dispersion) (1.2 equiv) in THF under nitrogen atmosphere. The mixture was stirred at room temperature for 1 h to form the appropriate alkoxide. The mixture was again cooled in an ice-bath and to this solution was added a solution of the tosylate compound 2 (1 equiv) in THF, and the mixture was stirred at room temperature for 5 to 15 h (the reaction process was monitored by thin layer chromatography). The mixture was diluted with ethyl acetate (20 parts) and the solution washed three times with a small amount of water and once with brine. After drying the extract over $MgSO_4$, the organic solvent was evaporated under reduced pressure, and the remaining oil was purified by silica gel column chromatography.

Synthesis of compound 5

General procedure D:

Synthesis of compound 4 (m + n = 7-12)

i) Compound 3d was dissolved in ethanol and a catalytic amount of concentrated hydrochloric acid was added to the solution, before the mixture was set aside for 10 to 15 h. A saturated aqueous solution of sodium hydrogen carbonate was added to the mixture, which was then extracted with dichloromethane. After drying the extract over $MgSO_4$, the dichloromethane was evaporated under reduced pressure. The residue was used for the following step without further purification.

ii) The crude alcohol (1 equiv) obtained in process (i) and p-toluenesulfonyl chloride (1.2 equiv) were dissolved in dichloromethane. Triethylamine (1.5 equiv) was added to the mixture, which was stirred at room temperature for 10 to 15 h. The mixture was poured into a small amount of water and extracted thoroughly with ethyl acetate several times. The extract was washed once with brine and dried over $MgSO_4$. The extract was taken to dryness under vacuum and the obtained residue was purified by silica gel column chromatography.

General procedure E:

Synthesis of compound 5a

Compound 5a was synthesized from compound 4 with 3’,5’-bis(trifluoromethyl)benzylamine, by a method similar to that used to synthesize compound 3a.

General procedure F:

Synthesis of compounds 5b and 5c

Compounds 5b and 5c were synthesized from compound 4 by a method similar to that for compounds 3b and 3c, respectively.

The ^{19}F and ^{1}H nuclear magnetic resonance (NMR) spectra of some of the compounds prepared for this work are summarized in Supplementary Table 1.

Reference

[1] Loiseau FA, Hii KK, Hill AM (2004) Multigram synthesis of well-defined extended bifunctional polyethylene glycol (PEG) chains. *J Org Chem* **69**, 639-647.

Supplementary Table 1.

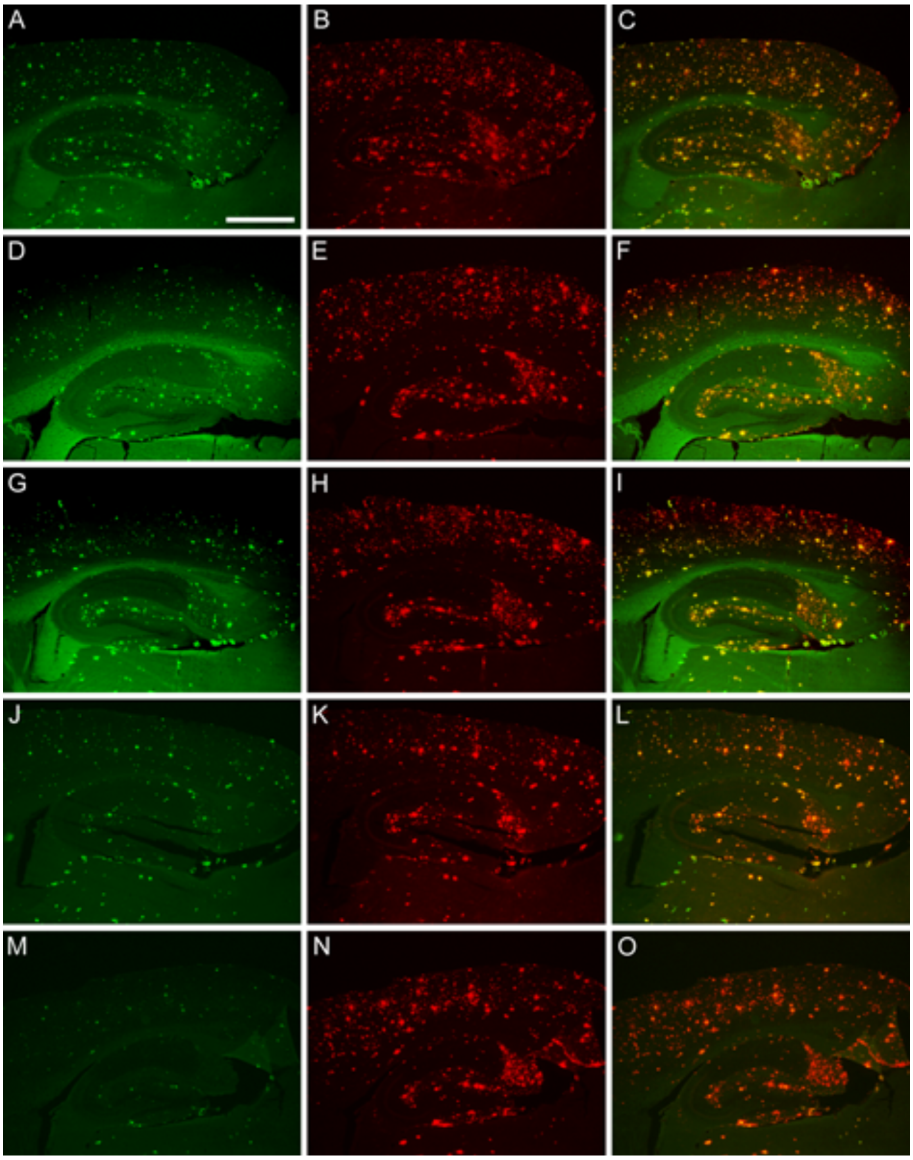
^{19}F and ^{1}H nuclear magnetic resonance (NMR) spectra (in $CDCl_3$)

Compound	^{19}F NMR Chemical Shift (δ)	^{1}H NMR Chemical Shift (δ)	
		$N(CH_3)_2$	Other Signals
2, m = 2		3.03 (6H,s)	2.40 (3H,s), 3.80 (4H,m), 4.10 (2H,m), 4.21 (2H,m), 6.72 (1H,d,J=9Hz), 6.81 (1H,d,J=16Hz), 6.89 (1H,dd,J=2Hz,9Hz), 7.02 (1H,d,J=2Hz), 7.30 (2H,d,J=8Hz), 7.48 (2H,d,J=9Hz), 7.53(2H,d,J=9Hz), 7.65 (1H,d,J=16Hz), 7.81 (2H,d,J=8Hz)
2, m = 3		3.03 (6H,s)	2.42 (3H,s), 3.6-3.8 (6H), 3.86 (2H,m), 4.1-4.2 (4H), 6.71 (1H,d,J=9Hz), 6.80 (1H,d,J=16Hz), 6.90 (1H,dd,J=2Hz,9Hz), 7.04 (1H,d,J=2Hz), 7.32 (2H,d,J=8Hz), 7.47 (2H,d,J=9Hz), 7.52 (2H,d,J=9Hz), 7.63 (1H,d,J=16Hz), 7.79 (2H,d,J=8Hz)
2, m = 4		3.03 (6H)*	2.43 (3H,s), 3.5-4.2 (16H), 6.2-7.1 (5H), 7.32 (2H,d,J=8Hz), 7.79 (2H,d,J=8Hz), 7.4-7.9 (4H)
2, m = 5		3.03 (6H)*	2.43 (3H,s), 3.5-3.8 (14H), 3.89 (2H,m), 4.1-4.2 (4H), 6.2-7.1 (5H), 7.32 (2H,d,J=8Hz), 7.79 (2H,d,J=8Hz), 7.4-7.9 (4H)
2, m = 6		3.03 (6H)*	2.44 (3H,s), 3.5-3.8 (18H). 3.89 (2H,m), 4.1-4.3 (4H), 6.2-7.1 (5H), 7.3-7.9 (8H)
3a, m = 4	-64.05 (s)	3.03 (6H)*	2.7-2.9 (2H), 3.5-4.2 (16H), 6.2-7.1 (5H), 7.4-7.9 (4H), 7.75 (1H,s), 7.83 (2H,s)
3a, m = 5	-64.04 (s)	3.03 (6H)*	2.75-2.84 (2H), 3.5-4.2 (20H), 6.2-7.1 (5H), 7.4-7.9 (4H), 7.67 (1H,s), 7.83 (2H,s)
3a, m = 6	-64.07 (s)	3.03 (6H)*	2.80 (2H,t,J=5Hz), 3.5-3.8 (18H), 3.8-4.0 (4H), 4.1-4.3 (2H), 6.2-7.1 (5H), 7.4-7.9 (7H)
3b, m = 2	-75.54 (t,J=9Hz)	3.03 (6H,s)	3.65-3.95 (10H), 3.91 (2H,q,J=9Hz), 4.1-4.3 (2H), 6.2-7.1 (5H), 7.4-7.9 (4H)
3b, m = 4	-75.56 (t,J=9Hz)	3.03 (6H)*	3.6-3.8 (16H), 3.90 (2H,q,J=9Hz), 3.85-3.95 (2H), 4.17 (2H,q,J=5Hz), 6.2-7.1 (5H), 7.4-7.9 (4H)
3b, m = 5	-75.54 (t,J=9Hz)	3.03 (6H)*	3.6-3.8 (20H), 3.8-3.9 (2H), 3.90 (2H,q,J=9Hz), 4.1-4.2 (2H), 6.2-7.1 (5H), 7.4-7.9 (4H)
3b, m = 6	-75.55 (t,J=9Hz)	3.03 (6H)*	3.6-4.0 (26H), 3.91 (2H,q,J=9Hz), 4.17 (2H,q,J=5Hz), 6.2-7.1 (5H), 7.4-7.9 (4H)
3c, m = 6	-75.52 (d,J=6Hz)	3.03 (6H)*	3.6-3.8 (18H), 3.90 (2H,m), 3.98 (2H,m), 4.18 (2H,m), 4.51 (1H,sep,J=6Hz), 6.2-7.1 (5H), 7.4-7.9 (4H)
4, m + n = 7		3.03 (6H)*	2.44 (3H,s), 3.5-3.8 (22H), 3.89 (2H,m), 4.1-4.2 (4H), 6.2-7.1 (5H), 7.4-7.9 (4H), 7.33 (2H,d,J=8Hz), 7.79 (2H,d,J=8Hz)
4, m + n = 8		3.03 (6H)*	2.44 (3H,s), 3.5-3.8 (26H), 3.89 (2H,m), 4.1-4.3 (4H), 6.2-7.1 (5H), 7.4-7.9 (8H)
4, m + n = 10		3.03 (6H)*	2.44 (3H,s), 3.5-3.8 (32H), 3.89 (2H,m), 4.12 (2H,q,J=6Hz), 4.1-4.2 (4H), 6.2-7.1 (5H), 7.4-7.9 (4H), 7.34 (2H,d,J=8Hz), 7.80 (2H,d,J=8Hz)
5b, m + n = 7	-75.54 (t,9Hz)	3.03 (6H)*	3.6-3.8 (28H), 3,8-3.9 (2H), 3.90 (2H,q,J=9Hz), 4.17 (2H,q,J=5Hz), 6.2-7.1 (5H), 7.4-7.9 (4H)
5b, m + n = 8	-75.55 (t,J=9Hz)	3.03 (6H)*	3.5-3.8 (32H), 3.88 (2H,m), 3.91 (2H,q,J=9Hz), 4.17 (2H,q,J=5Hz), 6.2-7.1 (5H), 7.4-7.9 (4H)
5b, m + n = 10	-75.56 (t,J=9Hz)	3.03 (6H)*	3.5-3.9 (40H), 3.85-3.95 (2H), 3.91 (2H,q,J=9Hz), 4.17 (2H,q,J=5Hz), 6.2-7.1 (5H), 7.4-7.9 (4H)

5b, m + n = 12	-75.56 (t,J=9Hz)	3.03 (6H)*	3.5-4.0 (50H), 3.93 (2H,q,J=9Hz), 4.17 (2H,q,J=5Hz), 6.2-7.1 (5H), 7.4-7.9 (4H)
5c, m + n = 8	-75.52 (d,J=6Hz)	3.03 (6H)*	3.5-3.8 (26H), 3.89 (2H,m), 3.99 (2H,m), 4.17 (2H,m), 4.51 (1H,sep,J=6Hz), 6.2-7.1 (5H), 7.4-7.9 (4H)

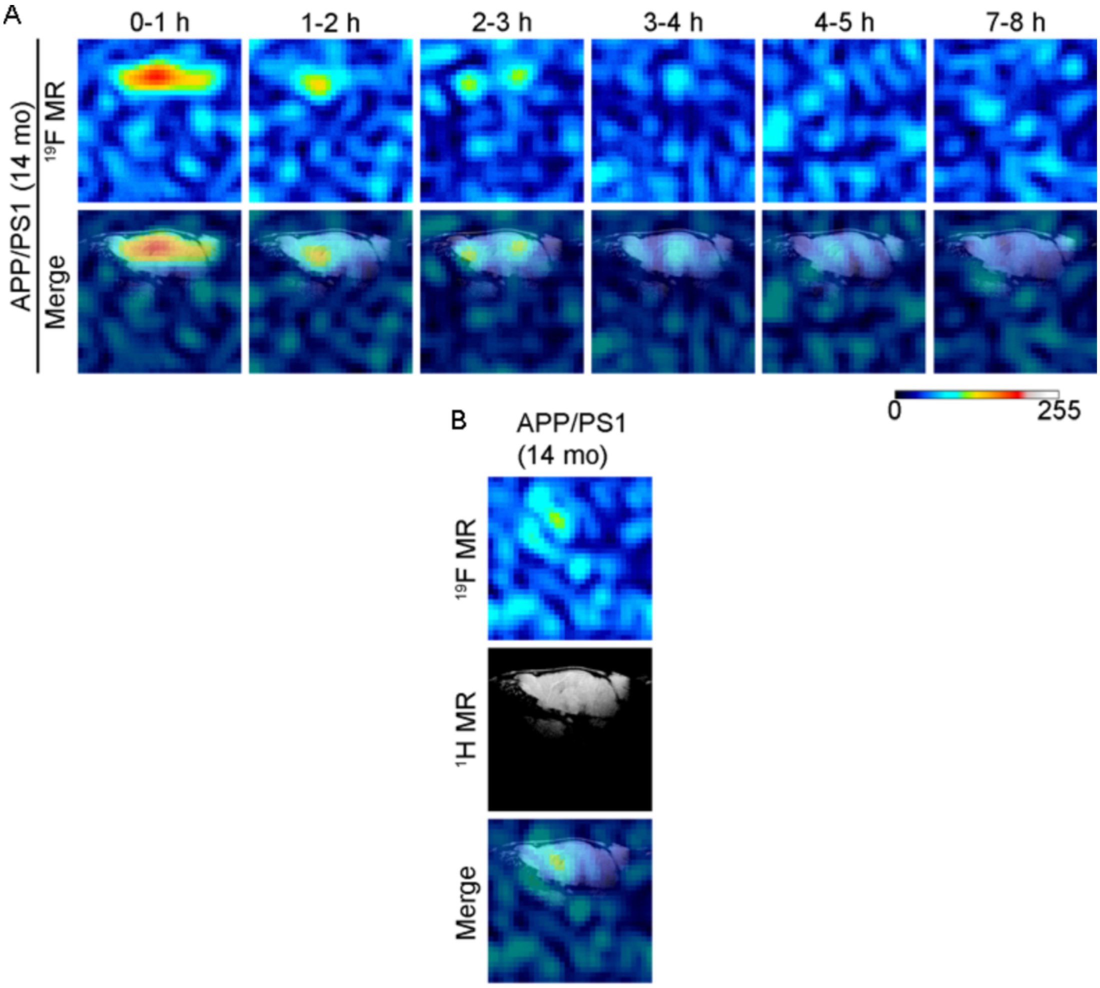
*The signal at δ3.03 was accompanied by a singlet peak at δ3.04, because the products were obtained as a mixture of trans and cis isomers. Hence, the signals in the aromatic region showed a complex pattern.

Supplementary Figure 1



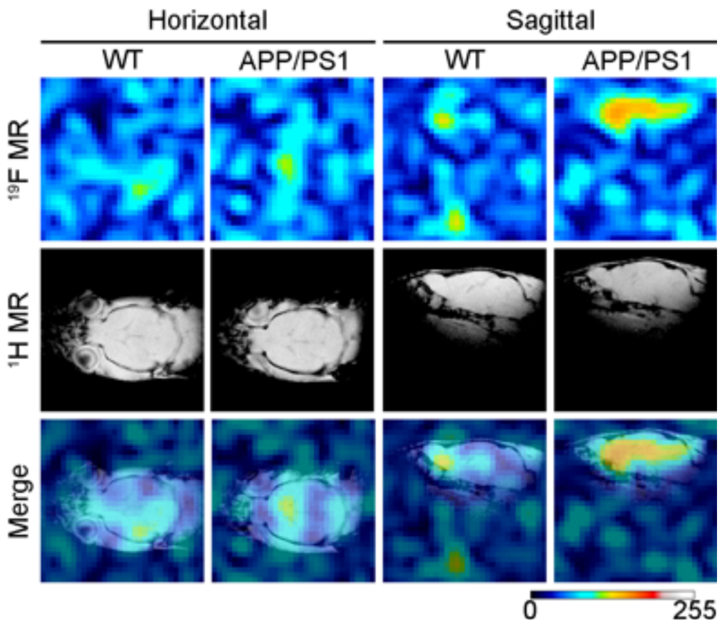
Ex vivo labeling of amyloid plaques with intravenously injected compounds. Fluorescence microscopic analysis was performed in sagittal brain sections of AβPPswe/PS1dE9 double-transgenic mice that received an intravenous injection of compound 3b (m = 4) (A-C), compound 3b (m = 5) (D-F), compound 3b (m = 6) (G-I), compound 5b (m + n = 10) (J-L) or compound 5b (m + n = 12) (M-O). Fluorescence of compounds and immunoreactivity for amyloid-β is shown as green (A, D, G, J, M) and red (B, E, H, K, N), respectively. Merged images (C, F, I, L, O). Scale bars are 1 mm (A).

Supplementary Figure 2



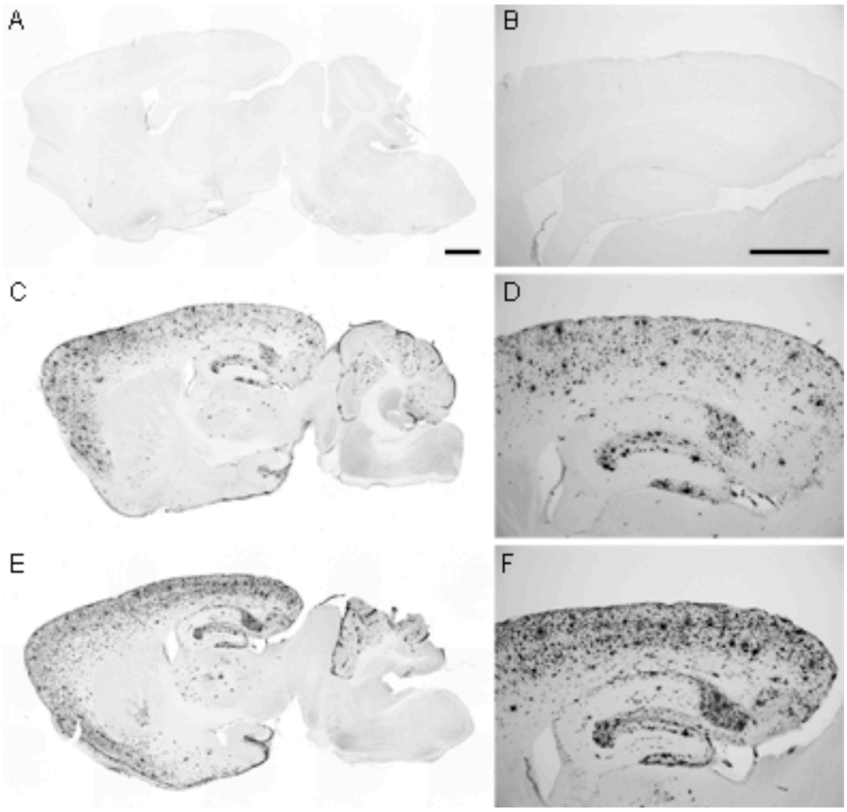
Fluorine-19 magnetic resonance (^{19}F MR) images in living 14-month-old AβPPswe/PS1dE9 double-transgenic (AβPP/PS1) mice that were injected with compound 3b (m = 6). (A) Time course of changes in ^{19}F MR signals after injection. (B) ^{19}F MR image constructed by adding the data collected from 3 h to 8 h after the intravenous injection.

Supplementary Figure 3



Fluorine-19 magnetic resonance (^{19}F MR) images of the brain of compound 3b (m = 6)-injected wild-type (WT) and AβPPswe/PS1dE9 double-transgenic (AβPP/PS1) mice that were perfused with saline 3 h post-injection.

Supplementary Figure 4



Distribution pattern of amyloid deposits in AβPPswe/PS1dE9 double-transgenic (AβPP/PS1) mice. Representative photographs show brain sections immunostained with anti-amyloid-β antibody in wild-type mice (A, B) and AβPP/PS1 mice at 14 months (C, D) and 20 months (E, F) of age. Scale bars are 1 mm (A, B).