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Technetium-99m Labeled Pyridyl Benzofuran Derivatives as Single Photon Emission Computed Tomography Imaging Probes for β-Amyloid Plaques in Alzheimer's Brains

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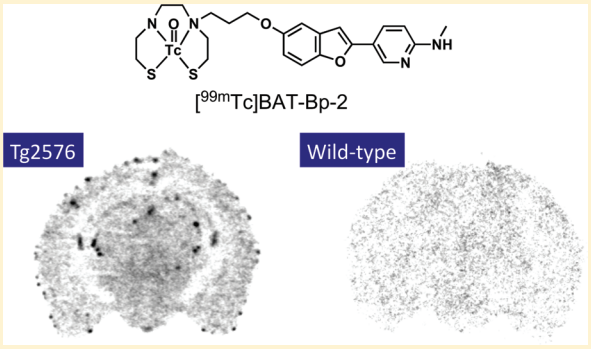
ABSTRACT: Three novel ^{99m}Tc-labeled pyridyl benzofuran derivatives were tested as potential probes for imaging β-amyloid plaques using single photon emission computed tomography (SPECT). The ^{99m}Tc and corresponding rhenium complexes were synthesized with bis(aminoethanethiol) (BAT) as a chelating ligand. All Re complexes showed affinity for Aβ(1−42) aggregates (K_i = 13.6−149.6 nM). Biodistribution experiments in normal mice revealed that the ^{99m}Tc-labeled derivatives displayed sufficient uptake in the brain (1.41−1.80% ID/g at 2 min postinjection). Notably, [^{99m}Tc]BAT-Bp-2 showed a good initial uptake (1.80% ID/g at 2 min) and a reasonable washout from the brain (0.79% ID/g at 60 min). Ex vivo autoradiography with [^{99m}Tc]BAT-Bp-2 revealed substantial labeling of β-amyloid plaques in sections of brain tissue from Tg2576 transgenic mice but not in the age-matched controls. [^{99m}Tc]BAT-Bp-2 may be a potential SPECT probe for imaging β-amyloid plaques in Alzheimer's brains.



INTRODUCTION

Alzheimer's disease (AD) is an age-related brain disorder with the symptoms of memory loss and dementia. Senile plaques (SPs) and neurofibrillary tangles (NFTs), which were first identified in the post-mortem brain of a demented patient by Alois Alzheimer in 1907, have been regarded as the most defining hallmarks of AD.[1] SPs consist of β-amyloid peptides (Aβ) that aggregate into fibrillar, β-pleated sheet structures.[1−3] As β-amyloid plaques deposit years before the onset of AD, their detection in vivo may facilitate the diagnosis of β-amyloidosis in the brain, improve monitoring of the progression of AD, and increase the effectiveness of antiamyloid therapies currently under intense development throughout the world.[1,3]

Over the past several years, remarkable progress in the imaging of β-amyloid plaques in vivo with noninvasive techniques such as positron emission tomography (PET) has brought promise to the early clinical diagnosis of AD. On the basis of the structure of the amyloid-staining agent Thioflavin T or Congo red, several PET imaging probes have been designed and evaluated for imaging β-amyloid plaques such as [^{11}C]4-*N*-methylamino-4′-hydroxystilbene (SB-13),[4,5] [^{11}C]2-(4′-(methylaminophenyl)-6-hydroxybenzothiazole (PIB),[6,7] (2-(3-[^{18}F]-fluoro-4-methyamino-phenyl)benzothiazol-6-ol (GE-067, flutemetamol),[8] (*E*)-4-(*N*-methylamino)-4′-(2-(2-(2-[^{18}F]-fluoroethoxy)ethoxy)ethoxy)-stilbene (BAY94−9172, florbetaben),[9,10] and (*E*)-4-(2-(6-(2-(2-(2-([^{18}F]-fluoroethoxy)-ethoxy)ethoxy)pyridyn-3-ylvinyl)-*N*-methyl benzenamine (AV-45, florbetapir).[10,11] Although these preliminary studies showed promising results, routine clinical use might be hampered by the compounds' short half-lives, high costs, and limited applicability (radioactive nuclide production from a cyclotron, PET camera). More suitable Aβ imaging probes are needed for potential clinical use.

Among medical radioisotopes, ^{99m}Tc ($T_{1/2}$ = 6.01 h, 141 keV) has been most commonly used in diagnostic imaging by single photon emission computed tomography (SPECT) for several reasons: it is readily produced by a ^{99}Mo/^{99m}Tc generator and its physical half-life is compatible with the biological localization and residence time required for imaging. Thus, the development of ^{99m}Tc-labeled Aβ imaging probes will provide simple, convenient, and cost-effective methods for the detection of cerebral β-amyloid plaques in potentially pre-AD conditions such as in cognitively normal elderly subjects and in mild cognitive impairment (MCI) subjects long before the diagnosis of AD.

Initially, ^{99m}Tc-labeled Congo red and chrysamine G derivatives were evaluated as potential Aβ imaging probes, but these large, charged molecules failed to penetrate the blood−brain barrier (BBB).[12,13] Smaller, neutral ^{99m}Tc-labeled ligands have since been reported, such as derivatives of biphenyl,[14] benzothiazle aniline (BTA),[15] chalcone,[16] flavone, aurone,[17] and curcumin[18] (Figure 1), but none of them showed specific binding to β-amyloid plaques in vivo due to either low affinity or low uptake in the brain.

Previously, we designed a series of ^{18}F-labeled benzofuran derivatives and evaluated their biological potential for imaging β-amyloid plaques in AD.[19−21] One of the pyridyl benzofuran derivatives, [^{18}F]FPYBF-2, showed selective affinity for Aβ and good pharmacokinetics in the brain (Figure 2). On the basis of

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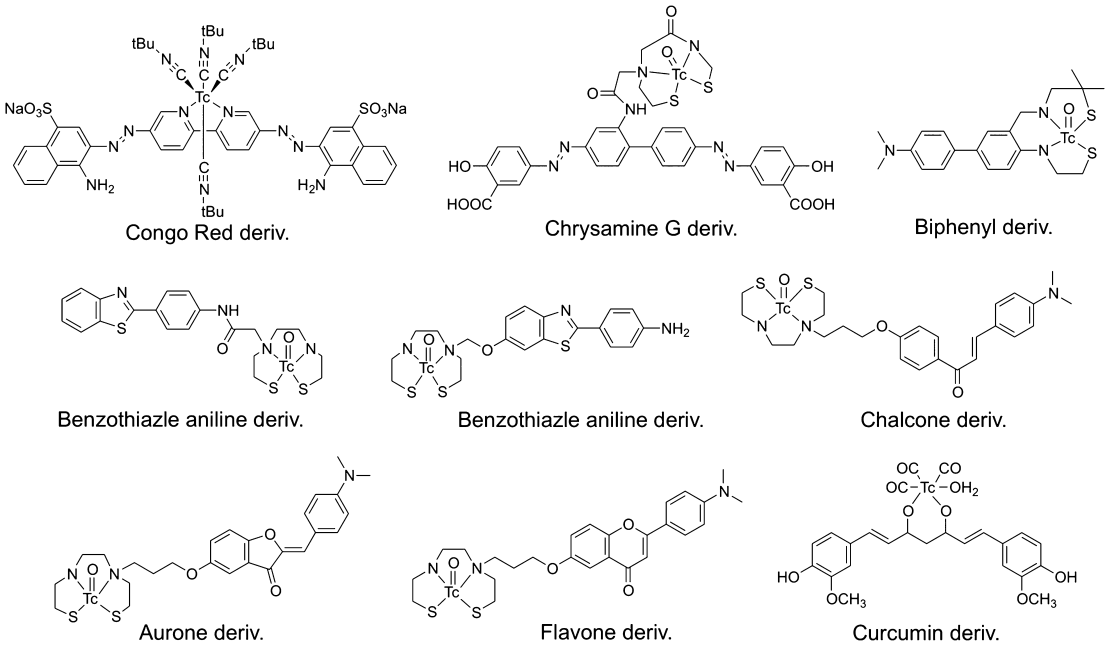

Figure 1. Chemical structure of ^{99m}Tc-labeled Aβ imaging probes reported previously.

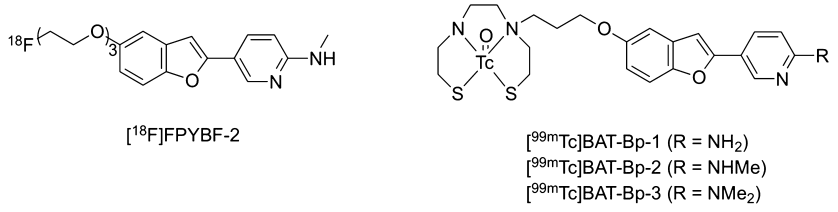

Figure 2. Chemical structure of [^{18}F]FPYBF-2 and ^{99m}Tc-labeled pyridyl benzofuran derivatives reported in the present study.

this encouraging result, we attempted to design novel ^{99m}Tc-labeled Aβ imaging probes with the backbone of pyridyl benzofuran. Different from ^{18}F, a chelating structure is necessary for the transition of metal ^{99m}Tc to an organic molecule. In consideration of the permeability of the BBB, we chose bis(aminoethanethiol) (BAT) as a compact chelating ligand to form a neutral complex with ^{99m}Tc (Figure 2).[16,17,22]

The aim of this study was to synthesize ^{99m}Tc/Re complexes based on pyridyl benzofuran with BAT as the chelating ligand and to study their binding to Aβ and pharmacokinetics in the brain. Herein we report the synthesis and biological evaluation of these novel ^{99m}Tc-labeled pyridyl benzofuran derivatives as potential SPECT agents for imaging β-amyloid plaques in AD.

RESULTS AND DISCUSSION

The synthesis of the ^{99m}Tc/Re pyridyl benzofuran derivatives is outlined in Schemes 1, 2, and 3. The key step in the formation of the pyridyl benzofuran backbone was accomplished by Suzuki coupling between 5-methoxybenzofuran-2-boronic acid and 2-amino-5-iodopyridine.[23] Suzuki coupling afforded the desired compound **1** in a yield of 52.1%. Conversion of **1** to the corresponding monomethylamino derivative **3** was achieved by monomethylation with paraformaldehyde and NaOMe (yield 92.1%).[24] Conversion of **1** to the corresponding dimethylamino derivative **9** was achieved by dimethylation with paraformaldehyde and sodium cyanoborohydride (yield 62.0%).[25] A methoxy group of **1**, **3**, and **9** was converted to a hydroxyl group using BBr_3/CH_2Cl_2, which afforded **2**, **4**, and **10** in yields of 98.0%, 99.0%, and 98.9%, respectively.[24] The thiol-protected chelation ligand (TRT-Boc-BAT) was synthesized according to methods reported previously.[16] TRT-Boc-BAT-Br was then synthesized by reacting TRT-Boc-BAT with 1,3-dibromopropane (yield 53.0%). A BAT group was introduced into **2** or **4** by reacting them with TRT-Boc-BAT-Br (**5**, yield 75.2%; **7**, yield 70.3%). A trimethylene group was introduced into **10** as a linker by reacting it with 1,3-dibromopropane (**11**, yield 73.2%) and the chelating ligand BAT was then conjugated with **11** (**12**, yield 45.0%).[16,17] As there is no stable technetium isotope, rhenium, the congener of technetium, has been widely used as a nonradioactive surrogate for the structural identification of technetium complexes.[14,16,17,26,27] Then, we tried to prepare the Re complexes. After deprotection of the thiol groups in **5**, **7**, or **12** in TFA and triethylsilane, the Re complex (**6**, **8**, or **13**) was prepared through a reaction with $(PPh_3)_2ReOCl_3$.

The corresponding ^{99m}Tc complex, **6′** ([^{99m}Tc]BAT-Bp-1), **8′** ([^{99m}Tc]BAT-Bp-2), or **13′** ([^{99m}Tc]BAT-Bp-3), was prepared by a ligand exchange reaction employing the precursor **5**, **7**, or **12** and ^{99m}Tc-glucoheptonate (GH) (Scheme 3).[16,17] The resulting mixture was analyzed by reversed-phase HPLC, showing that a single radioactive complex formed with radiochemical purity higher than 99% after purification by HPLC. The radiochemical identity of the ^{99m}Tc complex was verified by comparative HPLC by using the corresponding Re complex as a reference. The retention times for **6′** ([^{99m}Tc]-BAT-Bp-1), **8′** ([^{99m}Tc]BAT-Bp-2), and **13′** ([^{99m}Tc]BAT-Bp-3) on HPLC (radioactivity) were 9.1, 20.9, and 29.0 min,

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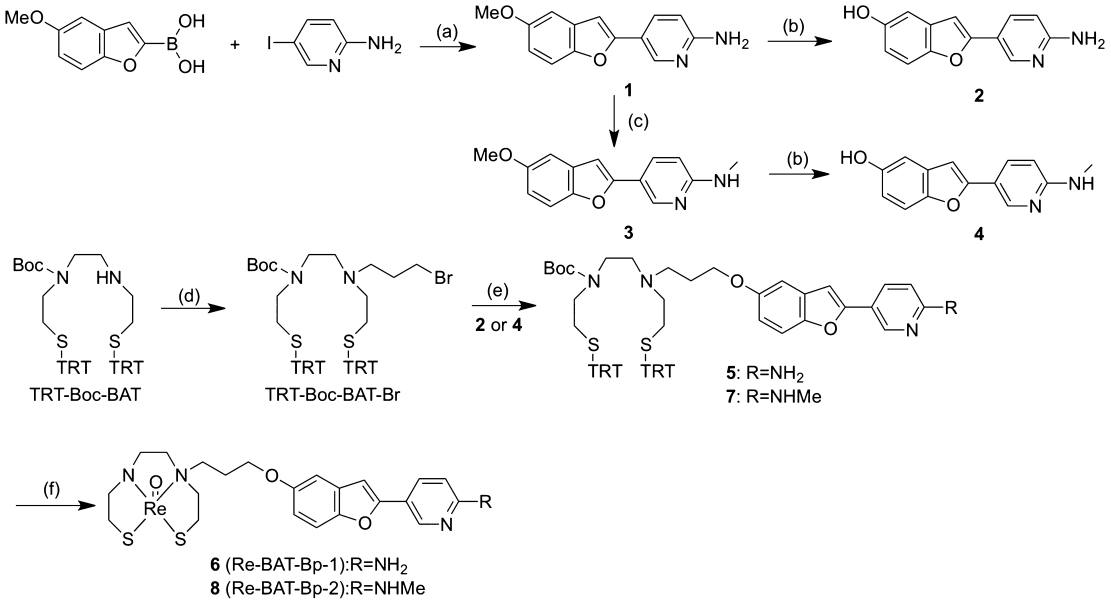
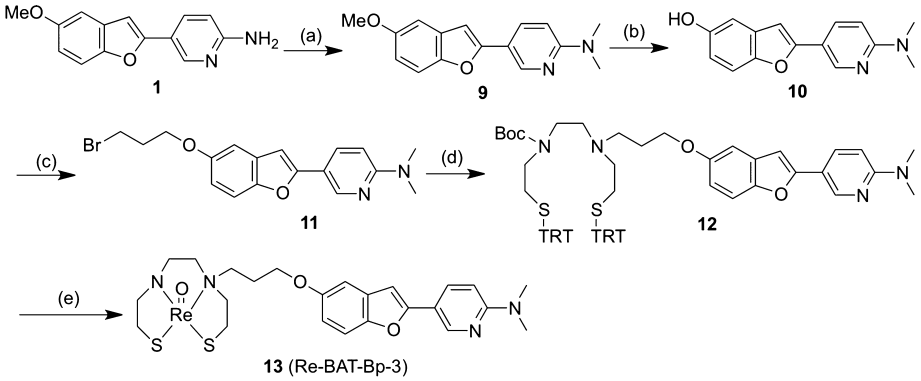
Scheme 1[a]



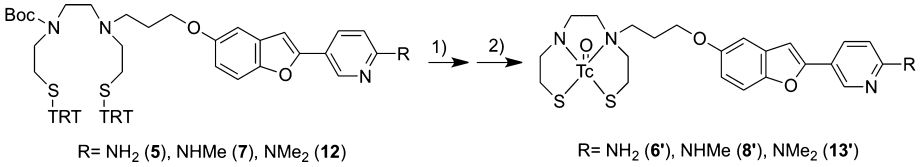
[a]Reagents and conditions: (a) $Pd(Ph_3P)_4$, 2 M Na_2CO_3 (aq)/dioxane; (b) BBr_3, CH_2Cl_2; (c) (1) NaOMe, MeOH, $(CHO)_n$, (2) $NaBH_4$; (d) $Br(CH_2)_3Br$, CH_3CN, K_2CO_3; (e) Cs_2CO_3, acetone, reflux; (f) (1) TFA, Et_3SiH, (2) $(Ph_3P)_2ReOCl_3$, AcONa, CH_2Cl_2/MeOH.

Scheme 2[a]



[a]Reagents and conditions: (a) $(CHO)_n$, $NaBH_3CN$, AcOH; (b) BBr_3, CH_2Cl_2; (c) $Br(CH_2)_3Br$, CH_3CN, K_2CO_3; (d) TRT-Boc-BAT, CH_3CN, DIPEA; (e) (1) TFA, Et_3SiH, (2) $(Ph_3P)_2ReOCl_3$, AcONa, CH_2Cl_2/MeOH.

Scheme 3[a]



[a]Reagents and conditions: (1) TFA, Et_3SiH; (2) CH_3CN, 0.1 N HCl, ^{99m}Tc-glucoheptonate.

respectively. The retention times of the corresponding Re complexes on HPLC (UV detection) were 8.2, 19.4, and 28.1 min, respectively. The proximate retention times between ^{99m}Tc-labeled tracers and the corresponding Re complexes suggest that the desired ^{99m}Tc-labeled pyridyl benzofuran derivatives were successfully synthesized. Two stereoisomers are known to be generated in ^{99m}Tc complexation reactions of *N*-alkylated BAT complexes.[12,28−30] Although we have not carried out structural analyses by X-ray crystallography of the corresponding rhenium complexes in this study, we presume the major product to be a *syn* isomer because: (1) a single peak of radioactivity was observed in the reaction mixture after ^{99m}Tc-labeling, and (2) most known examples of ^{99m}Tc/Re complexes of this type are predominantly produced as a *syn* isomer.[12,30]

Binding affinity for Aβ aggregates was evaluated with rhenium complexes **6** (Re-BAT-Bp-1), **8** (Re-BAT-Bp-2), and **13** (Re-BAT-Bp-3) according to conventional methods using [^{125}I] 6-iodo-2-(4′-dimethylamino)phenyl-imidazo[1,2-*a*]-pyridine (IMPY).[31,32] Compounds **6**, **8**, and **13** all inhibited the binding of [^{125}I]IMPY in a dose-dependent manner, with

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K_i values of 149.6, 32.8, and 13.6 nM, respectively (Table 1). The affinity for Aβ aggregates increased in the order of **13** > **8** > **6**.

Table 1. Inhibition Constants for the Binding of [^{125}I]IMPY to Aβ(1−42) Aggregates

compd	K_i (nM)[a]
Re-BAT-Bp-1 (**6**)	149.6 ± 34.4
Re-BAT-Bp-2 (**8**)	32.8 ± 5.80
Re-BAT-Bp-3 (**13**)	13.6 ± 1.60
IMPY[b]	10.5 ± 1.05
PIB[b]	9.00 ± 1.31

[a]Values are the means ± standard errors of the mean of three independent determinations. [b]Data from ref 21.

The result of the binding study was consistent with that of previous reports,[33−36] indicating that the affinity increased in the order of the *N*,*N*-dimethylated derivative > *N*-monomethylated derivative > primary amino derivative. The binding affinity of the *N*,*N*-dimethylated derivative (**13**) and *N*-monomethylated derivative (**8**) was close to that of PIB or IMPY, indicating that they possess sufficient affinity for the imaging of Aβ(1−42) aggregates in vivo. However, the K_i values of these Re complexes were higher than those of fluorinated benzofuran derivatives reported previously.[19−21,24,32] This result suggests that the introduction of the bulky BAT into the pyridyl benzofuran backbone interferes with the binding to Aβ(1−42) aggregates.

To evaluate the pharmacokinetics of ^{99m}Tc-labeled pyridyl benzofuran complexes in the brain, biodistribution experiments were performed in normal mice (Table 2). A biodistribution study provides critical information on penetration of the BBB. Previous papers reported that the optimal measured log *P* value of compounds ranged from 0.1 to 3.5.[1,37] The log *P* values for **6**′ ([^{99m}Tc]BAT-Bp-1), **8**′ ([^{99m}Tc]BAT-Bp-2), and **13**′ ([^{99m}Tc]BAT-Bp-3) were 0.68, 1.35, and 2.09, respectively, indicating that these complexes should penetrate the BBB. As expected, the three ^{99m}Tc-labeled complexes showed uptake into the brain within 10 min ([^{99m}Tc]BAT-Bp-1 and [^{99m}Tc]-BAT-Bp-2 peaked at 2 min postinjection while [^{99m}Tc]BAT-Bp-3 peaked at 10 min), and the radioactivity of all complexes in the brain cleared with time. Among the three, [^{99m}Tc]BAT-Bp-2 showed the highest initial uptake at 2 min postinjection (1.80% ID/g). Although the value was much lower than that of [^{11}C]PIB (7.0% ID/g),[7] [^{18}F]AV-45 (7.33% ID/g),[10,11] or [^{123}I]IMPY (2.88% ID/g),[38] which are currently under clinical trials, it was superior to that of any other ^{99m}Tc-labeled tracer for Aβ imaging reported previously (0.2−1.48% ID/g).[12−18,26]

Table 2. Biodistribution of Radioactivity after Injection of [^{99m}Tc]Tracers in Normal Mice[a]

organ	2 min	10 min	30 min	60 min
		[^{99m}Tc]BAT-Bp-1		
brain	1.59 (0.21)	1.02 (0.03)	0.89 (0.07)	0.97 (0.13)
blood	5.59 (0.53)	2.52 (0.15)	1.83 (0.18)	1.62 (0.24)
liver	15.98 (2.98)	26.01 (3.20)	24.16 (3.56)	22.58 (4.16)
kidney	13.24 (1.17)	13.03 (1.11)	7.96 (1.15)	5.56 (0.87)
pancreas	5.59 (0.58)	5.20 (0.24)	2.83 (0.37)	1.93 (0.29)
spleen	3.54 (0.32)	2.90 (0.16)	1.99 (0.27)	1.51 (0.23)
stomach[b]	1.52 (0.16)	2.89 (0.31)	5.02 (0.46)	5.38 (1.02)
intestine[b]	5.72 (0.57)	12.44 (0.72)	28.94 (2.00)	41.59 (4.31)
lung	11.89 (2.81)	5.33 (0.83)	3.19 (0.36)	2.84 (0.64)
heart	9.83 (1.66)	3.41 (0.16)	1.88 (0.31)	1.35 (0.20)
		[^{99m}Tc]BAT-Bp-2		
brain	1.80 (0.16)	1.30 (0.07)	0.99 (0.09)	0.79 (0.04)
blood	6.01 (0.47)	3.09 (0.13)	2.10 (0.22)	1.60 (0.30)
liver	19.14 (3.78)	30.57 (2.22)	31.29 (2.24)	27.08 (1.11)
kidney	13.96 (1.10)	12.55 (0.72)	8.20 (0.57)	5.62 (0.63)
pancreas	4.36 (0.49)	4.54 (0.13)	2.69 (0.26)	1.55 (0.28)
spleen	3.93 (0.51)	3.80 (0.23)	2.44 (0.23)	1.78 (0.32)
stomach[b]	1.50 (0.24)	3.68 (1.05)	6.06 (0.72)	7.86 (2.14)
intestine[b]	5.60 (0.38)	10.53 (1.02)	23.91 (2.98)	33.24 (2.61)
lung	12.23 (0.94)	6.01 (0.73)	3.98 (0.40)	3.14 (0.61)
heart	13.94 (1.41)	4.12 (0.28)	2.23 (0.37)	1.42 (0.18)
		[^{99m}Tc]BAT-Bp-3		
brain	1.41 (0.17)	1.64 (0.27)	1.08 (0.12)	0.79 (0.07)
blood	8.11 (2.00)	3.90 (0.41)	3.50 (0.80)	1.69 (0.21)
liver	33.85 (4.81)	38.71 (4.35)	37.30 (2.61)	34.75 (5.47)
kidney	19.32 (1.13)	14.61 (1.93)	11.62 (0.93)	6.74 (0.69)
pancreas	6.61 (1.02)	7.16 (0.66)	4.04 (0.70)	3.18 (1.90)
spleen	6.53 (1.70)	5.82 (0.64)	4.15 (0.76)	2.48 (0.32)
stomach[b]	2.78 (0.45)	6.13 (1.22)	11.62 (1.79)	11.90 (2.51)
intestine[b]	6.79 (0.31)	14.70 (4.01)	34.88 (1.83)	45.93 (5.12)
lung	19.17 (2.93)	9.98 (3.06)	6.97 (0.78)	3.87 (0.34)
heart	19.24 (2.77)	7.43 (0.93)	4.03 (0.51)	1.99 (0.18)

[a]Expressed as % injected dose per gram. Each value represents the mean (SD) for five animals at each interval. [b]Expressed as % injected dose per organ.

The brain$_{2min}$/brain$_{60min}$ ratio is generally used as an index for evaluating radioactivity pharmacokinetics in vivo. The brain$_{2min}$/brain$_{60min}$ ratio of [^{99m}Tc]BAT-Bp-1, [^{99m}Tc]BAT-Bp-2, and [^{99m}Tc]BAT-Bp-3 was 1.64, 2.28, and 1.78, respectively, indicating that [^{99m}Tc]BAT-Bp-2 provided the best profile of radioactivity in the brain among not only the three ligands tested here but also all ^{99m}Tc ligands reported previously.[13−17,26] Although the brain$_{2min}$/brain$_{60min}$ ratio of [^{99m}Tc]BAT-Bp-2 was lower than that of [^{11}C]PIB (11.7),[6] [^{18}F]AV-45 (3.89),[11] and [^{123}I]IMPY (14.4),[38] it was similar to that of [^{18}F]FPYBF-2 (2.32), which was previously reported as a ^{18}F-labeled pyridyl benzofuran derivative.[21] On the basis of the results of binding affinity in vitro and biodistribution in normal mice, [^{99m}Tc]BAT-Bp-2 was further evaluated for binding to β-amyloid plaques in Tg2576 transgenic mice.

Next, ex vivo autoradiography was carried out using Tg2576 transgenic mice (27-month-old) and wild-type mice (27-month-old) as age-matched controls (Figure 3). Tg2576 transgenic mice have been widely used for in vitro and in vivo evaluations of Aβ imaging agents because of marked Aβ deposition in the brain.[39] The brain was removed at 30 min postinjection for autoradiography. Autoradiographic images revealed extensive labeling of β-amyloid plaques in the transgenic mice (Figure 3A) but not the age-matched controls (Figure 3B). Furthermore, we confirmed that the radioactive spots of [^{99m}Tc]BAT-Bp-2 corresponded with those of thioflavin-S staining in vitro in the same brain sections (Figure 3C), while there was no marked staining in the sections of wild-type mouse brain. The results suggest that [^{99m}Tc]BAT-Bp-2 had sufficient binding affinity to image β-amyloid plaques in vivo, although [^{99m}Tc]BAT-Bp-2 displayed lower affinity than [^{18}F]FPYBF-2.[21] However, some β-amyloid plaques, which were not labeled with [^{99m}Tc]BAT-Bp-2 in the brain sections, were also observed. This may be attributable to the lower brain uptake and lower affinity for β-amyloid plaques of [^{99m}Tc]BAT-Bp-2 than [^{18}F]FPYBF-2.

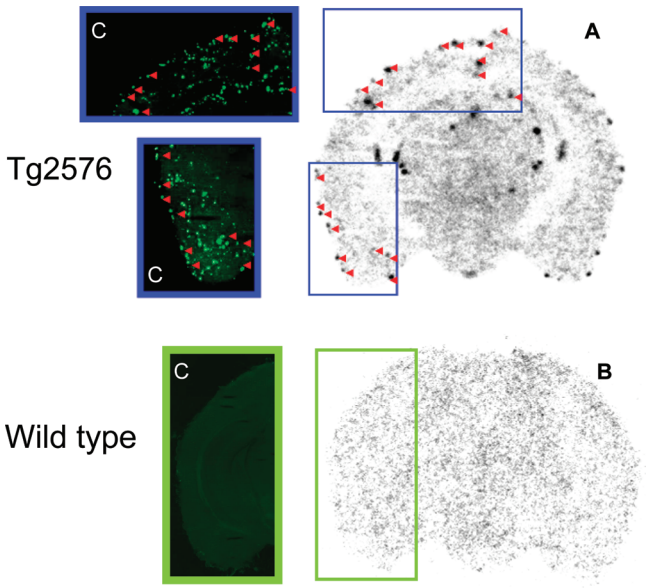

Figure 3. Labeling of β-amyloid plaques was visualized by autoradiography ex vivo with [^{99m}Tc]BAT-Bp-2 in brain sections of Tg2576 (A) and wild-type (B) mice. The same section was also stained with thioflavin-S (C), a pathological dye commonly used for staining β-amyloid plaques .

This is the first report that a ^{99m}Tc-labeled probe has successfully detected β-amyloid plaques in vivo. Therefore, [^{99m}Tc]BAT-Bp-2 should be investigated further as a potentially useful β-amyloid imaging probe.

CONCLUSION

In conclusion, three ^{99m}Tc-labeled pyridyl benzofuran derivatives and their corresponding rhenium complexes were successfully synthesized. All complexes showed affinity for Aβ in vitro and good uptake in the brain. Among them, [^{99m}Tc]BAT-Bp-2 not only displayed the highest initial uptake in the brain and a reasonable washout from the brain, but also showed extensive labeling of β-amyloid plaques in vivo. Taken together, [^{99m}Tc]BAT-Bp-2 may be a potential SPECT probe for imaging β-amyloid plaques in Alzheimer's brains.

EXPERIMENTAL SECTION

General Remarks. All chemicals used in synthesis were commercial products used without further purification. ^{1}H NMR spectra were obtained at 400 MHz on JEOL JNM-AL400 NMR spectrometers at room temperature with TMS as an internal standard. Chemical shifts are reported as δ values relative to the internal TMS. Coupling constants are reported in hertz. Multiplicity is defined by s (singlet), d (doublet), t (triplet), and m (multiplet). Mass spectra were acquired with a Shimadzu GC-MS-QP2010 Plus (ESI). HPLC was performed with a Shimadzu system (a LC-10AT pump with a SPD-10A UV detector, λ = 254 nm) with a Cosmosil C18 column (Nacalai Tesque, 5C$_{18}$-AR-II, 4.6 mm × 150 mm) using a mobile phase (water/acetonitrile: 0 min 3/2 to 30 min 3/7) delivered at a flow rate of 1.0 mL/min. Fluorescent observation was performed by microscope (Nikon Eclipse 80i) with a BV-2A filter set (excitation, 400−440 nm; diachronic mirror, 455 nm; long pass filter, 470 nm). All key compounds were proven to show ≥99% purity by HPLC.

Chemistry. *5-(5-Methoxybenzofuran-2-yl)pyridin-2-amine (**1**).* A solution of 5-methoxybenzofuran-2-boronic acid (576 mg, 3.0 mmol), 2-amino-5-iodopyridine (660 mg, 3.0 mmol), and Pd(Ph$_3$P)$_4$ (366 mg, 0.3 mmol) in 2 M Na$_2$CO$_3$ (aq)/dioxane (1:1, 150 mL) was stirred overnight under reflux. The mixture was allowed to cool to room temperature, and 1 M NaOH (20 mL) was added. After extraction with ethyl acetate, the organic phase was dried over Na$_2$SO$_4$ and filtered. The filtrate was concentrated, and the residue was purified by silica gel chromatography (hexane:ethyl acetate = 1:1) to give 374 mg of **1** (52.1%). ^{1}H NMR (400 MHz, CDCl$_3$): δ 3.85 (s, 3H), 4.67 (s, 2H), 6.59 (d, 1H, J = 8.8 Hz), 6.76 (s, 1H), 6.82 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.4 Hz), 7.07 (d, 1H, J = 2.4 Hz), 7.36 (d, 1H, J = 8.8 Hz), 7.86 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.4 Hz), 8.67 (d, 1H, J = 2.4 Hz). MS: m/z 241 (M$^+$ + H).

*2-(6-Aminopyridin-3-yl)benzofuran-5-ol (**2**).* BBr$_3$ (8.0 mL, 1 M solution in CH$_2$Cl$_2$) was added to a solution of **1** (370 mg, 1.54 mmol) in CH$_2$Cl$_2$ (20 mL) dropwise in an ice bath. The mixture was allowed to warm to room temperature and stirred for 1 h. Water (20 mL) was added while the reaction mixture was cooled in an ice bath. After extraction with ethyl acetate, the organic phase was dried over Na$_2$SO$_4$ and filtered. The filtrate was concentrated, and the residue was purified by silica gel chromatography (hexane:ethyl acetate = 1:1) to give 221 mg of **2** (98.0%). ^{1}H NMR (400 MHz, CDCl$_3$): δ 4.67 (s, 2H), 6.59 (d, 1H, J = 8.8 Hz), 6.76 (s, 1H), 6.82 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.4 Hz), 7.07 (d, 1H, J = 2.4 Hz), 7.36 (d, 1H, J = 8.8 Hz), 7.86 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.4 Hz), 8.67 (d, 1H, J = 2.4 Hz). MS: m/z 227 (M$^+$ + H).

*5-(5-Methoxybenzofuran-2-yl)-N-methylpyridin-2-amine (**3**).* Sodium methoxide (275 mg, 5.0 mmol) was added to **1** (240 mg, 1.0 mmol) in methanol (15 mL) followed by paraformaldehyde (101 mg, 4.0 mmol). The solution was heated to reflux for 2 h and cooled to 0 °C with an ice bath. Sodium borohydride (128 mg, 4.0 mmol) was added. The reaction mixture was brought to reflux again for 1 h and poured onto crushed ice. After a standard workup with ethyl acetate,

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the residue was purified by silica gel chromatography (hexane:ethyl acetate = 1:1) to give 234 mg of **3** (92.1%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.98 (d, 3H, J = 5.2 Hz), 3.84 (s, 3H), 4.78 (s, 1H), 6.47 (d, 1H, J = 8.8 Hz), 6.76 (s, 1H), 6.84 (dd, 1H, J_1 = 8.8 Hz, J_2 = 4.4 Hz), 7.00 (d, 1H, J = 2.4 Hz), 7.36 (d, 1H, J = 8.4 Hz), 7.87 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.4 Hz), 8.59 (d, 1H, J = 2.8 Hz). MS: m/z 255 (M^+ + H).

*2-(6-(Methylamino)pyridin-3-yl)benzofuran-5-ol (**4**).* The same reaction as described above to prepare **2** was used, and 218 mg of **4** was obtained in a 99.0% yield from **3**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.98 (d, 3H, J = 5.2 Hz), 4.78 (s, 1H), 6.47 (d, 1H, J = 8.8 Hz), 6.76 (s, 1H), 6.84 (dd, 1H, J_1 = 8.8 Hz, J_2 = 4.4 Hz), 7.00 (d, 1H, J = 2.4 Hz), 7.36 (d, 1H, J = 8.4 Hz), 7.87 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.4 Hz), 8.59 (d, 1H, J = 2.8 Hz). MS: m/z 241 (M^+ + H).

tert-Butyl 2-((3-Bromopropyl)(2-(tritylthio)ethyl)amino)ethyl (2-(tritylthio)ethyl)carbamate (TRT-Boc-BAT-Br). To a solution of TRT-Boc-BAT[18] (399.8 mg, 0.52 mmol) in CH_3CN (20 mL) were added K_2CO_3 (200 mg, 1.45 mmol) and 1,3-dibromopropane (110 μL, 1.0 mmol). The mixture was heated to reflux for 18 h, and after cooling to room temperature, evaporated dry. The residue was redissolved in $CHCl_3$ and washed with brine. The organic layers were dried with Na_2SO_4 and evaporated dry. The crude product was chromatographed on silica gel (ethyl acetate:hexane = 3:7) to give 244 mg of desired compound (53.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (s, 9H), 1.71–1.73 (m, 2H), 2.25–2.42 (m, 10H), 2.87–3.03 (m, 4H), 3.90–3.93 (m, 2H), 7.14–7.29 (m, 19H), 7.38–7.40 (m, 12H). MS: m/z 887 (M^+ + H).

*tert-Butyl 2-((3-(2-(6-Aminopyridin-3-yl)benzofuran-5-yloxy)-propyl)(2-(tritylthio)ethyl)amino)ethyl(2-(tritylthio)ethyl)carbamate (**5**).* To a solution of **2** (18 mg, 0.08 mmol) and Boc-BAT-Br (70 mg, 0.08 mmol) in acetone (10 mL) was added cesium carbonate (31 mg, 0.10 mmol). The reaction mixture was heated to reflux for 5 h. After evaporation of the solvent, a saturated NaCl solution was added, and after extraction with $CHCl_3$, the organic layers were combined, dried with Na_2SO_4, and evaporated dry. The crude product was chromatographed on silica gel (ethyl acetate:hexane = 1:1) to give 62 mg of **5** (75.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37 (s, 9H), 1.71–1.73 (m, 2H), 2.25–2.42 (m, 10H), 2.87–3.03 (m, 4H), 3.90–3.93 (m, 2H), 4.80 (s, 2H), 6.55 (d, 1H, J = 8.8 Hz), 6.70 (s, 1H), 6.77 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.4 Hz), 6.95 (d, 1H, J = 2.8 Hz), 7.14–7.29 (m, 19H), 7.38–7.40 (m, 12H), 7.82 (J_1 = 8.8 Hz, J_2 = 2.4 Hz), 8.54 (d, 1H, J = 2.0 Hz). HRMS (FAB): m/z calcd for $C_{65}H_{66}N_4O_4S_2$ (M^+), 1030.4423; found, 1030.4417.

*Compound **6** (Re-BAT-Bp-1).* To a solution of **5** (25 mg, 0.02 mmol) in TFA (1 mL) was added triethylsilane (0.29 mL) and mixed for 10 min, and then the solvent was removed under a stream of nitrogen gas. The residue was resolved in 10 mL of CH_2Cl_2, $(Ph_3P)_2ReOCl_3$ (33 mg, 0.04 mmol), and 1 M sodium acetate in methanol (1 mL) was added. The reaction mixture was heated to reflux for 4 h. The mixture was filtered after cooling to room temperature. Evaporation of the solvent gave a residue which was purified with silica gel chromatography ($CHCl_3$:CH_3OH = 10:1), to give 6.1 mg of **6** (46.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.27–2.34 (m, 2H), 2.98–3.06 (m, 2H), 3.28–3.47 (m, 2H), 3.78–3.93 (m, 2H), 4.07–4.33 (m, 6H), 6.56 (d, 1H, J = 8.8 Hz), 6.75 (s, 1H), 6.80 (d, 1H, J = 6.4 Hz), 6.98 (s, 1H), 7.36 (d, 1H, J = 8.8 Hz), 7.86 (d, 1H, J = 9.2 Hz), 8.65 (s, 1H). HRMS (FAB): m/z calcd for $C_{22}H_{28}N_4O_3ReS_2$ (M^+), 675.1471; found, 675.1469.

*tert-Butyl 2-((3-(2-(6-(Methylamino)pyridin-3-yl)benzofuran-5-yloxy)propyl)(2-(tritylthio)ethyl)amino)ethyl(2-(tritylthio)ethyl)-carbamate (**7**).* The same reaction as described above to prepare **5** was used, and 220.5 mg of **7** was obtained in a 70.3% yield from **4**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.36 (s, 9H), 1.69–1.75 (m, 2H), 2.25–2.40 (m, 10H), 2.87–3.03 (m, 4H), 2.97 (s, 3H), 3.89–3.93 (m, 2H), 5.03 (s, 1H), 6.44 (d, 1H, J = 8.8 Hz), 6.68 (s, 1H), 6.76 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.4 Hz), 6.95 (d, 1H, J = 2.8 Hz), 7.14–7.25 (m, 19H), 7.38–7.39 (m, 12H), 7.85 (J_1 = 8.8 Hz, J_2 = 2.4 Hz), 8.56 (d, 1H, J = 2.0 Hz). HRMS (FAB): m/z calcd for $C_{66}H_{68}N_4O_4S_2$ (M^+), 1044.4580; found, 1044.4587.

*Compound **8** (Re-BAT-Bp-2).* The same reaction as described above to prepare **6** was used, and 30.1 mg of **8** was obtained in a 26.7% yield from **7**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.27–2.34 (m, 2H), 2.97 (s, 3H), 2.98–3.06 (m, 2H), 3.28–3.47 (m, 2H), 3.78–3.93 (m, 2H), 4.07–4.33 (m, 6H), 6.56 (d, 1H, J = 8.8 Hz), 6.75 (s, 1H), 6.80 (d, 1H, J = 6.4 Hz), 6.98 (s, 1H), 7.36 (d, 1H, J = 8.8 Hz), 7.86 (d, 1H, J = 9.2 Hz), 8.65 (s, 1H). HRMS (FAB): m/z calcd for $C_{23}H_{30}N_4O_3ReS_2$ (M^+), 661.1314; found, 661.1312.

*5-(5-Methoxybenzofuran-2-yl)-N,N-dimethylpyridin-2-amine (**9**).* A mixture of **1** (360 mg, 1.5 mmol), paraformaldehyde (450 mg, 15 mmol), and sodium cyanoborohydride (284 mg, 4.5 mmol) in acetic acid (20 mL) was stirred at room temperature overnight and then poured into 100 mL of water. Sodium bicarbonate was added to adjust the pH to 8–9. After a standard workup with ethyl acetate, the residue was purified by silica gel chromatography (hexane:ethyl acetate = 3:1) to give 249 mg of **9** (62.0%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.15 (s, 6H), 3.85 (s, 3H), 6.59 (d, 1H, J = 8.8 Hz), 6.76 (s, 1H), 6.82 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.4 Hz), 7.07 (d, 1H, J = 2.4 Hz), 7.36 (d, 1H, J = 8.8 Hz), 7.86 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.4 Hz), 8.67 (d, 1H, J = 2.4 Hz). MS: m/z 269 (M^+ + H).

*2-(6-(Dimethylamino)pyridin-3-yl)benzofuran-5-ol (**10**).* The same reaction as described above to prepare **2** was used, and 234 mg of **10** was obtained in a 98.9% yield from **9**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.15 (s, 6H), 4.89 (s, 1H), 6.59 (d, 1H, J = 8.8 Hz), 6.76 (s, 1H), 6.82 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.4 Hz), 7.07 (d, 1H, J = 2.4 Hz), 7.36 (d, 1H, J = 8.8 Hz), 7.86 (dd, 1H, J_1 = 8.8 Hz, J_2 = 2.4 Hz), 8.67 (d, 1H, J = 2.4 Hz). MS: m/z 255 (M^+ + H).

*5-(5-(3-Bromopropoxy)benzofuran-2-yl)-N,N-dimethylpyridin-2-amine (**11**).* To a solution of **10** (324.9 mg, 1.27 mmol) in CH_3CN (20 mL) were added K_2CO_3 (216 mg, 1.57 mmol) and 1,3-dibromopropane (0.65 mL, 6.4 mmol). The mixture was heated to reflux for 18 h, and after cooling to room temperature, evaporated dry. The residue was redissolved in $CHCl_3$ and washed with brine. The organic layers were dried with Na_2SO_4 and evaporated dry. The crude product was chromatographed on silica gel (ethyl acetate:hexane = 3:7) to give 275 mg of **11** (73.2% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.09 (t, 2H, J = 6.4 Hz), 3.09 (s, 6H), 3.60(t, 2H, J = 6.4 Hz), 4.08 (t, 2H, J = 5.8 Hz), 6.48 (s, 1H), 6.68 (s, 1H), 6.80 (d, 1H, J = 6.4 Hz), 6.96 (s, 1H), 7.33 (d, 1H, J = 8.8 Hz), 7.79 (d, 1H, J = 9.2 Hz), 8.64 (s, 1H). MS: m/z 375 (M^+ + H).

*tert-Butyl-2-((3-(2-(6-(dimethylamino)pyridin-3-yl)benzofuran-6-yloxy)propyl)(2-(tritylthio)ethyl)amino)ethyl(2-(tritylthio)ethyl)-carbamate (**12**).* To a solution of **11** (272 mg, 0.72 mmol) and *tert*-butyl 2-(tritylthio)ethyl(2-(2-(tritylthio)ethylamino)ethyl)carbamate (TRT-Boc-BAT)[18] (550 mg, 0.72 mmol) in acetonitrile (30 mL) was added DIPEA (225 μL, 1.45 mmol). The reaction mixture was heated to reflux for 12 h. When the solvent had evaporated, a saturated NaCl solution was added, and after extraction with $CHCl_3$, the organic layers were combined, dried with Na_2SO_4, and evaporated dry. The crude product was chromatographed on silica gel (ethyl acetate:hexane = 3:7) to give 343.6 mg of **12** (45.0% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.26 (s, 9H), 1.61 (s, 2H), 2.16–2.30 (m, 10H), 2.77–2.87 (m, 4H), 2.97 (s, 6H), 3.81 (s, 2H), 6.40 (d, 1H, J = 9.2 Hz), 6.55 (s, 1H), 6.66 (d, 1H, J = 6.4 Hz), 6.84 (s, 1H), 7.03–7.20 (m, 19H), 7.21–7.31 (m, 12H), 7.71 (d, 1H, J = 9.2 Hz), 8.55 (s, 1H). HRMS (FAB): m/z calcd for $C_{67}H_{71}N_4O_4S_2$ (M^+), 1059.4917; found, 1059.4910.

*Compound **13** (Re-BAT-Bp-3).* The same reaction as described above to prepare **6** was used, and 29 mg of **13** was obtained in a 33.0% yield from **12**. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.27–2.34 (m, 2H), 2.98–3.06 (m, 2H), 3.16 (s, 6H), 3.28–3.47 (m, 2H), 3.78–3.93 (m, 2H), 4.07–4.33 (m, 6H), 6.56 (d, 1H, J = 8.8 Hz), 6.75 (s, 1H), 6.80 (d, 1H, J = 6.4 Hz), 6.98 (s, 1H), 7.36 (d, 1H, J = 8.8 Hz), 7.86 (d, 1H, J = 9.2 Hz), 8.65 (s, 1H). HRMS (FAB): m/z calcd for $C_{24}H_{32}N_4O_3ReS_2$ (M^+), 675.1471; found, 675.1469.

Binding Assays Using the Aggregated Aβ Peptides in Solution. Aβ(1–42) was purchased from Peptide Institute (Osaka, Japan). Aggregation was carried out by gently dissolving the peptide (0.25 mg/mL) in a buffer solution (pH 7.4) containing 10 mM sodium phosphate and 1 mM EDTA. The solution was incubated at 37 °C for 42 h with gentle and constant shaking. A mixture containing 50 μL of Re complex (**6**, **8**, or **13**) (10^{-5}–10^{-10} M in 10% EtOH),

50 μL of [^{125}I] IMPY (50000−100000 cpm), 50 μL of Aβ(1−42) aggregates (28 nM), and 850 μL of 10% EtOH was incubated at room temperature for 3 h. The mixture was then filtered through Whatman GF/B filters using a Brandel M-24 cell harvester, and the radioactivity of the filters containing the bound ^{125}I ligand was measured in a γ counter. Values for the half-maximal inhibitory concentration (IC_{50}) were determined from displacement curves of three independent experiments using GraphPad Prism 5.0, and those for the inhibition constant (K_i) were calculated using the Cheng−Prusoff equation: $K_i = IC_{50}/(1 + [L]/K_d)$, where [L] is the concentration of [^{125}I]IMPY used in the assay and K_d is the dissociation constant of IMPY.

^{99m}Tc Labeling Reaction and Analysis by RP-HPLC. To a solution of sodium heptonate dehydrate (2 g, 7.04 mmol) in nanopure water (25 mL) was added 0.75 mL of a $SnCl_2{\cdot}2H_2O$ solution [12 mg of Tin(II) chloride dehydrate (53.2 mmol) dissolved in 15 mL of 0.1 M HCl]. This solution was adjusted to pH 8.5−9.0 using a small amount of 0.1 M NaOH and then lyophilized to give Sn glucoheptonate (SnGH) kit. SnGH kit (1 mg) was added to a $Na^{99m}TcO_4$ solution (200 μL) and reacted at room temperature for 10 min to give a ^{99m}TcGH solution. To a solution of precursor (**5**, **7**, or **12**) (0.5 mg) in TFA (200 μL) was mixed in triethylsilane (10 μL), and the solvents were removed under a stream of nitrogen gas. The residue was resolved in acetonitrile (200 μL), followed by addition of 0.1 M HCl (15 μL) and the ^{99m}TcGH solution (200 μL). The reaction mixture was heated to 85 °C for 20 min. After cooling to room temperature, sodium bicarbonate was added to adjust the pH to 8−9. The mixture was purified with RP-HPLC. The ^{99m}Tc-labeled pyridyl benzofuran complex was analyzed by analytical RP-HPLC on a Cosmosil C_{18} column ($5C_{18}$-AR-II, 4.6 mm × 150 mm) with a solvent of H_2O/acetonitrile (0 min 3/2 to 30 min 3/7) at a flow rate of 1.0 mL/min. The absorption of the complexes was measured at 254 nm, and the radioactivity of the ^{99m}Tc-labeled form was recorded for 60 min.

log *P* Measurement. The experimental determination of partition coefficients was performed in 1-octanol and PBS buffer (pH 7.4). The two phases were presaturated with each other. 1-Octanol (3.0 mL) and PBS (3.0 mL) were pipetted into a 12 mL test tube containing 1.11 MBq of [^{99m}Tc]tracer. The test tube was vortexed for 2 min and centrifuged (5 min, 1000*g*). Aliquots (500 μL) from the 1-octanol and PBS phases were transferred into two test tubes for counting. One milliliter of the remaining 1-octanol phase was transferred into a new test tube. New 1-octanol (2.0 mL) and PBS (3.0 mL) were pipetted into the same test tube. The vortexing, centrifuging, and counting were repeated. The amount of radioactivity in each tube was measured with a γ counter and corrected for decay. The partition coefficient was calculated using the equation: $\log P = \log[\text{count}_{1\text{-octanol}}/\text{count}_{PBS}]$

Biodistribution in Normal Mice. Experiments with animals were conducted in accordance with our institutional guidelines and approved by the Kyoto University Animal Care Committee. While under isoflurane anesthesia, ddY mice (5 weeks old, 22−25 g, male) were injected intravenously with 100 μL of a 10% ethanol in saline solution containing [^{99m}Tc]tracers (148 kBq) via the tail. The mice (n = 5 for each time point) were sacrificed at 2, 10, 30, and 60 min postinjection. The organs of interest were removed and weighed, and radioactivity was measured with an automatic γ counter (COBRAII, Packard). The percent dose per organ was calculated by comparing the tissue counts with suitably diluted aliquots of the injected material. The %dose/g of samples was calculated by comparing the sample counts with the count for the diluted initial dose.

Ex Vivo Autoradiography Using Tg2576 Mice. Tg2576 transgenic mice (27 months, male) and wild-type mice (27 months, male) were used as an Alzheimer's model and an age-matched control, respectively. After anesthetization with 1% isoflurane, 16.6 MBq of [^{99m}Tc]BAT-Bp-2 in 250 μL of a 10% ethanol solution was injected through the tail. The animals were allowed to recover for 30 min and then killed by decapitation. The brains were immediately removed and frozen in a dry ice/hexane bath. Sections of 20 μm were cut and exposed to a BAS imaging plate (Fuji Film, Tokyo, Japan) overnight. Ex vivo film autoradiograms were thus obtained. After autoradiographic examination, the same sections were stained by thioflavin-S to confirm the presence of β-amyloid plaques. For the staining of thioflavin-S, sections were immersed in a 0.125% thioflavin-S solution containing 50% EtOH for 5 min and washed in 50% EtOH. After drying, the sections were examined using a microscope (Nikon, Eclipse 80i) equipped with a B-2A filter set (excitation, 450−490 nm; diachronic mirror, 505 nm; long-pass filter, 520 nm).

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

AD, Alzheimer's disease; SP, senile plaque; NFT, neurofibrillary tangle; Aβ, β-amyloid peptide; PET, positron emission tomography; SPECT, single photon emission computed tomography; MCI, mild cognitive impairment; BAT, bis(aminoethanethiol); BBB, blood−brain barrier; GH, glucoheptonate; PIB, 2-(4′-(methylaminophenyl)-6-hydroxybenzothiazole; IMPY, 6-iodo-2-(4′-dimethylamino)phenylimidazo[1,2-*a*]pyridine

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