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Synthesis and Evaluation of Novel Chalcone Derivatives with ^{99m}Tc/Re Complexes as Potential Probes for Detection of β-Amyloid Plaques

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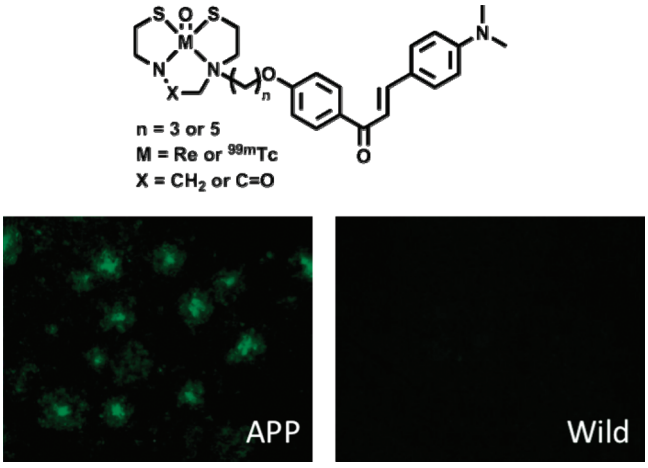
Abstract



Four ^{99m}Tc-labeled chalcone derivatives and their corresponding rhenium analogues were tested as potential probes for imaging β-amyloid plaques. The chalcones showed higher affinity for Aβ(1−42) aggregates than did ^{99m}Tc complexes. In sections of brain tissue from an animal model of AD, the four Re chalcones intensely stained β-amyloid plaques. In biodistribution experiments using normal mice, ^{99m}Tc-BAT-chalcone ([^{99m}Tc]**17**) displayed high uptake in the brain (1.48% ID/g) at 2 min postinjection. The radioactivity washed out from the brain rapidly (0.17% ID/g at 60 min), a highly desirable feature for an imaging agent. [^{99m}Tc]**17** may be a potential probe for imaging β-amyloid plaques in Alzheimer's brains.

Keywords: Alzheimer's disease, β-amyloid plaque, ^{99m}Tc, single photon emission computed tomography (SPECT) imaging

Alzheimer's disease (AD), the most common senile dementia, is characterized by β-amyloid (Aβ) plaques, vascular amyloid, neurofibrillary tangles, and progressive neurodegeneration. The formation of plaques composed of β-amyloid peptides (Aβ) in the brain is considered the initial neurodegenerative event in AD (*1*). Thus, the detection of individual plaques *in vivo* by single photon emission tomography (SPECT) or positron emission tomography (PET) should improve diagnosis and also accelerate the discovery of effective therapeutic agents for AD (*2*−*4*).

Many radiolabeled probes for imaging β-amyloid based on Congo Red, thioflavin T, and DDNP have been reported. Among them, [^{11}C]PIB (*5*, *6*), [^{11}C]SB-13 (*7*, *8*), [^{18}F]BAY94-9172 (*9*, *10*), [^{11}C]BF-227 (*11*), [^{18}F]FDDNP (*12*, *13*), [^{123}I]IMPY (*14*−*16*), and [^{18}F]-AV-45 (*17*, *18*) have been tested clinically and demonstrated potential utility.

Recently, while searching for novel imaging probes, we found that ^{125}I-, ^{11}C-, and ^{18}F-labeled chalcone derivatives showed high affinity for Aβ aggregates and good uptake into and rapid clearance from the brain (*19*−*21*). In this study, we planned the development of novel chalcone derivatives labeled with technetium-99m (^{99m}Tc). ^{99m}Tc ($T_{1/2}$ = 6.01 h, 141 keV) has become the most commonly used radionuclide in diagnostic nuclear medicine for several reasons: it is readily produced by a ^{99}Mo/^{99m}Tc generator, the medium gamma-ray energy it emits is suitable for detection, and its physical half-life is compatible with the biological localization and residence time required for imaging. It is ready availability, essentially 24 h/day, and ease of use make it the radionuclide of choice. New ^{99m}Tc-labeled imaging agents will provide simple, convenient, and widespread SPECT-based imaging methods for detecting and eventually quantifying β-amyloid plaques in living brain tissue.

Kung et al. reported that a dopamine transporter imaging agent, [^{99m}Tc]TRODAT-1, is useful to detect the loss of dopamine neurons in the basal ganglia associated with Parkinson's disease. This is the first example of a ^{99m}Tc imaging agent that can penetrate the blood−brain barrier via a simple diffusion mechanism and localize at sites in the central nervous system. Based on this success, efforts were made to search for comparable ^{99m}Tc imaging

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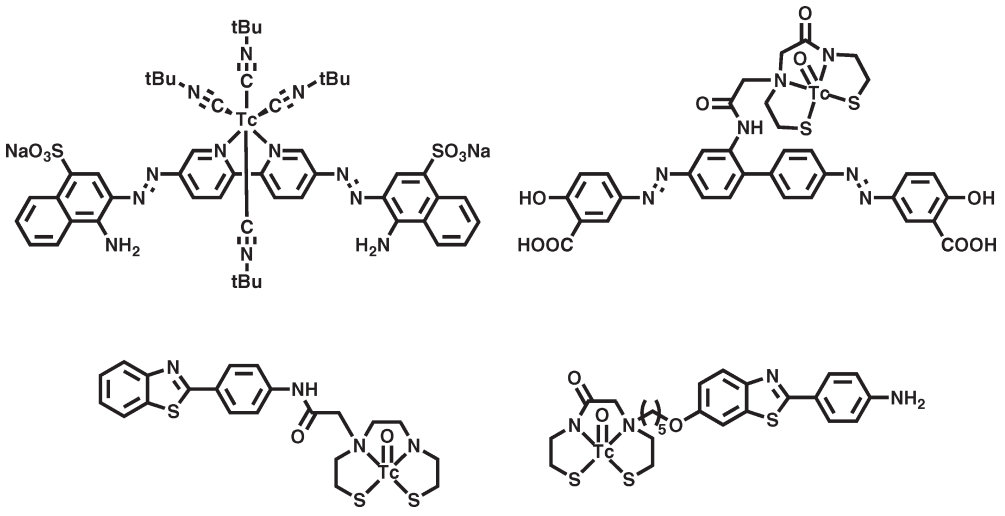
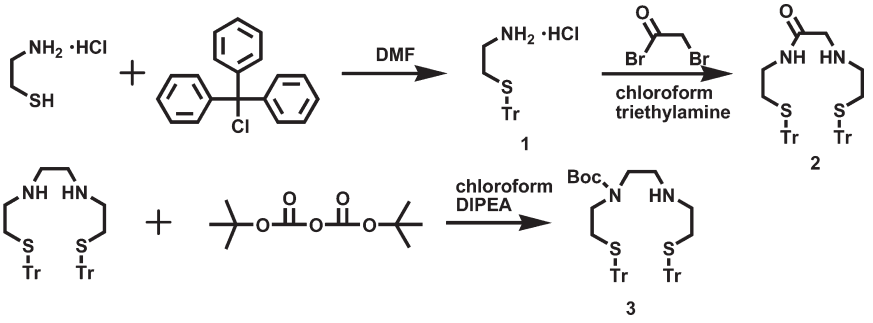


Figure 1. Chemical structure of ^{99m}Tc-labeled Aβ imaging probes reported previously.

Scheme 1. Synthesis of Chelation Ligands



agents that target binding sites on β-amyloid plaques in the brain of AD patients. Several ^{99m}Tc-labeled imaging probes have been developed (Figure 1), but no clinical study of them has been reported (*22*−*25*).

In the present study, to develop more useful ^{99m}Tc-labeled probes for clinical diagnosis, we synthesized four chalcone derivatives with monoamine−monoamide dithiol (MAMA) and bis-amino-bis-thiol (BAT). MAMA and BAT were selected as chelation ligands taking into consideration the permeability of the blood−brain barrier, because they form an electrically neutral complex with ^{99m}Tc (*26*). We then evaluated their biological potential as probes by testing their affinity for Aβ aggregates and β-amyloid plaques in sections of brain tissue from Tg2576 mice and their uptake by and clearance from the brain in biodistribution experiments using normal mice. To our knowledge, this is the first time chalcones with ^{99m}Tc/Re complexes have been proposed as probes for the detection of β-amyloid plaques in the brain.

Results and Discussion

The synthesis of the ^{99m}Tc/Re chalcone derivatives is outlined in Schemes 1−3. The chelation ligands (MAMA and BAT) were synthesized according to methods reported previously with some slight modifications (Scheme 1) (*26*). The most useful method of preparing chalcones is the condensation of acetophenones with benzaldehydes(*19*). In this process, 4-hydroxybenzaldehyde was reacted with 4-dimethylaminobenzaldehyde in the presence of a basic catalyst (10% KOH) in ethanol at room temperature to form 4-dimethylamino-4′-hydroxy-chalcone (**4**) in a yield of 70%. The reaction of dibromo with **4** produced two chalcone derivatives (**5** and **6**) with alkyl groups (n = 3 or 5) of different lengths. Then, **5** (n = 5) or **6** (n = 3) was joined to **2** (Tr-MAMA) or **3** (Tr-Boc-BAT) to generate compounds **7** (n = 5) and **13** (n = 3) (Tr-MAMA-chalcones) or compounds **10** (n = 5) and **16** (n = 3) (Tr-Boc-BAT). Compounds **8**, **11**, **14**, and **17** (the precursors for ^{99m}Tc labeling) were obtained by deprotection of the thiol groups in **7**, **10**, **13**, and **16**, respectively. The Re complexes (**9**, **12**, **15**, and **18**) were prepared by the reaction of **7**, **11**, **13**, and **17** with $(PPh_3)_2ReOCl_3$. The corresponding ^{99m}Tc complexes, [^{99m}Tc]**8**, [^{99m}Tc]**11**, [^{99m}Tc]**14**, and [^{99m}Tc]**17**, were prepared by a ligand exchange reaction employing the precursor ^{99m}Tc-gluco-heptonate (GH). The resulting mixture was analyzed by reversed-phase HPLC, showing that a single radioactive complex formed with radiochemical purity higher than

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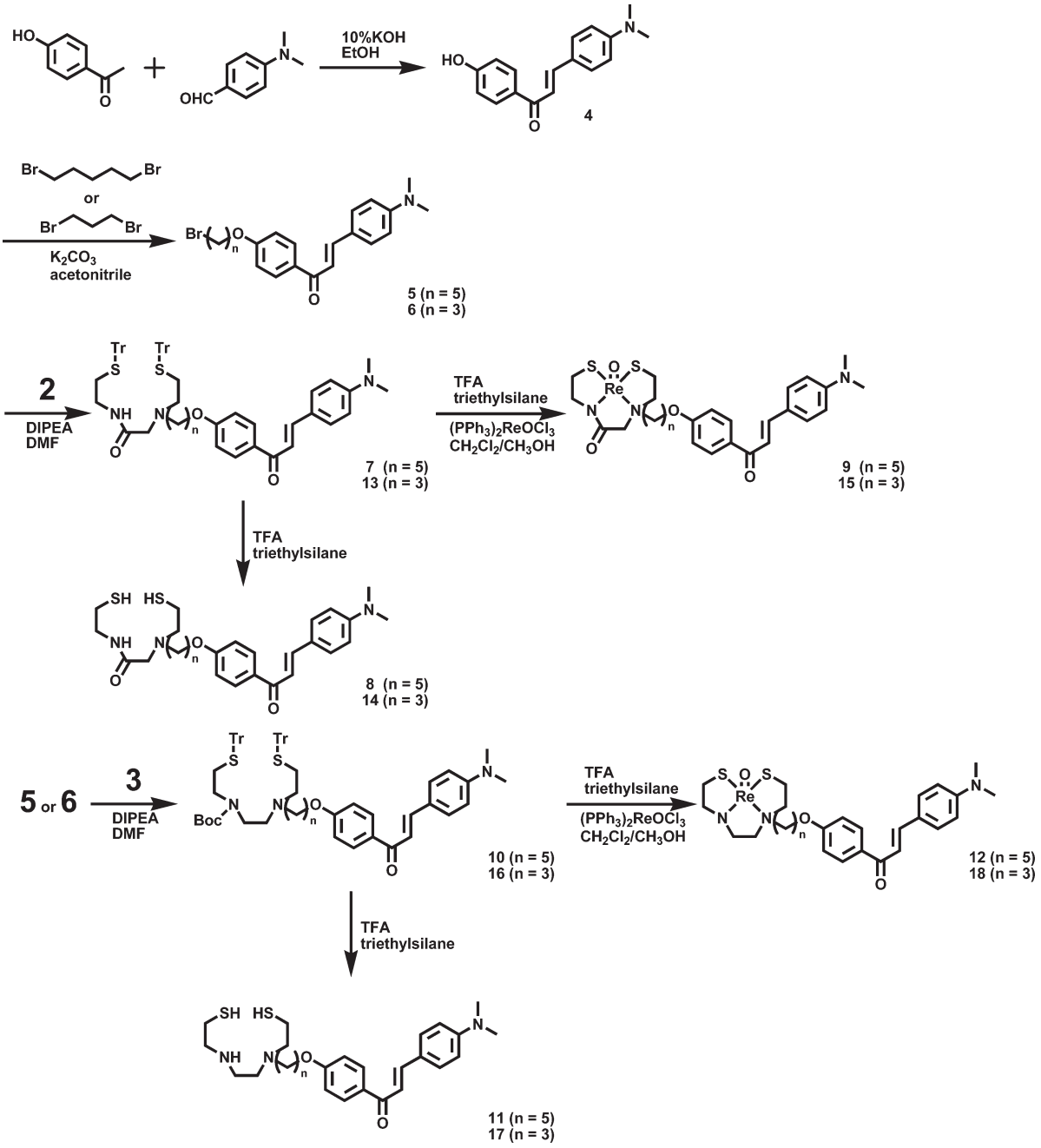
Scheme 2. Synthesis of Chalcone Derivatives



95% after purification by HPLC. The identity of the complex was established by comparative HPLC using the corresponding Re complexes as a reference (Table 1). The retention times for [^{99m}Tc]**8**, [^{99m}Tc]**11**, [^{99m}Tc]**14**, and [^{99m}Tc]**17** on HPLC (radioactivity) were 14.2, 20.3, 9.3, and 12.4 min, respectively. The retention times of the corresponding Re complexes on HPLC (UV detection) were 13.5, 18.4, 8.6, and 11.2 min, respectively.

In vitro binding experiments to evaluate the affinity of the ^{99m}Tc-labeled chalcones for Aβ aggregates were carried out in solutions (Figure 2). To make quantitative comparisons with ^{99m}Tc-BAT and ^{99m}Tc-MAMA, we showed the binding affinity as total Aβ(1−42) aggregate-bound radioactivity (%) at different concentrations of Aβ aggregates. In this assay, we also confirmed that the nonspecific Aβ(1−42) aggregate-bound radioactivity (%) was 1.9−3.2% in the four ^{99m}Tc-labeled chalcones, indicating that nearly all of the radioactivity occupied the specific binding site for Aβ aggregates. The percent radioactivity of the chalcones bound to aggregates increased dependent on the dose of Aβ(1−42). In terms of Aβ(1−42) aggregate-bound radioactivity, the derivatives ranked in the following

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Scheme 3. ^{99m}Tc Labeling of Chalcone Derivatives

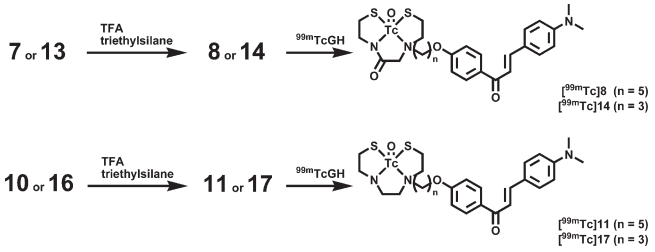

Table 1. HPLC Retention Times of ^{99m}Tc-Labeled Chalcones and Their Re Analogues and log *P* Values of ^{99m}Tc-Labeled Chalcones

^{99m}Tc compound	retention time (min)[a]	Re compound	retention time (min)[a]	log *P* of ^{99m}Tc compounds[b]
[^{99m}Tc]**8**	14.2	**9**	13.5	2.55 ± 0.19
[^{99m}Tc]**11**	20.3	**12**	18.4	2.73 ± 0.16
[^{99m}Tc]**14**	9.3	**15**	8.6	1.51 ± 0.09
[^{99m}Tc]**17**	12.4	**18**	11.2	2.51 ± 0.05

[a] Reversed-phase HPLC using a mixture of H_2O/acetonitrile (2/3) as a mobile phase. [b] The measurement was done in triplicate and repeated three times. Each value represents the mean ± SD for three independent experiments.

order: [^{99m}Tc]**11** > [^{99m}Tc]**8** > [^{99m}Tc]**14** > [^{99m}Tc]**17**. ^{99m}Tc complexes (^{99m}Tc-MAMA and ^{99m}Tc-BAT) showed no marked binding to Aβ(1−42) aggregates. This result suggests that the length of the alkyl chain between ^{99m}Tc complexes and the chalcone backbone played an important role in the binding of Aβ(1−42) aggregates, and the difference in ligand (MAMA and BAT) did not affect the affinity.

To confirm the affinity for β-amyloid plaques in the mouse brain, neuropathological fluorescent staining with Re-chalcone derivatives (**9**, **12**, **15**, and **18**) was carried out using Tg2576 mouse brain sections (Figure 3). Many β-amyloid plaques were clearly stained with the derivatives (Figure 3A,C,E,G), as reflected by the high affinity for Aβ aggregates in binding assays *in vitro*, while only minimum labeling was observed in the wild-type mouse brain (Figure 3I). The labeling pattern was consistent with that observed with thioflavin S (Figure 3B,D,F,H). These results suggest that the ^{99m}Tc-chalcones bind to β-amyloid plaques in the mouse brain in addition to having affinity for synthetic Aβ(1−42) aggregates.

Four ^{99m}Tc-labeled chalcones ([^{99m}Tc]**8**, [^{99m}Tc]**11**, [^{99m}Tc]**14**, [^{99m}Tc]**17**) were examined as to their biodistribution in normal mice (Table 2). A biodistribution study provides important information on brain uptake. The ideal β-amyloid imaging probe should have good blood−brain barrier penetration to deliver a sufficient dose into the brain while achieving rapid clearance from normal regions to result in a higher signal-to-noise ratio in the AD brain. Previous studies suggested that the

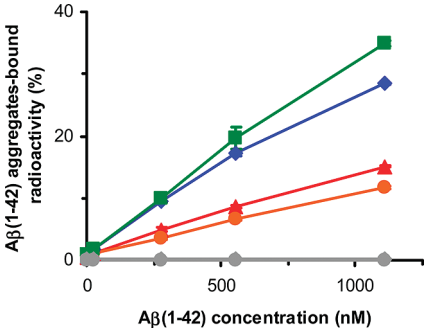

Figure 2. Binding assay of ^{99m}Tc-labeled chalcone derivatives (◆, blue) [^{99m}Tc]**8**, (■) [^{99m}Tc]**11**, (▲) [^{99m}Tc]**14**, (●, orange) [^{99m}Tc]**17**, (●, gray) ^{99m}Tc-MAMA, (◆, gray) ^{99m}Tc-BAT with Aβ(1−42) aggregates. Values are the mean ± standard error of the mean for three independent experiments.

optimal lipophilicity for entry into the brain is obtained with log *P* values of between 1 and 3 (*27*). All four ligands examined in the present study displayed optimal lipophilicity as reflected by their log *P* values (Table 1). Among the four ^{99m}Tc-labeled chalcones, [^{99m}Tc]**17** showed the highest uptake (1.48% ID/g at 2 min postinjection), but [^{99m}Tc]**8**, [^{99m}Tc]**11**, and [^{99m}Tc]**14** did not show sufficient uptake (0.32−0.78% ID/g at 2 min postinjection) despite their lipophilicity. The reason for the low uptake remains unknown. The uptake of [^{99m}Tc]**17** was greater than that of ^{99m}Tc-labeled Aβ imaging agents reported previously, supporting the feasibility of developing ^{99m}Tc-labeled probes for the detection of β-amyloid plaques *in vivo*. Thereafter, the radioactivity of [^{99m}Tc]**17** that accumulated in the brain was rapidly eliminated (0.17% ID/g at 60 min postinjection), a highly desirable property for imaging agents. The pharmacokinetics of [^{99m}Tc]**17** in the brain appears superior to that of any other ^{99m}Tc-labeled probe reported previously, indicating that this chalcone should be investigated further for the imaging of β-amyloid. [^{99m}Tc]**8**, [^{99m}Tc]**11**, and [^{99m}Tc]**14** showed no marked initial uptake, 0.32−0.78% ID/g, and were washed out from the brain relatively slowly (0.11−0.16% ID/g at 60 min). The uptake of [^{99m}Tc]**8**, [^{99m}Tc]**11**, and [^{99m}Tc]**14** appears insufficient for the imaging of β-amyloid plaques in the brain, and additional structural changes are needed to further improve the properties of these chalcone derivatives.

In conclusion, we successfully designed and synthesized novel chalcone derivatives conjugated with ^{99m}Tc or Re complexes for the detection of β-amyloid plaques in the brain. When *in vitro* plaque labeling was carried out using sections of brain from Tg2576 mice, four Re-chalcones intensely stained β-amyloid plaques. In addition, [^{99m}Tc]**17** displayed good uptake into and a rapid washout from the brain after its injection into normal mice. The combination of affinity for β-amyloid plaques, good uptake, and rapid

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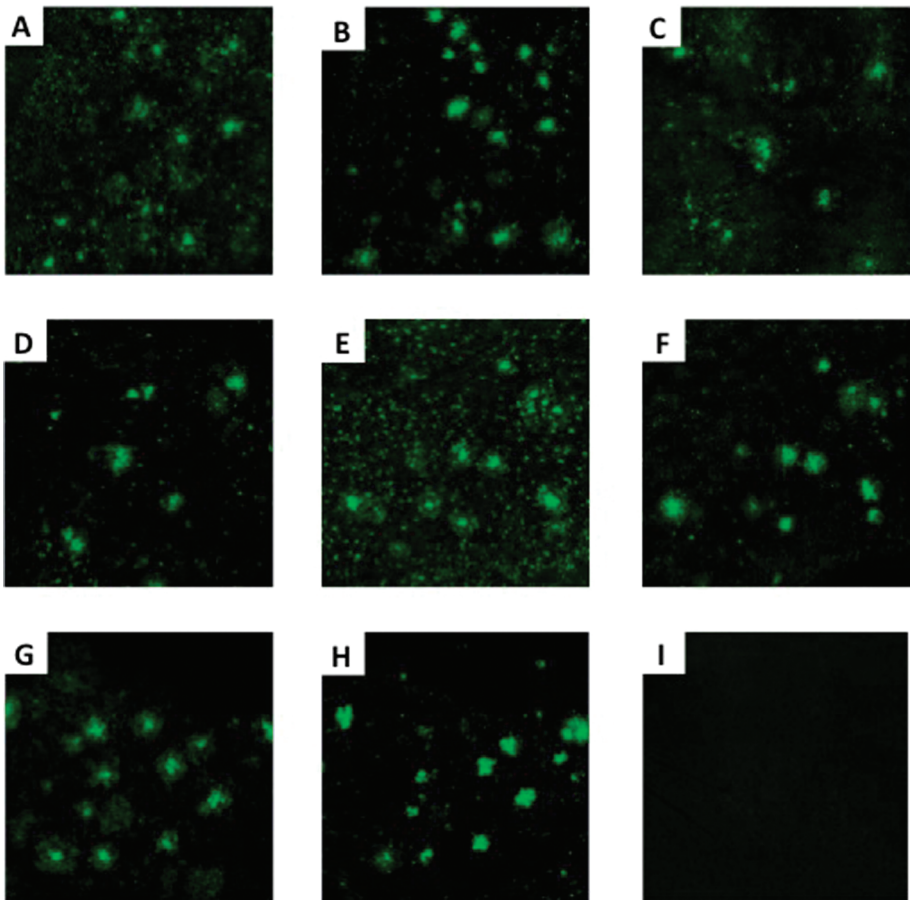

Figure 3. Fluorescent staining of chalcone derivatives **9** (A), **12** (C), **15** (E), and **18** (G) in 10-μm Tg2576 mouse brain sections. Labeled plaques were confirmed by staining of the adjacent sections with thioflavin-S (B, D, F, H). No apparent staining of **18** (I) was observed in the age-matched control mouse brain section.

clearance makes [^{99m}Tc]**17** a promising probe for the detection of β-amyloid plaques in the brain. The results of the present study should provide information useful to the development of ^{99m}Tc-labeled probes for the imaging of β-amyloid plaques in the brain.

Methods

General

All reagents were obtained commercially and used without further purification unless otherwise indicated. ^{1}H NMR spectra were obtained on a Varian Gemini 300 spectrometer with TMS as an internal standard. Coupling constants are reported in hertz. Multiplicity was defined by s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), and m (multiplet). Mass spectra were obtained on a JEOL IMS-DX. HPLC was performed with a Shimadzu system (a LC-10AT pump with a SPD-10A UV detector, λ = 254 nm) using a Cosmosil C18 column (Nakalai Tesque, 5C18-AR-II, 4.6 mm × 150 mm) and acetonitrile/H_2O (3/2) as the mobile phase at a flow rate of 1.0 mL/min. All key compounds were proven by this method to show >95% purity.

Chemistry

2-(Tritylthio)ethanamine Hydrochloride (1). A solution of 2-mercaptoethylamine hydrochloride (1.14 g, 10 mmol) and triphenylmethyl chloride (2.79 g, 10 mmol) in DMF (10 mL) was stirred at room temperature for 72 h. After the evaporation of DMF, the residue was redissolved in ethyl acetate and added to a 5% $NaHCO_3$ solution in an ice bath. The white precipitate that formed was filtered to yield **1** (3.50 g, 98% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.31 (t, J = 6.8 Hz, 2H), 2.60 (t, J = 6.8 Hz, 2H), 7.18−7.31 (m, 9H), 7.43 (d, J = 7.2 Hz, 6H).

2-(2-(Tritylthio)ethylamino)-*N*-(2-(tritylthio)ethyl)acetamide (Tr-MAMA) (2). To a stirring solution of **1** (2.50 g, 7.02 mmol) in $CHCl_3$ (20 mL) and triethylamine (5 mL) was slowly added bromoacetyl bromide (0.5 mL, 5.76 mmol) in an ice bath, and the mixture was stirred at 0 °C for 3 h. The mixture was washed with a diluted H_2SO_4 (pH 3), a saturated $NaHCO_3$, and a saturated NaCl solution, sequentially. The organic layers were combined and dried with Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by silica gel chromatography using $CHCl_3$ and then ethyl acetate to give 1.39 g of **2** (58% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.35 (q, J = 6.6 Hz, 4H), 2.45 (t, J = 5.9 Hz, 2H), 3.02 (s, 2H), 3.07 (q, J = 6.3 Hz, 2H), 7.18−7.29 (m, 18H), 7.38−7.42 (m, 12H). MS m/z 679 (MH^+).

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

	time after injection (min)			
organ	2	10	30	60
		[^{99m}Tc]**8**		
blood	1.85(0.31)	0.91(0.15)	0.62(0.16)	0.28(0.02)
liver	18.92(2.03)	24.48(1.34)	26.63(5.57)	17.05(1.52)
kidney	9.45(1.24)	7.62(3.79)	9.85(1.35)	5.25(0.87)
intestine [b]	4.71(0.63)	12.45(2.90)	34.90(3.01)	36.49(6.04)
spleen	4.16(0.52)	3.17(0.52)	2.37(0.48)	1.28(0.09)
lung	16.13(1.34)	6.59(1.23)	3.61(1.00)	1.44(0.17)
stomach [b]	0.76(0.10)	1.31(0.15)	2.06(0.65)	1.67(0.29)
pancreas	3.78(0.49)	5.28(0.33)	4.71(0.96)	2.27(0.14)
heart	11.05(1.99)	5.10(1.00)	2.16(0.63)	0.87(0.22)
brain	0.22(0.05)	0.32(0.14)	0.19(0.030)	0.11(0.01)
		[^{99m}Tc]**11**		
blood	2.49(0.24)	0.92(0.05)	0.50(0.11)	0.35(0.13)
liver	23.89(2.51)	24.03(4.51)	23.18(3.67)	21.95(4.58)
kidney	11.26(0.62)	9.66(0.58)	7.64(0.88)	6.39(1.51)
intestine [b]	6.27(0.31)	15.99(0.87)	37.18(2.54)	54.09(10.94)
spleen	3.15(0.22)	1.64(0.33)	0.69(0.16)	0.35(0.15)
lung	15.71(4.59)	4.54(0.57)	1.89(0.24)	1.28(0.49)
stomach [b]	0.95(0.14)	1.37(0.19)	1.77(0.62)	2.41(0.99)
pancreas	4.93(0.87)	3.94(0.84)	1.71(0.30)	0.80(0.32)
heart	13.17(1.42)	3.03(0.36)	1.30(0.35)	0.78(0.10)
brain	0.78(0.16)	0.55(0.06)	0.28(0.09)	0.16(0.09)
		[^{99m}Tc]**14**		
blood	7.84(2.85)	5.37(2.42)	1.55(0.62)	0.41(0.19)
liver	18.35(1.93)	24.89(2.27)	21.29(3.34)	12.96(3.14)
kidney	10.50(1.36)	11.03(2.66)	9.62(1.54)	4.65(0.75)
intestine [b]	3.70(0.55)	9.60(1.35)	31.67(4.85)	41.40(8.54)
spleen	5.87(2.07)	4.44(0.69)	2.71(0.92)	1.42(0.85)
lung	16.33(4.74)	7.87(1.60)	3.16(0.68)	4.12(4.25)
stomach [b]	0.85(0.21)	1.42(0.28)	2.29(0.61)	2.32(0.81)
pancreas	3.14(1.32)	5.30(2.14)	4.64(0.76)	1.63(0.37)
heart	13.26(2.46)	4.96(1.83)	2.54(0.56)	1.23(0.28)
brain	0.62(0.27)	0.47(0.13)	0.38(0.11)	0.16(0.08)
		[^{99m}Tc]**17**		
blood	2.81(0.76)	0.95(0.45)	0.56(0.30)	0.29(0.13)
liver	21.26(2.50)	27.33(2.45)	22.08(3.93)	14.34(0.60)
kidney	11.21(1.46)	8.54(0.64)	4.18(0.52)	1.92(0.41)
intestine [b]	6.22(0.40)	21.95(2.50)	42.24(3.78)	53.39(4.78)
spleen	2.91(0.61)	2.37(0.55)	0.74(0.16)	0.30(0.04)
lung	10.33(1.80)	5.92(1.38)	2.47(0.62)	0.73(0.27)
stomach [b]	1.14(0.26)	1.80(0.22)	1.93(0.19)	1.68(0.67)
pancreas	6.91(2.23)	4.45(0.54)	1.44(0.33)	0.47(0.06)
heart	11.71(2.13)	3.01(0.70)	0.98(0.26)	0.44(0.10)
brain	1.48(0.44)	1.09(0.20)	0.35(0.14)	0.17(0.06)

[a] Each value represents the mean (SD) for 3−6 mice at each interval. Expressed as % injected dose per gram. [b] Expressed as % injected dose per organ.

***tert*-Butyl-2-(2-(tritylthio)ethylamino)ethyl-2-(tritylthio)ethylcarbamate (Tr-BAT) (3).** To a solution of N^1,N^2-bis(2-(tritylthio)ethyl)ethane-1,2-diamine (3.33 g, 5 mmol) and DIPEA (0.86 mL, 5 mmol) in CH_2Cl_2 (80 mL) was added dropwise a solution of $(BOC)_2O$ (1.09 g, 5 mmol) in CH_2Cl_2 (20 mL) in an ice bath. The mixture was stirred at 0 °C for 1 h, concentrated, and purified by column chromatography ($CHCl_3/CH_3OH$ = 99:1) to give 3.59 g of **3** (94%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (s, 9H), 1.54 (s, broad, 1H), 2.46−2.28 (m, 8H), 2.96 (s, broad, 4H), 7.17−7.30 (m, 18H), 7.37−7.43 (m, 12H).

(*E*)-3-(4-(Dimethylamino)phenyl)-1-(4-hydroxyphenyl)prop-2-en-1-one (4). *p*-Hydroxyacetophenone (1.36 g, 10 mmol) and *p*-dimethylaminobenzaldehyde (1.49 g, 10 mmol) were dissolved in EtOH (15 mL). A 30-mL aliquot of a 10% aqueous KOH solution was then slowly added dropwise to the reaction mixture. The mixture was stirred for 12 h at 100 °C and then poured into a 1 M aqueous HCl solution and extracted with ethyl acetate. 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 ($CHCl_3/CH_3OH$ = 49:1) to give 1.86 g of **4** (70%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.05 (s, 6H), 6.70 (d, J = 8.7 Hz, 2H), 6.91 (d, J = 9.0 Hz, 2H), 7.34 (d, J = 15.3 Hz, 1H), 7.55 (d, J = 9.0 Hz, 2H), 7.79 (d, J = 16.2 Hz, 1H), 7.99 (d, J = 9.0 Hz, 2H).

(*E*)-1-(4-(5-Bromopentyloxy)phenyl)-3-(4-(dimethylamino)-phenyl)prop-2-en-1-one (5). To a solution of **4** (1.51 g, 5.64 mmol) in acetonitrile (15 mL) were added 1,5-dibromopentane (1.54 mL, 11.4 mmol) and K_2CO_3 (1.5 g). The reaction mixture was heated to reflux for 4 h. After the acetonitrile was evaporated, the residue was poured into water and extracted with $CHCl_3$. The organic layer was combined and dried with Na_2SO_4. After the mixture was concentrated, the residue was redissolved in DMSO and washed using hexane. The DMSO layer was concentrated and purified by silica gel chromatography using $CHCl_3$ to give 1.48 g of **5** (63% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.62−1.70 (m, 2H), 1.83−1.90 (m, 2H), 1.91−2.01 (m, 2H), 3.05 (s, 6H), 3.56 (t, J = 6.8 Hz, 2H), 4.06 (t, J = 6.3 Hz, 2H), 6.70 (d, J = 9.0 Hz, 2H), 6.95 (d, J = 8.7 Hz, 2H), 7.36 (d, J = 15.3 Hz, 1H), 7.55 (d, J = 8.7 Hz, 2H), 7.79 (d, J = 15.6 Hz, 1H), 8.02 (d, J = 9.0 Hz, 2H).

(*E*)-1-(4-(3-Bromopropoxy)phenyl)-3-(4-(dimethylamino)-phenyl)prop-2-en-1-one (6). To a solution of **4** (890 mg, 3.33 mmol) in acetonitrile (25 mL) were added 1,3-dibromopropane (0.679 mL, 6.66 mmol) and K_2CO_3 (1.0 g). The reaction mixture was heated to reflux for 4 h. After the acetonitrile was evaporated, the residue was poured into a saturated NaCl solution and extracted with $CHCl_3$. The organic layer was combined and dried with Na_2SO_4. After the mixture was concentrated, the residue was redissolved in DMF and washed using hexane. The DMF layer was concentrated and purified by silica gel chromatography ($CHCl_3/CH_3OH$ = 199:1) to give 981 mg of **6** (73% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.35 (quin, J = 6.1 Hz, 2H), 3.04 (s, 6H), 3.61 (t, J = 6.5 Hz, 2H), 4.18 (t, J = 5.7 Hz, 2H), 6.69 (d, J = 9.0 Hz, 2H), 6.97 (d, J = 8.7 Hz, 2H), 7.35 (d, J = 15.3 Hz, 1H), 7.55 (d, J = 8.7 Hz, 2H), 7.79 (d, J = 15.6 Hz, 1H), 8.02 (d, J = 9.0 Hz, 2H).

Compound 7 (Tr-MAMA-C5-CH). To a solution of **5** (485 mg, 1.13 mmol) and **2** (830 mg, 1.22 mmol) in DMF (10 mL) was added DIPEA (1 mL). The reaction mixture was heated to reflux

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for 12 h. After the evaporation of the solvent, water was added. The mixture was extracted with $CHCl_3$. The organic layers were combined and dried with Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by silica gel chromatography ($CHCl_3/CH_3OH$ = 199:1) to give 330 mg of **7** (29% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 1.39 (s, 4H), 1.69−1.71 (m, 2H), 2.27−2.41 (m, 8H), 2.87 (s, 2H), 2.95−3.01 (m, 2H), 3.04 (s, 6H), 3.93 (t, J = 6.5 Hz, 2H), 6.70 (d, J = 9.0 Hz, 2H), 6.89 (d, J = 8.7 Hz, 2H), 7.14−7.27 (m, 18H), 7.32−7.39 (m, 13H), 7.55 (d, J = 9.0 Hz, 2H), 7.79 (d, J = 15.6 Hz, 1H), 7.99 (d, J = 9.0 Hz, 2H). MS m/z 1014 (MH^+).

Compound 8 (MAMA-C5-CH). To a solution of a little **7** in TFA (200 mL) was added triethylsilane (10 mL), and the solution was mixed. The solvent was removed under a stream of nitrogen gas to give **8**. MS m/z 530 (MH^+).

Compound 9 (Re-MAMA-C5-CH). To a solution of **7** (82 mg, 0.081 mmol) in TFA (4 mL) was added triethylsilane (0.2 mL), and the solution was mixed, and then the solvent was removed under a stream of nitrogen gas. The residue was resolved in a mixed solvent (22 mL, CH_2Cl_2/CH_3OH = 10:1), to which were added $(Ph_3P)_2ReOCl_3$ (135 mg, 0.162 mmol) and 1 M sodium acetate in methanol (4 mL). The reaction mixture was heated to reflux for 6 h and, after cooling to room temperature, mixed with ethyl acetate (60 mL) and filtered. Evaporation of the solvent gave a residue, which was purified by silica gel chromatography ($CHCl_3/CH_3OH$ = 49:1) and then RP-HPLC (acetonitrile/H_2O = 4:1) to give 20 mg (35%) of **9**. ^1H NMR (300 MHz, $CDCl_3$) δ 1.60−1.67 (m, 2H), 1.89−1.92 (m, 4H), 2.82−2.91 (m, 1H), 3.05 (s, 6H), 3.14−3.27 (m, 3H), 3.33−3.42 (m, 1H), 3.52−3.65 (m, 1H), 3.95−4.16 (m, 6H), 4.56−4.62 (m, 1H), 4.67 (d, J = 16.5 Hz, 1H), 6.70 (d, J = 8.7 Hz, 2H), 6.95 (d, J = 8.7 Hz, 2H), 7.35 (d, J = 15.6 Hz, 1H), 7.55 (d, J = 9.0 Hz, 2H), 7.79 (d, J = 15.6 Hz, 1H), 8.03 (d, J = 8.7 Hz, 2H). HRMS m/z $C_{28}H_{37}N_3O_4ReS_2$ found 730.1748, calcd 730.1783 (MH^+).

Compound 10 (Tr-BAT-C5-CH). To a solution of **5** (630 mg, 1.51 mmol) and **3** (855 mg, 1.12 mmol) in DMF (20 mL) was added DIPEA (2 mL). The reaction mixture was heated to reflux for 12 h. After the evaporation of the solvent, the residue was purified by silica gel chromatography using $CHCl_3$ and then ethyl acetate/hexane (1:2) to give 126 mg of **10** (10% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 1.32−1.37 (m, 12H), 1.70−1.77 (m, 2H), 2.20−2.35 (m, 10H), 2.85−3.01 (m, 4H), 3.04 (s, 6H), 3.98 (t, J = 6.5 Hz, 2H), 6.69 (d, J = 9.3 Hz, 2H), 6.93 (d, J = 8.7 Hz, 2H), 7.15−7.29 (m, 18H), 7.33−7.42 (m, 13H), 7.55 (d, J = 8.7 Hz, 2H), 7.79 (d, J = 15.6 Hz, 1H), 8.01 (d, J = 9.0 Hz, 2H). MS m/z 1100 (MH^+).

Compound 11 (BAT-C5-CH). To a solution of **10** in TFA (200 mL) was added triethylsilane (10 mL), and the solution was mixed. The solvent was removed under a stream of nitrogen gas to give **11**. MS m/z 516 (MH^+).

Compound 12 (Re-BAT-C5-CH). To a solution of **10** (110 mg, 0.100 mmol) in TFA (4 mL) was added triethylsilane (0.2 mL), and the solution was mixed, and then the solvent was removed under a stream of nitrogen gas. The residue was resolved in a mixed solvent (22 mL, CH_2Cl_2/CH_3OH = 10:1), to which were added $(Ph_3P)_2ReOCl_3$ (167 mg, 0.200 mmol) and 1 M sodium acetate in methanol (4 mL). The reaction mixture was heated to reflux for 6 h and, after cooling to room temperature, mixed with ethyl acetate (60 mL) and filtered. Evaporation of the solvent gave a residue, which was purified by silica gel chromatography ($CHCl_3/CH_3OH$ = 49:1) and then RP-HPLC (acetonitrile/H_2O = 4:1) to give 44 mg (61%) of **12**. ^1H NMR (300 MHz, $CDCl_3$) δ 1.60−1.73 (m, 2H), 1.87−1.92 (m, 4H), 2.72−2.78 (m, 1H), 2.96−3.00 (m, 2H), 3.05 (s, 6H), 3.21−3.41 (m, 4H), 3.53−3.63 (m, 1H), 3.76−3.92 (m, 3H), 4.06−4.17 (m, 5H), 6.70 (d, J = 8.7 Hz, 2H), 6.96 (d, J = 9.0 Hz, 2H), 7.36 (d, J = 15.6 Hz, 1H), 7.56 (d, J = 9.0 Hz, 2H), 7.79 (d, J = 15.6 Hz, 1H), 8.03 (d, J = 9.0 Hz, 2H). HRMS m/z $C_{28}H_{39}N_3O_3ReS_2$ found 716.1990, calcd 716.1990 (MH^+).

Compound 13 (Tr-MAMA-C3-CH). To a solution of **6** (981 mg, 2.53 mmol) and **2** (1.72 g, 2.53 mmol) in DMF (30 mL) was added DIPEA (3 mL). The reaction mixture was heated to reflux for 12 h. After the evaporation of the solvent, water was added. The mixture was extracted with $CHCl_3$. The organic layers were combined and dried with Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by silica gel chromatography (ethyl acetate/hexane = 3:2) to give 714 mg of **13** (29% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 1.80−1.87 (m, 2H), 2.28 (t, J = 6.3 Hz, 2H), 2.33−2.43 (m, 4H), 2.51 (t, J = 6.9 Hz, 2H), 2.91 (s, 2H), 2.99−3.03 (m, 2H), 3.05 (s, 6H), 3.99 (t, J = 5.9 Hz, 2H), 6.70 (d, J = 9.0 Hz, 2H), 6.86 (d, J = 9.0 Hz, 2H), 7.14−7.27 (m, 18H), 7.29−7.38 (m, 13H), 7.54 (d, J = 8.7 Hz, 2H), 7.78 (d, J = 15.3 Hz, 1H), 7.93 (d, J = 9.0 Hz, 2H). MS m/z 986 (MH^+).

Compound 14 (MAMA-C3-CH). To a solution of **13** in TFA (200 mL) was added triethylsilane (10 mL), and the solution was mixed. The solvent was removed under a stream of nitrogen gas to give **14**. MS m/z 502 (MH^+).

Compound 15 (Re-MAMA-C3-CH). To a solution of **13** (99 mg, 0.100 mmol) in TFA (4 mL) was added triethylsilane (0.2 mL), and the solution was mixed, and then the solvent was removed under a stream of nitrogen gas. The residue was resolved in a mixed solvent (22 mL, CH_2Cl_2/CH_3OH = 10:1), to which were added $(Ph_3P)_2ReOCl_3$ (167 mg, 0.200 mmol) and 1 M sodium acetate in methanol (4 mL). The reaction mixture was heated to reflux for 6 h and, after cooling to room temperature, mixed with ethyl acetate (60 mL) and filtered. Evaporation of the solvent gave a residue, which was purified with silica gel chromatography ($CHCl_3/CH_3OH$ = 49:1) and then RP-HPLC (acetonitrile/H_2O = 4:1) to give 20 mg (29%) of **15**. ^1H NMR (300 MHz, $CDCl_3$) δ 1.65−1.75 (m, 1H), 2.32−2.37 (m, 2H), 2.90−2.98 (m, 1H), 3.05 (s, 6H), 3.15−3.34 (m, 3H), 3.40−3.52 (m, 1H), 3.81−3.86 (m, 1H), 4.09−4.19 (m, 5H), 4.58−4.64 (m, 1H), 4.74 (d, J = 16.2 Hz, 1H), 6.70 (d, J = 9.0 Hz, 2H), 6.96 (d, J = 8.7 Hz, 2H), 7.40 (d, J = 15.3 Hz, 1H), 7.56 (d, J = 8.7 Hz, 2H), 7.79 (d, J = 15.3 Hz, 1H), 8.03 (d, J = 9.0 Hz, 2H). HRMS m/z $C_{26}H_{33}N_3O_4ReS_2$ found 702.1476, calcd 702.1470 (MH^+).

Compound 16 (Tr-BAT-C3-CH). To a solution of **6** (243 mg, 0.626 mmol) and **3** (479 mg, 0.626 mmol) in DMF (10 mL) was added DIPEA (1 mL). The reaction mixture was heated to reflux for 12 h. After the evaporation of the solvent, a saturated NaCl solution was added. The mixture was extracted with $CHCl_3$. The organic layers were combined and dried with Na_2SO_4. Evaporation of the solvent gave a residue, which was purified by silica gel chromatography

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using $CHCl_3$ and then ethyl acetate/hexane (1:2) to give 128 mg of **16** (19% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.37 (s, 9H), 1.73 (t, J = 6.0 Hz, 2H), 2.24−2.38 (m, 10H), 2.88−2.95 (m, 4H), 3.04 (s, 6H), 3.97 (t, J = 6.2 Hz, 2H), 6.70 (d, J = 9.0 Hz, 2H), 6.89 (d, J = 8.7 Hz, 2H), 7.15−7.31 (m, 19H), 7.37−7.41 (m, 12H), 7.54 (d, J = 9.0 Hz, 2H), 7.78 (d, J = 15.6 Hz, 1H), 7.96 (d, J = 8.7 Hz, 2H). MS m/z 1072 (MH^+).

Compound 17 (BAT-C3-CH). To a solution of **16** in TFA (200 mL) was added triethylsilane (10 mL), and the solution was mixed. The solvent was removed under a stream of nitrogen gas to give **17**. MS m/z 488 (MH^+).

Compound 18 (Re-BAT-C3-CH). To a solution of **16** (104 mg, 0.097 mmol) in TFA (4 mL) was added triethylsilane (0.2 mL), and the solution was mixed, and then the solvent was removed under a stream of nitrogen gas. The residue was resolved in a mixed solvent (22 mL, CH_2Cl_2/CH_3OH = 10:1), to which were added $(Ph_3P)_2ReOCl_3$ (167 mg, 0.200 mmol) and 1 M sodium acetate in methanol (4 mL). The reaction mixture was heated to reflux for 6 h and, after cooling to room temperature, mixed with ethyl acetate (60 mL) and filtered. Evaporation of the solvent gave a residue, which was purified with silica gel chromatography ($CHCl_3/CH_3OH$ = 49:1) and then RP-HPLC (acetonitrile/H_2O = 4:1) to give 44 mg (66%) of **18**. ^{1}H NMR (300 MHz, $CDCl_3$) δ1.75−1.85 (m, 1H), 2.31−2.38 (m, 2H), 2.76−2.82 (m, 1H), 2.91−3.08 (m, 2H), 3.05 (s, 6H), 3.24−3.49 (m, 4H), 3.76−4.00 (m, 3H), 4.12−4.33 (m, 5H), 6.70 (d, J = 9.0 Hz, 2H), 6.95 (d, J = 9.0 Hz, 2H), 7.35 (d, J = 15.3 Hz, 1H), 7.56 (d, J = 9.0 Hz, 2H), 7.79 (d, J = 15.3 Hz, 1H), 8.03 (d, J = 8.7 Hz, 2H). HRMS m/z $C_{26}H_{35}N_3O_3ReS_2$ found 688.1631, calcd 688.1677 (MH^+).

^{99m}Tc Labeling Reaction

To a solution of sodium heptonate dehydrate (2.0 g, 7.04 mmol) in nanopure water (25 mL) was added a 0.75 mL aliquot of a $SnCl_2 \cdot 2H_2O$ solution [12 mg of Tin(II) chloride dehydrate (53.2 mmol) was dissolved in 0.1 M HCl (15 μL)]. This solution was adjusted to pH 8.5−9.0 with a small amount of 0.1 M NaOH and then lyophilized to give tin 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 solutions of **7**, **10**, **13**, and **16** (0.5 mg) in TFA (200 μL) was added triethylsilane (10 μL), and the solutions were mixed, and then the solvents were removed under a stream of nitrogen gas. The residues were resolved in acetonitrile (200 μL), to which were added a 0.1 M HCl solution (15 μL) and the ^{99m}TcGH solution (200 mL). The reaction mixtures were heated to 80−90 °C for 10 min. The residue taken up in 100 μL of acetonitrile was purified by a reversed-phase HPLC system with an isocratic solvent of acetonitrile/H_2O (3/2) as the mobile phase at a flow rate of 1.0 mL/min. After cooling to room temperature, the mixtures were purified with RP-HPLC to give [^{99m}Tc]**8**, [^{99m}Tc]**11**, [^{99m}Tc]**14**, and [^{99m}Tc]**17**. The desired fraction was collected in a flask and evaporated dry. The radiochemical identity of [^{99m}Tc]**8**, [^{99m}Tc]**11**, [^{99m}Tc]**14**, and [^{99m}Tc]**17** was verified with the corresponding Re-complex from the HPLC profiles. The final ^{99m}Tc-complexes showed a single peak of radioactivity at retention times of 14.2, 20.3, 9.3, and 12.4 min, respectively, close to those of the Re complexes. The four ^{99m}Tc-complexes were obtained in 46−95% radiochemical yields with a radiochemical purity of >95% after HPLC.

Partition Coefficient

Partition coefficients were measured by mixing the [^{99m}Tc]tracer with 3 mL each of 1-octanol and buffer (0.1 M phosphate, pH 7.4) in a test tube. The test tube was vortexed for 3 min at room temperature, then centrifuged for 5 min. Two weighed samples (0.5 mL) from the 1-octanol and buffer layers were measured in a well counter. The partition coefficient was determined by calculating the ratio of counts per minute per gram of 1-octanol to that of buffer. Samples from the 1-octanol layer were repartitioned until consistent partitions of coefficient values were obtained. The measurement was done in triplicate and repeated three times.

Binding Assays Using the Aggregated Aβ Peptides in Solution

A solid form of 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 solutions were incubated at 37 °C for 42 h with gentle and constant shaking. The binding assay was performed by mixing 50 μL of Aβ(1−42) aggregates (0−100 μg/mL), 50 μL of an appropriate concentration of the ^{99m}Tc-labeled form ([^{99m}Tc]**8**, [^{99m}Tc]**11**, [^{99m}Tc]**14**, [^{99m}Tc]**17**), and 900 μL of 30% ethanol in 12 mm × 75 mm borosilicate glass tubes. Nonspecific binding was defined in the presence of 1 μM of chalcone derivative (4-dimethylamino-4′-iodo-chalcone) (*20*). After incubation for 3 h at room temperature, the mixture was filtered through GF/B filters (Whatman, Kent, U.K.) using an M-24 cell harvester (Brandel, Gaithersburg, MD). Filters containing the bound ^{99m}Tc-labeled form were examined in a γ counter (Perkin-Elmer, WIZARD^2 2470).

Biodistribution in Normal Mice

The experiments with animals were conducted in accordance with our institutional guidelines and approved by the Nagasaki University Animal Care Committee. A saline solution (100 μL) of ^{99m}Tc-chalcones (1.0×10^7 cpm/mL) containing ethanol (30 μL) was injected intravenously directly into the tail of ddY mice (5 weeks old, 22−25 g). The mice were sacrificed at various time points postinjection. The organs of interest were removed and weighed, and radioactivity was measured with an automatic γ counter (Perkin-Elmer, WIZARD^2 2470).

Neuropathological Staining of Mouse Brain Sections

The Tg2576 transgenic (female, 30-month-old) and wild-type mice (female, 30-month-old) mice were used as the Alzheimer's model and control, respectively. After the mice were sacrificed by decapitation, the brains were immediately removed and frozen in powdered dry ice. The frozen blocks were sliced into serial sections, 10 μm thick. Each slide was incubated with a 50% EtOH solution (2.5−10 μM) of **9**, **12**, **15**, and **18** for 2−9 h. The sections were washed in 50% EtOH for 5 min two times and examined for **9**, **12**, **15**, and **18** with excitation of 458 nm using a microscope (Carl Zeiss, LSM710). Thereafter, the serial sections were also stained with thioflavin S, a pathological dye commonly used for staining β-amyloid plaques in the brain.

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Note Added after ASAP Publication

This paper was published on the Web on July 8, 2010, with an error in Figure 3. The corrected version was reposted on July 13, 2010.

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