Supporting Information

Structure-activity Relationships of Radioiodinated 6,5,6-Tricyclic Compounds for the Development of Tau Imaging Probes

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Materials and Methods

General remarks.

All reagents were obtained commercially and used without further purification unless otherwise indicated. High-performance liquid chromatography (HPLC) was performed with a Shimadzu system (an LC-20AD pump with an SPD-20A UV detector, λ = 254 nm; Shimadzu, Kyoto, Japan) using a Cosmosil C_{18} column (5C_{18}-AR-II, 4.6ID × 150 mm, Nacalai Tesque, Kyoto, Japan). ^{1}H NMR and ^{13}C NMR spectra were obtained on a JEOL JNM400 (JEOL, Tokyo, Japan) with TMS as an internal standard. Coupling constants are reported in hertz. Multiplicity was defined by s (singlet), d (doublet), and m (multiplet). Mass spectra were obtained on a SHIMADZU LCMS-2020 EV. High-resolution mass spectrometry (HRMS) was carried out with a JEOL JMS-700 (JEOL).

Synthesis of 4'-bromo-*N,N*-dimethyl-2'-nitro-[1,1'-biphenyl]-4-amine (1).

A mixture of 1,4-dibromo-2-nitrobenzene (281 mg, 1.00 mmol), *N,N*-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (247 mg, 1.00 mmol), $Pd(PPh_3)_4$ (46 mg, 0.04 mmol), and $NaHCO_3$ (2 mL of 1 M aqueous solution, 2.00 mmol) in toluene (10 mL) and EtOH (6 mL) was stirred under reflux for 18 h. The

mixture was extracted with ethyl acetate (60 mL), and the organic phase was separated and dried over Na_2SO_4. The solvent was removed and the residue was purified by silica gel chromatography (ethyl acetate/hexane = 1/4) to give **1** (184 mg, 57.6%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (s, 1H), 7.65 (d, J = 4.0 Hz, 1H), 7.30 (d, J = 8.4 Hz, 1H), 7.16 (d, J = 8.0 Hz, 2H), 6.72 (d, J = 9.2 Hz, 2H), 2.98 (s, 6H). MS (ESI) *m/z* calcd. for $C_{14}H_{13}BrN_2O_2$ (MH^+) 321.0, found 321.0.

Synthesis of 4-(5-bromo-3-nitropyridin-2-yl)-*N,N*-dimethylaniline (2).

The same reaction described above to prepare **1** was used, and 266 mg of **2** was obtained from 2,5-dibromo-3-nitropyridine and *N,N*-dimethyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline at a yield of 33.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.80 (s, 1H), 8.14 (s, 1H), 7.49 (d, J = 2.8 Hz, 1H), 7.47 (d, J = 1.6 Hz, 1H), 6.72 (d, J = 9.2 Hz, 2H), 3.03 (s, 6H). MS (ESI) *m/z* calcd. for $C_{13}H_{12}BrN_3O_2$ (MH^+) 322.0, found 322.0.

Synthesis of 5-bromo-*N,N*-dimethyl-3-nitro-[2,3'-bipyridin]-6'-amine (3).

The same reaction described above to prepare **1** was used, and 321 mg of **3** was obtained from 2,5-dibromo-3-nitropyridine and

N,N-dimethyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridine-2-amine at a yield of 66.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.77 (s, 1H), 8.36 (s, 1H), 8.15 (s, 1H), 7.57 (s, 1H), 6.47 (s, 1H), 3.09 (s, 6H). MS (ESI) *m/z* calcd. for $C_{12}H_{11}BrN_4O_2$ (MH^+) 323.0, found 322.9.

Synthesis of 7-bromo-*N,N*-dimethyl-9*H*-carbazol-2-amine (4).

A mixture of **1** (184 mg, 0.58 mmol) and PPh_3 (378 mg, 1.44 mmol) in *o*-dichlorobenzene (12 mL) was stirred under reflux for 5 h. The mixture was extracted with chloroform (60 mL), and the organic phase was separated and dried over Na_2SO_4. The solvent was removed and the residue was purified by silica gel chromatography (ethyl acetate/hexane = 1/4) to give **4** (55.9 mg, 33.4%). ^{1}H NMR (400 MHz, acetone-d_6) δ 10.1 (s, 1H), 7.87 (d, J = 9.6, 1H), 7.82 (d, J = 8.4 Hz, 1H), 7.53 (s, 1H), 7.20 (d, J = 8.4 Hz, 1H), 6.75 (s, 1H), 6.73 (d, J = 4.8 Hz, 1H), 3.00 (s, 6H). MS (ESI) *m/z* calcd. for $C_{14}H_{13}BrN_2$ (MH^+) 289.0, found 289.2.

Synthesis of 3-bromo-*N,N*-dimethyl-5*H*-pyrido[3,2-b]indol-7-amine (5).

The same reaction described above to prepare **4** was used, and 70 mg of **5** was obtained from **2** at a yield of 29.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 8.10 (d,

J = 9.2 Hz, 1H), 7.84 (s, 1H), 7.70 (s, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 6.60 (s, 1H), 3.08 (s, 6H). MS (ESI) *m/z* calcd. for $C_{13}H_{12}BrN_3$ (MH^+) 290.0, found 290.1.

Synthesis of 3-bromo-*N*,*N*-dimethyl-5*H*-pyrrolo[2,3-b:4,5-b']dipyridin-7-amine (6).

The same reaction described above to prepare **4** was used, and 169 mg of **6** was obtained from **3** at a yield of 57.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.6 (s, 1H), 8.34 (s, 1H), 8.18 (d, *J* = 8.8 Hz, 1H), 7.79 (s, 1H), 6.64 (d, *J* = 8.8 Hz, 1H), 3.15 (s, 6H). MS (ESI) *m/z* calcd. for $C_{12}H_{11}BrN_4$ (MH^+) 291.0, found 290.9 [MH^+].

Synthesis of *N*,*N*-dimethyl-7-(tributylstannyl)-9*H*-carbazol-2-amine (7).

A mixture of **4** (55.9 mg, 0.19 mmol), bis(tributyltin) (194 μL, 0.38 mmol), and $Pd(PPh_3)_4$ (96.4 mg, 0.083 mmol) in a mixed solvent (22.0 mL, dioxane/Et_3N/DMF = 6/3/2) was stirred under reflux for 3 h. The solvent was removed, and the residue was purified by silica gel chromatography (ethyl acetate/hexane = 1/4) to give **7** (5.6 mg, 5.9%). ^{1}H NMR (400 MHz, acetone-d_6) δ 9.73 (s, 1H), 7.76 (d, *J* = 7.6 Hz, 1H), 7.73 (d, *J* = 8.8 Hz, 1H), 7.39 (s, 1H), 7.06 (d, *J* = 7.2 Hz, 1H), 6.63 (s, 1H), 6.60 (d, *J* = 8.0 Hz, 1H), 2.88 (s, 6H), 1.51-1.46 (m, 6H), 1.27-1.23 (m, 6H), 1.02-0.98 (m, 6H), 0.78-0.74 (m, 9H). MS (ESI) *m/z* calcd. for $C_{26}H_{40}N_2Sn$ (MH^+) 501.2, found 501.2.

Synthesis of *N,N*-dimethyl-3-(tributylstannyl)-5*H*-pyrido[3,2-b]indol-7-amine (8).

The same reaction described above to prepare **7** was used, and 11.3 mg of **8** was obtained from **5** at a yield of 9.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.35 (s, 1H), 8.08 (d, J = 4.0 Hz, 1H), 7.77 (s, 1H), 7.54 (s, 1H), 6.73 (d, J = 8.8 Hz, 1H), 6.55 (s, 1H), 3.00 (s, 6H), 1.52-1.48 (m, 6H), 1.30-1.25 (m, 6H), 1.07-1.03 (m, 6H), 0.83-0.80 (m, 9H). MS (ESI) *m/z* calcd. for $C_{25}H_{39}N_3Sn$ (MH^+) 502.2, found 502.1 [MH^+].

Synthesis of *N,N*-dimethyl-3-(tributylstannyl)-5*H*-pyrrolo[2,3-b:4,5-b']dipyridin-7-amine (9).

The same reaction described above to prepare **7** was used, and 50.2 mg of **9** was obtained from **6** at a yield of 17.1%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.27 (s, 1H), 8.42 (s, 1H), 8.34 (d, J = 8.8 Hz, 1H), 7.65 (s, 1H), 6.54 (d, J = 8.4 Hz, 1H), 3.22 (s, 6H), 1.60-1.52 (m, 6H), 1.37-1.31 (m, 6H), 1.13-1.09 (m, 6H), 0.90-0.86 (m, 9H). MS (ESI) *m/z* calcd. for $C_{24}H_{38}N_4Sn$ (MH^+) 503.2, found 502.9.

Synthesis of 7-iodo-*N,N*-dimethyl-9*H*-carbazol-2-amine (10).

To a solution of **7** (5.6 mg, 0.0112 mmol) in chloroform (5.0 mL) was added a

solution of iodine (3.7 mg in 5.0 mL chloroform) at room temperature. The mixture was stirred for 1.5 h, and saturated $NaHSO_3$ aq. was added to quench the reaction. The mixture was extracted with chloroform (60 mL), and the organic phase was separated and dried over Na_2SO_4. The solvent was removed and the residue was purified by silica gel chromatography (ethyl acetate/hexane = 1/2) to give **10** (2.1 mg, 62.4%). ^{1}H NMR (400 MHz, acetone-d_6) δ 9.94 (s, 1H), 7.75 (d, J = 8.8 Hz, 1H), 7.62 (d, J = 1.2 Hz, 1H), 7.60 (s, 1H), 7.26 (d, J = 4.0 Hz, 1H), 6.64-6.61 (m, 2H), 2.89 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 150.2, 141.8, 140.9, 126.6, 122.8, 120.8, 120.5, 118.5, 112.5, 106.9, 93.1, 87.3, 40.6. HRMS (FAB) *m/z* calcd. for $C_{14}H_{14}IN_2$ (M^+) 337.0202, found 337.0210.

Synthesis of 3-iodo-*N*,*N*-dimethyl-5*H*-pyrido[3,2-b]indol-7-amine (11).

The same reaction described above to prepare **10** was used, and 4.70 mg of **11** was obtained from **8** at a yield of 63.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.0 (s, 1H), 8.41 (s, 1H), 8.00 (s, 1H), 7.90 (d, J = 4.8 Hz, 1H), 6.75 (d, J = 7.6 Hz, 1H), 6.64 (s, 1H), 3.00 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 151.4, 145.2, 143.2, 141.2, 134.0, 123.8, 120.8, 111.2, 107.6, 92.8, 85.9, 40.4. MS (ESI) *m/z* calcd. for $C_{13}H_{12}IN_3$ (MH^+) 338.0, found 337.9 [MH^+].

Synthesis of 3-iodo-*N*,*N*-dimethyl-5*H*-pyrrolo[2,3-b:4,5-b']dipyridin-7-amine (12).

The same reaction described above to prepare **10** was used, and 25.1 mg of **12** was obtained from **9** at a yield of 82.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.5 (s, 1H), 8.45 (s, 1H), 8.15 (d, J = 8.8 Hz, 1H), 7.91 (s, 1H), 6.61 (d, J = 8.8 Hz, 1H), 3.13 (s, 6H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 159.8, 153.5, 142.6, 137.0, 132.9, 130.6, 126.3, 101.9, 100.8, 84.2, 38.1. HRMS (FAB) *m/z* calcd. for $C_{12}H_{12}IN_4$ (M+) 339.0107, found 339.0103.

Synthesis of 2-bromo-*N*,*N*-dimethylpyridin-4-amine (13).

To a solution of 2-bromopyridin-4-amine (418 mg, 2.41 mmol) in dry DMF (15 mL) was added NaH (116 mg, 4.83 mmol) on an ice bath. The reaction mixture was stirred under an argon atmosphere for 30 min. Then, CH_3I (587 μL, 7.24 mmol) was added to the solution. The mixture was stirred for 3 h at room temperature, and deionized water was added to quench the reaction. The mixture was extracted with ethyl acetate (60 mL), and the organic phase was separated and dried over Na_2SO_4. The solvent was removed and the residue was purified by silica gel chromatography (ethyl acetate/hexane = 1/1) to give **13** (284 mg, 58.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92

(d, J = 3.2 Hz, 1H), 6.62 (s, 1H), 6.42 (d, J = 6.4 Hz, 1H), 2.98 (s, 6H). MS (ESI) *m/z* calcd. for $C_7H_9BrN_2$ (MH^+) 201.0, found 201.0 [MH^+].

Synthesis of 6-bromo-*N*,*N*-dimethylpyrimidin-4-amine (14).

The same reaction described above to prepare **13** was used, and 282 mg of **14** was obtained from 2-bromopyrimidin-4-amine at a yield of 59.2%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 6.50 (s, 1H), 3.01 (s, 6H). MS (ESI) *m/z* calcd. for $C_6H_8BrN_3$ (MH^+) 202.0, found 201.9 [MH^+].

Synthesis of 3-bromo-*N*,*N*-dimethylimidazo[1,2-a:5,4-b']dipyridin-7-amine (15).

A mixture of **13** (284 mg, 1.42 mmol), 2,5-dibromopyridin-3-amine (537 mg, 2.13 mmol), CuI (54.5 mg, 0.28 mmol), Cs_2CO_3 (1.40 g, 4.29 mmol), and 1,10-phenanthroline (103 mg, 0.57 mmol) in xylene (20.0 mL) was stirred under reflux for 24 h. The solvent was removed, and the residue was purified by silica gel chromatography (ethyl acetate/hexane = 1/1) to give **15** (22.7 mg, 7.8%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J = 8.0 Hz, 1H), 8.23 (s, 1H), 8.03 (s, 1H), 6.63 (d, J = 7.6 Hz 1H), 6.41 (s, 1H), 3.15 (s, 6H). MS (ESI) *m/z* calcd. for $C_{12}H_{11}BrN_4$ (MH^+) 291.0, found 290.9 [MH^+].

Synthesis of 7-bromo-*N*,*N*-dimethylbenzo[4,5]imidazo[1,2-c]pyrimidin-3-amine (16).

The same reaction described above to prepare **15** was used, and 21.7 mg of **16** was obtained from **14** and 2,5-dibromoaniline at a yield of 7.48%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.91 (s, 1H), 7.82 (s, 1H), 7.63 (d, J = 8.4 Hz, 1H), 7.32 (d, J = 3.2, 1H), 6.19 (s, 1H), 3.18 (s, 6H). MS (ESI) *m/z* calcd. for $C_{12}H_{11}BrN_4$ (MH^+) 291.0, found 290.9 [MH^+].

Synthesis of 3-bromo-*N*,*N*-dimethylpyrido[3',2':4,5]imidazo[1,2-c]pyrimidin-7-amine (17).

The same reaction described above to prepare **15** was used, and 50.1 mg of **17** was obtained from **14** and 2,5-dibromopyridin-3-amine at a yield of 10.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.09 (s, 1H), 8.16 (s, 1H), 7.93 (s, 1H), 6.05 (s, 1H), 3.14 (s, 6H). MS (ESI) *m/z* calcd. for $C_{11}H_{10}BrN_5$ (MH^+) 292.0, found 292.0 [MH^+].

Synthesis of *N*,*N*-dimethyl-3-(tributylstannyl)imidazo[1,2-a:5,4-b']dipyridin-7-amine (18).

The same reaction described above to prepare **7** was used, and 6.80 mg of **18** was obtained from **15** at a yield of 17.3%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.50 (d, J = 7.6 Hz, 1H), 8.20 (s, 1H), 8.04 (s, 1H), 6.60 (d, J = 8.0 Hz, 1H), 6.42 (s, 1H), 3.14 (s, 6H), 1.59-1.55 (m, 6H), 1.37-1.31 (m, 6H), 1.16-1.11 (m, 6H), 0.90-0.86 (m, 9H). MS (ESI) *m/z* calcd. for $C_{24}H_{38}N_4Sn$ (MH^+) 503.2, found 503.2 [MH^+].

Synthesis of *N,N*-dimethyl-7-(tributylstannyl)benzo[4,5]imidazo[1,2-c]pyrimidin-3-amine (19).

The same reaction described above to prepare **7** was used, and 4.41 mg of **19** was obtained from **16** at a yield of 12.5%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.94 (s, 1H), 7.83 (s, 1H), 7.76 (d, J = 8.0 Hz, 1H), 7.29 (d, J = 8.0 Hz, 1H), 6.20 (s, 1H), 3.18 (s, 6H), 1.60-1.53 (m, 6H), 1.37-1.31 (m, 6H), 1.12-1.08 (m, 6H), 0.90-0.86 (m, 9H). MS (ESI) *m/z* calcd. for $C_{24}H_{38}N_4Sn$ (MH^+) 503.2, found 503.2 [MH^+].

Synthesis of *N,N*-dimethyl-3-(tributylstannyl)pyrido[3',2':4,5]imidazo[1,2-c]pyrimidin-7-amine (20).

The same reaction described above to prepare **7** was used, and 13.4 mg of **20**

was obtained from **17** at a yield of 15.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.22 (s, 1H), 8.21 (s, 1H), 8.02 (s, 1H), 6.14 (s, 1H), 3.21 (s, 6H), 1.61-1.53 (m, 6H), 1.37-1.31 (m, 6H), 1.16-1.12 (m, 6H), 0.90-0.87 (m, 9H). MS (ESI) *m/z* calcd. for $C_{23}H_{37}N_5Sn$ (MH^+) 504.2, found 503.9 [MH^+].

Synthesis of 3-iodo-*N*,*N*-dimethylimidazo[1,2-a:5,4-b']dipyridin-7-amine (21).

The same reaction described above to prepare **10** was used, and 3.28 mg of **21** was obtained from **18** at a yield of 71.7%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.45 (d, J = 6.8 Hz, 1H), 8.37 (s, 1H), 8.23 (s, 1H), 6.64 (d, J = 6.8 Hz, 1H), 6.42 (s, 1H), 3.15 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 152.4, 152.0, 144.0, 141.5, 140.0, 132.1, 124.2, 102.9, 90.1, 87.8, 40.1. HRMS (FAB) *m/z* calcd. for $C_{12}H_{12}IN_4$ (M^+) 339.0107, found 339.0115.

Synthesis of 7-iodo-*N*,*N*-dimethylbenzo[4,5]imidazo[1,2-c]pyrimidin-3-amine (22).

The same reaction described above to prepare **10** was used, and 2.75 mg of **22** was obtained from **19** at a yield of 92.9%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.83 (s, 1H), 8.00 (s, 1H), 7.45 (d, J = 8.0 Hz, 1H), 7.41 (d, J = 8.4 Hz, 1H), 6.12 (s, 1H), 3.11 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.4, 149.3, 137.7, 128.3, 127.1, 110.9, 90.4, 82.2,

38.3, 29.6, 29.3. HRMS (FAB) *m/z* calcd. for $C_{12}H_{11}IN_4$ (M^+) 339.0107, found 339.0113.

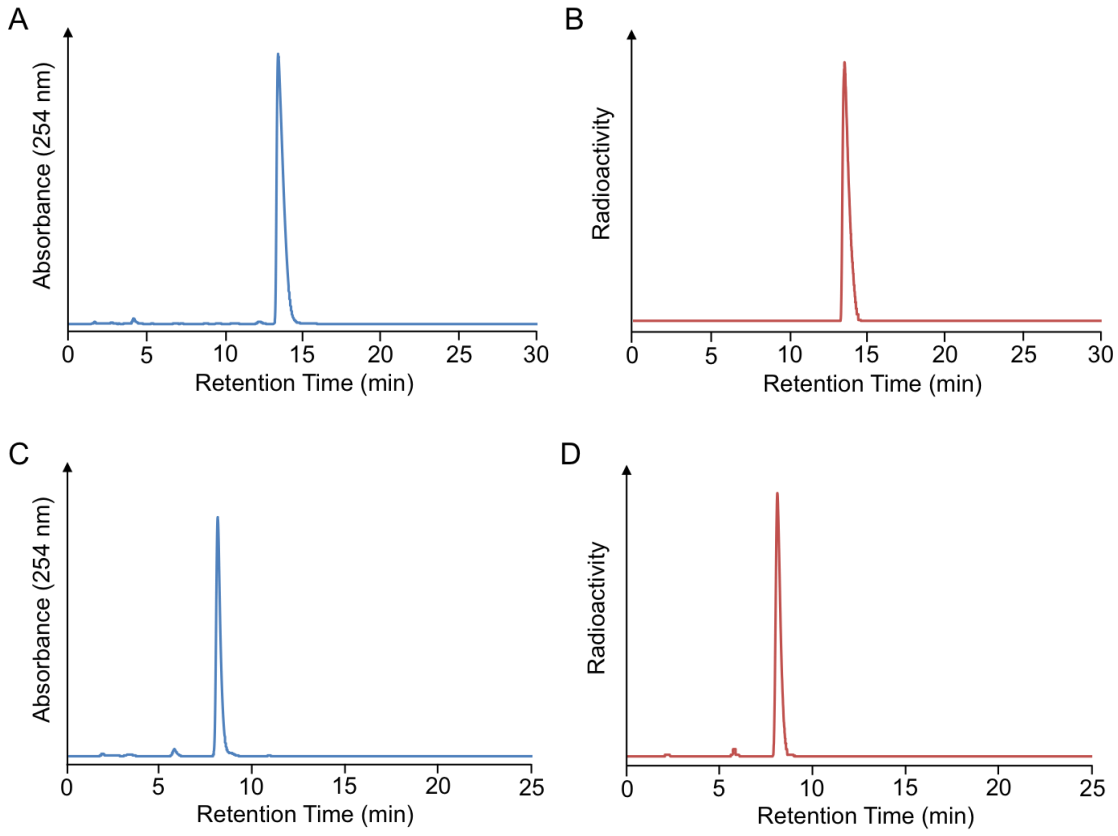
Synthesis of 3-iodo-*N,N*-dimethylpyrido[3',2':4,5]imidazo[1,2-c]pyrimidin-7-amine (23).

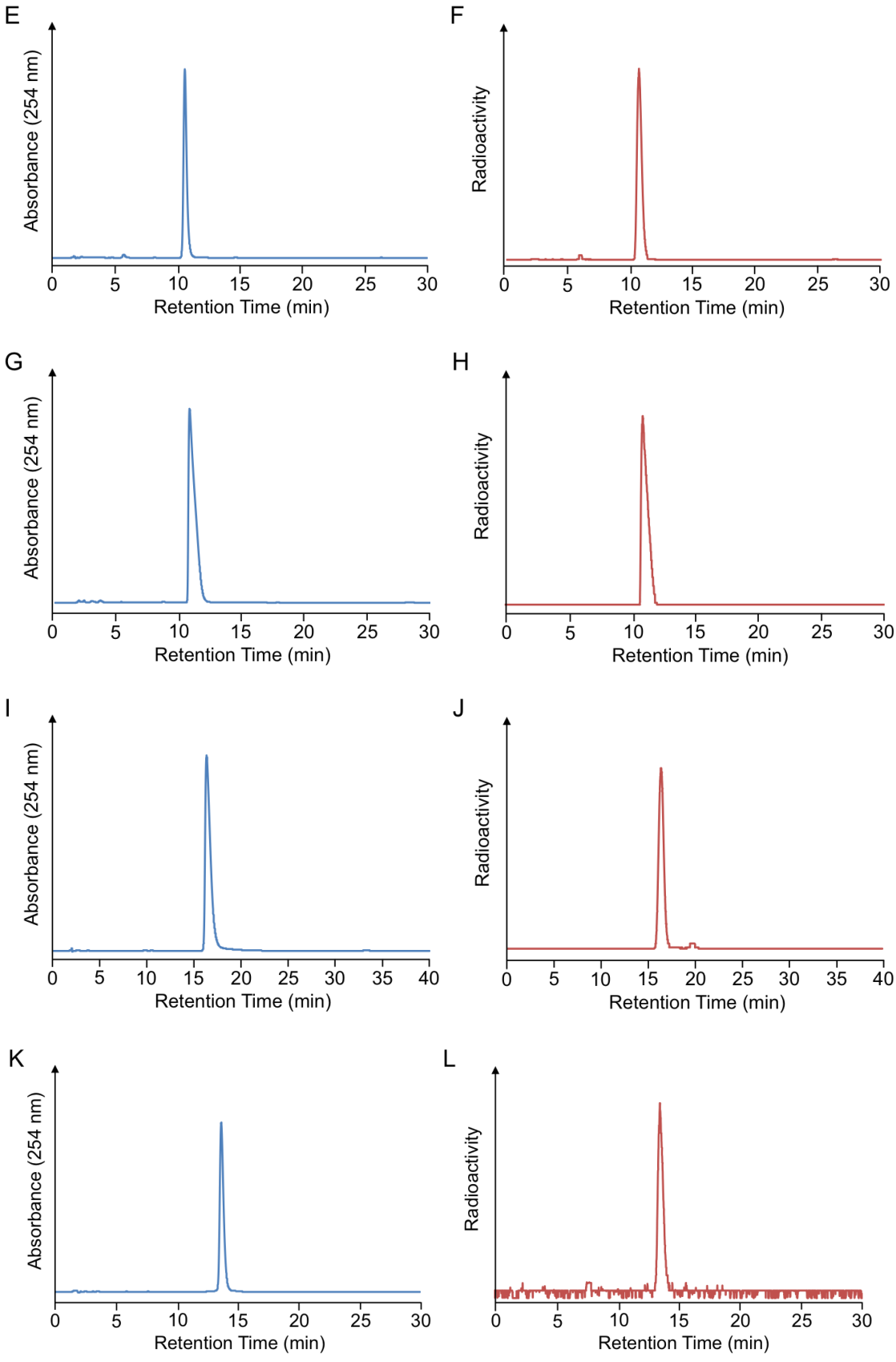
The same reaction described above to prepare **10** was used, and 8.00 mg of **23** was obtained from **20** at a yield of 88.6%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.17 (s, 1H), 8.38 (s, 1H), 8.21 (s, 1H), 6.14 (s, 1H), 3.21 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.4, 145.1, 140.4, 140.1, 137.3, 132.6, 89.5, 81.9, 38.3, 29.7. HRMS (FAB) *m/z* calcd. for $C_{11}H_{11}IN_5$ (M^+) 340.0059, found 340.0052.

Radiolabeling

The radioiodinated forms of ligands were prepared from the corresponding tributyltin precursors by iododestannylation. Briefly, to initiate the reaction, a tributyltin precursor (200 μg/100 μL EtOH) was added to a mixture of [^{125}I]NaI (3.70-7.40 MBq, specific activity: 81.4 TBq/mmol) in 20 μL EtOH, 50 μL of 3% H_2O_2 aq., and 50 μL of 1 N HCl aq. in a sealed vial. The reaction to obtain radioiodinated ligands was allowed

to proceed at room temperature for 5 min and terminated by the addition of saturated $NaHSO_3$ aq. (100 μL). After neutralization with $NaHCO_3$ and extraction with ethyl acetate, the organic phase was dried by passing through a column filled with anhydrous Na_2SO_4. The solution was gas-dried with a stream of nitrogen gas. The crude radioiodinated ligands were purified by HPLC on a COSMOSIL $5C_{18}$-AR-II column with an isocratic solvent of acetonitrile/H_2O (with TFA) at a flow rate of 1.0 mL/min.

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Absorbance (254 nm)
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Radioactivity
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Retention Time (min)

Figure S1. Representative HPLC profiles. UV chromatogram at 254 nm of **10** (A), **11** (C), **12** (E), **21** (G), **22** (I), and **23** (K). Radio-chromatogram of [^{125}I]**10** (B), [^{125}I]**11** (D), [^{125}I]**12** (F), [^{125}I]**21** (H), [^{125}I]**22** (J), and [^{125}I]**23** (L). These data were obtained under the following HPLC mobile phase conditions: MeCN (0.1% TFA) / H_2O (0.1% TFA) = 30 / 70 (A and B), MeCN (0.1% TFA) / H_2O (0.1% TFA) = 23 / 77 (C and D), M MeCN (0.1% TFA) / H_2O (0.1% TFA) = 20 / 80 (E, F, G, H, I, and J), MeCN (0.1% TFA) / H_2O (0.1% TFA) = 18 / 82 (K and L), respectively.

Immunohistochemical staining

We performed immunohistochemical staining of Alzheimer's disease brain sections according to a method reported previously.[1]

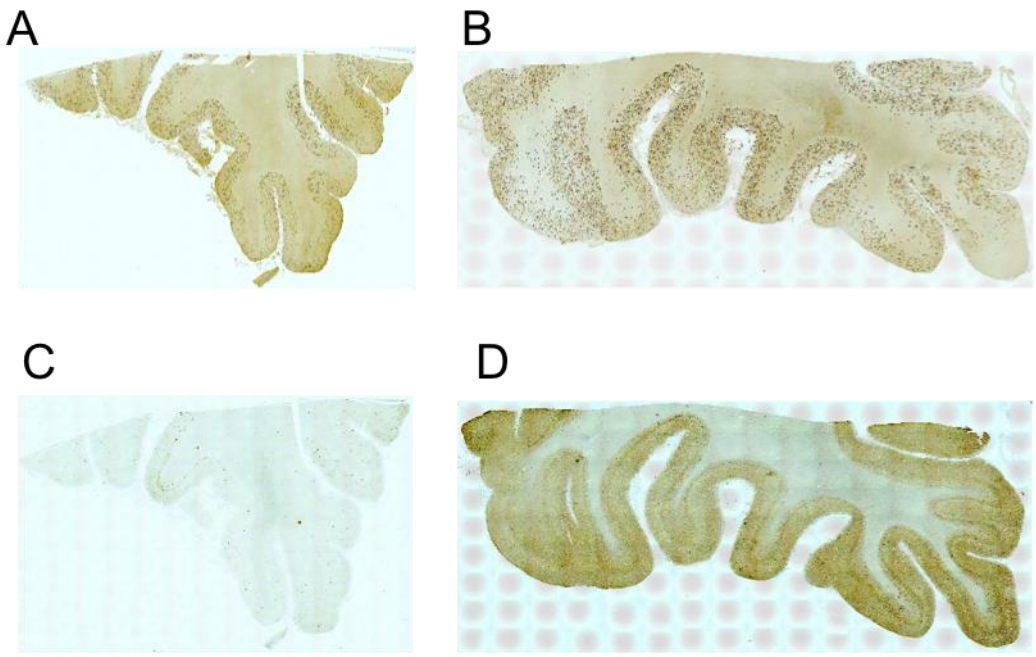

Figure S2. Immunohistochemical staining with antibodies against Aβ (A and B) and phosphorylated tau (C and D) of Alzheimer's disease brain sections.

***In vitro* autoradiography**

[^{125}I]IMPY and [^{125}I]BIP-NMe$_2$ were synthesized according to a method reported previously.[2-3] Six-micrometer-thick serial sections of paraffin-embedded blocks were used for staining. The sections were subjected to two 15-min incubations in xylene, two 1-min incubations in 100% EtOH, one 1-min incubation in 90% EtOH, and one 1-min incubation in 70% EtOH to completely deparaffinize them, followed by two 2.5-min washes in water. The sections were incubated with radioiodinated ligands (370 kBq/mL in 10% EtOH) for 2 h at room temperature. They were then dipped in 50% EtOH for 1 h and washed with H_2O for 1 min. After drying, the ^{125}I-labeled sections were exposed to a BAS imaging plate (Fuji Film, Tokyo, Japan) overnight. Autoradiographic images were obtained using an American Typhoon scanner system (GE Healthcare Life Sciences, Illinois, USA).

***In vitro* blocking study**

We used the same postmortem brain tissues as employed for *in vitro* autoradiography. The sections were incubated with radioiodinated ligands (370 kBq/mL in 10% EtOH) for 2 h at room temperature in the presence of nonradioactive ligands (100 μM). After incubation, we dipped the sections and exposed them to a BAS imaging plate with the same methods as *in vitro* autoradiographic studies.

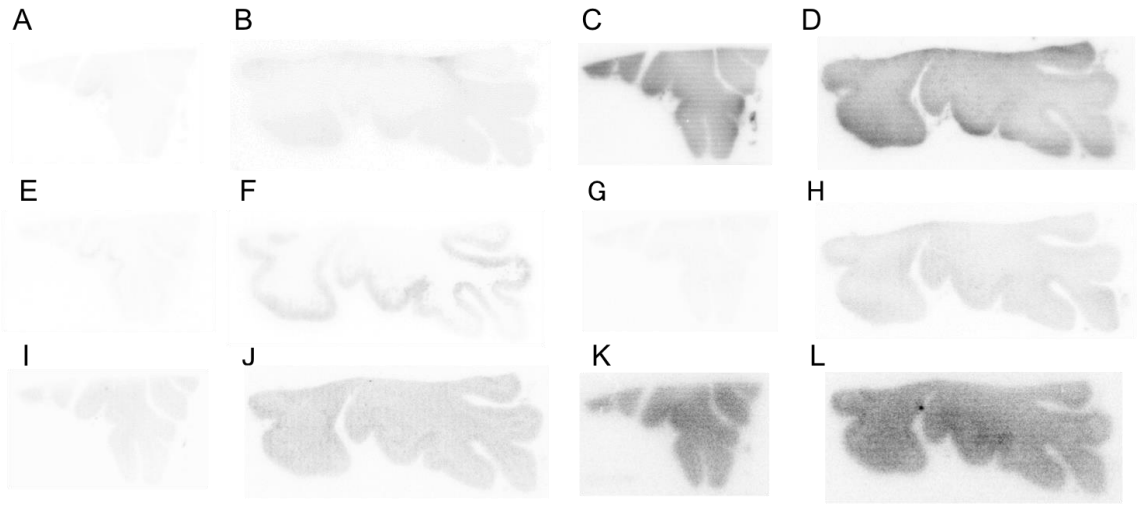

Figure S3. Comparison of *in vitro* autoradiography of [^{125}I]**10** (A and B), [^{125}I]**11** (C and D), [^{125}I]**12** (E and F), [^{125}I]**21** (G and H), [^{125}I]**22** (I and J), and [^{125}I]**23** (K and L) in the presence of nonradioactive ligands (10 μM). A, C, E, G, I, and K show results in Aβ(+)/tau(-) brain sections. B, D, F, H, J, and L show results in Aβ(+)/tau(+) brain sections.

Saturation binding assay with [^{125}I]22 using AD brain sections

A solution of [^{125}I]**22** (final conc., 0.0625–2 nM) was prepared by mixing with nonradioactive **22**. Nonspecific binding was defined in the presence of 10 μM nonradioactive **22**. The Aβ(+)/tau(+) brain sections were incubated with the solution (200 μL) described above for 2 h at room temperature. They were then dipped in 50% EtOH for 1 h and washed with H_2O for 1 min. After drying, the ^{125}I-labeled sections were exposed to a BAS imaging plate (Fuji Film, Tokyo, Japan) overnight. Autoradiographic images were obtained using an American Typhoon scanner system (GE Healthcare Life Sciences, Illinois, USA). The dissociation constants (K_d) of **22** for were determined by Scatchard analysis using GraphPad Prism 6.0 (Graphpad Software, California, USA).

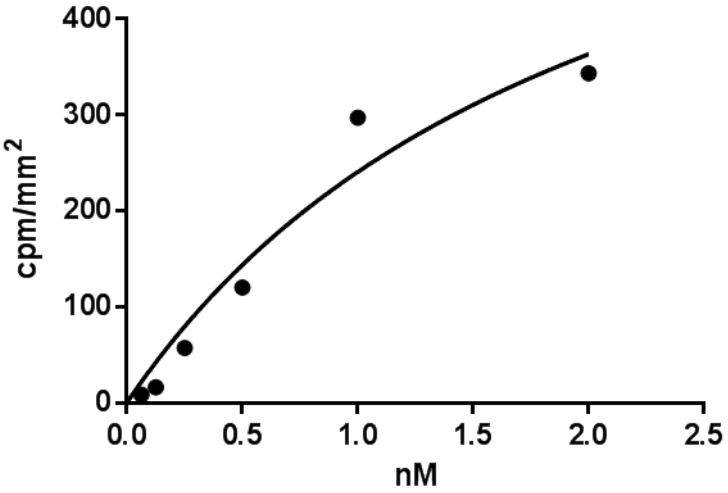

Figure S4. Saturation curve of [^{125}I]**22** in AD brain sections.

Brain uptake and clearance in normal mice.

A saline solution (100 μL) of each ^{125}I-labeled compound (27.8 kBq) containing ethanol (10 μL) and Tween 80 (0.1 μL) was injected intravenously directly into the tails of ddY mice (5 weeks old, male). The mice were sacrificed at various time-points postinjection. The brains were removed and weighed, and the radioactivity was measured with an automatic γ counter (Wallac WIZARD 1470, PerkinElmer, MA, USA).

Table S1. Biodistribution of radioactivity after intravenous injection of [^{125}I]**10**, [^{125}I]**11**, [^{125}I]**12**, [^{125}I]**21**, [^{125}I]**22**, and [^{125}I]**23** in normal mice[a]

Tissue	Time after injection (min)			
	2	10	30	60
		[^{125}I]**10**		
Blood	5.89 (0.61)	5.22 (0.71)	3.80 (0.41)	3.58 (0.38)
Liver	13.82 (2.72)	14.02 (1.50)	10.28 (1.26)	7.03 (1.17)
Kidney	9.90 (1.59)	7.36 (1.08)	5.51 (1.26)	3.92 (0.74)
Intestine	1.57 (0.57)	4.65 (1.35)	10.82 (0.56)	14.56 (3.72)
Spleen	3.28 (0.76)	2.98 (0.67)	1.94 (0.56)	1.80 (0.20)
Pancreas	4.67 (0.71)	2.91 (0.50)	1.00 (0.10)	0.70 (0.09)
Heart	7.50 (1.62)	3.01 (0.36)	1.49 (0.09)	1.32 (0.19)
Lung	8.01 (1.42)	5.48 (0.71)	2.85 (0.38)	2.35 (0.25)
Stomach[b]	1.10 (0.10)	2.25 (0.81)	3.72 (1.58)	4.95 (2.51)
Brain	3.11 (0.49)	2.56 (0.36)	0.95 (0.11)	0.40 (0.06)
Thyroid[b]	0.07 (0.03)	0.09 (0.07)	0.11 (0.08)	0.12 (0.09)
		[^{125}I]**11**		
Blood	5.61 (0.58)	5.70 (1.58)	3.25 (0.46)	2.33 (0.19)
Liver	13.45 (1.66)	14.78 (1.46)	6.87 (0.86)	4.10 (0.37)
Kidney	10.80 (0.96)	8.41 (0.69)	4.51 (1.09)	3.74 (2.42)
Intestine	2.48 (0.52)	7.29 (0.68)	13.42 (3.22)	18.87 (2.17)
Spleen	2.16 (0.45)	2.64 (0.41)	1.45 (0.13)	1.23 (0.23)
Pancreas	4.62 (0.70)	1.90 (0.27)	0.78 (0.07)	0.50 (0.07)
Heart	6.10 (0.53)	2.61 (0.43)	1.31 (0.18)	0.96 (0.09)
Lung	7.54 (1.19)	5.04 (0.64)	2.77 (0.50)	2.00 (0.31)
Stomach[b]	1.85 (0.24)	4.22 (0.48)	6.87 (1.25)	7.80 (1.30)
Brain	3.59 (0.33)	1.08 (0.23)	0.27 (0.01)	0.16 (0.02)
Thyroid[b]	0.70 (0.17)	0.22 (0.03)	0.32 (0.03)	0.52 (0.02)
		[^{125}I]**12**		
Blood	5.24 (0.36)	4.09 (0.54)	2.31 (0.17)	1.43 (0.18)
Liver	12.43 (1.60)	11.64 (2.39)	5.53 (2.70)	3.54 (1.19)
Kidney	11.19 (0.57)	8.69 (1.35)	4.58 (1.10)	2.23 (0.33)
Intestine	3.17 (0.92)	7.01 (2.24)	11.51 (3.31)	16.15 (4.93)
Spleen	2.79 (0.58)	3.45 (0.95)	1.46 (0.46)	1.00 (0.29)
Pancreas	5.32 (0.63)	2.46 (0.26)	1.13 (0.34)	0.66 (0.07)

Heart	6.14 (0.93)	2.44 (0.45)	1.23 (0.32)	0.77 (0.08)
Lung	6.89 (1.51)	4.42 (0.68)	1.84 (0.78)	1.50 (0.95)
Stomach[b]	2.11 (0.39)	5.88 (1.45)	6.81 (2.50)	5.67 (1.68)
Brain	3.69 (0.52)	0.96 (0.25)	0.16 (0.03)	0.09 (0.01)
Thyroid[b]	0.23 (0.08)	0.12 (0.02)	0.19 (0.10)	0.40 (0.13)
		[^{125}I]**21**		
Blood	5.01 (0.57)	5.13 (0.57)	3.46 (0.20)	2.80 (0.22)
Liver	8.65 (0.93)	5.22 (0.88)	2.37 (0.07)	1.63 (0.13)
Kidney	10.53 (0.90)	5.99 (0.86)	2.88 (0.25)	2.54 (0.53)
Intestine	4.04 (0.36)	5.41 (1.13)	8.32 (1.36)	7.27 (0.59)
Spleen	4.21 (0.76)	4.71 (0.64)	2.35 (0.23)	1.85 (0.32)
Pancreas	5.62 (0.71)	2.97 (0.39)	1.53 (0.11)	1.29 (0.15)
Heart	6.01 (0.66)	2.76 (0.43)	1.42 (0.14)	1.22 (0.14)
Lung	7.48 (0.69)	4.90 (0.83)	3.12 (0.16)	2.45 (0.28)
Stomach[b]	2.93 (0.69)	13.23 (1.62)	22.03 (2.74)	27.85 (4.99)
Brain	5.73 (0.66)	1.40 (0.32)	0.21 (0.01)	0.14 (0.02)
Thyroid[b]	0.06 (0.02)	0.03 (0.01)	0.03 (0.01)	0.04 (0.01)
		[^{125}I]**22**		
Blood	5.30 (0.57)	3.78 (0.35)	2.24 (0.54)	1.56 (0.27)
Liver	14.30 (0.36)	11.10 (1.52)	4.93 (1.62)	2.49 (0.13)
Kidney	14.96 (0.71)	7.67 (0.51)	3.68 (0.89)	2.41 (0.99)
Intestine	4.07 (0.68)	7.45 (2.91)	15.10 (3.55)	15.67 (3.34)
Spleen	3.02 (0.17)	4.27 (0.84)	1.62 (0.31)	1.17 (0.19)
Pancreas	6.92 (1.21)	2.42 (0.25)	1.14 (0.16)	0.86 (0.10)
Heart	6.76 (0.49)	2.35 (0.05)	1.01 (0.23)	0.68 (0.10)
Lung	7.73 (0.61)	4.30 (0.43)	2.40 (0.50)	1.80 (0.27)
Stomach[b]	2.46 (0.41)	6.90 (1.57)	11.19 (0.95)	12.18 (2.37)
Brain	5.66 (0.34)	1.00 (0.09)	0.19 (0.04)	0.10 (0.01)
Thyroid[b]	0.12 (0.03)	0.04 (0.01)	0.03 (0.02)	0.02 (0.01)
		[^{125}I]**23**		
Blood	5.63 (0.49)	4.72 (0.36)	3.54 (0.39)	2.98 (0.38)
Liver	11.51 (1.62)	5.84 (0.58)	3.10 (0.36)	2.38 (0.26)
Kidney	7.96 (0.70)	7.46 (1.09)	3.68 (1.03)	3.00 (1.39)
Intestine	3.47 (0.24)	6.14 (0.56)	7.66 (1.28)	7.58 (1.05)
Spleen	3.32 (0.41)	2.60 (0.34)	2.05 (0.36)	1.86 (0.27)
Pancreas	4.61 (0.35)	2.90 (0.20)	1.73 (0.20)	1.54 (0.17)

Heart	5.30 (0.72)	2.56 (0.22)	1.46 (0.08)	1.21 (0.17)
Lung	6.34 (0.73)	4.27 (0.38)	3.00 (0.47)	2.67 (0.35)
Stomach[b]	2.61 (0.26)	10.21 (2.70)	16.58 (2.60)	17.71 (5.95)
Brain	4.12 (0.68)	0.97 (0.17)	0.20 (0.03)	0.10 (0.01)
Thyroid[b]	0.08 (0.02)	0.05 (0.01)	0.03 (0.01)	0.04 (0.03)

[a]Expressed as %ID/g. Each value represents the mean (SD) of 5 animals.

[b]Expressed as %ID.

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