Supplemental data

Chemical synthesis

General remarks

4-(4*H*-1,2,4-Triazol-4-ylamino)benzonitrile (**6**) and 4-{(4-Bromobenzyl)(4*H*-1,2,4-triazol-4-yl)amino}benzonitrile (**12**, YM511) were prepared according to the reported method (*1*).

4-Fluorobenzonitrile (**4**) (Cat. No. 329-70013), 4-amino-4*H*-1,2,4-triazole (**5**) (Cat. No. 326-41573), potassium *tert*-butoxide (Cat. No. 161-08421), 4-methylbenzyl bromide (**7**) (Cat. No. 021-03131), potassium carbonate (Cat. No. 162-03495), 4-bromobenzyl alcohol (**13**) (Cat. No. 320-77123), tributyltin(IV) chloride (Cat. No. 202-08981), methanesulfonyl chloride (Cat. No. 131-01583), triethylamine (Cat. No. 202-02646), and bis(pinacolato)diboron (Cat. No. 329-56970) were purchased from Wako Pure Chemical Industries Ltd., Japan. $(Ph_3P)_4Pd$ (Cat. No. 216666), $(n\text{-}Bu_3Sn)_2$ (Cat. No. 251127), and 4-iodobenzyl alcohol (**8**) (Cat. No. 523496) were purchased from Sigma-Aldrich Japan K.K. $PdCl_2(dppf)\cdot CH_2Cl_2$ (Cat. No. B2064) was purchased from Tokyo Chemical Industry Co., Ltd., Japan. A solution of *n*-butyllithium in *n*-hexane (Cat. No. 04937-25), potassium bromide (Cat. No. 32319-30), and potassium

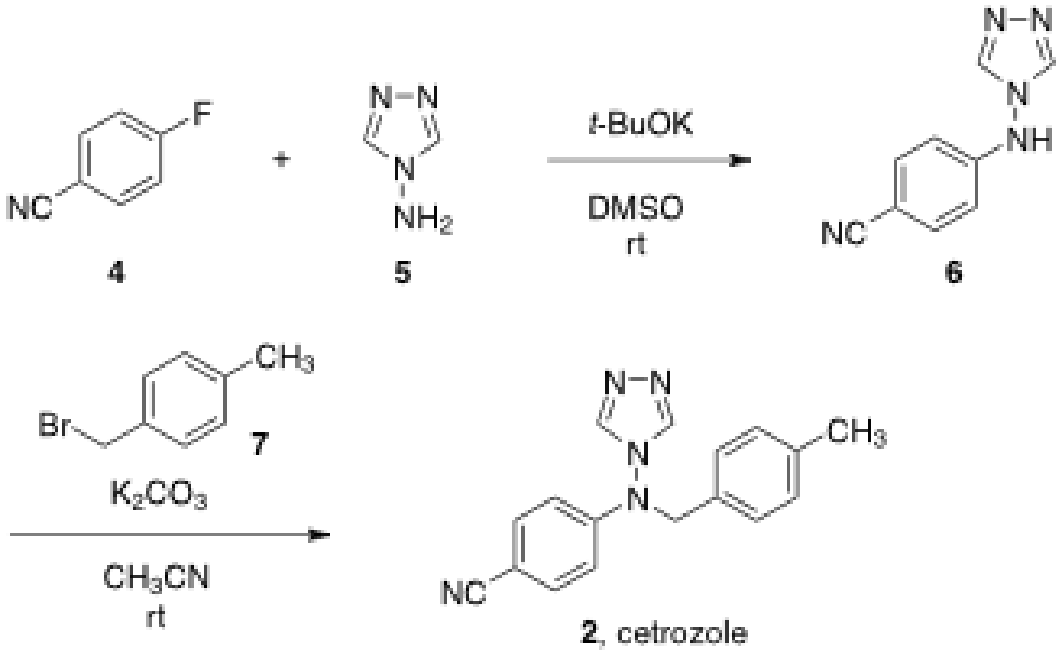
acetate (32299-30) were purchased from Kanto Chemical Co., Inc., Japan. All other chemical reagents used were commercial grade and were used as received.

Analytical thin-layer chromatography (TLC) was performed on precoated (0.25 mm) silica gel plates (Merck Chemicals, Silica Gel 60 F_{254}, Cat. No. 1.05715). Flash column chromatography was conducted using silica gel (Kanto Chemical Co., Inc., Silica Gel 60N, spherical neutral, particle size 40–50 μm, Cat. No. 37563-85 or particle size 63–210 μm, Cat. No. 37565-85). Melting points (Mp) were measured on a YANACO MP-J3 instrument and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained with a Varian MERCURY 300 spectrometer at 300 and 75.5 MHz, respectively. $CDCl_3$ (CIL, Cat. No. DLM-7TB) and DMSO-d_6 (CIL, Cat. No. DLM-10) were used as solvents for obtaining NMR spectra. Chemical shifts (δ) are given in parts per million (ppm) downfield from $(CH_3)_4Si$ (δ 0.00 for ^{1}H NMR in $CDCl_3$) or the solvent peak (δ 2.49 for ^{1}H NMR, δ 39.5 for ^{13}C NMR in DMSO-d_6, and δ 77.0 for ^{13}C NMR in $CDCl_3$) as an internal reference with coupling constants (*J*) in hertz (Hz). The abbreviations s, d, t, m, and br signify singlet, doublet, triplet, multiplet, and broad, respectively. IR spectra were measured by the diffuse reflectance method on a SHIMADZU IRPrestige-21 spectrometer equipped with DRS-8000A with the absorption band given in cm^{-1}. Elemental analyses were performed with a YANACO CHN CORDER MT-5 at the

Center for Advanced Materials Analysis (Suzukakedai), Technical Department, Tokyo Institute of Technology. High-resolution mass spectra (HRMS) were measured on a JEOL JMS-700 mass spectrometer under positive fast atom bombardment (FAB^+) conditions at the Center for Advanced Materials Analysis (Suzukakedai), Technical Department, Tokyo Institute of Technology, or a Bruker micrOTOF mass spectrometer under positive electrospray ionization (ESI^+) conditions at the Institute of Biomaterials and Bioengineering, Tokyo Medicinal and Dental University.

*Synthesis of cetrozole (**2**)*

Non-radiolabeled cetrozole was prepared by N-alkylation of 4-(4*H*-1,2,4-triazol-4-ylamino)benzonitrile (**6**) (*1*) with 4-methylbenzyl bromide (**7**).



4-{(4-Methylbenzyl)(4*H*-1,2,4-triazol-4-yl)amino}benzonitrile (**2**, cetrozole)

Under an argon atmosphere, a mixture of 4-(4*H*-1,2,4-triazol-4-ylamino)benzonitrile (**6**) (*1*) (2.00 g, 10.8 mmol), 4-methylbenzyl bromide (**7**) (2.00 g, 10.8 mmol), and potassium carbonate (2.99 g, 21.6 mmol) in acetonitrile (80 mL) was stirred at room temperature for 2 h. To this was added water (200 mL) and the mixture was extracted with CH_2Cl_2 (100 mL × 3). The combined

organic extracts were washed with water (30 mL × 2), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel 200 g, CH_2Cl_2/EtOAc = 1/1 to 1/4) to give 4-{(4-methylbenzyl)(4*H*-1,2,4-triazol-4-yl)amino}benzonitrile (**2**, cetrozole) (1.60 g, 51.2%) as a colorless solid. Recrystallization from EtOAc (64 mL) afforded colorless plates (1.27 g, 40.6%);

TLC R_f = 0.30 (CH_2Cl_2/EtOAc = 1/1);

Mp 201-202 °C;

^{1}H NMR (300 MHz, DMSO-d_6) δ 2.25 (s, 3H, CH_3), 4.99 (s, 2H, benzylic CH_2), 6.70-6.78 (AA'BB', 2H, aromatic), 7.06-7.14 (AA'BB', 2H, aromatic), 7.14-7.22 (AA'BB', 2H, aromatic), 7.70-7.79 (AA'BB', 2H, aromatic), 8.76 (br s, 2H, triazole);

^{13}C NMR (75.5 MHz, DMSO-d_6) δ 20.7, 56.9, 102.8, 113.7 (2C), 119.1, 128.5 (2C), 129.3 (2C), 131.2, 133.9 (2C), 137.4, 143.4 (2C), 151.6;

IR (KBr, cm^{-1}) 548, 606, 667, 741, 814, 833, 858, 870, 1069, 1180, 1207, 1234, 1265, 1287, 1302, 1389, 1456, 1501, 1512, 1605, 2220, 2340, 2359, 3121;

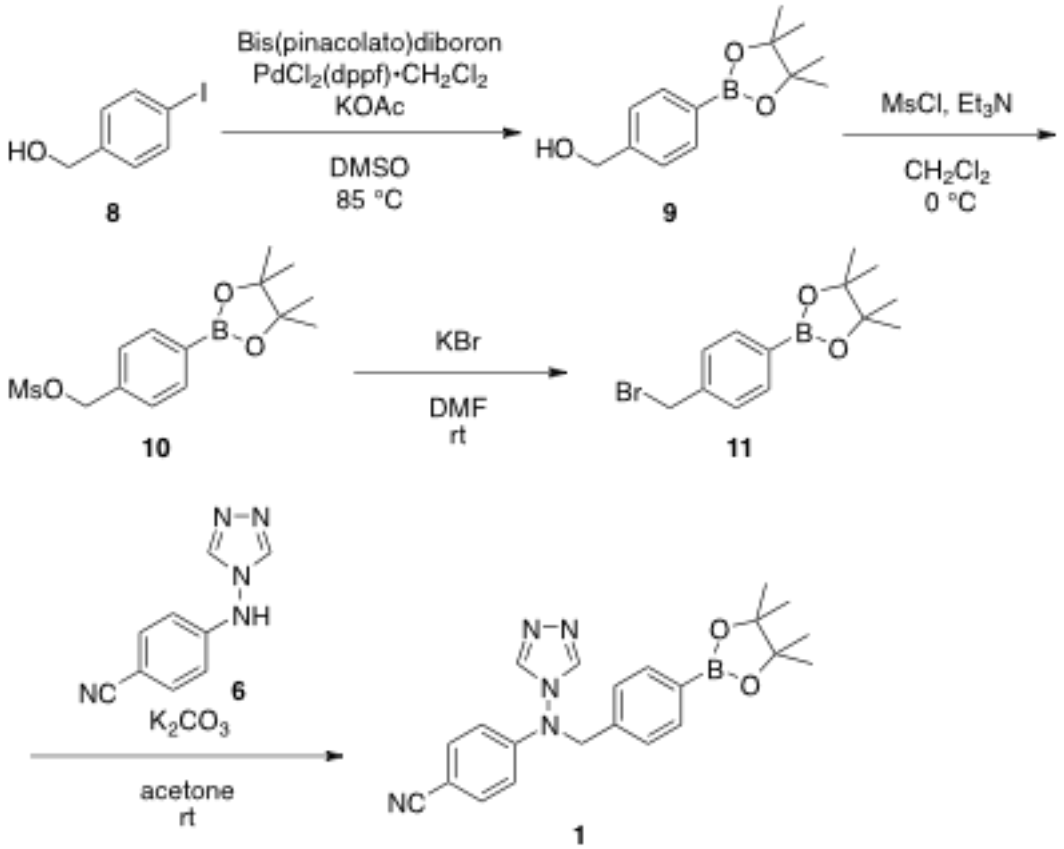
Anal. Calcd. for $C_{17}H_{15}N_5$: C, 70.57; H, 5.23; N, 24.21. Found: C, 70.41; H, 5.10; N, 24.48;

HRMS (FAB$^+$/NBA+NaI) *m/z* 312.1220 (M+Na, $C_{17}H_{15}N_5Na$ requires 312.1225).

*Synthesis of pinacol boranyl precursor **1** for [^{11}C]cetrozole*

The pinacol boranyl precursor **1** was prepared by N-alkylation of 4-(4*H*-1,2,4-triazol-4-ylamino)benzonitrile (**6**) (*1*) with 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl bromide (**11**) (*2*) (Method A) or by Miyaura–Ishiyama borylation (*3*) of YM511(**12**) (*1*) (Method B).

[Method A]



4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl alcohol (**9**) (*2*)

Under an argon atmosphere, to a solution of 4-iodobenzyl alcohol (**8**) (2.34 g, 10.0 mmol) in DMSO (30 mL) were successively added $PdCl_2(dppf)\cdot CH_2Cl_2$ (245 mg, 300 μmol), potassium acetate (2.94 g, 30.0 mmol), and bis(pinacolato)diboron (2.79 g, 11.0 mmol) at room temperature, and the mixture was heated with stirring at 85 °C for 18 h. After cooling the mixture to room temperature, to this was added water (250 mL), and the mixture was extracted with EtOAc (100 mL × 3). The combined organic extracts were successively washed with water (100 mL × 3) and brine (100 mL × 1), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel 60 g, *n*-hexane/EtOAc = 5/1) to give 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl alcohol (**9**) (2.24 g, 95.7%) as a colorless solid;

TLC R_f = 0.41 (*n*-hexane/EtOAc = 2/1);

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (s, 12H, $4CH_3$), 4.72 (s, 2H, benzylic CH_2), 7.34-7.41 (AA'BB', 2H, aromatic), 7.78-7.84 (AA'BB', 2H, aromatic).

4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl methanesulfonate (**10**) (*2*)

Under an argon atmosphere, to a solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl alcohol (**9**) (3.48 g, 14.9 mmol)

in CH_2Cl_2 (60 mL) were successively added triethylamine (3.15 mL, 22.6 mmol) and methanesulfonyl chloride (1.40 mL, 18.1 mmol) at 0 °C, and the mixture was stirred at the same temperature for 2 h. To this was added water (150 mL), and the mixture was extracted with CH_2Cl_2 (100 mL × 3). The combined organic extracts were successively washed with water (70 mL × 3) and brine (70 mL × 3), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel 110 g, *n*-hexane/EtOAc = 3/1) to give 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl methanesulfonate (**10**) (3.55 g, 76.5%) as a colorless solid, which was used in the next step without further purification; TLC 0.41 (*n*-hexane/EtOAc = 2/1).

4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl bromide (**11**) (*2*)

Under an argon atmosphere, to a solution of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl methanesulfonate (**10**) (3.55 g, 11.4 mmol) in DMF (25 mL) was added potassium bromide (2.03 g, 17.1 mmol) at room temperature, and the mixture was stirred at the same temperature for 19 h. To this was added water (150 mL), and the mixture was extracted with EtOAc (100 mL × 3). The combined organic extracts were washed with water (100 mL × 3), dried (Na_2SO_4),

filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel 100 g, *n*-hexane/EtOAc = 9/1) to give 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl bromide (**11**) (2.85 g, 84.4%) as a colorless solid;

TLC R_f = 0.73 (*n*-hexane/EtOAc = 3/1);

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 12H, $4CH_3$), 4.49 (s, 2H, benzylic CH_2), 7.36-7.43 (AA'BB', 2H, aromatic), 7.75-7.83 (AA'BB', 2H, aromatic).

4-[{4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl}(4*H*-1,2,4-triazol-4-yl)amino]benzonitrile (**1**)

Under an argon atmosphere, a mixture of 4-(4*H*-1,2,4-triazol-4-ylamino)benzonitrile (**6**) (*1*) (1.36 g, 7.33 mmol), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl bromide (**11**) (2.61 g, 8.79 mmol), and potassium carbonate (2.03 g, 14.7 mmol) in acetone (50 mL) was stirred at room temperature for 5 h. To this was added water (150 mL), and the mixture was extracted with EtOAc (100 mL × 3). The combined organic extracts were successively washed with water (50 mL × 1) and brine (50 mL × 1), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash column

chromatography (silica gel 110 g, CH_2Cl_2/CH_3OH = 40/1) to give 4-[{4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl}(4*H*-1,2,4-triazol-4-yl)amino]benzonitrile (**1**) (2.02 g, 68.5%) as a colorless solid;

TLC R_f = 0.57 (CH_2Cl_2/CH_3OH = 9/1);

Mp 193-194 °C

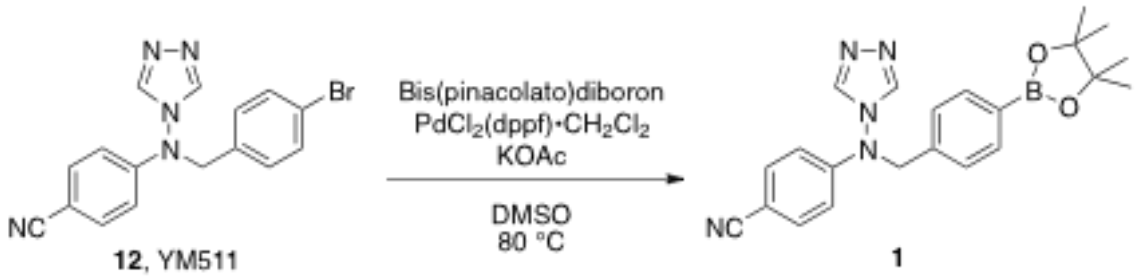
^{1}H NMR (300 MHz, $CDCl_3$) δ 1.34 (s, 12H, $4CH_3$), 4.91 (s, 2H, benzylic CH_2), 6.63-6.70 (AA'BB', 2H, aromatic), 7.18-7.25 (AA'BB', 2H, aromatic), 7.55-7.63 (AA'BB', 2H, aromatic), 7.75-7.83 (AA'BB', 2H, aromatic), 8.11 (s, 2H, triazole);

^{13}C NMR (67.8 MHz, $CDCl_3$) δ 24.7 (4C), 58.2, 83.9 (2C), 104.8, 113.3 (2C), 118.5, 127.2 (2C), 133.9 (2C), 135.5 (2C), 136.3, 142.5 (2C), 150.4 (the carbon adjacent to boron was not observed);

IR (KBr, cm^{-1}) 546, 656, 671, 733, 826, 858, 912, 962, 1020, 1065, 1088, 1142, 1179, 1213, 1271, 1325, 1360, 1398, 1508, 1603, 2222, 2978, 3115, 3401;

HRMS (ESI^+) *m*/*z* 424.1912 ($[M+Na]^+$, $C_{22}H_{24}BN_5NaO_2^+$ requires 424.1915).

[Method B]



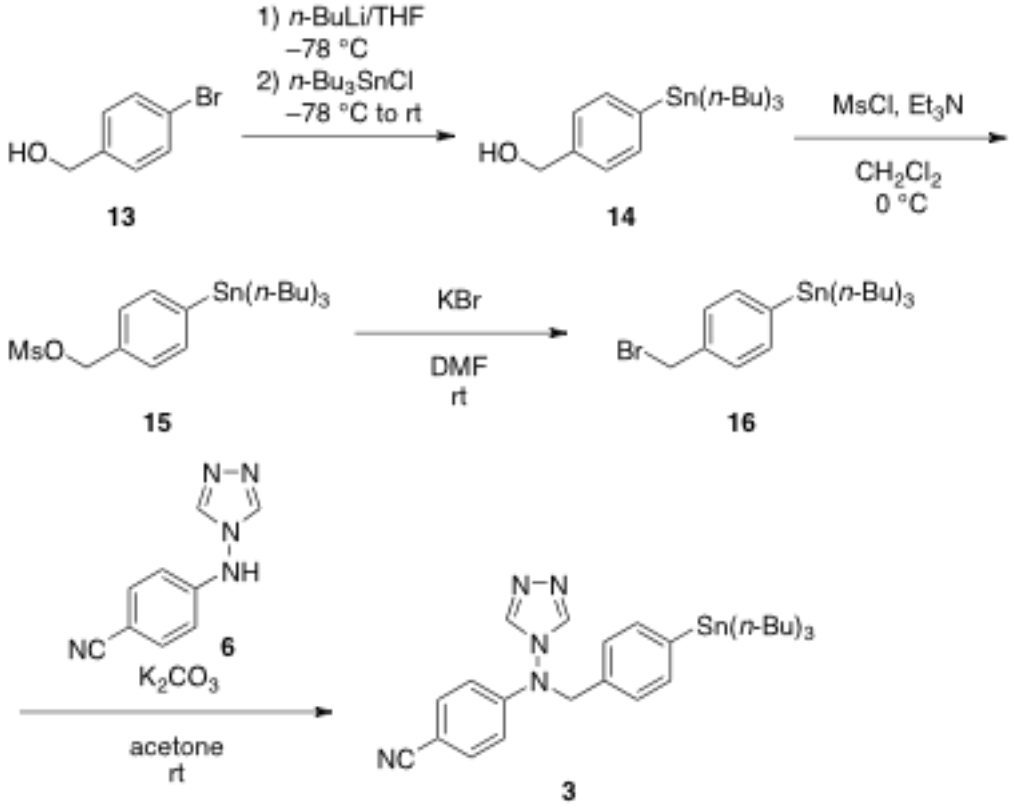
4-[{4-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl}(4*H*-1,2,4-triazol-4-yl)amino]benzonitrile (**1**)

Under an argon atmosphere, to a solution of YM511 (**12**) (*1*) (324 mg, 915 μmol) in DMSO (5.5 mL) were successively added $PdCl_2(dppf)\cdot CH_2Cl_2$ (22.5 mg, 27.5 μmol), potassium acetate (270 mg, 2.75 mmol), and bis(pinacolato)diboron (256 mg, 1.01 mmol) at room temperature, and the mixture was heated with stirring at 80 °C for 2 h. After cooling the mixture to room temperature, to this was added water (80 mL) and the mixture was extracted with EtOAc (50 mL × 3). The combined organic extracts were successively washed with water (50 mL × 3) and brine (50 mL × 1), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel 30 g, CH_2Cl_2/CH_3OH = 20/1) to give 4-[{4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl}(4*H*-1,2,4-triazol-4-yl)amino]benzonitrile (**1**) (330 mg, 89.9%) as a slightly gray solid.

*Synthesis of tributylstannyl precursor **3** for [^{11}C]cetrozole*

The tributylstannyl precursor **3** was prepared by N-alkylation of 4-(4*H*-1,2,4-triazol-4-ylamino)benzonitrile (**6**) (*1*) with 4-(tributylstannyl)benzyl bromide (**16**) (*4*) (Method A) or by palladium-catalyzed tributylstannylation of YM511 (**12**) (*1*) (Method B).

[Method A]



4-(Tributylstannyl)benzyl alcohol (**14**) (*5*, *6*)

Under an argon atmosphere, to a solution of 4-bromobenzyl alcohol (**13**) (4.15

g, 22.2 mmol) in anhydrous THF (150 mL) was slowly added a solution of *n*-butyllithium (1.63 M, 30.0 mL, 48.9 mmol) in *n*-hexane at −78 °C. After stirring the mixture for 50 min at the same temperature, to this was added tributyltin(IV) chloride (13.3 mL, 48.9 mmol) at −78 °C, and the mixture was allowed to warm to room temperature. After stirring for 1.5 h, the reaction mixture was concentrated under reduced pressure. To the residue was added water (200 mL) and the mixture was extracted with CH_2Cl_2 (80 mL × 2). The combined organic extracts were successively washed with water (150 mL × 3) and brine (150 mL × 1), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel 200 g, *n*-hexane/EtOAc = 8/1) to give 4-(tributylstannyl)benzyl alcohol (**14**) (7.14 g, 81.0%) as a colorless oil;

TLC R_f = 0.44 (*n*-hexane/EtOAc = 4/1), R_f = 0.50 (*n*-hexane/CH_2Cl_2/EtOAc = 4/1/1);

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, 9H, *J* = 7.2 Hz, $3CH_3$), 0.93-1.16 (m, 6H, $3CH_2$), 1.26-1.39 (m, 6H, $3CH_2$), 1.43-1.67 (m, 6H, $3CH_2$), 4.68 (d, 2H, *J* = 5.8 Hz, benzylic CH_2), 7.30-7.37 (AA'BB', 2H, aromatic), 7.39-7.56 (AA'BB', 2H, $^3J(^{119/117}Sn\text{-}^1H)$ = 37.4 Hz, aromatic);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ 9.3 (3C, $^1J(^{119}Sn\text{-}^{13}C)$ = 339.2 Hz, $^1J(^{117}Sn\text{-}^{13}C)$ = 324.5 Hz), 13.5 (3C), 27.2 (3C, $^3J(^{119/117}Sn\text{-}^{13}C)$ = 54.7 Hz), 28.9 (3C, $^2J(^{119/117}Sn\text{-}^{13}C)$ = 19.7

Hz), 64.2, 126.5 (2C, $^{3}J(^{119/117}\text{Sn-}^{13}\text{C})$ = 41.3 Hz), 136.2 (2C, $^{2}J(^{119/117}\text{Sn-}^{13}\text{C})$ = 31.4 Hz), 140.2, 140.5;

IR (KBr, cm^{-1}) 514, 598, 621, 662, 689, 743, 791, 833, 864, 961, 1015, 1069, 1207, 1290, 1375, 1393, 1418, 1462, 2851, 2870, 2924, 2955, 3296;

Anal. Calcd. for $C_{19}H_{34}Sn$: C, 57.46; H, 8.63. Found: C, 57.29; H, 8.88;

HRMS (FAB^{+}/NBA+NaI) *m*/*z* 421.1536 (M+Na, $C_{19}H_{34}O^{120}SnNa$ requires 421.1529).

4-(Tributylstannyl)benzyl methanesulfonate (**15**) (*6*)

Under an argon atmosphere, to a solution of 4-(tributylstannyl)benzyl alcohol (**14**) (6.90 g, 17.4 mmol) in CH_2Cl_2 (100 mL) were successively added triethylamine (3.63 mL, 26.0 mmol) and methanesulfonyl chloride (1.61 mL, 20.8 mmol) at 0 °C, and the mixture was stirred at the same temperature for 35 min. To this was added water (200 mL) and the mixture was extracted with CH_2Cl_2 (80 mL × 3). The combined organic extracts were washed with brine (150 mL × 3), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel 200 g, *n*-hexane/EtOAc = 4/1) to give 4-(tributylstannyl)benzyl methanesulfonate (**15**) (7.17 g, 86.8%) as a colorless oil;

TLC 0.45 (*n*-hexane/EtOAc = 4/1), R_f = 0.57 (*n*-hexane/CH_2Cl_2/EtOAc = 4/1/1);

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, 9H, *J* = 7.2 Hz, $3CH_3$), 0.94-1.19 (m, 6H, $3CH_2$), 1.23-1.41 (m, 6H, $3CH_2$), 1.42-1.67 (m, 6H, $3CH_2$), 2.90 (s, 3H, CH_3), 5.23 (s, 2H, benzylic CH_2), 7.33-7.41 (AA'BB', 2H, aromatic), 7.43-7.60 (AA'BB', 2H, $^3J(^{119/117}Sn\text{-}^1H)$ = 36.5 Hz, aromatic);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ 7.0 (3C, $^1J(^{119}Sn\text{-}^{13}C)$ = 340.7 Hz, $^1J(^{117}Sn\text{-}^{13}C)$ = 325.5 Hz), 13.3 (3C), 26.9 (3C, $^3J(^{119/117}Sn\text{-}^{13}C)$ = 55.4 Hz), 28.7 (3C, $^2J(^{119/117}Sn\text{-}^{13}C)$ = 20.0 Hz), 37.3, 71.4, 127.8 (2C, $^3J(^{119/117}Sn\text{-}^{13}C)$ = 40.3 Hz), 132.9, 136.4 (2C, $^2J(^{119/117}Sn\text{-}^{13}C)$ = 30.8 Hz), 143.1;

IR (KBr, cm^{-1}) 513, 529, 664, 691, 816, 862, 932, 1069, 1175, 1356, 1396, 1416, 1464, 2851, 2870, 2924, 2955;

Anal. Calcd. for $C_{20}H_{36}O_3SSn$: C, 50.54; H, 7.63. Found: C, 50.32; H, 7.80;

HRMS (FAB^+/NBA+NaI) *m*/*z* 499.1301 (M+H, $C_{20}H_{36}O_3S^{120}SnNa$ requires 499.1305).

4-(Tributylstannyl)benzyl bromide (**16**) (*4*)

Under an argon atmosphere, to a solution of 4-(tributylstannyl)benzyl methanesulfonate (**15**) (6.91 g, 14.5 mmol) in DMF (100 mL) was added potassium bromide (3.46 g, 29.1 mmol) at room temperature, and the mixture was stirred at the

same temperature for 12 h. To this was added water (100 mL) and the mixture was extracted with Et_2O (80 mL × 3). The combined organic extracts were successively washed with water (150 mL × 3) and brine (150 mL × 1), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by with flash column chromatography (silica gel 200 g, *n*-hexane/Et_2O = 49/1) to give 4-(tributylstannyl)benzyl bromide (**16**) (6.43 g, 96.1%) as a colorless oil;

TLC R_f = 0.39 (*n*-hexane);

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, 9H, *J* = 7.2 Hz, $3CH_3$), 0.92-1.18 (m, 6H, $3CH_2$), 1.25-1.40 (m, 6H, $3CH_2$), 1.42-1.64 (m, 6H, $3CH_2$), 4.49 (s, 2H, benzylic CH_2), 7.30-7.39 (AA'BB', 2H, aromatic), 7.39-7.54 (AA'BB', 2H, $^3J(^{119/117}Sn\text{-}^1H)$ = 36.6 Hz, aromatic);

^{13}C NMR (75.5 MHz, $CDCl_3$) δ 7.3 (3C, $^1J(^{119}Sn\text{-}^{13}C)$ = 340.0 Hz, $^1J(^{117}Sn\text{-}^{13}C)$ = 324.8 Hz), 13.6 (3C), 27.3 (3C, $^3J(^{119/117}Sn\text{-}^{13}C)$ = 55.4 Hz), 29.0 (3C, $^2J(^{119/117}Sn\text{-}^{13}C)$ = 19.3 Hz), 33.4, 128.2 (2C, $^3J(^{119/117}Sn\text{-}^{13}C)$ = 41.2 Hz), 136.6 (2C, $^2J(^{119/117}Sn\text{-}^{13}C)$ = 30.5 Hz), 137.2, 142.2;

IR (KBr, cm^{-1}) 627, 669, 777, 797, 839, 937, 1011, 1086, 1113, 1406, 1462, 1485, 2857, 2884, 2928, 2953;

Anal. Calcd. for $C_{19}H_{33}BrSn$: C, 49.60; H, 7.23. Found: C, 49.44; H, 7.41.

4-[(4*H*-1,2,4-Triazol-4-yl){4-(tributylstannyl)benzyl}amino]benzonitrile (**3**)

Under an argon atmosphere, a mixture of 4-(4*H*-1,2,4-triazol-4-ylamino)benzonitrile (**6**) (*1*) (1.17 g, 6.33 mmol), 4-(tributylstannyl)benzyl bromide (**16**) (3.49 g, 7.59 mmol), and potassium carbonate (1.76 g, 12.7 mmol) in acetone (50 mL) was stirred at room temperature for 8 h. To this was added water (100 mL) and the mixture was extracted with CH_2Cl_2 (100 mL × 3). The combined organic extracts were successively washed with water (100 mL × 1) and brine (100 mL × 1), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel 110 g, CH_2Cl_2/CH_3OH = 20/1) to give 4-[(4*H*-1,2,4-triazol-4-yl){4-(tributylstannyl)benzyl}amino]benzonitrile (**3**) (2.62 g, 73.5%) as a colorless solid. Recrystallization from *n*-hexane (100 mL) afforded colorless plates (2.26 g, 63.4%);

TLC R_f = 0.57 (CH_2Cl_2/CH_3OH = 9/1);

Mp 93-94 °C;

^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, 9H, *J* = 7.2 Hz, $3CH_3$), 0.93-1.18 (m, 6H, $3CH_2$),

1.23-1.41 (m, 6H, $3CH_2$), 1.44-1.58 (m, 6H, $3CH_2$), 4.88 (s, 2H, benzylic CH_2), 6.62-6.70 (AA'BB', 2H, aromatic), 7.09-7.19 (AA'BB', 2H, aromatic), 7.36-7.53 (AA'BB', 2H, $^{3}J(^{119/117}Sn\text{-}^{1}H)$ = 36.0 Hz, aromatic), 7.55-7.62 (AA'BB', 2H, aromatic), 8.12 (br s, 2H, triazole);

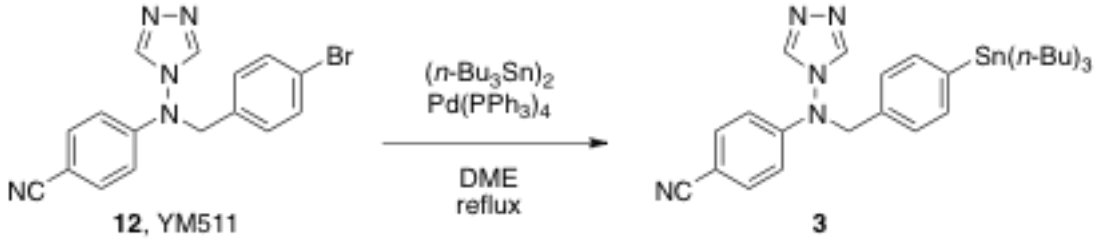
^{13}C NMR (75.5 MHz, DMSO-d_6) δ 9.1 (3C, $^{1}J(^{119}Sn\text{-}^{13}C)$ = 338.8 Hz, $^{1}J(^{117}Sn\text{-}^{13}C)$ = 324.2 Hz), 13.6 (3C), 26.7 (3C, $^{3}J(^{119/117}Sn\text{-}^{13}C)$ = 53.7 Hz), 28.6 (3C, $^{2}J(^{119/117}Sn\text{-}^{13}C)$ = 20.3 Hz), 57.3, 102.8, 113.6 (2C), 119.1, 127.9 (2C, $^{3}J(^{119/117}Sn\text{-}^{13}C)$ = 40.9 Hz), 133.9 (2C), 134.7, 136.4 (2C, $^{2}J(^{119/117}Sn\text{-}^{13}C)$ = 31.9 Hz), 141.0, 143.3 (2C), 151.5;

IR (KBr, cm^{-1}) 669, 826, 1065, 1179, 1292, 1335, 1395, 1462, 1508, 1605, 2222, 2851, 2870, 2924, 2955;

Anal. Calcd. for $C_{28}H_{39}N_5Sn$: C, 59.59; H, 6.97; N, 12.41. Found: C, 59.47; H, 6.65; N, 12.44;

HRMS (FAB^{+}/NBA) *m*/*z* 566.2318 (M+H, $C_{28}H_{40}N_5{}^{120}Sn$ requires 566.2306).

[Method B]



4-[(4*H*-1,2,4-Triazol-4-yl){4-(tributylstannyl)benzyl}amino]benzonitrile (**3**)

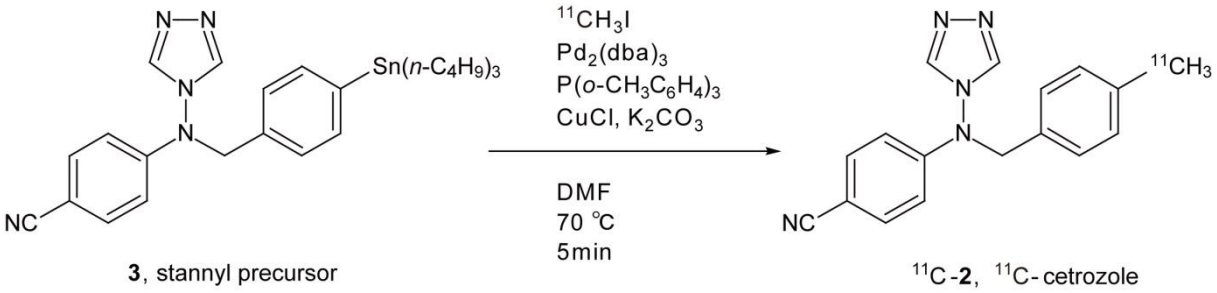
Under an argon atmosphere, to a solution of YM511 (**12**) (*1*) (685 mg, 1.93 mmol) in DME (20 mL) were successively added $(Ph_3P)_4Pd$ (66.9 mg, 57.9 μmol) and $(n\text{-}Bu_3Sn)_2$ (2.50 mL, 2.93 mmol) at room temperature, and the mixture was refluxed at 100 °C for 20 h. After cooling the mixture to room temperature, to this was added saturated aqueous potassium fluoride solution (50 mL), and the resulting precipitate was removed by filtration. The filtrate was extracted with EtOAc (100 mL × 2), and the combined organic extracts were successively washed with saturated aqueous potassium fluoride solution (100 mL × 1), water (100 mL × 1), and brine (100 mL × 1), dried (Na_2SO_4), filtered, and concentrated under reduced pressure. The residue was purified by flash column chromatography (silica gel 50 g, CH_2Cl_2/EtOAc = 9:1) to give 4-[(4*H*-1,2,4-triazol-4-yl){4-(tributylstannyl)benzyl}amino]benzonitrile (**3**) (551 mg, 50.5%) as a colorless solid.

*Synthesis of [^{11}C]cetrozole from tributylstannyl precursor **3** (Supplemental Fig. 1)*

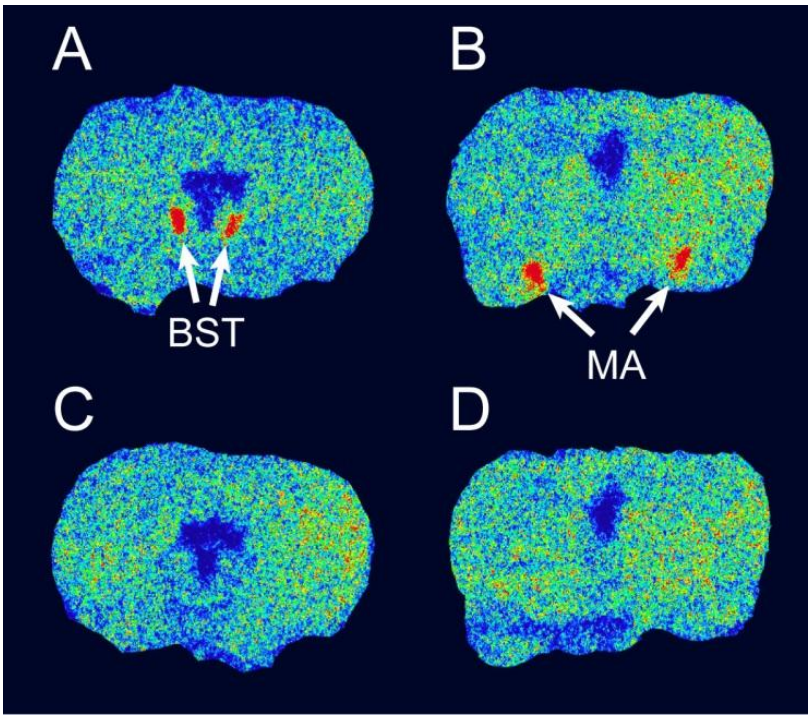
[^{11}C]Carbon dioxide was produced by a ^{14}N(p,α)^{11}C reaction using a Sumitomo CYPRIS HM-18 cyclotron (Sumitomo Heavy Industries Ltd., Tokyo, Japan) and then converted to [^{11}C]methyl iodide by treatment with lithium aluminum hydride followed by hydriodic acid using a RIKEN original automated radiolabeling system. The obtained [^{11}C]methyl iodide was used for palladium(0)-mediated rapid [^{11}C]methylation (*7*) as follows.

[^{11}C]Methyl iodide was transferred by a stream of He gas (30 mL/min) into a solution of $Pd_2(dba)_3$ (2.0 mg, 1.8 μmol) and P(*o*-tolyl)$_3$ (3.2 mg, 10.1 μmol) in DMF (0.3 mL) in a reaction vessel (A) at room temperature. The mixture was transferred to a reaction vessel (B) containing stannyl precursor **3** (2.5 mg, 4.4 μmol), CuCl (2.0 mg, 20 μmol), and K_2CO_3 (2.5 mg, 19 μmol). The inside of the reaction vessel (A) was rinsed in DMF (0.5 mL), and the solution was successively transferred to the reaction vessel (B). The resulting mixture was heated at 70 °C for 5 min. Salts and palladium residue in the reaction mixture were removed by a solid phase extraction, washing with 1 mL of a solution of CH_3CN:30 mM CH_3COONH_4 (35:65). The combined elutes were injected into preparative HPLC with a γ-detector (mobile phase, CH_3CN:30 mM CH_3COONH_4 (35:65); column, Nacalai COSMOSIL AR-II C18, 10 mm × 250 mm, 5 mm; guard

column, Sumika SUMIPAK Filter PG-ODS; flow rate, 6 mL/min; UV detection, 254 nm; retention time of [^{11}C]-**2**, 13.5 min). The desired fraction was collected into a flask, and then the organic solvent was removed under reduced pressure. The desired ^{11}C-labeled compound was dissolved in a mixture of polysorbate 80 (0.05 mL), propylene glycol (0.3 mL), and saline (4 mL). The total synthesis time including HPLC purification and radiopharmaceutical formulation for intravenous administration was 38 min. The radioactivity of [^{11}C]-**2** prepared for administration was 1.5–2.3 GBq, and the specific radioactivity was 50–120 GBq/μmol. Identification of [^{11}C]-**2** was confirmed by co-injection with the unlabeled authentic sample on analytical HPLC with a γ-detector (mobile phase, CH_3CN:30 mM CH_3COONH_4 (40:60); column, Nacalai COSMOSIL, AR-II C18, 4.6 mm × 100 mm; flow rate, 1 mL/min; UV detection, 254 nm; retention time of 10, 4.5 min). The chemical purity and the radiochemical purity were greater than 95%. Decay-corrected yield based on $^{11}CH_3I$ in a reaction vessel B was 25-35%.

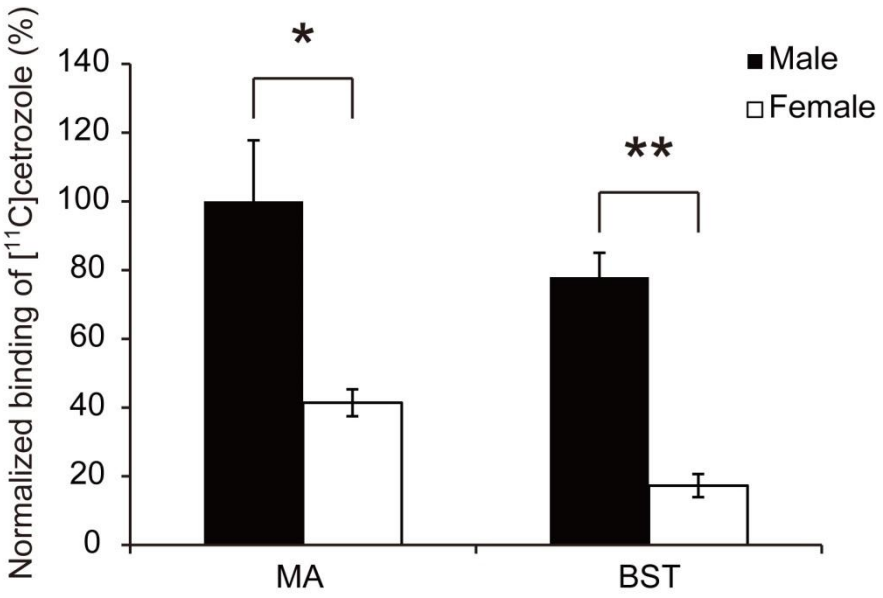


Supplemental Figure 1. Radiosynthesis of [^{11}C]cetrozole ([^{11}C]-**2**) by rapid [^{11}C]methylation using tributylstannyl precursor **3**.

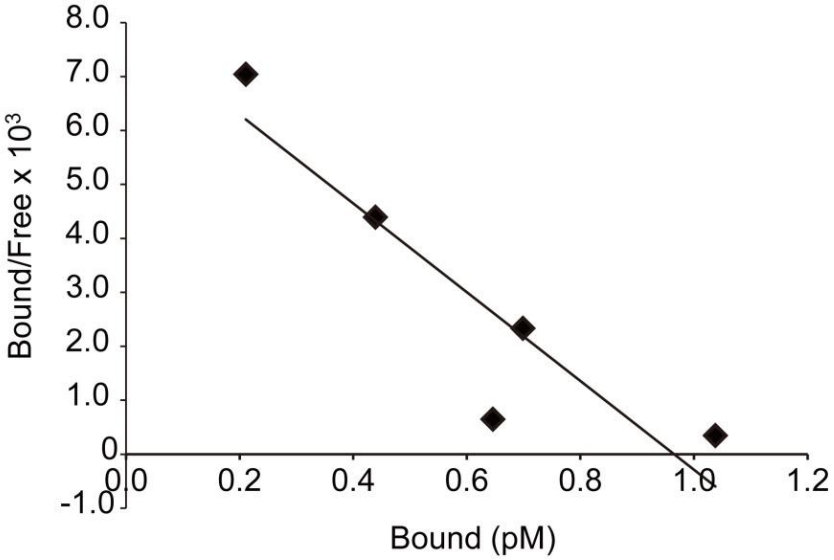


Supplemental Figure 2. Autoradiograms of [^{11}C]cetrozole (2 nM) in male rat brain sections containing the bed nucleus of stria terminalis (BST) (A and C) and the medial amygdala (MA) (B and D). Frozen rat brain sections (25 μm thick) were pre-incubated for 10 min in Tris-HCl (50 mM, pH 7.4) buffer and then incubated in 2 nM [^{11}C]cetrozole in the presence or absence of unlabeled vorozole (1 μM) in the same buffer at room temperature for 30 min. The sections were washed, rapidly dried, and exposed to imaging plates (BAS-SR2040, Fuji Photo Film Co., Tokyo, Japan) for 40 min. The exposed imaging plates were scanned with a bioimaging analyzer (FLA-7000IR, Fuji Photo Film Co.).

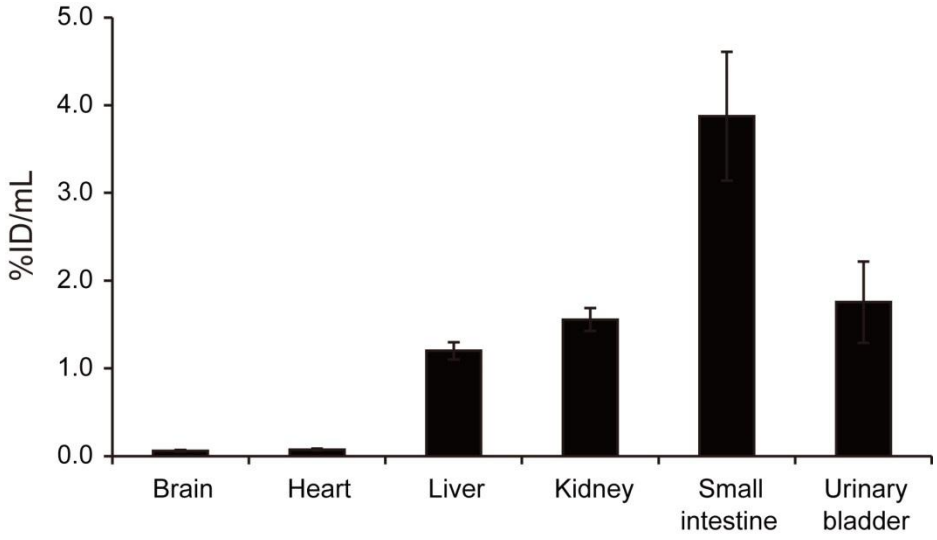
The upper images show the total binding of [^{11}C]cetrozole, and the lower ones show nonspecific binding obtained by adding unlabeled vorozole (1 μM). High binding in the bed nucleus of stria terminalis and amygdala was blocked with unlabeled vorozole.



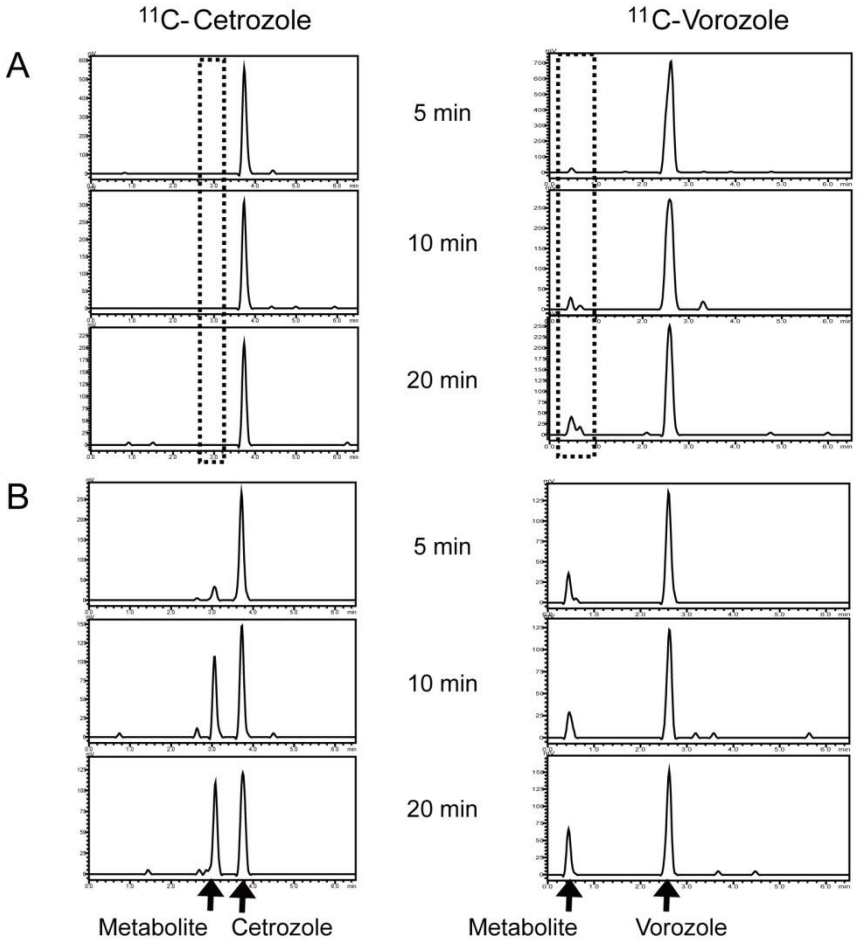
Supplemental Figure 3. Sex difference of [^{11}C]cetrozole binding in the rat brain (MA, medial amygdala; BST, bed nucleus of stria terminalis) (mean ± SEM, n = 3) . In vitro autoradiography was performed as described in the text using male and female Sprague-Dawley rats (11 w). The values were normalized to the value in the medial amygdala of male rats (* $P < 0.05$, ** $P < 0.01$).



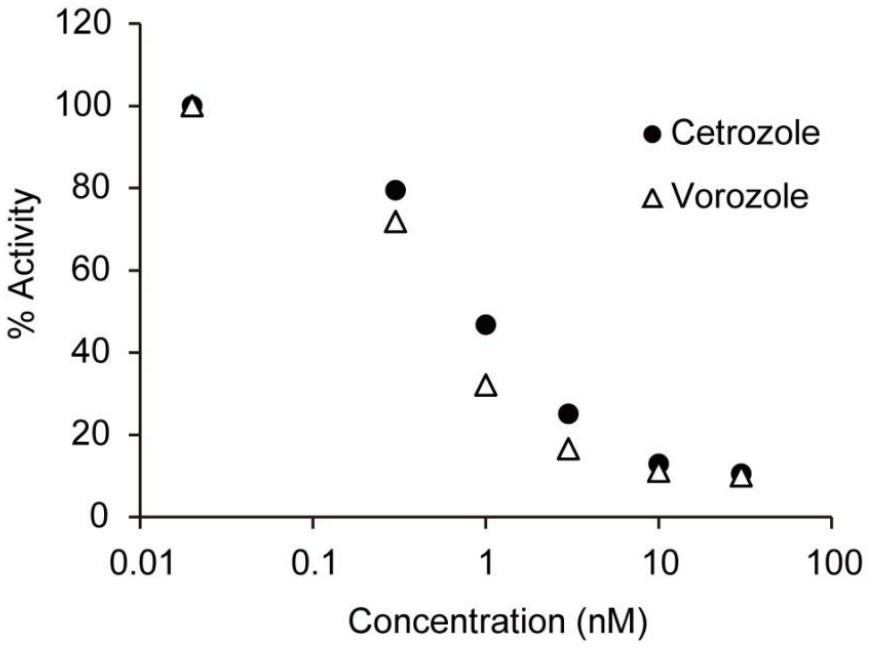
Supplemental Figure 4. A representative Scatchard plot from a binding assay with [^{11}C]cetrozole. Duplicates of homogenized tissue from male rat amygdala were incubated with ascending concentrations of [^{11}C]cetrozole (0.03, 0.10, 0.30, 1.00, and 3.00 nM) at room temperature for 30 min. The experiments were repeated three times, and the dissociation constant (K_d) was calculated as 0.11 ± 0.02 nM with Scatchard plot analysis.



Supplemental Figure 5. Biodistribution of [^{11}C]cetrozole in rats. PET scans were performed with male Sprague-Dawley rats (11 w, n = 4) using [^{11}C]cetrozole. The rats were anesthetized and maintained using a mixture of 1.5% isoflurane and nitrous oxide/oxygen (7:3) and positioned in the PET scanner. After a bolus injection of [^{11}C]cetrozole (injected radioactivity, 80.5-112.6 MBq) into the tail vein, a 60-min emission scan was performed. Whole-body scans were performed with 180-s duration for 1 bed pass. The scanner bed was moved continuously in a reciprocating motion to ensure scanning of the entire rat body. Volumes of interest were delineated in the brain, heart, liver, kidney, small intestine, and urinary bladder. Percentage injected dose (%ID) at 30 min after the injection of [^{11}C]cetrozole is presented (mean ± SEM).



Supplemental Figure 6. Representative radiochromatograms of [^{11}C]cetrozole and [^{11}C]vorozole and their metabolites in the brain (A) and plasma (B) of rat. The areas delineated by dashed lines indicate the latency at which each metabolite was detected in plasma. The [^{11}C]cetrozole metabolite detected in plasma was not detected in the brain. In contrast, the [^{11}C]vorozole metabolite was detected both in the brain and plasma.



Supplemental Figure 7. Inhibitory activity of unlabeled cetrozole and vorozole to aromatase in the marmoset placenta. Marmoset placentas (2 g) were homogenized in 0.25 M sucrose (15 mL) with Complete protease inhibitor cocktail (Roche Applied Science, Rotkreuz, Switzerland). The homogenate was centrifuged, and the microsomal pellet was resuspended in 0.05 M potassium phosphate buffer, pH 7.4. The suspension was centrifuged again at 148,000 ×*g* for 60 min, and the resulting pellet was resuspended in 1.6 mL of potassium phosphate buffer. The suspension was dispensed and stored at −80°C until use.

The aromatase inhibitory assay was performed by measuring the inhibition of estrogen synthesis activity, which was measured according to the method described by Steele et al. (*8*) with suitable modifications. Estrogen synthesis activity was measured using

[4-^{14}C]testosterone as a substrate. The incubation mixture contained 110–120 nM [4-^{14}C]testosterone (1.96 GBq/mmol, GE Healthcare Japan, Tokyo, Japan), 0.24 mM NADPH (Sigma-Aldrich, St. Louis, MO, USA), 0.3, 1, 3, 10, or 30 nM unlabeled cetrozole, and 10 μL microsomal protein in a total volume of 400 μL at 37°C. [4-^{14}C]Testosterone and unlabeled cetrozole were added as a solution in 50% ethanol in 0.05 M potassium phosphate buffer so that the final concentration of ethanol was 0.5%. The reaction was started by adding the substrate and was stopped after 20 min by adding 800 μL ethyl acetate. The mixture was stirred with a Vortex mixer and centrifuged at 2,000 ×*g* for 2 min. The aqueous phase was evaporated to dryness using a centrifugal concentrator (CC-105, TOMY, Tokyo, Japan) and a low temperature trap (TU-500, TOMY). Over 95% of the radioactivity was recovered. The obtained residue was dissolved in 10 μL ethyl acetate, and 5-μL aliquots were applied to Silica 60 thin-layer chromatography plates (Merck KGaA, Darmstadt, Germany). Plates were developed at room temperature with ethyl acetate/isooctane (140:60, v/v) as a mobile phase. After migration, plates were dried and exposed to BAS-SR2040 imaging plates overnight. The distribution of radioactivity on the imaging plates was determined with digital PSL autoradiography using a FLA-7000IR analyzer (Fuji Photo Film Co.), and

the data were analyzed with the MultiGauge image analysis program (Fuji Photo Film Co.).

The IC_{50} of cetrozole and vorozole were determined as 1.27 and 0.66 nM, respectively.

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