Electronic Supplementary Information

Stable aluminum fluoride chelate with triazacyclononane derivatives proved by X-ray crystallography and implications in PET for one step ^{18}F-labeling

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Electronic Supplementary Material (ESI) for Chemical Communications

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Experimental Section

General: NOTA and 1,4,7 triazacyclononane were purchased from ChemaTech (Dijon, France). All other chemicals were purchased from Sigma/Aldrich (St. Louis, MO, U.S.A.). ^{18}F was produced on the PET cyclotron, CYCLONE® 18/9 (IBA, Louvain-la-Neuve, Belgium) by the ^{18}O (p, n) ^{18}F nuclear reaction according to standard procedure. ^{1}H and ^{13}C NMR spectra were recorded on 300-MHz, AL-300 FTNMR spectrometer JEOL (Tokyo, Japan). Chemical shifts (δ) were reported in ppm downfield from tetramethylsilane and multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), and br (broad). ^{1}H and ^{13}C NMR spectra were acquired in $CDCl_3$ and reference to residual $CHCl_3$ at 7.26 and 77.00 ppm respectively or in D_2O referenced to residual DOH at 4.65 ppm. Electrospray ionization mass spectra (ESI-MS) were acquired on a Waters ESI ion trap spectrometer (Milford, U.S.A.) for both positive and negative ion detection. The samples were diluted 1 to 100 with methanol and injected directly into the source. Data are reported in the form of (*m/z*) versus intensity.

Preparative purification of the compound was performed on a X-terra 10 *C*m RP18 (19 × 250 mm) column. Waters Sep-Pak Light Accell Plus QMA cartridge (Milford, U.S.A.) was used to obtain purified $^{18}F^-$ solution. The gamma scintillation counter was a Packard Cobra II (GMI, MN, U.S.A.). Radio-thin layer chromatography (TLC) was counted using a Bio-Scan AR-2000 System imaging scanner (Bioscan, DC, U.S.A.). Instant TLC-silica gel (ITLC-SG) plates were purchased from Varian Inc. (Agilent Technologies, Wilmington, U.S.A.) and the alumina-N cartridge was from Waters (Milford, U.S.A.). Autoradiography images were captured using FUJIFILM Bio-imaging Analyzer System (BAS), FLA-2000 (Tokyo, Japan). PET images were obtained using a small-animal PET/CT scanner (GE Healthcare, Princeton, NJ, U.S.A.). The biodistribution experiments were performed in Seoul National University Hospital, Seoul, Korea, which is fully accredited by AAALAC International (2007, Association for Assessment and Accreditation of Laboratory Animal Care International).

Synthesis of 1,4-bis(tert-butoxycarbonylmethyl)-1,4,7-triazanonane (1): Solution of *tert*-butyl bromoacetate (8.31 g, 42.59 mmol) in $CHCl_3$ (50 mL) was added to triazacyclononane (2.5 g, 19.36 mmol) in $CHCl_3$ (25 mL) slowly over 40 min. The resulting mixture was stirred at room temperature for 24 h. After monitoring the completion of the starting material using thin layer

chromatography (CH_2Cl_2/MeOH; 9/1), reaction mixture was filtered, and the filtrate was evaporated. The residue was treated with deionized (DI) water (15 mL) and the resulting solution was adjusted to pH 3 using 1 M HCl and extracted with ether (50 mL × 2). Organic layer was evaporated and dried to obtain trisubstituted product. The aqueous layer was then adjusted to pH 8 using 1 M NaOH and extracted with CH_2Cl_2 (25 mL × 2). Organic layer was evaporated and resulting residue was treated with DI water (5 mL). Solution pH was adjusted to 10 using 1 M NaOH, and extracted with ether (30 mL × 2). Organic layer was evaporated and dried. Hexane (5 mL) was added to resulting solution and kept in a freezer for 6 h. Disubsubstituted product was obtained as solid and evaporation of decanted hexane layer gave trisubstituted product. Aqueous layer was further adjusted to pH 8 using 1 M HCl and extracted with CH_2Cl_2 (25 mL × 2). Organic layer collected was evaporated and dried to obtain required disubstituted product **1**. Overall yield: 4.6 g (66%). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.46 (s, 18H), 2.78 (s, 4H), 3.08 (t, 4H, J = 4 Hz), 3.28 (t, 4H, J = 4 Hz), 3.39 (s, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 27.9, 44.4, 48.6, 51.4, 56.4, 81.7, 170.6 (*C*O) ppm. ESI-MS: m/z = 358.4 for $[M+H]^+$.

Synthesis of 4,7-bis(tert-butoxycarbonylmethyl)-1,4,7-triazanonane-1-propionic acid: Solution of 3-bromo prapionic acid (0.021 g, 0.14 mmol) in acetonitrile (1 mL) was added to mixture of **1** (0.05 g, 0.139 mmol) and Et_3N (0.028 g, 0.279 mmol) in acetonitrile (2 mL) slowly, and stirred for 36 h at room temperature. Completion of the reaction was checked by TLC (CH_2Cl_2/MeOH; 9/1). Solvent was removed using a rotary evaporator and the crude product in CH_2Cl_2 (5 mL) was extracted with water (3 mL) and brine (3 mL). Organic layer was concentrated *in vacuo* and purified by flash column chromatography (CH_2Cl_2/MeOH). Product was eluted with 8% methanol. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.34-1.25 (m, 2H), 1.44 (s, 18H), 2.64 (t, 2H, J = 6 Hz), 2.76 (s, 4H), 3.16-3.04 (m, 4H), 3.46-3.30 (m, 8H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 8.6, 28.1, 45.3, 51.4, 52.9, 54.0, 57.8, 81.3, 171.0 (*C*O), 174.2 (*C*O) ppm. ESI-MS: m/z = 430.5 for $[M+H]^+$.

General Procedure I: Ethyl 4-bromobutyrate (0.030 g, 0.153 mmol) or ethyl 5-bromovalerate (0.032 g, 0.153 mmol) solution in acetonitrile (1 mL) was added to a solution of **1** (0.05 g, 0.139 mmol) and K_2CO_3 (0.039 g, 0.279 mmol) in acetonitrile (2 mL) slowly, and stirred for 20 h at room temperature. Completion of the reaction was checked by TLC (CH_2Cl_2/MeOH; 9/1).

Reaction mixture was filtered and concentrated *in vacuo*. Crude product was purified by flash column chromatography (CH_2Cl_2/MeOH). Products were eluted with 5 to 6% methanol.

4,7-bis(tert-butoxycarbonylmethyl)-1,4,7-triazanonane-1-butyric acid ethyl ester (n= 3): ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.26 (t, 3H, J = 9 Hz, 6 Hz), 1.46 (s, 18H), 1.84 (qn, 2H), 2.35 (t, 2H, J = 6 Hz), 2.66- 2.62 (m, 2H), 3.0-2.71 (m, 12H), 3.33 (s, 4H), 4.12 (q, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 14.2, 28.2, 31.9, 51.5, 54.8, 55.1, 59.7, 60.3, 80.8, 171.4 (*C*O), 173.5 (*C*O). ESI-MS: m/z = 472.5 for $[M + H]^+$.

4,7-bis(tert-butoxycarbonylmethyl)-1,4,7-triazanonane-1-pentanoic acid ethyl ester (n = 4): ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.19 (t, 3H, J = 6 Hz), 1.38 (s, 18H), 1.69-1.59 (m, 2H), 1.83-1.76 (m, 2H), 2.31 (t, 2H, J = 6 Hz), 2.71-2.66 (m, 2H), 3.80-3.03 (m, 16H), 4.06 (q, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 14.1, 21.8, 23.9, 28.1, 33.3, 48.8, 52.1, 55.5, 57.6, 60.4, 81.6, 170.6 (*C*O), 172.8 (*C*O). ESI-MS m/z = 486.5 for $[M + H]^+$.

General Procedure II: LiOH (5 eq) was added to a solution of protected compounds (1 eq) in EtOH (0.5 mL) and stirred at 50^{o}C for 24 h. ESI-mass analysis of reaction mixture revealed the completion of the cleavage. Reaction mixture was filtered through Whatman syringe filter (0.45 µm) and concentrated *in vacuo*. Final products were purified by RP-HPLC (10 mM HCl and EtOH; 0 to 40 % for 20 min). Collected fractions were lyophilized to obtain the required products.

4,7-bis(carboxymethyl)-1,4,7-triazanonane-1-butyric acid (5b): ^{1}H NMR (D_2O, 300 MHz, 25 °C): δ 1.45 (qn, 2H), 1.88 (t, 2H, J = 4 Hz, 6 Hz), 2.80-2.40 (m, 14H), 3.25 (s, 4H). ^{13}C NMR (D_2O, 75 MHz, 25 °C): 23.9, 36.1, 52.1, 52.4, 53.1, 58.1, 62.0, 182.2 (*C*O), 183.7 (*C*O). ESI-MS: m/z = 332.2 for $[M + H]^+$.

4,7-bis(carboxymethyl)-1,4,7-triazanonane-1-pentanoic acid (5c): ^{1}H NMR (D_2O, 300 MHz, 25 °C): δ 1.51-1.35 (m, 4H), 2.08 (t, 2H, J = 4 Hz), 2.85-2.35 (m, 12H), 3.16 (s, 4H) ^{13}C NMR (D_2O, 75 MHz, 25 °C): 17.4, 18.6, 24.3, 37.9, 52.0, 53.2, 58.1, 168.1 (*C*O), 181.7 (*C*O), 184.2 (*C*O). ESI-MS: m/z = 346.2 for $[M + H]^+$.

Synthesis of 1-benzyl-4,7-bis(tert-butoxycarbonylmethyl)-1,4,7-triazanonane.: Benzyl bromide (0.096 g, 0.559 mmol) solution in THF (2 mL) was added to a solution of **1** (0.2 g,

0.559 mmol) and K_2CO_3 (0.077 g, 0.559 mmol) in THF (7 mL) slowly, and stirred for 12 h at room temperature. Completion of the reaction was checked by TLC (CH_2Cl_2/MeOH; 9/1). Reaction mixture was filtered and concentrated *in vacuo*. Crude product was purified by flash column chromatography (CH_2Cl_2/MeOH). Product was eluted with 8% methanol. ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.39 (s, 18H), 3.10-2.61 (m, 8H), 3.21 (s, 4H), 3.68-3.40 (m, 4H), 4.47 (s, 2H), 7.35- 7.32 (m, 3H), 7.66-7.63 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 28.0, 49.7, 51.7, 53.3, 58.0, 59.4, 81.6, 129.0, 129.4, 130.7, 170.6 (*C*O). ESI-MS: *m*/*z* = 448.2 for $[M + H]^+$.

Synthesis of 4,7-bis(tert-butoxycarbonylmethyl)-1,4,7-triazanonane-1-oxo-butyric acid: Succinic anhydride (0.031 g, 0.336 mmol) solution in $CHCl_3$ (1 mL) was added to a solution of **1** (0.1 g, 0.278 mmol) and Et_3N (0.034 g, 0.336 mmol) in $CHCl_3$ (3 mL) slowly, and stirred for 24 h at room temperature. Completion of the reaction was analyzed by TLC (CH_2Cl_2/MeOH; 9/1). Reaction mixture was extracted with water (2 mL) and brine (2 mL). Organic layer was evaporated *in vacuo* and purified by flash column chromatography (CH_2Cl_2/MeOH). ^{1}H NMR ($CDCl_3$, 300 MHz, 25 °C): δ 1.46 (s, 18H), 2.70-2.66 (m, 2H), 3.21-2.82 (m, 8H), 3.27-3.22 (m, 2H), 3.47 (s, 4H), 3.70-3.59 (m, 4H). ^{13}C NMR ($CDCl_3$, 75 MHz, 25 °C): 28.0, 28.7, 30.0, 45.0, 50.4, 50.7, 58.2, 58.7, 81.7, 171.0 (*C*O), 173.5 (*C*O). ESI-MS: *m*/*z* = 458.2 for $[M + H]^+$.

General Procedure III: Protected compounds were dissolved in 1,4-dioxane (3 mL). Conc HCl (0.3 mL) was added to the solution dropwise and stirred at room temperature. Completion of the cleavage was analyzed by ESI-Mass spectral analysis. Solvent and acid were removed *in vacuo*. Repeated washing with 1,4-dioxane and with diethyl ether gave products as hydrochloride salts.

4,7-bis(carboxymethyl)-1,4,7-triazanonane-1-propionic acid (5a): ^{1}H NMR (D_2O, 300 MHz, 25 °C): δ 2.79 (t, 2H, J = 6 Hz), 3.15-3.110 (m, 4H), 3.35-3.22 (m, 4H), 3.52-3.45 (m, 4H), 3.54 (s, 4H), 3.75-3.72 (m, 2H). ^{13}C NMR (D_2O, 75 MHz, 25 °C): 29.3, 50.0, 51.0, 51.8, 53.8, 57.4, 63.0, 173.3 (*C*O), 174.5 (*C*O). ESI-MS: *m*/*z* = 318.2 for $[M + H]^+$.

1-benzyl-4,7-bis(carboxymethyl)-1,4,7-triazanonane (3): ^{1}H NMR (D_2O, 300 MHz, 25 °C): δ 3.98-2.26 (m, 16H), 4.33 (s, 4H), 7.33 (br, 3H), 7.96 (br, 2H). ^{13}C NMR (D_2O, 75 MHz, 25 °C): 50.0, 50.1, 51.0, 57.1, 67.1, 81.2, 129.1, 130.1, 131.8, 173.2 (*C*O). ESI-MS: *m*/*z* = 336.3 for $[M + H]^+$.

4,7-bis(carbxymethyl)-1,4,7-triazanonane-1-oxo-butyric acid (4): ^{1}H NMR (D_2O, 300 MHz, 25 °C): δ 2.53-2.41 (m, 2H), 2.99-2.82 (m, 4H), 3.22-3.14 (m, 2H), 3.48-3.30 (m, 4H), 3.52 (s, 4H), 3.61-3.53 (m, 4H). ^{13}C NMR (D_2O, 75 MHz, 25 °C): 29.1, 29.5, 46.4, 47.2, 54.0, 55.2, 56.1, 170.2 (*C*O), 175.3 (*C*O), 176.7 (*C*O). ESI-MS: *m/z* = 346.2 for $[M + H]^+$.

Synthesis of Complex 6: A solution of **3** (0.07 g, 0.1886 mM) and $AlCl_3$ (0.029 g, 0.226 mM) in water was adjusted to pH 3.5 using 1 M sodium acetate buffer. Reaction mixture was heated on boiling water bath for 30 min. NaF (0.039 g, 0.943 mM) was added to the above reaction mixture, and heated for another 30 min. Complex formation was confirmed by ESI-Mass analysis of reaction mixture. Product was purified by RP-HPLC (Water/EtOH; 0 to70 % of EtOH for 30 min; Rt ~ 15 min). Collected fraction was lyophilized after removing the organic solvent to obtain white fluffy solid. Crystals were obtained by slow evaporation of above product solution in water/EtOH mixture (1:9). (ESI-MS)**:** *m/z* = 380.1 for $[M+H]^+$, 402.2 for $[M+Na]^+$.

Radiolabeling Procedure: Stock solution of 2 mM $AlCl_3$ was prepared by dissolving $AlCl_3 \cdot 6H_2O$ in 0.1 M sodium acetate buffer (pH 4). Sep-Pak Light Accell Plus QMA cartridge was pre-conditioned by eluting with 0.4 M $KHCO_3$ (5 mL), followed by water (10 mL). ^{18}F was loaded to the cartridge by eluting aqueous ^{18}F solution produced by a cyclotron through the cartridge and followed by washing with water (5 mL). The ^{18}F loaded cartridge was eluted with saline (0.4 mL) to obtain ^{18}F solution in saline. $Al^{18}F$ was prepared by mixing 45 nmol stock $AlCl_3$ solutions (22.5 µL) and the eluted ^{18}F saline solution (50 µL, 66.6 to 115.81 MBq). After adjusting pH to 4 by adding glacial acetic acid, the mixture was incubated at room temperature for 10 min. The prepared $Al^{18}F$ solution was added to solutions of synthesized ligands (30 to 100 nM) in 0.1 M sodium acetate buffer (1 mL). Reaction mixture was placed on 110 ^{o}C heating block for 10 min. The labeled compounds were passed through alumina-N cartridge to remove the unlabeled $Al^{18}F$.

Labeling efficiency was measured by ITLC-SG, eluted with 75% MeCN solution. Chromatography strips were monitored using a TLC scanner. To capture the autoradiography images, these strips were placed on FUJIFILM imaging plates (IP) and kept for exposure. Exposed IP's were set on IP STAGE, which was placed on the loading unit of bio-imaging analyzer. IP's were scanned automatically and image analysis was carried out on personal computer. Stabilities in prepared medium at room temperature and in human serum at 37˚C were

checked for 120 min. Extent of decomposition was checked by ITLC eluted with 70% MeCN solution in water.

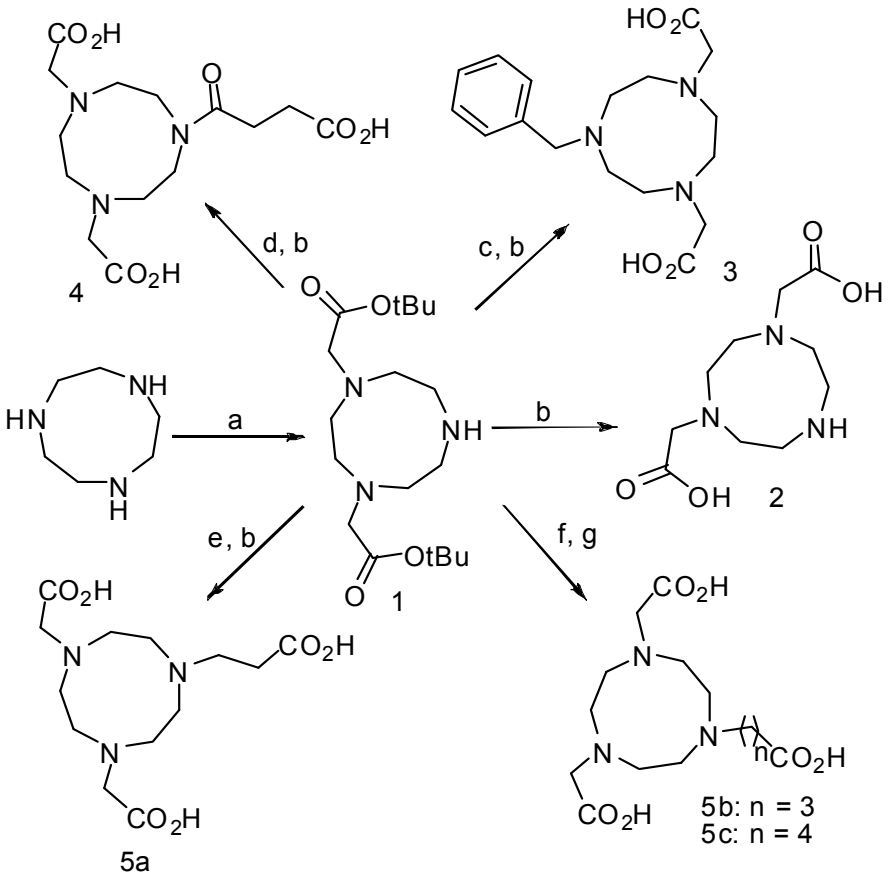
Protein Binding Assay: To measure serum protein binding percentage, labeled compounds were incubated with human serum (1 mL) at 37^{o}C for 10 min and 60 min. After incubation, these samples were loaded onto a PD-10 column (pre-conditioned with 1 mL of 1% BSA/0.1 M DTPA), and eluted with PBS solutions. Thirty fractions of each 0.5 mL were collected using test tubes. Radioactivity of each fraction was measured as cpm using gamma counter. Two μL aliquot from each test tube was spotted on filter paper and the presence of protein was checked by Coomassie blue dye staining. Percentage protein binding was calculated from the activity curve of the fractions.

Crystallographic Study: Data collection and structure analysis were conducted at the Organometallic Laboratory, Seoul National University. Single crystal diffraction data were measured by an Enraf-Nonius CCD single-crystal X-ray diffractometer at room temperature using graphite-monochromated MoKα radiation (λ = 0.71073 Å). Preliminary orientation matrices and unit cell parameters were obtained from the peaks of the first 10 frames and then refined using the whole data set, using setting angles in the range $3^{o} < \theta < 27^{o}$. A total of 7515 reflections were collected for the complex and 4371 reflections were unique. The structure was solved by direct methods using SHELXS-97 and refined by full-matrix least-squares with SHELXL-97. All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were found during refinement.

Biodistribution in Normal Mice: Al^{18}F-**3** (0.074 MBq/0.1 mL) and Al^{18}F-**5b** (0.148 MBq/0.1 mL) were injected intravenously into each mouse through tail vein. Mice were sacrificed at different time intervals (10 and 60 min) after injection. Blood, muscle, bone and other organs were separated immediately and weighed. Counts were obtained with γ-scintillation counter.

PET Imaging in Normal Mice: Normal balb/c mice were injected with Al^{18}F-**5b** (4.81 MBq/0.1 mL) through a tail vein. After inducing anesthesia with 2% isoflurane, PET images were obtained using a small-animal PET/CT scanner. The acquired 3-dimensional emission data were reconstructed to temporally framed sonograms by Fourier rebinning using an ordered-subsets

expectation maximization reconstruction algorithm without attenuation correction. ASIPro software (Concorde Microsystems Inc.) was used for image visualization.



Scheme S1. Synthesis of NODA derivatives for $Al^{18}F$ labeling. a) *tert*-butyl bromoacetate, $CHCl_3$; b) 1,4-dioxane/Conc.HCl; c) benzyl bromide, THF; d) succinic anhydride, Et_3N, $CHCl_3$; e) 3-bromopropionic acid, Et_3N; f) ethyl 4-bromobutyrate/ethyl 5-bromovalerate, K_2CO_3, CH_3CN; g) LiOH, MeOH.

Table S1 Crystal data and structural refinement for **6**·$2H_2O$

Empirical formula	$C_{17}H_{27}Al\ FN_3O_6$
Formula weight	415.40
Temperature	293(2) K
Crystal size	0.31 x 0.28 x 0.13 mm
Wavelength	0.71073 Å
Crystal system	Monoclinic
Space group	P 2_1/a
Unit cell dimensions	a = 13.9007(6) Å; α = 90° b = 7.1927(5) Å; β = 106.473(3)° c = 19.9931(12) Å; γ = 90°
Volume	1916.93(19) $Å^3$
Z	4
Calculated density	1.439 Mg/m^3
Absorption coefficient	0.156 mm^{-1}
F(000)	880
θ range for data collection	3.02 to 27.52°
Index ranges	-17<=h<=17, -9<=k<=8, -25<=l<=25
Reflections collected	7515
Unique reflections	4371 [R(int) = 0.0471]

Completeness to θ = 27.52°	99.2 %
Absorption correction	Empirical
Max. and min. transmission	0.9800 and 0.9533
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	4371 / 0 / 361
Goodness-of-fit on F^2	1.008
Final R indices [I>2sigma(I)]	R1 = 0.0499, wR2 = 0.1051
R indices (all data)	R1 = 0.0905, wR2 = 0.1222
Largest diff. peak and hole	0.220 and -0.251 $e.A^{-3}$

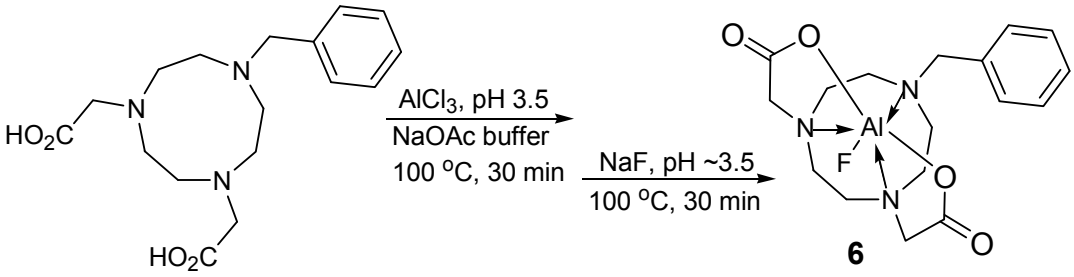


Fig.S1 Synthesis of model $Al^{19}F$-**3** complex.

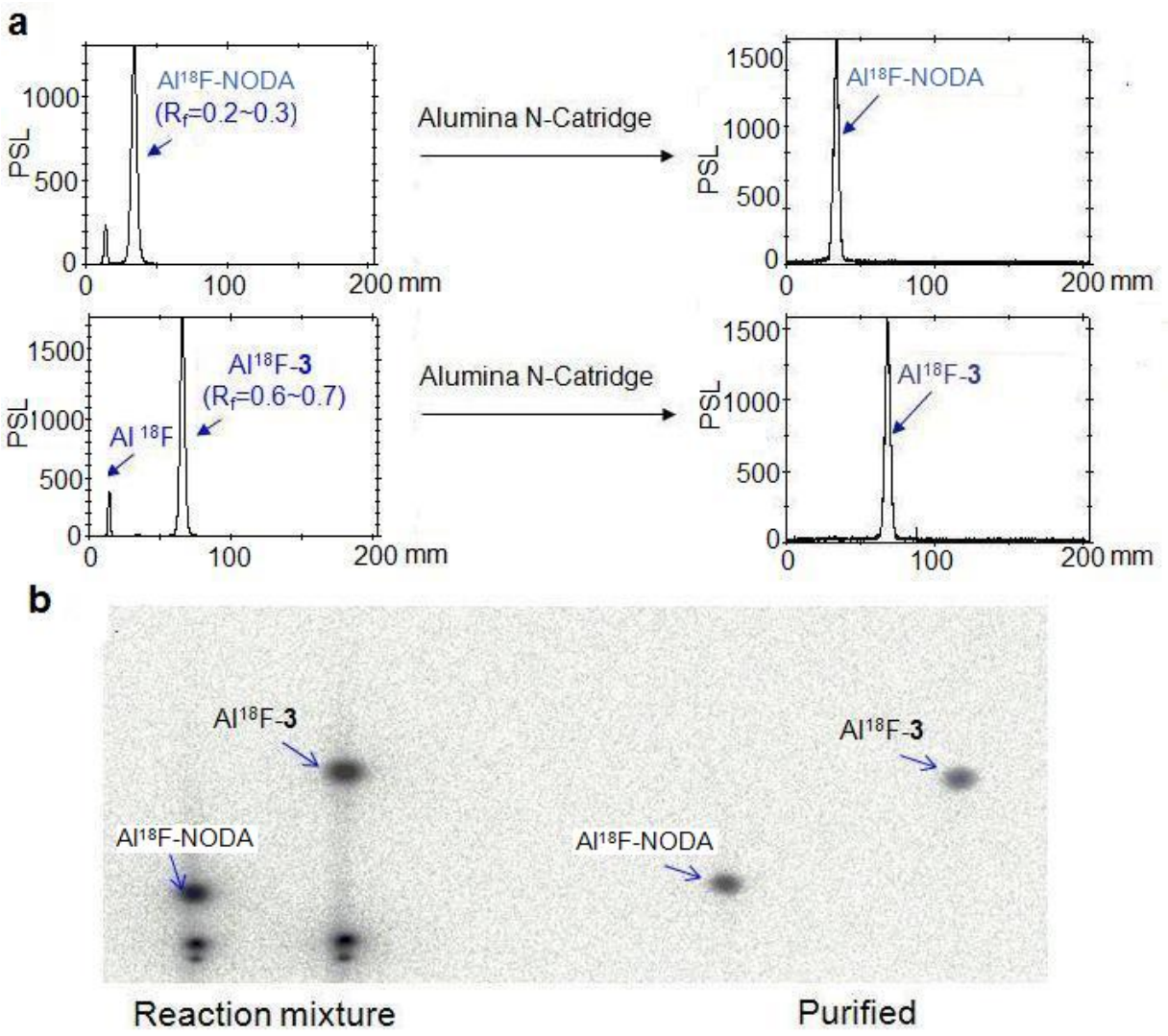


Fig. S2 Labeling efficiecy analysis. (a) ITLC analysisof $Al^{18}F$-NODA and $Al^{18}F$-**3** before and after purification. (b) Autoradiography analysis of the same.

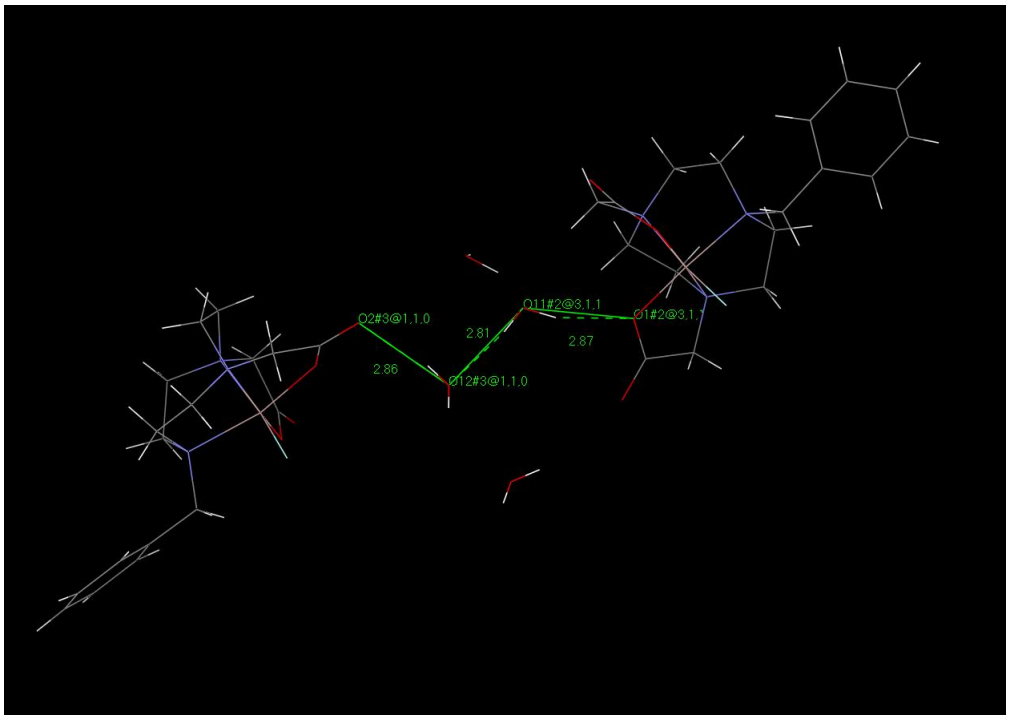


Fig. S3 Inter and Intra molecular hydrogen bonding observed in complex **6**

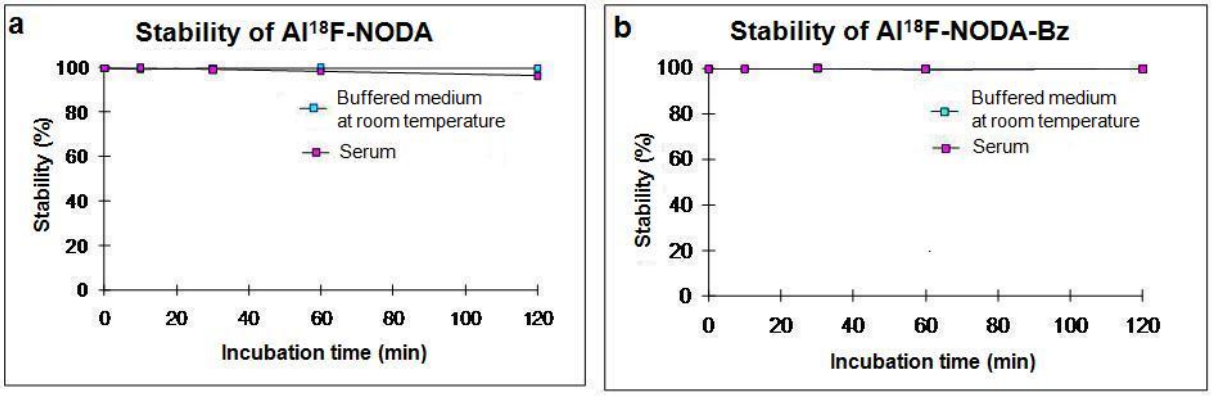


Fig. S4 Stability study of the labeled compounds. $Al^{18}F$-NODA and $Al^{18}F$-**3** in (a) buffered medium at room temperature and (b) in human serum at 37˚C.

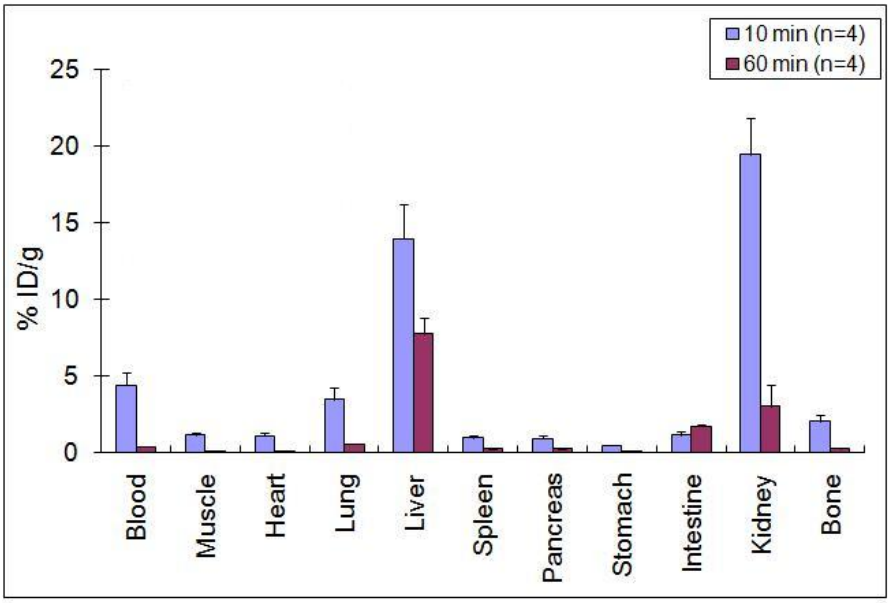


Fig. S5 Biodistribution of ^{18}F-Al-**3** in normal balb/c mice.

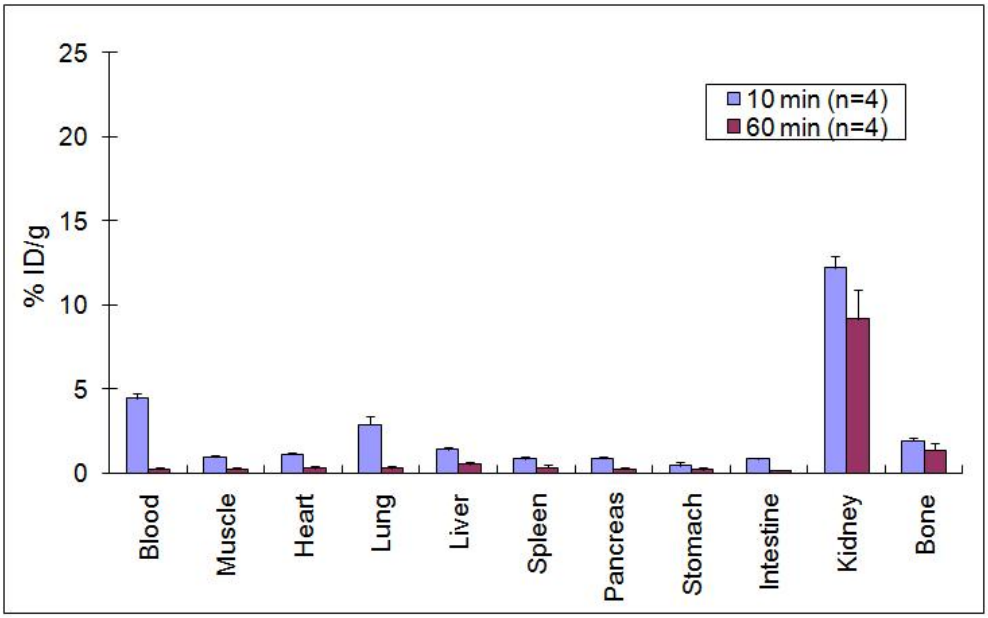


Fig. S6 Biodistribution of ^{18}F-Al-**5b** in normal balb/c mice