

Electronic Supplementary Information

**New chelators for low temperature Al¹⁸F-labeling of
biomolecules**

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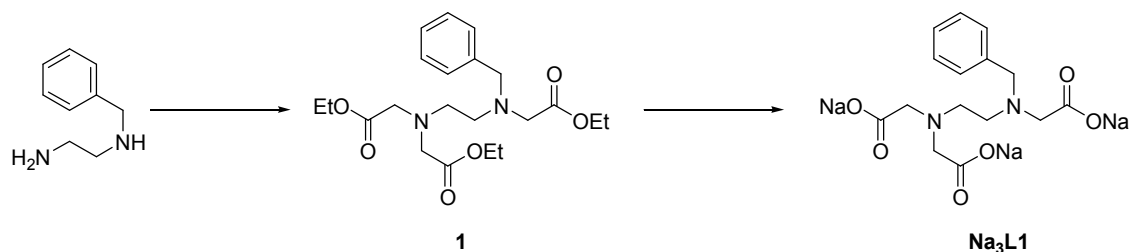
Table S3: Biodistribution (SUV) of Al¹⁸F-Glu-NH-CO-NH-Lys(Ahx)L3 in male NMRI mice30

Table S4: Biodistribution (%ID) of Al¹⁸F-Glu-NH-CO-NH-Lys(Ahx)L3 in male NMRI mice.....30

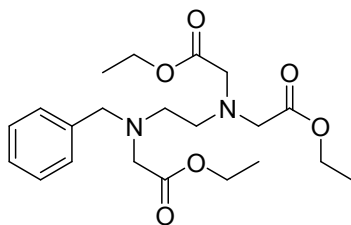
Figure S14: Radio-iTLC analysis of mouse urine samples31

1. Synthesis of H₃L1-8

1.1 Synthesis of ligand H₃L1 (S1)

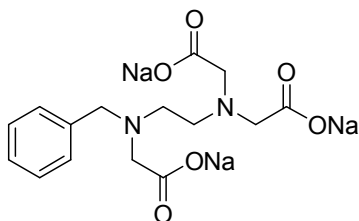


Step 1: Synthesis of compound **1** (diethyl 2,2'-((2-(benzyl(2-ethoxy-2-oxoethyl)amino)ethyl)azanediyl)diacetate).



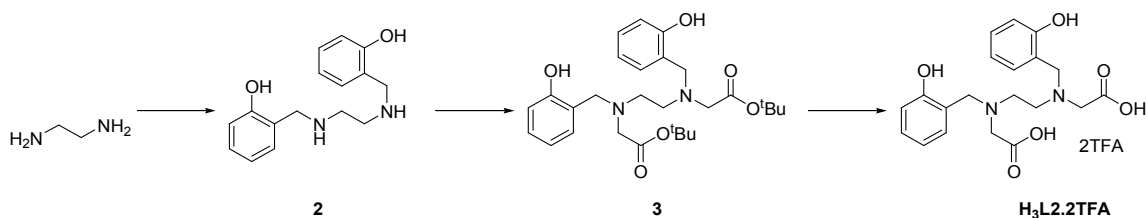
To a solution of *N*-benzylethylenediamine (0.7 g, 4.66 mmol) in anhydrous tetrahydrofuran (4 mL), potassium carbonate (1.9 g, 13.7 mmol) and ethyl bromoacetate (3.0 mL, 27.1 mmol) were successively added. The mixture was stirred for 6 h at room temperature (RT). The solvent was evaporated and ethyl acetate (20 mL) was added. The organic phase was washed with water (3 x 10 mL), dried over magnesium sulfate and the solvent was evaporated under reduced pressure. Flash column chromatography on silica gel (ethyl acetate/heptane, 1:5) afforded pure product, **1** (3.1 g, 80%). **HRMS (ESI):** Calcd. for C₂₁H₃₁N₂O₆ [M+H⁺] 408.2254 ; found 408.2286. **¹H NMR (400 MHz, CDCl₃)** δ 7.39 – 7.19 (m, 5H), 4.14 (q, *J* = 7.1 Hz, 6H), 3.80 (s, 2H), 3.57 (s, 4H), 3.37 (s, 2H), 2.86 (tt, *J* = 7.3, 3.7 Hz, 4H), 1.27 (s, 9H). **¹³C NMR (101 MHz, CDCl₃)** δ 171.5, 171.3 (2C), 138.8, 129.0 (2C), 128.3 (2C), 127.1, 60.4 (2C), 60.2, 58.5, 55.3 (2C), 54.2, 52.1, 52.0, 14.3, 14.2 (2C).

Step 2: Synthesis of compound **Na₃L1** (trisodium 2,2'-((2-(benzyl(carboxylatomethyl)amino)ethyl)azanediyl)triacetate).

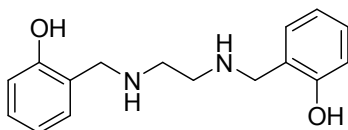


Compound **1** (0.10 g, 0.25 mmol) was stirred in a mixture consisting of an aqueous solution of sodium hydroxide (1 M) and methanol (1:3, 3 mL) for 12 h at RT. Then, the solvent was removed by evaporation. The residue was washed with absolute ethanol and dry diethyl ether, affording **Na₃L1** as a yellow pale solid (61 mg, 63%). **HRMS (ESI)**: Calcd. For C₁₅H₂₀N₂O₆ [M+H⁺], 325.1394 ; found.: 325.1391. **¹H NMR (400 MHz, D₂O)** δ 7.46 – 7.29 (m, 5H), 3.67 (s, 2H), 3.05 (s, 2H), 3.02 (s, 4H), 2.62 (s, 4H). **¹³C NMR (101 MHz, D₂O)** δ 180.2 (2C), 179.9, 138.0, 130.8 (2C), 129.1 (2C), 128.2, 59.2 (2C), 58.5, 58.1, 51.9, 51.2.

1.2 Synthesis of ligand H₃L2 (S2)

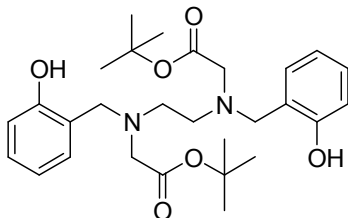


*Step 1: Synthesis of compound 2 (2,2'-((ethane-1,2-diylbis(azanediyl))bis(methylene))diphenol)*¹



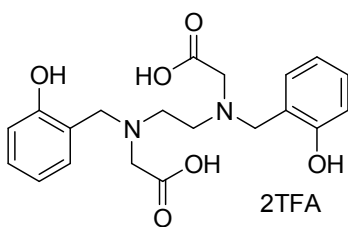
To a solution of ethylenediamine (0.835 mL, 12.5 mmol) in methanol (3 mL), salicylaldehyde (2.36 mL, 25 mmol) was added. A yellow precipitate was formed. The reaction mixture was heated at 80 °C for 1 h. The mixture was then cooled on an ice bath to 0 °C. Next, sodium borohydride (0.88 g, 12.5 mmol) was gradually added to the mixture and the mixture was allowed to reach RT. A white precipitate was formed after stirring for 6 h. The solid was filtered and recrystallized in methanol affording **2** (2.5 g, 73%). **HRMS (ESI)**: Calcd. for C₁₆H₂₀N₂O₂ [M+H⁺] 273.1598 ; found 273.1602. **¹H NMR (400 MHz, CDCl₃)** δ 7.23 – 7.14 (m, 2H), 6.98 (d, *J*=7,1 Hz, 2H), 6.91 – 6.71 (m, 4H), 3.98 (s, 4H), 2.83 (s, 4H).

Step 2: Synthesis of compound 3 (di-tert-butyl 2,2'-(ethane-1,2-diylbis((2-hydroxybenzyl)azanediyl))diacetate).



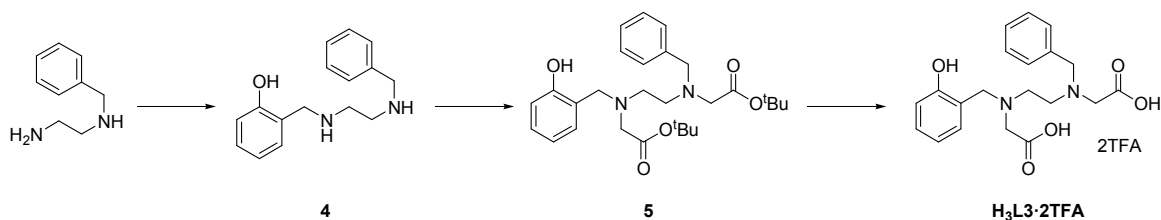
Compound **2** (0.052 g, 0.19 mmol) was dissolved in anhydrous tetrahydrofuran (2 mL). To this solution disodium phosphate (0.080 g, 0.56 mmol) was added. The mixture was stirred at 0 °C and *tert*-butylbromoacetate (0.057 mL, 0.39 mmol) was slowly added to the mixture over 30 min. The reaction mixture was stirred at RT overnight. Next, the solvent was evaporated and an aqueous solution (10 mL, pH 8.5) was added. The product was extracted three times using equal volumes of dichloromethane. The combined organic layers were then dried with sodium sulfate and evaporated under reduced pressure. The yellow oil obtained was purified by flash chromatography on silica gel (ethyl acetate/heptane, 1:2) affording **3** (66 mg, 70%). **HRMS (ESI)**: Calcd. for C₂₈H₄₀N₂O₆ [M+H⁺] 501.2959 ; found 501.2979. **¹H NMR (400 MHz, CDCl₃)** δ 7.17 (t, *J* = 7.6 Hz, 2H), 6.87 (dd, *J* = 20.1, 7.7 Hz, 4H), 6.76 (t, *J* = 7.3 Hz, 2H), 3.72 (s, 4H), 3.17 (s, 4H), 2.69 (s, 4H), 1.46 (s, 18H) **¹³C NMR (101 MHz, CDCl₃)** δ 170.2 (2C), 157.6 (2C), 129.4 (2C), 129.3 (2C), 121.8 (2C), 119.3 (2C), 116.6 (2C), 82.2 (2C), 58.1 (2C), 55.6 (2C), 50.3 (2C), 28.3 (6C).

Step 3: Synthesis of ligand H₃L2.2TFA (2,2'-(ethane-1,2-diylbis((2-hydroxybenzyl)azanediyl))diacetic acid trifluoroacetate salt).

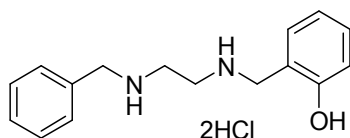


Compound **3** (0.066 g, 0.13 mmol) was stirred in trifluoroacetic acid (1 mL) at RT for 12 h and the deprotection was monitored using LC-MS. Afterwards the solvent was evaporated under reduced pressure affording **H₃L2.2TFA** (20 mg, 39%). **HRMS (ESI)**: Calcd. for C₂₀H₂₄N₂O₆ [M+H⁺] 389.1707 ; found 389.1710. **¹H NMR (400 MHz, D₂O)** δ 7.35 (t, *J*=7.8 Hz, 2H), 7.29 (d, *J*=7.5 Hz, 2H), 7.00 – 6.92 (m, 4H), 4.33 (s, 4H), 3.70 (s, 4H), 3.54 (s, 4H). **¹³C NMR (101 MHz, D₂O)** δ 171.0, 156.1, 133.3, 132.8, 132.8, 121.4, 116.6, 116.2, 56.5, 55.5, 50.1.

1.3 Synthesis of ligand H₃L3 (S3)

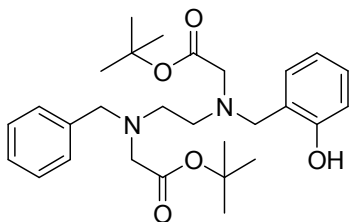


Step 1: Synthesis of compound 4 (2-(((2-(benzylamino)ethyl)amino)methyl)phenol)



A mixture of 2-hydroxybenzaldehyde (0.58 g, 4.8 mmol) and *N*-benzylethylenediamine (0.72 g, 4.8 mmol) in methanol (7 mL) was heated at 50 °C for 30 min. The solvent was then evaporated under reduced pressure, and the residue was suspended in ethanol (10 mL). Then, sodium borohydride (95 mg, 2.5 mmol) was added. The reaction mixture was stirred at RT for 1 h and the solvent was evaporated. Water (7 mL) was added and concentrated hydrochloric acid was used to acidify the solution to pH 1. The crystalline dihydrochloride salt was collected by filtration, washed with cold absolute ethanol and vacuum-dried to yield **4** (0.84 g, 70%). **HRMS (ESI)**: Calcd. for C₁₆H₂₀N₂O [M+H⁺] 257.1648 ; found 257.1658. **¹H NMR (400 MHz, D₂O)** δ 7.56 – 7.44 (m, 5H), 7.42 – 7.30 (m, 2H), 7.03 – 6.95 (m, 2H), 4.30 (s, 4H), 3.61 – 3.35 (m, 4H). **¹³C NMR (101 MHz, D₂O)** δ 158.5, 140.4, 128.8, 128.6, 128.4, 128.2, 127.2, 122.7, 119.0, 116.5, 53.9, 52.6, 48.2, 48.1.

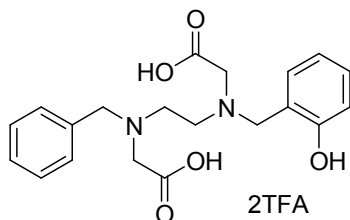
Step 2: Synthesis of compound 5 (tert-butyl 2-(benzyl(2-((2-(tert-butoxy)-2-oxoethyl)(2-hydroxybenzyl)amino)ethyl)amino)acetate).



To a solution of compound **4** (0.174 g, 0.53 mmol) in anhydrous tetrahydrofuran (3 mL), distilled *N,N*-diisopropylethylamine (390 μl, 2.12 mmol) and *tert*-butyl bromoacetate (0.228 g, 1.06 mmol) were successively and slowly added at 0 °C. The mixture was then stirred at RT for 12 h. The solution was filtered and the solvent removed under vacuum. The obtained oil was purified using flash chromatography on silica gel (ethyl acetate/heptane, 1:3) affording pure product **5** (0.144 g, 56%).

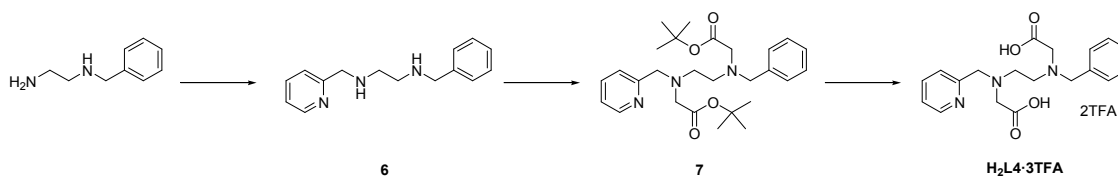
HRMS (ESI): Calcd. for $C_{28}H_{40}N_2O_5$ $[M+H^+]$ 485.3010 ; found 485.3028. **1H NMR (400 MHz, $CDCl_3$)** δ 7.36 – 7.22 (m, 5H), 7.22 – 7.14 (m, 1H), 6.97 (m, 3H), 3.80 (s, 2H), 3.79 (s, 2H), 3.27 (s, 2H), 3.23 (s, 2H), 2.84 (t, $J = 6.2$ Hz, 2H), 2.76 (t, $J = 6.1$ Hz, 2H), 1.48 – 1.44 (m, 17H) **^{13}C NMR (101 MHz, $CDCl_3$)** δ 170.7, 170.2, 157.9, 138.8, 129.3, 129.3, 128.4, 127.3, 122.1, 119.1, 116.5, 81.8, 81.1, 57.4, 55.0, 51.0, 50.9, 28.3, 28.2.

Step 3: Synthesis of ligand **H₃L3.2TFA** (2-(benzyl(2-((carboxymethyl)(2-hydroxybenzyl)amino)ethyl)amino)acetic acid trifluoroacetate salt).



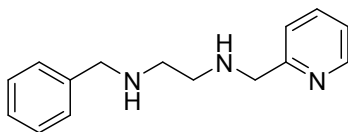
Compound **5** (0.108 g, 0.22 mmol) was stirred in trifluoroacetic acid (2 mL) at RT for 12 h. The deprotection was monitored using LC-MS. Afterwards, the solvent was evaporated under reduced pressure affording pure compound **H₃L3.2TFA** (0.113 g, 90% yield). **HRMS (ESI):** Calcd. for $C_{20}H_{24}N_2O_5$ $[M+H^+]$ 373.1758 ; found 373.1762. **1H NMR (400 MHz, D_2O)** δ 7.35 (t, $J=7.8$ Hz, 2H), 7.29 (d, $J=7.5$ Hz, 2H) 7.00 – 6.91 (m, 5H), 4.33 (s, 4H), 3.70 (s, 4H), 3.54 (s, 4H). **^{13}C NMR (101 MHz, D_2O)** δ 171.0, 156.1, 133.3, 132.8, 121.4, 116.6, 116.2, 56.5, 55.5, 50.1.

1.4 Synthesis of ligand **H₂L4 (S4)**



Step 1: Synthesis of **6** (*N*¹-benzyl-*N*²-(pyridin-2-ylmethyl)ethane-1,2-diamine)

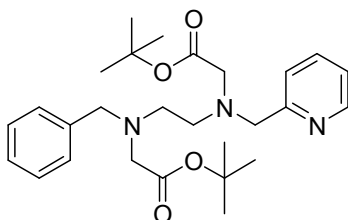
According to Sundaravel et al. 2008².



To a solution of *N*-benzylethylenediamine (200 μ L; 1.33 mmol) in methanol (5.0 mL) was slowly added 2-pyridinecarboxaldehyde (127 μ L; 1.33 mmol). The mixture was then stirred at RT for 24 h.

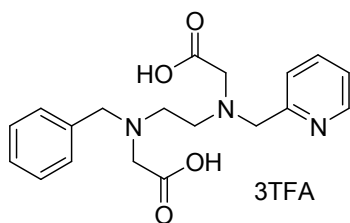
Sodium borohydride (76 mg; 2.00 mmol) was added portionwise and the mixture was stirred at RT overnight. The solvent was then evaporated under reduced pressure. The residue was taken up in dichloromethane and an aqueous solution of sodium hydroxide (0.1 M) was added. After three extractions with dichloromethane, the combined organic phases were dried over magnesium sulfate, filtered and evaporated under reduced pressure to give compound **6** (308 mg; 96%) as a yellow oil. **HRMS (ESI)** calculated for $C_{15}H_{19}N_3$ $[M+H^+]$ 242.1652, found 242.1667; **1H NMR (400 MHz, $CDCl_3$)** δ 8.54 (d, 1H, $J = 4.3$ Hz), 7.62 (t, 1H, $J = 7.6$ Hz), 7.21-7.32 (m, 6H), 7.14 (m, 1H), 3.90 (s, 2H), 3.78 (s, 2H), 2.78 (s, 4H), 2.03 (bs, 2H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 160.0, 149.3, 140.5, 136.5, 128.4 (2C), 128.2 (2C), 126.9, 122.3, 122.0, 55.2, 54.0, 49.1, 48.9.

Step 2: Synthesis of **7** (tert-butyl 2-(benzyl(2-((2-(tert-butoxy)-2-oxoethyl)(pyridin-2-ylmethyl)amino)ethyl)amino)acetate)



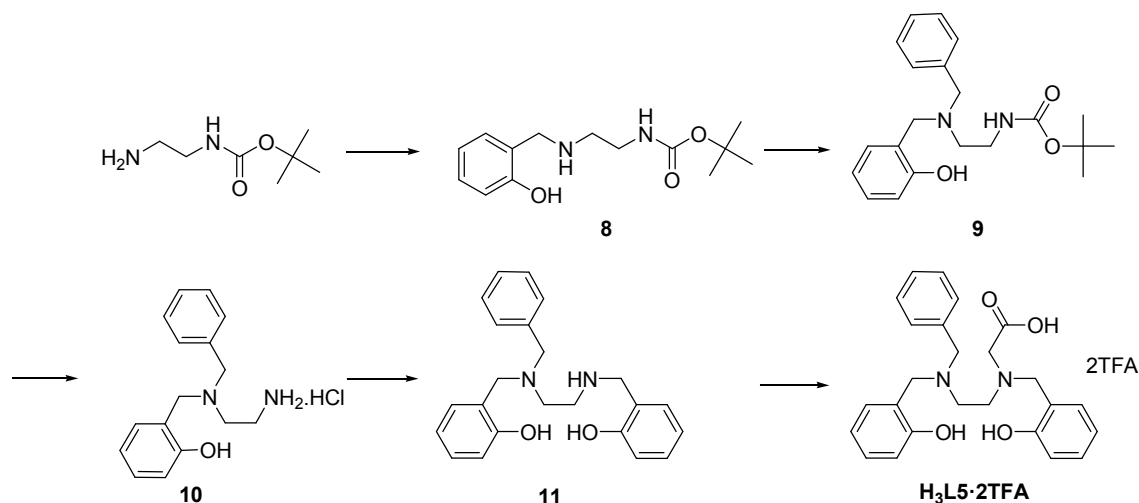
To a solution of **6** (127 mg; 0.53 mmol) in methanol (5 mL) were successively added *N,N*-diisopropylethylamine (183 μ L; 1.05 mmol) and *tert*-butyl bromoacetate (155 μ L; 1.05 mmol), at 0 °C. The mixture was stirred at RT overnight. The solvent was then evaporated under reduced pressure. The residue was taken up in dichloromethane and an aqueous solution of sodium hydroxide (0.1 M) was added. After three extractions with dichloromethane, the combined organic phases were dried over magnesium sulfate, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (silica, DCM/EtOH 96:4) to give **7** (149 mg; 61%) as a yellow oil. Rf (silica, DCM/EtOH 95:5) 0.73; **HRMS (ESI)** calculated for $C_{27}H_{39}N_3O_4$ $[M+H^+]$ 470.3013, found 470.3040; **1H NMR (400 MHz, $CDCl_3$)** δ 8.51 (d, 1H, $J = 4.1$ Hz), 7.61 (td, 1H, $J = 7.7$ Hz, $J = 1.4$ Hz), 7.50 (d, 1H, $J = 7.8$ Hz), 7.22-7.31 (m, 5H), 7.13 (t, 1H, $J = 6.6$ Hz), 3.92 (s, 2H), 3.77 (s, 2H), 3.34 (s, 2H), 3.23 (s, 2H), 2.82 (s, 4H), 1.45 (s, 9H), 1.44 (s, 9H); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 171.1, 171.0, 160.1, 149.1, 139.3, 136.5, 129.1 (2C), 128.3 (2C), 127.1, 123.1, 122.0, 81.0, 80.8, 60.7, 58.5, 56.3, 55.3, 52.2, 52.0, 28.3 (6C).

Step 3: Synthesis of **H₂L4.3TFA** (2-(benzyl(2-((carboxymethyl)(pyridin-2-ylmethyl)amino)ethyl)amino)acetic acid trifluoroacetate salt)

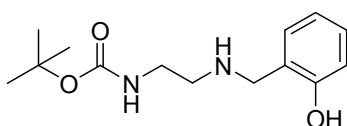


A solution of **7** (53 mg; 0.11 mmol) in trifluoroacetic acid (5 mL) was stirred at RT overnight. The solvent was then evaporated under reduced pressure. The residue was taken up in water (5 mL) and the solvent was evaporated under reduced pressure. The residue was then dried under vacuum to give **H₂L4.2TFA** (65 mg; 82%) as a brown oil. **HRMS (ESI)** calculated for C₁₉H₂₃N₃O₄ [M+H⁺] 358.1761, found 358.1772; **¹H NMR (400 MHz, D₂O)** δ 8.64 (d, 1H, *J* = 5.9 Hz), 8.48 (t, 1H, *J* = 7.9 Hz), 7.93 (t, 1H, *J* = 6.9 Hz), 7.83 (d, 1H, *J* = 8.0 Hz), 7.39-7.49 (m, 5H), 4.46 (s, 2H), 4.12 (s, 2H), 4.08 (s, 2H), 3.45 (t, 2H, *J* = 5.8 Hz), 3.41 (s, 2H), 3.13 (t, 2H, *J* = 5.2 Hz); **¹³C NMR (101 MHz, D₂O)** δ 175.2, 169.3, 153.1, 148.0, 141.8, 132.0 (2C), 131.2, 130.1 (2C), 128.8, 127.3, 126.9, 60.1, 55.3, 54.9, 54.4, 52.3, 50.0.

1.5 Synthesis of ligand H₃L5 (S5)

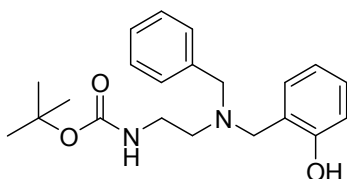


Step 1: Synthesis of compound **8** (tert-butyl (2-((2-hydroxybenzyl)amino)ethyl)carbamate)



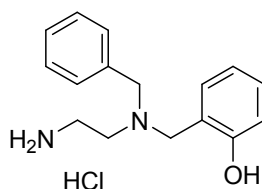
To a solution of *N*-Boc-ethylenediamine (988 μL , 6.24 mmol) in methanol (5.0 mL) was slowly added salicylaldehyde (732 μL , 6.87 mmol). The mixture was heated at 65 $^{\circ}\text{C}$ for 1 h, and then cooled to 0 $^{\circ}\text{C}$. Sodium borohydride (236 mg, 6.24 mmol) was added portionwise and the mixture was stirred at RT for 4 h. The solvent was then evaporated under reduced pressure. The residue was taken up in dichloromethane and an aqueous solution of sodium hydroxide (1N) was added. After five extractions with dichloromethane, the combined organic phases were dried over magnesium sulfate, filtered and evaporated under reduced pressure to give compound **8** (1.627 g, 98%) as yellow crystals. Rf (silica; dichloromethane/ethanol 95:5) 0.57; **HRMS (ESI)** calculated for $\text{C}_{14}\text{H}_{22}\text{N}_2\text{O}_3$ $[\text{M}+\text{H}^+]$ 267.1703, found 267.1652; **^1H NMR (400 MHz, CDCl_3)** δ 7.15 (t, 1H, $J = 7.7$ Hz), 6.97 (d, 1H, $J = 7.3$ Hz), 6.73-6.84 (m, 2H), 4.89 (bs, 1H), 3.98 (s, 2H), 3.26 (d, 2H, $J = 5.5$ Hz), 2.76 (t, 2H, $J = 5.7$ Hz), 1.43 (s, 9H); **^{13}C NMR (101 MHz, CDCl_3)** δ 158.1, 156.3, 128.8, 128.5, 122.4, 119.2, 116.4, 79.6, 52.4, 48.4, 40.1, 28.4 (3C).

Step 2: Synthesis of **9** (tert-butyl (2-(benzyl(2-hydroxybenzyl)amino)ethyl)carbamate)



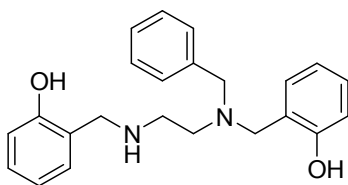
To a solution of **8** (200 mg; 0.75 mmol) in anhydrous tetrahydrofuran (4 mL) were successively added potassium carbonate (104 mg; 0.75 mmol) and benzyl bromide (98 μL ; 0.83 mmol) at 0 $^{\circ}\text{C}$. The mixture was stirred at RT for 7 d. After filtration and evaporation under reduced pressure, the residue was purified by column chromatography (silica, DCM/EtOH 98:2) to give **9** (192 mg; 72%) as a yellow oil. Rf (silica; dichloromethane) 0.25; **HRMS (ESI)** calculated for $\text{C}_{21}\text{H}_{28}\text{N}_2\text{O}_3$ $[\text{M}+\text{H}^+]$ 357.2173, found 357.2183; **^1H NMR (400 MHz, CDCl_3)** δ 7.28-7.36 (m, 5H), 7.16 (td, 1H, $J = 7.3$ Hz, $J = 1.3$ Hz), 6.98 (d, 1H, $J = 7.0$ Hz), 6.83 (d, 1H, $J = 8.0$ Hz), 6.78 (t, 1H, $J = 7.4$ Hz), 4.69 (bs, 1H), 3.78 (s, 2H), 3.68 (s, 2H), 3.30 (d, 2H, $J = 5.8$ Hz), 2.63 (t, 2H, $J = 6.2$ Hz), 1.41 (s, 9H); **^{13}C NMR (101 MHz, CDCl_3)** δ 158.2, 156.6, 137.3, 130.3 (2C), 129.6, 129.5, 129.4 (2C), 128.5, 122.6, 120.0, 116.9, 80.0, 59.1, 58.3, 54.2, 38.5, 29.1 (3C).

Step 3: Synthesis of **10** (2-(((2-aminoethyl)(benzyl)amino)methyl)phenol hydrochloride)



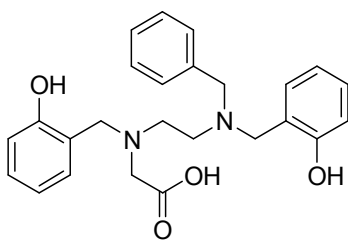
A solution of **9** (175 mg; 0.49 mmol) in hydrochloric acid (1 M, 10 mL) was stirred at RT overnight. The solvent was then evaporated under reduced pressure. The residue was twice taken up in absolute ethanol (15 mL) and the mixture evaporated under reduced pressure to give compound **10** (140 mg; 99%) as a pale-pink solid. **HRMS (ESI)** calculated for $C_{16}H_{20}N_2O$ $[M+H^+]$ 257.1648, found 257.1663; **1H NMR (400 MHz, MeOD- d_4)** δ 7.65 (m, 2H), 7.49 (m, 3H), 7.39 (d, 1H, $J = 7.5$ Hz), 7.32 (t, 1H, $J = 8.2$ Hz), 6.96 (d, 1H, $J = 8.1$ Hz), 6.91 (t, 1H, $J = 7.5$ Hz), 4.52 (s, 2H), 4.42 (s, 2H), 3.52 (s, 4H); **^{13}C NMR (101 MHz, MeOD- d_4)** δ 157.9, 134.1, 133.1, 132.6 (2C), 131.3, 130.4 (2C), 130.3, 121.3, 116.7, 116.6, 59.5, 54.2, 50.7, 35.4.

Step 4: Synthesis of **11** (2-((benzyl(2-((2-hydroxybenzyl)amino)ethyl)amino)methyl)phenol)



To a solution of **10** (150 mg; 0.51 mmol) in methanol (8 mL) were successively added *N,N*-diisopropylethylamine (178 μ L; 1.02 mmol) and salicylaldehyde (60 μ L; 0.56 mmol). The mixture was then heated at 70 °C for 1 h, and cooled to 0 °C. Sodium borohydride (20 mg; 0.51 mmol) was added portionwise and the mixture was stirred at RT for 4 h. The solvent was then evaporated under reduced pressure. The residue was taken up in dichloromethane and an aqueous solution of sodium hydroxide (1 M) was added. After three extractions with dichloromethane, the combined organic phases were dried over magnesium sulfate, filtered and evaporated under reduced pressure to give compound **11** (163 mg; 88%) as a yellow oil. **HRMS (ESI)** calculated for $C_{22}H_{26}N_2O_2$ $[M+H^+]$ 363.2067, found 363.2084; **1H NMR (400 MHz, $CDCl_3$)** δ 7.27-7.37 (m, 5H), 7.12-7.20 (m, 2H), 6.98 (d, 1H, $J = 6.5$ Hz), 6.72-6.86 (m, 5H), 3.78 (s, 2H), 3.76 (s, 2H), 3.63 (s, 2H), 2.79 (t, 2H, $J = 6.3$ Hz), 2.66 (t, 2H, $J = 6.3$ Hz); **^{13}C NMR (101 MHz, $CDCl_3$)** δ 158.7, 158.1, 137.1, 130.3 (2C), 129.8, 129.5, 129.5 (2C), 129.5, 129.2, 128.7, 122.8, 122.5, 120.2, 119.8, 117.1, 116.9, 59.6, 58.8, 53.2, 52.8, 46.0.

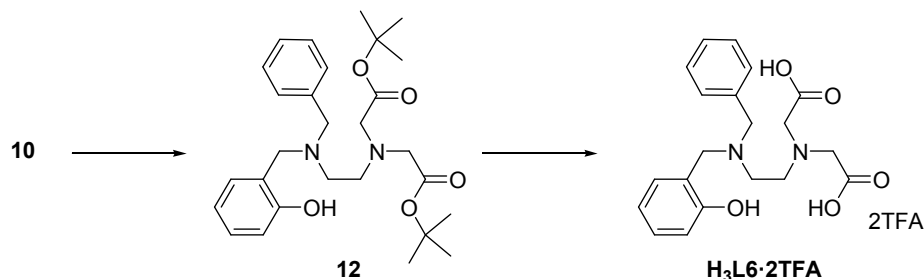
Step 5: Synthesis of **H₃L5** (2-((2-((benzyl(2-hydroxybenzyl)amino)ethyl)(2-hydroxybenzyl)amino)acetic acid)



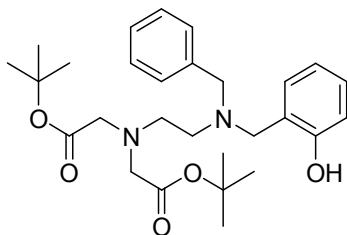
According to a slightly modified procedure from Baffert, C. *et al*³

To a solution of **11** (123 mg; 0.34 mmol) in absolute ethanol (5 mL) were successively added *N,N*-diisopropylethylamine (59 μ L; 0.34 mmol) and bromoacetic acid (47 mg; 0.34 mmol). The solution was refluxed overnight under N₂. The solvent was then evaporated under reduced pressure. The residue was taken up in dichloromethane and an aqueous solution of sodium hydroxide (1 M) was added. After extractions, the pH of the aqueous layer was lowered to 6 with hydrochloric acid (1 M). The aqueous layer was then evaporated under reduced pressure. The residue was taken up in absolute ethanol. After removal of undissolved sodium chloride by filtration, the filtrate was evaporated to dryness under vacuum to give **H₃L5** (96 mg; 68%) as a white solid. **HRMS (ESI)** calculated for C₂₅H₂₈N₂O₄ [M+H⁺] 421.2122, found 421.2124; **¹H NMR (400 MHz, MeOD-d₄)** δ 7.13-7.39 (m, 8H), 6.79-6.86 (m, 5H), 3.98 (s, 2H), 3.77 (s, 2H), 3.73 (s, 2H), 3.19 (m, 4H), 2.89 (t, 2H, *J* = 5.7 Hz); **¹³C NMR (101 MHz, MeOD-d₄)** δ 157.2, 156.8, 136.6, 132.6, 132.1, 131.3, 129.9, 130.4 (2C), 129.1 (2C), 128.4, 122.1, 120.2, 120.1, 118.5, 116.0, 115.8, 58.5, 55.8, 54.2, 53.2, 51.0, 48.3.

1.6 Synthesis of H₃L6 (S6)



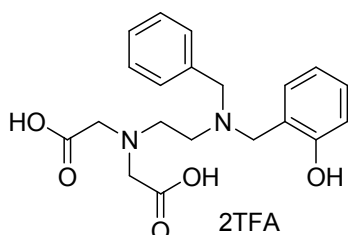
Step 1: Synthesis of **12** (di-tert-butyl 2,2'-((2-(benzyl(2-hydroxybenzyl)amino)ethyl)azanediyl)diacetate)



To a solution of **10** (222 mg; 0.76 mmol) in methanol (5 mL) were successively added *N,N*-diisopropylethylamine (396 μ L; 2.28 mmol) and *tert*-butyl bromoacetate (224 μ L; 1.52 mmol), at 0 °C. The mixture was stirred at RT for 24 h. The solvent was then evaporated under reduced pressure. The residue was taken up in dichloromethane and an aqueous solution of sodium hydroxide (0.1 M) was added. After three extractions with dichloromethane, the combined organic phases were dried over magnesium sulfate, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (silica, DCM/EtOH 100:0 \rightarrow 98/2) to give **12** (147 mg; 40%) as a colorless

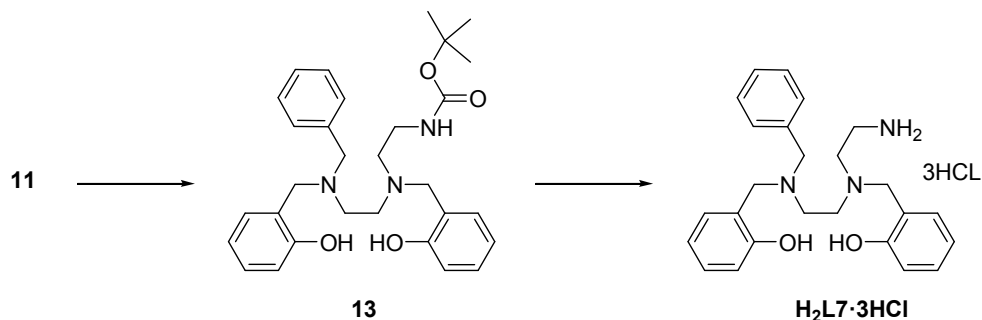
oil. Rf (silica, DCM/EtOH 98:2) 0.45; **HRMS (ESI)** calculated for $C_{28}H_{40}N_2O_5$ $[M+H]^+$ 485.3010, found 485.3037; 1H NMR (400 MHz, $CDCl_3$) δ 7.21-7.34 (m, 5H), 7.15 (t, 1H, $J = 7.1$ Hz), 7.00 (d, 1H, $J = 7.1$ Hz), 6.84 (d, 1H, $J = 8.0$ Hz), 6.76 (t, 1H, $J = 7.3$ Hz), 3.80 (s, 2H), 3.62 (s, 2H), 3.32 (s, 4H), 2.96 (t, 2H, $J = 6.8$ Hz), 2.61 (t, 2H, $J = 6.8$ Hz), 1.41 (s, 18H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.3 (2C), 157.7, 137.2, 129.7 (2C), 129.2, 128.8, 128.6 (2C), 127.6, 122.5, 119.1, 116.3, 81.1 (2C), 58.5, 57.0, 55.8 (2C), 50.8, 50.5, 28.2 (6C).

Step 2: Synthesis of **H₃L6.2TFA** (2,2'-((2-(benzyl(2-hydroxybenzyl)amino)ethyl)azanediyl)diacetic acid trifluoroacetate salt)

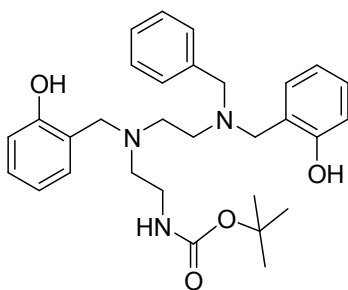


A solution of **12** (111 mg; 0.23 mmol) in trifluoroacetic acid (5 mL) was stirred at RT overnight. The solvent was then evaporated under reduced pressure. The sample was taken up in water (5 mL) and the solvent was evaporated under reduced pressure. The residue was then dried under vacuum to give **H₃L6.2TFA** (90 mg; 70%) as a pale-pink gum. **HRMS (ESI)** calculated for $C_{20}H_{24}N_2O_5$ $[M+H]^+$ 373.1758, found 373.1780; 1H NMR (400 MHz, D_2O) δ 7.38 (s, 5H), 7.26 (t, 1H, $J = 7.7$ Hz), 7.15 (d, 1H, $J = 7.4$ Hz), 6.83-6.88 (m, 2H), 4.29 (s, 2H), 4.16 (s, 2H), 3.26 (s, 4H), 3.22 (t, 2H, $J = 5.6$ Hz), 3.02 (t, 2H, $J = 5.5$ Hz); ^{13}C NMR (101 MHz, D_2O) δ 174.0 (2C), 155.9, 133.1, 132.7, 131.7 (2C), 130.8, 129.9 (2C), 129.4, 121.3, 116.6, 116.3, 59.0, 54.9, 54.4 (2C), 50.3, 49.2.

1.7 Synthesis of ligand **H₂L7** (S7)

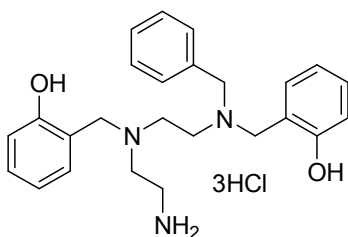


Step 1: Synthesis of **13** (tert-butyl (2-((2-(benzyl(2-hydroxybenzyl)amino)ethyl)(2-hydroxybenzyl)amino)ethyl)carbamate)



To a solution of **11** (151 mg; 0.42 mmol) in anhydrous tetrahydrofuran (5 mL) were successively added *N,N*-diisopropylethylamine (218 μ L; 1.25 mmol) and 2-(Boc-amino)ethyl bromide (103 mg; 0.46 mmol) at 0 °C. The mixture was stirred at RT for 5 d, then at 50 °C for 8 d. Then 2 more equivalents of *N,N*-diisopropylethylamine were added and the mixture was stirred at 50 °C for 7 d. The solvent was evaporated under reduced pressure. The residue was taken up in dichloromethane and an aqueous solution of sodium hydroxide (0.1 M) was added. After three extractions with dichloromethane, the combined organic phases were dried over magnesium sulfate, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (silica, *n*-heptane/ethyl acetate 75:25) to give **13** (31 mg; 15%) as a pale-yellow oil. Rf (silica, *n*-heptane/ethyl acetate 75:25) 0.25; **HRMS (ESI)** calculated for C₃₀H₃₉N₃O₄ [M+H⁺] 506.3013, found 506.3028; **¹H NMR (400 MHz, CDCl₃)** δ 7.13-7.35 (m, 8H, H-Ar), 6.74-6.96 (m, 5H, H-Ar), 3.73 (s, 2H), 3.65 (s, 2H), 3.58 (s, 2H), 3.18 (m, 2H), 2.71 (s, 4H), 2.51 (t, 2H, *J* = 5.5 Hz), 1.41 (s, 9H); **¹³C NMR (101 MHz, CDCl₃)** δ 157.5, 157.4, 156.1, 136.1, 129.8 (2C), 129.2, 129.2, 129.0, 128.9, 128.8 (2C), 128.0, 121.7, 121.6, 119.6, 119.6, 116.4, 116.3, 79.6, 58.5, 58.1, 53.8 (2C), 51.0, 50.1, 38.0, 28.5 (3C).

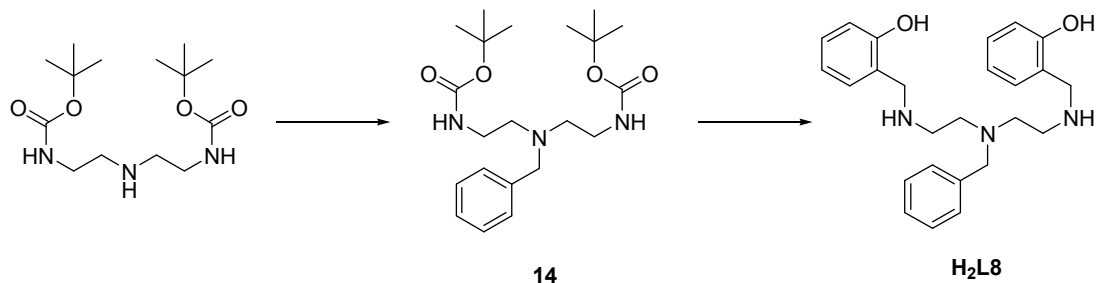
Step 2: Synthesis of **H₂L7.3HCl** (2-(((2-aminoethyl)(2-(benzyl(2-hydroxybenzyl)amino)ethyl)amino)methyl)phenol trihydrochloride)



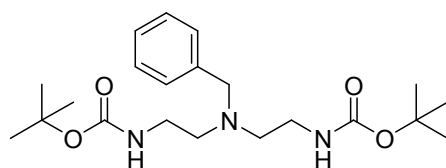
A solution of **13** (31 mg; 61 μ mol) in hydrochloric acid (1 M, 5 mL) was stirred at RT overnight. The solvent was then evaporated under reduced pressure. The sample was taken up in absolute ethanol (2 x 15 mL) and evaporated under reduced pressure to give compound **H₂L7.3HCl** (27 mg; 99%) as a pale-pink solid. **HRMS (ESI)** calculated for C₂₅H₃₁N₃O₂ [M+H⁺] 406.2489, found 406.2494; **¹H NMR (400 MHz, MeOD-d₄)** δ 7.63 (m, 2H), 7.48 (m, 3H), 7.30-7.39 (m, 4H), 6.88-6.97 (m, 4H), 4.54 (s, 2H), 4.44 (s, 2H), 4.41 (s, 2H), 3.84 (s, 4H), 3.67 (m, 2H), 3.55 (m, 2H); **¹³C NMR (101 MHz,**

MeOD-d₄) δ 157.9, 157.7, 134.2, 134.0, 133.5, 133.2, 132.6 (2C), 131.4, 130.5 (2C), 130.2, 121.5, 121.4, 116.9, 116.7, 116.6, 115.9, 59.9, 55.0, 54.5, 52.1, 49.9, 49.4, 35.4.

1.8 Synthesis of ligand **H₂L8** (**S8**)

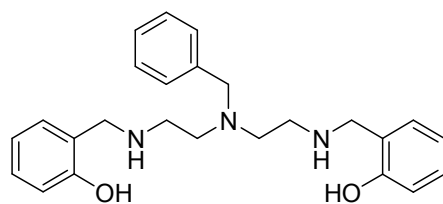


*Step 1: Synthesis of compound **14** (tert-butyl N-(2-(benzyl(2-((tert-butoxycarbonyl)amino)ethyl))amino)ethyl)carbamate)*



To a solution of 1,7-bis-boc-1,4,7-triazaheptane (0.229 g, 0.76 mmol) in anhydrous tetrahydrofuran (2 mL), potassium carbonate (0.156 g; 1.13 mmol) and benzyl bromide (0.259 g; 1.51 mmol) were successively and slowly added at 0 °C. The mixture was then stirred at RT overnight. The solution was filtered and the solvent removed under vacuum. The obtained oil was purified using flash chromatography on silica gel using a gradient of heptane (A) and ethyl acetate (B) (0-1 min: 0% B, 1-9 min: linear gradient 0% B to 80% B) at a flow rate of 15 mL/min affording pure product **14** (0.236 g; 80%). **HRMS (ESI)**: Calcd. for C₂₁H₃₅N₃O₄ [M+H⁺] 394.2700 ; found 394.2698. **¹H NMR (400 MHz, CDCl₃)** δ 7.39 – 7.18 (m, 5H), 3.58 (s, 2H), 3.20 (br. s, 4H), 2.55 (br. s, 4H), 1.45 (s, 18H). **¹³C NMR (101 MHz, CDCl₃)** δ 156.27, 128.93, 128.34, 127.15, 79.09, 58.67, 53.75, 38.27, 28.46.

*Step 2: Synthesis of compound **H₂L8** (2-[(2-[benzyl(2-[(2-hydroxyphenyl)methyl]amino)ethyl]amino)methyl]phenol)*



Compound **14** (0.236 g; 0.60 mmol) was stirred in trifluoroacetic acid (2 mL) at RT. The deprotection was monitored using LC-MS and after 2 hours, the deprotection was complete. The solvent was removed under vacuum and the residue was washed three times with dichloromethane. Salicylaldehyde (0.41 g; 3.32 mmol) and methanol (7 mL) were added and the reaction mixture was heated at 50 °C for 60 min. Then, sodium borohydride (95 mg; 2.5 mmol) was slowly added at 0 °C and the mixture was stirred at RT overnight. The solvent was then evaporated under reduced pressure. The residue was taken up in dichloromethane and an aqueous solution of sodium hydroxide (0.1 M) was added. After three extractions with dichloromethane, the combined organic phases were dried over magnesium sulfate, filtered and evaporated under reduced pressure. The residue was dissolved in a minimal volume of hot methanol and the mixture immediately filtered. The filtrate was placed in an ice bath and after a short period of time crystals started to form. The filtrate was stored at 6 °C overnight to complete the crystallization. The crystals were filtered off and washed twice with ice-cold methanol (2 x 30 mL), affording pure **H₂L8** (0.153 g; 0.38 mmol) with 63% yield. **HRMS (ESI)**: Calcd. for C₂₅H₃₁N₃O₂ [M+H⁺] 406.2489 ; found 406.2496. **¹H NMR (400 MHz, MeOD)** δ 7.36 – 7.18 (m, 5H), 7.06 (br. t, *J* = 7.7 Hz, 2H), 6.96 (d, *J* = 7.2 Hz, 2H), 6.79 – 6.67 (m, 4H), 3.71 (s, 4H), 3.45 (s, 2H), 2.64 – 2.56 (m, 4H), 2.56 – 2.49 (m, 4H). **¹³C NMR (101 MHz, MeOD)** δ 158.65, 140.23, 130.53, 130.23, 129.63, 129.38, 128.15, 124.84, 119.90, 116.76, 60.11, 54.00, 51.33, 46.62.

Table S1: {Al¹⁸F}-complexation efficiency of H₃L5-8 at different temperatures. The results are expressed as RCY in percentage.

temperature	H ₃ L5	H ₃ L6	H ₂ L7	H ₂ L8
RT	0.0	0.0	0.0	0.0
40 °C	0.0	2.9	0.0	0.0
60 °C	0.0	6.1	0.0	0.0
80 °C	0.0	7.2	0.0	0.0
110 °C	0.0	8.1	0.0	0.0

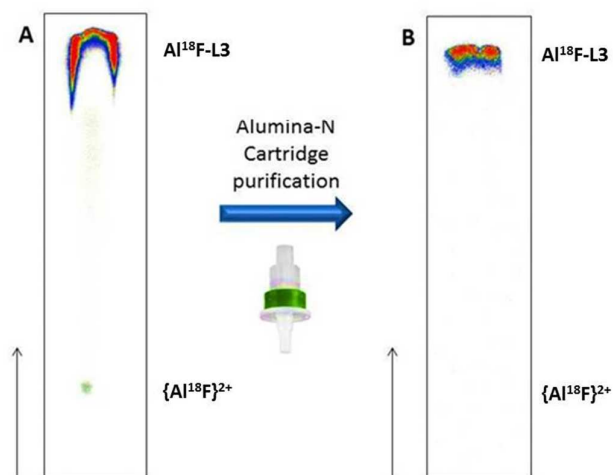


Figure S1: iTLC-SG analysis of $\text{Al}^{18}\text{F-L3}$, before (A) and after (B) alumina-N-cartridge purification. $\text{H}_3\text{L3}$ at a concentration of $150\ \mu\text{M}$ was labeled with $\{\text{Al}^{18}\text{F}\}^{2+}$ in sodium acetate buffer (pH 4) at $40\ ^\circ\text{C}$ for 12 min and passed through an alumina-N cartridge to remove $\{\text{Al}^{18}\text{F}\}^{2+}$. Labeling efficiency was 91% and the radiochemical purity after purification was 98%, both determined by iTLC-SG followed by autoradiography

2. Complexation of $\{\text{Al}^{18}\text{F}\}^{2+}$

$\{\text{Al}^{18}\text{F}\}^{2+}$ solution

$[\text{F}^{18}]\text{F}^-$ was separated from $[\text{O}^{18}]\text{H}_2\text{O}$ by trapping on a SepPak Light Accel plus QMA anion exchange cartridge (Cl^- form; Waters). The cartridge was washed with water (3 mL) and $[\text{F}^{18}]\text{F}^-$ (148-333 MBq) was eluted from the cartridge with an aqueous solution of sodium chloride 0.9% (0.3 mL). The obtained $[\text{F}^{18}]\text{F}^-$ solution (50 μl) was added to 22.5 μL of 2 mM aluminum chloride (AlCl_3) in sodium acetate buffer (0.1 M, pH 4). The solution was incubated at RT for 5 min to form $\{\text{Al}^{18}\text{F}\}^{2+}$.

Synthesis and purification of $[\text{Al}^{18}\text{F}]$ -complexes

A solution of each ligand (1 mL, $150\ \mu\text{M}$) in sodium acetate buffer (0.1 M, pH 4) was added to separate vials which contained a freshly prepared $\{\text{Al}^{18}\text{F}\}^{2+}$ solution. The closed vials were heated in an oil bath at different temperatures for 12 min and the RCY's were determined by iTLC-SG ($\text{H}_3\text{L1}$ and $\text{H}_3\text{L3}$ $n=3$, $\text{H}_3\text{L2}$, $\text{H}_3\text{L4-8}$ and N-benzyl-NODA $n=1$).

The resulting mixtures were purified using a Sep-Pak Plus Alumina-N-Cartridge (Waters, pre-rinsed with an aqueous solution of sodium chloride 0.9% (5 mL)). After loading of the crude reaction mixture, the cartridge was eluted with an aqueous solution of sodium chloride 0.9% (3 mL). The eluate was collected and each of the purified $[\text{Al}^{18}\text{F}]$ -complexes was analysed using iTLC-SG and/or radio-HPLC-HRMS (Acquity UPLC BEH C_{18} , 1.7 μm , 2.1 mm x 150 mm, Waters) with gradient elution at a flow rate of 0.6 mL/min A: H_2O B: ACN, 0-2 min 5% B, 2 to 8 min 5% B to 95% B, 8-10 min 95% B, 10 to 12 min 95% B to 5% B).

2.1 $\text{Al}^{18}\text{F-L1}$

$\text{H}_3\text{L1}$ was labeled with $\{\text{Al}^{18}\text{F}\}^{2+}$ in a non-optimized yield of 96% (12 min $40\ ^\circ\text{C}$, pH 4). The radio-HPLC-HRMS confirms the presence of the aluminum fluoride complex (ESI-MS (m/z): found, 367.0851 [M] $^-$ calculated for $\text{C}_{15}\text{H}_{17}\text{AlFN}_2\text{O}_6$ 367.0892), and the aluminum hydroxide complex (ESI-MS (m/z): found, 365.0935 [M] $^-$ calculated for $\text{C}_{15}\text{H}_{18}\text{AlN}_2\text{O}_7$ 365.0935). The proposed structures of AlOH-L1 and $\text{Al}^{18}\text{F-L1}$ are shown in Figure S2.

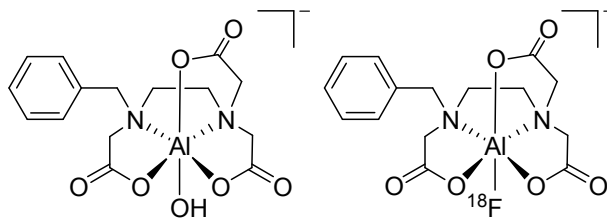


Figure S2: The proposed structures of **AlOH-L1** and **Al¹⁸F-L1**

2.2 Al¹⁸F-L2

H₃L2 was labeled with {Al¹⁸F}²⁺ in a non-optimized yield of 96% (12 min 40 °C, pH 4). The Radio-HPLC-HRMS confirmed the presence of the aluminum fluoride complex (ESI-MS (m/z): found, 431.0751 [M]⁻ calculated for C₂₀H₂₁AlFN₂O₆ 431.1205), an aluminum complex (ESI-MS (m/z): found, 411.1008 [M]⁻ calculated for C₂₀H₂₀AlN₂O₆ 411.1142) and the free chelator (ESI-MS (m/z): found, 387.1166 [M-H]⁻ calculated for C₂₀H₂₄N₂O₆ 387.1561). The proposed structures of **Al-L2** and two possible structures of **Al¹⁸F-L2** are shown in Figure S3.

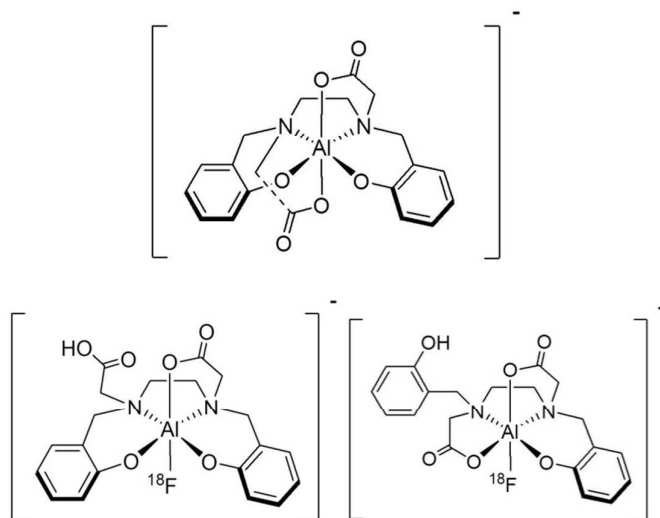


Figure S3 : The proposed structure of **Al-L2** and two possible structures of **Al¹⁸F-L2**

2.3 Al¹⁸F-L3

H₃L3 was labeled with {Al¹⁸F}²⁺ in a non-optimized yield of 89 % (12 min 40 °C, pH 4). The Radio-HPLC-HRMS confirmed the presence of the aluminum fluoride complex (ESI-MS (m/z): found, 415.1217 [M]⁻ calculated for C₂₀H₂₁AlFN₂O₅ 415.1255), an aluminum hydroxide complex (ESI-MS (m/z): found, 413.1141 [M]⁻ calculated for C₂₀H₂₂AlN₂O₆ 413.1299) and the free chelator (ESI-MS (m/z): found, 371.1587 [M-H]⁻ calculated for C₂₀H₂₄N₂O₅ 371.1612). The proposed structures of **AlOH-L3** and **Al¹⁸F-L3** are shown in Figure S4.

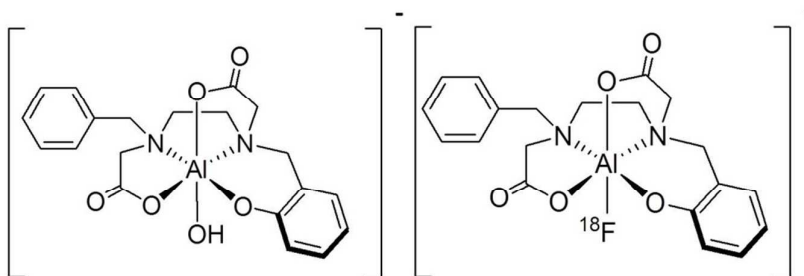


Figure S4: The proposed structures of **AlOH-L3** and **Al¹⁸F-L3**

2.4 Al¹⁸F-L4

H₂L4 was labeled with {Al¹⁸F}²⁺ in a non-optimized yield of 94 % (12 min 80 °C, pH 4). Radio-HPLC-HRMS confirmed the presence of the aluminum fluoride complex (ESI-MS (m/z): found, 402.1397 [M+H] calculated for C₁₉H₂₁AlFN₃O₄ 402.1404), and an aluminum hydroxide complex (ESI-MS (m/z): found, 400.1405 [M+H] calculated for C₂₀H₂₂AlN₃O₅ 400.1448). The proposed structures of **AlOH-L4** and **Al¹⁸F-L4** are shown in Figure S5.

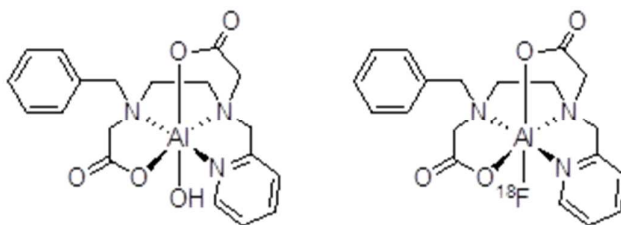
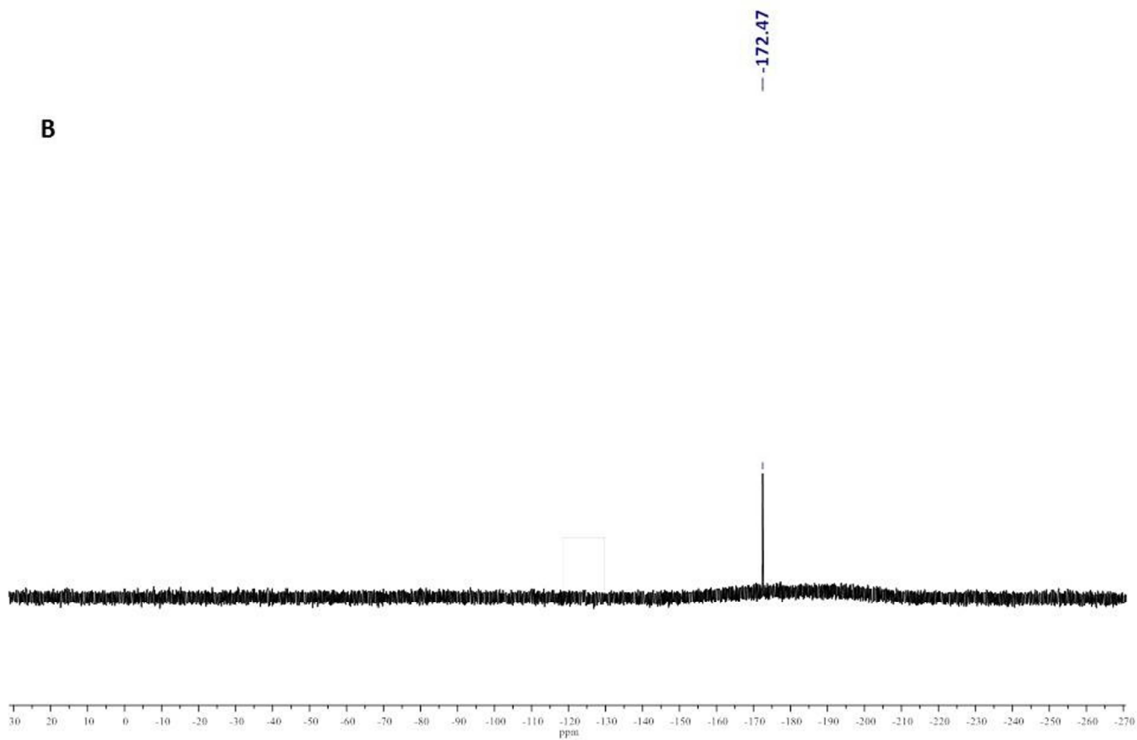
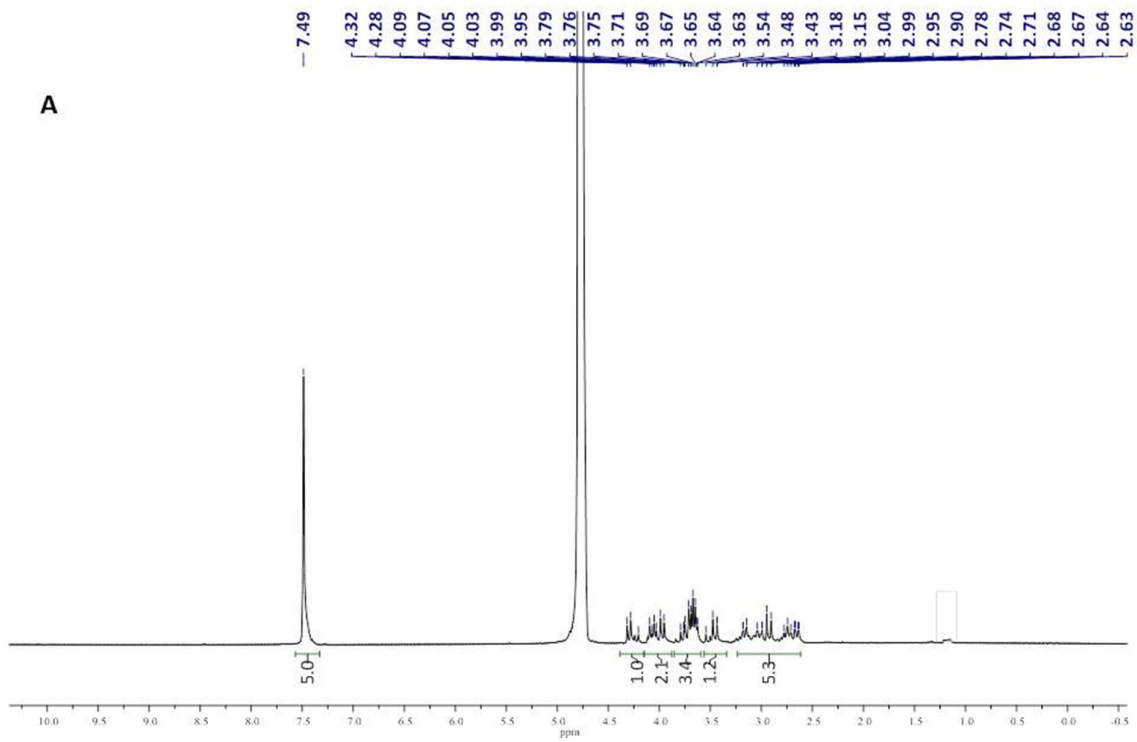


Figure S5: The proposed structures of **AlOH-L4** and **Al¹⁸F-L4**

3. Identification of reference compounds, Al¹⁹F-L_n and Al¹⁹F-N-benzyl

NODA

Na¹⁹F (42 mg, 1 mmol) was added to an aqueous solution of AlCl₃ (1 M, 1 mL). The pH was adjusted to 4 using an aqueous solution of sodium hydroxide (0.1 M) and the mixture was stirred for 10 min. Then, an aqueous solution of the corresponding ligand (1 M, 1 mL) was slowly added and the mixture was heated at 40 °C for 1 h. The reaction was monitored by LC-MS. Al¹⁹F-L1, **HRMS (ESI)**: Calcd. for C₁₅H₁₇AlFN₂O₆⁻ [M]⁻ 367.0892; found 367.0862. Al¹⁹F-L2, **HRMS (ESI)**: Calcd. for C₂₀H₂₁AlFN₂O₆⁻ [M]⁻ 431,1205; found 431.1210. Al¹⁹F-L3, **HRMS (ESI)**: Calcd. for C₂₀H₂₁AlF N₂O₅⁻ [M]⁻ 415.1255; found 415.1262. Al¹⁹F-L4, **HRMS (ESI)**: Calcd. for C₁₉H₂₁AlFN₃O₄ [M+H]⁺ 402.1407; found 402.1397. Al¹⁹F-N-benzyl-NODA, **HRMS (ESI)**: Calcd. for C₁₇H₂₃AlFN₃O₄ [M+H]⁺ 380.1561; found 379.1541



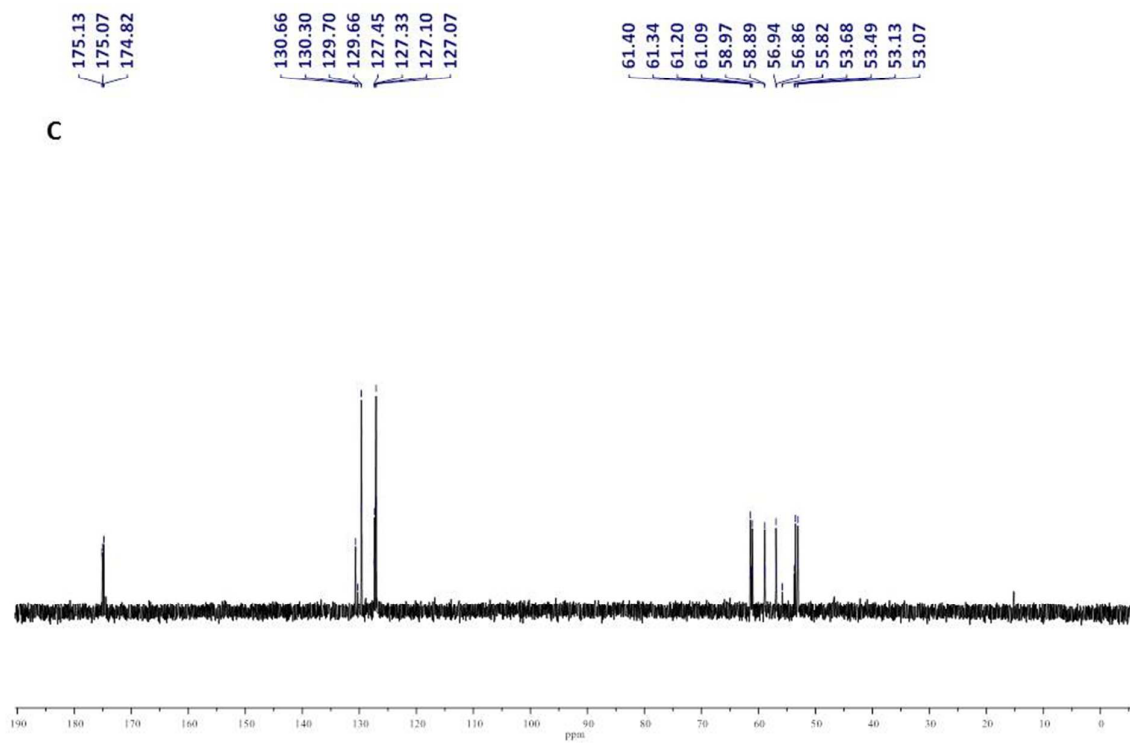
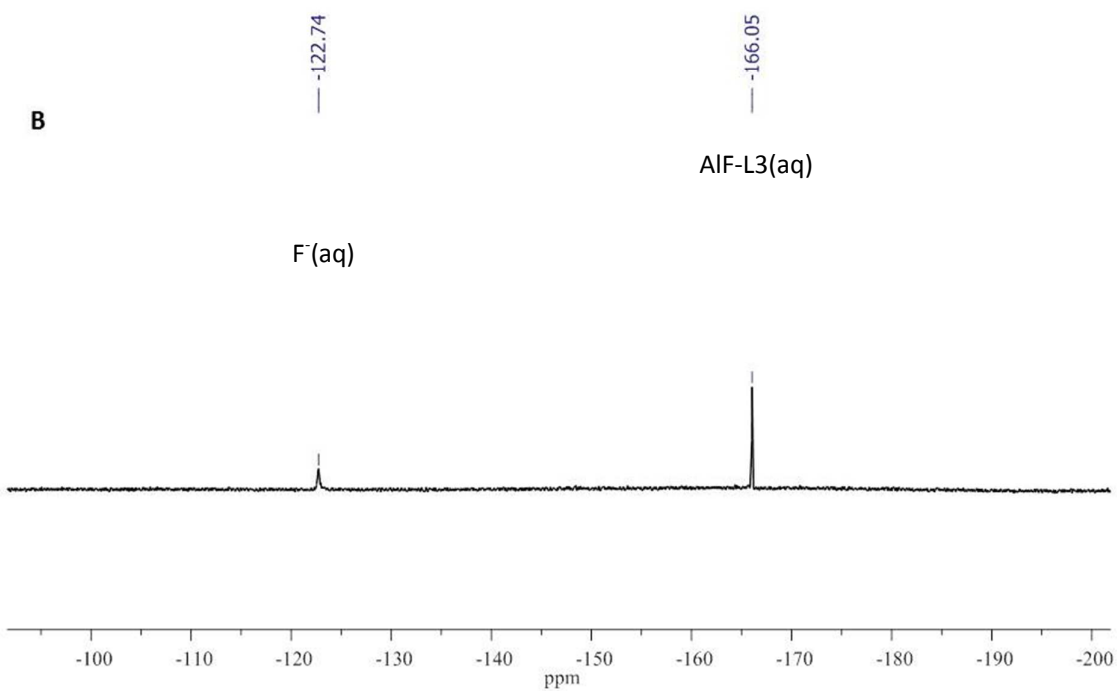
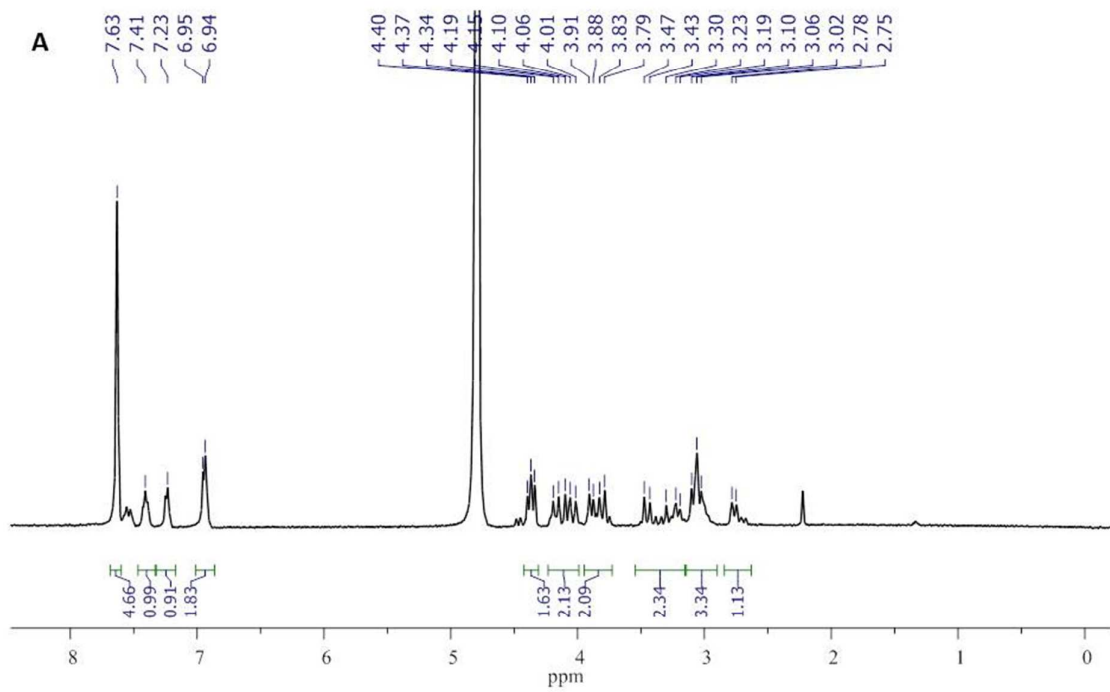


Figure S6: ^1H -NMR (A), $^{19}\text{F}\{^1\text{H}\}$ -NMR (B) and ^{13}C NMR (C) of $\text{Al}^{19}\text{F-L1}$ in D_2O at room temperature



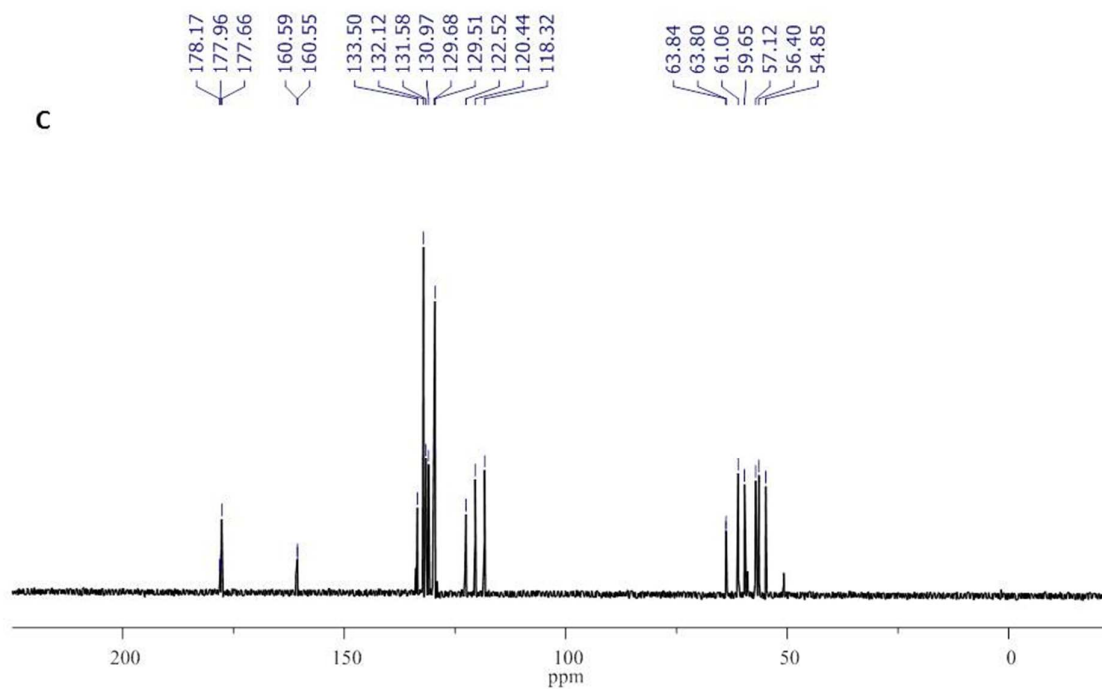


Figure S7: ^1H -NMR (A), $^{19}\text{F}\{^1\text{H}\}$ -NMR (B) and ^{13}C NMR (C) of $\text{Al}^{19}\text{F-L3}$ in D_2O at room temperature

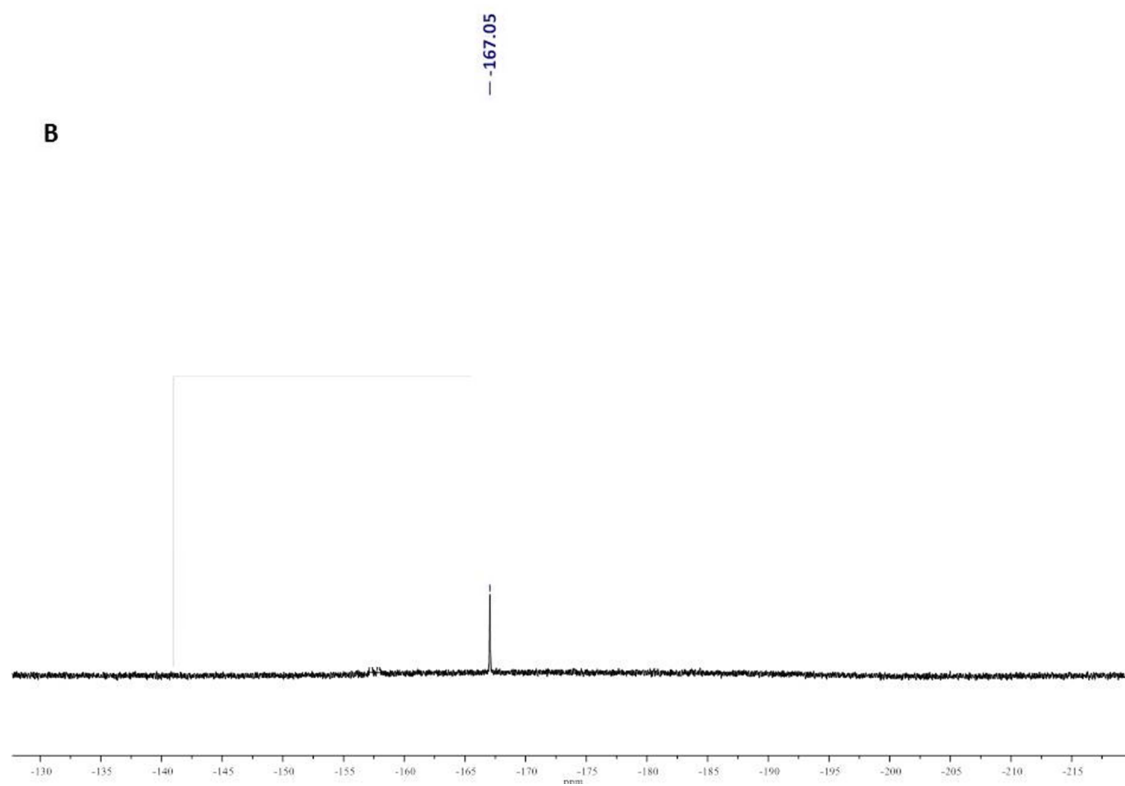
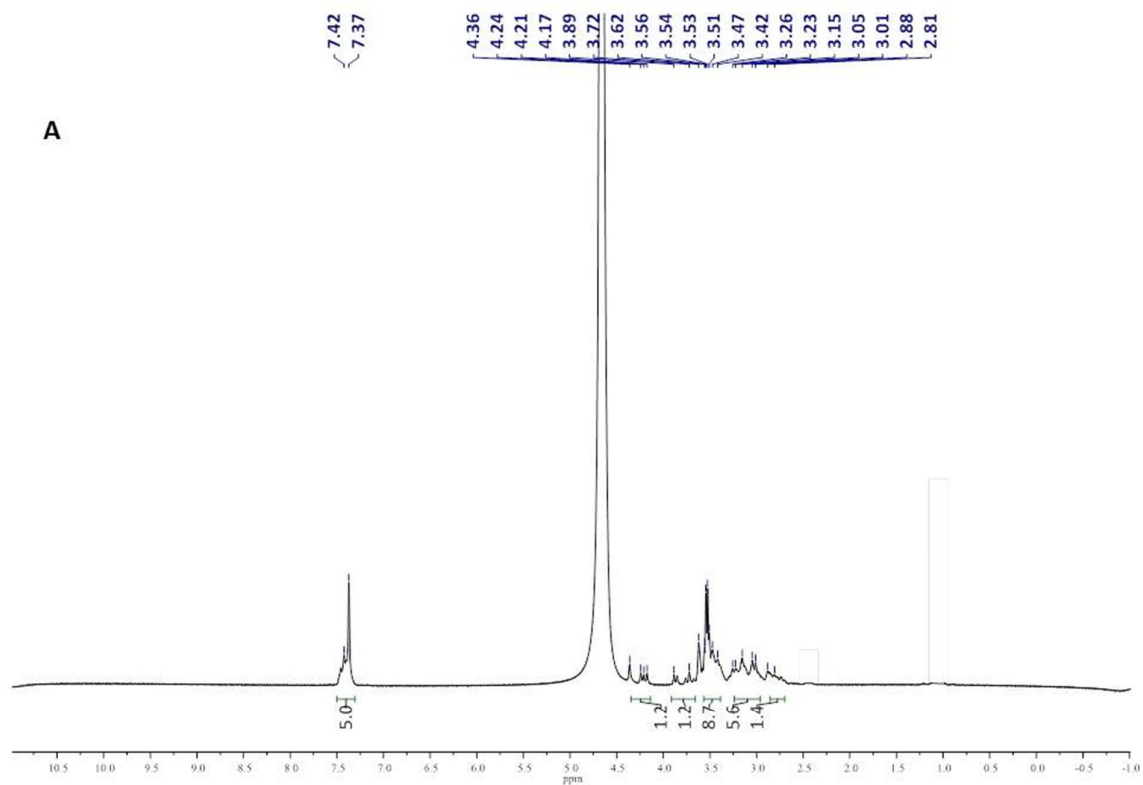


Figure S8: ^1H -NMR (A) and $^{19}\text{F}\{^1\text{H}\}$ -NMR (B) of Al^{19}F -N-Benzyl-NODA in D_2O at room temperature

Table S2: Biodistribution (%ID) of Al¹⁸F-L3 and {Al¹⁸F}²⁺ in male NMRI mice at 10 minutes (N=4) and 60 minutes (N=4) p.i.

	Al ¹⁸ F-L3				{Al ¹⁸ F} ²⁺			
	10 min p.i.	SD	60 min p.i.	SD	10 min p.i.	SD	60 min p.i.	SD
blood	1.1	0.30	0.3	0.03	5.0	0.52	0.6	0.13
bone	1.6	0.28	2.5	0.64	60.0	11.94	83.3	10.65
kidneys	2.2	0.30	1.0	1.17	2.2	0.25	0.5	0.32
bladder	13.2	4.41	17.8	5.40	20.7	2.28	30.6	11.53
liver	47.83	5.14	13.53	3.70	3.71	0.43	0.55	0.10
intestines	25.17	3.69	59.48	4.94	4.22	0.74	1.91	0.29

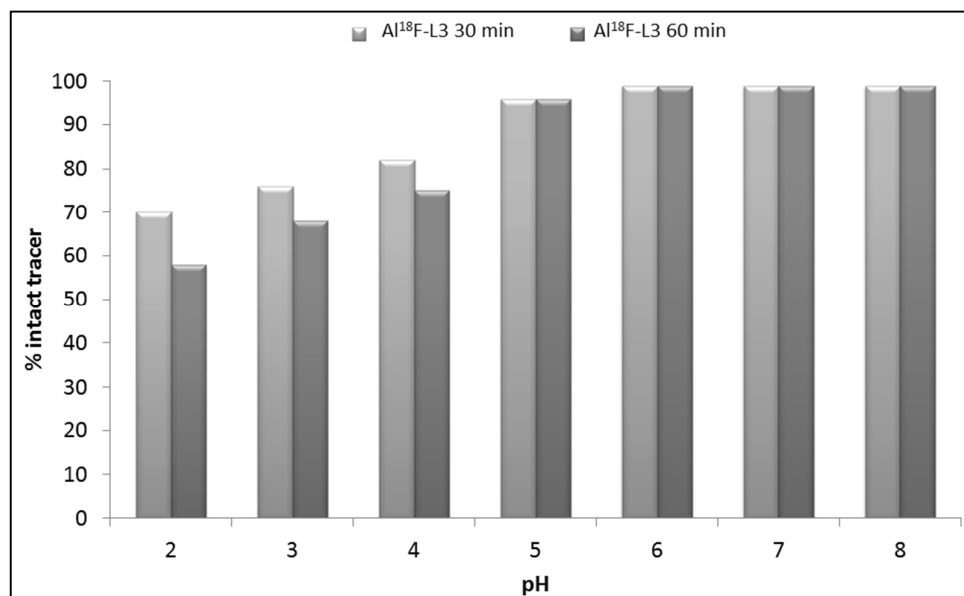


Figure S9: Stability results of Al¹⁸F-L3 at different pH values for 30 min or 60 min

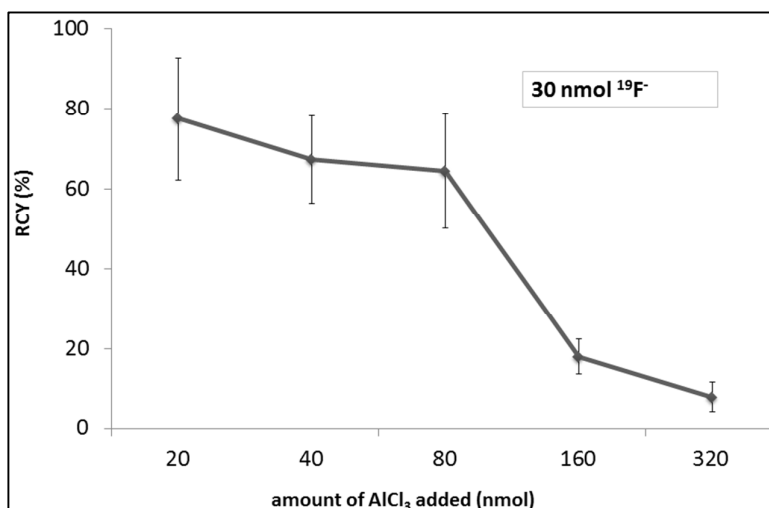
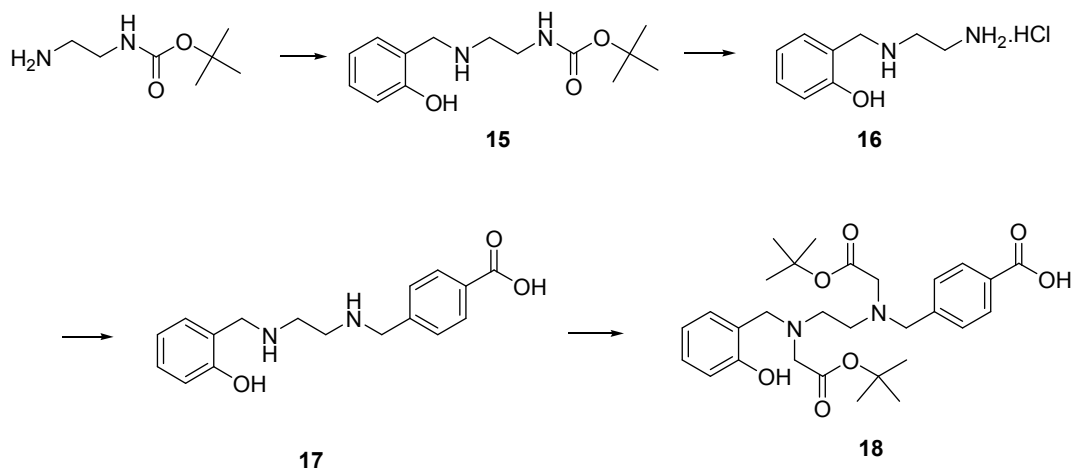


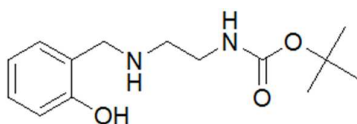
Figure S10: Effect of the amount of AlCl₃ added to the reaction mixture on the RCY (%) of Al¹⁸F-L3 in the presence of 30 nmol ¹⁹F (N=3)

4. Synthesis of Glu-NH-CO-NH-Lys(Ahx)L3 (S9)

a) Synthesis of *4-(((2-(tert-butoxy)-2-oxoethyl)(2-((2-(tert-butoxy)-2-oxoethyl)(2-hydroxybenzyl)amino)ethyl)amino)methyl)benzoic acid*



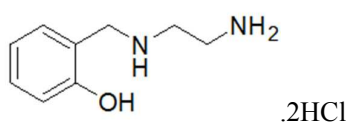
Step 1: Synthesis of compound 15 (tert-butyl (2-((2-hydroxybenzyl)amino)ethyl)carbamate)



To a solution of *N*-Boc-ethylenediamine (988 μ L, 6.24 mmol) in methanol (5.0 mL) was slowly added salicylaldehyde (732 μ L, 6.87 mmol). The mixture was heated at 65 $^{\circ}$ C for 1 h, and then cooled to 0 $^{\circ}$ C. Sodium borohydride (236 mg, 6.24 mmol) was added portionwise and the mixture was stirred at RT for 4 h. The solvent was then evaporated under reduced pressure. The residue was taken up in

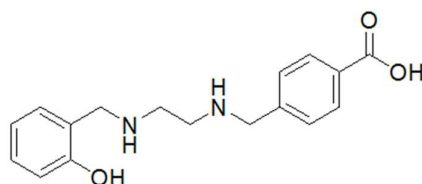
dichloromethane and an aqueous solution of sodium hydroxide (1N) was added. After five extractions with dichloromethane, the combined organic phases were dried over magnesium sulfate, filtered and evaporated under reduced pressure to give compound **15** (1.627 g, 98%) as yellow crystals. Rf (silica; dichloromethane/ethanol 95:5) 0.57; **HRMS (ESI)** calculated for C₁₄H₂₂N₂O₃ [M+H⁺] 267.1703, found 267.1652; **¹H NMR (400 MHz, CDCl₃)** 7.15 (t, 1H, *J* = 7.7 Hz), 6.97 (d, 1H, *J* = 7.3 Hz), 6.73-6.84 (m, 2H), 4.89 (s, 1H), 3.98 (s, 2H), 3.26 (d, 2H, *J* = 5.5 Hz), 2.76 (t, 2H, *J* = 5.7 Hz), 1.43 (s, 9H); **¹³C NMR (101 MHz, CDCl₃)** 158.1, 156.3, 128.8, 128.5, 122.4, 119.2, 116.4, 79.6, 52.4, 48.4, 40.1, 28.4 (3C).

Step 2: Synthesis of **16** (2-(((2-aminoethyl)amino)methyl)phenol (dihydrochloride salt))



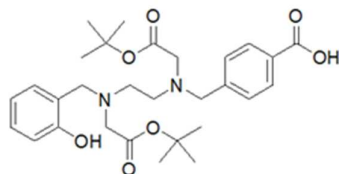
A solution of **15** (600 mg; 2.25 mmol) in hydrochloric acid (1 M, 10 mL) was stirred at RT overnight. The mixture was then evaporated under reduced pressure. Twice, the residue was taken up in absolute ethanol (15 mL) and evaporated under reduced pressure to give compound **16** (455 mg; 99%) as a white solid. **HRMS (ESI)** calculated for C₉H₁₄N₂O [M+H⁺] 167.1179, found 167.1190; **¹H NMR (400 MHz, D₂O)** 7.23-7.34 (m, 2H), 6.86-6.97 (m, 2H), 4.25 (s, 2H), 3.31-3.43 (m, 4H); **¹³C NMR (101 MHz, D₂O)** 155.7, 132.5, 132.4, 121.3, 117.4, 116.3, 48.1, 44.1, 36.1.

Step 3: Synthesis of **17** (4-(((2-((2-hydroxybenzyl)amino)ethyl)amino)methyl)benzoic acid)



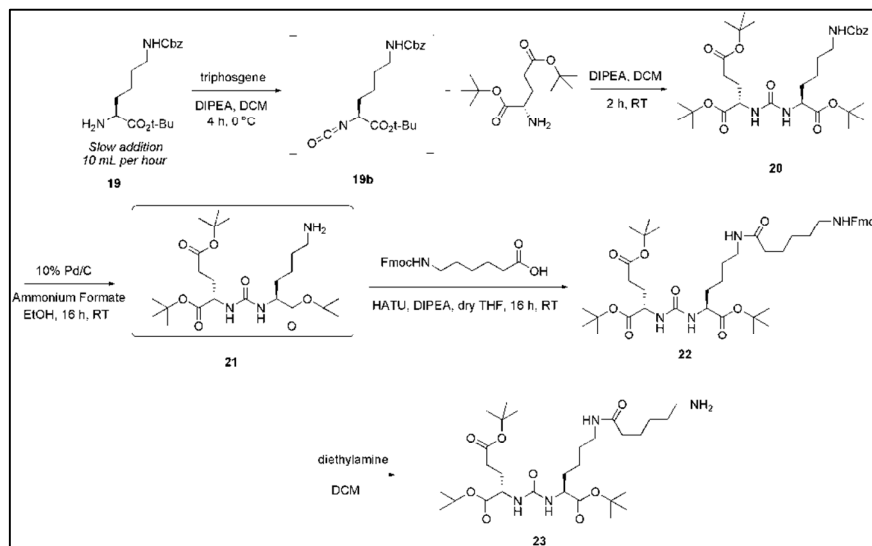
To a solution of **16** (370 mg; 1.83 mmol) in methanol (5.0 mL) were successively added *N,N*-diisopropylethylamine (318 μL; 1.83 mmol) and 4-carboxybenzaldehyde (302 mg; 2.01 mmol) portionwise. The mixture was heated at 70 °C for 1 h, and then cooled to 0 °C. Sodium borohydride (69 mg; 1.83 mmol) was added portionwise and the mixture was stirred at RT for 4 h. The solvent was then evaporated under reduced pressure to give **17** as a pink oil, which was used without further purification. **HRMS (ESI)** calculated for C₁₇H₂₀N₂O₃ [M+H⁺] 301.1547, found 301.1554; **¹H NMR (400 MHz, MeOD-d₄)** 7.89 (d, 2H, *J* = 8.0 Hz), 7.34 (d, 2H, *J* = 8.0 Hz), 7.22-7.25 (m, 2H), 6.83-6.89 (m, 2H), 4.10 (s, 2H), 3.85 (s, 2H), 3.02 (t, 2H, *J* = 5.8 Hz), 2.91 (t, 2H, *J* = 5.9 Hz); **¹³C NMR (101 MHz, MeOD-d₄)** 174.8, 45.8, 47.1, 53.6, 55.9, 116.5, 120.5, 121.0, 129.2 (2C), 130.8 (2C), 131.9, 132.3, 138.1, 141.9, 157.7.

Step 4: Synthesis of **18** (4-(((2-(tert-butoxy)-2-oxoethyl)(2-((2-(tert-butoxy)-2-oxoethyl)(2-hydroxybenzyl)amino)ethyl)amino)methyl)benzoic acid)



To a solution of **17** (490 mg; 1.63 mmol) in methanol (5 mL) were successively added *N,N*-diisopropylethylamine (853 μ L; 4.89 mmol) and *tert*-butyl bromoacetate (482 μ L; 3.26 mmol), at 0 $^{\circ}$ C. The mixture was stirred at RT overnight. The solvent was then evaporated under reduced pressure. The residue was taken up in dichloromethane and an aqueous solution of sodium hydroxide (1 M) was added. After three extractions, the pH of the aqueous layer was lowered to 7 with hydrochloric acid (3 M). The aqueous layer was then evaporated under reduced pressure. The residue was taken up in methanol and the mixture stirred at RT for 1 h. After filtration, the filtrate was evaporated under reduced pressure to give **18** as a pale-orange solid (579 mg; 68%). **HRMS (ESI)** calculated for $C_{29}H_{40}N_2O_7$ $[M+H]^+$ 529.2908, found 529.2948; **1H NMR (400 MHz, MeOD- d_4)** 7.91 (d, 2H, $J = 8.0$ Hz), 7.27 (d, 2H, $J = 7.7$ Hz), 7.14 (t, 1H, $J = 7.7$ Hz), 7.04 (d, 1H, $J = 7.4$ Hz), 6.83 (d, 1H, $J = 8.0$ Hz), 6.76 (t, 1H, $J = 7.3$ Hz), 3.68 (s, 2H), 3.66 (s, 2H), 3.25 (s, 2H), 3.16 (s, 2H), 2.67 (s, 4H), 1.44 (s, 18H); **^{13}C NMR (101 MHz, MeOD- d_4)** 175.3, 173.2 (2C), 158.8, 140.8, 138.3, 132.0, 130.4 (2C), 130.1 (2C), 127.1, 124.2, 120.1, 117.4, 83.0, 82.8, 59.4, 58.1, 57.4, 56.5, 52.6, 52.3, 28.3 (6C).

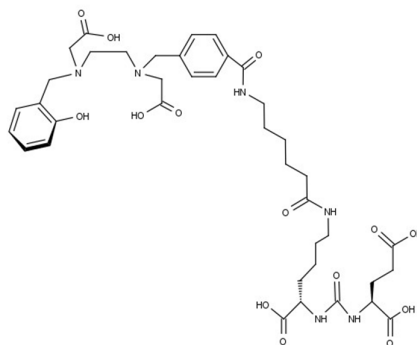
b) Synthesis of Glu-NH-CO-NH-Lys(Ahx)



Compound **21** was synthesized following the reported procedure by Maresca et al⁴. In short **21** was prepared *via* the isocyanate (**19b**), which was generated *in situ* from **19** upon treatment with

trisphosgene. Subsequent reaction with Cbz-Lys-*Or*-Bu followed by removal of the Cbz protecting group by hydrogenolysis afforded **21**. The following coupling of the aminohexanoic moiety was performed using **21** (0.650 g, 1.33 mmol, 1 eq), Fmoc-protected 6-amino-hexanoic acid (471 mg, 1.33 mmol, 1 eq), diisopropylethylamine (0.35 mL, 2.0 mmol, 1.5 eq) and 1-[Bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluorophosphate (HATU) (0.608 g, 1.60 mmol, 1.2 eq) in a final volume of 10 ml dry THF. Purification on silica column gave **22** as a colorless powder (0.840 g, 76%). **HRMS (ESI)** calculated for C₄₅H₆₆N₄O₁₀ [M+H⁺] 823.4852, found 823.4861; **¹H NMR (CDCl₃, 400 MHz)**: δ 1.43-1.46 (m, 6H, 3CH₂), 1.47 (s, 27H, 9CH₃), 1.58-1.62 (m, 3H), 1.68-1.78 (m, 2H, CH₂), 1.98-2.02 (m, 3H), 2.14-2.20 (m, 2H, CH₂), 2.30-2.34 (m, 2H, CH₂), 3.09-3.16 (m, 4H, 2CH₂), 4.10-4.19 (m, 3H), 4.33 (d, 2H, *J* 4.0, CH₂), 7.30 (t, 2H, *J* 8.0, Ar), 7.39 (t, 2H, *J* 8.0, Ar), 7.64 (d, 2H, *J* 8.0, Ar), 7.79 (d, 2H, *J* 8.0, Ar). **¹³C NMR (CDCl₃, 400 MHz)**: 23.95, 26.70, 27.37, 28.29, 28.31, 28.35, 29.03 (9CH₃), 29.98, 30.57, 32.47, 33.20, 38.87, 40.06, 54.15 (CH), 54.81 (CH), 67.58 (CH₂), 81.73 (C), 82.55 (C), 82.78 (C), 120.93 (Ar), 126.17 (Ar), 128.13 (Ar), 128.77 (Ar), 142.60 (Ar), 145.35 (Ar), 158.89 (CO), 159.93 (CO), 173.46 (CO), 173.74 (CO), 173.91 (CO), 176.03 (CO). Compound **23** was obtained by removal of the Fmoc-protecting group with 10% diethylamine in dichloromethane. Afterwards, the solvent was evaporated under reduced pressure affording the *tert*-butyl protected compound **23** (0.420 g, 95%). **HRMS (ESI)** calculated for C₃₀H₅₆N₄O₈ [M+H⁺] 601.4171, found 601.4183

Step 5: Synthesis of Glu-NH-CO-NH-Lys(Ahx)L3



To a solution of **23** (71.5 mg; 0.12 mmol) in dichloromethane (2 ml) were successively added *N,N*-diisopropylethylamine (41.5 μL; 0.238 mmol), *O*-(*N*-Succinimidyl)-*N,N,N,N*-tetramethyluronium tetrafluoroborate (TSTU) (90 mg; 0.299 mmol) and **18** (64 mg; 0.12 mmol). The mixture was stirred at RT overnight. The solvent was then evaporated under reduced pressure and the concentrate was diluted with a mixture of water/MeCN (50;50, 1 ml) and purified by preparative RP-HPLC. After HPLC purification, the compound was stirred in trifluoroacetic acid (2 mL) at RT for 12 h. The deprotection was monitored using LC-MS. Afterwards, the solvent was evaporated under reduced

pressure affording Glu-CO-NH-Lys(Ahx)-L3 (12.9 mg). **HRMS (ESI)**: Calcd. for $C_{39}H_{54}N_6O_{14}$ $[M+H^+]$ 831.3771; found 831.3773

5. Identification of reference compound $Al^{19}F$ -Glu-NH-CO-NH-Lys(Ahx)L3

A solution of $Na^{19}F$ (12.9 μ l, 100 mM) in sodium acetate buffer (0.1 M, pH 4) was added to a solution of $AlCl_3$ (64.51 μ l, 20 mM) in sodium acetate buffer (0.1 M, pH 4) and the mixture was stirred at RT for 10 min. Then, an aqueous solution of **Glu-NH-CO-NH-Lys(Ahx)L3** (100 μ l, 2.58 mM) was slowly added and the mixture was heated at 80 °C for 1 h. The reaction was monitored by LC-MS.

HRMS (ESI): calcd. for $C_{39}H_{51}AlFN_6O_{14}$ $[M]^-$ 873.3268; found 873.3265

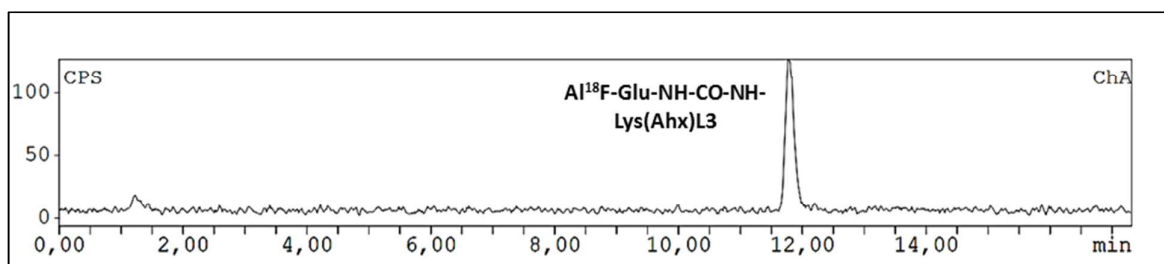


Figure S11: Radiochromatogram of $Al^{18}F$ -Glu-NH-CO-NH-Lys(Ahx)L3 (RT 11.8 min). *Radio-HPLC (XBridge C_{18} , 3.5 μ m, 3.0 mm x 100 mm, Waters) was performed using gradient elution at a flow rate of 0.8 ml/min (A: ammonium acetate 0.05M pH 5.5 B: acetonitrile, 0 to 25 min 0% B to 20% B)*

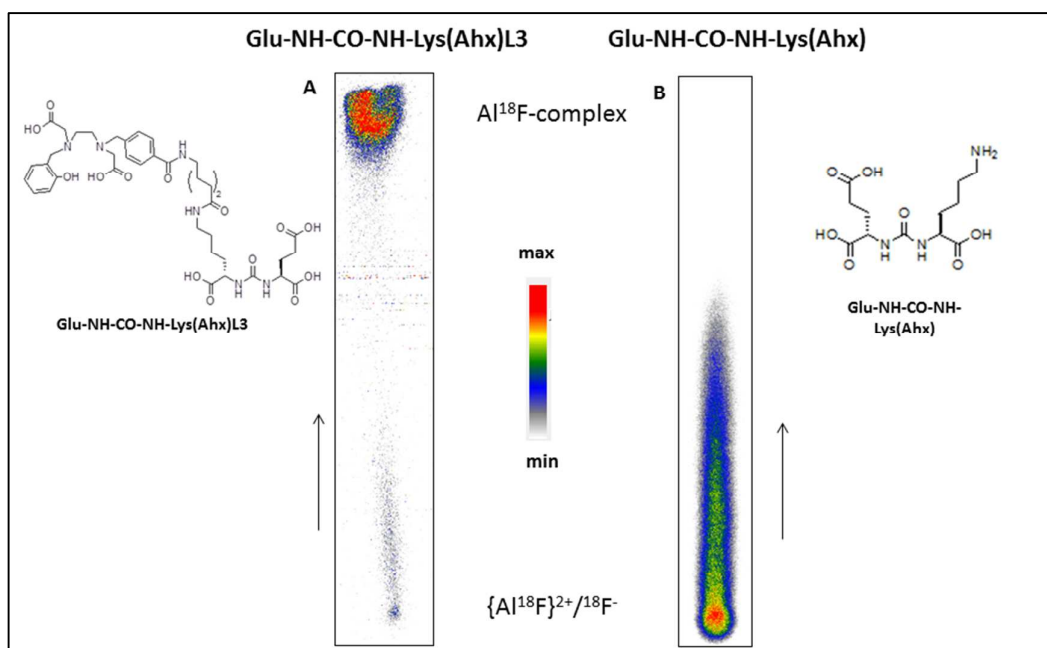


Figure S12: iTLC-SG analysis of crude reaction mixture of Al^{18}F -Glu-NH-CO-NH-Lys(Ahx)L3 (A) and Al^{18}F -Glu-NH-CO-NH-Lys(Ahx) (B). Glu-NH-CO-NH-Lys(Ahx)L3 or Glu-NH-CO-NH-Lys(Ahx) at a concentration of 300 μM were labeled with $\{\text{Al}^{18}\text{F}\}^{2+}$ (3.7 MBq) in sodium acetate buffer (pH 4) at 25 °C for 12 min. Labeling efficiency was 86% for Glu-NH-CO-NH-Lys(Ahx)L3 and 0% for Glu-NH-CO-NH-Lys(Ahx), determined by iTLC-SG followed by autoradiography. The results show that the PSMA fragment as such cannot bind the $\{\text{Al}^{18}\text{F}\}^{2+}$ core under the condition tested.

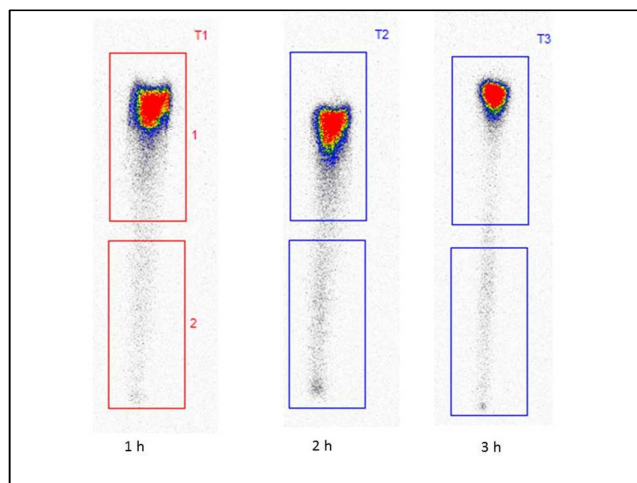


Figure S13: Stability of Al^{18}F -Glu-NH-CO-NH-Lys(Ahx)L3 in saline at RT 1,2 and 3 hours after incubation at RT, determined by iTLC-SG followed by autoradiography

Table S3: Biodistribution of Al^{18}F -Glu-NH-CO-NH-Lys(Ahx)L3 in male NMRI mice at 10 minutes (N=4) and 60 minutes p.i. with (N=4) or without (N=4) blocking with 2-PMPA. The results are presented as standard uptake value (SUV).

	10 min p.i.	SD	60 min p.i.	SD	60 min p.i. blocking	
blood	0.90	0.09	0.17	0.02	0.15	0.03
bone	0.25	0.04	0.36	0.06	1.19	0.38
muscle	0.26	0.10	0.08	0.01	0.07	0.01
kidneys	26.73	3.00	25.12	2.64	0.57	0.08
liver	1.16	0.11	0.89	0.08	0.32	0.08
pancreas	0.36	0.05	0.11	0.08	0.06	0.00
lungs	1.16	0.15	0.56	0.22	0.11	0.03
spleen	3.65	0.50	2.19	0.16	0.07	0.02

Table S4: Biodistribution (%ID) of Al^{18}F -Glu-NH-CO-NH-Lys(Ahx)L3 in male NMRI mice at 10 minutes and 60 minutes p.i. (N=4).

	10 min p.i.	SD	60 min p.i.	SD

blood	6.28	0.63	1.16	0.16
bone	3.03	0.45	4.29	0.78
muscle	11.93	3.29	3.63	0.70
brain	0.02	0.00	0.01	0.00
kidneys	48.55	3.03	45.75	5.27
bladder	4.60	2.10	26.52	8.11
liver	5.04	0.48	3.79	0.48
intestines	2.58	0.46	2.05	0.22
stomach	0.48	0.06	0.21	0.09
pancreas	0.21	0.02	0.06	0.05
lungs	0.79	0.22	0.32	0.16
spleen	0.82	0.18	0.57	0.11
carcass	31.70	4.14	19.48	4.64

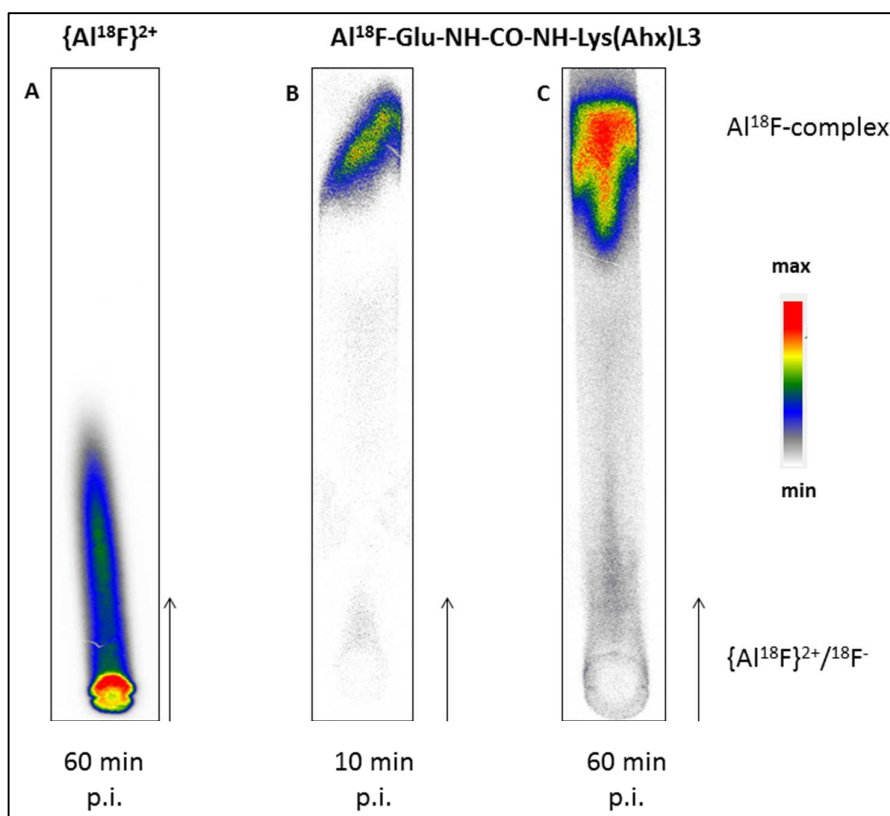


Figure S14: Radio-iTLC-SG analysis of mouse urine samples (A) 60 min p.i. of $\{\text{Al}^{18}\text{F}\}^{2+}$ (B) 10 min p.i. of Al^{18}F -Glu-NH-CO-NH-Lys(Ahx)L3 or (C) 60 min p.i. of Al^{18}F -Glu-NH-CO-NH-Lys(Ahx)L3, determined by iTLC-SG followed by autoradiography

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- (2) Sundaravel, K., Dhanalakshmi, T., Suresh, E., and Palaniandavar, M. (2008) Synthesis, structure, spectra and reactivity of iron(III) complexes of facially coordinating and sterically hindering 3N ligands as models for catechol dioxygenases. *Dalton Trans.* 7012–7025.
- (3) Baffert, C., Collomb, M., Deronzier, A., Kjærgaard-knudsen, S., Latour, J., Lund, K. H., Mckenzie, C. J., Mortensen, M., Preuss, L., and Thorup, N. (2003) Biologically relevant mono- and di-nuclear manganese II / III / IV complexes of mononegative pentadentate ligands *Dalton Trans.* 1765–1772.
- (4) Maresca, K. P., Hillier, S. M., Femia, F. J., Keith, D., Barone, C., Joyal, J. L., Zimmerman, C. N., Kozikowski, a. P., Barrett, J. a., Eckelman, W. C., and Babich, J. W. (2009) A series of halogenated heterodimeric inhibitors of prostate specific membrane antigen (PSMA) as radiolabeled probes for targeting prostate cancer. *J. Med. Chem.* 52, 347–357.