Bridged Cyclams as Imaging Agents for Chemokine Receptor 4 (CXCR4)

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Supplementary Information

Methods:

Synthesis of 2-nitro-*N*-(pyridin-2-ylmethyl)benzenesulfonamide (3) A solution of 2-(amino methyl)pyridine (**1**, 2.0 g, 18.5 mmol) was added drop wise to a stirred solution of 2-nitrobenzenesulfonyl chloride (6.0 g, 27.1 mmol) and Et_3N (3.7 g, 37.0 mmol) in dry CH_2Cl_2 (60 mL) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 3.5 h, and then quenched with water (50 mL). The aqueous layer was separated and extracted with EtOAc (5 X 50 mL). The combined organic layers were dried over $MgSO_4$ and evaporated to give a white solid which was collected by filtration and washed with cold CH_2Cl_2 to give desired product **3** as a white solid in 74% yield. **^{1}H NMR** (400 MHz, acetone-d_6): **δ** 4.45 (s, 2H), 7.20 (dd, J_1 = 7.4 Hz, J_2 = 4.5 Hz, 1H), 7.40 (d, J = 7.7 Hz, 1H), 7.68 (ddd, J_1 = 7.7 Hz, J_2 = 7.4 Hz, J_3 = 1.6 Hz, 1H), 7.76 (t, J = 7.6 Hz, 1H), 7.82 (t, J = 7.6 Hz, 1H), 7.94 (dd, J_1 = 7.7 Hz, J_2 = 1.5 Hz, 1H), 8.05 (dd, J_1 = 7.5 Hz, J_2 = 1.7 Hz, 1H), 8.38 (d, J = 4.6 Hz, 1H); **^{13}C NMR** (100 MHz, acetone-d_6): **δ** 48.09, 121.62, 121.68, 122.43, 124.96, 132.64, 133.51, 133.57, 136.57, 148.84, 148.93, 155.91; **ESI-MS** *m/z*: 294.0 [M^+1].

Synthesis of 2-nitro-*N*-(pyrimidin-2-ylmethyl)benzenesulfonamide (4) A mixture of 2-pyrimidinemethaneamine (**2**, 0.1g, 0.916 mmol), 2-nitrobenzene sulfonylchloride (0.31 g, 0.137 mmol) and Et_3N (0.18 g, 1.83 mmol) in dry CH_3CN was stirred under N_2 for 4 h at room temperature, and then quenched with H_2O. The aqueous layer was separated and extracted with EtOAc. The combined organic layer was dried over $MgSO_4$ and evaporated to small volume. The crude product was purified by column chromatography on silica gel (10% MeOH/CH_2Cl_2 with 0.1% Et_3N) to give **4** as a white solid (0.134 g,

50% yield). **^{1}H NMR** (400 MHz, acetone-D_6): **δ** 4.57 (s, 2H), 7.13 (s, 1H, NH), 7.28 (t, *J* = 4.8 Hz, 1H), 7.79 (t, *J* = 9.2 Hz, 1H), 7.85 (t, *J* = 8.0 Hz, 1H), 7.95 (d, *J* = 8.0 Hz, 1H), 8.02 (d, *J* = 8.0 Hz, 1H), 8.86 (d, *J* = 4.6 Hz, 1H); **ESI-MS** *m/z*: 295.07 [M^+1].

Synthesis of *N*-(4-(hydroxymethyl)benzyl)-2-nitro-*N*-(pyridin-2-ylmethyl) benzene sulfonamide (5) A mixture of 2-nitro-*N*-(pyridin-2-ylmethyl) benzenesulfonamide **3** (0.3 g, 1 mmol), 4-bromo(methyl)benzyl alcohol (0.2g, 1 mmol), and K_2CO_3 (0.28 g, 2 mmol) in dry CH_3CN (10 mL) was heated at 60°C for 4 h with stirring under atmospheric nitrogen. The reaction mixture was then allowed to cool down to room temperature, the solvent evaporated and the residue was partitioned between water and CH_2Cl_2. The separated aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$ and concentrated in vacuo. The residue was suspended in hexane overnight and collected by filtration to get the desired product **5** in 78% yield (0.32 g) as a white solid. **^{1}H NMR** (400 MHz, $CDCl_3$): **δ** 1.78 (bs, 1H), 4.55 (s, 1H), 4.58 (s, 1H), 4.61 (s, 1H), 7.13-7.22 (m, 6H), 7.48-7.57 (m, 2H), 7.65-7.68 (m, 2H), 7.96 (d, *J* = 4.2 Hz, 1H); **^{13}C NMR** (100 MHz, $CDCl_3$): **δ** 51.46, 52.12, 64.94, 122.38, 122.50, 124.22, 127.20, 128.85, 131.07, 131.63, 132.01, 133.34, 134.12, 134.56, 136.65, 140.62, 149.23, 155.77; **ESI-MS** *m/z*: 414.1 [M^+1].

Synthesis of *N*-(4-(hydroxymethyl)benzyl)-2-nitro-*N*-(pyrimidin-2-ylmethyl) benzene sulfonamide (6) A mixture of 2-nitro-*N*-(pyrimidin-2-ylmethyl)benzenesulfonamide **4** (0.3 g, 1 mmol), 4-bromo(methyl)benzyl alcohol (0.2 g, 1 mmol), and K_2CO_3 (0.28 g, 2 mmol) in dry CH_3CN (10 mL) was heated at 60°C for 4 h with stirring under nitrogen.

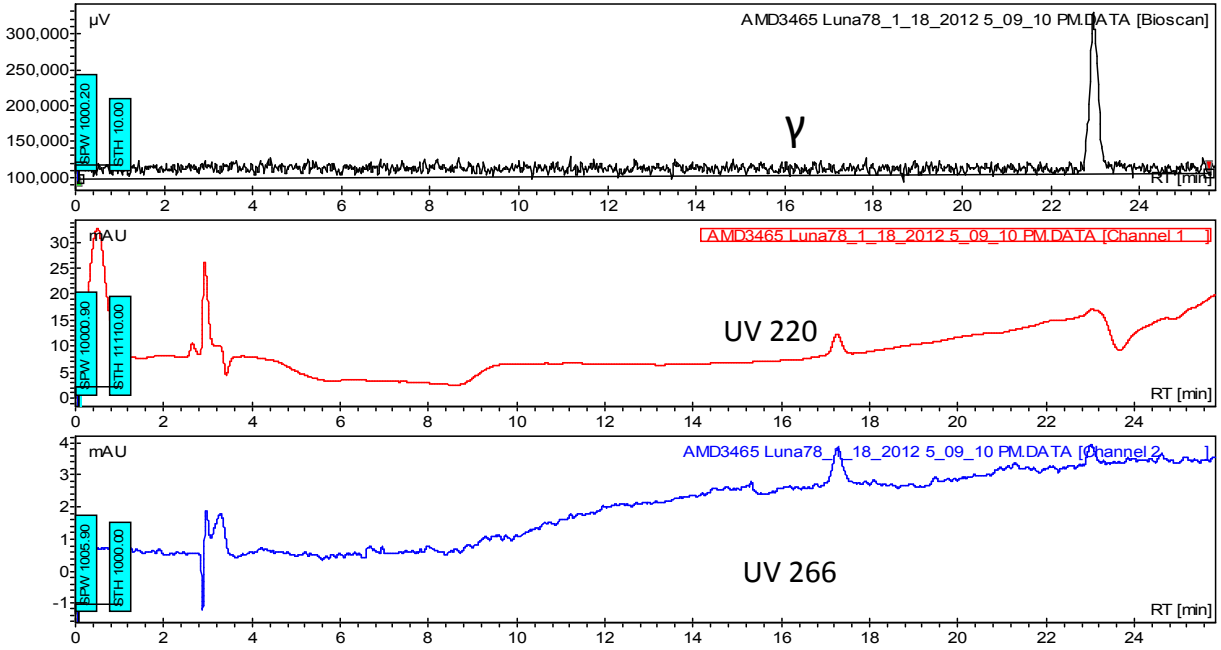
The reaction mixture was then allowed to cool down to room temperature, the solvent was evaporated and the residue partitioned between water and CH_2Cl_2. The separated aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried over $MgSO_4$, concentrated in vacuo, and purified by column chromatography on silica gel (10% MeOH/CH_2Cl_2 with 0.1% Et_3N) to give **6** as a white solid (47% yield). **^{1}H NMR** (400 MHz, acetone-d_6): **δ** 4.55 (s, 2H), 4.59 (s, 2H), 4.73 (s, 2H), 7.15 (d, *J* = 7.7 Hz, 2H), 7.25-7.21 (m, 3H), 7.81-7.69 (m, 3H), 8.09 (d, *J* = 7.7 Hz, 1H), 8.52 (d, *J* = 4.6 Hz, 1H); **ESI-MS** *m/z*: 414.0 [M^+1].

Synthesis of *N*-(4-(chloromethyl)benzyl)-2-nitro-*N*-(pyridin-2-ylmethyl) benzene sulfonamide (7) To a mixture of **5** (0. 1 g, 0.24 mmol) and Et_3N (0.12 mL, 0.72 mmol) in dry CH_2Cl_2 (5 mL) cooled in an ice bath under nitrogen atmosphere was added methanesulfonylchloride (0.045 mL, 0.58 mmol), and the reaction mixture was heated to reflux for 6 h. The solution was cooled down to room temperature, diluted with CH_2Cl_2 and washed with aqueous $NaHCO_3$ and H_2O then dried over $MgSO_4$ and concentrated in vacuo to give **7** as an orange oil (0.1 g, 98% yield). **7** was used without further purification. **^{1}H NMR** (400 MHz, $CDCl_3$): **δ** 4.48 (s, 2H), 4.52-4.54 (two singles closely overlap, 4H), 7.12-7.27 (m, 6H), 7.55 (m, 2H), 7.66 (d, *J* = 4.0 Hz, 2H), 7.92 (d, *J* = 8.0 Hz, 1H), 8.39 (d, *J* = 3.9 Hz, 1H); **ESI-MS** *m/z*: 432.0 [M^+1].

Compound **8** was synthesized using the same general procedure as compound 7. The identity of compound 8 was verified using mass spectrometry (exact mass: 432.07, observed mass (M+H): 432.84), and was subsequently used without further purification.

Synthesis of 1,4,8,11-tetraazabicyclo[10.2.2]hexadecane (17) DIBAL-H in hexanes (20 mL, 20 mmol) was added drop wise to *cis*-glyoxal cyclam (**16**, 0.15 g, 0.67 mmol) at 0ºC. The reaction mixture was stirred and allowed to come back to room temperature over an hour. The hexanes were removed on a rotary evaporator and replaced with 10 mL of toluene. The solution was refluxed for 10 days before workup at 0ºC with benzene, sodium fluoride and water. The mixture was filtered and washed with ethanol to yield **17** as a colorless oil (0.10 g, 0.44 mmol, 66%). Our ^{1}H and ^{13}C NMR results are consistent with literature values [23].

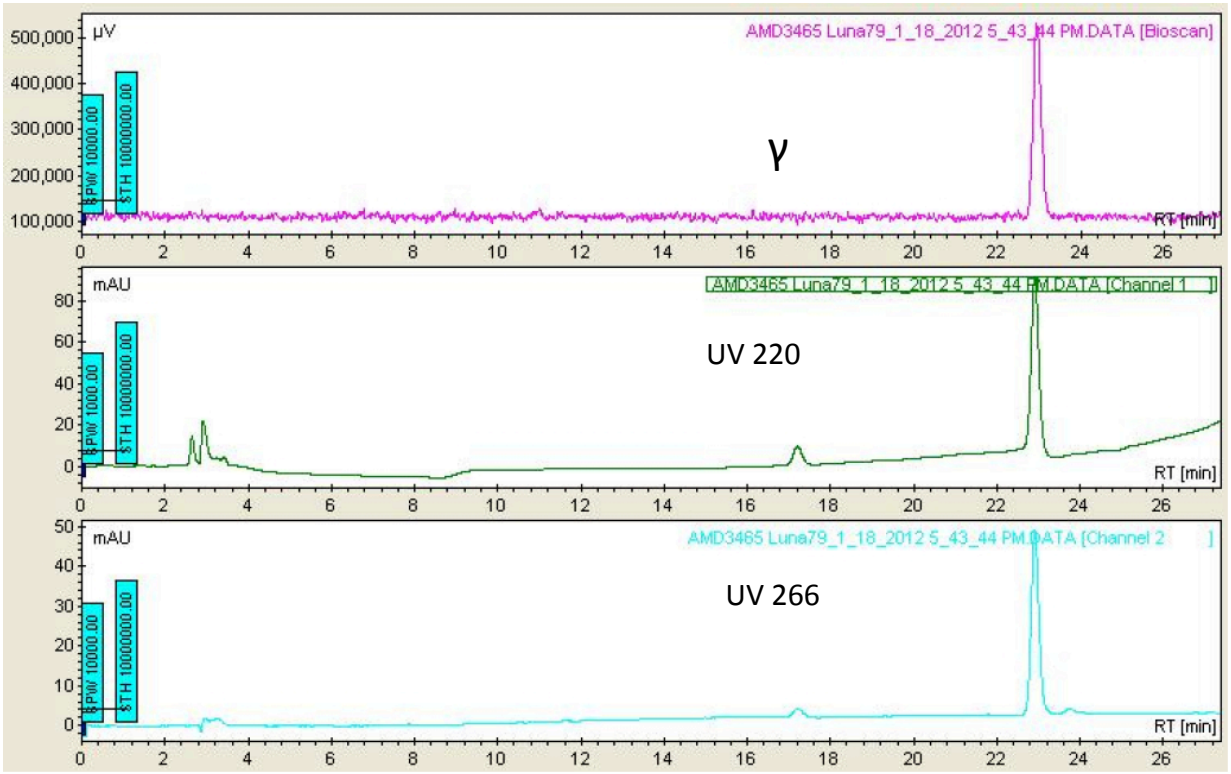
Results



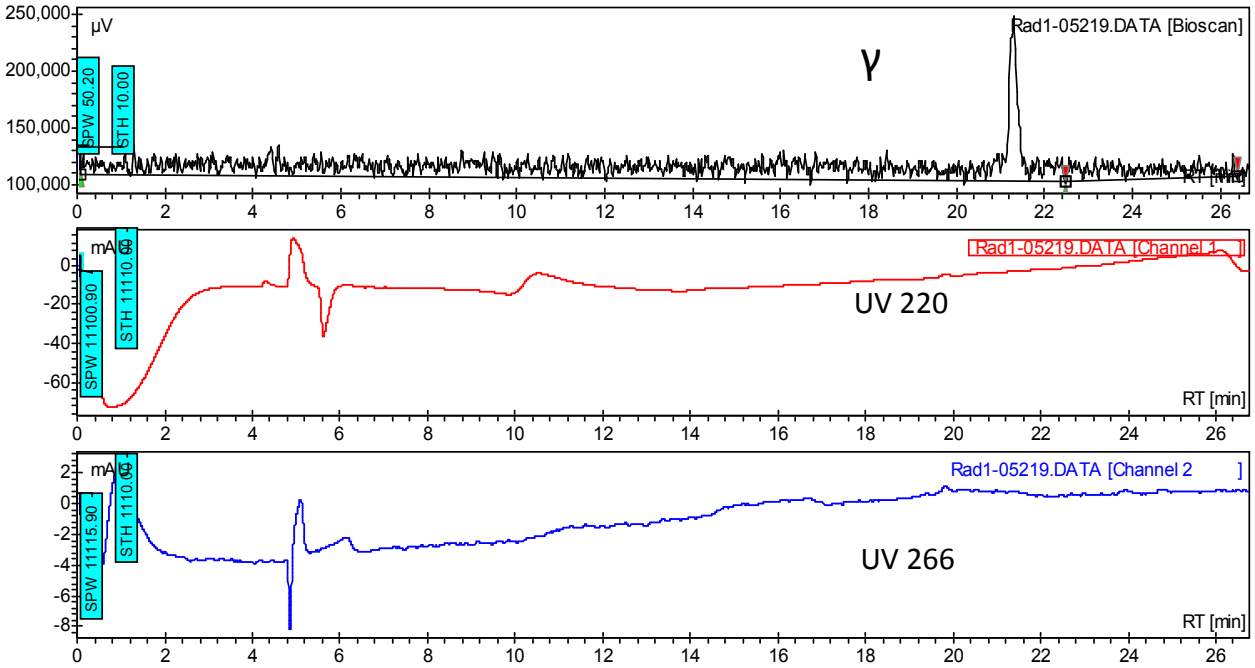
Supp. Figure 1: Radiochemical purity of pyrimidine [Cu-64]**RAD1-24**

Solvent A: Water+ 0.1% Trifluoroacetic acid
Solvent B: Methanol+ 0.1% Trifluoroacetic acid

Time	Flow rate(mL/min)	Mobile phase
Prerun	5	A=90%, B=10%
5	5	A=90%, B=10%
30	5	A=50%, B=50%
55	5	A=50%, B=50%



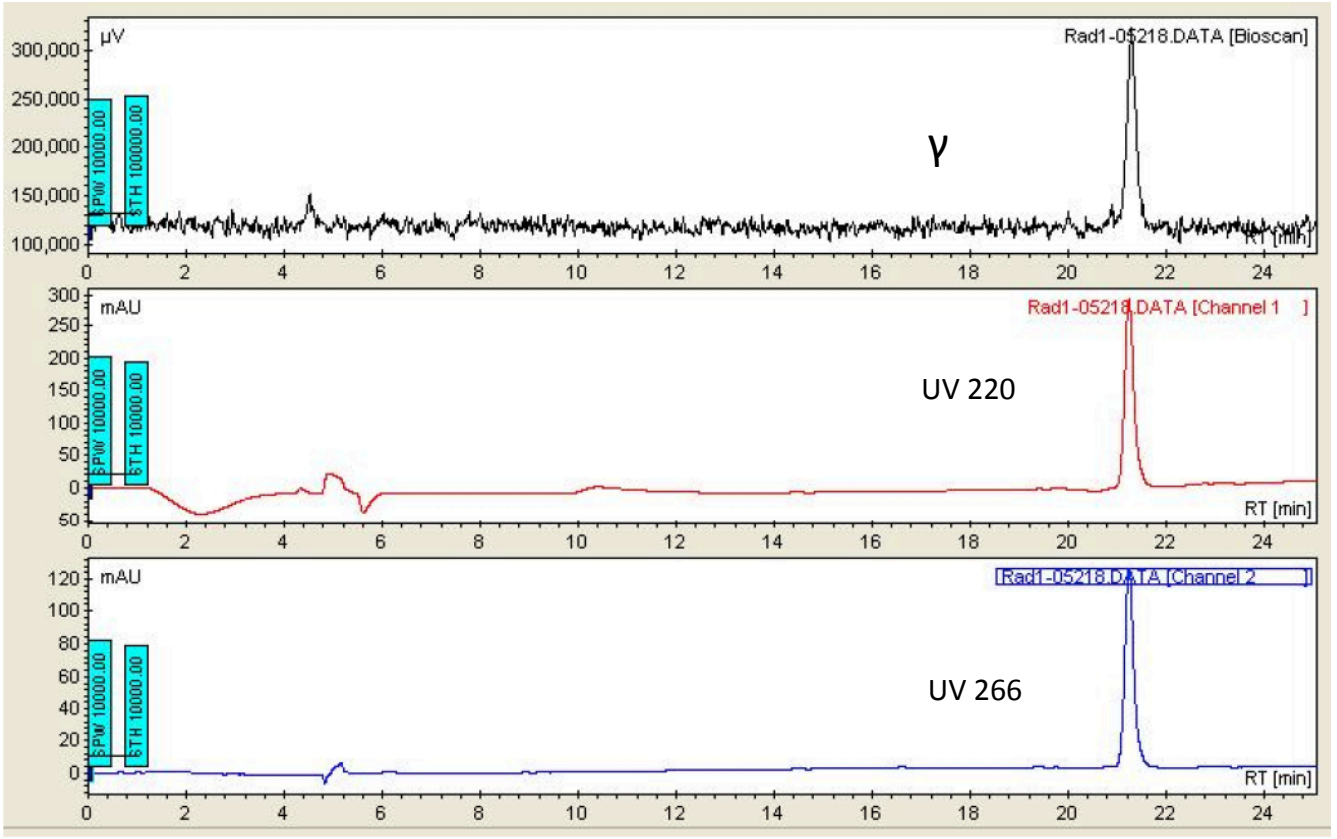
Supp. Figure 2: Co-injection of cold pyridine Cu(II)-**RAD1-24** and [Cu-64]**RAD1-24**



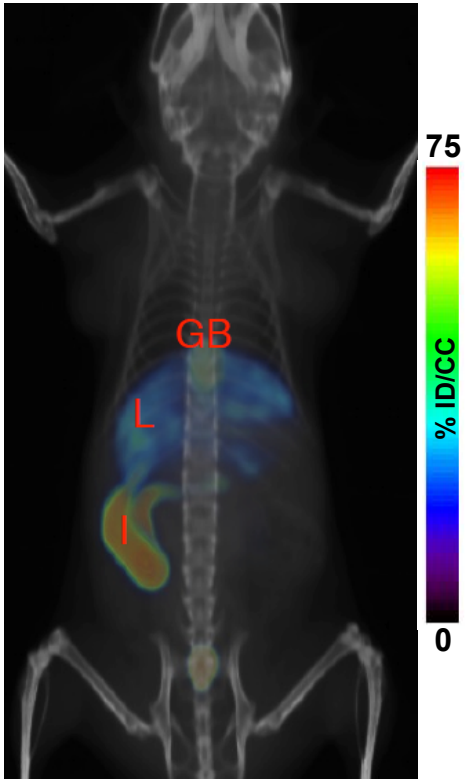
Supp. Figure 3: Radiochemical purity of pyrimidine [Cu-64]**RAD1-52**

Solvent A: Water+ 0.1% Trifluoroacetic acid
Solvent B: Methanol+ 0.1% Trifluoroacetic acid

Time	Flow rate(mL/min)	Mobile phase
Prerun	3	A=95%, B=5%
0-5	3	A=95%, B=5%
10	5	A=85%, B=15%
15	5	A=75%, B=25%
55	5	A=10%, B=90%



Supp. Figure 4: Co-injection of cold pyrimidine Cu(II)-**RAD1-52** and [Cu-64]**RAD1-52**



Supp. Figure 5: Whole body PET/CT scan at 90 minutes post-injection of ~250 μCi of [Cu-64]**RAD1-39**; GB, gallbladder; L, liver; I, intestines.