Supplementary Material (ESI) for Chemical Communications

SUPPLEMENTARY DATA

Fluorescent CXCR4 chemokine receptor antagonists: metal activated binding

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Materials and Methods

1. Cell cultures

Human leukaemic T cell lymphoblasts (Jurkat) expressing CXCR4 were obtained from the Medical Research Laboratory (University of Hull, Hull, UK). These cells were cultured in RPMI medium (Invitrogen, Paisley, UK) containing 10% (v/v) heat inactivated fetal bovine serum (FBS, Biowest, France), 2mM glutamine, 100 i.u./ml penicillin and streptomycin. The cell cultures were maintained at 37^{o}C in a humidified, CO_2 (5%) controlled atmosphere and subcultivations were carried out every 2-3 days.

2. Antibody stainings and flow cytometry

The antibodies used in this study were: unconjugated mouse anti-human CXCR4 mAbs clone 44716.111 and PE-conjugated clone 44717.111(R&D Systems Europe, Abingdon, UK). Cells were preincubated with the compound for 30 mins (10 µl of **5** at 25 µM or Cu**5**Cl_2 at 32 µM). Thereafter cells were incubated with each of the mAbs (10 µgmL^{-1} at 4^{o}C) for a further 60 mins. Cells were then incubated with a secondary fluorescein isothiocyanate-conjugated anti-mouse antibody (IgG-FITC) for 30 mins (Serotec, UK; 1:100) Cell samples were analysed by a FACSCalibur flow cytometer (BD Biosciences, Oxford, UK). Data were acquired and analysed with CellQuest software (Becton Dickinson).

3. Rhodamine stainings

Rhodamine B (BDH, UK) and macrocyclic compounds were incubated with Jurkat cells for 1 hour at 4^{o}C, washed twice with PBS/BSA/azide and analysed by flow cytometry

4. Microscopy stainings

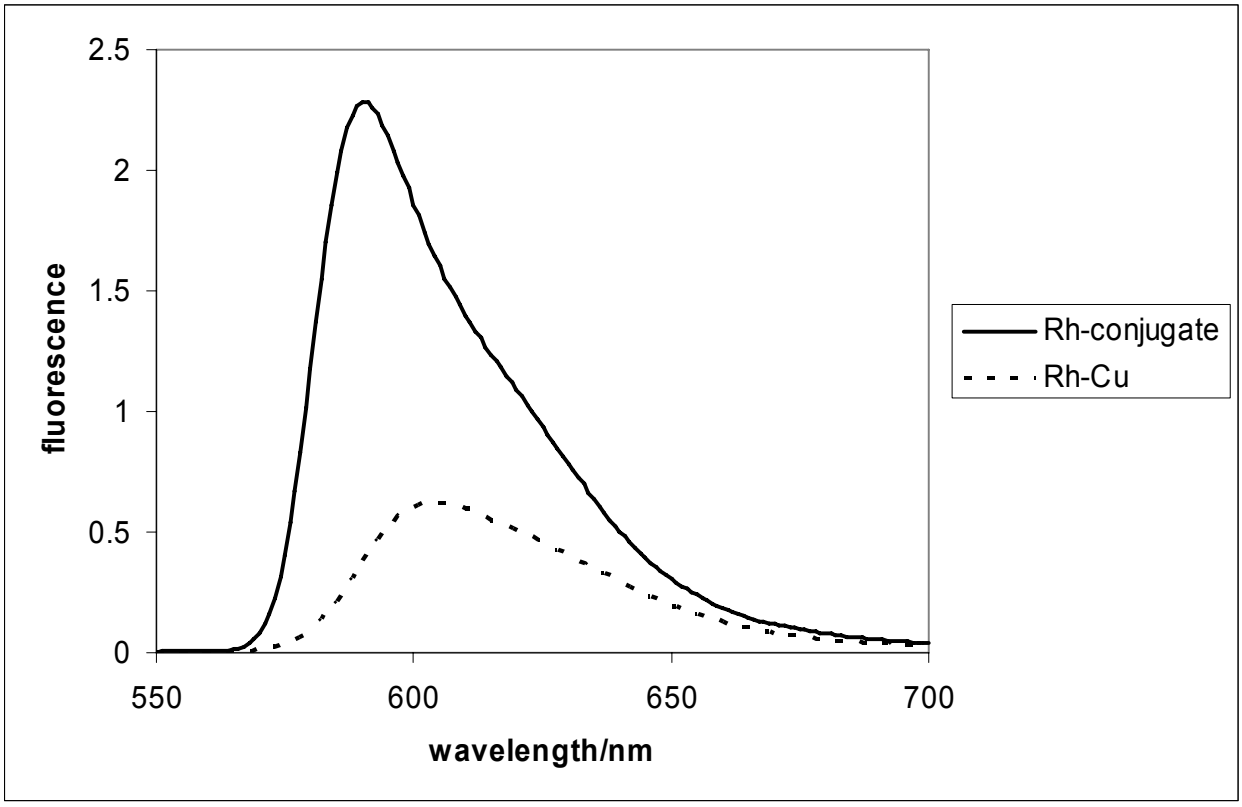
Jurkat cells were preincubated with mouse anti-human CD3 FITC (Serotec, UK) or PBS (Invitrogen) containing 2% (w/v) milk powder and 1% (w/v) bovine serum albumin (Sigma, UK). Thereafter cells were washed with PBS/BSA/azide and incubated with relevant compounds (10 µl of rhodamine at 40 µM, **5** at 25 µM or Cu**5**Cl_2 at 32 µM) for 1 hour at 4^{o}C. Cell samples were then washed twice and analysed by confocal microscopy.

4.1 Image acquisition

Images were obtained using a Bio-Rad Radiance 2100 confocal laser scanning microscope (Bio-Rad Laboratories, Hemel Hempstead, UK) equipped with Ar (488nm), Green HeNe (563nm) and Red diode (637nm) laser lines and connected to a Nikon TE 2000E inverted microscope (Nikon, Japan). Images were collected using Lasersharp2000 software under the following conditions; laser excitation line Ar (488nm), fluorescence from samples passed through 560 and 650nm dichroic filters and was collected in photomultiplier tubes (PMT) equipped with the following emission filters, 515/30 and 570nm long pass. The laser scan speed was set at 166 lines per sec, and the viewable area was between 20 and 200µm^2 when using a 60x oil objective.

5. Fluorescence spectroscopy

Fluorescence emission spectra were collected on an Aminco-Bowman Series 2 Luminescence Spectrometer, at an excitation wavelength of = 470 nm and a sensitivity of 650 V, using aqueous solutions of 800 μM concentration.



Supplementary Material (ESI) for Chemical Communications

Synthesis: Experimental

cis-3a,5a,8a,10a-tetraazaperhydropyrene, 1
This compound was prepared following literature procedures.[1]

3a-(4-nitrobenzyl)-decahydro-3a,5a,8a,10a-tetraazapyrenium bromide, 2
4-Nitrobenzyl bromide (653 mg, 3.02 mmol) was added to a stirred solution of **1** (160 mg, 720 µmol) in dry acetonitrile (40 mL). The solution was stirred for 24 h. The solvent was removed, the residue was washed with toluene (4 x 40 mL) and dried to yield a yellow solid (275 mg, 87 %). ^{1}H NMR (DMSO-d_6): δ 8.34 and 7.84 (AB, 4H, *J* 9.0 Hz, H(Ar)), 5.37 and 4.75 (AB, 2H, *J* 13.0 Hz, CH_2-Ar), 4.22 (br s, 1H, CH), 4.19 (td, 1H, *J* 13.0, 3.5 Hz, CH_2-N), 3.76 (br s, 1H, CH), 3.59 (td, 1H, *J* 13.0, 3.5 Hz, CH_2-N), 3.47 (td, 1H, *J* 11.5, 2.5 Hz, CH_2-N), 3.38-3.26 (m, 4H, CH_2-N), 3.18-3.12 (m, 2H, CH_2-N), 2.61 (td, 1H, 12.0, 3.5 Hz, CH_2-N), 2.37 (td, 1H, CH_2-N), 2.28-2.19 (m, 2H, CH_2-N), 2.11-1.99 (m, 2H, CH_2-N), 1.66 (br d, 1H, *J* 13.5 Hz, CH_2-β-N), 1.26 (br d, 1H, *J* 13.5 Hz, CH_2-β-N). ^{13}C NMR (DMSO-d_6): δ 148.5 (*C*(Ar)-CH_2), [134.8, 134.3] (C(Ar)), 123.7 (C(Ar)-NO_2), [82.5, 69.0] (NCN) 59.2 (CH_2-Ar), [59.2, 53.7, 53.6, 51.9, 48.3, 46.0, 42.1] (CH_2-N), [18.7, 18.5] (CH_2-β-N).

5-(4-nitrobenzyl)-1,5,8,12-tetraazabicyclo[10.2.2]hexadecane, 3
Sodium borohydride (470 mg, 12.4 mmol) was added in small portions to a stirred solution of **2** (217 mg, 495 µmol) in ethanol (20 mL). The mixture was stirred at 25°C for 30 min then heated to reflux for 2 h. Water (10 mL) was added to decompose excess $NaBH_4$, and solvents were removed under reduced pressure. The residue was taken up into water (30 mL), and the aqueous solution was made strongly basic (pH 14, KOH). The basic solution was extracted with dichloromethane (4 x 50 mL), and the combined organic extracts were dried over $MgSO_4$, and evaporated to dryness under reduced pressure to yield a yellow oil (101 mg, 56 %). δ_H (400 MHz; solvent DMSO-d_6) 8.21 - 8.16 (2H, m), 7.54 - 7.41 (2H, m), 3.72 (1H, s), 3.39 - 3.18 (2H, m), 3.12 - 3.07 (2H, m), 2.85 - 2.75 (4H, m), 2.70 - 2.64 (4H, m), 2.59 - 2.27 (4H, m), 1.87 (1H, p, *J* 5.0 Hz), 1.69 (1H, p, *J* 5.0 Hz). δ_C (100 MHz; solvent DMSO-d_6) 147.0, 146.5, 129.7, 123.3, 63.1, 57.4, 56.4, 54.8, 54.5, 54.4, 51.1, 49.9, 48.5, 47.9, 24.9, 23.4. ES-MS: m/z 362 (M^+). HRMS: calc. 362.2551, found 362.2551.

5-(4-aminobenzyl)-1,5,8,12-tetraazabicyclo[10.2.2]hexadecane, 4
A solution of **3** (214 mg, 592 µmol) in methanol (20 mL) was added to a stirred suspension of 5% palladium on calcium carbonate, poisoned with lead (100 mg), in methanol (5 mL) and the mixture was stirred under hydrogen for 16 hr. Solids were removed by filtration through a pad of Hyflo Super-cel filter aid, and solvent was removed from the filtrate to yield a yellow oil (196 mg, 100%). ^{1}H NMR ($CDCl_3$): 8.12 and 7.47 (AB, 4H, *J* 9.0 Hz, H(Ar)), 3.65 (s, 2H, CH_2-Ar), 3.18-3.11 (m, 2H, CH_2-N), 3.08-3.02 (m, 2H, CH_2-N), 2.86-2.63 (m, 6H, CH_2-N), 2.60-2.54 (m, 4H, CH_2-N), 2.51 (t, 2H, *J* 5.0 Hz, CH_2-N), 2.44-2.22 (m, 4H, CH_2-N), 1.89-1.82 (m, 2H, CH_2- -N), 1.69-1.61 (m, 2H, CH_2- -N). ^{13}C NMR ($CDCl_3$): 147.2 (C(Ar)-*C*H_2), [129.8, 129.6] (C(Ar)), 123.5 (C(Ar)-NH_2), 57.7 (CH_2-Ar), [56.5, 55.0, 54.7, 51.2, 50.1, 48.7, 48.0] (CH_2-N), [26.1, 23.5] (CH_2- -N). MS: 332 (M+). HRMS: calcd. 332.2809, found 332.2810.

[9-(2-Carboxy-4-{3-[4-(1,5,8,12-tetraaza-bicyclo[10.2.2]hexadec-5-ylmethyl)-phenyl]-thioureido}-phenyl)-6-diethylamino-xanthen-3-ylidene]-diethyl-ammonium chloride, 5
Rhodamine isothiocyanate (40.0 mg, 74.6 µmol) was added to a stirred solution of **4** (22.5 mg, 67.9 µmol) and triethylamine (95 µL) in methanol (5 mL). The mixture was stirred for 16 hr. Solvents were removed under reduced pressure, and the residue was purified by size exclusion chromatography (Sephadex LH-20, MeOH) to yield a purple solid (46.4 mg, 74%). ^{1}H HMR (CD_3OD): 7.89 - 7.07 (m, 7H), 7.04 - 6.60 (m, 6H), 4.02 - 3.40 (br m, 12H), 3.32 - 3.24 (m, 4H), 3.17 (q, 8H, *J* 7.3 Hz), 1.27 (t, 12H, *J* 7.3 Hz), 1.28 - 1.16 (m, 8H). ES-MS: 831 (M+). HRMS: calcd. 831.4738, found 831.4734.

Copper(II)chloride complex of 5, Cu**5**Cl_2
A solution of copper(II) chloride dihydrate (5.0 mg, 29.4 μmol) in methanol (2 mL) was added dropwise to a stirred solution of **5** (22.5 mg, 27.1 μmol) in methanol (3 mL). The pink solution was heated to reflux for 2 h, allowed to cool, concentrated to < 1 mL and purified by size exclusion chromatography (Sephadex LH-20, MeOH). Solvent was removed from the eluent to afford a dark pink solid (21.7 mg, 83 %). Elemental analysis (%) calcd for $C_{48}H_{63}N_8O_3Cu.HN(CH_2CH_3)_3.MeOH.5H_2O$: C 53.91, H 7.57, N 10.29; found C 53.59, H 7.94, N 10.03. ES-MS, *m/z*: 641 $[M\text{-}C_{21}H_{26}N_2O]^+$, 100%

5-(4-phenylmethyl)-1,5,8,12-tetraazabicyclo[10.2.2]hexadecane, 6
This compound was prepared from 3a-(phenylmethyl)-decahydro-3a,5a,8a,10a-tetraazapyrenium bromide[2] following literature procedures.[3]

Copper(II) complex of 6, [Cu**6**Cl]($CuCl_2$)
A solution of copper(II) chloride dihydrate (53.2 mg, 312 μmol) in methanol (5 mL) was added dropwise to a stirred solution of **6** (98.8 mg, 312 μmol) in methanol (10 mL). The solution turned deep blue upon initial addition of copper(II) salt. The solution was heated to reflux for 2 h. The solution was filtered through Hyflo filter-aid and solvent was removed to yield a blue/ green solid (128 mg, 75 %). UV/vis (MeCN): $_{max}$: 683 nm, = 231 M^{-1} cm^{-1}. ES-MS, *m/z*: 414 $[M]^+$, 80 %; 378 $[M+H]^+$, 100 %.

1. M. Le Baccon, F. Chuburu, L. Toupet, H. Handel, M. Soibinet, I. Dechamps-Olivier, J. P. Barbiere and M. Aplincourt, *New J. Chem.*, 2001, **25**, 1168-1174.
2. E. H. Wong, G. R. Weisman, D. C. Hill, D. P. Reed, M. E. Rogers, J. S. Condon, M. A. Fagan, J. C. Calabrese, K. C. Lam, I. A. Guzei and A. L. Rheingold, *J. Am. Chem. Soc.*, 2000, **122**, 10561-10572.
3. R. A. Kolinski, *Pol. J. Chem.*, 1995, **69**, 1039-1045.

Crystallographic data

Table 1. Crystal data and structure refinement for [Cu**6**Cl](CuCl$_2$)

Empirical formula	$C_{19}H_{32}Cl_3Cu_2N_4$	
Formula weight	549.92	
Temperature	150(2) K	
Wavelength	0.71073 Å	
Crystal system	monoclinic	
Space group	*P* $2_1/c$	
Unit cell dimensions	a = 6.8348(7) Å	α= 90°.
	b = 18.7560(14) Å	β= 91.838(8)°.
	c = 17.8251(18) Å	γ = 90°.
Volume	2283.9(4) Å^3	
Z	4	
Density (calculated)	1.599 Mg/m^3	
Absorption coefficient	2.226 mm^{-1}	
F(000)	1132	
Crystal size	0.54 x 0.52 x 0.09 mm^3	
Theta range for data collection	2.98 to 30.00°.	
Index ranges	-9<=h<=9, -26<=k<=26, -23<=l<=25	
Reflections collected	21632	
Independent reflections	6350 [R(int) = 0.0544]	
Completeness to theta = 25.00°	96.8 %	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	6350 / 0 / 256	
Goodness-of-fit on F^2	0.956	
Final R indices [I>2sigma(I)]	R1 = 0.0429, wR2 = 0.1027	
R indices (all data)	R1 = 0.0719, wR2 = 0.1130	
Largest diff. peak and hole	1.133 and -0.648 e.Å^{-3}	

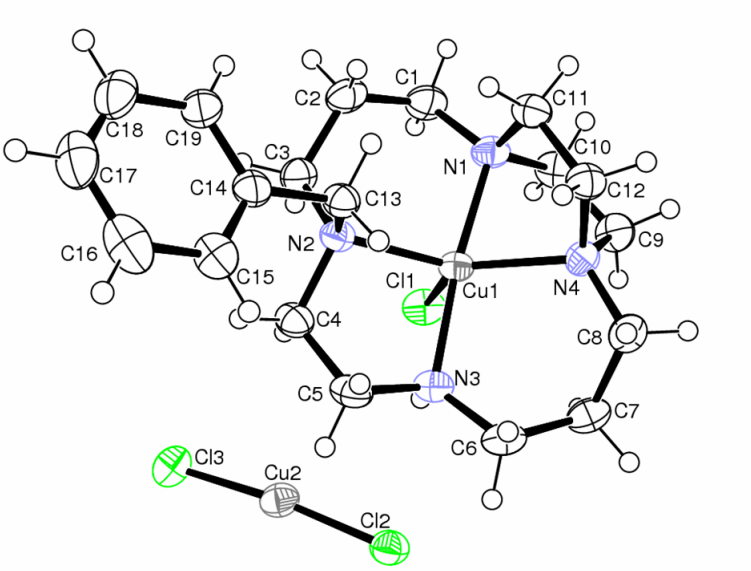


Figure: Thermal ellipsoids are shown at the 50% probability level

Table 2: bond lengths (Å) and angles for [Cu**6**Cl](CuCl$_2$)

C(1)-N(1)	1.481(4)	Cu(1)-N(1)	2.014(2)	N(1)-Cu(1)-N(2)	97.63(11)
C(1)-C(2)	1.508(6)	Cu(1)-N(4)	2.044(3)	N(4)-Cu(1)-N(2)	141.26(10)
C(2)-C(3)	1.526(5)	Cu(1)-N(2)	2.072(3)	N(3)-Cu(1)-Cl(1)	87.29(9)
C(3)-N(2)	1.487(4)			N(1)-Cu(1)-Cl(1)	94.34(8)
C(4)-N(2)	1.491(4)	N(1)-C(1)-C(2)	115.2(3)	N(4)-Cu(1)-Cl(1)	115.83(8)
C(4)-C(5)	1.530(5)	C(1)-C(2)-C(3)	116.7(3)	N(2)-Cu(1)-Cl(1)	102.29(7)
C(5)-N(3)	1.469(5)	N(2)-C(3)-C(2)	114.1(3)	Cl(2)-Cu(2)-Cl(3)	173.61(4)
C(6)-N(3)	1.477(4)	N(2)-C(4)-C(5)	110.3(3)	C(11)-N(1)-C(1)	114.1(3)
C(6)-C(7)	1.518(5)	N(3)-C(5)-C(4)	107.9(3)	C(11)-N(1)-C(10)	106.2(3)
C(7)-C(8)	1.522(5)	N(3)-C(6)-C(7)	112.1(3)	C(1)-N(1)-C(10)	112.1(3)
C(8)-N(4)	1.475(4)	C(6)-C(7)-C(8)	112.6(3)	C(11)-N(1)-Cu(1)	105.52(19)
C(9)-N(4)	1.477(5)	N(4)-C(8)-C(7)	111.9(3)	C(1)-N(1)-Cu(1)	115.2(2)
C(9)-C(10)	1.544(5)	N(4)-C(9)-C(10)	107.8(3)	C(10)-N(1)-Cu(1)	102.70(19)
C(10)-N(1)	1.481(5)	N(1)-C(10)-C(9)	107.3(3)	C(3)-N(2)-C(4)	110.7(2)
C(11)-N(1)	1.477(4)	N(1)-C(11)-C(12)	107.0(2)	C(3)-N(2)-C(13)	112.9(2)
C(11)-C(12)	1.543(4)	N(4)-C(12)-C(11)	107.8(3)	C(4)-N(2)-C(13)	111.6(2)
C(12)-N(4)	1.493(4)	C(14)-C(13)-N(2)	116.4(2)	C(3)-N(2)-Cu(1)	109.7(2)
C(13)-C(14)	1.503(5)	C(15)-C(14)-C(19)	118.1(3)	C(4)-N(2)-Cu(1)	100.93(19)
C(13)-N(2)	1.503(4)	C(15)-C(14)-C(13)	120.6(3)	C(13)-N(2)-Cu(1)	110.36(18)
C(14)-C(15)	1.389(5)	C(19)-C(14)-C(13)	121.2(3)	C(5)-N(3)-C(6)	114.7(3)
C(14)-C(19)	1.404(5)	C(14)-C(15)-C(16)	121.1(4)	C(5)-N(3)-Cu(1)	108.65(19)
C(15)-C(16)	1.400(6)	C(17)-C(16)-C(15)	119.5(4)	C(6)-N(3)-Cu(1)	118.6(2)
C(16)-C(17)	1.395(7)	C(18)-C(17)-C(16)	119.8(4)	C(8)-N(4)-C(9)	112.8(3)
C(17)-C(18)	1.353(7)	C(17)-C(18)-C(19)	121.5(4)	C(8)-N(4)-C(12)	112.1(2)
C(18)-C(19)	1.391(6)	C(18)-C(19)-C(14)	120.1(4)	C(9)-N(4)-C(12)	107.9(3)
Cl(1)-Cu(1)	2.4904(9)	N(3)-Cu(1)-N(1)	174.09(12)	C(8)-N(4)-Cu(1)	118.6(2)
Cl(2)-Cu(2)	2.0975(11)	N(3)-Cu(1)-N(4)	100.30(11)	C(9)-N(4)-Cu(1)	103.43(19)
Cl(3)-Cu(2)	2.1043(10)	N(1)-Cu(1)-N(4)	73.87(11)	C(12)-N(4)-Cu(1)	100.77(19)
Cu(1)-N(3)	1.998(2)	N(3)-Cu(1)-N(2)	87.56(11)		