Electronic Supplementary Material (ESI) for Chemical Communications

Gd^{3+} cFLFLFK conjugate for MRI: a targeted contrast agent for Formyl Peptide Receptor 1 in inflammation

Graeme J. Stasiuk,[a,b] Helen Smith,[b] Marzena Wylezinska-Arridge,[c] Jordi L. Tremoleda,[c] William Trigg,[d] Sajinder Kaur Luthra,[d] Veronique Morisson Iveson,[d] Felicity N. E. Gavins[b*] and Nicholas J. Long[a*]

a) *Department of Chemistry, Imperial College London, South Kensington Campus, London SW7 2AZ, UK*

b) *Division of Brain Sciences, Department of Medicine, Imperial College London, Du Cane Road, London, W12 ONN*

c) *Biological Imaging Centre, Imperial College London, Du Cane Road, London, W12 ONN*

d) *GE Healthcare, The Grove Centre, White Lion Road, Amersham, Buckinghamshire, HP7 9LL UK*

General procedures. NMR spectra were recorded on an Advance DMX 400 Bruker spectrometer. Chemical shifts are reported in ppm with solvent as internal reference. HPLC was carried out on a HP 3000 system with a reverse phase c18 semi prep column. Electronic absorption spectra were recorded on a Varian CARY 50 probe UV/vis. spectrometer.

Materials. Solvents and starting materials were obtained from Aldrich, Cambridge biochemicals, Fluka, Acros, and Alfa. They were used without further purification unless otherwise stated. Water and H_2O refer to high purity water with resistivity value of 18 MΩ·cm, obtained from the "Millipore/MilliQ" purification system. Lanthanide chloride salts were purchased from Aldrich.

Ligand Synthesis

1,4,7,10-Tetra(*tert*-butoxycarbonylmethyl)-1,4,7,10-tetraazacyclododecane, (^{t}BuDOTA) 1.

Tert-butyl bromoacetate (0.965 cm^3, 6.55 mmol) was dissolved in chloroform (50 cm^3) and added dropwise under nitrogen over 4 h at room temperature to a stirred solution of 1,4,7,10-tetraazacyclododecane (0.25 g, 1.45 mmol) and K_2CO_3 (0.902g, 1.45 mmol) in $CHCl_3$ (125 cm^3). After the addition was complete the mixture was stirred for 3 days. After this the inorganic salts were filtered off and the solution was concentrated *in vacuo*. The crude product was purified by silica column chromatography, with a gradient DCM 100% to MeOH 10%, 1% ammonia and DCM 89% giving a white solid (0.267 g, 29%) δ_H (400 MHz, $CDCl_3$)

1.43 (36H, s, ~C(CH_3)$_3$~) 2.11-2.85 (16H, bm, ~NCH_2CH_2N~) 2.90-3.50 (12H, bm, ~NCH_2CO_2). *m/z* (ESMS+) 629[M + H]$^+$.

1,4,7,10-tetra(carbonylmethyl)- 1,4,7,10-tetraazacyclododecane 2.

1 (0.100 g, 0.15 mmol), was dissolved in TFA (2 ml) and DCM (2 ml) and stirred for 12 hours, the solvent was concentrated *in vacuo*. This was followed by the addition and concentration of DCM (3 x2 ml) and diethyl ether (3 x 2 ml) yielding a hydroscopic white solid **2** (0.078g, 89%). δ_H (400 MHz, D_2O) 3.16 (16H, bm, ~NCH_2CH_2N~) , 3.95 (8H, bm, ~ NCH_2CO_2~). *m/z* (ESMS+) 405 [M + H]$^+$

Synthesis of NHS-DOTA 3

2 (1.05 mg, 0.0026 mmol) was dissolved in H_2O (1 ml) at pH 8 to which EDC (1.06 mg, 0.0059 mmol) and sulfonyl NHS (0.84 mg, 0.0038 mmol) were added sequentially at 0^{o}C. This solution was stirred for 1 hour after which half was taken and used in the formation of **3**, the other half was stored in the freezer.

cFLFLFK-DOTA 4

To a solution of **3** (0.5 mg, 0.0013 mmol) in H_2O (0.5 ml), **cFLFLFK** (5 mg, 0.0013 mmol) was added in Na borate buffer (0.5 ml) and this solution was stirred at 4^{o}C for 12 hours. The solvent was removed and the crude solid was purified via HPLC using a c18 reverse phase column with a gradient of H_2O 0.1% TFA: MeCN starting at 50:50 to 5:95 over 28 mins. δ_H (400 MHz, CD_3OD) 0.68 (6H, bm, ~CH_2CH~), 0.90 (4H, bm, ~CH_2CH~), 1.31 (18H, s, ~CH_3C~), 1.69 (1H, m, ~NHCHC~), 1.75 (1H, m, ~NHCHC~), 1.85 (1H, m, ~NHCHC~), 2.10 (1H, m, ~NHCHC~), 2.25 (1H, m, ~NHCHC~), 2.36 (1H, m, ~NHCHC~), 3.10 (12H, bm, ~NCH_2CH_2N~), 3.20 (4H, bm, ~NCH_2CH_2N~), 3.30 (2H, s, ~NCH_2CONH~) 3.65 (2H, bm, ~ NCH_2CO_2~),3.95 (4H, s, ~ NCH_2CO_2~), 4.11 (2H, m, ~CHCH2CH2CH2CH2NH~), 4.31(2H, m, ~CHCH2CH2CH2CH2NH~), 4.49 (2H, m, ~CHCH2CH2CH2CH2NH~), 4.61 (2H, m, ~CHCH2CH2CH2CH2NH~), 6.68 (1H, d, ~HC=CH~), 6.72 (1H, d, ~CH=CH~), 7.29 (20H, m, phenyl) (MALDI-TOF+) 1394.8 [M + K]$^+$

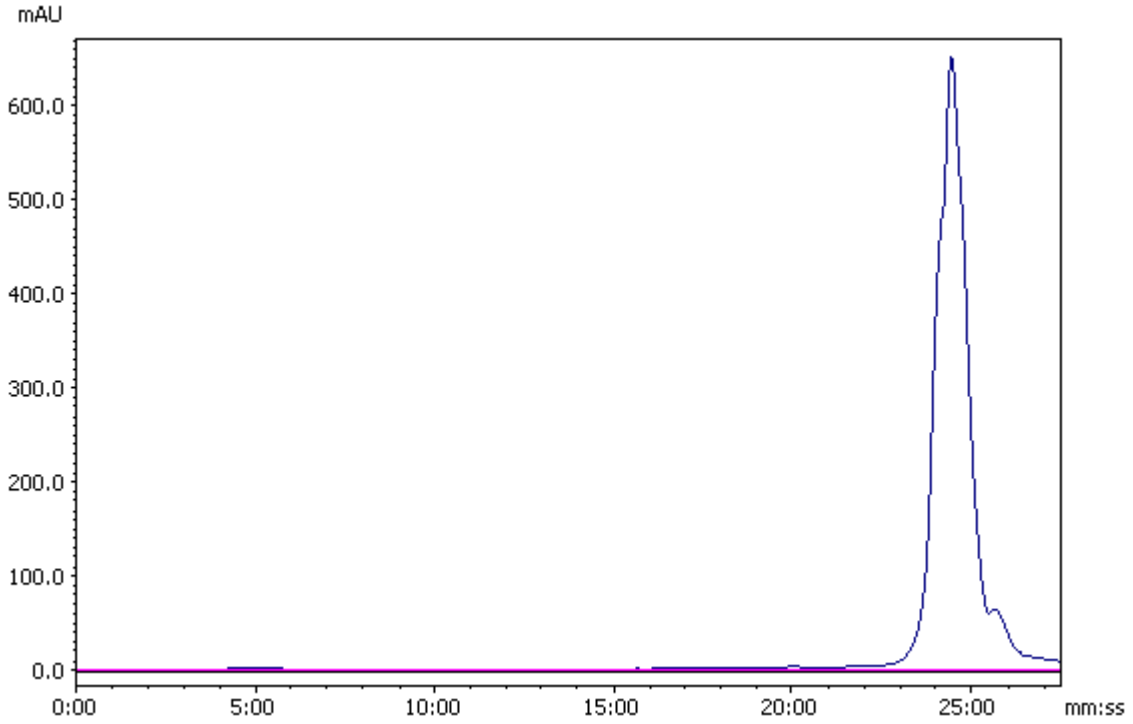

Figure S1. HPLC of **4**, H_2O 0.1% TFA: MeCN starting at 50:50 to 5:95 over 28 mins, λ = 254 nm.

Gd cFLFLFLK-DOTA Gd.1

4 (5 mg, 0.001 mmol) was dissolved in water (2 ml) and the pH was adjusted to 5.5 with small aliquots of 1.0 M NaOH. $GdCl_3.6H_2O$ (0.46 mg, 0.001 mmol) was dissolved in 1 ml of water at pH 5.5. The two solutions were combined and the pH re-adjusted to 5.5. The solution was then stirred for 3 hours. The solvent was removed to give a hygroscopic white powder, this was purified *via* Sephadex G25 resin (equilibrated with water) to remove inorganic salts. The resulting aliquots containing the complex were combined and the solvent removed, yielding the desired **Gd.1** complex as white hygroscopic solid, 6 mg, 88% yield. *m/z* (MALDI-TOF+) $[M+H]^+$ 1511.0

Tb cFLFLFLK-DOTA Tb.1

4 (5 mg, 0.001 mmol) was dissolved in water (2 ml) and the pH was adjusted to 5.5 with small aliquots of 1.0 M NaOH. $TbCl_3.6H_2O$ (0.46 mg, 0.001 mmol) was dissolved in 1 ml of water at pH 5.5. The two solutions were combined and the pH re-adjusted to 5.5. The solution was then stirred for 3 hours. The solvent was removed to give a hygroscopic white powder, this was purified *via* Sephadex G25 resin (equilibrated with water) to remove inorganic salts. The resulting aliquots containing the complex were combined and the solvent removed, yielding the desired **Tb.1** complex as white hygroscopic solid, 2 mg, 48% yield. *m/z* (MALDI-TOF+) $[M+H]^+$ 1513.3.

Relaxivity measurements

The **Gd.1** was prepared *in situ* by mixing the appropriate amounts of ligand and $GdCl_3.6H_2O$ (99.99%; Aldrich) in H_2O followed by adjustment of the pH with NaOH aqueous solution (pH = 7.4). The resulting solution was placed in a 1.7 mm diameter capillary which was

sealed. The absence of free gadolinium was checked in all samples by the xylenol orange test. The $1/T_1$ measurements were performed on a Bruker Avance 400 spectrometer (400 MHz).

In vivo MRI studies

Experiments were performed using male C57BL/6 mice weighing 23-25 g. All animal procedures were carried out under licence and complied with Home Office regulations (Guidance on the Operation of Animals, Scientific Procedures Act, 1986). Mice were maintained on a 12 h light/dark cycle during which temperature was maintained at 21-23 °C, and had access to a standard chow pellet diet and tap water *ad libitum*. Vehicle (saline) or lipopolysaccharide (LPS; 10 μg/mouse) was administered i.p., prior to imaging using **Gd.1** or **Gd.DOTA**. The following day, animals were anaesthetised via a facemask using isoflurane (induced with 4% and maintained with 1.5-3% isoflurane delivered in 100% O2 (L/min)). The left jugular vein was cannulated with PE 10 tubing for administration of Gd.1 and Gd.DOTA (150 μl). Tissue adhesive (Vetbond™ Tissue Adhesive, 3M UK plc, Bracknell, UK) was used to close the skinbefore mice were transferred to the magnetic resonance imaging (MRI) scanner.

Mice were placed in a stereotactic frame within a 72 mm, volume quadrature coil, with a respiratory cuff system (SA Instruments, Stony Brook, NY, USA) was used for respiration monitoring and body temperature was monitored via a rectal probe and maintained at 37^{o}C using an air-heated system (SA Instruments, USA). Each of these parameters was recorded from a control room and anaesthesia adjusted according to fluctuations in the stability of each mouse.

Once animals were securely positioned, the coil was placed in the centre of the MRI machine a 9.4T Varian Direct Drive system (Oxford, Uk) and quad 72 400Mhz volume coil (Varian,Inc. UK). The MRI protocol consisted of dynamic contrast acquisitions with a multi-slice T1 weighted gradient echo with a fixed 30° flip angle. Baseline acquisitions of the whole body (7 slices, 6 averages over 1 min 30 sec), brain (12 slices, 6 averages over 4 min 23 sec) and abdomen (12 slices, 6 averages over 4 min 23 sec) were taken, 24 h after initial LPS injection. 1 mmol/kg contrast agent was injected over 5 min, and subsequent acquisitions taken continuously post-injection, from the whole body, brain and abdomen in sequential rotation.

Receptor binding studies

HEK cells stably expressing FPR1 were cultured for one week. The cells were plated at a concentration of 100,000 cells/ml on a 24 well plate, (1 ml). Once the cells were confluent the media was removed and the appropriate amount of HEPS buffer including $CaCO_3$ $MgCO_3$, BSA (1%) was added. To this 50 ul of ^{125}I-fmlp analogue at 1000 counts per second was added followed by various concentrations of **Gd.1**. This was incubated at room temperature for 90 minutes, the cells were scraped of the plate and placed in ependorfs (1.5 ml). The tubes were centrifuged for 2 minutes at 21000 rcf. The supernatant was discarded. The pellet was re-dispersed in 0.5 ml buffer solution and centrifuged for a further 2 minutes at 21000 rcf, followed by discarding the supernatant. The radiation was measured on Nuclear Enterprises NE1600 Counters for 240 seconds. The Kd was determined using an exponential decay fit in Prism (Figure **S2**)

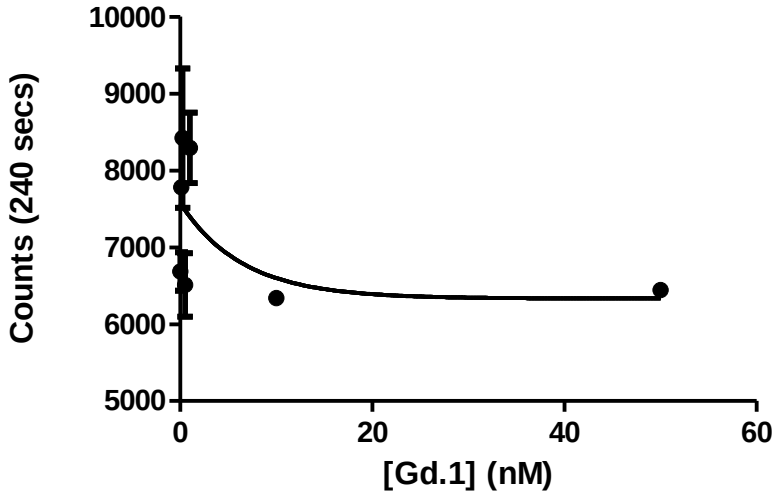

Figure S2. Representative saturation curve of ^{125}I-fMLP analogue vs **Gd.1**, specifically bound to HEK cells expressing FPR1. Binding affinity was computed to be 4.5 nM.

Fluorescence Measurements

Luminescence data was recorded using a Cary Varian luminescence spectrometer (using SCAN for Windows). Samples were held in a 10x10 nm or 10x4 nm quartz Hellma cuvette.

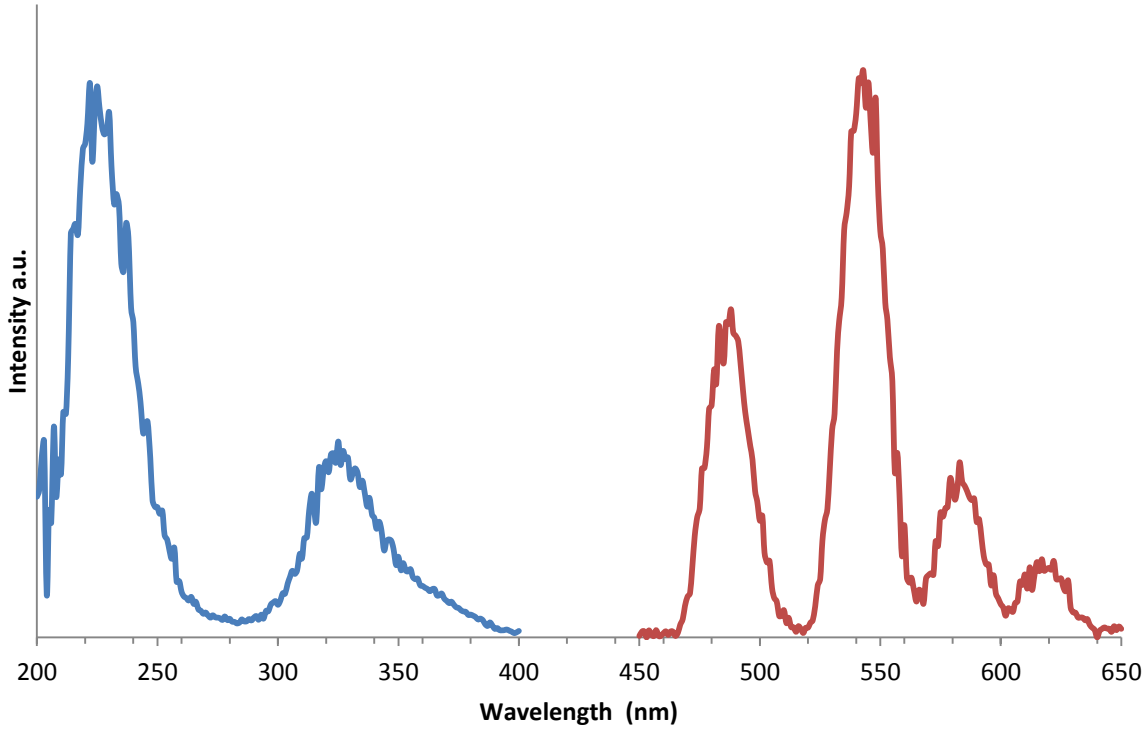

Figure S3. Luminescence spectra for Tb.1, (Left) excitation spectra with λ_{em} = 545 nm (dark blue; (Right) : emission spectra with λ_{ex} = 225 nm. (H_2O, 298K)

Lifetimes

Lifetimes were measured by direct excitation (225, 325 and 377 nm) of the sample with a short 40 ms pulse of light (50 pulses per point) followed by monitoring the integrated intensity of light (546 nm) emitted during a fixed gate time of 0.1 ms, at a delay time later. Delay times were set at 0.1 ms intervals, covering 4 or more lifetimes. Excitation and emission slits were set to 10:10 nm bandpass respectively. The obtained decay curves were fitted to a simple mono exponential first-order decay curve using Microsoft Excel. *q*-values were calculated using the following equation.

$$q = 5[k\mathrm{H2O} - k\mathrm{D2O} - 0.06] \text{ for Tb}$$

λ_{ex}	λ_{em}	K_{H2O}	K_{D2O}	q
225, 325, 377 nm	545 nm	1.387	1.114	1.06

Table S1 Lifetime measurements for **Tb.1**