Supplementary Information for:

A two step fluorinase mediated fluorine-18 labelling of an RGD peptide for positron emission tomography

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General experimental

All reagents and solvents from commercial sources were of the highest grade available, and were used without further purification, unless otherwise specified. c(RGDfK(N_3)) **12** was purchased from Peptides International, USA. NMR spectra were recorded on Bruker Advance 300, 400 or 500 instruments. ^{1}H and ^{13}C NMR spectra were recorded using deuterated solvent as the lock and residual solvent as the internal standard. ^{19}F NMR spectra were referenced to $CFCl_3$ as an external standard. Chemical shifts are reported in parts per million (ppm) and coupling constants (*J*) are given in Hertz (Hz). The abbreviations for the multiplicity of the proton, and fluorine signals are as follows: s singlet, d doublet, dd doublet of doublets, ddd doublet of doublet of doublets, t triplet, dt doublet of triplets, q quartet, m multiplet, br s broad singlet. Compounds are numbered according to customary purine numbering. When necessary, resonances were assigned using two-dimensional experiments (COSY, TOCSY). MALDI MS was acquired using a 4800 MALDI TOF/TOF Analyser (ABSciex) equipped with a Nd:YAG 355 nm laser and calibrated using a mixture of peptides. HPLC analyses/semi-preparations were performed using either a Shimadzu Prominence or a Varian Prostar system equipped with a Varian 400 autosampler, Varian Prostar 230 solvent delivery system, Varian Prostar 235 UV-Vis detector (for cold assays) and an Agilent 1100 Series system equipped with a binary pump, a degasser, a diode array detector or 321 Gilson HPLC system equipped with a binary pump, a UV detector and radioactivity detector (for hot assays) with reverse phase column as indicated in individual experiment. LCMS analysis was performed on a Waters 2795 HPLC coupled in parallel to a Waters 2996 photodiode array detector and Micromass LCT TOF mass spectrometer in ESI in positive mode. Protein concentrations were measured on a NanoDrop 1000 spectrophotometer at 280 nm using an extinction coefficient of 10.42 as calculated by ExPASy ProtParam tool.[1]

Synthesis and characterisation of compounds and NMR spectra

Preparation of 10 mM $CuSO_4$.TBTA (55% DMSO solution)

Copper (II) sulfate pentahydrate (50 mg) was dissolved in water (10 mL), and to this, a solution of TBTA (tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine) (116 mg) in DMSO (11 mL) was added. The solution was well mixed to generate the complex, which was stored at room temperature until use.

Synthesis of ClDEA 6

Synthesised as reported in reference 2.

Synthesis of FDEA 8

Synthesised as reported in reference 2.

Synthesis of ClDEA-RGD 14

ClDEA **6** (0.49 mg, 0.0016 mmol, 1 eq) and cRDGfK(N_3) **12** (1.3 mg, 0.0020 mol, 1.25 eq) were dissolved in water (9.5 mL), and fresh sodium ascorbate solution (20 mM stock in water, 798 µL, 10 eq) and $CuSO_4$.TBTA solution (10 mM stock in 55% DMSO, 319 µL, 2 eq) was added and the mixture left to react for 30 minutes, at which point HPLC sampling showed all the alkyne to be consumed. The reaction mixture was passed through an ExtractClean C_{18}-HC (1000 mg) cartridge (preconditioned with water), and the product eluted with 1:1 water:acetonitrile (2 × 10 mL). The water:acetonitrile fractions were combined and concentrated to dryness under a stream of air, and the crude product purified by preparative HPLC (Shimadzu Prominence, using Phenomenex Kingsorb C_{18} (250 × 10.00 mm, 5µ)) column; mobile Phase: A (H_2O + 0.05% TFA), B (MeCN + 0.05% TFA); linear gradient: 20% B to 42 %B in 18 min, 42% B to 95% B in 1 min; hold at 95% B for 5 min, 95% B to 20% B in 1 min, and re-equilibration at 20%B for 10 min; flow rate: 2.5 mL/min; detection 220 nm). Fractions containing product were collected, concentrated under a stream of air, and lyophilised from a 20% t-BuOH in water solution affording **14** (0.72 mg, 48%) as the TFA salt.

δ_H (500.0 MHz, d_6-DMSO + 15% D_2O): 8.48 (1 H, s, *H*-8), 8.32 (1 H, s, triazole-*H*), 7.19–7.09 (5 H, D-Phe Ar*H*), 5.97 (1 H, d, *J* 5.7, *H*-1′), 4.80 (1 H, dd, *J* 5.4, 5.4, *H*-2'), 4.59 (1 H, dd, *J* 9.6, 5.5, Asp α-*H*), 4.49 (1 H, dd, *J* 7.5, 5.4, Arg α-*H*), 4.37 (2 H, t, *J* 6.8, Lys ε), 4.34–4.28 (2 H, m, Lys α-*H*, *H*-3'), 4.16–4.10 (3 H, m, *H*-4', D-Phe α-*H*, Gly α-H_a), 3.99 (1 H, dd, *J* 11.7, 4.8, *H*-5'$_a$), 3.92 (1 H, dd, *J* 11.7, 6.4, *H*-5'$_b$), 3.38–3.32 (2 H, m, Gly α-H_b, Asp β-H_a), 3.06 (2 H, t, *J* 7.1, Arg δ-CH_2), 2.77–2.68 (1 H, m, D-Phe β-H_a), 2.58 (1 H, dd, *J* 14.4, 9.6, Asp β-H_b), 2.08–1.97 (1 H, m, D-Phe β-H_b), 1.89–1.79 (2 H, m, Lys β-CH_2), 1.73–1.52 (4 H, m, Lys δ-CH_2, Arg β-CH_2), 1.50-1.33 (2 H, m, Arg δ-CH_2), 1.28–1.12 (2 H, m, Lys γ-CH_2).

m/z (MALDI-TOF) calc. for $C_{39}H_{52}ClN_{16}O_{10}$ $[M+H]^+$: 939.37, found 939.37.

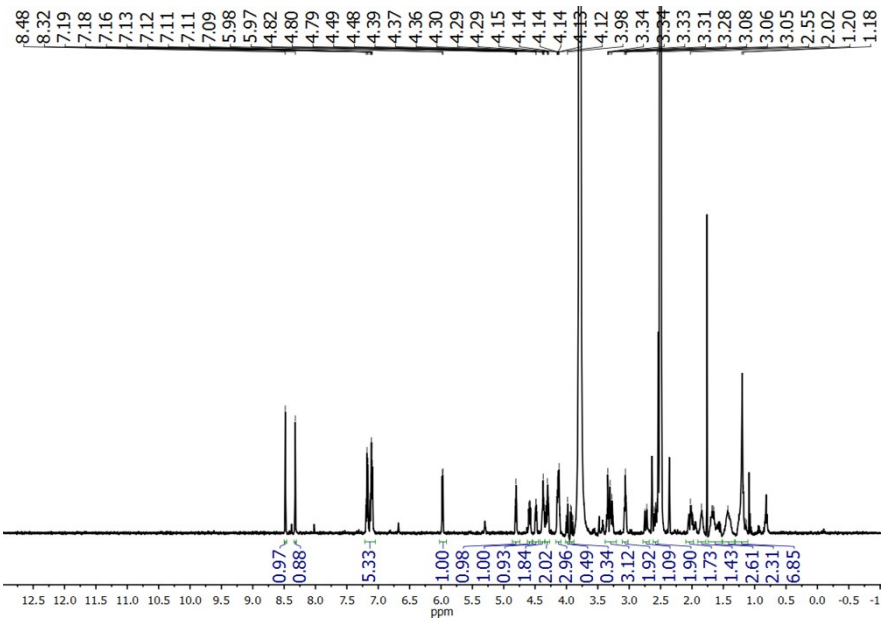

Fig. S1. ^{1}H NMR spectrum (500.0 MHz, d_6-DMSO + 15% D_2O) of ClDEA-RGD **14**.

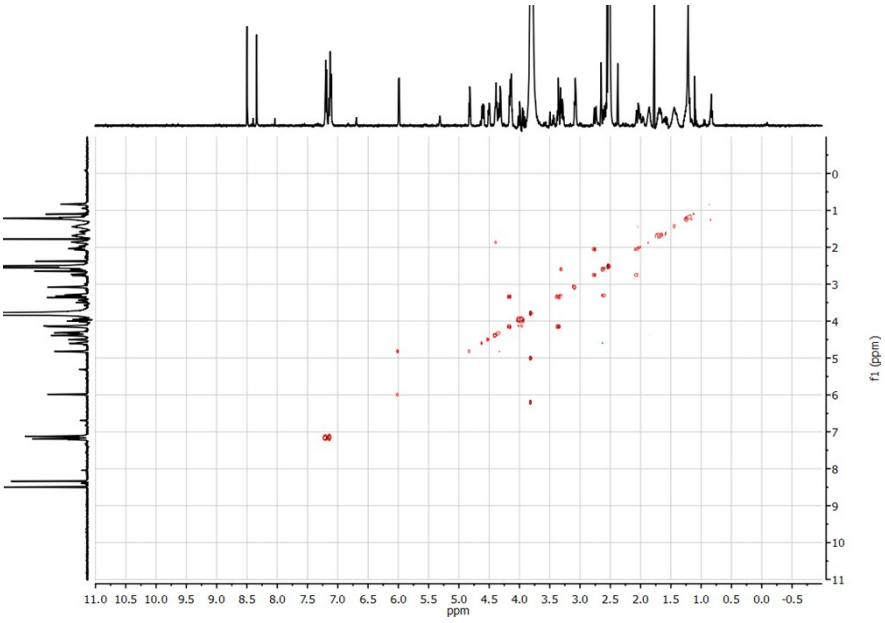

Fig. S2. COSY NMR spectrum (500.0 MHz, d_6-DMSO + 15% D_2O) of ClDEA-RGD **14**.

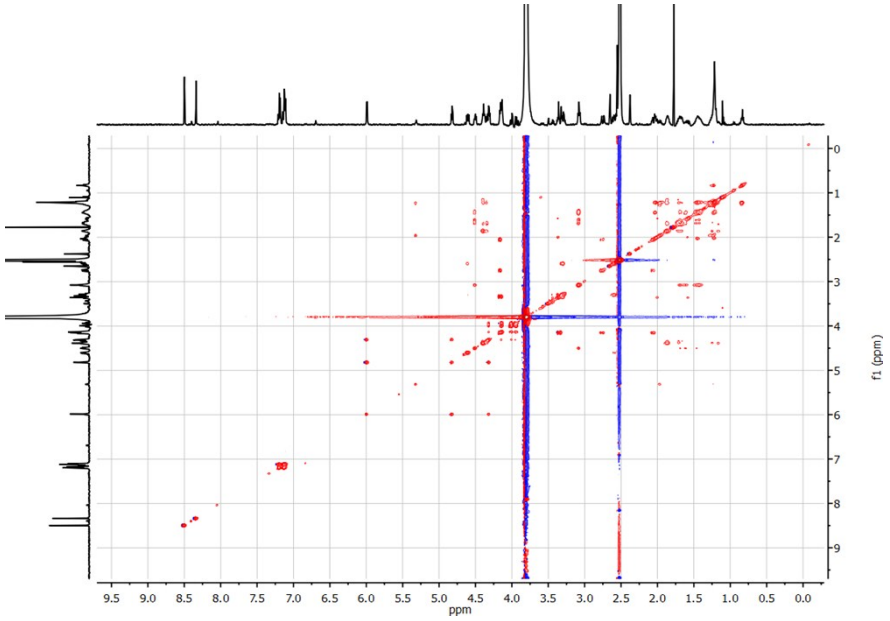

Fig. S3. TOCSY NMR spectrum (500.0 MHz, d_6-DMSO + 15% D_2O. mixing time 90 ms) of ClDEA-RGD **14**.

Synthesis of FDEA-RGD 13

FDEA **8** (0.48 mg, 0.0016 mmol, 1 eq) and cRDGfK(N_3) (1.3 mg, 0.0020 mol, 1.25 eq) were dissolved in a mixture of DMSO (3 mL) and water (6.7 mL), and fresh sodium ascorbate solution (20 mM stock in water, 813 µL, 10 eq) and $CuSO_4$.TBTA solution (10 mM stock in 55% DMSO, 325 µL, 2 eq) was added and the mixture left to react for 70 minutes, at which point HPLC sampling showed all the alkyne to be consumed. The reaction mixture diluted with water (2 mL) and was passed through an ExtractClean C_{18}-HC (1000 mg) cartridge (preconditioned with water), washed with water (10 mL), and the product eluted with 1:1 water:acetonitrile (2 × 10 mL). The water:acetonitrile fractions were combined and concentrated to dryness under a stream of air, and the crude product purified by preparative HPLC (Shimadzu Prominence, using Phenomenex Kingsorb C_{18} (250 × 10.00 mm, 5µ)) column; mobile phase: A (H_2O + 0.05% TFA), B (MeCN + 0.05% TFA); linear gradient: 20% B to 42 %B in 18 min, 42% B to 95% B in 1 min; hold at 95% B for 5 min, 95% B to 20% B in 1 min, and re-equilibration at 20%B for 10 min; flow rate: 2.5 mL/min; detection 220 nm). Fractions containing product were collected, concentrated under a stream of air, and lyophilised from a 20% t-BuOH in water solution affording **13** (0.74 mg, 49%) as the TFA salt.

δ_H (500.0 MHz, d_6-DMSO + 15% D_2O): 8.48 (1 H, s, *H*-8), 8.26 (1 H, s, triazole-*H*), 7.21–7.04 (5 H, D-Phe Ar*H*), 5.99 (1 H, d, *J* 5.7, *H*-1′), 4.75–4.70 (1 H, m, ½ *H*-5_a', ½ *H*-5_b'), 4.67–4.61 (2 H, m, *H*-2' ½ *H*-5_a', ½ *H*-5_b'), 4.59 (1 H, dd, *J* 9.6, 5.5, Asp α-*H*), 4.49 (1 H, dd, *J* 7.4, 5.5, Arg α-*H*), 4.37 (2 H, t, *J* 6.9, Lys ε), 4.34–4.29 (2 H, m, Lys α-*H*, *H*-3'), 4.19–4.09 (3 H, m, *H*-4', D-Phe α-*H*, Gly α-H_a), 3.35–3.23 (2 H, m, Gly α-H_b, Asp β-H_a), 3.06 (2 H, t, *J* 7.1, Arg δ-CH_2), 2.76–2.69 (1 H, m, D-Phe β-H_a), 2.58 (1 H, dd, *J* 14.2, 9.6, Asp β-H_b), 2.08–1.99 (1 H, m, D-Phe β-H_b), 1.90–1.80 (2 H, m, Lys β-CH_2), 1.74–1.51 (4 H, m, Lys δ-CH_2, Arg β-CH_2), 1.49-1.32 (2 H, m, Arg δ-CH_2), 1.30–1.10 (2 H, m, Lys γ-CH_2).

δ_F (470.5 MHz, d_6-DMSO + 15% D_2O): 227.01 (1 F, dt, *J* 47.4, 24.2, CH_2F).

m/z (MALDI-TOF) calc. for $C_{39}H_{52}FN_{16}O_{10}$ $[M+H]^+$: 923.40, found 923.41.

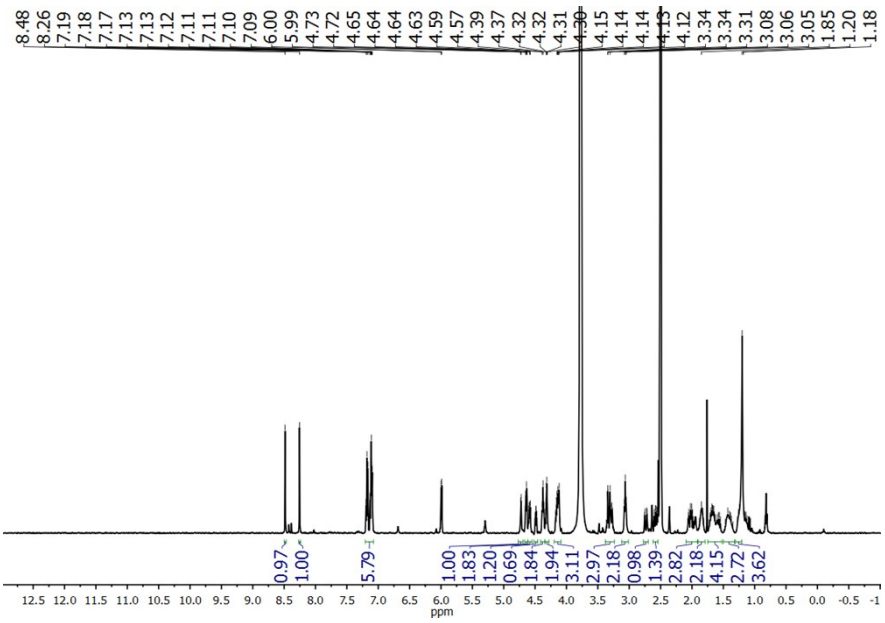

Fig. S4. ^{1}H NMR spectrum (500.0 MHz, d_6-DMSO + 15% D_2O) of FDEA-RGD **13**.

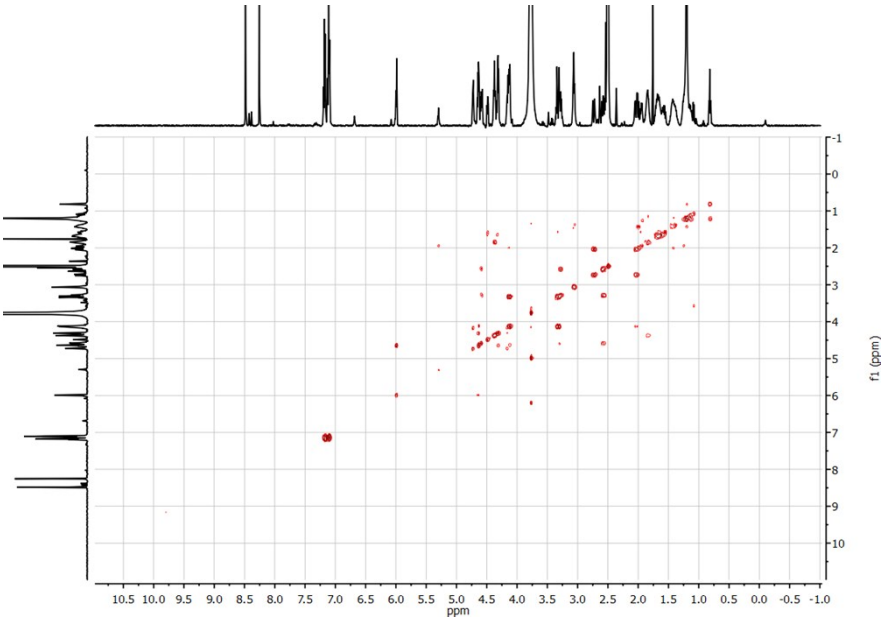

Fig. S5. COSY NMR spectrum (500.0 MHz, d_6-DMSO + 15% D_2O) of FDEA-RGD **13**.

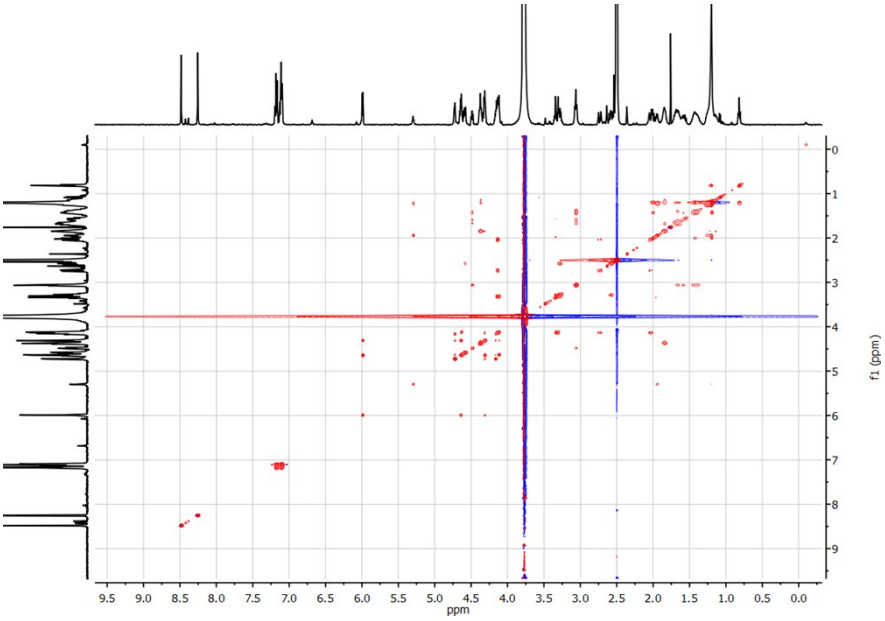

Fig. S6. TOCSY NMR spectrum (500.0 MHz, d_6-DMSO + 15% D_2O, mixing time 90 ms) of FDEA-RGD **13**.

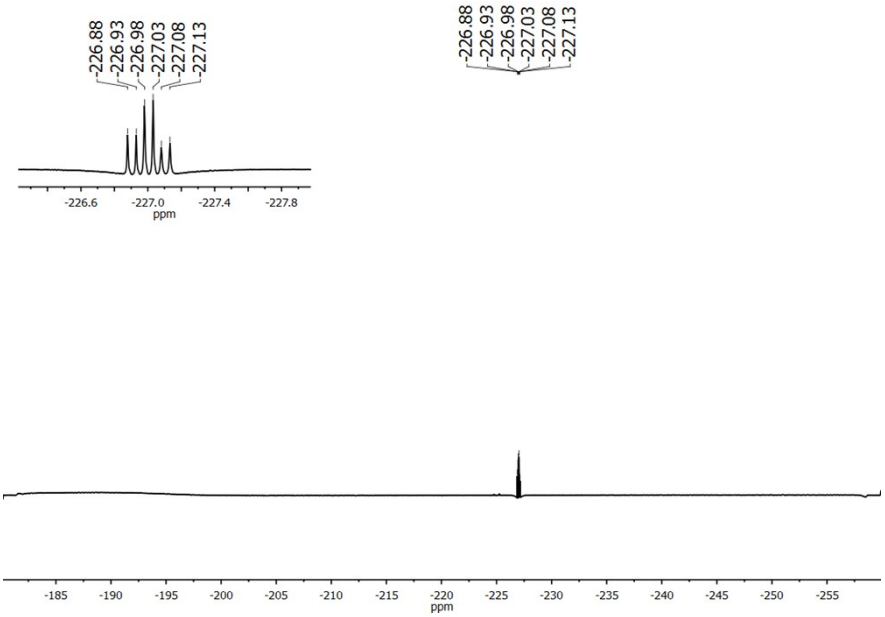

Fig. S7. ^{19}F NMR spectrum (470.5 MHz, d_6-DMSO + 15% D_2O) of FDEA-RGD **13**.

Fluorinase Overexpression

Recombinant fluorinase was overexpressed in *E. coli*, and purified by Ni^{2+} affinity chromatography, and treated with calf spleen adenosine deaminase (Sigma Aldrich), as reported previously.[2] After the final Ni^{2+} column, the fluorinase was dialysed overnight, against phosphate buffer (2 × 5 L, 50 mM, pH 7.8). Fluorinase was then concentrated, using an Amicon Ultra 15 spin concentrator (10 kDa cut off, Merck), to the desired concentration. When freeze dried fluorinase was used, the enzyme solution was aliquotted into portions (5 mg or 10 mg, based on fluorinase), before being lyophilised, and stored at −78 °C until needed.

ClDEA 6 and FDEA 8 Reactions

Fluorinase Assay of ClDEA 6 to FDEA 8, and click reaction to give FDEA-RGD (Figure 1)

ClDEA **6** (103 µL of 0.58 mM stock, final conc. 0.2 mM), L-SeMet (15 µL of 2 mM stock, final conc. 0.1 mM), potassium fluoride (500 mM stock, 45 µL, 75 mM final conc.), phosphate buffer (6 µL, 1 M pH 7.8 stock, final conc. 20 mM) and fluorinase (125 µL of 12 $mg.mL^{-1}$ stock, final conc. 5 $mg.mL^{-1}$) were combined and made up to 300 µL with water ("t = 0 min" sample removed (50 µL) and heated to 95°C for 5 minutes, and centrifuged at 13 000 rpm for 10 minutes, trace **A** in Figure 1), and left to react at 37°C for 2 hours. The mixture was heated to 95°C for 5 minutes, and centrifuged at 13 000 rpm for 10 minutes. Supernatant was used for the subsequent click reaction.

Enzymatically produced FDEA **8** (115 µL of 0.2 mM stock, final conc. 0.1 mM) and cRDGfK(N_3) **12** (46 µL of 1.0 mM stock, final conc. 0.2 mM) and fresh sodium ascorbate solution (23 µL of 5 mM stock, final conc. 0.5 mM) was added to water (23 µL) ("t = 0 min" sample removed (50 µL) and analysed by HPLC, trace **B** in Figure 1) before $CuSO_4$.TBTA solution (23 µL of 10 mM stock, final conc. 1.0 mM) and the mixture left to react for 70 minutes. A "t = 70 min" sample removed (50 µL) and analysed by HPLC, trace **C** in Figure 1.

HPLC analysis was performed on a using the Varian instrument, using Phenomenex Kingsorb C_{18} (150 mm × 4.6 mm, 5µm) column and guard cartridge; mobile phase: A (H_2O + 0.05% TFA), B (MeCN + 0.05% TFA); linear gradient: 5% B for 2 min, 5% to 25% B in 10 min, 25% to 50% B in 6 min, 50% B to 95% B in 2 min; hold at 95% B for 3 min, 95% B to 5% B in 2 min, and reequilibration at 95% B for 10 min; flow rate: 1 mL/min; detection 220 and 254 nm); injection volume: 20 µL.

LCMS of Click Reaction with 0.5 eq. of cRGDfK(N_3) 12

An enzymatic mixture of FDEA **8**, (with residual ClDEA **6**) (500 µL of 0.3 mM stock, final conc. 0.2 mM) and cRDGfK(N_3) **12** (75 µL of 1.0 mM stock, final conc. 0.1 mM) and fresh sodium ascorbate solution (11 µL of 5 mM stock, final conc. 0.075 mM) was added to water (158 µL) before $CuSO_4$.TBTA solution (6 µL of 10 mM stock, final conc. 0.075 mM) was added and the mixture left to react for 15 hours to go to completion. A sample (50 µL + 100 µL water) was analysed by LCMS (Figure S8).

LCMS analysis was performed on a using the Waters/Micromass instrument, using Phenomenex Kinetix C_{18} (150 mm × 4.6 mm, 5µm) XB-C_{18} 100A column and guard cartridge; mobile phase: A (H_2O + 0.1% formic acid), B (MeCN + 0.1% formic acid); linear gradient: 5% B for 2 min, 5% to 25% B in 10 min, 25% to 50% B in 6 min, 50% B to 95% B in 2 min; hold at 95% B for 3 min, 95% B to 5% B in 2 min, and reequilibration at 95% B for 10 min; flow rate: 0.5 mL/min; injection volume: 50 µL.

As the reaction was run with limiting amounts of azide, unreacted FDEA **8** (t_R = 10.7 min, **Fig. S9**) and ClDEA **6** (t_R = 12.1 min, **Fig. S10**) are seen, along with the anticipated triazole products, FDEA-RGD **13** (t_R = 12.8 min, **Fig. S11**) and ClDEA-RGD **14** (t_R = 13.5 min, **Fig. S12**). Also observed is SeMetDEA-RGD **15** (t_R = 14.0 min , **Fig. S13**), produced by click reaction between the azide and selenomethyl-ethynyldeoxyadenosine (produced by heat-catalysed decomposition of ethynyl-SeSAM).

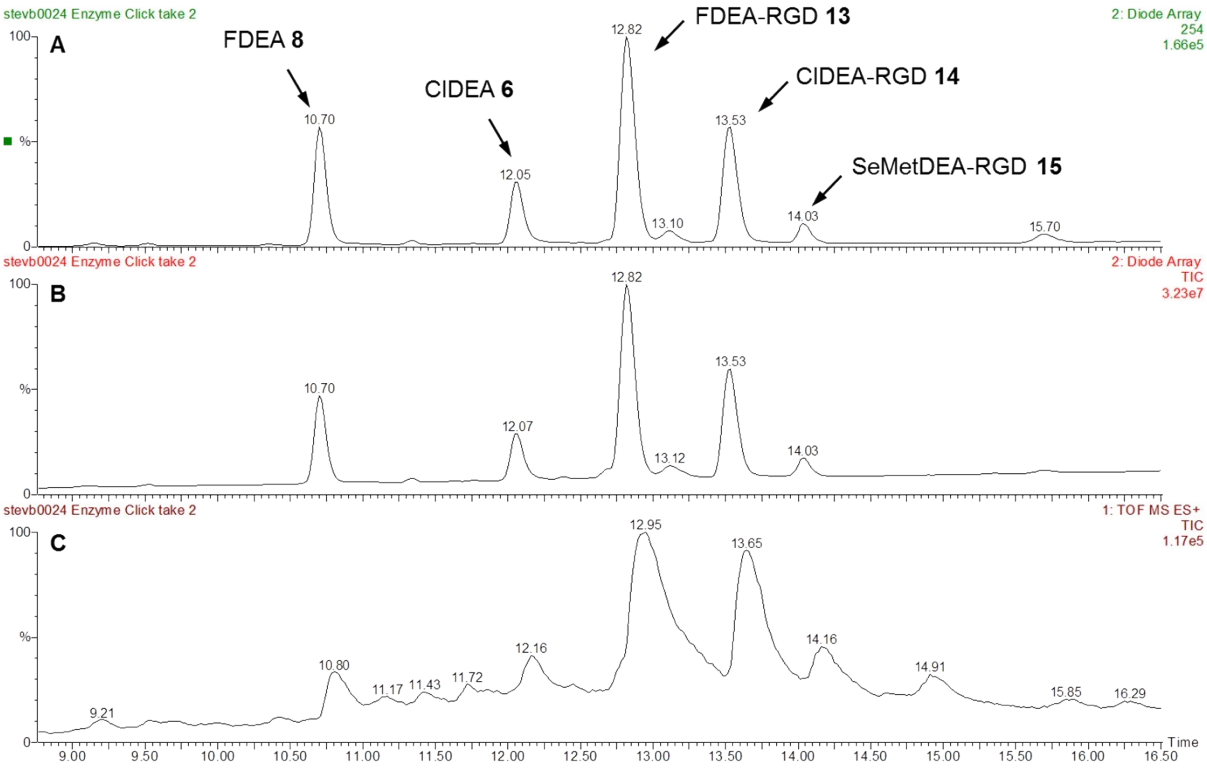

Fig. S8. LCMS trace of a click reaction enzymatically synthesised FDEA **8** and cRGDfK(N_3) **12** showing detection at 254 nm (**A**), 220 nm (**B**) and the total ion chromatogram (**C**).

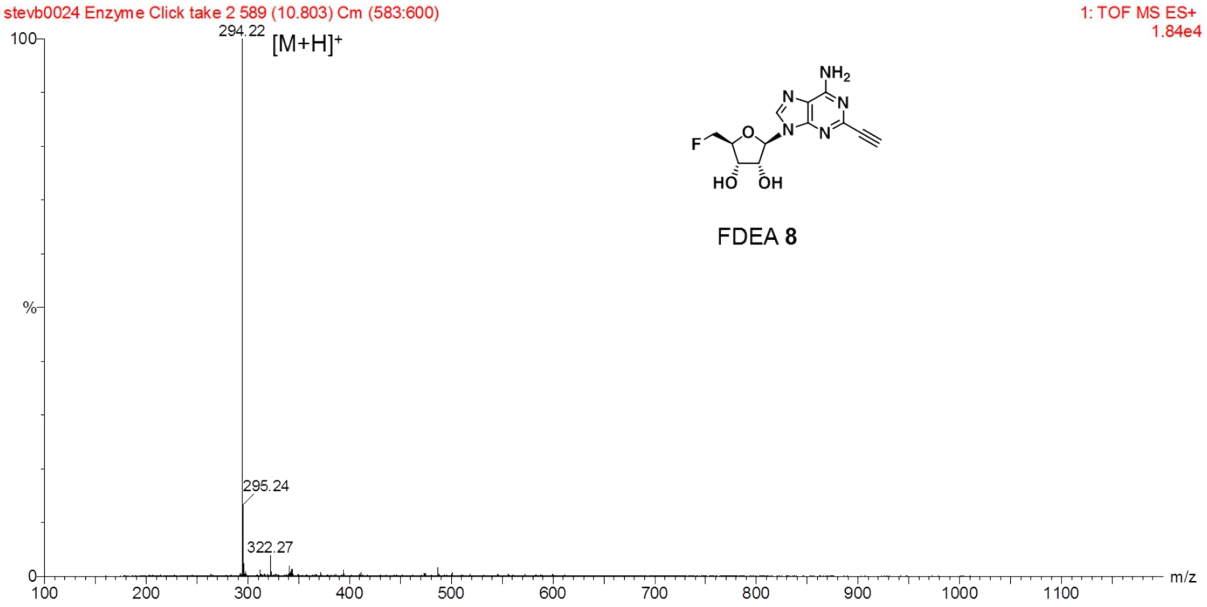

Fig. S9. Combined mass spectra of the peak in the total ion chromatogram at t_R = 10.8 min (**Figure S8**), corresponding to FDEA **8**.

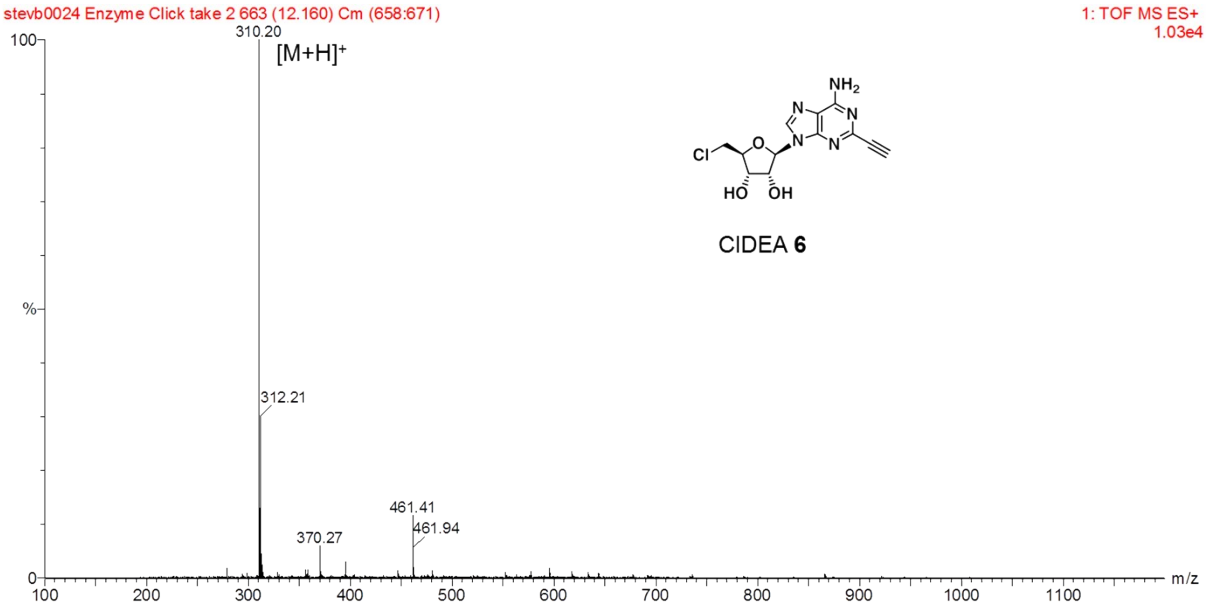

Fig. S10. Combined mass spectra of the peak in the total ion chromatogram at t_R = 12.1 min (**Figure S8**), corresponding to ClDEA **6**.

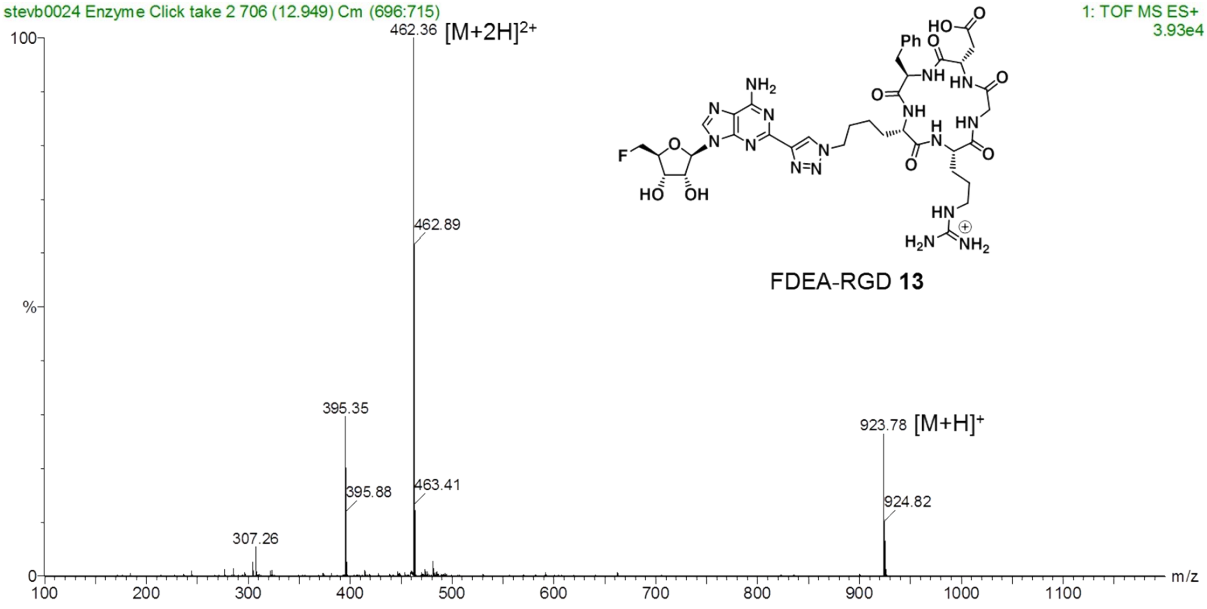

Fig. S11. Combined mass spectra of the peak in the total ion chromatogram at t_R = 12.8 min (**Figure S8**), corresponding to FDEA-RGD **13**.

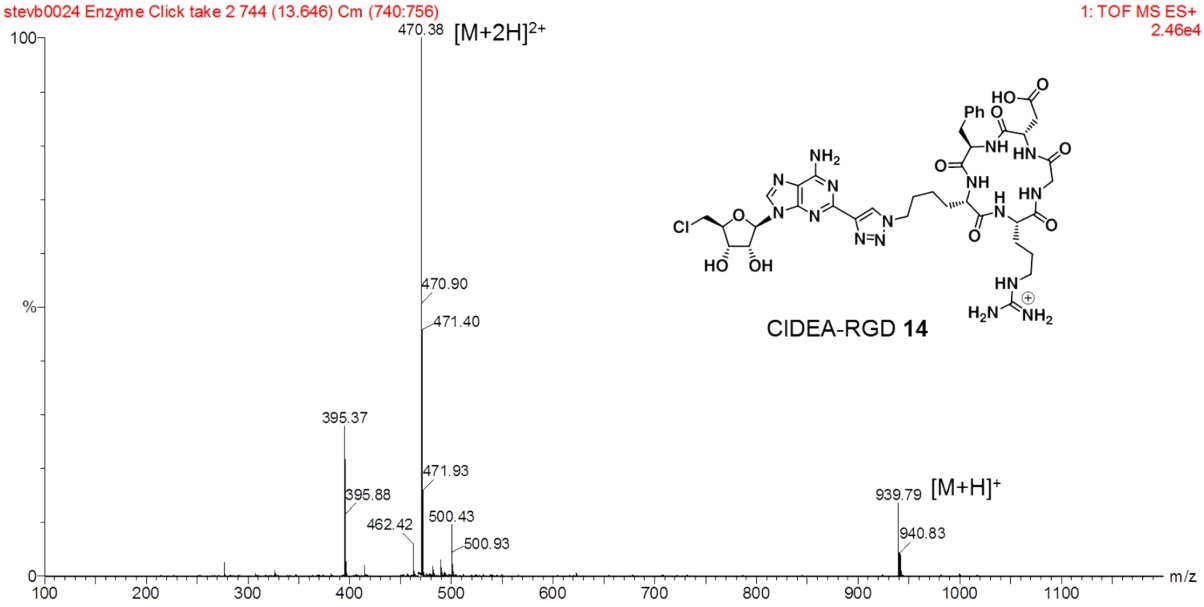

Fig. S12. Combined mass spectra of the peak in the total ion chromatogram at t_R = 13.5 min (**Figure S8**), corresponding to ClDEA-RGD **14**.

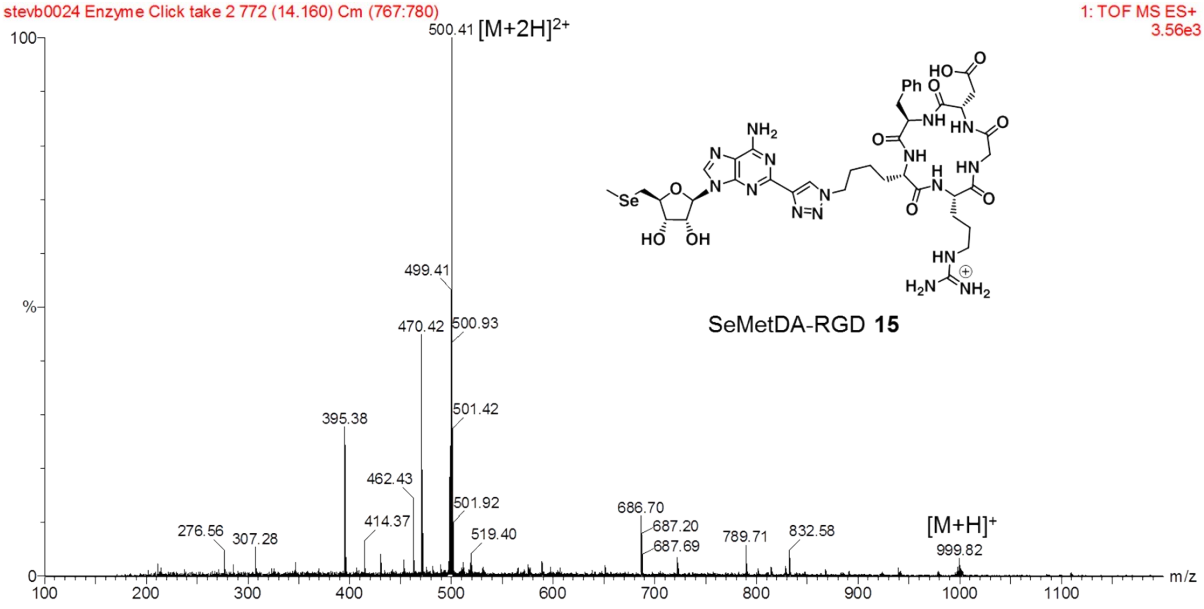

Fig. S13. Combined mass spectra of the peak in the total ion chromatogram at t_R = 14.0 min (**Figure S8**), corresponding to SeMetDEA-RGD **15**.

Radiolabelling experiements

[^{18}F]Fluoride was produced by the ^{18}O(p,n)^{18}F reaction using a Siemens RDS-111 Eclipse cyclotron equipped with a fluoride target loaded with oxygen-18 enriched water. The [^{18}F]fluoride solution in oxygen-18 enriched water was transferred with a sweep of argon gas from the cyclotron target to the hot cell containing the reaction equipment. Radioactivity was measured using an ion chamber (ISOMED 2000 Dose Calibrator).

200 µL of [^{18}F]fluoride solution in [^{18}O]water (up to 525 MBq) was added to an eppendorf containing ClDEA **6** (approx. 0.1 mg), L-selenomethionine (10 µL, 20 mM) and freeze dried

fluorinase/phosphate buffer (5 mg protein). The contents were well mixed and incubated at 37 °C for 30 min. After this time, the reaction mixture was heated at 95 °C for 5 min and the denatured protein was removed by centrifugation (13,000 rpm, 5 min). An aliquot of the supernatant was analysed by HPLC (trace shown in **Fig. 2A**) on the Agilent system using a Phenomenex Kinetex 5 μm, XB-C18 100A (150 × 4.6 mm) column and a guard cartridge; Mobile phase: 0.05% TFA in water (solvent A) and 0.05% TFA in acetonitrile (solvent B); Linear gradient: 5% B for 2 min, 45% B at 10 min and then return to initial conditions (5% B); Flow rate: 1 $mL.min^{-1}$. The supernatant was also compared against a synthetic standard of FDEA **8** confirming the production of [^{18}F]FDEA **[^{18}F]-8** (trace shown in **Fig. 2B**).

The supernatant containing [^{18}F]FDEA **[^{18}F]-8** was added to a solution containing solid RGDfK(N_3) **12** peptide (0.3 mg), freshly prepared sodium ascorbate (50 uL of 100 mM) and $CuSO_4$.TBTA solution (100 uL of 10 mM). The reaction was incubated at room temperature for 30 min.

Crude [^{18}F]FDEA-RGD **[^{18}F]-13** was purified by semi-preparative HPLC using the Gilson HPLC system using a Phenomenex Luna 5 μm, C18 100A (250 × 10 mm) column; Mobile phase: 0.05% TFA in water (solvent A) and 0.05% TFA in acetonitrile (solvent B); Linear gradient: 5% B for 2 min, 25% B at 12 min, 50% B at 18 min and 90% B at 20 min; Flow rate: 5 $mL.min^{-1}$. The fraction containing [^{18}F]FDEA-RGD **[^{18}F]-13** (r.t. = 16 min approx.) was collected and diluted in water before loading onto a C_{18} Classic SepPak. The cartridge was washed with water (10 mL) prior to elution with ethanol (0.5 mL) into a vial containing saline to give a final formulation of 10% ethanol in saline. An aliquot of the final [^{18}F]FDEA-RGD **[^{18}F]-13** dose was analysed by HPLC (following the method described above) to confirm identity and radiochemical purity (see **Fig. 2C** and **2D**). Conversions of up to 20% (non-decay-corrected) from [^{18}F]fluoride were observed, providing up to 95 MBq of reformulated [^{18}F]FDEA-RGD **[^{18}F]-13** (n = 2).

Rodent Imaging Experiment

Animal Information: Species: Male Sprague-Dawley Rat of weight 382 to 430 g, obtained from Charles River Laboratories. Rats were acclimated for more than 3 days prior to use for the study with food and water given *ad libitum*.

All the experiments described in this protocol were performed in accordance with the U.K. Animals (Scientific Procedures) Act 1986. The procedures used in the present study are approved by the Animal Ethical Review Committee of Imperial College.

Two male Sprague-Dawley rats (Charles River Ltd., Kent, UK), were anaesthetised and maintained under terminal isoflurane anaesthesia. Each animal received an intravenous bolus injection of the radiotracer [^{18}F]FDEA-RGD **[^{18}F]-13** formulated in 10% ethanol in saline.

One animal was placed in the Siemens Inveon™ DPET/MM PET-CT scanner, with the thorax within the field of view. A CT scan was collected for attenuation correction and to provide structural information. A dynamic PET scan was performed for 60 min following *i.v.* administration of *ca.* 3 MBq of [^{18}F]FDEA-RGD **[^{18}F]-13**. The body temperature of each rat was maintained throughout the procedure with a heating mat, and monitored using a rectal probe. A respiration pad was used to monitor the respiration rate of the rat in the scanner.

The images of the rat in the PET-CT scanner (**Fig. S14**) showed no evidence of radioactivity uptake into the bone indicating that [^{18}F]FDEA-RGD **[^{18}F]-13** was stable to [^{18}F]-fluoride ion release over the duration of the scan.

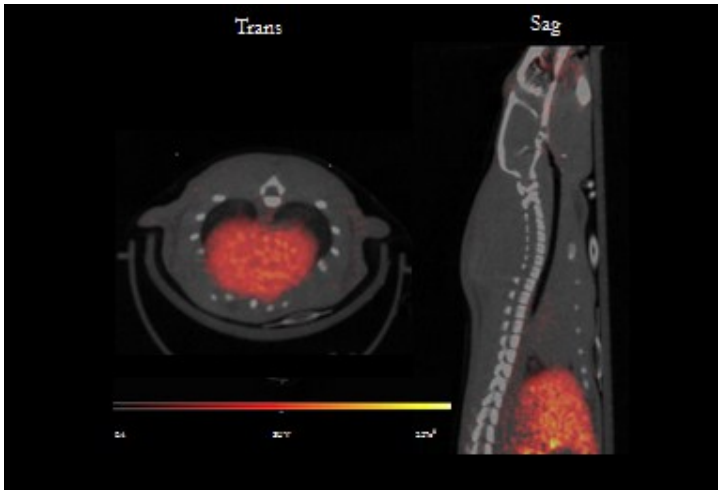

Fig. S14. Summed transaxial (transaxial, L) and saggital (saggital, R) PET images of a rat, 5-60 min following administration of 2.5MBq of [^{18}F]FDEA-RGD **[^{18}F]-13**, co-registered with corresponding CT images (greyscale). PET images are presented as standard uptake values {(tissue activity/mL)/(injected activity/animal weight[g])}.

Metabolites HPLC Analysis Procedure and Data Analysis.

Both rats received an intravenous administration of [^{18}F]FDEA-RGD **[^{18}F]-13** (approximately 3 MBq) *via* a tail vein cannula. Discrete arterial blood samples (at 5, 15, 30 and 60 minutes post [^{18}F]FDEA-RGD **[^{18}F]-13** administration) were taken from each rat, *via* an arterial cannula. Each sample was collected into a heparinized tube. The radioactivity associated with an aliquot of the blood was determined by gamma counting. The remaining blood was centrifuged (7500 x g for 3 min at +4 °C) to obtain plasma, an aliquot of which was also counted.

Whole blood of collected samples (at 5, 15, 30 and 60 minutes post [^{18}F]FDEA-RGD **[^{18}F]-13** administration) was separated by centrifugation at 7500 x g for 3 min at 4 °C to obtain plasma. Typically a sample of 300 µL of plasma was added to an equal volume of 50 mM ammonium formate (adjusted to pH 8) before injection for HPLC analysis. The resulting solution was mixed and 0.5 mL was injected onto the HPLC system consisting of a pump, degasser, UV-vis/diode array detector and fraction collector. The HPLC conditions used were as follows: 0.05% TFA in water (solvent A) and 95:5 acetonitrile/water (solvent B) at 5 mL/min; gradient method: 5% B for 2 min then 5% acetonitrile to 60% acetonitrile thereafter. The HPLC column used was a Phenomenex Luna 5 µm C18 semi-preparative column (100 Å; 10 µm, 250 x 10 mm). 30 sample fractions were collected over a 10 min run after passing through the UV detector. A radio-chromatogram was reconstructed from the collected HPLC fractions and the percent of unchanged radioligand candidate ([^{18}F]FDEA-RGD **[^{18}F]-13**) was calculated. Results for the two animals, Rat A and Rat B, at 5 min and 60 min are shown below in **Fig. S15** to **Fig. S18**.

Rat A

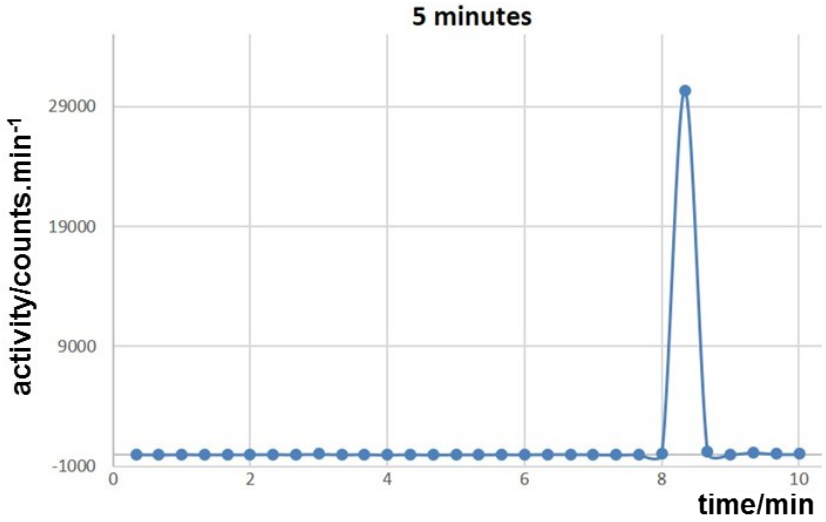

Fig. S15. Reconstructed radiochromatogram of HPLC fractions (30 fractions collected over 10 minutes) of acetonitrile plasma extracts of arterial blood from rat A, taken 5 min post injection showing a single peak, corresponding to unmetabolised [^{18}F]-FDEA-RGD **[^{18}F]-13**.

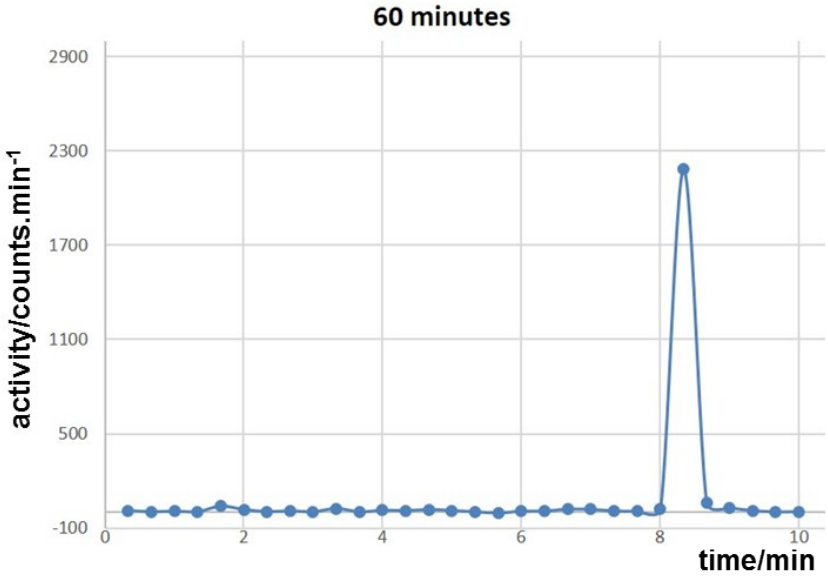

Fig. S16. Reconstructed radiochromatogram of HPLC fractions (30 fractions collected over 10 minutes) of acetonitrile plasma extracts of arterial blood from rat A, taken 60 min post injection showing a single peak, corresponding to unmetabolised [^{18}F]-FDEA-RGD **[^{18}F]-13**.

Rat B

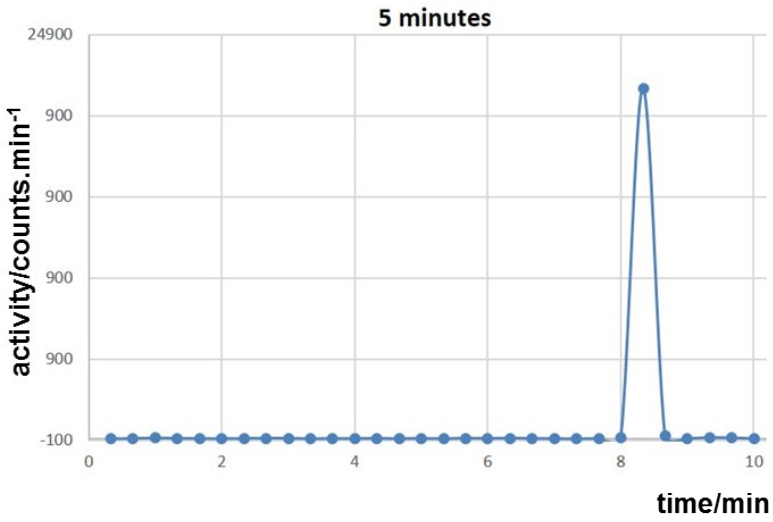

Fig. S17. Reconstructed radiochromatogram of HPLC fractions (30 fractions collected over 10 minutes) of acetonitrile plasma extracts of arterial blood from rat B, taken 5 min post injection showing a single peak, corresponding to unmetabolised [^{18}F]-FDEA-RGD **[^{18}F]-13**.

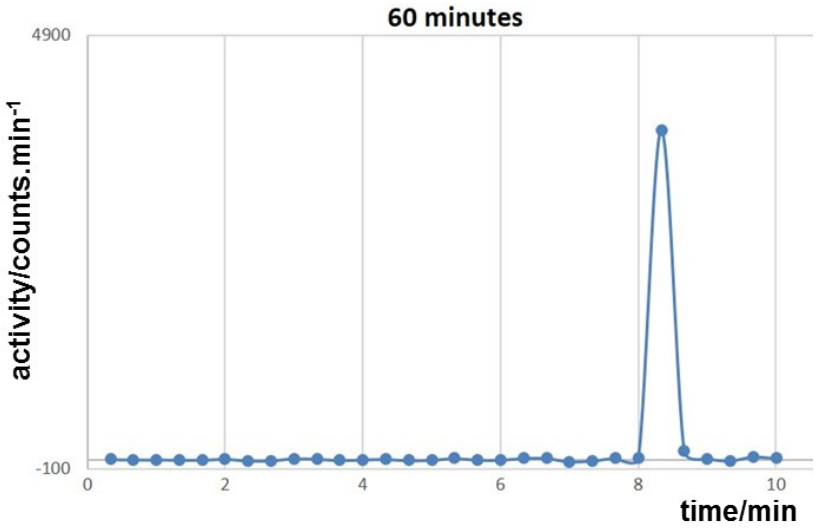

Fig. S18. Reconstructed radiochromatogram of HPLC fractions (30 fractions collected over 10 minutes) of acetonitrile plasma extracts of arterial blood from rat A, taken 60 min post injection showing a single peak, corresponding to unmetabolised [^{18}F]-FDEA-RGD **[^{18}F]-13**.

Biodistribution of [^{18}F]FDEA-RGD [^{18}F]-13

At 60 minutes post [^{18}F]FDEA-RGD **[^{18}F]-13** administration, each rat was exsanguinated under terminal anaesthesia (isofluorane). The tissues of interest were removed, rinsed, weighed and counted for radioactivity concentration using a gamma counter. The following tissues were collected: thymus, lung, heart, bladder content, bladder wall, fat, testis, adrenal, kidney, spleen, liver, muscle, stomach, small intestine content, small intestine wall, large intestine content, large intestine wall, red bone marrow, bone surface and brain.

Blood, plasma and tissue radioactive counts were decay corrected to the time of radioligand injection (biodistribution data) for each rat and expressed as the percentage dose per gram of wet tissue (%ID/g) and standardised uptake values (SUV). The resultant mean tissue radioactivity distribution is shown in **Fig. S19**.

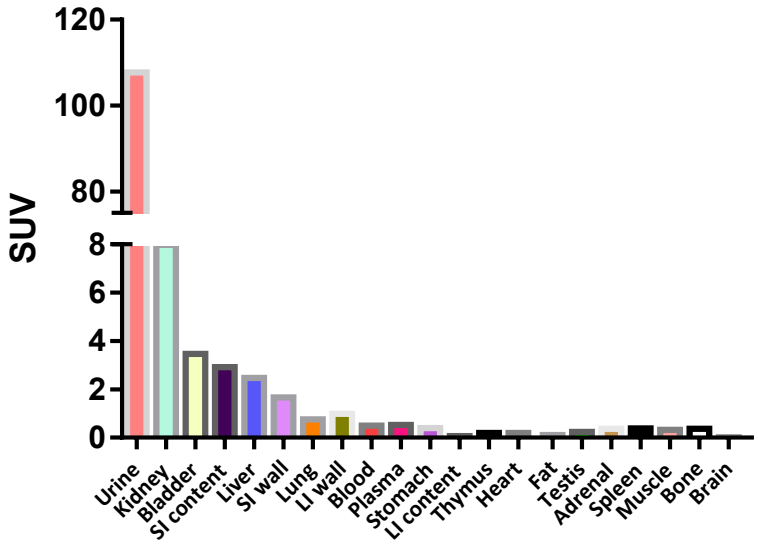

Fig. S19. The mean biodistribution of radioactivity in the tissues of healthy rats (n=2) at 60 minutes post intravenous administration of [^{18}F]FDEA-RGD **[^{18}F]-13**. The distribution is consistent with that previously seen for other $\alpha_v\beta_3$-targeting compounds.

Binding affinity of 5′-FDEA-RGD 13 to $\alpha_V\beta_3$ integrin

Binding affinity of cold bioconjugates to purified $\alpha_V\beta_3$ integrin was determined by ELISA as described previously.[3]

References

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3. M. Piras, I. Fleming,W. Harrison, M. Zanda, *Synlett* 2012, 2899–2902.