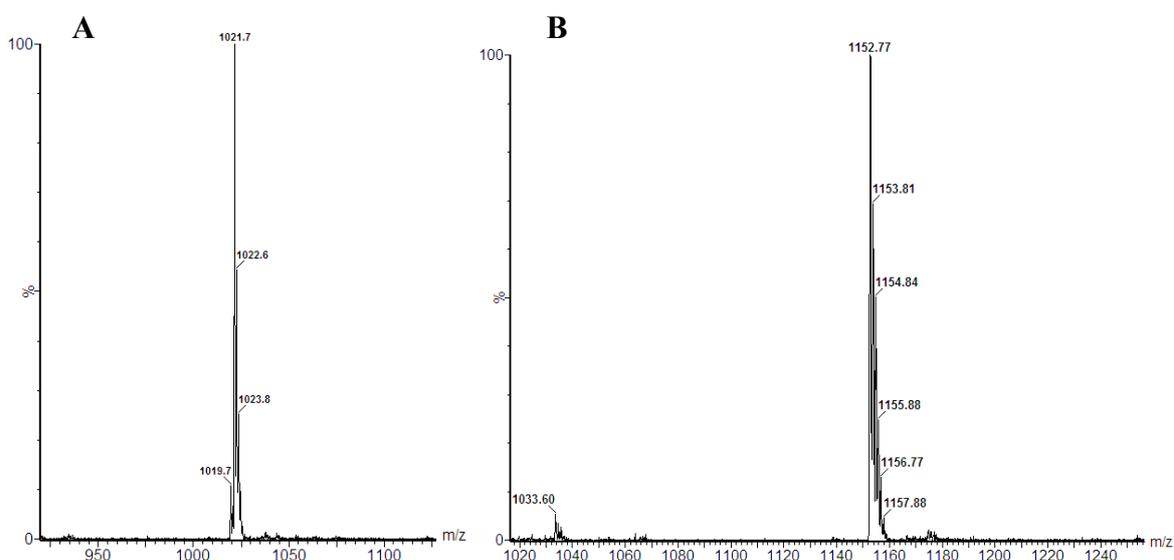


Supplementary Information

A dual optical and nuclear imaging reagent for peptide labelling *via* disulfide bridging

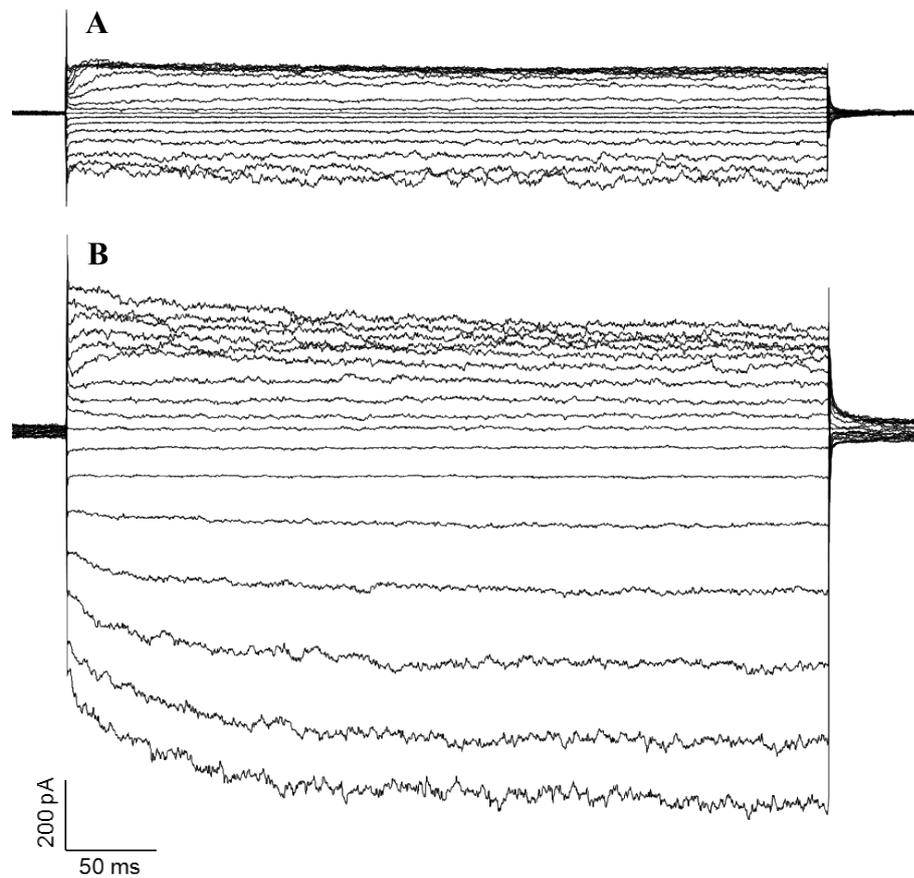
Sally A. Fletcher, Pak Kwan Brian Sin, Muriel Nobles, Erik Årstad, Andrew Tinker,
James R. Baker

Supplementary Figure 1: ESI-mass spectroscopy of reduced octreotide and modified octreotide 2.



ESI mass spectrum of **A**) peak at m/z 1021.7 corresponding to reduced octreotide (expected mass = m/z 1021.3) and **B**) peak at m/z 1152.8 corresponding to alkyne modified octreotide **2** (expected mass = m/z 1151.55).

Supplementary Figure 2: Typical current recordings from GIRK1/2a channel at rest and after activation.



Current recordings of **A**) the resting GIRK1/2a channel and **B**) GIRK1/2a channel activation by octreotide (100 nM). Current was recorded over 20 mV voltage steps, over a range of -120 to +40 mV from a holding potential of -50 mV.

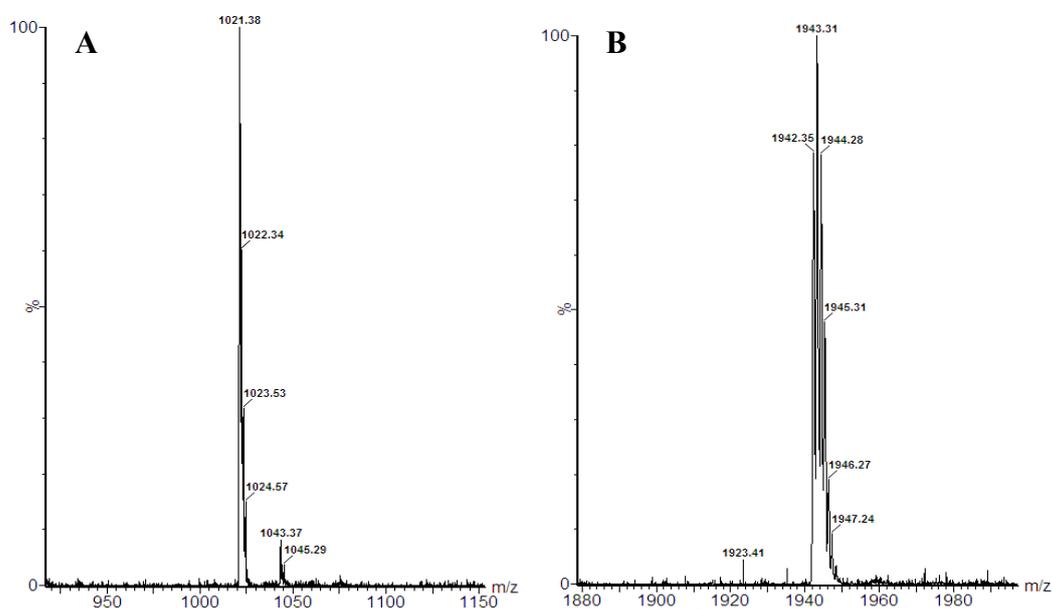
Supplementary Table 1: Data for the dose-response curve of native octreotide (0-100 nM)

| <i>Concentration (nM)</i> | <i>Log Concentration</i> | <i>Absolute Current Activation (pA/pF)</i> | <i>No. of experiments</i> | <i>Standard Error of the Mean (pA/pF)</i> |
|---------------------------|--------------------------|--|---------------------------|---|
| 0 | -6.00 | -12.237 | 7 | 1.372 |
| 0.01 | -5.00 | -15.730 | 4 | 1.129 |
| 0.10 | -4.00 | -24.606 | 7 | 1.203 |
| 1.00 | -3.00 | -41.777 | 5 | 4.063 |
| 10.0 | -2.00 | -59.441 | 4 | 5.164 |
| 100 | -1.00 | -62.824 | 4 | 4.429 |

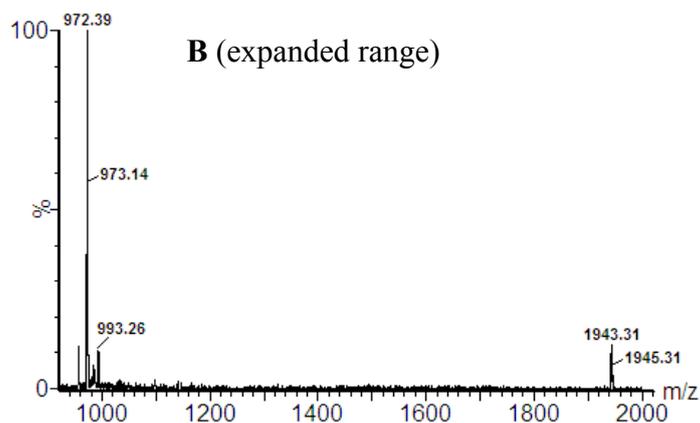
Supplementary Table 2: Data for the dose-response curve of modified octreotide 2 (0-100 nM)

| <i>Concentration (nM)</i> | <i>Log Concentration</i> | <i>Absolute Current Activation (pA/pF)</i> | <i>No. of experiments</i> | <i>Standard Error of the Mean (pA/pF)</i> |
|---------------------------|--------------------------|--|---------------------------|---|
| 0 | -6.00 | -17.303 | 7 | 3.030 |
| 1.00 | -3.00 | -18.079 | 5 | 4.436 |
| 10.00 | -2.00 | -24.193 | 6 | 3.506 |
| 100.00 | -1.00 | -40.784 | 8 | 3.582 |
| 1 x10 ³ | 0 | -62.323 | 6 | 5.454 |
| 1 x10 ⁴ | 1.00 | -65.167 | 5 | 5.934 |

Supplementary Figure 3: ESI-mass spectroscopy of reduced octreotide and octreotide MOMIA.



ESI mass spectrum of **A**) peak at m/z 1021.4 corresponding to reduced octreotide (expected mass = m/z 1021.3) and **B**) peak at m/z 1943.31 corresponding to modified octreotide and (expected mass = m/z 1942.51). Note: see below for expanded range of spectrum **B**, confirming the absence of remaining starting material; peak at 972 is the M^{2+} peak.



Supplementary Methods

General Experimental

Synthetic reactions were all carried out at room temperature and under an inert atmosphere unless otherwise stated. All commercially available reagents were used as received without further purification. Octreotide was obtained from LKT Laboratories.

^1H and ^{13}C NMR spectra were recorded on either a Bruker Avance-500 machine or a Bruker Avance-600 machine (as stated), ran at a frequency of 500 MHz and 600 MHz respectively for ^1H spectra and 125 MHz and 150 MHz respectively for ^{13}C spectra. Deuterated solvents used were obtained from Sigma Aldrich. Peaks are assigned as singlet (s), doublet (d), triplet (t) or multiplet (m) and are sharp peaks unless denoted as broad (br). Chemical shifts are recorded in parts per million (ppm) denoted by δ . Proton coupling constants (J values) are reported in Hertz (Hz). Where necessary, assignments were confirmed with the aid of DEPT spectra.

Mass spectra were recorded on a VG70-SE mass spectrometer running in EI or CI mode.

Infra-red (IR) spectra were recorded on a PerkinElmer Spectrum 100 FT-IR spectrometer operating in ATR mode.

LC-MS measurements were taken on an Acquity Ultra Performance LC instrument. LC data was recorded at a wavelength of 280 nm and MS data obtained in ES^+ and ES^- mode with a detection range between m/z 90 – 2000. LC solvent solutions: A - H_2O (0.1% TFA); B – MeCN (0.1% TFA); running conditions: Gradient 5-95% B in 5 min; injection volume: 10 μL . Masses are assigned as a mass to charge ratio (m/z).

Octreotide was obtained from LKT Laboratories. Lyophilised octreotide was resolubilised in buffer (2 mL, 50 mM NaHPO_4^- , pH 6.2, 40% MeCN, 2.5 % DMF) and stored at 4 $^\circ\text{C}$ until use.

Peptide concentrations were determined using a nanodrop ND-1000 spectrophotometer.

‘Peptide buffer’ employed in experiments consisted of pH 6.2 phosphate buffer (50 mM) with 40 % MeCN and 2.5 % DMF.

For peptide purification by RP-HPLC, a Shimadzu LC-10AT instrument fitted with a C_{18} column, 150 x 4.60 mm (Phenomenex) was used for all purification procedures.

The mobile phases were water, 0.1% TFA (solvent A) and acetonitrile, 0.1 % TFA (solvent B). Flow rate of 1 mL/min. Species eluting from the column was monitored by measuring the absorbance at 280 nm. A fraction collector was used to collect the desired peaks (1 mL fractions) and identical fractions were pooled before being lyophilised using a Scanvac vacuumed centrifuge (Labmode). The residual peptide was reconstituted in distilled water (RP-HPLC-grade, Fisher Chemicals).

RP-HPLC purification method:

| <i>Time (min)</i> | <i>Solvent B (%)</i> |
|-------------------|----------------------|
| 1.0 | 0 |
| 15.0 | 60 |
| 19.0 | 60 |
| 22.0 | 90 |
| 24.50 | 0 |
| 30.0 | 0 |

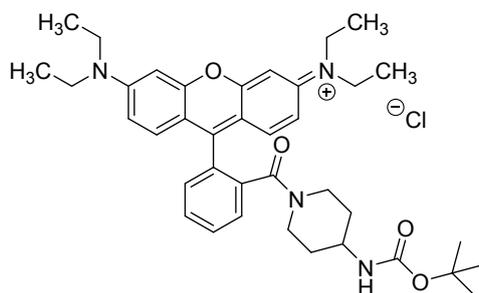
Protocol for the modification of octreotide

Octreotide (140 μ L, 643 μ M) was diluted to a concentration of 300 μ M using peptide buffer (160 μ L). TCEP (5.93 mg) was prepared at a concentration of 30 mM using peptide buffer (690 μ L) before 1.2 equivalents (3.60 μ L, 0.108 μ mol) of this was added to the octreotide solution and mixed. The peptide was left to reduce for 1 h before an aliquot (30 μ L) was removed and analysed by LC-MS to ensure complete disulfide reduction. A solution of the desired maleimide reagent was prepared at a concentration of 30 mM using DMF. 1.2 equivalents (3.24 μ L, 0.097 μ mol) of the maleimide solution were added to the reduced octreotide and mixed. After 10 min, an aliquot (30 μ L) of this reaction mixture was removed and analysed by LC-MS to ensure full conversion to the bridged product.

For modification of octreotide with maleimide **1**, LC retention time of the bridged product **2** (m/z 1152.8) was 1.10 min.

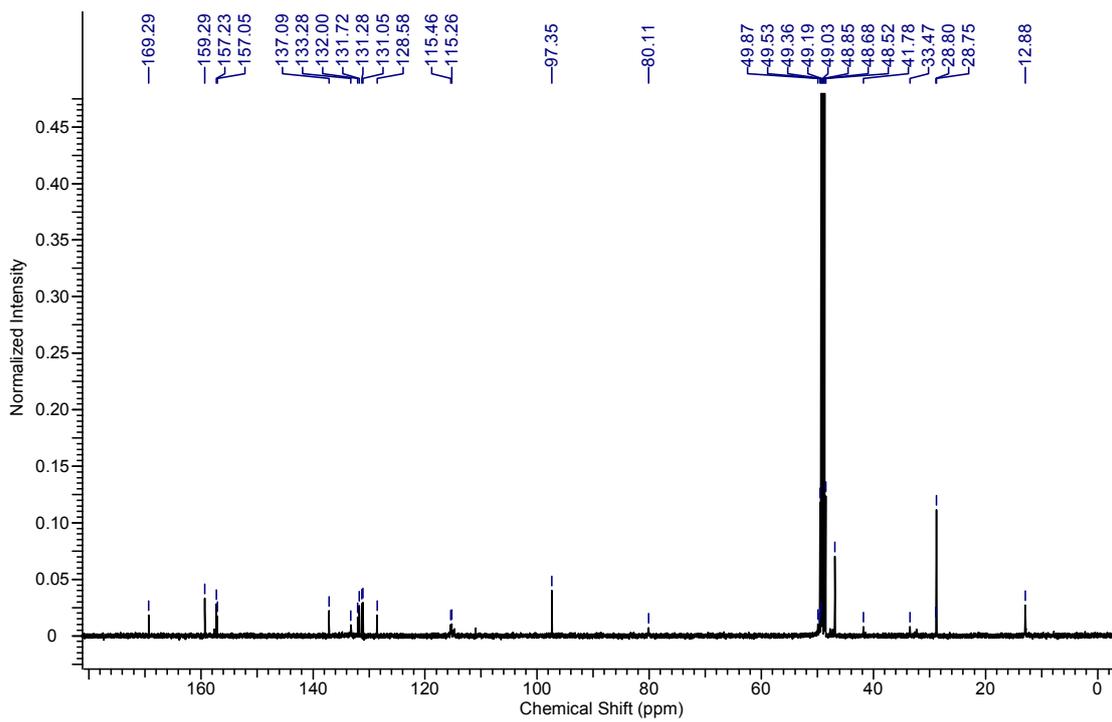
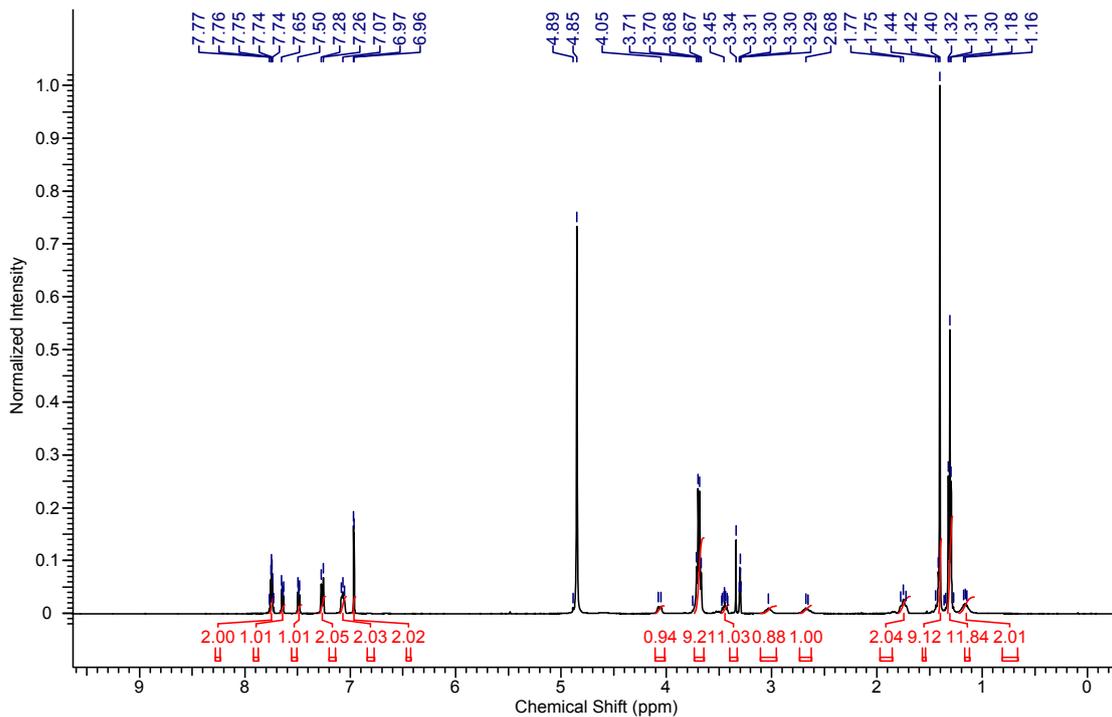
For modification of octreotide with maleimide **4**, LC retention time of the bridged product (m/z 1943.31) was 1.75 min.

***N*-(9-(2-(4-((*tert*-butoxycarbonyl)amino)piperidine-1-carbonyl)phenyl)-6-(diethylamino)-3*H*-xanthen-3-ylidene)-*N*-ethylethanaminium chloride (**5**)¹**

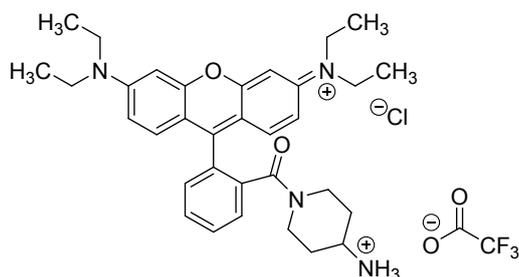


Rhodamine B (427 mg, 0.891 mmol) was dissolved in DCM (30 mL), oxalyl chloride (2.5 mL) was added and the reaction mixture stirred for 2 h. The solvent and excess oxalyl chloride was removed *in vacuo*. The purple residue was re-dissolved in DCM (15 mL) before being slowly added to a solution of Cs₂CO₃ (1.50 g, 4.56 mmol) and 4-(*N*-Boc-amino)-piperidine (913 mg, 4.56 mmol) in DCM (15 mL). The mixture was stirred for 18 h before the solvent was removed *in vacuo* to yield the crude product as a purple solid. This was purified by flash chromatography on silica gel (DCM : MeOH, gradient elution from 98 : 2 to 90 : 10) to afford the product **5** as a dark purple solid (331 mg, 59%).

IR ν_{\max} (oil): 2977 (w), 2910 (w), 1702 (m), 1584 (m) cm⁻¹; ¹H NMR (500 MHz, MeOD): δ = 7.77-7.74 (m, 2H), 7.64 (dd, 1H, *J* = 6.0, 3.0 Hz), 7.49 (dd, 1H, *J* = 6.0, 2.5 Hz), 7.27 (d, 2H, *J* = 9.5 Hz), 7.08-7.07 (m, 2H), 6.97 (d, 2H, *J* = 2.5 Hz), 4.08-4.05 (m, 1H), 3.71-3.67 (m, 9H), 3.45-3.42 (m, 1H), 3.03 (bs, 1H), 2.68-2.65 (m, 1H) 1.77-1.73 (m, 2H), 1.40 (s, 9H), 1.31 (t, 12H, *J* = 7.5 Hz), 1.18-1.15 (m, 2H); ¹³C NMR (125 MHz, MeOD): δ = 169.3 (C), 159.3 (C), 157.6 (C), 157.2 (C), 157.1 (C), 137.1 (C), 133.3 (CH), 132.0 (C), 131.7 (CH), 131.3 (CH), 131.0 (CH), 128.6 (CH), 115.5 (CH), 115.3 (C), 97.4 (CH), 80.1 (C), 48.9 (CH), 48.5 (CH₂), 46.9 (CH₂), 41.8 (CH₂), 33.5 (CH₂), 32.3 (CH₂), 28.8 (CH₃), 12.9 (CH₃); MS (EI+) *m/z*: 625 (100%, M⁺), 372 (30%); Mass calculated for [C₃₈H₄₉N₄O₄]: 625.3754; Found: 625.3761.

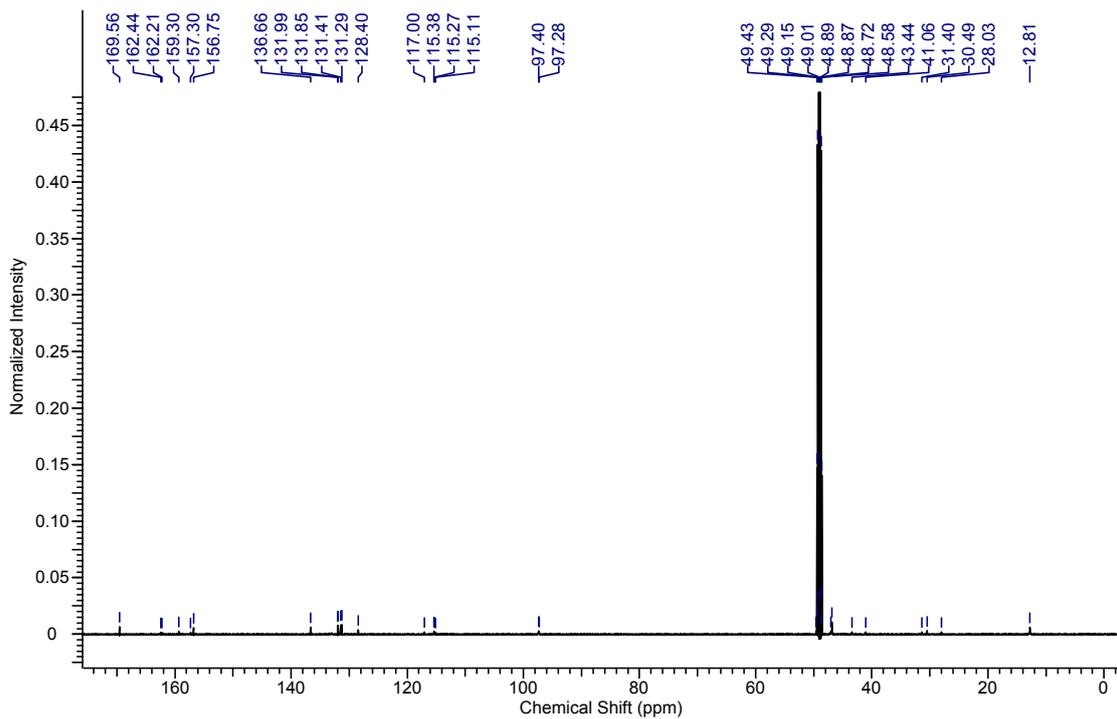
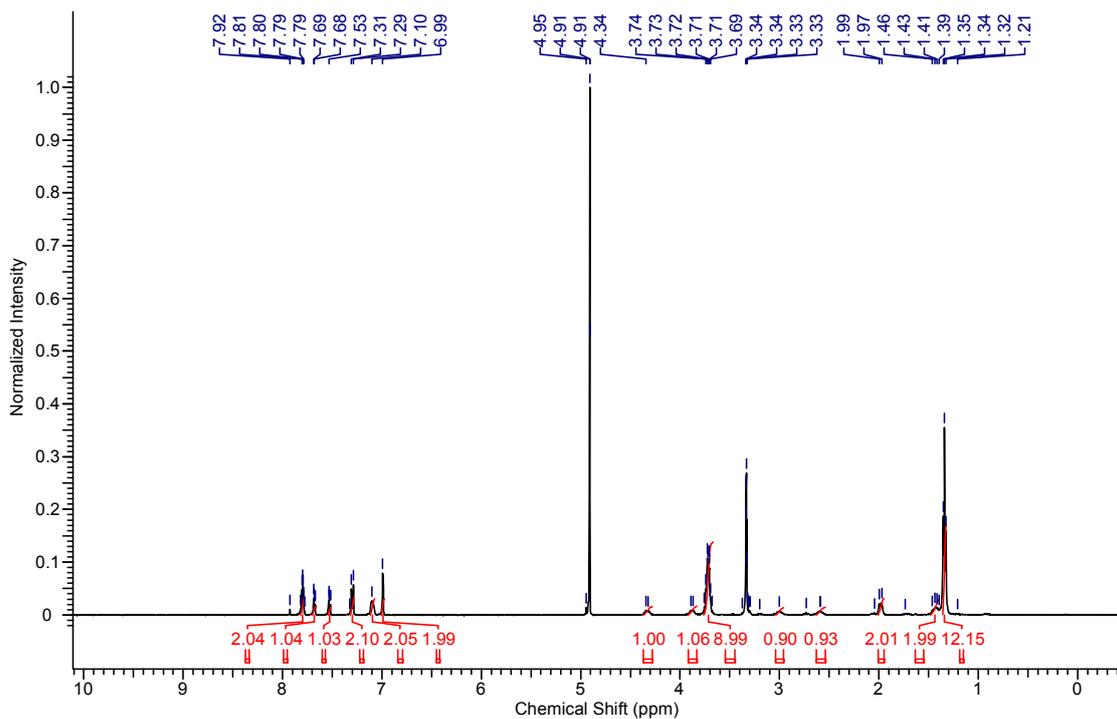


1-(2-(6-(diethylamino)-3-(diethyliminio)-3*H*-xanthen-9-yl)benzoyl)piperidin-4-aminium 2,2,2-trifluoroacetate chloride (6)¹

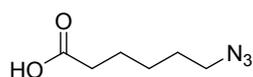


TFA (15 mL) was added to a stirred solution of Boc-protected rhodamine **5** (257 mg, 0.410 mmol) in DCM (15 mL) and the solution stirred for 4 h. The solvent was then removed azeotropically using toluene (3 x 15 mL) followed by chloroform (4 x 10 mL) to yield the product **6** as a purple solid (402 mg, quantitative yield).

m.p. 231-234 °C; ¹H NMR (500 MHz, MeOD): δ = 7.81-7.79 (m, 2H), 7.69-7.68 (m, 1H), 7.53-7.52 (m, 1H), 7.30 (d, 2H, *J* = 9.5 Hz), 7.15-7.05 (m, 2H), 7.0-6.96 (m, 2H), 4.34-4.31 (m, 1H), 3.90-3.84 (m, 1H), 3.74-3.69 (m, 9H), 3.05-2.95 (m, 1H), 2.60-2.55 (m, 1H), 1.99-1.97 (m, 2H), 1.46-1.39 (m, 2H), 1.34 (t, 12H, *J* = 7.5); ¹³C NMR (125 MHz, MeOD): δ = 169.6 (C), 162.4 (C), 159.3 (C), 157.3 (C), 156.8 (C), 136.7 (C), 133.4 (CH), 132.0 (C), 131.9 (CH), 131.4 (CH), 131.3 (CH), 128.4 (CH), 117.0 (C), 115.4 (CH), 115.3 (CH), 115.1 (C), 97.4 (CH), 46.9 (CH₂), 31.4 (CH₂), 30.5 (CH₂), 12.8 (CH₃); MS (EI⁺) *m/z*: 525 (100%, M⁺), 338 (10%); Mass calculated for [C₃₈H₄₁N₄O₂]: 525.3230; Found: 525.3238.

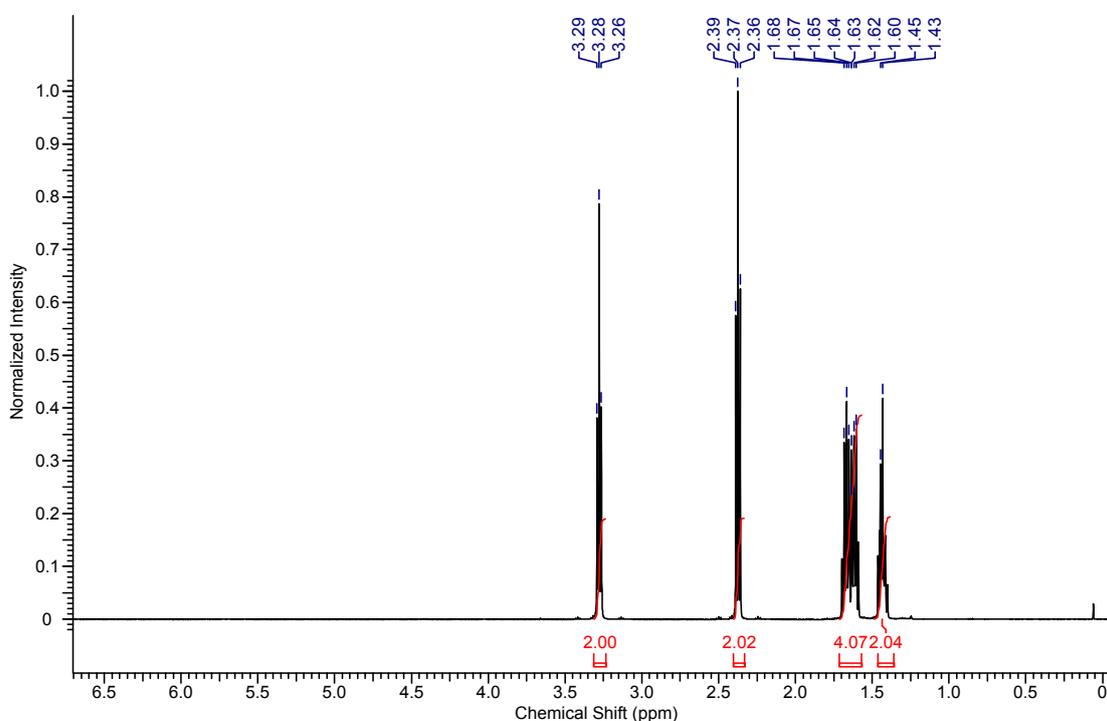


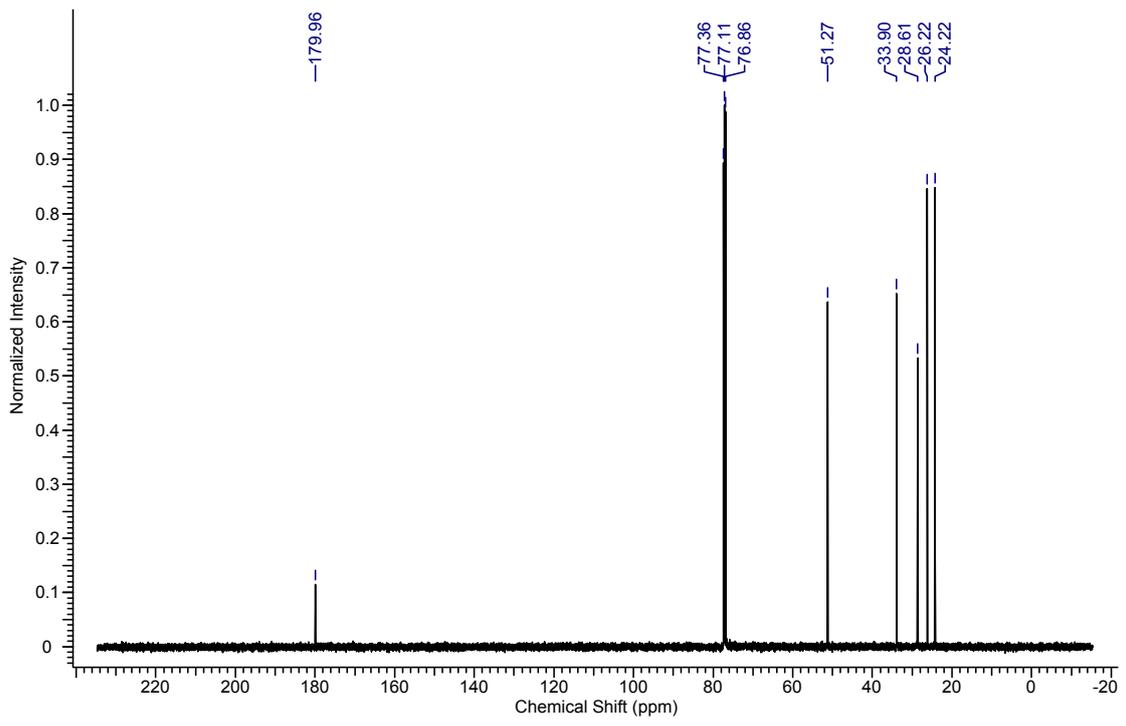
6-Azidohexanoic acid (**7**)²



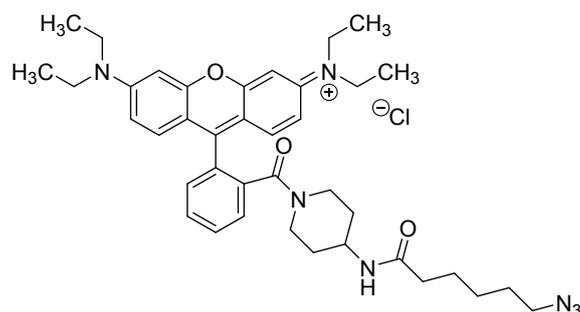
A mixture of 6-bromohexanoic acid (402 mg, 2.06 mmol) and sodium azide (668 mg, 10.3 mmol) in DMF (1.5 mL) was stirred at 50°C for 3 h. The mixture was cooled to room temperature and DCM (30 mL) added. The organic phase was washed with water (2 x 30 mL), saturated aqueous LiCl (5 x 30 mL) and brine (2 x 30 mL), dried (MgSO₄) and the solvent removed *in vacuo*. The crude product was purified by flash chromatography on silica gel (petroleum ether : ethyl acetate, gradient elution from 8 : 2 to 6 : 4) to afford the product **7** as a colourless liquid (124 mg, 39 %).

IR ν_{max} (oil): 3200 (m), 2925 (m), 2095 (m), 1702 (s)cm⁻¹; ¹H NMR (500 MHz, CDCl₃): δ = 3.28 (t, 2H, J = 7.0 Hz), 2.37 (t, 2H, J = 7.5 Hz), 1.68-1.60 (m, 4H), 1.45-1.43 (m, 2H); ¹³C NMR (125 MHz, CDCl₃): δ = 180.0 (C), 51.3 (CH₂), 33.9 (CH₂), 28.6 (CH₂), 26.2 (CH₂), 24.2 (CH₂); MS (EI⁺) as sodium adduct m/z : 180 (100%, M+Na⁺); Mass calculated for [C₆H₁₁N₃O₂Na]: 180.0749; Found: 180.0733.



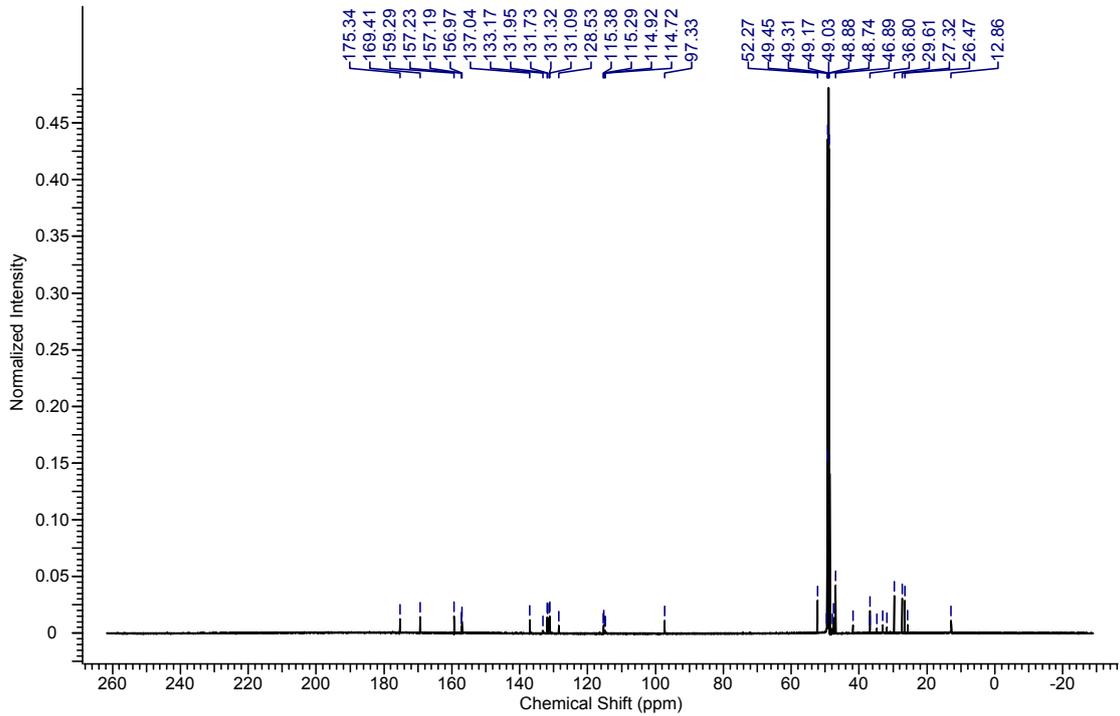
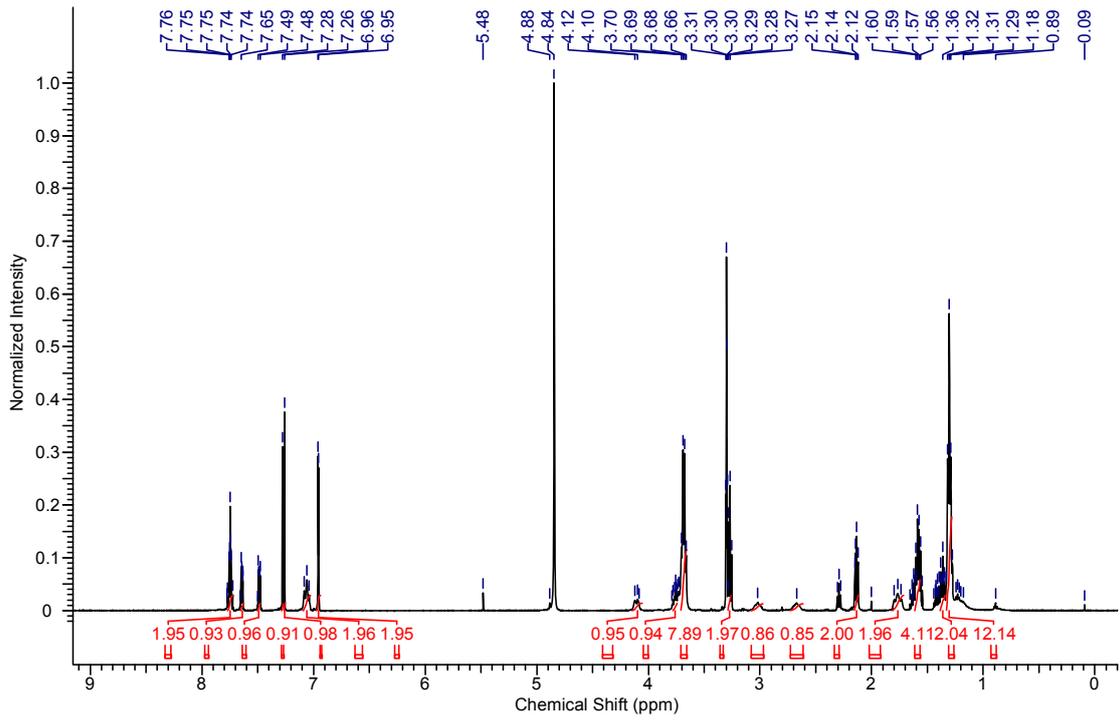


***N*-(9-(2-(4-(6-azidohexanamido)piperidine-1-carbonyl)phenyl)-6-(diethylamino)-3*H*-xanthen-3-ylidene)-*N*-ethylethanaminium chloride (3)**

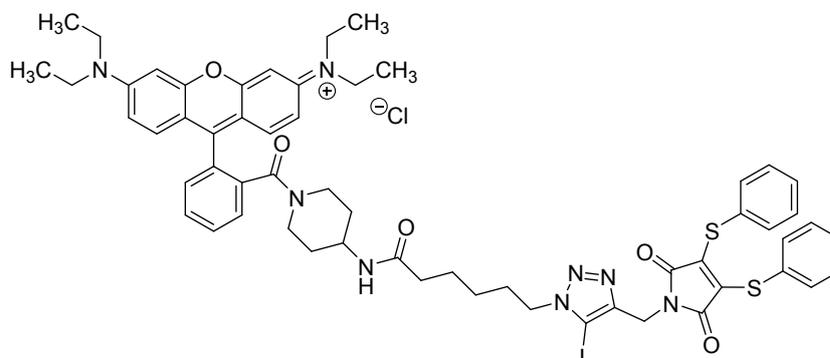


To a solution of 6-azidohexanoic acid **7** (29.5 mg, 0.189 mmol), HBTU (71.7 mg, 0.189 mmol), and HOBT (25.5 mg, 0.189 mmol) in DMF (2 mL) was added DIPEA (99.0 μ L, 0.576 mmol) in DMF (2 mL). The reaction mixture was stirred for 20 min before a solution of rhodamine B-amine **6** (99.3 mg, 0.189 mmol) in DMF (2 mL) was added drop-wise and the mixture stirred for 3 h. The solvent was then removed *in vacuo* and the crude product re-dissolved in DCM (10 mL) before being washed with saturated aqueous LiCl (3 x 10 mL), 15 % aqueous K_2CO_3 (2 x 10 mL), 15 % aqueous citric acid (2 x 10 mL) and water (2 x 10 mL). The resulting product was dried ($MgSO_4$) and the remaining solvent removed *in vacuo* before being purified by flash chromatography on silica gel (DCM : MeOH, gradient elution from 100 : 0 to 96 : 4) to yield the product **3** as a purple solid (96.9 mg, 77 % yield).

IR ν_{max} (solid): 2963 (w), 2928 (w), 2875 (w), 2090 (s) 1582 (m) cm^{-1} ; 1H NMR (500 MHz, MeOD): δ = 7.76-7.74 (m, 2H), 7.65-7.64 (m, 1H), 7.49-7.48 (m, 1H), 7.27 (d, 2H, J = 9.5 Hz), 7.08-7.05 (m, 2H), 6.96-6.95 (d, 2H, J = 2.5 Hz), 4.12-4.10 (m, 1H), 3.76-3.75 (m, 2H), 3.70-3.68 (q, 8H, J = 7.5 Hz), 3.31 (t, 2H, J = 7.0 Hz), 3.04-3.02 (m, 1H), 2.69-2.65 (m, 1H), 2.15-2.12 (m, 2H), 1.80-1.70 (m, 2H), 1.62-1.56 (m, 4H), 1.41-1.39 (m, 2H), 1.38-1.36 (m, 1H), 1.31 (t, 12H, J = 6.5 Hz), 1.29-1.22 (m, 1H); ^{13}C NMR (125 MHz, MeOD): δ = 175.3 (C), 169.4 (C), 159.3 (C), 157.2 (C), 157.0 (C), 137.0 (C), 133.2 (CH), 132.0 (C), 131.7 (CH), 131.3 (CH), 131.1 (CH), 128.5 (CH), 115.4 (CH), 114.8 (C), 97.3 (CH), 52.3 (CH_2), 47.6 (CH), 46.9 (CH_2), 41.8 (CH_2), 36.8 (CH_2), 33.0 (CH_2), 31.9 (CH_2), 29.6 (CH_2), 27.3 (CH_2), 26.5 (CH_2), 25.7 (CH_2), 12.9 (CH_3); MS (EI+) m/z : 664 (100%, M^+); Mass calculated for $[C_{39}H_{50}N_7O_3]$: 664.3975; Found: 664.3950.



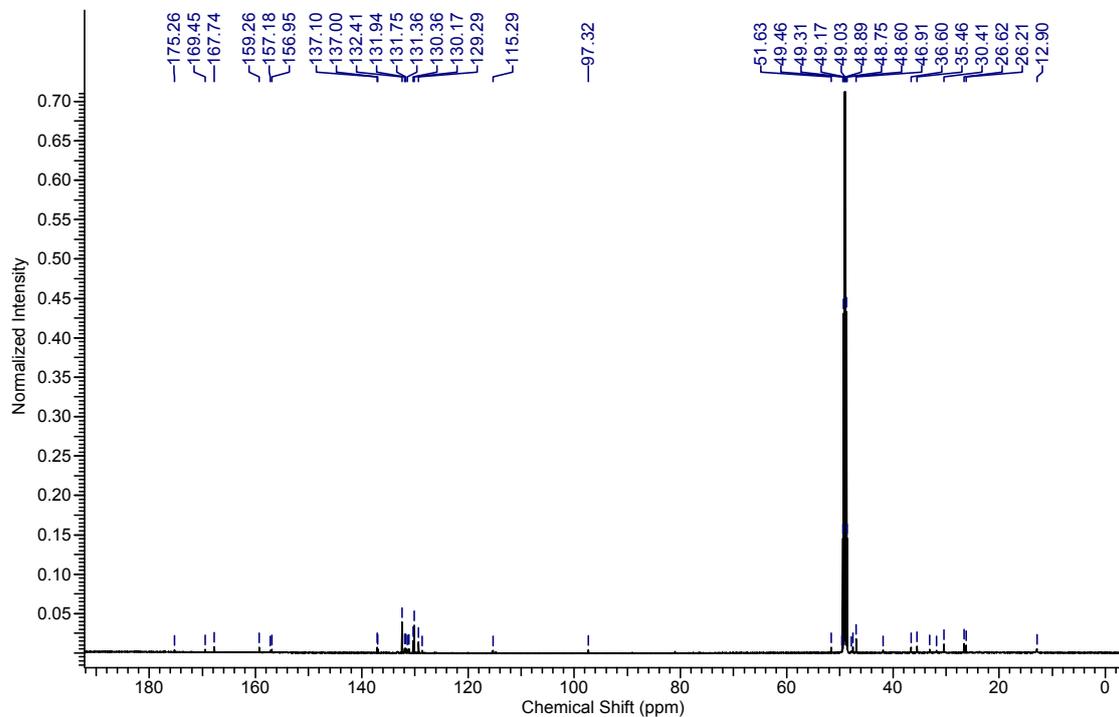
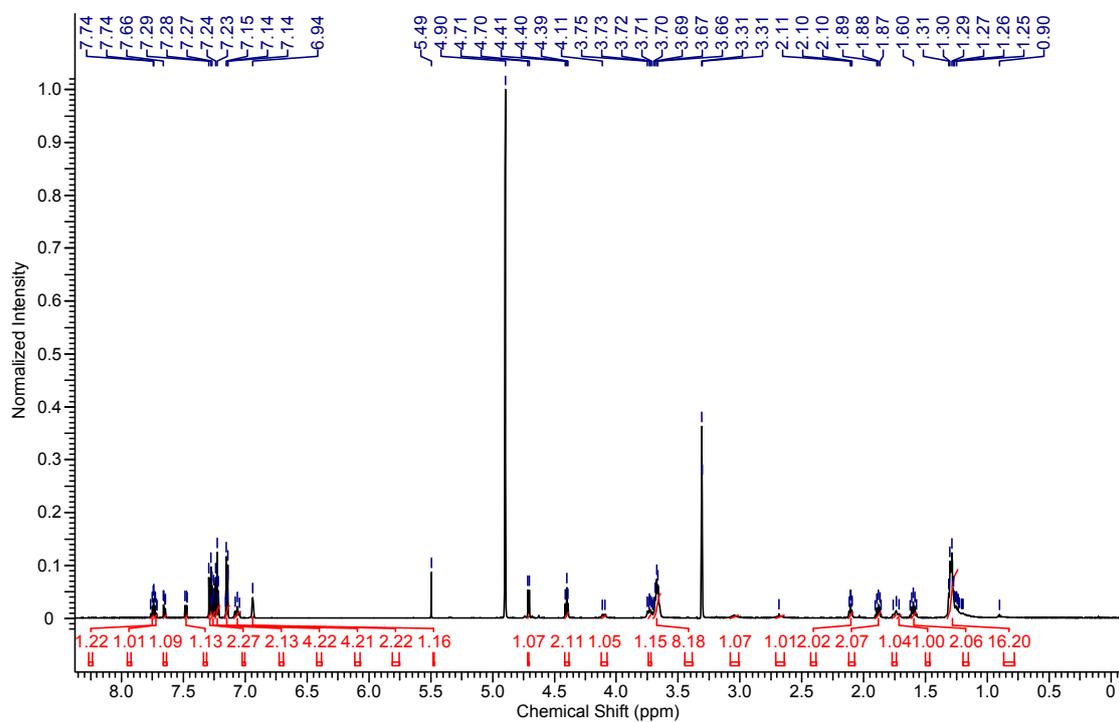
***N*-(6-(diethylamino)-9-(2-(4-(6-(4-((2,5-dioxo-3,4-bis(phenylthio)-2,5-dihydro-1*H*-pyrrol-1-yl)methyl)-5-iodo-1*H*-1,2,3-triazol-1-yl)hexanamido)piperidine-1-carbonyl) phenyl)-3*H*-xanthen-3-ylidene)-*N*-ethylethanaminium (4)³**



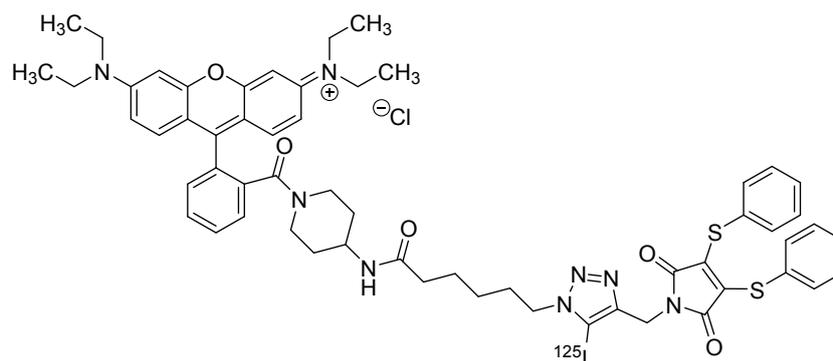
To a solution of copper (I) iodide (8.00 mg, 0.0420 mmol) in dry acetonitrile (5 mL) was added *N*-alkyne dithiophenolmaleimide **1⁴** (13.4 mg, 0.0380 mmol), rhodamine-azide **3** (27.9 mg, 0.0420 mmol), triethylamine (5.3 μ L, 0.0380 mmol) and *N*-chlorosuccinimide (6.10 mg, 0.0460 mmol) and the reaction mixture stirred overnight. The copper was removed by gravity filtration and the filtrate concentrated *in vacuo*. The crude product was purified by flash chromatography on silica gel (DCM : MeOH, gradient elution from 100 : 0 to 95 : 5) to afford the product **4** as a purple solid (15.9 mg, 37 % yield).

IR ν_{\max} (solid): 2969 (m), 2904 (m), 1709 (m), 1583 (m) cm^{-1} ; ¹H NMR (600 MHz, MeOD): δ = 7.74 (td, 1H, J = 7.6 Hz, 1.6), 7.73 (td, 1H, J = 7.6, 1.6 Hz), 7.66-7.64 (m, 1H), 7.50-7.48 (m, 1H), 7.28 (d, 2H, J = 9.6 Hz), 7.28-7.26 (m, 2H), 7.24-7.23 (m, 4H), 7.15-7.14 (m, 4H), 7.10-7.07 (m, 2H), 6.94 (d, 1H, J = 2.0 Hz), 6.93 (d, 1H, J = 2.0 Hz), 4.71 (d, 1H, J = 15.7 Hz), 4.70 (d, 1H, J = 15.7 Hz), 4.40 (t, 2H, J = 6.8 Hz), 4.11-4.09 (m, 1H), 3.75-3.73 (m, 1H), 3.72-3.71 (m, 1H), 3.67 (bq, 8H, J = 7.0 Hz), 3.08-3.02 (m, 1H), 2.68-2.64 (m, 1H), 2.11 (td, 2H, J = 7.1, 2.0 Hz), 1.89-1.87 (m, 2H), 1.76 (d, 1H, J = 12.5 Hz), 1.75 (d, 1H, J = 12.5 Hz), 1.60-1.58 (m, 2H), 1.30 (t, 12H, J = 7.0 Hz), 1.29-1.27 (m, 1H), 1.27-1.25 (m, 2H), 1.25-1.20 (m, 1H); ¹³C NMR (125 MHz, MeOD): δ = 175.3 (C), 169.5 (C), 167.7 (C), 159.3 (C), 157.2 (C), 157.0 (C), 147.2 (C), 137.1 (C), 137.0 (C), 133.3 (CH), 132.4 (CH), 131.9 (C), 131.8 (CH), 131.4 (CH), 131.1 (CH), 130.4 (C), 130.2 (CH), 129.3 (CH), 128.6 (CH), 115.4 (CH), 114.8 (C), 97.3 (CH), 81.1 (C), 51.6 (CH₂), 47.8 (CH₂), 47.6 (CH), 46.9 (CH₂), 41.9 (CH₂), 36.6 (CH₂), 35.5 (CH₂), 33.1 (CH₂), 31.8 (CH₂), 30.4 (CH₂), 26.6

(CH₂), 26.2 (CH₂), 12.9 (CH₃); MS (EI+) *m/z*: 1141 (100%, M⁺), 285 (20%); Mass calculated for [C₅₈H₆₂IN₈O₅S₂]: 1141.33; Found: 1141.3103.



Synthesis of rhodamine B-¹²⁵I]-dithiophenol maleimide ([¹²⁵I]4)³



Radio-HPLC analysis was performed with an Agilent 1200 HPLC system equipped with a GABI Star NaI(Tl) scintillation detector and a Knauer Smartline fixed wavelength 254 nm UV-detector. A ZORBAX column (300SB-C18, 9.4 x 250 mm) was used. Reductant free [¹²⁵I]NaI was purchased from Perkin Elmer with a concentration of 370 mCi/mL (13.69 GBq/mL) and specific activity of 629 GBq/mg in 10⁻⁵ M NaOH (pH 8-11) aqueous solution.

To copper(II) chloride (13.4 mg, 0.100 mmol) was added anhydrous acetonitrile (4 mL) followed by anhydrous triethylamine (20.9 μL, 0.150 mmol), and the mixture was mixed by vortex.

To the *N*-alkyne dithiophenolmaleimide **1**⁴ (0.35 mg, 1.0 μmol) was added the above solution of the CuCl₂/triethylamine complex in acetonitrile (40 μL) and the mixture vortexed. After 5 min, Na¹²⁵I in water (6.00 μL, 1.438 MBq) was added to the mixture. Azide **3** (6.64 mg, 10.0 μmol) was dissolved in MeCN (200 μL) before 20 μL (1.00 μmol) was added to the reaction mixture. The tube was capped and the reaction was heated at 60 °C for 90 min. The reaction was quenched with acetonitrile and water (1:1, 400 μL) and the resulting solution was purified by HPLC (Buffer A: water + 0.1 % TFA, Buffer B: methanol + 0.1 % TFA, gradient elution from 60-90 % Buffer B over 30 min, flow rate 3 mL/min). The labelled compound [¹²⁵I]**4** was identified by co-injection and co-elution with the non-radioactive reference compound **4**. The isolated RCY of [¹²⁵I]**4** was 47%.

Cell Culture for Patch Clamp Experiments

Cell-culture methods and the generation of stable cell lines were carried out as described.⁵ HEK293 (Human Embryonic Kidney cell line) stably expressing Kir3.1 and Kir3.2A channels were maintained in minimum essential medium (MEM) supplemented with 10% foetal calf serum and G-418 (800 µg/mL) (Invitrogen), at 37 °C in a humidified atmosphere (95% O₂, 5% CO₂).

The BON-1 cell line is an immortalised human cell line derived from a metastatic carcinoid tumour of the pancreas and was kindly donated by Professor Meyer (UCL). The cell line was maintained in MEM : Ham's F-12 K Nutrient Mixture (F-12K) (1 : 1) supplemented with 10% foetal calf serum and 1% penicillin/streptomycin (Life Technologies).

The Phosphate Buffered Saline (PBS) and 0.25% trypsin solutions used were obtained from Gibco Life Technologies.

When cells were required for patch clamp experiments they were passaged directly onto glass coverslips (10 mm diameter, borosilicate glass, VWR International). The cells were incubated at 37 °C for 2 days before use.

Transfection of Cells

Cells were transiently co-transfected with 800 ng SSTR2 plasmid DNA (Missouri S&T cDNA Resource Center) along with 50 ng eGFP plasmid DNA (Clontech) for visualization of transfected cells using epifluorescence. Transfections were performed with 5 µl of Fugene HD (Roche).

Electrophysiology

Patch Clamp Equipment Setup

Whole cell patch-clamp current recordings were performed with an Axopatch 200B amplifier (Axon Instruments) using pipettes with a resistance of 3-4 M Ω pulled from filamented borosilicate glass capillaries (Harvard Apparatus, 1.5 mm OD x 1.17 mm ID). Data was acquired and analysed via a Digidata 1440 interface (Axon Instruments) and captured and analysed using pClamp software (version 10.0, Axon Instruments). Voltage commands were generated using pClamp10 software. A gravity driven system was used to apply octreotide and modified analogues. From a holding potential of -50 mV, 20 mV steps were applied from -120 mV to +40 mV. The extracellular solution was: KCl (20 mM), NaCl (120 mM), CaCl₂ (2 mM), MgCl₂ and HEPES (10 mM), pH7.4 while the intracellular solution was: KCl (130 mM), NaCl (10 mM), MgCl₂ (1 mM), MgATP (2 mM), EGTA (2 mM), GTP (0.3 mM) and HEPES (10 mM), pH7.4.

Activation of the GIRK current was achieved by sequential perfusion with 0.01, 0.1, 1, 10 and 100 nM octreotide or 1, 10, 100, 10³ and 10⁴ nM of bridged octreotide **2**.

Confocal Microscopy Imaging

General Information

For these experiments, HEK293 cells stably expressing the GIRK1/2a channel and BON-1 cells were used.

Tyrode's solution used washing was: NaCl (135 mM), KCl (5.4 mM), CaCl₂ (2 mM), MgCl₂ (1 mM), HEPES (5 mM) and glucose (10 mM), pH 7.4. The solution was stored at 4 °C and warmed to room temperature before use.

Microscopy Imaging

Live cells were imaged using a Zeiss LSM510 confocal microscope and a Plan-Apochromat 63x oil lens objective (1.4 numerical aperture). eGFP was visualized using a multi-line Argon laser (wavelength 488 nm) and filtered using a BP505-530 (band pass) emission filter. Rhodamine B was visualised using a helium/neon laser (wavelength 543 nm) and filtered using a BP560-615 emission filter. To avoid 'cross-talk' between eGFP and rhodamine B signals, images were acquired sequentially.

Images were taken at 1024×1024 frame size, a bit depth of 16 bit, and were averaged twice.

Cells were seeded and transfected with SSTR2 and eGFP onto Mattek petri dishes (Mattek Corporation). Cells were imaged in control conditions (in Tyrode's solution), and after incubation for 20 min at 37°C with octreotide-MOMIA (1 μM).

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