Electronic Supplementary Material (ESI) for Dalton Transactions.

Facile solid phase peptide synthesis with a Re-lysine conjugate generated via a one-pot procedure

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Supplementary Information

Experimental

Synthesis of Compound 2: Pyridine-2-carboxaldehyde (0.012 mL, 0.125 mmol) and FMOC-Lys-OH (46 mg, 0.125 mmol) were mixed and refluxed in methanol (15 mL) for an hour. Upon the change of the color of the reaction mixture from very pale yellow to a light brown, $Re(CO)_5Br$ (50 mg, 0.125 mmol) was added to the reaction mixture. The solution was further refluxed for 8 hours. After cooling the orange solution to room temperature, the solvent was removed and the orange solid was dried under vacuum for a few days. The resultant brown-orange solid was washed with ethyl ether and air-dried. Compound **2** was obtained in 79% yield (80 mg, 0.099 mmol).

^{1}H-NMR (500 MHz in DMSO-d_6): 9.21 (s, 1H, *H*C=N), 9.02 (m, 1H, H on py), 8.23-8.28 (m, 2H, H on py), 7.87-7.89 (m, 2H, H on py and N*H*), 7.31-7.76 (m, 8H, H on FMOC), 4.20 (m, 1H, H on FMOC), 4.26 (m, 2H, O-CH_2), 3.95 (m, 1H, *H*C-NH), 4.05 (m, 2H, CH_2-CH-NH), 1.87-2.01 (m, 2H, =N-CH_2), , 1.66-1.81 (m, 2H, =N-CH_2-CH_2), 1.30-1.45 (m, 2H, CH_2-CH_2-CH-NH)

^{13}C-NMR (125 MHz in DMSO-d_6): 197.7, 197.5, 187.5, 174.3, 169.9, 156.6, 155.3, 153.6, 144.3, 141.2, 129.6, 128.1, 127.5, 125.7, 120.6, 66.1, 64.4, 54.3, 47.1, 30.7, 29.3, 23.1 ppm

Synthesis of Compound 1: Compound **1** can be synthesized in an analogous way as **2** by using $Re(CO)_5Cl$ instead of $Re(CO)_5Br$.

^{1}H-NMR (500 MHz in DMSO-d_6): 9.23 (s, 1H, *H*C=N), 8.98 (m, 1H, H on py), 8.19-8.28 (m, 2H, H on py), 7.88 (m, 2H, H on py and N*H*), 7.60-7.76 (m, 4H, H on FMOC), 7.28-7.43 (m, 4H, H on FMOC), 4.25 (m, 2H, O-CH_2), 4.20 (m, 1H, *H*C-NH), 4.03 (m, 2H, CH_2-CH-NH), 3.95 (m, 1H, H on FMOC), 1.90-1.95 (m, 2H, =N-CH_2), 1.69-1.75 (m, 2H, =N-CH_2-CH_2), 1.42 (m, 2H, CH_2-CH_2-CH-NH).

^{13}C-NMR (125 MHz in DMSO-d_6): 198.2, 197.9, 188.2, 174.3, 170.0, 156.6, 155.2, 153.4, 144.3, 141.2, 129.7, 129.5 128.1, 127.5, 125.7, 120.5, 66.1, 64.3, 54.2, 47.1, 30.7, 29.2, 23.1 ppm

Synthesis of peptide conjugates 3 and 4: Rhenium bioconjugates **3** and **4** were synthesized using standard SPPS methods using the FMOC protecting group strategy, as shown in Scheme S1. The completeness of each coupling reaction was monitored by the Kaiser Test.[1] FMOC-Leu-Wang resin (50 mg, substitution=0.8 mmol/g) was used for each synthesis. The resin was first swelled in DMF for 1 h. The FMOC protecting group removal between each cycle was performed by 20-minute treatments with 20% piperidine in DMF followed by washing with DMF several times. Five equimolar equivalents of

FMOC-Ile-OH, FMOC-Tyr(tBu)-OH, FMOC-Pro-OH and FMOC-Arg(Pbf)-OH along with 4.5 eq. of HBTU and HOBt as coupling reagents and 4-methylmorpholine (10 eq.) as base were used in this synthesis. The coupling process was performed for 2 hours followed by washing and performance of the Kaiser Test before starting each subsequent cycle. All reactions were performed on a mechanical shaker. In some cases, excess unreacted amine termini remained present on the resins, which required that the coupling process be repeated. After completion of five cycles with the FMOC-protected amino acids, 5 eq. of the rhenium complex **1** were reacted overnight with use of HBTU/HOBt as the activator and 4-methylmorpholine in the same equimolar ratio as previously used. For compound **4**, further coupling of **3** (on-resin) with 5(6)-carboxyfluorescein yields the fluorescently modified peptide. For both **3** and **4**, the completed coupled resin turned orange after completion of the rhenium cycle. The resin was washed with DMF, moved to a clean round bottom flask and air-dried. DCM was added to the flask and the resin was allowed to swell (20-30 min). DCM was removed under vacuum and then the TFA (trifluoroacetic acid) cocktail cleavage method was started.

The TFA/water/triisopropylsilane (95:2.5:2.5) solution (7 mL) was added to dry peptide resin. The resultant mixture was gently stirred for 90 minutes. The resin-solvent mixture was filtered on a glass frit and resin was washed 2 times with a small amount of fresh cocktail solution. The filtrate was collected and the volume was reduced to be approximately ¼ of the original volume. An excess amount of ethyl ether (> 10 times the initial TFA volume) was added which initiated coagulation of the yellow peptide. The suspension was centrifuged at cold temperature for 5 minutes at 5000 rpm and the clear layer of ether was discarded. The resultant peptides were solubilized in acetonitrile/water/TFA (50:50:0.1) and was lyophilized to give yellow-orange powders. Mass spectrum data showed *m/z* = 1606.6 $[M]^+$ and 1526.7 $[M\text{-}Br]^+$.

Cell uptake experiments using Human Umbilical Vein Endothelial Cells (HUVECs)

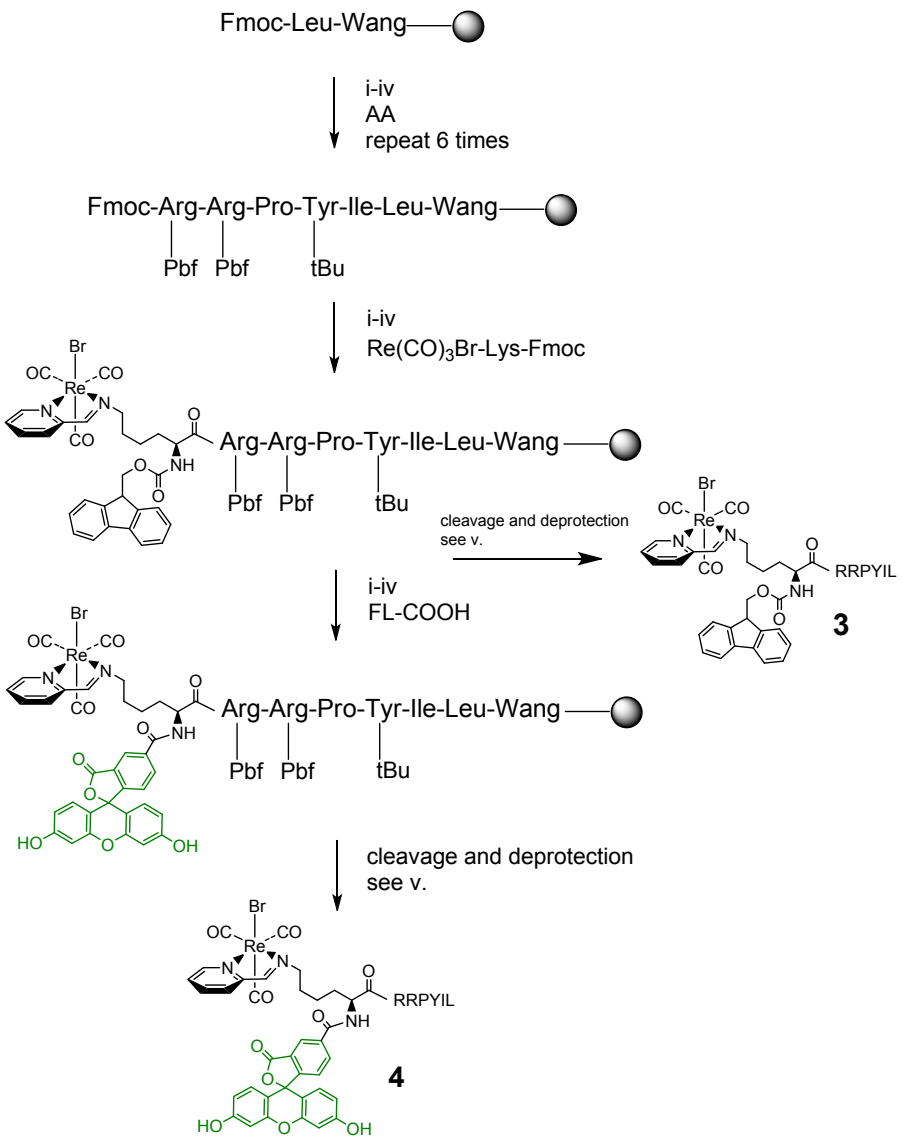
Cell culture and imaging: Human umbilical vascular endothelial cells (HUVECs) were maintained in EBM2 medium with EGM2 Bullet supplements and 10% fetal bovine serum at 37^{o}C in a humidified 5% CO_2 environment. The cells were seeded on 24-well 0.1% gelatin coated plate in a concentration of 75,000 cells per well and incubated for 24 h prior to incubation with the rhenium compounds at different concentrations.

Incubations of compounds were carried out on adherent cells. Concentrated DMSO stock solutions of the compounds were diluted using cell culture medium to desired final concentrations. After incubations, the cells were washed three times with PBS (pH 7.4) and covered with medium for microscopic examination. The imaging experiments were carried out on an AMG EVOS All-in-one digital inverted microscope by using GFP Fluorescent light cube (excitation- 470 nm and emission-525).

HUVECs (75,000 cells/well) in EGK2 media were incubated with Re-neurotensin (**3**), FL-Re-neurotensin (**4**), **1** and 5(6)-carboxyfluorescein. All four compounds were dissolved in a mixture of DMSO and PBS, using as little DMSO as possible. The concentration of all compounds was varied from 80, 40, 20, 10 and 1 μM. After 24 hours of incubation, cells were washed 5 times and media was replaced to allow the further imaging experiment. We saw no fluorescence in cells at all concentrations in the cases of **3**, **1** and 5(6)-carboxyfluorescein, and in the last case this observation was made in spite of the strong emission of 5(6)-carboxyfluorescein in the initial incubation solution. This result confirmed our hypothesis that the fluorescence in cell incubated with **4** does not result from trace FL-COOH or compound **1** that were left either as starting material or as a result of peptide cleavage in the media.

In the case of **4**, we observed increasing emission from cells as the compound concentration increased. At the highest concentration (80 μM), most of the cell fluoresced and were alive. The accumulation is specifically in the nuclei, which can be clearly seen from the figure. The pictures derived from 1 μM indicated that this concentration is too low to observe good fluorescence in cells. Concentrations ranging between 10-80 μM afforded cells with good fluorescence emission.

All fluorescence micrographs were obtained under the same conditions including exposure time, collecting methods, and sensitivity. The light intensity was fixed at 30% for all micrographs captured.



Scheme S1. Solid phase peptide synthesis of compounds **3** and **4**. i. **FMOC-deprotection**: 2 mL of piperidine (20% in DMF) was taken into the reaction column and the reaction was carried out for 2 x min with shaking. ii. **Washing**: The deprotection solution was pushed out of the reaction column into the waste container and the resin washed with 3 × 2 mL of DMF, 1 × 2 mL of MeOH, 1 × 2 mL of DCM and 1 × 2 mL of DMF. iii. **Coupling**: To the solution of the protected amino acid, HBTU and HOBt in DMF (approx. 1 mL) and 10x mole equivalents of *N*-methylmorpholine was added. The mixture was allowed to react for several minutes, added into the reaction column and the reaction column was shaken for 2 hours. iv. **Washing**: Repeat step ii. v. **TFA Cleavage**: TFA (95 %, 2.5% triisopropylsilane, 2.5% water) for 90 min. The TFA was removed by evaporation and the peptide was precipitated by addition of cold diethyl ether (10x excess).

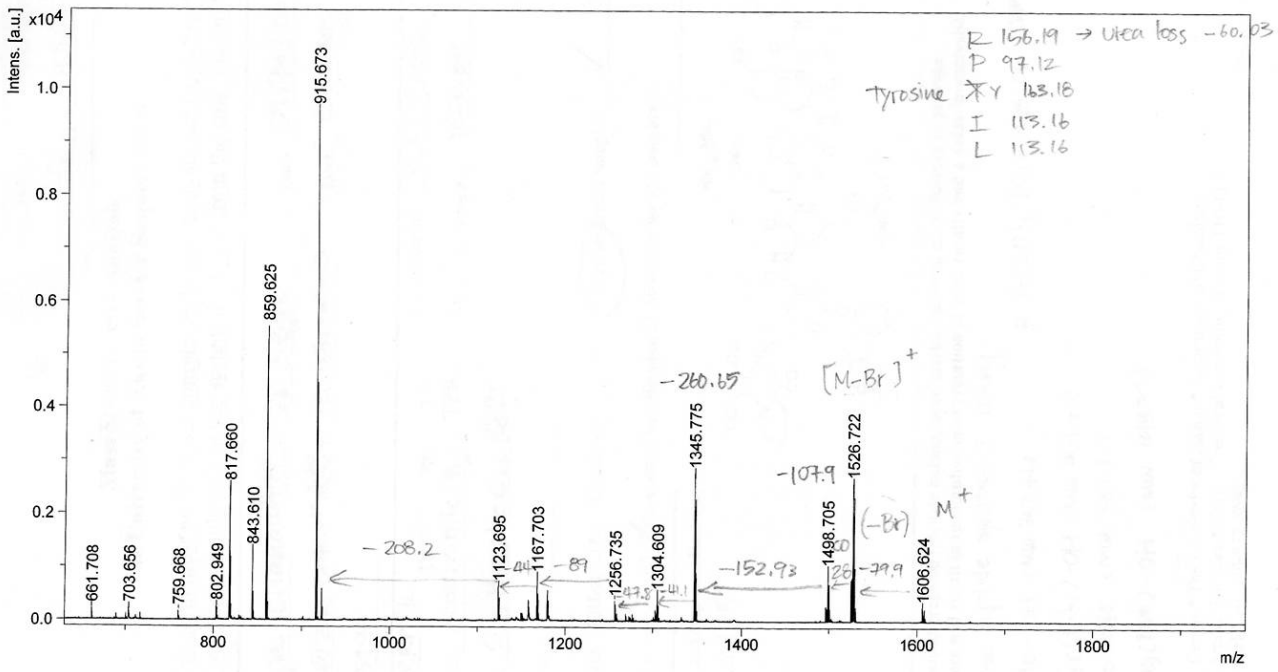


Figure S1. MS (MALDI-TOF) of **3**. *m/z* = 1606.6 $[M]^+$, 1526.7 $[M\text{-}Br]^+$

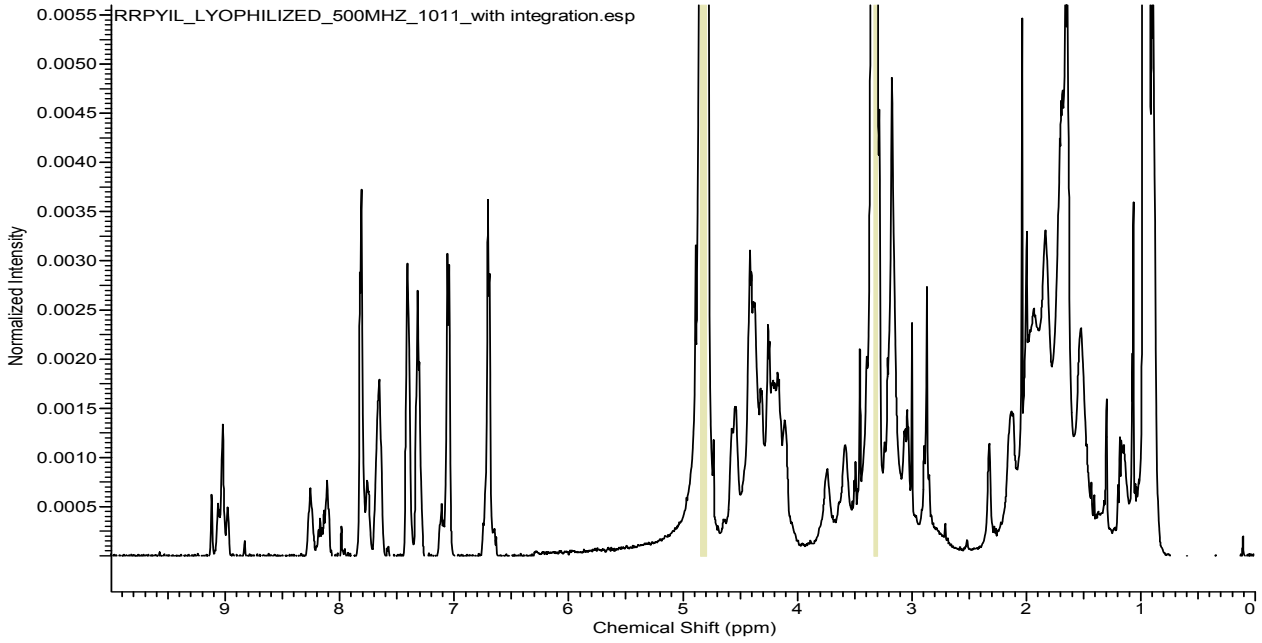


Figure S2. ^{1}H NMR of lyophilized **3**

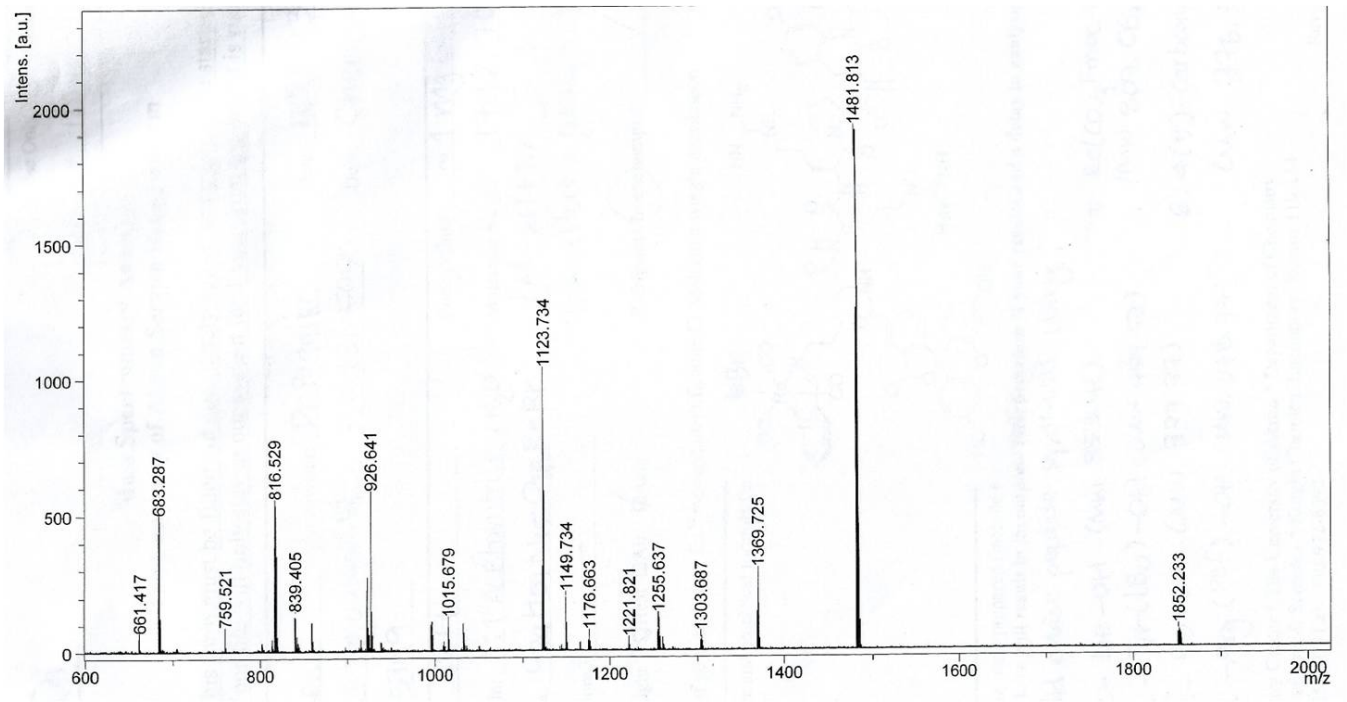


Figure S3. MS (MALDI-TOF) of **4**. *m/z* = 1852.23 $[M-2H+TFA]^+$

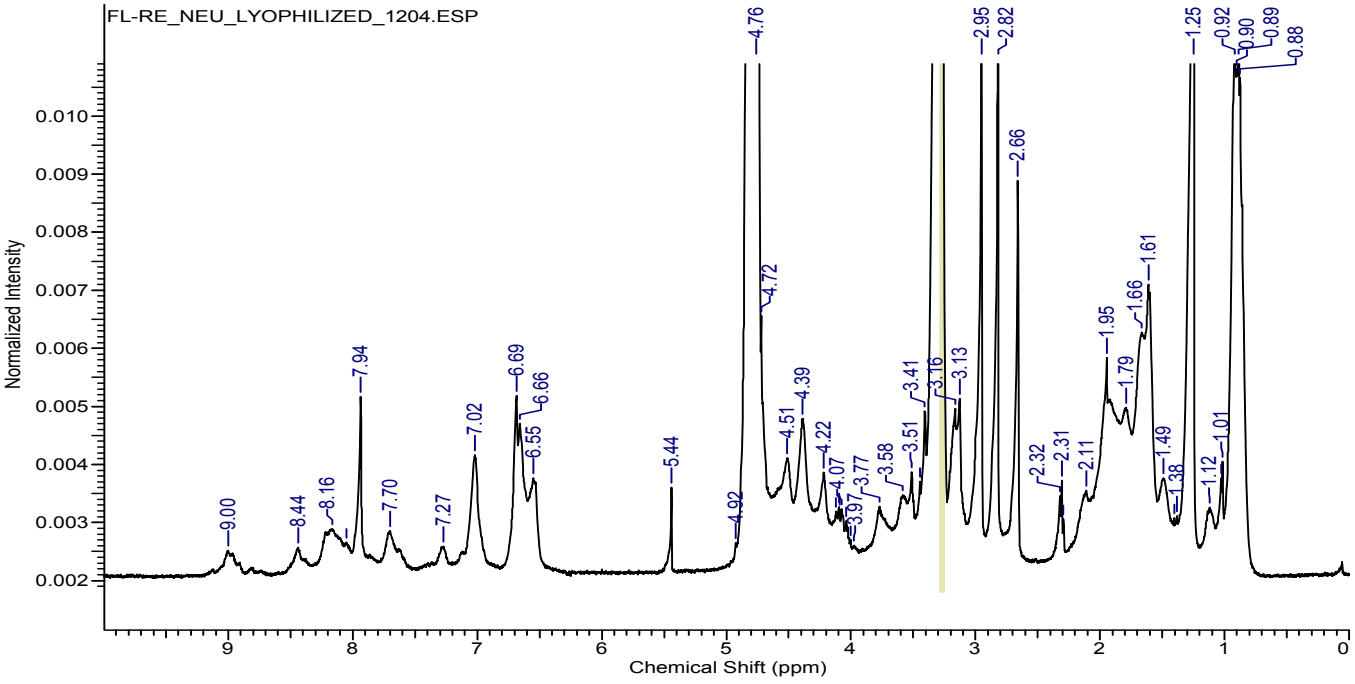


Figure S4. ^{1}H NMR of lyophilized **4**

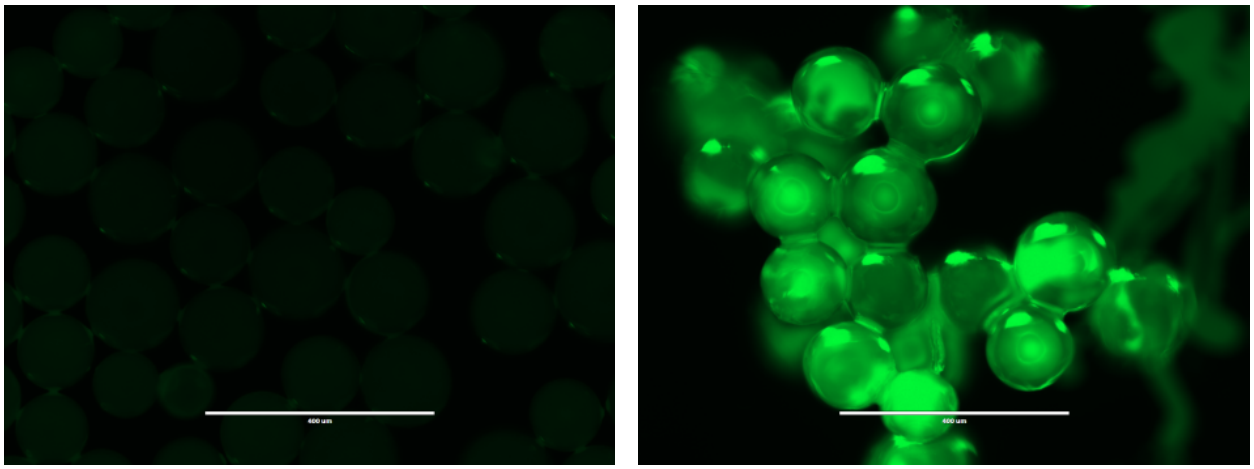


Figure S5. Fluorescent images of peptide appended beads: GFP: 470 nm excitation, 20% light, 10X Re-neurotensin **3** (left), Fluorescein-Re-neurotensin **4** (right).

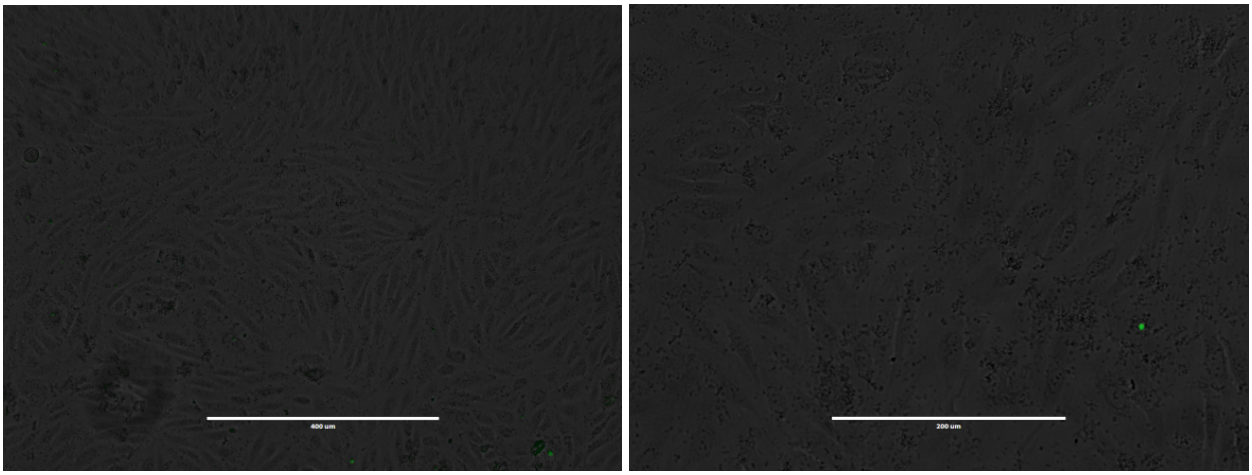


Figure S6. Overlaid images (transmitted and GFP mode) of HUVECs incubated with 40 μM 5(6)-carboxyfluorescein (FL-COOH), excited at 470 nm 10x (left) 20x (right). Trace FL-COOH was present in the media and could not be removed by washing.

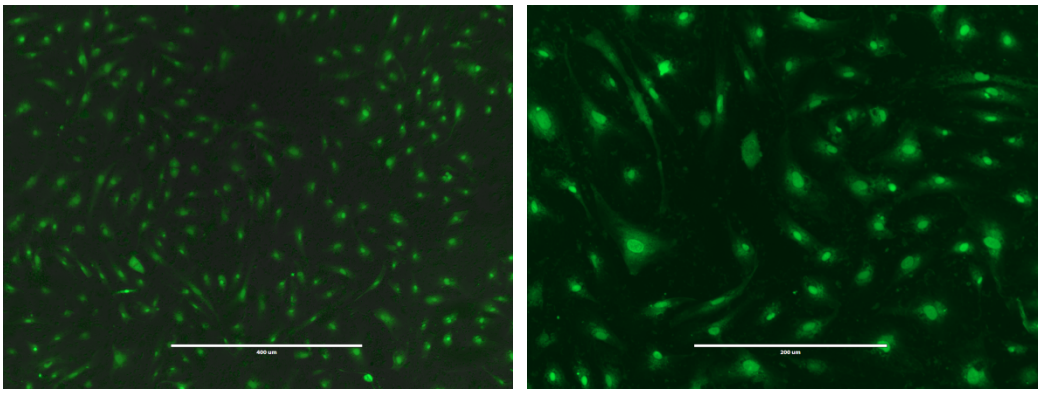
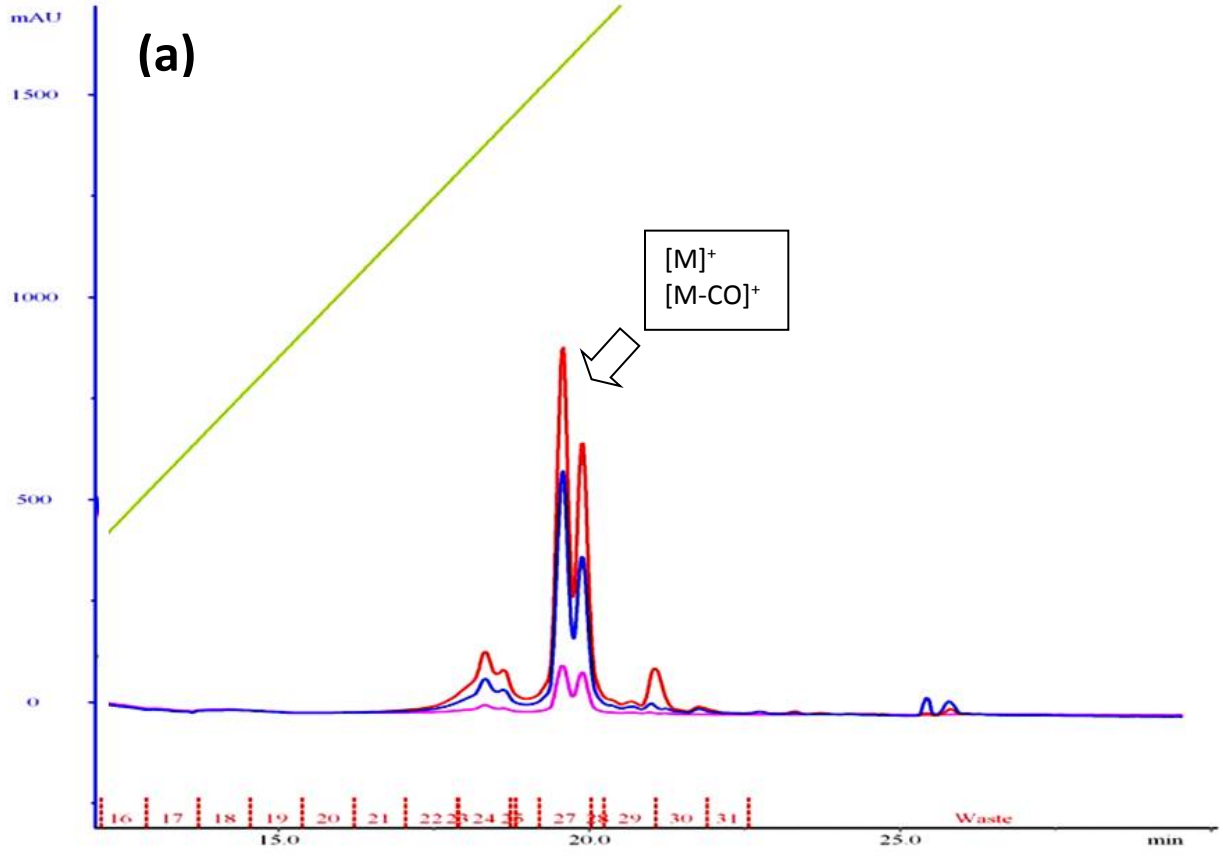


Figure S7. GFP mode images of HUVECs incubated with 40 μM of **4**, excited at 470 nm 10x (left), 20x (right).

HPLC was performed on a GE Aktamicro equipped with UV900 detector using a Vydac RP-C18 column. Buffers system is 99.9% water+0.1% TFA (A) and 95%acetonitrile+5%water+0.1%TFA (B).



(b)

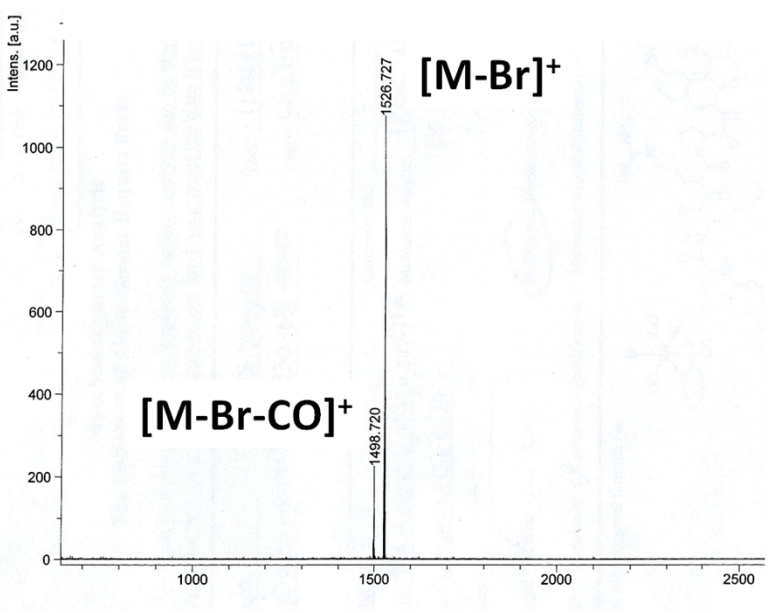


Figure S8. **(a)** HPLC traces of **3** (100% A → 100% B in 25 min): 19 min. Fraction 27 was used in cell experiment. Red, blue, and pink traces represent the absorption at 254, 280, and 380 nm (MLCT), respectively. **(b)** MALDI-TOF of fraction 27 showed no impurity.

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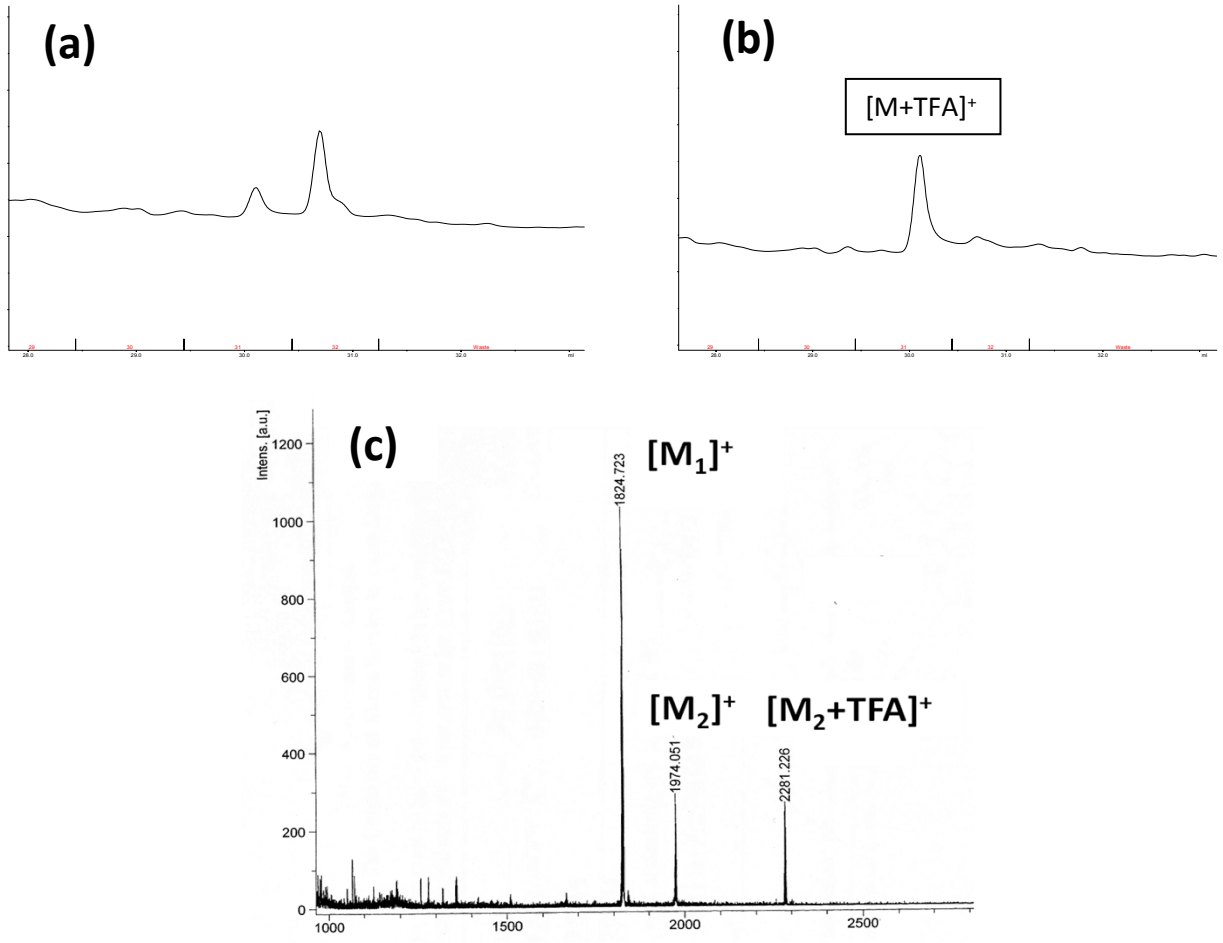

Figure S9. (a) Crude HPLC traces of **4** showed two species. (b) HPLC trace of purified **4** showed only $[M+TFA]^+$ fraction. (100% A → 100% B in 33 min): 30 min (c) MALDI-TOF of purified **4** (M_1 = M-H-CO+TFA, $M_2 = M_1+TFA+2H_2O$)

After HPLC purification, the success of $Re(CO)_3$-modified lysine chemistry as well as the purity improvement of the peptides were able to be confirmed by MALDI-TOF.[2] Both purified **3** and **4** were used in cell experiment.

[1] Stewart, J. M.; Young, J. D. Solid Phase Peptide Synthesis, 2nd Ed. Pierce, **1979**, 105-107.
[2] Gasser, G., Sosniak, A. M., Leonidova, A., Braband, H., and Metzler-Nolte, N. *Aust. J. Chem.* **2011**, *64*, 265–272.