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Supplementary Information

Synthesis of porphyrin with histidine-like chelate: an efficient path towards molecular PDT/SPECT theranostic

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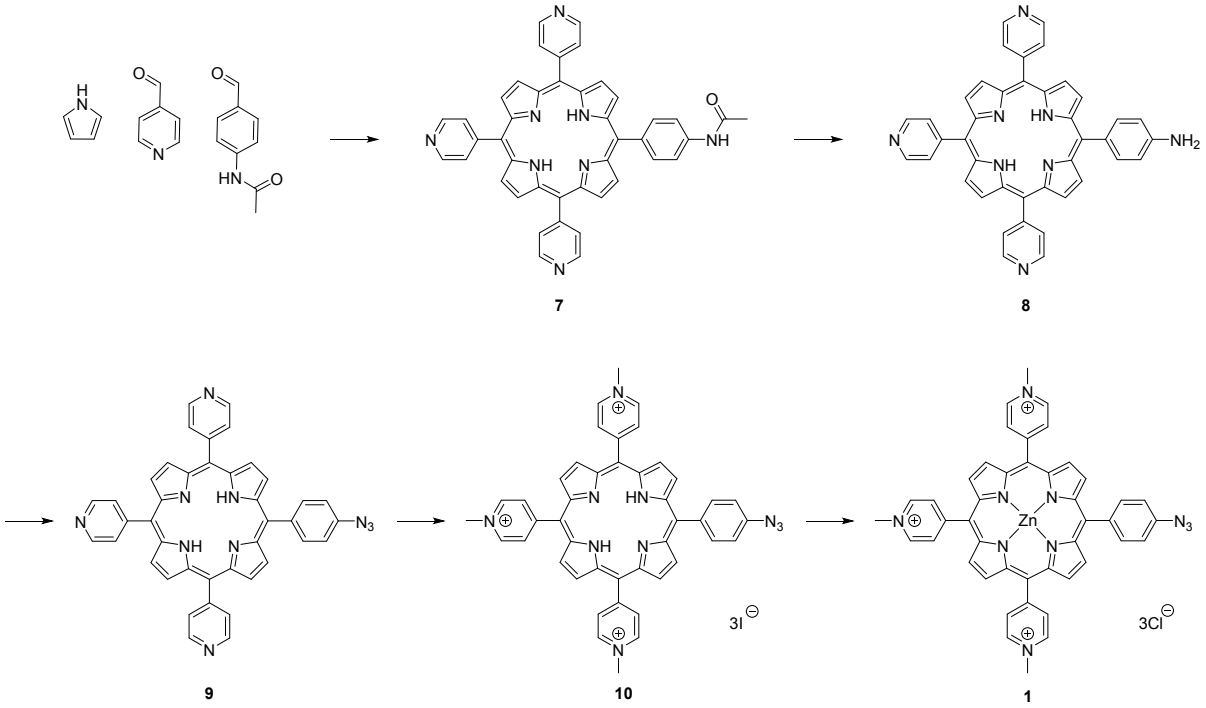
Experimental section

General remarks

NMR spectra were recorded on a JEOL ECZ 400S spectrometer at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR, with residual protic solvent as the internal reference. Chemical shifts are given in ppm (δ) and coupling constants (J) are given in Hertz (Hz). Mass spectrometry data were obtained from the EPSRC National Mass Spectrometry Facility at Swansea University. UV-vis spectroscopy was carried out on a Varian Cary 50 Bio UV-vis spectrophotometer. All commercially available starting material used in synthesis were obtained from Sigma Aldrich, Fluorochem, and Alfa Aesar and were used without further purification. Deionised water was obtained from a Millipore Milli-Q reagent water system. All solvents were obtained from Fisher Scientific, VWR, and Honeywell. Solvents were dried according to the procedure by William *et al*.[1]

HPLC analysis were performed on Agilent HPLC system. The separations were performed on a Gemini® 5µm C18 110 Å LC column 150×4.6 mm (Phenomenex, UK) at a flow rate of 1 mL min^{-1}, with a mobile phase consisting of 0.1% TFA in water (solvent A) and 0.1% TFA in acetonitrile (solvent B). Gradient [time/min](solvent A:solvent B): [0-2](85:15). [2-17](85:15-40:60). [17-18](40:60-5:95). [18-23](5:95). [23-24](5:95-85:15). [24-26](85:15)

Synthesis



5-[4-acetamidophenyl]-10,15,20-tri-(4-pyridyl)porphyrin (7)[2]

To a stirred solution of 4-acetamidobenzaldehyde (3.53 g, 21.5 mmol) and 4-pyridinecarboxaldehyde (4.89 mL, 52 mmol) in propionic acid (500 mL) was added pyrrole (5 mL, 72 mmol) dropwise. The reaction mixture was refluxed at 170 °C for 1 hour. Propionic acid was removed under reduced pressure. The crude was purified using column chromatography (silica, 95:5 DCM:MeOH) and recrystallizes from MeOH over DCM to yield a purple solid (764 mg, 1.13 mmol, 6.3%).

^{1}H NMR (400 MHz, $CDCl_3$): δ2.39 (s, 3H, -CH_3), 7.95 (d, 2H, m-Ph), 8.16 (m, 8H, βH), 8.83 (m, 6H, o-Py), 8.94 (d, 2H, o-Ph), 9.05 (m, 6H, m-Py). ^{13}C NMR (100 MHz, $CDCl_3$): δ24.98 ($\underline{C}H_3$-C=O), 117.15, 117.54, 118.22, 121.20, 129.45 (βC), 135.23, 137.44, 138.14, 148.47 (βC), 150.09, 168.75 (C=O). MS: (ESI) m/z 675 $[M+H]^+$. HRMS: calcd. for $C_{43}H_{31}N_8O_1$ 675.2615 found 675.2605. UV-vis [CH_2Cl_2, nm] 418, 513, 590, 645. ε (418 nm) = 417000 M^{-1} cm^{-1}.

5-[4-Aminophenyl]-10,15,20-tri-(4-pyridyl)porphyrin (8)[2]

Porphyrin **7** (670 mg, 0.99 mmol) was taken up in aqueous HCl (300 mL, 6 M) and stirred at reflux for 3 h. The solvent was removed under reduced pressure and redissolved in a mixture of dichloromethane and triethylamine (400 mL, 9:1). The solution was washed with water (3x200 ml), the organic layer dried ($MgSO_4$), and solvent removed under reduced pressure. The porphyrin product was precipitated from dichloromethane over methanol to yield a shiny purple powder (518 mg, 0.79 mmol, 78%).

^{1}H NMR (400 MHz, $CDCl_3$): δ4.08 (s, 2H, NH_2), 7.08 (d, 2H, o-Ph), 7.98 (d, 2H, m-Ph), 8.15 (m, 6H, o-Py), 8.82 (m, 6H, m-Py), 9.03 (m, 8H, βH). ^{13}C NMR (100 MHz, $CDCl_3$): δ113.65, 116.74, 117.38, 122.66, 129.47 (βC), 131.68, 135.92, 146.54, 148.46 (βC), 148.51, 150.11, 150.21. UV-vis [CH_2Cl_2, nm] 420, 515, 555, 590, 650. ε (420 nm) = 662000 M cm^{-1}. MS: (ESI) m/z 633 $[M+H]^+$. HRMS: calcd. for $C_{41}H_{28}N_8$ 633.2510, found 633.2512.

5-[4-Azidophenyl]-10,15,20-tri-(4-pyridyl)porphyrin (9)[2]

To a stirred solution of porphyrin **8** (580 mg, 0.917 mmol) in TFA (6 mL) at 0 °C was added a solution of sodium nitrite (132 mg, 1.91 mmol) in water. The reaction mixture was allowed to proceed for 15 mins at 0 °C. A solution of sodium azide (249 mg, 3.83 mmol) in water was added and the reaction mixture was allowed to proceed for 1 hour at 0 °C. The reaction mixture was diluted with water and neutralised using saturated sodium hydrogen carbonate solution. The solution was extracted using DCM (3×50 mL), the organic layer dried and removed under reduced pressure. The crude was purified using column chromatography (silica, 3% MeOH:DCM). The crude was recrystallized from MeOH over DCM to yield the product as a purple solid (438 mg, 0.665 mmol, 73%).

^{1}H NMR (400 MHz, $CDCl_3$) δ -2.90 (s, 2H, NH), 7.44 (d, *J* = 8.3 Hz, 2H, o-Ph), 8.16 (d, *J* = 5.9 Hz, 6H, o-Py), 8.18 (d, *J* = 8.3 Hz, 2H, m-Ph), 8.87 (m, 8H, βH), 9.05 (d, *J* = 5.8 Hz, 6H, m-Py). ^{13}C NMR (100 MHz, $CDCl_3$): δ117.32, 117.64, 117.74, 120.57, 129.46 (βC), 131.24, 135.81, 138.26, 140.45, 148.49 (βC), 148.51, 150.05. MS: (ESI) m/z 659 $[M+H]^+$, HRMS: calcd. for $C_{41}H_{27}N_{10}$ 659.2415 found 659.2408. UV-vis (CH_2Cl_2, nm): 418, 514, 550, 590, 644. ε (418 nm) = 484000 M^{-1} cm^{-1}.

5-[4-Azidophenyl]-10,15,20-tri-(*N*-methyl-4-pyridinium)porphyrin triiodide (10)[2]

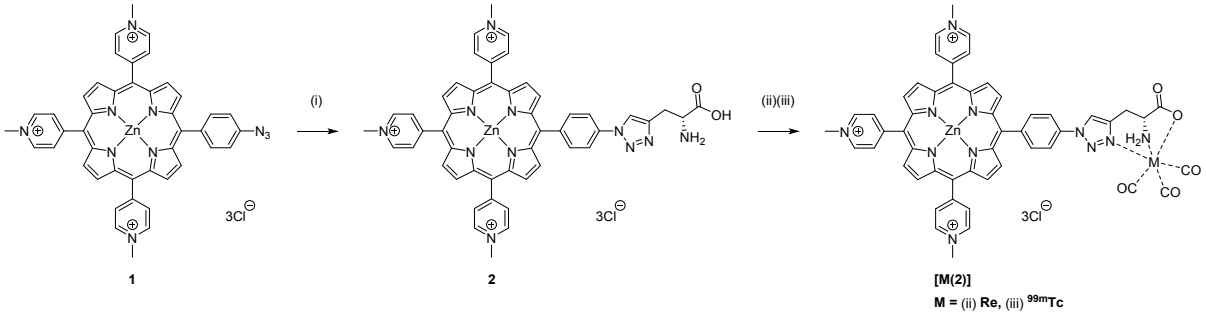
To a stirred solution of porphyrin **9** (588 mg, 0.893 mmol) in DMF (60 mL) was added methyl iodide (6 mL, 0.096 mol) dropwise via a syringe. The reaction mixture was heated to 40 °C and allowed to proceed overnight. Once reaction mixture had cooled to room temperature, diethyl ether was added to promote precipitation the precipitate was filtered through cotton wool. The crude was recrystallized from diethyl ether over methanol to yield a purple solid (941 mg, 0.868 mmol, 97%).

^{1}H NMR (400 MHz, DMSO-d_6) δ -3.06 (s, 2H, NH), 4.68 (s, 9H, N-CH_3), 7.61 (d, *J* = 8.2 Hz, 2H, o-Ph), 8.23 (d, *J* = 8.0 Hz, 2H, m-Ph), 8.97 (d, *J* = 5.4 Hz, 6H, o-Py), 9.06 (m, 8H, βH), 9.44 (d, *J* = 5.8 Hz, 6H, m-Py). ^{13}C NMR (100 MHz, DMSO-d_6) δ 48.42 (N-CH_3), 115.14, 115.88, 118.67, 122.46, 132.64 (βC), 136.26, 137.56, 140.57, 144.71 (βC), 157.07. MS: (ESI) m/z 243 $[M-3I]^{3+}$, HRMS calcd. for $C_{44}H_{33}N_{10}^{3+}$ 243.4337, found 234.4343. UV-vis (DMSO, nm): 425, 516, 560, 595, 650, ε (425 nm) = 339000 M^{-1} cm^{-1}.

Zinc 5-[4-Azidophenyl]-10,15,20-tri-(*N*-methyl-4-pyridinium)porphyrin trichloride (1)[2]

To a stirred solution of porphyrin **10** (300 mg, 0.277 mmol) in water (30 mL) was added zinc (II) acetate (300 mg, 1.64 mmol). The reaction mixture was allowed to proceed at 40 °C overnight. The reaction mixture was diluted with water and was added a solution of 10% ammonium hexafluorophosphate in water. The precipitate was filtered and redissolved in acetone. A solution of 10% tetrabutylammonium chloride was added and the precipitate was filtered. The residue was precipitated from diethyl ether over methanol to yield a green solid (235 mg, 0.270 mmol, 98%).

HPLC: R_f = 10.8 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 4.67 (s, 9H, N-CH_3), 7.56 (d, J = 7.5 Hz, 2H, o-Ph), 8.16 (d, J = 7.9 Hz, 2H, m-Ph), 8.88 (m, 14H, o-Py, βH), 9.38 (m, 6H, m-Py). ^{13}C NMR (100 MHz, DMSO-d_6) δ 48.25 (N-CH_3), 115.25, 116.02, 118.20, 132.65 (βC), 136.06, 139.23, 139.87, 144.14 (Cβ), 148.39, 148.68, 148.86, 150.74, 158.91. MS: (ESI) m/z 255 $[M-3Cl]^{3+}$, HRMS calcd. for $C_{44}H_{33}N_{10}Zn^{3+}$ 255.0722 found 255.0725. UV-vis (DMSO, nm): 440.0, 565.0, 615.0, ε (440 nm) = 71000 $M^{-1}cm^{-1}$.



Porphyrin-glycine conjugate (2)

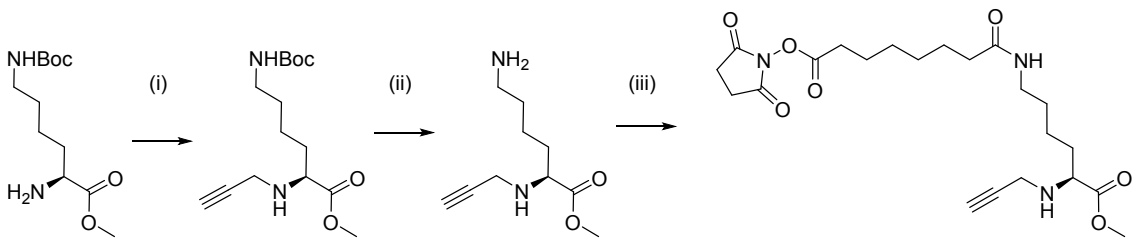
Porphyrin **1** (29.8 mg, 38.8 μmol) and D-propargyl glycine (7.0 mg, 61.4 μmol) were dissolved in a mixture of t-butanol and water (1:1, 20 mL) and was added a solution of copper(II) sulphate (500 μL, 10 mM), followed by a solution of sodium ascorbate (500 μL, 100 mM). To the resulting solution was added tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (TBTA) (1 mg). The reaction mixture was heated in a microwave reactor (20 mins, 75 W, 50 °C). The reaction was concentrated under reduced pressure, diluted with water and was added a solution of 10% ammonium hexafluorophosphate in water. The precipitate was filtered and redissolved in acetone. A solution of 10% tetrabutylammonium chloride was added and the precipitate was filtered. The crude was precipitated from diethyl ether over MeOH to give a purple solid. (32.0 mg, 32.4 μmol, 83.6%).

HPLC: R_f = 7.5 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.119 (s, 2H, α-CH_2), 4.680 (s, 9H, N-CH_3), 7.853 (m, 4H, Ph), 8.327 (s, 1H) 8.844-8.946 (m, 15H, βH, o-Py, triazole-H), 9.422 (s, 6H, m-Py). ^{13}C NMR (100 MHz, DMSO-d_6) δ 48.160 (N-CH_3), 115.188, 115.955, 118.168, 122.681, 132.014, 132.503, 132.647 (βC), 132.771, 133.596, 136.049, 139.412, 144.136 (βC), 148.381, 148.659, 148.851, 150.709, 159.046. MS: (ESI) m/z 293 $[M\text{-}3Cl]^{3+}$, HRMS calcd. for $C_{49}H_{40}N_{11}O_2Zn^{3+}$ 292.7547, found 292.7547. UV-vis (DMSO, nm): 436, 564, 610, ε (436 nm) = 190000 M^{-1} cm^{-1}.

[Re(2)]

To a stirred solution of conjugate **2** (25 mg, 0.025 mmol) in 0.1 M pH 7 phosphate buffer (3 mL) was added $[Re(CO)_3Br_3][NEt_4]_2$ (20 mg, 0.026 mmol). The reaction mixture was allowed to stir at 65 °C for 2 hours. The reaction mixture was diluted with water and was added a solution of 10% ammonium hexafluorophosphate in water. The precipitate was filtered and redissolved in acetone. A solution of 10% tetrabutylammonium chloride was added and the precipitate was filtered. The crude was precipitated from diethyl ether over methanol to give a purple solid (24 mg, 0.019mmol, 76%).

HPLC: R_f = 10.2 min. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.16 (dd, J = 14.2, 6.9 Hz, 2H, C$\underline{H}_2$-αCH), 3.98 (s, 1H, αCH), 4.67 (s, 9H, N-CH_3), 8.34 (d, J = 7.2 Hz, 2H, o-Ph), 8.42 (d, J = 7.0 Hz, 2H, m-Ph), 8.91 (m, 14H, βH, o-Py), 9.28 (s, 1H, triazole-H), 9.41 (m, 6H, m-Py). ^{13}C NMR (100 MHz, DMSO-d_6) δ 26.80 (C$\underline{H}_2$-αCH), 48.19 (N-CH_3), 51.21 (αCH), 115.45, 116.09, 119.69, 121.69, 125.21, 132.27, 132.65 (βC), 132.86, 133.62, 135.62, 136.01, 144.16 (βC), 144.44, 148.50, 148.75, 148.88, 150.39, 158.97, 180.93 (O-C=O), 197.38 (Re-CO), 197.93 (Re-CO), 199.04 (Re-CO). MS: (ESI) m/z 382 $[M\text{-}3Cl+H]^{3+}$ HRMS calcd. for $C_{52}H_{39}N_{11}O_5ReZn$ 382.0647 $[M\text{-}3Cl+H]^{3+}$, found 382.0649. UV-vis (DMSO, nm) 436.0, 565.9, 607.9. ε (436 nm) = 106000 $M^{-1}cm^{-1}$.



11 **12** **5**

***N*(α)-propargyl-*N*(ε)-Boc-Lys(OMe) (11)**

To a stirred solution of *N*(ε)-Boc-Lys(OMe).HCl (1 g, 3.36 mmol) in DMF (30 mL) was added with potassium carbonate (953 mg, 6.89 mmol). The mixture was stirred under argon for 5 minutes. To the resulting solution was added propargyl bromide in toluene (80% wt., 599 µL, 5.38 mmol). The reaction mixture was stirred for 48 h at room temperature. The solution was filtered, and excess solvent was removed under reduced pressure. The crude was purified using column chromatography (silica, 99:1 DCM:MeOH) to yield the product as a yellow oil (724 mg, 2.43 mmol, 72%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (m, 13H, $C(CH_3)_3$, CH_2CH_2), 1.75 (m, 2H, CH_2), 2.25 (m, 1H, CCH), 3.10 (m, 2H, CH_2), 3.52 (m, 2H, CH_2), 3.74 (s, 3H, CH_3), 4.57 (s, 1H, α-CH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.85, 28.50 ($C(\underline{C}H_3)_3$), 29.84, 32.63, 36.90, 40.33, 52.06, 59.76, 72.34, 79.18, 156.03 (C=O). MS: (ESI) m/z 299.2 $[M+H]^+$, HRMS: calcd. for $C_{15}H_{27}N_2O_4$ 299.1965 found 299.1969.

***N*(α)-propargyl-Lys(OMe) (12)**

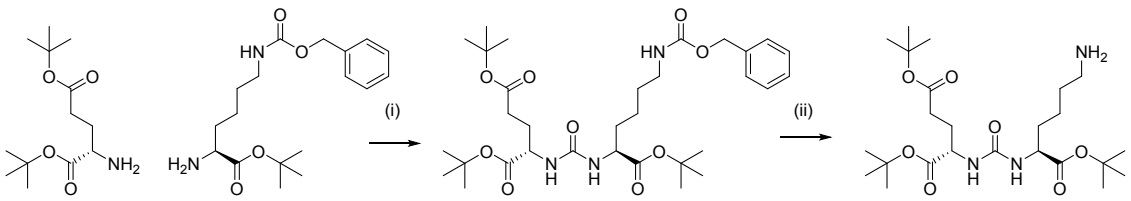
11 (120 mg, 0.402 mmol) was taken up in 1 mL DCM and was added 1 mL TFA. The reaction mixture was allowed to proceed for 2 hours and solvent was removed under reduced pressure, triturated with diethyl ether and was dried further under reduced pressure to yield the product as a yellow oil (75 mg, 0.379 mmol, 95%).

^{1}H NMR (400 MHz, DMSO-d_6) δ 1.52 (m, 6H, CH_2), 2.73 (m, 2H, CH_2), 3.69 (s, 1H, α-CH), 3.73 (s, 3H, CH_3), 3.89 (s, 2H, CH_2), 3.97 (m, 1H, CCH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 15.70, 21.83, 27.04, 28.99, 35.45, 38.91, 53.53, 58.47, 65.45, 158.88. MS: (ESI) m/z 199.4 $[M+H]^+$.

***N*(α)-propargyl-*N*(ε)-NHS-SA-Lys(OMe) (5)**

Disuccinimidyl suberate (500 mg, 1.36 mmol) was taken up in dry DMF (3 mL), was added TEA (191 µL, 1.36 mmol) and a solution of **12** (128 mg, 0.65 mmol) in DMF (3 mL) dropwise. The reaction mixture was allowed to proceed at room temperature under inert atmosphere overnight. Solvent was removed under reduced pressure. The crude was purified using column chromatography (silica, 96:4 DCM:MeOH) to yield the product as a yellow oil (258 mg, 0.57 mmol, 88%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (m, 6H, CH_2), 1.46 (m, 2H, CH_2), 1.58 (m, 2H, CH_2), 1.69 (m, 4H, CH_2), 2.14 (t, *J* = 7.5 Hz, 2H, $O{=}CCH_2$), 2.25 (t, *J* = 2.4 Hz, 1H, $C{\equiv}C\underline{H}$), 2.55 (t, *J* = 7.3 Hz, 2H, $O{=}CCH_2$), 2.80 (s, 4H, $O{=}CCH_2CH_2C{=}O$), 3.18 (m, 2H, ε-$C\underline{H}_2NH$), 3.47 (m, 3H, $HC{\equiv}CC\underline{H}_2$, α-CH), 3.69 (s, 3H, O-$CH_3$). ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.52, 24.43, 25.39, 25.69 ($O{=}C\underline{C}H_2\underline{C}H_2C{=}O$), 28.13, 28.40, 28.93, 30.95, 31.78, 36.22, 36.52, 39.04 (ε-$\underline{C}H_2NH$), 52.35 (O-CH_3), 59.16 (αCH), 73.37 ($\underline{C}{\equiv}CH$), 79.42 ($C{\equiv}\underline{C}H$), 168.76 (C=O), 169.59 (C=O), 173.07 (C=O), 173.73 (C=O), 174.06 (C=O). MS: (ESI) m/z 452.2 $[M+H]^+$, HRMS: calcd. for $C_{22}H_{34}N_3O_7$ 452.2391 found 452.2389.



Glu(OtBu)$_2$-urea-*N*(ε)-Cbz-(OtBu)Lys^3 (13)

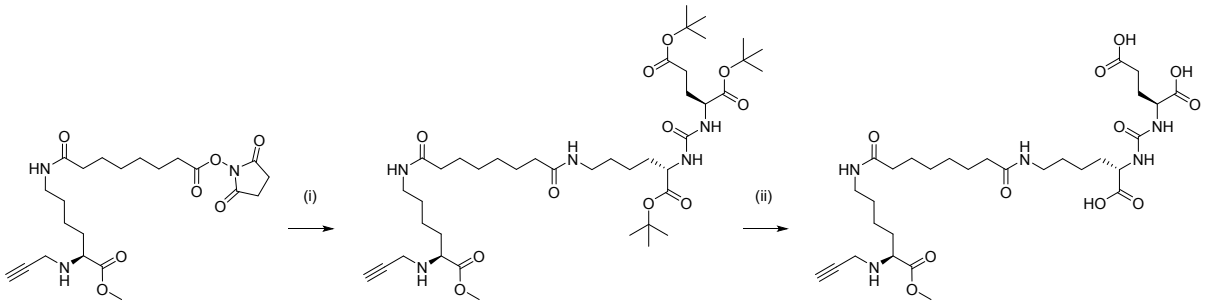
Triphosgene (0.83 g, 2.8 mmol) was dissolved in anhydrous dichloromethane (10 mL) and cooled to 0 °C. A mixture of L-glutamic acid di-*tert*-butyl ester hydrochloride (2.24 g, 7.6 mmol), triethylamine (2.1 mL, 15.2 mmol) and anhydrous dichloromethane (30 mL) was added dropwise during 1 hours at 0 °C, and was allowed to stir for a further 1 hour. A solution of *N*(ε)-benzoyloxycarbonyl-L-lysine-*tert*-butyl ester hydrochloride (2.82 g, 7.6 mmol), triethylamine (2.1 mL, 15.2 mmol), and anhydrous dichloromethane (25 mL) was added in one portion and the reaction mixture was allowed to stir at room temperature for 1 hour. The solution was evaporated under reduced pressure and ethyl acetate (50 mL) was added. The organic phase was washed with 2 M $NaHSO_4$ (2 x 50 mL), brine (40 mL), and dried over magnesium sulphate. The solvent was removed under reduced pressure to give a colourless oil. The crude was purified using column chromatography (silica, 7:3 hexane:ethyl acetate) to yield the product as a waxy white solid (1.6 g, 2.6 mmol, 34%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.24 (m, 3H), 1.39 (m, 27H, C(CH_3)$_3$), 1.65 (m, 4H), 1.99 (m, 1H), 2.21 (m, 2H), 3.11 (m, 2H, Lys-εC$\underline{H}_2$), 4.31 (d, *J* = 25.0 Hz, 2H, αC$\underline{H}$-NH-CO-NH-αC$\underline{H}$), 5.05 (q, *J* = 12.4 Hz, 2H, Ph-C$\underline{H}_2$), 7.28 (m, 5H, C_6H_5). ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.48, 28.06 (C($\underline{C}H_3$)$_3$), 28.12 (C($\underline{C}H_3$)$_3$), 28.38, 29.40, 31.63, 32.67, 40.78 (Lys-ε$\underline{C}H_2$), 52.88 (αCH-NH-CO-NH-α$\underline{C}$H), 53.34 (α$\underline{C}$H-NH-CO-NH-αCH), 66.50 (Ph-$\underline{C}H_2$), 80.51, 81.61, 82.23, 128.01 (Ph-C), 128.07 (Ph-C), 128.49 (Ph-C), 136.83 (Ph-C), 156.77 (C=O), 157.28 (C=O), 172.40 (C=O), 172.67 (C=O), 173.07 (C=O). MS: (ESI) m/z 623.3 $[M+H]^+$.

Glu(OtBu)$_2$-urea-(OtBu)Lys^3 (14)

Glu(OtBu)$_2$-urea-*N*(ε)-Cbz-(OtBu)Lys (13) (610 mg, 0.98 mmol) was dissolved in MeOH (10 mL) and was added Pd on C (50 mg), and hydrazine monohydrate (500 µL, 10 mmol). The reaction mixture was allowed to proceed at room temperature for overnight under argon. Reaction was analysed using mass spectrometry. The reaction mixture was filtered through celite and washed with MeOH. The volatile part was removed under reduced pressure, with co-evaporation with DCM (3×50 mL) and diethyl ether (3×50 mL). The crude was dried under high vacuum to yield the product as a transparent oil (425 mg, 0.87 mmol, 89%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.05 (m, 3H), 1.24 (d, *J* = 11.3 Hz, 27H, C(CH_3)$_3$), 1.54 (m, 4H), 1.86 (m, 1H), 2.11 (m, 2H), 2.50 (t, *J* = 6.6 Hz, 2H), 4.17 (m, 2H, , αC$\underline{H}$-NH-CO-NH-αC$\underline{H}$), 5.75 (s, 2H, , αCH-N$\underline{H}$-CO-N$\underline{H}$-αCH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.37, 27.93 (C($\underline{C}H_3$)$_3$), 27.97 (C($\underline{C}H_3$)$_3$), 28.39, 31.54, 32.60, 32.71, 41.42 (Lys-ε$\underline{C}H_2$), 52.74 (αCH-NH-CO-NH-α$\underline{C}$H), 53.31 (α$\underline{C}$H-NH-CO-NH-αCH), 80.19, 81.22, 81.58, 157.36 (C=O), 172.27 (C=O), 172.51 (C=O), 172.77 (C=O). MS: (ESI) m/z 488.7 $[M+H]^+$.



5 **15** **6**

Glu(OtBu)$_2$-urea-(OtBu)Lys-SA-*N*(α)-propargyl-Lys(OMe) (15)

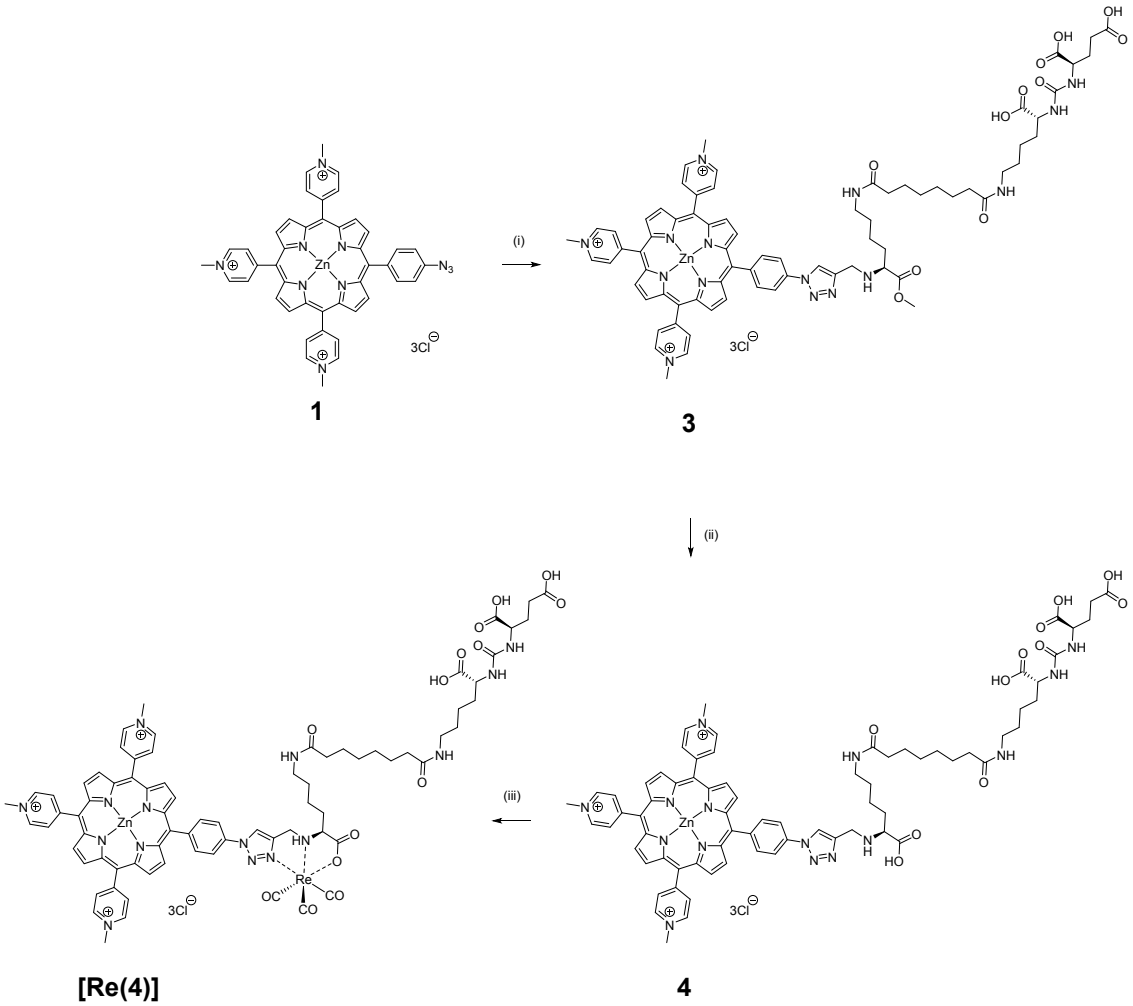
To a solution of **Glu(OtBu)$_2$-urea-(OtBu)Lys (14)** (158 mg, 0.35 mmol) in DMF (1 mL) was added TEA (115 µL, 0.8 mmol) and **5** (85 mg, 0.175 mmol) in DMF (4 mL). The reaction mixture was allowed to proceed at room temperature overnight. Solvent was removed under reduced pressure. The crude was purified using column chromatography (97:3-95:5 DCM:MeOH), to yield the product as a transparent oil (90 mg, 0.109 mmol, 62%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (m, 8H), 1.37 (m, 27H, C(CH_3)$_3$), 1.55 (m, 13H), 2.16 (m, 8H, C≡CH), 3.14 (m, 4H, Glu-urea-Lys-ε-C$\underline{H}_2$, Lys-ε-C$\underline{H}_2$), 3.38 (m, 3H, Lys-αC$\underline{H}$, HC≡CC$\underline{H}_2$), 3.66 (s, 3H, O-CH_3), 4.21 (m, 2H, αC$\underline{H}$-NH-CO-NH-αC$\underline{H}$), 5.76 (d, *J* = 8.0 Hz, 1H, αCH-NH-CO-N$\underline{H}$-αCH), 5.87 (d, *J* = 8.2 Hz, 1H, αCH-N$\underline{H}$-CO-NH-αCH), 6.33 (t, *J* = 5.6 Hz, 1H, NH-linker-N$\underline{H}$), 6.70 (t, *J* = 5.5 Hz, 1H, N$\underline{H}$-linker-NH). ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.65, 22.79, 25.54, 25.62, 28.05 (C($\underline{C}H_3$)$_3$), 28.11 (C($\underline{C}H_3$)$_3$), 28.24, 28.64, 28.72, 28.84, 29.16, 29.73, 31.70, 32.39, 32.48, 36.35, 36.48, 36.76, 39.02, 39.11, 52.12 (O-$\underline{C}H_3$), 53.00 (αCH-NH-CO-NH-α$\underline{C}$H), 53.39 (α$\underline{C}$H-NH-CO-NH-αCH), 59.53 (Lys-α$\underline{C}$H), 72.50 ($\underline{C}$≡CH), 80.61(C≡$\underline{C}$H), 81.52, 82.07, 157.60, 172.40 (C=O), 172.59 (C=O), 172.80 (C=O), 172.91 (C=O), 173.74 (C=O), 173.99 (C=O), 174.71 (C=O). MS: (ESI) m/z 825.2 [M+H]$^+$.

Glu-urea-Lys-SA-*N*(α)-propargyl-Lys(OMe) (6)

15 (150 mg, 0.182 mmol) was dissolved in DCM (2 mL) and was added TFA (2 mL). The reaction mixture was allowed to stir at room temperature for 3 hours. Solvent was removed under reduced pressure. The crude was washed with DCM (3×50 mL) and evaporated, followed by diethyl ether (3×50 mL) and evaporated, to yield the product as a yellow oil (115 mg, 0.175 mmol, 96%).

^{1}H NMR (400 MHz, D_2O) δ 1.22 (m, 17H), 1.53 (m, 2H), 1.87 (m, 8H), 2.29 (m, 2H), 2.81 (m, 1H), 2.95 (d, *J* = 6.5 Hz, 4H), 3.63 (m, 3H, O-CH_3), 3.78 (m, 2H, HC≡CC$\underline{H}_2$), 3.97 (m, 1H, Lys-αCH), 4.05 (m, 2H, αC$\underline{H}$-NH-CO-NH-αC$\underline{H}$). ^{13}C NMR (100 MHz, D_2O) δ 21.28, 22.30, 25.10, 25.23, 26.30, 27.76, 27.80, 28.19, 29.95, 30.63, 35.39, 35.60, 38.58, 38.95, 52.44 (O-$\underline{C}H_3$), 53.11 (αCH-NH-CO-NH-α$\underline{C}$H), 53.61 (α$\underline{C}$H-NH-CO-NH-αCH), 58.54 (Lys-α$\underline{C}$H), 72.40 ($\underline{C}$≡CH), 78.69 (C≡$\underline{C}$H), 114.70, 117.58, 159.13 (C=O), 162.29 (C=O), 162.65 (C=O), 169.53 (C=O), 175.99 (C=O), 176.85 (C=O), 177.00 (C=O). MS: (ESI) m/z 656.3 [M+H]$^+$, HRMS: calcd. for $C_{30}H_{50}N_5O_{11}$ 656.3501 found 656.3498.



1 **3** **4** **[Re(4)]**

Porphyrin-Lys(OMe)-SA-Lys-urea-Glu (3)

Porphyrin **1** (100 mg, 0.115 mmol) and **6** (115 mg, 0.175 mmol) was taken up in 1:1 t-butanol:water (10 mL). The reaction mixture was added aq. $CuSO_4$ (10 mM, 500 µL), sodium ascorbate (20 mg), and TBTA (1 mg). The reaction mixture was heated in a microwave reactor (3 hours, 75 W, 70 °C). The reaction mixture was concentrated under reduced pressure. The crude was diluted with water, was added ammonium hexafluorophosphate, and the precipitate isolated via filtration. The residue collected was redissolved in acetone, was added tetrabutylammonium chloride, and the precipitate isolated via filtration. The crude was precipitated from diethyl ether over methanol to yield the product as a purple solid (135 mg, 0.089 mmol, 77%).

HPLC: R_f = 8.6 mins. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.30 (m, 20H), 1.59 (s, 2H), 1.97 (m, 6H), 2.93 (m, 5H), 3.67 (s, 3H, O-CH_3), 3.80 (m, 3H, Lys-αCH), 3.97 (m, 2H, αC<u>H</u>-NH-CO-NH-αC<u>H</u>), 4.68 (s, 9H, N-CH_3), 6.25 (d, J = 38.2 Hz, 2H, αCH-N<u>H</u>-CO-N<u>H</u>-αCH), 7.76 (d, J = 52.2 Hz, 2H, N<u>H</u>-suberate-N<u>H</u>), 8.34 (s, 4H, Ph-H), 8.91 (m, 15H, triazole-H, βH, o-Py), 9.41 (m, 6H, m-Py). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.11, 23.30, 25.73, 25.82, 28.99, 29.35, 29.55, 31.61, 32.23, 32.91, 35.85, 35.95, 38.72, 42.96, 48.20 (N-CH_3), 52.07 (O-CH_3), 52.53 (αCH-NH-CO-NH-α<u>C</u>H), 52.86 (α<u>C</u>H-NH-CO-NH-αCH), 60.43, 115.35, 116.06, 118.68, 121.82, 122.21, 132.20, 132.67 (βC), 132.85, 133.64, 135.78, 136.93, 142.62, 144.18 (βC), 148.14, 148.47, 148.75, 148.88, 150.56, 157.66, 158.99, 172.39 (C=O), 172.52 (C=O), 174.61 (C=O), 174.69 (C=O), 175.11 (C=O), 175.68 (C=O). MS: (ESI) m/z 473.5 $[M\text{-}3Cl]^{3+}$, HRMS: calcd. for $C_{74}H_{82}N_{15}O_{11}Zn$ 473.5198 found 473.5191. UV-vis (H_2O, nm): 437, 565, 611. ε (437 nm) = 183000 M cm^{-1}.

Porphyrin-Lys-SA-Lys-urea-Glu (4)

3 (100 mg, 0.065 mmol) was taken up in water (10 mL) and was added LiOH (25 mg, 1.04 mmol). The reaction mixture was allowed to proceed for 3 hours. The reaction was acidified using 1 M HCl to pH 2-3, was added ammonium hexafluorophosphate, and the precipitate isolated via filtration. The residue collected was redissolved in acetone, was added tetrabutylammonium chloride, and the precipitate isolated via filtration. The crude was precipitated from diethyl ether over methanol to yield the product as a purple solid (89 mg, 0.059 mmol, 90%).

HPLC: R_f = 8.2 mins. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.37 (m, 22H), 2.00 (m, 6H), 2.92 (m, 5H), 4.00 (m, 5H, α-CH), 4.67 (s, 9H, N-CH_3), 6.31 (m, 2H, αCH-N<u>H</u>-CO-N<u>H</u>-αCH), 7.80 (d, *J* = 25.5 Hz, 2H, N<u>H</u>-suberate-N<u>H</u>), 8.33 (s, 4H, o,m-Ph), 8.92 (m, 14H, o-Py, βH), 9.06 (s, 1H, triazole-H), 9.41 (s, 6H, m-Py). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.12, 25.72, 25.77, 28.87, 29.33, 29.58, 31.41, 31.86, 32.32, 35.80, 35.89, 38.73, 42.35, 48.18 (N-CH_3), 52.64 (αCH-NH-CO-NH-α<u>C</u>H), 53.00 (α<u>C</u>H-NH-CO-NH-αCH), 60.85, 115.35, 116.06, 118.75, 122.18, 122.87, 132.22, 132.66 (βC), 132.83, 133.68, 135.81, 136.82, 142.75, 144.16 (βC), 148.46, 148.75, 148.86, 150.55, 157.77, 158.97, 172.45 (C=O), 172.52 (C=O), 174.17 (C=O), 174.69 (C=O), 174.77 (C=O), 175.25 (C=O). MS: (ESI) m/z 490.8 $[M-3HCl+3Na]^{3+}$, HRMS: calcd. for $C_{73}H_{77}N_{15}O_{11}ZnNa_3$ 490.8298 found 490.8288. UV-vis (H_2O, nm): 437, 565, 609. ε (437 nm) = 175000 M cm^{-1}.

Porphyrin-[Re(Lys)]-SA-Lys-urea-Glu [Re(4)]

4 (40 mg, 0.026 mmol) was taken up in phosphate buffer (4 mL, pH 7.4, 0.1 M) and was added $[Re(CO)_3Br_3][NEt_4]_2$ (30 mg, 0.039 mmol). The reaction mixture was allowed to proceed at 65 °C for 30 mins. Quantitative conversion was observed on TLC (eluate: 2:1:1 MeCN:H_2O:sat. aq. KNO_3) and HPLC. The reaction mixture was acidified using 1M HCl, was added ammonium hexafluorophosphate, and the precipitate isolated via filtration. The residue collected was redissolved in acetone, was added tetrabutylammonium chloride, and the precipitate isolated via filtration. The crude was precipitated from diethyl ether over methanol to yield the product as a purple solid (35 mg, 0.020 mmol, 75%).

HPLC: R_f = 16.0 mins. ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.65 (m, 30H), 3.06 (m, 5H), 4.26 (m, 3H, α-CH), 4.66 (s, 9H, N-CH_3), 8.39 (s, 4H, o,m-Ph), 8.92 (m, 14H, o-Py, βH), 9.22 (m, 1H, triazole-H), 9.33 (d, *J* = 5.4 Hz, 6H, m-Py). ^{13}C NMR (100 MHz, DMSO-d_6) δ 23.12, 23.52, 25.74, 28.91, 29.10, 29.30, 29.42, 31.53, 32.39, 32.75, 35.88, 35.92, 38.55, 38.75, 48.32 (N-CH_3), 52.75, 52.97, 53.19, 65.98, 115.46, 116.13, 119.58, 121.83, 122.74, 132.48, 132.66 (βC), 133.01, 133.76, 135.86, 135.93, 144.13 (βC), 148.51, 148.81, 148.89, 149.90, 150.45, 157.75, 158.83, 172.83 (C=O), 172.90 (C=O), 173.06 (C=O), 174.71 (C=O), 175.07 (C=O), 175.31 (C=O), 182.65 (C=O), 197.04 (Re-C=O), 197.20 (Re-C=O), 198.52 (Re-C=O). MS: (ESI) m/z 558 $[M-3Cl]^{3+}$, HRMS: calcd. for $C_{76}H_{79}N_{15}O_{14}ReZn$ 558.8252 found 558.8249. UV-vis (H_2O, nm): 437, 565, 615. ε (437 nm) = 127000 M cm^{-1}.

Synthesis of $[Re(CO)_3Br_3][NEt_4]_2$[4]

NEt_4Br tetraethyl ammonium bromide (230 mg, 1.09 mmol) was slurried in 2,5,8-trioxanonane (diglyme) (40 mL) under dry nitrogen and heated to 80 °C. A suspension of $[ReBr(CO)_5]$ (200 mg, 0.49 mmol) in warm 50 °C diglyme (2.7 mL) was slowly added. The mixture was left at 115 °C for 5 hours during which time a white precipitate formed. The reaction mixture was filtered whilst hot and washed with several portions of cold diglyme, diethyl ether and dried under suction filtration. The resulting white powder was then slurried in ethanol (3 cm^3) to remove unreacted NEt_4Br. Filtration and drying in vacuo yielded the product as a white powder (315 mg, 0.410 mmol, 84%).

Elemental analysis calcd. (%) for $C_{19}H_{40}Br_3N_2O_3Re$ C, 29.62; H, 5.23; N, 3.64 found (%) C, 29.86; H, 5.17; N, 3.66.

Radiolabelling with ^{99m}Tc

Synthesis of $[^{99m}Tc(CO)_3(H_2O)_3]^+$

$[^{99m}Tc(CO)_3(H_2O)_3]^+$ was prepared as described previously.[5] Briefly, sodium tetraborate decahydrate (2.9 mg, 7.6 µmol), sodium carbonate (7.8 mg, 73.6 µmol), potassium sodium tartrate tetrahydrate (9.0 mg, 31.9 µmol) and disodium boranocarbonate (4.5 mg, 43.3 µmol) was purged with argon for 10 minutes, after which $Na[^{99m}Tc]TcO_4$ in saline (1 mL, 407-658 MBq) was added and heated at 99 °C for 20 minutes. $[^{99m}Tc(CO)_3]^+$ was analysed using radio-HPLC and radio-TLC. Radio-TLC was carried out on Kieselgel 60 F_{254} plates (1×10 cm, Merck) with mobile phase of 1% HCl in methanol (R_f = 0.2-0.8). For reactions at pH 7.4, $[^{99m}Tc(CO)_3]^+$ was neutralised with 1 M HCl (160 µL) and was buffered with PBS 1× (pH 7.4, 100 µL). pH was measured using universal indicator paper.

^{99m}Tc radiolabelling

Porphyrin-conjugate (500 µL, 0.1 mM and 500 µL, 1 mM) in saline was degassed for 10 minutes using argon. Previously prepared $[^{99m}Tc(CO)_3(H_2O)_3]^+$ (40-45 MBq, 500 µL) was added to the degassed ligand solution and heated at 90 °C for 30 minutes. After cooling, radio-HPLC and radio-TLC analysis were carried out to determine radiochemical yield. Radio-TLC was carried out on Kieselgel 60 F_{254} plates (1×10 cm, Merck) with mobile phase of 0.1 M aqueous 1:1 trisodium citrate:citric acid. This mobile phase gives clear separation between labelled-complex (R_f = 0) and unreduced $[^{99m}TcO_4]^-$ and unreacted $[^{99m}Tc(CO)_3]^+$ (both with R_f = 1).

Determination of partition coefficient ($LogD_{7.4}$)

Lipophilicity was determined using the shake-flask method. Briefly, a solution of tracer (ca. 0.1 MBq) in PBS was diluted to 500 µL in PBS and 500 µL of octanol was added. The solution was vortex mixed at room temperature for 15 minutes. The solutions were centrifuged, a portion of each layer was read in a gamma counter (Wallac). The results are presented as a Log of the ratio of counts in the water:octanol layers and as an average and standard deviation of experiments in triplicate.

FACS protocol

PSMA expression was determined by flow cytometry. 70-80% confluent cells were harvested, aliquoted at 3 x 10^5 cells per sample and centrifuged for 5 min at 200 x g. The supernatant was removed and the cell pellet was resuspended in 97 µL of FACS buffer (1X PBS, 5 mM $MgCl_2$, 1 mM $CaCl_2$) supplemented with 2% FBS. Following the addition of 3 µL of the relevant antibody (for a final volume of 100 µL), cells were incubated for 1 h at room temperature and in the dark. Cells were incubated with either phycoerythrin (PE)-conjugated anti-PSMA monoclonal antibody (GCP-05, Abcam) or PE-conjugated mouse IgG1 (R&D Systems) isotype control antibody to account for non-specific binding. After the incubation, unbound antibody was removed with three washes (200 µL) with FACS buffer. After the last wash, the cell pellet was resuspended in 400 µL of FACS buffer supplemented with 1% formaldehyde and transferred into FACS tubes. Acquisition was carried out on a BD FACSCaliburTM flow cytometer. For each sample, a gate was manually drawn around the population of interest and 10000 events were acquired. The data was analysed with BD CellQuestTM Pro.

In vitro toxicity and phototoxicity evaluation

A stock solution of **2**, **[Re(2)]**, **4**, and **[Re(4)]** were made by dissolving in medium (1-2 mL). The stock was sterilized by filtration through 0.22 µm PES syringe filter unit (Millex-GP). The concentration of the stock was calculated by UV-vis spectroscopy using the extinction coefficient of the conjugate. The stock was diluted further with medium to give the desired concentration range. Phototoxicity and toxicity of **2** and **[Re(2)]** were evaluated on human colorectal adenocarcinoma (HT-29) cells, by incubation of varying concentration of nanoparticles for 1 hour. Phototoxicity and toxicity of **4** and **[Re(4)]** were evaluated on native and transfected prostate carcinoma cells, DU145 and DU145-PSMA, by incubation of varying concentration for 30 mins. 800 µl of the appropriate cells, adjusted to a concentration of $1x10^6$ cells /ml in medium with L-glutamine, was added to 200 µL conjugate solution in a 12×75 mm polystyrene FACS tube (Falcon). The cells were allowed to incubate in the dark for 1 hour at 37 °C and 5% CO_2, after which they were centrifuged with 3× excess of medium to remove unbound compounds. The pellet of cells was resuspended in 1 ml medium and 4 x100 µl of each concentration was put in two 96 wells plates. One plate was irradiated with white light to a dose of 20 J cm^{-2} while the other serves as a dark control. After irradiation, 5 µl of foetal bovine serum (FBS) was added to each well and the plates are returned to the incubator overnight. After 18 to 24 hours, the cell viability was determined using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) colorimetric assay. 10 µL of 12 mM MTT solution was added to each well and incubated between 1 and 4 hours at 37 °C to allow MTT metabolisation. The crystals formed were dissolved by adding 150 µL of acid-alcohol mixture (0.04M HCl in absolute 2-propanol). The absorbance at 570 nm was measured on a Biotek ELX800 Universal Microplate Reader. The results were expressed with respect to control values.

UV-HPLC

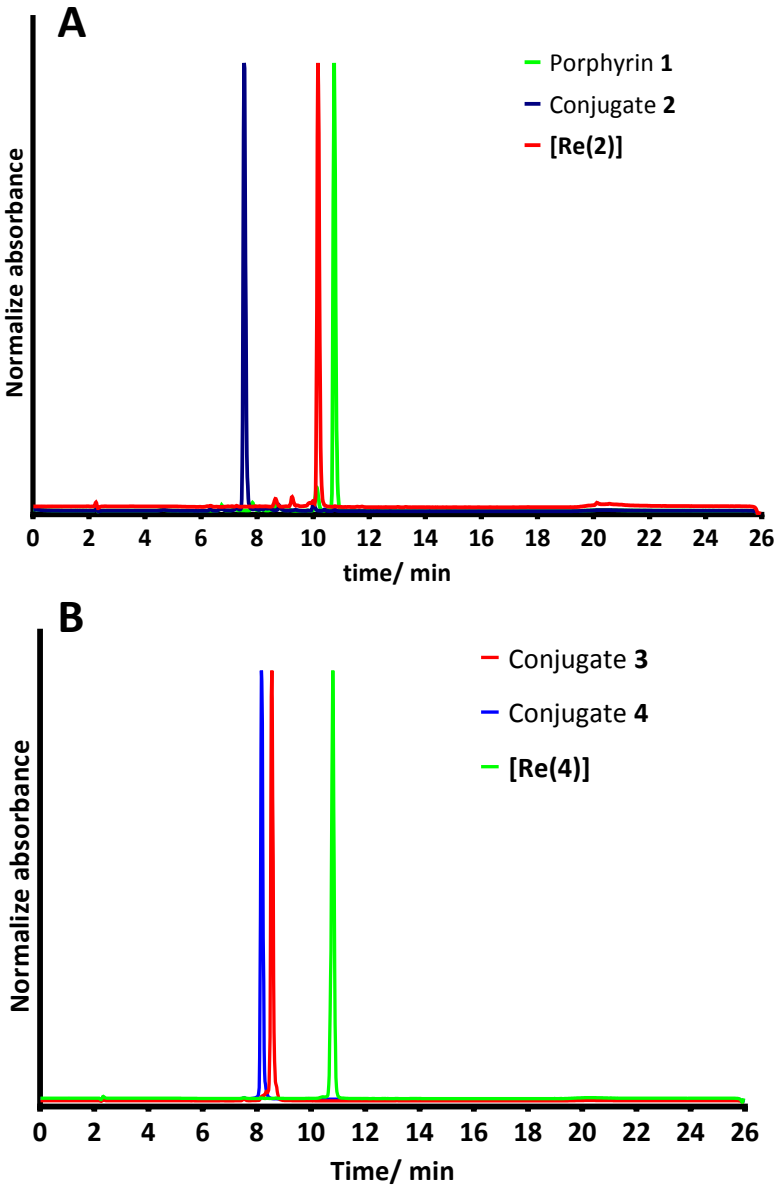


Figure S 1 Analytical-HPLC chromatogram of (A) porphyrin **1**, conjugate **2**, **[Re(2)]**, (B) conjugate **3**, conjugate **4**, and **[Re(4)]**.

Radio-HPLC

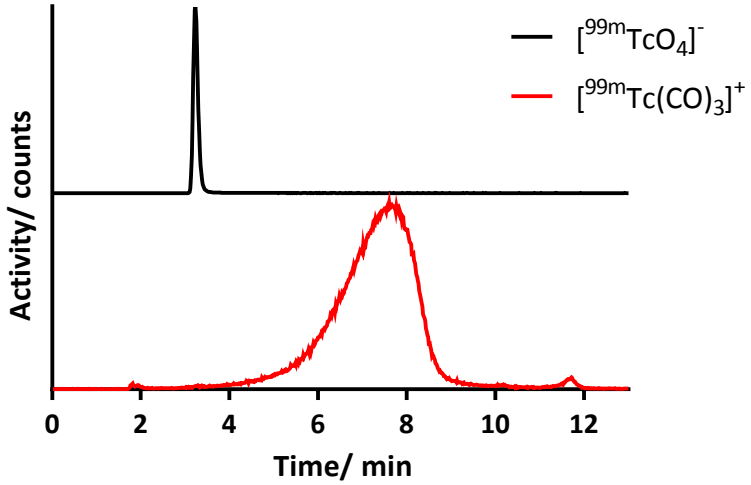


Figure S 2 Radio-HPLC chromatogram of $[^{99m}TcO_4]^-$ and $[^{99m}Tc(CO)_3]^+$.

Radio-TLC

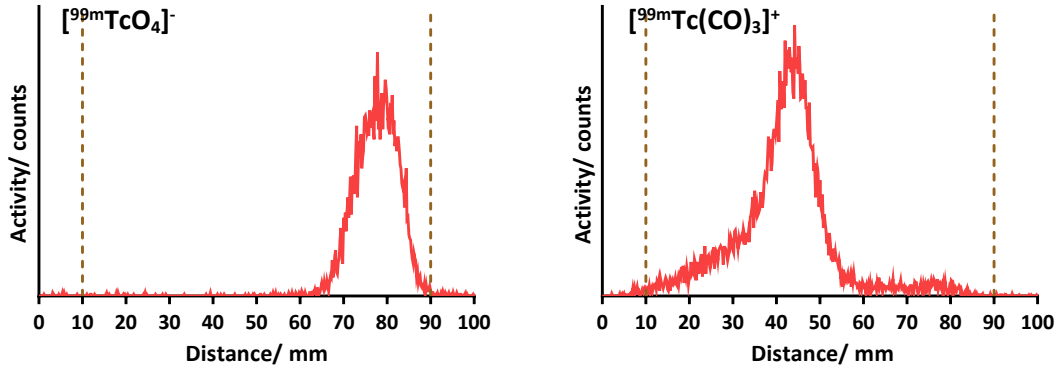


Figure S 3 Radio-TLC chromatograms of reduction of $[^{99m}TcO_4]^-$ to $[^{99m}Tc(CO)_3]^+$. Dotted lines indicate baseline (10 cm, $R_f = 0$) and solvent front (90 cm, $R_f = 1$). Radio-TLC were carried out on aluminium-backed silica TLC plates with 1% HCl in methanol as the mobile phase.

Radiolabelling efficiency with varying ligand concentration

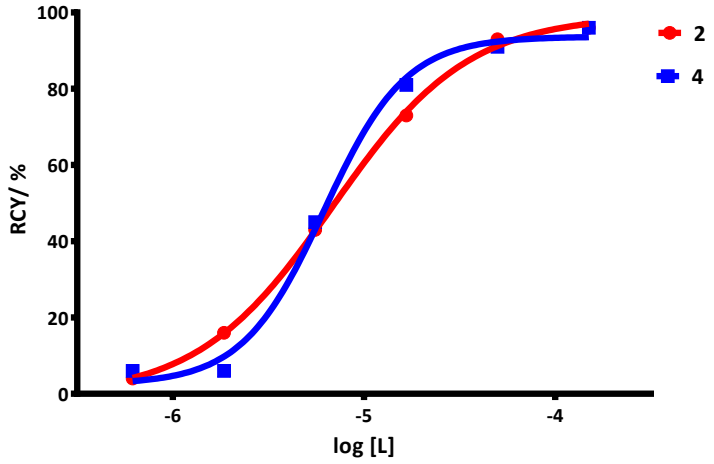
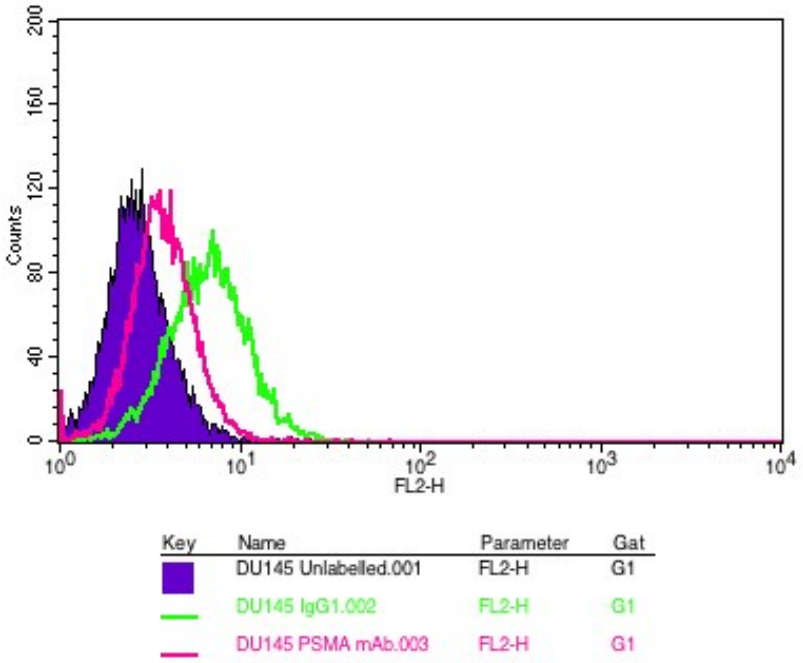


Figure S 4 RCY for the $[^{99m}Tc(CO)_3]^+$ radiolabelling of conjugate **2** and conjugate **4** with varying concentration. Reaction conditions: t = 30 mins, T = 99 °C, pH 7.4.

FACS analysis

DU145



PSMA-DU145

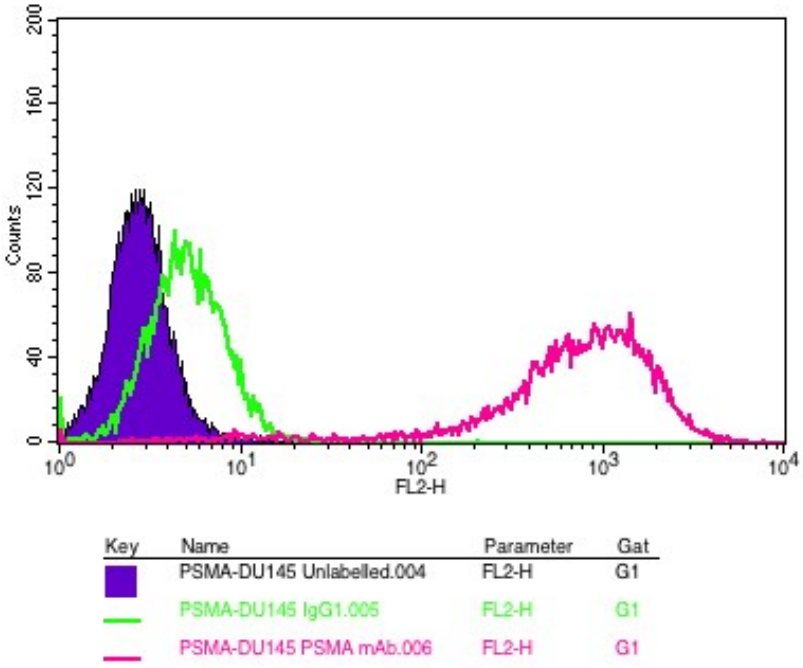


Figure S 5 FACS analysis of cell line DU145 and PSMA-DU145.

In vitro toxicity and phototoxicity evaluation

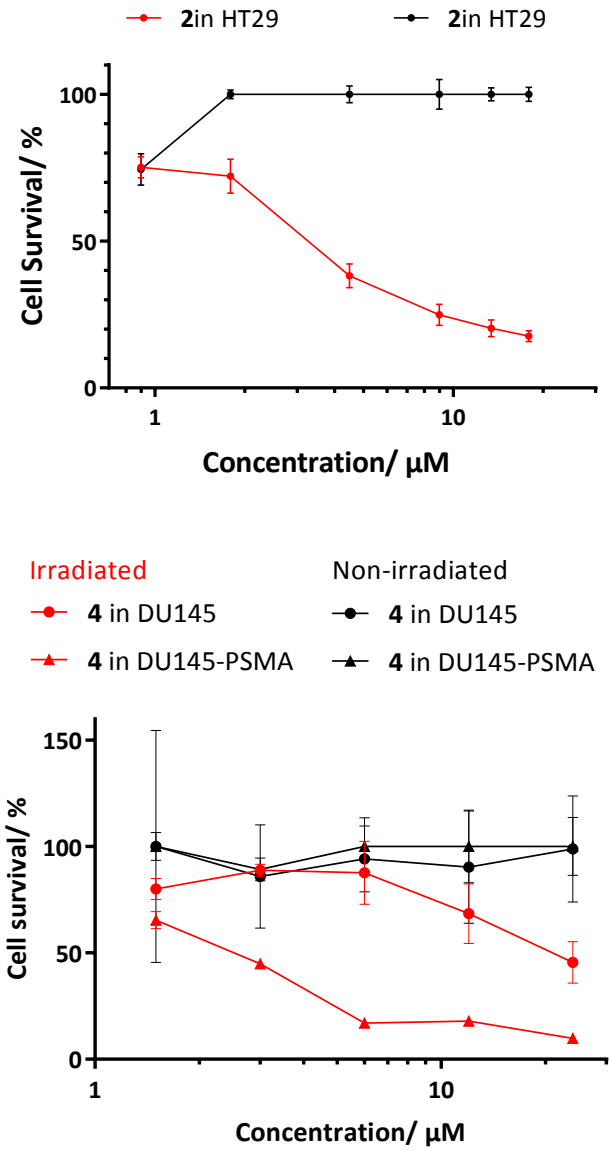
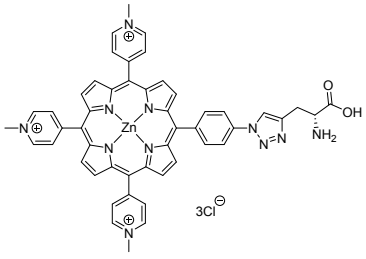
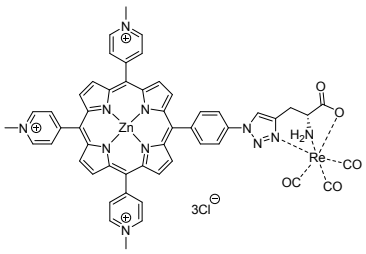


Figure S 6 Percentage cell survival of HT-29 (top) and DU145 and DU145-PSMA (bottom), irradiated (red) and non-irradiated (black) cells, as determined using MTT assay. Cells were incubated with varying concentration of conjugate **2** (top) and **4** (bottom) and irradiated cells received 20 J cm^{-1} white light.

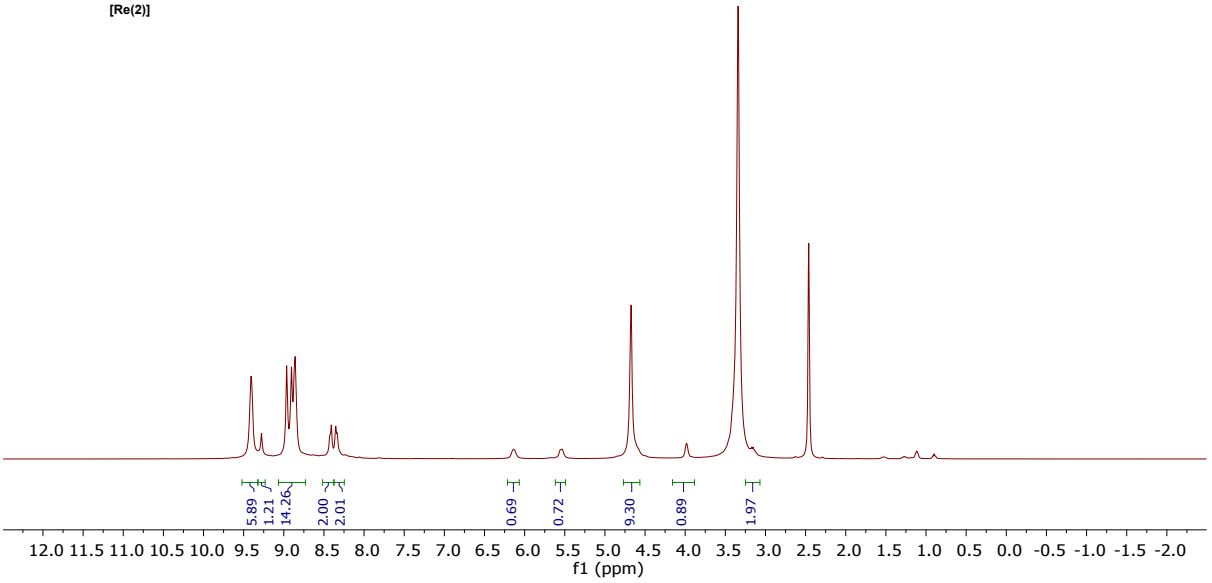
^{1}H NMR spectra

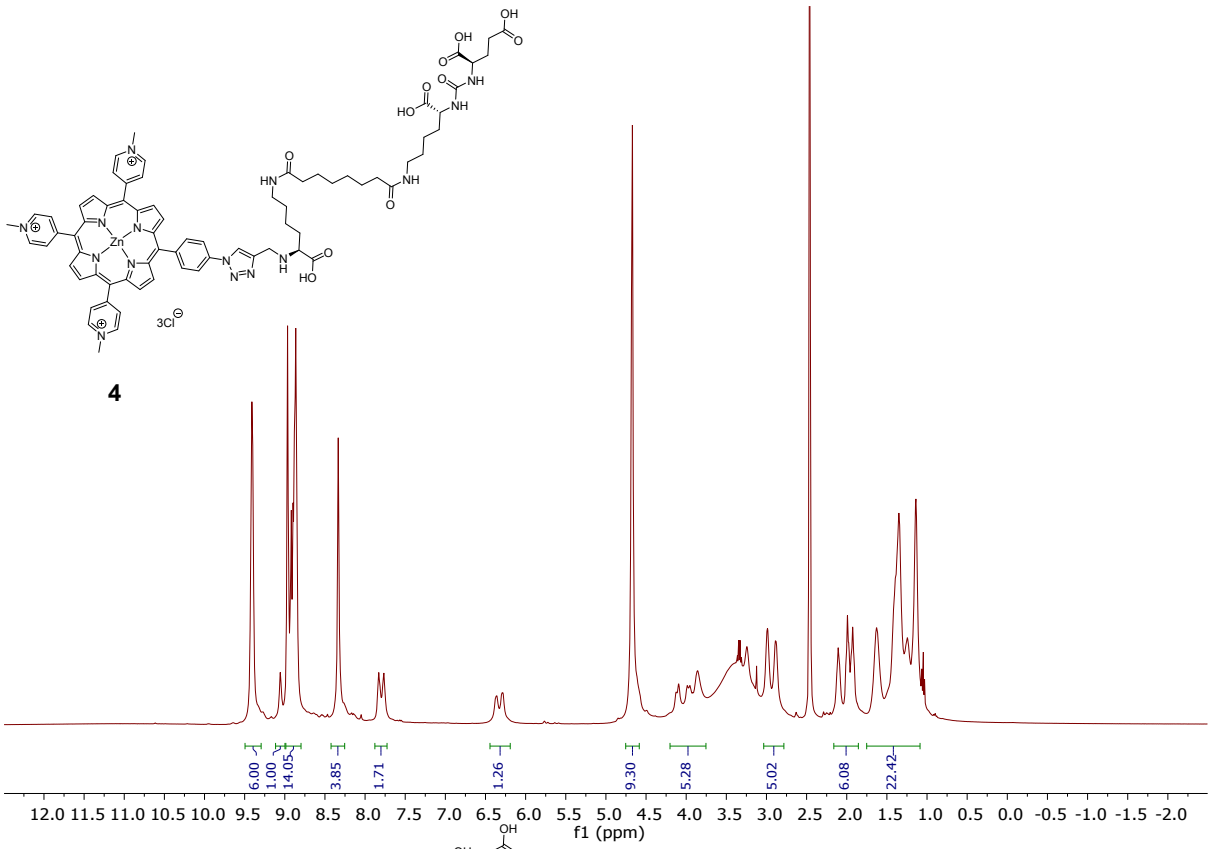


2



[Re(2)]

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5.28
5.02
6.08
22.42
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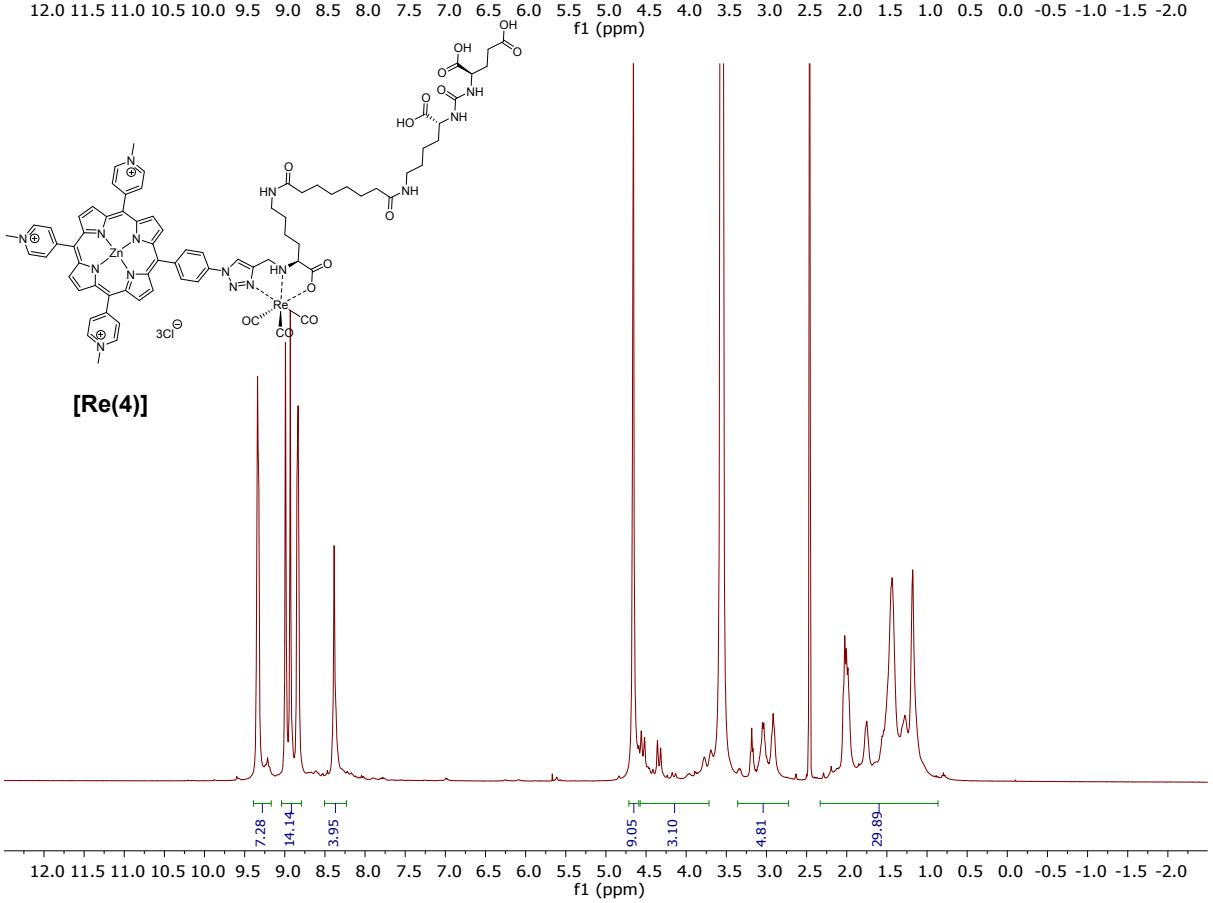
f1 (ppm)

4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3.95
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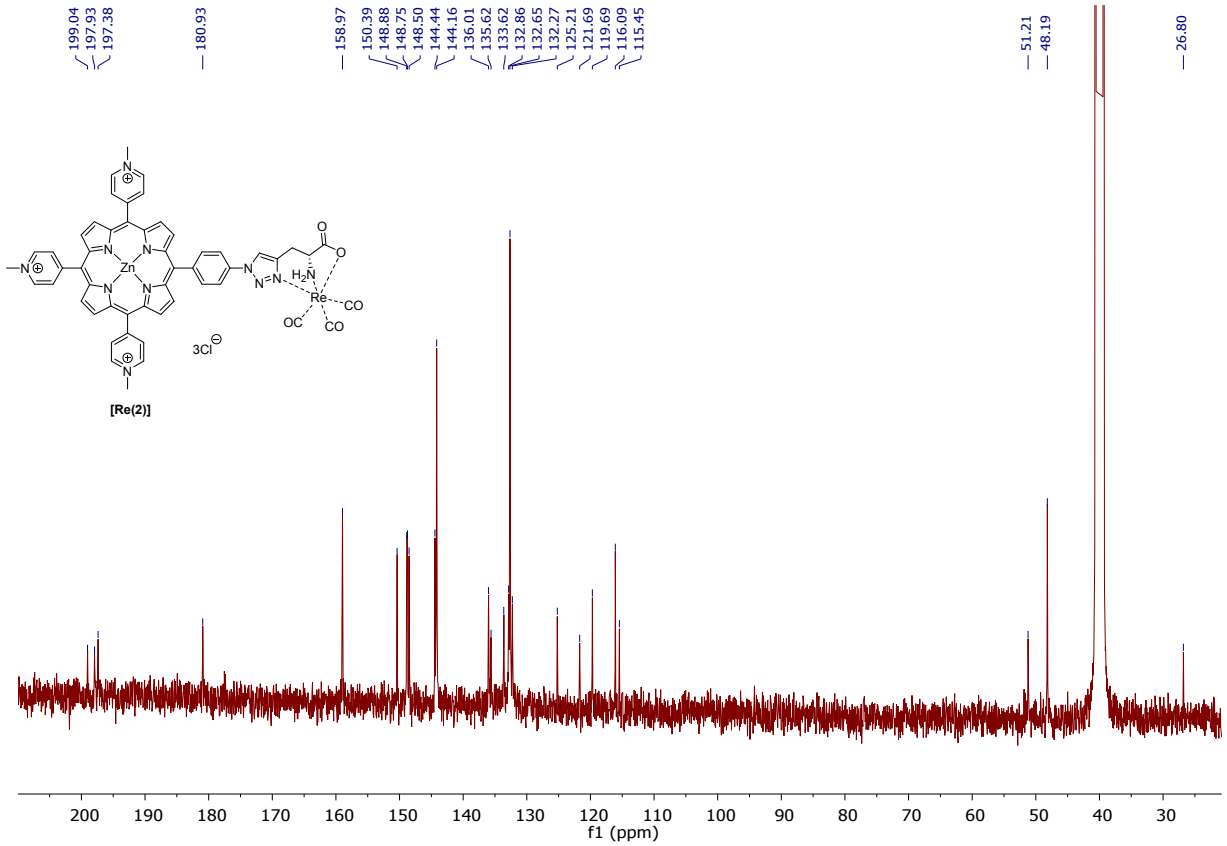
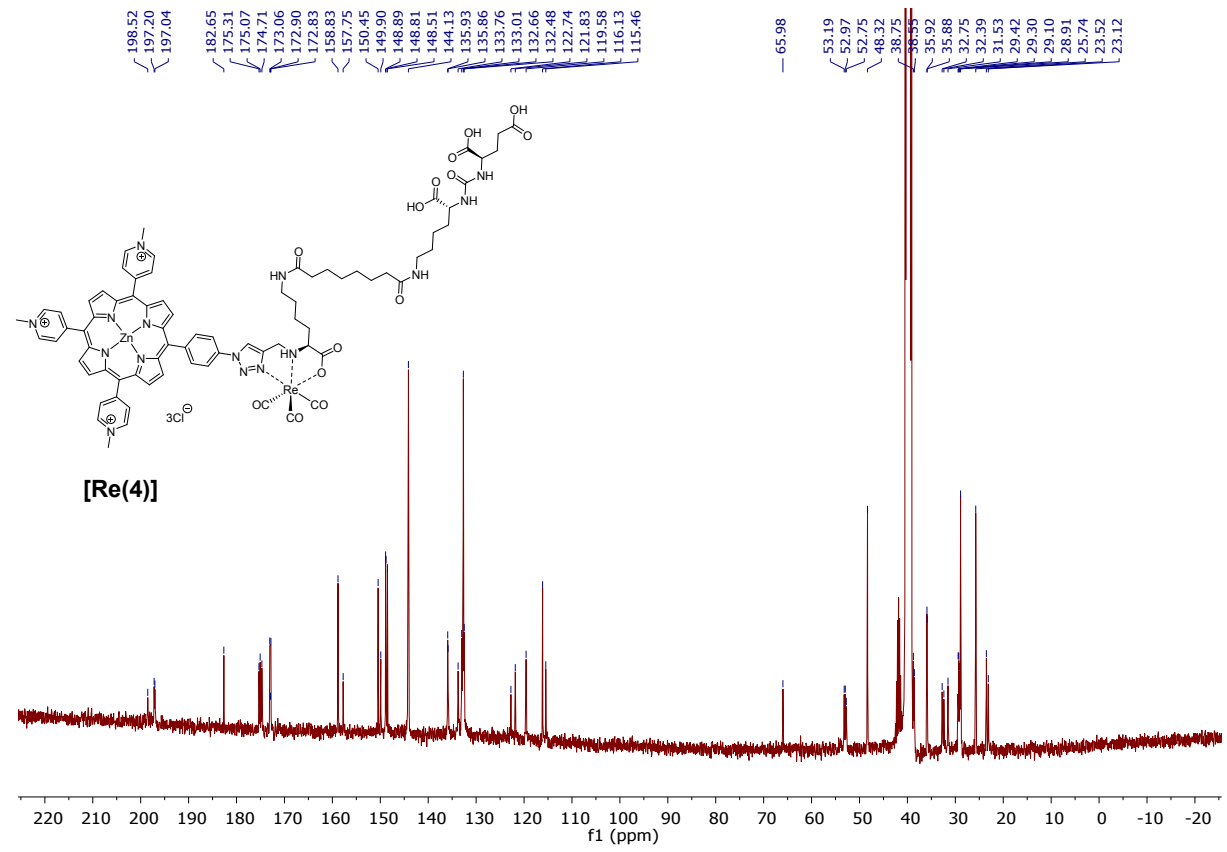
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0 -1.5 -2.0
f1 (ppm)

[Re(4)]

^{13}C NMR spectra



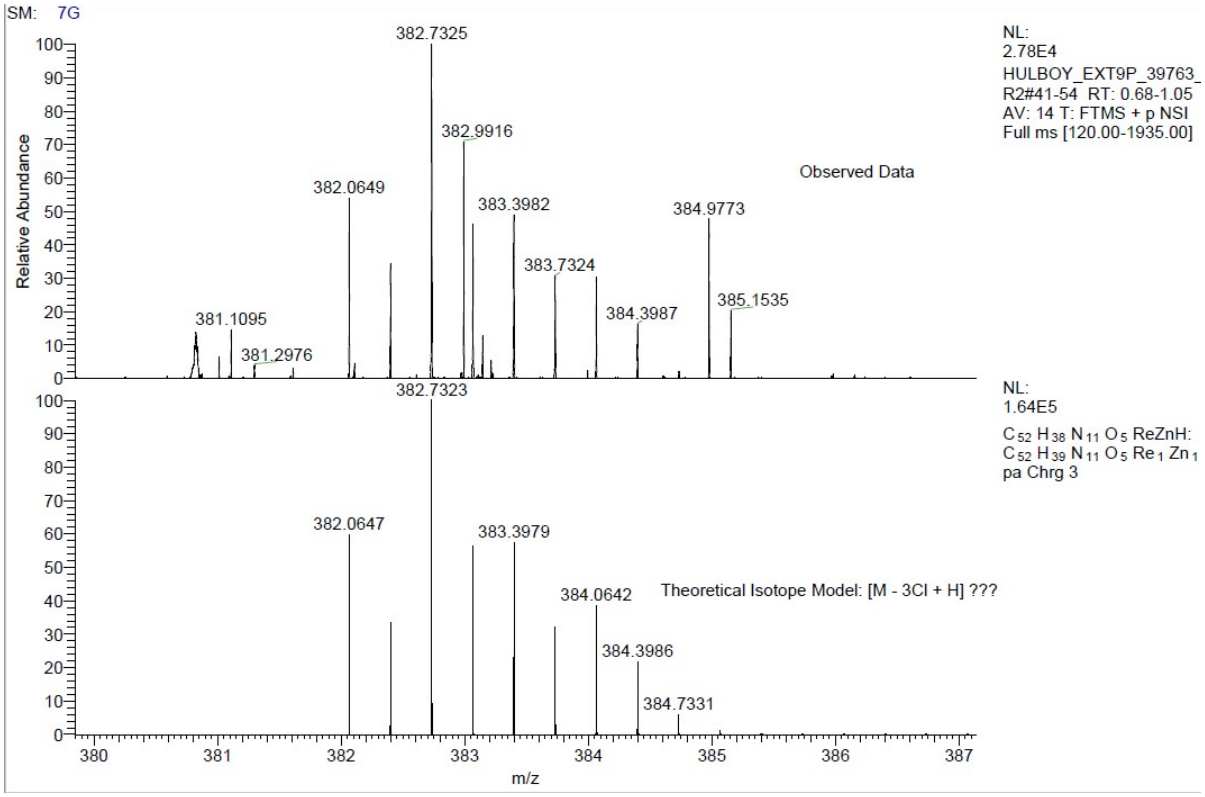
[Re(2)]



[Re(4)]

High resolution mass spectra

[Re(2)]



[Re(4)]

O:\HULBOY_F7934_49307_R_3
(MeOH:H2O)//H2O:MeOH 80:20 + 1%FA
YY212

EPSRC UK National MS Facility
LTQ Orbitrap XL

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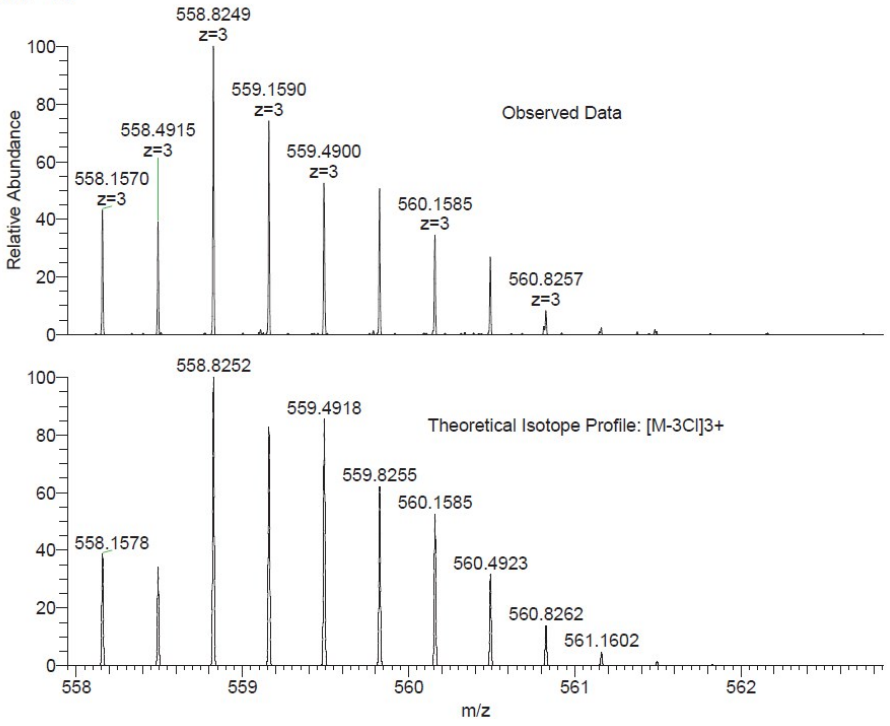
08/11/2018 12:23:32

SM: 7G



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