# Selective radiolabelling with 68Ga under mild conditions: a route towards a porphyrin PET/PDT theranostic agent

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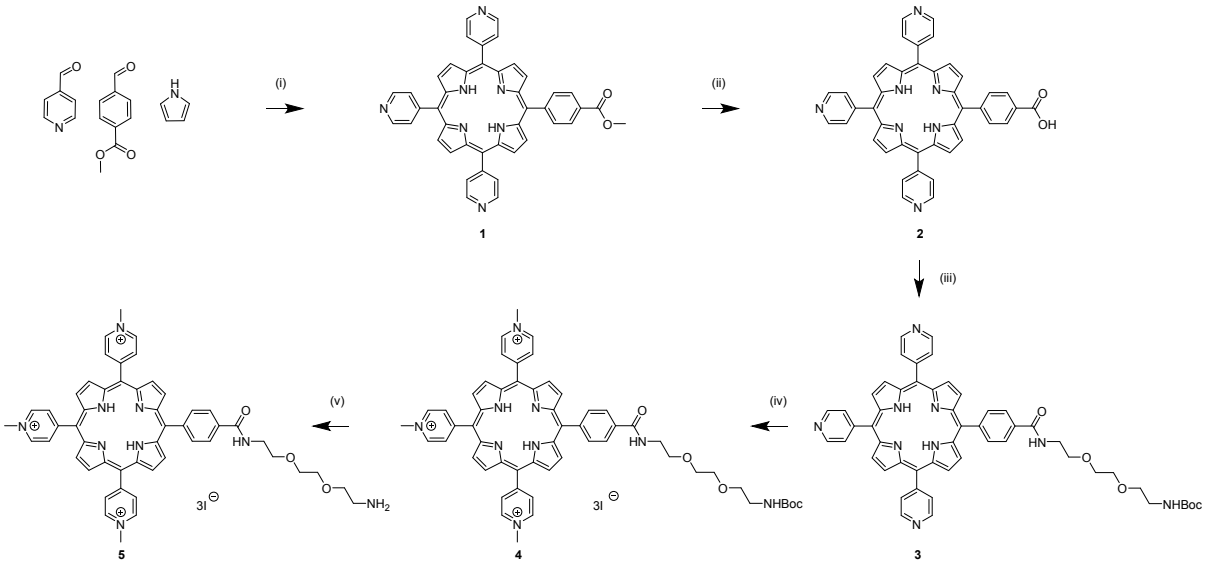
## Experimental section

### General remarks

NMR spectra were recorded on a JEOL ECZ 400S spectrometer at 400 MHz for $^{1}$H NMR and 100.6 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 and VWR. 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](95:5). [2-17](95:5-5:95).[17-19](5:95). [19-21](5:95-95:5). [21-23](95:5).



## Synthesis

**Scheme S1** Synthesis of amine-appended porphyrin **5**. (i) Propionic acid, 170 °C, 1 hr. (ii) EtOH/$H_2O$, KOH, 40 °C, overnight. (iii) DMF, EDC, HOBt, DMAP, r.t., overnight. (iv) DMF, $CH_3I$, 40 °C, overnight. (v) DCM, TFA, r.t., 3 hrs.

### Synthesis of 5-(methyl 4-formylphenyl)-10,15,20-tris-(4-pyridyl)porphyrin (1)

To a stirred solution of methyl 4-formylbenzoate (5.90 g, 36 mmol) and 4-pyridinecarboxaldehyde (10.16 mL, 108 mmol) in propionic acid (500 mL) was added pyrrole (10 mL, 144 mmol) dropwise. The reaction mixture was refluxed at 170 °C for 1 hour. Propionic acid was removed under reduced pressure. The crude was purified by column chromatography (silica, 95:5 DCM:MeOH) and recrystallizes from MeOH over DCM to yield a purple solid (2.03g, 3 mmol, 8.3%).

$^1$H NMR (400 MHz, $CDCl_3$, 298 K), δ: 4.12 (s, 3H, O-$CH_3$), 8.16 (d, 6H, o-Py), 8.29 (d, 2H, o-Ph), 8.46 (d, 2H, m-Ph), 8.86 (m, 8H, βH), 9.05 (m, 6H, m-Py). $^{13}$C NMR (100 MHz, $CDCl_3$, 298 K), δ: 52.62 (O-$CH_3$), 117.52, 117.71, 120.22, 128.17, 129.44 (β-C), 130.08, 134.60, 146.30, 148.44 (β-C), 150.03, 167.24. MS (ESI), m/z: 676 ([M+H]$^+$), HRMS: calcd. for $C_{43}H_{30}N_7O_2$ 676.2455 found 676.2450. UV-vis ($CH_2Cl_2$, nm) 416, 513, 547, 588, 644. ε (416 nm) = 485574 M cm$^{-1}$.

### Synthesis of 5-(4-carboxyphenyl)-10,15,20-tris-(4-pyridyl)porphyrin (2)

To a stirred solution of **1** (1.0 g, 1.48 mmol) in ethanol (100 ml) was added a solution of KOH (4.0 g, 78 mmol) in water (10 ml) and the mixture stirred at 40 °C overnight. The solvent was concentrated under reduced pressure, and the residue neutralised with 1 M HCl. The mixture was filtered, and the crude precipitated from MeOH over DCM to yield the product as a purple solid (957 mg, 1.45 mmol, 97%).

$^1$H NMR (400 MHz, 9:1 $CDCl_3$:$CD_3OH$, 298 K), δ: 8.11 (m, 6H, o-Py), 8.17 (d, 2H, o-Ph), 8.36 (d, 2H, m-Ph), 8.75 (bs, 8H, βH), 8.88 (m, 6H, m-Py). $^{13}$C NMR (100 MHz, 9:1 $CDCl_3$:$CD_3OH$, 298 K): δ: 117, 117.27, 120.52, 128.29, 129.68 (β-C), 130.47, 134.45, 145.98, 147.62 (β-C), 150.74, 168.83 (C=O). MS: (ESI) m/z 660 ( [M-H]$^-$), HRMS: calcd. for $C_{42}H_{26}N_7O_2$ 660.2153 found 660.2139. UV-vis ($CH_3OH$, nm) 413, 510, 546, 591, 641. ε (413 nm) = 82876 M cm$^{-1}$.

### Synthesis of 5-[4-(2-(2-(2-Boc-aminoethoxy)ethoxy)ethaneaminocarbonyl)phenyl]-10,15,20-tris-(4-pyridyl)porphyrin (3)

To a stirred solution of **2** (300 mg, 0.454 mmol) in DMF (30 mL) was added 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (174 mg, 0.907 mmol), 1-hydroxybenzotriazole (HOBt) (123 mg, 0.907 mmol), *N*-Boc-2,2'-(ethylenedioxy)-diethylamine (225 mg, 0.907 mmol), and 4-dimethylaminopyridine (DMAP) (111 mg, 0.907 mmol). The reaction mixture was allowed to stirred overnight at room temperature. Solvent was co-evaporated with toluene under reduced pressure. The crude was redissolved in DCM and washed with brine (3×50 mL). The organic layer was dried and the solvent removed under reduced pressure. The crude was precipitated from hexane over DCM to yield a purple solid (361 mg, 0.405 mmol, 89%).

$^1$H NMR (400 MHz, $CDCl_3$, 298 K), δ: -2.91 (s, 2H, NH), 1.39 (s, 9H, $C(CH_3)_3$), 3.28 – 3.41 (m, 2H, $CH_{2\ peg}$), 3.55 – 3.64 (m, 2H, $CH_{2\ peg}$), 3.74 (m, 4H, $CH_{2\ peg}$), 3.80 – 3.90 (m, 4H, $CH_{2\ peg}$), 8.12 – 8.17 (m, 6H, o-Py), 8.22 (d, J = 7.9 Hz, 2H, o-Ph), 8.28 (d, J = 7.7 Hz, 2H, m-Ph), 8.85 (q, J = 4.7 Hz, 8H, βH), 8.97 – 9.09 (m, 6H, m-Py). $^{13}$C NMR (100 MHz, $CDCl_3$, 298 K), δ: 28.49, 40.14, 40.44, 70.41, 117.68, 125.74, 129.44 (C-β), 131.23, 134.70, 148.49 (C-β), 149.98. MS: (ESI) m/z 892 ($[M+H]^+$), HRMS: calcd. for $C_{53}H_{50}N_9O_5$ 892.3929 found 892.3933. UV-vis ($CH_2Cl_2$, nm): 417, 513, 549, 588, 643.

**Synthesis of 5-[4-(2-(2-(2-Boc-aminoethoxy)ethoxy)ethaneaminocarbonyl)phenyl]-10,15,20-tris-(N-methyl-4-pyridinium)porphyrin triiodide (4)**

To a stirred solution of **3** (350 mg, 0.393 mmol) in DMF (35 mL) was added methyl iodide (4 mL, 0.064 mol) *via* a syringe. The reaction mixture was heated to 40 °C and was allowed to proceed overnight. The reaction mixture was allowed to cool to room temperature and diethyl ether added to promote precipitation and the precipitate was filtered through cotton wool. The residue was redissolved in MeOH and diethyl ether was added. Precipitate formed was filtered and collected to yield a purple solid (426 mg, 0.323 mmol, 82%).

$^1$H NMR (400 MHz, DMSO-$d_6$, 298 K), δ: -3.07 (s, 2H, NH), 3.07 (m, 2H, $CH_{2\ peg}$), 3.40 (m, 2H, $CH_{2\ peg}$), 3.60 (m, 8H, $CH_{2\ peg}$), 4.69 (s, 9H, N-$CH_3$), 8.32 (m, 4H, o,m-Ph), 9.01 (m, 14H, βH, o-Py), 9.45 (m, 6H, m-Py). $^{13}$C NMR (100 MHz, DMSO-$d_6$, 298 K), δ: 15.72, 28.77, 48.45, 65.46, 69.57, 69.76, 70.10, 70.18, 78.16, 115.29, 115.93, 122.39, 126.62, 132.64, 134.74, 143.59, 144.72, 156.16, 157.04, 166.61. MS: (ESI) m/z 312 ($[M-3I]^{3+}$), HRMS: calcd. for $C_{56}H_{58}N_9O_5$ 312.1515 found 312.1518. UV-vis ($H_2O$, nm): 422, 519, 560, 585, 656. ε (422 nm) = 207367 M cm$^{-1}$.

**Synthesis of 5-[4-(2-(2-(2-aminoethoxy)ethoxy)ethaneaminocarbonyl)phenyl]-10,15,20-tris-(*N*-methyl-4-pyridinium)porphyrin (5)**

**4** (50 mg, 0.0380 mmol) was added dry DCM (5 mL) and TFA (190 µL, 283 mg, 2.48 mmol). The reaction mixture was allowed to stir at room temperature for 3 hours. The solvent was removed under reduced pressure to give the product as a purple solid. The product formed was immediately redissolved in dry DMF and was used for subsequent reaction.

$^1$H NMR (400 MHz, DMSO-$d_6$, 298 K), δ: -3.07 (s, 2H, NH), 2.95 (t, J = 5.3 Hz, 2H, $CH_{2\ peg}$), 3.63 (m, 10H, $CH_{2\ peg}$), 4.72 (s, 9H, N-$CH_3$), 8.29 (d, J = 8.2 Hz, 2H, o-Ph), 8.38 (d, J = 8.2 Hz, 2H, m-Ph), 9.02 (m, 14H, βH, m-Py), 9.53 (m, 6H, o-Py). $^{13}$C NMR (100 MHz, DMSO-$d_6$, 298 K), δ: 38.98, 48.33, 67.19, 69.51, 70.06, 70.29, 115.32, 115.96, 122.39, 126.70, 131.56, 132.64 (Cβ), 134.72, 143.58, 144.78 (Cβ), 146.07, 156.93, 157.00, 166.61. MS: (ESI) m/z 278 ($[M-3Cl]^{3+}$), HRMS: calcd. for $C_{51}H_{50}N_9O_3$ 278.8007 found 278.8008. UV-vis ($H_2O$, nm): 422, 518, 558, 585, 656. ε (422 nm) = 200671 M cm$^{-1}$.

**Synthesis of *N*-Boc-2,2'-(ethylenedioxy)-diethylamine**

2,2'-(ethane-1,2-diylbis(oxy))diethaneamine (20 mL, 0.136 mol) was dissolved in dry DCM (50 mL) and cool to 0 °C under nitrogen. Di-*tert*-butyl dicarbonate (9.97 g, 0.045 mol) was dissolved in dry DCM (50

mL) was added dropwise over a period of 1 hour. The reaction mixture was allowed to warm up to room temperature and stirred overnight under nitrogen. The solution was diluted with DCM (100 mL) and washed with water (3×200 mL). The aqueous layer was extracted with DCM and the organic layer dried and the solvent removed under reduced pressure. The crude was purified using column chromatography (silica, 5-25% MeOH:DCM) to yield a colourless oil (8.4 g, 33.8 mmol, 75%)

$^1$H NMR (400 MHz, $CDCl_3$, 298 K), δ: 1.43 (s, 9H), 2.87 (t, J = 5.2 Hz, 2H), 3.31 (q, J = 5.2 Hz, 2H), 3.52 (dt, J = 10.5, 5.2 Hz, 4H), 3.61 (s, 4H). $^{13}$C NMR (100 MHz, $CDCl_3$, 298 K), δ: 28.50, 40.40, 41.49, 70.27, 72.51. MS: (ESI) m/z 248 ($[M+H]^+$), HRMS: calcd. for $C_{11}H_{25}N_2O_4$ 249.1809 found 249.1808.

**Synthesis of $H_4$Dpaa.ga (6)**

$H_4$Dpaa.ga was synthesised as described in previous literature.[2]

**Synthesis of porphyrin-$H_3$Dpaa conjugate (9b)**

To $H_4$Dpaa.ga, **6** (79 mg, 0.19 mmol) was added acetic anhydride (29 mg, 27 µL, 0.29 mmol), dry pyridine (45 mg, 46 µL, 0.57 mmol), and dry acetonitrile (500 µL). The reaction mixture was allowed to proceed at room temperature for 30 mins. Dry diethyl ether (1 mL) was added dropwise to promote precipitation. The resulting suspension was filtered through celite and washed with dry diethyl ether (1 mL). $H_2$Dpaa-anhydride **7** was eluted from celite using dry DMF (1 mL) and was collected in the reaction flask. To this solution was added dry TEA (57 mg, 79 µL, 0.57 mmol) and *N*-hydroxysuccinimide (75 mg, 0.65 mmol). The reaction mixture was allowed to proceed at room temperature for 2 hours to give $H_3$Dpaa-NHS **8**. The product was not isolated, and the reaction mixture was used immediately for subsequent reaction.

**5** was redissolved in dry DMF (2 mL) and to this was added TEA (57 mg, 79 µL, 0.57 mmol) and $H_3$Dpaa-NHS **8** reaction mixture above. The reaction mixture was allowed to proceed at room temperature overnight. The solvent was co-evaporated with toluene under reduced pressure. The crude was redissolved in 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 residue was precipitate from diethyl ether over MeOH to yield a clay red solid (42 mg, 0.031 mmol, 82%).

$^1$H NMR (400 MHz, DMSO-$d_6$, 298 K), δ: -3.10 (s, 2H, NH), 1.86 (m, 2H, O=C-$CH_2$C$\underline{H}_2$αCH), 2.28 (m, 2H, O=C-C$\underline{H}_2$$CH_2$αCH), 3.50 (m, 19H, $CH_2$PEG, αC-H, residual $H_2O$), 3.83 (m, 4H, Py-$CH_2$-N), 4.72 (s, 9H, N-$CH_3$), 7.44 (d, J = 7.1 Hz, 2H, dpaa-Py), 7.63 (dq, J = 17.6, 7.7 Hz, 4H, dpaa-Py), 8.26 (d, J = 7.8 Hz, 2H, o-Ph), 8.36 (d, J = 7.8 Hz, 2H, m-Ph), 9.05 (m, 14H, βH, porphyrin-o-Py), 9.52 (s, 6H, porphyrin-m-Py). $^{13}$C NMR (100 MHz, DMSO-$d_6$, 298 K) δ 1.44, 15.71, 23.81, 25.77, 32.41, 48.32, 56.62, 57.07, 63.02, 65.45, 69.50, 69.65, 69.99, 70.11, 70.22, 123.12, 125.68, 126.67, 132.66, 134.83, 137.81, 144.81, 149.23, 157.01, 159.98, 166.58, 166.93, 167.10, 172.20, 172.37, 174.50, 174.83. MS: (ESI) m/z 411 ($[M-3Cl]^{3+}$), HRMS: calcd. for $C_{70}H_{67}N_{12}O_{10}$ 411.8362 found 411.8362. UV-vis ($H_2O$, nm): 426, 520, 561, 589, 643. ε (424 nm) = 216288 M $cm^{-1}$.

**Synthesis of porphyrin-[Ga(Dpaa)] conjugate (10)**

To porphyrin-$H_3$Dpaa conjugate **9** (25 mg, 18.7 µmol) was added a solution of $GaCl_3$ (550 µL, 51 mM, 28.1 µmol) in acetate buffer (1 mL, 0.1 M, pH 4.5). The reaction was allowed to proceed at room temperature overnight. $NH_4PF_6$ was added to the reaction mixture, and the precipitate isolated via filtration. The residue collected was redissolved in acetone, was added tetrabutylammonium chloride, and the precipitate isolated via filtration. Removal of excess Ga(III) was confirmed by xylenol orange

assay. The residue was precipitated from diethyl ether over MeOH to yield a purple solid (19 mg, 13.3 μmol, 71%).

$^{1}$H NMR (400 MHz, DMSO-$d_6$, 298 K) δ: -3.09 (s, 2H, NH), 1.96 (m, 4H, O=C-C$\underline{H}_2$C$\underline{H}_2$αCH), 3.07 (m, 3H, $CH_2$PEG, αC-H), 3.60 (m, 12H, $CH_2$PEG, Py-$CH_2$-N), 4.71 (s, 9H, N-$CH_3$), 8.10 (m, 10H, Dpaa-Py, o,m-Ph), 9.04 (m, 14H, βH, porphyrin-o-Py), 9.50 (s, 6H, porphyrin-m-Py). $^{13}$C NMR (100 MHz, DMSO-$d_6$, 298 K) δ: 15.71, 48.42, 65.46, 69.50, 69.67, 70.13, 70.23, 115.23, 115.98, 122.32, 126.58, 132.65 (βC), 134.69, 144.78 (βC), 144.91, 151.80, 156.84, 157.03, 166.61, 172.34. MS: (ESI) m/z 650 ([M-3Cl-H]$^{2+}$), HRMS calcd. for $C_{70}H_{63}GaN_{12}O_{10}$ 650.2017 found 650.2013. UV-vis ($H_2O$, nm): 424, 519, 557, 588, 642. ε (424 nm) = 195424 M cm$^{-1}$.

## Radiochemistry

The IGG100 generator was eluted with 0.6 M aq. HCl (4 mL). This eluate (300–200 MBq) was diluted with $H_2O$ (20 mL) and passed through a Strata-X-C 33 μM Cation Mixed-mode polymeric support. The activity was liberated from the column using 98:2 acetone:0.1 M aq. HCl (1 mL). Aliquots (∼30 MBq) of this solution were dried at 90 °C and allowed to cool before use.

1 mL of porphyrin-$H_3$Dpaa conjugate **9** (0.1 mM) in buffered solution was added to the cool and dried $^{68}$Ga and shaken. 5 μL aliquots were taken for analysis by TLC and 20 μL aliquots for analysis by HPLC. TLC analysis was performed on Kieselgel 60 F254 plates (Merck) with an eluate of 0.1 M aqueous 1:1 trisodium citrate:citric acid. Radiochemical yield (RCY) was determined using radio-HPLC via integration on the intensity of the peak with reference to free gallium which eluted at solvent front.

## Cytotoxicity assays

A stock solution of **10** was made by dissolving in medium (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. 800 μl of the appropriate cells (HT-29 colon adenocarcinoma, 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 **10**. 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.

**HPLC trace**

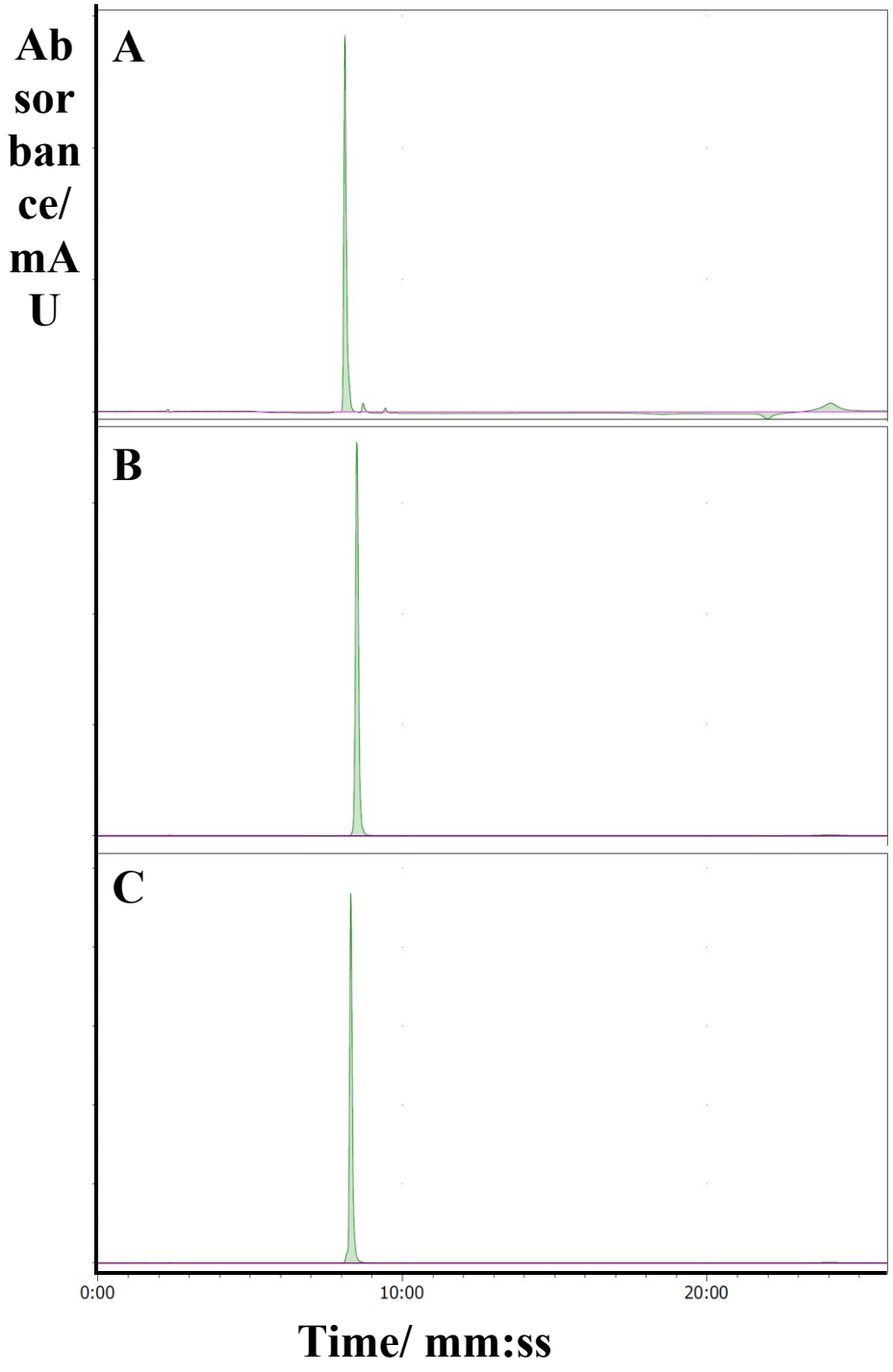



Figure S 1 HPLC trace of (A) **5**, (B) **9**, and (C) **10**.

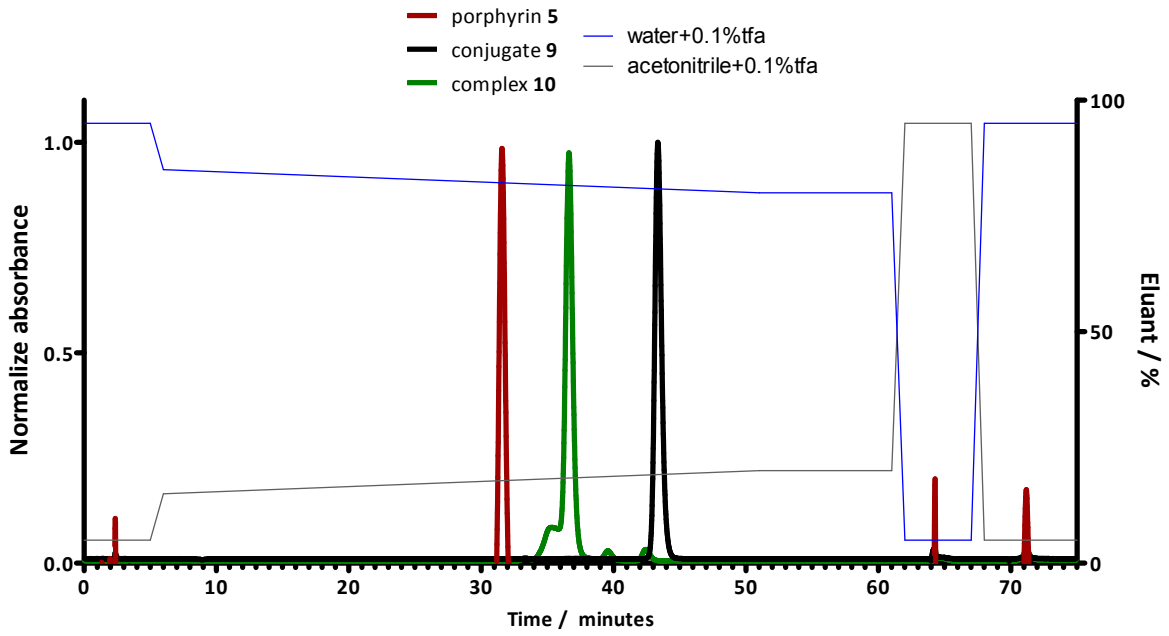



Figure S 2 HPLC chromatograms of porphyrin **5**, conjugate **9**, and complex **10** in longer HPLC conditions.

## Radio-HPLC

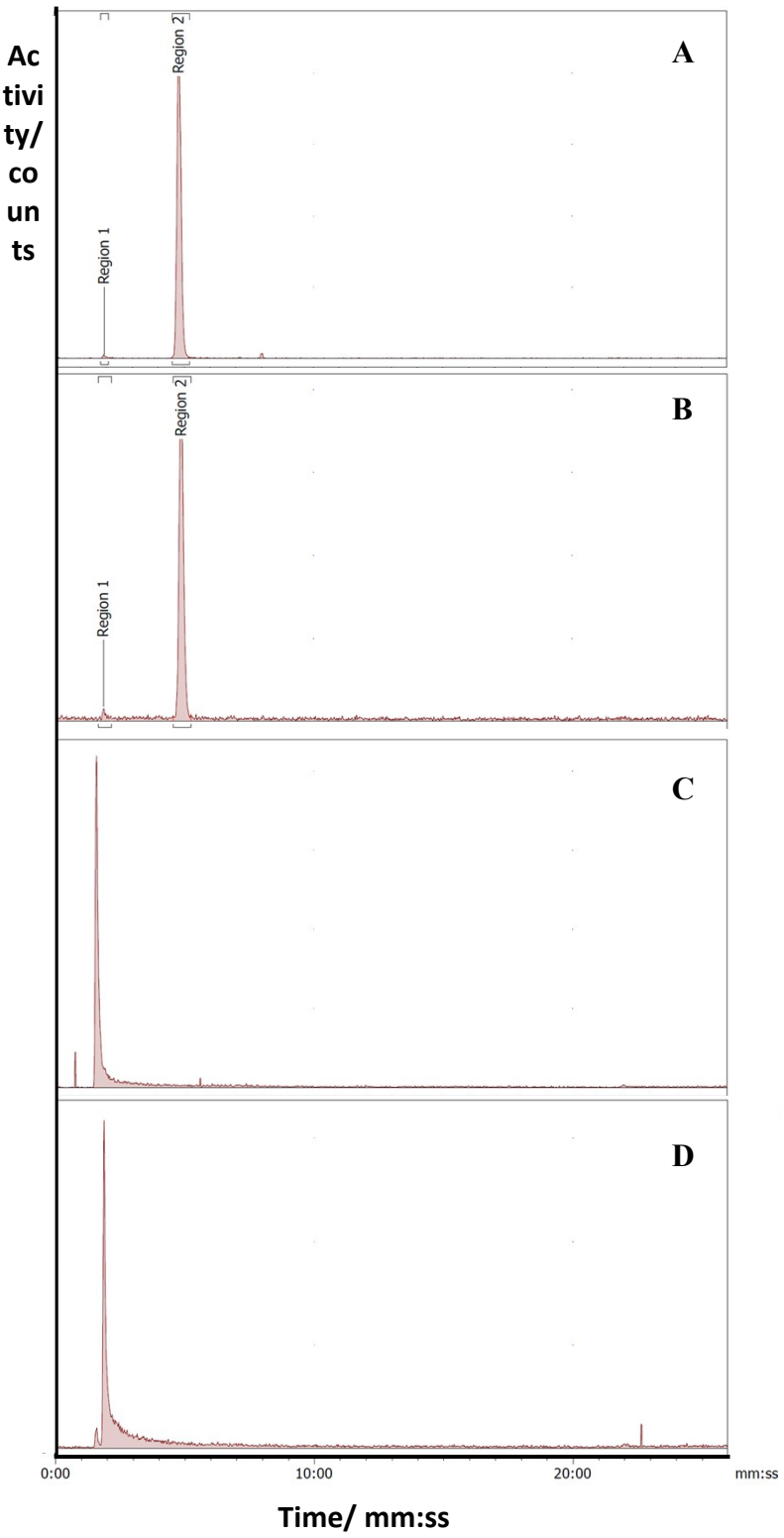



Figure S 3*:* Radio-HPLC chromatograms of crude reaction mixtures. A) $^{68}GaCl_3$ incubated with **6** for 15 minutes, pH 4.5, RT. B) $^{68}GaCl_3$ incubated with **6** for 15 minutes, pH 7.4, RT. C) $^{68}GaCl_3$ incubated with **5** for 15 minutes, pH 4.5, 99 °C, D) $^{68}GaCl_3$ incubated with **5** for 15 minutes, pH 7.4, 99 °C.

## Radio-TLC

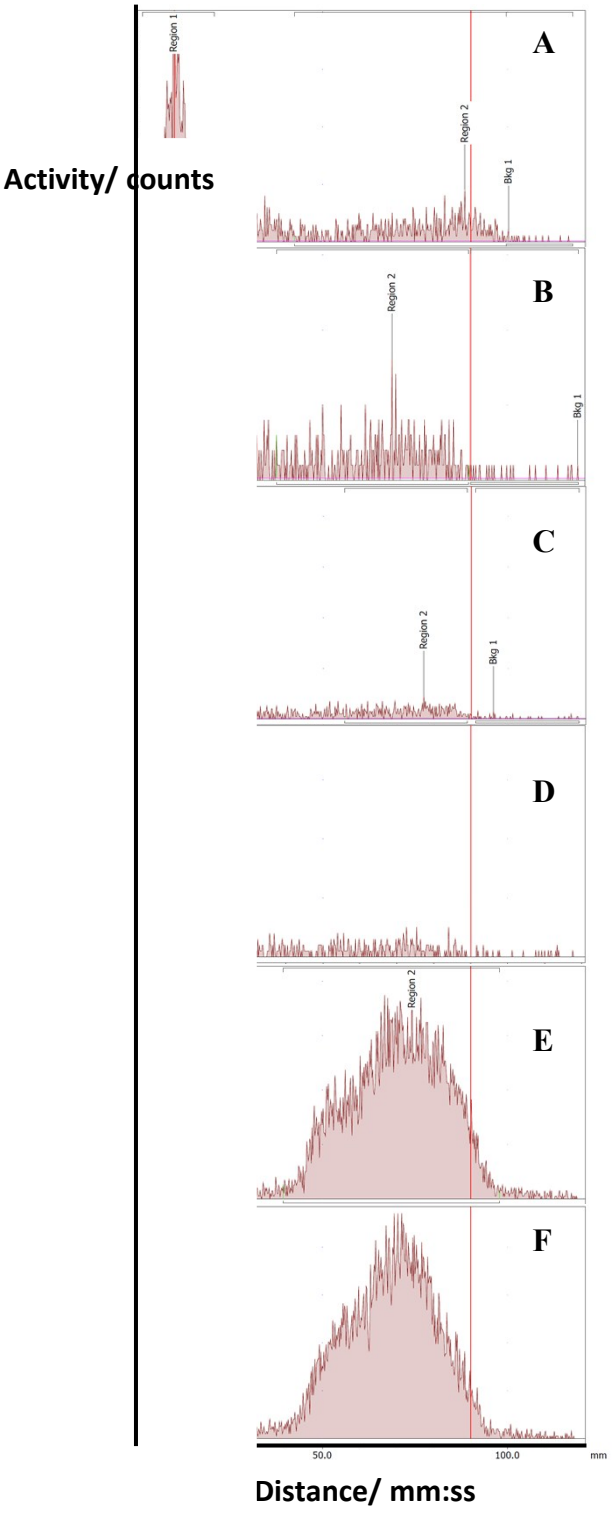



Figure S 4 Radio-TLC for radiolabelling of **9** at pH 4.5 (A) and pH 7.4 (B), **6** at pH 4.5 (C) and pH 7.4 (D), and **5** at pH 4.5 (E) and pH 7.4 (F).

## NMR spectra

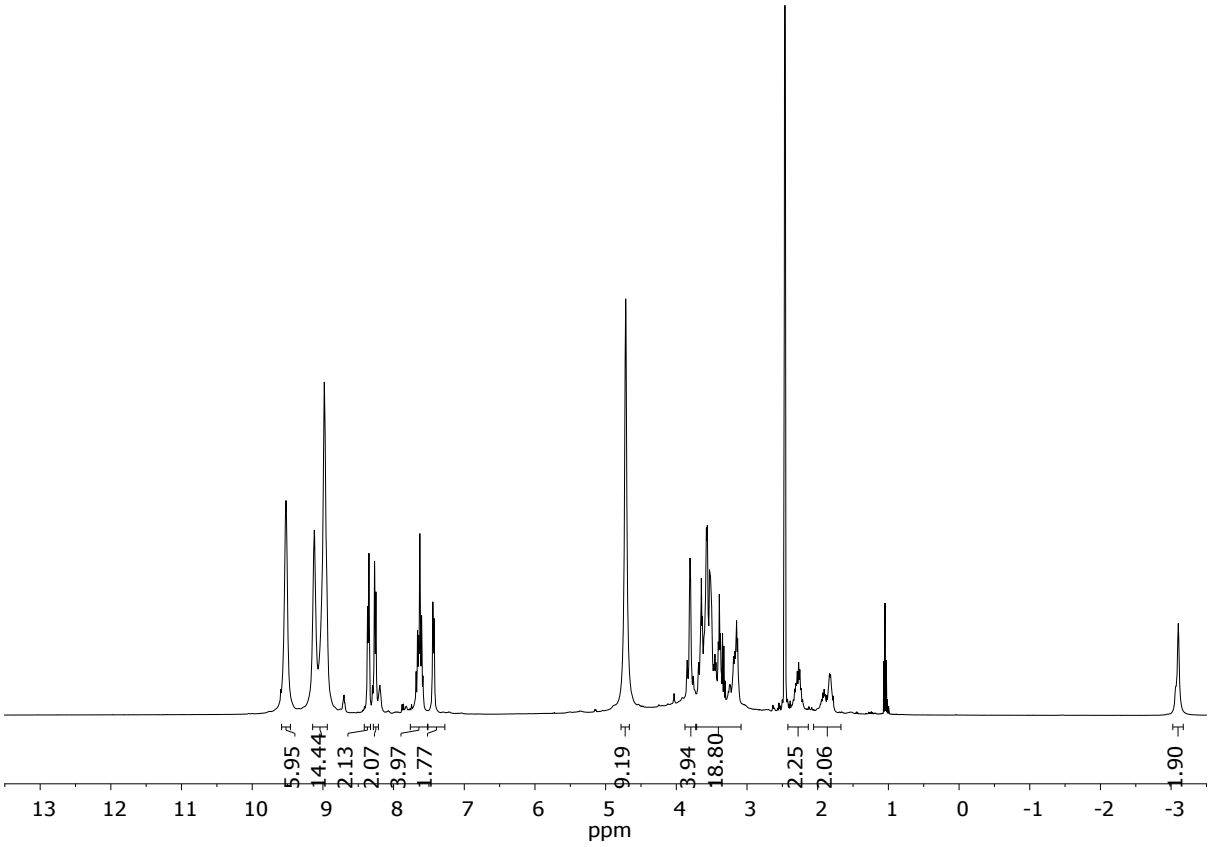



Figure S 5 $^{1}$H NMR of porphyrin-$H_3$Dpaa **9**.

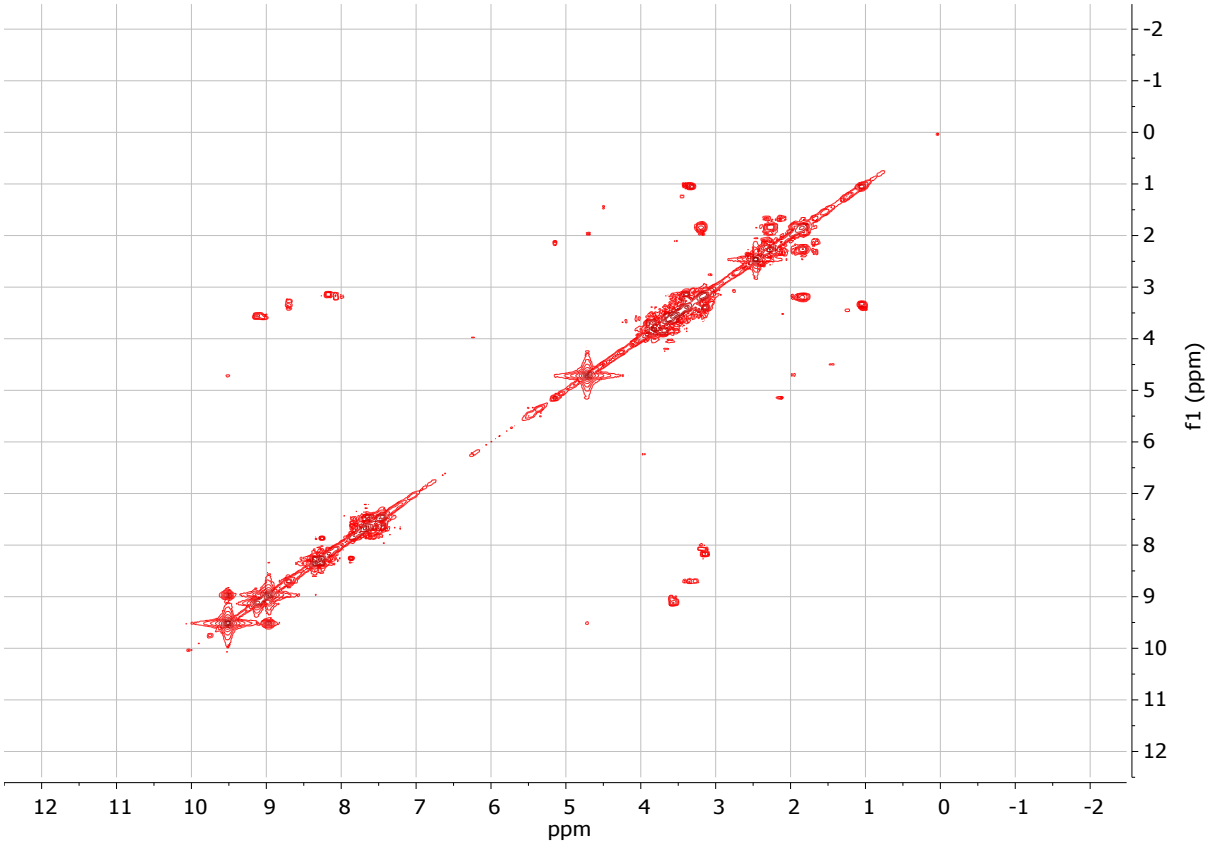



Figure S 6 COSY of porphyrin-$H_3$Dpaa **9**.

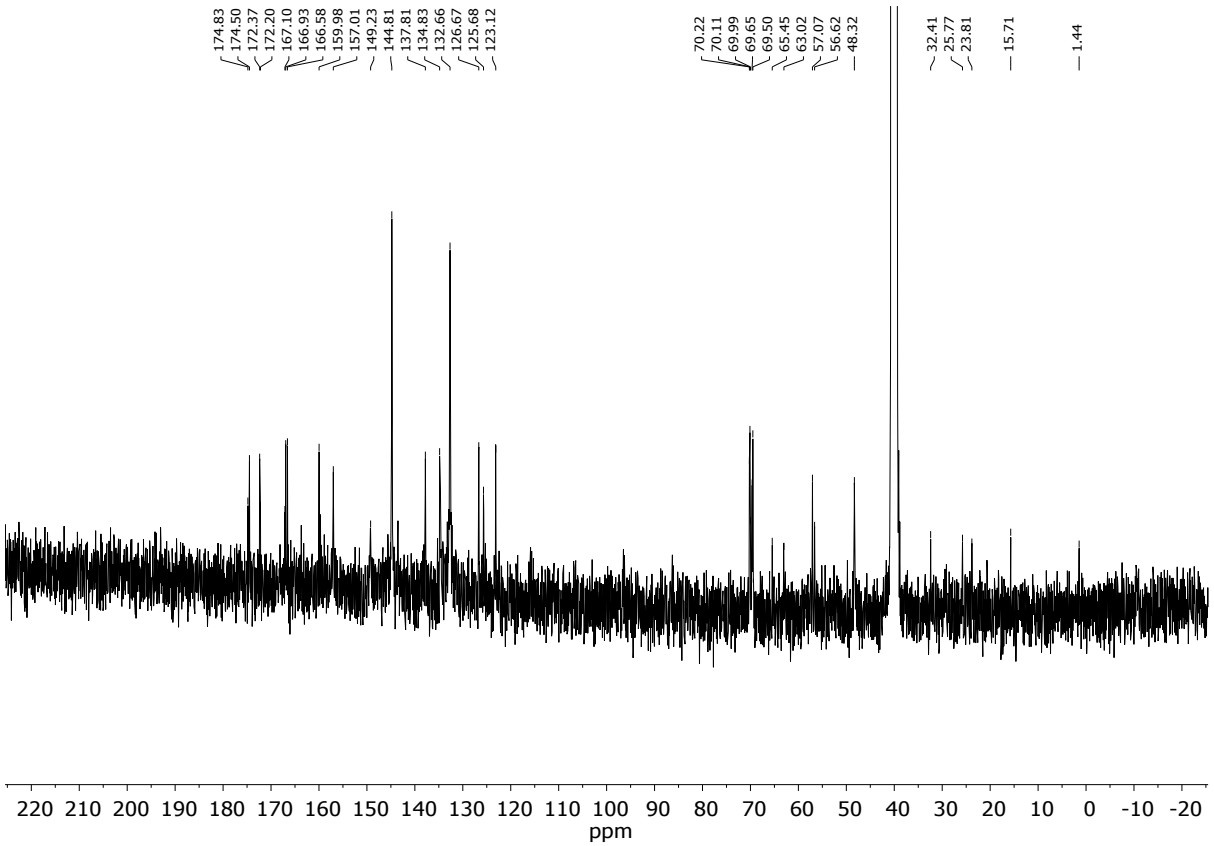



Figure S 7 $^{13}C$ NMR of porphyrin-$H_3$Dpaa **9**.

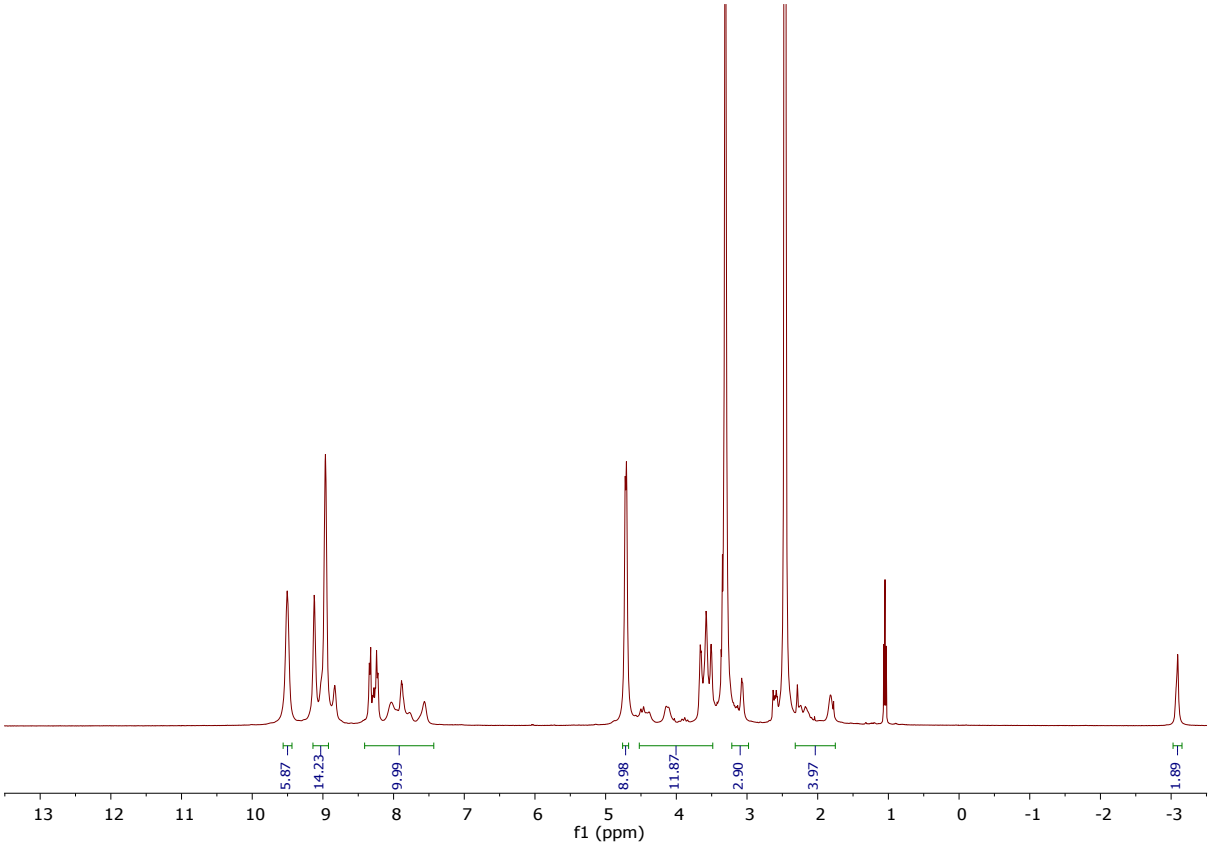



Figure S 8 $^{1}$H NMR of porphyrin-[Ga(Dpaa)] **10**.

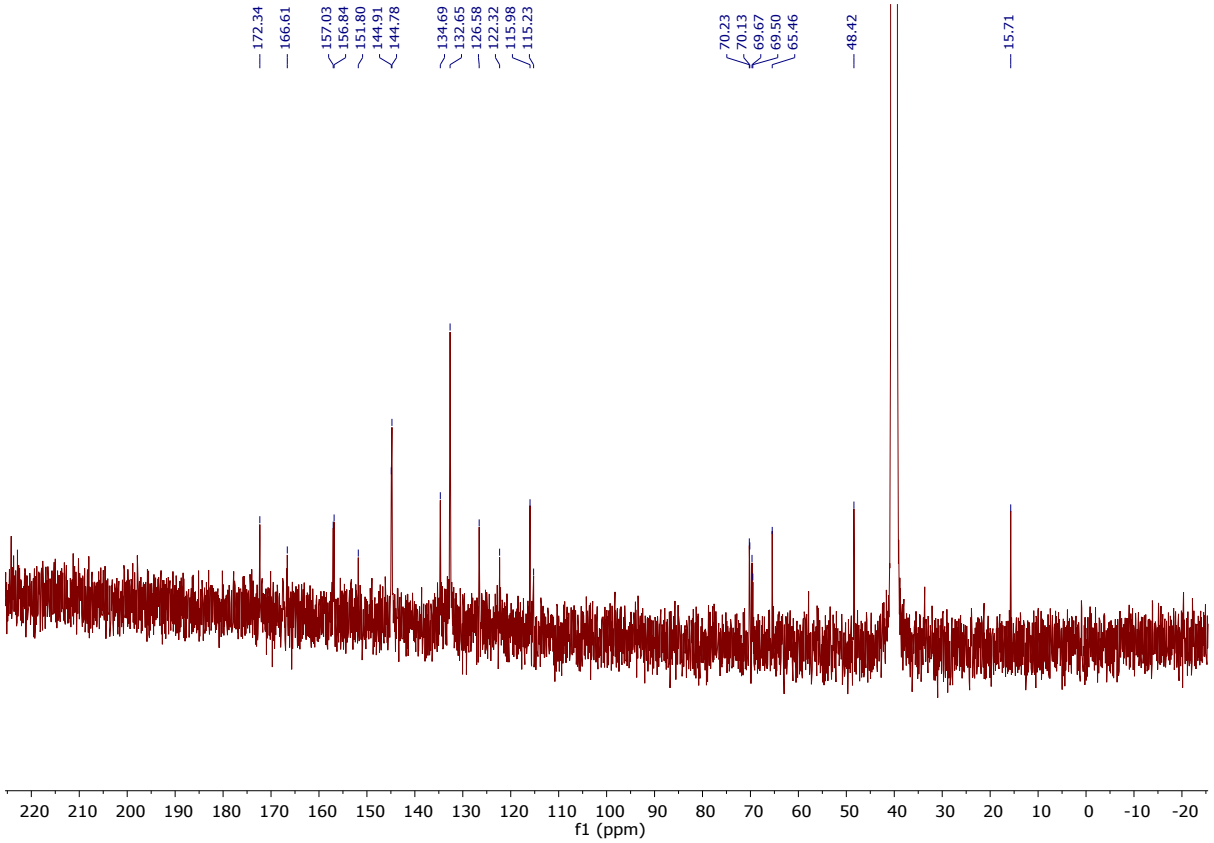



Figure S 9 $^{13}C$ NMR of porphyrin-[Ga(Dpaa)] **10**.

## UV-vis spectrum

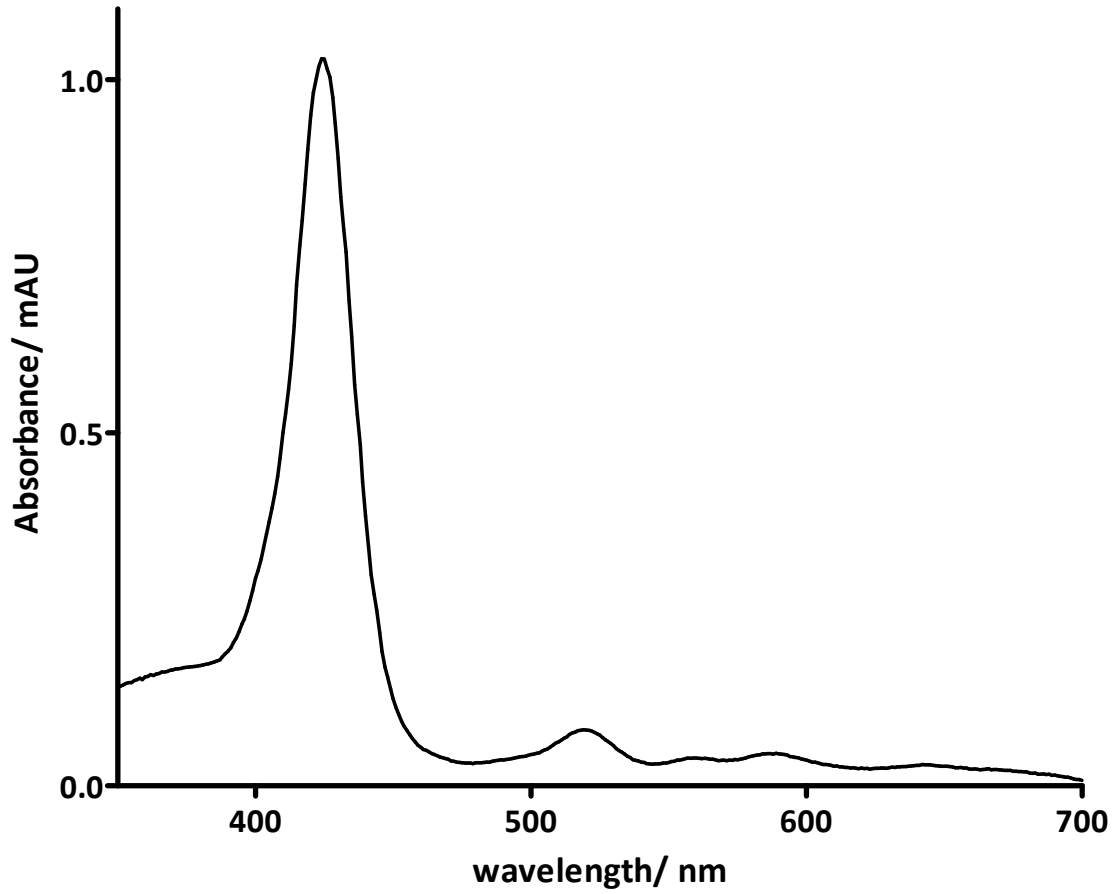



Figure S 10 UV-vis spectrum of **10** in $H_2O$.

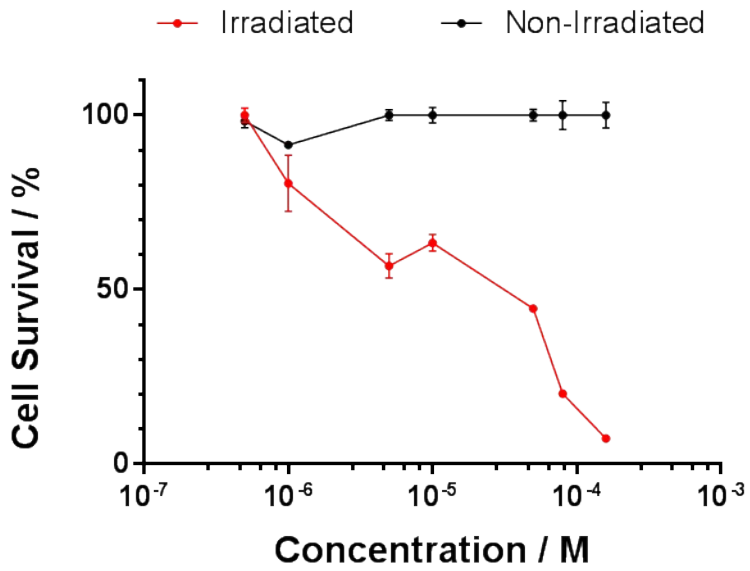



Figure S 11 %cell survival of HT-29, irradiated cell and non-irradiated cells (control), determined using MTT assay. Cells were incubated with varying concentration of **9** for 1 hour and irradiated cells received 20 J $cm^{-1}$ white light.

## Reference

1 D. B. G. Williams and M. Lawton, *J. Org. Chem.*, 2010, **75**, 8351–8354.
2 T. W. Price, J. Gallo, V. Kubíček, Z. Böhmová, T. J. Prior, J. Greenman, P. Hermann and G. J. Stasiuk, *Dalton Trans.*, 2017, **46**, 16973–16982.