

# Supporting Information

## Novel porphyrin-psoralen conjugates: synthesis, DNA interaction and cytotoxicity studies

Dalip Kumar,<sup>\*a</sup> Bhupendra Mishra,<sup>a</sup> K. P. Chandrashekar,<sup>a</sup> Anil Kumar,<sup>a</sup> Kanako Akamatsu<sup>b</sup>  
Ryohsuke Kurihara,<sup>b</sup> Takeo Ito<sup>\*b</sup>

<sup>a</sup> Department of Chemistry, Birla Institute of Technology and Science, Pilani- 333 031, India, Tel.: +91-1596-245073-5238; Fax: +91-1596-244183; E-mail: dalipk@pilani.bits-pilani.ac.in

<sup>b</sup> Department of Energy and Hydrocarbon Chemistry, Graduate School of Engineering, Kyoto University, Kyoto 615-8510, Japan; e-mail: takeoit@scl.kyoto-u.ac.jp

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## General

The reagents and solvents were procured from commercial sources and used as such unless otherwise mentioned. Hexane was distilled prior to use to remove any higher boiling fractions. *N,N'*-Dimethylformamide (DMF) was distilled *in vacuo* over calcium hydride prior to use. Chloroform (CHCl<sub>3</sub>) and dichloromethane (DCM) were kept over anhydrous calcium chloride overnight and distilled over calcium hydride prior to use. Diethyl ether was stored over anhydrous calcium chloride for 8 h and passed over activated basic alumina prior to use. Silica gel for chromatography and TLC plates (60 F<sub>254</sub>) were procured from Merck. Fourier transform-infrared (FTIR) spectra were performed on Shimadzu IRPrestige-21 spectrometer. <sup>1</sup>H NMR spectra were recorded on Bruker Advance II 400 MHz/500 MHz spectrometer in CDCl<sub>3</sub>/DMSO-*d*<sub>6</sub> using tetramethylsilane (TMS) as internal standard. Spectral data are presented as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant (*J*) in Hertz (Hz). High resolution mass spectrometry was performed using 2,5-dihydroxybenzoic acid (DHB) as the matrix on Bruker ProFLEX III MALDI-TOF/ESI-TOF mass spectrometer. Steady-state absorption spectra were recorded on a Perkin-Elmer model Lambda25 absorption spectrophotometer. Waters high performance liquid chromatography (HPLC) system with sunfire-C18 column was used to establish the purity of compounds. HPLC grade acetonitrile was procured from Merck. Mobile phase: isocratic 0.05% TFA/acetonitrile: 80/20 was found optimum for the porphyrin conjugate **11**.

## DNA Cleavage Assay

Photoirradiation was carried out using a high pressure Xe-arc through a band-path filter (UTVAF-36U, Sigma-Koki, Tokyo, Japan) ( $\lambda = 300\text{-}390$  nm, 4 mW, UV-A) or a white LED light source (ISL-150×150-WW, CCS, Kyoto, Japan) ( $\lambda = 400\text{-}800$  nm, 2 mW, visible). A gel electrophoresis apparatus (Mupid-exu, Advance, Tokyo, Japan) was used for agarose gel electrophoresis. DNA cleavage studies were performed by the use of supercoiled, covalently closed, circular  $\Phi$ X174 RF I DNA (Form I) (New England Biolabs, USA). Typically, solution of  $\Phi$ X174 RF I DNA (0.5  $\mu$ g) and the drugs in 20 mM Tris-HCl buffer (pH 7.2) containing 20 mM

NaCl and 2.5 vol% DMSO (total volume 20  $\mu$ L) was exposed to UV-A or visible light at ambient temperature. The resultant mixtures were then analyzed by gel electrophoresis (1% agarose gel, ethidium bromide stain). DNA cleavage was determined by the formation of relaxed circular DNA (Form II) and linear DNA (Form III). The gels were visualized on a UV transilluminator ( $\lambda_{\text{ex}} = 312$  nm, ETX-35.M, Vilber-Lourmat, France).

### Restriction Enzyme Assay for Detecting DNA Cross-Links

Restriction enzymes *Mun I* and *Ssp I* were purchased from Takara Bio (Japan) and New England Biolabs, respectively.  $\Phi$ X174 RF I DNA and the drugs in 20 mM Tris-HCl buffer (pH 7.2) containing 20 mM NaCl and 2.5 % DMSO was exposed to UV-light at ambient temperature. Mixed solution of the irradiated sample and the restriction enzyme (*Mun I* or *Ssp I*, 2-5 units) was incubated in the supplied buffer solution at 37 °C for 1 h. The reaction was quenched by the addition of gel loading buffer. The resultant mixtures were then analyzed by gel electrophoresis (1% agarose gel, ethidium bromide stain).

### Cell Viability Assay

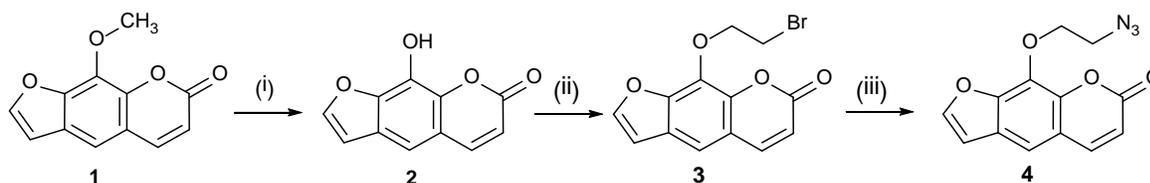
A549, a human epithelial cell line derived from a lung carcinoma (doubling time; 20–24 h), was obtained from American Type Culture Collection. A549 were grown in Dulbecco's modified Eagle medium with high concentrations of glucose (Invitrogen, Carlsbad, CA, USA) containing 10% fetal bovine serum (FBS), 100 U/mL penicillin and 100  $\mu$ g streptomycin. Cell line was incubated at 37 °C in a humidified atmosphere consisting of 5% CO<sub>2</sub>/air. Porphyrins were dissolved in DMSO (final concentration: 0.1% DMSO in culture medium). A549 cells were seeded (5000 cells/well) on a 96 well plates the day before chemicals treatment. After 24 h of incubation in the presence of the porphyrins (0–5  $\mu$ M), cells were washed with phosphate buffered saline (PBS), and incubated in new PBS for 10 min at 37 °C. Cells were exposed to UV-A or visible light for 10 min, and then PBS was replaced to FBS containing medium. After 48 h incubation in the dark, cell viability was determined by the Cell Counting Kit-8 (Dojin, Kumamoto, Japan) using a spectrophotometer (xMark; Bio-Rad, Hercules, CA, USA). IC<sub>50</sub> values were determined using 4-parameter equations. Half-maximal inhibitory concentrations (IC<sub>50</sub>) were determined from mean values using the 4-parameter model. The following formula

for the 4-parameter logistic model was used:  $Y = \{[a-d]/[1 + (X/c)^b]\} + d$ , where  $Y$  is the response,  $X$  is the concentration,  $a$  is the lower plateau,  $d$  is the upper plateau,  $b$  is the slope factor.

### Nuclear Staining

A549 cells were seeded ( $1 \times 10^5$  cells/well) on a 12 well plate the day before chemical treatment. After 24 h of incubation in the presence of the porphyrins (0.1  $\mu$ M), cells were rinsed with PBS and then incubated in new PBS for 10 min. Cells were photoexposed to UV-A or visible light for 10 min, and then PBS was replaced to FBS containing medium. After 24 h incubation in the dark, cells were washed twice with PBS, then stained with Hoechst 33342 (Dojin, Kumamoto, Japan), and imaged by a fluorescence microscope (Keyence BZ-8000, Osaka, Japan).

### Experimental Procedures



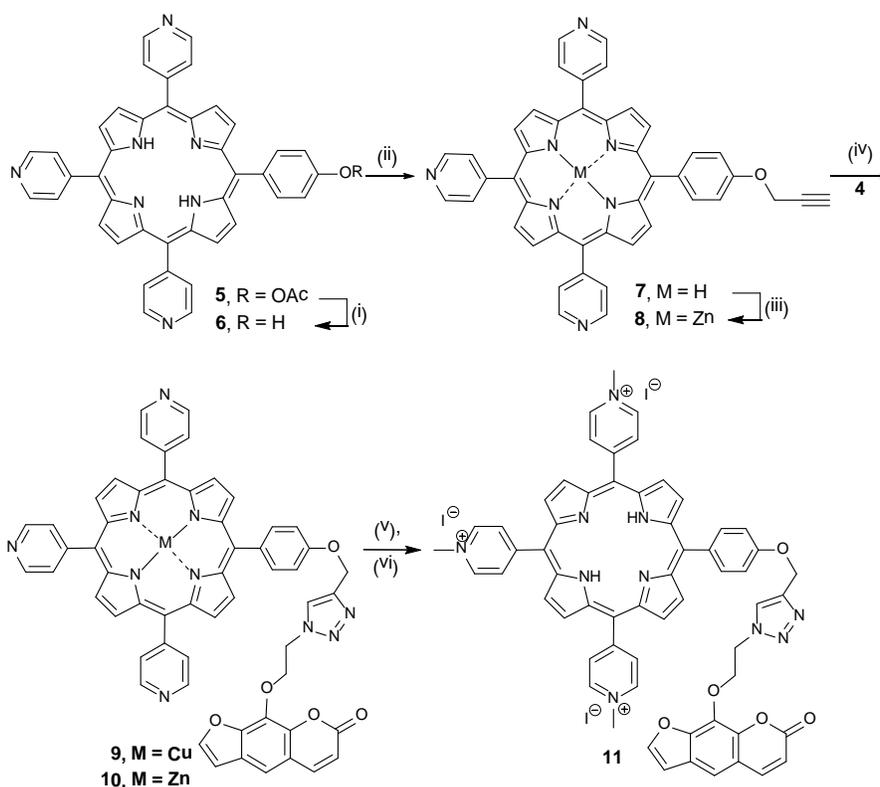
Reaction conditions: (i)  $\text{AlCl}_3$ ,  $\text{CH}_2\text{Cl}_2$ , rt, 5 h; (ii) 1,2-dibromoethane,  $\text{K}_2\text{CO}_3$ , DMF, rt, 6 h; (iii)  $\text{NaN}_3$ , DMF:H<sub>2</sub>O (3:1), 40 °C, 8 h.

#### Scheme S1: Synthesis of psoralen azide 4

**8-Hydroxy psoralen (2)** To a stirred solution of 8-methoxy psoralen (0.5 g, 2.31 mmol) at 0 °C was added anhydrous aluminium chloride (0.92 g, 6.93 mmol) in dichloromethane (50 mL) and allowed to stir for 5 h. After completion, the reaction contents were cooled in an ice-bath and 50% aq. HCl (30 mL) was added and layers were separated. The organic layer was washed with water (20 mL), brine (20 mL), dried over anhydrous sodium sulfate, and concentrated *in vacuo* to afford a pale yellow solid **2** (0.39 g, yield 85%).

**9-(2-Bromoethoxy)-7H-furo[3,2-g]chromen-7-one (3)** To stirred solution of hydroxy psoralen **2** (0.35 g, 1.73 mmol) in a mixture of 1,2-dibromoethane (2 mL) and dry DMF (0.5 mL) was added finely powdered fused potassium carbonate (0.311 g, 2.25 mmol) and allowed to stir for 6 h. After completion, the reaction contents were adsorbed over silica gel and chromatographed. Elution with hexane (100 mL) removed dibromoethane and DMF, and then ethylacetate/hexane (2/8 v/v) afforded **3** as a pale yellow solid (0.4 g, yield 75%).

**9-(2-Azidoethoxy)-7H-furo[3,2-g]chromen-7-one (4)** To a solution of bromoethoxy psoralen **3** (0.39 g, 1.27 mmol) in DMF (5 mL) was added sodium azide (0.24 g, 3.79 mmol) and the mixture was stirred at 40 °C for 8 h. After completion of the reaction, water (50 mL) was added and the aqueous layer was extracted with dichloromethane (2 × 40 mL). The combined organic layer was washed with water (4 × 20 mL), brine (40 mL), dried over anhydrous sodium sulfate and solvents were evaporated under reduced pressure. The residue thus obtained was purified by chromatography over silica gel to afford of psoralen azide **4** as a pale yellow solid (0.24 g, yield 70 %). m.p. 193–195 °C; IR (KBr) 2912, 2119 (–N<sub>3</sub>), 1739 (–C=O), 1601 (ArC=C), 1435 cm<sup>-1</sup>; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 7.77 (d, *J* = 10.9 Hz, 1H, C<sub>3</sub>H), 7.73 (d, *J* = 8.8 Hz, 1H, C<sub>7</sub>H), 7.45 (s, 1H, C<sub>5</sub>H), 7.02 (d, *J* = 8.9 Hz, 1H, C<sub>6</sub>H), 6.43 (d, *J* = 10.8 Hz, 1H, C<sub>4</sub>H), 4.72 (t, *J* = 7.6 Hz, 2H, –OCH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>), 3.73 (t, *J* = 7.5 Hz, 2H, –OCH<sub>2</sub>CH<sub>2</sub>N<sub>3</sub>); MALDI-MS: *m/z* calcd for C<sub>13</sub>H<sub>9</sub>N<sub>3</sub>O<sub>4</sub> [M]<sup>+</sup>: 271.2193, found: 271.2177.



Reaction conditions: (i) KOH, MeOH–H<sub>2</sub>O, reflux, 5 h; (ii) propargyl bromide (1.2 equiv), K<sub>2</sub>CO<sub>3</sub> (1.2 equiv), DMF, 0–27 °C, 24 h; (iii) Zn(OAc)<sub>2</sub>, CHCl<sub>3</sub>:MeOH, reflux, 2 h; (iv) CuSO<sub>4</sub>·5H<sub>2</sub>O, sodium ascorbate, DMF:H<sub>2</sub>O, 80 °C, 32–168 h; (v) **10**, CHCl<sub>3</sub>, aq HCl (25%), 1 h; (vi) MeI (120 equiv), DMF, rt, 36 h.

**Scheme S2:** Synthesis of 5-(4'-propargyloxyphenyl)-10,15,20-triphenylporphyrin

**5-[4'-(Methylcarbonyloxy)]phenyl]-10,15,20-tri(4'-pyridyl)porphyrin 5:** To a stirred solution of 4-acetyloxybenzaldehyde (4.25 g, 35 mmol), propionic acid (280 mL), and acetic anhydride (16 mL) was heated at 110 °C with stirring. Gradually, to this solution were

simultaneously added 4-pyridinecarboxaldehyde (10 mL, 113.6 mmol) and pyrrole (9 mL, 135.7 mmol). The resulting mixture was refluxed for 1.5 h. The volume of solvent was reduced under low pressure to 80 mL and the mixture was neutralized with a saturated sodium bicarbonate solution, filtered and washed several times with water. The crude material was extracted with a mixture of dichloromethane/methanol (8.5:1.5 v/v) and purified by chromatography using chloroform/methanol (9.5:0.5 v/v), silica-gel was neutralized with triethylamine prior to loading compound. The (acetyloxyphenyl)porphyrin **5** was obtained as purple solid (0.57 g, yield 5%). m.p. >300 °C. IR (KBr) 3380 (–NH stretching), 3222, 2933, 1740 (C=O), 1597 (Ar C=C), 1285 cm<sup>-1</sup>.

**5-(4'-Hydroxyphenyl)-10,15,20-tri(4'-pyridyl)porphyrin 6:** Porphyrin **5** (0.56 g, 0.82 mmol) was dissolved in 25 mL of ethanol and 45 mL of 2 M NaOH. The solution was refluxed for 1 h, cooled the reaction mixture to room temperature and 90 mL of 1N HCl was added. The resulting solution (pH = 7) was then extracted with chloroform (4 × 80 mL). The solvents were evaporated under reduced pressure to give **6** as purple solid (0.49 g, yield 85%) which was used as such without further purification.

**5-(4'-Propargyloxyphenyl)-10,15,20-tri(4'-pyridyl)porphyrin 7:** To a stirred solution of **6** (0.3 g, 0.47 mmol) and fused potassium carbonate (0.078 g, 0.57 mmol) at 0–5 °C in dry DMF (20 mL) was gradually added propargyl bromide (0.064 g, 0.057 mmol) over a period of 15 min. The reaction mixture was allowed to stir at room temperature for 24 h. After completion of the reaction, contents were cooled to room temperature, diluted with DCM (50 mL) and filtered. To the filtrate, 70 mL of water was added and organic phase was separated, then dried over anhydrous sodium sulfate and concentrated *in vacuo*. Chromatography (9:1, chloroform: methanol) of the residual mass afforded **7** as purple solid (0.17 g, yield 53%). m.p. >300 °C; IR (KBr) 3210 (–NH str.), 3033 (–C–H) 2967, 2945, 2875, 2110 (–C≡C) cm<sup>-1</sup>; <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 9.00–8.98 (m, 6H), 8.88–8.81 (m, 8H), 8.27–8.26 (m, 6H), 8.17 (d, *J* = 8.0 Hz, 2H), 7.45 (d, *J* = 8.0 Hz, 2H), 5.10 (s, 2H), 2.88 (s, 1H), –3.02 (s, 2H); ESI-MS: *m/z* calcd for C<sub>44</sub>H<sub>30</sub>N<sub>7</sub>O [M+H]<sup>+</sup>: 672, found: 672.

**5-(4'-Propargyloxyphenyl)-10,15,20-tri(4'-pyridyl)porphyrinato Zinc(II) 8:** To a stirred solution of **7** (0.15 g, 0.22 mmol) in a mixture chloroform–methanol (3:1, 15 mL) was added Zn(OAc)<sub>2</sub> (0.051 g, 0.23 mmol) and the contents were refluxed for 1h. After completion of the reaction, the contents were diluted with chloroform (30 mL) and saturated sodium bicarbonate (50 mL) (pH = 8). The organic layer was separated, washed with water and dried over anhydrous sodium sulphate. The excess of solvent was distilled off *in vacuo* to produce **8** (0.15 g, yield 91%) as a purple-green solid. The compound was sufficiently pure and used for the next step without further purification.

**Preparation of porphyrin–psoralen conjugate 9:** To a stirred solution of free base porphyrin **7** (0.10 g, 0.15 mmol) in DMF:H<sub>2</sub>O (1:1; 40 mL) was added CuSO<sub>4</sub>.5H<sub>2</sub>O (0.037 g, 0.15 mmol), sodium ascorbate (0.0594 g, 0.30 mmol) followed by psoralen azide **4** (0.121g, 0.45 mmol). The

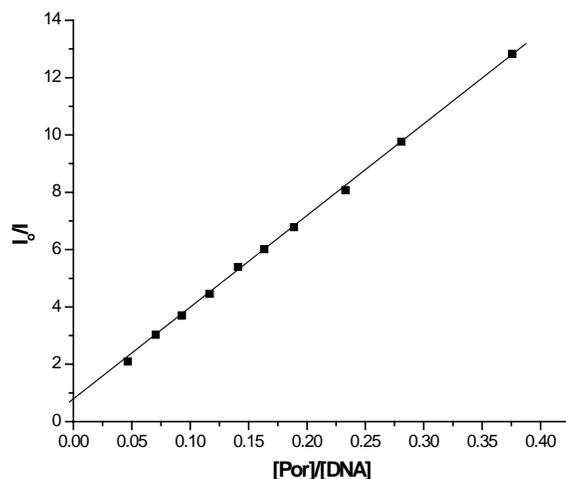
reaction mixture was heated at 80 °C and stirred for 32 h. After completion of the reaction, the contents were diluted with chloroform (50 mL) and ammonia solution (20%, 15 mL). The resulting solution filtered over thin celite bed and washed thoroughly with chloroform. The combined organic phase was washed with water, dried over anhydrous sodium sulfate and distilled off under reduced pressure. The residue so obtained was purified by column chromatography using chloroform–methanol (9:1) as eluent to afford the porphyrin conjugate **9** (0.095 g, yield 67%) as red solid. m.p. >300 °C; ESI-TOF:  $m/z$  calcd for  $C_{57}H_{36}CuN_{10}O_5 [M]^+$ : calcd: 1003; found: 1003.

**Preparation of porphyrin–psoralen conjugate 10:** To a stirred solution of metallated porphyrin **8** (0.12 g, 0.163 mmol) in DMF:  $H_2O$  (1:1; 70 mL) was added  $CuSO_4 \cdot 5H_2O$  (0.163 g, 0.653 mmol), sodium ascorbate (0.259 g, 1.306 mmol) followed by psoralen azide **4** (0.132 g, 0.49 mmol). The reaction mixture was allowed to stir at 80 °C for 168 h. After completion of the reaction, the contents were diluted with chloroform (4 × 80 mL) and ammonia solution (20%, 30 mL). The resulting solution filtered over a thin celite bed and washed thoroughly with chloroform. The combined organic phase was washed with water, dried over anhydrous sodium sulfate and distilled off *in vacuo*. The residue thus obtained was purified by column chromatography using chloroform/methanol (8:2) as eluent to afford pure porphyrin conjugate **10** (0.082 g, yield 52%) as a red solid. m.p. >300 °C;  $^1H$  NMR (500 MHz,  $DMSO-d_6$ )  $\delta$  9.39–9.38 (m, 6H, 2',6' *N*-pyridiniumyl H), 9.34–9.18 (m, 8H,  $\beta$ -pyrrole H), 8.72–8.70 (m, 6H, 3',5' *N*-pyridiniumyl H), 8.17–8.11 (m, 2H, psoralen  $C_3H$  & psoralen  $C_7H$ ), 7.83 (s, 1H, triazolyl  $C_5H$ ), 7.40 (d,  $J = 8.1$  Hz, 2H, 3',5' *meso*-phenyl H), 7.31 (s, 1H, psoralen  $C_5H$ ), 7.18–7.17 (m, 3H, 2',6' *meso*-phenyl H & psoralen  $C_6H$ ), 6.82 (d,  $J = 9.8$  Hz, 1H, psoralen  $C_4H$ ), 5.77 (s, 2H,  $-C_6H_4OCH_2$ -triazol), 5.00 (t,  $J = 7.3$  Hz, 2H, triazolyl- $CH_2CH_2O$ -), 3.76 (t,  $J = 7.3$  Hz, 2H, triazolyl- $CH_2CH_2O$ -); ESI-TOF:  $m/z$  calcd for  $C_{57}H_{35}CuN_{10}O_5Zn [M]^+$ : calcd: 1004; found: 1004.

**Preparation of cationic porphyrin–psoralen conjugate 11:** To a cooled solution (5–10 °C) of Zn(II) conjugate **10** (0.091 g) in chloroform (80 mL) was added hydrochloric acid (25%, 20 mL) and stirred for 1 h at 27 °C. After complete demetallation, the temperature was lowered to 0–2 °C and the reaction mixture was basified with ammonia solution, separated the organic layer and dried over anhydrous sodium sulfate. The solvents were distilled off *in vacuo* to afford demetallated porphyrin triazole (0.085 g), which was dissolved in dichloromethane: methanol (9:1), precipitated by adding hexane, filtered, dried and used as such for further reaction. To a solution of demetallated porphyrin triazole (0.085 g, 0.091 mmol) in dry *N,N'*-dimethylformamide (20 mL) was added methyl iodide (0.75 mL, 11.73 mmol) and allowed to stir at room temperature until starting material consumed (36 h). The solvent was removed *in vacuo* and methanol (5 mL) was added. The solution was precipitated by adding diethyl ether (15 mL), and the resulting solid was filtered. This procedure was repeated four times to afford compound **11** (0.071 g, yield 65%) as brown solid. m.p. >300 °C  $^1H$  NMR (500 MHz,  $DMSO-d_6$ )  $\delta$  9.48–9.47 (m, 6H, 2',6'  $Me^+N$ -pyridiniumyl H), 9.32–9.01 (m, 14H, 3',5'  $Me^+N$ -

pyridiniumyl H &  $\beta$ -pyrrole H), 8.25 (d,  $J = 9.9$  Hz, 1H, psoralen C<sub>3</sub>H), 8.20 (d,  $J = 8.9$  Hz, 1H, psoralen C<sub>7</sub>H), 7.88 (s, 1H, triazolyl C<sub>5</sub>H), 7.48 (d,  $J = 8.2$  Hz, 2H, 2',6' *meso*-phenyl H), 7.33 (s, 1H, psoralen C<sub>5</sub>H), 7.24–7.23 (m, 3H, 3',5' *meso*-phenyl H & psoralen C<sub>6</sub>H), 6.75 (d,  $J = 9.9$  Hz, 1H, psoralen C<sub>4</sub>H) 5.83 (s, 2H, –C<sub>6</sub>H<sub>4</sub>OCH<sub>2</sub>–triazol), 5.22 (t,  $J = 7.5$  Hz, 2H, triazolyl–CH<sub>2</sub>CH<sub>2</sub>O–), 4.73 (s, 9H, 3 × –N<sup>+</sup>CH<sub>3</sub>), 3.99 (t,  $J = 7.4$  Hz, 2H, triazolyl–CH<sub>2</sub>CH<sub>2</sub>O–), –3.01 (s, 2H, pyrrole NH); <sup>13</sup>C NMR (126 MHz, DMSO-*d*<sub>6</sub>)  $\delta$  161.10 (psoralen OC=O) 159.05, 151.66, 151.00, 148.58, 148.08, 147.10, 144.28, 143.27, 142.75, 140.44, 138.45, 133.18, 132.13, 131.89, 131.18, 127.35, 126.94, 124.18, 122.49, 118.81, 116.11, 114.98, 114.88, 107.19, 67.21 (–OCH<sub>2</sub>–triazol), 56.97 (–OCH<sub>2</sub>CH<sub>2</sub>–), 47.30 (–N<sup>+</sup>CH<sub>3</sub>); MALDI–TOF: *m/z* calcd for C<sub>60</sub>H<sub>47</sub>N<sub>10</sub>O<sub>5</sub> [M<sup>+</sup>–3Γ]: calcd: 987.2814; found: 987.2841; HPLC purity: 99.24%.

### Stern-Volmer plot of EB-ctDNA.



**Fig. S1.** Fluorescence quenching plots of DNA-bound EB by **11**, 5 mM Tris-HCl, 0.1 M NaCl, (pH 8.0). EB (5  $\mu$ M), DNA (12  $\mu$ M)

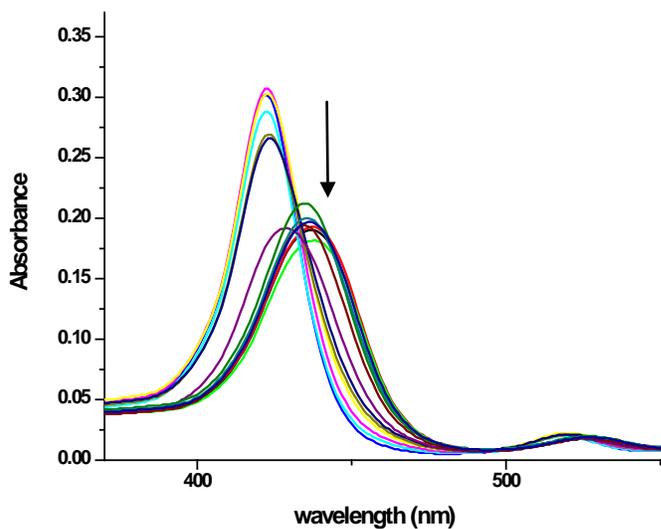
The Stern-Volmer equation<sup>1</sup>

$$\frac{I_0}{I} = 1 + K_{sv}r$$

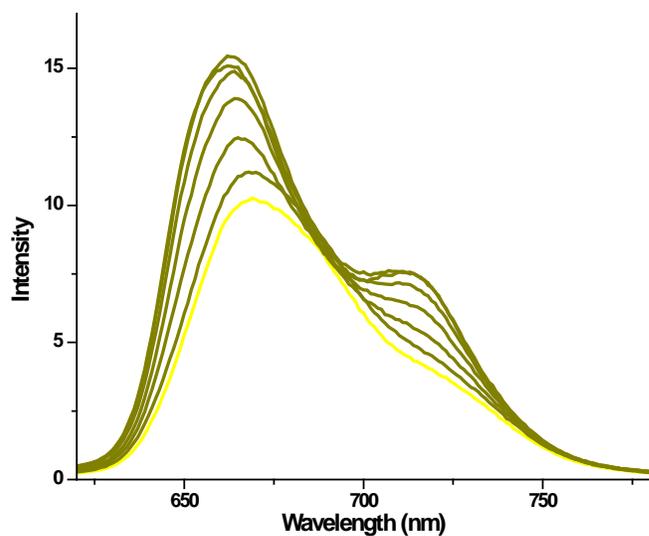
where  $I_0$  and  $I$  are the fluorescence intensities of ethidium bromide (EB) in the absence and the presence of porphyrin, respectively,  $K_{sv}$  is a linear Stern-Volmer quenching constant,  $r$  is the ratio of total concentration of porphyrin to that of ctDNA,  $[Por]/[DNA]$ . The quenching plot illustrates that the quenching of EB bound to DNA by **11** is in good agreement with the linear Stern-Volmer equation, which additionally proves that the **11** binds to DNA. In the plot of  $I_0/I$

vs. [porphyrin]/[DNA],  $K_{sv}$  is given by the ratio of the slope to the intercept which was found out to be 3.31 for cationic porphyrin-psoralen conjugate **11** (Fig. S1).

### Absorption and Emission Studies of TMPyP with ctDNA

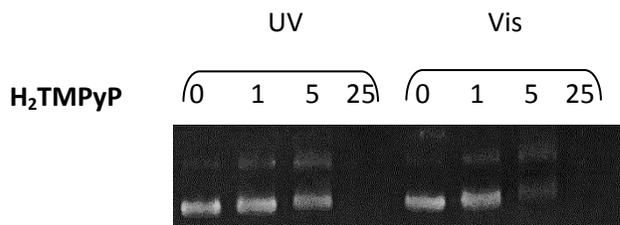


**Fig. S2** Absorption spectra of TMPyP (2 μM) with increasing ctDNA concentrations (5 mM Tris-HCl, 0.1 M NaCl, pH 8.0, 25 °C).



**Fig. S3.** Fluorescence plots of ctDNA-bound TMPyP (2 μM), 5 mM Tris-HCl, 0.1 M NaCl, (pH 8.0).

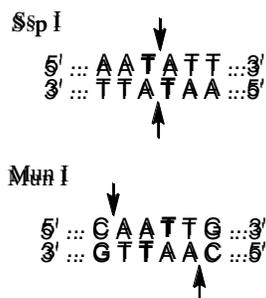
## DNA photocleavage assay for Tetrakis(*N*-methylpyridinium-4-yl)porphyrin (TMPyP)



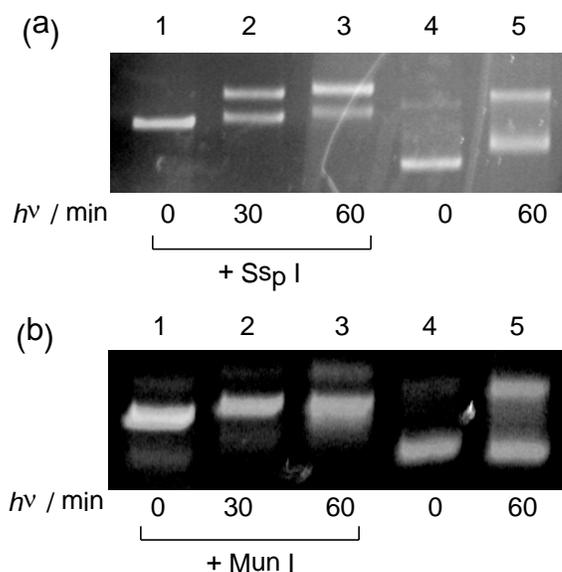
**Fig. S4:** Electrophoresis of  $\Phi$ X174 plasmid DNA on agarose gel after UV- or visible-light irradiation (10 min.) in the presence of various concentrations of porphyrins (0–25  $\mu$ M).

## Restriction Enzyme Assay for Detecting DNA Cross-Links

It has been demonstrated that psolarens interact with DNA and induce interstrand crosslinking between thymines in a close proximity.<sup>2</sup> Crosslink formation by the psolaren-porphyrin conjugates **11** can be accessed by the use of restriction enzymes which recognize AT-containing DNA sequences.<sup>3</sup> *Ssp I* and *Mun I* recognize and cleave 5'-AATATT / 3'-TTATAA and 5'-CAATTG / 3'-GTTAAC in DNA at the indicated sites in Fig. S5, respectively.  $\Phi$ X174 plasmid DNA has single recognition sites for each enzyme. As shown in Fig. S6a, lane 1, quantitative double strand scission in the non-irradiated  $\Phi$ X174 (Form III) was observed after *Ssp I*-treatment. On the other hand, *Ssp I* treatment of the UV-irradiated DNA (lanes 2 and 3) in the presence of **11** yielded Form II and Form III. This result suggests that crosslinking between **11** and thymines in the 5'-AATATT / 3'-TTATAA sequence inhibited enzymatic double strand cleavage. Strand scission by *Mun I* was fairly inhibited by UV-irradiation in the presence of **11** (Fig. S6b, lanes 2 and 3). It has been demonstrated that psolaren preferentially forms interstrand TT crosslinks at 5'-TA / 3'-AT that is involved in the *Ssp I* recognition sequence, but not in the *Mun I* recognition site.<sup>4</sup>



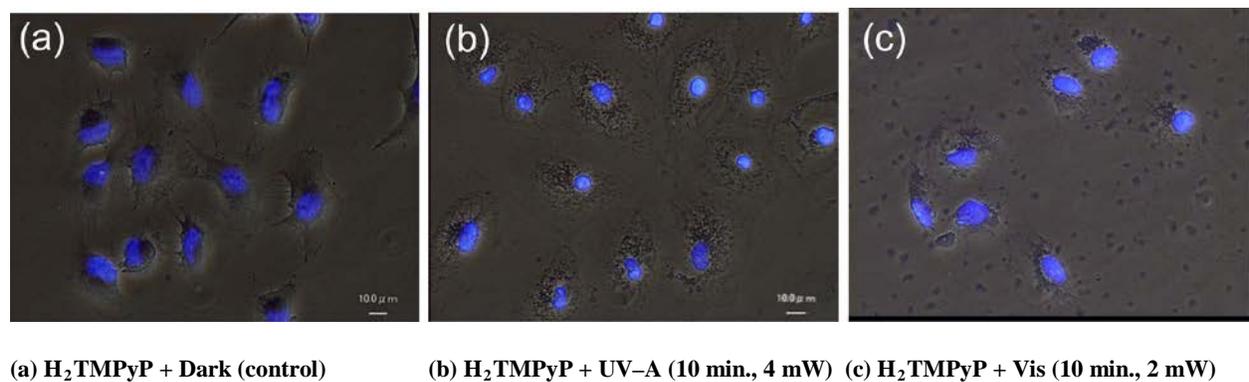
**Fig. S5.** Restriction sites of *Ssp I* and *Mun I*. The arrows pointing at the recognition sites indicate where the enzymes will cleave the DNA.



**Fig. S6.** Photoinduced DNA damage formation by **11**.  $\Phi$ X174 supercoiled DNA (0.5  $\mu$ g) was incubated with **11** (40 mM) in 20 ml of Tris-HCl (20 mM, pH 7.2) containing NaCl (20 mM), DMSO (2.5 vol%) at ambient temperature in the dark for 30 min, and then exposed to UV-light (310~390 nm). The samples were further treated with (a, lanes 1-3) *Ssp I* or (b, lanes 1-3) *Mun I*. (a) Lane 1, DNA+*Ssp I*; lane 2, UV 30 min + *Ssp I*; lane 3, UV 60 min + *Ssp I*. lane 4, DNA alone; lane 5, DNA + UV 60 min. (b) Lane 1, DNA + *Mun I*; lane 2, UV 30 min + *Mun I*; lane 3, UV 60 min + *Mun I*. lane 4, DNA alone; lane 5, DNA + UV 60 min.

### Cell morphology studies of H<sub>2</sub>TMPyP by fluorescent microscopy

Photoexcited TMPyP seems to disrupt cell membranes (Fig S7, b and c). In addition, like in the case of the cationic porphyrin–psoralen conjugate **11**, TMPyP induced shrinking of nuclei especially after UV-A irradiation.



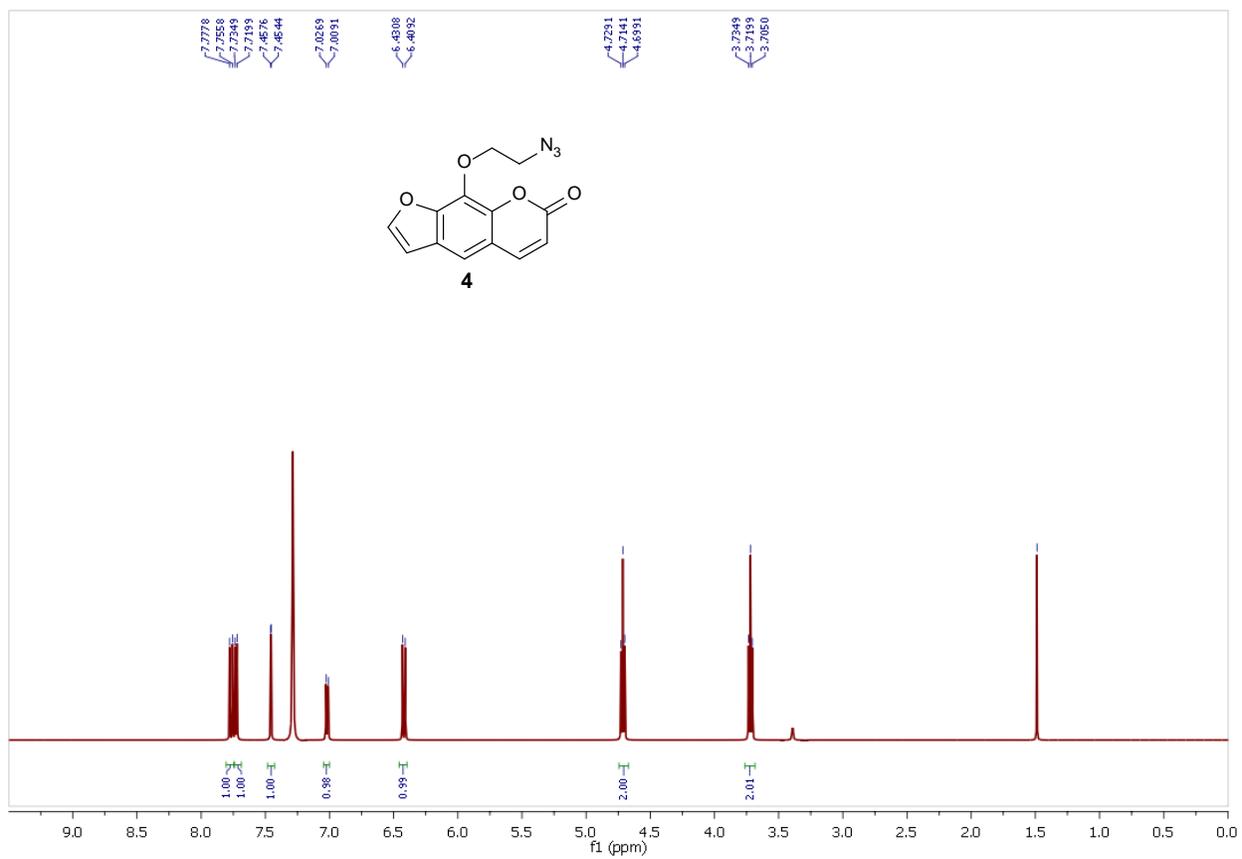
**Fig. S7** Fluorescence along with phase contrast microscopic images of A549 cells. TMPyP (1  $\mu$ M) treated A549 cells were stained with Hoechst 33342 to identify nuclear morphology.

## References

1. B. C. Baguley and M. Le Bret, *Biochemistry*, 1984, **23**, 937.
2. (a) D. W. Ussery, R. W. Hoepfner, R. R. Sinden, *Method Enzymol.*, 1992, **212**, 242-262; (b) D. Bethea, B. Fullmer, S. Syed, G. Seltzer, J. Tiano, C. Rischko, L. Gillespie, D. Brown and F. P. Gasparro, *J. Dermatol. Sci.*, 1999, **19**, 78.
3. L. Kittler, G. Löber, *J. Photochem. Photobiol. B: Biol.*, 1995, **27**, 161.
4. (a) V. Boyer, E. Moustacchi and E. Sage *Biochemistry*, 1988, **27**, 3011; (b) E. Sage and E. Moustacchi, *Biochemistry* 1987, **26**, 3307.

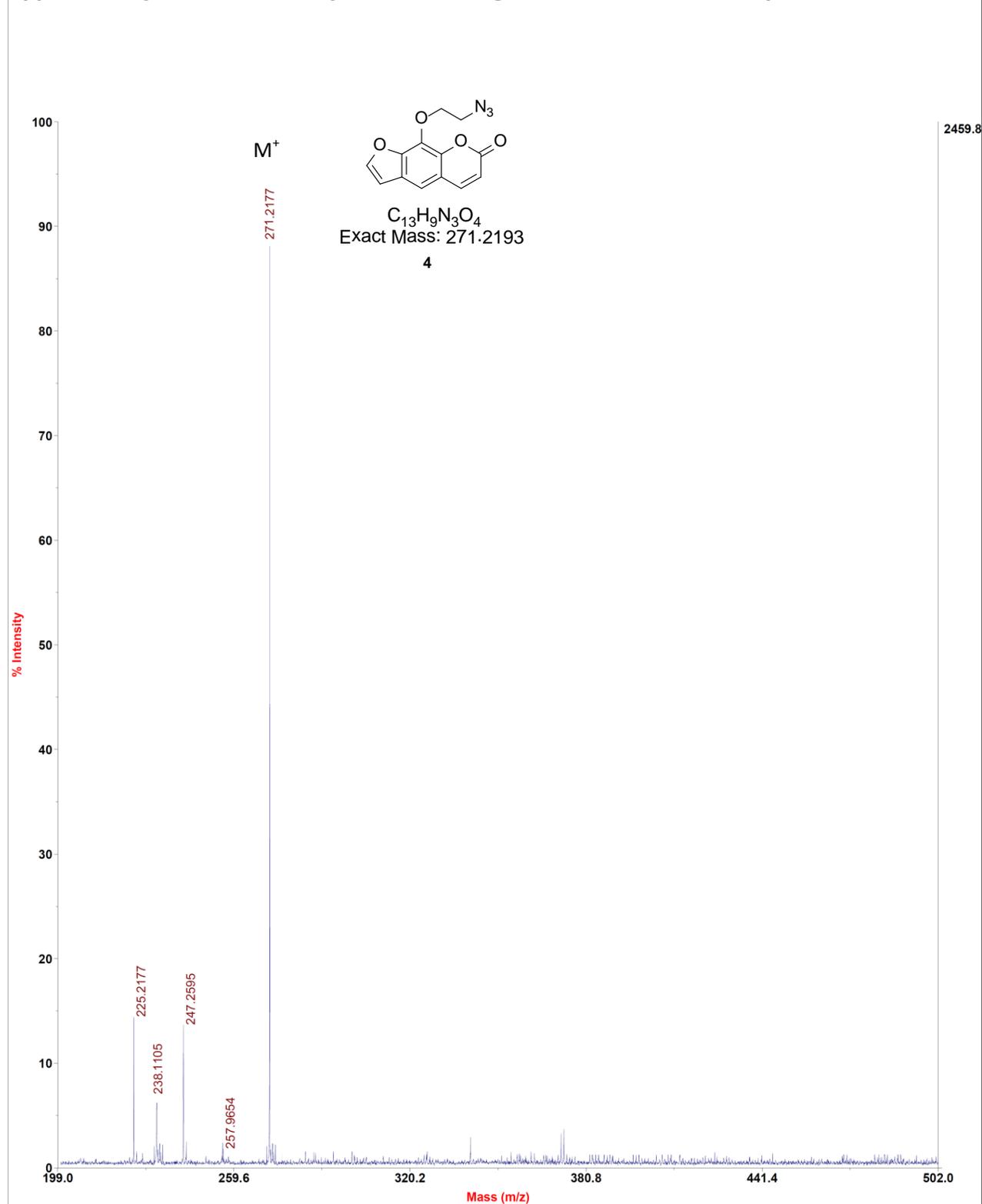
## Copies of NMR and mass spectral data of synthesized compounds

<sup>1</sup>H NMR spectrum of psoralen azide **4**



MALDI-TOF MS spectrum of psoralen azide **4**

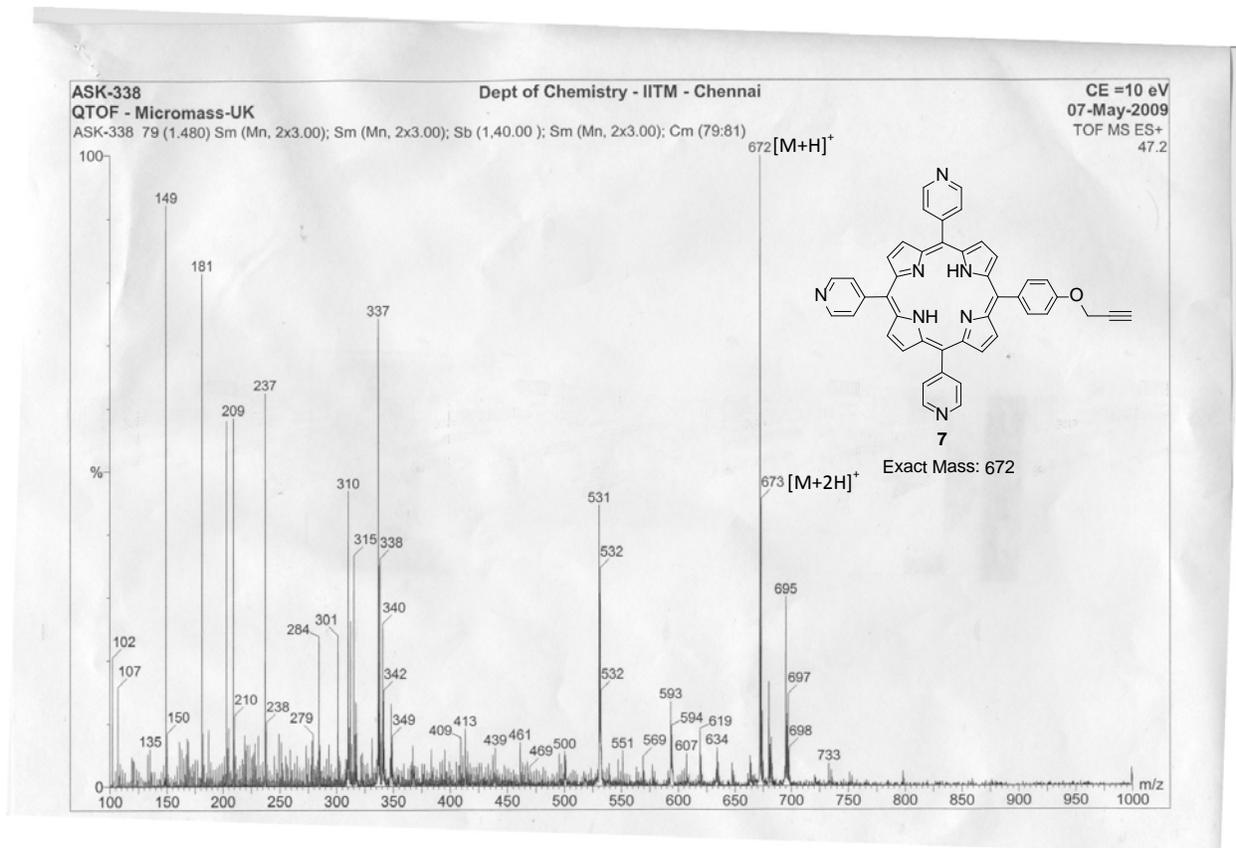
Applied Biosystems MDS Analytical Technologies TOF/TOF™ Series Explorer™ 72027



C:\Applied Biosystems MDS Analytical Technologies\TOFTOF Data\ExportT2D\AIRF-MASS\2805112J1\_MS.t2d

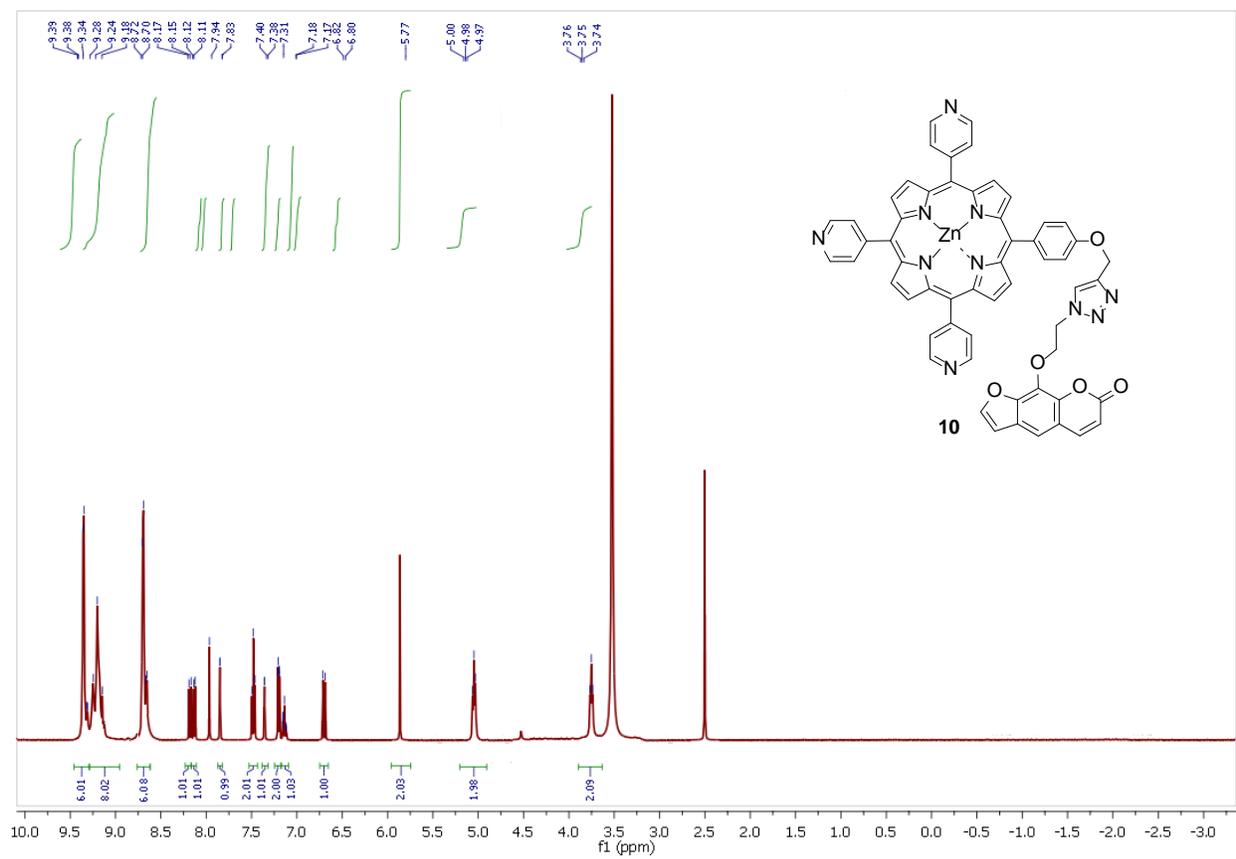
Printed: 12:44, June 11, 2012

ESI-TOF MS spectrum of (propargyloxyphenyl)porphyrin **7**

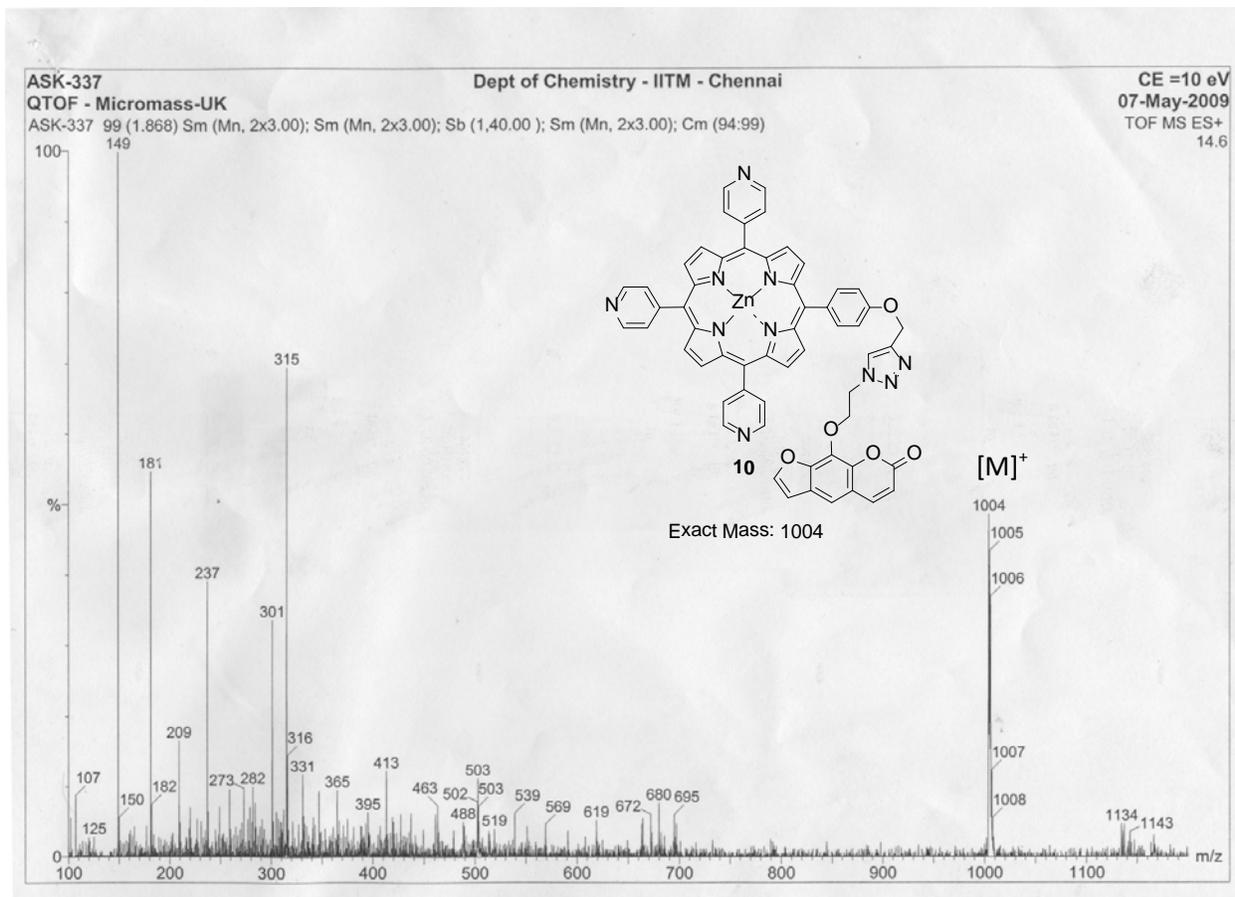




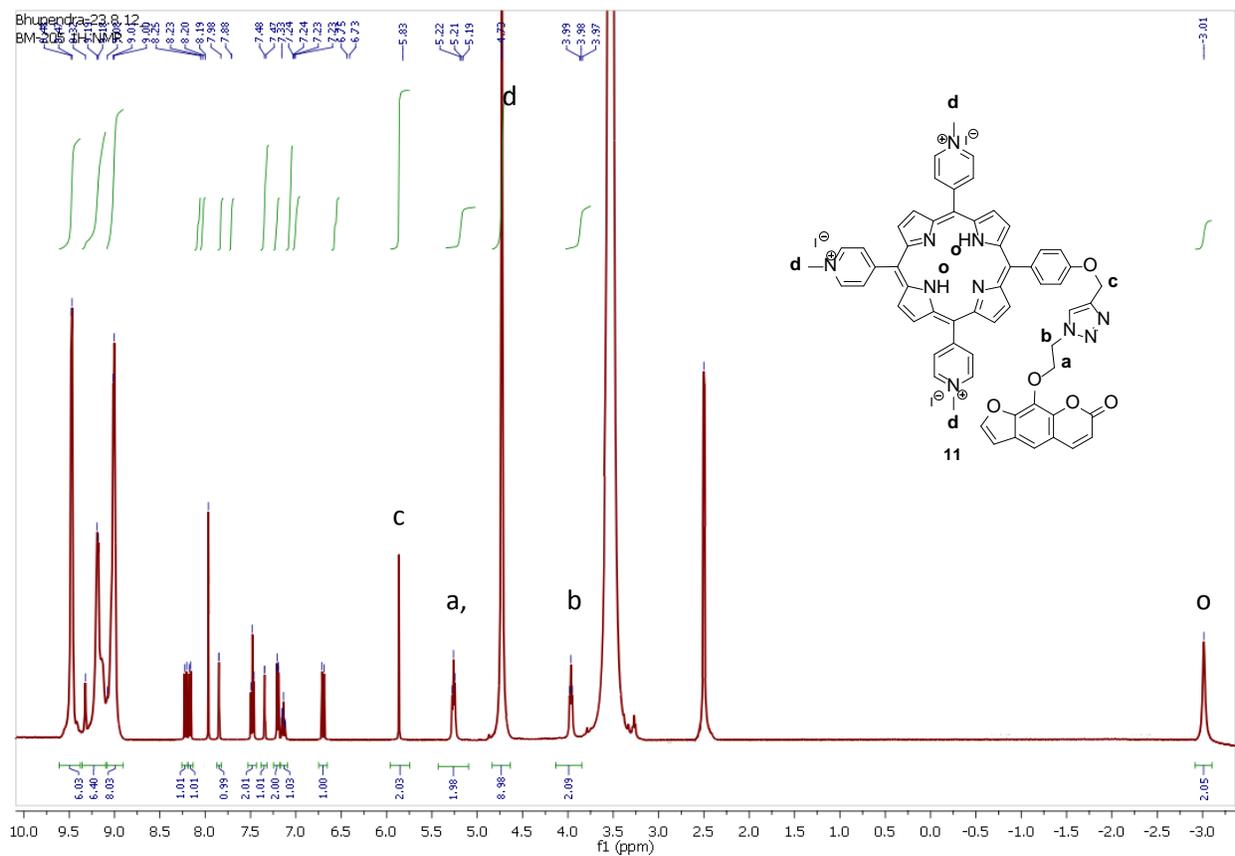
$^1\text{H}$  NMR spectrum of Zn (II) porphyrin conjugate **10**



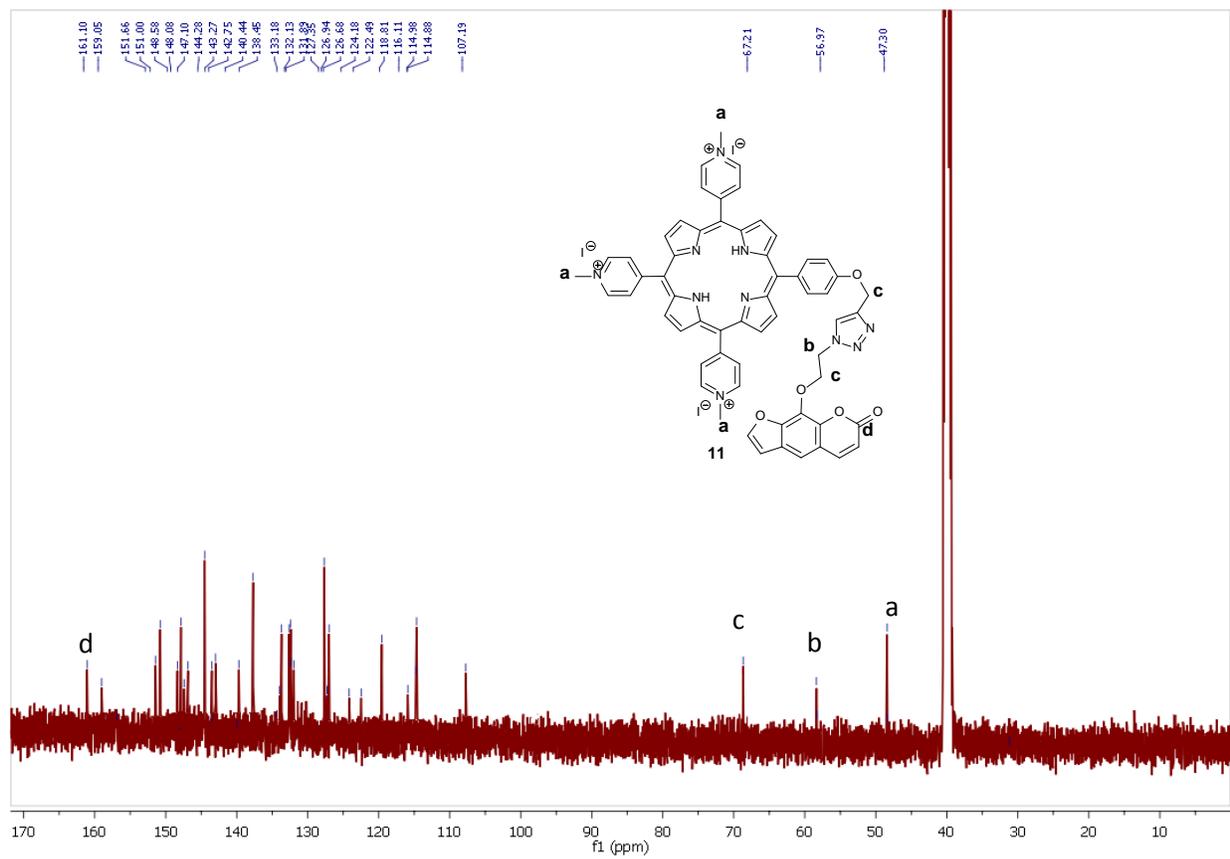
ESI-TOF MS spectrum of Zn (II) porphyrin conjugate **10**



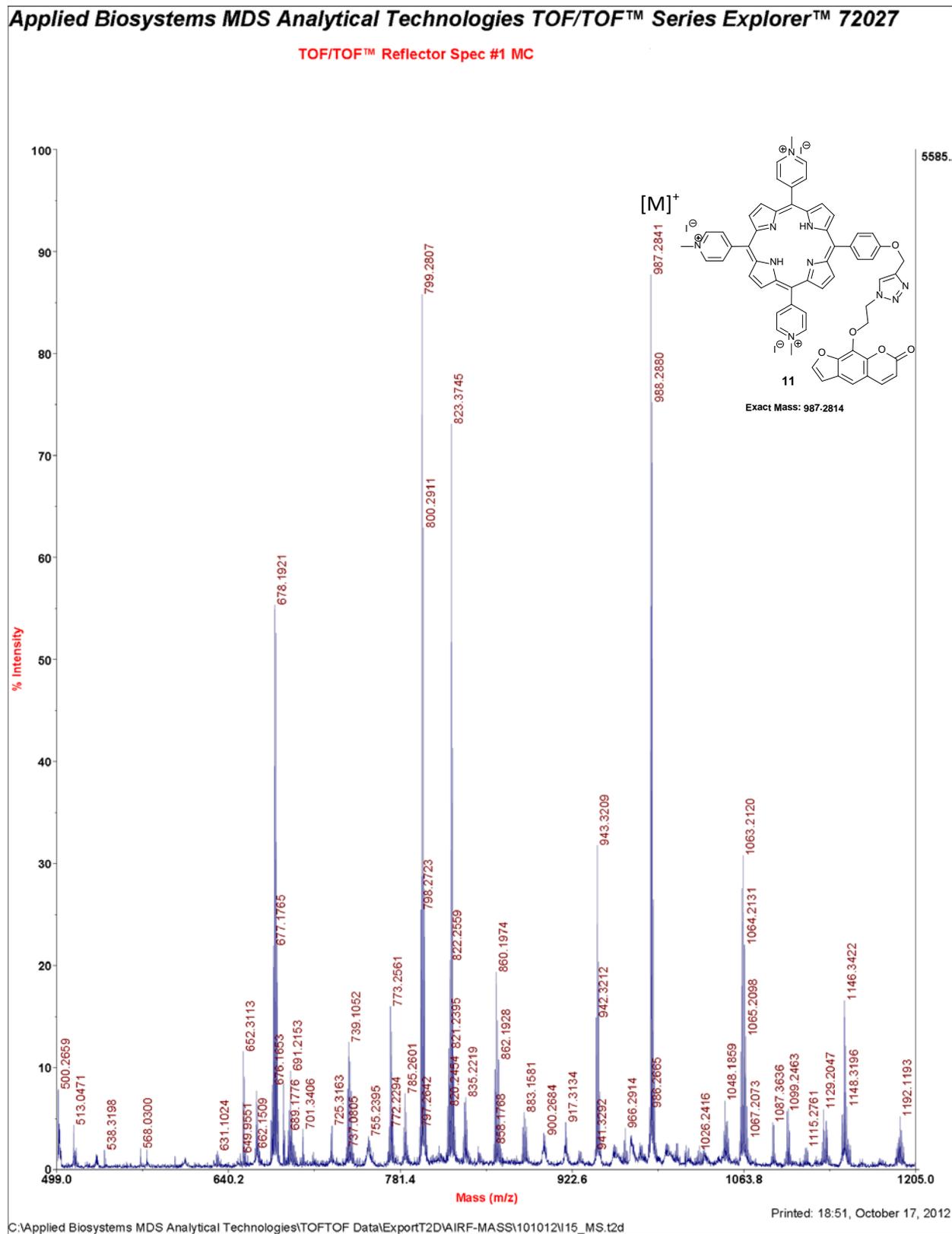
$^1\text{H}$  NMR spectrum of cationic porphyrin conjugate **11**



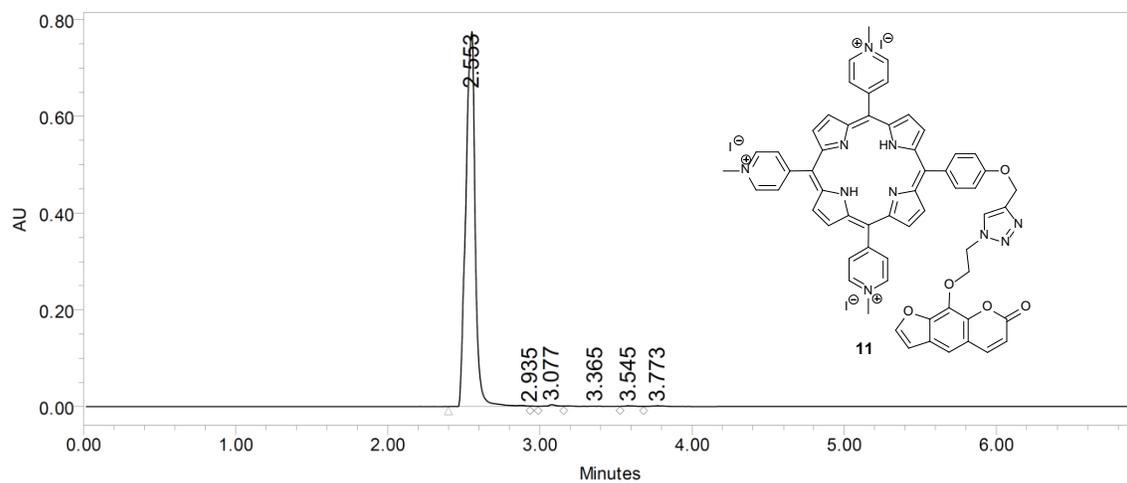
$^{13}\text{C}$  NMR spectrum of cationic porphyrin conjugate **11**



### MALDI-TOF MS spectrum of cationic porphyrin conjugate **11**



### HPLC Trace for cationic porphyrin conjugate **11**



#### Processed Channel Descr.: PDA 400.0 nm

	Processed Channel Descr.	RT	Area	% Area	Height
1	PDA 400.0 nm	2.553	3303471	99.24	775786
2	PDA 400.0 nm	2.935	3132	0.09	1132
3	PDA 400.0 nm	3.077	17513	0.50	3765
4	PDA 400.0 nm	3.365	2584	0.08	1001
5	PDA 400.0 nm	3.545	1758	0.05	654
6	PDA 400.0 nm	3.773	1406	0.04	513