Synthesis and *in vitro* investigation of cationic 5,15-diphenyl porphyrin-monoclonal antibody conjugates as targeted photodynamic sensitisers

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Abstract. The synthesis of three cationic 5,15-diphenyl porphyrins, bearing an isothiocyanate group for conjugation to proteins is described. The potential of these compounds as targeted photosensitisers for use in photodynamic therapy (PDT) is demonstrated by their efficient conjugation to monoclonal antibodies and the ability of the bioconjugates to bind and photodynamically inactivate cancer cells that express the corresponding antigen.

Introduction

Photodynamic therapy (PDT) is now becoming accepted as a clinical alternative to radiation and chemotherapy in the treatment of cancer (1) having already become established as a treatment regime in other diseases, most notably age related macular degeneration (2). Generally, two mechanisms of PDT activity are thought to operate. The first of these involves direct damage to blood vessels which, in the case of cancer treatment, leads to inhibition of blood flow into the tumour mass eventually causing necrosis and/or apoptosis; such a mechanism is also exploited to photodynamically destroy the neovasculature associated with macular degeneration. In the second mechanism, photosensitisers associate with, and often are internalised within tumour cells. Subsequent activation with light then causes oxidative damage to the cells, and again cell death. The proportion of each mechanism that is operating in any given clinical situation is difficult to ascertain precisely, but it is recognised that certain PDT drugs favour one mechanism over the other. Photofrin™ , possibly the most investigated PDT drug, has been shown to exert its activity primarily via vascular damage and shutdown (3), although some proportion of 'direct' damage to the cancer cells has also been demonstrated (4). Exploitation of photodynamic damage to the vasculature, while effective for well developed solid tumours and conditions in which inappropriate vascular growth is implicated, limits the treatment of conditions where damage to vasculature may have less effect, such as the treatment of small, early-stage tumours and metastases.

Tumour targeted PDT is an area that has recently been receiving considerable interest as a method for increasing the proportion of administered photosensitiser that has a direct cell killing effect on the tissue being treated (5). Targeting can involve the manipulation of substituents on the photosensitiser to affect the biodistribution and pharmacokinetics of the drug (6) or conjugation of photosensitisers to biologically-active species with affinity for the target tissue (5). In the latter category, the conjugates can be divided into those formed by non-covalent interactions, such as loading low density lipoproteins with photosensitiser, and covalent interactions, including conjugation to proteins (7). We reported an efficient method for conjugation of porphyrin-based photosensitisers to proteins, using bovine serum albumin (BSA) as a model (8). While BSA is a good model for developing bioconjugation strategies, it is of little value in targeting conjugates to specific tissues.

Monoclonal antibodies (Mab) represent ideal targeting moieties for small, early-stage tumours and metastases where penetration into the tumour mass is not a limiting factor, and many Mabs which target antigens overexpressed on tumours are now commercially available. Furthermore, with the advent of efficient humanisation and recombinant antibody technologies a growing number of therapeutic antibodies are gaining FDA approval, following good phase III clinical trial data, and are moving into the clinic (9). We now wish to report the application of our conjugation methods for porphyrins to Mab, and demonstrate that the bioconjugates formed in this way retain both the antigen recognition characteristics of the native antibody and the photosensitising abilities of the porphyrin. Combining these new derivatives in the reliable and efficient manner described here with the ever-growing panel of Mab available for tissue targeting offers an excellent way of further exploiting the potential of PDT across oncology.

Materials and methods

General. Melting points were measured in glass capillary tubes using Gallenkamp melting point apparatus and are uncorrected.

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IR spectra were recorded on a Perkin-Elmer 882 IR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on a JEOL JNM-LA-400 spectrometer (at 400 MHz) and are referenced downfield to tetramethylsilane. UV/vis spectra were recorded on an Agilent 8453 UV-visible spectrophotometer. Nominal mass spectra were obtained using a Bruker Reflex IV MALDI-TOF spectrometer or Shimadzu QP 5050A EI-MS. Accurate masses were obtained from the EPSRC Mass Spectrometry Service, Swansea, Wales. All commercial chemicals and solvents were of reagent grade or higher and were used as received, unless otherwise specified. All experiments with moisture- or air-sensitive compounds were performed in anhydrous solvents under a nitrogen atmosphere. TLC analyses were performed on Merck silica gel 60 plates (F_{254}, 0.2 mm thick). Flash column chromatography was performed with MP silica gel 60 (32-63) with all of the crude reaction mixtures being pre-adsorbed onto silica gel prior to separation, unless otherwise stated. All purified compounds were found to contain only one component by TLC analysis.

Synthesis

5-(4-Nitrophenyl)dipyrromethane 1. Dipyrromethane 1 was synthesized according to the method of Boyle *et al* (10). The mixture of freshly distilled pyrrole (36.8 ml, 0.53 mol, 40 equiv) and 4-nitrobenzaldehyde (2 g, 13.24 mmol) was purged with nitrogen for 15 min, then trifluoroacetic acid (TFA) (0.1 ml, 1.32 mmol, 0.1 equiv) was added under nitrogen and the mixture was stirred for another 30 min at room temperature. The reaction mixture was quenched by adding solid Na_2CO_3 (0.208 g, 1.96 mmol). This solid was then removed by filtration and the excess pyrrole was removed *in vacuo*. The residue was purified by column chromatography on silica with CH_2Cl_2 as eluent to afford 1 as yellow crystals (2.31 g, 65%); R_f=0.47 (CH_2Cl_2, silica); mp 155-156˚C; ^{1}H NMR ($CDCl_3$): δ 8.18-8.15 (m, 2H, *J*=8.7 Hz, Ar*H*), 7.99 (br s, 2H, N*H*), 7.39-7.36 (m, 2H, *J*=8.7 Hz, Ar*H*), 6.75-6.74 (m, 2H, *1-H*), 6.18 (q, 2H, *J*=2.8 Hz, *2-H*), 5.87-5.78 (m, 2H, *3-H*), 5.58 (s, 1H, *5-H*); ^{13}C NMR ($CDCl_3$): δ 150.6, 145.8, 130.9, 128.7, 122.7, 117.1, 107.1, 106.6, 43.1; EI-MS m/z 267 ([M]$^+$, 100%).

5-(4-Aminophenyl)dipyrromethane 2. Dipyrromethane 1 (4.36 g, 16.3 mmol) was dissolved in anhydrous tetrahydrofuran (90 ml) and 10% Pd/C (2 g) was added. The mixture was stirred in the dark under a hydrogen atmosphere at room temperature overnight. The solution was filtered through Celite; the flask and the Celite layer were rinsed with CH_2Cl_2. The solvent was removed *in vacuo* to afford 2 as a white solid (3.64 g, 94%); R_f=0.51 (EtOAc, silica); mp 139-140˚C; ^{1}H NMR ($CDCl_3$ + DMSO-*d*6): δ 8.82 (br s, 2H, N*H*), 6.95-6.92 (m, 2H, *J*=8.4 Hz, Ar*H*), 6.64-6.62 (m, 2H, *1-H*), 6.61-6.59 (m, 2H, *J*=8.4 Hz, Ar*H*), 6.04 (q, 2H, *J*=2.8 Hz, *2-H*), 5.82-5.81 (m, 2H, *3-H*), 5.33 (s, 1H, *5-H*); ^{13}C NMR ($CDCl_3$ + DMSO-d_6): δ 145.0, 133.2, 132.2, 128.9, 116.7, 114.7, 107.4, 106.4, 42.8; IR (KBr, cm^{-1}): υ 3384, 3352, 3318, 3223, 3111, 1615, 1560, 1515, 1439, 1264; EI-MS m/z 237 ([M]$^+$, 100%).

5-(4-Fluorenylmethylaminophenyl)dipyrromethane 3. Solid $NaHCO_3$ (3.9 g, 46.5 mmol) was added to a stirred solution of dipyrromethane 2 (1.86 g, 7.8 mmol) in anhydrous 1,4-dioxane (95 ml). To this mixture was then added a solution of 9-fluorenylmethyl chloroformate (4.05 g, 1.56 mmol, 2 equiv) in dioxane (42 ml) under nitrogen. The mixture was stirred at room temperature for 4 h. The solvent was removed *in vacuo*, and the solid residue was dissolved in CH_2Cl_2 and washed with water and brine. The organic layer was dried over Na_2SO_4, the solvent was then removed *in vacuo*, and the product was purified by column chromatography on silica with CH_2Cl_2 as eluent to give 3 as a pale yellow solid (3.48 g, 97%); R_f=0.25 (CH_2Cl_2, silica); mp 147˚C; ^{1}H NMR ($CDCl_3$): δ 7.93 (br s, 2H, N*H*), 7.78 (d, 2H, *J*=7.3 Hz, Ar*H*), 7.61 (d, 2H, *J*=7.6 Hz, Ar*H*), 7.43-7.31 (m, 6H, *J*=7.6 Hz, *J*=7.3 Hz, Ar*H*), 7.15 (d, 2H, *J*=8.4 Hz, Ar*H*), 6.70-6.69 (m, 2H, *1-H*), 6.16 (q, 2H, *J*=2.8 Hz, *2-H*), 5.91-5.90 (m, 2H, *3-H*), 5.44 (s, 1H, *5-H*), 4.55 (d, 2H, *J*=6.5 Hz, fluoreno-C*H*$_2$), 4.27 (t, 1H, *J*=6.5 Hz, fluoreno-C*H*), 3.70 (s, 1H, N*H*); ^{13}C NMR ($CDCl_3$): δ 143.7, 141.4, 137.4, 136.5, 132.5, 129.0, 127.8, 127.2, 124.9, 120.1, 119.8, 119.1, 117.3, 108.5, 107.2, 66.8, 47.2, 43.4; IR (KBr, cm^{-1}): υ 3335, 3140, 3064, 2969, 1703, 1521, 1416, 1299, 1227; EI-MS m/z 459 ([M]$^+$, 12%), 237 (39), 178 (100).

5-(4-Pyridyl)dipyrromethane 4. Dipyrromethane 4 was synthesized and described previously by Gryko and Lindsey (11).

5-[4-(2-Pyridyl)phenyl]dipyrromethane 5. Dipyrromethane 5 was synthesized according to the method of Boyle *et al* (10). The mixture of freshly distilled pyrrole (37.9 ml, 0.55 mol, 40 equiv) and 4-pyridin-2-yl-benzaldehyde (2.5 g, 13.65 mmol) was purged with nitrogen for 15 min, then trifluoroacetic acid (TFA) (0.1 ml, 1.36 mmol, 0.1 equiv) was added under nitrogen. The reaction mixture was stirred overnight at room temperature and then quenched by adding solid Na_2CO_3 (0.21 g, 2 mmol). This solid was removed by filtration and the excess pyrrole was removed *in vacuo*. The crude reaction mixture was purified by column chromatography on silica with CH_2Cl_2/EtOAc (7:1) as eluent to afford 5 as a yellow solid (2.78 g, 68%); R_f=0.55 (4:1 CH_2Cl_2:EtOAc, silica); mp 124˚C; ^{1}H NMR ($CDCl_3$): δ 8.69-8.68 (m, 1H, Pyr*H*), 7.99 (br s, 2H, N*H*), 7.95-7.93 (m, 2H, *J*=8.4 Hz, Ar*H*), 7.79-7.70 (m, 2H, Pyr*H*), 7.34 (d, 2H, *J*=8.4 Hz, Ar*H*), 7.25-7.23 (m, 1H, Pyr*H*), 6.72-6.71 (m, 2H, *1-H*), 6.17 (q, 2H, *J*=2.8 Hz, *2-H*), 5.96-5.95 (m, 2H, *3-H*), 5.54 (s, 1H, *5-H*); ^{13}C NMR ($CDCl_3$): δ 156.5, 148.5, 143.8, 138.2, 136.7, 132.1, 129.1, 127.5, 122.4, 121.2, 117.4, 108.5, 107.4, 43.8; EI-MS m/z 299 ([M]$^+$, 91%), 233 (40), 154 (32), 145 (100).

5-(4-N,N'-Dimethylaminophenyl)dipyrromethane 6. The mixture of freshly distilled pyrrole (50 ml, 0.72 mol, 45 equiv) and 4-dimethylamino-benzaldehyde (2.38 g, 16 mmol) was purged with nitrogen for 15 min, then trifluoroacetic acid (TFA) (0.3 ml, 4 mmol, 0.25 equiv) was added under nitrogen and the mixture was stirred for another 30 min at room temperature. The reaction mixture was quenched by adding solid Na_2CO_3 (0.6 g, 5.65 mmol). This solid was then removed by filtration and the excess pyrrole was removed *in vacuo*. The residue was purified by column chromatography on silica with CH_2Cl_2 as eluent to afford 6 as pale-yellow

solid (3.01 g, 71%); R_f=0.23 (CH_2Cl_2, silica); mp 124-125˚C; ^{1}H NMR ($CDCl_3$): δ 7.93 (br s, 2H, N*H*), 7.74 (d, 2H, *J*=8.7 Hz, Ar*H*), 7.08 (d, 2H, *J*=8.7 Hz, Ar*H*), 6.72-6.68 (m, 2H, *1-H*), 6.15 (q, 2H, *J*=2.8 Hz, *2-H*), 5.94-5.93 (m, 2H, *3-H*), 5.40 (s, 1H, *5-H*), 2.93 (s, 6H, N(CH_3)$_2$); EI-MS m/z 265 ([M]$^+$, 100%).

5-(4-Flourenylmethylaminophenyl)-15-(4-pyridyl)porphyrin 7. Porphyrin 7 was synthesized according to the method of Boyle *et al* (10) from dipyrromethanes 3 and 4, the ratio of dipyrromethanes used was 3:4, 1:3 equivalents respectively. To a stirred solution of dipyrromethane 3 (0.792 g, 1.725 mmol) and dipyrromethane 4 (1.17 g, 5.175 mmol) in CH_2Cl_2 (1040 ml), trimethylorthoformate (27 ml, 0.245 mol) was added by syringe under nitrogen. Then, trichloroacetic acid (13.245 g, 0.08 mol) in CH_2Cl_2 (250 ml) was added dropwise over 15 min. The reaction was allowed to stir protected from light for 4 h at room temperature. The reaction was then quenched with pyridine (23.4 ml, 0.289 mol) and left to stir protected from light for a further 17 h at room temperature. The solution was purged with compressed air for 15 min and finally stirred open to air and light for 4 h at room temperature. Excess solvent was removed *in vacuo*, and the porphyrin mixture was purified by column chromatography on silica with CH_2Cl_2 (1% of triethylamine was used to make the slurry for the column), then with CH_2Cl_2/EtOAc (4:1) as eluent. The solid obtained was washed with a minimum amount of ethanol to afford 7 as a purple-brown solid (45 mg, 4%); R_f=0.41 (4:1 CH_2Cl_2:EtOAc, silica); ^{1}H NMR ($CDCl_3$): δ 10.36 (s, 2H, meso-*H*), 9.62 (br s, 1H, N*H*), 9.46-9.42 (dd, 4H, *J*=4.8 Hz, *J*=4.5 Hz, ß-*H*), 9.12 (d, 2H, *J*=4.8 Hz, ß-*H*), 9.04 (d, 2H, *J*=4.5 Hz, ß-*H*), 8.21 (d, 2H, *J*=7.8 Hz, Pyr*H*), 7.86 (d, 4H, *J*=7.3 Hz, Ar*H*), 7.76 (d, 4H, *J*=7.3 Hz, Ar*H*), 7.52-7.41 (m, 6H, Ar*H* + Pyr*H*), 4.74 (d, 2H, *J*=7.6 Hz, fluoreno-CH_2), 4.43 (t, 1H, *J*=7.6 Hz, fluoreno-C*H*), -3.14 (br s, 2H, N*H*); ^{13}C NMR ($CDCl_3$): δ 147.6, 145.5, 145.3, 143.8, 141.5, 137.7, 135.5, 132.5, 131.9, 131.4, 130.1, 127.9, 127.3, 125.0, 120.2, 105.8, 67.1, 47.3; IR (KBr, cm^{-1}): υ 3423, 2926, 1717, 1653, 1593, 1529, 1407, 1314, 1222, 1053, 957; UV/vis (CH_2Cl_2, nm): λ 408, 503, 538, 576, 631; MALDI-MS m/z (CH_2Cl_2) 701.6 ([M]$^+$, 100%).

5-(4-Aminophenyl)-15-(4-pyridyl)porphyrin 8. Porphyrin 7 (60 mg, 0.086 mmol) was dissolved in anhydrous CH_2Cl_2 (15 ml), and piperidine (0.42 ml, 4.28 mmol, 50 equiv) was added under nitrogen. The reaction mixture was stirred overnight at room temperature, protected from light. The solvent and the excess of piperidine were removed *in vacuo*, and the crude amine was purified by column chromatography on silica with EtOAc (1% of triethylamine was used to make the slurry for the column) to give 8 as a red-brown solid (26 mg, 63%); R_f=0.23 (EtOAc, silica); ^{1}H NMR ($CDCl_3$): δ 10.34 (s, 2H, meso-*H*), 9.44 (d, 2H, *J*=4.5 Hz, ß-*H*), 9.41 (d, 2H, *J*=4.8 Hz, ß-*H*), 9.19 (d, 2H, *J*=4.8 Hz, ß-*H*), 9.08 (d, 2H, *J*=5.6 Hz, Pyr*H*), 9.03 (d, 2H, *J*=4.5 Hz, ß-*H*), 8.25 (d, 2H, *J*=5.6 Hz, Pyr*H*), 8.07 (d, 2H, *J*=8.4 Hz, Ar*H*), 7.15 (d, 2H, *J*=8.4 Hz, Ar*H*), 3.50 (br s, 2H, NH_2), -3.09 (br s, 2H, N*H*); UV/vis (CH_2Cl_2, nm): λ 409, 505, 542, 577, 635; MALDI-MS m/z (CH_2Cl_2) 479.6 ([M+1]$^+$, 100%).

5-(4-Isothiocyanatophenyl)-15-(4-pyridyl)porphyrin 9. Porphyrin 9 was synthesized from porphyrin 8 according to the method described by Sutton *et al* (8). Porphyrin 8 (22.32 mg, 46.64 μmol) was dissolved in anhydrous CH_2Cl_2 (15 ml), stirred under nitrogen and protected from light. 1,1'-Thiocarbonyldi-2,2'-pyridone (TDP) (21.66 mg, 93.28 μmol) was dissolved in CH_2Cl_2 (1-2 ml) and injected by syringe. The mixture was stirred for 2 h at room temperature. Excess solvent was removed *in vacuo* and the porphyrin mixture was purified by column chromatography on silica with CH_2Cl_2/EtOAc (4:1) as eluent to afford 9 as a violet solid (17 mg, 70%); R_f=0.30 (EtOAc, silica); ^{1}H NMR ($CDCl_3$): δ 10.36 (s, 2H, meso-*H*), 9.45-9.43 (m, 4H, ß-*H*), 9.05-9.04 [m (overlapping), 6H, ß-*H* + Pyr*H*], 8.26 (d, 2H, *J*=8.2 Hz, Ar*H*), 8.22 (d, 2H, *J*=5.6 Hz, Pyr*H*), 7.69 (d, 2H, *J*=8.2 Hz, Ar*H*), -3.18 (br s, 2H, N*H*); UV/vis (CH_2Cl_2, nm): λ 406, 502, 537, 575, 630; MALDI-MS m/z (CH_2Cl_2) 520.5 ([M]$^+$, 100%).

5-(4-Isothiocyanatophenyl)-15-(4-N-methylpyridiniumyl) porphyrin chloride 10. Porphyrin 9 (17 mg, 32.65 μmol) was dissolved in anhydrous DMF (15 ml), and iodomethane (0.45 ml, 7.2 mmol) was added under nitrogen. The reaction mixture was stirred overnight at room temperature, protected from light. The solvent and the excess of iodomethane were removed *in vacuo*, and the solid obtained was dissolved in anhydrous MeOH (30 ml). Amberlite IRA 400 (Cl) (0.9 g) was added and the mixture was stirred for 1 h at room temperature. Amberlite resin was removed by filtration, and the volume of the porphyrin filtrate was reduced *in vacuo*. The product was then collected by precipitation from Et_2O to give 10 as a dark violet solid (7.5 mg, 40%); R_f=0.60 (4:1 CH_3CN:5 M KNO_3(aq), silica); ^{1}H NMR (DMSO-d_6): δ 10.54 (s, 2H, meso-*H*), 9.65-9.51 [m (overlapping), 6H, ß-*H* + Pyr*H*], 9.12-9.09 (m, 4H, ß-*H*), 8.96 (d, 2H, *J*=6.2 Hz, Pyr*H*), 8.30 (d, 2H, *J*=8.4 Hz, Ar*H*), 8.01-7.99 (m, 2H, Ar*H*), 2.97 (s, 3H, N-CH_3), -3.12 (br s, 2H, N*H*); UV/vis (CH_2Cl_2, nm): λ 407, 504, 545, 575, 634; MALDI-MS m/z (CH_2Cl_2) 535.4 ([M]$^+$, 100%). ES-HRMS Anal. Calcd. for $C_{33}H_{23}N_6S$ ([M]$^+$), 535.1699; found, 535.1697.

5-(4-Flourenylmethylaminophenyl)-15-[4-(2-pyridyl)phenyl] porphyrin 11. Porphyrin 11 was synthesized following the method described above for porphyrin 7, from dipyrromethanes 3 and 5, using 1:1 equivalents. To a stirred solution of dipyrromethane 3 (0.792 g, 1.725 mmol) and dipyrromethane 5 (0.516 g, 1.725 mmol) in CH_2Cl_2 (1040 ml), trimethylorthoformate (27 ml, 0.245 mol) was added by syringe under nitrogen. Then, trichloroacetic acid (13.245 g, 0.08 mol) in CH_2Cl_2 (250 ml) was added dropwise over 15 min. The reaction was allowed to stir protected from light for 4 h at room temperature. The reaction was then quenched with pyridine (23.4 ml, 0.289 mol) and left to stir protected from light for a further 17 h at room temperature. The solution was purged with compressed air for 15 min and finally stirred open to air and light for 4 h at room temperature. Excess solvent was removed *in vacuo*, and the porphyrin mixture was purified by column chromatography on silica with *n*-hexane/CH_2Cl_2/EtOAc (6:10:1) as eluent to afford 11 as a dark violet solid

(90 mg, 7%); R_f=0.66 (EtOAc, silica); ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 10.35 (s, 2H, meso-*H*), 9.45-9.42 (m, 4H, *J*=4.8, ß-*H*), 9.15-9.14 (m, 4H, ß-*H*), 8.93 (br s, 1H, N*H*), 8.51-8.41 (m, 4H, Ar*H*), 8.24-7.97 (m, 6H, Ar*H* + Pyr*H*), 7.86-7.83 (m, 4H, Ar*H*), 7.50-7.43 (m, 4H, Ar*H*), 7.08-7.06 (m, 2H, Ar*H*), 4.71 (d, 2H, *J*=7.5 Hz, fluoreno-CH_2), 4.44 (t, 1H, *J*=7.5 Hz, fluoreno-C*H*), -3.08 (br s, 2H, N*H*); UV/vis (CH_2Cl_2, nm): λ 410, 505, 540, 579, 631; MALDI-MS m/z (CH_2Cl_2) 776.7 ([M]$^+$, 100%).

5-(4-Aminophenyl)-15-[4-(2-pyridyl)phenyl]porphyrin 12. Porphyrin 12 was synthesized from porphyrin 11 following the same procedure as described above for porphyrin 8. Porphyrin 11 (57 mg, 0.073 mmol) was dissolved in anhydrous CH_2Cl_2 (15 ml), and piperidine (0.36 ml, 3.66 mmol, 50 equiv) was added under nitrogen. The reaction mixture was stirred overnight at room temperature, protected from light. The solvent and the excess of piperidine were removed *in vacuo*, and the crude amine was purified by column chromatography on silica with *n*-hexane/CH_2Cl_2/EtOAc (3:5:1) as eluent to afford 12 as a dark brown solid (24 mg, 60%); R_f=0.48 (EtOAc, silica); ^{1}H NMR ($CDCl_3$): δ 10.61 (s, 2H, meso-*H*), 9.67-9.64 (dd, 4H, *J*=4.8 Hz, *J*=4.5 Hz, ß-*H*), 9.17 (d, 2H, *J*=4.5 Hz, ß-*H*), 9.11 (d, 2H, *J*=4.8 Hz, ß-*H*), 8.88-8.87 (m, 1H, *J*=0.84 Hz, Pyr*H*), 8.62 (d, 2H, *J*=7.9 Hz, Ar*H*), 8.48 (d, 1H, *J*=3.1 Hz, Pyr*H*), 8.41 (d, 2H, *J*=7.9 Hz, Ar*H*), 8.36-8.33 (m, 1H, *J*=7.9 Hz, *J*=3.1 Hz, Pyr*H*), 8.10-8.05 (m, 1H, *J*=7.9 Hz, *J*=0.84 Hz, Pyr*H*), 7.96 (d, 2H, *J*=8.2 Hz, Ar*H*), 7.08 (d, 2H, *J*=8.2 Hz, Ar*H*), 3.35 (br s, 2H, NH_2), -3.11 (br s, 2H, N*H*); UV/vis (CH_2Cl_2, nm): λ 412, 505, 543, 580, 637; MALDI-MS m/z (CH_2Cl_2) 554.4 ([M]$^+$, 100%).

5-(4-Isothiocyanatophenyl)-15-[4-(2-pyridyl)phenyl] porphyrin 13. Porphyrin 13 was synthesized from porphyrin 12 following the same procedure as described for porphyrin 9. Porphyrin 12 (20 mg, 36.06 μmol) was dissolved in anhydrous CH_2Cl_2 (15 ml) and stirred under nitrogen and protected from light. 1,1'-Thiocarbonyldi-2,2'-pyridone (TDP) (16.75 mg, 72.12 μmol) was dissolved in CH_2Cl_2 (1-2 ml) and injected by syringe. The mixture was stirred for 2 h at room temperature. Excess solvent was removed *in vacuo* and the porphyrin mixture was purified by column chromatography on silica with CH_2Cl_2 as eluent to afford 13 as a pink-violet solid (15.7 mg, 73%); R_f=0.34 (CH_2Cl_2, silica); ^{1}H NMR ($CDCl_3$): δ 10.35 (s, 2H, meso-*H*), 9.44-9.43 (dd, 4H, *J*=4.5 Hz, *J*=4.4 Hz, ß-*H*), 9.15 (d, 2H, *J*=4.5 Hz, ß-*H*), 9.03 (d, 2H, *J*=4.4 Hz, ß-*H*), 8.97-8.79 (m, 2H, Pyr*H*), 8.51 (d, 2H, *J*=7.9 Hz, Ar*H*), 8.43 (d, 2H, *J*=7.9 Hz, Ar*H*), 8.28-8.08 (m, 4H, *J*=8.1 Hz, Ar*H* + Pyr*H*), 7.69 (d, 2H, *J*=8.1 Hz, Ar*H*), -3.12 (br s, 2H, N*H*); UV/vis (CH_2Cl_2, nm): λ 410, 504, 539, 577, 632; MALDI-MS m/z (CH_2Cl_2) 596.4 ([M]$^+$, 100%).

5-(4-Isothiocyanatophenyl)-15-[4-(2-N-methylpyridiniumyl) phenyl]porphyrin chloride 14. Porphyrin 13 (15.7 mg, 26 μmol) was dissolved in anhydrous CH_2Cl_2 (15 ml), and iodomethane (0.4 ml, 6.4 mmol) was added under nitrogen. The reaction mixture was heated at reflux, and stirred for 3 days, protected from light. The solvent and the excess of iodomethane were removed *in vacuo*, and the solid obtained was dissolved in anhydrous MeOH (27 ml). Amberlite IRA 400 (Cl) (0.9 g) was added and the mixture was stirred for 1 h at room temperature. Amberlite resin was removed by filtration, and the volume of the porphyrin filtrate was reduced *in vacuo*. The product was then collected by precipitation from Et_2O to give 14 as a dark violet solid (6.2 mg, 37%); R_f=0.73 (4:1 CH_3CN:5 M KNO_3(aq), silica); ^{1}H NMR ($CDCl_3$ + DMSO-d_6): δ 10.69 (s, 2H, meso-*H*), 9.74-9.71 (m, 4H, ß-*H*), 9.33 (d, 1H, *J*=6.2 Hz, Pyr*H*), 9.17-9.12 (m, 4H, ß-*H*), 8.87-8.83 (m, 1H, Pyr*H*), 8.58 (d, 2H, *J*=8.2 Hz, Ar*H*), 8.51 (d, 2H, *J*=7.3 Hz, Ar*H*), 8.34-8.18 (m, 4H, Ar*H* + Pyr*H*), 7.90 (d, 2H, *J*=8.2 Hz, Ar*H*), 4.54 (s, 3H, N-CH_3), -3.21 (br s, 2H, N*H*); UV/vis (CH_2Cl_2, nm): λ 405, 502, 536, 575, 468; MALDI-MS m/z (MeOH) 611.3 ([M]$^+$, 100%). ES-HRMS Anal. Calcd. for $C_{39}H_{27}N_6S$ ([M]$^+$), 611.2012; found, 611.2018.

5-(4-Flourenylmethylaminophenyl)-15-(4-N,N'-dimethyl-aminophenyl)porphyrin 15. Porphyrin 15 was synthesized following the same method as described above for porphyrin 7, from dipyrromethanes 3 and 6, using 1:1 equivalents. To a stirred solution of dipyrromethane 3 (0.792 g, 1.725 mmol) and dipyrromethane 6 (0.458 g, 1.725 mmol) in CH_2Cl_2 (1040 ml), trimethylorthoformate (27 ml, 0.245 mol) was added by syringe under nitrogen. Then, trichloroacetic acid (13.245 g, 0.08 mol) in CH_2Cl_2 (250 ml) was added dropwise over 15 min. The reaction was allowed to stir protected from light for 4 h at room temperature. The reaction was then quenched with pyridine (23.4 ml, 0.289 mol) and left to stir protected from light for a further 17 h at room temperature. The solution was purged with compressed air for 15 min and finally stirred open to air and light for 4 h at room temperature. Excess solvent was removed *in vacuo*, and the porphyrin mixture was purified by column chromatography on silica with CH_2Cl_2 as eluent to afford 15 as a violet solid (160 mg, 12.5%); R_f=0.43 (CH_2Cl_2, silica); ^{1}H NMR ($CDCl_3$): δ 10.29 (s, 2H, meso-*H*), 9.39-9.38 (dd, 4H, *J*=4.8, *J*=4.5 Hz, ß-*H*), 9.19 (d, 2H, *J*=4.8 Hz, ß-*H*), 9.08 (d, 2H, *J*=4.5 Hz, ß-*H*), 8.22-8.15 [m (overlapping), 5H, Ar*H* + N*H*], 7.87-7.76 (m, 6H, Ar*H*), 7.52-7.41 (m, 4H, Ar*H*), 7.22-7.17 (m, 2H, Ar*H*), 4.74 (d, 2H, *J*=6.8 Hz, fluoreno-CH_2), 4.43 (t, 1H, *J*=6.8 Hz, fluoreno-C*H*), 3.27 [s, 6H, N(CH_3)$_2$], -3.07 (br s, 2H, N*H*); UV/vis (CH_2Cl_2, nm): λ 409, 508, 549, 579, 638; MALDI-MS m/z (CH_2Cl_2) 742.8 ([M]$^+$, 100%).

5-(4-Aminophenyl)-15-(4-N,N'-dimethylaminophenyl) porphyrin 16. Porphyrin 16 was synthesized from porphyrin 15 following the same procedure as described for porphyrin 8. Porphyrin 15 (56.9 mg, 0.076 mmol) was dissolved in anhydrous CH_2Cl_2 (13 ml), and piperidine (0.37 ml, 3.8 mmol, 50 equiv) was added under nitrogen. The reaction mixture was stirred overnight at room temperature, protected from light. The solvent and the excess of piperidine were removed *in vacuo*, and the crude amine was purified by column chromatography on silica with CH_2Cl_2 as eluent to afford 16 as a dark violet solid (23.7 mg, 60%); R_f=0.32 (CH_2Cl_2, silica); ^{1}H NMR ($CDCl_3$): δ 10.54 (s, 2H, meso-*H*), 9.62-9.60 (m, 4H, ß-*H*), 9.13 (d, 2H, *J*=4.5 Hz, ß-*H*), 9.11 (d, 2H, *J*=4.5 Hz, ß-*H*),

8.11 (d, 2H, *J*=8.7 Hz, Ar*H*), 7.94 (d, 2H, *J*=8.4 Hz, Ar*H*), 7.23 (d, 2H, *J*=8.7 Hz, Ar*H*), 7.07 (d, 2H, *J*=8.4 Hz, Ar*H*), 3.34 (br s, 2H, NH_2), 3.22 (s, 6H, N(CH_3)$_2$), -3.06 (br s, 2H, N*H*); UV/vis (CH_2Cl_2, nm): λ 414, 511, 575, 485, 576; MALDI-MS m/z (CH_2Cl_2) 520.4 ([M]$^+$, 100%).

5-(4-Isothiocyanatophenyl)-15-(4-N,N'-dimethylaminophenyl)porphyrin 17. Porphyrin 17 was synthesized from porphyrin 16 following the same procedure as described for porphyrin 9. Porphyrin 16 (23 mg, 44.2 μmol) was dissolved in anhydrous CH_2Cl_2 (15 ml) and stirred under nitrogen and protected from light. 1,1'-Thiocarbonyldi-2,2'-pyridone (TDP) (20.53 mg, 88.4 μmol) was dissolved in CH_2Cl_2 (1-2 ml) and injected with syringe. The mixture was stirred for 2 h at room temperature. Excess solvent was removed *in vacuo* and the The porphyrin mixture was purified by column chromatography on silica with *n*-hexane/CH_2Cl_2 (1:1) as eluent to afford 17 as a pink-violet solid (17.4 mg, 70%); R_f=0.37 (1:1 *n*-hexane:CH_2Cl_2, silica); ^{1}H NMR ($CDCl_3$): δ 10.31 (s, 2H, meso-*H*), 9.43-9.39 (dd, 4H, *J*=4.8 Hz, *J*=4.5 Hz, ß-*H*), 9.15 (d, 2H, *J*=4.8 Hz, ß-*H*), 9.02 (d, 2H, *J*=4.5 Hz, ß-*H*), 8.27 (d, 2H, *J*=8.2 Hz, Ar*H*), 7.72-7.67 (m, 4H, *J*=8.2 Hz, *J*=7.8 Hz, Ar*H*), 7.31-7.29 (m, 2H, Ar*H*), 3.36 [s, 6H, N(CH_3)$_2$], -3.09 (br s, 2H, N*H*); UV/vis (CH_2Cl_2, nm): λ 408, 508, 550, 573, 638; MALDI-MS m/z (CH_2Cl_2) 562.5 ([M]$^+$, 100%).

5-(4-Isothiocyanatophenyl)-15-(4-N,N',N''-trimethylammoniophenyl)porphyrin chloride 18. Porphyrin 17 (10 mg, 17.8 μmol) was dissolved in anhydrous CH_2Cl_2 (3 ml), then MeOH (0.5 ml) and iodomethane (0.5 ml, 8 mmol) were added under nitrogen. The reaction mixture was stirred for 2 days at room temperature, protected from light. The solvent and excess iodomethane were removed *in vacuo*, and the solid obtained was dissolved in anhydrous MeOH (20 ml). Amberlite IRA 400 (Cl) (0.6 g) was added and the mixture was stirred for 1 h at room temperature. Amberlite resin was removed by filtration, and the volume of the porphyrin filtrate was reduced *in vacuo*. The product was then collected by precipitation from Et_2O to give 18 as a dark violet solid (9.5 mg, 87%); R_f=0.64 [4:1 CH_3CN:5 M KNO_3(aq), silica]; ^{1}H NMR (DMSO-d_6): δ 10.45 (s, 2H, meso-*H*), 9.53-9.51 (dd, 4H, *J*=4.8 Hz, *J*=4.5 Hz, ß-*H*), 9.15-9.02 (dd, 4H, *J*=4.8 Hz, *J*=4.5 Hz, ß-*H*), 8.53-8.46 (m, 4H, *J*=7.3 Hz, Ar*H*), 8.29 (d, 2H, *J*=8.2 Hz, Ar*H*), 7.76 (d, 2H, *J*=8.2 Hz, Ar*H*), 3.95 [s, 9H, N(CH_3)$_3$], -3.12 (br s, 2H, N*H*); UV/vis (MeOH, nm): λ 404, 501, 535, 575, 468; MALDI-MS m/z (MeOH, HABA matrix) 577.45 ([M]$^+$, 100%). ES-HRMS Anal. Calcd. for $C_{36}H_{29}N_6S$ ([M]$^+$), 577.2169; found, 577.2171.

Biological protocols

Cell lines and antibodies. LoVo human colon adenocarcinoma and CORL23 human lung large cell carcinoma cell lines (ECACC) were grown in DMEM and RPMI respectively. All media was supplemented with 10% v/v fetal calf serum, 2 mM L-glutamine, 100 μg/ml streptomycin and 100 μg/ml penicillin (Invitrogen). All cells were maintained at 37˚C in a humidified incubator with a 5% CO_2 atmosphere.

The murine antibodies, anti-EpCAM and anti-CD104 were purchased from Serotec. Anti-EpCAM recognises a 40-kDa cell-cell adhesion molecule up-regulated on some carcinomas, particularly colorectal. Anti-CD104 binds to a 205-kDa glycoprotein involved in cell-cell adhesion and is up-regulated in colorectal and bladder carcinomas.

Conjugation to monoclonal antibodies. Conjugation was carried out in a 1-ml reaction volume containing 1 mg/ml antibody in 0.5 M bicarbonate buffer (pH 9.2) and a 20x molar excess of 10, 14 or 18. The reaction vessels were agitated gently at room temperature for 1 h and protected from light. The conjugates were purified using Sephadex G25 columns (Amersham) and eluted with phosphate-buffered saline (PBS) pH 7.4. The degree of labelling (the number of moles of porphyrins 10, 14 and 18 conjugated per mole of antibody) was calculated using spectroscopic methods.

Flow cytometry. Cells were removed from culture vessels with 5 mM EDTA in PBS. After washing in PBS cells were counted, resuspended in PBS/0.25% w/v BSA and 2x10^5 cells were added to each tube. The cells were labelled with 50 μl (5 μg/ml) of either anti-EpCAM or anti-CD104 (conjugated/unconjugated) for 1 h at 4˚C. After washing with PBS/BSA, cells were labelled with 5 μl (10 μg/ml) of rabbit anti-mouse IgG-FITC (Serotec) for 1 h at 4˚C. A further wash with PBS/BSA was performed before analysis of cells in a FACScalibur flow cytometer (BD Biosciences).

In vitro cytotoxicity. Cells were removed from culture vessels with 5 mM EDTA in PBS, washed, counted and 1x10^5 cells were added to 5 ml polypropylene tubes. Conjugated and unconjugated porphyrin was diluted in serum-free media, added to the cells, and incubated at 37˚C for 6 h. Cells were washed three times with media to remove unbound porphyrin, resuspended in complete media and plated out in duplicate in two 96-well plates (2.5x10^4 cells/well). One plate acted as the dark control (i.e. no irradiation) whilst the other was irradiated with 15 J/cm^2 of cooled and filtered red light (630 nm) delivered by a Patterson light system (Phototherapeutics Ltd: Patterson Lamp BL1000A, bandpass 630±15-nm filter). The plates were incubated at 37˚C overnight before a commercial MTS assay (Promega) was performed. The percentage of cell survival was calculated in proportion to the number of cells incubated without photosensitiser. All assays were performed in quadruplicate.

Results

The required dipyrromethane precursors 1-6 were synthesised and condensed with trimethylorthoformate under acidic conditions to yield, after chromatography, porphyrins 7, 11 and 15. Subsequent functional group modifications then gave cationic isothiocyanato diphenyl porphyrins (DPPs) 10, 14 and 18 (Fig. 1). DPPs 10, 14 and 18 were then used to investigate the conjugation of cationic isothiocyanato DPPs to monoclonal antibodies.

Conjugation of both antibodies (anti-EpCAM, and anti-CD104) was successfully carried out with all porphyrins. The average degree of labelling (moles porphyrin per mole antibody) was calculated from three experiments (Table I).

To assess any possible change in antibody binding specificity following conjugation, binding of conjugated and

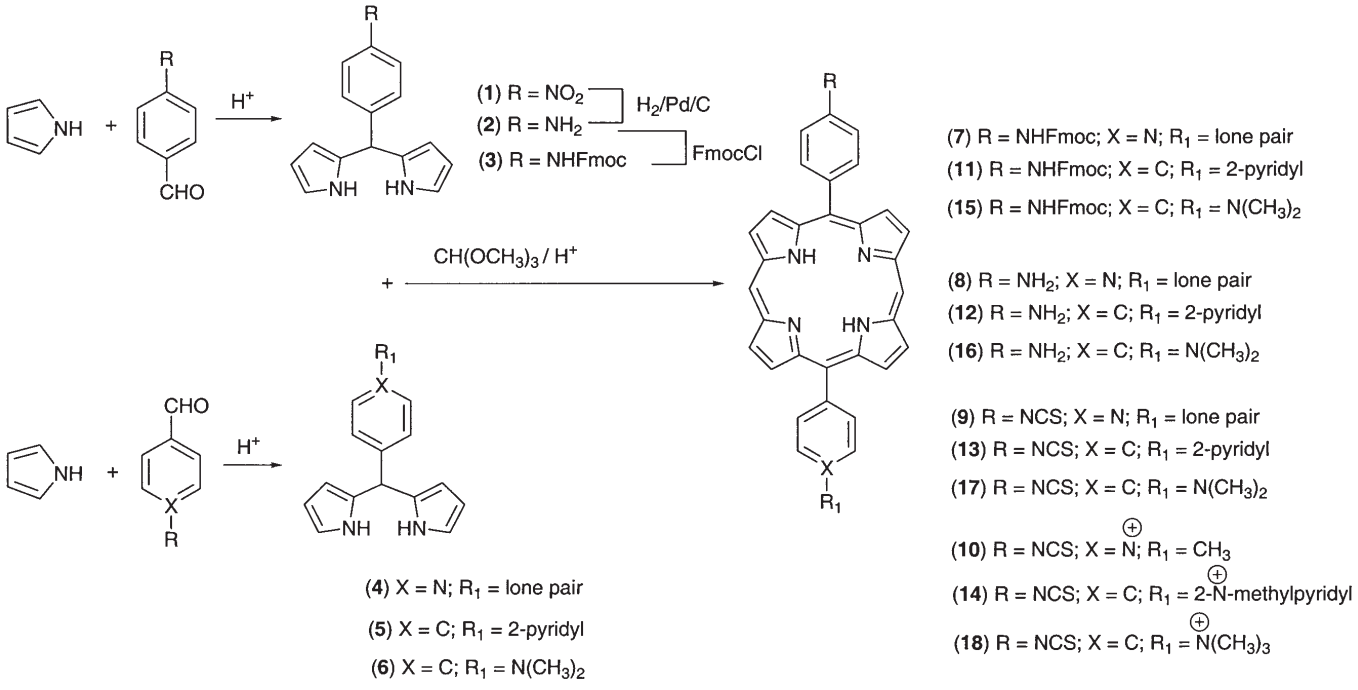

Figure 1. Synthetic routes to cationic isothiocyanato 5,15-diphenyl porphyrins.

Table I. Average degree of labelling for Mabs with porphyrins 10, 14, and 18.

Mab	10 (SD)	14 (SD)	18 (SD)
Anti-EpCAM	0.81 (0.05)	0.29 (0.075)	0.99 (0.045)
Anti-CD104	1.37 (0.328)	0.69 (0.15)	1.74 (0.121)

Table II. Photocytotoxicity IC_{50} values for CORL23 cells.

Porphyrin	IC_{50} value (μM)		
	Unconjugated	Anti-EpCAM conjugate	Anti-CD104 conjugate
10	5.6	0.78	0.62
14	9.68	0.52	0.93
18	6.25	0.17	0.38

unconjugated antibodies to the CORL23 (Fig. 2) and LoVo (Fig. 3) cell lines was assessed by flow cytometry.

The binding of the conjugated and unconjugated antibodies was very similar as demonstrated by the overlays on the relevant histograms. This confirms that conjugation of the antibody with any of the compounds did not affect the pattern of binding displayed by the two antibodies.

The toxicity of the photosensitisers and conjugates on the CORL23 (Table II) and LoVo (Table III) cell lines was examined using an MTS assay. IC_{50} values are given for both porphyrin and antibody conjugates. The IC_{50} values are

Table III. Photocytotoxicity IC_{50} values for LoVo cells.

Porphyrin	IC_{50} value (μM)		
	Unconjugated	Anti-EpCAM conjugate	Anti-CD104 conjugate
10	4.2	1.11	0.67
14	9.95	0.31	1.26
18	3.23	0.33	0.85

significantly lower for the antibody conjugates as compared with the free porphyrin. The porphyrins and their conjugates were not toxic to cells in the absence of light (data not shown).

Discussion

In our previous studies on the conjugation of isothiocyanato porphyrins to proteins (8) we identified cationic character as being important for efficient covalent binding to BSA. Cationic character of PDT sensitisers has also been associated with mitochondrial localisation (12) and induction of cell death by apoptosis upon activation with visible light (13). 5-(4-Isothiocyanatophenyl)-10,15, 20-tris-(N-methylpyridiniumyl) porphyrin gave the most efficient conjugation of all the compounds tested; however, the synthesis of this compound requires the unsymmetrically substituted tetra-*meso*-aryl porphyrin to be synthesised by a mixed condensation reaction of two different aromatic aldehydes, 4-acetamidobenzaldehyde and 4-pyridine carboxaldehyde, with pyrrole to give a statistical mixture of six compounds, two of which exist as regioisomers. The required precursor, 5-(4-acetamidophenyl)-10,15, 20-tri-

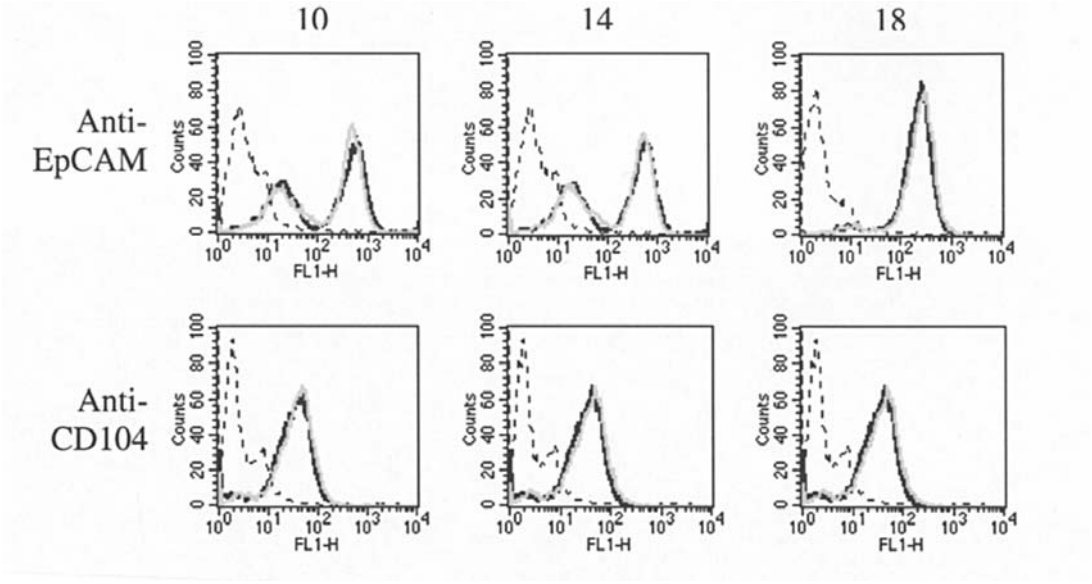

Figure 2. Representative flow cytometric analysis of CORL23 cells with conjugated and unconjugated antibody (dotted line, isotype-matched irrelevant negative control antibody; solid line, unconjugated antibody; grey line, conjugated antibody).

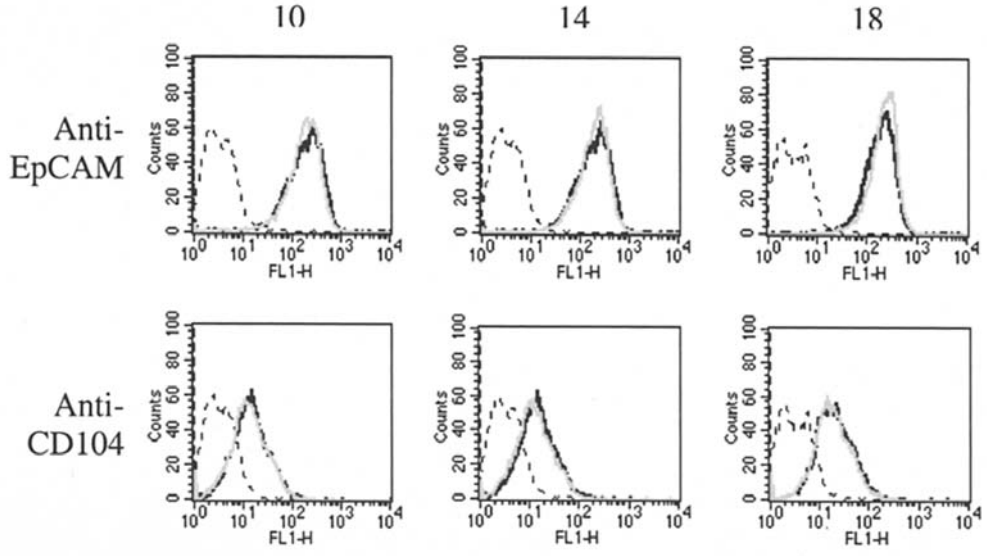

Figure 3. Representative flow cytometric analysis LoVo cells with conjugated and unconjugated antibody (dotted line, negative control; solid line, unconjugated antibody; grey line, conjugated antibody).

(4-pyridyl) porphyrin must then be isolated by exhaustive chromatography before being subjected to several further reactions to achieve the cationic isothiocyanato porphyrin used for bioconjugation. DPPs allow much greater flexibility and efficiency in terms of synthesis (14), as they can be assembled from two differently substituted dipyrromethanes, which can be prepared in multi-gram quantities (15). The subsequent 2+2 condensation of dipyrromethanes with trimethylorthoformate leads to only three compounds, which are easily separated. We therefore set out to investigate if cationic character and the isothiocyanate group required for conjugation to proteins could be incorporated into the DPP framework.

Immediate dipyrromethane precursors (3, 5 and 6) to the required 5,15-diphenyl porphyrins were prepared by condensation with excess pyrrole under acidic conditions (10) and, where required, subsequent functional group modifications. DPP 4 was prepared as described by Gryko and Lindsey (11). Reaction of dipyrromethanes 3, 4, 5 and 6, in the appropriate combinations, with trimethylorthoformate afforded DPPs 7, 11, and 15. DPPs 11 and 15 were formed from a [2+2] condensation of dipyrromethanes in equimolar amounts but it was found that, for porphyrin 7, dipyrromethane 4 was required in excess (3 equivalents) to obtain optimal yields. All three DPPs were then Fmoc deprotected to unmask the amino groups which were converted into isothiocyanates. DPPs 8, 12 and 16, which all have free amino groups, proved to be sensitive to handling so, apart from a sample for characterisation, the crude amino porphyrins were routinely used in the following synthetic step without

purification. Conversion of the amino group into an isothiocyanate group afforded DPPs 9, 13 and 17, which proved less sensitive and were consequently more easy to purify. Finally, the nitrogens on the rings in the 15 position, opposing that now bearing the isothiocyanate group, were quaternised with methyl iodide to introduce cationic character. In order to increase water solubility, the cationic isothiocyanato DPPs were all treated with ion exchange resin to convert the counter anions from iodide to chloride.

Recently, we have reported that the tricationic isothiocyanato tetraphenyl porphyrin, 5-(4-isothiocyanatophenyl)-10,15, 20-tris-(N-methylpyridiniumyl) porphyrin, could be conjugated to monoclonal antibodies (16). However, as described above, the synthetic route to this compound is limited in that: a) only small quantities can be prepared due to the exhaustive chromatic separation involved and b) there is little scope for preparing analogues in order to study the relationship between structure and photodynamic activity of cationic porphyrin-monoclonal antibody conjugates. DPPs offer a 'tinker toy' approach, where bulk quantities of dipyrromethanes can be made and mixed to produce cationic isothiocyanato porphyrins varying in the nature of the cationic group and lipophilicity. We sought further to demonstrate the versatility of this approach by conjugation of the three DPPs described above with two different monoclonal antibodies, and investigation of binding properties and photodynamic activity against two cell lines that express the relevant antigens at the cell surface.

Conjugations were performed using anti-EpCAM and anti-CD104 Mabs for the three cationic isothiocyanato DPPs 10, 14 and 18 as described above. Molar ratios of porphyrin to Mab were then determined (Table I). The significantly lower loading ratio for 14 seen with both anti-EpCAM and anti-CD104 antibodies is likely to be due to the lower hydrophilic character for this compound, a factor we have previously shown affects the efficiency of bioconjugation of isothiocyanato porphyrins to proteins (8). It has been reported that conjugation of porphyrins to Mabs can result in loss of antigen binding, due to conjugated material blocking the antigen recognition region, and/or loss of photodynamic activity due to quenching of porphyrin excited states by the amino acid residues on the antibody (17). As both Mab specificity and photocytotoxicity are crucial for effective targeted PDT, these parameters were investigated. Binding of Mab-cationic porphyrin conjugates was quantified by flow cytometry, Fig. 2 shows these results for the CORL23 cell line, while Fig. 3 shows the same data for LoVo cells. In all cases binding of conjugates to antigen positive cells is superimposable on the curve for unconjugated antibody, while the irrelevant, negative control antibody trace is significantly to the left of both the conjugated and unconjugated antibodies, indicating that recognition of target antigen positive cells by the Mabs has been unaffected by the conjugation process in all cases. It is also worth noting that the conjugation process has not caused enhanced, non-specific, binding of the immunoconjugates to cells due to aggregation.

Photocytotoxicity studies were then performed with all conjugates using both CORL23 and LoVo cells. In order to allow meaningful comparisons to be made between porphyrins 10, 14 and 18, and to normalise for the different degrees of labelling with these compounds, concentrations were adjusted spectroscopically using the Soret band of the porphyrins to ensure the same amount of photosensitiser was being delivered in all cases, although there would be differences in the amount of immunoconjugate bound to the cell due to the level of surface expressed antigen, i.e. EpCAM is expressed at a higher level then CD104 on both cell types. The most immediate effect which is obvious for all Mab conjugates is the decrease in drug dose, relative to the unconjugated analogues, required to achieve the inactivation of fifty percent of cells (IC_{50}) with a constant light dose (15 J/cm^2; 630 nm), these range from a factor of 3.8 for 10/anti-EpCAM conjugate and 18/anti-CD104 conjugate, both against LoVo cells, to a factor of 36.8 for 18/ anti-EpCAM conjugate against CORL23 cells. It is clear from these results that the antigen recognition characteristics of the antibody are retained for Mab-cationic porphyrin conjugates, and also that the photodynamic activity is not only retained, but enhanced, relative to unconjugated photosensitiser. It is very interesting that, although the LoVo cells express over 10-fold the amount of EpCam as compared with CD104, the photocytoxicity data is quite similar, with the former immunoconjugates only being 3- to 4-fold more effective. This suggests that the biology of the target molecule plays an important part in the photocytoxicity process.

The fact that conjugation and functionality has been demonstrated for three different cationic isothiocyanato porphyrins with two monoclonal antibodies on two different cell lines suggests the procedure has general applicability. In conclusion, we have devised a convenient method for the synthesis of porphyrins that have both cationic character and an isothiocyanate group suitable for conjugation to proteins. We have demonstrated the applicability of this method for the formation of Mab-porphyrin conjugates with potential for use as targeted PDT drugs, and the generality on different antibodies and cell lines.

Acknowledgements

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