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# Towards Synthetic-Porphyrin/Monoclonal Antibody Conjugates

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*Abstract*: the synthesis of an unsymmetrically *meso*-aminoalkoxyphenyl-substituted porphyrin, and its conjugation to a monoclonal antibody (mAb), are described.

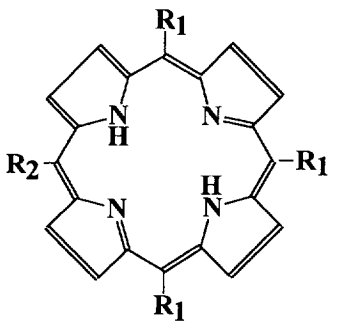
Photodynamic therapy (PDT) is based on the ability of certain sensitisers, especially porphyrins[1] to selectively accumulate in tumour cells when introduced into the body. Upon light activation (usually via laser endoscopy) of a particular wavelength,[2] photonecrosis[3] is induced in the tumour cell by, it is thought, singlet oxygen, $^1O_2$. This is generated via quenching[4] of the triplet excited state of the sensitiser (itself a product of intersystem crossing from the singlet excited state) by ground-state triplet oxygen, $^3O_2$. The singlet oxygen is thought to attack cell components such as membrane molecules and DNA.[5]

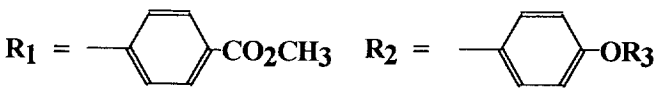
The most common photosensitising agent has been haematoporphyrin derivative, HpD. Although used to treat a variety of lung, bladder, breast and some occular tumours, HpD has serious shortcomings as a PDT-agent. It is an irreproducible mixture of PDT-active and inactive compounds,[6] it localises in healthy, as well as cancerous tissue, causing photosensitivity, and it is excited at a wavelength (630 nm) that is not the most efficient for producing photodamage or for penetration of the skin by an external light-source.

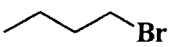
This has led to the enunciation of criteria[7] for what would constitute an ideal PDT photosensitiser. New PDT-agents attempt to combine some of these criteria; water-soluble phthalocyanines, for example, having good absorption at long wavelengths, phototoxicity, non-toxicity in the dark, and low skin photosensitivity.[8] However, their ability (and that of porphyrin- and chlorin-derived photosensitisers) to accumulate selectively in tumours is not ideal, selectivity ratios over healthy tissue varying from 2-10:1. In order to limit photodamage to healthy tissue, therefore, lower concentrations of photosensitiser have to be used, along with increased light doses. This means lower levels of tumour necrosis, so that PDT, at the moment, is effectively limited to the treatment of superficial tumours, e.g., in the bladder and in airways.[9]

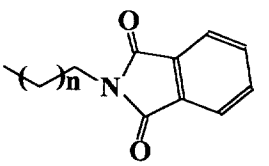
More effective targetting of tumours would permit higher concentrations of photosensitiser to be used, leading to greater tumour necrosis. The photosensitiser must localise selectively in the tumour, must clear rapidly from the body after tumour photonecrosis, and must be soluble in body fluid and tumour-cell membranes, so that it can travel through the vascular system to reach its target.

Monoclonal antibodies offer such an ideal tumour-targetting system, having been suggested previously as tumour targetting agents for covalently-bound aza-crown ether-radionucleide complexes.[10] There are also reports of mAbs conjugated with porphyrins and chlorins derived from natural sources.[11] However, as far as we are aware, there are no reports of monoclonal antibodies conjugated to *synthetic* porphyrins. This




1. a; $R_3$ = H: b; $R_3$ = 


2. $R_3$ = 


a; n = 1: b; n = 2: c; n = 3

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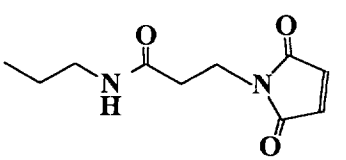
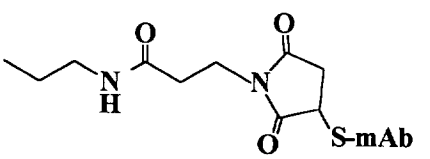
4. $R_3$ = 


5. $R_3$ = 


**Scheme**

would afford sensitisers of precise and reproducible composition, coupled to a highly selective targetting system. We describe here a simple strategy for binding synthetic porphyrins to mAb's, involving preparation of an unsymmetrical *meso*-aminoalkoxyphenyl-substituted tetraarylporphyrin.[12] This is coupled to a modified mAb, thiolated on its lysine residues via a bifuntional linker molecule.

## RESULTS AND DISCUSSION

The mAb B.72.3 was chosen for porphyrin conjugation because it has been raised to specifically recognise colorectal cancer, which is easily accessible to the laser endoscopy used in PDT.[2] The synthetic strategy adopted to conjugate a porphyrin to the mAb was determined by the protocol for thiolating lysine residues on the mAb. The thiolated lysine residues on B.72.3 can react with maleimide groups, so that a suitable synthetic target was a porphyrin with a side chain ending in a maleimide moiety. Such a compound may be obtained by synthesising an unsymmetrically *meso*-substituted tetraarylporphyrin with one phenyl group carrying an alkoxyamino side chain. This may then be reacted with the bifunctional linker molecule N-succinimydyl-3-maleimidopropionate (SMP) to give the desired porphyrin-maleimide compound ready for conjugation to the thiolated antibody.

The unsymmetrical porphyrin **1a**[12] was chosen as the initial synthetic target because, (a) it is easy to prepare via the Adler route[13], (b) the phenolic hydroxy group is a suitable site[14] on which to build an aminoalkyl side-chain via a Gabriel-type synthesis, (c) the 4-methoxycarbonyl side-chains of the other *meso*-substituents may be deesterified to convert a hydrophobic porphyrin into a hydrophilic one.

Thus, addition of pyrrole to refluxing propionic acid containing equimolar amounts of 4-methoxycarbonylbenzaldehyde and 4-hydroxybenzaldehyde gave two main products separated by column chromatography on alumina. The first porphyrin to be eluted from the column was the symmetric *meso*-tetrakis(4-methoxycarbonyphenyl)-porphyrin, followed by the desired unsymmetrical porphyrin **1a**. The next step involves one-pot generation of the porphyrin-phenoxide,[14] using potassium carbonate in DMF, followed by Williamson alkylation[15] with a bromoalkylphthalimide to yield the phthalimidoalkoxyphenylporphyrins **2**. Compounds **2a**, **2b**, and **2c** represent porphyrins with, respectively, $C_2$, $C_3$, and $C_4$ alkyl chains joining the aryl oxygen atom to the phthalimide nitrogen. An alternative two-step reaction to **2b** involved generation of the porphyrin-phenoxide, followed by Williamson alkylation with 1,3-dibromopropane, to yield the bromopropoxyphenylporphyrin, **1b**. This was separated and reacted with potassium phthalimide to yield **2b.**

Reaction of porphyrins **2** with ethanolic methylamine[16] opens the phthalimide ring to yield the aminoalkoxyphenylporphyrins **3**. The aminoethoxyphenylporphyrin **3a** was further reacted with SMP to yield the maleimidopropionamidoethoxyphenylporphyrin **4**. This was incubated with the thiolated mAb B.72.3. The number of thiolated lysine residues on the antibody is assayed by reaction with 4,4'-dithiodipyridine (DTDP) and observing quantitatively the uv-absorption at 324 nm from the released thiopyridine. Thus, assay of the thiol groups on the mAb, before and after incubation with the porphyrin, indicated that approximately one molecule of porphyrin had been conjugated to each antibody molecule.

## CONCLUSIONS

In a relatively small number of steps (five), we have shown it is possible to synthesise and characterise a conjugate of an unsymmetrical porphyrin with a monoclonal antibody. In its present form, however, the particular porphyrin/mAb conjugate synthesised here is unlikely to be of use as a therapeutic agent in photodynamic therapy. Preliminary investigation of the photophysical properties of the conjugate indicate that the fluorescence of the porphyrin moiety is strongly quenched after its attachment to the mAb. We believe this may be due to the hydrophobic nature of the porphyrin - the methyl ester groups were not deesterified prior to its attachment to the mAb. In aqueous solution, it is likely that hydrophobic interactions

between the porphyrin and the protein lead to the fluorescence quenching of the former. This means that it is highly unlikely that the porphyrin/mAb conjugate will be able to generate the photonecrotic agent, $^1O_2$, on light irradiation. We are currently investigating conversion of the hydrophobic porphyrin trimethyl ester **4** into a hydrophilic porphyrin (by deesterification of the methyl ester groups at the amine stage, prior to coupling with the bifunctional linker SMP) and its conjugation to the mAb. We are also investigating the coupling of reduced porphyrins (e.g., chlorins and bacteriochlorins) based on **1**.

## EXPERIMENTAL

Uv/visible spectra were recorded on a Cecil CF 5500 double beam UV spectrophotometer, using spectroscopic grade $CHCl_3$ as solvent. $^1$H-NMR spectra were recorded on a JEOL JNM FX 200 instrument in $CDCl_3$ using TMS (tetramethylsilane) as an internal reference. FABS mass spectra were recorded by the SERC Mass Spectroscopy Service at Swansea, on a Vacuum Generators ZAB 2e double sector spectrometer, using 3-nitrobenzyl alcohol (3-NOBA) and chloroform as co-solvents.

Tlc was performed on Aldrich aluminium-backed silica-gel 60 $F_{254}$ plates, and aluminium-backed neutral alumina 60 $F_{254}$ type E. Porphyrins were separated via column chromatography on neutral alumina (Brockmann grade 3), supplied ready-made by ICN Biomedicals or as Brockmann grade 1 (150 mesh) supplied by Aldrich and converted to grade 3 by addition of, and vigorous shaking with 7% (w/v) water.

Solvents and reagents were purchased from Aldrich and used as supplied. The bifunctional linker molecule, N-succinimidyl-3-maleimidopropionate (SMP) was also supplied by Celltech. Yields of porphyrins have not been optimised.

*Synthesis of 5,10,15-tris(4-methoxycarbonylphenyl)-20-(4-hydroxyphenyl)porphyrin* **1a** - 4-Methoxycarbonyl-benzaldehyde (8.2 g; 0.05 mole) and 4-hydroxybenzaldehyde (6.1 g; 0.05 mole) were dissolved in propionic acid (500 ml) and brought to reflux. Pyrrole (6.7 gm, 0.1 mole) was added and reflux continued during 2 hours. At the end of this time, half the propionic acid was distilled off and the remainder was removed by rotary evaporation under reduced pressure. The solid residue was triturated with acetone (500 ml) several times and filtered. The insoluble purple residue consisted of *meso*-tetrakis(4-methoxycarbonylphenyl)porphyrin, while the soluble fraction contained the crude product, **1a**. This was evaporated to dryness, taken into dichloromethane (DCM) and subjected to column chromatography on neutral alumina (Brockmann grade 3). After elution of a front-running band (consisting of the symmetrically-substituted porphyrin), the eluting solvent was changed to chloroform. A second band was eluted from the column that was filtered, concentrated and precipitated with n-hexane to give the porphyrin **1a** (780 mg; 4.1%) as purple microcrystals (Found: C, 73.8; H, 4.6; N, 6.9. $C_{50}H_{36}N_4O_7.{}^1/_2H_2O$ requires C, 73.62; H, 4.50; N, 6.79%). $\lambda_{max}$nm( $\varepsilon$mmol $l^{-1}$) 423(346.5), 518(14.6), 552(8.3), 594(5.1), 652(4.9). FAB-MS(3-NOBA, $CHCl_3$): found m/z = 805; $[M+H]^+$ requires m/z = 805. $\delta_H$(ppm): 8.88, 8.82, 8.73, 8.67 (quartet centred on 8.78, 4H, AB spin-system from pyrrole-ß-H, $J_{AB}$ = 6.8 Hz); 8.73 (s, 4H, other pyrrole-ß-Hs); 8.42, 8.32, 8.25, 8.14 (quartet centred on 8.28, 12H, AB spin-system from 4-methoxycarbonylphenyl-Hs, $J_{AB}$ = 5.7 Hz); 8.02, 7.92, 7.16, 7.06 (quartet centred on 7.54, 4H, AB spin-system of 4-hydroxyphenyl-Hs, $J_{AB}$ = 60.4 Hz); 5.24 (s, 1H, phenolic -OH); 4.07 (s, 9H, $-CO_2CH_3$); -2.70 (broad singlet, 2H, porphyrin-NH).

*Synthesis of 5,10,15-tris(4-methoxycarbonylphenyl)-20-(4-{2-phthalimidoethoxy}phenyl)porphyrin* **2a** - Porphyrin **1a** (150 mg, $1.87*10^{-4}$ mol) was dissolved in DMF and warmed to 50°C. To this solution was added excess potassium carbonate (1 g; $7.25*10^{-3}$ mol) and excess N-(2-bromoethyl)phthalimide (1 g; $3.94*10^{-3}$), and the mixture stirred at 50°C for three days. Fresh 1 gram batches of potassium carbonate were added each day. After three days, the dark red mixture was suspended several times between DCM and water, and the DCM layer washed with water three times and dried over sodium sulphate. After filtration, the DCM solution was concentrated and applied to an alumina (grade 3) column, eluting with a mixture of DCM and n-hexane (7:3). The collected eluants were concentrated and crystallised with n-

hexane to yield porphyrin **2a** (120 mg; 66%) as purple microcrystals (Found: C, 72.2; H, 4.6; N, 6.9. $C_{60}H_{43}N_5O_9.H_2O$ requires C, 72.36; H, 4.52; N, 7.04%) $\lambda_{max}$nm($\varepsilon$ mmol $l^{-1}$) 422(403), 518(15.1), 551 (7.8), 593(4.8), 651(4.4). FAB-MS(3-NOBA, $CHCl_3$); Found: m/z = 978; $[(M+H]^+$ requires m/z = 978.$\delta_H$(ppm): 8.91, 8.82, 8.73, 8.67 (quartet centred on 8.79, 4H, AB spin-system from pyrrole-ß-H, $J_{AB}$ = 6.9 Hz); 8.73 (s, 4H, other pyrrole-ß-Hs); 8.44, 8.34, 8.26, 8.16 (quartet centred on 8.30, 12H, AB spin-system from 4-methoxycarbonylphenyl-Hs, $J_{AB}$ = 5.8 Hz); 7.77 (m, 4H, aromatic-H on phthalimido group); 8.06, 7.96, 7.26, 7.15 (quartet centred on 7.61, 4H, AB spin-system of 4-alkoxyphenyl-Hs, $J_{AB}$ = 55.8 Hz); 4.37 (t, 2H, -$CH_2$- bonded to aryl oxygen); 4.07 (s, 9H, -$CO_2CH_3$); 3.8 ((t, 2H, -$CH_2$- bonded to phthalimido nitrogen); -2.76 (broad singlet, 2H, porphyrin-NH).

*Synthesis of 5,10,15-tris(4-methoxycarbonylphenyl)-20-(4-{3-phthalimidopropoxy}phenyl)porphyrin* **2b** - This porphyrin was synthesised from **1a** (60 mg, $0.75*10^{-4}$ mol), excess potassium carbonate (1 g; $7.25*10^{-3}$ mol) and excess N-(3-bromopropyl)phthalimide (1 g; $3.73*10^{-3}$ mol) by the same protocol as **2a** to yield porphyrin **2b** (25 mg; 34%) as purple microcrystals (Found: C, 73.6; H, 4.5; N, 6.9. $C_{61}H_{45}N_5O_9$ requires C, 73.62; H, 4.54; N, 7.06%) $\lambda_{max}$nm($\varepsilon$ mmol $l^{-1}$) 423(406), 517(15.0), 550 (7.7), 592(4.7), 651(4.3). FAB-MS(3-NOBA, $CHCl_3$); Found: m/z = 992; $[(M+H]^+$ requires m/z = 992. $\delta_H$(ppm): 8.87, 8.81, 8.74, 8.69 (quartet centred on 8.77, 4H, AB spin-system from pyrrole-ß-H, $J_{AB}$ = 5.5 Hz); 8.74 (s, 4H, other pyrrole-ß-Hs); 8.45, 8.35, 8.27, 8.17 (quartet centred on 8.31, 12H, AB spin-system from 4-methoxycarbonylphenyl-Hs, $J_{AB}$ = 5.9 Hz); 7.72 (m, 4H, aromatic-H on phthalimido group); 8.06, 7.95, 7.20, 7.08 (quartet centred on 7.57, 4H, AB spin-system of 4-alkoxyphenyl-Hs, $J_{AB}$ = 60.5 Hz); 4.30 (t, 2H, -$CH_2$- bonded to aryl oxygen); 4.08 (s, 9H, -$CO_2CH_3$); 3.95 ((t, 2H, -$CH_2$- next to phthalimido nitrogen); 2.35 (m, 2H, -$CH_2$- propoxy group); -2.75 (broad singlet, 2H, porphyrin-NH).

*Alternative synthesis of 5,10,15-tris(4-methoxycarbonylphenyl)-20-(4-{3-phthalimidopropoxy}phenyl)-porphyrin* **2b** - Porphyrin **1a** (400 mg, $5.00*10^{-4}$ mol) was dissolved in DMF and warmed to 50°C. To this solution was added excess potassium carbonate (1 g; $7.25*10^{-3}$ mol) and a 100-fold molar excess of 1,3-dibromopropane (1 g; $5.00*10^{-2}$ mol), and the mixture stirred at 50°C for three days. Fresh 1 gram batches of potassium carbonate were added each day. After three days, the dark red mixture was suspended several times between DCM and water, and the DCM layer washed with water three times and dried over magnesium sulphate. After filtration, the DCM solution was concentrated and applied to an alumina (grade 3) column, eluting with DCM. The collected eluant was concentrated and crystallised with methanol to yield *5,10,15-tris(4-methoxycarbonylphenyl)-20-(4-{3-bromopropoxy}phenyl)porphyrin* **1b** (200 mg; 45%) as purple microcrystals (Found: C, 67.6; H, 4.6; N, 5.7. $C_{53}H_{41}N_4O_7Br.H_2O$ requires C, 67.44; H, 4.56; N, 5.94%) $\lambda_{max}$nm($\varepsilon$ mmol $l^{-1}$) 421(394), 516(14.2), 549 (6.9), 591(3.9), 649(3.4). FAB-MS(3-NOBA, $CHCl_3$); Found: m/z = 926; $[(M+H]^+$ requires m/z = 926. $\delta_H$(ppm): 8.91, 8.82, 8.73, 8.67 (quartet centred on 8.79, 4H, AB spin-system from pyrrole-ß-H, $J_{AB}$ = 6.9 Hz); 8.73 (s, 4H, other pyrrole-ß-Hs); 8.44, 8.34, 8.26, 8.16 (quartet centred on 8.30, 12H, AB spin-system from 4-methoxycarbonylphenyl-Hs, $J_{AB}$ = 5.8 Hz); 8.08, 7.98, 7.28, 7.17 (quartet centred on 7.63, 4H, AB spin-system of 4-alkoxyphenyl-Hs, $J_{AB}$ = 55.8 Hz); 4.36 (t, 2H, -$CH_2$- bonded to aryl oxygen); 4.07 (s, 9H, -$CO_2CH_3$); 3.74 ((t, 2H, -$CH_2$- bonded to bromine); 2.48 (m, 2H, middle -$CH_2$- in propoxy groupbonded -2.73 (broad singlet, 2H, porphyrin-NH).

The forgoing porphyrin **1b** (190 mg; $2.1*10^{-4}$ mol) was dissolved in DMF (10 ml) and warmed to 50°C. To this solution was added a 25-molar excess of potassium phthalimide (1 g; $5*10^{-3}$ mol) and the mixture stirred at 50°C for two days. Two further 1 g portions of potassium phthalimide were added during this time. The mixture was suspended several times between DCM and water, and the DCM layer washed with water three times and dried over magnesium sulphate. After filtration, the DCM solution was concentrated and applied to an alumina (grade 3) column, eluting with DCM. The collected eluant was concentrated and crystallised with methanol to give porphyrin **2b** (100 mg; 49%) as purple microcrystals. Elemental and spectral analysis of this material were the same as before.

*Synthesis of 5,10,15-tris(4-methoxycarbonylphenyl)-20-(4-{4-phthalimidobutoxy}phenyl)porphyrin* **2c** - This porphyrin was synthesised from **1a** (60 mg, $0.75*10^{-4}$ mol), excess potassium carbonate (1 g; $7.25*10^{-3}$ mol) and excess N-(4-bromobutyl)phthalimide (1 g; $3.55*10^{-3}$) by the same protocol as **2a** to yield porphyrin **2c** (38 mg; 52%) as purple microcrystals (Found: C, 72.0; H, 4.9; N, 6.6. $C_{62}H_{47}N_5O_9.{}^3/_2H_2O$requires C, 72.09; H, 4.84; N, 6.78%) $\lambda_{max}$nm( $\varepsilon$ mmol $l^{-1}$) 422(398), 518(15.0), 552 (8.0), 593(4.9), 652(4.6). FAB-MS(3-NOBA, $CHCl_3$); Found: m/z = 1006; $[(M+H]^+$ requires m/z = 1006. $\delta_H$(ppm): 8.87, 8.80, 8.74, 8.69 (quartet centred on 8.78, 4H, AB spin-system from pyrrole-ß-H, $J_{AB}$ = 5.4 Hz); 8.74 (s, 4H, other pyrrole-ß-Hs); 8.44, 8.34, 8.27, 8.17 (quartet centred on 8.31, 12H, AB spin-system from 4-methoxycarbonylphenyl-Hs, $J_{AB}$ = 5.7 Hz); 7.72 (m, 4H, aromatic-H on phthalimido group); 8.06, 7.95, 7.20, 7.08 (quartet centred on 7.57, 4H, AB spin-system of 4-alkoxyphenyl-Hs, $J_{AB}$ = 60.5 Hz); 4.31 (t, 2H, -$CH_2$- bonded to aryl oxygen); 4.08 (s, 9H, -$CO_2CH_3$); 3.89 ((t, 2H, -$CH_2$- bonded to phthalimido nitrogen); 2.10 (m, 4H, -$CH_2$- two in middle of butoxy group); -2.79 (broad singlet, 2H, porphyrin-NH).

*Synthesis of 5,10,15-tris(4-methoxycarbonylphenyl)-20-(4-{2-aminoethoxy}phenyl)porphyrin* **3a** - Porphyrin **2a** (110 mg; 0.113 mol) in benzene (10 ml) was added to an ethanolic solution of methylamine (3 ml; 33%), and the mixture stirred for two days at 50°C. The mixture was then evaporated to dryness and the solid residue taken into chloroform (5 ml) and applied to a column of neutral alumina (grade 3), eluting with chloroform (to remove any starting porphyrin, **2a**). The product was eluted from the column using 1% methanol/chloroform. The eluant was concentrated and crystallised with n-hexane to yield porphyrin **3a** (80 mg; 87%) as purple microcrystals (Found: C, 72.0; H, 5.1; N, 8.0. $C_{52}H_{41}N_5O_7.H_2O$ requires C, 72.14; H, 4.97; N, 8.09%) $\lambda_{max}$nm( $\varepsilon$ mmol $l^{-1}$) 423(390), 519(14.9), 553(7.9), 592(5.0), 650(4.6). FAB-MS(3-NOBA, $CHCl_3$); Found: m/z = 848; $[(M+H]^+$ requires m/z = 848. $\delta_H$(ppm): 8.88, 8.82, 8.75, 8.70 (quartet centred on 8.79, 4H, AB spin-system from pyrrole-ß-H, $J_{AB}$ = 5.9 Hz); 8.75 (s, 4H, other pyrrole-ß-Hs); 8.45, 8.34, 8.27, 8.17 (quartet centred on 8.31, 12H, AB spin-system from 4-methoxycarbonylphenyl-Hs, $J_{AB}$ = 5.7 Hz); 8.11, 8.00, 7.30, 7.21 (quartet centred on 7.66, 4H, AB spin-system of 4-alkoxyphenyl-Hs, $J_{AB}$ = 56.4 Hz); 4.25 (t, 2H, -$CH_2$- bonded to aryl oxygen); 4.08 (s, 9H, -$CO_2CH_3$); 2.90 ((t, 2H, -$CH_2$- bonded to amino nitrogen); -2.79 (broad singlet, 2H, porphyrin-NH).

*Synthesis of 5,10,15-tris(4-methoxycarbonylphenyl)-20-(4-{3-aminopropoxy}phenyl)porphyrin* **3b** - A similar protocol was used for the preparation and separation of **3b**, using porphyrin **2b** (50 mg; 0.05 mol) in benzene (10 ml) and ethanolic methylamine (3 ml; 33%). Porphyrin **3b** (42 mg; 93%) separated as purple microcrystals of the hydrochloride salt (Found: C, 70.5; H, 4.9; N, 7.4. $C_{53}H_{43}N_5O_7.HCl.{}^1/_4H_2O$ requires C, 70.51; H, 4.93; N, 7.76%). Spectroscopic measurements were performed on the free-base by addition of a few drops of triethylamine. $\lambda_{max}$nm( $\varepsilon$mmol $l^{-1}$) 422(393), 518(15.2), 552(8.0), 591(5.1), 651(4.8). FAB-MS(3-NOBA, $CHCl_3$); Found: m/z = 862; $[(M+H]^+$ requires m/z = 862. $\delta_H$(ppm): 8.87, 8.81, 8.74, 8.68 (quartet centred on 8.78, 4H, AB spin-system from pyrrole-ß-H, $J_{AB}$ = 6.0 Hz); 8.74 (s, 4H, other pyrrole-ß-Hs); 8.44, 8.34, 8.26, 8.16 (quartet centred on 8.30, 12H, AB spin-system from 4-methoxycarbonylphenyl-Hs, $J_{AB}$ = 5.7 Hz); 8.10, 7.99, 7.28, 7.22 (quartet centred on 7.65, 4H, AB spin-system of 4-alkoxyphenyl-Hs, $J_{AB}$ = 57.1 Hz); 4.32 (t, 2H, -$CH_2$- bonded to aryl oxygen); 4.07 (s, 9H, -$CO_2CH_3$); 2.88 (t, 2H, -$CH_2$- bonded to amino nitrogen); 2.10 (m, 2H, -$CH_2$- middle of propoxy group); -2.79 (broad singlet, 2H, porphyrin-NH).

*Synthesis of 5,10,15-tris(4-methoxycarbonylphenyl)-20-(4-{4-aminobutoxy}phenyl)porphyrin* **3c** - A similar protocol was used for the preparation and separation of **3c**, using porphyrin **2c** (60 mg; 0.06 mol) in benzene (10 ml) and ethanolic methylamine (3 ml; 33%). Porphyrin **3c** (31 mg; 60%) separated as purple microcrystals (Found: C, 72.5; H, 5.1; N, 7.7. $C_{54}H_{45}N_5O_7.H_2O$ requires C, 72.56; H, 5.26; N, 7.83%) $\lambda_{max}$nm( $\varepsilon$ mmol $l^{-1}$) 423(396), 520(15.3), 552(8.2), 593(5.0), 652(4.7). FAB-MS(3-NOBA, $CHCl_3$); Found: m/z = 876; $[(M+H]^+$ requires m/z = 876. $\delta_H$(ppm): 8.88, 8.82, 8.75, 8.68 (quartet centred on 8.80, 4H, AB spin-system from pyrrole-ß-H, $J_{AB}$ = 6.0 Hz); 8.75 (s, 4H, other pyrrole-ß-Hs); 8.45, 8.34, 8.26, 8.16 (quartet centred on 8.30, 12H, AB spin-system from 4-methoxycarbonylphenyl-Hs, $J_{AB}$ = 5.7

Hz); 8.10, 7.99, 7.28, 7.22 (quartet centred on 7.65, 4H, AB spin-system of 4-alkoxyphenyl-Hs, $J_{AB}$ = 57.1 Hz); 4.30 (t, 2H, -$CH_2$- bonded to aryl oxygen); 4.07 (s, 9H, -$CO_2CH_3$); 2.90 (t, 2H, -$CH_2$- bonded to amino nitrogen); 2.10 (m, 4H, -$CH_2$- middle two of butoxy group); -2.79 (broad singlet, 2H, porphyrin-NH).

*Synthesis of 5,10,15-tris(4-methoxycarbonylphenyl)-20-{4-[2-[3-maleimido]propionamido}phenyl)porphyrin* **4** - To porphyrin **3a** (20 mg; 0.024 mmol) in dry DCM were added N-methylmorpholine (2.59 $\mu$l; 2.38 mg; 0.024 mmol; 1 eq) and N-succinimydyl-3-maleimidopropionate (SMP, 7.53 mg; 0.029 mmol; 1.2 eq). The mixture was stirred at room temperature for two hour, after which it was evaporated to dryness and the solid residue applied to an alumina column (grade 3) and eluted with chloroform containing 1% methanol. The eluant was concentrated and precipitated with n-hexane to give the porphyrin **4** (9 mg; 21%) as purple microcrystals (Found: C, 70.7; H, 4.8; N, 8.3. $C_{59}H_{47}N_6O_{10}$ requires C, 70.87; H, 4.70; N, 8.41%). $\lambda_{max}$nm( $\varepsilon$ mmol $l^{-1}$) 420(433), 516(15.4), 550(7.6), 592(4.6), 648(3.8). FAB-MS(3-NOBA, $CHCl_3$); Found: m/z = 1000; $[(M+H]^+$ requires m/z = 1000. $\delta_H$(ppm): 8.92, 8.87, 8.80, 8.72 (quartet centred on 8.84, 4H, AB spin-system from pyrrole-ß-H, $J_{AB}$ = 6.0 Hz); 8.80 (s, 4H, other pyrrole-ß-Hs); 8.53, 8.42, 8.34, 8.22 (quartet centred on 8.38, 12H, AB spin-system from 4-methoxycarbonylphenyl-Hs, $J_{AB}$ = 5.7 Hz); 8.15, 8.04, 7.33, 7.27 (quartet centred on 7.70, 4H, AB spin-system of 4-alkoxyphenyl-Hs, $J_{AB}$ = 57.1 Hz); 6.75 (s, 2H, maleimido protons); 6.25 (t, H, amide proton); 4.30 (t, 2H, -$CH_2$- bonded to aryl oxygen); 4.10 (s, 9H, -$CO_2CH_3$); 3.95 (t, 2H, -$CH_2$- bonded to maleimide nitrogen); 3.85 (q, 2H, -$CH_2$- bonded to amide nitrogen); 2.68 (t, 2H, -$CH_2$- bonded to amide carbonyl); -2.75 (broad singlet, 2H, porphyrin-NH).

*Thiolation of monoclonal antibody B72.3* - The mAb B72.3 (molecular weight, approximately 150K) was first thiolated on its lysine residues by reaction with Traut's reagent (2-iminothiolane hydrochloride) according to the following protocol:-

An aqueous solution of the mAb (5 ml; 5 mg/ml) was buffer exchanged with sodium bicarbonate solution (O.1M; pH 7.96) and then concentrated using an AMICON concentration cell fitted with a YM 10 membrane, to give a final mAb concentration of 10.5 mg/ml. The concentration of the mAb solution was determined quantitatively via uv spectroscopy (observation of absorption at 280 nm from aromatic amino-acid residues in the peptide chains).

To the antibody solution (1 ml) was added a solution (21 $\mu$l; 50 mM) containing a 15-fold excess of Traut's reagent, and the mixture allowed to incubate for 30 minutes. This mixture was then purified on a PD-10 Sephadex column, the fourth fraction containing the thiolated antibody at a concentration of 9.04 mg/ml (found by measuring the absorption at 280 nm). The number of thiolated lysine residues on the antibody was then assayed. Thus, an aqueous solution of 4,4'-dithiodipyridine (DTDP, 10 $\mu$l; 5mM) was added to the thiolated antibody solution (90 $\mu$l) and incubated for 10 minutes. The difference in absorbance at 324 nm between this solution and a control (i.e. 90 $\mu$l of buffer solution containing 10 $\mu$l of DTDP) was used to calculate the number of thiol groups introduced into the mAb by the Traut's reagent. This was found to be approximately 1.58 thiol groups/antibody molecule.

*Conjugation of Porphyrin* **4** *to thiolated monoclonal antibody B72.3* - To the thiolated antibody solution (1 ml) was added a solution of porphyrin **4** in DMF (47.59 $\mu$l; 10 mM; 5-fold excess of porphyrin/antibody thiol residue). A precipate formed and the reaction mixture was allowed to incubate at 37°C during 2 hours. The mixture was then centrifuged, the supernatent collected and purified on a Sephadex PD-10 column as before, and the fourth fraction containing the porphyrin-conjugated mAb collected. Uv/visible spectroscopy on this solution showed the presence of a porphyrin B band at 420 nm. A thiol assay with DTDP was then performed as before to demonstrate that the porphyrin had been conjugated onto a thiol residue and was not simply associating with the antibody protein. The thiol assay indicated only 0.52 thiol residues/antibody molecule, i.e., one porphyrin molecule/antibody molecule had been conjugated.

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## REFERENCES

1. Dougherty, T.J., *Photochem. Photobiol.*, 1987, **45**, 879.
2. a, Moan, J., and K. Berg, K., *Photochem. Photobiol.*, 1992, **55**, 931; b, Chan, W.S., MacRobert, A., Phillips, D., and Hart, I.R., *Photochem. and Photobiol.*, (1989) **50**, 617; c, Barr, H., Tralau, C.J., MacRobert, A.J., Morrison, I., Phillips, D., and Bown, S.G., *Lasers Med. Sci.* (1988), **3**, 81.
3. a, Dougherty, T.J., Grindley, G.B., Fiel, R., Weishaupt, K.R., and Boyle, D.G., *J. Natl. Cancer Inst.*, (1975), **55**, 1; b, Berenbaum M.C., and Bonnett, R., in *Photodynamic Therapy of Neoplastic Disease,* ed. Kessel, D., vol. 2, CRC Press, Boca Raton, Boston, 1990, p169.
4. See Jari, G., Reddi, E., and Tomio, L., "Porphyrin Photosensitisation" in *Advances in Experimental Medicine and Biology*, Vol. 160, ed., D. Kessel and T.J. Dougherty, 1988, p193.
5. Stenstrom, A.G.K., Moan, J., Brunborg, G., Eklund, T., *Photochem. Photobiol.*, 1980, **32**, 349.
6. See Milgrom, L.R. and O'Neill, F., "Porphyrins" in *The Chemistry of Natural Products*, 2nd Edition, ed., R.H. Thomson, Blackie Academic and Professional, 1993, p357, and references therein.
7. MacRobert, A.G., Bown, S.G., and Phillips, D., *Photosensitising Compounds: Their Chemistry, Biology, and Clinical Uses* (Ciba Foundation Symposium 146, Wiley) 1989, p4.
8. Rosenthal, I., *Photochem. Photobiol.*, 1991, **53**, 859.
9. Dougherty, T.J., *Semin. Surg. Oncol.*, 1986, **2**, 24.
10. Cox, J.P.L., Jankowski, K.J., Kataky, R., Parker, D., Beeley, N.R.A., Harrison, A., and Walker, C., *J. Chem. Soc., Chem. Commun.*, 1989, 797, and references therein.
11. a, Jiang, F.N., Jiang, S., Liu, D.J., Richter, A., and Levy, J.G., *J. Immun. Meth.*, 1990, **134**, 139; b, Jiang, F.N., Liu, D., Neyndorff, H., Chester, M., Jiang, S., and Levy,J.G., *J. Natl. Cancer Inst.*, 1991, **83** 1218; c, Egorov, S. Yu., Krasnovskii, Jr. A.A., Papkovskii, D.B., Ponomarev, G.V., and Savitskii, A.P., *Bull. Exp. Biol. Med.*, (1990),**109**, 454.
12. a, Little, R.G., Anton, J.A., Loach, P.A., and Ibers, J.A., *J. Heterocyclic Chem.*, 1975, **12**, 543; b, Anton, J.A., and Loach, P.A., *ibid.* 573; c, Chan, A.C., Dalton, J., and Milgrom, L.R., *J. Chem. Soc., Perkin Trans. 1.*, 1982, 707; d, Milgrom, L.R., *J. Chem. Soc., Perkin Trans. 1.*, 1984, 1483; e, Milgrom, L.R., Mofidi, N., Jones, C.C., and Harriman, A., *J. Chem. Soc., Perkin Trans. 2.*, 1989, 301; f, Milgrom, L.R., Mofidi, N., Harriman, A., *ibid.* 805; g, Milgrom, L.R., Mofidi, N., and Harriman, A., *Tetrahedron*, (1989), **45**, 7431.
13. Adler, A.D., Longo, F.R., Finarelli, J.D., Goldmacher, J., Assour, J., and Korsakoff, L., *J. Org. Chem.*, 1967, **32**, 476.
14. Milgrom, L.R., *J. Chem. Soc., Perkin Trans 1.*, 1983, 2535.
15. See "Advanced Organic Chemistry", by March, J., (Wiley), 3rd Edition, 1985, p342.
16. See Wolf, S., and Hasan, S.K., *Can. J. Chem.*, (1970), **48**, 3572.

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