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Synthesis of new glycosylated neutral and cationic porphyrin dimers

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Abstract

The synthesis and characterisation of symmetrical glycosylated neutral and cationic porphyrin dimers linked at the *meso*-position via a flexible hydrocarbon chain to improve targeting on malignant cells is reported. Photocytotoxicity of these compounds against the K562 leukemia cell line compared to the effect of hematoporphyrin is also presented.

Photofrin®, a purified form of hematoporphyrin derivative (Hpd), has demonstrated considerable promise in photodynamic therapy (PDT).[1] However, as it is a mixture of monomeric, dimeric and trimeric porphyrins joined either by ether or ester linkages, its complexity has precluded the elucidation of the most biologically active components and has lead to the development of other photosensitizers for use in PDT. Indeed, in the last decade, a number of porphyrin dimers used in PDT for the treatment of cancers have been reported in the literature,[2] including dimeric and trimeric species containing ether linkages showing significant biological activity.[3]

The presence of carbohydrate moieties on porphyrins is known to exhibit specific cancer cell targeting[4] and to increase plasmatic life time.[5] Furthermore the presence of a charge on cationic porphyrin dimers not only increases water solubility, but also favors interaction with DNA.[6] As porphyrins are retained longer by tumour cells than by normal cells,[7] they lead to highly selective, light induced mitochondrial damage and cell killing.[8] In the present work, which is part of an on-going research program on glycosylated porphyrins, we report the synthesis of symmetrical *O*-glycosyl neutral porphyrin dimers with ether linkages **5a**,**b** and cationic porphyrin dimer **8**. The presence of such substituents could increase

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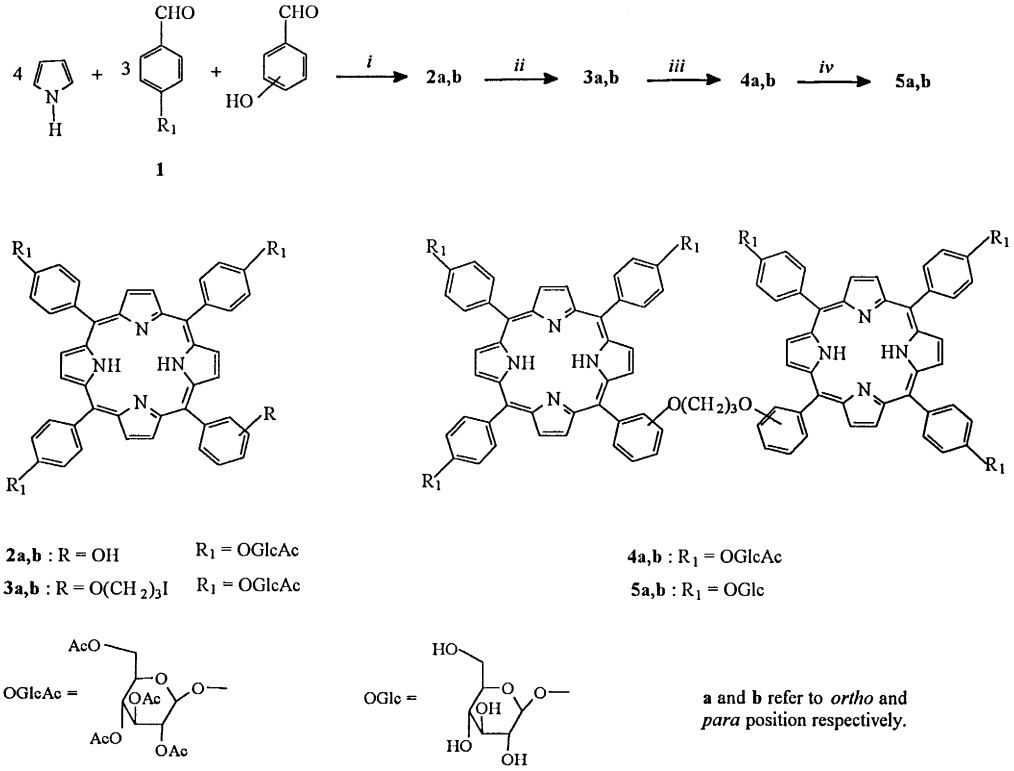
cancer cell specific recognition. Aryl-substituted porphyrins monomers **2a**,**b** were prepared by Lindsey's method.[9] The condensation reaction was carried out by treatment of a mixture of 4-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-glucopyranosyloxy)-benzaldehyde[10] **1** (1.4 g, 3 equiv.), *ortho* or *para* hydroxybenzaldehyde (122 mg, 1 equiv.) and pyrrole (0.29 mL, 4 equiv.) in dry CH_2Cl_2 (400 mL) with BF_3/etherate (10^{-3} M) as catalyst under argon at room temperature (Scheme 1). Oxidation of the porphyrinogen intermediates with chloranil, followed by flash chromatography and purification on silica gel PLC gave porphyrins **2a**,**b** in 5–13% yields, respectively. These latter compounds **2a**,**b** were also obtained according to the Little method[11] by condensation of pyrrole (4 equiv.) with glucosyl aldehyde **1** (3 equiv.) and *ortho* or *para* hydroxybenzaldehyde (1 equiv.) in propionic acid (yields 5–7%).



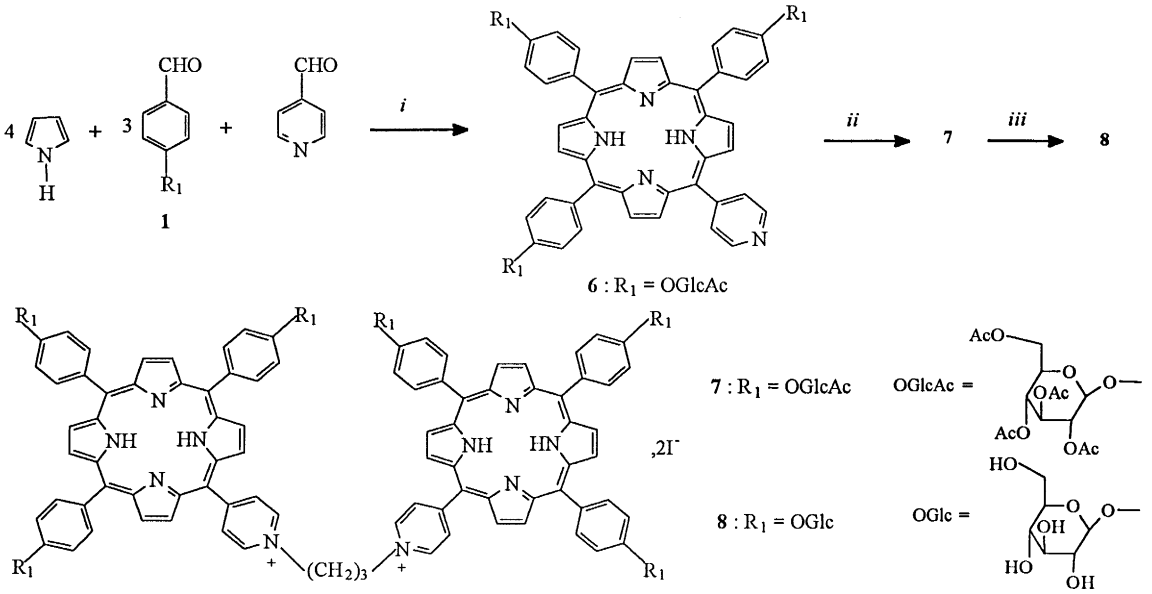
Scheme 1. (i) (a) CH_2Cl_2, BF_3/etherate, rt, 1 h; (b) *p*-chloranil, reflux, 1 h; (ii) $I(CH_2)_3I/K_2CO_3$/DMF, 6 h; (iii) 2a,b/K_2CO_3/DMF, 24 h; (iv) NaOMe/MeOH

The neutral porphyrins dimers **4a**,**b** were formed from **2a**,**b** in two steps by ether linkage[12] with acceptable yields (Scheme 1): compounds **2a**,**b** reacted with a 10-fold excess of 1,3-diiodopropane in distilled dimethylformamide in the presence of potassium carbonate under reflux for 6 h to yield **3a**,**b** (80–85%).

These latter products then reacted with a two fold excess of **2a**,**b** to give, after purification on silica gel PLC, the bisporphyrins **4a**,**b** (yield 50–54%).

For the cationic porphyrin dimer, we adapted the previously reported work of Fleischer et al.[13] The porphyrins were covalently linked through a pyridine nitrogen to form 1,3-dipyridiniumylpropane-linked bisporphyrins **7** (Scheme 2). The synthesis was performed in one-step by reacting **6** (102 mg, 2 equiv.) with 1,3-diiodopropane (56 mg, 1 equiv.) to yield 14% of compound **7** after separation on SiO_2 PLC and purification using size exclusion chromatography (Sephadex LH20, MeOH:THF 6:4). The monomeric porphyrin intermediate **6** bearing one pyridyl and three glucosyl units was obtained with the same

conditions as compounds **2a**,**b** by the Little method[11] by condensation of pyrrole (4 equiv.) with aldehyde **1** (3 equiv.) and 4-pyridine carboxyaldehyde (1 equiv.) (yield 7%) (Scheme 2). In that case, Lindsey's method did not give significant conversion.



Scheme 2. (i) EtCOOH/reflux, 1 h; (ii) $I(CH_2)_3I$/DMF, 20 h; (iii) NaOMe/MeOH

Absorption, ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra[14] of these compounds showed the expected signals. MALDI-TOF mass spectra exhibited the molecular ion signal (MH^+, *m/z* 3381.4) for the neutral compounds only; di-cationic species probably decompose in the gas phase giving fragment ions. Deacetylation of **4a**,**b** and **7** was carried out at room temperature (1 h) with NaOMe[15] in MeOH:CH_2Cl_2 (8:2) to give the deacetylated porphyrins **5a**,**b** and **8** in 88%, 80% and 82% yields, respectively. As for protected compounds, characteristic MALDI-TOF mass spectra exhibiting the molecular ion signal (MH^+, *m/z* 2273.6) were only obtained for the neutral compounds. Fluorescence spectra of compounds **4a**,**b**, **7** bands recorded in CH_2Cl_2 (excitation at 422 nm, about 10^{-6} M concentration, at room temperature) were characterised by two emission bands (λ_{max}: 654 nm, 718 nm for porphyrin **4a**; 675 nm, 718 nm for **4b** and 672 nm, 725 nm (shoulder) for **7**). The fluorescence emission wavelengths of **5a**, **5b** and **8** in aqueous solutions showed that their intensities were strongly quenched. This decay of fluorescence can be explained by aggregate formation[16] and confirmed by marked changes in absorption spectra of these compounds.

Thus, in aqueous solution, the Soret band of **5a**, **5b**, **8** is blue-shifted and split into two bands (**5a**: 404–420 nm, **5b**: 406–422nm and **8**: 406–418 nm). This blue-shift is consistent with a face to face (H) aggregation[16] along with its splitting into two bands as observed previously.[17] This result is due to the combination of cofacial and edge to edge interaction[18] of self-assembled aggregates.

Synthetic porphyrin dimers **5a**,**b** and **8** were tested for their photocytotoxicity against the promyelocytary cell line K562. These cells were incubated in a RPMI medium in presence of 2.10^{-6} M porphyrins and irradiated with fluorescent bulbs (fluence=50 watt/m^2); cells irradiated in presence of haematoporphyrin at the same concentration were used as a control. Dead cells were identified as propidium iodide (PI) permeable ones; they were counted by flux cytometry immediately after irradiation and after a further 24 h of incubation in the dark. The symmetrical glycosylated neutral and cationic porphyrin dimers tested did not induce significant cell death either just after irradiation or after a 24 h in

the dark, contrary to other neutral glycosyl or glycosylated amino acid porphyrin monomer derivatives.[17b] Since we have checked, these compounds produced singlet oxygen (for methodology see Ref. 17b), we presume that their lack of efficacy is the consequence of a low cellular permeability.[19]

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14. Compound **4a**: ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ (ppm)=8.88 (s broad, 8H, H-12,13,1.7,18 β-pyrrole), 8.74 (d, 4H, J=4.8 Hz, H-2,8 β-pyrrole), 8.58 (d, 4H, J=4.8 Hz, H-3,7 β-pyrrole), 8.15 (m, 12H, H-2,6 aryl), 7.77 (dd, 2H, J=7.4–1.4 Hz, H-6 phenyl-*o*-link), 7.41 (d broad, 12H, J=8.4 Hz, H-3,5 aryl), 6.97 (t broad, 2H, J=7.4 Hz, H-5 phenyl-*o*-link), 6.61 (td, 2H, J=8.4–1.4 Hz, H-4 phenyl-*o*-link), 5.80 (d broad, 2H, J=8.4 Hz, H-3 phenyl-*o*-link), 5.47 (m, 18H, H-1′,2′,3′ ose), 5.32 (t, 6H, J=9.4 Hz, H-4′ ose), 4.44 (dd, 6H, J=12.4–5.1 Hz, H-6′a ose), 4.32 (dd, 6H, J=12.4–2.2 Hz, H-6′b ose), 4.06 (m, 6H, H-5′ ose), 2.74 (m, 4H, α-link), 2.23 (s, 6H, CH_3CO), 2.20 (s, 6H, CH_3CO), 2.18 (s, 6H, CH_3CO), 2.14 (s, 6H, CH_3CO), 2.12 (s, 6H, CH_3CO), 2.10 (s broad, 36H, CH_3CO), 2.08 (s, 6H, CH_3CO), 0.58 (m, 2H, β-link), −2,77 (s, 4H, NH-pyrrole). ^{13}C NMR ($CDCl_3$, 100.13 MHz): δ (ppm)=170.5 (6CH_3*CO*), 170.3 (6CH_3*CO*), 169.5 (12CH_3*CO*), 157.8 (1C, C-2 phenyl-*o*-link), 156.7 (6C, C-4 aryl), 146.5 (16C, C_α pyrrole), 137.3 (1C, C-1 phenyl-*o*-link), 137.2 (6C, C-1 aryl), 135.7 (12C, C-2,6 aryl), 135.2 (1C, C-6 phenyl-*o*-link), 130.9 (16C, C_β pyrrole), 129.3 (1C, C-4 phenyl-*o*-link), 119.3 (2C, C-5 *meso* porphyrin), 119.0 (6C, C-10,15,20 *meso* porphyrin), 118.8 (1C, C-5 phenyl-*o*-link), 115.2 (12C, C-3,5 aryl), 115.0 (1C, C-3 phenyl-*o*-link), 99.2 (6C, C-1′ ose), 72.8 (6C, C-3′ ose), 72.3 (6C, C-5′ ose), 71.4 (6C, C-2′ ose), 68.4 (6C, C-4′ ose), 63.2 (2C, C_α-link), 62.1 (6C, C-6′ ose), 27.7 (1C, C_β-link), 20.8 (12C, *CH*$_3$CO), 20.7 (6C, *CH*$_3$CO), 20.6 (6C, *CH*$_3$CO). Compound **4b**: ^{1}H NMR ($CDCl_3$, 400.13 MHz): δ (ppm)=8.92 (d, 4H, J=4.7 Hz, H-2,8 β-pyrrole), 8.84 (d, 4H, J=4.7 Hz, H-3,7 β-pyrrole), 8.84 (s, 8H, H-12,13,17,18 β-pyrrole), 8.18 (d, 4H, J=8.5 Hz, H-2,6 phenyl-*o*-link), 8.13 (d, 12H, J=8.5 Hz, H-2,6 aryl), 7.42 (d, 4H, J=8.5 Hz, H-3,5 phenyl-*o*-link), 7.38 (d, 12H, J=8.5, H-3,5 aryl), 5.46 (m, 18H, H-1′,2′,3′ ose), 5.30 (m, 6H, H-4′ ose), 4.65 (t, 4H, J=6.0 Hz, α-link), 4.41 (dd, 6H, 12.4–5.6 Hz, H-6′a ose), 4.30 (m, 6H, H-6′b, ose), 4.05 (ddd, 6H, J=9.6–5.6–2.4 Hz, H-5′ ose), 2.70 (quintuplet, 2H, J=6.0Hz, β-link), 2.22 (s, 6H, CH_3CO), 2.20 (s, 12H, CH_3CO), 2.12 (s, 6H, CH_3CO), 2.11 (s, 24H, CH_3CO), 2.10 (s, 12H, CH_3CO), 2.09 (s, 12H, CH_3CO), −2.77 (s, 4H, NH-pyrrole). ^{13}C NMR ($CDCl_3$, 100.13 MHz): δ (ppm)=170.6 (6CH_3*CO*), 170.3 (6CH_3*CO*), 169.5 (12CH_3*CO*), 158.9 (2C, C-4 phenyl-*o*-link), 156.6 (6C, C-4 aryl), 146.5 (16C, C_α pyrrole), 137.2 (6C, C-1 aryl), 135.7 (4C, C-2,6 phenyl-*o*-link), 135.6 (12C, C-2,6 aryl), 134.6 (2C, C-1 phenyl), 130.9 (16C, C_β pyrrole), 120.3 (2C, C-5 *meso* porphyrin), 119.3 (4C, C-10,20 *meso* porphyrin), 119.1 (2C, C-15 *meso* porphyrin), 115.1 (12C, C-3,5 aryl), 112.9 (4C, C-3,5 phenyl), 99.2 (6C, C-1′ ose), 72.9 (6C, C-2′ ose), 72.3 (6C, C-5′ ose), 71.4 (6C,C-3′ ose), 68.4 (6C, C-4′ ose), 65.0 (2C, C_α-link), 62.1 (6C, C-6′ ose), 29.7 (1C, C_β-link), 20.9 (6C, *CH*$_3$CO), 20.8 (6C, *CH*$_3$CO), 20.7 (6C, *CH*$_3$CO), 20.6 (6C, *CH*$_3$CO). Compound **7**: ^{1}H NMR (DMSO-d_6, 400.13 MHz): δ (ppm)=9.52 (d, 4H, J=5.1 Hz, H-2,6 pyridyl), 9.01 (d, 4H, J=5.1 Hz, H-3,5 pyridyl), 8.97 (m, 4H, H-3,7 β-pyrrole), 8.90 (m, 4H, H-2,8 β-pyrrole), 8.89 (s, 8H, H-12,13,17,18 β-pyrrole), 8.19 (d, 12H, J=8.1 Hz, H-2,6 aryl), 7.47 (d, 12H, J=8.1 Hz, H-3,5 aryl), 5.96 (d, 6H, J=7.8 Hz, H-1′ ose), 5.56 (t, 6H, J=9.5 Hz, H-3′ ose), 5.28

(t, 6H, J=9.3 Hz, H-2′ ose), 5.14 (t, 6H, J=9.5 Hz, H-4′ ose), 5.02 (m, 4H, α-link), 4.44 (m, 6H, H-5′ ose), 4.33 (dd, 6H, J=12.2–5.3 Hz, H-6′a ose), 4.20 (d broad, 6H, J=12.2 Hz, H-6′b ose), 3.76 (m, 2H, β-link), 2.17 (s, 16H, CH_3CO), 2.07 (s, 16H, CH_3CO), 2.06 (s, 16H, CH_3CO), 2.04 (s, 16H, CH_3CO), −2.91 (s, 4H, NH-pyrrole). ^{13}C NMR (DMSO-d_6, 100.13 MHz): δ (ppm)=170.0 ($6CH_3\mathit{CO}$), 169.7 ($6CH_3\mathit{CO}$), 169.4 ($6CH_3\mathit{CO}$), 169.3 ($6CH_3\mathit{CO}$), 157.6 (16C, C_α pyrrole), 156.3 (6C, C-4 aryl), 143.4 (4C, C-2,6 pyridyl), 135.4 (12C, C-2,6 aryl), 135.3 (8C, C-4 pyridyl and C-1 aryl), 132.8 (4C, C-3,5 pyridyl), 131.5 (16C, C_β pyrrole), 120.2 (6C, C-10,15,20 *meso* porphyrin), 119.6 (2C, C-5 *meso* porphyrin), 114.8 (12C, C-3,5 aryl), 97.2 (6C, C-1′ose), 72.1 (6C, C-3′ ose), 71.0 (6C, C-5′ ose), 70.9 (6C, C-2′ ose), 68.2 (6C, C-4′ ose), 65.9 (2C, C_α-link), 61.7 (6C, C-6′ ose), 30.4 (1C, C_β-link), 20.5 (12C, $\mathit{CH_3}CO$), 20.4 (6C, $\mathit{CH_3}CO$), 20.3 (6C, $\mathit{CH_3}CO$).

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