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Toward Glycosylated Peptidic Porphyrins : a New Strategy for PDT ?

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Abstract : New porphyrins bearing glycosyl groups and alanine moieties to improve targeting on malignant cells were synthesised in four steps. Fluorescence properties were extensively determined and indicated aggregate formation in water. These compounds produced $^1O_2$. Photocytotoxicity of these molecules against K562 Leukemia cell line was compared to the effect of hematoporphyrin.

In the last few years, many efforts have been devoted to the synthesis of structurally modified porphyrins used as photosensitizers in the photodynamic therapy (PDT) of cancer.[1] Although the exact mechanism of PDT is unknown, it seems important that porphyrins exhibit excellent hydrophilic properties[2] and could improve the specific cancer cells targeting[3] to have an efficient photocytotoxic effect. It occurred that water soluble porphyrin based sensitizers in combination with cellular recognition element could appear promising candidates for an application in PDT. Thus, porphyrins with sugar moieties are known to have a good solubility in water, in addition the glycosyl substituents also increase plasmatic life time[4] and should be functional components involved in cell recognition.[5] Furthermore the presence of an amino acid residue with a free α-amino group should improve specific cancer cells targeting.[6] In connection with our research program on glycosylated porphyrins, we report here the synthesis of a series of *O*-glycosyl L-alanyl aryl porphyrins **9a,b** and **10a,b**. These unsymmetrical neutral *meso*-glycosylporphyrins contain one or three *meso*-phenyl groups bearing α-L-alanyl moiety in the *ortho* or *para* position (scheme). The presence of such substituents could increase cancer cells specific recognition.

According to Lindsey's method,[7] the condensation of pyrrole (4 eq) with 4 - (2',3',4',6'-tetra-*O*-acetyl-β-D glucopyranosyloxy)-benzaldehyde[8] (3 eq) **1b** and *ortho* or *para* nitrobenzaldehyde (1 eq) **2a,b** (scheme) gave compounds **4a,b** (14-16% yield after purification by SiO_2 PLC). With the same conditions porphyrins **3a,b** bearing three NO_2 and one glucosyl units were obtained by condensation of pyrrole with aldehydes **1a,b** and *para* nitrobenzaldehyde **2b** in the relative proportion 4/1/3 (yield 14-16%). The same compounds were also synthesised by Alder-Longo method[9] (4-6%). As sugar moieties are sensitive under strongly acidic conditions, usual reduction of nitro function with $SnCl_2/HCl$[10] could not be used. The amino functions were obtained by reduction with H_2/Pd-C[11] in THF for 5 h. Then the mixture was filtered through Celite and the solvent was removed *in vacuo*. Purification of the resulting solid on PLC (eluent CH_2Cl_2/EtOH 95/5) gave the porphyrin **5a,b** and **6a,b** (50-70%). These compounds reacted with 9-fluorenylmethoxycarbonyl-L-Alanine (Fmoc-L-Alanine)(3 eq/NH_2) in presence of dicyclohexylcarbodiimide (DCC) (3 eq/NH_2) in CH_2Cl_2.[12] After 15 h in darkness at room temperature, the solution was concentrated *in vacuo* and EtOAc was added to the residue in order to remove insoluble dicyclohexylurea by filtration then the solution was concentrated *in vacuo*. Purification of the residue gave **7a,b** and **8a,b** (90% yield).

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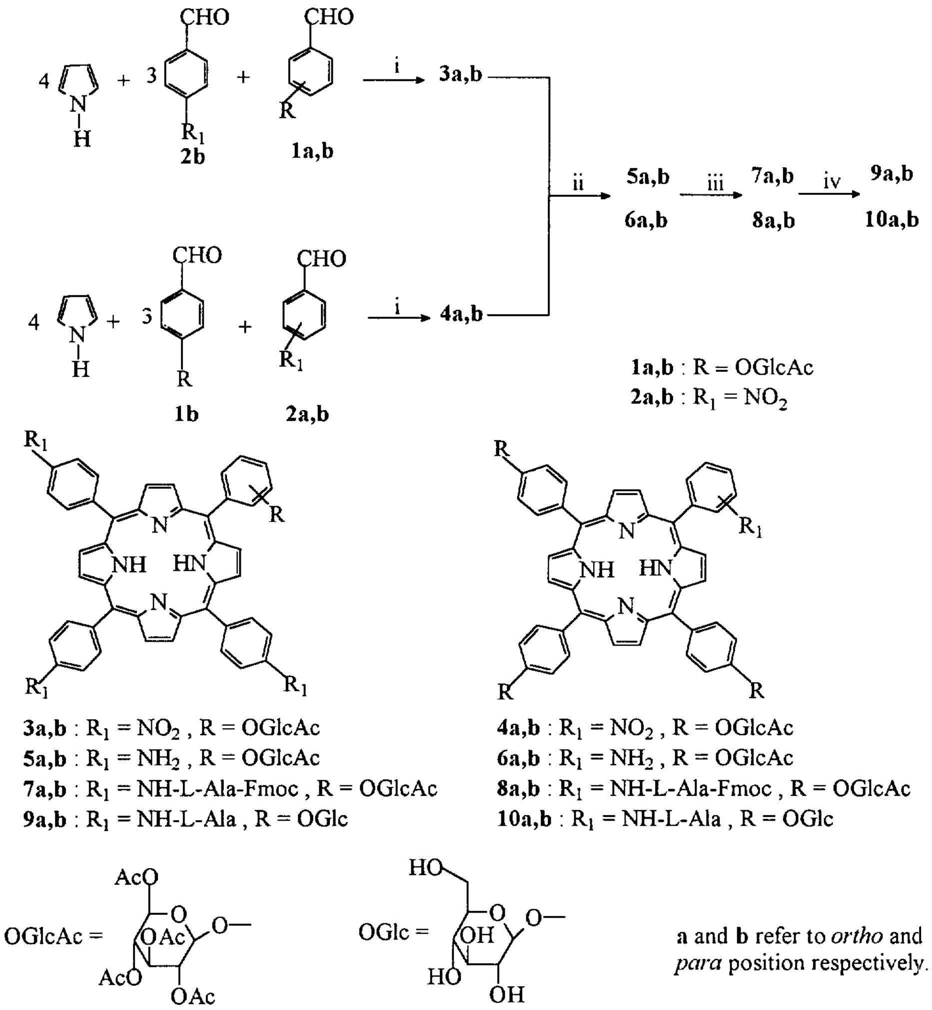

1a,b : R = OGlcAc
2a,b : $R_1 = NO_2$

3a,b : $R_1 = NO_2$, R = OGlcAc
5a,b : $R_1 = NH_2$, R = OGlcAc
7a,b : R_1 = NH-L-Ala-Fmoc , R = OGlcAc
9a,b : R_1 = NH-L-Ala , R = OGlc

4a,b : $R_1 = NO_2$, R = OGlcAc
6a,b : $R_1 = NH_2$, R = OGlcAc
8a,b : R_1 = NH-L-Ala-Fmoc , R = OGlcAc
10a,b : R_1 = NH-L-Ala , R = OGlc

a and b refer to *ortho* and *para* position respectively.

i) BF_3OEt_2, CH_2Cl_2, 18h then p-chloranil, reflux, 1h; ii) H_2, 10% Pd-C, THF, room temp, 5h, 50-70%; iii) CH_2Cl_2, DCC, Fmoc-L-Ala, 15h, room temp, 90%; iv) morpholine then NaOMe/MeOH.

Scheme

Absorption, MS MALDI, ^1H (200 MHz) and ^{13}C (50 MHz) RMN spectra of these compounds showed the expected signals.

Finally, the protecting groups were removed in two steps. Firstly, the neutral Fmoc protective group of the α-amino function was selectively removed in high yield with a weak base[13] (50% morpholine in CH_2Cl_2, rt,1h), then, the acetate groups of the carbohydrate moieties were removed easily by treatment at room temperature (1h) with NaOMe[14] in MeOH/CH_2Cl_2 (8/2) yielding compounds **9a,b** and **10a,b** in 70-60 %.

All fluorescence spectra of compounds with a protected sugar in CH_2Cl_2 (excitation at 422 nm, about 10^{-6} M concentration, at room temperature) were characterised by two emission bands (λ_{max} : 652 nm, 716 nm for porphyrins **4b**; **3a,b**; **6a,b**; **7a,b**; **8a,b** and 662 nm, 723 nm for **5a,b**). The only porphyrin **4a** bearing an NO_2 group in the *ortho* position gave no fluorescence emission most likely due to specific interaction of the NO_2 function with the porphyrin ring. The isomer **4b** with the NO_2 group in *para* position showed a significant fluorescence emission. The fluorescence emission wavelengths of **10a** and **10b** in aqueous solutions were identical to those obtained in THF but the emission intensity was strongly quenched. This decay of fluorescence can be explained by aggregate formation. Marked changes in absorption spectra of these compounds allowed to confirm this conclusion. Thus, in aqueous solution, the Soret band of **10b** (416 nm) is blue-shifted that is consistent to face to face (H) aggregation.[15] For the **10a** compound the Soret band is split into two bands (396-416 nm) as observed previously.[16] This result is due to the combination of cofacial and edge to edge interaction[17] of self-assembled aggregates.

In order to determine the photosensitising properties of porphyrins **9a,b** and **10a,b**, the trapping reactions of $^1O_2$ with ergosterol acetate[18] were carried out. Reference experiments with eosin and hematoporphyrin (HP) as sensitizers gave ergosterol acetate endoperoxide with nearly quantitative yields. In the same experimental conditions, porphyrins **9a,b** and **10a,b** were almost as efficient as HP which is known as a photosensitizer that produces singlet oxygen.

The photocytotoxicity of synthetic porphyrins was evidenced against K562 Human Chronic Myelogenous Leukemia cell line. Exponentially growing K562 cells were suspended in a RPMI medium containing 2.10^{-6}M porphyrin. The suspensions were irradiated with fluorescent light (fluence = 50 watt / m^2) for various times. Dead cells were identified as propidium iodide (PI) permeable ones; dead cell counts were measured by flow cytometry (Facscan, Becton Dickinson, San Jose, USA).

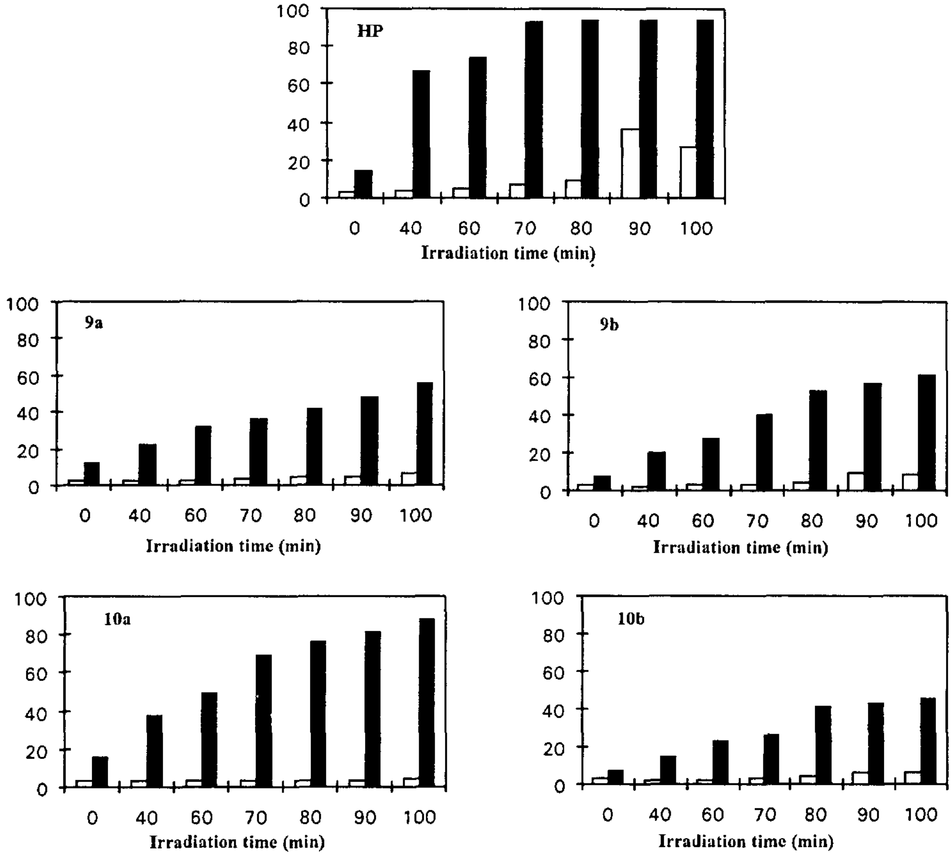

Figure 1 : percentage of PI stained cells *vs* time
- void bars : dead cell count after indicated irradiation time.
- solid bars : dead cell count after a further 24 h incubation in the dark.

Figure 1 displays dead cell counts in function of irradiation time and the subsequent increase following a further 24 hour incubation in the dark. These results can be compared to the effect of 2.10^{-6} M of hematoporphyrin (HP). The immediate dead cell counts were always lower than those observed with HP. However, the delayed effects, observed after a 24 hour incubation in the dark at 37 °C were always comparable to those observed with HP, provided that the irradiation time is greater than 60 minutes; this remark holds especially for porphyrin **10a**.

In conclusion, this paper presents promising compounds for photodynamic therapy. The approach described therein could be applied to other glycosylated peptidic porphyrins.

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