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Synthesis of Glycosyl Strapped Porphyrins

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Abstract : In this paper, we present the synthesis of two glycosylated strapped porphyrins. Structural elucidation is described (^{1}H NMR, MS Maldi and UV visible). The photocytotoxicity of these glycosyl strapped porphyrins against carcinogenic K562 cells is compared to hematoporphyrin as reference.

Strapped porphyrins have received great interest in the past years in relation to their 3-dimensionnal organization which confers to these structures stereoselective catalysis[1] and biomimetic behaviors such as enzymatic like properties.[2-3-4] For example, strapped porphyrins have been designed as models to mimic the oxygen binding to hemoglobin and myoglobin and the oxidations that are catalyzed by cytochrome P-450 enzymes. Furthermore, porphyrins with sugar moieties appear promising candidates for an application in Photodynamic therapy (PDT) of cancer owing to their good solubility in aqueous solutions and their possible specific interaction with membranes.[5] In connection with our research program regarding glycosylated porphyrins[6], we have decided to place a glycosyl strap at the periphery of porphyrins in order to use these glycosyl strapped porphyrins as photosensitizers in PDT. We report here the synthesis of glycosyl strapped porphyrins **6a** (*trans*-strapped porphyrin) and **6b** (*cis*-strapped porphyrin).

The general procedure of synthesis of the strapped porphyrins is given in the scheme. The strategy of synthesis of modified trehalose **3** is based on selective hydrolysis of trimethylsilyl groups at C_6 and $C_{6'}$ of the *per*trimethylsilyl trehalose[7] **1.** The reaction of potassium carbonate (4.5g.L^{-1}/MeOH) with **1** gave the 2,2',3,3',4,4'-hexa-*O*-trimethylsilyl-α,α-trehalose[7] **2** in 95% yield. The benzaldehyde linkage to **2** was achieved by an esterification[8-9] reaction with 3-carboxybenzaldehyde in toluene and DMF by use of dicyclohexylcarbodiimide (DCC)[10] as the activating agent of the carboxyl group and 4-dimethylaminopyridine (DMAP)[11] as a catalyst of acylation. The reaction mixture, initially cooled, was allowed to stand for 24 hours at room temperature. Compound **3** was obtained in 30% yield after purification with flash column chromatography on silica gel (CH_2Cl_2/Petroleum ether). The *meso*-(*p*-tolyl)dipyrromethane[12] **4** was obtained at room temperature by reaction of *p*-tolualdehyde with excess pyrrole (40 eq) using TFA as a catalyst (0,1 eq). After 15 minutes, the complete disappearance of *p*-tolualdehyde was observed. The crude product was dissolved in CH_2Cl_2 and washed with dilute NaOH (1%). Excess pyrrole was removed by vacuum distillation at room temperature. Column chromatography on silica gel (toluene/triethylamine : 100/1) of the resulting brownish solid afforded the green *meso*-(*p*-tolyl)dipyrromethane in 75% yield. Following Lindsey's method[13], the condensation of *meso*-(*p*-tolyl)dipyrromethane (2 eq) **4** with the glycosyl bisaldehyde 6,6'-di-*O*-(3 formyl)benzoyl,2,3,4,2',3',4'-hexa-*O*-trimethylsilyl-α,α-trehalose (1 eq) **3** gave compounds **5a** and **5b**. After elimination of a brown impurity by column chromatography on silica gel, the mixture of **5a** and **5b** was used without further purification and **6a** and **6b** were obtained in 8% and 5% yields respectively after hydroxyl groups deprotection and purification on PLC (dichloromethane / methanol) and Sephadex LH20 columns (methanol).

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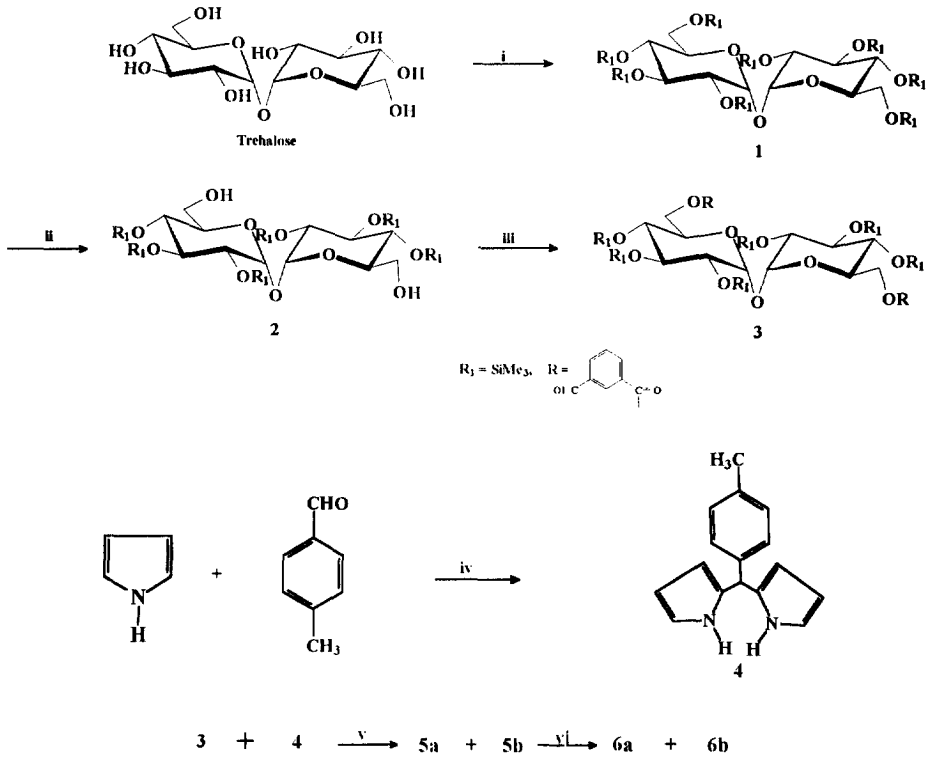
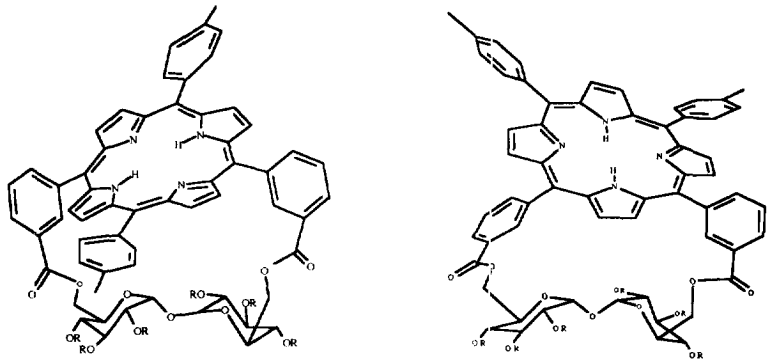




5a, R = -$SiMe_3$
6a, R = H

5b, R = -$SiMe_3$
6b, R = H

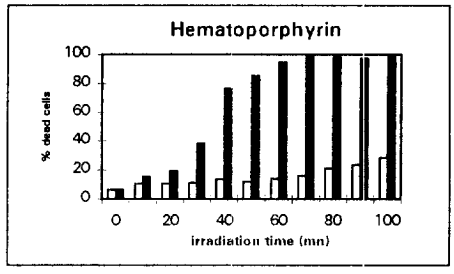
i) HMDS (1,1,1,3,3,3-hexamethyldisilazane), TMSCl (trimethylsilane), pyridine ; ii) K_2CO_3/MeOH ; iii) 3-carboxybenzaldehyde, DMAP (4-dimethylaminopyridine), DCC (dicyclohexylcarbodiimide), toluene, DMF ; iv) TFA, pyrrole ; v) BF_3Et_2O, *p*-chloranil, dichloromethane; vi) tetrabutylammonium fluoride.

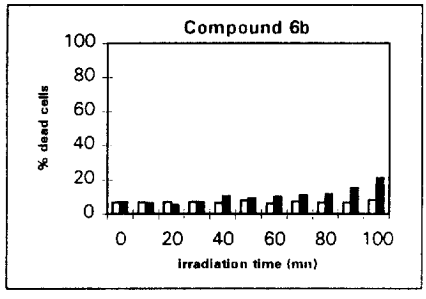
Scheme

The UV-vis, IR, MS MALDI, ^1H (400 MHz) RMN spectra showed the expected signals. In order to identify isomers **6a** and **6b**, we examined the ^1H NMR[14] results. The β-pyrrole region of compound **6b** shows two singlets and a multiplet at 9.00 - 8.84 ppm ($CDCl_3/CD_3OD$). In contrast **6a** shows only doublets at 8.87 - 8.71 ppm ($CDCl_3/CD_3OD$). For isomer **6b**, the NH groups show a broad singlet at -2.98 ppm ($CDCl_3$) while for isomer **6a**, the NH moiety shows a broad singlet at -1.48 ppm ($CDCl_3$) ; these results strongly support the distortion of the macrocycle. Furthermore, the UV-vis data[15] display a Soret band at 426 nm for the *trans*-strapped porphyrin and at 420 nm for the *cis*-strapped porphyrin.

In order to determine the photosensitizing properties of porphyrins **6a** and **6b**, the trapping reactions of $^1O_2$ with ergosterol acetate[16] were carried out. Reference experiments with eosin and hematoporphyrin (HP) as sensitizers gave ergosterol acetate endoperoxide in nearly quantitative yields. In the same experimental conditions, porphyrins **6a** and **6b** were almost as efficient as HP.

The photocytotoxicity of these synthetic porphyrins against K562 human chronic myelogenous leukemia cell line was evidenced. Exponentially growing cells were suspended in 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 those which became permeable to propidium iodide ; the dead cell counts were measured by flow cytometry.





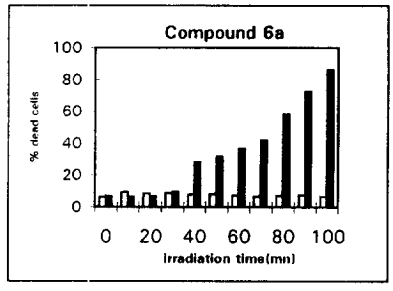


Figure : percentage of PI stained cells vs time

- 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 cells counts as a function of irradiation time and the subsequent increase following a further 24 hours incubation in the dark at 37°C. These results can be compared to the effect of 2.10^{-6} M hematoporphyrin. The *trans*-strapped porphyrin **6a** has an interesting activity despite a more delayed response time than the hematoporphyrin.

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14. Selected data : For **6a**, ^{1}H NMR ($CDCl_3$, 400MHz) δ -1.48(2H, br s, NH). ^{1}H NMR ($CDCl_3$/CD_3OD 400MHz) δ 1.71 (2H, dd, *J*=3.3-9.7Hz, H-2,2', ose) ; 2.60(2H, t, *J*=9.6Hz, H-4,4', ose) ; 3.21(2H, t, *J*=9.3Hz, H-3,3', ose) ; 3.5(2H, dt, *J*=10.6Hz, H-5,5', ose) ;3.61(2H, d, *J*=3.4Hz, H-1,1', ose) ;4.02(4H, m, H-6_a,6'$_a$,6_b,6'$_b$, ose) ; 2.68(6H, s, H-CH_3, tolyl) ; 7.56(4H, d, *J*=7.9Hz, H tolyl) ; 8.09(4H, br s, H tolyl) ; 6.83(2H, br s, H phenyl) ; 8.05(2H, t, *J*=7.8Hz, H phenyl) ; 8.18(2H, br d, *J*=8.1Hz, H phenyl) ; 9.22(2H, br d, *J*=7.6Hz, H phenyl) ; 8.71(2H, d, *J*=4.8Hz, H-β pyrrole) ; 8.77(2H, d, *J*=4.8Hz, H-β pyrrole) ; 8.85(2H, d, *J*=4.8Hz, H-β pyrrole) ; 8.87(2H, d, *J*=4.8Hz, H-β pyrrole). MS(MALDI) m/z : 1038.0 $(M+H)^+$.
   For **6b** ^{1}H NMR ($CDCl_3$, 400MHz) δ -2.98(2H, br s, NH). ^{1}H NMR ($CDCl_3$/CD_3OD 400MHz) δ 3.28(2H, t, *J*=9.6Hz, H-4,4', ose) ; 3.61(2H, dd, *J*=9.8-3.7Hz, H-2,2', ose) ; 3.75(2H, m, H-3,3', ose) ; 4.28(2H, m, H-5,5', ose) ;4.78(4H, m, H-6_a,6'$_a$,6_b,6'$_b$, ose) ; 5.16(2H, d, *J*=3.4Hz, H-1,1', ose) ; 2.72(6H, s, H-CH_3, tolyl) ; 7.58(2H, br s, H tolyl) ; 7.61(2H, br s, H tolyl) ; 7.72(2H, dd, *J*=2.3-8.9Hz, H tolyl) ; 8.11(2H, br d, *J*=7.1Hz, H tolyl) ; 7.88(2H, t, *J*=7.7Hz, H phenyl) ; 7.97(2H, t, *J*=7.7Hz, H phenyl) ; 8.32(1H, d, *J*=7.22Hz, H phenyl) ; 8.45(1H, d, *J*=7.8Hz, H phenyl) ; 8.52(1H, d, *J*=7.8Hz, H phenyl) ; 8.62(1H, d, *J*=7.3Hz, H phenyl) ; 8.84(2H, s, H-β pyrrole) ; 8.91(4H, m, H-β pyrrole) ; 9.00(2H, s, H-β pyrrole). MS(MALDI) m/z : 1038.0 $(M+H)^+$.
15. UV-vis. **6a**, CH_2Cl_2 : λ_{max}, nm(ε, $L.cm^{-1}.mol^{-1}x10^{-3}$) : 426(143.9) ; 524(7.3) ; 560(3.9) ; 596(3.1) ; 652(1.9). **6b**, CH_2Cl_2 : λ_{max}, nm(ε, $L.cm^{-1}.mol^{-1}x10^{-3}$) : 420(259.2) ; 518(13.8) ; 554(8.1) ; 590(5.1) ; 646(4.1).
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