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Nitroglycosylated *meso*-arylporphyrins as Photoinhibitors of Gram positive Bacteria.

V. Sol, P. Branland, R. Granet, C. Kaldapa, B. Verneuil, P. Krausz*.

Université de Limoges, Laboratoire de Chimie des Substances Naturelles, 123 Avenue Albert Thomas F-87060 Limoges.

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Abstract: Novel porphyrins bearing nitro groups and glucosyl moieties were synthesized. The antibacterial activity of these compounds on *Escherichia coli* and *Staphylococcus aureus* is described. Results reveal that their photocytotoxicity is markedly dependent on the nature, the number and the linking position of the glycosyl moieties and nitro groups controlling their amphiphilic characters. © 1998 Elsevier Science Ltd. All rights reserved.

The importance of photoactivated porphyrins as tumor localizers and phototoxic agents for tumor is well etablished,[1] and the development of photodynamic therapy (PDT) has allowed for many years the study of the antimicrobial effect of metal free porphyrins in their light excited state.[2] It was found that Gram positive bacteria are affected by porphyrins (HPD, HP, DP or TMPyP) and light.[2,3] On the other hand, Gram negative bacteria are resistant to such treatment.[4] The later bacteria were inhibited only when the treatment was combined with small nonapeptide (PMNP).[5] Recently, many porphyrins linked to sugar moieties have been synthesized and some derivatives have proved to possess an activity in PDT of cancer cells.[6] Indeed, porphyrins with sugar moieties are known to have a good solubility in water, in addition glycosyl substituents should be functional components for cell recognition.[7] However, the selectivity of these compounds could also be due to the hydrophilic, lipophilic balance (HLB).[8] In connection with our research program on glycosylated porphyrins[6] (with the aim of studying the photoinhibition of Gram positive bacteria), we report here the synthesis of a series of *O*-glycosyl-nitrophenylporphyrins **8-11** (scheme 1). These unsymmetrical neutral *meso* porphyrins contain one, two or three nitrophenyl groups and three, two or one phenyl groups bearing glucosyl moiety in the *ortho* or *para* position (scheme 1). In such compounds, the number of glycosidic residues linked to the macrocycle might allow large changes in the hydrophilic or hydrophobic characters controlling their global amphiphilic properties.

Chemistry. Glycosylated nitrophenylporphyrins **2-5** were synthesized following Lindsey's method,[9] this procedure involved the use of BF_3/etherate as Lewis acid catalyst in CH_2Cl_2 which permitted to obtain the porphyrinogen as intermediate product under mild conditions. Porphyrinogens were then oxidized by *p*-chloranil to afford porphyrins **2-5** which were purified by a silica gel column and PLC. Thus, the condensation of pyrrole (4 eq) with 4-(2',3',4',6'- tetra-O-acetyl-β-D-glucopyranosyloxy)benzaldehyde[10] (3 eq) and *ortho* or *para* nitrobenzaldehyde (1 eq, n=1) gave compounds **2a,b** (14 and 16% yield respectively)(scheme 1). Porphyrin derivatives **3,4** bearing two nitro and two glucosyl units were obtained by condensation of pyrrole with **1b** and *para* nitrobenzaldehyde (n=2) in the relative proportion 4/2/2 (scheme 1). The yield of *trans* **3** and *cis* **4** compounds were respectively 14 and 10%. When the relative proportions of reagents (pyrrole/**1a**/*para* nitrobenzaldehyde, n=3) was 4/1/3, glycosylated nitrophenyl porphyrins **5a,b** were obtained in 14-16% yield respectively. In same conditions, we synthetized mono 4-hydroxyphenyltriglucosylporphyrin **6** and mono 4-nitrophenyltritolylporphyrin **7** (14% yields) (scheme 2).

* Fax : 33(0)5 55 45 72 02 E.mail : krausz@unilim.fr

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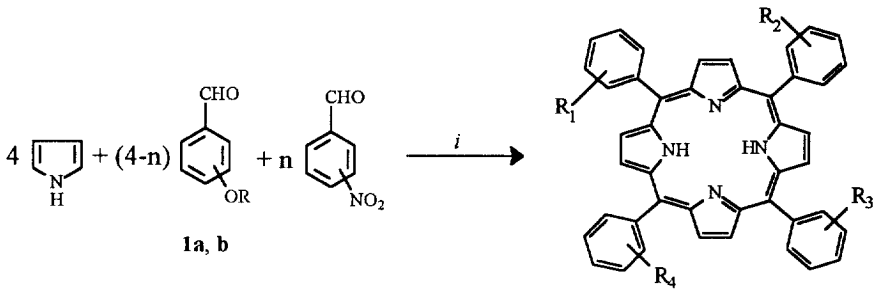
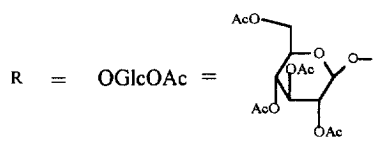
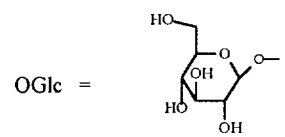
UV-visible absorption, MS MALDI, ^{1}H (200MHz) and ^{13}C (50MHz) RMN spectra of these compounds showed the expected signals.[11] Finally, the acetate groups of the carbohydrate moieties were removed by treatment at room temperature (1h) with NaOMe[12] in $MeOH/CH_2Cl_2$ (8/2) yielding compounds **8-11** (scheme1) in 80% after purification on Sephadex LH20 column. The same treatment was used to obtain porphyrin derivative **12** (85% yield).



1a, b

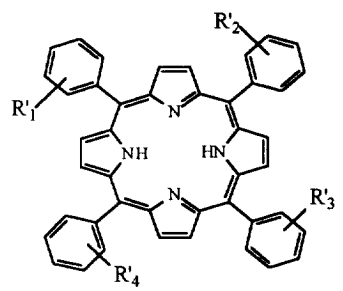
2a : R_1= *o* NO_2 ; R_2, R_3, R_4= *p* OGlcAc
2b : R_1 = *p* NO_2 ; R_2, R_3, R_4= *p* OGlcAc
3 : R_1=R_2= *p* NO_2 ; R_3=R_4= *p* OGlcAc
4 : R_1=R_3= *p* NO_2 ; R_2=R_4= *p* OGlcAc
5a : R_1= *o* OGlcAc ; R_2=R_3=R_4= *p* NO_2
5b : R_1= *p* OGlcAc ; R_2=R_3=R_4= *p* NO_2





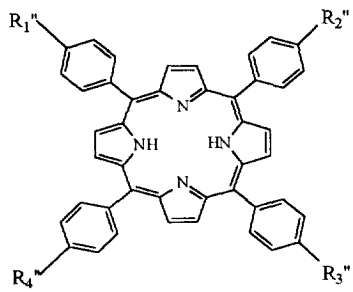
a and **b** refer to *ortho* and *para* position respectively.

ii



8a : R'_1= *o* NO_2 ; R'_2, R'_3, R'_4= *p* OGlc
8b : R'_1 = *p* NO_2 ; R'_2, R'_3, R'_4= *p* OGlc
9 : R'_1=R'_2= *p* NO_2 ; R'_3=R'_4= *p* OGlc
10 : R'_1=R'_3= *p* NO_2 ; R'_2=R'_4= *p* OGlc
11a : R'_1= *o* OGlc ; R'_2=R'_3=R'_4= *p* NO_2
11b : R'_1= *p* OGlc ; R'_2=R'_3=R'_4= *p* NO_2

Scheme 1 : *i*) BF_3OEt_2/CH_2Cl_2, 18h, then *p*-chloranil ; *ii*) NaOMe (1.5 eq/OAc) in $MeOH/CH_2Cl_2$ (8/2), 1h,rt.



6: R_1"=OH; R_2"=R_3"=R_4"=OGlcAc
7: R_1"=NO_2; R_2"=R_3"=R_4"=CH_3
12: R_1"=OH; R_2"=R_3"=R_4"=OGlc

Scheme 2: Structures of mono 4-hydroxyphenyltriglucosylporphyrin acetylated **6**, unprotected **12** and mono 4-nitrophenyltritolylporphyrin **7**.

Photoinhibition of bacteria growth. The two strains used in this study were obtained from "Institut Pasteur, Paris" : a) *Escherichia coli* CIP 548T and b) *Staphylococcus aureus* CIP 53156. This strain is DNAse positive, Phosphatase positive. The antibiotic resistance profiles are described in the Table . These two strains were grown 24H at 37°C in 10 mL peptone water medium. A dilution to 1/20 of the broth is used as inoculum of a gelose nutritive agar agar Petri dish. A stock solution of each porphyrins was prepared in pure DMSO at a concentration of 20 $mg.mL^{-1}$ and kept in the dark until use. The tests were realized at room temperature with illumination by two 60 watts tungsten lamps placed 30 cm away and 45° above both sides of the plates. Cultures treated in the dark as reference were covered with alumina foil during incubation. A Wathman paper disk, impregnated by 0.1 mL of a 100 $\mu g.mL^{-1}$ porphyrin solution, is placed on the gelose nutritive agar agar seeded with the target strain. A DMSO reference was experimented in the same conditions. Inhibition was quantified by measuring the halo diameter formed around the paper disk (a diameter larger than 1cm is considered as positive response). CI_{50} and CMI of the strain giving a positive reaction were quantified by measuring the absorbance at 660 nm for different porphyrin concentrations.

Table : Antibiotic Resistance Profiles of *E. Coli* and *Staph. aureus*.

Antibiotics	Str	Cm	Sul	Ery	Rif	Tc	Pen	Kan	Nal	Van	Amo	Ceph	Mn	Pri
Strain CIP 548T	+	+	+	∓	+	+	/	/	/	/	+	∓	+	–
Strain CIP 53156	+	∓	+	+	+	+	+	+	–	+	/	/	/	/

+:sensitive, –:resistant. Str:streptomycin, Cm:chloramphenicol, Sul :sulfamid Ery:erythromycin, Rif:rifampicin, Tc:tetracyclin, Pen:penicillin, Kan:kanamycin, Nal: nalidixic acid, Van:vancomycin, Amo:amoxillin, Ceph:cephalothin, Mn:cephamandol, Pri:pristinamycin.

Results Fifteen original glycosylated porphyrins were tested with the two bacterial strains. Two glycosylated porphyrins **8b** and **9** are associated with a significant antimicrobial activity under illumination for *Staphylococcus aureus*. The Gram negative bacteria, *Escherichia coli*, were not affected even by large dose of porphyrins (100 $\mu g.mL^{-1}$) and light. In order to analyse this activity on *Staphylococcus aureus*, the CI_{50} and CMI were determined, the values obtained for porphyrins **8b** and **9** are respectively 10 $\mu g.mL^{-1}$ and 100 $\mu g.mL^{-1}$ and these results are in accordance with precedent studies.[3-4] Moreover the incubation under illumination is a necessary condition, confirming the hypothesis of a photodynamic effect for these two original glycosylated porphyrins. This study allowed us to correlate the antimicrobial activities of all compounds **2 - 12** with their chemical structure. Thus, porphyrins derivatives **2 - 5** with acetylated sugar moities were inactive and among the unprotected porphyrins **8 - 12**, only compounds **8b**, **9** were active. These two porphyrins were substituted by three (**8b**) or two (**9**) glycosyl group linked at the *para* position of two adjacent *meso* phenyls. In contrast, compound **10** having glycosyl moieties in diagonally opposed phenyl rings exhibited no activity. In

addition, the presence of glycosyl is essential because unglycosylated porphyrin 7 induces no growth inhibition. On the other hand unnitrated porphyrins **6,12** have no activity. It is noteworthy that the antimicrobial activity is associated to the presence of at least two glucose units and one nitro group in *para* position.

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11. Selected data : For **2a**, ^{1}H NMR ($CDCl_3$, 200MHz) δ -2.71(2H, br s, NH); 2.11(9H, s, -OAc); 2.12(9H, s, -OAc); 2.13(6H, s, OAc); 2.14(3H, s, -OAc); 2.23(9H, s, -OAc); 4.08(3H, ddd, *J*=9.7-5.3-2.8Hz, H-5' ose); 4.31(3H, dd, *J*=12.2-2.2, H-6_b' ose); 4.43(3H, dd, *J*=12.2-5.3Hz, H-6_a ose); 5.34(3H, br d,*J*=9.7Hz, H-4' ose); 5.48(9H, m, H-1',2',3' ose); 7.40(6H, br d, *J*=7.3Hz, H-3,5 aryl); 7.96 (2H, m, H-4,5 *o*-nitrophenyl); 8.14 (6H, brd, *J*=7.5Hz, H-2,6 aryl); 8.25(1H, dd, *J*=7.1-2.1Hz, H-6 nitrophenyl); 8.44(1H, dd, *J*=7.3-2.1Hz, H-3 nitrophenyl); 8.67(2H, d, *J*=4.9Hz, H-β pyrrole); 8.85(2H, d, *J*=4.8Hz, H-β pyrrole); 8.86(4H, s, H-β pyrrole). ^{13}C NMR ($CDCl_3$, 50MHz) 20.6(6C, $\underline{C}H_3CO$); 20.7(6C, $\underline{C}H_3CO$); 62.1(3C, C-6' ose); 68.4(3C, C-4' ose); 71.3(3C, C-2' ose or C-3' ose); 72.3(3C, C-5' ose); 72.6(3C, C-2' ose or C-3' ose); 99.1(3C, C-1'ose); 113.9(1C, C *meso*); 115.1(6C, C-3,5 aryl); 119.6(2C, C *meso*); 120,0(1C, C *meso*); 124,0(1C, C-3 nitrophenyl); 129.5(1C, C-4 nitrophenyl); 130.9(1C, C-5 nitrophenyl); 131.5(8C, C-β pyrrole); 135.5(6C, C-2,6 aryl); 136.5(1C, C-6 nitrophenyl); 136.8(1C, C-1 nitrophenyl); 136.9(2C, C-1 aryl); 137.1(1C, C-1 aryl); 146.5(8C, C-α pyrrole); 169.4 (6C, $CH_3\underline{C}O$); 170.2(4C, $CH_3\underline{C}O$); 170.5(2C, $CH_3\underline{C}O$). UV-visible spectrum in CH_2Cl_2: λ_{max} , nm (ε, L.cm^{-1}.mol^{-1}x10^3): 420(233.3); 516(13.3); 552(8.2); 592(5.0); 648(4.2). MS(MALDI) m/z : 1699.5 $(M+H)^+$.
For **2b**, ^{1}H NMR ($CDCl_3$, 200MHz) δ -2.77(2H, br s, NH); 2.12(9H, s, -OAc); 2.13(18H, s, -OAc); 2.23(9H, s, -OAc); 4.08(3H, ddd, *J*=9.8-5.3-2.6Hz, H-5' ose); 4.32(3H, dd, *J*=12.1-2.1Hz, H-$6'_b$ ose); 4.42(3H, dd, *J*=12.1-5.1Hz, H-$6'_a$ ose); 5.35(3H, m, H-4' ose); 5.49(9H, m, H-1',2',3' ose); 7.39(6H, d, *J*=8.5Hz, H-3,5 aryl); 8.14(6H, d, *J*=8.4Hz, H-2,6 aryl); 8.39(2H, d, *J*=8.6Hz, H-2,6 nitrophenyl); 8.64(2H, d, *J*=8.6Hz, H-3,5 nitrophenyl); 8.75(2H, d, *J*=4.9Hz, H-β pyrrole); 8.88(4H, s, H-β pyrrole); 8.90(2H, d, *J*=4.9Hz, H-β pyrrole). ^{13}C NMR ($CDCl_3$, 50MHz) 20.6(8C, $\underline{C}H_3CO$); 20.8(4C, $\underline{C}H_3CO$); 62.1(3C, C-6' ose); 68.4(3C, C-4' ose); 71.4(3C, C-2' or C-3' ose); 72.3(3C, C-5' ose); 72.8(3C, C-3' or C-2' ose); 99.1(3C, C-1' ose); 115.2(6C, C-3,5 aryl); 116.8(1C, C *meso*); 119.2(2C, C *meso*); 120.2(1C, C *meso*); 121.7(2C, C-3,5 nitrophenyl); 131.0(8C, C-β pyrrole); 135.1(2C, C-2,6 nitrophenyl); 135.5(6C, C-2,6 aryl); 136.7(3C, C-1 aryl); 146.2(8C, C-α pyrrole); 147.8(1C, C-4 nitrophenyl); 149.1(1C, C-1 nitrophenyl); 156.7(3C, C-4 aryl); 168.4(6C, $CH_3\underline{C}O$); 170.3(3C, $CH_3\underline{C}O$); 170.6(3C, $CH_3\underline{C}O$). UV-visible spectrum in CH_2Cl_2: λ_{max} , nm (ε, L.cm^{-1}.mol^{-1}x10^3): 420(335.9); 516(13.9); 552(6.8); 592(4.3); 648(2.9). MS(MALDI) m/z : 1699.5 $(M+H)^+$.
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