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Synthesis of Porphyrins with Pendant Arms : Participation of the Ancillary Ligands to the Complexation Process in Proteic Medium

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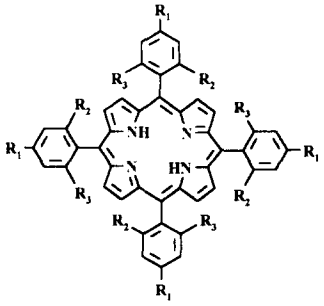
Abstract: Ortho substituted porphyrins are able to complex copper (+II) in a neutral aqueous medium containing an excess of bovine albumine.

Since the first observation by Policard in 1924 of preferential accumulation of hematoporphyrin in neoplastic tissues, a number of investigators have used the fluorescence of porphyrins for the detection and destruction of tumors.[1-3] More recently, the use of radiolabeled metalloporphyrins has significantly improved the efficiency of these compounds for tumor detection by γ-camera imaging.[4,5] This method can be improved by associating a radioactive metal complex and an antibody in order to deliver the reagent to a specific target. This technique has been considerably improved with the advent of monoclonal antibodies.[6] The problem often arising with porphyrins is the rather high temperature required for metal insertion that prevent preliminary coupling of the macrocycle with the antibody. Among all the radionucleides available, ^{64}Cu and ^{67}Cu are particularly suited for porphyrins because of their great affinity for this metal.[7] Their relatively short period (respectively 12.7 h and 61.9 h) does not allow complex and time wasting synthetic and purification procedures. In order to propose an easy-to-use kit, we report herein the synthesis of new porphyrins and the insertion of stable isotope of copper in proteic medium.The general formula of the ligand studied is outlined forward.



1: R_1= H. R_2 =H. R_3 = -O-CH_2-COOH
2: R_1= H. R_2 = R_3 = -O-CH_2-COOH
3: R_1= R_2 = -O-CH_2-COOH. R_3=H
4: R_1= O-$(CH_2)_3$-COOH .R_2= R_3=H

These derivatives were obtained by a four step procedure: a) classical condensation of pyrrole and methoxy substituted benzaldehyde in propionic acid b) demethylation of the ether functions by boron tribromide according to a previously described procedure[8] c) alkylation of the phenolic functions by ethyl bromoacetate d) saponification of the ester groups.[9] Porphyrin **4** was obtained by direct condensation of pyrrole with the corresponding aldehyde in propionic acid followed by saponification. Metallation was performed in a 25 mM Tris-HCl buffer (pH 7.4, 25°C) in the presence of $CuSO_4$(100 μM). The porphyrin (100μM) was added, and the metallation was followed by visible spectroscopy. Porphyrins **1**, **2** and **3** were

metallated instantaneously, even though it took about 10 min to be completed in the case of **4**. More interesting was the fact that insertion of the metal into the macrocycles **1**, **2** or **3** could be done even in the presence of bovine albumine (150µM). This metallation did not occur with **4** or with *meso*-5,10,15,20-Tetrakis[4-(carbonylmethoxy) phenyl]porphyrin.[10] We also successfully insert cobalt (as Co^{2+}) and manganese (as Mn^{2+}) into **1** even in the presence of bovine albumine. This reaction does not proceed when porphyrin **4** was used.[11] Participation of covalently linked carboxylate groups allowed considerable acceleration of metal incorporation in the porphyrin ring, as previously described with *meso*-αααα-tetra(2-maleamoylphenyl)porphyrin.[12] This atropisomer posseses the ideal geometry for a pre-complexation of the metal by the four carboxylate functions. In the case of **1** and **3**, we use a mixture of the four atropisomers in which at least two carboxylic functions are on the same side of the porphyrin ring. Participation of at least two carboxylate pendant arms seemed to be sufficient to allow specific transport of the metal and incorporation in the porphyrin ring. This procedure does not necessitate the preliminary activation of the nitrogen atom by alkylation as described by Cole *et al.*[13] Moreover, specific insertion of the metal in the porphyrin even in the presence of a good copper chelator like bovine albumine allows preliminary coupling of these ortho substituted porphyrins with antibodies before ^{64}Cu insertion. As the metal seemed to be not diluted on the protein if a stoichiometric amount of copper was used, we could avoid the tedious purification of radioactive material and *in vivo* exchange of radioactive copper with proteins. We are presently attempting to link covalently these substituted porphyrins to monoclonal antibodies, and testing their stability towards demetallation processes.

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9. Analytical data of **1**, **2**, **3** and **4**

   UV-Visible spectra :λ (nm), ε x 10^{-3}($mol^{-1}.l.cm^{-1}$)

   1(pyridine) : 420 (36.5), 514 (17.3), 547.5(5.4), 590 (5.5), 644.5 (2.7); **2**(H_2O) : 413 (245.6), 512.5 (11.5), 544 (2.6), 579 (4.4), 631 (1.4); **3**(H_2O) : 417.5 (179.1), 517 (9.9), 554.5 (4.3); 581 (4.1), 637 (2.4)

   ^1H NMR chemical shifts (ppm)

   1 (atropisomeric mixture) (DMSO d6) : 8.77 (s, 8H,pyrrolic) ; 7.94 (m, 4H) ; 7.78 (m, 4H) ; 7.36 (m, 8H) ; 4.57 / 4.56 / 4.53 (m, 8H) ; -2.87 (m, 2H)

   2 D_2O : 9.17 (m, 8H) ; 7.86 (t, 4H) ; 7.05 (d, 8H) ; 4.23 (s 16H)

   3 (atropisomeric mixture) (D_2O) : 8.96 (m, 8H) ; 7.95 (m, 4H) ; 6.95 (m, 4H) ; 6.76 (m, 4H) ; 4.66 (s, 8H) ; 4.32 / 4.31 / 4.27 / 4.25 (m, 8H)

   4 (DMSO d^6) 8.81 (s, 8H); 8.03 (d,8Hz, 8H); 7.26 (d, 8Hz, 8H), 4.22 (t, 6Hz, 8H); 2.55 (t, 6Hz, 8H); 2.11 (m,8H)
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11. The reaction proceeded more slowly as it was completed in ½ hour in the presence of a 5-fold excess of metal salt (used as chloride). When Mn (II) was added to **4**, slow precipitation occured, probably due to external complexation by carboxylate groups and formation of aggregates. With Co(II), we could observe a partial insertion in the macrocycle **4** , but the solution became also turbid. In the presence of bovine albumine this metallation process was completly inhibited.
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