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Synthesis, Stereochemical and Photophysical Studies of Chiral Mesoporphyrins

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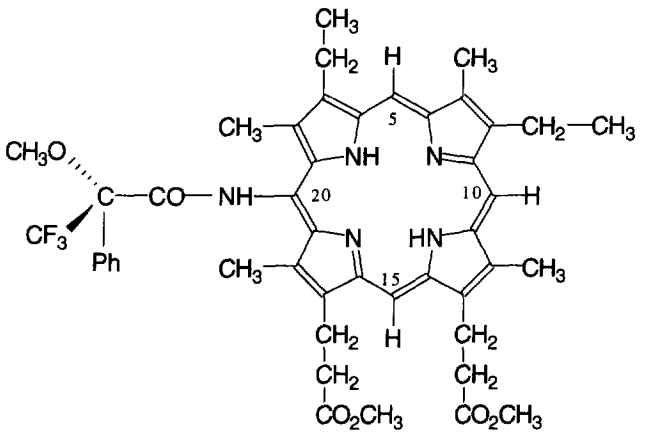
Abstract. The synthesis and NMR characterization of chiral mesoporphyrins bearing α-methoxy-α-(trifluoromethyl) phenylacetyl residues are reported. The phototoxicity with circular polarized light and intracellular localization in L1210 cells are also described as preliminary results.

Many naturally occuring porphyrins have enantiotopic faces. It was recently demonstrated that etioporphyrin can be converted into chiral porphyrins by the presence of amide substituents in the *meso* position because the ring is distorded from planarity[1]. This distorsion is due to the steric repulsion between the amide group and the neighboring substituents. We report herein the synthesis of diastereomeric mesoporphyrin systems in which the enantiopic faces of the macrocycle are directly linked to optically active Mosher acid[2]. Such compounds can be of interest as chemical substrates for photoasymmetric processes[3] and for photodynamic therapy (PDT)[4] with circular-polarized light. The cytotoxicity, phototoxicity and subcellular localization are also reported as preliminary results.

Our starting material was mesoporphyrin dimethyl ester **1** which is ready available and whose structure is closely related to the different components of Photofrin-II, the only drug currently use for treatment of various tumors using PDT. The synthesis of the mixture of mono-amino-mesoporphyrins **3** from **1** involves i) a nitration of the zinc(II) complex of mesoporphyrin with $AgNO_2$ to give the nitro-mesoporphyrins **2**[5] and, after demetalation with HCl, ii) an electroreduction in a special redox cell with porous electrodes (graphite felt), as we previously reported with deuteroporphyrin[6]. Using the theoretical amount of electricity at the porous cathode (6 moles of electrons per mole of the mixture of the regioisomers) nitro-mesoporphyrins **2** were converted into amino-mesoporphyrins **3** (yield: 60 %). Three of the four possible regioisomers were separated on preparative silica gel plates (eluent: $CHCl_3$/diethylether/hexane: 10/45/45) to give in order of elution, 5-amino (20%), 10-amino (15%) and 20-amino-mesoporphyrin IX dimethyl ester (45%). The 15-amino

regioisomer was not detected. The structure of the 20-amino-mesoporphyrin, compound **3a**, was determined by 2D intramolecular ^{1}H-nuclear Overhauser effect (NOESY) and COSY spectra, using the previously reported conditions with aminodeuteroporphyrins[7]. Thus the three meso protons were assigned to the 5, 10 and 15 positions, giving evidence for the 20-NH_2 substitution.



4a

After insertion of zinc into **3a**, the condensation of **3a**-Zn with optically active Mosher acid chloride ($[\alpha]^{25}D$= - 135°) (c 4.1, CCl_4)[2] gave the expected amide compound **4a**-Zn as a mixture (1/1) of two diastereomers with a good yield (85 %). Two bands in thin layer chromatography were observed at 10 °C but not completely resolved ($CHCl_3$/diethylether: 95/5) due to a facile exchange (see below). The presence of both the two enantiotopic faces of the porphyrin and the Mosher amide group on the *meso* position explains the two diastereomer derivatives. After demetalation, evidence for the diastereomeric nature of **4a** came also from the presence of two CF_3 peaks in the ^{19}F NMR spectrum and the presence of six *meso* protons. Full assignment of the unmetalled **4a** was accomplished through the help of 2-D proton-carbon heteronuclear correlation spectroscopy (HMBC). Thus the methoxy protons of the chiral Mosher group were assigned to 4.09 ppm through a cross-peak between these protons and a carbon at 55 ppm. A facile exchange was also detected by the presence of negative cross-peaks in the phase sensitive NOESY experiment between signals corresponding to the same proton but belonging to each diastereomer (40 °C, toluene). Such correlations were observed for the three pairs of meso protons as well as several CH_2 and CH_3 protons which are even better resolved in the toluene solvent. In contrast, coupling of pivaloyl chloride to the 20-amino regioisomer **3a** yielded to a racemic compound **5a** showing only one t-butyl group and three meso-protons in the ^{1}H NMR spectrum. The data for representative compounds are mentioned in reference section[8].

The intracellular localization patterns of porphyrin **4a** (as the mixture of the two diastereomers) were determined by confocal laser microspectrofluorometer, showing fluorescence staining in L1210 cells[9]. Figure 1 shows the spectral modification of fluorescence in the cytoplasm as a function of time, with a maximum of

fluorescence after 4 hours (λ_{ex} = 488nm,c = 50 μg/ml). Compound **4a** was also tested for in-vivo photosensitizing activity and it was found to have two times higher tumorcidal activity with circularly polarized light than with planar polarized light (3.1 μg/ml; 10 J/cm^2). Further developments and improvements of this approach are in progress using various chiral acid chlorides, and more extensive biological studies will be reported elsewhere.

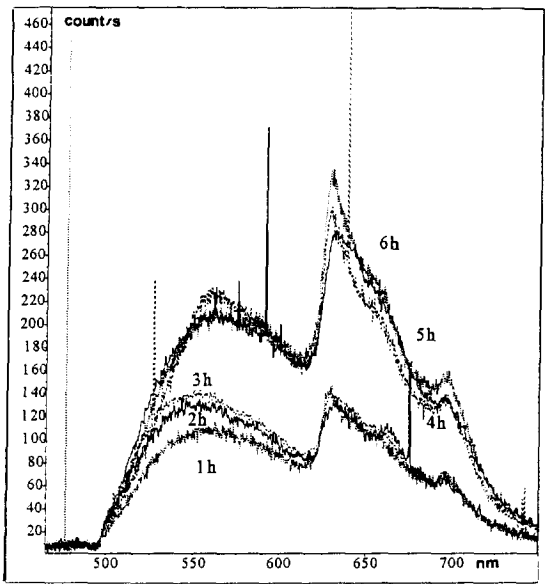


Figure 1. Change in **4a** fluorescence in L1210 cells as a function of time.

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References and Notes

1. Konishi, K.; Miyazaki, K.; Aida, T.; Inoue, S. *J. Am. Chem. Soc.* **1990**, *112*, 5639-5640.
2. Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543-2549.
3. Balavoine, G.; Moradpour, A.; Kagan, H. B. *J. Am. Chem. Soc.* **1974**, *96*, 5152-5158.
4. (a) Pandey, R. K.; Dougherty, T. J.; Smith, K. M. *Tetrahedron Lett.* **1988**, *29*, 4657-4660. (b) Bonnett, R. *Chem. Soc. Rev.* **1995**, *24*, 19-33.
5. Crossley, M. J.; Gosper, J. J.; Wilson, M. G. *J. Chem. Soc., Chem. Commun.* **1985**, 1798-1799.

6. Moinet, C.; Autret, M.; Floner, D.; Le Plouzennec, M.; Simonneaux, G. *Electrochim. Acta* **1994**, *39*, 673-679.

7. Bondon, A.; Autret, M.; Simonneaux, G. *Magn. Reson. Chem.* **1994**, *32*, 78-82.

8. Selected spectroscopic data:

for **3a**, Vis : λ_{max} (nm) (ε $M^{-1}cm^{-1}$) ($CHCl_3$) 414 (163 000), 518 (10100), 555 (5300), 588 (3300), 646 (6300) ; ^1H NMR ($CDCl_3$) δ 9.45 (s, *meso*-H-5), 9.44 (s, *meso*-H-15), 9.15 (s, *meso*-H-10), 5.58 (s, NH_2), - 1.38 (s, NH) ;

for **4a**, Vis : λ_{max} (nm) (ε $M^{-1}cm^{-1}$) ($CHCl_3$) 407 (100600), 506 (13200), 538 (9300), 576 (8600), 626 (6600) ; ^1H NMR ($CDCl_3$) δ 10.60 (s, NHCO), 10.09, 10.07, 10.01, 9.99 (s, *meso*-H), 9.89 (s, *meso*-H, 2H), 7.87 (m, ortho-H), 7.52 (m, meta + para-H), 4.09 (s, O-CH_3), -3.37 (s, NH) ; ^{19}F NMR δ -65.46, -65.50 (s, CF_3) ;

for **5a**, Vis : λ_{max} (nm) (ε $M^{-1}cm^{-1}$) ($CHCl_3$) 401 (114800), 503 (9800), 536 (5400), 573 (4400), 625 (2400) ; ^1H NMR ($CDCl_3$) δ 9.97, 9.95, 9.86 (s, *meso*-H), 8.66 (s, NHCO), 1.56 (s, t-Bu), -3.6 (s, NH).

9. Morlet, L.; Vonarx-Coinsmann, V.; Lenz, P.; Foultier, M. T.; de Brito, L. X.; Stewart, C.; Patrice, T. J. *J. Photochem. Photobiol. B* **1995**, *28*, 25-32.

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