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SYNTHESIS AND PRELIMINARY DNA-INTERACTION STUDIES OF A NEW CATIONIC PORPHYRIN

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Summary: *A new water-soluble porphyrin containing benzyl-trimethylammonium groups was synthesized in a two-step sequence from tetraphenylporphyrin and preliminary studies show that it binds strongly to DNA in an outside manner and gives fairly stable complexes with nucleotides and nucleosides.*

Water-soluble porphyrins were recently found of great interest because of their strong affinity for biological macromolecules or cells. Cationic porphyrins interact with DNA either by intercalation between the G-C base-pairs or by outside binding in the minor groove with an A-T sites selectivity.[1,2] Because of their strong affinity for DNA, cationic porphyrins have been used to induce single- and double-strand scissions of DNA, in general by photochemical oxidation.[3] The mode of binding to DNA depends on many factors: ionic strength, porphyrin to base-pair ratio and mainly the porphyrin geometry. The inability of the porphyrin to attain a planar configuration due to the steric bulk of the meso substituents prevents an intercalative interaction. The number and position of the positive charges are also of importance.Thus, H_2TMPyP-4, 1, (Fig.1) is found to intercalate into DNA, as shown by its ability to unwind supercoiled DNA[4] and to display, in the presence of DNA, a large negative induced CD spectrum,[5] whereas H_2TMAPP, 2, (Fig.1) binds to the outside of DNA with self-stacking.[6] 1 and some of its metal derivatives were also found to form moderately stable molecular complexes with nucleosides and nucleotides as a result of stacking and electrostatic interactions.[7]

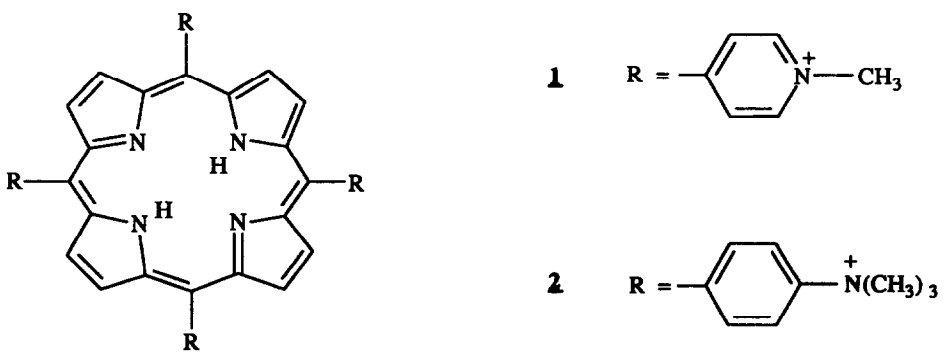

Figure 1

Therefore, it is of interest to find new cationic porphyrins differing from H_2TMPyP or H_2TMAPP by their geometry and/or by the position of the positive charges. We describe here the synthesis of *meso* tetrakis(4-(trimethylammonium)benzylporphyrin), H_2TMABP, 3, a new cationic water-soluble porphyrin, and preliminary results of its association with DNA, nucleotides and nucleosides.

Synthesis of H_2TMABP 3: The synthesis was achieved using two sequences (Fig.2).

In the first one, *meso* tetrakis(4-(carboxymethyl)phenyl)porphyrin 4 was prepared from methyl 4-formylbenzoate and pyrrole and reduced in dry THF by $LiAlH_4$ to give *meso* tetrakis(4-hydroxybenzyl)porphyrin 5, as described by Datta Gupta *et al.*.[8] 5 is transformed into *meso* tetrakis(4-chlorobenzyl)porphyrin 6 by treatment with $SOCl_2$. Reaction of 6 with trimethylamine and recristallisation in ethanol/acetone lead to pure water-soluble H_2TMABP 3 (overall yield from 4: 49%).

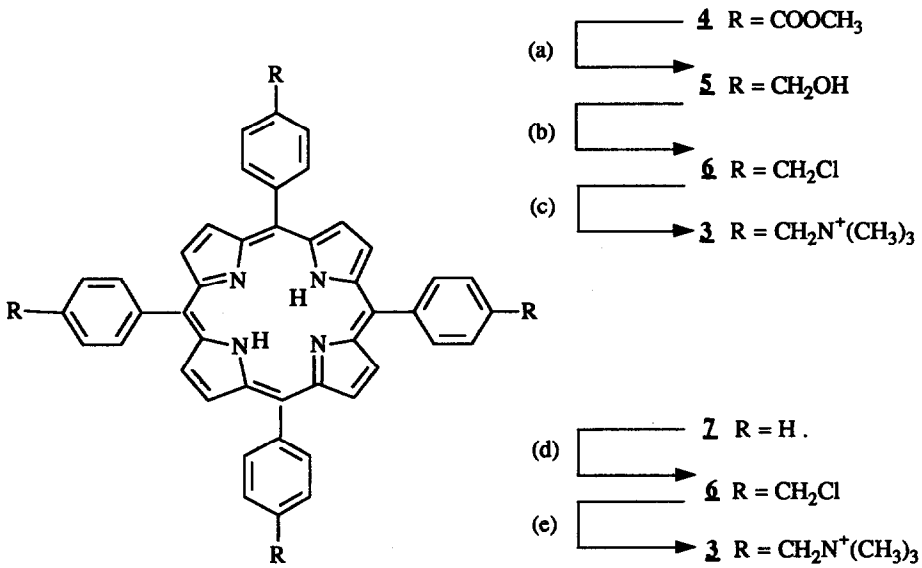

Figure 2

(a): refluxing anhydrous THF, $LiAlH_4$, 2 h, 60% ; (b): $SOCl_2$, 25°C, 10 h, 100% ; (c) (e): $N(CH_3)_3$ aqueous solution, $CHCl_3$, 25°C, 15 h, 90%.; (d): $ClSO_3H$, NaCl, HCHO, 0°C, 10 min, 50%.

While we were achieving this synthesis, Petrova and coworkers[9] described the direct synthesis of 6 using chloromethylation of *meso* tetraphenylporphyrin H_2TPP, 7, with chlorosulfonic acid, formaldehyde and sodium chloride at 0°C. Purification of the crude reaction products on a silicagel column yields pure tetrakis(4-chlorobenzyl)porphyrin 6 which can thus be obtained in two steps from the inexpensive *meso* tetraphenylporphyrin 7 (overall yield from 7: 52%) Metallation of 3 (Cu, Mn, Zn and Ni) was achieved as described in the literature.[10] All analysis and spectra were in agreement with the proposed structures.[11]

Visible spectroscopy, fluorimetry and T-Jump experiments all indicate that H_2TMABP does not aggregate between 10^{-7} and 10^{-4} M. Above 10^{-4}M, it aggregates but the auto-association phenomenon is more complex than usually observed with self-associating porphyrins as shown by ^1H NMR spectroscopy.[12]

Studies of the interaction of 3 and its metal derivatives with DNA, nucleosides and nucleotides have been performed using visible spectroscopy, circular dichroism and electrophoresis. The first results are indicative of a strong but non-intercalative binding with calf thymus DNA, poly(dG-dC) and poly(dA-dT), similar to that observed with H_2TMAPP 2. Further studies by circular dichroism and electrophoresis of DNA binding of 3 and its metal derivatives are currently done in order to elucidate the mode and specificity of the interactions. Spectrophotometric titrations in the Soret band of the differences in absorbance of a porphyrin solution in the presence and absence of the nucleoside (or nucleotide) were conducted. The data, analyzed by a general minimization routine, SIMPLEX,[13] indicate that H_2TMABP and its metal derivatives (Cu, Ni and Zn) give sligthly less stable complexes with nucleosides and mononucleotides than H_2TMPyP 1 and its metal derivatives. As an example, $K_{ATP/H2TMABP} = 1200\ M^{-1}$ whereas $K_{ATP/H2TMPYP} = 2900\ M^{-1}$.

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11. 3: RMN (CD_3OD) ppm: 8.98 (s:8H); 8.46 (d:8H); 8.10 (d:8H); 4.99 (s:8H); 3.48 (s:36H)
    Visible nm: 412 (100), 516 (5.2), 552 (2.6), 579 (1.6), 634 (1.2).
    Anal.: Calcd: + $16H_2O$ C% 54.04; H% 7.71; N% 8.40;Cl% 10.65
    Found: C% 53.85; H% 7.80; N% 8.41; Cl% 9.88.
    4: RMN ($CDCl_3$) ppm: 8.80 (s:8H); 8.43 (d:8H); 8.28 (d:8H); 4.10 (s:8H); -2.82 (s:2H).
    Visible nm: 419 (100), 514 (6.9), 548 (4.3), 590 (3.5), 646 (3.0).
    Anal.: Calcd: C% 73.75; H% 4.52; N% 6.62,
    Found: C% 73.60; H% 4.33; N% 6.46.
    5: RMN (DMSO D6) ppm: 8.85 (s:8H); 8.15 (d:8H); 7.75 (d:8H); 5.55 (t:4H); 4.85 (d:8H); -2.95 (s:2H).
    Visible nm: 418 (100), 515 (4.2), 551 (3.3), 590 2.0), 646 (1.4).
    6: RMN ($CDCl_3$) ppm: 8.83 (s:8H); 8.19 (d:8H); 7.76 (d:8H); 4.93 (s:8H); -2.82 (s:2H).
    Visible nm: 419 (100), 515 (4.2), 551 (1.8), 590 1.2), 646 (0.9).
    Anal.: Calcd: C% 71.28; H% 4.24; N% 6.93; Cl% 17.55
    Found: C% 71.13; H% 4.48; N%.6,93; Cl% 17.60.
    CuTMABP Visible nm: 412 (100), 538 (6.5), 576 (2.6).
    ClMnTMABP Visible nm: 462 (100), 560 (11.7), 593 (6.9).
    ZnTMABP Visible nm: 420 (100), 556 (6.2), 595 (3.3).
    NiTMABP Visible nm: 408 (100), 523 (7.1).
12. N. Robic, C. Bied-Charreton, M. Perrée-Fauvet, E. Amouyal, A. Gaudemer and R.F. Pasternack, results to be published.
13. Copyrighted by J.P. Chandler, Department of Physics, University of Indiana, Bloomington, IN, 1965. The routine was further expanded by T. Needham, Department of Chemistry, University of Illinois, Urbana, IL.

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