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Synthesis and Spectroscopic Properties of a Water-Soluble Porphyrin-Modified β-Cyclodextrin Compound

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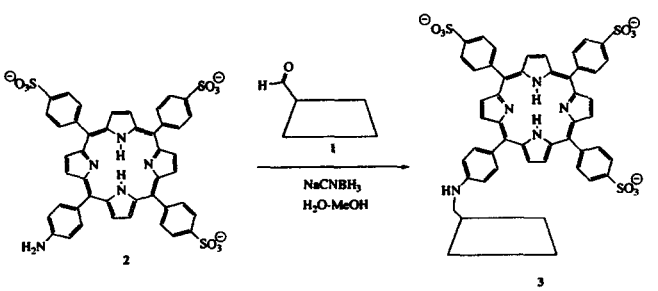
Abstract: Reductive amination of 6-deoxy-6-formyl-β-cyclodextrin, **1**, with 5-(*p*-aminophenyl)-10,15,20-tris(*p*-sulfonatophenyl)porphyrin, **2**, in the presence of an excess of sodium cyanoborohydride, affords the water-soluble compound **3** (23% yield) as confirmed by NMR spectroscopy and mass spectrometry techniques. © 1997 Elsevier Science Ltd.

The synthesis of covalently linked porphyrin-cyclodextrin adduct is an elegant approach to biomimetic models for some fundamental natural processes. Among the most successful results so far reported in this field noteworthy are the Kuroda's cyclodextrin-sandwiched porphyrin[1], which shows a lypoxygenase-like activity[1c] and mimics the characteristics of the natural photoreaction center[1d], and the Breslow's artificial cytochrome P-450 enzyme[2]. In these systems, the cyclodextrin plays both the role of hydrophobic recognition site and that of hydrophilic pendant such as to allow the covalent adduct to be soluble in water. As a matter of fact, in order to fully exploit the interactions between the host and hydrophobic substrates, it is of paramount importance to employ aqueous media, other solvents being not suitable for the type of host-guest interactions that are sought. Water solubility may be achieved by designing adducts with more than one cyclodextrin units[3] although this complicates the synthesis and does not guarantee that much large improvements may be achieved. An alternative approach is the use of hydrophilic porphyrins such as those carrying ionic groups as *meso* or *exo* substituents.

Herein we describe the attachment of a trisulphonatophenyl porphyrin moiety to the primary face of β-cyclodextrin to produce the new compound **3** (Scheme 1) which is remarkably soluble in aqueous milieus.



Scheme 1

The key step is the formation of a stable amino-linkage through the reductive amination of 6-deoxy-6-formyl-β-cyclodextrin[5], **1**, with 5-(*p*-amino-phenyl)-10,15,20-tris (*p*-sulphonatophenyl)porphyrin[6], **2**, in the presence of an excess of sodium cyanoborohydride in aqueous methanol. The product was purified by preparative TLC and isolated as ammonium salt (23% yield). The MALDI-TOF spectrum of compound **3** (matrix: 2,5-dihydroxybenzoic acid) shows a peak at m/z 1986 due to the $[M+H]^+$ ion.

For the most part, the ^{1}H- and ^{13}C-NMR spectra of compound **3** in DMSO-d_6[7] display a plenty of featureless or non attributable signals. Nevertheless, the combined use of various 1D, 2D and NOE techniques allowed the assignment of fundamental diagnostic resonances which will be reported in full detail and discussed elsewhere. The main evidence that the CD and porphyrin subunits are covalently linked stems from the following observations: (i) A broad ^{1}H resonance at 5.67 ppm, which does not show any ^{13}C direct correlation in the HMQC[8] (^{1}H detected Heteronuclear Multiple Quantum Coherence) spectrum may be attributed to the amino proton NH. (ii) The COSY spectrum (reported in Figure 1) reveals that the NH signal correlates with a resonance at 3.62 ppm, hidden under a broad featureless signal. In turn, this signal correlates with an isolated one at 4.01 ppm. These two signals, which show a HMQC correlation with a single ^{13}C resonance, are attributed to the diasterotopic protons of the methylene group (in the cyclodextrin-porphyrin junction), referred to as H-6 and H-6' respectively. The absence of a significant correlation between the signals of NH and H-6' indicates that in the most stable rotamer the amino hydrogen is antiperiplanar to the H-6 methylenic proton.

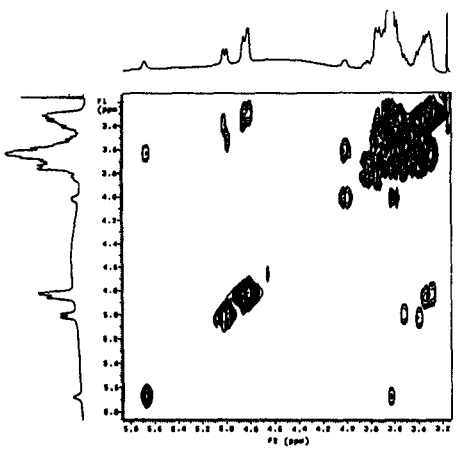


Figure 1: ^{1}H-^{1}H COSY spectrum of **3** in DMSO-d_6

(iii) NOE experiments confirmed this hypothesis. The adduct **3** has a molecular weight of 1985 Daltons, which puts the system in the negative NOE region. In order to control spin diffusion, TOE (truncated driven NOE)[9] experiments gave the cross relaxation rates σ_{IS} (between the investigated I and the saturated S protons).[10] The perturbation of the amino proton reveals a significantly large through-space dipolar interaction with the proton H-6' (σ_{IS} = -0.19) and a smaller one with the geminal H-6 proton (σ_{IS} = -0.08). (iv) Other important dipolar interactions have been measured for the amino and methylenic resonance upon perturbation of the *ortho* protons of the aniline ring. For NH, σ_{IS} = -0.77, and for H-6 and H6', σ_{IS} = -0.11 and -0.12 respectively indicate that the NH proton lies in the plane of aniline ring.

Unbound cyclodextrins and water soluble porphyrins may form exceptionally stable inclusion compounds in aqueous solution, as we recently reported[11]. Not surprisingly compound **3** gives rise to aggregated species in

water. The spectrophotometric analysis of solutions of 3 in 0.1 M ammonium carbonate (pH=8.5) at 410 nm (Soret band) showed that the Beer's law was obeyed up to *ca.* 1 x 10^{-6} M; the marked deviations from the expected linear curve observed for more concentrated solutions were taken as diagnostic of the onset of self-aggregation of 3. Quantitative analysis[12] of such deviations allowed to evaluate a dimerization constant of about 3.2 x 10^4 M^{-1}. Further evidence of the self-assembly of 3 were provided by a variety of techniques such as circular dichroism, fluorescence lifetime and electrospray mass spectrometry: (1) Figure 2 shows the UV-Vis and circular dichroism spectra of 3 in aqueous solution (ammonium carbonate 0.1 M, pH=8.5) in the Soret band region.

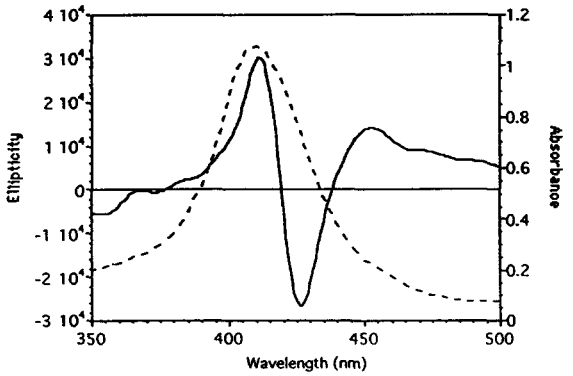
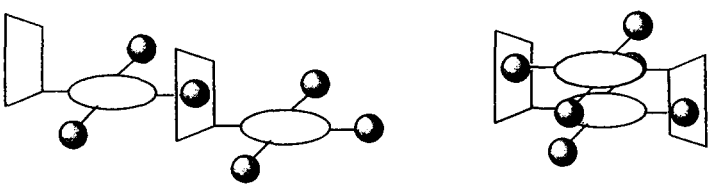


Figure 2: Circular dichroism (continuous line), and UV-Vis (dotted line) spectra of 3 (5.1 x 10^{-5} M in 0.1 M ammonium carbonate, pH=8.5)

The strong signal of induced circular dichroism (ICD) shows split Cotton effects centered at 419 nm consistent with a chiral exciton-coupling between the Soret band transitions of two or more porphyrin chromophores spatially correlated.

In Figure 3(a) the probable structure for a dimer of 3 that justifies the circular dichroism behavior observed is schematically depicted. As will be reported elsewhere, a more detailed analysis of both UV-Vis and ICD data allows to establish that also a face-to-face dimer (Figure 3(b)) is present in solution, albeit in a small amount.[13] Quite telling, in DMSO where the hydrophobic aggregation is unfavorable, the ICD signal disappears and a sharpening of the Soiret band results.



(a) (b)

Figure 3

(2) The fluorescence emission spectrum of compound 3 (ammonium carbonate 0.1 M, pH=8.5, excitation at λ=420 nm) presents a strong band at 660 nm and two weaker signals at 612 and 707 nm. The fluorescence dynamical parameters of 3 have been measured in different experimental conditions using a phase-modulation system.[15] In methanol, where 3 exists primarily as a monomeric species, the fluorescence decay is

monoexponential with a lifetime of 8.14 ns. In ammonium carbonate solution [0.1 M, pH=8.5] a biexponential behavior in observed for compound 3: the major (0.83 ± 0.23 ns, relative amplitude 74.6 %) component is assigned to aggregated species of 3, and a minor one (5.22 ± 0.36 ns, relative amplitude 25.4 %) to the monomeric form. The electrospray spectrum of 3 in methanol (5 x 10^{-5} M) shows two signals at m/z 1003 [M^{3-}] and 661 [$(M+Na)^{2-}$] for the monomeric species and a less intense signal at m/z 798 corresponding to a dimeric species [$(M_2+Na)^{5-}$]. Unfortunately, no meaningful electrospray mass spectra could be obtained so far in aqueous solution.

In conclusion, the present paper focuses on the synthesis and spectroscopic characterization of a new cyclodextrin-porphyrin adduct which displays a remarkable tendency to form supramolecular aggregates in aqueous solution. Further structural features and the dynamic behavior of such aggregates as well as the applications of 3 in catalysis and as an optical sensor are currently under investigation in these laboratories.

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14. See for example: Kalyanasundaram, K.; Spallart, M.N, *J. Phys. Chem.*, **1982**, *86*, 5163.
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