# Synthesis, Characterization, and Supramolecular Properties of a Hydrophilic Porphyrin−*β*-Cyclodextrin Conjugate

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*Received July 14, 2000*

Reductive amination of 6-deoxy-6-formyl-*β*-cyclodextrin with 5-(*p*-aminophenyl)-10,15,20-tris(*p*-sulfonatophenyl)porphyrin in the presence of an excess of sodium cyanoborohydride affords the hydrophilic cyclodextrin−porphyrin conjugate **3** in 23% yield. The structure of **3** was confirmed by NMR spectroscopy and mass spectrometry techniques. Compound **3** showed a marked tendency to dimerize in aqueous conditions via the formation of intermolecular porphyrin−cyclodextrin inclusion complexes and/or through electrostatic interactions. Information on the structure of these aggregates has been obtained by the use of circular dichroism and UV−vis spectroscopy. Aggregation can be avoided by the use of heptakis(2,3,6-tri-*O*-methyl)-*β*-cyclodextrin (TM*β*CD) that forms a 1:1 inclusion complex with compound **3**.

## Introduction

Porphyrins and porphyrin-like species participate in a wide diversity of biochemical functions including dioxygen transport and activation and electron- and energy-transfer.[1] In the attempt to unravel the mechanisms behind these biological processes, biomimetic systems, made of a porphyrin moiety covalently attached to a recognition element that mimics the hydrophobic pocket of natural enzymes, have been investigated. Cyclodextrins[2] have been extensively employed for this purpose, as they possess a well defined, hydrophobic, chiral cavity and since well-determined methods for their selective chemical modification are now available.[3]

Among the most successful results in this field is a noteworthy example that was recently reported by Breslow[4] describing an artificial cytochrome P-450 enzyme which features manganese(III) porphyrins covalently linked to the primary hydroxyl side of four cyclodextrin rings. A substrate, such as an olefin or a steroid bearing two hydrophobic ends, can bind to two cyclodextrin moieties in a rather rigid conformation and undergoes a regioselective oxidation with a good catalytic turnover.

To fully exploit the interactions between host and hydrophobic substrates in enzyme models, it is of paramount importance to employ species that are soluble in aqueous media, since other solvents are much less suitable for the type of host−guest interactions that are sought for. In the Breslow model, as well as in other systems reported in the literature,[5] water solubility is provided by the cyclodextrin rings that act as hydrophilic pendants. However, the synthesis of adducts with more than one cyclodextrin unit is not necessarily the only available option. We have recently reported[6] an alternative strategy based on the use of hydrophilic porphyrins carrying ionic groups as *meso* substituents. In particular, the attachment of a trisulfonatophenyl porphyrin moiety to the primary face of *β*-cyclodextrin leads to a compound which is remarkably soluble in aqueous media. Here we report details of the synthesis and characterization of this covalent adduct.

Unbound cyclodextrins and water soluble anionic porphyrins may form exceptionally stable supramolecular species in aqueous solution.[7,8] Not surprisingly, the porphyrin−cyclodextrin-conjugated **3** described here gives rise to aggregate species in water. This paper reports spectroscopic evidence of such aggregation and gives reasonable hypotheses concerning the morphology of the supramolecular complexes formed in solution. Finally, we will indicate a route to avoid self-aggregation phenomena based on the use of unbound permethylated cyclodextrin

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10.1021/jo0010678 CCC: $19.00 
Published on Web 11/30/2000

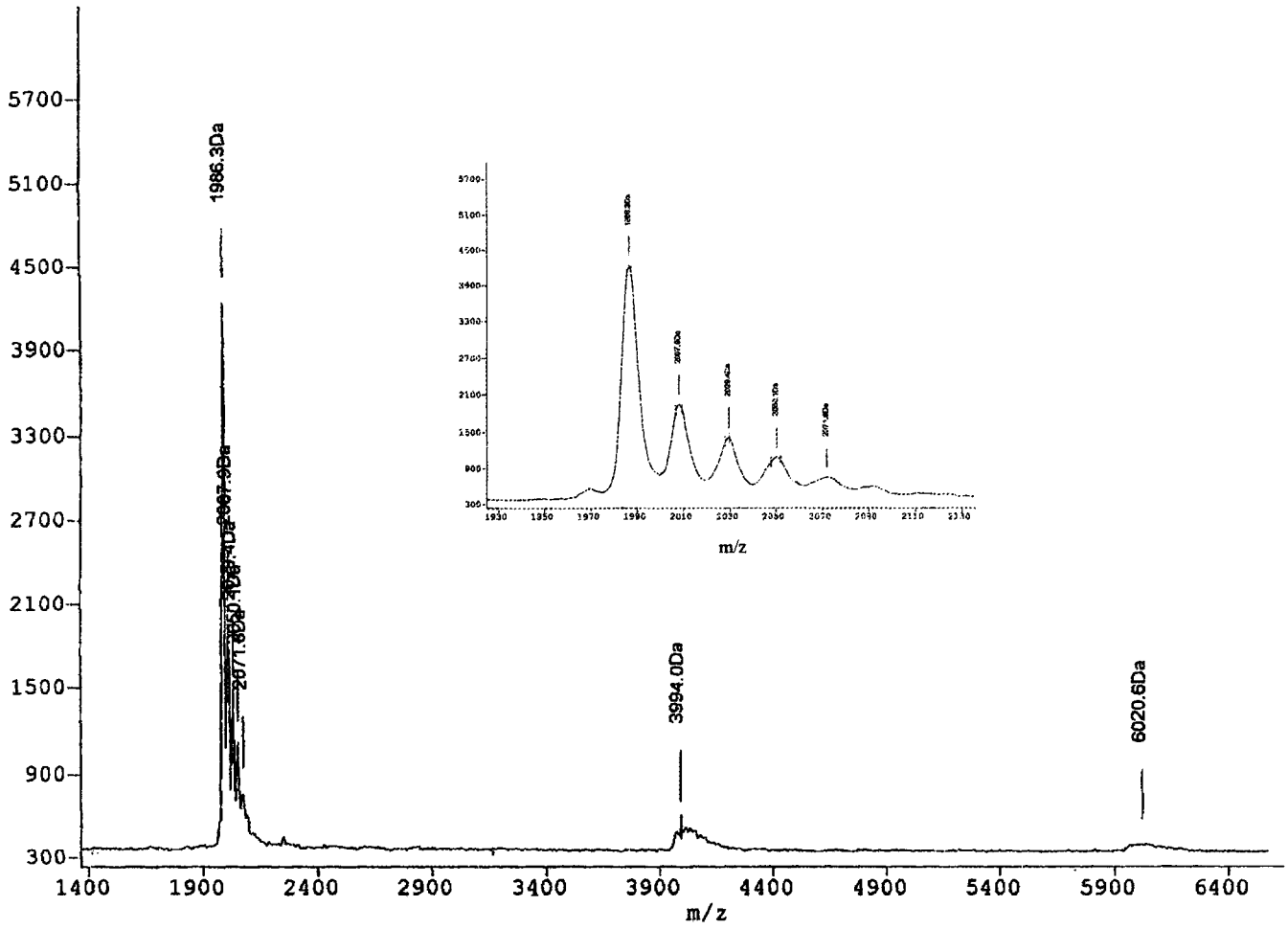
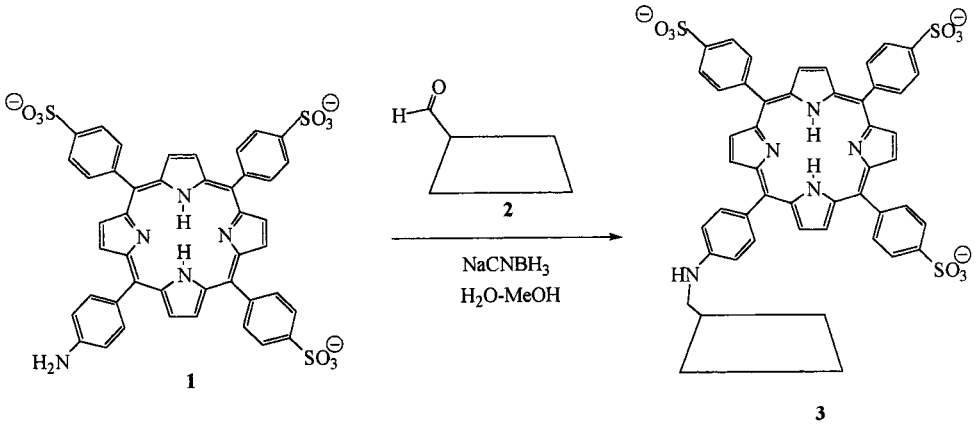



**Figure 1.** MALDI-TOF spectrum of compound **3** in 2,5-dihydroxybenzoic acid matrix. Inset: details of peak at $m/z$ = 1986 Da.

**Scheme 1. Synthesis of Compound 3**




1 2 3

which effectively interacts with **3** and keeps it in a pseudo-monomeric form.

## Results and Discussion

**Synthesis.** The synthesis of the porphyrin−cyclodextrin covalent adduct **3** was accomplished by the route outlined in Scheme 1, whereas the preparation of 5-(*p*-aminophenyl)-10,15,20-tris(*p*-sulfonatophenyl)porphyrin, **1**,[9] and 6-deoxy-6-formyl-*β*-cyclodextrin, **2**,[10] was based upon literature procedures.

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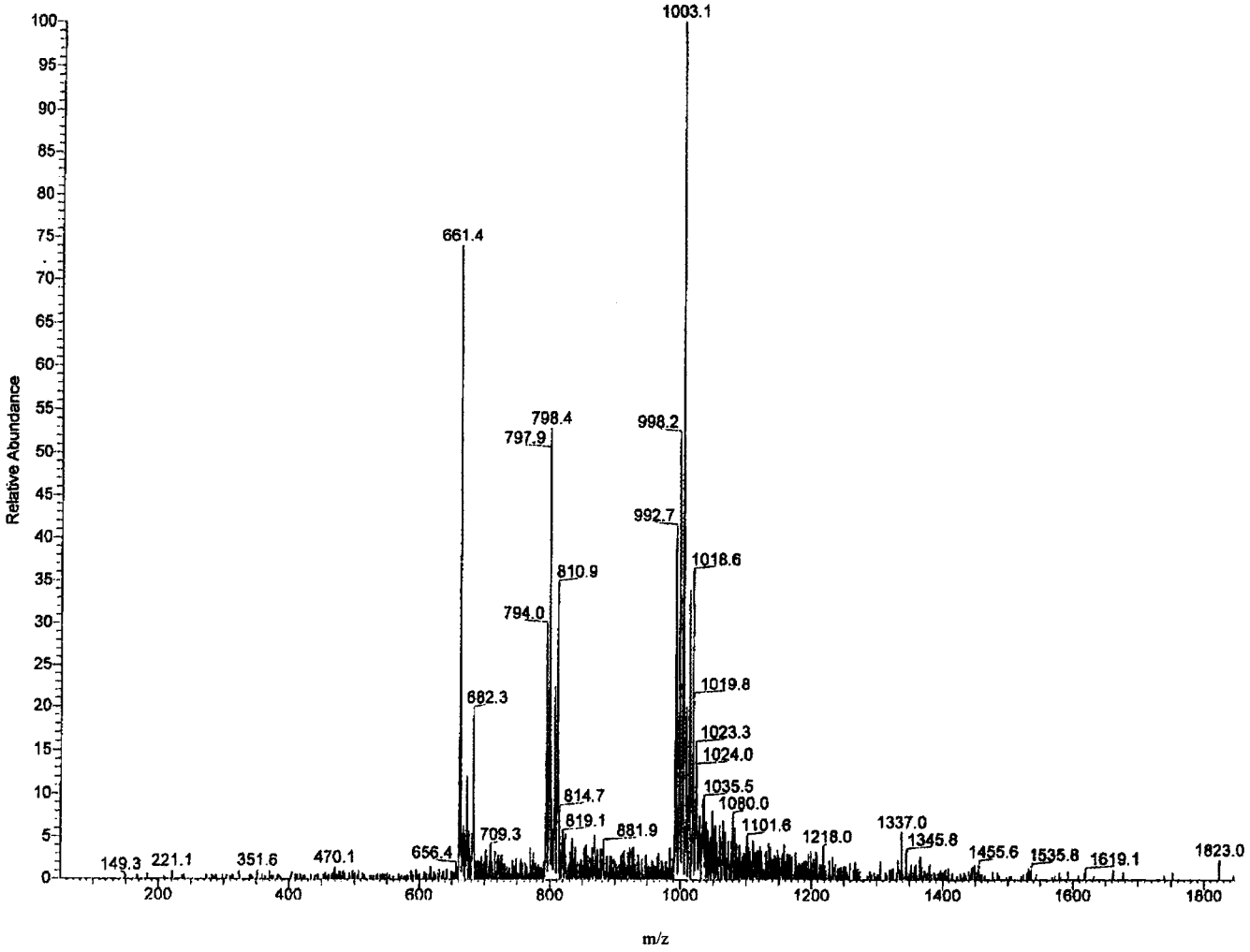


**Figure 2.** Negative ESI-MS spectrum of compound **3** ($5.0 \times 10^{-5}$ M) in methanol.

The key step in the synthesis of compound **3** is the formation of an amino-linkage through the reductive amination of **2** with **1** in the presence of sodium cyanoborohydride in aqueous methanol (apparent pH = 6). The reaction was very slow (one week, at room temperature). After the workup of the reaction mixture (see Experimental Section), product **3** was obtained in pure form by means of preparative TLC (23% yield).

**Definition of the Structure of Compound 3**

**Mass-Spectrometry.** The identity of compound **3** was confirmed by matrix-assisted laser desorption ionization/time-of-flight mass-spectrometry (MALDI-TOF) in a matrix of 2,5-dihydroxybenzoic (Figure 1).

The major peak at *m*/*z* of 1986 Daltons corresponds to the expected quasi-molecular $[\mathbf{3} + H]^+$ ion. The inset of Figure 1 is an expanded view of the peak, showing the presence of exogenous sodium cations among the counterions.

Compound **3** has been also analyzed by electrospray ionization mass spectrometry (ESI-MS). Figure 2 reports the negative ESI mass spectrum of compound **3** in methanol.

The two signals at *m*/*z* 1003 and *m*/*z* 661 have been assigned to the $[\mathbf{3} + Na]^{2-}$ and $[\mathbf{3}]^{3-}$ ions, respectively. The weaker signal at *m*/*z* 798 can be ascribed to a dimer of formula $[\{\mathbf{3}\}_2 + Na]^{5-}$, thus confirming the expected propensity of **3** to form aggregates. The observation of dimers of **3** in methanol is rather surprising since this kind of solvent normally hinders the formation of inclusion compounds. On the other hand, there is a dispute in the literature about the effectiveness of ESI-MS to distinguish between genuine inclusion complexes and electrostatic aggregates[11] formed during the electrospray process.

The ESI-MS spectrum of **3** has been also recorded in 0.1 M aqueous ammonium carbonate (pH = 8.5). Unfortunately, the spectrum collected in this medium is not very informative due to a low signal-to-noise ratio. At any rate, a detailed view of the peak at *m*/*z* 992, shown in Figure 3, indicates the presence of a $[\mathbf{3} + H]^{2-}$ ion as confirmed by the agreement between the observed and the simulated isotopic distribution calculated for $[\mathbf{3} + H]^{2-}$ ion (reported as inset in Figure 3).

**NMR Spectroscopy.** The $^1H$ NMR spectrum of compound **3** in $D_2O$ shows a severe broadening of signals as a consequence of aggregation phenomena (vide infra). Since polar solvents such as DMSO and DMF cause disruption of cyclodextrin inclusion complexes,[2] DMSO-$d_6$ has been the solvent of choice for our NMR studies. In the $^1H$ spectrum (Figure 4), besides a series of sharp signals which may be attributed to compound **3**, other broad signals are observed at 7.27 and 6.67 ppm and in

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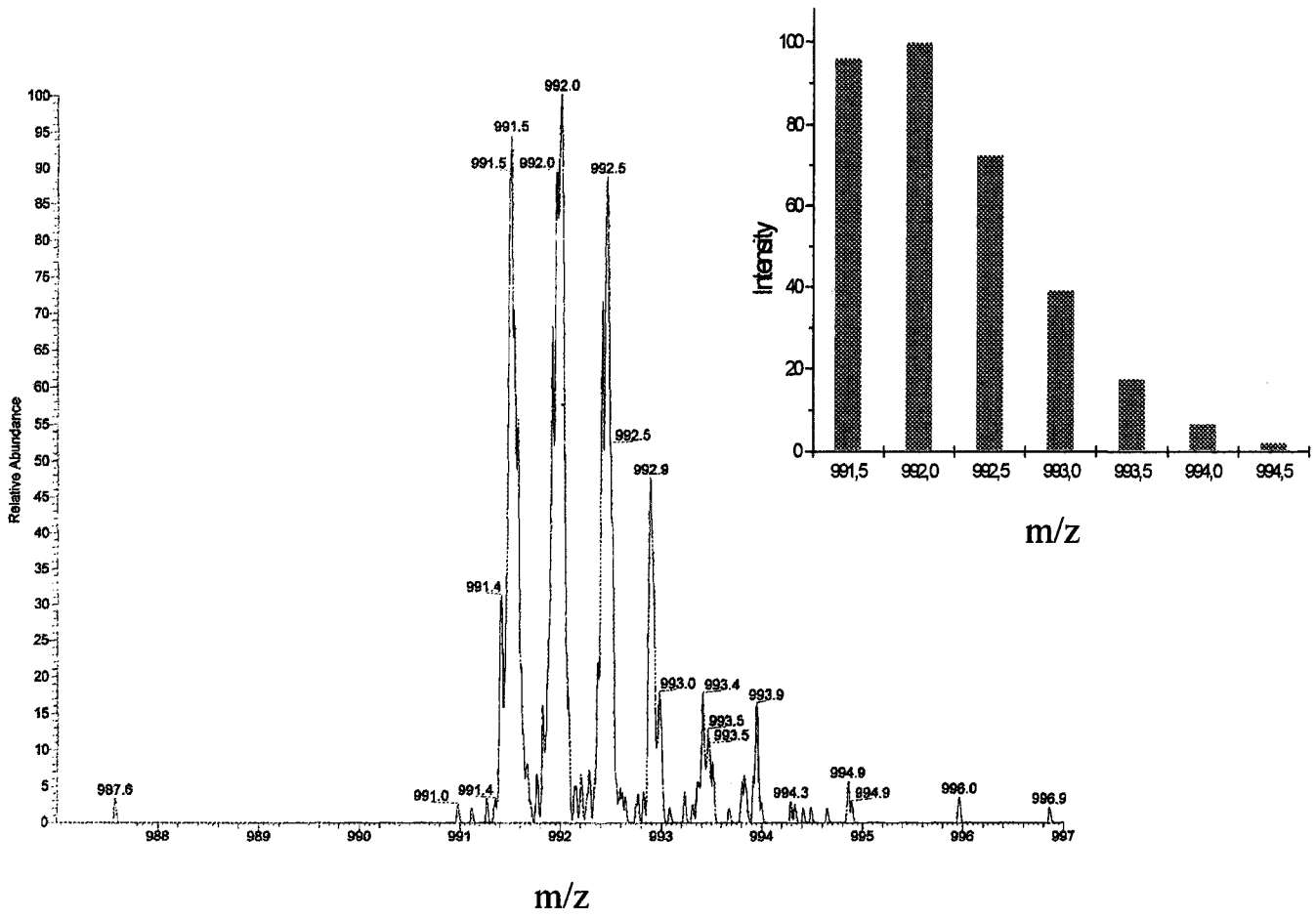


**Figure 3.** Negative ESI-MS spectrum of compound **3** ($5.0 \times 10^{-5}$ M) in 0.1 M ammonium carbonate, pH = 8.5. Inset: simulated isotopic distribution for $[\mathbf{3} + H]^{2-}$ anion.

the 6.2−3.9 ppm interval. The NMR experiments described below did not show any scalar or dipolar interaction between the two series of signals, which therefore indicate distinct and noninteracting systems. We will not attempt to define the system described by the series of broad signals belonging to the glucose units (all magnetically different because of the cyclodextrin ring monosubstitution), and we will focus on the series of sharp signals, to establish the presence of a covalent bond between the CD and the porphyrin subunit of **3**.

The assignments of some $^1$H resonances utilizes the numbering layout shown in Scheme 2a.

For the most part, the $^1$H spectrum of **3** is characterized by a complicated and featureless system of signals. However, it is possible to distinguish a *cyclodextrin region* (6−3 ppm range) and a *porphyrin region* (9−7 ppm range) and assign the signal resonating at −2.86 ppm to the pyrrolic protons. Notwithstanding the presence of broad interfering signals, the correct integration validates both the 1:1 stoichiometry of compound **3** and its purity. The combined utilization of the COSY 2D[12] technique and of NOE experiments allowed the recognition of diagnostic resonances and the determination of significant scalar or vectorial (dipolar) interactions between them. The high molecular weight of **3** (2036 Da), and the fact that the NOE measurements have been taken using the viscous DMSO solvent, confines the system in the negative NOE region. To avoid spin diffusion phenomena, experiments have been run with the truncate driven NOE (TOE)[13a] modality. The cross relaxation rates, $\sigma_{IS}$ (see Experimental Section), which are relevant for the present discussion are reported in Table 1.

In the $^1$H spectrum of **3**, the set of two multiplets at 8.17 and 8.04 ppm and the set of two pseudo-doublets at 7.92 and 7.12 ppm, which are in the 3:1 integral ratio, can be attributed to the three benzenesulfonic aromatic rings and to the aniline aromatic ring, respectively. From inspection of Table 1, the signal at 7.12 ppm gives a strong dipolar interaction with a broad resonance at 5.68 ppm, while the signal at 7.92 ppm gives a smaller interaction with the same resonance. This resonance can only be attributed to the NH aniline proton. Conversely, the resonances at 7.12 and 7.92 ppm can be assigned to the *ortho* ($H_8$ and $H_{12}$ in Scheme 2a) and *meta* ($H_9$ and $H_{11}$) aromatic aniline protons, respectively.

The COSY spectrum (Supporting Information, Figure A1) reveals that the NH signal correlates scalarly with a resonance at 3.62 ppm, hidden under a broad featureless signal. In turn, this resonance correlates with an isolated signal at 4.01 ppm. It is safe to attribute these latter signals to the diastereotopic protons $H_{6a}$ and $H_{6b}$ (in the order) of the adjacent methylenic group. This fact definitely confirms that the two subunits (CD and porphyrin) are covalently linked. The fact that any scalar

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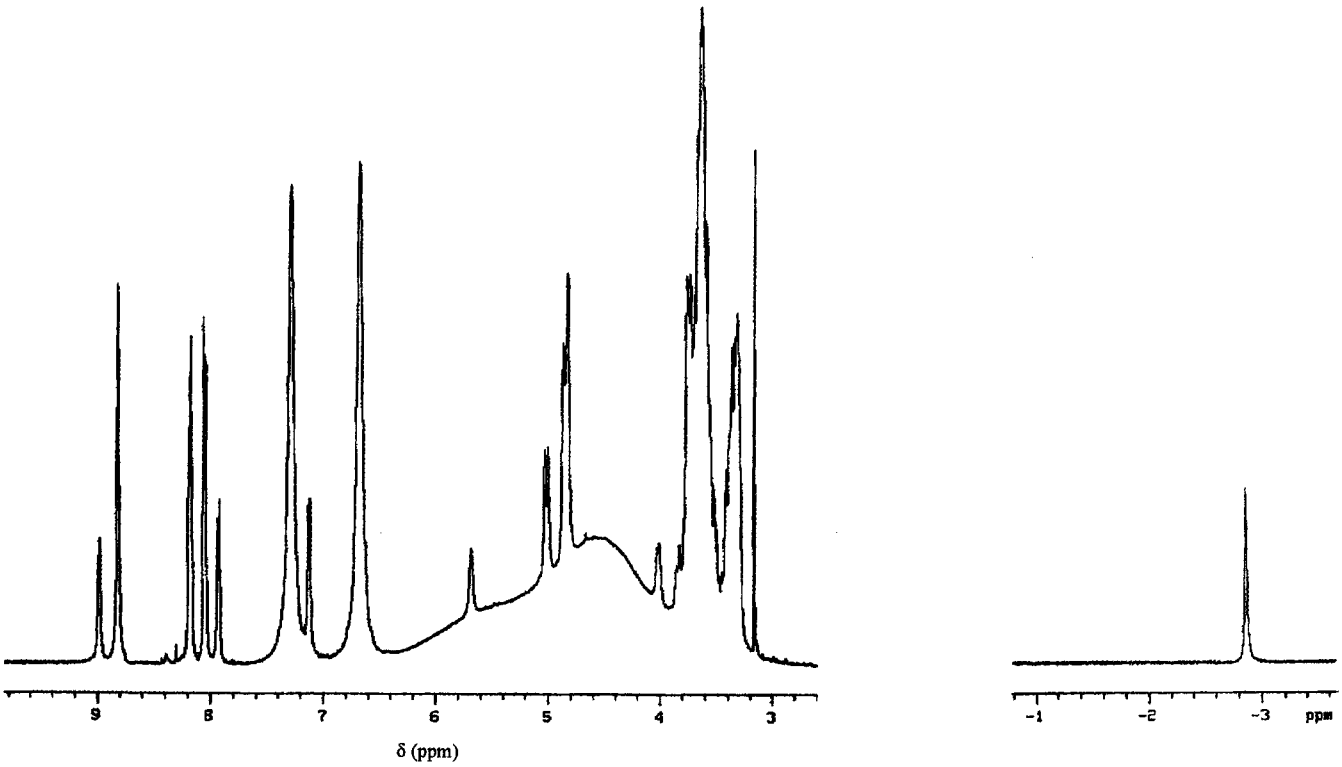
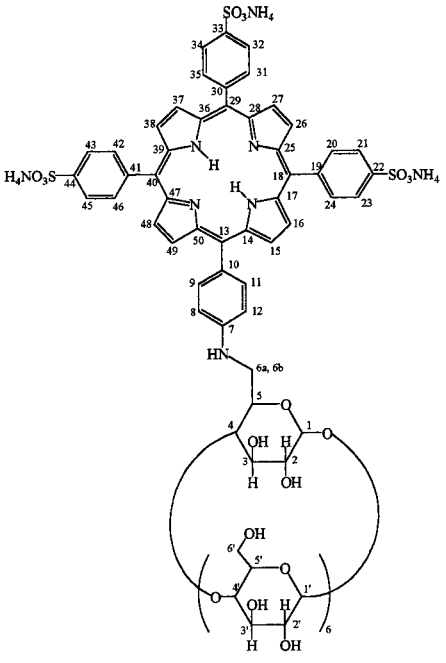
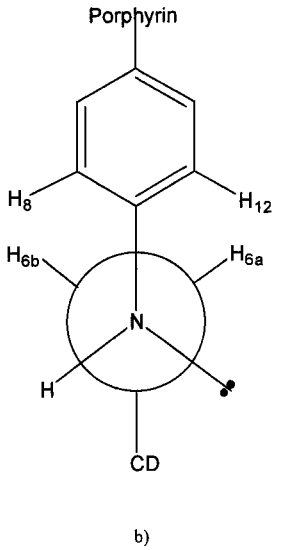



**Figure 4.** $^1$H NMR spectrum of compound **3** in DMSO-$d_6$.

**Scheme 2. (a) Labeling Used for Discussion of NMR Data. (b) Labeling Used for Discussion of NOE Data**




correlation is found between the NH signal and the $H_{6b}$ signal at 4.01 ppm suggests that in the most stable (or unique) rotameric conformation of **3** the two protons are in a gauche reciprocal orientation, while the $H_{6a}$ proton is trans to the NH proton. This hypothesis is confirmed by the inspection of the other dipolar interactions reported in Table 1. The interaction upon saturation of the NH resonance is greater with the gauche $H_{6b}$ proton than with the trans $H_{6a}$ proton. Finally, the interactions of the aniline *ortho* protons $H_8$ and $H_{12}$ with the methylenic protons $H_{6a}$ and $H_{6b}$ are similar. Thus the two protons are both gauche-oriented relative to the aniline ring, and the CD and the porphyrin subunits are, quite reasonably, in the trans reciprocal orientation (Scheme 2b).

**Aggregation Properties of 3.** Cyclodextrins and their O-methylated derivatives can form exceptionally stable inclusion complexes with water-soluble porphyrins in aqueous solution.[7,8] The formation of stable inclusion

**Scheme 3. Types of Aggregation for Compound 3. (a) Anti-dimer, (b) Syn-dimer, (c) Face-to-face Dimer, (d) Electrostatic Dimer**

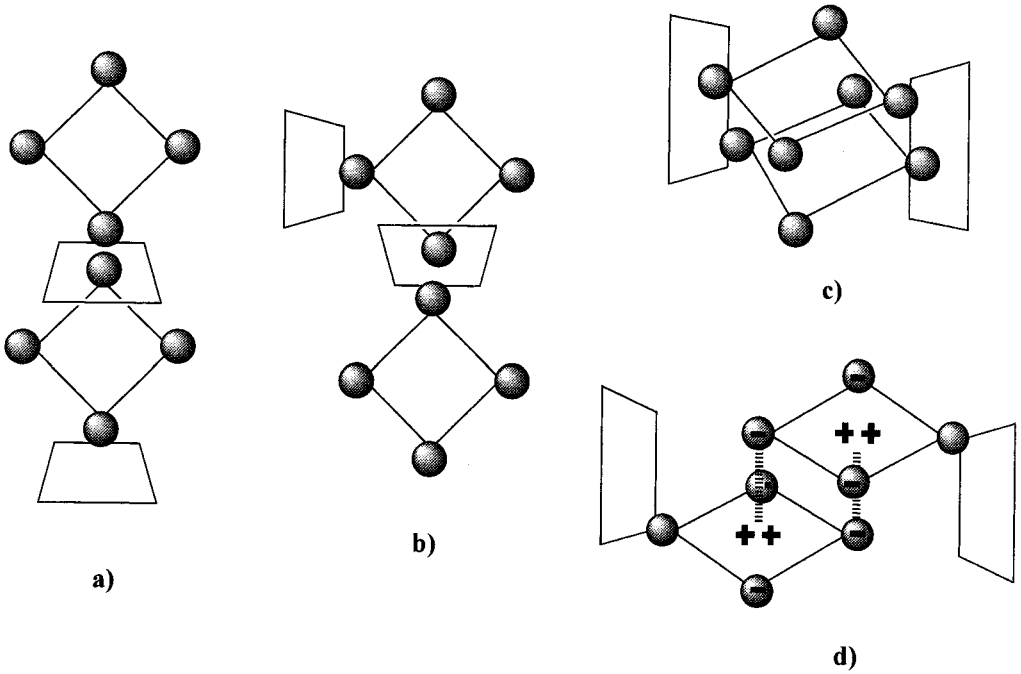



**Table 1. Rates for $\delta_{IS}$ Cross Relaxation between Protons in the Neighborhood of the Cyclodextrin−Porphyrin Junction**

| saturated | | observed ($\sigma_{IS}$) values | | | | |
|---|---|---|---|---|---|---|
| H | $\delta$ | $H_{9,11}$ | $H_{8,12}$ | NH | $H_{6b}$ | $H_{6a}$[a] |
| $H_{9,11}$ | 7.92 | − | −0.69 | −0.07 | − | − |
| $H_{8,12}$ | 7.12 | −0.79 | - | −0.77 | −0.12 | −0.11 |
| NH | 5.68 | −0.07 | −0.48 | - | −0.19 | −0.08 |
| $H_{6a}$ | 4.02 | − | −0.06 | −0.19 | − | −0.22 |
| $H_{6b}$ | 3.75 | − | −0.37 | −0.35[b] | −0.60 | − |

[a] This resonance is hidden under other signals. The evaluation of the intensity loss with the differential procedure is subjected to more relevant errors. [b] The intensity loss may arise from the saturation of other protons isochronous with $H_{6a}$.

compounds seems to be confined to aryl-porphyrins carrying anionic substituents in the *para*-position. The need of aryl substituents can be easily explained assuming that their hydrophobicity is the driving force for the noncovalent interaction with the cyclodextrin cavity. On the other hand, the role of a negative charge on the periphery of the porphyrin macrocycle, as suggested by Kano,[14] is ascribed to a large dipole moment of the cyclodextrin cavity pointing along the symmetry $C_n$ axis ($n$ = number of glucose units in the cyclodextrin considered), the secondary hydroxyls side being at the negative end of the dipole. This polarization favors the inclusion of anionic porphyrins as the aryl group enters in the cavity from the secondary side (negative pole of the cavity dipole) and the anionic sulfonate group protrudes from the primary side (positive pole of the cavity dipole) thus favoring formation of the inclusion complex. On the other hand, for a porphyrin carrying cationic substituents, a charge matching would be possible only if the aryl group enters in the cavity from the primary side. However, since the primary side is narrower than the secondary one, the hydrophobic interaction is decreased thus thwarting the electrostatic advantage.

Quite relevant, water-soluble porphyrins, in particular those carrying negative charges as the porphyrin moiety in **3**, have a marked propensity to self-aggregate in aqueous media, especially under acidic conditions.[15] In fact, at pH lower than 4 they have a zwitterionic nature (the pyrrolic nitrogen centers of the macrocycle are protonated whereas the anionic groups are still deprotonated), and the porphyrin−porphyrin aggregation is due to electrostatic interactions. On the other hand, the weak aggregation generally found in neutral or alkaline conditions is likely to be due to $\pi-\pi$ stacking of porphyrin rings.

In the case of compound **3**, assuming the above factors at play, there are several possible modes of supramolecular intra- or intermolecular interactions (Scheme 3).

The possibility of an intramolecular inclusion of the porphyrin (not reported in Scheme 3) has been discounted from inspection of CPK models, which indicate that the linking between the two units is not flexible enough to allow self-inclusion. The formation of dimeric aggregates by intermolecular inclusion of two molecules of **3** is instead quite likely, and Scheme 3 depicts the possible arrangements. An arylsulfonic group of a molecule may penetrate the cyclodextrin cavity of another molecule of **3** through the secondary hydroxyl side and, as a result, one can predict an anti (Scheme 3a) or a syn (Scheme 3b) arrangement. It is also possible to envisage the formation of a dimer in which the two porphyrins enter in the cyclodextrin cavity through the primary face of the saccharide (Scheme 3c). Finally, electrostatic dimers, like that depicted in Scheme 3d, can be formed in acidic conditions when the porphyrins are present as zwitterions.

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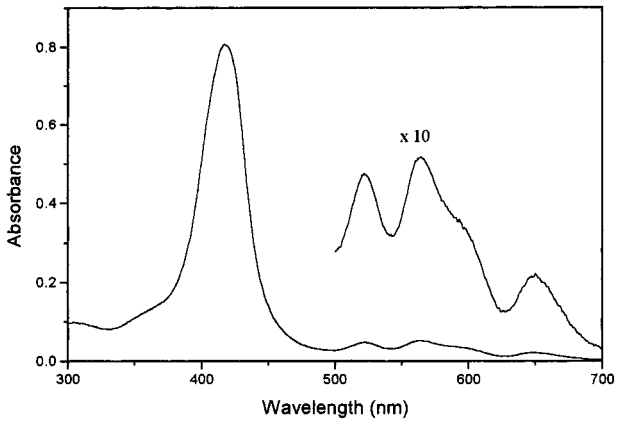


**Figure 5.** UV−vis spectrum of compound **3** ($4.24 \times 10^{-6}$ M) in 0.1 M ammonium carbonate, pH = 8.5.

**Aggregation in Alkaline Conditions.** The aggregation properties of **3** have been first studied in alkaline solution (ammonium carbonate 0.1 M, pH = 8.5) in order to exclude the electrostatic type of aggregation, thus focusing on hydrophobic and, possibly, on $\pi-\pi$ interactions.

Porphyrin−porphyrin aggregation has been conveniently studied by UV−vis spectroscopy, taking advantage of some very intense absorption bands exhibited by these molecules in the spectral region between 380 and 700 nm. The UV−vis spectrum of **3** (4.2 $\mu$M) in 0.1 M ammonium carbonate (pH = 8.5), reported in Figure 5, shows a strong absorption band at 418 nm ($\epsilon$ = 190400, Soret band) and four weaker absorptions (Q-bands) at 523 ($\epsilon$ = 11085), 564 ($\epsilon$ = 13300), 600 ($\epsilon$ =7850), and 652 nm ($\epsilon$ =5710). Beer's law experiments were carried out monitoring the absorbance at 418 nm versus the concentration of **3** (Supporting Information, Figure A2). The marked deviations from the linearity observed at concentrations of **3** above $1 \times 10^{-6}$ M are diagnostic of the onset of self-aggregation phenomena. The absorbance data were analyzed in terms of a dimerization model using a fitting equation reported by Pasternack.[15b,c] A dimerization constant of $3.2 \times 10^4$ $M^{-1}$ was obtained. The same measurements carried out using porphyrin **1** gave a lower dimerization constant of $9.3 \times 10^3$ $M^{-1}$. Since it is likely that, in alkaline conditions, the compound **1** dimerizes only via $\pi-\pi$ stacking interactions, the higher tendency of **3** to dimerize can be ascribed to the occurrence of intermolecular hydrophobic interactions.

More direct evidence for the inclusion of the porphyrin within a cyclodextrin cavity came from some circular dichroism spectroscopy measurements.[16]

From the electronic interaction of the porphyrin with the asymmetric environment provided by the cyclodextrin cavity, one expects an induced circular dichroism (ICD) signal in the region corresponding to the Soret band of the porphyrin. Figure 6 shows the UV−vis and the circular dichroism spectra of compound **3** ($5.1 \times 10^{-6}$ M) in 0.1 M aqueous ammonium carbonate solution (pH = 8.5).

A very strong ICD signal showing split Cotton effects centered at 419 nm is indeed observed. This chiral exciton coupling phenomenon[18] can be explained, admitting that

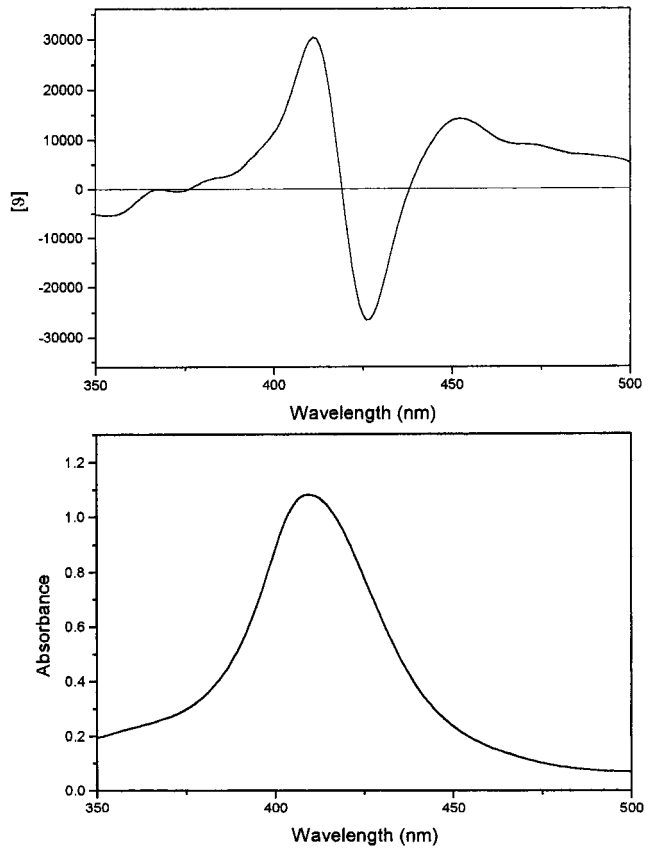


**Figure 6.** Circular dichroism spectrum (top) and UV−vis spectrum (bottom) of compound **3** ($5.1 \times 10^{-6}$ M) in 0.1 M ammonium carbonate, pH = 8.5.

the Soret transition of the porphyrin included into the cyclodextrin cavity is electronically perturbed by the Soret transition of another porphyrin in the neighborhood, like in Scheme 3, structures a and b. Quite telling, the ICD signal disappears in DMSO, because inclusion is inhibited. On the other hand, on the basis of the exciton coupling theory, the excitation of a parallel arrangement of dipoles, such as in the dimer shown in Scheme 3c, should lead to a blue shift of the Soret band with respect to the band of the monomer. Furthermore, the exciton type ICD is not expected. Thus, the above results apparently point to the presence in solution of a mixture of dimers having an "open" structure (Scheme 3a or Scheme 3b) rather than a "closed" structure (Scheme 3c).

**Aggregation in Acidic Conditions.** Figure 7 shows the absorption spectra and circular dichroism trace of **3** (7.0 $\mu$M) in acidic conditions (0.1 M sodium acetate buffer, pH = 3.5).

In acidic aqueous solution compound **3** forms a zwitterionic species in which all the nitrogens of the porphyrin inner core are protonated and the sulfonic functions are negatively charged. The UV−vis spectrum shows two absorption bands at 424 ($\epsilon$ = 88500) and 467 nm ($\epsilon$ = 85700). Comparison with literature data[19] of the similar

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(17) (a) Kobayashi, N.; Akiba, U.; Takatori, K.; Ueno, A.; Osa, T. *Heterocycles* **1982**, *19*, 2011. (b) Gonzalez, M. C.; Macintosh, A. R.; Bolton, J. R.; Weedon, A. C. *J. Chem. Soc., Chem. Commun.* **1984**, 1138. (c) Gonzalez, M. G.; Weedon, A. C. *Can. J. Chem.* **1985**, *63*, 602.

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(19) Akins, D. L.; Özçelik, S.; Zhu, H.-R.; Guo, C. *J. Phys. Chem.* **1996**, *100*, 14390−14396.

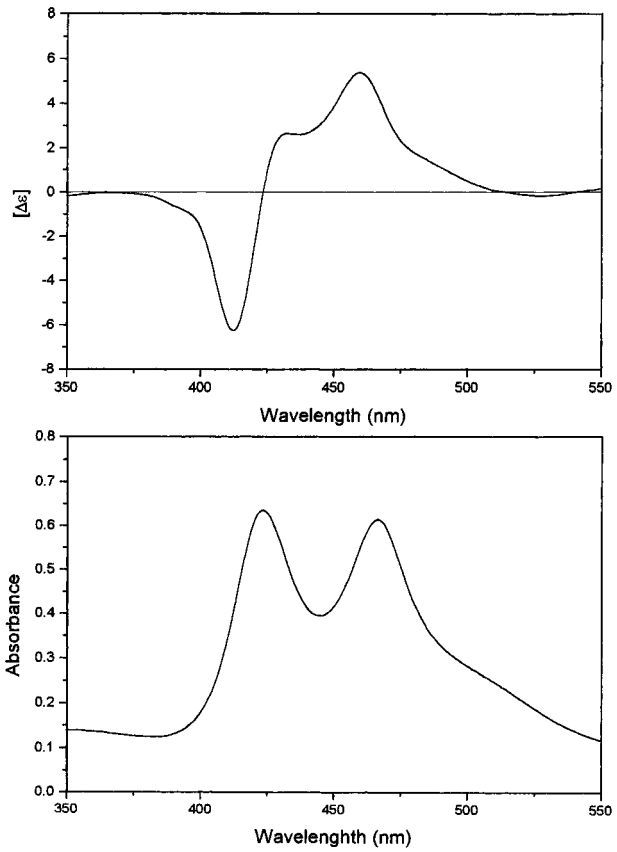



**Figure 7.** Circular dichroism spectrum (top) and UV−vis spectrum (bottom) of compound **3** ($7.0 \times 10^{-6}$ M) 0.1 M acetate buffer, pH = 3.5.

5,10,15,20-tetrakis(4-sulfonatophenyl-porphyrin), TPPS, allows assignment of the band at 424 nm to the acidic form of the porphyrin of **3** and the second one to the electrostatic aggregate of the acidic species. Strong ICD signals are associated with these absorptions. The shoulder near the crossover wavelength suggests an overlap between two induced pair bands associated to the two Soret bands observed in the UV−vis spectrum. The splitting of the 424 nm band into two ICD bands suggests that two protonated porphyrins are electronically coupled together in an arrangement similar to that already reported in alkaline conditions (Scheme 3a and Scheme 3b). On the other hand, the induced pair band around 447 nm may be due to the exciton coupling of a dimeric (or higher) electrostatic aggregate of the protonated porphyrin as depicted in Scheme 3d.

**Interaction of Compound 3 with TM*β*CD.** The self-assembly of supramolecular complexes of **3** via hydrophobic interactions is an appealing event. However, there are also some drawbacks associated with aggregation. The most important ones are the reduced availability of the cyclodextrin moiety as a molecular receptor and the alteration of the catalytic and photophysical properties of the porphyrin unit. These problems can eventually compromise the ability of **3** to perform the functions for which it was designed.

It is well-known[7,8] that *β*-cyclodextrin and its O-methylated derivatives are able to interfere with the self-aggregation of water-soluble porphyrins. In particular the binding strength increases in the order TM*β*CD ≫ DM*β*CD ≫ *β*CD. Therefore, we reasoned that TM*β*CD should be able to bind to the porphyrin moiety of **3** keeping it in a pseudo-monomeric form. This was indeed the case. Figure 8a shows both sharpening and a red-shift of the porphyrin Soret band the in the UV−vis spectrum of **3** ($3.0 \times 10^{-6}$ M in 0.1 M ammonium carbonate, pH = 8.5) upon addition of increasing amounts of a TM*β*CD solution.

The titration plot (Figure 8b), obtained by reporting the absorbance measured at 417 nm versus the number of equivalents of TM*β*CD added to the solution, clearly demonstrates the formation of a supramolecular complex between TM*β*CD and compound **3** having a 1:1 stoichiometry. Furthermore, the sharpness of the titration curve indicates that the microscopic binding constant is very large. The isosbestic point testifies the occurrence of a chemical equilibrium among two species. Due to the large values of the dimerization constant of **3** and of its inclusion in TM*β*CD, the presence in solution of compound **3** in its monomeric form can be ruled out, and it may be safely assumed that the two species in equilibrium are the dimer of **3** and its 1:1 supramolecular adduct with TM*β*CD.

For the above adduct there are two possible isomers having geometry anti and syn, as reported in Scheme 4a and Scheme 4b, respectively.

The presence of an isomeric mixture could also explain the spectroscopic shape of the Soret band, which is not sharp, as expected, but shows a shoulder around 425 nm.

## Conclusions

The condensation of *β*-cyclodextrin with a hydrophilic porphyrin produces an adduct which is remarkably soluble in aqueous media. This species shows a marked propensity to form supramolecular dimers (or higher aggregates), whose nature depends on the pH of the solution. In neutral or basic conditions hydrophobic interactions are the driving force for the formation of dimers. On the other hand, in acidic conditions where the porphyrin moiety assumes a zwitterionic form, aggregation takes place also because of electrostatic interactions. A pseudo-monomeric form of **3** can be obtained in the presence of TM*β*CD that forms a very stable inclusion complex with **3**:TM*β*CD 1:1 stoichiometry. We are currently investigating functions and applications of **3** such as the photoelectron transfer from the porphyrin moiety to a substrate included into the cyclodextrin cavity, and the benefits of the cyclodextrin residue in the production of porphyrin doped sol−gels. Results of these studies will be reported in due course.

## Experimental Section

**General Methods**. Chemicals were purchased from Aldrich and used as received. Solvents (HPLC grade) have been used without any purification. The compounds **1**[10] and **2**[9] were prepared as described previously in the literature. Analytical thin-layer chromatography (TLC) was carried out on glass sheets coated with silica gel (Merck F-254) using ethyl acetate/2-propanol/25% ammonium hydroxide/water (7/7/5/4 volume) as eluent. The spray agent used for spot detection was a mixture composed as follows: *p*-anisaldehyde (9.2 mL)/ethanol-(338 mL)/acetic acid (3.75 mL)/sulfuric acid(12.5 mL). The plate was heated to 180 °C for 15 min to visualize the spots. Preparative thin-layer chromatography was performed on glass sheets (1 mm thickness, Merck) using the same eluent as analytical TLC. $^1$H NMR spectra were recorded at 250 MHz. COSY[12] and TOE[13] experiments were performed at 400 MHz as follows: for every saturated nucleus (S), six experiments

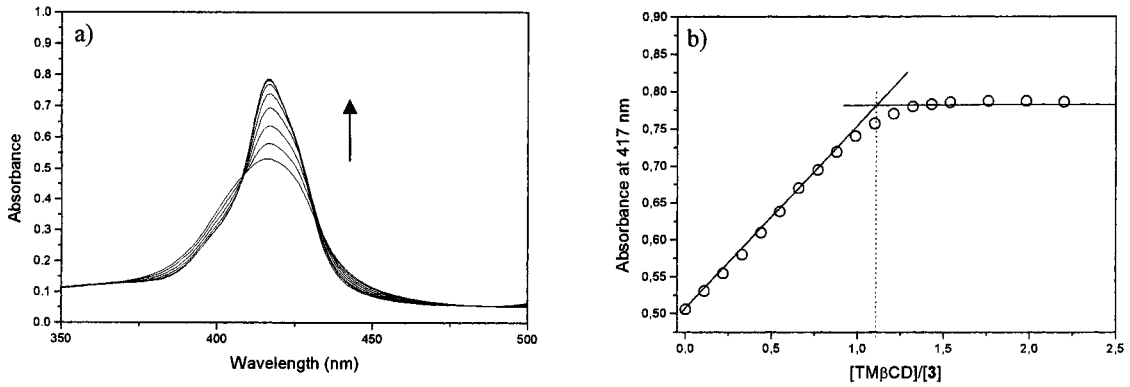
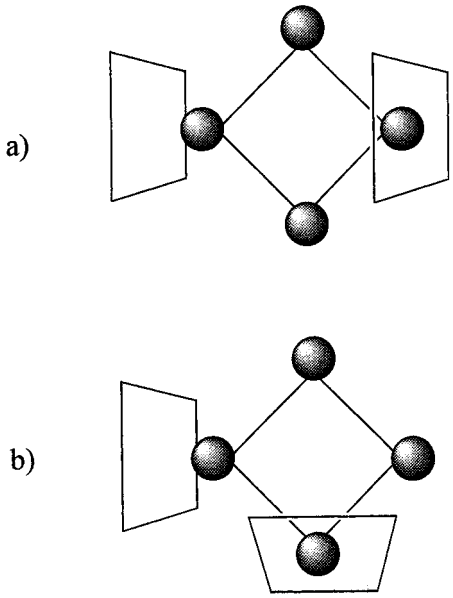



**Figure 8.** (a) evolution of UV−vis spectrum of compound **3** ($3.0 \times 10^{-6}$ M in 0.1 M ammonium carbonate, pH = 8.5) upon addition of TM$\beta$CD (from 0 to 2.2 equivalents). (b) Titration plot at 417 nm.

**Scheme 4. Complexes between Compound 3 and TM$\beta$CD: (a) Anti-complex, (b) Syn-complex**




were performed, with saturation times of 0.1, 0.2, 0.3, 0.4, 0.6, and 0.8 s. The intensity losses of the investigated nucleus (I) were measured in the differential mode, by calculating the coefficient which makes the signal of the perturbed spectrum to exactly match the corresponding signal of the control unperturbed. The unitary coefficients, $f_{I\{S\}}$, correlate with the saturation times, $t$, following the relationship:[13b]

$$f_{I\{S\}} = (\sigma_{IS}/\rho_{IS})(1 + \exp(-\rho_{IS}t))$$

The fitting into this equation gave the $\sigma_{IS}$ cross relaxation rates, related to the internuclear distance between I and S. All chemical shifts were quoted in ppm using the solvent as an internal standard. Circular dichroism spectra were recorded using the following parameters: sensitivity = 5 mdeg; resolution = 1 nm; band amplitude = 2 nm; response time = 2 s; scan rate = 20 nm/min; scan number = 4. MALDI analysis was performed in a 2,5-dihydroxybenzoic acid matrix. The equipment utilized for the MALDI-TOF experiments reported here did not allow the determination of the exact mass of compound **3**. ESI-MS measurements were carried out using the following parameters: spray voltage = −4 kV; capillary voltage = −10 V; capillary temperature = 180 °C; nebulizing gas = nitrogen (40 units flow rate).

**Synthesis of Compound 3**. To a solution of cyclodextrin derivative **1** (0.560 g, 0.494 mmol) and porphyrin compound **2** (0.307 g, 0.332 mmol) in aqueous methanol (60%, 33 mL) was added $NaCNBH_3$ (100 mg, 1.59 mmol). The mixture was stirred at room temperature for 7 days. The pH of the solution was adjusted every 24 h to 6.0 with 0.01 M sodium hydroxide solution, and a portion of $NaCNBH_3$ (20 mg, 0.32 mmol) was added. The pH was lowered to 3 with 1 M HCl solution, to destroy the excess of $NaCNBH_3$, and the solution was neutralized with 1 M NaOH solution and then poured into acetone (200 mL), resulting in a dark-red crude solid. That solid was recovered by filtration and subjected to exhaustive washing with acetone to remove the unreacted porphyrin **2**. The crude solid was purified by preparative TLC (silica, ethyl acetate/2-propanol/25% ammonium hydroxide/water 7/7/5/4 v/v/v/v). Yield 155 mg (23%). An attempt to ensure uniformity of porphyrin countercations by isolating the corresponding ammonium salt was unsuccessful, as some residual (exogenous) sodium cations were always present. This fact, together, the hygroscopic nature of compound **3** resulted in incorrect elemental analysis results. UV−vis (0.1 M ammonium carbonate, pH = 8.5, $\lambda_{max}$) 418, 523, 564, 600, 652 nm. $^1$H NMR (DMSO-$d_6$, $\delta$): 8.98 (2H, d), 8.82 (6H, m), 8.17 (2H, m), 8.05 (2H, m), 7.92 (2H, m), 7.12 (2H, m), 5.7 (1H, m), 5.02÷5.00 (1H, m), 4.83 (6H, m), 4.44 (1H, m), 3.75÷3.35 (35H, m), −2.86 (2H, s).

**Acknowledgment.** We thanks CNR and the MURST for financial support, and Regione Veneto, Department of Industry and Energy, for financing the purchase of the 400 MHz spectrometer. Furthermore, Dr. Pietro Traldi and Dr. Roberta Bertani (CNR Area di Ricerca Padova, Italy) are gratefully acknowledged for performing mass spectroscopy measurements of compound **3**.

**Supporting Information Available:** $^1$H−$^1$H COSY spectrum and Lambert−Beer plot of **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

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