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Self-Assembly of Cyclodextrins with *meso*-Tetrakis(4-sulfonatophenyl)porphyrin in Aqueous Solution

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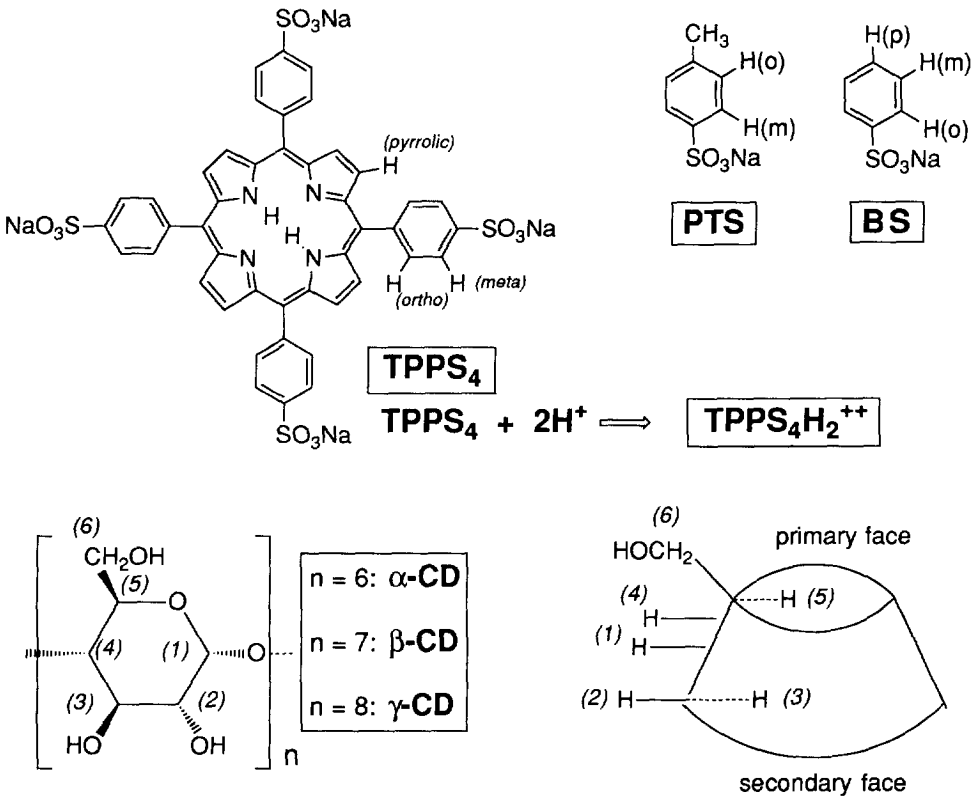
Abstract.: The ability of α-CD, β-CD and γ-CD to break the aggregates in the solution of the title porphyrin ($TPPS_4$) and to form inclusion self-assemblies has been studied by UV/Vis and ^{1}H-NMR (ROESY). The behaviour of $TPPS_4$ in the presence of α-CD, β-CD and γ-CD has been compared to that of sodium *p*-toluenesulfonate and sodium benzenesulfonate. $TPPS_4$ in neutral media forms inclusion assemblies, through its *meso*-phenyl groups, with β-CD and γ-CD, but not with α-CD. Diprotonated $TPPS_4$, which has a different geometry from the free base, forms an inclusion assembly only with β-CD. The inclusion assemblies of $TPPS_4$ with β-CD and γ-CD show different geometry: introduction of the phenyl substituent through the cyclodextrin secondary face for β-CD and through the primary face for γ-CD.

Introduction

Water-soluble porphyrins and metalloporphyrins are interesting materials in many applied fields. From a synthetic and economic point of view *meso*-tetrakis(4-sulfonatophenyl)porphyrin ($TPPS_4$) is the most accessible water soluble porphyrin. Several potential applications have been reported for $TPPS_4$, *e.g.* as photosensitiser in photodynamic therapy,[1] and for its metalloporphyrins as catalysts in oxidation processes.[2] The interest of the encapsulation of water-soluble porphyrins with cyclodextrins lies in: 1) the modification of the porphyrin properties; 2) the hindrance of porphyrin aggregation; 3) the protection of the porphyrin macro cycle and its phenyl substituents from the active species originating during oxidation processes. The self-assembly of 2,6-dimethoxy β-CD with non-water soluble meso-phenyl substituted porphyrins has shown that the phenyl substituent is introduced into the cyclodextrin cavity through the secondary face.[3] This type of self- -assembly has been used to synthesise supramolecular complexes between substituted tetraphenylporphyrin and substituted β-CD,[3b] which have been proposed as artificial analogues of heme proteins.[3c] However, in spite of the growing literature on the interaction between cyclodextrins and porphyrins or metalloporphyrins,[4] there are few studies concerning cyclodextrins and water-soluble tetraphenylporphyrins. They are mentioned[5,6] in a patent on optical recording materials[5a] and, very recently, in a report of the formation of an external assembly between *meso*-tetrakis(4-carboxyphenyl)porphyrin and a derivative of β-cyclodextrin.[5b] It has been reported that, in aqueous solutions, β-cyclodextrin (β-CD) can break the aggregates of the free base of $TPPS_4$ and also the μ-oxo dimer of the corresponding Fe(III) metalloporphyrin ($FeTPPS_4$), because of the formation of an association with the monomeric

metalloporphyrin.[6] Further, it has been reported that β-CD induces modification on the properties of the soluble metalloporphyrins of $TPPS_4$.



Formula Scheme

Here we present a study of the interaction in water solution of α-CD, β-CD and γ-CD with the tetrasodium salt of the free base of $TPPS_4$ and of the diprotonated $TPPS_4$ ($TPPS_4H_2^{++}$).

Results and Discussion

UV/Vis:

$TPPS_4$ above the concentration of $4\ 10^{-5}$ mol l^{-1} at pH = 7.0 and above $2\ 10^{-5}$ mol l^{-1} at pH = 13.0 shows, small Lambert-Beer law deviations due to the formation of aggregates. At a concentration of $1\ 10^{-4}$ mol l^{-1} $TPPS_4$ and pH = 7.0 or pH = 13.0, a ten-fold excess of β-CD or γ-CD results in changes of the UV/Vis spectra corresponding to a decrease or elimination of porphyrin aggregation. In contrast, α-CD does not show any effect on the UV/Vis spectra. However, the changes in the UV/Vis absorption spectra due to $TPPS_4$ aggregation are small, both in the intensity and in the position of the Soret and Q bands, and in consequence the effect of cyclodextrins on aggregation leads to UV/Vis spectrum changes of low significance.

$TPPS_4$ in acidic solutions, (*i. e.* $TPPS_4H_2^{++}$) and above 4 10^{-5} mol l^{-1} aggregates through intermolecular zwitterionic interactions. This aggregate shows a characteristic red-shifted Soret band (489 nm) compared to the monomeric $TPPS_4H_2^{++}$ (433 nm) and also shows red-shifted Q bands.[7] At a $TPPS_4H_2^{++}$ concentration of 8 10^{-5} mol l^{-1} (acetic acid, pH =3.5) addition of a ten-fold molar excess of β-CD results in a decrease in the absorption bands corresponding to the associated $TPPS_4H_2^{++}$ and an increase in the monomeric $TPPS_4H_2^{++}$ (see Fig. 1): addition of the same excess of α-CD or γ-CD does not result in the decrease of the absorption corresponding to the associated form of $TPPS_4H_2^{++}$. In summary these results show the association of $TPPS_4H_2^{++}$ with β-CD but not with α-CD or γ-CD, and suggest the association of $TPPS_4$ with β-CD, in agreement with previous reports,[6d] and γ-CD, but not with α-CD.

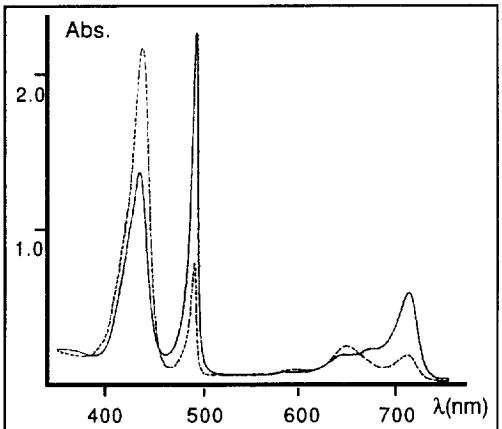


Fig. 1. UV/Vis spectra of $TPPS_4H_2^{++}$ ($TPPS_4$ 8 10^{-5} mol l^{-1}, in acetic acid pH=3.5); ---- alone; - - - with β-CD(10:1). In the presence of α-CD or γ-CD no significant changes were observed

^{1}H-NMR:

If cyclodextrins and $TPPS_4$ form inclusion assemblies, two types of effect could be expected on the ^{1}H-NMR spectra of their mixtures: *a)* a change in the $TPPS_4$ part of the spectrum, originated by the increase of the monomeric complexed form at the expense of the aggregated form,[6d,8,9] *b)* a change in the cyclodextrin part of the spectrum because of the magnetic anisotropy of the aromatic host (phenyl group inside the cyclodextrin cavity and porphyrin ring near the cyclodextrin). Further, Nuclear Overhauser Effect (NOE) experiments can detect the proximity of the internal protons of the cyclodextrin to the protons of the phenyl substituent of the guest molecule.[3a] In our case the size of a complex between the porphyrin and cyclodextrins indicates that the rotating frame NOE (ROESY) is a more suitable experiment than the simple NOE method.[10]

The influence of cyclodextrin on the aggregation of $TPPS_4$ was studied (200 MHz) by the addition of cyclodextrins to a neutral D_2O solution of $TPPS_4$ 10 mmol l^{-1}, *i. e.* at concentrations at which the monomeric form is about 40 %. ^{1}H-NMR experiments on $TPPS_4H_2^{++}$could not be performed, even at concentrations close to the limit of sensitivity, because of its colloidal nature.[7] ROESY (400 MHz) experiments were performed in a four-fold molar excess of cyclodextrin in $TPPS_4$ 7 mmol l^{-1} D_2O solutions. In addition, the same series of ROESY experiments were performed on sodium *p*-toluenesulfonate (PTS) and sodium benzenesulfonate (BS). Tables 1 and 2 show a summary of the results obtained.

Table 1. NOE (ROESY) [presence (+) or absence (-)] in D_2O solution between $TPPS_4$ (7 mmol l^{-1}), TPS (30 mmol l^{-1}) or BS (30 mmol l^{-1}) and α-CD, β-CD and γ-CD (30 mmol l^{-1}).

	Internal cyclodextrin protons					
	H-3			H-5		
	α-CD	β-CD	γ-CD	α-CD	β-CD	γ-CD
$TPPS_4^{++}$						
*o*H	(-)	(++)	(++)	(-)	(++)	(++)
*m*H	(-)	(++)	(++)	(-)	(++)	(++)
pyrrolicH [a]	(-)	(-)	(-)	(-)	(-)	(+)
PTS						
*o*H	(++)	(++)	(++)	(++)	(++)	(++)
*m*H	(+)	(++)	(++)	(+)	(++)	(++)
CH_3	(++)	(++)	(++)	(++)	(++)	(++)
BS						
*m*H	(++)	(++)	(++)	(++)	(++)	(++)
*o*H	(+)	(++)	(++)	(+)	(++)	(++)
*p*H	(++)	(++)	(++)	(++)	(++)	(++)

a) A small NOE was detected with H6 of γ-CD.

α-CD cannot dissociate the $TPPS_4$ aggregates. The absence of NOE between the protons of α-CD and those of $TPPS_4$ rules out the formation of an inclusion complex between α-CD and $TPPS_4$. In the presence of $TPPS_4$ the α-CD protons show a significant diamagnetic shift but of similar value for all protons, which could be attributed to the effect of the porphyrin on the volume magnetic susceptibility of the medium. In the case of mixtures of α-CD with PTS or BS the modification of the chemical shift of α-CD protons is very small and does not provide any information on the formation of inclusion assemblies. In contrast, these mixtures show NOE corresponding to the inclusion complexes: NOE is detected only between the internal protons H3 and H5 of α-CD and the aromatic protons of PTS and BS or the benzylic protons of PTS. The relative intensity of the NOE signals of the *ortho* signal compared to the *meta* and *para* signals suggests that the phenyl ring of PTS and BS is only partially enclosed in the cyclodextrin cavity.

These results show that the primary face of α-CD does not allow the crossing of the sulfonate group of $TPPS_4$: the α-CD inclusion complexes of PTS and BS should be formed *via* the introduction of the phenyl group through the cyclodextrin secondary face, which does not allow the crossing of the porphyrin ring in the case of $TPPS_4$ (see Figure 2).

ROESY experiments show that PTS and BS give inclusion assemblies with β-CD and γ-CD (see Table 1). NOE are shown between the internal H3 and H5 of cyclodextrins with the aromatic and methyl protons of BS or PTS. However, in contrast to the case of α-CD, the NOE relative intensities do not allow us to infer the geometry of the inclusion complexes. The fact that β-CD and γ-CD dissociate the aggregates of $TPPS_4$, and that NOE is observed between the internal protons of both cyclodextrins and the *ortho* and *meta* protons of $TPPS_4$ shows the formation of the inclusion complexes between both cyclodextrins and the porphyrin. Comparison of the changes in chemical shift of the cyclodextrin protons shows highly significant differences between the two cyclodextrins (see Table 2).

In the case of γ-CD the protons H5, H6 and H'6, placed in the primary face, show a high diamagnetic shift (see Fig. 3). For β-CD the changes are smaller than for γ-CD, and the higher diamagnetic shifts are shown by the protons H3 (internal secondary face) and H2. Since the diamagnetic shifts of H5 and H'6 in the complexes PTS:γ-CD and BS:γ-CD are small, the larger diamagnetic shifts of H5, H6 and H'6 in the complex $TPPS_4$:γ-CD may be attributed to an effect of the magnetic anisotropy of the porphyrin macrocycle[11] on the primary face of γ-CD. For β-CD, only NOE between the internal H3 and H5 with the phenyl protons of $TPPS_4$

Table 2. Chemical shift changes ($\Delta\partial$ ppm: negative values correspond to diamagnetic shifts) of the ^{1}H-NMR signals of cyclodextrins (30 mmol l^{-1}) in mixtures with $TPPS_4$ (7 mmol l^{-1}), PTS (30 mmol l^{-1}) or BS (30 mmol l^{-1}) compared to the solutions without guest substrate.[a)]

	Internal cyclodextrin protons						*External cyclodextrin protons*														
	H3			H5			H1			H2			H4			H6			H'6		
	α-CD	β-CD	γ-CD	α-CD	β-CD	γ-CD	α-CD	β-CD	γ-CD	α-CD	β-CD	γ-CD	α-CD	β-CD	γ-CD	α-CD	β-CD	γ-CD	α-CD	β-CD	γ-CD
$TPPS_4^{++}$	-0.12	-0.1	-0.02	-0.13	0.0	-0.30	-0.19	-0.04	-0.07	-0.16	-0.07	-0.06	-0.19	-0.04	-0.04	-0.13	0.0	-0.18	-0.15	-0.02	-0.50
PTS	-0.04	-0.10	-0.04	+0.05	-0.05	-0.02	+0.02	-0.05	-0.03	+0.01	-0.05	-0.02	+0.02	-0.04	-0.03	+0.03	-0.06	-0.01	+0.06	-0.16	-0.03
BS	-0.02	-0.10	-0.02	+0.05	-0.04	-0.03	+0.02	-0.04	-0.02	+0.01	-0.04	-0.02	+0.02	-0.04	-0.02	+0.03	-0.02	-0.01	+0.06	-0.14	-0.03

a) These $\Delta\partial$ changes do not correspond to the highest observed change, which is shown at molar ratio 1:2; *e. g.* -1.0 ppm for γ-CD H'6 at 3.3 mmol l^{-1} $TPPS_4$ and 6.6 mmol l^{-1} γ-CD.

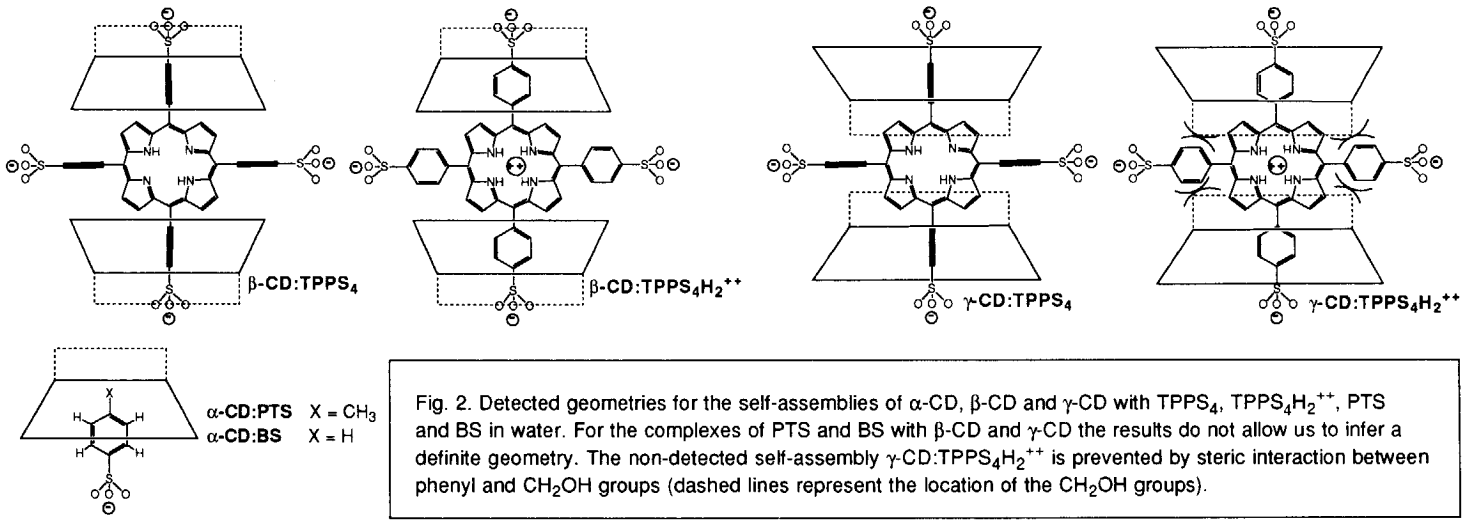


Fig. 2. Detected geometries for the self-assemblies of α-CD, β-CD and γ-CD with $TPPS_4$, $TPPS_4H_2^{++}$, PTS and BS in water. For the complexes of PTS and BS with β-CD and γ-CD the results do not allow us to infer a definite geometry. The non-detected self-assembly γ-CD:$TPPS_4H_2^{++}$ is prevented by steric interaction between phenyl and CH_2OH groups (dashed lines represent the location of the CH_2OH groups).

are detected. For γ-CD, in addition to these NOE other small ones are also detected between the pyrrolic protons with the H5 and one of the protons at C6 (H6).[12]

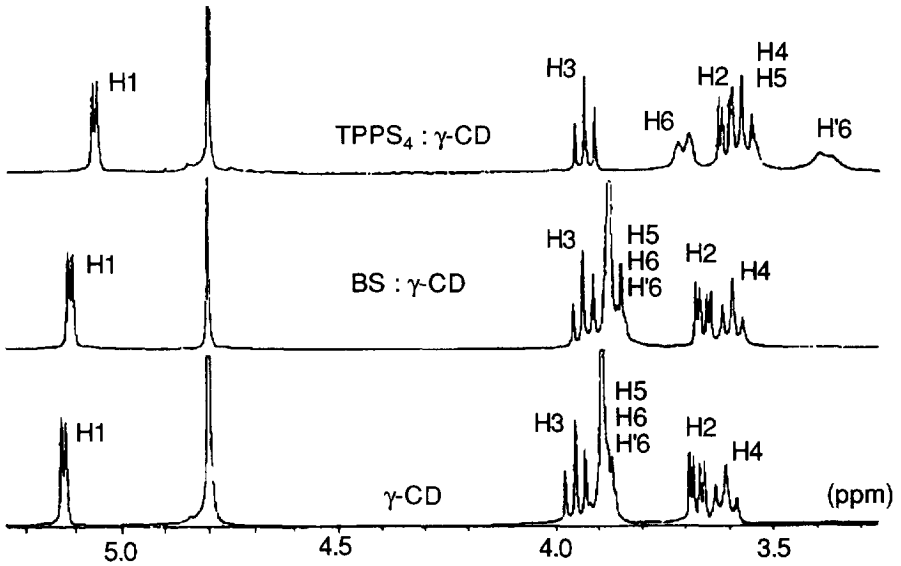


Fig. 3. ^{1}H-NMR spectra of γ-CD (30 mmol l^{-1}) in D_2O; alone: with TPPS4 (7 mmol l^{-1}): with BS (30 mmol l^{-1})

These results show that the inclusion complex of $TPPS_4$ with β-CD has the cyclodextrin secondary face close to the porphyrin ring, in contrast to the complex with γ-CD, which has its primary face close to the porphyrin ring. The modification of chemical shifts and the NOE detected suggest that the -CH_2OH groups of γ-CD are closer to the porphyrin ring than the secondary ring carbon atoms of β-CD. This suggests that the phenyl penetrates to a similar extent in both cases, which is probably determinated by the fact that the sulfonate group remains water solvated outside the cavity.

The effect of β-CD and γ-CD on the aggregation of $TPPS_4$ (10 mmol l^{-1}) shows that at a molar ratio of 2:1 there is still a small amount of aggregate (≈ 5%), which disappears at a molar ratio 3:1. In this respect no difference was detected between β-CD and γ-CD. The changes in chemical shifts with the cyclodextrin:$TPPS_4$ ratio, at constant porphyrin concentration and at constant total concentration, suggest a 2:1 stoichiometric ratio, as proposed for other tetraphenylporphyrins and β-cyclodextrins.[3,4,6]

The different geometry of the self-assemblies of β-CD and γ-CD with $TPPS_4$ explains the different effect of the two cyclodextrins on $TPPS_4H_2^{++}$ ($TPPS_4$ in acidic medium). It is well known that tetraphenylporphyrins show different geometry for the free base and for the diprotonated form.[13a,b] In the case of the free base, the phenyl groups are nearly orthogonal to the porphyrin ring, while in the diprotonated form the phenyl rings are almost planar to the porphyrin ring. Molecular models[14] using X-ray

crystallographic data of β-CD and γ-CD and of the free base and the diprotonated form of tetraphenylporphyrin[13] show that the self-assembly of $TPPS_4H_2^{++}$ to γ-CD through its primary face is prevented by steric hindrance between the cyclodextrin CH_2OH groups and the nearly coplanar phenyl rings (see Fig. 2). In contrast, the nearly orthogonal phenyl rings of the free base porphyrin do not prevent its self-assembly to γ-CD through its primary face.

Experimental

The tetrasodium salt of $TPPS_4$ was obtained by sulfonation of 5,10,15,20-tetraphenylporphyrin[15] as described elsewhere:[16] the product of sulfonation was neutralised with sodium carbonate and purified of inorganic sodium salts by methanol precipitation and chromatography through an MCI: CHP20P (Mitsubishi Kasei Corp.) column using water as eluent. α-CD, β-CD and γ-CD were of commercial origin (99 %, hydrates; Janssen Chim.): for the ^{1}H-NMR experiments they were equilibrated with D_2O and lyophilised (twice).

UV/Vis spectra were recorded in a Perkin-Elmer Lambda 5. ^{1}H-NMR experiments on the effect of cyclodextrins in aggregation were performed in a Geminis 200 Varian (200 MHz). Conventional 400 MHz ^{1}H-NMR spectra and ROESY experiments were recorded in a Bruker ARX-400 spectrometer equipped with an inverse broadband probehead incorporating a shielded Z-gradient coil. The ROESY experiments were carried out with the introduction of a water suppression gradient tailored excitation (WATERGATE)[17] between spin-lock time and the acquisition period, obtaining a selective and near complete elimination of water resonance. The spin lock time was optimized to 500 ms.

The influence of cyclodextrin on the aggregation of $TPPS_4$ was studied by ^{1}H-NMR (200 MHz) following the addition of cyclodextrins to a D_2O solution of $TPPS_4$ 10 mmol l^{-1} at molar ratios ($TPPS_4$:CD) 10:1, 10:3, 10:8, 1:1, 1:5 and 1:10. ROESY experiments (400 MHz) were performed in a four-fold molar excess of cyclodextrin over $TPPS_4$ 7 mol l^{-1} D_2O solutions: the cyclodextrin chemical shifts performed by $TPPS_4$ were evaluated in these ^{1}H-NMR spectra. In addition, the same series of NMR experiments were performed on sodium *p*-toluenesulfonate (PTS) and sodium benzenesulfonate (BS). Differentiation between NOE intensities has been performed only between signals in the same experiment and only significant differences in intensity were taken into account. Also series of experiments on the changing of chemical shifts with the $TPPS_4$:CD ratio at 10 mmol l^{-1} total concentration (Job plot) were performed.

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References and Notes

1 Kessel, D.; Thompson, P.; Saatio, K.; Nantwi, K. D. *Photochem. Photobiol.* **1987**, *45*, 787.

2 Labat, G.; Séris, J. L.; Meunier, B. *Angew. Chem. Int. Ed.*, **1990**, *29*, 1471.

3 a) Manka, J. S.; Lawrence, D. S. *Tetrahedron Lett.* **1989**, *30*, 7341. b) Manka, J. S.; Lawrence, D. S. *J. Am. Chem. Soc.* **1990**, *112*, 2440. c) Dick, D. L.; Venkata S. Rao, T.; Sukumaran, D.; Lawrence, D. S. *J. Am. Chem. Soc.* **1992**, *114*, 2664.

4 See *e. g.* a) Matile, S.; Hansen, T.; Stocker, A.; Woggon, W.-D. *Helv. Chim. Acta* **1994**, *77*, 1087. b) Hirai, H.; Toshima, N.; Fujii, Y. *Chem. Lett.* **1983**, 643.

5 a) Toshihiro, N.; Koichi, A.; Hiroaki, H. *Jpn. Kokai Tokkyo Koho JP* 61,159,634 [86,159,634], 19 Jul. 1986, Appl. 85/185: *CA*, **1987**, *106*, 93726m. b) Zao, S.; Luong, J. H. T. *J. C. S. Chem. Comm.* **1994**, 2307.

6 a) Mosseri, S.; Mialocq, J. C.; Perly, B. *J. Phys. Chem.* **1990**, *94*, 98. b) Mosseri, S.; Mialocq, J. C.; Perly, B.; Hambright, P. *J. Phys. Chem.* **1991**, *95*, 2196. c) Mosseri, S.; Mialocq, J. C.; Perly, B.; Hambright, P. *J. Phys. Chem.* **1991**, *95*, 4659.d) Mosseri, S.; Mialocq, J. C.; Perly, B.*Radiat. Phys. Chem.* **1992**, *39*, 223.

7 a) Ohno, O.; Kaizu, Y.; Kobayashi, H. *J. Chem. Phys.* **1994**, *99*, 4128. b) Ribó, J. M.; Crusats, J.; Farrera, J.-A.; Valero, M. L. *J.C.S. Chem. Comm.* **1994**, 681. c) Pasternack, R. F., Shaefer, R. F.; Hambright, P. *Inorg. Chem.* **1994**, *33*, 2062.

8 Although the ^{1}H-NMR spectrum of the free base TPPS4 has already been described (see ref.[6d,9]) the assignation of the signals was not complete: variable temperature and aggregate breaking experiments led to the following assignment: (≈ 1 mmol l^{-1} D_2O,DSS, ∂, ppm, 200 MHz, 20°C): 8.9 (broad s, monomer β-pyrrolic H), 7.4 (broad s, aggregated β-pyrrolic H, chemical shift depending on the degree of aggregation); these signals collapse at about 30-35°C depending on concentration; 7.8 (apparent d, ortho-H, collapsed signals of monomer, 8.2, and aggregate, ≈ 7.3), 8.3 (apparent d, meta-H, collapsed signals of monomer, ≈ 8.4, and aggregate, ≈ 8.3); monomer-to-aggregate ratio, 5:6.

9 Corsini, A.; Herrmann, O. *Talanta* **1986**, *33*, 335.

10 a) Bothner-By, A. A.; Stephens, R. L.; Lee, J.; Warren, C. D.; Jeanloz, R. W.; *J. Am. Chem. Soc.* **1984**, *106*, 811. b) Duchene, D.; *New trends in cyclodextrins and derivatives*; Editions de Santé, Paris, 1991.

11 a) Scheer, H.; Katz, J. J. *Nuclear Magnetic Resonance of Porphyrins and Metalloporphyrins in Porphyrins and Metalloporphyrins*, Smith, K. Ed., Elsevier: Amsterdam, 1975: pp. 402-403. b) Jason, T. R. *The Ring-Current Effect of the Phtalocyanine Ring*, Dissertation, Case Western University. Univ. Microfilms, Ann Arbor, 72-51, 1971.

12 For H6, which shows NOE with the pyrrolic protons, a smaller diamagnetic shift is shown than for H'6 (-0.2 compared to -0.5, see Table 2). This suggests a conformation at C6 with its two hydrogens at different positions in respect to the porphyrin ring: one H (H'6) near to the porphyrin ring and other H (H-6) near to the pyrrolic H. For the anisotropy of the porphyrin ring see references 11.

13 a) Lindner, K.; Saenger, W. *Acta Cryst. B* **1982**, *38*, 203. b) Lindner, K.; Saenger, W. *Angew. Chem. Int. Ed.* **1978**, *17*, 203. c) Harata, K. *Bull. Chem. Soc. Japan* **1987**, *60*, 2763. d) Stone, A.; Fleischer, E. B. *J. Am. Chem. Soc.*, **1968**, *90*, 2735. e) Hamor, M. J.; Hamor, T. A.; Hoard, J. L. *J. Am. Chem. Soc.*, **1964**, *86*, 1938. f) Hamor, M. J.; Hamor, T. A.; Hoard, J. L. *J. Am. Chem. Soc.*, **1964**, *86*, 1938. g) Stone, A., Fleischer, E. B. *J. Am. Chem. Soc.*, **1968**, *90*, 2735. h) coordinated files *TPHPOR10*, *TPPFEC*, *TPHPOR10*, *TPPFEC*, *CHXAMH03*, *BCDEXD* and *CIWMIE10* from Cambridge Data Base: Allen, F. H.; Kennard, O.; Taylor, R. *Acc. Chem. Res.*, **1983**, *16*, 146.

14 Müller, N.; Falk, A. *Ball & Stick 3.0*, Cherwell Scientific, Oxford.

15 Lindsey, J. S.; Screiman, I. C; Shu, H. C.; Kearnay, P. C.; Marguerettaz, A. M. *J. Org. Chem.*, **1987**, *52*, 827.

16 Fleischer, E. G.; Palmer, J. M.; Srivastana, T. S.; Chatterjee, A. *J. Am. Chem. Soc.*, **1971**, *93*, 3162.

17 Piotto, M.; Saudek, V.; Sklenar, V.; *J. Biomol. NMR*, **1992**, *2*, 661.

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