Porphyrin-Based Peptide Receptors: Syntheses and NMR Analysis

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Abstract: The synthesis and purification of a water-soluble host compound that contains three pyridinium units and one spacer-connected benzocrown ether unit in the *meso*-positions of porphyrin and of its Zn^{II} or Cu^{II} complexes is described. Metalation leads to small (compared to the *apo*-derivative) changes of selectivities with different peptides, with complexation constants in water of above $10^5\,M^{-1}$. One complex containing the tripeptide Gly-Gly-Phe is analyzed in detail by COSY, HSQC, HMBC, and NOESY NMR experiments. Temperature-dependent spectra show activation energies for a intramolecular hydrogen exchange of amide protons with valence isomerization of the porphyrin ring, in accordance with the literature. Sharp signals for the spin system are only found at elevated temperature. Vicinal coupling constants within the crown ether moiety indicate stronger puckering than that reported for benzocrowns. All NMR signals of the complexed peptide are shielded, in particular those of the terminal phenylalanine unit, in line with its stacking on the porphyrin surface. A corresponding structural model, obtained by CHARMm simulation, is also in line with the observed intermolecular NOE cross peaks.

Keywords: conformation analysis · crown compounds · peptides · porphyrinoids · receptors

Introduction

Complexation of peptides by artificial receptors is of great current interest.[1] The combination of a porphyrin or other moieties with a crown ether for association with the peptide N terminus has been shown to be one of the most promising strategies for length- and sequence-selective recognition of natural peptides in aqueous medium, partially with unprecedented sensitivity.[2] Most of the artificial receptors for peptides reported until now rely on hydrogen bonds; thus they require aprotic solvents as medium and therefore the use of protected peptides.[1a] The receptors described to date, including the few adapted to aqueous environment,[1b,c] are symmetric and therefore unable to align peptide analytes in one direction from the N to the C terminus, which is a prerequisite for sequence selectivity.[1] Very efficient complexation in aqueous systems—occurring, however, on the surface of water-insoluble polymers—has been reported by Still et al,[1f,g,j]. This paper describes the syntheses of our porphyrin-derived host compounds, which achieve the goals of water solubility and sequence selectivity, together with a detailed NMR characterization of a corresponding complex with a tripeptide.

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Results

Syntheses: The syntheses of the porphyrin derivatives were carried out by means of a slightly modified version of the Adler–Longo method, by mixing suitable precursor aldehydes (Scheme 1 and Scheme 2) to yield mixtures of regioisomers that could be separated by column chromatography, although the yields were low, as is usual with such statistical syntheses.[3] After methylation of the condensation products **1**, **2**, and **3**—obtained from pyridine and benzoic acid ester with carbaldehyde moieties in their 4-positions—the methyl esters **4**, **5**, and **6** could be isolated in acceptable yield in pure form; they may be used for complexation studies with Z-protected peptides (low affinities, therefore not studied here). In all cases the pyridine units were methylated after chromatographic separation in order to obtain water-soluble host compounds. The free acids could be of interest as they should be able to form additional salt bridges or hydrogen bonds to the N termini of peptides. All attempts to hydrolyze the esters, however, led to insoluble material or decomposition of the porphyrins.

Condensation with the benzocrown ether carbaldehyde proved to be suitable for the preparation of the most effective peptide receptor **8**, via the pyridine derivative **7**. Metalation of **8** with zinc(II) or copper(II) acetate afforded the corresponding complexes in 50 % yield. Such metal complexes are of interest not only with respect to possible changes in association constants, but also as possible catalysts for the hydrolysis and/or oxidation of suitable peptides.

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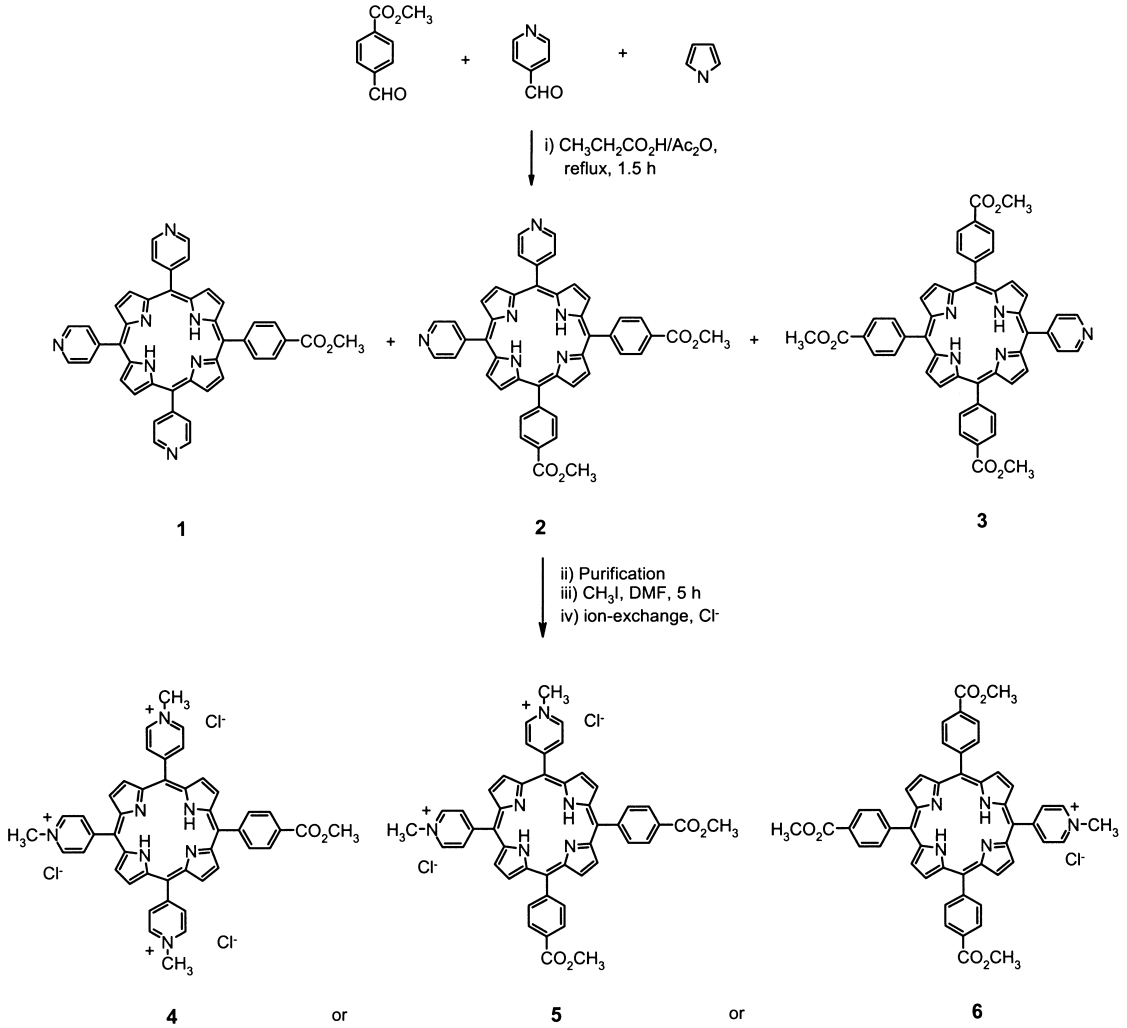

Scheme 1. Synthesis of porphyrins with carboxylic side chains.

Complexation constants: These were measured by following the changes of Soret band extinctions as a function of the concentration of added peptide; the absorption maxima were red-shifted by 8–10 nm, and the apparent extinction coefficients $\Delta\varepsilon$ were 30000 to 70000 $mol^{-1}dm^3cm^{-1}$ for porphyrin **8** (see Table 1). The titration curves showed isosbestic points, in line with the formation of 1:1 complexes, which were supported by very good nonlinear least-squares fits to a 1:1 calculated model. The high extinction coefficients of the porphyrins, which could be used at 5 μM concentrations, offer the advantage of completely negligible self-association of the receptors. Metalation of the hosts often led to affinity increases or decreases, which, interestingly, depended on the sequence of the applied peptide (Table 1, entries 1, 2, 8–10, 11, 12); at the same time the observed $\Delta\varepsilon$ values changed even more. It is noteworthy that although metalation of porphyrin macrocycle **8** in receptors **8**(Zn) and **8**(Cu) (entries 1, 7, and 13) does not change the length selectivity for peptides, the sequence selectivity is reversed in **8**(Zn) and **8**(Cu), albeit with a lower preference. As with the metal-free host, these receptors show the highest affinities ever reported for natural, unprotected peptides in water, with complexation constants of almost $10^6\,M^{-1}$.

Table 1. Logarithms of association constants ($\log K\,M^{-1}$) of peptides with receptors **8**, **8**(Zn), and **8**(Cu).[a,b]

	Peptide	**8**		**8**(Zn)		**8**(Cu)	
		$\log K$	$\Delta\varepsilon \times 10^{-3}$	$\log K$	$\Delta\varepsilon \times 10^{-3}$	$\log K$	$\Delta\varepsilon \times 10^{-3}$
1	Gly-Gly	2.93	15.7	3.30	14.5	3.50	17.50
2	Gly-Phe	4.71	22.0	4.10	14.9	4.38	17.7
3	Phe-Gly	4.36	18.0	4.62	15.1	4.60	20.7
4	Ala-Phe	4.60	10.2	4.07	7.9	4.18	9.20
5	Asp-Phe	4.05	14.0	3.72	3.77	4.17	8.02
6	Phe-Phe	4.52	13.1	3.48	15.8	4.20	10.80
7	Gly-Gly-Gly	3.41	7.8	4.33	3.5	4.71	7.00
8	Gly-Gly-Phe	4.39	20.7	4.74	2.72	4.54	15.38
9	Gly-Phe-Gly	4.35	12.5	4.15	4.01	4.15	15.3
10	Phe-Gly-Gly	4.48	12.3	4.60	1.34	4.32	9.56
11	Gly-Gly-Trp	3.52	15.4	4.33	5.24	4.07	14.81
12	Trp-Gly-Gly	4.48	16.9	4.20	6.27	4.21	11.10
13	Gly-Gly-Gly-Gly	5.02	18.9	5.00	3.27	5.08	8.76

[a] Measured by UV/visible titration of **8**, **8**(Zn), or **8**(Cu) with natural peptides at 25 °C. Titrations were carried out in phosphate buffer (5 mM, pH 6.9 ± 0.2) by adding concentrated stock solutions of peptide ([peptide] = 10 mM) containing about 5 μM of **8**, **8**(Zn), or **8**(Cu) to equally concentrated solutions of **8**, **8**(Zn), or **8**(Cu) in a 10 mm cuvette. Error limits: $\log K$ ± 5 %. [b] $\Delta\varepsilon$ [$mol^{-1}dm^3cm^{-1}$]. Extinction coefficient changes at [ligand]/[host] = 200, the values agree within ± 5 % with the $\Delta\varepsilon$ from nonlinear fit (ε: **8** (410 nm = 91200 $mol^{-1}dm^3cm^{-1}$), **8**(Zn) (425 nm = 48567 $mol^{-1}dm^3cm^{-1}$), **8**(Cu) (410 nm = 62010 $mol^{-1}dm^3cm^{-1}$. Error limits: ± 5 %).

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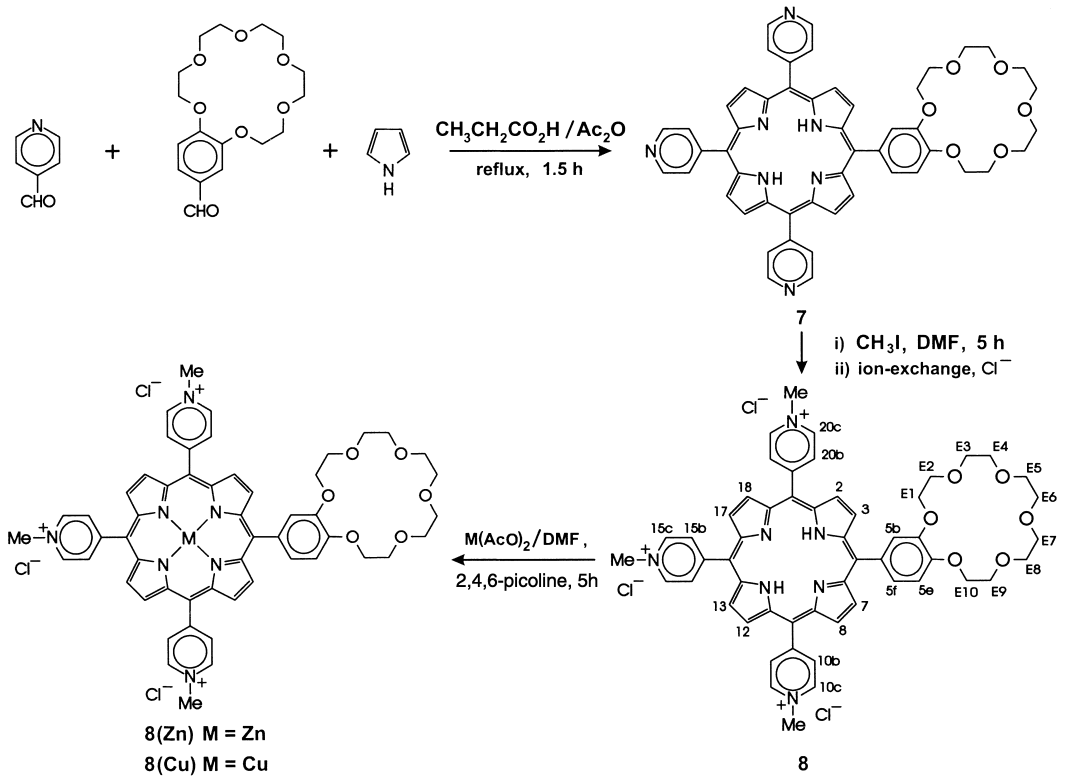

Scheme 2. Synthesis of the porphyrin crown ether host and its metal complexes.

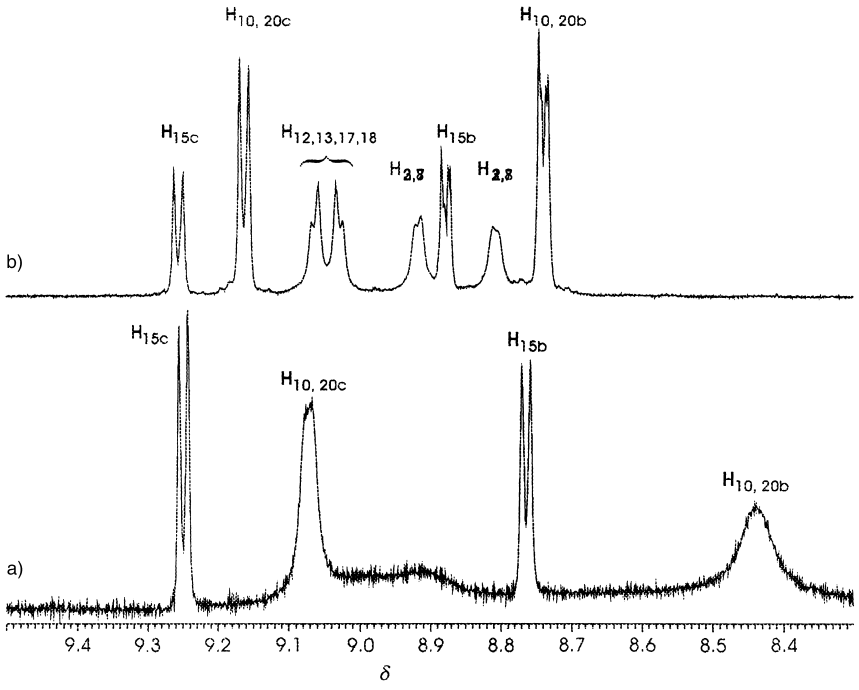

Figure 1. Aromatic region of the ^{1}H NMR spectrum of receptor **8** recorded a) at room temperature and b) at 347 K (D_2O, 500 MHz).

NMR studies: NMR spectra of the porphyrin receptor **8** show significant broadening of aromatic-proton signals at room temperature, particularly for the pyrrole protons (see Figure 1). The broadening is smaller for α- and β-pyridinium protons attached to positions 10 and 20 of the porphyrin system, as well as for their methyl groups. Almost regular narrow lines are observed for protons H15b and H15c of the pyridinium cycle, remote from the crown ether moiety. Variation of the temperature leads to changes of NMR line shapes typical for a degenerate two-site exchange phenomenon. With increasing temperature, all broadened signals became sharper, and, at 347 K, the aromatic region of the spectrum consists of only two well-resolved AB-type subsystems with $J_{AB} = 4.9$ Hz for the pyrrolic protons, and two AA′XX′-type multiplets with integral intensities corresponding to one pyridinium unit (attached to C15) and to two equivalent pyridinium moieties attached to C10 and C20. This observation agrees well with data for parent porphyrins containing *meso*-substituted aryl and/or pyridyl functional groups.[4, 5] The dynamic NMR phenomenon has already been interpreted, by Abraham et al.[6] and by others,[7] for example, in terms of slow amine-proton hydrogen exchange accompanied by valence isomerization of the porphyrin moiety.

At 500 MHz, the protons pairs H2(H8) and H3(H7) showed a coalescence point at 307 K, with an estimated chemical shift

difference of 0.18 ppm under slow exchange. This gives a free energy of activation $\Delta G^{\ddagger}_{307}$ of 14.7 kcal mol^{-1}, in reasonable agreement with the reported[6] $\Delta G^{\ddagger}_{308}$ values of 12.3 and 13.9 kcal mol^{-1} for tetraphenylporphyrin and its deuterated counterpart *N,N′*-dideuterio-tetraphenylporphyrine. Receptor **8** in D_2O certainly exists in a protonated (deuterated) form, with minor dynamic differences of the pyridinium-containing porphyrin derivative **8** from the tetraphenyl analogue. It is interesting to note that the valence isomerism in the parent porphyrin, measured in the solid state, is significantly faster at $\Delta G^{\ddagger} = 9.3$ kcal mol^{-1}.[7b] Possibly the slow tautomerization observed in the present case is due to aggregation of the porphyrins[5] at the higher concentrations needed for the NMR analyses. In contrast, UV measurements for the peptide associations could be made at concentrations considerably below that at which any self-aggregation of the host compound occurs.

The appearance of sharp signals at higher temperature is crucial for the NMR analyses and allows rigorous assignment of ^{1}H and ^{13}C NMR signals both for the receptor molecule and for the complex. Initial assignments were made by using COSY and HSQC techniques. The signals of the crown ether moiety reveal well-resolved characteristic multiplets for distinguishable protons of all ten nonequivalent CH_2-groups (see Figure 2). Analysis of the multiplet structure for every -OCH_2-CH_2O- moiety was carried out in terms of AA′BB′

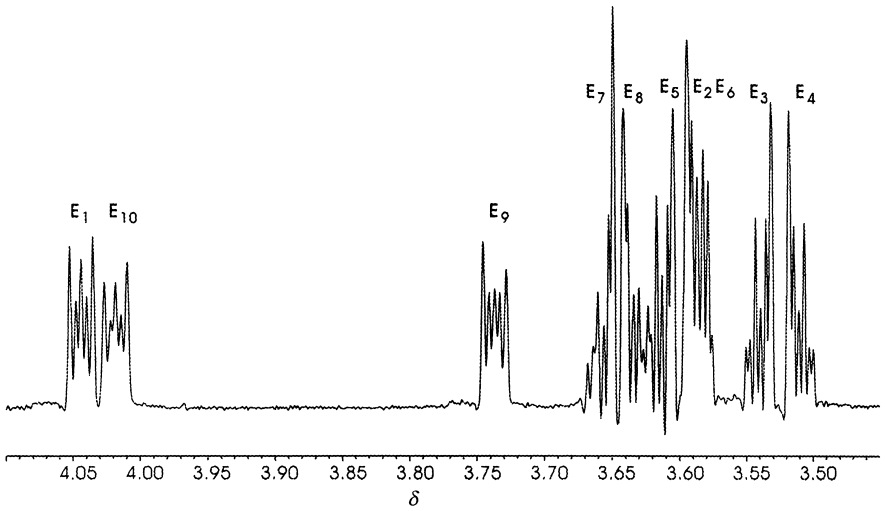

Figure 2. Crown ether region of the ^{1}H NMR spectrum of the receptor **8** (D_2O, 500 MHz, 347 K).

spin systems with fixed values of geminal spin–spin couplings of 10.0 Hz, by using the LAOCN-type program PAREMUS.[8] This procedure allowed neighboring CH_2 groups to be assigned and also provided values for vicinal spin–spin couplings in the crown ether moiety, which were of interest with respect to the conformation of the macrocycle (see Table 2). Recent studies have once more shown that such couplings provide a good measure of conformation, even for these flexible macrocycles.[9] It is interesting that the values of both ^{cis}J and $^{trans}J$ vary only slightly along the O-CH_2-CH_2-O sequence in **8**. The conversion of *J* values into mean dihedral angles for a sequence of CH_2 groups can be done with the traditional "*R* value" method developed initially by Lam-

Table 2. Vicinal spin–spin coupling constants $^{cis}J_{ij}$ and $^{trans}J_{ij}$ [Hz], *R* factor values (see text) and O-C-C-O dihedral angles ψ [°] for the crown ether moiety of receptor **8** (D_2O solution at 347 K)[a] and benzo[18]crown-6 (data from ref. [7]).

Protons[b]	$^{cis}J_{ij}$	$^{trans}J_{ij}$	*R*	ψ
E1,E2	2.37 (0.05)	6.30 (0.05)	2.66	60.8
E3,E4	2.45 (0.03)	6.34 (0.03)	2.59	63.4
E5,E6	2.36 (0.07)	6.46 (0.07)	2.74	61.2
E7,E8	2.25 (0.04)	6.32 (0.04)	2.81	61.6
E9,E10	2.30 (0.04)	6.23 (0.04)	2.71	61.1
B18C6	3.05	5.9	1.98	56.7

[a] Evaluated in terms of AA′BB′ spin systems for 12 to 22 assigned lines for each subspectrum; RMS errors 0.07–0.15 Hz. Standard errors of coupling constants are given in brackets. [b] See Scheme 4 for the notation.

bert[10] and refined by Buys.[11] Our numerical results for the O-C-C-O dihedral angles and the corresponding *R* values are also shown in Table 2. The crown ether moiety has a rather regular puckering of the whole aliphatic chain. From the values in Table 2, it can be seen that the degree of puckering is very similar for all O-CH_2-CH_2-O fragments. One can see only slight flattening of the macrocycle for the E1-E2 and E9-E10 CH_2 groups adjacent to the aromatic ring. Comparison with literature data for benzo[18]crown-6[12] shows that all the O-CH_2-CH_2-O fragments of the crown ether moiety of receptor **8** are significantly more puckered (by 4.1–6.7°) than those of the parent crown ether; this probably arises from the positively charged porphyrin (see also the discussion in ref. [13]).

Final assignments of the NMR signals were based on NOE measurements (Table 3). Most of the observed NOEs are positive, in line with close contacts between aromatic *ortho*-protons as well as between protons of neighboring CH_2 groups. Significant NOEs of resorcinol protons H5b, H5e, and H5f secure the assignment of the pyrrolic H3 and H7 signals, as well as of the terminal E1 and E10 CH_2 groups in the crown ether moiety. At the same time, there are also NOEs corresponding to nontrivial contacts between H5b and E4-CH_2 as well as H5e and E7-CH_2. These almost symmetrical long-range contacts obviously support the idea of high flexibility of the crown ether moiety.

The assignment of ^{1}H and ^{13}C NMR signals for the tripeptide Gly-Gly-Phe (Table 4) used for the NMR analysis in the complex with host **8** was based on COSY, HSQC, and HMBC experiments. Crucial information was obtained from long-range ^{13}C-H connectivities (see, e.g., reference data in ref. [14]), particularly on HMBC experiments for the carbonyl carbons and methylene protons of both peptide Gly and Phe subfragments. Hence, the carbonyl carbon C1α of the

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Table 3. NOE Effects between protons H_i (monitored) and H_j of receptor **8**.[a,b]

H_i	H_j	NOE[c]
5b	E1	++
	E4	++
	3, 7	+
5e	5f	+
	E1, E10	+++
	E7	++
5f	3, 7	++
	5e	+++
10c, 20c	10b, 20b	++
	10, 20-N-CH_3	+
15b	15c	+
15c	15b	+
	15-CH_3	++
E1	E2	++
	5b	++
	10b,20b	+
E2	E1	++
E9	E10	++
E10	E9	++
	5e	++
	5f	+

[a] Measured by integration of two-dimensional phase-sensitive NOESY spectra (4K by 4K data points, with $t_{mix} = 0.4$ s) in D_2O at 347 K. [b] See Scheme 4 for the notation. [c] All observed NOEs are positive and denoted by + ++ for strong (> 8%), ++ for medium (3 to 8%), and + for weak (1 to 3%) effects, respectively.

Table 4. ^{1}H chemical shifts[a] for the free peptide **P** (Gly-Gly-Phe), (δ_P); observed shifts (δ_{PR}^{obs})[b] for the mixture of the host **R**(**8**) and **P**; and complexation-induced shifts (CIS).[c]

	δ_P	δ_{PR}^{obs}	CIS
H1′	3.83	3.62	−0.4
H2′a	3.93	3.73	−0.4
H2′b	3.89	3.68	−0.4
H3′	4.47	4.17	−0.6
H4′a	3.18	2.60	−1.2
H4′b	2.95	2.40	−1.1
$H_{o\text{-Phe}}$	7.25	6.88	−0.7
$H_{m\text{-Phe}}$	7.36	6.88	−1.0
$H_{p\text{-Phe}}$	7.30	6.71	−1.2

[a] All shifts in ppm, δ_P and δ_{PR}^{obs} from internal DSS; measured in D_2O at 303 K. [b] Measured with a mixture of free peptide **P** (10.0 mM), receptor **R** (5.0 mM); concentrations after equilibrium: complex **RP** [**P**] = 5.0 mM; [**R**] = 0.04 mM and [**RP**] = 5.0 mM; signal numbering see Figure 3. [c] CIS values calculated for 100% complexation with known *K* value (see Table 1 and text).

terminal Gly revealed connectivity to both methylene groups through the geminal spin–spin coupling constant of the same Gly (^2J(C1α,H1)) and the relatively weak peak with protons of the CH_2 group of the central Gly through the vicinal spin–spin coupling ^3J(C1α,H2). At the same time, the carbonyl carbon C2α of the central Gly showed connectivity to only one methylene group (^2J(C2α,H2)) as well as to the methine proton of the neighboring Phe moiety through the vicinal ^3J(C2α,H3). The terminal carbonyl carbon C3α of Phe showed connectivity only to the methine and methylene protons of the Phe residue, due to geminal ^2J(C3α,H3) and vicinal ^3J(C3α,H4a) and ^3J(C3α,H4b) spin–spin coupling.

The shift changes in the fully complexed peptide (CIS values, Table 4) were obtained directly from single measurements at one set of concentrations, by taking into account the known complexation constants. Addition of the peptide Gly-Gly-Phe to a solution of the receptor **8** had negligible effects on the porphyrin moiety both with respect to peak positions and to shapes, in line with a low level of self-aggregation with porphyrin **8** and/or the same tendency for its complex. Some broadening (by 2 to 3 Hz) of the crown ether multiplets only prevents exact determination of *J* couplings. In contrast, almost all peptide signals show strong upfield shifts, indicating the placement of the peptide within the ring current of the porphyrin moiety. Such a geometry is in line with a gas-phase simulation of the complex with the CHARMm[15] force field (see Figure 3). The most significant upfield shift is observed

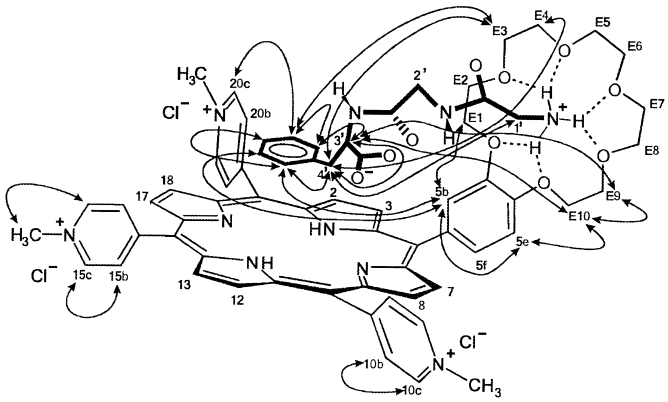

Figure 3. A projection of the three-dimensional structure for a complex of the receptor **8** with peptide Gly-Gly-Phe. Significant NOEs are depicted by arrows.

for protons of the Phe residue, which is expected to stack well with the porphyrin surface, in line with the higher affinity observed for the Phe-containing peptides in comparison with, for example, Gly-Gly-Gly. The largest shielding was observed for protons H4a, H4b ($\delta = -1.1$ to -1.2) and the Phe protons ($\delta = -0.7$ to -1.2). It is interesting that the shielding of aromatic protons is maximal for H_{para} and diminishes markedly for *meta*- and even more for *ortho*-protons. A minimum value of a negative CIS of $\delta = -0.4$ was observed for the terminal Gly (protons H1a and H1b). Intermediate shielding effects were observed for protons H2 of the central Gly and the methine proton H3 of the Phe residue ($\delta = -0.4$ to -0.6). The data show that both the methylene protons and the phenyl group of the peptide reside over the middle part of the porphyrin moiety of receptor **8**. At the same time, the aliphatic protons of both the terminal and central Gly residues experience much smaller but significant shielding effects, resulting most probably from both the benzene ring of the benzocrown moiety and the neighboring pyridinium fragments.

The observed NOE cross peaks from the two-dimensional NOESY spectra (Table 5) vary from strongly negative to equally strongly positive values; this indicates significant differences in segmental motions and therefore correlation times within the complex of receptor **8** and peptide Gly-Gly-Phe, which has a molecular mass of 1.284 kDa—see, for example, ref. [16]. Vicinal protons usually show the expected

Table 5. NOE Effects between protons H_i (monitored) and H_j of the complex **RP**.[a]

H_i	H_j	NOE[c]
3′	4′A	+
	4′B	−
	o-Phe	+
	E1, E10	+ (inter)
4′A	1′	−−−
	4′B	+
4′B	4′A	+++
	o-Phe	++
o-Phe	3′	+
	4′A, 4′B	+
	m, *p*-Phe	−−−
m, *p*-Phe	*o*-Phe	++
5b	*m*,*p*-Phe	++ (inter)
	o-Phe	−− (inter)
	5e	++
	5f	−
	E1	−−−
10b, 20b	10c, 20c	−
10c, 20c	*o*-Phe	−− (inter)
	m, *p*-Phe	−− (inter)
	10b, 20b	−−−
15b	15c	−
15c	15b	−−−
	15-CH_3	−
E1, E10	5b	−−
	5e	−
	3′	−− (inter)
E3, E4	4′A	+ (inter)
	4′B	+ (inter)
	m, *p*-Phe	+ (inter)
E9	3′	−− (inter)
	E10	−−−

[a] Measured by integration of two-dimensional phase-sensitive NOESY spectra (4 K by 4 K data points, with $t_{mix} = 0.4$ s) in D_2O at 303 K. See also footnote [b] in Table 4. [b] Negative NOEs denoted by −−− if strong ($\leq -8\%$), −− if medium (-3 to -8%), and − if weak (-1 to -3%); positive NOEs denoted accordingly +++, ++, and + for strong ($\geq 8\%$), medium (3 to 8%), and weak (1 to 3%) effects , respectively. Inter: intermolecular NOEs.

stronger effects, with the exception of some signals, for example in the crown ether moiety, which may be due to unfavorable correlation times in these cases. Most informative are the intermolecular NOEs, which generally support the conformation shown in Figure 3. Thus, the effects between the pyridyl and the aromatic protons of peptide Gly-Gly-Phe indicate a rather close proximity (distance in CHARMm-simulated structure: 3.5–4.1 Å). Although the cross peaks between the remote protons E3 and E4 of the crown ether moiety and the peptide aromatics, as well as those to Phe methylene protons H4a and H4b, are weak, the force field simulation indicates sufficient proximity. Complexation of receptor **8** with the peptide Gly-Gly-Phe relies significantly on the interaction of the terminal R-NH_3 group with the crown ether (see Figure 3). As a consequence, one might expect some higher rigidity for the crown ether moiety. However, gas phase dynamics simulations at least showed it to retain a high flexibility. For many reasonable conformations, the protons E1, E9, and E10 appear at distances from the H3′ of the peptide of between 4.0 and even 3.5 Å, in line with the observed NOEs.

Experimental Section

NMR experiments: These were carried out with Bruker AM 400 or DRX 500 NMR spectrometers either in $CDCl_3$ and DMSO with TMS or in D_2O with trace amounts of 3-(trimethylsilyl) 1-propanesulfonic acid (DSS) as internal reference. Standard two-dimensional COSY, NOESY, HSQC, and HMBC experiments were performed with a PFG module in a fashion and with parameters as described in the literature.[17, 18] HMBC experiments were done with a D2 delay of 0.05 s, optimal for spin–spin coupling constants of 10 Hz. NOE effects were evaluated by integration of the phase-sensitive NOESY spectra with 4 K by 4 K data points and mixing time $t_{mix} = 0.4$ s. Measuring conditions and results are given in Tables 2 to 5, or below under the synthetic details.

Materials: All chemicals were of reagent grade and were purchased from either Aldrich or Fluka. Solvents were freshly distilled before use. Column chromatography was carried out on 60 grade silica gel obtained from Merck. For TLC, Macherey–Nagel 0.25 mm silica gel 60 precoated plates with fluorescent indicator were used. The porphyrins were very hygroscopic, and/or difficult to remove from traces of silica; obtaining satisfactory CHN analyses of such porphyrins proved to be nearly impossible. Melting points were generally above 300 °C. Mass spectra were recorded on a FAB-MS Varian Mat 311 instrument. UV/Vis spectra were obtained with a Varian BioCary 1 UV/Vis spectrophotometer.

4-Formyl-benzo[18]crown-6 was synthesized according to a literature procedure.[19] The "*cis*" and "*trans*" dipyridyl porphyrin **2** (**5**) isomers could not be separated.

NMR of the free tripeptide Gly-Gly-Phe: ^{1}H NMR (500 MHz, D_2O, 303 K): $\delta = 7.36$ (m, 2H; $H_{m\text{-}Phe}$), 7.30 (m, 1H; $H_{p\text{-}Phe}$), 7.25 (m, 2H; $H_{o\text{-}Phe}$), 4.47 (dd, $J_d = 8.2$ Hz, $J_d = 5.1$ Hz, 1H; H3), 3.93 (d, $J = 16.9$ Hz, 1H; H2a), 3.89 (d, $J = 16.9$ Hz, 1H; H2b), 3.83 (s, 2H; H1), 3.18 (dd, $J_d = 13.9$ Hz, $J_d = 5.1$ Hz, 1H; H4a), 2.95 (dd, $J_d = 13.9$ Hz, $J_d = 8.2$ Hz, 1H; H4b); ^{13}C NMR (D_2O, 303 K): $\delta = 180.43$ (C3α), 172.69 (C2α), 170.28 (C1α), 140.32 ($C_{i\text{-}Phe}$), 132.03 ($C_{o\text{-}Phe}$), 131.27 ($C_{m\text{-}Phe}$), 129.51 ($C_{m\text{-}Phe}$), 58.99 (C3), 44.96 (C1), 43.15 (C2), 40.32 (C4).

Synthesis of the porphyrins 5-(4-carboxymethylphenyl)-10,15,20-tris(4-pyridyl)porphyrin (1), 5,10-bis(4-carboxymethylphenyl)-15,20-bis(4-pyridyl)porphyrin (2), and 5-(4-pyridyl)-10,15,20-tris(4-carboxymethylphenyl)-porphyrin (3): The three porphyrins **1**, **2**, and **3** were obtained from a single reaction as described below.

A mixture of methyl 4-formyl-benzoate (4.27 g, 0.026 mol), propionic acid (180 mL), and acetic anhydride (15 mL) was heated to 110 °C with stirring. Pyridine-4-carbaldehyde (4.27 mL, 0.045 mol) and pyrrole (4.13 mL, 0.06 mol) were slowly added to this solution. The resulting mixture was heated under reflux for 1.5 h, then allowed to cool to room temperature and evaporated to dryness. The residue was washed with water and neutralized with 1M aqueous ammonia. After filtration, the black powder was dissolved in hot methanol (150 mL) and stored overnight in the freezer. The mixture was filtered, and the precipitate was washed with cold methanol before extraction with CH_2Cl_2/EtOH (95:5) in a Soxhlet extractor. The organic phase was then concentrated and purified by column chromatography on dry silica gel.

Porphyrin 1: Yield: 0.5 g (5%); $R_f = 0.44$ (CH_2Cl_2/EtOH 92:8); ^{1}H NMR (400 MHz, $CDCl_3$, 300 K, TMS): $\delta = 9.03$ (m, 6H; 3,5-pyridine), 8.86 (m, 8H; β-pyrrole), 8.46 (d, $J = 7.8$ Hz, 2H; 2,6-phenyl), 8.29 (d, $J = 8.4$ Hz, 2H; 3,5-phenyl), 8.15 (m, 6H; 2,6-pyridine), 4.12 (s, 3H; CH_3), −2.86 (s, 2H; NH pyrrole); ^{13}C NMR ($CDCl_3$, 294 K): $\delta = 167.14$, 149.92, 148.39, 134.53, 131.38, 130.08, 129.36, 128.08, 120.14, 117.69, 117.48, 71.29, 70.54, 61.88, 52.49, 31.69; UV/Vis (CH_2Cl_2): $\lambda_{max} = 643$, 587, 545, 512, 415 nm; MS CI (NH_3): m/z: 675 [M^+].

Porphyrin 2: Yield: 6%; $R_f = 0.55$ (CH_2Cl_2/EtOH 92:8); ^{1}H NMR (400 MHz, $CDCl_3$, 300 K, TMS): $\delta = 9.03$ (d, $J = 5.9$ Hz, 4H; 3,5-pyridine), 8.84 (q, $J = 6.4$ Hz, 8H; β-pyrrole), 8.44 (d, $J = 7.4$ Hz, 4H; 3,5-phenyl), 8.28 (d, $J = 7.8$ Hz, 2H; 2,6-phenyl), 8.15 (d, $J = 5.9$ Hz, 4H; 2,6-pyridine), 4.10 (s, 6H; CH_3), −2.87 (s, 2H; NH pyrrole); ^{13}C NMR ($CDCl_3$, 294 K): $\delta =$ 167.20, 150.18, 148.25, 146.43, 134.53,130.02, 129.41, 128.09, 120.03, 117.28, 52.49, 29.73; UV/Vis (CH_2Cl_2): $\lambda_{max} = 644$, 588, 547, 512, 416 nm; MS CI (NH_3): m/z: 733 [M^+].

Porphyrin 3: Yield: 5%; $R_f = 0.71$ (CH_2Cl_2/EtOH 92:8); ^{1}H NMR (400 MHz, $CDCl_3$, 300 K, TMS): $\delta = 9.03$ (d, $J = 5.4$ Hz, 2H; 3,5-pyridine),

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8.84 (m, 8H; β-pyrrole), 8.45 (d, $J = 8.4$ Hz, 6H; 3,5-phenyl), 8.29 (d, $J = 7.9$ Hz, 6H; 2,6-phenyl), 8.15 (d, $J = 5.9$ Hz, 2H; 2,6-pyridine), 4.12 (s, 9H; CH_3), −2.82 (s, 2H; NH pyrrole); ^{13}C NMR ($CDCl_3$, 294 K): $\delta = 167.17$, 150.33, 148.10, 146.49, 134.50,131.44, 129.91, 129.42, 128.03, 119.82, 119.59, 117.03, 52.43 ; UV/Vis (CH_2Cl_2): $\lambda_{max} = 645$, 589, 548, 513, 417 nm; MS CI (NH_3): m/z: 790 [M^+].

General procedure for methylation of porphyrins 1, 2, and 3: A large excess of methyl iodide (2 mL) was added to a stirred solution of porphyrin **1**, **2**, or **3** (0.1 mmol) in DMF (5 mL). The mixture was stirred at 40 °C for 5 h, the solvent was evaporated to dryness under reduced pressure. The residue was taken up in methanol and, after being stirred at room temperature for 5 h, purified in chloride form by ion-exchange with Dowex-1X8-200 resin. The solution was filtered, and the filtrate was concentrated under vacuum and precipitated by slow addition of diethyl ether to the methanol solution. Yield 90%.

5-(4-Carboxymethylphenyl)-10,15,20-tris(4-methylpyridinium)porphyrin (4): ^{1}H NMR (400 MHz, [D$_6$]DMSO, 300 K, TMS): $\delta = 9.47$ (d, $J = 4.4$ Hz, 6H; 3,5-pyridine), 9.17 (br, 4H; β-pyrrole), 9.08 (br, 2H; β-pyrrole), 9.00 (m, 6H; 2,6-pyridine), 8.98 (d, 2H; $J = 5.2$ Hz, β-pyrrole), 8.45 (d, $J = 7.9$ Hz, 2H; 3,5-phenyl), 8.38 (d, $J = 6.4$ Hz, 2H; 2,6-phenyl) 4.70 (s, 9H; NCH_3), 4.06 (s, 3H; OCH_3), −3.05 (s, 2H; NH pyrrole); ^{13}C NMR (DMSO, 294 K): $\delta = 166.43$, 156.59, 144.27, 133.95, 132.20, 115.48, 114.89, 54.63, 54.59, 54.55, 48.05; UV/Vis (H_2O): λ_{max} (ε) = 640 (1862), 583 (6026), 556 (5495), 518 (13183), 420 nm (346737 mol^{-1} dm^3 cm^{-1}); MS EI (+ve): m/z: 722 [$M^+ + 1 - 3I^-$].

***cis*-5,10-Bis(4-carboxymethylphenyl)-15,20-bis(4-methylpyridinium)porphyrin (5)**: ^{1}H NMR (400 MHz, [D$_6$]DMSO, 300 K, TMS): $\delta = 9.49$ (d, $J = 6.5$ Hz, 4H; 3,5-pyridine), 9.16 (d, $J = 6.5$ Hz, 2H; β-pyrrole), 9.07 (br, 2H; β-pyrrole), 9.02 (d, $J = 6.6$ Hz, 4H; 3,5-pyridine), 8.98 (d, $J = 4.1$ Hz, 2H; β-pyrrole), 8.90 (br, 2H; β-pyrrole), 8.42 (d, $J = 8.2$ Hz, 4H; 3,5-phenyl), 8.38 (d, $J = 8.7$ Hz, 4H; 2,6-phenyl), 4.74 (s, 6H; NCH_3), 4.06 (s, 6H; OCH_3), −2.95 (s, 2H; NH pyrrole); ^{13}C NMR (DMSO, 294 K): $\delta = 157.10$, 145.66, 144.47, 134.88, 132.48, 129.97, 128.21, 126.47, 114.66, 52.84, 48.22; UV/Vis (CH_3OH): λ_{max} (ε) = 646 (1860), 590 (5130), 551 (6160), 515 (13490), 420 nm (169825 mol^{-1} dm^3 cm^{-1}); MS EI (+ve): m/z: 763 [$M^+ + 1 - 2I^-$].

10,15,20-Tris(4-carboxymethylphenyl)-5-(4-methylpyridinium)porphyrin (6): ^{1}H NMR (400 MHz, [D$_6$]DMSO, 300 K, TMS): $\delta = 9.44$ (d, $J = 6.0$ Hz, 2H; 3,5-pyridine), 9.01 (d, $J = 4.9$ Hz, 4H; 2,6-pyridine), 8.99 (br, 2H; β-pyrrole), 8.95 (br, 2H; β-pyrrole), 8.85 (s, 4H; β-pyrrole), 8.41 (d, $J = 7.9$ Hz, 6H; 3,5-phenyl), 8.36 (d, $J = 5.4$ Hz, 6H; 2,6-phenyl) 4.68 (s, 3H; NCH_3), 4.04 (s, 9H; OCH_3), −2.97 (s, 2H; NH pyrrole); UV/Vis (DMF): λ_{max} (ε) = 645 (3467), 589 (5755), 550 (7585), 515 (17378), 419 nm (316228 mol^{-1} dm^3 cm^{-1}); MS, EI (+ve): m/z: 805 [$M^+ - I^-$].

5-[3,4-(1,4,7,10,13,16-Hexaoxahexadecano)phenyl]-10,15,20-tris(4-pyridyl)porphyrin (7): A mixture of 4-formyl-benzo[18]crown-6 (1.29 g, 0.0038 mol), propionic acid (100 mL), and acetic anhydride (10 mL) was heated at 110 °C with stirring. Pyridine-4-carbaldehyde (1.07 mL, 0.011 mol) and pyrrole (1.04 mL, 0.015 mol) were successively and slowly added to this solution. The resulting mixture was heated under reflux for 1.5 h, allowed to cool to room temperature, and evaporated to dryness. The residue was neutralized with 1M aqueous ammonia, filtered through a glass frit, and washed several times with water. The crude material was extracted with a mixture of $CHCl_3$/EtOH (95:5) and purified by chromatography on a dry silica gel column. The desired product was isolated on elution with $CHCl_3$/CH_3OH (90:10). Evaporation of the solvent afforded **7** as purple powder (0.1 g; 3%). $R_f = 0.10$ (CH_2Cl_2/EtOH 9:8); ^{1}H NMR (400 MHz, $CDCl_3$, 300 K, TMS): $\delta = 9.04$ (m, 6H; 3,5-pyridine), 8.98 (d, $J = 4.8$ Hz, 2H; β-pyrrole), 8.84 (s, 4H; β-pyrrole), 8.82 (d, $J = 4.8$ Hz, 2H; β-pyrrole), 8.16 (m, 6H; 2,6-pyridine), 7.76 (d, $J = 2.4$ Hz, 1H; *o*-phenyl), 7.71 (dd, $J = 2.1$ Hz, 1H; *o*-phenyl), 7.22 (d, $J = 8.1$ Hz, 1H; *m*-phenyl), 4.45 (m, 2H; crown-CH_2), 4.30 (m, 2H; crown-CH_2), 4.13 (m, 2H; crown-CH_2), 3.98 (m, 2H; crown-CH_2), 3.92 (m, 2H; crown-CH_2), 3.83 (m, 4H; crown-CH_2), 3.78 (m, 6H; crown-CH_2), −2.86 (s, 2H; NH pyrrole); ^{13}C NMR ($CDCl_3$, 294 K): $\delta = 149.98$, 149.95, 149.23, 148.40, 148.38, 147.22, 135.92, 134.43, 129.38, 129.33, 128.05, 121.54, 120.93, 117.41, 116.96, 112.09, 71.03, 70.97, 70.89, 70.85, 70.81, 69.81, 69.70, 69.34; UV/Vis (CH_2Cl_2): λ_{max} (ε) = 645 (2290), 589 (4365), 549 (5248), 514 (13805), 417 nm (288403 mol^{-1} dm^3 cm^{-1}); MS EI (+ve): m/z: 853 [$M^+ + 1$].

5-[3,4-(1,4,7,10,13,16-Hexaoxahexadecano-2-enyl)phenyl]-10,15,20-tris(*N*-methyl-4-pyridinium)porphyrin (8): A large excess of methyl iodide (2 mL) was added to a stirred solution of porphyrin **7** (0.1 mmol) in DMF (5 mL). The mixture was stirred at 40 °C for 5 h, and the solvent was evaporated to dryness under reduced pressure. The residue was taken up in methanol and purified by ion-exchange with Dowex-1X8-200 resin (chloride form) after stirring at room temperature for 5 h. The solution was filtered, and the filtrate was concentrated under vacuum and precipitated by slow addition of diethyl ether to the methanol solution to obtain **8** (90%). ^{1}H NMR (600 MHz, D_2O, 347 K): $\delta = 9.25$ (d, $J = 7.0$ Hz, 2H; H15c), 9.17 (d, $J = 7.0$ Hz, 4H; H10,20c), 9.06, 9.03 (AB, $J_{AB} = 4.9$ Hz, 4H; H12,13,17,18), 8.95 (d, $J = 4.8$ Hz, 2H; H3,7), 8.88 (d, $J = 7.0$, 2H; H15b), 8.82 (d, $J = 4.9$ Hz, 2H; H2,8), 8.76 (d, $J = 7.0$, 4H; H10,20b), 7.68 (d, $J = 1.9$ Hz, 1H; H5b), 7.22 (dd, $J = 8,1$ and 1.9 Hz, 1H; H5f), 6.79 (8.13, $J = 8.1$ Hz, 1H; H5e), 4.78 (s, 3H; 15-NCH_3), 4.74 (s, 6H; 10,20-NCH_3), 4.044 (m, 2H; E1-CH_2), 4.018 (m, 2H; E10-CH_2), 3.737 (m, 2H; E9-CH_2), 3.656 (m, 2H; E7-CH_2), 3.635 (m, 2H; E8-CH_2), 3.612 (m, 2H; E5-CH_2), 3.588 (m, 2H; E2-CH_2), 3.587 (m, 2H; E6-CH_2), 3.539 (m, 2H; E3-CH_2), 3.511 (m, 2H; E4-CH_2); ^{13}C NMR (D_2O, 300 K): $\delta = 157.53$, 157.46, 148.19, 146.05, 143.57, 133.19, 132.73, 132.61, 132.49, 128.23, 123.39, 120.46, 114.73, 113.92, 111.42, 69.68, 69.56, 69.49, 69.31, 68.59, 68.52, 68.14, 68.02, 48.19, 48.12; UV/Vis (H_2O): λ_{max} (ε) = 643 (2345), 584 (6310), 563 (6166), 522 (11220), 425 nm (158490 mol^{-1} dm^3 cm^{-1}); MS EI (+ve): m/z: 899 [$M^+ + 2 - 3Cl^-$].

Metalated porphyrins:

Zn{5-[3,4-(1,4,7,10,13,16-hexaoxahexadecano)phenyl]-10,15,20-tris(N-methyl-4-pyridinium)porphyrin} (8(Zn)): Zinc acetate dihydrate, (26 mg, 117 μmol) and 2,4,6-collidine (34 μL, 240 μmol) were added to a solution of porphyrin **8** (10 mg, 11.7 μmol) in DMF (1 mL); the contents were heated in a sealed Eppendorf tube for 1 h at 90 °C. The mixture was allowed to cool to room temperature, a small amount of precipitate was removed by centrifugation, and the supernatant was precipitated with diethyl ether (5 mL). The ensuing pellet was dissolved in methanol (0.5 mL). Some material insoluble in methanol was removed. The soluble material was precipitated with diethyl ether (2 mL). This process was repeated . After drying, a dark green powder was obtained. Yield: 5 mg (50%); UV/Vis (H_2O): λ_{max} (ε) = 564 (8912), 437 nm (102330 mol^{-1} dm^3 cm^{-1}).

Cu{5-[3,4-(1,4,7,10,13,16-hexaoxahexadecano)phenyl]-10,15,20-tris(N-methyl-4-pyridinium)porphyrin} (8(Cu)): The synthesis of the copper derivative was carried out in a similar manner to that of the zinc derivative, with copper acetate in place of zinc acetate. Yield: 50%. UV/Vis (H_2O): λ_{max} (ε) = 548 (8910), 425 nm (123027 mol^{-1} dm^3 cm^{-1}).

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