Synthesis and Properties of New Cationic-Periphery Porphyrins, Tetrakis(*p*-(aminomethyl)phenyl)porphyrin and *N*-Methyltetrakis(*p*-(aminomethyl)phenyl)porphyrin

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Two new cationic periphery porphyrins, tetrakis(*p*-(aminomethyl)phenyl)porphyrin, TAMPP, and *N*-methyltetrakis(*p*-(aminomethyl)phenyl)porphyrin, *N*-MTAMPP, have been synthesized. These porphyrins are protonated in a pH range suitable for studies of binding with nucleic acids. The structure of the N-methylated analogue is significantly different, resulting in a phenonmenon that has not been previously reported: the aryl rings at the methine bridge positions of the porphyrin ring undergo restricted rotational motion. The ability of the aryl rings to rotate may be crucial to allow intercalation of cationic periphery porphyrins. The acidity constants and associated pK_a's for the porphyrins TAMPP and *N*-MTAMPP were determined by a combination of spectrophotometric and potentiometric methods. The acid–base properties of these new porphyrins are profoundly altered by N-methylation. The pyrroleninic nitrogen of the free base N-methylated analogue is significantly more basic (at an ionic strength of 0.12 M, the apparent pK_a's for deprotonation of TAMPP with all four central nitrogen atoms protonated and the form with three protonated are the same, 8.8, while for *N*-MTAMPP, these pK_a's are 2.4 and 8.1, respectively). As a result, the N-methylated species bears an additional positive charge in the neutral pH region where binding studies with nucleic acids are normally carried out. The peripheral amino groups of the N-methylated porphyrin are more basic as well, by 1.5 pH units at an ionic strength of 0.12 M.

The interactions of a variety of drugs and other relatively small molecules with DNA have been the focus of numerous studies.[1-3] Among these molecules, porphyrins with cationically charged peripheries have received particular attention.[4-11] We have been interested in the possibility of delivering specific alkyl groups to DNA by means of a unique porphyrin dealkylation process that can be selectively triggered by metal ions.[12] Our previous work has demonstrated that the cationic porphyrins currently available are unsuitable (vide infra). Hence, we have designed and synthesized a new cationic porphyrin which can be readily alkylated. In addition to its interactions with nucleic acids, which is the subject of another report, the acid–base and structural properties of these new porphyrins are of intrinsic interest and form the basis of this article.

Results

The synthetic scheme used to obtain two new cationic-periphery porphyrins, tetrakis(*p*-(aminomethyl)phenyl)-porphyrin (TAMPP) and *N*-methyltetrakis(*p*-(aminomethyl)phenyl)porphyrin (*N*-MTAMPP), is shown in Scheme I. It proceeds with an overall yield of 10% for TAMPP, **5a**, and 3% for *N*-MTAMPP, **5b**.

The ^{1}H NMR spectrum of *N*-MTAMPP at temperatures from 30 °C to 90 °C is given in Figure 1. All assignments were made by decoupling studies. It should be noted that peaks labeled S^1, D_3, and D_4 in the spectrum taken at 30 °C coalesce and are then resolved into a singlet and two doublets, S, D_c and D_a, as the temperature is raised to 90 °C. All resonances of aromatic protons move downfield with increasing temperature.

Figure 2 shows the series ^{1}H NMR spectra of a D_2O solution of TAMPP in a temperature range of 30–90 °C. The aromatic resonances move downfield with increasing temperature, as for *N*-MTAMPP. Assignments were made using decoupling experiments as well as spectral resolution that occurred with increasing temperature.

Acid–Base Properties. The acidity constants and associated pK_a's for the porphyrins TAMPP and *N*-MTAMPP were determined by a combination of spectrophotometric and potentiometric methods. From Figure 3, the plot of the absorbances at the wavelength maxima of the diprotonated and neutral forms of **5a**, TAMPP, vs pH, pH at the midpoint = 4.4, giving an apparent pK_a of 8.8. A similar plot at an ionic strength less than 0.01 M gives a midpoint pH of 3.6 and apparent pK_a of 7.2.

In contrast to the process observed for TAMPP, dissociation of diprotonated *N*-MTAMPP is a two-step process, as evidenced from the fact that two isosbestic points appear during dissociation of the diprotonated

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(1) *Chemistry and Physics of DNA–Ligand Interactions*; Kallenbach, N. R., Ed.; Adenine Press: Schnectady, NY, 1989.
(2) *Metal–DNA Chemistry*; Tullius, T. D., Ed.; ACS Symposium Series 402; American Chemical Society: Washington, DC, 1989.
(3) *DNA–Ligand Interactions From Drugs to Proteins*; Guschlbauer, W., Saenger, W., Eds.; NATO ASI Series 137; Plenum Press: New York, 1987.
(4) Fiel, R. J.; Howard, J. C.; Mark, E. H.; Datta Gupta, N. *Nucleic Acids Res.* **1979**, *6*, 3093.
(5) File, R. J.; Munson, B. R. *Nucleic Acids Res.* **1980**, *8*, 2835.
(6) Pasternack, R. F.; Gibbs, E. J.; Villafranca, J. J. *Biochemistry* **1983**, *22*, 2406.
(7) Pasternack, R. F.; Gibbs, E. J.; Villafranca, J. J. *Biochemistry* **1983**, *22*, 5409.
(8) Pasternack, R. F.; Antebi, A.; Ehrlich, B.; Sidney, D.; Gibbs, E. J.; Bassner, S. L.; Depoy, L. M. *J. Mol. Catal.* **1984**, *23*, 235.
(9) Carvlin, M. J.; File, R. J. *Nucleic Acids Res.* **1983**, *11*, 6121.
(10) Carvlin, M. J.; Mark, E.; File, R. *Nucleic Acids Research* **1983**, *11*, 6141.
(11) Sari, M. A.; Battioni, J. P.; Mansuy, D.; Lepecq, J. B. *Biochem. Biophys. Res. Commun.* **1986**, *141*, 643.
(12) Lavallee, D. K. *The Chemistry and Biochemistry of N-Substituted Porphyrins and Metalloporphyrins*; VCH Publishers: New York, 1987, 313 pp.

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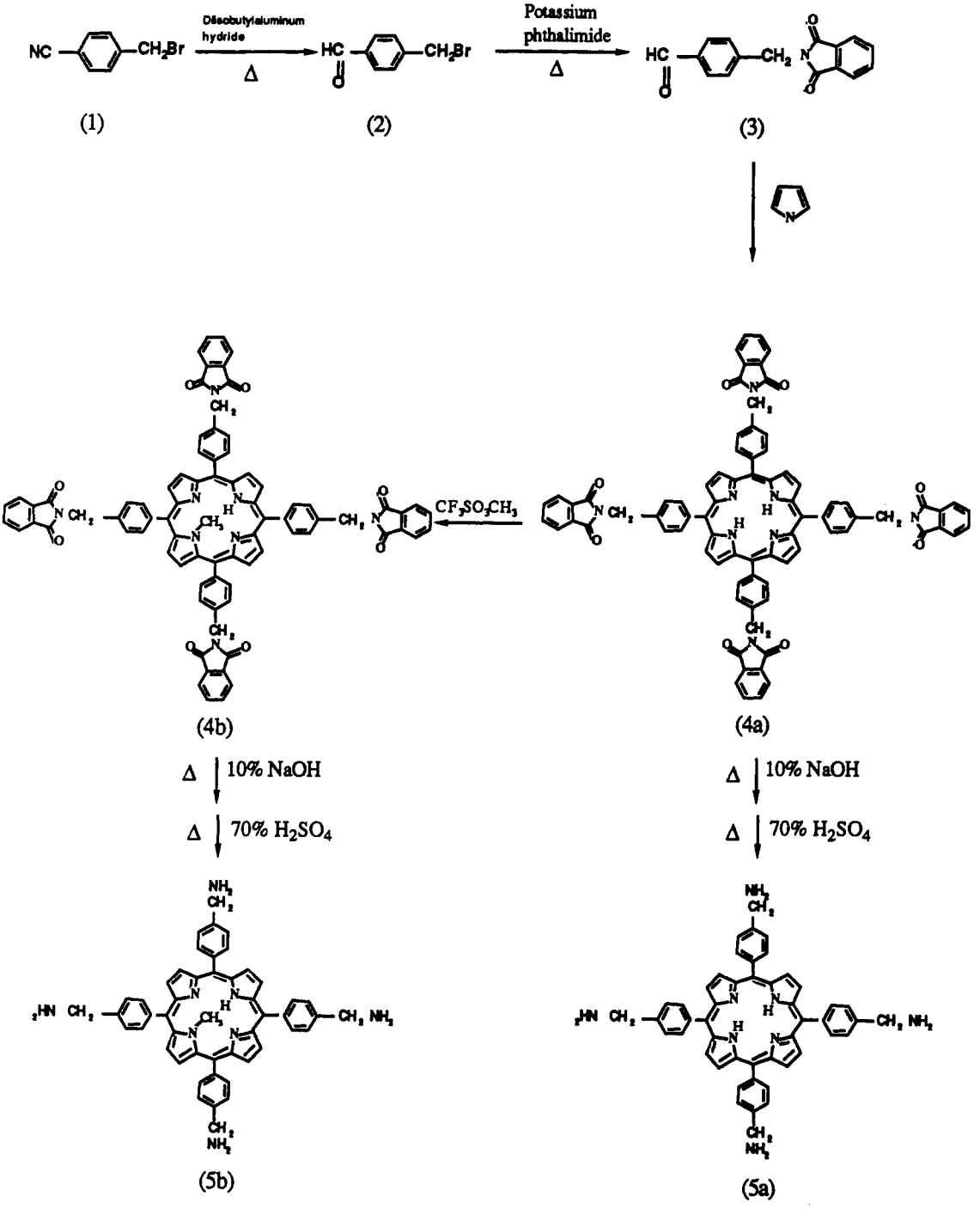
Scheme I



porphyrin to form the free porphyrin (Figure 4). From the spectral changes in Figure 4, the pK_a for the deprotonation of the porphyrin with all four porphyrin ring nitrogens protonated, pK_4, = 3.1 and the pK_a for the deprotonation of the porphyrin with three porphyrin ring nitrogens protonated, pK_3, = 8.1 when the ionic strength = 0.12 M, and pK_4 = 2.4 and pK_3 = 7.4 at an ionic strength less than 0.01 M.

The potentiometric titration for TAMPP is shown in Figure 5 The apparent pK_a of TAMPP is 8.5 ± 0.3, at an ionic strength of 0.12 M and 6.9 ± 0.3 at an ionic strength less than 0.01 M.

For *N*-MTAMPP, interpretation of the potentiometric titration is somewhat more complex because of the separation and overlap of the hydrolysis of the porphyrin ring nitrogen atoms and the peripheral amines. At the first titration end point, the hydrolysis of MPH_2^+ is significant and $[Por\text{-}NH_3^+] \approx 4C_{tot}(N\text{-MTAMPP}) - 0.5[H^+]$ and $[MPH_2^+] \approx C_{tot}(N\text{-MTAMPP}) - 0.5[H^+]$. The extent of dissociation of MPH_2^+ (pK_3 = 8.1 at an ionic strength of 0.12 M and 7.4 when the ionic strength is <0.01 M) and of $Por\text{-}NH_3^+$ is quite similar (pK_a = 7.7 at an ionic strength of 0.12 M and 7.0 at an ionic strength <0.01 M).

The Acidity Constant of Peripheral Amino Groups. The acidities of the peripheral *p*-(aminomethyl)phenyl moieties were determined potentiometrically using the expression for TAMPP and the expression

$$K_a = \frac{K_w(4[TAMPP]_{tot} - [OH^-])}{[OH^-]^2}$$

for *N*-MTAMPP, in which the basicities of the central

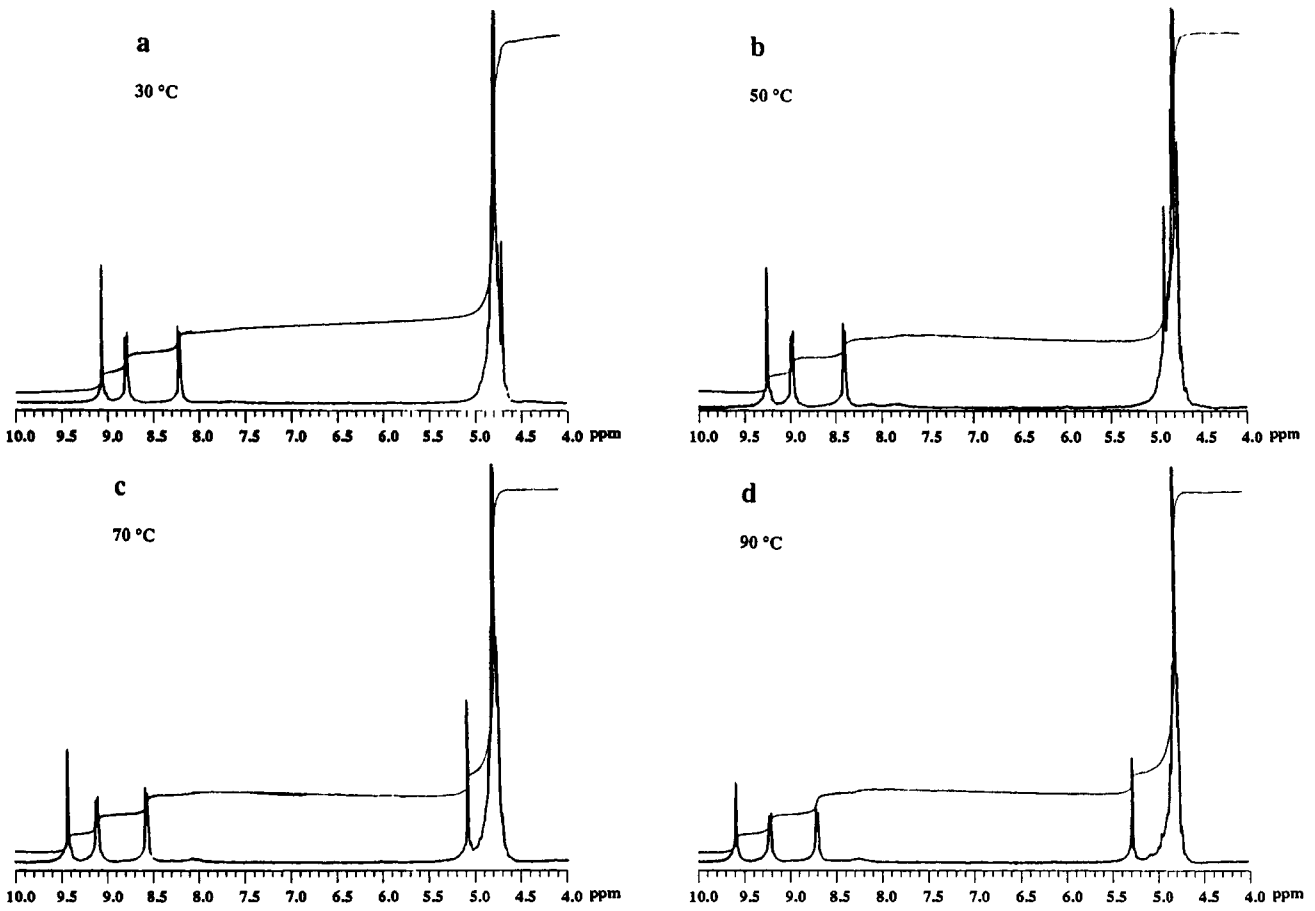


Figure 1. 300-MHz ^{1}H NMR spectra (downfield region) of 5,10,15,20-tetrakis(*p*-(aminomethyl)phenyl)porphine in D_2O at 30, 50, 70, and 90 °C.

nitrogen atoms and the peripheral amino groups are similar.

$$K_a = \frac{K_w(4[\text{N-MTAMPP}]_{tot} - 0.5[OH^-])}{[OH^-](0.5[OH^-] - [H^+])}$$

The expressions are based on the following assumptions: (1) the dissociation of four groups must be treated as an average, (2) for TAMPP, the amount of OH^- contributed by hydrolysis of PH_2 can be neglected compared with the hydrolysis of Por-NH_2, and (3) for *N*-MTAMPP, the amounts of OH^- contributed by hydrolysis of PH_2 and of Por-NH_2 are about same. The results are given in Table II. The *p*-(aminomethyl)phenyl groups of the methylated porphyrin are considerably more basic: by 1.5 units at an ionic strength of 0.12 M and by 0.6 units at an ionic strength less than 0.01 M.

The average apparent pK_a, pK_3, pK_4, and pK_a values for TAMPP and *N*-MTAMPP at ionic strength 0.12 M and less than 0.01 M based on the spectrophotometric and potentiometric methods are summarized in Table III. The numbers shown after "±" are the corresponding uncertainties which are specified as the average deviation from the mean for the various acidity constants. The pK_3 and pK_a values of *N*-MTAMPP may not be as accurate because of the occurrence of deprotonation equilibria of the pyrroleninic nitrogen and peripheral amine positions in the same pH region. However, the data are sufficiently definitive for us to identify the nature of species present in the studies of binding with nucleic acids.

Discussion

Synthesis. The synthetic scheme to produce a new cationic-periphery porphyrin that could be readily modified both at the porphyrin ring nitrogens and at the periphery was based on literature precedents as follows. The initial step, conversion of commercially available α-bromo-*p*-tolunitrile to the corresponding aldehyde by reduction of the tolunitrile[13] followed by replacement of the bromine atom with a phthalimide group, provided a precursor for condensation with pyrrole to produce a tetrakis(*p*-(phthalimidomethyl)phenyl)porphyrin.[14] The pyrroleninic nitrogen atoms of this porphyrin can be alkylated, in this case methylated by methyl trifluoromethanesulfonate,[15] without reaction at the periphery. After this step, the phthalimido groups were removed by hydrolysis. In this case, the typical method using hydrazine[16-18] was unsuccessful, and a combination of alkaline followed by acidic hydrolysis[19] to convert the imide to an amide to an amine sequentially was required.

Structure: Aryl Substitutent Rotation. The complex NMR spectrum of *N*-MTAMPP required a greater number of inequivalent protons than could be rationalized for a system in rapid rotational equilibrium. In most cases,

(13) Baker, J. W.; Brieux, J. A. L.; Saunders, D. G. *J. Chem. Soc.* **1956**, 404
(14) Adler, A. D.; Longo, F. R.; Finorelli, J. D.; Goldmacher, J.; Assour, J.; Korsakoff, L. *J. Org. Chem.* **1976**, *32*, 476.
(15) Lavallee, D. K.; Gebala, A. E. *Inorg. Chem.* **1974**, *19*, 2004.
(16) Smith, L. I.; Emerson, O. H. *Org. Synth. Collect.* **1955**, *3*, 151.
(17) Sheehan, J. C.; Bolhofer, W. A. *J. Am. Chem.Soc.* **1950**, *72*, 2786.
(18) Sasaki, T.; Minomato, K.; Itoh, H. *J. Org. Chem.* **1978**, *43*, 2320.
(19) Buethler, C. A.; Pearson, D. E. *Surv. of Org. Synth.* **1970**, 438.

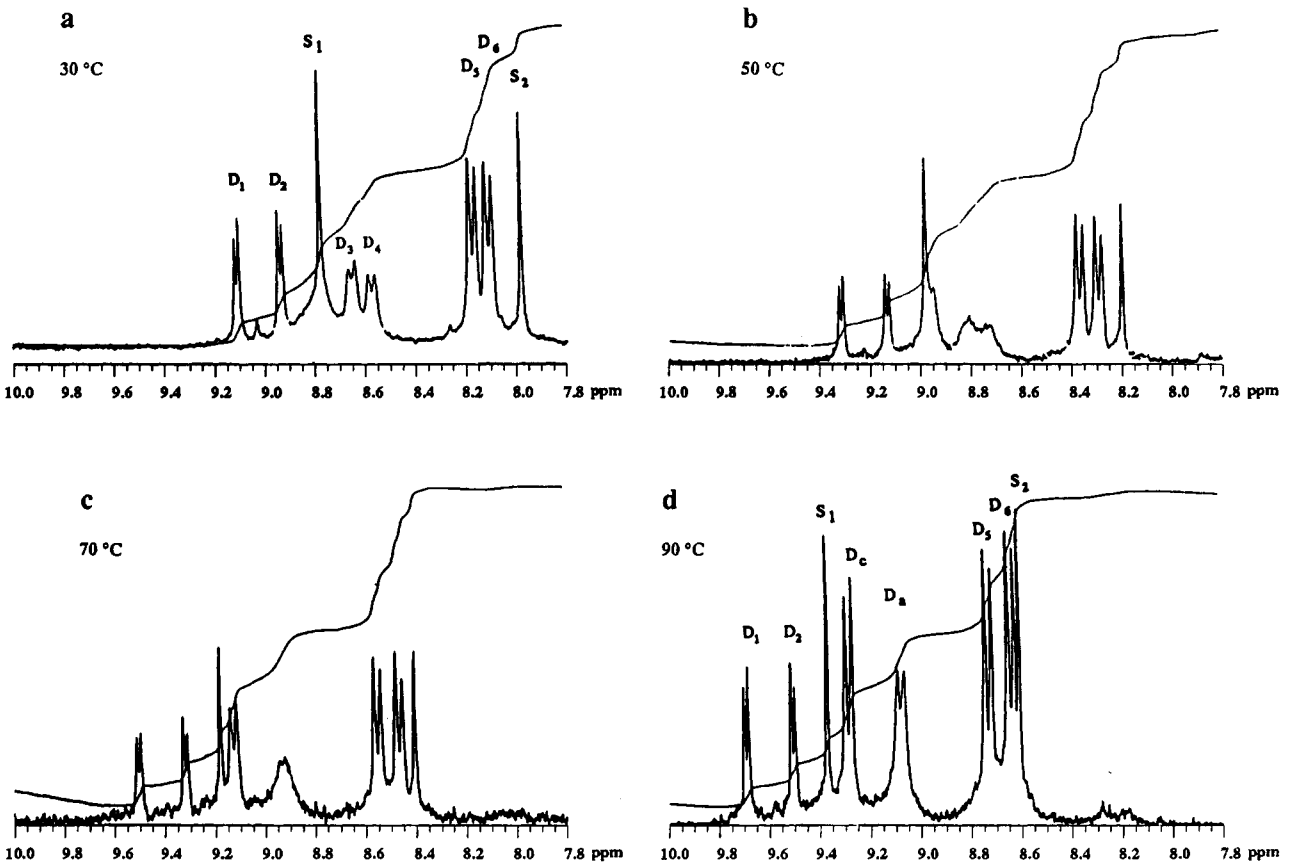


Figure 2. 300-MHz ^{1}H NMR spectra (downfield region) of *N*-21-methyl-5,10,15,20-tetrakis(*p*-(aminomethyl)phenyl)porphine in D_2O at 30, 50, 70, and 90 °C.

aryl ring rotation is too fast at ambient temperature to be observed by broadening of signals in the NMR spectrum, but a number of examples have been reported in which the rotation is slowed because of special steric hindrance.[20] For example, a large metal ion can force the porphyrin ring system to become nonplanar, leading to a constriction of the region available to the aryl substituents.[21] The series of ^{1}H NMR spectra of a D_2O solution of TAMPP (5a) in a temperature range of 30–90 °C (Figure 1) demonstrates that, when the temperature is increased, the chemical shifts move downfield, consistent with the benzyl groups of **5a** preferentially remaining nearly perpendicular to the porphyrin ring at room temperature and spending more time more nearly parallel to the aromatic porphyrin ring at higher temperatures. The ^{1}H NMR spectrum of TAMPP at any temperature can be interpreted as arising from a system of high symmetry in which the aryl rings rotate rapidly on the NMR time scale.

The complexity of the ^{1}H NMR spectra of the N-methylated porphyrin at ambient temperature, Figure 2, however, requires a greater number of dissimilar protons, a situation consistent with relatively slow rotation of the benzyl groups on the exterior of the porphyrin ring. The proton chemical shift differences of the two ortho hydrogen atoms of each benzyl group should be different if one occupies a position on the side of porphyrin ring where the methyl group is located and the other is on the side without the methyl group. The meta hydrogen atoms are less affected by the difference due to the N-substitution because they are further from the porphyrin ring.

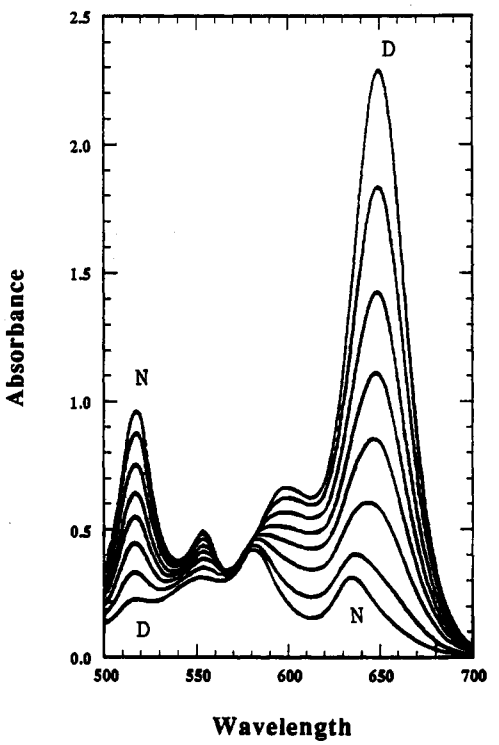


Figure 3. Visible absorption spectra during the titration of 5,10,15,20-tetrakis(*p*-(aminomethyl)phenyl)porphine at an ionic strength of 0.12 M (N = neutral, D = fully protonated central nitrogens).

Peak assignments made from decoupling experiments and comparison of spectra taken over a temperature range of 30–90 °C are consistent with restricted aryl group

(20) Eaton, S. S.; Eaton, G. R.; Holm, R. H. *J. Organomet. Chem.* **1972**, *139*, 179.
(21) Eaton, S. S.; Eaton, G. R. *J. Am. Chem. Soc.* **1979**, *99*, 6594.

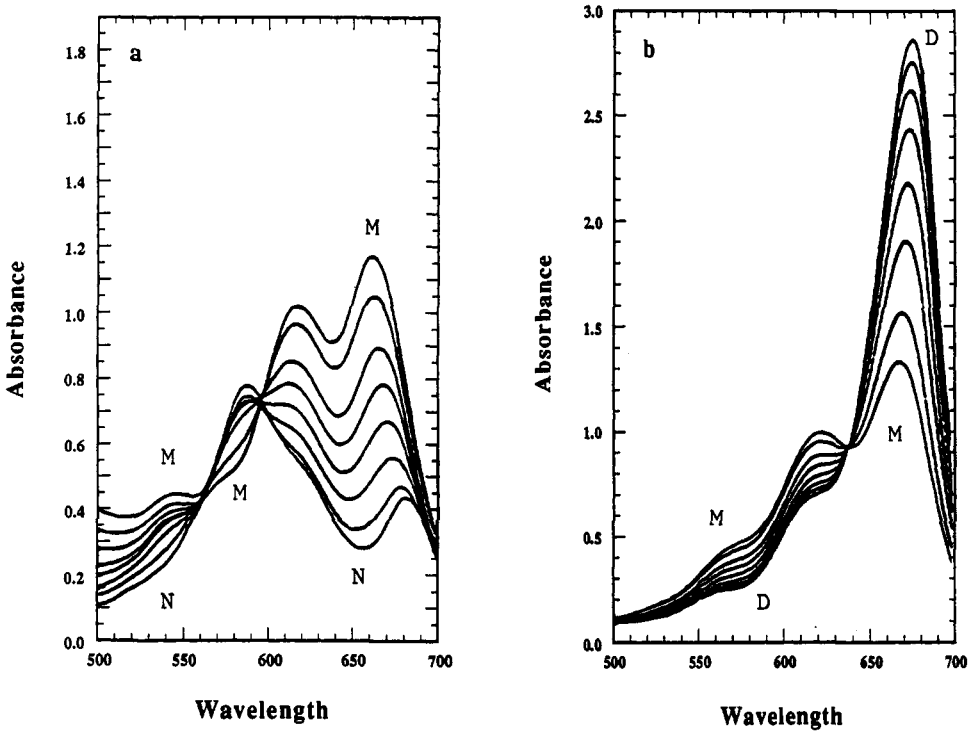


Figure 4. Visible absorption spectra during the titration of *N*-21-methyl-5,10,15,20-tetrakis(*p*-(aminomethyl)phenyl)porphine, [*I*] = 0.12 M (N = neutral, M = one additional protonated central nitrogen, D = two additional protonated central nitrogens).

Table I. Acidity Constants K_{app} and K_a for TAMPP

	[*I*] = 0.12 M				[*I*] < 0.01 M				
ionic strength expl methods	spectrophotometric	potentiometric			spectrophotometric		potentiometric		
pK_{app}	9.0	8.1	8.7	8.7	7.4	7.2	6.4	7.3	7.0
($PH_4^{2+} \overset{K_{app}}{\rightleftharpoons} PH_2 + 2H^+$)									
pK_a		6.0	6.5	6.2			5.7	6.9	6.6
($Por\text{-}NH_3^+ \overset{K_a}{\rightleftharpoons} Por\text{-}NH_2 + H^+$)									

rotation. From the structure of N-methylated porphyrin (for lettering scheme, see Figure 6), which can be accurately predicted from the crystallographic structure determination for a very similar porphyrin, *N*-methyl-5,10,15,20-tetrakis(*p*-bromophenyl)porphine,[22] it is very possible that hydrogen atoms a and a′ are responsible for the signal of the D_3 and D_4 peaks while hydrogen atoms b are responsible for the signal of the D_6 peak, since when D_3 or D_4 was decoupled the doublet peak D_6 was only partly affected (only half of the b hydrogen atoms experience a difference environment after hydrogen atoms a and a′ were decoupled) and when D_6 was decoupled both D_3 and D_4 changed to singlets.

One assignment, however, is not evident from these spectra, i.e., the location of the peak or peaks due to hydrogen atoms c and c′. This assignment can be made from a comparison of the spectra of the N-methylated intermediate which has the peripheral amine groups protected as phthalimide groups, **4b** in Scheme I, and *N*-MTAMPP (**5b**). This spectral comparison and decoupling and high temperature experiments demonstrate that hydrogen atom c is different from hydrogen atom c′ but that this difference is much smaller than the difference between hydrogen atoms a and a′.

The benzyl groups which are farther away from the *N*-methyl group and give rise to peak D_c in the NMR spectrum of **4b** change from slow rotation at room temperature (a broad D_c peak) to a faster rotation at higher temperature (a "doublet" D_c peak), while the benzyl groups giving rise to D_3 and D_4 change from rotation that is slower than the NMR time scale at room temperature (two doublet peaks) to rotation at the rate corresponding to the NMR time scale at higher temperature (a broad peak D_a).

Restricted rotation is responsible for the appearance of D_c, D_3, and D_4 in the NMR spectrum of *N*-MTAMPP at ambient temperature. Comparison of the series of ^1H NMR spectra of D_2O solutions of *N*-MTAMPP (**5b**) recorded at 70 and 90 °C with that at 30 °C (Figure 2) shows that the D_c resonance is hidden by D_2 at ambient temperature but is separate and occurs as a doublet at higher temperature, while D_3 and D_4 gradually converge and eventually combine to give a doublet. The coupling relationship between D_c, D_5 and D_a, D_6 pairs were confirmed by high-temperature decoupling experiments. When D_c was decoupled, D_5 became a singlet, and when D_a was decoupled, D_6 became a singlet. As temperature is increased, all the ^1H chemical shifts of porphyrin *N*-MTAMPP (**5b**) move gradually downfield, as in the case of TAMPP.

(22) Lavallee, D. K.; Anderson, O. P. *J. Am. Chem. Soc.* **1982**, *68*, 1112.

Table II. Aciditiy Constants K_4, K_3, and K_a for *N*-MTAMPP

	[I] = 0.12 M					[I] < 0.01 M				
ionic strength exptl methods	spectrophotometric		potentiometric			spectrophotometric		potentiometric		
pK_4	3.1	3.1				2.4	2.4			
$(PH_4^{2+} \overset{K_4}{\rightleftharpoons} PH_3^+ + H^+)$										
pK_3	8.1	8.1				7.4	7.4			
$(PH_3^+ \overset{K_3}{\rightleftharpoons} PH_2 + H^+)$										
first end point pH[a]	6.1	6.1	6.1	6.0	6.0	5.8	5.8	6.0	6.0	6.0
pK_a			7.8	7.7	7.5			6.9	7.3	6.9
$(Por\text{-}NH_3^+ \overset{K_a}{\rightleftharpoons} Por\text{-}NH_2 + H^+)$										

[a] For the spectrophotometric titration method: the first end point is calculated from the corresponding pK_4 and pK_3 by the equation

$$[H^+]^2 = \frac{K_w + (C_{tot} - 1/2[H^+])K_3}{1/2 + (C_{tot} - 1/2[H^+])/K_4}$$

For the potentiometric titration method, the first titration end point is read from experimental curves of pH vs V_{NaOH} and $\Delta pH/\Delta V'$ NaOH vs V' NaOH.

Table III. Average Acidity Constants K_4, K_3, K_{app}, and K_a for TAMPP and *N*-MTAMPP

	TAMPP		N-MTAMPP	
porphyrin ionic strength	[I] = 0.12 M	[I] < 0.01 M	[I] = 0.12 M	[I] < 0.01 M
pK_4			3.1 ● (0.1)	2.4 ± (0.1)
pK_3			8.1 ● (0.1)	7.4 ± (0.1)
pK_{app}	8.6 ● (0.3)	7.0 ± (0.3)	11.2 ± (0.2)	9.8 ± (0.2)
pK_a	6.2 ± (0.2)	6.4 ● (0.4)	7.7 ± (0.1)	7.0 ± (0.2)

From these experiments we conclude that at ambient temperature (1) two of the four benzyl groups of *N*-MTAMPP, probably those closest to the *N*-methyl group, rotate slower than the NMR time scale, (2) the other two benzyl groups of *N*-MTAMPP rotate slowly on the NMR time scale, and (3) the average position of the benzyl rings of TAMPP and *N*-MTAMPP is more nearly perpendicular to the porphyrin ring at room temperature than at higher temperature.

Acid–Base Properties. In this work, both spectrophotometric and potentiometric titration methods were applied to determine apparent pK_a (for TAMPP), pK_3, and pK_4 (for *N*-MTAMPP) values. These values are of intrinsic interest with respect to other cationic-periphery porphyrins and with respect to the effect of N-alkylation. In addition, they are necessary to establish the nature of the species present in the binding studies with polynucleic acids.[28] Data were obtained at two ionic strengths, 0.12 M and less than 0.01 M, to allow comparison with other porphyrins that have been used in nucleic acid binding studies and porphyrins that have been studied for other reasons.

Potentiometric titrations were used to confirm the results derived from spectrophotometric methods and also to determine the acidity of amino groups outside of the porphyrin ring. Protonation of these peripheral groups does not result in useful spectroscopic changes in the UV–visible region.

It is clear from Tables I and II that the experimental results are satisfactorily reproducible and the two titration methods give consistent results. The two cationic periphery porphyrins, TAMPP and *N*-MTAMPP, differ significantly in their basicity and the stability of their monoprotonated species. *N*-Alkyl substituents can cause an appreciable increase in basicity, as observed for the coproporphyrin pair,[23] tetraphenylporphyrin pair,[24,25] and the octaethylporphyrin pair,[26] as well ($pK_3 = 0$ for non-N-methylated octaethylporphyrin and $pK_3 = 4.1$ for its N-methylated derivative). The increase in basicity is attributed to the fact that an N-alkylated porphyrin is distorted from planarity and loses less resonance energy upon protonation than a more planar non-N-alkylated porphyrins.

The average pK_a for the peripheral amino groups of *N*-MTAMPP is about one unit larger than the pK_a of TAMPP (Table III). The structural difference between the folded porphyrin ring of *N*-MTAMPP and the flatter ring of TAMPP causes the rotational energy barriers of the *meso* aryl groups of the N-MTAMPP ring to be larger than those of the *meso* aryl groups of TAMPP. The *meso* aryl groups of TAMPP are more likely to exhibit configurations in which the phenyl ring is nearly coplanar with the aromatic porphyrin ring than is the case for *N*-MTAMPP. The greater conjugation of the lone pair electrons on the nitrogens of the amino groups of TAMPP with its porphyrin ring leads to the weaker basicity for free amino group of TAMPP.

The third characteristic shown by Tables III is that the apparent K_a value of TAMPP and pK_3, pK_4 values of *N*-MTAMPP are about one unit less when the ionic strength of solution is less than 0.01 M than when the ionic strength of solution is equal to 0.12 M. The stabilization of the protonated periphery by a greater concentration of chloride ions could lead to the greater basicity at a higher ionic strength.

The presence or absence of an *N*-methyl group changes the absolute value of the basicity of the peripheral

(23) Neuberger, A.; Scott, J. J. *Proc. R. Soc. London* **1952**, *A213*, 307.
(24) Aronoff, S. *J. Phys. Chem*, **1958**, *62*, 428.
(25) Lavallee, D. K.; Gebala, A. E. *Inorg. Chem.* **1974**, *13*, 2004.
(26) Grigg, R.; Hamilton, R. J.; Josefowicz, M. L.; Rochester, C. H.; Terrell, R. J.; Wicker, H. *J. Chem. Soc., Perkin Trans. 2* **1973**, 407.
(27) Conant, J. B.; Chow, B. F.; Dietz, E. M. *J. Am. Chem. Soc.* **1934**, *56*, 2185
(28) Submitted for publication and International Microfiche, Ann Arbor, MI; Z. Xu, PhD Dissertation, City University of New York, 1990.

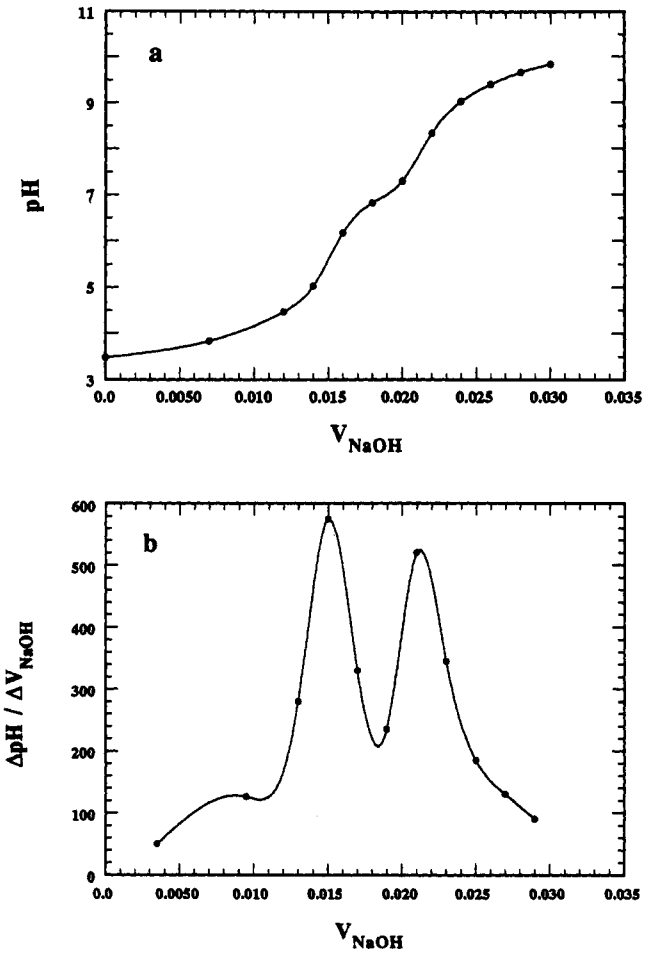


Figure 5. (a) Potentiometric titration curve of tetrakis(*p*-(aminomethyl)phenyl)porphyrin (TAMPP) and (b) differential curve of a, [*I*] = 0.12 M; (•) experimental data, (–) calculated curve.

(aminomethyl)phenyl groups, but no large differential effect due to changes in ionic strength is expected. The observed difference in the average acidity constants for the peripheral amino groups because of the change in ionic strength is, in fact, within the range of reproducibility of measurement at either ionic strength condition.

Conclusion

Two new cationic periphery porphyrins have been synthesized and fully characterized. These porphyrins are protonated in a pH range suitable for studies of binding with nucleic acids. Addition of a methyl group to one of the pyrroleninic nitrogen atoms has several important effects that could bear upon the interaction of these species with nucleic acids.

First, the structure of the N-methylated analogue is significantly different. The porphyrin ring is undoubtedly distorted in a manner similar to other N-alkylated porphyrins. In this case there is an additional consequence of N-alkylation that has not been previously reported: the aryl rings at the methine bridge positions of the porphyrin ring undergo restricted rotational motion. In the cases of porphyrins which intercalate into DNA, such as those reported by Pasternack and co-workers, *vide infra*, the ability of the aryl rings to rotate may be crucial to allow intercalation.

The acid–base properties of the porphyrin reported herein are profoundly altered by N-methylation. The

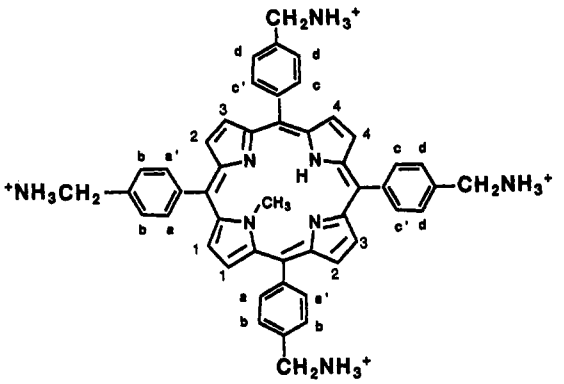


Figure 6. Numbering scheme of *N*-21-methyl-5,10,15,20-tetrakis(*p*-(aminomethyl)phenyl)porphine, protonated form.

pyrroleninic nitrogen of the free base N-methylated analogue is significantly more basic. As a result, the N-methylated species bears an additional positive charge in the neutral pH region where binding studies with nucleic acids are normally carried out.

Experimental Section

The following starting materials: α-bromo-*p*-tolunitrile, calcium hydride, diisobutylaluminum hydride as a 1 M solution in methylene chloride, pyrrole, and barium oxide were purchased from Aldrich Chemical Co., Inc. Barium hydroxide, methylene chloride, as well as other organic solvents were purchased form Fisher Scientific Co.

Proton NMR spectra were run on a GE QE-300 spectrometer, equipped with a decoupling unit and a variable-temperature probe. Spectra were obtained by locking onto the specific solvent resonances for different solvents using the frequency lock system of the instrument. Typical parameters were as follows: LB, 0.5; Tip angle, 60; scan number, 80; NA, 80; L_2, 2830; solvent, D_2O or $CDCl_3$.

To determine the apparent molecular weights of the porphyrins from NMR line intensities, tetrakis(1-methyl-4-pyridyl) porphine as the tetra-*p*-tosylate salt (TMPyP-4), purchased from Aldrich Chemical Co., Inc., was used as a standard.

Visible absorption spectra were taken on the Beckman Du-8 spectrophotometer. Mass spectrometry experiments were run by Mass Spectrometric Biotechnology Resource of Rockefeller University. The elemental analysis experiments were conducted by Analytical Laboratories GMBH in Engelskirchen, Germany.

Synthesis of Tetrakis(*p*-(aminomethyl)phenyl)porphyrin and *N*-methyltetrakis(*p*-(aminomethyl)phenyl)porphyrin. Synthesis of the 4-Formylbenzyl Bromide (2) from α-Bromo-*p*- tolunitrile (1). α-Bromo-*p*-tolunitrile was dissolved in freshly distilled methylene chloride (dried overnight with CaH_2) at the ratio of 1 g/3 mL (for example, 50 g of α-bromo-*p*-tolunitrile in 150 mL of methylene chloride). After N_2 was bubbled into the stirred solution for 30 min, an equimolar amount of diisobutylaluminum hydride (1.0 M in methylene chloride) solution (255 mL) was added slowly using a syringe. *Care must be taken to avoid contact with moisture, including moist air, or the solution will ignite.* The solution was cooled in an ice bath during mixing. The reaction solution was warmed at 45 °C for 4 h. The reaction mixture was cooled, and the remaining diisobutylaluminum hydride can be decomposed by acidifying the solution carefully with 10% H_2SO_4 until the pH is below 4. The mixture was stirred overnight at room temperature, and the aldehyde was extracted with CH_2Cl_2 several times. The organic mixtures were combined and dried over $MgSO_4$, filtered, and concentrated under reduced pressure. This crude product was recrystallized with hexane. The purified yield is about 60%.

The resulting 4-formylbenzyl bromide (2) is obtained as white needle crystals, and its melting point is 98–99°C (lit.[13] 96 °C). ^1H NMR spectrum peak assignments: aldehyde, 1H, s, 10.06 ppm; benzyl, 2 2H, d, 7.92 and 7.61 ppm methylene, 2H, s, 4.56

ppm. Anal. Calcd for C_8H_7OBr: C, 48.3; H, 3.5; O, 8.0; Br, 40.2%. Found: C, 48.3; H, 3.4; O, 7.9; Br, 40.1.

Synthesis of 4-(Formylbenzyl)phthalimide (3) from 4-formylbenzyl Bromide (2). Equimolar amounts of 4- formylbenzyl bromide (5 g) and potassium phthalimide (4.6 g) were dissolved completely in a minimum amount of *N,N*- dimethyl formamide (DMF) (about 200 mL) that was dried overnight (by BaO) and freshly distilled. The system was refluxed for 4 h and then cooled to room temperature. Because the product is soluble in DMF as well as in CH_2Cl_2, but not in water, water was added and the product extracted with CH_2Cl_2 several times. After most of the CH_2Cl_2 was removed by rotary evaporation, the CH_2Cl_2 layer was extracted with water several times to remove the remaining DMF. Then, the product was dried and recrystallized from a mixture of hexane and methylene chloride (9:1). Yield: ~90%. Mp: 132–133 °C. ^{1}H NMR peak assignments: aldehyde, 1H, s, 10.06 ppm; methylene, 2H, s, 4.97 ppm; benzyl, 2 2H, d, 7.90 and 7.63 ppm; phthalimido, 2 2H, q, 7.91 and 7.80 ppm. Anal. Calcd for $C_{16}H_{11}O_3N$ (**3**): C, 72.4; H, 4.2; N, 5.3; O, 18.1%. Found: C, 72.4; H, 4.2; N, 5.4; O, 18.0.

Synthesis of Tetrakis(*p*-(phthalimidomethyl)phenyl)-porphyrin (4a) from (4-Formylbenzyl)phthalimide (3). The standard method of porphyrin synthesis[14] was used. The crude porphyrin was purified by alumina column chromatography (1 g of crude porphyrin required ~0.5 L of aluminum gel) slowly eluted with CH_2Cl_2 solution. The yield was 26%.

The molecular weight of tetrakis(*p*-(phthalimidomethyl)-phenyl) porphyrin (**4a**) determined from mass spectrometry is 1251.7, which agrees well with the molecular weight (1251.3) calculated from its structure. ^{1}H NMR peak assignments: β-pyrrolenine, 8H, s, 8.81 ppm; pyrrolenine central nitrogen, 2H, s, −2.85 ppm. Extinction coefficients of product **4a**: $\epsilon_{647.3} = 4.58 \times 10^3$ M^{-1} cm^{-1}, $\epsilon_{590.1} = 4.66 \times 10^3$ M^{-1} cm^{-1}, $\epsilon_{549.8} = 7.53 \times 10^3$ M^{-1} cm^{-1}; $\epsilon_{515.6} = 1.54 \times 10^4$ M^{-1} cm^{-1}. Anal. Calcd for $C_{80}H_{50}N_8O_8$ (**4a**): C, 76.8; H, 4.0; N, 9.0; O, 10.2. Found: C, 76.5; H, 4.2; N, 8.8; O, 10.4.

Synthesis of *N*-methyltetrakis(*p*-phthalimidomethyl)-phenyl)porphyrin (4b) from Tetrakis(*p*-(phthalimidomethyl)phenyl)porphyrin (4a). The porphyrin (1 g) was dissolved completely in CH_2Cl_2 (about 500 mL). Then 15% molar excess of methyl trifluoromethanesulfonate ($CF_3SO_3CH_3$) in CH_2Cl_2 (0.1 mL $CF_3SO_3CH_3$ in 5 mL CH_2Cl_2) was added slowly drop by drop while the porphyrin solution was stirred. The mixture was refluxed for about 8 h and then cooled and reduced in volume using a rotary evaporator. The best separation of non-, mono-, and dimethylated porphyrins was obtained using two columns. First, a silica gel column was used (1 g of crude product to 150 mL of silica gel slurry). The nonmethylated porphyrin was eluted first using 2% acetone/98% methylene chloride solution. After the first band (unreacted porphyrin) was removed from the column, the percentage of acetone was increased to 5% and then 10% to elute mostly mono- and some dimethylated porphyrin. Second, the crude mono-N-methylated porphyrin obtained from silica gel was dissolved in CH_2Cl_2 containing about 5 mL of 2,6-lutidine and was eluted from an alumina column. The crude product was eluted first with pure methylene chloride to remove any remaining lutidine and second with a mixture of acetone and methylene chloride solution in a 1:19 ratio. Under these conditions, the neutral mono-methylated porphyrin was eluted first. The yield was about 30%.

^{1}H NMR peak assignments: positions 1 and 4 β-pyrrolenine, 2H, s, 7.40 and 8.78 ppm; position 2 and 3 β-pyrrolenine, 2H, d, 8.41 and 8.59 ppm; methylene, 8H, s, 5.20 ppm; *N*-methyl, 3H, s, −4.20 ppm, from *N*-methyl hydrogen atoms. The molecular weight of **4b** calculated from its structure is 1265.3 and the one determined from mass spectrometry is 1265.2. Extinction coefficients of product **4b**: $\epsilon_{677.3} = 5.32 \times 10^3$ M^{-1} cm^{-1}, $\epsilon_{576.4} = 1.45 \times 10^4$ M^{-1} cm^{-1}, $\epsilon_{534.8} = 8.35 \times 10^3$ M^{-1} cm^{-1}. Anal. Calcd for $C_{81}H_{52}N_8O_8$ (**4b**): C, 76.9; H, 4.1; N, 8.9; O, 10.1. Found: C, 76.1; H, 4.4; N, 8.6; O, 11.0.

Synthesis of Tetrakis(*p*-(aminomethyl)phenyl)porphyrin (TAMPP) (5a) from Its Corresponding Phthalimido-Protected Porphyrin 4a. The phthalimido groups in compound **4a** was removed in two steps. In the first hydrolysis step, the phthalimido porphyrin **4a** (500 mg) was dissolved in dioxane (500 mL). Then a 1:1 mixture of methanol and water was added (1/3 of the volume of dioxane). The solution was stirred and heated to about 50 °C and all solid material completely dissolved. Then, 5 N NaOH solution (74 mL) was added drop-by-drop into the reaction system until the final concentration of NaOH was 0.5 N. The system was refluxed for 15–30 min and then cooled. About 370 mL of water was added. Then the unreacted original porphyrin and dioxane were removed by extracting the solution with methylene chloride several times. The water layer was evaporated to dryness with a rotary evaporator.

In the second hydrolysis step, the crude phthalimido porphyrin was dissolved in a minimum amount (not more than 50 mL) of 70% H_2SO_4 and refluxed for 2–3 h. The system was cooled to room temperature and neutralized by adding saturated barium hydroxide solution slowly. During this process, the mixture was immersed in an ice bath and constantly stirred until the solution reached neutrality, precipitating barium sulfate. As the system was acidified with 5% HCl solution, the color of the upper solution gradually changed from colorless to green as the porphyrin dissolved from the surface of the precipitate. The supernatant was decanted and the large volume of precipitate washed several times with 5% HCl solution until the water layer showed only a slight green coloration. All of the acidic aqueous washes were collected and the water removed using a rotary evaporator. The resulting paste was dissolved with 5% HCl solution and filtered through a fine mesh sintered glass funnel. The filtrate was made basic by adding concentrated aqueous ammonia. This basic solution was extracted with CH_2Cl_2 several times until the water layer was almost colorless. After rotary evaporation, the CH_2Cl_2 layer was reduced to a small volume (about 50 mL) and washed with distilled water several times to remove all salts and dissolved ammonia. The CH_2Cl_2 layer was reduced to dryness using a rotary evaporator and acidified with a 10% aqueous HCl solution. The acidic aqueous solution was washed with CH_2Cl_2 again to remove unhydrolyzed porphyrin and any other organic material. Then the water layer was reduced to a small volume (about 2 mL) and lyophilized to dryness. The tetrakis(*p*-(aminomethyl)-phenyl)porphyrin (TAMPP) is obtained by the above purification procedure as its diprotonated chloride salt. The neutral porphyrin is obtained by exchanging the order of the above purification steps, i.e., washing the acidic aqueous porphyrin aqueoussolution first with CH_2Cl_2 and then washing the methylene chloride porphyrin solution withwater. The yield after purification is about 70%.

The TAMPP is obtained in its fully protonated state as the hydrochloride together with water (TAMPP·6HCl·xH$_2$O). To determine the extinction coefficient, the apparent molecular weight was determined by a ^{1}H NMR spectral method using the tetra-*p*-tosylate salt of tetrakis-4-(*N*-methylpyridyl)porphine (TMPyP-4), a well-defined compound, as the standard. TMPyP-4 and TAMPP were weighed into the same beaker and D_2O and two drops of DCl added. The apparent molecular weight of TAMPP was derived from the integrals of assigned peaks in the ^{1}H NMR spectrum. The molecular weight of TAMPP·6HCl·xH$_2$O determined from the ^{1}H NMR spectrum is 1217.9. Using this molecular weight, at pH = 1, $\epsilon_{648} = 3.64 \times 10^4$ M^{-1}cm^{-1}.

The molecular weight of neutral TAMPP determined from mass spectrometry matches the calculated value, 730.4 amu.

Anal. Calcd for TAMPP·6HCl ($C_{48}H_{48}N_8Cl_6$): C, 60.8; H, 5.1; N, 10.1; Cl, 22.5. Found: C, 43.0; H, 4.7; N, 9.9; Cl, 25.4. It is common for charged porphyrins to precipitate with significant amounts of solvent, rendering elemental analysis indeterminate, as found for this case.

Synthesis of *N*-Methyltetrakis(*p*-(aminomethyl)phenyl)-porphyrin (*N*-MTAMPP) (5b) from Its Corresponding Phthalimido-Protected Porphyrin 4b. The same two hydrolysis steps and purification procedures were used to derive *N*-MTAMPP. In this work, the N- MTAMPP is obtained also in its diprotonated salt. The yield of this hydrolysis step is about 60%.

^{1}H NMR assignments at 90 °C: positions 1 and 4 β-pyrrolenine, 2 2H s, 8.61 and 9.37 ppm; positions 2 and 3 β-pyrrolenine, 2 2H d, 9.51 and 9.69 ppm; positions b, a′, a benzyl: 2 2H d at 8.64 ppm and 9.08 ppm; positions d, c′, c benzyl, 2 2H d, 8.73 ppm and 9.29 ppm.

The *N*-MTAMPP is also obtained in its fully protonated salt form with some structure water (*N*-MTAMPP·6HCl·yH_2O). The method used to determine the apparent molecular weight of *N*-MTAMPP was similar to that used for TAMPP except that DCl is not added in the mixture of *N*-MTAMPP and TMPyP-4 in deuterium oxide solution. The apparent molecular weight of *N*-MTAMPP determined from its ^1H NMR spectrum is 1254.8. Using this molecular weight, at pH = 1, $\epsilon_{669} = 4.47 \times 10^4\ M^{-1}\ cm^{-1}$ for *N*-MTAMPP. The molecular weight of neutral *N*-MTAMPP determined from mass spectrometry is the calculated value, 744.5 amu.

Anal. Calcd for fully protonated *N*-MTAMPP·6HCl, ($C_{49}H_{50}N_8Cl_6$): C, 61.2; H, 5.2; N, 10.0; Cl, 22.1. Found: C, 53.4; H, 5.3; N 9.7; Cl, 18.4. As in the case of the unmethylated analogue, the elemental analysis for the charged porphyrin is in poor agreement with predicted values because of charged porphyrins tend to precipitate with significant amounts of solvent.

Acid–Base Studies. Two ionic strength conditions in aqueous solution were examined for all of the p*K* determinations: 0.12 M adjusted by NaCl, which is in the same ionic strength as was used in the binding characteristic studies of porphyrins with nucleic acids,[28] and <0.01 M to allow comparison with the acidity constants of other porphyrins and to investigate the sensitivity of the acidity constants to changes in ionic strength. The spectrophotometric titration and potentiometric titration were each done using 4 mL cuvettes. Titrations were either carried out beginning at about pH 3 and adding 0.092 M sodium hydroxide to raise the pH or by adding 0.01 M nitric acid to reduce the pH.

Absorbancies at different wavelengths were read from a Hewlett Packard 8452A diode array spectrophotometer, and visible spectra were taken from Beckman DU-8 spectrophotometer. The pH values of solutions at different titration points were detected by a Radiometer Copenhagen PHM84 research pH meter.

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