# Coordination of N-Donor Ligands by the Monomeric Ferric Porphyrin *N*-Acetylmicroperoxidase-8

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The monomeric ferric hemepeptide from cytochrome *c*, *N*-acetylmicroperoxidase-8 (NAcMP8), retains the proximal His ligand and has a coordinated water molecule in the second axial site. It provides an opportunity for quantitatively studying in aqueous solution the coordination chemistry of an iron porphyrin with a single accessible coordination site. An examination of the dependence of the spectrophotometrically determined equilibrium constants for coordination of imidazole and cyanide as a function of pH provides a procedure for correcting conditional equilibrium constants for the ionization of coordinated $H_2O$ in NAcMP8 and protonation of the entering ligand, L. An examination of the coordination by NAcMP8 of L = primary amines and imidazoles (25 °C, $\mu$ = 0.1 M) and pyridines (as a function of temperature, $\mu$ = 0.1 M) has shown that the process of binding L to NAcMP8 may be complex. With some ligands such as pyridines and some primary amines there is evidence for formation of an outer-sphere complex before displacement of $H_2O$ occurs. In some cases, a third process occurs at high concentrations of L which may entail substitution of the proximal His ligand. Pyridines and imidazoles fall into different classes in their basicity−affinity for Fe(III) relationships; primary amines fall into two distinctive classes, with one class having a higher affinity for Fe(III) than expected from basicity alone. It is suggested that neutral or cationic functional groups in the side chain of these ligands provide a second site of attachment to the iron porphyrin by hydrogen bonding. A compensating effect between $\Delta H$ and $\Delta S$ values for coordination of pyridines to NAcMP8 is demonstrated; hence log *K* values, rather than the individual thermodynamics parameters, were used to explore the possible influence of the electronic structure of the ligands on the magnitude of their affinity for Fe(III). Log *K* for coordination of pyridines and imidazoles correlates linearly, and statistically meaningfully, with the energies of the frontier orbitals of $\sigma$ and $\pi$ symmetry (determined by ab initio or semiempirical molecular orbital methods at the RHF level of theory using, respectively, the 6-31G* split-valence polarized basis set and the PM3 model), which have large amplitude on the donor N atom. There is only a weak correlation between log *K* and the energy of the frontier $\sigma$ orbital for primary amines. These observations suggest that both $\sigma$ bonding and $\pi$ bonding between planar aromatic ligands and Fe(III) play a significant role in determining the magnitude of the binding constant of these ligands to a ferric porphyrin.

## Introduction

The widespread occurrence of the hemoproteins in nature has drawn considerable attention to the coordination chemistry of iron porphyrins.[1] Many of these studies have been prompted, at least partially, by an expectation that understanding the fundamental properties of protein-free iron porphyrins will lead to a better understanding of the ways the protein can control and modify the properties of the prosthetic group. One way of delimiting the influence of the protein on the properties of the prosthetic group is to explore the chemistry of realistic protein-free models. Studies with iron porphyrins themselves, such as ferriprotoporphyrin-IX, are compromised by the porphyrins' well-known insolubility in acidic media, the formation of $\mu$-oxo dimers, and the tendency to aggregate in alkaline aqueous solution.[2] Furthermore, an exploration of their coordination chemistry is complicated by the availability of two axial coordination sites. Provided the first axial ligand that coordinates is not an exceptionally good $\pi$ acceptor (CO, NO), a second ligand almost always coordinates in the trans position,[3] and only rarely have conditions been found for observing the monoligated intermediate.[1h,4]

The hemeoctapeptide, microperoxidase-8 (MP8, Figure 1), derived by proteolytic degradation of cytochrome *c*, retains the proximal His-18 ligand above pH 4 and provides an ideal

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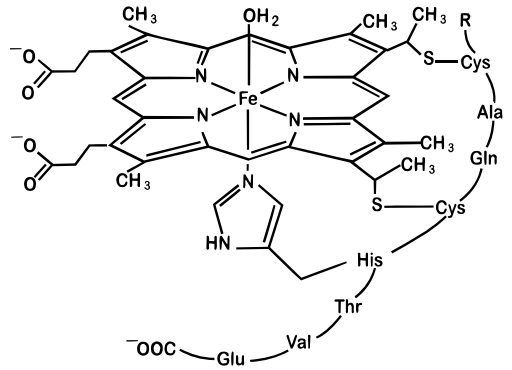


**Figure 1.** The hemeoctapeptide derived by proteolysis of cytochrome *c*, microperoxidase-8 (MP8). In MP8, R = $NH_3^+$; in NAcMP8, R = $NHCOCH_3$.

opportunity for investigating the fundamental properties of the prosthetic group of the hemoproteins (e.g., hemoglobin, myoglobin, the peroxidases) which have histidine as axial ligand,[5] although a propensity to aggregation limits studies to aqueous alcohol mixtures. More recently,[6] we have conducted a systematic investigation into the solution properties and electronic structure of *N*-acetylmicroperoxidase-8 (NAcMP8, Figure 1) in which the N-terminus is acetylated. Protection of the amino group prevents its participation in intermolecular coordination, the major cause of aggregation of MP8 in aqueous solution. Studies are now possible in strictly aqueous solution since, at low ionic strengths (<0.1 M), NAcMP8 is monomeric up to approximately 30 $\mu$M.[6a,b]

NAcMP8 has six spectroscopically active pH-dependent transitions: (i) Glu-21 C-terminal carboxylate binds heme iron at low pH (p$K_a$ = 2.1), but is (ii) substituted by His-18 (p$K_a$ = 3.12) as the pH is increased; the two heme propanoate substituents ionize with p$K_a$s of (iii) 4.95 and (iv) 6.1, respectively; (v) Fe(III)-bound $H_2O$ ionizes with p$K_a$ = 9.59; and (vi) His-18 forms the histidinate complex (p$K_a$ = 12.71).[6a] EPR and Mössbauer spectra and magnetic susceptibility data[6c] show that the aqua complex has thermally accessible a quantum-mechanically admixed spin state ($S = {}^3/_2$, ${}^5/_2$; 12−22% $S = {}^3/_2$ character) and a low-spin state ($S = {}^1/_2$), with the former the ground state.

In an endeavor to explore the coordination chemistry of NAcMP8, we report on its coordination of N-donor ligands (pyridines, amines, and imidazoles) in aqueous solution as a function of temperature (for pyridines) and at 25 °C (for amines and imidazoles), together with correlations between the binding constants and properties of the ligands determined from ab initio and semiempirical MO calculations.

## Experimental Section

NAcMP8 was prepared as previously described.[6a] The following reagents were used as received: 3-aminopropanoic acid ($\beta$-alanine), ethyl 3-aminopropanoate hydrochloride ($\beta$-alanine ethyl ester), 2-aminoethanesulfonic acid (taurine), 3-amino-1-propanesulfonic acid, 3-cyanopyridine, 3-methylpyridine, 4-chloropyridine hydrochloride, 4-cyanopyridine, 5-benzimidazolecarboxylic acid, dimethylamine hydrochloride, 1,2-diaminoethane (ethylenediamine), methyl 2-aminoethanoate hydrochloride (glycine methyl ester), methyoxylamine hydrochloride, 1-acetylimidazole, *n*-propylamine hydrochloride, and trimethylamine hydrochloride (Aldrich); 1-methylimidazole, ammonium chloride, and 2-aminoethanoic acid (glycine) (Merck); 4(5)-methylimidazole, 4-(dimethylamino)pyridine, and hydroxylamine hydrochloride (Sigma); and sodium cyanide (BDH). 3-Chloropyridine, 3,4-dimethylpyridine, 3,5-dimethylpyridine, 4-ethylpyridine, and 4-methylpyridine (Aldrich), ethanolamine (Merck), and pyridine (BDH) were redistilled. The following reagents were recrystallized: 1,2-dimethylimidazole (Aldrich, from toluene); 2-methylimidazole (Merck, from toluene); 4-aminobutanoic acid (Sigma, from ethanol); and imidazole (Merck, from toluene). 2,6-Lutidine (2,6-dimethylpyridine, BDH) was distilled three times from $AlCl_3$[7] and analyzed by GC (methyl silicone capillary column 50 × 0.25 mm; flame ionization detector; temperature programming from 25 °C for 10 min, linearly ramped to 200 °C at 20 min, then constant at 200 °C to 30 min total run time). The purity (area integration) was 98.8%, with 0.66% 3- and/or 4-methylpyridine contaminants, and 0.67% unidentified contaminants (which were not pyridine, 3,4-dimethylpyridine, or 3,5-dimethylpyridine).

Water was purified using a Millipore RO unit and further purified using a Millipore MilliQ unit (18 MΩ cm). The pH of solutions was determined using a Metrohm 601 pH meter and 6.0201 glass electrode calibrated against standard buffers.

The acid dissociation constants of some ligands were determined by glass electrode potentiometry in a thermostated cell under high purity $N_2$, essentially as described by Martell and Motekaitis.[8] The titration data were analyzed using the program PKAS;[8] the p$K_a$ values obtained, and reported p$K_a$ values used, are listed in Table S1 of the Supporting Information.

The equilibrium constants for the coordination of the ligands by NAcMP8 were determined by addition of aliquots of a stock solution of the appropriate ligand to a solution of between 4 and 6 $\mu$M NAcMP8 contained in a 1.00 cm path length cuvette housed in the thermostated cell block of a Cary 2300, a Cary 1E, or a Cary 3E spectrophotometer. The temperature of the cell block was maintained (± 0.1 °C) by a water-circulating bath and measured with a thermistor device. The absorbance changes at 530 and 397.2 nm were monitored, and all absorbance readings were corrected for dilution. The solutions were buffered with MES (pH 5.5−7), MOPS (pH 7−8), Tris/HCl (pH 8−9), CHES (pH 9−10), or CAPS (pH 10−11), and the ionic strength was maintained at 0.100 M using NaCl.

The reported equilibrium constants are the averages of the values obtained at the two monitoring wavelengths in at least three separate determinations. They were determined as a function of temperature for pyridines, and at 25.0 °C for imidazoles and amines. $\Delta H$ and $\Delta S$ values were obtained from linear least-squares fits to plots of ln $K$ against $T^{-1}$.

Molecular orbital calculations were performed with HYPERCHEM version 5.0.[9] Semiempirical calculations were performed using the PM3 model.[10] Ab initio calculations were performed at the restricted Hartree−Fock (RHF) level of theory[11] using the 6-31G* split-valence

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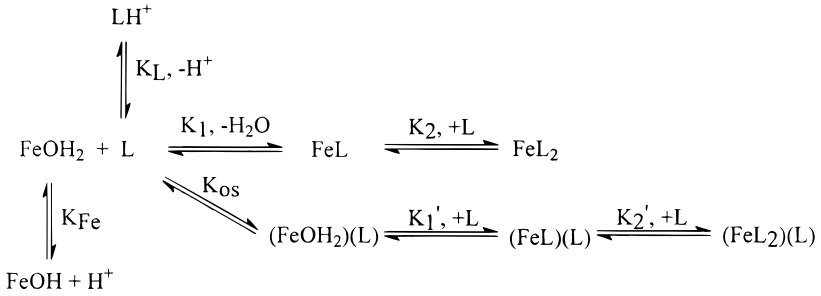
**Scheme 1**




polarized basis set.[12] Full geometry optimizations were performed with a convergence criterion of 0.05 kcal Å$^{-1}$ mol$^{-1}$ root-mean-square gradient.

## Results

We have found that up to three successive processes can be observed by UV−visible spectrophotometery when a ligand, L, is coordinated by NAcMP8. (a) There is often an initial process at low ligand concentrations that involves little or no shift in the band positions and accounts for 5−8% of the total absorbance change at the monitoring wavelength. We attribute this to formation of an outer-sphere complex between incoming L and NAcMP8 (vide infra). (b) This is followed by a process that involves the major spectral change and where the bands in the spectrum shift from those typical for a predominantly high-spin Fe(III) porphyrin[13] (Soret 397 nm; $Q_v$ 480 nm; $Q_o$ 525 nm (sh); charge transfer 625 nm) to a predominantly low-spin complex (Soret 405 nm; $Q_v$ 530 nm; $Q_o$ 560 nm (sh); charge transfer at 625 nm absent), and is clearly associated with the displacement of $H_2O$ from the coordination sphere of Fe(III) by L. (c) With a pyridine as incoming ligand, a third process was observed at high ligand concentrations that involved small shifts in band positions toward longer wavelength, and typically accounted for 20−30% of the total absorbance change. The origin of this process is considered below. Our observations are summarized in Scheme 1.

If we make the reasonable assumption that the conjugate acid $LH^+$ is assumed incapable of binding to the metal ion, and, as we found for cyanide and a number of imidazoles, there is no evidence for formation of an outer-sphere complex or for further addition of a ligand at high ligand concentrations, then only the equilibria indicated by $K_{Fe}$, $K_L$, and $K_1$ in Scheme 1 are relevant. The experimentally determined equilibrium constant, $K_{obs}$, is a conditional constant; it is a function of pH and is related to $K_1$ by eq 1.

$$K_{obs} = \frac{K_1 K_L [H^+]}{([H^+] + K_{Fe})(K_L + [H^+])} \qquad (1)$$

The dependence of $K_{obs}$ on pH for coordination of cyanide and imidazole was investigated; the results are listed in Table S2 of the Supporting Information and plotted in Figure 2. The solid lines in Figure 2 are fits to eq 1 as objective function, with $K_L$ fixed at the experimentally determined values (9.04 for HCN[14] and 7.004 (this work) for imidazole), and $K_{Fe}$ and

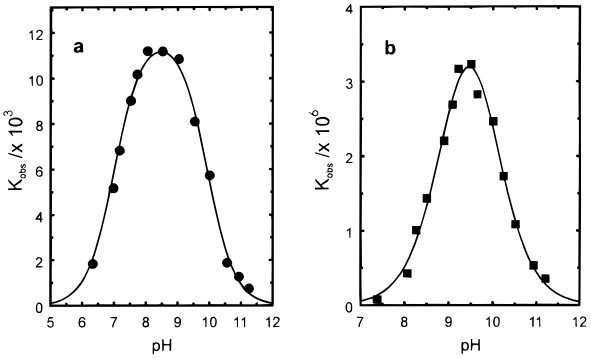



**Figure 2.** The pH dependence of the observed equilibrium constant, $K_{obs}$, for coordination of (a) imidazole and (b) cyanide by NAcMP8. The solid lines are fits of eq 1 to the experimental data.

$K_1$ as variable parameters. From the fits, log $K_1 = 4.076 \pm 0.007$ and p$K_{Fe} = 9.93 \pm 0.04$, in reasonable agreement with the experimentally determined[6a] value of 9.59. For cyanide, log $K_1 = 6.76 \pm 0.02$ and p$K_{Fe} = 9.91 \pm 0.02$. The results (Table S2) for other imidazoles were determined at a single pH value, and eq 1 was applied to correct for the effect of pH.

With some amines, only a single process was observed, and eq 1 was applied to determine $K_1$ values. With other amines, an initial process was observed before the displacement of $H_2O$ from the coordination sphere of the metal and formation of a low-spin complex. The appropriate equilibria are those identified as $K_{Fe}$, $K_L$, $K_{os}$, and $K_1'$ in Scheme 1. An expression relating the absorbance, $A$, at the monitoring wavelength to $A_0$, $A_1$, and $A_2$ (the absorbance due to the starting complex, $(FeH_2O)(L)$, and $(FeL)(L)$, respectively), and $K_{os}$ and $K_1'$ (eq 2), and taking explicitly into account the free ligand concentration rather than assuming that this is approximately equal to the total concentration of added ligand, was derived (see Supporting Information).

$$A = \frac{A_0 + (K_{os}[L]_{free})A_1 + (K_{ox}K_1'[L]_{free}')A_2}{1 + K_{os}[L]_{free} + K_{os}K_1'[L]_{free}^{\ 2}} \qquad (2)$$

In the case of pyridines, the formation of the outer-sphere complex, displacement of coordinated $H_2O$, and the further addition of a ligand at high ligand concentrations was observed. Hence, $K_{Fe}$, $K_L$, $K_{os}$, $K_1'$, and $K_2'$ are all pertinent and the binding isotherm is given by eq 3. Fits to eqs 2 and 3 were by nonlinear least-squares methods.

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$$A = \frac{A_0 + (K_{os}[L]_{free})A_1 + (K_{os}K_1'[L]_{free}^2)A_2 + (K_{os}K_1'K_2'[L]_{free}^3)A_3}{1 + K_{os}[L]_{free} + K_{os}K_1'[L]_{free}^2 + K_{os}K_1'K_2'[L]_{free}^3} \quad (3)$$

If, as we surmise below, $K_2'$ entails the displacement of the proximal His ligand, then only L, and not $LH^+$, will react. Hence $K_2'$ values were corrected for the effect of pH. The results are listed in Table S2.

In all instances fits to the experimental data were attempted with a model involving the minimum number of binding processes; further processes were introduced only if there was a systematic deviation of the residuals. Only a single process, corresponding to ligand substitution of $H_2O$, was required to fit the experimental data for imidazoles. In the reactions with some amines ($NH_3$, $MeONH_2$, $HONH_2$, n-$PrNH_2$, 3-aminopropanesulfonate, $Me_3N$, glycine methyl ester, and $\beta$-alanine ethyl ester) the initial process was not observed, while with other amines (taurine, ethanolamine, monocationic ethylenediamine, glycine, $\beta$-alanine, 4-aminobutanoate) two processes were clearly present. The very substantial overlap between them means that the values for the first equilibrium constant are only approximate. In the case of all pyridines, three binding processes were required for adequate fitting. As an example, Figure 3 shows the experimental data for the coordination of 3,5-dimethylpyridine fitted with an equation that accounts for the binding of a single ligand, and by one in which three successive processes are assumed. Clearly, the data cannot be adequately explained by a single process, and this is particularly noticeable at low ligand concentrations.

We attribute the initial process to formation of an outer-sphere complex between incoming L and NAcMP8. In the case of the pyridines this could involve binding by donor−acceptor $\pi-\pi$ interactions with the porphyrin system. The formation of such donor−acceptor complexes between aromatic systems and iron porphyrins in general[15] and hemepeptides in particular[1i,16] is well-established. These processes all occur without significant shifts in band positions. In the case of the amines, the outer-sphere complex could arise from formation of an ion pair (as recently observed, for example, between CNG and methylcobalamin[17]) between the residual positive charge at the metal center and the negative functionality on ligands such as taurine, glycine, $\beta$-alanine, and 4-aminobutanoate (although no evidence for an initial process was observed with 3-aminopropanesulfonate) or by an ion−dipole interaction (ethanolamine). Clear evidence for the initial process was also observed with monocationic ethylenediamine, so a purely Coulombic effect cannot be the only mechanism operating in forming the outer-sphere complex.

The process is unlikely to entail the formation of a hydrogen bond between L and the proximal His-18 aromatic N−H group, or coordinated $H_2O$. This would increase the imidazolate character of the proximal and the hydroxo character of the distal ligand and would be expected to cause shifts in the band positions to longer wavelengths.[6a]

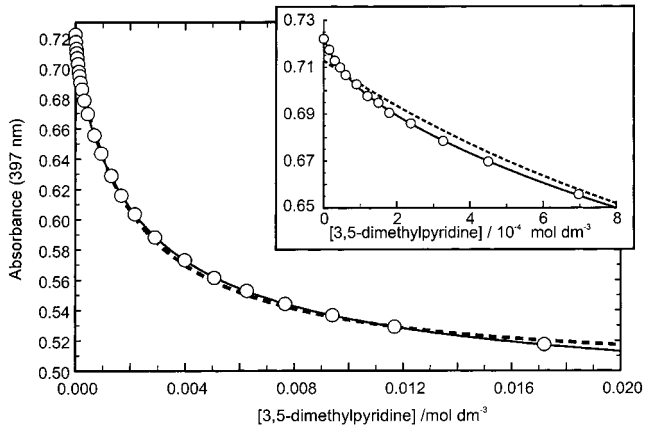


**Figure 3.** Absorbance change at the Soret maximum of the aqua complex on titration of NAcMP8 (4.6 $\mu$M) with 3,5-dimethylpyridine, pH 8.5, 25.0 °C, $\mu = 0.1$ M (NaCl). The inset to the figure shows the titration at low concentrations of added ligand. The solid line takes into account three successive binding processes (see text for details); the dashed line is a fit of the data to a single binding process.

Supporting evidence for the formation of a donor−acceptor complex between NAcMP8 and pyridines comes from the behavior of the system with low concentrations of 2,6-lutidine (2,6-dimethylpyridine). Because of the steric hindrance of the methyl substituents, coordination is disfavored, and only the initial process is observed.[18]

The second process is clearly the coordination of the ligand to Fe(III) with displacement of $H_2O$; the spectral changes are consistent with a change from a predominantly high-spin species to a predominantly low-spin species. Such spectral changes have been reported previously for coordination of many moderate- and strong-field ligands, including amines,[1i,j] imidazoles, pyridines, and cyanide.[19]

The third process could entail a further donor−acceptor interaction between a pyridine and the porphyrin ring, hydrogen bonding to the proximal histidine by pyridine, or possibly displacement of the proximal histidine as the concentration of L becomes large. In our studies of the binding of imidazoles to aquacobalamin[20] we showed by HPLC that, at imidazole concentrations >0.1 M, the exogenous ligand displaces 5,6-dimethylbenzimidazole from the proximal coordination site of Co(III) with log $K \approx 1$. This may occur with NAcMP8 and the pyridines as well, although, perhaps because of the lability of Fe(III), we found no evidence by HPLC for an additional species at high ligand concentrations.

A summary of the equilibrium constants determined is listed in Table 1; a complete listing is given in Table S2 of the Supporting Information.

## Discussion

**Log *K* and Ligand Basicity.** The ligand substitution process of the hemepeptides with simple ligands such as $CN^-$ and $SCN^-$

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**Table 1.** Summary of Equilibrium Constants (25 °C) for the Binding of the Ligand, L, by Monomeric *N*-Acetylmicroperoxidase-8 Determined by Spectrophotometric Titration[a]

| ligand, L | $K_{os}$ | $K_{1,obs}$ | $K_1$ | $K_{1,obs}'$ | $K_1'$ | $K_{2,obs}'$ | $K_2'$ | log $K_1$ or log $K_1'$ at 25 °C | $\Delta H$, kJ mol$^{-1}$ | $\Delta S$, J K$^{-1}$ mol$^{-1}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| imidazole | | $1.84(3) \times 10^3$ | $1.19(2)\ 10^4$ | | | | | 4.076(7) | | |
| cyanide | | $7.50(3) \times 10^4$ | $5.86(15) \times 10^6$ | | | | | 6.76(2) | | |
| 3-cyanopyridine | $1(1) \times 10^4$ | | | 10.9(8) | 10.9(8) | | | 1.04 | −28(3) | −73(9) |
| 4-cyanopyridine | $6(8) \times 10^3$ | | | 32.6(4) | 32.6(4) | | | 1.51 | −22(2) | −44(9) |
| 3-chloropyridine | $3(1) \times 10^4$ | | | 32.6(2) | 32.6(2) | | | 1.51 | −14(1) | −18(4) |
| 4-chloropyridine | *b* | | | 65(2) | 65(2) | | | 1.84 | −49(3) | −130(9) |
| pyridine | *b* | | | $4.02(5) \times 10^2$ | $4.21(5) \times 10^2$ | 0.7(1.5) | 0.7(1.6) | 2.62 | −20.7(3) | −19.3(9) |
| 3-methylpyridine | *b* | | | $4.5(7) \times 10^2$ | $4.8(7) \times 10^2$ | 5(2) | 5(2) | 2.67 | −19(2) | −13(3) |
| 4-methylpyridine | $8(3) \times 10^4$ | | | $9.02(10) \times 10^2$ | $9.53(11) \times 10^2$ | 5.5(4) | 5.8(4) | 2.97 | −23(2) | −20(5) |
| 4-ethylpyridine | $4(3) \times 10^4$ | | | $8.4(5) \times 10^2$ | $9.3(5) \times 10^2$ | 6(8) | 7(9) | 2.98 | −19.7(6) | −9(2) |
| 3,5-dimethylpyridine | $1.4(1) \times 10^4$ | | | $4.3(2) \times 10^2$ | $4.7(2) \times 10^2$ | 11(5) | 12(5) | 2.72 | −17(2) | −5(6) |
| 3,4-dimethylpyridine | $2.5(5) \times 10^4$ | | | $9.5(2) \times 10^2$ | $1.04(2) \times 10^3$ | 7(3) | 8(3) | 3.03 | −18.5(1.3) | −4(4) |
| 4-(dimethylamino)-pyridine | $1.6(6) \times 10^5$ | | | $9.97(8) \times 10^3$ | $3.81(3) \times 10^4$ | 20(9) | 79(36) | 4.61 | −57(11) | −160(4) |
| 2-methylimidazole | | $1.45(1) \times 10^2$ | $1.94(1) \times 10^2$ | | | | | 2.288(2) | | |
| 1,2-dimethylimidazole | | $1.0(2) \times 10^2$ | $1.4(3) \times 10^2$ | | | | | 2.15(8) | | |
| benzimidazole-5-carboxylate | | $9.9(7) \times 10^1$ | $1.08(8) \times 10^2$ | | | | | 2.03(3) | | |
| 1-acetylimidazole | | $1.09(6) \times 10^4$ | $1.18(6) \times 10^4$ | | | | | 4.07(2) | | |
| 1-methylimidazole | | $1.31(2) \times 10^4$ | $1.48(2) \times 10^4$ | | | | | 4.17(1) | | |
| 4(5)-methylimidazole | | $1.08(5) \times 10^4$ | $1.29(6) \times 10^4$ | | | | | 4.11(2) | | |
| methoxylamine | | $1.3(5) \times 10^2$ | $1.4(5) \times 10^2$ | | | | | 2.14(13) | | |
| hydroxylamine | | $8(3) \times 10^2$ | $8(3) \times 10^2$ | | | | | 2.9(1) | | |
| ethylenediammine (monocationic form) | $3(1) \times 10^4$ | $2.26(1) \times 10^3$ | $2.55(1) \times 10^3$ | | | | | 3.406(2) | | |
| glycine methyl ester | | $1.4(1) \times 10^3$ | $3.9(3) \times 10^3$ | | | | | 3.59(3) | | |
| $\beta$-alanine ethyl ester | | $3.1(6) \times 10^3$ | $1.3(2) \times 10^4$ | | | | | 4.13(7) | | |
| ethanolamine | $3.0(6) \times 10^4$ | $1.287(8) \times 10^3$ | $4.784(9) \times 10^3$ | | | | | 3.680(1) | | |
| 2-aminopropane-sulfonate (taurine) | $2.0(3) \times 10^4$ | $1.74(7) \times 10^3$ | $3.9(1) \times 10^3$ | | | | | 3.59(3) | | |
| ammonia | | $2.95(4) \times 10^2$ | $1.59(2) \times 10^2$ | | | | | 3.203(6) | | |
| glycine | $1.7(2) \times 10^4$ | $7(2) \times 10^2$ | $2.8(1) \times 10^3$ | | | | | 3.44(2) | | |
| 3-aminopropane-sulfonate | | $8.7(6) \times 10^2$ | $3.4(2) \times 10^3$ | | | | | 3.53(3) | | |
| $\beta$-alanine | $1.5(3) \times 10^4$ | $4.71(6) \times 10^2$ | $3.76(2) \times 10^3$ | | | | | 3.576(3) | | |
| 4-aminobutanoic acid | $1.5(3) \times 10^4$ | $4.1(5) \times 10^2$ | $4.6(5) \times 10^3$ | | | | | 3.66(5) | | |
| *n*-propylamine | | $6(2) \times 10^2$ | $1.1(3) \times 10^4$ | | | | | 4.04(10) | | |
| dimethylamine | *c* | | | | | | | | | |
| trimethylamine | | 1.9(5) | 11(3) | | | | | 1.0(1) | | |

[a] A complete listing is given in Table S2 of the Supporting Information. [b] Overlapped too closely with $K_1'$ to be resolved. [c] Reduction to Fe(II) too fast to permit determination of log *K*.

as incoming ligands proceeds with very well defined isosbestic points;[21] we also observed this in the titrations with imidazoles during the present study. On the other hand, small, but discernible, shifts in the isosbestic points were detected when titrating NAcMP8 with pyridines and many of the amines, undoubtedly a consequence of the multiple equilibria we have observed with these ligands. There is a clear relationship between the basicity of L (as measured by its p$K_a$) and log *K* for the binding of that ligand by NAcMP8 (Figure 4), i.e., the affinity of L toward Fe(III) in an iron porphyrin parallels its affinity toward the proton. This is evident for primary amines, imidazoles, and pyridines. A similar observation has been made by Pratt et al. for MP8 itself in aqueous methanol solutions.[1j−l]

**The Binding of Primary Amines.** Although the ability of primary amines to coordinate iron porphyrins has been doubted,[22] the known coordination of Lys in cytochrome *f*,[23] as well as its likely coordination in the alkaline form of cytochrome *c*,[24] seems to discount such an assertion. Moreover, we have shown[1h] that the bis(ethanolamine) complex of hematohemin is readily formed in methanol solutions; Silver and co-workers[25] have recently prepared a wide range of complexes between hemin and aliphatic amines; and Pratt and co-workers[1i,j] have studied

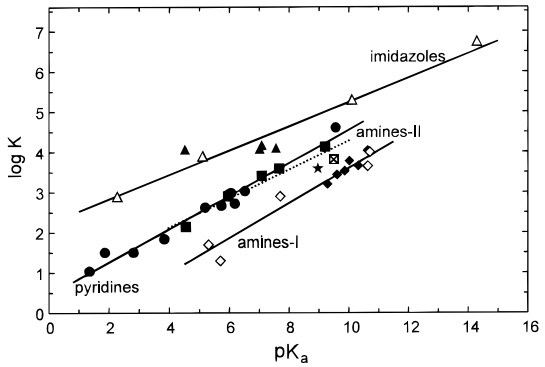


**Figure 4.** Dependence of log $K_1$ (or log $K_1'$, see Scheme 1 for definitions) for coordination of imidazoles (−▲−), pyridines (−●−), and amines (···■··· and −◆−) on the p$K_a$ ($BH^+$) of the conjugate acid. Data from this work (filled symbols) and selected data from refs 11−13 (open symbols). There is uncertainty (see text) in which class of amines to place taurine (★) and ethanolamine (× in □).

(21) Marques, H. M.; Baldwin, D. A.; Pratt, J. M. *J. Inorg. Biochem.* **1987**, *29*, 77.
(22) Hwang Y. C.; Dixon, D. W. *Inorg. Chem.* **1986**, *197*, 253.
(23) Martinez, S. E.; Huang, D.; Szczepaniak, A.; Cramer, W. A.; Smith, J. L. *Structure (London)* **1994**, *2*, 95.
(24) Gadsby, P. M. A.; Peterson, J.; Foote, N.; Greenwood, C.; Thomson, A. J. *Biochem. J.* **1988**, *246*, 43.

quantitatively the binding of a wide range of aliphatic amines with MP8 in aqueous methanol solution. The binding of primary amines to an Fe(III) porphyrin is further confirmed by the present work.

In a study of the coordination of aliphatic amines by MP8 in aqueous methanol solution, Pratt and co-workers[1j] showed that although there is a good linear correlation between the p$K_a$ of the aliphatic amines $NH_2CH_2CN$, $NH_2CH_2CH_2CN$, $NH_2CH_2CH_2Br$, $NH_3$, and $NH_2CH_2CH_3$ and log $K$ for their coordination to Fe(III), a number of amines did not fall onto the correlation line. The binding constants of ligands such as $NHMe_2$ and $NH_2{}^i$Pr are depressed below values expected on the basis of basicity alone because of steric hindrance. We have confirmed the low binding constant for $NMe_3$ (Table 1), but have been unable to measure a reliable value for $NHMe_2$ because of rapid reduction of Fe(III) to Fe(II), as has been reported for other iron porphyrins.[26] A putative donor−acceptor interaction between the aromatic substituent on, for example, $NH_2CH_2Ph$ and Trp and the porphyrin enhances the binding constant above that expected.[1j] Two other noteworthy deviations from this simple linear correlation were reported; $NH_2NH_2$ and $NH_2OH$ both have larger log $K$ values than expected. Their anomalous behavior was attributed to the so-called α effect. This effect is usually kinetic in nature[27] but has also been observed at the thermodynamic level (see ref 1j for a summary), causing an excess of reactivity of a species above that expected from basicity alone. It is attributed to the presence of an electronegative atom bearing one or more lone pairs in a position α to the donor atom. We have been unable to measure a value for $NH_2NH_2$ because over the pH range 5−8 coordination is rapidly followed by the reduction of the metal. We can, however, confirm the value reported for $NH_2OH$.

Figure 4 shows that there is no simple linear dependence of log $K_1$ (or log $K_1$) on the p$K_a$ of the conjugate acid of a primary amine. *N*-Propylamine, ammonia, 3-aminopropanesulfonate, glycine, *β*-alanine, 4-aminobutanesulfonic acid—all determined in this work—and, chosen from the values of Pratt *et al.*[1j] to extend the range of p$K_a$ values and to demonstrate the fairly close overlap with the results reported here, aminoacetonitrile, 3-aminopropionitrile, methylamine, ethylamine, and 2,2,2-trifluoroethylamine, appear to form a set. We have termed these class I amines, and the relationship described is a reasonable straight line ($R^2 = 0.92$). A second set of ligands which we term class II amines (methoxylamine, glycine methyl ester, *β*-alanine ethyl ester, hydroxylamine, ethylenediamine) yield an excellent straight line between log $K_1$ and p$K_a$ ($R^2 = 0.99$). They all have significantly larger log $K_1$ values for a given p$K_a$ value than the corresponding class I amines. In only two of these five ligands could their enhanced affinity for NAcMP8 be due to the α effect. We conclude that the α effect is not responsible for the enhanced affinity of these ligands for Fe(III).

It is not obvious into which of the two classes either taurine or ethanolamine should be placed. There are structural differences that distinguish class I from class II amines. The former have unsubstituted side chains or carry a functional group which, at the pH at which the log $K_1$ values were determined, is negatively charged. The latter are neutral or positively charged at the pH in question, and they carry a heteroatom in the side chain capable of acting as a hydrogen bond donor or acceptor.

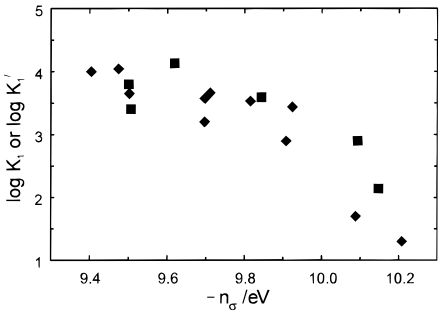


**Figure 5.** Dependence of log $K_1$ (or log $K_1'$) for coordination of class I (◆) and class II (■) amines on the ionization potential from the highest occupied molecular orbital with $\sigma$ symmetry and large amplitude on N.

We tentatively suggest that class II amines may be hydrogen bonded, possibly through intermediacy of solvent molecule(s), to a negatively charged functionality on the hemepeptide such as a heme propanoate, and are in effect doubly anchored to the porphyrin. NMR studies of the interaction of amino acids with water-soluble Co(III) porphyrins indicate that the side chains of the amino acids may interact with the porphyrin ring by hydrophobic and/or donor−acceptor interaction.[28] Analogous secondary interactions may be responsible for the deviation of a significant number of amine ligands from a strictly linear relationship between their affinity for the Lewis acids $H^+$ and Fe(III)(porphyrin). If this interpretation is correct, then taurine should be placed in class I, and ethanolamine in class II. This does not change the linear relationship noted for the former, but that for the class II amines becomes significantly poorer ($R^2 = 0.92$).

Ramsey and Walker[29] have argued that if, for a series of bases B:, the "rehybridization energies" and homolytic bond dissociation energies of $BH^+$ either are constant or are linear functions of the vertical ionization potentials, IP, and differences in solvation energies between B: and $BH^+$ are also linear functions of IP, then a linear relationship should exist between the p$K_a$ of $BH^+$ and the IP of the "lone pair" on B:. The $\sigma$-donor properties of an axial ligand will depend on the relative energies of the metal d orbitals with $\sigma$ symmetry and the ligand orbital with $\sigma$ symmetry corresponding in essence to the nitrogen lone pair.[30]

Figure 5 shows a plot of the dependence of log $K_1$ (or log $K_1$) on the energy of the highest occupied $\sigma$ orbital, $n_\sigma$, with large amplitude on nitrogen (Table S3) determined by semiempirical MO methods. There is a clear, albeit weak, dependence of log $K_1$ on $n_\sigma$; this dependence may saturate as the IP decreases.[31] There is no clear difference between the dependence on basicity for class I and class II amines, as expected if the difference in log $K_1$ values for the two classes is unrelated to the electronic properties of the donor atom, and due to the secondary interactions suggested.

**The Binding of Pyridines.** The binding of pyridines by NAcMP8 has been determined as a function of temperature (Table S2), and values of $\Delta H$ and $\Delta S$ were determined from plots of log $K_1$ (or log $K_1$) against $T^{-1}$. There is a linear, compensating effect ($R^2 = 0.95$) between $\Delta H$ and $\Delta S$ (Figure

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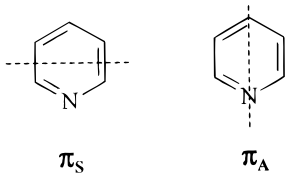
(30) Safo, M. K.; Gupta, G. P.; Watson, C. T.; Simonis, U.; Walker, F. A.; Scheidt, W. R. *J. Am. Chem. Soc.* **1992**, *114*, 7066.
(31) The orbital energies were not determined by ab initio methods because of the inordinate computational time required; the studies with pyridines and imidazoles show that the relationships found remain essentially identical whether PM3 or ab initio MO energies are used.

S1). The most satisfactory explanation of which we are aware for the phenomenon is provided by Williams and co-workers.[32] When an associated state lies in a deep enthalpic well ($-\Delta H > 40RT$ is suggested[32a]), the density of states is low and there is little motion compared to the unassociated state; conversely, if the enthalpic well is relatively small (say, $-\Delta H < 10RT$), the larger amplitude vibrations close to the lip of the well (where the density of states is greatest) become accessible. Hence, in attempting to explore factors affecting the affinity of a ligand for an Fe(III) porphyrin, attention has to focus on log $K$ (or $\Delta G$) rather than on either $\Delta H$ or $\Delta S$.

The occupied frontier orbitals of pyridines have been identified[29] as an orbital, n, with $\sigma$ symmetry corresponding essentially to the "lone pair" on N, and two orbitals of $\pi$ symmetry, designated $\pi_S$ and $\pi_A$. There is an excellent linear relationship




between the p$K_a$ of the conjugate acid of the pyridine, $BH^+$, and the $\sigma_n$ ionization potentials.[29] The energies of the frontier orbitals, as determined by ab initio and semiempirical molecular orbital calculations, are listed in Table S3 of the Supporting Information.[33] In view of the linear correlation between the energy of $n_\sigma$ and the p$K_a$ of $BH^+$, a correlation between p$K_a$ and the $\sigma$-donor properties of an axial ligand, as reflected in the log $K_1'$ values, is expected. However, other interactions may be important as well. Pyridines are potentially both $\pi$ donors to and $\pi$ acceptors from low-spin Fe(III). The most likely $\pi$ orbital on the ligand to participate in such an interaction would be $\pi_S$, which has appreciable amplitude on N, and a $\pi^*$ orbital also with a large amplitude on N. The energy of $\pi_S$ can be calculated by standard MO methods (Table S3), but not that of $\pi^*$ since, as recently pointed out,[34] the virtual orbitals of any self-consistent field calculation are an artifact of the computational method used in solving the Hartree−Fock equations, and it is very doubtful whether any physical meaning can be attached to their energies.

There is evidence that planar aromatic ligands such as pyridines and imidazoles can participate in $\pi$ bonding with metal ions. The stability imparted to the Fe−$O_2$ bond in compounds such as oxyhemoglobin and oxymyoglobin may be due in part to the trans imidazole of the proximal His ligand acting as a $\pi$ donor toward the metal ion.[35] The equilibrium constants for uptake of $O_2$ by the Co(II) porphyrin, [Co(T(*p*-OMe)PP)L], increase in the order of $\pi$-donor ability, pyridine < piperidine < 4-methylpyridine < 4-(dimethylamino)pyridine < 1-methylimidazole.[36] The rate constants for the dissociative displacement of $O_2$ by CO in [Fe(TPP)($O_2$)(L)] (−79 °C, $CH_2Cl_2$) increase from $5 \times 10^{-6}$ $s^{-1}$, to $5 \times 10^{-5}$ $s^{-1}$, to $5 \times 10^{-4}$ $s^{-1}$ as L is changed from 1-methylimidazole, to piperidine, to pyridine.[37] When two planar ligands coordinate an iron porphyrin, it is reasonable to expect, on steric grounds, that $\phi$, the angle between the projection of the ligand onto the porphyrin plane and an Fe−$N_{porphyrin}$ bond, should be 45°.[38] The value of $\phi$ for bis(imidazole) and bis(pyridine) complexes is considerably smaller.[39] Iterative extended Hückel MO calculations[39] show that metal p$\pi$−ligand p$\pi$ and metal d$\pi$−ligand p$\pi$ bonding is strongest when the projection of the axial ligand on the porphyrin plane eclipses an Fe−$N_{porphyrin}$ bond, so explaining the unfavorable steric orientation of these ligands.

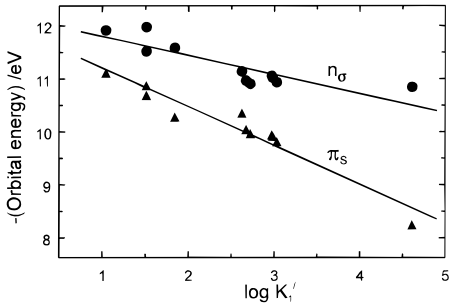



**Figure 6.** Dependence of log $K$ for coordination of pyridines by NAcMP8 on the energies of $n_\sigma$ and $\pi_s$ frontier molecular orbitals.

The magnitude of log $K_1'$ correlates linearly with the ionization potentials of the $n_\sigma$ and the $\pi_S$ orbitals (Figure 6). If log $K_1'$ is treated as linearly dependent on both the energies of $n_\sigma$ and $\pi_S$, then fitting the data to a multiple regression equation of the form $\log K_1' = aE(n_\sigma) + bE(\pi_S) + c$ gives $R^2 = 0.96$, and the root-mean-square difference (rmsd) between the predicted and the observed values is 0.15. The $F$ statistic for the regression is 108.20; since for a single-tailed test with $\alpha = 0.01$, 2 variables, and 11 observations the $F_{critical}$ value is 8.65, there is less than a 1% chance that the correlation is accidental. For $\alpha = 0.01$ and 8 degrees of freedom, the single-tail $t_{critical}$ value is 2.90; $t$ values for the energies of $n_\sigma$ and $\pi_S$ are greater than $t_{critical}$, so both are important variables in predicting log $K_1'$. Hence both $\sigma$ and $\pi$ bonding play a role in determining the affinity of a pyridine for Fe(III) in NAcMP8.[40]

**The Binding of Imidazoles.** Studies of the dependence of log $K_1$ on the p$K_a$ of imidazoles are compromised by the limited range of basicities available for these ligands. By making a number of assumptions, and determining spectrophotometrically the p$K_a$ for ionization of a coordinated imidazole ligand to MP8, Pratt *et al.*[11] were able to estimate equilibrium constants for the coordination of the imidazolate and 1,2,4-triazolate anions,

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(33) There is a reasonably good linear correlation between the experimentally determined ionization potentials and the orbital energies as determined by either ab initio ($R^2 = 0.94$) or semiempirical ($R^2 = 0.95$) methods; therefore the relationships noted hold whether ab initio or semiempirical MO energies are used.

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(38) (a) Collins, D. M.; Countryman, R.; Hoard, J. L. *J. Am. Chem. Soc.* **1972**, *94*, 2066. (b) Safo, M. K.; Walker, F. A.; Raitsimring, A. M.; Walters, W. P.; Dolata, D. P.; Debrunner, P. G.; Scheidt, W. R. *J. Am. Chem. Soc.* **1994**, *116*, 7760. (c) Munro, O. Q.; Marques, H. M.; Debrunner, P. G.; Mohanrao, K.; Scheidt, W. R. *J. Am. Chem. Soc.* **1995**, *117*, 935.

(39) Scheidt, W. R.; Chipman, D. M. *J. Am. Chem. Soc.* **1986**, *108*, 1163.

(40) As expected because of the compensation between $\Delta H$ and $\Delta S$, neither of these parameters correlates with the energy of either frontier orbital (not shown). There are, however, statistically meaningful multivariate correlations between each of $\Delta H$ and $\Delta S$, and the energies of *both* $n_\sigma$ and $\pi_S$: $\Delta H = -35(5)n_\sigma + 25(3)\pi_S + 117(72)$, $R^2 = 0.76$, $F_{stat} = 12.8$, $|t_{stat}| = 6.1$, 7.9, and 1.6 for the coefficients of $n_\sigma$, $\pi_S$, and the constant, respectively; $\Delta S = -162(20)n_\sigma + 98(11)\pi_S + 790(256)$, $R^2 = 0.80$, $F_{stat} = 16.2$, $|t_{stat}| = 7.9$, 8.7, and 3.1 for the coefficients of $n_\sigma$, $\pi_S$, and the constant, respectively. The rmsd between the predicted and observed values of $\Delta H$ is 4.6 kJ $mol^{-1}$ (the rmsd of the experimental uncertainties in the measurements of $\Delta H$ is a comparable 2.6 kJ $mol^{-1}$), and 17.6 J $K^{-1}$ $mol^{-1}$ for $\Delta S$ (rmsd experimental = 8.4 J $K^{-1}$ $mol^{-1}$).

thereby considerably extending the range of p$K_a$ values over which a linear free energy relationship between p$K_a$ and log $K_1$ could be explored. In the present work, we have used their values of log $K$ (and assumed them to be analogous to our log $K_1$ values) for 1,2,4-triazole, 5-chloro-*N*-methylimidazole, imidazolate, and 1,2,4-triazolate. A plot of log $K_1$ against p$K_a$ is a reasonably good straight line ($R^2 = 0.94$, Figure 4). Sterically hindered imidazoles (imidazoles with a substituent α to the donor atom) such as 2-methylimidazole and 1,2-dimethylimidazole do coordinate NAcMP8, but log $K_1$ is some 2 orders of magnitude smaller than for the corresponding sterically unhindered analogues (Table 1).

As with pyridines, there is reason to suppose that $\pi$ bonding between an axially coordinated imidazole and Fe(III) will be important. Silver et al.[25] have presented evidence from Mössbauer spectroscopy of $\pi$ donation from coordinated imidazoles to the $(d_{xz},d_{yz})^3$ hole in Fe(III) porphyrins. On the basis of the magnitude of differences in $\Delta E_Q$ between Fe(II) and Fe(III) porphyrins with axially coordinated amines, pyridines, and imidazoles, they further argued that L → M $\pi$ bonding is much more significant in imidazoles than in pyridines.

There appears to be a strong linear correlation between the energies of the frontier orbitals with the same symmetry as the $n_\sigma$ and $\pi_S$ frontier orbitals on imidazoles and log $K_1$, but only if the values for 1,2,4-triazole and its anion are excluded (Figure 7). It is difficult to determine the validity of the apparently strong regression because of the paucity of data between the neutral imidazoles and imidazolate. We tentatively suggest, therefore, that the energies of both orbitals correlate with log $K_1$, but that 1,2,4-triazole (and its anion) may belong to a different ligand class; if this is correct, then the apparently good correlation between p$K_a$ and log $K_1$ (Figure 4), insofar as these two species are concerned, is fortuitous.

In conclusion, we have demonstrated that the apparently simple process of substituting coordinated $H_2O$ in monomeric NAcMP8 by an N-donor ligand, L, may be preceded by the formation of an outer-sphere complex between the porphyrin and the ligand. At high ligand concentrations, there may be a further reaction, probably due to displacement of the proximal His ligand by L. The values of the binding constants for the coordination of L depend on a number of factors, including ligand basicity, secondary interactions between the ligand and the porphyrin, and, in the case of aromatic L, $\pi$ bonding with the metal.

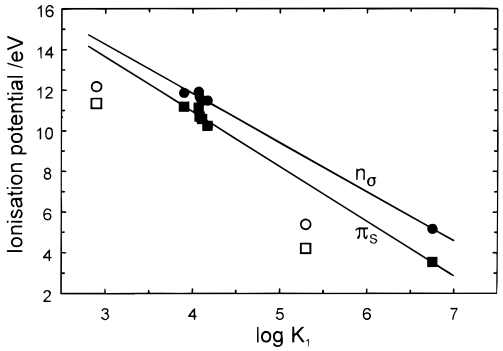


**Figure 7.** Dependence of log $K$ for coordination of imidazoles by NAcMP8 on the energies of the $n_\sigma$ and $\pi_S$ frontier molecular orbitals of the ligand. The open symbols are for 1,2,4-triazole and its anion and have been excluded from the regressions.

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**Supporting Information Available:** Table S1, the acid dissociation constants of ligands used in this work; Table S2, equilibrium constants for the binding of a ligand, L, by monomeric NAcMP8 determined by spectrophotometric titration; Table S3, frontier molecular orbital energies of some pyridines, imidazoles, and primary amines from ab initio and semiempirical molecular orbital methods; derivation of equations for the analysis of titration data; Figure S1, plot of $\Delta H$ against $\Delta S$ for the binding of pyridines by NAcMP8. This material is available free of charge via the Internet at http://pubs.acs.org.

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