Effects of a triangular nanocage structure on the binding of neutral and anionic ligands to Co\textsuperscript{II} and Zn\textsuperscript{II} porphyrins

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ABSTRACT
Interactions of neutral pyridine ligands and anionic benzoate ligands with the metalloporphyrin walls of M\textsubscript{6}L\textsubscript{3} nanocages (M = [(tmeda)Pt]\textsuperscript{2+}, L = tetra(3-pyridyl)porphyrin-M', M' = Co\textsuperscript{II}, Co\textsubscript{3-1}; Zn\textsuperscript{II}, Zn\textsubscript{3-1}) were evaluated in CD\textsubscript{3}CN. The binding of ligands to simple tetra(N-methyl-3-pyridinium)porphyrin-M' complexes Co-2 and Zn-2 was also evaluated. It was found that pyridine ligands bind with generally similar strength to the monomeric porphyrins as to the outer faces of the corresponding cage. Coordination is enhanced inside the cages for the binding of one equivalent of pyridine, 3-methylpyridine, or 3,5-dimethylpyridine. In contrast, 4-(4-methoxyphenyl)pyridine cannot bind inside the cages, and 4-methylpyridine shows weaker binding inside than outside. Steric effects play a complex role in influencing ligand binding since 4-tert-butylpyridine binds more strongly in the cages than 4-methylpyridine. These latter two ligands also reveal a subtle allosteric effect in which binding of ligands inside Zn\textsubscript{3-1} becomes increasingly favored over external binding once some of the external sites are occupied. It was confirmed that benzoate and 3,5-di-tert-butylbenzoate can also coordinate to the walls of M\textsubscript{3-1}, but quantitative analysis of these interactions was complicated by competing affinity of the anions for other sites of the cages, limiting detailed insight.

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1. Introduction

Porphyrin-based macrocycles [1–3] and nanocages [4–6] have been of interest for decades owing to their remarkable 3D structures [7], ability to serve as supramolecular hosts [2, 4, 5, 8–12], and myriad of potential applications (e.g. catalysis [13–18], molecular separations [4, 5, 8], etc. [2, 9, 19]). The longstanding interest in these structures has led to numerous examples in which the porphyrins are either covalently linked [1, 2, 4, 9, 15] or assembled via coordination chemistry [3, 5, 6, 8, 11, 13]. The latter class includes structures in which the porphyrins are linked by coordination of their peripheral substituents to various metal complexes, such as those of Re [3], Ru [13, 19], Pd [6, 11, 12], or Pt [10, 20–22], and structures in which assembly occurs via coordination of multitopic ligands to metals (commonly Zn) bound in the porphyrins themselves (Figure 1A) [23, 24]. Coordination chemistry is also important to the host-guest properties of porphyrinic cages and macrocycles in which the preorganization of metalloporphyrin walls facilitates multivalent recognition of ligands that can bind to multiple metal sites simultaneously (Figure 1B) [1, 3].

Despite the importance of coordination chemistry to the synthesis and function of porphyrinic nanostructures, the binding of simple monodentate ligands to the metalloporphyrin walls has been relatively neglected as an area of study. It might seem, upon cursory consideration, that the porphyrin walls would exhibit similar interactions with monodentate ligands as seen for monomeric metalloporphyrins since the binding of monodentate ligands cannot benefit from the preorganization of multiple porphyrin binding sites. However, other factors might influence the coordination chemistry of metalloporphyrins embedded in nanocages or macrocycles (Figure 1C). Most obviously, porphyrin cages and macrocycles feature internal cavities that can influence the endo vs. exo binding preferences of ligands, either via steric limitations on endo coordination of bulky ligands or by providing secondary noncovalent interactions that might favor endo binding of ligands [25]. Other potential factors are less obvious, such as the potential for a nanostructure to enforce bent versus planar conformations of its porphyrin components, which in turn, could influence the strength of ligand association [26]. Additionally, many porphyrin nanocages and macrocycles are positively charged [6, 10–14, 19], potentially promoting the binding of anionic ligands [27–29].

Understanding the nuances of ligand coordination to porphyrinic nanostructures is important since simple axial ligands can tune the reactivity of metalloporphyrins, either by modifying reactivity at the opposite face of the porphyrin [28–33] or by blocking reactivity at the occupied site [25]. Thus, as part of our work to examine and

![Figure 1. (A) Assembly of multitopic components into a porphyrin-based cage structure [23]. (B) Recognition of a multivalent guest by a tris-porphyrin macrocycle [1]. (C) Potential influences on the binding of monodentate ligands (L) in porphyrin nanocages.](image-url)
control the reactivity of porphyrin nanocages, we set out to examine the coordination of neutral ligands to trigonal prismatic porphyrin nanocages \( \text{M}_{3}-1 \) \((M = \text{Co}^{II}, \text{Zn}^{II})\), that we recently reported [34]. In this study, we compare the binding of a series of neutral pyridine ligands and anionic benzoate ligands to the Co- and Zn-metallated derivatives of these dodecacationic cages as well as to simple tetracationic porphyrins \( \text{M-2} \) representing individual walls of the cages (Scheme 1). Our results reveal a range of influences of the cages on ligand binding, in some cases providing only slight changes relative to the monomeric porphyrins while other examples show large increases or decreases in the strength of ligand binding and similarly varied preferences for endo/exo association of ligands.

2. Results and discussion

2.1. Comparing the cages and monomeric metalloporphyrins for binding 4-methylpyridine

We began our studies by comparing coordination of a simple neutral ligand 4-methylpyridine (4-MePy, Scheme 1) to the porphyrin cages and related monomeric porphyrin complexes. The results are summarized in Table 1. The cobalt-metallated derivatives \( \text{Co}_{3}-1 \) and \( \text{Co}-2 \) showed the greatest similarity in their affinity for 4-MePy. Titration of 4-MePy into a 3 mM solution of \( \text{Co}-2 \) in CD$_3$CN resulted in gradual shifts of most of the \(^1\)H NMR resonances of \( \text{Co}-2 \) (Figures S5–S7), revealing fast exchange between complexes with and without the axial ligand coordinated. Shifts of one of the aromatic pyridinium resonances of \( \text{Co}-2 \) provided an excellent fit to a 1:1 binding isotherm (Figure S8), revealing a \( K_a \) of 636 ± 15 M$^{-1}$. These results are consistent with those
reported for coordination of pyridines to neutral CoII porphyrins in toluene [35], except the association is slightly weaker for Co-2 in CD3CN, presumably because the added ligand must compete with the solvent for binding to cobalt. Similar results were obtained for titration of 4-MePy into a 1 mM solution of Co3-1 in CD3CN (Figures S25–S27), providing a $K_a$ of 720 ± 16 M$^-1$/C0 per porphyrin face under the assumption that the three CoII sites exhibit non-cooperative binding of the pyridine ligand. It must be cautioned, however, that it can be challenging to determine host-guest stoichiometry in systems that feature multiple binding sites [36]. We have assumed a maximum stoichiometry of 1:3 throughout our analyses of pyridine ligands binding to M3-1, but a limit of 1:2 stoichiometry cannot be ruled out by the experimental data in most cases. For example, the NMR data obtained for interactions between 4-MePy and Co3-1 also fits well to a 1:2 non-cooperative binding model (see Figure S28), which is less intuitive than 1:3 stoichiometry but can be rationalized if the binding of the first two ligands inhibits binding of a third.

The possibility of endo vs. exo coordination represents an additional complicating factor in evaluating the coordination of ligands to M3-1. Thus, it is notable that after the first addition of 4-MePy to Co3-1 (0.1 equiv of 4-MePy per porphyrin face), the $^1$H NMR resonance of the 4-methyl group of 4-MePy appeared as a broad signal at 4.15 ppm (Figure 2A), which is downfield from where this resonance is observed (~5.93 ppm) after the first 0.1 equiv addition of 4-MePy to Co-2 (Figure 2B). The appearance of this signal in the negative region of both spectra is attributed to the paramagnetism of the CoII centers to which 4-MePy binds. Since the other paramagnetic CoII sites and the aromatic ring-currents of the other walls of the cage would also induce upfield shifts for a CH$_3$ group that is inside Co3-1 [37], the more downfield shift of the CH$_3$ signal in the titration of Co3-1 versus Co-2 suggests that 4-MePy preferentially binds to the external faces of Co3-1, though a small fraction of the ligand associating inside the cage cannot be ruled out. As discussed below, comparisons between the association of 4-MePy and other pyridine ligands to Zn3-1 and Co3-1 revealed that 4-MePy indeed has an anomalously low affinity for coordination to the internal metal sites of the cages despite being small enough to fit inside these structures.

More substantial differences were observed for the binding of 4-MePy to the zinc-metallated cage Zn3-1 vs. the monomeric porphyrin Zn-2. First, a $K_a$ of
5,297 ± 143 M⁻¹ was determined for 1:1 association of 4-MePy to Zn-2 (Figures S16 and S17), which is consistent with the preference of Zn II porphyrin complexes for binding a single axial ligand [26, 38, 39] and the higher Lewis acidity typically observed for such complexes relative to their CoII congeners [26]. However, while Co3-1 and Co-2 showed comparable affinity to each other for binding 4-MePy, the coordination of 4-MePy to cage Zn3-1 was weaker than observed for the monomeric complex Zn-2. At low concentrations of Zn3-1 (0.1 mM), shifts of the ¹H NMR signals of the cage provided an excellent fit to a 1:1 binding isotherm assuming a 0.3 mM concentration of independent porphyrin faces (Figures S51–S53), providing a $K_a$ (1,887 ± 27 M⁻¹) that is about 35% of the association constant observed for Zn-2. Thus, unlike for the cobalt derivatives, the cage structure appears to weaken the binding of 4-MePy to ZnII porphyrin sites, though the overall affinity was still larger than that of the CoII derivative Co3-1.

Titration of 4-MePy into a higher concentration solution of Zn3-1 (1 mM) resulted in similar shifts of the ¹H NMR signals of the cage as were seen for the lower concentration sample, but new signals were also clearly evident. A new singlet was observed about 0.1–0.2 ppm upfield of the singlet corresponding to the 2-position CH bonds of the 3-pyridyl groups of exo-(4-MePy)-Zn3-1, a red star marks a smaller new signal that is attributed to this CH position in endo-(4-MePy)-Zn3-1, and a white diamond marks a signal attributed to the 4-methyl position of internally bound 4-MePy in endo-(4-MePy)-Zn3-1. Note that the upfield region of the spectra are magnified relative to the downfield region.
main CH$_3$ signal of 4-MePy shifted from 1.46 ppm to 2.26 ppm over the course of adding 0.3–30 equiv of the ligand. This latter methyl signal clearly corresponds to 4-MePy in fast exchange between bulk solution and bound to the exterior of the cage, while the distinct upfield methyl signal observed for endo-(4-MePy)-Zn$_3$-1 indicates slower exchange between internally bound 4-MePy and the free ligand in solution. It is worth noting that, as depicted in Scheme 2, the external faces of endo-(4-MePy)-Zn$_3$-1 likely also accommodate additional equivalents of 4-MePy.

The new 2-position CH resonance was integrated to quantify the relative preference for endo versus exo coordination of 4-MePy to the Zn$^{II}$ sites of the cage. At the point in the titration when roughly a third of the external sites were estimated to be occupied, the new 2-position resonance accounted for only ca. 15% of the cage in solution. This amount corresponds to the occupancy of only 5% of internal Zn$^{II}$ sites since only one pyridine ligand can bind internally, which is evident from steric considerations as well as integration of the CH$_3$ resonance near ~5.8 ppm that corresponds to internally bound 4-MePy. These measurements indicate a seven-fold preference for exo coordination of 4-MePy to Zn$_3$-1, consistent with the data for Co$_3$-1 that indicates a preference for exo coordination of 4-MePy. Interestingly, as 4-MePy is added past ~3 equiv (corresponding to about two-thirds of external Zn$^{II}$ sites occupied), the 2-position signal of endo-(4-MePy)-Zn$_3$-1 grows more quickly than the signals of exo-(4-MePy)$_n$-Zn$_3$-1 shift, suggesting increasing favorability of endo vs. exo coordination. One possible explanation is that 4-MePy can bind to an internal Zn$^{II}$ site even as the external sites become saturated, providing a (4-MePy)$_4$-Zn$_3$-1 complex in which one Zn$^{II}$ center is six-coordinate (Scheme 2). Consistent with this interpretation, the two distinct 2-position CH signals coalesce into a single broad signal as increasing amounts of 4-MePy are added (Figure 3). However, zinc porphyrin complexes only rarely bind more than one axial ligand [26, 38–42], arguing against formation of (4-MePy)$_4$-Zn$_3$-1. Another possibility is that allosteric effects increase the relative favorability of endo coordination once one or two pyridine ligands bind to the exo position of the other Zn$^{II}$ sites. As described below, the titration of Zn$_3$-1 with 4-$t$BuPy provides compelling evidence of this latter possibility rather than formation of any substantial concentrations of (4-MePy)$_4$-Zn$_3$-1. However, (4-MePy)$_4$-Zn$_3$-1 might still play a role as a transient intermediate that facilitates interconversion of the observed endo-(4-MePy) and
exo-(4-MePy) complexes, which would explain the coalescence of the NMR signals of the exo and endo isomers at high 4-MePy concentrations.

2.2. Influence of substituents on the association of pyridine ligands with Zn₃⁻¹ and Co₃⁻¹

It was surprising that 4-methylpyridine appears to show a preference for binding to the external faces of Co₃⁻¹ and Zn₃⁻¹ since the internal cavities of these cages should have sufficient space to accommodate this ligand. To better understand steric influences on the binding of pyridine ligands, we examined the association between the cages and a range of pyridines, beginning with the combination of Zn₃⁻¹ and 3-methylpyridine (3-MePy). Titration of 3-MePy into solutions of Zn₃⁻¹ in CD₃CN revealed host-guest behavior that differed substantially from that observed for the 4-MePy isomer. New aromatic ¹H NMR signals assigned to endo-(3-MePy)⁻¹-Zn₃⁻¹ were evident beginning from the addition of just 0.1 equiv of 3-MePy to a 1 mM solution of the cage (Figure 4). The new resonances grew to represent about 80% of the cage in solution once 1 equiv of 3-MePy was added. The assignment of the new signals was confirmed by the concomitant growth of a resonance at -3.29 ppm that integrates appropriately to correspond to the CH₃ group of the encapsulated ligand (Figures 4 and S47). Thus, 3-MePy exhibits substantially higher affinity than 4-MePy for coordination to the internal metal sites of Zn₃⁻¹. An association constant of 2.3 ± 0.1 × 10⁴ M⁻¹ (Table 2) was determined based on integration of the resonances of Zn₃⁻¹ vs. endo-(3-MePy)⁻¹-Zn₃⁻¹ in seven spectra acquired between the addition of 1.1–1.7 equiv of 3-MePy to the cage (Figure S48). The endo-(3-MePy)⁻¹-Zn₃⁻¹ complex was also...
observable by ESI-MS, giving the most intense peaks in the mass spectrum (Figures S74–S76).

Upon further additions of 3-MePy to Zn$_3$-1, gradual shifts of some of the aromatic $^1$H NMR signals of endo-(3-MePy)-Zn$_3$-1 were observed (Figure S46), consistent with binding of 3-MePy to the external faces of the initially formed endo complex. These shifts provided a good fit to a 1:2 non-cooperative binding model (Figure S50), yielding an association constant $K_{a-endo1}$ of 1,100 ± 50 M$^{-1}$ (Table 2) for the binding of 3-MePy to the first external site of the cage. The spectra acquired during this titration also display the growth of a new upfield methyl resonance at $\delta$ 3.05 ppm (Figures 4 and S46) that can be attributed to the association of a second 3-MePy ligand in the cage to provide endo-(3-MePy)$_2$-Zn$_3$-1. The ratio of endo-(3-MePy)$_2$-Zn$_3$-1 to endo-(3-MePy)-Zn$_3$-1 was less than 1:10 after 1 equiv of 3-MePy had been added to Zn$_3$-1, but the new methyl signal continued to grow as 3-MePy was added until the endo-(3-MePy)$_2$ and endo-(3-MePy)$_1$ complexes reached a constant ratio of about 1:3 when ≥10 equiv of the ligand had been added (Figure S47). This ratio corresponds to the equilibrium constant for movement of one ligand from an external face of endo-(3-MePy)$_1$-exo-(3-MePy)$_2$-Zn$_3$-1 to an internal site (Scheme 3), and thus, external binding of 3-MePy appears to be about three times more favorable than binding a second 3-MePy ligand in the cage. Nevertheless, it is notable that up to two 3-MePy ligands can bind in Zn$_3$-1 considering that this cage has weak affinity for encapsulating even a single 4-MePy ligand.

Titration of a 1 mM solution of Zn$_3$-1 with 3,5-dimethylpyridine (3,5-Me$_2$Py) initially resulted in very similar changes to the $^1$H NMR spectrum of the cage (Figure 5) as were observed during titration with 3-MePy. Several aromatic signals of Zn$_3$-1 decreased while new aromatic signals corresponding to endo-(3,5-Me$_2$Py)-Zn$_3$-1 appeared. Likewise, a new signal appeared near $\delta$ -2.7 ppm that corresponds to the methyl groups of the encapsulated 3,5-Me$_2$Py ligand (Figures S55 and S56). However,
growth of the host-guest signals was more gradual than observed during the titration of \( \text{Zn}_{3-1} \) with 3-MePy, indicating that the additional methyl substituent in 3,5-Me₂Py weakens the binding of this ligand inside the cage. Indeed, integration of fifteen spectra acquired between the addition of 0.3–3.0 equiv of 3,5-Me₂Py revealed an association constant of \( 11,000 \pm 1,000 \text{M}^{-1} \), which is about half that for the binding of a single 3-MePy ligand in \( \text{Zn}_{3-1} \). The extra methyl substituent also prevented the binding of a second 3,5-Me₂Py ligand even after adding 15 equiv of the ligand to \( \text{Zn}_{3-1} \) (see Figures S55 and S56). In contrast, \( \text{exo} \) association of 3,5-Me₂Py was comparable in strength \( (K_a-\text{exo}_{1} = 880 \pm 50 \text{M}^{-1}) \) to \( \text{exo} \) association of 3-MePy (Table 2), as expected since the 3- and 5-positions are too far from the pyridine nitrogen to sterically influence its coordination to metal sites on the external faces of \( \text{Zn}_{3-1} \).

Examining the binding of unsubstituted pyridine (py) in \( \text{Zn}_{3-1} \) provided confirmation of the trend that methyl substituents inhibit the \( \text{endo} \) association of pyridine ligands (Figure 5). The unsubstituted pyridine binds strongly enough that the empty cage was not detectable by \(^1\text{H} \) NMR spectroscopy once 1.6 equiv of the ligand had been added to a 1 mM solution of \( \text{Zn}_{3-1} \) (see Figures S51 and S52) whereas > 3 equiv of 3-MePy were required to induce full occupancy under comparable conditions. The strength of association of py in \( \text{Zn}_{3-1} \) was measured to provide a \( K_{a-\text{endo}_{1}} \) of \( \left(4.4 \pm 0.6\right) \times 10^4 \text{M}^{-1} \), but it should be noted that this value is based on a limited number (five) of spectra acquired during the titration in which the concentration of \( \text{Zn}_{3-1} \), \( \text{endo-py-Zn}_{3-1} \), and free py could be quantified effectively by NMR integration. As a result, the association constant for py has a larger error than that determined for 3-MePy and 3,5-Me₂Py, but nevertheless, the strength of \( \text{endo} \) association follows a clear trend of \( \text{py} > 3\text{-MePy} > 3,5\text{-Me}_2\text{Py} \) (Table 2), confirming that the methyl groups weaken association of the substituted pyridines in \( \text{Zn}_{3-1} \).

Further analysis of the binding of py in \( \text{Zn}_{3-1} \) is complicated by the possibility of a second equivalent of the ligand associating strongly in the cage, as might be expected based on the trend established for \( \text{endo} \) association of 3-MePy and 3,5-Me₂Py. Unfortunately, the unsubstituted pyridine lacks a clear NMR handle for discerning one vs. two equivalents bound in the cage. Indirect evidence for an \( \text{endo-}(\text{py})_2 \) complex was obtained when attempting to evaluate \( \text{exo} \) association of py to the initially

![Figure 5. \(^1\text{H} \) NMR spectra (CD₃CN, 298 K, 500 MHz) of 1 mM samples of \( \text{Zn}_{3-1} \) after adding 0.1, 1.0, and 2.0 equiv of py (left), 3-MePy (middle), or 3,5-Me₂Py (right). Blue triangles mark the 2-position CH signal of the 3-pyridyl groups of the empty cage and red stars mark the corresponding signal of the cage when occupied by a pyridine guest.](image-url)
formed endo-py-Zn₃⁻¹ complex. A large error was obtained when attempting to analyze exo association of py via a 1:2 non-cooperative binding model ($K_{a,exo1} = (6 ± 2) \times 10^3$ M⁻¹, Figure S44), while a better fit was obtained for a 1:1 binding model under the assumption that the first two equivalents of py bind inside the cage (Figure S43). This analysis revealed an association constant $K_{a,exo}$ of $900 ± 50$ M⁻¹, which is comparable to those determined for the exo binding of 3-MePy and 3,5-Me₂Py. It is important to caution that these results represent only limited evidence for the binding of two py ligands in Zn₃⁻¹, and it was not possible to better verify that an endo-(py)₂ complex forms prior to substantial occupancy of the exo coordination sites. Only a 1:1 adduct between py and Zn₃⁻¹ could be observed by ESI-MS (Figures S71–S73), suggesting against the binding of two pyridines inside the cage, though it is also possible that the second bound pyridine simply dissociates too readily in the gas phase to observe.

The relative strengths of endo association of py, 3-MePy, 3,5-Me₂Py, and 4-MePy in Zn₃⁻¹ can reasonably be attributed to steric effects of the methyl substituents, suggesting that 4-position substitution has a particularly strong influence since 4-MePy binds much weaker in Zn₃⁻¹ than the other pyridines do. To probe this possible effect, 4⁻¹BuPy was examined as a ligand, with the expectation that binding in Zn₃⁻¹ might be prevented entirely by the bulky 4-tert-butyl group. Surprisingly, 4⁻¹BuPy showed stronger endo association than was observed for 4-MePy. Like the other endo-bound pyridine complexes, formation of endo-(4⁻¹BuPy)-Zn₃⁻¹ was evident from the appearance of several new host resonances in the aromatic region of the $1^H$ NMR spectrum after addition of 4⁻¹BuPy to Zn₃⁻¹ (Figure 6). Likewise, a single upfield resonance near $-5.7$ ppm arises from the tert-butyl group of the encapsulated ligand. Approximately 50% conversion of Zn₃⁻¹ to endo-(4⁻¹BuPy)-Zn₃⁻¹ was evident after 1.2 equiv of 4⁻¹BuPy had been added to a 1 mM sample of the cage (Figure 6), whereas 4-MePy provided only 15% conversion under comparable conditions, thereby confirming that the bulkier ligand binds more strongly inside the cage. As the titration was continued,

**Figure 6.** $1^H$ NMR spectra (CD₃CN, 298 K, 500 MHz) of a 1 mM sample of Zn₃⁻¹ upon titration with 4⁻¹BuPy. Blue triangles mark signals arising from the host without an internally bound guest and red stars mark signals of endo-(4⁻¹BuPy)-Zn₃⁻¹. Note that the upfield regions of the spectra are magnified relative to the downfield regions.
endo-(4-tBuPy)-Zn₃₋₁ reached a steady concentration representing about 90% of the cage in the sample (Figure 6), indicating about a 9-fold preference for the binding of 4-tBuPy inside the cage vs. binding on the external zinc sites.

Quantifying the strength of endo and exo association of 4-tBuPy with Zn₃₋₁ revealed unexpected complexity to the interactions between the cage and this ligand. The strength of exo association was determined by titrating a 0.1 mM sample of Zn₃₋₁ with 4-tBuPy and fitting the resulting shifts of one of the 1H NMR signals of the cage to a 1:1 binding isotherm assuming a 0.3 mM concentration of independent porphyrin faces (Figures S59–S61). This analysis provided a $K_{a\text{-exo}}$ of $3,400 \pm 100 \text{ M}^{-1}$ for binding 4-tBuPy, which lies between the association constants determined for binding of 4-MePy to Zn₃₋₁ and Zn₋₂, consistent with the expectation that the remote tert-butyl group should not substantially affect exo coordination of 4-tBuPy. The spectra acquired between the addition of 1–2 equiv of ligand (Figure S60) were used to assess the strength of 4-tBuPy binding inside the cage, revealing a $K_{a\text{-endo}}$ of $6,300 \pm 500 \text{ M}^{-1}$. This value is only about twice that determined for exo binding of 4-tBuPy, which is much less than the nine-fold preference for endo association that was indicated from titrations at higher concentrations. Additionally, these exo and endo association constants are not directly comparable since $K_{a\text{-exo}}$ was determined per porphyrin face while $K_{a\text{-endo}}$ was calculated for the cage acting as a single receptor, which exaggerates the $K_{a\text{-endo}}$ value 3-fold relative to $K_{a\text{-exo}}$. For a more direct comparison, the ratio of occupied exo and endo zinc sites was estimated to exceed 2:1 in the early stages of the titration when fewer than a third of external binding sites are occupied. Thus, it would appear that exo coordination of 4-tBuPy is favored in the early stages of the titration, while the relative favorability of endo binding must increase later in the titration as the zinc sites become saturated.

The change in the preference of exo vs. endo association over the course of titrating Zn₃₋₁ with 4-tBuPy is an interesting result that merits further discussion. In the initial stages of the titration, the exo:endo ratio predominantly reflects equilibration between exo-(4-tBuPy)₁₋₁-Zn₃₋₁ and endo-(4-tBuPy)₁₋₁-Zn₃₋₁ since these are the statistically favored complexes when there is less than one equivalent of ligand in solution (Scheme 4A). As a large excess of ligand is added, a stable exo:end endo ratio is reached that corresponds to equilibration between exo-(4-tBuPy)₃₋₁-Zn₃₋₁ and endo-(4-tBuPy)₃₋₁.

Scheme 4. Endo and exo isomers of 4-tBuPy bound to Zn₃₋₁.
exo-(4'-BuPy)$_2$-Zn$_3$-1 (Scheme 4B). An entropic contribution provides a 3-fold increase in the favorability of the endo isomer in this latter equilibrium, but this accounts only partially for the increased favorability of endo association. It is conceivable that the apparent increase in favorability of endo-binding reflects a misestimate of the exo:endo ratio earlier in the titration (i.e. the increase may not be as large as suggested by the interpretation of the data above). It is unlikely that there is any major error in determining the number of endo sites that are occupied during the titration since the internally occupied cage has distinct $^1$H NMR signals that were integrated relative to those of the empty cage. In contrast, the amount of 4'-BuPy bound outside the cage may be overestimated since we employed a method for analyzing exo binding that assumes each successive coordination of ligand produces the same change in the chemical shift of the signals of the cage. In the extreme limit of assuming instead that only the first equivalent of exo-bound ligand causes shifts of the cage signals (or that only one exo position can be occupied at all), then the number of exo sites that are occupied in the early stages of the titration may be as low as one-third of that estimated above. Nevertheless, even in this extreme case, the ratio of exo:endo sites that are occupied would be only a little less than 1:1 in the early stage of the titration, in contrast to the nearly 10-fold favorability of endo-(4'-BuPy)$_1$-exo-(4'-BuPy)$_2$-Zn$_3$-1 at the end of the titration. Thus, it would appear that exo coordination of 4'-BuPy ligands produces an allosteric effect that favors binding of the final equivalent of the ligand inside the cage.

The titration of Zn$_3$-1 with 4'-BuPy sheds light on the peculiar behavior described above in Section 2.1 for the titration of this cage with 4-MePy. The constant ratio of exo to endo isomers that was reached upon addition of a large excess of 4'-BuPy to the cage rules out any possibility that (4'-BuPy)$_4$-Zn$_3$-1 plays a substantial role in ligand binding since increasing concentrations of ligand would continually shift the equilibrium from exo-(4'-BuPy)$_3$-Zn$_3$-1 to (4'-BuPy)$_4$-Zn$_3$-1 if the latter was meaningfully accessible. This finding argues against the possibility that (4-MePy)$_4$-Zn$_3$-1 forms in any substantial concentration in the interactions of 4-MePy with the cage. Instead, the increasing ratio of endo to exo isomers during titration with 4-MePy is likely due to a similar cooperative effect between internal and external coordination as appears to be present in the 4'-BuPy complexes. Comparisons between 4'-BuPy and 4-MePy also shed light on the nuances of steric effects on the binding of pyridine ligands inside Zn$_3$-1. The 4-MePy ligand is certainly not too large to fit inside the cage since the bulkier 4'-BuPy ligand binds inside effectively, and thus, an alternative explanation is needed for the anomalously weak binding of 4-MePy to the endo zinc sites. It is conceivable that the 4-methyl group may be too small to provide good van der Waals contacts with the inside of the cage, thus leading to weaker endo binding than observed for the bulkier 4'-BuPy ligand.

Since all of the pyridines thus far examined have at least some affinity for binding in Zn$_3$-1, we sought to identify a pyridine that is large enough to fully prevent association inside the cage. Titration of a 1 mM solution of Zn$_3$-1 with 4-(4'-methoxyphenyl)-pyridine (4-ArPy) resulted in shifts of the $^1$H NMR signals of the cage that are consistent with the changes observed for exo association of 4-MePy and 4'-BuPy, while no new signals appeared that could be attributed to the formation of an endo-(4-
ArPy) complex (see Figures S63 and S64). Thus, 4-ArPy appears to bind exclusively to the external faces of the cage. Quantification of \textit{exo} association provided a $K_{a-exo}$ of $2,000 \pm 200 \text{M}^{-1}$ under the assumption of three independent \textit{exo} binding sites (Figure S65). However, a 1:2 non-cooperative model provided an even better fit to the data (Figure S66), providing $K_{a-exo1}$ of $1,700 \pm 50 \text{M}^{-1}$. This observation provides evidence that coordination of a third ligand is inhibited, especially since the absence of competing \textit{endo} association enabled the titration to be performed at higher concentrations that render the data analysis more sensitive to the stoichiometry of the binding model. It seems unlikely that a third ligand would be prevented entirely from binding to the last available \textit{exo} site of the cage, but it is particularly challenging to measure the weaker equilibria in host-guest systems featuring multiple possible stoichiometries that vary in their favorability, preventing further interrogation of the number of 4-ArPy ligands that bind to $\text{Zn}_3^{-1}$. It is worth noting that allosteric effects that weaken but do not entirely inhibit the binding of a third ligand outside $\text{Zn}_3^{-1}$ would explain the apparent change in the relative favorability of \textit{exo} vs. \textit{endo} association that was noted above for the titration of this cage with 4-MePy and 4-$t$BuPy.

Interactions of all the alkylpyridine ligands with $\text{Co}_3^{-1}$ were also evaluated, producing a similar trend in the favorability of \textit{endo} association as was observed for the interactions of these pyridines with $\text{Zn}_3^{-1}$. As can be seen in Figure 7, the addition of each pyridine to 1 mM solutions of $\text{Co}_3^{-1}$ resulted in the appearance of new aromatic $^1\text{H}$ NMR signals that can be attributed to the binding of the ligands inside the cage. Overlap of the two sharp aromatic resonances of $\text{Co}_3^{-1}$ with other, broader signals of the cage prevented precise quantification of the strength of binding of pyridines, but it is clear from inspection of the titration spectra that the strengths of association qualitatively follow the order $\text{py} > \text{3-MePy} > \text{3,5-Me}_2\text{Py} > \text{4-$t$BuPy}$. In particular, the addition of 1 equiv of py or 3-MePy to $\text{Co}_3^{-1}$ results in more than half of the host being occupied, while 1 equiv of 3,5-Me$_2$Py leads to ca. 50% occupancy, and 1 equiv of 4-$t$BuPy produces only a small amount of the host-guest complex. Likewise, full occupancy of the cage is observed once 3 equiv of py or 3-MePy have been added, while much larger amounts of the empty host remain after adding 3 equiv of either of the two bulkier ligands. It is notable that distinct signals for the \textit{endo} host-guest complex can be observed for this series of ligands since similar signals were not observed at any point when titrating $\text{Co}_3^{-1}$ with 4-MePy. These observations confirm that
4-MePy has uniquely poor affinity for binding inside this cage. We did not extend our experiments to examine interactions of 4-ArPy with Co$_3$-1, but it is assumed that this ligand also has no affinity for endo association in this cage since it has no affinity for binding inside Zn$_3$-1.

2.3. Coordination of benzoate ligands to M$_3$-1 and M-2

Benzoate anions (see Scheme 1), in the form of their tetrabutylammonium (TBA) salts, were chosen for examining the coordination of anionic ligands to M$_3$-1 and M-2 in CD$_3$CN. Titration of [TBA][BzO] into a 3 mM solution of Co-2 resulted in shifts of all the $^1$H NMR signals of the complex, with the $\beta$ CH signal of the porphyrin ring showing a particularly dramatic downfield change from 12.58 to 23.43 ppm (Figures 8 and S9). The nearly 11 ppm shift of this signal is a useful diagnostic for the binding of the anionic BzO$^-$/Co$^{II}$ ligand, whereas this resonance is affected much less by coordination of the neutral ligand 4-MePy (Figure S7). Fitting the movement of the $\beta$ CH signal to a 1:1 binding isotherm provided a $K_a$ of 3,700 ± 400 M$^{-1}$ for coordination of BzO$^-$ (Figure S11), which is substantially higher than that determined for the neutral ligand 4-MePy. Additionally, small new signals in the aromatic region and $N$-methyl region of the spectrum grew over the course of the titration and are attributed to the oxidation of the Co$^{II}$ complex to a diamagnetic Co$^{III}$ derivative (Figures S9 and S10). These signals were also observed when just 1 equiv of BzO$^-$ was added to a solution of Co-2, with the oxidized complex representing about 13% of the sample after 12 h (Figures S88–S90). The Co$^{III}$ assignment was also confirmed by UV-vis spectroscopy (Figure S87), which displayed the growth of a new Soret band at a longer wavelength ($\lambda_{\text{max}} \approx 443$ nm) than that for the Co$^{II}$ state ($\lambda_{\text{max}} = 417$ nm), consistent with formation of a Co$^{III}$ porphyrin [34]. We did not attempt to further characterize the Co$^{III}$ product, but a Co$^{III}$-O-O-Co$^{III}$ dimer seems plausible based on precedent established for other axially ligated cobalt porphyrins [35].

Interestingly, titration of the cage Co$_3$-1 with BzO$^-$ produced substantially different results than observed with the monomeric cobalt complex. First, the $^1$H NMR signals of the cage were affected only slightly by the addition of up to 2.1 equiv of BzO$^-$, followed by much more substantial shifts upon further addition of benzoate (Figures 9 and S33–S35). Since the $\beta$ CH resonances of the porphyrins are highly sensitive to

![Figure 8](image-url)
coordination of the anionic ligand to cobalt (see above), the minimal change of these signals until after >2 equiv of BzO$^-$ have been added to Co$_3$-1 suggests that the first two equivalents of the anion do not bind to the Co$^{II}$ centers. We attribute this behavior to the ability of the cage to host two benzoates in anion-binding pockets located just inside the cage apertures (Scheme 5). These sites host PF$_6^-$ in our previously reported solid-state structure of the free-base-porphyrin version of this cage (H$_6$-1) [20], and closely related Pd-linked cages also bind various anions (e.g. PF$_6^-$ or NO$_3^-$) [6, 12]. We are presently conducting a thorough investigation of the binding of arylsulfonate and benzoate anions by H$_6$-1, and preliminary results confirm that such guests interact more strongly ($K_a > 10^5$ M$^{-1}$) with the anion-binding sites of the cage than expected for coordination to the Co$^{II}$ sites of Co$_3$-1.

Since the internal anion-binding pockets in the cages complicate comparisons of benzoate coordination to the metal sites of M$_3$-1 and M-2, a substituted benzoate ligand, 3,5-di-tert-butylbenzoate (3,5-tBu$_2$BzO$^-$), was selected for further study since it was expected to be too bulky to bind inside the cages. Coordination of 3,5-tBu$_2$BzO$^-$ to Co-2 was found to occur with a $K_a$ of 2,200 ± 200 M$^{-1}$, which is weaker than for the unsubstituted benzoate ligand but within the same order of magnitude (Table 3). Likewise, the presence of 3,5-tBu$_2$BzO$^-$ induces partial oxidation of the porphyrin complex to Co$^{III}$ (see Figures S92–S95), much as was observed for samples of Co-2 containing the smaller BzO$^-$ anion. Thus, the properties of the substituted and unsubstituted benzoates are comparable with respect to binding to the monomeric complex Co-2.

**Figure 9.** Aromatic region of the $^1$H NMR spectra (CD$_3$CN, 298 K, 500 MHz) of a 1 mM sample of Co$_3$-1 upon titration with [TBA][BzO]. Changes to the signals of the cage are tracked with dotted lines and the $\beta$ CH resonance of the porphyrins are labeled.
Titration of $\text{Co}_3\cdot 1$ with 3,5-$t$Bu$_2$BzO$^-$ resulted in gradual changes of all the $^1$H NMR signals of the cage, including the $\beta$ CH resonances, well before 2 equiv of 3,5-$t$Bu$_2$BzO$^-$ had been added to a 1 mM solution of $\text{Co}_3\cdot 1$ in CD$_3$CN (Figures S36–S38). These results appear to confirm that the two $t$-butyl groups of 3,5-$t$Bu$_2$BzO$^-$ inhibit this benzoate anion from interacting with the two internal anion-binding pockets of the cage, allowing the anion to interact with the Co$^{II}$ centers earlier in the titration. However, as can be seen in Figures S37–S40, the different resonances of $\text{Co}_3\cdot 1$ show inconsistent behavior during the titration, with some signals nearing their final values earlier than others. Changes of one of the sharper aromatic signals of $\text{Co}_3\cdot 1$ could be fitted reasonably well to an isotherm for 1:1 association between the benzoate ligand and the Co$^{II}$ sites (Figure S39), but the movement of this signal begins to taper off at a point in the titration when the $\beta$ CH signals of the cage still shift considerably (Figures S37–S40). Since movement of the broad $\beta$ CH signals is diagnostic of benzoates binding to Co$^{II}$, it is unlikely that movement of the sharper aromatic signal corresponds primarily to binding of the anions to cobalt. It is conceivable that the exteriors [43] of the cationic apertures of the cage retain some competing affinity with the Co$^{II}$ sites for binding 3,5-$t$Bu$_2$BzO$^-$, resulting in the contradictory changes observed in the $^1$H NMR spectra during titration. Thus, it was not possible to determine a reliable association constant for binding of 3,5-$t$Bu$_2$BzO$^-$ to the Co$^{II}$ sites of $\text{Co}_3\cdot 1$, though the substantial shifts of the $\beta$ CH signals of the cage suggest that binding of the anion to the cobalt sites must not be severely inhibited relative to binding of this anion to Co-2.

Unlike the monomeric cobalt complex, the $^1$H NMR and UV-vis spectra of $\text{Co}_3\cdot 1$ did not show significant formation of Co$^{III}$ species in the presence of either the substituted or unsubstituted benzoate anions. Thus, $\text{Co}_3\cdot 1$ appears to be more resistant to oxidation to Co$^{III}$ than its monomeric congener. The internal Co–Co spacing of $\text{Co}_3\cdot 1$ is expected to be between 8–9 Å based on solid-state structures determined for closely related cages [6, 12, 20]. This distance is too large to facilitate O$_2$ binding between two internal cobalt sites of a single cage, which would require a Co–Co distance of $<5$ Å [14, 44–46], while coordination of the benzoate ligands to the external faces of the cage prevents the intermolecular binding of O$_2$ between two cages. These factors likely contribute to the observed preference for $\text{Co}_3\cdot 1$ to remain in its Co$^{II}$ state.

The association of 3,5-$t$Bu$_2$BzO$^-$ with Zn-2 and Zn$_3\cdot 1$ was also examined, revealing that these show substantial differences between the cage and monomeric porphyrin for the association of this benzoate anion. The titration of 3,5-$t$Bu$_2$BzO$^-$ to Zn-2

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**Table 3.** Association constants for coordination of benzoate ligands to porphyrin complexes and cages.

| Metalloporphyrin derivative and benzoate ligand | $K_a$ (M$^{-1}$) |
|-----------------------------------------------|-----------------|
| Co-2 + BzO$^-$                                 | 3,700 ± 400     |
| Co-2 + 3,5-$t$Bu$_2$BzO$^-$                   | 2,200 ± 200     |
| Co$_3\cdot 1$ + 3,5-$t$Bu$_2$BzO$^-$          | NA$^b$          |
| Zn-2 + 3,5-$t$Bu$_2$BzO$^-$                   | (2.3 ± 0.6)×10$^3$ |
| Zn$_3\cdot 1$ + 3,5-$t$Bu$_2$BzO$^-$          | 5,000 ± 600     |

$^a$Association constant per metal site for 1:1 coordination of benzoate anions in CD$_3$CN.

$^b$Metal-ligand association could not be quantified reliably due to competing interactions of the anions with other sites of the cage.
produced near saturation of the zinc sites when 1 equiv of ligand was added, even for low concentration (0.03 mM) samples of Zn-2 (Figures S18 and S19), thus revealing very strong association of the anionic ligand. A $K_a$ of $(2.3 \pm 0.6) \times 10^5 \text{M}^{-1}$ was determined, with the rather high error resulting from the difficulty of analyzing titration data for such strong binding.

Greater complexity was found for interaction of 3,5-$t$Bu$_2$BzO$^-$ with Zn$_3$-1. However, while the $^1$H NMR data for titration of Co$_3$-1 with 3,5-$t$Bu$_2$BzO$^-$ was challenging to interpret, the titration data for Zn$_3$-1 provided better insight into the interactions of the anionic ligand with the cage. Most of the aromatic signals of the cage exhibited similar patterns of change, with the exception of the 6-position CH resonance of the pyridyl groups of the cage (Figures 10, S67, and S68). This signal showed only slight changes until after the other aromatic resonances of the cage had neared their final shifts, at which point the 6-position signal moved dramatically as more 3,5-$t$Bu$_2$BzO$^-$ was added. The signals that moved initially in the titration provided a good fit to an isotherm for independent binding of 3,5-$t$Bu$_2$BzO$^-$ to the three Zn sites of Zn$_3$-1 (Figure S70), revealing the cage has a lower affinity ($K_a = 5,100 \pm 600 \text{M}^{-1}$) for 3,5-$t$Bu$_2$BzO$^-$ than was observed with the monomeric zinc complex. Subsequent changes to the 6-position signal must arise from the association of additional benzoate anions with other parts of the cage. The 6-position of the pyridyl groups of Zn$_3$-1 are among the most outward-facing CH bonds of the cage, so the unique movement of these resonances strongly implies interactions of 3,5-$t$Bu$_2$BzO$^-$ with the exterior of the cage. These findings confirm that the benzoate anion is not limited to association with the metal sites of Zn$_3$-1. Nevertheless, since these additional interactions are weaker than coordination of 3,5-$t$Bu$_2$BzO$^-$ to the zinc sites (i.e. the zinc sites are nearly saturated before the additional interactions become evident), they do not interfere

Figure 10. Aromatic region of the $^1$H NMR spectra (CD$_3$CN, 298 K, 500 MHz) of a 1 mM sample of Zn$_3$-1 upon titration with [TBA][3,5-$t$Bu$_2$BzO]. Dotted lines mark changes to the 2-position and 6-position CH resonances of the pyridyl groups of Zn$_3$-1, showing the different behavior of these signals. The other aromatic signals are labeled and show similar relative changes as the 2-position signal.
with analysis of benzoate coordination to the metal sites. Thus, it can be reliably concluded that the cage structure weakens coordination of the benzoate anions to the zinc sites.

3. Summary and conclusions

These studies reveal that the cage structures of $\text{M}_3\cdot1$ can have a range of influences on the binding of monodentate ligands to metal centers supported in the porphyrin walls. Neutral pyridine ligands bind to the outer faces of the cages with comparable or slightly lower affinity than binding to monomeric metalloporphyrins, at least with respect to the first one or two equivalents of the ligand that bind to the cages. Examining interactions of 4-MePy and 4-tBuPy with $\text{Zn}_3\cdot1$ revealed a subtle allosteric effect in which endo coordination of these ligands becomes increasingly favorable relative to exo coordination after one or two exo zinc sites are occupied. This behavior could be explained by negative cooperativity for ligands binding at the exo sites, which would decrease the stepwise association constants for successive ligands binding to the cage. However, it can be challenging to determine the weaker association constants in such equilibria, especially for systems with more than two receptor sites, preventing further resolution of how allosteric effects impact the strength and stoichiometry of ligand binding to the outer surfaces of $\text{M}_3\cdot1$.

The coordination of pyridine ligands to the internal metal sites of $\text{M}_3\cdot1$ could be scrutinized in greater detail than the binding of ligands outside the cages, revealing considerable variability in the strength of endo ligand binding. Unsubstituted pyridine, 3-MePy, and 3,5-Me$_2$Py all show enhanced binding in the cages to give well-defined 1:1 complexes, with this association enhanced for the less substituted ligands. Pyridine ligands substituted in the 4-position (4-MePy, 4-tBuPy, and 4-ArPy) showed lower affinity for binding in the cages, with the endo association of 4-ArPy completely inhibited by the length of this ligand. However, 4-tBuPy binds more strongly than 4-MePy in either $\text{Co}_3\cdot1$ or $\text{Zn}_3\cdot1$, demonstrating that the strength of endo coordination is not always inversely proportional to the size of the pyridine ligands. It is likely the 4-tBuPy better matches the size of the internal cavity of the cages, strengthening the binding of this ligand relative to the smaller 4-MePy ligand.

Examining the coordination of benzoate ligands to $\text{M}_3\cdot1$ was complicated by competing affinity of these anions for sites of the cages other than the metal centers. For unsubstituted benzoate, $\text{Co}_3\cdot1$ provides anion recognition sites in its interior that compete with the metal sites for binding the anionic ligand. The much bulkier 3,5-tBu$_2$BzO$^-$ ligand eliminates competition from the internal anion binding pockets of $\text{Co}_3\cdot1$, but other interactions of the anion with the cage still compete with binding to the Co$^{II}$ sites. Only the combination of $\text{Zn}_3\cdot1$ and 3,5-tBu$_2$BzO$^-$ provided data that were clear enough to use to quantify the strength of interactions of the anions with the metal sites of the cage. Remarkably, the cage considerably weakens the metal-ligand interactions relative to the binding of 3,5-tBu$_2$BzO$^-$ to a monomeric zinc porphyrin complex. Together, our data show that 3D assemblies of porphyrins can exhibit a myriad of complex influences on the binding of simple ligands to the metalloporphyrins, leading in some cases to
substantially strengthened or weakened ligand binding as well as allosteric effects that alter the relative favorability of endo and exo coordination of ligands.

4. Experimental

4.1. General considerations

Unless otherwise specified, commercially available chemicals and solvents were used as received from (1) Fisher: acetonitrile, pyridine (py); (2) Acros Organics: ammonium hexafluorophosphate, zinc acetate dihydrate, tetrabutylammonium hydroxide 40 wt% (1.5 M) aqueous solution; (3) Cambridge Isotopes: acetonitrile-d$_3$ (CD$_3$CN, D-99.8%); (4) TCI Chemicals: 4-methylpyridine (4-MePy); (5) Ambeed: 3,5-di-tert-butylbenzoic acid. (6) Alfa Aesar: 3-methylpyridine (3-MePy), 3,5-dimethylpyridine (3,5-Me$_2$Py); (7) Accela: 4-tert-butylpyridine (4-tBuPy). Nanocages Co$_3$-1 and Zn$_3$-1, and porphyrins Co-2 and [tetra(N-methyl-3-pyridinium)porphyrin]$_2$PF$_6$ (2) were prepared as we have previously described [34, 47]. Tetrabutylammonium benzoate ([TBA][BzO]) and 4-(4'-methoxyphe- 

NMR spectra were recorded on samples in CD$_3$CN at 298 K using a Bruker AVANCE Neo 500 MHz spectrometer or a Varian VNMRS 500 MHz spectrometer. $^1$H NMR spectra were referenced using the residual proteo signal of CD$_2$HCN and $^{13}$C{$^1$H} NMR spectra were referenced to the nitrile $^{13}$C{$^1$H} signal of CD$_3$CN. High resolution mass spectra were obtained via direct infusion of acetonitrile solutions of the analyte into a Waters Xevo G2-XS QTOF mass spectrometer.

4.2. Evaluation of metal-ligand association constants

4.2.1. Representative procedure for systems showing only exo association

A sample of Co$_3$-1 (3.16 mg, 0.56 μmol) was dissolved in 560 μL CD$_3$CN to provide a 1.0 mM solution. After an initial $^1$H NMR spectrum was acquired, 3 μL aliquots of a 0.056 M solution of 4-methylpyridine (4-MePy) were titrated into the solution of the cage, providing 0.3 equiv of the ligand per addition, with the $^1$H NMR spectrum of the sample acquired after each addition. After twenty additions of 4-MePy (6 equiv total), the sizes of the additions were increased steadily through the rest of the experiment, ending with 30 equiv of 4-MePy added in total. Changes to the chemical shift of a resonance of Co$_3$-1 starting at 10.16 ppm in the initial spectrum were analyzed using Bindfit (supramolecular.org) [36]. A 1:1 binding model was employed with a 3.0 mM concentration of host representing three independent cobalt sites of the 1 mM concentration sample of Co$_3$-1. This model provided an excellent fit to the experimental data, revealing a binding constant of 720 ± 20 M$^{-1}$. The titration data were also analyzed using a 1:2 non-cooperative binding model and a 1.0 mM concentration of host, also providing a good fit to give a binding constant $K_{aexo}$ of 1,770 ± 30 M$^{-1}$.

4.2.2. Representative procedure for systems showing strong endo association

A sample of Zn$_3$-1 (2.26 mg, 0.40 μmol) was dissolved in 400 μL CD$_3$CN to provide a 1.0 mM solution. After an initial $^1$H NMR spectrum was acquired, 1 μL aliquots of a 0.040 M solution of 3-methylpyridine (3-MePy) were titrated into the solution of the
cage, providing 0.1 equiv of the ligand per addition until 2.0 equiv had been added. The $^1$H NMR spectrum of the sample was acquired after each addition, and the ratio of $\text{Zn}_3\text{-1}$ to endo-(3-MePy)-$\text{Zn}_3\text{-1}$ was determined by integration of the 2-position CH resonance of the 3-pyridyl groups of the porphyrin walls of the cage and endo host-guest complex. The concentration of unbound 3-MePy was calculated as the difference between the amount of 3-MePy added and the amount encapsulated. The concentrations of the empty cage, occupied host, and free ligand were used to calculate the association constant for binding of 3-MePy inside the cage, using only the spectra acquired between the addition of 1.1–1.7 equiv of ligand since these samples had sufficient concentrations of each component of the equilibrium to quantify reliably. This analysis produced $K_{a-\text{endo}_1}$ values ranging from 18,000 M$^{-1}$ to 27,000 M$^{-1}$ with an average of $(2.3 \pm 0.1) \times 10^{-4}$ M$^{-1}$ for the seven data points used.

For analyzing exo coordination of 3-MePy to the initially formed endo-(3-MePy)-$\text{Zn}_3\text{-1}$ complex, a 1.0 mM sample of $\text{Zn}_3\text{-1}$ was prepared as described above and then titrated with 3-MePy in 0.5–1.0 equiv increments up to a total of 15 equiv. After the first 1.0 equiv of 3-MePy was added, shifts of the 2-position CH resonance of the 3-pyridyl groups of the porphyrin walls of endo-(3-MePy)-$\text{Zn}_3\text{-1}$ were measured and fitted to a 1:2 non-cooperative binding isotherm with the concentration of added 3-MePy adjusted to account for the amount of the ligand encapsulated in the host. This analysis provided a $K_{a-\text{exo}_1}$ of 1,100 ± 50 M$^{-1}$. The data also fit well to a 1:1 binding model, providing $K_{a-\text{exo}}$ of 705 ± 18 M$^{-1}$.

### 4.2.3. Representative procedure for systems showing competing endo and exo association

A 1.0 mM solution of $\text{Zn}_3\text{-1}$ was prepared as described above, and a 40 µL aliquot of this solution was diluted to 400 µL with CD$_3$CN to provide a 0.1 mM sample of the cage. This solution was titrated with 4-tBuPy in 0.1–1.0 equiv increments until a total of 15 equiv had been added. Shifts of the 2-position CH resonance of the 3-pyridyl groups of the porphyrin walls of $\text{Zn}_3\text{-1}$ were fitted to a 1:1 binding model employing a 0.3 mM concentration of host representing three independent zinc sites for the 0.1 mM sample of the cage. This analysis provided a $K_{a-\text{exo}}$ of 3,400 ± 100 M$^{-1}$. The data were also analyzed with a 1:2 non-cooperative binding model and a 0.1 mM concentration of host, providing a $K_{a-\text{exo}_1}$ of 3,800 ± 100 M$^{-1}$. For both binding models, the concentration of 4-tBuPy in solution was not corrected to account for the amount of this ligand that becomes encapsulated in the cage. The resulting error caused by ignoring this consideration is expected to be small owing to the low concentration (0.1 mM) of the host that was used in the titration.

For analyzing endo coordination of 4-tBuPy in $\text{Zn}_3\text{-1}$, the ratio of unoccupied cage to endo-(4-tBuPy)-$\text{Zn}_3\text{-1}$ was determined by integration of the spectra acquired between the addition of 1.0–2.0 equiv of ligand to the sample described above. The concentration of free 4-tBuPy in solution was calculated as the difference between the amount added and the amount encapsulated by the cage. Association constants ($K_{a-\text{endo}}$) of 5,700 M$^{-1}$ to 8,700 M$^{-1}$ were determined, with an average of 6,300 ± 500 M$^{-1}$ for the six spectra that were analyzed.
4.2.4. Special considerations for titrations with anions

Upon titrating $\text{M}_3^-$ and $\text{M}_2^-$ with $\text{BzO}^-$ or $3,5^\text{-tBu}_2\text{BzO}^-$, the first additions of the ligands produced much smaller changes to the spectra of the porphyrin cages and complexes than observed for subsequent additions (see Figure S9 for an example). We attribute this behavior to the presence of trace impurities (either an acid or metal cations) that protonate or precipitate the benzoate anion from solution after the first addition. Thus, the spectra acquired after the first addition of the ligands was treated as the initial point of the titration when fitting the resulting spectral changes to binding isotherms, providing much better fitting than without this correction.

4.3. Synthetic procedures

4.3.1. Zn-2·4PF$_6$

[tetra($N$-methyl-$3$-pyridinium)porphyrin]-4PF$_6$ (2, 32.6 mg, 25.9 µmol) was dissolved in 50 mL of MeCN in a 100 mL round bottom flask to give a dark purple solution. Zn(OAc)$_2$·2H$_2$O (28.4 mg, 129.5 µmol) was added as a solid and stirred at room temperature for 16 h, resulting in a dark green-purple solution. This solution was concentrated to 20 mL under reduced pressure, followed by addition of solid NH$_4$PF$_6$ (147.8 mg, 906.5 µmol) and then 200 mL of water, resulting in a fine green precipitate. The supernatant was removed by vacuum filtration on a fine mesh frit, and the solids were washed with water (3×15 mL) and then Et$_2$O (15 mL). The product was washed through the frit with 10 mL of MeCN and the resulting solution was dried by rotary evaporation, providing 26.7 mg of Zn-2·4PF$_6$ as a dark green-purple solid (78% yield).

$^1$H NMR (500 MHz, CD$_3$CN): $\delta$ 9.48 (s, 4 H), 9.22 (d, $J = 7.7$ Hz, 4 H), 9.13 (d, $J = 6.2$ Hz, 4 H), 9.02 (s, 8 H), 8.46 (t, $J = 7.06$ Hz, 4 H), 4.64 (s, 12 H). $^{13}$C($^1$H) NMR (125 MHz, CD$_3$CN): $\delta$ 151.10, 149.12, 147.88, 145.71, 143.42, 133.79, 127.43, 114.02, 49.63.

4.3.2. [TBA][3,5-tBu$_2$BzO]

Adapting a literature procedure [48], 3,5-di-tert-butylbenzoic acid (256.8 mg, 1.10 mmol) was dissolved in 10 mL ethanol. Tetrabutylammonium hydroxide (730.5 µL, 1.5 M, 1.10 mmol) was added, and the resulting solution was stirred for 5 minutes. Solvent was removed by rotary evaporation, providing a pale-yellow oil, which was further dried overnight under vacuum at 80°C to afford 496.8 mg of [TBA][3,5-tBu$_2$BzO] as a white powder (95.3% yield). $^1$H NMR (500 MHz, CD$_3$CN): $\delta$ 7.81 (m, 2 H), 7.38 (t, $J = 2.0$ Hz, 1 H), 3.12 (m, 8 H), 1.59 (q, $J = 8.07$ Hz, 8 H), 1.38-1.29 (m, 26 H), 0.95 (t, $J = 7.3$ Hz, 12 H). $^{13}$C NMR (125 MHz, CD$_3$CN): $\delta$ 170.69, 149.97, 142.71, 124.16, 122.83, 59.24, 53.30, 31.89, 24.30, 20.29, 13.77.

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Disclosure statement

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References

[1] S.J. Webb, J.K.M. Sanders. Inorg. Chem., 39, 5912 (2000).
[2] H. Nobukuni, Y. Shimazaki, H. Uno, Y. Naruta, K. Ohkubo, T. Kojima, S. Fukuzumi, S. Seki, H. Sakai, T. Hasobe, F. Tani. Chemistry, 16, 11611 (2010).
[3] M. Boccalon, E. Iengo, P. Tecilla. Org. Biomol. Chem., 11, 4056 (2013).
[4] C. Zhang, Q. Wang, H. Long, W. Zhang. J. Am. Chem. Soc., 133, 20995 (2011).
[5] C. García-Simón, A. Monferrer, M. García-Borrás, I. Imaz, D. Maspoch, M. Costas, X. Ribas. Chem. Commun. (Camb.), 55, 798 (2019).
[6] A.K. Bar, S. Mohapatra, E. Zangrando, P.S. Mukherjee. Chemistry, 18, 9571 (2012).
[7] S. Durot, J. Taesch, V. Heitz. Chem. Rev., 114, 8542 (2014).
[8] W. Brenner, T.K. Ronson, J.R. Nitschke. J. Am. Chem. Soc., 139, 1098 (2021).
[9] D.A. Rothschild, W.P. Kopcha, A. Tran, J. Zhang, M.C. Lipke. Chem. Sci., 13, 5325 (2022).
[10] P.T. Smith, B.P. Benke, Z. Cao, Y. Kim, E.M. Nichols, K. Kim, C.J. Chang. Angew. Chem. Int. Ed., 40, 1718 (2001).
[11] Y. Hatakeyama, T. Sawada, M. Kawano, M. Fujita. Angew. Chem. Int. Ed. Engl., 48, 8695 (2009).
[12] A.N. Oldacre, A.E. Friedman, T.R. Cook. J. Am. Chem. Soc., 139, 1424 (2017).
[13] M.R. Crawley, D. Zhang, A.N. Oldacre, C.M. Beavers, A.E. Friedman, T.R. Cook. J. Am. Chem. Soc., 143, 1098 (2021).
[14] P.T. Smith, B.P. Benke, Z. Cao, Y. Kim, E.M. Nichols, K. Kim, C.J. Chang. Angew. Chem. Int. Ed. Engl., 57, 9684 (2018).
[15] P.T. Smith, Y. Kim, B.P. Benke, K. Kim, C.J. Chang. Angew. Chem. Int. Ed. Engl., 59, 4902 (2020).
[16] P.F. Kuipers, M. Otte, M. Dürr, I. Ivanović-Burmazović, J.N.H. Reek, B. de Bruin. ACS Catal., 6, 3106 (2016).
[17] V. Mourarrawis, E.O. Bobylev, B. de Bruin, J.N.H. Reek. Chemistry, 27, 8390 (2021).
[18] N.P.E. Barry, O. Zava, P.J. Dyson, B. Therrien. Aust. J. Chem., 63, 1529 (2010).
[19] K.G. Dutton, D.A. Rothschild, D.B. Pastore, T.J. Emge, M.C. Lipke. Inorg. Chem., 59, 12616 (2020).
[20] Y.-R. Zheng, Z. Zhao, M. Wang, K. Ghosh, J.B. Pollock, T.R. Cook, P.J. Stang. J. Am. Chem. Soc., 132, 16873 (2010).
[21] A.K. Bar, R. Chakrabarty, G. Mostafa, P.S. Mukherjee. Angew. Chem. Int. Ed. Engl., 47, 8455 (2008).
[22] R.F. Kelley, S.J. Lee, T.M. Wilson, Y. Nakamura, D.M. Tiede, A. Osuka, J.T. Hupp, M.R. Wasielewski. J. Am. Chem. Soc., 130, 4277 (2008).
[23] S.J. Lee, K.L. Mulfert, X. Zuo, A.J. Goshe, P.J. Wesson, S.T. Nguyen, J.T. Hupp, D.M. Tiede. J. Am. Chem. Soc., 130, 836 (2008).
[24] J. Elemans, E.J.A. Bijsterveld, A.E. Rowan, R.J.M. Nolte. Chem. Commun., 2443 (2000).
[25] T. Kojima, T. Nakanishi, T. Honda, R. Harada, M. Shiro, S. Fukuzumi. Eur. J. Inorg. Chem., 2009, 727 (2009).
[27] Z. Zhang, W.-Y. Gao, L. Wojtas, S. Ma, M. Eddaoudi, M.J. Zaworotko. Angew. Chem. Int. Ed. Engl., 51, 9330 (2012).
[28] D. Solomon, P. Peretz, M. Faraggi. J. Phys. Chem., 86, 1842 (1992).
[29] D.J. Martin, J.M. Mayer. J. Am. Chem. Soc., 143, 11423 (2021).
[30] T.L. Poulos. J. Biol. Inorg. Chem., 1, 356 (1996).
[31] J. Chlistunoff, J.-M. Sansiñena. J. Phys. Chem. C, 118, 19139 (2014).
[32] Y. Zhou, Y.-F. Xing, J. Wen, H.-B. Ma, F.-B. Wang, X.-H. Xia. Sci. Bull., 64, 1158 (2019).
[33] D. Balcells, C. Raynaud, R.H. Crabtree, O. Eisenstein. Inorg. Chem., 47, 10090 (2008).
[34] P.T. Blackburn, I.F. Mansoor, K.G. Dutton, A.M. Tyryshkin, M.C. Lipke. Chem. Commun. (Camb.), 57, 11342 (2021).
[35] F.A. Walker. J. Am. Chem. Soc., 95, 1150 (1973).
[36] D.B. Hibbert, P. Thordarson. Chem. Commun. (Camb.), 52, 12792 (2016).
[37] G.N. La Mar, F.A. Walker. J. Am. Chem. Soc., 95, 1790 (1973).
[38] D.M. Collins, J.L. Hoard. J. Am. Chem. Soc., 92, 3761 (1970).
[39] D.L. Cullen, E.F. Meyer. Jr. Acta Crystallogr. B Struct. Crystallogr. Cryst. Chem., 32, 2259 (1976).
[40] C.K. Schauer, O.P. Anderson, S.S. Eaton, G.R. Eaton. Inorg. Chem., 24, 4082 (1985).
[41] B. Puttaiah, V. Veerapandian. J. Chem. Sci., 127, 663 (2015).
[42] A.D. Shukla, P.C. Dave, E. Suresh, A. Das, P. Dastidar. J. Chem. Soc., Dalton Trans., 4459 (2000).
[43] C. Sgarlata, J.S. Mugridge, M.D. Pluth, B.E.F. Tiedemann, V. Zito, G. Arena, K.N. Raymond. J. Am. Chem. Soc., 132, 1005 (2010).
[44] L. Nurdin, D.M. Spasyuk, L. Fairburn, W.E. Piers, L. Maron. J. Am. Chem. Soc., 140, 16094 (2018).
[45] M. Volpe, H. Hartnett, J.W. Leeland, K. Wills, M. Ogunsun, B.J. Duncombe, C. Wilson, A.J. Blake, J. McMaster, J.B. Love. Inorg. Chem., 48, 5195 (2009).
[46] D.B. Leznoff, M.J. Katz, L.K.L. Cheng, N.D. Draper, R.J. Batchelor. J. Mol. Struct., 796, 223 (2006).
[47] M.B. Berezin, N.M. Berezina, M.I. Bazanov, A.I. Vyugin, A.S. Semeikin, A.V. Glazunov. Russ. J. Phys. Chem., 84, 1449 (2010).
[48] H. Jiang, L. Xie, Z. Duan, K. Lin, Q. He, V.M. Lynch, J.L. Sessler, H. Wang. Chemistry, 27, 15006 (2021).
[49] Y. Zhang, T.-Y. Zhou, K.-D. Zhang, J.-L. Dai, Y.-Y. Zhu, X. Zhao. Chem. Asian J., 9, 1530 (2014).