Tuning of Binding Selectivity: Metal Control of Organic Guest Binding and Allosteric Perturbation of Fluorescent Metal Sensor

Fen Wang and Alan W. Schwabacher*

Department of Chemistry, University of Wisconsin—Milwaukee, Milwaukee, Wisconsin 53211

Received August 5, 1999

Ligand **1**, bearing two ethylenediamine groups, was designed to form a hydrophobic cavity upon binding to metals. The shape of its nonpolar cavity depends on the metal: a reversal in binding preference for naphthalene or biphenyl groups is found when the metal is changed from zinc to copper, with a selectivity change of 260-fold. In the presence of dansylated amino acids, the new ligand constitutes a fluorescent sensor for zinc ion. Variations are seen in affinity for dansylamino acid with minor structural changes, and organic selectivity changes with complexes of variant metals. These findings suggest that sensor tuning of affinity and selectivity for metal is possible by choice of simple organic guest and for organic guest by choice of metal.

Introduction

Sensors for determination of metals and organic analytes are of great technical significance.[1,2] Biosensors[3] offer exquisite selectivities because of their subtle positioning of functional groups. One of the important areas in contemporary sensor development is the design and synthesis of functional molecules as fluorescent receptors for the spectrometric determination of metal ions with high sensitivity and selectivity.[4,5] Imperiali has recently demonstrated[6] that hybrid peptide structures are advantageous: the high metal affinity of simple organic ligands can be tuned by incorporation into peptide structures.

Synthesis of self-assembled structures has received considerable attention in the field of molecular recognition,[7] due to the efficiency provided for construction of well-defined large structures.[8,9] The ability of a metal ion to orient, as well as to gather organic fragments around its coordination sphere, provides a versatile way to build specific recognition sites.[7a,10,11] The metal coordination geometry restricts the conformation, varying the geometry and cavity shape of a macrocyclic receptor. Schneider and DeShayes have studied metal-organized cyclophane receptors that enhance the fluorescence intensity of a fluorophore.[12]

Metal tuning in such a structure would be of particular interest. We decided to investigate the ability of a metal to tune the selectivity for organic ligand binding and its converse, tuning of metal binding by an organic guest. We report here that flexible bis diamine **1** forms a complex with both zinc and copper (Figure 1) and in doing so closes the macrocycle. Furthermore, the distinct coordination geometries of these metals perturb the shape of the nonpolar cavity. Our studies were undertaken to investigate the effect of the metal identity on the organic binding selectivity and organic guest on metal affinity. Here we confirm that the identity of the metal can have a profound effect on the selectivity of the derived receptor and also demonstrate a new approach to the tuning of metal selectivity in sensors.[13]

Results and Discussion

Synthesis of Ligand 1. We have designed and studied several diarylphosphinic acid derivatives because of their favorable properties: the aryl groups provide a hydrophobic concave surface for the cavity, and the phosphinate anion provides water solubility and a hydrophilic

* To whom correspondence should be addressed. E-mail: awschwab@uwm.edu.

(1) *Chemosensors of Ion and Molecular Recognition*; Desvergne, J.-P., Czarnik, A. W., Ed.; Kluwer Academic Press: Dordrecht, The Netherlands, 1997; Vol. 492.

(2) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515−1566.

(3) *Advances in Biosensors*; Turner, A. P. F., Yevdokimov, Y. M., Ed.; JAI Press: Greenwich, CT, 1995; Vol. 3.

(4) Dujols, V.; Ford, F.; Czarnik, A. W. *J. Am. Chem. Soc.* **1997**, *119*, 7386-7387. Corradini, R.; Dossena, A.; Galaverna, G.; Marchelli, R.; Panagia, A.; Sartor, G. *J. Org. Chem.* **1997**, *62*, 6283−6289. Mortellaro, M. A.; Nocera, D. G. *J. Am. Chem. Soc.* **1996**, *118*, 7414−7415. Czarnik, A. W. *Chem. Biol.* **1995,** *2*, 423−428.

(5) Czarnik, A. W. *Acc. Chem. Res.* **1994**, *27*, 302−308. Valeur, B.; Bardez, E. *Chem. Brit.* **1995**, 216−220.

(6) Torrado, A.; Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1998**, *120*, 609−610. Walkup, G. K.; Imperiali, B. *J. Am. Chem. Soc.* **1997**, *119*, 3443−3450. Cheng, R. P.; Fisher, S. L.; Imperiali, B. *J. Am. Chem. Soc.* **1996**, *118*, 11349−11356.

(7) (a) Linton, B.; Hamilton, A. D. *Chem. Rev.* **1997**, *97*, 1669−1680. (b) Conn, M. M.; Rebek, J., Jr. *Chem. Rev.* **1997**, *97*, 1647−1668.

(8) Zeng, F.; Zimmerman, S. C. *Chem. Rev.* **1997**, *97*, 1681−1712. Seto, C. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **1993**, *115*, 905−916. Lindsey, J. S. *New J. Chem.* **1991**, *15*, 153−180.

(9) Wyler, R.; de Mendoza, J.; Rebek, J., Jr. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1699. Zimmerman, S. C.; Zeng, F.; Reichert, D. E. C.; Kolotuchin, S. V. *Science* **1996**, *271*, 1095−1098.

(10) Fujita, M.; Aoyagi, M.; Ibukuro, F.; Ogura, K.; Yamaguchi, K. *J. Am. Chem. Soc.* **1998**, *120*, 611−612. Rivera, J. M.; Martin, R.; Rebek, J., Jr. *Science* **1998**, *279*, 1021−1023. Kuroda, Y.; Kawashima, A.; Hayashi, Y.; Ogoshi, H. *J. Am. Chem. Soc.* **1997**, *119*, 4929−4933. Olenyuk, B.; Whiteford, J. A.; Stang, P. J. *J. Am. Chem. Soc.* **1996**, *118*, 8221−8230. Cole, K. L.; Farran, M. A.; Deshayes, K. *Tetrahedron Lett.* **1992**, *33*, 599−602. Maverick, A. W.; Ivie, M. L.; Waggenspack, J. H.; Fronczek, F. R. *Inorg. Chem.* **1990**, *29*, 2403−2409.

(11) Fox, O. D.; Dulley, N. K.; Harrison, R. G. *J. Am. Chem. Soc.* **1998**, *120*, 7111−7112.

(12) Baldes, R.; Schneider, H. J. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 321−323. Schneider, H. J.; Ruf, D. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1159−1160. Cole, K. L.; Farran, M. A.; Deshayes, K. *Tetrahedron Lett.* **1992**, *33*, 599−602.

(13) Part of this work has been communicated: Wang, F.; Schwabacher, A. W. *Tetrahedron Lett.* **1999**, *40*, 7641−7644.

10.1021/jo991246t CCC: $18.00
Published on Web 11/26/1999

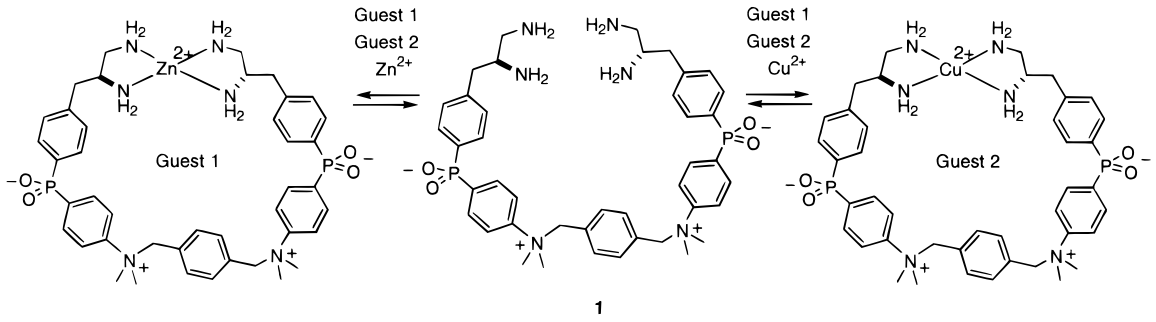
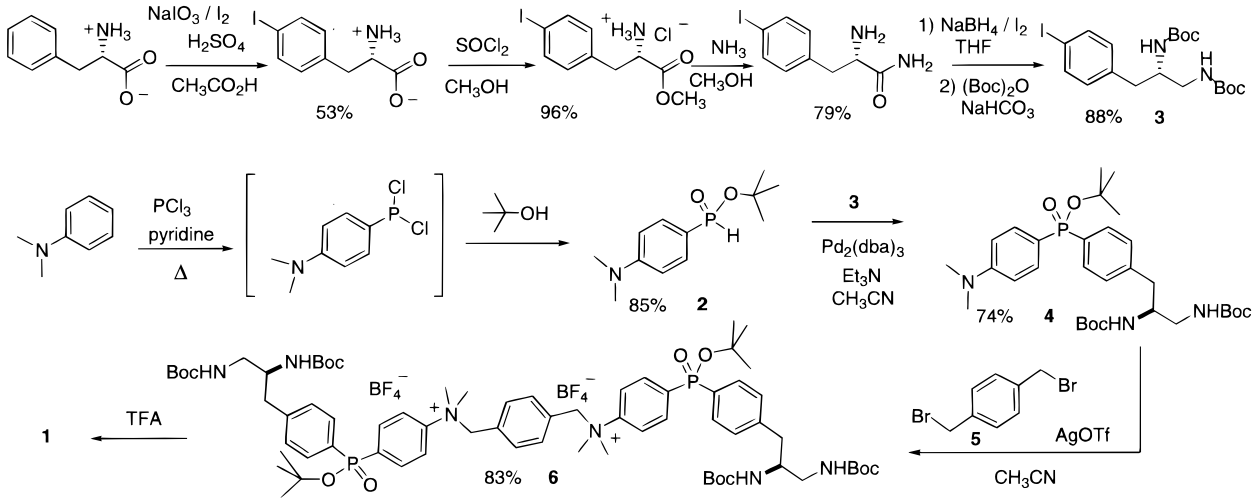


Figure 1.

Scheme 1



convex surface.[14] A Co^{2+}-assembled bis amino acid macrocycle formed a cavity of ideal shape for indole and naphthalene derivatives.[14a,b] A related structure with ethylenediamine groups was found to ligate Zn^{2+} to form a complex that showed substantial discrimination upon binding to naphthalene guests.[14c] The metals play both structural and functional roles, as substrates bind best that can simultaneously fill the cavity and interact directly with the metal. Variation of the metal causes a perturbation of the binding abilities,[14] but relatively few metals cause the formation of good binding sites. With the expectation that the requirements for a single metal would be less stringent than those placed upon metals that must both assemble and organize a receptor, we now report a related molecule that binds a single metal ion to form a hydrophobic binding site. Dibromoxylene was chosen as an appropriate sized spacer because of its ready reactivity. Simply by changing the spacer, aniline, or iodoaryl group, a family of related structures could be prepared similarly.

The desired ligand **1** was prepared as shown in Scheme 1. *N*,*N*-Dimethylaniline is converted to *tert*-butyl arylphosphinate **2** by heating in a sealed tube with PCl_3 and pyridine, followed by quenching with *tert*-butyl alcohol. Iodide **3** was prepared in enantiomerically pure form from phenylalanine as we have reported.[14c] Palladium-catalyzed coupling[15] of phosphinate **2** with iodide **3** leads to a diastereomeric mixture of diarylphosphinates **4**. Alkylation with dibromide **5** in the presence of AgOTf gave **6**, a protected form of **1**. Compound **6**, formed initially as a triflate salt, was isolated as the tetrafluoroborate salt by chromatography on silica gel with $NaBF_4$. Deprotection with acid provides the desired ligand.

The syntheses of dansylamino[16] acids were carried out in the usual way with dansyl chloride in the presence of excess amino acid under basic conditions, followed by acidification.

Complex Formation. Association of ligand **1** with metals is readily discerned: changes in NMR and CD spectra are consistent with 1:1 complexes (Figure 2) with Zn^{2+} and Cu^{2+}, as well as a 2:1 complex in the presence of excess Cu^{2+}. The shape of the hydrophobic cavity, as well as the relative orientation of the nearby metal center, should be distinctly different for these two complexes since Cu^{2+} should lead to square planar coordination, while Zn^{2+} is probably octahedral.

Metal Effects on Organic Binding Selectivity. NMR titrations of diamagnetic zinc complex **1**·Zn with (1-naphthoxy)acetate **7** and with 2-phenylphenoxyacetate **8** provided strong evidence for a two-state binding event. In each titration, resonances of host metal complex were fit by multidimensional nonlinear least squares to a single K_d. Titration of paramagnetic copper complex **1**·

(14) (a) Schwabacher, A. W.; Lee, J.; Lei, H. *J. Am. Chem. Soc.* **1992**, *114*, 7597−7598. (b) Lee, J., Schwabacher, A. W. *J. Am. Chem. Soc.* **1994**, *116*, 8382−8383. (c) Schwabacher, A. W.; Stefanescu, A. D.; Rehman, A. *J. Org. Chem.* **1999**, *64*, 1784−1788.

(15) Lei, H.; Stoakes, M.; Schwabacher, A. W. *Synthesis* **1992**, 1255−1260. Schwabacher, A. W.; Stefanescu, A. D. *Tetrahedron Lett.* **1996**, *37*, 425−428.

(16) Dansyl = *N*-(5-dimethylaminonaphthalene-1-sulfonyl).

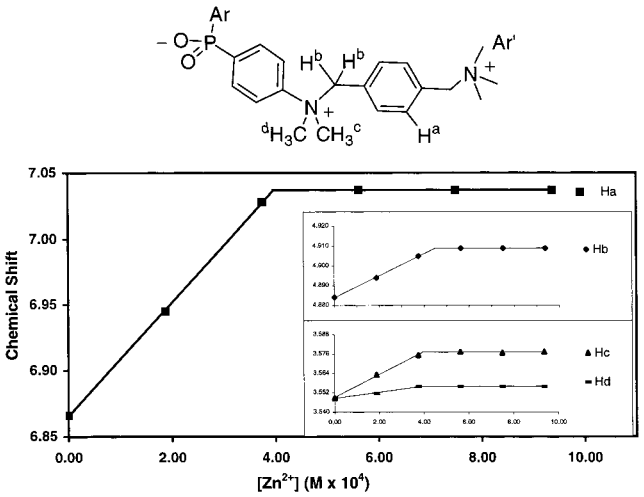


Figure 2. NMR titration of 4×10^{-4} M **1** at 23 °C with Zn^{2+} in 10^{-2} M pH 9 borate. Linear changes until saturation indicate high Zn affinity and a 1:1 stoichiometry.

Table 1.

guest	**1**·Zn Kd[a] (M)	**1**·Cu Kd[b] (M)
7, 2-(1-naphthoxy)-acetate	$(1.69 \pm 0.11) \times 10^{-4}$	$(7.25 \pm 1.42) \times 10^{-5}$
8, 2-(2-phenylphen-oxy)acetate	$(6.00 \pm 0.43) \times 10^{-3}$	$\leq(9.73 \pm 2.50) \times 10^{-6}$ [c]
9, 1-naphthyl phosphate	$(5.04 \pm 0.18) \times 10^{-4}$	$(1.66 \pm 0.54) \times 10^{-4}$

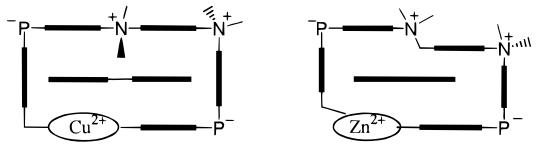
[a] Measured by NMR titration. [b] Measured by CD titration. [c] [Host] = 10^{-5} M, so this value is an upper limit. Error bars reflect only random error.

Cu was monitored by changes in circular dichroism (CD) and fit in the same manner.

As shown in Table 1, a modest 2.3-fold stronger binding of naphthalene **7** by copper complex **1**·Cu compared to zinc complex **1**·Zn becomes a ≥600-fold stronger binding by the Cu complex on replacement of the aromatic portion of the guest with biphenyl **8**. Phosphate **9** rather than carboxylate slightly decreases the affinity, which highlights the nonpolar component to the binding. This corresponds to a reversal in aromatic group preference for these two metal complexes. Approximately a 35-fold preference for the naphthalene **7** over the biphenyl **8** is found for complex **1**·Zn; in contrast, **1**·Cu prefers the biphenyl substrate by at least a factor of 7.4. Metal causes a very substantial nonpolar shape selectivity with a change of 260-fold.

These results can be rationalized using models. Simple CPK model-building exercises lead to the prediction that bis diamine **1** appears capable of forming an unstrained box-like Zn^{2+} complex **1**·Zn with a cavity that readily accommodates naphthalenes. In contrast, square planar coordination by **1** provides a somewhat strained and lengthwise macrocyclic Cu^{2+}complex **1**·Cu that should better accommodate biphenyl than naphthalene in its cavity. A favorable conformation places an inward-pointing methyl group toward the center of one side (Scheme 2). This conformation would be compatible with biphenyl binding, as the central twist prevents steric interference. However the methyl group could interfere with naphthalene binding, so a different conformation would be anticipated. That different conformations of Zn and Cu species are present is indicated by the difference

Scheme 2[a]



[a] Schematic diagram showing how octahedral Cu leads to cavity where methyl interferes with naphthalene, but not biphenyl guest.

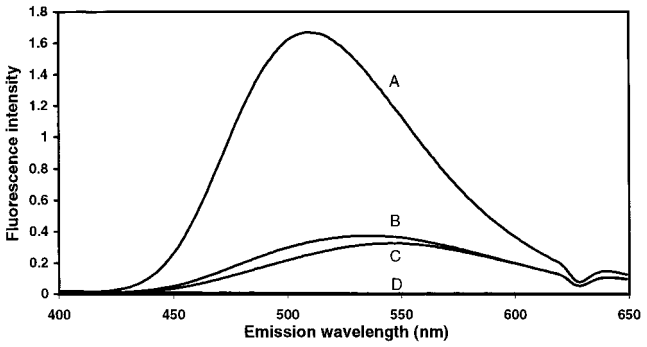


Figure 3. Fluorescence spectra of 2.0×10^{-4} M dansyl glycine at 23 °C in 10^{-2} M pH 9 borate in the presence and absence of 2.0×10^{-4} M **1** and 2.0×10^{-4} M $ZnSO_4$: (A) Dans-Gly + **1** + Zn; (B) Dans-Gly + **1**; (C) Dans-Gly + Zn^{2+} (spectrum of Dans-Gly alone is identical); (D) **1** + Zn^{2+} (**1** alone is identical).

between the CD spectra of **1**·Zn and **1**·Cu. **1**·Zn shows little if any CD, while **1**·Cu exhibits a distinct negative extremum at 234 nm. These data confirm a significant difference between the Zn and Cu complex conformations, but do not allow detailed structure assignment.

We believe that the metal centers interact with the anionic portions of the guest molecules.[14] While both naphthalene and biphenyl guests bear the same anionic groups, the polar groups will be presented differently to the metal upon binding of the nonpolar aromatic group into the cavity. It is the cooperativity between the two types of binding interactions that leads to the observed selectivity.

Organic Substrate Affects Metal Affinity. Complexation of dansyl glycine and 1·Zn causes a substantial increase in fluorescence (Figure 3), because of the well-documented sensitivity of dansylamide fluorescence to environmental polarity. The increase in dansyl amino acid fluorescence intensity was dependent on the presence of both **1** and Zn^{2+} and reversed upon the addition of a strong chelating agent such as ethylenediamine tetraacetic acid (EDTA) or iminodiacetate.

This fluorescence change allowed determination (Figure 4) of the affinity of the Zn complex of **1** for a series of dansyl amino acids, shown in Table 2. The fluorescence intensities at 10 distinct wavelengths were simultaneously fit by multidimensional nonlinear least squares to obtain a dissociation constant.

The α-carboxylate of the dansyl amino acid guest most likely interacts with metal on binding, since lengthening its distance from the naphthyl group by two carbons decreases binding 39-fold (compare **10a**−**c**). Further evidence that the carboxylate interacts with the metal on binding is found in the λ_{max} of the emission: dansylglycine emission shifts from 555 nm to 520 nm, equivalent to a solvent change from H_2O to MeOH. In contrast,

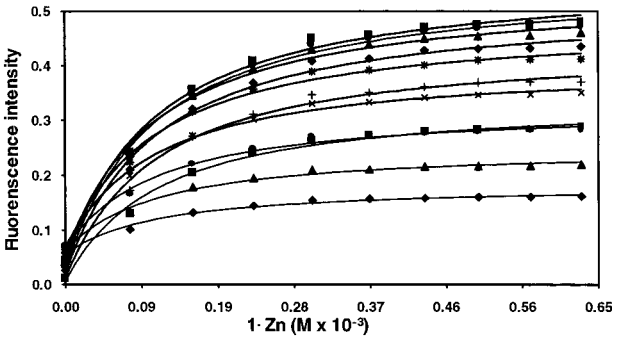


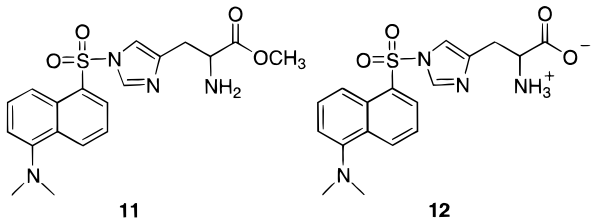
Figure 4. Titration of 2.0×10^{-5} M dansyl glycine with **1**·Zn at 23 °C in pH 9 borate at 340 nm excitation. Emission wavelengths (nm) from top to bottom at right: 510, 500, 520, 490, 530, 480, 540, 470, 550, 560, 570. All curves are theoretically fit to a single K_d.

Table 2.

	guest	dissoc constant (M)
10a	dansylglycine	$(9.70 \pm 0.71) \times 10^{-5}$
10b	dansyl-β-alanine	$(1.27 \pm 0.08) \times 10^{-3}$
10c	dansyl-4-aminobutyric acid	$(3.77 \pm 0.33) \times 10^{-3}$
10d	dansyl-aminomalonic acid	$(5.77 \pm 0.51) \times 10^{-5}$
10e	dansyl-L-aspartic acid	$(1.03 \pm 0.07) \times 10^{-4}$
10f	dansyl-D-aspartic acid	$(1.09 \pm 0.06) \times 10^{-4}$
10g	dansyl-L-glutamic acid	$(2.48 \pm 0.10) \times 10^{-4}$
10h	dansyl-D-glutamic acid	$(8.05 \pm 0.38) \times 10^{-4}$
10i	dansyliminodiacetic acid	$(1.45 \pm 0.15) \times 10^{-4}$
10j	dansyl-L-alanine	$(1.10 \pm 0.11) \times 10^{-4}$
10k	dansyl-D-alanine	$(2.60 \pm 0.18) \times 10^{-4}$
10l	dansyl-L-serine	$(1.15 \pm 0.88) \times 10^{-4}$
10m	dansyl-*o*-phospho-D,L-serine	$(2.05 \pm 0.14) \times 10^{-5}$
10n	dansyl-*o*-phospho-L-serine	$(2.14 \pm 0.12) \times 10^{-5}$
10o	dansyl aminomethylphosphonate	$(2.07 \pm 0.11) \times 10^{-4}$
10p	dansyl l-ala-l-ala	$(5.67 \pm 0.70) \times 10^{-4}$
10q	dansyl L-proline	$(5.17 \pm 0.90) \times 10^{-4}$
10r	dansyl D-proline	$(1.81 \pm 0.53) \times 10^{-3}$
10s	dansyl l-his methyl ester	$(6.30 \pm 0.46) \times 10^{-5}$
10t	dansyl L-histidine	$(8.65 \pm 0.68) \times 10^{-5}$

dansyl-β-alanine emission shifts upon binding only to 540 nm, indicating a more polar environment, and suggesting a different orientation of the naphthalene in the cavity. Presumably this conformation allows metal ligation. A second carboxylate provides limited assistance: substrate **10d** is bound only 2 times better than **10e**, and 4-fold stronger than **10g** which is consistent with the conclusion that the shorter the tether between carboxylate and naphthalene, the better the binding, as long as an appropriate conformation can be attained. Charge is not the dominant factor: although dansyl-*O*-phosphoserine (**10m** and **10n**), bearing three negative charges, has a 10-fold higher affinity than dansylaminomethylphosphonic acid (**10o**) with two negative charges, substrate **10o** is bound two times less strongly than dansyl L-serine (**10l**), which has only one negative charge. Moreover, there is no significant different between the ester and acid form of dansyl-L-histidine (**10s** and **10t**). Therefore, it is not the charge, but a specific structural feature, presumably metal ligation, that is important. This must be geometrically compatible with the hydrophobic component of the binding.

If both aromatic group and carboxylate interact with host, enantioselective binding is reasonable. The enantioselectivity of host **1**·Zn has been tested, and a small 2.4-fold preference for (*S*)-dansylalanine is observed (**10j** and **10k**). The dansyl amino acid guest molecules



11 **12**

Figure 5.

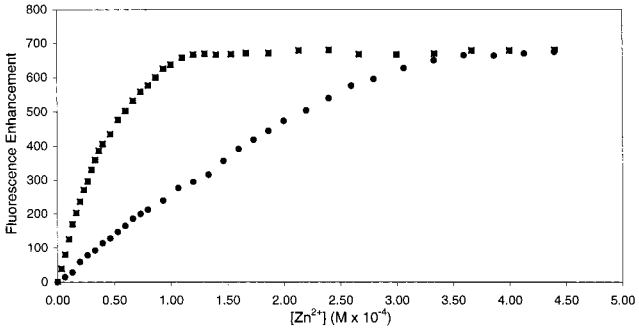


Figure 6. Fluorescence of 10^{-5} M dansyl glycine (■) or dansyl β-alanine (●) and 5×10^{-4} M **1** in 100 mM pH 9 borate at 23 °C as Zn^{2+} is added. Background fluorescence of each dansyl amino acid has been subtracted: 199 arbitrary units for dansyl glycine and 553 units for dansyl β-alanine.

constitute a very narrow class of substrates; nonetheless, a 180-fold range of affinities is observed. Interestingly, **11** and **12** (Figure 5), the sulfonyl imidazole isomers of **10s** and **10t**, behave totally differently than the other dansyl derivatives: no changes in fluorescence intensity are observed upon the addition of **1**·Zn up to 5×10^{-4} M, indicating a dissociation constant well above 10^{-3} M. As the fluorescence of these derivatives is also sensitive to solvent polarity, this structural change appears to have had a profound effect on binding, consistent with a quite specific geometric preference of the host for binding both aromatic and carboxylate groups.

Sensing Metal Ions by Organic Substrates. These complexes that act as hosts for organic substances can be used as sensors for metals.[6,7] A mixture of **1** and **10a** constitutes a fluorescent sensor, as it becomes substantially more fluorescent as Zn^{2+} is added and a complex is formed. The significance of such behavior is that a metal sensor composed of **1** and a dansyl amino acid can be tuned by choice of readily available dansyl amino acid. This is analogous to the tuning of a protein active site by second-sphere ligand interactions that restrict conformation.[6] However, our approach is operationally much simpler. One could incorporate the fluorophore into a large molecule and sense the metal ion upon binding to the molecule. Perturbation of metal binding affinity and selectivity would require structural changes to a large molecule. In contrast, we simply change the small substrates that are much more easily accessible.

Figure 6 shows the fluorescence of a mixture of **1** and two different dansyl amino acids as they are titrated with Zn^{2+}. Each of these mixtures acts as a fluorescent sensor for zinc, but dansylglycine causes a much more rapid increase in fluorescence than does dansyl-β-alanine, even though each is present at the same concentration. Eventually, each enhances fluorescence to a similar extent when all the dansyl groups are bound. In this way,

the different amino acids lead to sensors whose affinity can be tuned. The photophysics remains constant, as the chromophore is not substantially perturbed. It is likely that the metal selectivity can also be tuned in the same way as the affinity, since we have demonstrated[13] above that complexes of different metals bind with substantially different selectivity for organic molecular shape. Since these are equilibria, the variation of organic ligand will cause a similar variation in metal binding selectivity. Such variations may be particularly useful in arrays of sensors, where a pattern of changes can lead to very specific information.[17]

In this example, the sensitivity difference is due directly to a difference in binding of dansylamino acid to the same host complex. Some of the benefits of tunable sensors derive from an actual difference in the metal affinity for sensor. For instance, if the intracellular zinc concentration of a living cell were to be measured, a sensor species must provide a signal at the concentration range of interest, but too strong binding would be deleterious. This is because a sufficiently strongly binding species would compete for bound zinc, and not simply signal the presence of the free ion. As the total intracellular zinc concentration is several orders of magnitude higher than the concentration of free zinc, unsatisfactory behavior would result.

Variation of detection sensitivity, involving the formation of the receptor complex described here, would not appear to be as useful as a tunable sensor where the metal affinity varies. Note, however, that there are two equilibria involved: first the binding of metal to **1** to form a host complex, and then the binding of dansyl amino acid to this host with fluorescence enhancement. If the metal binding equilibrium is driven by binding to the signal-causing organic substrate, an effective variation in metal affinity will be achieved.

The specific sensors described here are not likely to be appropriate for intracellular studies because of the high pH optimum and difficulties associated with a three-component sensor. Endogenous aromatic anionic species can compete with fluorophore for binding, and the ratio of sensor components could vary. Both difficulties could be addressed to some extent by linkage of **1** and fluorophore with a flexible tether. Information readily obtained by evaluation of separated molecules could lead to an appropriate choice of groups.

Conclusion

We have presented an effective synthesis of the new ligand **1** that forms a metal complex of 1:1 stoichiometry. The hydrophobic cavity shape upon binding to metal depends on the metal with the selectivity as hoped: the octahedral complex **1**·Zn prefers naphthalene, while the square planar **1**·Cu better binds biphenyl. Complexation of the metal ion with ligand **1**, and subsequent dansyl amino acid inclusion in the cavity were demonstrated by NMR, CD, and fluorescence spectra. A substantial change is seen in the hydrophobic binding selectivity with change of metal, and a variation of affinity with position of ancillary binding groups. The convergent synthesis of **1** allows ready structural variation.

Experimental Section

General Procedures. Melting points were determined without correction. ^{1}H NMR spectra were recorded at 300 MHz, ^{13}C NMR at 75 MHz. All chemical shifts are reported downfield as δ values (ppm) from TMS in organic solvents or DSS in D_2O. ^{13}C NMR spectra are ^{1}H decoupled; multiplets are ^{31}P coupled. ^{31}P NMR are reported vs external 85% H_3PO_4. TLC was performed on 0.25 mm Merck silica gel 60-F and flash chromatography on 230−400 mesh Merck Kieselgel 60 as described.[18] Substituted naphthalenes and biphenyl used as binding substrates were purchased or prepared by literature procedures. CH_3CN was freshly distilled from P_4O_{10}; CH_2Cl_2, *tert*-butyl alcohol, and triethylamine were freshly distilled from CaH_2. Other chemicals were used as obtained from commercial suppliers unless otherwise specified.

***tert*-Butyl (4-*N*,*N*-Dimethylamino)phenylphosphinate (2).** Freshly distilled *N*,*N*-dimethylaniline (1.0 mL, 1.89 mmol) under N_2 in a screw cap tube was cooled to −5 °C, and PCl_3 (2.0 mL, 23 mmol) was added, followed by freshly distilled pyridine (1.8 mL, 22 mmol), and the tube was capped. The mixture was heated at 115 °C behind a shield for 4 h and cooled to room temperature, and the excess reagents were removed under reduced pressure. The solid was redissolved in 2 mL of CH_2Cl_2, the solution under N_2 was cooled to −10 °C, and *tert*-butyl alcohol (4.4 mL, 46 mmol) was added dropwise. After 1 h at room temperature, the excess reagents were removed by rotary evaporation, and the crude product was redissolved in CH_2Cl_2 (100 mL), washed with satd $NaHCO_3$ (3 × 30 mL) and satd NaCl, and purified by flash chromatography (silica, 20:1 CH_2Cl_2/MeOH) to yield 1.60 g (85%) of *tert*-butyl 4-(dimethylamino)phenylphosphinate as a white solid: mp 77.8−79.0 °C; ^{1}H NMR ($CDCl_3$) δ 7.70 (d, J = 547.3 Hz, 1H), 7.59 (dd, J = 13.0, 8.9 Hz, 2H), 6.73 (dd, J = 8.9, 2.7 Hz, 2H), 3.02 (s, 6H), 1.55 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 152.86 (d, J = 2.7 Hz), 132.30 (d, J = 13.0 Hz), 116.70 (d, J = 149.7 Hz), 111.36 (d, J = 14.6 Hz), 82.01 (d, J = 8.1 Hz), 40.07, 30.46 (d, J = 4.8 Hz); ^{31}P NMR ($CDCl_3$) δ 16.55; HRMS calcd for $C_{12}H_{20}NO_2P$ 241.1232, found 241.1253. Anal. Calcd for $C_{12}H_{20}NO_2P$: C, 59.74; H, 8.36; N, 5.81. Found: C, 59.99; H, 8.40; N, 5.80.

***tert*-Butyl 4-(*N*,*N*-Dimethylamino)phenyl-4-((2S)-2,3-di(*tert*-butoxycarbonylamino)propyl)phenyl)phosphinate (4).** A suspension of iodoarene **3** (0.45 g, 0.94 mmol), phosphinate **2** (0.217 g, 0.90 mmol), PPh_3 (0.012 g, 0.046 mmol), and Pd_2(dibenzylideneacetone)$_3$ (0.020 g, 0.022 mmol) in CH_3CN (8 mL) and Et_3N (1.3 mL, 9.37 mmol) was sealed in a screw-capped tube under N_2 and heated in a 90 °C oil bath for 36 h. Solvent removal by rotary evaporation and flash chromatography (silica, 50:3 CH_2Cl_2/2-propanol) gave 0.278 g (53%) **4** as a white solid: mp = 96.5−98 °C; ^{1}H NMR ($CDCl_3$) δ 7.66 (dd, J = 12.0, 7.8 Hz, 2H), 7.610 ± 0.0035 (dd, J = 11.4, 9.0 Hz 2H, diastereomers resolved), 7.21 (dd, J = 8.0, 3.0 Hz, 2H), 6.67 (dd, J = 8.8, 2.5 Hz, 2H), 5.13 (br m, 2H), 3.85 (br m, 1H), 3.15 (br m, 2H), 2.98 (s, 6H), 2.83 (br m, 1H), 2.74 (dd, J = 13.5, 6.8 Hz, 1H), 1.48 (s, 9H), 1.46 (s, 9H), 1.37 (s, 9H); ^{13}C NMR ($CDCl_3$) δ 156.71, 155.87, 152.18 (d, J = 2.5 Hz), 141.01, 133.90 (d, J = 146.9 Hz), 132.85 (d, J = 11.5 Hz), 131.21 (d, J = 10.3 Hz), 129.10 (d, J = 13.4 Hz), 119.51 (d, J = 148.7 Hz), 111.15 (d, J = 13.8 Hz), 82.58 (d, J = 8.3 Hz), 79.36, 79.20, 52.46, 43.67, 39.99, 38.86, 30.95 (d, J = 4.0 Hz), 28.35; ^{31}P NMR ($CDCl_3$) δ 28.23; HRMS calcd for $C_{31}H_{48}N_3O_6P$ 589.3281, found 589.3282. Anal. Calcd for $C_{31}H_{48}N_3O_3P$: C, 63.14; H, 8.20; N, 7.13. Found: C, 63.16; H, 7.89; N, 7.16.

Bis-phosphinate (6). A mixture of amine **4** (0.632 g, 1.07 mmol), α,α′-dibromo-*p*-xylene (0.113 g, 0.430 mmol), and AgOTf (0.276 g, 1.07 mmol) was evacuated and flushed three times with N_2 before CH_3CN (15 mL) and 2,6-di-*tert*-butylpyridine (500 μL, 2.23 mmol) were added by syringe. An ash-colored precipitate formed upon heating in an oil bath at 38 °C. After 12 h, the solvent was removed by rotary evaporation,

(17) Dickinson, T. A.; Michael, K. L.; Kauer, J. S.; Walt, D. R. *Anal. Chem.* **1999**, *71*, 2192−2198.

(18) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923−2925.

and crude **6** was applied to a silica gel column, rinsed with 10:3 CH_2Cl_2/MeOH, and eluted as the tetrafluoroborate with 10:3 MeOH/0.33 M aqueous $NaBF_4$. The eluent was removed by rotary evaporation, and the solid was partitioned between H_2O and CH_2Cl_2 and extracted with CH_2Cl_2 several times. Organic aliquots were combined, and the solvent was removed, affording 0.412 g (83%) **6** as a white solid mp > 200 °C dec; ^{1}H NMR ($CDCl_3$) δ 7.90 (br dd, 4H), 7.86 (br dd, 4H), 7.62 (br dd, 4H), 7.30 (br dd, 4H), 6.97 (s, 4H), 5.29 (br m, 4H), 4.87 (s, 4H), 3.87 (br m, 2H), 3.48 (s, 12H), 2.99 (br m, 4H), 2.80 (br m, 4H), 1.45 (s, 18H), 1.40 (s, 18H), 1.33 (s, 18H); ^{13}C NMR ($CDCl_3$) δ 155.83, 154.93, 146.11, 141.96, 136.98 (d, J = 136.2 Hz), 132.40, 132.03, 130.48 (d, J = 9.7 Hz), 129.87 (d, J = 142.4 Hz), 128.73 (d, J = 7.5 Hz), 128.68, 120.43 (d, J = 12.5 Hz), 84.01 (d, J = 8.2 Hz), 78.37, 78.18, 71.17, 51.66, 42.76, 38.00, 29.84 (d, J = 3.9 Hz), 27.37, 27.33; ^{31}P NMR ($CDCl_3$) δ 26.02; FAB-MS calcd for $[C_{70}H_{104}N_6O_{12}P_2BF_4]^+$ 1370, found 1370. Anal. Calcd for $C_{70}H_{104}N_6O_{12}P_2B_2F_8\cdot 2CH_2Cl_2$: C, 53.15; H, 6.69; N, 5.17. Found: C, 53.21; H, 6.63; N, 5.56.

Bis-diamine (1). A solution of tetrafluoroborate **6** (0.200 g, 0.137 mmol) in CH_2Cl_2 (50 mL) and CF_3CO_2H (0.3 mL, 3.89 mmol) was stirred at room temperature for 0.5 h, and excess reagents were removed under reduced pressure. After three cycles of dissolution in H_2O and rotary evaporation, the residue was precipitated from 1 mL of H_2O by addition of 30 mL of MeOH. The white precipitate was isolated by centrifugation and dried in vacuo to yield 0.154 g of **1** (96%): mp > 200 °C dec; ^{1}H NMR (D_2O) δ 7.79 (dd, J = 10.9, 8.9 Hz, 4H), 7.67 (dd, J = 11.7, 8.0 Hz, 4H), 7.54 (dd, J = 7.4, 1.6 Hz, 4H), 7.43 (dd, J = 8.0, 2.4 Hz, 4H), 6.89 (s, 4H), 4.91 (s, 4H), 3.97 (ddd, J = 10.4, 10.4, 6.4 Hz, 2H), 3.57 (s, 12H), 3.42 (dd, J = 13.7, 5.7 Hz, 2H), 3.36 (d, J = 13.0, 4.7 Hz, 2H), 3.23 (dd, J = 14.3, 6.2 Hz, 2H), 3.06 (dd, J = 14.3, 8.6 Hz, 2H); ^{13}C NMR (D_2O) δ 146.14 (d, J = 3.1 Hz), 138.75 (d, J = 2.8 Hz), 137.31 (d, J = 140.0 Hz), 133.03 (d, J = 138.2 Hz), 133.27 (d, J = 11.1 Hz), 132.91, 132.05 (d, J = 10.7 Hz), 130.15 (d, J = 13.4 Hz), 129.85, 121.95 (d, J = 13.1 Hz), 73.25, 53.35, 50.86, 41.04, 36.46; ^{31}P NMR (D_2O) δ 22.82; FAB-MS calcd for $[C_{42}H_{56}N_6O_4P_2BF_4]^+$ 857, found 857.

General Procedure for Syntheses of Dansyl Amino Acids (10a−t, 11, and 12). Compounds **10a**,[19] **10b**,[20] **10c**,[21] **10e**,[22] **10f**,[22] **10g**,[23] **10h**,[23] **10j**,[20] **10k**,[20] **10*l***,[24] **10q**,[25] **10r**,[25] **10s**,[20] **10t**,[20] **11**,[26] and **12**[26] were prepared as described. A 0.65 mmol portion of amino acid was dissolved in 2.5 mL of solvent along with an equivalent amount of base, 0.13 mmol of dansyl chloride in 2.5 mL of CH_3CN was added dropwise, and the mixture was stirred at rt until TLC indicated dansyl chloride reacted completely. For dansyl diethylaminomalonate (solvent, CH_2Cl_2; base, pyridine), **10i**, **10p** (solvent, H_2O; base, Na_2CO_3), the solution was acidified to pH 4−5 and extracted with EtOAc. After removal of EtOAc, the crude product was purified by flash chromatography. **10d** was obtained by hydrolysis of its ethyl ester. For **10m**−**o** (solvent, H_2O; base, Et_3N), the solution was extracted with CH_2Cl_2 several times to remove excess dansyl chloride. The unreacted amino acid was precipitaed from the H_2O layer by adding CH_3CN and removed by centrifuge. The precipitation was repeated until unreacted amino acid was removed completely. The solvent was evaporated and the product was dried under vacuum.

Dansyl diethylaminomalonate: ^{1}H NMR ($CDCl_3$) δ 8.56 (d, J = 8.5 Hz, 1H), 8.30 (d, J = 8.6 Hz, 1H), 8.24 (dd, J = 7.4, 1.0 Hz, 1H), 7.60 (dd, J = 8.1, 7.8 Hz, 1H), 7.51 (dd, J = 8.4, 7.6 Hz, 1H), 7.20 (d, J = 7.5 Hz, 1H), 5.88 (d, J = 8.3 Hz, 1H), 4.65 (d, J = 8.3 Hz, 1H), 3.96 (m, 4H), 2.88 (s, 6H), 1.05 (t, J = 7.1 Hz, 6H); ^{13}C NMR ($CDCl_3$) δ 165.39, 134.29, 130.93, 129.84, 129.63, 129.30, 128.58, 128.52, 123.17, 119.06, 115.47, 62.70, 58.92, 45.46, 13.67.

Dansylaminomalonic acid (10d): ^{1}H NMR (DMSO) δ 9.16 (d, J = 9.3 Hz, 1H), 8.75 (d, J = 8.5 Hz, 1H), 8.58 (d, J = 8.3 Hz, 1H), 8.23 (d, J = 7.2 Hz, 1H), 7.70 (dd, J = 16.2, 7.6 Hz, 2H), 4.49 (d, J = 9.1 Hz, 1H), 3.66 (d, J = 6.0 Hz, 1H), 3.05 (s, 6H).

Dansyliminodiacetic acid (10i): ^{1}H NMR (D_2O) δ 8.47 (d, J = 8.7 Hz, 1H), 8.42 (d, J = 7.3 Hz, 1H), 8.32 (d, J = 8.7 Hz, 1H), 7.68 (dd, J = 8.0, 7.8 Hz, 2H), 7.43 (d, J = 7.2 Hz, 1H), 4.06 (s, 4H), 2.90 (s, 6H); ^{13}C NMR (D_2O) δ 176.13, 150.19, 135.23, 129.98, 129.89, 129.79, 129.20, 128.68, 124.46, 120.68, 116.37, 51.06, 45.37.

Dansyl *O*-phospho-D,L-serine tris(triethylamine) salt (10m): ^{1}H NMR (D_2O) δ 8.60 (dd, J = 4.8, 4.7 Hz, 1H), 8.41 (d, J = 8.6 Hz, 1H), 8.32 (d, J = 7.3 Hz, 1H), 7.76 (m, 3H), 4.03 (m, 1H), 3.92 (m, 2H), 3.23 (s, 6H), 3.20 (q, J = 7.4 Hz, 18H), 1.28 (t, J = 7.3 Hz, 27H); ^{13}C NMR (D_2O) δ 174.57, 143.35, 135.30, 130.19, 129.18, 128.52, 127.67, 127.11, 126.04, 124.27, 118.37, 66.46 (d, J = 4.7 Hz), 58.79 (d, J = 8.8 Hz), 46.97, 46.36, 8.57; ^{31}P NMR (D_2O) δ 1.15; FAB-MS calcd for $[C_{15}H_{20}N_2O_8PS\cdot 2Et_3N]^+$ 621, $[C_{15}H_{20}N_2O_8PS\cdot Et_3N]^+$ 520, $[C_{15}H_{20}N_2O_8PS]^+$ 419, found 621, 520, 419.

Dansyl *O*-phospho-L-serine tris(triethylamine) salt (10n): ^{1}H NMR (D_2O) δ 8.56 (d, J = 8.3 Hz, 1H), 8.41 (d, J = 8.6 Hz, 1H), 8.32 (d, J = 7.3 Hz, 1H), 7.75 (m, 3H), 4.04 (m, 1H), 3.95 (m, 2H), 3.19 (q, J = 7.2 Hz, 18H), 3.16 (s, 6H), 1.28 (t, J = 7.3 Hz, 27H); ^{13}C NMR (D_2O) δ 174.63, 144.08, 135.24, 130.13, 129.20, 128.57, 127.93, 127.33, 125.88, 123.84, 118.17, 66.49 (d, J = 4.45 Hz), 58.82, 46.97, 46.25, 8.57; ^{31}P NMR (D_2O) δ 1.14; FAB-MS calcd for $[C_{15}H_{20}N_2O_8PS\cdot 2Et_3N]^+$ 621, $[C_{15}H_{20}N_2O_8PS\cdot Et_3N]^+$ 520, $[C_{15}H_{20}N_2O_8PS]^+$ 419, found 621, 520, 419.

Dansyl aminomethylphosphonic bis(triethylamine) salt (10o): ^{1}H NMR (D_2O) δ 8.44 (d, J = 8.7 Hz, 1H), 8.35 (d, J = 8.8 Hz, 1H), 8.27 (d, J = 7.3 Hz, 1H), 7.67 (dd, J = 8.3, 8.3 Hz, 2H), 7.34 (d, J = 7.7 Hz, 1H), 3.17 (q, J = 7.3 Hz, 12H), 2.99 (s, 1H), 2.94 (s, 1H), 2.79 (s, 6H), 1.27 (t, J = 7.3 Hz, 18H); ^{13}C NMR (D_2O) δ 150.98, 133.57, 130.25, 129.81, 129.32, 129.20, 129.09, 124.37, 119.49, 116.30, 46.85, 45.18, 41.27(d, J = 140.07 Hz), 8.52; ^{31}P NMR (D_2O) δ 14.73; FAB-MS calcd for $[C_{13}H_{18}N_2O_5PS\cdot Et_3N]^+$ 446, $[C_{13}H_{18}N_2O_5PS]^+$ 345, found 446, 345.

Dansyl L-Ala-L-Ala:. ^{1}H NMR ($CDCl_3$) δ 8.50 (br s, 1H), 8.49 (d, J = 8.5 Hz, 1H), 8.31 (d, J = 8.6 Hz, 1H), 8.21 (d, J = 7.1 Hz, 1H), 7.48 (ddd, J = 12.0, 11.9, 8.0 Hz, 2H), 7.17 (dd, J = 7.7, 7.6 Hz, 2H), 6.53 (d, J = 7.6 Hz, 1H), 4.17 (dd, J = 6.9, 6.9 Hz, 1H), 3.88 (dd, J = 7.4, 7.4 Hz, 1H), 2.86 (s, 6H), 1.10 (s, 3H), 1.08 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 175.35, 172.10, 150.76, 134.50, 130.46, 129.92, 129.43, 129.40, 128.58, 123.58, 119.39, 116.50, 115.71, 52.55, 48.37, 45.49, 19.00, 17.37.

Titration Procedure. ^{1}H NMR titrations were performed at 20.0 ± 0.5 °C in 0.1 M borate buffer in D_2O, prepared from anhydrous $Na_2B_4O_7$. In each titration, the host concentration was kept constant by adding a solution of host and guest to a solution of host at the same concentration. All distinguishable resonances of host were fit to yield a single dissociation constant, using multidimensional nonlinear least squares using Scientist (MicroMath) version 2.01. Error limits are 95% confidence (s plane).

Resonances were fit using the following equation:

$$\delta_i = \delta_{0i} + \frac{\Delta\delta_{maxi}}{H_0}\left(S - \sqrt{S^2 - 4H_0G_0}\right)$$

where K_d = dissociation constant, H_0 = total host added, G_0 = total guest added, and $S = H_0 + G_0 + K_D$, all in M.

Fluorescence titrations were performed at 23 °C in 0.1 M borate. Guests at 10^{-5}−10^{-4} M (always [guest] < K_d/5) were titrated with a solution of host, and the fluorescence intensities

(19) Malamas, M. S.; Sestanj, K.; Millen, J. *J. Med. Chem. Chim. Ther.* **1991**, *26*, 369−374.
(20) Froelich, P. M.; Murphy, L. D. *Anal. Chem.* **1977**, *49*, 1606−1608.
(21) Hartkoorn, A.; Lunden, R. *J. Med. Chem.* **1974**, *17*, 649−651.
(22) Nakamura, M.; Ikeda, T.; Nakamura, A.; Ikeda, H.; Ueno, A.; Toda, F. *Chem. lett.* **1995**, *5*, 343−344.
(23) Corradini, R.; Sartor, G.; Marchelli, R.; Dossena, A.; Spisni, A. *J. Chem. Soc., Perkin Trans. 2* **1992**, *11*, 1979−1983.
(24) Armstrong, D. W.; Tang, Y.; Chen, S.; Zhuo, Y.; Bagwill, C.; Chen, J. *Anal. Chem.* **1994**, *66*, 1473−1484.
(25) Reitsma, B. H.; Yeung, E. S. *Anal. Chem.* **1987**, *59*, 1059−1061.
(26) Mostovnikov, V. A.; Nechaev, C. V.; Solonenko, M. G. *J. Appl. Spectrosc.* **1990**, *53*, 712−717.

at 10 distinct wavelengths between 470 and 570 nm were fit to yield a K_d, as described for NMR titrations.

CD titrations were performed at 23 °C in 0.1 M borate. As in NMR titrations, hosts (always [host] < K_d/5) were titrated with a solution of guest and host, and ellipticities at 6−10 wavelengths (320−330 nm for naphthalene guests, 240−260 nm for biphenyl) were simultaneously fit to yield a K_d, as described above.

(27) Herath, K. P. B. *Synthesis of Arylphosphinic Acid Derivatives as Building Blocks for Binding Sites*; MS Thesis, Iowa State University. Ames, 1994.

Acknowledgment. This work was supported by NIH grant GM47736-02. We acknowledge Kamal P. B. Herath for preliminary studies in a related synthesis.[27]

Supporting Information Available: Copies of ^{1}H and ^{13}C NMR spectra for **1** and ^{1}H NMR spectra for **1**·Zn. This material is available free of charge via the Internet at http://pubs.acs.org.

JO991246T