

A computer-designed macrocyclic zinc receptor†

Haidong Huang and Dale G. Drueckhammer*

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A macrocycle containing a pair of bipyridine moieties with a linker designed using the computer program CAVEAT exhibits unique selectivity in the binding of zinc ion relative to other metals.

The development of specific receptors and sensors for metal ions, especially Zn^{2+} , has attracted much recent interest.^{1–12} The features of a practical sensor include high selectivity for the molecule or ion of interest, solubility in the medium of interest (usually aqueous) and a practical sensing mechanism, most often fluorescence. While aqueous solubility can be achieved by incorporation of polar or charged functional groups and much progress has been made in the incorporation of fluorescent signaling mechanisms into available receptors, the primary challenge in this field continues to be selectivity.¹³ This is especially problematic in the development of metal ion sensors as different metals may have very similar coordination preferences.

Based on ongoing work in this lab on the use of the computer program CAVEAT as a design tool in molecular recognition,¹⁴ we undertook the design of a receptor for Zn^{2+} as a model for pursuing selectivity in metal ion binding. The approach was to join a pair of bipyridine groups *via* a rigid linker to provide an optimal cavity for tetra-coordinate binding of Zn^{2+} . The Zn^{2+} bipyridine complex **1** was optimized using AM1 calculations, with the resulting 3-D structure **2** having approximate tetrahedral coordination of zinc (Fig. 1). The calculated Zn–N bond lengths of 2.088 Å were in excellent agreement with those in experimentally determined structures of Zn–bipyridine complexes,¹⁵ providing support for the reliability of this predicted structure. The bipyridine moieties in this model complex incorporated 1-propenyl groups for

defining vectors for the CAVEAT search, with a vision towards replacing the alkenes with amide bonds in the final structure.

The computer program CAVEAT searches databases of three-dimensional molecular structures to identify those having bonds matching a defined set of vectors.^{16,17} A CAVEAT search defined by the vinyl–CH₃ bonds of **2** (in bold in **1**) was conducted of the TRIAD database of computer-generated tricyclic hydrocarbons.¹⁶ Among the structures identified, **3** (Fig. 2) was chosen for development due to its symmetry and simplicity. The bonds to the hydrogens shown match the defined vectors and represent the point of attachment of the bipyridine moieties. Incorporation of linker **3** into complex **1** and converting the olefins to amide bonds gives the potential macrocyclic receptor **4**. Further modeling of the zinc complex of **4** showed good overlap with the unlinked complex **2**.

The synthesis of the linker **3** was based on the known hydrocarbon structure **8**, as shown in Scheme 1.^{18–21} Following published procedures, reaction of dimethylaminofulvene **5** with lithium metal and catalytic naphthalene formed **6**, shown in the *s-cis* conformation that reacts in the next step.^{18–21} The electrocyclic reaction of **6** is so facile that **6** can be isolated and purified only at low temperature.^{18–21} No attempt was made to purify **6** in this work but the product mixture from reductive coupling of **5** was left at room temperature to undergo the electrocyclic reaction to form **7**, which was then purified. Heating of **7** at 80 °C for 3 h induced a pair of sigmatropic hydrogen shifts to form **8**.^{18–21} Compound **8** was then subjected to asymmetric hydroboration using (–)-diisopinocampheylborane (Ipc₂BH) followed by oxidation to form the diol **9**. This reaction was expected to give primarily the (*S*)-alcohol at each position.²² NMR analysis of the isolated product indicated an approximately 3 : 1 mixture of diastereomers, which could not be separated by chromatography. The diol mixture was converted to the bis-Mosher esters, which were separated by column chromatography.²³ The major isomer gave only a single signal in the ¹⁹F-NMR spectrum, indicating the same configuration at both stereocenters while the minor isomer gave two signals indicating it was derived from the meso (*R,S*)-diol. The assignment of the absolute configuration was not

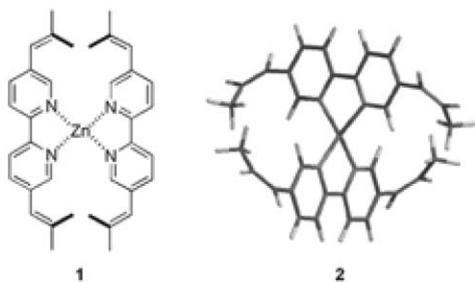


Fig. 1 The Zn(II) bipyridine complex used in receptor design.

Department of Chemistry, Stony Brook University, Stony Brook, New York, USA. E-mail: dale.drueckhammer@sunysb.edu; Fax: +1 631 632 7960; Tel: +1 631 632 7923

† Electronic supplementary information (ESI) available: Experimental procedures and spectral data for all compounds, Job plots for metal–ligand complexes and full description of the calculated geometry of **2**. See <http://dx.doi.org/10.1039/b508542j>

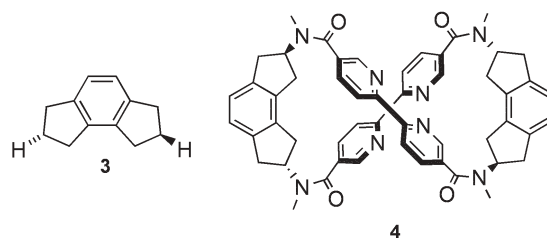
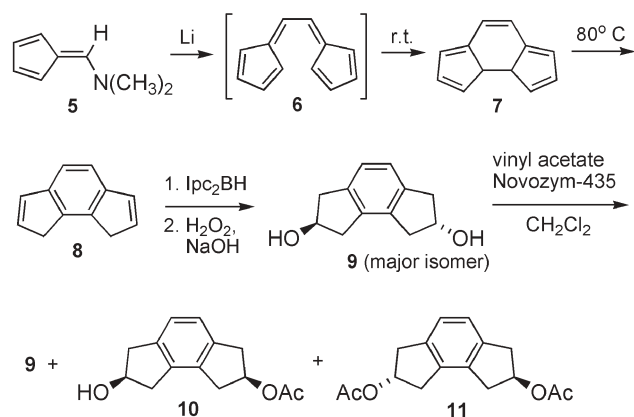


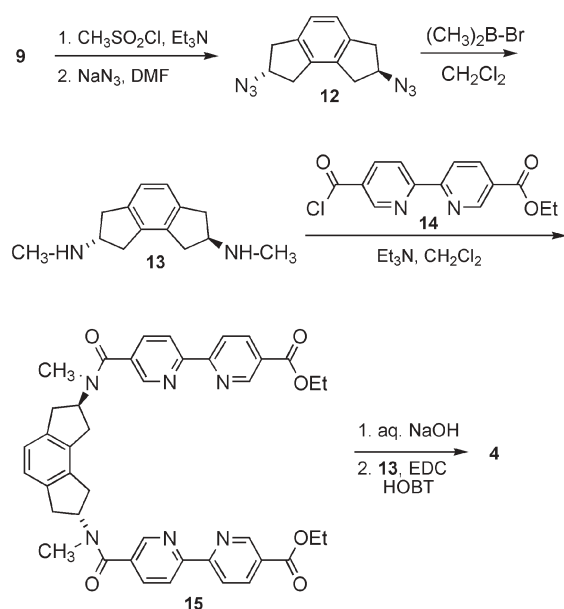
Fig. 2 A linker structure **3** and the zinc receptor **4** based on this linker.



Scheme 1 Synthesis and isomeric enrichment of the diol form of the linker moiety.

essential for this work, but literature precedence provides a high level of confidence that the major product is the (*S,S*) isomer as shown.²²

An enzymatic method was developed for more efficient preparative separation of the isomers. The lipase Novozym-435 was found to catalyze the partial acylation of the diol mixture by vinyl acetate. The resulting mixture of unreacted diol and monoester were readily separated and NMR analysis of the Mosher ester of the diol indicated a single isomer identical to the major isomer from the crude diol. Apparently the meso (*R,S*) diastereomer was acylated on the hydroxyl group at the *R* stereocenter to form **10**. The very small amount of (*R,R*) isomer is probably converted to the diester **11**, though it was not isolated. The stereochemically enriched **9** was converted to the bis-azide **12**, which was then converted to the bis-*N*-methylamine **13** by reaction with dimethylborane (Scheme 2).²⁴ The *N*-methyl groups were included to avoid potential difficulty in the formation of *cis*-secondary amides in the cyclic product. **13** was coupled with two equivalents of the acid chloride of the monoester of



Scheme 2 Final assembly of the Zn receptor.

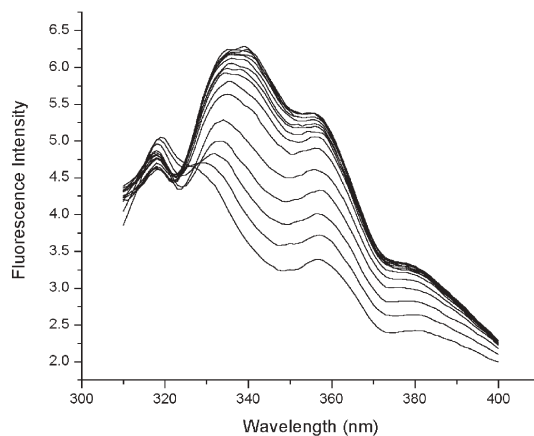


Fig. 3 Increasing fluorescence of **4** (0.23 mM in CH₃CN) with increasing [Zn(OTf)₂]. Curves (in order of increasing intensity) correspond to [Zn(OTf)₂] concentrations (mM) of 0, 0.012, 0.024, 0.035, 0.047, 0.059, 0.071, 0.082, 0.094, 0.106, 0.118, 0.141, 0.165, 0.188, 0.212, and 0.235.

2,2'-bipyridine-5,5'-dicarboxylic acid **14** to form **15**.²⁵ Ester hydrolysis and coupling with another equivalent of the diamine **13** formed the macrocycle **4**.

As **4** was insoluble in aqueous solution, metal ion binding to **4** was investigated in acetonitrile. **4** exhibited fluorescence with an emission maximum at 357 nm upon excitation at 290 nm. The fluorescence intensity increased and a shorter wavelength peak appeared upon addition of zinc triflate, with a 60% increase in intensity at 357 nm reached at saturation (Fig. 3). A binding constant of $2 \times 10^5 \text{ M}^{-1}$ was obtained from a plot of fluorescence vs. Zn²⁺ concentration. A Job plot based on fluorescence verified the 1 : 1 stoichiometry of binding (see supplementary information†). Similar Job plots indicated 1 : 1 stoichiometry for Ni but showed binding of two equivalents of Co²⁺ and Cu²⁺. Binding constants and stoichiometries for all four metal ions are given in Table 1, along with formation constants in aqueous solution for the 1 : 1 complexes of each metal ion with 2,2'-bipyridine (bipy) and *N,N,N',N'*-tetrakis(2-pyridylmethyl)-ethylenediamine (TPEN). Fluorescence titration of **4** with copper triflate gave an irregular curve so the binding constant in Table 1 was determined by isothermal titration calorimetry (ITC). ITC was also performed with zinc triflate and gave a similar binding constant to the fluorescence result. The binding constant for Zn(OTf)₂ to the diethyl ester of 2,2'-bipyridine-5,5'-dicarboxylic acid in acetonitrile was 10-fold lower than the literature value for bipyridine, making the binding constant for **4** about 20-fold greater than that of an electronically similar simple bipyridine. The 1 : 1 complex formation with Zn and Ni is consistent with binding of the metal ion in the cavity created by the two bipyridine moieties. The 2 : 1 complex formation with cobalt and copper indicates that these

Table 1 Metal ion binding to **4** (in CH₃CN) and other ligands

Salt	Metal : 4	$K(\mathbf{4})/\text{M}^{-1}$	$K(\text{bipy})/\text{M}^{-1a}$	$K(\text{TPEN})/\text{M}^{-1b}$
Zn(OTf) ₂	1 : 1	3×10^5	1.3×10^5	10^{18}
Ni(OTf) ₂	1 : 1	2×10^5	1.1×10^7	10^{18}
Co(OTf) ₂	2 : 1	2×10^4	6.5×10^5	—
Cu(OTf) ₂	2 : 1	2×10^5	2.1×10^6	4×10^{20}

^a Data from ref. 26. ^b Data from ref. 27.

ions do not form a complex analogous to the zinc and nickel complexes but presumably only bind to one bipyridine unit in a conformation of the receptor in which the bipyridine moiety is rotated so that the nitrogen atoms point outwards.

Of the four metals studied, Zn^{2+} has the lowest inherent affinity for bipyridine. While **4** exhibits similar affinity to Ni(II) and Cu(II) relative to Zn(II), and only modest selectivity relative to Co(II), it does overcome the inherent preference of the bipyridine ligand for the other three metals. The bond distances in bipyridine complexes of the four metal ions studied differ by $<0.1 \text{ \AA}$, and receptor **4** is probably not sufficiently rigid to exhibit selectivity based on the small bond length preferences.¹⁵ It seems more likely that the observed preferences are due to the geometric constraints that enforce an approximately tetrahedral geometry of nitrogen atoms around the metal in a 1 : 1 complex. Co(II) exhibits a modest preference for octahedral vs. tetrahedral coordination while Ni(II) and Cu(II) prefer octahedral and square planar coordination respectively.^{28,29} The formation of a 1 : 1 complex of **4** with Ni(II) is consistent with the observation of a few tetrahedral Ni(II) complexes, though square planar, trigonal bipyramidal, and octahedral are all more common.²⁹ The Ni(II) complex of **4** in acetonitrile exhibits an absorbance maximum at 636 nm and is visibly greenish-blue. This is consistent with tetrahedral coordination rather than either a square planar coordination or octahedral coordination with solvent or counterion as additional ligands.^{30,31} The observation that the greater affinity of bipyridine for Ni(II) relative to Zn(II) does not translate to **4** is consistent with the Ni(II) complex not being an optimal one.

Imperiali and coworkers have made zinc sensors based on synthetic peptides incorporating amino acids having bipyridine side chains, and have compared zinc vs. cobalt binding.^{4,5,11} While peptides incorporating only a single bipyridine formed 2 : 1 ligand to metal complexes with cobalt, peptides incorporating two bipyridines appear to form 1 : 1 complexes with both cobalt and zinc.⁴ The constraints of macrocycle **4** that disfavor 1 : 1 complex formation with cobalt are thus not observed in the peptide-based systems. Results ranging from 9-fold selectivity for zinc over cobalt to slight selectivity for cobalt have been observed with bipyridine and phenanthroline-containing peptides.^{4,11} The receptor **4** thus has equal or greater selectivity for Zn^{2+} than the best of these bipyridine-containing peptides.

A number of fluorescence-based zinc sensors have been built upon the *N,N,N',N'*-tetrakis(2-pyridylmethyl)-ethylenediamine (TPEN) ligand and other tetrakis(2-pyridylmethyl) diamines.¹⁻³ The TPEN ligand however shows 400-fold greater affinity for Cu relative to Zn. Ni, Co, and Cu all interfere significantly with Zn measurement using a TPEN-based sensor (see Fig. S8 in ref. 2), demonstrating the need for more selective sensors for applications in solutions containing multiple metal ions. Receptor **4** overcomes the inherent greater affinity of Cu for typical nitrogen ligands, thus offering significant progress towards selectivity.

This work demonstrates a CAVEAT-based strategy for the design of structurally defined ligands for metal ions that may be generally applicable to a variety of metals. This approach holds promise for addressing the key selectivity issue in metal

ion receptors that may be applicable to the design of molecules possessing all the desirable properties of a practical metal ion sensor.

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