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Colorimetric Discrimination between Important Alkaloid Nuclei Mediated by a Bis-Salphen Chromophore

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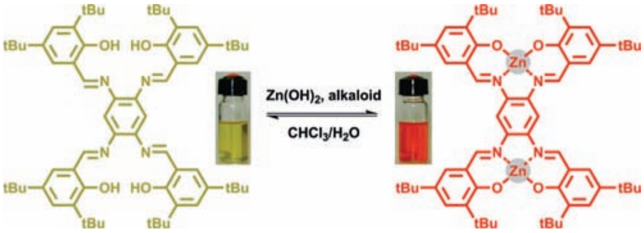
ABSTRACT



A new colorimetric method is presented that allows for simple and effective discrimination between (iso)quinoline nuclei pertinent to many alkaloid species. The method is based on the reversible incorporation of Zn^{2+} ions into a bis-salphen chromophore under aqueous conditions. The metalation process is a function of the stability of the Zn−N (acceptor−donor) interaction and is accompanied by a clear colorimetric response.

Alkaloids are secondary plant metabolites that are among the most widely studied natural products due to their ease of isolation and bioavailablity.[1] They are primarily used as biologically active, medicinal compounds, and prominent examples of these include derivatives that are applied as anesthethics (morphine, codeine), antimalarial drugs (quinine), or as mammal stimulants (nicotine, caffeine). Some classes of alkaloids have also recently attracted widespread interest as natural sources for asymmetric organocatalysis.[2] The compounds that consist of (iso)quinoline nuclei (Figure 1) are found in many plant metabolites and represent the largest class of alkaloid structures.[3] In addition, such nuclei may serve as valuable precursors in natural product synthesis.[4]

In most zinc-containing enzymes, the Zn^{2+} ion is tetrahedrally coordinated by three amino acid residues and one

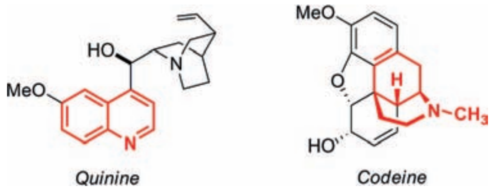


Figure 1. Line drawings of some important alkaloid derivatives with highlighted (iso)quinoline nuclei.

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[‡] ICREA.

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(2) For some recent examples, see: (a) Dickerson, T. J.; Janda, K. D. *J. Am. Chem. Soc.* **2002**, *124*, 3220. (b) Vakulya, B.; Varga, S.; Csámpai, A.; Soós, T. *Org. Lett.* **2005**, *7*, 1967. (c) Marcelli, T.; van der Haas, R. N. S.; van Maarseveen, J. H.; Hiemstra, H. *Angew. Chem., Int. Ed.* **2006**, *118*, 943.

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water molecule.[5] The Lewis acidic Zn(II) center can activate the coordinated water molecule toward deprotonation by means of increasing its Brønsted acidity.[6] We recently discovered that Zn(II)salphen complexes [where salphen stands for *N*,*N′*-phenylenebis(salicylideneimine)] also possess a remarkable high Lewis acid character,[7] which allows strong axial binding of nitrogen-containing donor molecules.[8] Lately, this feature has proven to be extremely valuable in the construction of various supramolecular systems,[9] as well as in the chemical sensing and discrimination of nitroaromatics.[10] Furthermore, these Zn(II)salphen derivatives are known to be unstable in the presence of relatively acidic solvents such as $CHCl_3$[7] and by activation of relatively acidic ligands leading to demetalated structures.[11]

Herein, we describe a reversible demetalation process of (multinuclear) Zn(II)salphen complexes; demetalation occurs under aqueous conditions by activation of the ligated H_2O molecule, while incorporation of Zn^{2+} ions may be facilitated in the presence of sufficiently strong coordinating N-heterocycles pertinent to alkaloid species, thereby giving a clear colorimetric reponse. As the strength of the Zn−N coordination motif is a function of the alkaloid structure, this feature may be used as a simple and effective colorimetric discriminator for related alkaloid compounds at concentration regimes as low as 1−10 μM.

First, the stability of Zn(II)salphens **2** and **4** (Scheme 1) was studied in detail by NMR and UV−vis spectroscopy in noncoordinating solvents ($CDCl_3$ and CH_2Cl_2, respectively). In the presence of water, both complexes decomposed readily leading to the demetalated structures **1** and **3**, whereas in the absence of water virtually no demetalation was noted. Most interestingly, NMR samples of complexes **2** and **4** could be effectively stabilized in the presence of pyridine-*d*$_5$, and demetalated samples could be fully remetalated in the presence of small amounts (2% v/v) of this cosolvent (see the Supporting Information).

Scheme 1. Schematic Representation of the Free Base and Zn(II)-Centered Salphen Derivatives **1**−**4**

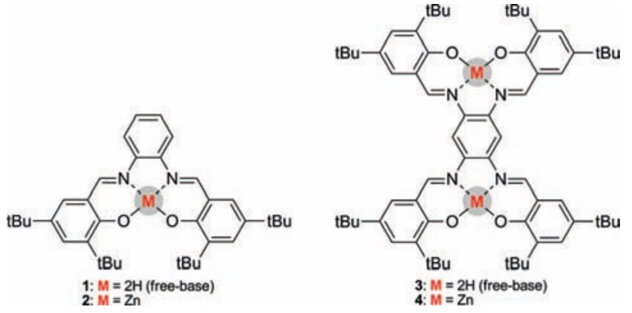
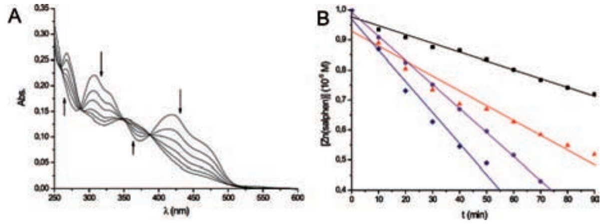


Figure 2. (A) UV−vis absorption spectra of a 1.0×10^{-5} M solution of **2** in CH_2Cl_2 at 20 min intervals with $[H_2O] = 9.8$ mM. (B) Reaction rate plots for identical solutions with $[H_2O] = 1.9$ mM (■), 2.9 mM (▲), 5.6 mM (●), or 9.8 mM (◆).



The demetalation of **2** is accompanied by a color change from orange ($\lambda_{max} = 420$ nm) to yellow ($\lambda_{max} = 355$) and was monitored in time by UV−vis spectroscopy using four different concentrations of water (see Figure 2). From the corresponding rate plots a linear, first-order dependence on the concentration of water was derived (see the Supporting Information). Similar studies were conducted with **4**, for which decomplexation gives a very clear color change from red ($\lambda_{max} = 490$ nm) to yellow ($\lambda_{max} = 380$ nm). Remarkably, the initial concentration dependence proved to be higher-order in nature and appears to level off to a pseudo-first-order kinetic profile (see the Supporting Information). This can be explained by assuming that the demetalation of the first Zn(II) center in **4** is faster since both centers are electronically connected. After monodemetalation, the Lewis acidity of the remaining Zn^{2+} ion will decrease, and kinetics related to the demetalation of **2** are observed. Based on these studies, we propose that the mechanism of this reversible demetalation is centered around the activation of an axially bound water molecule (Scheme 2). Upon coordination, the water protons will become acidic enough to protonate the interior phenolic oxygen atoms of the Zn(II)salphen structure, eventually leading to the free-base salphen ligands and $Zn(OH)_2$.[12] The demetalation event occurs only in the absence of a stabilizing axial ligand, while in the presence

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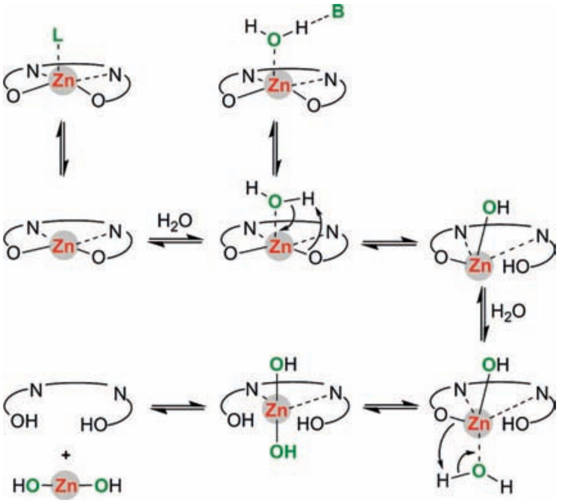
(12) An insoluble white precipitate was isolated from an NMR sample of **2** and characterized as $Zn(OH)_2$ by IR analysis ($\nu_{OH} = 3300$ cm^{-1}, see the Supporting Information). The formation of free ligand **1** was supported by separate NMR analysis.

Scheme 2. View of the Salphen N_2O_2 Metal-Binding Pocket Showing the Activation of H_2O Followed by Demetalation[a]

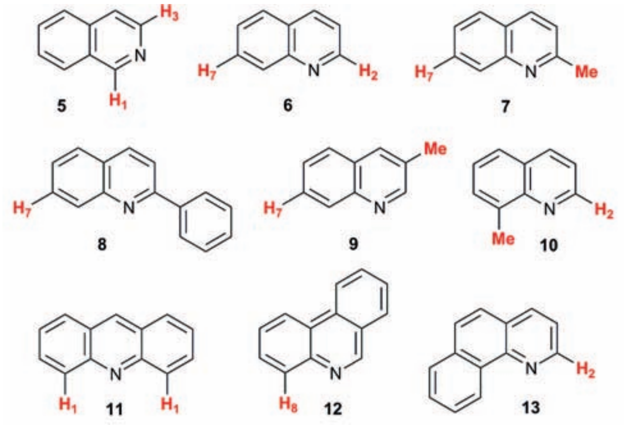


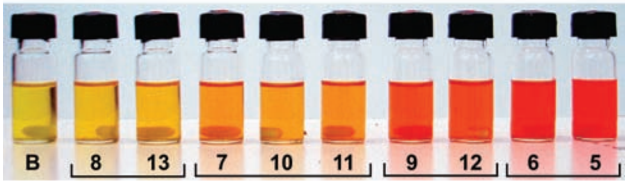
[a] Other species may arise from the displacement of H_2O by another axial ligand (L) or the generation of a nucleophilic hydroxide ligand that interacts with a basic residue (B).

of a coordinating ligand a stable five-coordinated Zn(II) complex is trapped.

We then studied the remetalation of **1** and **3** in the presence of various structurally related (iso)quinolines (see Table 1) in wet $CHCl_3$. As a typical colorimetric experiment, a mixture of **3** (0.1 mM) and $Zn(OH)_2$ in a 1:20 ratio was stirred in the presence of an excess of N-heterocycles **5**−**13**. It was anticipated that the suitability of these ligands to act as a stabilizing ligand for the metalated complex **4** is directly related to the steric impact upon coordination to the Zn(II) center,[13] and subtle changes within the ligand framework may thus be used to tune the Zn−L interaction and the colorimetric response. This will provide a method to discriminate between structurally related alkaloids based on these (iso)quinoline nuclei.

Table 1. Selected NMR Chemical Shifts (Δδ)[14] Observed for N-Heterocycles **5**−**13** upon Mixing with **2** and Maximum UV−vis Absorptions (λ_{max}) for Equilibrated Combinations of **3**/$Zn(OH)_2$ with the Indicated Ligands[a] (Some Typical Colorimetric Responses Are Shown Below, B = Blank Reaction without Added Ligand)





L	Δδ (ppm)[b]	λ_{max} (nm)[c]
		380[d] 490[e]
5	− 0.09 (H_1) − 0.24 (H_3)	489
6	− 0.07 (H_2) − 0.17 (H_7)	457
7	0.00 (Me, H_7)	377
8	0.00 (H_7)	n.d.[f]
9	− 0.10 (Me) − 0.14 (H_7)	452
10	0.00 (Me, H_2)	n.d.[f]
11	− 0.01 (H_1)	n.d.[f]
12	− 0.13 (H_8)	450
13	0.00 (H_2)	379

[a] After stirring for 2 h in the presence of 20 equiv of $Zn(OH)_2$, [**3**] = 0.1 mM, and [L] = 0.1 M in wet $CHCl_3$, detection limit 1−10 μM, B = blank reaction containing only **3** and $Zn(OH)_2$. [b] Measured in acetone-d_6, all upfield shifts. [c] Measured at 10 μM in $CHCl_3$. [d] UV−vis data for ligand **3**. [e] UV−vis data for complex **4**. [f] Not determined due to overlap of the λ_{max} with the absorption band of the ligand.

The combined results of ^{1}H NMR studies of **2** with **5**−**13** and UV−vis spectroscopic analyses of the remetalation of **3** in the presence of these ligands revealed an unambiguous relationship between the alkaloid structure and the Zn−N coordination motif (Table 1). The 2- and 8-substituted N-heterocycles **7**, **8**, **10**, **11**, and **13** showed (almost) no signal displacement in the NMR spectra when combined with **2**, while their analogues **5**, **6**, **9**, and **12** displayed significant upfield shifts that support axial ligation to complex **2**. Likewise, the treatment of **3** with $Zn(OH)_2$ and (iso)quinolines **5**−**13** gave only a fast color change from yellow to red for the N-heterocycles that caused signal displacements in the NMR spectra. This is also illustrated in the position of the maximum absorptions (Supporting Information), which are near to that of the fully metalated complex **4**. As can be expected, the colorimetric response is most pronounced in the presence of nonsubstituted ligands **5** and **6**, for which

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(13) Recently, Dalla Cort et. al. reported on the influence of steric effects on the coordination of tertairy amines: Dalla-Cort, A.; Mandolini, L.; Pasquini, C.; Rissanen, K.; Russo, L.; Schiaffino, L. *New. J. Chem.* **2007**, *31*, 1633.

(14) Note that the chemical shift changes for the ligand upon coordination to complex **2** depends on its relative position with respect to the salphen plane/phenyl groups.

(15) Selected bond distances (Å)/angles (deg) for **2·11**: Zn(1B)−O(1WB) = 2.053(2), Zn(1B)−O(1B) = 2.0103(18), Zn(1)−O(2B) = 1.9656(16), Zn(1B)−N(1B) = 2.152(2), Zn(1B)−N(2B) = 2.0660(19), Zn(1A)−O(1A) = 1.9624(17), Zn(1A)−O(2A) = 1.9798(18), Zn(1A)−N(1A) = 2.1111(19), Zn(1A)−N(2A)=2.0749(19), Zn(1A)−O(1W)=2.065(2), O(1B)−Zn(1B)−O(2B) = 92.29(6), N(1B)−Zn(1B)−N(2B) = 77.75(6), O(1B)−Zn(1B)−N(1B) = 150.60(7), O(2B)−Zn(1B)−N(2B) = 150.71(7), O(1WB)−Zn(1B)−O(1B) = 102.36(7), O(1A)−Zn(1A)−O(2A) = 99.71(6), N(1A)−Zn(1A)−N(2A) = 79.80(6), O(1A)−Zn(1A)−N(1A) = 87.62(6), O(2A)−Zn(1A)−N(2A) = 87.90(6), O(1A)−Zn(1A)−O(1W) = 98.58(7).

Zn^{2+} incorporation was almost complete after 2 h. In contrast, the more sterically demanding ligands **8** and **13** induced no color change and hence no metalation of **3**. The slight color shift to orange for **7**, **10**, and **11** may be ascribed to stabilizing hydrogen-bond interactions between the ligand acting as a basic residue and H_2O (see Scheme 2).

The solid state features were additionally investigated by X-ray diffraction for combinations of **2** with **6**, **11**, and **12**. The molecular structures are presented in Figure 2 and Figures S1−S3 (Supporting Information. Combinations **2·6** and **2·12** crystallized as the expected 1:1 complexes with the N-heterocyclic ligand bonded to the Zn(II) complex via its respective N-donor atom. In contrast, the structure determined for **2·11** revealed no direct interaction of the N-atom with the Zn(II) salphen unit (Figure 3). Instead, the Zn complex is axially ligated by a water molecule, which in turn is associated with the N-heterocycle via hydrogen bonding. Remarkably, here two different Zn(II)salphen complexes are found in the unit cell; the first one has a hydrogen-bond pattern between an acridine molecule and the H_2O ligand, while the second Zn(salphen) unit contains an axial water ligand showing H-bond interactions with the phenolic O-atoms of the first Zn(salphen) complex. As a result, for both complexes in the crystal distinct bond distances/angles are observed. The solid state findings are fully in line with the spectroscopic results, i.e. no coordination via the N-donor atom of **11** to **2** or **4** is feasible due to too much steric repulsion upon enforcement of the Zn−N coordination pattern.

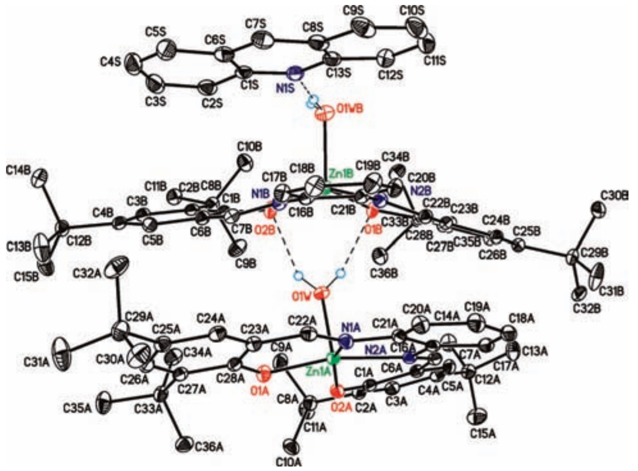

Figure 3. X-ray molecular structures for **2·11** showing the repeating unit.[15] Hydrogen atoms, numbering scheme, and cocrystallized solvent molecules are omitted for clarity.

In summary, we have shown that the bis-salphen chromophore **3** can be reversibly metalated by Zn^{2+} ions in wet $CHCl_3$ in the presence of suitable N-donor ligands. In the absence of these ligands, the Zn(II)salphen complexes **2** and **4** are insufficiently stabilized and rapid demetalation occurs by activation of axially coordinated water. The entrapment of the stable five-coordinated Zn(II) complex depends on the strength of the Zn−N interaction, and our results have clearly demonstrated that subtle differences in the backbone of the N-heterocycle can have a dramatic effect on their stabilization ability in relation to **4**. Since (iso)quinoline nuclei are found in many different alkaloid structures, this molecular colorimetric differentiation method may be projected onto a range of structurally related alkaloids. To our knowledge, this is the first example of a synthetic system that is able to discriminate between such alkaloid components. Furthermore, as structural modifications of the bis-salphen chromophore are easily accomplished and experimental conditions optimized, combinatorial screening methods come in reach for different classes of alkaloids. Our current activities focus on the development of new sal(ph)en chromophores that are equipped with groups that allow for chiral recognition and improved sensitivity. Consequently, this offers a huge potential in the colorimetric sensing and molecular differentiation of important classes of biologically active molecules.

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Supporting Information Available: Experimental details, relevant ^1H NMR and UV−vis spectra, X-ray crystallographic data (CIF), and displacement ellipsoid plots of all structures. This material is available free of charge via the Internet at http://pubs.acs.org.

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