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Zinc and Copper Complexes of Prodigiosin: Implications for Copper-Mediated Double-Strand DNA Cleavage

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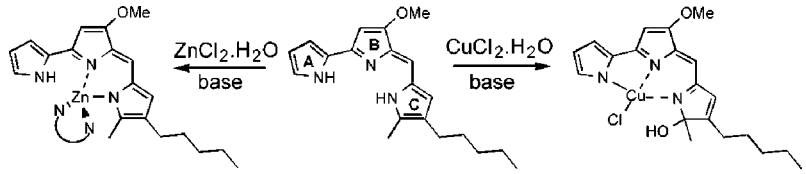
ABSTRACT



Zn(1)$_2$, **8** prodigiosin, **1** Cu(1), **5**

Zinc(II) and copper(II) complexes of prodigiosin (1) have been characterized. All N-atoms of 1 bind Cu(II) to generate 5: the complex exhibits regiospecific oxidation of the C-pyrrole. In contrast, coordination by Zn(II) to 1 produces Zn(1)$_2$ (8), a 4-coordinate tetrahedral complex. The influence of these binding geometries on Cu-mediated double-strand (ds) DNA cleavage by 1 is discussed.

Prodigiosin (**1**, Figure 1) is the parent member of a family of red pigments produced by microorganisms such as *Streptomyces* that possess a pyrrolylpyrromethene skeleton with a **B**-ring methoxy group.[1,2] They are noted for their promising immunosuppressive[3] and anticancer activities[4] where they facilitate cell death by apoptosis in a number of human cancer cell lines.[5]

The therapeutic potential of prodigiosins has stimulated research into their mechanism of action. Thus, recent efforts show that an N-containing heterocyclic **A**-ring along with the lone-pair N-electrons in conjugation with the tricyclic frame[6] and the **B**-ring methoxy group[6,7] are critical for cytotoxic potency. The electron density of the **A**-pyrrole ring also plays an important role, as attachment of electron-

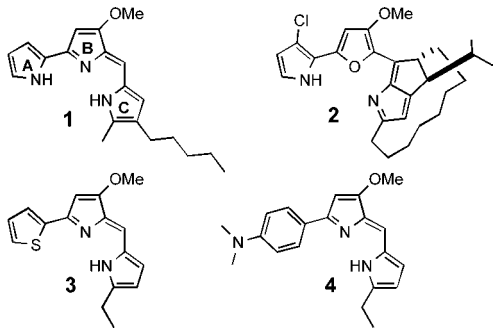


Figure 1. Structures of prodigiosin (**1**), roseophilin (**2**), and synthetic analogues **3** and **4**.

(1) For reviews see: (a) Gerber, N. N. *Crit. Rev. Microbiol.* **1974**, *3*, 469−485. (b) Bennett, J. W.; Bentley, R. *Adv. Appl. Microbiol.* **2000**, *47*, 1−32. (c) Manderville, R. A. *Curr. Med. Chem.-Anti-Cancer Agents* **2001**, *1*, 195−218.

(2) Rapoport, H.; Holden, K. G. *J. Am. Chem. Soc.* **1962**, *84*, 635−642.

(3) (a) Magae, J.; Yamashita, M.; Nagai, K. *Ann. N.Y. Acad. Sci.* **1993**, *685*, 339−340. (b) Lee, M. H.; Yamashita, M.; Tsuji, R. F.; Kataoka, T.; Magae, J.; Nagai, K. *J. Antibiot.* **1998**, *51*, 92−94. (c) Mortellaro, A.; Songia, S.; Gnocchi, P.; Ferrari, M.; Fornasiero, C.; D'Alessio, R.; Isetta, A.; Colotta, F.; Golay, J. *J. Immunol.* **1999**, *162*, 7102−7109. (d) Stepkowski, S. M.; Erwin-Cohen, R. A.; Behbod, F.; Wang, M. E.; Qu, X.; Tejpal, N.; Nagy, Z. S.; Kahan, B. D.; Kirken, R. A. *Blood* **2002**, *99*, 680−689.

(4) The NCI has screened **1** for cytotoxic potency in their 60-cell-line panel. The average LC_{50} value for **1** was 2.1 μM. This information is available on the Internet at www.dtp.nci.nih.gov, the NSC number for **1** is 47147-F.

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donating substituents enhances potency, whereas electron-withdrawing groups affect a marked decrease in activity.[6]

In our studies prodigiosin (**1**) and related 4-methoxypyrrolic natural products have been shown to bind DNA effectively[8,9] and facilitate oxidative DNA cleavage in the presence of Cu(II) without the need of an added reductant.[9−11] This mechanism of action is triggered by oxidation of the electron-rich pyrrolylpyrromethene moiety, and here it was noted that prodigiosin (**1**) is capable of promoting double-strand (ds) DNA cleavage.[11] Since dsDNA cleavage is thought to be responsible, at least in part, for the cytotoxicity of the clinically used bleomycins,[12] it is conceivable that this mechanism of action is important for the anticancer properties of **1**.

In analogy to the role played by Fe·bleomycin in strand scission,[12] it is anticipated that a Cu-bound prodigiosin species is critical for dsDNA cleavage. In this regard, Fürstner has shown that the related cytotoxic agent roseophilin (**2**, Figure 1) is incapable of promoting oxidative strand scission in the presence of Cu(II).[13] Since in **2** the **B**-pyrrole ring of **1** has been replaced by the weaker Cu-ligating furan, it was suggested that the biological activity of the prodigiosins stems from their ability to complex Cu(II).[13] We have also found that replacement of the **A**-pyrrole ring with a phenyl,[14] furan-2-yl,[14] or thiophen-2-yl[11,14] moiety inhibits Cu-mediated DNA cleavage[11,14] and cytotoxic potency.[11,15] Accordingly, the synthetic analogue **3** (Figure 1) is incapable of generating Cu-mediated strand breaks even though it possesses the metal-coordinating pyrromethene entity.

To establish a basis for dsDNA cleavage by **1**, its direct interaction with Cu(II) and Zn(II) was studied, with the latter metal used as a model for Cu(I).[16] For comparison to Cu(II) coordination by **1**, we also examined Cu(II) binding by the synthetic analogues **3** and **4** (Figure 1). In this Letter we report our preliminary results from these studies wherein the key findings include the isolation and X-ray crystallographic characterization of a 1:1 Cu(**1**) complex **5** containing an oxidized **C**-pyrrole ring and a 1:2 $Zn(\mathbf{1})_2$ complex **8** where the prodigiosin molecule has not undergone oxidation. These complexes represent the first structurally characterized metalloprodigiosins and provide insight into the 1:1 stoichiometry of copper-mediated DNA cleavage by prodigiosin.[11,13]

Blue prisms of the copper complex **5** ($CuCl(C_{20}H_{24}N_3O_2)$) (Figure 2)[17] were obtained in ∼17% yield from vapor diffusion of pentane into an ethyl acetate solution of a 1:1 Cu(prodigiosin) mixture prepared by pretreating **1** with 3 molar equiv of potassium *tert*-butoxide in *tert*-butyl alcohol, followed by the addition of 2 molar equiv of $CuCl_2·2H_2O$. The structure **5** (Figure 2) revealed that all three pyrrolic N-atoms of **1** coordinated Cu to form a distorted square-planar structure. Interestingly, the **C**-pyrrole ring was oxidized and contained an OH group (O = O_2, H = H_2O) attached to C1 to generate an sp^3-hydridized carbon atom and a new double bond between N1 and C4 was present. A methanolic solution of **5** had UV−vis absorbances at λ_{max}, nm (ϵ, M^{-1} cm^{-1}): 406 (1656), 562 (3893), and 605 (3965)

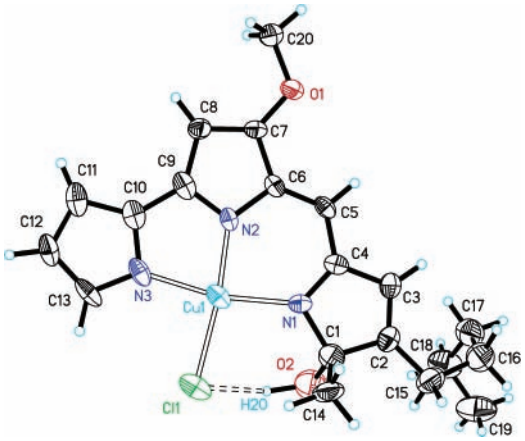


Figure 2. ORTEP plot (50% probability thermal ellipsoids) of **5**. The Cu atom is represented by the pale blue ellipsoid, N atoms are in dark blue, O atoms are in red, and the Cl atom is in green. Hydrogen atoms are represented by small open spheres and the H-bonding interaction is shown with a dashed-open bond. Selected bond distances (Å): Cu−N1 2.015(5); Cu−N2 1.903(5); Cu−N3 2.005(5); Cu−Cl 2.2645(19); N1−C1 1.510(9); N1−C4 1.314(7); C1−O2 1.404(8); C1−C2 1.500(9); C2−C3 1.322(8); C3−C4 1.462(8); C4−C5 1.428(8); C5−C6 1.354(8). Selected bond angles (deg): N2−Cu−N3 80.2(2); N2−Cu−N1 87.5(2); N3−Cu−Cl 93.1(2); N1−Cu−Cl 99.3(2); N3−Cu−N1 167.6(2); N2−Cu−Cl 173.3(2).

(5) (a) Yamamoto, C.; Takemoto, H.; Kuno, K.; Yamamoto, D.; Tsubura, A.; Kamata, K.; Hirata, H.; Yamamoto, K.; Kano, H.; Seki, T.; Inoue, K. *Hepatology* **1999**, *30*, 894−902. (b) Yamamoto, D.; Kiyozuka, Y.; Uemura, Y.; Yamamoto, C.; Takemoto, H.; Hirata, H.; Tanaka, K.; Hioki, K.; Tsubura, A. *J. Cancer Res. Clin. Oncol.* **2000**, *126*, 191−197. (c) Montaner, B.; Navarro, S.; Piqué, M.; Vilaseca, M.; Martinell, M.; Giralt, E.; Gil, J.; Perez-Tomas, R. *Br. J. Pharmacol.* **2000**, *131*, 585−593. (d) Diaz-Ruiz, C.; Montaner, B.; Perez-Tomas, R. *Histol. Histopathol.* **2001**, *2*, 415−421. (e) Montaner, B.; Perez-Tomas, R. *Life Sci.* **2001**, *68*, 2025−2026.

(6) D'Alessio, R.; Bargiotti, A.; Carlini, O.; Colotta, F.; Ferrari, M.; Gnocchi, P.; Isetta, A.; Mongelli, N.; Motta, P.; Rossi, A.; Rossi, M.; Tibolla, M.; Vanotti, E. *J. Med. Chem.* **2000**, *43*, 2557−2565.

(7) Boger, D. L.; Patel, M. *J. Org. Chem.* **1988**, *53*, 1405−1415.

(8) Melvin, M. S.; Ferguson, D. C.; Lindquist, N.; Manderville, R. A. *J. Org. Chem.* **1999**, *64*, 6861−6869.

(9) Melvin, M. S.; Wooton, K. E.; Rich, C. C.; Saluta, G. R.; Kucera, G. L.; Lindquist, N.; Manderville, R. A. *J. Inorg. Biochem.* **2001**, *87*, 129−135.

(10) Borah, S.; Melvin, M. S.; Lindquist, N.; Manderville, R. A. *J. Am. Chem. Soc.* **1998**, *120*, 4557−4562.

(11) Melvin, M. S.; Tomlinson, J. T.; Saluta, G. R.; Kucera, G. L.; Lindquist, N.; Manderville, R. A. *J. Am. Chem. Soc.* **2000**, *122*, 6333−6334.

(12) Keck, M. V.; Manderville, R. A.; Hecht, S. M. *J. Am. Chem. Soc.* **2001**, *123*, 8690−8700 and references therein.

(13) Fürstner, A.; Grabowski, E. J. *Chem. Bio. Chem.* **2001**, *9*, 706−709.

(14) Melvin, M. S.; Calcutt, M. W.; Noftle, R. E.; Manderville, R. A. *Chem. Res. Toxicol.* **2002**, *15*, 742−748.

(15) Melvin, M. S.; Tomlinson, J. T.; Park, G.; Day, C. S.; Saluta, G. R.; Kucera, G. L.; Manderville, R. A. *Chem. Res. Toxicol.* **2002**, *15*, 734−741.

(16) Zn(II) derivatives are often used as structural models of analogous Cu(I) species, see: (a) Coggin, D. K.; Gonzalez, J. A.; Kook, A. M.; Stanbury, D. M.; Wilson, L. J. *Inorg. Chem.* **1991**, *30*, 1115−1125. (b) Murthy, N. N.; Karlin, K. D. *Chem. Commun.* **1993**, 1236−1238. (c) Yashiro, M.; Ishikubo, A.; Komiyama, M. *Chem. Commun.* **1997**, 83−84.

(17) Crystal data (198 K) for $CuCl(C_{20}H_{24}N_3O_2)$, **5**: M_r = 437.41, monoclinic space group, *C*2/*c* − *C* (No. 15), a = 29.413(8) Å, b = 6.757-(2) Å, c = 20.154(5) Å, β = 100.385(5)°, V = 3940.1(18) Å^3, Z = 8, GOF = 0.916. Final R values (all 2405 data): R1 = 0.1175, wR2 = 0.1214.

(Figure S1, Supporting Information) and its EPR spectrum in MeOH at 10 K (Figure S2, Supporting Information) was typical of a mononuclear square-planar Cu(II) complex.[18]

To gain insight into the transformation of **1** into **5**, the in situ reaction of **1** with $Cu(OAc)_2$ under an ambiently oxygenated atmosphere was examined. Figure 3a shows a UV−

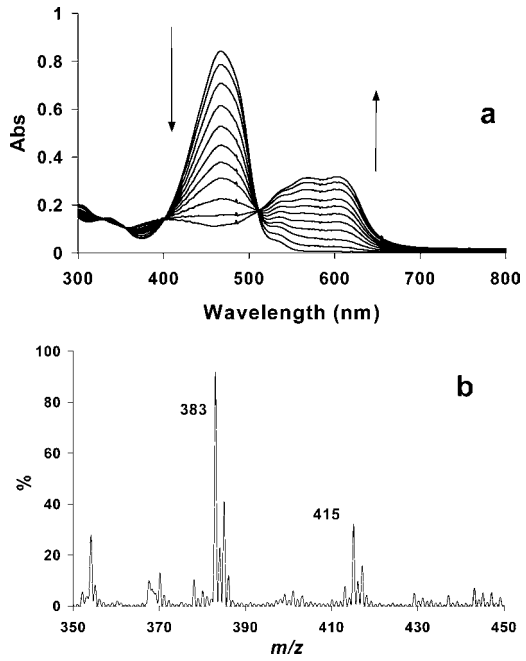
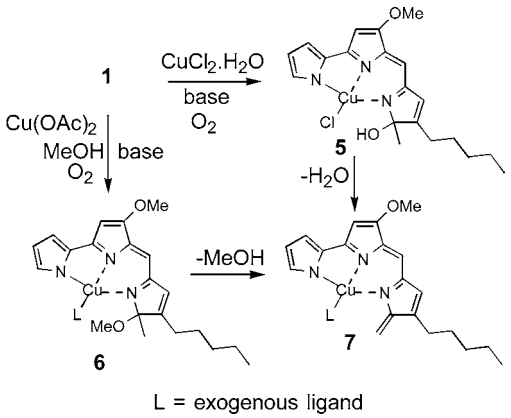


Figure 3. (a) UV−vis titration of **1** (19 μM) with $Cu(OAc)_2$ added in 0.1 equiv at 25 °C in MeOH containing 10 mM 1:1 *tert*-butoxide/acetic acid, apparent pH 10. (b) ES^+ spectrum of the 1:1 Cu(**1**) mixture in MeOH.

vis titration of **1** with $Cu(OAc)_2$ in MeOH containing 10 mM *tert*-butoxide/acetic acid, apparent pH ~10. In the figure arrows point to loss of the bright yellow prodigiosin free-base absorbance (463 nm, ϵ = 26 300 M^{-1} cm^{-1}) and gain of absorbances at λ_{max} = 562 and 605 nm to generate a blue solution with spectral features almost identical to **5** (Figure S1). Scatchard analysis of the titration in Figure 3a indicated 1:1 stoichiometry from which an apparent association constant of 2350 M^{-1} was obtained. Examination of the blue solution ascribed to the 1:1 Cu(**1**) complex by ES^+-MS showed the presence of Cu-bound species with *m*/*z* 383 and 415 (Figure 3b). Collision-induced dissociation of the complex with *m*/*z* 415 indicated loss of 32 mass units (CH_3OH) to generate the species with *m*/*z* 383. Thus, as shown in Scheme 1, the in situ reaction between **1** and $Cu(OAc)_2$ in MeOH appeared to generate the 1:1 Cu-bound prodigiosin species **6** with an attached OMe group in place of the OH group in **5**. Loss of MeOH from **6**, or H_2O from **5**, would yield the azafulvenic species **7** with an M^+ peak at *m*/*z* 383. These observations suggested strongly that the bound OH group in **5** was derived from adventitious H_2O in the solvent.

(18) Solomon, E. I.; Baldwin, M. J.; Lowery, M. D. *Chem. Rev.* **1992**, *92*, 521−542.

Scheme 1



In contrast to the results obtained with Cu(II), prodigiosin **1** binds Zn(II) and Co(II) to generate 2:1 complexes, i.e., Zn(**1**)$_2$.[19] Figure 4 shows a perspective drawing of a solid-

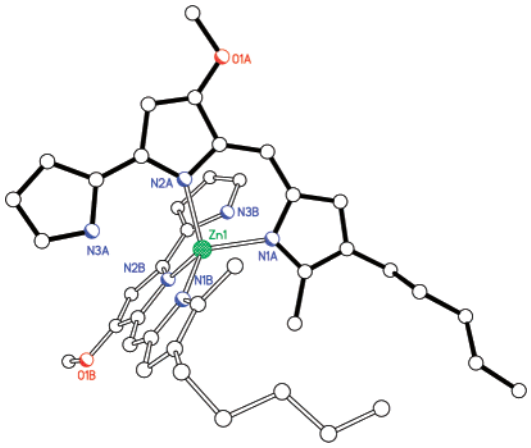


Figure 4. Perspective drawing of the solid-state structure of Zn(**1**)$_2$, **8**. The Zn atom is represented by a large crosshatched green sphere; O and N atoms are represented by medium-sized red spheres and blue spheres, respectively; H atoms are omitted for clarity. Selected bond distances (Å): Zn1−N1A 1.978(9); Zn1−N2A 2.013(8); Zn1−N1B 1.953(9); Zn1−N2B 1.994(8). Selected bond angles (deg): N1B−Zn1−N1A 117.3(4); N1B−Zn1−N2B 95.3-(4); N1A−Zn1−N2B 115.9(4); N1B−Zn1−N2A 119.4(4); N1A−Zn1−N2A 96.6(4); N2B−Zn1−N2A 113.8(3).

state structure of Zn(**1**)$_2$, **8**,[20] prepared by treatment of **1** with 2 equiv of potassium *tert*-butoxide and 2 equiv of $ZnCl_2$ in hot *tert*-butyl alcohol. Dissolving the purified product in a 2:1 acetone:water mixture generated orange-red prisms (λ_{max} = 532 nm) of the complex **8**, in which the prodigiosin

(19) Wasserman, H. H.; McKeon, J. E.; Smith, L. A.; Forgione, P. *Tetrahedron* **1966**, *Suppl. 8*, *Part II*, 647−662, contains elemental analyses of Zn and Co complexes of **1**.

(20) Crystal data (193 K) for $C_{40}H_{48}N_6O_2Zn$, **8**: M_r = 710.21, triclinic space group, V = 1848.7(7) Å^3, Z = 2, GOF = 0.873. Final R values (all 4215 data): R1 = 0.2214, wR2 = 0.1600. See Supporting Information for full details.

molecule has not undergone oxidation and the **A**-pyrrole ring does not participate in Zn(II) binding. The complex **8** is a 4-coordinate neutral species with the pyrromethene entity (**B**- and **C**-pyrrole rings) of two prodigiosin molecules bound to Zn(II) to form a distorted tetrahedral complex. The ^{1}H NMR spectrum of **8** in $CDCl_3$ (Figure S3, Supporting Information) showed a single set of resonances, indicating the symmetrical nature of the Zn complex, with the NH proton of the **A**-pyrrole ring resonating at δ 9.27 ppm. This chemical shift is upfield by ~3 ppm of the corresponding NH proton in protonated prodigiosin,[21] suggesting that the **A**-ring NH proton is shielded by the aromatic nucleus of the other Zn-bound prodigiosin molecule.

On the basis of the results for metal coordination by **1** it was predicted that the synthetic analogues **3** and **4** (Figure 1) would bind Cu(II) to form 1:2 complexes, i.e., Cu(**3**)$_2$. This was confirmed by UV−vis titration studies and LC/MS analyses (Figures S4 and S5, Supporting Information). The interaction of Cu(II) with **3** proceeded cleanly to yield the anticipated Cu(**3**)$_2$ complex as the sole Cu-bound species in solution (Figure S4).[22] However, the interaction of Cu(II) with **4** produced several Cu(**4**)$_2$ complexes that suggested ligand oxidation during the binding process (Figure S5). This finding was not unexpected, as the synthetic derivative **4** possesses an apparent pK_a that is nearly identical to that of **1** in 1:1 acetonitrile:H_2O (pK_a = 7.98 for **1**, 7.99 for **4**).[15] Thus the electron distribution of **4** and **1** should be similar; **4** also contains an **A**-ring with lone-pair N-electrons (NMe_2 group) in conjugation with the pyrromethene moiety. In contrast, replacement of the **A**-pyrrole in a prodigiosin analogue with thiophen-2-yl lowers the apparent pK_a of the pyrromethene entity by ~1 pK unit,[15] and raises the half-peak oxidation potential ($E_{p/2}$) of the first oxidative process by ~230 mV.[14] Thus, **3** is more resistant to oxidation, as observed in its interaction with Cu(II) (Figure S4).

Hence it is interesting that **4** undergoes an oxidative process in the presence of Cu(II) (Figure S5), yet, like other prodigiosin analogues that contain alternative **A**-ring systems,[11,13,14] it fails to promote strand scission in the presence of Cu(II). This strongly suggests that a 1:2 binding arrangement is inactive in Cu-mediated DNA cleavage. Fürstner has alluded to this aspect of the DNA cleavage chemistry, noting that a prodigiosin analogue containing two pharmacophore units is less active in cleaving the plasmid than the 1:1 mixture.[13]

We anticipate that steps for the conversion of **1** into **5** will shed light on the mechanism of dsDNA cleavage by the prodigiosin/Cu(II) nuclease system. Our current results suggest that the hydroxyl group in **5** is derived from H_2O, which differs from hydroxylation by copper monooxygenases, such as dopamine β-monooxygenase or peptidylglycine α-hydroxylating monooxygenase, that utilize copper to reversibly bind and activate O_2 for insertion into the C−H bond.[23] As noted with isoporphyrin formation,[24] two-electron oxidation of **1** followed by attachment of H_2O to yield **5** appears consistent with our experimental evidence. Redox cycling through Cu(I) and Cu(II) is expected to facilitate prodigiosin oxidation and convert O_2 into H_2O_2, which is critical for dsDNA cleavage.[11] Experiments designed to test these predictions are currently underway.

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Supporting Information Available: Experimental details, Figures S1−S5 described in the text, and X-ray data. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) Rizzo, V.; Morelli, A.; Pinciroli, V.; Sciangula, D.; D'Allessio, R. *J. Pharm. Sci*. **1999**, *88*, 73−78.

(22) The ES$^+$ spectra of Cu(**3**)$_2$ and Cu(**4**)$_2$ exhibit parent ions at *m*/*z* 628 and 702, respectively. Like Zn(**1**)$_2$, **8**, these complexes should be neutral species and an $[M + H]^+$ peak would be found at *m*/*z* 630 and 704 respectively: two mass units heavier than the observed parent ions. However, oxidation of a **C**-ring into the azafulvenic structure (i.e **7**, Scheme 1) during the ionization process would yield species with M^+ 628 and 702.

(23) Klinman, J. P. *Chem. Rev*. **1996**, *96*, 2541−2561.

(24) Dolphin, D.; Felton, R. H. *Acc. Chem. Res.* **1974**, *7*, 26−32.