Methylation of Tethered Thiolates in $[(bme\text{-}daco)Zn]_2$ and $[(bme\text{-}daco)Cd]_2$ as a Model of Zinc Sulfur-Methylation Proteins

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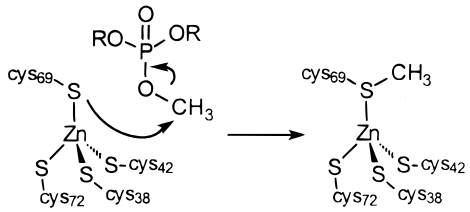
The dimeric dithiolate complex [1,5-bis(mercaptoethyl)-1,5-diazacyclooctanato]zinc(II), $[(bme\text{-}daco)Zn]_2$ or **Zn-1**, and its cadmium analogue, **Cd-1**, were investigated as models for the active site of zinc-dependent methylation proteins. The key issue addressed was whether alkylation of a thiolate in a relatively rigid tetradentate ligand would result in coordination of the thioether product to the metal. On the basis of ^{1}H and ^{13}C NMR spectroscopy and similar reactivity toward alkylating agents, the newly synthesized cadmium complex, **Cd-1**, is proposed to be isostructural with the previously reported **Zn-1** complex, which is known from X-ray crystallography to be dimeric in the solid state (Tuntulani, T.; Reibenspies, J. H.; Farmer, P. J.; Darensbourg, M. Y. *Inorg. Chem.* **1992**, *31*, 3497). Iodomethane reacts with **Zn-1** in hot CH_3OH/CH_3CN to produce a thioether which dissociates, replaced by coordination of iodide in the pseudotetrahedral complex, $(Me_2bme\text{-}daco)ZnI_2$ or **Zn-2**. Complex **Zn-2** crystallizes in the triclinic $P\bar{1}$ space group with $a = 7.911(2)$ Å, $b = 10.675(2)$ Å, $c = 12.394(2)$ Å, $\alpha = 75.270(10)°$, $\beta = 75.270(10)°$, $\gamma = 82.12(2)°$, $V = 998.270$ Å^3, and $Z = 2$. An analogous reaction was observed for the cadmium derivative, **Cd-1**, which displays a ^{1}H NMR spectrum identical to that of **Zn-2**. In attempts to promote thioether binding, the iodide was displaced by addition of $AgBF_4$ to solutions of **Zn-2** or the BF_4^- analogue was synthesized directly from $Zn(BF_4)_2$ and methylated ligand, Me_2bme-daco, to yield **Zn-3**. Similar reactions with the cadmium analogue yielded a product identified as **Cd-3** that was indistinguishable from **Zn-3** by ^{1}H NMR. The ^{113}Cd NMR spectra of **Cd-3** displayed a single resonance at 88 ppm consistent with a hard donor environment and inconsistent with sulfur binding. As a further attempt to induce thioether binding to zinc, the macrocyclization reagent 1,3-dibromopropane was added to **Zn-1**. The resulting product, $[BrZn(macrocycle)]^+$, was only slightly soluble in pyridine and identified by +FAB/MS as the desired macrocyclic product with a large amount of free macrocycle ligand. Recrystallization from pyridine/ether resulted in loss of the zinc as $Zn(py)_2Br_2$, which was obtained as colorless crystals and characterized by X-ray crystallography. Complex $Zn(py)_2Br_2$ crystallizes in the monoclinic $P2_1/c$ space group with $a = 8.534(2)$ Å, $b = 18.316(4)$ Å, $c = 8.461(2)$ Å, $\beta = 101.07(3)°$, $V = 1297.9(5)$ Å^3, and $Z = 4$.

Introduction

Zinc, the second most abundant transition metal in biology, functions as the active site of hydrolytic enzymes, such as carboxypeptidase and carbonic anhydrase where it is in a hard donor coordination environment of nitrogen and oxygen.[1] While cysteine coordination to zinc was long thought to exist solely as a structural motif, most notably in zinc finger proteins,[1] recent discoveries of zinc-dependent thiolate methylation proteins, including methionine synthase,[2] methyl Co-M,[3,4] farnysyl transfersase,[5] and the much studied Ada repair protein,[6−9] have implicated a chemical role as well. The Ada protein contains a zinc ion coordinated to four cysteine residues and serves as a transcriptional regulator through conformational changes induced by methylation of the metal-bound cys69 residue, Scheme 1.[9] A series of ^{113}Cd NMR studies of the catalytically active cadmium analogue[10] conclude that cys69 remains coordinated following methylation.[8,11] Since the binding of thioether to zinc is uncommon, studies of model systems are warranted.

Scheme 1



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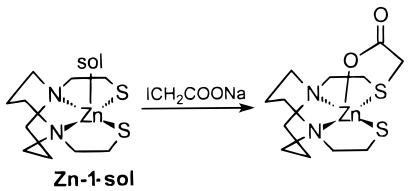
Scheme 2

$$Zn(SPh)_4^{2-} \xrightarrow{DMSO} Zn(DMSO)(SPh)_3^- + PhS^-$$

$$PhS^- \xrightarrow{(CH_3O)_3PO} PhSCH_3 + (CH_3O)_2PO_2^-$$

The leading model study for zinc−thiolate alkylation is that of the methylation of $Zn(SPh)_4^{2-}$ by $(MeO)_3P{=}O$, explored by Lippard *et al.*[12,13] The reaction releases MeSPh replacing the thiolate donor with $[O_2P(OMe)_2^-]$ as demonstrated in Scheme 2. A mechanism invoking thiolate dissociation and alkylation of free thiolate is consistent with the analysis of kinetic data. The substitution of one or more thiophenolate ligands with a neutral methylimidazole (MeIm) donor decreased the rate of thiolate methylation, attributable to the diminished amount of free thiolate through dissociation.[12,13] Alternatively, retention of thiolate coordination in the complex of higher positive charge, $(MeIm)Zn(SPh)_3^-$ or $(MeIm)_2Zn(SPh)_2$, would also result in reduced rates as bound zinc−thiolates are less nucleophilic than free thiolate. Unlike the Ada protein, alkylation of the monodentate thiolate of the model complex resulted in free thioether and bound phosphodiester. Lippard postulated that in the Ada protein zinc may serve as a supply depot of cysteine thiolate which in the absence of the metal would be largely protonated at physiological pH and hence unreactive toward alkylation.[12] This conclusion is consistent with the role played by zinc in methionine synthase.[2] Whether methylation in these proteins occurs at a metal-bound or dissociated thiolate remains an unanswered question.

The tetradentate ligand 1,5-bis(mercaptoethyl)-1,5-diazacyclooctane, bme-daco, binds a variety of transition metals including Ni,[14] Pd,[15] Fe,[16] Zn,[17] and Cu[18] in all cases maintaining relative planarity of the N_2S_2 donor set. Sulfur-based alkylation of (bme-daco)Ni, either with methyl iodide or macrocyclization agents, yields stable metal-bound thioether complexes.[14,19] Functionalized alkylation agents, such as sodium iodoacetate, a well-known cysteine modification agent, also add to the thiolate producing in the case of $[(bme\text{-}daco)Zn]_2$ a structurally characterized N_2S_2O complex with one S-thiolate and one S-thioether, eq 1.[20] Although the thioether donor is tied into a



Zn-1·sol (1)

pentacoordinate anionic ligand, its existence suggested the potential of thioether coordination in such a sterically restricted ligand. The study reported below focuses on the methylation of $[(bme\text{-}daco)Zn]_2$ and the analogous complex $[(bme\text{-}daco)Cd]_2$. The cadmium analogue will also serve to further examine the validity of substituting Cd^{2+} for Zn^{2+} in the study of zinc-containing enzymes.

Table 1. Summary of X-ray Crystal Structure Data for **Zn-2** and $Zn(py)_2Br_2$

	Zn-2	$Zn(py)_2Br_2$
fw	581.6	383.39
space group	$P\bar{1}$	$P2_1/c$
a (Å)	7.911(2)	8.534(2)
b (Å)	10.675(2)	18.316(4)
c (Å)	12.394(2)	8.461(2)
α (deg)	75.270(10)	90
β (deg)	82.84(2)	101.07(3)
γ (deg)	82.12(2)	90
V (Å^3)	998.270(1)	1297.9(5)
Z	2	4
ρ(calcd) (g/cm^3)	1.935	1.962
temp (K)	296	193
radiation (λ, Å)	Mo Kα (0.710 73)	Mo Kα (0.710 73)
abs coeff (mm^{-1})	4.515	9.598
R	0.046	0.0526
R_w	0.043	0.1574

Experimental Section

Materials. Reagent-grade solvents were dried using standard techniques and distilled under nitrogen prior to use. Cadmium acetylacetonate, cadmium iodide, and cadmium tetrafluoroborate (50% aqueous solution) were purchased from Strem Chemical Co. and used with no further purification. Deuterated pyridine (C_5D_5N, 99.5%) was obtained from Cambridge Isotope Laboratories. 1,3-Dibromopropane was supplied by Lancaster Synthesis and used as received. All other reagents were purchased from Aldrich Chemical Co. Standard Schlenk techniques using nitrogen and an argon-filled glovebox were used in synthesis of the complexes, although once obtained the pure products were stable to air.

Physical Measurements. All NMR spectra for **Zn-1**, **Zn-2**, **Cd-1**, and **Cd-2** were obtained on a Varian XL-400 FT NMR. For **Zn-3** and **Cd-3** a Varian XL-200 FT NMR was sufficient to record quality ^{1}H and ^{13}C NMR spectra while the ^{19}F and ^{113}Cd NMR spectra of **Cd-3** were obtained on a Varian XL-400 FT NMR. All spectra were recorded in pyridine-d_5 at 100 °C. The ^{1}H and ^{13}C NMR spectra were referenced versus TMS internally to solvent while the ^{113}Cd and ^{19}F NMR spectra were externally referenced to $Cd(ClO_4)_2$ and CCl_3F, respectively. Assignments of the ^{1}H NMR were made by selective decoupling of the **Zn-1** spectrum while selective proton decoupling of the ^{13}C spectrum of **Zn-2** was used to assign the carbon spectra. The T_1 relaxation times for the carbon resonances of **Zn-2** and **Zn-3** were determined by the inversion recovery method.[21]

Mass spectra were determined at the Center for Chemical Characterization and Analysis at Texas A&M University. Positive ion fast atom mass spectra were recorded in a nitrobenzyl alcohol matrix using a VG-70S spectrometer with a xenon source having particle energy of 10 keV. Data were collected by a VG11-250J data system. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN.

X-ray crystal structures were determined at the Crystal & Molecular Structure Laboratory of the Center for Chemical Characterization and Analysis at Texas A&M University. X-ray crystallographic data were obtained on a Rigaku AFC5R single-crystal X-ray diffractometer operating with an oriented graphite monochromator (Mo Kα (λ = 0.710 73 Å) radiation). A single crystal was mounted on a glass fiber with epoxy cement at room temperature. The structure was solved by direct methods using the SHELXTL-PLUS program package. X-ray experimental conditions and data collections for **Zn-2** and $Zn(py)_2Br_2$ are shown in Table 1.

Syntheses. The syntheses of the ligand 1,5-bis(mercaptoethyl)-1,5-diazacyclooctane, H_2bme-daco, and the nickel complex, (bme-daco)-Ni, have been described previously.[14,22]

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[(bme-daco)Zn]$_2$, Zn-1. The following synthesis of [(bme-daco)Zn]$_2$ is a revision of the published method.[17] Under anaerobic conditions, a toluene solution (100 mL) of zinc acetylacetonate (3.95 g, 15 mmol) was added to a toluene solution (25 mL) of H_2bme-daco (3.50 g, 15 mmol). A white solid precipitated immediately, and the mixture was stirred overnight at 22 °C. The supernatant was separated from the precipitate by cannulation and discarded. The white solid was washed with 2 × 50 mL portions of toluene and dried under vacuum (3.50 g, 78%). An analytically pure sample was obtained by recrystallization of the white solid from a hot 1:1 mixture of methanol and acetonitrile. Calcd (found) for $Zn_2N_4S_4C_{20}H_{40}$: C, 40.3 (40.6); H, 6.77 (6.58); N, 9.41 (9.58). NMR measurements were carried out in pyridine-d_5 at 100 °C on a Varian XL400 FT NMR. ^{1}H NMR: δ 3.18 (m, 3.8 H), 3.00 (t, 4.3 H), 2.90 (m, 3.9 H), 2.45 (m, 3.9 H), 2.35 (m, 2.1 H), 1.83 (m, 1.9 H). ^{13}C NMR: δ (ppm) 64, 54, 28, 26.

[(bme-daco)Cd]$_2$, Cd-1. Under anaerobic conditions, a cloudy solution of cadmium acetylacetonate (1.50 g, 4.82 mmol) in 100 mL of toluene was added to a toluene solution (50 mL) containing H_2-bme-daco (1.78 g, 7.60 mmol). The mixture was stirred overnight. A white solid precipitated and was isolated by cannula transfer of the supernatant. The solid was washed with 2 × 50 mL portions of toluene and dried under vacuum (400 mg, 16%). An analytically pure sample was obtained by recrystallization of the white solid from a hot 1:1 mixture of methanol and acetonitrile. Calcd (found) for $Cd_2N_4S_4C_{20}H_{40}$: C, 34.8 (34.7); H, 5.85 (5.77); N, 8.12 (7.89). NMR measurements were carried out in pyridine-d_5 at 100 °C on a Varian XL400 FT NMR. ^{1}H NMR: δ 3.06 (t, 4.1 H), 2.94 (m, 4.0 H), 2.69 (t, 3.9 H), 2.42 (m, 4.1 H), 1.95 (m, 2.0 H), 1.82 (m, 1.9 H) ^{113}Cd NMR: δ (ppm) 538.

(Me$_2$bme-daco)ZnI$_2$, Zn-2. A 250 mL three-necked round-bottomed flask equipped with a reflux condenser was charged with **Zn-1** (206 mg, 0.351 mmol). A 1:1 mixture (100 mL) of MeOH/MeCN was added and warmed to 70 °C, at which point the solution became homogeneous. Iodomethane (0.22 mL, 0.351 mmol) was added followed by 1 h of reflux. The solution was slowly cooled to room temperature with stirring. The solvent was reduced to a minimum under vacuum, and a white solid was obtained by addition of diethyl ether. Following filtration, the solid was washed with 2 × 25 mL of diethyl ether (266 mg, 65%). Crystals suitable for X-ray crystallography were obtained from ether diffusion into a concentrated MeOH/MeCN solution of **Zn-2**. NMR measurements were carried out in pyridine-d_5 at 100 °C on a Varian XL-400 FT NMR. ^{1}H NMR: δ 2.82 (m, 12.6H), 2.68 (t, 3.7H), 2.05 (s, 5.9 H) 1.55 (q, 4.0 H). ^{13}C NMR δ (ppm): 57, 52, 33, 28, 16.

Me$_2$bme-daco. To a 50 mL Schlenk flask containing (bme-daco)-Ni (100 mg, 0.34 mmol) dissolved in 20 mL of MeCN was added iodomethane (100 μL mL, 1.6 mmol). The green solution was stirred at 22 °C for 10 h. The solvent was removed under vacuum, and the residue was dissolved in 10 mL of distilled water. Potassium cyanide (90 mg, 1.4 mmol) was added yielding a colorless solution upon stirring. After 2 h, the solution was transferred to a separatory funnel and the ligand Me$_2$bme-daco was extracted with 3 × 50 mL of diethyl ether. The ether extracts were dried over Na_2SO_4 and transferred to a 250 mL round-bottom flask. Removal of solvent by rotary evaporation yielded Me$_2$bme-daco as an oil (91.2 mg, 0.34 mmol).

(Me$_2$bme-daco)CdI$_2$, Cd-2. (Me$_2$bme-daco)CdI$_2$ can be synthesized by the procedure similar to the zinc analogue. However, the insolubility of **Cd-1** make this route impractical for preparative synthesis. The following procedure is preferable. Under anaerobic conditions, a solution of CdI_2 (127 mg, 0.35 mmol) in methanol (5 mL) was added to Me$_2$bme-daco (see above) in a 50 mL Schlenk flask resulting in a white precipitate. The mixture was stirred overnight. The supernatant was removed via cannula and discarded. The remaining white solid was washed with 2 × 20 mL of diethyl ether and dried under vacuum to yield (Me$_2$bme-daco)CdI$_2$, **Cd-2** (128 mg, 60%). An analytically pure sample was obtained by recrystallization from hot methanol. Anal. Calcd (found) for $C_{12}H_{26}N_2S_2CdI_2$: C, 22.9 (23.0); H, 4.17 (4.30); N, 4.46 (4.12). NMR measurements were performed in pyridine-d_5 at 100 °C. ^{1}H NMR [δ (ppm)]: 2.80 (m, 12.6 H), 2.65 (m, 4.4 H); 2.08 (s, 5.2 H); 1.62 (q, 4.0 H). ^{13}C NMR [δ (ppm)]: 56, 52, 33, 27, 14. ^{113}Cd NMR [δ (ppm)]: 266.

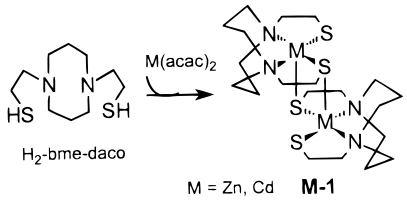
[Me$_2$bme-daco)Zn][BF$_4$]$_2$, Zn-3. The ligand Me$_2$bme-daco was obtained by the procedure described above. Under anaerobic conditions, $Zn(BF_4)_2$ (117 mg, 0.49 mmol) was dissolved in methanol (5 mL) and added to a methanolic solution (10 mL) of Me$_2$bme-daco (128 mg, 0.49 mmol). The reaction was stirred for 12 h. The volume of the reaction was reduced, and dry diethyl ether was added to precipitate **Zn-3** as a white solid (124 mg, 50%). NMR measurements were performed in pyridine-d_5 at 100 °C. ^{1}H NMR [δ (ppm)]: 3.08 (m, 13.0 H), 2.75 (t, 4.0 H); 2.04 (s, 5.5 H); 1.88 (q, 4.2 H). ^{13}C NMR [δ (ppm)]: 56, 53, 29, 23, 14.

[Me$_2$bme-daco)Cd][BF$_4$]$_2$, Cd-3. The [Me$_2$bme-daco)Cd][BF$_4$]$_2$ complex was prepared in 52% yield from $Cd(BF_4)_2$ in analogy to the corresponding zinc complex, **Zn-3**. ^{1}H NMR [δ (ppm)]: 3.10 (M, 12.1 H), 2.78 (t, 3.9 H); 2.05 (s, 5.8 H); 1.90 (q, 3.9 H). ^{113}Cd NMR [δ (ppm)]: 88.

[BrZn(macrocycle)]Br, Zn-4. To a 100 mL three-necked round-bottom flask equipped with a N_2 inlet and a water-cooled condenser was added **Zn-1** (225 mg, 0.377 mmol). A 1:1 mixture (100 mL) of MeOH/MeCN was added and heated to reflux to yield a homogeneous solution. An excess of 1,3-dibromopropane (1.5 mL, 14.7 mmol) was added and the solution refluxed for 1 h. The reaction was allowed to slowly cool to room temperature, and with stirring overnight a white precipitate formed. The solvent was removed by cannula, and the solid was washed with 2 × 50 mL portions of dry diethyl ether. Drying under vacuum yielded **Zn-4** as a white, insoluble solid (263 mg, 70%). The product is insoluble in all common solvents except pyridine which slowly removes the zinc upon stirring. FAB/MS (*m*/*z*): 419 (RI = 10%, **Zn-4**, $ZnC_{13}H_{26}N_2S_2Br$), 275 (RI = 100%, free macrocycle ligand, $C_{13}H_{26}N_2S_2$).

Results and Discussion

The synthesis and X-ray crystal structure for **Zn-1** have been reported previously.[17] An alternate synthesis is expressed by eq 2 and described above. The zinc adopts a distorted square



(2)

pyramidal N_2S_3 geometry with thiolate sulfurs from the adjacent (bme-daco)Zn unit occupying the apical site resulting in a dimer with a 2 Zn−2 S core. The dimeric nature of **Zn-1** underscores the ligand-imposed planarity of the N_2S_2 donors which does not permit tetrahedral geometry. A dimer of similar structure has been observed in the iron derivative of bme-daco.[16] Both **Zn-1** and **Fe-1** show the pentacoordinated M^{2+} to be displaced from the N_2S_2 basal plane by ca. 0.6 Å. To investigate the validity of substituting Cd for Zn for NMR characterizations, the cadmium analogue, **Cd-1**, has been synthesized. Recrystallization from hot methanol/acetonitrile yields thin plates unsuitable for X-ray crystallographic analysis, requiring NMR studies to delineate the structure of **Cd-1**.

Solution Structure of Zn-1 and Cd-1. The ^{1}H NMR spectrum of **Zn-1** measured in pyridine-d_5 displays six distinct resonances as shown in Figure 1a. The multiplets at 3.18 ppm (H3, 3.8 H) and 2.45 ppm (H4, 3.9 H) are assigned to the protons on the diazacyclooctane (daco) backbone on the carbon α to nitrogen while the broad multiplets at 2.35 ppm (H5, 2.1 H)

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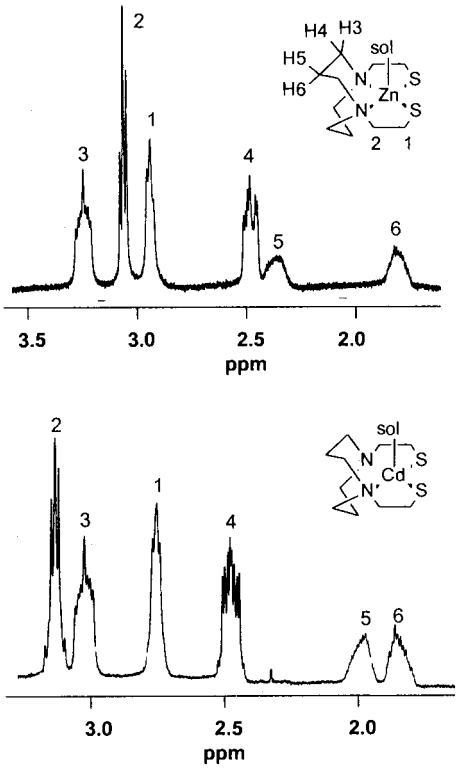


Figure 1. (a) Top: ^{1}H NMR of **Zn-1** in pyridine-d_5 at 100 °C. (b) Bottom: ^{1}H NMR of **Cd-1** in pyridine-d_5 at 100 °C.

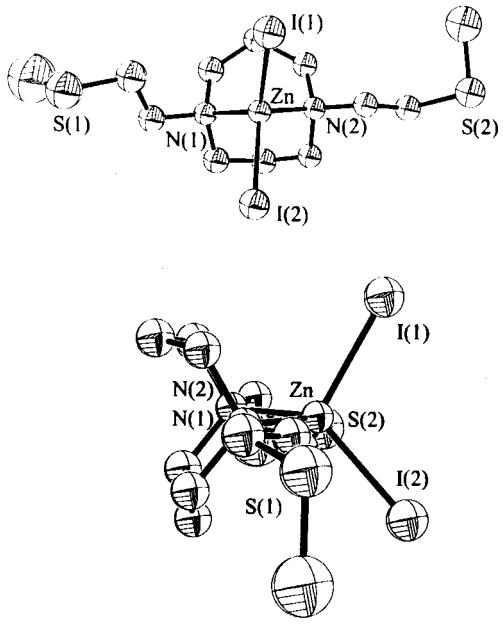


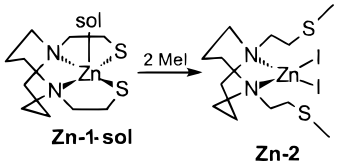
Figure 2. Two views of the molecular structure of (Me_2bme-daco)-ZnI_2, **Zn-2**. Hydrogen atoms have been omitted. Selected bond lengths (Å): Zn(1)−I(1) 2.555(2); Zn(1)−I(2) 2.560(2); Zn(1)−N(1) 2.099-(9); Zn(1)−N(2) 2.09(1); S(1)−C(12) 1.78(1); S(2)−C(11) 1.78(1). Selected bond angles (deg): I(1)−Zn(1)−I(2) 109.7(1); I(1)−Zn(1)−N(1) 124.7(2); I(2)−Zn(1)−N(1) 106.1(3); I(1)−Zn(1)−N(2) 114.1-(2); I(2)−Zn(1)−N(2) 112.0(3); N(1)−Zn(1)−N(2) 88.9(4); Zn(1)−N(1)−C(2) 111.9(7); Zn(1)−N(2)−C(9) 106.9(6).

and 1.85 ppm (H6, 1.9 H) are assigned to the apical hydrogens on the daco backbone. These assignments are consistent with spin-decoupling experiments which show that resonances H3, H4, H5, and H6 are in the same spin system. The daco backbone forms two fused six-membered metalloheterocycle rings which can adopt chair or boat conformations. The splitting of the protons on the daco backbone is consistent with conformationally constrained cyclohexane rings in which axial and equatorial protons can be separated by 0.1−0.7 ppm.[23] The resonances at 3.00 and 2.90 ppm are assigned to the protons adjacent to the nitrogen and sulfur, respectively. The ^{13}C NMR spectrum of **Zn-1** shows four distinct resonances at 25, 28, 54, and 63 ppm. The downfield resonances are assigned to the C α to the N; *vide infra*.

Since X-ray-quality crystals of **Cd-1** were unobtainable, NMR spectroscopy was used for characterization of **Cd-1**. The ^{1}H NMR spectrum of **Cd-1** in pyridine-d_5 at 100 °C displays six resonances, Figure 1b, and is similar to that observed for **Zn-1.** On the basis of these similarities, **Zn-1** and **Cd-1** are most likely isostructural. The key differences in the ^{1}H NMR spectra is the smaller splitting of the protons on the daco backbone of **Cd-1** relative to **Zn-1** which indicates greater flexibility in **Cd-1** versus **Zn-1**. The ^{113}Cd NMR spectrum of **Cd-1** displays a single resonance at 538 ppm, consistent with a nitrogen/sulfur donor environment.[24,25]

The degree of aggregation of **Zn-1** and **Cd-1**, i.e., dimeric with bridging thiolates or monomeric with terminal thiolates, in solution is of importance to S-alkylation reactions. In the ^{13}C NMR spectrum, also measured in pyridine-d_5 at 100 °C, a monomer would display four resonances while a dimer would show more resonances as the C α to the terminal thiolate would be shifted from the bridging thiolate. Since only four ^{13}C resonances are observed, **Zn-1** is likely a monomer with solvent coordinated in the apical site. The similarity of solution spectra suggests that **Cd-1** in pyridine is identical to **Zn-1**. Square planar cadmium complexes have recently been reported for sterically hindered phenolates in noncoordinating solvents, but in pyridine a five coordinate complex is observed.[26,27]

Methylation Studies. Reaction of **Zn-1** with iodomethane in warm methanol/acetonitrile yields the sulfur-based alkylation product, **Zn-2**, eq 3. Attempts to isolate the monomethylated



(3)

product by addition of 1 equiv of methyl iodide yielded only **Zn-2** and unreacted **Zn-1**. Crystals suitable for X-ray crystallography were obtained by ether diffusion into a MeOH/MeCN solution of **Zn-2**.

The X-ray crystal structure of **Zn-2**, Figure 2, shows the zinc coordinated to two nitrogens and two iodides in a tetrahedral geometry. The average Zn−I bond distance, 2.558 Å, is typical for Zn−I bond lengths.[28] The average Zn−N distance, 2.095 Å, is significantly shorter than that of the **Zn-1** dinuclear

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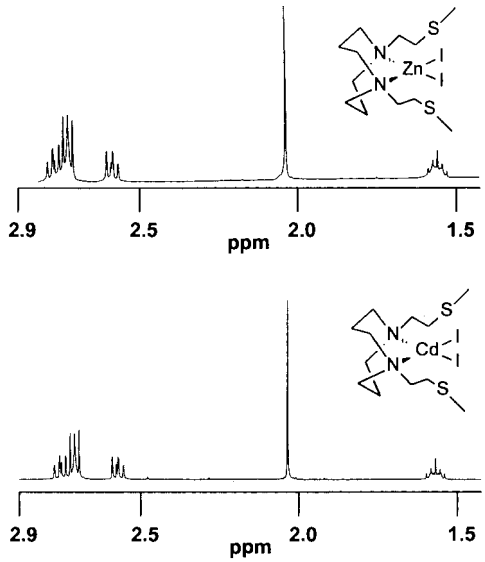


Figure 3. (a) Top: ^{1}H NMR of (Me$_2$bme-daco)ZnI$_2$, **Zn-2**, in pyridine-d_5 at 100 °C. (b) Bottom: ^{1}H NMR of **Cd-2** in pyridine-d_5 at 100 °C.

Table 2. NMR Assignments for **Zn-2** and **Zn-3** with T_1 Relaxation Times

NMR shifts[a] (ppm)			T_1 relaxation (s)		
^{1}H	^{13}C	assignt	**Zn-2**	**Zn-3**	**Zn-2/Zn-3**
2.05	16	S−*C*H$_3$	7.8 ± 0.5	4.4 ± 0.4	0.57
2.68	28	CH$_2$*C*H$_2$CH$_2$	1.4 ± 0.2	0.8 ± 0.3	0.56
2.80	33	CH$_2$*C*H$_2$S	1.8 ± 0.3	2.1 ± 0.1	1.2
2.77	53	N*C*H$_2$CH$_2$CH$_2$	1.48 ± 0.05	0.79 ± 0.05	0.53
1.55	57	N*C*H$_2$CH$_2$S	1.63 ± 0.09	1.00 ± 0.07	0.61

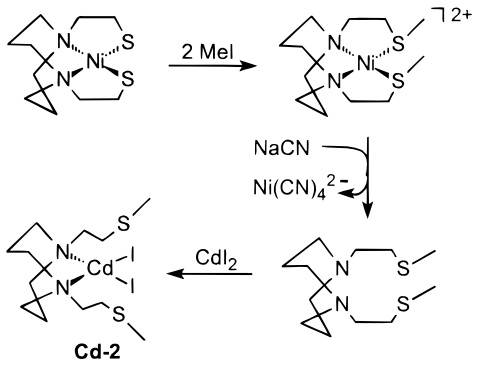
[a] NMR shifts are for **Zn-2**; the corresponding **Zn-3** resonances are slightly shifted (see text).

complex, 2.243 Å. The I−Zn−I bond angle of 109.7(1)° is as expected for tetrahedral geometry, while the daco backbone dictates the N−Zn−N bond angle, 88.9°. Diazacyclooctane complexes for a wide variety of metals display N−M−N bond angles very near 90°. The long Zn−S(CH$_3$) distance, 3.8 Å, unambiguously establishes that the thioether is unbound.

The ^{1}H NMR of **Zn-2**, Figure 3a, shows a sharp resonance at 2.05 ppm assignable to the thioether methyl protons. The protons on the daco backbone resonate at 1.55 ppm for the apical position and 2.78 ppm for the protons on the C α to the N. The resonance at 2.78 ppm overlaps a resonance at 2.82 assignable to the protons on the ethylene side arm. The protons adjacent to the sulfur are observed at 2.68 ppm. On the basis of these assignments, selective decoupling was used to assign the ^{13}C spectrum, Table 2. The methyl group displays the resonance furthest upfield, 16 ppm, while the carbons α to N resonate most downfield, 53 ppm for the daco carbon and 57 ppm for the carbon on the ethylene side arm. The carbon α to sulfur displays a resonance at 33 ppm, with the resonance at 28 ppm assigned to the apical carbon of the daco.

Since **Zn-2** does not show thioether coordination, it is of interest to compare the Cd analogue. Due to the low solubility of **Cd-1**, the methylation reaction is an unattractive preparative route to **Cd-2**. As shown in Scheme 3, S-methylation of (bme-daco)Ni and subsequent removal of nickel by addition of cyanide yields the free ligand, Me$_2$bme-daco. Addition of CdI$_2$ to the

Scheme 3



free ligand yields **Cd-2** as a white solid. The ^{1}H NMR spectrum of **Cd-2**, Figure 3b, confirms that **Cd-2** and **Zn-2** are, at least in solution, isostructural. The ^{113}Cd NMR spectrum shows a single resonance at 266 ppm which is consistent with Cd^{2+} in an N_2I_2 environment.[24,29]

Ion Exchange. The **Zn-2** complex demonstrates that the low coordinating ability of thioether to Zn^{2+}, coupled with the tetrahedral geometrical preferences of Zn^{2+}, results in preferential binding of iodide anion over the neutral thioether sulfur. An additional driving force in facilitating anion binding is the relatively low polarity of the solvent system (MeOH/MeCN). Neither does Cd^{2+} coordinate thioether in this ligand system.

To establish whether a poorly coordinating counterion could induce thioether binding, BF_4^- analogues were prepared, [(Me$_2$-bme-daco)Zn][BF$_4$]$_2$ or **Zn-3** and **Cd-3.** Crystals suitable for X-ray crystallography have not been obtained for either complex. The solution structures of **Zn-3** and **Cd-3** were therefore investigated by NMR spectroscopy.

As with the other complexes described heretofore, the ^{1}H NMR spectra of **Zn-3** and **Cd-3** are identical and the complexes are assumed to be isostructural. The ^{1}H NMR spectrum of **Zn-3**, not shown, displays resonances shifted downfield by approximately 0.3 ppm with respect to **Zn-2**. The ^{13}C NMR spectrum of **Zn-3** shows only minor shifts from that of **Zn-2**. Neither the ^{1}H or the ^{13}C NMR spectra suggest significant structural differences between **Zn-2** and **Zn-3**.

Coordination of the thioether sulfur in **Zn-2** or **Zn-3** would influence the T_1 relaxation time of the carbons α to the sulfur. As shown in Table 2, all of the T_1 relaxation times for **Zn-3** are approximately 60% of those for **Zn-2** with the notable exception of the CH$_2$ α to the sulfur which is unchanged. The relative increase in the T_1 relaxation for this C indicates a change in the relaxation mode, which could be due to coordination of the sulfur. However, S-coordination should similarly influence the relaxation of the methyl carbon, which it does not. Alternately, it is the CH$_2$ α to the sulfur which most directly would interact with ligands substituted on the zinc and thus would be most affected by changes in the metal's coordination sphere, such as binding of solvent or changes in the coordinated counterion.

As a further probe to delineate the coordination of **Zn-3**, the ^{113}Cd NMR spectrum of **Cd-3** was recorded in hot pyridine-d_5. Too far upfield for S-coordination, the single resonance at 88 ppm suggests coordination of the counterion or solvent, i.e., in the range of a hard-donor ligand set.[24] The possibility of coordinated BF_4^-, which has been observed previously,[30] was

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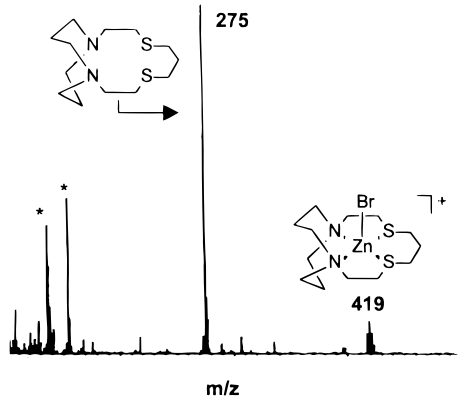
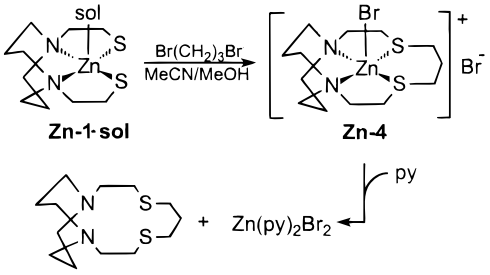


Figure 4. +FAB/MS spectra of [BrZn(macrocycle)]$^+$, **Zn-4**, recorded in an NBA matrix (matrix denoted by asterisks).

Scheme 4



also investigated. The ^{19}F NMR spectrum of **Zn-3** in pyridine shows a resonance at −152 ppm identical to that of $NaBF_4$ in D_2O. These results suggest that BF_4^- is only a counterion and that solvent, either pyridine or trace water, is bound to the metal in **Zn-3** and **Cd-3**. Alternatively, there is a fluxional ligated BF_4^- ion with a coincidental resonance. The ^{113}Cd resonance was unaffected by addition of imidazole or thiophene.

Alkylation with 1,3-Dibromopropane. In the Ada protein, the protein organization may facilitate thioether binding by holding the alkylated cysteine in close proximity to the zinc. To model this effect and induce thioether binding, **Zn-1** was reacted with the macrocyclization alkylation reagent, 1,3-dibromopropane, to yield a white precipitate, **Zn-4**. The **Zn-4** complex has very limited solubility in pyridine precluding NMR analysis. The FAB/MS spectrum, Figure 4, shows a peak at $m/z = 419$ assignable to the monocation [BrZn(macrocycle)]$^+$. A more intense peak at $m/z = 275$ is attributed to the free macrocyclic ligand. Crystals suitable for X-ray crystallography were obtained by ether diffusion into a pyridine solution of **Zn-4**. The X-ray structure revealed that the solvent had abstracted the zinc from the ligand as $Zn(py)_2Br_2$, Scheme 4. Although the X-ray structures of $Zn(py)_2Cl_2$[31,32] and $Zn(py)_2I_2$[33] have been previously reported, this is the first report of $Zn(py)_2Br_2$, Figure 5. The ease of removal of zinc from **Zn-4** indicates that thioether only weakly coordinates zinc even when the ligand encourages S-coordination.

Conclusions

The S-alkylation of zinc−thiolates has recently been found to be of biological significance in numerous proteins.[2−9] For

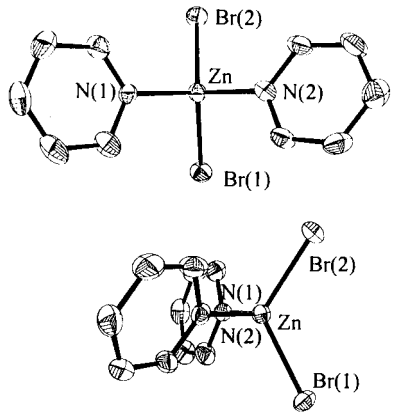
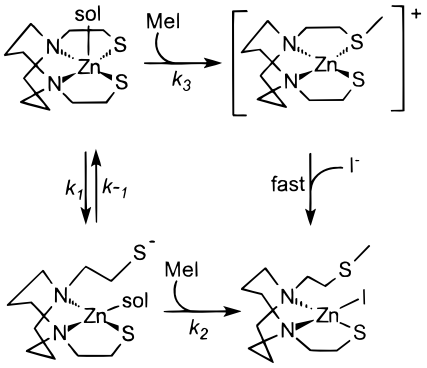


Figure 5. Two views of the molecular structure of $Zn(py)_2Br_2$. Hydrogen atoms have been omitted. Selected bond lengths (Å): Zn(1)−N(1); Zn(1)−N(2); Zn(1)−Br(1); Zn(1)−Br(2). Selected bond angles (deg): N(1)−Zn(1)−N(2) 107.2(2); N(2)−Zn(1)−Br(2) 108.6(2); N(1)−Zn(1)−Br(2) 107.7(2); N(2)−Zn(1)−Br(1) 106.4(2); N(1)−Zn(1)−Br(1) 105.4(2); Br(1)−Zn(1)−Br(2) 120.9(5).

Scheme 5



labile monodentate thiolate complexes, ligand dissociation is kinetically competent to account for S-alkylation, and Lippard et al. suggest such cysteine dissociation may occur in the Ada protein.[12] In contrast, work of Verdine and Ohkuba on the Cd analogue of the Ada protein indicates that the methylated cysteine product is bound to zinc.[8,11] Taken together, these results raise the interesting issue of just how reasonable is the supposition that the protein provides an environment which facilitates lability of the borderline hard thiolate at the hard zinc ion while allowing coordination of the soft thioether. The answer may lie in conformational changes at the Zn site upon alkylation[6,11] although this possibility is far from being fully addressed in *in vivo* studies. An alternate possibility is that the thiolate remains bound prior to alkylation, a situation less appropriate to the monodentate $Zn(SPh)_4^{2-}$ model.

Shown in Scheme 5 are two possible mechanisms for S-based methylation utilizing metal-bound and free thiolate in the first alkylation step. The evidence presented by Lippard et al. for the methylation of $Zn(SPh)_4^{2-}$ strongly supports a thiolate dissociation pathway.[13] However, for the neutral $Zn(MeIm)_2(SPh)_2$ complex, dissociation does not occur to any measurable extent and a slow alkylation rate is observed.[12,13] While the dissociated thiolate produced in the k_1 step is expected to be more nucleophilic than the metal-bound thiolate, it is also expected that k_{-1} would be large for **Zn-1** and that the equilibrium would lie on the side of metal-bound thiolate. In the **Zn-1** system, it is not possible to distinguish between the pathways shown in Scheme 5, that is, if the rate is limited by dissociation of thiolate or by the lessened nucleophilicity of the bound thiolate.

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Regardless of the alkylation mechanism, the difficulty of binding thioether to zinc is further substantiated. Thus methylation of thiolate in both the monodentate tetrathiophenolate[12] and the sterically restricted tetradentate bme-daco zinc model complexes yields dissociated thioether. While the former model serves as a better mimic of the expected geometry about the zinc in the protein, the dissociation of the thiolate prior to alkylation foreshadows the noncoordination of the neutral thioether product. We have used the **Zn-1** chelated complex in order to bias coordination of thioether by tethering it onto the ligand. This assistance was not sufficient, perhaps because the rigidity of the bme-daco ligand enforces a N_2S_2 square plane upon coordination of all four donors requiring a bound-thioether product to adopt a square pyramidal geometry. Dissociation of the thioether and binding of the iodide counterion, or solvent in the case of poorly coordinating counterions, results in the familiar tetrahedral geometry for zinc. We have, however, demonstrated that higher restrictions, such as macrocyclization and the production of **Zn-4** or creation of the pentacoordinate complex shown in eq 1, result in thioether S-binding to zinc. In zinc-dependent thiolate alkylation enzymes, such as methionine synthase,[2] the release of thioether is the desired goal of the process. This would suggest a restrictive protein environment in the Ada protein is required to enforce thioether binding. The macrocyclic **Zn-4** complex suggests that thioether coordination can occur when sulfur is held close to the metal. Throughout this study the softer Cd^{2+} mirrors the harder Zn^{2+} in geometry and in spectroscopy, supportive of assumption that Cd^{2+} is a legitimate structural probe for zinc in the Ada protein.

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Supporting Information Available: Tables of crystallographic data collection parameters, atomic coordinates and equivalent isotropic displacement parameters, complete bond lengths and bond angles, and anisotropic displacement parameters and packing diagrams for **Zn-2** and $Zn(py)_2Br_2$ (17 pages). Ordering information is given on any current masthead page.

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