Inclusion Complexation of Acridine Red Dye by Calixarenesulfonates and Cyclodextrins: Opposite Fluorescent Behavior

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Introduction

Calixarene chemistry is currently developing rapidly. It was stimulated by the pioneering work[1] about two decades ago by Gutsche et al., who optimized the synthetic procedures for *p-tert*-butylcalix[*n*]arene (*n* = 4, 6, and 8)[2] in multigram amounts on a laboratory scale in a relatively simple condensation reaction starting from inexpensive materials. Derivatization on the upper rim (*para*-positions) and the lower rim (the phenolic hydroxy groups) has been conducted to obtain a large number of calixarene derivatives in the past 20 years.[3] As the third generation of host molecules,[4] calixarenes possess the merits of crown ethers and cyclodextrins. However, in contrast to their attractive architecture, complexation studies have been limited, especially on alkaline cations and alkaline earth cations in organic solvents due to their low solubility in aqueous solution. Therefore, it was difficult to compare the complexation behaviors between the cyclodextrins and the calixarenes. Fortunately, the sulfonation of calixarenes on their *para*-positions produced calixarenesulfonates, which are readily soluble in water.[5] Subsequently, although some other water-soluble calixarene derivatives have been prepared, calixarenesulfonates have attracted the most attention. Shinkai[6a–d] and Barra[6e] have reported the complexation of several ammonium and dyes with calix[*n*]arenesulfonates (*n* = 4, 6, and 8) and their alkylated derivatives. Kaifer et al. have qualitatively and quantitatively studied the complexation of metallocenium (ferrocenium and cobaltocenium) with calix[*n*]arenesulfonates, and metallocene

(ferrocene, and cobaltocene) with cyclodextrins, respectively.[7] A general conclusion has been drawn that the complexation behaviors of the calixarenes are largely driven by electrostatic interaction, rather than the hydrophobic interaction in the cyclodextrin's complexation. Furthermore, Shinkai has stated that the calix[6]-arene cavity is more hydrophobic than that of β-cyclodextrin.[8]

It is difficult to select a guest molecule which shows strong binding with both cyclodextrins and water-soluble calixarenes, which possess a hydrophobic cavity; however, their cavities are different in nature. Although there are many documents that focus on the inclusion complexation of modified cyclodextrins, or water-soluble calixarene derivatives, respectively, there are few studies on the complexation of a guest molecule with two host compounds.[9]

In our project, acridine red dye was found to be suitable for comparison purposes. It could form stable complexes with two kinds of host compounds, showing distinctly opposite fluorescent behavior upon the complexation of cyclodextrins and calixarenesulfonates. Complexation and thermodynamic research would help to investigate and compare the cavity hydrophobicity, the interaction nature, and the complexation behavior between cyclodextrins and water-soluble calixarenes. Herewith, we present the results on the complexation behaviors and thermodynamics of calix[*n*]arenesulfonates and α-, β-, and γ-cyclodextrins, employing acridine red dye as the guest molecule (Chart 1).

Results and Discussion

It is interesting that the fluorescent behaviors of the acridine red dye molecule were unprecedentedly, to the best of our knowledge, found to be distinctly opposite upon the complexation with two host compounds that possess a hydrophobic cavity. The fluorescence intensity of acridine red gradually decreased upon the addition of calix[*n*]arenesulfonates. On the contrary, it increased upon the addition of cyclodextrins. Figures 1 and 2 show the opposite fluorescence spectral changes of acridine red dye molecule, showing the different characteristics of the host compounds employed. Furthermore, the alkylated derivative of calix[6]arenesulfonate caused the fluorescence enhancement (Figure 3), which is opposite to that of its parent. The fluorescence behaviors are different from the results obtained by Warner et al., who have researched the complexation of auramine O dye with the cyclodextrins and the water-soluble calix[6]arene, both of which enhance the fluorescence intensity.[9]

As shown in Figure 1, the fluorescence intensity of acridine red dye decreased markedly upon the addition

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(1) (a) Gutsche, C. D.; Dhawan, B.; No, K. H.; Muthukrishnan, R. *J. Am. Chem. Soc.* **1981**, *103*, 3782–3792. (b) Gutsche, C. D. In *Progress in Macrocyclic Chemistry. Vol. 3. Synthesis of Macrocycles. The Design of Selective Complexing Agents*; Izatt, R. M., Christensen, J. J., Eds.; Wiley-Interscience: New York, 1987; pp 93–165.

(2) (a) Gutsche, C. D.; Iqbal, M. *Org. Synth.* **1990**, *68*, 234–237. (b) Gutsche, C. D.; Dhawan, B.; Leonis, M.; Stewart, D. *Org. Synth.* **1990**, *68*, 238–242. (c) Munch, J. H.; Gutsche, C. D. *Org. Synth.* **1990**, *68*, 234–246.

(3) Böhmer, V. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 713–745.

(4) (a) Shinkai, S. *Tetrahedron* **1993**, *49*, 8933–8968. (b) Takeshita, M.; Shinkai, S. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 1088–1097.

(5) Shinkai, S.; Mori, S.; Koreishi, H.; Tsubaki, T.; Manabe, O. *J. Am. Chem. Soc.* **1986**, *108*, 8, 2409–2416.

(6) (a) Koh, K. N.; Araki, K.; Ikeda, A.; Otsuka, H.; Shinkai, S. *J. Am. Chem. Soc.* **1996**, *118*, 755–758. (b) Shinkai, S.; Araki, K.; Kubota, M.; Arimura, T.; Matsuda, T. *J. Org. Chem.* **1991**, *56*, 295–300. (c) Shinkai, S.; Araki, K.; Matsuda, T.; Manabe, O. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 3856–3862. (d) Shinkai, S.; Araki, K.; Manabe, O. *J. Chem. Soc., Chem. Commun.* **1988**, 187–189. (e) Tao, W.; Barra, M. *J. Chem. Soc., Perkin Trans. 2* **1998**, 1957–1960.

(7) (a) Zhang, L.; Macias, A.; Lu, T.; Gordon, J. I.; Gokel, G. W.; Kaifer, A. E. *J. Chem. Soc., Chem. Commun.* **1993**, 1017–1019. (b) Castro, R.; Godínez, L. A.; Criss, C. M.; Kaifer, A. E. *J. Org. Chem.* **1997**, *62*, 4928–4935. (c) Wang, Y.; Alvarez, J.; Kaifer, A. E. *Chem. Commun.* **1998**, 1457–1458. (d) Godínez, L. A.; Patel, S.; Criss, C. M.; Kaifer, A. E. *J. Phys. Chem.* **1995**, *99*, 17449–17455.

(8) Arimura, T.; Nagasaki, T.; Shinkai, S.; Matsuda, T. *J. Org. Chem.* **1989**, *54*, 3766–3768.

(9) (a) Zhang, Y.; Agbaria, R. A.; Warner, I. M. *Supramol. Chem.* **1997**, *8*, 309–318. (b) Mwalupindi, A. G.; Rideau, A.; Agbaria, R. A.; Warner, I. M. *Talata* **1994**, *41*, 599–609.
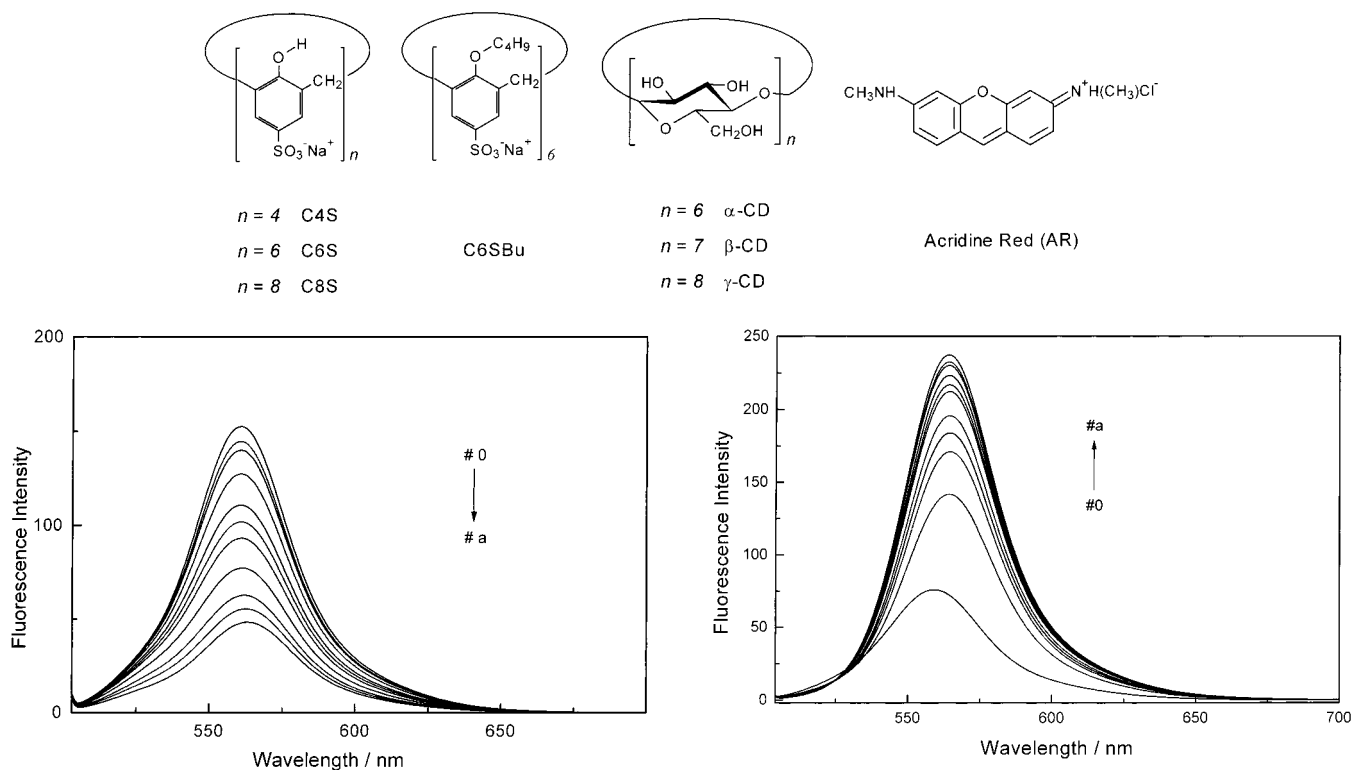
Chart 1

$n = 4$ C4S

$n = 6$ C6S

$n = 8$ C8S

C6SBu

$n = 6$ α-CD

$n = 7$ β-CD

$n = 8$ γ-CD

Acridine Red (AR)

Figure 1. Fluorescence spectra of acridine red dye (6.6×10^{-6} mol dm^{-3}) in the presence and absence of calix[6]arenesulfonate (10^{-4} mol dm^{-3}): (0) 0; (1) 0.0599; (2) 0.120; (3) 0.300; (4) 0.600; (5) 0.899; (6) 1.199; (7) 2.398; (8) 4.796; (9) 7.194; (a) 11.99, in aqueous citrate buffer solution (pH 6.00) at 25.0 °C.

Figure 3. Fluorescence spectra of acridine red dye (3.93×10^{-6} mol dm^{-3}) in the presence and absence of C6SBu (10^{-4} mol dm^{-3}): (0) 0; (1) 0.339; (2) 0.458; (3) 0.568; (4) 0.687; (5) 0.911; (6) 1.13; (7) 1.38; (8) 2.26; (9) 2.73; (a) 3.64, in aqueous citrate buffer solution (pH 6.00) at 25.0 °C.
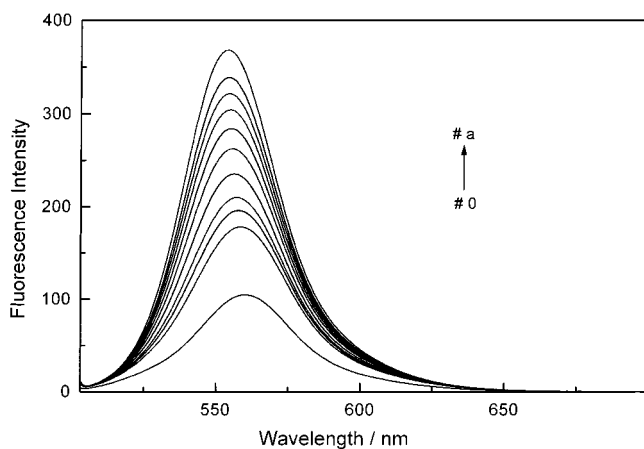

Figure 2. Fluorescence spectra of acridine red dye (6.77×10^{-6} mol dm^{-3}) in the presence and absence of β-cyclodextrin (10^{-4} mol dm^{-3}): (0) 0; (1) 0.427; (2) 0.640; (3) 0.853; (4) 1.280; (5) 1.706; (6) 2.133; (7) 2.560; (8) 2.986; (9) 3.413; (a) 4.266, in aqueous citrate buffer solution (pH 6.00) at 25.0 °C.

of calix[n]arenesulfonates, and the order of the fluorescence intensity changes is consistent with the size of the calixarene ring, i.e., calix[8]arenesulfonate > calix[6]-arenesulfonate > calix[4]arenesulfonate. Although sulfonates could yield quenching effect, it was found that, in the control titration experiment of acridine red with 4-phenolsulfonate, i.e., the monomeric unit of calix[n]-arenesulfonates (equiv: 0−1270), the quenching effect is limited and/or negligible, as compared with the changes in fluorescence intensity upon the addition of calix[n]-arenesulfonates. Furthermore, the emission peak at 561

nm does not shift at all. Therefore, the decreases in fluorescence intensity of acridine red upon the addition of calixarenesulfonates were mainly attributed to the inclusion complexation, not just to the simple quenching effect of sulfonate groups. The electrostatic interaction between the the positively charged acridine red molecule and the negatively charged substituent groups in the flexible calixarene ring, as well as the hydrogen bonding, restricted the internal rotation, which should lead to fluorescence enhancement. However, the polarity or hydrophilicity around the dye molecule that resulted from the hydrogen bonding and electrostatic interaction afforded a much larger quenching effect. From the observed results, the cavity of the water-soluble calixarenes was found to display little hydrophobicity. This is confirmed by the slight and slow bathochromic shift (3 nm: 560 → 563 nm) for the calix[6]arenesulfonate system. From the bathochromic shift and the decrease in fluorescence intensity, it could be drawn that the acridine red dye molecule formed a host−guest inclusion complex with calixarene[n]sulfonates, although we do not have any direct evidence for the inclusion by calixarene hosts and we could not rule out the possibility of the formation of an "external-type" complex.[10]

It is clearly seen that the fluorescence intensity of acridine red dye was enhanced upon the addition of butylated calix[6]arenesulfonate, and the emission peak showed a larger and faster bathochromic shift (Figure 3), as compared with calix[6]arenesulfonate. The larger

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(10) Thank a reviewer for his/her suggestion, the data could also be rationalized by the formation of "external-type" complexes.

and faster shift may be attributed to the stronger binding of acridine red with C6SBu. It is interesting that the changes in fluorescence intensity of acridine red upon complexation of C6SBu are different from those of the parent C6S. It is well-known that the fluorescence intensity (F) is proportional to the concentration of the fluorophore (c) in dilute solution (eq 1),[11] in which the coefficient is called as molar fluorescence intensity (ϵ').[12] The change in ϵ' value results from changes in fluorescence quantum yield (ϕ_F) and the molecular extinction (absorption) coefficient (ϵ) under certain experimental conditions in which the intensity of incident light (I_0) and the thickness of the sample (l) are kept constant (eq 2).[11]

$$F = \epsilon' c \quad (1)$$

$$\epsilon' = (\ln 10)\phi_F I_0 \epsilon l \quad (2)$$

The alkylation in the lower rim enlarges the hydrophobic cavity of the calixarene ring, which would result in a great increase in ϵ values, and thus fluorescence enhancement. Although the enhancement was canceled in part due to the decrease in fluorescence quantum yield as in the case of C6S, the C6SBu system showed increases in the fluorescence intensity.

On the other hand, the fluorescence intensity of acridine red dye increased evidently, and the emission peak gradually shifted from 560 to 553 nm (β-cyclodextrin) upon the complexation with the cyclodextrins (Figure 2). However, the order of the fluorescence intensity changes is not the same as that of calixarenesulfonates, i.e., β-cyclodextrin > γ-cyclodextrin > α-cyclodextrin. Apparently, the acridine red dye molecules insert into the hydrophobic cavity of cyclodextrins, and the resulting hydrophobic interaction led to the hypsochromic shift and the fluorescence enhancement, due to the increases in both ϕ_F and ϵ values.

The 1:1 stoichiometry was determined by continuous molar variation (Job) plots of the fluorescence intensity changes (the concentration) of the complexes C6S-AR and β-CD-AR vs mole fraction of acridine red (f_{guest}) under conditions where ([host] + [AR]) was kept constant; the maximum occurs at $f_{guest} = 0.5$.

The 1:1 complexation of the acridine red dye molecule (AR) with the host compounds (cyclodextrins or calixarenesulfonates) is expressed by eq 3, and the complex stability constant (K_s) is given by eq 4.

$$H + AR \overset{K_s}{\rightleftharpoons} H \cdot AR \quad (3)$$

$$K_s = \frac{[H \cdot AR]}{[H][AR]} \quad (4)$$

$$\Delta F = \Delta \epsilon' [H \cdot AR] \quad (5)$$

where ΔF and $\Delta \epsilon'$ denote the changes in the fluorescence intensity and molar fluorescence intensity of the acridine red dye molecule upon complexation with cyclodextrins or calixarenesulfonates.

Using the nonlinear curve-fitting approach,[13] the complex stability constant could be calculated according to

(11) Rendell, D. *Fluorescence and Phosphorescence*; John Wiley & Sons: London, 1987; pp 91–93.
(12) Wang, Y.; Ikeda, T.; Ikeda, H.; Ueno, A.; Toda, F. *Bull. Chem. Soc. Jpn.* **1994**, *67*, 1598–1607.
(13) Liu, Y.; Han, B.-H.; Sun, S.-X.; Wada, T.; Inoue, Y. *J. Org. Chem.* **1999**, *64*, 1487–1493.

Table 1. Stability Constants (log K_s) and Thermodynamic Parameters (in kJ mol^{-1}) for the Complexation of Acridine Red Dye Molecule with Water-Soluble Calix[n]arene (n = 4, 6, and 8) and α-, β-, and γ-Cyclodextrins in Aqueous Citrate Buffer Solution (0.10 mol dm^{-3}, pH = 6.00) at 25.0 °C

host	K_s	log K_s	$-\Delta G$	$-\Delta H$	$T\Delta S$
C4S	1660	3.22	18.4		
C6S	9550	3.98	22.7	34.7	−11.8
C6SBu	22600	4.35	25.5	24.4	1.1
C8S	146000	5.16	29.5		
α-CD	49.7	1.70	9.68		
β-CD	1380	3.14	17.9	0	17.8$_5$
γ-CD	117	2.07	11.8		

*Values are the averages of two or more independent runs; errors are less than 5% of the values reported in the table.

eq 6. Good curve fits further verify the 1:1 complex stoichiometry for each acridine red−calix[n]arenesulfonate and acridine red−cyclodextrin system. The results are summarized in Table 1.

$$\Delta F = \frac{1}{2}\left\{ \Delta\epsilon'\left([H]_0 + [AR]_0 + \frac{1}{K_s}\right) \pm \sqrt{\Delta\epsilon'^2\left([H]_0 + [AR]_0 + \frac{1}{K_s}\right)^2 - 4\Delta\epsilon'^2[H]_0[AR]_0} \right\} \quad (6)$$

The complexation thermodynamic parameters (ΔH and ΔS) were determined by the slope and ordinate-intercept of $\ln K_s \sim 1/T$ plots applying eq 7. The results obtained are also listed in Table 1.

$$\ln K_s = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (7)$$

Inspection of Table 1 indicates that the cyclodextrin series show cavity selectivity for β-cyclodextrin over its analogues upon complexation with acridine red, giving the stability constant order of β-cyclodextrin > γ-cyclodextrin > α-cyclodextrin. The relatively rigid cavity of β-cyclodextrin ($\sim$7.8 Å) is best size-fitted to the acridine red dye molecule, while the cavity of α-cyclodextrin ($\sim$5.7 Å) is small as compared to the dye molecule. However, the complex stability constant monotonically increases with the number of phenolic units in the calixarene ring, which is attributed mainly to the electrostatic interaction, rather than to the cavity size. Therefore, the electrostatic interaction plays a determining role in the complex stability. Furthermore, alkylation in the lower rim of calix[6]arenesulfonate (C6SBu) enhances the complex stability, which is 2 times that of calix[6]arenesulfonate (C6S).

The complexation thermodynamic parameters in Table 1 indicate that the complexation of acridine red molecule with calix[6]arenesulfonate is mainly driven by the favorable enthalpic change with a small entropic loss, which is attributed to the intermolecular hosts−guest interactions, such as electrostatic interaction of guest's cationic moiety with sulfonates and hydrogen bonding of the guest's CH$_3$NH moiety with the phenolic hydroxy groups. This is consistent with Barra's results.[6e] Meanwhile, the complexation behavior of C6SBu is also driven by the enthalpic change, but with little entropic gain. Although C6SBu could not form effective hydrogen bonding with the lower rim of alkyloxy groups, hydrophobic interaction and van der Waals forces, as well as the electrostatic interactions with sulfonate groups, would

contribute to the favorable enthalpy change, which is less than that of C6S, because the hydrophobic interaction and van der Waals forces are weaker than hydrogen bonding. The entropy change originates from the entropic gain from the rearrangement of water molecules originally surrounding the host and guest molecules, and the entropic loss from the decrease in the motion freedom upon the complexation. In the case of C6SBu, the entropic gain term should be larger than that of C6S due to the guest molecules included into the enhanced hydrophobic cavity (lower rim), and it would be expected to balance the entropic loss due to the restricted mobility of the guest molecules to a much greater extent than in the case of C6S, resulting in the slight overall entropic gain. However, the complexation of the acridine red dye molecule with β-cyclodextrin is wholly driven by the entropic increase. This is the typical case driven by the classical hydrophobic interaction in the inclusion complexation.[14] The hydrophobic interaction seems to be the main binding contribution in the cyclodextrin−acridine red complexation system. A reasonable explanation is the release of high energy water molecules from the cavity upon complexation and the desolvation of acridine red dye molecules.

Experimental Section

Calix[*n*]arenesulfonates (*n* = 4, 6, and 8) and a butylated derivative (C6SBu) were synthesized according to the literature procedures from the calix[*n*]arenes.[15,9a,6b] α-, β-, and γ-Cyclodextrins were commercially available from Tokyo Kasei, which were dried under reduced pressure before use. 4-Phenolsulfonic acid, sodium salt dihydrate, was purchased from Acros Organics. Acridine red was purchased from Chroma-Gesellschaft Schmid & Co. Citrate buffer solution (0.10 mol dm^{-3}) at pH 6.00 was used throughout the measurements.

Fluorescence spectra were measured using a JASCO spectrofluorometer model FP-715 using a conventional 1×1 cm quartz cell in a thermostated compartment, which was kept at constant temperature in the water bath. The excitation and emission bandwidths were set at 5 nm. The sample solutions containing acridine red dye at a concentration of approximately 5×10^{-6} mol dm^{-3} were excited at 493 nm to afford a strong emission at ca. 560 nm, and the fluorescence intensity at a wavelength near the emission maximum was used to determine the complex stability constants. The spectrofluorometric titrations were performed at 20.0, 25.0, 30.0, 35.0, 40.0, 45.0, and 50.0 °C to give the complexation thermodynamic parameters.

Supporting Information Available: Curve-fitting plots of ΔF versus [H]$_0$ and van't Hoff plots of log K_s versus $1/T$ for the complexation of acridine red dye with C6S and β-CD. The material is available free of charge via the Internet at http://pubs.acs.org.

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(14) (a) Connors, K. A. *Chem. Rev.* **1997**, *97*, 1325−1357. (b) Rekharsky, M. V.; Inoue, Y. *Chem. Rev.* **1998**, *98*, 1875−1917. (c) Harrison, J. C.; Eftink, M. R. *Biopolymers* **1982**, *21*, 1153−1166.

(15) Atwood, J. L.; Orr, G. W.; Means, N. C.; Hamada, F.; Zhang, H.; Bott, S. G.; Robinson, K. D. *Inorg. Chem.* **1992**, *31*, 603−606.