Chromomycin A_3 as a Blueprint for Designed Metal Complexes

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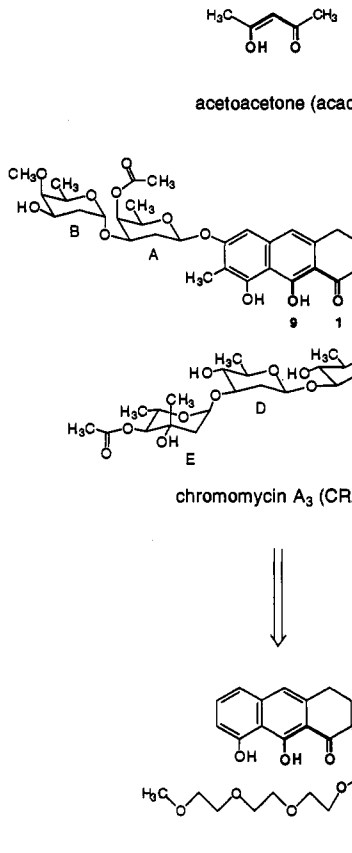
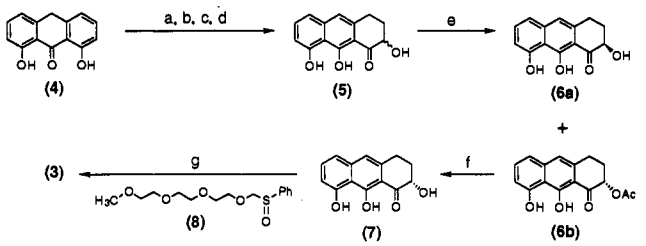
In 1989, Patel and co-workers showed that the antitumor antibiotic chromomycin A_3 (CRA_3, **2**) binds to DNA as an octahedral 2:1 drug–Mg^{2+} complex.[1] In the complex the drug acts as an acetylacetonate (acac) ligand (Scheme 1, **1**), coordinating Mg^{2+} through an ionized phenolate (O_9) and the neighboring ketone oxygen (O_1). Interestingly, the closest analogies to CRA_3 among simple acac ligands prefer to form 1:1 octahedral complexes with Mg^{2+}, apparently because their 2:1 octahedral complexes are extremely hindered (2:1 = $Mg(acac)_2(solvent)_2$; 1:1 = $[Mg(acac)(solvent)_4]^+$).[2] This fact led us to wonder whether DNA binding stabilizes the 2:1 CRA_3–Mg^{2+} complex[3] or whether there is something unusual about CRA_3 that overrides the effects of steric hindrance. Accordingly, we studied the Mg^{2+} complexes formed by CRA_3 and its aglycone in methanol in the absence of DNA. We found that the chromomycin aglycone forms a 1:1 complex with Mg^{2+} as expected for a hindered acac ligand; however, the more hindered parent drug **2** forms a 2:1 complex.[4] Further work indicated that the CDE trisaccharide side chain of CRA_3 stabilizes the 2:1 complex in some way.[5] We have now designed a minimalist acac ligand based on CRA_3 in which the algycone has been radically simplified and the CDE trisaccharide has been replaced by a triethylene glycol chain.[6] This TEG-linked ligand forms a stable 2:1 complex with Mg^{2+} even though the parent compound **7** forms a 1:1 complex. The ability of **3** to form a 2:1 complex like CRA_3 thus provides insight into how the CDE trisaccharide in CRA_3 stabilizes the 2:1 CRA_3–Mg^{2+} complex.

The TEG-linked ligand **3** and the parent compound **7** were synthesized as shown in Scheme 2. **5** was synthesized in four steps from the commercially available anthralin **4**. The racemic tricyclic α-hydroxy ketone **5** was resolved by enzymatic acylation with *Pseudomonas fluorescens* lipase in vinyl acetate (>95% ee).[7,8] This is the first example of an enzymatic resolution of an α-hydroxy ketone.[9] Subsequent enzymatic hydrolysis of the (2*S*)-α-acetoxy ketone **6b** in aqueous buffer provided the desired (2*S*)-α-hydroxy ketone **7** (>95% ee).

Scheme 1. Minimalist Approach to the Design of a Ligand



Scheme 2. Synthesis of the TEG–Chromophore Conjugate **3**[a]



[a] Conditions: (a) H_2, 50 psi, Raney Ni, 4% aqueous NaOH, 83%; (b) TMSOTf (5 equiv), NEt_3 (10 equiv), CH_2Cl_2, 25 °C; (c) mCPBA (1.2 equiv), anhydrous $NaHCO_3$, CH_2Cl_2, −78 °C to −20 °C; (d) MeOH, reflux (50% in three steps); (e) PFL, vinyl acetate, 25 °C, 5 h; (f) PFL, 0.1 M phosphate buffer, pH 7.0, 25 °C, 5 h; (g) **7** (1 equiv), **8** (3 equiv), Tf_2O (1.5 equiv), CH_2Cl_2, −78 °C, 5 min, 30%.

(1) In the 2:1 complex, the other two ligands for Mg^{2+} are water molecules. (a) Gao, X.; Patel, D. J. *Biochemistry* **1989**, *28*, 751. (b) Gao, X.; Patel, D. J. *Biochemistry* **1990**, *29*, 10940. (c) Gao, X.; Mirau, P.; Patel, D. J. *J. Mol. Biol.* **1992**, *223*, 259.

(2) (a) Cotton, F. A.; Wilkinson, G. *Advanced Inorganic Chemistry*; John Wiley & Sons: New York, 1988. (b) Graddon, D. P. *Coord. Chem. Rev.* **1969**, *4*, 1–28 and references therein. (c) Poonia, N. S.; Bajaj, A. V. *Chem. Rev.* **1979**, *79*, 389.

(3) Others have suggested that the 2:1 complex forms only upon binding to DNA: Stankus, A.; Goodisman, J.; Dabrowiak, J. *Biochemistry* **1992**, *31*, 9310.

(4) Silva, D. J.; Goodnow, R.; Kahne, D. *Biochemistry* **1993**, *32*, 463. Earlier studies on CRA_3 were also carried out in methanol because CRA_3 aggregates in water even at micromolar concentration. Although the energetics of complex formation are undoubtedly affected by solvent, there is plenty of evidence that the results in methanol are relevant for understanding structure–function relationships in CRA_3.

(5) Silva, D. J.; Kahne, D. *J. Am. Chem. Soc.* **1993**, *115*, 7962.

(6) For other work on designed metal complexes and their various uses, see *inter alia*: (a) Barton, J. K. *Science* **1986**, *233*, 727. (b) Sitlani, A.; Long, E. C.; Pyle, A. M.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 2303. (c) Sigman, D. S.; Chen, C-h. B. *Annu. Rev. Biochem.* **1990**, *59*, 207. (d) Cuenoud, B.; Schepartz, A. *Science* **1993**, *259*, 510. (e) Schwabacher, A. W.; Lee, J.; Lei, H. J. *J. Am. Chem. Soc.* **1992**, *114*, 7597. (f) Margalit, R.; Pecht, I.; Gray, H. B. *J. Am. Chem. Soc.* **1983**, *105*, 301. (g) Ghadiri, M. R.; Fernholz, A. K. *J. Am. Chem. Soc.* **1990**, *112*, 9633. (h) Piguet, C.; Bernardinelli, G.; Bocquet, B.; Quattropani, A.; Williams, A. F. *J. Am. Chem. Soc.* **1992**, *114*, 7440. (i) Bowler, B. E.; Ahmed, K. J.; Sundquist, W. I.; Hollis, L. S.; Whang, E. E.; Lippard, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 1299.

(7) Roberts, S. M.; Shoberu, K. A. *J. Chem. Soc., Perkin Trans. 1* **1989**, 1206. The enantiomeric excesses (ees) of the enzymatic reactions were determined by HPLC using a chiral AGP column (supplementary material).

(8) The absolute configurations of the reaction products were determined using exciton-coupling CD analysis of the enzymatically synthesized benzoate corresponding to the acetate. (a) Harada, N.; Nakanishi, K.; Tatsuoda, S. *J. Am. Chem. Soc.* **1969**, *91*, 5896. (b) Harada, N.; Nakanishi, K. *Circular Dichroic Spectroscopy—Exciton Coupling in Organic Stereochemistry*; University Science Books: Mill Valley, CA, 1983.

(9) There are several methods for asymmetric hydroxylation of ketones, but they give low conversion yields for hindered substrates such as **7**: (a) Hashiyama, T.; Morikawa, K.; Sharpless, K. B. *J. Org. Chem.* **1992**, *57*, 5067. (b) Davis, F. A.; Chen, B.-C. *Chem. Rev.* **1992**, *92*, 919. (c) Reddy, D. R.; Thornton, E. R. *J. Chem. Soc., Chem. Commun.* **1992**, 172. (d) Zhang, W.; Jacobsen, E. N. *J. Org. Chem.* **1991**, *56*, 2296.

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Initial attempts to attach the TEG side chain to the hydroxyl group of **7** using standard methods[10] proved unsuccessful because the molecule racemizes rapidly in base. However, we have shown that anomeric sulfoxides activated with triflic anhydride glycosylate unreactive alcohols under mild conditions.[11] We therefore concluded that it might be possible to alkylate the sensitive alcohol in **7** using an activated α-alkoxy sulfoxide. In fact, the desired product **3** was isolated in 30% yield from the reaction of activated TEG sulfoxide **8** and the α-hydroxy ketone **7**.[12,13] Chiral HPLC analysis indicated that there was no detectable epimerization of the sensitive asymmetric center.

We examined the behavior of **7** and the corresponding TEG–chromophore conjugate **3** in the presence of Mg^{2+} in methanol using UV–vis spectroscopy. Addition of Mg^{2+} to both **3** and **7** produces a hyperchromic effect around 400 nm, indicating complex formation. A Job plot derived for the chromophore **7** shows a plateau from x_{ligand} (mole fraction of drug) = 0.70 to 0.50 (Figure 1), indicating that **7** forms a mixture of 2:1 and 1:1 complexes as Mg^{2+} is added.[14] Conversion to the 1:1 complex is complete upon the addition of 1 mol equiv of Mg^{2+}. This behavior is also seen for the chromomycin aglycone and is typical for a congested acac ligand. In contrast to that of **7**, the Job plot generated for the TEG–chromophore conjugate **3** shows a maximum at $x_{ligand} = 0.68 \pm 0.02$ (Figure 1), indicating the formation of a stable 2:1 ligand–metal complex ($K_f = (1.1 \pm 0.2) \times 10^9$ M^{-2}).[15] We have evidence that this 2:1 complex is octahedral.[16] Therefore, like CRA_3, **3** forms a very stable, octahedral 2:1 ligand–Mg^{2+} complex in methanol even though it is *more* sterically hindered near the metal center than the corresponding chromophore **7**.

Further information about the structure of the 2:1 **3**–Mg^{2+} complex was obtained from ^1H NMR spectroscopy. The most interesting features of the ^1H NMR spectrum are several upfield chemical shift changes in the TEG side chain upon complex formation.[17] Unusual upfield shifts are also observed in several protons in the CDE trisaccharide in the 2:1 CRA_3–Mg^{2+} complex.[4,5] We have previously proposed that an interaction between the CDE trisaccharide in one CRA_3 molecule and the aromatic chromophore in the other CRA_3 molecule stabilizes the 2:1 CRA_3–Mg^{2+} complex.[5] We now find that a simple TEG moiety can mimic that interaction and stabilize the 2:1 complex of a hindered acac ligand that resembles the chromomycin aglycone.

The TEG side chain and the CDE trisaccharide have little in common except for the presence of alternating polar oxygens and

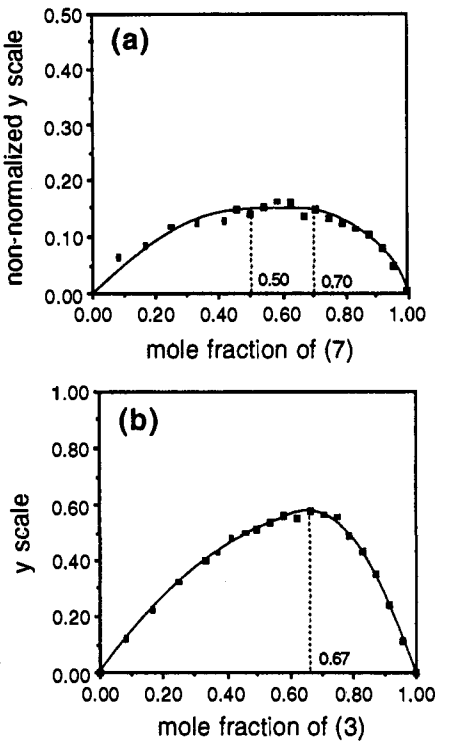

Figure 1. (a) Job titration for the **7**–Mg^{2+} system in methanol (25 °C, 440 nm). (b) Job titration for the **3**–Mg^{2+} system in methanol (25 °C, 440 nm).

nonpolar hydrocarbon units. A reasonable hypothesis to explain the 2:1 stoichiometry of the hindered acac complexes formed by **3** and CRA_3 is that lipophilic portions of the TEG side chain (in **3**) and the CDE trisaccharide (in CRA_3) interact with the lipophilic chromophore while some of the polar functionality is solvent-exposed. These non-specific intermolecular interactions must override the nonbonded steric interactions that favor the 1:1 complex. It is potentially very useful that the structures of certain types of acac metal complexes can be controlled by simple modifications.

Studies of the chromomycin–Mg^{2+} complex have revealed that it is possible to form very stable octahedral 2:1 metal complexes from hindered acac ligands. We have abstracted from chromomycin the minimal structural elements required for dimer formation. In so doing, we have provided insight into one of the roles that the sugars play in the natural system. We have also provided a starting point for the design of a new family of metal complexes with the potential to bind to DNA.[18]

Acknowledgment. This work was supported by the National Institutes of Health.

Supplementary Material Available: Experimental details for the preparation of **6a**, **6b**, and **7**, and HPLC traces to establish the enantioselectivity of the resolution; CD data showing how the absolute configurations were assigned and UV data on the 2:1 **3**:Co^{2+} complex used to determine the coordination geometry (12 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(10) *Poly(ethylene glycol) Chemistry: Biotechnical and Biomedical Applications*; Harris, J. M., Ed.; Plenum Press: New York, 1992.

(11) (a) Kahne, D.; Walker, S.; Cheng, Y.; Van Engen, D. *J. Am. Chem. Soc.* **1989**, *111*, 6881. (b) Raghavan, S.; Kahne, D. *J. Am. Chem. Soc.* **1993**, *115*, 1580.

(12) **8** was prepared by mCPBA oxidation of the sulfide produced by treating triethylene glycol monomethyl ether (1.7 equiv) with chloromethyl phenyl sulfide (1 equiv) and sodium hydride (1.7 equiv) in DMF at 25 °C.

(13) The reaction also yielded 25% of Pummerer rearrangement products.

(14) (a) Angelici, R. *Synthesis and Technique in Inorganic Chemistry*, 2nd ed.; W. B. Saunders, Co.: Philadelphia, PA, 1977. (b) Cantor, C.; Schimmel, P. *Biophysical Chemistry*; Freeman: New York, 1980; Part III.

(15) In the Job titration the total concentration of **3** plus metal was kept constant at 125 μM. The formation constant of the 2:1 complexes was calculated using the continuous variations method.[4] The formation contant of the 2:1 CRA_3–Mg^{2+} complex in methanol is $(5.9 \pm 2.9) \times 10^9$ M^{-2}.[4,5]

(16) Co^{2+} is a good model for Mg^{2+}.[2,5] UV–vis binding assays show that a stable 2:1 **3**–Co^{2+} complex is formed in the presence of 1 mol equiv of Co^{2+}. The UV–vis spectrum of the 2:1 **3**–Co^{2+} complex (2.5 mM) shows a broad shoulder around 550 nm, characteristic of an octahedral or slightly distorted octahedral complex in solution.

(17) Silva, D. J.; Kahne, D., unpublished results.

(18) Preliminary studies show that **3** interacts with DNA. We are pursuing studies to establish the mode and stoichiometry of binding of **3** and related compounds.