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Use of Triethylene Glycol to Mimic Oligosaccharides: Design and Synthesis of a Ligand Based on Chromomycin A_3

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Abstract—Chromomycin A_3 (CRA_3) is an antitumor antibiotic that binds to DNA. It contains an acac-like metal binding site and forms a 2:1 complex with Mg^{2+}. Interestingly, acac ligands similar to CRA_3 form 1:1 complexes with Mg^{2+}. We have previously shown that the unusual stability of the 2:1 CRA_3–Mg^{2+} complex is related to a favorable intermolecular interaction between the CDE trisaccharide of one CRA_3 molecule and the chromophore of the other. We have used this knowledge to design and synthesize a very simple molecule in which a triethylene glycol chain mimics the CDE trisaccharide of CRA_3. This minimalist ligand behaves like CRA_3 with respect to dimer formation. This result sheds light on how the CRA_3 sugars function to stabilize the dimer. At the same time, the work provides a starting point for investigating the relationship between dimer formation and DNA binding. Starting from these relatively simple metal complexes, it should be possible to develop a better understanding of the structural requirements for DNA binding by CRA_3 and related molecules.

Introduction

Many antitumor antibiotics that bind to DNA contain sugars as components of their structures.[1] In several instances, these sugars have been shown to be important for DNA binding. Nevertheless, in most cases little is actually known about how the sugars function in the binding process.[2] A better understanding of the roles of the carbohydrates in natural DNA binding glycoconjugates could lead to the ability to design synthetic DNA binders.

Chromomycin A_3 (CRA_3, **1**, Fig. 1) is a glycosylated antitumor antibiotic of the aureolic group.[1g] It was first isolated from fermentation broths of *Streptomyces griseus* in 1960.[3] Studies carried out over many years showed that CRA_3 binds tightly to GC-rich regions of DNA[4] in the presence of Mg^{2+},[5] inhibiting the action of DNA and RNA polymerases.[6] It was also shown that sequential removal of the sugars from CRA_3 causes a progressive decrease in DNA binding.[7] The aglycone of CRA_3, chromomycinone (CRN), does not bind to DNA at all.[8] These studies established that the chromomycin sugars are critical for DNA binding but did not provide insight into how the sugars are involved in the binding process. In 1989 Gao and Patel reported an NMR study of a CRA_3–Mg^{2+}–DNA complex showing that CRA_3 binds in the minor groove of DNA as a dimeric complex held together by Mg^{2+}.[9] In the complex, the drug acts as a bidentate ligand, coordinating the metal through an ionized phenolate (O_9) and the β-ketone oxygen (O_1). A crystal structure of a CRA_3–Mg^{2+}–DNA complex shows that the Mg^{2+} ion occupies an octahedral site, coordinated to two CRA_3 molecules and two water molecules.[10] The structural work established a role for the Mg^{2+} ion in DNA binding. However, only a few contacts between the CRA_3 sugars and the DNA duplex were observed in the complex, raising questions about how the chromomycin sugars contribute to DNA binding.

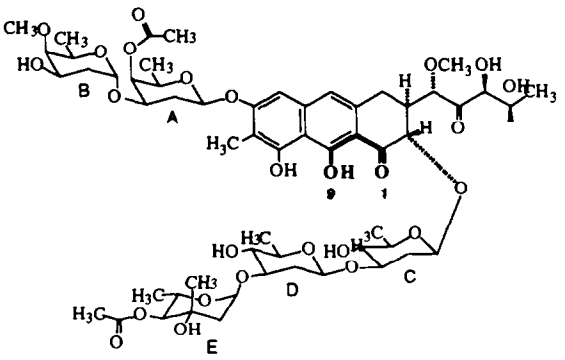


Figure 1. Chromomycin A_3 (CRA_3, **1**).

The structural work also raised other questions about the relationship between structure and function in the CRA_3 molecule. The data showed that the drug behaves like an acetoacetone (acac) ligand when it coordinates Mg^{2+}. Acac ligands[11] are known to form either 2:1 or 1:1 octahedral complexes with Mg^{2+}, i.e. $Mg(acac)_2(solvent)_2$ or $[Mg(acac)(solvent)_4]^+$. It is thought that the size of an acac ligand is the major factor determining the stoichiometry of the corresponding Mg^{2+} complex.[12] Hindered acac ligands generally form 1:1 complexes rather than 2:1 complexes. CRA_3 is a hindered acac ligand, and the closest analogies to it among simple acac ligands are known to prefer to form 1:1 complexes rather than 2:1 complexes. This fact led us to wonder whether the DNA acts as a template that stabilizes the 2:1 CRA_3–Mg^{2+} complex,[13] or whether there are some structural elements in the CRA_3 molecule itself that override the effects of steric hindrance and stabilize the hindered 2:1

complex relative to the 1:1 complex. We thought that the CRA_3 sugars might play a role in stabilizing the unusual 2:1 complex that forms with Mg^{2+}.

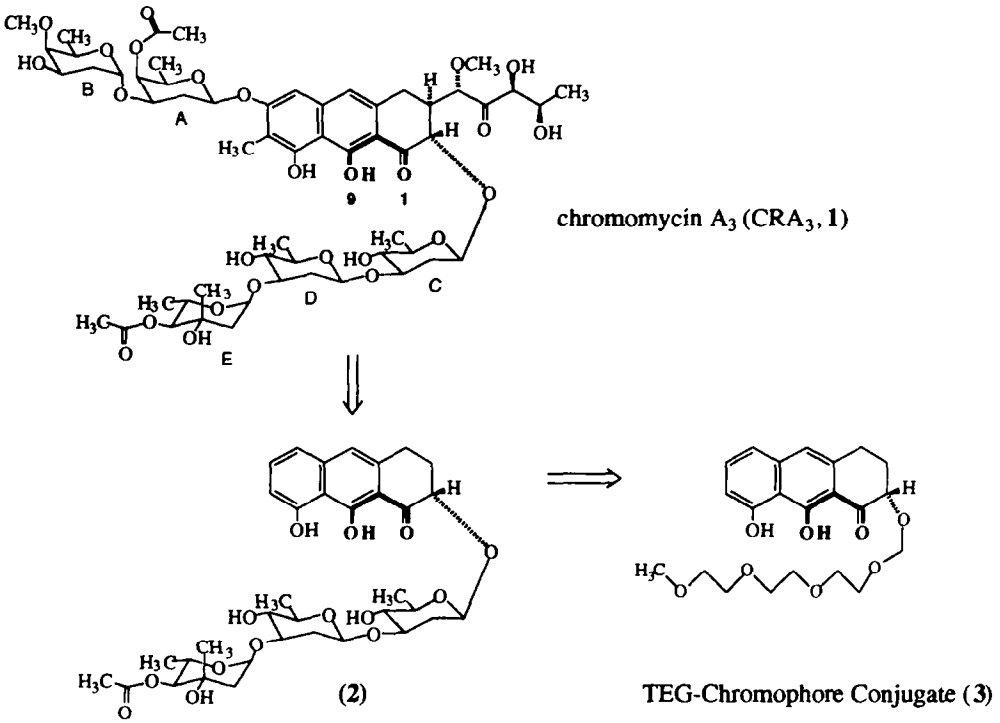
We recently reported studies showing that CRA_3 forms a stable 2:1 drug–Mg^{2+} complex in solution but the aglycone, CRN, does not.[14] NMR studies of the 2:1 CRA_3–Mg^{2+} complex in solution have revealed interactions between the CDE trisaccharide side chain of one CRA_3 molecule and the chromophore of the other CRA_3.[15] These interactions, which are also observed in the complex that binds to DNA, apparently override the effects of steric hindrance, stabilizing the 2:1 complex relative to the 1:1 complex. We have therefore concluded that one of the crucial functions of the CRA_3 sugars is to stabilize the 2:1 complex that binds to DNA.[16] This finding provided us with a starting point for designing synthetic model compounds based on CRA_3. We now report the design and synthesis of a very simple acac ligand which behaves like CRA_3 with respect to dimer formation.[17] This synthetic molecule provides further insight into how the CDE trisaccharide stabilizes the 2:1 complex in the natural system. At the same time, this work points the way towards the design of new metal complexes with the potential to bind to DNA.

Results and Discussion

Design of a ligand based on CRA_3

Our previous studies showed that the trisaccharide side chain of CRA_3 stabilizes the 2:1 complex with Mg^{2+}.[14] The stabilization apparently derives from intermolecular interactions between the trisaccharide of one CRA_3 molecule and the chromophore of the other CRA_3 molecule in the complex. There do not appear to be any intermolecular stabilizing interactions involving the hydrophilic side chain or the AB disaccharide.[15] We have concluded that the structural features responsible for dimer formation in CRA_3 are the tricyclic chromophore with the β-keto phenolate plus the CDE trisaccharide.[15] Based on this analysis, one might wonder whether **2** forms a stable 2:1 complex in solution (Scheme I).

Even though the biophysical studies on CRA_3 showed that the trisaccharide–chromophore interaction stabilizes the 2:1 complex in solution, they did not indicate the physical basis of this interaction. This information is crucial for the design of CRA_3 mimics which could be tested for dimer formation and DNA binding. Therefore, in order to obtain insight about the role of the trisaccharide in dimer stabilization, the associative behavior of the drug and its 2:1 complex in methanol was analyzed. NMR concentration studies indicated that uncomplexed CRA_3 undergoes widespread aggregation in methanol at concentrations above 3 mM.[15] Most of the resonances affected by aggregation are located around the chromophore, suggesting that this moiety plays an active role in self-association. The hydrophobic chromophore apparently prefers to be solvated by other CRA_3 molecules rather than to be exposed to the solvent molecules. However, in the case of the 2:1 CRA_3–Mg^{2+} complex, NMR concentration studies did not show significant aggregation in methanol at concentrations as high as 8 mM, suggesting that the chromophore is appropriately solvated in the dimer and has less tendency to aggregate. Since an intermolecular trisaccharide–



Scheme I. Simplification of CRA_3 to an acac ligand containing the features necessary for dimer formation.

chromophore interaction was detected in the 2:1 complex but not in the uncomplexed drug, these observations suggest that the trisaccharide 'solvates' the chromophore in the 2:1 complex, stabilizing it with respect to the 1:1 complex and other aggregation states.

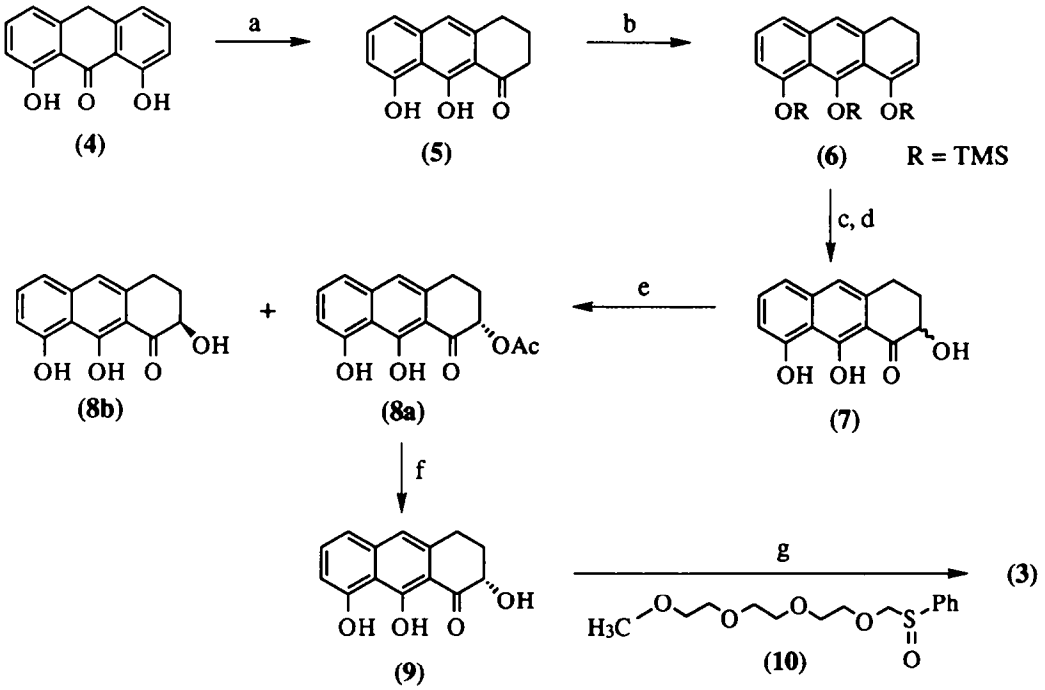
This model helps explain the role of the CDE trisaccharide in dimer formation but raises questions about the specificity of the intermolecular interaction. Why did Nature choose a trisaccharide to solvate the chromophore? Is the interaction between the chromophore and trisaccharide specific? If not, the CDE trisaccharide could be replaced by an appropriate solvating group without affecting dimer stability. The study of a model system where the CDE trisaccharide is replaced by another solvating group should thus shed light on the role of the sugars in CRA_3. One of the most commonly used solvating groups is a polyethylene glycol (PEG) chain.[18] Although the CDE trisaccharide and a PEG chain have little structural analogy,[19] they share an interesting property: amphiphilicity. The CDE trisaccharide is a naturally occurring amphiphile. Its α-face has only lipophilic groups (mainly C–H bonds), whereas its β-face has some hydrophilic groups. Similarly, PEGs are constituted of alternating lipophilic ethylene groups and hydrophilic ether groups.[20] It is thus possible that the PEG chain may reproduce the solvating properties of the CDE trisaccharide, stabilizing the 2:1 ligand–Mg^{2+} complex. CPK models indicate that a triethylene glycol (TEG) has a comparable length to the trisaccharide and should be able to span the whole length of the chromophore.

Based on these considerations, the molecule **3a** was chosen as a suitable minimalist ligand based on CRA_3 (Scheme I). Compound **3** has the same basic structural elements of **2**: a tricyclic chromophore, with an *acac* chelating site, and a 'solvating' side chain. Below we report details of the synthesis and studies showing that **3** behaves like CRA_3 in several key respects. To our knowledge this is the first time that a polyethylene glycol unit has been used to mimic the function of a carbohydrate in a glycoconjugate.

Synthesis of the TEG-chromophore conjugate

The synthesis of **3** is shown in Scheme II. The principal challenges in the synthesis were to obtain the appropriate enantiomerically pure α-hydroxy ketone from the precursor ketone **5** and to alkylate it with the TEG chain without causing racemization or decomposition. There are several procedures for the asymmetric α-hydroxylation of ketones.[21] For most ketone substrates, these methods afford high yields with good enantiomeric excesses (*e.e.*'s). However, cyclic ketones such as **5** are more hindered, leading to low conversion yields. Moreover, **5** is not soluble in the polar solvent systems required for some of these procedures.[21a] It was not possible to derivatize **5** using reported methods for asymmetric α-hydroxylation. Instead, we decided to use an enzymatic resolution to separate the enantiomers of **7**.[22]

The tricyclic ketone **5** was readily prepared from the commercially available anthralin **4** by hydrogenation over Raney Ni,[23] then converted to its trimethylsilyl enol ether **6** and oxidized to give the desired α-hydroxy ketone **7**. Resolution of racemic **7** was accomplished by treatment with *Pseudomonas fluorescens* lipase in vinyl acetate at room temperature.[22b] The enzymatic acylation yields a 50 % conversion to the α-acetoxy



Scheme II. Conditions: (a) H_2, 50 psi, Ni Raney, 4 % aq. 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 (95 % in 3 steps from **5**); (e) PFL, vinyl acetate, 25 °C, 5 h; (f) PFL, 0.1 M phosphate buffer, pH 7.0, 25 °C, 5 h; (g) **9** (1 equiv.), **10** (3 equiv.), Tf_2O (1.5 equiv.), CH_2Cl_2, −78 °C, 5 min, 30 %.

ketone in 3–5 h. Preparative TLC afforded the α-acetoxy ketone **8a** and the α-hydroxy ketone **8b** in high enantiomeric excess (greater than 95 %, as judged by chiral HPLC). To establish the absolute configurations of the products, **7** was treated with *P. fluorescens* lipase in vinyl benzoate to obtain the α-benzoyloxy ketone **8c**.[22c] Exciton coupling CD analysis showed that **8c** has the (*S*) configuration.[24] After confirming that the acetate **8a** and the benzoate **8c** have the same configuration, **8a** was assigned to be (*S*) and **8b** to be (*R*). Subsequent enzymatic hydrolysis of **8a** with *P. fluorescens* lipase in aqueous buffer yielded the desired (2*S*)-α-hydroxy ketone **9**. This is the first example of an enzymatic resolution of an α-hydroxy ketone. The *e.e.*'s for this enantiomeric resolution are excellent. Furthermore, we were able to obtain both enantiomers of the α-hydroxy ketone **8b** and **9** in gram quantities, making the synthetic route amenable to the construction of metal complexes with different configurations.

Attachment of the TEG chain to **9** was the next challenge. In general, PEG chains are attached by alkylation or acylation in basic media,[18] conditions that readily epimerize the α-hydroxy ketone. Furthermore, most of the PEG attachment protocols are intended for amines and are not applicable to the hindered secondary alcohol **9**. In order to complete the synthesis, we needed a milder method to attach the PEG side chain. In 1989 we reported a method for glycosylation that involves treating an anomeric sulfoxide with triflic anhydride to generate a highly reactive glycosyl donor.[25] This reaction is conducted at low temperatures (–78 °C) and under virtually neutral conditions. Since a glycosyl sulfoxide is an α-alkoxy sulfoxide, we thought that it might be possible to alkylate the alcohol in **9** by treating the α-alkoxy sulfoxide derived from triethylene glycol with triflic anhydride under conditions similar to those used for glycosylation reactions. Accordingly, the TEG sulfoxide **10**, synthesized in two steps from triethylene glycol monomethyl ether, was reacted with triflic anhydride in the presence of **9** at –78 °C in CH_2Cl_2. After 5 min the reaction was quenched and the desired product **3** was isolated in 30 % yield. (A 25 % yield of Pummerer products was also obtained.) Chiral HPLC analysis indicated no detectable epimerization of the sensitive asymmetric center. Therefore, activated sulfoxides appear to be good alkylating agents, without the problems normally encountered with alkyl halides (long reaction times, extremely basic conditions, product over-reaction and decomposition).

Binding studies with the TEG-chromophore conjugate

We compared the ability of the acac ligand **9** and the corresponding TEG–chromophore conjugate **3** to form complexes with Mg^{2+} in methanol, using UV-vis spectroscopy. UV-vis spectra of **9** and **3** were recorded at different concentrations of Mg^{2+}. Absorbances of the drugs were monitored at varying concentrations of metal and plotted to give the titration curves shown in Figure 2.

The titration of acac ligand **9** shows a single transition, complete upon the addition of 1 mole equivalent of metal (Fig. 2a). The Job plot for **9** (Fig. 3a) shows a plateau from x_{drug} = 0.50 to 0.70, consistent with the formation of 2:1 and 1:1 complexes upon addition of Mg^{2+}.[26] The 1:1 complex predominates once 1 mole equivalent of metal is added. This behavior is also observed for CRN and is characteristic of hindered acac ligands.[14]

The TEG–chromophore conjugate **3** displays similar behavior to CRA_3 upon titration with Mg^{2+}. There is a distinct transition to another species which is complete upon the addition of 0.50 mole equivalent of Mg^{2+} (Fig. 2b). The Job plot for **3** (Fig. 3b) shows a maximum at x_{ligand} = 0.68±0.02, indicating the formation of a stable 2:1 complex. From the normalized Job plot y_{max} was found to be (0.582±0.016), corresponding to a formation constant of (1.1±0.2) × 10^9 M^{-2}. We have previously shown that the formation constant for the 2:1 CRA_3–Mg^{2+} complex is (5.9±2.9) × 10^9 M^{-2}.[15] The 2:1 complex formed by **3** with Mg^{2+} is thus of comparable stability to the 2:1 complex formed by CRA_3 with Mg^{2+}. Despite differences in the structures of the two ligands, the TEG side chain of **3** stabilizes the 2:1 complex relative to the 1:1 complex just as the CDE trisaccharide does in CRA_3.

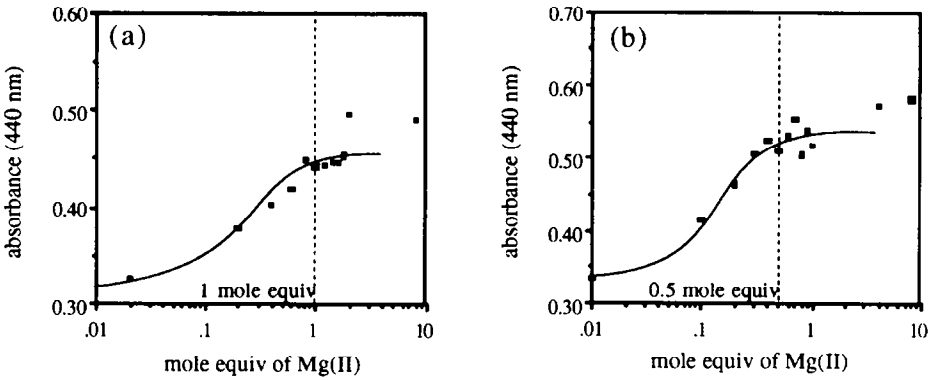


Figure 2. (a) Absorbance at 440 nm monitored for the Mg^{2+} titration of **9** (100 μM) in methanol (25 °C). (b) Absorbance at 440 nm monitored for the Mg^{+2} titration of **3** (100 μM) in methanol (25 °C).

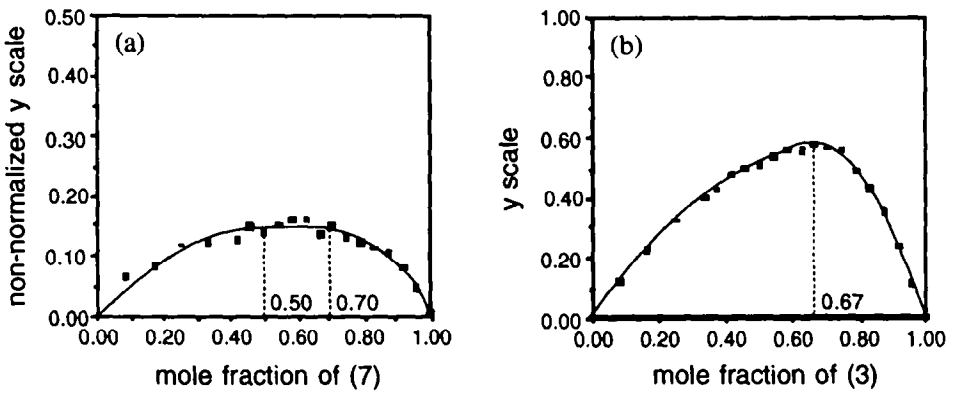


Figure 3. (a) Job titration for the 9-Mg^{2+} system in methanol (k = 125 μM, 25 °C, 440 nm). (b) Job titration for the 3-Mg^{2+} system in methanol (k = 125 μM, 25 °C, 440 nm).

UV-vis studies indicate that the 2:1 3–Mg^{2+} complex is octahedral like the 2:1 CRA_3–Mg^{2+} complex.[27] Further information about the structure of the 2:1 3–Mg^{2+} complex has been obtained from ^1H NMR spectroscopy.[28] There are upfield chemical shifts in the TEG chain upon complex formation. Upfield shifts are also seen in the CDE trisaccharide of CRA_3 upon complex formation. These shifts may be due to an interaction between the trisaccharide of one CRA_3 molecule and the chromophore of the other. This interaction is related to the stability of the 2:1 complex formed by CRA_3 with Mg^{2+}. The upfield shifts in the TEG chain in the complex formed by **3** with Mg^{2+} therefore suggest that the TEG chain of one molecule can interact with the chromophore of another to stabilize the 2:1 complex relative to the 1:1 complex in a manner analogous to that observed with CRA_3.

Conclusions

Biophysical studies on CRA_3 indicated that the drug forms a stable 2:1 complex with Mg^{2+} in solution. The dimeric complex is stabilized by an intermolecular trisaccharide–chromophore interaction. We suggested that the interaction consists primarily of solvation of the chromophore by the trisaccharide and is non-specific. We tested this idea by replacing the CDE trisaccharide with a simple TEG chain. Our binding studies on **3** indicate that it also forms a stable 2:1 complex with Mg^{2+}, while the parent ligand **9** does not. The PEG mimics the ability of the CDE trisaccharide to stabilize the 2:1 complex with Mg^{2+}, presumably via similar intermolecular solvation interactions. We have thus been able to isolate one of the roles that the sugars of CRA_3 play. We are now in a position to investigate the structural requirements for DNA binding. We believe it may be possible to design new metal complexes that are much simpler than CRA_3 but that bind to DNA. The studies reported highlight the importance of correlating structure and function in natural systems and identifying principles which can be tested with synthetic models.

Experimental Procedures

NMR spectra were recorded on a JEOL GSX 270 NMR spectrometer. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS) with reference to internal solvent. Coupling constants (J) are reported in hertz (Hz). Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q) and multiplet (m).

Mass spectra were obtained at the Princeton University Department of Chemistry Mass Spectrometry Facility from a Kratos MS 50 spectrometer. UV-vis spectra were recorded on a Varian Cary 1 UV-visible spectrophotometer. UV-vis binding assays were conducted according to procedures described elsewhere.[14,15]

Analytical thin-layer chromatography (TLC) was performed using Silica Gel 60 F254 precoated plates (0.25 mm thickness) with a fluorescent indicator. Flash chromatography was performed using Silica Gel 60 (230–400 mesh) from EM Science. Preparative thin-layer chromatography (PTLC) was performed using Silica Gel GF precoated plates (20 × 20 cm; 1,000 μm) from Analtech.

All reactions were carried out under an argon atmosphere with dry, freshly distilled solvents under anhydrous conditions unless otherwise noted. For moisture-sensitive reactions the glassware was thoroughly flame dried on a vacuum line, followed by cooling under argon, and the starting materials were azeotroped with toluene three times before reaction.

8,9-Dihydroxy-3,4-dihydro-1(2H)-anthracenone (5)

To a solution of anthralin **4** (Spectrum Chemical; 3.0 g, 13.3 mmol) in 50 mL of aqueous 4 % NaOH solution was added Ni Raney catalyst (4.0 g of 50 % w/w aqueous suspension). The system was agitated under 50 psi of hydrogen in a Parr hydrogenation apparatus for 5.5 h. The resulting suspension was filtered and the dark filtrate was discarded. The yellow solid was dissolved in

methanol. The methanolic solution was acidified to pH 7 with aqueous 4 N HCl, treated with propylene oxide (20 mL) and filtered through Celite. The solvent was removed under reduced pressure and the residue was recrystallized from ethyl acetate to afford 2.5 g (83 %) of **5**. R_f (TLC) = 0.30 (CH_2Cl_2). ^1H NMR (270 MHz, $CDCl_3$): 16.14 (s, 1H, OH-9), 9.75 (s, 1H, OH-8), 7.44 (t, J = 8.0, 8.0 Hz, 1H, H-6), 7.11 (d, J = 8.0 Hz, 1H), 6.96 (s, 1H, H-10), 6.80 (d, J = 8.0 Hz, 1H), 2.95 (t, J = 5.8, 5.8 Hz, 2H), 2.74 (t, J = 6.5, 6.5 Hz, 2H), 2.10 (m, J = 5.8, 6.5 Hz, 2H, H-3). ^{13}C NMR (67.9 MHz, $CDCl_3$): 204.88, 165.72, 157.96, 139.26, 137.73, 132.47, 117.97, 116.92, 112.51, 110.48, 110.26, 38.08, 29.56, 22.68. HRMS calcd for $C_{14}H_{12}O_3$ (M^+): 228.0787, found 228.0773.

1,8,9-Tris(trimethylsilyloxy)-3,4-dihydro-anthracene (6)

To a solution of ketone **5** (1.0 g, 4.39 mmol) in 200 mL of CH_2Cl_2 was added triethylamine (6.1 mL, 43.9 mmol). Under agitation trimethylsilyl trifluoromethylsulfonate (4.2 mL, 22.0 mmol) was added. After 5 min the reaction was quenched with saturated aqueous $NaHCO_3$. The organic layer was extracted with aqueous $NaHCO_3$ (2 × 100 mL), dried over anhydrous Na_2SO_4, filtered and concentrated under vacuum. The dark residue was dissolved in petroleum ether and filtered through Celite. The resulting solution was concentrated, yielding crude **6**, which was used immediately in the next step. R_f (TLC, alumina) = 0.60 (petroleum ether). ^1H NMR (270 MHz, C_6D_6/CCl_4): 7.12 (d, J = 8.0 Hz, 1H), 7.04 (t, J = 8.0, 8.0 Hz, 1H, H-6), 7.00 (s, 1H, H-10), 6.60 (d, J = 8.0 Hz, 1H), 5.20 (t, J = 4.0, 4.0 Hz, 1H, H-2), 2.60 (m, 2H, H-4), 2.10 (m, 2H, H-3), 0.18 (s, 9H), 0.15 (s, 9H), 0.12 (s, 9H). ^{13}C NMR (67.9 MHz, C_6D_6/CCl_4): 152.56, 150.56, 148.56, 138.04, 136.77, 126.36, 123.49, 122.69, 121.31, 119.59, 116.09, 109.26, 31.13, 23.13, 0.81, 0.77, 0.55. HRMS calcd for $C_{23}H_{36}Si_3O_3$ (M^+): 444.1973, found 444.1955.

2,8,9-Trihydroxy-3,4-dihydro-1(2H)-anthracenone (7)

To a solution of **6** (assumed 4.39 mmol) in 200 mL of CH_2Cl_2 was added solid anhydrous $NaHCO_3$ (0.1 g). The suspension was cooled to –78 °C and mCPBA (64 % purity, 1.4 g, 5.21 mmol) was added. The system was allowed to warm slowly to 0 °C over 1 h and quenched with saturated $NaHCO_3$. The organic layer was extracted with saturated $NaHCO_3$ (2 × 50 mL), dried over Na_2SO_4 and concentrated under vacuum. The dark residue was dissolved in petroleum ether and filtered through Celite. The resulting solution was concentrated and the residue (crude epoxide) was dissolved in 20 mL of CH_2Cl_2. MeOH (60 mL) and citric acid (100 mg) were added. The system was heated to reflux for 0.5 h and cooled to 25 °C. The reaction mixture was diluted with water. The organic layer was washed with saturated $NaHCO_3$ (2 × 100 mL), dried over anhydrous Na_2SO_4 and concentrated under vacuum. The residue was dissolved in CH_2Cl_2/petroleum ether and filtered through Celite. The resulting solution was concentrated. Recrystallization from ethyl acetate yielded **7** (1.01 g, 95 % in 3 steps from **5**). R_f (TLC) = 0.30 (50 % ethyl acetate/petroleum ether). ^1H NMR (270 MHz, $CDCl_3$): 14.80 (s, 1H, OH-9), 9.43 (s, 1H, OH-8), 7.47 (t, J = 7.9, 7.9 Hz, 1H, H-6), 7.12 (d, J = 8.2 Hz, 1H), 7.00 (s, 1H, H-10), 6.83 (d, J = 7.9 Hz, 1H), 4.44 (dd, J = 5.3, 12.5 Hz, 1H, H-2), 3.68 (s, 1H, OH-2), 3.04 (m, 2H, H-4), 2.49 (m, 1H, H-3), 2.04 (m, 1H, H-3). ^{13}C NMR (67.9 MHz, $CDCl_3$): 204.15, 164.46, 157.69, 139.60, 137.17, 132.94, 118.27, 117.94, 112.31, 110.99, 108.56, 72.99, 30.94, 27.31. HRMS calcd for $C_{14}H_{12}O_4$ (M^+): 244.0736, found 244.0733.

Phenyl 1-thio-2,5,8,11-tetraoxa-dodecane

Triethylene glycol monomethyl ether (261 mg, 1.59 mmol) was azeotroped three times with toluene and dissolved in DMF. NaH (38 mg, 1.59 mmol) was added under intense agitation. When the evolution of gas ceased, chloromethyl phenyl sulfide (213 µL, 1.59 mmol) was added. After 1 h the solvent was removed under vacuum and the residue was directly submitted to flash chromatography (50 % ethyl acetate/petroleum ether), yielding 50 mg (11 %) of phenyl 1-thio-2,5,8,11-tetraoxa-dodecane. R_f (TLC) = 0.60 (ethyl acetate). ^1H NMR (270 MHz, $CDCl_3$): 7.45 (m, 2H), 7.25 (m, 3H), 5.06 (s, 2H, OCH_2O), 3.80 (m, 2H), 3.65 (m, 8H), 3.55 (m, 2H), 3.38 (s, 3H, OCH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): 135.87, 130.06, 128.79, 126.55, 76.27, 71.85, 70.52, 70.49, 70.46, 70.17, 67.40, 58.95. HRMS calcd for $C_{14}H_{22}SO_4$ (M^+): 286.1240, found 286.1215.

Phenyl 1-sulfinyl-2,5,8,11-tetraoxa-dodecane (10)

Phenyl 1-thio-2,5,8,11-tetraoxa-dodecane (237 mg, 0.827 mmol) was dissolved in CH_2Cl_2 (50 mL) and cooled to –78 °C. mCPBA (64 % purity, 223 mg, 0.827 mmol) was added and the reaction mixture was slowly warmed to –20°C over a period of 2 h. The reaction was quenched with dimethyl sulfide (1 mL). The organic layer was extracted with aqueous $NaHCO_3$ (2 × 50 mL), dried over Na_2SO_4 and concentrated under vacuum. Flash chromatography (5 % methanol/ethyl acetate) afforded 232 mg (93 %) of **10**. R_f (TLC) = 0.13 (ethyl acetate). ^1H NMR (270 MHz, $CDCl_3$): 7.35 (m, 2H), 7.25 (m, 3H), 4.24 (s, 2H, OCH_2SO), 3.72 (m, 2H), 3.40 (m, 8H), 3.25 (m, 2H), 3.37 (s, 3H, OCH_3). ^{13}C NMR (67.9 MHz, $CDCl_3$): 140.48, 130.64, 128.63, 123.81, 92.28, 72.00, 71.28, 70.01, 69.93, 69.87, 69.77, 58.33. HRMS calcd for $C_{14}H_{23}SO_5$ (M+H): 303.1266, found 303.1259.

Enzymatic resolution of 7 with Pseudomonas fluorescens *lipase; preparation of 8a and 8b*

Compound **7** (250 mg, 1.025 mmol) and *Pseudomonas fluorescens* lipase (215 mg) were added to 20 mL of vinyl acetate at 25 °C. The progress of the reaction was followed by ^1H NMR spectroscopy. After 5 h (50 % conversion as judged by NMR) the suspension was filtered and the insoluble enzyme was recovered. The solution was concentrated under vacuum. PTLC

(HOAc:EtOAc: petroleum ether, 1:30:70 v/v/v) afforded 106 mg (85 %) of **8b** and 144.7 mg (99 %) of **8a**.

8a: R_f (TLC) = 0.70 (50 % ethyl acetate/petroleum ether). ^{1}H NMR (270 MHz, $CDCl_3$): 15.26 (s, 1H, OH-9), 9.55 (s, 1H, OH-8), 7.48 (t, J = 7.9, 7.9 Hz, 1H, H-6), 7.13 (d, J = 7.8 Hz, 1H), 7.01 (s, 1H, H-10), 6.84 (d, J = 7.8 Hz, 1H), 5.65 (dd, J = 5.6, 12.5 Hz, 1H, H-2), 3.11 (dd, J = 3.6, 7.9 Hz, 2H, H-4), 2.35 (m, 2H, H-3), 2.23 (s, 3H, CH_3CO). ^{13}C NMR (67.9 MHz, $CDCl_3$): 198.92, 170.07, 165.13, 157.67, 139.29, 136.06, 132.84, 118.09, 117.47, 112.19, 110.92, 109.24, 72.79, 28.65, 27.35, 20.72. HRMS calcd for $C_{16}H_{14}O_5$ (M^+): 286.0841, found 286.0847.

8b: identical R_f, ^{1}H NMR, ^{13}C NMR and HRMS to **7**.

2(S),8,9-Trihydroxy-3,4-dihydro-1(2H)-anthracenone (9)

Pseudomonas fluorescens lipase (80 mg) was dissolved in 50 mM phosphate buffer (pH 7, 50 mL) and added to a solution of **8a** (90 mg, 315 mmol) in CH_2Cl_2 (5 mL). After 5 h CH_2Cl_2 (50 mL) was added and the layers were separated. The aqueous layer was lyophilized, affording crude recovered enzyme. The organic layer was dried over anhydrous Na_2SO_4 and concentrated. PTLC (acetic acid: ethyl acetate:petroleum ether 1:30:69 v/v/v) afforded 72.3 mg (94 %) of **9** and 5.3 mg (6 %) of recovered **8a**.

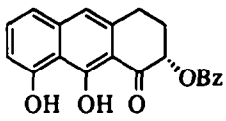
2-O-(3',6',9',12'-Pentaoxa-dodecacyl)-2,8,9-trihydroxy-3,4-dihydro-1(2H)-anthracenone (3)

Compounds **9** (67 mg, 0.275 mmol) and **10** (290 mg, 0.960 mmol) were azeotroped three times with toluene and dissolved in CH_2Cl_2 (50 mL). The system was cooled to –78 °C and trifluoromethylsulfonic anhydride (80 μL, 0.480 mmol) was slowly added. After 5 min the reaction was quenched with saturated aqueous $NaHCO_3$. The organic layer was extracted with aqueous $NaHCO_3$ (2 × 100 mL), dried over Na_2SO_4 and concentrated under vacuum. PTLC (1 % acetic acid/ethyl acetate) yielded 36 mg (30 %) of **3**. R_f (TLC) = 0.50 (1 % acetic acid/ethyl acetate). ^{1}H NMR (270 MHz, $CDCl_3$): 15.65 (s, 1H, OH-9), 9.62 (s, 1H, OH-8), 7.46 (t, J = 8.0, 8.0 Hz, 1H, H-6), 7.11 (d, J = 8.0 Hz, 1H), 6.98 (s, 1H, H-10), 6.82 (d, J = 8.0 Hz, 1H), 5.04 (d, J = 7.2 Hz, 1H, H-2'), 4.95 (d, J = 7.2 Hz, 1H, H-2'), 4.50 (dd, J = 4.4, 11.8 Hz, 1H, H-2), 3.82 (m, 2H), 3.65 (m, 2H), 3.51 (m, 2H), 3.34 (s, 3H, OCH_3), 3.11 (dt, J = 4.4, 4.4, 16.5 Hz, 1H, H-4), 3.00 (td, J = 3.8, 11.6, 16.5 Hz, 1H, H-4), 2.38 (m, 1H, H-3), 2.17 (qd, J = 4.2, 11.5, 11.6, 11.8 Hz, 1H, H-3). ^{13}C NMR (67.9 MHz, $CDCl_3$): 202.43, 165.76, 157.92, 139.44, 136.79, 132.84, 118.12, 117.31, 112.51, 110.85, 109.56, 95.04, 76.30, 75.65, 71.92, 70.61, 70.55, 70.42, 67.45, 59.02, 29.66, 27.10. HRMS calcd for $C_{22}H_{28}O_8$ (M^+): 419.1706, found 419.1728.

Enzymatic benzoylation of 7

Compound **7** was added to a suspension of PFL immobilized on HyFlo Super Cell in vinyl benzoate.[20d] The reaction mixture was filtered after stirring at 60 °C for 72 h. PTLC (ethyl acetate:petroleum ether:acetic acid, 30:70:1 v/v/v) afforded pure chiral 2-(benzoyl)oxy ketone **8c** for CD analysis. R_f (TLC) = 0.40 (30 % ethyl acetate: petroleum ether). ^{1}H NMR (270 MHz, $CDCl_3$): 15.30 (s, 1H, OH-9), 9.54 (s, 1H, OH-8), 8.13 (d, J = 8.4 Hz, 2H), 7.60 (bt, J = 8.1 Hz, 8.0 Hz, 1.0 Hz, 1H), 7.49 (m, 3H), 7.15 (d, J = 8.4 Hz, 1H), 7.05 (s, 1H, H-10), 6.85 (d, J = 8.0 Hz, 1H), 5.91 (dd, J = 5.4 Hz, 12.3 Hz, 1H, H-2), 3.19 (dd, J = 3.6 Hz, 6.6 Hz, 2H, H-4), 2.50 (m, 2H, H-3). ^{13}C NMR (67.9 MHz, $CDCl_3$): 199.01, 165.79, 165.51, 157.91, 139.51, 136.22, 133.47, 133.06, 130.00, 129.40, 128.51, 118.24, 117.69, 112.49, 111.12, 109.53, 73.36, 29.69, 29.69. HRMS calcd for $C_{21}H_{16}O_5$ (M^+): 348.0998, found 348.1008.

Spin–spin couplings between H-2 and H-3/H-3' (J_{2-3} = 5.4 Hz and $J_{2-3'}$ = 12.3 Hz) suggest that the benzoyl group occupies an equatorial position. Its CD spectrum in MeOH exhibits two strong exciton-coupled Cotton effects, λ_{ext} 265 nm, $[\theta] = +26.8 \times 10^6$ mdeg.m^{-1}.M^{-1} and 232 nm, $[\theta] = -58.4 \times 10^6$ mdeg.m^{-1}.M^{-1}. The positive sign of the longer wavelength Cotton effect indicates that the chirality between the long axes of benzoate and the naphthalene chromophores is positive. Therefore, the C2 carbon in the chiral benzoate must have the (*S*) configuration. HPLC analysis showed that the alcohols left unreacted by the enzymatic acetylation and benzoylation reactions of **7** are identical, indicating that the benzoate **8c** and the acetate **8a** have the same configuration.



(8c)

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28. Silva, D. J.; Kahne, D. preliminary results.

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