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New DNA binding ligands as a model of chromomycin A_3

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Abstract—Small molecules with DNA-binding affinity within the minor groove have become of great interest. In this study, new DNA-binding ligands were designed to mimic Chromomycin A_3 (CRA_3), which contains a hydroxylated tetrahydroanthracene chromophore substituted with di and trisaccharides. The trisaccharide part of CRA_3 that is supposed to contribute to form the Mg^{2+}-coordinated dimer was expected to be mimicked by a simple alkyl group attached to the chromophore part as new model compounds. The present study has successfully demonstrated that the new ligands form Mg^{2+}-coordinated dimer complexes to exhibit DNA-binding affinity.

Chromomycin A_3 **1** (CRA_3) is a potent antitumour antibiotic, and is a part of the aureolic acid group including plicamycin, mithramycin and olivomycins.[1] It has been shown that CRA_3 binds in the minor groove of GC-rich regions of duplex DNA to show inhibition against DNA-dependent DNA polymerases as well as RNA polymerases.[2] Although the aureolic family of drugs are not currently used for therapeutic purposes, the new compounds of this family are reevaluated as potential candidates in cancer therapy.[3] CRA_3 contains a hydroxylated tetrahydroanthracene chromophore substituted with various sugars. Studies on the structure–activity relationship have shown that the sugar parts contribute to DNA-binding properties as well as biological activities.[4] The aglycone of CRA_3, chromomycinone, does not bind to DNA.[5] It has been established by structural studies with NMR and X-ray crystallography that CRA_3 binds in the minor groove of the DNA as an Mg^{2+}-coordinated dimer and G-specific hydrogen bonds between CRA_3 and DNA provide the G-rich sequence specificity. It has been also shown from the crystal structure that Mg^{2+} adopts an octahedral coordination to O1 and O9 atoms of two chromophores and two water molecules, as schematically shown in **2**. The keto-phenol structure of O1 and O9 is responsible for Mg^{2+} binding, and mutual stackings of the aromatic part of the chromophore of one monomer with the C–D glycosidic linkage of the other monomer stabilize dimer structure.[6] Triethyleneglycol was used to mimic the C/D/E trisaccharide part for the formation of Mg^{2+}-coordinated dimer, but the role of the triethyleneglycol part for DNA binding of the model compound remains unclear.[7] We hypothesized that an alkyl chain would produce higher stacking interaction to stabilize the dimer structure complexed with Mg^{2+},[8] and designed a new model compound **3**. In this study, the hexyl group was chosen as an alkyl chain to mimic the C–D part stacking with the aromatic part (Fig. 1). Herein we describe that the model compound **3** binds to DNA as an Mg^{2+}-coordinated dimer.

The synthesis of **3** is shown in Scheme 1. We planned to construct the *peri*-hydroxy aromatic skeleton by strong-base-induced [4 + 2] cycloaddition of homophthalic anhydrides with α-sulfinyl-substituted derivatives of enolizable enones, which was developed by Kita's group.[9] Homophthalic anhydride derivative **6** was obtained from the corresponding homophthalic acid derivative **5**.[10] The α-sulfinyl-substituted cyclohexenone **8** was synthesized from commercially available (*R*)-3-methylcyclohexanone. The disulfinylated derivative **7** was obtained via a two-step reaction by regioselective sulfinylation with LDA–PhS–SPh,[11] followed by reaction with potassium *tert*-butoxide and $PhSSO_2Ph$. Oxidation of **7** with an equivalent of *m*-CPBA produced the 2-phenylthiocyclohexenone, which was further exposed to another equivalent of *m*-CPBA to furnish the enone **8** in good

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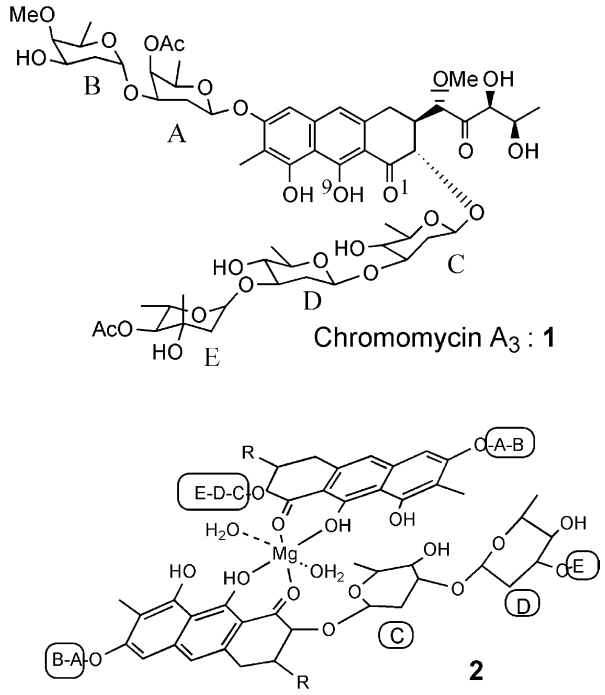
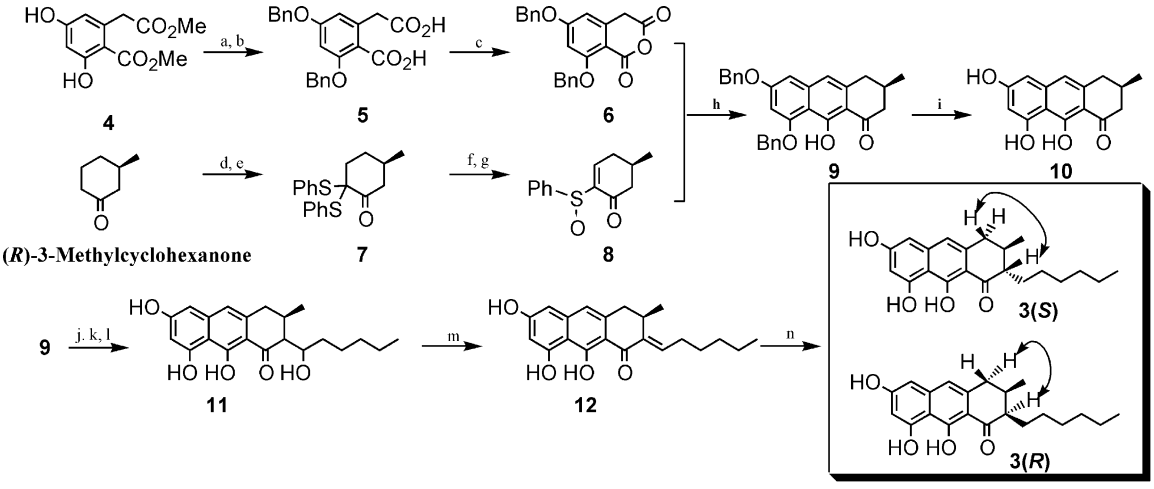


Figure 1. Chromomycin A_3 (CRA_3, **1**) and the schematic structure of Mg^{2+}-coordinated dimer (**2**).

yield. The [4 + 2] cycloaddition was performed by using **6** and **8** in the presence of NaH in refluxing dioxane to produce **9** in 20–30% yield. After a number of attempts, we found that molecular sieves catalyze the [4 + 2] cycloaddition to produce **9** in good yield.[12] The hexyl group was introduced by aldol reaction, followed by deprotection of the benzyl ethers by hydrogenolysis with H_2-palladium hydroxide to give **11**. Dehydroxylation was accomplished under acidic condition, and the produced *exo*-olefin was hydrogenated with H_2-palladium hydroxide to furnish a mixture of **3**(*S*) and **3**(*R*) isomers. The diastereomers were separated by normal phase HPLC using ChiralPak-AS as a column, and their stereochemistry was determined by ^1H COSY and NOESY spectra.[13]

The Mg^{2+}-binding properties of the compounds were investigated by measurement of the UV spectra (Fig. 2) in methanol. The UV spectra of **3**(*S*) was changed by the addition of $MgCl_2$ to produce a hyperchromic effect around 420 nm (Fig. 2A) with the isosbestic points. Stoichiometry of the complex was evaluated by the Job plot, and the 2:1 **3**(*S*) to Mg^{2+} complex was proven (Fig. 2B). The ability of the dimer complex of **3**(*S*) with Mg^{2+} resembles the TEG-chromophore conjugate previously reported.[7] It has been also shown that the **3**(*R*) isomer forms the 2:1 **3**(*R*) to Mg^{2+} complex. The UV titration data (Fig. 2A) was analyzed based on the 2:1 complex to produce a stability constant of $K_s = 1.0 \times 10^6\,M^{-1}$. In contrast, the Job plots with the nonalkylated compound **10** (Fig. 2C) were not clear compared to Figure 2B, probably because **10** forms a mixture of 1:1 and 2:1 complexes. These results have shown that the alkyl chain of **3** contributes to the formation of the dimer complex.

We also analyzed the complex compositions by positive ESI-MS measurements. The mass spectrum of **3**(*S*) in the presence of $MgCl_2$ indicated 2:1 ligand to Mg^{2+} complexes ($m/z = 707$) in addition to 1:1 complexes (429, 415, 401) (Fig. 3B). In the MS spectra of **10** with $MgCl_2$, there are the major peaks ($m/z = 345$, 331, 317) due to the 1:1 ligand to Mg^{2+} complexes together with the minor peak corresponding to the 2:1 complex (Fig. 3C). There is a small tendency for the formation of 2:1 complexes with Ca^{2+} (Fig. 3D). These results by the positive ESI-MS measurements agree with those obtained by UV measurements in that **3**(*S*) forms 2:1 complexes with $MgCl_2$. It is also indicated that the alkyl chain of **3**(*S*) plays an important role for dimer formation. The **3**(*R*) isomer produced similar ESI-MS spectrum.



Scheme 1. Reagents and conditions: (a) NaH, BnBr, 93%; (b) KOH, EtOH/H_2O, 96%; (c) TMS ethoxyacetylene, 91%; (d) PhS–SPh, LDA, THF, −78 °C to rt, 82%; (e) $PhSSO_2Ph$, *tert*-BuOK, THF, 0 °C, 77%; (f) *m*-CPBA, CH_2Cl_2, −50 °C, quant.; (g) *m*-CPBA, CH_2Cl_2, −60 °C, 73%; (h) MS 4 Å, rt, 75%; (i) $Pd(OH)_2$, H_2 gas, quant.; (j) TBSOTf, TEA, CH_2Cl_2, quant.; (k) hexanal, BF_3Et_2O, −70 °C, quant.; (l) $Pd(OH)_2$, H_2 gas, 55%; (m) TsOH, benzene, 40 °C, 56%; (n) $Pd(OH)_2$, H_2 gas, 96%.

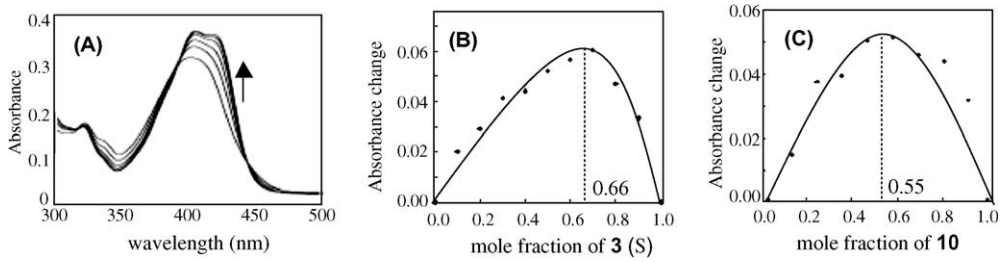


Figure 2. The UV spectrum change of **3**(*S*) by the addition of $MgCl_2$ (A), and Job plot with **3**(*S*) (B) and **10** (C).

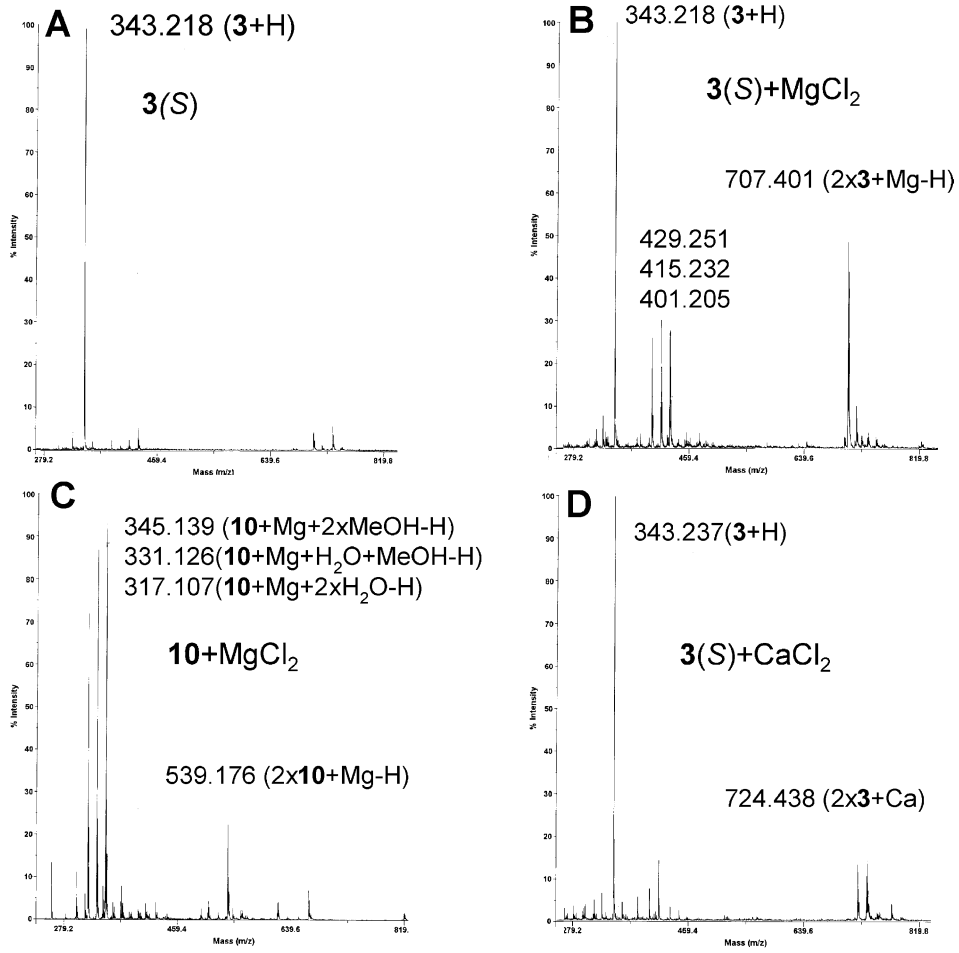


Figure 3. Analysis of the complexes by ESI-MS measurements: (A) **3**(*S*) only; (B) **3**(*S*) + $MgCl_2$; (C) **10** + $MgCl_2$, (D) **3**(*S*) + $CaCl_2$.

The binding affinity of the ligand with the duplex DNA was evaluated by a competitive displacement assay using fluorescent ethidium bromide (ETBr). The emission intensity of ethidium bromide is increased by binding with duplex DNA, and is inhibited by another competitive binder. Since competitive DNA binders displace DNA-bound ETBr to decrease emission regardless of their binding modes, although the binding mode of ETBr is intercalation, displacement assay with ETBr is widely used for primary estimation of the binding affinity of the compounds.[14] In this study, self-complementary 12 base pair DNA duplexes, CT12 and CA12, were used for measurement of the binding affinity.[15] CA12 includes an AATT region, while CT12 has a GCGC region at the middle of the duplex. Displacement assay was carried out at 25 °C using a solution of the duplex DNA (CT12 or CA12, 1 μM) in 20 mM Tris–HCl buffer. ETBr was effectively displaced by **3**(*S*) in the buffer containing 1 mM $MgCl_2$ (Fig. 4A), clearly indicating the relatively high affinity of **3**(*S*) to DNA. Conversely, displacement was not observed with use of **10** in the buffer containing $MgCl_2$ (Fig. 4B), or with use of **3**(*S*) in the buffer containing NaCl instead of $MgCl_2$ (Fig. 4C), showing low DNA-binding affinity of the ligands under these conditions. Moderate displacements were observed with **3**(*S*) in the buffer containing $CaCl_2$ (Fig. 4D). These results have indicated that the DNA-binding affinity of **3**(*S*) correlates with the tendency of dimer complex formation mediated by Mg^{2+}, and that the alkyl chain of **3**(*S*) is also an important contributor for DNA binding. The **3**(*R*)

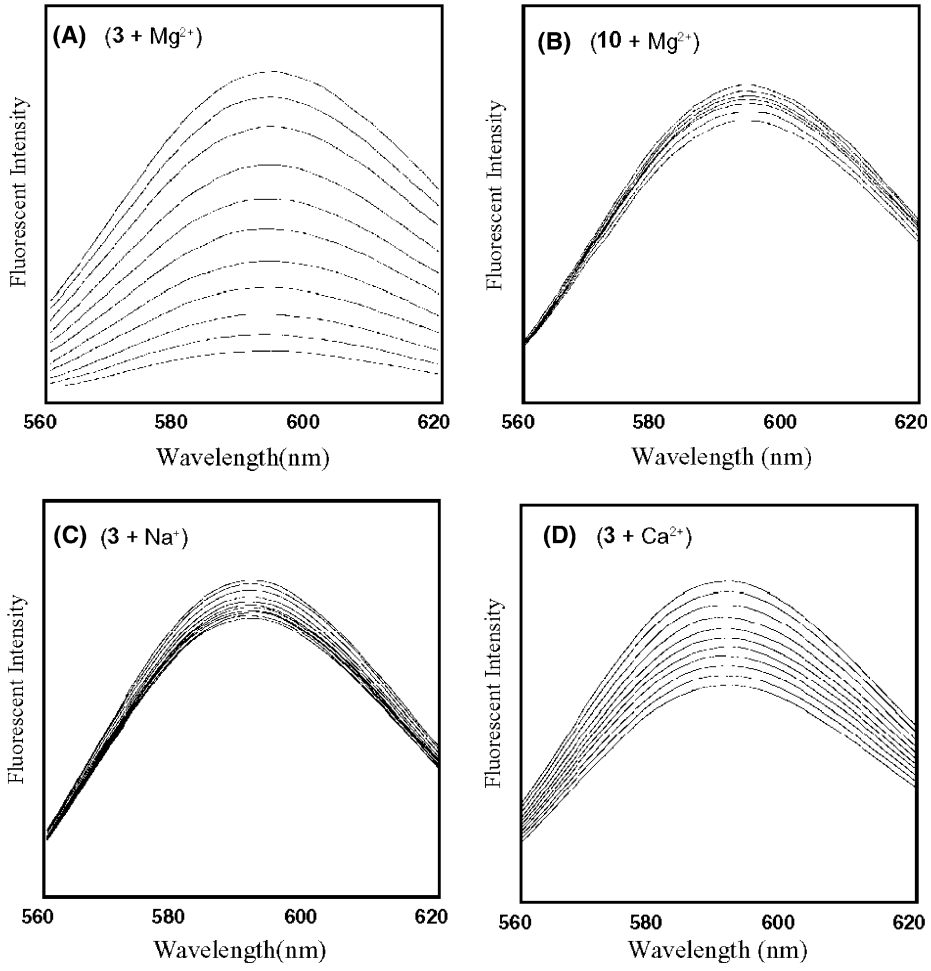
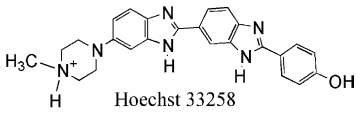


Figure 4. The ethidium bromide displacement assay with **3**(*S*) and **10**: (A) **3**(*S*) in the presence of 1 mM $MgCl_2$, (B) **10** in the presence of 1 mM $MgCl_2$, (C) **3**(*S*) in the presence of 2 mM NaCl, (D) **3**(*S*) in the presence of 1 mM $CaCl_2$. One micromolar each of CT12 and ethidium bromide were used. The ligand concentration was increased from 0 to 10 μM. λ_{ex} = 546 nm.

isomer showed almost the same results in the ethidium displacement assay. Concentrations of the ligand to inhibit 50% of fluorescence intensity of ETBr are expressed as IC_{50} values, and correspond to the relative binding affinity of the ligand (Table 1). Selective binding of Hoechst 33258[16] to the AATT region of DNA has been proven in this ethidium displacement assay (the IC_{50} values for CT12 and CA12; >20 vs 1 μM, respectively). Displacement profile of CRA_3 is somewhat complexed, because only one of two ethidium bromide molecules bound to CT12 was displaced. This is probably because the limited region of CT12 is tightly bound with CRA_3.[17] The stereochemistry of **3** did not affect the binding affinity, and the ligand **3** showed slightly weaker binding affinity to CA12 than Hoechst 33258. The IC_{50} values of the new ligands are not different towards CT12 or CA12, and sequence selectivity of the new ligands was not clear in this ethidium displacement assay.[18]



Hoechst 33258

Table 1. Concentrations to displace 50% DNA-bound ethidium bromide (IC_{50}, μM)[a]

Compounds	IC_{50} (μM)[b]	
	CT12[c]	CA12[d]
CRA_3	3[e]	9
3(*S*)	3	3
3(*R*)	3	2.5
3(*R* + *S*)	3.5	3
10	No inhibition	No inhibition
11	6.5	4.5
12	2.5	2.5
Hoechst 33258	>20	1

[a] 1 μM each of the duplex DNA and ethidium bromide were used in 20 mM Tris–HCl containing 1 mM $MgCl_2$ at pH 8.0. λ_{ex} = 546 nm, λ_{em} = 590 nm.
[b] Concentration to displace 50% DNA-bound ethidium bromide is expressed as the dimer complex except for Hoechst 33258.
[c] CT12: ($^{5'}$CGTAGCGCTACG)$_2$.
[d] CA12: ($^{5'}$CGCGAATTCGCG)$_2$.
[e] IC_{50} value to displace one of two ethidium bromide molecules bound to CT12.[17]

In conclusion, we have developed the new DNA binding molecules as a model of Chromomycin A_3. The model ligands **3**(*S* and *R*) have shown a tendency to form 2:1

ligand-to-Mg^{2+} complexes in methanol. In comparison with **10** without containing the alkyl chain, it has been revealed that the alkyl chain is responsible for the formation of the dimeric complex of **3** with Mg^{2+}. The DNA binding experiments have indicated that the alkyl chain of **3** plays a key role for Mg^{2+}-mediated DNA binding. There is no significant difference in binding properties examined in this study between the **3**(*S*) and the **3**(*R*) isomer. The present study has shown that the simple alkyl chain can mimic some roles of the trisaccharide part for Mg^{2+}-dimer complex formation and Mg^{2+}-mediated DNA binding. Further efforts are currently underway to develop new ligands that bind to DNA more strongly based on the structure of **3**.

Acknowledgements

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12. Details will be published soon.
13. Selected data for compound **3**(*S*) and **3**(*R*). **3**(*S*): ^{1}H NMR: δ (ppm) 16.64 (1H, b s), 10.02 (1H, s), 6.72 (1H, s), 6.46 (1H, d, J = 2.3 Hz), 6.36 (1H, d, J = 2.3 Hz), 3.04 (1H, dd, J = 16.0, 3.5 Hz), 2.59 (1H, dd, J = 16.0, 7.5 Hz), 2.36–2.33 (1H, m), 2.24–2.19 (1H, m), 1.89–1.83 (1H, m), 1.71–1.67 (1H, m), 1.37–1.27 (8H, m), 1.08 (3H, d, J = 6.7 Hz), 0.87 (3H, t, J = 6.9 Hz), IR (cm^{-1}, KBr) 3384, 1635, 1596, HR-ESIMS (*m*/*z*) calcd for $C_{21}H_{27}O_4$ $(M+H)^+$ 343.1904, found 343.1879. **3**(*R*): ^{1}H NMR: δ (ppm) 16.44 (1H, b s), 9.95 (1H, s), 6.74 (1H, s), 6.47 (1H, d, J = 2.3 Hz), 6.37 (1H, d, J = 2.3 Hz), 2.97 (1H, dd, J = 16.2, 3.6 Hz), 2.79 (1H, dd, J = 16.2, 6.7 Hz), 2.60 (1H, dt, J = 6.7, 4.0 Hz), 2.47–2.43 (1H, m), 1.88–1.84 (1H, m), 1.49–1.30 (9H, m), 0.97 (3H, d, J = 6.7 Hz), 0.88 (3H, t, J = 6.9 Hz), IR (cm^{-1}, KBr) 3384, 1635, 1596, HR-ESIMS (*m*/*z*) calcd for $C_{21}H_{27}O_4$ $(M+H)^+$ 343.1904, found 343.1890. The stereochemistry of **3**(*S*) and **3**(*R*) was determined by ^{1}H COSY and NOESY as illustrated in Scheme 1.
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17. Two ethidium bromide molecules bind to one CT12 or CA12 duplex. We have observed that some minor groove binders such as distamycin displace only one of two ethidium bromide molecules.[15]
18. DNA-binding affinity of the dimer complex of **3**-Mg^{2+} was estimated from the IC_{50} value to be ca. $K_s = 2 \times 10^6\,M^{-1}$ to CT12, and $7 \times 10^6\,M^{-1}$ to CA12. Several attempts to estimate DNA binding by footprinting experiments were not successful so far, probably because of insufficient DNA-binding affinity of **3**. Further modification of **3** for higher affinity to DNA is now in progress.