# Actinomycin D Binding to Oligonucleotides with 5′d(GCGC)3′ Sequences. Definitive $^1H$ and $^{31}P$ NMR Evidence for Two Distinct d(GC) 1:1 Adducts and for Adjacent Site Binding in a Unique 2:1 Adduct

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DNA binding of proteins and anticancer drugs and the communication along the DNA chain between such bound species are subjects of significant current interest.[1-6] Actinomycin D (ActD), a highly cytotoxic anticancer drug, is a rare example of an uncharged intercalator.[3,6-9] Intercalation of the phenoxazone ring is likely facilitated by interactions of DNA with the two cyclicpentapeptide lactones which are attached to the ring and bestow near *C*2 symmetry on ActD.[3,6-12] Specifically, H-bonding interactions with the two $NH_2$ groups of $(GC)_2$ have been proposed to account for the high GC sequence selectivity of the drug.[9] As such, ActD may be viewed as a model for selectivity in protein binding. However, some reports[13] have suggested that other types of selectivity, particularly for CG, are possible and have pointed out that NMR studies with ActD have generally used short oligomers which did not contain CG sequences.

We present here the first detailed $^1H$ (imino proton) and $^{31}P$ NMR titration studies of the dependence of ActD binding on oligonucleotide length (tetramers to 14-mers) and GC vs. CG site composition as a function of ratio (*R*) of ActD to duplexes.[14] The following self-complementary oligomers (5′→3′) have been studied up to $R = 2$: $d(GCGC)_2$; $d(TGCGCA)_2$ (**1**); d(TATGCGCA-

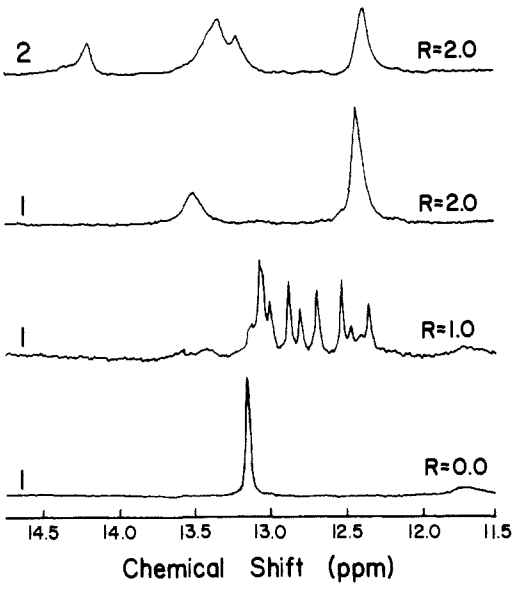


**Figure 1.** Imino proton spectra, 360 MHz, for **1**, ActD–**1**, and, at the top, ActD–**2** at 5 °C. The signals for the two different G imino protons of **1** are at 13.1 ppm in the $R = 0$ spectrum and the T imino proton is too broad to observe at 5 °C. At the $R = 2.0$ ratio, signals for the two G protons are at 12.4 ppm, the A·T bp is stabilized, and the T imino proton signal is shifted downfield (13.5 ppm). The G proton signals are at a similar chemical shift in the ActD–**2** complex but the A·T bp adjacent to the G·C bp's is additionally stabilized by the terminal A·T bp's and its imino proton signal is farther downfield. This T proton resonance is shifted significantly downfield relative to the $R = 0$ spectrum for **2** but the other T proton signals are shifted slightly upfield by ActD.

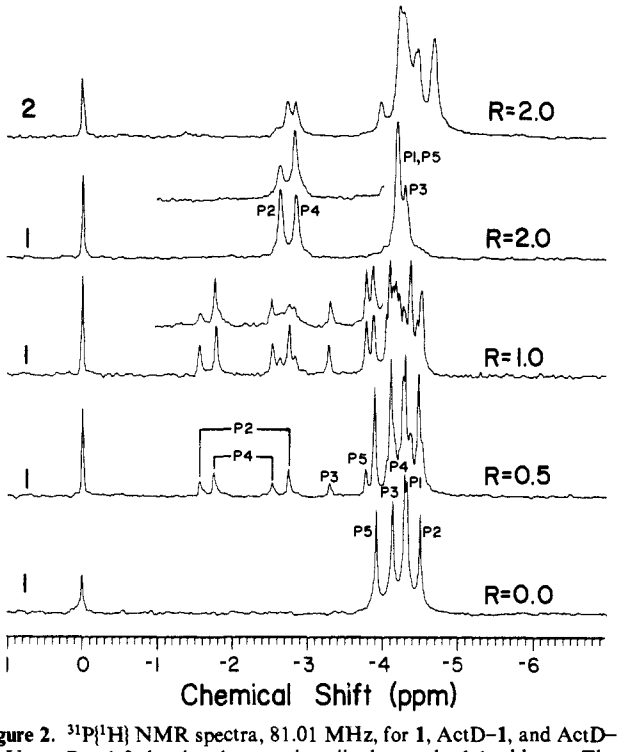


**Figure 2.** $^{31}P\{^1H\}$ NMR spectra, 81.01 MHz, for **1**, ActD–**1**, and ActD–**2**. Up to $R = 1.0$ the signals are primarily due to the 1:1 adducts. The insets are for **1** labeled at P2 with $^{17}O$ and illustrate the method, which is based on broadening and loss in intensity of the $^{31}P$ signal of the phosphate substituted with $^{17}O$, used for deriving the assignments given in the figure.[18,19] The top trace is for ActD–**2** at $R = 2.0$. Spectra were obtained at 5 °C and are referenced to trimethyl phosphate.

(1) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984.
(2) *Biological Macromolecules and Assemblies. Vol. 2: Nucleic Acids and Interactive Proteins*; Jurnak, F. A., McPherson, A., Eds.; Wiley: New York, 1985.
(3) Waring, M. In *The Molecular Basis of Antibiotic Action*; Gale, F. F., Cundliffe, E., Reynolds, P. E., Richmond, M. H., Waring, M. J., Eds.; Wiley: London, 1981; pp 314-333.
(4) Wilson, W. D.; Jones, R. L. *Adv. Pharmacol. Chemother.* **1981**, *18*, 177-222.
(5) Berman, H. M.; Young, P. R. *Annu. Rev. Biophys. Bioeng.* **1981**, *10*, 87-114.
(6) Early, T. A.; Kearns, D. R.; Burd, J. F.; Larson, J. E.; Wells, R. D. *Biochemistry* **1977**, *16*, 541-551.
(7) Müller, W.; Crothers, D. M. *J. Mol. Biol.* **1968**, *35*, 251-290.
(8) (a) Krugh, T. R. In *Molecular and Quantum Pharmacology*; Bergmann, E., Pullman, B., Eds.; Reidel: Dordrecht, 1974; pp 465-471. (b) Krugh, T. R.; Nuss, M. E. In *Biological Applications of Magnetic Resonance*; Schulman, R. G., Ed.; Academic Press: New York, 1979; Chapter 3.
(9) (a) Jain, S. C.; Sobell, H. M. *J. Mol. Biol.* **1972**, *68*, 1-20. (b) Sobell, H. M.; Jain, S. C. *J. Mol. Biol.* **1972**, *68*, 21-34. (c) Sobell, H. M. *Progr. Nucl. Acid Res.* **1973**, *13*, 153-190.
(10) (a) Patel, D. J. *Biochemistry* **1974**, *13*, 2396-2402. (b) Patel, D. J. *Biopolymers* **1976**, *15*, 533-558. (c) Patel, D. J.; Kozlowski, S. A.; Rice, J. A.; Broka, C.; Itakua, K. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 7281-7284.
(11) Brown, S. C.; Mullis, K.; Levenson, C.; Shafer, R. H. *Biochemistry* **1984**, *23*, 403-408.
(12) Reid, D. G.; Salisbury, S. A.; Williams, D. H. *Biochemistry* **1983**, *22*, 1377-1385.
(13) (a) Allen, F. S.; Moen, R. P.; Hollstein, U. *J. Am. Chem. Soc.* **1976**, *98*, 864-865. (b) Allen, F. S.; Jones, M. B.; Hollstein, U. *Biophys. J.* **1977**, *20*, 69-78. (c) Allen, F. S.; Gray, D. M. *Biopolymers* **1984**, *23*, 2661-2668.
(14) Oligonucleotides were synthesized, purified, and studied by NMR as previously described.[15-17] The buffer used in all experiments was 0.01 M PIPES, $10^{-3}$ EDTA, 0.1 M $NaNO_3$, pH 7, 90% $H_2$/10% $D_2O$, and the oligomer concentration was 20 mM in bases.
(15) Stec, W. J.; Zon, G.; Egan, W.; Byrd, R. A.; Phillips, L. R.; Gallo, K. A. *J. Org. Chem.* **1985**, *50*, 3908-3913.

0002-7863/86/1508-7113$01.50/0 

TA)$_2$; d(TATATGCGCATATA)$_2$ (**2**); d(ATACGCGTAT)$_2$; d(ATATACGCGTATAT)$_2$. The spectral changes induced by ActD, after accounting for the flanking sequences, are extremely similar for all oligomers with the GCGC sequence. In Figure 1 imino proton spectra of **1** at various ratios of ActD are shown and are compared to a spectrum for **2** at the 2:1 ratio. Spectral changes at ratios of 1.0 and below are quite complex and indicate the presence of two 1:1 complexes. At the 2.0 ratio the spectra simplify and indicate the presence of a unique 2:1 complex for both **1** and **2**. In contrast, titrations of duplexes containing the sequence CGCG give very similar spectra with evidence for formation of only a single 1:1 species at the GC site.

$^{31}$P NMR titrations of **1** with ActD are shown in Figure 2 and a spectrum for **2** at a ratio of 2.0 is included for comparison. Four of five phosphodiester groups of **1** were labeled with $^{17}$O and the assignments (Figure 2) employ the numbering scheme T-1-G-2-C-3-G-4-C-5-A. As ActD is added to **1**, a complex pattern of downfield peaks appears which are identified in the 0.5 ratio spectrum. As can be seen, there are two sets of downfield signals for P2 and P4 which represent two different 1:1 complexes at GC base pairs.

All 1:1 complexes with ActD studied here and described previously[8,10,18,19] have downfield $^{31}$P signals between −1 and −3 ppm and between −2 and −3 ppm. With the CGCG oligomers, as with most other oligomers investigated by NMR to this time, the GC binding site is on the duplex *C*2 symmetry axis and orientation of the unsymmetric ActD in either possible direction, with the peptides in the minor groove, produces an identical complex. With **1** and **2**, however, binding of a single ActD at either GC site gives two different complexes, which are a consequence of the two possible orientations of ActD in an intercalation site,[8] and this accounts for the two sets of downfield peaks obtained in the $^{31}$P spectra in Figure 2 at ratios up to 1.0. The peak areas for the two adducts suggest similar, but not identical, energetics in their DNA binding with the driving force largely a result of the peptide–DNA interactions. At the 2.0 ratio only single signals for P2 and P4 are shifted significantly downfield (−2 to −3 ppm region). In principle three different 2:1 complexes, two with *C*2 symmetry, are possible but the spectral simplification (relative to the 1:1 complexes) indicates that a unique complex with *C*2 symmetry is formed. It seems likely that steric constraints on the actinomycin cyclic peptides force the 2:1 complex into a single bound configuration. The shift differences among the 1:1 and 2:1 complexes in $^{31}$P spectra are, no doubt, a consequence of differences in ring current effects, torsional angles, hydrogen bonding, and other similar factors which are not yet fully understood.[8,10,18,19]

Several new points are quite clear from these results. First, with these oligomers there is no significant length dependence in the observed effects of ActD binding. Comparison of Figures 1 and 2 illustrates that the six bp's of **1** exhibit imino and $^{31}$P shifts on addition of ActD which are similar to the shifts for the central six bp's of **2**. Indeed d(GCGC)$_2$, which has no flanking sequences, also gives similar results. Second, ActD binds to GC sequences with a much higher preference than to CG or any other sequence in these oligonucleotides. All oligomers which have two CG and a single GC site, for example, form only a 1:1 complex with ActD and the binding site is the GC site as evidenced by imino proton shifts induced by the anisotropic ring current of the phenoxazone ring. Both **1** and **2**, on the other hand, have two GC sites and one CG site and form 1:1 and 2:1 complexes. The $^{31}$P shifts for the five phosphodiesters of **1** indicate that the 1:1 complexes are at either of the GC sites while the 2:1 complex has ActD bound at both GC sites. Third, the spectra in Figures 1 and 2 contain the first direct evidence for formation of two 1:1 complexes by the unsymmetric phenoxazone ring of ActD as suggested by Krugh and co-workers based on chemical shift analysis of ActD–dinucleotide complexes.[8] This is the first direct evidence for multiple 1:1 complexes for unsymmetrical intercalators and raises important questions about multiple binding orientations of intercalators, in general. Fourth, this finding indicates that the exclusion limit of ActD is quite small (it can intercalate at adjacent GC sites). DNAse I footprinting studies have suggested that binding of ActD at a GC site can inhibit binding at other GC sites up to four bp's away.[20] The NMR and footprinting results may not be in disagreement, however, since the more stable 1:1 complexes are almost completely formed before any significant amount of the 2:1 complex is seen (Figures 1 and 2). We are currently investigating oligomers with longer runs of adjacent GC sites to define the exclusion limit and structural effects of ActD binding in more detail.

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(16) Marzilli, L. G.; Banville, D. L.; Zon, G.; Wilson, W. D. *J. Am. Chem. Soc.* **1986**, *108*, 4188–4192.
(17) Wilson, W. D.; Jones, R. L.; Zon, G.; Banville, D. L.; Marzilli, L. G. *Biopolymers*, in press.
(18) Petersheim, M.; Mehdi, S.; Gerlt, J. A. *J. Am. Chem. Soc.* **1984**, *106*, 439–440.
(19) Gorenstein, D. G.; Lai, K.; Shah, D. O. *Biochemistry* **1984**, *23*, 6717–6723.
(20) Fox, K. R.; Waring, M. J. *Nucl. Acids Res.* **1984**, *12*, 9271–9285.

## Enantioselective Conjugate Addition of Rationally Designed Chiral Cuprate Reagents to 2-Cycloalkenones

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Evidence has been obtained recently that the conjugate addition reaction of Gilman reagents with $\alpha,\beta$-enones can follow a pathway involving (1) reversible d,$\pi^*$-complexation of nucleophilic copper with the enone, (2) $\beta$-cuprio adduct formation, and (3) reductive elimination to give the $\beta$-carbon adduct.[1,2] With this mechanistic guidance it became of interest to evaluate appropriate chiral reagents which might deliver copper enantioselectively to one face of an $\alpha,\beta$-enone, both as a test of the mechanistic hypothesis and a step toward more powerful synthetic methodology. Described herein are results of an initial investigation that demonstrate for the first time the possibility of achieving useful enantioselectivities (75–95%) with a simple chiral controller ligand (**1**), obtainable in one step in either enantiomeric form from inexpensive (+)- or (−)-ephedrine.

Reaction of (1*R*,2*S*)-(−)-ephedrine (**2**) with 1.16 equiv of (2-chloroethyl)dimethylamine hydrochloride and 2 equiv of powdered potassium carbonate in ethanol at reflux for 4 h afforded after vacuum concentration, extractive isolation, filtration through silica gel (20:1 ethyl acetate–triethylamine), and Kugelrohr distillation [160 °C (0.1 torr)] 82% yield of amino alcohol **1**, $[\alpha]^{23}_D$ +1.86° (*c* 1.2, chloroform). This ligand was then deprotonated (1 equiv of RLi), complexed with cuprous iodide (dissolved in tetrahydrofuran (THF)–dimethyl sulfide), and treated with additional RLi to generate the complexed cuprate reagent.

Experiments on the conjugate addition of *n*-butyl to 2-cyclohexenone revealed a strong dependence of the results on the purity of the organolithium reagent. Thus, with a fresh bottle of the purest *n*-butyllithium available (as a clear solution in hexane) to make the chiral reagent, an 80% yield of 1,4-adduct of 88% ee was obtained. Otherwise, identical experiments with older bottles

(1) (a) Corey, E. J.; Boaz, N. *Tetrahedron Lett.* **1985**, *26*, 6015. (b) Corey, E. J.; Boaz, N. *Ibid.* **1984**, *25*, 3063.
(2) (a) Hallnemo, G.; Olsson, T.; Ullenius, C. *J. Organomet. Chem.* **1985**, *282*, 133. (b) Hallnemo, G.; Ullenius, C. *Tetrahedron Lett.* **1986**, *27*, 395.

0002-7863/86/1508-7114$01.50/0 