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Communications

Manipulating the Stoichiometry and Strength of Phosphodiester Binding to a Bisguanidine Cleft in DMSO/Water Solutions

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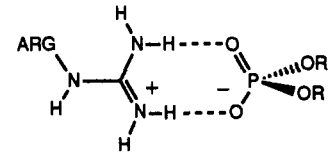
Summary: A cleft with two aminoimidazoline groups preorganized to form a V-shaped cavity was found to bind dibenzyl phosphate in mixtures of DMSO and water.

The molecular recognition,[1] transport,[2] and catalytic hydrolysis[3] of phosphodiesters are currently attracting attention due to applications in nucleotide chemistry. A typical strategy for complexing phosphate esters is ion pairing with positive amine salts such as ammonium[4] or guanidinium.[5] Guanidinium groups on the side chains of arginines are used commonly by proteins to recognize the phosphates of nucleotides via a two-point hydrogen-bonding motif.[1,6]



Compound 1 contains two amino imidazoline groups in which the guanidinium moieties are preorganized to converge toward each other and complex a phosphodiester via four point hydrogen bonding (two interactions of the type shown above) in a similar manner to that found in the active site of staphylococcal nuclease.[7] Our goal is to enhance the hydrolysis of phosphodiesters via stabilization of the dianionic phosphorane intermediate within the dicationic[8] positively charged cleft.[9]

The synthesis of 1 began with the known compound 2[10] (Scheme I). Compound 2 was reductively aminated[11] to

(1) The molecular recognition of phosphate groups by an arginine-rich RNA-binding motif has been identified in several RNA-binding proteins: Lazinski, E.; Grzadzielska, A. D. *Cell* **1989**, *59*, 207.

(2) (a) Furuta, H.; Furuta, K.; Sessler, J. L. *J. Am. Chem. Soc.* **1991**, *113*, 4706–4707. (b) Benzing, T.; Tjivikua, T.; Wolfe, J.; Rebek, J., Jr. *Science* **1988**, *242*, 266–268. (c) Tabushi, I.; Kobuke, Y.; Imuta, J. *J. Am. Chem. Soc.* **1980**, *102*, 1744–1745.

(3) (a) Kluger, R.; Taylor, S. D. *J. Am. Chem. Soc.* **1990**, *112*, 6669–6671. (b) Lim, C.; Karplus, M. *J. Am. Chem. Soc.* **1990**, *112*, 5872–5873. (c) Hengge, A. C.; Cleland, W. W. *J. Am. Chem. Soc.* **1990**, *112*, 7421–7422. (d) Anslyn, E. V.; Breslow, R. *J. Am. Chem. Soc.* **1989**, *111*, 4473. (e) Breslow, R.; Berger, D.; Huang, D.-L. *J. Am. Chem. Soc.* **1990**, *112*, 3686–3687. (f) Hendry, P.; Sargeson, A. M. *J. Am. Chem. Soc.* **1989**, *111*, 2521–2527. (g) Chin, J.; Banaszczyk, M. *J. Am. Chem. Soc.* **1989**, *111*, 4103–4105. (h) Chin, J.; Banaszczyk, M.; Jubian, V.; Zou, X. *J. Am. Chem. Soc.* **1989**, *111*, 186–190. (i) Moser, H. E.; Dervan, P. B. *Science* **1987**, *238*, 645–650. (j) Basile, L. A.; Barton, J. K. *J. Am. Chem. Soc.* **1987**, *109*, 7548–7550. (k) Basile, L. A.; Raphael, A. L.; Barton, J. K. *J. Am. Chem. Soc.* **1987**, *109*, 7550–7551.

(4) For a good summary of examples, see ref 8 in: Furuta, H.; Magda, D.; Sessler, J. L. *J. Am. Chem. Soc.* **1991**, *113*, 978–85. For other examples see: (a) Kodama, M.; Kimura, E.; Yamaguchi, S. *J. Chem. Soc., Dalton Trans.* **1980**, 2536. (b) Kimura, E.; Sakonaka, A.; Yatsunami, T.; Kodama, M. *J. Am. Chem. Soc.* **1981**, *103*, 3041. (c) Dietrich, B.; Hosseini, M. W.; Lehn, J.-M.; Sessions, R. B. *J. Am. Chem. Soc.* **1981**, *103*, 1282. (d) Marecek, J. F.; Fisher, P. A.; Burrows, C. J. *Tetrahedron Lett.* **1988**, *29*, 6231.

(5) (a) Galâm, A.; Pueyo, E.; Slamerôn, A.; de Mendoza, J. *Tetrahedron Lett.* **1991**, *32*, 1827–1830. (b) Dietrich, B.; Fyles, D. L.; Fyles, T. M.; Lehn, J.-M. *Helv. Chim. Acta* **1979**, *62*, 2763. (c) Dietrich, B.; Fyles, T. M.; Lehn, J.-M.; Pease, L. G.; Fyles, D. L. *J. Chem. Soc., Chem. Commun.* **1978**, 934. (d) Schmidtchen, F. P. *Tetrahedron Lett.* **1989**, *30*, 4493.

(6) (a) Calnan, B. J.; Tidor, B.; Biancalana, S.; Hudson, D.; Frankel, A. D. *Science* **1991**, *252*, 1167 and references cited therein. (b) Saenger, W. *Principles of Nucleic Acid Structure*: Springer Verlag: New York, 1984; p 387.

(7) (a) Tucker, P. W.; Hazen, E. E., Jr.; Cotton, F. A. *Mol. Cell. Biochem.* **1979**, *23*, 67–86. (b) Cotton, F. A.; Hazan, E. E., Jr.; Legg, M. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 2551-2555. (c) Loll, P. J.; Lattman, E. E. *Proteins* **1989**, *5*, 183–201.

(8) Substituted guanidines are known to be very basic: (a) Leffek, K. T.; Pruszynski, P.; Thanapaalasingham, K. *Can. J. Chem.* **1989**, *67*, 590. (b) Wirth, T. H.; Davidson, N. *J. Am. Chem. Soc.* **1964**, *86*, 4325.

(9) Electrostatic transition-state stabilization is discussed in: Fersht, A. *Enzyme Structure and Mechanism*, 2nd ed.; W. H. Freeman: New York, 1985; Chapter 12.

(10) Huang, C. Y.; Cabell, L. A.; Anslyn, E. V. *Tetrahedron Lett.* **1990**, *31*, 7411.

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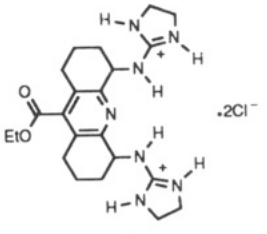
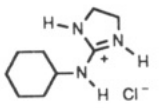
Table I. Composite Binding Constants for a Mixture of *meso*/*d*,*l*-1 and Other Receptors with Dibenzyl Phosphate with Various Solvent Conditions. Two Significant Figures Are Appropriate and an Error of 15% Is Estimated

host	solvent	ionic strength (M)	K_1 (M^{-1})	K_2 (M^{-1})
 1	DMSO	0.045	800	100
	SMXO/D_2O (90/10)	0.017	300	60
	DMSO/D_2O (80/20)	0.027	60	20
	DMSO/D_2O (67/33)	0.013	30	
	DMSO/D_2O (67/33)	0.178	140	
	DMSO/D_2O (67/33)	0.325	360	
	DMSO/D_2O (67/33)	0.516	700	
 6	DMSO	0.030	330	
	DMSO/D_2O (67/33)	0.321	no binding	
guanidinium chloride	DMSO/D_2O (67/33)	0.33	no binding	

form a mixture of *meso*- and *d*,*l*-**3**. Attempts to form **1** directly via reaction of 2-(methylthio)-2-imidazoline[12] with **3** resulted in very low yields, and thus a more lengthly but higher yield route was developed. Protection of ethylenediamine with BOC,[13] followed by reaction with carbon disulfide and DCC,[14] forms thioisocyanate **4**. When **3** and **4** were allowed to react followed by alkylation with ethyl bromide and deprotection of the BOC groups,[13] cyclization occured under slightly basic conditions to produce free base **1** in an overall 36% yield from **2**. Free base **1** was crystallized as the dipicrate salt and converted to the di-HCl salt by anion exchange chromatography.[15]

Binding studies were performed by both ^{31}P NMR and ^{1}H NMR titrations.[16] Based on the shape of the ^{31}P NMR titration curves versus the host/guest ratio, it was concluded that two complexes were forming, both a 2:1 and a 1:1 guest to host complex. Upon introducing water to the solvent system, the binding became weaker as expected due to better solvation of the host and guest charges (Table I). In a 67/33 DMSO/D_2O mixture, only 1:1 binding was observed. To solve for the two binding constants from the ^{31}P NMR titration, we used an algorithm based upon following the chemical shift of the species whose stoichiometry is two in a 2:1 complex.[17] In order to confirm these binding constants, ^{1}H NMR titrations were performed, and a curve-fitting program which follows the species whose stoichiometry is one in a 2:1 complex was used.[18] The binding constants calculated by the two different methods were within 10% of each other. The

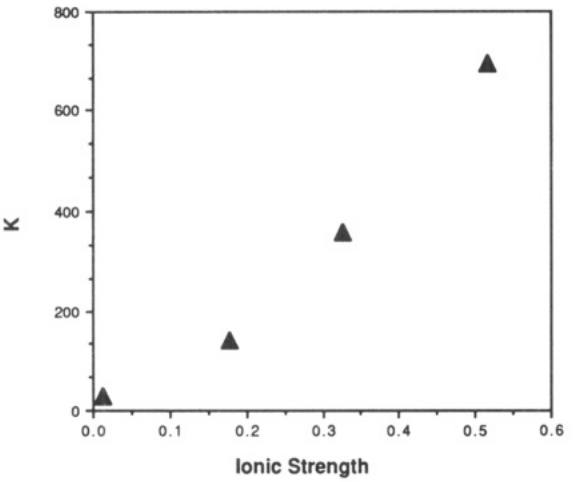


Figure 1. Effect of ionic strength on 1:1 binding constant K M^{-1}.

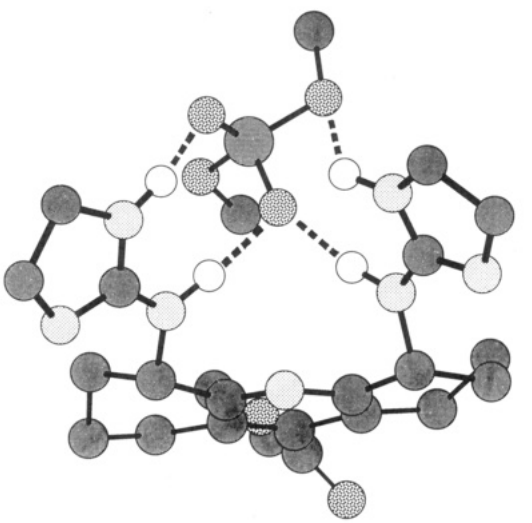
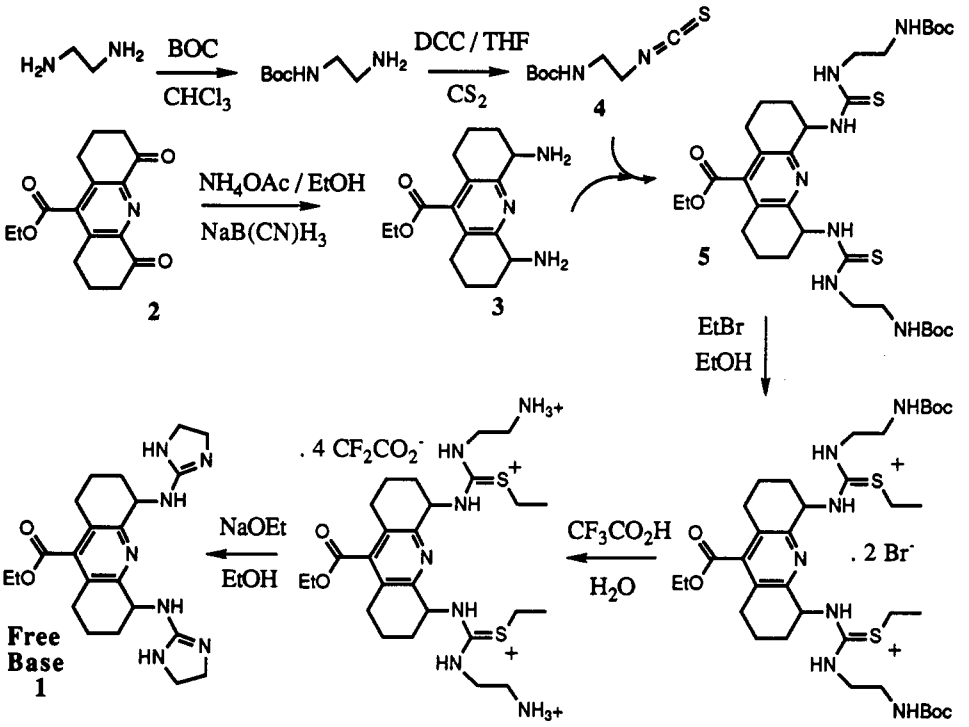


Figure 2. Molecular mechanics generated structure for the complexation of dimethyl phosphate to **1**. Hydrogens deleted for clarity. Dashed lines are hydrogen bonds with heteroatom–heteroatom distances less than 3.2 Å.

(11) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897.
(12) Short, J. H.; Biermacher, U.; Dunnigan, D. A.; Leth, T. D. *J. Med. Chem.* **1963**, *6*, 275.
(13) Tarbell, D. S.; Yamamoto, Y.; Pope, B. M. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 730.
(14) Jochins, J. C.; Seeliger, A. *Angew. Chem.* **1967**, *6*, 174.
(15) Free base **1**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.30 (t, 3 H), 1.7–2.2 (m, 8 H), 2.64 (m, 4 H), 3.63 (s, 8 H), 4.37 (q, 2 H), 4.68 (m, 2 H), 7.7–9.0 (bs, 2 H), 8.73 (t, 2 H); ^{13}C {^{1}H} NMR (MeOH-d_4, 75 MHz) δ 14.55 (20.27, 20.21), 26.39, (30.31, 30.36), 44.18, 54.86, 63.04, 129.57, (144.07, 144.17) (153.42, 153.49), 161.67, 168.33. Resonances in parentheses are doubled due to a mixture of meso and *d*,*l*. Anal. Calcd for HCl salt of **1**, $C_{22}H_{33}N_7O_2Cl_2{\cdot}4(H_2O)$: C, 46.31; H, 7.24; N, 17.19. Found: C, 46.30; H, 7.17; N, 16.77. Picrate salt of **6**: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 1.0–1.9 (m, 10 H), 3.24 (m, 1 H), 3.56 (s, 4 H), 7.3–8.5 (bs, 2 H), 8.13 (d, 1 H), 8.58 (s, 2 H); ^{13}C {^{1}H} NMR (DMSO-d_6, 75 MHz) δ 24.17, 24.64, 32.28, 42.35, 51.40, 124.22, 125.16, 141.81, 158.26, 160.80. Anal. Calcd $C_{15}H_{20}N_6O_7$: C, 45.45; H, 5.09; N, 21.20. Found: C, 45.44; H, 5.02; N, 21.38.
(16) Since ^{31}P chemical shifts change significantly with ion strength, we confirmed that over the range of ionic strength that occurs in any binding study, no significant changes in ^{1}H and ^{31}P NMR chemical shifts occur. Costello, A. J. R.; Glonek, T.; Van Wazer, J. R. *Inorg. Chem.* **1976**, *15*, 972.
(17) Lenkinski, R. E.; Elgavish, G. A.; Reuben, J. *J. Mag. Reson.* **1978**, *32*, 367–376.
(18) Sheridan, R. E., Whitlock, H. W., Jr. *J. Am. Chem. Soc.* **1986**, *108*, 7120–7121.

Scheme I. Synthesis of Compound 1



cooperativity of the two symmetrical halves of compound 1 for phosphodiester binding was established by checking the binding of the control compounds guanidinium chloride and **6**. Under the same experimental conditions that 1 binds dibenzyl phosphate with a binding constant of 3.6 $\times 10^2$ M^{-1}, no complexation[19] with guanidinium chloride and **6** was observed.

The effect of ionic strength (adjusted with NaCl) on the 1:1 binding constant with the DMSO/D_2O (67/33) solvent mixture is shown in Figure 1. Under all conditions, the ^{31}P NMR titrations showed simple 1:1 binding. Although the addition of chloride ion to the solution might be expected to compete with the phosphates for the positive amino imidazoline groups and thus lower the phosphate binding constants, the opposite was observed. The binding constants of the phosphates increased with increasing amounts of NaCl. We attribute this effect to a "salting out" phenomenon.[20] We propose that as increasing amounts of NaCl are added to the solution, the solvent becomes more tied up solvating the NaCl and the solvent becomes a worse solvator of the amino imidazoline groups. Further studies to elucidate the origin of the ionic strength effect are in progress.

Figure 2 shows a molecular mechanics[21] derived structure for the complexation of dimethyl phosphate (benzyls removed for clarity) to **1**. As expected, the binding in this hypothetical structure involves four hydrogen bonds between the phosphodiester and the host. The amino imidazoline groups are twisted away from the saturated carbons of the octahydroacridine linker forming a V-shaped cleft which complements the divergent oxygen lone pairs on the phosphodiester. Only the meso form of **1** is shown. The *d,l* form was calculated to adopt a similar structure except with different conformations of the CH_2 groups in the octahydroacridine linker. Phosphodiester hydrolysis studies along with attempts to further increase the strength of complexation in water containing solvents are in progress.

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Registry No. *cis*-**1**, 137743-40-9; *trans*-**1**, 137743-41-0; *cis*-**1**·2HCl, 137743-42-1; *trans*-**1**·2HCl, 137743-43-2; **2**, 132844-22-5; *cis*-**3**, 137743-44-3; *trans*-**3**, 137743-45-4; **4**, 137743-46-5; *cis*-**5**, 137743-47-6; *trans*-**5**, 137743-48-7; *cis*-**5**·2EtBr, 137743-49-8; *trans*-**5**·2EtBr, 137743-50-1; *cis*-**5** (diamine; *S,S'*-diethyl derivative·4CF_CO_2H), 137743-52-3; *trans*-**5** (diamine; *S,S'*-diethyl derivative·4CF_3CO_2H), 137743-54-5; **6**·picrate, 137743-55-6; **6**·HCl, 50993-83-4; $NH_2(CH_2)_2NH_2$, 107-15-3; $BOCNH(CH_2)_2NH_2$, 57260-73-8.

(19) Binding constant detection limit is about 10 M^{-1}.

(20) (a) Long, F. A.; McDevitt, W. F. *Chem. Rev.* **1952**, *51*, 119. (b) Gordon, J. E. *The Organic Chemistry of Electrolyte Solutions*; Wiley: New York, 1975. (c) Kool, E. T.; Breslow, R. *J. Am. Chem. Soc.* **1988**, *110*, 1596–1597.

(21) Still, C. MACROMODEL Version 2.5, Columbia University.