proceeds via a stereospecific olefin-forming pathway.[21] Tetramethylene-d_2 generated from a 1,2-diazene decomposition[23] has the properties k(cleavage)/k(closure) = 2.2 and k(rotation)/k(closure) = 12.[25]

Acknowledgment. The authors are grateful to the National Science Foundation (CHE-75-06776) for generous support.

Supplementary Material Available: Reactions of compounds **2–11** (2 pages). Ordering information is given on any current masthead page.

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(9) Epoxide **7** allowed NMR analysis of the deuterium content of each olefinic position and confirmed the cis-3,4-d_2 assignment in **6**.

(10) 10 ft × 0.125 in., 30% SE-30 on 100/120 Chromosorb P; flame ionization detector; electronic integration. Assignment of the products were made by coinjection techniques using authentic samples. The ethylene/cyclobutane ratio was corrected for detector response.

(11) Perkin-Elmer Model 180 infrared spectrophotometer. We thank Dr. George R. Rossman, Division of Geological and Planetary Sciences, California Institute of Technology, for allowing us to use this instrument.

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(13) Ethylene products were analyzed[11] using the 842-cm^{-1} (and 724-cm^{-1}) bands for *cis*- (and *trans*-) ethylene-*1,2-d*$_2$. Ethylene-d_1 and ethylene-d_0 (half the ethylene from **2** is d_0) have bands at 809 and 945 cm^{-1}, respectively. These peaks did not interfere with the analyses.

(14) *cis*-Cyclobutane-*1,2-d*$_2$ (**3**): IR (gas) 2990 (C–H), 2200 (C–D), 1450 (CH_2), 1307 (CHD), 569, 562, (1294 < 2% *trans*-**4**-d_2); mass spectrum (9.0 eV), d_2/d_1 = 95.5:4.5 ± 1.[16] *trans*-Cyclobutane-*1,2-d*$_2$ (**4**): IR (gas) 2950 (C–H), 2190 (C–D), 1450 (CH_2), 1294 (CHD), 579, 543 (562 < 2% *cis*-**3**-d_2); mass spectrum, d_2/d_1 = 95.5:4.5 ± 1.[16]

(15) For an alternative synthesis and thermolysis of *cis*- and *trans*-cyclobutane-*1,2-d*$_2$, see recent work of Chickos, J. S. *J. Org. Chem.* **1979,** *49*, 780.

(16) The percent deuterium incorporation in the cyclobutane-*1,2-d*$_2$ was obtained by comparison with cyclobutane-d_0 sample using ion cyclotron resonance spectroscopy.[17]

(17) We thank Peter Armentrout and Professor J. L. Beauchamp for their generous assistance. See Beauchamp, J. L. *Ann. Rev. Phys. Chem.* **1971,** *22*, 527.

(18) Controls: (a) The hydrazone and the hydrazine **10** corresponding to *cis*-**2**-*3,4-d*$_2$ afforded hydrocarbon products in <1% yield under identical pyrolysis conditions as used for **2**. (b) *trans*-ethylene-*1,2-d*$_2$ was shown not to isomerize under the pyrolysis conditions. (c) Cyclobutane was stable under the reaction conditions. (d) Surface and pressure effects were checked. None were found.

(19) Recently, evidence[5d,e] was provided that *cis*- and *trans*-3,4-dimethyltetrahydropyridazines, six-membered cyclic 1,2-diazenes, undergo a stereospecific fragmentation reaction to olefin competitive with the generation of 3-methyl-1,4-pentanediyl, a 1,4-biradical intermediate which was *identical in behavior* with the intermediate(s) from the pyrolyses of 1,2-dimethylcyclobutanes.[3b]

(20) Neglecting deuterium isotope effects.

(21) In the case of 3,4-dimethyltetrahydropyridazines, a 36% stereospecific olefin-forming reaction in competition with a 64% 1,4-diradical pathway was found. Whether these stereospecific fragmentation reactions are [2 + 2 + 2] cycloreversions[22] or the decomposition of diazenyl biradicals that do not lose their stereochemical integrity cannot be distinguished from this data.

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(24) Private communication: Professor M. J. Goldstein, Cornell University.

(25) Benson[2a] estimates that A(cleavage) = $10^{13.07}$ and A(closure) = $10^{12.30}$ from parent tetramethylene. From the k(cleavage)/k(closure) ratio reported here (2.2), one calculates that E_a(cleavage) > E_a(closure) by 1.4 kcal mol^{-1} at 712 K.

(26) A. P. Sloan Research Fellow, 1977–1979. Camille and Henry Dreyfus Teacher–Scholar, 1978–.

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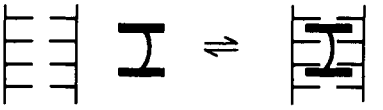
Molecular Recognition of Nucleic Acid by Small Molecules. Binding Affinity and Structural Specificity of Bis(methidium)spermine

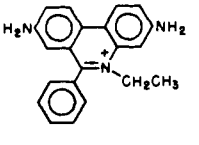
Sir:

Nucleic acids are biologically important receptors sufficiently characterized to encourage the syntheses of site specific probes. Molecules capable of binding to nucleic acid templates and interfering with processes in which nucleic acids participate are important in both antibiotic and cancer chemotherapy.[1] Some drugs bind to nucleic acids by intercalation, the insertion of a flat molecule between the base pairs of a double helix.[2] In the absence of unfavorable entropic or steric con-

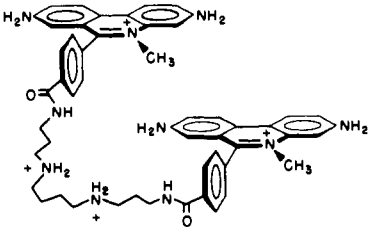


straints, an increase in binding affinity and sequence specificity would be expected for polyintercalators[3] which are capable of inserting two or more intercalating units into the nucleic acid double helix.

We report the *quantitative* determination of the nucleic acid binding affinity and specificity which result when two intercalating monomers of ethidium bromide (EB),[4] connected by a spermine[5] link, are incorporated into the same molecule, bis(methidium)spermine (BMSp).[3f] The results presented in this paper clearly demonstrate that dimers constructed from



EB



BMSp

two intercalating monomers can bind nucleic acids with a free energy approaching the sum of the free energies of the monomeric constituents resulting in substantial increases in both binding affinity and specificity.

BMSp (a) has a binding site size which is always twice that of EB,[3f,6] (b) increases the length of double helical DNA 1.6

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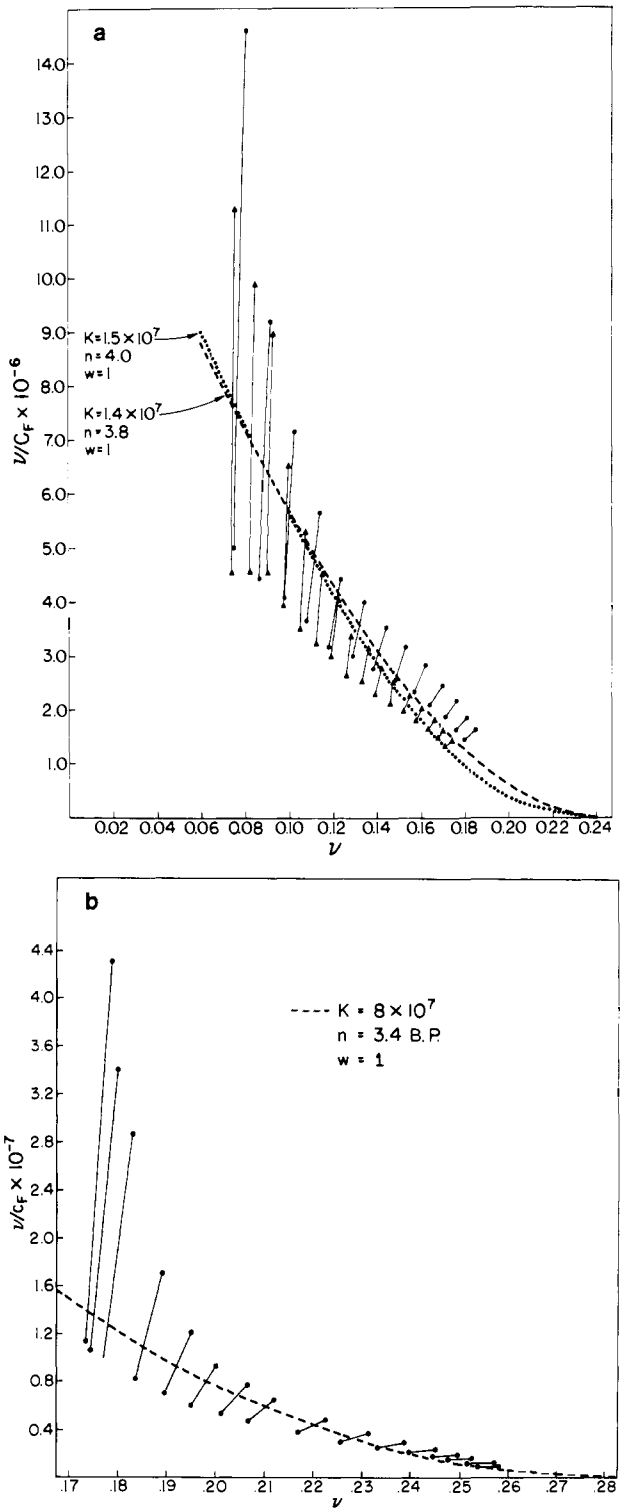

Figure 1. Scatchard plots of the binding of BMSp to (a) calf thymus DNA and (b) poly(dG-dC)·poly(dG-dC) at 1 M Na^+. The results of two titrations (●—●, ▲—▲) are shown for calf thymus DNA. The binding density, concentration of bound drug per base pair (ν), is plotted against the ratio ν/C_F where C_F equals the concentration of free drug. Dotted lines are theoretical plots generated by the von Hippel-McGhee binding equation[12] for the indicated binding affinity, K, binding site size, n, and binding cooperativity, w. For noncooperative binding, $w = 1$.

times more per bound molecule than EB as determined by viscometric titrations of "rigid-rod" DNA,[7] and (c) unwinds DNA 1.5 times more per bound molecule than EB as determined by viscometric titrations of closed circular PM2 phage DNA.[3f] Taken together these data suggest that both EB moieties in BMSp simultaneously intercalate. The observations that the extension and unwinding of the DNA helix caused by

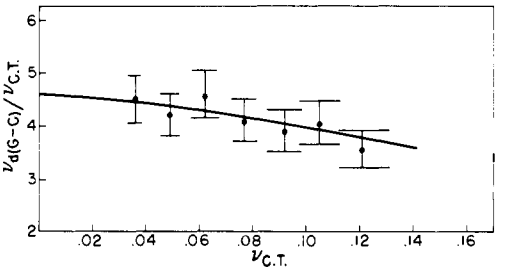

Figure 2. Equilibrium dialysis competition plot at [Na^+] = 1.0 M. The BMSp binding density to calf thymus DNA, ν_{CT}, is plotted against the poly(dG-dC)·poly(dG-dC)/calf thymus binding density ratio.

BMSp binding are less than twice that observed for EB suggest that the intercalation geometry of the EB groups may be altered for BMSp under conditions of low salt (0.075 M^+).

We measured the binding affinity of BMSp to four nucleic acids and compared these affinities to those measured for EB[9,4c] under nearly identical conditions. Binding measurements were carried out at 1.0 M Na^+ because at this salt concentration electrostatic contributions to the observed binding affinity[10] and the intercalation geometry[8] are minimized. Thus, the nucleic acid affinities measured for BMSp, a tetracation at pH 7, can be directly compared to EB, a monocation.

Two approaches have been taken toward determining the binding affinities of BMSp for different nucleic acids. The first method takes advantage of the spectral shift that occurs in the visible spectrum of BMSp when it binds nucleic acids.[3f] The concentrations of bound and free BMSp can be determined from such measurements assuming the formation of one complex.[11] This data is then plotted in terms of a Scatchard plot (Figure 1).[12] Comparison of the experimentally observed Scatchard plot to theoretical Scatchard plots generated by the binding equations of von Hippel-McGhee[13] allows estimation of the binding affinity. This technique was used to determine the binding affinity of BMSp for calf thymus DNA, and poly(dG-dC)·poly(dG-dC).[14]

The second approach used to determine the binding affinity of BMSp involves competition equilibrium dialysis.[15] In this method, a three-part dialysis cell containing two different DNA species in the outer compartments is stirred to equilibrium. At equilibrium the two DNA-BMSp complexes are in simultaneous equilibrium with the same concentration of free BMSp. The ratio of the association constants of BMSp for two nucleic acids i and j (K_i/K_j) can then be determined directly from the observed ratio of binding densities (ν_i/ν_j) in the limit of low binding density ($\nu_i, \nu_j \rightarrow 0$). Unlike Scatchard plots, the competition approach allows relative binding affinities to be determined without specifying the binding site size and binding cooperativity of each complex (see Figure 2).

We have used this competition dialysis approach to determine the binding affinity of BMSp to poly(dG-dC)·poly(dG-dC) and poly(rA)·poly(dT). By conducting competition dialysis between several different pairs of nucleic acids, we have been able to internally verify the binding affinities obtained by the competition approach as well as the relative binding affinities obtained by the titration approach. Combination of the two methods allows an estimation of the binding affinity to within an accuracy of ±10% for calf thymus DNA, (dG-dC)·(dG-dC), and rA·dT. The results are summarized in Table I.

At 1.0 M Na^+, for the four nucleic acids studied, we find that $K_{BMSp} = (K_{EB})^{1.4-1.8}$, or the free energy of dimer binding is 1.4-1.8 times that of the monomer: $\Delta G_{BMSp} = 1.4\text{-}1.8\Delta G_{EB}$.

The binding affinity of BMSp for nucleic acid at salt concentrations other than 1 M Na^+ can be estimated from a consideration of the binding process in terms of the polyelec-

Table I

nucleic acid	Na^+(M)	K_{EB}	K_{BMSp}
calf thymus	0.075	2×10^5 M^{-1} [b]	$\geq 2 \times 10^{11}$ M^{-1}
calf thymus	1.0	4.2×10^4 M^{-1}	1.5×10^7 M^{-1}
(dG-dC)·(dG-dC)	1.0	2.0×10^4 M^{-1}	7.5×10^7 M^{-1}
dA·dT	1.0	2×10^3 M^{-1} [a]	4.4×10^4 M^{-1}
rA·dT	1.0	2.3×10^5 M^{-1} [a]	2.3×10^8 M^{-1}

[a] From Bresloff and Crothers.[9] [b] Data of LePecq[4c] reanalyzed in terms of von Hippel-McGhee equations.

trolyte theory of Manning.[16] As shown by Record et al.,[10] the observed binding affinity, K_{obsd}, of a ligand at a monovalent cation concentration equal to M^+ can be estimated by the equation $K_{obsd} = K_0[M^+]^{n\psi}$, where K_0 is the binding affinity at 1 M Na^+, n is the number of ion pair interactions which the ligand makes with nucleic acid, and ψ is the charge density parameter which is known for a variety of nucleic acids. For example, from Table I, at 1 M Na^+ the binding affinity of BMSp is 1.5×10^7 M^{-1} or 3.6×10^2 times greater than EB. At low salt, 0.075 M, where electrostatic contributions become more important, the estimated affinity of BMSp for calf thymus is 1×10^{11} M^{-1} or 10^6 times greater than EB. This estimate compares favorably with the estimated affinity, $K \geq 2 \times 10^{11}$ M^{-1}, determined experimentally from spectrophotometric titrations.[17]

In addition, the binding specificity of BMSp compared to EB is substantially increased. From the work of Crothers,[9] it is known that the binding of the monomer EB to the RNA-DNA hybrid rA·dT is favored over the DNA-DNA duplex dA·dT by a factor of 100. This 100-fold specificity exhibited by EB increases to 5200 for BMSp. Since the only difference between rA·dT and dA·dT is the presence of the 2′-hydroxyl group on the sugar ring and not base sequence, these results indicate that the specificity which BMSp and EB[9] exhibit for certain nucleic acids can arise from *preferential recognition of different nucleic acid conformations.*

Acknowledgment. We are grateful to the National Institutes of Health (GM-22909), Biomedical Research Support Funds (RR-07003), and the Eli Lilly and Company for generous support.

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(17) See footnote 32 in ref 3f. Spectrophotometric data revealed the percent bound BMSp at BMSp/BP = 0.25 to be ≥97% at 0.075 M Na^+.
(18) National Institutes of Health Trainee (GM-01262).
(19) Alfred P. Sloan Research Fellow, 1977–1979. Camille and Henry Dreyfus Teacher-Scholar Grant Recipient, 1978–.

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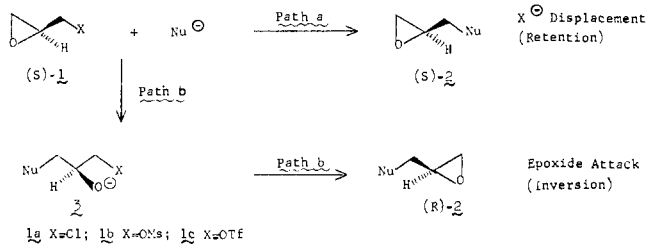
Mode of Nucleophilic Addition to Epichlorohydrin and Related Species: Chiral Aryloxymethyloxiranes

Sir:

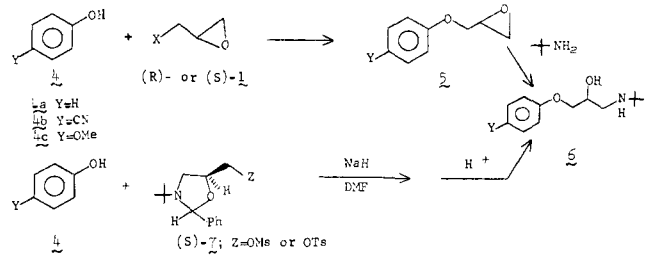
Nucleophilic attack on epichlorohydrin (**1a**) or a related methyloxirane (**1**) generally results in the formation of a new methyloxirane **2**.[1-8] In principle, **2** may be derived from **1** via two distinct processes: (1) direct displacement of the leaving



group (path a) or (2) initial epoxide attack (**3**) followed by extrusion of the leaving group (path b). In spite of considerable effort, the mechanism of such a nucleophilic addition has yet to be conclusively established.[4-7]

Since the stereochemistry of the products obtainable from chiral **1**[9,10] according to paths a or b would not be identical, a determination of the absolute configuration and chiral purity of **2** would establish the mode of nucleophilic addition. Our results from the reactions of chiral **1**[9,10] with various phenols to give chiral aryloxymethyloxiranes reported herein indicate that the mode of nucleophilic addition depends on the leaving group involved and the conditions used.

The reactions of (*R*)- and/or (*S*)-**1** with phenols **4a–c** have been examined using two sets of conditions: (1) refluxing in acetone or CH_2Cl_2 in the presence of K_2CO_3 and (2) stirring **1** with the preformed phenoxide in DMF or THF. The *S*/*R* ratios presented were determined by an examination of the ^1H NMR spectra of **5** in the presence of a chiral shift reagent, Eu(hfbc)$_3$,[9,12,13] and by optical rotation.



The absolute configuration of **5** obtained from (*R*)-**1a** under acetone-K_2CO_3 conditions was established by the reaction with *tert*-butylamine to give **6**, which was then compared with chirally pure (*S*)-**6** synthesized from (*S*)-**7**.[14] The unambiguous assignment of the predominant configuration of **5** and **6** derived from (*R*)-**1a** as (*S*)[15] was thus possible. The *S*/*R* ratios

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