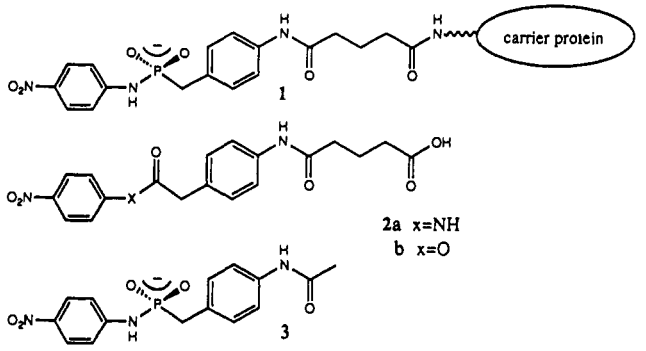


Figure 1. Transition-state analogue phosphonamidate **1** used to induce antibodies capable of hydrolyzing **2**. **3** is an inhibitor of the reaction.

the number of different antibodies which can be studied and in many cases can even bypass the need for immunization.[2,3] However, before these procedures could be generally adopted, it remained to be demonstrated that one could obtain catalytic antibodies from combinatorial libraries. We now report this accomplishment.

An Fab[4] combinatorial library in phage λ was generated[2b] from the spleen of a mouse immunized with phosphonamidate **1** (Figure 1) conjugated to keyhole limpet hemocyanin (KLH). The library was screened for binding to **1** conjugated to bovine serum albumin (BSA). Twenty-two phage clones were isolated that bound to **1**. Purified Fab's from six of these clones were assayed for catalytic activity, and three were found to be active for hydrolysis of the substrate **2b** (Figure 1).

In order to obtain enough antibody to carry out kinetic studies, we expressed one clone (1D) in the baculovirus expression system.[5] By transferring the antibody-encoding genes into insect cells, we increased the expression level approximately 10-fold from >0.5 to 5 mg/L. This increase in yield allowed us to obtain enough antibody for rigorous purification and kinetic characterization. The cell line producing the greatest amount of this Fab, as judged by the intensity of the ELISA signal, was selected, amplified, and used for large-scale expression. The purified Fab 1D was judged to be >95% pure as determined by silver staining of SDS-polyacrylamide gels and cross-reacted with anti-murine Fab and anti-κ antibodies on Western blots (data not shown). Control cells infected with wild-type virus and uninfected cells did not yield protein bound to the **1**-BSA conjugate.

Antibody activity was measured by monitoring the release of *p*-nitrophenol at 404 nm. Assays were performed in PBS (10 mM sodium phosphate, pH 7.2, 160 mM NaCl) containing 1-4 μM antibody, 31.25-500 μM substrate **2b** in 5% dioxane in a final volume of 800 μL. The initial rate of Fab 1D catalyzed hydrolysis measured as a function of substrate **2b** was observed to follow Michaelis-Menten kinetics (K_m = 115 μM, k_{cat} = 0.25 min^{-1}). The gene encoding this antibody was sequenced (data not shown) and found to differ from genes encoding catalysts isolated previously (**1**-KLH was again the immunogen) from our laboratory by conventional hybridoma procedures.[6] Furthermore, 1D did not hydrolyze amide **2a** (Figure 1), unlike some other antibodies we isolated previously.[6] Nevertheless, hapten **3** (Figure 1) was a potent inhibitor of the reaction.[7] Potent inhibition by the immunogen has been observed with other catalytic antibodies.[6]

We have isolated a catalytic antibody by cloning the immunological repertoire of a hyperimmunized mouse into phage λ.[2b] While early combinatorial antibody libraries, including the one studied here, utilized an immunization procedure, these methods are rapidly being replaced by semisynthetic libraries.[3c] Such libraries can provide a uniform and reproducible source of antibody molecules as starting materials. Thus, we envision that future catalytic antibody experiments will begin with screening of either immunized or semisynthetic combinatorial libraries for binding to selected haptens. Once catalysts are found, their rates and substrate specificities can be altered by mutagensis or genetic selection procedures.

Acknowledgment. We thank Charles Hasemann and Donald Capra for providing the vector pAC360E. Y.-C.J.C. is a fellow of the Jane Coffin Childs Memorial Fund for Medical Research. This research was supported in part by the National Institutes of Health (GM-43858, K.D.J.).

Supplementary Material Available: Listing of relevant molecular biological and kinetic assay procedures (5 pages). Ordering information is given on any current masthead page.

(2) (a) Sastry, L.; Alting-Mees, M.; Huse, W. D.; Short, J. M.; Sorge, J. A.; Hay, B. N.; Janda, K. D.; Benkovic, S. J.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 5728. (b) Huse, W. D.; Sastry, L.; Iverson, S. A.; Kang, A. S.; Alting-Mees, M.; Burton, D. R.; Benkovic, S. J.; Lerner, R. A. *Science* **1989**, *246*, 1275.

(3) (a) Kang, A. S.; Jones, T. M.; Burton, D. R. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 11120. (b) Barbas, C. F.; Bain, J. D.; Hoekstra, D. M.; Lerner, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 4457.

(4) Defined here as one heavy and light chain that make up a single antibody binding site.

(5) (a) Hasemann, C. A.; Capra, J. D. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3942. (b) zu Putlitz, J.; Kubasek, W. L.; Duchêne, M.; Marget, M.; von Specht, B.-U.; Domdey, H. *Bio/Technology* **1990**, *8*, 651. (c) Burand, J. P.; Summers, M. D.; Smith, G. E. *Virology* **1980**, *101*, 286. (d) Volkman, L. E.; Summers, M. D.; Hsieh, C.-H. *J. Virol.* **1976**, *19*, 820. (e) Smith, G. E.; Fraser, M. J.; Summers, M. D. *J. Virol.* **1983**, *46*, 584. (f) Smith, G. E.; Summers, M. D.; Fraser, M. J. *Mol. Cell. Biol.* **1983**, *3*, 2156. (g) Miyamoto, C.; Smith, G. E.; Farrel-Towt, J.; Chizzonite, R.; Summers, M. D.; Ju, G. *Mol. Cell. Biol.* **1985**, *5*, 2860.

(6) Janda, K. D.; Schloeder, D.; Benkovic, S. J.; Lerner, R. A. *Science* **1988**, *241*, 1188.

(7) Potent inhibition by **3** was demonstrated by the following experiment: A 1 μM solution of Fab 1D (for assay conditions, see supplementary material) was completely inhibited by the addition of 1 μM **3** in the presence of 150 μM substrate **2b**.

Synthesis and Structure of a Transition-Metal-Substituted Silylene Complex, $(CO)_4OsSi(STol\text{-}p)[Ru(\eta^5\text{-}C_5Me_5)(PMe_3)_2]$

Steven D. Grumbine and T. Don Tilley*

Department of Chemistry, 0506
University of California at San Diego
9500 Gilman Drive
La Jolla, California 92093-0506

Arnold L. Rheingold*

Department of Chemistry
University of Delaware
Newark, Delaware 19716

Received September 18, 1992

Unsaturated silicon species are important reactive intermediates in silicon chemistry.[1] In addition, it is often assumed that metal complexes containing unsaturated silicon centers (as in silylene complexes L_nMSiX_2) are key intermediates in transition-metal-promoted transformations of silicon compounds, although little conclusive evidence exists to support such hypotheses.[2] Pannell,[3] Ogino,[4] and Turner[5] have reported mechanistic studies which

(1) See, for example: (a) Gaspar, P. P. In *Reactive Intermediates*; Jones, M., Jr., Moss, R. A., Eds.; Wiley: New York, 1978; Vol. 1, p 229; (b) 1981; Vol. 2, p 335; (c) 1985; Vol. 3, p 333. (d) Wiberg, N. *J. Organomet. Chem.* **1984**, *273*, 141. (e) Raabe, G.; Michl, J. *Chem. Res.* **1985**, *85*, 419. (f) West, R. *Pure Appl. Chem.* **1984**, *56*, 163. (g) Chojnowski, J.; Staficzyk, W. *Adv. Organomet. Chem.* **1990**, *30*, 243. (h) Brook, A. G.; Baines, K. M. *Adv. Organomet. Chem.* **1986**, *25*, 1 and references in the above.

(2) (a) Tilley, T. D. In *The Chemistry of Organic Silicon Compounds*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1989; Chapter 24, p 1415. (b) Tilley, T. D. In *The Silicon-Heteroatom Bond*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1991; Chapters 9, 10, pp 245, 309.

(3) (a) Pannell, K. H.; Cervantes, J.; Hernandez, C.; Cassias, J.; Vincenti, S. *Organometallics* **1986**, *5*, 1056. (b) Pannell, K. H.; Rozell, J. M., Jr.; Hernandez, C. *J. Am. Chem. Soc.* **1989**, *111*, 4482. (c) Pannell, K. H.; Wang, L.-J.; Rozell, J. M. *Organometallics* **1989**, *8*, 550.

0002-7863/93/1515-358$04.00/0

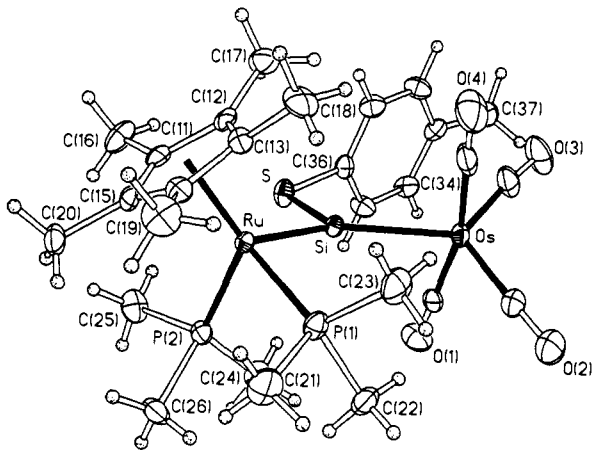


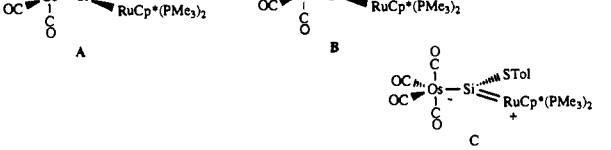
Figure 1. ORTEP view of **1**. Important bond distances (Å) and angles (deg): Os-Si 2.419 (2), Ru-Si, 2.286 (2), Si-S 2.172 (4), S-C(36), 1.779 (8), Os-C(1) 1.949 (11), Os-C(2) 1.876 (11), Os-C(3), 1.918 (10), Os-C(4), 1.926 (12), Ru-P(1), 2.285 (3), Ru-P(2) 2.303 (3), Ru-CNT 1.931 (9); Os-Si-Ru 140.4 (1), Os-Si-S 113.2 (1), Ru-Si-S 106.5 (1), C(1)-Os-C(4) 166.9 (4), C(3)-Os-C(4) 90.8 (5), C(2)-Os-C(3) 118.5 (5), C(1)-Os-C(3) 93.1 (4), C(1)-Os-C(2) 93.3 (5), Si-Os-C(1) 83.6 (3), Si-Os-C(2) 117.4 (3), Si-Os-C(3) 124.0 (4), Si-Os-C(4) 83.9 (3), Si-S-C(36) 109.4 (3), P(1)-Ru-P(2) 92.0 (1), P-(1)-Ru-Si 92.2 (1), P(2)-Ru-Si 89.7 (1), CNT-Ru-P(2) 125.7 (3), CNT-Ru-P(1), 125.0 (3), CNT-Ru-Si 122.2 (2). CNT is the centroid of the Cp* ring.

convincingly implicate silyl(silylene) complexes Cp(CO)Fe-(=SiR_2)(SiR'_3) as intermediates in photochemical silylene-elimination reactions; and over the past five years numerous examples of base-stabilized silylene complexes, containing tetracoordinate silicon, have been prepared and structurally characterized.[6-12] In 1991, we reported the synthesis and characterization of base-free silylene complexes of the type $[Cp^*(PMe_3)_2Ru{=}Si(SR)_2]BPh_4$.[13] Although such complexes are stable and crystalline, repeated attempts to obtain X-ray-quality crystals have been unsuccessful. Given the elusive nature of silylene complexes[2,7f] and their potential relevance to catalysis,[2,7f,14] it is of interest to learn more about the nature of metal–silylene bonding interactions. Here we report the first structural characterization of a transition-metal complex containing an sp^2-hybridized silicon center. This compound, $(CO)_4OsSi(STol\text{-}p)[Ru(\eta^5\text{-}C_5Me_5)(PMe_3)_2]$ (**1**; $Cp^* = \eta^5\text{-}C_5Me_5$), may be regarded as either a silylene or a bridging silylyne[15] complex. Interestingly, in 1980 Aylett predicted that the first isolated silylene complexes might be sterically and electronically stabilized by transition-metal substituents.[16]

Compound **1** was synthesized by reaction of $Na_2Os(CO)_4$ with $Cp^*(PMe_3)_2RuSi(STol\text{-}p)(OTf)_2$ (OTf = OSO_2CF_3) in 1,2-dimethoxyethane over 12 h. Orange crystals of the product were obtained from diethyl ether solution in 23% yield. Spectroscopic and analytical data established the stoichiometry of the compound.[17]

The molecular structure of **1**[18] (Figure 1) reveals a somewhat distorted trigonal bipyramidal geometry, with C(1) and C(4) leaning toward the equatorial silylene ligand (the axial C(1)–Os–C(4) angle is 166.9 (4)°). Note that in Zybill's $(CO)_4FeSiR_2(HMPA)$ complexes, the base-stabilized silylene ligands are *axially* coordinated.[6h] The inequivalent angles about Si (∠Os-Si-S = 113.2 (1)°; ∠Os-Si-Ru = 140.4 (1)°; ∠Ru-Si-S = 106.5 (1)°) probably reflect steric crowding in the molecule, but their sum (360.1°) is consistent with sp^2 hybridization (displacements from the Os-Si-S-Ru least-squares plane are ≤0.005 Å).

The planar geometry at silicon suggests π-bonding to one or more of the substituent atoms. Simply on the basis of electron counting, an important resonance structure would seem to be A, with an Os–Si double bond. However, the Os–Si distance of 2.419 (2) Å is typical for an Os–Si single bond.[19] The Si–S bond distance, 2.172 (4) Å, is somewhat shorter than the comparable distances in $Cp^*(PMe_3)_2RuSi(STol\text{-}p)_3$ (2.223 (1), 2.195 (1), and 2.196 (1) Å),[20] implying some degree of Si–S π-bonding (B). Stereochemically this π-bonding appears possible, given that the Si–S–C(36) plane is tilted by only 11.1° with respect to the Os–Si–S–Ru least-squares plane. The Ru–Si bond (2.286 (2) Å) also appears to be "shortened" when compared to the Ru–Si distance of 2.350 (1) Å in $Cp^*(PMe_3)_2RuSi(STol\text{-}p)_3$.[20] Thus, it appears that silicon is π-bonded most strongly to ruthenium, making C a more important resonance hybrid than either A or B. Note that the latter structure may be described equivalently



in terms of π-donation from Ru to Si, and coordination of silicon to osmium via donation from a "silylene" lone pair (Os←:Si←Ru). Consistent with this view, there is no stereochemical evidence for π-bonding between Os and Si, as the Ru–Si–S plane is not coplanar with $Os(CO_{ax})_2$ or $Os(CO_{eq})_2$,[21] but is canted by 28.5° with respect to the plane of $Os(CO_{eq})_2$ (defined by the C(2), Os, and C(3) atoms). Additionally, the infrared spectrum of **1** reflects

(4) (a) Tobita, H.; Ueno, K.: Ogino, H. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2797. (b) Ueno, K.; Tobita, H.; Ogino, H. *Chem. Lett.* **1990**, 369.
(5) Haynes, A.; George, M. W.; Haward, M. T.; Poliakoff, M.; Turner, J. J.; Boag, N. M.; Green, M. *J. Am. Chem. Soc.* **1991**, *113*, 2011.
(6) (a) Straus, D. A.; Tilley, T. D.; Rheingold, A. L.; Geib, S. J. *J. Am. Chem. Soc.* **1987**, *109*, 5872. (b) Straus, D. A.; Zhang, C.; Quimbita, G. E.; Grumbine, S. D.; Heyn, R. H.; Tilley, T. D.; Rheingold, A. L.; Geib, S. J. *J. Am. Chem. Soc.* **1990**, *112*, 2673. (c) Grumbine, S. D.; Chadha, R. K.; Tilley, T. D. *J. Am. Chem. Soc.* **1992**, *114*, 1518.
(7) (a) Zybill, C.; Müller, G. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 669. (b) Zybill, C.; Wilkinson, D. L.; Müller, G. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 583. (c) Zybill, C.; Müller, G. *Organometallics* **1988**, *7*, 1368. (d) Zybill, C.; Wilkinson, D. L.; Leis, C.; Müller, G. *Angew. Chem., Int. Ed. Engl.* **1989**, *28*, 203. (e) Leis, C.; Zybill, C.; Lachmann, J.; Müller, G. *Polyhedron* **1991**, *11*, 1163. (f) Zybill, C. *Top. Curr. Chem.* **1991**, *160*, 1. (g) Probst, R.; Leis, C.; Gamper, S.; Herdtweck, E.; Zybill, C.; Auner, N. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1132. (h) Leis, C.; Wilkinson, D. L.; Handwerker, H.; Zybill, C.; Müller, G. *Organometallics* **1992**, *11*, 514.
(8) (a) Ueno, K.; Tobita, H.; Shimoi, M.; Ogino, H. *J. Am. Chem. Soc.* **1988**, *110*, 4092. (b) Tobita, H.; Ueno, K.; Shimoi, M.; Ogino, H. *J. Am. Chem. Soc.* **1990**, *112*, 3415. (c) Takeuchi, T.; Tobita, H.; Ogino, H. *Organometallics* **1991**, *10*, 835. (d) Koe, J. R.; Tobita, H.; Ogino, H. *Organometallics* **1992**, *11*, 2479.
(9) Corriu, R.; Lanneau, G.; Priou, C. *Angew. Chem., Int. Ed. Engl.* **1991**, *9*, 1130.
(10) Woo, L. K.; Smith, D. A.; Young, V. G., Jr. *Organometallics* **1991**, *10*, 3977.
(11) Lee, K. E.; Arif, A. M.; Gladysz, J. A. *Chem. Ber.* **1991**, *124*, 309.
(12) Jutzi, P.; Möhrke, A. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 893.
(13) Straus, D. A.; Grumbine, S. D.; Tilley, T. D. *J. Am. Chem. Soc.* **1990**, *112*, 7801.
(14) Tilley, T. D. *Comments Inorg. Chem.* **1990**, *10*, 37.
(15) A base-stabilized bridging silylyne ligand, supported by an Fe–Fe bond, is observed in $[Cp_2Fe_2(CO)_2(\mu\text{-}CO)(\mu\text{-}Si^tBu\text{-}N\text{-}methylimidazole)]I$. See: Kawano, Y.; Tobita, H.; Ogino, H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 843.
(16) Aylett, B. J. *J. Organomet. Chem. Libr.* **1980**, *9*, 327.
(17) For **1**: ^1H NMR (dichloromethane-d_2, 300 MHz) δ 1.65 (vir t, 18 H, PMe_3), 1.95 (t, J_{PH} = 1.4 Hz, 15 H, Cp*), 2.40 (s, 3 H, C_6H_4Me), 7.20 (d, J = 8 Hz, 2 H, C_6H_4Me), 7.39 (d, J = 8 Hz, 2 H, C_6H_4Me); $^{31}P\{^1H\}$ NMR (dichloromethane-d_2, 121.5 MHz) δ 2.34, ^{29}Si NMR (dichloromethane-d_2, 59.6 MHz) δ 19.43 (t, $^2J_{SiP}$ = 23 Hz). Anal. C, H.
(18) Crystal data for **1**: $C_{27}H_{40}O_4OsP_2RuSSi$, monoclinic, $P2_1/c$, a = 9.839 (2) Å, b = 32.228 (6) Å, c = 10.854 (2) Å, β = 110.62 (3)°, V = 3221.2 (11) Å^3, Z = 4, D_x = 1.736 g cm^{-3}, T = 233 K, μ(Mo Kα) = 46.32 cm^{-1}. Of 7040 absorption-corrected data (Siemens P4, 2θ(max) = 53°), 6686 were independent, and 3986 were observed ($6\sigma F_o$). All non-hydrogen atoms were anisotropically refined; hydrogen atoms were idealized. $R(F)$ = 3.95%, $R(wF)$ = 4.37% (all data 5.74%). SHELXTL PLUS software was used for all calculations (G. Sheldrick, Siemens Corp., Madison, WI).
(19) A search of the Cambridge Structural Database revealed a mean Os–Si bond length of 2.42 (4) Å. The Os–Si distance in the base-stabilized silylene complex (*meso*-tetra-*p*-tolylporphyrin)Os=$SiEt_2$·THF is 2.325 (8) Å (ref 10).
(20) Heyn, R. H.; Grumbine, S. D.; Tilley, T. D. Unpublished results.
(21) Rossi, A. R.; Hoffmann, R. *Inorg. Chem.* **1975**, *14*, 365.

little Os-Si π-bonding, since the observed ν(CO) stretching frequencies (2024, 1940, and 1900 cm^{-1}) are much closer to those for $(CO)_4Os(PMe_3)$ (2060, 1977, and 1936 cm^{-1})[22] than to those for $(CO)_4Os(CH_2{=}CH_2)$ (2111, 2023, and 1993 cm^{-1}).[22] In summary, the structural evidence points toward very little of the expected π-bonding between Si and Os, and significant, delocalized π-bonding between silicon, sulfur, and ruthenium. This is consistent with stronger π-donating properties for the electron-rich $Cp^*(PMe_3)_2Ru$ fragment (relative to $(CO)_4Os$) and with calculations which indicate that thiolate groups should be relatively effective at stabilization of an sp^2 Si center.[23]

Preliminary reactivity studies show that **1** is remarkably stable toward nucleophiles, as no reactions are observed with $OPPh_3$, *p*-(dimethylamino)pyridine, $CN(2,6\text{-}Me_2C_6H_3)$, or $NEt_4^+Cl^-$ (by ^1H NMR spectroscopy).

Acknowledgment is made to the National Science Foundation for financial support. T.D.T. also thanks the Alfred P. Sloan Foundation for a research fellowship (1988-1992), Union Carbide for an Innovation Recognition Award (1991-1992), and the Mobile Foundation for a financial contribution.

Supplementary Material Available: Tables of crystal, data collection, and refinement parameters, bond distances and angles, anisotropic displacement parameters, and hydrogen atom coordinates for **1** (8 pages); listings of observed and calculated structure factors for **1** (15 pages). Ordering information is given on any current masthead page.

(22) Carter, W. J.; Kelland, J. W.; Okrasinski, S. J.; Warner, K. E.; Norton, J. R. *Inorg. Chem.* **1982**, *21*, 3955.
(23) (a) Apeloig, Y.; Godleski, S. A.; Heacock, D. J.; McKelvey, J. M. *Tetrahedron Lett.* **1981**, *22*, 3297. (b) Lambert, J. B.; Schulz, W. J., Jr.; McConnell, J. A.; Schilf, W. *J. Am. Chem. Soc.* **1988**, *110*, 2201.

Binding of Two Different DNA Sequences by Conformational Switching

Ethel Rubin, Timothy L. McKee, and Eric T. Kool*

Department of Chemistry, University of Rochester
Rochester, New York 14627

Received October 5, 1992

Proteins can recognize varied structures by making use of conformational flexibility. For example, DNA-binding proteins can have affinity for multiple sequences using a single binding pocket, by changing conformation in response to varied structures. To date, we know of no synthetic ligand which can recognize multiple sequences in this way. We now wish to report the design and synthesis of a macrocyclic ligand which can bind to two distinct DNA sequences in a mutually exclusive way by changing conformation on binding.

We constructed the 36-base oligonucleotide **1** by a template-directed cyclization of the linear precursor, 5′-dTCTCTTTTTTTTTTTCTCTCTCTTTTTTTTTTTCTCp.[1,2] The template oligomer 5′-dAAAGAGAGAGAAA (1 equiv) was used to align the reactive ends in an aqueous esterification reaction, as described previously.[3,4] The yield of purified circular **1** was

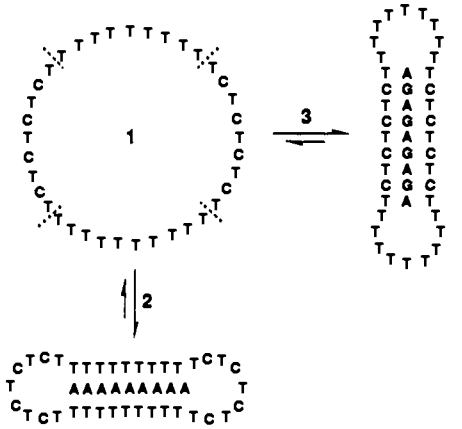


Figure 1. Sequence of macrocycle **1** and the conformational changes involved in the binding of **1** to the sequences **2** and **3**. Dotted lines demarcate the four domains in **1**. When both sequences are present, the macrocycle binds to one of the two as shown, but cannot bind to both simultaneously.

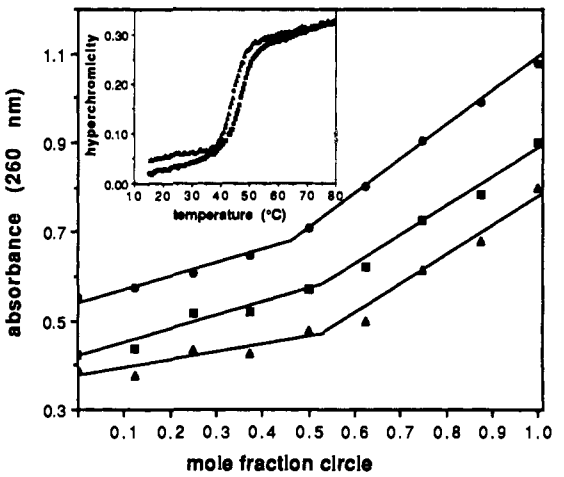


Figure 2. Mixing plots of mole fraction vs absorbance at 260 nm for circle **1** with purine complementary strands. Total DNA strand concentration was 3 μM, with molar ratios being varied as shown. Plots show **1** mixed with dA_9 (▲), **1** with d(AG)4A (■, offset 0.1 AU), and **1** with a 1:1 mixture of dA_9 and $d(AG)_4A$ (●, offset 0.3 AU). From the plots, mole fractions for full complexation are 0.52, 0.53, and 0.47, respectively. (Inset: Melting profiles of hyperchromicity vs temperature for 1:1 complexes of circle **1** with dA_9 (T_m = 48.0 °C) and **1** with $d(AG)_4A$ (T_m = 44.6 °C) at pH 7.0. Experiments were carried out as described.[7]

48%.[5,6] Also prepared were two nine-base purine oligomers, dAAAAAAAAA (**2**) and dAGAGAGAGA (**3**) (Figure 1).

As expected from its non-self-complementary sequence, compound **1** alone shows no significant temperature-dependent melting behavior when examined at 260 nm in a pH 7.0 buffer containing 100 mM NaCl and 10 mM $MgCl_2$. In the presence of 1 equiv of the oligomer dA_9 (**2**), however, there is a sharp, apparently two-state transition showing a hyperchromicity of 23% and a

(1) The phosphorylated 36-mer was purchased from Midland Certified Reagents (Midland, TX) and was prepared using the standard β-cyanoethyl phosphoramidite method.[2] Other oligomers were synthesized on a Pharmacia-LKB instrument, using this same chemistry.
(2) Beaucage, S. L.; Caruthers, M. H. *Tetrahedron Lett.* **1981**, *22*, 1859-1862.
(3) The BrCN/imidazole/Ni^{2+} ligation method has been shown to give the natural DNA phosphodiester linkage: (a) Kanaya, E.; Yanagawa, H. *Biochemistry* **1986**, *25*, 7423-7430. (b) Ferris, J. P.; Huang, C.-H.; Hagan, W. J. *Nucleosides Nucleotides* **1989**, *8*, 407-414. (c) Luebke, K. J.; Dervan, P. B. *J. Am. Chem. Soc.* **1989**, *111*, 8733-8735.
(4) (a) Kool, E. T. *J. Am. Chem. Soc.* **1991**, *113*, 6265-6266. (b) Prakash, G.; Kool, E. T. *J. Chem. Soc., Chem. Commun.* **1991**, 1161-1163. (c) Prakash, G.; Kool, E. T. *J. Am. Chem. Soc.* **1992**, *114*, 3523-3527.
(5) Oligonucleotide concentrations were measured by absorbance at 260 nm. Extinction coefficients were calculated by the nearest-neighbor method.[6] The reaction was allowed to proceed for 12 h at 23 °C, and the product was isolated by preparative denaturing gel electrophoresis. The mobility of the circular product on a 20% denaturing polyacrylamide gel was 0.90 that of the 36-nucleotide linear precursor. Conversion to circular product was ≥95% in 12 h, as judged by UV shadowing. The circularity of the product was confirmed by complete resistance to cleavage by exonuclease activity (T4 polymerase, Promega) under conditions that completely cleave a linear sequence to mononucleotides.
(6) Borer, P. N. In *Handbook of Biochemistry and Molecular Biology*; Fasman, G. D., Ed.; CRC Press: Cleveland, 1975; p 589.

0002-7863/93/1515-360$04.00/0