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).

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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.

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Binding of Two Different DNA Sequences by Conformational Switching

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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′-dTCTCTTTTTTTTTTCTCTCTCTTTTTTTTTTCTCp.[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

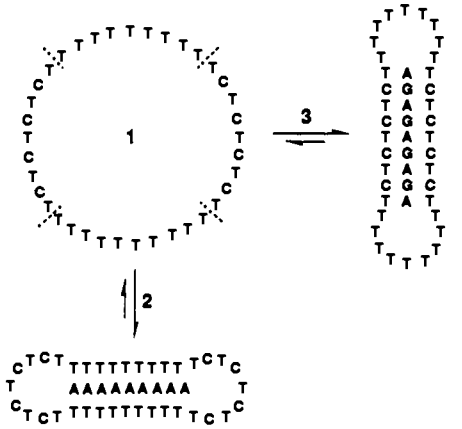

Figure 1. Sequence of macrocycle **1** and the conformational changes involved in the binding of **1** to the sequences **2** and **3**. Dotted lines demarcate the four domains in **1**. When both sequences are present, the macrocycle binds to one of the two as shown, but cannot bind to both simultaneously.

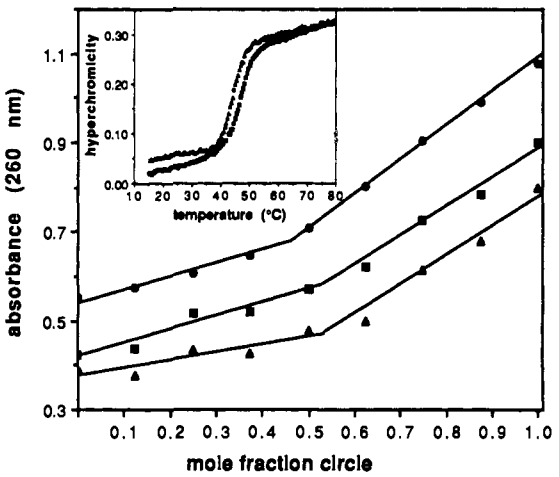

Figure 2. Mixing plots of mole fraction vs absorbance at 260 nm for circle **1** with purine complementary strands. Total DNA strand concentration was 3 μM, with molar ratios being varied as shown. Plots show **1** mixed with dA_9 (▲), **1** with d(AG)4A (■, offset 0.1 AU), and **1** with a 1:1 mixture 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.
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(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.

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melting temperature (T_m) of 48.0 °C (Figure 2 (inset)).[7] This is a considerably higher T_m than that observed for the duplex $dA_9 \cdot dT_9$ (T_m = 28.7 °C) under identical conditions. This high affinity is consistent with the bimolecular triple helical structure expected for this complex,[4] with the purine oligomer held between opposing dT_9 domains in the macrocycle.

Significantly, the macrocycle displays almost identical binding behavior with a second, distinctly different sequence. When added to the oligomer dAGAGAGAGA (**3**), a melting transition having a 22% hyperchromicity and a T_m of 44.6 °C is seen at pH 7 (Figure 2). Once again, this is significantly higher than the corresponding duplex $d(AG)_4A \cdot d(TC)_4T$, which has a T_m of 32.7 °C under these conditions.

Several observations support the involvement of two opposing binding domains, resulting in triplexes, for the two complexes. Mixing plots carried out at 25 °C between the circle and **2** (Figure 2) show that the stoichiometry of the complex is 1:1; if both dT_9 domains were involved in duplexes, the expected stoichiometry would be 2:1 dA_9:circle. Similarly, a mixing plot for the circle and dAGAGAGAGA reveals a stoichiometry of 1:1 as well (Figure 2). In addition, the denaturation of complex **1·2** shows a significant hyperchromicity of 13% at 284 nm, consistent with reported properties of $T_n \cdot A_n \cdot T_n$ triple helices and not duplexes.[8] Finally, the melting transition for complex **1·3** is pH dependent, with a higher T_m of 56.2 °C being observed at pH 6.0; this is again consistent with a triplex protonated at cytosines in the Hoogsteen strand.[9]

The formation of these two different complexes requires that the macrocycle adopt two opposite conformations in the binding of **2** and **3** (Figure 1). In forming a complex, two opposed nine-base domains move inward and align themselves with the complementary purine strand on opposite sides, forming two or three hydrogen bonds on each side of a base. The unbound nine-base domains then act as loops for bridging the bound domains. In order to bind the other sequence, the pairs of domains reverse roles, with the former binding domains acting as bridging loops and vice versa.

Interestingly, a further mixing experiment reveals that this complexation is mutually exclusive: the act of binding to one sequence precludes the binding of the other, even though the necessary bases are unpaired in the loops. When the circle is mixed at varied molar ratios with a one-to-one mixture of the two sequences (Figure 2), the mixing plot shows a stoichiometry of 1:1. This indicates that a given complex cannot bind the second sequence with its loops at 25 °C. While this macrocycle at all times carries the bases necessary for forming complexes with the two sequences, the act of binding causes a conformational switch, holding the loops in an inaccessible or unproductive conformation.[10]

To confirm that the macrocycle can recognize these two sequences within a longer strand of DNA, we synthesized a 33-nucleotide oligomer having the sequence 5′-dCACAAGAGAGAGAATCCCTAAAAAAAAAAACAC (**4**). This oligomer contains the two recognition sequences separated by seven bases. When mixing experiments are performed (pH 7.0, 25 °C) with this oligomer and the macrocycle **1**, a plot of absorbance versus mole fraction (not shown) clearly demonstrates a binding stoichiometry of two macrocycles to 1 equiv of **4**; this confirms that the unusual binding property is also seen with two sites in one strand.

The results demonstrate a new strategy for the binding of two or more specific nucleic acid sequences with a single ligand. Such a strategy may be useful in the design of multifunctional biosensors or of therapeutics directed to multiple medicinally important targets.

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(7) Thermal denaturations were performed on a Cary 1 spectrophotometer in 1 cm path length stoppered quartz microcells under an N_2 atmosphere. The cells were monitored at 260 nm, with a temperature rise of 0.5 °C/min. Mixtures were 3 μM in each DNA strand and contained 10 mM Na·PIPES buffer (pH 7.0), 100 mM NaCl, and 10 mM $MgCl_2$. T_m's were taken as the inflection point in the denaturation curve and are estimated to be accurate to within ±0.5 °C.

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Gambierol: A New Toxic Polyether Compound Isolated from the Marine Dinoflagellate *Gambierdiscus toxicus*

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We have previously isolated ciguatoxin congeners and maitotoxin from natural blooms of the marine dinoflagellate *Gambierdiscus toxicus* and assumed that *G. toxicus* is the biogenetic origin of toxins implicated in ciguatera fish poisoning, which is prevalent in tropical regions.[1,2] Despite efforts by many scientists over a decade, no toxins other than maitotoxin could be isolated from *G. toxicus* cultures.[3] Gambieric acids produced by the GII-1 strain of *G. toxicus* were practically nontoxic and structurally different from ciguatoxin.[4] Thus, a troubling question of the true origin of ciguatoxins persisted. We recently collected and tested several new clones for possible genetic variation and found a clone that produced toxins other than maitotoxin. In this communication we report the structure of gambierol (**1**), a new toxin reminiscent of ciguatoxin by its ladder-shaped polyether skeleton.

G. toxicus (RGI-1 strain) collected at Rangiroa Atoll, Tuamotu Archipelago, French Polynesia, was cultured in seawater medium enriched with ES-1 nutrients at 25 °C for 21 days.[5] Cultured cells were extracted with MeOH, and the extract was partitioned between CH_2Cl_2 and MeOH/H_2O (6:4). The toxin was extracted into the organic phase and was further purified guided by mouse bioassay.[6] From 1100-L cultures was obtained 1.2 mg of **1** as an amorphous solid: HR-FABMS $[M + Na]^+$ m/z 779.4348, calcd for $[C_{43}H_{64}O_{11}Na]^+$ 779.4346; UV_{max} (MeOH) 237 nm (ε 15 800; LD_{50} in mouse (ip) 50 μg/kg. The ^1H NMR spectrum showed five singlet methyls, three hydroxyls, and four olefins (two conjugated). Analyses of ^1H–^1H COSY and 2D HOHAHA spectra allowed us to connect protons H1–H6, H8–H10, H12–H18, H19–H20, H24–H29, and H31–H38.[7] Protons H_2-18 and

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