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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(6) The $CH_2Cl_2$ extract was successively chromatographed over Florisil ($Me_2CO$/MeOH, 9:1); Toyopearl HW-40 (MeOH); and Asahipak ODP-50 (MeCN/$H_2O$, 45:55).

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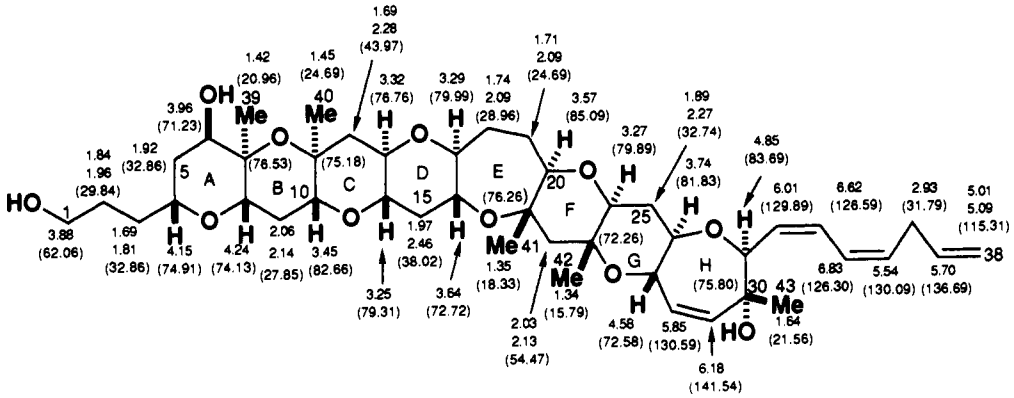

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**Figure 1.** Structure and NMR assignments of **1**. $^1H$ and $^{13}C$ chemical shifts in parentheses are those in $C_5D_5N$.

$H_2$-19 were difficult to connect by COSY because of the closeness of their chemical shifts. However, a 2D HOHAHA spectrum clarified their connectivity by a cross peak due to a multiple relayed coupling between H17 and H20. The number of methylenes present between C17 and C20 was determined to be two by HSQC measurements. Connectivities of protons H17-H20 were thus established. Five oxygen-bearing quaternary carbons observed in the $^{13}C$ NMR spectra explained the disconnection of spin systems. Clearly, five singlet methyls (1.34-1.64 ppm) observed in the $^1H$ NMR spectrum resided on these quaternary carbons and were useful for HMBC experiments. Cross peaks due to $^{2,3}J_{CH}$ from methyl groups were shown for Me-39 vs C6/C7/C8, Me-40 vs C10/C11/C12, Me-41 vs C20/C21/C22, Me-42 vs C22/C23/C24, and Me-43 vs C29/C30/C31. Connectivities around the quaternary carbons were thus clarified and allowed us to assemble partial structures into a skeletal structure (Figure 1).

NOEs between angular protons or between an angular proton and a singlet methyl, as observed in NOESY and NOE difference spectra, supported the notion that ether rings A-H were trans-fused. No NOE between H16 and Me-41 was observed at room temperature, probably due to perturbation of ring E, but was clearly detected at -20 °C, as had been the case with ciguatoxin.[2] Coupling constants of angular protons (10 Hz) also supported trans-fusion of rings.[2] Positions of 1-OH and 6-OH were deduced from couplings of the hydroxyl protons with $H_2$-1 and H6, respectively. HMBC experiments clarified the position of 30-OH, by revealing a cross peak due to $^3J_{CH}$ coupling between the hydroxyl proton and C43. NOE difference spectra showed NOEs between H6 and Me-39, and between H27 and Me-43, thereby indicating that both 6-OH and Me-43 are in $\beta$ configuration. The geometry of double bonds C32=C33 and C34=C35 was determined to be *Z* from proton coupling constants (11 Hz). The above results led to **1** as the structure of gambierol, including relative stereochemistry. Assignment of all $^1H$ and $^{13}C$ signals of **1** was achieved by analysis of HSQC spectra.

Production of **1** by cultured *G. toxicus* and the resemblance between gambierol (**1**) and ciguatoxin in molecular size, chromatographic properties, and symptoms caused in mice strongly support our hypothesis that *G. toxicus* is the true cause of ciguatera. Structural elucidation was accomplished with only 1.5 $\mu$mol of the material. The ring system of **1** (6/6/6/6/7/6/6/7) differs from previously known polyethers, e.g., brevetoxins,[8] yessotoxin,[9] and ciguatoxins,[2] thus demonstrating the diversity of ciguatera toxins and the complex biosynthesis of polyether compounds in dinoflagellates.

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**Supplementary Material Available:** 1D NMR, 2D HOHAHA, $^1H$-$^1H$ COSY, HSQC, and HMBC spectra (8 pages). Ordering information is given on any current masthead page.

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## Enhanced Imidazole-Catalyzed RNA Cleavage Induced by a Bis-Alkylguanidinium Receptor

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DNA and RNA cleavage agents have received considerable attention for multiple purposes,[1,2] including such health-related goals as the coupling of an RNA hydrolytic catalyst to antisense DNA[3] for use in mRNA gene therapy.[4] RNA hydrolytic catalysts have typically been metal complexes and amines. Metal complexes are quite efficient,[5,6] but they can be complicated by lability and toxicity.[6] Simple amine catalysts yield appreciable cleavage,[7] but they often require elevated concentrations and/or

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