(10.44 Å vs 10.72 Å[28b] or 10.64 Å in the Watson-Crick pair of **2**). The geometry of the Watson-Crick base pair between the G and the C2 ring is, except for the somewhat larger propeller twist (11.9°), very similar to that observed in (9-EtGH)·(1-MeC)[28a] or $(GpC)_2$.[28b] The base pair between the neutral and the protonated C3 rings is normal.[29] The latter is virtually parallel to the guanine (dihedral angle 1.5°) and almost parallel to the platinated C1 ring and the hydrogen-bonded C2 ring (dihedral angles 9° and 12.1°, respectively). Within the crystal, platinated base triples and the (1-MeC)(1-MeCH)$^+$ base pairs occur in alternating layers, with considerable stacking between the heterocyclic rings. The chloride anions as well as the water molecules are involved in extensive hydrogen bonding with no unusual features apparent.

The (metal-modified) nucleobase triple observed in **2** represents, to the best of our knowledge, the first example of its kind and is different from tertiary base pairs found in tRNAs.[30] The results of the X-ray structure determination strongly suggest that covalent binding of a pyrimidine oligonucleotide strand to a DNA duplex via a linear *trans*-a_2Pt^{II} entity (a = NH_3 or amine) is sterically feasible. We assume that, provided the oligonucleotide is sufficiently long, recognition and H-bond formation with the target sequence will be much faster than covalent binding of the Pt to the target. Thus, specific rather than unspecific binding of a platinated oligonucleotide to DNA appears to be possible. Work in our laboratory is in progress to apply this binding principle to oligonucleotides.

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Supplementary Material Available: Experimental details for the structure determination of **2** and tables of atomic positional and thermal parameters, bond distances and angles, intermolecular distances and angles, and least-squares planes for **2** (13 pages); listing of F_o and F_c for **2** (29 pages). Ordering information is given on any current masthead page.

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Enantiomeric Cholesterol as a Probe of Ion-Channel Structure

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ent-Cholesterol has been prepared for the first time as a single isomer to probe the role of sterols in ion-channel formation.[2,3] It was prepared by enantioselective total synthesis and shown to have >97% ee by optical rotation and Mosher's ester analysis[4] of the intermediate *ent*-testosterone.[5] Cholesterol is a vital component of mammalian membranes that is required for proper membrane protein function[6,7] and plays an important role in human health. Its primary activity is to stabilize membranes and mediate their fluidity.[8] *ent*-Cholesterol can be used to probe the role of cholesterol in biological systems. Wherever cholesterol binding is important, substitution by *ent*-cholesterol will lead to diastereomeric interactions resulting in measurably different behavior.

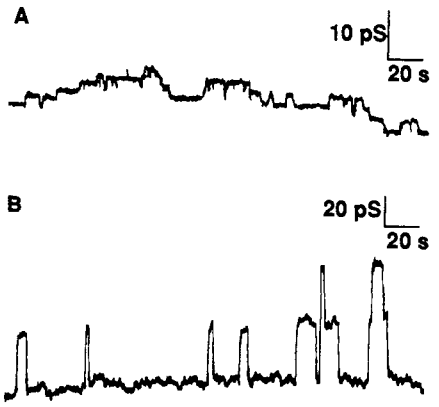

Figure 1. Amphotericin B ion channels in soy azolecithin with cholesterol. (A) Cholesterol, 5% in azolecithin, 2×10^{-8} M amphotericin B, 2 M KCl, 0.1 M HEPES buffered to pH 7.0, 120 mV. (B) *ent*-Cholesterol, 5% in azolecithin, 2×10^{-7} M amphotericin B, 2 M KCl, pH 7.0, 120 mV. Membranes were formed by painting lipid solutions across a 0.1-mm hole in a Teflon partition. Membrane-forming solutions were 1-5% lipid in decane (w/v), doped with 5% (w/w) cholesterol to lipid. Membranes were formed in the presence of amphotericin B. All records were filtered at 20 Hz.

Enantiomers can be used to distinguish between specific binding interactions and nonspecific associations. Enantiomers will have identical physical properties in an achiral environment, but can often be distinguished through diastereomeric complex formation with a chiral probe molecule. This is the basis for enantiomer analysis by NMR spectroscopy using chiral shift reagents[9] and for chromatographic resolutions using chiral stationary phases.[10] The same strategy can be used to test for binding between chiral components in a complex system. If each enantiomer of a biologically active compound has identical properties in a complex environment like a cell, then the biological activity does not result from a specific binding interaction. For example, the two enantiomers of the antibiotic lasalocid A have identical biological properties, and thus their biological activity does not involve specific binding to a receptor or any other chiral cellular component.[11] On the other hand, the *R* and *S* enantiomers of carvone smell like spearmint and caraway, respectively, and this alone demonstrates that the sense of smell involves specific binding.[12,13]

Amphotericin B is a polyene macrolide antibiotic used to treat life-threatening systemic fungal infections that are often found in patients with impaired immune systems. Its activity is attributed to the formation of ion channels in cell membranes containing sterols.[14] In the most widely accepted model, amphotericin B and the membrane sterol from a complex, and several complexes assemble in the membrane to form an ion channel.[15,16] This model

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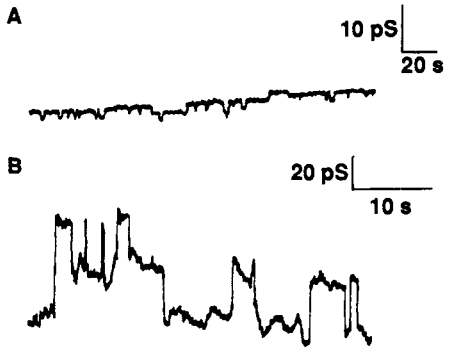

Figure 2. Amphotericin B ion channels in racemic glycerol monooleate with cholesterol. (A) Cholesterol, 5% in glycerol monooleate, 2×10^{-8} M amphotericin B, 2 M KCl, 0.1 M HEPES buffered to pH 7.0, 120 mV. (B) *ent*-Cholesterol, 5% in glycerol monooleate, 2×10^{-7} M amphotericin B, 2 M KCl, pH 7.0, 120 mV. Membranes were formed as in Figure 1. All records were filtered at 20 Hz.

nicely accounts for the sterol requirement[17] in ion-channel formation, and sterol binding provides a rational explanation for the greater sensitivity of ergosterol-containing fungal cells than of cholesterol-containing mammalian cells. A good deal of circumstantial evidence supports this model,[18] but there is no direct evidence that distinguishes between ion-channel formation mediated by sterol modification of membrane properties and sterol binding with amphortericin B. This distinction is significant because the two models lead to different strategies for increasing the therapeutic index of amphotericin B.

We have found that amphotericin B forms different ion channels in the presence of natural cholesterol and *ent*-cholesterol. We measured two-sided, single-channel conductances in black lipid membranes using soy azolecithin containing 5% cholesterol or *ent*-cholesterol.[19] The initial experiments were carried out using coded samples of cholesterol and its enantiomer to avoid operator bias. Single channels of 1-3 pS conductance were observed in natural cholesterol membranes at an amphotericin B concentration of 2×10^{-8} M, in accord with previous reports.[20] Membranes containing *ent*-cholesterol did not support any ion channels at these amphotericin B concentrations, but ion channels were observed at a 10-fold higher amphotericin B concentration. These new ion channels had a much higher conductance, 30-35 pS, than those formed in the presence of natural cholesterol (Figure 1). At this amphotericin B concentration, natural cholesterol membranes show higher bulk conductances than *ent*-cholesterol membranes. Membranes without sterols did not form ion channels, even at 10-fold higher amphotericin B concentration than required with *ent*-cholesterol membranes. Amphotericin B samples obtained commercially and those purified to homogeneity by C8 reverse-phase MPLC gave the same results, demonstrating that amphotericin B is required for both ion channels.

Soy azolecithin is composed of a mixture of enantiomerically pure chiral phospholipids that form diastereomeric membranes when combined with natural cholesterol and *ent*-cholesterol. Previous studies suggest that the lipid chiral center does not affect membrane properties; indeed membranes composed of natural cholesterol and the two enantiomers of dioleoyllecithin are indistinguishable.[21] We tested membranes prepared from soy azolecithin and the two enantiomers of cholesterol by comparing their ability to support gramicidin ion channels. Gramicidin A forms identical ion channels in membranes with 5% natural cholesterol or *ent*-cholesterol in soy azolecithin, demonstrating that gramacidin ion channels do not bind cholesterol,[22] and that the two diastereometric membranes are indistinguishable in this simple test.

Studies with racemic lipids confirm the stereochemical dependence of ion-channel formation. Synthetic, racemic glycerol monooleate was used to prepare membranes with 5% natural cholesterol or *ent*-cholesterol. In accord with the soy azolecithin membrane studies, purified amphotericin B produced low-conductance channels with natural cholesterol and high conductance channels with *ent*-cholesterol (Figure 2). The two glycerol monooleate membranes are exact mirror images of each other and have identical physical properties, so the differences observed cannot be attributed to macroscopic membrane properties.

Amphotericin B produces different ion channels in the presence of natural cholesterol or *ent*-cholesterol, and the distinction cannot be attributed to differences in membrane properties. Amphotericin B specifically binds the enantiomers of cholesterol, thus producing diastereomeric ion channels that have measurably different properties. This is the first direct proof that amphotericin B binds to cholesterol *in the ion-channel structure*. Cholesterol plays a vital role in biochemical systems throughout the body, and *ent*-cholesterol will be a valuable new probe to explore its function.

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Terminal Difluoro Olefin Analogues of Squalene Are Time-Dependent Inhibitors of Squalene Epoxidase

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Squalene epoxidase (EC 1.14.99.7) catalyzes the conversion of squalene to (3*S*)-2,3-oxidosqualene,[1] an essential step in the biosynthesis of sterols in mammals, plants, and microorganisms. The chemical and kinetic mechanisms of squalene epoxidase are not known, but the enzyme requires O_2, NADPH, and FAD for full activity;[2] it is not a cytochrome P-450.[3,4] Many reversible squalene epoxidase inhibitors have been described;[5-8] some are useful antifungal[6,7] and hypolipidemic[8] agents. We report the time-dependent inhibition of squalene epoxidase from rat liver

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