The ready reversibility of the sulfametallacycle (**10a**)-terminal sulfide (**9a**) transformation suggests that $Cp_2^*Zr{=}S$ is more stable than $Cp_2^*Zr{=}O$ with respect to cycloaddition with alkynes. We are presently exploring the reactivity of these terminal oxo and sulfido complexes with other unsaturated organic fragments such as olefins, aldehydes, and ketones. Also, metallacyclobutenes such as **3** and **10** promise to exhibit rich M-C and perhaps M-O(S) insertion chemistry (as do their nitrogen-containing analogues),[13] and experiments designed to test this expectation will be reported in due course.

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Supplementary Material Available: Spectroscopic and analytical data for complexes **2-10c** and details of the structure determinations for **9a** and **10b** including experimental descriptions, ORTEP drawings showing full atomic numbering, crystal and data collection parameters, general temperature factor expressions (B's), positional parameters and their estimated standard deviations, and intramolecular distances and angles (30 pages); listing of observed and calculated structure factors for **9a** and **10b** (34 pages). Ordering information is given on any current masthead page.

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Sugars as DNA Binders: A Comment on the Calicheamicin Oligosaccharide

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Preorganization is one of the first principles of tight binding.[1] In general, the more the conformation of a free ligand resembles its bound conformation, the better it binds. Small molecules that bind in the minor groove of DNA tend to be extended structures that present an essentially acyclic array of functionality to the groove and nature uses a variety of strategies to rigidify these molecules.[2,3] The recent discovery of calicheamicin γ^1, a small antitumor antibiotic that cleaves DNA site specifically, has drawn attention to the important role that oligosaccharides may play in DNA binding.[4] Oligosaccharides are components of many natural DNA binders, but their contributions to the energetics and specificity of binding are only beginning to be explored.[5] Below we report the results of NMR studies on the oligosaccharide portion of calicheamicin. We suggest that oligosaccharides may be well suited to function as minor groove binders because they are substantially preorganized. Some of the unusual structural features of calicheamicin are discussed in relation to their possible role in DNA binding.

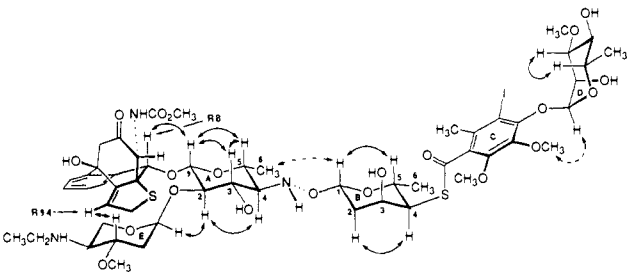

Figure 1. Calicheamicin ϵ. Selected through-space connectivities are indicated by bold (strong NOEs) or dashed (weak NOEs) arrows. ROESY experiments were carried out with 200-300-ms mixing times and an approximately 3-KHz spin-lock field.

NMR studies of calicheamicin ϵ (Figure 1), the rearrangement product of γ^1,[6] were carried out in CD_3OD, $CDCl_3$, and DMSO-d_6, three solvents that differ greatly in their hydrogen-bonding capabilities and dielectric constants. Double-quantum-filtered COSY was used to assign chemical shifts, and rotating frame nuclear Overhauser enhancement spectroscopy (ROESY)[7] was used to determine through-space connectivities (a table of ^{1}H NMR chemical shifts, J coupling constants, and NOEs for the oligosaccharide portion of calicheamicin ϵ is provided as supplementary material). The coupling constants and intraresidue NOEs of three of the sugar rings are virtually identical in all three solvents and are consistent with a single chair conformer for each monosaccharide: $^4C_1$ for the A and B rings and $^1C_4$ for the D ring. Temperature experiments conducted in CD_3OD and $CDCl_3$ indicate that neither the coupling constants nor the chemical shifts of the three sugars change significantly from -50 to +50 °C. Taken together, these results imply that there is a large free energy difference between the lowest energy ring conformers and all others. Conformational rigidity is a general feature of substituted six-membered rings and may make them ideal building blocks for DNA binders. While no experimental information about the bound conformation of the calicheamicin oligosaccharide has been obtained yet, it is clear that the energy cost of significantly distorting the A, B, or D rings upon binding would be substantial.[8]

The room-temperature coupling constants and NOEs of the remaining sugar, the E ring, are consistent with a chair conformation with an axial glycosidic linkage. However, the H1-H2e couplings differ notably in $CDCl_3$ and the more polar solvents,[9] suggesting that there is a degree of conformational flexibility in the E ring. Consistent with this interpretation, the E-ring resonances broaden and shift significantly below 0 °C in CD_3OD.

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(9) ^{1}H NMR ($CDCl_3$) H1 (d, J = 3 Hz); (CD_3OD, DMSO) H1 (t, J = 3 Hz).

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Information about the disposition of the ring systems relative to one another and to other parts of calicheamicin can in principal be obtained from interresidue NOEs. Calicheamicin is an extended molecule and more than one atom intervenes between monosaccharides in several of the linkages, so in practice few interresidue NOEs are observed in the ROESY spectra. The strong NOEs between E1 and A2, and E3 and R14, and the weak NOE from A1 to R8 in both $CDCl_3$ and CD_3OD help define two of the glycosidic linkages and the orientation of the rearranged aglycon to the oligosaccharide. The fact that the NOEs are similar in these different solvents suggests that the molecule is rigid in this region. Such an interpretation is consistent with studies showing that torsional oscillations of glycosidic linkages to secondary alcohols tend to be confined to narrow regions of conformational space.[10-12] Thus, not only are the individual sugars in oligosaccharides rigid, but many of the glycosidic linkages are conformationally restricted as well.

Perhaps the most interesting feature in the calicheamicin oligosaccharide is the N–O linkage between the A and B rings; N–O linkages are quite rate in oligosaccharides. Obviously, the N–O bond could play a role in hydrogen bonding to polar functionalities in the minor groove. It may also be an important structural element that enforces an extended conformation in the central portion of the molecule. Studies by others on hydroxylamine derivatives show that rotation and inversion barriers around N–O bonds can be high, as much as 15 kcal/mol in some instances.[13] However, even at −50 °C, the resonance lines of the A- and B-ring protons in the vicinity of the N–O linkage of calicheamicin do not show signs of slow exchange in either $CDCl_3$ or CD_3OD. Although the temperature studies are equivocal because we do not know either the barrier height or the population distribution around the N–O bond, the results could indicate that there is a preferred conformer of the N–O bond. The existence of a weak NOE between B1 and the A6 methyl group and the fact that the protons in the vicinity of the N–O linkage resonate at almost identical frequencies in all three solvents strongly support this interpretation.

Finally, it is worth noting that these studies were carried out in organic solvents because neither calicheamicin ϵ nor calicheamicin γ^1 is soluble in water at millimolar concentrations. In fact, the calicheamicin oligosaccharide is remarkably hydrophobic—all the sugars are 6-deoxy[14] and there are only four free hydroxyls. It is likely that this hydrophobicity plays a significant role in DNA binding.

In conclusion, NMR studies indicate that the calicheamicin oligosaccharide is substantially preorganized. The ability to adopt a rigid, extended conformation makes oligosaccharides potentially ideal DNA binders. The calicheamicin oligosaccharide may provide insight into additional features necessary to design oligosaccharide-based DNA-binding molecules. In particular, the N–O linkage and the notable hydrophobicity may be important design elements.

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Supplementary Material Available: A table of ^1H NMR chemical shifts, coupling constants, and NOEs for the oligosaccharide portion of calicheamicin ϵ (1 page). Ordering information is given on any current masthead page.

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Enantioselective Total Synthesis of a Protosterol, 3β,20-Dihydroxyprotost-24-ene

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The enzymic cyclization of 2,3-oxidosqualene[1] in sterol biosynthesis is considered to form a primary tetracyclic skeleton (protostane, fusidane), which must undergo rearrangement before common sterols such as lanosterol, the precursor of cholesterol, can be produced.[2] We report herein the first direct total synthesis of the protosterol system, specifically protostenediols **1a** and **1b**, which are of biosynthetic interest, by an effective and enantiospecific route.[3]

Enone **2**[4] and 2-methyl-1,3-cyclohexanedione were converted to the Michael coupling product (Et_3N in dimethoxyethane at 23 °C),[5] which underwent enantioselective aldol cyclization[6] with 1 equiv of (*S*)-phenylalanine and 0.5 equiv of (+)-camphorsulfonic acid in dimethylformamide at 23 °C for 24 days to form **3** [77% yield, 95% ee as determined by ^1H NMR analysis in C_6D_6 with added shift reagent Eu(hfc)$_3$ (Aldrich Co.)]. Recrystallization from ether at −20 °C afforded pure (*S*)-(+)-enedione **3**; $[\alpha]^{23}_D$ + 110.3° (*c* = 4, $CHCl_3$), mp 67–68 °C, 81% recovery. Position-selective and stereoselective annulation of **3** was effected by the following sequence: (1) addition of **3** to a premixed solution of potassium hexamethyldisilazide and Et_3B (1.1:1) in THF at −78 °C, warming to −25 °C, and reaction at −25 °C with diethyl 3-iodopropynephosphonate[7] for 2 h to afford (after sgc) the desired monoalkylation product (86%); (2) hydration of C≡C to give a β-ketophosphonate using 1 equiv of $HgCl_2$ and 1.5 equiv of pyridine in aqueous THF at 23 °C for 36 h; and (3) cyclization of the crude product with 2 equiv of Cs_2CO_3 in THF at 23 °C for 16 h to give stereospecifically the pure tricyclic enone **4** (72%). Reduction of **4** with lithium trisiamylborohydride in THF at −40

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(5) Abbreviations used herein: THF, tetrahydrofuran; sgc, silica gel chromatography; MOM, methoxymethyl; TBS, *tert*-butyldimethylsilyl; TES, triethylsilyl.

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