Communications to the Editor

DNA–Carbohydrate Interactions. Specific Binding of the Calicheamicin γ_1^I Oligosaccharide with Duplex DNA

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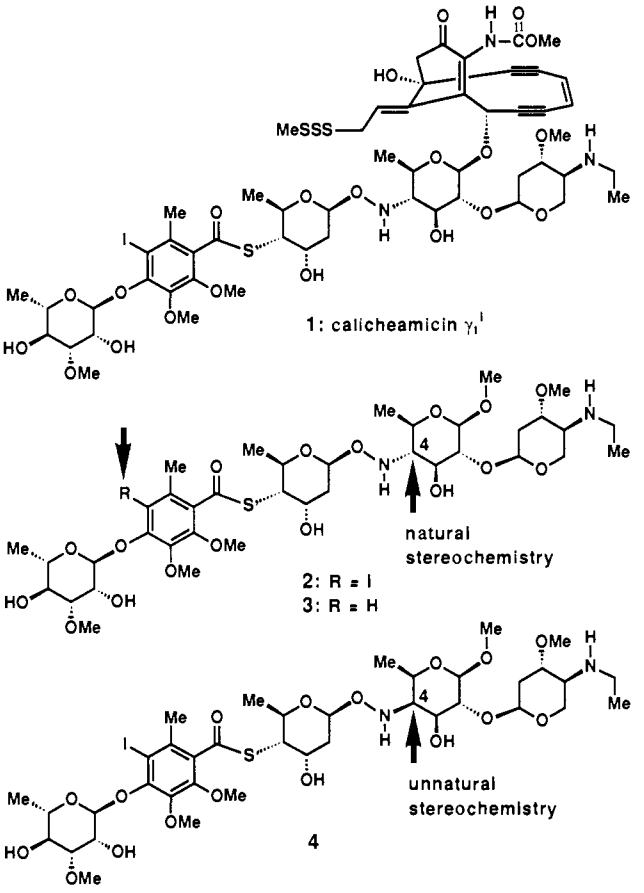
In contrast to DNA-protein[1] and DNA-DNA[2] interactions, DNA–carbohydrate complexation phenomena are rather poorly understood.[3] Due to the importance of such binding in biological systems and due to the recent emergence of several powerful DNA-interacting molecules containing carbohydrate moieties such as the enediyne antibiotics[4] (e.g., calicheamicin γ_1^I (**1**),[5] Chart I), we initiated a program directed toward the understanding of such interactions. Here we describe the first observation of specific DNA binding of the calicheamicin γ_1^I oligosaccharide[6] to DNA sequences and the dependence of this binding on both its iodine content and hydroxylamine nitrogen stereochemistry.

Chart I



Previous studies by the Ellestad,[7] Schreiber,[8] Kahne,[9] and Danishefsky[10] groups suggested selective interactions of the calicheamicin γ_1^I oligosaccharide with duplex DNA along specific sequences within the minor groove. In particular, Schreiber et al.[8] proposed a binding model for this interaction based on both the stereochemistry of the oligosaccharide bonds and the potential of its iodine group to bind to the DNA's nitrogen atoms. Footprinting experiments with synthetic compounds **2–4** (Chart I), designed to probe these questions, demonstrate that although the calicheamicin oligosaccharide binds specifically to DNA, the preferred binding sites do not coincide precisely with those observed for intact calicheamicin.

Oligosaccharides **2–4** were obtained by chemical synthesis as previously described.[6] The 93-base-pair *SalI-SphI* double-stranded DNA fragment of pBR322 was obtained enzymatically and was 5′-^{32}P-labeled on one strand.[11] The oligosaccharides were

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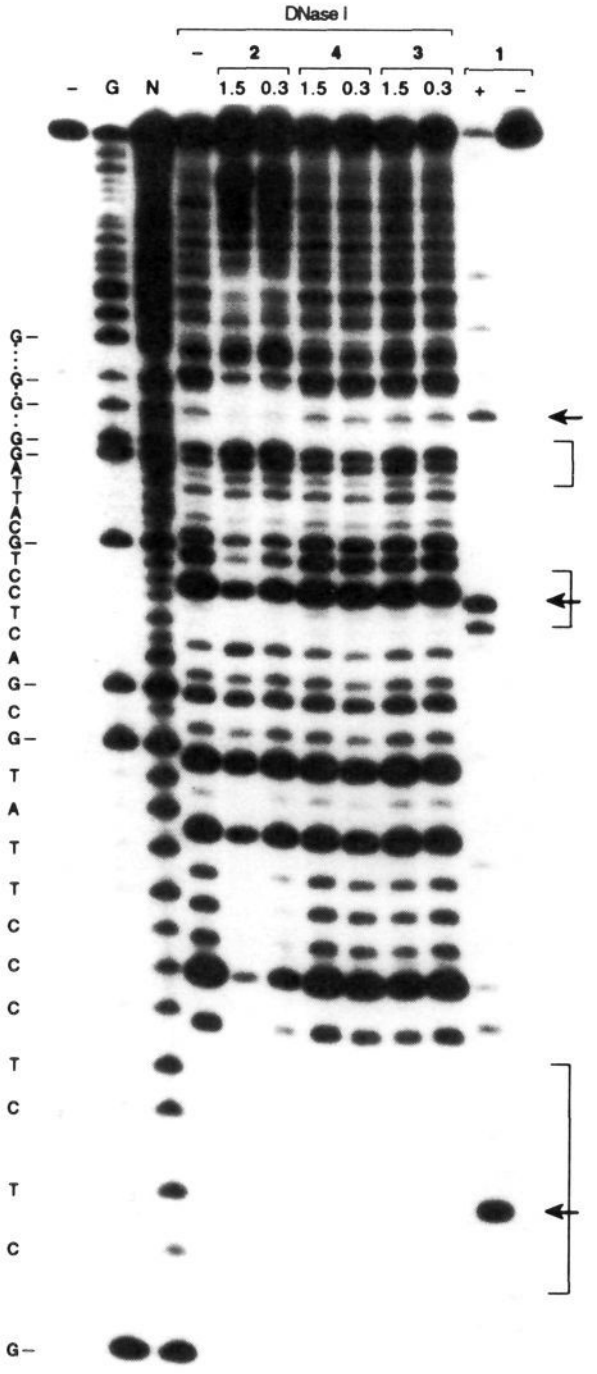

Figure 1. DNase I footprinting of oligosaccharides **2**, **3**, and **4** bound to the 5′-^{32}P-end-labeled *Sal*I–*Sph*I fragment of pBR322: –, unincubated DNA; G, guanosine residues determined by Maxam–Gilbert chemical sequencing; N, piperidine cleavage ladder. The nucleotide sequence of the labeled strand of the DNA fragment is shown at the left. DNase I digestion was carried out in either the absence (–) or presence (**2**, **4**, **3**) of either 1.5 or 0.3 mM oligosaccharide. Oligosaccharide and DNA fragment were preincubated in the presence of 10 mM KCl, 10 mM $MgCl_2$, 5 mM $CaCl_2$, 10 mM Tris·HCl (pH 7.5), and 2 μg/μL salmon testes DNA at 37 °C for 30 min; 0.025 U/μL of DNase I was added, and incubation was continued at 37 °C for 2 min. For comparison, 3.7 μM calicheamicin γ_1^I (**1**) was incubated with end-labeled DNA fragment, 4.5 mM Tris·HCl (pH 7.5), 1% DMSO (v/v), and 0.25 μg/μL salmon testes DNA, in either the presence (+) or absence (–) of 5 mM dithiothreitol; DNA cleavage occurs under the former condition. Expected calicheamicin γ_1^I binding sites (brackets) and cleavage positions (arrows) are indicated at the right. Materials were separated by electrophoresis in a 20% polyacrylamide/8 M urea gel, an autoradiogram of which is shown.

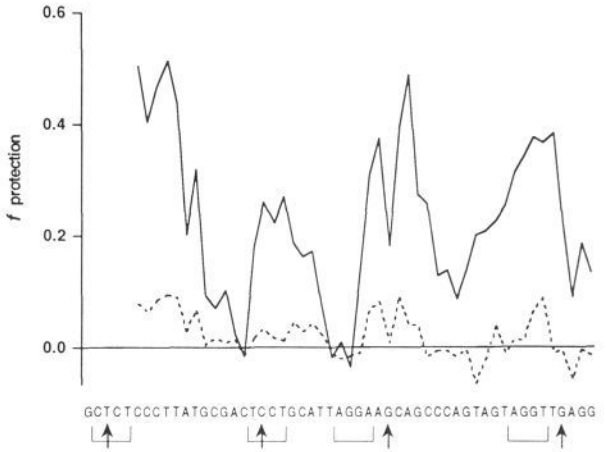

Figure 2. DNase I footprinting data for interaction of carbohydrates **2** (solid line) and **4** (dashed line) with the *Sal*I–*Sph*I fragment of pBR322. *Replicate footprinting experiments were carried out as described in the* caption below Figure 1. Individual bands were cut from the gel and measured by Cerenkov counting. Fractional protection was calculated for each nucleotide position by [ln (cpm, absence) – ln (cpm, presence)]/ln (cpm, absence), where *absence* refers to the control with DNase I alone and *presence* refers to the addition of 1.5 mM carbohydrate. The nucleotide sequence of the labeled strand of the DNA fragment is shown at the bottom. Expected calicheamicin γ_1^I binding sites (brackets) and cleavage positions (arrows) are indicated.

preincubated with the DNA fragment at 37 °C for 30 min, and DNase I was added to cleave the DNA and generate footprinting patterns such as that shown in Figure 1. As seen from the autoradiogram, the nautral oligosaccharide **2** provides concentration-dependent protection, presumably due to binding, in the region of three calicheamicin γ_1^I binding sites: CTCT, TCCT, and AGGA (reading 5′→3′ along the labeled strand). The oligosaccharide **4**, with the unnatural stereochemistry at the hydroxylamine nitrogen-bearing C-4 position, offers no protection at the same concentrations. Similar lack of protection under the same conditions was observed with the oligosaccharide **3**, in which the iodine atom has been replaced by a hydrogen.

Our concerns that the protection pattern did not coincide precisely with the observed calicheamicin cleavage sites prompted us to carry out replicate footprinting experiments over a 48-base-pair segment of the DNA fragment. The degree of protection against DNase I digestion offered by both the natural and unnatural stereoisomers was determined by comparing the amount of radioactivity in adjacent lanes along the electrophoresis gel (Figure 2). The protected regions coincide only roughly with calicheamicin γ_1^I recognition sequences[7,8] and observed calicheamicin γ_1^I cleavage sites (Figure 1). The disparity may be due to (1) imprecise DNA recognition by the carbohydrate moiety alone; (2) impaired access of DNase I at sites adjacent to bound carbohydrate; and (3) distortion of the DNA duplex adjacent to bound carbohydrate which prevents functional recognition by DNase I. Nonetheless, this data does demonstrate selective binding of oligosaccharide **2** with specific base sequences of DNA and defines as crucial the natural stereochemistry at C-4.

These results show that some, but not all, of the binding specificity of calicheamicin γ_1^I resides in its carbohydrate domain. The data are consistent with Schreiber's hypothesis concerning the importance of the iodine and carbohydrate stereochemistry to DNA binding. However, it appears that the enediyne moiety confers added specificity to the calicheamicin/DNA interactions. Furthermore, it should be emphasized that while reduced binding

(11) pBR322 DNA was cleaved at the unique *Sal*I site, dephosphorylated using calf intestine phosphatase, 5′-^{32}P-phosphorylated using T4 polynucleotide kinase and [γ-^{32}P]ATP (4.5 μCi/pmol), and cleaved again at the unique *Sph*I site. This generated a 93-base-pair double-stranded fragment, 5′-^{32}P-labeled at the *Sal*I end, which was purified by gel electrophoresis in a nondenaturing 10% polyacrylamide gel and subsequent column chromatography on Sephadex G-25.

affinity is demonstrated for compounds **2** and **3**, no conclusions can be drawn regarding the role of iodine or the C-4 stereochemistry in the sequence specificity of calicheamicin γ_1^I. The simple footprinting techniques used to determine these interactions and the power of synthesis to produce a variety of oligosaccharides and glycopeptides should facilitate further studies in this new and important area of molecular recognition.[12]

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(12) A number of partial oligosaccharide fragments of calicheamicin γ_1^I were studied using the footprinting techniques described in this paper, and in each case no specific binding was observed.

Reversible Formation of $(\mu\text{-H})Ru_4(CO)_{10}(\mu\text{-PPh}_2)[\mu_4\text{-}\eta^1(P),\eta^1(P),\eta^1(P),\eta^1,\eta^2\text{-}\{C_6H_4\}PPh]$ from the Electron-Rich Cluster $Ru_4(CO)_{13}(\mu\text{-PPh}_2)_2$: The First Example of a Molecule with a Five-Coordinate Bis(aryl)phosphido Bridge

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Bridging five-coordinate phosphines and phosphido ligands have long been implicated as intermediates in organometallic processes, including ligand exchange.[1] Yet to date only one example of a μ_2- or μ_3-PR_3 ligand has been reported.[2] By analogy, five-coordinate μ_3-phosphido (μ_3-PR_2) groups should be accessible from their μ_2-counterparts. While exploring the chemistry of the electron-rich cluster $Ru_4(CO)_{13}(\mu_2\text{-PPh}_2)_2$ (**1**),[3,4] we discovered a remarkably facile transformation via overall two-electron loss to the novel molecule $(\mu\text{-H})Ru_4(CO)_{10}(\mu\text{-PPh}_2)[\mu_4\text{-}\eta^1(P),\eta^1(P),\eta^1(P),\eta^1,\eta^2\text{-}\{C_6H_4\}PPh]$ (**2**). Cluster **2** contains an example of a five-coordinate phosphido bridge bound simultaneously to three metal atoms. We also describe a unique, reversible phosphido orthometalation and η^2-arene coordination process.

Heating an *n*-heptane suspension of **1** at reflux for 5–6 h results in a remarkably smooth conversion to a single brown product **2**[5] in near-quantitative yield (90%) (Scheme I). The $^{31}P\{^1H\}$ NMR spectrum[6] of **2** at 298 K consists of two singlet resonances, one at low field (δ = 214.31 ppm) the other at high field (δ = 59.57 ppm) relative to those of the parent cluster **1** (δ = 119 ppm). The high-field resonance in **1** reflects a considerable elongation of the

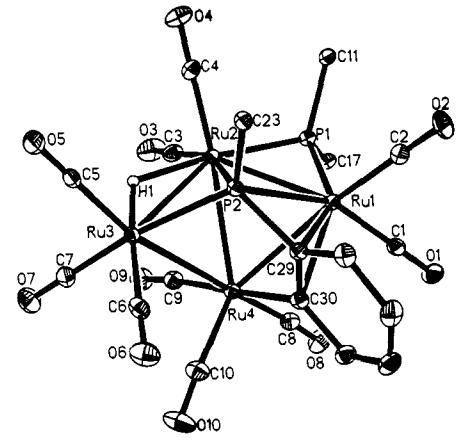
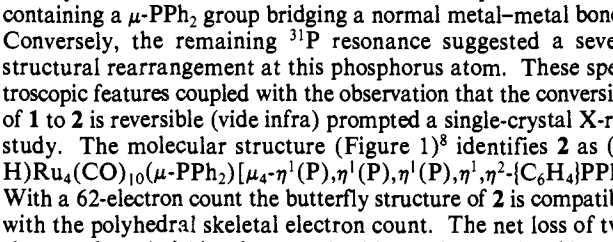


Figure 1. Perspective view of the molecular structure of $(\mu\text{-H})Ru_4(CO)_{10}(\mu\text{-PPh}_2)[\mu_4\text{-}\eta^1(P),\eta^1(P),\eta^1,\eta^2\text{-}\{C_6H_4\}PPh]$ (**2**) showing the atomic numbering. For clarity, only the ipso carbon atoms of the noninteracting phenyl rings are shown. Selected bond lengths and angles: Ru(1)–Ru(2) = 2.829 (1) Å, Ru(1)–Ru(4) = 2.905 (1) Å, Ru(3)–Ru(4) = 2.885 (1) Å, Ru(2)–Ru(4) = 2.916 (1) Å, Ru(2)–Ru(3) = 2.908 (1) Å; Ru(1)–P(2)–Ru(3) = 121.6 (1)°, Ru(1)–P(2)–Ru(2) = 68.3 (1)°, Ru(2)–P(2)–Ru(3) = 74.6 (1)°, Ru(1)–P(2)–C(29) = 61.4 (1)°, Ru(3)–P(2)–C(20) = 111.9 (1)°.

Scheme I[a]



1 **2**

[a] Reagents: (i) 97 °C, *n*-heptane; (ii) CO, 60 °C, *n*-hexane.

Ru–Ru vectors bridged by the phosphido ligands,[3] a fact consistent with the unusual electronic structure of this flat 64-electron butterfly cluster.[7] Thus, the lower field ^{31}P shift of **2** was confidently attributed to the formation of an electron-precise structure containing a μ-PPh_2 group bridging a normal metal–metal bond.[4] Conversely, the remaining ^{31}P resonance suggested a severe structural rearrangement at this phosphorus atom. These spectroscopic features coupled with the observation that the conversion of **1** to **2** is reversible (vide infra) prompted a single-crystal X-ray study. The molecular structure (Figure 1)[8] identifies **2** as $(\mu\text{-H})Ru_4(CO)_{10}(\mu\text{-PPh}_2)[\mu_4\text{-}\eta^1(P),\eta^1(P),\eta^1(P),\eta^1,\eta^2\text{-}\{C_6H_4\}PPh]$. With a 62-electron count the butterfly structure of **2** is compatible with the polyhedral skeletal electron count. The net loss of two electrons from **1** yields a framework with metal–metal bond lengths [2.828 (1)–2.910 (1) Å] and a dihedral angle [117.9°] more consistent with other butterfly structures.[9] The reduction in the phosphido-bridged Ru–Ru bond length [Ru(1)–Ru(2) = 2.828 (1) Å] accounts for the low-field ^{31}P shift of the μ-PPh_2 bridge P(1) [δ = 214.31].[10]

The most unusual features of **2** are the triply bridging nature of the phosphido ligand P(2) [Ru(1)–P(2) = 2.567 (1), Ru-

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(5) Data for **2**: ^1H NMR ($CDCl_3$, 298 K, δ) 8.12 (d, 1 H, $^3J_{PH}$ = 7.9 Hz, H ortho), 7.67 (m, 6 H, H ortho), 7.25 (m, 12 H, phenyl), −14.06 (broad t, 1 H, M*H*M); ^1H NMR (CD_2Cl_2, 196 K, δ) 6.9–8.2 (m, phenyl), −11.13 (t, $^2J_{PH}$ = 23.0 Hz, M*H*M), −20.05 (d, $^2J_{PH}$ = 16.4 Hz, M*H*M). Anal. Calcd for $C_{34}H_{20}O_{10}P_2Ru_4$: C, 38.72; H, 1.91. Found: C, 38.72; H, 1.90.

(6) Data for **2**: $^{31}P\{^1H\}$ NMR (CD_2Cl_2, 298 K, δ) 214.31 (s, br, μ-PPh_2), 59.57 (s, br, $P(C_6H_4)Ph$); (193 K, δ) isomer A 211.83 (s, μ-PPh_2), 38.34 (s, μ-$PPhC_6H_4$), isomer B 199.26 (d, $^2J_{PP}$ = 26.5 Hz, μ-PPh_2), 91.64 (d, $^2J_{PP}$ = 26.5 Hz, μ-$PPhC_6H_4$).

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(8) Deep brown crystals of $(H)Ru_4(CO)_{10}[P\{C_6H_4\}Ph]$ **2** from $(C_2H_5)_2O$ at 298 K are triclinic, space group $P\bar{1}$, with a = 10.287 (1) Å, b = 11.122 (1) Å, c = 15.934 (1) Å, α = 89.67 (1)°, β = 81.66 (1)°, and γ = 81.11 (1)° at 150 K, V = 1781.3 (4) Å^3, d_{calcd} = 1.966 g cm^{-3}, and Z = 2. Data were collected via ω scans on an LT2-equipped Nicolet-Siemens R3m/V diffractometer with graphite monochromated Mo Kα (λ = 0.71073 Å) radiation in the 2θ range 4.0–55°. A total of 8238 reflections were collected, of which 7350 were observed [$F > 6.0\sigma(F)$]. The structure was solved by Patterson and Fourier methods and refined by full-matrix least-squares techniques to yield R = 0.0202 and R_w = 0.0261.

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