The Molecular Basis for Pyrimidine-Selective DNA Binding: Analysis of Calicheamicin Oligosaccharide Derivatives by Capillary Electrophoresis

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Received February 11, 2000

Abstract: Synthetic derivatives of the calicheamicin oligosaccharide were designed to probe the molecular basis for pyrimidine recognition. Binding affinities of the oligosaccharide derivatives for a range of DNA sequences were determined using capillary electrophoresis. The results show that having an iodo-substituted C-ring is neither necessary nor sufficient for pyrimidine-selective binding. Pyrimidine-selective binding depends on maintaining the overall shape and rigidity of the calicheamicin oligosaccharide. These experiments support the proposal that the novel pyrimidine selectivity of calicheamicin is the result of a shape-dependent induced-fit interaction with DNA sequences.

Introduction

Calicheamicin γ_1^I (**1**, Figure 1) is an enediyne antibiotic that is currently in advanced clinical trials for the treatment of different kinds of cancer.[1] It is believed to kill cells by effecting double stranded cleavage of DNA. Calicheamicin has been shown to bind in the minor groove of duplex DNA at pyrimidine tracts including TCCT, TTTT, and CTCT. Following DNA binding, the enediyne moiety rearranges in the presence of reducing agents to form a diradical that abstracts hydrogen atoms from both strands of the DNA backbone, leading to double strand scission.[2]

Calicheamicin has attracted a great deal of attention over the years for several reasons. In addition to having an unusual structure and mechanism of action, it is the first known example of a DNA binder that utilizes a carbohydrate moiety to recognize DNA.[3] It is also the first example of a minor groove binder that is selective for pyrimidine sequences. Most other natural small molecules that bind in the minor groove are selective either for AT-rich regions of DNA or GC-rich regions of DNA.[4] The topology of the minor groove at AT-rich and GC-rich sites is quite different, with AT sites containing a deep minor groove that forms a cleft for flat, aromatic molecules, and GC sites containing a wider and shallower groove suited to larger molecules that can hydrogen bond to the exocyclic amino groups of guanine that protrude into the floor of the groove.[5] Calicheamicin's intriguing sequence selectivity has been the focus of many investigations.

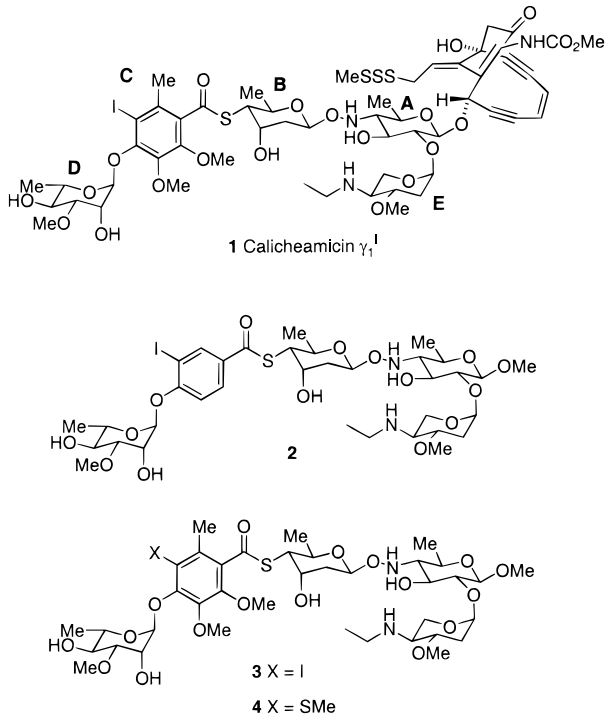

Figure 1. Structures of calicheamicin γ_1^I and synthetic oligosaccharides.

Initial work on the binding selectivity of calicheamicin focused on the role of the iodine atom on the aromatic ring in the recognition of TCCT sites. Schreiber and co-workers suggested that favorable interactions between this iodine atom and the exocyclic guanine amino groups in the floor of the minor

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10.1021/ja000519v CCC: $19.00
Published on Web 08/10/2000

groove are critical for sequence recognition.[6] Removal of the iodine caused a dramatic decrease in binding to TCCT sites, supporting Schreiber's hypothesis.[7] NMR studies showed, however, that the iodine is in close proximity only to the 5′ guanine amino group, implying that only one of the guanine amino groups can interact with the iodine.[8,9] Nucleotide replacement studies confirmed that only the 5′ guanine amino group is essential for binding.[7,10,11] While these experiments implicated the aromatic iodine in the recognition of one G−C base pair, they did not shed any light on the underlying basis for the pyrimidine selectivity.

In fact, the molecular basis for pyrimidine recognition is still unclear. Only a few studies have addressed how calicheamicin can bind to recognition sites as different as TCCT and TTTT with similar affinities. In the preceeding contribution NMR studies on several different calicheamicin−DNA complexes have shown that calicheamicin binds in the same orientation with respect to all pyrimidine recognition sites.[8,12] These results imply that pyrimidine tracts share some common structural features that calicheamicin is able to sense. We have proposed that pyrimidine sequences may be able to distort at a lower energetic cost to accommodate the drug.[8] In independent experiments with nucleosomes and A-tract DNA, Dedon and Townsend have suggested that the drug recognizes some "structural perturbations" associated with 3′ junctions of oligopurine tracts.[13] According to both hypotheses, the shape of the drug is the key determinant of the pyrimidine selectivity.[14] If so, changes to the drug that do not alter the shape significantly should not destroy pyrimidine selectivity. Changes that alter the shape or increase the flexibility substantially should cause a degradation in selectivity.

To evaluate the relative importance of specific interactions versus shape-selective recognition of DNA by the calicheamicin oligosaccharide, we synthesized two synthetic analogues of the oligosaccharide designed to probe these issues. Below we present results demonstrating that shape complementarity plays a more important role than specific attractive interactions in determining pyrimidine-selective binding.

Results

Design. The aryl rhamnoside moiety of calicheamicin (C and D rings, **1**, Figure 1) plays an important role in oligopyrimidine recognition.[15] We synthesized analogues of the calicheamicin oligosaccharide (**3**, Figure 1) in order to test the role of substituents on the aromatic ring in pyrimidine-selective binding. The two analogues were designed (**2** and **4**, Figure 1) to test the individual contributions of overall shape and electronic interactions to the overall binding affinities of the oligosaccharides. Analogue **2** contains the iodine functionality but lacks other substituents on the aromatic ring. This change alters the steric surface that the oligosaccharide presents to the minor groove. It also increases the flexibility of the molecule in the vicinity of the C−D rings compared to the natural oligosaccharide. This analogue enables a direct estimate of the importance of the iodine substituent in binding selectivity in comparison to maintaining the shape of the molecule. We also wanted to determine whether the iodine per se is crucial, or whether it can be replaced by a substituent of comparable size. Therefore, we synthesized an oligosaccharide analogue (**4**) in which the iodine was replaced with a methylthio substituent. Analogue **4** presents the same steric surface[16] and maintains the rigidity of the natural system but has different electronic properties along one edge of the aromatic ring. If pyrimidine selectivity depends on maintaining the overall shape and rigidity of the oligosaccharide, then one would predict that **4** should be a pyrimidine selective binder that binds preferred recognition sites with similar affinity as the natural calicheamicin oligosaccharide. Conversely, if attractive interactions involving the iodine are critical, then the binding affinity and pyrimidine selectivity should decrease substantially.[17]

Synthesis of Calicheamicin Oligosaccharide Analogues. Analogue **2** was synthesized following a convergent route previously developed for the natural oligosaccharide **3** (Figure 1),[18] using the known compound 3-iodo-4-hydroxy-benzaldehyde[19] as the modified C-ring.

The synthesis of analogue **4** is described in Scheme 1. The protected phenolic rhamnoside **5**[18] was converted to **7** by the exchange of protecting groups, followed by the substitution of the iodine with a methylthio functionality by treatment with methyllithium at −78 °C and subsequent quenching with dimethyl disulfide. Protecting-group exchange followed by deprotection of the benzylic alcohol with buffered pyridinium hydrofluoride[20] and Swern oxidation[21] revealed the aromatic aldehyde **10**. Oxidation of the aldehyde functionality to the

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(17) The dipolar interaction between the exocyclic nitrogen of guanine and the iodine should be significantly stronger than that between the external methyl group of the methylthio functionality and the guanine amino group. The internal sulfur atom is not expected to contact the DNA due to steric hindrance from the external methyl group and the two ortho-functionalities on the aromatic ring (see Figure 1).

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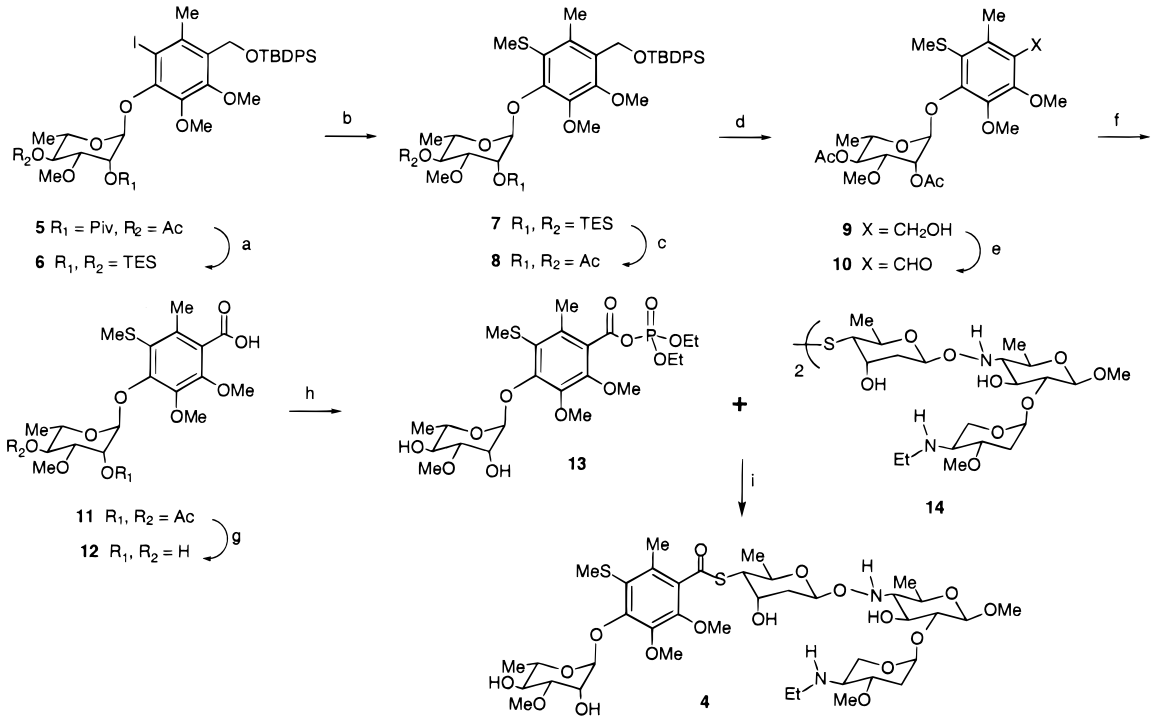
Scheme 1. Synthesis of the Calicheamicin Oligosaccharide Analogue 4[a]



[a] Reagents and conditions: (a) DIBAL-H, Et_2O, −78 °C; TESOTf, Et_3N, CH_2Cl_2 (70%, 2 steps); (b) MeLi, Et_2O, −78 °C, then MeSSMe, −78 °C to −40 °C, then Et_3N (74%); (c) AcOH, THF, H_2O (3:1:1); Ac_2O, pyr (82%, 2 steps); (d) HF·pyr (88%); (e) DMSO, $(COCl)_2$, CH_2Cl_2, then Et_3N, −78 °C (88%); (f) NBS, AIBN, CCl_4, 80 °C (74%); (g) K_2CO_3, MeOH (74%); (h) $ClPO(OEt)_2$, Et_3N, THF (62%); (i) *n*-Bu_3P, THF; then DMAP, **13** (33%).

carboxylic acid in the presence of the thioether moiety proved to be troublesome. After model studies with 4-(methylthio)-benzaldehyde and various oxidizing agents, the desired transformation was achieved by treatment of the aldehyde with *N*-bromosuccinimide in the presence of a catalytic amount of the radical initiator AIBN (2,2′-azobisisobutyronitrile). This led to the in situ formation of an acid bromide,[22] which was hydrolyzed to the carboxylic acid **11** on workup. **11** was subsequently deprotected and converted to **4**, as described previously.[18]

Estimation of Pyrimidine Selectivity. Analogue **2** was tested for its ability to inhibit the cleavage of a radiolabeled DNA duplex containing one calicheamicin binding site.[7] The 20-mer DNA was incubated with intact calicheamicin and various concentrations of either analogue **2** or natural oligosaccharide **3** at 37 °C. Enediyne-induced cleavage was triggered by the addition of β-mercaptoethanol, and the resulting products were resolved by gel electrophoresis. Analogue **2** was found to inhibit calicheamicin-induced DNA cleavage at much higher concentrations (approximately 2 orders of magnitude) than the natural oligosaccharide **3**. Similar results were obtained with both the TCCT (Figure 2) and the TTTT sites (data not shown).[23] This synthetic modification of the C ring thus leads to a significant loss in affinity for pyrimidine sequences. We concluded that selective DNA recognition requires, in addition to the iodine, at least one or more of the three other substituents on the aromatic ring.

Capillary Electrophoresis. To compare the binding of the native oligosaccharide **3** and the methylthio analogue **4** to various DNA sequences, we needed a better binding assay than we had used for **2**. The cleavage-based assay allowed us to determine that **2** binds poorly to DNA. However, it is not possible to measure accurately the binding affinity of similar ligands to closely related DNA sequences using inhibition of calicheamicin-induced DNA cleavage. Calicheamicin binds to and cuts the different recognition sequences at different rates.[11] Therefore, the use of cleavage inhibition to estimate and compare differential binding affinities will introduce errors in the measurement. Alternative methods that have been used to measure DNA binding affinities of calicheamicin include quantitative hydroxyl radical footprinting, circular dichroism, and microcalorimetry.[11,24] Of these, microcalorimetry is the most direct method for evaluating binding constants, but it requires large amounts of material and is not practical for evaluating analogues produced through multistep syntheses. We wanted a

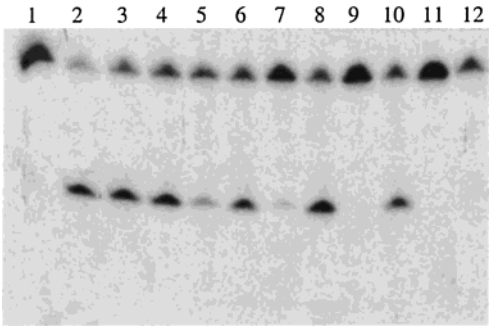


Figure 2. Cleavage inhibition reactions with calicheamicin oligosaccharide **3** and analogue **2**. Lane 1: 5′-end labeled d(GCTGAGTGTC-CTGCACTGCC). Lane 2: cleavage of TCCT duplex with 3 μM calicheamicin γ_1^I (**1**). Lanes 3, 5, 7, 9, 11: cleavage of TCCT duplex with 3 μM calicheamicin γ_1^I in the presence of 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} M, 10^{-2} M native oligosaccharide **3**. Lanes 4, 6, 8, 10, 12: cleavage of TCCT duplex with 3 μM calicheamicin γ_1^I in the presence of 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} M, 10^{-2} M analogue **2**.

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rapid, direct method that did not require invasive labeling of the oligosaccharide, that was suitable for small amounts of material, and that could be used under a wide range of conditions. Capillary electrophoresis (CE) seemed appropriate for our needs because it requires nanogram quantities of material and the measurements are direct and fast.

Capillary electrophoresis has developed in the 1990s as a highly sensitive technique for the separation of charged analytes. Affinity capillary electrophoresis has been used extensively for measuring protein−ligand affinities.[25] Because both DNA itself and most DNA ligands are charged, CE would seem to be ideal for studying the differential DNA binding affinities of synthetic ligands. Although preliminary investigations have validated CE as a technique for measuring DNA−ligand affinities,[26] it has not yet been applied to address specific questions. Below, we describe a CE-based assay for the quantitative measurement of DNA binding affinities of synthetic oligosaccharides. This method should be applicable to the study of other ligand−DNA complexes.

CE is based on the mobility of charged and neutral species through a buffer-filled capillary upon the application of voltage. The different analytes are separated due to differences in their charge-to-mass ratio, and the concentrations are measured by online UV detection. Affinity capillary electrophoresis involves measuring the change in mobility of a ligand (or receptor) in the presence of different amounts of a receptor (or ligand) in the buffer. The mobility change can be used to estimate binding constants.[25a] We tried to validate affinity capillary electrophoresis as a method for measuring ligand−DNA interactions using oligosaccharide **3** as the test case because a dissociation constant for the binding of this oligosaccharide to DNA had been reported.[7] We chose to use 15-mer duplexes containing a single binding site because 15-mers are long enough to be stable and yet short enough to minimize undesired interactions with the flanking sequences. Because the component in the buffer is needed in larger quantities, we first examined the mobility of ligand **3** in a capillary containing DNA in the buffer. The presence of DNA in the buffer led to problems with detector response, leading us to investigate alternative methods. Li and Martin have reported the utility of capillary zone electrophoresis for measuring the binding of small peptides to DNA.[27] In capillary zone electrophoresis, a pre-equilibrated mixture of ligand and receptor is injected into the buffer-filled capillary. Since most DNA ligands are positively charged, the separation of the free ligand from the negatively charged free DNA and the ligand−DNA complex is very efficient, leading to an accurate estimation of the free ligand concentration in the equilibrium mixture. Comparison of this value with the total ligand concentration (obtained from a measurement in the absence of DNA) affords an estimate of the bound ligand concentration for each data point. The affinity constant can be obtained from a Scatchard analysis of the above data (Figure 3).[28]

Using the above conditions, we measured the affinity of **3** to the TCCT duplex (Figure 3). There is one report regarding the binding affinity of **3** to a TCCT site. Using a cleavage inhibition assay, Nicolaou and co-workers estimated a dissociation constant of 4.1 μM.[7] With the CE assay, we found the association constant (K_a) for the binding of **3** to TCCT to be 4.74×10^4 M^{-1} ($K_d = 21.07$ μM, Figure 4a). This 5-fold discrepancy could be due to different experimental conditions or to different flanking sequences. However, the cleavage inhibition assay may be less accurate because it is indirect and rests on the validity of a number of assumptions. Townsend and Tullius noted a similar discrepancy between K_d values obtained by cleavage inhibition and other methods (i.e., footprinting and microcalorimetry).[11] To further calibrate our technique, we examined the binding of the polyamide antibiotic netropsin to an AT-rich duplex by capillary electrophoresis, and compared the affinity constant to a reported literature value measured with DNase footprinting.[29] We obtained a dissociation constant of 1.66 μM, in close agreement with the literature value of 1.0 μM.[30]

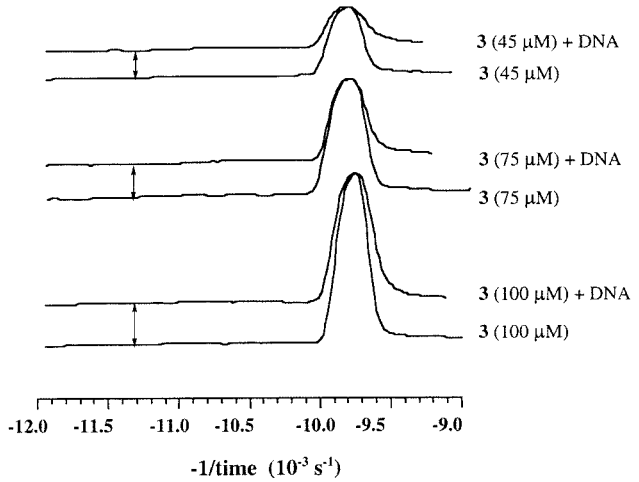

Figure 3. Electropherogram sections from CE experiments involving calicheamicin oligosaccharide (**3**) and the TCCT 15-mer duplex, showing *only* the free ligand peak in the presence and absence of DNA (UV absorbance is plotted against −1/time). Various concentrations of ligand were titrated against a fixed concentration of DNA(25 μM) in a 20mM NaCl, 30 mM Tris buffer at pH 7.5 (with 1% DMSO). The difference between the ligand peak in the absence and presence of DNA (as shown by the arrows) represents the estimate of the bound ligand concentration for each data point. The concentrations of the free and bound ligands were used to obtain the association constant by Scatchard analysis.

After validating our technique, we measured the interaction of the calicheamicin oligosaccharide **3** with the other duplexes, and repeated the experiments with the synthetic analogue **4** as the ligand. Association constants were obtained from the CE data (Figure 4). We compared the affinities of the methylthio analogue **4** to the TCCT and TTTT sequences, and to the corresponding affinities exhibited by the native iodo-oligosaccharide **3** (Table 1). The substitution of methylthio for iodine resulted in a 3-fold decrease in affinity for TCCT, and had no effect on the affinity for TTTT. Thus, analogue **4** has a similar affinity for these known calicheamicin binding sequences as **3**.

A comparison of the binding affinities to TCCT and TTTT sites does not, by itself, permit us to draw any conclusions about pyrimidine selectivity. There was the formal possibility that we had just designed a better non-specific binder to DNA.

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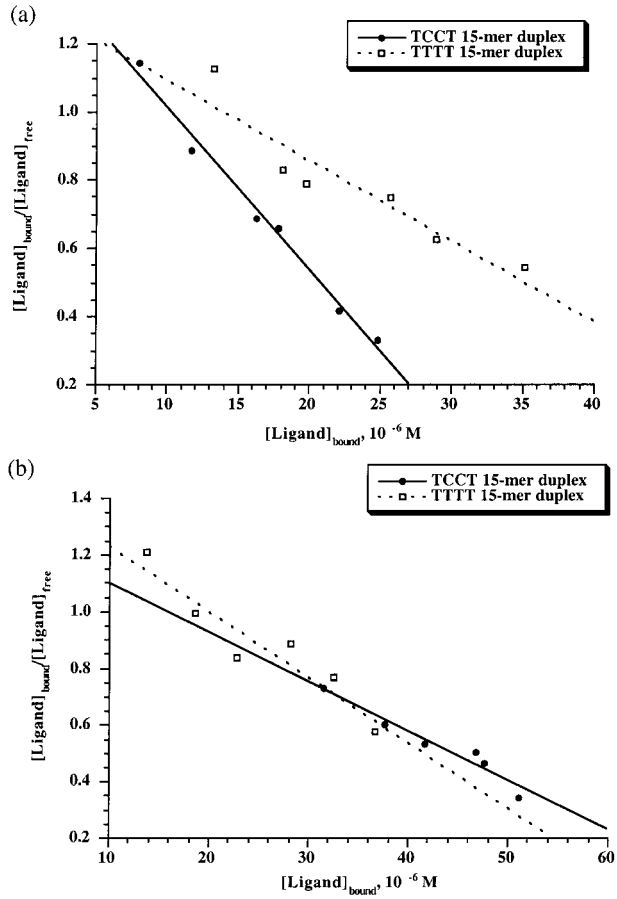
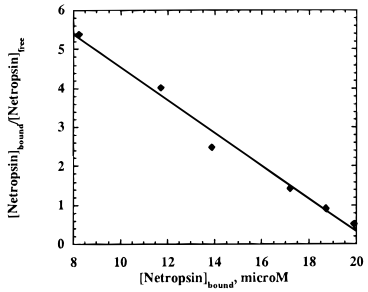


Figure 4. Scatchard plots of the binding of (a) the calicheamicin oligosaccharide **3** and (b) the methylthio analogue **4** to DNA 15-mer duplexes with TCCT or TTTT as the binding site. The data were fit to eq 1 using Kaleidagraph (see Experimental Section).

Therefore, we also evaluated the binding of oligosaccharides **3** and **4** to 15-mer duplexes containing TATA, TAAT, and TGGT, respectively, as the binding sites. These DNA sequences have similar base pair compositions as the TTTT and TCCT sites, but they do not contain sequential pyrimidines, the common feature of all calicheamicin binding sites. CE experiments showed that neither the native oligosaccharide **3** nor the synthetic analogue **4** bound to these DNA sequences (the free ligand peak height remained the same in the presence and absence of the duplexes). The association constants could not be determined due to the absence of any measurable binding (the binding affinities of analogue **2** to DNA could not be quantitated by capillary electrophoresis due to a similar reason). Thus, **4** maintains the oligopyrimidine selectivity exhibited by the native oligosaccharide.

(30) The experiments with netropsin and a TATA-containing 15-mer duplex were performed in DNase I buffer (10 mM Tris·Cl, 8 mM $MgCl_2$, 2 mM $CaCl_2$) in order to replicate the conditions used in the DNase titration experiment. The DNA sequence was 5′-CGTCGGTATACCACG-3′, incorporating the 8-mer duplex used in the footprinting experiments in the center. Netropsin concentrations were determined spectroscopically from the published extinction coefficient ($\epsilon_{296} = 20200\ M^{-1}\ cm^{-1}$). Histamine was used as a positive marker in all the separations to monitor changes in EOF. The DNA and the ligand were initially equilibrated at 25 °C in the running buffer for 15 min before injection into the capillary. The experiments were performed in a procedure similar to the experiments with the synthetic oligosaccharides. Different concentrations of netropsin were titrated with 10 μM DNA to obtain the affinity constant by Scatchard analysis.



We obtained an affinity constant of $(6.0 \pm 0.23) \times 10^5\ M^{-1}$ ($K_d = 1.66\ \mu M$), in close agreement to the value obtained by footprinting, $10^6\ M^{-1}$ ($K_d = 1.0\ \mu M$).

Table 1. Association Constants for the Binding of the Calicheamicin Oligosaccharide (**3**) and the Synthetic Analogue (**4**) to 15-mer DNA Duplexes

	K_a ($10^4\ M^{-1}$)				
ligand	TCCT	TTTT	TATA	TAAT	TGGT
3 (I)	4.74 ± 0.25	2.36 ± 0.44	*a*	*a*	*a*
4 (SCH_3)	1.73 ± 0.20	2.31 ± 0.41	*a*	*a*	*a*

[a] For low affinity sites, association constants could not be determined

Discussion

Role of the Iodine in Pyridine Selectivity. Previous studies have attributed the role of a specific contact to the aromatic iodine. NMR studies have shown that the iodine is in close proximity to a guanine amino group present in some of the sites.[8,9] Studies with synthetic derivatives and nucleotide replacements have also shown that the iodine plays a critical role in the recognition process.[7,10,11] In an effort to quantitate the electronic contribution due to the iodine, the interaction with the guanine amino group has been measured by different techniques. Using their cleavage inhibition assay, Nicolaou and co-workers have estimated that the iodine−amino group interaction is worth ~2.3 kcal/mol. This estimate was based on a comparison of the affinities of the native oligosaccharide **3** and the corresponding des-iodo analogue for a TCCT site.[7] Townsend and co-workers concluded that the iodine−amino group interaction is only worth ~1 kcal/mol based on comparing the extent of cleavage of DNA sequences containing guanine or inosine by calicheamicin.[11]

Capillary electrophoresis gave us an opportunity to evaluate the non-covalent iodine−amino group interaction by a direct biophysical technique. By comparing the binding of **3** and analogue **4** to both TCCT and TTTT sites, we have concluded that the iodine−amino group interaction is worth about 0.6 kcal/mol.[31] Furthermore, both **3** and **4** bind only to oligopyrimidine sequences, demonstrating that the role of the iodine in pyrimidine selectivity is largely steric and that it can be successfully substituted by a functionality of similar size. The large decrease in binding affinity to the TCCT site when the iodine is replaced by hydrogen may be due to at least three factors. First, substitution of the iodine by hydrogen creates a gap along one edge of the C ring that would be a void in a complex with DNA. Second, the change also reduces the rigidity of the molecule because rotation around the C−D linkage is less hindered. Increased flexibility could well reduce specificity for TCCT sites. Finally, the loss of the iodine−nitrogen interaction also decreases the affinity, albeit modestly.

Role of Molecular Shape in Pyrimidine Selectivity. We have suggested that the pyrimidine-selective binding exhibited by calicheamicin is due to an induced-fit interaction between the oligosaccharide and the DNA duplex.[8a] This hypothesis was based on two observations. First, the pyrimidine selectivity cannot be explained on the basis of specific directional contacts because the functional group arrays at different pyrimidine sites

(31) The $\Delta\Delta G$ was calculated from the measured K_a's at 298 K.

differ markedly. Second, the conformation of the DNA changes in a consistent way in all the specific complexes we have examined.

Induced-fit recognition is one way that proteins achieve selectivity in DNA binding. For example, TATA binding protein binds to a range of AT sites in the regulatory regions of genes.[32] The recognition sites share an ability to adapt to the shape of the protein. Von Hippel and Berg have noted that induced-fit recognition of DNA requires a relatively rigid protein.[33] The calicheamicin oligosaccharide contains a number of unusual structural features that enforce a particular shape.[34−36] NMR studies on calicheamicin have shown that the oligosaccharide has a well-defined conformation and that this conformation is maintained on binding.[8,12] Hence, the conformational changes in the DNA and the lack thereof in calicheamicin are consistent with the hypothesis that recognition involves an induced-fit process in which the DNA adapts to the ligand.

In this work we have examined the relative importance of specific functional groups versus overall shape in the selective recognition of pyrimidine sequences. As shown with the oligosaccharide analogue **2**, which contains an iodine attached to the aromatic ring but lacks the other three substituents, there is a loss in binding affinity to DNA. These substituents may contribute to the energetics of binding by making favorable contacts to the DNA, but none of them have been singled out as making particularly strong contacts. It seems more likely that the substituents on the aromatic ring play a more subtle—but essential—role in binding. Ortho substituents restrict rotation around bonds to aromatic rings.[37] The presence of ortho substituents on the aromatic ring likely enforces a particular conformation of the calicheamicin oligosaccharide. Removing all three substituents would not only leave a gap along one edge of the C ring, but it would permit the C and D rings to adopt a number of other conformations. If the binding selectivity is largely determined by the oligosaccharide inducing an appropriate fit in the DNA duplex, increased flexibility would be expected to destroy binding selectivity. For entropic reasons, the flexibility could also lead to a decrease in binding affinity. In contrast, analogue **4**, in which the iodine is replaced by a substitutent of comparable size that differs in its electronic properties, maintains pyrimidine selectivity. Apparently, maintaining the overall shape and rigidity of the oligosaccharide is more important in determining pyrimidine selectivity than any specific contacts between the oligosaccharide and the DNA.

Conclusions

In this work, we report the use of capillary electrophoresis to address specific questions about small molecule−DNA molecular recognition. We show that the calicheamicin oligosaccharide binds to TCCT and TTTT sequences with similar affinity. Furthermore, we show that synthetic changes to the oligosaccharide that alter the shape of the molecule cause a degradation in selectivity, while analogues that maintain the overall shape, even while removing specific contacts, demonstrate a similar oligopyrimidine selectivity. The modest preference (2-fold) that the oligosaccharide displays for TCCT over TTTT sequences is due to the iodine, but this functionality plays no role in the general selectivity for pyrimidine sites.

Experimental Section

General Methods. Unless otherwise stated, all chemicals were purchased from Aldrich or Sigma and used without further purification. Pyridinium hydrofluoride (70% HF) was purchased from Fluka. Methylene chloride, toluene, pyridine, and triethylamine were distilled from calcium hydride under dry argon. Diethyl ether and tetrahydrofuran were distilled from potassium benzophenone ketyl under dry argon. All reactions were carried out under argon atmosphere with freshly distilled solvents under anhydrous conditions unless otherwise noted.

Analytical thin-layer chromatography (TLC) was performed using silica gel 60 F254 precoated plates (0.25 mm thickness) with a fluorescent indicator. The developed plates were examined under short-wave UV light and stained with anisaldehyde. Flash column chromatography was performed using silica gel 60 (230−400 mesh) from EM Science.

NMR spectra were recorded on a JEOL GSX 270 or a Varian Inova 500 Fourier transform NMR spectrometer. Chemical shifts (δ) are reported in parts per million (ppm) downfield from tetramethylsilane (TMS). Coupling constants (J) are reported in hertz (Hz). Multiplicities are abbreviated as follows: singlet (s), doublet (d), triplet (t), quartet (q), multiplet (m), doublet of doublets (dd), and broad singlet (bs). High-resolution mass spectra were obtained on a VG ZAB under conditions of fast atom bombardment by Dr. Ron New at the University of California at Riverside Mass Spectrometry Facility.

Oligosaccharide 2. Methyl 4,6-Dideoxy-4-(((2,6-dideoxy-4-*S*-(4-((6-deoxy-3-*O*-methyl-α-L-manno-pyranosyl)oxy)-3-iodobenzoyl)-4-thio-*β*-D-*ribo*-hexopyranosyl)oxy)amino)-2-*O*-(2,4-dideoxy-4-(*N*-ethylamino)-3-*O*-methyl-α−L-xylopyranosyl)-*β*-D-glucopyranoside (2). R_f 0.2 (10% MeOH/$CHCl_3$ with 1% NH_3); ^{1}H NMR (CD_3OD, 270 MHz) δ 8.36 (d, J = 2.0 Hz, 1 H, ArH), 8.01 (dd, J = 8.6, 2.0 Hz, 1 H, ArH), 7.26 (d, J = 8.6 Hz, 1 H, ArH), 5.69 (d, J = 2.0 Hz, 1 H, D-1), 5.37 (m, 1 H, E-1), 5.08 (dd, J = 10.3, 1.9 Hz, 1 H, B-1), 4.40 (t, J = 2.0 Hz, 1 H, D-2), 4.21 (m, 3 H, D-5, B-3 and A-1), 4.06 (dq, J = 10.6, 6.3 Hz, 1 H, B-5), 3.91 (t, J = 9.8 Hz, 1 H, A-3), 3.86 (t, J = 10.9 Hz, 1 H, E-5_{axial}), 3.72−3.61 (m, 4 H, A-5, B-4, D-3 and E-$5_{equatorial}$), 3.58 (t, J = 9.2 Hz, 1 H, D-4), 3.54 (m, 4 H, E-3 and OCH_3), 2.76−2.66 (m, 3 H, E-4 and NCH_2), 2.38 (ddd, J = 12.7, 2.9, 1.5 Hz, 1 H, E-$2_{equatorial}$), 2.24 (t, J = 9.8 Hz, 1 H, A-4), 1.95 (dt, J = 12.4, 2.9 Hz, 1 H, B-$2_{equatorial}$), 1.74 (ddd, J = 12.7, 10.3, 2.9 Hz, 1 H, B-2_{axial}), 1.44 (m, 1 H, E-2_{axial}), 1.36 (d, J = 5.9 Hz, 3 H, B-6), 1.27 (d, J = 5.3 Hz, 3 H, A-6), 1.21 (d, J = 5.3 Hz, 3 H, D-6), 1.15 (t, J = 7.2 Hz, 3 H, NCH_2CH_3); HRFABMS calcd for $C_{35}H_{55}IN_2O_{15}S$ $(M)^+$ 902.2369, found 902.2380.

Synthesis of Oligosaccharide 4. 2-((*tert*-Butyldiphenylsiloxy)-methyl)-5-((6-deoxy-3-*O*-methyl-2,4-bis-*O*-(triethylsilyl)-α-L-mannopyranosyl)oxy)-3,4-dimethoxy-6-iodotoluene (6). DIBAL-H (5.85 mL of a 1.0 M solution in toluene, 5.85 mmol, 5.0 equiv) was added to a solution of **5** (1.0 g, 1.17 mmol) in 45 mL of Et_2O at −78 °C. The reaction was warmed to −20 °C over 30 min, quenched with 5.0 mL methanol, and poured into a solution of 1 N HCl (50 mL). The product was extracted with Et_2O (4 × 50 mL), dried over Na_2SO_4, concentrated, and purified by flash chromatography (50% EtOAc/petroleum ether) to give 700 mg (72%) of the rhamnoside diol as a white solid: R_f 0.2 (50% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.70−7.67 (m, 4 H, ArH), 7.42−7.35 (m, 6 H, ArH), 5.64 (d, J = 1.3 Hz, 1 H, H-1), 4.76 (s, 2 H, benzylic CH_2), 4.51 (dd, J = 3.3, 1.6 Hz, 1 H, H-2), 4.32 (dq, J = 9.8, 6.0 Hz, 1 H, H-5), 3.84 (dd, J = 9.3, 3.3 Hz, 1 H, H-3), 3.76 (s, 3 H, OCH_3), 3.64 (t, J = 9.7 Hz, 1 H, H-4), 3.64 (s, 3 H, OCH_3), 3.57 (s, 3 H, OCH_3), 2.50 (s, 3 H, $ArCH_3$), 1.32 (d, J = 6 Hz, 3 H, H-6), 1.04 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 152.8, 149.7, 142.9, 137.9, 135.9, 133.4, 129.5, 128.7, 127.5, 102.7, 93.8, 80.9, 71.7, 71.1, 70.2, 67.0, 61.2, 60.6, 58.6, 57.1, 57.0, 26.8, 25.5, 19.3, 17.5, 17.5; HRFABMS calcd for $C_{33}H_{43}O_8SiI$ $(MNa)^+$ 745.1669, found 745.1650.

To a solution of the diol (254.2 mg, 0.35 mmol) in 10 mL CH_2Cl_2 at 0 °C was added Et_3N (233 μL, 1.75 mmol, 5.0 equiv), followed by

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(36) Walker, S.; Gange, D.; Gupta, V.; Kahne, D. *J. Am. Chem. Soc.* **1994**, *116*, 3197−3206.
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the addition of triethylsilyl trifluoromethanesulfonate (240 μL, 1.05 mmol, 3.0 equiv). The reaction was maintained at 0 °C for 20 min, quenched with 1.0 mL methanol, diluted with 10 mL Et_2O, and washed with water (20 mL), saturated aqueous $NaHCO_3$ (20 mL), and brine (20 mL). The organic layer was dried over Na_2SO_4, concentrated, and purified by flash chromatography (2% *tert*-butyl methyl ether/petroleum ether) to give 314.3 mg (94%) of **6** as a colorless oil: R_f 0.45 (5% *tert*-butyl methyl ether/petroleum ether); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.70−7.67 (m, 4 H, ArH), 7.45−7.33 (m, 6 H, ArH), 5.31 (d, J = 1.3 Hz, 1 H, H-1), 4.79 (d, J = 10.9 Hz, 1 H, benzylic H), 4.72 (d, J = 10.9 Hz, 1 H, benzylic H), 4.47 (dd, J = 2.6, 1.3 Hz, 1 H, H-2), 4.21 (dq, J = 9.8, 6 Hz, 1 H, H-5), 3.74 (t, J = 9.8 Hz, 1 H, H-4), 3.73 (s, 3 H, OCH_3), 3.63 (s, 3 H, OCH_3), 3.56 (dd, J = 9.8, 2.6 Hz, 1 H, H-3), 3.42 (s, 3 H, OCH_3), 2.50 (s, 3 H, $ArCH_3$), 1.25 (d, J = 6 Hz, 3 H, H-6), 1.04 (s, 9 H, $C(CH_3)_3$), 1.01−0.89 (m, 18 H, $Si(CH_2CH_3)_3$), 0.73−0.54 (m, 12 H, $Si(CH_2CH_3)_3$); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 152.8, 150.7, 143.2, 137.8, 135.7, 133.5, 129.5, 128.5, 127.5, 104.8, 94.3, 81.4, 72.3, 72.2, 68.6, 61.2, 60.6, 58.6, 57.1, 26.8, 25.6, 19.3, 18.0, 7.0, 6.8, 5.2, 4.9; HRFABMS calcd for $C_{45}H_{71}O_8Si_3I$ $(MNa)^+$ 973.3399, found 973.3435.

2-((*tert*-Butyldiphenylsiloxy)methyl)-5-((6-deoxy-3-*O*-methyl-2,4-bis-*O*-(triethylsilyl)-α-L-mannopyranosyl)oxy)-3,4-dimethoxy-6-methylthio-toluene (7). MeLi (65 μL of a 1.4 M solution in Et_2O, 0.085 mmol, 2.5 equiv) was added to a solution of **6** (32.5 mg, 0.034 mmol) in 1 mL Et_2O at −78 °C, followed by the addition of MeSSMe (20 μL, 0.17 mmol, 5.0 equiv) after 5 min. The reaction temperature was maintained at −78 °C for 10 min, quenched with 200 μL Et_3N, and poured into 5 mL of saturated aqueous $NaHCO_3$. The aqueous layer was extracted with Et_2O (3 × 5 mL), the organic layers were combined, dried over Na_2SO_4, concentrated, and purified by flash chromatography (2% EtOAc/petroleum ether) to give 22 mg (74%) of the des-iodo methylthio compound **7** as a colorless oil: R_f 0.4 (5% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.71−7.68 (d, J = 7.2 Hz, 4 H, ArH), 7.42−7.34 (m, 6 H, ArH), 5.27 (d, J = 2.0 Hz, 1 H, H-1), 4.76 (d, J = 11.2 Hz, 1 H, benzylic H), 4.69 (d, J = 11.2 Hz, 1 H, benzylic H), 4.47 (t, J = 2.0 Hz, 1 H, H-2), 4.25 (dq, J = 9.2, 6.0 Hz, 1 H, H-5), 3.75 (s, 3 H, OCH_3), 2.72 (t, J = 9.2 Hz, 1 H, H-4), 3.65 (s, 3 H, OCH_3), 3.49 (dd, J = 9.2, 2.6 Hz, 1 H, H-3), 3.41 (s, 3 H, OCH_3), 2.54 (s, 3 H, $ArCH_3$), 2.27 (s, 3 H, $ArSCH_3$), 1.27 (d, J = 6.5 Hz, 3 H, H-6), 1.04 (s, 9 H, $C(CH_3)_3$), 1.02−0.91 (m, 18 H, $Si(CH_2CH_3)_3$), 0.70−0.59 (m, 12 H, $Si(CH_2CH_3)_3$); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 152.8, 152.5, 144.1, 138.6, 135.7, 133.6, 129.5, 128.8, 127.5, 125.6, 104.7, 81.7, 72.5, 71.8, 68.5, 61.1, 60.5, 58.2, 56.9, 26.9, 19.3, 18.9, 18.2, 17.3, 7.0, 6.8, 5.2, 4.9; HRFABMS calcd for $C_{46}H_{74}O_8SSi_3$ $(MNa)^+$ 893.4309, found 893.4316.

2-((*tert*-Butyldiphenylsiloxy)methyl)-5-((6-deoxy-2,4-di-*O*-acetyl-3-*O*-methyl-α-L-mannopyranosyl)oxy)-3,4-dimethoxy-6-methylthio-toluene (8). 7 (284.2 mg, 0.32 mmol) was dissolved 30 mL of AcOH:THF:H_2O (3:1:1), and the reaction mixture was maintained at 25 °C for 12 h. After dilution with 30 mL ice-cold water, the reaction was quenched with the careful addition of solid $NaHCO_3$ until the solution was shown to be basic by pH paper. The aqueous layer was extracted with EtOAc (3 × 40 mL), and the organic layers were combined, dried over Na_2SO_4, and concentrated. The crude diol was azeotroped with toluene (3 × 20 mL) and taken to the next step without further purification.

To a solution of the diol in 20 mL of pyridine was added DMAP (8 mg, 0.06 mmol, 0.2 equiv), followed by acetic anhydride (155 μL, 1.63 mmol, 5.0 equiv). The reaction mixture was maintained at 25 °C for 3 h, followed by the removal of pyridine in vacuo. The residue was dissolved in 30 mL of CH_2Cl_2 and washed with 1 N HCl (40 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL), and the organic layers were combined, dried over Na_2SO_4, concentrated, and purified by flash chromatography (25% EtOAc/petroleum ether) to give 194 mg (82%, 2 steps) of diacetate **8** as a colorless oil: R_f 0.6 (30% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 7.71−7.68 (m, 4 H, ArH), 7.42−7.34 (m, 6 H, ArH), 5.80 (dd, J = 3.3, 1.9 Hz, 1 H, H-2), 5.51 (d, J = 1.9 Hz, 1 H, H-1), 5.08 (t, J = 9.9 Hz, 1 H, H-4), 4.75 (d, J = 10.5 Hz, 1 H, benzylic H), 4.70 (d, J = 10.5 Hz, 1 H, benzylic H), 4.49 (dq, J = 9.9, 5.9 hz, 1 H, H-5), 3.96 (dd, J = 9.9, 3.3 Hz, 1 H, H-3), 3.78 (s, 3 H, OCH_3), 3.65 (s, 3 H, OCH_3), 3.43 (s, 3 H, OCH_3), 2.55 (s, 3 H, $ArCH_3$), 2.29 (s, 3 H, $ArSCH_3$), 2.16 (s, 3 H, $OCOCH_3$), 2.13 (s, 3 H, $OCOCH_3$), 1.21 (d, J = 5.9 Hz, 3 H, H-6), 1.04 (s, 9 H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 170.2, 170.1, 152.9, 151.2, 143.6, 138.9, 135.7, 133.6, 129.5, 129.2, 127.5, 125.0, 100.6, 72.3, 68.5, 67.9, 61.2, 60.6, 58.2, 57.6, 26.8, 21.0, 19.3, 19.0, 17.5, 17.3; HRFABMS calcd for $C_{38}H_{50}O_{10}SSi$ $(MNa)^+$ 749.2791, found 749.2819.

5-((6-Deoxy-2,4-di-*O*-acetyl-3-*O*-methyl-α-L-mannopyranosyl)-oxy)-3,4-dimethoxy-4-hydroxymethyl-6-methylthio-toluene (9). To a plastic vial containing **8** (50 mg, 0.06 mmol) was added 1.5 mL of a freshly prepared solution of buffered pyridinium hydrofluoride (10 mL THF, 5.7 mL pyridine and 2.1 g pyridinium hydrofluoride).[20] The reaction mixture was quenched after 3 h with 5 mL of saturated aqueous $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (3 × 5 mL), and the organic layers were combined, dried over Na_2SO_4, concentrated, and purified by flash chromatography (45% EtOAc/petroleum ether) to give 29.6 mg (88%) of benzylic alcohol **9** as a white solid: R_f 0.2 (45% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 5.77 (dd, J = 3.3, 1.9 Hz, 1 H, H-2), 5.54 (d, J = 1.3 Hz, 1 H, H-1), 5.07 (t, J = 9.9 Hz, 1 H, H-4), 4.72 (s, 2 H, benzylic H), 4.46 (dq, J = 9.9, 5.9 Hz, 1 H, H-5), 3.96 (dd, J = 9.9, 3.3 Hz, 1 H, H-3), 3.91 (s, 3 H, OCH_3), 3.84 (s, 3 H, OCH_3), 3.43 (s, 3 H, OCH_3), 2.60 (s, 3 H, $ArCH_3$), 2.29 (s, 3 H, $ArSCH_3$), 2.16 (s, 3 H, $OCOCH_3$), 2.13 (s, 3 H, $OCOCH_3$), 1.20 (d, J = 5.9 Hz, 3 H, H-6); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 170.2, 170.1, 153.1, 151.5, 143.7, 137.7, 129.1, 125.4, 100.5, 72.2, 68.6, 67.9, 61.4, 60.7, 57.8, 57.6, 21.0, 18.9, 17.5, 17.1; HRFABMS calcd for $C_{22}H_{32}O_{10}S$ $(MNa)^+$ 511.1613, found 511.1636.

4-((6-Deoxy-2,4-di-*O*-acetyl-3-*O*-methyl-α-L-mannopyranosyl)-oxy)-2,3-dimethoxy-6-methyl-5-methylthio-benzaldehyde (10). To a solution of dimethyl sulfoxide (24 μL, 0.34 mmol, 2.2 equiv) in 6 mL CH_2Cl_2 at −78 °C was added oxalyl chloride (85 μL of a 2.0M solution in CH_2Cl_2, 0.17 mmol, 1.1 equiv). After 6 min, the reaction mixture was treated with a solution of **9** (75.5 mg, 0.15 mmol) in 2 mL of CH_2Cl_2. The temperature was allowed to rise to −60 °C over 15 min, followed by the addition of Et_3N (100 μL, 0.75 mmol, 5.0 equiv). The temperature was further raised to −40 °C over 45 min and the reaction quenched with 10 mL of saturated aqueous $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL), and the organic layers were combined, dried over Na_2SO_4, concentrated, and purified by flash chromatography (30% EtOAc/petroleum ether) to give 65.7 mg (88%) of aromatic aldehyde **10** as a white solid: R_f 0.45 (30% EtOAc/petroleum ether); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 10.45 (s, 1 H, CHO), 5.76 (dd, J = 3.3, 2.0 Hz, 1 H, H-2), 5.72 (d, J = 1.9 Hz, 1 H, H-1), 5.09 (t, J = 9.9 Hz, 1 H, H-4), 4.37 (dq, J = 9.9, 6.0 Hz, 1 H, H-5), 3.98 (dd, J = 9.9, 3.3 Hz, 1 H, H-3), 3.97 (s, 3 H, OCH_3), 3.88 (s, 3 H, OCH_3), 3.43 (s, 3 H, OCH_3), 2.78 (s, 3 H, $ArCH_3$), 2.29 (s, 3 H, $ArSCH_3$), 2.18 (s, 3 H, $OCOCH_3$), 2.14 (s, 3 H, $OCOCH_3$), 1.19 (d, J = 5.9 Hz, 3 H, H-6); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 191.3, 170.0, 158.6, 155.6, 143.4, 141.2, 126.9, 125.4, 100.3, 72.0, 68.9, 67.7, 62.1, 60.9, 60.8, 57.7, 21.0, 18.8, 18.1, 17.4; HRFABMS calcd for $C_{22}H_{30}O_{10}S$ $(MNa)^+$ 509.1457, found 509.1462.

4-((6-Deoxy-2,4-di-*O*-acetyl-3-*O*-methyl-α-L-mannopyranosyl)-oxy)-2,3-dimethoxy-6-methyl-5-methylthio-benzoic acid (11). To a solution of aromatic aldehyde **10** (56.5 mg, 0.11 mmol,) in 10 mL CCl_4 was added *N*-bromosuccinimide (45 mg, 0.24 mmol, 2.1 equiv) and 2,2′-azobisisobutyronitrile (2 mg, 0.01 mmol, 0.1 equiv). The reaction mixture was heated to reflux for 90 min, cooled to room temperature, and washed with brine (15 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL), the organic layers combined, dried over Na_2SO_4, concentrated, and purified by flash chromatography (5% MeOH/$CHCl_3$ with 1% AcOH) to give 43 mg (74%) of aromatic acid **11** as a white solid: R_f 0.2 (10% MeOH/$CHCl_3$); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 5.78 (dd, J = 3.3, 2.0 Hz, 1 H, H-2), 5.54 (d, J = 1.9 Hz, 1 H, H-1), 4.99 (t, J = 9.9 Hz, 1 H, H-4), 4.48 (dq, J = 9.9, 5.9 Hz, 1 H, H-5), 3.99 (dd, J = 9.9, 3.3 Hz, 1 H, H-3), 3.89 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3), 3.41 (s, 3 H, OCH_3), 2.47 (s, 3 H, $ArCH_3$), 2.31 (s, 3 H, $ArSCH_3$), 2.14 (s, 3 H, $OCOCH_3$), 2.11 (s, 3 H, $OCOCH_3$), 1.14 (d, J = 5.9 Hz, 3 H, H-6); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 171.3, 170.8, 152.8, 151.2, 144.6, 134.7, 126.2, 101.2, 77.7, 72.9, 69.3, 68.5, 61.3, 60.7, 57.4, 20.1, 20.0, 18.3, 17.4, 17.1; HRFABMS calcd for $C_{22}H_{30}O_{11}S$ $(MNa)^+$ 525.1406, found 525.1418.

4-((6-Deoxy-3-*O*-methyl-α-L-mannopyranosyl)oxy)-2,3-dimethoxy-

6-methyl-5-methylthio-benzoic Acid (12). To a solution of diacetate **11** (32.8 mg, 0.06 mmol) in 1.5 mL of MeOH:THF (2:1) was added K_2CO_3 (27 mg, 0.19 mmol, 3.0 equiv). The reaction mixture was maintained at 25 °C for 6 h and quenched by the careful addition of acetic acid until the solution was shown to be acidic by pH paper. All solvents were removed in vacuo, and the residual acetic acid was removed by means of a toluene azeotrope (3 × 2 mL). The crude product was purified by flash chromatography (5% MeOH/$CHCl_3$ with 1% AcOH) to give 20.2 mg (74%) of the diol **12** as a white solid: R_f 0.3 (5% MeOH/$CHCl_3$ with 1% AcOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.46 (d, J = 1.5 Hz, 1 H, H-1), 4.47 (dd, J = 3, 1.5 Hz, 1 H, H-2), 4.32 (dq, J = 9.5, 6.0 Hz, 1 H, H-5), 3.92 (s, 3 H, OCH_3), 3.85 (s, 3 H, OCH_3), 3.69 (dd, J = 9.5, 3 Hz, 1 H, H-3), 3.56 (t, J = 9.5 Hz, 1 H, H-4), 3.54 (s, 3 H, OCH_3), 2.51 (s, 3 H, $ArCH_3$), 2.28 (s, 3 H, $ArSCH_3$), 1.98 (bs, 2 H, OH), 1.26 (d, J = 6.0 Hz, 3 H, H-6); ^{13}C NMR (CD_3OD, 125 MHz) δ 175.4, 151.9, 150.1, 144.9, 133.4, 125.8, 104.3, 81.4, 71.8, 71.2, 67.5, 61.0, 60.6, 56.6, 18.3, 17.7, 17.4; HRFABMS calcd for $C_{18}H_{26}O_9S$ $(MNa)^+$ 441.1195, found 441.1194.

Methyl 4,6-Dideoxy-4-(((2,6-dideoxy-4-*S*-(4-((6-deoxy-3-*O*-methyl-α-L-mannopyranosyl)oxy)-2,3-dimethoxy-6-methyl-5-methylthiobenzoyl)-4-thio-*β*-D-*ribo*-hexo-pyranosyl)oxy)amino)-2-*O*-(2,4-dideoxy-4-(*N*-ethylamino)-3-*O*-methyl-α-L-xylo-pyranosyl)-*β*-D-glucopyranoside (4). To a solution of aromatic acid **12** (20 mg, 0.047 mmol) in 1.5 mL of THF were added Et_3N (9 μL, 0.061 mmol, 1.3 equiv) and $ClPO(OEt)_2$ (9 μL, 0.059 mmol, 1.25 equiv). The reaction was maintained at 25 °C for 20 min and filtered through Celite to remove insoluble Et_3NûHCl. The solvent was removed in vacuo, and the residue was purified by filtration through a short silica column (5% MeOH/EtOAc) to give 16.0 mg (62%) of **13** which was used immediately for the next coupling step.

To a solution of disulfide **14** (2.9 mg, 3 μmol) in 1 mL of deoxygenated THF was added excess n-Bu_3P (34 μL, 0.13 mmol, 45 equiv). Disulfide reduction was shown to be complete in 20 min by TLC (3:1 Et_2O:MeOH). The activated acid **13** (16.0 mg, 28 μmol, 9 equiv) in 0.5 mL THF and DMAP (5 mg, 0.036 mmol, 12 equiv) were added, and the reaction mixture was maintained at 25 °C for 4 h. The solvent was removed in vacuo, and the crude product was purified by flash chromatography (6:1 Et_2O:MeOH) to give 1.7 mg (32%, 2 steps) of aryl tetrasaccharide **4** as a white solid: R_f 0.25 (3:1 Et_2O:MeOH); ^{1}H NMR (CD_3OD, 500 MHz) δ 5.53 (d, J = 1.5 Hz, 1 H, D-1), 5.38 (bs, 1 H, E-1), 5.07 (dd, J = 10.3, 1.8 Hz, 1 H, B-1), 4.46 (t, J = 2.0 Hz, 1 H, D-2), 4.28 (dq, J = 9.5, 6.5 Hz, 1 H, D-5), 4.21 (m, 2 H, B-3 and A-1), 4.04 (dq, J = 10.6, 6.5 Hz, 1 H, B-5), 3.92 (t, J = 9.5 Hz, 1 H, A-3), 3.90 (s, 3 H, OCH_3), 3.86 (s, 3 H, OCH_3), 3.84 (t, J = 10.0 Hz, 1 H, E-5_{axial}), 3.70 (dd, J = 9.5, 2.2 Hz, 1 H, D-3), 3.69−3.63 (m, 3 H, A-5, B-4 and E-$5_{equatorial}$), 3.57 (t, J = 9.5 Hz, 1 H, D-4), 3.54 (s, 3 H, OCH_3), 3.51 (m, 4 H, E-3 and OCH_3), 3.38 (s, 3 H, OCH_3), 3.37 (dd, J = 8.8, 7.5 Hz, 1 H, A-2), 2.74−2.65 (m, 3 H, E-4 and NCH_2), 2.43 (s, 3 H, $ArCH_3$), 2.40 (ddd, J = 12.8, 2.9, 1.5 Hz, 1 H, E-$2_{equatorial}$), 2.31 (s, 3 H, $ArSCH_3$), 2.25 (t, J = 9.5 Hz, 1 H, A-4), 1.97 (dt, J = 12.5, 2.9 Hz, 1 H, B-$2_{equatorial}$), 1.77 (ddd, J = 12.8, 10.3, 2.8 Hz, 1 H, B-2_{axial}), 1.46 (ddd, J = 13.2, 11.0, 3.7 Hz, 1 H, E-2_{axial}), 1.39 (d, J = 6.5 Hz, 3 H, B-6), 1.37 (d, J = 6.0 Hz, 3 H, A-6), 1.26 (d, J = 6.5 Hz, 3 H, D-6), 1.14 (t, J = 7.0 Hz, 3 H, NCH_2CH_3); ^{13}C NMR (CD_3OD, DEPT, 30 unique non-quaternary carbons) δ 104.4, 103.1, 100.7, 99.0, 81.3, 79.6, 76.2, 71.4, 70.5, 69.7, 68.6, 67.5, 61.6, 60.7, 56.7, 56.1, 55.4, 52.5, 42.0, 38.2, 34.3, 30.0, 27.4, 18.7, 18.3, 17.8, 17.4, 16.9, 13.7, 13.5; HRFABMS calcd for $C_{39}H_{64}N_2O_{17}S$ $(MH)^+$ 897.3724, found 897.3703.

Duplex DNA. Purified synthetic oligonucleotides were obtained from the Princeton Synthesis Facility. Following dialysis to remove the TFA salts, the strands were lyophilized and dissolved in deionized water. Absorbances were measured, and the concentrations were determined from the calculated extinction coefficients.[38] The samples were stored at 4 °C for further use.

Inhibition of Cleavage Assay.[7] The DNA oligomers (20-mers) were 5′-end labeled in a 10 μL reaction mixture containing 1−5 pmol of oligonucleotide, 5−7× excess of [γ-^{32}P] ATP (3000 Ci/mmol), kinase buffer (10 mM $MgCl_2$; 70 mM Tris·HCl, pH 7.6; 5 mM DTT), and 5−10 units of T4 polynucleotide kinase, which were incubated at 37 °C for 1 h. The enzyme was denatured by heating the reaction mixture at 90 °C for 5 min. The reaction mixture was mixed with 100 μL of 1× TE buffer (pH 7.2) and excess ATP was removed on a G25 sephadex spin column. The 5′-labeled oligomer (1 pmol) and 20 times excess of the "cold" complementary strand were hybridized in a volume of 100 μL containing 10 mM Tris·HCl, pH 7.6, which was incubated at 90 °C for 5 min and slow-cooled to 25 °C over 90 min. The DNA sequence was d(GCTGAGTGNNNNGCACTGCC), where NNNN represented a calicheamicin binding site (TCCT or TTTT).

Different concentrations of the inhibitor, native oligosaccharide **3** or the mono-iodo analogue **2** (10^{-6} M to 10^{-2} M) in DMSO were incubated with 3 μM calicheamicin, also in DMSO, in a 20 mM NaCl, 30 mM Tris·HCl buffer (pH 7.5), along with 1 fmol of labeled DNA duplex. The final DMSO concentration was 10% (v/v). The reaction mixture was incubated at 37 °C for 1 h. Calicheamicin-dependent DNA cleavage was initiated by the addition of 1 μL of β-mercaptoethanol (prewarmed to 37 °C) to attain a final concentration of 100 mM. The cleavage reaction was allowed to proceed for 11 min at 37 °C, and quenched by heating at 95 °C for 4 min. An equal volume of gel loading buffer, containing 96% formamide, 7.5 mM EDTA, 0.05% (w/v) bromophenol blue and 0.05% (w/v) xylene cyanol, was added to the reaction mixture and the cleavage products separated on a 19% denaturing polyacrylamide gel (containing 6.2 M urea).

Capillary Electrophoresis. CE experiments were conducted on a Beckman P/ACE 5510 capillary electrophoresis system, with the anode on the injection side and cathode on the detection side. Uncoated fused silica capillaries (50 μm i.d.), obtained from Polymicro Technologies Inc. (Phoenix, AZ), were used with a total length of 27 cm, the length to the detector being 20 cm. The running buffer was 20 mM NaCl, 30 mM Tris·Cl (pH 7.5). The capillary was initially rinsed with the following reagents: 1 N HCl for 5 min, deionized water for 2 min, 0.1 N NaOH for 15 min, deionized water for 2 min, and finally, running buffer for 5 min. The applied separation voltage was 10 kV, at 25 °C. The capillary was rinsed with 0.1 N NaOH for 2 min, deionized water for 2 min, and running buffer for 2 min between successive separations. Fresh capillaries were prepared each day. The analytes were quantitated by UV detection at 214 nm.

The nucleotide sequence of the 15-mer duplexes were 5′-CGTGCGNNNNGCACG-3′ where NNNN represents the various binding sites (TCCT, TTTT, TATA, TGGT, and TAAT, respectively). The sequence contains only one binding site per duplex. Equimolar amounts of the two complementary strands were annealed for each duplex, by heating to 95 °C for 5 min, followed by slow cooling to room temperature over 3−5 h. The DNA and the ligand were initially equilibrated at 25 °C in the running buffer (with 1% DMSO) for 15 min before injection into the capillary. To measure the affinity of the synthetic oligosaccharides for the different DNA 15-mer duplexes, successive samples were run with a fixed concentration of DNA and varying concentrations of the ligands. Absorbances were plotted against −1/time.[39] For each ligand concentration, a run was performed in the absence of the DNA, to calibrate the ligand concentration (peak heights were found to be directly proportional to concentration). The difference between total ligand concentration and the amount of free ligand in the presence of DNA gave the concentration of the bound ligand. To obtain the association constants by Scatchard analysis, the data were fit to eq 1 using Kaleidagraph software (version 3.08d, Synergy Software, Reading, PA).

$$[\text{Ligand}]_{\text{bound}}/[\text{Ligand}]_{\text{free}} = K_a[\text{DNA}]_{\text{total}} - K_a[\text{Ligand}]_{\text{bound}} \quad (1)$$

Acknowledgment. This work was supported by Grants GM 53066 and GM 42733 from the National Institutes of Health. K.B. acknowledges a graduate fellowship from Bristol-Myers Squibb. We thank Drs. George Ellestad and Wei-Dong Ding of Lederle Laboratories for generously providing us with calicheamicin.

JA000519V

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