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DNA Cleaving Activities of 9-Membered Masked Enediyne Analogues Possessing DNA Intercalator and Sugar Moieties

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Abstract: The DNA cleaving properties of various enediyne analogues possessing sugar moieties and DNA-intercalators were investigated. The DNA cleaving experiments show that these hybrids analogues induced sequence-selective DNA cleavage and the simple sugars in the enediyne serve as a DNA recognition element for DNA cleavage.

The mode of action of the enediyne antibiotics envisions initial noncovalent binding to double-stranded DNA.[1] Previous studies of the enediyne antibiotics such as calicheamicin γ_1 suggested selective interactions of the calicheamicin γ_1 oligosaccharide with duplex DNA along specific sequences within the minor groove.[2] However, in contrast to DNA-protein and DNA-DNA interactions, DNA-carbohydrate binding[3] is not well understood. Due to the importance of DNA-carbohydrate binding in biological systems and to gain some insight into the role of the carbohydrate moiety in DNA recognition, we initiated a program directed to aid our understanding of how the carbohydrate moiety recognizes a DNA duplex. In the preceding communication,[4] we reported a new method for the introduction of the sugar moiety into a labile enediyne structure using S-alkylation of a bromoacetate derivative with a sugar-containing thiol without the need for protection of the sugar moiety. Here, we describe DNA cleaving studies of the masked enediynes **1**, **2**, **3**, **4** and **5** conjugated with a sugar moiety and/or naphthoate moiety. This provides the first example of enediyne analogues in which the DNA sequence selectivity is observed by introducing a simple sugar moiety.

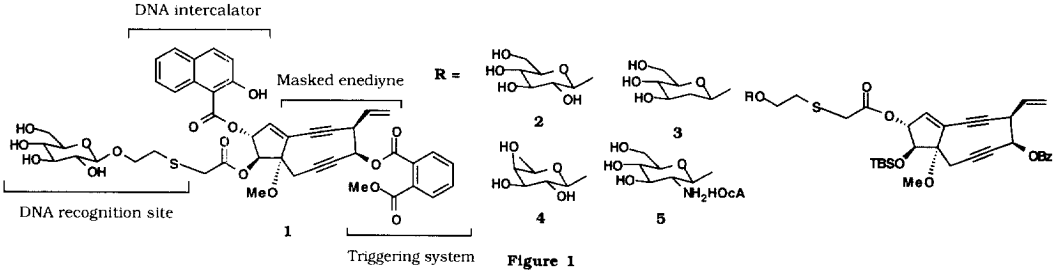

Figure 1

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For comparison of the effect of the sugar moiety on DNA cleavage, we examined the sequence selectivity of the enediyne analogues **6** and **7**, which have no sugar moiety. Analogues **6** and **7** were obtained by chemical synthesis as previously described.[5] To assay for DNA cleavage, 1 mM of the analogues **6** and **7** containing 5'-end ^{32}P labeled pBR322 Bam HI-Sal I fragment (275 bp), sonicated calf thymus DNA in 10% MeOH in 20mM Tris-HCl buffer (pH 7.5) was incubated at 37 °C for 18 h. Analogues **6** and **7** do not have any sequence selectivity, as demonstrated by the rather uniform cleavage ladder. (Figure 2) We have already shown that analogue **7** possessing a naphthoate moiety increases the DNA cleaving activity with respect to the core diol **6** by about one order of magnitude. These results suggest that the naphthoate moiety increases the binding affinity to the DNA duplex and the 9-membered enediyne core structure and naphthoate moiety do not contribute to sequence specific recognition.

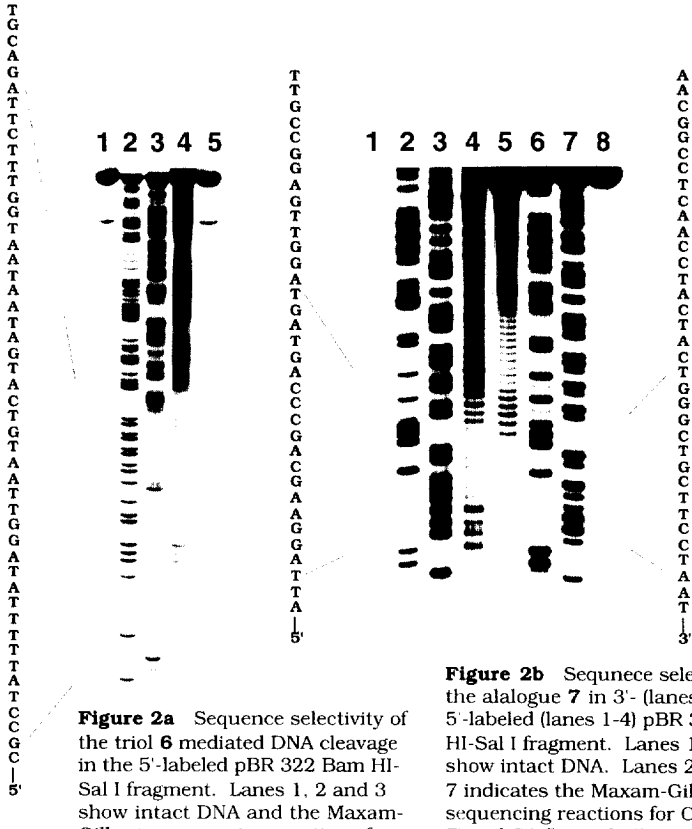

Figure 2a Sequence selectivity of the triol **6** mediated DNA cleavage in the 5'-labeled pBR 322 Bam HI-Sal I fragment. Lanes 1, 2 and 3 show intact DNA and the Maxam-Gilbert sequenceing reactions for AG and CT, respectively. Lane 4 shows DNA cleavage pattern by **6**.

Figure 2b Sequnece selectivity of the alalogue **7** in 3'- (lanes 5-8) and 5'-labeled (lanes 1-4) pBR 322 Bam HI-Sal I fragment. Lanes 1 and 8 show intact DNA. Lanes 2, 3, 6 and 7 indicates the Maxam-Gilbert sequencing reactions for CT (lanes 2, 7) and GA (lanes 3, 6), respectively. Lanes 4 and 5 show DNA cleavage patterns by **7** (1 mM).

DNA cleavage site selectivity of the masked enediyne analogues **1** and **2** was examined with the 5'-end ^{32}P labeled pBR322 Bam HI-Sal I fragment (275 bp). The 5'-^{32}P-labeled DNA was incubated with 1.0 mM of **1** and **2**, and sonicated calf thymus DNA in 10% MeOH in 20 mM Tris-HCl buffer (pH 7.5) at 37 °C for 18 h. Both analogues **1** and **2** possessing a glucose moiety showed identical sequence selectivity. (Figure 3a) To elucidate the sequence selectivity of the various sugars, the DNA-cleaving activities of the hybrids **3**, **4** and **5** possessing 2-deoxyglucose, fucose and glucosamine, respectively, were examined with 5'-end ^{32}P labeled

323-base pair (SalI-NruI) pBR322 DNA fragment. The pBR322 DNA was incubated with 1 mM of **3**, **4** and **5**, and 1 mg of sonicated calf thymus carrier DNA in 10% MeOH in 20 mM Tris-HCl buffer (pH 7.0) at 37 °C for 18 h. Figure 3b shows autoradiographic results for the DNA strand scission by analogues **3**, **4** and **5**. All of the analogues clearly induced DNA breakage at guanine and adenine residues, although the cutting of **5** was weak. The preferential cleavage sites of the analogues were 5'-CGG, 5'-CAG and 5'-CGC segments. This result demonstrated for the first time the feasibility of using simple sugars as DNA recognition elements for the DNA cleavage by enediyne analogues.

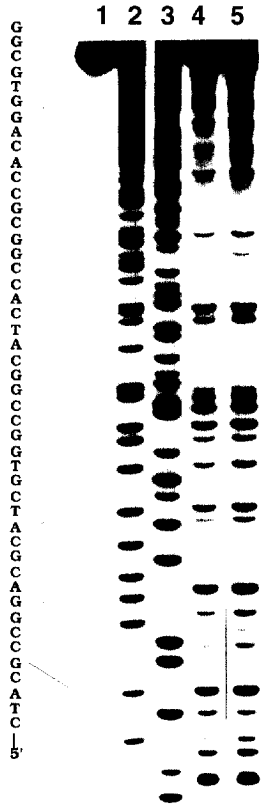

Figure 3a Sequence-selective DNA-cleavages by the analogues **1** and **2**. Lanes 1, 2 and 3 show intact DNA and the Maxam-Gilbert sequencing reactions for GA and CT, respectively. The 5'-end labeled DNA from pBR322 was incubated with **1** (lane 4) and **2** (lane 5).

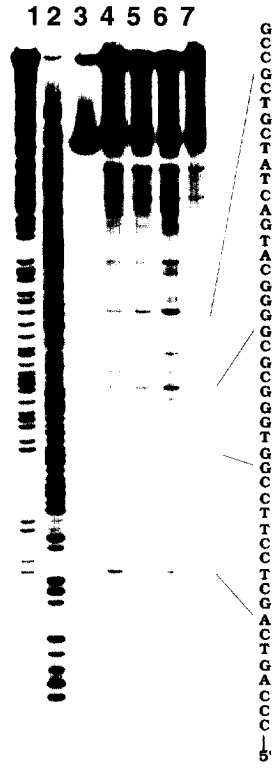

Figure 3b Sequence-selective DNA-cleavages by the analogues **3**, **4** and **5**. The 5'-end labeled DNA from pBR322 was incubated with **4** (lanes 4 and 5), **3** (lane 6) and **5** (lane 7). Lanes 1, 2 and 3 show the Maxam-Gilbert sequencing reactions for GA, CT, and intact DNA, respectively.

These masked enediynes with sugar moieties could serve as "probes" for elucidating the binding affinity of oligosaccharides to DNA. At this stage, details of the mechanism of DNA recognition by **1**, **2**, **3**, **4** and **5** are not clear. The power of synthesis to produce a variety of oligosaccharide-enediyne analogues should facilitate further studies in this important area of molecular recognition. Further studies to elucidate the precise mechanism of the DNA recognition are currently under investigation in our laboratory.

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