Design and Synthesis of a Novel DNA−DNA Interstrand Adenine−Guanine Cross-Linking Agent

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DNA interstrand cross-linking agents that interact within the minor groove have attracted considerable interest in the past few years,[1] and much progress has been made in the design and synthesis of these compounds.[2] These cross-linking agents belong to two broad classes: natural products that cross-link 2−3 base pairs apart[1,3] and synthetic agents that cross-link 4−7 base pairs apart.[1,4] Both groups generally target either guanines (e.g., mitomycin C and DSB-120)[1,3,4] or adenines (e.g., bizelesin).[1,5] What is missing is the availability of minor groove DNA−DNA interstrand cross-linking agents that alkylate guanines on one strand and adenines on the opposite strand. This would provide new agents capable of recognizing and binding to more extended and mixed A·T and G·C sequence tracts of DNA to uniquely define individual gene targets and hence exert biological specificity. We report here the design and synthesis of the agent UTA-6026, which contains two different alkylation moieties with the potential to alkylate G or A.

We chose a DNA sequence (sequence I in Figure 1) containing an adenine and guanine six base pairs apart on opposite strands as the potential DNA cross-linking target template. To achieve the cross-linking, (+)-cyclopropapyrroloindole [(+)-CPI] (the DNA−DNA alkylating moiety of (+)-CC-1065 that selectively alkylates N3 of adenine[6]) and DC-81 (one member of the pyrrolo-

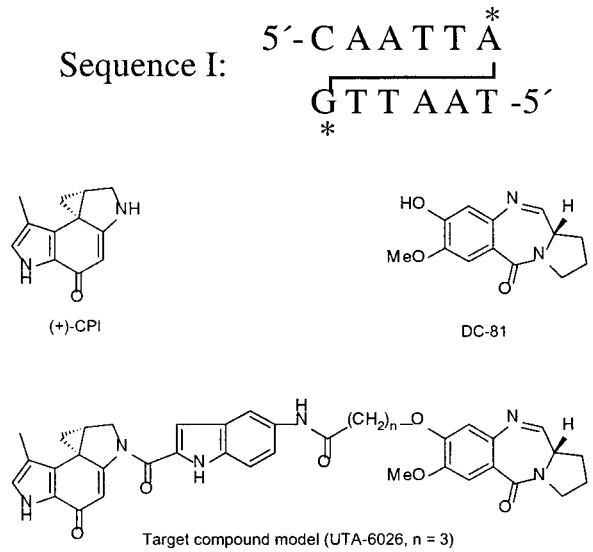

Figure 1. Structures of (+)-CPI, DC-81, and target compound.

[1,4]benzodiazepine (P[1,4]B) family that is capable of covalently reacting with the exocyclic 2-NH_2 group of guanine[7]) were chosen as the two monoalkylating subunits. By tethering them with a suitable linker, a potential adenine−guanine interstrand cross-linking agent could be synthesized. The linker was designed with two objectives in mind. First, the indole was included to increase the minor groove binding affinity without affecting the sequence selectivity, which is primarily determined by the (+)-CPI alkylating subunit.[8] Second, the flexible alkyl chain was chosen to facilitate the isohelical accommodation of the P[1,4]B alkylating subunit.[2c] The structure of the target compound is shown in Figure 1. To achieve the six-base-pair span, molecular modeling was used to determine the alkyl chain length between the two alkylating subunits. The modeling results (unpublished) show that when $n = 3$ (UTA-6026), the two units have an optimal chance to alkylate the two desired sites, A* and G*, on opposite DNA strands in sequence I to form the DNA−DNA interstrand cross-link.

The strategy for the synthesis of compound UTA-6026 is shown in Scheme 1. The main synthetic challenge is to form the active cyclopropyl subunit in the (+)-CPI unit and the acid-sensitive N10−C11 imine moiety in the P[1,4]B subunit in one molecule at the same time. We used Thurston's approach[2e] for synthesizing the nucleophile-sensitive/acid-sensitive P[1,4]B analogues by protecting the N10 with Fmoc and, after the remainder of the molecule is assembled, removing the Fmoc group to form the imine bond of the P[1,4]B subunit in the last step. Vanillic acid (**1**), the starting material, was reacted with ethyl bromobutyrate, followed by hydrolysis of the ester under basic conditions to give compound **2**. Nitration of **2** with nitric acid gave the nitro compound **3**. Selective esterification of **3** afforded the monoester **4**, which was coupled with (2s)-(+)-pyrrolidine-2-carbaldehyde diethyl thioacetal **12** to give **5**. Reduction of **5** yielded the amino compound **6**. The amine was protected by the Fmoc group followed by deprotection and cyclization of **7** with $HgCl_2/CaCO_3$ to give an Fmoc-protected P[1,4]B with an ether alkyl chain (**8**). Hydrolysis of the methyl ester **8** under acidic conditions gave the key intermediate acid **9**.[9a,b] Compound **9** was coupled with (+)-seco-CPI-indole **13**[9c,d,e] (which was converted from *N*-mesyl-

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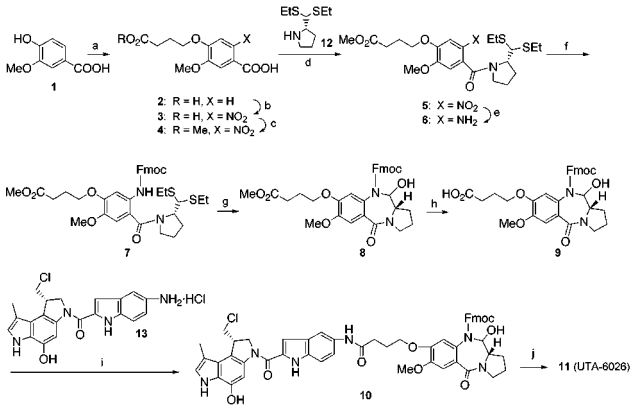
Scheme 1[a]



[a] (a) [i] $BrCH_2CH_2CH_2COOEt/K_2CO_3/DMF$; [ii] $NaOH/H_2O/EtOH$/reflux; (b) 70% HNO_3; (c) MeOH/*p*-TsOH/rt; (d) [i] $(COCl)_2/DMF/THF$; [ii] 12/Et_3N/CH_2Cl_2; (e) $SnCl_2 \cdot 2H_2O$/MeOH/reflux; (f) Fmoc-Cl/aq Na_2CO_3/H_2O/dioxane; (g) $HgCl_2/CaCO_3/CH_3CN/H_2O$; (h) 10% aq HCl/THF/rt; (i) **13**/EDCI/DMA; (j) $Bu_4N^+F^-$/DMF/rt.

seco-CPI via six steps) by EDCI/DMA to give compound **10**. Fortuitously, removal of the Fmoc-protecting group and cyclization of the seco-CPI part were finished in one step with TBAF in DMF to give the final target compound **11** (UTA-6026).[2d] The overall yield from vanillic acid **1** to the target compound **11** was 6%.

Nondenaturating gel electrophoresis was used to explore the DNA−DNA interstrand cross-linking ability of UTA-6026 on the sequence shown in Figure 2C. The results in Figure 2, A and B, show that UTA-6026 has a concentration-dependent cross-linking ability when either strand is radiolabeled.[10] To identify the sites of alkylation on the top strand, a thermal cleavage assay was performed.[11] As expected, DNA cleavage was found at adenines 9 and 19 (Figure 2C).[12] Because the P[1,4]B subunit alkylates at N2 of guanine, no cleavage was demonstrated on the bottom strand; however, upon substitution of the guanines purportedly alkylated by the P[1,4]B end by inosine, which lacks the 2-amino group, no cross-linking band equivalent to that shown in Figure 2 was found (unpublished results).

Preliminary in vitro tests showed that UTA-6026 has remarkably potent cytotoxicity to several tumor cell lines (IC_{50} = 0.28 nM in human non-small cell breast tumor cell line MCF-7, IC_{50} = 0.047 nM in colon tumor cell line SW480, and IC_{50} = 5.1 nM in human lung tumor cell line A549).

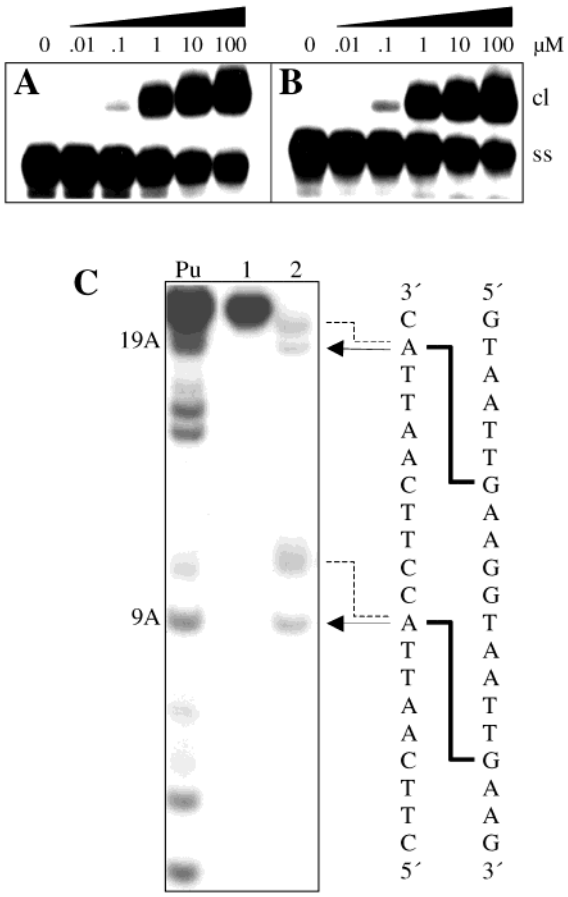


Figure 2. Concentration dependency of DNA cross-linkage by UTA-6026 on the top (A) and bottom (B) strands. Cross-linked species = cl; single-stranded species = ss. (C) Autoradiograph of products from thermal strand breakage assay to demonstrate (+) alkylation sites at N3 of adenines 9 and 19 by UTA-6026 on the upper strand of the sequence. Lane Pu: Maxam−Gilbert A/G sequencing reaction. Lane 1: DNA without added drug. Lane 2: UTA-6026-reacted DNA. Arrows point to the product of thermal cleavage, while the dashed lines indicate incomplete products from the same reaction.[11]

In summary, a unique heterobifunctional compound that forms interstrand cross-linking between adenine and guanine six base pairs apart was designed and synthesized. It shows mixed sequence-specific alkylation selectivity and demonstrates potent antitumor activity against several tumor cell lines. In vivo tests and further cytotoxicity studies are in progress.

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Supporting Information Available: Experimental details and analytical data for the preparation of **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, **10**, and **11** are provided (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(10) UTA-6026 at the concentrations shown in Figure 2, A and B, was incubated for 6 h at an ambient temperature in pH 7.2 buffer. Each reaction was terminated with 5 μg/μL calf thymus DNA, followed by addition of 3 M sodium acetate, pH 7.2, 5 μg/μL tRNA, and ethanol precipitation. Dried pellets were resuspended in strand separation buffer (30% w/w DMSO in 1 mM EDTA). Denaturation for 1 min at 70 °C was followed by immediate chilling. Electrophoresis was performed on a 12% native polyacrylamide gel at 150 V for 12 h at 4 °C in Tris-acetate running buffer.

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(12) Drug reactions were carried out using 100 μM drug with 50 ng ^{32}P end-labeled DNA for 6 h at ambient temperature in a final volume of 10 μL buffer (1 mM Tris, pH 7.2, 10 mM NaCl). Reactions were stopped with 5 μg calf thymus DNA, followed by ethanol precipitation with 3 M sodium acetate pH 7.2 and 10 μg/μL tRNA. Ethanol precipitates were dried, resuspended in piperidine, and boiled for 20 min to induce strand breakage. The strand breakage products were then ethanol precipitated, resuspended in alkaline dye, and resolved on a 20% sequencing gel.