Sequence-Specific DNA Alkylation by Hybrid Molecules between Segment A of Duocarmycin A and Pyrrole/Imidazole Diamide**

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Sequence-specific DNA-alkylating agents have received much current interest due to their significant potential in molecular biology and human medicine.[1, 2] Duocarmycins, highly potent antitumor antibiotics, are among the most intriguing classes of such molecules that bind to AT-rich sequences and selectively alkylate N3 of adenine (A) at the 3′ end of three or more consecutive AT base pairs in DNA.[2] Duocarmycin A (Duo) has the highest reactivity among the duocarmycin family and, in some cases, can alkylate N3 of guanine (G).[3] Recently, we found that the addition of distamycin A (Dist) markedly modulates the Duo alkylation site in DNA fragments where alkylation occurs predominantly at the G residues in GC-rich sequences.[4] We also demonstrated by NMR spectroscopy that the molecular mechanism of such a G alkylation involves cooperative formation of a heterodimer (Figure 1).[5]

Polyamides containing *N*-methylimidazole (Im) and *N*-methylpyrrole (Py) developed by Dervan and co-workers as sequence-specific DNA-binding ligands have attracted much current attention.[6] These polyamides bind cooperatively as an antiparallel dimer to the minor groove of the DNA helix. A simple binary code has been developed to correlate the reading DNA sequence with the side-by-side pairing between

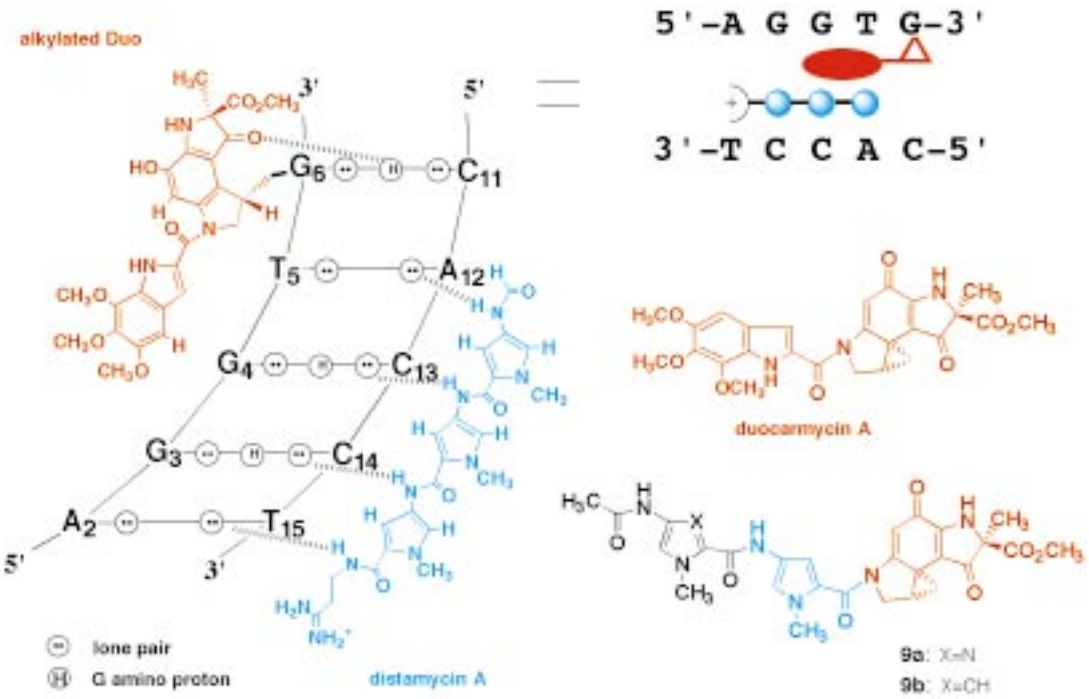

Figure 1. Schematic representation of the heterodimeric binding model of duocarmycin A (Duo) and distamycin A (Dist) to the minor groove of 5′-d(AGGTG)-3′5′-d(CACCT)-3′ as well as the structures of Duo, Dist, and the hybrid compounds **9a** and **9b**. Hydrogen bonds between the drugs and DNA are illustrated by dashed lines.

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[**] Duocarmycin B_2 was obtained from Kyowa Hakko through the courtesy of Dr. C. Murakata.

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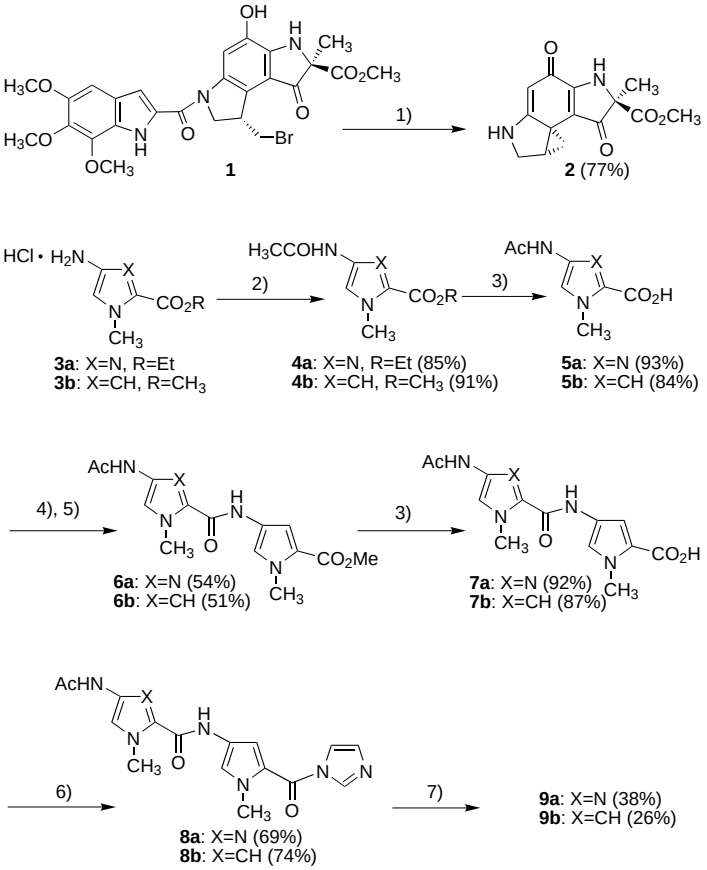
Py and Im carboxamides; that is, Im/Py recognizes GC base pairs and Py/Py recognizes AT base pairs.[7] Our structure of a Duo-Dist-DNA octamer complex, as determined by NMR spectroscopy, clearly demonstrated that the heterodimer of Duo and Dist precisely binds to the minor groove, in that Dist recognizes the minor groove of one strand according to the binary code of Im/Py polyamides.[5] In fact, our preliminary studies revealed that substitution of the Py unit of Dist with Im dramatically modulates the sequence specificity of Duo in a predictable manner. Therefore, based on these findings, a new class of tailor-made sequence-specific DNA-alkylating agents can be designed through the incorporation of the Py/Im polyamide subunit into the antitumor antibiotic Duo. Herein we describe the synthesis of novel hybrid molecules between segment A of Duo and Py/Im diamides and their ability to alkylate DNA in the presence and absence of Dist.

  1433-7851/99/3805-0650 $ 17.50+.50/0

Hybrids **9a** and **9b** were prepared by the routes shown in Scheme 1. The hydrolysis of duocarmycin B_2 (**1**) readily produced the segment **2** as reported previously.[8] Activated amides of the diamides (**8**) were prepared in five steps from amine hydrochloride salts **3**.[9] The key step was the coupling of the activated amides **8** with the segment **2** to afford target compounds **9a** and **9b**, whose structures were fully characterized by ^{1}H NMR spectroscopy and high-resolution fast atom bombardment (FAB) mass spectrometry.



Scheme 1. Synthesis of hybrids **9a** and **9b**. 1) NaOMe, MeOH, CH_3CN; 2) pyridine, *N*,*N*-diisopropylethylamine (DIEA), Ac_2O; 3) NaOH, MeOH, H_2O; 4) dicyclohexyl carbodiimide (DCC), 1-hydroxy-1*H*-benzotriazole (HOBt), DMF; 5) **3b**, DIEA, DMF; 6) 1,1′-carbonyldiimidazole, DMF; 7) NaH, DMF, **2**.

Oligodeoxynucletides (ODNs) 1 and 2 were initially selected to investigate DNA-alkylation modes of hybrids **9a** and **9b** in the presence and absence of Dist. As shown in Figure 2, ODNs 1 and 2 were match sequences for **9a** and **9b**, respectively. Analysis by HPLC of the reaction mixture of these ODNs incubated with **9a** and **9b** showed no appreciable alkylation products in the absence of Dist even after 46 h. In contrast, these new hybrids alkylated the target G^6 of their match sequences in the presence of Dist efficiently and with high selectivity. For example, after 46 h of incubation 72% of ODN 1 was alkylated by **9a**, whereas only 8% of ODN 2 was alkylated by **9a** under the same conditions, because A^4T^{13} of ODN 2 is a mismatch base pair to **9a** (Figure 2). In clear contrast, ODN 2 was smoothly alkylated by **9b**, and ODN 1 was only alkylated to 26% by **9b** after 22 h of incubation. Direct observations of the three- and fourfold negatively

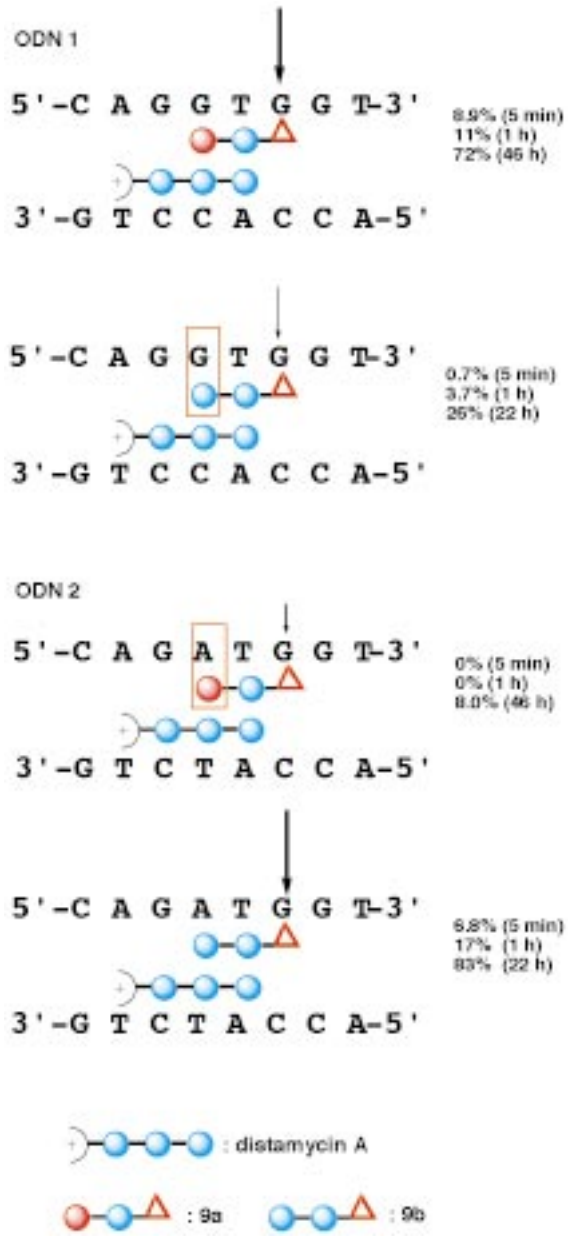


Figure 2. Sequence-specific alkylation of ODN 1 and ODN 2 by hybrids **9a** and **9b** in the presence of Dist. The arrows represent the location and the extent of alkylation after the indicated incubation times. The imidazole and pyrrole rings are represented by red and blue circles, respectively. According to the binary code of Im/Py polyamides, Im/Py recognizes GC base pairs and Py/Py recognizes AT base pairs;[7] hydrogen-bond mismatches are also highlighted by red boxes. A reaction mixture (50 μL) containing a new hybrid compound (0.1 mM), distamycin A (0.1 mM), and the DNA octamer (1 mM base concentration) in 50 mM sodium cacodylate buffer (pH 7.0) was incubated at 0 °C for various times. The reaction was monitored by HPLC using a Chemcobond 5-ODS-H column (4.6 × 150 mm). Elution was performed with 0.05 M ammonium formate and a 0–50% acetonitrile linear gradient (0–40 min) at a flow rate of 1.0 mL min^{-1}. Detection was at 254 nm. Different peaks were assigned on the basis of the UV absorption spectrum. The production of compound–guanine adduct together with the abasic site-containing nucleotides upon brief heating (90 °C for 5 min) was established with previous procedures.[3, 4]

charged ions for **9a**-Dist-ODN1 and **9b**-Dist-ODN2, respectively, by electrospray ionization mass spectroscopy indicated that the alkylation occurs through cooperative formation of a heterodimer.[10]

  1433-7851/99/3805-0651 $ 17.50+.50/0

The sequence-selective alkylation by these hybrids was further confirmed using a TexasRed-labeled 450 base pair DNA fragment. Alkylated DNA fragments were cleaved at the alkylated sites by heating at 90 °C, and the cleaved fragments were analyzed with a DNA sequencer.[11] As shown in Figure 3, in the absence of Dist **9a** and **9b** selectively alkylated the 3′ end of A in AT-rich sequences, although **9b** is much more reactive than **9a**. The narrower the minor groove, the more efficient the alkylation, which is consistent with previously reported alkylation by a monomer.[12] In contrast, in the presence of Dist, alkylation by **9a** predominantly occurred at the 3′ end of guanosine in 5′-GTG-3′ sequences within a 450 base pair DNA fragment. These results are in good agreement with the previously reported binary code for base pair recognition by Py/Im polyamides.[7] The specificity of T over A at the 5′ side of the reacting G residue observed in both hybrids can be explained by steric interaction with H5 protons of Duo, as observed in the previous study.[5] Similarly, the **9b**–Dist heterodimer specifically alkylated the 3′ end of G in the 5′-(T/A)TG-3′ sequence (Figure 3). These results clearly indicated that the addition of Dist dramatically modulates the sequence selectivity of these hybrid molecules in a predictable manner. This is the first demonstration that the sequence-specific DNA alkylation at a specific atom can be accomplished by Py/Im polyamide ligands that bind DNA.

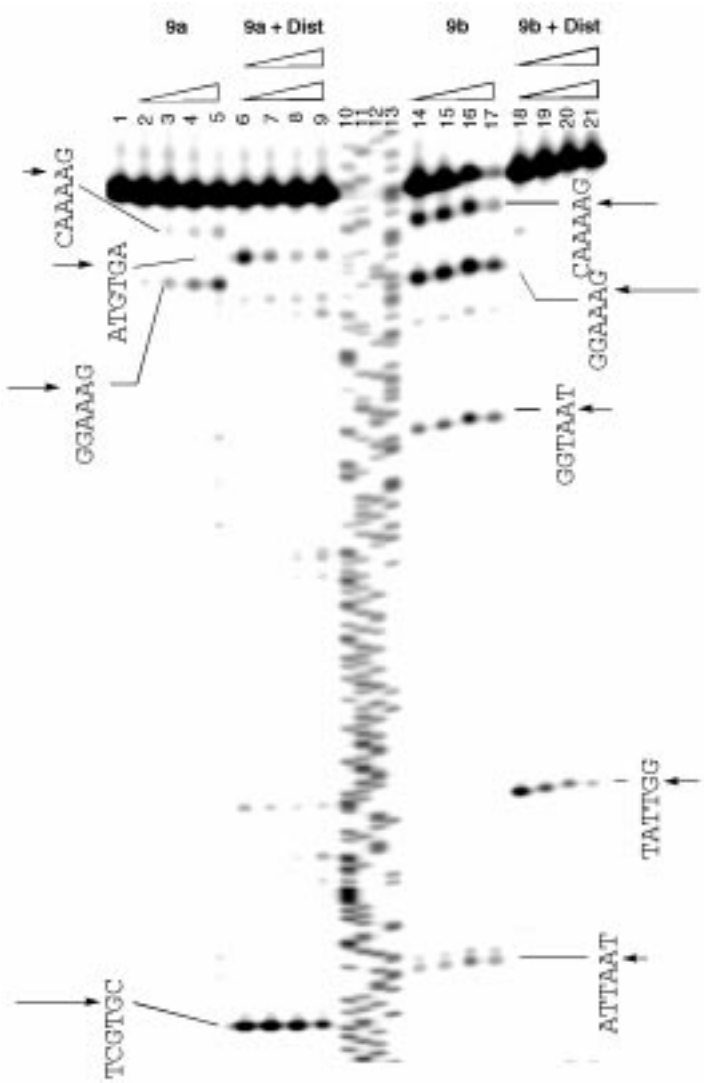


Figure 3. Thermally induced strand cleavage of a 5′-TexasRed-labeled pUC18F378–827 DNA fragment incubated with hybrids **9a** and **9b** in the presence and absense of Dist. Lane 1: DNA control; lanes 2–5: 4, 8, 16, and 32 μM **9a**, respectively; lanes 6–9: 4, 8, 16, and 32 μM **9a** with 8, 16, 32, and 64 μM Dist, respectively; lanes 10–13: Sanger G, C, T, and A sequencing standards; lanes 14–17: 4, 8, 16, and 32 μM of **9b**, respectively; lanes 18–21: 4, 8, 16, and 32 μM **9b** with 8, 16, 32, and 64 μM Dist, respectively. A singly 5′-TexasRed-labeled 450 base pair fragment was prepared by polymerase chain reaction (PCR) using 5′-TexasRed-modified 5′-TGTAAAACGACGGCCAGT-3′ (pUC 18 forward 378–395), and 5′-TGCTGGCCTTTTGCTCACATG-3′(pUC 18 reverse 1861–1881) as primers. The 5′-TexasRed-labeled DNA fragment (75 nM) was alkylated in 8 μL of 12.5 mM Na phosphate buffer (pH 7.0) at room temperature overnight. The reaction was quenched by addition of calf thymus DNA (5 mM, 1 μL) and heated for 5 min at 90 °C. DNA was collected by precipitation with ethanol. The pellet was resolved in 8 μL of loading dye (formamide with fushin red) and heated at 94 °C for 20 min and then immediately cooled at 0 °C. An aliquot (2 μL) was subjected to electrophoresis on 6% denaturing polyacrylamide gel with a 5500-S DNA sequencer.

In conclusion, the present study outlines the preparation of novel hybrid molecules between segment A of Duo and Py/Im diamides. These hybrids primarily alkylate the 3′ end of A in AT-rich sequences, as does the parent Duo. More importantly, these hybrids alkylate G residues of predetermined DNA sequences efficiently and with high specificity by formation of a heterodimer with Dist. Recently, Dervan and co-workers have demonstrated that synthetic Py/Im polyamides have a strong affinity and a full range of specificity to DNA sequences which are comparable to that in naturally occurring DNA-binding proteins.[13] These polyamides are cell-permeable and inhibit transcription of specific genes in cell cultures.[14] Results from the present investigation suggest a promising approach for developing a new generation of DNA-alkylating agents that can alkylate purine bases at any desired sites.

Received: August 31, 1998 [Z12351IE]
German version: *Angew. Chem.* **1999**, *111*, 692–695

Keywords: alkylations · antibiotics · antitumor agents · DNA cleavage · DNA recognition

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[10] Alkylation by a heterodimer was directly confirmed by electrospray ionization mass spectrometry: **9a**-Dist-ODN 1: calcd: 5863.3, found: 5863.2; **9b**-Dist-ODN 2: calcd: 5861.4, found: 5860.8.
[11] Subsequent treatment with hot piperidine (90 °C, 20 min) did not further increase the intensity of the cleavage bands, indicating that the present heating condition is sufficient to cleave all alkylated DNA.
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An Open-Framework Germanate with Polycubane-Like Topology**

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The ability of germanates to form extended structures with GeO_4 tetrahedra, GeO_5 trigonal bipyramids, and GeO_6 octahedra, coupled with their tendency to adopt a lower M-O-M (M = Ge) minimum angle than that of silicate-based solids, implies that a high number of possible structures with open-framework topologies can be potentially accessed.[1–3] Unexpectedly, however, only a few porous germanates have been reported thus far.[4–9] By using synthetic methods analogous to those employed for the production of zeolites and related crystalline materials,[10] we have synthesized and structurally characterized an open-framework germanate $[Ge_9O_{18}(OH)_4]\cdot 2H_2ppz\cdot 0.5H_2O$ (ASU-14, ppz = piperazine = HNC_4H_8NH), which is constructed from Ge_9 body-centered parallelepiped building blocks. These are linked together at each of their eight vertices to give the rare polycubane topology with an intersecting channel system of ten- and eight-membered rings in which the piperazinium cations and water molecules reside.

ASU-14 was prepared by heating a mixture of germanium dioxide, piperazine, water, pyridine, and HF in the molar ratio 1.0:2.4:31.0:27.7:0.80 to 165 °C for four days. A crystalline colorless solid was recovered in 76% yield (based on germanium dioxide) upon cooling this mixture. Elemental microanalysis performed on a bulk sample of this material gave the composition $[Ge_9O_{18}(OH)_4]\cdot(H_2ppz)_2(H_2O)_{0.5}$ (calcd: C 8.04, H 2.45, N 4.69, Ge 54.68, F 0.00%; found: C 7.70, H 2.47, N 4.61, Ge 53.06, F 0.19%).

An X-ray diffraction analysis of a single crystal isolated from the reaction product revealed a three-dimensional open framework constructed from the $[Ge_9O_{18}(OH)_4]$ units shown in Figure 1. A GeO_6 octahedral germanium center links two Ge_4 units that are related by an inversion center. Each of these

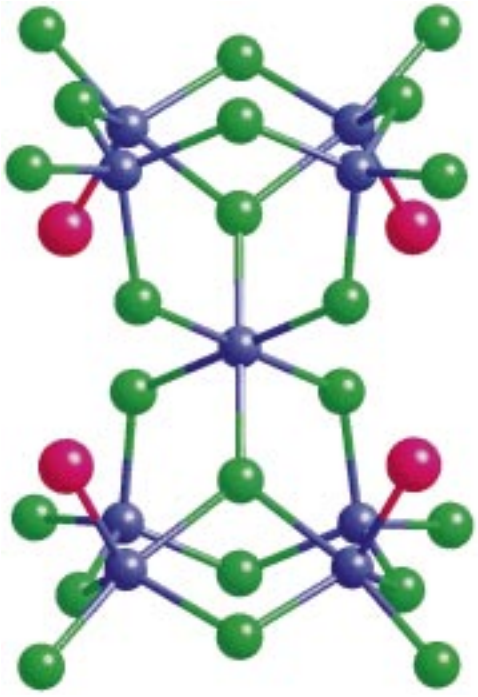

Figure 1. The building block unit present in crystalline $[Ge_9O_{18}(OH)_4]\cdot 2H_2ppz\cdot 0.5H_2O$ (ASU-14), with atoms represented by spheres: blue: Ge, green: O, pink: OH.

units is constructed from a pair of GeO_4 tetrahedra and a pair of $GeO_4(OH)$ trigonal bipyramids. These are linked together through doubly bridging oxides to yield eight germanium centers that are positioned at what can be considered as the corners of a body-centered parallelepiped building block. The tetrahedral and trigonal bipyramidal Ge atoms are connected to the Ge atom at the center through doubly and triply bridging oxides, respectively. All Ge – O distances for tetrahedral germanium centers (mean: 1.734(9) Å) are similar to those reported for the quartz modification of GeO_2

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[**] This work was supported by the National Science Foundation (CHE-9702131). We thank Prof. M. O'Keeffe for invaluable discussions.