Sequence-Specific DNA Interstrand Cross-Linking by Imidazole−Pyrrole CPI Conjugate

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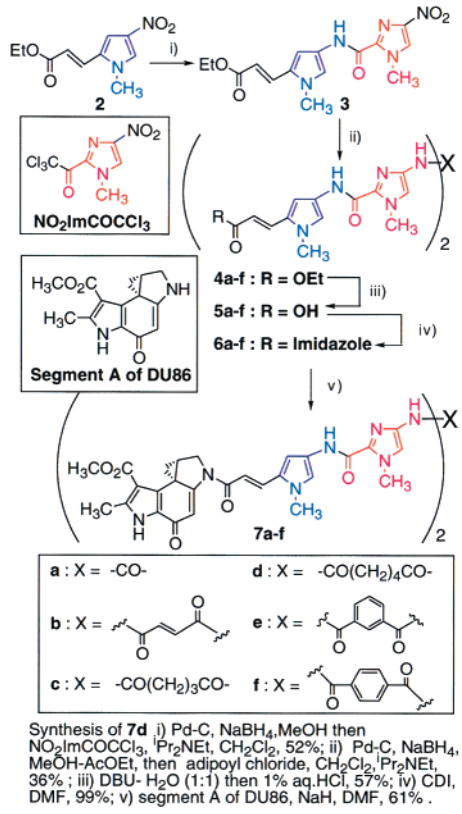
DNA interstrand cross-linking inhibits both DNA replication and gene expression and therefore has considerable potential for molecular biology and human medicine.[1] Cross-linking agents, including antitumor antibiotics such as mitomycin C and carzinophilin A as well as synthetic antitumor agents such as Bizelesin and nitrogen mustard derivatives, show intrinsic sequence-selectivity in the formation of interstrand cross-links.[2] However, an interstrand cross-linking agent that targets a predetermined base-pair sequence has not been achieved. Minor-groove binding polyamides that contain *N*-methylimidazole (Im)-*N*-methylpyrrole (Py)-hydroxylpyrrole (Hp),[3] which uniquely recognize each of the four Watson−Crick base pairs, can be used as novel recognition parts of sequence-specific DNA alkylating agents. Indeed, we demonstrated that hybrid molecules between segment A of duocarmycin A[4] and Im/Py diamides and hairpin polyamides specifically alkylate at predetermined base-pair sequences.[5] Dervan and collegues have recently achieved similar sequence-specific DNA alkylations[6] by the conjugation of hairpin polyamide and seco-CBI.[7] We also demonstrated that Im/Py diamide-CPI conjugate with a vinyl linker, ImPyLDu86, alkylates double-stranded DNA at predetermined sequences through highly cooperative homodimer formation.[8] Herein we describe the synthesis of a covalent dimer of ImPyLDu86 connected with various linkers and their DNA interstrand cross-linking abilities.

Scheme 1



Synthesis of **7d** i) Pd-C, $NaBH_4$,MeOH then $NO_2ImCOCCl_3$, $^iPr_2NEt$, CH_2Cl_2, 52%; ii) Pd-C, $NaBH_4$, MeOH-AcOEt, then adipoyl chloride, CH_2Cl_2, $^iPr_2NEt$, 36% ; iii) DBU- H_2O (1:1) then 1% aq.HCl, 57%; iv) CDI, DMF, 99%; v) segment A of DU86, NaH, DMF, 61% .

We have designed an interstrand cross-linking system by forming a 1:2 complex of an alkylating dimer component with partner Im/Py triamides to achieve efficient interstrand cross-linking by synthesizing dimers of ImPyLDu86 possessing various linkers (**7a**−**f**). Scheme 1 shows that the dimeric units (**4a**−**f**) were synthesized by the reduction of **3** followed by coupling with six different linkers. After subsequent deprotection, carboxyl groups were activated by CDI to form **6a**−**f**, which were coupled with segment A of Du86,[9] to give **7a**−**f**.[10]

The interstrand cross-linking abilities of **7a**−**f** were examined by denaturing polyacrylamide gel electrophoresis using a 5′-TexasRed-labeled DNA oligomer and its complementary strand as shown in Figure 1. Compounds **7c** and **7d** generated slow migrating bands only in the presence of ImImPy (lane 6 and 7), while other compounds such as **7a**,**b**,**e**, and **f** did not generate such slow migrating bands under the same conditions. When a longer complementary 21 mer was employed in the same reaction, a slower migrating band appeared, suggesting that these slow migrating bands are due to cross-linked products (Figure 1S). These results suggest that the combination of **7d** and ImImPy efficiently produces DNA interstrand cross-links. Interestingly, rigid linker compounds did not produce slow migrating bands, and only tri- and tetramethylene linker compounds gave cross-

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(10) The purity of **7d** was more than 85% as determined by HPLC analysis (Wakopak 5C18, 0.05 M ammonium formate containing 0−100% acetonitrile, linear gradient, 20 min, at a flow rate of 1.0 mL/min; retention time **7d**: 14.4 min). ^{1}H NMR (DMSO-d_6) δ 1.29 (m, 2H), 1.58 (s, 4H), 2.09 (m, 2H), 2.33 (s, 4H), 2.47 (s, 6H), 3.45 (m, 2H), 3.72 (s, 6H), 3.73 (s, 6H), 3.95 (s, 6H), 4.18 (m, 2H), 4.28 (m, 2H), 6.57 (d, J = 14.5 Hz, 2H), 6.83 (brs, 2H), 6.99 (s, 2H), 7.41 (s, 2H), 7.44 (s, 2H), 7.58 (d, J = 14.5 Hz, 2H), 9.98 (s, 2H), 10.23 (s, 2H), 12.36 (brs, 2H); ESIMS *m/e* calcd for $C_{60}H_{61}N_{14}O_{12}$ (M + H) 1169.5, found 1169.6. The synthesis of other compounds **7a**−**c** and **7e**−**f** were performed in a manner similar to that for **7d**.

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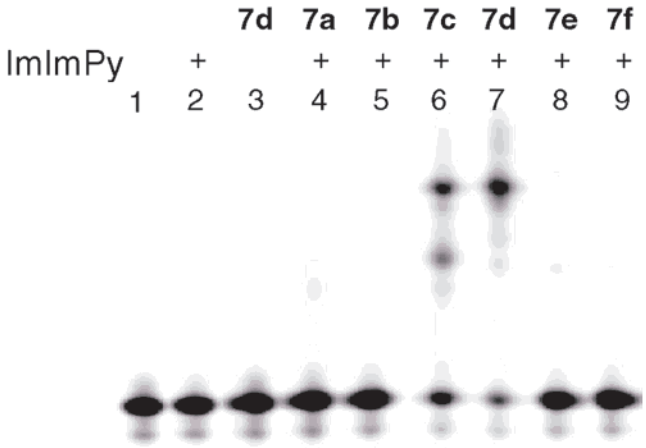

Figure 1. Denaturing polyacrylamide gel electrophoresis of 5′-TexasRed-labeled 5′-[TR]-TTACAGTGGCTGCCAGCA-3′ and 5′-TGCTGGCAGC-CACTG-3′ cross-linked by **7a**−**f** in the presence and absence of ImImPy. (a) Lane 1, DNA control; lane 2, 30 μM ImImPy; lane 3, 24 μM **7d**; lanes 4−9, 30 μM ImImPy and 24 μM **7a**−**f**, respectively. DNA fragment (3 μM) labeled with 5′-TexasRed was incubated in 10 μL of 5 μM Na cacodylate buffer (pH 7.0) at 37 °C for 15 h. The reaction was quenched by adding 1 μL of 1 μM calf thymus DNA. To this solution was added 89 μL of H_2O. A 1 μL of aliquot was concentrated, and the resulting residue was redissolved in 8 μL of loading dye (formamide with New Fuchsine). A 2 μL aliquot was electrophoresed on 15% denaturing polyacrylamide gel using a Hitachi 5500-S DNA sequencer.

linking products. The compound with a pentamethylene linker gave less cross-linked product, indicating that tetramethylene linker is an optimal spacer for the formation of interstrand cross-linking (Figure 3S). The reason for an efficient cross-linking by **7d** is not known; however, flexibility of the linker region would be important for the reaction. The band which appeared just below the cross-linking product observed in the case of **7c** was assumed to be a mono-alkylated product, since this band was predominantly formed when the same reaction by **7c** was carried out with 5′-[TR]-TTACAGTGGCTGCC**A**GCA-3′/5′-TGCTGGCAGCCTCTG-3′ in which target A in the lower strand was substituted with T.

To elucidate the site of interstrand cross-linking, we chemically degraded the products using two set of oligomers in which the upper or lower strand was labeled. Both systems provided slow migrating bands (Figure 2, lanes 2 and 7). Heating with piperidine (90 °C, 20 min) converted these bands to a faster migrating product (lanes 3, and 8). The faster bands were converted into slightly slower migrating bands by dephosphorylation with alkaline phosphatase (lanes 4 and 9), the mobilities of which were identical to that of the authentic fragments (lanes 5 and 10). These results demonstrated that interstrand cross-linking selectively occurred at target A's colored in red.[11] For efficient cross-linking, one A·T base pair between two recognition moieties was required to accommodate the linker region. Deletion of the A·T base pair and insertion of two A·T base pairs significantly and slightly reduced cross-linking, respectively. Substitution of the A·T linker with a G·C base pair almost completely inhibited the cross-linking. These results suggest that the linker region possesses A·T base-pair preference.[12]

We examined the sequence specificity of the interstrand cross-linking using **7d** in the presence of various triamides. In contrast to ImImPy, significant cross-linked bands were not observed for ImImIm, ImPyPy, and PyImPy. Densitometric analysis of gel electrophoresis indicated that the efficiency of cross-linking by these compounds was less than 10%. These results indicate that interstrand cross-linking proceeds sequence-specifically according to Dervan's base-pair recognition rule.[3]

(11) It was found that **7d** also cross-linked to the corresponding oligomers possessing G's at cross-link sites in the presence of ImImPy with a slightly low efficiency (Figure 2S).

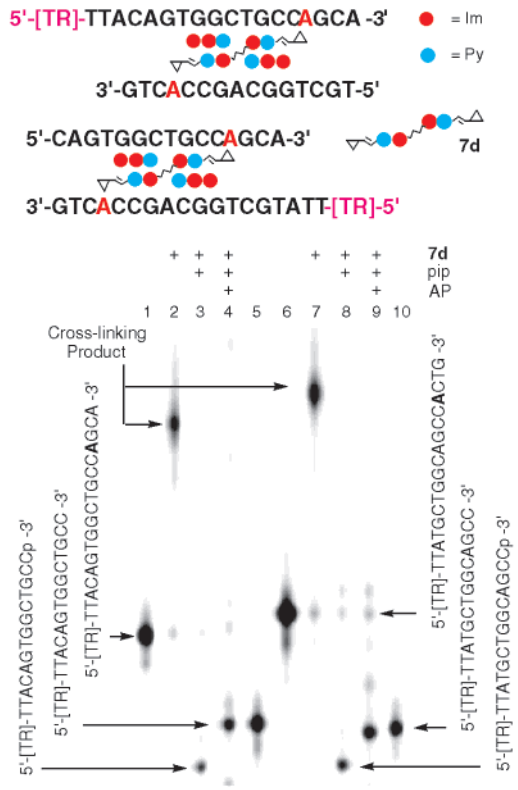

Figure 2. Denaturing polyacrylamide gel electrophoresis of 5′-TexasRed-labeled oligonucleotides. Lanes 1−5 and lanes 6−10 use 5′-[TR]-TTAC-AGTGGCTGCCAGCA-3′ and 5′-[TR]-TTATGCTGGCAGCCACTG-3′, respectively. Lanes 1 and 6, DNA controls; lanes 2 and 7, reaction mixture; lanes 3 and 8, reaction mixture after exposure to heat and hot piperidine; lanes 4 and 9, samples of lane 3 and 8 after exposure to alkaline phosphatase. Lanes 5 and 10, 5′-[TR]-TTACAGTGGCTGCC-3′ and 5′-[TR]-TTATGCTGGCAGCC-3′. DNA cross-linking was carried out as described in the legend to Figure 1. Heat degradation of cross-linking product was performed at 90 °C for 20 min and followed by heating in 0.1 M piperidine at 90 °C for 20 min. Dephosphorylation was carried out with calf intestine alkaline phosphatase at 37 °C for 1 h. The samples were analyzed as described in the legend to Figure 1.

In conclusion, we developed a novel DNA interstrand cross-linking agent **7d** that cross-linked double strands only in the presence of ImImPy at a nine-base-pair sequence, 5′-PyGGC(T/A)GCCPu-3′. The present system will provide a promising approach for the design of novel sequence-specific DNA interstrand cross-linking agents. Targeting specific sequences in the human genome by such sequence-specific cross-linking agent would constitute a powerful gene-regulating tool.[3g,13] Further studies on the applicability of this novel class of cross-linking agents are currently in progress.

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Supporting Information Available: Figures 1S−3S show denaturing polyacrylamide gel electrophoresis of 5′-[TR]-TTACAGTGGCTGCC**A**-GCA-3′/5′-**TTTTTA**TGCTGGCAGCC**A**CTG-3′ and 5′-[TR]-TTACA-GCGGCTGCC**G**GCA-3′/5′-TGCCGGCAGCC**G**CTG-3′ cross-linked by **7c**, **7d**, and pentamethylene linker compound in the presence of ImImPy (PDF). This material is available via the Internet at http://pubs.acs.org.

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