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Site-Selective DNA Alkylation of GG Steps by Naphthaldiimide Derivatives Possessing Enantiomeric Epoxide

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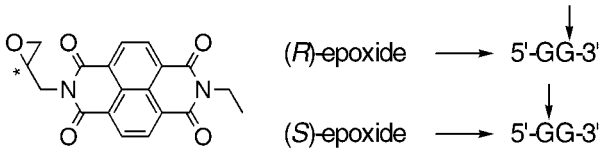
ABSTRACT



We have synthesized an enantiomeric pair of novel DNA alkylating agents consisting of a naphthaldiimide intercalator and a chiral epoxy side chain. These naphthaldiimide derivatives have high DNA binding affinity and selectively alkylate 5′G of the GG steps for (*S*)-epoxide and 3′G for (*R*)-epoxide.

Guanine N7 is one of the most nucleophilic sites in DNA and is readily alkylated by naturally occurring antitumor antibiotics and carcinogens. A large number of guanine N7 alkylating agents are known such as aflatoxin B_1 oxide,[1] altromycin B,[2] psorospermin,[3] ptaquiloside,[4] kapurimycin A_3,[5] dimethyl sulfate,[6] nitrogen mustards,[7] alkylnitrosoureas,[8] and bromoacetate derivatives.[9] These guanine alkylating agents are classified into two categories in terms of DNA alkylation selectivity: DNA alkylators that equally alkylate all guanine residues, such as ptaquiloside analog[4b] and dimethyl sulfate, and sequence selective DNA alkylators that recognize two or more base pairs and subsequently alkylate N7 of guanine in a sequence specific manner. For example, pluramycin aglycons preferentially alkylate the 5′G of GG steps.[10] On the other hand, the 3′G of GG or AG steps is selectively modified by aflatoxin B_1 oxide[1] and altromycin B,[2] respectively. To get a clear insight into the molecular basis for the sequence selective G alkylation, it is important to employ a structurally simplified system as DNA alkylator. Herein we report the synthesis of naphthaldiimide (NDI) derivatives possessing an optically active epoxy side chain and demonstrate for the first time that the selectivity of GG

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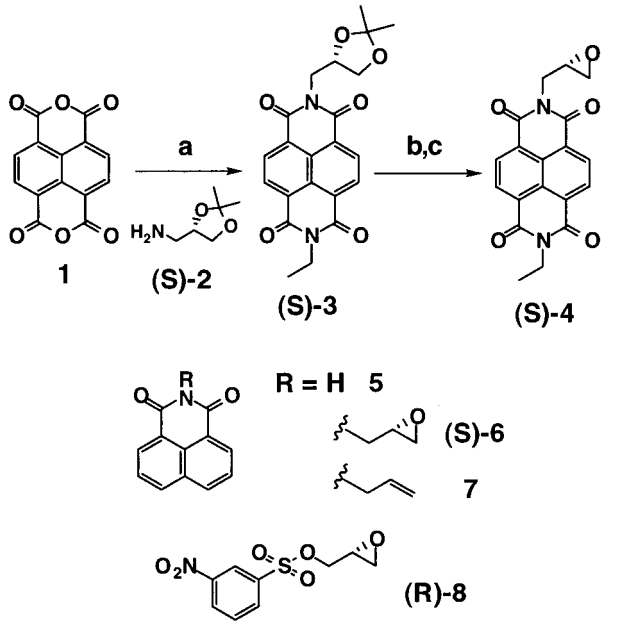
alkylation was completely modulated solely by the chirality of the epoxide on the side chain.

DNA alkylating agents were synthesized as shown in Scheme 1. The condensation of naphthalenetetracarboxylic dianhydride (**1**) with a 1:1 mixture of optically active amine **2**[11] and ethylamine followed by deprotection of the resulting diol and the stereospecific conversion from diol to epoxide[12] afforded the chiral naphthaldiimide (NDI) derivative **4**. Chiral naphthalimide (NI) **6** was also obtained by treatment of naphthalimide (**5**) with optically active glycidyl 3-nitrobenzenesulfonate (**8**).

Scheme 1[a]



[a] Reagents and conditions: (a) (*S*)-**2** (1 equiv), $EtNH_2$·HCl (1 equiv), THF, reflux, 6 h (34%); (b) HCl, AcOH, THF, r.t., 16 h (90%); (c) (i) $MeC(OMe)_3$, PPTS, CH_2Cl_2, r.t., 30 min, (ii) AcBr, CH_2Cl_2, r.t, 1 h, (iii) K_2CO_3, MeOH, rt, 2 h (91%).

Sequence selectivity of DNA alkylation induced by **4** and **6** was investigated using 38-mer oligodeoxyribonucleotide (ODN) duplexes, which consisted of ^{32}P-5′-end-labeled strands containing GN sequences (N = G, A, C, T) and the complementary strand. The duplex ODN was incubated with **4** or **6** in 10 mM sodium cacodylate buffer (pH 7.0) at 37 °C for 40 min. The alkylated guanine sites were detected as cleavage bands by hot piperidine treatment on polyacrylamide gel electrophoresis (PAGE). A typical result of PAGE was shown in Figure 1. Treatment of the duplex ODN with **4** gave 3−10-fold stronger cleavage bands at the GG step than any other GN step. In (*R*)-**4** a highly selective cleavage was observed at 3′G of the GG step (lane 4), whereas (*S*)-**4** preferentially cleaved ODN at the 5′G of the GG step (lane 5). The sequence selectivity indicates that the chirality of

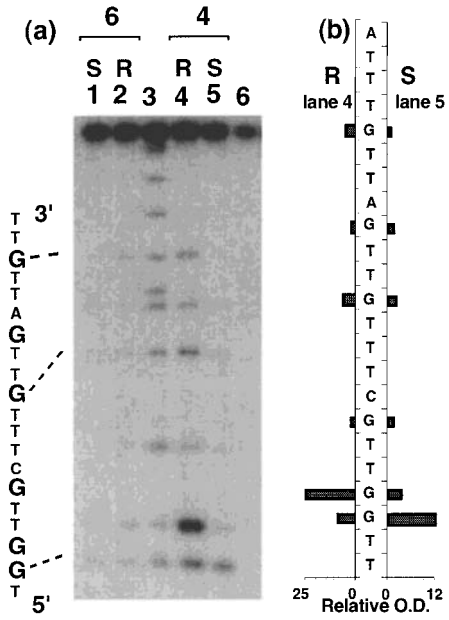


Figure 1. Cleavage of ^{32}P-5′-end-labeled 38-mer ODN 5′-AGTCTATTGGTTGCTTTGTTGATTGTTTATTTACTTAT-3′ via guanine alkylation by enantiomeric isomers of **4** and **6**. ^{32}P-5′-end-labeled ODN 38-mer was hybridized to the complementary strand (2.5 μM, strand concentration) in 10 mM sodium cacodylate buffer, pH 7.0, and the duplex was incubated with drug (50 μM) at 37 °C for 40 min. After piperidine treatment (90 °C, 20 min), the sample was analyzed on 12% denatured polyacrylamide gel electrophoresis. (a) The result of PAGE: lane 1, (*S*)-**6**; lane 2, (*R*)-**6**; lane 3, Maxam−Gilbert sequencing reactions G + A; lane 4, (*R*)-**4**; lane 5, (*S*)-**4**; lane 6, no drug. A partial sequence was shown on the left. (b) The densitometric analysis of lanes 4 and 5. The height of bars in this histogram shows the percentage of strand breakage at a given site relative to the total strand cleavage.

epoxide **4** is very important in determining 5′G and 3′G selectivity for GG alkylation. In contrast to the site-selective guanine alkylation by **4**, DNA alkylation by **6** was inefficient and almost nonselective regardless of the chirality of the epoxide (lanes 1 and 2). Alkylation of ODN containing NG sequences was also examined (see Supporting Information). NDI **4** preferentially alkylated the 5′G of the GG step for (*S*)-epoxide and the 3′G for (*R*)-epoxide. The cleavage bands at the GG step of **4** were stronger than any other NG steps.

To evaluate DNA binding abilities of these drugs, UV−vis titration was examined using racemic NDI *rac*-**3** and NI **7** in the presence of herring sperm DNA. The UV spectral change was shown in Figure 2. The absorption of *rac*-**3** showed a pronounced hypochromic effect in the presence of herring sperm DNA, whereas the absorption band of **7** showed no significant change upon addition of herring sperm DNA. The binding curve of *rac*-**3** was well fitted with the noncooperative site exclusion model of McGhee and von Hippel ($r < 0.2$).[13] The apparent neighbor n and the resulting binding constants K for *rac*-**3** were 4.5 and 1.1×10^6 M^{-1}, respectively. The result is consistent with the threading

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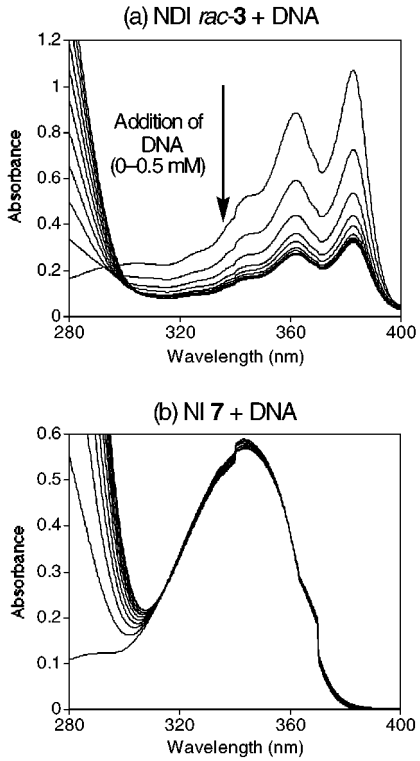

Figure 2. UV−vis titration of (a) *rac*-**3** (50 μM) and (b) **7** (50 μM) with herring sperm DNA in 10 mM sodium cacodylate buffer (pH 7.0). Spectra were recorded at ambient temperature. Small aliquots of herring sperm DNA stock solution (1.5 mM base concentration) in 10 mM sodium cacodylate buffer (pH 7.0) were added. The concentrations of DNA in the mixture were 0, 0.07, 0.14, 0.20, 0.25, 0.30, 0.35, 0.39, 0.43, 0.47, and 0.50 mM for (a) and 0, 0.29, 0.55, 0.8, 1.00, 1.20, 1.39, 1.56, and 1.71 mM for (b) from top to bottom, consecutively.

intercalation into DNA as reported previously for other NDI derivatives[14] and indicates that a tight intercalated complex is formed between NDI *rac*-**3** and DNA, whereas NI **7** has no such strong DNA binding affinity.

Guanine alkylation of ODN containing GN or NG steps by **4** site-selectively occurred at the 5′G of the GG step for (*S*)-epoxide and the 3′G for (*R*)-epoxide. It has been demonstrated in our previous studies that many DNA intercalators preferentially intercalate into GG steps rather than any GN or NG steps.[10b] According to this proposal, we built a binding model for the intercalation of **4** into the GG step to rationalize the remarkable difference in the selectivity of GG alkylation by enantiomers of **4**. In modeling studies for (*R*)-**4** that are intercalated into the GG step, the S_N2 type backside attack of the 5′G N7 on the epoxide was sterically

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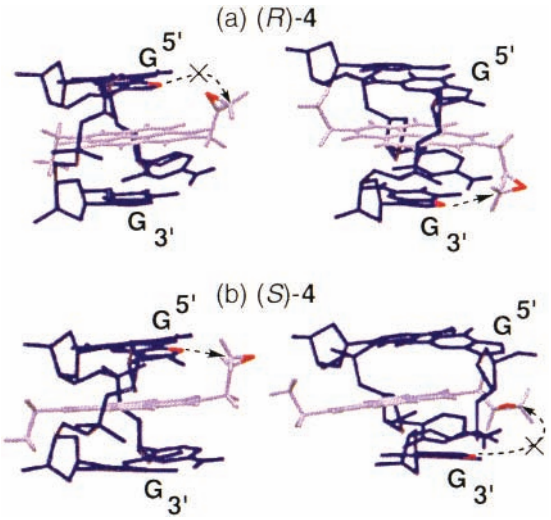

Figure 3. Molecular models of **4**-[d(GG)/d(CC)] complex. (a) (*R*)-**4**-[d(GG)/d(CC)] complex; (b) (*S*)-**4**-[d(GG)/d(CC)] complex. These models were obtained from optimization of **4**-[d(TGGT)/d(ACCA)] complex by AMBER* force field in water by using MacroModel version 5.0. After energy minimization, A/T base pairs were removed. [d(GG)/d(CC)] and **4** are shown in dark blue and light blue, respectively. Oxygen atom of epoxide and guanine N7 to be alkylated are shown in red.

unfeasible, whereas the epoxide was well oriented for S_N2 attack by 3′G N7 (Figure 3a). On the contrary, the conformation of the epoxide in the (*S*)-**4**-[d(GG)/d(CC)] complex was well suited for the epoxide ring-opening by 5′G N7, whereas for the (*S*)-**4**-[d(GG)/d(CC)] complex S_N2 backside attack was unfeasible for 3′G N7 (Figure 3b). Thus, the absolute configuration of the epoxide on the side chain plays a crucial role in determining the site-selectivity of the GG step in the DNA alkylation.

The selectivity of guanine alkylation of GG steps by NDI **4** was controlled by the chirality of the epoxide on the side chain. In (*S*)-**4** the highly selective G cleavage was observed at the 5′G of the GG step, whereas (*R*)-**4** preferentially cleaved DNA at the 3′G of the GG step. The present results on the site-selective alkylation of GG steps by simple NDI enantiomers afford useful information for the mechanism of highly site-selective alkylation of GG steps by antitumor antibiotics and other DNA alkylators possessing an optically active epoxy side chain by assuming strong binding of NDI by threading intercalation into the GG step.

Supporting Information Available: Experimental procedures and spectral data for reaction products and an autoradiogram showing site-selective alkylation of ODN containing NG sequences. This material is available free of charge via the Internet at http://pubs.acs.org.

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