Supporting Information

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

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Experimental Section

General: ^{1}H NMR spectra were measured with Varian Mercury 400 (400 MHz) spectrometer. ^{13}C NMR spectra were measured with JEOL JNM α-500 (125 MHz) spectrometer. Coupling constants (*J* values) are represented in hertz. The chemical shifts are shown in ppm downfield from tetramethylsilane using residual chloroform (δ = 7.24 in ^{1}H NMR, δ = 77.0 in ^{13}C NMR) and dimethylsulfoxide (δ = 2.48 in ^{1}H NMR, δ = 39.5 in ^{13}C NMR) as an internal standard. Melting points were obtained on a Yanaco micro melting point apparatus. EI mass and FAB mass were recorded on HX-110 and JEOL JMS HX-110 spectrometer, respectively. Wakogel C-200 was used for silica gel flash chromatography. Precoated TLC plates Merck silica gel 60 F_{254} was used for monitoring reactions. Herring sperm DNA was purchased from GIBCO BRL, LIFE TECHNOLOGIES. The oligodeoxynucleotides were purchased from Amersham Pharmacia Biotech. T4 polynucleotide kinase (10 units/μL) was purchased from NIPPON GENE and γ-[^{32}P]-ATP (10 mCi/ml) was from Amersham. A GIBCO BRL Model S2 sequencing gel electrophoresis apparatus was used for polyacrylamide gel electrophoresis (PAGE).

(*S*)-*N*-[(3,3-Dimethyl-2,4-dioxolanyl)methyl]-*N'*-ethyl-1,4,5,8-naphthaldiimide (3). To a suspension of ethylamine hydrochloride (150 mg, 1.84 mmol) in tetrahydrofuran

(10 mL) was added triethylamine (0.26 mL, 3.54 mmol) at ambient temperature, and the mixture was stirred for 30 min. To this mixture was added 1,4,5,8-naphthalenetetracarboxylic dianhydride (**1**) (250 mg, 0.93 mmol) and (*S*)-1-amino-2,3-propanediol isopropylidene ketal (**2**)[11] (183 mg, 0.87 mmol) at ambient temperature and the mixture was stirred at reflux for 6 h. The resulting precipitate was filtered off and the filtrate was concentrated *in vacuo*. The crude product was purified by column chromatography on silica gel, eluting with 5% methanol in chloroform to give **3** (130 mg, 34%) as a yellow solid: mp 201 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (s, 4H), 4.60 (dd, 1H, J = 7.4, 12.4 Hz), 4.56 (dddd, 1H, J = 4.0, 5.2, 6.0, 7.4 Hz), 4.25 (q, 2H, J = 7.1 Hz), 4.15 (dd, 1H, J = 4.0, 12.4 Hz), 4.12 (dd, 1H, J = 6.0, 8.8 Hz), 3.90 (dd, 1H, J = 5.2, 8.8 Hz), 1.47 (s, 3H), 1.34 (t, 3H, J = 7.1 Hz), 1.47 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.97, 162.57, 131.13, 130.86, 126.81, 126.79, 126.67, 126.45, 109.85, 73.32, 67.70, 43.46, 36.07, 26.69, 25.50, 13.29; MS (FAB) *m*/*e* (%) 409 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{22}H_{21}O_6N_2$ [$(M+H)^+$], 409.1398; found, 409.1403.

(*S*)-*N*-(2-Oxiranylmethyl)-*N'*-ethyl-1,4,5,8-naphthaldiimide (4). To a solution of **3** (60 mg, 0.15 mmol) in tetrahydrofuran (5 mL) and acetic acid (5 mL) was added 0.4 M hydrochloric acid (0.5 mL) at 0 °C, and the mixture was stirred at ambient temperature for 16 h. The resulting precipitate was filtered off and the residue was washed with chloroform and dried *in vacuo* to give the corresponding diol (48.5 mg, 90%) as a pale

red solid: mp 284 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.64 (s, 4H), 4.84 (d, 1H, J = 5.2 Hz), 4.63 (t, 1H, J = 5.8 Hz), 4.23 (dd, 1H, J = 8.4,12.4 Hz), 4.09 (q, 2H, J = 7.1 Hz), 4.00 (dd, 1H, J = 4.8, 12.4 Hz), 3.91 (m, 1H), 3.37–3.46 (2H), 1.23 (t, 3H, J = 7.1 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 162.78, 162.34, 130.29, 126.34, 126.17, 126.04, 126.00, 68.28, 43.69, 35.20, $_{1}$2.94; MS (FAB) *m*/*e* (%) 369 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{19}H_{17}O_6N_2$ [$(M+H)^+$], 369.1085; found, 369.1099. To a suspension of diol (20.0 mg, 54 μmol) and pyridinium *p*-toluene sulfonate (1.0 mg, 3.0 μmol) in dichloromethane (5 mL) was added trimethyl orthoacetate (40 μL, 0.31 mmol) at ambient temperature, and the mixture was stirred for 30 min. The volatiles were evaporated and residual methanol was removed *in vacuo*. To the resulting residues was added dichloromethane (5 mL) and acetyl bromide (20 μL, 0.23 mmol) at ambient temperature and the mixture was stirred at ambient temperature for 1 h. The volatiles were evaporated. To the resulting residues was added methanol (10 mL) and potassium carbonate (113 mg, 0.82 mmol) at ambient temperature and the mixture was stirred vigorously at ambient temperature for 2 h. The mixture was poured into saturated aqueous ammonium chloride (20 mL) and extracted with chloroform (50 mL × 3). The combined organic layers were dried over magnesium sulfate, filtered and evaporated. The crude product was purified by column chromatography on silica gel, eluting with 10% methanol in chloroform to give **4** (17.4 mg, 91%) as a pale yellow solid: mp 232 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 4.51 (dd, 1H, J = 5.2, 13.6 Hz), 4.33

(dd, 1H, *J* = 4.8, 13.6 Hz), 4.27 (q, 2H, *J* = 7.1 Hz), 3.35 (m, 1H), 2.83 (dd, 1H, *J* = 4.0, 5.0 Hz), 2.77 (dd, 1H, *J* = 2.4, 5.0 Hz), 1.34 (t, 3H, *J* = 7.1 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 162.93, 162.56, 131.26, 130.92, 126.99, 126.82, 126.76, 126.30, 49.07, 46.48, 42.23, 36.10, 13.30; MS (FAB) *m*/*e* (%) 351 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{19}H_{15}O_5N_2$ [$(M+H)^+$], 351.0980; found, 351.0983.

(*S*)-*N*-[Oxiranylmethyl]-1,8-naphthalimide (6). To a suspension of sodium hydride (60%, 26.8 mg, 1.12 mmol) in *N*,*N*-dimethylformamide (5 mL) was added a solution of 1,8-naphthalimide (**5**) (200 mg, 1.01 mmol) in *N*,*N*-dimethylformamide (5 mL) at 0 °C, and the mixture was stirred at 70 °C for 30 min. After cooling to ambient temperature, to the mixture was added a solution of (2*R*)-(–)-glycidyl 3-nitrobenzenesulfonate (526 mg, 2.02 mmol) in *N*,*N*-dimethylformamide (4 mL) at ambient temperature, and the mixture was stirred at 50 °C for 1 h. After cooling to ambient temperature, the mixture was diluted with *sat. aq*. ammonium chloride and extracted with chloroform (50 mL × 3). The combined organic layers were dried over magnesium sulfate, filtered and evaporated. The crude product was purified by column chromatography on silica gel, eluting with 10% methanol in chloroform to give **6** (190 mg, 74%) as a white solid: mp 169 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, 2H, *J* = 8.0 Hz), 8.22 (d, 2H, *J* = 8.4 Hz), 7.76 (t, 2H, *J* = 8.0 Hz), 4.52 (dd, 1H, *J* = 4.8, 5.2 Hz), 4.28 (dd, 1H, *J* = 5.2, 5.6 Hz), 3.35 (m, 1H), 2.77-2.81(2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.26, 134.23,

131.65, 131.53, 128.26, 126.99, 122.41, 49.31, 46.57, 41.78; MS (EI) *m*/*e* (%) 253 (M^+,39), 222 (100), 210 (64), 180 (61), 152 (49), 126 (51); HRMS (EI) calcd for $C_{15}H_{11}O_3N$ (M^+), 253.0738; found, 253.0735.

***N*-(2-propenyl)-1,8-naphthalimide (7).** To a suspension of 1,8-naphthalic anhydride (1.00 g, 5.07 mmol) in toluene (20 mL) was added allylamine (434 mg, 7.61 mmol), and the mixture was stirred at reflux for 5.5 h. The reaction mixture was concentrated *in vacuo*, and the residue was purified by column chromatography on silica gel, eluting with 10% ethyl acetate in toluene to give **7** (1.05 g, 88%) as a light orange solid: mp 137 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.62 (d, 2H, J = 7.3 Hz), 8.22 (d, 2H, J = 8.4 Hz), 7.76 (t, 2H, J = 8.4 Hz), 6.00 (ddt, 1H, J = 17.2, 10.3, 5.7 Hz), 5.33 (dd, 1H, J = 17.0, 1.3 Hz), 4.80 (dt, 2H, J = 5.7, 1.3 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.94, 134.02, 132.18, 131.63, 131.35, 128.22, 126.95, 122.62, 117.57, 42.41; MS (FAB) *m*/*e* (%) 238 [$(M+H)^+$]; HRMS (FAB) calcd for $C_{15}H_{12}O_2N$ [$(M+H)^+$], 238.0790; found, 238.0860.

Preparation of ^{32}P-5'-End-Labeled ODN. The oligonucleotides (400 pmol) was 5'-end-labeled by phosphorylation with [γ-^{32}P]ATP (4 μL, Amersham, 370 MBq/mL) and T4 polynucleotide kinase (4 μL, Takara, 10 units/mL) using standard procedures. The 5'-end-labeled DNA were recovered by ethanol precipitation, purified by 15% nondenatured gel electrophoresis, and then isolated by a crush and soak method.

Cleavage of ^{32}P-5'-End-Labeled DNA. The isolated ^{32}P-5'-end-labeled ODNs was incubated with the complementary strand (2.5 μM, strand concentration) in 10 mM sodium cacodylate buffer (pH 7.0) at 90 ˚C for 5 min and slowly cooled to ambient temperature for duplex formation. The ^{32}P-5'-end-labeled duplex solutions (40 μL) were added to a 1.5 mL Eppendorf tube containing 55 μL of water, 5 μL of 1 mM drug in acetonitrile (50 μM, final concentration). After incubation at 37 °C for the period indicated, the solutions were diluted with 10 μL of calf thymus DNA (50 μM, base pair conc) and 10 μL of 3 M sodium acetate buffer (pH 5.2). After precipitation with cold ethanol (800 μL) for 20 min at –80 °C followed by centrifugation (15 min at 0 °C, 1.5 × 10^4 rpm), the resulting DNA pellets were washed with cold 80% ethanol and dried under vacuum. After the DNA pellets were treated with 1 M piperidine for 20 min at 90 °C, the solution was concentrated *in vacuo*, and the residual piperidine was removed by coevaporation with water twice. The radioactivity of the samples was assayed using an Aloka 1000 liquid scintillation counter, and the dried samples were dissolved in 80% formamide loading buffer (a radiation density of 2000-3000 cpm/μL) and electrophoresed through a denaturing 12% polyacrylamide/7 M urea gel (1900 V, 1.5 h). The gels were exposed to X-ray film with an intensifying screen at –70 °C.

Determination of Binding Constants of NDI *rac*-3 to Herring Sperm DNA. A buffered solution (10 mM sodium cacodylate buffer at pH 7.0) of NDI *rac*-**3** (50 μM in a 1.0 cm cuvette) was titrated with a mixture solution of NDI *rac*-**3** (50 μM) and herring sperm DNA (1.5 mM, base concentration). The optical density of the solution at 382.6 nm was measured initially and after each addition on a JASCO V-550 UV/VIS spectrophotometer. Scatchard plot for the binding of NDI *rac*-**3** to DNA ($r < 0.2$) was fitted by nonlinear least square analysis to the McGhee and von Hippel equation governing random noncooperative binding to a lattice.[13]

Alkylation of ODN containing NG sequences

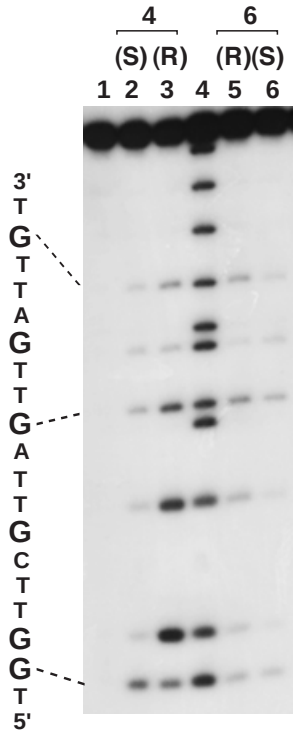
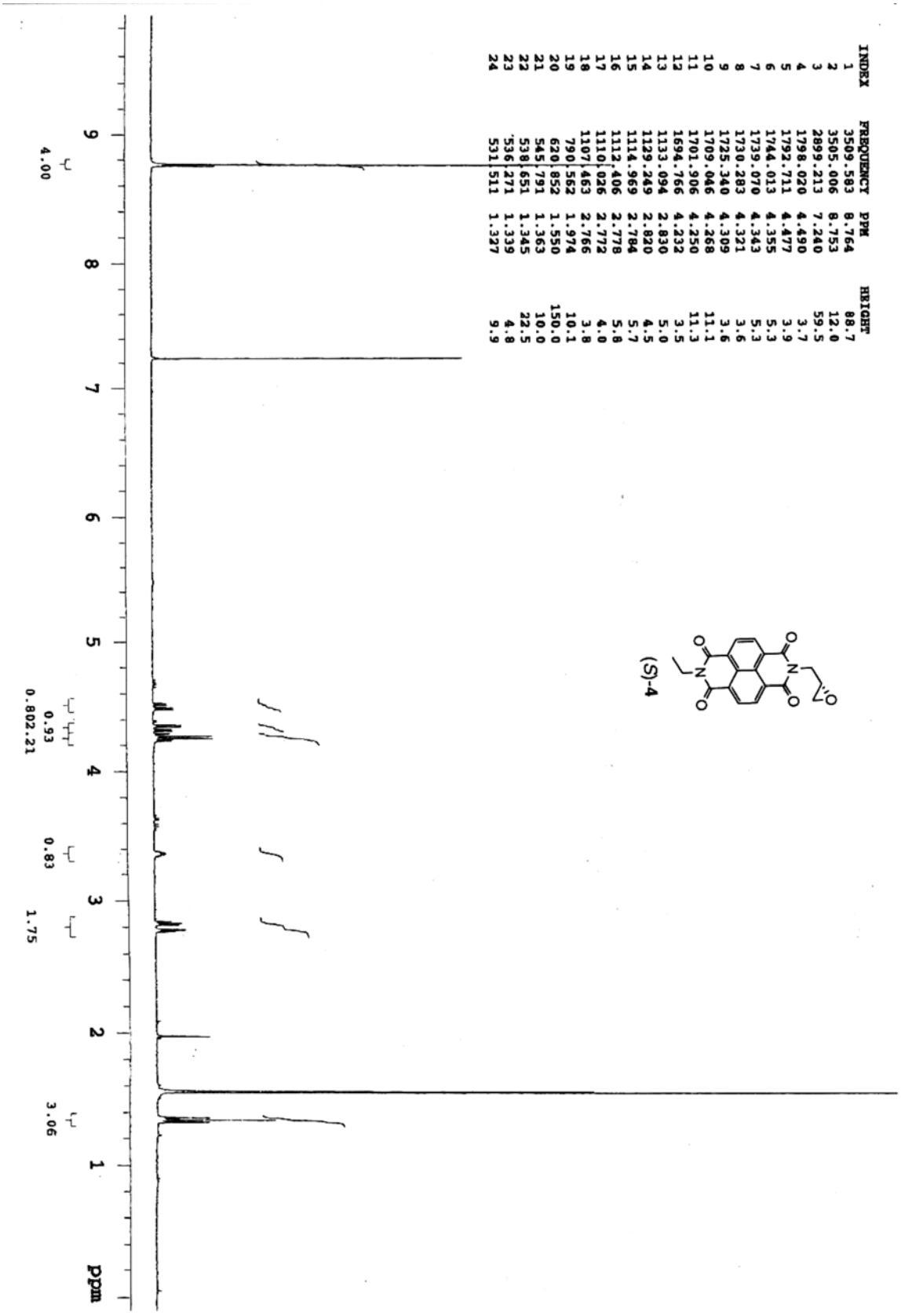


Figure S1. Cleavage of ^{32}P-5'-end-labeled 38-mer ODN 5'-AGTCTATTGGTTCGTTAGTTGATTGTTTATTTACTTAT-3' via guanine-alkylation by enantiomeric isomers of **4** and **6**. ^{32}P-5'-end-labeled ODN 38-mer was hybridized to the complementary strand (2.5 μM, strand concentration) in 10 mM sodium cacodylate 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. Lane 1, no drug; lane 2, (*S*)-**4**; lane 3, (*R*)-**4**; lane 4, Maxam–Gilbert sequencing reactions G+A; lane 5, (*R*)-**6**; lane 6, (*S*)-**6**. A partial sequence was shown on the left.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2 3505.006 8.753 12.0
3 2899.213 7.240 59.5
4 1798.020 4.490 3.7
5 1792.711 4.477 3.9
6 1744.013 4.355 5.3
7 1739.070 4.343 5.3
8 1730.283 4.321 3.6
9 1725.340 4.309 3.6
10 1709.046 4.268 11.1
11 1701.906 4.250 11.3
12 1694.766 4.232 3.5
13 1133.094 2.830 5.0
14 1129.249 2.820 4.5
15 1114.969 2.784 5.7
16 1112.406 2.778 5.8
17 1110.026 2.772 4.0
18 1107.463 2.766 3.8
19 790.562 1.974 10.1
20 620.852 1.550 150.0
21 545.791 1.363 10.0
22 538.651 1.345 22.5
23 536.271 1.339 4.8
24 531.511 1.327 9.9
(S)-4
9
8
7
6
5
4
3
2
1
ppm
4.00
0.93
0.83
1.75
3.06
0.80
2.21

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INDEX	FREQUENCY(PPM)	Hz	HEIGHT
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4	8.603	3444.9788	5.6
5	8.235	3297.7870	5.8
6	8.234	3297.2378	5.7
7	8.233	3296.8716	5.7
8	8.214	3289.3656	6.0
9	7.779	3114.8956	5.6
10	7.759	3107.2065	7.0
11	7.740	3099.3343	4.7
12	7.241	2899.6001	21.9
13	7.240	2899.2339	22.2
14	4.528	1813.2363	2.2
15	4.507	1804.8149	2.7
16	4.495	1799.8719	2.9
17	4.308	1725.1775	3.1
18	4.295	1720.0515	3.4
19	4.275	1711.8131	2.4
20	4.262	1706.5039	2.6
21	2.802	1122.1303	4.6
22	2.793	1118.2858	5.3
23	2.789	1117.0042	5.2
24	2.783	1114.2581	5.1
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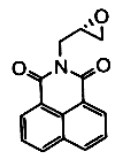
27	1.549	620.3233	45.5



(*S*)-6