Recognition of a Single Guanine Bulge by 2-Acylamino-1,8-naphthyridine

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Abstract: 2-Acylamino-1,8-naphthyridine (**1**), which possesses hydrogen bonding groups fully complementary to guanine (G), selectively binds to a single G bulge of duplex DNA. The melting temperature (T_m) of the duplex containing a G bulge was increased by the presence of **1**, whereas no increase of T_m was observed for the duplexes containing adenine (A) and thymine (T) bulges as well as for normal duplex. Riboflavin-sensitized photooxidation of DNA containing GG steps opposite to G and A bulges was selectively inhibited by the presence of **1** at the G bulge. DNase I footprinting titration indicated a selective binding of **1** to the G bulge with an association constant of $3.4 \pm 1 \times 10^4$ M^{-1}. In the presence of **1**, CD spectra of the G bulge-containing duplex noticeably changed, being accompanied by the induced CD at 300−350 nm, whereas no CD spectral change was observed for the duplex containing A bulge. Both the hydrogen bonding groups complementary to G and the planar bicyclic ring system are essential for the complex formation between G bulge and **1**.

Unpaired or bulged bases in duplex DNA can arise from replication error or from recombination of single stranded DNAs that are not fully complementary to each other. These irregular DNA sites containing bulges are considered to play an important role in frame-shift mutagenesis.[1,2] DNA repair proteins MutS[3] and RecA[4] bind to bulge structures much more tightly than to regular duplex, and MutS can differentiate DNA structures by the recognition of a single nucleotide bulge.[3] The structures of DNA containing single nucleotide bulges have been studied by NMR[5] and X-ray crystallography,[6] showing that bulged bases are looped-out into the solution or stacked into the helix depending upon the type of base, temperature, flanking sequence, and other conditions. Gel electrophoretic studies have also indicated that bulges introduce kinks into the DNA helix.[7]

In view of the importance of bulges in biological systems,[1,2,8] numerous molecular probes that bind to bulges and induce strand cleavage nearby have been reported.[9−11] However, structural diversities of bulges make it difficult to design a bulge specific probe. Furthermore, specific recognition of a bulged base that is indispensable for sensitive detection and accurate read-out of genetic defects still remains to be investigated. To develop a molecular device for detecting specific bulges with high sensitivity and accuracy, we attempted to design a bulge-recognition molecule that can differentiate the type of bulged

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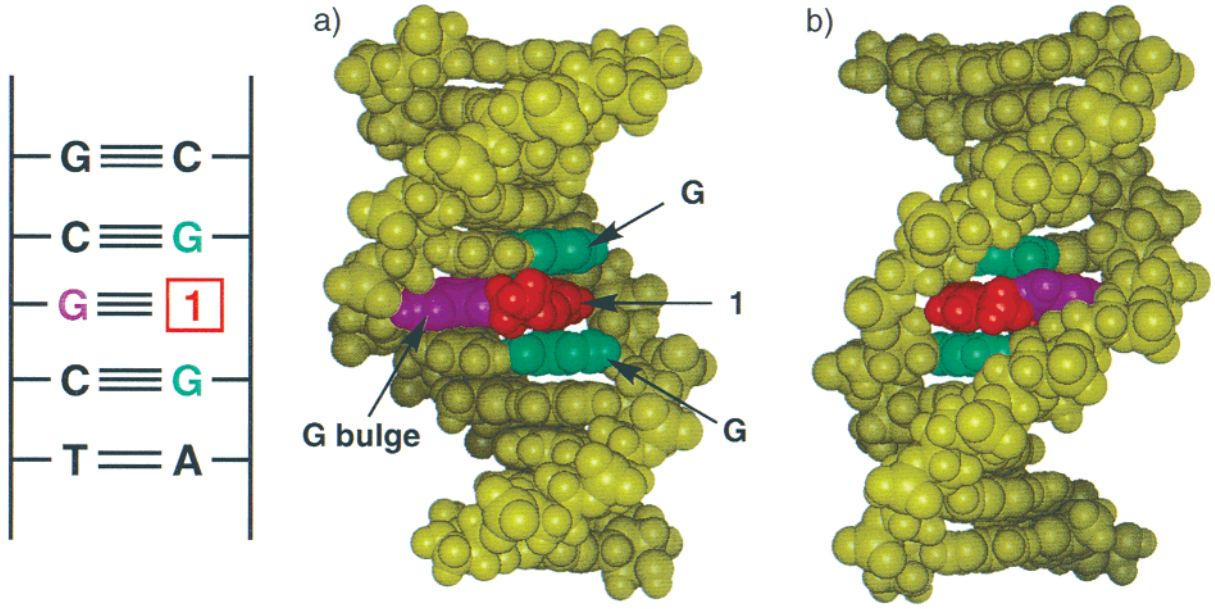

Figure 1. Proposed structure of the complex between G bulge and **1**. Molecular modeling simulations of the complex of d(TCCAG_GCAAC)/d(GTTGC*G*CTGGA) containing G bulge with **1** were carried out with MacroModel (version 6.0) with Amber* force field. Initial structure of the complex was obtained by manually inserting **1** into the G bulge of duplex d(TCCAG_GCAAC)/d(GTTG*C*GCTGGA). Energy minimization was done for the initial structure of the complex with GB-SA treatment of water. Complexes viewed from the a) major goove and the b) minor groove are shown. Bulged G and flanking Gs are colored purple and light green, respectively. Naphthyridine **1** is colored red.

bases. We herein report a specific recognition of a single guanine (G) bulge in the d(G_G)/(C*G*C) sequence by 2-acylamino-1,8-naphthyridine (**1**),[12] which was elaborated by the concept of thermodynamic stabilization of a single bulge.[13]

It is well documented that intercalating agents bind selectively to bulges compared to regular duplex.[9,13,14] NMR studies also showed that hydrogen bonding sites of bulged bases mostly remain unoccupied.[5] These facts suggest that thermodynamic stabilization of a single bulge by a small ligand may be accomplished by a complex formation wherein the bulged base is captured by the ligand through multiple hydrogen bonds and the resulting ligand-base pair is incorporated into the helix π stack. Intercalating agents possessing hydrogen bonding groups fully complementary to the target bulged base seem to be ideal for such purpose. 2-Acylamino-1,8-naphthyridines have been shown to form hydrogen bonds to guanine in organic solvents with an association constant comparable to GC base pairing.[12] Molecular modeling studies indicated that the formation of such **1**-G hydrogen bonded complex is feasible (Figure 1).

Base selective bulge stabilization was first examined by measuring the melting temperature (T_m) of four oligomer duplexes d(TCCAG_GCAAC)/d(GTTGC*N*CTGGA) containing a single nucleotide bulge (N = A, C, G, or T) in the presence of **1** (Table 1). GC base pairs were used for flanking both 5′ and 3′ sides of the bulge not only to stabilize bulged structure[7a,15] but also to gain a strong stacking stabilization for **1** by both side Gs in the complex.[16] Duplexes containing the bulge showed much lower T_m compared to the fully complementary 10-mer duplex under these conditions.[17] In the presence of **1** (100 μM), T_m was increased by 5.0 °C for the G bulge duplex and by 1.9 °C for the C bulge duplex. However, no increase of T_m was observed for A and T bulge duplexes as well as for the fully complementary duplex. To get more information on the structural requirement for stabilizing the G bulge, T_m measurements were examined in the presence of reference compounds **2** and **3**. 2-Aminoquinoline derivative **2** has only two of the three hydrogen bonding groups of **1** and cytosine derivative **3** possesses hydrogen bonding groups fully complementary to G

Table 1. Melting Temperature (T_m) of Bulge-Containing Duplexes in the Presence and Absence of Drug[a]

duplex[b]	$T_{m(-)}$	drug[c]	$T_{m(+)}$	ΔT_m
5'-TCCAG_GCAAC-3' 3'-AGGTC**G**CGTTG-5'	32.3	**1**	37.3 (41.5)[d]	5.0 (9.2)[d]
		2	33.2 (33.5)[d]	0.9 (1.2)[d]
		3	33.0 (34.1)[d]	0.7 (1.8)[d]
5'-TCCAG_GCAAC-3' 3'-AGGTC**C**CGTTG-5'	33.2	**1**	35.1	1.9
5'-TCCAG_GCAAC-3' 3'-AGGTC**A**CGTTG-5'	32.6	**1**	32.5	–0.1
5'-TCCAG_GCAAC-3' 3'-AGGTC**T**CGTTG-5'	31.2	**1**	31.0	–0.2
5'-TCCAGGCAAC-3' 3'-AGGTCCGTTG-5'	45.3	**1**	45.3	0.0

[a] The UV−melting curve was measured at a total base concentration of 100 μM in 10 mM sodium cacodylate buffer (pH 7.0) containing 0.1 M NaCl. Melting temperature in the absence ($T_{m(-)}$) or presence ($T_{m(+)}$) of the drug was calculated as the maximum in a plot of $\Delta Abs_{260}/\Delta T$ *vs* temperature. Temperature was increased at a rate of 1 °C/min. [b] Bulge bases are shown in boldface. [c] Drug concentration was 100 μM unless otherwise noted. [d] T_m shown in the parentheses was measured with 300 μM of the drug.

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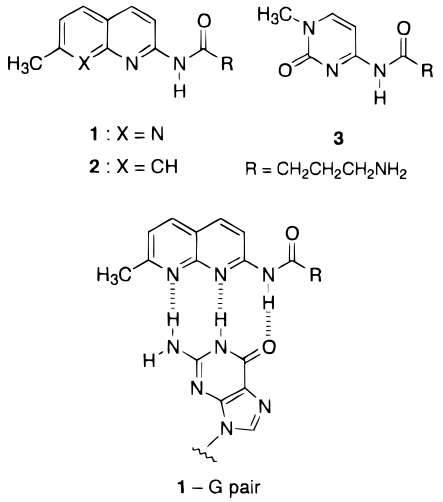

but in a monocyclic ring system. ΔT_m values obtained in the presence of these reference compounds (300 μM) were 1.2 and 1.8 °C for **2** and **3**, respectively, in drastic contrast to the ΔT_m of 9.2 °C in the presence of **1** (300 μM). These results indicated that both the bicyclic ring system and the hydrogen bonding groups are indispensable for **1** to stabilize the G bulge duplex.

Having established that naphthyridine **1** stabilizes the G bulge duplex, we have examined the photoinduced cleavage of DNA containing GG steps opposite to the G and A bulges using riboflavin (Rf) as a photosensitizer in the presence of **1**. Photoinduced cleavage of regular duplex DNA by Rf occurred selectively at the 5′ G of the 5′GG3′ step via a single electron transfer.[18] We anticipated that Rf-induced photocleavage of the bulge-containing duplex is inhibited site selectively at the GG step opposite to the G bulge, since **1** binds to the G bulge much more strongly than Rf. A single stranded 52-mer was 5′-^{32}P-end labeled and annealed with a 54-mer complementary strand to produce the duplex containing GG steps opposite to both G and A bulges in the d(AG_GC)/d(GC*N*CT) sequence (N = G and A). The duplex also contains a GG step in the d(AGGC)/d(GCCT) sequence as an internal standard. The duplex was photoirradiated at 366 nm in the presence of Rf (100 μM) under various concentrations of **1** (0−63 μM) (Figure 2). Molar absorption coefficients of Rf and **1** at 366 nm are 8870 and 280, respectively, indicating that more than 96% of the incident light is absorbed by Rf under the experimental conditions. Without **1** (lane 2), strand cleavage after hot piperidine treatment occurred selectively at GG steps opposite to both G and A bulges. The normal GG step in the regular duplex region was not oxidized under these conditions, suggesting that Rf selectively binds to both G and A bulges and oxidized the neighboring Gs upon photoirradiation.[19] With an increase in the concentration of **1** (lanes 3−5), the intensities of the cleavage bands decreased at the GG step opposite to the G bulge. The cleavage at the stacked Gs opposite to the G bulge was almost completely (ca. 95%) inhibited at the concentration of 16 μM of **1** (lane 5), whereas the cleavage at the GG step opposite to the A bulge was not inhibited even in the presence of 63 μM of **1** (lane 7).

The results of Rf sensitized photooxidation were further supported by quantitative DNase I footprinting titration (Figure 3).[19] An apparent association constant for the binding of **1** to the G bulge was determined as $3.4 \pm 1 \times 10^4$ M^{-1} by an average of three data sets (Figure 4),[20] whereas no measurable footprints

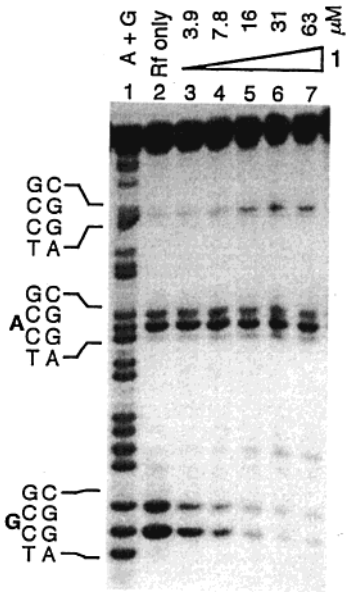

Figure 2. Cleavage of a duplex containing both G and A bulges by photoexcited riboflavin (Rf) in the presence of various concentrations of **1**. Lane 1, Maxam−Gilbert A + G sequencing reaction; lanes 2−7, 0.0, 3.9, 7.8, 16, 31, and 63 μM of **1**, respectively. Sequences of three GG steps are shown on the left side.

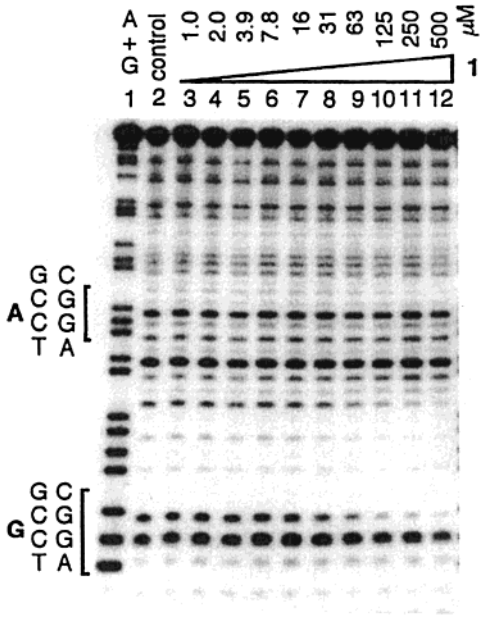

Figure 3. DNase I footprinting analysis of 5′-^{32}P-end labeled 52-mer DNA containing both G and A bulges with naphthyridine **1**. Lane 1, Maxam−Gilbert A + G sequencing reaction; lanes 2−12, 0.0, 1.0, 2.0, 3.9, 7.8, 16, 31, 63, 125, 250, and 500 μM of **1**, respectively. Bulge sites are shown on the left side.

were observed at the A bulge. Formation of a stable complex (MW = 6625) between **1** and a G bulge-containing duplex d(TCCAG_GCAAC)/d(GTTGC*G*CTGGA) in a 1:1 stoichiometry was also supported by electrospray ionization mass spectrometry (ESI-MS) (Figure 5).[21,22]

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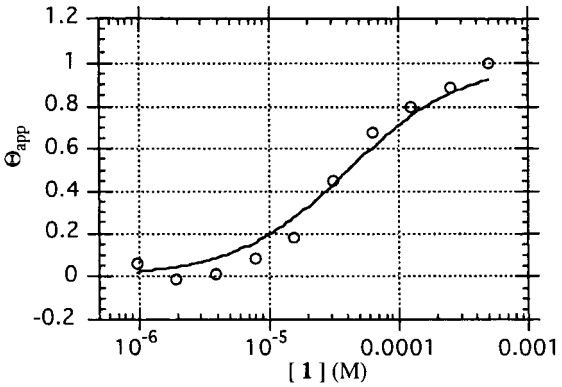

Figure 4. Data for the quantitative DNase I footpronting titration experiment for **1** with the G bulge site of 5′-CAG_GCC-3′/3′-GTC*G*CGG-5′. The Θ_{app} points were obtained using autoradiography shown in Figure 3. The solid line is the best-fit Langmuir binding titration isotherm obtained from a nonlinear least-squares algorithm as reported.[20]

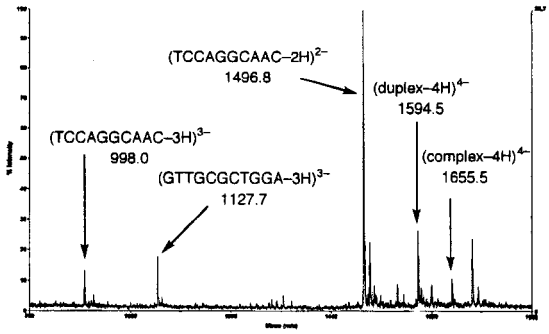

Figure 5. ESI-TOF MS spectra obtained from a mixture of duplex d(TCCAG_GCAAC)/d(GTTGC*G*CTGGA) (225 μM) and **1** (225 μM).

To gain further insight into the structure of the **1**−G bulge complex, CD spectra of duplexes containing either G or A bulge were measured in the presence and absence of **1** (Figure 6). In the presence of **1**, CD spectra of the G bulge duplex noticeably changed being accompanied by the induced CD in the region of 300−350 nm, whereas virtually no CD spectral change was observed for the A bulge duplex under the same conditions. These are fully consistent with the results obtained from T_m measurements, Rf sensitized photooxidation, and DNase I footprinting.

Effects of sequences flanking the G bulge on the stabilization of the complex with **1** were examined by DNase I footprinting analysis. A duplex ODN used for these experiments contains G bulges at four different sequences, d(C_C)/d(G*G*G), d(T_T)/d(AGA), and d(A_A)/d(T*G*T) in addition to the standard d(G_G)/d(C*G*C) sequence (Figure 7). As is apparent from the figure, detectable footprints were observed only at the sequence of d(G_G)/d(C*G*C) under these conditions. These results strongly suggested that π-stacking of **1** by both 5′ and 3′ side bases is important for stabilization of the complex.

In summary, we disclosed for the first time that naphthyridine **1** produces a stable complex with a G bulge with high selectivity. The hydrogen bonding groups complementary to G and the planar bicyclic ring system are essential for the formation of thermodynamically stable complex. This strategy for G bulge recognition may be applicable to the recognition of other single nucleotide bulges.

Experimental Section

General. Reagents and solvents were purchased from standard suppliers without further purification. Reactions were monitored with TLC plates precoated with Merck silica gel 60 F_{254}. Wakogel C-200 was used for silica gel flash chromatography. ^{1}H and ^{13}C NMR spectra were measured on a JEOL JNM α-400 (^{1}H spectra at 400 MHz; ^{13}C spectra at 100 MHz) spectrometer. Coupling constants (*J* values) are represented in hertz. ELI and FAB mass spectra were recorded on a JEOL JMS HX-110 spectrometer. ESI-MS spectra were recorded on a Perseptive Mariner ESI-TOF mass spectrometer. The CD spectrum of the oligomer was recorded on a Jasco J-720 instrument at 25 °C in a buffer of 10 mM sodium cacodylate (pH 7.0) and 100 mM NaCl. Photoirradiation at 366 nm was carried out using a Funakoshi TEL-33 transilluminator. Gel electrophoresis was carried out on a Gibco BRL Model S2 apparatus. All enzymes used in the studies were from commercial sources. [γ-^{32}P]ATP (6000 Ci/mmol) was obtained from Amersham. All DNA oligomers were purchased from Greiner Japan CO., Ltd.

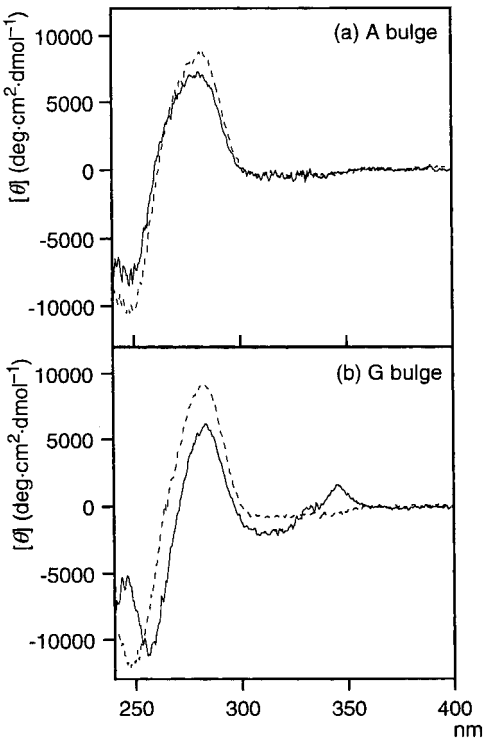

Figure 6. CD spectra of DNA duplexes containing (a) A and (b) G bulges in the absence (shown in a dotted line) and presence (a solid line) of **1** (100 μM). CD spectra of duplex d(TCCAG_GCAAC)/d(GTTGC*N*CTGGA) (*N* = A or G) (100 μM base concentration) were measured in 10 mM sodium cacodylate buffer (pH 7.0) and 100 mM NaCl.

Synthesis of 1, 2, and 3: 4-((*tert*-Butoxy)carbonylamino)-*N*-(7-methylpyridino[3,2-e]pyridin-2-yl)butanamide (Boc-Protected 1). To a solution of 2-amino-7-methylnaphthyridine[23] (260 mg, 1.63 mmol) in dry $CHCl_3$ (15 mL) was added *N*-Boc-4-aminobutyric acid succinimidyl ester (736 mg, 2.45 mmol) and the mixture was stirred at ambient temperature for 12 h. Solvent was evaporated to dryness and the crude residue was purified by silica gel column chromatography ($CHCl_3$/MeOH = 50/1) to give Boc-protected **1** (299 mg, 53%) as a white solid: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.58 (s, 1 H), 8.43 (d, 1 H, J = 8.8 Hz), 8.12 (d, 1 H, J = 8.8 Hz), 7.99 (d, 1 H, J = 8.4 Hz), 7.26 (d, 1 H, J = 8.4 Hz), 4.72 (s, 1 H), 3.23 (m, 2 H), 2.74 (s, 3 H), 2.51 (t, 2 H, J = 7.6 Hz), 1.93 (m, 2 H), 1.42 (s, 9 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.2, 163.4, 156.1, 154.1, 153.4, 139.2, 136.5, 121.7, 118.5, 114.3, 79.3, 39.8, 34.9, 28.4, 25.6, 25.5; FABMS (NBA), *m*/*e* (%) 345 [(M + H)$^+$], (100), 261 (40); HRMS calcd for $C_{18}H_{25}O_3N_4$ [(M + H)$^+$] 345.1925, found 345.1924.

4-Amino-*N*-(7-methylpyridino[3,2-e]pyridin-2-yl)butanamide (1). To a $CHCl_3$ (5 mL) solution of Boc-protected **1** (100 mg, 0.29 mmol) was added ethyl acetate containing 4 M HCl (1.5 mL) and the mixture was stirred at room temperature for 3 h. Solvent was evaporated to dryness to give **1** (quantitative yield) as a white solid: ^{1}H NMR (CD_3-OD, 400 MHz) δ 8.92 (d, 1 H, J = 8.4 Hz), 8.70 (d, 1 H, J = 8.8 Hz),

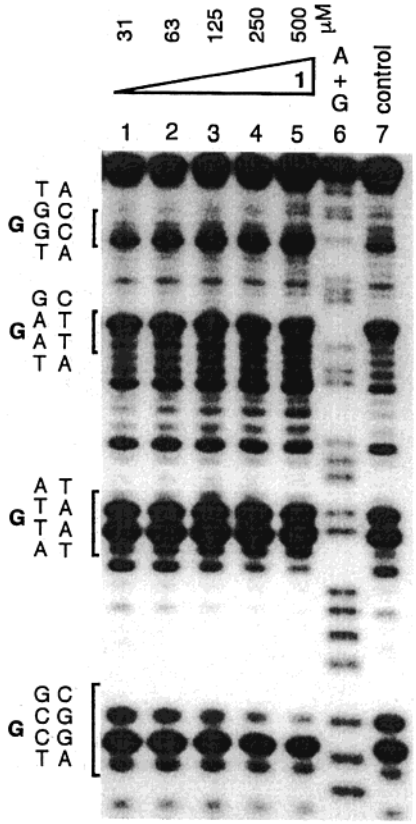

Figure 7. DNase I footprinting analysis of 5′-^{32}P-end labeled 52-mer DNA containing four G bulges with different flanking sequence in the presence of naphthyridine **1**. Lanes 1−5, 31, 63, 125, 250, and 500 μM of **1**, respectively; lane 6, Maxam−Gilbert A + G sequencing reaction; lane 7, DNase I only. Bulge sites are shown on the left side of the gel.

8.63 (d, 1 H, J = 8.8 Hz), 7.83 (d, 1 H, J = 8.4 Hz), 3.06 (t, 2 H, J = 8.0 Hz), 2.97 (s, 3 H), 2.78 (t, 2 H, J = 6.8 Hz), 2.06 (m, 2 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 174.2, 161.2, 158.6, 148.7, 147.4, 141.4, 123.5, 121.4, 118.8, 40.2, 34.6, 23.5, 20.7; FABMS (NBA), *m/e* (%) 245 [(M + H)$^+$], (100); HRMS calcd for $C_{13}H_{17}ON_4$ [(M + H)$^+$] 245.1401, found 245.1411.

4-((*tert*-Butoxy)carbonylamino)-*N*-(4-methyl-3-oxo(2,4-diazinyl))-butanamide (Boc-Protected 2). Cytosine derivative **2** was synthesized from 1-methylcytosine.[24] ^{1}H NMR ($CDCl_3$, 400 MHz) δ 10.46 (s, 1 H), 7.58 (d, 1 H, J = 7.2 Hz), 7.41 (d, 1 H, J = 7.2 Hz), 5.16 (s, 1 H), 3.52 (s, 3 H), 2.55 (m, 2 H), 2.26 (t, 2 H, J = 6.8 Hz), 1.84 (m, 2 H), 1.39 (s, 9 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 173.7, 162.9, 156.3, 155.8, 149.1, 96.8, 78.8, 39.8, 38.5, 34.5, 28.5, 25.1; FABMS (NBA), *m/e* (%) 311 [(M + H)$^+$], (100); HRMS calcd for $C_{14}H_{23}O_4N_4$ [(M + H)$^+$] 311.1718, found 311.1727.

4-Amino-*N*-(4-methyl-3-oxo(2,4-diazinyl))butanamide (2). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.48 (d, 1 H, J = 7.2 Hz), 6.58 (d, 1 H, J = 7.2 Hz), 3.61 (s, 3 H), 3.08 (t, 2 H, J = 7.6 Hz), 2.81 (t, 2 H, J = 7.2 Hz), 2.04 (m, 2 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.5, 159.6, 157.8, 148.6, 94.9, 39.8, 38.9, 34.8, 22.7; FABMS (NBA), *m/e* (%) 211 [(M + H)$^+$] (50), 154 (100); HRMS calcd for $C_9H_{15}O_2N_4$ [(M + H)$^+$] 211.1194, found 211.1189.

4-((*tert*-Butoxy)carbonylamino)-*N*-(7-methyl(2-qunolyl))butanamide (Boc-Protected 3). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.62 (s, 1 H), 8.37 (m, 1 H), 8.13 (m, 1 H), 7.76 (m, 1 H), 7.63 (m, 1 H), 7.42 (m, 1 H), 4.75 (s, 1 H), 3.21 (m, 2 H), 2.47 (t, 2 H, J = 6.8 Hz), 1.92 (m, 2 H), 1.40 (s, 9 H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 171.6, 156.0, 150.8, 146.2, 138.4, 129.8, 127.4, 127.1, 126.1, 125.0, 114.2, 79.2, 39.9, 34.9, 28.5, 25.8; FABMS (NBA), *m/e* (%) 330 [(M + H)$^+$] (90), 274 (40), 145 (100); HRMS calcd for $C_{18}H_{24}O_3N_3$ [(M + H)$^+$] 330.1816, found 330.1812.

(23) Brown, E. V. *J. Org. Chem.* **1965**. *30*, 1607−1610.
(24) Hosmane, R. S.; Leonard, N. J. *Synthesis* **1981**, 2, 118−119.

4-Amino-*N*-(7-methyl(2-qunolyl))butanamide (3). ^{1}H NMR (CD_3OD, 400 MHz) δ 8.91 (m, 1 H), 8.18 (m, 1 H), 8.16 (m, 1 H), 8.05 (m, 1 H), 7.83 (m, 1 H), 7.58 (m, 1 H), 3.11 (t, 2 H, J = 7.6 Hz), 2.89 (t, 2 H, J = 6.8 Hz), 2.13 (m, 2 H); ^{13}C NMR (CD_3OD, 100 MHz) δ 176.9, 150.8, 148.3, 136.4, 135.6, 130.2, 129.6, 126.2, 121.2, 114.8, 39.9, 34.5, 23.0; FABMS (NBA), *m/e* (%) 230 [(M + H)$^+$] (60), 154 (100); HRMS calcd for $C_{13}H_{16}O_1N_3$ [(M + H)$^+$] 230.1293, found 230.1289.

Measurements of the Melting Temperature of Bulge-Containing Duplex. Compounds **1**−**3** (100 μM) were taken in a solution containing bulge duplex (100 μM, base concentration), sodium cacodylate (10 mM, pH 7.0), and NaCl (100 mM). The mixture was heated for 5 min at 70 °C and cooled slowly to make sure that the starting oligodeoxynucleotide is in a duplex state. The thermal denaturation profile was obtained with a Jasco V-550 spectrometer equipped with a Peltier temperature controller. The absorbance of the sample was monitored at 260 nm from 2 to 70 °C with a heating rate of 1 °C/min. The T_m value was determined from a plot of absorbance (A_{260}) versus temperature and assigned as the temperature at 1/2(ΔA_{260}).

Riboflavin Footprinting Experiments. All reactions were executed in a total volume of 50 μL with final concentrations of each species as indicated. 5′-^{32}P-end-labeled 52-mer ODN 5′-d(GTC GTA GAA TCA GGC AGA ACT AAT AGG CTT AAC ATT CAG GCT TAC CAG TGT C)-3′ (10×10^3 cpm, 1 μM, strand concentration) was annealed with 54-mer ODN 5′-d(GAC ACT GGT AAG CCT GAA TGT TAA GCA CTA TTA GTT CTG CGC TGA TTC TAC GAC)-3′ (1 μM, strand concentration) in a sodium cacodylate buffer (10 mM, pH 7.0) containing NaCl (100 mM). Naphthyridine **1** (0−63 μM) was added to the solution and the mixture was kept at 4 °C for 12 h. Riboflavin (100 μM) was added to the resulting solution and the mixture was irradiated with a transilluminator (366 nm) at a distance of 5 cm at 0 °C for 40 min. After irradiation, the reaction mixture was ethanol precipitated with 900 μL of ethanol. The precipitated DNA was washed with 100 μL of 80% cold ethanol and dried in vacuo. The precipitated DNA was dissolved in 100 μL of 10% piperidine (v/v) and heated at 90 °C for 30 min. The solution was concentrated to dryness using a vacuum rotary evaporator and resuspended in 10 μL of 80% formamide loading buffer (a solution of 80% v/v formamide, 1 mM ETD, 0.1% xylene cyanol, and 0.1% bromophenol blue). All DNA samples and the Maxam−Gilbert G + A sequencing marker were heat denatured at 90 °C for 1 min and quick-chilled on ice. The samples (1 μL, 1×10^3 cpm) were loaded onto 12% (19:1) polyacrylamide and 7 M urea sequencing gel and electrophoresed at 1500 V for approximately 2.5 h. The gel was dried and exposed to X-ray film with an intensifying sheet at −70 °C.

DNase I Footprinting Experiments. 5′-^{32}P-end-labeled 52-mer ODN 5′-d(GTC GTA GAA TCA GGC AGA ACT AAT AGG CTT AAC ATT CAG GCT TAC CAG TGT C)-3′ (20 pmol) was annealed to a 2-fold excess of 54-mer ODN 5′-d(GAC ACT GGT AAG CCT GAA TGT TAA GCA CTA TTA GTT CTG CGC TGA TTC TAC GAC)-3′ in a Tris−HCl buffer (10 mM, pH 7.6) containing NaCl (100 mM). The duplex was purified by nondenaturing polyacrylamide gel electrophoresis. All reactions were executed in a total volume of 50 μL with final concentrations of each species as indicated. The 5′-^{32}P-end-labeled DNA duplex (4 nM, 10×10^3 cpm) was incubated with various concentrations of naphthyridine **1** (0−500 μM) at 4 °C for 12 h in a Tris−HCl buffer (10 mM, pH 7.6) containing NaCl (100 mM) and $MgCl_2$ (5 mM). The mixture was incubated with DNase I (0.2 U) for 8 min at 25 °C. The reaction was quenched by addition of a 10 μL solution containing EDTA (70 mM), NaOAc (0.6 M), and calf thymus DNA (5 μM). After quenching, the reaction mixture was ethanol precipitated with 900 μL of ethanol. The precipitated DNA was washed with 100 μL of 80% cold ethanol, dried in vacuo, and resuspended in 10 μL of 80% formamide loading buffer (a solution of 80% v/v formamide, 1 mM EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue). All DNA samples obtained above and the Maxam−Gilbert G + A sequencing marker were heat denatured at 90 °C for 1 min and quick-chilled on ice. The samples (1 μL, 1×10^3 cpm) were loaded onto 12% (19:1) polyacrylamide and 7 M urea sequencing gel and electrophoresed at 1500 V for approximately 2.5 h. The gel was dried and exposed to X-ray film with an intensifying sheet at −70 °C.

The sequences of ODNs used for experiments described in Figure 7 are as follows: 52-mer, ODN 5′-(GTC GTA GAA TCA GGC AGA ACT AAT AGG CTT AAC ATT CAG GCT TAC CAG TGT C)-3′, and 56-mer, ODN 5′-(GAC ACT GGG TAA GCC TGA GAT GTT AAG CCT ATG TAG TTC TGC GCT GAT TCT ACG AC)-3′.

Analysis of Quantitative DNase I Footprinting Experiments. The equilibrium association constant was determined as previously described.[20] The goodness-of-fit of the binding curve to the data points is evaluated by the correlation coefficient, with $R > 0.97$ as the criterion for an acceptable fit. The apparent association constant of **1** to the G bulge was determined as an average of three data sets.

Measurements of CD Spectra. Naphthyridine **1** (100 μM) was taken in a sodium cacodylate (10 mM, pH 7.0) buffer containing bulge duplex (100 μM, base concentration) and NaCl (100 mM). The mixture was heated for 5 min at 70 °C and cooled slowly to make sure that the starting oligodeoxynucleotide is in a duplex state. CD spectra of the solution was recorded at 25 °C using a 1 cm path length cell.

ESI-MS Measurements. ESI-MS spectra were obtained from a mixture of duplex d(TCCAG_GCAAC)/d(GTTGCGCTGGA) containing guanine bulge (225 μM) and naphthyridine **1** (225 μM).

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