Molecular recognition of a thymine bulge by a high affinity, deazaguanine-based hydrogen-bonding ligand†

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7-Deazaguanine (7-DeG) was developed as a hydrogen-bonding module capable of enhanced recognition of uracil (U) and thymine (T); a water-soluble derivative displayed high affinity and selectivity toward DNA and RNA duplexes containing single T- and U-bulges.

Single-base bulges in DNA are irregularities that may arise from replication errors or from recombination between imperfectly homologous sequences.[1] Owing to their inherent thermodynamic instability, bulges are susceptible targets for small molecules and proteins with recognition capabilities. A number of ligands have been developed that can recognize adenine (A), guanine (G), or cytosine (C) at a DNA bulge or mismatch site. These ligands consist of a bicyclic ring system for intercalation and hydrogen-bonding groups for base-specificity.[2] To our knowledge, there are no known ligands that bind a thymine (T) bulge in preference to the other base bulges.[3] This may in part be attributed to its acceptor–donor–acceptor (ADA) hydrogen-bonding motif pairing weakly.[4]

Herein we introduce 7-deazaguanine (7-DeG) as a module capable of binding thymine and uracil with higher affinity than most modules containing the donor–acceptor–donor (DAD) motif. The 7-DeG unit with an intramolecularly hydrogen-bonded amide group at N-2 (purine numbering) contains a rigid bicyclic structure that preorganizes the hydrogen-bond donor and acceptor groups.[5] The rigidification occurs partly through the formation of an intramolecular hydrogen-bond with NH-1 (Fig. 1) and is clearly seen in the X-ray analysis of **1**.[6] Possibly as a result of the greater degree of preorganization, ^{1}H NMR binding studies in $CDCl_3$ reveal a comparatively high stability for the **1**·U complex (K_a = 5800 M^{-1}) (Table 1). Typical diamidopyridine derivatives such as **3–4** exhibit weaker affinity toward thymine and uracil ($K_a \approx 10^1$–10^3 M^{-1}).[4c,7] Other factors that may play a role in the enhanced binding exhibited by **1** include enhanced acidity or basicity of the hydrogen-bond donor and acceptor sites and the orientation of the NH-7 group.

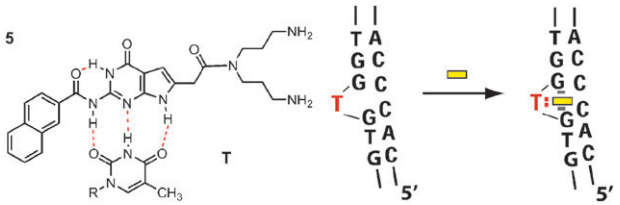
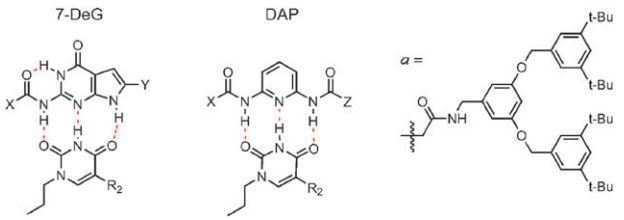


Fig. 1 Hydrogen-bonded complex of ligand **5** to thymine (T). Schematic illustration of T-bulge recognition by **5**.

Table 1 Equilibrium association constants of various 7-DeG and DAP derivatives in $CDCl_3$. The association constants are averages of at least two experiments by titration or dilution at 20 °C in which values agreed within ±15%



Compound	X	Y	Z	R_2	K_a/M^{-1}
1 (7-DeG)	*t*-Bu	CH_2CO_2Et	—	–H	5800
2 (7-DeG)		*a*	—	–H	2860
2 (7-DeG)		*a*	—	$-CH_3$	2560
3 (DAP)	C_7H_9	—	C_7H_9	–H	160
4 (DAP)		—	C_7H_9	–H	40

To illustrate the utility of 7-DeG, ligand **5** was synthesized[6] as a water-soluble derivative for recognition of thymine and uracil bulges in oligonucleotides (Fig. 1). A naphthoyl unit was incorporated to introduce a hydrophobic aromatic group extending the ligand's intercalative ability while still retaining affinity toward the target thymine or uracil. The design was validated by our studies in chloroform, which showed comparable affinity of **2** towards thymine and uracil (Table 1).

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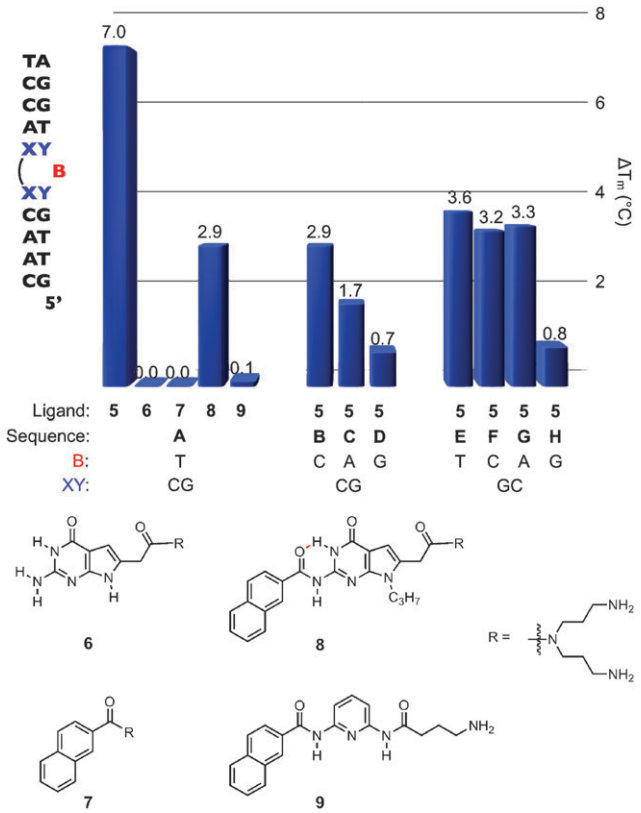

Fig. 2 Graphical summary of thermal denaturation studies with ligands **5–9** with various single base bulges flanked with purines and pyrimidines. 20 μM ligand, 5 μM duplex, 10 mM sodium cacodylate pH 7.0, 100 mM NaCl, 1 mM EDTA.

A qualitative structure–function relationship was established by thermal denaturation studies of DNA oligonucleotides containing a single T-bulge (Fig. 2). In the presence of ligand **5**, DNA duplex **A** containing a single T-bulge flanked by guanines was stabilized by +7.0 °C. Control ligands, lacking one or more features of ligand **5**, were also studied by thermal denaturation. Control **6** lacks the intercalative naphthoyl group, while retaining its hydrogen-bonding capability. Control **7** lacks the 7-DeG unit entirely. Neither **6** nor **7** stabilized duplex **A**, suggesting that both aromatic groups are essential for stabilization. Alkyl substitution of NH-9 (**8**) stabilizes duplex **A** by +2.9 °C, suggesting that blocking the DAD motif weakens the stabilization. Compound **9** provided no stabilization, despite having both the naphthalene and a recognition group that is fully complementary to T; however, an increased melting temperature was observed at higher concentrations. It should be noted that **9** is not a perfect comparison because it contains only a single basic amino group. No stabilization of fully complementary duplex d(TCCACCCAAC) was observed in the presence of **5**.

Quantitative determination of the affinity of ligand **5** for the single-base bulge sequences **A–J** was carried out by isothermal titration calorimetry (ITC) (Fig. 3). Each dataset was fit to several models and in each case it was found that a sequential two-site model gave the best fit,[6] whereas a single-site model fit the data poorly. The K_2 values varied considerably in magnitude, but, with one exception, were always less than the K_1

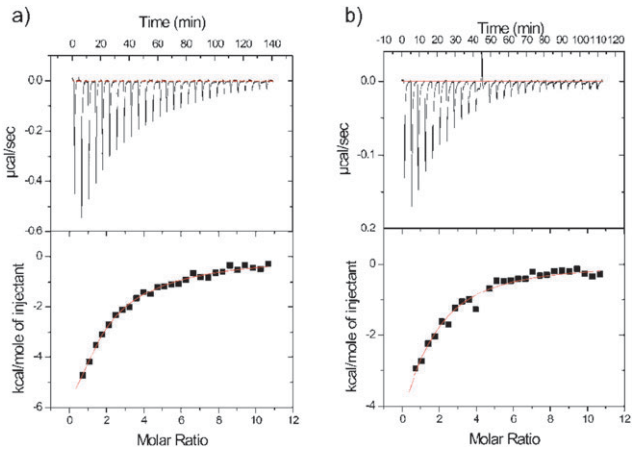

Fig. 3 ITC binding isotherms **5** to (a) DNA **A** and (b) RNA **J**. In (b) one anomalous point resulting from an air bubble has been removed (see ESI†). For (a) and (b) ligand was added to 5–40 μM solution of DNA/RNA in 10 mM sodium cacodylate pH 7.0, 100 mM NaCl and 1.0 mM EDTA.

values. It is proposed that the K_1 value (henceforth called K_d) represents specific binding at the bulge, whereas the second complexation event represents nonspecific complexation, including potentially at the bound bulge site. Similar findings have been reported by others.[8] Using a 2-aminopurine containing oligonucleotide with fluorescence titration, it was confirmed that the binding with **5** was 1:1 with a K_d similar to that determined by ITC.[6]

Ligand **5** was determined to bind deoxyoligonucleotide **A** with a K_d of 4.0 ± 0.6 μM. Ligand **5** showed a 5-, 19-, and 13-fold decrease in affinity towards duplexes containing C- (**B**), A- (**C**), and G- (**D**) bulged bases flanked by guanines, respectively (Table 2). To examine the effect of flanking pyrimidines (C), which can increase the equilibrium of a T-bulge towards its extrahelical state,[9] ITC studies were performed with duplexes **E–H**. High affinity of **5** towards **E** was observed in addition to the same selectivity pattern towards other base bulges (**F–G**) indicating that selectivity towards a T-bulge containing DNA duplex is accomplished regardless of whether the bulge is flanked by guanine or cytosine. No binding was detected for **H** or the normal duplex d(TCCACCCAAC) indicating the K_d to be >100 μM. Diamidopyridine derivative **9** binding duplex **A** provided an ~25-fold decrease in affinity (K_d = 95 μM) when compared to

Table 2 Equilibrium dissociation constants of ligand **5** to various nucleic acid secondary structures determined by ITC

Base bulge	Duplex	Flanking bases	K_d/μM[a]
T	**A**	G	4.0
C	**B**	G	21
A	**C**	G	74
G	**D**	G	51
T	**E**	C	7.4
C	**F**	C	37
A	**G**	C	61
G	**H**	C	>100
Duplex	**I**	—	>100
U (RNA)	**J**	G	9.9

[a] Average of 2–4 measurements. Repeat experiments were within 20% error.

5 binding to **A**. These results are in accordance with both the ΔT_m and chloroform studies and indicate that the special features of the 7-DeG unit are partially responsible for the observed affinity. Lastly, ITC binding analysis showed that **5** could bind RNA **J** with a K_d of 9.9 $\pm$ 1.2 μM (Fig. 3b, Table 2), thus confirming the ligand's ability to bind both T- and U-bulges.

In summary, we have introduced 7-DeG as a high affinity recognition unit for the selective binding of thymine and uracil. Selective recognition of single base bulges in DNA could provide use in single nucleotide polymorphism (SNP) analysis,[10] and in RNA, where bulges are ubiquitous and play functional roles in cellular regulatory processes. The 7-DeG unit may also find application in supramolecular chemistry, where stronger DAD·ADA motifs are needed. Structural elucidation studies of **5**·**A** and **5**·**J** are currently underway.

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Notes and references

1 (*a*) E. C. Friedberg, G. C. Walker and W. Siede, *DNA Repair and Mutagenesis*, ASM Press, Washington, 1995; (*b*) Y. Ionov, M. A. Peinado, S. Malkhosyan, D. Shibata and M. Perucho, *Nature*, 1993, **363**, 558; (*c*) G. Streisinger, Y. Okada, J. Emrich, J. Newton, A. Tsugita, E. Terzaghi and M. Inouye, *Cold Spring Harbor Symp. Quantum Biol.*, 1966, **31**, 77.

2 Selected examples: (*a*) K. Nakatani, S. Sando and I. Saito, *J. Am. Chem. Soc.*, 2000, **122**, 2172; (*b*) L. S. Kappen, Z. Xi and I. H. Goldberg, *Biochemistry*, 2001, **40**, 15378; (*c*) B. T. Patterson, J. G. Collins, F. M. Foley and F. R. Keene, *J. Chem. Soc., Dalton Trans.*, 2002, 4343; (*d*) A. Kobori, T. Murase, H. Suda, I. Saito and K. Nakatani, *Bioorg. Med. Chem. Lett.*, 2004, **14**, 3431; (*e*) H. Suda, A. Kobori, J. Zhang, G. Hayashi and K. Nakatani, *Bioorg. Med. Chem.*, 2005, **13**, 4507; (*f*) F. Takei, H. Suda, M. Hagihara, J. Zhang, A. Kobori and K. Nakatani, *Chem.–Eur. J.*, 2007, **13**, 4452; (*g*) B. M. Zeglis, J. A. Boland and J. K. Barton, *J. Am. Chem. Soc.*, 2008, **130**, 7530.

3 There are ligands that are pyrimidine selective, binding both C and T: *e.g.*, see ref. 2*d*.

4 (*a*) J. Pranata, S. G. Wierschke and W. L. Jorgensen, *J. Am. Chem. Soc.*, 1991, **113**, 2810; (*b*) T. J. Murray and S. C. Zimmerman, *J. Am. Chem. Soc.*, 1992, **114**, 4010; (*c*) S. C. Zimmerman and T. J. Murray, *Tetrahedron Lett.*, 1994, **35**, 4077; (*d*) J. R. Quinn, S. C. Zimmerman, J. E. Del Bene and I. Shavitt, *J. Am. Chem. Soc.*, 2007, **129**, 934.

5 A urea of 7-deazaguanine (7-DeUG) has been used as a component in a highly stable ADDA·DAAD quadruply hydrogen-bonded complex: H. C. Ong and S. C. Zimmerman, *Org. Lett.*, 2006, **8**, 1589.

6 See ESI† for details.

7 (*a*) A. D. Hamilton and D. Van Engen, *J. Am. Chem. Soc.*, 1987, **109**, 5035; (*b*) Triaminotriazine·cyanuric acid (ADA·DAD) complexes are more stable: A. G. Bielejewska, C. E. Marjo, L. J. Prins, P. Timmerman, F. de Jong and D. N. Reinhoudt, *J. Am. Chem. Soc.*, 2001, **123**, 7518; (*c*) Adenine itself binds T weakly, K_a = 130 M^{-1} (see ref. 3*c* for this and many more examples).

8 (*a*) M. Kaul and D. S. Pilch, *Biochemistry*, 2002, **41**, 7695; (*b*) M. Kaul, C. M. Barbieri and D. S. Pilch, *J. Mol. Biol.*, 2005, **346**, 119; (*c*) J. R. Thomas, X. Liu and P. J. Hergenrother, *Biochemistry*, 2006, **45**, 10928.

9 Pyrimidine bulges have a propensity to be in an intra- and extrahelical equilibrium when flanked by purines (temperature dependent), and extrahelical when flanked by pyrimidines: (*a*) M. W. Kalnik, D. G. Norman, M. G. Zagorski, P. F. Swann and D. J. Patel, *Biochemistry*, 1989, **28**, 294; (*b*) M. W. Kalnik, D. G. Norman, B. F. Li, P. F. Swann and D. J. Patel, *J. Biol. Chem.*, 1990, **265**, 636; (*c*) K. M. Morden, B. M. Gunn and K. Maskos, *Biochemistry*, 1990, **29**, 8835; (*d*) J. Zhu and R. M. Wartell, *Biochemistry*, 1999, **38**, 15986.

10 K. Nakatani, *ChemBioChem*, 2004, **5**, 1623.

