Ab Initio Evaluation of the Substitution Effect of the Hydrogen Bond Energy of the Watson−Crick Type Base Pair between 1-Methyluracil and Substituted 9-Methyladenine Derivatives

Shun-ichi Kawahara,[†] Tadafumi Uchimaru,*[,‡] Kazunari Taira,[†] and Mitsuo Sekine[§]

Research Center of Gene Discovery, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki 305-8562, Japan, Research Institute for Computational Sciences, National Institute of Advanced Industrial Science and Technology, Tsukuba, Ibaraki 305-8568, Japan, and Department of Life Science, Faculty of Bioscience and Biotechnology, Tokyo Institute of Technology, Nagatsuta, Midori-ku, Yokohama 226-8501, Japan

Received: November 9, 2000; In Final Form: February 5, 2001

The substitution effect on hydrogen bond energy of the Watson−Crick type base pair between uracil and chemically modified adenine derivatives was evaluated by ab initio molecular orbital theory. Predicted hydrogen bond energies were compared with experimental binding constants in some cases, and the calculated hydrogen bond energies correlated well with the experimental binding constants. Thus, ab initio calculation is an effective method to estimate the stability of the base pair between chemically modified nucleic acid bases. In contrast to the substitution effect in uracil on hydrogen bond energy, no remarkable trend was observed in the relation between the substituent in adenine derivatives and the hydrogen bond energies. The adenine derivatives, which have a nitro group on the 8-position or an amino group on the 2-position, can form the most stable hydrogen bonds with uracil.

Introduction

The hydrogen bond formation of a Watson−Crick type base pair (Figure 1) is fundamental for molecular recognition in the duplex formation of nucleic acid.[1] It is essential for transmission of genetic information, i.e., the processes of transcription from DNA to mRNA,[2] and of translation from mRNA to protein via tRNA.[3] The molecular recognition via highly selective Watson−Crick base pairing has widely attracted much attention; for example, it has been applied to construction of artificial supermolecular systems[4] and to template synthesis.[5]

On the other hand, antisense technology,[6] which is an attractive topic from the standpoint of control of expression of genetic information, is based on the selective hydrogen bond formation of nucleic acid bases. A molecule that can selectively form a stable complex is needed for the antisense technique. Many chemically modified nucleic acid analogues have been studied for the antisense strategy,[6b,c] most of which focused on modification of sugar/phosphodiester moieties, because nuclease resistance is also required for the antisense molecule. Modification of base moieties should also be taken into account in the design of an antisense molecule, considering that formation of hydrogen bonds between base moieties is essential for recognition of the targeted sequence. Modification of base moieties has been studied by some groups;[7] however, no systematic study has been made in pursuit of improvement of the base pair stability.

Recently, new types of base pairs, which use nonnatural bases, have been developed by some groups.[8] It is expected that these new types of base pairs will provide some possibilities: expansion of the genetic code,[9] probes for some enzyme assays,[10] and so on. Understanding the characteristics of the base pair formation via hydrogen bonds, especially base pairs between the nonnatural (chemically modified) nucleic acid bases, is important in this new research field.

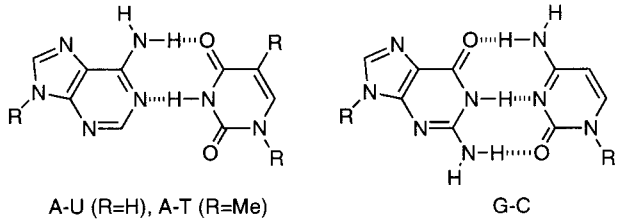

Figure 1. Watson−Crick base pairs.

We have already reported an ab initio molecular orbital study of the substitution effect on hydrogen bond energy in the base pair between 9-methyl adenine (A) and modified 1-methyl uracil derivatives (U^X).[11] In the case of the substituent effect on uracil in the A−U^X base pair, we have observed a remarkable tendency for U^X:U^X possessing a stronger electron-withdrawing group (EWG) to form a more stable base pair.

Although there are many theoretical studies on the hydrogen bond energy of the Watson−Crick type base pair between natural nucleic acid bases,[12] no systematic ab initio molecular orbital studies on modified base pairs have been reported, except for our studies.[11] Moreover, there is no study comparing the theoretically and experimentally estimated stability of the base pair of nucleic acid base analogues. Theoretical studies are important for understanding the nature of the hydrogen bond in the base pair and are useful for applications such as those described above. We report herein an ab initio study regarding the substitution effect on hydrogen bond energy in the base pair between modified 9-methyl adenine derivatives (A^X) and 1-methyl uracil (U).

* Corresponding author: Tadafumi Uchimaru. Tel.: +81-298-61-4522. Fax: +81-298-61-4487. E-mail: t_uchimaru@home.nimc.go.jp.
[†] Research Center of Gene Discovery.
[‡] Research Institute for Computational Sciences.
[§] Tokyo Institute of Technology.

10.1021/jp004117l CCC: $20.00
Published on Web 03/24/2001

Computational Methods

In most theoretical studies, the hydrogen bond energies of the Watson−Crick type base pairs were evaluated at the second-order Møller−Plesset (MP2) level of theory using double-ζ basis sets with polarization.[12] Rablen et al. showed[13] that hydrogen bond energies of small molecules calculated at the level of B3LYP/6-31++G(2d(X+),p)//B3LYP/6-31++G(d(X+),p)[14] were in good agreement with the results of the complete basis set approach (CBS-Q[15]). Sponer et al. reported[12m] that the hydrogen bond energies of some model compounds in MP2/6-31G*(0.25)//MP2/6-31G*(0.25)[16] reproduced relatively well the result of much larger basis sets. They also found[17] that the contribution of higher-level electron correlation was small on hydrogen bond energy, and that MP2 interaction energies were close to the results of coupled cluster electron correlation (CCSD(T)[18]) data. Hydrogen bond energy is mainly characterized by electrostatic contribution,[19] so the contribution of electron correlation should be relatively small. Thus, the conclusion of Sponer et al. would be quite reasonable and also be generally applicable to various types of hydrogen bonding systems. We already reported an ab initio study regarding the basis set effect on the calculated hydrogen bond energies of Watson−Crick type base pairs at the MP2 levels of theory.[20] The values of hydrogen bond energies of A−U and G−C base pairs, evaluated at the computational levels of MP2/6-31+G(2d′,p′)[15]//HF/6-31G(d,p), were in excellent agreement not only with the values calculated at MP2/6-311++G(3df,p)//HF/6-311++G(3d,p) but also with the values reported by Rablen et al.[13] Thus, the MP2/6-31+G(2d′,p′)//HF/6-31G(d,p) level calculation was employed for estimation of the hydrogen bond energies of the Watson−Crick type base pairs in this report. Recently, Dunning's triple-ζ basis sets were applied to the base pair,[12n,o] and triple-, quadruple- and quintuple-ζ basis sets were applied to the model complex of the base pair, for the discussion about the basis set effect on the hydrogen bond energy.[12n] From the results of model compounds, Sponer et al. pointed out that double-ζ basis sets should underestimate the hydrogen bond energies by about 2.5 kcal·mol^{-1}, comparing quintuple-ζ basis sets in the base pair which contains two hydrogen bonds. However, we consider that the error, which originates from the basis set, should be comparable for all A^X-U base pairs. Thus, the substituent effects in nucleic acid bases on the hydrogen bond energy for base pair formation can be discussed, at least qualitatively, based on the energy estimates derived from MP2/6-31+G(2d′,p′)//HF/6-31G(d,p) calculations.

The hydrogen bond energies of the Watson−Crick type base pairs were evaluated by a supermolecular method. The basis set super position error (BSSE) for hydrogen bond energies was corrected by using the counterpoise method.[21] Hereafter, we refer to the molecular interaction energy without BSSE correction as δE and the energy with BSSE correction as ΔE^{HB} (eqs 1 and 2). Thus, the more negative ΔE^{HB} means the more stable hydrogen bond. $\Delta\Delta E$ was defined as the substitution effect on ΔE^{HB} (eq 3). As shown in eqs 1 and 2, $\Delta E^{HB}(A^X\text{-U})$ includes the total interaction energy, and the deformation energy was not separated from $\Delta E^{HB}(A^X-U)$, because of our standpoint in this research: The substitution effect on the interaction energy, including the deformation energy, is important for the purpose in this work.

$$\delta E(A^X-U) = E(A^X-U) - (E(A^X) + E(U)) \quad (1)$$

$$\Delta E^{HB}(A^X-U) = \delta E(A^X-U) + \mathrm{BSSE} \quad (2)$$

$$\Delta\Delta E = \Delta E^{HB}(A^X-U) - \Delta E^{HB}(A-U) \quad (3)$$

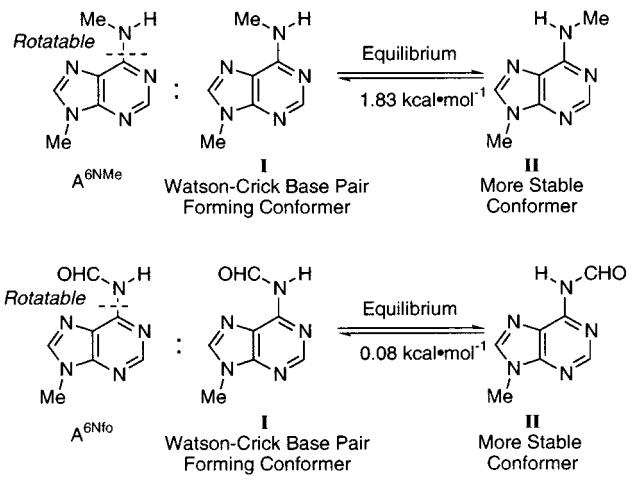

Figure 2. Rotatable exocyclic bonds in A^{6NMe} amd A^{6Nfor}.

The structures of A^X-U, as well as those of nucleic acid bases A^X and U, were optimized in the 6-31G(d,p) basis set at the HF level of theory. In all cases, C_s symmetry was assumed: all atoms, except for hydrogen atoms in the methyl group(s), were placed on the plane of the symmetry. The energies of the optimized structures were evaluated with single-point calculations with the 6-31+G(2d′,p′) basis set at the MP2 level of theory. A preliminary conformer search with HF/3-21G calculations was carried out in some cases. Additionally, energy estimation of the two important conformers in MP2/6-31+G(2d′,p′)//HF/6-31G(d,p) was carried out for 6-*N*-methyl-9-methyl adenine (A^{6NMe}) and for 6-*N*-formyl-9-methyl adenine (A^{6Nfo}). In the case of these A derivatives, which have a substituent on the exocyclic amino moiety on the 6-position, there are conformational isomers because of the rotation of the amino group and the substituent (Figure 2). $\Delta E^{HB}(A^X-U)$ of these derivatives were calculated based on the hydrogen bond forming conformer (**I**). For both A^{6NMe} and A^{6Nfo}, conformer (**I**) was found to be higher in energy than conformer (**II**). We refer to the molecular interaction energies calculated based on the conformer (**II**) as $\Delta E^{total}(A^X-U)$.

Conformer search calculations of some derivatives were carried out using the SPARTAN program.[22] Structure optimization and energy estimation calculations were both carried out using the GAUSSIAN 94 program.[23]

Result and Discussion

In the present work, we studied 15 adenine derivatives (A^X), whose structures and abbreviations are shown in Figure 3. The adenine derivatives shown in Figure 3 were classified into the following four groups. Group A: unmodified adenine (A). Group B: a substitution group was introduced at the 8-position on adenine[24] or at the exocyclic amino moiety of adenine. Position and number of hydrogen bonds were the same as in the A−U base pair. The structure of the purine ring also remained unchanged; EWG was introduced on the 8-position of adenine (A^{8F}, A^{8oxo}, and A^{8NO2}), an electron-donating group (EDG) was introduced on the 8-position of adenine (A^{8NH2}), and a formyl group was introduced as an EWG (A^{6Nfo}) or a methyl group was introduced as an EDG (A^{6NMe}) on the exocyclic amino moiety on the 6-position. Group C: Position or number of the hydrogen bonds was changed (see Figure 4); changing the position of the hydrogen bond (H−Bond A to H−Bond C, P^{2NH2}), adding a new hydrogen bond (H−Bond C) on the A−U base pair (A^{2NH2}), and deleting H−Bond A (P). Group D: Position and number of hydrogen bonds were

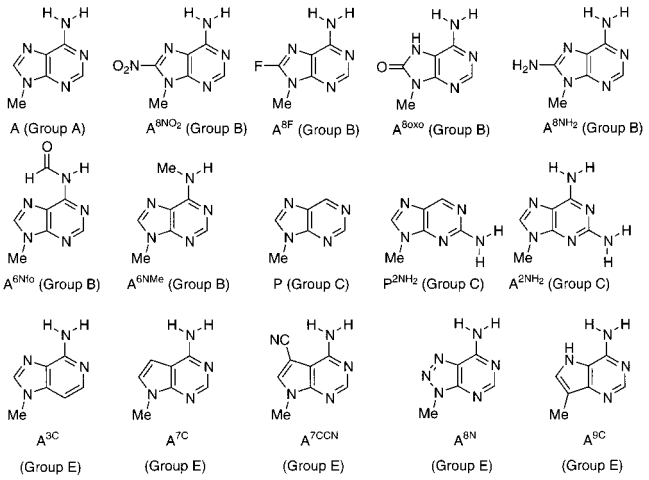

Figure 3. Substituent introduced 9-methyl adenine derivatives (A^X) in this study.

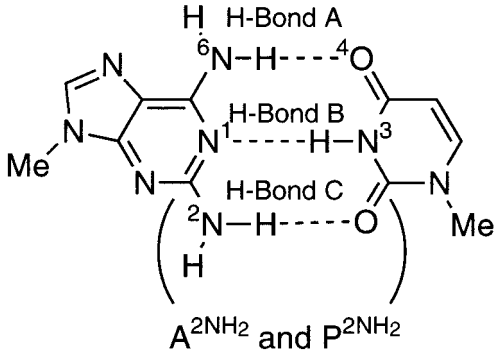

Figure 4. Hydrogen bond between A^X and U.

unchanged, but a nitrogen or carbon atom in the purine ring was changed, replacing a carbon atom with a nitrogen (A^{8N}) or a nitrogen atom with a carbon (A^{3C}, A^{7C}, A^{7CCN}, and A^{9C}), which constructs the purine ring.

Table 1 shows the results of theoretically estimated ΔE^{HB} of each A^X. By examining the change in the N−H stretching mode in the IR spectum, Kyogoku et al. derived the binding constants (k) between uracil and some adenine derivatives (A, A^{2NH2}, P^{2NH2}, A^{8Br} [25], and A^{6NMe}).[26] First, we compared theoretically predicted hydrogen bond energies to log of k. As shown as

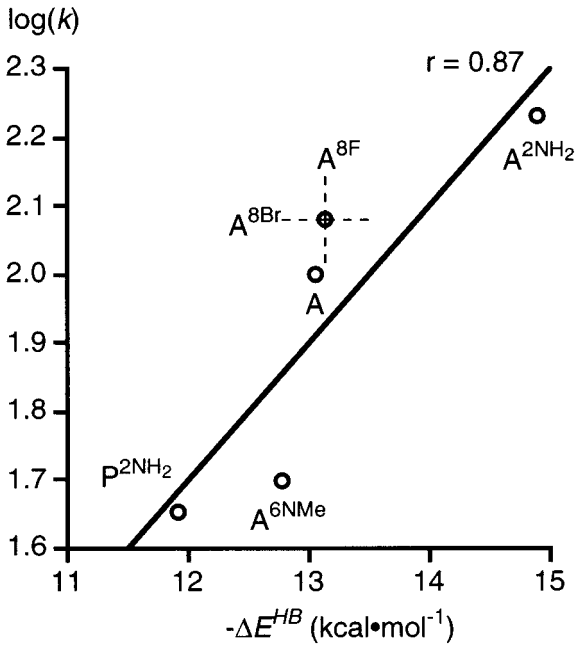

Figure 5. Relationship between theoretically estimated ΔE^{HB} and log of binding constant of some A^X (see ref 25 for A^{8Br}/A^{8F} and ref 27 for A^{6NMe}).

Figure 5, theoretically estimated substitution effect on ΔE^{HB} reproduced the substitution effect on k: $A^{2NH2} > A^{8Br}/A^{8F} \approx A > A^{6NMe} \approx P^{2NH2}$. As described in ref 27, A^{6NMe} has conformers that cannot form a base pair. ΔE^{HB} of A^{6NMe}−U was calculated based on the energy of the conformer, which can form the base pair (conformer **I** in Figure 2). The calculated value of $\Delta E^{total}(A^{6NMe}-U)$ is shown in Table 1.[28]

Oligonucleotides possessing some A^X were prepared, and the duplex stability of A^X introduced oligonucleotides was studied.[7c,i,j,k,m] The duplex stability of oligonucleotides was observed as melting temperature (T_m). An increase in T_m shows an increase in the duplex stability of A^X introduced oligonucleotides, and vice versa. Thus, the difference in T_m ($\Delta T_m = T_m-(A^X)-T_m(A)$) is the index of the substitution effect in duplex stability. So experimentally observed ΔT_m, reported in refs 7c,i,j,k, and m, are also shown in Table 1. However, T_m and ΔT_m are highly sensitive to the experimental conditions, i.e., length and sequence of the oligonucleotides, A^X introduced

TABLE 1: δE, ΔE^{HB}, $\Delta\Delta E$, and BSSE (kcal·mol^{-1}) of Each A^X Calculated at MP2/6-31+G(2d′,p′)//HF/6-31G(d,p) Level, the Binding Constants (k, l·mol^{-1}) and ΔT_m (°C)

	δE	BSSE	ΔE^{HB}	k[a]	$-\Delta\Delta E$	ΔT_m[c]
A	−15.31	2.13	−13.11	100	0.00	
A^{8F}	−15.37	2.17	−13.20	140[b]	0.09	
A^{8oxo}	−15.32	2.16	−13.16		0.05	
A^{8NO_2}	−17.58	2.21	−15.37		2.26	
A^{8NH_2}	−16.41	2.16	−14.25		1.14	
A^{6Nfo}	−13.21	2.33	−10.87[d]		−2.24	
ΔE^{total}			−10.79[e]			
A^{6NMe}	−15.13	2.30	−12.83[d]	50	−0.28	
ΔE^{total}			−11.00[e]			
P	−10.15	1.73	−8.42		−4.69	−6 °C (1/7,35 °C)
P^{2NH_2}	−14.15	2.19	−11.96	45	−1.15	
A^{2NH_2}	−17.46	2.50	−14.96	170	1.85	+1.5 °C (2/15,60 °C), +3.5 °C (5/15,60 °C)
A^{3C}	−14.50	2.20	−12.31		−0.80	−6 °C (1/10,42 °C), −1 °C (1/12,50 °C)
A^{7C}	−15.62	2.19	−13.43		0.32	+3 °C (6/6,33 °C)
A^{7CCN}	−15.19	2.21	−12.98		−0.13	
A^{8N}	−15.49	2.19	−13.30		0.19	
A^{9C}	−15.09	2.16	−12.93		−0.18	

[a] Reference 26. [b] The value of k for A^{8Br}. [c] See ref 29 and corresponding references in ref 7. [d] Without rotational energy of exocyclic amino group. See the conformer **I** in Figure 2. [e] With rotational energy of exocyclic amino group. See the conformer **II** in Figure 2.

position, concentration of salt in the solution, and so on. The experimental conditions of the abovementioned refs were different from each other. Thus, only the tendency (plus or minus sign) of the ΔT_m can be compared with $\Delta\Delta E$. The signs of the calculated values of $-\Delta\Delta E$ are in accord with those of ΔT_m without exception. The amount of experimental data available is quite limited. As far as these experimental values are concerned, our ab initio estimations are in reasonable agreement with experimental results.

In contrast to the substitution effect in uracil on hydrogen bond energy,[11a] no remarkable trend was observed in the relation between the substituent in adenine derivatives and the hydrogen bond energies. The substitution effects of each group are discussed as follows. Group B: Both EWG (A^{8F}, A,^{8oxo} and A^{8NO2}) and EDG (A^{8NH2}) on the 8-position of A stabilize the hydrogen bond with U, but the substituent effects are not so large ($-\Delta\Delta E = 0.05$ to 0.09 kcal·mol^{-1}) except for A^{8NH2} ($-\Delta\Delta E = 1.14$ kcal·mol^{-1}) and A^{8NO2} ($-\Delta\Delta E = 2.26$ kcal·mol^{-1}). The methyl group on the exocyclic amino group on the 6-position (A^{6NMe}) has little effect ($-\Delta\Delta E = -0.28$ kcal·mol^{-1}) on the hydrogen bond stability. On the other hand, a formyl group at the same position (A^{6Nfo}) destabilizes the hydrogen bond by 2.24 kcal·mol^{-1}. The hydrogen bond stability of A^{6NMe}−U and A^{6Nfo}−U should be overestimated because they have conformers which are unsuitable for base pair formation.[27] Group C: ΔE^{HB} of P^{2NH2}−U is 1.15 kcal·mol^{-1} less negative than that of A−U, though both A and P^{2NH2} form two hydrogen bonds in base pair formation with U. As expected, ΔE^{HB} of A^{2NH2}−U, which forms three hydrogen bonds, is 1.85 kcal·mol^{-1} more negative than A−U. ΔE^{HB} of P−U, which forms only one hydrogen bond, is 4.69 kcal·mol^{-1} less negative than in the case of A−U. Group D: The effects of these substitutions on ΔE^{HB} were not so large ($-\Delta\Delta E$ of this group was less than 1 kcal·mol^{-1}). ΔE^{HB} of A^{7C}-U and A^{8N}-U become more negative and the others become less negative, than ΔE^{HB} of A−U.

There are two hydrogen bonds between A^X and U, except for A^{2NH2}−U and P−U base pairs (Figure 4). A^X acts as electron acceptor in H−Bond A and acts as electron donor in H−Bond B. Thus, considering the fact that the hydrogen bond is mainly characterized by electrostatic contribution,[19] a decrease in the electron population of the purine ring enforces the H−Bond A and weakens the H−Bond B. On the other hand, an increase in the electron population of the purine ring weakens the H−bond A and enforces the H−bond B. Figure 6 shows the hydrogen bond length (Å) of the base pairs of 8-substituted A derivatives. As expected, the introduction of an EWG results in shorter and longer bond length of H−Bond A and B, respectively. The introduction of an EDG results in the opposite trends, namely, the substitution effects on the strengths of H−bond A and B may cancel each other out. Thus, the substitution effect on total hydrogen bond energy of A^X-U is complicated and difficult to forecast from the structure.

Conclusion

The substitution effect on hydrogen bond energy of the Watson−Crick type base pair between U and A^X was estimated by ab initio molecular orbital theory. The substitution effect on hydrogen bond energy of A^X−U base pairs, calculated by ab initio method, was in good agreement with the substitution effect on experimentally observed binding constants of A^X−U base pairs. Among the modified adenines studied in the present work, the adenine derivatives, which have a nitro group on the 8-position or an amino group on the 2-position, can form the most stable base pair with uracil. In contrast to the substitution effect in uracil on hydrogen bond energy, no remarkable trend was observed in the relation between the substituent in adenine derivatives and the hydrogen bond energies, so it is difficult to forecast the substitution effect from the structure. Thus, it is important that ab initio calculation is an effective method to estimate the base pair stability between chemically modified nucleic acid bases, described in this report.

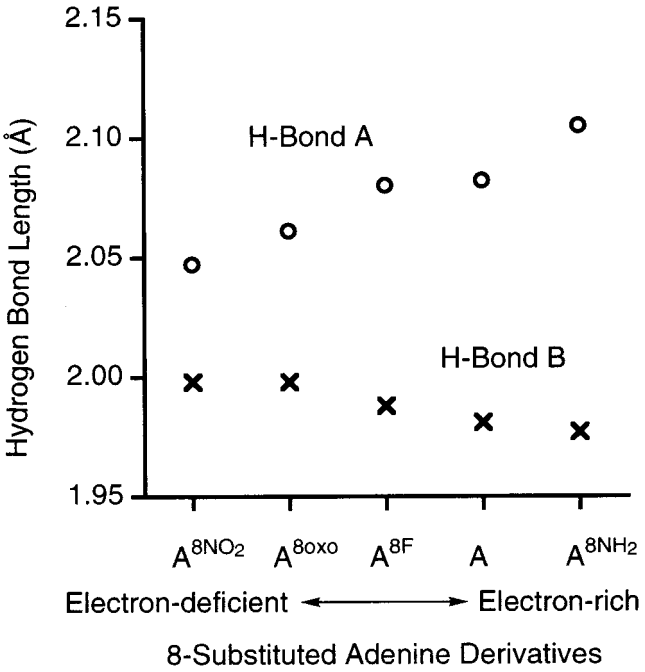

Figure 6. Hydrogen bond length (Å) of 8-substituted adenine derivatives. The lengths between the hydrogen and oxygen atoms for H−Bond A (○) and those between the hydrogen and nitrogen atoms for H−Bond B (×) are plotted.

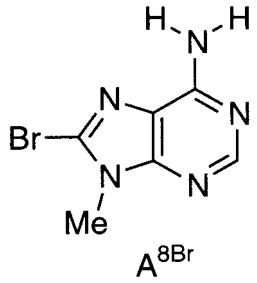

Figure 7. Structure of A^{8Br} (see ref 25).

Acknowledgment. The services and computational time made available by National Institute of Materials and Chemical Research and Tsukuba Advanced Computing Center (TACC) have been essential to this study and are gratefully acknowledged. We also thank the Science and Technology Agency for financial support.

References and Notes

(1) Watson, J. D.; Crick, H. C. *Nature* **1953**, *171*, 737.

(2) Brenner, S.; Jacob, F.; Meselson, M. *Nature* **1961**, *190*, 576.

(3) Hopfield, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 4135.

(4) (a) Sessler, J. L.; Wang, R. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 1726. (b) González, J. J.; Prados, P.; de Mendoza, J. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 525.

(5) (a) Tjivikua, T.; Ballester, P.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1990**, *112*, 1249. (b) Nowick, J. S.; Feng, Q.; Tjivikua, T.; Ballester, P.; Rebek, J., Jr. *J. Am. Chem. Soc.* **1991**, *113*, 8831. (c) Menger, F. M.; Eliseev, A. V.; Khanjin, N. A.; Sherrod, M. J. *J. Org. Chem.* **1995**, *60*, 2870.

(6) (a) Murray, J. A. H., Ed. *Antisense RNA and DNA*; Wiley-Liss: New York, 1992. (b) Uhlmann, E.; Peyman, A. *Chem. Rev*. **1990**, *90*, 543. (c) Englisch, U.; Gauss, D. H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 613.

(7) For A^{6NMe}: (a) Littlefield, J. W.; Dunn, D. B. *Nature*, **1958**, *181*, 254. (b) Johnson, J. A.; Thomas, H. J.; Schaeffer, H. J. *J. Am. Chem. Soc*. **1958**, *80*, 699. For P: (c) Eritja, R.; Horowitz, D. M.; Walker, P. A.; Ziehler-Martin, J. P.; Boosalis, M. S.; Googman, M. F.; Itakura, K.; Kaplan, B. E. *Nucleic Acid Res*. **1986**, *14*, 8135. For P^{2NH2}: (d) Freese, E. *Proc. Natl. Acad. Sci. U.S.A.* **1959**, *45*, 622. (e) Watanabe, S. M.; Goodman, M. F. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 2864. (f) Seela, F.; Becher, G. *Hevl. Chim. Acta* **2000**, *83*, 928. For A^{2NH2}: (g) Gaffney, B. L.; Markey, L. A.; Jones, R. A. *Tetrahedron*, **1984**, *40*, 3. (h) Diekmann, S.; von Kitzing, E.; McLaughlin, L.; Ott, J.; Eckstein, F. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8257. (i) Chollet, A.; Kawashima, E. *Nucleic Acid Res*. **1988**, *16*, 305. For A^{3C}. (j) Ono, A.; Ueda, T. *Nucleic Acid Res*. **1987**, *15*, 3059. (k) Cosstick, R.; Li, X.; Tuli, D. K.; Williams, D. A.; Connolly, B. A.; Newman, P. C. *Nucleic Acid Res*. **1990**, *18*, 4771. For A^{7C}: (l) Seela, F.; Berg, H.; Rosemeyer, H. *Biochemistry* **1989**, *28*, 6193. (m) Seela, F.; Zulauf, M. *Chem. Eur. J*. **1998**, *4*, 1781. (n) Seela, F.; Zulauf, M.; Suer, M.; Deimel, M. *Hevl. Chim. Acta* **2000**, *83*, 910. For A^{8N}: (o) Wierzchowsli, J.; Wielgus-Kutrowska, B.; Shuger, D. *Biochim. Biophys. Acta* **1996**, *1290*, 9. (p) Rosemeyer, H.; Zulauf, M.; Ramzaeva, N.; Becher, G.; Feiling, E.; Mühlegger, K.; Münster, I.; Lohmann, A.; Seela, F. *Nucleosides Nucleotides* **1997**, *16*, 821. For A^{9C}, (q) Otmar, M.; Masojidkova, M.; Holy, A. *Collect. Czech. Chem. Commun*. **1996**, *61*, S49. For a comprehensive review see (r) Limbach, P. A.; Crain, P. F.; AcCloskey, J. A. *Nucleic Acid Res*. **1994**, *22*, 2183.

(8) (a) Bain, J. D.; Switzer, C.; Chamberlin, A. R.; Benner, S. A. *Nature* **1992**, *356*, 537. (b) Switzer, C.; Moroney, S. E.; Benner, S. A. 1989 *J. Am. Chem. Soc*., **111**, 8322. (c) Switzer, C. Y.; Moroney, S. E.; Benner, S. A. *Biochemistry* **1993**, *32*, 10489. (d) Piccirilli, J. A.; Krauch, T.; Moroney, S. E.; Benner, S. A. *Nature* **1990**, *343*, 33. (e) Horlacher, J.; Hottiger, M.; Podust, V. N.; Hubscher, U.; Benner, S. A. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 6329. (f) Lutz, M. J.; Held, H. A.; Hottiger, M.; Hubcher, U.; Benner, S. A. *Nucleic Acids Res*. **1996**, *24*, 1308. (g) Voegel J. J.; Benner, S. A. *Helv. Chim. Acta* **1996**, *79*, 1881. (h) Ren, R. X. F.; Chaudhuri, N. C.; Paris, P. L.; Rumney, S.; Kool, E. T. *J. Am. Chem. Soc*. **1996**, *118*, 7671. (i) Morales J. C.; Kool, E. T. *Nature Struct. Biol*. **1998**, *5*, 950. (j) Morales J. C.; Kool, E. T. *J. Am. Chem. Soc*. **1999**, *121*, 2323. (k) Kool, E. T.; Morales J. C.; Guckian, K. M. *Angew. Chem., Int. Ed. Engl*. **2000**, *39*, 990. (l) Ogawa, A. K.; Wu, Y.; McMinn; D. L.; Liu, J.; Schultz, P. G.; Romesberg, F. E. *J. Am. Chem. Soc*. **2000**, *122*, 3274.

(9) For a comprehensive review of genetic code expansion using "natural base pair" see: (a) Cornishi, V. W.; Mendel, D.; Schultz, P. G. *Angew. Chem., Int. Ed. Engl*. **1995**, *34*, 621. (b) Sisido, M.; Hohsaka, T. *Bull. Chem. Soc. Jpn*. **1999**, *72*, 1409.

(10) Morales, J. C.; Kool, E. T. *J. Am. Chem. Soc*. **2000**, *122*, 1001.

(11) (a) Kawahara, S.-I.; Wada, T.; Kawauchi, S.; Uchimaru, T.; Sekine, M. *J. Phys. Chem. A* **1999**, *103*, 8516. (b) Kawahara, S.-I.; Uchimaru, T.; Sekine, M. *J. Mol. Struct. (THEOCHEM)* **2000**, *530*, 109.

(12) (a) Scheiner, S.; Kern, C. W. *Chem. Phys. Lett.* **1978**, *57*, 331. (b) Scheiner, S.; Kern, C. W. *J. Am. Chem. Soc*. **1979**, *101*, 4081. (c) Hobza, P.; Sandorfy, C. *J. Am. Chem. Soc*. **1987**, *109*, 1302. (d) Trollope, K. I.; Gould, I. R.; Hiller, I. H. *Chem. Phys. Lett*. **1993**, *209*, 113. (e) Destexhe, A.; Smets, J.; Adamowicz, L.; Maes, G. *J. Phys. Chem*. **1994**, *98*, 1506. (f) Gould, I. R.; Kollman P. A. *J. Am. Chem. Soc*. **1994**, *116*, 2493. (g) Hobza, P.; Sponer, J.; Polásek, M. *J. Am. Chem. Soc*. **1995**, *117*, 792. (h) Sponer, J.; Leszczynski, J.; Hobza, P. *J. Biomol. Struct. Dyn*. **1996**, *14*, 117. (i) Sponer, J.; Hobza, P. *Chem. Phys*. **1996**, *204*, 365. (j) Sponer, J.; Hobza, P. *Chem. Phys. Lett*. **1996**, *261*, 379. (k) Sponer, J.; Hobza, P. *Chem. Rev*. **1999**, *99*, 3247. (l) Sponer, J.; Leszczynski, J.; Hobza, P. *J. Biomol. Struct. Dyn*. **1996**, *14*, 117. (m) Sponer, J.; Leszczynski, J.; Hobza, P. *J. Phys. Chem*. **1996**, *100*, 1965. (n) Sponer, J.; Hobza, P. *J. Phys. Chem. A* **2000**, *104*, 4592. (o) Guerra, C. F.; Bickelhaupt, F. M.; Snijders, J. G.; Baerends, E. J. *J. Am. Chem. Soc*. **2000**, *122*, 4117.

(13) Rablen, P. R.; Lockman, J. W.; Jorgensen, W. L. *J. Phys. Chem. A* **1998**, *102*, 3782.

(14) X/A//Y/B shows energy evaluation at the level of theory X with basis set A at the geometry optimized at the level of theory Y with basis set B.

(15) (a) Petersson, G. A.; Bennett, A.; Tensfeldt, T. G.; Al-Laham, M. A.; Sheirley, W. A.; Mantzaris, J. *J. Chem. Phys*. **1988**, *89*, 2193. (b) Petersson, G. A.; Al-Laham, M. A. *J. Chem. Phys*. **1991**, *94*, 6081.

(16) Kroon-Batenburg, L. M. J.; van Duijneveldt, F. B. *J. Mol. Struct*. **1985**, *121*, 185.

(17) Sponer, J.; Hobza, P. *Chem. Phys. Lett.* **1997**, *267*, 263.

(18) Pople, J. A.; Head-Gordon, M.; Raghavachari, K. *J. Phys. Chem.* **1987**, *87*, 5968.

(19) (a) Morokuma, K. *Acc. Chem. Res.* **1977**, *10*, 294. (b) Rendell, A. P. L.; Bacskay, G. B.; Hush, N. S. *Chem. Phys. Lett.* **1985**, *117*, 400. (c) Chen, W.; Gordon, M. S. *J. Phys. Chem.* **1996**, *100*, 14316.

(20) Kawahara, S.-I.; Uchimaru, T. *Phys. Chem. Chem. Phys*. **2000**, *2*, 2869.

(21) Boys, S. F.; Bernardi, F. *Mol. Phys.* **1970**, *19*, 553.

(22) Deppmeir; D. J.; Driessen, A. J.; Hehere, T. S.; Hehere, W. J.; Johnson, J. A.; Klunzinger, P. E.; Lou, L.; Yu, J.; Baker, J.; Carpenter, J. E.; Dixon, R. W.; Fielder, S. S.; Johnson, H. C.; Kahn, S. D.; Leonard J. M.; Piero, W. J.; Wavefunction, Inc., Irvine CA, 1998.

(23) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzalez, C.; Pople; J. A. Gaussian 94; Gaussian, Inc.: Pittsburgh, PA, 1995.

(24) Looking at B-DNA standard geometry structure, close contacts between the substituent at the 8-position and backbone are obvious. Because of the steric hindrance, the nucleic acid base orientation of most of the 8-substituted A^X is syn in nucleotides.[30] Thus, the modified base possessing a substituent at the 8-position may not form a favorable hydrogen bonding base pair in the standard DNA duplex structure, and these derivatives would not be suitable for antisense application. However, if the structure of the backbone is drastically altered, e.g., in PNA,[31] the substituent at the 8-position will not be unfavorable for base pair formation via hydrogen bonds.

(25) A^{8F} as the model of A^{8Br}: The structure of 1-methyl-8-bromoadenine (A^{8Br}) is shown in Figure 7. The electrostatic property of A^{8Br} will be similar to A^{8F}, so A^{8F} was considered the model of A^{8Br} in this section.

(26) Kyogoku, Y.; Lord, R. C.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1967**, *57*, 250. They guessed that in addition to Watson−Crick type base pairs, a Hoogsteen type base pair is partially involved.

(27) The adenine derivative A^{6NMe} in the solution phase should be a mixture of the rotational conformers (**I**) and (**II**). The conformers (**I**) can form Watson−Crick type base pair, while the conformers (**II**) cannot. The values of $\Delta E^{HB}(A^{6NMe}-U)$ and $\Delta E^{total}(A^{6NMe}-U)$ are calculated based on conformers (**I**) and conformers (**II**), respectively. Thus, the either ΔE^{HB}-$(A^{6NMe}-U)$ value or the $\Delta E^{total}(A^{6NMe}-U)$ value cannot be compared directly with the experimentally derived binding constant. However, noteworthy is that the differences between $\Delta E^{HB}(A^X-U)$ and $\Delta E^{total}(A^X-U)$ are not so large and that the values of $\Delta E^{HB}(A^X-U)$ reproduce well the trend of the strength of base pair formation of A derivatives.

(28) The experimental values (k and T_m) should be compared with the difference in free energy, but the discussion in the present work does not include the entropic contribution at all. Because the structures of adenine derivatives studied in this work resemble each other, the entropic effect is likely to remain almost constant. However, due to the rotational freedom of the amino group, the magnitude of entropic contribution to the base pair formation of A^{6NMe} and A^{6Nfo} will possibly be slightly different from that of other derivatives, but the difference should not be so important.

(29) Here, + or − x° C (n/m, y °C) means as follows. x: ΔT_m (+ shows increase in T_m duplex of oligonucleotide was stabilized, whereas − shows decrease in T_m: duplex of oligonucleotide was destabilized), n: number of introduced A^X in the oligonucleotide, m: number of chain length of oligonucleotide, y: Tm of "standard" oligonucleotide (Tm(A)).

(30) Saenger, W. *Principals of Nucleic Acid Structure*; Springer-Verlag: New York, Berlin, Heidelberg, Tokyo, 1984; Chapter 4.

(31) (a) Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497. (b) Hyrup, B.; Nielsen, P. E. *Bioorg. Med. Chem*. **1996**, *4*, 5. (c) Uhlmann, E.; Peyman, A.; Breipohl, G.; Will; D. W. *Angew. Chem., Int. Ed. Engl*. **1998**, *37*, 2796.