Theoretical and Experimental Study of Isoguanine and Isocytosine: Base Pairing in an Expanded Genetic System

Christopher Roberts, Rajanikanth Bandaru, and Christopher Switzer*

Contribution from the Department of Chemistry, University of California, Riverside, California 92521

Received January 15, 1997[⊗]

Abstract: The stabilities of all possible purine/pyrimidine pairings between the isomeric nucleobases isocytosine (iso-C) and isoguanine (iso-G) and standard genomic bases are reported for two independent oligonucleotide duplexes. Additionally, results are given from ab initio calculations performed on iso-C and iso-G. The calculations are used as an aid in the interpretation of thermodynamic data obtained from duplex denaturation studies. The unnatural iso-C/iso-G pair is found to be as stable as a C/G Watson−Crick pair in both duplex systems. The next most stable unnatural pair is that formed by C/iso-G and is observed to be isoenergetic with a U/A Watson−Crick pair. Ab initio data suggest iso-G may adopt an unprecedented imino oxo tautomer which could explain the unusual stability of the C/iso-G pair. The stability of the other possible unnatural pairs are reported and similarly interpreted in terms of ab initio and other available experimental data. Finally, the fitness is discussed of a six-component genetic system that includes iso-C, iso-G, and four standard genomic bases.

Introduction

Nucleosides and nucleotides bearing an isomeric backbone,[1] sugar,[2] or base[3,4] exist naturally, but others may have existed in the past.[5,6] By studying the properties of the many alternatives to nucleic acids, it may be possible to further an understanding of their origins. Once they are prepared in the laboratory, the relative fitness of "unnatural" and natural structures can be assessed through comparative functional assays. Isomeric structures, in particular, comprise an important starting point for the investigation of biopolymer evolution.

An increasing number of isomeric oligonucleotides have been characterized. Ribose isomers for which oligonucleotides have been created include pyranosylribose,[7] arabinose,[8] xylose,[9] α-anomeric nucleosides,[10−12] and L-ribose.[13,14] Backbone isomers of DNA and RNA have been prepared bearing a 2′,5′ rather than a 3′,5′ phosphodiester linkage.[15−21] Nucleobase isomers incorporated into oligomers include pseudouridine,[22−24] pseudoisocytidine,[25] isocytosine (iso-C),[26−32] and isoguanine (iso-G).[26−30,33−35] Interestingly, in nearly all of the above cases,

[⊗] Abstract published in *Advance ACS Abstracts,* May 1, 1997.

(1) Kerr, I. M.; Brown, R. E. *Proc. Natl. Acad. Sci. U.S.A.* **1978**, *75*, 256−260.
(2) Shimada, N.; Hasegawa, S.; Harada, T.; Tomisawa, T.; Fujii, A.; Takita, T. *J. Antibiot.* **1986**, *39*, 1623−1625.
(3) Jeanteur, P.; Amaldi, F.; Attardi, G. *J. Mol. Biol.* **1968**, *33*, 757−770.
(4) Cherbuliez, E.; Bernhard, K. *Helv. Chim. Acta* **1932**, *15*, 978−980.
(5) Joyce, G. F.; Schwartz, A. W.; Miller, S. L.; Orgel, L. E. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 4398−4402.
(6) Joyce, G. F.; Orgel, L. E. *The RNA World*; Cold Spring Harbor Press: Plainview, NY, 1993; 1−25.
(7) Krishnamurthy, R.; Pitsch, S.; Minton, M.; Miculka, C.; Windhab, N.; Eschenmoser, A. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 1537−1541.
(8) Giannaris, P. A.; Damha, M. J. *Can. J. Chem.* **1994**, *72*, 909−918.
(9) Schoppe, A.; Hinz, H. J.; Rosemeyer, H.; Seela, F. *Eur. J. Biochem.* **1996**, *239*, 33−41.
(10) Debart, F.; Tosquellas, G.; Rayner, B.; Imbach, J.-L. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1041−1046.
(11) Thuong, N. T.; Asseline, U.; Roig, V.; Takasugi, M.; Helene, C. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5129−5133.
(12) Damha, M. J.; Giannaris, P. A.; Marfey, P. *Biochemistry* **1994**, *33*, 7877−7885.
(13) Fujimori, S.; Shudo, K. *J. Am. Chem. Soc.* **1990**, *112*, 7436−7438.
(14) Garbesi, A.; Capobianco, M. L.; Colonna, F. P.; Tondelli, L.; Arcamone, F.; Manzini, G.; Hilbers, C. W.; Aelen, J. M. E.; Blommers, M. J. J. *Nucleic Acids Res.* **1993**, *21*, 4159−4165.
(15) Hashimoto, H.; Switzer, C. *J. Am. Chem. Soc.* **1992**, *114*, 6255−6256. (b) Jung, K.-E.; Switzer, C. *J. Am. Chem. Soc.* **1994**, *116*, 6059−6061. (c) Robinson, H.; Jung, K.-E.; Switzer, C.; Wang, A. H.-J. *J. Am. Chem. Soc.* **1995**, *117*, 837−838. (d) Prakash, T. P.; Jung, K.-E.; Switzer, C. *Chem. Commun.* **1996**, 1793−1794.
(16) Dougherty, J. P.; Rizzo, C. J.; Breslow, R. *J. Am. Chem. Soc.* **1992**, *114*, 6254−6255. (b) Jin, R. Z.; Chapman, W. H.; Srinivasan, A. R.; Olson, W. K.; Breslow, R.; Breslauer, K. J. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10568−10572. (c) Sheppard, T. L.; Breslow, R. *J. Am. Chem. Soc.* **1996**, *118*, 9810−9811.
(17) Kierzek, R.; He, L.; Turner, D. H. *Nucleic Acids Res.* **1992**, *20*, 1685−1690.
(18) (a) Giannaris, P. A.; Damha, M. J. *Nucleic Acids Res.* **1993**, *21*, 4742−4749. (b) Damha, M. J.; Meng, B.; Wang, D. G.; Yannopoulos, C. G.; et al. *Nucleic Acids Res.* **1995**, *23*, 3967−3973.
(19) Alul, R.; Hoke, G. D. *Antisense Res. Dev.* **1995**, *5*, 3−11.
(20) Sawai, H.; Seki, J.; Ozaki, H. *J. Biomol. Struct. Dyn.* **1996**, *13*, 1043−1051.
(21) (a) Michelson, A. M.; Monny, C. *Biochem. Biophys. Acta* **1967**, *149*, 107−127. (b) Hirao, I.; Ishido, Y.; Miura, K.-I. *Nucleic Acids Res., Symp. Ser.* **1983**, *12*, 193−196.
(22) Bandaru, R.; Hashimoto, H.; Switzer, C. *J. Org. Chem.* **1995**, *60*, 786−787.
(23) Trapane, T. L.; Christopherson, M. S.; Roby, C. D.; Tso, P. O. P.; et al. *J. Am. Chem. Soc.* **1994**, *116*, 8412−8413.
(24) Hall, K. B.; McLaughlin, L. W. *Nucleic Acids Res.* **1992**, *20*, 1883−1889.
(25) Ono, A.; Ts'o, P. O. P.; Kan, L.-S. *J. Am. Chem. Soc.* **1991**, *113*, 4032−4033.
(26) (a) Switzer, C.; Moroney, S. E.; Benner, S. A. *J. Am. Chem. Soc.* **1989**, *111*, 8322−8323. (b) Switzer, C. Y.; Moroney, S. E.; Benner, S. A. *Biochemistry* **1993**, *32*, 10489−10496.
(27) Bain, J. D.; Switzer, C.; Chamberlin, A. R.; Benner, S. A. *Nature* **1992**, *356*, 537−539.
(28) Roberts, C.; Bandaru, R.; Switzer, C. *Tetrahedron Lett.* **1995**, *36*, 3601−3604.
(29) Tor, Y.; Dervan, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 4461−4467.
(30) Horn, T.; Chang, C. A.; Collins, M. L. *Tetrahedron Lett.* **1995**, *36*, 2033−2036.
(31) Strobel, S. A.; Cech, T. R.; Usman, N.; Beigelman, L. *Biochemistry* **1994**, *33*, 13824−13835.
(32) Musier-Forsyth, K.; Shi, J.-P.; Henderson, B.; Bald, R.; Fürste, J. P.; Erdmann, V. A.; Schimmel, P. *J. Am. Chem. Soc.* **1995**, *117*, 7253−7254.
(33) (a) Kazimierczuk, Z.; Mertens, R.; Kawczynski, W.; Seela, F. *Helv. Chim. Acta* **1991**, *74*, 1742−1748. (b) Seela, F.; Mertens, R.; Kazimierczuk, Z. *Helv. Chim. Acta* **1992**, *75*, 2298−2306. (c) Seela, F.; Frohlich, T. *Helv. Chim. Acta* **1994**, *77*, 399−408.
(34) Ng, M. M. P.; Benseler, F.; Tuschl, T.; Eckstein, F. *Biochemistry* **1994**, *33*, 12119−12126.

S0002-7863(97)00123-6 CCC: $14.00

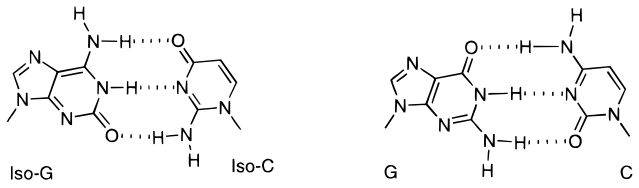


Figure 1.

some level of function is retained by the isomeric replacement and in some instances new functions found.

Thirty-five years ago Rich hypothesized that isomers of cytidine and guanosine involving transposition of the amino and carbonyl functions ought to comprise a third Watson−Crick base pair (Figure 1).[36] Initial characterization of the iso-C/iso-G base pair involved an in vitro polymerase experiment where a template bearing iso-C directed the incorporation of 2′-d-iso-GMP into a product strand.[26a] In addition to an iso-C/iso-G base pair, this study also found evidence for pairing of iso-G with T. This latter result was interpreted as consistent with the reported tendency of iso-G to adopt two different tautomeric forms, N1-H and O2-H.[37] Evidence for multiple iso-G tautomers was also seen in ribosomal translation experiments.[27] Only recently has support been gained for iso-C and iso-G pairing in the absence of enzymes in two limited studies.[28,30] Reported herein are the stabilities of all possible purine/pyrimidine pairings of iso-C, iso-G, and the standard genomic bases in two independent DNA/RNA duplexes (one with a purine-rich DNA strand, the other with a pyrimidine-rich DNA strand) and results from ab initio calculations on tautomers of the nonstandard bases. Ab initio data are used in combination with thermodynamic data to develop models for base-pair structures. As discussed below, an RNA strand was chosen to bear iso-C out of necessity owing to glycosidic linkage stability, whereas iso-G was incorporated into a DNA strand for the sake of synthetic simplicity.

Results

iso-G Synthesis. The synthesis of iso-G in protected form suitable for solid-phase synthesis is shown in Scheme 1 and was conducted as described recently.[28] Model deprotection studies with **6** indicated that the diphenylcarbamoyl and dibutylformamidine groups were cleanly removed using standard ammonium hydroxide deprotection. The protected nucleoside was also stable to conditions for automated DNA synthesis.

iso-C Stability Studies. Determining a suitable iso-C derivative for the present studies was troublesome. It was known at the outset that N^2-benzoyl-2′-d-iso-C does not tolerate deprotection with ammonium hydroxide, giving significant hydrolytic deamination to dU (∼15%) along with d-iso-C after 17 h.[26a] Use of dimethylformamidine[38] for protection of N-2 was attempted to limit the time of exposure to ammonium hydroxide to only 1 h. However, it was discovered that the dimethylformamidine group activated 2′-d-iso-C toward depyrimidination (Table 1, entry B vs A). 2′-Deoxy-5-methylisocytidine[29] was explored as a possible solution. Both free nucleoside (Table 1, entry C) and dimethylformamidine-protected 2′-deoxy-5-methylisocytidine (Table 1, entry D) were found to be much more stable during ammonium hydroxide treatment. Nevertheless, samples of both 2′-deoxyisocytidine and 2′-deoxy-5-methylisocytidine were observed to depyrimidinate upon short-term storage in the pH 5.5 phosphate buffer employed for HPLC analysis. As a result, a more thorough study of the acid stability of iso-C derivatives was undertaken (Table 1, entries G−I). As can be seen in Table 1, whereas both of the 2′-deoxy-iso-C nucleosides (5-methyl and desmethyl) are unstable in the pH 5.5 phosphate buffer, both r-iso-C (r = ribo) and 2′-dC are stable. Stability of r-iso-C may be rationalized on the basis of the inductive effect of the 2′-hydroxyl group leading to destabilization of an incipient C1′ carbocation that would form during depyrimidination. Finally, N^2-dimethylformamidine-r-iso-C was synthesized and subjected to RNA deprotection conditions (Table 1, entry F). No depyrimidination or deamination was observed in this case.

iso-C Synthesis. The riboisocytidine monomer was synthesized compatible with standard solid-phase RNA synthesis (Scheme 2). Worthy of note is that conditions reported for the introduction of a 2′-TBDMS group in cytidine[39a] resulted in the undesired (3′) regioisomer for iso-C **12**. Isomerization of the undesired isomer with triethylamine enabled recovery of the desired isomer **13**. Attempts at formation of a phosphoramidite from **13** using optimal conditions for natural RNA[39b] involving *N*-methylimidazole and collidine failed. A modification of the procedure commonly employed for DNA phosphoramidite synthesis was ultimately employed where an excess of chlorophosphine reagent was used in order to swiftly drive the reaction to completion, and thus avoid isomerization of the silyl group.

Oligomer Synthesis. Oligodeoxyribonucleotides **15**−**17** and **21**−**24** and oligoribonucleotides **18**−**20** and **25**−**28** (Figure 2) were synthesized with an automated DNA/RNA synthesizer using the iso-C and iso-G phosphoramidites **8** and **14** along with the appropriate natural phosphoramidites. All oligomers were analyzed by polyacrylamide gel electrophoresis (PAGE) as well as enzymatic digestion to constituent nucleosides followed by HPLC analysis employing authentic standards.

Denaturation Experiments. Melting temperatures and free energies for duplexes derived from oligomers **15**−**28** are given in Table 2 (in order of decreasing free energy of duplex formation). Melting curves for four of the entries in Table 2 are displayed in Figure 3. Duplex free energy values were determined from melting curves by nonlinear regression using a two-state model where $\Delta H°$ and $\Delta S°$ are parameters according to the method of Turner.[40a] As a check to confirm two-state melting behavior, the free energy for the duplex in entry 13 of Table 2 was determined by plotting $1/T_m$ versus the natural logarithm of total oligonucleotide concentration.[40] With this procedure a $\Delta G°_{37}$ was found that differs from the value listed in Table 2 by less than 5%. Less than 15% deviation between the two methods has been taken as evidence in favor of two-state melting behavior.[40a]

Theoretical Calculations. Results from ab initio calculations are summarized in Tables 3 and 4. Geometry optimization was carried out at the 6-31G* and 6-31G** ab initio levels. Electron correlation effects on iso-G were probed by MP-2/6-31G** single-point calculations. Frequency calculations were per-

(35) Sugiyama, H.; Ikeda, S.; Saito, I. *J. Am. Chem. Soc.* **1996**, *118*, 9994−9995.

(36) Rich, A. On the Problems of Evolution and Biochemical Information Transfer. In *Horizons in Biochemistry*; Kasha, M., Pullman, B., Eds.; Academic Press: New York, 1962; pp 103−126.

(37) (a) Seela, F.; Wei, C. F., Kazimierczuk, Z., *Helv. Chim. Acta* **1995**, *78*, 1843−1854. (b) Sepiol, J.; Kazimierczuk, Z.; Shugar, D. *Z. Naturforsch.* **1976**, *31*, 361−370.

(38) McBride, L. J.; Kierzek, R.; Beaucage, S. L.; Caruthers, M. H. *J. Am. Chem. Soc.* **1986**, *108*, 2040−2048.

(39) (a) Hakimelahi, G. H.; Proba, Z. A.; Ogilvie, K. K. *Can. J. Chem.* **1982**, *60*, 1106−1113. (b) Scaringe, S. A.; Francklyn, C.; Usman, N. *Nucleic Acids Res.* **1990**, *18*, 5433−5441.

(40) (a) Longfellow, C. E.; Kierzek, R.; Turner, D. H., *Biochemistry* **1990**, *29*, 278−285. (b) Marky, L. A.; Breslauer, K. J. *Biopolymers* **1987**, *26*, 1601−1620.

(41) (a) Eschenmoser, A. *Pure Appl. Chem.* **1993**, *65*, 1179−1188. (b) Hunziker, J.; Roth, H. J.; Bohringer, M.; Giger, A.; Diederichsen, V.; Göbel, M.; Krishnan, R.; Jaun, B.; Leumann, C.; Eschenmoser, A. *Helv. Chim. Acta* **1993**, *76*, 259−352.

(42) Wong, M. W.; Wiberg, K. B.; Frisch, M. J. *J. Am. Chem. Soc.* **1992**, *114*, 1645−1652.

Scheme 1. Synthesis of 2′-Deoxyisoguanosine Phosphoramidite

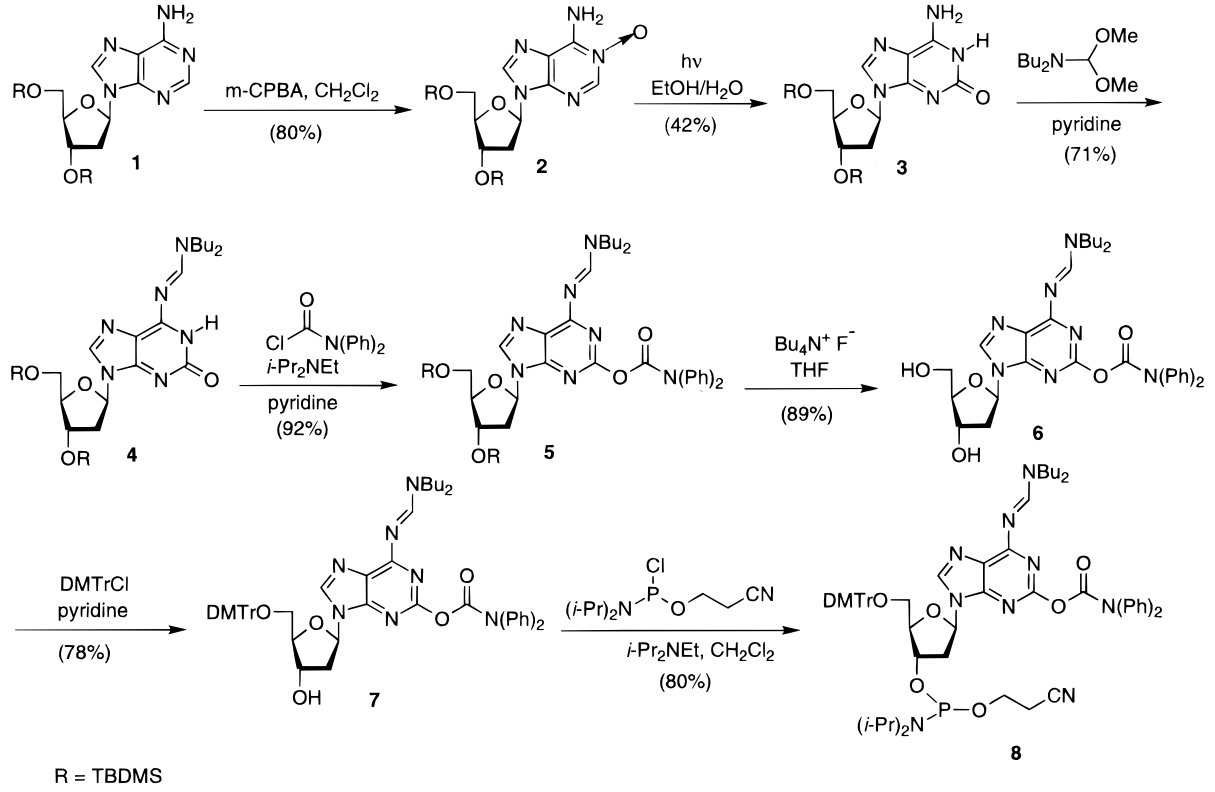
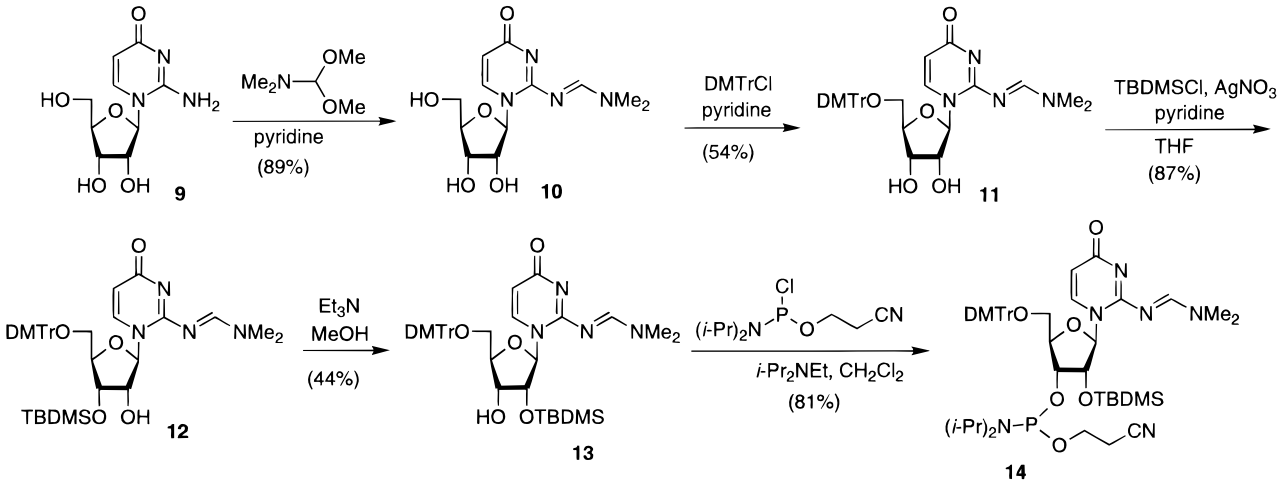


Table 1. Isocytidine Stability (Product Compositions Determined by HPLC and ^{1}H NMR)

entry	nucleoside	conditions	% (deprotected) nucleobase	% (deprotected) nucleoside	% uridine
A	2′-deoxyisocytidine	*a*	0	99	1
B	DMF-2′-deoxyisocytidine[d]	*a*	36.3	63.7	0
C	2′-deoxy-5-methylisocytidine	*a*	2.6	97.4	0
D	DMF-2′-deoxy-5-methylisocytidine	*a*	1.7	98.3	0
E	riboisocytidine	*a*	0	100	0
F	DMF-riboisocytidine	*b*	0	100	0
G	2′-deoxyisocytidine	*c*	38.6	61.4	0
H	2′-deoxy-5-methylisocytidine	*c*	67.8	32.2	0
I	riboisocytidine	*c*	0	100	0
J	2′-deoxycytidine	*c*	0	100	0

[a] NH_4OH (conc)/55 °C/1 h. [b] NH_4OH (conc)/EtOH (3:1)/55 °C/2 h (RNA deprotection). [c] 20 mM KH_2PO_4 (pH 5.5)/55 °C/16 h. [d] DMF = N^4-dimethylformamidyl.

Scheme 2. Synthesis of Isocytidine Phosphoramidite



formed at the 6-31G* level (iso-G) or 6-31G** level (iso-C). No imaginary frequencies were observed. A gross estimate of the effect of solvent on the tautomer energies was obtained by geometry optimization using the Onsager reaction field model.[42]

Discussion

Nucleobase Tautomerism: Isoguanine. Past Work. The existence of both N1-H and O2-H tautomers of isoguanine

d-5'-AGGGAGXGAAAG-3'	r-5'-CUUUCYCUCCCU-3'
15: X=iG	**18**: Y=iC
16: X=G	**19**: Y=C
17: X=A	**20**: Y=U
d-5'-CTTTXTTTG-3'	r-5'-CAAAYAAAG-3'
21: X=iG	**25**: Y=iC
22: X=G	**26**: Y=C
23: X=A	**27**: Y=U
24: X=T	**28**: Y=G

Figure 2.

Table 2. Duplex Stabilities[a]

(12-mer)
d-5′-AGGGAGXGAAAG-3′
r-3′-UCCCUCYCUUUC-5′

(9-mer)
d-5′-CTTTXTTTG-3′
r-3′-GAAAYAAAC-5′

entry	dX	rY	T_m (°C)	$-\Delta G°_{37}$ (kcal/mol)	$-\Delta\Delta G°_{37}$ (kcal/mol)
			12-mer		
1	iG	iC	50.3	11.5	0.1
2	G	C	49.8	11.4	0
3	iG	C	44.1	9.9	−1.5
4	A	U	42.8	9.6	−1.8
5	iG	U	39.6	8.9	−2.5
6	G	iC	37.8	8.6	−2.8
7	G	U	37.0	8.4	−3.0
8	A	iC	35.2	8.1	−3.3
9	A	C	27.4	6.9	−4.5
			9-mer		
10	iG	iC	33.6	7.6	0.2
11	G	C	31.4	7.4	0
12	iG	C	22.6	6.0	−1.4
13	A	U	24.0	5.9	−1.5
14	iG	U	19.4	5.3	−2.1
15	T	G	18.5	4.9	−2.5
16	G	iC	17.3	4.2	−3.2
17	A	iC	14.3	3.9	−3.5
18	G	U	13.2	3.2	−4.2
19	A	C	<0		

[a] All melts were performed with 5 μM total strands in 1 M NaCl, 10 mM sodium phosphate, and 0.1 mM EDTA at pH 7. Other conditions were as noted in the Experimental Section. Errors in free energies are estimated to be less than 5%.

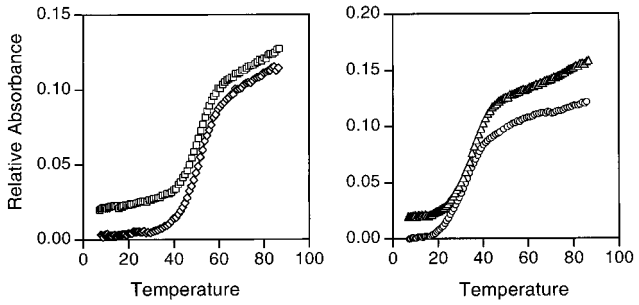


Figure 3. Denaturation curves. See the Experimental Section for details. Example profiles corresponding to entry 1 (□), entry 2 (◇), entry 10 (△), and entry 11 (○) of Table 2.

(Figure 4) has been implicated in separate studies on isoguanine[37b] and isoguanosine.[37a] In this work, tautomerically fixed model derivatives were prepared to represent each tautomer, and UV[37] and/or NMR[37a] spectra of the derivatives were then compared against the parent isoguanine structure. The main conclusions of these studies are that the N1-H form of isoguanine is the predominant one in polar solvents such as water, and in nonpolar environments (e.g., dioxane solvent), the O2-H form prevails. Whereas neither of the two aforementioned studies found support for a third, N3-H tautomer of isoguanine (Figure 4), recent work in the laboratory of Eschenmoser[7,41] is consistent with the existence of such a tautomer. Thermodynamics of

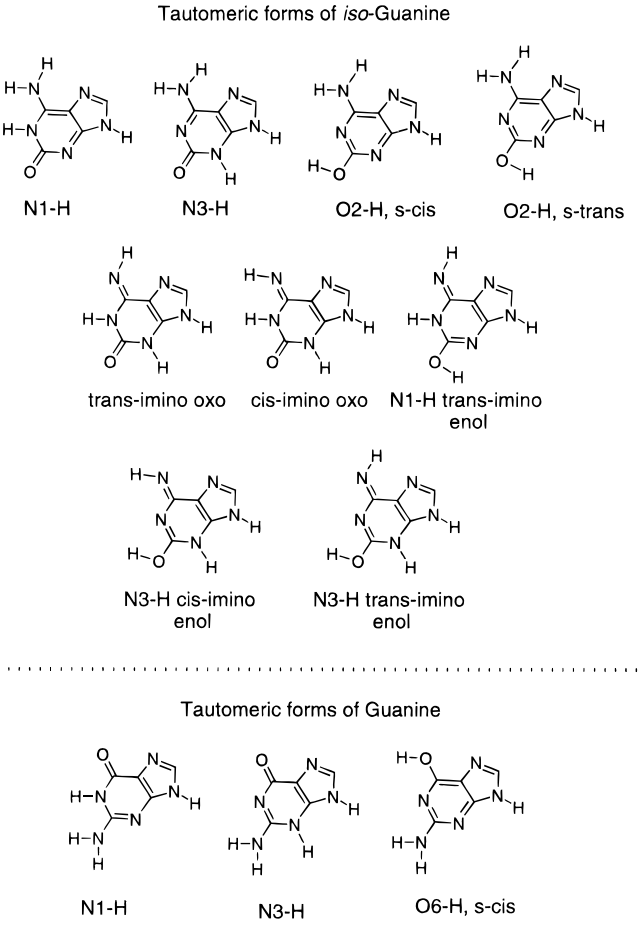


Figure 4.

duplex formation in two unnatural nucleotide systems investigated in his laboratory, dideoxyhexose-DNA[41] and pyranosyl-RNA,[7] provide evidence of iso-G/G Watson−Crick pairing, which is most consistently explained by invoking an iso-G N3-H tautomer.

Gas-Phase Calculations. In the present study, ab initio calculations (Table 3) have been carried out on the nine isoguanine tautomers given in Figure 4 to supplement what is known experimentally. The 6-31G* calculations predict the iso-G O2-H, s-cis and s-trans tautomers to be most stable in the gas phase ($\epsilon = 1$), separated from the N1-H and N3-H tautomers by an energy difference of approximately 6 kcal/mol. Surprisingly, the trans-imino oxo tautomer rivals the N1-H and N3-H tautomers in stability, being separated from them by ∼0.9 kcal/mol. This difference increases somewhat (to ∼1.5 kcal/mol) upon zero-point energy correction. To our knowledge, this tautomer has never been suggested to be important in any published study. Beyond the trans-imino oxo tautomer falls the cis-imino oxo and various enol tautomers. In investigating the energies of "rare" isoguanine tautomers (those apart from N1-H, N3-H, and O2-H), in each tautomer series (i.e., imino oxo, N1-H imino enol, N3-H imino enol), that tautomeric form maximizing attractive electrostatic interactions (and to a first approximation maximizing stability) was pursued to conserve computational efforts. All of the rare tautomers with the exception of the cis-imino oxo form maximize attractive electrostatic interactions. The cis-imino oxo tautomer stability was determined due to the high stability seen for its trans parent. The effect of an expanded basis set and electron correlation was probed for the five most stable tautomeric forms by performing geometry optimizations at the 6-31G** level and then single-point calculations using second-order Møller−Plesset perturbation (MP-2/6-31G**). No change in the relative order

Table 3. Ab Initio Data for Isoguanine and Guanine

		6-31* ($\epsilon = 1$)				6-31G* ($\epsilon = 40$)			6-31G**		MP-2/6-31G**	
entry		$E_{SCF(rel)}$	E_{SCF}	ZPE_{SCF}	dipole moment	$E_{SCRF(rel)}$	E_{SCRF}	dipole moment	$E_{SCF(rel)}$	E_{SCF}	$E_{SCF(rel)}$	E_{SCF}
	isoguanine											
1	N1-H	6.35	−539.385 305	79.2	6.31	2.47	−539.393 787	8.34	8.07	−539.403 360	8.70	−541.070 568
2	N3-H	6.18	−539.385 572	79.0	7.39	0.487	−539.396 954	9.62	7.78	−539.403 816	7.64	−541.072 251
3	O2-H (s-cis)	0.00	−539.395 430	79.4	2.49	0.631	−539.396 724	3.24	0.00	−539.416 229	0.00	−541.084 444
4	O2-H (s-trans)	0.165	−539.395 167	79.3	3.42	0.00	−539.397 731	4.67	0.16	−539.415 969	0.139	−541.084 222
5	trans-imino oxo	7.17	−539.383 996	79.7	7.29	1.77	−539.394 912	9.35	8.89	−539.402 052	8.27	−541.071 258
6	cis-imino oxo	13.0	−539.374 772	79.4	5.61	10.2	−539.381 414	7.39				
7	N1-H trans-imino enol	13.3	−539.374 170	80.1	5.24	11.2	−539.379 904	6.82				
8	N3-H trans-imino enol	32.0	−539.344 367	79.2	8.75	23.3	−539.360 510	11.45				
9	N3-H cis-imino enol	32.0	−539.344 313	79.0	8.38	24.2	−539.359 090	11.00				
	guanine											
10	N1-H	0.00	−539.394 731	79.7	6.79	0.00	−539.404 226					
11	N3-H	20.4	−539.362 137	79.3	11.1	9.13	−539.389 658					
12	O6-H (s-cis)	1.53	−539.392 285	79.6	3.04	6.31	−539.394 164					

Table 4. Ab Initio Data for Isocytosine and Cytosine (Calculations in the Table from This Work Unless Otherwise Noted)

		6-31G* ($\epsilon = 1$)		6-31G* ($\epsilon = 40$)		6-31G** ($\epsilon = 1$)				6-31G** ($\epsilon = 78.5$)[g]		
entry		$E_{SCF(rel)}$	E_{SCF}	$E_{SCRF(rel)}$	E_{SCRF}	$E_{SCF(rel)}$	E_{SCF}	ZPE_{SCF}	dipole moment	$E_{SCRF(rel)}$	E_{SCRF}	dipole moment
	isocytosine											
1	N1-H amino oxo	4.16	−392.600 026	0.00	−392.617 969	4.23	−392.615 552	66.87	8.43	0.00	−392.644 379	13.04
2	trans-imino oxo	0.00	−392.606 654	5.72	−392.608 851	0.00	−392.622 301	67.28	3.12			
3	cis-imino oxo	1.44	−392.604 363	3.10	−392.613 018	1.42	−392.620 041	67.20	6.13	7.15	−392.632 981	8.29
4	amino enol, s-cis					−8.95[a,b]						
5	N3-H amino oxo					−6.52[a,b]						
6	trans-imino enol, s-cis					9.13[a,b]			2.61[a]			
	cytosine											
7	N1-H amino oxo	0.0[c]		0.0[c]		0.00[d,e]			7.12[f]	0.00[e]		
8	trans-imino oxo	0.5[c]		4.1[c]		0.61[d,e]			5.19[f]	5.86[e]		
9	cis-imino oxo					2.32[d]			2.59[f]			

[a] Reference 49. [b] Reference 48. [c] Reference 43. [d] Reference 56. [e] Reference 57. [f] Reference 58. [g] For cytosine, ϵ = water.

of tautomer energies was observed in the case of the 6-31G** method, but MP-2/6-31G** calculations altered the relative ordering of entries 1 and 5 in Table 3.

Reaction Field Calculations. Owing to the the relatively large dipole moments found for the oxo tautomers of iso-G as compared to the enol ones, the effects of medium polarity were investigated with self-consistent reaction field (SCRF) theory (Table 3). This theory has been applied to 2-pyridone tautomerism[42] and guanine·cytosine base pairing.[43] A dielectric constant of 40 was chosen for the reaction field calculations, the same value as that used in the study by Florian and Leszczynski.[43] This value corresponds roughly to the dielectric constant of *N*,*N*-dimethylformamide (36.71)[44] or acetonitrile (37.5)[44] and is taken to approximate the interior of a nucleic acid double helix. As may be seen in Table 3, proceeding from a dielectric value of 1 to a value of 40 causes the N1-H, N3-H, and trans-imino oxo tautomers of iso-G to increase in stability relative to the O2-H tautomers to a point where the N3-H tautomer becomes intermediate in stability between the two enol forms. This change in relative energies may be attributed to the favorable interactions with solvent of the lactams compared to the lactims due to greater dipole moments and polarizability of the former. The computational prediction that the iso-G N3-H tautomer is stable at intermediate dielectric values is intriguing, as is the relatively high stability predicted for trans-imino oxo tautomer. It is not clear at this time why the N3-H tautomer has not been implicated in the two experimental studies of isoguanine tautomerism noted above since reference compounds corresponding to this structure were investigated. No reference compound was synthesized for the imino oxo form of iso-G, and so its presence in these experiments cannot be ruled out. Nevertheless, the N3-H tautomer has been implicated indirectly in past experimental work,[7,41] and in experimental work reported here, the imino oxo tautomer has been invoked to account for an unusually stable pairing interaction (vide infra). At a dielectric of 40, the stability of the N1-H form of iso-G appears to be underestimated compared to the enol form in light of the approximate equilibrium constants of 8 and 2 in favor of the latter tautomer in DMSO and acetonitrile, respectively, determined with N^6,N^6,N^9-trimethyliso-G.[37b] A possibly related issue is the difference in SCRF stabilities of the N1-H and N3-H tautomers. This difference is apparently due in part to the N1-H form having a notably smaller dipole moment than the N3-H form, resulting in disparate stabilities in solvent. By integrating theoretical and experimental information relating to iso-G tautomer energies, it is proposed that the first five tautomers (four excepting rotational isomerism) listed in Table 3 (i.e., entries 1−5) are important in solution. The energy gap separating these five tautomers proposed to be populated is not out of line with the agreement between theory and experiment for guanine. Nevetheless, as discussed below, a different trend is seen in relative energies of guanine tautomers.

iso-G and G Compared. It is of interest to compare iso-G and G in that considerable theoretical and experimental work has been done on G, the congruence of which has bearing on the treatment of iso-G. Guanine tautomer energies have been investigated by the ab initio method in past work.[43,45] We have reproduced these calculations (Table 3) for internal reference purposes to investigate the effect of medium polarity on the

(43) Florian, J.; Leszczynski, J. *J. Am. Chem. Soc*. **1996**, *118*, 3010−3017.

(44) Riddick, J. A.; Bunger, W. B. In *Techniques of Chemistry*, 3rd ed.; Weissberger, A., Ed.; Wiley: New York, 1970; Vol. II.

(45) (a) Gould, I. R.; Burton, N. A.; Hall, R. J.; Hillier, I. H. *J. Mol. Struct*. **1995**, *331*, 147−154. (b) Sabio, M.; Topiol, S.; Lumma, W. C. *J. Phys. Chem*. **1990**, *94*, 1366−1372. (c) Leszczynski, J. *Chem. Phys. Lett*. **1990**, *174*, 347−354. (d) Gould, I. R.; Hillier, H. *Chem. Phys. Lett*. **1989**, *161*, 185−187.

N3-H tautomer of guanine, since this had not been reported previously.[43] The N1-H tautomer is predicted by the 6-31G* calculations to be most stable irrespective of dielectric value. However, in the gas phase, the O6-H enol form is relatively close in energy to the N1-H form and could be anticipated to also be populated as previously discussed.[45] In fact, it is known that, at the 6-31G** ab initio level,[45] the enol tautomer is favored slightly (by 0.23 kcal/mol). However, inclusion of electron correlation again favors the N1-H oxo tautomer (by 0.84 kcal/mol).[45a] Experimental evidence[46] for both of these tautomers, O6-H enol and N1-H oxo, in an approximate 1:1 ratio, has been observed in FT-IR spectra of matrix-isolated guanine at low temperatures, consistent with ab initio predictions. In contrast, X-ray crystallography shows only the N1-H oxo form to be present in guanosine monophosphate.[47] It is noteworthy that the same energy gap separating the guanine N1-H oxo and (s-cis) O6-H enol tautomers in gas-phase calculations (∼1.5 kcal/mol) also separates the (s-cis) O2-H enol form of isoguanine from three other tautomeric forms at a dielectric of 40, the N1-H oxo, N3-H oxo, and trans-imino oxo (Table 3, note that relative energies for isoguanine are rooted to the s-trans enol form in the table and not the s-cis form as discussed here). Thus, in general, the calculations predict and experiments support a reciprocal relationship between tautomer multiplicity and medium polarity for guanine and isoguanine: isoguanine populates multiple tautomers, and guanine exists (principally) as a single tautomer in polar media, whereas the opposite holds for nonpolar media.

Nucleobase Tautomerism: Isocytosine. Past and Present Experiment and Theory. Both ab initio and spectroscopic data are available for isocytosine tautomerism. Results from previous[48,49] and selectively reproduced[50] ab initio calculations on isocytosine in the gas phase are given in Table 4 and Figure 5 along with new data from SCRF calculations. With the proviso of N1 substitution, these data suggest that the trans-imino oxo tautomer of iso-C is most stable in the gas phase, irrespective of basis set. However, in condensed phases, the cis-imino oxo and N1-H amino oxo tautomers would be expected to dominate due to their relatively large dipole moments, where the N1-H amino oxo form may predominate owing to its being most polar. These expectations are reflected in the SCRF calculations at the 6-31G* level ($\epsilon = 40$) where the N1-H amino oxo tautomer is found to be most stable by ∼3 kcal/mol. This margin increases to 7 kcal/mol at the 6-31G** level with $\epsilon = 78.5$ (note: this calculation was performed to allow comparison with data from ref 57, Table 4). The theoretical predictions are also born out in various experiments. IR spectroscopy of matrix-isolated isocytosine shows predominantly the amino enol form along with the N3-H amino oxo form.[51] When the 1-position bears a substituent, as with matrix-isolated 1-methylisocytosine, the imino oxo tautomer is observed by IR spectroscopy,[52]

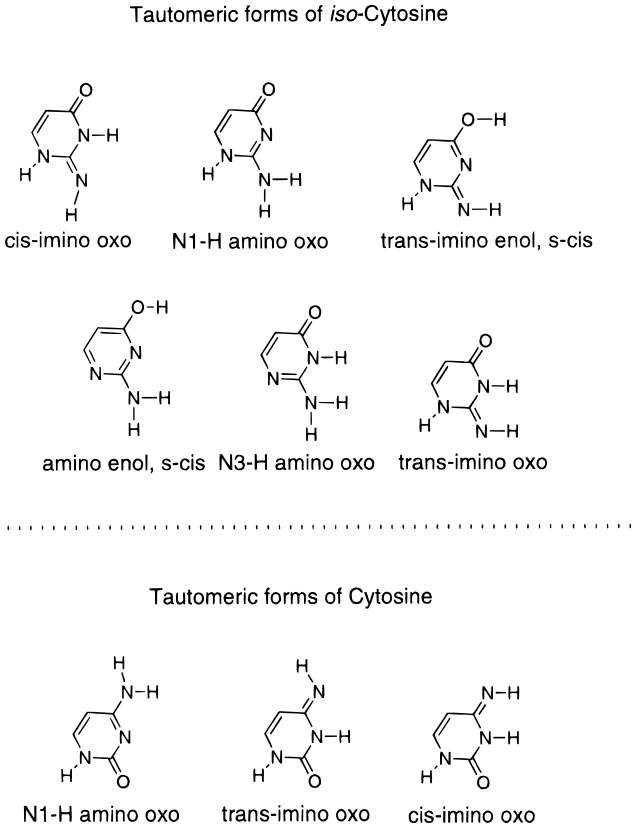


Figure 5.

predicted to be most stable of the N1-substituted derivatives in the gas-phase calculations given in Table 4 and the exhaustive study of Ha et al.[49,50] X-ray crystallography has shown isocytosine to exist as an exact 1:1 mixture of N1-H and N3-H amino oxo tautomers.[53] Studies of the solution structure of isocytosine by ^{1}H NMR and UV spectroscopy yield results similar to the crystal structure where the N1-H and N3-H amino oxo tautomers are together observed.[54,55] The solution and crystallographic data support, and the theoretical data are consistent with, iso-C adopting the N1-H amino oxo tautomer when bearing a 1-substituent in environments of intermediate to high polarity.

iso-C and C Compared. As with isocytosine, ab initio calculations (6-31G*, $\epsilon = 1$) on cytosine indicate that the two possible imino oxo tautomers lie near in energy to the canonical N1-H amino oxo form (+0.6 and +2.3 kcal/mol, respectively, Table 4).[43,56−58] The principal difference between cytosine versus isocytosine computationally is that with the isomeric base imino tautomers are more rather than less stable in the gas phase compared to the N1-H amino oxo form. However, the N1-H amino oxo tautomers of cytosine and isocytosine are similar in that they are both more polar than their respective imino oxo tautomers. As a consequence, in the presence of a reaction field and by extension solvent, the N1-H amino oxo form dominates in both isocytosine and cytosine and by nearly the same margin (3.1 vs 4.1 kcal/mol, iso-C vs C). Experiments on cytosine

(46) (a) Sheina, G. G.; Stephanian, S. G.; Radchenko, E. D.; Blagoi, Y. P. *J. Mol. Struct*. **1987**, *158*, 275−292. (b) Szczepaniak, K.; Szczesniak, M.; Person, W. B. *Chem. Phys. Lett*. **1988**, *153*, 39−44. (c) Szczepaniak, K.; Szczesniak, M.; Szajda, W.; Person, W. B.; Leszynski, J. *Can. J. Chem*. **1991**, *69*, 1705−1720.

(47) Thewalt, U.; Bugg, C. E.; Marsh, R. E. *Acta Crystallogr*. **1971**, *B27*, 2358−2363.

(48) Smets, J.; Adamowicz, L.; Maes, G. *J. Mol. Struct*. **1994**, *322*, 113−130.

(49) Ha, T.-K.; Keller, H. J.; Gunde, R.; Gunthard, H. H. *J. Mol. Struct*. **1996**, *376*, 375−397.

(50) Note that the 6-31G** energies for the N3-H and N1-H amino oxo tautomers of isocytosine in refs 48 and 49 differ by 10.8 kcal/mol. Examination of the data for the other tautomers in ref 49 suggested that the data for the N3-H and N1-H amino oxo tautomers of isocytosine were transposed. This was confirmed by reproducing the calculations (Table 4). Calculations in Table 4 were performed in this work unless otherwise noted.

(51) (a) Vranken, H.; Smets, J.; Maes, G.; Lapinski, L.; Nowak, M. *Spectrochim. Acta* **1994**, *50A*, 875−889. (b) Stepanian, S. G.; Radchenko, E. D.; Sheina, G. G.; Blagoi, Y. P. *J. Mol. Struct*. **1990**, *216*, 77−90.

(52) Szczesniak, M.; Nowak, M. J.; Szczepaniak, K. *J. Mol. Struct*. **1984**, *115*, 221−224.

(53) Sharma, B. D.; McConnel, J. F. *Acta Crystallogr*. **1965**, *19*, 797−806.

(54) Stolarski, R.; Remin, M.; Shugar, D. *Z. Naturforsch.* **1977**, *32e*, 894−900.

(55) Morita, H.; Nagakura, S. *Theor. Chim. Acta* **1968**, *11*, 279−295.

(56) Ha, T.-K.; Keller, H. J.; Gunde, R.; Gunthard, H. H. *J. Mol. Struct*. **1992**, *276*, 209−249.

(57) Gould, I. R.; Green, D. V. S.; Young, P.; Hillier, I. H. *J. Org. Chem.* **1992**, *57*, 4434−4437.

(58) Ha, T.-K.; Gunthard, H. H. *J. Mol. Struct*. **1993**, *300*, 619−635.

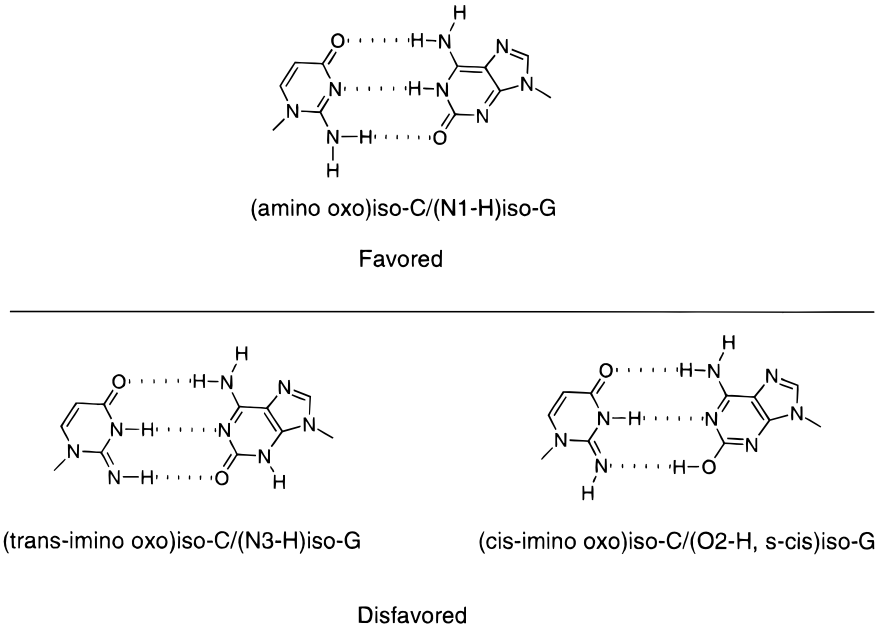


Figure 6.

support this computational picture. In the gas phase, the N1-H amino oxo form of cytosine predominates, but ~10% of the imino oxo form is present, as evidenced from IR spectroscopy on matrix-isolated N1-methylcytosine.[59] X-ray crystallographic and solution studies of cytosine[60] support a single tautomer, the N1-H amino oxo form, in agreement with SCRF theory.

Duplex Thermodynamics and Base-Pair Structure. Isomeric Nucleotide Selection. For the sake of synthetic simplicity, the deoxyriboside of isoguanine was selected as the form of isoguanine to be incorporated into oligonucleotides for the present stability studies. Because of its robust nature, riboisocytidine has been utilized for the complementary base. The result of these choices is the investigation of free energy relationships within families of DNA/RNA hybrid duplexes. Such hybrid duplexes are reported to have thermodyamic behaviors similar to those of DNA/DNA and RNA/RNA duplexes in that their thermodynamic stabilities follow nearest-neighbor model predictions.[61]

The iso-G/iso-C Base Pair. The most stable base pairs formed in both duplex systems as summarized in Table 2 are the iso-G/iso-C and G/C (entries 1 and 10; entries 2 and 11), consistent with the formation of three-hydrogen bonds between iso-G and iso-C. As proposed above, any of the first five iso-G tautomers in Table 3 (entries 1−5) could be taken to enter into a base pair due to their similar energies, coupled with mitigating experimental data for the N1-H tautomer. In the case of iso-C, both SCRF theoretical and solution experimental data support the amino oxo tautomer as most stable in environments of intermediate to high polarity. Nevertheless, whereas no direct experimental data support the imino forms of iso-C, it is not possible to rigorously exclude these tautomers. The proposed mode for the interaction of iso-G and iso-C given in Figure 6 reflects the above arguments. The situation in this figure may be amplified by two additional observations: (i) pairs between either the N3-H or O2-H (s-cis) forms of iso-G and the imino oxo forms of iso-C may be ruled out since their existence would imply stable iso-C/A Watson−Crick pairing, and this is observed in neither Table 2 nor earlier work,[31] and (ii) existence of a stable O2-H (s-cis) form of iso-G implies stable iso-G/U Watson−Crick pairing, which likewise is not observed (Table 2).

The iso-G/C Base Pair. The second most stable pair involving an isomeric base is that formed between iso-G and C (Table 2, entries 3 and 12). This pair rivals the natural A/U pair in stability. This result is surprising given that neither iso-G nor C in a template directed the incorporation of the other in initial experiments with a DNA (or RNA) polymerase.[26] On the surface, the polymerase result could be taken as evidence favoring a non-Watson−Crick pairing geometry. However, Watson−Crick pairing could be said to be a necessary, but not sufficient condition for nucleotide incorporation, as documented by ideosyncratic recognition of nucleotides bearing unnatural bases by a variety of different polymerases.[62] In point of fact, a mammalian polymerase has recently been found to incorporate iso-G across from C in a DNA template[63] where the bacterial polymerase used in earlier work failed.[26] As a consequence, not only the thermodynamic but also enzymatic data are consistent with an iso-G/C Watson−Crick pair. As summarized in Figure 7, it would appear that the trans-imino oxo iso-G tautomer, as supported by SCRF ab initio data, is in a singular position to form such a pair with C. In this case it is imagined that water or cations would complex to one or both carbonyls to diminish electrostatic repulsion. Another means to ameliorate this clash would involve tautomerization to the less stable s-cis rotamer of the N1-H, trans-imino enol form of iso-G (Figure 4). However, the N1-H, trans-imino enol tautomer would appear energetically too far removed from the trans-imino oxo tautomer as evidenced by the low stability of the s-trans isomer of the former (+9.43 kcal/mol removed from the latter, entries 7 vs 5, Table 3, 6-31G*, $\epsilon = 40$). The two possible wobble pairs of iso-G to C may be invoked. These pairs can seemingly be excluded solely on the basis of this geometry not being consistent with the unusual stability observed for this pair. Yet, one theoretical study concluded that a wobble geometry need

(59) (a) Szczesniak, M.; Leszczynski, J.; Person, W. B. *J. Am. Chem. Soc*. **1992**, *114*, 2731−2733. (b) Smets, J.; Adamowicz, L.; Maes, G. *J. Phys. Chem*. **1996**, *100*, 6434−6444.

(60) For a review, see: Kwiatkowski, J. S.; Pullman, B. In *Advances in Heterocyclic Chemistry*; Katritzky, A. R., Boulton, A. J., Eds.; Academic Press: New York 1975; Vol. 18, pp 200−327.

(61) (a) Sugimoto, N.; Nakano, S.; Katoh, M.; Matsumura, A.; Nakamuta, H.; Ohmichi, T.; Yoneyama, M.; Sasaki, M. *Biochemistry* **1995**, *34*, 11211−11216. (b) Sugimoto, N.; Katoh, M.; Nakano, S.; Ohmichi, T.; Sasaki, M. *FEBS Lett*. **1994**, *354*, 74−78.

(62) (a) Horlacher, J.; Hottiger, M.; Podust, V. N.; Hübscher, U.; Benner, S. A. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 6329−6333. (b) Lutz, M. J.; Held, H. A.; Hottiger, M.; Hübscher, U.; Benner, S. A. *Nucleic Acids Res*. **1996**, *24*, 1308−1313.

(63) Kamiya, H.; Kasai, H. *J. Biol. Chem*. **1995**, *270*, 19446−19450.

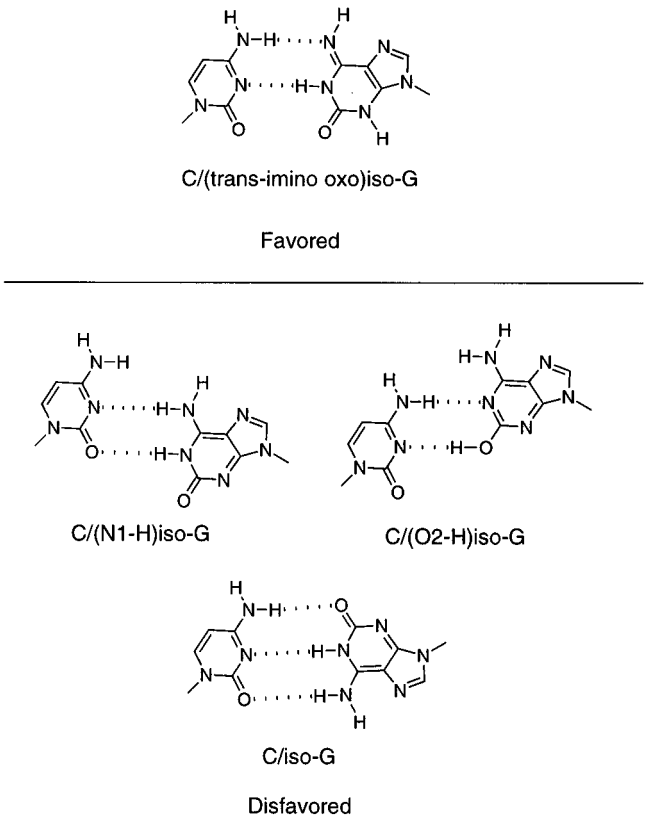


Figure 7.

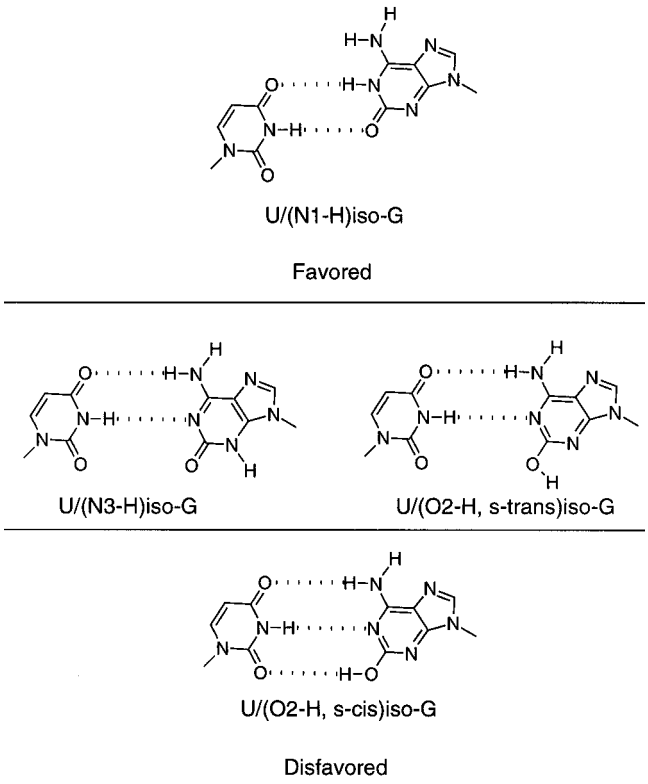


Figure 8.

not be intrinsically less stable than a three hydrogen bond Watson−Crick pair.[64] It appears that the additional stability for wobble pairing in this published study may derive from simultaneously forming two bifurcated hydrogen bonds.[64] In contrast to this special case, the C/N1-H iso-G pair may form only one bifurcated hydrogen bond. The C/O2-H iso-G pair is disfavored on the basis of the argument applied to this tautomer in the discussion above related to Figure 6. As a final note, it is formally possible for iso-G to form a reverse-Watson−Crick pair with C by adopting a syn orientation about its glycosidic bond, but strain engendered during the attendant backbone reorganization makes this an unlikely alternative.

The iso-G/U Base Pair. The least stable iso-G pair is that formed with U (entries 5 and 14 of Table 2), which has stability intermediate between the standard wobble pairs in this study, G/U and T/G[65] (entries 7 and 15 of Table 2), and an A/U Watson−Crick pair. Four possible U/iso-G pairs are given in Figure 8. The data in Table 2 do not easily allow the given pairs to be distinguished, nor does the ab initio or other data (vide supra). Because of its being less stable than an A/U pair, the most obvious geometry to invoke for iso-G/U would be wobble (top of Figure 8). However, to be consistent with polymerase work, one of the three Watson−Crick motifs should be accessible. There is of course no restriction on the coexistence of wobble and Watson−Crick pairing modes. Indeed, the simultaneous presence of both wobble and Watson−Crick geometries has been reported.[66,67] Paradoxically, the Watson−Crick iso-G/U pairs given in the lower two sections of Figure 8 would seem most favorable, all other factors being equal, yet these pairs appears too stable to be consistent with the data in Table 2. This need not be the case if the path taken to the Watson−Crick pairs includes perturbation of an unfavorable iso-G tautomeric equilibrium by the complementary U base. A variety of other contextual factors could serve to reorder the narrowly spaced energies of the lowest lying iso-G tautomers as well. As a case in point, the energy gap between the s-cis and s-trans rotamers of the O2-H tautomer of iso-G may be accentuated in favor of the s-trans rotamer when paired with U—the compensating electrostatic attraction between the O2-H and the lone pairs on N1 and N3 of iso-G in the unpaired state would be masked at N1 when so paired. In addition, an unfavorable repulsive secondary electrostatic interaction between the O2-H of iso-G and the N3-H of U is also avoided in the U/(O2-H, s-trans)iso-G pair as compared to its s-cis counterpart.[68] The positions of the various iso-G/U pairs in Figure 8 reflect these arguments. The Watson−Crick pairs in the middle of Figure 8 are anticipated to lie close in energy to the wobble pair such that, minimally, they would be accessible in the context of a polymerase active site, and therefore, they are classified as neither favored nor disfavored.

The iso-C/G and iso-C/A Base Pairs. The remaining two unnatural pairs to be discussed are iso-C/G and iso-C/A. Both are similar in energy to a U/G or G/T[65] wobble pair (Table 2). As a consequence, the geometries of these unnatural pairs are likely wobble. Similar conclusions were reached by Strobel et al.[31] with the exception that two additional pairing possibilities were considered by these authors for iso-C/G: reverse-Watson−Crick pairing via a syn-G and Watson−Crick pairing by way of the imino enol form of iso-C. Given that the imino enol form is destabilized in the gas phase[49] (Table 4, entry 6) with little chance to alter this situation in solvent due to a relatively

(64) Czerminski, R.; Szczepaniak, K.; Person, W. B.; Kwiatkowski, J. S. *J. Mol. Struct.* **1990**, *237*, 151−163.

(65) The free energy difference for the U/G pair in the nonamer duplex (entry 18, Table 2) appears to be anomalously large (4.2 kcal/mol). It is not clear at this time why this is so, but further investigation did show that inverting the U/G pair to G/T (entry 15) yielded a free energy difference similar to that obtained in the dodecamer duplex for U/G (3.0 vs 2.5 kcal/mol, entry 7 vs 15). The fact that the free energy differences between the standard Watson−Crick pairs G/C and A/U (entries 11 and 13) is as expected, serves as a control for the integrity of the G and U bearing oligomers used for entry 18 of Table 2.

(66) Moore, M.; van Meervelt, L.; Salisbury, S. A.; Kong, T. L.; Brown, D. M. *J. Mol. Biol.* **1995**, *251*, 665−673.

(67) Fazakerley, G. V.; Gdaniec, Z.; Sowers, L. C. *J. Mol. Biol*. **1993**, *230*, 6−10.

(68) Jorgensen, W. L.; Pranata, J. *J. Am. Chem. Soc*. **1990**, *112*, 2008−2010.

small dipole moment, we suggest that the latter pair is unlikely. While a syn-G, reverse-Watson−Crick pair would be unusual, our data, like that of Strobel et al.,[31] are also consistent with this interpretation. Wobble pairing between 2-aminoadenine and iso-C is supported by aminoacylation studies carried out by Musier-Forsyth et al.,[32] where this pair effectively replaces a "required" U/G pair. In this same work weak aminoacylation of a substrate bearing an iso-C/G pair in place of U/G was interpreted as consistent with either an amino oxo iso-C/G pair or a cis-imino oxo iso-C/G pair.

Fitness of the Expanded Genetic System. The results in Table 2 can be used to assess the viability of an expanded genetic system with iso-C, iso-G, and the natural bases. This is perhaps best approached by first considering the two alternative four-component systems with iso-C and iso-G as two of the components. The two unnatural bases in combination with C and G can be argued viable on thermodynamic grounds, where viability is judged on the basis of the free energy separating the least stable pair from the most stable mispair. Pairs between C/G and their isomeric counterparts are approximately as stabilized compared to the most stable mispair, iso-G/C, as are the two natural pairs compared to a G/U wobble. A four-component system based on the two unnatural bases in combination with A and U may also be said to be thermodynamically viable. After the iso-G/iso-C and A/U pairs falls the iso-G/U pair, which is destabilized relative to A/U, but not to the same degree as a G/U wobble pair. Consequently, the unnatural four-component system incorporating A and U is less optimal than the one incorporating G and C. Combination of these two four-component systems into a single six-component system does not appear to engender any special problems. While it is true that iso-G/C is isoenergetic with A/U, no serious consequence can be foreseen for having a mispair and a pair with similar energies if they are composed of different bases. A serious shortcoming for the expanded system probably lies in the discrimination of mispairs during replication. Whereas the most stable natural mispair, G/U, adopts a wobble geometry, the two lowest energy unnatural mispairs, iso-G/C and iso-G/U, have the apparent tendency to adopt Watson−Crick geometries. This situation makes the fidelity of replication inherently more difficult to control for any genetic system bearing iso-G and iso-C.

Experimental Section

General Methods. See Supporting Information.

Synthesis. 3′,5′-*O*-(Di-*tert*-butyldimethylsilyl)-2′-deoxyadenosine N^1-Oxide (2). *m*-Chloroperbenzoic acid (50−60%, 8.06 g, 23.4 mmol) was dissolved in CH_2Cl_2 (67 mL), dried over $MgSO_4$, and filtered. To this solution was added a solution of 3′,5′-*O*-(*Di*-*tert*-butyldimethylsilyl)-2′-deoxyadenosine[69] (5.62 g, 11.7 mmol) in CH_2Cl_2 (56 mL). The reaction was stirred at room temperature for 6 h. The solution was diluted with 150 mL of CH_2Cl_2 and washed with 5% aqueous NaI. The organic layer was clarified by addition of solid $NaHSO_3$ and then extracted with saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$, and concentrated. Flash chromatography (SiO_2, MeOH (0−5%)/(CH_2Cl_2) provided **2**: 4.64 g (9.4 mmol), 80% yield. ^{1}H NMR ($CDCl_3$): δ 0.09 (s, 6 H), 0.1 (s, 6 H), 0.91 (s, 18 H), 2.41−2.49 (m, 1 H), 2.57−2.65 (m, 1 H), 3.78 (dd, 1 H, J = 11.26 Hz, 3.02 Hz), 3.86 (dd, 1 H, J = 11.26 Hz, 3.99 Hz), 4.02 (m, 1 H), 4.60 (m, 1 H), 6.39 (t, 1 H, J = 6.28 Hz), 7.09 (br s, 2 H), 8.25 (s, 1 H), 8.68 (s, 1 H).

3′,5′-*O*-(Di-*tert*-butyldimethylsilyl)-2′-deoxyisoguanosine (3). To a solution of **2** (2.01 g, 4.05 mmol) in 95% ethanol (800 mL) was added water (800 mL). The mixture was irradiated at 252 nm in a quartz reaction vessel with mixing carried out by a N_2 bubbler. After 24 h, the reaction mixture was removed, and the pH was adjusted to 10.0 with 28% ammonium hydroxide and stirred at room temperature overnight. The pH was then adjusted to 7.0 with 10% HCl and the ethanol removed by rotary evaporation. To the aqueous solution were added CH_2Cl_2 (400 mL) and water (200 mL). The aqueous layer was washed twice more with CH_2Cl_2 (200 mL). The combined organic layers were washed with saturated NaCl, dried over $MgSO_4$, and concentrated. Flash chromatography (SiO_2, EtOAc (100%), then MeOH (20%)/CH_2Cl_2) afforded **3**: 850 mg (1.71 mmol), 42% yield. ^{1}H NMR: ($CDCl_3$) δ 0.09 (s, 12 H), 0.9 (m, 18 H), 2.39 (br s, 2 H), 3.75−3.86 (m, 2 H), 3.96 (d, 1 H, J = 2.25 Hz), 4.54 (d, 1 H, J = 3.74 Hz), 6.23 (br s, 1 H), 7.84 (br s, 1 H).

3′,5′-*O*-(Di-*tert*-butyldimethylsilyl)-N^6-(*N*,*N*-dibutylformamidine)-2′-deoxyisoguanosine (4). To a solution of **3** (500 mg, 1.01 mmol) in pyridine (6.1 mL) was added *N*,*N*-dibutylformamidine dimethyl acetal (0.690 mL, 3.03 mmol) at room temperature. The reaction was stirred for 24 h, then CH_2Cl_2 (50 mL) was added and the mixture was extracted with saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$, and concentrated. Flash chromatography (SiO_2, MeOH (3%)/CH_2Cl_2) provided **4**: 454 mg (0.72 mmol), 71% yield. ^{1}H NMR: ($CDCl_3$) δ 0.07 (s, 6 H), 0.1 (s, 6 H), 0.9 (m, 18 H), 0.97 (m, 6 H), 1.37 (m, 4 H), 1.64 (m, 4 H), 2.37 (m, 2 H), 3.43 (t, 2 H, J = 7.2 Hz), 3.56 (t, 2 H, J = 7.6 Hz), 3.8 (m, 2 H), 3.94 (d, 1 H, J = 3.35 Hz), 4.5 (d, 1 H, J = 4.77 Hz), 6.35 (t, 1 H, J = 6.21 Hz), 7.9 (s, 1 H), 8.43 (s, 1 H), 9.47 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ -5.61, −5.5, −4.99, −4.76, 13.46, 13.67, 17.85, 18.28, 19.5, 19.96, 25.64, 25.87, 28.97, 30.54, 41.67, 45.25, 52.16, 56.67, 62.63, 71.48, 83.51, 87.37, 114.3, 138.89, 153.92, 157.53, 157.75, 161.35. MS (FAB^+): *m/z* 635. HRMS (FAB^+): calcd for $C_{31}H_{58}N_6O_4Si_2$ 635.4137 (MH^+), found 635.4117.

3′,5′-*O*-(Di-*tert*-butyldimethylsilyl)-N^6-(*N*,*N*-dibutylformamidine)-O^2-(*N*,*N*-diphenylcarbamoyl)-2′-deoxyisoguanosine (5). To a solution of **4** (360 mg, 0.57 mmol) in pyridine (5.5 mL) was added diisopropylethylamine (0.148 mL, 0.85 mmol) followed by diphenylcarbamoyl chloride[70] (263 mg, 1.13 mmol) at room temperature. After 3 h, 100 mL of CH_2Cl_2 was added and the mixture was extracted with saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$, and concentrated. Flash chromatography (SiO_2, MeOH (4%)/CH_2Cl_2) afforded **5**: 431 mg (0.52 mmol), 92% yield. ^{1}H NMR: ($CDCl_3$) δ 0.07 (s, 6 H), 0.09 (s, 6 H), 0.93 (m, 24 H), 1.37 (m, 4 H), 1.62 (m, 4 H), 2.41 (m, 1 H), 2.54 (m, 1 H), 3.38 (t, 2 H, J = 7.39 Hz), 3.66−3.86 (m, 4 H), 3.97 (d, 1 H, J = 3.54 Hz), 4.58 (d, 1 H, J = 5.48 Hz), 6.43 (t, 1 H, J = 6.44 Hz), 7.22 (m, 2 H), 7.31−7.41 (m, 8 H), 8.12 (s, 1 H), 8.92 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ −5.52, −5.40, −4.87, −4.71, 13.62, 13.85, 17.95, 18.35, 19.69, 20.12, 25.73, 25.93, 29.13, 29.62, 30.83, 41.26, 45.07, 51.72, 62.81, 71.84, 83.96, 87.68, 124.76, 126.24, 126.86, 128.84, 140.11, 142.27, 152.19, 152.32, 156.04, 158.61, 161.25. MS (FAB^+): *m/z* 830. HRMS (FAB^+): calcd for $C_{44}H_{67}N_7O_5Si_2$ 830.4821 (MH^+), found 830.4806.

N^6-(*N*,*N*-Dibutylformamidine)-O^2-(*N*,*N*-diphenylcarbamoyl)-2′-deoxyisoguanosine (6). To a solution of **5** (357 mg, 0.43 mmol) in THF (9 mL) was added tetrabutylammonium fluoride (1.0 M solution in THF, 3.54 mL, 3.54 mmol). After $1^1/_2$ h the reaction was concentrated under vacuum. The residue was dissolved in 150 mL of CH_2Cl_2 and extracted with saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$, and concentrated. Flash chromatography (SiO_2, MeOH (5%)/CH_2Cl_2) provided **6**: 231 mg (0.38 mmol), 89% yield. ^{1}H NMR: ($CDCl_3$) δ 0.89−1.04 (m, 6 H), 1.29−1.45 (m, 4 H), 1.57−1.67 (m, 4 H), 2.23 (dd, 1 H, J = 13.3 Hz, 5.3 Hz), 2.93 (m, 2 H), 3.19 (t, 1 H, J = 8.4 Hz), 3.38 (t, 2 H, J = 7.32 Hz), 3.68 (m, 2 H), 3.85 (d, 1 H, J = 12.3 Hz), 4.0 (d, 1 H, J = 12.3 Hz), 4.16 (s, 1 H), 4.72 (s, 1 H), 6.06 (m, 1 H), 7.21 (t, 2 H, J = 7.2 Hz), 7.35 (t, 4 H, 7.7 Hz), 7.47 (d, 4 H, J = 7.5 Hz), 7.76 (s, 1 H), 8.9 (s, 1 H). ^{13}C NMR ($CDCl_3$): δ 13.42, 13.56, 13.75, 19.46, 19.66, 19.79, 20.02, 23.63, 25.95, 29.02, 30.77, 41.16, 51.88, 53.07, 58.44, 63.35, 73.05, 87.6, 88.55, 125.75, 126.75, 127.38, 128.93, 141.98, 142.32, 150.94, 152.40, 154.62, 158.74, 161.39. MS (FAB^+): *m/z* 602. HRMS (FAB^+): calcd for $C_{32}H_{39}N_7O_5$ (MH^+) 602.3091, found 602.3108.

5′-*O*-(4,4′-Dimethoxytrityl)-N^6-(*N*,*N*-dibutylformamidine)-O^2-(*N*,*N*-diphenylcarbamoyl)-2′-deoxyisoguanosine (7). To a solution of **6** (151 mg, 0.29 mmol) in pyridine (3 mL) was added 4,4′-dimethoxytrityl chloride (51 mg, 0.15 mmol) at room temperature. After 2 h, another portion of 4,4′-dimethoxytrityl chloride (51 mg, 0.15 mmol) was added and the reaction was stirred for an additional 2 h, at which time 150 mL of CH_2Cl_2 was added. The mixture was then extracted with saturated $NaHCO_3$ and saturated NaCl, dried over Na_2SO_4, and concentrated. Flash chromatography (SiO_2, pyridine (1%)/MeOH (1%)/

(69) Ogilvie, K. K. *Can. J. Chem.* **1973**, *51*, 3799−3807.

(70) Kamimura, T.; et al. *J. Am. Chem. Soc.* **1984**, *106*, 4552−4557.

CH_2Cl_2) afforded **7**: 174 mg (0.19 mmol), 77% yield. ^{1}H NMR: ($CDCl_3$) δ 0.94 (dd, 6 H, J = 14.2 Hz, 7.2 Hz), 1.36 (m, 4 H), 1.6 (m, 4 H), 2.16 (d, 1 H, J = 2.74 Hz), 2.54−2.63 (m, 2 H), 3.8 (m, 4 H), 3.69 (m, 2 H), 3.77 (s, 6 H), 4.05 (d, 1 H, J = 4.1 Hz), 4.59 (br s, 1 H), 6.45 (t, 1 H, J = 6.36 Hz), 6.8 (d, 4 H, J = 8.76 Hz), 7.18−7.4 (m, 19 H), 7.98 (s, 1 H), 8.92 (s, 1 H). ^{13}C NMR ($CDCl_3$) δ 13.57, 13.79, 19.61, 20.03, 29.06, 30.75, 40.91, 45.07, 51.76, 55.06, 63.86, 72.09, 83.51, 85.91, 86.34, 113.05, 124.39, 126.32, 126.71, 127.74, 127.98, 128.84, 129.86, 129.88, 135.53, 135.63, 139.67, 142.05, 144.44, 152.21, 152.24, 156.01, 158.33, 158.62, 161.18. MS (FAB^+:) *m/z* 904. HRMS (FAB^+): calcd for $C_{53}H_{57}N_7O_7$ (MH^+) 904.4391, found 904.4398.

5′-*O*-(4,4′-Dimethoxytrityl)-*N*^{6}-(*N*,*N*-dibutylformamidine)-*O*^{2}-(*N*,*N*-diphenylcarbamoyl)-2′-deoxyisoguanosine 2-Cyanoethyl *N*,*N*′-Diisopropylphosphoramidite (8). To a solution of **7** (166 mg, 0.18 mmol) in CH_2Cl_2 (2 mL) was added diisopropylethylamine (0.064 mL, 0.37 mmol) followed by (2-cyanoethoxy)(*N*,*N*′-diisopropylamino)chlorophosphine (0.053 mL, 0.22 mmol). After 1 $^1/_2$ h at room temperature, 75 mL of CH_2Cl_2 was added and the mixture extracted with saturated $NaHCO_3$ and saturated NaCl, dried over Na_2SO_4, and concentrated. Flash chromatography (SiO_2, pyridine (0.5%)/MeOH (0.7%)/CH_2Cl_2) provided **8**: 161 mg (0.15 mmol), 80% yield. ^{1}H NMR: ($CDCl_3$) δ 0.9−0.98 (m, 12 H), 1.06−1.5 (m, 48 H), 2.42 (m, 2 H), 2.54 (m, 2 H), 2.66 (m, 2 H), 3.38 (m, 8 H), 3.6−3.72 (m, 12 H), 3.76 (s, 6 H), 3.77 (s, 6 H), 4.25 (m, 2 H), 4.7 (m, 2 H), 6.45 (m, 2 H), 6.79 (m, 8 H), 7.19−7.41 (m, 38 H), 8.02 (s, 1 H), 8.07 (s, 1 H), 8.92 (s, 1 H). ^{31}P NMR (acetone-d_6) δ 149.35, 149.53. MS (FAB^-): *m/z* 1105. HRMS (FAB^-): calcd for $C_{62}H_{74}N_9O_8P_1$ (M^-) 1105.5482, found 1104.5427.

***N*^{2}-(*N*,*N*-Dimethylformamidine)isocytidine (10).** To a solution of isocytidine[71] (**9**) (1.02 g, 4.3 mmol) in pyridine (28 mL) was added *N*,*N*-dimethylformamidine dimethyl acetal (0.917 mL, 6.5 mmol). After stirring overnight, the reaction mixture was concentrated under vacuum. Flash chromatography (SiO_2, MeOH (25%)/CH_2Cl_2) afforded **10**: 1.14 g (3.82 mmol), 89% yield. ^{1}H NMR: (CD_3OD) δ 3.15 (s, 3 H), 3.19 (s, 3 H), 3.69 (dd, 1 H, J = 12.4 Hz, 2.9 Hz), 3.83 (dd, 1 H, J = 12.4 Hz, 2.4 Hz), 3.98 (m, 1 H), 4.09 (m, 2 H), 5.93 (d, 1 H, J = 7.7 Hz), 6.34 (d, 1 H, J = 2.68 Hz), 8.2 (d, 1 H, J = 7.7 Hz), 8.63 (s, 1 H). ^{13}C NMR (DMSO-d_6) δ 35.0, 40.82, 60.53, 69.43, 74.69, 84.46, 89.64, 108.13, 138.39, 158.06, 170.21.

5′-*O*-(4,4′-Dimethoxytrityl)-*N*^{2}-(*N*,*N*-dimethylformamidine)isocytidine (11). To a solution of **10** (1.02 g, 3.43 mmol) in pyridine (51 mL) was added a mixture of 4,4′-dimethoxytrityl chloride (533 mg, 1.51 mmol) in pyridine (3 mL) at 0 °C. The mixture was allowed to warm to room temperature, and after 30 min, another portion of 4,4′-dimethoxytrityl chloride (533 mg, 1.51 mmol) in pyridine (3 mL) was added. Thirty minutes later the final portion of 4,4′-dimethoxytrityl chloride (533 mg, 1.51 mmol) in pyridine (3 mL) was added. The reaction was then stirred at room temperature for 6 h when 250 mL of CH_2Cl_2 was added. The mixture was extracted with saturated $NaHCO_3$ and saturated NaCl, dried over Na_2SO_4, and concentrated. Flash chromatography (SiO_2, pyridine (1%)/MeOH (5%)/CH_2Cl_2) provided **11**: 1.11 g (1.85 mmol), 54% yield. ^{1}H NMR: ($CDCl_3$) δ 3.10 (s, 1 H), 3.17 (s, 1 H), 3.41 (dd, 1 H, J = 10.6 Hz, 3.1 Hz), 3.5 (dd, 1 H, J = 10.6 Hz, 2.4 Hz), 3.78 (s, 7 H), 4.28−4.31 (m, 4 H), 5.81 (d, 1 H, J = 7.73 Hz), 6.24 (d, 1 H, J = 3.79 Hz), 6.83 (d, 4 H, J = 8.55 Hz), 7.21−7.29 (m, 7 H), 7.38 (d, 2 H, J = 7.76 Hz), 8.82 (s, 1 H). ^{13}C NMR ($CDCl_3$) δ 35.34, 41.13, 55.06, 62.16, 69.61, 75.92, 83.24, 86.59, 91.22, 107.92, 113.07, 126.82, 127.78, 127.99, 128.86, 129.98, 135.19, 135.41, 138.59, 144.36, 157.97, 158.39, 172.71. MS (FAB^+): *m/z* 601. HRMS (FAB^+): calcd for $C_{33}H_{36}N_4O_7$ (MH^+) 601.2584, found 601.2676.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-(*N*,*N*-dimethylformamidine)isocytidine (12). To a solution of **11** (1.92 g, 3.2 mmol) in THF (33 mL) was added pyridine (1.34 mL, 16.8 mmol), silver nitrate (854 mg, 5.03 mmol), and *tert*-butyldimethylsilyl chloride (885 mg, 5.7 mmol). The reaction was stirred at room temperature for 8 h, at which time the reaction was added to 5% aqueous $NaHCO_3$. This mixture was extracted twice with 75 mL of CH_2Cl_2. The combined organic layers were washed with saturated NaCl, dried over Na_2SO_4, and concentrated. Flash chromatography (SiO_2, pyridine (1%)/MeOH (3%)/CH_2Cl_2) gave 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-(*N*,*N*-dimethylformamidine)isocytidine (**12**) as a white foam: 1.19 g (1.67 mmol), 87% yield.

A mixture of **12** (1.19 g, 1.67 mmol), triethylamine (1 mL), and MeOH (10 mL) was stirred at room temperature for 2 $^1/_2$ h, at which time the mixture was concentrated. Flash chromatography (SiO_2, pyridine (1%)/MeOH (3%)/CH_2Cl_2) afforded **13**: 503 mg (0.7 mmol), 42% yield (22% yield from **11**). ^{1}H NMR: ($CDCl_3$) δ −0.06 (s, 3 H), 0.06 (s, 3 H), 0.87 (s, 9 H), 2.865 (d, 1 H, J = 1.51 Hz), 3.09 (s, 3 H), 3.14 (s, 3 H), 3.42 (dd, 1 H, J = 10.6 Hz, 2.14 Hz), 3.49 (dd, 1 H, J = 10.6 Hz, 1.87 Hz), 3.79 (s, 6 H), 4.19 (br s, 1 H), 4.27 (d, 1 H, J = 5.06 Hz), 4.45 (t, 1 H, J = 5.8 Hz), 5.75 (d, 1 H, J = 7.72 Hz), 6.68 (d, 1 H, J = 6.48 Hz), 6.83 (d, 4 H, J = 8.58 Hz), 7.24−7.38 (m, 9 H), 7.76 (d, 1 H, J = 7.73 Hz), 8.8 (s, 1 H). ^{13}C NMR ($CDCl_3$): δ 17.8, 25.43, 35.22, 41.19, 54.98, 55.34, 63.64, 71.98, 72.02, 76.37, 83.73, 83.96, 87.14, 88.16, 88.39, 109.67, 109.8, 113.17, 113.25, 127.04, 127.86, 127.96, 128.01, 129.90, 129.93, 130.04, 130.07, 134.94, 135.08, 137.61, 137.65, 144.19, 158.61, 158.67, 158.98, 159.11, 171.6. MS (FAB^-): *m/z* 714.

5′-*O*-(4,4′-Dimethoxytrityl)-2′-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-(*N*,*N*-dimethylformamidine)isocytidine 2-Cyanoethyl *N*,*N*′-Diisopropylphosphoramidite (14). To a solution of **13** (274 mg, 0.38 mmol) in CH_2Cl_2 (2.5 mL) was added diisopropylethylamine (0.270 mL, 1.54 mmol) followed by (2-cyanoethoxy)(*N*,*N*′-diisopropylamino)chlorophosphine (0.300 mL, 1.25 mmol). The reaction was stirred at room temperature for 1 h, at which time the mixture was added to 50 mL of 5% aqueous $NaHCO_3$. This mixture was then extracted with 50 mL of CH_2Cl_2. The organic layer was washed with saturated NaCl, dried over Na_2SO_4, and concentrated. Flash chromatography (SiO_2, pyridine (1%)/EtOAc to remove H-phosphonate followed by pyridine (1%)/MeOH (10%)/CH_2Cl_2) to elute **14**: 285 mg (0.31 mmol), 81%. ^{1}H NMR: ($CDCl_3$) δ −0.11−0.06 (m, 12 H), 0.8−0.85 (m, 18 H), 1.04 (m, 4 H), 1.17 (m, 10 H), 2.31 (m, 1 H), 2.65 (m, 2 H), 3.08 (m, 12 H), 3.35−3.52 (m, 2 H), 3.54−3.64 (m, 4 H), 3.79 (s, 6 H), 3.8 (s, 6 H), 3.9−3.97 (m, 4 H), 4.18 (s, 2 H), 4.29−4.5 (m, 2 H), 4.52 (m, 1 H), 5.77 (d, 1 H, J = 7.72 Hz), 5.87 (d, 1 H, J = 7.88 Hz), 6.72 (m, 2 H), 6.85 (m, 6 H), 7.21−7.42 (m, 20 H), 7.81 (d, 2 H, J = 7.78 Hz), 8.78 (s, 2 H). ^{31}P NMR ($CDCl_3$) δ 149.93, 153.02. MS (FAB^+): *m/z* 914. HRMS (FAB^+): calcd for $C_{48}H_{67}N_6O_8PSi$ (MH^+) 914.4327, found 914.4276.

DNA/RNA Synthesis and Melting Experiments. See Supporting Information.

Ab Initio Calculations. Calculations were performed using Gaussian 94 (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.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; 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*, Revision B.1; Gaussian, Inc.: Pittsburgh, PA, 1995) on a Cray C98/8128 at the San Diego Supercomputer Center. In the Onsager reaction field calculations, a value of a_o = 4.1[43] was used for iso-G and G and values of 3.9[43] (ϵ = 40) and 3.53[57] (ϵ = 78.5) were used for iso-C.

Acknowledgment. We thank Professors Albert Eschenmoser, Andrew H.-J. Wang, and Thomas Hellman Morton for helpful discussions. Support for this work by the National Institutes of Health (GM-47375) and the National Aeronautics and Space Administration (NAGW-4184) is gratefully acknowledged. The San Diego Supercomputer Center is also gratefully acknowledged.

Supporting Information Available: A listing of general experimental procedures, procedures for the synthesis of 2′-d-iso-G and isocytosine with supporting spectral data, DNA and RNA synthesis methods, and protocols for duplex denaturation experiments (3 pages). See any current masthead page for ordering and Internet access instructions.

JA970123S

(71) Kimura, J.; Yagi, K.; Suzuki, H.; Mitsunobu, O. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 3670−3677.

(72) Hashimoto, H.; Nelson, M. G.; Switzer, C. *J. Am. Chem. Soc.* **1993**, *115*, 7128−7134.