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Novel Benzopyrimidines as Widened Analogues of DNA Bases

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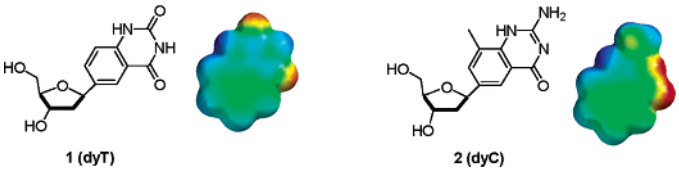

We report on the synthesis, stacking, and pairing properties of a new structural class of size-expanded pyrimidine nucleosides, abbreviated dyT and dyC. Their bases are benzo-homologated variants of thymine and cytosine and have a design that is distinct from a previously described class of size-expanded (xDNA) pyrimidines, with a different vector of expansion relative to the sugar. We term this new base geometry "yDNA" (a mnemonic for "wide DNA"). Both C-glycosides were prepared using Pd-mediated coupling of iodinated base derivatives with a deoxyribose precursor. As free deoxynucleosides, both dyT and dyC displayed robust fluorescence, with emission maxima at 375 and 390 nm, respectively. Both widened pyrimidines could be incorporated readily as protected phosphoramidite derivatives into synthetic oligonucleotides. Experiments in "dangling end" DNA contexts revealed that both yT and yC stack more favorably than their natural counterparts. When opposite natural bases in the context of Watson−Crick DNA were paired, the yT nucleotide formed a pair with A that was equally stable as a T−A pair, despite the mismatch in size with the neighboring natural pairs. The yC nucleotide (paired opposite G) was destabilizing by a small amount in the same context. Despite the large size of the pairs, both yT and yC were selective for their Watson−Crick complementary partners A and G, respectively. The pairing properties and fluorescence of yDNA nucleotides may lead to useful applications in the study of steric effects in DNA-protein interactions. In addition, the compounds may serve as building blocks for a large-sized artificial genetic system.

Introduction

We have recently undertaken a series of studies aimed at design of new nucleotide analogues in which the bases are larger than those in natural nucleic acids.[1] This approach was inspired by Leonard's early studies with a benzo-fused analogue of adenine ribonucleotide, which was useful as a tool in studies of ATP-dependent enzymes.[2] A larger set of size-expanded bases and nucleotides may serve more generally as useful tools in biophysical studies of DNA and proteins that interact with it. In addition, size-expanded bases can be paired with natural complements to generate base pairs that are also increased in size.[1] Early studies of oligomeric strands composed of sized-expanded nucleotides have shown that they can bind complementary nucleic acids with high affinity. Thus, such DNA homologues might find useful applications in new biotechnologies.

A number of laboratories have examined properties of nonnatural nucleosides in which the nucleobases are larger than natural ones. One advantage of increased size is extended conjugation, which can lead to useful fluorescence. For example, after early work by Robins,[3] Moreau and co-workers have constructed bicyclic and tricyclic nucleobase analogues in an effort to yield useful fluorescent reporters of triple helix formation in DNA.[4] Because these benzo- and naphthopyrimidines are attached as N-nucleosides, they would not form expanded-

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size pairs. Several related heterobicyclic compounds reported by Pfleiderer and by Hawkins are notable for their useful fluorescence properties,[5,6] although they are analogues of purines, and so are not appreciably expanded in size.

In addition to the above examples, Seley has recently reported a thienyl-bridged tricyclic analogue of deoxyadenosine in an effort to explore enzyme inhibition properties.[7] The compound has not been incorporated into oligonucleotides. Finally, Matsuda has recently reported the synthesis of tricyclic bases capable of forming four hydrogen bonds and studied pairing properties in oligonucleotides.[8] They were designed to pair with other nonnatural bases and display no pairing selectivity among the natural bases.

Our initial approach to the design of size-expanded purines and pyrimidines was the linear extension of length by insertion of a benzene ring (analogous to the Leonard approach[2]), yielding 2.4 Å of added size. This yielded the xDNA nucleosides (dxT and dxA have been reported to date) and helices containing them.[1] Because of their larger sizes, the xDNA nucleosides are fluorescent, and experiments showed that they stack with high affinity on neighboring DNA sequences.[1c] Single substitutions of xDNA base pairs in natural DNAs were somewhat destabilizing, due to the mismatch in size with the neighboring DNA. However, when strands were designed to form helices in which every base pair was expanded, the resulting duplexes were exceptionally stable.[1a,e]

Studies of the properties of the xDNA nucleosides are continuing. However, examination of models suggested to us that other geometries might be equally viable for extension of bases and pairs. An adenine-homologous nucleoside having a new "yDNA" geometric expansion was described recently.[9] Here we describe the design, preparation, and properties of a new class of widened pyrimidine analogues, also having the new yDNA classification. These compounds are shown to exhibit a number of potentially useful properties, including strong stacking affinity in DNA, selective pairing with Watson−Crick complements, and robust fluorescence. Similarities and differences with previous expanded pyrimidine analogues are discussed.

Results

Design. The design of the current widened pyrimidines (yT and yC, Figure 1) is different from previous extended pyrimidines (xT and xC)[1,10] by their vector of extension (Figure 2). The length of extension of the bases in yT and yC is essentially the same as xT and xC, at 2.4 Å, but the extension vector orientation is altered by 60°. Simple models of the expected base pairs (Figures 1C and 2B) shows that the overall effects of these two extensions on base pair length are similar but are predicted to yield different groove depths. More specifically, the stacking geometry with neighboring bases is altered, and yDNA pairs are expected to project further into the major groove, while the previous xDNA pairs project further into the minor groove. Such simple models suggested that the yDNA-widened pyrimidine design merited testing both as the free nucleosides or nucleotides and in DNA helices. A very recent study reported a widened form of a purine base (yA), which is also benzo-fused and has a similar vector of extension as yT and yC.[9] It remained to be seen whether pyrimidine-like compounds would behave with similarly favorable properties as did the purine analogue yA.

It may be noted that, formally, the base yT as prepared here lacks a methyl group and thus is technically a closer analogue of uracil than thymine. Similarly, the yC base, with its methyl group, is a close analogue of 5-methyl-C. The presence or absence of methyl was expected to have little influence on biophysical properties;[11] we chose the different methylation states to evaluate the two different synthetic routes, which later were shown to be nearly equally efficient (see below).

Space-filling models (Figure 1B) illustrate the close relationships between natural T/C nucleobases and the yT/yC analogues, which are enlarged by addition of the benzo group. Electrostatic potentials mapped as colors on the surfaces are extremely similar (T vs yT and C vs yC) along the Watson−Crick edges, suggesting similar hydrogen-bonding ability. However, the benzo groups are significantly less polar than the rest of the molecules.

Synthesis. The preparation of the yT nucleoside started with iodoanthranilic acid (**3**) and proceeded to the free dyT nucleoside in nine steps and in generally high yield (Scheme 1). The critical C-glycoside bond formation was achieved in 50% yield under Heck conditions, giving the desired β anomer exclusively. The anomeric geometry was confirmed by proton NOE data. The free nucleoside was thus obtained in eight steps and 20% yield. Two subsequent steps following standard conditions were needed to prepare the 5′-*O*-dimethoxytrityl, 3′-*O*-phosphoramidite derivative as required for standard oligonucleotide synthesis (see the details in the Supporting Information). Overall yield for the total of 10 steps was 18%. Based on precedent with a different isomer of this nucleoside derivative,[1a,b] we opted not to protect the oxygens for DNA synthesis; later oligomer synthesis added support to this decision (see below).

The synthesis of the dyC nucleoside followed a similar strategy, making use of Heck chemistry to install the glycosidic bond (Scheme 2). The synthesis in this case began with 5-methylanthranilic acid (**13**), and the benzopyrimidine heterocycle was protected at the exocyclic amine prior to glycoside bond formation. This did not add steps to the overall approach, since the same protecting group was needed for oligonucleotide synthesis. In general, as for dyT, the dyC synthesis proceeded with high yield and the latter required fewer steps overall as

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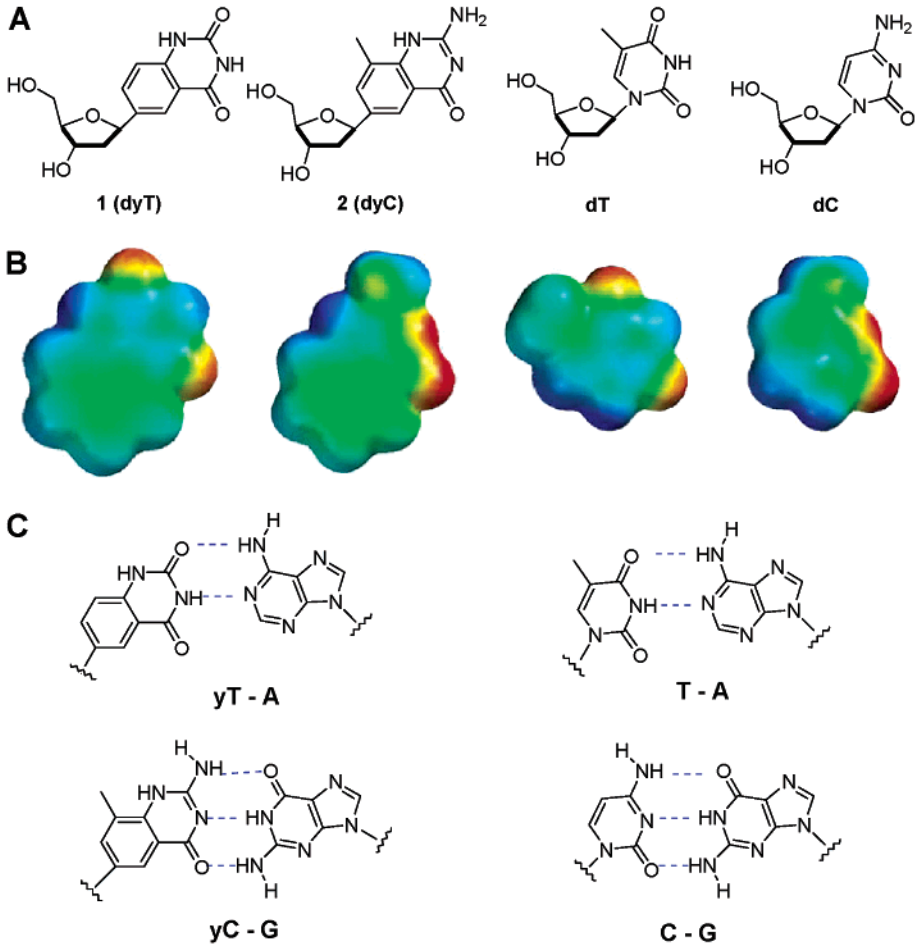

FIGURE 1. Structures of widened nucleobases and nucleosides in this study. (A) Nucleosides dyT and dyT shown next to the natural nucleosides dT and dC. (B) Space-filling models of the yT and yC nucleobases with electrostatic potentials mapped on the surfaces. Natural T and C bases are shown for comparison (methyl groups are omitted for clarity). Calculated using AM1 with Spartan (Wavefunction, Inc.). (C) The designed structures of widened base pairs involving yT and yC, with comparison to natural analogous pairs.

compared to the former. Glycoside bond formation for yC was accomplished in 59% yield through the use of Heck chemistry, and if desired, the free nucleoside could be obtained from amino-protected intermediate **19**, thus yielding this dyC nucleoside **2** in seven steps and 26% yield overall. The entire synthesis of the protected phosphoramidite derivative proceeded in 22% yield (eight steps) from starting compound **13**. Details are given in the Experimental Section and in the Supporting Information.

Properties. Because other isomers of benzopyrimidines are fluorescent,[1b,d,4] we tested the fluorescence of the dyT and dyC free nucleosides. Excitation and emission spectra were recorded for the two in methanol (Figure 3). Emission maxima for dyT and dyC were 375 and 390 nm, respectively, which are nearly the same as the structurally related dxT and dxC analogues.[1d] Absorption maxima for dyT were at 221 nm (ϵ = 38 100) and a long-wavelength band at 315 nm (ϵ = 3040); the corresponding maxima for and dyC were 231 nm (ϵ = 33 900) and 312 nm (ϵ = 2780). Florescence quantum yields for the two nucleosides were measured to be 0.54 (dyT) and 0.40 (dyC) in water. Thus, both are relatively efficient violet-colored fluorophores.

We then tested the ability of the ydT and dyC nucleotide precursors to be incorporated into DNA by synthesis of short (trimeric) oligomers, followed by characterization. Proton NMR spectra of the trimers T−yT−T and T−yC−T in crude form after deprotection showed clean and intact incorporation of the new nucleosides. The two oligomers were also characterized by electrospray mass spectra, which also confirmed the expected masses (Supporting Information).

Size-widened nucleobase analogues such as yT and yC may be useful as fluorescent probes of biophysical interactions and enzymatic mechanisms involving DNA. We therefore characterized the basic stacking and pairing properties of these new size-expanded nucleoside derivatives in the context of natural DNA duplexes. Table 1 shows dangling-end thermal denaturation data,[12] measuring stacking tendencies of the compounds as compared with standard-sized T and C, and Table 2 lists pairing data, with each of the two new benzopyrimidines separately placed opposite natural bases in the center of a 12mer DNA duplex. Results of the stacking studies showed that both yT and yC in a 5′-dangling position of the self-complementary duplex formed by the sequence 5′-dCGCGCG stabilized the helices quite considerably

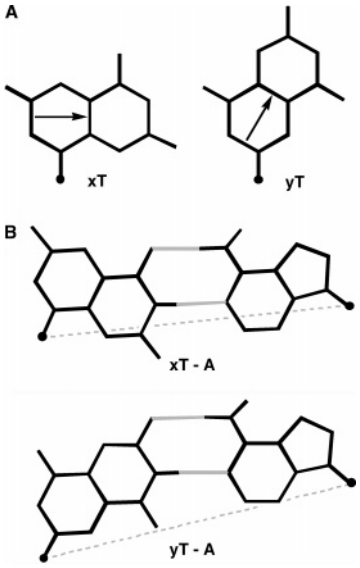

FIGURE 2. Comparison of the designs of yDNA and previous xDNA nucleobase designs. (A) Skeletons of yT and xT, showing the vectors of extension (arrows) by insertion of a benzo-ring. (B) Illustration comparing the framework of expanded-size T−A base pairs involving xT (top) and yT (bottom). Dashed lines connect the glycosidic points of attachment. Note that total degree of extension is similar; the chief difference is the shapes of the grooves (the major groove is above the pairs and the minor groove is below).

over the core duplex lacking a dangling base, suggesting stacking on the neighboring base pairs that is considerably more favorable in duplex form than in the single-stranded state. Both yT and yC gave greater stabilization than their natural smaller counterparts; however, the advantage of yT over T was larger than that of yC over C, which showed only a small difference. A comparison with data for earlier xDNA variants of these widened compounds (Table 1) shows that the previous expanded analogues xT and xC stabilize the duplex more dramatically,[1d] suggesting more efficient stacking.

In the pairing studies we tested each of the two widened pyrimidines in both a purine context (5′-AGAAXGAAAAG) and a pyrimidine context (5′-CTTTT-CYTTCTT), where X and Y are widened or natural bases. Data are listed in Table 2, and examples of melting profiles are shown in Figure 4; additional data are available in the Supporting Information. In general, the results were similar regardless of the difference in the two contexts. The thermal denaturation data showed (Table 2) that, when paired opposite A, yT showed equal stability as the control, T paired with A. The margin of base pairing selectivity of yT for A (as compared to "mismatches" of yT with C, G, or T) was 0.9−3.1 kcal/mol, which is smaller than the selectivity shown by natural T in the control experiments (Supporting Information). The yC analogue displayed somewhat different performance in these contexts (Table 2). The yC substitution, when paired opposite G, was mildly destabilizing, at approximately 0.6−2.2 kcal/mol relative to C. However, the pairing selectivity was similar to that seen for yT; the data for yC mismatches showed a penalty of 1.0−4.1 kcal/mol relative to the yC−G pair. This is smaller than the pairing selectivity displayed by natural C in the control duplexes.

The pairing thermodynamic data established that the yT−A and yC−G pairs were not strongly destabilizing and were selective. Although these facts suggested a hydrogen-bonded geometry in the duplex (see Figure 1C), confirmation of this must await future structural studies. Nevertheless, to obtain more information about possible structural distortions we used CD spectroscopy to evaluate 12mer duplexes containing these pairs. The data are shown in the Supporting Information. The results showed that spectra of duplexes containing a central yT−A pair closely resembled those containing a native T−A pair (Figure S1, Supporting Information). Similarly, the CD spectra of duplexes containing yC−G or C−G pairs were also virtually the same. Thus, the preliminary data suggest that although the widened pairs must by necessity cause some localized backbone distortion due to the added size, the overall duplex conformation is not significantly distorted.

Discussion

The syntheses of the current benzopyrimidine nucleoside analogues are in general relatively short and efficient. One may compare the ease of preparation of dyT and dyC with the syntheses of the previously described dxT and dxC nucleoside analogues,[1b,10] which are essentially isomers of the present compounds. The new synthetic data show that the earlier isomers were prepared in similar yields. The free dxT nucleoside was prepared in eight steps (34% yield), while the dxC nucleoside was prepared in a longer route starting from the dxT nucleoside (11 steps overall, 19% total). Thus, the new dyT nucleoside is synthesized in the same number of steps with slightly lower overall yield as dxT, while the dyC nucleoside is prepared in fewer steps and higher yield than the previous dxC.

A comparison of stacking and pairing properties of dyT and dyC, which are pyrimidine analogues, with the recently reported first member of the yDNA class, dyA (a purine analogue),[9] shows that dyA, a larger, tricyclic compound, stacks considerably more strongly. This is not surprising, as the size of a nucleobase or base analogue generally correlates with stacking ability in DNA.[12d] In addition, it is consistent with the fact that natural adenine stacks with higher affinity than thymine and cytosine do. As for pairing properties of dyA in natural DNA, the earlier data showed that yA is destabilizing when paired opposite T to a similar degree as dyC is here.[9] The selectivity of yA for T was also similar in magnitude to the selectivities of yT and yC, which were measured in the same sequence context here. This parallel behavior is not unexpected, as the vector of widening (by benzo-homologation) is almost the same for yA as for the new pyrimidines.

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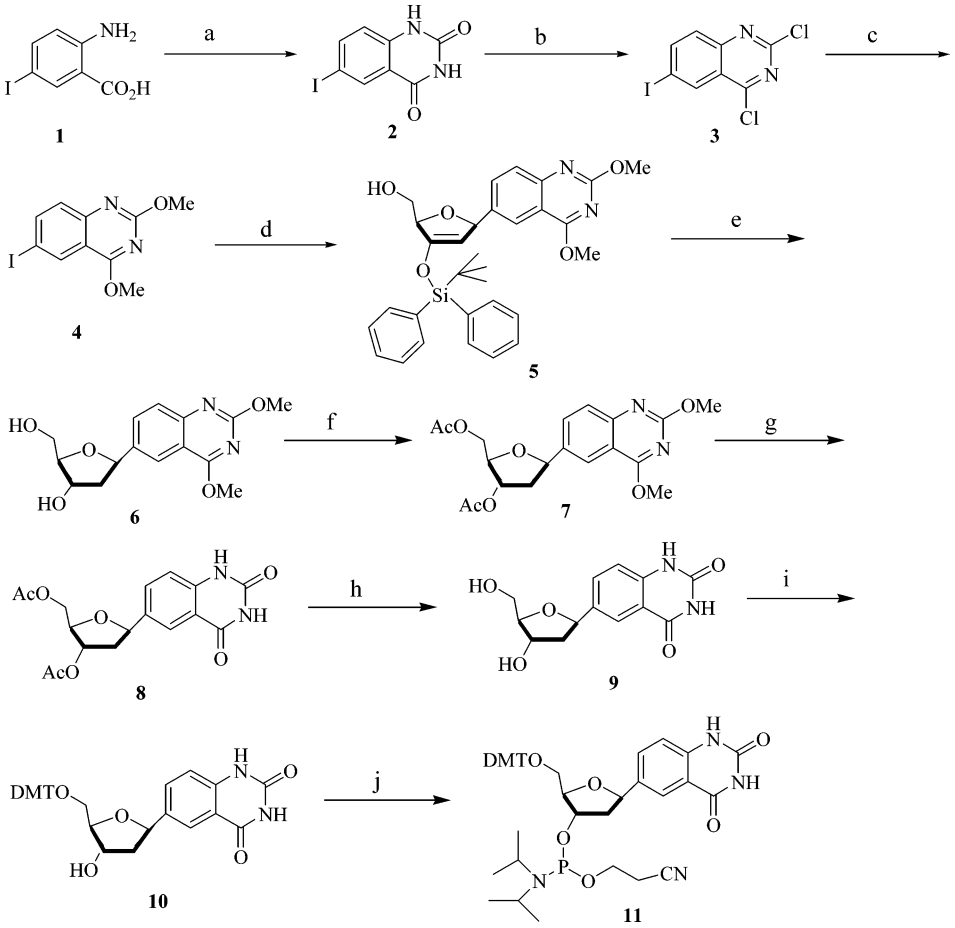
SCHEME 1[a]



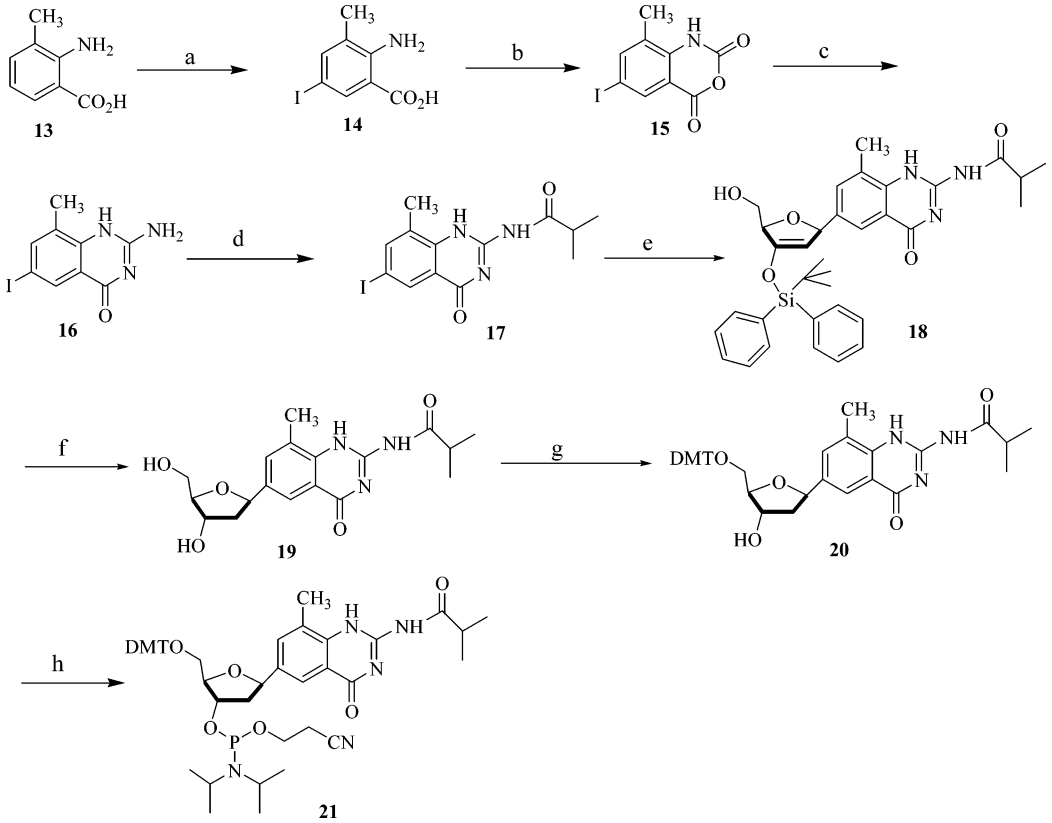
[a] Reagents and conditions: (a) urea, 160 °C, 96%; (b) $POCl_3$, DEA, reflux, 75%; (c) 0.5 M NaOMe/MeOH, reflux, 91%; (d) 1,2-dehydro-3-*O*-(*tert*-butyldiphenylsilyl)-5-hydroxymethylfuran, $Pd(OAc)_2$, Ph_3P, Bu_3N, CH_3CN, 85 °C, 50%; (e) (i) 1.0 M TBAF, THF, cat. HOAc, 0 °C, (ii) $NaB(OAc)_3H$, $CH_3CN-HOAc$, 4:1 v/v, −15 °C, 85%; (f) Ac_2O, pyridine, 25 °C, 87%; (g) NaI, HOAc, 60 °C, 83%; (h) NH_3, MeOH, 25 °C, 12 h, 99%; (i) DMTrCl, DIPEA, pyridine, 25 °C, 95%; (j) *N,N*-Diisopropylammonium tetrazole, 2-cyanoethyl tetraisopropylphosphoramidite, CH_2Cl_2, 25 °C, 2 h, 94%.

A comparison of the pairing behavior of the yT/yC family in DNA with the previous xT/xC isomers (which have different geometries of glycosidic attachment) shows, in general, quite similar properties.[1d,10] All of these expanded/widened bases retain the possibility of hydrogen bonding that is analogous to that of Watson−Crick pairs. Because of their size expansions, however, the bases are expected to be somewhat destabilizing when surrounded by normally-sized DNA. The new data generally support this idea. When paired with adenine, the previous xT was slightly destabilizing, while the current data show that substitution of yT for T is energetically neutral. As for the cytosine analogues, yC in the present study is destabilizing, while the earlier xC was less destabilizing. In terms of pairing selectivity for Watson−Crick partners, xT displayed lower selectivity than the current data show for yT. A study of xC pairing selectivity showed that it was somewhat higher than that seen for yC here. Thus, overall, the differences between the yDNA pyrimidines and the xDNA pyrimidines in the context of natural DNA are small, with minor variations that are likely due, at least in part, to differences in stacking stability.

Because of their generally favorable pairing properties and their fluorescence, yDNA nucleotides may be useful as tools in the study of size and steric effects in protein−DNA recognition. If such compounds are to be used as probes in a base-paired context, it will be useful in future studies to examine their structures in DNA at high resolution to test whether they are, in fact, paired as expected (see Figure 1) and what backbone distortions result from the mismatch in size with the neighboring pairs. It is encouraging in this regard that the yDNA bases are only weakly destabilizing at most and retain selectivity in pairing. Thus, they likely represent a minimal and localized perturbation to the overall DNA structure (this is supported by the CD spectra as well) and give little alteration to the stability as well.

Beyond the possible use of yT and yC nucleotides as probes of biophysical interactions, yDNA nucleosides are also useful building blocks for construction of a new, artificial genetic system (Lee, A. H. F.; Kool, E. T. Manuscript in preparation). The yDNA nucleotide geometry is distinct from that of the earlier xDNA design.[1] It remains to be seen how helices fully constructed from the yDNA design compare to those of the xDNA design. In addition, work will be needed to explore whether other properties of a functioning genetic system, such as transcription or replication, can be exhibited by such artificial information-encoding systems.

SCHEME 2[a]



[a] Reagents and conditions: (a) ICl, 6% HCl, Na_2SO_3, NaOH, 95%; (b) (i) Ethyl chloroformate, THF, reflux, (ii) PBr_3, Et_2O, reflux, 95%; (c) *S*-methylisothiourea hemisulfate, CH_3CN-H_2O, Na_2CO_3, reflux, 90%; (d) isobutyric anhydride, reflux, 69%; (e) 1,2-dehydro-3-*O*-(*tert*-butyldiphenylsilyl)-5-hydroxymethylfuran, $Pd(dba)_2$, $AsPh_3$, Bu_3N, CH_3CN, 85 °C, 59%; (f) 1.0 M TBAF, THF, cat. HOAc, 0 °C, (ii) NH4OH, 0 °C, (iii) $NaB(OAc)_3H$, $CH_3CN-HOAc$, 4:1 v/v, −15 °C, 84%; (g) DMTrCl, DIPEA, pyridine, 25 °C, 98%; (h) *N,N*-diisopropylammonium tetrazole, 2-cyanoethyl tetraisopropylphosphoramidite, CH_2Cl_2, 25 °C, 82%.

Experimental Section

6-Iodo-1*H*-quinazoline-2,4-dione (4). 2-Amino-5-iodobenzoic acid **3** (6.52 g, 24.79 mmol) and urea (15.00 g, 0.25 mol) were heated to 150 °C in a round-bottom flask, and the melt was stirred overnight. The mixture was then allowed to cool to 100 °C, and a volume equivalent of water was added. The resulting mixture was stirred for 10 min to allow unreacted urea to dissolve. The solid was filtered, resuspended in 150 mL 0.5 M NaOH (aq), and then heated to boiling to allow formation of the sodium salt. The mixture was neutralized with 10% diluted hydrochloric acid. The precipitate was suction-filtered, and the filter cake was washed with a water−methanol mixture. The product **4** (6.85 g, 96%) was obtained after vacuum-drying without further purification as a yellow powder. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.25 (s, 1H), 8.11 (s, 1H), 7.93 (dd, 1H, J = 7.2, 2.0 Hz), 6.98 (d, 1H, J = 8.4 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 161.9, 150.6, 142.8, 141.1, 134.9, 118.2, 116.7, 84.8. HREIMS *m/z*: $[M]^+$ calcd for $C_8H_5N_2O_2I$ 287.9397, found 287.9402.

6-Iodo-2,4-dichloroquinazoline (5). A mixture of iodoquinazolinedione **4** (7.64 g, 26.52 mmol) and phosphorus oxychloride (30.0 mL, 323 mmol) was stirred in an oven-dried round-bottom flask fitted with a condenser. To this solution was added *N,N*-diethylaniline (8.8 mL, 68.97 mmol). The mixture was heated at reflux for 4 h. Then the solution was allowed to cool to room temperature, and volatiles were removed in vacuo. The residue was dissolved in CH_2Cl_2 (150 mL) and washed with water (2 × 120 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The resulting residue was purified by silica column chromatography (hexane/EtOAc 15:1) to yield **3** (6.44 g, 75%) as an orange powder. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.63 (dd, 1H, J = 2.4, 0.4 Hz), 8.23 (dd, 1H, 8.8, 2.0 Hz), 7.73 (d, 1H, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 162.3, 155.4, 151.3, 144.9, 134.6, 129.3, 132.6, 94.6. HREIMS *m/z*: $[M]^+$ calcd for $C_8H_3N_2Cl_2I$ 323.8718, found 323.8706.

6-Iodo-2,4-dimethoxyquinazoline (6). 6-Iodo-2,4-dichloroquinazoline **5** (5.00 g, 15.43 mmol) was suspended in 0.5 M sodium methoxide in methanol (77.0 mL). The reaction mixture was heated at reflux under argon atmosphere for 18 h. The reaction mixture was then cooled to room temperature and neutralized with 10% HCl (aq). The quenched mixture was concentrated in vacuo. The resulting red solution was dissolved in EtOAc (200 mL) and washed with water (2 × 70 mL). The aqueous phase was washed with CH_2Cl_2 (50 mL) again, and the combined organics were dried (Na_2SO_4) and concentrated in vacuo. The orange residue was purified by silica column chromatography (hexane/ EtOAc 20:1) to yield **6** (4.45 g, 91%) as a white solid. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.41 (d, 1H, J = 2.0 Hz), 7.95 (dd, 1H, J = 8.4, 2.0 Hz), 7.46 (d, 1H, J = 8.8 Hz), 4.17 (s, 1H), 4.09 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 168.1, 162.3, 151.4, 142.4, 132.7, 127.9, 115.4, 87.6, 54.9, 54.7. HREIMS *m/z*: $[M]^+$ calcd for $C_{10}H_9N_2O_2I$ 315.9709, found 315.9708.

2,4-Dimethoxy-6-{2′*R*-*cis*-3-[2′3′-dehydro-3′-(*tert*-butyldiphenylsilyloxy)]-5′-hydromethyl-2′-furanyl}quinazole (7). The dimethoxyquinazoline **6** (1.67 g, 5.28 mmol) and 1,2-dehydro-3-*O*-(*tert*-butyldiphenylsilyl)-5-hydroxymethylfuran[13] (1.44 g, 4.07 mmol) were charged in an oven-dried round-bottom flask equipped with a septum. Freshly distilled CH_3CN

(13) Zhang, H. C.; Daves, G. D. J. *J. Org. Chem.* **1992**, *57*, 4690−4696.

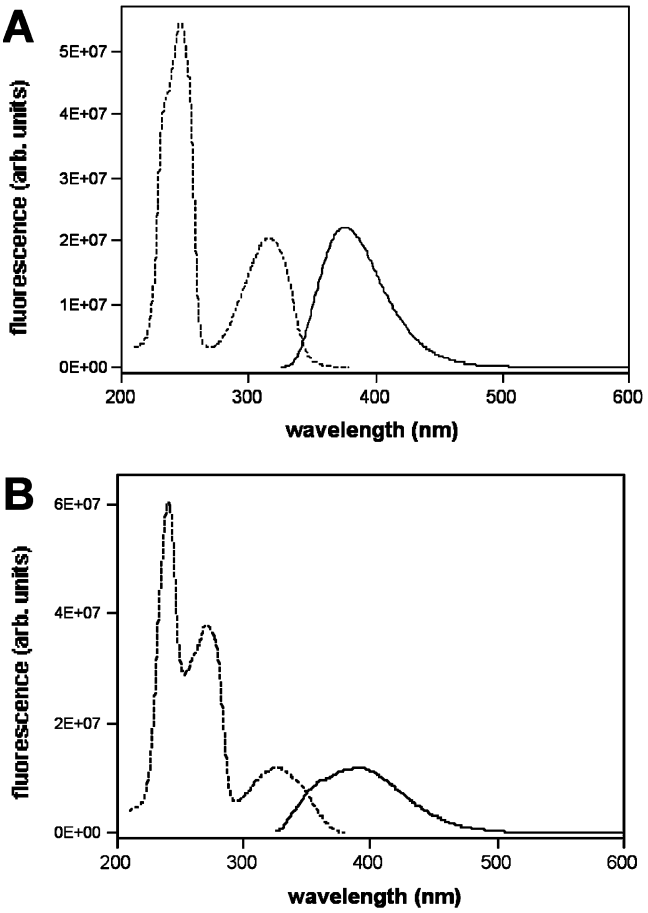


FIGURE 3. Normalized fluorescence excitation and emission spectra of free deoxynucleosides yT (A) and dyC (B). Spectra were measured in methanol; excitation for emission spectra was 315 nm; excitation spectra (dashed lines) were monitored at 400 nm.

(50 mL) was added to the flask to form a suspension, and the mixture was bubbled with argon for 30 min. $Pd(OAc)_2$ (45 mg, 0.21 mmol) and PPh_3 (105 mg, 0.41 mmol) were charged into a separated round-bottom flask and suspended in freshly distilled CH_3CN (30 mL). The catalyst−ligand mixture was stirred at room temperature and degassed with argon bubbling before being transferred to the glycal−quinazoline suspension via hypodermic syringe under inert atmosphere. Tri(*n*-butyl)-amine (1.38 mL, 5.81 mmol) was added to the reaction mixture in a portion. The septum-sealed flask was heated to 85−90 °C for 24 h and allowed to cool to room temperature. After removal of the volatiles, the residue was purified with silica column chromatography (hexane/EtOAc 3:1 initially and 1:1 subsequently) to afford **7** (1.30 g 50%) as yellow foam. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.83 (m, 3H), 7.76 (m, 2H), 7.57 (d, 1H, J = 8.4 Hz), 7.43 (m, 7H), 5.67 (dd, 1H, J = 16.8, 5.6 Hz), 4.79 (m, 1H), 4.37 (m, 1H), 4.15 (s, 3H), 4.07 (s, 3H), 3.88 (bs, 2H), 1.10 (s, 9H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.2, 162.2, 152.2, 151.1, 138.1, 135.9, 135.7, 135.3, 133.2, 133.0, 131.3, 131.0, 130.2, 127.9, 127.8, 126.6, 126.2, 122.5, 121.6, 113.1, 102.6, 84.5, 83.3, 62.7, 54.7, 54.5, 26.3, 19.2, 14.0. HRFABMS *m/z*: $[M + H]^+$ calcd for $C_{31}H_{35}N_2O_5Si$ 543.2315, found 543.2302.

1′-β-[6-(2,4-Dimethoxyquinazoline)]-2′-deoxy-d-ribofuranose (8). A solution of compound **7** (0.84 g, 1.55 mmol) in anhydrous THF (20 mL) was chilled in an ice−water bath. Glacial acetic acid (0.9 mL) and 1 M TBAF solution in THF (6.0 mL) were added sequentially via hypodermic syringe. The reaction mixture was allowed to stir at 0 °C for 5 min, and the volatiles were removed in vacuo. The crude intermediate of 2,4-dimethoxy-6-(β-D-*glycero*-pentofuran-3′-ulos-1′yl)-6-methylquinazoline was characterized by NMR. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.10 (d, 1H, J = 2.0 Hz), 7.80 (m, 2H), 5.33 (dd, 1H, J = 16.8, 5.6 Hz), 4.18 (s, 3H), 4.10 (s, 3H), 4.08 (m, 1H), 3.99 (m, 2H), 2.94 (m, 1H), 2.58 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 213.35, 169.19, 162.41, 152.34, 135.58, 131.87, 126.73, 121.40, 113.32, 82.48, 61.45, 54.81, 54.62, 45.27, 33.16. This crude product was dissolved in a solution of CH_3CN (12 mL) and glacial acetic acid (3 mL). The resulting mixture was chilled to −15 °C in an ice−salt bath followed by the addition of sodium triacetoxyboronhydride (0.82 g, 3.88 mmol) in one portion. The reaction mixture was allowed to stir at the low temperature for 10 min and was then concentrated in vacuo. The residue was purified by silica column chromatography (hexane/EtOAc 2:1 initially and EtOAc/methanol 10:1 subsequently) to give **8** (1.01 g, 85%) as a yellow syrup. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.95 (s, 1H), 7.67 (s, 1H), 7.28 (s,1H), 5.25 (dd, 1H, J = 10.0, 5.6 Hz), 4.44 (1H, m), 4.15 (m, 1H), 4.13 (s, 3H), 4.07 (s, 3H), 3.79 (m, 1H), 2.29 (m,1H), 2.04 (m, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 169.1, 162.1, 151.8, 137.3, 132.0, 126.1, 120.8, 113.1, 87.4, 79.7, 73.4, 63.2, 54.7, 54.5, 43.6. HRFABMS *m/z*: $[M + H]^+$ calcd for $C_{15}H_{19}N_2O_5$ 307.1294, found 307.1300.

1′-β-[6-(2,4-Dimethoxyquinazoline)]-3′,5′-*O*-(diacetyl)-2′-deoxy-d-ribofuranose (9). A mixture of **8** (0.41 g, 1.34 mmol), acetic anhydride (0.6 mL, 5.40 mmol), and pyridine (5 mL) was refluxed for 4 h. The mixture was dissolved in EtOAc (30 mL), and the solution was extracted with saturated sodium bicarbonate (3 × 50 mL), dried (Na_2SO_4), and concentrated in vacuo. The residue was purified by silica column chromatography (hexane/EtOAc 1:2) to afford **9** (0.46 g, 87%) as a yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.02 (s, 1H), 7.69 (s, 1H), 7.26 (s, 1H), 5.21 (m, 2H), 4.42 (m, 1H), 4.28 (m, 2H), 4.25 (s, 3H), 4.06 (s, 3H), 2.37 (m, 1H), 2.09 (m, 7H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.7, 170.5, 169.1, 162.1, 152.0, 136.6, 131.7, 126.3, 120.8, 113.3, 82.7, 80.2, 76.7, 76.6, 64.3, 54.7, 54.5, 41.3, 21.0, 20.7. HRFABMS *m/z*: $[M + H]^+$ calcd for $C_{19}H_{23}N_2O_7$ 391.1505, found 391.1500.

1′-β-[6-(Quinazoline-2,4-dione)]-3′,5′-*O*-(diacetyl)-2′-deoxy-d-ribofuranose (10). To a solution of **9** (0.34 g, 0.86 mmol) in glacial acetic acid (10 mL) was added sodium iodide (0.59 g, 4 mmol). The reaction mixture was heated to 60−65 °C for 45 min, and then the volatiles were removed in vacuo. The residue was dissolved in EtOAc (50 mL) and extracted with saturated Na_2SO_3 (aq) (3 × 30 mL) and saturated sodium bicarbonate solution (2 × 20 mL). The aqueous layers were extracted with EtOAc (2 × 30 mL). The combined organics were dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica column chromatography (EtOAc) to yield **10** (0.26 g, 83%) as a white solid. ^{1}H NMR (CD_3OD, 400 MHz): δ 8.04 (s, 1H), 7.65 (m, 1H), 7.16 (d, 1H, J = 8.4 Hz), 5.25 (m, 1H), 5.13 (dd, 1H, J = 10.8, 4.8 Hz), 4.35 (m, 1H), 4.25 (m, 2H), 2.40 (m, 1H), 2.11 (s, 3H), 2.09 (s, 3H), 2.05 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 172.6, 172.3, 165.0, 152.5, 141.8, 137.5, 134.5, 125.9, 116.7, 115.79, 84.3, 81.3, 78.2, 65.5, 42.2, 20.9, 20.8. HRFABMS *m/z*: $[M + H]^+$ calcd for $C_{17}H_{19}N_2O_7$ 363.1192, found 363.1190.

1′-β-[6-(Quinazoline-2,4-dione)]-2′-deoxy-d-ribofuranose (1). A solution of **10** (0.39 g, 1.1 mmol) in saturated NH_3/methanol (15 mL) was stirred at room temperature for 18 h. The volatiles were removed in vacuo, and the residue was purified by silica column chromatography (EtOAc/methanol 10:1) to yield **1** (0.33 g, 99%) as a slightly yellow solid. ^{1}H NMR (CD_3OD, 400 MHz): δ 8.02 (s, 1H), 7.70 (m, 1H), 7.16 (d, 1H, J = 8.4 Hz), 5.15 (dd, 1H, J = 10.8, 5.6 Hz), 4.33 (m, 1H), 3.96 (m, 1H), 3.69 (d, 2H, J = 4.8 Hz), 2.23 (m, 1H), 1.94 (m, 1H). ^{13}C NMR (CD_3OD, 100 MHz): δ 165.2, 152.6, 141.6, 138.6, 134.7, 125.8, 116.6, 115.6, 89.4, 80.7, 74.4, 64.0, 44.9. HRFABMS *m/z*: $[M + Na]^+$ calcd for $C_{13}H_{14}N_2O_5Na$ 301.0800, found 301.0795.

Phosphoramidite Derivative of Nucleoside Analogue 1. Subsequent synthetic steps and characterization are given in the Supporting Information.

TABLE 1. Thermodynamic Data for Self-Complementary Duplexes Containing a 5′ Dangling Base in the Duplex Core Sequence 5′-dCGCGCG[a]

dangling base	T_m[b] (°C)	$\Delta G°_{37}$[c] (kcal/mol)	$\Delta\Delta G°_{37}$[d] (kcal/mol)	$\Delta H°$ [c] (kcal/mol)	$\Delta S°$ [c] (eu)
(none)	44.8 ± 0.5	−8.5 ± 0.2		−43 ± 11	−113 ± 35
T	50.0 ± 0.5	−9.2 ± 0.5	−0.4 ± 0.2	−51 ± *9*	−135 ± 29
C	47.8 ± 0.5	−9.3 ± 0.2	−0.4 ± 0.3	−52 ± 3	−137 ± 9
yT	56.6 ± 0.5	−10.9 ± 0.1	−0.7 ± 0.2	−57 ± 1	−150 ± 3
yC	53.4 ± 0.5	−9.7 ± 0.2	−0.6 ± 0.2	−45 ± 1	−114 ± 4
xT[e]	57.7 ± 0.5	−11.2 ± 0.2	−0.9 ± 0.2		
XC[e]	53.6 ± 0.5	−10.1 ± 0.3	−0.8 ± 0.3		

[a] Conditions: 1 M NaCl, 10 mM phosphate (pH 7.0) with 0.1 mM EDTA. [b] T_m values are for 5.0 μM oligonucleotide. [c] Values from van't Hoff plots with at least five data points. [d] Values per each dangling base, relative to the core duplex. [e] Data from ref ld,e.

TABLE 2. Thermodynamic Data for 12mer DNA Duplexes Containing a Widened Base Pair or Mismatch (X−Y) in a Central Position[a,b]

base pair X−Y	T_m[c] (°C)	$\Delta G°_{37}$[d] (kcal/mol)	$\Delta H°$ [d] (kcal/mol)	$\Delta S°$ [d] (eu)
A−T	40.4 ± 0.5	−9.4 ± 0.1	−94 ± 12	274 ± 39
T−A	41.2 ± 0.5	−9.4 ± 0.1	−84 ± 5	241 ± 15
yT−A	41.5 ± 0.5	−9.4 ± 0.2	−82 ± 6	234 ± 20
yT−G	28.5 ± 0.5	−6.3 ± 0.1	−73 ± 3	215 ± 12
yT−C	31.8 ± 0.5	−6.9 ± 0.2	−82 ± 5	244 ± 17
yT−T	32.3 ± 0.5	−7.0 ± 0.2	−86 ± 9	256 ± 32
yT−ϕ[e]	25.4 ± 0.5	−5.7 ± 0.6	−69 ± 16	207 ± 54
A−yT	40.6 ± 0.5	−9.1 ± 0.2	−78 ± 5	222 ± 20
G−yT	31.9 ± 0.5	−7.2 ± 0.3	−73 ± 9	214 ± 30
C−yT	36.8 ± 0.5	−8.2 ± 0.1	−79 ± 2	231 ± 8
T−yT	36.4 ± 0.5	−8.1 ± 0.2	−73 ± 6	211 ± 20
ϕ−T	27.3 ± 0.5	−6.2 ± 0.3	−67 ± 6	197 ± 21
C−G	43.0 ± 0.5	−9.7 ± 0.1	−69 ± 6	192 ± 18
G−C	46.5 ± 0.5	−11.1 ± 0.4	−95 ± 6	271 ± 18
yC−G	40.6 ± 0.5	−9.1 ± 0.1	−66 ± 3	185 ± 10
yC−A	28.3 ± 0.5	−5.8 ± 0.4	−79 ± 7	237 ± 26
yC−C	36.0 ± 0.5	−8.1 ± 0.1	−74 ± 6	212 ± 19
yC−T	33.1 ± 0.5	−7.4 ± 0.1	−76 ± 4	223 ± 14
yC−ϕ	26.0 ± 0.5	−6.1 ± 0.1	−63 ± 4	185 ± 14
G−yC	39.6 ± 0.5	−8.9 ± 0.1	−44 ± 14	112 ± 44
A−yC	26.5 ± 0.5	−5.8 ± 0.2	−73 ± 2	215 ± 8
C−yC	31.9 ± 0.5	−7.1 ± 0.1	−77 ± 2	224 ± 6
T−yC	28.2 ± 0.5	−6.1 ± 0.2	−77 ± 5	229 ± 16
ϕ−C	23.7 ± 0.5	−5.2 ± 0.6	−66 ± 18	197 ± 59

[a] Conditions: 100 mM NaCl, 10 mM $MgCl_2$, 10 mM PIPES−Na (pH 7.0). [b] Sequence is 5′-AAGAAXGAAAAG·5′-CTTTTCYT-TCTT. [c] T_m values are for 5.0 μM oligonucleotide. [d] Averages of values from van't Hoff and curve fitting methods. [e] "ϕ" is tetrahydrofuran abasic analogue.

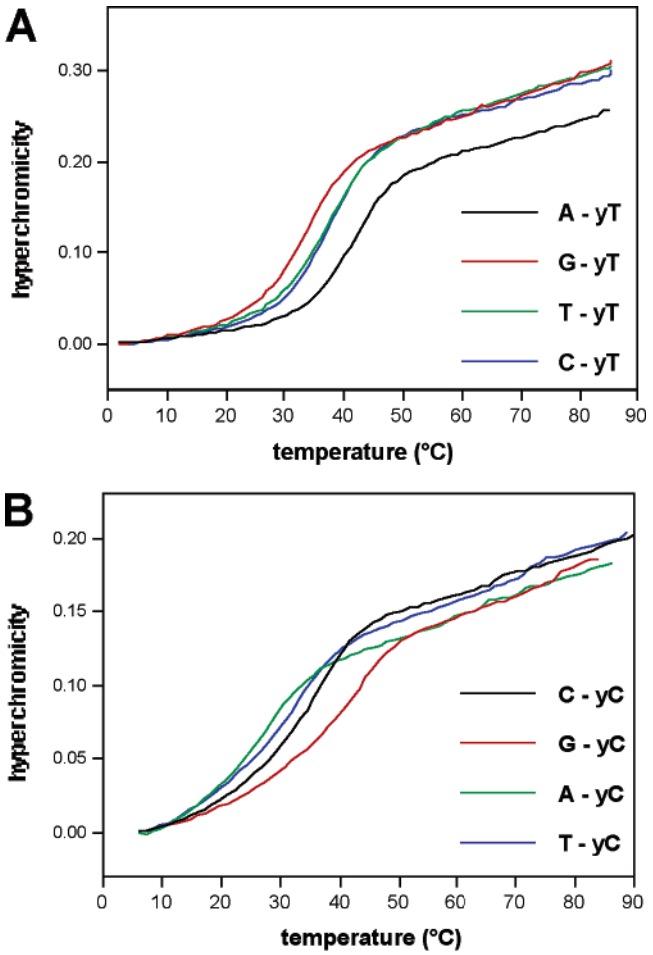


FIGURE 4. Examples of thermal denaturation curves for 12mer duplexes containing single widened bases paired opposite each of the four natural bases. (A) Plots for X−yT pairs, where X = A, T, G, or C. (B) Plots for X−yC pairs. Thermodynamics are listed in Table 2. Conditions: pH 7.0, 100 mM NaCl, 10 mM $MgCl_2$, 10 mM Na·PIPES buffer, [DNA] = 5 μM; monitoring at 260 nm.

2-Amino-5-iodo-3-methylbenzoic Acid (14). Iodine monochloride (5.1 mL, 0.10 mol) was added to a solution of 2-amino-3-methylbenzoic acid **13** (5.16 g, 34 mmol) and 6% aqueous HCl (60 mL) at 50−60 °C. The mixture was stirred at that temperature for 1 h and cooled to room temperature. Then the mixture was poured onto sodium metabisulfate (19.0 g, 0.10 mol) in crushed ice and neutralized with potassium hydroxide pellets cautiously with ice bath cooling. The pale brown precipitate was filtered, washed with ice−water and methanol (10 mL), and then dried in vacuo to give **14** (9.34 g 95%) as a brown powder. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.86 (d, 1H, J = 2.0 Hz), 7.44 (d, 1H, J = 2.4 Hz), 2.08 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 168.8, 149.4, 141.6, 136.8, 126.4, 111.7, 74.5, 17.1. HREIMS *m/z*: $[M]^+$ calcd for $C_8H_8NO_2I$ 276.9600, found 276.9608.

6-Iodo-8-methyl-1*H*-benzo-1,3-oxazine-2,4-dione (15). Intermediate **14** (8.10 g, 31.50 mmol) was dissolved in anhydrous THF (50 mL), to which was added ethyl chloroformate (10.0 mL, 97.50 mmol) dropwise at room temperature. After addition, the mixture was heated at reflux for 22 h under argon. The mixture was allowed to cool to room temperature, and the volatiles were removed in vacuo to yield a dark brown suspension. The residue was redissolved in anhydrous diethyl ether (50 mL), to which was added PBr_3 (3.1 mL, 32.50 mmol) cautiously. After being refluxed for 24 h under Ar, the mixture was chilled in ice−water. The yellow precipitate formed was filtered by suction filtration and washed with petroleum ether and a solvent mixture of CH_2Cl_2−methanol (1:1). The filter cake was dried in vacuo to give **15** (9.20 g, 95%) as a yellowish brown powder. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 11.06 (bs, 1H), 7.99 (s, 1H), 7.92 (s, 1H), 2.29 (s, 3H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 158.9, 147.0, 145.4, 139.6, 134.2, 127.3, 112.5, 86.3, 16.7. HREIMS *m/z*: $[M]^+$ calcd for $C_9H_6NO_3I$ 302.9392, found 302.9399.

2-Amino-6-iodo-8-methyl-1*H*-quinazolin-4-one (16). A mixture of **15** (8.10 g 26.70 mmol), sodium carbonate (5.00 g, 40.00 mmol), and *S*-methylisothiourea hemisulfate (4.47 g, 32.00 mmol) was dissolved in a solvent mixture of CH_3CN-H_2O (40 mL/10 mL). The mixture was then heated at reflux for 2 h and cooled to room temperature. The brown precipitate was filtered with suction filtration, and the filter cake was washed with a solution of CH_3CN-H_2O (5:1, 20 mL). As the vacuum-dried yellow solid was poorly soluble in various solvents, the crude product **16** (7.25 g, 90%) was subjected to the next step without further purification. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.98 (s, 1H), 7.70 (s, 1H), 6.63 (bs, 1H), 2.31 (s, 3H). HREIMS *m/z*: $[M]^+$ calcd for $C_9H_8N_3OI$ 300.9712, found 300.9722.

***N*-(6-Iodo-8-methyl-4-oxo-1,4-dihydroquinazolin-2-yl)-isobutyramide (17).** Isobutyric anhydride (7.0 mL, 42.21 mmol) was added to a solution of **16** (4.30 g, 14.30 mmol) in distilled pyridine (15 mL), and the solution was stirred at room temperature for 18 h. The resulting mixture was concentrated in vacuo and purified by silica column chromatography (CH_2Cl_2/EtOAc 6:1 initially, 3:1 subsequently) to give **17** (3.68 g, 69%) a yellow powder. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 8.07 (d, 1H, J = 2.0 Hz), 7.86 (d, 1H, 2.0 Hz), 2.80 (m, 1H), 2.38 (s, 3H), 1.10 (d, 3H, J = 6.8 Hz), 1.01 (d, 3H, J = 6.8 Hz). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 180.6, 177.4, 146.6, 142.8, 136.8, 131.9, 121.4, 88.8, 34.7, 34.5, 18.91, 18.8, 16.9. HREIMS *m/z*: $[M]^+$ calcd for $C_{13}H_{14}N_3O_2I$ 371.0130, found 371.0128.

{(2′*R*)-*cis*-3-[2′,3′-Dehydro-3′-(*tert*-butyldiphenylsilyloxy)]-5′-hydroxymethyl-2′-furanyl}-6-[*N*-(8-methyl-4-oxo-1,4-dihydroquinazolin-2-yl)]isobutyramide (18). Isobutyramide **17** (2.24 g, 6.04 mmol) and 1,2-dehydro-3-*O*-(*tert*-butyldiphenylsilyl)-5-hydroxymethylfuran [13] (2.56 g, 7.25 mmol) were charged into an oven-dried round-bottom flask equipped with a septum. Freshly distilled CH_3CN (50 mL) was added to the flask to form a suspension, and the mixture was bubbled with argon for 30 min. $Pd(dba)_2$ (580 mg, 0.64 mmol) and $AsPh_3$ (350 mg, 1.20 mmol) were charged into a separated round-bottom flask and suspended in freshly distilled CH_3CN (25 mL). The catalyst−ligand mixture was stirred at room temperature and degassed with argon bubbling before being transferred to the glycal−isobutyramide suspension via hypodermic syringe under inert atmosphere. Tri(*n*-butyl)amine (1.8 mL, 7.85 mmol) was added to the reaction mixture in portions. The septum-sealed flask was heated to 85−90 °C for 22 h and allowed to cool to room temperature. After the removal of the volatiles, the residue was purified with silica column chromatography (hexane/ EtOAc 2:1 initially and 1:1 subsequently) to afford **18** (1.30 g, 59%) as slightly yellow foam. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.84 (m, 3H), 7.76 (m, 2H), 7.48 (m, 6H), 7.19 (s, 1H), 5.60 (dd, 1H, J = 2.0, 1.6 Hz), 4.74 (s, 1H), 4.41 (s, 1H), 3.93 (s, 2H), 2.66 (m, 1H), 2.18 (s, 3 H), 1.29 (dd, 6H, J = 6.8, 2.4 Hz), 1.11 (s, 9H). ^{13}C NMR (DMSO-d_6, 100 MHz): δ 178.6, 160.9, 151.2, 145.6, 137.8, 135.4, 134.6, 134.2, 131.4, 131.1, 130.4, 130.3, 128.0, 128.0, 123.2, 119.4, 101.9, 84.6, 83.4, 62.8, 36.7, 26.4, 21.1, 20.7, 19.3, 19.0, 14.2, 13.5. HRFABMS *m/z*: $[M + H]^+$ calcd for $C_{34}H_{40}N_3O_5Si$ 598.2737, found 598.2720.

1′-β-{6-[*N*-(8-Methyl-4-oxo-1,4-dihydroquinazolin-2-yl)]-isobutyramide}-2′-deoxy-d-ribofuranose (19). A solution of **18** (1.07 g, 1.79 mmol) in anhydrous THF (15 mL) was chilled in an ice−water bath. Glacial acetic acid (0.6 mL) and 1 M TBAF solution in THF (2.8 mL, 2.69 mmol)) were added sequentially via hypodermic syringe. The reaction mixture was allowed to stir at 0 °C for 10 min, ammonium hydroxide (2 mL) was added to quench the reaction, and the volatiles were removed in vacuo. The intermediate was dissolved in a solution of CH_3CN (15 mL) and glacial acetic acid (3 mL). The resulting mixture was chilled to −15 °C in an ice−salt bath followed by the addition of sodium triacetoxyborohydride (0.95 g, 4.48 mmol) in one portion. The reaction mixture was allowed to stir at low temperature for 7 min and concentrated in vacuo. The residue was purified by silica column chromatography (EtOAc/methanol 25:1) to afford **19** (0.54 g, 84%) as a white powder. ^{1}H NMR (CD_3OD, 400 MHz): δ 7.85 (s, 1H), 7.52 (s, 1H), 5.12 (dd, 1H, J = 10.4, 5.2 Hz), 4.34 (m, 1H), 3.97 (m, 1H), 3.71 (d, 2H, J = 4.8 Hz), 2.72 (m, 1H), 2.38 (s, 3H), 2.23 (m, 1H), 1.96 (m, 1H), 1.23 (s, 6H). ^{13}C NMR (CD_3OD, 100 MHz): δ 182.0, 175.5, 139.8, 134.4, 122.1, 120.1, 89.3, 80.9, 74.4, 64.1, 44.8, 36.9, 20.9, 19.4, 19.4, 14.4, 13.9. HRFABMS *m/z*: $[M + H]^+$ calcd for $C_{18}H_{24}N_3O_5$ 362.1716, found 362.1700.

1′-β-6-[2-Amino-(8-methyl-4-oxo-1,4-dihydroquinazolin-2-yl)]-2′-deoxy-d-ribofuranose (2). A solution of compound **19** (0.25 g, 0.66 mmol) in 5 mL of ammonium hydroxide was heated to 55 °C overnight. The volatiles were removed in vacuo, and the residue was purified by washing with CH_2Cl_2 to afford pure free deoxyriboside dyC (**2**) (0.18 g, 95%) as a white to slightly yellow solid. ESIMS *m/z*: $[M + H]^+$ calcd for $C_{14}H_{17}N_3O_4$ 292.3, found 292.3.

Protected Phosphoramidite Derivative of Nucleoside Analogue 2. Subsequent synthetic steps and characterization are given in the Supporting Information.

Oligonucleotide Synthesis and Characterization. 5′-*O*-Tritylated phosphoramidite derivatives of **1** and **2** were prepared as described. Oligonucleotides were synthesized on DNA synthesizers using standard β-cyanoethyl phosphoramidite chemistry. The coupling time for the modified nucleoside phosphoramidites was extended from 100 to 700 s to increase the likelihood of high yields; shorter coupling times were not tested. Stepwise coupling yields for the extended bases were all greater than 98% as determined by trityl cation response. All extended oligonucleotides were DMT-off on the 5′-end and deprotected from CPG (alkyl-controlled pore glass) supports in concentrated ammonia overnight at 55 °C. They were purified by preparative 20% denaturing polyacrylamide gel electrophoresis (PAGE) and isolated by excision and elution from gel. The recovered material was subsequently quantified by absorption at 260 nm with molar extinction coefficients determined by the nearest neighbor method. All synthesized oligonucleotides were confirmed by MALDI mass spectroscopy (5′-TyTT, 5′-TyCT were analyzed with ESI-MS). Data are given in the Supporting Information.

Thermal Denaturation Studies. Solutions for the thermal denaturation studies contained the indicated concentrations of self-complementary or non-self-complementary strands containing nucleoside analogues. The buffer contained NaCl/$MgCl_2$/Na·PIPES; (100 mM/10 mM/10 mM) at pH 7.0. After the solutions were prepared they were heated to 95 °C and annealed in the UV−vis spectrophotometer equipped with a thermoprogrammer at the rate of 1.0 °C /min. Melting studies were carried out in Teflon-stoppered 1 cm path length quartz cuvettes under a steady flow of nitrogen current to prevent moisture condensation on quartz wall at low temperature. Absorbance was monitored while the temperature was raised from 5 to 95 °C or declined from 95 to 5 °C at a rate of 1 °C/min. All sequences were monitored at 260 nm except the marked ones. Computer fitting of the melting data with MeltWin software generated melting temperature (T_m), free energies (ΔG), and other thermodynamic data for the duplexes. The curve fit values were compared with those calculated from van't Hoff plots by plotting $1/T_m$ verse $\ln(C_T/4)$ and matched with good agreement.

Optical Measurements. Solutions, instruments, and methods for fluorescence and CD spectra are given in the Supporting Information.

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Supporting Information Available: Experimental details of nucleoside analogue synthesis, oligonucleotide synthesis and characterization data, and thermodynamics methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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