Zwitterionic DNA

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Abstract: As a strategy to make DNA net charge neutral, oligodeoxynucleotides bearing pyrimidine 5-ω-aminohexyl substituents have been synthesized and characterized. The resultant zwitterionic oligomers bind to natural DNA at low ionic strength as well or better than does natural DNA with itself, even when all of the nucleotides in a given single strand are rendered zwitterionic. As would be expected, stabilities of duplexes bearing zwitterionic strands are relatively insensitive to changes in solution ionic strength as compared with natural DNA. Somewhat surprising, however, is the finding that a DNA duplex composed of a fully zwitterionic strand and a natural complementary strand exhibits no change in stability over a 20-fold change in ionic strength. Thus, double- and single-stranded states in this case have equivalent charge densities, consistent with the zwitterionic strand contributing no net charge. Stabilization due to the ammonium ions was verified by comparing free energies of DNA duplexes bearing hexyl tethered ammonium ions with those bearing simply hexyl groups devoid of ammonium ions. This comparison showed that without an added positive ammonium ion, the hexyl tether itself has a severe and cumulative unfavorable effect on duplex stability. Finally, zwitterionic nucleotides are found to distinguish matched from mismatched nucleotides in complementary DNA strands to a degree that rivals natural DNA.

Introduction

Activities of biological macromolecules frequently find use outside of their natural contexts, but often such use is hampered by a shortfall in some property not selected for naturally. As a result, it is often necessary to introduce ancillary properties into natural structures by either the addition of new structural features or the conservative replacement of existing ones. These ideas can be seen in the design of antisense oligonucleotides capable of inhibiting protein translation through hybridization with mRNA.[1] Here the ability of natural DNA to bind complementary RNA is to be preserved and the ancillary properties of membrane permeability and nuclease resistance introduced.

Most efforts to design antisense oligonucleotides have sought replacements for the negatively charged phosphodiester group of the DNA backbone while leaving the remainder of the structure unchanged.[1] In connection with our ongoing investigations on the properties of nonstandard oligonucleotides,[2,3] a different design strategy is described that instead seeks to neutralize the phosphate negative charge by attaching a lipophilic cation to each nucleotide base. Base-tethered cations should displace metal ions normally neutralizing negative phosphate charges, making DNA more membrane permeable and nuclease resistant as a result of attendant changes in charge and size of monomer units. Interestingly, nature has partially implemented a design of this general type in φW-14 bacteriophage DNA where half of the thymines are replaced with positively charged α-putrescinylthymine, resulting in one positively charged base every eight nucleotides on average.[4] These and other hypermodified bacteriophage DNAs have been shown to resist a variety of endonucleases and some exonucleases as well.[4,5] While double-stranded øW-14 bacteriophage DNA itself exhibits greater thermal stability than unmodified DNA,[4b] in complete contrast, duplexes of short synthetic oligomers bearing α-putrescinylthymine have been reported much less stable than those of natural oligomers.[6] In light of this last fact and to achieve uniform DNA charge neutralization, we have undertaken an effort to evaluate a base aminoalkyl substituent of a different type.

Herein is reported the synthesis and characterization of oligodeoxynucleotides bearing ω-aminohexyl groups at the 5-position of the natural pyrimidine nucleotides. Not only do these zwitterionic nucleotides not diminish duplex formation but also in several cases they enhance its stability. Significantly, even in the full implementation of our design where every nucleotide in an oligomer is rendered zwitterionic, duplex stability is preserved. This degree of DNA major groove modification without any deleterious effect on stability is unprecedented.[7]

Results

DNA that is zwitterionic due to ω-aminohexyluridylate and -cytidylate zwitterions **1** and **2** has been prepared (Figure 1). As a control, DNA containing only the hexyl tether used in these two compounds, in the form of 5-hexyl-2′-deoxyuridylate **3**, was also prepared (Figure 1). Syntheses of the phosphoramidite precursors **16**, **17**, and **18** for oligonucleotides bearing these modified nucleotides are shown in Figure 2. ω-(Trifluoroacetyl)-aminohexynyl- and hexynylnucleosides **6**, **7**, and **8** were prepared

(1) For reviews, see: (a) Uhlmann, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 543. (b) Riordan, M. L.; Martin, J. C. *Nature* **1991**, *350*, 442.

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(3) Bain, J. D.; Switzer, C.; Chamberlin, A. R.; Benner, S. A. *Nature* **1992**, *356*, 537.

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(5) (a) Fleischman, R. A.; Campbell, J. L.; Richardson, C. C. *J. Biol. Chem.* **1976**, *251*, 1561. (b) Kaplan, D. A.; Nierlich, D. P. *J. Biol. Chem.* **1975**, *250*, 2395.

(6) Takeda, T.; Ikeda, K.; Mizuno, Y.; Ueda, T. *Chem. Pharm. Bull.* **1987**, *35*, 3558.

(7) Oligodeoxynucleotides bearing base tethered ω-amino groups have been prepared routinely in the past to attach dyes, reactive groups, and other macromolecules to DNA, usually where one such group is introduced per oligonucleotide chain but never where all of the bases in a DNA strand bears such a modifying group without any intervening unmodified bases. See, for example: (a) Povsic, T. J.; Strobel, S. A.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 5934. (b) MacMillan, A. M.; Verdine, G. L. *Tetrahedron* **1991**, *47*, 2603. (c) Telser, J.; Cruickshank, K. A.; Morrison, L. E.; Netzel, T. L.; Chan, C. *J. Am. Chem. Soc.* **1989**, *111*, 7226. (d) Telser, J.; Cruickshank, K. A.; Schanze, K. S.; Netzel, T. L. *J. Am. Chem. Soc.* **1989**, *111*, 7221. (e) Allen, D. J.; Darke, P. L.; Benkovic, S. J. *Biochemistry* **1989**, *28*, 4601. (f) Gebeyehu, G.; Rao, P. Y.; SooChan, P.; Simms, D. A.; Klevan, L. *Nucleic Acids Res.* **1987**, *15*, 4513. (g) Haralambidis, J.; Chai, M.; Tregear, G. W. *Nucleic Acids Res.* **1987**, *15*, 4857. (h) Jablonski, E.; Moomaw, E. W.; Tullis, R. H.; Ruth, J. L. *Nucleic Acids Res.* **1986**, *14*, 6115. (i) Smith, L. M.; Fung, S.; Hunkapiller, M. W.; Hunkapiller, T. J.; Hood, L. E. *Nucleic Acids Res.* **1985**, *13*, 2399. (j) Bodnar, J. W.; Zempsky, W.; Warder, D.; Bergson, C.; Ward, D. C. *J. Biol. Chem.* **1983**, *258*, 15206.

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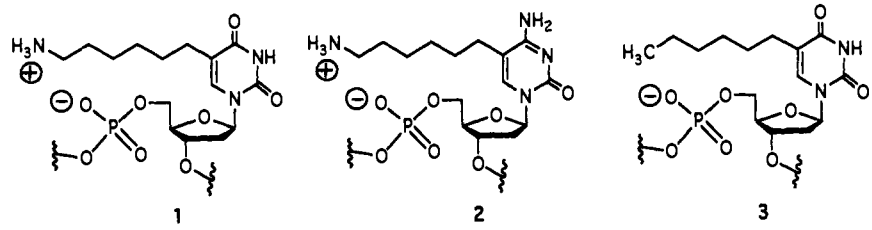

Figure 1

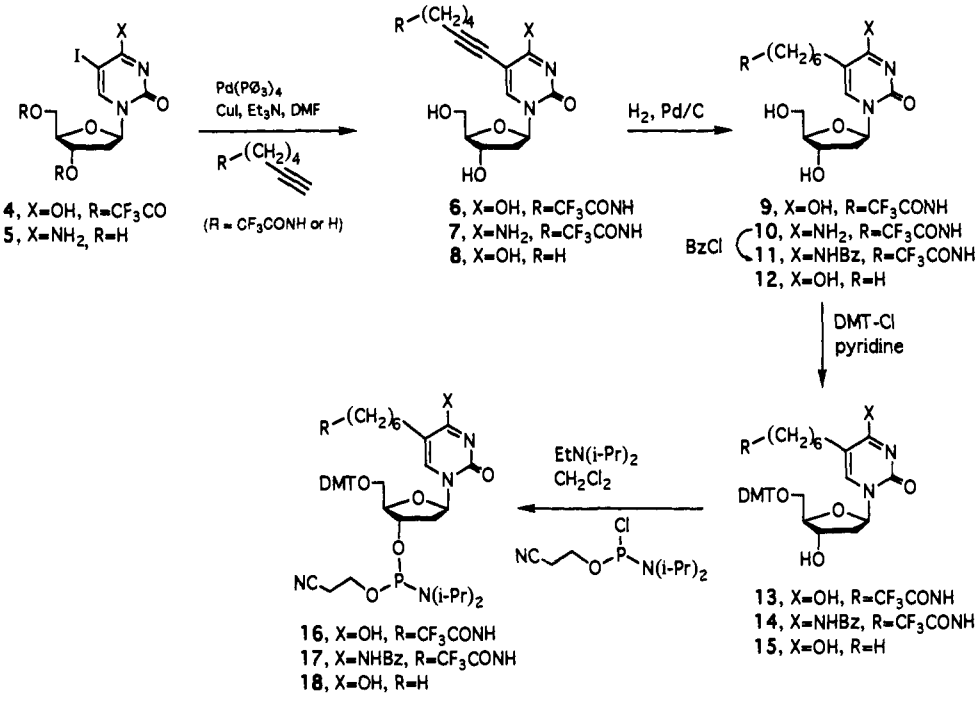

Figure 2

using the palladium(0) coupling procedure described by Hobbs.[8] In the syntheses of **6** and **8**, transient protection of the 3′- and 5′-hydroxyl groups of 5-iododeoxyuridine as trifluoroacetyl esters was done to reduce the formation of side products[9] observed during the palladium coupling reaction. The remaining steps proceeded by conventional means.

Dodecanucleotides **20–27** (Table I) containing **1**, **2**, and **3** were prepared from appropriately protected phosphoramidites using an automated DNA synthesizer. All oligomers were characterized by digestion[10] to constituent bases followed by HPLC analysis, 5′-end labeling followed by PAGE, and also by laser desorption mass spectrometry.

Gel electrophoretic mobilities (PAGE mobility) of the pure oligomers, their duplex melting temperatures and free energies with complementary natural DNA under low (50 mM NaCl) and high (1 M NaCl) ionic strength conditions, and their duplex melting temperatures with mismatched natural DNA under low ionic strength conditions are summarized in Table I. Duplex free energy values were determined in two different ways. In all cases where a value is reported, one of the values was derived 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.[11] This procedure also gave the T_m values. The second method, which was used only in six cases, involved plots of $1/T_m$ versus the natural logarithm of the total oligonucleotide concentration.[11,12] An example of such a plot is shown in Figure 3. As can be seen in Table I, there is excellent agreement in general between the free energy values derived by the two different methods. Comparison of the $\Delta H°$ values derived from these two procedures has been used in the past to determine whether two-state melting behavior is obeyed, where a 15% difference serves as an approximate limit.[11] With this definition, two-state behavior is observed in five of the six instances where $\Delta H°$ from the two methods is available ($\Delta H°$ derived by the nonlinear regression procedure not shown). Only in the duplex incorporating oligonucleotide **27** was a deviation from two-state behavior observed. Nevertheless, even in this latter case reasonable agreement is still seen in the $\Delta G°$ values obtained from nonlinear regression fitting of melting curves and plots of $1/T_m$ versus concentration.

Gel electrophoretic data summarized in Table I show an expected correspondence between the degree of zwitterionic nucleotide substitution and electrophoretic mobility. Thus, completely zwitterionic oligomer **24** migrates only a small fraction of the distance traveled by partially zwitterionic oligomers **20–23**, and the zwitterionic oligomers as a group migrate to less of an extent than the nonzwitterionic oligomers **19** and **25–27**. The fact that the fully zwitterionic oligomer travels any distance at all may be ascribed to the relatively high pH conditions of the electrophoresis (pH 8.5–9). The observation that oligomers **25** and **27**, containing one and four 5-hexyldeoxyuridylates, respectively, migrate a distance greatly disproportionate to their zwitterionic counterparts **20** and **22** clearly shows that neutralization of the phosphate charge through the accumulation of tethered ammonium ions is primarily responsible for the mobility trends observed in the zwitterionic oligomers rather than an increase in size.

Duplexes of zwitterionic oligomers **20–24** with complementary DNA exhibit approximately equal or greater negative free energy

(8) Hobbs, F. W. *J. Org. Chem.* **1989**, *54*, 3420.
(9) Robins, M. J.; Barr, P. J. *J. Org. Chem.* **1983**, *48*, 1854.
(10) Adams, R. L. P.; McKay, E. L.; Craig, L. M.; Burdon, R. H. *Biochim. Biophys. Acta* **1979**, *563*, 72.
(11) Longfellow, C. E.; Kierzek, R.; Turner, D. H. *Biochemistry* **1990**, *29*, 278.
(12) Marky, L. A.; Breslauer, K. J. *Biopolymers* **1987**, *26*, 1601.

Table I

oligodeoxynucleotide[a]	PAGE mobility[b]	50 mM NaCl[c] $-\Delta H°$ (kcal/mol)	$-\Delta S°$ (eu)	$-\Delta G°_{37}$ (kcal/mol)	$-\Delta G°_{37}$[f] (kcal/mol)	T_m (°C)	T_m (°C) A-mismatch[g]	T_m (°C) G-mismatch[h]	1 M NaCl[d] T_m (°C)	$-\Delta G°_{37}$[f] (kcal/mol)	$-\Delta\Delta G°_{37}$[f] (kcal/mol)
dCTTTCTCTCCCT (**19**)	21.9	83.9	241.0	9.1	9.0	39.4	20.9	32.0	53.3	12.5	3.5
dCTTTCTCTCCCT (**20**)	20.8				8.9	39.0			50.6	11.7	2.8
dCTTTCTCTCCCT (**21**)	20.8				9.9	42.8			54.5	12.8	2.9
dCTTTCTCTCCCT (**22**)	16.6	81.9	234.9	9.0	8.9	39.1		32.7	45.0	10.3	1.4
dCTTTCTCTCCCT (**23**)	16.2	84.5	238.2	10.6	10.6	45.4	26.0		54.1	12.7	2.1
dCTTTCTCTCCCT (**24**)	3.8	82.6	235.2	9.6	9.4	41.5	21.0	36.9	41.5	9.4	0
					9.2	40.8[i]					
dCTTTCtCTCCCT (**25**)	21.6				7.8	34.9			49.0	11.6	3.8
dCTTTCtCtCCCT (**26**)	21.4	75.3	221.0	6.7	6.6	30.3			44.6	10.4	3.8
dCtTtCtCtCCCT (**27**)	20.8	67.0	199.1	5.2	4.2	24.2			36.1	8.2	4.0
dCTTTCTCTCCCT (**19**)					9.1	39.7[j]					
dCTTTCTCTCCCT (**19**)					9.6	41.5[k]					
dCTTTCTCTCCCT (**19**)					9.2	39.9[l]					

[a] "T" and "C" are **1** and **2**, respectively. "t" corresponds to **3**. [b] Polyacrylamide gel electrophoretic mobility is given in cm from the origin of the gel. The samples were electrophoresed in a 20% polyacrylamide, 7 M urea denaturing gel using Tris-Borate-EDTA buffer at pH 8.5–9.0. [c] Sample contained 50 mM NaCl, 10 mM sodium phosphate, 0.1 mM EDTA, 2.5 μM 5′-dAGGGAGAGAAAG, and 2.5 μM of **19–27** in H_2O at pH 7. [d] Same conditions as *c* except 1 M NaCl was used in place of 50 mM NaCl. [e] Derived from plot of $1/T_m$ versus ln C_T, errors in $\Delta H°$ and $\Delta S°$ are estimated as ±10%. [f] Derived from the average fitted parameters of melting curves from two different samples, errors in $\Delta H°$ and $\Delta S°$ (from which $\Delta G°$ is calculated) are estimated as ±10%. [g] The following oligomer containing an A-mismatch was used in place of the complementary one: 5′-dAGGGAAAGAAAG. [h] The following oligomer containing a G-mismatch was used in place of the complementary one: 5′-dAGGGAGGGAAAG. [i] Performed at pH 5.5. [j] Performed in the presence of 13.8 μM putrescine-2HCl.[21] [k] Performed in the presence of 1.0 mM putrescine-2HCl.[21] [l] Performed in the presence of 1.0 mM ethylamine-HCl.

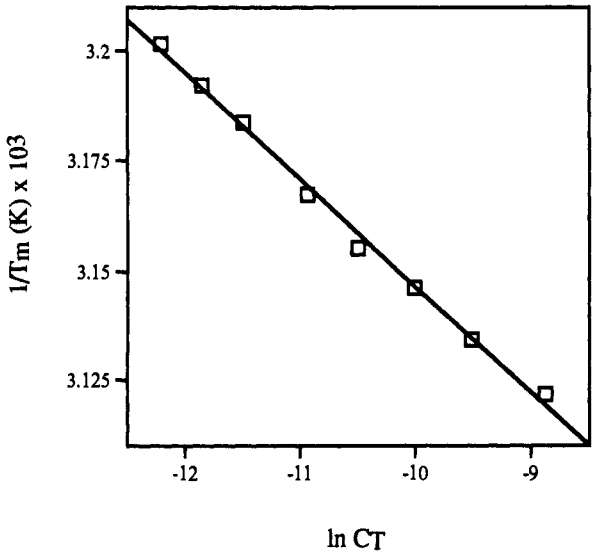

Figure 3. Example of a plot of $1/T_m \times 10^3$ versus ln C_T for 5′-dCTTTCTCTCCCT (**22**). Similar plots were obtained for oligonucleotides **19**, **23**, **24**, **26**, and **27**.

values than the corresponding natural oligomer **19** in low ionic strength buffer at pH 7 (Table I). Under these conditions, uridylate zwitterion **1** is neither stabilizing nor destabilizing in duplexes of oligomers **20** and **22**, whereas cytidylate zwitterion **2** has a marked stabilizing effect in duplexes of oligomers **21** and **23**. These trends are altered only somewhat when **1** and **2** are combined to give fully zwitterionic oligomer **24**. Here the observed negative free energy of the duplex is still slightly greater than that of natural oligomer **19**. Importantly, duplex stability of fully zwitterionic oligomer **24** remains essentially unaltered when the buffer is changed from pH 7 to pH 5.5 (Table I), providing evidence that all of the amino groups are in their protonated forms at pH 7.

Enthalpic and entropic contributions to duplex stabilities where one of the strands bears zwitterionic nucleotides are not appreciably different from those of the natural, reference duplex (Table I). Negative enthalpy values for duplexes incorporating zwitterionic oligomers **22** and **24** are slightly less favorable than for the natural duplex derived from **19**, whereas the negative enthalpy term is slightly more favorable for the duplex derived from **23** bearing four cytidylate zwitterions. However, without exception, negative entropy values of duplexes derived from zwitterionic oligomers **22–24** are slightly more favorable than that of the natural parent. The net result in all cases is that duplexes wherein one strand is one of the zwitterionic oligomers **22–24** are of equal or greater thermodynamic stability than the unmodified parent duplex. In general, it is somewhat astonishing that grossly nonlinear behavior is not encountered in duplex stability due to neighbor effects when zwitterion **1** and zwitterion **2** are combined to give fully zwitterionic oligomer **24**.

The importance of the ammonium ions in stabilizing duplexes of zwitterionic oligomers **20–24** at low ionic strength can be seen from a comparison of free energies for these duplexes bearing hexyl tethered ammonium ions with those containing oligomers **25–27** bearing hexyl groups alone, without ammonium ions. From this comparison it is clear that without a positively charged ammonium ion, the hexyl tether itself severely destabilizes duplex structure. Further, destabilization is cumulative where each addition of a hexyluridylate diminishes stability by ~1.2 kcal/mol. When viewed against these results, the fact that neither zwitterion **1** nor **2** has an unfavorable effect on duplex stability is remarkable.

The tethered ammonium ions clearly have a substantial electrostatic influence as a 20-fold change in buffer ionic strength (0.05–1 M NaCl) has no effect on the stability of the duplex between fully zwitterionic oligomer **24** and its natural DNA complement ($-\Delta\Delta G°_{37} = 0$, Table I), whereas the same shift in ionic strength results in a ~4 kcal/mol increase in the duplex stabilities of the unmodified oligomer **19** and the hexyluridylate modified oligomers **25–27** ($-\Delta\Delta G°_{37} = 3.5$–$4.0$ kcal/mol, Table I).[13] Changes in stability intermediate between the above extremes are observed for duplexes of the partially zwitterionic oligomers **20–23** ($-\Delta\Delta G°_{37} = 1.4$–$2.9$ kcal/mol, Table I). Significantly, the complete insensitivity to solution ionic strength

(13) A plot of T_m versus log [Na^+] for the duplex of natural oligomer **19** using the two sodium concentrations given in Table I yielded a $dT_m/d(\log [Na^+])$ value of 10.7 (contributions to [Na^+] from the 10 mM sodium phosphate buffer were ignored). It is unlikely that a comparison of this value with those derived from more extensive data for polynucleotides and oligonucleotides obtained at low Na^+ concentrations would be meaningful since $dT_m/d(\log [Na^+])$ is generally found to be linear only in the range of [Na^+] from 10^{-3} to 10^{-1} M. Even in cases where linearity is known to extend beyond this range, the effect of sodium ions on T_m is saturated well before 1 M Na^+. See: (a) Record, M. T.; Woodbury, C. P.; Lohman, T. M. *Biopolymers* **1976**, *15*, 893. (b) Gruenwedel, D. W.; Hsu, C.-H. *Biopolymers* **1969**, *7*, 557.

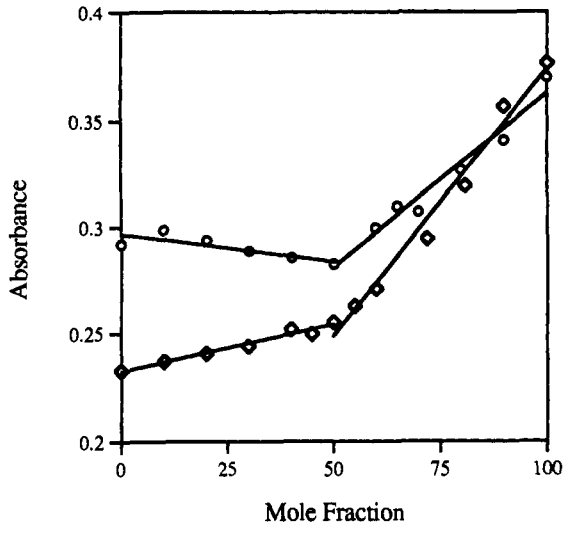

Figure 4. UV mixing curves of 5'-dCTTTCTCTCCCT (**19**) [○] and 5'-dCTTTCTCTCCCT (**24**) [◇] with the complementary oligomer 5'-dAGGGAGAGAAAG in 50 mM NaCl, 10 mM sodium phosphate, and 0.1 mM EDTA at pH 7. The UV absorbance at 260 nm for the mole fraction of dAGGGAGAGAAAG relative to **19** and **24** was monitored (100 = pure complementary oligomer, 0 = pure **19** or **24**).

found for stability of the duplex between fully zwitterionic oligomer **24** and its natural DNA complement suggests that there is no difference in the charge density between the double- and single-stranded states for this particular case and is consistent with the zwitterionic strand contributing no net charge to the complex.

Zwitterionic nucleotides are found to distinguish matched from mismatched bases in complementary DNA to a degree that is comparable with natural DNA. Thus, zwitterionic oligomers **22–24** give depressed T_m values that nearly mirror those of natural oligomer **19** when zwitterions **1** or **2** are opposed by a G or an A mismatch in an otherwise complementary DNA strand (after accounting for differential duplex stabilities in the nonmismatched cases, Table I). Interestingly, in all cases a cytidylate zwitterion **2**/dA mismatch is somewhat more unfavorable (ΔT_m = 19.4 and 20.5 °C), while a uridylate zwitterion **1**/dG mismatch is somewhat less unfavorable (ΔT_m = 6.4 and 4.6 °C) than seen for the natural dC/dA (ΔT_m = 18.5 °C) and natural T/dG (ΔT_m = 7.4 °C) mismatches, respectively.

The final three entries in Table I address the effect an external ammonium ion has on the duplex stability of natural oligomer **19** and are based on a referee suggestion. In the presence of the divalent ammonium ion from putrescine, no duplex stabilization is observed with an ammonium ion to phosphate ion ratio of 1:2 (13.8 μM putrescine-2HCl)—the same ratio present in the duplex of zwitterionic oligomer **24**. However, when the putrescine concentration is increased to 1 mM—where the ammonium ion to phosphate ion ratio is 36:1—the duplex of **19** is stabilized to approximately the same extent as the duplex of fully zwitterionic oligomer **24**. In comparison, the monovalent ammonium ion from ethylamine, arguably a more relevant control, leads to little duplex stabilization for the natural oligomer **19** at a 1 mM concentration.

The stoichiometry for the complex of **24** with its DNA complement was determined as 1:1 from a UV mixing curve (Figure 4).[14] Several UV absorbance versus temperature profiles from which T_m and thermodynamic data were derived for duplexes of zwitterionic oligomers **22–24** and natural oligomer **19** are presented in Figure 5. It may be seen that the curves for zwitterionic oligomers are identical in shape with that of the corresponding natural oligomer.

(14) Felsenfeld, G.; Rich, A. *Biochim. Biophys. Acta* **1957**, *26*, 457.

Discussion

It is interesting to speculate about the basis for the observed behaviors of zwitterionic nucleotides **1** and **2** in duplexes at low ionic strengths. The observation that uridylate zwitterion **1** has no effect on duplex stability is the apparent sum of two opposing influences, one destabilizing from the tether itself and the other stabilizing due to the positive ammonium ion, and the outcome as the sum of these two evenly balanced effects is observed as no net change. This is seen by comparing free energies of duplex formation for oligomer **22** bearing four uridylate zwitterions **1**, oligomer **27** bearing four hexyluridylates **3** in the same positions, and unmodified natural oligomer **19** (Table I). These effects translate into a ~1 kcal/mol destabilization in the duplex $-\Delta G°_{37}$ per hexyl group in **27** relative to **19** and ~1 kcal/mol stabilization in the duplex $-\Delta G°_{37}$ per ammonium group in **22** relative to **27**. The net result being that **22** has the same value of $-\Delta G°_{37}$ as **19**. Duplex destabilization by the hexyl tether might be due to van der Waals strain or a disruption of the hydration of the bases, each effect manifested in the major groove of the double helix. Duplex stabilization by the ammonium ions is consistent with some combination of more effective charge neutralization by this group than by the normal complement of free counterions in the double-stranded state and binding interactions with inter- or intrastrand functional groups. The above picture should also apply to the case of cytidylate zwitterion **2**, except that either the ammonium ion is slightly more stabilizing or the hexyl substituent slightly less destabilizing than seen in the case of **1** to account for the greater duplex stability with oligomer **23** relative to **19**. The structural basis for the observed differences in behavior between **1** and **2** is not clear.

Polyelectrolyte ion condensation theory[15] provides a framework for understanding the electrostatic effects on the oligomers. This theory considers a polyelectrolyte in terms of its average linear charge density and makes two predictions. First, it predicts equivalent intrastrand phosphate distances for the natural oligonucleotide complement to oligomer **24** in both double-stranded and single-stranded states, as a consequence of the lack of a duplex stability dependence on salt concentration (Table I).[16] The second prediction is a reduction or elimination of an entropic contribution to duplex destabilization present in natural oligonucleotides. Namely, during the denaturation of a natural DNA duplex to single strands, a portion of the counterions bound territorially to DNA are lost to the bulk solvent due to an attendant reduction in charge density. In contrast, during denaturation of a DNA duplex in which one of the strands is zwitterionic, either no counterions should be lost (predicted for the duplex of **24**), or only a fraction of the counterions should be lost compared to the natural case, since charge densities of double-stranded and single-stranded states are balanced due to the (nondiffusible) tethered ammonium ions.[15,16] Such a process if operative would amount to a novel stabilization mechanism accessed uniquely through intramolecular phosphate neutralization.

Some analogies may be drawn between the well-known interactions of biogenic polyamines with DNA and the interactions of tethered amines in the present case. While extensive studies have been made of putrescine, spermidine, and spermine association with DNA duplexes,[17] there is no general agreement about the principal site for polyamine binding. However, recent experiments support polyamine/minor groove interaction for B-DNA and major groove interaction for A-DNA.[17c] With respect to major groove association, very different modes of polyamine/DNA interaction are apparent in X-ray crystal structures of two different sequences of duplex DNA with a single bound spermine.[17g,k] In one example spermine bridges two

(15) (a) Manning, G. S. *Acc. Chem. Res.* **1979**, *12*, 443. (b) Manning, G. S. *Q. Rev. Biophys.* **1978**, *2*, 179.

(16) This follows in analogy with previous work accounting for the behavior of titrated polynucleotides and higher order (e.g., triplex) complexes. See ref 13a.

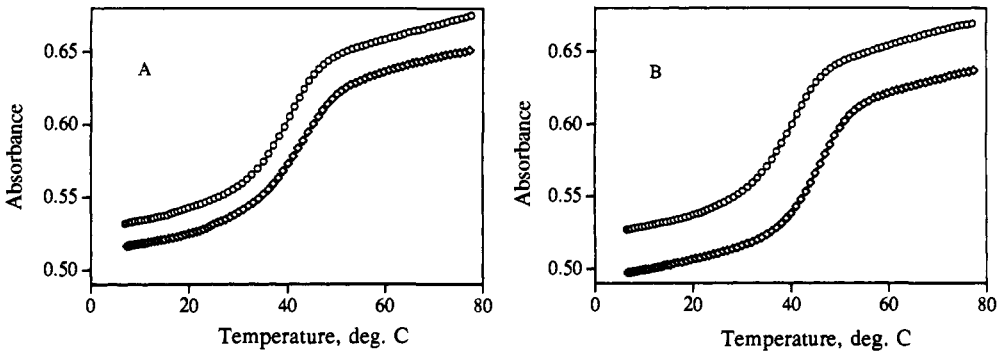

Figure 5. (A) UV absorbance profiles versus temperature for 5′-dCTTTCTCTCCCT (**19**) [○] and 5′-dCTTTCTCTCCCT (**24**) [◇] in the presence of complementary 5′-dAGGGAGAGAAAG, and (B) UV absorbance profiles versus temperature for 5′-dCTTTCTCTCCCT (**22**) [○] and 5′-dCTTTCTCTCCCT (**23**) [◇], also in the presence of 5′-dAGGGAGAGAAAG. T and C are **1** and **2**, respectively. Experimental conditions in all cases were as noted in the legend of Table I, note *c*.

interstrand phosphates across the major groove,[17k] whereas in the other binding occurs exclusively to bases along the floor of the major groove.[17g] For hexyl tethered amines we cannot rule out one or the other of these types of major groove interactions—ammonium ion/phosphate ion or ammonium ion/base—as even the latter sort might be anticipated to have a sizable electrostatic component.

Summary

Replacement of the natural pyrimidine nucleotides with unnatural ω-aminohexyl, zwitterionic ones leads to oligodeoxynucleotides with diminished charge but undiminished ability to complex with DNA at low ionic strengths. Our findings underscore that appreciable changes in DNA primary structure do not necessarily manifest themselves unfavorably in its secondary structure. The fact that DNA can be made fully zwitterionic by introducing tethered ammonium ions without affecting duplex formation could have important implications for enhancing biological activity of oligonucleotides in vivo, and we are actively investigating this possibility.

Experimental Section

General Methods. All air sensitive reactions were carried out under N_2 or Ar. Melting points were taken on a Thomas-Hoover Uni-melt capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a GE QE-300 (300 MHz) spectrometer. ^{13}C NMR spectra were recorded on either a GE QE-300 (75 MHz) or GN-500 (125 MHz) spectrometer. ^{31}P NMR spectra were recorded on a GE GN-500 (202 MHz, H_3PO_4 external standard) spectrometer. Infrared spectra were taken on a Nicolet 5-DX FT-IR spectrophotometer. Ultraviolet-visible spectra were recorded on a Hewlett-Packard 8452A diode-array spectrophotometer. Elemental analyses were carried out at Desert Analytics Organic Microanalysis Laboratory (Tucson, AZ). Mass spectra and high-resolution mass spectra were recorded on a VG-ZAB-2FHF mass spectrometer using FAB ionization at the Southern California Regional Mass Spectrometry Facility (University of California, Riverside). Laser desorption mass spectra were recorded on a Finnegan Lasermat mass spectrometer at the Biotechnology Instrumentation Facility (University of California, Riverside). Chemicals were purchased from either Sigma or Aldrich Chemical Companies. Solvents were purchased from Fisher Scientific. AG1X8 (–OH) anion exchange resin was purchased from Biorad. T4 polynucleotide kinase was purchased from Boehringer Mannheim. 5′-(γ-^{32}P)ATP (~3000 Ci/mmol) was purchased from Amersham. Controlled pore glass support was purchased from CPG, Inc. (Fairfield, NJ). Tetrakis(triphenylphosphine)palladium(0) was prepared by a literature method.[18] Pyridine and CH_2Cl_2 were distilled from CaH_2 and stored over molecular sieves. DMF was stored over molecular sieves prior to use.

Synthesis. 5-(6-*N*-(Trifluoroacetyl)amino-1-hexynyl)-2′-deoxyuridine (6). To a suspension of 5-iodo-2′-deoxyuridine (650 mg, 1.84 mmol) in CH_2Cl_2 (7.0 mL) was added trifluoroacetic anhydride (2.35 mL, 16.6 mmol) at room temperature. The mixture was stirred overnight. After concentration of the mixture, the residue was dried in vacuo at room temperature to give as a solid foam 1.30 g of 3′,5′-di-*O*-trifluoroacetyl-5-iodo-2′-deoxyuridine **4**.

To a mixture of **4**, *N*-trifluoroacetyl-1-amino-5-hexyne[19] (1.066 g, 5.52 mmol), tetrakis(triphenylphosphine)palladium(0) (150 mg, 0.13 mmol), and copper(I) iodide (50 mg, 0.26 mmol) were added dry DMF (10 mL) and Et_3N (0.768 mL, 5.52 mmol). The mixture was stirred at room temperature for 24 h and then concentrated under a vacuum. The residue was purified by flash chromatography (SiO_2, MeOH (5–10%)/CH_2Cl_2). The fraction containing **6** was concentrated and treated with anion exchange resin AG1X8 (HCO_3^-, 1.58 g, 3.0 equiv) in 5 mL of 1/1, CH_2Cl_2/MeOH at room temperature for 30 min. Evaporation of the solvent gave **6**: white solid, 605 mg, 78% yield; mp 154–157 °C (MeOH/Et_2O); ^{1}H NMR (DMSO-d_6) δ 1.48 (m, 2 H), 1.58 (m, 2 H), 2.10 (m, 2 H), 2.38 (t, 2 H, J = 6.9 Hz), 3.20 (m, 2 H), 3.57 (m, 2 H), 3.77 (m, 1 H), 4.21 (m, 1 H), 5.08 (t, 1 H, J = 4.6 Hz), 5.24 (d, 1 H, J = 3.8 Hz), 6.10 (t, 1 H, J = 6.6 Hz), 8.10 (s, 1 H), 9.43 (br s, 1 H), 11.56 (br s, 1 H); ^{13}C NMR (DMSO-d_6) δ 18.61, 25.73, 27.73, 38.87, 40.20, 61.20, 70.38, 73.30, 84.77, 87.75, 93.06, 99.16, 116.19 (CF_3, J_{C-F} = 288.9 Hz), 142.96, 149.68, 156.41, ($COCF_3$, J_{C-F} = 36.4 Hz), 162.00; UV (MeOH) nm (ε × 10^3) 230 (11.9), 292 (12.3); IR (KBr) cm^{-1} 3521, 3407, 3320, 2239, 1697, 1285, 1214, 1195, 1032; MS (FAB$^+$) m/z 420 (MH^+), 330, 304; HRMS (FAB$^+$) calcd for $C_{17}H_{21}N_3O_6F_3$ 420.1382 (MH^+), found 420.1382. Anal. Calcd for $C_{17}H_{20}N_3O_6F_3$: C, 48.69; H, 4.81; N, 10.02. Found: C, 48.51; H, 4.73; N, 9.92.

5-(6-*N*-(Trifluoroacetyl)amino-1-hexynyl)-2′-deoxycytidine (7). To 5-iodo-2′-deoxycytidine (**5**) (1.496 g, 4.24 mmol), tetrakis(triphenylphosphine)palladium(0) (490 mg, 0.42 mmol), copper(I) iodide (163 mg, 0.86 mmol), and *N*-trifluoroacetyl-1-amino-5-hexyne[19] (2.455 g, 12.7 mmol) were added DMF (21 mL) and Et_3N (1.2 mL, 8.63 mmol). After stirring for 12 h, AG-1X8 anion exchange resin (HCO_3^- form, 3.0 equiv), 20 mL of MeOH, and 20 mL of CH_2Cl_2 were added, and the suspension stirred for 1 h. The reaction was filtered through a sintered glass funnel, and the DMF removed in vacuo. Flash chromatography (SiO_2, MeOH (10–20%)/CH_2Cl_2) provided 7:1.389 g, 78% yield; mp 146–148 °C dec (EtOAc); ^{1}H NMR (DMSO-d_6) δ 1.55 (m, 4 H), 1.96 (m, 1 H), 2.11 (ddd, 1 H, J = 3.5, 5.7, 13.0 Hz), 2.41 (t, 2 H, J = 6.5 Hz), 3.20 (m, 2 H), 3.56 (m, 2 H), 3.77 (m, 1 H), 4.18 (m, 1 H), 5.03 (t, 1 H, J = 5.0 Hz), 5.18 (d, 1 H, J = 4.1 Hz), 6.10 (t, 1 H, J = 6.5 Hz), 6.71 (br s, 1 H), 7.67 (br s, 1 H), 8.05 (s, 1 H), 9.42 (m, 1 H); ^{13}C NMR (DMSO-

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d_6) δ 18.67, 25.26, 27.61, 38.73, 40.73, 61.06, 70.18, 72.27, 85.26, 87.41, 90.40, 95.31, 115.99 (CF_3, J_{C-F} = 288.6 Hz), 143.55, 153.52, 156.21 ($COCF_3$, J_{C-F} = 35.6 Hz), 164.41; UV (H_2O) nm (ε × 10^3) 298 (8.1), 236 (15.7), 208 (25.8); IR (KBr) cm^{-1} 3441, 3275, 1706, 1636, 1506, 1176, 1094; MS (FAB+) m/z 419 (MH^+); HRMS (FAB+) calcd for $C_{17}H_{22}F_3N_4O_5$ 419.1542 (MH^+), found 419.1558. Anal. Calcd for $C_{17}H_{21}F_3N_4O_5$: C, 48.81; H, 5.06; N, 13.39. Found: C, 48.46; H, 4.98; N, 13.32.

5-(6-*N*-(Trifluoroacetyl)aminohexyl)-2′-deoxyuridine (9). A mixture of **6** (480 mg, 1.14 mmol) and 10% Pd/C (95 mg) in MeOH (6 mL) was stirred under H_2 pressure (50 psi) at room temperature for 3 days. The mixture was filtered through Celite and concentrated to give **9**: 475 mg, 98% yield; mp 136–140 °C (Et_2O); ^1H NMR (DMSO-d_6) δ 1.2–1.3 (m, 4 H), 1.3–1.5 (m, 4 H), 2.07 (m, 2 H), 2.16 (m, 2 H), 3.15 (t, 2 H, J = 6.9 Hz), 3.56 (m, 2 H), 3.76 (m, 1 H), 4.23 (br s, 1 H), 5.02 (br s, 1 H), 5.23 (br s, 1 H), 6.16 (t, 1 H, J = 6.8 Hz), 7.68 (s, 1 H), 9.39 (br s, 1 H), 11.22 (br s, 1 H); ^{13}C NMR (DMSO-d_6) δ 26.14, 26.44, 28.07, 28.40, 28.40, 39.56, 39.98, 61.49, 70.65, 84.10, 87.51, 113.76, 116.20 (CF_3, J_{C-F} = 288.5 Hz), 136.33, 150.60, 156.33 ($COCF_3$, J_{C-F} = 35.6 Hz), 163.68; UV (MeOH) nm (ε×10^3) 212 (15.9), 268 (13.6); IR (KBr) cm^{-1} 3552, 3384, 3328, 1701, 1684, 1559, 1203, 1180, 1170; MS (FAB+) m/z 424 (MH^+); HRMS (FAB+) calcd for $C_{17}H_{25}N_3O_6F_3$ 424.1695 (MH^+), found 424.1706. Anal. Calcd for $C_{17}H_{24}N_3O_6F_3$: C, 48.23; H, 5.71; N, 9.92. Found: C, 48.19; H, 5.68; N, 10.00.

5-(6-*N*-(Trifluoroacetyl)aminohexyl)-2′-deoxycytidine (10). A mixture of **7** (1.379 g, 3.30 mmol) and 10% Pd/C (70 mg) in MeOH (6.6 mL) was stirred under H_2 pressure (50 psi). After 20 h the mixture was filtered through Celite and concentrated. Flash chromatography (SiO_2, MeOH (20%)/CH_2Cl_2) gave **10**: 1.235 g, 89% yield; mp 126–129 °C (MeOH/CH_3CN); ^1H NMR (DMSO-d_6) δ 1.21–1.33 (m, 4 H), 1.33–1.52 (m, 4 H), 1.95 (m, 1 H), 2.06 (ddd, 1 H, J = 3.3, 5.9, 13.0 Hz), 2.20 (t, 2 H, J = 7.1 Hz), 3.15 (m, 2 H), 3.54 (m, 2 H), 3.74 (m, 1 H), 4.20 (m, 1 H), 4.97 (t, 1 H, J = 5.0 Hz), 5.16 (d, 1 H, J = 4.1 Hz), 6.15 (t, 1 H, J = 6.7 Hz), 6.63–6.99 (br s, 1 H), 6.99–7.35 (br s, 1 H), 7.60 (s, 1 H), 9.38 (m, 1 H); ^{13}C NMR (DMSO-d_6) δ 26.01, 26.59, 27.77, 28.12, 28.24, 39.18, 40.32, 61.32, 70.39, 84.77, 87.12, 105.40, 118.28 (CF_3, J_{C-F} = 288.4 Hz), 138.10, 154.91, 156.11 ($COCF_3$, J_{C-F} = 35.6 Hz), 164.71; UV (H_2O) nm (ε ×10^3) 280 (7.9); IR (KBr) cm^{-1} 3423, 3309, 1713, 1668, 1615, 1493, 1187, 1155, 1101; MS (FAB+) m/z 423 (MH^+); HRMS (FAB+) calcd for $C_{17}H_{26}F_3N_4O_5$ 423.1855 (MH^+), found 423.1878. Anal. Calcd for $C_{17}H_{25}F_3N_4O_5$: C, 48.34; H, 5.97; N, 13.26. Found: C, 48.21; H, 5.77; N, 13.19.

4-*N*-Benzoyl-5-(6-*N*-(trifluoroacetyl)aminohexyl)-2′-deoxycytidine (11). To a mixture of **10** (1.133 g, 2.68 mmol) and benzoic anhydride (618 mg, 2.73 mmol) was added pyridine (18 mL). The mixture was stirred for 3 h, and 20 mL of MeOH was then added. The mixture was concentrated and flash chromatographed (SiO_2, MeOH (5%)/CH_2Cl_2) to give **11**: 960 mg, 70% yield: mp 176–177 °C (MeOH); ^1H NMR (DMSO-d_6) δ 1.20–1.40 (m, 4 H), 1.48 (m, 2 H), 1.58 (m, 2 H), 2.18 (m, 2 H), 2.46 (t, 2 H, J = 7.7 Hz), 3.16 (m, 2 H), 3.61 (m, 2 H), 3.83 (m, 1 H), 4.27 (m, 1 H), 5.13 (t, 1 H, J = 4.8 Hz), 5.29 (d, 1 H, J = 4.2 Hz), 6.16 (t, 1 H, J = 6.4 Hz), 7.48 (m, 2 H), 7.58 (t, 1 H, J = 7.0 Hz), 8.09 (s, 1 H), 8.15 (d, 2 H, J = 7.4 Hz), 9.39 (m, 1 H), 12.75–13.25 (br s, 1 H); ^{13}C NMR (DMSO-d_6) δ 25.93, 26.82, 27.93, 28.11, 28.19, 39.12, 40.06, 60.88, 69.94, 85.12, 87.73, 113.89, 115.97 (CF_3, J_{C-F} = 288.3 Hz), 128.26, 129.22, 132.44, 136.86, 139.08, 147.23, 156.09 ($COCF_3$, J_{C-F} = 37.1 Hz), 159.11, 178.19; UV (MeOH) nm (ε ×10^3) 332 (24.1), 262 (9.4); IR (KBr) cm^{-1} 3520, 3295, 1699, 1681, 1559, 1279, 1182, 1099; MS (FAB+) m/z 527 (MH^+); HRMS (FAB+) calcd for $C_{24}H_{30}F_3N_4O_6$ 527.2117 (MH^+), found 527.2101. Anal. Calcd for $C_{24}H_{29}F_3N_4O_6$: C, 54.75; H, 5.55; N, 10.64. Found: C, 54.61; H, 5.54; N, 10.40.

5-Hexyl-2′-deoxyuridine (12). To a mixture of **4** (1.257g, 2.16 mmol, prepared as outlined for the synthesis of **6** above), tetrakis(triphenylphosphine)palladium(0) (250 mg, 0.22 mmol), copper(I) iodide (82 mg, 0.43 mmol), and 1-hexyne (0.750 mL, 6.53 mmol) were added DMF (10.7 mL) and Et_3N (0.903 mL, 6.47 mmol). After stirring for 20 h, the reaction was concentrated in vacuo. A mixture of the crude product and 10% Pd/C (179 mg) in 25 mL of MeOH was stirred under H_2 pressure (50 psi) at room temperature for 48 h. The mixture was filtered through Celite, concentrated, and crystallized from CH_3CN to yield **12**: 247 mg, 47% yield; ^1H NMR (DMSO-d_6) δ 0.85 (t, 3 H, J = 6.1 Hz), 1.15–1.35 (m, 6 H), 1.35–1.50 (m, 2 H), 2.06 (m, 2 H), 2.16 (t, 2 H, J = 7.3 Hz), 3.55 (m, 2 H), 3.75 (m, 1 H), 4.23 (m, 1 H), 4.98 (t, 1 H, J = 4.6 Hz), 5.20 (d, 1 H, J = 3.8 Hz), 6.16 (t, 1 H, J = 6.7 Hz), 7.67 (s, 1 H), 11.20 (br s, 1 H); ^{13}C NMR (CD_3OD) δ 14.42, 23.69, 27.77, 29.57, 29.98, 32.74, 41.32, 62.80, 72.26, 86.31, 88.87, 115.92, 138.18, 152.25, 166.01; UV (MeOH) nm (ε ×10^3) 268 (6.41); MS (FAB+) m/z 313 (MH^+); HRMS (FAB+) calcd for $C_{15}H_{25}N_2O_5$ 313.1763 (MH^+), found 313.1768.

5-(6-*N*-(Trifluoroacetyl)aminohexyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine (13). To a solution of **9** (490 mg, 1.16 mmol) in dry pyridine (10 mL) was added 4,4′-dimethoxytrityl chloride (430 mg, 1.27 mmol) in small portions over 30 min at room temperature. The reaction mixture was stirred at room temperature for 2 h. The mixture was then concentrated, and the residue was purified by flash chromatography (SiO_2, MeOH (3–10%)/pyridine (0.5%)/CH_2Cl_2) to give **13**: white amorphous solid, 753 mg, 89% yield; ^1H NMR ($CDCl_3$) δ 1.0–1.1 (m, 4 H), 1.15–1.3 (m, 2 H), 1.3–1.4 (m, 2 H), 1.71 (m, 1 H), 1.93 (m, 1 H), 2.32 (m, 1 H), 2.41 (m, 1 H), 2.64 (br s, 1 H), 3.31 (dd, 1 H, J = 2.4 Hz, 10.5 Hz), 3.38 (m, 2 H), 3.53 (dd, 1 H, J = 2.6 Hz, 10.5 Hz), 3.79 (s, 6 H), 4.05 (1 H), 4.57 (m, 1 H), 6.44 (dd, 1 H, J = 6.2 Hz, 7.5 Hz), 6.71 (br s, 1 H), 6.83 (d, 4 H, J = 8.6 Hz), 7.2–7.5 (m, 7 H), 7.39 (2 H), 7.57 (s, 1 H), 9.22 (s, 1 H); ^{13}C NMR ($CDCl_3$) δ 25.71, 26.40, 28.22, 28.32, 28.32, 39.90, 40.97, 55.27, 63.50, 72.44, 84.70, 86.39, 86.77, 113.27, 115.82, 115.97 (CF_3, J_{C-F} = 287.4 Hz), 127.14, 127.98, 128.20, 130.11, 135.48, 135.90, 144.30, 150.75, 157.26 ($COCF_3$, J_{C-F} = 36.8 Hz), 158.65, 164.10; UV (MeOH) nm (ε ×10^3) 234 (22.1), 270 (10.6); IR (KBr) cm^{-1} 3329, 1701, 1680, 1508, 1252, 1178; MS (FAB+) m/z 726 (MH^+), 725 (M^+), 303; HRMS (FAB+) calcd for $C_{38}H_{42}N_3O_8F_3$ 725.2924 (M^+), found 725.2964. Anal. Calcd for $C_{38}H_{42}N_3O_8F_3$: C, 62.89; H, 5.83; N, 5.79. Found: C, 62.65; H, 5.77; N, 6.05.

4-*N*-Benzoyl-5-(6-*N*-(trifluoroacetyl)aminohexyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine (14). To a mixture of **11** (854 mg, 1.62 mmol) and 4,4′-dimethoxytrityl chloride (585 mg, 1.73 mmol) was added pyridine (15 mL). After stirring for 4 h, 80 mL of CH_2Cl_2 was added, and the mixture was extracted with 5% aqueous Na_2CO_3, dried over Na_2SO_4, and concentrated. Flash chromatography (SiO_2, pyridine (1%)/MeOH (2%)/CH_2Cl_2) provided **14**: 1.130 g, 84% yield: ^1H NMR ($CDCl_3$) δ 1.00–1.18 (m, 4 H), 1.20–1.45 (m, 4 H), 1.94 (m, 1 H), 2.23 (m, 1 H), 2.34 (m, 1 H), 2.49 (ddd, 1 H, J = 3.2, 5.6, 13.4 Hz), 3.16 (m, 2 H), 3.32 (dd, 1 H, J = 2.3, 10.5 Hz), 3.58 (dd, 1 H, J = 2.7, 10.5), 3.80 (s, 6 H), 4.07 (m, 1 H), 4.59 (m, 1 H), 6.25 (m, 1 H), 6.43 (t, 1 H, J = 6.6 Hz), 6.85 (d, 4 H, J = 7.7 Hz), 7.26–7.38 (m, 6 H), 7.38–7.48 (m, 5 H), 7.53 (m, 1 H), 7.76 (s, 1 H), 8.26 (d, 2 H, J = 7.3 Hz), 13.1–13.6 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 26.06, 27.37, 28.45, 28.62, 28.77, 39.78, 41.23, 55.12, 63.17, 71.89, 85.16, 86.31, 86.60, 113.13, 115.74 (CF_3, J_{C-F} = 287.9 Hz), 116.31, 126.98, 127.84, 127.98, 128.05, 129.61, 129.95, 132.36, 135.33, 136.94, 136.99, 144.14, 147.87, 157.05 ($COCF_3$, J_{C-F} = 36.6 Hz), 158.48, 159.32, 179.45; UV (MeOH) nm (ε ×10^3) 332 (21.4), 268 (10.0), 236 (25.5); IR (KBr) cm^{-1} 3331, 1709, 1567, 1509, 1252, 1176; MS (FAB+) m/z 829 (MH^+); HRMS (FAB+) calcd for $C_{45}H_{48}F_3N_4O_8$ 829.3424 (MH^+), found 829.3436. Anal. Calcd for $C_{45}H_{47}F_3N_4O_8$: C, 65.21; H, 5.72; N, 6.76. Found: C, 65.57; H, 5.80; N, 6.55.

5-Hexyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine (15). To a mixture of **12** (220 mg, 0.71 mmol) and 4,4′-dimethoxytrityl chloride (265 mg, 0.782 mmol) was added pyridine (15 mL). After stirring for 12 h, 20 mL of CH_2Cl_2 was added, and the mixture extracted with 5% aqueous Na_2CO_3, dried over Na_2SO_4, and concentrated. Flash chromatography (SiO_2, pyridine (1%)/MeOH (2%)/CH_2Cl_2) provided **15**: 333 mg, 77% yield; ^1H NMR ($CDCl_3$) δ 0.79 (t, 3 H, J = 7.1 Hz), 1.00–1.10 (m, 4 H), 1.10–1.25 (m, 4 H), 1.70–1.85 (m, 1 H), 1.95–2.05 (m, 2 H), 2.25–2.45 (m, 2 H), 3.35 (dd, 1 H, J = 3.0, 10.4 Hz), 3.48 (dd, 1 H, J = 3.5, 10.4 Hz), 3.79 (s, 6 H), 4.03 (m, 1 H), 4.56 (m, 1 H), 6.39 (dd, 1 H, J = 6.2, 7.4 Hz), 6.83 (d, 4 H, J = 8.8 Hz), 7.21–7.35 (m, 7 H), 7.35–7.45 (m, 3 H), 8.10 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 13.97, 22.45, 26.89, 28.82, 28.97, 31.35, 40.79, 55.10, 63.50, 72.40, 84.66, 86.26, 86.62, 113.11, 116.05, 126.96, 127.83, 128.04, 129.97, 135.34, 144.23, 150.71, 158.51, 163.64; UV (MeOH) nm (ε ×10^3) 270 (14.4), 234 (26.3); IR (KBr) cm^{-1} 3400, 2930, 1688, 1510, 1463, 1252, 1177, 1035; MS (FAB+) m/z 614 (M^+); HRMS (FAB+) calcd for $C_{36}H_{42}N_2O_7$ 614.2992 (MH^+), found 614.2966. Anal. Calcd for $C_{36}H_{42}N_2O_7$: C, 70.35; H, 6.84; N, 4.56. Found: C, 70.21; H, 6.73; N, 4.40.

5-(6-*N*-(Trifluoroacetyl)aminohexyl)-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine 2-Cyanoethyl *N*,*N*-Diisopropylphosphoramidite (16). To a solution of **13** (300 mg, 0.41 mmol) in CH_2Cl_2 (3.0 mL) was added diisopropylethylamine (0.216 mL, 1.24 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphine (0.120 mL, 0.54 mmol) successively at room temperature. The mixture was stirred for 1 h, followed by dilution with 100 mL of CH_2Cl_2, extraction with 5% aqueous $NaHCO_3$, drying over Na_2SO_4, and concentration. Purification by flash chromatography (SiO_2, EtOAc (30%)/Et_3N (1%)/CH_2Cl_2) gave phos-

phoramidite **16**: 367 mg as a mixture of two diastereomers, with a small amount of $HPO(OCH_2CH_2CN)N(iPr)_2$ as an inseparable impurity (^{31}P NMR δ 14.28), 88:12 of **16**:H-phosphonate, yield of **16** 93%; ^1H NMR ($CDCl_3$) selected signals of diastereomers δ 2.40 (t, 2 H, J = 3.2 Hz, $-OCH_2CH_2CN$), 2.62 (t, 2 H, J = 3.2 Hz, $-OCH_2CH_2CN$), 3.15 (m, 4 H, $CF_3CONHCH_2-$), 3.79 (s, 3 H, OMe), 3.80 (s, 3 H, OMe), 4.11 (m, 1 H, H_4'), 4.16 (m, 1 H, H_4'), 4.6–4.7 (m, 2 H, H_3'), 6.40 (m, 1 H, H_1'), 6.43 (m, 1 H, H_1'), 6.56 (m, 2 H, CF_3CONH-); ^{31}P NMR ($CDCl_3$) δ 148.57, 148.93; MS (FAB$^+$) m/z 926 (MH^+), 818, 708, 404, 303; HRMS (FAB$^+$) calcd for $C_{47}H_{60}N_5O_9F_3P$ 926.4081 (MH^+), found 926.4065.

5-(6-*N*-(Trifluoroacetyl)aminohexyl)-4-*N*-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxycytidine 2-Cyanoethyl *N,N*-Diisopropylphosphoramidite (17). To a solution of **14** (917 mg, 1.11 mmol) in CH_2Cl_2 (10 mL) was added diisopropylethylamine (0.580 mL, 3.33 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphine (0.275 mL, 1.23 mmol). After 3 h, 50 mL CH_2Cl_2 was added. The mixture was extracted with saturated aqueous $NaHCO_3$, dried over Na_2SO_4, and then concentrated. Flash chromatography (SiO_2, pyridine (1%)/MeOH (1%)/CH_2Cl_2) gave **17**: 944 mg, 83% yield; ^1H NMR ($CDCl_3$) δ 1.00–1.10 (m, 7 H), 1.10–1.20 (m, 8 H), 1.20–1.40 (m, 5 H), 1.80–1.97 (m, 1 H), 2.17–2.30 (m, 1 H), 2.30–2.37 (m, 1 H), 2.39 (m, 1 H), 2.50–2.59 (m, 1 H), 2.62 (m, 1 H), 3.13 (m, 2 H), 3.21–3.32 (m, 1 H), 3.45–3.69 (m, 4 H), 3.69–3.90 (m, 7 H), 4.12–4.22 (m, 1 H), 4.62–4.73 (m, 1 H), 6.15–6.28 (br s, 1 H), 6.43 (m, 1 H), 6.80–6.90 (m, 4 H), 7.28–7.38 (m, 6 H), 7.38–7.48 (m, 5 H), 7.48–7.58 (m, 1 H), 7.75–7.83 (m, 1 H), 8.23–8.30 (m, 2 H), 13.20–13.50 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 20.12, 20.17, 20.37, 20.42, 24.41, 24.49, 24.55, 24.60, 26.12, 27.48, 28.55, 28.72, 28.75, 28.86, 39.84, 40.37, 43.10, 43.21, 43.31, 55.30, 57.97, 58.12, 58.27, 62.63, 62.79, 73.08, 73.20, 73.43, 73.57, 84.96, 85.07, 85.39, 85.43, 85.74, 85.76, 86.67, 113.23, 113.26, 115.82 (CF_3, J_{C-F} = 288.4 Hz), 116.37, 116.39, 117.32, 117.54, 127.13, 127.96, 128.08, 128.21, 128.27, 129.78, 130.11, 132.39, 135.42, 136.75, 136.83, 137.23, 144.20, 147.77, 147.80, 156.99 ($COCF_3$, J_{C-F} = 37.1 Hz), 158.66, 159.39, 179.55; ^{31}P ($CDCl_3$) δ 148.62, 149.12; MS (FAB$^+$) m/z 1029 (MH^+); HRMS (FAB$^+$) calcd for $C_{54}H_{65}F_3N_6O_9P$ 1029.4503 (MH^+), found 1029.4551.

5-Hexyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-deoxyuridine 2-Cyanoethyl *N,N*-Diisopropylphosphoramidite (18). To a solution of **15** (125 mg, 0.20 mmol) in CH_2Cl_2 (3 mL) was added diisopropylethylamine (0.105 mL, 0.60 mmol) and 2-cyanoethyl *N,N*-diisopropylchlorophosphine (0.078 mL, 0.31 mmol). After 30 min, CH_2Cl_2 was added, and the mixture was extracted with 5% aqueous $NaHCO_3$, dried over Na_2SO_4, and then concentrated. Flash chromatography (SiO_2, Et_3N (1%)/EtOAc (25%)/CH_2Cl_2) gave **18**: 147 mg, 90% yield; ^1H NMR ($CDCl_3$) δ 0.79 (m, 3 H), 1.00–1.10 (m, 8 H), 1.10–1.20 (m, 12 H), 1.65–1.75 (m, 1 H), 1.90–2.00 (m, 1 H), 2.25–2.35 (m, 1 H), 2.35–2.65 (m, 3 H), 3.25–3.35 (m, 1 H), 3.45–3.70 (m, 5 H), 3.70–3.90 (m, 6 H), 4.10–4.20 (m, 1 H), 4.60–4.70 (m, 1 H), 6.40 (m, 1 H), 6.80–6.90 (m, 4 H), 7.20–7.35 (m, 7 H), 7.35–7.55 (m, 3 H), 7.94 (br s, 1 H); ^{13}C NMR ($CDCl_3$) δ 14.00, 20.09, 20.15, 20.32, 20.37, 22.48, 24.39, 24.45, 24.50, 24.53, 24.58, 26.94, 28.91, 29.00, 31.38, 39.93, 43.07, 43.16, 43.26, 55.17, 58.03, 58.19, 58.35, 62.87, 63.09, 73.31, 73.45, 73.74, 73.88, 84.49, 85.21, 85.25, 85.50, 85.52, 86.67, 113.14, 116.00, 116.05, 117.33, 117.53, 127.05, 127.88, 128.12, 128.17, 130.06, 135.20, 135.26, 135.29, 144.16, 150.26, 150.33, 158.61, 163.32; ^{31}P NMR ($CDCl_3$) δ 148.44, 148.90; MS (FAB$^-$) m/z 813 ($M - H^-$); HRMS (FAB$^+$) calcd for $C_{45}H_{58}N_4O_8P$ 813.3992 ($M - H^-$), found 813.3981.

DNA Synthesis. All oligodeoxynucleotides were synthesized trityl-on using a controlled pore glass solid support via the phosphite-triester method with an Applied Biosystems 391EP DNA synthesizer (1 μmol scale). For the synthesis of oligomer **24**, controlled pore glass support derivatized with **13** was prepared by standard methodology.[20] Cleavage from the solid support and deprotection (except for the 5′-trityl group) was accomplished by treatment with concentrated NH_4OH for 15 hours at 55 °C. The solution was then lyophilized with the addition of Et_3N every hour to inhibit detritylation. The residue was then taken up in 1 mL of 100 mM triethylammonium acetate (TEAA), pH 7, and purified by reverse phase HPLC (Hamilton PRP-1, 300 mm × 7 mm, Eppendorf CH-30 column heater, 60 °C, 23-33% CH_3CN/100 mM TEAA, pH 7, 20 min, monitored at 260 nm). The fractions were lyophilized to dryness followed by repeated lyophilization to dryness with H_2O (2 × 1 mL) to remove any residual TEAA. Detritylation was accomplished by treatmentwith 80% AcOH (0.3 mL) for 20 min. After lyophilization with EtOH (0.3 mL), the residue was taken up in H_2O (1 mL), extracted with diethyl ether (3 × 1 mL), and then lyophilized to dryness. H_2O (1 mL) was added to the dry DNA pellet, and the solution was quantified by UV absorbance at 260 nm at 70 °C. The extinction coefficients (at 260 nm) of the natural nucleotides used for calculations were as follows: dAMP, 15 200; dCMP, 7700; TMP, 8830; dGMP, 11 500. The extinction coefficients of the unnatural nucleosides at 260 nm were determined to be the following: 5-(6-aminohexyl)-2′-deoxycytidine, 5170 (prepared by hydrolysis of **11**); 5-(6-aminohexyl)-2′-deoxyuridine, 9200 (prepared by hydrolysis of **9**). The extinction coefficient for 5-hexyldeoxyuridine was taken to be that of thymidine. All oligonucleotide base compositions were confirmed by formic acid hydrolysis[10] followed by HPLC analysis monitored at 260 or 270 nm (Alltech, HS, C-18; 20 mM K_2HPO_4, pH 5.6 (A), MeOH (B), 100% A to 40% B, 20 min).

Laser Desorption Mass Spectral Data for Oligonucleotides 20–27. 5′-d(CTTTCTCTCCCT)-3′ (**20**): calculated mass, 3582; observed mass, 3590. 5′-d(CTTTCTCTCCCT)-3′ (**21**): calculated mass, 3596; observed mass, 3595. 5′-(dCTTTCTCTCCCT)-3′ (**22**): calculated mass, 3838; observed mass, 3841. 5′-d(CTTTCTCTCCCT)-3′ (**23**): calculated mass, 3894; observed mass, 3892. 5′-d(CTTTCTCTCCCT)-3′ (**24**): calculated mass, 4504; observed mass, 4509. 5′-d(CTTTCtCTCCCT)-3′ (**25**): calculated mass, 3567; observed mass, 3565. 5′-d(CTTTCtCtCCCT)-3′ (**26**): calculated mass, 3638; observed mass, 3637. 5′-d(CtTtCtCtCCCT)-3′ (**27**): calculated mass, 3778; observed mass, 3779. "T" and "C" are **1** and **2**, respectively. "t" corresponds to **3**.

General Procedure for Kinasing and Gel Electrophoresis. H_2O (5 μL), ATP (1 μL, 660 mM), spermidine (1 μL, 10 mM), 10X kinase buffer (1 μL, 700 mM Tris-HCl pH 7.6, 100 mM $MgCl_2$, 1 mM KCl, 50 mM dithiothreitol), $\gamma^{32}P$ ATP (1 μL, 1 μCi), and T4 polynucleotide kinase (1 μL, 30 units) were added to the lyophilized DNA sample (200 pmol), and the mixture was incubated at 37 °C for 30 min. Stop solution (10 μL, 7 M urea, 1X TBE, 0.1% bromophenol blue, 0.1% xylene cyanol) was added, and the sample was run through a 20% polyacrylamide, 7 M urea denaturing gel with Tris-Borate-EDTA (TBE, pH 8.6) as the buffer. Autoradiograms were developed in 1–3 h with an intensifying screen.

Melting Experiments. UV absorbance versus temperature profiles were measured on an HP 8452A diode-array UV spectrophotometer in a temperature controlled cell holder with an HP 89090A peltier temperature controller. The temperature of the cell holder was increased from 0 °C to 90 °C in 1 °C increments at a heating rate of 1 deg/min. The temperature of the solution was monitored by a thermocouple placed in the cell solution (10-mm path length only). N_2 gas (ice-cold) was passed over the cell at low temperatures to avoid the condensation of moisture. Experiments were performed a minimum of twice with different samples and T_m's were averaged. Free energy values (in part) and melting temperatures were obtained by nonlinear regression using a two-state model.[11] An excellent fit of the experimental data was seen in all cases. Reverse melting experiments (90 °C to 0 °C, 1 deg/min) and reduced rate forward melting experiments (0 °C to 90 °C, 0.5 deg/min) were also performed and found to give T_m's within 0.5 °C. Plots of $1/T_m$ versus ln C_T were used to obtain thermodynamic parameters.[11,12] For the T_m versus concentration studies, the following total oligonucleotide concentrations were employed: 140, 70, 46, 28, 18, 12, 8.0, and 5.0 μM. A 1.0-mm path length cell was used for 140–12 μM concentrations, and a 10-mm path length cell for 8.0 and 5.0 μM concentrations.

UV Mixing Curves. Mixing curve experiments were performed in a 10-mm path length cell at 20 °C monitoring absorbance at 260 nm. The sample contained 50 mM NaCl, 0.1 mM EDTA, 10 mM NaH_2PO_4, and the appropriate molar ratio of oligomers in H_2O at pH 7.

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