Solid-Phase Synthesis of Deoxynucleic Guanidine (DNG) Oligomers and Melting Point and Circular Dichroism Analysis of Binding Fidelity of Octameric Thymidyl Oligomers with DNA Oligomers

Barry A. Linkletter, Istvan E. Szabo, and Thomas C. Bruice*

Contribution from the Department of Chemistry, University of California, Santa Barbara, California 93106

Received December 7, 1998

Abstract: A practical solid-phase synthesis of deoxynucleic guanidine (DNG), a positively charged DNA backbone analogue, is reported. The nucleoside coupling step in the solid-phase synthesis of DNG involves the attack of a terminal 3′-amine upon an electronically activated 5′-carbodiimide to create a protected guanidinium internucleoside linkage. The activated carbodiimide is synthesized in situ by the mercury(II) abstraction of sulfur from an unsymmetrically substituted thiourea in which one substituent is an electron-withdrawing protecting group and the other is the 5′-nucleoside monomer. This produces, in addition to the carbodiimide, a mercury sulfide precipitate which accumulates in the pores of the solid support, restricting solvent and reagent access and reducing the coupling yields with each successive cycle. This obstacle is overcome by a simple washing step involving a thiophenol solution which readily removes the mercury salt. The addition of this step to the cycle enables DNG oligomers to be synthesized using standard macroporous SPS supports. Coupling yields of 98% were estimated from the HPLC analysis of the product mixtures. An octameric thymidyl oligomer (**II**) was synthesized and the fidelity of binding to octameric adenyl DNA oligomers containing cytidyl mismatches was determined. Binding was studied by thermal denaturation (Tm), Job plots, and circular dichroism spectrophotometry. The DNG oligomer (**II**) formed a 2:1 complex with octameric adenyl DNA (**III**) with a melting temperature of 63 °C. Each cytidyl mismatch induced a penalty of 4 to 5 °C in the observed melting temperatures. DNA sequences with four or more mismatches showed no base pairing in the presence of **II**. No association was observed between **II** and octameric cytidyl DNA. These observations demonstrate that DNG oligomers of moderate length are able to discriminate between complementary and mismatched DNA oligomers.

Introduction

Oligonucleoside analogues capable of arresting cellular processes at the translational and transcriptional levels via recognition and binding to complementary RNA or DNA are known, respectively, as antisense and antigene agents.[1−3] Important goals in designing antisense compounds include increasing the binding affinity to the base sequence while maintaining fidelity of recognition, resistance to degradation by nucleases, and effective membrane permeability. A recurring theme in many of these antisense compounds is the incorporation of neutral internucleoside linkages which eliminate the mutual repulsion between the negatively charged phosphate diester backbones in duplex DNA. These unnatural linkages are likely to be resistant to nucleases and may also be membrane permeable. Oligonucleosides linked by amides,[4] phosphonates,[5] carbamates,[6] methylenemethylimino (MMI),[7] heterocycles,[8] and acetals[9] are representative of this approach. Another approach is to replace the phosphoribose backbone entirely, such as in the cases of PNA,[10,11] PHONA,[12] or PNAA.[13]

In recent years, approaches involving the incorporation of positive charges in antisense oligomers have been developed. Positive charges can be added to the bases[14] or the sugar rings to give zwitterionic DNA. The phosphate backbone can be alkylated with alkylamines to produce positively charged phosphate triester linkages between the nucleosides.[15] Another promising approach is to replace the internucleoside phosphate linkage with a positively charged, achiral guanidinium group.[16] The guanidinium linkage is resistant to nucleases[17] and the positive charges of the backbone may give rise to cell membrane permeability through electrostatic attraction of the oligonucleoside to the negatively charged phosphate groups of the cell surface. Recently, micelles featuring positively charged surfaces

(1) Agrawal, S.; Zhao, Q. Y. *Curr. Opin. Chem. Biol.* **1998**, *2*, 519.
(2) Temsamani, J. G. P. *Biotechnol. Appl. Biochem.* **1997**, *26*, 665.
(3) De Mesmaeker, A.; Häner, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res.* **1995**, *28*, 366.
(4) De Mesmaeker, A.; Waldner, A.; Lebreton, J.; Hoffmann, P.; Fritsch, V.; Wolf, R. M.; Freier, S. M. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 226.
(5) Tseng, B. Y.; Ts'o, P. O. P. *Antisense Res. Dev.* **1995**, *5*, 251.
(6) Waldner, A.; De Mesmaeker, A.; Lebreton, J.; Fritsch, V.; Wolf, R. M. *Synlett* **1994**, 57.
(7) Morvan, F.; Sangvhi, Y. S.; Perbost, M.; Vasseur, J.-J.; Bellon, L. *J. Am. Chem. Soc.* **1996**, *118*, 255.
(8) von Matt, P.; Altmann, K.-H. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1553.
(9) Wang, J.; Matteucci, M. D. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 229.
(10) Uhlmann, E.; Peyman, A.; Breipohl, G.; Will, D. W. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2796.
(11) Nielsen, P. E. *Pure Appl. Chem.* **1998**, *70*, 105.
(12) Peyman, A.; Uhlmann, E.; Wagner, K.; Augustin, S.; Breipohl, G.; Will, D. W.; Schäfer, A.; Wallmeier, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2636.
(13) Fujii, M.; Yoshida, K.; Hidaka, J. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 637.
(14) Ueno, Y.; Mikawa, M.; Matsuda, A. *Bioconj. Chem.* **1998**, *9*, 33.
(15) Skibo, E. B.; Xing, C. *Biochemistry* **1998**, *37*, 15199.
(16) Dempcy, R. O.; Almarsson, Ö.; Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 7864.
(17) Barawkar, D. A.; Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 11047.

10.1021/ja984212w CCC: $18.00
Published on Web 04/09/1999

have been reported to carry impermeable compounds such as DNA oligomers through the lipid bilayer of cells.[18]

Short pentameric thymidyl oligomers of DNG (**I**) with four positively charged internucleoside guanidinium groups (Chart 1), have been synthesized by solution phase methods[19,20] and have been found to bind strongly to polyadenylic DNA [poly(dA)] in a 2:1 (DNG:DNA) complex.[21−23] No interaction of polyguanylic [poly(dG)], polycytidylic [poly(dC)], or polythymidylic [poly(dT)] acids with **I** was observed.[21−23] A detailed study of the binding of **I** with short strand DNA oligomers containing adenine and guanine tracts established that these short DNG oligomers bind with fidelity in a triple helical fashion.[22]

The pentameric DNG **I** was the longest oligomer that could be synthesized by solution-phase synthesis. To be an effective antisense or antigene agent, DNG must be available in longer sequences that can recognize unique sites in the human genome. A solid-phase synthetic method was developed to make longer oligomers.[24] The single most important feature of solid-phase synthesis is that purification after each synthetic cycle is accomplished by washing the solid support to which the growing oligomer is covalently attached. This eliminates losses of the product while enabling the use of greater excesses of the reagents, increasing coupling yields. Using this method, the octameric thymidyl DNG oligomer **II** (Chart 1) was synthesized.[24] This paper presents, for the first time, the detailed solid-phase synthesis of an octameric DNG oligomer and investigates the binding of this new, much more positively charged oligomer to complementary and mismatched short DNA oligomers.

Results and Discussion

Solid-Phase Synthesis of DNG. Monomers for the various solid-phase synthesis approaches were synthesized as outlined in Scheme 1. The syntheses of **1**, **2,** and **3** have been described previously[16] and the details for the synthesis of **4**, **5**, **6,** and **7** are described in the Experimental Section.

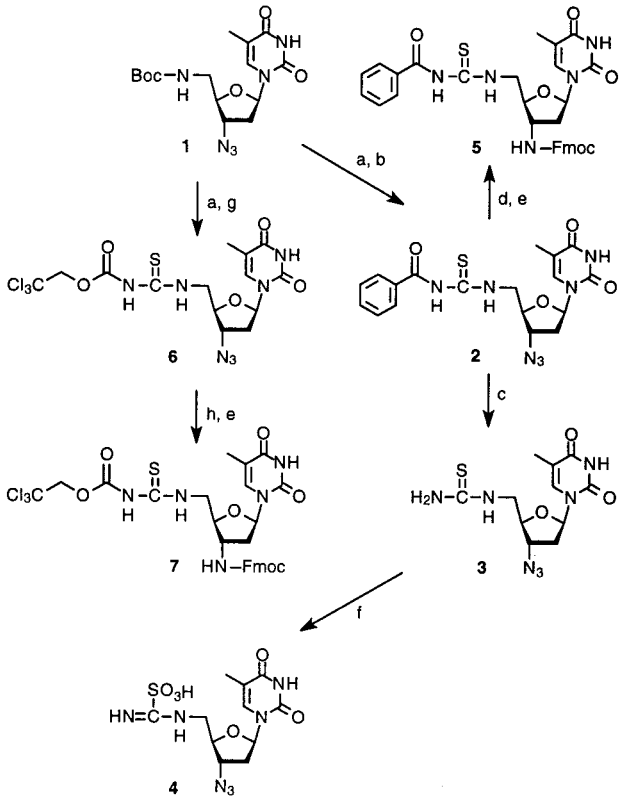
Initially, the synthetic approach was based on the origional solution-phase synthesis.[16] The oligomer was extended by the addition of **4** to a Rink peptide amide resin[25] loaded with a 3′-amino thymidyl nucleside. The coupling reaction involved the displacement of the sulfonyl group to produce a guanidinium linkage.[26] After coupling, the 3′-azide was reduced with H_2S in pyridine[16] and the cycle was repeated. However, the coupling yields dropped substantially as the oligomer was extended and the maximum length of oligomer possible by this solid-phase approach did not surpass that of the intial solution-phase method.

It was decided to protect the guanidinium linkages in a manner that would enhance the solubility of the growing oligomer in organic solvents and prevent the basic guanidinium group from interfering with the coupling chemistry. A new synthetic approach was adopted which results in protected guanidinium linkages being formed in the coupling step. The protecting group can then be removed after the synthesis is completed. This reaction was adapted from a method developed by Kim and Qian for the synthesis of terminal guanidines[27] which involves converting a *N*,*N*′-bis-Boc-protected thiourea to a carbodiimide by addition of $HgCl_2$ followed by substitution of an alkylamine. The two t-Boc groups on the carbodiimide intermediate electronically activate it to the nucleophilic attack of the amine without the need for protonation or any catalyst. The resulting diprotected guanidinium group is then deprotected by acid to produce a terminal guanidinium.

We have found that a single electron-withdrawing group is sufficient to activate the carbodiimide to attack by an amino group. A thiourea with an electron-withdrawing protecting group on one nitrogen and a nucleoside on the other can be used as a precursor to an activated carbodiimide suitable for producing a protected guanidinium internucleoside linkage when reacted with an 3′-amino nucleoside derivative as shown in Scheme 2.

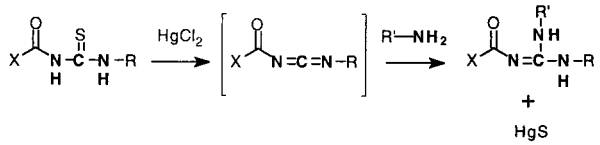
The monomer, **5**, was easily obtained by the reduction and Fmoc protection of **2**, an intermediate in the synthesis of **4**.

Scheme 1. Synthesis of Monomers for Solid-Phase Synthesis of DNG Oligomers[a]



[a] Key: (a) 20% TFA/DCM; (b) benzoyl isothiocyanate, DCM; (c) Na_2CO_3, 2:1 MeOH/H_2O; (d) H_2S, pyridine; (e) 9-fluorenylmethylchloroformate, DCM, TEA; (f) peracetic acid; (g) trichloroethoxycarbonyl isothiocyanate, TEA, DCM; (h) H_2, 10% Pd/C, EtOH.

Scheme 2. Formation of an Electronically Activated Carbodiimide from an Acyl-Protected Thiourea Enables Synthesis of a Protected Guanidine Group



(18) Huang, C. Y.; Uno, T.; Murphy, J. E.; Lee, S.; Hamer, J. D.; Escobedo, J. A.; Cohen, F. E.; Radhakrishnan, R.; Dwarki, V.; Zuckermann, R. N. *Chem. Biol.* **1998**, *5*, 345.

(19) Dempcy, R. O.; Browne, K. A.; Bruice, T. C. *J. Am. Chem. Soc.* **1995**, *117*, 6140.

(20) Dempcy, R. O.; Luo, J.; Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 4326.

(21) Blaskó, A.; Dempcy, R. O.; Minyat, E. E.; Bruice, T. C. *J. Am. Chem. Soc.* **1996**, *118*, 7892.

(22) Blaskó, A.; Minyat, E. E.; Dempcy, R. O.; Bruice, T. C. *Biochemistry* **1997**, *36*, 1821.

(23) Brown, K. A.; Dempcy, R. O.; Bruice, T. C. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 7051.

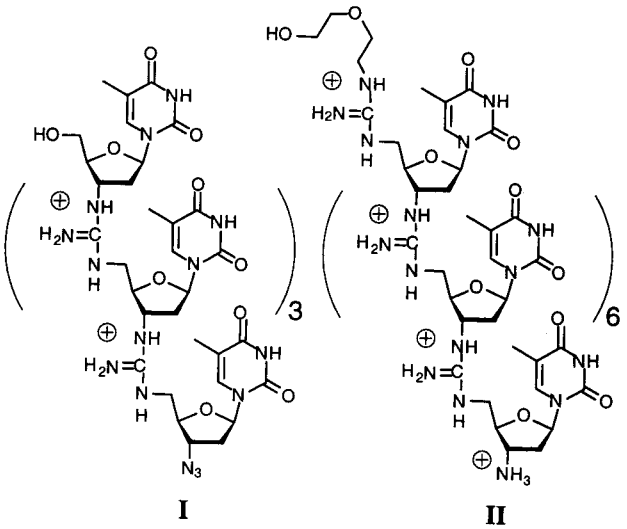
(24) Linkletter, B.; Bruice, T. C. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1285.

(25) Rink, H.; Sieber, P. *Peptides* **1988**, 139.

(26) Maryanoff, C. A.; Stanzione, R. C.; Plampin, J. N. *Phosphorus Sulfur* **1986**, *27*, 221.

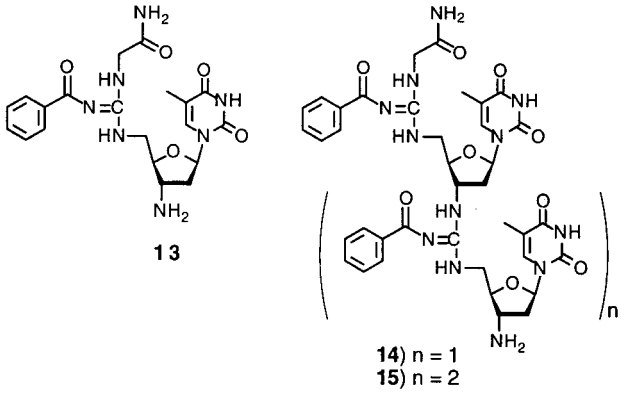
(27) Kim, K. S.; Qian, L. *Tetrahedron Lett.* **1993**, *34*, 7677.

Chart 1



I **II**

Chart 2



13

14) n = 1
15) n = 2

Rink amide resin was again employed and loaded with glycine to give a terminal primary amine to react with the activated carbodiimide. This method allows loading of the first nucleoside using the same chemistry as all the coupling steps in the synthesis. The $HgCl_2$-mediated coupling of **6** with the resin-bound 3′-amino group was very successful. A trimeric oligomer with benzoyl-protected guanidinium linkages, **15**, was synthesized with coupling yields of 95% as measured by Fmoc colorimetry methods and HPLC analysis of cleaved resin samples. A trisubstituted guanidine-linked DNA analogue such as **15** may be of interest as it has a slightly positive character (as evidenced by its retention on the cation exchange HPLC column, Figure 1) and is soluble in organic solvents.

The trichloroethoxycarbonyl group was found to be more suitable than the benzoyl group as a guanidinium protecting group for the synthesis of longer oligomers. It is stable to the coupling conditions and is easily removed by zinc powder and acetic acid.[28] A commercially available 2% cross-linked polystyrene 2-(2-aminoethoxy)ethanol 2-chlorotrityl resin was used as the solid support. The amino functionality allows the loading step to use the same chemistry as the subsequent coupling steps and the chlorotrityl linker is cleavable under mild acidic conditions. The synthesis cycle proceeds in the 5′−3′ direction as outlined in Scheme 3.

In the solid-phase synthesis of **II**, reported in our preliminary communication, the coupling chemistry suffered from the presence of the mercury sulfide precipitate.[24] As the precipitate

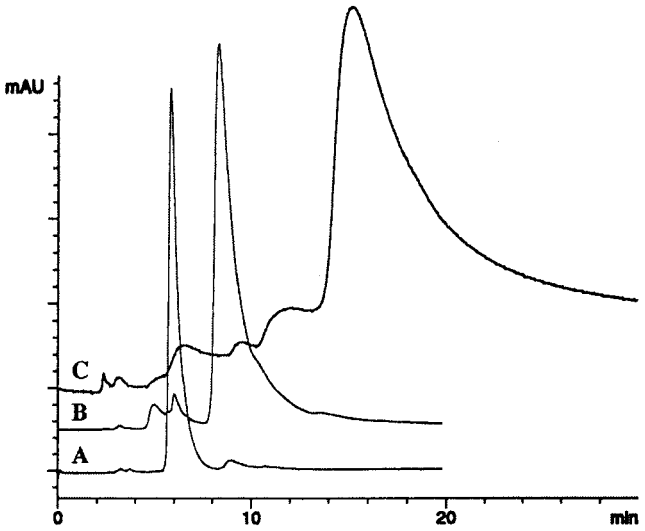


Figure 1. Cation exchange HPLC of the crude cleavage mixture for the synthesis of **13** (A), **14** (B), and **15** (C): (A) cleaved after the first coupling/loading step and subsequent deblocking—peak at 4.9 min is **13**; (B) cleaved after the second coupling reaction and subsequent deblocking—peak at 8.3 min is **14**; and (C) cleaved after coupling the third 3′-azido nucleoside and subsequent deblocking—peak at 15.2 min is **15**.

collected in the pores of the resin, the beads became black in color and no longer shrunk in solvents, such as methanol, that normally deswell polystyrene resin. To avoid the problem of pore blockage, it is necessary to remove the precipitate from the resin after each coupling cycle. When a 20% thiophenol solution in DMF was filtered through the precipitate-blocked resin, the dark color immediately disappeared and the resin returned to its original yellow color and swelling behavior. The resin was then washed with copious amounts of DMF and agitated in 20% piperidine in DMF for 20 min to de-block the Fmoc-protected 3′-amine. Then the cycle was repeated until the desired number of nucleosides had been coupled.

The chlorotrityl linker used in this SPS method is cleavable by 3% TFA in DCM or AcOH/TFE/DCM.[29] The latter cleavage protocol is mild enough to prevent depurination of adenine nucleosides, making this methodology practical for any base sequence of DNG. In the case of thymidyl oligomers, cleavage from the resin can be accomplished in 5 min with 3% TFA in DCM. The product was precipitated from cold ether and purified by reverse phase preparative LC. The fractions containing the product were evaporated to dryness to obtain the purified protected oligomer **16**. Analysis of the crude cleavage product showed that the major component was approximately 75% of the integration signal (Figure 2). The peaks preceding the major product were shorter oligomers resulting from incomplete coupling reactions. No peaks indicative of longer oligomers, caused by double additions, were observed. From this information, the average coupling yields were estimated to be 95 to 98%.

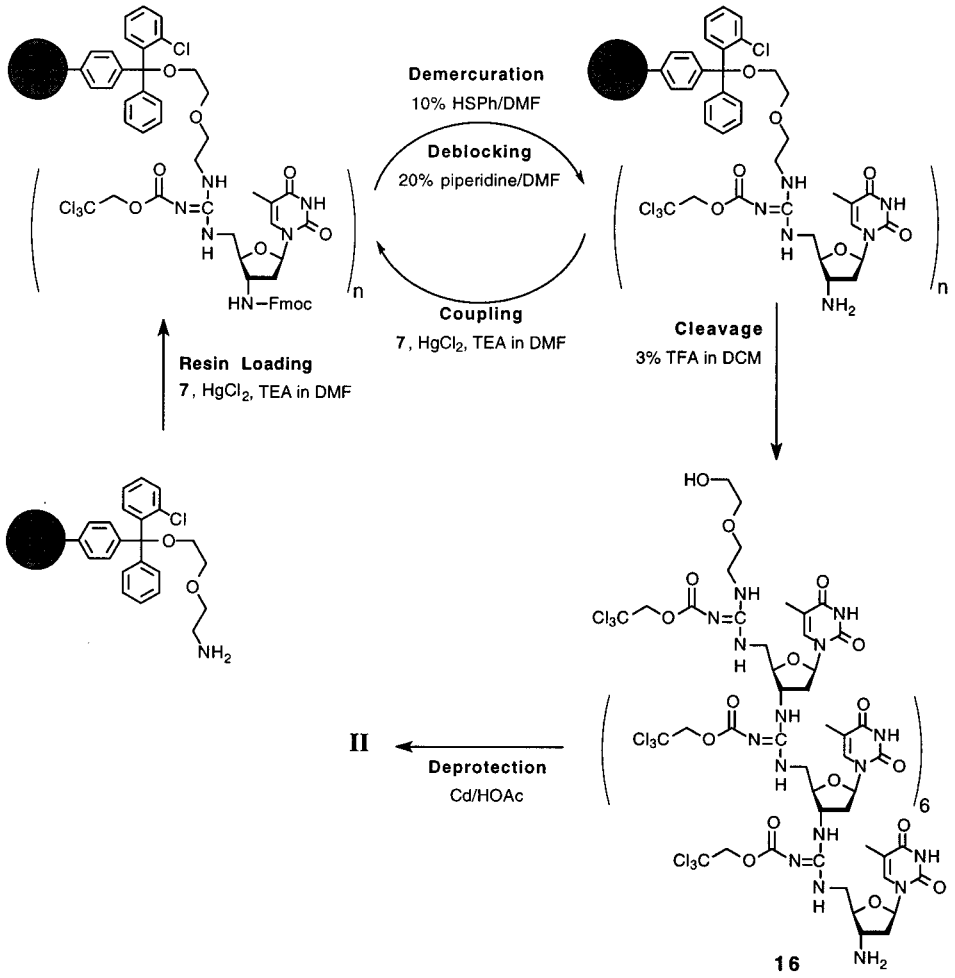
The deprotection of **16** was performed using cadmium powder in acetic acid. This procedure produces superior results to the usual zinc deprotection method used for trichloroethyl protecting groups.[30] The deprotection reaction proceeded in a quantitative yield with no detected side products. The final product was desalted by preparative cation exchange HPLC and, after evaporation and sublimation of the ammonium acetate buffer,

(28) Just, G.; Grozinger, K. *Synthesis* **1976**, 457.

(29) Barlos, K.; Gatos, D.; Kallitsis, J.; Papaphotiu, G.; Sotivic, P.; Wenging, Y.; Schäfer, W. *Tetrahedron Lett.* **1989**, *30*, 3943.

(30) Hancock, G.; Galpin, I. J.; Morgan, B. A. *Tetrahedron Lett.* **1982**, 249.

Scheme 3



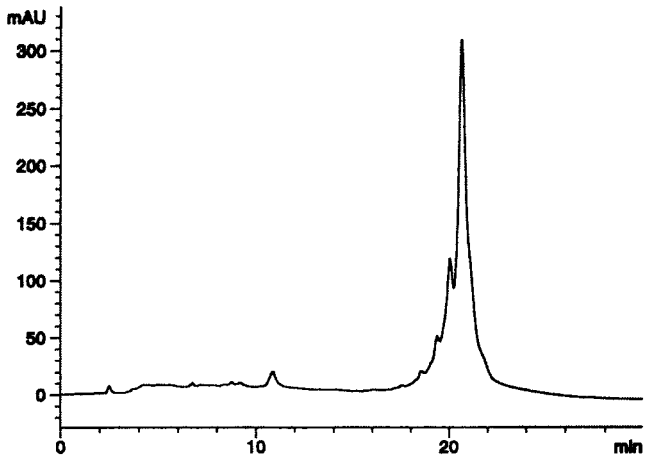


Figure 2. Reverse phase analytical HPLC of the crude cleavage mixture in the synthesis of **16** showing few side products or truncated oligomers. Percent area of major peak is ca. 70% of the total integrated region.

was stored as a solution in water at 4 °C. Electrospray mass spectroscopic analysis indicates the expected masses for the triply charged [*m*/*z* 743.2, calcd for $C_{92}H_{134}N_{41}O_{26}$ $(M + 3H)^{3+}$ 743.4] and quadruply charged [*m*/*z* 557.7, calcd for $C_{92}H_{135}N_{41}O_{26}$ $(M + 4H)^{4+}$ 557.8] forms of the oligomeric DNG **II**.

Effect of Mismatches on Thermal Melting Behavior. Melting studies were performed to determine if **II** would bind with fidelity to DNA oligomers. Table 1 shows the DNA oligomers chosen and the thermal denaturation temperatures (Tm) recorded for each DNA oligomer after complexation with 2 equiv of **II**. The Tm for the 2:1 association of octameric DNG **II** associated with the complementary octameric adenyl DNA **III** is 62.8 °C in the presence of 0.1 M KCl. The Tm for the A:T duplex of **III** and octameric thymidyl DNA is less than 10 °C under the same conditions. The large increase in the Tm is due to the opposite charge attraction between the positively charged DNG and negatively charged DNA strands. The thermal denaturation plots of 2 equiv of **II** in the presence of 1 equiv of various DNA oligomers containing mismatches at the terminal ends in 0.1 M KCl are presented in Figure 3. As mismatches are incorporated in the DNA oligomer, the Tm and the magnitude of the hyperchromic effect are reduced. When two cytidine mismatches are present at each end of the oligomer (as in DNA **VI**), complex formation is no longer significant as measured by the hyperchromic effect.

Internal mismatches have a much more pronounced effect. The single internal mismatch substitution in DNA oligomer **VIII** reduced the Tm from 62.8 to 48 °C and inclusion of two internal mismatches (**IX**) eliminated base pairing association (Figure 4). The large positive charge is not enough to overcome the disruption in base pairing that two internal mismatches cause. The observed Tm for the DNG:DNA association drops when the salt concentration is increased from 0.1 M KCl to 0.3 M KCl since increased ionic strength reduces the importance of the opposite charge attraction.[21,23] Figures 5 and 6 show the thermal denaturation plots for the DNA oligomers possessing terminal and internal mismatches with DNG **II** in the presence of 0.3 M KCl. The Tm measurements are lower and the

Table 1. Melting Points of Mixtures of DNA Oligomers and DNG **II**[a]

DNA oligomer	Tm, 0.1M KCl	Tm, 0.3M KCl
AAAAAAAA **III**	62.8	56.3
AAAAAAAC **IV**	59.7	52.0
CAAAAAAC **V**	55.1	47
CCAAAACC **VI**	-	-
CCCAACCC **VII**	-	-
AAAACAAA **VIII**	48	46.1
AAACCAAA **IX**	-	-
AAACCCAA **X**	-	-
AACCCCAA **XI**	-	-
ACCCCCCA **XII**	-	-
ACACACAC **XIII**	-	-
AACCAACC **XIV**	-	-
CCCCCCCC **XV**	-	-

[a] Samples consist of 1.25 μM DNA oligomer, 2.5 μM **II**, and 10 mM $KHPO_4$ buffer at pH 7.4 and the indicated ionic strength controlled by KCl. Melting points were determined by first derivative analysis of samples that showed melting behavior.

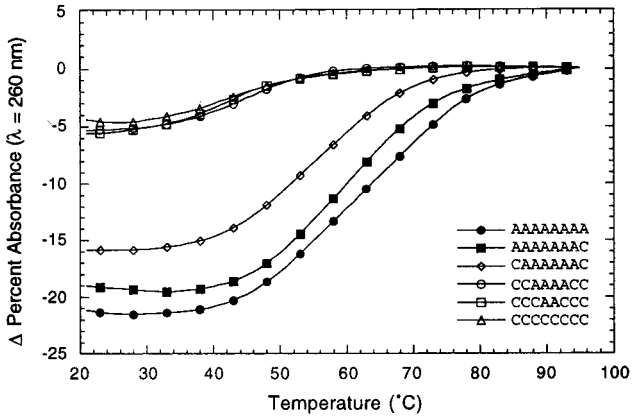


Figure 3. Thermal denaturation plots for DNA:DNG association (1.25 μM DNA oligomer, 2.5 μM DNG **II**, 10 mM $KHPO_4$ buffer, pH 7.4, 0.1 M KCl). Rate of heating was 0.2 °C/min DNA oligomer. Absorbance measurements taken every 1 °C (for clarity only one in five data points are represented by a symbol) in plots 1 through 4. DNA oligomer: **III**, ●; **IV**, ■; **V**, ◇; **VI**, ○; **VII**, □; and **XV**, △.

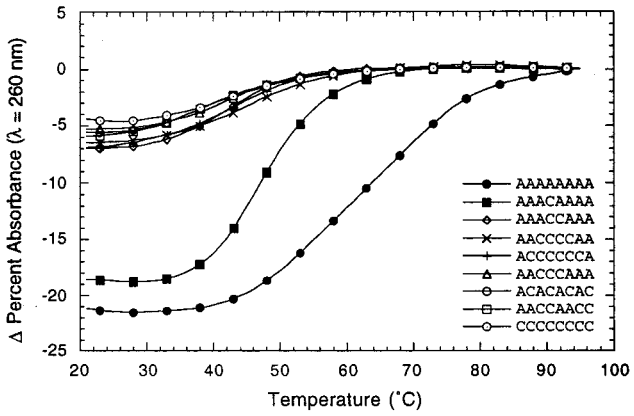


Figure 4. Thermal denaturation plots for DNA:DNG association (1.25 μM DNA oligomer, 2.5 μM DNG **2**, 10 mM $KHPO_4$ buffer, pH 7.4, 0.1 M KCl). Rate of heating was 0.2 °C/min DNA oligomer: **III**, ●; **VIII**, ■; **IX**, ◇; **X**, ○; **XI**, ×; **XII**, +; **XIII**, ○; **XIV**, □; and **XV**, ◆.

hyperchromicities are smaller than in the case of 0.1 M KCl. Figure 7 shows the relationship between the number of mismatches (terminally or internally placed) and the Tm

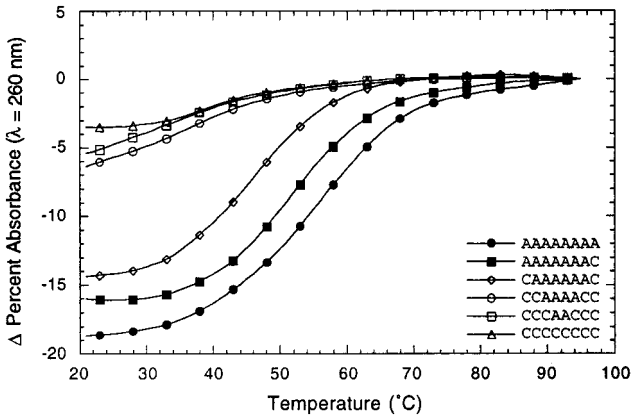


Figure 5. Thermal denaturation plots for DNA:DNG association (1.25 μM DNA oligomer, 2.5 μM DNG **2**, 10 mM $KHPO_4$ buffer, pH 7.4, 0.3 M KCl). Rate of heating was 0.2 °C/min DNA oligomer: **III**, ●; **IV**, ■; **V**, ◇; **VI**, ○; **VII**, □; and **XV**, △.

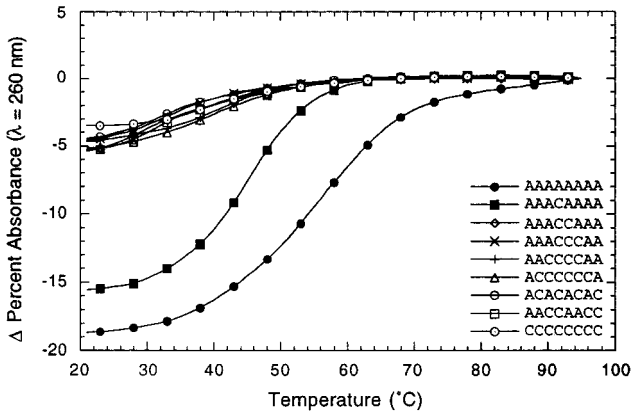


Figure 6. Thermal denaturation plots for DNA:DNG association (1.25 μM DNA oligomer, 2.5 μM DNG **2**, 10 mM $KHPO_4$ buffer, pH 7.4, 0.3 M KCl). Rate of heating was 0.2 °C/min DNA oligomer: **III**, ●; **VIII**, ■; **IX**, ◇; **X**, ○; **XI**, ×; **XII**, +; **XIII**, ○; **XIV**, □; and **XV**, ◆.

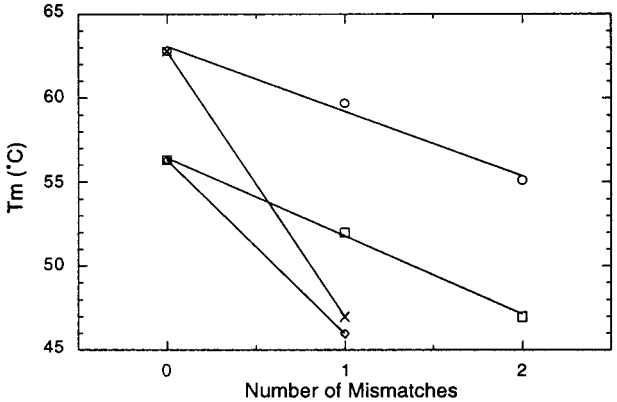


Figure 7. DNA:DNG thermal melting point (T_m) in relation to the number of mismatches at the terminals (○, 0.1 M KCl; □, 0.3 M KCl) and internal mismatches (×, 0.1 M KCl; ◇, 0.3 M KCl). [DNA oligomer] = 1.25 μM, [**II**] = 2.5 μM, [$KHPO_4$] = 10 mM, pH 7.4, rate of heating 0.2 °C/min. Data from Table 1.

determined for all oligomers which showed a significant hyperchromicity in the presence of **II**. In all the nonbinding thermal plots a very weak hyperchromicity is seen in the range of 35 to 45 °C. This is probably due to a nonspecific ionic association of the backbones and not base pairing effects.

Effect of Mismatches and Ionic Strength on Continuous Variation Plots. Job[31] plot analyses were performed to measure the stoicheometry of association. The absorbance at 260 nm

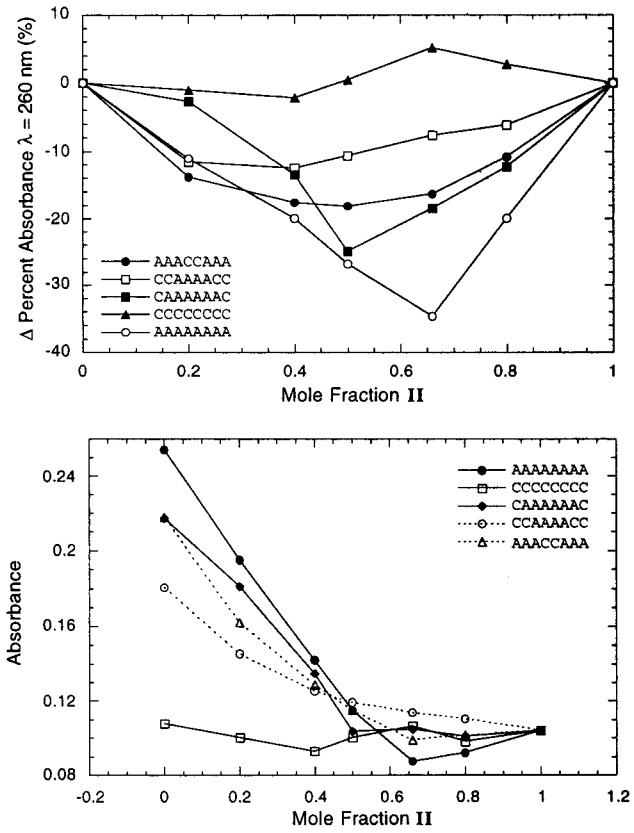


Figure 8. Job plots for DNG **II** with DNAs **III**, **V**, **VI**, **IX**, and **XV** plotted by percent hyperchromicity (top) and absorbance (bottom). [oligomer] = 2.0 uM, [$KHPO_4$] = 10 mM, [KCl] = 0.1 M, pH 7.4, observed at 260 nm.

was measured for samples containing a constant concentration of 2 μM oligonucleside varying between 0 and 100% DNG **II** with a given DNA oligomer making up the remainder. The inflection point in the plot indicates the stoicheometry of the DNG:DNA complexes. Job plots of oligomers **III**, **V**, **VI**, **IX**, and **XV** complexed with DNG **II** in 10 mM phosphate buffer and 0.1 M KCl salt for ionic strength are shown in Figure 8. The Job plot for oligomer **III** with DNG **II** shows a clear inflection point at 64% DNG indicating that **III** binds in a 1:2 ratio with DNG **II**. Oligomer **V**, with two terminal mismatches, has an inflection point at 50% DNG **II**. Oligomer **IX** displays a weaker hyperchromic effect with DNG **II** and the Job plot in this case does not show a clear inflection point. Oligomer **VI** has an even weaker hyperchromic effect and also displays no clear break point in the Job plot. The maximum hyperchromicity, as determined by the percent difference between the observed optical density (OD) and that calculated for the sum of the two separate oligomers at the given concentrations, is greatest for the complementary oligomer **III**, with a hyperchromicity of 33% at 66% DNG **II**. As mismatches are included, the hyperchromicity is weakened and the point of maximum deflection from the calculated OD shifts toward 50% DNG **II** for oligomers **V**, **IX,** and **VI**, with maximum hyperchromicities of 24%, 18%, and 10%, respectively. The noncomplementary oligomer **XV** shows little hyperchromic effect in the presence of DNG **II**.

It has been reported that the pentameric DNG **I** binds to homopurine strands in a 2:1 DNG:DNA ratio even in the presence of mismatches, although the mismatches significantly reduce the strength of association.[21,22] We see from the above

(31) Job, P. *Ann. Chim.* **1928**, *9*, 113.

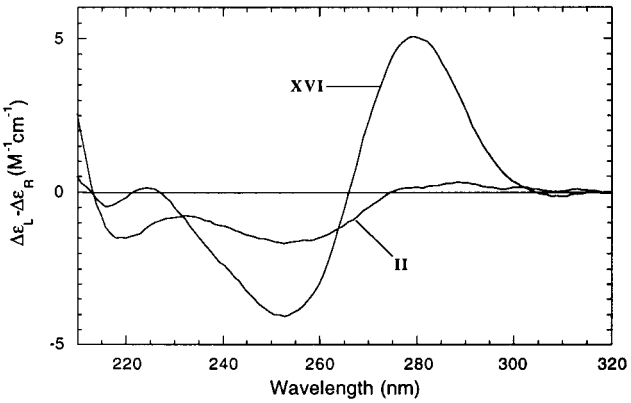


Figure 9. CD spectra of HO-T(pT)$_7$−OH (○) compared to DNG **II** (●). Solution conditions: 2.0×10^{-6} M oligomer, 0.1 M KCL, 10 mM phosphate buffer, pH 7.3 at 20 °C.

that mismatches due to inclusion of pyrimidine bases do not support the formation of a triplex under the experimental conditions.

Circular Dichroism Spectroscopy of Complementary and Mismatched DNA Oligomers with II. The CD spectrum of an oligomer in solution can give valuable information about its conformation as a single strand or in association with other DNA oligomers. The bases of DNA are planar and have no intrinsic CD signal of their own. Any CD signals observed in the absorbance band of the bases (230−300 nm) are caused by the spatial organization of the bases in a chiral structure, such as a helix, under the influence of the chiral sugar backbone. Base stacking interactions magnify this effect and give rise to the strong CD signals observed for DNA and RNA oligomers and polymers.[32] The CD spectra of the octameric thymidyl DNA oligomer HO-T(pT)$_7$-OH (**XVI**) and DNG **II** are compared in Figure 9. The DNA thymidyl oligomer shows a CD profile consistent with polymeric thymidyl DNA CD spectra.[33] The DNG, however, is very different. The positive CD band at 280 nm is not present in the CD spectrum of **II**, and the negative band at 255 nm is greatly attenuated. This seems to indicate that the nucleobases interact with each other more weakly in the oligothymidyl DNG **II** than in the case of the oligothymidyl DNA **XVI**. This may be due to the steric effect of the guanidinium linkage. The angle between the bonds of the tetrahedral phosphate diesters in the backbone of DNA is ∼109° whereas the angle between the bonds of a guanidium group is ∼120°. This wider angle may lead to a greater distance between the nucleoside bases in single-stranded DNG and, consequently, the bases would not stack as effectively as in single-stranded helical DNA. If this is the case, the positive charge attraction of the guanidinium backbone to the DNA target seems to overcome the penalty for the lack of preorganization and any strain induced in the sugar ring by the geometry of the guanidinium.

When DNG **II** is associated with complementary DNA, the CD spectrum does not match that of the theoretical spectrum calculated from the weighted sums of the CD spectra for the individual oligomers. The CD spectrum of the triple helical complex of DNG **II** with DNA **III** and the spectrum calculated for the weighted combination of uncomplexed **II** and **III** are shown in Figure 10. The differences in the spectra indicate that

(32) Johnson, W. C., Jr. In *Circular Dichroism and the Conformational Analysis of Biomolecules*; Fasman, G. D., Ed.; Plenum Press: New York, 1996; p 433.

(33) Gray, D. M.; Ratcliff, R. L.; Vaughan, M. R. *Methods Enzymol.* **1992**, *211*, 389.

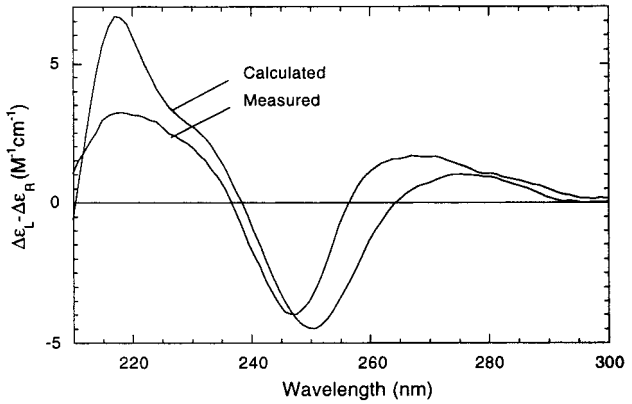


Figure 10. CD spectra of a 2:1 mixture of DNG **II** and DNA **III** compared to the calculated CD spectra for the combined unassociated oligomers.

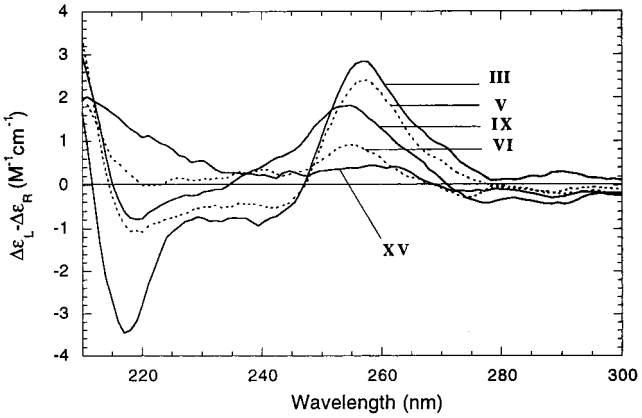


Figure 11. Difference CD spectra resulting from the subtraction of the calculated CD spectrum from that measured for 2:1 mixtures of DNG **II** and DNA oligomers **III**, **V**, **VI**,**IX**, and **XV**.

structural changes have taken place in the two oligomers because of their association. Difference spectra obtained from subtracting the measured CD for samples containing DNG **II** in the presence of DNA **III**, **V**, **VI**, **IX**, and **XV** from the calculated CD for the corresponding oligomer combinations are shown in Figure 11. The difference spectra show the largest changes for the complementary sequence **III** with DNG **II**. As mismatches are included, the difference spectra drop in amplitude, indicating that the strength of association is weakening. For the non-complementary oligomer **XV**, the difference indicates no association.

Computer modeling studies of an octameric thymidyl DNG oligomer with an octameric adenyl DNA oligomer for both double and triple helical binding modes have been reported in a previous study.[34] These studies suggested that the duplex and Watson−Crick portion of the triplex maintain an overall B-like conformation. This is consistent with the CD spectra of complementary DNA **III** with DNG **II** (Figure 10) which indicates a normal B-DNA based triple helix with a strong negative band at 247 nm and a weaker positive band above 260 nm.[33]

Conclusions

An effective and convenient solid-phase synthesis for oligomeric DNG has been developed. It features an efficient coupling reaction and a mild cleavage and deprotection protocol that will be compatible with all DNA bases. This method for synthesizing oligomeric guanidyl-linked thymidines is not limited to nucleosides and could, in principle, be useful for many oligomeric guanidine compounds. It is now possible to synthesize DNG oligomers of many different lengths and sequences in useful quantities.

Binding studies with short complementary and mismatched DNA oligomers indicate that the highly positively charged DNG oligomer **II** is able to discriminate between complementary and noncomplementary base pairs. Cytidyl mismatches in octameric adenyl DNA oligomers lower the Tm melting points by approximately 4 to 5 °C for each terminal mismatch and weaken the hyperchromic effect. Four terminal mismatches result in the loss of the hyperchomicity due to base pairing, leaving only weak nonspecific ionic association. Internal mismatches have a more profound effect with the majority of the hyperchromic effect removed after two internal mismatches. A single substitution near the center of the octameric adenyl oligomer **VIII** results in a reduction in the Tm of 15 °C in 0.1 M KCl. The CD spectrum of single-stranded **II** indicates that the thymidyl bases are not stacked in an organized helix, as in the case of the analogous octameric thymidine. CD spectra of **II**, in the presence of complementary and mismatched oligomers, compared to the calculated CD signal for the nonassociated oligomers indicate that the complex of **II** with the complementary oligomer **III** has the greatest change in CD spectra and the difference is reduced with increasing mismatches until no significant difference is seen for the association with non-complementary oligomer **XV**.

Experimental Section

Materials. HPLC grade solvents and sodium carbonate were obtained from Fisher Scientific. Anhydrous solvents, trichloroethoxychloroformate, triethylamine, 9-fluorenylmethylchloroformate, mercury(II) chloride, and potassium thiocyanate were purchased from Aldrich and used without further purification. Novabeads Rink amide MBHA resin (0.30 mequiv/g substitution) and 2% cross-linked polystyrene 2-(2-aminoethoxy)ethanol 2-chlorotrityl resin (0.31 meq/g substitution) was purchased from Novabiochem. All DNA oligomers were purchased prepurified from the Biological Resource Center at UCSF. Compounds **1**, **2**, and **3** were synthesized as described previously.[16]

General. Hydrogenations were carried out with a Parr hydrogenator equipped with a 500 mL hydrogenation vessel. Analytical reverse phase HPLCs were performed on a Hewlett-Packard 1050 system equipped with a quaternary solvent delivery system and UV detector set at 260 nm and a 2.1 × 250 mm C18 ODS-hypersil reverse phase column purchased from Altech. A gradient from 100% eluent A (100 mM triethylammonium acetate, pH 7.0) to 50% eluent A, 50% eluent B (acetonitrile) over 15 min with a flow rate of 1.3 mL/min was used. Analytical cation exchange HPLCs were performed with the same system with a 2.1 × 250 mm SCX ion exchange column purchased from Altech. A gradient from 100% eluent A (100 mM TRIS buffer, pH 7.0) to 100% eluent B (100 mM TRIS, 1.0 M guanidinium hydrochloride, pH 7.0) over 20 min with a flow rate of 1.3 mL/min was used. Preparative reverse phase HPLC were performed on the same system with a 10 × 250 mm C8 ODS-hypersil reverse phase column purchased from Altech. A gradient from 66% eluent A (100 mM triethylammonium acetate, pH 7.0), 33% eluent B (acetonitrile) to 20% eluent A, 50% eluent B over 15 min with a flow rate of 3.0 mL/min was used. Preparative cation exchange HPLCs were performed with the same system with a 10 × 250 mm SCX ion exchange column purchased from Altech. A gradient from 66% eluent A (water), 34% eluent B (3 M ammonium acetate) to 100% eluent B over 15 min with a flow rate of 3.0 mL/min was used. ^{1}H and ^{13}C NMR spectra were obtained on a Varian Unity 400 spectrometer at 400 and 100 MHz, respectively. UV spectra were obtained on a Cary 100 Bio UV/vis spectrophotometer equipped with a temperature programmable cell block. IR spectra were in KBr pellets with a Perkin-Elmer 1300

(34) Luo, J.; Bruice, T. C. *J. Chem. Soc.* **1998**, *120*, 1115.

spectrophotometer. TLC was carried out on silica gel (Kieselger 60 F_{254}) glass backed commercial plates and visualized by UV light.

Melting Studies. Thermal denaturation (Tm) plots were obtained by observing the absorbance at 260 nm of a solution of the oligomers in 1 cm path-length quartz cuvettes as the temperature was raised 0.2 °C/min from 20 to 95 °C. All samples had been previously annealed by cooling from 90 to 10 °C at 0.2 °C/min and stored at 10 °C for 2 days. Samples consisted of 2.5 μM DNG **II** and 1.25 μM DNA oligomer with 10 mM potassium phosphate buffer at pH 7.4 and either 0.1 or 0.3 M KCl for ionic strength control. For Tm determinations hyperchromicity was used. Data were recorded every 1 °C. Samples were covered with mineral oil to prevent evaporation.

Job Plots. Job[31] or continuous variation plots were obtained by mixing six samples at various ratios of DNG oligomer to DNA oligomer while maintaining the total concentration of nucleoside base at 2 μM. All solutions contained 10 mM potassium phosphate buffer at pH 7.4 and 0.1 M KCl. Solutions were mixed and left to equilibrate at room temperature for 24 h. DNA oligomer concentrations were determined spectrophotometrically using extinction coefficients provided by the manufacturer. The value of $\epsilon_{268} = 8700\ M^{-1}\ cm^{-1}$ was used for the extinction coefficient (per nucleoside base) of DNG **II**.[21]

Circular Dichroism Spectra. CD spectra were obtained on an OLIS RSM circular dichroism spectrophotometer. Scans were run from 350 to 210 nm taking a measurement every 1 nm. The integration time for each data point was 1.4 s. Ten scans were made of each sample and then averaged and smoothed using a 15 point exponential fitting algorithm. Samples were held in a 1 cm path-length cuvette and the temperature was maintained at 20 °C.

Methods: 5′-Benzoylthiourea-3-(9-fluorenylmethoxycarbamoyl)-3′,5′-deoxythymidine (5). Hydrogen sulfide gas was bubbled through a solution of **2** (2.05 g, 4.8 mmol) in 80 mL of 1:1 pyridine/water for 5 min until saturated, then the mixture was left to stir for 3 h. Argon gas was then bubbled through the mixture to remove the hydrogen sulfide. A fine yellow precipitate formed which was removed by filtration through a pad of Celite. The filtrate was evaporated to dryness and the residue was dissolved in 100 mL of dioxane. TEA (1.0 mL) was added and then 9-fluorenylmethoxychloroformate (1.15 g, 4.4 mmol). After 30 min TLC analysis indicated the reaction was complete. The mixture was evaporated to dryness and 100 mL of water was added, and the resulting slurry was extracted with ethyl acetate (3 × 150 mL). The organic extracts were dried with sodium sulfate, evaporated, and then purified by silica gel flash chromatography (3 × 20 cm silica column with 75% to 100% gradient of ethyl acetate in hexanes). TLC (EtOAc) $R_f = 0.62$; ^{1}H NMR (400 MHz DMSO-d_6) δ (ppm) 1.79 (3 H, s, thymine-CH_3), 2.20 (m, 1 H; 2′-H), 2.33 (m, 1 H; 2′-H), 3.90 (m, 1 H; 5′-H), 4.00 (m, 2 H; 5′-H, 4′-H), 4.16 (t, $J = 8$ Hz, 1 H; 3′-H), 4.22 (t, $J = 7$ Hz, 1 H; Fmoc-CH), 4.35 (m, 2 H; Fmoc-CH_2), 6.18 (t, $J = 7$ Hz, 1 H; 1′-H), 7.32 (t, $J = 7$ Hz, 2 H; Fmoc-2″-H), 7.40 (t, $J = 7$ Hz, 2 H; Fmoc-3″-H), 7.48 (t, $J = 8$ Hz, 2 H, benzoyl-*m*-H), 7.58 (s, 1 H; 6-H), 7.61 (t, $J = 8$ Hz, 1 H, benzoyl-*p*-H), 7.68 (m, 2 H; Fmoc-1″-H), 7.77 (d, $J = 8$ Hz, 1 H; Fmoc-NH), 7.88 (d, $J = 7$ Hz, 4 H; Fmoc-4″-H, benzoyl-*o*-H), 11.06 (t, $J = 5$ Hz, 1 H; 5′-NH), 11.36 (s, 1 H; thymine-NH), 11.46 (s, 1 H; Troc-NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.1, 36.0, 46.7, 51.7, 65.5, 73.8, 80.7, 83.3, 94.9, 109.9, 120.1, 125.1, 127.1, 127.6, 136.2, 140.8, 143.8, 150.4, 152.0, 155.8, 163.7, 179.6; IR (KBr pellet) 3034, 2956, 2857, 1704 (C═O), 1658 (C═O), 1516 (C═S), 1449, 1252, 1167, 1077, 740; MS (FAB) *m*/*z* 626 $(M + H)^+$; HRMS (FAB) *m*/*z* 626.20820, calcd for $C_{33}H_{31}N_5O_6S$ 626.20733.

5′-(Aminoiminomethanesulfonic acid)-3′-azido-3′,5′-deoxythymidine (4). To a 37% (w/w) solution of peracetic acid in acetic acid in an ice bath was added, by spatula, 250 mg of **3**. All the material dissolved and over the following 10 min a white precipitate appeared. The mixture was poured into 50 mL of cold ether and the precipitate was collected by filtration and dried under vacuum. While solid (227 mg) was collected (79% yield). ^{1}H NMR (400 MHz DMSO-d_6) δ (ppm) 1.79 (3 H, s, thymine-CH_3), 2.29 (m, 1 H; 2′-H), 2.34 (m, 1 H; 2′-H), 3.58 (m, 1 H; 5′-H), 3.62 (m, 1 H; 5′-H), 3.93 (dd, 1 H, 4′-H, $J = 6$, 10 Hz,), 4.38 (m, 1 H, 3′-H), 6.08 (t, $J = 7$ Hz, 1 H; 1′-H), 7.36 (d, 1 H, 6-H, $J = 1$ Hz), 9.31 (s, 1 H, SO_3H), 9.41 (s, 1H, imine-NH), 9.78 (t, $J = 6$ Hz, 1 H, 5′-NH), 11.37 (s, 1 H; thymine-NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.2, 35.2, 43.5, 60.7, 80.2, 83.1, 110.4, 135.2, 150.5, 163.8, 166.5; IR (KBr pellet) 3197, 3074, 2108 (azide), 1687 (C═O), 1479, 1269, 1055 (−SO_3H); MS (FAB) *m*/*z* 374 $(M + H)^+$; HRMS (FAB) *m*/*z* 374.09001, calcd for $C_{11}H_{15}N_7O_6S$ 374.08828.

5′-Trichloroethoxycarbonylthiourea-3′-azido-3′,5′-deoxythymidine (6). First, trichloroethoxycarbonylisothiocyanate (Troc-NCS) was synthesized using an adapted literature procedure.[35] A solution of potassium thiocyanate (10 g, 0.10 mole), potassium acetate (25 mg, 25 mmol), and pyridine (0.05 mL, 0.06 mmol) in 10 mL of water was made to which was added trichloroethylchloroformate (10 mL, 47 mmol) slowly over a period of 10 min while the temperature was maintained at 7 °C by a water bath. The resulting heterogeneous mixture was stirred vigorously for 9 h at 7 °C, and the progress of the reaction was monitored by gas chromatography. When all starting material was consumed, the reaction mixture was extracted once with 100 mL of DCM and the organic layer was collected, dried with sodium sulfate, and evaporated to a red oil. The product proved to be too unstable to purify or store effectively so the crude material was made as needed and used immediately without further purification. To a solution of **1** (1.1 g, 3.0 mmol) in 100 mL of dichloromethane was added 30 mL of trifluoroacetic acid. The solution was stirred for 20 min at room temperature and then evaporated to dryness using a rotary evaporator and kept under high vacuum overnight. The residue was a pale yellow foam. This was slurried in 50 mL of dichloromethane and cooled in an ice bath and then 1.3 mL of triethylamine (TEA) was added. Upon addition of the TEA the mixture became clear. To this mixture was added 0.70 g of the crude Troc-NCS and the solution was left to stir in the ice bath for 10 min. Then the solution was washed with 100 mL of water and the water layer was extracted with dichloromethane (2 × 50 mL). The combined organic layers were dried over sodium sulfate and concentrated to a foam using a rotary evaporator. The residue was purified by silica gel flash column chromatography (5 × 20 cm column with 75% to 100% gradient of ethyl acetate in hexanes). Fractions containing the product, **6**, were collected and evaporated under vacuum to give 0.98 g of yellow foam (65%). TLC (3:1, EtOAc:hexanes) $R_f = 0.72$; ^{1}H NMR (400 MHz DMSO-d_6) δ (ppm) 1.78 (3 H, s, thymine-CH_3), 2.33 (m, 1 H; 2′-H), 2.46 (m, 1 H; 2′-H), 3.75 (dt, 1 H; 5′-H, $J = 4$, 14 Hz), 4.00 (m, 1 H, 4′-H), 4.08 (m, 1 H; 5′-H), 4.38 (q, 1 H, 3′-H, $J = 7$ Hz), 4.91 (dd, 2 H; Troc-CH_2, $J = 12.4$, 16.8 Hz), 6.10 (t, $J = 7$ Hz, 1 H; 1′-H), 7.47 (d, 1 H, 6-H, $J = 1$ Hz), 9.85 (t, $J = 6$ Hz, 1 H, 5′-NH), 11.37 (s, 1 H; thymine-NH), 11.70 (s, 1 H; Troc-NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.1, 35.2, 45.7, 60.5, 73.8, 80.1, 83.5, 94.9, 110.0, 135.8, 150.4, 151.9, 163.8, 180.0; IR (KBr pellet) 3044, 2953, 2830, 2499, 2116 (azide), 1700 (C═O), 1524 (C═S), 1470, 1369, 1276, 1218, 1077, 1036, 959, 738; MS (FAB) *m*/*z* 500 $(M + H)^+$; HRMS (FAB) *m*/*z* 500.006931, calcd for $C_{14}H_{16}H_7O_5SCl_3$ 500.00775.

5′-(*N*′-Trichloroethoxycarbonylthiourea)-3′-(9-fluorenylmethoxycarboxamide)-3′,5′-deoxythymidine (7). To a solution of **6** (0.25 g, 0.50 mmol) in 50 mL of 95% ethanol was added 10 mg of 10% Pd/C and the mixture was hydrogenated at 50 PSI for 2 h then filtered through Celite. The filtrate was evaporated to dryness using a rotary evaporator and kept under high vacuum overnight. The solid residue was dissolved in a mixture of 20 mL of dioxane and 10 mL of 10% sodium carbonate in water. This mixture was cooled in an ice bath and 9-fluorenylmethylchloroformate (0.23 g, 0.89 mmol) was added slowly. The mixture was left to stir in the ice bath for 1 h until TLC analysis indicated that the reaction was complete. A crystalline precipitate had appeared during the reaction and 10 mL of water were added until it completely dissolved. Then the mixture was extracted with ethyl acetate (3 × 100 mL) and the organic layer was dried with sodium sulfate and evaporated to a white foam which was purified by silica gel flash column chromatography (5 × 20 cm column with 0 to 5% gradient of methanol in ethyl acetate). Fractions containing the product, **7**, were collected and evaporated to give 0.21 g of white foam (60% yield). TLC (3:1, EtOAc:hexanes) $R_f = 0.35$; ^{1}H NMR (400 MHz DMSO-d_6) δ (ppm) 1.79 (3 H, s, thymine-CH_3), 2.16 (m, 1 H; 2′-H), 2.30 (m, 1

(35) Wang, S. S.; Magliocco, L. G. U.S. Patent, American Cyanimid Company, 5194673, **1993**.

(36) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.

H; 2′-H), 3.83 (m, 1 H; 5′-H), 3.95 (m, 2 H; 5′-H, 4′-H), 4.10 (p, J = 7 Hz, 1 H; 4′-H), 4.22 (t, J = 7 Hz, 1 H; Fmoc-CH), 4.35 (m, 2 H; Fmoc-CH_2), 4.91 (dd, 2 H; Troc-CH_2, J = 12, 16.8 Hz), 6.13 (t, J = 6.8 Hz, 1 H; 1′-H), 7.32 (t, J = 7 Hz, 2 H; Fmoc-2″-H), 7.40 (t, J = 7 Hz, 2 H; Fmoc-3″-H), 7.52 (s, 1 H; 6-H), 7.69 (m, 2 H; Fmoc-1″-H), 7.75 (d, J = 8 Hz, 1 H; Fmoc-NH), 7.88 (d, J = 7 Hz, 2 H; Fmoc-4″-H), 9.83 (t, J = 5 Hz, 1 H; 5′-NH), 11.35 (s, 1 H; thymine-NH), 11.66 (s, 1 H; Troc-NH); ^{13}C NMR (100 MHz, DMSO-d_6) δ 12.1, 36.0, 46.7, 51.7, 65.5, 73.8, 80.7, 83.3, 94.9, 109.9, 120.1, 125.1, 127.1, 127.6, 136.2, 140.8, 143.8, 150.4, 152.0, 155.8, 163.7, 179.6; IR (KBr pellet) 3302, 3179, 3047, 2957, 1703 (C = O), 1683 (C═O), 1528 (C═S), 1451, 1257, 1218, 1080, 1036, 738; MS (FAB) *m*/*z*:: 696 $(M + H)^+$ HRMS *m*/*z* 696.0844, calcd for $C_{29}H_{29}N_5O_7SCl_3$ 696.0853.

Solid-Phase Syntheses. (a) Synthesis of Benzoyl-Protected Guanidyl Trimer (15). Rink amide resin (15 mg) was swelled in DMF for 2 h. Then the resin was loaded with N-α-Fmoc-glycine by addition of 1.0 mL of a 0.6 M solution of the peptide in DMF and 1.0 mL of a 1.6 M solution of DCC in DMF. The vial was agitated for 1 h and the resin was filtered and washed. **5** (16 mg, 0.025 mmol) was dissolved in 1 mL of DMF and poured over the beads. Then 0.5 mL of a 50 mM $HgCl_2$ solution in DMF and 0.5 mL of a 100 mM TEA solution in DMF were added rapidly and simultaneously to the monomer/resin mixture in the reaction tube via two syringes. A fine white precipitate formed immediately and the solution turned pale yellow. The tube was capped tightly and agitated for 2 h at room temperature. After this interval a black precipitate had replaced the white. The supernatant in the reaction tube was removed by filtration. Most of the fine black precipitate in this mixture passed through the filter. Repeated washing with DMF (3 × 2 mL) was able to remove all visible precipitate, but the resin beads were darkened from the precipitate contained within. A solution of 10% piperidine in DMF was poured over the beads and the reaction vial was agitated for 20 min. The supernatant was removed by filtration and the beads were washed with DMF (3 × 2 mL). At this point one nucleoside unit had been added to the resin and the 3′-terminal amine deprotected (cleavage would give **10**, see Scheme 3). The coupling/deprotection cycle was repeated to give **11** and repeated again to give **16**. Resin samples were cleaved after each piperidine deprotection step of the cycle to analyze the products. HPLC indicated high coupling yields and FAB MS confirmed the presence of the expected molecular ions for the products. Figure 1 shows the HPLC chromatogram for each step of the trimer synthesis. *m*/*z* for **10**, 444; *m*/*z* for **11**, 813; *m*/*z* for **16**, 1182.

(b) Synthesis of Troc-Protected Guanidyl Octamer (16). Fifteen milligrams of the commercially available 2% cross-linked polystyrene 2-(2-aminoethoxy)ethanol 2-chlorotrityl resin were placed in a screw-capped reaction tube fitted with a coarse glass frit filter and a stopcock and swelled in dry DMF for 2 h. Then the resin was filtered and enough DMF was added to just barely cover the beads. **7** (22 mg) was placed in a small vial and dissolved in 1 mL of dry DMF. This was transferred via syringe to the reaction tube. Then 0.5 mL of a 50 mM $HgCl_2$ solution in DMF and 0.5 mL of a 100 mM TEA solution in DMF were added as described above. After 2 h the resin was filtered and washed and the beads were gray from the mercury salt within. Then the resin beads were briefly treated with a 20% thiophenol solution in DMF (2 mL for 10 s) and the beads instantly returned to their original yellow color as the black precipitate was removed. The supernatant was removed by filtration of the beads and the resin was washed with DMF repeatedly (5 × 2 mL). The coupling/washing/deprotection cycle was repeated seven more times to give the octameric thymidyl oligomer with protected guanidinium linkages.

(c) Cleavage and Deprotection of Troc-Protected Guanidyl Octamer. The resin was washed with DCM (2 × 2 mL), acetic acid (2 × 2 mL), and methanol (2 × 2 mL) and dried under vacuum in the presence of KOH desiccant. Then the beads were swelled in 2 mL of DCM for 1 h. Then 0.5 mL of TFA was added and the beads immediately turned dark red in color indicating the formation of the chlorotrityl cation. The reaction tube was capped and agitated for 10 min then filtered. The filtrate was added dropwise to 30 mL of cold ether. A white precipitate immediately formed and was collected by centrifugation of the ether mixture. The supernatant was poured off and the pellet was dried by a stream of nitrogen. Reverse-phase HPLC showed this crude product to be approximately 75% pure indicating an average coupling yield of 97%. This crude mixture was purified preparative HPLC (10 × 250 mm Macrosphere 300A 7μ C8 silica column, 0.1 M TEAA, pH 7.0 with a gradient of 0 to 80% acetonitrile over 20 min, 3.0 mL/min) and the fractions containing the product were evaporated using a centrifugal evaporator. The residue was then dissolved in 2 mL of glacial acetic acid, 100 mg of cadmium powder was added, and the mixture was vigorously shaken for 1 h. The mixture was centrifuged and the supernatant was decanted and lyophilized. Analytical cation exchange HPLC (4.6 × 100 mm Macrosphere 300A 7μ SCX column, buffer A: 50 mM Tris, pH 7.0; buffer B: 50 mM Tris, 1 M guanidinium hydrochloride, gradient 0 to 50% B over 15 min, 1.3 mL/min) showed complete deprotection with no side products. The deprotected product mixture was purified by cation exchange HPLC on a 10 × 250 mm SCX column (10 × 250 mm Macrosphere 300A 7μ SCX column, 0 to 3 M ammonium acetate gradient over 15 min, 3.0 mL/min). The fractions containing the product were lyophilized, redissolved in water, and lyophilized again to evaporate the ammonium acetate buffer. The residue was dissolved in 2 mL of water and optical density indicated that 1.6 mg of purified **II** was present.

Acknowledgment. This research was supported by grants from the National Institute of Health and Genelabs Technologies, Inc.

Supporting Information Available: Details of the initial solid-phase synthesis via oxidized thiourea **4** is described in detail and the cation exchange HPLC of purified DNG **II** and CD spectra of a 2:1 solution of DNG **II** and DNA oligomers **V**, **VI**, **IX**, and **XV** and their comparison to the CD spectrum calculated from the CD spectra of the constituent oligomers (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

JA984212W