determined by 360-MHz ^{1}H NMR[4] (74%). Also, a 21% yield of benzyl alcohol was obtained from the preparative plate.

Cyclization of 44 with Bromine. A solution of 220 mg of **44** (0.43 mmol) in 2 mL of CCl_4 was treated with 25 μL of bromine (0.45 mmol). After 1 h, the solution was loaded onto a 2000-μm preparative TLC plate and eluted with EtOAc/hexane, 1:9. This resulted in 140 mg (65%) of pure **6a** and **6b** in a ratio of 10:90 (by 360-MHz ^{1}H NMR) and 45 mg (60%) of benzyl bromide.

Attempted Cyclization of 44 with Mercuric Acetate. A solution of 200 mg (0.394 mmol) of **44** in 4 mL of THF was treated with 200 mg of mercuric acetate (200 mg, 0.63 mmol) and 90 mg of $CaCO_3$. After refluxing for 16 h, very little change in the TLC of the solution had occurred; starting material was largely unchanged.

Attempted Cyclization of 44 with *N*-Bromosuccinimide. A solution of 100 mg (0.2 mmol) of **44** and 71 mg of freshly recrystallized NBS (0.4 mmol) in 10 mL of methylene chloride was heated at reflux. After 24 h, the predominant spot by TLC was still starting **44**.

3,4,6-Tri-*O*-benzyl-5-(dichlorobenzyl)-1,2-dideoxy-D-gluc-1-enitol (50). This material was prepared in the same manner as described for **44**, giving **50** (30% yield) after recrystallization from MeOH/H_2O (mp 69–79 °C): 90-MHz ^{1}H NMR δ 3.6–4.2 (m, 5 H), 4.2–4.9 (m, 8 H), 4.9–6.2 (m, 3 H), 6.9–7.3 (m, 18 H); fast atom bombardment (FAB-MS), *m/e* 577. Anal. Calcd for $C_{34}H_{34}Cl_2O_4$: C, 70.71; H, 5.93. Found: C, 70.01; H, 5.87.

Cyclization of 50 with Bromine. To a solution of **50** (20 mg, 0.035 mmol) in 0.5 mL of methylene chloride was added 2.2 μL of bromine (0.043 mmol) via syringe. After 10 min, the solution was loaded onto a 1000-μm preparative TLC plate and chromatographed with EtOAc/hexane (15/85) to give 16 mg (90% yield) of a 3.7:96.3 ratio of **6a**:**6b**.

Acknowledgment. We thank Dr. M. Blumenstein, M. Mutter, and S. Campbell for NMR spectral data and Dr. G. Caldwell and J. Masucci for mass spectral data. Experimental details for the preparation of **27** were kindly provided by Prof. W. R. Rousch prior to publication. Valuable discussions with K. Robarge and Prof. F. Freeman (University of California at Irvine) are greatly appreciated.

Registry No. 1, 57445-90-6; **5**, 102208-53-7; **6a**, 102208-56-0; **6b**, 102093-88-9; **7a**, 109065-80-7; **7b**, 109121-38-2; **8a**, 109065-81-8; **8b**, 109121-39-3; **9a**, 109065-82-9; **9b**, 109121-40-6; **10a**, 109065-83-0; **10b**, 109065-84-1; **12a**, 102208-55-9; **12b**, 102208-54-8; **13**, 80959-53-1; **14b**, 100590-12-3; **15b**, 109066-14-0; **16a**, 109066-00-4; **16b**, 109066-01-5; **17**, 109065-85-2; **18**, 109066-05-9; **19**, 109066-06-0; **20b**, 109065-92-1; **21b**, 109066-15-1; **22a**, 109066-02-6; **22b**, 109066-03-7; **23**, 109065-86-3; **24a**, 109065-93-2; **25a**, 109066-16-2; **26a**, 109066-04-8; **27**, 109065-87-4; **28**, 88406-01-3; **29**, 109121-41-7; **30** (isomer 1), 109066-07-1; **30** (isomer 2), 109121-42-8; **31**, 109066-08-2; **32a**, 109066-09-3; **32b**, 109121-43-9; **33a**, 109066-17-3; **33b**, 109121-45-1; **34a**, 109066-10-6; **34b**, 109121-44-0; **35**, 51174-44-8; *cis*-**36**, 109066-11-7; *trans*-**36**, 109066-12-8; **37a**, 109065-89-6; **37b**, 109065-88-5; **38a**, 109065-94-3; **38b**, 109065-95-4; **39a**, 109065-97-6; **39b**, 109065-98-7; **40**, 109065-91-0; **41a**, 109065-90-9; **42a**, 109065-96-5; **43a**, 109065-99-8; **44**, 102093-89-0; **50**, 109066-13-9; acetic acid, 64-19-7; acrolein, 107-02-8; 3-hydroxypropanenitrile, 109-78-4; 3-(methoxymethoxy)propanenitrile, 52406-33-4; vinylmagnesium bromide, 1826-67-1; (trimethylsilyl)acetylene, 1066-54-2; methyltriphenylphosphonium bromide, 1779-49-3; ethyltriphenylphosphonium bromide, 1530-32-1.

Uncharged Stereoregular Nucleic Acid Analogues. 1. Synthesis of a Cytosine-Containing Oligomer with Carbamate Internucleoside Linkages

Eugene P. Stirchak* and James E. Summerton

Antivirals, Inc., Corvallis, Oregon 97333

Dwight D. Weller

Department of Chemistry, Oregon State University, Corvallis, Oregon 97331

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The uncharged, stereoregular nucleic acid analogue **18**, with carbamate internucleoside linkages, was prepared. A block synthesis scheme was used to prepare **18**, using the dimer **11** as the basic unit. The oligomer **18** was shown to strongly bind to p(dG_6) and poly(G).

A variety of nucleic acid analogues containing wholly or substantially uncharged backbones have been shown to enter living animal cells and to be resistant to nucleolytic degradation therein.[1-3] Paul Miller has shown that one class of uncharged nucleic acid analogues significantly inhibit the intracellular activity of genetic sequences to which the analogues are complementary.[3] These results suggest that uncharged nucleic acid analogues may be of value for the study of genetic mechanisms, for the treatment of viral diseases, and possibly as anticancer agents. Further, suitable nucleic acid analogues lacking charges on their backbones can be exploited to considerable advantage in a diagnostic system having high specificity and sensitivity.

For analogues of nucleic acids to be suitable for such applications as mentioned above, we postulated that certain structural criteria are required to effect efficient hybridization of the analogue with its targeted genetic sequence. These criteria include a stereoregular backbone (required for a homogeneous binding constant between the analogue and its complementary genetic sequence), proper spacing and orientation of the component bases for hybridization, and analogue affinities for targeted genetic sequences that are sufficient to effect the desired biological or physical action. We believe certain internucleoside linkages containing carbonyl moieties, particularly carbamates **4**, satisfy the above criteria and should constitute

(1) Pitha, P. M.; Teich, N. M.; Lowy, D. R.; Pitha, J. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 1204.
(2) Karpopva, G. G.; Knorre, D. G.; Ryte, A. S.; Stephanovich, L. E. *FEBS Lett.* **1980**, *122*, 21.
(3) (a) Jayarman, K.; McParland, K.; Miller, P. S.; Ts'o, P. O. P. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *78*, 1537. (b) Blake, K. R.; Murakami, A.; Spitz, S. A.; Glave, S. A.; Reddy, M. P.; Ts'o, P. O. P.; Miller, P. S. *Biochemistry* **1985**, *24*, 6139. (c) Miller, P. S.; Agris, C. H.; Aurelian, L.; Blake, K. R.; Murakami, A.; Reddy, M. P.; Spitz, S. A.; Ts'o, P. O. P. *Biochim.* **1985**, *67*, 769.

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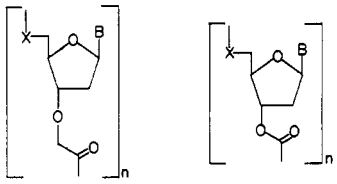
a valuable class of nucleic acid analogues.

Several examples of carbonyl-linked oligonucleosides have appeared in the literature. Most complete is the report by Jones on the acetate-linked analogues **1**, which have been shown to interact weakly with polynucleotides and to interfere with protein biosynthesis in a cell-free system.[4] Unfortunately, **1** is rapidly degraded in neutral aqueous solutions to its component nucleosides. The corresponding acetamido-linked derivatives **2**, while of increased stability compared to **1**, failed to hybridize to complementary polynucleotides.[5] Carbonate-linked oligonucleotides **3** have also been prepared[6] but their interaction with nucleic acids has not been reported. Dimeric



1, X = O
2, X = NH

3, X = O
4, X = NH

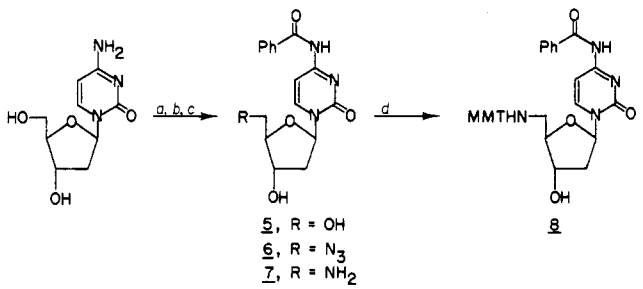
B = Purine or Pyrimidine

and trimeric carbamate-linked derivatives **4**, prepared by Jones[7] and later by Mungall,[8] were reported to have excellent resistance to both chemical and enzymatic degradation. However, neither Jones or Mungall reported binding studies of their carbamate-linked oligonucleosides to complementary nucleic acids and there remained a serious question as to whether such nucleic acid analogues could effectively bind their targeted genetic sequences.

While the carbamate bond lengths and angles vary from those of the phosphodiester, our CPK molecular modeling suggested that there was sufficient flexibility in the carbamate-linked deoxyribose backbone to accommodate Watson–Crick pairing of the analogue to a complementary nucleic acid. A hexamer of cytosine (**18**) was chosen as our synthetic objective to confirm this prediction. The choice of cytosine follows from the expected substantial avidity of **18** for guanine-containing polynucleotides, which allows analysis of oligocarbamate binding to nucleic acids using relatively shorter oligomers than would be possible with thymine- or adenine-containing species. Described below is the preparation, characterization, and preliminary binding studies of the cytosine-containing, carbamate-linked oligomer **18**.

As in previous syntheses, we planned to prepare the carbamate linkage by the reaction of a 5′-amino nucleoside with a 3′-carbonate derivative.[8] In the earlier work an unmodified nucleoside was used for the 5′ terminal unit.[8] We chose to incorporate a 5′-amino nucleoside at the 5′ terminus. With the choice of a suitable amino protecting group this strategy allows for efficient block synthesis of the oligomers and simplifies the adoption of solid-phase synthetic methodology. Additionally, a free amine at the 5′ terminus of carbamate-linked oligomers, protonated at physiological pH, would increase the solubility of the oligomers.[9] The basic subunit was configured from 2′-deoxycytidine as follows (Scheme I). The exocyclic amine of 2′-deoxycytidine was protected with benzoyl chloride[10] and the 5′-hydroxyl converted to azide **6**.[11] Reduction of nucleoside azides via catalytic hydrogenation had literature procedence[12] but afforded in this case mixtures of the desired product **7** and materials derived from cleavage of the N-4 benzoyl group. The presence of an excess of acetic acid to the hydrogenolysis of the azide avoided these side reactions. However, while the amine remained protonated under these conditions at ambient temperature, heating solutions of the amine acetate salt in order to evaporate DMF and excess acetic acid resulted in transacylation products. Addition of 1 equiv of *p*-toluenesulfonic acid at the completion of the azide reduction, but prior to the workup, afforded clean amine **7** as the tosic acid salt. The tosylate of 5′-amino-2′,5′-dideoxycytidine **7** was not purified but carried on directly to the monomethoxytrityl (MMT) amine **8**.[13] Although the MMT group is very sensitive to acid, purification of **8** may be achieved by silica gel chromatography in the presence of a weak base such as dimethylaniline.

Scheme I[a]



5, R = OH
6, R = N_3
7, R = NH_2

8

[a] (a) TMS-Cl, PhC(O)Cl; (b) LiN_3, Ph_3P, CBr_4; (c) H_2, Pd/C, HOAc; (d) MMT-Cl, Et_3N, pyridine. MMT = monomethoxytrityl.

In order to introduce the carbamate linkage between two subunits the nucleoside 3′-hydroxyl substituent was activated by conversion of **8** to 4-nitrophenyl carbonate **9**. This was achieved in 67% yield by the reaction of **8** with bis(4-nitrophenyl) carbonate in DMF in the presence of triethylamine. While *N*-methylimidazole and 4-(dimethylamino)pyridine also served as efficient catalysts, triethylamine was employed due to its ease of removal by evaporation. The activated monomer **9** was carefully chromatographed to remove unactivated monomer **8**. Compound **9** proved to be moderately stable and could be stored under anhydrous conditions.[14] An alternative strategy involving reaction of **8** with carbonyldiimidazole was less attractive due to the instability of the active carbamate **10** to chromatography. Removal of the monomethoxytrityl group in **8** was achieved by using glacial acetic acid in MeOH/THF (5:5:1). Amine **7** was again

(4) (a) Bleaney, R. C.; Jones, A. S.; Walker, R. I. *Nucleic Acid Res.* **1975**, *2*, 699. (b) Jones, A. S.; MacCoss, M.; Walker, R. I. *Biochim. Biophys. Acta* **1973**, *365*, 365.

(5) Gait, M. J.; Jones, A. S.; Jones, M. D.; Shepard, M. J.; Walker, R. T. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1389.

(6) (a) Mertes, M. P.; Coats, E. A. *J. Med. Chem.* **1969**, *12*, 154. (b) Jones, D. S.; Tittensor, J. R. *J. Chem. Soc. D* **1969**, 1240. (c) Tittensor, J. R. *J. Chem. Soc. C* **1971**, 2656.

(7) Gait, M. J.; Jones, A. S.; Walker, R. J. *J. Chem. Soc., Perkin Trans. 1* **1974**, 1684.

(8) Mungall, W. S.; Kaiser, J. K. *J. Org. Chem.* **1977**, *42*, 703.

(9) Paul S. Miller, personal communication. Professor Miller found that incorporation of a charge on phosphonate oligomers (one phosphodiester linkage per oligomer) did not adversely affect the ability of the oligomer to enter cells or inhibit hybridization of the nucleoside with its target.

(10) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316.

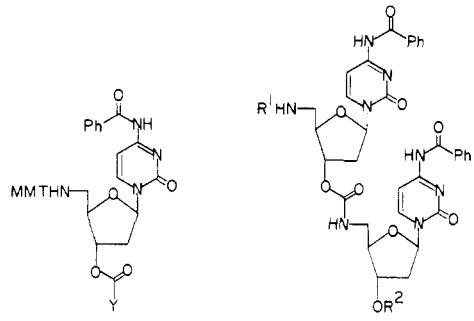
(11) Yamamoto, I.; Sekine, M.; Hata, T. *J. Chem. Soc., Perkin Trans. 1* **1980**, 306.

(12) Horwitz, J. P.; Tomson, A. J.; Urbanski, J. A.; Chua, J. *J. Org. Chem.* **1962**, *27*, 3045.

(13) Lapidot, Y.; de Groot, N.; Weiss, M.; Pelep, R.; Wolman, Y. *Biochim. Biophys. Acta* **1967**, *138*, 241.

(14) All failure sequences found in the desired oligomers after coupling reactions could be traced back to the presence of unactivated material in the 3′-(4-nitrophenyl) carbonates. The care taken in purification and storage of the activated species, especially in the preparation of longer oligomers (>dimers), is critical.

isolated as the tosylate and used without further purification. The coupling of the activated nucleoside **9** with the tosylate of **7** was effected at room temperature in DMF containing diisopropylethylamine. The coupling occurred selectively at the amine terminus of **7**, giving **11** in 73% yield. Only a trace of the 3′–3′ coupled product was isolated.



9, Y = -O-(p-NO$_2$-Ph)

10, Y = -N(imidazolyl)

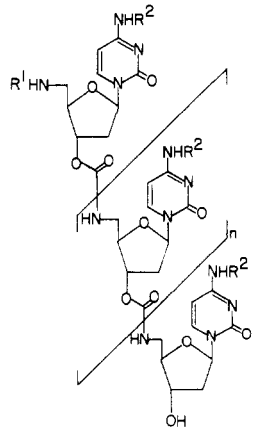
11, R^1 = MMT, R^2 = H

12, R^1 = MMT, R^2 = C(O)OPNP

13, R^1 = R^2 = H

PNP = p-Nitrophenyl

Our basic building block for the preparation of the hexamer was dimer **11**, which could be homologated from either the 3′ or the 5′ terminus. Activation of **11** was effected in the manner as for **8** in satisfactory yield (75%), but due to the sensitivity of **12** toward hydrolysis, greater care was required in its handling.[14,15] Removal of the 5′ blocking group of **11** with acetic acid/methanol proceeded rapidly to afford the 3′ terminus for the growing oligomer, **13**. Coupling of the activated dimer **12** with an excess of amine **13** proceeded smoothly at ambient temperature and furnished the tetramer **14** in 62% yield. The sequence of removing the 5′ blocking group of the growing oligomer **14** followed by coupling of the resulting amine **15** and **12** was repeated to afford the hexamer **16**. Structural verification of **11**, **14**, and **16** was found in the ^{1}H NMR and mass spectra of the individual compounds. Elemental analyses of these oligomers were consistent with molecular formulas that included one water molecule per residue of the oligomer.



14, R^1 = MMT, R^2 = C(O)Ph, n = 2

15, R^1 = H, R^2 = C(O)Ph, n = 2

16, R^1 = MMT, R^2 = C(O)Ph, n = 4

17, R^1 = MMT, R^2 = H, n = 4

18, R^1 = H, R^2 = H, n = 4

(15) Extensive decomposition of the 3′-(4-nitrophenyl) carbonate **9** occurred over 5 min upon the addition of D_2O to a sample of **9** in DMSO-d_6.

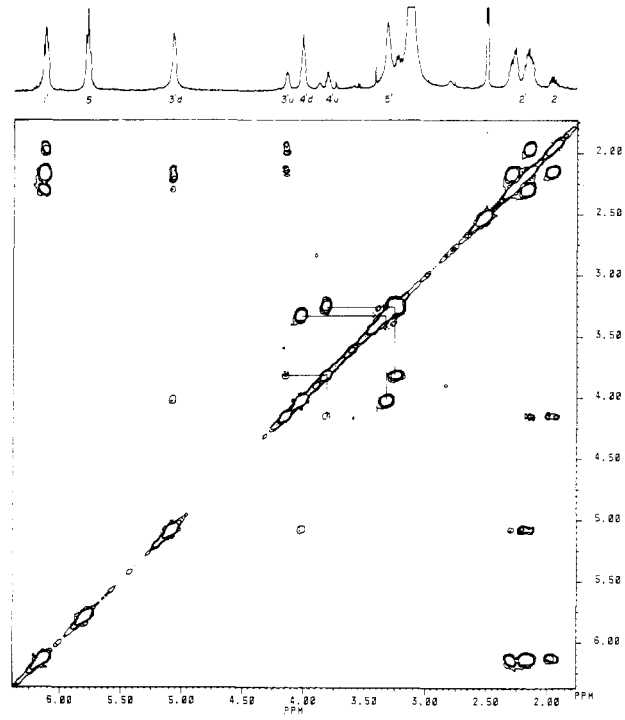


Figure 1. COSY spectrum of **18**. u = upfield signal of set; d = downfield signal of set.

Hexamer **16** was subjected to aqueous ammonia in DMSO for 24 h at 30 °C to effect complete removal of the benzoyl groups. The use of ethylenediamine as a deacylating reagent[16] was also explored. While cleavage of the base protecting groups was rapid (5 h at room temperature), transamination at N4 of cytosine by ethylenediamine occurred to an unacceptably large extent. The de-tritylation of hexamer **17** with acetic acid/methanol (2/1) proceeded smoothly to give the fully deprotected hexamer **18**.

Initial purification of the deprotected hexamer **18** was secured by precipitation of the hexamer from 0.01 N HCl by the addition of pH 7.4 buffer. Final purification was achieved by reverse-phase HPLC (RP-18), eluting with pH 4 acetate buffer/methanol gradients. Structural characterization of **18** was achieved by 400-MHz ^{1}H NMR studies. A COSY[17] plot of **18** (see Figure 1) revealed two sets of signals each for the 2′, 3′, and 4′ protons. Furthermore, the upfield signals of each of the sets appeared to account for only one proton and were coupled to each other but not to the other 3′ and 4′ protons, indicating that these protons are attached to the same deoxyribose. The NMR spectra of the precursors to **18** showed resonances for the 3′ and 4′ protons upfield for sugar residues which were not acylated at the 3′ hydroxyl relative to those that were acylated (e.g., in **8** the 3′H, 4′H occur at 4.15 and 3.94 ppm compared to **9** with 3′H, 4′H at 5.38 and 4.37 ppm, respectively). This led to assigning the upfield set of signals in **18** to the 3′ terminal deoxyribose unit. Integration of the simple ^{1}H NMR spectrum of **18** and comparison of the integrals, for example, of 1′-protons to the 3′ terminal proton (6 to 1), defined the length of the oligomer as 6 units (see Table I). The mass spectrum of **18** confirmed the structure assignment.

A thermal melt study of **18** with p(dG$_6$) and poly(G) gave melt temperatures of 70 and 79 °C, respectively (p(dC$_6$)

(16) Miller, P. S.; Reddy, M. P.; Murakami, A.; Blake, K. R.; Lin, S.-B.; Agris, C. H. *Biochemistry* **1986**, *25*, 5092.

(17) For a general reference to COSY, see: Bax, A. *Two-Dimensional Nuclear Magnetic Resonance in Liquids*, Reidel: London, 1982.

Table I. Integrations of ^{1}H NMR Signals of 18[a]

proton	chem shift (ppm)[b]	integrn[c]	proton	chem shift (ppm)[b]	integrn[c]
6	7.65	6.00	3'u	4.10	1.29
1'	6.11	6.00	4'd	3.97	4.69
5	5.78	6.00	4'u	3.77	1.09
3'd	5.01	5.15			

[a] Standard nucleic acid numbering. u = upfield signal of set; d = downfield signal of set. [b] Chemical shifts relative to DMSO-d_6 (2.49 ppm). [c] Integrations relative to the 1' proton signal.

with p(dG_6) and poly(G) gave melts of 32 and 40 °C, respectively). Full binding studies of 18 will be reported elsewhere. Work in progress includes the preparation of carbamate-linked heteropolymers.

Note Added in Proof. After submission of this manuscript a paper describing the preparation and physical studies of a carbamate-linked oligonucleoside hexamer of thymidine was published (Coull, J. M.; Carlson, D. V.; Weith, H. L. *Tetrahedron Lett.* **1987**, *28*, 745). The hexamer of thymidine was found not to interact with either p(A_6) of p(dA_6). This results serves to reinforce the need for employing cytosine-containing oligomers when working with relatively short sequences.

Experimental Section

General. NMR spectra were obtained with a Bruker 400-MHz NMR. All samples were DMSO-d_6 solutions with a few drops of D_2O. Chemical shifts are reported (ppm) relative to DMSO (2.49 ppm). IR spectra were obtained on a Perkin-Elmer Model 237 infrared spectrophotometer. The IR spectra of these oligomers were not particularly insightful with the exception of the *p*-nitrophenyl carbonates **9** and **12**. Negative ion mass spectra were obtained with a Kratos MS-50TC high resolution mass spectrometer. The sample matrix consisted of the oligonucleoside dissolved in thioglycerol. The primary xenon atom, with 8 keV kinetic energy, was directed at a target sample of 5 mm^2. Calibration was achieved with dry cesium iodide. HPLC purifications were performed on a Altex Beckmann Model 332 HPLC system, employing an Ultrasphere C-18 semipreparative column and eluting with gradients of 0.1 N acetic acid–sodium acetate buffer adjusted to pH 4)/methanol where methanol varies from 20% to 45%. Elemental analyses were performed by Micnal Inc., Tuscon, AZ.

TLC was preformed on Baker silica gel 1BF flex plates. Column chromatography was carried out on silica gel, Merck, grade 60, 230–400 mesh, with all eluting solvents containing 0.1% dimethylaniline (DMA). Triethylamine, dimethylformamide (DMF), and pyridine were distilled from CaH_2 under N_2 and stored over activated sieves. Tetrahydrofuran (THF) was distilled from lithium aluminum hydride. DEAE cellulose was prepared for use by treatment in succession with methanol (MeOH), H_2O, concentrated ammonia, H_2O, MeOH, and THF/MeOH (3/1).

The physical studies of the synthetic oligomer were performed by using a double-beam 100-80A Hitachi UV–vis spectrophotometer. The temperature was regulated by circulating water from a constant-temperature bath through the cell holder and monitored by a probe immersed in the cell holder block.

5'-Amino-N^4-benzoyl-2',5'-dideoxycytidine (7). Azide **6** (1.980 g, 5.56 mmol) was dissolved in EtOH (100 mL) and DMF (100 mL). Glacial acetic acid (10 mL) was added to the hydrogenation vessel followed by the 10% Pd/C catalyst (260 mg). The reaction vessel was charged with hydrogen (35 psi) and shaken for 18.5 h. After the heterogenous solution was filtered, *p*-toluenesulfonic acid monohydrate (1.060 g, 5.56 mmol) was added to the reaction flask as a methanol solution. The filtrate was evaporated to near dryness, the residue taken up in water, and the resulting solution extracted three times with ethyl acetate. The aqueous solution was evaporated and the residue twice evaporated from DMF. The amine was usually carried on directly; however, for analytical analysis a small amount of the crude amine salt was treated with DEAE cellulose in THF/MeOH (3/1), and the solution was filtered and evaporated to dryness without heating. The residue was dissolved in a minimum of THF (ca. 100 mg in 0.5 mL) and precipitated by addition of the THF solution to an exces of hexanes/toluene (4/1, ca. 50 mL). The amine was collected and dried under vacuum to afford a noncrystalline solid. **7**: mp 142–143.5 °C (lit.[18] mp 143 °C, dec); ^{1}H NMR δ 8.39 (1 H, d, J = 7.46 Hz), 8.00 (2 H, d, J = 7.42 Hz), 7.62 (1 H, t, J = 7.36 Hz), 7.51 (2 H, t, J = 7.70 Hz), 7.35 (1 H, d, J = 7.51 Hz), 6.12 (1 H, d, J = 6.42 Hz), 4.19 (1 H, m), 3.79 (1 H, m), 2.77 (2 H, d, J = 5.22 Hz), 2.30 (1 H, m), 2.07 (1 H, m).

N^4-Benzoyl-2',5'-dideoxy-5'-(4''-monomethoxytrityl)-aminocytidine (8). The tosylate of amine **7** (crude material from the reduction step, ca. 5.3 mmol) was evaporated twice from pyridine. After the amine was suspended in pyridine (26 mL), triethylamine (2.2 mL, 15.9 mmol) and then *p*-anisylchlorodiphenylmethane (3.342 g, 10.8 mmol) were added to the reaction vessel. The solution was stirred for 4 h and then diluted with $CHCl_3$ to 200 mL. The resulting solution was extracted with 2% aqueous $NaHCO_3$ and then with water. The organic fraction was separated, dried (Na_2SO_4), and evaporated to dryness. The residue was chromatographed on SiO_2 (300 g) by elution with 5% MeOH/95% $CHCl_3$. The eluant was evaporated to dryness and the residue was dissolved in 3 mL of THF and the product precipitated by addition of the THF solution to an excess of hexanes/toluene (3/1) to afford 1.728 g (55% from azide **6**) of **8**. While this afforded material suitable for further use, an analytical sample was prepared by repeated precipitation of **8** as above. **8**: ^{1}H NMR δ 8.12 (1 H, d, J = 7.46 Hz), 8.00 (2 H, d, J = 7.36 Hz), 7.70–7.15 (13 H, m), 6.86 (2 H, d, J = 8.80 Hz), 6.12 (1 H, dd, J = 5.99, 5.99 Hz), 4.15 (1 H, m), 3.94 (1 H, m), 3.72 (3 H, s), 2.49–2.00 (4 H, m); mass spectrum, M – 1 601 (100), 214 (67).

Anal. Calcd for $C_{36}H_{34}N_4O_5 \cdot H_2O$: C, 69.64; H, 5.85; N, 9.03. Found: C, 69.99; H, 5.44; N, 8.83.

***p*-Nitrophenyl N^4-Benzoyl-2',5'-dideoxy-5'-(4''-monomethoxytrityl)aminocytidine 3'-Carbonate (9).** Bis(4-nitrophenyl) carbonate (1.594 g, 5.24 mmol) was evaporated from DMF (20 mL) twice. The carbonate was dissolved in DMF (8 mL) and to this was added triethylamine (0.4 mL, 2.9 mmol). Tritylamine **8** (1.587 g, 2.64 mmol) (twice evaporated from 25 mL of DMF) was added to the reaction vessel as a DMF solution (5 mL). After being stirred for 2.5 h, the solution volume was reduced under vacuum. The residue was dissolved in $CHCl_3$ and the resulting solution extracted twice with 0.01 N aqueous NaOH and once with H_2O and then dried (Na_2SO_4). The solvent was evaporated and the residue was chromatographed on SiO_2 (100 g) eluting initially with 1% MeOH/99% $CHCl_3$ (250 mL) and then with 3% MeOH/97% $CHCl_3$. After the eluant was evaporated, the residue was dissolved in 8 mL of THF and this solution was added to an excess of hexanes/toluene (3/1). The precipitate was collected and dried to afford 1.363 g (67%) of **9**. **9**: IR ν 1770 cm^{-1}; ^{1}H NMR δ 8.34 (2 H, d, J = 8.86 Hz), 8.25 (1 H, d, J = 7.53 Hz), 8.00 (2 H, d, J = 7.69 Hz), 7.70–7.10 (18 H, m), 6.84 (2 H, d, J = 8.66 Hz), 6.17 (1 H, m), 5.38 (1 H, m), 4.37 (1 H, m), 3.71 (3 H, s), 2.80–2.20 (4 H, m).

N^4-Benzoyl-2',5'-dideoxy-5'-(4''-monomethoxytrityl)-aminocytidylyl-(3'–5'')-carbamoyl)-5'-amino-N^4-benzoyl-2',5'-dideoxycytidine (11). Tritylated amine **8** (930 mg, 1.54 mmol) was taken up in THF (2 mL), glacial acetic acid (10 mL), and MeOH (10 mL). After 5 h at room temperature, *p*-toluenesulfonic acid monohydrate (290 mg, 1.54 mmol) was introduced into the reaction vessel as a methanol solution. The solvent was removed under vacuum and the residue was twice evaporated from DMF (20 mL each). The amine salt was redissolved in DMF (5 mL) and to this solution was added triethylamine (0.5 mL, 3.6 mmol) and the activated monomer **9** (837 mg, 1.09 mmol). The solution was stirred overnight and then evaporated to dryness. The residue was taken up in $CHCl_3$ and this solution extracted twice with 0.01 N aqueous NaOH and once with water and finally dried (Na_2SO_4). The solvent was evaporated and the residue chromatographed on SiO_2 (50 g) eluting with 5% MeOH/95% $CHCl_3$. After the eluant was evaporated to dryness, the residue was dissolved in a minimum of THF and this solution

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was added to an excess of hexanes/toluene (3/1). The precipitate was collected and dried to yield 835 mg (73%) of dimer **11**. **11**: ^{1}H NMR δ 8.30 (1 H, d, J = 7.14 Hz), 8.23 (1 H, d, J = 7.00 Hz), 7.99 (4 H, m), 7.80–7.10 (20 H, m), 6.85 (2 H, d, J = 8.70 Hz), 6.13 (2 H, m), 5.19 (1 H, m), 4.16 (1 H, m), 4.10 (1 H, m), 3.90 (1 H, m), 3.71 (3 H, s), 3.35–3.20 (2 H, m), 2.49–2.00 (6 H, m); mass spectrum, M − 1 957 (31), 601 (38), 355 (22), 214 (100).

Anal. Calcd for $C_{53}H_{50}N_8O_{10}\cdot 2H_2O$: C, 63.97; H, 5.47; N, 11.26. Found: C, 64.11; H, 5.13; N, 11.24.

4-Nitrophenyl N^4-Benzoyl-2′,5′-dideoxy-5′-(4″-monomethoxytrityl)aminocytidylyl-(3′–5′-carbamoyl)-5′-amino-N^4-benzoyl-2′,5′-dideoxycytidine 3′-Carbonate (12). The dimer **11** (388 mg, 405 μmol) was treated with bis(4-nitrophenyl)-carbonate (365 mg, 1200 μmol) and triethylamine (80 μL, 530 μmol) in the same manner as the monomer **8**, except that the chromatography solvent was 5% MeOH/95% $CHCl_3$, and afforded 343 mg (75%) of the activated dimer **12**. **12**: IR ν 1765 cm^{-1}; ^{1}H NMR δ 8.40–6.80 (32 H, m), 6.15 (2 H, m), 5.32 (1 H, m), 5.20 (1 H, m), 4.32 (1 H, m), 4.10 (1 H, m), 3.70 (3 H, s), 3.38 (4 H, m), 2.30–2.00 (4 H, m); mass spectrum, M − 1 1122 (20), 601 (30), 355 (32), 214 (90).

N^4-Benzoyl-2′,5′-dideoxy-5′-(4″-monomethoxytrityl)-aminocytidylyl-(3′–5′-carbamoyl)-[5′-amino-N^4-benzoyl-2′,5′-dideoxycytidylyl-(3′–5′-carbamoyl)]$_2$-5′-amino-N^4-benzoyl-2′,5′-dideoxycytidine (14). The dimer **12** (174 mg, 181 μmol) was detritylated in the identical manner as **8**. Coupling of the *p*-toluenesulfonic acid salt of amino dimer **13** and the activated dimer **12** (199 mg, 177 μmol) using the method described the preparation of **11** afforded 183 mg (62%) of the tetramer **14**. **14**: ^{1}H NMR δ 8.26 (4 H, m), 7.98 (8 H, m), 7.80–7.10 (28 H, m), 6.83 (2 H, m), 6.13 (4 H, m), 5.12 (3 H, m), 4.13 (4 H, m), 3.91 (1 H, m), 3.70 (3 H, s), 3.35 (8 H, m), 2.30–2.00 (8 H, m); mass spectrum, M − 1 1669 (15), 957 (30), 711 (20), 601 (100).

Anal. Calcd for $C_{87}H_{82}N_{16}O_{20}\cdot 4H_2O$: C, 59.92; H, 5.20; N, 12.85. Found: C, 59.77; H, 5.01; N, 12.39.

N^4-Benzoyl-2′,5′-dideoxy-5′-(4″-monomethoxytrityl)-aminocytidylyl-(3′–5′-carbamoyl)-[5′-amino-N^4-benzoyl-2′,5′-dideoxycytidylyl-(3′–5′-carbamoyl)]$_4$-5′-amino-N^4-benzoyl-2′,5′-dideoxycytidine (16). The tetramer **14** (163 mg, 97.6 μmol) was detritylated by using glacial acetic acid/MeOH (4/1, 6 mL) in the same manner as **8**. Coupling of the tosylate of amino tetramer **15** and the activated dimer **12** (105 mg, 93.5 μmol) in the presence of triethylamine (40 μL, 289 μmol) was carried out as for the preparation of **11**, except that the chromatography solvent was 7% MeOH/93% $CHCl_3$. This procedure afforded 163 mg (73%) of hexamer **16**. **16**: ^{1}H NMR δ 8.26 (6 H, m), 7.97 (12 H, m), 7.80–6.90 (38 H, m), 6.82 (2 H, m), 6.12 (6 H, m), 5.11 (5 H, m), 4.14 (7 H, m), 3.68 (3 H, s), 3.30 (12 H, m), 2.30–2.00 (12 H, m); mass spectrum, M − 1 2381 (10), 957 (22), 711 (60), 601 (100).

Anal. Calcd for $C_{121}H_{114}N_{24}O_{30}\cdot 6H_2O$: C, 58.73; H, 5.05; N, 13.58. Found: C, 58.74; H, 4.86; N, 13.15.

5-Amino-2′,5′-dideoxycytidylyl-(3′–5′-carbamoyl)-[5′-amino-2′,5′-dideoxycytidylyl-(3′–5′-carbamoyl)]$_4$-5′-amino-2′,5′-dideoxycytidine (18). The hexamer **16** (51.4 mg, 21.6 μmol) was dissolved in DMSO (4 mL). Concentrated ammonia (4 mL) was carefully layered on top of the DMSO solution. The vessel was sealed and the layers were mixed. The vessel was kept at 30 °C for 40 h. The solvent was evaporated and the residue was taken up in a minimum of trifluoroethanol (TFE, 0.5 mL). The TFE solution was added to ether (20 mL) and the etheral solution was diluted with hexanes (10 mL). The precipitate was collected and dried to afford **17** in nearly a quantitative yield.

Oligomer **17** was detritylated by treatment with glacial acetic acid/methanol (2/1) for 2 h. The solvent was evaporated and the residue twice evaporated from H_2O. The residue was taken up in TFE (0.5 mL) and this solution was added to ether (20 mL). The etheral solution was diluted with hexanes (10 mL) and the precipitate collected. This solid was purified by HPLC and the eluant was concentrated by evaporation. The residue was taken up in 0.01 N HCl and precipitated by addition to pH 7.4 buffer. The residue was washed with H_2O and dried to give **18**. **18**: ^{1}H NMR δ 7.65 (6 H, m), 6.11 (6 H, m), 5.78 (6 H, m), 5.01 (5 H, m), 4.10 (1 H, m), 3.97 (5 H, m), 3.77 (1 H, m), 3.27 (12 H, m), 2.40–2.05 (11 H, m), 2.00–1.80 (1 H, m).

Hybridization Studies of 18. Oligomer **18** (0.2 mg) was dissolved in 0.1 mL of 0.1 N hydrochloric acid. The solution was diluted with 4.0 mL of 0.1 N pH 7.4 phosphate buffer and then treated with 0.1 mL of 0.1 N sodium hydroxide. The oligomer (1 mL of the above solution) was treated with 1 equiv of p(dG_6) in 0.24 mL of pH 7.4 buffer (total volume of the solution was 1.24 mL). This solution showed a maximum hypochromic shift relative to unmixed components of 23% at 273 nm at 20 °C. A thermal melt study was preformed with the above solution and an analogous solution of p(dC_6) and p(dG_6). The temperature was increased in 5 °C increments from 10 to 85 °C allowing 10 min for equilibration at a given temperature. The p(dC_6)/p(dG_6) duplex Tm was found to be 30 °C while the **18**/p(dG_6) duplex Tm was determined to be 71 °C.

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