Diisopropylsilyl-Linked Oligonucleotide Analogs: Solid-Phase Synthesis and Physicochemical Properties

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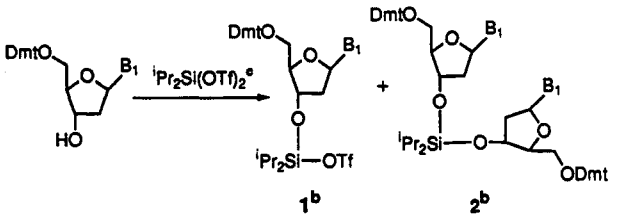
A novel synthetic method has been developed for efficient preparation of silyl-linked oligodeoxyribonucleotide analogs. The method allows, for the first time, automated solid-phase synthesis of long oligomers uniformly linked with the silyl internucleoside bridge. Synthesis of a thymidylate decanucleotide analog (14) illustrates this advance. The preparation of chimeric oligodeoxyribonucleotides containing single or multiple diisopropylsilyl backbone structures along with natural phosphodiester links is also described. These mixed backbone DNA strands were soluble and chemically stable in buffered aqueous solutions, as required for physicochemical study. These oligomers demonstrated excellent stability toward cleavage by 3′-exonuclease and good binding affinity with complementary oligonucleotides.

Introduction

Analogs of antisense oligodeoxyribonucleotides are of interest as potential antiviral, antibacterial, and anticancer agents.[1] To overcome the enzyme lability and drug delivery limitations of natural phosphate-linked antisense oligomers, various oligonucleotide analogs have been prepared.[2] Practical utility requires these analogs to have good cell penetration properties, resistance to degradation by nucleases, sequence specific hybridization to target nucleic acids, and an accessible chemical synthesis. The dialkylsilyl internucleoside linkage, first described in 1985,[3a] is attractive due to its neutral, achiral, and lipophilic properties and apparently simple synthesis. The development of this backbone, however, has been hampered for two reasons. The reported synthesis gives low yields of even relatively short oligomers, and the silyl ether products are insoluble in aqueous systems.

We have developed a method for high yielding solution synthesis of short oligomers and automated solid-phase synthesis of long oligomers of any sequence containing the diisopropylsilyl internucleoside linkage. This method involves preparation of intermediate 3′-*O*-diisopropylsilyl triflate **1** free from 3′,3′ dimers **2** which result from self-condensation. The formation of this self-condensation product in the initial silylation step[3b] has been a major impediment to successful solid-phase synthesis of oligonucleotide analogs uniformly linked by the dialkylsilyl internucleoside bridge. To permit evaluation of the physicochemical and biological properties of the dialkylsilyl internucleoside linkage, we have also developed a method for synthesis of mixed dialkylsilyl–phosphodiester backbone DNA strands, which are soluble and chemically stable in buffered aqueous systems and suitable for physicochemical study.

Scheme I



[a]Dmt = dimethoxytrityl, iPr = isopropyl, OTf = trifluoromethanesulfonyl. [b]**a**, B_1 = T; **b**, B_1 = A^{Bz}; **c**, B_1 = C^{Bz}; **d**, B_1 = G^{iB}; Bz = benzoyl, iB = isobutyryl. [c]Method 1: imidazole; ratio 1:2 = 1:1. Method 2: 2,6-di-*tert*-butyl-4-methylpyridine (Dtbp); ratio 1:2 > 20:1.

Results and Discussion

Our initial synthesis of the diisopropylsilyl-linked dinucleotide analog **3a** involved silylation of 5′-*O*-(dimethoxytrityl)thymidine with bis(trifluoromethanesulfonyl)diisopropylsilane and imidazole (Scheme I). This reaction gave significant amounts of the undesired 3′,3′ symmetrically-linked dinucleoside **2** as evidenced by TLC. Introduction of thymidine (Scheme II) followed by chromatographic isolation provided only 20% of the desired 3′,5′-linked dinucleoside **3a** along with 3′,3′-dimers **2a** and **4a** in 60% and 10% yields, respectively. Variations of reaction conditions including temperature, addition rate, stoichiometry, and concentration were applied for optimization of the ratio 1:2 with little success. Thus, it was necessary to develop a new silylation protocol that minimized formation of the self-condensation product **2**.

Since the symmetric dimer **2** results from a relatively hindered transition state involving attack of a secondary

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(1) For reviews, see: (a) Uhlmann, E.; Peymann, A. *Chem Rev.* **1990**, *90*, 543. (b) Helene, C.; Touleme, J. *J. Biochem. Biophys. Acta* **1990**, *1049*. (c) Miller, P. S. in *Topics in Molecular and Structural Biology: Oligonucleotides–Antisense Inhibitors of Gene Expression*; Cohen, J. S., Ed., CRC Press Inc: Boca Raton, FL, 1989. (d) Cook, P. D. *Anticancer Drug Design* **1991**, *6*, 585. (e) Chrisey, L. A. *Antisense Res. Develop* **1991**, *1*, 65.

(2) (a) Matteucci, M. D. *Tetrahedron Lett.* **1990**, *31*, 2385. (b) Venneman, G. H.; Van Der Marel, G. A; Van Der Elst, H.; Van Boom, J. H. *Tetrahedron* **1991**, *47*, 1547. (c) Huang, Z.; Schneider, K. C.; Benner, S. A. *J. Org Chem.* **1991**, *56*, 3869. (d) Reynolds, R. C.; Crooks, P. A.; Maddry, J. A.; Akhtar, M. S.; Montgomery, J. A.; Secrist, J. A., III. *J. Org. Chem.* **1992**, *57*, 2983. (e) Vassuer, J.-J.; Debart, F.; Sangvi, Y. S.; Cook, P. D. *J. Am. Chem. Soc.* **1992**, *114*, 4006. (f) Matteucci, M. D.; Lin, K.-Y.; Butcher, S.; Moulds, C. *J. Am. Chem. Soc.* **1991**, *113*, 7767.

(3) (a) Ogilvie, K. K.; Cormier, J. F. *Tetrahedron Lett.* **1985**, *26*, 4159. (b) Cormier, J. F.; Ogilvie, K. K. *Nucleic Acids Res.* **1988**, *16*, 4583.

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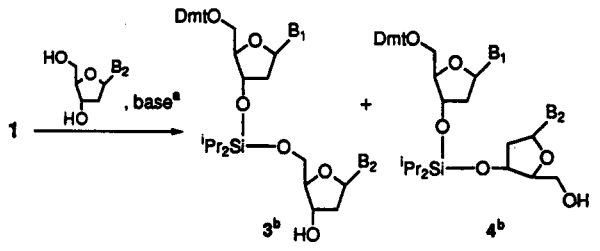
Scheme II



[a]Method 1: imidazole; ratio 3:4 = 2:1, yield of 3 20%. Method 2: Dtbp; ratio 3:4 > 20:1, yield of 3 70%. [b]**a**, $B_1 = B_2 = T$; **b**, $B_1 = T$, $B_2 = A^{Bz}$; **c**, $B_1 = T$, $B_2 = C^{Bz}$; **d**, $B_1 = T$, $B_2 = G^{iB}$; **e**, $B_1 = A^{Bz}$, $B_2 = T$; **f**, $B_1 = C^{Bz}$, $B_2 = T$; **g**, $B_1 = G^{iB}$, $B_2 = T$.

hydroxyl group on the 3′-silyl intermediate **1**, we reasoned that use of a hindered base as proton scavenger might slow the rate of formation of the 3′,3′ dimer **2** by increasing the activation energy for this undesired reaction path. The same effect should also enhance formation of the 3′,5′ coupling product, when using unprotected nucleosides in the coupling step. Indeed, when imidazole was replaced with the hindered base 2,6-di-*tert*-butyl-4-methylpyridine, almost none of the self-condensation product **2a** (<5%) was formed. Coupling by introduction of unprotected thymidine resulted in the formation of the dinucleoside **3a** in high yield. Thin layer chromatographs of this reaction show nearly quantitative formation of the dimers **3**. The product **4a** corresponding to reaction at the unprotected secondary hydroxyl was not observed. The advantage of not using 3′-OH protection can be exploited by continuing the reaction to trinucleoside **7** in a single pot. Thus the oligonucleoside **3a** was silylated without isolation and coupled with another molecule of unprotected thymidine to give, after chromatographic purification, the 3′,5′-linked trinucleoside **7** (Scheme III) in 55% yield. Higher yields (76%) of the trimer **7** were obtained in a stepwise (2 + 1) reaction that involved isolation and purification of the dimer unit. Isolation of diisopropylsilyl-linked nucleosides by silica gel chromatography is accompanied by some loss, presumably due to hydrolytic cleavage of silyl–ether linkages. Small-scale purification can be performed by reverse-phase HPLC. Unequivocal proof of the 3′,5′ nature of the silyl internucleoside link was obtained by ^1H–^{29}Si long range heteronuclear multiple quantum correlation NMR spectroscopy.[4]

The hindered base procedure was similarly useful for silylation of N^6-benzoyl-2′-deoxy-5′-*O*-(dimethoxytrityl)-adenosine, N^4-benzoyl-2′-deoxy-5′-*O*-(dimethoxytrityl)-cytidine, and N^2-isobutyryl-2′-deoxy-5′-*O*-(dimethoxytrityl)-guanosine. In each case, the 3′-silylated species **1** was the sole product. Coupling to unprotected thymidine gave the 3′,5′-linked heterodinucleosides A-Si-T **3e**, C-Si-T **3f**, and G-Si-T **3g**, respectively, in good yields (60–75%). Similar syntheses of T-Si-A **3b**, T-Si-C **3c**, and T-Si-G **3d** were also accomplished. Our next aim was to chain-extend the silyl-linked oligonucleosides. A tetrathymidylate oligomer **12** was synthesized by 3′-O-silylation of 5′,3′-linked trimer **7** followed by coupling with thymidine. The silylation reaction of trimer proceeded smoothly to give a single 3′-O-silylated species. No trace of the self-condensation product was observed. Stoichiometric reaction with thymidine gave a 50–60% condensation, leading to a 30% isolated yield of the tetrathymidylate analog after preparative reverse-phase HPLC.

(4) Hewitt, J. M.; Lenhart, W. C.; Moore, R. N.; Saha, A. K.; Weis, A. L. *Nucleosides Nucleotides*, **1992**, *11*(9), 1661–1666.

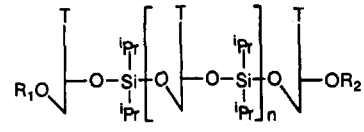
Syntheses of penta- and hexathymidine analogs were planned as [3 + 2] and [3 + 3] sequences, the first component being the 5′-protected trinucleoside **7** and the second component the detritylated di- or trinucleosides **5** or **8** (Scheme II). The latter compounds were prepared by reaction of dinucleoside **3a** and trinucleoside **7**, respectively, with 3% trichloroacetic acid in methylene chloride followed by flash chromatography on silica gel. Yields in these detritylation reactions were high (70–80%), indicating good acid stability of the diisopropylsilyl link. The 3′-O-silylation of the 5′-*O*-dimethoxytrityl trimer **7** proceeded well, as described for the tetramer above. However, coupling efficiency to the deprotected di- and trinucleosides **5** and **8**, respectively, was low and only minor amounts (20–30% by TLC) of the desired oligomers were formed. Evidence for formation of these oligomers was obtained from good intensity molecular and fragmentation ions in their FAB mass spectra as well as NMR of partially purified products.

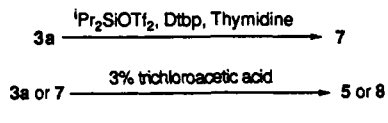
Solution syntheses of higher chain length (>5–6 nucleobases) diisopropylsilyl-linked oligodeoxynucleotide analogs, though feasible, tend to be tedious and complex. This problem was solved by developing a scheme for solid-phase automated synthesis (Scheme III), which includes high purity synthesis of the monomeric synthon, its stabilization for room temperature handling and storage, and rapid coupling reactions. The 3′-*O*-diisopropylsilyl triflate intermediate **1a**, prepared at −40 °C, underwent some detritylation when warmed to room temperature. Stability against detritylation was achieved by adding an excess of imidazole (2 equiv) to the reaction mixture prior to warming to room temperature. The mixture was diluted with acetonitrile to 0.1 M to reduce the viscosity of the medium. The reagent solution remained colorless for several weeks and was stored under anhydrous conditions at −20 °C until use in a DNA synthesizer. Purity analysis for this synthon was according to high resolution NMR whereby no trace of the 3′,3′-dimer was observed.

Strands of varying lengths up to a 10-mer of thymidines containing the all-silicon backbone were synthesized reproducibly by solid-phase methodology (Scheme IV). The overall coupling efficiency based on the DMT assay was 96.3%. A synthesis was performed whereby the end-capping procedure after every coupling was omitted. This alteration to the synthesis cycle produced a mixture having shorter (9, 8, 7, etc.) lengths of the intended 10-mer strand. This mixture produced a good sizing ladder and retention time marker on reverse-phase HPLC. After synthesis the CPG solid support was cleaved with 6:3:1 NH_4OH (30% aqueous)/2-propanol/ CH_3CN with good recoveries as determined from the 260-nm absorbance reading (16–20 optical density units from 1-μm scale). The crude product from each synthesis was analyzed by reverse-phase HPLC in 0.01 M TEAA (pH 7.5)/acetonitrile gradient. A major peak was observed for each synthesis. The retention time increased as the chain length of the synthesized product increased: 5′-TSiTSiT-3′ 12.72 min; 5′ TSiTSiTSiTSiT-3′ 16.06 min; 5′ TSiTSiTSiTSiTSiTSiTSiTSiT-3′ 21.01 min. The trimer was identical in all respects to the same compound prepared by solution-phase methodology. The penta- and the decanucleosides were also characterized by proton NMR and FAB–mass spectroscopy.

The silyl-linked decathymidylate molecule **14** synthesized by solid phase was soluble in polar organic solvents but insoluble in aqueous systems. For determination of the physicochemical properties of the diisopropylsilyl

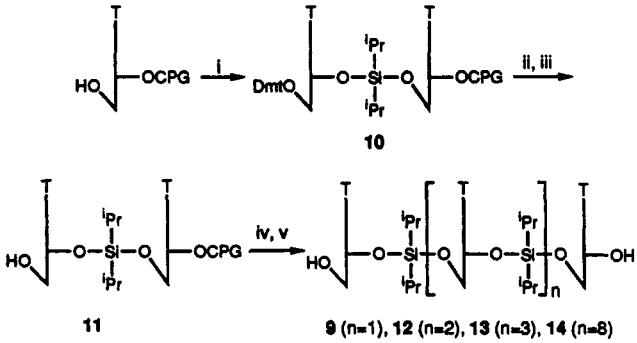
Scheme III





Product	R_1	n	R_2
3a	Dmt	0	H
7	Dmt	1	H
5	H	0	H
8	H	1	H
6	Dmt	0	$P(OCH_2CH_2CN)N^iPr_2$
9	Dmt	1	$P(OCH_2CH_2CN)N^iPr_2$

Scheme IV



[a]CPG = controlled pore glass, Dmt = dimethoxytrityl. [b](i) 1a, Dtbp, imidazole; (ii) Ac_2O, *N*-methylimidazole; (iii) 3% trichloroacetic acid; (iv) cycle repeat (i) to (iii); (v) aqueous NH_3, ^iPrOH–CH_3CN.

linkage as a phosphodiester mimic, we synthesized phosphodiester-linked DNA oligomers containing single or multiple units of the silyl-linked di- and trithymidylates. The building blocks **6** and **9** for automated synthesis were prepared from **3a** and **7** respectively, by reaction with 2-cyanoethyl *N,N* diisopropylphosphoramidochloridite (Scheme III). The yields of isolated products ranged from 60 to 80%. The following 11-mer oligonucleotides were prepared[5] incorporating the T-Si-T dimer and the T-Si-T-Si-T trimer at the indicated positions within the strands:

1. 5′ TTT TTT TTTSiT T-3′
2. 5′ TTT TTT TTSiT TT-3′
3. 5′ TTT TTTSiT TTT T-3′
4. 5′ TTT TTT TTSiTSiT T-3′
5. 5′ TTT TTSiTSiT TTT T-3′

The mean coupling yield for these incorporations was >97%. The synthesized products were cleaved from the CPG support with NH_4OH (30% aqueous)/2-propanol/CH_3CN (6:3:1) and the crude overall product yield was determined on the basis of the OD 260 nm measurement. A preparative reverse-phase HPLC procedure allowed isolation of the major peak from the failure sequences and molecular weight was verified by FAB/MS. The purified compounds were also analyzed by ion exchange HPLC and a repeat reverse-phase HPLC. A single major peak was seen by both methods indicating good purity. Chemical stability in buffered media [(0.01 M TEAA (pH 7.5)/acetonitrile (4–20%)] was monitored by reverse-phase HPLC and no significant degradation was detected for the 6 weeks tested.

A nuclease stability assay, analyzed by anion exchange HPLC for the above five chimeric oligothymidylate compounds, was run in the presence of 10% FBS (fetal bovine serum which contains 3′-exonucleases) media, with control standards run in parallel. Retention times of the standards were used to identify fragment lengths. The control T_{11} strand was completely digested in 3 min. For each of the five oligomers, the exonuclease cleaved the phosphodiester bonds in the 3′-5′ direction but stopped at the siloxane link.

Thermodynamic melting[6] data (T_m) were acquired by mixing the above oligomers with complementary dA_{11} strands in equimolar amounts and annealing at 4° in a phosphate buffer (0.1 M NaCl) for 24 h. The UV absorbance at 260 nm was measured every 0.25° while the temperature was ramped from 20 to 70°. The T_m value for dissociation of the duplex was obtained from the first derivative of the absorbance vs temperature plot. In all cases, the T_m values were less than the control T_{11}/dA_{11} duplex by 2–5°, depending on extent of the modification.[7] The melting curve shapes were however normal, indicating good duplex formation. Full details of these studies will be reported elsewhere.

In conclusion, a versatile and efficient synthetic method has been developed for solution- and automated solid-phase synthesis of dialkylsilyl-linked oligonucleotide analogs. This method employs minimal protection and avoids problems of self-coupling. Our solid-phase automated synthesis allows preparation of uniformly dialkylsilyl-linked DNA analogs of any sequence. This method also allows high yield solution synthesis of dialkylsilyl-linked dimers and trimers of any sequence. We have also accomplished the synthesis of mixed phosphodiester-silyl-linked oligonucleotide analogs containing 1–2 incorporations of diisopropylsilyl-linked thymidylate dimers or trimers. These mixed backbone oligomers were soluble and chemically stable in buffered aqueous systems. Evidence for the potential utility of the siloxane modification in antisense and related research was also obtained.

Experimental Section

General. All chemicals used in this study were reagent grade unless otherwise stated. Nucleosides were purchased from Raylo Chemical Company, Edmonton, Canada, or Pharma-Waldorf,

(5) Gait, M. J., Ed. *Oligonucleotide Synthesis: A Practical Approach*; IRL Press: Washington, DC, 1984.

(6) (a) Cantor, C. R.; Schimmel, P. R., Eds. *Biophysical Chemistry*, III; W. H. Freeman and Company: New York, 1980; pp 1134–1160. (b) Tinoco, I.; Puglisi, J. D. in *Methods in Enzymology*; Dahlberg, J. E., Abelson, J. N., Eds.; Academic Press: San Diego, 1989, Vol. 180 (A), 304.

(7) Replacement of the natural phosphodiester linkage with modified counterparts leads to a minimum of 1–3 °C drop in T_m value. See, for example: (a) Gao, X.; Brown, F. K.; Jeffs, P.; Bischofpberger, N.; Lin, K.-Y.; Pipe, A. J.; Noble, S. A. *Biochemistry* **1992**, *31*, 6228. (b) Kibler-Herzog, L.; Zon, G.; Uznanski, B.; Whittier, G.; Wilson, W. D. *Nucleic Acids Res.* **1991**, *19*, 2979.

Dusseldorf, Germany. Bis(trifluoromethanesulfonyl)diisopropylsilane was purchased from Petrarch Systems Inc., Bertram, PA, or Fluka Chemical Corporation, Ronkonkoma, NY, and distilled from K_2CO_3 prior to use. Phosphoramidites were purchased from Applied Biosystems, Foster City, CA. All other reagents were purchased from Aldrich and dried as appropriate, by standard procedures prior to use. All solvents were purchased from Aldrich (<0.0005% H_2O) and used as received. All nucleosides were dried by coevaporation from a mixture of pyridine and acetonitrile *in vacuo* and furthur dried over P_2O_5 in high vacuum prior to use.

Nuclear magnetic resonance (NMR) spectra were recorded on a Varian XL-300, Varian FX-270, or Bruker AMX 360 spectrometer and data are presented as ppm downfield from either tetramethylsilane or 85% H_3PO_4. The FAB–mass spectra were recorded on a Finnigan MAT TSQ70 triple quadrupole instrument or on a VG Analytical ZAB 2-SE instrument. UV absorbance and T_m studies were carried out in a Perkin-Elmer spectrophotometer equipped with a temperature controller. HPLC was carried out on a Waters HPLC apparatus Model 3000 with a Waters 481 UV detector set at 254 nm. Reverse-phase chromatography was done on Beckman Ultrasphere ODS (10 mm × 25 cm) and Vydac (51 mm × 250 mm) columns. Normal-phase HPLC was performed on a Zorbax Sil (21 mm × 25 cm) column, purchased from Dupont. Silica gel used for flash chromatography was Merck Silica Gel 60 (70–230 mesh). Elemental analysis was obtained from Microanalysis Inc., Wilmington, DE.

General Procedure for Preparation of 3′-*O*-(Diisopropylsilyl)-2′-deoxynucleoside Triflate Intermediates 1. Bis(trifluoromethanesulfonyl)diisopropylsilane (2 mmol, 0.60 mL) was added via syringe to a solution of 2,6-di-*tert*-butyl-4-methylpyridine (2 mmol, 0.41 g) in CH_3CN (5 mL) in a 100-mL round-bottom flask under N_2. The clear solution was cooled to −40 °C (dry ice–CH_3CN) and to it a solution of 5′-*O*-(dimethoxytrityl)-2′-deoxynucleoside (1.84 mmol, 1.0 g) and 2,6-di-*tert*-butyl-4-methylpyridine (0.46 mmol, 94 mg) in DMF (5 mL) was added dropwise via syringe over 10 min. The reaction was stirred at −40 °C for 1 h. For characterization, a small amount was isolated by precipitation from water followed by chromatography on a small silica column eluting with 60% to 100% EtOAc/hexanes. Yield of 3′-*O*-diisopropylsilanols: 95–100%. R_fs: 0.56–0.90 (0.5% MeOH/EtOAc). Data for 1c: R_f 0.71 (0.5% MeOH/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.43 (d, J = 7.6 Hz, 1 H), 7.88 (d, J = 8.2 Hz, 2 H), 7.60–7.26 (m, 13 H), 6.87 (d, J = 8.4 Hz, 4 H), 6.25 (t, J = 6.5 Hz, 1 H), 4.71 (m, 1 H), 4.10 (m, 1 H), 3.80 (s, 6 H), 3.48 (ABq, J = 3 Hz, 11 Hz, Δν = 30 Hz, 2 H), 2.67 (m, 1 H), 2.35 (m, 1 H), 0.97 (m, 14 H); MS (FAB) m/z 764.7 (M + H)$^+$.

5′-*O*-(Dimethoxytrityl)-3′-*O*-(5′-*O*-thymidyldiisopropylsilyl)thymidine (3a). Thymidine (0.8 mmol, 193 mg) was added to a solution of intermediate 1a (0.92 mmol) prepared as above. The reaction was stirred for 1 h and then added dropwise into a vigorously-stirred ice–water mixture (500 mL). The mixture was filtered to give a white solid which was air dried and subjected to column chromatography (SiO_2, gradient of 60% to 100% EtOAc/hexanes). Yield: 0.581 g, 70%; R_f 0.45 (5% MeOH/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.85 (s, 1 H, NH), 9.44 (s, 1 H, NH), 7.64 (s, 1 H), 7.41–7.24 (m, 10 H), 6.84 (d, J = 7.8 Hz, 4 H), 6.33 (m, 2 H), 4.65 (s, 1 H), 4.43 (d, J = 2.2 Hz, 1 H), 4.11 (d, J = 2.56, 1 H), 4.00 (d, J = 3.36, 1 H), 3.93 (ABq, J = 3.7 Hz, 11.0 Hz, Δν = 24.6 Hz, 2 H), 3.79 (s, 6 H), 3.39 (ABq, J = 3.0 Hz, 10.8 Hz, Δν = 49.5 Hz, 2 H), 2.50–2.38 (m, 2 H), 2.30–2.07 (m, 2 H), 1.88 (s, 3 H), 1.56 (s, 3 H), 1.05–0.98 (m, 14 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.84, 164.80, 159.40, 151.72, 151.25, 144.89, 136.16, 136.01, 135.86, 130.60, 128.58, 127.75, 113.80, 112.10, 111.42, 87.48, 87.38, 85.76, 85.48, 73.90, 71.70, 63.82, 63.48, 60.75, 55.57, 41.71, 40.85, 21.24, 17.52, 17.47, 17.39, 14.35, 12.69, 12.18, 12.10, 11.85; ^{29}Si NMR (53.5 MHz) δ −8.09; MS (FAB): m/z 899 (M + H)$^+$; HR-MS (FAB) calcd for $C_{47}H_{58}N_4O_{12}Si$ 898.3821, obsd 898.3748.

3′-*O*-(5′-*O*-Thymidyldiisopropylsilyl)thymidine (5). A solution of dimer 3a (0.22 mmol, 200 mg) in CH_2Cl_2 (4 mL) was added to 3% trichloroacetic acid in CH_2Cl_2 (6 mL). The bright orange solution was stirred at room temperature for 10 min. The reaction mixture was poured into 5% aqueous $NaHCO_3$ (5 mL) and extracted into 5% MeOH/EtOAc. The organic layer was washed with brine (10 mL) and dried over Na_2SO_4. The crude product was purified by column chromatography (SiO_2, gradient of 60:40 EtOAc/hexanes to 10% MeOH/EtOAc): yield 90 mg, 70%; R_f 0.40 (10% MeOH/EtOAc); ^{1}H NMR (300 MHz, CD_3OD) δ 7.54 (s, 1 H), 7.28 (s, 1 H), 6.03 (m, 2 H), 4.46 (m, 1H), 4.18 (m, 1 H), 3.82–3.69 (m, 3 H), 3.50 (m, 4 H), 2.06–1.97 (m, 4 H), 1.62 (s, 6 H), 0.85 (m, 14 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 167.0, 152.6, 138.6, 138.2, 112.1, 89.8, 88.7, 86.8, 86.5, 74.6, 72.3, 64.6, 63.1, 42.0, 41.2, 18.0, 17.9, 13.4, 13.3, 12.8, 12.7; ^{29}Si NMR (53.5 MHz) δ −8.2; MS (FAB): m/z 597.3 (M + H)$^+$; HR-MS (FAB) calcd for $C_{26}H_{41}N_4O_{10}Si$ 597.2592, obsd 597.2612.

5′-*O*-(Dimethoxytrityl)-3′-*O*-[(3′-*O*-5′-*O*-thymidyldiisopropylsilyl)-3′-*O*-[(3′-*O*-(5′-*O*-5′-*O*-thymidyl)diisopropylsilyl]thymidine (7). A solution of dimer 3a (1.11 mmol, 1.0 g) and 2,6-di-*tert*-butyl-4-methylpyridine (0.28 mmol, 60 mg) in DMF (3 mL) was added via syringe to a solution of bis(trifluoromethanesulfonyl)diisopropylsilane (1.22 mmol, 0.504 g, 0.360 mL) and 2,6-di-*tert*-butyl-4-methylpyridine (1.22 mmol, 0.25 g) in CH_3CN (3 mL) at −40 °C (dry ice–CH_3CN). The reaction was stirred for 1 h at −40 °C. A solution of imidazole (1.22 mmol, 0.16 g) in CH_3CN (2.5 mL) was added and the reaction was warmed to room temperature. A solution of thymidine (1.11 mmol, 0.269 g) in DMF (2 mL) was added. The reaction was stirred for 1 h and added dropwise to a vigorously-stirred ice/water mixture (1 L) and stirred for 30 min. The precipitate was filtered and air dried to give a white solid (1.5 g). This crude product was triturated with hexanes (20 mL) to give pure product: isolated yield 1.05 g, 76%; R_f 0.38 (5% MeOH/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (s, 1 H), 7.36–7.22 (m, 11 H), 6.80 (d, J = 7.7, 4 H), 6.36–6.22 (m, 3 H), 4.62–4.54 (m, 2 H), 4.45 (m, 1 H), 4.06–3.83 (m, 7 H), 3.75 (s, 6 H), 3.36 (ABq, J = 10 Hz, Δν = 47.6 Hz, 2 H), 2.45–2.30 (m, 3 H), 2.28–2.14 (m, 1 H), 2.13–2.00 (m, 2 H), 1.85 (s, 3 H), 1.81 (s, 3 H), 1.48 (s, 3 H), 0.98 (m, 28 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 164.54, 164.45, 164.33, 159.00, 151.12, 150.99, 144.40, 135.73, 135.51, 135.40, 130.15, 128.16, 127.35, 113.37, 111.54, 111.32, 111.07, 87.50, 87.02, 85.23, 85.02, 73.35, 72.98, 71.24, 63.28, 63.02, 55.15, 41.33, 40.64, 40.25, 17.04, 17.00, 16.92, 12.26, 11.70, 11.59, 11.53, 11.47; ^{29}Si NMR (53.5 MHz) δ −7.9, −8.2; MS (FAB): m/z 1252.5 (M + H)$^+$. Anal. Calcd for $C_{63}H_{84}N_6O_{17}Si_2$: C, 60.38; H, 6.71; N, 6.71. Found: C, 60.44; H, 6.84; N, 6.56.

3′-*O*-[(3′-*O*-(5′-*O*-Thymidyldiisopropylsilyl)-(5′-*O*-thymidyl)diisopropylsilyl]thymidine (8). A solution of 5′-*O*-(dimethoxytrityl trimer 7 (0.638 mmol, 0.80 g) in CH_2Cl_2 (12 mL) was added to 3% trichloroacetic acid/CH_2Cl_2 (14 mL). The bright orange solution was stirred at room temperature for 1 h. The reaction mixture was poured into 5% aqueous $NaHCO_3$ (15 mL) and extracted into 5% MeOH/EtOAc. The organic layer was washed with brine (20 mL) and dried over Na_2SO_4. The crude product was purified by column chromatography (SiO_2, gradient of EtOAc/MeOH 100% to 95%): isolated yield 420 mg, 70%; R_f 0.50 (10% MeOH/EtOAc); ^{1}H NMR (360 MHz, DMSO-d_6) δ 11.3 (s, br, 2 H), 7.66 (s, 1 H), 7.37 (s, 2 H), 6.19 (m, 3 H), 5.29 (s, br, 1 H), 5.09 (s, br, 1 H), 4.56 (m, 2 H), 4.20 (m, 1 H), 3.9–3.8 (m, 7 H), 3.55 (m, 2 H), 2.26–2.1 (m, 6 H), 1.75 (s, 9 H), 1.04 (m, 28 H); MS (FAB) m/z 949.5 (M − H)$^-$; HR-MS (FAB) calcd for $C_{42}H_{66}N_6O_{18}Si_2Na$ 973.4022, obsd 973.3979.

5′-*O*-(Dimethoxytrityl)-3′-*O*-[(3-*O*′-[(3-*O*′-(5′-*O*-thymidyldiisopropylsilyl)-5-*O*-thymidyl)diisopropylsilyl)-5′-*O*-(thymidyldiisopropyl)silyl]thymidine (12). 5′-*O*-Dimethoxytrityl trimer 7 (20 μmol, 25 mg) and 2,6-di-*tert*-butyl-4-methylpyridine (40 μmol, 8.25 mg) were dissolved in DMF (200 μL) and added slowly via syringe to a solution of bis(trifluoromethanesulfonyl)diisopropylsilane (20 μmol, 6 μL) and 2,6-di-*tert*-butyl-4-methylpyridine (20 μmol, 4.1 mg) in DMF (100 μL) in a 5-mL round-bottom flask cooled to −40 °C (dry ice–CH_3CN). The reaction was stirred for 1 h and to it a solution of thymidine (20 μmol, 4.84 mg) and imidazole (40 μmol, 2.7 mg) in DMF (200 μL) was added. Stirring was continued at −40 °C for 30 min. The reaction was warmed to room temperature; 5% aqueous $NaHCO_3$ (1 mL) was added and the reaction mixture extracted into $CHCl_3$ (2 × 5 mL), washed with brine (1 mL), and dried over Na_2SO_4. The crude product was purified by preparative reverse-phase HPLC on Ultrasphere ODS (20% 0.01 M triethylammonium acetate pH 7.5/80% CH_3CN; t_R = 35.4 min): yield 10 mg, 31%; R_f 0.16 (5% MeOH/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.65 (s, 1 H), 7.39–7.26 (m, 12 H), 6.85 (d, J =

Chart I

A. Programming Cycles
1. All oxidation and subsequent iodine wash step/purges were disabled.
2. Tetrazole step was disabled by programming/hardwiring off.
3. Increased coupling times, increased monomer molarity, and double couple cycles were used to enhance coupling efficiency.

B. Reagents
1. Iodine solution was not used and removed from valveblock.
2. NH_4OH bottle position was used for 6:3:1 solution of NH_4OH:^{i}PrOH:CH_3CN.
3. Spare position 5 and X were used for synthon 1a on Models 380B and 381A, respectively, after accurate flowcheck.

7.7 Hz, 4 H), 6.38–6.27 (m, 4 H), 4.66–4.45 (m, 4 H), 4.10–3.85 (m, 10 H), 3.80 (s, 6 H), 3.39 (ABq, J = 11 Hz, $\Delta\nu$ = 45 Hz, 2 H), 2.45–2.08 (m, 8 H), 1.92 (s, 3 H), 1.90 (s, 3 H), 1.87 (s, 3 H), 1.56 (s, 3 H), 1.03 (m, 42 H); MS (FAB) calcd for $C_{79}H_{110}N_8O_{22}Si_3$ 1607.6770, obsd 1607.6765.

Solid-Phase Synthesis of Thymidine Decanucleoside 14. The monomeric synthetic unit **1a** was prepared from 5′-(dimethoxytrityl)thymidine according to the procedure described earlier with the exception that 2 equiv of imidazole was added prior to warming the reaction to room temperature and that acetonitrile was added to a final synthon concentration of 0.1 M. R_f of **1a**: 0.56, 60% EtOAc/hexanes. This intermediate was employed in solid-phase automated synthesis as described below (see text also).

All reagents used for synthesis were purchased from Applied Biosystems Inc. (Foster City, CA). Oligomer synthesis using 0.2 and 1 μmol of derivatized thymidine controlled pore glass support was used employing both ABI 380B and ABI 381A programmable DNA synthesizers. Synthesis cycles closely followed the high efficiency DNA cycles ABI001 and CE103A with modifications as indicated in Chart I. Step yields averaged 96.3% for 3 identical 10 base syntheses. Data for **14**: ^{1}H NMR (300 MHz, CD_3OD) δ 7.55–7.47 (m, 10 H), 6.23–6.33 (m, 10 H), 4.69 (s, br, 9 H), 4.40 (s, br, 1 H), 4.10–3.40 (m, 19 H), 3.25–3.08 (m, 1 H), 2.55–2.20 (m, 20 H), 1.87 (s, br, 27 H), 1.30 (s, br, 3 H), 1.08 (m, 126 H); MS (FAB) calcd for $C_{154}H_{249}N_{20}O_{50}Si_9$ 3430.55, obsd 3430.6.[8]

3′-*O*-(2′-Deoxy-*N*^{2}-isobutyryl-5′-*O*-guanosyl)diisopropylsilyl)-5′-*O*-(dimethoxytrityl)thymidine (3d). This compound was prepared according to the procedure described for **3a**, with purification by column chromatography (SiO_2, 3–4% MeOH/EtOAc): yield 40%; R_f 0.30 (5% MeOH/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.98 (s, 1 H), 7.67 (s, 1 H), 7.5–7.25 (m, 9 H), 6.84 (d, J = 8.5 Hz, 4 H), 6.3 (t, J = 6 Hz, 1 H), 6.21 (t, J = 6 Hz, 1 H), 4.83 (m, 1 H), 4.72 (m, 1 H), 4.61 (m, 1 H), 4.07 (m, 2 H), 3.90 (m, 2 H), 3.77 (s, 6 H), 3.47–3.29 (m, 2 H), 2.85 (m, 1 H), 2.55–2.20 (m, 4 H), 1.48 (s, 3 H), 1.2 (d, J = 6.0 Hz, 6 H), 1.0–0.9 (m, 14 H); MS (FAB) m/z 992.4 (M − H)$^-$; HR-MS (FAB) calcd for $C_{51}H_{63}N_7O_{12}SiNa$ 1016.4202, obsd 1016.4238.

N^6-Benzoyl-2′-deoxy-5′-*O*-(dimethoxytrityl)-3′-*O*-(5′-*O*-thymidyldiisopropylsilyl)adenosine (3e). This compound was prepared according to the procedure described for **3a**, with purification by preparative TLC (SiO_2, 3% MeOH/EtOAc): yield 78%; R_f 0.32 (2% MeOH/EtOAc); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.99 (s, 1 H, NH), 8.76 (s, 1 H), 8.22 (s, 1 H), 8.09 (d, J = 7.8 Hz, 2 H), 7.55–7.13 (m, 13 H), 6.74 (m, 4 H), 6.42 (t, J = 5.8 Hz, 1 H), 6.25 (t, J = 6.0 Hz, 1 H), 4.96 (d, J = 5.4 Hz, 1 H), 4.48 (s, 1 H), 4.19 (d, J = 3.8 Hz, 1 H), 3.94 (s, 1 H), 3.83–3.72 (m, 2 H), 3.72 (s, 6 H), 3.38 (d, J = 3.4 Hz, 2 H), 2.83–2.76 (m, 1 H), 2.60–2.52 (m, 1 H), 2.45–2.38 (m, 1 H), 2.05–1.95 (m, 1 H), 1.79 (s, 3 H), 0.95 (m, 14 H); MS (FAB): m/z 1011.1 (M − H)$^-$.

N^4-Benzoyl-2′-deoxy-5′-*O*-(dimethoxytrityl)-3′-*O*-(5′-*O*-thymidyldiisopropylsilyl)cytidine (3f). This compound was prepared according to the procedure described for **3a**, with purification by preparative TLC (SiO_2, 3% MeOH/EtOAc): yield 70%; R_f 0.40 (2% MeOH/EtOAc); ^{1}H NMR ($CDCl_3$) δ 9.54 (s, 1 H, NH), 8.21 (d, J = 7.6 Hz, 1 H), 7.94 (d, J = 8.2 Hz, 2 H), 7.59–7.24 (m, 14 H), 6.83 (d, J = 8.4 Hz, 4 H), 6.25 (m, 2 H), 4.58 (s, 1 H), 4.46 (s, 1 H), 4.17 (s, 1 H), 4.07–3.80 (m, 3 H), 3.77 (s, 6 H), 3.39 (ABq, J = 3.0 Hz, 10.9 Hz, $\Delta\nu$ = 29.1, 2 H), 2.84–2.78 (m, 1 H), 2.46–2.41 (m, 1 H), 2.14–1.79 (m, 2 H), 1.84 (s, 3H), 0.98 (m, 14 H); MS (FAB) m/z 987.2 (M − H)$^-$.

5′-*O*-(Dimethoxytrityl)-3′-*O*-(5′-*O*-thymidyldiisopropylsilylthymidine-3′-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite (6). 5′-*O*-Dimethoxytrityl dimer **3a** (coevaporated from THF (4 mL)/pyridine (2 mL) twice, 0.1 mmol, 90 mg) was dissolved in THF (500 μL) and added dropwise via syringe to a stirred solution of 4-(dimethylamino)pyridine (cat., 4 mg), diisopropylethylamine (distilled from CaH_2, 0.4 mmol, 87 μL), and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.15 mmol, 28.77 μL) in THF (500 μL) under N_2 flow at room temperature. The reaction was allowed to stir for 2 h. To remove trace amount of **5**, additional 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.025 mmol, 5 μL) was added. The reaction was stirred 1 h and added to EtOAc (10 mL, prewashed with 5 mL of brine), washed with brine (2 × 2 mL), and dried over Na_2SO_4. This crude product was purified by column chromatography (SiO_2, 1:1 EtOAc/hexanes): isolated yield 82 mg, 74.5%; R_f 0.70 (1% MeOH/EtOAc); RP-HPLC t_R = 38.6 and 39.7 min (mixture of diastereomers); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.20 (s, br, 1 H, NH), 7.64 (s, 1 H), 7.40–7.22 (m, 10 H), 6.84 (d, J = 8.8, 4 H), 6.40 (t, J = 6.5 Hz, 1 H), 6.27 (t, J = 6.5 Hz, 1 H), 4.67 (m, 1 H), 4.53 (m, 1 H), 4.10 (m, 2 H), 3.93 (m, 1 H), 3.87 (m, 1 H), 3.80 (s, 6 H), 3.72 (m, 1 H), 3.61 (m, 1 H), 3.39 (ABq, J = 4 Hz, 10 Hz, $\Delta\nu$ = 42 Hz, 2 H), 2.64 (m, 2 H), 2.53–2.05 (m, 4 H), 1.86 (s, 3 H), 1.51 (s, 3 H), 1.26 (m, 2 H), 1.17 (m, 12 H), 1.01 (m, 14 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 163.6, 163.4, 158.7, 150.1, 144.2, 135.5, 135.2, 130.0, 128.0, 127.2, 117.6, 113.3, 111.2, 111.0, 110.9, 87.0, 86.2, 85.8, 85.7, 84.9, 84.7, 84.6, 73.4, 73.2, 63.4, 62.9, 62.7, 58.2, 58.2, 58.0, 57.9, 55.29, 43.4, 43.2, 41.5, 39.7, 39.5, 24.5, 24.4, 23.0, 20.4, 20.3, 17.2, 17.0, 12.4, 11.9, 11.7, 11.6. ^{31}P NMR ($CDCl_3$) δ 149.16; MS (FAB): m/z 1098.8 (M + H)$^+$.

5′-*O*-(Dimethoxytrityl)-3′-*O*-[(3′-*O*-(5′-*O*-thymidyldiisopropylsilyl)(5′-*O*-thymidyl)diisopropylsilyl]thymidine 3′-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite (9). Trimer **7** (0.44 mmol, 550 mg) was dissolved in CH_2Cl_2 (2 mL) and added dropwise via syringe to a stirred solution of 4-(dimethylamino)-pyridine (cat., 20 mg), diisopropylethylamine (distilled from CaH_2; 1.69 mmol, 370 μL), and 2-cyanoethyl *N,N*-diisopropylphosphoramidochloridite (0.64 mmol, 120 μL) in CH_2Cl_2 (2.0 mL) under N_2 flow at 0 °C. The reaction mixture was brought to room temperature, stirred for 1 h, poured into EtOAc (prewashed with 25 mL brine; 50 mL), washed with brine (2 × 20 mL), and dried over Na_2SO_4. The crude product was purified by column chromatography (10 g of SiO_2, EtOAc): isolated yield 320 mg, 64%; R_f 0.76 (EtOAc); RP-HPLC t_R = 47.7 and 48.7 min (mixture of diastereomers); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1 H), 7.41–7.22 (m, 11 H), 6.83 (d, J = 7.8 Hz, 4 H), 6.40–6.24 (m, 3 H), 4.67–4.54 (m, 3 H), 4.13–3.85 (m, 7 H), 3.75 (s, 6 H), 3.55 (m, 2 H), 3.48–3.28 (m, 2 H), 2.74 (t, J = 6 Hz, 2H) 2.45–2.03 (m, 6H), 1.88 (s, 3 H), 1.83 (s, 3 H), 1.50 (s, 3 H), 1.28–1.13 (m, 14 H), 1.00 (m, 28 H); MS (FAB): m/z 1453 (M − H)$^-$.

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Supplementary Material Available: NMR and mass spectral data for all compounds, HPLC data for selected compounds including silyl-linked decathymidylate **14**, and mass spectrum of mixed backbone oligomer **5** (48 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(8) The sample was analyzed by negative ion FAB mass spectrometry with an instrument resolution of 1500. An exact mass analysis was not conducted due to poor sensitivity observed at higher resolution.