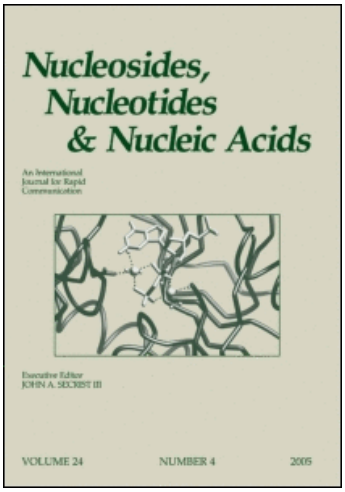

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Kenneth D. James[a]; Guenter von Kiedrowski[b]; Andrew D. Ellington[a]
[a] Department of Chemistry, Indiana University, Bloomington, IN [b] Lehrstuhl fuer Organische Chemie I-Bioorganische Chemie -Ruhr-Universitaet Bochum Bochum, Germany

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INCORPORATION OF 5'-N-BOC-2',5'-DIDEOXYNUCLEOSIDE-3'-O-PHOSPHORAMIDITES INTO OLIGONUCLEOTIDES BY AUTOMATED SYNTHESIS

Kenneth D. James[†], Guenter von Kiedrowski[‡], and Andrew D. Ellington[†]*

[†]Department of Chemistry, Indiana University, Bloomington, IN

[‡]Lehrstuhl fuer Organische Chemie I- Bioorganische Chemie -Ruhr-Universitaet Bochum Bochum, Germany

ABSTRACT. An efficient method for synthesizing 5'-Boc-5'-amino-2',5'-dideoxynucleoside phosphoramidites and conditions for their incorporation in solid-phase oligonucleotide synthesis are presented.

INTRODUCTION

Chemically modified nucleotide bases, sugars, and phosphates have been synthesized and introduced into RNA and DNA molecules. Such modified oligomers have frequently proven to be useful for probing the chemistry and function of natural RNA and DNA molecules[1] and for developing effective antisense therapies.[2-3] Modified nucleic acids have also been used to study the origin and evolution of life.[4-5] In particular, oligonucleotides with terminal amines have been used to examine the kinetics of chemical ligation reactions.[6-8] Since a 5'-amine has the advantage of being 6000 times more reactive than a 5'-hydroxyl,[9] the possibilities for chemistry and catalysis in aqueous solution are correspondingly enhanced. Previous studies of chemical ligation have focused on the reactivity of a few specific 5'-amino oligonucleotide sequences. In contrast, we hoped to examine the chemical reactivity of a wide variety of 5'-amino oligonucleotide sequences. To this end, we have synthesized 5'-protected-5'-amino-2',5'-dideoxynucleoside phosphoramidites and built these into oligonucleotide libraries. These random sequence pools should eventually be useful for *in vitro* selection experiments.[10-11]

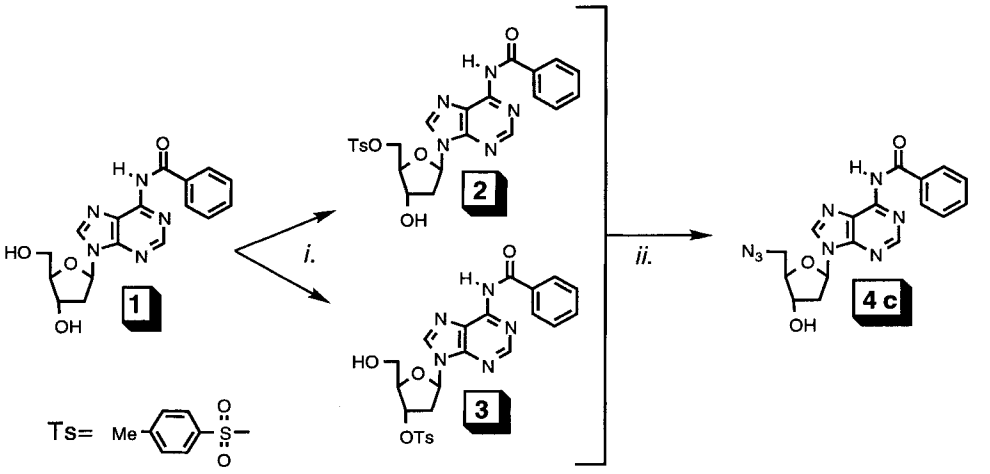
The monomethoxytrityl (MMT) group has previously been used for the protection of the amino functionality.[12-13] Its hydrophobic character aids in the organic synthesis of

the phosphoramidite and it can be removed during automated synthesis by extending the length of the deblocking step (3% trichloroacetic acid (TCA)). However, in the course of our synthesizing large amounts of 5' MMT-protected purine aminonucleosides for future phosphitylation and incorporation into oligonucleotides, we observed changes in their NMR spectra and TLC mobilities following storage.

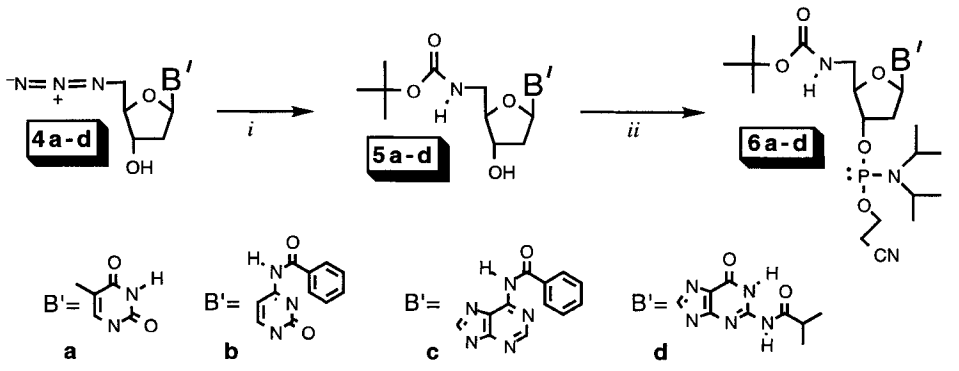
We were thus prompted to examine other protective groups for the 5' amine that would be more stable than the MMT group, yet still be compatible with automated oligonucleotide synthesis. The *t* -butoxycarbonyl (Boc) group was an excellent candidate: it is standardly used in automated peptide synthesis, is easily attached to amines, and can be quantitatively removed with a 1:1 mixture of TFA and CH_2Cl_2. Unfortunately, while these acidic deprotection conditions are compatible with peptide synthesis, they can result in extensive depurination of oligonucleotides. Consequently, the Boc group has not previously been considered for use in oligonucleotide synthesis. However, some studies have shown that the Boc group can be removed under milder acidic conditions.[14-15] Building on this work, we demonstrate here the successful use of the Boc group in oligonucleotide synthesis.

RESULTS AND DISCUSSION

Synthesis of 5'-amino nucleoside phosphoramidites. A series of 5'-azido nucleoside phosphoramidites were initially prepared according to literature procedures. Briefly, the bases of cytidine, adenosine, and guanosine were protected via transient hydroxyl silylation.[16] Cytidine and adenosine were N-acylated with benzoyl chloride in yields of 84% and 81%, respectively. Isobutyric anhydride was used to protect guanosine in 80% yield. The azide functionality was introduced into the pyrimidine nucleosides using the one-pot method of Yamamoto *et al.*;[17] the 5'-hydroxyl was activated with triphenyl phosphine and carbon tetrabromide and then displaced with lithium azide in anhydrous DMF. The azide functionality was introduced into the purine nucleosides in two steps using a modified procedure of Mag and Engels;[13] the 5'-hydroxyls were converted to 5'-tosylates which were then displaced with lithium azide. In the case of adenosine (SCHEME 1), some 3'-tosylate (**3**) was always formed along with the 5'-tosylate (**2**) in a 1:5 ratio, regardless of the concentration of reagents. Separation proved to be extremely difficult, so both compounds were carried through to the next reaction. Only the 5'-tosylate was found to be displaced by lithium azide, generating the desired 5'-azido-2',5'-dideoxyadenosine (**4c**). This compound was then separated from the 3'-tosylate by silica gel chromatography.



SCHEME 1. Introduction of the azido functionality to the 5' position of N^6-bzl-2'-deoxyadenosine. i. TsCl, pyridine ii. LiN_3, DMSO



SCHEME 2. i. Boc anhydride, 10% (w/w) Pd/C, H_2 (22 psi) ii. β-cyanoethyl-N,N-diisopropylamino-chlorophosphite, N,N-diisopropylethylamine

Azides can be directly converted to Boc-protected amines.[18] Conditions for the reduction and protection of the azidonucleosides are presented below (SCHEME 2). This route proved to be faster and led to a more facile purification of the products than when the MMT protective group was used. Synthetic yields for the one-pot reduction and protection are shown in TABLE 1. As was expected, the Boc group proved to be quite stable and very selective. No evidences of diacylation or of the formation of the wrong regioisomer were observed either during synthesis or upon storage for several months. The

TABLE 1. Results of one-pot conversions of 5'-azido-2',5'-dideoxynucleosides to 5' Boc-5'-amino-2',5'-dideoxynucleosides.

Nucleoside	Reaction Time (h)	Equiv. Boc Anhydride	Yield (%)
T	5.0	2.0	94
C	5.0	1.5	91
A	5.5	1.5	83
G	6.0	1.5	88

corresponding phosphoramidites were synthesized by the standard route using β-cyanoethyl-N,N'-diisopropylaminochlorophosphite with Huenig's base catalyst.[19]

Efficiency of incorporation into oligonucleotides. The 5'-amino nucleoside phosphoramidites were incorporated into oligonucleotides using an automated DNA synthesizer (391 PCR Mate, Applied Biosystems, Foster City, CA). The efficiencies of internal incorporation were assessed by monitoring the amount of trityl protecting groups released prior to and following their incorporation, with yields as high as 84-86% over the two steps. Assuming that the addition of the aminonucleoside phosphoramidite and the addition of the residue following were equally efficient, the step-wise coupling efficiencies for each step would have been on the order of 92 - 93%. However, it is apparent that the two steps were not equally efficient. When the capping and deblocking steps were omitted just after the addition of the aminonucleoside phosphoramidite, no further chain elongation occurred. These results suggested that the decreased yield was not a result of inefficient coupling of the modified phosphoramidite. Rather, it might have been due to depurination and/or

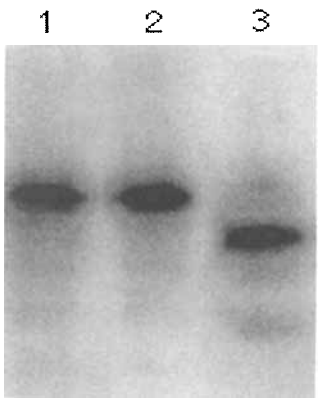


FIGURE 1. Polyacrylamide gel of a phosphoramidate-containing oligomer. Radiolabelled $^{5'}$CGTAGCATGCAT^{N}CGTA$^{3'}$ (Lane 1) as compared to a standard oligomer of the same sequence (Lane 2) and the phosphoramidate-containing oligomer after overnight treatment with 80% acetic acid (Lane 3).

insufficient deprotection. The purity of synthetic oligonucleotides that contained the phosphoramidate linkages was assayed by polyacrylamide gel electrophoresis; an example is shown in FIGURE 1. It was clear that internal purines are protected from depurination, even during the rather harsh deprotection step that is necessary to remove the Boc group. This observation is illustrated in FIGURE 2, in which various standard oligonucleotides were subjected to the conditions used for Boc removal for a time twice as long as necessary. The HPLC traces reveal that loss of adenosine only occurs when this purine is at the 5' terminus. While the use of 10% TFA in CH_2Cl_2 does result in acceptable yields, the use of an oxyphilic Lewis acid may be more appropriate for some applications. Several candidates are currently being investigated.

Pool synthesis. In order to examine the kinetics of oligonucleotide self-replication we wished to synthesize random sequence oligonucleotide pools of the form 5' XNN 3' where X is an equimolar mixture of 5'-aminonucleosides and N is a mixture of natural nucleosides. Resins containing each of the four canonical nucleosides (G, A, T, and C) were pre-mixed and divided into four separate columns. Internal residues were randomized by automated mixing on the DNA synthesizer. The terminal aminonucleosides were then added separately, one per column. Preliminary experiments had revealed that differences in the reactivities of the aminonucleoside phosphoramidites precluded their prior mixing. The final pools were essentially stochastic, as again demonstrated by HPLC analyses of enzymatic digestions (FIGURE 3).

CONCLUSION

The Boc protective group for amines can be used in oligonucleotide synthesis as an alternative to the MMT group. The Boc group is more stable than the MMT group and greatly facilitates the syntheses of monomer phosphoramidites. Deprotection times of 3 - 4 minutes using 10% TFA in CH_2Cl_2 are optimal. However, acid deprotection of the Boc group can result in the loss of some of the 5'-terminal purine nucleosides. Internal purines are safe during the deblocking step and the full-length product can easily be isolated by gel electrophoresis. This method has been used to construct random pools of oligonucleotides that feature primary amines at the 5' termini.

EXPERIMENTAL

General

Automated syntheses of the short oligonucleotides were performed using an ABI 391 DNA Synthesizer. The phosphoramidites were purchased from Glen Research.

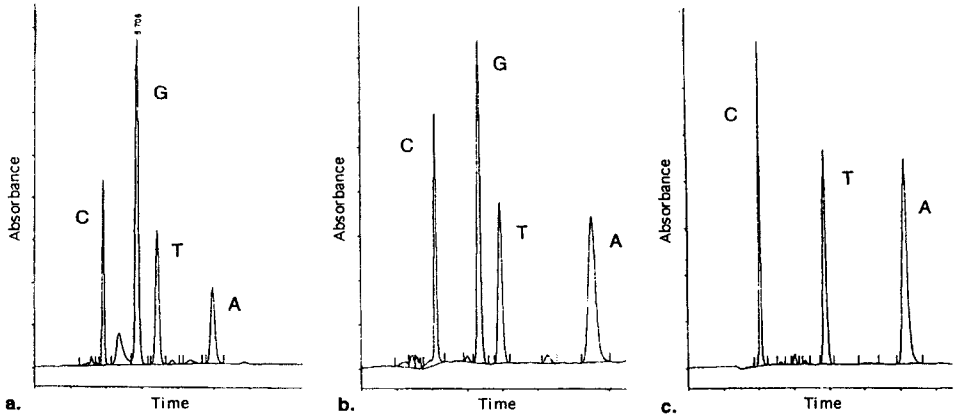


FIGURE 2. Enzymatic digestion analyses of short oligonucleotides that had been subjected to dilute TFA for 7.5 minutes before they were cleaved from the column. a. $^{5'}$ACTG$^{3'}$ b. $^{5'}$TCGA$^{3'}$ c. $^{5'}$TTAACC$^{3'}$ Depurination is evident only when the purine is at the 5' terminus (trace a.).

Columns were purchased from Cruachem. HPLC plots were obtained using a Thermo Separation Products SpectraSystem P2000 pump coupled to a SpectraFocus diode array detector. The reversed phase (C_{18}) column was purchased from Alltech. NMR spectra were obtained using a Varian VXR 400S spectrometer. ^{1}H spectra were obtained at 400 MHz; ^{13}C spectra were obtained at 100 MHz. ^{31}P spectra were obtained at 360 MHz using a Nicolet NIC 1180E spectrometer. IR spectra were obtained with a Galaxy 4020 Series FT-IR. Mass spectra were measured at 1000 resolution using a Kratos MS80 RFAQ with a double focusing magnetic sector. The ion gun was made by Ion Tech and used a Xenon light source. UV spectra were obtained with a Shimadzu UV160U spectrophotometer. Nucleosides were purchased from Farma Waldhof. Nuclease P1 and alkaline phosphatase were obtained from Boehringer Mannheim. Trifluoroacetic acid was purchased from Kodak. All other chemicals were purchased from Aldrich. DMF was vacuum distilled after overnight stirring over Sicapent. Pyridine was distilled over benzophenone; CH_2Cl_2 was distilled over CaH_2. Triphenyl phosphine was recrystallized from toluene.

Syntheses of Phosphoramidites

Lithium azide.[20] Sodium azide (13.0 g; 0.20 mol) and lithium sulfate (14.1 g; 0.13 mol) were dissolved in 90 ml of distilled water and were stirred vigorously at room temperature. After 5 minutes, 400 ml of absolute ethanol were added steadily over 20 minutes. The

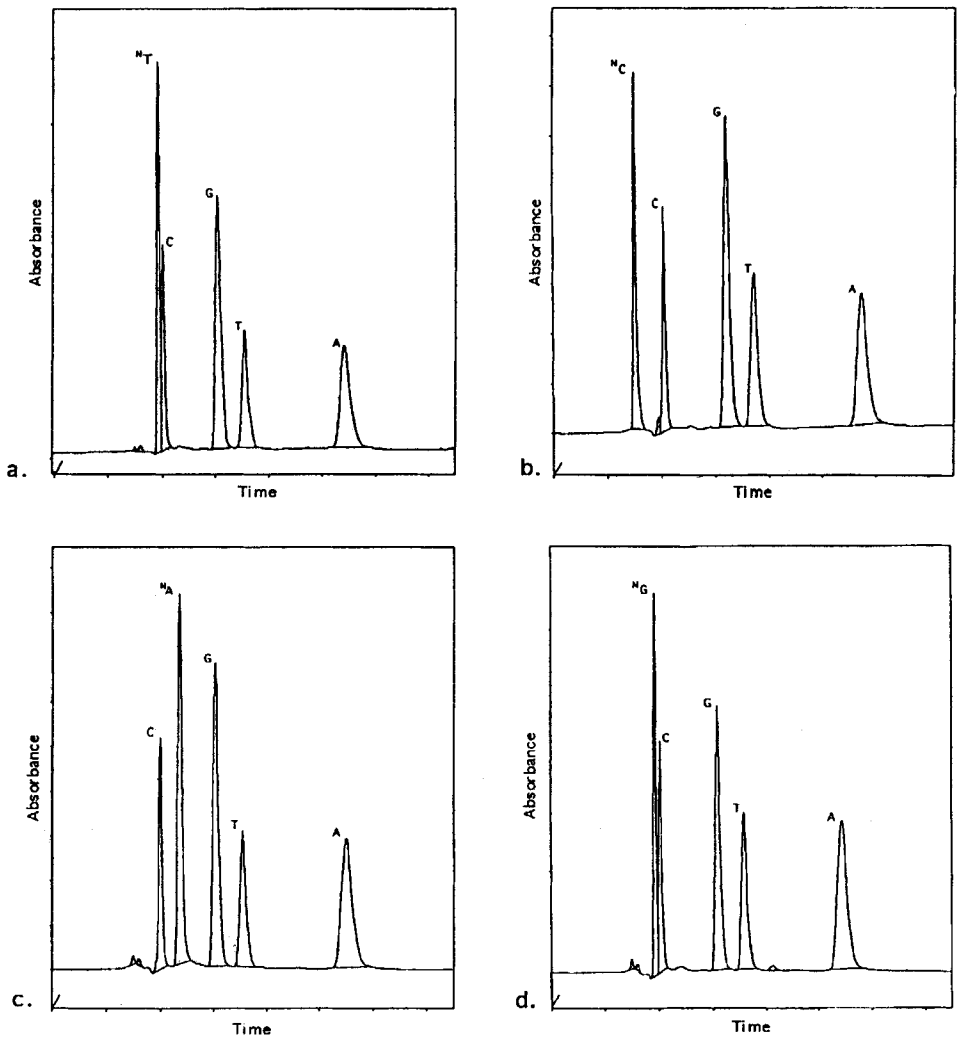


FIGURE 3. Randomized pools with a specific aminodideoxynucleoside (^{N}X) at the 5' termini. a. Thymidine (^{N}T). b. Cytidine (^{N}C). c. Adenosine (^{N}A). d. Guanosine (^{N}G). The pools were made separately so they could be used individually or combined.

contents were left stirring at room temperature for 10 minutes, at which time the white precipitate was removed by filtration with a coarse sintered-glass funnel. The precipitate was washed once with 75 ml of absolute ethanol and the filtrate was concentrated to yield a white powder. With the aid of an ultrasonic bath and heating to 45°C, this powder was suspended in 30 ml of absolute ethanol. The insoluble solid was removed by filtration and was washed twice with 80 ml of absolute ethanol. The filtrate was concentrated, dried on a vacuum manifold, and stored in a vacuum dessicator over P_4O_{10}. Yield: 78% IR: (KBr) 2100.3 (N_3).

5'-O-(4-Methylbenzenesulfonyl)-N^6-benzoyl-2'-deoxyadenosine (**2**). The protected nucleoside **1** (2.5 g; 7.0 mmol) was dried by coevaporation with pyridine (2x) in the reaction vessel. The powder was suspended in 22 ml of dry pyridine and TsCl (4.03 g, 21.0 mmol) was added. The yellow suspension was left stirring under argon at room temperature. After 50 min, the reaction was cooled to 0° C, quenched with 1 ml of water, and stirred for another 20 min. The solution was concentrated and partitioned between ethyl acetate (125 ml) and water (25 ml). The organic layer was washed with 25 ml of 5% $NaHCO_3$ (2x), 25 ml of water (2x), and 25 ml of saturated brine (2x). It was dried with Na_2SO_4 and was concentrated to a yellow powder. The product was isolated by silica gel chromatography using a step gradient of 0 - 7% MeOH in CH_2Cl_2. Yield: 73% Rf: 0.35 mp: 146-149°C ^1H NMR: (400 MHz, $CDCl_3$, TMS): δ 2.4 (s, 3H, CH_3); 2.5-2.6 (m, 1H, 2''); 2.8-2.9 (m,1H, 2'); 4.2-4.3 (m, 3H, 4', 5'); 4.75-4.85 (m, 1H, 3'); 6.45-6.55 (dd, 1H, 1'); 7.2-7.3 (d, 2H, H-$C^{3,5}$Ts); 7.45-7.55 (m, 2H, H-$C^{3,5}$bzl); 7.55-7.65 (m, 1H, H-C^4bzl); 7.65-7.70 (d, 2H, H-$C^{2,6}$Ts); 8.0-8.1 (dd, 2H, H-$C^{2,6}$bzl); 8.25 (s,1H, H-C^8); 8.7 (s, 2H, H-C^2) ^{13}C NMR: (100 MHz, $CDCl_3$, TMS): δ 21.64 (CH_3); 39.82 ($C^{2'}$); 69.08 ($C^{5'}$); 71.48 ($C^{3'}$); 84.49 ($C^{4'}$); 84.87 ($C^{1'}$); 123.26 (C^5); 127.86, 128.00 ($C^{2,3,5,6}$Ts); 128.91 ($C^{3,5}$bzl); 129.99 ($C^{2,6}$bzl); 132.28 (C^4Ts); 132.93 (C^4bzl); 133.65 (C^1bzl); 141.87 (C^8); 145.36 (C^1Ts); 149.53 (C^4); 151.36 (C^6); 152.47 (C^2); 165.09 (C=O) UV: λ_{min} 248.4, λ_{max} 279.4, $\lambda_{1/2max}$ 294.9.

5'-O-(4-Methylbenzenesulfonyl)-N^2-isobutyryl-2'-deoxyguanosine. The protected nucleoside (1.00 g; 3.97 mmol) was dried by coevaporation with pyridine (3x) in the reaction vessel. The powder was suspended in 9 ml of dry pyridine and TsCl (1.70 g, 8.91 mmol) was added. The yellow suspension was left stirring under argon at room

temperature. After 1.75 hrs, the reaction was cooled to 0° C, quenched with 1 ml of water, and stirred for another 20 min. The solution was concentrated and partitioned between ethyl acetate (125 ml) and water (25 ml). The organic layer was washed with 25 ml of 5% $NaHCO_3$ (2x), 25 ml of water (2x), and 25 ml of saturated brine (2x). It was dried with $MgSO_4$ and was concentrated to a yellow powder. The product was isolated by silica gel chromatography using a step gradient of 3 - 10% MeOH in CH_2Cl_2. Yield: 77% Rf: 0.25. ^1H NMR: (400 MHz, $CDCl_3$): δ 1.2-1.3 (dd, 6H, CH_3 (ibu)); 2.4 (s, 3H, CH_3 (Ts)); 2.4-2.5 (m, 1H, 2'); 2.8-2.9 (m,1H, 2''); 2.9-3.0 (m, 1H, CH (ibu)); 4.2-4.3 (m, 2H, 5'); 4.3-4.4 (m, 1H, 4'); 4.8 (m, 1H, 3'); 4.9 (s, 1H, OH); 6.2 (dd, 1H, 1'); 7.2-7.3 (d, 2H, H-$C^{3,5}$Ts); 7.6-7.7 (d, 1H, H-$C^{2,6}$Ts); 7.9 (s,1H, C^8); 10.2 (s, 1H, NH) ^{13}C NMR: (100 MHz, $CDCl_3$): δ 18.98 (CH_3 (ibu)); 21.65 (CH_3 (Ts)); 36.14 ($C^{2'}$); 39.17 (CH (ibu)); 69.81 ($C^{5'}$); 71.37 ($C^{3'}$); 84.46, 85.64 ($C^{4'}$, $C^{1'}$); 121.71 (C^5); 127.78 ($C^{3,5}$Ts); 130.00 ($C^{2,6}$Ts) 131.86 (C^8); 139.08 (C^4Ts); 145.53 (C^4); 147.81 (C^2); 148.35 (C^1Ts); 155.85 (C^6); 180.13 (C=O). MS (FAB): m/z 514 (m+Na). UV: λ_{min} 239.0, λ_{max} 254.4, 283.0.

5'-Azido-5'-deoxythymidine (**4a**). To a solution of thymidine (3.00 g; 12.4 mmol), triphenyl phosphine (3.41 g; 13.0 mmol), and lithium azide (3.65 g; 74.4 mmol) in 42 ml of dry DMF was added carbon tetrabromide (4.32 g; 13.0 mmol). After 24 hours of stirring at room temperature, the yellow solution was quenched with 5 ml of ddH_2O and stirred for an additional 2 hrs. The solution was dissolved in 200 ml CH_2Cl_2 and was extracted with saturated $NaHCO_3$ (40 ml) and saturated brine (2 x 60 ml). The water phase was back extracted with CH_2Cl_2 several times. The organic phase was concentrated and the product was isolated as a white powder on a silica column using a step gradient of 0 - 6% MeOH on CH_2Cl_2 with steps of 2%. Yield: 93% Rf: 0.33 mp: 163-165°C ^1H NMR: (400 MHz, $CDCl_3$/MeOD): δ 1.84 (d, J=1.2, 3H, CH_3); 2.05-2.13 (m, 1H, 2'); 2.21-2.28 (m, 1H, 2''); 3.46-3.48 (dd, J=13.2, J=4.0, 1H, 5'); 3.61-3.66 (dd, J=13.2, J=3.2, 1H, 5"); 3.89-3.93 (dd, J=7.2, J=4.0, 1H, 4'); 4.23-4.28 (m, 1H, 3'); 6.14-6.19 (t, J=6.8, 1H, 1'); 7.30-7.32 (q, J=1.2, 1H, H-C^6) ^{13}C NMR: (100 MHz, $CDCl_3$/MeOD): δ 12.78 (CH_3); 40.34 ($C^{2'}$); 52.49 ($C^{5'}$); 71.22 ($C^{3'}$); 84.51, 86.21 ($C^{1'}$, $C^{4'}$); 111.65 (C^5); 135.97 (C^6); 151.02 (C^2); 164.78 (C^4) MS (FAB): m/z 268 ($m+1^+$) IR: 3388 (OH); 2100 (N_3); 1722, 1654 (C=O) UV: λ_{min}=232.4; λ_{max}=263.6.

5'-Azido-N^4-benzoyl-2',5'-dideoxycytidine (**4b**). To a solution of base-protected cytidine (1.36 g; 4.11 mmol) in 36 ml of dry DMF was added triphenylphosphine (1.37 g; 5.22 mmol), lithium azide (0.66 g; 13.45 mmol), and carbon tetrabromide (1.76 g; 5.30 mmol). The reaction was left stirring under argon at room temperature. After 22 hrs, the solution was quenched with 5 ml of ddH_2O and stirred for an additional 2 hrs. The solution was dissolved in 100 ml CH_2Cl_2 and was extracted with 5% $NaHCO_3$ (30 ml) and sat'd brine (2 x 30 ml). The water phase was back extracted with CH_2Cl_2 several times. The organic phase was concentrated and the product was isolated as a white powder on a silica gel column using a step gradient of 0 - 6% MeOH in CH_2Cl_2. Yield: 73% Rf: 0.33 mp: 150-151°C ^{1}H NMR: (250 MHz, $CDCl_3$/MeOD, TMS): δ 2.21 (ddd, J=6.4, J=3.9, 1H, 2'); 2.59 (ddd, J=13.9, J=6.2, J=5.1, 1H, 2''); 3.73 (ddd, J=13.3, J=4.2, J=3.4, 2H, 5'); 4.11 (ddd, J=4.1, 1H, 4'); 4.32 (ddd, J=6.6, J=5.0, 1H, 3'); 6.22 (dd, J=6.1, 1H, 1'); 7.46-7.75 (m, 4H, H-C^5, H-$C^{3,4,5}$bzl); 8.00 (dt, J=6.8, J=1.4, 2H, H-$C^{2,6}$bzl); 8.22 (d, J=7.4, 1H, H-C^6) ^{13}C NMR: (100 MHz, $CDCl_3$, TMS): δ 39.66 ($C^{2'}$), 51.70 ($C^{5'}$), 70.61 ($C^{3'}$), 85.06 ($C^{1'}$), 86.15 ($C^{4'}$), 96.45 (C^5), 128.33, 128.38 ($C^{2,6,3,5}$ Ar), 132.60 (C^4Ar), 133.16 (C^1Ar), 144.87 (C^6), 154.17 (C^2), 162.94 (C^4), 167.43 (C=O) MS (FAB): m/z 356 (m^+) IR: 3438 (OH); 2110 (N_3); 1658, 1628 (C=O) UV (MeOH): λ_{max} 260.

5'-Azido-N^6-benzoyl-2',5'-dideoxyadenosine (**4c**). The tosylnucleosides **2** and **3** (2.5 g; 4.9 mmol) and lithium azide (1.68 g; 34.3 mmol) were dissolved in 24 ml of spec grade DMSO. After 20 hrs of stirring under argon at room temperature, the solution was dissolved in 200 ml of ethyl acetate and was extracted with 25 ml of 5% $NaHCO_3$ (2x) and 50 ml of saturated brine (2x). The organic layer was concentrated and the product was isolated on silica gel column using a gradient of 0 - 6.4% MeOH with steps of 0.4%. Yield: 75% Rf: 0.33 mp: 120-122°C ^{1}H NMR: (400 MHz, $CDCl_3$, TMS): δ 2.5-2.6 (m, 1H, 2''); 2.85-2.95 (m, 1H, 2'); 3.6-3.7 (dd, 2H, 5'); 4.1-4.2 (m, 1H, 4'); 4.5-4.6 (m, 1H, 3'); 6.5-6.6 (dd, 1H, 1'); 7.45-7.55 (m, 2H, H-$C^{3,5}$bzl); 7.55-7.65 (m, 1H, H-C^4bzl); 8.05-8.15 (dd, 2H, H-$C^{2,6}$bzl); 8.35 (s, 1H, C^8); 8.75 (s, 2H, C^2); 10.35 (s, 1H, NH) ^{13}C NMR: (100 MHz, $CDCl_3$, TMS): δ 39.92 ($C^{2'}$); 51.45 ($C^{5'}$); 70.55 ($C^{3'}$); 83.66 ($C^{4'}$); 84.86 ($C^{1'}$); 124.03 (C^5); 127.71 ($C^{2,3,5,6}$Ar); 131.70 (C^4Ar); 132.95 (C^1Ar); 141.23 (C^8); 149.44 (C^4); 151.10 (C^6); 151.26 (C^2); 164.83 (C=O) IR: 3380 (NH,OH); 2925 (CH); 2105 (NNN) UV: λ_{min} 247.5, λ_{max} 277.8, $\lambda_{1/2max}$ 303.5.

5'-Azido-N^2-isobutyryl-2',5'-dideoxyguanosine (**4d**). Tosylated guanosine (1.05 g; 2.14 mmol) and lithium azide (0.73 g; 15.0 mmol) were dissolved in 10 ml of spec grade DMSO. After 24 hrs of stirring under argon at room temperature, the solution was concentrated under high vacuum. The product was isolated by silica gel chromatography using a gradient of 3 - 10% MeOH with steps of 0.5%. Yield: 74% Rf: 0.23. ^{1}H NMR: (400 MHz, $CD_3OD/CDCl_3$): δ 1.2-1.3 (dd, 6H, CH_3 (ibu)); 2.4-2.5 (m, 1H, 2'); 2.6-2.8 (m, 2H, 2'', CH (ibu)); 3.6 (m, 2H, 5'); 4.0-4.1 (m, 1H, 4'); 4.5 (m, 1H, 3'); 6.3 (dd, 1H, 1'); 8.0 (s,1H, C^8). ^{13}C NMR: (100 MHz, $CD_3OD/CDCl_3$): δ 18.43 (CH_3 (ibu)); 35.65 ($C^{2'}$); 39.54 (CH (ibu)); 52.03 ($C^{5'}$); 71.20 ($C^{3'}$); 84.00, 85.43 ($C^{4'}$, $C^{1'}$); 120.45 (C^5); 137.87 (C^8); 148.19, 148.77 (C^2, C^4); 156.12 (C^6); 180.36 (C=O). MS (FAB): m/z 363 (m+1). IR: 3417 (NH,OH); 2101 (NNN); 1680 (C=O). UV: λ_{max} 253, 283.

5'-N-*t*-butoxycarbonyl-5'-deoxythymidine (**5a**). The azidonucleoside **4a** (350 mg, 1.31 mmol) was dissolved in 30 ml of absolute ethanol in a hydrogenation vessel. Pd/C activated catalyst (35 mg) and Boc anhydride (0.60 ml, 2.6 mmol) were added. The slurry was subjected to hydrogen (22 psi) for 5.0 hours. The vessel was then removed, capped, and allowed to sit overnight. The catalyst was removed by filtration through celite and a medium sintered-glass funnel. The product was obtained as a white solid after purification by silica gel flash chromatography. Yield: 94%. Rf (10% MeOH in CH_2Cl_2): 0.35. ^{1}H NMR (400 MHz, $CDCl_3$:MeOH (9:1)): δ 1.3 (s, 9H, Boc); 1.8 (s, 3H, CH_3(5)); 2.1 (m, 1H, 2'); 2.2 (m, 1H, 2''); 3.2 (m, 2H, 5'); 3.7 (m, 1H, 4'); 4.1 (m, 1H, 3'); 6.1 (t, 1H, 1'); 7.2 (s, 1H, 6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 12.2 (CH_3(5)); 28.1 (CH_3(Boc)); 39.1 (2'); 41.7 (5'); 70.7 (3'); 79.9 (C(Boc); 84.8, 85.0 (4',1'); 111.1 (5); 136.0 (6); 150.6 (2); 156.9 (C=O(Boc)); 164.4 (4). MS (FAB): m/z 342 (m+1). IR: 3382, 3205 (OH, NH); 2981, 2925 (CH); 1726, 1687, 1656 (C=O). UV: λ_{max} = 264.

5'-N-*t*-butoxycarbonyl-N^4-benzoyl-2',5'-dideoxycytidine (**5b**). The azidonucleoside **4b** (370 mg, 1.0 mmol) was dissolved in 30 ml of absolute ethanol in a hydrogenation vessel. Pd/C activated catalyst (37 mg) and Boc anhydride (0.36 ml, 1.6 mmol) were added. The slurry was subjected to hydrogen (22 psi) for 5.0 hours. The vessel was then removed, capped, and allowed to sit overnight. The catalyst was removed by filtration through celite and a medium sintered-glass funnel. The product was obtained as a white solid after purification by silica gel flash chromatography. Yield: 91%. Rf (10% MeOH in CH_2Cl_2):

0.35. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.4 (s, 9H, Boc); 2.1 (m, 1H, 2'); 2.5 (m, 1H, 2''); 3.3 (m, 2H, 5'); 3.9 (m, 1H, 4'); 4.1 (m, 1H, 3'); 6.1 (t, 1H, 1'); 7.4 (t, 2H, $C^{3,5}$bzl); 7.5 (t, 1H, C^{4}bzl); 7.6 (d, 1H, 5); 7.9 (d, 2H, $C^{2,6}$bzl); 8.0 (d, 1H, 6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.2 (CH_3(Boc)); 40.4 (2'); 41.8 (5'); 70.7 (3'); 80.0 (C(Boc)); 85.8 (1'); 87.2 (4'); 97.4 (5); 127.8 ($C^{3,5}$bzl); 128.7 ($C^{2,6}$bzl); 132.9 (C^{1}bzl); 133.0 (C^{4}bzl); 144.3 (6); 156.0 (2); 156.9 (C=O(Boc)); 163.1 (4); 167.4 (C=O(bzl)). MS (FAB): m/z 431 (m+1). UV: λ_{max} = 256.

5'-N-*t*-butoxycarbonyl-N^6-benzoyl-2',5'-dideoxyadenosine **(5c)**. The azidonucleoside **4c** (430 mg, 1.1 mmol) was dissolved in 30 ml of absolute ethanol in a hydrogenation vessel. Pd/C activated catalyst (43 mg) and Boc anhydride (0.40 ml, 1.7 mmol) were added. The slurry was subjected to hydrogen (22 psi) for 5.5 hours. The vessel was then removed, capped, and allowed to sit overnight. The catalyst was removed by filtration through celite and a medium sintered-glass funnel. The product was obtained as a white solid after purification by silica gel flash chromatography. Yield: 83%. Rf (10% MeOH in CH_2Cl_2): 0.30. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.4 (s, 9H, Boc); 2.4 (m, 1H, 2'); 2.9 (m, 1H, 2''); 3.6 (m, 2H, 5'); 4.1 (m, 1H, 4'); 4.5 (m, 1H, 3'); 6.3 (t, 1H, 1'); 7.5 (t, 2H, $C^{3,5}$bzl); 7.6 (t, 1H, C^{4}bzl); 8.0 (d, 2H, $C^{2,6}$bzl); 8.1 (s, 1H, 8); 8.8 (s, 1H, 2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 28.4 (CH_3(Boc)); 39.4 (2'); 42.0 (5'); 71.5 (3'); 79.8 (C(Boc); 86.2 (1'); 87.1 (4'); 123.8 (5); 128.1 ($C^{3,5}$bzl); 128.7 ($C^{2,6}$bzl); 132.8 (C^{4}bzl); 133.6 (C^{1}bzl); 142.3 (8); 150.2 (4); 150.9 (6); 152.4 (2); 157.5 (C=O(Boc)); 165.4 (C=O (bzl)). MS (FAB): m/z 455 (m+1). IR: 3355, 3271 (OH, NH); 2976, 2930 (CH); 1696 (C=O). UV: λ_{max} = 279.

5'-N-*t*-butoxycarbonyl-N^2-isobutyryl-2',5'-dideoxyguanosine **(5d)**. The azidonucleoside **4d** (400 mg, 1.1 mmol) was dissolved in 30 ml of absolute ethanol in a hydrogenation vessel. Pd/C activated catalyst (40 mg) and Boc anhydride (0.38 ml, 1.6 mmol) were added. The slurry was subjected to hydrogen (22 psi) for 6.0 hours. The vessel was then removed, capped, and allowed to sit overnight. The catalyst was removed by filtration through celite and a medium sintered-glass funnel. The product was obtained as a white solid after purification by silica gel flash chromatography. Yield: 88%. Rf (10% MeOH in CH_2Cl_2): 0.30. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.2 (m, 6H, CH_3(ibu)); 1.5 (s, 9H, Boc); 2.4 (dd, 1H, 2'); 2.8 (m, 1H, CH (ibu)); 3.0 (m, 1H, 2''); 3.7 (dd, 2H, 5'); 4.3 (m, 1H, 4'); 4.5 (m, 1H, 3'); 6.4 (dd, 1H, 1'); 7.8 (s, 1H, 8) . ^{13}C NMR (100 MHz,

$CDCl_3$): δ 18.7, 19.0 (CH_3(ibu)); 28.4 (CH_3(Boc)); 35.8 (2'); 40.3 (5'); 43.8 (CH (ibu); 73.5 (3'); 81.0 (C(Boc); 86.7, 87.5 (4',1'); 122.6 (5); 139.5 (8); 147.8, 148.7 (2, 4); 155.8 (C=O(Boc)); 158.2 (6); 180.5 (C=O (ibu)). MS (FAB): m/z 437 (m+1); 459 (m+Na), 481 (m-1+2Na). UV: λ_{max} = 254; 281.

5'-N-*t*-butoxycarbonyl- 5'-deoxythymidine-3-O-(2-cyanoethyl-N,N-diisopropyl-amino)phosphite **(6a)**. The nucleoside **5a** (200 mg, 0.59 mmol) was dried in a vacuum dessicator over P_4O_{10} for 4 days. It was then dissolved in 4.5 ml of CH_2Cl_2. Huenig's base (0.21 ml, 1.18 mmol) and β-cyanoethyl-N,N-diisopropylaminochlorophosphite (0.20 ml, 0.89 mmol) were added by syringe. After 4 hours at room temperature, the reaction was diluted to 10 ml and was then extracted with 12 ml of saturated $NaHCO_3$ and 12 ml of saturated brine. The organic phase was dried with $MgSO_4$, which was then removed by gravity filtration. The product diasteriomers were recovered as a white solid after silica gel flash chromatography using a solvent system of ethyl acetate, hexanes, and triethylamine (67:30:3). Yield: 60%. Rf: 0.60, 0.65. ^{31}P NMR (360 MHz, $CDCl_3$): δ 149.9; 150.7. MS (FAB): m/z 542 (m+1).

5'-N-*t*-butoxycarbonyl-N^4-benzoyl-2',5'-dideoxycytidine-3-O-(2-cyanoethyl-N,N-diisopropylamino)phosphite **(6b)**. The nucleoside **5b** (377 mg, 0.88 mmol) was dried in a vacuum dessicator over P_4O_{10} for one day. It was then dissolved in 8.5 ml of CH_2Cl_2. Huenig's base (0.31 ml, 1.75 mmol) and β-cyanoethyl-N,N-diisopropylaminochloro-phosphite (0.29 ml, 1.32 mmol) were added by syringe. After one hour at room temperature, the reaction was diluted to 10 ml and was then extracted once with 13 ml of saturated $NaHCO_3$ and twice with 10 ml of saturated brine. The organic phase was dried with $MgSO_4$, which was then removed by gravity filtration. The product diasteriomers were recovered as a pale yellow solid after silica gel flash chromatography using a solvent system of ethyl acetate, hexanes, and triethylamine (67:30:3). Yield: 76%. Rf: 0.60, 0.65. ^{31}P NMR (360 MHz, $CDCl_3$): δ 149.1; 150.2. MS (FAB): m/z 631 (m+1); 653 (m+Na).

5'-N-*t*-butoxycarbonyl-N^6-benzoyl-2',5'-dideoxyadenosine-3-O-(2-cyanoethyl-N,N-diisopropylamino)phosphite **(6c)**. The nucleoside **5c** (200 mg, 0.44 mmol) was dried in a vacuum dessicator over P_4O_{10} for one week. It was then dissolved in 4.5 ml of CH_2Cl_2. Huenig's base (0.15 ml, 0.88 mmol) and β-cyanoethyl-N,N-diisopropylaminochloro-phosphite (0.15 ml, 0.66 mmol) were added by syringe. After one hour at room

temperature, the reaction was diluted to 10 ml and was then extracted with 12 ml of saturated $NaHCO_3$ and 12 ml of saturated brine. The organic phase was dried with $MgSO_4$, which was then removed by gravity filtration. The product diasteriomers were recovered as a white solid after silica gel flash chromatography using a solvent system of ethyl acetate, hexanes, and triethylamine (67:30:3). Yield: 72%. Rf: 0.35, 0.40. ^{31}P NMR (360 MHz, $CDCl_3$): δ 152.7; 153.0. MS (FAB): m/z 637 (m+1); 659 (m+Na).

5'-N-*t*-butoxycarbonyl-N^2-isobutyryl-2',5'-dideoxyguanosine-3-O-(2-cyanoethyl-N,N-diisopropylamino)phosphite **(6d)**. The nucleoside **5d** (200 mg, 0.46 mmol) was dried in a vacuum dessicator over P_4O_{10} for 5 days. It was then suspended in 4.5 ml of CH_2Cl_2. Huenig's base (0.16 ml, 0.92 mmol) and β-cyanoethyl-N,N-diisopropylaminochloro-phosphite (0.15 ml, 0.66 mmol) were added by syringe. After three hours at room temperature, the reaction was diluted to 10 ml and was then extracted with 12 ml of saturated $NaHCO_3$ and 13 ml of saturated brine. The organic phase was dried with $MgSO_4$, which was then removed by gravity filtration. The product diasteriomers were recovered as a white solid after silica gel flash chromatography using a solvent system of ethyl acetate, hexanes, and triethylamine (82:15:3). Yield: 73%. Rf: 0.17, 0.20. ^{31}P NMR (360 MHz, $CDCl_3$): δ 148.8; 149.5. MS (FAB): m/z 655 (m+1).

Incorporation. A dry solution of 25mg of the modified phosphoramidite (X) in 0.5 ml of CH_3CN was sufficient for two couplings. The synthesizer was paused for 10 minutes for coupling X to the growing chain. Then the automated synthesis was resumed. At the end of the cycle, the acidic solution was then taken into a syringe and forced through the column containing the resin at a rate of 1 ml / min for four minutes at room temperature. Immediately afterward, the column was washed with CH_2Cl_2. For internal incorporation of the phosphoramidites, the column was placed back on the synthesizer to continue the automated synthesis. The synthesizer was again paused for 12 minutes as the next phosphoramidite coupled to the chains with X at the 5' terminus. After the coupling was complete, the capping step was extended to one minute to ensure that all unreacted 5' amino termini were blocked.

Enzymatic Digestion and HPLC Analysis. Nuclease P1 (1 unit) was added to 0.1 units (A_{260}) of the oligomer. After the addition of 20 μL of 0.5 M ammonium acetate (pH=5.3), distilled water was added to bring the final volume to 100 μL. The reaction was allowed to incubate for one hour at 37° C. Alkaline phosphatase (5 units) was added and

the reaction was again incubated for one hour at 37° C. The digested oligomers were analyzed by HPLC using a reversed phase (C_{18}) column and an isocratic solvent system of CH_3CN (5%) in 0.1 M triethyl-ammonium acetate (pH=7.0). The single-wavelength chromatograms were obtained at 254 nm.

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