N3′→P5′ Oligodeoxyribonucleotide Phosphoramidates: A New Method of Synthesis Based on a Phosphoramidite Amine-Exchange Reaction

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A new method for the synthesis of N3′→P5′ phosphoramidate oligodeoxynucleotides is demonstrated. Described herein is the synthesis of the monomers utilized in the phosphoramidite amine-exchange process and the experimental details pertaining to this new mode of chain assembly. The phosphoramidite amine-exchange method generates coupling yields in the 92−95% range per cycle and further enables the synthesis of chimeric phosphoramidate/phosphodiester or phosphoramidate/phosphorothioate oligonucleotides with no instrument modifications.

Introduction

The synthesis of nucleic acid analogues has assumed considerable importance as the chemical and pharmaceutical communities attempt to bring the promise of the antisense and antigene concepts to fruition. A plethora of chemical modifications have been investigated, in hopes of developing oligonucleotide-based therapeutics with improved stability and hybridization properties, as well as increased cell membrane permeability.[1] Many of the modified oligonucleotides reported in the literature arise from chemical modification of the native internucleoside phosphodiester linkage. Phosphorothioates, and the vast majority of "first generation" antisense agents which possess increased hydrolytic stability, are accompanied by weaker binding affinity for their RNA or DNA targets via duplex or triplex formation.[2]

The synthesis and hybridization properties of uniformly modified oligonucleotide N3′→P5′ phosphoramidates were recently described.[3] These analogues possess several advantageous features, including an achiral phosphorus-containing and negatively charged backbone, excellent water solubility, improved resistance to nuclease degradation, minimal adventitious protein binding, and high affinity and sequence-specific binding to native single-stranded RNA and DNA, as well as double-stranded DNA targets. These attributes render these analogues outstanding candidates for potential therapeutic and diagnostic applications, and in our opinion, represent one of the leading classes of "second generation" antisense and "first generation" antigene agents.

In our attempt to optimize and scale-up the oxidative phosphorylation method previously reported,[3] we observed severe limitations with this method, particularly in the case of purine rich sequences. Consequently, we undertook an effort to investigate alternative chemistries in hopes of developing an improved and scaleable method for the synthesis of N3′→P5′ phosphoramidate oligonucleotides (pnODNs). A number of synthetic strategies were investigated employing P(III) and P(V) chemistries in both the 3′→5′ and 5′→3′ direction. After carefully evaluating the merits and limitations of each approach, we focused our efforts on a method for the synthesis of pnODNs that employs a phosphoramidite amine-exchange reaction.[4] This improved method and the corresponding monomer syntheses are described herein.

Results and Discussion

The general strategy of the new method, unlike the oxidative phosphorylation approach, employs a 5′→3′ strategy and utilizes a trityl-protected 3′-aminonucleoside 5′-phosphoramidite, **4**, for the basic building block. Each coupling involves the exchange of the diisopropylamino group of the incoming 5′-phosphoramidite monomer for the (5′-succinylated and solid-support bound) 3′-amino-(oligo)nucleoside, via a phosphoramidite amine-exchange reaction.[5] The internucleotide phosphoramidite is then oxidized to the relatively stable phosphoramidate as shown in Scheme 1.

Synthesis of 3′-(trityl)amino-5′-phosphoramidite monomers. The syntheses of the 3′-(trityl)amino 5′-phosphoramidite monomers are shown below.

The synthesis of the thymidine ("T") monomer is shown in Scheme 2. Catalytic hydrogenation of azide **1t**[a],[6] and protection of the resulting 3′-amine with trityl chloride, afforded an 83% yield of 3′-(trityl)amino-5′-*O*-anisoyl-3′-deoxythymidine, **2t**[a]. The anisoyl protecting group was removed with sodium hydroxide, and the 5′-hydroxyl was phosphitylated to afford an 86% yield of 3′-(trityl)amino 5′-phosphoramidite "T" monomer, **4t** (71% overall yield from **1t**[a]).

The synthesis of the 2′-deoxycytidine ("C") monomer is shown in Scheme 3. Compound **2c** was found to be

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(1) Uhlman, E.; Peyman, A. *Chem. Rev.* **1990**, *90*, 544.

(2) Kibler-Herzog, L.; Zon, G.; Uzanski, B.; Whittier, G.; Wilson, W. D. *Nucleic Acids Res.* **1991**, *19*, 2979.

(3) (a) Gryaznov, S.; and Chen, J.-K. *J. Am. Chem. Soc.* **1994**, *116*, 3143. (b) Chen, J.-K.; Schultz, R. G.; Lloyd, D. H.; Gryaznov, S. M. *Nucleic Acids Res.* **1995**, *23*, 2661. (c) Gryaznov, S. M.; Lloyd, D. H.; Chen, J.-K.; Schultz, R. G.; DeDionisio, L. A.; Ratmeyer, L.; Wilson, W. D. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5798.

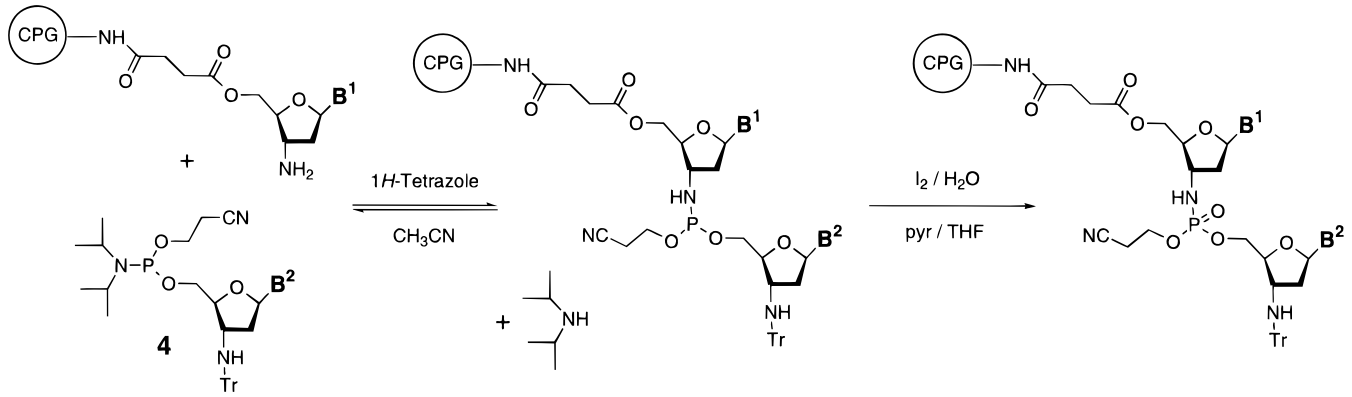
(4) McCurdy, S. N.; Nelson, J. S.; Hirschbein, B. L.; Fearon, K. L. *Tetrahedron Lett.* **1997**, *38*, 207.

(5) The phosphoramidite amine-exchange method has previously been used to form (from one to three) P3′→N5′ phosphoramidate oligonucleotide linkages. (a) Bannwarth, W. *Helv. Chim. Acta* **1988**, *71*, 1517. (b) Gryaznov, S. M.; Letsinger, R. L. *Nucleic Acids Res.* **1992**, *20*, 3403. The phosphoramidite amine-exchange approach has also been recently described for the synthesis of 2′-fluoro-2′-deoxynucleotide N3′→P5′ phosphoramidates in which the basicity of the 3′-amine is considerably lower than in this reported chemistry where the 2′-position is unfunctionalized. (c) Schultz, R. G.; Gryaznov, S. M. *Nucleic Acids Res.* **1996**, *24*, 2966.

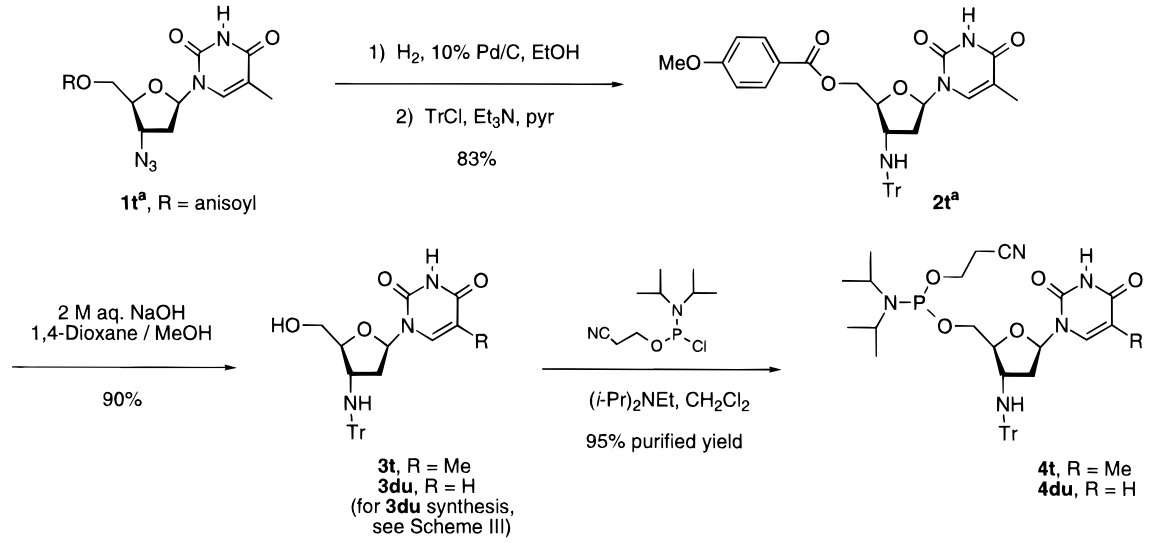
(6) Czernecki, S.; Valéry, J.-M. *Synthesis* **1991**, 239.

S0022-3263(97)00801-3 CCC: $14.00

Scheme 1



Scheme 2



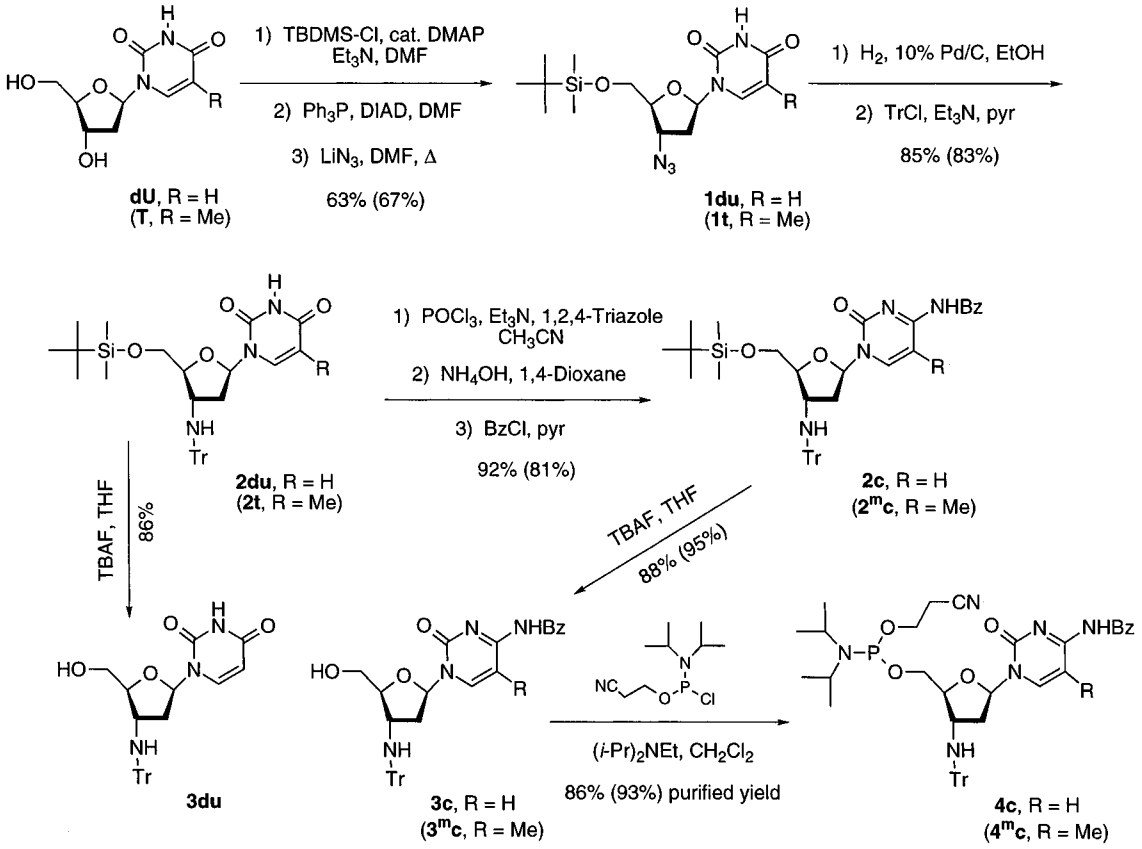
more readily and efficiently synthesized employing the dU → dC route depicted in Scheme 3, rather than via lithium azide ring-opening of the 2,3′-anhydro-2′-deoxycytidine derivative.[7] Selective silylation of the 5′-OH of 2′-deoxyuridine with *tert*-butyldimethylsilyl chloride,[8] Mitsonobu reaction facilitated ring-closure,[9] and subsequent ring-opening with lithium azide afforded a 63% yield of **1du**. Catalytic hydrogenation of **1du** and protection of the 3′-amine with trityl chloride afforded an 85% yield of **2du**. Pyrimidine ring functionalization by the method of Reese and co-workers[10] afforded **2c** in a 92% yield (in three steps). The silyl protecting group was cleanly removed in an 88% yield with TBAF, which was found to provide higher yields than $Et_3N{\cdot}3HF$.[11] The 5′-hydroxyl of **3c** was then phosphitylated to afford an 86% yield of 3′-(trityl)amino-5′-phosphoramidite "C" monomer, **4c** (37% overall yield from dU). In a similar fashion, **3du** and **3^{m}c** monomers have also been synthesized (for RNA decoy and triplex applications, respectively), as depicted in Scheme 3.

The synthesis of the 2′-deoxyguanosine ("G") monomers is shown in Schemes 4 and 5. Although we determined that protection of O^6 of guanosine is preferred (data not shown),[12] the syntheses of both **4g** and **4g^{dpc}** are provided in Scheme 4 and Scheme 5, respectively. Silylation of the 5′-OH of the xylodeoxyguanosine derivative, **^{x}g**,[3b] hydrolysis of the 3′-*O*-benzoate, and subsequent conversion to the 3′-azide proceeded smoothly. Because the 3′-azide was found to be difficult to purify, it was directly hydrogenated and purified as the 3′-amine, **1g**. Tritylation of **1g** afforded a 49% overall yield of **2g** (from **^{x}g**). Silyl protecting group removal with TBAF afforded a 93% yield of **3g**, and phosphitylation under the standard conditions yielded a 69% yield of monomer **4g** (31% overall yield from **^{x}g**).

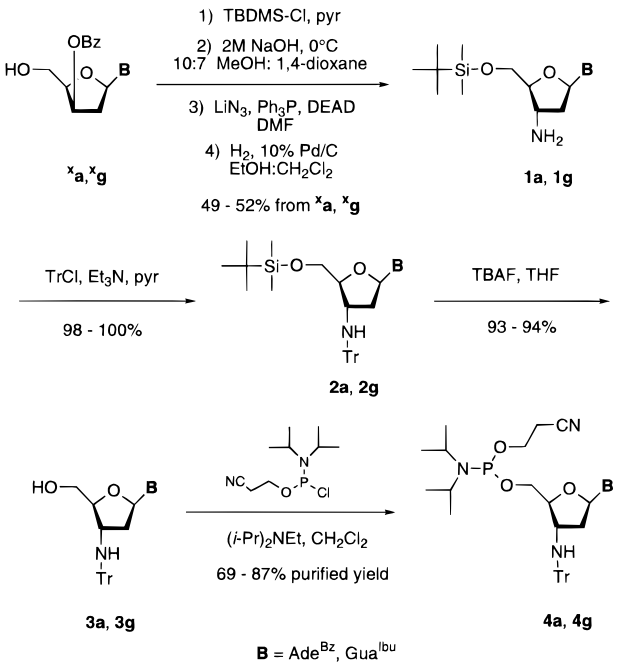
The O^6-protected 2′-deoxyguanosine ("G^{dpc}") monomer, **4g^{dpc}**, was synthesized as shown in Scheme 5. Protection of O^6 of **2g** with diphenylcarbamoyl chloride, and $Et_3N{\cdot}3HF$ mediated removal of the 5′-silyl protecting group (the dpc protecting group was found to be unstable to TBAF) provided an 82% yield of **3g^{dpc}**, which was then phosphitylated to afford a 92% yield of **4g^{dpc}** (32% overall yield from **^{x}g**). Purification of **4g^{dpc}** was found to be considerably easier than that of **4g** (see the Experimental Section), and **4g^{dpc}** was furthermore found to be significantly more stable in solution.

(7) Mikhailopulo, I. A.; Zaitseva, G. V.; Vaaks, E. V.; Rosemeyer, H.; Seela, F. *Nucleosides Nucleotides* **1992**, *11*, 273.
(8) Chaudhary, S. K.; Hernandez, O. *Tetrahedron Lett.* **1979**, *2*, 99.
(9) Mitsunobu, O. *Synthesis* **1981**, 1.
(10) Reese, C. B.; Skone, P. A. *J. Chem. Soc. Perkin Trans. I* **1984**, 1263.
(11) Due to the acidic nature of $Et_3N{\cdot}3HF$ and the sensitivity of the 3′-(trityl)amino group to acid exposure, TBAF was found to provide higher yields than $Et_3N{\cdot}3HF$. The only exception to this trend was in the case of **3g^{dpc}** (Scheme 5), in which TBAF was found to cleave the O^6-diphenylcarbamoyl protecting group. In this case the $Et_3N{\cdot}3HF$ reagent was found to be acceptable if the reaction is run in dichloromethane/pyridine.
(12) (a) Himmelsbach, F.; Schulz, B. S.; Trichtinger, T.; Charubala, R.; Pfleiderer, W. *Tetrahedron* **1984**, *40*, 59. (b) Pon, R. T.; Usman, N.; Damha, M. J.; Ogilvie, K. K. *Nucleic Acids Res.* **1986**, *14*, 6453. (c) Kamimura, T.; Tsuchiya, M.; Koura, K.; Sekine, M.; Hata, T. *Tetrahedron Lett.* **1983**, *24*, 2775.

Scheme 3



Scheme 4



The synthesis of the 2′-deoxyadenosine ("A") monomer is also shown in Scheme 4. The synthesis of **1a**, as well as compounds **2a**−**4a**, is analogous to the "G" monomer synthesis (**1g**−**4g**). The overall yield of **2a** (from ^{x}**a**)[13] was 53%, and as described above, the 3′-amino compound, **1a** (after catalytic hydrogenation), was found to be more easily purified than the 3′-azide. Desilylation with TBAF afforded a 94% yield of **3a**, and phosphitylation proceeded smoothly to afford an 87% yield of **4a** (43% overall yield from ^{x}**a**).

The synthesis of 5′-succinylates **5t**, **5c**, **5a**, and **5g**^{dpc} is shown in Scheme 6. The 5′-hydroxyl of **3t**, **3c**, **3a**, and **3g**^{dpc} was individually reacted with succinic anhydride and DMAP in dichloromethane to afford 5′-succinylates **5t**, **5c**, **5a**, and **5g**^{dpc} in 94%, 76%, 100%, and 78% yields, respectively. Each succinylate was then attached to the solid-support (aminopropyl CPG) by conventional HOBT/HBTU chemistry.[18]

Chain-Assembly of N3′→P5′ Phosphoramidates via Amine-Exchange. The phosphoramidate oligonucleotide chain-assembly cycle is outlined in Table 1. The cycle begins with detritylation of the 3′-(trityl)amino nucleoside bound to aminopropyl-CPG via a 5′-succinyl linker (1 μmol). The trityl group is used for protection of the 3′-amine because it is stable to the weakly acidic coupling solution, as well as the oxidation and capping reagents, yet is completely removed by a 60 s flow of 3% dichloroacetic acid in CH_2Cl_2. The resulting 3′-ammonium dichloroacetate salt is then coupled to 5′-phosphoramidite **4** in the presence 1*H*-tetrazole in acetonitrile, followed by immediate oxidation of the inter-

(13) (a) Herdewijn, P. A. M. *J. Org. Chem.* **1988**, *53*, 5050. (b) Herdewijn, P.; Van Aerschot, A. *Tetrahedron Lett.* **1989**, *30*, 855.

(14) 3-*O*-(Dimethoxytrityl)-2′-deoxynucleoside 5′-(2-cyanoethyl *N*,*N*-diisopropylphophoramidites) are available from Glen Research. A 90-s detritylation time with 3% dichloroacetic acid/DCM should be used.

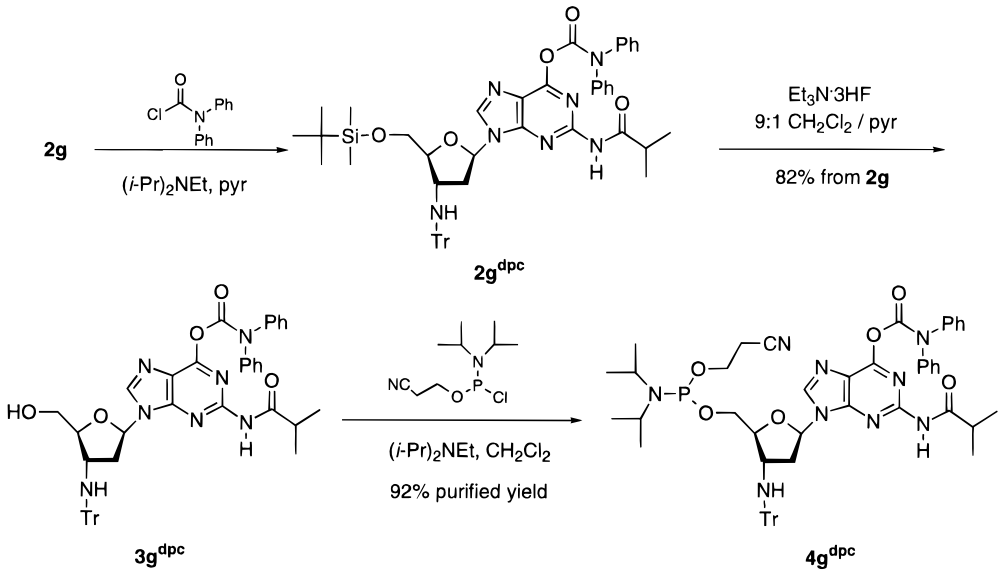
(15) Hirschbein, B. L.; *et al.* Unpublished results.

(16) (a) Brill, W. K.-D. *Tetrahedron Lett.* **1994**, *35*, 3041. (b) Scremin, C. L.; Zhou, L.; Srinivasachar, K.; Beaucage, S. L. *J. Org. Chem.* **1994**, *59*, 1963.

(17) Precaution! The heating of LiN_3 can result in violent decomposition (explosion) in the presence of heavy metals, and therefore, only LiN_3 with acceptably low levels (≤0.6% ppm) of heavy metals should be utilized.

(18) Carpino, L. A.; El-Faham, A.; Minor, C. A.; Albericio, F. *J. Chem. Soc., Chem. Commun.* **1994**, 201 and the references cited therein.

Scheme 5



Scheme 6

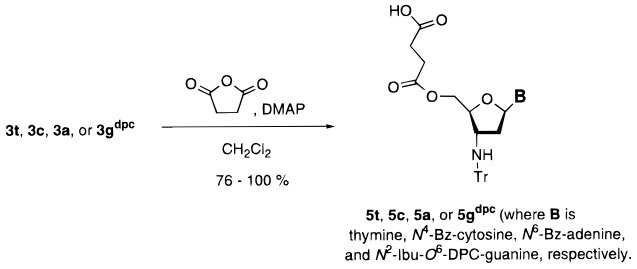


Table 1. Phosphoramidite Amine-Exchange Cycle for the Synthesis of Oligonucleotide N3′→P5′ Phosphoramidates

1) 3% Cl_2CHCO_2H in CH_2Cl_2 (60 s), then CH_3CN wash (6 × 10 s)
2) monomer (15 equiv; 0.1 M) + 1*H*-tetrazole (200 equiv; 0.5 M) in CH_3CN (5 min)
3) 0.1 M I_2 in 2:20:78 H_2O:pyridine:THF (0.8 mL; 2 min), then CH_3CN wash (6 × 10 s)
4) repetition of steps 2 and 3 (COCOA)
5) 1:1:8 IBA:2,6-lutidine:THF (0.65 mL) + 16.5% v/v NMI:THF (0.65 mL; 2 min)
6) repetition of steps 1−5
7) 3% Cl_2CHCO_2H in CH_2Cl_2 (60 s), then CH_3CN wash (6 × 10 s)
8) concentrated aqueous NH_3 (58 °C for 8−12 h)

nucleotide phosphoramidite with iodine/H_2O. After thorough washing of the support-bound oligonucleotide with acetonitrile, the coupling and oxidation steps are repeated (double couple/ox). Attempts to neutralize the amine salt with an amine solution and subsequently rinse with acetonitrile prior to coupling (as an alternative to the COCOA protocol) were found to be inferior to the method described herein (data not shown). Finally, unreacted 3′-amino groups are capped with isobutyric anhydride/*N*-methylimidazole (IBA/NMI). We found that the widely used acetic anhydride/*N*-methylimidazole capping reagents react to some extent with the terminal 3′-(trityl)amine, whereas the the bulkier isobutyric anhydride reagent does not exhibit this problem.

The synthesis cycle is repeated until the desired sequence is fully assembled, then the resulting oligonucleotide is cleaved from the support and deprotected in concentrated aqueous ammonia (1 mL) at 58 °C for 8−12 h. The pnODNs can be synthesized with either a terminal 3′-amino group by using the described monomers, **4**, or with a terminal 3′-hydroxyl group by using commercially available 5′-(2-cyanoethyl *N*,*N*-diisopropylphosphoramidite) 3′-*O*-(dimethoxytrityl)-2′-deoxynucleosides for the final cycle.[14] Chimeric oligonucleotides, with any combination of N3′→P5′ phosphoramidate, phosphodiester (*vide infra*), and/or phosphorothioate (data not reported) linkages in predetermined positions can be synthesized easily by choosing the appropriate 3′-(trityl)amino or 3′-*O*-DMT monomers and oxidation or sulfurization reagents. The pnDNAs are generally synthesized "trityl-off", because ammoniolytic removal of the cyanoethyl protecting groups renders the phosphoramidate oligomer relatively unstable to the acidic detritylation conditions, and are purified via preparative ion-exchange chromatography (IEC) at pH 12.

Table 2. Comparative Analysis of Couple/Oxidize (1× ~30 equiv) vs COCOA (2× ~15 equiv)

method	crude OD_{260}	×	HPLC purity	=	yield (OD units)
couple/oxidation	113		21.3%		24.1
COCOA	115		29.4%		33.8

The effect on product yield of a single couple/oxidation step was compared with that of a couple/oxidize-couple/oxidize approach (COCOA) as follows: the oligomer 5′-AAC-ATG-GAG-AGC-GTC-3′ was synthesized employing the 5′-diisopropylamino phosphoramidite monomers, **4**, and using the procedure described in the Experimental Section. The result was then compared to a second synthesis which was identical to that above except (1) a single couple/oxidation was used per cycle (instead of two) and (2) the concentration of monomer solution was 0.2 M (instead of 0.1 M). Therefore in the single couple/oxidation experiment 30 equiv of monomer were used in each synthesis cycle, whereas in the COCOA experiment 15 equiv of monomer were used in each of the two coupling steps, again totaling 30 equiv of monomer per cycle. The results, shown in Table 2, demonstrate the improved efficiency using COCOA, which is in accordance with equilibrium theory for an amine-exchange reaction. Presumably, the first oxidation "locks in" the equilibrium concentration of desired phosphoramidite from the first coupling as the stable phosphoramidate, and then any

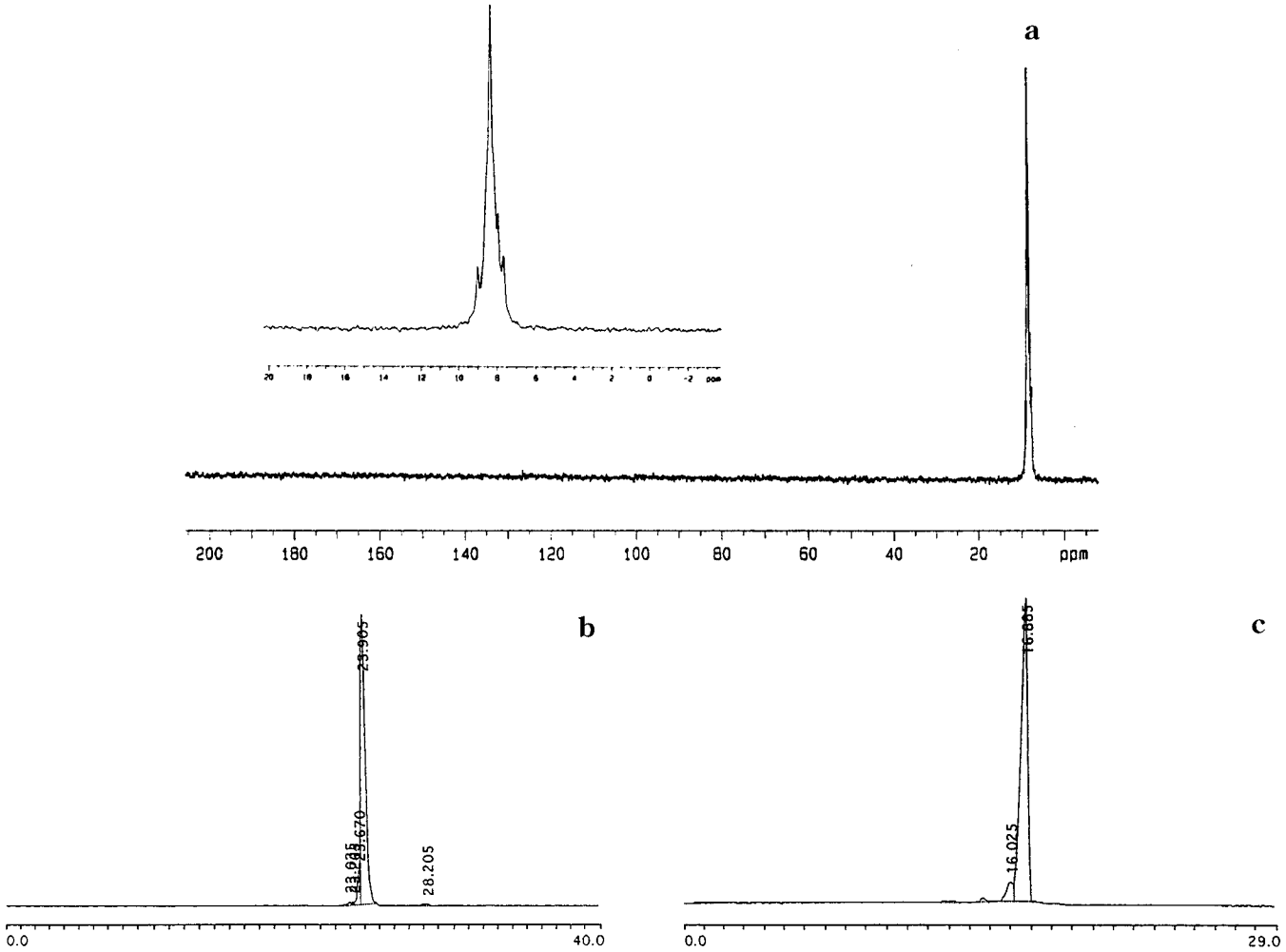


Figure 1. Representative data for phosphoramidate 5′-AAC GTT GAG GGG CAT (3′-OH). (a) ^{31}P NMR Spectrum. (b) Ion-exchange chromatographic profile. (c) CE profile.

unreacted amino groups can engage in a new equilibration with monomer during the second coupling.

The isolated yield of final product (in OD_{260} units) depends on both the length and the sequence composition but is typically in the 20−50% range (of total crude ODs) for oligomers 12−15 nucleotides in length. The current method affords a substantially improved yield in terms of crude and purified OD units (ca. 5-fold for purine rich 15mers)[4] and affords cleaner final products as compared to the prior method, although monomer purity appears to be an important determinant in the yield of final product obtained via this method. The stability of the 3′-(trityl)amino-5′-phosphoramidite monomers, **4**, in anhydrous CH_3CN is ∼5 days (except for **4g**, which is only stable for 1−2 days).

Representative ^{31}P NMR (a) as well as IEC (b) and CE (c) profiles of a phosphoramidate oligomer purified via ion-exchange chromatography (at pH 12) are provided in Figure 1 and correspond to the following sequence: 5′-AAC GTT GAG GGG CAT (3′-OH).

The final product purities (IEC and CE) of two representative phosphoramidate-containing ODNs synthesized via the phosphoramidite amine-exchange method are provided in Table 3. Oligonucleotide **A** is a uniformly modified pnDNA corresponding to 5′-AAC GTT GAG GGG CAT (3′-OH), and oligonucleotide **B** is a PN/PO/PN chimera ODN corresponding to 5′-CAG ATpCp GpTpCp CpApT GGT C (3′-OH), where p denotes phosphodiester linkages (the rest being N3′→P5′ phosphoramidate linkages).

Table 3. Final Product Purities of Representative Phosphoramidate-Containing ODNs

oligomer	IE purity	CE purity
A	91%	92%
B	95%	85%

We continue efforts to further optimize the new process and will describe soon a more efficient and economical version of this method for both research and large-scale (e.g., 200 μmol) pnODN syntheses (which utilizes significantly less equivalents of 5′-phosphoramidite monomer).[15] We also continue to investigate alternative strategies to enable hydrophobic purification (RP-HPLC) of the assembled phosphoramidate oligomers, as well as more highly convergent monomer syntheses, since the major cost associated with the synthesis of uniformly modified (and chimeric) pnODNs continues to be the cost of monomers.

Conclusions

The phosphoramidite amine-exchange method has been used to synthesize a vast array of oligonucleotide sequences on the research scale and has proven to be reliable and reproducible. The new method requires no instrument modifications, and final products possessing either a terminal 3′-amine (convenient for postlabeling,

etc.) or a terminal 3′-hydroxyl (employing commercially available 3′-*O*-DMT-5′-phosphoramidite monomers in the last coupling reaction) are readily accessible.[14] The new method also enables the synthesis of chimeric phophoramidate/phosphodiester or phosphoramidate/phosphorothioate oligonucleotides, by choosing the appropriate monomer and oxidation or sulfurization, respectively. Perhaps even more important from a process development perspective, the new (and fully optimized) method can also be efficiently and cost-effectively scaled,[15] and a portion of the unused excess monomers can be recycled.[16]

Experimental Section

General Procedures. Ion exchange (IE) HPLC was performed on a Perkin-Elmer Series 410 chromatograph. For analytical IE analysis a Dionex NucleoPac PA-100 column (4 × 250 mm) was used, with a gradient of 0−50% buffer B over 40 min at a flow rate of 1 mL/min. For purification of oligomers a Pharmacia MonoQ 10/10 column was used, with a 1%/min gradient of buffer B. [Buffer A = 0.01 M aqueous NaOH/0.01 M NaCl (pH 12); buffer B = 0.01 M aqueous NaOH/1.5 M NaCl (pH 12).] Capillary electrophoresis (CE) was performed on a Beckman P/ACE 5510 system with 10% microgel capillaries (0.1 × 500 mm) in 35 mM Tris-borate buffer, pH 9.0, in the presence of 15% ethylene glycol, with a 5-s injection at 10 kV and a running voltage of 25 kV.

Flash silica gel chromatography was performed with 230−400 mesh 60-Å silica from Merck and gravity chromatography with 70−230 mesh 60-Å silica from Aldrich. For acid sensitive (trityl-containing and phosphoramidite) compounds, columns were always packed and equilibrated (and in the case of phosphoramidite reagents, eluted) with 0.5−2% triethylamine in the appropriate eluant prior to sample loading.

TLC analysis was performed on 0.2-mm-thick precoated Merck silica gel 60 F_{254} plates, employing one of the following eluant mixtures (unless specified otherwise): (A) 5:95 MeOH/CH_2Cl_2, (B) 10:90 MeOH/CH_2Cl_2, (C) 80:20 EtOAc/hexane, or (D) 3:5:92 MeOH/Et_3N/toluene. TLC plates (for phophoramidite reagents) were preeluted with 10% triethylamine/dichloromethane and dried, prior to use.

^{1}H NMR spectra (400 MHz; with tetramethylsilane as internal standard) and ^{31}P NMR spectra (162 MHz; with H_3PO_4 as external standard) were recorded on a Bruker Avance DRX-400 spectrometer. FAB mass spectra were recorded at the Scripps Research Institute on a VG ZAB2-SE instrument, using nitrobenzyl alcohol and cesium iodide as the matrix. Microanalyses were performed by Desert Analytics, Tucson, AZ.

3′-Azido-5′-*O*-(4-methoxybenzoyl)-3′-deoxythymidine, **1t^a**, was synthesized by the method of Czernecki and Valéry.[6] N^6,3′-*O*-Dibenzoyl-2′-deoxyxyloadenosine, **^{x}a**, was synthesized by the method of Herdewijn.[13a] 3′-*O*-Benzoyl-N^2-isobutyryl-2′-deoxyxyloguanosine, **^{x}g**, was prepared as described previously.[3b,13b]

General Procedure for the Synthesis of 5′-Protected-3′-(trityl)amino-2′,3′-dideoxynucleosides (2t^a, 2t, 2du, 2g, and 2a), via Hydrogenation of 3′-Azido Compounds and Subsequent Tritylation of 3′-Aminonucleosides. Each 5′-protected-3′-azido-nucleoside (**1t^a, 1t, 1du**, and the analogous 3′-azido-containing A and G nucleosides) was dissolved in ethanolic solvent (specified below) and hydrogenated in the presence of 10% Pd/C (ca. 5−10% by mass of azide-containing starting material) for 16−18 h. Subsequent removal of the catalyst via filtration and evaporation of solvent *in vacuo* generally afforded 90−95% yield of the corresponding 3′-amine, which was taken on directly to the next reaction. Each 3′-amine (**1a**, **1g**, and the analogous 3′-amino-containing T and dU nucleosides) was routinely azeotroped from pyridine (2−3 times) prior to the tritylation reaction. Each tritylation reaction proceeded smoothly (amounts of reactants and reaction times specified below) and was complete upon stirring 2−16 h in pyridine with trityl chloride and triethylamine. Workup then typically involved removal of solvent and silica gel chromatography.

3′-(Trityl)amino-5′-*O*-(4-methoxybenzoyl)-3′-deoxythymidine (2t^a). 3′-Azido-5′-*O*-(4-methoxybenzoyl)-3′-deoxythymidine, **1t^a** (10.0 g, 24.9 mmol), was dissolved in ethanol (500 mL) and reduced via the general procedure described above to afford a 92% yield (8.6 g, 22.9 mmol) of 5′-*O*-(4-methoxybenzoyl)-3′-amino-3′-deoxythymidine (8.6 g, 22.9 mmol). The following amounts were used in the subsequent tritylation reaction: solvent, pyridine (50 mL), triethylamine (6.71 mL, 48.1 mmol), and trityl chloride (7.0 g, 25.2 mmol). This mixture was stirred for 2 h at ambient temperature, an additional portion of trityl chloride was added (1.9 g, 6.9 mmol), and the reaction was stirred an additional 2 h. Solvents were removed *in vacuo*, and the crude product was purified on silica (2−5% MeOH/CH_2Cl_2) to afford a 90% yield (12.7 g, 20.6 mmol) of 3′-(trityl)amino-5′-*O*-(4-methoxybenzoyl)-3′-deoxythymidine, **2t^a**. R_f (eluant A) = 0.45. ^{1}H NMR ($CDCl_3$/TMS): δ 8.26 (1 H, br s, exchanges with D_2O), 7.84, 6.90 (4 H, AB, J = 8.86 Hz), 7.55 (6 H, d, J = 7.45 Hz), 7.29 (6 H, t, J = 7.56 Hz), 7.21 (3 H, t, J = 7.27 Hz), 7.02 (1 H, s), 6.08 (1 H, t, J = 6.19 Hz), 4.59 (1 H, dd, J = 12.35, 2.35 Hz), 4.29 (1 H, dd, J = 12.43, 3.94 Hz), 3.98 (1 H, m), 3.88 (3 H, s), 3.41 (1 H, m), 1.97 (1 H, br, exchanges with D_2O), 1.65−1.75 (1 H, m), 1.58 (3 H, s), 1.30−1.40 (1 H, m). HRMS (FAB$^+$): calcd for [M + Cs]$^+$, 750.1580; observed, 750.1559.

3′-(Trityl)amino-3′-deoxythymidine (3t). The 5′-*O*-anisoyl protecting group was removed by dissolving **2t^a** (30.1 g, 48.7 mmol) in 57:43 1,4-dioxane/MeOH (150 mL), followed by the addition of 2 M aqueous NaOH (73.1 mL, 146.2 mmol). After stirring for 1.5 h at ambient temperature, the reaction mixture was neutralized with Dowex 50W-X8 cation exchange resin (ca. 150 g of dry pyridinium H^+-form, 1.6 mequiv/g). Once the pH was neutral (ca. 10 min), the resin was filtered and washed extensively with CH_2Cl_2 and MeOH, and the crude product was concentrated *in vacuo*. The residue was dissolved in EtOAc (500 mL) and extracted with saturated aqueous $NaHCO_3$ (2 × 250 mL), H_2O (250 mL), and saturated aqueous NaCl (250 mL). After drying over Na_2SO_4 and filtration, the solvents were removed *in vacuo*, and the resulting foam was dissolved in 95:5 CH_2Cl_2/MeOH (300 mL). This solution was added slowly to a rapidly stirring mixture of 1:1 Et_2O/hexane (1250 mL) to precipitate the pure 3′-(trityl)amino-3′-deoxythymidine, **3t**, in 90% yield (21.2 g, 43.8 mmol). R_f (eluant B) = 0.50. ^{1}H NMR ($CDCl_3$/TMS): δ 8.30 (1 H, br s, exchanges with D_2O), 7.52 (6 H, d, J = 7.46 Hz), 7.29 (6 H, t, J = 7.55 Hz), 7.21 (3 H, t, J = 7.25 Hz), 7.16 (1 H, s), 6.01 (1 H, t, J = 6.38 Hz), 3.85 (1 H, d, J = 11.71 Hz), 3.74 (1 H, m), 3.65 (1 H, dd, J = 11.99, 2.59 Hz), 3.34 (1 H, q, J = 6.54 Hz), 1.80−2.00 (1 H, br, exchanges with D_2O), 1.83 (3 H, s), 1.45−1.55 (1 H, m), 1.30−1.40 (1 H, m). HRMS (FAB$^+$): calcd for [M + Cs]$^+$, 616.1212; observed, 616.1226. Anal. Calcd for $C_{29}H_{29}N_3O_4 \cdot 0.5\ H_2O$: C, 70.85; H, 5.95; N, 8.26. Observed: C, 70.77; H, 5.71; N, 8.56.

3′-Azido-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxyuridine (1du). 2′-Deoxyuridine (11.4 g, 50 mmol) was thoroughly dried by coevaporating with anhydrous DMF (2 × 100 mL) *in vacuo*. DMF (100 mL) was then added, followed by triethylamine (8.36 mL, 60 mmol), 4-dimethylaminopyridine (0.31 g, 2.5 mmol), and *tert*-butyldimethylsilyl chloride (8.29 g, 55.0 mmol). The reaction mixture was stirred for 1 h at RT (room temperature), diluted with dichloromethane (600 mL), and extracted with H_2O (3 × 200 mL), and saturated aqueous NaCl (200 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The resulting residue was purified on silica (2−10% MeOH/CH_2Cl_2) to afford an 80% yield (13.7 g, 40.0 mmol) of 5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyuridine. The 5′-protected nucleoside (13.7 g, 40.0 mmol) and triphenylphosphine (16.8 g, 64.0 mmol) were dissolved in DMF (100 mL), and to this stirring mixture was added a solution of diisopropyl azodicarboxylate (12.6 mL, 64.0 mmol) in DMF (20 mL). After stirring 2 h at RT, the reaction mixture was concentrated *in vacuo* to ca. 30 mL and poured into Et_2O (1200 mL). The desired 2,3′-anhydro-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyuridine began precipitating out after 10 min of rapid stirring. The resulting mixture was placed in the refrigerator overnight, and then the precipitate was collected by filtration, washed with additional cold Et_2O (2 × 300 mL), and dried *in*

vacuo to afford a 90% yield (11.7 g, 36.0 mmol) of 2,3′-anhydro-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyuridine as a white solid. The 2,3′-anhydro-5′-*O*-(*tert*-butyldimethylsilyl)-2′-deoxyuridine (33.8 g, 104.2 mmol) was then reacted with LiN_3 (7.65 g, 156.3 mmol) in DMF (300 mL) at 95−100 °C for 48 h.[17] The resulting brown, homogeneous mixture was cooled to RT, concentrated *in vacuo* to an oil, dissolved in EtOAc (800 mL), and extracted with H_2O (200 mL). The aqueous layer was extracted twice more with EtOAc (75 mL), and the combined organics were washed with H_2O (3 × 250 mL) and once with saturated aqueous NaCl (250 mL). The EtOAc solution was dried over Na_2SO_4, filtered, and concentrated *in vacuo*, to afford an 87% yield (33.2 g, 90.3 mmol) of 3′-azido-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxyuridine, **1du**, as a brownish foam, which was taken on directly to hydrogenation. R_f (8% MeOH/CH_2Cl_2) = 0.57. ^{1}H NMR ($CDCl_3$/TMS): δ 8.87 (1 H, br s, exchanges with D_2O), 7.91 (1 H, d, J = 8.10 Hz), 6.23 (1 H, t, J = 5.88 Hz), 5.71 (1 H, d, J = 8.18 Hz), 4.25 (1 H, q, J = 5.91 Hz), 3.95−4.05 (2 H, m), 3.83 (1 H, dd, J = 11.40, 1.68 Hz), 2.45−2.55 (1 H, m), 2.25−2.35 (1 H, m), 0.95 (9 H, s), 0.15 (3 H, s), 0.14 (3 H, s). HRMS (FAB$^+$): calcd for [M + H]$^+$, 368.1754; observed, 368.1747. Anal. Calcd for $C_{15}H_{25}N_5SiO_4$: C, 49.02; H, 6.86; N, 19.06; Si, 7.64. Observed: C, 48.77; H, 6.96; N, 18.84; Si, 7.44.

3′-(Trityl)amino-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxyuridine (2du). Crude **1du** (33.2 g, 90.3 mmol) was dissolved in 2:1 EtOH/ CH_2Cl_2 (300 mL) and reduced via the general procedure described above to afford a quantitative yield (30.4 g, 89.8 mmol) of 5′-*O*-(*tert*-butyldimethylsilyl)-3′-amino-2′,3′-dideoxyuridine (30.4 g, 89.8 mmol). The following amounts were used in the subsequent tritylation reaction: solvent, mixture of CH_2Cl_2 (600 mL) and anhydrous pyridine (70 mL), triethylamine (25.0 mL, 179.6 mmol), and trityl chloride (35.0 g, 125.7 mmol). The reaction mixture was stirred for 2 h at ambient temperature, solvents were removed *in vacuo*, and the crude product was purified on silica (1−5% MeOH/CH_2Cl_2) to afford an 85% yield (44.3 g, 75.9 mmol) of 3′-(trityl)amino-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxyuridine, **2du**. R_f (eluant C) = 0.58. ^{1}H NMR ($CDCl_3$/TMS): δ 8.24 (1 H, br s, exchanges with D_2O), 7.73 (1 H, d, J = 8.25 Hz), 7.52 (6 H, d, J = 7.78 Hz), 7.31 (6 H, m), 7.23 (3 H, t, J = 7.23 Hz), 6.21 (1 H, t, J = 6.69 Hz), 5.60 (1 H, d, J = 8.17 Hz), 3.84 (1 H, m), 3.76 (1 H, dd, J = 11.34, 2.00 Hz), 3.48 (1 H, dd, J = 11.37, 2.27 Hz), 3.32 (1 H, m), 2.07 (1 H, br, exchanges with D_2O), 1.60−1.70 (1 H, m), 1.45−1.55 (1 H, m), 0.84 (9 H, s), 0.01 (3 H, s), −0.05 (3 H, s). HRMS (FAB$^+$): calcd for [M + Na]$^+$, 606.2764; observed, 606.2751.

***N*^{4}-Benzoyl-3′-(trityl)amino-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxycytidine (2c).**[10] Triethylamine (22.5 mL, 161.1 mmol) was added dropwise over a period of 10 min to a stirring mixture of 1,2,4-triazole (11.1 g, 161.1 mmol) and phosphorus oxychloride (3.5 mL, 37.1 mmol) in anhydrous acetonitrile (125 mL) at 0 °C. To this cold, stirring mixture was added **2du** (9.4 g, 16.1 mmol) as a solution in acetonitrile (50 mL). This mixture was stirred at RT for 2 h, triethylamine (30 mL) and H_2O (10 mL) were added to quench the reaction and promote dissolution, and solvents were removed *in vacuo*. The resulting brown solid was dissolved in CH_2Cl_2 (250 mL), extracted with saturated aqueous $NaHCO_3$ (3 × 150 mL), saturated aqueous NaCl, dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford a quantitative yield (10.2 g, 16.1 mmol) of 3′-(trityl)amino-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxy-4-(1,2,4-triazol-1-yl)uridine as an orange solid. This crude material was dissolved in 1,4-dioxane (200 mL) and cold, concentrated NH_4OH (50 mL) was added. The reaction mixture was stirred at RT for 4 h and concentrated *in vacuo* to afford a quantitative yield (9.4 g, 16.1 mmol) of 3′-(trityl)amino-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxycytidine as a beige solid. This crude material was then azeotroped from anhydrous pyridine (2 × 200 mL), redissolved in pyridine (200 mL), and cooled externally in a 0 °C ice bath. To this prechilled, stirring solution was added benzoyl chloride (2.2 mL, 19.3 mmol). The reaction was allowed to slowly warm to RT and stir an additional 16 h at RT, at which time the reaction mixture was externally cooled to 0 °C and quenched with H_2O (40 mL). After stirring 5 min, cold concentrated aqueous ammonia (40 mL) was added and the reaction mixture was stirred for an additional 15 min at 0 °C. The solvents were removed *in vacuo* and the residue was redissolved in CH_2Cl_2 (125 mL), extracted with saturated aqueous $NaHCO_3$ (3 × 75 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. This crude material was purified on silica (1−5% MeOH CH_2Cl_2) to afford a 92% yield (10.2 g, 14.8 mmol) of *N*^{4}-benzoyl-3′-(trityl)amino-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxycytidine, **2c**. R_f (eluant A) = 0.71. ^{1}H NMR ($CDCl_3$/TMS): δ 8.70 (1 H, d, J = 7.36 Hz, exchanges with D_2O), 8.27 (1 H, d, J = 7.36 Hz), 7.91 (2 H, d, J = 7.46 Hz), 7.62 (1 H, t, J = 7.20 Hz), 7.50−7.60 (8 H, m; with 6 H, d, J = 7.74 Hz at 7.52), 7.42 (1 H, br d, J = 7.41 Hz), 7.30 (6 H, t, J = 7.39 Hz), 7.22 (3 H, t, J = 7.39 Hz), 6.26 (1 H, t, J = 6.26 Hz), 3.80 (1 H, br m), 3.77 (1 H, br d, J = 11.39 Hz), 3.49 (1 H, dd, J = 11.24, 2.33 Hz), 3.30 (1 H, m), 1.90−2.10 (2 H, br m; 1 H exchanges in D_2O), 1.52 (1 H, dt, J = 13.57, 6.76 Hz), 0.86 (9 H, s), 0.04 (3 H, s), −0.01 (3 H, s). HRMS (FAB$^+$): calcd for [M + Cs]$^+$, 819.2343; observed, 819.2366. Anal. Calcd for $C_{41}H_{46}N_4SiO_4$: C, 71.69; H, 6.75; N, 8.16; Si, 4.09. Observed: C, 71.42; H, 6.66; N, 7.95; Si, 3.62.

General Procedure for the Synthesis of 3′-(Trityl)-amino-2′,3′-dideoxynucleosides (3du, 3c, 3g, and 3a), via Desilylation of 5′-TBDMS Protected Compounds (2du, 2c, 2g, and 2a) with TBAF. The 5′-TBDMS protecting group was cleanly removed by dissolving each 3′-(trityl)amino-2′,3′-dideoxynucleoside (amounts and reaction times specified below) in THF and reacting with TBAF (1 M in THF) for 16−24 h. Each reaction mixture was concentrated to a syrup, redissolved in CH_2Cl_2 (or specified solvent), and extracted four times with H_2O and once with saturated aqueous NaCl. The organic layer was dried over Na_2SO_4 and filtered, and the solvent was removed *in vacuo*. The crude 5′-OH monomers were then purified via silica gel chromatography.

***N*^{4}-Benzoyl-3′-(trityl)amino-2′,3′-dideoxycytidine (3c).** The following amounts were utilized in the desilylation reaction: Solvent, THF (15 mL), **2c** (2.0 g, 2.85 mmol), and TBAF (1M in THF, 15 mL). After stirring for 16 h, the reaction mixture was concentrated to a syrup, extracted as described above in the general procedure, and purified on silica (packed in 1% Et_3N in 3:7 EtOAc/hexane and eluted with 3:7−1:1 EtOAc/hexane) to afford an 88% yield (1.4 g, 2.50 mmol) of *N*^{4}-benzoyl-3′-(trityl)amino-2′,3′-dideoxycytidine, **3c**. R_f (eluant A) = 0.55. ^{1}H NMR ($CDCl_3$/TMS): δ 8.65 (1 H, br s, exchanges with D_2O), 8.19 (1 H, d, J = 7.36 Hz), 7.87 (2 H, d, J = 7.57 Hz), 7.62 (1 H, t, J = 7.37 Hz), 7.47−7.57 (9 H, m), 7.30 (6 H, t, J = 7.50 Hz), 7.23 (3 H, t, J = 7.24 Hz), 6.07 (1 H, dd, J = 6.66, 4.31 Hz), 3.91 (1 H, d, J = 12.00 Hz), 3.79 (1 H, m), 3.73 (1 H, d, J = 12.10 Hz), 3.30 (1 H, q, J = 6.38 Hz), 1.80−2.00 (2 H, m, 1 br H exchanges in D_2O), 1.40 (1 H, ddd, J = 13.89, 7.03, 4.41 Hz). HRMS (FAB$^+$): calcd for [M + Na]$^+$, 595.2321; observed, 595.2310. Anal. Calcd for $C_{35}H_{32}N_4O_4\cdot 2H_2O$: C, 69.06; H, 5.96; N, 9.20. Observed: C, 69.20; H, 5.59; N, 9.17.

3′-(Trityl)amino-2′,3′-dideoxyuridine (3du). This compound was obtained in 86% yield via desilylation of **2du** as shown in Scheme 3 (and as described in the syntheses of **3c**, **3a**, and **3g**). 3′-(Trityl)amino-2′,3′-dideoxyuridine, **3du**, R_f (eluant C) = 0.16. ^{1}H NMR ($CDCl_3$/TMS): δ 8.46 (1 H, br s, exchanges with D_2O), 7.54 (6 H, d, J = 7.32 Hz), 7.46 (1 H, d, J = 8.13 Hz), 7.31 (6 H, t, J = 7.56 Hz), 7.23 (3 H, t, J = 7.25 Hz), 6.03 (1 H, dd, J = 6.89, 5.18 Hz), 5.63 (1 H, d, J = 8.17 Hz), 3.89 (1 H, d, J = 11.73 Hz), 3.67−3.80 (2 H, m), 3.37 (1 H, q, J = 6.73 Hz), 1.80−1.90 (1 H, br, exchanges with H_2O), 1.58−1.62 (1 H, m), 1.27−1.33 (1 H, m). HRMS (FAB$^+$): calcd for [M + Na]$^+$, 492.1899; observed, 492.1890. Anal. Calcd for $C_{28}H_{27}N_3O_4$: C, 71.63; H, 5.80; N, 8.95. Observed: C, 71.41; H, 5.94; N, 8.56.

3′-(Trityl)amino-5′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxythymidine (2t). This compound was obtained in 83% yield by an analogous method described above in the synthesis of **2du** (as shown in Scheme 3), except thymidine was utilized as the starting material rather than deoxyuridine. 3′-(Trityl)-amino-5′-*O*-(*tert*-butyldimethylsilyl)-3′-deoxythymidine, **2t**, R_f (eluant C) = 0.67. ^{1}H NMR ($CDCl_3$/TMS): δ 8.57 (1 H, br s, exchanges with D_2O), 7.46 (1 H, s), 6.24 (1 H, t, J = 6.55 Hz), 4.26 (1 H, m), 3.93−4.02 (2 H, m), 3.83 (1 H, dd, J = 11.32,

2.06 Hz), 2.45 (1 H, ddd, J = 13.67, 6.09, 4.06 Hz), 2.24 (1 H, m), 1.94 (3 H, s), 0.95 (9 H, s), 0.15 (6 H, s). HRMS (FAB$^+$): calcd for [M + Cs]$^+$, 730.2077; observed, 730.2104.

***N*^{4}-Benzoyl-5-methyl-3′-(trityl)amino-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxycytidine (2^{m}c).** This compound was synthesized in 81% yield by an analogous method to **2c**, except **2t** was employed as the starting material rather than **2du**. *N*^{4}-Benzoyl-5-methyl-3′-(trityl)amino-5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxycytidine, **2^{m}c**, R_f (eluant C) = 0.84. ^{1}H NMR ($CDCl_3$/TMS): δ 13.25 (1 H, br s, exchanges in D_2O), 8.32 (2 H, d, J = 7.21 Hz), 7.53 (6 H, d, J = 7.56 Hz), 7.45 (2 H, t, J = 7.51 Hz), 7.27−7.42 (8 H, m), 7.23 (3 H, t, J = 7.23 Hz), 6.27 (1 H, dd, J = 8.00, 6.05 Hz), 3.89 (1 H, m), 3.75 (1 H, dd, J = 11.34, 1.73 Hz), 3.43 (1 H, dd, J = 11.37, 2.47 Hz), 3.32 (1 H, m), 2.06 (3 H, s), 1.50−1.70 (3 H, m), 0.84 (9 H, s), 0.01 (3 H, s), −0.04 (3 H, s). HRMS (FAB$^+$): calcd for [M + Cs]$^+$, 833.2499; observed, 833.2476.

***N*^{4}-Benzoyl-5-methyl-3′-(trityl)amino-2′,3′-dideoxycytidine (3^{m}c).** This compound was obtained in 95% yield via desilylation of **2**^{m}c as shown in Scheme 3 (and as described in the syntheses of **3c**, **3a**, and **3g**). *N*^{4}-Benzoyl-5-methyl-3′-(trityl)amino-2′,3′-dideoxycytidine, **3**^{m}c, R_f (1:1 EtOAc/hexane) = 0.50. ^{1}H NMR ($CDCl_3$/TMS): δ 13.2 (1 H, br s, exchanges with D_2O), 8.25 (2 H, d, J = 8.28 Hz), 7.58 (6 H, d, J = 8.08 Hz), 7.47 (1 H, s), 7.43 (2 H, d, J = 7.51 Hz), 7.30 (6 H, t, J = 7.50 Hz), 7.22 (3 H, t, J = 7.21 Hz), 6.03 (1 H, dd, J = 6.72, 5.36 Hz), 3.90 (1 H, ddd, J = 11.97, 5.42, 2.16 Hz), 3.76 (1 H, m), 3.70 (1 H, ddd, J = 11.98, 5.56, 3.36 Hz), 3.34 (1 H, m), 2.04 (3 H, s), 1.82 (1 H, br d, J = 8.47 Hz, exchanges with D_2O), 1.55−1.65 (1 H, m), 1.32−1.42 (1 H, m). HRMS (FAB$^+$): calcd for [M + Cs]$^+$, 719.1634; observed, 719.1653. Anal. Calcd for $C_{36}H_{34}N_4O_4$: C, 73.70; H, 5.84; N, 9.55. Observed: C, 73.66; H, 6.09; N, 9.15.

5′-*O*-(*tert*-Butyldimethylsilyl)-*N*^{2}-isobutyryl-3′-amino-2′,3′-dideoxyguanosine (1g). To a stirring solution of 3′-*O*-benzoyl-*N*^{2}-isobutyryl-2′-deoxyxyloguanosine, **^{x}g** (4.86 g, 11.0 mmol), in DMF (20 mL), was added triethylamine (3.4 mL, 24.2 mmol), 4-dimethylaminopyridine (54 mg, 0.44 mmol), and *tert*-butyldimethylsilyl chloride (3.31 g, 22.0 mmol). The reaction was stirred for 2 h at RT, methanol (10 mL) was added, and after stirring an additional 5 min, the reaction mixture was concentrated *in vacuo*. The residue was redissolved in CH_2Cl_2 (150 mL) and washed with H_2O (3 × 40 mL) and saturated aqueous NaCl (60 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford 6.40 g (>100% crude yield) of a reddish-colored foam. To this crude material was added a prechilled (ca. 5 °C) solution of 2 M aqueous NaOH in either 1:1 MeOH:1,4-dioxane or 65:30:5 pyridine:MeOH:H_2O (44.0 mL, 87.9 mmol) at 5 °C. The reaction mixture was stirred in an ice bath for 15−20 min and neutralized with 1 M aqueous HCl (97 mL) to pH 6−7. After the ice bath was removed, the reaction mixture was concentrated *in vacuo* to ca. 50 mL and extracted with CH_2Cl_2 (3 × 75 mL). The combined organics were washed with saturated aqueous $NaHCO_3$ (3 × 50 mL)] and saturated aqueous NaCl (2 × 50 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo* to afford an 82% yield (4.1 g, 9.1 mmol) of 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-isobutyryl-2′-deoxyxyloguanosine as a sandy-colored foam, which was taken on to the next reaction without further purification.

To crude 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-isobutyryl-2′-deoxyxyloguanosine (47.3 g, 104.7 mmol) was added LiN_3 (15.4 g, 314.1 mmol), triphenylphosphine (41.2 g, 157.1 mmol), and anhydrous DMF (1000 mL). Diethylazodicarboxylate (24.7 mL, 157.1 mmol) was added and the reaction mixture was stirred for 5 h at RT under argon. H_2O (20 mL) was added and the reaction mixture was concentrated *in vacuo*. The residue was dissolved in EtOAc (1500 mL), washed with H_2O (3 × 1000 mL) and saturated aqueous NaCl (1000 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was purified on silica (1−5% MeOH/CH_2Cl_2), although this afforded a >100% yield (112.7 g) of impure 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-isobutyryl-3′-azido-2′,3′-dideoxyguanosine. This crude (triphenylphosphine oxide-contaminated) product was not purified further and was taken on directly to hydrogenation (and purified as the 3′-amine).

The crude azide (≤104.7 mmol) was dissolved in (warm) ethanol (1600 mL) and reduced via the general method described above to afford the crude 3′-amine, which was purified on silica (2−6% MeOH/CH_2Cl_2 and then 1% Et_3N/6% MeOH/CH_2Cl_2) to afford a 60% yield (28.2 g, 63.2 mmol) of pure 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-isobutyryl-3′-amino-2′,3′-dideoxyguanosine, **1g**, as an off-white foam. R_f (eluant B) = 0.14. ^{1}H NMR ($CDCl_3$/TMS): δ 8.01 (1 H, s), 6.17 (1 H, dd, J = 6.77, 3.98 Hz), 3.80−3.90 (4 H, mm), 2.83 (1 H, septet, J = 6.80 Hz), 2.59 (1 H, ddd, J = 13.26, 6.16, 4.03 Hz), 2.33 (1 H, dt, J = 13.19, 6.79 Hz), 1.26 (6 H, dd, J = 6.86, 2.79 Hz), 0.88 (9 H, s), 0.07 (3 H, s), 0.06 (3 H, s). HRMS (FAB$^+$): calcd for [M + H]$^+$, 451.2489; observed, 451.2480.

5′-*O*-(*tert*-Butyldimethylsilyl)-*N*^{2}-isobutyryl-3′-(trityl)amino-2′,3′-dideoxyguanosine (2g). The following amounts were used in the tritylation reaction: Solvent, pyridine (500 mL), 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-isobutyryl-3′-amino-2′,3′-dideoxyguanosine, **1g** (28.5 g, 63.2 mmol), triethylamine (17.6 mL, 126.4 mmol), and trityl chloride (28.2 g, 101.1 mmol). After stirring 16 h at RT, solvents were removed *in vacuo* and the residue was purified on silica (1−5% MeOH/CH_2Cl_2) to afford a quantitative yield (43.8 g, 63.2 mmol) of 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-isobutyryl-3′-(trityl)amino-2′,3′-dideoxyguanosine, **2g**. R_f (eluant B) = 0.72. ^{1}H NMR ($CDCl_3$/TMS): δ 11.90 (1 H, br s, exchanges with D_2O), 8.01 (1 H, br s, exchanges with D_2O), 7.58 (1 H, s), 7.56 (6 H, d, J = 7.39 Hz), 7.31 (6 H, t, J = 7.58 Hz), 7.23 (3 H, t, J = 7.28 Hz), 6.00 (1 H, dd, J = 6.86, 4.63 Hz), 3.88 (1 H, dt, J = 5.91, 3.01 Hz), 3.75 (2 H, ABX, J_{AB} = 11.25 Hz), 3.52 (1 H, m), 2.57 (1 H, septet, J = 6.91 Hz), 2.00− 2.10 (1 H, br s, exchanges with D_2O), 1.72 (1 H, dt, J = 13.63, 6.89 Hz), 1.59 (1 H, ddd, J = 13.73, 6.69, 4.87 Hz), 1.28 (6 H, dd, J = 6.90, 3.22 Hz), 0.81 (9 H, s), −0.03 (3 H, s), −0.04 (3 H, s). HRMS (FAB$^+$): calcd for [M + Cs]$^+$, 825.2561; observed, 825.2540. Anal. Calcd for $C_{39}H_{48}N_6SiO_4$: C, 67.60; H, 6.98; N, 12.13. Observed: C, 67.89; H, 7.12; N, 12.01.

***N*^{2}-Isobutyryl-3′-(trityl)amino-2′,3′-dideoxyguanosine (3g).** The following amounts were utilized in the desilylation reaction: Solvent, THF (123.0 mL), 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-isobutyryl-3′-(trityl)amino-2′,3′-dideoxyguanosine, **2g** (42.6 g, 61.5 mmol), and TBAF (1 M in THF, 123.0 mL). The reaction mixture was stirred for 24 h, extracted as described above in the general desilylation procedure (albeit substituting EtOAc for CH_2Cl_2), and purified on silica (packed in 2% Et_3N in 2% MeOH/CH_2Cl_2 and eluted with 2−5% MeOH/CH_2Cl_2) to afford a 93% yield (33.1 g, 57.2 mmol) of *N*^{6}-benzoyl-3′-(trityl)amino-2′,3′-dideoxyguanosine, **3g**. R_f (eluant A) = 0.29. ^{1}H NMR ($CDCl_3$/TMS): δ 11.91 (1 H, br s, exchanges with D_2O), 7.89 (1 H, br s, exchanges with D_2O), 7.58 (1 H, s), 7.54 (6 H, d, J = 7.39 Hz), 7.29 (6 H, t, J = 7.60 Hz), 7.21 (3 H, t, J = 7.27 Hz), 5.98 (1 H, t, J = 6.67 Hz), 4.19 (1 H, dd, J = 9.26, 1.76 Hz, exchanges with D_2O), 3.86 (1 H, m), 3.82 (1 H, d, J = 12.26 Hz), 3.70 (1 H, m), 3.50 (1 H, ddd, J = 10.72, 9.92, 2.00 Hz), 2.55 (1 H, septet, J = 6.88 Hz), 1.95−2.03 (1 H, br s, exchanges with D_2O),1.91 (1 H, dt, J = 13.58, 6.93 Hz), 1.60 (1 H, ddd, J = 13.59, 6.48, 4.69 Hz), 1.24 (6 H, t, J = 6.83 Hz). HRMS (FAB$^+$): calcd for [M + Na]$^+$, 601.2539; observed, 601.2527. Anal. Calcd for $C_{33}H_{34}N_6O_4$: C, 68.50; H, 5.92; N, 14.52. Observed: C, 68.23; H, 6.28; N, 14.14.

5′-*O*-(*tert*-Butyldimethylsilyl)-*N*^{2}-isobutyryl-*O*^{6}-(diphenylcarbamoyl)-3′-(trityl)amino-2′,3′-dideoxyguanosine (2g^{dpc}). To 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-isobutyryl-3′-(trityl)amino-2′,3′-dideoxyguanosine, **2g** (30.3 g, 43.7 mmol), was added anhydrous pyridine (90 mL), *N*,*N*-diisopropylethylamine (11.4 mL, 65.6 mmol), and diphenylcarbamyl chloride (11.1 g, 48.1 mmol) under argon. After stirring for 1.5 h at RT, the intensely red/purple reaction mixture was concentrated *in vacuo*. The residue was redissolved in CH_2Cl_2 (600 mL), extracted with H_2O (2 × 400 mL) and saturated aqueous NaCl (400 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was then redissolved in CH_2Cl_2 and azeotroped with toluene (3×). This afforded a >100% yield (43.8 g) of impure 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-isobutyryl-*O*^{6}-diphenylcarbamoyl-3′-(trityl)amino-2′,3′-dideoxyguanosine, **2g^{dpc}**, which was generally taken on directly to desilylation (although it could also be purified on silica). R_f

(60% EtOAc/hexane) = 0.58. ^{1}H NMR ($CDCl_3$/TMS): δ 8.03 (1 H, s), 7.90 (1 H, br s, exchanges with D_2O), 7.55 (6 H, d, J = 7.63 Hz), 7.24−7.50 (16 H, mm), 7.21 (3 H, t, J = 7.22 Hz), 6.29 (1 H, t, J = 6.07 Hz), 3.89 (1 H, m), 3.75 (2 H, ABX, J_{AB} = 11.25 Hz), 3.49 (1 H, br m), 3.01 (1 H, br m), 2.77 (1 H, septet, J = 6.78 Hz), 2.00−2.10 (br s, exchanges with D_2O), 1.65−1.75 (2 H, m), 1.28 (6 H, d, J = 6.64 Hz), 0.83 (9 H, s), −0.01 (3 H, s), −0.02 (3 H, s). HRMS (FAB$^+$): calcd for [M + Cs]$^+$, 1020.3245; observed, 1020.3281.

***N*^{2}-Isobutyryl-*O*^{6}-(diphenylcarbamoyl)-3′-(trityl)amino-2′,3′-dideoxyguanosine (3g^{dpc}).** Crude 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{2}-isobutyryl-*O*^{6}-(diphenylcarbamoyl)-3′-(trityl)amino-2′,3′-dideoxyguanosine, **2g^{dpc}** (ca. 43.7 mmol), was dissolved in CH_2Cl_2 (200 mL), and pyridine (25 mL) was added. Triethylamine trihydrofluoride (49.8 mL, 305.8 mmol) was added, followed by a CH_2Cl_2 rinse (25mL), and the reaction mixture was stirred at RT under argon for 20 h. The reaction mixture was diluted with CH_2Cl_2 (600 mL) and extracted with H_2O (2 × 400 mL). The first aqueous layer was back-extracted with CH_2Cl_2 (50 mL), and the combined organics were dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The residue was redissolved in CH_2Cl_2, azeotroped with toluene (3×) to remove traces of pyridine, and purified on silica (packed in 2% Et_3N in 70:30 EtOAc/hexane and eluted with 70:30 EtOAc/hexane) to afford an 82% yield (27.6 g, 35.7 mmol) of *N*^{2}-isobutyryl-*O*^{6}-(diphenylcarbamoyl)-3′-(trityl)amino-2′,3′-dideoxyguanosine, **3g^{dpc}**. R_f (60% EtOAc/hexane) = 0.20. ^{1}H NMR ($CDCl_3$/TMS): δ 7.95 (1 H, s), 7.84 (1 H, br s, exchanges with D_2O), 7.55 (6 H, d, J = 7.85 Hz), 7.25−7.45 (16 H, mm), 7.21 (3 H, t, J = 7.25 Hz), 6.15 (1 H, t, J = 6.31 Hz), 3.77−3.87 (2 H, br m), 3.69 (1 H, m), 3.62 (1 H, m), 3.19 (1 H, m), 2.80 (1 H, septet, J = 6.86 Hz), 1.92−2.05 (2 H, mm, 1 H exchanges with D_2O), 1.65 (1 H, m), 1.24 (6 H, d, J = 6.86 Hz). HRMS (FAB$^+$): calcd for [M + Cs]$^+$, 906.2380; observed, 906.2350. Anal. Calcd for $C_{46}H_{43}N_7O_5\cdot H_2O$: C, 69.77; H, 5.73; N, 12.38. Observed: C, 69.98; H, 5.65; N, 12.25.

5′-*O*-(*tert*-Butyldimethylsilyl)-*N*^{6}-benzoyl-3′-amino-2′,3′-dideoxyadenosine (1a). Silylation was performed in an analogous fashion to that described above in the synthesis of **1g**. The following amounts were utilized in the silylation reaction: Solvent, pyridine (200 mL), *N*^{6},3′-*O*-dibenzoyl-2′-deoxyxyloadenosine, **^{x}a** (44.1 g, 96.1 mmol),[13] and *tert*-butyldimethylsilyl chloride (24.1 g, 160.0 mmol). The reaction was stirred for 16 h at RT, MeOH (50 mL) was added, and the reaction mixture was concentrated *in vacuo*. Following an extractive workup (as in the case of **1g**), the residue was purified on silica (packed in 1% Et_3N/EtOAc and eluted with EtOAc) to afford an 83% yield of 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{6},3′-dibenzoyl-2′-deoxyxyloadenosine (45.9 g, 80.1 mmol), as a colorless foam.

To a prechilled (0−4 °C) solution of 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{6},3′-dibenzoyl-2′-deoxyxyloadenosine (70.3 g, 122.6 mmol) in 7:10 MeOH:1,4-dioxane (560 mL) was added 2 M aqueous NaOH (180 mL) in one portion. The reaction mixture was stirred for 5 min, neutralized with pyridine hydrochloride (44.0 g) to pH 6−7, and concentrated *in vacuo* to ca. 200 mL. This concentrate was partitioned between CH_2Cl_2 (700 mL) and H_2O (300 mL). The organic layer was washed with H_2O (300 mL), saturated aqueous $NaHCO_3$ (2 × 300 mL), and saturated aqueous NaCl (300 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified on silica (2% MeOH/CH_2Cl_2) to afford a 94% yield (53.8 g, 114.7 mmol) of 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{6}-benzoyl-2′-deoxyxyloadenosine.

To 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{6}-benzoyl-2′-deoxyxyloadenosine (17.6 g, 37.5 mmol) was added, LiN_3 (5.5 g, 113.0 mmol), triphenylphosphine (14.8 g, 56.3 mmol), and anhydrous DMF (200 mL). Diethyl azodicarboxylate (8.9 mL, 56.3 mmol) was added and the reaction mixture was stirred for 6 h at RT under argon. H_2O (10 mL) was added and the reaction mixture was concentrated *in vacuo*. The residue was dissolved in EtOAc (500 mL), washed with H_2O (3 × 300 mL), and saturated aqueous NaCl (300 mL), dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was purified on silica (packed in CH_2Cl_2 and eluted with 0−2% MeOH/CH_2Cl_2), although this afforded a >100% yield (18.8 g) of impure 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{6}-benzoyl-3′-azido-2′,3′-dideoxyadenosine. This crude (triphenylphosphine oxide-contaminated) product was not purified further and was taken on directly to hydrogenation and purified as the 3′-amine.

Crude azide (18.8 g) was dissolved in 1:1 ethanol:CH_2Cl_2 (250 mL) and reduced via the general procedure described above to afford the crude 3′-amine, which was purified on silica (2−6% MeOH/CH_2Cl_2 and then 1%Et_3N/6% MeOH/CH_2Cl_2) to afford a 68% yield (12.0 g, 25.6 mmol) of pure 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{6}-benzoyl-3′-amino-2′,3′-dideoxyadenosine, **1a**, as an off-white foam. R_f (8% MeOH/CH_2Cl_2) = 0.30. ^{1}H NMR ($CDCl_3$/TMS): δ 8.95 (1 H, br s, exchanges with D_2O), 8.81 (1 H, s), 8.40 (1 H, s), 8.02 (2 H, d, J = 7.23 Hz), 7.62 (1 H, t, J = 7.43 Hz), 7.54 (2 H, t, J = 7.48), 6.49 (1 H, dd, J = 6.81, 3.68 Hz), 3.80−3.98 (4 H, mm), 2.76 (1 H, ddd, J = 13.27, 6.42, 3.69), 2.39 (1 H, dt, J = 13.43, 6.93), 0.92 (9 H, s), 0.11 (3 H, s), 0.00 (3 H, s). HRMS (FAB$^+$): calcd for [M + Cs]$^+$, 601.1360; observed, 601.1373. Anal. Calcd for $C_{23}H_{32}N_6SiO_3$: C, 58.95; H, 6.88; N, 17.93; Si, 5.99. Observed: C, 58.91; H, 6.89; N, 17.74; Si, 5.73.

5′-*O*-(*tert*-Butyldimethylsilyl)-*N*^{6}-benzoyl-3′-(trityl)amino-2′,3′-dideoxyadenosine (2a). The following amounts were used in the tritylation reaction: solvent, CH_2Cl_2 (350 mL), 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{6}-benzoyl-3′-amino-2′,3′-dideoxyadenosine, **1a** (29.5 g, 63.0 mmol), triethylamine (12.9 mL, 94.5 mmol), and trityl chloride (21.1 g, 75.6 mmol). After stirring 16 h at RT, the reaction mixture was diluted with additional CH_2Cl_2 (150 mL), extracted with H_2O (400 mL), saturated aqueous $NaHCO_3$ (3 × 300 mL), and saturated aqueous NaCl (2 × 300 mL), and concentrated *in vacuo* to a glassy foam. The crude product was purified on silica (packed in 1% Et_3N in 4:6 EtOAc/hexane and eluted with 4:6 EtOAc/hexane) to afford a 98% yield (44.1 g, 62.1 mmol) of 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{6}-benzoyl-3′-(trityl)amino-2′,3′-dideoxyadenosine, **2a**. R_f (eluant A) = 0.48. ^{1}H NMR ($CDCl_3$/TMS): δ 8.99 (1 H, br s, exchanges with D_2O), 8.76 (1 H, s), 8.12 (1 H, s), 8.00 (2 H, d, J = 7.29 Hz), 7.60 (1 H, t, J = 7.41 Hz), 7.54 (6 H, d, J = 7.44 Hz), 7.51 (2 H, t, J = 7.28 Hz), 7.28 (6 H, t, J = 7.56 Hz), 7.20 (2 H, t, J = 7.22 Hz), 6.36 (1 H, t, J = 5.96 Hz), 3.90 (1 H, m), 3.82 (1 H, dd, J = 11.28, 2.74 Hz), 3.67 (1 H, dd, J = 11.26, 3.10 Hz), 3.50 (1 H, br m), 2.00−2.10 (1 H, br s, exchanges with D_2O), 1.68−1.83 (2 H, mm), 0.82 (9 H, s), −0.02 (3 H, s), −0.03 (3 H, s). HRMS (FAB$^+$): calcd for [M + Cs]$^+$, 843.2455; observed, 843.2477. Anal. Calcd for $C_{42}H_{46}N_6SiO_3$: C, 70.96; H, 6.52; N, 11.82; Si, 3.95. Observed: C, 70.73; H, 6.70; N, 11.67; Si, 3.94.

***N*^{6}-Benzoyl-3′-(trityl)amino-2′,3′-dideoxyadenosine (3a).** The following amounts were utilized in the desilylation reaction: solvent, THF (123.0 mL), 5′-*O*-(*tert*-butyldimethylsilyl)-*N*^{6}-benzoyl-3′-(trityl)amino-2′,3′-dideoxyadenosine, **2a** (43.7 g, 61.5 mmol), and TBAF (1 M in THF, 123.0 mL). The reaction mixture was stirred for 24 h, extracted as described above in the general desilylation procedure (albeit substituting EtOAc for CH_2Cl_2), and purified on silica (packed in 2% Et_3N in 8:2 EtOAc/hexane and eluted with 8:2 EtOAc/hexane−100% EtOAc) to afford a 94% yield (34.5 g, 57.9 mmol) of *N*^{6}-benzoyl-3′-(trityl)amino-2′,3′-dideoxyadenosine, **3a**. R_f (eluant A) = 0.40. ^{1}H NMR ($CDCl_3$/TMS): δ 9.06 (1 H, br s, exchanges with D_2O), 8.68 (1 H, s), 8.02 (1 H, s), 8.01 (2 H, d, J = 7.33 Hz), 7.60 (1 H, t, J = 7.47 Hz), 7.53 (6 H, d, J = 7.38 Hz), 7.51 (2 H, t, J = 7.20 Hz), 7.29 (6 H, t, J = 7.58 Hz), 7.21 (3 H, t, J = 7.25 Hz), 6.24 (1 H, dd, J = 7.56, 6.30 Hz), 4.85 (1 H, dd, J = 9.87, 3.19 Hz, exchanges with D_2O), 3.65−3.82 (3 H, mm), 3.37 (1 H, t, J = 10.16 Hz), 2.38 (1 H, dt, J = 13.46, 7.02 Hz), 2.00−2.20 (1 H, br s, exchanges with D_2O), 1.75 (1 H, ddd, J = 13.28, 5.96, 2.97 Hz). HRMS (FAB$^+$): calcd for [M + Na]$^+$, 619.2434; observed, 619.2421. Anal. Calcd for $C_{36}H_{32}N_6O_3\cdot H_2O$: C, 70.34; H, 5.57; N, 13.67. Observed: C, 70.63; H, 5.65; N, 13.72.

General Procedure for the Synthesis of 3′-(Trityl)-amino-2′,3′-dideoxynucleoside 5′-(2-Cyanoethyl *N*,*N*-diisopropylphosphoramidite) (4a, 4c, 4g, 4g^{dpc}, and 4t). To the 3′-(trityl)amino-2′,3′-dideoxynucleoside (8.4 mmol of **3a**, **3c**, **3g**, **3g^{dpc}**, **3t**, **3du**, or **3^{m}c**; previously azeotroped thrice from anhydrous CH_3CN) in CH_2Cl_2 (25 mL) under argon was added *N*,*N*-diisopropylethylamine (2.0 mL, 11.8 mmol) and 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (2.1 mL, 9.4 mmol).

After stirring for 15 min, the reaction mixture was diluted with CH_2Cl_2 and extracted with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl. The organic layer was dried over Na_2SO_4, filtered, and concentrated *in vacuo*.

***N*⁶-Benzoyl-3′-(trityl)amino-2′,3′-dideoxyadenosine 5′-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (4a).** This compound was purified on silica (5% Et_3N/2% MeOH/toluene) to afford an 87% yield (5.82 g) of pure phosphoramidite. Overall yield of **4a** (from **^{x}a**) = 43%. ^{31}P NMR (CD_3CN): δ 148.6, 149.2.

***N*⁴-Benzoyl-3′-(trityl)amino-2′,3′-dideoxycytidine 5′-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (4c).** This compound was purified on silica (5% Et_3N/3% MeOH/toluene) to afford an 86% yield (5.58 g) of pure phosphoramidite. Overall yield of **4c** (from **dU**) = 37%. ^{31}P NMR (CD_3CN): δ 149.3, 149.6.

***N*²-Isobutyryl-3′-(trityl)amino-2′,3′-dideoxyguanosine 5′-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (4g).** This compound was precipitated under argon from CH_2Cl_2 (10 mL) into a rapidly stirring solution of 1:1 Et_2O/hexane (400 mL) at 4 °C. The solid was filtered, washed with hexane, and dried *in vacuo*. This precipitation step was repeated once, and the resulting solid was purified further on silica (10% Et_3N/CH_2Cl_2) to afford a 69% yield (4.51 g) of pure phosphoramidite. Overall yield of **4g** (from **^{x}g**) = 31%. ^{31}P NMR (CD_3CN): δ 148.7, 149.4.

***N*²-Isobutyryl-*O*⁶-(diphenylcarbamoyl)-3′-(trityl)amino-2′,3′-dideoxyguanosine 5′-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (4g^{dpc}).** This compound was purified on silica (3% Et_3N/60% EtOAc/hexane) to afford a 92% (7.53 g) of pure phosphoramidite. Overall yield of **4g^{dpc}** (from **^{x}g**) = 32%. ^{31}P NMR (CD_3CN): δ 148.8, 149.4.

3′-(Trityl)amino-3′-deoxythymidine 5′-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (4t). This compound was purified on silica (3% MeOH/5% Et_3N/toluene) to afford a 95% yield (5.43 g) of pure phosphoramidite and some mixed fractions. Overall yield of **4t** (from **1t^{a}**) = 71%. ^{31}P NMR (CD_3CN): δ 149.4, 149.5.

***N*⁴-Benzoyl-3′-(trityl)amino-2′,3′-dideoxyuridine 5′-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (4du).** This compound was precipitated under argon from CH_3CN (10 mL) into a rapidly stirring solution of 3:1 hexane/Et_2O (400 mL) at 4 °C. The solid was filtered, washed with hexane, and dried *in vacuo*. This precipitation step was repeated once, and the resulting solid was purified further on silica (2% MeOH/5% Et_3N/toluene) to afford a 53% yield (2.97 g) of pure phosphoramidite and several mixed fractions. ^{31}P NMR (CD_3CN): δ 149.4.

***N*⁴-Benzoyl-5-methyl-3′-(trityl)amino-2′,3′-dideoxycytidine 5′-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (4^{m}c).** This compound was purified on silica (2% MeOH/5% Et_3N/toluene) to afford a 93% yield (6.15 g) of pure phosphoramidite. ^{31}P NMR (CD_3CN): δ 149.6, 149.4.

General Procedure for the Synthesis of 3′-(Trityl)-amino-2′,3′-dideoxynucleoside 5′-Succinylates (5a, 5c, 5t, and 5g^{dpc}). To a solution of 3′-(trityl)amino-2′,3′-dideoxynucleoside **3t**, **3c**, **3a**, or **3g^{dpc}** (1.5 mmol) in CH_2Cl_2 (5 mL) was added 4-dimethylaminopyridine (0.22 g, 1.8 mmol) and then succinic anhydride (0.18 g, 1.8 mmol). After stirring at room temperature for 1 h the reaction was quenched by addition of methanol (0.6 mL), diluted with CH_2Cl_2, and extracted with cold 10% aqueous citric acid, H_2O, and saturated aqueous NaCl. The organic layer was dried (Na_2SO_4), filtered, and concentrated to a foam. *N*⁶-Benzoyl-3′-(trityl)-amino-2′-3′-dideoxyadenosine-5′-succinylate, **5a**, 100% yield (1.15 g). *N*⁴-Benzoyl-3′-(trityl)amino-2′,3′-dideoxycytidine-5′-succinylate, **5c**, 76% yield (0.77 g). 3′-(Trityl)amino-3′-deoxythymidine-5′-succinylate, **5t**, 94% yield (0.82 g). *N*²-Isobutyryl-*O*⁶-(diphenylcarbamoyl)-3′-(trityl)amino-2′,3′-dideoxyguanosine-5′-succinylate, **5g^{dpc}**, 78% yield (1.02 g).

General Procedure for the Synthesis of 3′-(Trityl)-amino-2′,3′-dideoxynucleoside 5′-Succinyl-Loaded CPG. To a solution of 3′-(trityl)amino-2′,3′-dideoxynucleoside-5′-succinylate **5a**, **5c**, **5t**, or **5g^{dpc}** (1 mmol) and 1-hydroxybenzotriazole (0.13 g, 0.95 mmol) in 1:1 *N*-methylpyrrolidine:DMSO (10 mL) was added *N,N*-diisopropylethylamine (0.35 mL, 2.0 mmol) and then 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (0.36 g, 0.95 mmol).[18] The solution was stirred 5 min, added to aminopropyl-CPG (10.0 g), and put on the shaker for 6 h. The CPG was filtered and washed successively with DMF, methanol, acetonitrile, and ethyl ether. Unreacted amino groups on the CPG were acetylated by using the standard PE-ABD capping solutions (Ac_2O/NMI) for 30 min. The nucleoside loadings, determined by trityl assay at 432 nm in 20% TFA/$CHCl_3$ using a molar extinction coefficient of 40.7 μmol^{-1} cm^{-1}, were 38.6 μmol/g for A, 33.6 μmol/g for C, 29.0 μmol/g for T, and 39.0 μmol/g for G^{dpc}.

General Procedure for 1 μmol Scale Synthesis of Oligo-2′-deoxynucleoside N3′→P5′ Phosphoramidates. Oligonucleotide N3′→P5′ phosphoramidates were prepared on an ABI 392 DNA synthesizer at the 1 μmol scale and purified by preparative ion-exchange chromatography. The synthesis is performed in the 5′ to 3′ direction (instead of the 3′ to 5′ direction, which commercially available synthesizers are programmed for) using 1 μmol of 3′-(trityl)amino-2′,3′-dideoxynucleoside-5′-succinyl-loaded CPG in the column. Because of the change in direction of the synthesis, the desired sequence must be entered into the automated synthesizer in backwards order. 3′-(Trityl)amino-5′-diisopropylphosphoramidite monomers were prepared as 0.1 M solutions in acetonitrile; the activation solution was 0.5 M tetrazole in acetonitrile (PE Applied Biosystems, Foster City, CA); the detritylation solution was 3% dichloroacetic acid (DCA) in dichloromethane (DCM); the oxidation solution was 0.1 M iodine in tetrahydrofuran/pyridine/water, 75/20/2, v/v/v solution (PE Applied Biosystems, Foster City, CA); and the capping solutions were 1/1/8 isobutyric anhydride/2,6-lutidine/THF (Cap A) and *N*-methylimidazole (NMI; Cap B). Oligonucleotide N3′→P5′ phosphoramidates were synthesized using the method shown in Table 1.

Upon completion of the chain-assembly, the support-bound 3′-detritylated pnODN was released from the CPG and base-deprotected in concentrated aqueous ammonia (1 mL) at 58 °C for 12 h. The cleaved and deprotected pnODN solution was filtered, and the CPG was washed 2 times with ammonia (0.2 mL). The combined ammonia washes were buffered to 0.01 M NaOH, and the ammonia was removed *in vacuo*. Following filtration, the crude oligonucleotide was purified on a preparative anion-exchange column (Pharmacia MonoQ 10/10) and the pure product-containing fractions (>85% purity) were pooled, concentrated, and preciptitated (3×) with absolute ethanol [or desalted on Sephadex G-25 (Pharmacia NAP-5), and lyophilized].

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