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Convenient Syntheses of 3′-Amino-2′,3′-dideoxynucleosides, Their 5′-Monophosphates, and 3′-Aminoterminal Oligodeoxynucleotide Primers

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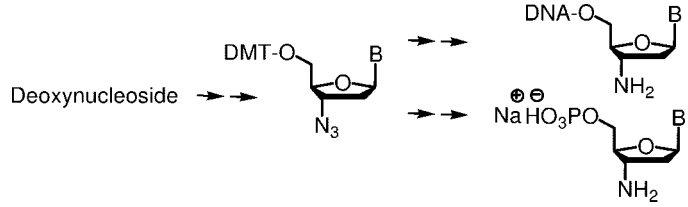

5′-Protected 3′-amino-2′,3′-dideoxynucleosides containing any of the four canonical nucleobases (A/C/G/T) were prepared via azides in five to six steps, starting from deoxynucleosides. For pyrimidines, the synthetic route involved nucleophilic opening of anhydronucleosides. For purines, an in situ oxidation/reduction sequence, followed by a Mitsunobu reaction with diphenyl-2-pyridylphosphine and sodium azide, provided the 3′-azidonucleosides in high yield and purity. For solid-phase synthesis of aminoterminal oligonucleotides, aminonucleosides were linked to controlled pore glass through a novel hexafluoroglutaric acid linker. These supports gave 3′-aminoterminal primers in high yield and purity via conventional DNA chain assembly and one-step deprotection/release with aqueous ammonia. Primers thus prepared were successfully tested in enzyme-free chemical primer extension, an inexpensive methodology for genotyping and labeling. Protected 5′-monophosphates of 3′-amino-2′,3′-dideoxynucleosides were also prepared, providing starting materials for the preparation of labeled or photolably protected monomers for chemical primer extension.

Introduction

Structurally modified 2′-deoxynucleosides are important as building blocks for the synthesis of oligonucleotides with novel properties and as antiviral and antitumor agents.[1,2] The 3′-position is frequently chosen for the modification, as removal or replacement of its hydroxy group renders the corresponding nucleoside triphosphate unsuitable for formation of phosphodiester linkages, preventing elongation of DNA chains during replication or reverse transcription after their incorporation.

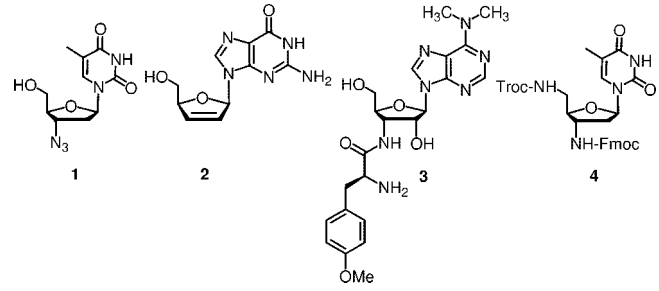

FIGURE 1. Selected 3′-deoxynucleoside derivatives.

Some well-known 3′-modified nucleosides with biological activity are shown in Figure 1. Among them is 3′-azido-3′-deoxythymidine (zidovudine or AZT, **1**),[3] a first-generation anti-HIV drug. Other members of this class of antiviral compounds

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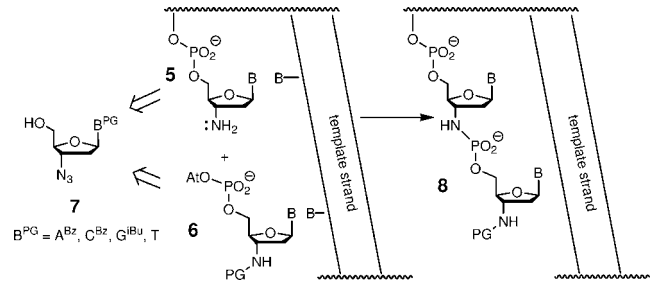
include 2′,3′-didehydro-2′,3′-dideoxyguanosine (carbovir, **2**),[4] 2′,3′-didehydro-3′-deoxythymidine (stavudine),[5] 2′,3′-dideoxy-3′-fluoro-4-thiothymidine,[6] and 2′,3′-dideoxy-3′-hydroxymethylcytidine.[7] A more distantly related, ribonucleoside antibiotic with an acylamido substituent replacing the 3′-OH group is puromycin (**3**). This dimethyladenosine derivative has also been used to set up in vitro selection of new proteins through synthetically modified mRNA-peptide fusions.[8]

As mentioned above, access to nucleosides with a non-natural functional group and/or the stereochemical configuration at the 3′-position is also important for the synthesis of novel oligonucleotides. This includes aminonucleosides, such as **4**, employed as building block for the solid-phase synthesis of cationic deoxynucleic oligomers. Other aminonucleosides serve as building blocks for the assembly of phosphoramidate-linked oligonucleotides,[9] which are important candidate structures for sequence-specific inhibition of gene expression.[10] For some applications, amino groups are attractive because aliphatic amines possess high nucleophilicity in aqueous solution, allowing for labeling or bioconjugation.[11] A conventional way of introducing aliphatic amino groups to oligonucleotides involves appending aminoalkyl substituents via phosphodiester linkages.[12] This approach has the advantage of producing sterically well accessible amino groups. It is unsuitable, however, for the generation of oligonucleotides with phosphoramidate linkages, which are isoelectronic to the natural phosphodiesters of DNA and RNA.

Chemical primer extension is the template-directed incorporation of mononucleotides in the absence of enzymes.[13] This reaction occurs in high yield and base selectivity when the 3′-terminal nucleoside of the primer (**5**, Scheme 1) is reacted with an oxyazabenzotriazolide (OAt) of a nucleoside 5′-monophosphate (**6**). Either can be traced back to azido building block **7**. Chemical primer extension generates oligodeoxyribonucleotides with a phosphoramidate linkage (**8**). It is suitable for genotyping single nucleotide polymorphisms[14] and is being developed as a technology for enzyme-free resequencing of short stretches of DNA[15] or possibly RNA.[16]

SCHEME 1. Template-Directed Primer Extension[a]



[a] An amino-terminal primer (**5**) reacts with an active ester of a 3′-amino-2′,3′-dideoxynucleoside monophosphate (**6**, PG = protecting group), as directed by a template strand to generate phosphoramidate-linked extended primer **8**. Both **5** and **6** are retrosynthetically linked to common precursor **7**.

Chemical primer extension avoids the need for polymerases in the sequence-determining step and employs inexpensive monophosphate substrates, rather than expensive triphosphates. It is therefore expected to be considerably less expensive than current, enzyme-based methods, making it a candidate technology for meeting the $1000 genome challenge.[17] Routine use of chemical primer extension requires streamlined syntheses of the aminonucleosides and automated, massively parallel syntheses of aminoterminal primers. These requirements motivated us to develop convenient syntheses of 3′-amino-2′,3′-dideoxynuclesides and -nucleotides that are rugged, require inexpensive starting materials, involve as few chromatographic steps as possible, and allow for standard methodology and reagents to be used for chain assembly of aminoterminal primers on solid support. In the process of developing such syntheses, we hoped to also gain convenient access to xylodeoxynucleosides and azidodideoxynucleosides. Either may serve as intermediates in the preparation of other functionalized nucleic acids. For example, 3′-azidonucleosides may be used in copper-catalyzed 1,3-dipolar cycloaddition reactions.[18]

Results and Discussion

Two routes to 3′-amino-terminal primer were tested. The first employs 5′- or "reversed" phosphoramidites of 2′-deoxynucleosides[19] for chain assembly on solid support and phthalimidate **10** in the last coupling step. Compound **10** was obtained through phosphitylation of **9**[20] in 72% yield and an overall yield of 14% from thymidine (Scheme 2). The deprotection of oligonucleotides thus prepared proved difficult, yielding no more than 20% of model oligodeoxyribonucleotide 5′-CTGT*-3′, where T* denotes the 3′-aminothymidine residue, when deprotecting with a mixture of 40% methylamine/ammonium hydroxide (1:1)[21] at elevated temperature. MALDI analysis suggested that breaking the second, amidic bond of the phthalimido group is a slow process. Ammonium hydroxide alone did not produce

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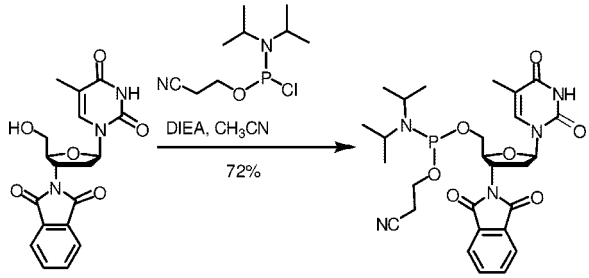
SCHEME 2. Phosphitylation of Phthalimidodeoxythymidine 9



9 10

the free amine, even after heating to 55 °C for 4 d. An Fmoc- or trifluoroactyl-protected version of **10** would have led to aminoterminal oligomers without a trityl or DMT group on the full-length product, a fact that was expected to complicate purification. This and the high cost of the reversed phosphoramidites prompted us to pursue the second route, which involves inexpensive, commercial 3′-phosphoramidites and solid support-bound 3′-aminonucleosides.

Aminonucleosides. Starting from thymidine, 5′-protected aminodeoxythymidine **11** (Scheme 3) was prepared in five steps via AZT, using literature-known transformations including reduction of the azido group with $NaBH_4$.[22,23] This route (Scheme S1, Supporting Information) proceeded in 51% overall yield and required no more than a single chromatographic step. The synthesis of 5′-protected 3′-amino-2′,3′-dideoxycytidine **20** started from commercial *N*4-benzoyl-2′-deoxycytidine (**12**, Scheme 3). A double-Mitsunobu reaction induced the cyclization and protected the 5′-hydroxy function.[24] The resulting anhydronucleoside was opened with azide, followed by exchange of the 5′-protecting group and reduction to the amine.

Attempts to induce these transformations without protecting the nucleobase failed. A series of 5′-acyl groups were tested to achieve high yields in the first part of the route, leading up to the 3′-azide. Anhydronucleoside **13** with an unsubstituted benzoyl group was too soluble for precipitation from ether, and chromatographic separation from the reagents proved difficult. A *p*-methoxybenzoyl moiety, the preferred protecting group in the AZT synthesis of Valery and co-workers,[22] gave no more than 25% overall yield for azide **18**, due to partial loss of the nucleobase protecting group during 5′-deprotection. A *p*-nitrobenzoyl group gave the anhydronucleoside **14** in 75% yield but unacceptably low yields during the subsequent nucleophilic ring opening with azide, most likely due to the high lability of the protecting group.[25] Protection with the *p*-chlorobenzoyl group led to low yields in the Mitsunobu reaction to anhydronucleoside **15** (40%). A *p*-bromobenzoyl group gave satisfactory results for all three steps (cyclization/protecting, ring opening, and hydrolytic 5′-deprotection), with yields of 80%, 67%, and 95%, respectively. With this protecting group, anhydronucleoside **16** could be readily separated from triphenylphosphine oxide and other contaminations through precipitation from diethylether. Interestingly, the addition of lithium chloride to the reaction mixture of the ring opening to compound **17**[26] lowered the yield by favoring opening at the anomeric position with concomitant loss of benzoylcytosine.

While 5′-monophosphates were generated without protecting group change at the 5′-position (vide infra), the aminonucleosides subsequently used for solid-phase DNA synthesis were provided with a 5′-DMT group to allow for chain assembly with standard instrumentation and reagents. The installation of the DMT group proceeded to give compound **19** in 83% yield in pyridine. The reduction of the azido group occurred in 93% yield when hydrogen and Pd/C were used. Amine **20** was thus obtained in 40% overall yield over five steps in high purity and with no more than two chromatographic purifications. The route proved convenient, rugged, and inexpensive and gives a slightly higher overall yield than literature-known protocols,[27] which also start from more costly starting materials.

We then turned to the purines. The route to the 3′-amino-2′,3′-dideoxynucleosides of guanine and adenine is shown in Scheme 4. Attempts to prepare the 3′-azides of the purines via the *N*3,3′-anhydronucleosides failed, due to their low reactivity in the ring opening reaction. Transglycosylation reactions with AZT as initial starting material were considered,[28] but not actively pursued due to the expected low yield and time-consuming chromatographic isolation of the desired β-nucleosides from the anomeric mixtures that makes the approach unattractive for large scale syntheses. A route via the 2′-deoxy*xylo*nucleosides, conversion of the 3′-hydroxy group to a leaving group, and S_N2 displacement with azide appeared more attractive, but published versions of this general route had significant drawbacks. The route of Robins and co-workers gives access to xylonucleosides,[29,30] starting from 2′-tosylated ribonucleosides, but it involves pyrophoric reagents and unconventional steps. The best known route, established for both deoxyadenosine and deoxyguanosine,[31,32] is that of Herdewijn and co-workers, involving intramolecular attack on a 3′-mesylate from the 5′-position to generate the xylo configuration at the 3′-position. The approach has also been studied for the deoxycytidine derivative.[33] After initial experiments, we decided not to pursue this route further, since the acyl migration accompanying the intramolecular attack leads to a mixture of isomers that limits the yield and requires an expensive and time-consuming chromatographic purification. Instead, we opted for inversion of configuration at C3′ via an oxidation/reduction sequence (Scheme 4). Related approaches are known for ribonucleosides.[34]

As expected, attempts to isolate the 3′-ketones of deoxyadenosine[35] and deoxyguanosine[36] were complicated by their lability toward depurination, which can lead to full decomposi-

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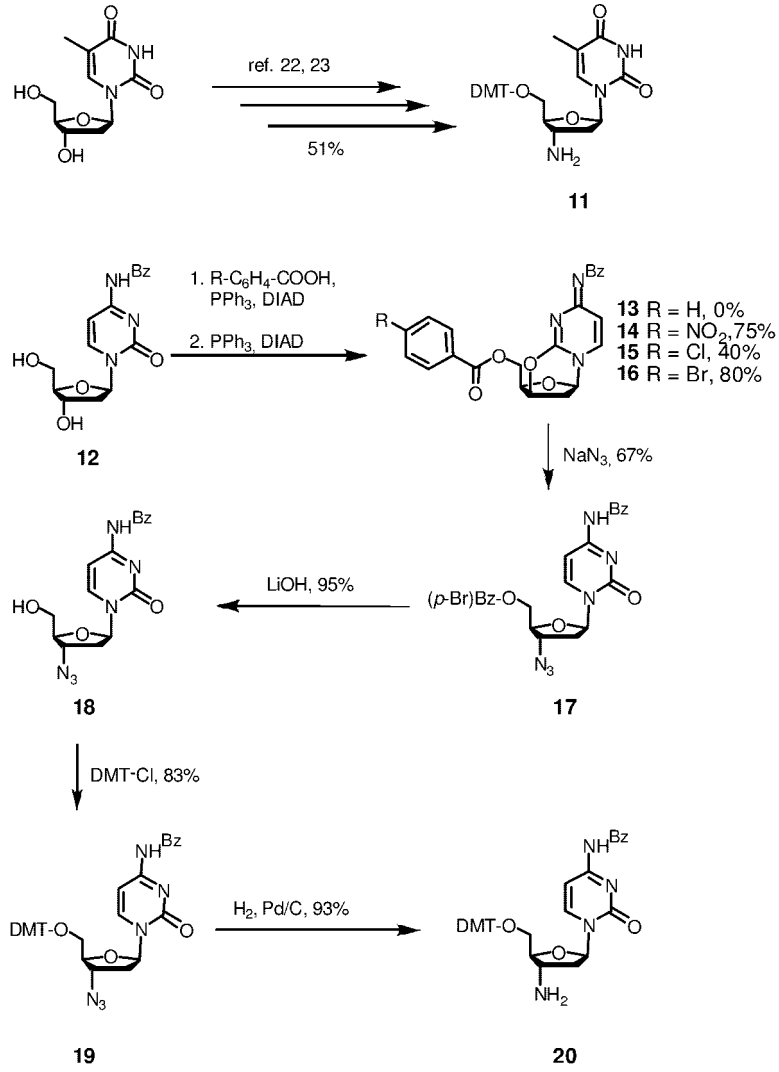
SCHEME 3. Synthesis of 3′-Amino-3′-deoxythymidine and 3′-Amino-2′,3′-dideoxycytidine



tion, even at room temperature. To avoid this, a one-pot oxidation/reduction approach with a low temperature regime was developed. We chose a TBDPS protecting group for the 5′-hydroxy group to ensure high regioselectivity and to prevent partial deprotection during the oxidation step, which was to be expected for trityl-based protecting groups. Oxidation at the 3′-position with the Dess−Martin reagent and immediate reduction of the ketone with $NaBH_4$ in ethanol at 0 °C gave a diastereomeric mixture of 4:1 (xylo to ribonucleoside). The initial yield for the two-step conversion of **21** to **22** was low (22%) due to partial depurination and cumbersome chromatographic separation of the polar mixture. Protecting groups for the nucleobase were introduced to address these issues. A dimethylformamidino group[37] at *N*6 worsened the depurination problem, so a more conventional benzoyl protection was used. Silylation of the 5′-hydroxy group of *N*6-benzoyl-2′-deoxyadenosine was higher yielding with TBS-Cl than with TBDPS-Cl and the separation of the epimeric mixture was easier, so the less sterically demanding reagent was used. Optimization of all three steps of the protection/oxidation/reduction sequence led to 70% overall yield for A. Key features of the optimized procedure are imidazole-free pyridine for silylation, addition of the solid nucleoside to a solution of Dess−Martin reagent at 0 °C, quenching of excess oxidation reagent with 2-propanol, which also serves as solvent for the reduction step, and lowering of the temperature for the latter step to −60 °C. The low temperature reduced the extent of depurination; it also improved the diastereoselectivity of the reduction step to 19:1 in favor of the desired xylonucleoside.

The oxidation/reduction route was also employed for generating the xylo derivative of 2′-deoxyguanosine. Amidine protecting groups for the exocyclic amino group of the nucleobase (dimethylformamidino or dibutylforamidino groups)[37] again fared more poorly than acyl protection (iBu group), due to the resulting lability and/or more complicated chromatographic separation of the epimers. For the *N*2-isobutyryl derivative, 5′-silylation proceeded in 84% yield and the two-step epimerization of **24g** to **25g** gave 77% overall yield.

Different approaches for generating the 3′-azides from the xylopurines were tested. Conversion of the 3′-hydroxy group to the corresponding triflate[31] followed by S_N2 displacement with NaN_3 in DMF gave no more than 30% yield, most probably due to competing sulfonation at the nucleobase protecting group

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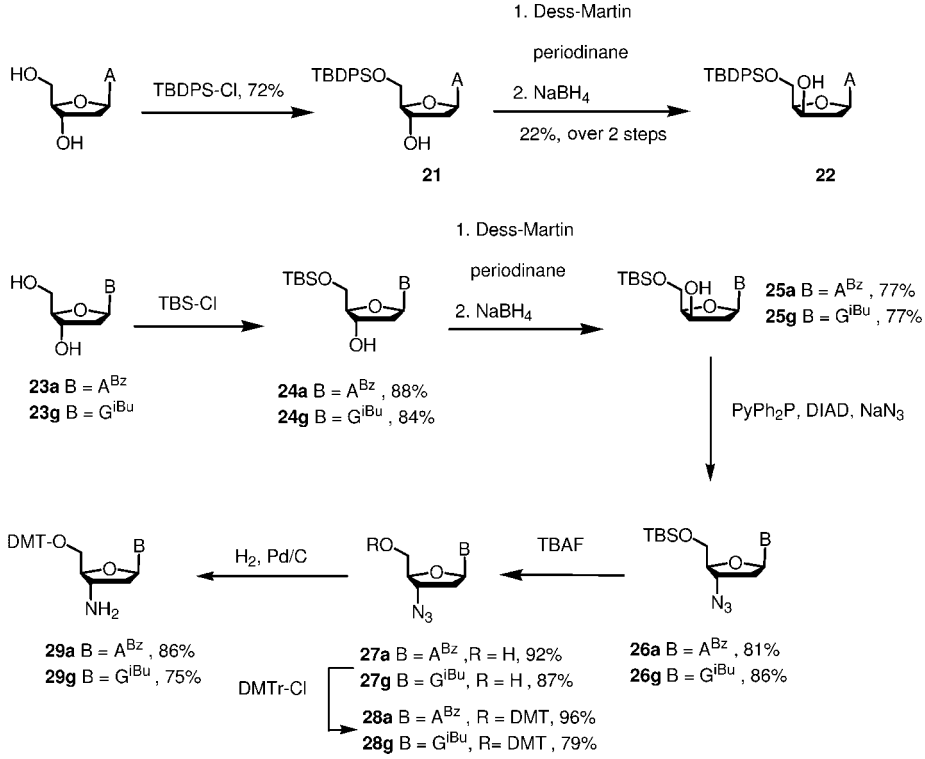
SCHEME 4. Synthesis of 3′-Amino-2′,3′-dideoxyguanosine and 3′-Amino-2′,3′-dideoxyadenosine



and subsequent loss of the benzoyl group. Mesylation at O3′ proved feasible,[38] but the S_N2 displacement with sodium azide was low yielding. Treatment of deoxyxyloguanosine **25g** with CBr_4, PPh_3, and NaN_3[32,39] gave azide **26g** in 56% yield, but the subsequent removal of the triphenylphosphine proved more than tedious. The same problem plagued initial attempts to use a Mitsunobu reaction with PPh_3, DEAD, and NaN_3 for the conversion of the xylopurine to the azidonucleoside via the Hirschbein procedure.[40] *tert*-Butylphosphine as reagent[41] did not give discernible conversion, and gas evolution upon addition of this reagent suggested that a Staudinger reduction of the azide occurred instead. Reactions with solid-phase bound triphenylphosphine[42] were also unsuccessful. The purification problem encountered with PPh_3 was overcome by employing diphenyl-2-pyridylphosphine. While removal of the corresponding phosphine oxide in an acidic extraction step was doable,[43] chromatographic removal proved more reliable, particularly when DIAD, rather than DEAD was used. The availability of pure azides (**27a** and **27g**) allowed us to avoid extensive purification on the stage of the free amine. With the optimized protocol, azides **27a** and **27g** were obtained in isolated yields of 81% and 86%, respectively (Scheme 4).

Though oligonucleotide syntheses involving 5′-silyl protecting groups are known,[44] we opted for DMT protection of the 5′-hydroxy groups of our building blocks to ensure compatibility with conventional DNA synthesizers. Desilylation with TBAF, followed by reprotection with DMT, and reduction of the azido functionality with H_2, Pd/C proceeded uneventfully in overall yields of 65−79%. Thus, the conversion of *N*6-benzoyl-2′-deoxyadenosine (**23a**) to amine **29a** requires six steps and proceeds in 44% overall yield. For the conversion of 2′-deoxy-*N*2-isobutyrylguanosine (**23g**) to the corresponding amine **29g**, the route requires six steps and gives 37% overall yield. These yields are 7% and 17%, higher, respectively, than those of the highest yielding route known previously.[27] We found the routes for either of the four nucleosidic amines easy-to-scale up to a gram scale. They have been successfully performed by undergraduate students in a teaching laboratory.

Aminoterminal Primers. We then developed a linker for immobilizing the aminonucleosides on controlled pore glass (cpg) such that standard DNA synthesis with conventional 3′-phosphoramidites generates aminoterminal primers of any given sequence without a single nonstandard reagent. This was to ensure that aminoterminal primers may be generated in automated and possibly massively parallel fashion. Supports for the solid-phase synthesis of aminoalkyloxyphosphoryl-terminated oligonucleotides based on phthalimido[45] or acetoxymethylbenzoyl[46] linkers are known from the literature. These were not pursued in the present case to avoid loss or migration of protecting groups. Immobilization of aminonucleosides has also been achieved[47] using linkers derived from the photolabile 2-(2-nitrophenyl)ethoxycarbonyl protecting group[48] or from phosphoramidate linkages.[49] The former leads to low loading and the latter to a two-stage chemical deprotection.

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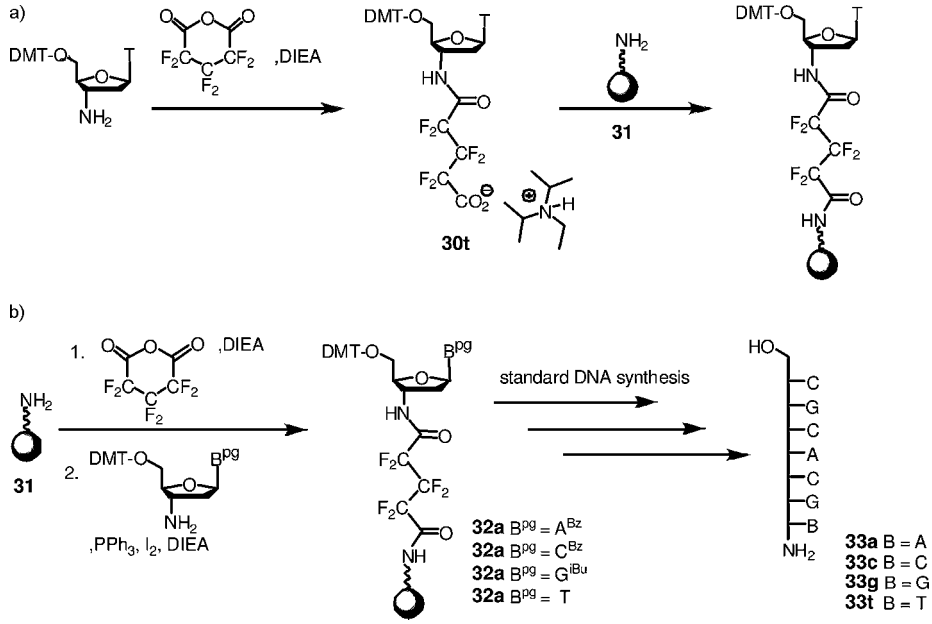
SCHEME 5. (a) Preparation of Solid Supports Loaded with Aminonucleosides and (b) Synthesis of Amino-Terminal Primer



Earlier work on lysyl-terminated oligonucleotides had shown that substantial strand cleavage can occur during deprotection in the presence of free aliphatic amino groups.[50,51] A trifluoroacetyl group protecting the ϵ-amino group of conjugated lysine residues gives oligonucleotides in high yields and crudes of excellent purity, probably because amino groups are released more slowly than the internucleosidic phosphodiester anions, which are much more resistant toward nucleophilic attacks than the initial phosphotriesters. This prompted us to test fluorinated derivatives of the succinyl linker employed in conventional DNA syntheses.[52] Hexafluorogutaric anhydride is an affordable, commercial reagent that creates sufficiently labile amide bonds. Using this anhydride, amide **30t** was obtained as diisopropylethylammonium salt (Scheme 5a). All attempts to couple **30t** to long-chain alkylamine controlled pore glass (LCAA-cpg, **31**) or simple model amines by using conventional amide-forming reagents, such as DCC, DIC, EDC·HCl, HATU[53] (HOAt), HBTU (HOBt), and TFFH[54] failed, probably because of the low reactivity of the carboxylate. Triphenylphosphine/I_2, an activation mixture developed for peptide synthesis,[55] was successful in model syntheses. For oligonucleotides, the highest yields of aminoterminal primers were obtained when this reagent was used for linking the aminonucleosides to the LCAA-cpg previously treated with hexafluoroglutaric anhydride, rather than coupling the previously acylated nucleoside (compare Scheme 5b).

A low apparent loading (10 and 14 μmol/g support) was measured after coupling the aminonucleosides under literature conditions for the I_2/PPh_3 mixture.[55] This was traced to partial loss of the DMT groups, on which the quantitation was based, probably through local release of HI during the coupling reaction. Using more equivalents of base and switching to pyridine as solvent did not eliminate this problem fully, but loadings in the range of 17−28 μmol/g were measured after changing the order of addition of the reagents (iodine was added last). This level of loading, which is comparable to that of commercial cpgs with unmodified nucleosides, was achieved with no more than 3 equiv of the respective aminonucleoside in the coupling reaction.

The average overall yield of aminoterminal primers **33a**−**t** was 40%, as determined by HPLC. This value is twice as high as that obtained using 5′-phosphoramidites and incorporation of an aminodeoxynucleoside in the last coupling cycle (21%). The purity of crude aminoterminal primers prepared from any of the four cpgs (**32a**−**t**) was determined by HPLC and MALDI-TOF MS and was found to be identical to that of unmodified control oligonucleotides prepared under the same conditions (compare parts a and b of Figure 2 for representative spectra). Chromatographically and spectrometrically pure primers were also obtained after simple cartridge purification. This was considered an important feature for routine, automated, and potentially parallel syntheses of primers for practical applications.

The ability of the aminoterminal primers to undergo chemical primer extension was also tested. Figure 3 shows representative

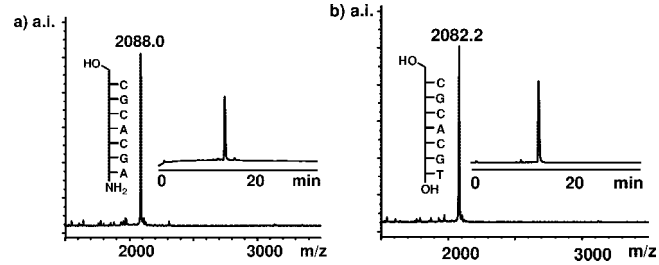


FIGURE 2. MALDI-TOF mass spectrum (lower part) and HPLC trace (insert) of (a) crude aminoterminal primer **33a** or (b) unmodified control oligonucleotide **34**, prepared using the same protocol and synthesizer, starting from either **32a** or conventional cpg.

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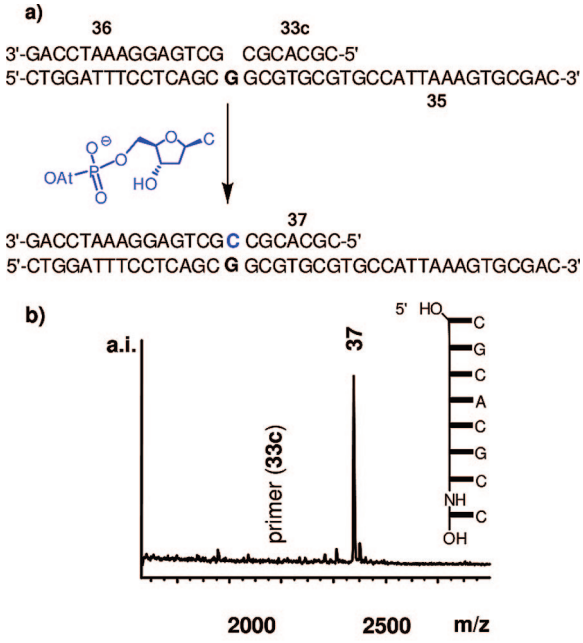


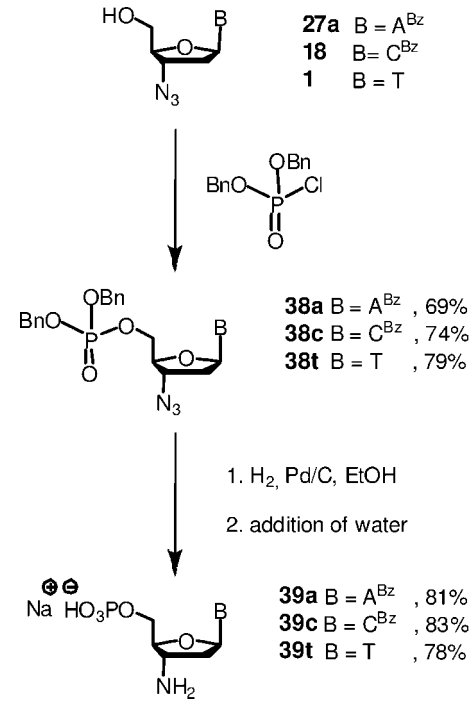
FIGURE 3. Chemical primer extension: (a) sequences of aminoterminal primer **33c**, DNA template **35**, and DNA "helper strand" GACCTAAAGGAGTCG (**36**) reacting with activated mononucleotide to form the extended primer **37**; (b) MALDI-TOF mass spectrum showing full primer extension.

MALDI spectra and the sequences of the strands involved. Full primer extension was observed in every case, confirming that the 3′-termini react in the expected, template-directed fashion. Additional data can be found in the Supporting Information.

Monophosphates of Aminonucleosides. Chemical primer extension by more than one nucleotide requires nucleosidic building blocks containing both a reactive nucleophilic group and a 5′-phosphate.[15] Aminonucleoside monophosphates are also of interest as starting materials for the synthesis of fluorophore-labeled monomers. Such fluorescent monomers allow for genotyping single nucleotide polymorphisms with optical detection of alleles.[56] Further, other forms of labeling nucleotides, e.g., with affinity ligands become feasible through the high reactivity of the aliphatic 3′-amino group of aminonucleotides toward active esters of carboxylic esters in aqueous solution.

We sought a short, convenient route for the 5′-monophospates of any of the four canonical aminonucleosides (A/C/G/T) that would avoid ionic intermediates, which are difficult to purify by conventional column chromatography. Further, the route should ideally provide intermediates with free 3′-amino groups but protected nucleobases to prevent side reactions during acylation, even under harsh reaction conditions. A route to 3′-amino-3′-deoxythymidine 5′-monophosphate (**39t**) is known which involves DCC-mediated coupling of the pyridinium salt of cyanoethylphosphate[57] to AZT.[58] The pyridinium salt of the phosphorylating reagent used is not commercially available though, and a purification of ionic intermediates cannot be avoided. Phosphorylation with $POCl_3$ suffers from modest yields and also requires ion-exchange chromatography or similar methods for the purification of intermediates. Phosphitylation

SCHEME 6. Synthesis of 3′-Aminonucleoside 5′-Monophosphates



of nucleobase-protected 3′-azidonucleosides, followed by oxidation and stepwise deprotection, was also considered, but not pursued due to the large number of steps required. Instead, azidonucleosides **1**, **18**, and **27a/g** were converted to triesters **38a**−**t** by reacting them with dibenzylphosphoryl chloride, which is commercially available. The benzyl groups of the resulting (uncharged) triester were hydrogenolytically removed without reduction of the nucleobases,[59,60] with concomitant reduction of the azido groups to the free amines (Scheme 6).

The reaction of azidonucleosides **1**, **18**, and **27a/g** with dibenzylphosphoryl chloride in pyridine at −30 °C gave yields of 69−79%. For **38g**, near-identical R_f values for educt and product led to low yields. Unless a more suitable eluant and/or stationary phase for chromatography can be identified, this route will probably remain problematic for this compound. Exploratory work shows that a satisfactory yield for the phosphorylation step can be achieved when position *O*6 is protected, as shown in Scheme 7. Substrate **41** may be obtained from **26g** through a combination of a Mitsunobu reaction with benzyl alcohol and desilylation. The former of the two steps has yet to be optimized. All three benzyl groups of **42** should be removable in a single hydrogenation step.[61]

The hydrogenation of **38a**−**t** proceeded smoothly when water was added after the initial phase of the reaction, which was run in ethanolic bicarbonate solution. This ensured that poorly soluble intermediates were brought back into solution and prevented incomplete conversion to the free aminonucleotides. The hydrogenation step thus produced nucleobase-protected amines **39a** and **39c**, in yields of 81 and 83%. The corresponding guanosine derivative **39g** also reacted smoothly in exploratory reactions. Aminothymidine monophosphate **39t** was obtained

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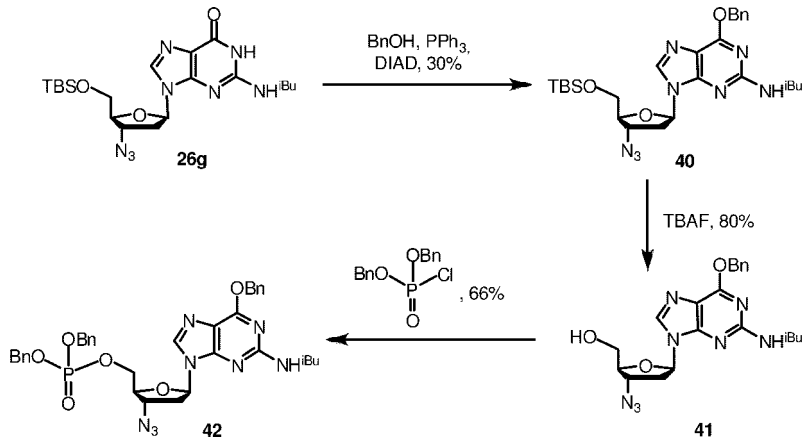
(60) Sim, M. M.; Kondo, H.; Wong, C. H. *J. Am. Chem. Soc.* **1993**, *115*, 2260–2267.
(61) Note added in proof: The hydrogenation has been performed successfully, with liberation of the free aminonucleotide in high yield.

SCHEME 7. Synthesis of Protected Azidodideoxyguanosine-5′-monophosphate 42



in 78% yield. For A, C, and T, filtration and lyophilization alone gave crude products that are pure by NMR, avoiding complicated purification steps of these zwitterionic compounds (see the Supporting Information for spectra). The overall yield from the commercial, nucleobase-protected (A and C) or unprotected (T) deoxynucleosides to the 5′-monophosphates of the aminonucleoside was 28−38%. Exploratory experiments with photolably protected aminonucleoside monophosphates led to doubly extended aminoterminal primers in high yield.[62]

Conclusions

The syntheses described here give access to aminodideoxynucleosides **11**, **20**, and **29a/g**, in five to six steps and 37−50% overall yield, starting from inexpensive deoxynucleosides. The route to the pyrimidines avoids HMPA as solvent and produces DMT-protected nucleosides compatible with trityl monitoring on conventional synthesizers. The route for the purines proceeds in 7−17% higher overall yield than that reported previously.[27] Further, the xylonucleosides were obtained in two rather than four steps and in 25−28% higher yield. Either route avoids difficult chromatographic steps, and either route proved easy to scale up.

Controlled pore glass loaded with aminonucleosides using the hexafluoroglutaric acid linker gives access to aminoterminal primers of any sequence via inexpensive, conventional DNA syntheses. The loading reaction via a seldom used redox reaction[55] requires no more than 3 equiv of the 5′- and nucleobase-protected aminonucleosides and generates supports with 17 and 28 μmol of nuclosides per gram of cpg. The aminoterminal primers obtained are of unusually high purity, suggesting that side reactions common for oligonucleotides with aliphatic amino groups[51] are successfully suppressed. The aminoterminal primers also passed all functional tests, involving template-directed chemical primer extension assays.

Finally, the results from our exploratory work on nucleobase-protected aminodideoxynucleoside-5′-monophosphates suggest that such charged and richly functionalized monomers may also be generated in convenient syntheses via our methodology. The monophosphates were obtained in analytically pure form after hydrogenation, again avoiding any unconventional or time-consuming purification steps.

The usefulness of aminodeoxynucleosides and oligonucleotides containing them is not limited to primer extension reactions. The high reactivity of the aliphatic amino group at the 3′-position can also be used to label nucleic acids with fluorescent residues.[15,56] Labeling[63] and immobilization on surfaces[64] may also be performed via azidonucleosides undergoing "Click"-type 1,3-dipolar cycloaddition[18] with underlying ligation.[65] Thus, azidonucleosides synthesized by the methodology described here may become valuable intermediates in the corresponding syntheses. Other intermediates, namely the xylonucleosides **25a** and **25g**, may also lead to other 3′-modified deoxynucleosides[66] that are pharmaceutically relevant.

Experimental Section

3′-Phthalimido-3′-deoxythymidine-5′-*O*-(2-cyanoethyl)diisopropylphosphoramidite (10). A slurry of 3′-phthalimido-3′-deoxythymidine[20] (**9**, 35 mg, 0.09 mmol) in acetonitrile (2 mL) and NEt_3 (45 μL, 0.27 mmol, 3 equiv) was treated with 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite (32 μL, 0.14 mmol, 1.5 equiv). After 2 h, the solution was poured into CH_2Cl_2, and the organic solution was washed with satd aqueous $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated in vacuo to a volume of 1 mL. The solution was poured into pentanes (20 mL) and the precipitate collected to give the title compound in 81% (47 mg, 0.08 mmol) yield: TLC (1% NEt_3 in toluene/acetone, 2/1, v/v) R_f = 0.52; ^{31}P NMR (101 MHz, CD_3CN) δ 150.3, 149.7; MS (MALDI-TOF) 594 $(M + Na)^+$.

***O*2,3′-Anhydro-*N*4-benzoyl-5′-*O*-(*p*-bromobenzoyl)-2′-deoxycytidine (16).** To a solution of *N*4-benzoyl-2′-deoxycytidine (**12**, 2 g, 6.04 mmol) in DMF (15 mL) were added PPh_3 (2.38 g, 9.06 mmol, 1.5 equiv) and *p*-bromobenzoic acid (1.82 g, 9.06 mmol, 1.5 equiv), followed by addition of DIAD (1.76 mL, 9.06 mmol, 1.5 equiv). The resulting yellow solution was stirred at rt for 30 min. A solution of PPh_3 (2.38 g, 9.06 mmol, 1.5 equiv) and DIAD (1.76 mL, 9.06 mmol, 1.5 equiv) in DMF (3 mL) was added dropwise, followed by stirring at rt for an additional 90 min. The

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deep red solution was poured into ice-cold Et_2O (150 mL), and the resulting suspension was kept at 4 °C for 2 h. The precipitate was collected, washed with ice-cold diethyl ether (5 × 20 mL), and dried in vacuo to afford a 80% yield of *O*2,3′-anhydro-*N*4-benzoyl-5′-*O*-(*p*-bromobenzoyl)-2′-deoxycytidine (**16**, 2.4 g, 4.83 mmol) as a colorless powder: ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.95−7.92 (m, 2H, Ar-*H*), 7.83−7.80 (m, 2H, Ar-*H*), 7.75−7.71 (m, 2H, Ar-*H*), 7.66 (d, J = 7.6 Hz, 1H, *H6*), 7.54−7.50 (m, 1H, Ar-*H*), 7.46−7.41 (m, 2H, Ar-*H*), 6.46 (d, J = 7.6, 1H, *H5*), 6.00 (bd, J = 3.8, 1H, *H1′*), 5.49−5.46 (m, 1H, *H3′*), 4.63−4.58 (m, 2H, *H5′* and *H5″*), 4.46−4.40 (m, 1H, *H4′*), 2.74−2.66 (m, 1H, *H2″*), 2.60−2.54 (m, 1H, *H2′*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 177.2, 164.6, 161.9, 152.8, 139.8, 136.5, 131.9, 131.5, 131.1, 128.8, 128.1, 128.0, 127.6, 105.7, 87.2, 81.8, 77.9, 62.3, 32.6; MS (FAB) *m*/*z* 496.2, 498.2 [M + H]$^+$.

3′-Azido-*N*4-benzoyl-5′-*O*-(*p*-bromobenzoyl)-2′,3′-dideoxycytidine (17). A slurry of *O*2,3′-anhydro-*N*4-benzoyl-5′-*O*-(*p*-bromobenzoyl)-2′-deoxycytidine (**16**, 2.2 g, 4.44 mmol) and NaN_3 (866 mg, 13.32 mmol, 3 equiv) in DMF (15 mL) was stirred at 130 °C. After 2 h, the slurry was poured into satd aqueous NH_4Cl solution (100 mL), and the resulting yellow precipitate was extracted with ethyl acetate. The organic phase was washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified on silica (0.1% H_2O in hexanes/ethyl acetate, 1/5, v/v) to afford the title compound in 67% yield (1.6 g, 2.98 mmol) as a yellow foam: TLC (hexanes/ethyl acetate, 1/2) R_f = 0.34; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.98 (d, J = 7.6 Hz, 1H, *H6*), 7.85−7.76 (m, 4H, Ar-*H*), 7.59−7.34 (m, 6H, Ar-*H* and *H5*), 6.05 (t, J = 5.6 Hz, 1H, *H1′*), 4.60−4.55 (m, 2H, *H4′* and *H3′*), 4.27−4.12 (m, 2H, *H5′* and *H5″*), 2.81−2.68 (m, 1H, *H2″*), 2.48−2.36 (m, 1H, *H2′*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 165.3, 162.4, 143.7, 133.2, 132.8, 132.0, 131.0, 129.0, 127.9, 127.5, 87.6, 82.6, 63.6, 60.0, 38.7; MS (FAB) *m*/*z* 514.5, 516.5 [M + H]$^+$.

3′-Azido-*N*4-benzoyl-2′,3′-dideoxycytidine (18). A stirred solution of 3′-azido-*N*4-benzoyl-5′-*O*-(*p*-bromobenzoyl)-2′,3′-dideoxycytidine (**17**, 716 mg, 1.39 mmol) in THF (8 mL) was treated with aqueous LiOH solution (1 M, 5.5 mL, 5.5 mmol, 4 equiv). After 40 min, the reaction mixture was added to $CHCl_3$. The solution was washed with satd aqueous NH_4Cl solution and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give the title compound in 93% yield (461 mg, 1.29 mmol) as a yellow solid, which was used in the subsequent step without further purification: TLC (CH_2Cl_2/MeOH, 95/5) R_f = 0.3; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.30 (s, 1H, N-*H*), 8.47 (d, J = 7.5 Hz, 1H, *H6*), 8.09−8.05 (m, 1H, Ar-*H*), 7.71−7.66 (m, 1H, Ar-*H*), 7.60−7.55 (m, 2H, Ar-*H*), 7.43 (d, J = 7.5 Hz, 1H, *H5*), 6.13 (t, J = 5.9 Hz, 1H, *H1′*), 5.36 (t, J = 5.3 Hz, 1H, *OH*), 4.44 (q, J = 6.1 Hz, 1H, *H4′*), 4.03−3.99 (m, 1H, *H3′*), 3.81−3.67 (m, 2H, *H5′* and *H5″*), 2.57−2.42 (m, 2H, *H2′* and *H2″*); ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.2, 163.1, 154.3, 144.9, 133.0, 132.7, 128.4, 95.9, 85.9, 84.8, 60.3, 59.4, 37.6; MS (FAB) *m*/*z* 357.3 [M + H]$^+$.

3′-Azido-*N*4-benzoyl-5′-*O*-(dimethoxytrityl)-2′,3′-dideoxycytidine (19). A stirred solution of 3′-azido-*N*4-benzoyl-2′,3′-dideoxycytidine (**18**, 300 mg, 0.85 mmol) in pyridine (5 mL), containing some molecular sieves (3 Å, 300 mg), was treated with DMTrCl (245 mg, 0.72 mmol, 1.3 equiv). After 1 h, methanol (500 μL) was added, and the reaction mixture was transferred into ethyl acetate. The solution was washed with satd aqueous $NaHCO_3$ solution, H_2O, and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting yellow foam was purified on silica (pretreated with 1% NEt_3 in CH_2Cl_2, elution with CH_2Cl_2 and then ethyl acetate) to give the title compound in 86% (476 mg, 0.73 mmol) yield. Spectroscopic data were in agreement with the literature.[47]

Reduction of Azides. 3′-Amino-*N*4-benzoyl-5′-*O*-(dimethoxytrityl)-2′,3′-dideoxycytidine (20) (General Protocol A). The following protocol is representative for the conversion of azidonucleosides **19** and **28a/g** to the aminonucleosides **20** and **29a/g**. To a solution of 3′-azido-*N*4-benzoyl-5′-*O*-(dimethoxytrityl)-2′,3′-dideoxycytidine (**19**, 300 mg, 0.46 mmol) in ethanol (10 mL) was added saturated aqueous $NaHCO_3$ solution (200 μL). The resulting slurry was placed under an argon atmosphere, and Pd/C (90 mg) was added. After the argon was replaced with a hydrogen atmosphere, generated by the hydrostatic pressure of a 20 cm water column, the slurry was stirred, with the hydrogen atmosphere being replaced every 60 min, until TLC indicated complete conversion (typically less than 6 h). The catalyst was removed by filtration over a bed of Celite and washed with ethanol. The combined solutions were concentrated in vacuo to give 3′-amino-*N*4-benzoyl-5′-*O*-(dimethoxytrityl)-2′,3′-dideoxycytidine (**20**) in 93% yield (269 mg, 0.42 mmol). Spectroscopic data were in agreement with the literature.[27]

***N*6-Benzoyl-5′-*O*-*tert*-butyldimethylsilyl-2′-deoxyadenosine (24a).** To a stirred slurry of *N*6-benzoyl-2′-deoxyadenosine (**23a**, 2.5 g, 7.04 mmol) in pyridine (15 mL) was added TBS-Cl (1.5 g, 9.86 mmol, 1.4 equiv). After 1 h, additional TBS-Cl (530 mg, 3.53 mmol, 0.5 equiv) was added. After 3 h, methanol (1 mL) was added, and the reaction mixture was transferred into ethyl acetate. The solution was washed with satd aqueous $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The residue was coevaporated twice from toluene and purified on silica (CH_2Cl_2/MeOH, 93/7, v/v) to give **24a** as a colorless foam in 88% yield (2.91 g, 6.19 mmol). Spectroscopic data were in agreement with the literature.[67]

***N*6-Benzoyl-5′-*O*-*tert*-butyldimethylsilyl-2′-deoxyxyloadenosine (25a).** To a chilled solution (0 °C) of Dess−Martin periodinane in CH_2Cl_2 (10 wt %, 24 mL, 11.56 mmol, 2.25 equiv) was added *N*6-benzoyl-5′-*O*-*tert*-butyldimethylsilyl-2′-deoxyadenosine (**24a**, 2.4 g, 5.13 mmol), and the resulting solution was stirred at 0 °C. After 30 min, the cooling bath was removed, and stirring was continued for another 90 min. After addition of 2-propanol (25 mL), the resulting slurry was cooled to −60 °C, and freshly powdered $NaBH_4$ (607 mg, 10.26 mmol, 2 equiv) was added. After 2 h of stirring at −60 °C, acetone (25 mL) was added, and the slurry was allowed to warm to rt and poured into ethyl acetate (500 mL). The solution was washed with satd aqueous $NaHCO_3$ solution, H_2O, and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting yellow foam was purified on silica (CH_2Cl_2/MeOH, 95:5, v/v) to give the title compound (1.85 g, 3.95 mmol, 77% yield) as a colorless foam: TLC (CH_2Cl_2/MeOH, 95/5) R_f = 0.26; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.32 (bs, 1H, N-*H*), 8.88 (s, 1H, *H8*), 8.35 (s, 1H, *H2*), 8.04 (d, J = 7.5 Hz, 2H, Ar-*H*), 7.62 (t, J = 7.5 Hz, 1H, Ar-*H*), 7.53−7.50 (m, 2H, Ar-*H*), 6.32 (dd, J = 2.1 Hz, J = 9.1 Hz, 1H, *H1′*), 5.91 (d, J = 6.9 Hz, 1H, O-*H*), 4.58−4−53 (bs, 1H, *H3′*), 4.13−3.99 (m, 3H, *H4′*, *H5′*, and *H5″*), 2.94−2.85 (m, 1H, *H2″*), 2.60−2.54 (m, 1H, *H2′*), 0.89 (s, 9 H, Si-CH-CH_3), 0.08 (s, 3H, Si−CH_3), 0.07 (s, 3H, Si−CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.7, 152.0, 150.5, 149.9, 143.0, 133.5, 132.8, 128.8, 127.9, 123.7, 84.8, 84.2, 71.1, 62.1, 40.9, 25.8, 18.3, −5.3; MS (FAB) *m*/*z* 495.1 [M + H]$^+$.

3′-Azido-*N*6-benzoyl-5′-*O*-*tert*-butyldimethylsilyl-2′,3′-dideoxyadenosine (26a). A stirred slurry of *N*6-benzoyl-5′-*O*-*tert*-butyldimethylsilyl-2′-deoxyxyloadenosine (**25a**, 710 mg, 1.50 mmol) and NaN_3 (293 mg, 4.52 mmol, 3 equiv) in DMF (5 mL) was treated with a solution of diphenyl-2-pyridylphosphine (590 mg, 2.26 mmol, 1.5 equiv) and DIAD (438 μL, 2.26 mmol, 1.5 equiv) in DMF (1.5 mL). After 4 h, H_2O (2 mL) was added, and the reaction mixture was transferred into ethyl acetate. The solution was washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting brown oil was dried overnight in vacuo and purified on silica (CH_2Cl_2/ethyl acetate, 3/2, *v*/*v*) to give **26a** in 81% yield (605 mg, 1.22 mmol): TLC (CH_2Cl_2/ethyl acetate, 3/2) R_f = 0.4; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H, *H8*), 8.32 (s, 1H, *H2*), 8.02 (d, J = 7.2 Hz, 2H, Ar-*H*), 7.59−7.55 (m, 1H, Ar-*H*), 7.50−7.46 (m, 2H, Ar-*H*), 6.43 (t, J = 6.0 Hz, 1H, *H1′*), 4.50 (q, J = 5.5 Hz, 1H, *H3′*), 4.08 (q, J = 3.9 Hz, 1H, *H4′*),

(67) Ogilvie, K. K. *Can. J. Chem.* **1973**, *51*, 3799–3807.

3.86−4.82 (m, 2H, *H5′* and *H5″*), 2.93−2.86 (m, 1H, *H2″*), 2.64−2.58 (m, 1H, *H2′*), 0.90 (s, 9 H, Si-CH-CH_3), 0.10 (s, 3H, Si−CH_3), 0.09 (s, 3H, Si−CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 164.9, 152.5, 151.1, 149.6, 141.4, 133.7, 132.7, 128.7, 127.9, 123.4, 85.0, 84.4, 62.7, 60.4, 38.0, 25.9, 18.4, −5.3, −5.4; MS (FAB) *m/z* 495.1 $[M + H]^+$.

3′-Azido-*N*6-benzoyl-2′,3′-dideoxyadenosine (27a). A stirred solution of 3′-azido-*N*6-benzoyl-5′-*O*-*tert*-butyldimethylsilyl-2′,3′-dideoxyadenosine (**26a**, 600 mg, 1.25 mmol) in THF (5 mL) was treated with TBAF in THF (1 M solution, 2.5 mL, 2.5 mmol, 2 equiv). After 90 min, methoxytrimethylsilane (120 μL) was added, and stirring was continued. After 1 h, the solvent was removed in vacuo. The resulting residue was purified on silica (CH_2Cl_2/MeOH, 95/5, v/v) to give the title compound in 92% yield (380 mg, 1.12 mmol). Spectroscopic data were in agreement with the literature.[39]

3′-Azido-*N*6-benzoyl-5′-*O*-dimethoxytrityl-2′,3′-dideoxyadenosine (28a). A stirred solution of 3′-azido-*N*6-benzoyl-5′-*O*-*tert*-butyldimethylsilyl-2′,3′-dideoxyadenosine (**27a**, 261 mg, 0.69 mmol) in pyridine (5 mL) was treated with DMTrCl (303 mg, 0.90 mmol, 1.3 equiv). After 4 h, methanol (1 mL) was added, and stirring was continued for another 30 min. The reaction mixture was added to CH_2Cl_2 and the solution washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The yellow foam was purified on silica (pretreated with 1% NEt_3 in CH_2Cl_2, eluted with 1% NEt_3 in CH_2Cl_2 and CH_2Cl_2/MeOH, 95/5, v/v) to give **28a** in 96% (450 mg, 0.66 mmol) yield: TLC (CH_2Cl_2/MeOH, 95/5) R_f = 0.6; ^{1}H NMR (400 MHz, CD_3CN) δ 8.58 (s, 1H, *H8*), 8.29 (s, 1H, *H2*), 8.00−7.94 (m, 2H, Ar-*H*), 7.59−7.55 (m, 1H, Ar-*H*), 7.48−7.43 (m, 2H, Ar-*H*), 7.36−7.35 (m, 2H, Ar-*H*), 7.28−7.15 (m, 7H, Ar-*H*), 6.82−6.76 (m, 4H, Ar-*H*), 6.39 (dd, J = 4.5, J = 4.7 Hz, 1H, *H1′*), 4.72 (q, J = 6.4 Hz, 1H, *H3′*), 4.10−4.06 (m, 1H, *H4′*), 3,72 (s, 6H, O−CH_3), 3.42−4.40 (m, 2, *H5′* and *H5″*), 3.15−3.08 (m, 1H, *H2″*), 2.63−2.54 (m, 1H, *H2′*); ^{13}C NMR (125 MHz, CD_3CN) δ 166.0, 159.3, 159.3, 145.6, 143.3, 136.4, 136.3, 133.2, 130.6, 130.6, 129.2, 128.8, 128.6, 128.5, 127.5, 125.4, 118.1, 113.7, 86.9, 84.8, 84.3, 63.8, 61.3, 55.6, 36.8; MS (FAB) *m/z* 683.4 $[M + H]^+$.

3′-Amino-*N*6-benzoyl-5′-*O*-dimethoxytrityl-2′,3′-dideoxyadenosine (29a). Compound **21a** was prepared according to general protocol A, using 3′-azido-*N*2-isobutyryl-5′-*O*-dimethoxytrityl-2′,3′-dideoxyguanosine (**28a**, 300 mg, 0.30 mmol), ethanol (5 mL), satd aqueous $NaHCO_3$ (100 μL), and Pd/C (60 mg). Compound **29a** was obtained as a slightly yellow foam in 90% (260 mg, 0.40 mmol) yield. Spectroscopic data were in agreement with the literature.[27]

5′-*O*-*tert*-Butyldimethylsilyl-*N*2-isobutyryl-2′-deoxyguanosine (24g). A stirred solution of *N*2-isobutyryl-2′-deoxyguanosine (**23g**, 1.5 g, 4.45 mmol) in pyridine (10 mL) was treated with TBS-Cl (773 mg 4.89 mmol, 1.1 equiv). After 16 h, the reaction mixture was transferred into ethyl acetate, and the solution was washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo to give a yellow gum. After coevaporation from toluene, the resulting foam was purified on silica (CH_2Cl_2/MeOH, 9/1, v/v) to give **24g** in 84% yield (1.68 g 3.67 mmol) as a colorless foam: TLC (CH_2Cl_2/MeOH, 9/1) R_f = 0.31; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (bs, 1H, *H8*), 6.19 (t, J = 6.6 Hz, 1H, *H1′*), 5.34 (d, J = 3.8 Hz, 1H, O-*H*), 4.37−4.31 (m, 1H, *H3′*), 3.85−3.81 (m, 1H, *H4′*), 3.70−3.64 (m, 2H, *H5′* and *H5″*), 2.74 (sept, J = 6.8 Hz, 1H, C*H*-CH_3, iBu), 2.59−2.51 (m, 1H, *H2″*), 2.35−2.25 (m, 1H, *H2′*), 1.10 (d, J = 6.8 Hz, 6H, CH-CH_3, iBu), 0.83 (s, 9H, Si−C−CH_3), 0.00 (s, 6H, Si−CH_3); ^{13}C NMR (100 MHz, DMSO-d_6) δ 180.0, 154.7, 148.2, 148.0, 137.0, 120.2, 87.1, 82.9, 70.0, 63.1, 34.7, 25.7, 18.8, 18.7, 17.9, −5.4, −5.5; MS (FAB) *m/z* 452.3 $[M + H]^+$.

5′-*O*-*tert*-Butyldimethylsilyl-*N*2-isobutyryl-2′-deoxyxyloguanosine (25g). To a chilled solution (0 °C) of Dess−Martin periodinane in CH_2Cl_2 (10 wt%, 11 mL, 5.32 mmol, 1.5 equiv) was added *N*2-isobutyryl-5′-*O*-*tert*-butyldimethylsilyl-2′-deoxyguanosine (**24g**, 1.6 g, 3.55 mmol) and the resulting solution stirred at 0 °C. After 60 min, the cooling bath was removed, and stirring was continued at rt. After 3 h, 2-propanol (11 mL) was added, the resulting white slurry cooled to −60 °C, and freshly powdered $NaBH_4$ (270 mg, 7.10 mmol, 2 equiv) added. After 13 h of stirring at −60 °C, acetone (11 mL) was added, and the slurry was allowed to warm to rt and poured into ethyl acetate (400 mL). The solution was washed with satd aqueous $NaHCO_3$, H_2O, and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting yellow foam was purified on silica (CH_2Cl_2/MeOH, 93/7, v/v) to give the title compound in 77% yield (1.23 g, 2.73 mmol) as a colorless foam: TLC (CH_2Cl_2/MeOH, 9/1) R_f = 0.33. ^{1}H NMR (500 MHz, $CDCl_3$) δ 12.17 (b, 1H, N-*H*), 8.23 (bs, 1H, *H8*), 6.14 (dd, J = 1.9 Hz, J = 8.5 Hz, 1H, *H1′*), 4.99 (b, 1H, O-*H*), 4.61 (bs, 1H, *H3′*), 4.09−3.99 (m, 3H, *H4′*, *H5′*, and *H5″*), 2.82 (sept., J = 6.9 Hz, 1H, C*H*-CH_3, iBu), 2.73−2.66 (m, 1H, *H2″*), 2.61−2.56 (m, 1H, *H2′*), 1.26−1.22 (m, 6H, CH-CH_3, iBu), 0.89 (s, 9H, Si−C−CH_3), 0.08 (s, 3H, Si−CH_3), 0.06 (s, 3H, Si−CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) 178.8, 154.8, 147.2, 146.9, 138.3, 119.3, 83.7, 82.1, 69.7, 61.3, 40.2, 35.0, 24.8, 18.0, 17.2, −6.3, −6.4; MS (FAB) *m/z* 452.2 $[M + H]^+$.

3′-Azido-5′-*O*-*tert*-butyldimethylsilyl-*N*2-isobutyryl-2′,3′-dideoxyguanosine (26g). To a stirred slurry of *N*2-isobutyryl-5′-*O*-*tert*-butyldimethylsilyl-2′-deoxyxyloguanosine (**25g**, 580 mg, 1.28 mmol) and NaN_3 (250 mg, 3.84 mmol, 3 equiv) in DMF (6 mL) was added a solution of diphenyl-2-pyridylphosphine (504 mg, 1.93 mmol, 1.5 equiv) and DIAD (374 μL, 1.93 mmol, 1.5 equiv) in DMF (1.5 mL). After 4 h, H_2O (2 mL) was added, and the reaction mixture was transferred into ethyl acetate. The organic solution was washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting brown oil was dried in vacuo and purified on silica (CH_2Cl_2/ethyl acetate, 2/3, v/v) to give the title compound in 86% yield (522 mg, 1.10 mmol): TLC (CH_2Cl_2/MeOH, 9/1) R_f = 0.5; ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.31 (bs, 1H, N-*H*), 10.56 (bs, 1H, N-*H*), 7.96 (bs, 1H, *H8*), 6.03 (t, J = 6.0 Hz, 1H, *H1′*), 4.31 (q, J = 5.7 Hz, 1H, *H3′*), 3.97−3.93 (m, 1H, *H4′*), 3.84−3.72 (m, 2H, *H5′* and *H5″*), 2.89 (sept., J = 6.8 Hz, 1H, C*H*-CH_3), 2.62−2.54 (m, 1H, *H2″*), 2.42−2.34 (m, 1H, *H2′*), 1.20−1.15 (m, 6H, CH-CH_3, iBu), 0.81 (s, 9H, Si−C−CH_3), 0.01 (s, 3H, Si−CH_3), 0.00 (s, 3H, Si−CH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ 180.0, 155.9, 148.2, 148.1, 136.9, 120.9, 84.9, 83.8, 62.8, 60.2, 38.3, 36.0, 25.8, 19.0, 18.9, 18.3, −5.4, −5.5; MS (FAB) *m/z* 477.1 $[M + H]^+$.

3′-Azido-*N*2-isobutyryl-2′,3′-dideoxyguanosine (27g). A stirred solution of 3′-azido-5′-*O*-*tert*-butyldimethylsilyl-*N*2-isobutyryl-2′,3′-dideoxyguanosine (**26g**, 200 mg, 0.42 mmol) in THF (2 mL) was treated with a solution of TBAF in THF (1 M, 844 μL, 0.844 mmol, 2 equiv). After 2 h, the solvent was removed in vacuo and the residue purified on silica (CH_2Cl_2/MeOH, 9/1, v/v) to give the title compound in 87% yield (133 mg, 0.37 mmol) as a colorless solid. Spectroscopic data were in agreement with the literature.[30]

3′-Azido-5′-*O*-dimethoxytrityl-*N*2-isobutyryl-2′,3′-dideoxyguanosine (28g). A stirred solution of 3′-azido-*N*2-isobutyryl-2′,3′-dideoxyguanosine (**27g**, 132 mg, 0.37 mmol) in pyridine (3 mL) was treated with DMTrCl (161 mg, 0.48 mmol, 1.3 equiv). After 3 h, MeOH (500 μL) was added, and stirring was continued. After 1 h, the reaction mixture was transferred into CH_2Cl_2. The solution was washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting yellow oil was purified on silica (pretreated with 1% NEt_3 in CH_2Cl_2, and eluted with 1% NEt_3 in CH_2Cl_2 to 1% NEt_3 in CH_2Cl_2/MeOH 95/5, v/v) to give the title compound in 79% yield (191 mg, 0.29 mmol) as a colorless foam. Spectroscopic data were in agreement with the literature.[30]

3′-Amino-5′-*O*-dimethoxytrityl-*N*2-isobutyryl-2′,3′-dideoxyguanosine (29g). Compound **29g** was prepared according to general protocol A using 3′-azido-*N*2-isobutyryl-5′-*O*-dimethoxytrityl-2′,3′-dideoxyguanosine (**28g**, 190 mg, 0.29 mmol), ethanol (5 mL), satd aqueous $NaHCO_3$ (100 μL), and Pd/C (60 mg,). A yield of 95% of **29g** (179 mg, 0.27 mmol) was obtained in the form of a slightly yellow foam. Spectroscopic were data in agreement with the literature.[27]

Loading of Nucleosides on Solid Supports. Solid Support 32a (General Protocol B). The following protocol is representative for the synthesis of the solid supports **32a**−**t**. LCAA-cpg (**31**, 1.4 g, loading 75 μmol/g) was coevaporated twice from toluene and dried

in vacuo for 12 h. Under an argon atmosphere, CH_2Cl_2 (12 mL) was added, followed by the addition of DIEA (406 μL, 1.3 mmol, 12 equiv compared to the NH_2-loading given by the manufacturer). Hexafluoroglutaric anhydride (145 μL, 1.1 mmol, 10 equiv) was added, and the slurry was shaken at rt for 12 h. The cpg was filtered off, washed with DMF containing 1% NEt_3, MeOH, and CH_2Cl_2 (5 × 10 mL each), and dried in vacuo. Aminonucleoside **29a** (260 mg, 315 μmol, 3 equiv) was added, and the resulting mixture was coevaporated twice from toluene, followed by drying in vacuo. After addition of DMF (8 mL), PPh_3 (173 mg, 665 μmol, 5 equiv), and DIEA (406 μL, 1.3 mmol, 12 equiv) to the residue, a solution of iodine (169 mg, 665 μmol, 5 equiv) in DMF (2 mL) was added dropwise while gently swirling the round-bottom flask. The resulting red slurry was swirled gently on a shaker. After 16 h, the cpg was filtered off and the slurry was washed with DMF containing 1% NEt_3, MeOH, and CH_2Cl_2 (5 × 10 mL each) and dried in vacuo. The resulting loading of the solid support **32a**, determined by DMT-cation release, was 18 μmol/g.

Solid Support 32c. Solid support **32c** was synthesized by following the general protocol B. The quantities used were LCAA-cpg (**31**, 1.32 g, loading 95 μmol/g), 3′-amino-*N*4-benzoyl-5′-*O*-dimethoxytrityl-2′,3′-dideoxycytidine (**20**, 240 mg, 376 μmol, 3 equiv), PPh_3 (162 mg, 627 μmol, 5 equiv), DIEA (406 μL, 1.5 mmol, 12 equiv), I_2 (156 mg, 626 μmol, 5 equiv), and DMF (10 and 2 mL for the iodine solution). The loading of the resulting solid support (**32c**) was determined to be of 24 μmol/g by trityl essay.

Solid Support 32g. Solid support **32g** was synthesized by following the general protocol B. The quantities used were LCAA-cpg (**31**, 1.1 g, loading 75 μmol/g), 3′-amino-5′-*O*-dimethoxytrityl-*N*2-isobutyryl-2′,3′-dideoxyguanosine (**29g**, 164 mg, 247 μmol, 3 equiv), PPh_3 (125 mg, 413 μmol, 5 equiv), DIEA (255 μL, 940 μmol, 12 equiv), I_2 (120 mg, 413 μmol, 5 equiv), and DMF (8 and 2 mL for the iodine solution). The loading of the resulting solid support (**32g**) was determined to be of 17 μmol/g by trityl essay.

Solid Support 32t. Solid support **32t** was synthesized by following the general protocol B. The quantities used were LCAA-cpg (**31**, 1.3 g, loading 75 μmol/g), 3′-amino-5′-*O*-dimethoxytrityl-3′-deoxythymidine (**11**, 160 mg, 293 μmol, 3 equiv), PPh_3 (128 mg, 488 μmol, 5 equiv), DIEA (317 μL, 1.17 mmol, 12 equiv), I_2 (126 mg, 488 μmol, 5 equiv), and DMF (10 and 2 mL for the iodine solution). The loading of the resulting solid support (**32t**) was determined to be of 28 μmol/g by trityl essay.

DNA Chain Assembly (General Procedure C). Starting from the support loaded with the 3′-amino-2′,3′-dideoxynucleoside (**32a**−**t**), the chain of the 3′-aminoterminal primers **33a**−**t** was generated by standard phosphoramidite-based DNA synthesis, following the protocol recommended by the manufacturer of the synthesizer (8909 Expedite DNA synthesizer, system software 2.01, 16 equiv of phosphoramidite per coupling cycle). The DNA chains were then fully deprotected and cleaved from the solid support by treatment with 30% aqueous ammonia at 55 °C for 20 h. Excess ammonia was removed with a gentle stream of air and the solution lyophilized to dryness. The resulting crudes were either analyzed by anion-exchange HPLC (IE-HPLC) using a gradient of 1 M KCl in 10 mM NaOH, starting at 0% KCl-containing solution for 5 min to 20% of the KCl-containing solution in 40 min (retention times are given as t_R) or bulk purified using SepPak-cartridge (Waters, Eschborn, Germany) with a stepwise gradient of CH_3CN in 0.1 M triethylammonium acetate, pH 7.

Analytical Data for Oligonucleotides. CGCACGA* (33a): t_R = 13.0 min; MALDI-TOF MS calcd for $[M - H]^-$ 2087.4, found 2087.9; yield (IE-HPLC) 37%; yield (cartridge) 24%.

CGCACGC* (33c): t_R = 13.9 min; MALDI-TOF MS calcd for $[M - H]^-$ 2063.4, found 2064.1; yield (IE-HPLC) 47%; yield (cartridge) 15%.

CGCACGG* (33g): t_R = 14.6 min; MALDI-TOF MS calcd for $[M - H]^-$ 2103.4, found 2105.2; yield (IE-HPLC) 43%; yield (cartridge) 21%.

CGCACGT* (33t): t_R = 13.8 min; MALDI-TOF MS calcd for $[M - H]^-$ 2078.4.4, found 2079.1; yield (IE-HPLC) 33%; yield (cartridge) 20%.

CGCACGT (34): t_R = 13.9 min; MALDI-TOF MS calcd for $[M - H]^-$ 2079.4, found 2082.2; yield 150 nmol (50%).

5′-Phosphorylation of Azidonucleosides. 3′-Azido-*N*6-benzoyl-2′,3′-dideoxyadenosine-5′-*O*-dibenzylphosphate (38a) (General Procedure D). The following protocol is representative for the synthesis of the phosphotriesters **38a**, **38c**, and **38t**. Azidonucleoside **29a** (150 mg, 0.42 mmol) was coevaporated twice from pyridine. The resulting oily residue was dissolved in pyridine (5 mL) and the solution cooled to −30 °C. After addition of freshly prepared dibenzylphosphoryl chloride[68] (400 μL, 1.89 mmol, 4.5 equiv), the solution was stirred at −30 °C for 5 h. Once TLC indicated complete conversion, the reaction mixture was added to ethyl acetate, and the resulting solution was washed with citric acid (5% aqueous solution) and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting crude was purified via column chromatography on silica (CH_2Cl_2/ethyl acetate, 6/4, v/v) to give 3′-azido-*N*6-benzoyl-2′,3′-dideoxyadenosine-5′-*O*-dibenzylphosphate (**38a**) in 69% (180 mg, 0.41 mmol) yield: TLC (CH_2Cl_2/ethyl acetate, 6/4) R_f = 0.27; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.22 (b, 1H, N-*H*),8.76 (s, 1H, *H8*), 8.20 (s, 1H, *H2*), 8.04 (d, J = 7.2 Hz, 2H, Ar-*H*), 7.64−7.59 (m, 1H, Ar-*H*), 7.55−7.51 (m, 2H, Ar-*H*), 7.36−7−32 (m, 10H, Ar-*H*), 6.37 (t, J = 6.4 Hz, 1H, *H1′*), 5.09−5.00 (m, 4, O-CH_2-Ph), 4.39−4,35 (m, 1H, *H3′*), 4.27−4.07 (m, 3H, *H4′*, *H5*,′ and *H5″*), 2.92−2.87 (m, 1H, *H2″*), 2.56−2.60 (m, 1H, *H2′*); ^{13}C NMR (125 MHz, $CDCL_3$) δ 164.7, 152.6, 151.2, 149.7, 141.5, 135.5, 135.4, 135.4, 135.4, 133.6, 132.8, 128.8, 128.8, 128.7, 128.6, 128.6, 128.1, 128.1, 127.9, 123.6, 82.8, 82.7, 69.7, 69.7, 69.7, 69.7, 66.2, 66.1, 60.7, 37.1; ^{31}P NMR (101 MHz, $CDCl_3$) δ −0.04 ppm; MS (MALDI-TOF) m/z 641.4 $[M + H]^+$.

3′-Azido-*N*4-benzoyl-2′,3′-dideoxycytidine-5′-*O*-dibenzylphosphate (38c). Phosphotriester **38c** was prepared according to general protocol D, using 3′-azido-*N*4-benzoyl-2′,3′-dideoxycytidine (**18**, 140 mg, 0.39 mmol), pyridine (4 mL), and dibenzylphosphoryl chloride (370 μL, 1,76 mmol, 4.6 equiv). The yield of **38c**, after column chromatography on silica (0.1% water in CH_2Cl_2/ethyl acetate, 6/4, v/v), was 74% (180 mg, 0.41 mmol): TLC (CH_2Cl_2/MeOH, 95/5) R_f = 0.38; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.74 (b, 1H, N-*H*), 8.04 (d, J = 7.5 Hz, 1H, *H6*), 7.96−7.91 (m, 2H, Ar-*H*), 7.67−7.63 (m, 1H, Ar-*H*), 7.59−7.50 (m, 3H, Ar-*H* and *H5*), 7.43−7−35 (m, 10H, Ar-*H*), 6.13 (t, J = 6.0 Hz, 1H, *H1′*), 5.13−5.03 (m, 4,O-*CH*$_2$-Ph), 4.26−4−11 (m, 2H, *H5′* and *H5″*), 4.02−3.99 (m, 1H, *H4′*), 3.97−3.92 (m, 1H, *H3′*), 2.67−2.61 (m, 1H, *H2″*), 2.20−2.14 (m, 1H, *H2′*); ^{13}C NMR (125 MHz, CDCL3) δ135.4, 135.4, 135.4, 135.3, 133.2, 129.1, 128.9, 128.8, 128.8, 128.3, 128.2, 86.9, 82.9, 82.9, 70.0, 69.9, 69.9, 69.9, 65.7, 65.7, 59.3, 38.6; ^{31}P NMR (101 MHz, $CDCl_3$) δ 0.35 ppm. MS (MALDI-TOF): 616 $[M+H]^+$.

3′-Azido-2′,3′-dideoxythymidine-5′-*O*-dibenzylphosphate (38t). Phosphotriester **38t** was prepared according to general protocol D, using 3′-azido-3′-deoxythymidine (**1**, 120 mg, 0.52 mmol), pyridine (3 mL), and dibenzylphosphoryl chloride (480 μL, 2.37 mmol, 4.6 equiv). The yield of **38t**, after column chromatography on silica (CH_2Cl_2/MeOH, 95/5, v/v), was 79% (186 mg, 0.41 mmol): TLC (CH_2Cl_2/MeOH, 95/5) R_f = 0.31; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.10 (b, 1H, N-*H*), 7.42−7−36 (m, 11H, *H6* and Ar-*H*), 6.15 (t, J = 6.0 Hz, 1H, *H1′*), 5.12−5.08 (m, 4, O-*CH*$_2$-Ph), 4.30−4.18 (m, 3H, *H3′*, *H5′*, and *H5″*), 4.02−3.98 (m, 1H, *H4′*), 2.39−2.25 (m, 2H, *H2′* and *H2″*) 1.79 (d, J = 1.3 Hz, 3H, *CH*$_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ 163.5, 150.1, 135.7, 135.6, 135.4, 128.2, 127.6, 110.2, 84.1, 81.5, 81.4, 69.0, 69.0, 68.9, 68.9, 66.1, 66.1, 59.6, 36.1, 11.2; ^{31}P NMR (101 MHz, $CDCl_3$) δ 0.46 ppm; MS (MALDI-TOF) = 538.3 $[M + H]^+$.

(68) Gao, F.; Yan, X.; Shakya, T.; Baettig, O. M.; Ait-Mohand-Brunet, S.; Berghuis, A. M.; Wright, G. D.; Auclair, K. *J. Med. Chem.* **2006**, *49*, 5273–5281.

Hydrogenation of Phosphotriester Azides. 3′-Amino-*N*6-benzoyl-2′,3′-dideoxyadenosine-5′-monophosphate (39a) (General Procedure E). The following protocol is representative for the synthesis of the aminophosphates **39a**, **39c**, and **39t**. A solution of 3′-azido-*N*6-benzoyl-2′,3′-dideoxyadenosine-5′-*O*-dibenzylphosphate (**38a**, 125 mg, 0.20 mmol) in ethanol (5 mL) was treated with a satd aqueous solution of $NaHCO_3$ (100 μL). The resulting slurry was placed under an argon atmosphere, and Pd/C (20 mg) was added. After introducing a hydrogen atmosphere, the slurry was stirred at the hydrostatic pressure generated by a 20 cm water column until TLC (CH_2Cl_2/MeOH, 9/1, v/v) indicated complete conversion of the starting material. Then, an equal volume of H_2O (v/v) was added, and the hydrogenation process was continued until only one spot was detectable by TLC (2-propanol/water/NEt_3, 7/2/1, v/v/v). The catalyst was removed by filtration over a bed of Celite and washed with H_2O. The combined solutions were lyophilized to dryness to give the title compound in 81% yield (66 mg, 0.16 mmol): TLC (2-propanol/water/NEt_3, 7/2/1, v/v/v) $R_f = 0.07-0.1$; ^{1}H NMR (600 MHz, D_2O) δ 8.65 (s,1H, *H8*), 8.58 (s,1H, *H2*), 7.86−7.83 (m, 2H, Ar-*H*), 7.57−7.53 (m, 1H, Ar-*H*), 7.45−7.42 (m, 2H, Ar-*H*), 6.50−6.48 (m, 1H, *H1′*), 4.20−4.17 (m, 1H, *H4′*), 4.95−3.88 (m, 2H, *H5′* and *H5″*), 4.20−4.17 (m, 1H, *H4′*), 4.04−4.00 (m, 1H, *H3′*), 2.88−2.83 (m, 1H, *H2″*), 2.61−2.56 (m, 1H, *H2′*); ^{13}C NMR (125 MHz, D_2O) δ 169.0, 151.7, 151.4, 148.9, 143.4, 133.3, 132.7, 128.7, 128.0, 123.8, 83.7, 63.5, 63.5, 50.9, 37.6; ^{31}P NMR (101 MHz, D_2O) δ 4.65; MS (MALDI-TOF) 433.2 $(M - H)^-$.

3′-Amino-*N*4-benzoyl-2′,3′-dideoxycytidine-5′-monophosphate (39c). Compound **39c** was prepared according to general protocol E, using 3′-azido-*N*4-benzoyl-2′,3′-dideoxycytidine-5′-*O*-dibenzylphosphate (**38c**, 170 mg, 0.27 mmol), ethanol (5 mL), satd aqueous $NaHCO_3$ (100 μL), and H_2O (5 mL). Aminonucleotide **39c** was obtained in 83% yield (102 mg, 0.22 mmol) as a white powder: TLC (2-propanol/water/NEt_3, 7/2/1, v/v/v) $R_f = 0.1-0.15$. ^{1}H NMR (400 MHz, D_2O) δ 8.33 (d, J = 7.6 Hz, 1H, *H6*), 7.78−7.75 (m, 2H, Ar-*H*), 7.58−7.53 (m, 1H, Ar-*H*), 7.46−7.41 (m, 2H, Ar-*H*), 7.38 (d, J = 7.6 Hz, 1H, *H5*), 6.15 (t, J = 6.1 Hz, 1H, *H1′*), 4.38−4.34 (m, 1H, *H4′*), 4.04−3.97 (m, 3H, *H3′*, *H5′*, and *H5″*), 2.72−2.52 (m, 1H, *H2′* and *H2″*); ^{13}C NMR (100 MHz, D_2O) 169.7, 163.2, 156.7, 145.7, 133.4, 132.6, 128.8, 128.7, 127.9, 127.3, 98.5, 86.9, 50.1, 36.4; ^{31}P NMR (101 MHz, D_2O) δ 3.33 ppm; MS (MALDI-TOF) 409.2 $(M - H)^-$.

3′-Amino-3′-deoxythymidine-5′-monophosphate (39t). Compound **39t** was prepared according to general protocol E, using 3′-azido-3′-deoxythymidine-5′-*O*-dibenzylphosphate (**38t**, 93 mg, 0.18 mmol), ethanol (5 mL), satd aqueous $NaHCO_3$ (100 μL), and H_2O (5 mL). Aminonucleotide **39t** was obtained in 78% yield (47 mg, 0.14 mmol) as a white powder: TLC (2-propanol/water/NEt_3, 7/2/1, v/v/v) $R_f = 0.1-0.13$; ^{1}H NMR (500 MHz, D_2O) δ 7.62 (s, 1H, *H6*), 6.20, (t, J = 6.6 Hz, 1H, *H1′*), 4.16−4.30 (m, 1H, *H4′*), 3.98−3.87 (m, 3H, *H3′*, *H5′*, and *H5″*), 2.55−2.48 (m, 1H, *H2′*), 2.46−2.39 (m, 1H, *H2″*); ^{13}C NMR (125 MHz, D_2O) δ 137.6, 84.7, 82.2, 82.2, 63.4, 63.4, 50.8, 35.6, 11.5; ^{31}P NMR (101 MHz, D_2O) δ 4.41 ppm; MS (MALDI-TOF) 320.3 $(M - H)^-$.

3′-Azido-*O*6-benzyl-5′-*O*-*tert*-butyldimethylsilyl-*N*2-isobutyryl-2′,3′-dideoxyguanosine (40). To a stirred solution of 3′-azido-5′-*O*-*tert*-butyldimethylsilyl-*N*2-isobutyryl-2′,3′-dideoxyguanosine (**26g**, 440 mg, 0.92 mmol) and benzyl alcohol (143 μL, 1.38 mmol, 1.5 equiv) in DMF (5 mL) was added a solution of PPh_3 (363 mg, 1.38 mmol, 1.5 equiv) and DIAD (197 μL, 1.38 mmol, 1.5 equiv) in DMF (2 mL). After 4 h, H_2O was added, and stirring was continued for another 2 h. The reaction mixture was transferred into ethyl acetate and the organic solution washed with H_2O and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The resulting yellow gum was purified on silica (column length 10 cm, eluant 0.1% water in petroleum ether/ethyl acetate, 1.5/1, v/v) to give the title compound **40** in 30% yield (167 mg, 0.29 mmol) as a yellow foam: TLC (0.1% water in petroleum ether/ethyl acetate, 1.5/1) $R_f = 0.2$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (s, 1H), 7.90 (bs, 1 h), 7.53−7.30 (m, 5H), 6.31 (t, J = 6.0 Hz, 1H), 5.62 (s, 2H), 4.64−4.57 (m, 1H), 4.07−4.02 (m, 1H), 3.96−3.80 (m, 2H), 3.15−3.01 (b, 1H), 2.91−2.80 (m, 1H), 2.61−2.50 (m, 1H), 1.28−1.25 (m, 6H), 0.89 (s, 9H), 0.08 (d, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 135.9, 128.5, 128.3, 128.2, 85.1, 84.2, 77.5, 77.0, 76.6, 68.8, 62.9, 60.3, 38.1, 25.9, 22.0, 19.3, 18.4, −5.4, −5.5; HRMS (ESI-Tof) *m*/*z* calcd for $C_{27}H_{38}N_8NaO_4Si$ $[M + Na]^+$ 589.2677, *m*/*z* obsd 589.2668.

3′-Azido-*O*6-benzyl-*N*2-isobutyryl-2′,3′-dideoxyguanosine (41). A stirred solution of 3′-azido-*O*6-benzyl-5′-*O*-*tert*-butyldimethylsilyl-*N*2-isobutyryl-2′,3′-dideoxyguanosine (**40**, 110 mg, 0.19 mmol) in THF (1 mL) was treated with TBAF in THF (1 M solution, 230 μL, 0.23 mmol, 1.2 equiv). After 2 h, the resulting yellow solution was directly objected to purification on silica (CH_2Cl_2/MeOH, 96/4, v/v) to give the title compound in 80% yield (69 mg, 0.15 mmol) as a colorless foam: TLC (CH_2Cl_2/MeOH, 95/5) $R_f = 0.43$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.96 (b, 1H), 7.89 (b, 1H), 7.43−7.37 (m, 2H), 7.31−7.22 (m, 3H), 6.10 (t, J = 6.5 Hz, 1H), 5.51 (s, 2H), 4.93−4.86 (m, 1H), 4.00−3.96 (m, 1H), 3.90−3.84 (m, 1H), 3.73−3.66 (m, 1H), 2.98−2.87 (m, 1H), 2.84−2.73 (m, 1H), 2.41−2.31 (m, 1H), 1.22−1.17 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.5, 159.8, 150.7, 150.5, 140.3, 134.6, 127.5, 127.3, 118.4, 84.9, 84.7, 75.6, 67.9, 61.4, 60.1, 36.8, 35.2, 24.6, 20.9, 18.3, 17.0; HRMS (ESI-Tof) *m*/*z* calcd for $C_{21}H_{24}N_8NaO_4$ $[M + Na]^+$ 475.1813, *m*/*z* obsd 475.1806.

3′-Azido-*O*6-benzyl-*N*2-isobutyryl-2′,3′-dideoxyguanosine-5′-*O*-dibenzylphosphate (42). Phosphotriester **42** was prepared according to general protocol D, using 3′-azido-*O*6-benzyl-5′-*O*-*tert*-butyldimethylsilyl-*N*2-isobutyryl-2′,3′-dideoxyguanosine (**41**, 69 mg, 0.15 mmol), pyridine (1.5 mL), and dibenzylphosphoryl chloride (145 μL, 0.69 mmol, 4.6 equiv). The yield of **42**, after column chromatography on silica (first 0.1% water in CH_2Cl_2/ethyl acetate, 4/1, v/v, then ethyl acetate), was 66% (70 mg, 0.1 mmol): TLC (CH_2Cl_2/MeOH, 95/5) $R_f = 0.27$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.69 (b, 1H), 7.83 (1H), 7.71−7.63 (m, 2H), 7.55−7.42 (m, 4H), 7.34−7.22 (m, 9H), 6.15 (t, j = 6.3 Hz, 1H), 5.63 (s, 2H), 5−06−4.95 (m, 4H), 4.46−4.37 (m, 1H), 4.17−4.05 (m, 2H), 3.09−2.98 (m, 1H), 2.95−2.83 (m, 1H), 2.47−2.37 (m, 1H) 1.27−1.23 (m, 4H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 175.6, 160.8, 152.0, 151.9, 140.8, 135.9, 135.6, 135.5, 135.4, 132.2, 132.0, 132.0, 131.9, 128.7, 128.6, 128.5, 128.4, 128.2, 128.1, 127.9, 119.1, 85.1, 83.0, 82.9, 69.7, 69.7, 69., 69.5, 68.8, 66.6, 66.5, 61.0, 36.6, 36.0, 19.4; ^{31}P NMR (121 MHz, $CDCl_3$) δ −0.78 ppm; HRMS (ESI-Tof) *m*/*z* calcd for $C_{35}H_{37}N_8NaO_7P$ $[M + Na]^+$ 735.2412, *m*/*z* obsd 735.2412.

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Supporting Information Available: ^{1}H NMR of compounds **1**, **9**−**11**, **14**−**22**, **24a**,**g**, **25a**,**g**, **26a**,**g**, **27a**,**g**, **28a**,**g**, **29a**,**g**, **30t**, **38a**,**c**,**t**, **39a**,**c**,**t**, and **40**−**42**. ^{13}C NMR spectra of compounds **16**−**18**, **25a**, **28a**, **24g**, **25g**, **26g**, **38a**,**c**,**t**, **39a**,**c**,**t**, and **40**−**42**. Procedures for the synthesis of compounds **11**, **15**, and **16**. MALDI time-of-flight spectra and HPLC trace-analysis chromatograms of 3′-aminoterminal primers **33a**−**t** and primer extension reactions involving them. This material is available free of charge via the Internet at http://pubs.acs.org.

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