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Full Papers

Novel Chemoenzymatic Protocol for the Synthesis of 3′-*O*-Dimethoxytrityl-2′-deoxynucleoside Derivatives as Building Blocks for Oligonucleotide Synthesis

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Abstract:

An easy, efficient, and scalable chemoenzymatic strategy for the synthesis of 3′-*O*-dimethoxytrityl-2′-deoxynucleosides has been developed. A key feature of this approach is the regioselective synthesis of 5′-*O*-levulinyl-2′-deoxynucleosides through enzymatic acylation in the presence of *Candida antarctica* lipase B. In addition, it was observed that the deblocking of levulinyl group from the 5′-position is perfectly compatible with conventional base protecting groups. To demonstrate the scalability of this method, 3′-*O*-dimethoxytritylthymidine (4a) was synthesized on 25-g scale. These monomers (4a−d) are useful building blocks for the synthesis of oligonucleotides.

Introduction

Synthetic oligonucleotides are an emerging class of chemotherapeutic agents with tremendous potential for treatment of a wide range of cardiovascular, inflammatory, metabolic and infectious diseases, and a variety of cancers.[1] Vitravene and Macugen are two shining examples of FDA approved oligonucleotide drugs along with 40 others that are advancing through human clinical trials at a fast pace. As a result, a number of pharmaceutical companies are actively engaged in the discovery and development of oligonucleotide drugs.[2] Assuming successful human clinical trials with these products and the possibility of their commercial launch, it is anticipated that soon very large quantities of therapeutically useful oligonucleotides may be required. During the past decade enormous efforts have been made in the development of synthetic methodologies for oligonucleotides, particularly for their large-scale synthesis.[3,4]

As modified oligonucleotides have become a major field of investigation for chemists, methods for their suitable protection/deprotection for the synthesis of nucleoside monomers have become equally important. Selective protection of a multifunctional compound is a challenging problem in organic synthesis.[5] Among the plethora of synthetic tools available to chemists, application of biocatalysts in organic chemistry has become one of the most attractive alternatives to the conventional chemical methods for a variety of reasons.[6] For example, enzymes are environmentally acceptable, work under mild conditions, are compatible in organic solvents, and demonstrate high chemo- and regio-selectivity during chemical transformations with recycling possibilities. In nucleoside chemistry, selective manipulation of the hydroxyl groups of carbohydrate moiety over amino groups of the bases is synthetically challenging and requires a multistep protocol.[7] Recently, we reported the use of enzymes for efficient synthesis of 3′- and 5′-*O*-levulinyl nucleosides avoiding several tedious chemical protection/deprotection steps.[8] We further demonstrated that enzymes are capable

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(1) (a) Crooke, S. T., Ed. *Antisense Drug Technology Principles, Stategies and Applications*; Marcel Dekker: New York, 2001. (b) Sanghvi, Y. S. In *Comprehensive Natural Products Chemistry*; Kool, E. T., Ed.; Elsevier: Amsterdam, 1999; Vol. 7, Chapter 7, pp 285−311 and references therein.
(2) ISIS Pharmaceuticals (www.isispharm.com) has the largest portfolio of antisense oligonucleotide products currently in clinical trials.
(3) (a) For a recent review of oligo- and poly-nucleotide synthesis, see: Reese, C. B. *Org. Biomol. Chem.* **2005**, *3*, 3851−3868. (b) Sanghvi, Y. S.; Andrade, M.; Deshmukh, R. R.; Holmberg, L.; Scozzari, A. N.; Cole, D. L. In *Manual of Antisense Methodology*; Hartmann, G.; Endres, S., Eds.; Kluwer Academic Publishers: Hingham, 1999; pp 3−23.
(4) (a) Gukathasan, R.; Massoudipour, M.; Gupta, I.; Chowdhury, A.; Pulst, S.; Sivakumar, R.; Sanghvi, Y. S.; Laneman, S. *J. Organomet. Chem*. **2005**, *690*, 2603−2607. (b) Wang, Z.; Cedillo, I.; Cole, D. L.; Sanghvi, Y. S.; Hinz, M.; Prukala, W.; Sobkowski, M.; Seliger, H.; Rimmler, M.; Ditz, R.; Hoffmeyer, J. In *Innovation and Perspectives in Solid-Phase Synthesis & Combinatorial Libraries*; Epton, R., Ed.; Mayflower Worldwide Ltd.: England, 2004; pp 118−122. (c) Sanghvi, Y. S.; Guo, Z.; Pfundheller, H. M.; Converso, A. *Org. Process Res.* Dev. **2000**, *4*, 175−181. (d) Pon, R. T.; Yu, S.; Guo, Z.; Sanghvi, Y. S. *Nucleic Acid Res.* **1999**, *27*, 1531−1538.
(5) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; 3rd ed.; Wiley: New York, 1999.
(6) (a) Klibanov, A. M. *Nature* **2001**, *409*, 241−246. (b) Koeller, K. M.; Wong, C.-H. *Nature* **2001**, *409*, 232−240. (c) Patel, R. N. *Stereoselective Biocatalysis*; Marcel Dekker: New York, 2000. (d) Carrea, G.; Riva, S. *Angew. Chem. Int. Ed.* **2000**, *39*, 2226−2254. (e) Bornscheuer, U. T.; Kazlauskas, R. J. *Hydrolases in Organic Synthesis. Regio- and Stereoselective Biotransformations*; Wiley-VCH: Weinheim, 1999.
(7) For reviews on enzymatic transformations in nucleosides, see: (a) Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319−4347. (b) Ferrero, M.; Gotor, V. *Monatsh. Chem.* **2000**, *131*, 585−616.
(8) (a) Lavandera, I.; García, J.; Fernández, S.; Ferrero, M.; Gotor, V.; Sanghvi, Y. S. In *Current Protocols in Nucleic Acid Chemistry*; Beaucage, S. L., Bergstrom, D. E., Glick, G. D., Jones, R. A., Eds.; John Wiley and Sons: New York, 2005; Chapter 2.11. (b) García, J.; Fernández, S.; Ferrero, M.; Sanghvi, Y. S.; Gotor, V. *Org. Lett.* **2004**, *6*, 3759−3762. (c) García, J.; Fernández, S.; Ferrero, M.; Sanghvi, Y. S.; Gotor, V. *Tetrahedron: Asymmetry* **2003**, *14*, 3533−3540. (d) García, J.; Fernández, S.; Ferrero, M.; Sanghvi, Y. S.; Gotor, V. *J. Org. Chem.* **2002**, *67*, 4513−4519.

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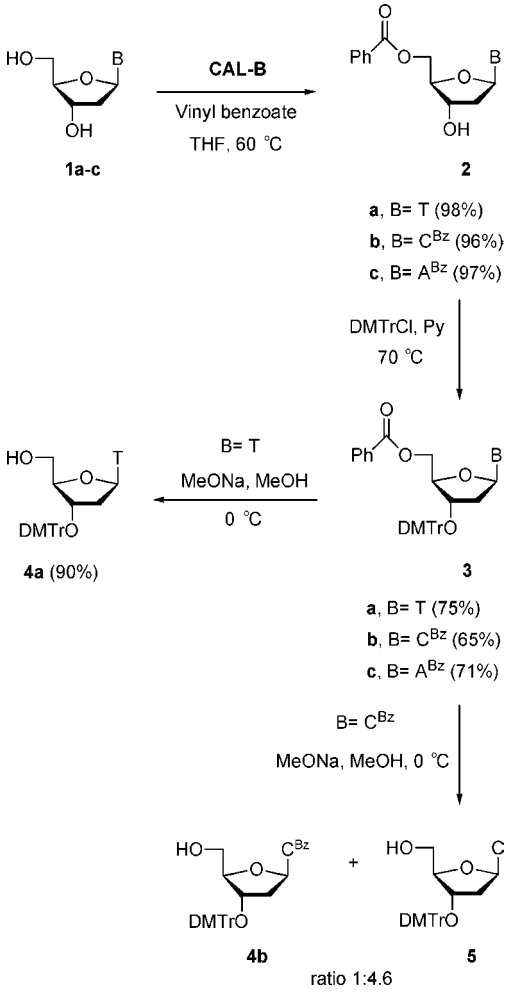
of regioselective enzymatic 5′-*O*-benzoylation of 2′-deoxynucleosides in high yields.[9]

In continuation of our efforts in utilizing enzymes for nucleoside transformations, we elected to synthesize 3′-*O*-dimethoxytrityl (DMTr)-protected nucleosides that are valuable building blocks for the assembly of oligonucleotides. The use of DMTr group for the protection of the primary 5′-hydroxyl group in nucleosides and oligonucleotide synthesis is well established.[10] The most common protocol for 5′-*O*-dimethoxytritylation of nucleosides is performed by the reaction of 4,4′-dimethoxytrityl chloride (DMTrCl) in pyridine with DMAP as a catalyst.[11] This procedure is also applicable to the solid-phase tritylation of nucleosides.[12] By contrast, efficient methods for the preparation of the 3′-*O*-DMTr-protected nucleosides are not available. To perform an inverse (5′→3′) oligodeoxyribonucleotide synthesis,[13] we needed large quantities of 3′-*O*-DMTr-protected nucleosides. Interestingly, the recently approved drug Macugen,[14] a vascular endothelial growth factor (VEGF) antagonist, possesses a 3′-thymidine residue that is inverted.[15] The incorporation of inverted residue in Macugen is accomplished via 3′-*O*-DMTr-protected thymidine.

The literature protocol for the installation of the 3′-*O*-DMTr group onto the nucleosides calls for the protection of the primary 5′-hydroxyl group with *tert*-butyldimethylsilyl chloride (TBDMSCl) and the subsequent reaction of 3′-hydroxyl group with DMTrCl.[13c,16] Next, treatment with tetrabutylammonium fluoride (TBAF) cleaved the 5′-*tert*-butyldimethylsilyl protecting group. The three-step protocol described in the literature is not suitable for scale-up due to a variety of reasons. First, the TBDMSCl is a corrosive and expensive reagent for large-scale applications. Second, regioselective protection of the 5′-hydroxyl group of nucleosides with TBDMSCl is tricky and requires chromatographic purification. Also, deprotection of the silyl group is accomplished by TBAF, which is difficult to remove from the desired product. In another approach, the 5′-position is blocked with the base-labile 2-dansylethoxycarbonyl group [2-((5-(dimethylamino)naphthalene-1-yl)sulfonyl)ethoxy)-carbonyl].[13b] The dansyl protecting group is not commercially available and must be synthesized in several steps. The use of 4,4′,4″-tris(benzoyloxy)trityl bromide (TBTrBr) as a blocking group is also reported.[17] This group is removed by the treatment with 0.5 M sodium hydroxide without affecting the DMTr group. However, TBTrBr is prepared from expensive rosolic acid.

Scheme 1



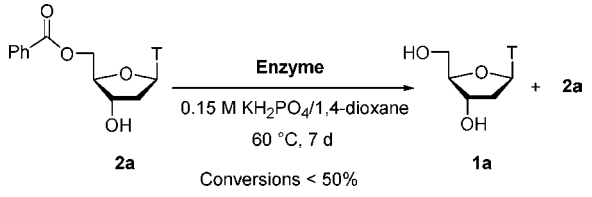
Discussion

These facts prompted us to investigate a chemoenzymatic protocol for the synthesis of 3′-*O*-DMTr nucleosides starting with readily available 5′-*O*-benzoyl derivatives **2**.[9] The protected nucleosides **2** are obtained in quantitative yield via selective monobenzoylation of the 5′-hydroxyl group with *Candida antarctica* B lipase (CAL-B). The scalability of this process and the fact that both the acylating agent and enzyme can be reclaimed and reused after each reaction makes these monomers very attractive starting materials.

The overall approach for the syntheses of 3′-*O*-DMTr derivatives is outlined in Scheme 1. Introduction of the DMTr group at the 3′-hydroxyl position is accomplished by treatment of 5′-*O*-benzoyl-2′-deoxynucleosides **2** with DMTrCl in pyridine at 70 °C. The choice of high reaction temperature furnished an improved reaction rate and better overall yield. Next, treatment of sodium methoxide in MeOH at 0 °C removed the 5′-*O*-benzoyl group. Under these conditions, 3′-*O*-DMTr-T (**4a**) is isolated in 90% yield after chroma-

(9) García, J.; Fernández, S.; Ferrero, M.; Sanghvi, Y. S.; Gotor, V. *Tetrahedron Lett.* **2004**, *45*, 1709−1712.
(10) (a) Chattopadhyaya, J. B.; Reese, C. B. *J. Chem. Soc., Chem. Commun.* **1978**, 639−640. (b) Barnett, W. E.; Needham, L. L.; Powell, R. W. *Tetrahedron* **1972**, *28*, 419−424.
(11) (a) McGee, D. P. C.; Martin, J. C.; Webb, A. S. *Synthesis* **1983**, 540−541. (b) Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316−1319. (c) Schaller, H.; Weiman, G.; Lerch, B.; Khorana, H. G. *J. Am. Chem. Soc.* **1963**, *85*, 3821−3827.
(12) Reddy, M. P.; Rampal, J. B.; Beaucage, S. L. *Tetrahedron Lett.* **1987**, *28*, 23−26.
(13) (a) D'Onofrio, J.; Montesarchio, D.; De Napoli, L.; Di Fabio, G. *Org. Lett.* **2005**, *7*, 4927−4930. (b) Wagner, T.; Pfleiderer, W. *Helv. Chim. Acta* **2000**, *83*, 2023−2035. (c) Esipov, D. S.; Esipova, O. V.; Korobko, V. G. *Nucleosides Nucleotides* **1998**, *17*, 1697−1704. (d) Robles, J.; Pedroso, E.; Grandas, A. *Nucleic Acids Res.* **1995**, *23*, 4151−4161.
(14) Macugen is developed and marketed by Eyetech Pharmaceuticals, Inc. and Pfizer, Inc.
(15) Nimjee S. M.; Rusconi C. P.; Sullenger B. A. *Annu. Rev. Med.* **2005**, *56*, 555−583.
(16) Wang, G.; Seifert, W. E. *Tetrahedron Lett.* **1996**, *37*, 6515−6518.
(17) Sekine, M.; Hata, T. *J. Org. Chem.* **1983**, *48*, 3011−3014.

Scheme 2



Enzyme: CAL-B, PSL-C, or PLE

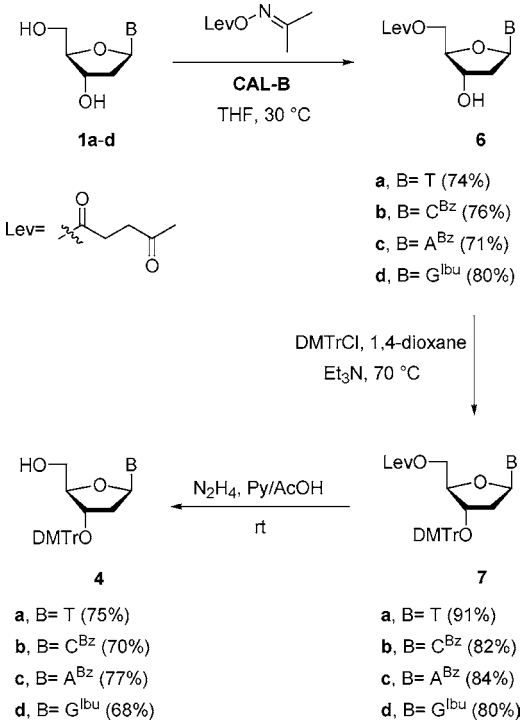
tography. However, under similar reaction conditions the cytidine derivative **3b** furnished a mixture of *N*-benzoyl-3′-*O*-DMTr-2′-deoxycytidine (**4b**) and the corresponding *N*-unprotected nucleoside **5** in 1:4.6 ratio, respectively. Attempts to avoid the formation of **5** with a weaker base such as K_2CO_3 in MeOH failed to improve the yield of the desired product **4b**. The unsuccessful outcome of chemical hydrolysis encouraged us to explore the enzymatic routes for the removal of the 5′-*O*-benzoyl group.

Among various hydrolytic enzymes available, lipases are well suited for the selective hydrolysis of an ester group vs an amide group. Thus, we studied the enzymatic hydrolysis of benzoates **3** with several commercial lipases: *Candida antartica* B (CAL-B), *Pseudomonas cepacia* (PSL-C), *Candida rugosa* (CRL), and *Chromobacterium viscosum* (CVL); and the pig liver esterase (PLE). The reactions were performed at 60 °C in 0.15 M phosphate buffer (pH 7) using 1,4-dioxane or acetone as cosolvent. In all cases, starting nucleosides were recovered unchanged after prolonged reaction times. These results suggested two possible explanations: the DMTr group could be too large to accommodate in the enzyme active site or the benzoyl group is not an appropriate acyl chain for hydrolysis with the enzymes tested.

To support our hypothesis, we attempted the enzymatic hydrolysis of 5′-*O*-benzoylthymidine (**2a**) as a model substrate (Scheme 2). The reaction is tested in the presence of CAL-B, PSL-C, or PLE as catalysts. The efficiency of benzoate hydrolysis in **2a** is ≤50% even after long reaction times (7 days). Thus, we postulate that the nucleosides with the sterically bulky group DMTr interfere with the binding site of the enzyme, leading to unfavorable hydrolysis reaction.

Therefore, we decided to explore other protecting groups to replace the 5′-*O*-benzoyl group in our synthetic strategy. Among the arsenal of protecting groups available, the levulinyl group is frequently chosen to protect the 3′- and/or 5′-hydroxyl of the nucleosides. This group is stable to coupling conditions and can be selectively cleaved under neutral pH conditions (without affecting other protecting groups in the molecule such as DMTr, acyl protecting groups on exocyclic amine bases, and internucleotide phosphate protecting groups[18]) with hydrazine hydrate in pyridine−acetic acid.[19] An alternative chemoenzymatic strategy of 3′-*O*-DMTr nucleosides involving the use of corresponding 5′-

Scheme 3



O-levulinyl derivatives is shown in Scheme 3. Effective 5′-*O*-levulinylation of various nucleosides has been accomplished by CAL-B-catalyzed regioselective acylation in organic solvents with acetonoxime levulinate as acylating agent.[8a,c] Using this protocol the 5′-*O*-Lev nucleosides **6** have been synthesized in 71−80% yield.[8a]

The dimethoxytritylation of **6** with DMTrCl in the presence of triethylamine and 1,4-dioxane as solvent at 70 °C furnished **7** in 80−91% yield. It is important to note that transformation of **6** to **7** is more efficient and easier to work up when 1,4-dioxane is used as a solvent compared to that with pyridine. Next, the 5′-*O*-levulinyl group is chemoselectively cleaved by treatment of **7** with 1 M hydrazine hydrate in pyridine−acetic acid (3:2 v/v) at room temperature without detectable loss of both the DMTr group or the acyl group at the exocyclic amine base, thereby confirming the orthogonality of these two hydroxyl protecting groups. DMTr derivatives **4** were purified by silica gel chromatography and isolated as pure solids in yields varying from 68% to 77%.

Large-Scale Studies. The industrial utility of this protocol is proven via synthesis of 3′-*O*-DMTr-T **4a** on large scale. Thus, 5’-*O*-levulinylthymidine (**6a**) is conveniently synthesized from 25 g (0.1 mol) of thymidine (**1a**), acetonoxime levulinate (0.3 mol), and CAL-B (1:1, w/w). The reaction is complete in 7 h (monitored by TLC). Reported enzymatic workup[8a] of the reaction provided 5′-*O*-Lev-T (**6a**) in pure state and 60% isolated yield via precipitation. An extra 19% product is recovered after dry-column chromatography[20] of the combined mother liquor obtained after precipitation step. The dry-column technique reduces the overall amount of

(18) (a) Eleuteri, A.; Cheruvallath, Z. S.; Capaldi, D. C.; Cole, D. L.; Ravikumar, V. T. *Nucleosides Nucleotides* **1999**, *18*, 1803−1807. (b) Krotz, A. H.; Klopchin, P.; Cole, D. L.; Ravikumar, V. T. *Nucleosides Nucleotides* **1997**, *16*, 1637−1640. (c) Iwai, S.; Toshiro, S.; Ohtsuka, E. *Tetrahedron* **1990**, *46*, 6673−6688.

(19) (a) Kumar, G.; Celewicz, L.; Chládek, S. *J. Org. Chem.* **1982**, *47*, 633−644. (b) van Boom, J. H.; Burguess, P. M. J.; Verdegaal, C. H. M.; Wille, G. *Tetrahedron* **1978**, *34*, 1999−2007.

(20) Pedersen, D. S.; Rosenbohm, C. *Synthesis* **2001**, 2431−2434.

solvent and silica gel usage, making the process cost-efficient for scale-up. Treatment of **6a** with DMTrCl in Et_3N/1,4-dioxane at 70 °C furnished the 3′-*O*-DMTr-5′-*O*-Lev-T (**7a**). After precipitation of the triethylamine hydrochloride salt by cooling and filtration, the filtrate is evaporated and subjected to hydrazinolysis of the 5′-*O*-levulinyl group. The final product 3′-*O*-DMTr-T is purified by crystallization of the brown viscous crude syrup from methylene chloride/hexane mixture which afforded the DMTr derivative **4a** in 73% overall yield from thymidine.

In summary, an efficient and high-yielding method for the preparation of 3′-*O*-dimethoxytrityl-2′-deoxynucleosides as versatile building blocks for oligonucleotide synthesis has been accomplished. The approach detailed herein is a significant improvement over reported protocols, thus providing an easy access to selectively protected nucleosides for other applications. A chemoenzymatic methodology has been shown, involving the regioselective enzymatic transesterification of 2′-deoxynucleosides with acetonoxime levulinate, subsequent introduction of the dimethoxytrityl group at the 3′-position, and selective cleavage of the 5′-*O*-levulinyl group. The *N*-protected-3′-*O*-dimethoxytrityl cytidine, adenosine, and guanosine derivatives were obtained in good yield, overcoming the limitations of our original strategy using the benzoyl group for the 5′-hydroxyl protection. The easy scalability of this process was demonstrated via large-scale synthesis of 3′-*O*-dimethoxytritylthymidine, making this procedure very attractive for industrial applications. In addition, enzyme-catalyzed reactions are less hazardous, less polluting, and less energy-consuming than the conventional chemistry-base methods using silyl reagents. Furthermore, CAL-B is available at a very reasonable cost, and since this lipase is immobilized, it can be reused to make the process further economical. The "green" nature of this protocol makes it an excellent alternative to existing chemical processes.[21]

Experimental Section

General. *Chromobacterium viscosum* lipase (CVL, 4100 U/mg) was a gift from Genzyme Co. *Candida* antarctica lipase B (CAL-B, Novozym 435, 10000 PLU/g) and immobilized *Pseudomonas cepacia* lipase (PSL-C, 904 U/g) were purchased from Novo Nordisk Co., and Amano Pharmaceuticals, respectively. *Candida rugosa* lipase (CRL, 1410 U/mg) and pig liver esterase (PLE, 24 U/mg) were purchased from Sigma. Silica gel chromatography has been performed, absorbing the crude product onto silica gel (methylene chloride). TLC plates were visualized with 2.5% *p*-anisaldehyde, 3.5% sulfuric acid, and 1% acetic acid in ethanol. To determine the purity of 3′-*O*-DMTr-T (**4a**) isolated from the large-scale reaction, HPLC analyses were carried out in a chromatograph with a UV detector at 254 nm, using a spherisorb W 5 μm column (0.46 cm × 25 cm); flow 1 mL/min; $T = 20$ °C; 2% ^{i}PrOH/CH_2Cl_2 as eluent; t_R for **4a** is 33.3 min. 5′-*O*-Benzoyl-2′-deoxynucleosides **2**[9] and 5′-*O*-levulinyl-2′-deoxynucleosides **6**[8a,c] have been prepared as previously described.

General Procedure for the Preparation of 5′-*O*-Benzoyl-3′-*O*-dimethoxytrityl-2′-deoxynucleosides 3a−c. To a solution of **2** (1 mmol) in anhydrous pyridine (10 mL) at 70 °C is added 4,4′-dimethoxytrityl chloride (212 mg, 0.625 mmol) in three equal portions. Progress of the reaction is monitored by TLC, which indicates complete disappearance of starting material in 16 h at 70 °C. The mixture is concentrated under reduced pressure and the crude product subjected to silica gel column chromatography (gradient elution with 67−100% EtOAc/hexane) to give DMTr derivatives **3** as pale-yellow solids (75% yield for **3a**, 66% yield for **3b**, and 71% yield for **3c**; yields are not optimized).

5′-*O*-Benzoyl-3′-*O*-dimethoxytritylthymidine (3a): mp 98−99 °C. R_f (4% CH_2Cl_2/MeOH): 0.32. $[\alpha]^{20}_D = -3.9$ (*c* 1.2, $CHCl_3$). IR (KBr): υ 3519, 3188, 3059, 2954, 1734−1643, 1606, 1251 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.71 (d, 3H, H_7, $^4J_{HH|}$ 1.1 Hz), 1.94 (m, 1H, H_2'), 2.30 (m, 1H, H_2'), 3.95 (2 s, 6H, OMe), 4.05 (dd, 1H, H_5', $^2J_{HH}$ 12.2 Hz, $^3J_{HH}$ 3.7 Hz), 4.28 (m, 1H, H_4'), 4.46−4.52 (m, 2H, $H_3'+H_5'$), 6.56 (dd, 1H, H_1', $^3J_{HH}$ 8.6, 5.4 Hz), 7.02 (2 d, 4H, $H_{m,2}+H_{m,2}''$, $^3J_{HH}$ 8.8 Hz), 7.40−7.82 (m, 13H, H_6, $H_{m,1}$, $H_{p,1}$, $H_{o,2}+H_{o,2}''$, $H_{o,3}$, $H_{m,3}$, $H_{p,3}$), 8.04 (dd, 2H, $H_{o,1}$, $^3J_{HH}$ 8.8 Hz, $^4J_{HH|}$ 1.1 Hz), and 8.72 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 12.7 (C_7), 40.3 (C_2'), 55.8 (2C, O*C*H$_3$), 64.9 (C_5'), 74.7 (C_3'), 84.4, 85.8 ($C_1'+C_4'$), 88.1 (C_t), 111.4 (C_5), 114.4 (4C, $C_{m,2}+C_{m,2}''$), 127.8−131.0 ($C_{m,1}+C_{o,1}+C_{i,1}+C_{o,2}+C_{o,2}''+C_{m,3}+C_{o,3}$), 134.1, 135.4 ($C_6+C_{p,1}$), 136.6 (2C, $C_{i,2}+C_{i,2}''$), 145.5 ($C_{i,3}$), 151.0 (C_2), 159.4 (2C, $C_{p,2}+C_{p,2}''$), 164.4 (C_4), and 166.6 (C═O). MS (ESI$^+$, *m*/*z*): 671 [(M + Na)$^+$, 100%], 687 [(M + K)$^+$, 50]. Anal. Calcd (%) for $C_{38}H_{36}N_2O_8$: C, 70.36; H, 5.59; N, 4.32. Found: C, 70.2; H, 5.6; N, 4.3.

***N*^{4},5′-Dibenzoyl-3′-*O*-dimethoxytrityl-2′-deoxycytidine (3b):** mp 111−112 °C. R_f (EtOAc): 0.45. $[\alpha]^{20}_D = +68.8$ (*c* 1.1, $CHCl_3$). IR (KBr): υ 3393, 3061, 2954, 2836, 1718, 1694, 1672, 1618, 1560, 1297 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.85 (m, 1H, H_2'), 2.71 (m, 1H, H_2'), 3.75 (2 s, 6H, OMe), 3.87 (dd, 1H, H_5', $^2J_{HH}$ 12.4 Hz, $^3J_{HH}$ 4.0 Hz), 4.07 (m, 1H, H_4'), 4.24 (dd, 1H, H_5', $^2J_{HH}$ 12.4 Hz, $^3J_{HH}$ 2.7 Hz), 4.35 (m, 1H, H_3'), 6.35 (apparent t, 1H, H_1', $^3J_{HH}$ 6.5 Hz), 6.82 (2 d, 4H, $H_{m,2}+H_{m,2}''$, $^3J_{HH}$ 8.8 Hz), 7.20−7.56 (m, 16H, H_5, H_m, H_P, $H_{m,1}$, $H_{p,1}$, $H_{o,2}+H_{o,2}''$, $H_{m,3}$, $H_{o,3}$, $H_{p,3}$), 7.75−7.90 (m, 4H, $H_o+H_{o,1}$), 7.96 (d, 1H, H_6, $^3J_{HH}$ 7.7 Hz), and 8.70 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 41.9 (C_2'), 55.92 (2C, O-CH_3), 64.7 (C_5'), 74.5 (C_3'), 85.2 ($C_1'+C_4'$), 88.2 (C_t), 88,6 ($C_4'+C_1'$), 96.7 (C_5), 114.1 (4C, $C_{m,2}+C_{m,2}''$), 127.8−130.9 ($C_o+C_m+C_{o,1}+C_{m,1}+C_{o,2}+C_{o,2}''+C_{o,3}+C_{m,3}+C_{p,3}+C_i$), 133.8, 134,1 (3C, $C_p+C_{i,1}+C_{p,1}$), 136.5, 136.7 (2C, $C_{i,2}+C_{i,2}''$), 144.3 (C_6), 145.6 ($C_{i,3}$), 153.2 (C_2+C_4), 159.5 (2C, $C_{p,2}+C_{p,2}''$), 162.7 (C═O), and 166.6 (C═O). MS (ESI$^+$, *m*/*z*): 739 [(M + H)$^+$, 50%], 761 [(M + Na)$^+$, 20]. Anal. Calcd (%) for $C_{44}H_{40}N_3O_8$: C, 71.53; H, 5.46; N, 5.69. Found: C, 71.5; H, 5.2; N, 5.8.

***N*^{6},5′-Dibenzoyl-3′-*O*-dimethoxytrityl-2′-deoxyadenosine (3c):** mp 101−102 °C. R_f (EtOAc): 0.56. $[\alpha]^{20}_D = -7.7$

(21) (a) Sanghvi, Y. S.; Ravikumar, V. T.; Scozzari, A. N.; Cole, D. L. *Pure Appl. Chem*. **2001**, *73*, 175−180. (b) *Green Chemical Syntheses and Processes*; Anastas, P. T.; Heine, L. G.; Williamson, T. C., Eds.; ACS Symposium Series 767; American Chemical Society: Washington, DC, 2000.

(*c* 1.1, $CHCl_3$). IR (KBr): υ 3407, 3060, 3001, 2954, 2836, 2047, 1968, 1719, 1608, 1581, 1509, 1251 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): δ 2.18 (m, 1H, H_2'), 2.48 (m, 1H, H_2'), 3.75 (2 s, 6H, OMe), 4.08 (dd, 1H, H_5', $^2J_{HH}$ 12.1 Hz, $^3J_{HH}$ 4.8 Hz), 4.32 (m, 1H, H_4'), 4.38 (dd, 1H, H_5', $^2J_{HH}$ 12.1 Hz, $^3J_{HH}$ 3.4 Hz), 4.60 (m, 1H, H_3'), 6.47 (apparent t, 1H, H_1', $^3J_{HH}$ 7.1 Hz), 6.83 (2 d, 4H, $H_{m,2}+H_{m,2}''$, $^3J_{HH}$ 11.1 Hz), 7.20−7.50 (m, 15H, H_m, H_P, $H_{m,1}$, $H_{p,1}$, $H_{o,2}+H_{o,2}''$, $H_{m,3}$, $H_{o,3}$, $H_{p,3}$), 7.80−8.08 (m, 4H, H_o, $H_{o,1}$), 8.10 (s, 1H, H_2+H_8), 8.70 (s, 1H, H_8+H_2), and 9.14 (s, 1H, NH). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 39.6 (C_2'), 55.9 (2C, O-*C*H_3), 64.8 (C_5'), 74.9 (C_3'), 84.9, 85.9 ($C_1'+C_4'$), 88.1 (C_t), 114.1 (4C, $C_{m,2}+C_{m,2}''$), 124.1 (C_5), 127.8−130.9 ($C_o+C_m+C_p+C_{o,1}+C_{m,1}+C_{o,2}+C_{o,2}''+C_{o,3}+C_{m,3}+C_{p,3}$), 133.4, 133.9, 134.3 ($C_{p,1}+C_{i,1}+C_i$), 136.6 (2C, $C_{i,2}+C_{i,2}''$), 142.1 (C_8), 145.6 ($C_{i,3}$), 150.1, 152.0, 153.3 ($C_6+C_2+C_4$), 159.5 (2C, $C_{p,2}+C_{p,2}''$), 165.3 (C═O), and 166.7 (C═O). MS (ESI^+, *m*/*z*): 761 [$(M + H)^+$, 100%], 783 [$(M + Na)^+$, 10]. Anal. Calcd (%) for $C_{45}H_{38}N_5O_7$: C, 71.04; H, 5.03; N, 9.2. Found: C, 71.1; H, 5.3; N, 9.1.

General Procedure for the Preparation of 3′-*O*-Dimethoxytrityl-2′-deoxynucleosides 4a−b. Fully protected deoxynucleosides **7a−d** (0.15 mmol) are dissolved in anhydrous pyridine (0.6 mL), and 1 M hydrazine hydrate in pyridine/acetic acid (3:2 v/v, 450 μL) is added to the solution, which is stirred during 1 h for **7a**, 2 h for **7b−c**, and 2.5 h for **7d**. The reaction mixture is then chilled in an ice−water bath, and pentane-2,4-dione (0.9 mmol) is added. After 10 min, the solution is partitioned between CH_2Cl_2/H_2O. The organic layer is separated and sequentially washed with 10% $NaHCO_3$ and H_2O, is dried over Na_2SO_4 and evaporated under reduced pressure. The residue is purified by flash chromatography (2% MeOH/CH_2Cl_2 for **4a−c** and gradient elution with 0.8−4% MeOH/CH_2Cl_2 for **4d**) to give **4** as pale-yellow solid (75% yield for **4a**; 70% yield for **4b**; 77% yield for **4c**; and 68% yield for **4d**).

Large-Scale Preparation of 3′-*O*-Dimethoxytritylthymidine (4a). A suspension of thymidine (25 g, 0.103 mol), acetonoxime levulinate (0.309 mol), and *Candida antarctica* lipase B (25 g) in anhydrous THF (490 mL) under nitrogen is stirred at 250 rpm for 7 h at 10 °C. Progress of the reaction is monitored by TLC (5% MeOH/CH_2Cl_2). The enzyme is filtered off and washed with THF, CH_2Cl_2, and MeOH. The solvents are distilled under vacuum, and the residue is dissolved in CH_2Cl_2 (200 mL). The organic layer is washed with $NaHCO_3$ (aq) (4 × 50 mL) to remove unreacted thymidine, dried over Na_2SO_4, and evaporated under reduced pressure. The residue is dissolved in CH_2Cl_2 (25 mL) and precipitated with cold (4 °C) diethyl ether (the precipitate is cooled at 4 °C overnight) to afford after filtration the 5′-*O*-levulinyl nucleoside **6a** as a white solid (60% yield). Dry column chromatography[20] of the ether mother liquors afforded a further 19% yield of **6a**. Et_3N (112 mL, 0.8 mmol), and DMTrCl (54 g, 0.16 mmol) are added to a solution of **6a** in anhydrous 1,4-dioxane (260 mL), and the reaction is stirred at 70 °C during 16 h. The reaction mixture upon cooling to room temperature precipitates the insoluble triethylamine hydrochloride that is removed by filtration. The filtrate is evaporated under reduced pressure to give dimethoxytrityl derivative **6a**. The crude residue is dissolved in anhydrous pyridine (145 mL), and hydrazine hydrate (209 mL, 1 M solution in pyridine/acetic acid, 3:2 v/v) is added to the solution, which is stirred for 1 h at room temperature. Part of the pyridine is evaporated under reduced pressure, the crude product is poured into CH_2Cl_2 (350 mL), and the organic layer is washed several times (4−6) with $NaHCO_3$ (aq). The organic phase is concentrated under reduced pressure, and the brown viscous syrup is precipitated. The residue is dissolved in the minimum amount of CH_2Cl_2 (first time ca. 150 mL is needed), and then hexane (ca. 300 mL) is added gradually until a gummy solid is precipitated. The solvents are poured off, and the residue is dried under vacuum. This procedure is repeated until the pure solid product is obtained. The yellow solid is obtained in 73% overall yield from thymidine and >98% purity (determined by HPLC).

Alternative Synthesis of 3′-*O*-Dimethoxytritylthymidine (4a). NaOMe (615 μL, 0.2 M solution in anhydrous MeOH) is added dropwise to a solution of **3a** (100 mg, 0.154 mmol) in anhydrous MeOH (7 mL) at 0 °C, and the reaction mixture is stirred at room temperature for 16 h. The mixture is neutralized with solid NH_4Cl and the solvent evaporated. The residue is purified by flash chromatography (gradient elution with 50−65% EtOAc/hexane), and the product is dried under vacuum to furnish **4a** as pale-yellow solid in 90% yield.

3′-*O*-Dimethoxytritylthymidine (4a): mp 54−55 °C. R_f (4% CH_2Cl_2/MeOH): 0.32. $[\alpha]^{20}_D = -16.7$ (*c* 1.0, $CHCl_3$). IR (KBr): υ 3257, 3058, 2929, 2836, 1690, 1607, 1509, 1250 cm^{-1}. ^1H NMR (MeOH-d_4, 300 MHz): δ 1.95 (m, 2H, H_2'), 1.99 (d, 3H, H_7, $^4J_{HH}$ 1.2 Hz), 3.46 (m, 1H, H_5'), 3.70 (m, 1H, H_5'), 3.95 (s, 6H, OMe), 4.03 (br s, 1H, H_4'), 4.54 (m, 1H, H_3'), 6.50 (apparent t, 1H, H_1', $^3J_{HH}$ 7.6 Hz), 7.03 (2d, 4H, $H_{m,2}+H_{m,2}''$, $^3J_{HH}$ 8.9 Hz), 7.45−7.64 (m, 9H, $H_{o,2}+H_{o,2}''$, $H_{o,1}$, $H_{m,1}$, $H_{p,1}$, $H_{o,1}$), and 7.85 (s, 1H, H_6). ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 12.5 (C_7), 40.5 (C_2'), 55.8 (2C, O*C*H_3), 63.1 (C_5'), 76.2 (C_3'), 86.5, 88.2 ($C_1'+C_4'$), 88.6 (C_t), 111.8 (C_5), 114.4 ($C_{m,2}+C_{m,2}''$), 128.1−131.6 ($C_{o,2}+C_{o,2}''+C_{o,3}+C_{m,3}+C_{p,3}$), 137.7 (2C, $C_{i,2}+C_{i,2}''$), 138.1 (C_6), 146.9 ($C_{i,3}$), 152.5 (C_2), 160.4 (2C, $C_{p,2}+C_{p,2}''$), and 166.4 (C_4). MS (ESI^+, *m*/*z*): 545 [$(M + H)^+$, 10%], 567 [$(M + Na)^+$, 20]. Anal. Calcd (%) for $C_{31}H_{32}N_2O_7$: C, 68.37; H, 5.92; N, 5.14. Found: C, 68.4; H, 5.9; N, 5.1.

***N*^{4}-Benzoyl-3′-*O*-dimethoxytrityl-2′-deoxycytidine (4b):** mp 116−117 °C. R_f (4% CH_2Cl_2/MeOH): 0.37. $[\alpha]^{20}_D = +48.5$ (*c* 1.2, $CHCl_3$). IR (KBr): υ 3342, 3061, 2932, 2836, 1701, 1654, 1608, 1560, 1485, 1396, 1250 cm^{-1}. ^1H NMR (MeOH-d_4, 300 MHz): δ 1.94 (m, 1H, H_2'), 2.36 (m, 1H, H_2'), 3.45 (m, 1H, H_5'), 3.71 (m, 1H, H_5'), 3.95 (s, 6H, OMe), 4.08 (m, 1H, H_4'), 4.54 (m, 1H, H_3'), 6.49 (dd, 1H, H_1', $^3J_{HH}$ 8.0, 5.4 Hz), 7.05 (2 d, 4H, $H_{m,2}+H_{m,2}''$, $^3J_{HH}$ 8.8 Hz), 7.40−7.65 (m, 13H, H_5, H_m, H_P, $H_{o,2}+H_{o,2}''$, $H_{m,3}$, $H_{o,3}$, $H_{p,3}$), 8.13 (d, 2H, H_o, $^3J_{HH}$ 7.7 Hz), and 8.54 (d, 1H, H_6, $^3J_{HH}$ 7.7 Hz). ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 42.1 (C_2'), 55.8 (2C, O-CH_3), 63.0 (C_5'), 76.2 (C_3'), 88.7 (C_t), 89.1, 89.2 ($C_1'+C_4'$), 98.9 (C_5), 114.4 (4C, $C_{m,2}+C_{m,2}''$), 128.1−132.6 (C_o+C_m+-

$C_{o,2}+C_{o,2}''+C_{o,3}+C_{m,3}+C_{p,3}$), 134.1 ($C_p$), 134.7 ($C_i$), 137.7 (2C, $C_{i,2}+C_{i,2}''$), 146.5 (C_6), 146.9 ($C_{i,3}$), 157.9 (C_2), 160.4 (2C, $C_{p,2}+C_{p,2}''$), 164.7 (C_4), and 169.1 (C═O). MS (ESI$^+$, *m*/*z*): 634 [(M + H)$^+$, 100%], 656 [(M + Na)$^+$, 40]. Anal. Calcd (%) for $C_{37}H_{35}N_3O_7$: C, 70.13; H, 5.57; N, 6.63. Found: C, 70.2; H, 5.4; N, 6.5.

***N*^{6}-Benzoyl-3′-*O*-dimethoxytrityl-2′-deoxyadenosine (4c):** mp 125−126 °C. R_f (4% CH_2Cl_2/MeOH): 0.28. $[\alpha]^{20}_D$ = +9.1 (*c* 0.4, $CHCl_3$). IR (KBr): υ 3328, 3192, 2930, 2836, 1718, 1639, 1608, 1509, 1299, 1251 cm^{-1}. ^{1}H NMR (MeOH-d_4, 300 MHz): δ 2.28 (m, 1H, H_2'), 2.63 (m, 1H, H_2'), 3.53−3.73 (m, 2H, H_5'), 3.96 (2 s, 6H, OMe), 4.17 (m, 1H, H_4'), 4.72 (m, 1H, H_3'), 6.73 (dd, 1H, H_1', $^3J_{HH}$ 8.5, 5.7 Hz), 7.07 (2 d, 4H, $H_{m,2}+H_{m,2}''$, $^3J_{HH}$ 8.8 Hz), 7.40−7.62 (m, 12H, H_m, H_P, $H_{o,2}+H_{o,2}''$, $H_{m,3}$, $H_{o,3}$, $H_{p,3}$), 8.24 (d, 2H, H_o, $^3J_{HH}$ 7.4 Hz), 8.72 (s, 1H, H_2+H_8), and 8.84 (s, 1H, H_8+H_2). ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 41.0 (C_2'), 55.8 (2C, O-*C*H$_3$), 63.6 (C_5'), 76.5 (C_3'), 87.4, ($C_1'+C_4'$), 88.8 (C_t), 89.2 ($C_4'+C_1'$) 114.4 (4C, $C_{m,2}+C_{m,2}''$), 128.1−131.7 ($C_5+C_o+C_m+$ $C_{o,2}+C_{o,2}''$ $+C_{o,3}+C_{m,3}+C_{p,3}$), 134.0 (C_p), 135.0 (C_i), 137.7 (2C, $C_{i,2}+C_{i,2}''$), 144.6 (C_8), 146.9 ($C_{i,3}$), 151.4 (C_2), 152.9 (C_6), 157.5 (C_4), 160.5 (2C, $C_{p,2}+C_{p,2}''$), and 168.2 (C═O). MS (ESI$^+$, *m*/*z*): 658 [(M + H)$^+$, 100%], 680 [(M + Na)$^+$, 10]. Anal. Calcd (%) for $C_{38}H_{35}N_5O_6$: C, 69.39; H, 5.36; N, 10.65. Found: C, 69.5; H, 5.1; N, 10.8.

3′-*O*-Dimethoxytrityl-*N*^{2}-isobutyryl-2′-deoxyguanosine (4d): mp 109−110 °C R_f (80% EtOAc/hexanol): 0.30. $[\alpha]^{20}_D$ = −7.6 (*c* 1.1, $CHCl_3$). IR (KBr): υ 3147, 3003, 1745, 1711, 1693 cm^{-1}. ^{1}H NMR (MeOH-d_4, 300 MHz): δ 1.38 (d, 6H, Me-Ibu, $^3J_{HH}$ 6.8 Hz), 2.11 (m, 1H, H_2'), 2.44 (m, 1H, H_2'), 2.88 (m, 1H, CH-Ibu), 3.56−3.65 (m, 2H, H_5'), 3.92 (2 s, 6H, OMe), 4.08 (m, 1H, H_4'), 4.61 (m, 1H, H_3'), 6.54 (dd, 1H, H_1', $^3J_{HH}$ 9.0, 5.7 Hz), 7.03 (2 d, 4H, $H_{m,2}+H_{m,2}''$, $^3J_{HH}$ 8.8 Hz), 7.40−7.65 (m, 9H, $H_{o,2}+H_{o,2}''$, $H_{o,3}$, $H_{m,3}$, $H_{p,3}$), and 8.30 (s, 1H, H_8). ^{13}C NMR (MeOH-d_4, 100.6 MHz): δ 19.4 (2C, *C*H$_3$-Ibu), 37.0 (CH-Ibu), 41.5 (C_2'), 55.8 (2C, O-*C*H$_3$), 63.2 (C_5'), 76.4 (C_3'), 85.8, 88.7 (3C, $C_1'+C_4'+C_t$), 114.4 (4C, $C_{m,2}+C_{m,2}''$), 120.9 (C_5), 128.1−131.6 ($C_{o,2}+C_{o,2}''+C_{m,3}+C_{o,3}+C_{p,3}$), 137.3 (2C, $C_{i,2}+C_{i,2}''$), 139.1(C_8), 146.9 ($C_{i,3}$), 149.8, 150.0 (C_2+C_4), 157.5 (C_6), 160.5 (2C, $C_{p,2}+C_{p,2}''$), and 181.7 (Ibu C═O). MS (ESI$^+$, *m*/*z*): 641 [(M + H)$^+$, 60%], 663 [(M + Na)$^+$, 10]. Anal. Calcd (%) for $C_{35}H_{38}N_5O_7$: C, 65.61; H, 5.98; N, 10.93. Found: C, 65.4; H, 6.1; N, 10.9.

General Procedure for the Preparation of 5′-*O*-Levulinyl-3′-*O*-dimethoxytrityl-2′-deoxynucleosides 7a−d. To a solution of **6a**−**d** (1.5 mmol) in anhydrous 1,4-dioxane (18 mL) is added Et_3N (2.1 mL, 15 mmol), and 4,4′-dimethoxytrityl chloride (1.52 g, 4.5 mmol), and the mixture is stirred at 70 °C for 12 h. The reaction mixture is poured into $NaHCO_3$ saturated aqueous solution and extracted with CH_2Cl_2. The combined organic extracts are dried over Na_2SO_4 and evaporated to dryness. The crude material is purified by silica gel chromatography (gradient elution with CH_2Cl_2−1% MeOH/CH_2Cl_2 for **7a**−**c**, and 0.5−3% MeOH/CH_2Cl_2 for **7d**) to afford **7** as pale-yellow solid (91% yield for **7a**; 82% yield for **7b**; 84% yield for **7c**; and 80% yield for **7d**).

3′-*O*-Dimethoxytrityl-5′-*O*-levulinylthymidine (7a): mp 66−67 °C. R_f (4% CH_2Cl_2/MeOH): 0.32. $[\alpha]^{20}_D$ = +21.3 (*c* 1.0, $CHCl_3$). IR (KBr): υ 3250, 2962, 1716, 1685, 1509, 1263 cm^{-1}. ^{1}H NMR (MeOH-d_4, 300 MHz): δ 2.00 (m, 1H, H_2'), 2.03 (d, 3H, H_7, $^4J_{HH}$ 1.1 Hz), 2.14 (m, 1H, H_5'), 2.30 (s, 3H, CH_3-Lev), 2.64 (t, 2H, CH_2-Lev, $^3J_{HH}$ 6.6 Hz), 2.92 (t, 2H, CH_2-Lev, $^3J_{HH}$ 6.6 Hz), 3.95 (s, 6H, OMe′), 4.05 (m, 2H, H_5'), 4.13 (m, 1H, H_4'), 4.48 (m, 1H, H_3'), 6.58 (dd, 1H, H_1', $^3J_{HH}$ 8.8, 5.7 Hz), 7.06 (apparent d, 4H, $H_{m,2}+H_{m,2}''$, $^3J_{HH}$ 8.8 Hz), and 7.45−7.57 (m, 10H, H_6, $H_{o,2}+H_{o,2}''$, $H_{o,3}$, $H_{m,3}$, $H_{p,3}$). ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 12.5 (C_7), 28.8 (CH_2-Lev), 29.6 (CH_3-Lev), 38.5 (CH_2-Lev), 39.8 (C_2'), 55.7 (2C, O-*C*H$_3$), 65.2 (C_5'), 75.5 (C_3'), 85.1, 86.5 ($C_1'+C_4'$), 88.7 (C_t), 111.8 (C_5), 114.4 (4C, $C_{m,2}+C_{m,2}''$), 128.1−135.5 ($C_{o,2}+C_{o,2}''$, $C_{m,3}$, $C_{o,3,}$ $C_{p,3}$), 137.3, 137.6 (3C, $C_6+C_{i,2}+C_{i,2}''$), 146.7 ($C_{i,3}$), 152.3 (C_2), 160.4 (2C, $C_{p,2}+C_{p,2}''$), 166.2 (C_4), 174.0 (C═O), and 209.1 (C═O). MS (ESI$^+$, *m*/*z*): 643 [(M + H)$^+$, 10%], 665 [(M + Na)$^+$, 100], 681 [(M + K)$^+$, 40]. Anal. Calcd (%) for $C_{36}H_{38}N_2O_9$: C, 67.28; H, 5.96; N, 4.36. Found: C, 67.1; H, 6.2; N, 4.4.

***N*^{4}-Benzoyl-3′-*O*-dimethoxytrityl-5′-*O*-levulinyl-2′-deoxycytidine (7b):** mp 72−73 °C. R_f (CH_2Cl_2/MeOH 2%): 0.40. $[\alpha]^{20}_D$ = +70.2 (*c* 1.3, $CHCl_3$). IR (KBr): υ 3411, 3062, 2956, 2931, 1718, 1696, 1608, 1509, 1485, 1251 cm^{-1}. ^{1}H NMR (MeOH-d_4, 300 MHz): δ 1.95 (m, 1H, H_2'), 2.30 (s, 3H, Me-Lev), 2.58 (m, 3H, CH_2-Lev+H_2'), 2.91 (t, 2H, CH_2-Lev, $^3J_{HH}$ 5.6 Hz), 3.97 (m, 7H, 2OMe+H_5'), 4.17 (m, 2H, $H_4'+H_5'$), 4.50 (m, 1H, H_3'), 6.45 (dd, 1H, H_1', $^3J_{HH}$ 7.5, 5.6 Hz), 7.07 (apparent d, 4H, $H_{m,2}+H_{m,2}''$, $^3J_{HH}$ 8.7 Hz), 7.40−7.80 (m, 13H, H_5, H_m, H_P, $H_{o,2}+H_{o,2}''$, $H_{m,3}$, $H_{o,3}$, $H_{p,3}$), 8.14 (d, 2H, H_o, $^3J_{HH}$ 6.9 Hz), and 8.34 (d, 1H, H_6, $^3J_{HH}$ 7.4 Hz). ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 29.7 (CH_2-Lev), 30.2 (CH_3-Lev), 40.2 (CH_2-Lev), 41.7 (C_2'), 55.9 (2C, O-CH_3), 65.1 (C_5'), 75.5 (C_3'), 86.1 ($C_4'+C_1'$), 88.8 (C_t), 89,3 ($C_1'+C_4'$), 98.7 (C_5), 114.5 (4C, $C_{m,2}+C_{m,2}''$), 128.6−132.1 ($C_o+C_m+C_{o,2}+C_{o,2}''$ $+C_{o,3}+C_{m,3}+C_{p,3}$), 134.2 (C_p), 134.7 (C_i), 137.3, 137.5 (2C, $C_{i,2}+C_{i,2}''$), 145.8 (C_6), 146.7 ($C_{i,3}$), 157.7 (C_2), 160.5 (2C, $C_{p,2}+C_{p,2}''$), 164.8 (C_4), 169.7 (C═O), 174.1 (C═O), and 209.3 (C═O). MS (ESI$^+$, *m*/*z*): 732 [(M + H)$^+$, 90%], 754 [(M + Na)$^+$, 40], 769 [(M + K)$^+$, 50]. Anal. Calcd (%) for $C_{42}H_{41}N_3O_9$: C, 68.93; H, 5.65; N, 5.74. Found: C, 69.0; H, 5.4; N, 5.7.

***N*^{6}-Benzoyl-3′-*O*-dimethoxytrityl-5′-*O*-levulinyl-2′-deoxyadenosine (7c):** mp 76−77 °C. R_f (EtOAc): 0.59. $[\alpha]^{20}_D$ = −11.3 (*c* 1.1, $CHCl_3$). IR (KBr): υ 3408, 3058, 2935, 2837, 1739, 1716, 1609, 1581, 1509, 1251 cm^{-1}. ^{1}H NMR (MeOH-d_4, 300 MHz): δ 2.25 (s, 3H, Me-Lev), 2.43 (m, 1H, H_2'), 2.52 (t, 2H, CH_2-Lev, $^3J_{HH}$ 6.4 Hz), 2.65 (m, 1H, H_2'), 2.80 (t, 2H, CH_2-Lev, $^3J_{HH}$ 6.4 Hz), 4.06 (2 s, 6H, OMe), 4.09−4.20 (m, 2H, H_5'), 4.30 (m, 1H, H_4'), 4.71 (m, 1H, H_3'), 6.69 (apparent t, 1H, H_1', $^3J_{HH}$ 6.4 Hz), 7.05 (apparent d, 4H, $H_{m,2}+H_{m,2}''$, $^3J_{HH}$ 8.8 Hz), 7.71−7.56 (m, 12H, H_m, H_P, $H_{o,2}+H_{o,2}''$, $H_{m,3}$, $H_{o,3}$, $H_{p,3}$), 8.24 (d, 2H, H_o, $^3J_{HH}$ 7.4 Hz), 8.58 (s, 1H, H_2+H_8), and 8.83 (s, 1H, H_8+H_2). ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 28.8 (CH_2-Lev), 29.7 (CH_3-Lev), 38.6 (CH_2-Lev), 39.9 (C_2'), 55.9 (2C, O-CH_3), 65.1 (C_5'), 75.5 (C_3'), 85.5, 86.6 ($C_4'+C_1'$), 88.8 (C_t), 114.5 (4C, $C_{m,2}+C_{m,2}''$), 125.4 (C_5), 128.2−133.9 ($C_o+C_m+C_{o,2}+C_{o,2}''$+-

$C_{o,3}$+$C_{m,3}$+$C_{p,3}$), 134.0 (C_p), 135.0 (C_i), 137.4, 137.5 (2C, $C_{i,2}$+$C_{i,2}''$), 144.2 (C_8), 146.8 ($C_{i,3}$), 151.1 (C_6), 153.0, 153.2 (C_2+C_4), 160.5 (2C, $C_{p,2}$+$C_{p,2}''$), 168.4 (C═O), 174.1 (C═O), and 209.2 (C═O). MS (ESI$^+$, *m*/*z*): 756 [(M + H)$^+$, 100%], 778 [(M + Na)$^+$, 80]. Anal. Calcd (%) for $C_{43}H_{41}N_5O_8$: C, 68.33; H, 5.47; N, 9.27. Found: C, 68.2; H, 5.7; N, 9.3.

3′-*O*-Dimethoxytrityl-*N*^{2}-isobutyryl-5′-*O*-levulinyl-2′-deoxyguanosine (7d): mp 116−117 °C. R_f (CH_2Cl_2/MeOH 4%): 0.23. $[\alpha]^{20}_D = -21.0$ (*c* 1.1, $CHCl_3$). IR (KBr): υ 3189, 2935, 2047, 1715, 1607, 1557, 1252 cm^{-1}. ^{1}H NMR (MeOH-d_4, 300 MHz): δ 1.39 (dd, 6H, Me-Ibu, $^3J_{HH}$ 6.8 Hz, $_{|}{}^4J_{HH|}$ 1.1 Hz), 2.26−2.28 (m, 4H, Me-Lev+H_2'), 2.47 (m, 1H, H_2'), 2.56 (t, 2H, CH_2-Lev), 2.84−2−89 (m, 3H, CH_2-Lev+CH-Ibu), 3.96 (2 s, 6H, OMe), 4.13−4.20 (m, 3H, H_4'+H_5'), 4.56 (m, 1H, H_3'), 6.52 (dd, 1H, H_1', $^3J_{HH}$ 8.0, 5.7 Hz), 7.05 (apparent d, 4H, $H_{m,2}$+$H_{m,2}''$, $^3J_{HH}$ 8.8 Hz), 7.30−7.55 (m, 9H, $H_{o,1}$, $H_{m,1}$, $H_{p,1}$, $H_{o,2}$+$H_{o,2}''$, $H_{p,2}$), and 8.20 (s, 1H, H_8). ^{13}C NMR (MeOH-d_4, 75.5 MHz): δ 19.3, 19.4 (2CH_3-Ibu), 28.7 (CH_2-Lev), 29.6 (CH_3-Lev), 37.0 (CH-Ibu), 38.5, 40.2 (C_2'+CH_2-Lev), 55.8 (2C, O-CH_3), 65.1 (C_5'), 75.6 (C_3'), 85.3 (C_4'), 86.0 (C_1'), 88.8 (C_t), 114.4 (4C, $C_{m,2}$+$C_{m,2}''$), 121.0 (C_5), 128.1−131.5 ($C_{o,2}$+$C_{o,2}''$+$C_{m,3}$+$C_{o,3}$+$C_{p,3}$), 137.3 (2C, $C_{i,2}$+$C_{i,2}''$), 139.0 (C_8), 146.7 ($C_{i,3}$), 149.7 (C_2+C_4), 157.4 (C_6), 160.5 (2C, $C_{p,2}$+$C_{p,2}''$), 174.1 (C═O), 181.8 (Ibu C═O), and 209.2 (C═O). MS (ESI$^+$, *m*/*z*): 738 [(M + H)$^+$, 40%], 760 [(M + Na)$^+$, 100], 776 [(M + K)$^+$, 20]. Anal. Calcd (%) for $C_{40}H_{43}N_5O_9$: C, 65.12; H, 5.87; N, 9.49. Found: C, 65.0; H, 5.8; N, 9.5.

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Supporting Information Available

The level of purity is indicated by the inclusion of copies of ^{1}H and ^{13}C NMR spectra for the new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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