Building Blocks for the Solution Phase Synthesis of Oligonucleotides: Regioselective Hydrolysis of 3′,5′-Di-*O*-levulinylnucleosides Using an Enzymatic Approach

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A short and convenient synthesis of 3′- and 5′-*O*-levulinyl-2′-deoxynucleosides has been developed from the corresponding 3′,5′-di-*O*-levulinyl derivatives by regioselective enzymatic hydrolysis, avoiding several tedious chemical protection/deprotection steps. Thus, *Candida antartica* lipase B (CAL-B) was found to selectively hydrolyze the 5′-levulinate esters, furnishing 3′-*O*-levulinyl-2′-deoxynucleosides **3** in >80% isolated yields. On the other hand, immobilized *Pseudomonas cepacia* lipase (PSL-C) and *Candida antarctica* lipase A (CAL-A) exhibit the opposite selectivity toward the hydrolysis at the 3′-position, affording 5′-*O*-levulinyl derivatives **4** in >70% yields. A similar hydrolysis procedure was successfully extended to the synthesis of 3′- and 5′-*O*-levulinyl-protected 2′-*O*-alkylribonucleosides **7** and **8**. This work demonstrates for the first time application of commercial CAL-B and PSL-C toward regioselective hydrolysis of levulinyl esters with excellent selectivity and yields. It is noteworthy that protected cytidine and adenosine base derivatives were not adequate substrates for the enzymatic hydrolysis with CAL-B, whereas PSL-C was able to accommodate protected bases during selective hydrolysis. In addition, we report an improved synthesis of dilevulinyl esters using a polymer-bound carbodiimide as a replacement for dicyclohexylcarbodiimide (DCC), thus considerably simplifying the workup for esterification reactions.

Introduction

The potential of antisense oligonucleotides for the treatment of a variety of diseases, through sequence-specific modulation of gene expression, has been well recognized.[1] Chemical modifications in oligonucleotides[2] have resulted in enhanced nuclease resistance, cellular uptake, and appropriate hybridization to mRNAs or target genes. Three principal methods—the phosphotriester,[3] phosphoramidite,[4] and *H*-phosphonate[5]—have been used for the synthesis of oligonucleotides. The phosphotriester approach has been used widely for solution-phase synthesis, whereas the phosphoramidite and *H*-phosphonate strategies have found application mainly in solid-phase synthesis. Recently, Reese and Song reported[6] a new approach for the synthesis of oligonucleotides via a solution-phase *H*-phosphonate coupling method.

With the recent success of various antisense oligonucleotides undergoing human clinical trials and the possibility of their commercial launch, soon very large quantities of therapeutically useful oligonucleotides may be required. Thus, development of methods for their large-scale synthesis has become a matter of urgency.[7] Particularly when multikilogram quantities of oligonucleotides are required, solution-phase synthesis appears to be an alternative method of choice instead of traditional solid-phase synthesis. The key building blocks for the solution-phase oligonucleotide synthesis are 3′- and/or 5′-protected nucleosidic monomers.

Among the limited protecting groups available, the levulinyl group is frequently chosen to protect the 3′- and/or 5′-hydroxyl of the nucleosides for solution-phase synthesis. This group is stable to coupling conditions and can be selectively cleaved, without affect other protecting groups in the molecule such as dimethoxytrityl (DMTr), acyl protections on exocyclic amine base, and internucleotide phosphate protecting groups,[8] with hydrazine hydrate in pyridine−acetic acid.[9] Until recently, the preparation of these building blocks has been carried out through several tedious chemical protection/deprotection

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(1) (a) Myers. K. J.; Dean, N. M. *Trends Pharmacol. Sci.* **2000**, *21*, 19−23. (b) Cook, P. D. *Nucleosides Nucleotides* **1999**, *18*, 1141−1162. (c) Crooke, S. T.; Bennett, C. F. *Annu. Rev. Pharmacol. Toxicol.* **1996**, *36*, 107−129. (d) Matteucci, M. D.; Wagner, R. W. *Nature* **1996**, *384*, 20−22.

(2) (a) Sanghvi, Y. S. In *Comprehensive Natural Products Chemistry*; Kool, E. T., Ed.; Elsevier: Amsterdam, 1999; Vol. 7, Chapter 7, 285−311 and references therein. (b) Matteucci, M. D. In *Perspective in Drug Discovery and Design*; Trainer, G., Ed.; ESCOM: Leiden, 1996; Vol. 4, 1−16.

(3) Reese, C. B. *Tetrahedron* **1978**, *34*, 3143−3179.

(4) Beaucage, S. L. In *Methods in Molecular Biology: Protocols for Oligonucleotides and Analogues*; Agrawal, S., Ed.; Humana Press: Totowa, 1993; Vol. 20, 33−61.

(5) Froehler, B. C. In *Methods in Molecular Biology: Protocols for Oligonucleotides and Analogues*; Agrawal, S., Ed.; Humana Press: Totowa, 1993; Vol. 20, 63−80.

(6) (a) Reese, C. B.; Song, Q. *Nucleic Acids Res.* **1999**, *27*, 963−971. (b) Reese, C. B.; Song, Q. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2787−2792.

(7) Pon, R. T.; Yu, S.; Guo, Z.; Sanghvi, Y. S. *Nucleic Acids Res.* **1999**, *27*, 1531−1538.

(8) (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.

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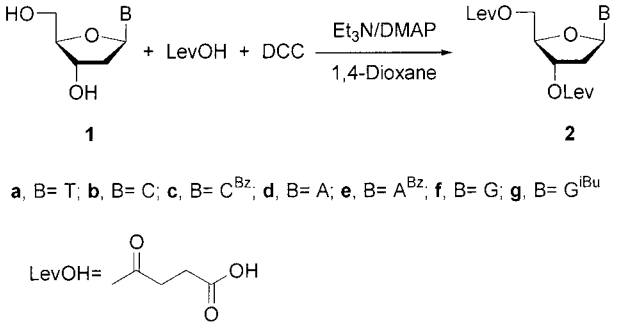
steps. The strategy for the synthesis of 3′-*O*-levulinylnucleosides (2′-deoxy or 2′-protected) involves the protection of the 5′-hydroxy group first as a 5′-*O*-DMTr derivative. Subsequent treatment with levulinic acid or levulinic anhydride and DCC followed by the removal of the 5′-*O*-DMTr group under acidic media affords the 3′-protected nucleosides.[6a,10] To obtain the 5′-*O*-levulinyl derivatives, parent nucleosides are treated with levulinic acid and 2-chloro-1-methylpyridinium iodide.[8c,11] In addition to the 5′-*O*-levulinylnucleoside, 3′-acyl and 3′,5′-diacyl derivatives are formed during this reaction. After separation of the 3′,5′-diacylated products by column chromatography, the residue was treated with DMTrCl to remove the 3′-acyl compound and purified again by column chromatography, furnishing the 5′-*O*-levulinyl derivatives in low yields.

Clearly, the current methods for the synthesis of 3′- and/or 5′- levulinyl-protected nucleosides are not useful for large-scale production of these key building blocks. Selective protection/deprotection of compounds containing multiple hydroxyl groups such as nucleosides is a challenging problem in organic synthesis. For the manipulation of protecting groups, application of biocatalysts in organic synthesis has become an attractive alternative to the conventional chemical methods.[12] Enzymes have been reported to catalyze reactions with high chemo-, regio-, and stereoselectivity.[13] Furthermore, enzyme-catalyzed reactions are less hazardous, polluting, and energy-consuming than the conventional chemistry-based methods. Among various commercial enzymes, we have had good success with the use of lipases as versatile biocatalysts in organic synthesis. Lipases are attractive because they are readily available, do not require cofactors, are inexpensive and highly stable, exhibit broad substrate specificity, and are well suited to retain a high degree of activity in organic media. Herein we report for the first time hydrolytic applications of commercial lipases resulting in a short and convenient chemoenzymatic procedure for the synthesis of both 3′-*O*-levulinyl- and 5′-*O*-levulinylnucleosides, from 2′-deoxy and 2′-*O*-alkylnucleosides.

Results and Discussion

3′,5′-Di-*O*-levulinyl-2′-deoxynucleosides (**2**) were prepared from the corresponding 2′-deoxynucleosides (**1**) by treatment with 5.2 equiv of levulinic acid (LevOH) and DCC in the presence of DMAP as catalyst (Scheme 1). The reaction takes place through activation of the LevOH with DCC via an *O*-acylurea intermediate. The excess of reactive intermediate was then transformed into more stable *N*-acylurea, which was subsequently isolated with

Scheme 1



DCU as a byproduct in the process. The structure of *N*-acylurea was confirmed by NMR studies. 3′,5′-Dilevulinyl derivatives **2** were obtained in high yields (70−95%) after flash chromatography column. During large-scale synthesis the chromatographic step could be avoided by washing the crude product first with Et_2O, which removes most of the *N*-acylurea, and a second wash with EtOAc, which dissolves the desired product. The byproduct DCU was separated by filtration of the EtOAc layer. Following the modified workup procedure, almost quantitative yields were achieved for all nucleosides. The purity for 3′,5′-dilevulinyl derivatives **2** made by this procedure was judged by ^{1}H NMR, which showed trace amounts of DCU and *N*-levulinylurea. Despite the presence of trace levels of byproducts, these nucleosides were found to be good substrates for the subsequent enzymatic hydrolysis step. It is noteworthy that under these acylation conditions the exocyclic amino groups of 2′-deoxyadenosine (**1d**) and 2′-deoxyguanosine (**1f**) were not *N*-acylated. However, the amino group of 2′-deoxycytidine (**1b**) was *N*-acylated. The *N*-acylation of the cytosine residue is not unusual, because of greater nucleophilicity and basic character (pK_a 4.25) compared to the amino groups in **1d** and **1f**. The undesirable *N*-acylation of **1b** was minimized by using fewer equivalents (3 vs 5.2) of LevOH and DCC. As a consequence, product formation was slower and some starting material was found to be unreacted. Despite of incomplete reaction, 68% isolated yield of 3′,5′-di-*O*-levulinyl-2′-deoxycytidine (**2b**) was obtained after flash chromatography.

Recently, Keck[14] described a macrolactonization procedure using a polymer-bound carbodiimide (PS-carbodiimide) as a replacement for DCC. Moreover, to prolong the lifetime of the activated acyl intermediate and suppress the formation of *N*-acylurea byproduct, DMAP hydrochloride was used as an additive.

With the aim to simplify the workup in the diacylation reactions, we carried out the reaction with PS-carbodiimide, instead of DCC, and used DMAP·HCl (Scheme 2), as recommended by Keck. Gratifyingly, the workup of these reactions was greatly simplified, since filtering off the polystyrene beads removes the urea and the *N*-levulinylurea derivatives, which are now polymer-bound. Using PS-carbodiimide, 3′,5′-di-*O*-levulinylthymidine (**2a**) and 3′,5′-di-*O*-levulinyl-2′-deoxyadenosine (**2d**) were prepared in 91% and 95% yield, respectively. Thus for industrial applications, use of PS-carbodiimide may completely avoid the handling of toxic DCC, and the

(9) (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.

(10) (a) Reese, C. B.; Song, Q. *J. Chem. Soc., Perkin Trans. 1* **1999**, 1477−1486. (b) Kumar, G.; Poonian, M. S. *J. Org. Chem.* **1984**, *49*, 4905−4912.

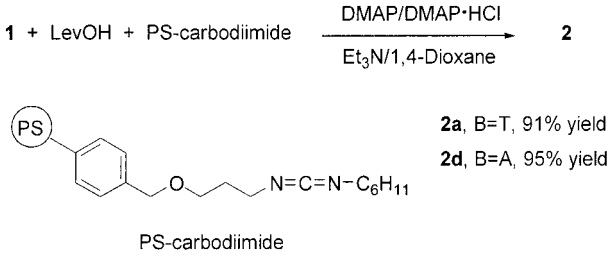
(11) Iwai, S.; Ohtsuka, E. *Nucleic Acids Res.* **1988**, *16*, 9443−9456.

(12) (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 and 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.

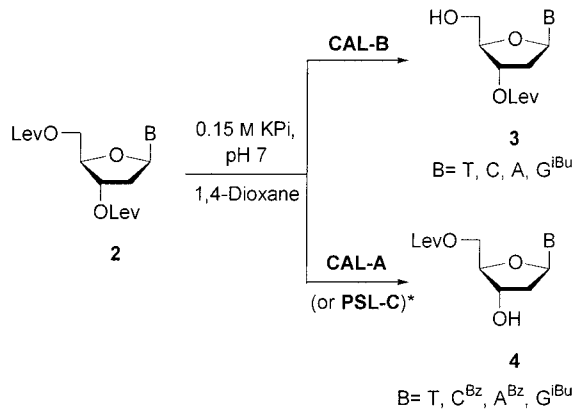
(13) For recent reviews on enzymatic transformations in nucleosides, see: (a) Ferrero, M.; Gotor, V. *Chem. Rev.* **2000**, *100*, 4319−4347. (b) Ferrero, M.; Gotor, V. *Monatsh. Chem.* **2000**, *131*, 585−616.

(14) Keck, G. E.; Sánchez, C.; Wager, C. A. *Tetrahedon Lett.* **2000**, *41*, 8673−8676.

Scheme 2



Scheme 3



*PSL-C was used for di-Lev-dG^{iBu} (**2g**) since **CAL-A** did not catalyze the hydrolysis.

expensive polymeric support could be recycled by an appropriate dehydration protocol.[15]

We previously reported[16] that *Candida antarctica* lipase B (CAL-B) catalyzes the acylation at the 5′-hydroxyl group of nucleosides with high selectivity, whereas *Pseudomonas cepacia* lipase (PSL) shows unusual regioselectivity toward the secondary alcohol at 3′-position of 2′-deoxynucleosides.

Taking into account the opposite preference showed for both lipases, diesters **2** were subjected to enzymatic hydrolysis (Scheme 3). When 3′,5′-di-*O*-levulinylthymidine (**2a**) was treated with CAL-B at 40 °C in 0.15 M phosphate buffer (pH 7) containing 18% of 1,4-dioxane, TLC showed after 62 h total disappearance of the starting material (entry 1, Table 1). After workup, ^{1}H NMR spectra clearly indicated the formation of 3′-*O*-levulinylthymidine (**3a**) as the only product, due to selective hydrolysis of the 5′-levulinate ester. HPLC analysis of the crude reaction mixture showed >95% purity of **3a** with traces of thymidine (**1a**) and unreacted **2a**. Crystalline **3a** was isolated in 85% yield without chromatography column. When the same process was carried out with substrates **2b**, **2d** and **2g**, excellent selectivity toward the 5′-position was observed (entries 2, 3, and 4, Table 1). It is noteworthy that the 5′-levulinyl group was completely and selectively hydrolyzed over the 3′-levulinyl group in high yields. In these three examples, HPLC showed traces of completely hydrolyzed nucleosides as the only side products. Again, all 3′-levulinyl compounds **3** were easily isolated as crystalline products.

(15) Weinshenker, N. M.; Shen, C. M.; Wong, J. Y. *Org. Synth., Coll. Vol. 6* **1988**, 951−954.

(16) (a) Moris, F.; Gotor, V. *J. Org. Chem.* **1993**, *58*, 653−660. (b) Gotor, V.; Moris, F. *Synthesis* **1992**, 626−628.

Table 1. Regioselective Enzymatic Hydrolysis of Dilevulinyl Esters 2[a]

entry	substrate	enzyme	T (°C)	*t* (h)	**1**	**2**	**3**[b]	**4**[b]
1	**2a**	CAL-B[c]	40	62	2	2	>95 (85)	
2	**2b**	CAL-B	30	62			100 (84)	
3	**2d**	CAL-B	40	28	1		99 (98)	
4	**2g**	CAL-B[d]	40	18	2		>95 (80)	
5	**2a**	PSL-C	40	82				100 (75)
6	**2a**	CAL-A	40	82				100 (71)
7	**2a**	PSL-C[e]	40	48				100 (70)
8	**2c**	CAL-A	40	100	3			97 (89)
9	**2e**	CAL-A	40	95	1			99 (84)
10	**2g**	PSL-C	40	96				>98 (80)
11	**2g**	PSL-C[e]	60	28				>98 (93)
12	**2a**	CAL-B[f]	40	24			100 (80)	
13	**2c**	CAL-A[f]	40	96	3			97 (98)
14	**2g**	CAL-B[g]	40	14	3		>97 (70)	
15	**2g**	CAL-B[g,h]	40	14	3		>97 (76)	

[a] Percentage of compounds calculated by HPLC after workup. [b] Isolated yields in percent are given in parentheses. [c] An extra fraction of lipase was added after 30 h. [d] Ratio of **2g**:CAL-B, 1:2 (w/w). [e] Ratio of **2**:PSL-C, 1:3 (w/w). [f] Reaction concentration is 0.25 M in 1 mmol scale. [g] 4.7 mmol scale. [h] Recycled CAL-B from entry 14.

Hydrolysis reaction catalyzed by CAL-B on *N*-benzoyl-di-*O*-levulinyl-2′-deoxycytidine (**2c**) and *N*-benzoyl-di-*O*-levulinyl-2′-deoxyadenosine (**2e**) afforded *N*-benzoyl-2′-deoxycytidine (**1c**) and *N*-benzoyl-2′-deoxyadenosine (**1e**), respectively. Although several reaction conditions were tried for hydrolysis, we could not achieve the desired regioselectivity with **2c** and **2e**. It seems that the active site of CAL-B was unable to accommodate the *N*-protected **2c** and **2e**, whereas hydrolysis of unprotected adenine and cytosine was acceptable. We postulate that the benzoyl protecting group may be sterically bulky and may interfere with the binding site of CAL-B, which led to unfavorable hydrolysis.

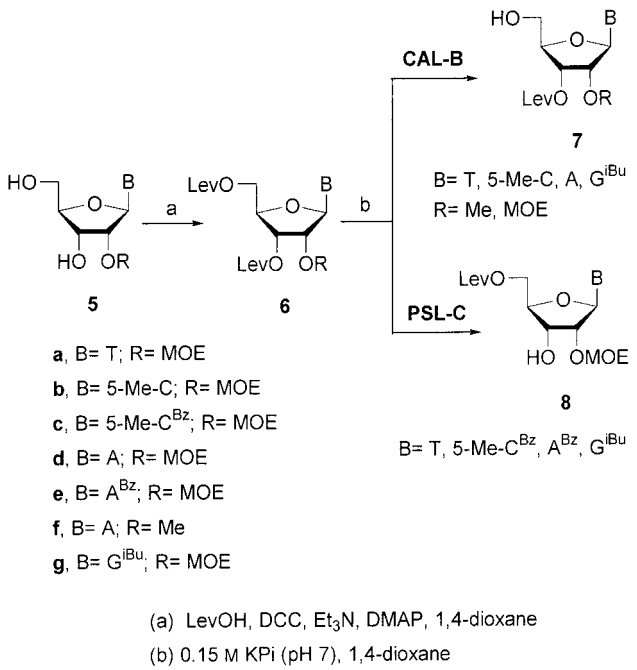
Gratifyingly, selective 3′-hydrolysis was accomplished by reaction of **2a** with immobilized *Pseudomonas cepacia* lipase (PSL-C) at 40 °C in 0.15 M phosphate buffer, furnishing 5′-*O*-levulinylthymidine (**4a**). *Candida antarctica* lipase A (CAL-A) also exhibited total selectivity toward the 3′-position (entries 5 and 6, Table 1). To shorten the reaction times of these processes, higher amounts of lipases can be used. This is not an inconvenience, since immobilized lipases can be reused. Thus, entry 7 in Table 1 indicates exclusively the formation of **4a** in 48 h with 70% isolated yield and indicated high purity by HPLC analysis. Similarly, CAL-A regioselectively hydrolyzed the 3′-*O*-levulinyl group of nucleosides **2c** and **2e**, furnishing *N*-benzoyl-5′-*O*-levulinyl-2′-deoxycytidine (**4c**) and *N*-benzoyl-5′-*O*-levulinyl-2′-deoxyadenosine (**4e**) in 89% and 84% yield, respectively (entries 8 and 9, Table 1). Importantly, no trace of the 3′-levulinyl derivative was detected by HPLC of the crude reaction mixture, except that a trace amount of parent nucleoside **1** was detected. Next, CAL-A-based enzymatic hydrolysis of *N*-isobutyryl-3′,5′-di-*O*-levulinyl-2′-deoxyguanosine (**2g**) led to the recovery of the starting material. Therefore, use of PSL-C after 96 h at 40 °C furnished *N*-isobutyryl-5′-*O*-levulinyl-2′-deoxyguanosine (**4g**) in 80% yield (entry 10, Table 1). The yield of **4g** was improved to 93% by employing a higher ratio of PSL-C (1:3, w/w). For efficient scale-up, the long reaction time was further reduced to 28 h by increasing the reaction temperature to 60 °C (entry 11, Table 1), without compromising the yield of **4g**.

All of the above lipase-mediated hydrolysis experiments were carried out at 0.1 M concentration, which is not ideal for scale-up in the industry. Therefore some of these reactions were repeated at a higher concentration (0.25 M) to evaluate tolerance of enzymes to high concentrations of substrate and product. For example, CAL-B selectively hydrolyzed the 5′-*O*-levulinyl group of **2a** (entry 12, Table 1) at 0.25 M concentration, furnishing **3a** (80% isolated yield) in 24 h. Similarly at higher concentration, CAL-A exhibited excellent selectivity toward hydrolysis of 3′-*O*-levulinyl group in **2c**, furnishing the 5′-*O*-levulinyl product **4c** in quantitative yield (entry 13, Table 1). The industrial productivity of lipase-mediated hydrolysis could be further increased via use of enzymes that are immobilized on a robust carrier. Gratifyingly, immobilized lipases are being used on commercial scale for the production of many drug intermediates on ton scale, and some are recycled to make the process more economical. For this reason, we explored the possibility of reusing enzyme in our applications. Preliminary results (entry 15, Table 1) indicate that CAL-B can be used more than once without loss of activity or product yield. Further studies are in progress to understand the reuse of immobilized lipases for our applications.

While the study with 2′-deoxynucleosides was in progress, we also became interested in the synthesis of 2′-*O*-alkylated nucleosides **7** and **8** using lipases. It is important to note that incorporation of 2′-*O*-Me- or 2′-*O*-$(CH_2)_2OCH_3$- (MOE) substituted nucleosides in an antisense oligonucleotide sequence provides enhanced stability toward nuclease digestion, higher binding affinity toward target mRNA, and improved potency relative to their 2′-deoxy predecessors in vivo.[17] Therefore, we decided to investigate whether 2′-*O*-alkylribonucleosides are suitable substrates for biocatalysis with CAL-B, CAL-A, and PSL-C. A series of 2′-*O*-Me and 2′-*O*-MOE derivatives (**6**) were subjected to enzymatic hydrolysis with lipases that worked well with 2′-deoxynucleosides.

The 3′,5′-di-*O*-levulinyl esters of 2′-*O*-alkylnucleosides (**6a**−**g**) were prepared according to the procedure previously described for 2′-deoxynucleosides (Scheme 4). As shown in Table 2, hydrolysis of **6a**,**b**,**d**,**f**, and **g** with CAL-B takes place with excellent selectivity toward the 5′-*O*-levulinyl group, isolating 3′-*O*-levulinyl-2′-*O*-alkylnucleosides **7a**,**b**,**d**,**f**, and **g** in high yields (entries 1, 2, 4, 6, and 7, Table 2). The crude product isolated from these reactions shows a high level of purity by HPLC analysis, and we found that no further purification was necessary for subsequent steps. As seen before with 2′-deoxynucleosides, if 5-methylcytosine and adenine bases were protected with a benzoyl group (**6c** and **6e**), CAL-B was unable to demonstrate the desired selectivity toward hydrolysis (entries 3 and 5, Table 2). Surprisingly, hydrolysis of 3′,5′-diLev-2′-*O*-MOE-T (**6a**) with CAL-A was slow and did not go to completion, even after 11 days. In this case, CAL-A also exhibited poor selectivity, giving rise to an undesirable product ratio (entry 8, Table 2). Similar results were obtained with adenosine analogues **6d** and **6f** (entries 12 and 15, Table 2) when CAL-A was employed for hydrolysis. Use of alternative enzyme

Scheme 4



(a) LevOH, DCC, Et_3N, DMAP, 1,4-dioxane
(b) 0.15 M KPi (pH 7), 1,4-dioxane

Table 2. Regioselective Enzymatic Hydrolysis of Dilevulinyl Esters 6

entry	substrate	enzyme	T (°C)	*t* (d)	5	6	7[c]	8[c]
1[a]	**6a**	CAL-B[d]	40	4	3		97 (84)	
2[a]	**6b**	CAL-B	40	6			>97 (70)	
3[b]	**6c**	CAL-B	40	1	41	35	24	
4[a]	**6d**	CAL-B	40	1.5	2		98 (84)	
5[b]	**6e**	CAL-B	40	2.5	49	1	50	
6[a]	**6f**	CAL-B	40	1.5			100 (97)	
7[b]	**6g**	CAL-B	40	1	2		98 (90)	
8[a]	**6a**	CAL-A	40	11	2	42	18	38
9[a]	**6a**	PSL-C[e]	40	7	3	5	6	86 (79)
10[a]	**6b**	PSL-C[e,f]	40	10	1	41		58
11[b]	**6c**	PSL-C[e]	40	1				>99 (81)
12[a]	**6d**	CAL-A[g]	40	14	8	44	27	21
13[a]	**6d**	PSL-C[h]	60	7	70	1	20	9
14[a]	**6d**	CVL	60	4	18	23	37	22
15[a]	**6f**	CAL-A[g]	40	7		48	21	31
16[a]	**6f**	PSL-C[g]	40	7		22	25	53
17[b]	**6e**	PSL-C[h]	50	3	1	2		97 (85)
18[b]	**6g**	PSL-C[e]	40	2	3			97 (85)

[a] Percentage of compounds calculated by HPLC after workup. [b] Percentage of compounds calculated by ^1H RMN. [c] Isolated yields in percent are given in parentheses. [d] An extra fraction of lipase was added after 2 d. [e] Ratio of **6**:PSL-C, 1:2 (w/w). [f] An extra fraction of lipase was added after 4 d. Then, 2 d later, the temperature was increased up to 60 °C. [g] An extra fraction of lipase was added after 5 d. [h] Ratio of **6**:PSL-C, 1:3 (w/w).

Chromobacterium viscosum lipase (CVL) (entry 14, Table 2) did not improve the outcome of hydrolysis.

Finally, selective synthesis of 5′-*O*-Lev-2′-*O*-MOE-T (**8a**) was successfully accomplished by using PSL-C in good yield (86%) (entry 9, Table 2). Application of PSL-C showed excellent selectivity toward the hydrolysis of 3′-levulinyl ester with *N*-protected 2′-*O*-MOE 5-methylcytidine and adenosine **6c** and **6e** (entries 11 and 17, Table 2). However, the selectivity of PSL-C was lost when hydrolysis of base unprotected nucleosides (**6b** and **6d**) or 2′-*O*-methyl (**6f**) was attempted (entries 10, 13, and 16, Table 2), whereas the selectivity of PSL-C was retained during hydrolysis of 3′,5′-diLev-2′-*O*-MOE-G^{iBu} (**6g**), furnishing **8g** in high yield (entry 18, Table 2).

(17) (a) Grotli, M.; Douglas, M.; Eritja, R.; Sproat, B. S. *Tetrahedron* **1998**, *54*, 5899−5914. (b) Altmann, K.-H.; Martin, P.; Dean, N. M.; Monia, B. P. *Nucleosides Nucleotides* **1997**, *16*, 917−926. (c) Martin, P. *Helv. Chim. Acta* **1995**, *78*, 486-504.

Conclusions

In summary, we have developed an efficient and regioselective method for synthesis of 3′- or 5′-*O*-levulinyl-protected nucleosides using a variety of lipases. As a result, these key building blocks needed for solution-phase synthesis of oligonucleotides are easily accessible in high yield and purity. Particularly, CAL-B was found to be selective in catalyzing the hydrolysis of 5′-*O*-levulinyl group in 3′,5′-dilevulinyl-2′-deoxynucleosides and 3′,5′-dilevulinyl-2′-*O*-alkylribonucleosides. In addition, regioselective hydrolysis of the 3′-*O*-levulinyl esters was accomplished with PSL-C in several examples. Similarly, CAL-A exhibited excellent selectivity toward the 3′-position in 2′-deoxynucleosides. Base-protected cytidine and adenosine derivatives were not appropriate substrates for the enzymatic hydrolysis with CAL-B, whereas PSL-C requires protected bases to perform the hydrolysis selectively. To our knowledge, this is the first example of the chemoenzymatic synthesis of appropriately protected levulinyl nucleosides, setting a path for large-scale production of these molecules. The results detailed herein for the synthesis of 3′-*O*-levulinyl protected 2′-deoxynucleosides **3** is a significant improvement over reported protocols, thus providing an easy access to selectively protected nucleosides for other applications. Additionally, an improved synthesis of dilevulinate esters has been developed using a polymer-bound carbodiimide, which simplifies the workup for these reactions. These enzymes are now available at a very reasonable cost and considered as industrial chemicals. The fact that these lipases are immobilized makes them more attractive reagents that can be used in a continuous mode for large-scale production. We are in process of scaling-up these reactions and making substantial amounts of levulinyl-protected nucleosides.

Experimental Section

General.[18] *Candida antarctica* lipase B (CAL-B, Novozym 435, 7300PLU/g) and *Chromobacterium viscosum* lipase (CVL, 3800 U/mg powder) were a gift. *Candida antarctica* lipase A (CAL-A, chirazyme L-5, c-f, lyo., 1000 U/g using tributyrin) and immobilized *Pseudomonas cepacia* lipase (PSL-C, 783 U/g) were purchased from suppliers. PS-carbodiimide and all other reagents were purchased from from commercial sources. Solvents were distilled over an adequate desiccant under nitrogen. High performance liquid chromatography analyses (HPLC) were carried out in a chromatograph with UV detection at 254 nm using a Spherisorb W 5 μm column (0.46 × 25 cm) and mixtures of MeOH/CH_2Cl_2 as eluent.

3′,5′-Di-*O*-levulinyl-2′-deoxynucleosides (2).

Method A. To a stirred mixture of **1** (2 mmol) and Et_3N (1.7 mL, 12 mmol) in 1,4-dioxane (20 mL) under nitrogen was added levulinic acid (1.21 g, 10.4 mmol), DCC (2.14 g, 10.4 mmol), and DMAP (20 mg, 0.16 mmol). The reaction was stirred at room temperature during 2 h. To minimize the formation of base-protected cytidine derivative, 6 mmol of LevOH and DCC and 5 mmol of Et_3N were used for **1b**. The insoluble material was collected by filtration and the filtrate was evaporated under vacuum. The residue was taken up in $NaHCO_3$ (aq) and extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and evaporated. Cold Et_2O was added, and the slurry was scratched until crystallization occurs. The solid was filtered, washed with cold Et_2O, and then was poured in EtOAc (MeOH in case of **2f**). The insoluble material was filtered and the filtrate was concentrated to afford the title compounds. The resulting materials were pure enough to be carried directly on to the enzymatic hydrolysis step. Further purification by flash chromatography (EtOAc) gives pure 3′,5′-di-*O*-levulinylnucleosides **2a**−**g**.

Method B. To a stirred mixture of **1** (0.4 mmol) and Et_3N (0.15 mL, 1 mmol) in 1,4-dioxane (5 mL) under nitrogen was added levulinic acid (0.14 g, 1.2 mmol), PS-carbodiimide (1.05 g, 1.2 mmol), DMAP (4 mg, 0.032 mmol), and DMAP·HCl (3 mg, 0.02 mmol). The reaction was stirred at room temperature during 3 h. The insoluble polymer was collected by filtration and the filtrate was evaporated under vacuum. The residue was taken up in $NaHCO_3$ (aq) and extracted with CH_2Cl_2. The combined organic extracts were dried over Na_2SO_4 and evaporated. The solid was washed with cold Et_2O to afford 3′,5′-di-*O*-levulinylnucleosides **2a** (91%) and **2d** (95%).

3′,5′-Di-*O*-levulinylthymidine (2a): >95% yield; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.88 (s, 3H), 2.14 (s, 3H), 2.15 (s, 3H), 2.18 (m, 1H), 2.41 (m, 1H), 2.54 (m, 4H), 2.73 (m, 4H), 4.19 (m, 1H), 4.31 (m, 2H), 5.18 (m, 1H), 6.28 (dd, 1H, $^3J_{HH}$ 8.5, $^3J_{HH}$ 5.4 Hz), 7.32 (s, 1H), and 9.99 (s, 1H); MS (ESI$^+$, *m*/*z*) 439 [(M + H)$^+$, 100] and 461 [(M + Na)$^+$, 50].

3′,5′-Di-*O*-levulinyl-2′-deoxycytidine (2b): 68% yield after flash chromatography; ^{1}H NMR (MeOH-d_4, 200 MHz) δ 2.39 (s, 3H), 2.40 (s, 3H), 2.43 (m, 1H), 2.75 (m, 1H), 2.79 (m, 4H), 3.02 (m, 4H), 4.51 (m, 3H), 5.46 (m, 1H), 6.17 (d, 1H, $^3J_{HH}$ 7.6 Hz), 6.45 (dd, 1H, $^3J_{HH}$ 8.6, $^3J_{HH}$ 5.7 Hz), and 7.98 (dd, 1H, $^3J_{HH}$ 7.3 Hz); MS (ESI$^+$, *m*/*z*) 446 [(M + Na)$^+$, 70] and 462 [(M + K)$^+$, 100].

***N*-Benzoyl-3′,5′-di-O-levulinyl-2′-deoxycytidine (2c):** >95% yield; ^{1}H NMR (MeOH-d_4, 200 MHz) δ 2.31 (s, 3H), 2.38 (s, 3H), 2.50 (m, 1H), 2.75 (m, 4H), 2.95 (m, 1H), 3.05 (m, 4H), 4.59 (m, 3H), 5.49 (m, 1H), 6.42 (dd, 1H, $^3J_{HH}$ 7.7, $^3J_{HH}$ 5.7 Hz), 7.75 (m, 4H), 8.15 (m, 2H), and 8.45 (d, 1H, $^3J_{HH}$ 7.6 Hz); MS (ESI$^+$, *m*/*z*) 528 [(M + H)$^+$, 100], 550 [(M + Na)$^+$, 30], and 566 [(M + K)$^+$, 40].

3′,5′-Di-*O*-levulinyl-2′-deoxyadenosine (2d): >95% yield; ^{1}H NMR (MeOH-d_4, 200 MHz) δ 2.33 (s, 3H), 2.39 (s, 3H), 2.79 (m, 5H), 3.00 (m, 4H), 3.25 (m, 1H), 4.52 (m, 3H), 5.65 (m, 1H), 6.61 (dd, 1H, $^3J_{HH}$ 6.0, $^3J_{HH}$ 7.9 Hz), 8.41 (s, 1H), and 8.50 (s, 1H); MS (ESI+, *m*/*z*) 448 [(M + H)$^+$, 20], 470 [(M + Na)$^+$, 80], and 486 [(M + K)$^+$, 100].

***N*-Benzoyl-3′,5′-di-*O*-levulinyl-2′-deoxyadenosine (2e):** >95% yield; ^{1}H NMR (MeOH-d_4, 300 MHz) δ 2.28 (s, 3H), 2.35 (s, 3H), 2.75 (m, 4H), 2.87 (m, 1H), 2.99 (m, 4H), 3.30 (m, 1H), 4.52 (m, 3H), 5.65 (m, 1H), 6.70 (apparent t, 1H, $^3J_{HH}$ 6.8 Hz), 7.75 (m, 3H), 8.25 (apparent d, 2H, $^3J_{HH}$ 7.4 Hz), 8.75 (s, 1H), and 8.88 (s, 1H); MS (ESI$^+$, *m*/*z*) 552 [(M + H)$^+$, 100] and 574 [(M + Na)$^+$, 17].

3′,5′-Di-*O*-levulinyl-2′-deoxyguanosine (2f): >95% yield; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 2.16 (s, 3H), 2.22 (s, 3H), 2.60 (m, 5H), 2.83 (m, 4H), 3.00 (m, 1H), 4.29 (m, 3H), 5.35 (m, 1H), 6.22 (dd, 1H, $^3J_{HH}$ 5.8, $^3J_{HH}$ 8.8 Hz), 6.69 (br s, 2H), and 8.00 (s, 1H); MS (ESI$^+$, *m*/*z*) 464 [(M + H)$^+$, 22], 486 [(M + Na)$^+$, 75], and 502 [(M + K)$^+$, 100].

***N*-Isobutyryl-3′,5′-di-O-levulinyl-2′-deoxyguanosine (2g):** >95% yield; ^{1}H NMR (DMSO-d_6, 200 MHz) δ 1.23 (d, 6H, $^3J_{HH}$ 6.5 Hz), 2.15 (s, 3H), 2.20 (s, 3H), 2.55−3.19 (several m, 11H), 4.32 (m, 3H), 5.35 (m, 1H), 6.35 (apparent t, 1H, $^3J_{HH}$ 7.2 Hz), 8.35 (s, 1H), 11.80 (br s, 1H), and 12.20 (br s, 1H); MS (ESI$^+$, *m*/*z*) 534 [(M + H)$^+$, 100], 556 [(M + Na)$^+$, 60], and 572 [(M + K)$^+$, 27].

General Procedure for the Enzymatic Hydrolysis of 3′,5′-Di-*O*-levulinyl-2′-deoxynucleosides 2. To a solution of **2** (0.2 mmol) in 1,4-dioxane (0.35 mL) was added 0.15 M phosphate buffer pH 7 (1.65 mL) and the corresponding lipase [ratio of **2**:enzyme was 1:1 (w/w)]. The mixture was allowed to react at 250 rpm for the time and at the temperature indicated in Table 1. The reactions were monitored by TLC (10% MeOH/CH_2Cl_2). The enzyme was filtered off and washed with CH_2Cl_2, the solvents were distilled under vacuum, and the residue

(18) For all products, full spectral data are given in the Supporting Information. ^{1}H NMR and MS spectral data are also presented in the Experimental Section. The purity of compounds was estimated by a combination of HPLC and NMR analysis. The level of purity is indicated by microanalysis and the inclusion of copies of ^{1}H NMR spectra and ^{13}C NMR spectra in the Supporting Information.

was taken up in $NaHCO_3$ (aq) and extracted with CH_2Cl_2. The combined organic layers were dried over Na_2SO_4 and evaporated to give monoacylated nucleosides **3** or **4**. In case of **3b**, the residue was purified by flash chromatography (gradient eluent 10−30% MeOH/EtOAc) instead of extraction.

3′-*O*-Levulinylthymidine (3a): this compound was previously reported;[10b] ^{1}H NMR (MeOH-d_4, 200 MHz) δ 2.09 (d, 3H, J_{HH} 1.3 Hz), 2.39 (s, 3H), 2.57 (m, 2H), 2.80 (t, 2H, $^3J_{HH}$ 6.0 Hz), 3.05 (t, 2H, $^3J_{HH}$ 6.2 Hz), 4.01 (m, 2H), 4.29 (m, 1H), 5.02 (m, 1H), 6.50 (dd, 1H, $^3J_{HH}$ 8.1, $^3J_{HH}$ 6.5 Hz), and 8.04 (d, 1H, $^3J_{HH}$ 1.3 Hz); MS (ESI$^+$, *m*/*z*) 363 [(M + Na)$^+$, 100] and 379 [(M + K)$^+$, 30].

3′-*O*-Levulinyl-2′-deoxycytidine (3b): ^{1}H NMR (MeOH-d_4, 200 MHz) δ 2.39 (s, 3H), 2.47 (m, 1H), 2.67 (m, 1H), 2.75 (m, 2H), 3.02 (m, 2H), 4.00 (m, 2H), 4.30 (m, 1H), 5.49 (m, 1H), 6.15 (d, 1H, $^3J_{HH}$ 6.8 Hz), 6.48 (apparent t, 1H, $^3J_{HH}$ 6.8 Hz), and 8.32 (d, 1H, $^3J_{HH}$ 7.3 Hz); MS (ESI$^+$, *m*/*z*) 326 [(M + H)$^+$, 10], 348 [(M + Na)$^+$, 50], and 364 [(M + K)$^+$, 12].

3′-*O*-Levulinyl-2′-deoxyadenosine (3d): ^{1}H NMR (MeOH-d_4, 200 MHz) δ 2.40 (s, 3H), 2.76 (m, 1H), 2.80 (t, 2H, $^3J_{HH}$ 6.2 Hz), 3.05 (t, 2H, $^3J_{HH}$ 6.2 Hz), 3.14 (m, 1H), 4.04 (m, 2H), 4.40 (m, 1H), 5.66 (d, 1H, $^3J_{HH}$ 6.0 Hz), 6.61(dd, 1H′, $^3J_{HH}$ 5.7, $^3J_{HH}$ 9.1 Hz), 8.39 (s, 1H), and 8.50 (s, 1H); MS (ESI$^+$, *m*/*z*) 350 [(M + H)$^+$, 100], 372 [(M + Na)$^+$, 100], and 388 [(M + K)$^+$, 60].

***N*-Isobutyryl-3′-*O*-levulinyl-2′-deoxyguanosine (3g):** this compound was previously reported;[8a] ^{1}H NMR (MeOH-d_4, 200 MHz) δ 1.41 (d, 6H, $^3J_{HH}$ 6.8 Hz), 2.38 (s, 3H), 2.70−3.09 (m, 7H), 3.98 (d, 2H′, $^3J_{HH}$ 3.6 Hz), 4.32 (m, 1H), 5.57 (m, 1H), 6.51 (dd, 1H, $^3J_{HH}$ 6.1, $^3J_{HH}$ 8.0 Hz), and 8.43 (s, 1H); MS (ESI$^+$, *m*/*z*) 436 [(M + H)$^+$, 15] and 458 [(M + Na)$^+$, 50].

5′-*O*-Levulinylthymidine (4a): ^{1}H NMR (DMSO-d_6, 200 MHz) δ 1.91 (s, 3H), 2.27 (s, 3H), 2.30 (m, 2H), 2.66 (m, 2H), 2.89 (t, 2H, $^3J_{HH}$ 6.2 Hz), 4.07 (m, 1H), 4.35 (m, 3H), 5.55 (d, 1H), 6.32 (t, 1H′, $^3J_{HH}$ 7.0 Hz), 7.6 (s, 1H), and 11.45 (s, 1H); MS (ESI$^+$, *m*/*z*) 341 [(M + H)$^+$, 40], 379 [(M + Na)$^+$, 100], and 379 [(M + K)$^+$, 80].

***N*-Benzoyl-5′-*O*-levulinyl-2′-deoxycytidine (4c):** ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.20 (s, 3H), 2.25 (m, 1H), 2.58 (m, 2H), 2.75 (m, 1H), 2.82 (m, 2H), 3.35 (s, 1H), 4.25 (m, 1H), 4.40 (m, 3H), 6.30 (apparent t, 1H′, $^3J_{HH}$ 6.2 Hz), 7.55 (m, 4H), 7.90 (apparent d, 2H, $^3J_{HH}$ 7.1 Hz), 8.20 (d, 1H, $^3J_{HH}$ 7.4 Hz), and 8.78 (s, 1H); MS (ESI$^+$, *m*/*z*) 430 [(M + H)$^+$, 20], 452 [(M + Na)$^+$, 65], and 468 [(M + K)$^+$, 40].

***N*-Benzoyl-5′-*O*-levulinyl-2′-deoxyadenosine (4e):** ^{1}H NMR (DMSO-d_6, 300 MHz) δ 2.06 (s, 3H), 2.45 (m, 3H), 2.66 (m, 2H), 2.90 (m, 1H), 4.03 (m, 1H), 4.20 (m, 2H), 4.51 (m, 1H), 5.55 (s, 1H), 6.50 (apparent t, 1H′, $^3J_{HH}$ 6.5 Hz), 7.58 (m, 3H), 8.03 (m, 2H), 8.69 (s, 1H), 8.74 (s, 1H), and 11.20 (s, 1H); MS (ESI$^+$, *m*/*z*) 476 [(M + Na)$^+$, 100] and 492 [(M + K)$^+$, 53].

***N*-Isobutyryl-3′-*O*-levulinyl-2′-deoxyguanosine (4g):** ^{1}H NMR (MeOH-d_4, 300 MHz) δ 1.42 (d, 6H, $^3J_{HH}$ 6.8 Hz), 2.32 (s, 3H), 2.59−3.07 (m, 7H), 4.32 (m, 1H), 4.50 (m, 2H), 4.75 (m, 1H), 6.30 (apparent t, 1H′, $^3J_{HH}$ 6.5 Hz), and 8.33 (s, 1H); MS (ESI$^+$, *m*/*z*) 436 [(M + H)$^+$, 20], 458 [(M + Na)$^+$, 100], and 474 [(M + K)$^+$, 50].

3′,5′-Di-*O*-levulinyl-2′-protected Ribonucleosides (6). The same procedure as that described in Method A was followed for **2**. The crude products were purified by flash chromatography (gradient eluent EtOAc−20%MeOH/EtOAc for **6a**, **6d**, **6f**, **6g**; gradient eluent 5−20% MeOH/CH_2Cl_2 for **6b**; gradient eluent 80−90% EtOAc/Hex for **6c**) to give 3′,5′-di-*O*-levulinylnucleosides **6a**−**g**.

3′,5′-Di-*O*-levulinyl-2′-*O*-methoxyethyl-5-methyluridine (6a): 95% yield; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.82 (s, 3H), 2.09 (s, 6H), 2.55 (m, 4H), 2.68 (m, 4H), 3.18 (s, 3H), 3.38 (m, 2H), 3.60 (m, 2H), 4.25 (m, 4H), 4.99 (m, 1H), 5.85 (d, 1H, $^3J_{HH}$ 4.8 Hz), 7.24 (s, 1H), and 10.04 (s, 1H); MS (ESI$^+$, *m*/*z*) 513 [(M + H)$^+$, 5] and 535 [(M + Na)$^+$, 100].

3′,5′-Di-*O*-levulinyl-2′-*O*-(2-methoxyethyl)-5-methylcytidine (6b): 57% yield; ^{1}H NMR (MeOH-d_4, 200 MHz) δ 2.18 (s, 3H), 2.35 (s, 3H), 2.36 (s, 3H), 2.81 (m, 4H), 3.02 (m, 4H), 3.46 (s, 3H), 3.66 (m, 2H), 3.88 (m, 2H), 4.52 (m, 4H), 5.33 (apparent t, 1H, $^3J_{HH}$ 5.0 Hz), 6.17 (d, 1H, $^3J_{HH}$ 4.9 Hz), and 7.75 (s, 1H); MS (ESI$^+$, *m*/*z*) 512 [(M + H)$^+$, 95], 534 [(M + Na)$^+$, 100], and 550 [(M + K)$^+$, 40].

***N*-Benzoyl-3′,5′-di-*O*-levulinyl-2′-*O*-(2-methoxyethyl)-5-methylcytidine (6c):** 88% yield; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.99 (s, 3H), 2.06 (s, 3H), 2.54 (m, 4H), 2.66 (m, 4H), 3.17 (s, 3H), 3.36 (m, 2H), 3.62 (m, 2H), 4.28 (m, 4H), 4.98 (m, 1H), 5.86 (d, 1H, $^3J_{HH}$ 4.4 Hz), 7.36 (m, 4H), and 8.17 (apparent d, 2H, $^3J_{HH}$ 6.9 Hz); MS (ESI$^+$, *m*/*z*) 616 [(M + H)$^+$, 80], 638 [(M + Na)$^+$, 80], and 654 [(M + K)$^+$, 100].

3′,5′-di-*O*-Levulinyl-2′-*O*-(2-methoxyethyl)adenosine (6d): 94% yield; ^{1}H NMR (MeOH-d_4, 300 MHz) δ 2.33 (s, 3H), 2.38 (s, 3H), 2.82 (m, 4H), 3.00 (m, 4H), 3.32 (s, 3H), 3.56 (m, 2H), 3.83 (m, 2H), 4.57 (m, 3H), 5.16 (apparent t, 1H, $^3J_{HH}$ 5.9 Hz), 5.68 (dd, 1H, $^3J_{HH}$ 2.9, $^3J_{HH}$ 5.2 Hz), 6.27 (d, 1H, $^3J_{HH}$ 6.5 Hz), 8.41 (s, 1H), and 8.42 (s, 1H); MS (ESI$^+$, *m*/*z*) 522 [(M + H)$^+$, 20] and 534 [(M + Na)$^+$, 100].

***N*-Benzoyl-3′,5′-di-*O*-levulinyl-2′-*O*-(2-methoxyethyl)-adenosine (6e):** 89% yield; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.14 (s, 3H), 2.17 (s, 3H), 2.58−2.76 (m, 8H), 3.15 (s, 3H), 3.38 (m, 2H), 3.66 (m, 2H), 4.40 (m, 3H), 4.93 (apparent t, 1H, $^3J_{HH}$ 5.5 Hz), 5.40 (apparent t, 1H, $^3J_{HH}$ 4.9 Hz), 6.13 (d, 1H, $^3J_{HH}$ 5.4 Hz), 7.47 (m, 3H), 8.01 (m, 2H), 8.28 (s, 1H), 8.75 (s, 1H), and 9.54 (s, 1H); MS (ESI$^+$, *m*/*z*) 626 [(M + H)$^+$, 45], 648 [(M + Na)$^+$, 100], and 664 [(M + K)$^+$, 80].

3′,5′-Di-*O*-Levulinyl-2′-*O*-methyladenosine (6f): 92% yield; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.17 (s, 3H), 2.18 (s, 3H), 2.64 (m, 4H), 2.76 (m, 4H), 3.38 (s, 3H), 4.40 (m, 3H), 4.69 (apparent t, 1H, $^3J_{HH}$ 5.4 Hz), 5.40 (t, 1H, $^3J_{HH}$ 4.3 Hz), 6.05 (d, 1H, $^3J_{HH}$ 5.6 Hz), 6.25 (br s, 2H), 8.05 (s, 1H), and 8.31 (s, 1H); MS (ESI$^+$, *m*/*z*) 478 [(M + H)$^+$, 20] and 500 [(M + Na)$^+$, 100].

***N*-Isobutyryl-3′,5′-di-*O*-levulinyl-2′-*O*-(2-methoxyethyl)-guanosine (6g):** 92% yield; ^{1}H NMR (MeOH-d_4, 300 MHz) δ 1.41 (d, 6H, $^3J_{HH}$ 6.8 Hz), 2.32 (s, 3H), 2.38 (s, 3H), 2.75−3.05 (several m, 9H), 3.37 (s, 3H), 3.58 (m, 2H), 3.81 (m, 2H), 4.57 (m, 3H), 4.98 (apparent t, 1H′, $^3J_{HH}$ 5.7 Hz) 5.59 (m, 1H), 6.20 (d, 1H′, $^3J_{HH}$ 6.3 Hz), and 8.36 (s, 1H); MS (ESI$^+$, *m*/*z*) 608 [(M + H)$^+$, 80], 630 [(M + Na)$^+$, 65], and 646 [(M + K)$^+$, 100].

General Procedure for the Enzymatic Hydrolysis of 3′,5′-Di-*O*-levulinyl-2′-protected Ribonucleosides 6. A similar procedure as that described for **2** was followed. Reaction time and temperature are indicated in Table 2. In the case of **8e**, the residue was purified by flash chromatography (gradient eluent 5−10% ^{i}PrOH/CH_2Cl_2) after extraction.

3′-*O*-Levulinyl-2′-*O*-methoxyethyl-5-methyluridine (7a): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.85 (s, 3H), 2.16 (s, 3H), 2.61 (m, 2H), 2.74 (m, 2H), 3.24 (s, 3H), 3.42 (m, 2H), 3.64 (m, 2H), 3.79 (m, 2H), 4.16 (m, 1H), 4.41 (apparent t, 1H, $^3J_{HH}$ 5.4 Hz), 5.28 (apparent t, 1H, $^3J_{HH}$ 4.6 Hz), 5.70 (d, 1H, $^3J_{HH}$ 5.6 Hz), and 7.48 (s, 1H); MS (ESI$^+$, *m*/*z*) 415 [(M + H)$^+$, 20] and 437 [(M + Na)$^+$, 100].

3′-*O*-Levulinyl-2′-*O*-(2-methoxyethyl)-5-methylcytidine (7b): ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.92 (s, 3H), 2.20 (s, 3H), 2.66 (m, 2H), 2.77 (m, 2H), 3.28 (s, 3H), 3.44 (m, 2H), 3.70 (m, 3H), 3.92 (m, 1H), 4.23 (m, 1H), 4.72 (apparent t, 1H, $^3J_{HH}$ 5.4 Hz), 5.38 (apparent t, 1H, $^3J_{HH}$ 4.6 Hz), 5.48 (d, 1H, $^3J_{HH}$ 5.7 Hz), and 7.41 (s, 1H); MS (ESI$^+$, *m*/*z*) 414 [(M + H)$^+$, 55] and 436 [(M + Na)$^+$, 100].

3′-*O*-Levulinyl-2′-*O*-(2-methoxyethyl)adenosine (7d): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.18 (s, 3H), 2.71 (m, 2H), 2.78 (m, 2H), 3.06 (s, 3H), 3.24 (m, 2H), 3.44 (m, 1H), 3.56 (m, 1H), 3.76 (m, 1H), 3.90 (m, 1H), 4.32 (s, 1H), 4.89 (dd, 1H, $^3J_{HH}$ 5.1, $^3J_{HH}$ 7.9 Hz), 5.62 (d, 1H, $^3J_{HH}$ 4.8 Hz), 5.85 (d, 1H, $^3J_{HH}$ 7.9 Hz), 6.51 (br s, 2H), 6.90 (br s, 1H), 7.86 (s, 1H), and 8.27 (s, 1H); MS (ESI$^+$, *m*/*z*) 424 [(M + H)$^+$, 80] and 446 [(M + Na)$^+$, 100].

3′-O-Levulinyl-2′-*O*-methyladenosine (7f): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.20 (s, 3H), 2.69 (m, 2H), 2.80 (m, 2H), 3.25 (s, 3H), 3.86 (m, 2H), 4.32 (s, 1H), 4.75 (dd, 1H, $^3J_{HH}$ 7.9,$^3J_{HH}$ 4.8 Hz), 5.64 (d, 1H, $^3J_{HH}$ 5.1 Hz), 5.83 (d, 1H, $^3J_{HH}$ 7.9 Hz), 6.36 (s, 2H), 6.90 (br s, 1H), 7.86 (s, 1H), and 8.31 (s, 1H); MS (ESI$^+$, *m*/*z*) 380 [(M + H)$^+$, 100] and 402 [(M + Na)$^+$, 90].

***N*-Isobutyryl-3′-*O*-levulinyl-2′-*O*-(2-methoxyethyl)guanosine (7g):** ^{1}H NMR (MeOH-d_4, 75.5 MHz) δ 1.41 (d, 6H, $^3J_{HH}$ 6.8 Hz), 2.38 (s, 3H), 2.84 (m, 2H), 2.92 (m, 1H), 3.03 (m,

2H), 3.36 (s, 3H), 3.56 (m, 2H), 3.80 (m, 2H), 3.98 (m, 2H), 4.41 (apparent d, 1H, $^3J_{HH}$ 2.9 Hz), 4.88 (apparent t, 1H, $^3J_{HH}$ 5.8 Hz), 5.64 (m, 1H), 6.21 (d, 1H′, $^3J_{HH}$ 6.4 Hz), and 8.50 (s, 1H); MS (ESI$^+$, *m*/*z*) 510 [(M + H)$^+$, 10] and 532 [(M + Na)$^+$, 100].

5′-*O*-Levulinyl-2′-[(2-methoxyethyl)oxy]thymidine (8a): ^{1}H NMR ($CDCl_3$, 75.5 MHz) δ 1.91 (s, 3H), 2.18 (s, 3H), 2.59 (m, 2H), 2.80 (m, 2H), 3.10 (br s, 1H), 3.37 (s, 3H), 3.75 (m, 2H), 3.75 (m, 1H), 4.01 (m, 2H), 4.13 (m, 2H), 4.42 (m, 2H), 5.89 (d, 1H, $^3J_{HH}$ 3.4 Hz), 7.38 (s, 1H), and 9.40 (s, 1H); MS (ESI$^+$, *m*/*z*) 415 [(M + H)$^+$, 20] and 437 [(M + Na)$^+$, 100].

***N*-Benzoyl-5′-*O*-levulinyl-2′-O-(2-methoxyethyl)-5-methylcytidine (8c):** ^{1}H NMR ($CDCl_3$, 75.5 MHz) δ 2.11 (s, 3H), 2.19 (s, 3H), 2.62 (m, 2H), 2.81 (m, 2H), 3.37 (s, 3H), 3.54 (m, 2H), 3.78 (m, 2H), 4.11 (m, 3H), 4.43 (m, 2H), 5.90 (d, 1H, $^3J_{HH}$ 2.8 Hz), 7.42 (m, 3H), 7.57 (s, 1H), 8.28 (apparent d, 2H, $^3J_{HH}$ 6.8 Hz), and 13.3 (s, 1H); MS (ESI$^+$, *m*/*z*) 518 [(M + H)$^+$, 100], 540 [(M + Na)$^+$, 80], and 556 [(M + K)$^+$, 90].

***N*-Benzoyl-5′-*O*-levulinyl-2′-O-(2-methoxyethyl)adenosine (8e):** ^{1}H NMR ($CDCl_3$, 200 MHz) δ 2.14 (s, 3H), 2.17 (s, 3H), 2.58−2.76 (m, 8H), 3.15 (s, 3H), 3.38 (m, 2H), 3.66 (m, 2H), 4.40 (m, 3H), 4.93 (apparent t, 1H, $^3J_{HH}$ 5.5 Hz), 5.40 (apparent t, 1H, $^3J_{HH}$ 4.9 Hz), 6.13 (d, 1H, $^3J_{HH}$ 5.4 Hz), 7.47 (m, 3H), 8.01 (m, 2H), 8.28 (s, 1H), 8.75 (s, 1H), and 9.54 (s, 1H); MS (ESI$^+$, *m*/*z*) 550 [(M + Na)$^+$, 100] and 566 [(M + K)$^+$, 10].

***N*-Isobutyryl-5′-*O*-levulinyl-2′-*O*-(2-methoxyethyl)guanosine (8g):** ^{1}H NMR (MeOH-d_4, 75.5 MHz) δ 1.43 (d, 6H, $^3J_{HH}$ 6.8 Hz), 2.35 (s, 3H), 2.77 (m, 2H), 2.98 (m, 3H), 3.52 (s, 3H), 3.76 (m, 2H), 4.02 (m, 2H), 4.41 (m, 1H), 4.60 (m, 3H), 4.71 (m, 1H), 6.24 (d, 1H, $^3J_{HH}$ 4.5 Hz), and 8.40 (s, 1H); MS (ESI$^+$, *m*/*z*) 510 [(M + H)$^+$, 70], 532 [(M + Na)$^+$, 50], and 548 [(M + K)$^+$, 10].

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Supporting Information Available: Complete ^{1}H and ^{13}C NMR spectral data in addition of microanalysis, mp, IR, and MS data for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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