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Improved Synthesis and Isolation of 2′-*O*-Methyladenosine: Effective and Scalable Enzymatic Separation of 2′/3′-*O*-Methyladenosine Regioisomers

Saúl Martínez-Montero,[a] Susana Fernández,[a] Tatiana Rodríguez-Pérez,[a] Yogesh S. Sanghvi,[b] Ke Wen,[c] Vicente Gotor,*[a] and Miguel Ferrero*[a]

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An efficient separation of a mixture of 2′/3′-*O*-methyladenosine regioisomers (**1** + **2**; 1:1) has been developed by selective enzymatic acylation using immobilized *Pseudomonas cepacia* lipase (PSL-C) in combination with acetonoxime levulinate as acyl donor. The 3′-hydroxy group of 2′-*O*-methyladenosine (**1**) was acylated with high selectivity (ca. 70 %), whereas an equal amount of 3′-*O*-methyladenosine (**2**) in the same solution resulted in minor acylation of 5′-hydroxy group (ca. 8 %). The differential behavior of both regioisomers towards enzymatic acylation allowed to develop a separation protocol. Upon extraction of the acylated products, the 3′-*O*-methyladenosine was isolated in 81 % yield and 97 % purity from the aqueous layer. Hydrolysis of acylated products in organic layer furnished 2′-*O*-methyladenosine in 67 % yield and 99 % purity. The separation process was successfully applied to the crude reaction mixture of methylated products (ca. 3:1 of **1**/**2**) on 5-g scale. We also report on the use of methyl *p*-toluenesulfonate as a safe reagent for 2′-*O*-methylation of adenosine.

Introduction

In recent years, several oligonucleotide-based drugs have entered human clinical trials for the treatment of a variety of viral, infectious or cancer-related diseases.[1] Among these, 2′-*O*-methylribonucleotides have been extensively used as a second-generation chemistry to elicit high nuclease resistance, cellular uptake and improved binding affinity for the RNA target.[2] Additionally, methylribonucleotides have found applications in studying pre-mRNA splicing, examining the structures of spliceosomes and preparing nuclease-resistant hammerhead ribozymes.[3] Also, 2′-*O*-alkylribonucleosides are present in RNAs as minor components.[4]

The importance of methylribonucleotides, which constitutes a significant portion of the raw material cost in the preparation of oligonucleotides, has stimulated large effort towards the synthesis of 2′-*O*-methylated nucleosides as a key building blocks using several synthetic approaches. Although some of these strategies have been successfully implemented for the production of pyrimidine-containing 2′-*O*-methyl nucleosides, these protocols are much less efficient for purine analogues.[5] In the latter case, the problems derive from the inherent reactivity of the purine bases towards alkylation and the need for selective protection of the 3′- and/or 5′-hydroxy groups.

Since the late 1950s, several protocols for the synthesis 2′-*O*-methylribonucleosides have been reported. In 1965, Broom and Robins reported the first direct synthesis of 2′-*O*-methyladenosine (**1**) by methylation with diazomethane in DME (Figure 1).[6] Alternatively, the use of methyl iodide in combination with sodium hydride[7] or silver oxide[8] has also been explored on nucleobase-protected ribonucleosides. However, these approaches suffer from the toxicity of the methylating reagents and their incompatibility with large-scale applications. Moreover, the low yields of the product obtained under these conditions and the concomitant formation of the 3′-*O*-methyl isomer (**2**) further complicates purification.

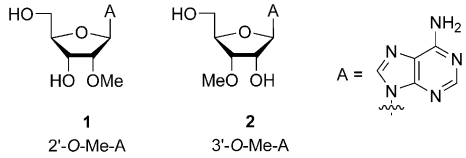

Figure 1. Structures of 2′-*O*-methyladenosine and 3′-*O*-methyladenosine.

Other procedures utilized metal catalysts (stannous chloride,[9] copper,[10] iron,[11] silver, and strontium acetylacetonates[5d]) to activate the *cis*-diol system in order to sup-

[a] Departamento de Química Orgánica e Inorgánica and Instituto Universitario de Biotecnología de Asturias, Universidad de Oviedo,
33006 Oviedo (Asturias), Spain
Fax: +34-98-510-3448
E-mail: vgs@uniovi.es
MFerrero@uniovi.es
[b] Rasayan Inc.,
2802 Crystal Ridge Road, Encinitas, CA 92024-6615, USA
[c] Chemistry Department, East China Normal University, Shanghai, China

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press methylation of the purine base. The acetylacetonates used herein are very expensive, thus hindering scale-up attempts. Alternative approaches toward the synthesis of 2′-*O*-alkylribonucleosides are based on a glycosylation reaction[12] that requires multistep synthesis of the carbohydrate precursors, where a mixture of α- and β-anomers was obtained. Advances in protecting group methodology using 3′,5′-di-*O*-(tetraisopropyldisiloxane-1,3-diyl) led to the synthesis of 2′-*O*-methyladenosine building block in seven steps from the 6-chloropurine nucleoside.[8b] Once again, the foregoing methodology required expensive reagents and complex scale-up processes.

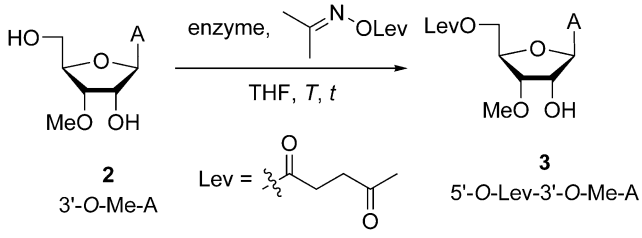
Although 3′-*O*-methyladenosine (**2**), the side product obtained in the direct alkylation protocols, has yet to find applications in oligonucleotide-based therapeutics, the 5′-triphosphates of 3′-*O*-methyladenosine and 3′-*O*-methylguanosine are chain terminators and are thus valuable tools for nucleic acid sequencing.[13] In addition, Sharma[14] reported that 3′-*O*-methyladenosine and to a lesser extent 3′-*O*-methylguanosine are potent inhibitors of vaccinia virus growth in L-cells and Vero cells. Early viral RNA synthesis was totally inhibited in 2.5 h post-infection adding 100 μM of 3′-*O*-methyladenosine to infected cells.

The increasing demand for **1** as a building block for oligonucleotide-based therapeutics and the interesting profile for **2** motivated us to develop an approach where both products could be harvested from a single process that is environmentally safe and scalable. Herein, we describe for the first time an economical methodology enabling the synthesis and efficient separation of the isomeric mixture of **1** and **2** formed during the direct methylation of inexpensive adenosine.

Results and Discussion

For the separation of isomeric and racemic mixtures, biocatalysts have become an attractive and powerful tool in organic synthesis due to their high selectivity, mild reaction conditions and low environmental impact. In our research group, we have developed methodologies for the separation of anomeric and racemic mixtures of nucleosides using lipases in organic solvents. For example, a convenient separation of α/β-mixtures of thymidine nucleosides from an industrial waste stream, an anomeric mixture of 2′-deoxy-2′-fluoro-α/β-arabinonucleosides[15] and a parallel kinetic resolution of D/L-nucleosides[16] have been accomplished. One common theme in these examples is the selection of levulinyl chemistry[17] due to: a) applications for orthogonal protection during the solution-phase synthesis of oligonucleotides; b) prior experience with the lipase-mediated levulinylation of nucleosides; c) inherently small, atom-efficient and inexpensive protecting group. Recently, we reported a general chemoenzymatic synthesis of monolevulinyl-protected nucleosides using commercial lipases.[18] In this study, we discovered that immobilized *Pseudomonas cepacia* lipase (PSL-C) was moderately regioselective towards the acylation of the 3′-hydroxy group of **1** (ca. 80%, 12% towards 5′, and 8% towards 3′,5′) and *Candida antarctica* lipase B (CAL-B) was found to be totally regioselective towards the acylation of 5′-hydroxy group.

In order to develop a biocatalytic process for the separation of a mixture of 2′/3′-*O*-methyladenosine, it is essential to know the selectivity of the pure 3′-isomer **2** under enzymatic protocols. Thus, treatment of 3′-*O*-Me-A (**2**) with levulinate oxime ester[19] in the presence of CAL-B at 45 °C in THF consumed all of the starting material in 12 h (TLC). After work-up, ^{1}H NMR spectroscopic data of the product confirmed the formation of 5′-*O*-levulinyl-3′-*O*-methyladenosine (**3**, Scheme 1) as the sole product confirming regioselective acylation of the 5′-hydroxy group. Nucleoside **3** was isolated in 86% yield as amorphous solid. Similar treatment using PSL-C as biocatalyst indicated that the reaction was much slower furnishing about 14% of **3** after 144 h.



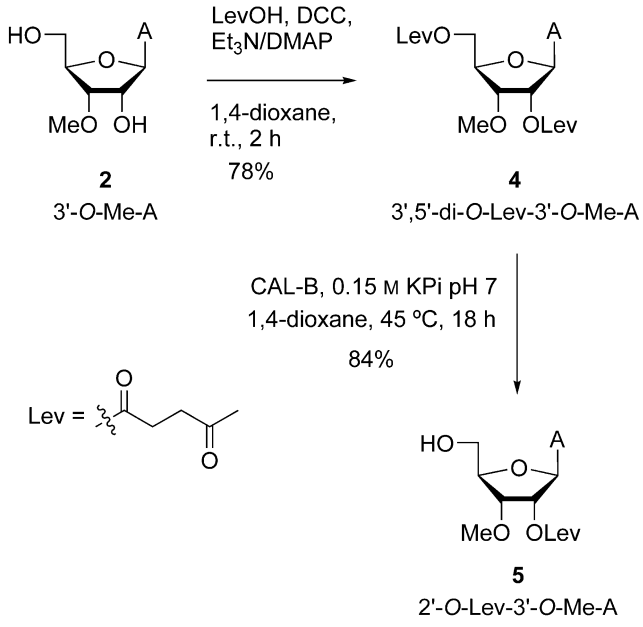
Enzyme	*T* [°C]	*t* [h]	% Conv.	% Yield
CAL-B	45	12	100	86
PSL-C	30	144	14	--

Scheme 1. Enzymatic synthesis of 5′-*O*-Lev-3′-*O*-Me-A.

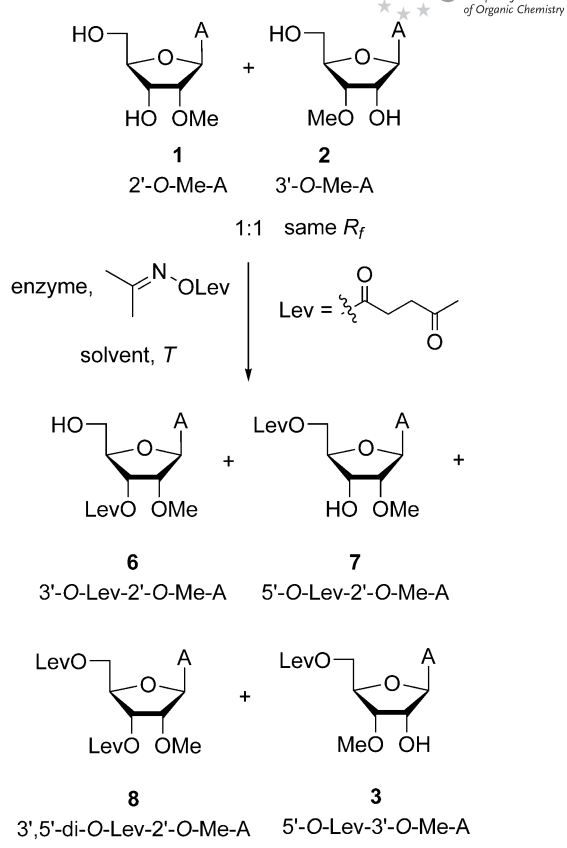
Next, we embarked on the synthesis of 2′-*O*-levulinyl-3′-*O*-methyladenosine (**5**) for two reasons. First, we wanted to prepare an authentic reference sample of **5** for comparison with its regio-isomer 5′-*O*-levulinyl-3′-*O*-methyladenosine (**3**). This exercise will enable us to conclusively demonstrate that the acylation of 3′-*O*-methyladenosine using PSL-C produced only **3** and none of **5**. Second, **5** would be a useful building block for the preparation of oligonucleotides. Therefore, we proposed a two-step protocol for the unequivocal synthesis of **5** shown in Scheme 2.

Treatment of 3′-*O*-methyladenosine (**2**) with 5.2 equiv. of levulinic acid (LevOH) and DCC in the presence of DMAP furnished 2′,5′-di-*O*-levulinyl-3′-*O*-methyladenosine (**4**) in 78% isolated yield. The hydrolysis of 5′-acyl group in **4** was carried out with CAL-B as catalyst due to its well-demonstrated selectivity with a variety of modified nucleosides.[19] When diester **4** was treated with CAL-B at 45 °C in 0.15 M phosphate buffer (pH 7) containing 18% of 1,4-dioxane, total regioselectivity toward the hydrolysis of the 5′-*O*-levulinyl group was observed, furnishing 2′-*O*-levulinyl-3′-*O*-methyladenosine (**5**) in 84% yield.

With a clear understanding of the lipase-mediated acylation of **2** and reference samples of related products **3**–**5** on hand, the stage was set for the separation of 2′/3′-*O*-



Scheme 2. Chemoenzymatic synthesis of 3′,5′-di-*O*-Lev-3′-*O*-Me-A and 2′-*O*-Lev-3′-*O*-Me-A.



Scheme 3. Enzymatic reaction of 1:1 mixture of 2′/3′-*O*-Me-A. For experimental details see Table 1.

methyladenosine mixture (Scheme 3). Treatment of an equimolar mixture[20] of 2′/3′-*O*-methyladenosine with acetonoxime levulinate and PSL-C (type II) afforded after 6 d 3′-*O*-Lev-2′-*O*-Me-A (**6**) as the major product, 5′-*O*-Lev-2′-*O*-Me-A (**7**), 3′,5′-di-*O*-Lev-2′-*O*-Me-A (**8**), and 5′-*O*-Lev-3′-*O*-Me-A (**3**) as minor products with unreacted 3′-*O*-Me-A (**2**) (entry 1, Table 1). As we observed earlier (Scheme 1), the 3′-*O*-Me isomer **2** reacted at a significantly slower rate furnishing only 5′-acylated product **3** under these conditions. Next, we altered several reaction conditions: temperature, solvent, acylation agent ratio, and different biocatalysts in order to increase the formation of **6** and reducing the amount of compound **3**.

The results from various experiments are summarized in Table 1. Lowering the temperature of the reaction to 30 °C using PSL-C, type I (a lot with 16% higher activity) the reaction was slower than PSL-C, type II, but selectivity was similar (entry 2, Table 1). Use of pure biocatalyst, *Chromobacterium viscosum* lipase (CVL), neither increased the selectivity nor the conversion (entry 3, Table 1). Increasing the enzyme and oxime ester ratios up to 1:3 using faster PSL-C (type I) offered shorter reaction times but lower selectivities (entry 4, Table 1). When the acylation was performed with PSL-C (type II), the best selectivity was observed for compound **6** (entry 5, Table 1). Increasing the reaction temperature to 40 °C resulted in reduced selectivity (entry 6, Table 1). Change of solvent to 1,4-dioxane provided no further improvement in the formation of **6** (entry 7, Table 1). We selected the reaction conditions described in entry 5 as the best case for further work. Because direct alkylation of adenosine offers about 3:1 ratio of 2′/3′-*O*-methyladenosine (**1/2**), we repeated this reaction using the same ratio of two products to validate the real utility of the system. Despite of the different ratio of the two products,

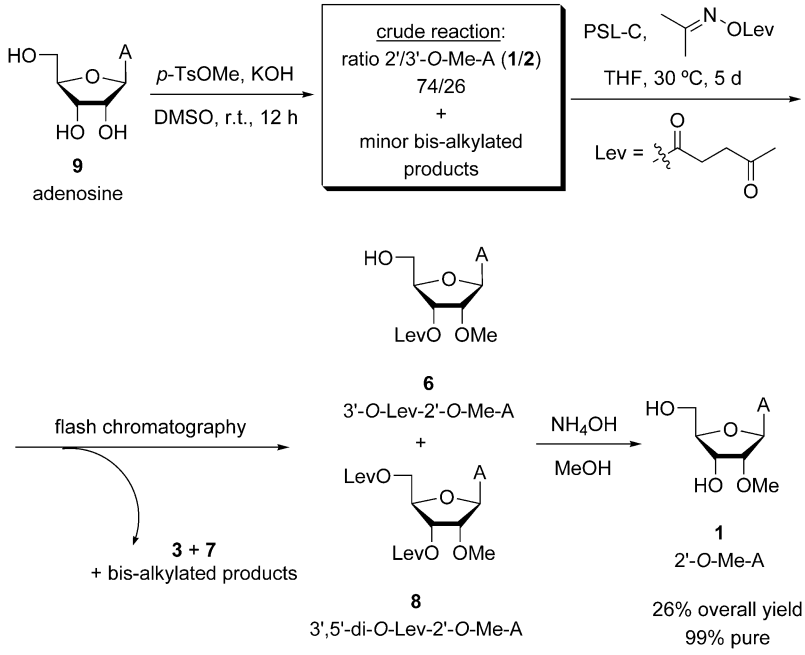
Table 1. Enzymatic acylation of 1:1 mixtures of 2′-*O*-Me-A (**1**) and 3′-*O*-Me-A (**2**).[a]

Entry	Enzyme	M/E[b]	M/OE[c]	*T* [°C]	*t* [d]	**3**[d]	**6**[d]	**7**[d]	**8**[d]
1	PSL-C	1:1.5	1:1.5	55	6	12	66	11	11
2	PSL-C′	1:1.5	1:1.5	30	7	8	69	11	12
3[e]	CVL	1:0.25	1:1.5	55	7	10	71	12	7
4	PSL-C′	1:3	1:3	30	4	16	55	10	19
5	PSL-C	1:3	1:3	30	5	10	72	11	7
6	PSL-C	1:3	1:3	40	5	17	62	12	9
7[f]	PSL-C	1:3	1:3	30	6	18	71	5	6
8[g]	**PSL-C**	**1:3**	**1:3**	**30**	**5**	**8**	**70**	**15**	**7**

[a] The reactions were carried out in THF, 0.20 M concentration, at 250 rpm with a 1:1 mixture of **1/2**. PSL-C′ = PSL-C type I, PSL-C = PSL-C type II. [b] Ratio mixture **1**+**2**/enzyme (w/w). [c] Mixture **1**+**2**/oxime ester. [d] Percentages of compounds in the crude reaction based on HPLC peak integration. [e] After 30 h at room temp., the temperature was increased up to 55 °C. [f] 1,4-dioxane as solvent. [g] The starting mixture **1/2** is 3:1.

we were pleased to see that the enzymatic reaction took place in a similar way as it was with the equimolar mixture (entry 8, Table 1).

We conclude that the reaction conditions shown in entry 5 of Table 1 are the best conditions, among the evaluated, to separate the isomeric mixture of **1** and **2**. Upon completion of the reaction, the reaction mixture is extracted with H_2O/CH_2Cl_2 providing unreacted 3′-*O*-Me regioisomer **2** (81% yield, 97% by HPLC)[21] in the aqueous layer. The two 5′-*O*-levulinylated compounds **3** and **7** were easily re-



Scheme 4. Synthesis and enzymatic separation of 2′-*O*-Me-A.

moved by flash chromatography column. The remaining 3′-*O*-Lev-2′-*O*-Me-A (**6**) and 3′,5′-di-*O*-Lev-2′-*O*-Me-A (**8**) were subjected to hydrolysis with NH_4OH/MeOH[22] providing the 2′-*O*-Me-A regioisomer (**1**) in 67% yield (99% by HPLC) starting from the initial isomeric mixture.

Scale Up of the Process

The utility of this enzymatic separation was then applied to a real mixture of regioisomers obtained via a direct alkylation process where products were used as such without chromatography. Therefore, methylation of 5 g of adenosine (**9**, Scheme 4) was carried out using safer conditions where methyl *p*-toluenesulfonate (*p*TosOMe) was employed as the alkylation reagent in the presence of KOH as base and DMSO as solvent. These conditions circumvent the use of hazardous reagents such as diazomethane or methyl iodide and NaH traditionally used for direct alkylation procedure. The ^{1}H NMR analysis of the crude product confirmed a mixture of monoalkylated (2′- and 3′-*O*-Me-A) as major products and trace amount of dialkylated products. The HPLC shows a 74:26 ratio of 2′/3′-*O*-Me-A, respectively. The enzymatic acylation was carried out directly on the crude products under the conditions described earlier [PSL-C (1:3, w:w), 30 °C, 3 equiv. of oxime ester, and THF as solvent]. In 5 d **1** was totally converted into acylated products. It is noteworthy that there was no difference in the rate of reaction between this protocol containing minor amount of other nucleoside related products and earlier procedure where we utilized a clean mixture of 2′/3′-*O*-Me-A regioisomers. This experiment indicates that efficiency of the immobilized enzyme was not compromised despite of the presence of impurities. After completion of the reaction, the products were extracted with organic solvent and subjected to a flash chromatography to remove 5′-*O*-acylated products **3** and **7**. Hydrolysis of the remaining mixture 3′-*O*-Lev-2′-*O*-Me-A (**6**) and 3′,5′-di-*O*-Lev-2′-*O*-Me-A (**8**) with NH_4OH/MeOH followed by flash chromatography furnished 2′-*O*-Me-A (**1**) in 26% yield and 99% of purity (HPLC).

Conclusions

Herein, we have described an efficient enzymatic separation of a 2′/3′-*O*-Me-A regioisomers mixture among other alkylated and dialkylated products which are usually obtained during the alkylation reactions of adenosine. Thus, using this methodology it is possible to avoid tedious purification steps that lead to lower yields. This protocol offers excellent separation of both regioisomers in good yields and high purities. PSL-C catalyzes the acylation of the 3′-hydroxy group in 2′-*O*-Me-A isomer (**1**) with high selectivity. However, 3′-*O*-Me-A isomer (**2**) is only acylated at the 5′-hydroxy group with a low conversion rate. As a result, a simple aqueous/organic extraction of the crude acylated products dissolved the polar 3′-*O*-Me-A (**2**) in the water layer and the non-polar acylated **6** and **8** were pulled in the organic phase. Subsequent hydrolysis of **6** and **8** followed by chromatography furnished 2′-*O*-Me-A (**2**) in 26% yield with 99% purity (HPLC). The usefulness of this process is clearly demonstrated when the enzymatic acylation was applied to a crude mixture after direct methylation of adenosine (**9**) using "green" reagents. Despite several impurities were present in the crude reaction mixture, the overall efficiency of enzyme was not compromised. This study constitutes another fine example of how immobilized enzymes

can be developed as key tools in organic synthesis offering economical processes under environmentally friendly conditions.

Experimental Section

General: *Pseudomonas cepacia* lipase immobilized on ceramic [PSL-C, Type I (1387 U/g) and Type II (1195 U/g) both from Amano] were purchased from Aldrich. *Chromobacterium viscosum* lipase (CVL, 4100 U/mg) was a gift from Genzyme Co. In order to prepare the artificial mixture of **1** and **2**, 3′-*O*-methyladenosine (**2**) was purchased from R. I. Chemical Inc. (www.richemical.com). HPLC analysis was performed at 254 nm using the following method: Mediterranea column (250 × 45 mm), 0.8 mL/min flow, 20 °C, and two mobile phases (A: MeCN; B: water); for gradient conditions see Table 2.

Table 2. Details for HPLC gradient.

Time [min]	A (%)	B (%)
0.0	8	92
15.0	10	90
20.0	20	80
25.0	50	50
30.0	70	30

Melting points were taken on samples in open capillary tubes and are uncorrected. IR spectra were recorded on an Infrared Fourier Transform spectrophotometer using KBr pellets. Flash chromatography was performed using silica gel 60 (230–400 mesh). For routine experiments, ^{1}H, ^{13}C NMR, and DEPT were obtained using 300.13 or 400.13 MHz for ^{1}H, and 75.5 or 100.61 MHz for ^{13}C. A spectrometer operating at 600.15 and 150.93 MHz for ^{1}H and ^{13}C, respectively, was used for the acquisition of ^{1}H-^{1}H homonuclear (COSY and NOESY) and ^{1}H-^{13}C heteronuclear (HSQC and HMBC) correlations. APCI$^+$ and ESI$^+$ were used to record mass spectra (MS). Microanalyses were performed on a Perkin–Elmer model 2400 instrument.

Enzymatic Synthesis of 5′-*O*-Levulinyl-3′-*O*-methyladenosine (3): A suspension of 3′-*O*-methyladenosine (**2**) (58 mg, 0.21 mmol), the oxime ester (106 mg, 0.62 mmol), and CAL-B (58 mg) in dry THF (1.1 mL) was stirred (250 rpm) under nitrogen for 12 h at 45 °C. The enzyme was filtered off and washed with CH_2Cl_2. The solvents were distilled under vacuum and the residue was purified by flash chromatography column (4% MeOH/CH_2Cl_2) to give 67 mg (86% yield) of 5′-*O*-levulinyl-3′-*O*-methyladenosine (**3**). R_f (10% MeOH/CH_2Cl_2) = 0.35; m.p. 123–125 °C. IR (KBr): $\tilde{\nu}$ = 3354, 3173, 2917, 1711, and 1662 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.17 (s, 3 H, *Me*-Lev), 2.56 (m, 2 H, C*H*$_2$-Lev), 2.74 (m, 2 H, C*H*$_2$-Lev), 3.53 (s, 3 H, OMe), 4.14 (t, $^3J_{HH}$ = 4.8 Hz, 1 H, 4′-H), 4.38 (m, 3 H, 3′-H+5′-H), 4.83 (t, $^3J_{HH}$ = 4.8 Hz, 1 H, 2′-H), 6.00 (m, 3 H, 1′-H +NH$_2$), 8.04 (s, 1 H, 2-H or 8-H), and 8.30 (s, 1 H, 8-H or 2-H) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 27.7 (*C*H$_2$-Lev), 29.8 (*Me*-Lev), 37.8 (*C*H$_2$-Lev), 58.6 (OMe), 63.6 (C-5′), 73.5 (C-3′), 79.7, 80.2 (C-2′+C-4′), 89.8 (C-1′), 120.0 (C-5), 139.3 (C-2 or C-8), 149.4 (C-4), 152.9 (C-8 or C-2), 155.6 (C-6), 172.5 (C=O), and 206.5 (C=O) ppm. MS (API$^+$): *m*/*z* = 380 (100) [M + H], 282 (10). $C_{16}H_{21}N_5O_6$ (379.37): calcd. C 50.66, H 5.58, N 18.46; found C 50.8, H 5.4, N 18.6.

Synthesis of 3′,5′-Di-*O*-Levulinyl-3′-*O*-methyladenosine (4): To a stirred mixture of 3′-*O*-methyladenosine (**2**) (80 mg, 0.28 mmol) and Et_3N (240 μL, 1.70 mmol) in 1,4-dioxane (2.8 mL) under nitrogen was added levulinic acid (170 mg, 1.45 mmol), DCC (298 mg, 1.45 mmol), and DMAP (2.8 mg, 0.02 mmol). The reaction was stirred at room temp. for 2 h. The insoluble material was collected by filtration and the filtrate was evaporated under vacuum. The crude was purified by flash chromatography column (gradient eluents 1–3% MeOH/CH_2Cl_2) to give 3′,5′-di-*O*-levulinyl-3′-*O*-methyladenosine (**4**) in 78% yield. R_f (10% MeOH/CH_2Cl_2) = 0.52. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.14 (s, 6 H, 2*Me*-Lev), 2.65 (m, 8 H, 4C*H*$_2$-Lev), 3.38 (s, 3 H, OMe), 4.36 (m, 4 H, 3′-H+4′-H+5′-H), 5.86 (dd, $^3J_{HH}$ = 3.1, $^3J_{HH}$ = 5.0 Hz, 1 H, 2′-H), 6.08 (d, $^3J_{HH}$ = 2.9 Hz, 1 H, 1′-H), 6.23 (br. s, 2 H, NH$_2$), 7.99 (s, 1 H, 2-H or 8-H), and 8.29 (s, 1 H, 8-H or 2-H) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 27.9 (*C*H$_2$-Lev), 29.9 (*Me*-Lev), 38.0 (*C*H$_2$-Lev), 59.3 (OMe), 63.3 (C-5′), 74.0 (C-3′), 78.1, 80.1 (C-2′+C-4′), 87.4 (C-1′), 119.9 (C-5), 139.4 (C-2 or C-8), 149.3 (C-4), 153.0 (C-8 or C-2), 155.8 (C-6), 171.7 (C=O), 172.4 (C=O), 206.2 (C=O), and 206.6 (C=O) ppm. MS (API$^+$): *m*/*z* = 478 (100) [M + H]$^+$, 380 (12), 282 (8).

Enzymatic Hydrolysis of (4). Synthesis of 2′-*O*-Levulinyl-3′-*O*-methyladenosine (5): To a solution of 3′,5′-di-*O*-levulinyl-3′-*O*-methyladenosine (**4**) (76 mg, 0.16 mmol) in 1,4-dioxane (0.28 mL) was added 0.15 M phosphate buffer pH 7 (1.30 mL) and CAL-B (76 mg). The stirred mixture (250 rpm) was allowed to react for 18 h at 45 °C. The enzyme was filtered off and washed with CH_2Cl_2, the solvents were distilled under vacuum, and the residue was dissolved in CH_2Cl_2 and washed with $NaHCO_3$ (aq.). The combined organic layers were dried with Na_2SO_4 and evaporated to give 51 mg (84%) of 2′-*O*-levulinyl-3′-*O*-methyladenosine (**5**). R_f (10% MeOH/CH_2Cl_2) = 0.45; m.p. 140–142 °C. IR (KBr): $\tilde{\nu}$ = 3236, 3125, 2928, 1745, 1713, 1691, and 1616 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 2.13 (s, 3 H, *Me*-Lev), 2.59 (m, 2 H, C*H*$_2$-Lev), 2.68 (m, 2 H, C*H*$_2$-Lev), 3.45 (s, 3 H, OMe), 3.72 (d, $^3J_{HH}$ = 12.0 Hz, 1 H, 5′-H), 4.00 (d, $^3J_{HH}$ = 12.0 Hz 1 H, 5′-H), 4.33 (m, 2 H, 3′-H + 4′-H), 5.78 (t, $^3J_{HH}$ = 6.0 Hz, 1 H, 2′-H), 6.01 (d, $^3J_{HH}$ = 7.0 Hz, 1 H, 1′-H), 6.39 (br. s, 2 H, NH$_2$), 7.85 (s, 1 H, 2-H or 8-H), and 8.26 (s, 1 H, 8-H or 2-H) ppm. ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 27.6 (*C*H$_2$-Lev), 29.7 (*Me*-Lev), 37.7 (*C*H$_2$-Lev), 58.6 (OMe), 62.9 (C-5′), 74.7 (C-3′), 79.3, 85.7 (C-2′+C-4′), 88.7 (C-1′), 120.8 (C-5), 140.4 (C-2 or C-8), 148.6 (C-4), 152.2 (C-8 or C-2), 155.8 (C-6), 171.6 (C=O), and 206.1 (C=O) ppm. MS (APCI$^+$): *m*/*z* = 380 (100) [M + H]$^+$, 282 (10). $C_{16}H_{21}N_5O_6$ (379.37): calcd. C 50.66, H 5.58, N 18.46; found C 50.4, H 5.5, N 18.5.

Procedure for the Enzymatic Separation of a 1:1 Mixture of 2′/3′-*O*-Methyladenosine: In a standard procedure, THF (5.1 mL) filled into an Erlenmeyer flask that contained the isomeric mixture of 2′/3′-*O*-methyladenosine (**1**/**2**) (300 mg, 1.07 mmol), oxime ester (547 mg, 3.20 mmol) and PSL-C (900 mg). The reaction mixture was stirred at 30 °C and 250 rpm under nitrogen and monitorized by HPLC. The enzyme was filtered off, washed with CH_2Cl_2 and MeOH, and the solvents evaporated under vacuum. The residue was poured in H_2O and extracted with CH_2Cl_2. The aqueous layer was concentrated and the solid was washed with CH_2Cl_2 to give 121 mg (81% yield, 97% by HPLC) of 3′-*O*-methyladenosine (**2**). The combined organic layers were dried with Na_2SO_4 and evaporated, and the residue was subjected to flash chromatography column (4% MeOH/CH_2Cl_2) in order to remove traces of 5′-*O*-levulinyl-3′-*O*-methyladenosine (**3**) and 5′-*O*-levulinyl-2′-*O*-methyladenosine (**7**).

On the other hand, the first fraction containing 3′-*O*-levulinyl-2′-*O*-methyladenosine (**6**) and 3′,5′-di-*O*-levulinyl-2′-*O*-methyladenosine (**8**) was dissolved in MeOH (9 mL) and then ammonia

(8.4 mL, 30% aq.) was added under continuous stirring. The reaction mixture was stirred at room temp. for 2 h. After evaporation of the solvents, the crude reaction mixture was separated by flash column chromatography (4% MeOH/CH_2Cl_2) to give 100 mg (67% yield from the initial isomeric mixture, 99% by HPLC) of 2′-*O*-methyladenosine (**1**).

2′-*O*-Methyladenosine (1): R_f (10% MeOH/CH_2Cl_2) = 0.21; m.p. 204–206 °C, ref.[9b] 203–204 °C or 202–203.5 °C.[9d] IR (KBr): $\tilde{\nu}$ = 3368, 3263, 3106, 2923, 1689, and 1611 cm^{-1}. ^1H NMR ([D_4]-MeOH, 300 MHz): δ = 3.42 (s, 3 H, OMe), 3.75 (dd, $^3J_{HH}$ = 2.8, $^3J_{HH}$ = 12.6 Hz, 1 H, 5′-H), 3.89 (dd, $^3J_{HH}$ = 2.7, $^3J_{HH}$ = 12.6 Hz, 1 H, 5′-H), 4.16 (q, $^3J_{HH}$ = 2.6 Hz, 1 H, 4′-H) 4.43 (t, $^3J_{HH}$ = 5.0 Hz, 1 H, 2′-H), 4.49 (apparent dd, $^3J_{HH}$ = 3.1, $^3J_{HH}$ = 5.0 Hz, 1 H, 3′-H), 6.06 (d, $^3J_{HH}$ = 5.9 Hz, 1 H, 1′-H), 8.19 (s, 1 H, 2-H or 8-H), and 8.34 (s, 1 H, 8-H or 2-H) ppm. ^{13}C NMR ([D_6]DMSO, 75.5 MHz)): δ = 57.4 (OMe), 61.4 (C-5′), 68.7 (C-3′), 82.4, 85.7, 86.3 (C-1′+C-2′+C-4′), 119.2 (C-5), 139.6 (C-2 or C-8), 148.9 (C-4), 152.4 (C-8 or C-2), and 156.0 (C-6) ppm. MS (EI): *m*/*z* (%) = 281 (5) [M^+], 192 (57), 164 (28), 146 (23), 135 (100). HRMS calcd. for $C_{11}H_{15}N_5O_4$: 281.1119; found 281.1111.

3′-*O*-Methyladenosine (2): R_f (10% MeOH/CH_2Cl_2) = 0.21; m.p. 175–177 °C, ref.[9b] 174–175 °C or 177–178 °C.[9d] IR (KBr): $\tilde{\nu}$ = 3365, 3266, 3135, 2924, 1689, and 1614 cm^{-1}. ^1H NMR ([D_4]-MeOH, 300 MHz): δ = 3.52 (s, 3 H, OMe), 3.73 (dd, $^3J_{HH}$ = 2.7, $^3J_{HH}$ = 12.6 Hz, 1 H, 5′-H), 3.90 (dd, $^3J_{HH}$ = 2.7, $^3J_{HH}$ = 12.6 Hz, 1 H, 5′-H), 3.99 (dd, $^3J_{HH}$ = 2.7, $^3J_{HH}$ = 5.1 Hz, 1 H, 3′-H), 4.25 (q, $^3J_{HH}$ = 2.7 Hz, 1 H, 4′-H), 4.83 (m, 1 H, 2′-H), 5.4 (d, $^3J_{HH}$ = 6.3 Hz, 1 H, 1′-H), 8.19 (s, 1 H 2-H or 8-H), and 8.32 (s, 1 H, 8-H or 2-H) ppm. ^{13}C NMR ([D_6]DMSO, 75.5 MHz): δ = 57.5 (OMe), 61.5 (C-5′), 72.7 (C-3′), 79.9, 83.3, (C-2′+C-4′), 87.9 (C-1′), 119.3 (C-5), 139.7 (C-2 or C-8), 148.9 (C-4), 152.3 (C-8 or C-2), and 156.0 (C-6) ppm. MS (EI): *m*/*z* (%) = 281 (3) [M^+], 266 (9), 192 (3), 178 (18), 164 (63), 148 (10), 135 (100). HRMS calcd. for $C_{11}H_{15}N_5O_4$: 281.1119; found 281.1116.

3′-*O*-Levulinyl-2′-*O*-methyladenosine (6): The isolated compound was identical with that described in ref.[18d]

5′-*O*-Levulinyl-2′-*O*-methyladenosine (7): The isolated compound was identical with that described in ref.[18b]

3′,5′-di-*O*-Levulinyl-2′-*O*-methyladenosine (8): The isolated compound was identical with that described in ref.[18d]

Methylation of Adenosine and Enzymatic Isolation of 2′-*O*-Methyladenosine (1): Adenosine (**9**) (5 g, 18.7 mmol) was dissolved in DMSO (75 mL). Then KOH (1.92 g, 34.2 mmol) was added under continuous stirring. After the KOH had dissolved, *p*TosOMe (4.2 g, 22.5 mmol) was added and the reaction mixture was stirred at room temp. for 12 h. Then the DMSO was evaporated under vacuum and acetone (75 mL) was added. The solid was filtered off and the filtrate evaporated to furnish 5.7 g of crude product. The crude product was placed in an Erlenmeyer flask and PSL-C (17.1 g), oxime ester (10.39 g, 60.8 mmol), and THF (100 mL) were added. The reaction mixture was stirred (250 rpm) at 30 °C under nitrogen atmosphere for 5 d. The enzyme was filtered off, washed with CH_2Cl_2 and MeOH, and the solvents evaporated under vacuum. The residue was taken up in H_2O and extracted with CH_2Cl_2. The aqueous layer contained 3′-*O*-methyladenosine (**2**), traces of 2′-*O*-methyladenosine (**1**), and *p*TosOK. The combined organic layers were dried with Na_2SO_4, evaporated, and the residue was subjected to flash chromatography column (4% MeOH/CH_2Cl_2) in order to remove traces of 5′-*O*-levulinyl-3′-*O*-methyladenosine (**3**) and 5′-*O*-levulinyl-2′-*O*-methyladenosine (**7**). The first fraction containing 3′-*O*-levulinyl-2′-*O*-methyladenosine (**6**) and 3′,5′-di-*O*-levulinyl-2′-*O*-methyladenosine (**8**) was dissolved in MeOH (65 mL), then ammonia (61 mL, 30% aq.) was added under continuous stirring. The reaction mixture was stirred at room temp. for 2 h. After evaporation of solvents crude reaction was subjected to flash chromatography column (4% MeOH/CH_2Cl_2) to obtain 1.36 g (26% yield, 99% by HPLC) of 2′-*O*-methyladenosine (**1**).

Supporting Information (see also the footnote on the first page of this article): General spectroscopic and experimental data are shown. Furthermore, copies of ^1H, ^{13}C, and DEPT NMR spectra in addition of some 2D NMR experiments are included. Some HPLC analyses are shown in support of the purity.

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[1] For selected recent reviews in this area, see: a) M. Sohail, *Drug Discovery Today* **2001**, *6*, 1260–1261; b) N. M. Dean, *Curr. Opin. Biotechnol.* **2001**, *12*, 622–625; c) S. T. Crooke, *Oncogene* **2000**, *19*, 6651–6659; d) D. W. Green, H. Roh, J. Pippin, J. A. Drebin, *J. Am. Coll. Surg.* **2000**, *191*, 93–105; e) E. Koller, W. A. Gaarde, B. P. Monia, *Trends Pharmacol. Sci.* **2000**, *21*, 142–148; f) Y. S. Sanghvi, in *Comprehensive Natural Products Chemistry* (Eds.: D. H. R. Barton, K. Nakanishi), vol. 7 (Ed.: E. T. Kool): *DNA and Aspects of Molecular Biology*, Pergamon Press, New York, **1999**, p. 285.

[2] *Antisense Drug Technology Principles: Strategies and Applications* (Ed.: S. T. Crooke), Marcel Dekker, New York, **2001**.

[3] a) L. Beigelman, J. A. McSwiggen, K. G. Draper, C. Gonzalez, K. Jensen, A. M. Karpeisky, A. S. Modak, J. Matulic-Admic, A. B. DiRenzo, P. Haeberli, D. Sweedler, D. Tracz, S. Grimm, F. E. Wincott, V. G. Thackray, N. Usman, *J. Biol. Chem.* **1995**, *270*, 25702–25708; b) A. I. Lamond, B. S. Sproat, U. Rider, *Cell* **1989**, *58*, 383–390; c) B. J. Blencowe, B. S. Sproat, U. Rider, S. Barbino, A. I. Lamond, *Cell* **1989**, *59*, 531–539.

[4] R. H. Hall, in: *The Modified Nucleosides in Nucleic Acids*, Columbia University Press, New York, **1971**.

[5] For recent accounts on 2′-*O*-methylation of guanosine and adenosine, see: a) A. R. Kore, G. Parmar, S. Reddy, *Nucleosides Nucleotides Nucleic Acids* **2006**, *25*, 307–314; b) S. Chow, K. Wen, Y. S. Sanghvi, E. A. Theodorakis, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1631–1634; c) K. Wen, S. Chow, Y. S. Sanghvi, E. A. Theodorakis, *J. Org. Chem.* **2002**, *67*, 7887–7889; d) L. Beigelman, P. Haeberli, D. Sweedler, A. Karpeisky, *Tetrahedron* **2000**, *56*, 1047–1056.

[6] A. D. Broom, R. K. Robins, *J. Am. Chem. Soc.* **1965**, *87*, 1145–1146.

[7] a) E. Wagner, B. Oberhauser, A. Holzner, H. Brunar, G. Issakides, G. Schaffner, M. Cotton, M. Knollmueller, C. R. Noe, *Nucleic Acids Res.* **1991**, *19*, 5965–5971; b) J. Yano, L. S. Kan, P. O. P. Ts'o, *Biochim. Biophys. Acta* **1980**, *629*, 178–183.

[8] a) R. P. Hodge, N. D. Sinha, *Tetrahedron Lett.* **1995**, *36*, 2933–2936; b) H. Inoue, Y. Hayase, A. Imura, S. Iwai, K. Miura, E. Ohtsuka, *Nucleic Acids Res.* **1987**, *15*, 6131–6148.

[9] a) H. Cramer, W. Pfleiderer, *Helv. Chim. Acta* **1996**, *79*, 2114–2136; b) T. Pathak, J. Chattopadhyaya, *Chem. Scr.* **1986**, *26*, 135–139; c) M. J. Robins, A. S. K. Lee, F. A. Norris, *Carbohydr. Res.* **1975**, *41*, 304–307; d) M. J. Robins, S. R. Naik, A. S. K. Lee, *J. Org. Chem.* **1974**, *39*, 1891–1899; e) M. J. Robins, S. R. Naik, *Biochim. Biophys. Acta* **1971**, *246*, 341–343.

[10] K. Yamauchi, K. Hattori, M. Kinoshita, *J. Chem. Soc. Perkin Trans. 1* **1985**, 1327–1330.

[11] K. Yamauchi, T. Nakagima, M. Kinoshita, *J. Org. Chem.* **1980**, *45*, 3865–3868.

[12] a) L. Beigelman, A. Karpeisky, J. Matulic-Adamic, N. Usman, *Nucleosides Nucleotides* **1995**, *14*, 421–424; b) B. S. Ross, R. H. Springer, G. Vasquez, R. S. Andrews, P. D. Cook, O. L. Acevedo, *J. Heterocycl. Chem.* **1994**, *31*, 765–769; c) G. Parmentier, G. Schmitt, F. Dolle, B. Luu, *Tetrahedron* **1994**, *50*, 5361–5368; d) C. Chavis, F. Dumont, R. H. Wightman, J. C. Zeigler, J. L. Imbach, *J. Org. Chem.* **1982**, *47*, 202–206; e) A. H. Haines, *Tetrahedron* **1973**, *29*, 2807–2810.

[13] V. D. Axelrod, M. R. Vartikyan, V. A. Aivazashvili, R. Beabealashvili, *Nucleic Acids Res.* **1978**, *15*, 3549–3561.

[14] B. B. Goswami, O. K. Sharma, *J. Virol.* **1983**, *45*, 1164–1167.

[15] J. García, A. Díaz-Rodríguez, S. Fernández, Y. S. Sanghvi, M. Ferrero, V. Gotor, *J. Org. Chem.* **2006**, *71*, 9765–9771.

[16] J. García, A. Díaz-Rodríguez, S. Fernández, Y. S. Sanghvi, M. Ferrero, V. Gotor, *Org. Lett.* **2004**, *6*, 9765–9771.

[17] a) For comprehensive literature, see: *Current Protocols in Nucleic Acid Chemistry* (Eds: S. L. Beaucage, D. E. Bergstrom, G. D. Glick, R. A. Jones), John Wiley & Sons, New York, **2007**, chapter 2; b) *Greene's Protective Groups in Organic Synthesis*, 4th ed. (Eds: T. W. Green, P. G. M. Wuts), John Wiley & Sons, New York, **2006**; c) *Protecting Groups*, 3rd ed. (Ed.: P. J. Kocienski), Georg Thieme, Stuttgart, **2005**.

[18] a) A. Díaz-Rodríguez, S. Fernández, Y. S. Sanghvi, M. Ferrero, V. Gotor, *Org. Process Res. Dev.* **2006**, *10*, 581–587; b) J. García, S. Fernández, Y. S. Sanghvi, M. Ferrero, V. Gotor, *Tetrahedron: Asymmetry* **2003**, *14*, 3533–3540; c) J. García, S. Fernández, M. Ferrero, Y. S. Sanghvi, V. Gotor, *Nucleosides Nucleotides Nucleic Acids* **2003**, *22*, 1455–1457; d) J. García, S. Fernández, M. Ferrero, Y. S. Sanghvi, V. Gotor, *J. Org. Chem.* **2002**, *67*, 4513–4519.

[19] I. Lavandera, J. García, S. Fernández, M. Ferrero, V. Gotor, Y. S. Sanghvi, in *Current Protocols in Nucleic Acid Chemistry*, (Eds: S. L. Beaucage, D. E. Bergstrom, G. D. Glick, R. A. Jones), John Wiley & Sons, New York, **2005**, chapter 2, pp. 2.11.1–2.11.36.

[20] The equimolar mixture of the two nucleosides was prepared by mixing the equal weight of 2′-*O*-methyladenosine (**1**) and 3′-*O*-methyladenosine (**2**) with an intention of giving both nucleosides an equal chance to compete for enzymatic acylation process.

[21] Presence of about 3% of starting material **1** was confirmed along with 97% of compound **3** in the HPLC analysis.

[22] C. B. Reese, H. Yan, *Tetrahedron Lett.* **2003**, *44*, 2501–2504.

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