Regioselective enzymatic acylation of pharmacologically interesting nucleosides in 2-methyltetrahydrofuran, a greener substitute for THF

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1-β-Arabinofuranosyl uracil, 9-β-arabinofuranosyl adenosine, 2′-*O*-(2-methoxyethyl)-5-methyl uridine, adenosine and uridine were enzymatically acylated with hexanoic anhydride and vinyl esters by CALB lipase (lipase B from *Candida antarctica*) with excellent regioselectivity in many cases and analytical reaction yields above 90%. The influence of the stereochemistry of the hydroxyl group on C-2′ was studied. Some of these esterifications were carried out in 2-methyltetrahydrofuran (MeTHF), which is described as an excellent substitute for THF in biocatalysed processes in organic media. This application for this green solvent is a proof-of-concept opening the use of MeTHF in biotransformations.

Introduction

Nucleosides and derivatives are relevant compounds in terms of pharmacological properties.[1] They have shown, among others, activity against some kinds of viruses (including the human immunodeficiency virus) and carcinogenic cells. For this reason, enormous efforts have been undertaken in the last 20 years to synthesize derivatives with potent antiviral and antitumor activities.[2] In this sense, the goal of the present research work was the regioselective synthesis of 1-β-arabinofuranosyl uracil (*ara*-U) and 9-β-arabinofuranosyl adenosine (*ara*-A) derivatives to be tested as prodrugs with antitumor activity.

Ara-U and *ara*-A are appealing compounds due to their cytotoxic activity. Nevertheless, they are extremely hydrophilic and, consequently, poorly absorbed enterically, so a different administration route would be desirable. For this reason, appropriate derivatives of the above-mentioned biologically active products should be developed. Different chemical structures can be proposed to produce nucleoside prodrugs, but ester formation is the most interesting reaction because of the high activity of human esterases, which can quickly liberate the drug. Besides, esters have higher lipophilicity, which facilitates the permeability through cell membranes. In fact, several esters from nucleoside analogues (such as acyclovir) have been prepared.[3] Among all possible derivatives, those with a small side chain in the OH-5′ position could give rise to products with enhanced pharmacokinetic properties. Indeed, the acylation on C-5′ would lead to a prodrug that could be administered transdermally or as a solid pharmaceutical preparation.

Although chemical acylations of nucleosides have been reported in few cases, this methodology usually implies the use of protecting groups and tedious separation processes, rendering low yields of the mono-*O*-acyl derivative.[4] In turn, regioselective enzymatic acylation of nucleosides[5] and hydrolysis[6] of the corresponding esters are being intensively investigated due to their feasibility and high efficiency. In general, biocatalysts used in these processes (lipases) are relatively cheap and environmentally friendly. Their use often avoids the need for protecting groups, the reaction conditions are mild, they can show high regioselectivity (reducing the appearance of side reactions) that can be modulated by modification of the reaction parameters and, in the case of immobilized enzymes, it is possible to reuse them,[7] so that the economical sustainability can be increased.

The benefits of using biotransformations can be further improved with a rational selection of the solvent. In this sense, we present an esterification study of the above-mentioned substrates with several vinyl esters as acylating agents and using different lipases and solvents. Remarkable results were obtained for the acylation of *ara*-U in 2-methyltetrahydrofuran (MeTHF), which is a versatile aprotic solvent that is being used more often in industrial synthetic processes because of its favorable properties.[8] In classical organic chemistry, MeTHF is increasingly being used as a THF substitute in the preparation of Grignard's reagents,[9a] for low-temperature lithiation,[9a,9b] for lithium aluminium hydride reductions,[9a] for the Reformatsky reaction,[9c] as well as for metal-catalyzed coupling reactions.[9d–9f] In fact, MeTHF has a higher log *P* value (0.99)[9g] compared to THF (0.49),[9g] and it is partially miscible with water (solubility of water in MeTHF ranges from 4% to 5% upon heating from 0 to 70.6 °C; solubility of MeTHF in water ranges from 21% to 6% in the same temperature range);[8] on the other hand, it has a boiling point of 89 °C, slightly higher than that of THF and, therefore, solvent evaporation during reaction is reduced. Besides, this solvent forms an azeotrope with 10.6% water, allowing the recycling of dry MeTHF; and gives clean water phase separations, useful for two phase reactions and product recovery. In many senses, MeTHF resembles toluene in terms of physical properties.[8] Most importantly, MeTHF

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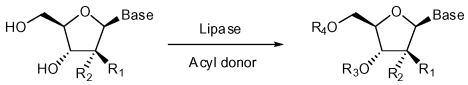
is the only aprotic solvent similar to THF that derives from renewable resources, *i.e.* it has low environmental impact, since it is produced from furfural, which is a chemical isolated from corn crops, sugar cane bagasse and oat hulls. Therefore, substitution of THF or other solvents like dichloromethane or dichloroethane for MeTHF will render greener processes, as the 3R desiderations—reduce, recycle and reuse—are all met by the introduction of MeTHF. As far as we know, and excluding a short statement in a old paper by Nakamura *et al.*[9g] (as one of many solvents and, of course, without emphasizing its green character at that time), this is the first description of MeTHF as a solvent in biotransformations.

Results and discussion

1-β-Arabinofuranosyl uracil (*ara*-U, **1**) and 9-β-arabinofuranosyl adenosine (*ara*-A, **15**), two cytotoxic compounds, were regioselectively acylated with vinyl esters by several lipases in different solvents with the aim of producing nucleoside derivatives with enhanced pharmacological properties (Scheme 1, Table 1). Most of the published research on enzymatic esterification of nucleosides is based on 2′-deoxy derivatives.[10] However, we show herein the broad applicability of lipase B from *Candida antarctica* (CALB) in such processes, since it is also capable of distinguishing among three hydroxyl groups.



Scheme 1

Acylation of 1-β-arabinofuranosyl uracil (*ara*-U)

Ara-U (**1**) was esterified at room temperature with hexanoic anhydride by Novozyme435, an immobilised CALB, using two solvents independently, THF and MeTHF. This process took place regioselectively at the hydroxyl group on C-5′, which is the preferred position for the lipase employed to react as demonstrated by 2′-deoxynucleosides.[11] These derivatives were characterized mainly by the ^{13}C NMR data, since the signal of the carbon supporting the acyl group (C-5′) moved downfield compared to the non-acylated. Thus, the neighboring carbon atom (C-4′) shifted upfield. The same regioselectivity has been obtained in the hydrolysis of nucleoside esters.[6]

Final conversion reached different values at the equilibrium with the different solvents, probably because of their different water miscibility and, therefore, also because of their different water activity. In fact, when using MeTHF, after 6 h, 90% of product 5′-*O*-hexanoyl-1-β-arabinofuranosyl uracil (**5**) was detected, but the yield decreased to 80% at 8 h, remaining stable after this time. The reaction in THF proceeded slightly slower but constantly increased up to a final value of 95% at 24 h. A similar behavior was observed for the esterification of uridine (**7**) under the same reaction conditions, in MeTHF the highest yield (95%) of 5′-*O*-hexanoyl uridine (**9**) was obtained after 4 h, decreasing then to 88% at 24 h; while in THF, the acylation proceeded slower but the product formation continuously increased up to 95% at 24 h. This fact may be caused by the different water miscibility of the solvents: thus, due to the liberation of hexanoic acid as the reaction is proceeding, the micropH in the enzyme surroundings may be altered, as described in ref. 12a, and causing a reversion in the equilibrium by promoting the acidolysis of the acylated product,[12b] this effect is more important when using MeTHF *vs.* THF.

With the aim of synthesizing derivatives of interesting cytotoxic agents *ara*-U and *ara*-A with short side chains in the molecule, the acylation of 1-β-arabinofuranosyl uracil with vinyl acetate (VA) by CALB in 2-methyltetrahydrofuran was performed. After 6 h, 93% of the corresponding derivative in the OH-5′ position was obtained (product **2**), along with 2% of starting material and 5% of 3′,5′-*O*-diacetyl-1-β-arabinofuranosyl uracil (**6**) (HPLC results, Table 2). This means that high regioselectivity was achieved with the above-mentioned lipase in only one step and short reaction time avoiding the use of a protection/deprotection strategy. Therefore, this procedure is atom economical and also environmentally friendly, since MeTHF was used. It is noteworthy that the reaction without enzyme did not work at all and only the initial substrate was detected.

Results with other vinyl esters such as vinyl propionate (VP) and butyrate (VB) largely paralleled those of vinyl acetate (Table 2). In the case of VP and VB, once the maximum reaction

Table 1 Structure of nucleosides and derivatives; U = uracil, 5-MU = 5-methyluracil, A = adenine

Base	R_1	R_2	R_3	R_4	Product
U	OH	H	H	H	**1**
U	OH	H	H	$COCH_3$	**2**
U	OH	H	H	$COCH_2CH_3$	**3**
U	OH	H	H	$CO(CH_2)_2CH_3$	**4**
U	OH	H	H	$CO(CH_2)_4CH_3$	**5**
U	OH	H	$COCH_3$	$COCH_3$	**6**
U	H	OH	H	H	**7**
U	H	OH	H	$COCH_3$	**8**
U	H	OH	H	$CO(CH_2)_4CH_3$	**9**
5-MU	H	$O(CH_2)_2OCH_3$	H	H	**10**
5-MU	H	$O(CH_2)_2OCH_3$	H	$COCH_3$	**11**
5-MU	H	$O(CH_2)_2OCH_3$	H	$COCH_2CH_3$	**12**
5-MU	H	$O(CH_2)_2OCH_3$	H	$CO(CH_2)_2CH_3$	**13**
5-MU	H	$O(CH_2)_2OCH_3$	H	$CO(CH_2)_4CH_3$	**14**
A	OH	H	H	H	**15**
A	OH	H	H	$COCH_3$	**16**
A	OH	H	H	$COCH_2CH_3$	**17**
A	OH	H	H	$CO(CH_2)_2CH_3$	**18**
A	OH	H	H	$CO(CH_2)_4CH_3$	**19**
A	H	OH	H	H	**20**
A	H	OH	H	$CO(CH_2)_4CH_3$	**21**

Table 2 Results obtained from the esterification of *ara*-U with CALB and several vinyl esters in MeTHF

	Ara-U (%)			5′-Ester (%)			3′,5′-Diester (%)		
Time/h	VA	VP	VB	VA	VP	VB	VA	VP	VB
4	13	6	4	83	88	91	4	6	5
6	2	—	1	93	93	94	5	7	5
8	3	—	1	91	93	94	6	7	5
24	—	—	—	90	93	94	10	7	6

Table 3 Comparison of results obtained from the esterification of *ara*-U with vinyl esters in MeTHF by *Pseudomonas fluorescens* and CALB

		Ara-U (%)			5′-Ester (%)			3′,5′-Diester (%)		
Time/h	Enzyme	VA	VP	VB	VA	VP	VB	VA	VP	VB
4	CALB	13	6	4	83	88	91	4	6	5
4	PFL	56	37	50	40	60	49	4	3	1
6	CALB	2	—	1	93	93	94	5	7	5
6	PFL	30	33	45	64	62	51	6	5	4
8	CALB	3	—	1	91	93	94	6	7	5
8	PFL	20	29	34	74	63	60	6	8	6
24	CALB	—	—	—	90	93	94	10	7	6
24	PFL	2	2	1	85	72	70	13	26	19

yield was achieved (6 h) it did not decrease with time, while with VA it was slightly lower.

To check the viability of using some other biocatalysts, acylation of *ara*-U with vinyl esters was also tested with other lipases, such as those from native lyophilized *Mucor javanicus* (MJL), *Candida rugosa* (CRL), *Pseudomonas cepacea* (PCL) and *Pseudomonas fluorescens* (PFL), but the yields were lower than 10%, probably due to aggregation of these native preparations in the organic solvent and the concomitant diffusion problems. This problem is overcome by the use of a lipase supported on a carrier, such as in the CALB derivative used in this study, in which all lipase molecules are more accessible to the substrate.[13] The only exception was PFL, although enzymatic esterification with this lipase led to lower yields than CALB and higher proportion of the diacyl derivative in both 5′ and 3′ positions, as shown in Table 3.

Nevertheless, it is difficult to draw a direct comparison between the two catalysts, since they are used in a different state (native lyophilized preparation (PFL) or immobilised derivative (CALB)), and also because of their different intrinsic declared activity (see Experimental). We would like to emphasise that not many crude lipases are able to work efficiently in such a polar solvent as THF, it is well known that more hydrophobic solvents are generally preferred;[14] in fact, only very stable catalysts such as CALB (or the recently described use of crude lipase from *Pseudomonas stutzeri* in THF)[15] can be employed. Anyhow, when the reaction time was extended to several days the proportion of the diester (**6**) notably increased (Table 4). Remarkably, 2′,5′-*O*-diacetyl-1-β-arabinofuranosyl uracil was not detected at all.

In order to prove if the second acylation took place through an acyl transfer process or if it was indeed an esterification, 5′-*O*-acetyl-1-β-arabinofuranosyl uracil (**2**) was dissolved in MeTHF and maintained with stirring for 192 h at room temperature, but no transformation occurred. This result confirms that both acetyl groups came directly from the reagent (VA). The marked preference for the hydroxyl group on C-3′ as the second place to acylate was attributed to the higher steric hindrance of the 2′-OH, which is situated on the β-position like the nitrogenated base. In fact, uridine was treated with vinyl acetate under the same reaction conditions, giving rise after 192 h to 39% of the derivative esterified in the 5′-position (**8**) and 56% of a 70 : 30 mixture (ratio estimated from the NMR spectra) of the 2′,5′-*O*- and 3′,5′-*O*-diacetyl compounds, which were impossible to separate by traditional analytical methods. In this case, the OH at the C-2′ is not hindered by the base and, therefore, is also accessible for acylation.

High yields, but lower regioselectivity, was reported by Uemura *et al.* for the acylation of 2′-deoxyuridine with hexanoic anhydride by PFL in several organic solvents (DMA, DMSO and DMF) at room temperature after 24 h.[16]

Table 4 Esterification of *ara*-U with vinyl acetate by *Pseudomonas fluorescens* in MeTHF at longer reaction times

Time/h	*Ara*-U (%)	5′-Ester (%) (**2**)	3′,5′-Diester (%) (**6**)
24	3	82	14
48	—	76	24
72	—	72	28
96	—	69	31
192	—	59	41

Acylation of 9-β-arabinofuranosyl adenosine (*ara*-A)

Likewise, a comparative study of the *ara*-A (**15**) esterification with hexanoic anhydride by CALB in MeTHF and THF was performed. In this case, THF turned to be a better solvent for the substrate, giving rise to higher yields than MeTHF. In fact, 81% of 5′-*O*-hexanoyl-9-β-arabinofuranosyl adenosine (**19**) was rendered after 28 h.

A similar behavior was observed for the acylation of adenosine (**20**) under the same reaction conditions, achieving the highest yield after 30 h for the esterification in THF (**21**). In general, longer reaction times are needed for nucleosides with a purine-type base.[17]

Taking into account that for the esterification of *ara*-A with hexanoic anhydride by CALB much higher yields were rendered in THF, this was the solvent of choice for the reactions using vinyl esters as acylating agents. As mentioned before, nucleosides with a purine-type base reacted with a lower conversion rate than those with a pyrimidine-type, *i.e.* longer reaction times were needed for *ara*-A.

In Table 5, it can be noticed that the highest yield was achieved after 24 h for VP and VB, and 28 h for VA (88%). This way, esterification of *ara*-A with vinyl propionate by CALB in THF gave rise to 98% of the 5′ derivative (compound **17**) with total regioselectivity (2% of starting material remained unreacted). In the case of vinyl butyrate, regioselectivity was also excellent, since 97% of 5′-*O*-butanoyl-9-β-arabinofuranosyl adenosine (**18**) was detected together with 2% of substrate and 1% of the corresponding 3′,5′-dibutyrate derivative. The reaction with vinyl acetate underwent slightly slower and rendered a lower yield (product **16**), but the regioselectivity was

Table 5 Results obtained from the esterification of *ara*-A with CALB and several vinyl esters in THF

	Ara-A (%)			5′-Ester (%)			3′,5′-Diester (%)		
Time/h	VA	VP	VB	VA	VP	VB	VA	VP	VB
4	47	46	41	53	54	59	—	—	—
6	43	41	40	57	59	60	—	—	—
8	38	31	29	62	69	71	—	—	—
24	19	2	2	79	98	97	2	—	1
28	10	—	2	88	95	92	2	5	6

Table 6 Results obtained from the esterification of 2′-*O*-methoxyethyl-5-methyluridine with CALB and several vinyl esters in MeTHF

	2′-*O*-methoxyethyl-5-methyluridine (%)			5′-Ester (%)		
Time/h	VA	VP	VB	VA	VP	VB
2	2	2	2	90	95	89
4	—	—	—	93	91	85
6	—	—	—	94	92	91
8	—	—	—	94	89	89
24	—	—	—	94	73	62

similar to the values measured for vinyl butyrate. Esterification of *ara*-A with the same vinyl esters but employing lipases from other microorganisms (MJL, CRL, PCL and PFL) was also tested. As occurred with the *ara*-U acylation, only PFL produced the 5′ ester derivative, but with rather irrelevant yields.

Acylation of 2′-*O*-(2-methoxyethyl)-5-methyl uridine

2′-*O*-Alkyl nucleosides are considered to be key building blocks of several second generation antisense oligonucleotides in clinical development. As promising therapeutic agents, much effort is being made towards the development of novel nuclease resistant oligonucleotides, which are capable of hybridizing with appropriate specificity and affinity to complementary sequences thus acting as effective inhibitors of gene expression.[18] However, these nucleotides should be modified to avoid rapid degradation by cellular nucleases. Therefore, we have esterified 2′-*O*-(2-methoxyethyl)-5-methyl uridine following the same strategy as for *ara*-A and *ara*-U.

Acylation of the latter with hexanoic anhydride in MeTHF gave rise directly to the diester in 3′ and 5′ position, achieving the highest yield after 6 h (89% of 2′-*O*-(2-methoxyethyl)-3′,5′-dihexanoyl-5-methyl uridine, **14**). However, the fact that the reaction was not regioselective and that 8% of compound **14** was formed in the sample without enzyme, prompted us to focus our attention again to vinyl esters. Reaction of this starting material with vinyl acetate rendered 94% of the 5′ acetyl derivative (compound **11**) after 6 h (Table 6). Slightly lower results were obtained with vinyl propionate (92%, product **12**) and vinyl butyrate (91%, compound **13**). This way, the regioselective synthesis of nucleoside derivatives with only the hydroxyl group at C-3′ free was accomplished.

Conclusion

The described enzymatic processes represent an excellent methodology to obtain valuable nucleoside derivatives. Reactions took place in many cases in short reaction times (6 h) and with total regioselectivity, which is arduous to achieve by pure chemical means due to the presence of various hydroxyl groups. A comparison between THF and environmentally friendly MeTHF was established, both solvents behaving in a similar way, while for the acylation of *ara*-U and uridine, MeTHF rendered better conversions. This green solvent can be really valuable in the biotransformations field.

Experimental

General

Ara-U and *ara*-A were kindly provided by Pro. Bio. Simt. S.p.A., 2′-*O*-methoxyethyl-5-methyl uridine by Ravi Chemicals and MeTHF by Penn Specialty Chemicals Inc. CALB (Novozyme435, immobilized lipase from *Candida Antarctica* B) was a gentle donation of Novozymes, Spain. Lipases from *Mucor javanicus* (MJL), *Candida rugosa* (CRL), *Pseudomonas cepacea* (PCL) and *Pseudomonas fluorescens* (Amano Lipase AK) were purchased from Sigma-Aldrich. All other chemicals were obtained from commercial sources. Reaction progress was followed by HPLC on an Agilent LC1200 using the C18 column Mediterranea Sea from Teknokroma, and by TLC on Kieselgel Plates 60 F254 (SDS). TLC plates were visualized under UV light or revealed with 10% H_2SO_4 in methanol and heating. Column chromatography was carried out, if necessary, on silica gel 60, AC, 40–63 μm (purchased from SDS). NMR spectra of samples in DMSO-d_6 were recorded on a Bruker Avance 250 (250 MHz) spectrometer. Compound assignments were based on ^{1}H, ^{13}C, HMQC and HMBC NMR experiments.

Experimental procedures

Esterification of *ara*-U with hexanoic anhydride

Ara-U (120 mg, 0.5 mmol) was dissolved in 50 mL of anhydrous MeTHF and 0.5 g of molecular sieves, 0.5 g of CALB (Novozyme435, 20% w/w loading,[19] 10 000 PLU g^{-1})[20] and 0.35 mL of hexanoic anhydride (1.5 mmol) were added, maintaining the reaction with orbital shaking for 24 h at room temperature. After that, MeOH was added, the reaction mixture was filtered, neutralized with saturated $NaHCO_3$ solution and dried with anhydrous Na_2SO_4. Then, silica gel was added, the solvent was evaporated at reduced pressure and the crude subsequently purified by column chromatography to give 75 mg (45%) of 5′-*O*-hexanoyl-1-β-arabinofuranosyl uracil (**5**): white solid; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.85 (3H, t, $J = 6.3$ Hz, $CH_3CH_2CH_2$), 1.26 (4H, m, $CH_3CH_2CH_2$), 1.54 (2H, t, $J = 6.7$ Hz, CH_2CH_2CO), 2.33 (2H, t, $J = 7.2$ Hz, CH_2CH_2CO), 3.95 (2H, m, H-3′ and H-4′), 4.01 (1H, br s, H-2′), 4.21 (1H, dd, $J_1 = 3.4$ Hz, $J_2 = 11.8$ Hz, 1H-5′), 4.30 (1H, dd, $J_1 = 7.2$ Hz, $J_2 = 11.8$ Hz, 1H-5′), 5.58 (1H, d, $J = 8.1$ Hz, H-5), 5.67 (1H, br s, OH-3′), 5.76 (1H, d, $J = 3.9$ Hz, OH-2′), 6.04 (1H, d, $J = 3.4$ Hz, H-1′), 7.51 (1H, d, $J = 8.1$ Hz, H-6), 11.34 (1H, br s, NH); ^{13}C NMR (DMSO-d_6, 63 MHz): δ 14.2 (CH_3), 22.2 ($CH_3CH_2CH_2$), 24.5 (CH_2CH_2CO), 31.0 ($CH_3CH_2CH_2$), 33.7 (CH_2CH_2CO), 63.8 (C-5′), 75.0 and 76.4 (C-2′ and C-3′), 82.1 (C-4′), 85.7 (C-1′), 100.5 (C-5), 142.6 (C-6), 150.8 (C-2), 163.6 (C-4), 173.2 (CO). Anal. Calcd. for $C_{15}H_{22}N_2O_7$: C, 52.63; H, 6.48; N, 8.18%. Found: C, 52.36; H, 6.44; N, 7.76%.

Acylation of uridine (7) with hexanoic anhydride

0.5 g of molecular sieves, 0.5 g of CALB and 0.35 mL of hexanoic anhydride were added to a solution of 120 mg uridine in 50 mL anhydrous MeTHF. After 24 h with orbital shaking at room temperature, MeOH was added, the reaction mixture filtered, neutralized with saturated $NaHCO_3$ solution and dried with anhydrous Na_2SO_4. Silica gel was added, the

solvent was evaporated at reduced pressure and the residue was chromatographed on a silica gel column to give 71 mg (42%) of 5′-*O*-hexanoyl uridine (**9**): white solid; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.87 (3H, m, CH_3), 1.27 (4H, m, $CH_3CH_2CH_2$), 1.52 (2H, m, CH_2CH_2CO), 2.34 (2H, t, J = 7.3, CH_2CH_2CO), 3.97 (2H, m, H-3′ and H-4′), 4.08 (1H, t, J = 5.0 Hz, H-2′), 4.18 (1H, dd, J_1 = 5.3 Hz, J_2 = 12.1 Hz, 1H-5′), 4.26 (1H, dd, J_1 = 3.6 Hz, J_2 = 12.1 Hz, 1H-5′), 5.33 (1H, br s, OH-3′), 5.52 (1H, br s, OH-2′), 5.67 (1H, d, J = 8.1 Hz, H-5), 5.76 (1H, d, J = 5.8 Hz, H-1′), 7.64 (1H, d, J = 8.1 Hz, H-6), 11.41 (1H, br s, NH); ^{13}C NMR (DMSO-d_6, 63 MHz): δ 14.2 (CH_3), 22.2 ($CH_3CH_2CH_2$), 24.5 (CH_2CH_2CO), 31.0 ($CH_3CH_2CH_2$), 33.6 (CH_2CH_2CO), 64.0 (C-5′), 70.1 (C-3′), 73.0 (C-2′), 81.4 (C-4′), 89.0 (C-1′), 102.3 (C-5), 141.1 (C-6), 151.0 (C-2), 163.4 (C-4), 173.1 (CO). Anal. Calcd. for $C_{15}H_{22}N_2O_7$: C, 52.63; H, 6.48; N, 8.18%. Found: C, 52.83; H, 6.56; N, 7.62%.

Esterification of *ara*-U with vinyl esters

Ara-U (120 mg, 0.5 mmol) was dissolved in 50 mL of anhydrous MeTHF and 0.5 g of molecular sieves, 0.5 g of CALB and 1.5 mmol of vinyl ester (138 μL vinyl acetate, 163 μL vinyl propionate or 190 μL vinyl butyrate) were added, maintaining the reaction with orbital shaking for 7 h at room temperature. After that, MeOH was added, the reaction mixture filtered, neutralized with saturated $NaHCO_3$ solution and dried with anhydrous Na_2SO_4. Then, silica gel was added and the solvent evaporated to dryness. Finally, the residue was purified by column chromatography over silica gel.

5′-*O*-acetyl-1-β-arabinofuranosyl uracil (2). 61 mg (43%): white solid; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 2.06 (3H, s, CH_3CO), 3.93 (2H, m, H-3′ and H-4′), 4.02 (1H, ddd, $J_1 = J_2$ = 4.5 Hz, J_3 = 7.0 Hz, H-2′), 4.21 (1H, dd, J_1 = 4.0 Hz, J_2 = 11.8 Hz, 1H-5′), 4.30 (1H, dd, J_1 = 7.1 Hz, J_2 = 11.8 Hz, 1H-5′), 5.60 (1H, d, J = 8.1 Hz, H-5), 5.71 (1H, d, J = 3.9 Hz, OH-3′), 5.78 (1H, d, J = 4.5 Hz, OH-2′), 6.05 (1H, d, J = 4.5 Hz, H-1′), 7.52 (1H, d, J = 8.1 Hz, H-6), 11.35 (1H, br s, NH); ^{13}C NMR (DMSO-d_6, 63 MHz): δ 21.5 (CH_3), 64.5 (C-5′), 75.5 and 77.0 (C-2′ and C-3′), 82.5 (C-4′), 86.2 (C-1′), 101.0 (C-5), 143.1 (C-6), 151.3 (C-2), 164.1 (C-4), 171.1 (CO). Anal. Calcd. for $C_{11}H_{14}N_2O_7$: C, 46.16; H, 4.93; N, 9.79%. Found: C, 46.17; H, 5.00; N, 9.45%.

5′-*O*-propanoyl-1-β-arabinofuranosyl uracil (3). 87 mg (58%): white solid; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 1.03 (3H, t, J = 7.5 Hz, CH_3CH_2CO), 2.34 (2H, dd, J_1 = 7.5 Hz, J_2 = 15.6 Hz, CH_3CH_2CO), 3.92 (2H, m, H-3′ and H-4′), 4.01 (1H, m, H-2′), 4.23 (2H, m, 2H-5′), 5.58 (1H, d, J = 8.1 Hz, H-5), 5.67 (1H, d, J = 3.4 Hz, OH-3′), 5.74 (1H, d, J = 4.5 Hz, OH-2′), 6.02 (1H, d, J = 4.2 Hz, H-1′), 7.49 (1H, d, J = 8.1 Hz, H-6), 11.30 (1H, br s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 9.3 (CH_3CH_2CO), 27.1 (CH_3CH_2CO), 63.8 (C-5′), 75.0 and 76.4 (C-2′ and C-3′), 82.0 (C-4′), 85.7 (C-1′), 100.5 (C-5), 142.6 (C-6), 150.8 (C-2), 163.7 (C-4), 174.0 (CO). Anal. Calcd. for $C_{12}H_{16}N_2O_7$: C, 48.00; H, 5.37; N, 9.33%. Found: C, 47.86; H, 5.38; N, 9.04%.

5′-*O*-butanoyl-1-β-arabinofuranosyl uracil (4). 84 mg (54%): white solid; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.90 (3H, t, J = 7.4 Hz, $CH_3CH_2CH_2CO$), 1.57 (2H, m, $CH_3CH_2CH_2CO$), 2.33 (2H, t, J = 7.2 Hz, $CH_3CH_2CH_2CO$), 3.95 (2H, m, H-3′ and H-4′), 4.03 (1H, m, H-2′), 4.23 (1H, dd, J_1 = 3.8 Hz, J_2 = 11.8 Hz, 1H-5′), 4.32 (1H, dd, J_1 = 7.1 Hz, J_2 = 11.8 Hz, 1H-5′), 5.60 (1H, d, J = 8.1 Hz, H-5), 5.72 (1H, br s, OH-3′), 5.80 (1H, d, J = 4.2 Hz, OH-2′), 6.05 (1H, d, J = 4.2 Hz, H-1′), 7.52 (1H, d, J = 8.1 Hz, H-6), 11.36 (1H, br s, NH); ^{13}C NMR (DMSO-d_6, 75 MHz): δ 13.7 ($CH_3CH_2CH_2CO$), 18.3 $CH_3CH_2CH_2CO$), 35.6 ($CH_3CH_2CH_2CO$), 63.7 (C-5′), 75.0 and 76.4 (C-2′ and C-3′), 82.1 (C-4′), 85.8 (C-1′), 100.5 (C-5), 142.6 (C-6), 150.8 (C-2), 163.7 (C-4), 173.1 (CO). Anal. Calcd. for $C_{13}H_{18}N_2O_7$: C, 49.68; H, 5.77; N, 8.91%. Found: C, 49.67; H, 5.77; N, 8.70%.

Synthesis of 3′,5′-*O*-acetyl-1-β-arabinofuranosyl uracil (6). 1 g of molecular sieves, 1 g of *Pseudomonas fluorescens* (activity ≥20 000 U g^{-1}, pH 8.0, 55 °C using triolein as substrate)[21] and 276 μL of vinyl acetate were added to a solution of 240 mg of *ara*-U in 100 mL of anhydrous MeTHF. After 8 days with orbital shaking at room temperature, MeOH was added, the reaction mixture filtered, neutralized with saturated $NaHCO_3$ solution and dried with anhydrous Na_2SO_4. Then, silica gel was added, the solvent evaporated at reduced pressure and the residue chromatographed on a silica gel column to give 158 mg (55%) of compound **2** and 139 mg (42%) of 3′,5′-*O*-acetyl-1-β-arabinofuranosyl uracil (**6**): white solid; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 2.06 (3H, s, CH_3CO), 2.10 (3H, s, CH_3CO), 4.15 (1H, ddd, $J_1 = J_2$ = 3.8 Hz, J_3 = 7.0 Hz, H-4′), 4.22 (1H, m, H-2′), 4.32 (2H, m, 2H-5′), 4.96 (1H, br s, H-3′), 5.63 (1H, d, J = 8.1 Hz, H-5), 6.02 (1H, d, J = 3.9 Hz, H-1′), 6.16 (1H, d, J = 4.3 Hz, OH-2′), 7.57 (1H, d, J = 8.1 Hz, H-6), 11.41 (1H, br s, NH); ^{13}C NMR (DMSO-d_6, 63 MHz): δ 21.0 (CH_3CO), 21.1 (CH_3CO), 63.5 (C-5′), 72.5 (C-2′), 78.4 (C-3′), 79.8 (C-4′), 85.8 (C-1′), 100.7 (C-5), 142.4 (C-6), 150.7 (C-2), 163.6 (C-4), 170.1 (CO), 170.6 (CO). Anal. Calcd. for $C_{13}H_{16}N_2O_8$: C, 47.56; H, 4.91; N, 8.53%. Found: C, 47.83; H, 5.62; N, 7.09%.

Acylation of uridine with vinyl acetate by *Pseudomonas fluorescens*

Uridine (240 mg, 1 mmol) was dissolved in 100 mL of anhydrous MeTHF and 1 g of molecular sieves, 1 g of *Pseudomonas fluorescens* and 276 μL of vinyl acetate (3 mmol) were added, maintaining the reaction with orbital shaking for 8 days at room temperature. After that, MeOH was added, the reaction mixture filtered, neutralized with saturated $NaHCO_3$ solution and dried with anhydrous Na_2SO_4. Then, silica gel was added and the solvent evaporated to dryness. Finally, the residue was purified by column chromatography over silica gel yielding 148 mg (53%) of a mixture consisting of 3′,5′-*O*-diacetyl uridine and 2′,5′-*O*-diacetyl uridine, and 106 mg (38%) of 5′-*O*-acetyl uridine (**8**): white solid; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 2.09 (3H, s, CH_3CO), 3.99 (2H, m, H-3′ and H-4′), 4.19 (3H, m, 2H-5′ and H-2′), 5.37 (1H, d, J = 4.5 Hz, OH-3′), 5.55 (1H, d, J = 4.9 Hz, OH-2′), 5.71 (1H, d, J = 8.0 Hz, H-5), 5.77 (1H, d, J = 4.7 Hz, H-1′), 7.67 (1H, d, J = 8.0 Hz, H-6), 11.42 (1H, br s, NH); ^{13}C NMR (DMSO-d_6, 63 MHz): δ 21.0 (CH_3), 64.1 (C-5′), 70.1 (C-3′), 73.0 (C-2′), 81.4 (C-4′), 89.0 (C-1′), 102.4 (C-5), 141.2 (C-6), 151.0 (C-2), 163.5 (C-4), 170.6 (CO). Elemental analysis (Found: C, 46.53; H, 5.19; N, 8.89%. Calc. for $C_{11}H_{14}N_2O_7$: C, 46.16; H, 4.93; N, 9.79%).

Esterification of *ara*-A with hexanoic anhydride

0.5 g of molecular sieves, 0.5 g of CALB and 0.35 mL of hexanoic anhydride were added to a solution of 135 mg of *ara*-A in 50 mL of anhydrous THF. After 24 h with orbital shaking at room temperature, MeOH was added, the reaction mixture filtered, neutralized with saturated $NaHCO_3$ solution and dried with anhydrous Na_2SO_4. Silica gel was added, the solvent was evaporated at reduced pressure and the residue was chromatographed on a silica gel column to give 58 mg (31%) of 5′-*O*-hexanoyl-9-β-arabinofuranosyl adenosine (**19**): yellow solid; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.67 (3H, t, $J = 6.9$ Hz, CH_3), 1.05 (4H, m, $CH_3CH_2CH_2$), 1.31 (2H, m, CH_2CH_2CO), 2.12 (2H, t, $J = 7.2$ Hz, CH_2CH_2CO), 3.78 (1H, ddd, $J_1 = J_2 = 3.6$ Hz, $J_3 = 7.5$ Hz, H-4′), 3.97 (2H, d, $J = 3.6$ Hz, H-2′ and H-3′), 4.08 (1H, dd, $J_1 = 3.6$ Hz, $J_2 = 11.7$ Hz, 1H-5′), 4.19 (1H, dd, $J_1 = 7.5$ Hz, $J_2 = 11.7$ Hz, 1H-5′), 5.62 (2H, br s, OH-2′ and OH-3′), 6.11 (1H, d, $J = 4.2$ Hz, H-1′), 7.10 (2H, br s, NH_2), 7.93 and 7.95 (2H, s, H-2 and H-8); ^{13}C NMR (DMSO-d_6, 63 MHz): δ 14.2 (CH_3), 22.2 ($CH_3CH_2CH_2$), 24.5 (CH_2CH_2CO), 31.0 ($CH_3CH_2CH_2$), 33.6 (CH_2CH_2CO), 64.1 (C-5′), 75.4 and 76.0 (C-2′ and C-3′), 81.3 (C-4′), 83.9 (C-1′), 118.5 (C-5), 140.7 (C-8), 149.7 (C-4), 152.9 (C-2), 156.3 (C-6), 173.2 (CO). Anal. Calcd. for $C_{16}H_{23}N_5O_5$: C, 52.59; H, 6.34; N, 19.17%. Found: C, 51.63; H, 6.01; N, 19.33%.

Acylation of adenosine with hexanoic anhydride

0.5 g of molecular sieves, 0.5 g of CALB and 0.35 mL of hexanoic anhydride were added to a solution of 135 mg of adenosine in 50 mL of anhydrous THF. After 24 h with orbital shaking at room temperature, MeOH was added, the reaction mixture filtered, neutralized with saturated $NaHCO_3$ solution and dried with anhydrous Na_2SO_4. Silica gel was added, the solvent was evaporated at reduced pressure and the residue was chromatographed on a silica gel column to give 62 mg (33%) of 5′-*O*-hexanoyl adenosine (**21**): white solid; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.82 (3H, t, $J_1 = J_2 = 6.8$ Hz, CH_3), 1.21 and 1.48 (6H, m, CH_2), 2.28 (2H, t, $J_1 = J_2 = 7.3$ Hz, CH_2CO), 4.08 (1H, m, H-4′), 4.26 (3H, m, H-3′ and 2H-5′), 4.68 (1H, m, H-2′), 5.41 (1H, d, $J = 4.6$ Hz, OH-3′), 5.62 (1H, d, $J = 5.8$ Hz, OH-2′), 5.91 (1H, d, $J = 4.9$ Hz, H-1′), 7.32 (2H, br s, NH_2), 8.15 (1H, s, H-8), 8.31 (1H, s, H-2); ^{13}C NMR (DMSO-d_6, 63 MHz): δ 14.1 (CH_3), 22.1 (CH_3CH_2), 24.4 (CH_2CH_2CO), 30.9 ($CH_3CH_2CH_2$), 33.6 (CH_2CO), 64.0 (C-5′), 70.6 (C-3′), 73.2 (C-2′), 81.8 (C-4′), 88.1 (C-1′), 119.5 (C-5), 140.1 (C-8), 149.7 (C-4), 153.0 (C-2), 156.4 (C-6), 173.1 (CO). Anal. Calcd. for $C_{16}H_{23}N_5O_5$: C, 52.59; H, 6.34; N, 19.17%. Found: C, 52.17; H, 6.20; N, 18.65%.

Esterification of *ara*-A with vinyl esters

Ara-A (135 mg, 0.5 mmol) was dissolved in 50 mL of anhydrous MeTHF and 0.5 g of molecular sieves, 0.5 g of CALB and 1.5 mmol of vinyl ester (138 μL vinyl acetate, 163 μL vinyl propionate or 190 μL vinyl butyrate) were added, maintaining the reaction with orbital shaking for 24 h at room temperature. After that, MeOH was added, the reaction mixture filtered, neutralized with saturated $NaHCO_3$ solution and dried with anhydrous Na_2SO_4. Then, silica gel was added and the solvent evaporated to dryness. Finally, the residue was purified by column chromatography over silica gel.

5′-*O*-acetyl-9-β-Arabinofuranosyl adenosine (16). 102 mg (65%): white solid; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 2.04 (3H, s, CH_3CO), 3.98 (1H, m, H-4′), 4.20 (2H, br s, H-2′ and H-3′), 4.33 (2H, m, H-5′), 5.80 (1H, d, $J = 4.1$ Hz, OH-3′), 5.85 (1H, d, $J = 4.5$ Hz, OH-2′), 6.32 (1H, d, $J = 4.2$ Hz, H-1′), 7.32 (2H, br s, NH_2), 8.16 and 8.17 (2H, s, H-2 and H-8); ^{13}C NMR (DMSO-d_6, 63 MHz): δ 21.0 (CH_3), 64.3 (C-5′), 75.4 and 76.0 (C-2′ and C-3′), 81.2 (C-4′), 83.9 (C-1′), 118.5 (C-5), 140.8 (C-8), 149.7 (C-4), 152.9 (C-2), 156.2 (C-6), 170.7 (CO). Anal. Calcd. for $C_{12}H_{15}N_5O_5$: C, 46.60; H, 4.89; N, 22.64%. Found: C, 45.96; H, 4.63; N, 22.51%.

5′-*O*-propanoyl-9-β-Arabinofuranosyl adenosine (17). 111 mg (68%): white solid; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 1.03 (3H, t, $J = 7.5$ Hz, CH_3), 2.35 (2H, dd, $J_1 = 7.5$ Hz, $J_2 = 14.9$ Hz, CH_3CH_2), 3.99 (1H, m, H-4′), 4.20 (2H, br s, H-2′ and H-3′), 4.34 (2H, m, H-5′), 5.79 (1H, d, $J = 3.7$ Hz, OH-3′), 5.85 (1H, d, $J = 4.1$ Hz, OH-2′), 6.31 (1H, d, $J = 4.0$ Hz, H-1′), 7.32 (2H, br s, NH_2), 8.15 and 8.16 (2H, s, H-2 and H-8); ^{13}C NMR (DMSO-d_6, 63 MHz): δ 9.3 (CH_3), 27.1 (CH_3CH_2), 64.1 (C-5′), 75.4 and 75.9 (C-2′ and C-3′), 81.2 (C-4′), 83.8 (C-1′), 118.5 (C-5), 140.7 (C-8), 149.7 (C-4), 152.9 (C-2), 156.2 (C-6), 174.0 (CO). Anal. Calcd. for $C_{13}H_{17}N_5O_5$: C, 48.29; H, 5.30; N, 21.66%. Found: C, 47.97; H, 4.90; N, 21.68%.

5′-*O*-Butanoyl-9-β-arabinofuranosyl adenosine (18). 107 mg (63%): white solid; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.88 (3H, t, $J = 7.3$ Hz, CH_3), 1.55 (2H, ddd, $J_1 = J_2 = 7.3$ Hz, $J_3 = 14.7$ Hz, CH_3CH_2), 2.32 (2H, t, $J_1 = J_2 = 7.1$ Hz, CH_2CO), 3.98 (1H, m, H-4′), 4.27 (2H, m, H-2′ and H-3′), 4.37 (2H, m, H-5′), 5.76 (1H, d, $J = 3.7$ Hz, OH-3′), 5.82 (1H, d, $J = 4.2$ Hz, OH-2′), 6.31 (1H, d, $J = 4.0$ Hz, H-1′), 7.31 (2H, br s, NH_2), 8.13 and 8.16 (2H, s, H-2 and H-8); ^{13}C NMR (DMSO-d_6, 63 MHz): δ 13.8 (CH_3), 18.3 (CH_3CH_2), 35.6 (CH_2CO), 64.1 (C-5′), 75.4 and 76.0 (C-2′ and C-3′), 81.3 (C-4′), 83.9 (C-1′), 118.5 (C-5), 140.7 (C-8), 149.7 (C-4), 152.9 (C-2), 156.3 (C-6), 173.1 (CO). Anal. Calcd. for $C_{14}H_{19}N_5O_5$: C, 49.85; H, 5.68; N, 20.76%. Found: C, 49.82; H, 5.57; N, 20.97%.

Acylation of 2′-*O*-(2-methoxyethyl)-5-methyl uridine with hexanoic anhydride

0.5 g of molecular sieves, 0.5 g of CALB and 0.35 mL of hexanoic anhydride were added to a solution of 160 mg 2′-*O*-(2-methoxyethyl)-5-methyl uridine in 50 mL of anhydrous MeTHF. After 24 h with orbital shaking at room temperature, MeOH was added, the reaction mixture filtered, neutralized with saturated $NaHCO_3$ solution and dried with anhydrous Na_2SO_4. Silica gel was added, the solvent was evaporated at reduced pressure and the residue was chromatographed on a silica gel column to give 86 mg (43%) of 2′-*O*-(2-methoxyethyl)-3′,5′-dihexanoyl-5-methyl uridine (**14**): yellow solid; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.88 (6H, m, $2 \times CH_3$), 1.28 (8H, m, $2 \times CH_3CH_2CH_2$), 1.52 (4H, m, $2 \times CH_2CH_2CO$), 1.81 (3H, s, CH_3), 2.19 (2H, t, $J = 7.3$ Hz, CH_2CH_2CO), 2.35 (2H, t, $J = 7.3$ Hz, CH_2CH_2CO), 3.23 (3H, s, OCH_3), 3.45 (2H, *t*, $J = 4.6$ Hz, $OCH_2CH_2OCH_3$), 3.67 (2H, m, $OCH_2CH_2OCH_3$), 4.04 (3H, m, H-2′, H-3′ and H-4′), 4.24 (1H, m, 2H-5′), 5.85 (1H,

d, $J = 4.9$ Hz, H-1′), 7.46 (1H, s, H-6), 11.43 (1H, br s, NH); ^{13}C NMR (DMSO-d_6, 63 MHz): δ 12.5 (CH_3), 14.1 and 14.2 ($2 \times CH_3CH_2CH_2$), 22.1 and 22.2 ($2 \times CH_3CH_2CH_2$), 24.4 and 24.6 (CH_2CH_2CO), 31.0 and 31.1 ($2 \times CH_3CH_2CH_2$), 33.7 and 34.1 (CH_2CH_2CO), 58.4 (OCH_3), 63.8 (C-5′), 69.0 (C-3′), 69.4 ($OCH_2CH_2OCH_3$), 71.6 ($OCH_2CH_2OCH_3$), 80.6 and 81.6 (C-2′ and C-4′), 87.0 (C-1′), 110.1 (C-5), 136.4 (C-6), 150.9 (C-2), 164.0 (C-4), 173.1 and 175.0 ($2 \times$ CO). Anal. Calcd. for $C_{25}H_{40}N_2O_9$: C, 58.58; H, 7.87; N, 5.47%. Found: C, 56.40; H, 7.52; N, 5.87%.

Esterification of 2′-*O*-(2-methoxyethyl)-5-methyl uridine with vinyl esters

2′-*O*-(2-methoxyethyl)-5-methyl uridine (120 mg, 0.5 mmol) was dissolved in 50 mL of anhydrous MeTHF and 0.5 g of molecular sieves, 0.5 g of CALB and 1.5 mmol of vinyl ester (138 μL vinyl acetate, 163 μL vinyl propionate or 190 μL vinyl butyrate) were added, maintaining the reaction with orbital shaking for 5 h at room temperature. After that, MeOH was added, the reaction mixture filtered, neutralized with saturated $NaHCO_3$ solution and dried with anhydrous Na_2SO_4. Then, silica gel was added and the solvent evaporated to dryness. Finally, the residue was purified by column chromatography over silica gel.

2′-*O*-(2-methoxyethyl)-5′-acetyl-5-methyl uridine (11). 128 mg, 70%: yellow solid; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 1.81 (3H, s, CH_3), 2.08 (3H, s, CH_3CO), 3.23 (3H, s, OCH_3), 3.48 (2H, m, $OCH_2CH_2OCH_3$), 3.68 (2H, m, $OCH_2CH_2OCH_3$), 4.05 (3H, m, H-2′, H-3′ and H-4′), 4.22 (2H, m, H-5′), 5.32 (1H, d, $J = 5.8$ Hz, OH-3′), 5.84 (1H, d, $J = 4.8$ Hz, H-1′), 7.47 (1H, s, H-6), 11.44 (1H, br s, NH); ^{13}C NMR (DMSO-d_6, 63 MHz): δ 12.5 (CH_3), 21.0 (CH_3CO), 58.5 (OCH_3), 63.9 (C-5′), 69.0 (C-3′), 69.4 ($OCH_2CH_2OCH_3$), 71.6 ($OCH_2CH_2OCH_3$), 80.6 and 81.6 (C-2′ and C-4′), 87.1 (C-1′), 110.2 (C-5), 136.4 (C-6), 150.9 (C-2), 164.1 (C-4) and 170.6 (CO). Anal. Calcd. for $C_{15}H_{22}N_2O_8$: C, 50.28; H, 6.19; N, 7.82%. Found: C, 50.50; H, 6.26; N, 7.28%.

2′-*O*-(2-methoxyethyl)-5′-propanoyl-5-methyl uridine (12). 130 mg, 68%: white solid; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 1.06 (3H, t, $J = 7.4$, CH_3CH_2CO), 1.81 (3H, s, CH_3), 2.37 (2H, dd, $J_1 = 7.5$ Hz, $J_2 = 14.9$ Hz, CH_3CH_2CO), 3.23 (3H, s, OCH_3), 3.48 (2H, m, $OCH_2CH_2OCH_3$), 3.68 (2H, m, $OCH_2CH_2OCH_3$), 4.05 and 4.11 (3H, m, H-2′, H-3′ and H-4′), 4.25 (2H, m, H-5′), 5.33 (1H, d, $J = 5.8$ Hz, OH-3′), 5.85 (1H, d, $J = 4.8$ Hz, H-1′), 7.46 (1H, s, H-6), 11.45 (1H, br s, NH); ^{13}C NMR (DMSO-d_6, 63 MHz): δ 9.3 (CH_3CH_2CO), 12.5 (CH_3), 27.0 (CH_3CH_2CO), 58.5 (OCH_3), 63.8 (C-5′), 69.0 (C-3′), 69.4 ($OCH_2CH_2OCH_3$), 71.6 ($OCH_2CH_2OCH_3$), 80.6 and 81.6 (C-2′ and C-4′), 87.1 (C-1′), 110.2 (C-5), 136.4 (C-6), 150.9 (C-2), 164.1 (C-4) and 173.9 (CO). Anal. Calcd. for $C_{16}H_{24}N_2O_8$: C, 51.61; H, 6.50; N, 7.52%. Found: C, 51.56; H, 6.48; N, 7.28%.

2′-*O*-(2-methoxyethyl)-5′-butanoyl-5-methyl uridine (13). 128 mg, 65%: white solid; ^{1}H NMR (DMSO-d_6, 250 MHz): δ 0.89 (3H, t, $J = 7.3$ Hz, $CH_3CH_2CH_2CO$), 1.57 (2H, ddd, $J_1 = J_2 = J_3 = 7.3$ Hz, $CH_3CH_2CH_2CO$), 1.81 (3H, s, CH_3), 2.35 (2H, t, $J = 7.2$ Hz, $CH_3CH_2CH_2CO$), 3.23 (3H, s, OCH_3), 3.48 (2H, m, $OCH_2CH_2OCH_3$), 3.67 (2H, m, $OCH_2CH_2OCH_3$), 4.05 (3H, m, H-2′, H-3′ and H-4′), 4.24 (1H, m, 2H-5′), 5.31 (1H, d, $J =$ 5.7 Hz, OH-3′), 5.84 (1H, d, $J = 4.8$ Hz, H-1′), 7.46 (1H, s, H-6), 11.44 (1H, br s, NH); ^{13}C NMR (DMSO-d_6, 63 MHz): δ 13.0 and 14.2 (CH_3 and $CH_3CH_2CH_2CO$), 18.7 $CH_3CH_2CH_2CO$), 36.1 ($CH_3CH_2CH_2CO$), 59.0 (OCH_3), 64.2 (C-5′), 69.5 and 69.9 (C-3′ and $OCH_2CH_2OCH_3$), 72.1 ($OCH_2CH_2OCH_3$), 81.1 and 82.1 (C-2′ and C-4′), 87.6 (C-1′), 110.7 (C-5), 136.9 (C-6), 151.4 (C-2), 164.6 (C-4), 173.5 (CO). Anal. Calcd. for $C_{17}H_{26}N_2O_8$: C, 52.84; H, 6.78; N, 7.25%. Found: C, 52.61; H, 6.80; N, 6.95%.

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21 Information from www.Sigma-Aldrich.com.

