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A Versatile Synthesis of 5′-Functionalized Nucleosides Through Regioselective Enzymatic Hydrolysis of Their Peracetylated Precursors

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We describe a chemo-enzymatic synthesis of modified nucleosides through lipase-catalyzed hydrolysis of their peracetylated precursors. It was found from screening of a large number of substrates that these enzymes' regioselectivities were affected by the sugar and the nucleobase structures. By selecting the best enzyme for each substrate in terms of activity and regioselectivity, we prepared a small library of differently monodeprotected purine and pyrimidine nucleosides useful as intermediates for the synthesis of high-value nucleosides and mononucleotides. By this approach, the chemo-enzymatic preparation of doxifluridine (**14**) and uridine 5′-monophosphate (5′-UMP, **15**) from peracetylated uridine **1** was carried out. Elimination of many of the processing stages associated with existing methods was achieved, and higher yields and products of increased purity were generated.

Introduction

The importance of nucleosides and nucleotides in different market sectors (pharmaceutical, food, biotechnology…) has led to various approaches for their synthesis. Current methodologies either employ natural nucleosides as starting materials or are based on convergent approaches involving condensation of the carbohydrate precursors and the heterocyclic bases. In both cases chemo-, regio-, and stereoselectivities are ensured through the use of protecting groups and specific deprotection reactions. As a result, chemical syntheses of nucleoside analogues involve many stages and are often low-yielding or unreliable, particularly for modified nucleosides.[1,2] Additionally, the resultant products may not be sufficiently clean for downstream reactions in spite of complex purification processes.

One of the main fields of nucleoside research explored in the past has involved nucleoside analogues as antitumor and antiviral agents.[3,4] However, interest in modified nucleosides, also used as synthons for the preparation of these drugs, is still growing, so it is not surprising that novel and more efficient syntheses are being sought.[5] Since nucleosides possess several functional groups with similar reactivities, selective methods that do not produce isomers would be desirable. This would also allow better control over by-product content in the final compounds.

Enzymatic syntheses have been generally shown to be an advantageous alternative both to chemical methods[6] and to the coupling of chemical methods and biochemical transformations. This strategy often provides synthetic routes with fewer steps and improved overall synthetic efficiency in yields and regio- and stereoselectivities. Enzymes' inherent selectivities, as well as their ability to function under mild conditions, can assist easier obtainment of pure products. Enzymes can be successfully exploited both for nucleoside functionalization[5] and for glycosidic bond formation.[6,7]

Suitable introduction and removal of protecting groups is often critical. In nucleoside chemistry, particularly in the sugar moieties, this problem is accentuated by the presence of multiple hydroxy functions with similar reactivities. In this case, stereo- and regioselective acylation and deacylation of the hydroxy groups can be achieved by using lipases as biocatalysts. Lipases (glycerol ester hydrolases, E.C. 3.1.1.3) are extremely versatile thanks to their efficiency, easy workup, and stability in aqueous and organic solvents.[8,9] Indeed, many examples of lipase-catalyzed deacylation of protected nucleosides are known.[5,10] In particular, when peracetylated nucleosides are used as substrates, it has been reported that ribonucleosides are generally deprotected at the primary hydroxy group, whereas in the case of the 2′-deoxyribo counterparts the 3′-position is preferentially hydrolyzed. However, only a few reports have so far been published on the influence either of non-natural sugars (e.g., arabinose) or of the nucleobase (mostly pyrimidines) on the enzymes' regioselectivities. Moreover,

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all those studies, with few exceptions,[11] have been performed with the native enzymes.

In this work, lipases from different biological sources were immobilized on hydrophobic supports[12] and used for the regioselective hydrolysis of several peracetylated nucleosides, including some substrates never tested before, such as arabinosyl derivatives. As a protective group, the acetyl moiety answers all the requisites for the development of a facile and scalable synthesis of monodeprotected nucleosides: it is recognized as a substrate by lipases and it can be introduced by a cheap, high-yielding, and quick reaction. Moreover, the use of immobilized enzymes increases the enzymes' stabilities under a wide range of experimental conditions and enables the reuse of the catalysts.

An extensive screening of substrates and immobilized enzymes provided both a small "enzymatic library" of easy to handle biocatalysts characterized by different regioselectivities and a small library of differently monodeprotected purine and pyrimidine nucleosides useful as intermediates for the synthesis of high-value modified nucleosides and mononucleotides.

The chemo-enzymatic approach described here has been successfully shown to be efficient and versatile. In fact, after selection of the best enzyme in terms of activity and regioselectivity for the 5′-position of peracetylated uridine (**1**, Scheme 1), this biocatalyst was used in the preparative synthesis of 5′-deoxy-5-fluorouridine (doxifluridine, **14**, Scheme 4, below) and 5′-uridinemonophosphate (5′-UMP, **15**, Scheme 5, below). Doxifluridine (**14**) is used in cancer chemotherapy as a 5-fluorouracil pro-drug,[13] whereas 5′-UMP (**15**) and the other natural 5′-monophosphates have attracted considerable attention on the food market as additives in infant dietary formulas.[14]

Results and Discussion

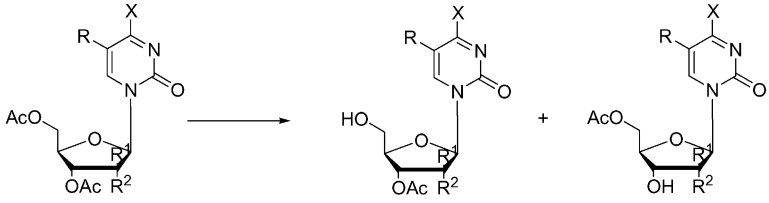
Regioselective Enzymatic Hydrolysis of Pyrimidine Nucleoside Derivatives

Lipases from different biological sources [*Candida rugosa* lipase (CRL), *Pseudomonas fluorescens* lipase (PFL), *Pseudomonas cepacia* lipase (PCL), and porcine pancreas lipase (PPL)] were immobilized on a hydrophobic support (octylsepharose) (see Materials and Methods). The enzyme preparations were then tested in the hydrolysis of different nucleoside esters, by studying the influence of the sugar and the base on each enzyme's regioselectivity. For this purpose, the screening of the lipases was performed with the aim of identifying the best catalysts in terms of activity and regioselectivity in the hydrolysis of peracetylated nucleosides, prepared by standard procedures (see Exp. Sect. for details). In order to investigate the role of the sugar components, the peracetylated substrates uridine (**1**), arabinosyluracil (**2**), and 2′-deoxyuridine (**3**) were considered first (Scheme 1).

These compounds share the same base (uracil) but have different sugar components. Enzymatic hydrolysis was carried out at room temperature in phosphate buffer (pH 7) containing acetonitrile (10% *v*/*v*).

As reported in Table 1, in the enzymatic deprotection of peracetylated uridine (**1**), CRL and PFL each exhibited a marked preference for the 5′-position. After 19 h, PFL had afforded an almost quantitative yield of 2′,3′-*O*-acetyluridine (**1a**, 93%), whereas the yield of the CRL-catalyzed hydrolysis was 85%. The relevance of 5′-monodeprotected peracetylated uridine **1a** lies in the use of this molecule as an intermediate for the synthesis of doxifluridine (**14**, Scheme 4, below) by 5′-deoxygenation and for its 5′-phosphorylation to give 5′-uridinemonophosphate (**15**, 5′-UMP, Scheme 5, below).

In the enzymatic hydrolysis of peracetylated arabinosyluracil (**2**), the lipases under investigation all displayed low regioselectivities with the exception of CRL, which afforded 2′,3′-*O*-acetylarabinosyluracil (**2a**) in 89% yield. It is interesting to note that both ribosyl- and arabinosyluracil are preferentially hydrolyzed at the primary hydroxy group. Some other data relating to the 5′-regioselectivities of free lipases with regard to peracetylated uridine (**1**) have been published;[5,10b] these data are in agreement with the results obtained with immobilized enzymes reported here. In contrast, to the best of our knowledge, few reports relating to the study of lipase regioselectivity on arabinosyl derivatives have so far been published.[15]



1 R = H R^1 = OAc R^2 = OAc X = OH
2 R = OAc R^1 = OAc R^2 = H X = OH
3 R = H R^1 = H R^2 = H X = OH
4 R = H R^1 = H R^2 = H X = OH
5 R = H R^1 = OAc R^2 = OAc X = NH_2
6 R = H R^1 = OAc R^2 = OAc X = NHAc

1a R = H R^1 = OAc R^2 = OAc
2a R = OAc R^1 = OAc R^2 = OH
3a R = H R^1 = H R^2 = H
4a R = CH_3 R^1 = H R^2 = H
5a R = H R^1 = OAc R^2 = OAc
6a R = H R^1 = OAc R^2 = OAc

3b R = H R^1 = H R^2 = H
4b R = CH_3 R^1 = H R^2 = H

Scheme 1. Enzymatic hydrolysis of peracetylated nucleosides **1**–**6**; experimental conditions: immobilized lipase, CH_3CN (10%) in KH_2PO_4 buffer (pH 7, 25 mM), room temp.

Table 1. Enzymatic screening of peracetylated pyrimidine nucleosides **1**–**6**.[a]

Substrate	Enzyme	*t* [h]	*Vh*[b]	% Conv.	Product (% yield)	
					5′-OH	3′-OH
1	CRL	24	0.09	>98	**1a** (85)	n.i.[c]
	PFL	19	0.24	100	**1a** (93)	
	PCL	29	0.04	95	**1a** (73)	
	PPL	48	0.02	64	**1a** (59)	
2	CRL	24	0.07	97	**2a** (89)	n.i.[c]
	PFL	24	<0.01	92	**2a** (2)	
	PCL	24	0.04	100	**2a** (66)	
	PPL	24	<0.01	39	**2a** (6)	
3	CRL	4	0.34	95	**3a** (24)	**3b** (65)
	PFL	30	0.02	6	**3a** (33)	**3b** (28)
	PCL	24	0.12	72	**3a** (6)	**3b** (59)
	PPL	48	0.01	8	**3a** (32)	**3b** (5)
4	CRL	24	0.10	>98	**4a** (4)	**4b** (91)
	PFL	2	1.07	96	**4a** (11)	**4b** (46)
	PCL	24	0.28	96	**4a** (13)	**4b** (73)
	PPL	48	0.02	34	**4a** (26)	**4b** (5)
5	CRL	120	0.02	97	**5a** (77)	n.i.[c]
	PFL	72	<0.01	39	**5a** (19)	
	PCL	98	<0.01	61	**5a** (39)	
	PPL	48	0.03	43	**5a** (34)	
6	CRL	22	0.17	100	**6a** (86)	n.i.[c]
	PFL	4	0.30	95	**6a** (93)	
	PCL	24	0.12	>98	**6a** (72)	
	PPL	48	0.02	19	**6a** (16)	

[a] Experimental conditions: CH_3CN (10%) in KH_2PO_4 buffer (pH 7, 25 mM), immobilized lipase (50 IU), reaction volume 2.5 mL, [substrate]: 10 mM, room temp. [b] μmol × min/IU. [c] n.i.: not isolated.

Enzymatic deacetylation of 3′,5′-di-*O*-acetyl-2′-deoxyuridine (**3**) was achieved only with low regioselectivity in all cases. The 3′-deprotected nucleoside, however, was formed as the major product, although only in moderate yield in comparison with its ribosyl and arabinosyl counterparts. In fact, the most efficient biocatalysts (CRL and PCL) afforded 5′-*O*-acetyl-2′-deoxyuridine (**3b**) in 65% and 59% yields, respectively. The other tested lipases showed poor activity with this substrate.

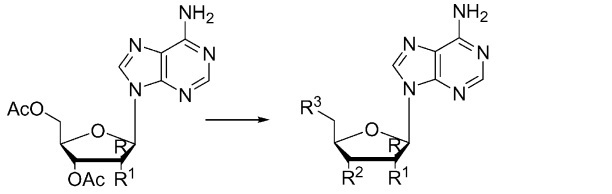
Surprisingly, when 3′,5′-di-*O*-acetylthymidine (**4**) was subjected to enzymatic hydrolysis by CRL the yield of the 3′-monodeprotected compound **4b** increased from 65% (for **3b**) up to 91%. The regioselectivity for the 3′-position generally observed for 2′-deoxynucleosides was preserved. This selectivity might be exploitable for easy preparation of starting materials for 3′-functionalized thymidines such as the well known anti-HIV drug 3′-azido-3′-deoxythymidine (zidovudine, AZT).[16]

Chemical acetylation of cytidine as substrate afforded the tri- and the tetraacetylated derivatives **5** and **6**, respectively, due to the presence of the exocyclic amino group. In the enzymatic hydrolysis of 2′,3′,5′-tri-*O*-acetylcytidine (**5**), CRL was identified as the best enzyme for the 5′-position (77% yield of **5a**, 120 h). Poorer regioselectivities were observed for all the other enzymes under the same experimental conditions. Interestingly, in the case of the tetraacetylated cytidine **6** the performance of CRL was even better (86%), but what is noteworthy is the result achieved with PFL: almost quantitative conversion (93%) of **6a** was achieved with a remarkable increase in the biotransformation rate (4 h). In contrast, 2′,3′,5′-tri-*O*-acetylcytidine (**5**) was poorly hydrolyzed by PFL (19% of **5a** after 72 h). The results relating to cytidine derivatives **5**, **6** and 2′-deoxyribonucleosides **3**, **4** reported here clearly show that the regioselectivity of a biocatalyst may strongly depend on the nature of the substrate. Even a minimal structural variation can play a crucial role in affecting the outcome of the reaction.

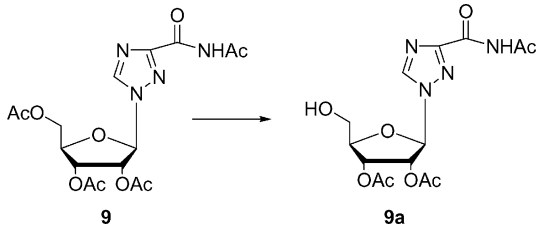
Products **1a**–**3a**, **3b**, **4a**, **4b**, **5a**, and **6a** were isolated, fully characterized by ^1H and COSY NMR, and then used as analytical standards. All the results are summarized in Table 1.

Regioselective Enzymatic Hydrolysis of Purine Nucleoside Derivatives

Regioselective lipase-catalyzed hydrolysis of the peracetylated purine nucleosides **7**–**9** was also investigated (see Schemes 2 and 3).



Scheme 2. Enzymatic hydrolysis of peracetylated nucleosides **7**–**8**; experimental conditions: immobilized lipase, CH_3CN (10%) in KH_2PO_4 buffer (pH 7, 25 mM), room temp.



Scheme 3. Enzymatic hydrolysis of peracetylated ribavirin (**9**); experimental conditions: immobilized lipase, CH_3CN (10%) in KH_2PO_4 buffer (pH 7, 25 mM), room temp.

For this purpose, adenosine, arabinosyladenine, and ribavirin were considered. The results are shown in Table 2.

The enzymatic deacylation of 2′,3′,5′-tri-*O*-acetyladenosine (**7**), consistently with the results reported above for ribonucleosides, was more selective for the 5′-hydroxy group. In fact, 2′,3′-*O*-acetyladenosine (**7a**) was synthesized in 89% yield after 24 h when PFL was used as biocatalyst.

2′,3′,5′-Tri-*O*-acetylarabinosyladenine (**8**) was selectively hydrolyzed at C-3′ (to afford **8b**) by CRL in up to 77% yield in 24 h. This result is very interesting in comparison with the data obtained for arabinosyluracil (Table 1): depending on the nucleobase, the regioselectivity of CRL

Table 2. Enzymatic screening of peracetylated nucleosides **7**–**9**.[a]

Substrate	Enzyme	*t* [h]	*Vh*[b]	% Conv.	Product (% yield) 5′-OH	3′-OH
7	CRL	6	0.25	>98	**7a** (59)	n.i.[c]
	PFL	24	0.14	>98	**7a** (89)	
	PCL	24	0.09	>98	**7a** (64)	
	PPL	48	0.01	38	**7a** (28)	
8	CRL	24	0.10	91	n.i.	**8b** (77)
	PFL	48	0.01	7		**8b** (6)
	PCL	48	0.03	28		**8b** (18)
	PPL	48	0.04	48		**8b** (43)
9	CRL	24	0.06	98	**9a** (13)	n.i.[c]
	PFL	3	0.58	97	**9a** (93)	
	PCL	24	0.09	>98	**9a** (44)	
	PPL	24	0.18	86	**9a** (7)	

[a] Experimental conditions: CH_3CN (10%) in KH_2PO_4 buffer (pH 7, 25 mM), immobilized lipase (50 IU), reaction volume 2.5 mL, [substrate]: 10 mM, room temp. [b] μmol × min/IU. [c] n.i.: not isolated.

switched from C-5′ for the peracetylated arabinosyluracil **2** to C-3′ for the peracetylated arabinosyladenine **8**.

Ribavirin, used for the treatment of hepatitis C infection,[17] is an example of a base-modified purine ribonucleoside. We studied the enzymatic hydrolysis of peracetylated ribavirin (**9**) because of the presence of the unnatural nucleobase. The regioselectivity of CRL, the most efficient biocatalyst (93% yield in 3 h), was not affected by the base. In fact, and consistently with most of the peracetylated ribonucleosides considered here (uridine, cytidine, adenosine), which were selectively deprotected at C-5′ by CRL, 2′,3′-di-*O*-acetylribavirin (**9a**) was also generated as the main product in the case of ribavirin.

Products **7a**, **8b**, and **9a** were isolated, fully characterized by ^1H and COSY NMR, and then used as analytical standards. All the results are summarized in Table 2.

Comparison between Immobilized and Native Enzymes

Figure 1 shows enzymatic hydrolyses of **1** and **2** with immobilized and native CRL. In both cases the use of the immobilized enzyme positively affected the reaction rate and the yield. In fact, the hydrolysis of **1** and **2** by immobilized CRL produced **1a** and **2a** in about 90% yields (in 24 h), whereas when native CRL was used the yields of the monodeprotected compounds (**1a** and **2a**, respectively) were 30% after the same reaction time. Additionally, it is well known that many applications can benefit from use of immobilized enzymes rather than their soluble counterparts – as reusable heterogeneous biocatalysts, for instance – through reduction of production costs by recycling and control of the process. Moreover, enzyme immobilization can result in improvement in enzyme performance: for example, in stability, selectivity, and activity. It is currently possible to draw the conclusion that immobilized enzymes, as in this case, can perform better than the native enzymes (if the immobilization method is correctly selected).[18]

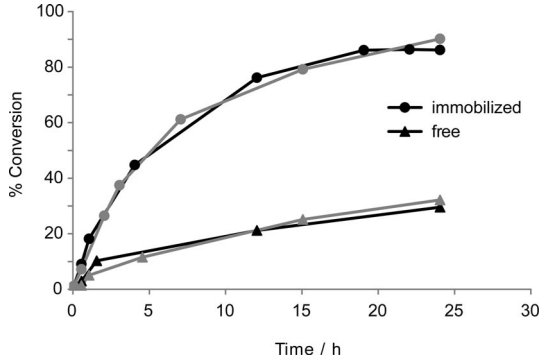
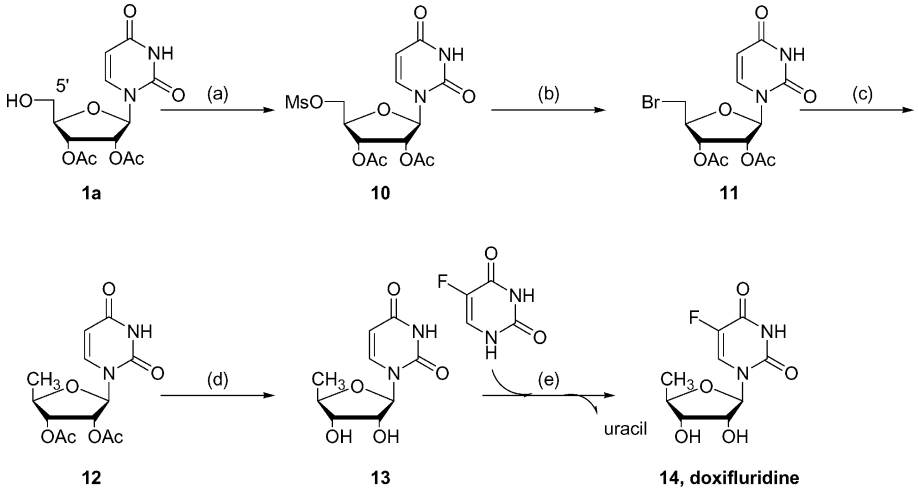


Figure 1. Enzymatic hydrolysis of peracetylated uridine, (**1**, black) and arabinosyluracil (**2**, gray) catalyzed by free CRL (triangle) and by immobilized CRL (circle).



Scheme 4. Chemo-enzymatic synthesis of doxifluridine (**14**); experimental conditions: a) $MeSO_2Cl$, pyridine, room temp. and 0 °C (yield 91%); b) $Br^-N^+Bu_4$, DMF, 130 °C (yield 60%); c) $(Bu)_3SnH$, AIBN, toluene, room temp. (yield 65%); d) MeOH/NaOMe, room temp. (yield 92%); e) immobilized uridine phosphorylase from *Bacillus subtilis* (UP),[21] KH_2PO_4 buffer (pH 7.5, 10 mM), room temp. (conv.: 38%, not optimized).

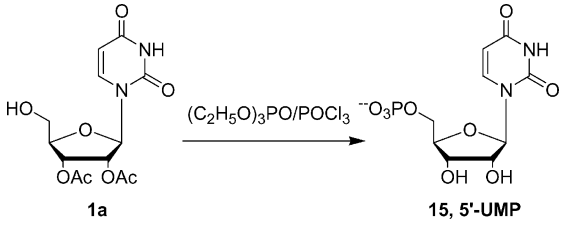
Chemo-Enzymatic Synthesis of 5′-Deoxy-5-fluorouridine (Doxifluridine, 14) and 5′-Uridinemonophosphate (5′-UMP, 15)

2′,3′,5′-Tri-*O*-acetyluridine (**1**) was subjected to PFL-catalyzed regioselective enzymatic hydrolysis to give **1a**. This nucleoside, bearing only one free hydroxy group, at 5′, was directly used without purification as an intermediate for functionalization (Scheme 4).

2′,3′-Di-*O*-acetyl-5′-*O*-mesyluridine (**10**) was prepared in 91% yield by treatment of 2′,3′-di-*O*-acetyluridine (**1a**) with mesyl chloride in pyridine.[19]

2′,3′-Di-*O*-acetyl-5′-bromouridine (**11**) was prepared in 60% yield from **10** by replacement of the mesyloxy group by bromine, accomplished by treatment with tetrabutylammonium bromide in DMF at 130 °C.[19] Subsequent tributyltin hydride reduction of **11** in a mixture of toluene and ethanol at reflux with initiation with AIBN afforded 2′,3′-di-*O*-acetyl-5′-deoxyuridine (**12**) in 65% yield.[19] Compound **12** was deprotected with sodium methoxide in anhydrous MeOH to afford 5′-deoxyuridine (**13**) in 92% yield.[20] This compound was used, after purification, for enzymatic (pyrimidine/pyrimidine) transglycosylation with 5-fluorouracil. This bioprocess, catalyzed by immobilized UP (E.C. 2.4.2.1) from *Bacillus subtilis*,[21] gave doxifluridine (**14**) in about 40% conversion (not optimized).

The phosphorylation of the intermediate **1a** (Scheme 5) was performed with phosphorus oxychloride ($POCl_3$) in triethyl phosphate (TEP)[22] to give the fully deprotected nucleoside 5′-monophosphate directly with 97% conversion. Uridine 5′-monophosphate (**15**, 5′-UMP) was obtained by a chemo-enzymatic two-step process starting from peracetylated uridine (**1**).



Scheme 5. Chemo-enzymatic synthesis of 5′-UMP (**15**).

Conclusions

Four immobilized lipases (CRL, PFL, PCL, PPL) were tested in the hydrolysis of a set of peracetylated nucleosides **1**–**9** to study the influence of different sugars and bases on the enzymes' regioselectivities.

The resulting products, generally deprotected at either 3′ or 5′, can be used as starting materials for the preparation of 3′- or 5′-functionalized nucleosides and mononucleotides. All these molecules are important in pharmaceuticals, the food industry, and biological fields.

This screening made it possible to find catalysts capable of regioselective deprotection of two crucial positions in nucleosides. By this chemo-enzymatic approach, therefore, a small library of monohydroxy acetylated nucleosides was prepared in good overall yields and high purities. The synthesis of those synthons by standard chemical procedures would require multi-stage protocols characterized by time-consuming protection/deprotection reactions and tedious purification steps.

Some conclusions can be drawn from the enzymatic screening described here, and these are summarized in Table 3.

Table 3. Enzymatic library for the regioselective deacylation of peracetylated nucleosides **1**–**9**.

Substrate	Enzyme	Deprotected position	% Yield
1	PFL	5′-OH	93
2	CRL	5′-OH	89
3	CRL	3′-OH	65
4	CRL	3′-OH	91
5	CRL	5′-OH	77
6	PFL	5′-OH	93
7	PFL	5′-OH	89
8	CRL	3′-OH	77
9	PFL	5′-OH	93

Out of the enzymes tested, CRL and PFL were the most efficient biocatalysts in terms of activity, whereas PPL displayed insignificant activity with respect to all the considered substrates.

With regard to the substrates, generally speaking, peracetylated ribonucleosides **1**, **6**, **7**, and **9** were preferentially hydrolyzed at C-5′ in very high yields by PFL, with the exception of 2′,3′,5′-tri-*O*-acetylcytidine (**5**), which was a better substrate for CRL. In the hydrolysis of peracetylated 2′-deoxyribonucleosides **3**–**4** the 3′-monodeprotected products were prevalently generated. Finally, in the case of the arabinonucleosides **2** and **8**, the same catalyst (CRL) displayed different regioselectivities depending on the nucleobase: the peracetylated arabinosyluracil **2** was deprotected at its 5′-position, peracetylated arabinosyladenine **8** at its 3′-position.

Additionally, the use of immobilized enzymes in place of the native ones under the same experimental conditions (Figure 1) clearly showed that the former give improvements in the enzymatic performances, such as activity and stability.[23]

In conclusion, as a result of the study reported here, a "ready to use" toolset of immobilized enzymes, fully characterized in terms of their regioselectivities with several peracetylated nucleosides, has been developed. This chemo-enzymatic strategy can provide an easy and versatile synthetic route for the preparation of various selectively protected nucleosides to be used for further functionalization. This has been successfully demonstrated by the application of this approach to the syntheses of doxifluridine (**14**) and 5′-UMP (**15**). In the case of doxifluridine (**14**, Scheme 4), it is worth mentioning that the synthetic process involves two enzymatic steps: the lipase-catalyzed hydrolysis of peracetylated uridine (**1**) to give 2′,3′-di-*O*-acetyluridine (**1a**), and the transglycosylation between 5′-deoxyuridine (**13**) and 5-fluorouracil. Transglycosylation was catalyzed in fully aque-

ous medium by immobilized uridine phosphorylase (UP, E.C. 2.4.2.1) from *Bacillus subtilis* by a procedure previously developed by our group for the synthesis of some purine 2′-deoxynucleosides.[24] In the case of doxifluridine (**14**), a further advantage of the transglycosylation is the obtainment of the fluorinated nucleoside **14** only in the final step: this minimizes the need for manipulation of toxic materials such as 5-fluorouracil and doxifluridine itself. This aspect should not be underestimated in view of process hazard evaluation.

Experimental Section

Materials and Methods: Lipases from *Candida rugosa* (CRL, 406 units g^{-1}), *Porcine pancreas* (PPL, 139 units g^{-1}), *Pseudomonas fluorescens* (PFL, 136 units g^{-1}), *Pseudomonas cepacia* (PCL, 250 units g^{-1}) and uridine phosphorylase (UP) from *Bacillus subtilis* were immobilized by Innovate Biotechnology s.r.l. Rivalta Scrivia (AL), Italy.[12,21] Uridine was kindly donated by Adorkem Technology s.p.a., Costa Volpino (BG), Italy, arabinosyluracil, 2′-deoxyuridine, and peracetylated ribavirin were kindly donated by Pro.bio.sint s.r.l. Varese, Italy, and cytidine, adenosine, doxifluridine (**14**), and uridine 5′-monophosphate (5′-UMP, **15**) were purchased from Sigma–Aldrich, Milano, Italy. Chromatographic purifications were performed on silica gel (Merck 60, 40–63 μm) with the solvent system indicated; TLC analyses were run on silica plates (Merck 60 F_{254}) and visualized with UV light (254 nm). HPLC analyses were run with a HPLC Merck Hitachi L-7100 (HPLC Multi HSM Manager Merck Hitachi D-7000) fitted with an L-7400 detector and an L-7300 oven.

^{1}H NMR spectra were recorded on a Bruker AC 400 MHz instrument with tetramethylsilane (Me_4Si, δ = 0.00 ppm) as internal standard. The chemical shifts are expressed in parts per millions (ppm) relative to the signal of the [D_6]DMSO at δ = 2.49 ppm as internal reference. The coupling constants ^{1}H,^{1}H (*J*, Hz) are in agreement with the proposed structures. The products obtained in the enzymatic hydrolyses were characterized by COSY 2D NMR spectroscopy.

Mass spectra were recorded on a LCQ-DECA Thermo Finnigan Spectrometer by the ESI (Electron Spray Ionization) ionization method with an ionic source and with use of Xcalibur 1.3 software (Thermo-Finnigan, San Jose, CA, USA). For sample injection, a flow of 5 μL min^{-1} was used. Analyses were run under positive modality and the experimental conditions were: voltage of the source 5.0 kV, voltage of the capillary 14 V, flow of the gas 35 (arbitrary units), temperature 200 °C.

The pH was kept constant during the enzymatic hydrolyses by automatic titration and the enzymatic activities were measured with a Metrohm pH-stat 718 Stat Tritino instrument (Herisau, Switzerland).

Synthesis of Peracetylated Nucleosides 1–8: A suspension of the nucleoside (12–20 mmol) in acetonitrile (1 mL/0.2 mmol of nucleoside) was treated with triethylamine (TEA, 4 equiv.) and acetic anhydride (4 equiv.) in the presence of a catalytic amount of 4-(dimethylamino)pyridine (DMAP). The resulting mixture was stirred at room temp. until the reaction was complete (TLC analysis) and was then diluted with chloroform and water (1:1). The organic phase was separated, washed with water (4 × 20–50 mL) and dried (Na_2SO_4), filtered, and concentrated in vacuo The residue was purified by silica gel column chromatography (CH_2Cl_2 100% to CH_2Cl_2/MeOH, 97:3) to afford the peracetylated nucleosides **1**–**8**.

2′,3′,5′-Tri-*O*-acetyluridine (1): Yield 96% (7.10 g); chromatographic conditions (CH_2Cl_2 100% to CH_2Cl_2/MeOH, 98:2); TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.61. ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 11.43 (s, 1 H, 3-NH), 7.90 (d, 1 H, 5-H), 6.01 (d, 1 H, 1′-H), 5.73 (d, 1 H, 6-H), 5.43–5.31 (m, 2 H, 2′-H, 3′-H), 4.20–4.10 (m, 3 H, 4′-H, 5′-H), 2.10–2.01 (s, 9 H, 3 OAc) ppm. MS: calcd. for [M + 1]$^+$: 371.30; found 370.00. HPLC: R_t = 25.64 min (method: 10 mM KH_2PO_4 buffer 80%/CH_3CN 20%, pH 4.2, flow: 1 mL min^{-1}, λ = 260 nm, RP-18 LiChrosphere select column).

2′,3′,5′-Tri-*O*-acetylarabinosyluracil (2): Yield 96% (4.20 g); chromatographic conditions (CH_2Cl_2 100% to CH_2Cl_2/MeOH, 98:2); TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.62. ^{1}H NMR (400 MHz, [D_6]-DMSO, 25 °C): δ = 11.50 (s, 1 H, 3-NH), 7.60 (d, 1 H, 5-H), 6.65 (d, 1 H, 1′-H), 5.75 (d, 1 H, 6-H), 5.37 (t, 1 H, 2′-H), 5.15 (t, 1 H, 3′-H), 4.25–4.50 (m, 3 H, 4′-H, 5′-H), 2.10–2.00 (s, 9 H, 3 OAc) ppm. MS: calcd. for [2 M + Na]$^+$: 763.61; found 763.00. HPLC: R_t = 17.31 min (A: 10 mM KH_2PO_4 buffer 90%/CH_3CN 10%, B: CH_3CN 90%/H_2O 10%, pH spontaneous; method: 0–2 min 100% A, 2–8 min 80% A to 20% B, 8–11 min 70% A to 30% B, 11–18 min 100% A, flow: 1 mL min^{-1}, λ = 260 nm, RP-18 Shiseido Capcell Pak column).

3′,5′-Di-*O*-acetyl-2′-deoxyuridine (3): Yield 94% (3.50 g); chromatographic conditions (CH_2Cl_2 100% to CH_2Cl_2/MeOH, 98:2); TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.53. ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 9.10 (s, 1 H, 3-NH), 7.50 (d, 1 H, 5-H), 6.10 (dd, 1 H, 1′-H), 5.60 (d, 1 H, 6-H), 5.15 (m, 1 H, 3′-H), 4.15 (m, 3 H, 4′-H, 5′-H), 2.25–2.35 (m, 2 H, 2′-H), 2.10–2.00 (s, 6 H, 2 OAc) ppm. MS: calcd. for [M + Na]$^+$: 335.27; found 335.00. HPLC: R_t = 15.92 min (A: 10 mM KH_2PO_4 buffer 90%/CH_3CN 10%, B: CH_3CN 90%/H_2O 10%, pH 4.2; method: 0–6 min 100% A, 6–14 min 85% A to 15% B, 14–22 min 100% A, flow: 1.3 mL min^{-1}, λ = 260 nm, RP-18 Zorbax SB-AQ column).

3′,5′-Di-*O*-acetylthymidine (4): Yield 90% (3.50 g); TLC (ethyl acetate/hexane, 7:3): R_f = 0.32. ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 10.70 (s, 1 H, 3-NH), 7.50 (s, 1 H, 6-H), 6.10 (dd, 1 H, 1′-H), 5.10 (m, 1 H, 3′-H), 4.20–4.00 (m, 3 H, 4′-H, 5′-H), 2.80 (m, 2 H, 2′-H), 2.10–2.00 (s, 6 H, 2 OAc), 190 (s, 3 H, 5-H) ppm. MS: calcd. for [2 M + Na]$^+$: 675.21; found 674.60. HPLC: R_t = 20.67 min (A: 10 mM KH_2PO_4 buffer 90%/CH_3CN 10%, B: CH_3CN 90%/H_2O 10%, pH 4.2; method: 0–6 min 100% A, 6–14 min 85% A to 15% B, 14–22 min 100% A, flow: 1.3 mL min^{-1}, λ = 260 nm, RP-18 Zorbax SB-AQ column).

2′,3′,5′-Tri-*O*-acetylcytidine (5): Yield 86% (3.80 g); chromatographic conditions (CH_2Cl_2 100% to CH_2Cl_2/MeOH, 97:3); TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.60. ^{1}H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 7.70 (d, 1 H, 5-H), 7.30 (s, 2 H, NH_2), 5.90 (d, 1 H, 1′-H), 5.80 (d, 1 H, 6-H), 5.40 (t, 1 H, 2′-H), 5.20 (t, 1 H, 3′-H), 4.50–4.40 (m, 3 H, 4′-H, 5′-H), 2.30–2.10 (s, 12 H, 3 OAc) ppm. MS: calcd. for [M + Na]$^+$: 411.35; found 411.10. HPLC: R_t = 17.40 min (A: 10 mM KH_2PO_4 buffer 90%/CH_3CN 10%, B: CH_3CN 90%/H_2O 10%, pH spontaneous; method: 0–3 min 100% A, 3–11 min 80% A–20% B, 11–14 min 70% A–30% B, 14–21 min 100% A, flow: 1 mL min^{-1}, λ = 260 nm, RP-18 Shiseido Capcell Pak column).

4-*N*-Acetyl-2′,3′,5′-tri-*O*-acetylcytidine (6): Yield 90% (4.45 g); TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.76. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 8.90 (s, 1 H, NHAc), 7.95 (d, 1 H, 5-H), 7.50 (d, 1 H), 6.15 (d, 1 H, 1′-H), 5.40 (t, 1 H, 2′-H), 5.30 (t, 1 H, 3′-H), 4.40 (m, 1 H, 4′-H) 4.20 (m, 2 H, 5′-H) 2.05 (s, 9 H, 4 OAc) ppm. MS:

calcd. for [M + Na]$^+$: 392.32; found 393.00. HPLC: R_t = 19.81 min (A: 10 mM KH_2PO_4 buffer 90%/CH_3CN 10%, B: CH_3CN 90%/H_2O 10%, pH spontaneous; method: 0–3 min 100% A, 3–11 min 80% A to 20% B, 11–14 min 70% A to 30% B, 14–21 min 100% A, flow: 1 mL min^{-1}, λ = 260 nm, RP-18 Shiseido Capcell Pak column).

2′,3′,5′-Tri-*O*-acetyladenosine (7): Yield 88% (4.15 g); chromatographic conditions (CH_2Cl_2 100% to CH_2Cl_2/MeOH, 97:3); TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.65. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 8.40 (s, 1 H, 2-H), 8.10 (s, 1 H, 6-H), 7.50 (s, 2 H, NH_2), 6.12 (d, 1 H, 1′-H), 6.05 (t, 1 H, 2′-H), 5.60 (t, 1 H, 3′-H), 4.30–4.20 (m, 3 H, 4′-H, 5′-H), 2.00–1.90 (s, 9 H, 3 OAc) ppm. MS: calcd. for [M + Na]$^+$: 416.34; found 416.00. HPLC: R_t = 19.66 min (A: 10 mM KH_2PO_4 buffer 90%/CH_3CN 10%, B: CH_3CN 90%/H_2O 10%, pH spontaneous; method: 0–3 min 100% A, 3–11 min 80% A to 20% B, 11–14 min 70% A to 30% B, 14–21 min 100% A, flow: 1 mL min^{-1}, λ = 260 nm, RP-18 Shiseido Capcell Pak column).

2′,3′,5′-Tri-*O*-acetylarabinosyladenine (8): Yield 86% (4.05 g); chromatographic conditions (CH_2Cl_2 100% to CH_2Cl_2/MeOH, 97:3); TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.54. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 8.20 (s, 1 H, 2-H), 8.10 (s, 1 H, 6-H), 7.30 (s, 2 H, NH_2), 6.50 (d, 1 H, 1′-H), 5.25 (t, 1 H, 2′-H), 5.00 (t, 1 H, 3′-H), 4.50–4.00 (m, 3 H, 4′-H, 5′-H), 2.00–1.50 (s, 9 H, 3 OAc) ppm. MS: calcd. for [M + Na]$^+$: 416.34; found 416.00. HPLC: R_t = 11.20 min (A: 10 mM KH_2PO_4 buffer 90%/CH_3CN 10%, B: CH_3CN 90%/H_2O 10%, pH spontaneous; method: 0–3 min 90% A to 10% B, 3–12 min 80% A to 20% B, 12–16 min 90% A to 10% B, flow: 1 mL min^{-1}, λ = 260 nm, RP-18 Shiseido Capcell Pak column).

Enzymatic Hydrolysis of Peracetylated Nucleosides 1–9: A solution of a peracetylated nucleoside (2–4 mmol) in acetonitrile (3.75 mL) was added to a solution of potassium phosphate buffer (pH 7, 25 mM, 33.75 mL). The pH was adjusted to 7.0 and the appropriate amount of immobilized lipase was added. The suspension was maintained under mechanical stirring at room temperature until the maximum hydrolysis of the substrate. During the reaction the pH was kept constant by automatic titration (Metrohm 718 STAT Tritino). Samples of the reaction mixture were analyzed at different times by TLC and HPLC. Finally, the enzyme was filtered off and washed with deionized water and a solution of acetonitrile (10%), and the filtrate was concentrated under reduced pressure and extracted with ethyl acetate (3 × 20 mL). The collected organic phases were dried with anhydrous Na_2SO_4, filtered, and dried under vacuum. The residue, when necessary, was further purified by silica gel column chromatography (CH_2Cl_2 100% to CH_2Cl_2/MeOH, 97:3) to afford the deprotected nucleosides **1a**–**3a**, **3b**, **4a**, **4b**, **5a**–**7a**, **8b** and **9a**.

2′,3′-Di-*O*-Acetyluridine (1a): TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.51. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 11.40 (s, 1 H, 3-NH), 7.89 (d, 1 H, 5-H), 6.00 (d, 1 H, 1′-H), 5.70 (d, 1 H, 6-H), 5.50 (s, 1 H, OH in 5′), 5.30 (m, 2 H, 2′-H, 3′-H), 4.14 (m, 1 H, 4′-H) 3.64 (m, 2 H, 5′-H) 2.10–2.02 (s, 6 H, 2 OAc) ppm. MS: calcd. for [M + 1]$^+$: 351.26; found 351.00. HPLC: R_t = 21.81 min (method: 10 mM KH_2PO_4 buffer 80%/CH_3CN 20%, pH 4.2, flow: 1 mL min^{-1}, λ = 260 nm, RP-18 LiChrosphere select column).

2′,3′-Di-*O*-Acetylarabinosyluracil (2a): TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.54. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 11.00 (s, 1 H, 3-NH), 7.85 (d, 1 H, 5-H), 6.25 (d, 1 H, 1′-H), 5.60 (d, 1 H, 6-H), 5.40–5.20 (m, 2 H, 2′-H, 3′-H), 5.10 (s, 1 H, OH at 5′), 4.10 (m, 1 H, 4′-H), 3.60 (m, 2 H, 5′-H), 2.10–1.90 (s, 6 H, OAc × 2) ppm. MS: calcd. for [M + 1]$^+$: 351.26; found 351.20. HPLC: R_t = 8.70 min (A: 10 mM KH_2PO_4 buffer 90%/CH_3CN 10%, B: CH_3CN 90%/H_2O 10%, pH spontaneous; method: 0–2 min 100% A, 2–8 min 80% A to 20% B, 8–11 min 70% A to 30% B, 11–18 min 100% A, flow: 1 mL min^{-1}, λ = 260 nm, RP-18 Shiseido Capcell Pak column). ^{1}H NMR of products **3a** and **4a** were in agreement with those previously reported.[25]

5′-Acetyl-*O*-2′-deoxyuridine (3b): TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.50. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 11.50 (s, 1 H, 3-NH), 7.90 (d, 1 H, 5-H), 6.10 (dd, 1 H, 1′-H), 5.80 (d, 1 H, 6-H), 5.20 (s, 1 H, OH in 3′), 5.15 (m, 1 H, 3′-H), 4.10 (m, 1 H, 4′-H), 3.60 (m, 2 H, 5′-H), 2.25 (m, 2 H, 2′-H), 2.00 (s, 3 H, OAc) ppm. MS: calcd. for [M + Na]$^+$: 293.23; found 293.00. HPLC: R_t = 4.93 min (A: 10 mM KH_2PO_4 buffer 90%/CH_3CN 10%, B: CH_3CN 90%/H_2O 10%, pH 4.2; method: 0–6 min 100% A, 6–14 min 85% A to 15% B, 14–22 min 100% A, flow: 1.3 mL min^{-1}, λ = 260 nm, RP-18 Zorbax SB-AQ column).

5′-*O*-Acetylthymidine (4b): TLC (ethyl acetate/hexane, 7:3). R_f = 0.20. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 8.40 (s, 1 H, 3-NH), 7.30 (s, 1 H, 6-H), 6.30 (dd, 1 H, 1′-H), 5.80 (s, 1 H, OH in 3′), 5.50 (m, 1 H, 3′-H), 4.50–4.10 (m, 4 H, 4′-H, 5′-H e OH in 5′), 2.50 (m, 2 H, 2′-H), 2.20 (s, 3 H, OAc), 2.10 (s, 3 H, 5-H) ppm. MS: calcd. for [M + Na]$^+$: 591.52; found 591.00. HPLC: R_t = 9.08 min (A: 10 mM KH_2PO_4 buffer 90%/CH_3CN 10%, B: CH_3CN 90%/H_2O 10%, pH 4.2; method: 0–6 min 100% A, 6–14 min 85% A to 15% B, 14–22 min 100% A, flow: 1.3 mL min^{-1}, λ = 260 nm, RP-18 Zorbax SB-AQ column).

2′,3′-Di-*O*-Acetylcytidine (5a): TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.50. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 7.80 (d, 1 H, 5-H), 7.30 (s, 2 H, NH_2), 6.00 (d, 1 H, 1′-H), 5.70 (d, 1 H, 6-H), 5.40 (m, 1 H, 2′-H, 3′-H, OH in 5′), 4.10 (m, 1 H, 4′-H), 3.70 (m, 2 H, 5′-H), 2.10–2.00 (s, 6 H, 2 OAc) ppm. MS: calcd. for [M + Na]$^+$: 350.28; found 350.10. HPLC: R_t = 9.80 min (A: 10 mM KH_2PO_4 buffer 90%/CH_3CN 10%, B: CH_3CN 90%/H_2O 10%, pH spontaneous; method: 0–3 min 100% A, 3–11 min 80% A to 20% B, 11–14 min 70% A to 30% B, 14–21 min 100% A, flow: 1 mL min^{-1}, λ = 260 nm, RP-18 Shiseido Capcell Pak column).

4-*N*-Acetyl-2′,3′-di-*O*-acetylcytidine (6a): TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.61. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 11.00 (s, 1 H, NHAc), 8.30 (d, 1 H, 5-H), 7.25 (d, 1 H, 6-H), 6.10 (d, 1 H, 1′-H), 5.20–5.30 (m, 1 H, 2′-H, 3′-H, OH in 5′), 4.30 (m, 1 H, 4′-H), 3.60–3.80 (m, 2 H, 5′-H), 2.10–2.00 (s, 9 H, 3 OAc) ppm. MS: calcd. for [M + Na]$^+$: 392.32; found 392.10. HPLC: R_t = 15.20 min (A: 10 mM KH_2PO_4 buffer 90%/CH_3CN 10%, B: CH_3CN 90%/H_2O 10% pH spontaneous; method: 0–3 min 100% A, 3–11 min 80% A to 20% B, 11–14 min 70% A to 30% B, 14–21 min 100% A, flow: 1 mL min^{-1}, λ = 260 nm, RP-18 Shiseido Capcell Pak column).

2′,3′-Di-*O*-acetyladenosine (7a): TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.53. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 8.40 (s, 1 H, 2-H), 8.20 (s, 1 H, 6-H), 7.50 (s, 2 H, NH_2), 6.12 (d, 1 H, 1′-H), 5.80 (t, 1 H, 2′-H), 5.60 (m, 1 H, OH in 5′), 5.50 (t, 1 H, 3′-H), 4.20 (m, 1 H, 4′-H), 3.70–3.60 (m, 2 H, 5′-H), 2.20–1.90 (s, 6 H, 2 OAc) ppm. MS: calcd. for [M + Na]$^+$: 374.30; found 374.00. HPLC: R_t = 16.31 min (A: 10 mM KH_2PO_4 buffer 90%/CH_3CN 10%, B: CH_3CN 90%/H_2O 10%, pH spontaneous; method: 0–3 min 100% A, 3–11 min 80% A to 20% B, 11–14 min 70% A to 30% B, 14–21 min 100% A, flow: 1 mL min^{-1}, λ = 260 nm, RP-18 Shiseido Capcell Pak column).

2′,5′-Di-*O*-acetylarabinosyladenine (8b): TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.40. ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C): δ = 8.10 (s, 1 H, 2-H), 8.00 (s, 1 H, 6-H), 7.40 (s, 2 H, NH_2), 6.50 (d, 1 H,

1′-H), 5.30 (t, 1 H, 2′-H), 5.00 (m, 1 H, OH in 3′), 4.50 (t, 1 H, 3′-H), 4.20–4.00 (m, 3 H, 4′-H, 5′-H), 2.00–1.50 (s, 6 H, 2 OAc) ppm. MS: calcd. for $[M + Na]^+$: 374.30; found 374.00. HPLC: R_t = 4.69 min (A: 10 mM KH_2PO_4 buffer 90%/CH_3CN 10%, B: CH_3CN 90%/H_2O 10%, pH spontaneous; method: 0–3 min 90% A to 10% B, 3–12 min 80% A to 20% B, 12–16 min 90% A to 10% B, flow: 1 mL min^{-1}, λ = 260 nm, RP-18 Shiseido Capcell Pak column).

2′,3′-Di-*O*-*N*-acetylribavirin (9a): TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.50. ^1H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 9.00 (s, 1 H, 2-H), 6.30 (d, 1 H, 1′-H), 5.70 (t, 1 H, 2′-H), 5.50 (m, 1 H, 3′-H), 5.10 (m, 1 H, OH in 5′), 4.25 (m, 1 H, 4′-H), 3.90 (s, 3 H, NHAc), 3.75–3.50 (m, 1 H, 5′-H), 3.40 (s, 1 H, NH), 2.20–2.00 (s, 6 H, 2 OAc) ppm. MS: calcd. for $[M + Na]^+$: 392.30; found 392.00. HPLC: R_t = 12.35 min (A: 10 mM KH_2PO_4 buffer 90%/CH_3CN 10%, B: CH_3CN 90%/H_2O 10%, pH spontaneous, T = 30 °C; method: 0–2 min 97% A to 3% B, flow 1 mL min^{-1}, 2–7 min 80% A to 20% B, flow 1 mL min^{-1}, 7–12 min 70% A to 30% B, flow 1.2 mL min^{-1}, 12–20 min 97% A to 3% B, flow 1.2 mL min^{-1}, λ = 220 nm, RP-18 Shiseido Capcell Pak column).

Synthesis of 2′,3′-Di-*O*-acetyluridine (1a): A solution of **1** (100 mM, 37 g L^{-1}) in acetonitrile (32 mL) was added to a solution of potassium phosphate buffer (pH 7, 25 mM, 128 mL). The pH was adjusted to 7.0 and the immobilized lipase (400 units) was added. The suspension was kept under mechanical stirring at room temperature until the maximum hydrolysis of the substrate. During the reaction, the pH was kept constant by automatic titration (Metrohm 718 STAT Tritino). Samples of the reaction mixture were analyzed at different times by TLC. Finally, the enzyme was filtered off and washed with deionized water and a solution of acetonitrile (20%), and the filtrate was extracted with ethyl acetate (3 × 70 mL) and dried in vacuo to give **1a** in 90% yield (4.75 g, 29.7 g/L).

Synthesis of 2′,3′-Di-*O*-acetyl-5′-*O*-mesyluridine (10): Mesyl chloride (1.2 mL) was added dropwise with stirring to an ice-cooled solution of 2′,3′-*O*-diacetyluridine (**1a**, 0.697 g, 2.13 mmol) in dry pyridine (50 mL). The solution was stirred at 25 °C for 1 h and was then kept in an ice-box overnight. The reaction mixture was concentrated and the residue was repeatedly coevaporated with a mixture of toluene and ethanol. The resultant residue was dissolved in ethyl acetate and washed with water (10 mL). The organic phase was extracted with ethyl acetate (3 × 15 mL), dried with anhydrous Na_2SO_4, and filtered. The filtrate was concentrated in vacuo to afford **10** in 90.5% yield (0.65 g). TLC (CH_2Cl_2/MeOH, 95:5): R_f = 0.62. ^1H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 11.29 (s, 1 H, 3-NH), 7.50 (d, 1 H, 5-H), 5.69 (d, 1 H, 1′-H), 5.49 (d, 1 H, 6-H), 5.40–5.00 (m, 2 H, 2′-H, 3′-H) 4.50–4.00 (m, 3 H, 4′-H, 5′-H) 3.10 (s, 3 H, CH_3S), 1.82 (s, 6 H, 2 OAc) ppm.

Synthesis of 2′,3′-Di-*O*-acetyl-5′-bromouridine (11): A mixture of 2′,3′-di-*O*-acetyl-5′-*O*-mesyluridine (**10**, 0.65 g, 1.59 mmol), tetrabutylammonium bromide (2.54 g, 7.95 mmol), and dimethylformamide (DMF, 27 mL) was heated at 130 °C for 1.5 h and concentrated, and the residue was dissolved in ethyl acetate (50 mL). The solution was washed with water (40 mL × 4), and the aqueous washings were combined and extracted with ethyl acetate (50 mL). The ethyl acetate solution was dried with anhydrous Na_2SO_4 and filtered, and the filtrate was concentrated. The final residue was chromatographed on a silica gel column (ethyl acetate/hexane, 70–30%); yield 60% (0.25 g). TLC (ethyl acetate/hexane, 7:3): R_f = 0.40. ^1H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 11.20 (s, 1 H, 3-NH), 7.40 (d, J = 8.0 Hz, 1 H, 5-H), 5.70 (d, 1 H, 1′-H), 5.50 (d, 1 H, 6-H), 5.30–5.00 (m, 2 H, 2′-H, 3′-H), 4.30–4.00 (m, 3 H, 4′-H, 5′-H), 1.90 (s, 6 H, 2 OAc) ppm.

Synthesis of 2′,3′-Di-*O*-acetyl-5′-deoxyuridine (12): 2,2′-Azobis(2-methylpropionitrile) (AIBN, 0.15 g) was added to 2′,3′-di-*O*-acetyl-5′-bromouridine (**11**, 0.25 g, 0.639 mmol) at reflux in toluene (20 mL) and absolute ethanol (5 mL). A solution of tributyltin hydride (0.794 g, 2.73 mmol) in toluene (5 mL) was then added and the reaction mixture was heated at reflux for 2 h. The mixture was filtered under reduced pressure, the filtrate was concentrated in vacuo, and the residue was crystallized from absolute ethanol to afford **12** in 65% yield (0.13 g). TLC (ethyl acetate/hexane, 7:3): R_f = 0.50. ^1H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 11.50 (s, 1 H, 3-NH), 7.80 (d, 1 H, 5-H), 5.80 (d, 1 H, 1′-H), 5.70 (d, 1 H, 6-H), 5.50 (m, 1 H, 2′-H), 5.00 (m, 1 H, 3′-H), 4.00 (m, 1 H, 4′-H), 2.00 (m, 6 H, 2 OAc), 1.30 (s, 3 H, 5′-H) ppm.

Synthesis of 5′-Deoxyuridine (13): Sodium methoxide (5 mL) was added to a solution of 2′,3′-di-*O*-acetyl-5′-deoxyuridine (**12**, 0.08 g, 0.256 mmol) in dry MeOH (10 mL) until pH 9–10 was reached. The mixture was kept at room temperature overnight and was then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off, and the filtrate was concentrated and coevaporated with dry CH_2Cl_2 (2 × 10 mL) to give 5′-deoxyuridine (**13**) in 92% yield (0.054 g). TLC (CH_2Cl_2/MeOH, 9:1): R_f = 0.36. ^1H NMR (400 MHz, [D_6]DMSO, 25 °C): δ = 10.60 (s, 1 H, 3-NH), 7.80 (d, 1 H, 5-H), 5.90 (d, 1 H, 6-H), 5.80 (d, 1 H, 1′-H), 5.70–5.20 (m, 2 H, OH in 2′ e 3′), 4.10 (m, 1 H, 2′-H), 3.90 (m, 1 H, 4′-H), 3.75 (m, 1 H, 3′-H), 1.30 (s, 3 H, 5′-H) ppm.

Synthesis of 5′-Deoxy-5-fluorouridine (Doxifluridine, 14): A solution of KH_2PO_4 buffer (pH 7.5, 10 mM, 1 mL) containing 5-fluorouracil (26 mg) and 5′-deoxyuridine (**13**, 9 mg) was maintained under mechanical stirring at 25 °C. Immobilized UP (2 U) was added to the solution. The reaction was monitored by HPLC (dilution samples 1:20 in 10 mM KH_2PO_4 buffer). The suspension was stirred for 24 h and then stopped by filtration of the immobilized enzyme under reduced pressure. Conversion 38%, R_t = 19.53 min (KH_2PO_4 buffer, 10 mM, pH 6.8 99%/MeOH 1%, flow: 1 mL min^{-1}, λ = 260 nm, T = 35 °C, LiChrocart RP-18 250 column).

Synthesis of Uridine 5′-Monophosphate (5′-UMP, 15): A mixture of 2′,3′-di-*O*-acetyluridine (**1a**, 0.40 g, 1.2 mmol) in water (0.025 mL) suspended in triethyl phosphate (3.8 mL, 3.6 mmol) was stirred at 0 °C for 10 minutes. Phosphorus oxychloride (0.4 mL, 3.6 mmol) was added to the cold mixture, which was maintained under stirring for 24 h. The solution was adjusted to pH 1.5 with a sodium hydroxide solution and heated at 70 °C for 2 h. Uridine 5′-monophosphate (**15**) was formed in 97% conversion, as determined by HPLC. R_t = 2.88 min [A: 2 g $(NH_4)H_2PO_4$, 0.5 g $(NH_4)_2HPO_4$ in 1 L and 30 mL of MeOH, B: CH_3CN 90%/H_2O 10%, method 0–8 min 100% A, 8–22 min 70% A to 30% B, 22–28 min 100% A], flow: 1 mL min^{-1}, λ = 260 nm, T = 25 °C, LiChrocart RP-18 column.

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