A Useful and Versatile Procedure for the Acylation of Nucleosides through an Enzymatic Reaction

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Lipase-mediated acylation of nucleosides with oxime esters in organic solvents has been achieved. *Candida antarctica* lipases (SP435 and SP435A) showed high regioselectivity toward the primary hydroxyl group of both deoxy- and ribonucleosides, whereas other lipases exhibited poor results for this goal. 2′-Deoxynucleosides, such as thymidine and 2′-deoxyadenosine, were acylated with oxime esters carrying saturated, unsaturated, aromatic, and functionalized chains, giving 5′-O-acylated compounds together with small quantities of the 3′-O-acylated regioisomer. Uridine, adenosine, and inosine, as representative ribonucleosides, were acylated exclusively at the 5′-OH by using the same methodology. Nucleosides bearing a cytosine ring were found to be unreactive with oxime esters under the same conditions. 2′-Deoxycytidine was acylated with acid anhydrides and *C. antarctica* lipase to give N,5′-O-diacylated compounds, whereas cytidine gave mixtures, reason for which it had to be previously chemically N-acylated and then subjected to the oxime esters and lipase, giving the same results as ribonucleosides.

Introduction

Modification of only one out of several identical functional groups in a molecule is a fundamental challenge to organic chemists. An important and synthetically relevant example of this problem is the regioselective acylation of polyhydroxy compounds. In the case of nucleosides, selective reactions on their functional groups is an interesting subject of study,[1] since successful developments in this area may lead to new methods for the synthesis of nucleoside analogues. Especially, nucleoside derivatives are compounds of high significance in some areas of medicinal chemistry,[2] showing antineoplasic[3] and antiviral activity,[4] and even just a single acylation can lead to compounds with antitumour activity, such as the 3′-*O*-acyl-*ara*-cytidines[5] or the antibiotic puromycin.[6] On the other hand, regioselective acylation of nucleosides represents a way of introducing protecting basic-labile groups,[7] interesting in oligonucleotide synthesis.[8]

Early work in this field was developed in the 1950s: it involved nonselective acylations,[9] with subsequent tedious separation processes, or time-consuming protection–deprotection steps in selective reactions on ribonucleosides[10] and mainly in the 3′-O-acylation of deoxyribonucleosides.[11] The strategy of peracylation followed by deprotection was also employed, but the selectivity was not satisfactory.[12]

By employing traditional chemical methods, one-step acylations with high selectivity was only achieved by means of using bulky acylating reagents, which leave secondary hydroxyls free due to steric hindrances.[13] Acylation at 3′-position of nonprotected deoxynucleosides was not reported, and in the case of ribonucleosides, the presence of 2′,3′-*cis*-diol system represented a problem, since acyl migrations could take place.[14] Other interesting contributions have been reported by Mitsunobu to aroylate the 5′-position[15] and by Moffat, who has used organotin derivatives to achieve regioselective acylations.[16]

Recently, enzymes such as lipases and proteases have proved to be effective tools to esterify polyhydroxy compounds with high regioselectivity.[17] However, few examples have been described with nucleosides. Klibanov used protease *Subtilisin* in anhydrous DMF to acylate

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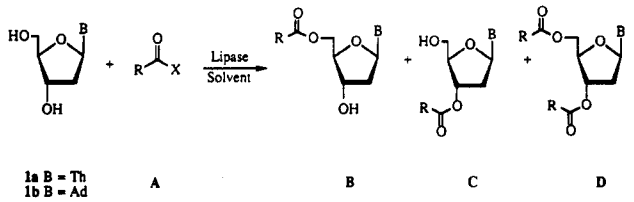
Scheme I



uridine and adenosine with trichloroethyl butyrate, but the regioselectivity was low.[18] In the same line of work, Wong et al. tried with a modified *Subtilisin* with enol esters as acylating agents:[19] regioselectivity was claimed to be complete, but enol esters are not versatile agents and the modified enzyme is hard to achieve. Lipases were employed by Nozaki et al. using acid anhydrides on deoxynucleosides, the results depending on the solvent: with highly polar solvents the primary hydroxyl group was acylated, accompanied by significative quantities of diacylated compounds,[20] but in THF or dioxane the preference was toward the 3′-position, although only in one case was the regioselectivity almost complete.[21]

As a part of our program to design new regioselective enzymatic transformations of polyhydroxy compounds with oxime derivatives, we have shown that *Pseudomonas cepacia* lipase (PSL) catalyzed the acylation[22] and alkoxycarbonylation[23] of 2′-deoxynucleosides in the 3′-position of the sugar moiety with total regioselectivity. Because of the immense potential of enzymes in regioselective and enantioselective processes, we believed that other lipases could catalyze the regioselective acylation of the primary hydroxyl group of nucleosides. In this paper we report some acylations of ribo- and deoxyribonucleosides using lipases.

Results and Discussion

Enzymatic Acylation of 2′-Deoxynucleosides. Since 2′-deoxynucleosides are hardly soluble in apolar organic solvents, usually employed in enzymatic esterifications,[24] we choose some polar solvents such as THF, 1,4-dioxane, and pyridine to run preliminary experiments, in which several lipases were tested in a standard reaction in order to get information about conversion and regioselectivity of the acylation (Scheme I). Reagents for this standard reaction were thymidine, **1a**, as a representative 2′-deoxynucleoside and acetonoxime butyrate (R = CH_3-$(CH_2)_2$, X = ON=C(Me)$_2$) as acylating agent, due to its utility in enzymatic acylations of polyhydroxy compounds.[22,25]

As one can see in Table I, whereas lipases from *P. cepacea* (PSL), porcine pancreas (PPL), and *Candida cylindracea* (CCL) exhibited a preference toward the 3′-position, lipases from *Aspergillus niger* and especially from *Candida antarctica* (CAL) acylated regioselectivity the 5′-hydroxy group. Lipase from *Humicola lanuginosa* gave no selective reaction, and other lipases tested were not capable of acylating thymidine with acetonoxime butyrate. Reactions were monitored by TLC, and since regioisomers show a good separation on the plates (R_f, 3′,5′-O-diacylated (**D**) > R_f 3′-O-acylated (**C**) > R_f 5′-O-acylated (**B**)), this method readily gives an idea of what kind of acylated compounds were formed. In order to quantify the conversions and relative rate of regioisomers, crudes were analyzed by ^{13}C-NMR spectroscopy. The solvent played an important role in the regioselectivity of the process: it is noteworthy that PSL was the most regioselective in pyridine[22] whereas PPL preferred dioxane and CCL tetrahydrofuran. CAL give the same results in THF and in dioxane, but poorer results were obtained in pyridine.

Another subject of interest is the choice of the acylating agent. Though oxime esters have already been employed by us with good results,[22,25] we decided to check several acylating agents, **A**, in order to compare them with regard to conversion and regioselectivity (Scheme I). Table II presents the results of this screening, for which acetylimidazole and alkyl, *p*-nitrophenyl, vinyl, and trifluoroethyl esters, together with acid anhydrides, were tested. Lipases were PSL, PPL, and CAL SP435A, and thymidine, **1a**, and 2′-deoxyadenosine, **1b**, served as substrates. Reactions were controlled in the same way as the screening for the lipases (vide supra) and did not take place in the absence of the enzyme. Alkyl esters, which we had used in the acylation of acyclonucleosides,[26] proved to be inadequate, due to lack of regioselectivity. Interestingly, no diacylated compounds could be detected, even when >95% conversion was reached (entry 2). Acetylimidazole (entry 4) had been described as acylating agent a long time ago,[27] but there are no reports on its use in enzymatic acylations: in this case, no conversion was found. With respect to vinyl esters, these reagents are well established as acylating agents in enzymatic reactions,[28] but the major inconvenience here is the low conversion (but high specificity), and when a high yield is achieved, low regioselectivity is found. Trifluoroethyl esters (used before to acylate sugars and nucleosides)[18] exhibited similar features to those of oxime esters but with lower conversions. Anhydrides (entries 12–14) showed unequal behavior: whereas with PSL and thymidine a total regioselectivity and good yield was achieved, with 2′-deoxyadenosine this goal was lost. Using DMF as the solvent, the regioselectivity of PSL was reversed, according to observations of Nozaki on 5-modified deoxynucleosides,[20] but the yield was low.

In view of these results, we decided to exploit the possibilities of CAL to acylate 5′-OH of nucleosides, since other lipases described in the literature to achieve this goal were not suitable for this purpose. Differences exhibited between SP435 and SP435A prompted us to use the latter, because of its higher regioselectivity. On the other hand, oxime esters **2a–h** had been chosen as

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Table I. Acylation with Several Lipases of Thymidine with Acetone Oxime Butyrate[a]

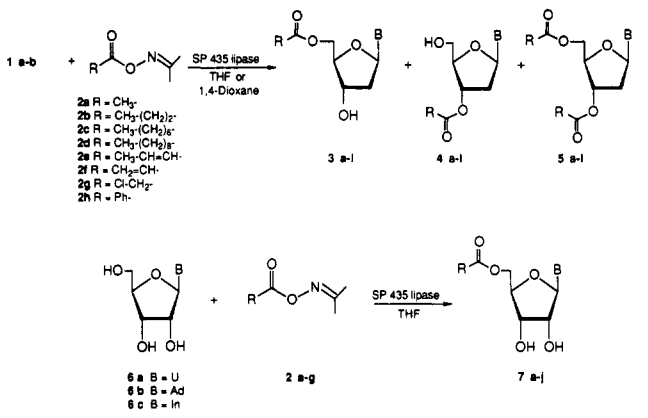
lipase[b]	T (°C)	t (h)	solvent	convn[c] (%)	rate[c] (%) B (5′)	C (3′)	D (5′,3′)
Aspergillus niger	40	72	THF	18	80	20	
	40	72	1,4-dioxane	11	80	20	
Candida antarctica (CAL-SP435)	0	8	THF	92	82	10	8
	30	9	1,4-dioxane	80	63	27	10
	60	48	pyridine	20	100		
Candida antarctica (CAL-SP435A)	30	10	THF	100	>95	<5	
	30	9	1,4-dioxane	90	92	8	
	60	72	pyridine				
Candida cylindracea (CCL)	50	48	THF	36		100	
	50	48	1,4-dioxane	40	8	92	
Humicola lanuginosa	60	72	THF	16	50	50	
	60	72	1,4-dioxane	12	40	60	
Porcine pancreas (PPL)	60	20	THF	93	<5	>95	
	60	24	1,4-dioxane	92		100	
	60	72	pyridine				
Pseudomonas cepacea (PSL)	60	24	THF	90	15	65	20
	60	24	1,4-dioxane	94	15	69	16
	60	48	pyridine	74		100	

[a] No reaction was observed in absence of enzymes. [b] Lipases from *Geotrichum candidum*, *Rhizopus arrhizus*, *Rhizopus javanicus*, and *Rhizopus niveus* gave no reaction under similar conditions. [c] Determined by ^{13}C NMR.

Table II. Selectivity of Acylating Reagents, RC(O)X

entry	lipase	B	R	X	solvent	T (°C)	t (h)	convn (%)	rate (%) B (5′)	C (3′)	D (3′,5′)
1	SP435	Th	Me	EtO	dioxane	60	24	50	90	10	
2	SP435	Th	$ClCH_2$	MeO	THF	60	5	>95	55	45	
3	PSL	Ad	$ClCH_2$	EtO	THF	30	48	37	41	51	8
4	SP435	Th	Me	imidazole	THF	30	96				
5	SP435	Th	Me	*p*-$NO_2C_6H_4O$	THF	30	24	>95	50	30	20
6	PPL	Th	$CH_3CH{=}CH$	$CH_2{=}CHO$	dioxane	30	24	40		100	
7	PPL	Ad	Me	$CH_2{=}CHO$	dioxane	60	2	50	<5	>95	
9	SP435A	Th	Me	$CH_2{=}CHO$	THF	30	24	>95	45	20	35
9	SP435A	Th	$CH_3(CH_2)_2$	CF_3CH_2O	dioxane	30	24	85	88	8	<5
10	PPL	Ad	$CH_3(CH_2)_2$	CF_3CH_2O	dioxane	30	24	50	<5	>95	
11	PSL	Th	$CH_3(CH_2)_2$	CF_3CH_2O	THF	30	24	60	<5	>95	
12	PSL	Ad	$CH_3(CH_2)_2$	$CH_3(CH_2)_2CO_2$	THF	30	24	62	10	80	10
13	PSL	Th	$CH_3(CH_2)_2$	$CH_3(CH_2)_2CO_2$	dioxane	30	24	60		100	
14	PSL	Th	$CH_3(CH_2)_2$	$CH_3(CH_2)_2CO_2$	DMF	30	24	40	89		11

Scheme II



acylating agents, due to their versatility and lack of better results with other reactives.

Target nucleosides were thymidine and 2′-deoxyadenosine, as representative of purine and pyrimidine nucleosides. Besides, 2′-deoxyadenosine possesses an exocyclic amino group, susceptible of acylation (Scheme II). Reactions were driven at the temperatures and times given in Table III, and conversions and rates were obtained as mentioned above. Solvents used were THF and dioxane, and especially 2′-deoxyadenosine gave better results in the latter than in THF. A variety of acyl moieties, such as saturated, unsaturated, aromatic, or functionalized, could be introduced in the nucleosides. The regioselectivity was overwhelming toward the 5′-position (**3a–l**), though when acetoneoxime chloroacetate was used, diacylated compounds (**5k–l**) appeared, probably due to the higher reactivity of the chloroacetyl moiety. It is of note that when acetoneoxime benzoate acted as acylating reagent the regioselectivity was total and the reaction time had to be increased. N-Acylation could not be found in 2′-adenosine. Products **3a–l** were purified by flash chromatography on silica gel (see the Experimental Section), and some of their characteristics as listed in Table IV. Structure elucidation was accomplished by NMR spectroscopy, especially ^{13}C NMR: the spectrum for compounds **3a–l** showed a shift on the C5′ of ca. 4 ppm toward lower fields with respect to the same carbon atom in the starting nucleosides. Besides, the C4′ showed a slightly upfield shift, according to the results found in monoacylated sugars (adjacent carbons to position of acylation bear shift towards high fields).[29] In addition, H5′,H5″ showed a shift of ca. 1.5 ppm downfield with respect to nucleosides **1a–b**. Complete ^1H and ^{13}C NMR spectral data, together with literature values for comparison, are given in Tables V and VI.

Enzymatic Acylation of Ribonucleosides. Enzymatic acylation of ribonucleosides have been only slightly investigated. Previous investigations were carried out with proteases, and results were poor[18] or the catalyst was hardly

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Table III. Reaction of Deoxynucleosides 1a–b with Oxime Esters and CAL SP435A

entry	B	R	solvent	T (°C)	t (h)	convn[a] (%)	rate[a] (%) 3	4	5
1	Th	CH_3	THF	0	6	>95	>95	<5	
2	Th	$CH_3(CH_2)_2$	THF	0	8	>95	>95	<5	
3	Ad[b]	$CH_3(CH_2)_2$	1,4-dioxane	30	5	85	90	10	
4	Th	$CH_3(CH_2)_6$	THF	0	20	82	92	8	
5	Th	$CH_3(CH_2)_8$	THF	0	20	81	93	7	
6	Th	$CH_3CH{=}CH$	THF	30	24	78	>95	<5	
7	Th	$CH_2{=}CH$	THF	0	8	82	88	12	
8	Ad[b]	$CH_2{=}CH$	1,4-dioxane	30	24	92	90	10	
9	Th	Ph	THF	60	48	60	100		
10	Ad[b]	Ph	1,4-dioxane	60	48	75	100		
11	Th	$ClCH_2$	THF	0	8	>95	66	22	12
12	Ad[b]	$ClCH_2$	1,4-dioxane	30	5	>95	72	18	10

[a] Determined by ^{13}C NMR [b] No acylation was observed at the exocyclic amino function of adenine.

Table IV. Compounds 3a–l Prepared with Oxime Esters and *Candida antarctica* Lipase (SP435A)

product	B	R	yield (%)	mp[a] (°C)	IR[b] ν(cm^{-1})	$[\alpha]^D_{25}$ (c, solvent)
3a	Th	CH_3	73	144–5[c]	1738	+0.51 (0.99, DMSO)[d]
3b	Th	$CH_3(CH_2)_2$	75	153–4	1737	+2.4 (0.90, DMSO)
3c	Ad	$CH_3(CH_2)_2$	83	122–3	1743	−14.3 (1, DMSO)
3d	Th	$CH_3(CH_2)_6$	72	145–6	1730	+1.1 (0.99, DMSO)
3e	Th	$CH_3(CH_2)_8$	69	145–6	1730	+2.5 (0.90, $CHCl_3$)
3f	Th	$CH_3CH{=}CH$	71	154–5	1720	−0.81 (0.99, DMSO)
3g	Th	$CH_2{=}CH$	69	145–6	1724	−0.6 (0.99, DMSO)
3h	Ad	$CH_2{=}CH$	78	110–5	1726	−17.4 (0.93, DMSO)
3i	Th	Ph	60	167–8[e]	1724	−5.38 (0.98, DMSO)
3j	Ad	Ph	75	203–4[f]	1730	−21.8 (0.8, DMSO)
3k	Th	$ClCH_2$	65	168–9	1747	+0.57 (0.7, DMSO)
3l	Ad	$ClCH_2$	66	144–5	1753	−17.3 (0.7, DMSO)

[a] Uncorrected. [b] Partial. [c] Lit. mps 146,[12b] 150,[9c] 151–2,[19] 151.5–152,[12f] 153–4.[15] [d] Lit. $[\alpha]^D_{18}$ = −7.1 (1.05, DMF).[12f] [e] Lit. mps 171–2,[15,13c] 167.[13d] [f] Lit. mps 188–190,[13d] 214–7.[13c]

Table V. ^1H NMR Spectral Data for Compounds 3a–l [δ (ppm)][a]

product	base ring NH	Me	H6	sugar moiety H1′	(H2′, H2″)	H3′	H4′	(H5′, H5″)	OH	acyl moiety
3a[12f,19]	11.38 (br)	1.80 (s)	7.46 (s)	6.20 (t)	2.10 (m), 2.20 (m)	3.97 (m)	4.20[b]	4.20[b]	5.40 (d)	2.05 (3 H, s)
3b	10.00 (br)	1.80 (s)	7.45 (s)	6.19(t)	2.10 (m), 2.20 (m)	3.91 (m)	4.20[b]	4.20[b]	5.45 (d)	0.92 (3 H, t), 1.55 (2 H, m), 2.31 (2 H, t)
3d	9.35 (br)	1.95 (s)	7.33 (s)	6.31 (t)	2.43 (m), 2.50 (m)	4.42[b]	4.18 (m)	4.28 (m), 4.42[b]	3.22 (br)	0.89 (3 H, t), 1.30 (8 H, m), 1.64 (2 H, m), 2.35 (2 H, t)
3e	9.15 (br)	1.91 (s)	7.33 (s)	6.30 (t)	2.44 (m), 2.50 (m)	4.41[b]	4.16 (m)	4.25 (m), 4.41[b]	3.05 (br)	0.89 (3 H, t), 1.30 (12 H, m), 1.62 (2 H, m), 2.32 (2 H, t)
3f	11.30 (s)	1.77 (s)	7.40 (s)	6.20 (t)	2.15 (m)	4.28 (m)	3.92 (m)	4.22 (m), 4.35 (m)	5.42 (d)	1.85 (3 H, d), 5.95 (1 H, d), 6.94 (1 H, m)
3g	11.31 (s)	1.76 (s)	7.38 (s)	6.20[b]	2.15 (m)	4.28[b]	3.95 (m)	4.28,[b] 4.40 (m)	5.40 (br)	5.98 (1 H, dd), 6.20,[b] 6.38 (1 H, dd)
3i[13c,d]	11.31 (s)	1.61 (s)	7.40 (s)	6.20 (t)	2.20 (m)	4.45[b]	4.05 (m)	4.45,[b] 4.51 (m)	5.50 (br)	7.55 (2 H, t), 7.67 (1 H, m), 7.99 (2 H, d)
3k	11.31 (s)	1.78 (s)	7.45 (s)	6.22 (t)	2.12 (m), 2.24 (m)	3.94 (m)	4.25 (m)	4.32 (m)	5.42 (d)	4.49 (2 H, s)

product	base ring NH_2	H2	H8	sugar moiety H1′	(H2′, H2″)	H3′	H4′	(H5′,H5″)	OH	acyl moiety
3c	7.39 (br)	8.19 (s)	8.30 (s)	6.39 (t)	2.38 (m), 2.88 (m)	4.52 (m)	4.04 (m)	4.16 (m), 4.27 (m)	5.54 (br)	0.78 (3 H, t), 1.45 (2 H, m), 2.20 (2 H, t)
3h	7.31 (br)	8.14 (s)	8.29 (s)	6.20[b]	2.34 (m), 2.85 (m)	4.51 (m)	4.04 (m)	4.25 (m), 4.35 (m)	5.52 (br)	5.93 (1 H, dd), 6.20,[b] 6.30 (1 H, dd)
3j[13c,d]	7.37 (br)	8.16 (s)	8.32 (s)	6.41 (t)	2.43 (m), 2.97 (m)	4.68 (m)	4.17 (m)	4.43 (m), 4.57 (m)	5.60 (br)	7.47 (2 H, t), 7.62 (1 H, m),7.90 (2 H, d)
3l	7.31 (br)	8.15 (s)	8.31 (s)	6.36 (s)	2.35 (m), 2.85 (m)	4.50 (m)	4.05 (m)	4.27 (m), 4.39[b]	5.51 (br)	4.39[b]

[a] All samples measured in DMSO-d_6 except **3d** and **3e** ($CDCl_3$). Signals of the sugar moiety assigned through selective irradiations. [b] Superimposed signals.

available.[19] For this reason, we decided to extend the foregoing strategy to study this subject. As in the case of deoxynucleosides, lipase from *C. antarctica* acylated preferably the 5′-hydroxyl group, but due to the presence of the 2′-OH, total regioselectivity and high yields were achieved. The triad of lipases with regioselectivity toward the 3′-position in deoxynucleosides gave mixtures and poor conversions. So, CAL proved to be a valuable tool to catalyze 5′-O-acylation of ribonucleosides **6a–c** with oxime esters **2a–g**. In this case, SP 435 lipase was employed with uridine, adenosine, and inosine and in all cases no other products than 5′-*O*-acyl nucleosides and starting material were detected. Reactions were carried out in THF as solvent (Scheme II) with the temperature and time specified in Table VII, where some characteristics of the products obtained, **7a–j**, are listed as well. As in the case of deoxynucleosides, acyl moieties of a different nature could be introduced (saturated, unsaturated, and functionalized chains). It is noteworthy that the results were homogeneous with regard to yields (with inosine slightly

Table VI. ^{13}C NMR Chemical Shifts of Compounds 3a–l [δ (ppm)][a]

	base ring					sugar moiety					acyl moiety	
product	C2	C4	C5	C6	Me	C1′	C2′	C3′	C4′	C5′	C=O	R
3a[19]	150.62	163.88	109.94	136.11	12.35	83.97	38.74	70.47	83.77	64.12	170.42	20.84
3b	150.63	163.89	109.93	136.07	12.34	84.01	38.79	70.47	83.83	63.91	172.84	13.58, 18.1, 35.45
3d	150.60	163.87	109.88	136.03	12.33	84.01	38.84	70.47	83.84	63.93	172.96	14.08. 22.21, 24.61, 28.53, 28.55, 31.30, 33.59
3e	150.61	163.87	109.88	136.00	12.36	84.02	38.87	70.49	83.86	63.95	172.95	14.13, 22.33, 24.62, 28.64, 28.91, 29.07, 31.50, 33.59
3f	150.64	163.90	109.95	135.98	12.30	84.06	38.92	70.60	83.95	63.88	165.55	17.95, 122.12, 146.16
3g	150.70	163.96	110.05	136.04	12.40	84.16	38.93	70.60	83.86	64.34	165.57	128.24, 132.30
3i	150.71	163.82	109.99	135.84	12.08	84.01	38.86	70.45	83.85	64.59	165.79	129.07, 129.40, 133.75
3k	150.69	163.96	110.11	136.05	12.38	84.03	38.78	70.43	83.60	65.65	167.52	41.29

	base ring					sugar moiety					acyl moiety	
product	C2	C4	C5	C6	C8	C1′	C2′	C3′	C4′	C5′	C=O	R
3c	152.82	149.36	119.48	156.28	139.80	83.66	38.84	70.87	84.36	64.01	172.88	13.54, 18.09, 35.41
3h	152.75	149.25	119.33	156.21	139.66	83.44	38.61	70.75	84.16	64.36	165.45	128.15, 132.08
3j	152.77	149.28	119.48	156.25	139.88	83.64	38.64	70.72	84.20	64.63	165.77	128.89, 129.39, 129.61, 133.58
3l	152.77	149.28	119.36	156.25	139.74	83.48	38.69	70.74	84.05	65.73	167.44	41.24

[a] All samples measured in DMSO-d_6. Distinction between C1′ and C4′ was made on the basis of the coupled ^{13}C NMR spectra, since $^1J_{C1',H-C1'}$ exhibit values around 165 Hz whereas the other $^1J_{C,H}$ of the sugar moiety, for example $^1J_{C4',H-C4'}$, are 15–20 Hz smaller (Seela et al. *Nucleosides Nucleotides* **1985**, *4*, 391).

Table VII. Regioselective Acylation of Ribonucleosides with Oxime Esters and *Candida antarctica* Lipase (SP435)

product	B	R	T (°C)	t (h)	yield[a] (%)	mp[b] (°C)	IR[c] ν (cm^{-1})	$[\alpha]^D_{25}$ (c, solvent)
7a	Ad	CH_3	60	17	92	131–2[d]	1741	−46.5 (0.99, DMSO)
7b	U	$CH_3(CH_2)_2$	60	10	93	119–120	1743	−2.2 (0.99, DMSO)
7c	In	$CH_3(CH_2)_2$	60	24	86	95–100	1722	−2.5 (0.20, DMSO)
7d	U	$CH_3(CH_2)_8$	60	4	96	137–8	1739	−1.9 (0.98, DMSO)
7e	Ad	$CH_3(CH_2)_6$	60	24	96	110–1	1743	−29.5 (0.98, DMSO)
7f	U	$CH_3CH{=}CH$	60	20	85	134–5	1728	−8.1 (0.99, DMSO)
7g	Ad	$CH_3CH{=}CH$	60	18	84	111–2	1730	−38.0 (0.98, DMSO)
7h	Ad	$CH_2{=}CH$	60	15	82	185 dec	1728	−19.2 (0.98, DMSO)
7i	In	$CH_2{=}CH$	60	15	80	190 dec	1737	−3.2 (0.50, DMSO)
7j	U	$ClCH_2$	30	24	90	151–2	1749	−11.3 (1.04, DMSO)

[a] Calculated with respect to 6 on pure isolated products 7. [b] Uncorrected. [c] Partial. [d] Lit. mps 135,[9b] 141–3.[19]

Table VIII. ^1H NMR Spectral Data for Compounds 7a–j [δ (ppm)][a]

	base ring				sugar moiety							acyl moiety
product	NH_2	NH	H2	H8	H1′	H2′	H3′	H4′	(H5′, H5″)	2′-OH	3′-OH	
7a[12f,19]	7.32 (br)		8.15 (s)	8.32 (s)	5.91 (d)	4.68 (m)	4.25[b]	4.05 (m)	4.25,[b] 4.31 (m)	5.58 (d)	5.38 (d)	2.02 (3 H, s)
7c		12.30 (br)	8.10 (s)	8.29 (s)	5.90 (d)	4.55 (m)	4.19[b]	4.10 (m)	4.19,[b] 4.33 (m)	5.62 (br)	5.42 (br)	0.85 (3 H, t), 1.52 (2 H, m), 2.30 (2 H, t)
7e	7.35 (br)		8.12 (s)	8.30 (s)	5.95 (d)	4.68 (m)	4.22[b]	4.08 (m)	4.22,[b] 4.31 (m)	5.60 (d)	5.38 (d)	0.84 (3 H, t), 1.21 (8 H, m), 1.48 (2 H, m), 2.28 (2 H, t)
7g	7.35 (br)		8.15 (s)	8.30 (s)	5.92 (d)	4.68 (m)	4.25[b]	4.10 (m)	4.25,[b] 4.38 (m)	5.61 (br)	5.42 (br)	1.82 (3 H, d), 5.88 (1 H, d), 6.88 (1 H, m)
7h	7.31 (s)		8.15 (s)	8.32 (s)	5.90 (d)	4.68 (m)	4.28[b]	4.10 (m)	4.28,[b] 4.43 (m)	5.60 (br)	5.40 (br)	5.92 (1 H, dd), 6.15 (1 H, dd), 6.31 (1 H, dd)
7i		12.10 (br)	8.06 (s)	8.27 (s)	5.93 (d)	4.57 (m)	4.26[b]	4.11 (m)	4.26,[b] 4.37 (m)	5.66 (br)	5.45 (br)	5.95 (1 H, dd), 6.21 (1 H, dd), 6.30 (1 H, dd)

	base ring			sugar moiety							acyl moiety
product	NH	H5	H6	H1′	H2′	H3′	H4′	(H5′, H5″)	2′-OH	3′-OH	
7b	11.40 (br)	5.68 (d)	7.59 (d)	5.78 (d)	4.08 (m)	3.95 (m)	4.00 (m)	4.18 (m), 4.27 (m)	5.43 (br)	5.22 (d)	0.90 (3 H, t), 1.51 (2 H, m), 2.28 (2 H, t)
7d	11.35 (br)	5.67 (d)	7.61 (d)	5.78 (d)	4.06 (m)	3.91 (m)	3.95 (m)	4.20 (m)	5.44 (br)	5.21 (br)	0.82 (3 H, t), 1.25 (12 H,m), 1.50 (2 H, m), 2.30 (2 H, t)
7f	11.30 (br)	5.66 (d)	7.60 (d)	5.72 (d)	4.07 (m)	3.95 (m)	4.00 (m)	4.25 (m), 4.32 (m)	5.45 (d)	5.30 (d)	1.85 (3 H, d), 5.92 (1 H, d), 6.92 (1 H, m)
7j	11.40 (br)	5.65 (d)	7.60 (d)	5.80 (d)	4.15 (m)	3.95 (m)	4.05 (m)	4.31 (m)	5.55 (br)	5.35 (br)	4.51 (2 H, s)

[a] All samples measured in DMSO-d_6. [b] Superimposed signals.

smaller), and in the case of adenosine the exocyclic amino group was not acylated. Structure elucidation was made on the basis of ^{13}C NMR spectra, in which C5′ carbon of the products exhibited a downfield shift of ca. 4 ppm with respect to the starting material, whereas C4′, the adjacent carbon, showed a slight upfield shift. Complete spectral data, both ^{13}C and ^1H, are given in Tables VIII and IX.

Acylation of Cytosine Nucleosides. Oxime esters were not able to acylate nucleosides bearing a cytosine ring. For this reason, another acylating agent had to be probed and we found that, with regards to deoxycytidine, **8a**, acid anhydrides gave N,5′-O-diacylated compounds as result when reacted with CAL SP435 in THF whereas PSL gave mixtures of regioisomers. On the other hand,

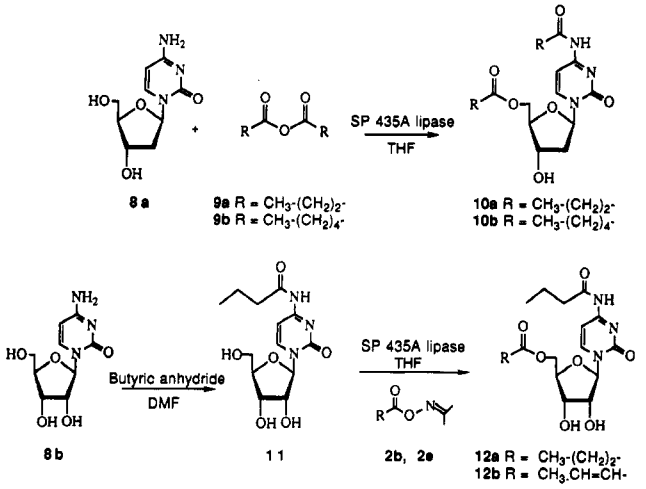
Table IX. ^{13}C NMR Spectral Data for Compounds 7a-j [δ (ppm)][a]

	base ring					sugar moiety					acyl moiety	
product	C2	C4	C5	C6	C8	C1′	C2′	C3′	C4′	C5′	C=O	R
7a	152.89	149.56	119.36	156.30	139.95	87.95	73.05	70.51	81.70	64.13	170.42	20.81
7c	146.09	148.36	124.66	156.72	138.97	87.88	73.48	70.38	81.84	63.78	172.77	13.54, 18.03, 35.33
7e	152.87	149.54	119.41	156.32	139.92	88.06	73.16	70.51	81.69	63.92	172.99	14.11, 22.23, 24.61, 28.57, 31.30, 33.56
7g	152.88	149.55	119.36	156.30	139.95	87.97	73.05	70.50	81.75	63.83	165.56	17.95, 122.07, 146.02
7h	152.91	149.56	119.37	156.31	139.95	87.99	73.08	70.49	81.66	64.28	165.52	128.22; 132.20
7i	146.08	148.34	124.80	156.68	138.98	87.87	73.42	70.36	81.80	64.15	165.43	128.14; 132.17

	base ring				sugar moiety					acyl moiety	
product	C2	C4	C5	C6	C1′	C2′	C3′	C4′	C5′	C=O	R
7b[18]	150.83	163.28	102.20	140.94	88.96	72.96	69.99	81.30	63.77	172.83	13.61, 18.12, 35.42
7d	150.86	163.32	102.22	140.91	89.03	73.11	70.03	81.33	63.85	172.97	14.19, 22.39, 24.70, 28.73, 28.96, 29.02, 29.16, 31.58, 33.64
7f	150.90	163.37	102.28	140.99	89.01	73.02	70.05	81.41	63.74	165.62	18.02, 122.07, 146.36
7j	150.84	163.34	102.35	141.09	88.91	72.93	69.90	81.17	65.47	167.51	41.32

[a] Samples measured in DMSO-d_6.

Scheme III



cytidine **8b** gave also diacylated compounds under the same conditions as CAL but mixtures appeared. Exploring the reason for this behavior, we prepared the corresponding N-acylated nucleosides[30] by means of acid anhydride in DMF.[31] Then, enzymatic reactions were carried out with anhydrides and with oxime esters, finding that the latter esterified the 5′-hydroxy groups exclusively, whereas with anhydride the results were those mentioned above, which could be interpreted as follows: first, chemical acylation took place and the N-acylated compounds underwent enzymatic acylation at the 5′-OH. Thus, *N*,5′-*O*-diacyl-2′-deoxycytidines **10a–b** could be prepared in "one-pot" with anhydrides, without isolation of N-acylated compound whereas, in contrast, *N*,5′-*O*-diacylcytidines **12a–b** had to be prepared in two steps (Scheme III), with oxime esters and lipase reacting on the isolated *N*-acyl intermediate. Reactions were monitored by TLC, and in the case of the deoxycytidine, intermediate *N*-acyl nucleoside disappeared completely. Yields were calculated on the pure isolated products with respect to the starting material. Results are summarized in Table X, together with some properties of the products. The method was particularly interesting in the case of the deoxynucleoside, since simultaneous protection of exocyclic amino group and primary hydroxyl could be achieved. As with the other acylated nucleosides along this paper, ^{13}C NMR proved the structure of these compounds; Table XI shows their spectral data.

Conclusions

Lipase from *C. antarctica* catalyzed regioselective acylation at the 5′-position of nucleosides with oxime esters as versatile acylating agents. Other acylating agents tested gave poorer results, and lipases usually described in acylation of polyhydroxy compounds were not able to acylate the primary hydroxyl group regioselectivity. In the case of 2′-deoxynucleosides, small quantities of 3′-acylated regioisomer were produced, whereas ribonucleosides were esterified exclusively at the primary hydroxyl group. For cytosine nucleosides, N-protected counterparts were necessary to carry out the lipase-mediated acylation. In conclusion, we have shown here the possibilities of lipase-catalyzed regioselective acylation of nucleosides. The versatility and simplicity of the procedure are noteworthy.

Experimental Section

General. Lipases from *P. cepacea*, *A. niger*, *H. lanuginosa*, *Geotrichum candidum*, *Rhizopus arrhizus*, *Rhizopus javanicus*, and *Rhizopus niveus* were purchased from Amano Pharmaceutical Co., and those from porcine pancreas and *C. cylindracea* were purchased from Sigma. *C. antarctica* lipase was kindly gifted by Novo Nordisk Co. Nucleosides were purchased from Aldrich Chemie. THF and 1,4-dioxane were distilled over $LiAlH_4$ in order to avoid moisture. Precoated TLC alumina sheets silica gel 60 F_{254} from Merck were used, and for column chromatography, Merck silica gel 60/230–400 mesh was used. Mp's were taken in open capillary tubes and are uncorrected. NMR spectra were recorded in $CDCl_3$, D_2O, and DMSO-d_6. Oxime esters **2** were prepared in almost quantitative yields by treating acetone oxime with the corresponding acyl chloride followed by vacuum distillation.

General Procedure for the Synthesis of Compounds 3a–l. A sample of 2 mmol of **1a–b**, 6 mmol of **2a–h**, and 0.4 g of lipase from *C. antarctica* SP 435A was suspended in 15 mL of THF or dioxane under nitrogen atmosphere. The mixture was allowed to react at 250 rpm during the time and at the temperature indicated in Table III. Then, the enzyme was filtered off and washed with MeOH, the residue was evaporated under vacuum, and the product was subjected to flash chromatography (AcOEt–MeOH, 100:1, for thymine nucleosides and CH_2Cl_2–MeOH, 9:1,

(30) For some preparations of these compounds, see: Watanabe, K. A.; Fox, J. J. *Angew. Chem.* **1966**, 589. Steinfeld, A. S.; Naider, F.; Becker, J. M. *J. Chem. Res. (S)* **1979**, 129. Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, 104, 1316. Kierzek, R. *Nucleosides Nucleotides* **1985**, *4*, 641.

(31) Bhat, V.; Ugarkar, B. G.; Sayeed, V. A.; Grimm, K.; Kosora, N.; Domenico, P. A.; Stocker, E. *Nucleosides Nucleotides* **1989**, *8*, 179.

Table X. Acylation of Citosine Nucleosides 8a–b with *Candida antarctica* Lipase in THF at 60 °C

product	R	t (h)	yield[a] (%)	mp[b] (°C)	IR[c] ν (cm^{-1})	$[\alpha]^{D}_{25}$ (c, solvent)
10a	$CH_3(CH_2)_2$	6	94	124–5	1745	+77.5 (1, MeOH)
10b	$CH_3(CH_2)_4$	12	90	80–1	1740	+60.3 (1.1, MeOH)
12a	$CH_3(CH_2)_2$	9	88	134–5	1741	+49.0 (0.79, MeOH)
12b	$CH_3CH{=}CH$	9	82	110–1	1722	+31.5 (1, MeOH)

[a] Calculated with respect to **8a** or **11** on pure isolated products. [b] Uncorrected. [c] Partial.

Table XI. ^{1}H NMR and ^{13}C NMR Spectral Data for Compounds 10a,b and 12a,b [δ (ppm)][a]

	base ring			sugar moiety						acyl moiety
product	NH	H5	H6	H1′	(H2′, H2″)	H3′	H4′	(H5′, H5″)	OH	
10a	10.86 (s)	7.26 (d)	8.06 (d)	6.11 (t)	2.07 (m), 2.32[b]	4.02 (m)	4.22[b]	4.22[b]	5.45 (d)	0.86 (6 H, m), 1.52 (4 H, m), 2.32[b]
10b	10.70 (br)	7.58 (d)	8.13 (d)	6.28 (t)	2.11 (m), 2.81 (m)	4.37[b]	4.37[b]	4.37[b]		0.89 (6 H, m), 1.31 (8 H, m), 1.65 (4 H, m), 2.34 (2 H, m), 2.51 (2 H, t)

	base ring			sugar moiety							acyl moiety
product	NH	H5	H6	H1′	H2′	H3′	H4′	(H5′, H5″)	2′-OH	3′-OH	
12a	10.87 (s)	7.25 (d)	8.07 (d)	5.77 (d)	4.10[b]	3.90 (m)	4.10[b]	4.31 (m)	5.57 (d)	5.26 (d)	0.88 (6 H, m), 1.55 (4 H, m), 2.35 (4 H, m)
12b	10.83 (br)	7.23 (d)	8.04 (d)	5.70 (d)	4.06[b]	3.95 (m)	4.06[b]	4.32 (m)	5.56 (br)	5.29 (br)	0.83 (3 H, t), 1.52 (2 H, m), 1.84 (3 H, d), 2.34 (2 H, t), 5.87 (1 H, d), 6.83 (1 H, m)

	base ring				sugar moiety					acyl moieties		
product	C2	C4	C5	C6	C1′	C2′	C3′	C4′	C5′	C(O)N	C(O)O	R, R′
10a	154.78	162.53	95.56	145.21	86.54	40.90	70.31	84.61	63.85	174.00	172.83	13.63, 18.09, 35.41, 38.42
10b	154.79	162.54	96.47	144.05	87.52	41.35	70.83	84.91	63.52	174.11	173.31	13.62, 22.00, 22.09, 24.24, 30.91, 31.01, 33.82, 33.96, 37.10 (amide), 41.34 (ester)
12a	154.78	162.63	95.76	145.34	91.20	74.02	69.48	80.96	63.43	174.04	172.80	13.63, 18.10, 35.44 (amide), 38.45 (ester)
12b	155.11	162.92	96.08	145.34	91.64	74.40	69.63	81.26	63.35	174.27	165.69	18.13, 38.72 (amide), 122.29, 146.34

[a] Samples measured in DMSO-d_6 except **10b** ($CDCl_3$).

for those from adenine). Crystallization was obtained from AcOEt or diethyl ether.

Characterization of Products 3a–l. Table IV shows the mp, IR data, and optical rotations. Tables V and VI present the ^{1}H and ^{13}C NMR spectral data and solvents used for their measurement.

3a: mass spectra (70 eV) *m/z* (relative intensity) 284 (M^+, 7), 159 (33), 127 (25), 81 (100), 43 (85). Anal. Calcd for $C_{12}H_{16}N_2O_6$: C, 50.70; H, 5.67; N, 9.85. Found: C, 50.85; H, 5.50; N, 9.71.

3b: mass spectra (70 eV) *m/z* (relative intensity) 312 (M^+, 6), 187 (22), 126 (23), 81 (100), 71 (91). Anal. Calcd for $C_{14}H_{20}N_2O_6$: C, 53.84; H, 6.45; N, 8.97. Found: C, 53.88; H, 6.65; N, 8.78.

3c: mass spectra (70 eV) *m/z* (relative intensity) 321 (M^+, 1), 135 (92), 71 (65), 43 (100). Anal. Calcd for $C_{10}N_{19}N_5O_4$: C, 43.95; H, 7.01; N, 25.63. Found: C, 43.82; H, 6.87; N, 25.78.

3d: mass spectra (70 eV) *m/z* (relative intensity) 368 (M^+, 3), 243 (32), 127 (36), 81 (100). Anal. Calcd for $C_{18}N_{28}N_2O_6$: C, 58.68; H, 7.66; N, 7.60. Found: C, 58.60; H, 7.54; N, 7.56.

3e: mass spectra (70 eV) *m/z* (relative intensity) 396 (M^+, 2), 271 (29), 155 (7), 127 (24), 81 (100). Anal. Calcd for $C_{20}H_{32}N_2O_6$: C, 60.59; H, 8.14; N, 7.07. Found: C, 60.41; H, 8.25; N, 6.98.

3f: mass spectra (70 eV) *m/z* (relative intensity) 310 (M^+, 4), 185 (40), 127 (11), 81 (96), 69 (100). Anal. Calcd for $C_{14}H_{18}N_2O_6$: C, 54.19; H, 5.85; N, 9.03. Found: C, 54.36; H, 5.79; N, 9.12.

3g: mass spectra (70 eV) *m/z* (relative intensity) 296 (M^+, 3), 171 (19), 127 (16), 81 (97), 55 (100). Anal. Calcd for $C_{13}H_{16}N_2O_6$: C, 52.70; H, 5.44; N, 9.45. Found: C, 52.58; H, 5.66; N, 9.21.

3h: mass spectra (70 eV) *m/z* (relative intensity) 234 (M^+ – 71, 1), 134 (100), 63 (51), 55 (51). Anal. Calcd for $C_{13}H_{15}N_5O_4$: C, 51.15; H, 4.95; N, 22.94. Found: C, 51.30; H, 4.73; N, 22.71.

3i: mass spectra (70 eV) *m/z* (relative intensity) 346 (M^+, 3), 221 (34), 126 (8), 105 (100), 81 (74), 77 (30). Anal. Calcd for $C_{17}H_{18}N_2O_6$: C, 58.96; H, 5.24; N, 8.09. Found: C, 59.08; H, 5.45; N, 8.30.

3j: mass spectra (70 eV) *m/z* (relative intensity) 354 (M^+ – 1, 1), 134 (76), 104 (100). Anal. Calcd for $C_{17}H_{17}N_5O_4$: C, 57.46; H, 4.82; N, 19.71. Found: C, 57.68; H, 4.75; N, 19.80.

3k: mass spectra (70 eV) *m/z* (relative intensity) 318 (M^+, 5), 193 (17), 126 (21), 110 (22), 77 (100), 49 (85). Anal. Calcd for $C_{12}H_{15}ClN_2O_6$: C, 45.22; H, 4.74; Cl, 11.12; N, 8.79. Found: C, 45.08; H, 4.55; Cl, 10.96; N, 8.55.

3l: mass spectra (70 eV) *m/z* (relative intensity) 215 (M^+ – 112, 25), 134 (52), 81 (64), 53 (100). Anal. Calcd for $C_{12}H_{14}ClN_5O_4$: C, 43.98; H, 4.31; Cl, 10.82; N, 21.37. Found: C, 44.12; H, 4.45; Cl, 10.98; N, 21.30.

Characterization of Products 4a–l. For compounds **4a–f**, see ref 22.

Compound **4g**: mp 137–8 °C; IR (KBr, cm^{-1}) 1737; $[\alpha]_{25}^{D}$ = –3.46 (*c* = 0.52, DMSO); ^{1}H NMR (DMSO-d_6) δ 11.35 (1 H, br, NH), 7.77 (1 H, s, H6), 6.42 (1 H, m, CH_2), 6.25 (2 H, m, CH_2 and H1′), 6.05 (1 H, m, CH), 5.36 (1 H, m, H3′), 5.22 (1 H, br, OH), 4.05 (1 H, m, H4′), 3.68 (2 H, m, H5′, H5″), 2.32 (2 H, m, H2′, H2″), 1.78 (3 H, s, Me); ^{13}C NMR (DMSO-d_6) δ 165.19 (C=O), 163.86 (C4), 150.07 (C2), 136.02 (C6), 132.51 (CH_2), 128.21 (CH), 109.94 (C5), 84.69 (C4′), 83.87 (C1′), 75.26 (C3′), 61.51 (C5′), 36.71 (C2′), 12.47 (CH_3); mass spectra (70 eV) *m/z* (relative intensity) 296 (M^+, 2), 171 (16), 126 (16), 99 (100), 69 (87), 55 (61). Anal. Calcd for $C_{13}H_{16}N_2O_6$: C, 52.70; H, 5.44; N, 9.45. Found: C, 52.50; H, 5.36; N, 9.41.

Compound **4h**: mp 225 °C dec; IR (KBr, cm^{-1}) 1718; $[\alpha]_{25}^{D}$ = –19.0 (*c* = 0.4, DMSO); ^{1}H NMR (DMSO-d_6) δ 8.35 (1 H, s, H8), 8.14 (1 H, s, H2), 7.36 (2 H, br, NH_2), 6.30 (3 H, m, H1′ and CH_2), 6.04 (1 H, m, CH), 5.46 (2 H, m, H3′ and OH), 4.13 (1 H, m, H4′), 3.64 (2 H, m, H5′, H5″), 2.90 (1 H, m, H2′), 1.92 (1 H, m, H2″); ^{13}C NMR (DMSO-d_6) δ 165.21 (C=O), 156.41 (C6), 152.66 (C2), 149.21 (C4), 139.67 (C8), 132.59 (CH_2), 128.29 (CH), 119.50 (C5), 85.50 (C4′), 84.35 (C1′), 75.78 (C3′), 61.97 (C5′), 36.72 (C2′); mass spectra (70 eV) *m/z* (relative intensity) 250 (M^+ – 55, 2), 134 (100), 55 (35). Anal. Calcd for $C_{13}H_{15}N_5O_4$: C, 51.15; H, 4.95; N, 22.94. Found: C, 51.32; H, 4.83; N, 22.81.

Compound **4k**: mp 144 °C; IR (KBr, cm^{-1}) 1745; $[\alpha]_{25}^{D}$ = +4.0 (*c* = 0.5, MeOH); ^{1}H NMR (DMSO-d_6) δ 10.10 (1 H, br, NH), 7.75 (1 H, s, H6), 6.22 (1 H, t, H1′), 5.35 (1 H, m, H3′), 5.30 (1 H, br, OH), 4.47 (2 H, s, $ClCH_2$), 4.10 (1 H, m, H4′), 3.63 (2 H, m, H5′, H5″), 2.29 (2 H, m, H2′, H2″), 1.78 (3 H, s, Me); ^{13}C NMR (DMSO-d_6) δ 167.18 (C=O), 163.86 (C4), 150.68 (C2), 135.98 (C6), 109.99 (C5), 84.48 (C4′), 83.85 (C1′), 76.75 (C3′), 61.50 (C5′), 41.42 ($ClCH_2$), 36.49 (C2′), 12.47 (CH_3); mass spectra (70 eV) *m/z* (relative intensity) 193 (M^+ – 125, 6), 126 (30), 99 (100), 77 (62), 69 (99). Anal. Calcd for $C_{12}H_{15}ClN_2O_6$: C, 45.22; H, 4.74; Cl, 11.12; N, 8.79. Found: C, 45.12; H, 4.82; Cl, 11.22; N, 8.58.

Compound **4l**: mp 145 °C; IR (KBr, cm^{-1}) 1749; $[\alpha]_{25}^{D} = -11.4$ (c = 0.7, DMSO); ^1H NMR (DMSO-d_6) δ 8.37 (1 H, s, H8), 8.15 (1 H, s, H2), 7.39 (2 H, br, NH_2), 6.35 (1 H, dd, H1'), 5.46 (2 H, m, H3' and OH), 4.48 (2 H, s, $ClCH_2$), 4.13 (1 H, m, H4'), 3.63 (2 H, m, H5', H5''), 3.00 (1 H, m, H2'), 2.50 (1 H, m, H2''); ^{13}C NMR (DMSO-d_6) δ 167.10 (C=O), 156.23 (C6), 152.20 (C2), 149.04 (C4), 139.67 (C8), 119.37 (C5), 85.17 (C4'), 84.10 (C1'), 77.12 (C3'), 61.82 (C5'), 41.40 ($ClCH_2$), 36.48 (C2'); mass spectra (70 eV) m/z (relative intensity) 215 (M^+ - 112, 16), 134 (24), 81 (75), 53 (100). Anal. Calcd for $C_{12}H_{14}ClN_5O_4$: C, 43.98; H, 4.31; N, 21.37. Found: C, 44.16; H, 4.34; N, 21.23.

General Procedure for the Synthesis of Compounds 7a–j. A sample of 2 mmol of **1a–b**, 6 mmol of **2a–g**, and 0.4 g of lipase from *C. antarctica* SP 435 was suspended in 15 mL of THF or dioxane under nitrogen atmosphere. The mixture reacted at 250 rpm during the time and at the temperature indicated in Table VII. Then, the enzyme was filtered off and washed with MeOH, the residue was evaporated under vacuum, and the product was subjected to flash chromatography (AcOEt–MeOH, 92:8, for uridine and AcOEt–MeOH–H_2O, 100:10:1, or CH_2Cl_2–MeOH, 9:1, for adenosine). Crystallization was obtained from diethyl ether.

Characterization of Products 7a–j. Table VII shows the mp, IR data, and optical rotations. Tables VIII and IX present the ^1H and ^{13}C NMR spectral data and solvents used for their measurement.

7a: mass spectra (70 eV) m/z (relative intensity) 250 (M^+ - 59, 2), 164 (71), 135 (65), 43 (100). Anal. Calcd for $C_{12}H_{15}N_5O_5$: C, 46.60; H, 4.89; N, 22.64. Found: C, 46.81; H, 4.77; N, 22.71.

7b: mass spectra (70 eV) m/z (relative intensity) 202 (M^+ - 84, 26), 113 (27), 71 (100), 43 (73). Anal. Calcd for $C_{13}H_{18}N_2O_7$: C, 49.68; H, 5.77; N, 8.91. Found: C, 49.88; H, 5.65; N, 8.78.

7c: mass spectra (70 eV) m/z (relative intensity) 273 (M^+ - 65, 3), 136 (22), 71 (92), 43 (100). Anal. Calcd for $C_{14}H_{18}N_4O_6$: C, 49.70; H, 5.36; N, 16.56. Found: C, 49.82; H, 5.47; N, 16.78.

7d: mass spectra (70 eV) m/z (relative intensity) 287 (M^+ - 111, 38), 155 (77), 113 (94), 43 (100). Anal. Calcd for $C_{19}H_{30}N_2O_7$: C, 57.27; H, 7.59; N, 7.03. Found: C, 57.30; H, 7.54; N, 7.06.

7e: mass spectra (70 eV) m/z (relative intensity) 393 (M^+, 1) 164 (100), 136 (87). Anal. Calcd for $C_{18}N_{27}N_5O_5$: C, 54.95; H, 6.92; N, 17.80. Found: C, 54.81; H, 7.05; N, 17.98.

7f: mass spectra (70 eV) m/z (relative intensity) 200 (M^+ - 112, 13), 113 (6), 69 (100). Anal. Calcd for $C_{13}N_{16}N_2O_7$: C, 50.00; H, 5.16; N, 8.97. Found: C, 50.16; H, 5.09; N, 9.12.

7g: mass spectra (70 eV) m/z (relative intensity) 334 (M^+ - 1, 1), 164 (38), 136 (34), 69 (100). Anal. Calcd for $C_{14}H_{17}N_5O_5$: C, 50.15; H, 5.11; N, 20.89. Found: C, 50.28; H, 5.16; N, 20.81.

7h: mass spectra (70 eV) m/z (relative intensity) 178 (M^+ - 143, 17), 164 (63), 135 (100), 55 (59). Anal. Calcd for $C_{13}H_{15}N_5O_5$: C, 48.60; H, 4.71; N, 21.08. Found: C, 48.70; H, 4.73; N, 21.01.

7i: mass spectra (70 eV) m/z (relative intensity) 135 (100). Anal. Calcd for $C_{13}H_{14}N_4O_6$: C, 48.45; H, 4.38; N, 17.38. Found: C, 48.38; H, 4.45; N, 17.30.

7j: mass spectra (70 eV) m/z (relative intensity) 302 (M^+ - 18, 2), 113 (17), 77 (100), 49 (91). Anal. Calcd for $C_{11}H_{13}ClN_2O_7$: C, 41.20; H, 4.09; Cl, 11.06; H, 8.74. Found: C, 41.28; H, 4.05; Cl, 10.88; N, 8.80.

Acylation of 2'-Deoxycytidine, 8a. A sample of 2 mmol of **8a**, 6 mmol of acid anhydride, and 0.4 g of lipase from *C. antarctica* SP 435 was suspended in 15 mL of THF under nitrogen atmosphere. The mixture reacted at 250 rpm at 60 °C and for the time indicated in Table X (when *N*-acyl-2'-deoxycytidine disappeared). Then, the enzyme was filtered off and washed with MeOH, the residue was evaporated under vacuum, and the product was subjected to flash chromatography (CH_2Cl_2–MeOH, 95:5). Crystallization was obtained from diethyl ether.

Acylation of Cytidine, 8b. Cytidine, **8b**, was previously N-acylated in DMF with equimolecular amounts of acid anhydride.[31] Then, 2 mmol of **11**, 6 mmol of oxime ester, **2b** or **2e**, and 0.4 g of lipase from *C. antarctica* SP 435 was suspended in 15 mL of THF under nitrogen atmosphere. The mixture was allowed to react at 250 rpm at 60 °C for the time in Table X. Then, the enzyme was filtered off and washed with MeOH, the residue was evaporated under vacuum, and the product was subjected to flash chromatography (CH_2Cl_2–MeOH, 9:1). Crystallization was obtained from diethyl ether.

Characterization of Products 10a–b and 12a–b. Table X shows the mp, IR data and optical rotations. Table XI presents the ^1H and ^{13}C NMR spectral data and solvents used for their measurement.

10a: mass spectra (70 eV) m/z (relative intensity) 367 (M^+, 1), 182 (12), 112 (30), 71 (71), 43 (100). Anal. Calcd for $C_{17}H_{25}N_3O_6$: C, 55.58; H, 6.86; N, 11.44. Found: C, 55.41; H, 6.77; N, 11.51.

10b: mass spectra (70 eV) m/z (relative intensity) 423 (M^+, 1), 210 (17), 112 (52), 81 (100). Anal. Calcd for $C_{21}H_{33}N_3O_6$: C, 59.56; H, 7.85; N, 9.92. Found: C, 59.68; H, 7.65; N, 9.78.

12a: mass spectra (70 eV) m/z (relative intensity) 365 (M^+ - 18, 1), 189 (20), 112 (100), 71 (63). Anal. Calcd for $C_{17}H_{25}N_3O_7$: C, 53.26; H, 6.57; N, 10.96. Found: C, 53.12; H, 6.47; N, 10.88.

12b: mass spectra (70 eV) m/z (relative intensity) 363 (M^+ - 18, 1), 190 (10), 112 (34), 69 (100). Anal. Calcd for $C_{17}H_{23}N_3O_7$: C, 53.54; H, 6.08; N, 11.02. Found: C, 53.33; H, 5.95; N, 10.86.

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