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Enzymatic Acylation and Alkoxycarbonylation of α-, Xylo-, Anhydro-, and Arabino-Nucleosides.

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Abstract: *5'-O-acyl and 5'-O-alkoxycarbonyl derivatives of α-, anhydro-, xylo - and arabinonucleosides could be obtained through a lipase-mediated reaction with SP 435 lipase (from Candida antarctica) by using acetoxime butyrate or butyric anhydride, together with benzyloxycarbonyl-O-acetoxime as acylating agents Alkoxycarbonylation gave poorer yields than acylation and other lipases tested gave non-selective reaction or not reaction at all*

INTRODUCTION

Selective protection and deprotection of polyfunctional molecules is a critical problem in organic synthesis.[1] In nucleoside chemistry this problem is accentuated by the presence of multiple hydroxy functions of very similar reactivity. For this reason, the achievement of selective reactions on them is an interesting challenge,[2] since successful advances in this field may lead to new synthetic methods of nucleoside analogues, which are compounds of high significance in some areas of medicinal chemistry,[3] and show antineoplasic[4] and antiviral activity.[5]

An important and synthetically relevant example of this problem is the selective acylation and alkoxycarbonylation of the sugar moiety of these compounds in order to obtain derivatives with antitumour activity,[6] and also represents a way of introducing protective basic-labile groups,[1] which play an important role in the synthesis of oligonucleotides.[7]

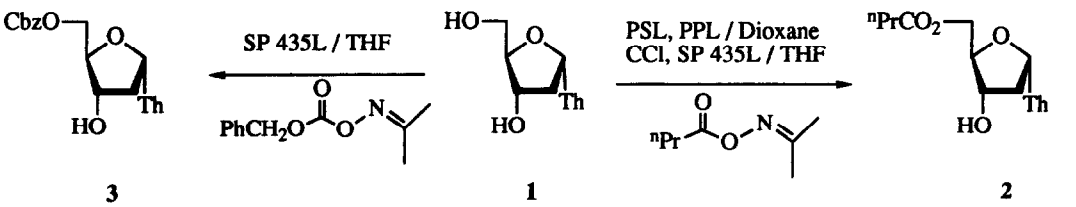
On the other hand, enzymatic approaches to natural products esterification have received keen attention in recent years.[8] This is due to the much higher regioselectivity of acylation achievable by enzymic as compared to conventional chemical methods.[9] In the case of nucleosides, few examples are known dealing with regioselective acylation. *Subtilisin*[10] and a modified derivative[11] was employed with trihaloethyl butyrates or enol esters, but selectivity was low or the catalytic material was difficult to achieve. Nozaki has tried lipases on 2'-deoxynucleosides, the results depended on the solvent and only in one case the regioselectivity was almost complete.[12] As a part of our ongoing program to design new regioselective enzymatic transformations of

polyhydroxy compounds, we have described recently the preparation of 3'-*O*-acylated derivatives[13] and 3'-*O*-carbonates[14] from unprotected 2'-deoxynucleosides through an enzymatic acylation or alkoxycarbonylation reaction using oxime esters or *O*-alkoxycarbonyloximes and lipase from *Pseudomonas cepacia* (PSL). We have also found that lipases from *Candida antarctica* (CAL) are able to acylate[15] or alkoxycarbonylate[16] both 2'-deoxynucleosides and ribonucleosides at 5'-OH.

In view of these results, we believed that nucleosides with different orientation of the hydroxyl groups, which have not been tested in enzyme-mediated reactions, could be an interesting subject of study from a synthetic standpoint as well as to get information about the preference of some lipases with regards to the geometry of the substrate employed. In this study we carried out a systematic investigation of the usefulness of this enzymatic approach for the synthsis of 5'-*O*-butyrates and 5'-*O*-carbonates of nucleosides bearing various configurations in the sugar moiety. This could be made with a lipase from *C. antarctica* using butyric anhydride or acetoxime butyrate and benzyloxycarbonyl-*O*-acetoxime. Other enzymes tested, like PSL, *Porcine pancreatic* lipase (PPL) and *Candida cylindracea* lipase (CCL) were not, in general, as effective as CAL in this kind of enzymatic processes.

RESULTS AND DISCUSSION

Enzymatic acylation and alkoxycarbonylation of α-Thymidine (1): To the best of our knowledge there is no report dealing with enzyme-mediated acylation of α-nucleosides. The α-configuration could involve severe steric hindrances to acylate 3'-OH whereas 5'-*O* position has no impediments to be esterified. Our previous experience have shown that the ß-counterpart is acylated at the primary hydroxyl group with a lipase from *Candida antarctica* (CAL SP435L)[17] whereas PPL, CCL and PSL present reversed regioselectivity, namely towards secondary hydroxyl group.[13,15] However, α-thymidine was tested with these four enzymes and none of them were able to acylate 3'-OH (Scheme I). In all cases acylation takes place exclusively at 5'-OH , though, as it can be seen in Table I, CAL and PSL gave the best results. The reactions were carried out in THF or 1,4-dioxane with oxime butyrate, although butyric anhydride can be also employed without affecting regioselectivity. It is of note that PSL changes its preference depending on configuration at the anomeric site. This effect is also observed with PPL and CCL but the conversion falls down with respect to that achieved with the ß-counterpart. With regards to CAL regioselectivity is now complete, in contrast with ß-thymidine, which gave little amounts of other regioisomers.[15] Thus, the bulky group in the anomeric site has a dramatic influence with regards to the specificity or even the conversion of the reaction.



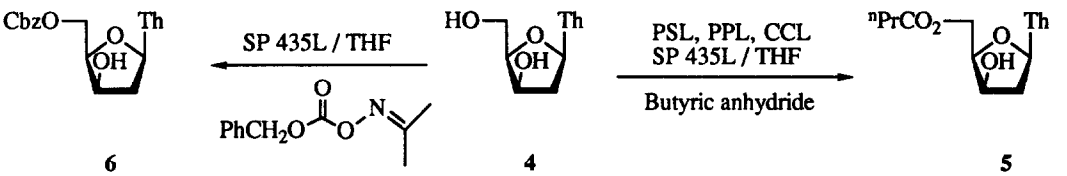
3 1 2

SCHEME I

In view of these results, PSL and CAL seemed the most suitable catalysts to perform the alkoxycarbonylation of α-thymidine. CAL was selected because the advantage of using an immobilized enzyme is the possibility of reusing the lipase. The alkoxycarbonylating reagent was acetone-*O*-(benzyloxy)carbonyloxime, which had been already employed by us to achieve Cbz-derivatives both of sugars[18] and nucleosides.[14,16] As it is shown in Scheme I the unique product was 5'-*O*-benzyloxycarbonyl-α-thymidine, **3**, whose characteristic data, together with those of 5'-*O*-butyryl-α-thymidine, **2**, are depicted in Table II. Tables III and IV give ^{1}H- and ^{13}C-NMR spectral data.

Enzymatic acylation and alkoxycarbonylation of *xylo*-Thymidine (4):[19] The 3'-"up" configuration of the secondary hydroxyl group in this nucleoside becomes it an interesting substrate. The secondary hydroxyl function is placed at the same face that the base moiety, so steric hindrances play an unfavorable role to achieve acylation at this position. Furthermore, this particular configuration becomes hydroxyl at 5' more hindered than in thymidine. The triad of lipases most widely used in synthesis, namely PPL, PSL and CCL when employed with thymidine exhibited preference towards 3'-OH and only CAL could acylate 5'-OH. In the case of *xylo*-thymidine only CAL was able to convert in good yield the substrate into 5'-*O*-butyryl*xylo*thymidine, **5** (Scheme II). As with α-thymidine, CCL, PPL and PSL gave poorer conversions with the same regioselectivity towards primary hydroxyl and acylation at 3'-OH was not observed in any case (see Table I). It is noteworthy that the selectivitiy of CCL, PPL and PSL is reversed due to the change in the configuration at 3' site, as it also occurrs in the case of α-thymidine and these three lipases (see above).

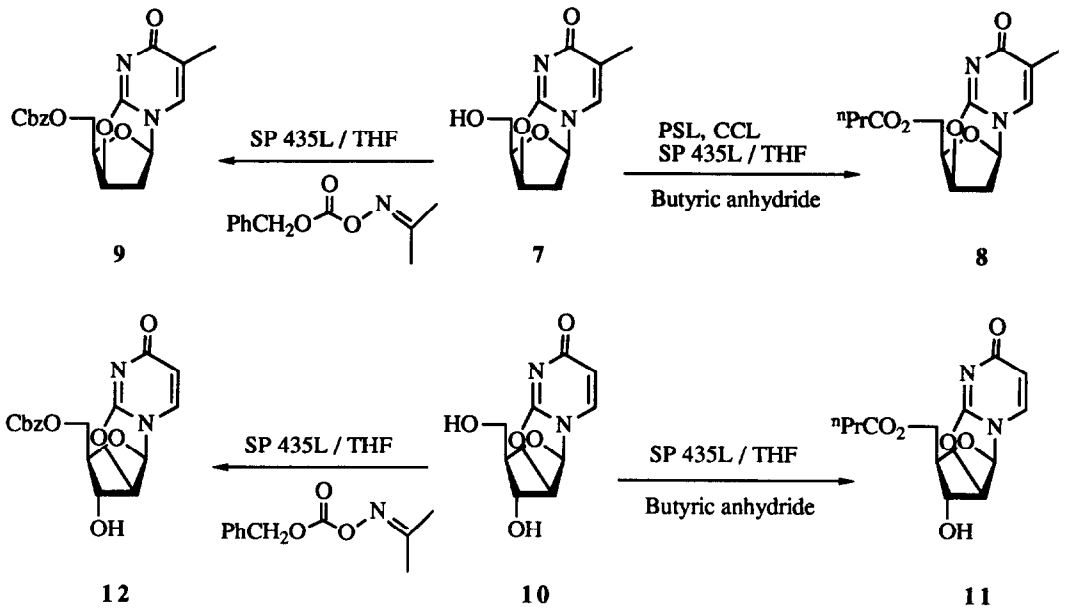
In view of the good result achieved for the acylation, the alkoxycarbonylation reaction was made with CAL using acetone-*O*-(benzyloxy)carbonyloxime, giving in high yield a unique product, 5'-*O*-benzyloxycarbonyl*xylo*thymidine, (**6**). Acetone oxime moiety played the role of leaving group and di- or 3'-*O*-acylated compounds could not be detected. Tables II, III and IV summarize physical and spectral properties of both compounds **5** and **6**.



6 **4** **5**

SCHEME II

Enzymatic acylation and alkoxycarbonylation of anhydronucleosides 7 and 10: 2,3'-anhydrothymidine, 7, was subjected to the action of the four aforementioned lipases. This nucleoside only possesses one alcoholic group but its structure represents an interesting target to test the capability of the lipases to acylate such a hindered hydroxyl. This case is similar to that of *xylo*thymidine with regards to the configuration of 3'-site but the presence of the base moiety fixed at this position involves more steric hindrances than in the *xylo*nucleoside. As it can be seen in Table I, PPL can not accept 2,3'-anhydrothymidine as substrate whereas CCL, PSL and CAL (higher conversion in this order), were able to convert it in the 5'-*O*-

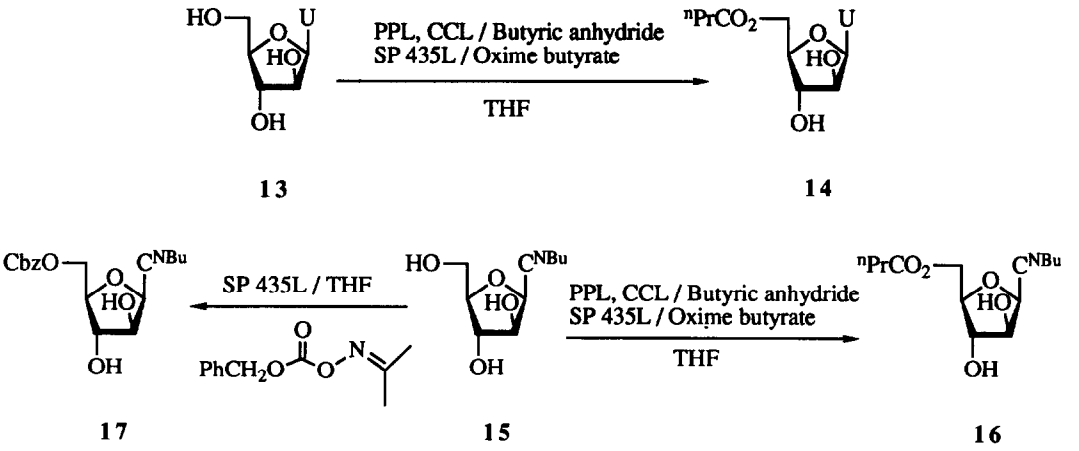


SCHEME III

butyryl-2,3'-anhydrothymidine, **8**. The low yields obtained were presumably due to the poor solubility of the substrate in organic solvents; THF was the most suitable and the acylating agent had to be butyric anhydride, since oxime butyrate is a weak one to accomplish this acylation. In the case of 2,2'-anhydrouridine, **10**, only CAL and PSL gave reaction with butyric anhydride. Again, the conversion was poor, because the anhydronucleoside is hardly soluble in 1,4-dioxane or THF. Selectivity towards 5'OH could only be achieved with CAL, since PSL yielded a mixture of 3'-*O*-butyrate and diacylated nucleoside. 2,2'-anhydrouridine has the same configuration that 2'-deoxynucleosides with regards to the possible sites of acylation so could be expected that PSL would acylate the secondary hydroxyl selectively. This is not possible due to the steric hindrances produced by the 2,2' linkage, which forces the nucleoside to an unfavorable position inside the binding pocket of PSL. PPL and CCL gave no conversion with this substrate.

With these results in mind, we choose CAL to alkoxycarbonylate selectively at primary hydroxyl group both **7** and **10** with yields similar to the acylation. The agent employed to achieve this goal was the same that in aforementioned cases, acetone-*O*-(benzyloxy)carbonyloxime. Cbz-nucleosides **9** and **12** are characterized in Table II, together with butyryl esters **8** and **11**. Tables III and IV show spectral data (^{1}H- and ^{13}C-NMR) of these compounds.

Enzymatic acylation and alkoxycarbonylation of *arabino*nucleosides 13 and 15:[20] *Arabino*nucleosides assayed were *ara*uridine,**13** and *N*-butyryl-*ara*cytidine, **15**. *Ara*cytidine was previously *N*-acylated[21] to be tested in enzymatic proccesses, since cytosine nucleosides remain unreactive under conditions throughout this work.[15] The shape of these substrates leaves 3'-OH in the same conditions that in 2'-deoxynucleosides, so it could be expected that PSL would acylate 3'-OH selectively. With regards to *ara*uridine



13 14 17 15 16

SCHEME IV

the enzymes tested were not only able to acylate this position but primary hydroxyl was the preferred site to all of them, though in the case of PSL this was accompanied by other byproducts including di- and triacylated derivatives. As it can be seen in Table I, CAL was the best catalyst to obtain 5'-*O*-butyryl*ara*uridine, **14**. In the case of *N*-butyryl-*ara*cytidine, PSL gave no conversion and only CAL converted the substrate in *N*,5'-*O*-dibutyryl*ara*cytidine, **16**. PPL and CCL gave a mixture of products and the conversion was low (30%).

Alkoxycarbonylation reaction was made as usually with CAL but in the case of *ara*uridine could not be effected with satisfactory yield. *N*-butyryl*ara*cytidine was converted into its 5'-Cbz derivative, **17**, albeit in low yield, showing that *ara*nucleosides are poor substrates for enzymatic alkoxycarbonylation. Tables II, III and IV depict the characteristic data of compounds **14**, **16** and **17**.

Table I. Acylation of nucleosides **1,4,7,10,13,15** with several lipases in organic solvents.[a]

Substrate	PSL				PPL				CCL				CAL			
	Solv.	T °C	t h	Yield %	Solv.	T °C	t h	Yield %	Solv.	T °C	t h	Yield %	Solv.	T °C	t h	Yield %
1[b]	Dioxane	60	4	95	Dioxane	60	24	22	THF	60	72	16	THF	30	3	97
4[c]	THF	60	72	12	THF	60	48	12	THF	60	72	19	THF	60	12	94
7[c]	THF	60	24	28	THF	60	12	-	THF	60	48	12	THF	60	12	41
10[c]	Dioxane	60	24	[d]	Dioxane	60	48	-	THF	60	48	-	THF	60	16	52
13[e]	THF	60	24	[d]	THF	60	20	15	THF	60	72	20	THF	60	7	81
15[c]	THF	60	40	-	THF	60	36	[d]	THF	60	36	[d]	THF	60	6	84

[a] Yields calculated with respect to substrates on pure isolated products (if >40%) or by ^{13}C-NMR analysys of crude reactions. [b] Oxime butyrate. [c] Butyric anhydride [d] Mixture of products. [e] Butyric anhydride for PSL, PPL, CCL; oxime butyrate for CAL.

Table II. Compounds **2-17** prepared with *Candida antarctica* lipase.[a]

Product	R	t (h)	Yield[b] (%)	m.p.[c] (°C)	IR[d] υ (cm^{-1})	$[\alpha]_{25}^{D}$ (c, solvent)
2	CH_3-$(CH_2)_2$-	3	97	syrup	1695	+8.24 (1.65, MeOH)
3	Ph-CH_2-O-	12	94	syrup	1747	+30.5 (10, MeOH)
5	CH_3-$(CH_2)_2$-	12	94	syrup	1697	+16.0 (1.20, MeOH)[e]
6	Ph-CH_2-O-	20	90	syrup	1753	+10.9 (1.0, MeOH)[e]
8	CH_3-$(CH_2)_2$-	12	41	187-8	1734	-24.6 (0.45, DMSO)[e]
9	Ph-CH_2-O-	24	32	187-8	1753	+7.70 (0.13, MeOH)
11	CH_3-$(CH_2)_2$-	16	52	179-80	1739	-38.4 (0.51, DMSO)[e]
12	Ph-CH_2-O-	24	30	155-6	1751	-2.50 (0.40, MeOH)
14	CH_3-$(CH_2)_2$-	7	81	127-8	1736	+122 (0.50, MeOH)
16	CH_3-$(CH_2)_2$-	6	84	160-1	1710	+116 (0.30, MeOH)[e]
17	Ph-CH_2-O-	24	25	145-6	1749	+104 (0.41, MeOH)[e]

[a] All reactions carried out at 60 °C except that for **1a** (30 °C). [b] Calculated with respect to **1-6** on pure isolated products. [c] Uncorrected. [d] Partial. [e] Measured with Hg lamp (λ = 578).

The structure of all the products were determined on the basis of their spectral data. For example, their ^{13}C-NMR spectra showed a shift on C5' of *ca.* 3 ppm. towards lower fields with respect to the same carbon atom in the starting nucleosides. In addittion, H5', H5" showed a shift of *ca.* 1 ppm. downfield with respect to the nucleosides without modification. Assignment of C1' and C4' was readily made on the basis of the coupled ^{13}C-NMR spectra, since $^{1}J_{C1',H\text{-}C1'}$ exhibit values around 165 Hz whereas the other $^{1}J_{C,H}$ of the sugar moiety (for example, $^{1}J_{C4',H\text{-}C4'}$) are 15-20 Hz smaller.[22]

CONCLUSION

In the present work we described a convenient method to obtain 5'-*O*-carbonates and butyrates of some unusual-configurated nucleosides under mild conditions through a lipase-mediated reaction. The procedure represents an easy way to introduce modifications on these kind of nucleosides, which can be used as intermediates in synthesis of novel derivatives with potential antiviral and antitumour activities. On the other hand, we have screened the possibilities of some unrelated lipases to achieve this goal and this could be useful to get more knowledge about the active sites of these enzymes in regard with the shape of the substrate. Interestingly the stereochemistry at C1', C2' and C3' seems to have no influence in the regioselectivity of the reaction in the case of CAL. Since the procedure offers versatility in order to introduce different groups in the nucleoside, and the commercial availability of the enzymes in large quantities (hundred of grams) and relatively

Table III. ^{1}H-NMR spectral data for compounds **2-17** (only non-interchangable signals) δ (ppm).[a]

Product	Base ring		Sugar moiety					Acyl moiety
	Me	H6	H1'	(H2',H2")	H3'	H4'	(H5',H5")	
2	1.73(s)	7.70(s)	6.08(dd)	2.53(m) 1.94(m)	4.18(m)	4.27(m)	4.00(m)	0.82 (3H, t); 1.45 (2H, m); 2.18(2H, t)
3	1.78(s)	7.48(s)	6.07(t)	2.61(m) 2.45 (m)	4.35(m)	4.52 (m)	4.13 (m)	5.15 (2H, s); 7.35 (5H, s)
5	1.78(s)	7.76(s)	6.08(dd)	1.90(m) 2.61(m)	4.33[b]	4.00(m)	4.27(m) 4.33[b]	0.89 (3H, t); 1.56 (2H, m); 2.29 (2H, t)
6	1.73(s)	7.61(s)	6.00(dd)	2.28(m) 2.50(m)	4.45[b]	4.02(m)	4.36(m) 4.45[b]	5.09 (2H, s); 7.28 (5H, s)
8	1.75(s)	7.58(s)	5.86(d)	2.59(m) 2.50[c]	5.30(m)	4.42(m)	4.29(m) 4.08(m)	0.83 (3H, t); 1.47 (2H, m); 2.17 (2H, t)
9	1.76(s)	7.51(s)	5.85(d)	2.58(m) 2.50[b]	5.31(m)	4.47(m)	4.17(m) 4.37(m)	5.13 (2H, s); 7.37 (5H, s)

Product	Base ring		Sugar moiety					Acyl moiety
	H5	H6	H1'	H2'	H3'	H4'	(H5', H5")	
11	5.86(d)	7.87(d)	6.33(d)	5.25(d)	4.37(m)	4.29(m)	3.96(m)	0.82 (3H, t); 1.45 (2H, m); 2.18 (2H, t)
12	6.20(d)	7.93(d)	6.56(d)	5.50(d)	4.70(m)	4.60(m)	4.33(m)	5.26 (2H, d); 7.55 (5H, s)
14	5.55(d)	7.49(d)	6.02(d)	4.03(m)	3.93[b]	3.93[b]	4.26(m)	0.89 (3H, t); 1.56 (2H, m); 2.30 (2H, t)
16	7.64(d)	8.30(d)	6.41(d)	4 46(m)	4.26(m)	4.35(m)	4.39(m) 4.75(m)	1.15 (6H, m); 1.87 (4H, m); 2.55 (4H, m)
17	7.50[b]	8.30(d)	6.41(d)	4.48(m)	4.23(m)	4.35(m)	4.53(m) 4.78(m)	5.41 (2H, s); 7.50[b]; 1.16(3H, t);1.87 (2H, m); 2.60 (2H, t)

[a] All samples measured in DMSO-d_6 except **3**, **6** ($CDCl_3$) and **12**, **16**, **17** (CD_3OD). Signals of the sugar moiety assigned through selective irradiations.[b] Superimposed signals. [c] Overlapped by DMSO-d_6

unexpensive, this method appears suitable to preparative regioselective acylation of nucleosides, which are compounds of importance in some areas of medicinal chemistry.

EXPERIMENTAL

Lipase from *Candida antarctica* SP 435L[17] was kindly gifted by Novo Nordisk Company. Nucleosides were purchased from Sigma. THF and 1,4-dioxane were distilled over $LiAlH_4$ in order to avoid moisture. Pre-coated 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. Melting points were taken on samples in open capillary tubes using a Büchi melting-point apparatus and are uncorrected. Optical rotations were measured using a Perkin-Elmer 241 polarimeter. IR spectra were recorded on a Mattson 3000 FT spectrometer. NMR spectra

Table IV. ^{13}C-NMR chemical shifts of compounds **2-17** δ (ppm).[a]

Product	Base ring					Sugar moiety					Acyl or carbonate moiety	
	C2	C4	C5	C6	Me	C1'	C2'	C3'	C4'	C5'	C=O	R
2	150.72	164.08	109.17	137.07	12.52	85.23[b]	[c]	70.71	85.23[b]	64.00	172.82	13.58; 18.13; 35.47
3	150.82	164.76	109.01	137.39	12.16	87.92	40.31	71.43	86.44	67.25	154.68	69.83; 128.22; 128 44; 128.49; 134.66
5	150.74	163.97	109.14	137.12	12.61	83.88	[c]	69.10	81.44	63.12	172.93	13.58; 18.06; 35.43
6	150.93	164.58	109.85	137.90	12.37	85.94	40.77	69.50	82.14	65.88	155.15	69.98; 128.14; 128.30; 128.53; 134.73
8	153.69	171.09	116.21	136.94	13.21	87.08	32.91	77.42	81.99	61.86	172.58	13.58; 18.02; 35.22
9	153.42	171.02	116 27	136.84	13.21	87 08	32.82	77.19	81 91	66 05	154.27	69.37; 128.37; 128.57; 128.66; 135.39
11	159.81	171.27	108.92	137.05	-	90.21	88.84	75.02	85.79	63.20	172.72	13.51; 18.13; 35.07
12	161.88	175.43	109.99	138.47	-	92.45	91 14	76.89	88.09	67.67	156.01	70.71; 129.4; 136.85
14	150.72	163.61	100.40	142.50	-	85.70	76 38	74.91	82.00	63.70	173.04	13.67; 18.22; 35.53
16	157.79	164.10	97.02	147.80	-	89.74	78.35	75.43	84.99	64 71	175 00	13.90; 19.38; 36.79; 39.93; 175.70 (CON)
17	157.80	164.06	97.09	147.83	-	89.70	78.02	75.84	84.73	68.22	156.54	13.89;19.39; 39.93;70.74; 129.20; 129.46; 129.57; 136.90; 175.64 (CON)

[a] Samples measured in DMSO-d_6 except **3**, **6** ($CDCl_3$) and **12**, **16** and **17** (CD_3OD). [b] Superimposed signals. [c] Overlapped by DMSO-d_6

were recorded using a Bruker AC300 spectrometer. Mass spectra were obtained on a Hewlett-Packard 5897A spectrometer. Microanalyses were performed on a Perkin-Elmer model 240 instrument. Acetoxime butyrate and benzyloxycarbonyl-*O*-acetoxime, were prepared as described in aforementioned papers.

General procedure for the synthesis of compounds 2-17 : 0.5 mmol of **1, 4, 7, 10, 13** or **15**, 1.5 mmol of the corresponding oxime ester, anhydride or carbonate and 0.1g of lipase from *Candida antarctica* SP 435 A or SP 435 L was suspended in 5 mL of THF or dioxane. The mixture was allowed to react at 60°C for the time indicated in Table I. 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 for a- and *xylo*-thymidine and *arabino*uridine; CH_2Cl_2 : MeOH 92 : 8 for anhydronucleosides and *N*-butyryl-*arabino*cytidine). Crystallization was obtained from AcOEt or diethyl ether.

Compound **2** Mass spectra (70eV) m/z,%: 312 (M+, 1), 187 (2), 127 (15), 81 (100), 71 (46); Anal. Calcd for $C_{14}H_{20}N_2O_6$: C, 53.84; H, 6.45; N, 8.97. Found: C, 53.70; H, 6.56; N, 9.01.

Compound **3**: Mass spectra (70eV) m/z,%: 376 (M+, 1), 251 (2), 127 (10), 91 (100); Anal. Calcd for

$C_{18}H_{20}N_2O_7$: C, 57.44; H, 5.36; N, 7.44. Found: C, 57.40; H, 5.56; N, 7.31.

Compound **5**: Mass spectra (70eV) m/z,%: 312 (M+, 2), 187 (36), 126 (19), 81 (100), 71 (49); Anal. Calcd for $C_{14}H_{20}N_2O_6$: C, 53.84; H, 6.45; N, 8.97. Found: C, 53.74; H, 6.56; N, 8.69.

Compound **6**: Mass spectra (70eV) m/z,%: 376 (M+, 1), 251 (1), 126 (21), 91 (100); Anal. Calcd for $C_{18}H_{20}N_2O_7$: C, 57.44; H, 5.36; N, 7.44. Found: C, 57.34; H, 5.44; N, 7.31.

Compound **8**: Mass spectra (70eV) m/z,%: 294 (M+, 2), 223 (5), 127 (63), 81 (100), 71 (46); Anal. Calcd for $C_{14}H_{18}N_2O_5$: C, 57.10; H, 6.15; N, 9.54. Found: C, 57.05; H, 6.16; N, 9.61.

Compound **9**: Mass spectra (70eV) m/z,%: 358 (M+, 3), 224 (2), 126 (33), 91 (100); Anal. Calcd for $C_{18}H_{18}N_2O_6$: C, 60.33; H, 5.06; N, 7.82. Found: C, 60.20; H, 5.16; N, 7.71.

Compound **11**: Mass spectra (70eV) m/z,%: 296 (M+, 1), 225 (10), 185 (30), 97 (56), 71 (100); Anal. Calcd for $C_{13}H_{16}N_2O_6$: C, 52.70; H, 5.44; N, 9.45. Found: C, 52.71; H, 5.46; N, 9.31.

Compound **12**: Mass spectra (70eV) m/z,%: 112 (13), 108 (82), 91 (100), 77 (65); Anal. Calcd for $C_{17}H_{16}N_2O_7$: C, 56.67; H, 4.48; N, 7.77. Found: C, 56.59; H, 4.34; N, 7.41.

Compound **14**: Mass spectra (70eV) m/z,%: 314 (M+, 1), 202 (23), 113 (36), 71 (100); Anal. Calcd for $C_{13}H_{18}N_2O_7$: C, 49.68; H, 5.77; N, 8.91. Found: C, 49.79; H, 5.66; N, 9.11.

Compound **16**: Mass spectra (70eV) m/z,%: 365 (M+-18, 1), 182 (30), 150 (50), 112 (100), 71 (75); Anal. Calcd for $C_{17}H_{25}N_3O_7$ C, 53.26; H, 6.57; N, 10.96. Found: C, 53.08; H, 6.56; N, 11.15.

Compound **17**: Mass spectra (70eV) m/z,%: 336 (M+-111, 1), 182 (21), 151 (42), 112 (100), 91 (79); Anal. Calcd for $C_{21}H_{25}N_3O_8$: C, 56.27; H, 5.65; N, 9.33. Found: C, 56.08; H, 5.56; N, 9.35.

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