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Selective Aminoacylation of Nucleosides through an Enzymatic Reaction with Oxime Aminoacyl Esters

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Abstract: *N-protected aminoacyl acetoxime esters are useful agents for enzymatic aminoacylation of 2'-deoxy- and ribo-nucleosides. In both cases, 5'-O-derivatives can be prepared if the lipase used is SP 435 (from Candida antarctica) whereas 3'-O-isomers from 2'-deoxynucleosides are obtained with Pseudomonas cepacia.*

INTRODUCTION

Aminoacyl nucleosides themselves and their oligonucleotide derivatives are compounds of high significance in biological chemistry. It has been well established for more than 30 years that the 3'-terminus of all transfer ribonucleic acids (tRNAs) contain the common C-C-A sequence and that its 3'-terminal adenosine *cis*-diol system is a site of attachment of the amino acid, so aminoacylated derivatives of oligoribonucleotides are important tools to study the specific incorporation of aminoacid into proteins.[1] The conjugates of short oligonucleotides and peptides also play a role as materials for transmembrane delivery of active nucleotides to the cell.[2] Aminoacyl components bound to deoxyribonucleic acids were detected in tumours.[3] On the other side, aminoacylated derivatives of adenine nucleosides have been used to prevent deamination *in vitro*.[4] Also, D-penicillinaminenucleoside conjugates were synthesized and evaluated against HIV,[5] and antitumour activity of cytosine and adenine nucleosides of unsaturated 5-(aminoacyl)aminopentofuranoses was tested.[6]

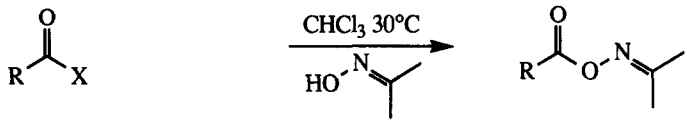
Common methods employed for the introduction of aminoacids into nucleosides involve direct condensation of appropriately *N*-protected aminoacids (together with the most known groups benzyloxycarbonyl, CBZ, and *tert*-butyloxycarbonyl, BOC, also 2-(4-biphenylyl)isopropyloxycarbonyl BPOC,[7] Phtalimido,[4] and *N*-formylthiazolidine derivatives[5] have been used) under basic conditions using various agents to catalyze the condensation: Dicyclohexylcarbodiimide (DCC), (mesitylenesulfonyl)tetrazole (MST),[7] *N,N*-bis-(2-oxooxazolidin-3-yl)phosphorodiamidic chloride (BOP-Cl)[5]. Aminoacyl anhydrides[8] with pyridine and *N,N'*-thiocarbonyl diimidazole[9] were also used to obtain 2'(3')-*O*-aminoacylnucleotides.

On the other hand, enzymatic approaches to the synthesis of aminoacyl conjugates are limited to the obtention of peptides, through papain[10] or subtilisin[11] catalyzed reaction. Recently there was a report oulining an enzymatic approach to the aminoacylation of nucleosides, but the results are quite poor using BOC-PheOH activated as *N*-Succinimidyl ester with subtilisin: in the case of adenine bases, exocyclic *N*-acylation took place.[12] As part of our ongoing program to design new regioselective enzymatic transformations of polyhydroxy compounds, we have described 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, ribonucleosides and other related nucleosides[17] at 5'-OH.

With these results in mind, we have developed a convenient method to obtain aminoacylated nucleosides in a regioselective manner by using lipases from *Pseudomonas cepacia* and *Candida antarctica*, employing *N*-protected acetoxime aminoacyl esters as acylating agents, the results are described here.

RESULTS AND DISCUSSION

Synthesis of *N*-protected acetoxime aminoacyl esters (1b-9b): Since lipases need activated ester forms to carry out acylations, we chose commercially available *N*-protected *p*-nitrophenyl- (PNP) or *N*-succinimidyl- (Succ) esters (**1a-9a**), but the results obtained with thymidine were not satisfactory, none of the enzymes tested (PSL, CAL, PPL, papain, pancreatin from hog pancreas, acylase from *Aspergillus sp.*, protease from *Aspergillus oryzae*) gave appreciable conversion in the solvents usually employed in these kind of biotransformations (THF or 1,4-dioxane). In view of this, we prepared the corresponding acetoxime esters by incubating overnight in $CHCl_3$ the aforementioned activated esters and acetoxime (Scheme I).

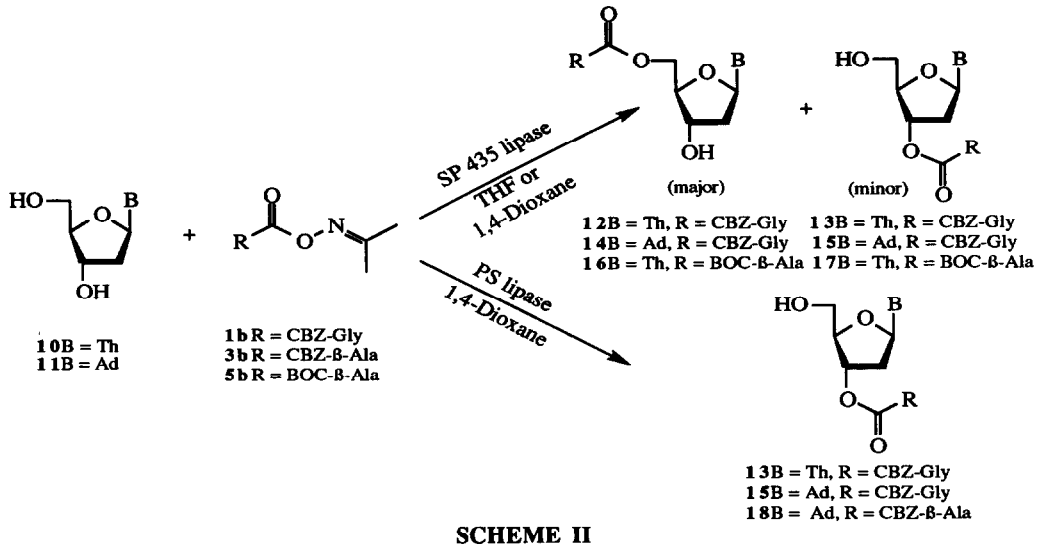


1a R = CBZ-Gly, X = PNP
2a R = BOC-Gly, X = Succ
3a R = CBZ-ß-Ala, X = PNP
4a R = CBZ-L-Ala, X = Succ
5a R = BOC-ß-Ala, X = Succ
6a R = CBZ-D-NorLeu, X = PNP
7a R = di-CBZ-L-Lys, X = PNP
8a R = CBZ-D-Phe, X = PNP
9a R = CBZ-L-Phe, X = PNP

1b R = CBZ-Gly
2b R = BOC-Gly
3b R = CBZ-ß-Ala
4b R = CBZ-L-Ala
5b R = BOC-ß-Ala
6b R = CBZ-D-NorLeu
7b R = di-CBZ-L-Lys
8b R = CBZ-D-Phe
9b R = CBZ-L-Phe

SCHEME I

In none of the cases was cleavage of CBZ or BOC protecting groups detected. In general, yields were high. The extraction step must be carefully carried out in order to prevent ester hydrolysis in the presence of NaOH (see Experimental Part for yields, physical data and ^{1}H-NMR and ^{13}C-NMR spectra).

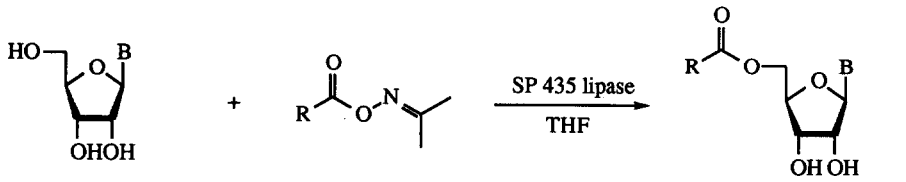


SCHEME II

Enzymatic synthesis of *N*-protected aminoacylated nucleosides, 12-18 and 21-22: Thymidine (**10**) and 2'-deoxyadenosine (**11**) were used as representative 2'-deoxynucleosides. We assayed the oxime esters with the lipases that had given good results in previous acylations of polyhydroxylated compounds. Such lipases are those from *Pseudomonas cepacia* (PSL), useful for acylation at the 3'-hydroxy function, and from *Candida antarctica* (CAL), for regioselective modification of 5'-hydroxyl group (Scheme II). Reactions were carried out in polar solvents such as THF or 1,4-Dioxane at 60°C and were monitored by TLC during the time indicated in Table I.

As can be seen in Table I, yields are moderated, perhaps due to the steric requirements of the protecting groups at the amino function. The behaviour of lipase from *Candida antarctica* is the same as with short acyl chains, so 3'-aminoacylated compounds appeared, whereas *Pseudomonas cepacia* lipase gave only a product, as a consequence of exclusively aminoacylation at the 3' position of the deoxynucleosides. It should be noted that in no case was acylation observed at the exocyclic amino group with adenine as the base moiety. With regards to the blocking group CBZ or BOC, it seemed that CBZ was better accepted by the enzymes than the bulky BOC, which gave reaction only with CAL in ß-Alanine oxime ester. The shape of the chain in the aminoacyl moiety seems to this moment,prevents extension of the versatility of the reaction to a variety of aminoacyl groups. Only glycil- and ß-Alanil- derivatives were introduced, and the branched oxime aminoacyl esters tested gave no satisfactory results with the lipases employed.

In the case of ribonucleosides (Scheme III), regioselective acylations mediated by lipases gave good results only at 5'-hydroxyl and by means of lipase from *Candida antarctica*. Both uridine, (**19**) and adenosine (**20**) were aminoacylated with lipase CAL SP435 L yielding only 5'-*O*-aminoacylated derivatives. Once again, adenosine underwent only *O*-acylation, the remaining exocyclic amino group being unreactive. Table I depicts the physical data of the products **21** and **22** and tables II and III show their spectral data.



19B = U
20B = Ad

1b R = CBZ-Gly
3b R = CBZ-ß-Ala

21B = U, R = CBZ-Gly
22B = Ad, R = CBZ-ß-Ala

SCHEME III

Table I. Aminoacyl nucleoside derivatives prepared.

Prod.	B	R	Lipase / Solvent	t (h)	Yield[a] (%)	m.p.[b] (°C)	IR[c] υ (cm^{-1})	$[\alpha]_{25}^{D}$ (c, solvent)
12	Th	CBZ-Gly / (5')	CAL / THF	32	43	91-2	1764	+7.69 (0.13, MeOH)
13	Th	CBZ-Gly / (3')	PSL / Dioxane	36	38	syrup	1695	+13.5 (2.5, MeOH)
14	Ad	CBZ-Gly / (5')	CAL / Dioxane	48	52	syrup	1695	-16.5 (1.5, MeOH)
15	Ad	CBZ-Gly / (3')	CAL / Dioxane	48	24	syrup	1705	-8.94 (0.85, MeOH)
16	Th	BOC-ß-Ala / (5')	CAL / THF	16	30	152-3	1694	+9.7 (0.7, MeOH)
17	Th	BOC-ß-Ala / (3')	CAL / THF	16	15	syrup	1696	+9.2 (1.7, $CHCl_3$)
18	Ad	CBZ-ß-Ala (3')	PSL / Dioxane	46	32	syrup	1705	+4.16 (1.8, MeOH)
21	U	CBZ-Gly / (5')	CAL / THF	40	71	74-5	1701	-0.46 (3, MeOH)
22	Ad	CBZ-ß-Ala (5')	CAL / THF	15	58	syrup	1697	-12.5 (0.2, MeOH)

[a] Calculated on isolated pure compounds. [b] Uncorrected. [c] Partial.

The structures 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' or C3' of *ca.* 5 ppm. towards lower fields with respect to the same carbon atom in the starting nucleosides. In addittion, H5', H5" or H3' showed a shift of 0.5-1 ppm. downfield with respect to the nucleosides without modification.

CONCLUSION

In the present work we describe a convenient method to obtain *N*-protected aminoacylated derivatives of both 2'-deoxy- and ribonucleosides through a lipase-mediated reaction. The procedure represents an easy route to introduce aminoacids into nucleosides. It is noteworthy that,in contrast to other reported methods, the exocyclic amino function in adenine nucleosides remains without reacting. Aminoacids protected with benzyloxycarbonyl groups are better substrates than those derivatized as *tert*-butyloxycarbonyl ones. With respect to α-branched aminoacids, both L and D, seem not to fit the steric requirements of the enzyme's active sites.

Table II. ^{1}H-NMR spectral data for compounds **1-7** (only non-interchangable signals) δ (ppm).[a]

Product	Base ring		Sugar moiety					Aminoacyl moiety
	Me / H5	H6	H1'	(H2',H2")	H3'	H4'	(H5',H5")	
12	1.77(s)	7.74(s)	6.20(t)	2.05-2.35(m)	4.27[b]	3.91(m)	4.27[b]	7.35 (5H, s); 5.04 (2H, s); 3.80 (2H, d)
13	1.86(s)	7.59(s)	6.21(t)	2.36(m)	5.80(m)	3.85[b]	3.85[b]	7.32 (5H, s); 5.11 (2H, s); 3.97 (2H, d)
16	1.78(s)	7.43(s)	6.17(t)	2.05-2.22(m)	4.20[b]	3.90(m)	4.20[b]	1.35 (9H,s); 3.16 (2H, q); 2.46[c]
17	1.81(s)	7.74(s)	6.18(t)	2.24(m) 2.48[b]	5.22(m)	3.98(m)	3.62(s)	1.33 (9H, s); 3.10 (2H, q); 2.33 (2H, t)
21	5.94(d)	7.82(s)	6.01(d)	4.25-4.37[b]	4.25-4.37[b]	4.25-4.37[b]	4.59 (m)	7.50 (5H, m); 5.29 (2H, s); 4.13 (2H, s)

Product	Base ring		Sugar moiety					Aminoacyl moiety
	H2	H8	H1'	(H2',H2")	H3'	H4'	(H5', H5")	
14	8.39(s)	8.43(s)	6.60(t)	2.99(d) 2.64(m)	4.75(m)	4.36(m)	4.57(d)	7.50 (5H, m); 5.25 (2H, s); 4.06 (2H, s)
15	8.37(s)	8.45(s)	6.57(dd)	3.13(m) 2.72(m)	5.72(d)	4.42(m)	4.02(s)	7.50 (5H, m); 5.28 (2H, s); 4.14 (2H, s)
18	8.37(s)	8.45(s)	6.57(dd)	3.07(m)2.82(m)	5.65(m)	4.41(m)	4.01(m)	7.50 (5H, m); 5.25 (2H, s); 3.56 (2H, q); 2.68 (2H, t)
22	8.39(s)	8.41(s)	6.20(d)	4.67(t)	4.26(t)	4.11(m)	4.20(m) 4.34(m)	7.50 (5H, m); 5.26 (2H, s); 3.56 (2H, q); 2.69 (2H, t)

[a] All samples measured in CD_3OD except **13**, **17** ($CDCl_3$) and **12**, **16** and **17** (DMSO-d_6). Signals of the sugar moiety assigned through selective irradiations.[b] Superimposed signals. [c] Superimposed with DMSO-d_6.

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

Product	Base ring					Sugar moiety					Aminoacyl moiety
	C2	C4	C5	C6	Me / C8	C1'	C2'	C3'	C4'	C5'	R
12	150.53	164.38	111.20	136.66	12.47	85.24	39.63	71.04	83.78	64.51	170.18; 156.67; 135.94; 128.48; 128.20; 128.00; 67.15; 42.67
13	150.65	164.49	111.18	136.67	12.39	85.68	37.08	75.93	84.88	62.11	169.97; 156.63; 136.06; 128.43; 128.13; 128.07; 67.13; 42.50
14	154.57	151.12	121.29	158.04	141.82	86.84[b]	41.56	73.25	86.54[b]	66.54	172.55; 159.92; 138.87; 130.29; 129.86; 128.67; 68.60; 44.25
15	154.34	151.20	121.20	158.42	142.51	88.43[b]	39.62	78.73	88.16[b]	64.54	172.30; 160.04; 139.07; 130.36; 129.89; 129.72; 68.70; 44.11
16	153.10	167.17	112.67	138.46	13.51	87.35[b]	41.42	73.12	86.57[b]	66.01	173.93; 159.13; 81.08; 38.20; 36.43; 29.62
17	150.63	164.42	111.22	136.69	12.44	85.91[b]	37.21	74.99	85.06[b]	62.21	172.09; 155.90; 79.64; 35.97 34.67; 28.29
18	154.34	151.24	121.33	158.43	142.50	88.48[b]	39.30	78.19	88.24[b]	64.57	173.72; 159.63; 139.21; 130.34; 129.67; 68.31; 38.53; 36.63
21	153.10	166.95	104.02	143.11	-	92.13	76.00	71.90	83.79	65.98	172.52; 159.92; 138.86; 130.36; 129.36; 128.80; 68.73; 44.38
22	154.76	151.18	121.30	158.45	142.11	91.24	75.98	72.67	84.23	65.86	173.92; 159.78; 139.11; 130.33; 129.83; 129.67; 68.31; 38.72; 36.31

[a] Samples measured in CD_3OD except **1** and **2** ($CDCl_3$). [b] Tentative assignements.

EXPERIMENTAL

Lipase from *Candida antarctica* SP 435L was kindly provided by Novo Nordisk Company. Nucleosides were purchased from Aldrich. 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 were recorded using a Bruker AC300 spectrometer. Microanalyses were performed on a Perkin-Elmer model 240 instrument.

General procedure for the synthesis of *N*-protected aminoacyl oxime esters: 3 mmol of starting *N*-protected aminoacyl *p*-nitrophenyl or succinyl esters are incubated at room temperature in 5 ml of $CHCl_3$ overnight with 6 mmol of acetone oxime. The reaction mixture is extracted with CH_2Cl_2/H_2O and washed twice with NaOH 1 mM. Evaporation of the organic layer yield *N*-protected aminoacyl oxime esters:

N-Benzyloxycarbonyl glycine acetoxime ester (**1b**): 82%, white solid, mp 111-2°C. IR (KBr, cm^{-1}) 1766. ^{1}H-NMR ($CDCl_3$) δ 7.35 (5H, s, Ph); 5.42 (1H, bs, NH); 5.13 (2H, s, CH_2-Ph); 4.13 (2H, d, CH_2-NH); 2.03 (3H, s, Me); 1.98 (3H, s, Me). ^{13}C-NMR ($CDCl_3$) δ 168.37 (C=O); 164.79 (C=N); 156.19 (CON); 136.07, 128.46, 128.16, 128.05 (four peaks from Ph); 67.08 (*C*H_2-Ph); 41.94 (*C*H_2-NH); 21.82 (*cis*-Me); 16.94 (*trans*-Me). Anal. Calcd for $C_{13}H_{16}N_2O_4$: C, 59.07; H, 6.11; N, 10.06. Found: C, 59.10; H, 6.16; N, 10.01.

N-tert-Butyloxycarbonyl glycine acetoxime ester (**2b**): 78%, colorless oil, IR (neat, cm^{-1}) 1770. ^{1}H-NMR ($CDCl_3$) δ 5.30 (1H, bs, NH); 3.97 (2H, d, CH_2-NH); 1.97 (3H, s, Me); 1.94 (3H, s, Me); 1.37 (9H, s, $(Me)_3$). ^{13}C-NMR ($CDCl_3$) δ 168.53 (C=O); 164.53 (C=N); 155.56 (CON); 79.78 (*C*-$(Me)_3$); 41.35 (*C*H_2-NH); 28.02 (Me_3); 21.59 (*cis*-Me); 16.73 (*trans*-Me). Anal. Calcd for $C_{10}H_{18}N_2O_4$: C, 52.15; H, 7.88; N, 12.17. Found: C, 52.20; H, 7.76; N, 12.01.

N-Benzyloxycarbonyl ß-Alanine acetoxime ester (**3b**): 75%, colorless oil. IR (neat cm^{-1}) 1755. ^{1}H-NMR ($CDCl_3$) δ 7.34 (5H, s, Ph); 5.43 (1H, bs, NH); 5.09 (2H, s, CH_2-Ph); 3.56 (2H, q, ß-CH_2); 2.67 (2H, t, α-CH_2); 2.03 (3H, s, Me); 1.97 (3H, s, Me). ^{13}C-NMR ($CDCl_3$) δ 170.12 (C=O); 164.16 (C=N); 156.26 (CON); 136.26, 128.39, 127.98, 127.91 (four peaks from Ph); 66.58 (*C*H_2-Ph); 36.33 (ß-CH_2); 32.99 (α-CH_2); 21.80 (*cis*-Me); 16.92 (*trans*-Me). Anal. Calcd for $C_{14}H_{18}N_2O_4$: C, 60.40; H, 6.52; N, 10.07. Found: C, 60.60; H, 6.56; N, 10.10.

N-Benzyloxycarbonyl L-Alanine acetoxime ester (**4b**): 82%, white solid, mp 63-4°C. IR (KBr, cm^{-1}) 1763. $[\alpha]_{25}^{D}$ = +22.4 (c = 0.5, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ 7.33 (5H, s, Ph); 5.43 (1H, d, NH); 5.11 (2H, s, CH_2-Ph); 4.52 (1H, m, C*H*-NH); 2.05 (3H, s, Me); 1.99 (3H, s, Me); 1.47 (3H, d, *Me*-CH).

^{13}C-NMR ($CDCl_3$) δ 170.60 (C=O); 165.20 (C=N); 155.50 (CON); 136.09, 128.47, 128.12, 128.03 (four peaks from Ph); 66.89 (*C*H_2-Ph); 48.66 (*C*H-NH); 21.84 (*cis*-Me); 18.86 (*Me*-CH); 16.96 (*trans*-Me). Anal. Calcd for $C_{14}H_{18}N_2O_4$: C, 60.40; H, 6.52; N, 10.07. Found: C, 60.56; H, 6.46; N, 10.12.

N-tert-Butyloxycarbonyl ß-alanine acetoxime ester (**5b**): 83%, colorless oil, IR (neat, cm^{-1}) 1757. ^{1}H-NMR ($CDCl_3$) δ 5.23 (1H, bs, NH); 3.45 (2H, q, ß-CH_2); 2.65 (2H, t, α-CH_2); 2.05 (3H, s, Me); 2.01 (3H, s, Me); 1.43 (9H, s, $(Me)_3$). ^{13}C-NMR ($CDCl_3$) δ 169.91 (C=O); 163.86 (C=N); 155.54 (CON); 78.98 (*C*-$(Me)_3$); 35.67 (ß-CH_2); 32.95 (α-CH_2); 28.04 (Me_3); 21.61 (*cis*-Me); 16.70 (*trans*-Me). Anal. Calcd for $C_{11}H_{20}N_2O_4$: C, 54.07; H, 8.26; N,11.47. Found: C, 54.10; H, 8.51; N, 11.33.

N-Benzyloxycarbonyl D-Norleucine acetoxime ester (**6b**): 88%, colorless oil. IR (neat, cm^{-1}) 1760. $[\alpha]_{25}^{D}$ = -14.0 (c = 0.43, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ 7.33 (5H, s, Ph); 5.45 (1H, d, NH); 5.11 (2H, s, C*H*$_2$-Ph); 4.48 (1H, q, C*H*-NH); 2.04 (3H, s, Me); 1.98 (3H, s, Me); 1.86 and 1.71 (2H, m, C*H*$_2$-CH); 1.35 (4H, m, C*H*$_2$-C*H*$_2$-CH$_2$-CH); 0.88 (Me). ^{13}C-NMR ($CDCl_3$) δ 170.16 (C=O); 165.06 (C=N); 155.71 (CON); 136.04, 128.34, 127.98, 127.89 (four peaks from Ph); 66.78 (*C*H_2-Ph); 52.81 (*C*H-NH); 32.36 (CH$_2$-CH$_2$-*C*H$_2$-CH); 27.07 (CH$_2$-*C*H$_2$-CH$_2$-CH); 22.07 (*C*H$_2$-CH$_2$-CH$_2$-CH) 21.74 (*cis*-Me); 16.92 (*trans*-Me); 13.67 (Me). Anal. Calcd for $C_{17}H_{24}N_2O_4$: C, 63.72; H, 7.55; N, 8.75. Found: C, 63.70; H, 7.56; N, 9.01.

Nα,Nε-Dibenzyloxycarbonyl L-Lysine acetoxime ester (**7b**): 96%, colorless oil. IR (neat, cm^{-1}) 1761. $[\alpha]_{25}^{D}$ = +8.6 (c = 1.6, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ 7.32 (10H, s, 2xPh); 5.61 (1H, d, α-NH); 5.01 (2H, s, α–C*H*$_2$-Ph); 5.05 (2H, s, ε-C*H*$_2$-Ph); 4.98 (1H, bs, ε-NH); 4.46 (1H, q, C*H*-NH); 3.16 (2H, q, ε-CH_2); 2.03 (3H, s, Me); 1.96 (3H, s, Me); 1.86 and 1.73 (2H, m, C*H*$_2$-CH); 1.49 and 1.41 (4H, m, C*H*$_2$-C*H*$_2$-CH$_2$-CH). ^{13}C-NMR ($CDCl_3$) δ 170.02 (C=O); 165.23 (C=N); 156.41 and 155.89 (2xCON); 136.40, 136.01, 128.36, 128.03, 127.95 (five peaks from 2xPh); 66.87 and 66.46 (2x*C*H$_2$-Ph); 52.67 (*C*H-NH); 40.29 (ε-CH$_2$); 32.07 (CH$_2$-CH$_2$-*C*H$_2$-CH); 29.16 (CH$_2$-*C*H$_2$-CH$_2$-CH); 22.08 (*C*H$_2$-CH$_2$-CH$_2$-CH); 21.74 (*cis*-Me); 16.92 (*trans*-Me). Anal. Calcd for $C_{25}H_{31}N_3O_6$: C, 63.94; H, 6.66; N, 8.95. Found: C, 63.73; H, 6.53; N, 8.81.

N-Benzyloxycarbonyl D-Phenylalanine acetoxime ester (**8b**): 76%, white solid. mp 81-2°C. IR (KBr, cm^{-1}) 1748. $[\alpha]_{25}^{D}$ = -12.4 (c = 0.51, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ 7.35-7.15 (10H, m, 2xPh); 5.36 (1H, d, NH); 5.10 (2H, s, C*H*$_2$-Ph); 4.75 (1H, q, C*H*-NH); 3.14 (2H, d, C*H*$_2$-CH); 2.02 (3H, s, Me); 1.79 (3H, s, Me). ^{13}C-NMR ($CDCl_3$) δ 169.31 (C=O); 165.22 (C=N); 155.53 (CON); 136.07, 135.45, 129.27, 128.59, 128.45, 128.12, 128.01, 127.07 (eight peaks from 2xPh); 66.94 (*C*H_2-Ph); 54.06 (*C*H-NH); 38.72 (*C*H$_2$-CH); 21.82 (*cis*-Me); 16.81 (*trans*-Me). Anal. Calcd for $C_{20}H_{22}N_2O_4$: C, 67.77; H, 6.26; N, 7.91. Found: C, 67.70; H, 6.46; N, 8.11.

N-Benzyloxycarbonyl L-Phenylalanine acetoxime ester (**9b**): 77%, white solid. $[\alpha]_{25}^{D}$ = +15.1 (c =

0.76, $CHCl_3$). Anal. Calcd for $C_{20}H_{22}N_2O_4$: C, 67.77; H, 6.26; N, 7.91. Found: C, 67.70; H, 6.46; N, 8.11.

General procedure for the synthesis of compounds 12-18 and 21-22: 0.5 mmol of **10**, **11**, **19**, or **20**, 1.5 mmol of the corresponding oxime aminoacy ester, and 0.2g of lipase from *Candida antarctica* SP 435 L or 1.5g of PSL were suspended in 5 mL of THF or dioxane. The mixture was allowed to react at 60ºC 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 thymidine and uridine nucleosides, CH_2Cl_2:MeOH 9:1 for adenosine ones). Table I shows physical data of the products and Tables II and III depicted their ^{1}H-NMR and ^{13}C-NMR spectra respectively.

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