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Novel and efficient regioselective enzymatic approach to 3'-, 5'- and 3',5'-di-*O*-crotonyl 2'-deoxynucleoside derivatives

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Regioselective syntheses of several *O*-crotonyl 2'-deoxynucleoside derivatives have been efficiently achieved using a biocatalytic methodology

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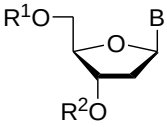

R1= $CH_3CH=CHCO$; **R2=** H
R1= H; **R2=** $CH_3CH=CHCO$
R1= R2= $CH_3CH=CHCO$

B= T, C^{Bz}, A^{Bz}

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TETRAHEDRON LETTERS

Novel and efficient regioselective enzymatic approach to 3'-, 5'-, and 3',5'-di-*O*-crotonyl 2'-deoxynucleoside derivatives

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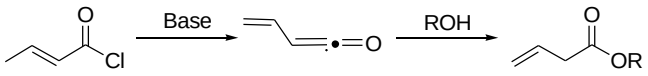
Abstract—Regioselective syntheses of several *O*-crotonyl 2'-deoxynucleoside derivatives have been efficiently achieved using a biocatalytic methodology. While *Candida antarctica* lipase B (CAL-B) afforded the 5'-*O*-acylated compounds, immobilized lipase from *Pseudomonas cepacia* (PSL-C) provided the 3'-*O*-crotonylated analogues. Since classical chemical approaches did not work appropriately due to side isomerization reactions, a mixture of both lipases was used to achieve a useful synthetic route toward diacylated nucleosides.

It has been described that in some cases acylation of one hydroxyl group of the sugar moiety in a nucleoside derivative can increase its biological activity compared with the unmodified analogue.[1] In this sense, lipase-catalyzed transformations have become simple and standard processes for regioselective acylation of nucleosides, since they avoid the time-consuming protection and deprotection steps required in non-enzymatic approaches.[2] Thus, in our research group we have developed efficient enzymatic reactions to obtain 5'-*O*-acylated nucleosides using the lipase B from *Candida antarctica* (CAL-B),[3] or 3'-*O*-acylated derivatives using the immobilized lipase from *Pseudomonas cepacia* (PSL-C).[3c,3d,4]

The crotonyl group is present in different biological active compounds as COTC [2-crotonyloxymethyl-(4*R*,5*R*,6*R*)-4,5,6-trihydroxy-2-cyclohexenone][5] or COMC (2-crotonyloxymethyl-2-cyclohexenone),[6] important antitumor agents. The activity of this type of derivatives can be ascribed to the presence of the α,β-unsaturated ester which can undergo Michael-type additions of nucleophiles within an enzyme.[7] However, the introduction of this moiety on nucleosides has been scarcely studied.[8] Previously, 3'-amino-5'-crotonylamino-3',5'-dideoxythymidine was synthesized,[9] and preliminary biological studies have shown that inhibits the *in vitro* replication of HIV-1 and HIV-2.[10] Due to this compound can not be 5'-phosphorilated, it may suffer a Michael-type addition from a specifically enzyme. Moreover, the presence of this moiety on nucleosides would afford excellent starting compounds for the synthesis of, e.g., β-amino acid analogues of potential interest.[11]

Nevertheless, the synthesis of *O*-crotonyl derivatives is not trivial since it is known that in the usual conditions to obtain them (base-catalyzed process with crotonyl chloride), mixtures of desired compounds and β,γ-unsaturated analogues are provided due to the deconjugation of the double bond. This fact, firstly described in 1966 by Ozeki and Kusaka,[12] depends on several factors such •as the alcohol, the solvent, the amine, and the temperature.[13] The mechanism for this transformation is assumed to pass through a ketene intermediate (Scheme 1).[13,14]



Scheme 1. Proposed mechanism for the deconjugation of the double bond in the crotonylation of an alcohol.

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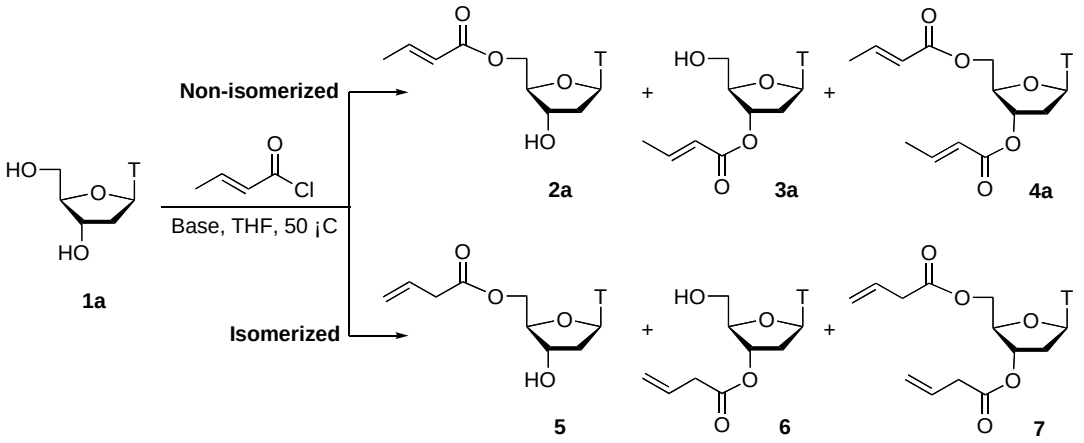


Scheme 2. Base-catalyzed crotonylation of thymidine with crotonyl chloride.

Table 1. Ratios of non-isomerized (**2a**–**4a**) and isomerized (**5**–**7**) derivatives in base-catalyzed crotonylation of **1a** at 50 °C.[a]

Entry	Base[b]	*t* (h)	conv (%)	**2a** (%)	**3a** (%)	**4a** (%)	**5** (%)	**6** (%)	**7** (%)	**8**+**9** (%)
1[c]	Et_3N	23	100	-	2	8	1	11	63	15
2	Collidine	68	88	2	50	4	2	25	2	3
3	Lutidine	68	80	5	47	6	4	15	-	3
4	Pyridine	68	88	15	29	40	1	-	-	3
5	Quinoline	68	90	19	53	14	1	-	-	3

[a] These percentages were obtained by gas chromatography (GC). [b] pK_a values: Et_3N (10.7); collidine (9.6); lutidine (6.8); pyridine (5.2); and quinoline (4.8). [c] Process was carried out at room temperature.

This process has been also observed in other α,β-unsaturated derivatives, as in the deprotonation of carboxylic acids or esters in the presence of strong bases as LDA.[15] Reactions must be highly selective since further purification of desired compound is not possible. Thus, other reaction conditions has been used, as phase-transfer catalyzed processes.[16] Herein we show a regioselective enzymatic approach to obtain *O*-crotonyl analogues derived from nucleosides avoiding the isomerization side reaction of the double bond.

We started this acylation study using the classical chemical conditions, that is, with crotonyl chloride (1.5 equiv.) and a base to catalyze it (Scheme 2). As was expected, in all cases mixtures of desired and isomerized compounds were obtained although in different ratios (Table 1). To identify them, GC was used.[17]

When the reaction was performed with triethylamine at room temperature (entry 1, Table 1), mainly isomerized derivatives were obtained (90% of total). This is in agreement with previous similar results,[13] which have shown that strong bases with pK_a>10 favor the deconjugation of the double bond. This is consistent with the proposed mechanism, since stronger bases can make easily the γ-deprotonation of the acylating agent favoring the formation of the ketene intermediate. 3',5'-Diisomerized nucleoside **7** was obtained as major derivative, mixed with the 3'-isomerized compound **6**, and the monoisomerized diacylated products **8** and **9** (Chart 1).

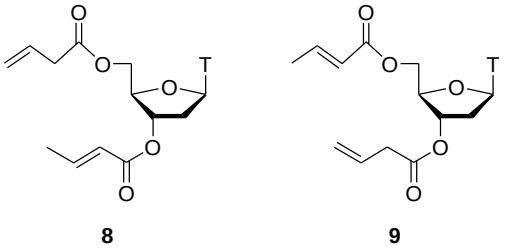
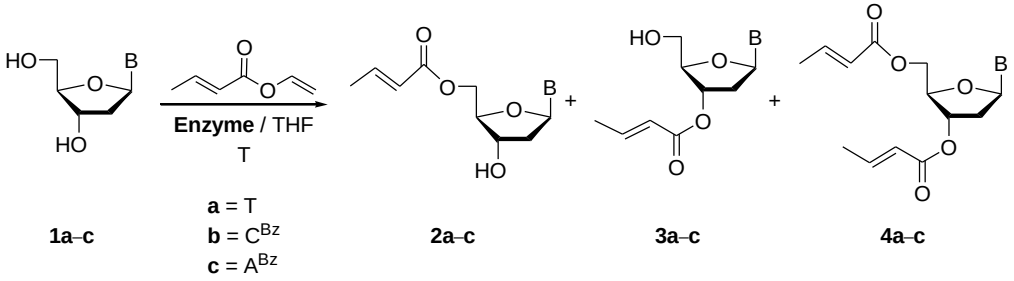


Chart 1. Monoisomerized diacylated derivatives.

Processes using less potent bases (lower pK_a) required 50 ºC to progress the reaction significantly. In these conditions, the percentage of the β,γ-unsaturated derivatives decreased as pK_a of the base diminished, corroborating previous results. Thus, bases with values of pK_a between 6-10 as collidine and lutidine afforded mixtures of compounds with a ratio of isomerized nucleosides between 22-32% (entries 2-3, Table 1), and bases with pK_a<6 as pyridine and quinoline (entries 4-5, Table 1), provided the α,β-unsaturated products almost quantitatively, although the regioselectivity was poor.



Scheme 3. Regioselective enzymatic acylation on 2'-deoxynucleosides **1a**–**c**.

Table 2. Enzymatic acylations catalyzed by CAL-B and PSL-C on 2'-deoxynucleosides **1a**–**c**.[a]

Entry	Enzyme	B	T (ºC)	*t* (h)	conv (%)[b]	**5'-acylation** (%)[b]	**3'-acylation** (%)[b]	**3',5'-diacylation** (%)[b]
1	CAL-B	T	60	43	97[c]	83[c] (75)[d]	6[c]	8[c]
2	CAL-B	T	40	47	94[c]	81[c]	7[c]	6[c]
3	PSL-C	T	60	12	100[c]	-	94[c] (86)[d]	6[c]
4[e]	CAL-B+PSL-C	T	60	96	100[c]	-	-	100[c] (78)[d]
5	CAL-B	C^{Bz}	60	40	97	91 (80)[d]	2	4
6	PSL-C	C^{Bz}	60	23	100	-	94 (90)[d]	6
7[e]	CAL-B+PSL-C	C^{Bz}	60	150	100	-	4	96 (93)[d]
8	CAL-B	A^{Bz}	60	64	100	97 (80)[d]	-	3
9	PSL-C	A^{Bz}	60	25	100	-	100 (97)[d]	-
10[e]	CAL-B+PSL-C	A^{Bz}	60	132	100	-	-	100 (83)[d]

[a] In a typical procedure, 2'-deoxynucleoside (**1a**–**c**, 0.4 mmol), and lipase [CAL-B (1:1 w/w substrate) and/or PSL-C (3:1 w/w substrate)] were suspended in THF (4.5 mL), and finally vinyl crotonate (1.2 mmol) was added. [b] Calculated by ^{1}H NMR. [c] Calculated by GC. [d] Isolated yield. [e] 2.0 mmol of vinyl crotonate were added.

Since base-catalyzed acylations with the acid chloride did not avoid isomerization products, other reaction conditions were studied. Thus, to synthesize diacylated compound **4a**, we employed crotonic acid with DMAP, Et_3N, and dicyclohexylcarbodiimide (DCC) since we had previously obtained good results using this methodology.[18] However, purification of **4a** was complex and the yield did not overcome 60%.

Due to our experience in biocatalytic processes on nucleosides,[3,4,18] lipase-catalyzed acylations were studied to provide the desired esterified derivatives with high yields and regioselectivities. Thus, we have already reported that CAL-B acylates 2'-deoxynucleosides with good 5'-selectivity using vinyl esters as acyl donor and THF as the best solvent (Scheme 3).[3a] Since thymidine (**1a**) is the simplest nucleoside, crotonylation study was started with it. Thus, when 1.5 equiv. of vinyl crotonate were used at 60 ºC (entry 1, Table 2), good regioselectivity toward 5'-OH was achieved, and after 43 h **2a** was isolated with 75% yield after flash chromatography, although small quantities of the other regioisomer **3a** and diacylated compound **4a** were obtained. To decrease the rate of later byproducts, the reaction was performed at lower temperature (entry 2, Table 2), but similar regioselectivities were observed. Changes in the amount of the lipase or the acylating agent did not provide better results (data not shown).

When PSL-C was used as biocatalyst at 60 ºC with 1.5 equiv. of vinyl crotonate, regioselectivity toward the more hindered 3'-hydroxyl group was even better (94%), affording **3a** with a yield of 86% after 12 h. 5'-Regioisomer **2a** was not detected and only **4a** was observed as a minor byproduct (entry 3, Table 2). As classical methods did not allow an efficient synthesis of diacylated compound **4a**, a similar enzymatic approach was used. In a first attempt, acylations catalyzed with CAL-B or PSL-C were allowed to react during several days, but conversion to **4a** were too low. Taking advantage of the complementarity shown by both lipases toward the crotonylation of **1a**, we designed a process where CAL-B and PSL-C were present in the reaction medium simultaneously (entry 4, Table 2). Although the acylation was slower, after 96 h only dicrotonyl nucleoside **4a** was formed with 78% of isolated yield.

In an attempt to confer versatility to these enzymatic preparations, another pyrimidine nucleoside, such as *N*-benzoyl-2'-deoxycytidine (**1b**), and a purine nucleoside such as *N*-benzoyl-2'-deoxyadenosine (**1c**) were used. All these processes showed a very similar behavior. Thus, CAL-B kept its excellent regioselectivity in the acylation of the 5'-position, isolating exclusively compounds **2b** and **2c** (entries 5 and 8, Table 2); PSL-C acylated exclusively the 3'-OH affording **3b** and **3c** with excellent yields (entries 6 and 9, Table 2); and the mixture of both lipases allowed to

synthesize diacylated derivatives **4b** and **4c** with high efficiency (entries 7 and 10, Table 2). Interestingly, in all these lipase-catalyzed reactions, isomerized or Michael-type addition derivatives were not detected.[19]

Herein we have shown a novel, efficient, and complementary methodology to afford in a regioselective manner *O*-crotonyl esters without isomerization. The enzymatic methodology has been applied in order to synthesize nucleoside analogues with potential anti-HIV properties. To obtain them, enzymatic conditions proved to be the most useful. The biological activity of these derivatives will be tested and the results will be reported in a due course.

Acknowledgments

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17. GC conditions: A capillary column TRACSIL TRB-5A (30 m × 0.32 mm × 0.50 μm) was used with nitrogen as carrier gas and a flame ionization detector. Injector and detector temperatures were set at 300 °C, head column pressure at 13.1 psi and split 50:1; column initial temperature 220 °C (3 min), rate 5 °C/min until 260 °C, then 15 min at 260 °C, followed by heating rate 5 °C/min until 300 °C, column final temperature was 300 °C (10 min). Samples (100 μL) were silylated as described in the literature (Sweeley, C. C.; Bentley, R.; Makita, M.; Wells, W. W. *J. Am. Chem. Soc.* **1963**, *85*, 2497-2507). 3',5'-di-*O*-TMS-thymidine appears at 12.3 min; 5'-*O*-crotonyl-3'-*O*-TMS-thymidine at 17.9 min; 3'-*O*-crotonyl-5'-*O*-TMS-thymidine at 18.8 min; **4a** at 29.7 min; 5'-*O*-but-3''-enoyl-3'-*O*-TMS-thymidine at 16.1 min; 3'-*O*-but-3''-enoyl-5'-*O*-TMS-thymidine at 17.2 min; **7** at 23.0 min; **8** and **9** at 25.6 and 26.7 min.
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Supplementary Material

Experimental procedures are described. Complete ^{1}H, ^{13}C, and DEPT NMR spectral data and some 2D NMR experiments are shown in addition to mp, IR, microanalysis, optical rotation, and MS data. The level of purity is indicated by the inclusion of copies of ^{1}H and ^{13}C NMR spectra.