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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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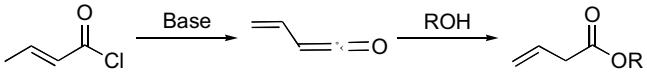
Dedicated to Professor Joaquín Plumet on the occasion of his 60th birthday

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 analogs. 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 biologically activity compared with the unmodified analog.[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,d,4]

The crotonyl group is present in different biological active compounds such as anti-tumor agents COTC [2-crotonyloxymethyl-(4*R*,5*R*,6*R*)-4,5,6-trihydroxy-2-cyclohexenone][5] and COMC (2-crotonyloxymethyl-2-cyclohexenone).[6] The activity of this type of derivative 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 it inhibits the in vitro replication of HIV-1 and HIV-2.[10] Due to the fact that this compound cannot be 5′-phosphorylated, it may suffer a Michael-type addition from a specific enzyme. Moreover, the presence of this moiety on nucleosides would afford excellent starting compounds for the synthesis of β-amino acid analogs 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 analogs are obtained due to the deconjugation of the double bond. This fact, first 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 occur 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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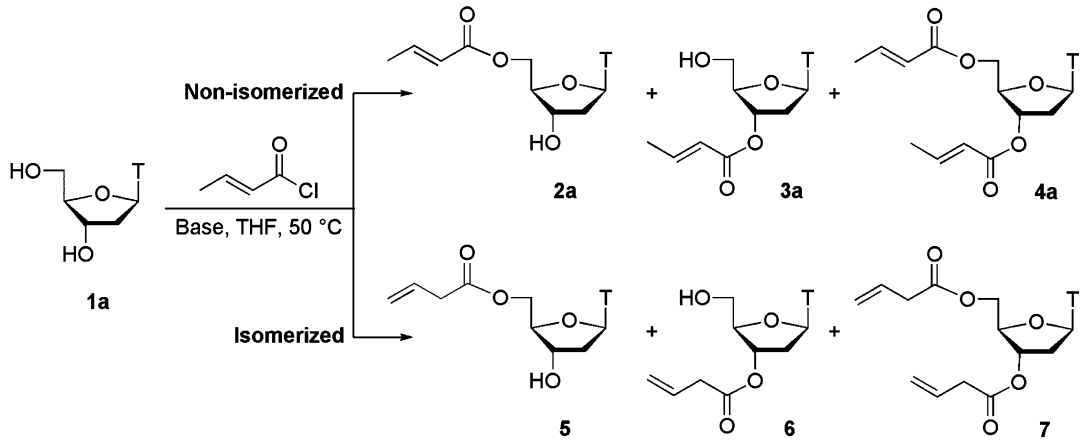


Scheme 2. Base-catalyzed crotonylation of thymidine with crotonyl chloride.

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 such as LDA.[15] Reactions must be highly selective since further purification of desired compound is not possible. Thus, other reaction conditions have been used, such as phase-transfer catalyzed processes.[16] Herein, we show a regioselective enzymatic approach to obtain *O*-crotonyl analogs 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 (Table 1, entry 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 effect the γ-deprotonation of the acylating agent favoring the formation of the ketene intermediate. 3′,5′-Diisomerized nucleoside **7** was obtained as a major derivative, mixed with the 3′-isomerized compound **6**, and the monoisomerized diacylated products **8** and **9** (Chart 1).

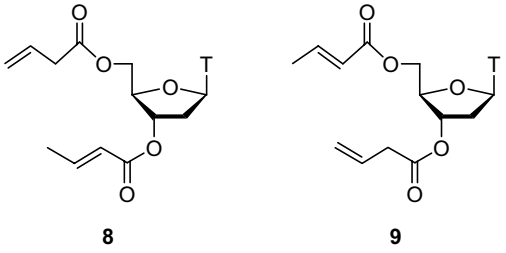


Chart 1. Partially isomerized diacylated derivatives.

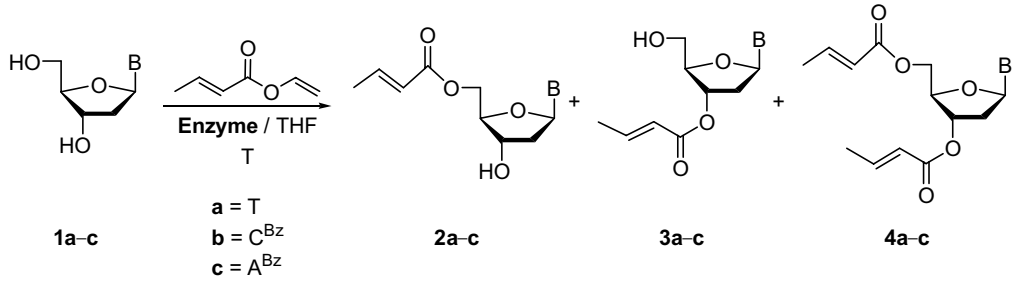
Processes using weaker bases (lower pK_a) required 50 °C to effect significant reaction conversions. In these conditions, the percentage of the β,γ-unsaturated derivatives decreased as pK_a of the base diminished, corroborating previous results.[13] Thus, bases with values of pK_a between 6 and 10 such as collidine and lutidine afforded mixtures of compounds with a ratio of isomerized nucleosides between 22% and 32% (Table 1, entries 2 and 3), and bases with pK_a <6 such as pyridine and quinoline (Table 1, entries 4 and 5), provided the α,β-unsaturated products almost quantitatively, although the regioselectivity was poor.

Since base-catalyzed acylations with the acid chloride did not avoid isomerized 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

Table 1. Ratios of non-isomerized (**2a**–**4a**) and fully or partially isomerized (**5**–**9**) 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.



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

had previously obtained good results using this methodology.[18] However, purification of **4a** was complex and the yield did not exceed 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 donors and THF as the best solvent (Scheme 3).[3a] Since thymidine (**1a**) is the simplest nucleoside, the crotonylation study was started with it. Thus, when 1.5 equiv of vinyl crotonate was used at 60 °C (Table 2, entry 1), 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. In an attempt to decrease the formation of the latter byproducts, the reaction was performed at lower temperature (Table 2, entry 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 °C 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 (Table 2, entry 3). 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** was 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 simultaneously present in the reaction medium (Table 2, entry 4). Since the acylation was slower, 2.5 equiv of vinyl crotonate was used, and 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 of these processes showed very similar behaviors. Thus, CAL-B kept its excellent regioselectivity in the acylation of the 5′-position, isolating exclusively compounds **2b** and **c** (Table 2, entries 5 and 8); PSL-C acylated exclusively the 3′-OH affording **3b** and **c** with excellent yields (Table 2, entries 6 and 9); and the mixture of both lipases allowed the synthesis of diacylated derivatives **4b** and **c** with high efficiency (Table 2, entries 7 and 10). Interestingly, in all of these lipase-catalyzed reactions, isomerized or Michael-type addition derivatives were not detected.[19]

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

Entry	Enzyme	B	*T* (°C)	*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.

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 proven to be the most useful for the synthesis of nucleoside analogs with potential anti-HIV properties. The biological activity of these derivatives will be tested and the results will be reported in due course.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version at doi:10.1016/j.tetlet.2005.06.138. Experimental procedures are described. Complete ^{1}H, ^{13}C, 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.

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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; compound **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; compounds **8** and **9** at 25.6 and 26.7 min.
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