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Regioselective Enzymatic Acylation of β-L-2′-Deoxynucleosides: Application in Resolution of β-D/L-2′-Deoxynucleosides

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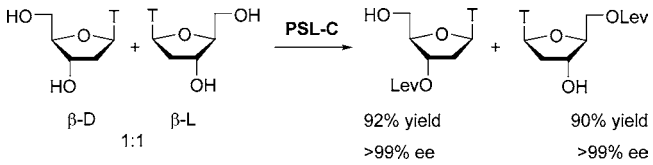
ABSTRACT



A practical synthesis of β-L-3′- and β-L-5′-*O*-levulinyl-2′-deoxynucleosides has been described for the first time through enzymatic acylation and/or hydrolysis processes. It is noteworthy that the different behavior exhibited by *Pseudomonas cepacia* lipase in the acylation of D- and L-nucleosides allows the parallel kinetic resolution of D/L-nucleosides.

Modified nucleosides have attracted much attention as antiviral and anticancer agents.[1] Consequently, extensive changes have been made to both the heterocyclic base and the sugar moiety to reduce the toxicity and viral drug resistance associated with certain nucleoside analogues.[2]

Until recently, only nucleosides possessing the natural β-D-configuration have been studied as chemotherapeutic agents due to their easy access. However, the discovery of lamivudine (L-2′,3′-dideoxy-3′-thiacytidine, 3TC, **1**, Figure 1), the first compound with the unnatural β-L-configuration approved by the FDA for use in combination therapy against human immunodeficiency virus type 1 (HIV-1) and hepatitis B virus (HBV), has sparked tremendous interest in the synthesis of β-L-nucleosides.[3] As a result, several L-nucleosides are currently undergoing clinical trials as potential

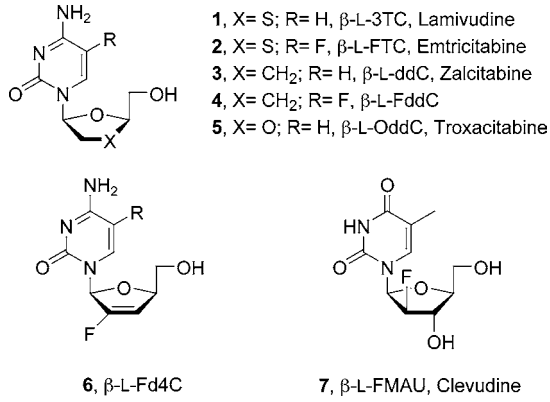


Figure 1. Structures of relevant β-L-nucleosides.

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antiviral or antitumor agents.[4] Some of the promising candidates are the 5-fluorinated analogue of 3TC (emtricitabine, **2**),[5] β-L-ddC (zalcitabine, **3**),[6] β-L-Fd4C (**6**),[7] and β-L-FddC (**4**).[8]

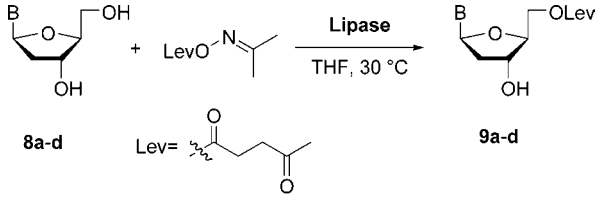
Favorable features of L-nucleosides include lower toxicity, while maintaining an antiviral activity comparable to and sometimes greater than their D-counterparts, and higher metabolic stability. For example, 1-(β-D-2-fluoro-2-deoxyarabino-furanosyl)-5-methyluracil (FMAU) showed promising anti-HBV activity, but its development was interrupted in phase I clinical trials due to severe neurological toxicity. Whereas, L-FMAU (clevudine, **7**) is reported to be nontoxic and more active.[9] The dioxolane analogue L-OddC (troxacitabine, **5**)[10] is the first L-nucleoside showing antitumor activity, and unlike its D-form, it is not metabolized by cytidine deaminase, which can prolong its effect as a drug. Furthermore, β-L-2′-deoxynucleosides have been described to inhibit the replication of HBV and woodchuck hepatitis viruses (WHV).[4b,c] In addition, the L-series of nucleosides are also of interest as precursors to nuclease-stable L-oligonucleotides.[11] The tremendous therapeutic potential of L-nucleosides has stimulated interest in their synthesis. Formation of a mixture of D- and L-nucleosides is a common occurrence during the synthesis of these compounds. This results in a challenging separation of the racemic mixtures.

Surprisingly, the separation of D/L-nucleosides remains an under-explored area of research. Two relevant examples in the literature are (i) a pig liver esterase-mediated hydrolysis, which resulted in enzymatic kinetic resolution of β-D- and L-configured dideoxynucleosides,[12] and (ii) the separation of D/L-adenosine via deamination reaction using an unknown cell culture PS-264.[13]

Although enzymatic-catalyzed reactions are becoming standard procedures for the preparation of enantiomerically pure compounds, they have not been fully exploited as a separation tool in the racemic mixture of nucleosides.[14] Due to the increased therapeutic applications of L-nucleosides, there is a need for a separation method that permits easy isolation of D-nucleosides from L-nucleosides. Recently, we have described the enzyme-catalyzed regioselective acylation[15] of unprotected β-D-nucleosides and the hydrolysis of acylated β-D-nucleosides. Herein, we report an extension of our studies in this area demonstrating for the first time a regioselective enzymatic acylation of L-nucleosides and its application in the resolution of D/L-mixtures. Enzymatic hydrolysis of acylated L-nucleosides is also reported.

For the acylation of L-nucleosides, we chose the levulinyl group due to its ease of cleavage under neutral conditions and the potential use of these levulinyl protected nucleosides in the solution-phase synthesis of oligonucleotides.[16] Acetonoxime levulinate was selected as the acylating agent since oxime esters have proven to be excellent reagents toward the regioselective enzymatic acylation of nucleosides.[15] The selective 5′-O-acylation of L-2′-deoxynucleosides **8a**−**d**[17] was accomplished using CAL-B at 30 °C (Scheme 1). Thus,

Scheme 1 [a]



[a] See Table 1 for details.

entries 1−4 in Table 1 indicate exclusively the formation of 5′-*O*-levulinyl derivatives except in the case of β-L-dA^{Bz} (**8c**),

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Table 1. Enzymatic Acylation of β-L-Nucleosides **8**[a]

entry	substrate	lipase	*t* (h)	**8** (%)[b]	**9** (%)[b,c]
1	**8a**	CAL-B	0.5	nd	>97 (91)
2	**8b**	CAL-B	2	nd	>97 (88)
3	**8c**	CAL-B	1	nd	95 (89)[d]
4	**8d**	CAL-B	5	nd	>97 (91)
5	**8a**	PSL-C	10	nd	>97 (95)
6	**8b**	PSL-C	2	nd	>97 (88)
7	**8c**	PSL-C	10.5	nd	>97 (92)
8	**8d**	PSL-C	6	>97	
9	**8d**	PSL-C	120	86	14
10[e]	**8d**	PSL-C	192	85	15

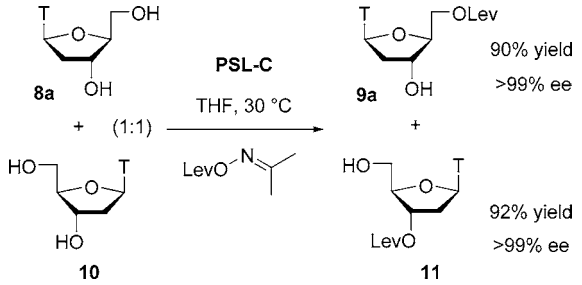
[a] Performed with 3 equiv of acetonoxime levulinate; ratio of **8**:CAL-B was 1:1 (w/w); ratio of **8**:PSL-C was 1:3 (w/w); 0.1 M concentration. [b] Based on ^{1}H NMR signal integration. [c] Percentages of isolated yields are given in parentheses. [d] Also, 5% 3′,5′-di-*O*-levulinyl-dA^{Bz} was isolated as a derivative. [e] Reaction run at 60 °C; nd = not detected.

in which traces of corresponding dilevulinyl compound were also observed. Attempted efforts to avoid the formation of minor bis-acylated product by lowering the reaction temperature (20 °C) or reducing the amount of lipase were unsuccessful. Gratifyingly, changing the reaction conditions did not have significant impact on the regioselectivity, and the reaction was completed in 0.5−5 h with excellent yields (88−91%).

Surprisingly, PSL-C showed the same behavior as CAL-B by catalyzing the acylation at the primary 5′-hydroxyl group of T (**8a**), dC^{Bz} (**8b**), and dA^{Bz} (**8c**) (entries 5−7, Table 1) in excellent yield. In general, the rate of acylation was slower with no detectable (^{1}H NMR) amounts of 3′,5′-di-*O*-Lev-dA^{Bz} in the crude reaction. On the other hand, the acylation reaction catalyzed by PSL-C on dG^{Ibu} (**8d**) led to the recovery of the starting material (entry 8, Table 1). We also tested this lipase at higher temperatures and longer reaction times (entries 9 and 10, Table 1), but the reaction stalled after 15% conversion. This limitation was circumvented when CAL-B afforded the desired derivative in 91% yield (entry 4, Table 1).

With a better understanding of the lipase-mediated acylation of L-nucleosides, the possibility of separation of a mixture of D/L-nucleosides was studied next (Scheme 2).

Scheme 2



Treatment of an equimolecular mixture of D/L-thymidine with acetonoxime levulinate afforded after 8 h a mixture of β-L-5′-*O*-levulinylthymidine (**9a**) and β-D-3′-*O*-levulinylthymidine (**11**)[15c] in good yields. The L-isomer **8** was regioselectively acylated at the 5′-hydroxyl group with PSL-C. The same lipase exhibited an opposite selectivity for the D-isomer, catalyzing the acylation of the 3′-hydroxyl group. This example constitutes a parallel kinetic resolution of nucleosides, since the different behavior of D/L-thymidine resulted in the formation of two different products.[18] The two acylated products have different R_f values and are separated easily from the reaction crude mixture by flash chromatography to give **9a** and **11** in 90 and 92% yields (based on the amount of D and L starting material), respectively, and are enantiomerically pure. The purity of the products was further corroborated with chiral HPLC data (Figure 2).

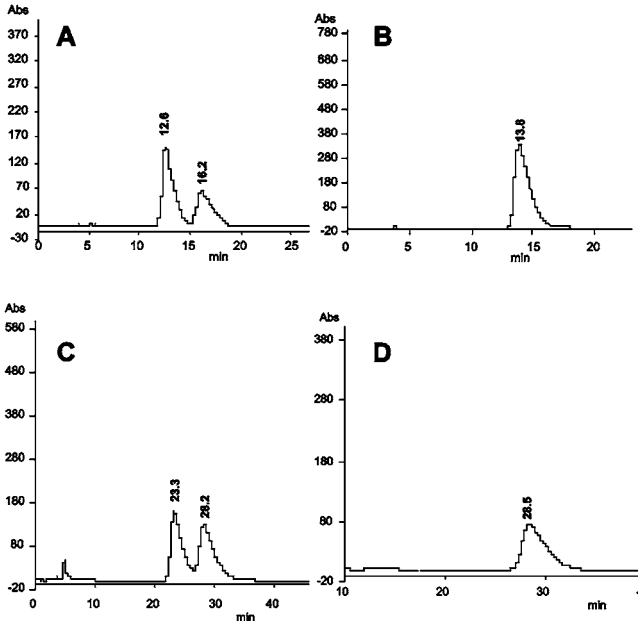


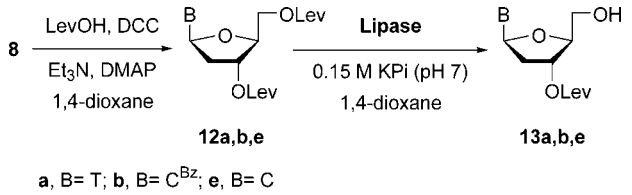
Figure 2. Chiral HPLC chromatograms. **A**: 3′-*O*-Lev-β-D/L-T. **B**: 3′-*O*-Lev-β-D-T from the enzymatic reaction. **C**: 5′-*O*-Lev-β-D/L-T. **D**: 5′-*O*-Lev-β-L-T from the enzymatic reaction. Conditions: Chiralcel OJ (250 × 46 mm), 0.78 mL/min, 10 °C, hexane/EtOH (1:1).

Having established a route for the synthesis of β-L-5′-*O*-levulinyl-protected nucleosides, we embarked on the synthesis of β-L-3′-*O*-levulinyl-protected nucleosides next (Scheme 3). The latter compounds are very useful building blocks for the solution-phase synthesis of L-oligonucleotides. On the basis of our previous work, we envisioned a two-step protocol that involves acylation followed by regioselective hydrolysis on the 5′-group.[15]

Therefore, the β-L-3′,5′-di-*O*-levulinyl derivatives of thymidine and 2′-deoxycytidine were conveniently prepared by the reaction of 2′-deoxynucleosides with levulinic acid and DCC in the presence of DMAP. Screening with a variety of

(18) For a review of the parallel kinetic resolution of racemic mixtures, see: Dehli, J. R.; Gotor, V. *Chem. Soc. Rev.* **2002**, *31*, 365−370.

Scheme 3 [a]



[a] See Table 2 for details.

lipases indicated that CAL-B had an excellent selectivity toward the hydrolysis of the 5′-levulinyl ester of 3′,5′-di-*O*-Lev-T (**12a**) furnishing 3′-*O*-Lev-T (**13a**) in 90% isolated yield (entry 1, Table 2).

Table 2. Enzymatic Hydrolysis of β-L-Di-*O*-Lev-dB **12**[a]

entry	diester	lipase	*T* (°C)	*t* (h)	**8** (%)[b]	**12** (%)[b]	**13** (%)[b,c]
1	**12a**	CAL-B	30	28.5	7		93 (90)
2	**12a**	PSL-C	30	117		90	10
3	**12a**	PSL-C	60	96	13	15	72
4	**12b**	CAL-B	30	166	19	15	66
5	**12b**	PSL-C	30	144	12	40	48
6	**12b**	CVL	30	25.5	13		87 (80)
7	**12e**	CAL-B	30	127		4	96 (89)

[a] Ratio of **12**:CAL-B was 1:1 (w/w); ratio of **12**:PSL-C was 1:3 (w/w); ratio **12**:CVL was 1:0.3 (w/w); 0.1 M concentration. [b] Based on HPLC signal integration. [c] Percentages of isolated yields are given in parentheses.

Similarly, PSL-C also exhibited total selectivity toward the 5′-acyl group, although low conversion was achieved even after the extended reaction time. Increasing the reaction temperature to 60 °C improved the conversion, but the selectivity was compromised (entries 2 and 3, Table 2). When the same process was carried out with 3′,5′-di-*O*-Lev-dC^{Bz} (**12b**), both CAL-B and PSL-C showed moderate selectivity, whereas hydrolysis of base-unprotected derivative **12e** took place exclusively at the 5′-position (entries 4 and 5 vs entry 7, Table 2). Nevertheless, *Chromobacterium viscosum* lipase (CVL) was found to hydrolyze the 5′-*O*-levulinyl ester with high selectivity furnishing the 3′-*O*-acyl derivative **13b** in 80% yield (entry 6, Table 2).

In summary, we have demonstrated that the 3′- and 5′-*O*-levulinyl-protected derivatives of β-L-2′-deoxynucleosides can be prepared through selective acylation and/or deacylation processes catalyzed by enzymes. Furthermore, utilizing this approach, we have accomplished a parallel kinetic resolution of a 1:1 mixture of D/L-nucleosides via an acylation reaction to furnish easily separable compounds. We envision that this separation methodology would have a huge potential for the isolation of pure isomers from a racemic mixture of nucleosides. Thus, enzymes continue to play an important role in expanding our understanding and knowledge of substrate specificity. The high efficiency, safety, and "green" nature of these processes makes them an attractive alternative to existing chemistry-based methods.

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Supporting Information Available: Experimental procedures and complete ^{1}H and ^{13}C NMR spectral data in addition to mp, IR, optical rotation, microanalysis, and MS data for the new compounds; the level of purity is indicated in the included copies of ^{1}H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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