Pure Appl. Chem., Vol. 77, No. 1, pp. 237–243, 2005.
DOI: 10.1351/pac200577010237

Selective transacylation reactions on 4-aryl-3,4-dihydropyrimidin-2-ones and nucleosides mediated by novel lipases*

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Abstract: Different (±)-4-(3/4-acetoxyaryl)-5-ethoxycarbonyl-6-methyl-3,4-dihydropyrimidin-2-ones have been synthesized and subjected to enantioselective deacetylation reactions mediated by different lipases in organic media. Novozyme 435 in tetrahydrofuran:diisopropyl ether was found to be the catalyst of choice for efficient enantioselective deacetylation of dihydropyrimidinones under study. Further, we discovered that lipase isolated from *Pseudomonas aeruginosa* can be used for selective acylation of secondary hydroxyl groups in nucleosides. This observation can be very useful for selective manipulation of different hydroxyl groups in nucleosides.

INTRODUCTION

Enzymes hold great potential for carrying out selective reactions in organic synthesis with the added advantage of environmental friendliness [1,2]. Important in this context is the enantioselective synthesis/chiral resolution of various biologically active compounds as it is a common observation that one enantiomer may be active and the other inactive/toxic [3,4]. Interest in 4-aryl-3,4-dihydropyrimidinones lies in their close structural relationship to clinically important 1,4-dihydropyridine-based calcium channel modulators of the nifedipine type (Fig. 1) [5] and also because of the interesting biological properties of several marine alkaloids based upon the structure of dihydropyrimidine, viz. crambine, batzelladine (potent HIV inhibitor), and ptilomycalin A (Fig. 1) [6,7]. In contrast to the achiral nifedipine, dihydyropyrimidinones are inherently asymmetric. The calcium channel modulation activity of 4-aryl-3,4-dihydropyrimidinones is dependent on the absolute configuration at the stereogenic center at C-4 [5]. The orientation of the C-4 aryl group acts as a molecular switch between calcium channel-blocking and -activating activity. For example, it is the (*R*)-enantiomer of SQ 32926 (Fig. 1) that carries the therapeutically desired calcium channel-blocking (antagonistic) activity [5].

*Paper based on a presentation at the 24th International Symposium on the Chemistry of Natural Products and the 4th International Congress on Biodiversity, held jointly in Delhi, India, 26–31 January 2004. Other presentations are published in this issue, pp. 1–344.

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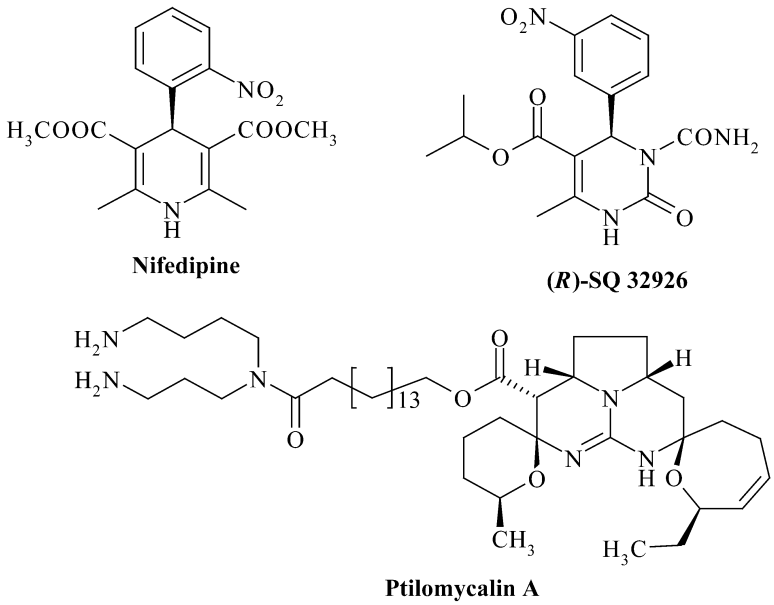


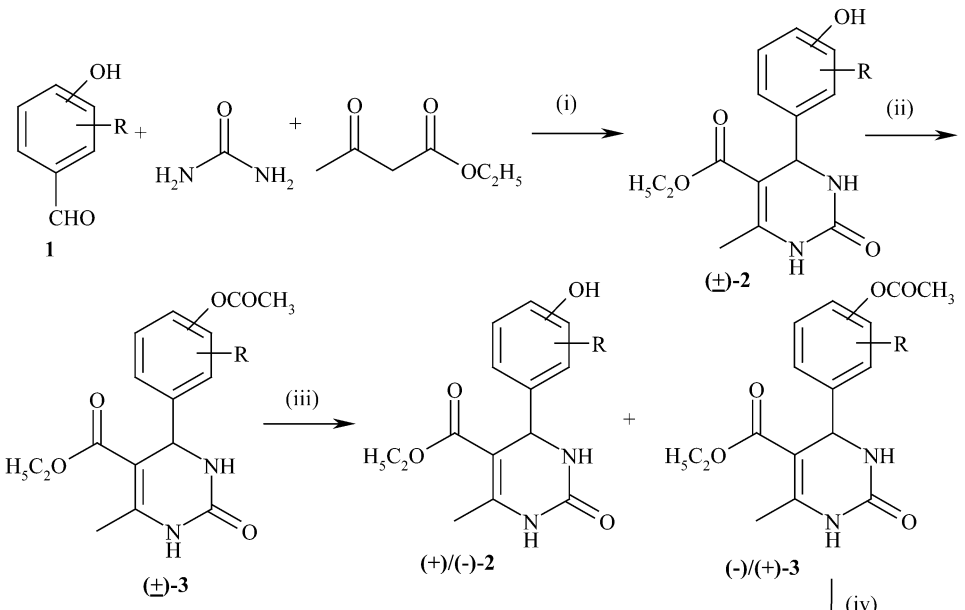
Fig. 1 Structures of some important dihydropyrimidine-based compounds.

The current interest therefore lies in the synthesis of enantiomerically pure dihydropyrimidinones. In the past, optically pure dihydropyrimidinones were obtained by classical resolution of the corresponding carboxylic acids [8], by separation of diastereomeric derivatives bearing chiral auxiliaries at N-3 [9] or by HPLC using chiral stationery phases [10]. In this article, we present a simple biocatalytic approach toward the enantioselective resolution of (±)-4-(3/4-acetoxyaryl)-5-ethoxycarbonyl-6-methyl-3,4-dihydropyrimidin-2-ones **3a–3e**.

Another important application of enzymes is regioselective acylation of polyhydroxy compounds [11–14], e.g., nucleosides, which are the building blocks of nucleic acids and play a crucial role in storage and transfer of genetic information. Glycosyl-transferring enzymes have also been used for the synthesis of enantiomerically pure bioactive nucleosides [15 and references therein]. The recent focus on nucleoside chemistry is due to the discovery of 2',3'-dideoxynucleosides like AZT, ddC, and d4T as anti-HIV agents and the emergence of antisense and antigene oligonucleotides as selective inhibitors of gene expression [16–19]. The intrinsic problem faced in nucleic acid chemistry is the selective protection and deprotection of hydroxyl groups of nucleosides for oligonucleotide and modified nucleoside synthesis [20]. Although several chemical methods are available for the regioselective acylation of the hydroxyl groups, the enzymatic methods offer significant advantages with respect to selectivity, novelty, and efficiency. Herein, we report the rare selective acylation of secondary hydroxyl group in nucleosides in the presence of a novel lipase from *Pseudomonas aeruginosa.*

SYNTHESIS AND ENANTIOSELECTIVE RESOLUTION OF (±)-4-(3/4-ACETOXYARYL)-5-ETHOXYCARBONYL-6-METHYL-3,4-DIHYDROPYRIMIDIN-2-ONES 3a–3e

Racemic 4-(3/4-hydroxyaryl)-5-ethoxycarbonyl-6-methyl-3,4-dihydropyrimidin-2-ones **2a–2e** were prepared in 80–90 % yields by Biginelli cyclocondensation of ethyl acetoacetate, urea, and the corresponding aromatic hydroxyaldehydes **1a–1e** in the presence of ferric chloride under microwave conditions following the modified literature procedure [21]. The compounds **2a–2e** were acetylated to afford in quantitative yields the (±)-4-(3/4-acetoxyaryl)-5-ethoxycarbonyl-6-methyl-3,4-dihydropyrimidin-2-ones **3a–3e** using acetic anhydride-pyridine and a catalytic amount of dimethylaminopyridine (DMAP) (Scheme 1). All of the hydroxy- and acetoxy-4-aryl-3,4-dihydropyrimidin-2-ones **2a–2e** and **3a–3e**



	1	(±)-2	(+)/(-)-2	(-)/(+)-2	(±)-3	(-)/(+)-3	(+)/(-)-3
		OH	R		$OCOCH_3$		R
a		C-3	H		C-3		H
b		C-3	C-4 OCH_3		C-3		C-4 OCH_3
c		C-4	H		C-4		H
d		C-4	C-3 OCH_3		C-4		C-3 OCH_3
e		C-4	C-3 OC_2H_5		C-4		C-3 OC_2H_5

Reagents and Conditions: i) microwave 1-2 min, $FeCl_3$, SiO_2; ii) acetic anhydride, DMAP, 60 min, 25-30°C; iii) Novozyme 435, THF:DIPE (2:1), butan-1-ol, 10-35h, 50-55°C; iv) sat. methanolic ammonia, 30-45 min, 25-30°C

Scheme 1

were unambiguously characterized on the basis of their spectral data (IR, ^1H, and ^{13}C NMR and MS) analysis. The structures of known compounds **2a–2e** were further confirmed by comparison of their melting points and/or spectral data with those reported in the literature [21].

Different lipases, i.e., porcine pancreatic lipase (PPL), *Candida rugosa* lipase (CRL), Novozyme 435 (immobilized CAL-B) and *P. aeruginosa* lipase were screened for enantioselective deacetylation of (±)-4-(3/4-acetoxyaryl)-5-ethoxycarbonyl-6-methyl-3,4-dihydropyrimidin-2-ones **3a–3e**. None of the five 3,4-dihydropyrimidin-2-ones under study were accepted as substrates by PPL and *P. aeruginosa* lipase. Although CRL in tetrahydrofuran:diisopropyl ether (THF:DIPE) catalyzed the deacetylation of 3,4-dihydropyrimidin-2-ones, the reaction was very slow and of no practical use. On the basis of the screening tests, Novozyme 435 in THF:DIPE (2:1) was selected for further deacetylation studies on 3,4-dihydropyrimidin-2-ones **3a–3e**. The mixture of solvents, i.e., THF:DIPE (2:1) was selected on the basis of many test reactions in different mixtures of solvents and on the basis of our experience of better efficiency of CAL in relatively nonpolar solvents.

In a typical reaction, the racemic 3,4-dihydropyrimidin-2-one (**3a–3e**, 1 mmol) was treated in an incubator shaker with Novozyme 435 (~150 mg) in THF:DIPE (2:1, 20–25 ml) at 50–55 °C in the presence of butan-1-ol (2 equiv). The reaction was monitored by TLC and stopped by filtering off the enzyme after about 50 % conversion of the starting acetate to a slow moving product on TLC. The solvent was removed from the reaction mixture and residue was subjected to column chromatography to afford the enzymatically deacetylated 4-hydroxyaryl-3,4-dihydropyrimidin-2-ones **2a–2e** and unreacted 4-acetoxyaryl-3,4-dihydropyrimidin-2-ones **3a–3e** in pure forms in 39–48 and 35–45 % yields, respectively (Table 1). The enzymatically deacetylated compounds **2a–2e** and recovered, unreacted acetates **3a–3e** had significant optical rotation values (Table 2).

Table 1 Enantioselective deacetylation of 4-(3/4-acetoxyaryl)-5-ethoxycarbonyl-6-methyl-3,4-dihydropyrimidin-2-ones mediated by Novozyme 435 in THF:DIPE (2:1) at 50–55 °C in the presence of butan-1-ol[a,b].

Substrate	Time (h)	Products[c]	Yield (%)
(±)-3'-OAc (**3a**)	10	(+)-3'-OH (**2a**)	42
		(–)-3'-OAc (**3a**)	45
(±)-3'-OAc-4'-OMe (**3b**)	35	(–)-3'-OH-4'-OMe (**2b**)	43
		(+)-3'-OAc-4'-OMe (**3b**)	40
(±)-4'-OAc (**3c**)	8	(–)-4'-OH (**2c**)	48
		(+)-4'-OAc (**3c**)	42
(±)-4'-OAc-3'-OMe (**3d**)	15	(–)-4'-OH-3'-OMe (**2d**)	39
		(+)-4'-OAc-3'-OMe (**3d**)	45
(±)-4'-OAc-3'-OEt (**3e**)	18	(–)-4'-OH-3'-OEt (**2e**)	41
		(+)-4'-OAc-3'-OEt (**3e**)	35

[a]All of these reactions, when performed under identical conditions but without adding lipase, did not yield any product.
[b]All deacetylation reactions were stopped by filtering off the enzyme after about 50 % conversion of the starting racemic acetoxy dihydropyrimidin-4-ones to the products, i.e., corresponding hydroxy compounds.
[c]Deacetylated dihydropyrimidin-4-ones and recovered, unreacted, acetylated dihydropyrimidin-4-ones.

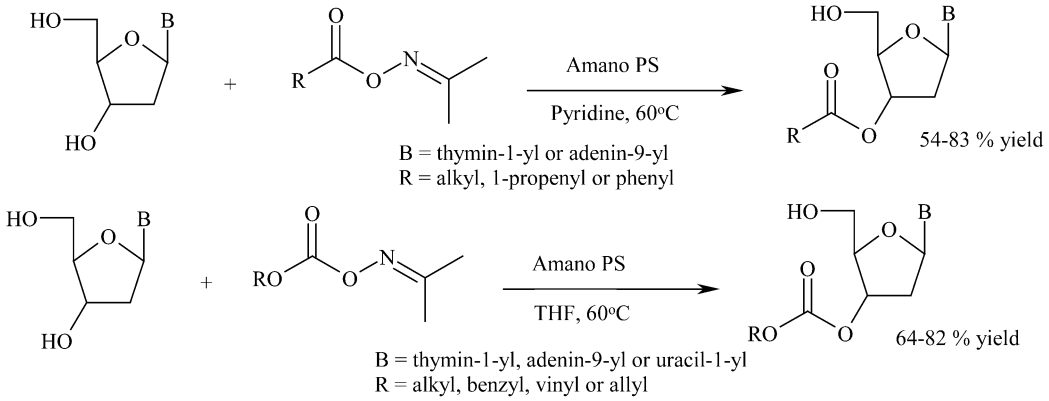
Table 2 Optical rotation values of hydroxy dihydropyrimidinones (+)/(–)-**2a**–**2e** obtained by Novozyme 435-catalyzed deacetylation of (±)-**3a**–**3e**, recovered, unreacted acetates (–)/(+)-**3a**–**3e**, and the hydroxy dihydropyrimidinones (–)/(+)-**2a**–**2e** obtained by chemical deacetylation of (–)/(+)-**3a**–**3e**.

Substrate (racemic)	$[\alpha]^{25}_D$ values		
	Hydroxy dihydropyrimidinones (+)/(–)-**2a**–**2e**	Recovered, unreacted acetoxy dihydropyrimidinones (–)/(+)-**3a**–**3e**	Dihydropyrimidinones (–)/(+)-**2a**–**2e** obtained by chemical deacetylation of (–)/(+)-**3a**–**3e**
(±)-**3a**	(+)-**2a**: +21.0	(–)-**3a**: –18.0	(–)-**2a**: –18.0
(±)-**3b**	(–)-**2b**: –33.5	(+)-**3b**: +12.0	(+)-**2b**: +30.0
(±)-**3c**	(–)-**2c**: –38.0	(+)-**3c**: +32.0	(+)-**2c**: +30.0
(±)-**3d**	(–)-**2d**: –3.0	(+)-**3d**: +8.9	(+)-**2e**: +3.1
(±)-**3e**	(–)-**2e**: –11.0	(+)-**3e**: +14.2	(+)-**2d**: +8.8

In order to determine enantiomeric excess values of the deacetylated (+)/(–)-hydroxy-4-aryl-3,4-dihydropyrimidin-2-ones **2a–2e** and recovered, unreacted (–)/(+)-acetoxy-4-aryl-3,4-dihydropyrimidin-2-ones **3a–3e**, their ^{1}H NMR spectra were recorded in the presence of different concentrations of chiral-shift reagent, (*S*)-(+)-2,2,2-trifluoro-1-(9-anthryl)-ethanol [(+)-TFAE], but the separation of the signals in the ^{1}H NMR spectra of these compounds was not observed. However, to know the extent of optical enrichment of enzymatically deacetylated (+)/(–)-4-hydroxyaryl-3,4-dihydropyrimidin-2-ones **2a–2e**, chemical deacetylation of recovered, unreacted acetates (–)/(+)-**3a–3e** was affected with saturated methanolic ammonia and optical rotation values of enzymatically deacetylated (+)/(–)-**2a–2e** and chemically deacetylated (–)/(+)-**2a–2e** were compared. These were quite comparable and were of opposite signs (Table 2).

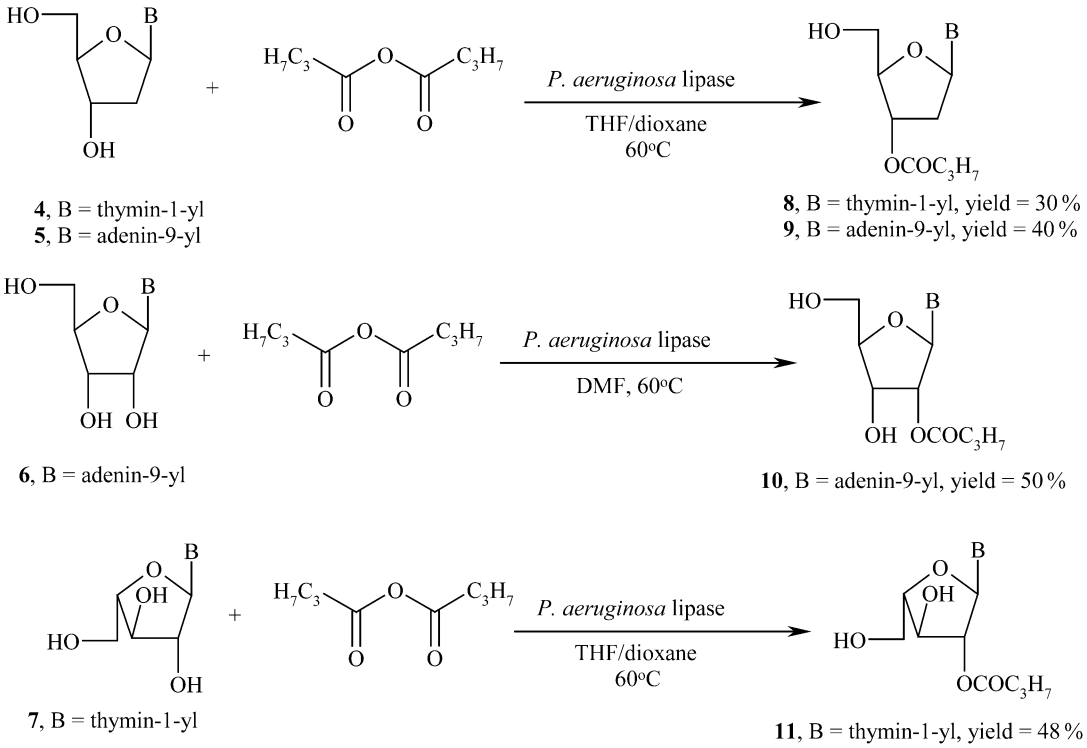
LIPASE-CATALYZED SELECTIVE ACYLATION OF DEOXYRIBO- AND RIBONUCLEOSIDES 4–7

There are numerous reports of lipase-mediated acylation of primary hydroxyl groups over secondary hydroxyl group(s) in nucleosides [22,23]. However, only a few examples of selective acylation of secondary hydroxyl group(s) over primary hydroxyl group of nucleosides are reported [24,25]. For example, Gotor and his colleagues have reported the selective protection of 3'-hydroxyl group of thymidine, 2'-deoxyadenosine, and 2'-deoxyuridine through the catalysis by Amano PS lipase. Oxime esters in pyridine and alkoxycarbonyl oxime esters in THF were used as acyl and alkoxycarbonyl transferring agents for the synthesis of 3'-*O*-acylated/3'-*O*-alkoxycarbonylated thymidine, 2'-deoxyadenosine, or 2'-deoxyuridine in 54 to 83 % yields [24] (Scheme 2).



Scheme 2

In the present study, a novel bacterial lipase (from *P. aeruginosa*) was screened for regioselective acylation of hydroxyl groups of deoxyribo- and ribonucleosides, e.g., thymidine, 2'-deoxyadenosine, adenosine, and α-L-arabinosylthymine using different aliphatic acid anhydrides as acylating agents. On screening of different acylating agents, butanoic anhydride was found to be the best acylating agent and THF or DMF was found to be the solvent of choice. In a typical reaction, a mixture of nucleoside (**4–7**, 1 mmol) and butanoic anhydride (1.1 mmol) in THF (DMF in case of **6**, 12–15 ml) was shaken with *P. aeruginosa* lipase in an incubator shaker at 60 °C and the progress of the reaction was monitored on TLC (Scheme 3). All nucleoside acylation reactions almost stopped after 45–60 % conversion of the starting compound into a fast moving product on TLC in 10–12 h. After establishment of equilibrium in acylation reaction, the enzyme was filtered off and the solvent removed under reduced pressure. The crude product obtained was purified by loading on a silica gel column to afford the unreacted **4–7** and the monoacylated nucleosides **8–11** in 30–50 % yields.



Scheme 3

Spectral analysis of the acylated products **8** and **9** obtained from lipase-catalyzed acylation of thymidine (**4**) and 2'-deoxyadenosine (**5**) revealed that the lipase exclusively acylates the lone secondary hydroxyl group of the nucleoside over the primary hydroxyl group at 5'-position. Thus, the chemical shift values of C-3'H in 3'-*O*-butanoylated thymidine (**8**) and 3'-*O*-butanoylated 2'-deoxyadenosine (**9**) exhibited downfield shift of 1.1 and 1.2 ppm in their ^{1}H NMR spectra, and the corresponding C-3 carbon showed a downfield shift of 4 ppm in their ^{13}C NMR spectra with respect to the chemical shift values of the same proton/carbon in thymidine (**4**) and 2'-deoxyadenosine (**5**), respectively. Furthermore, to extend the selective lipase-catalyzed acylation studies on nucleosides having two secondary and one primary hydroxyl groups, adenosine (**6**) and α-L-arabinosylthymine (**7**) were incubated with *P. aeruginosa* lipase in DMF and THF, respectively, in the presence of butanoic anhydride. It was observed that the lipase selectively catalyzes the acylation of 2'-hydroxyl group in both adenosine and α-L-arabinosylthymine leading to the formation of 2'-*O*-butanoyladenosine (**10**) and 2'-*O*-butanoyl-α-L-arabinosylthymine (**11**). This was established on the basis of downfield shift in the chemical shift values of C-2' protons of butanoylated nucleosides **10** and **11** with respect to the chemical shift values of the same protons in the corresponding unprotected nucleosides **6** and **7**, respectively. The structures of all the acylated nucleosides **8–11** were unambiguously established on the basis of their spectral analysis (IR, ^{1}H, and ^{13}C NMR and MS).

This is the first report of enzyme-catalyzed selective acylation studies on nucleosides of ribo- and arabino- series and a rare example of selective acylation of secondary hydroxyl group over primary hydroxyl group in nucleosides.

CONCLUSIONS

This study has clearly demonstrated the enantioselective capabilities of Novozyme 435 for chiral resolution of racemic mixtures of (±)-4-(3/4-acetoxyaryl)-5-ethoxycarbonyl-6-methyl-3,4-dihydropyrimidin-2-ones, an important class of compounds with calcium channel-blocking activity. Further, a novel lipase isolated from *P. aeruginosa* has exhibited exclusive selectivity for the acylation of secondary hydroxyl groups over primary hydroxyl groups in nucleosides. More importantly, the lipase can selectively acylate the C-2' hydroxyl group of adenosine and α-L-arabinosylthymine over the other secondary and a primary hydroxyl group present in the molecule. This methodology can be useful for the efficient synthesis of building blocks for preparation of novel oligonucleotides.

ACKNOWLEDGMENT

We thank the Council of Scientific and Industrial Research (CSIR, New Delhi) and the Danish National Research Foundation for financial assistance.

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