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Lipase-Catalyzed Regio- and Stereoselective Acylation of Hydroxy Groups in Steroid Side Chains

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Summary. Lipase from *Pseudomonas cepacia* (PCL) catalyzes the regio- and stereoselective acylation of primary and secondary hydroxy groups in steroid side chains under irreversible transesterification conditions with vinyl acetate in organic solvents.

Keywords. *Pseudomonas cepacia* lipase; Stereoselective acylation; Irreversible transesterification; Steroid side chain; Organic solvents.

Introduction

The lipase-catalyzed acylation of hydroxylated substrates in organic solvents is a well established method extensively applied to the synthesis of enantiomerically pure compounds [1–4]. For instance, under the conditions of irreversible transesterification in an organic solvent [5,6] we have successfully achieved the enantioselective resolution of a variety of 2-substituted alkanols [2]. In principle, the enzymatic method could be satisfactorily applied to substrates such as steroids that are highly insoluble in water. At the time we started our research on the regio- and stereoselective enzymatic acylation of functionalized steroid side chains, the only available examples of acylation on steroid alcohols referred almost exclusively to hydroxy groups located in the steroid rings [7–13].

Results and Discussion

We have reported for the first time that *Pseudomonas cepacia* lipase (PCL or lipase PS if the enzyme is from the Amano *Pseudomonas* species) catalyzes the stereoselective acylation of a hydroxy groups in a steroid side chain. The substrate was the (20*R*,*S*)-22-hydroxy steroid **1** which was acetylated with vinyl acetate in chloroform [14]. Starting from a 2:1 20*S*/20*R* ratio, in 68 h the epimeric ratio of the

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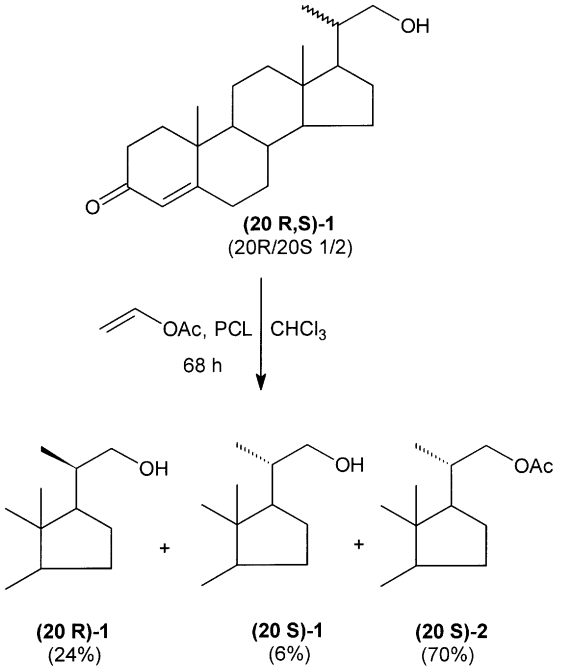
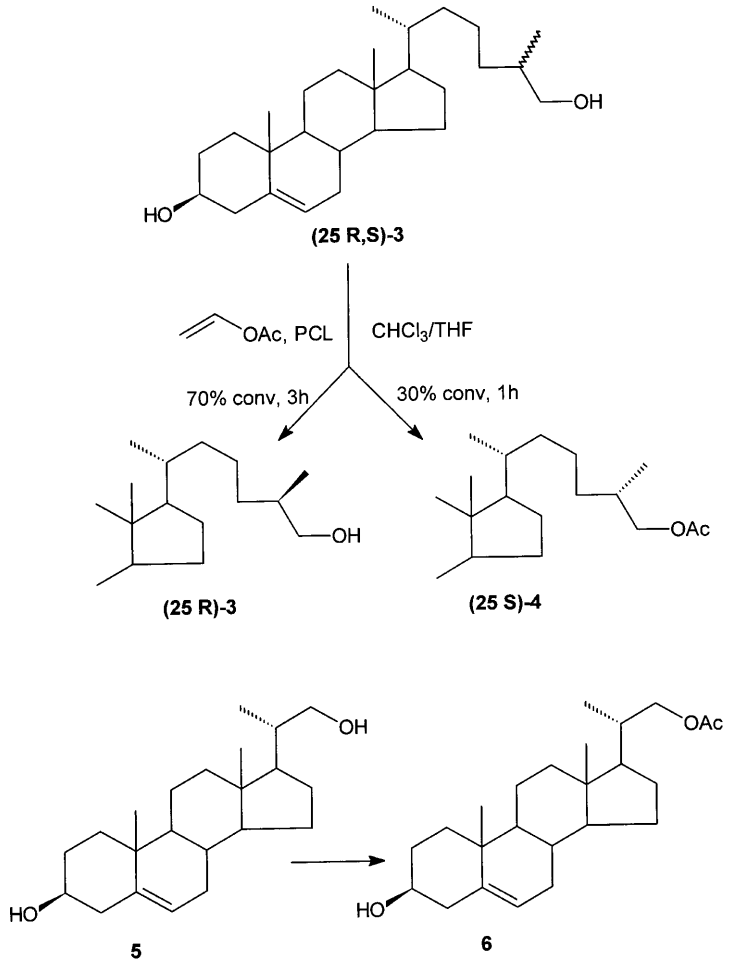


Scheme 1

unreacted alcohol **1** became 1:4. The preferential acetylation of the (20*S*)-isomer afforded the diastereomerically pure (20*S*)-acetate **2** (Scheme 1). For configurational analysis, a sample of epimerically pure (20*S*)-alcohol **1** was prepared from commercially available stigmasterol in a few steps [14].

The relatively slow reaction of the (20*R*)-alcohol and the stereochemical outcome of the enzymatic reaction may be, at least in part, determined by the steric hindrance of the 13-β methyl group. In fact, chemical transformations at the 22,23-position are influenced in the same way, as shown by the epimerization of the 22-aldehyde that, on reduction, determines the $20S/20R = 2{:}1$ ratio of the substrate **1**. Starting from the above considerations, we have examined a further primary alcohol function located in a steroid side chain at position 25 with the same stereogenic center as **1**. (25*R*,*S*)-26-Hydroxycholesterol (**3**) was subjected to the reaction with lipase and vinyl acetate in a chloroform/tetrahydrofuran mixture [15]. After 1 h, 30% of the (25*S*)-26-acetate **4** was formed, thus showing that the enzymatic reaction was also highly regioselective, since no trace of the 3-acetate was observed. Similarly, although slower, the (20*S*)-3β,22-dihydroxy derivative **5** was regioselectively converted in 30 h (30% yield) to the 22-acetate **6** (Scheme 2).

A 70% conversion to **4** was reached in 3 h, and the unreacted (25*R*)-**3** was isolated. The configuration of the enzymatic products **3** and **4** was assigned by comparison with published data [16], and the results were in agreement with the configurational outcome of the enzymatic reaction on other 2-methyl alkanols as substrates [17] within the model recently proposed for the interpretation of the enantiopreference of *Pseudomonas capacia* lipase toward primary alcohols [18]. Finally, comparing the hindered C-22 with the more accessible C-26 alcohol, the

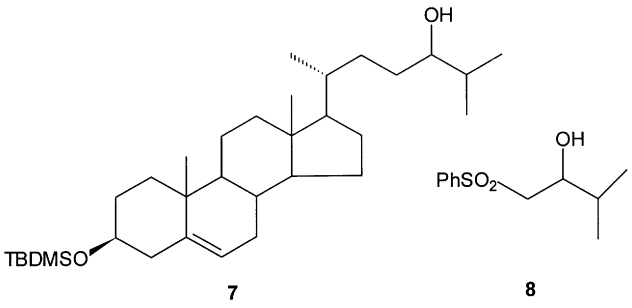


Scheme 2

enzymatic formation of the (25*S*)-acetate **4** is faster than the corresponding reaction on (20*R*,*S*)-22-hydroxy steroid **1**. This structural difference was confirmed by ^{1}H NMR analysis of the diastereomeric ratio of the two alcohols. In fact, in order to establish the 20*R*/20*S* ratio in **1** no derivatization was necessary, whereas the 26-alcohol **3** required the analysis of the corresponding *MTPA* ester [19].

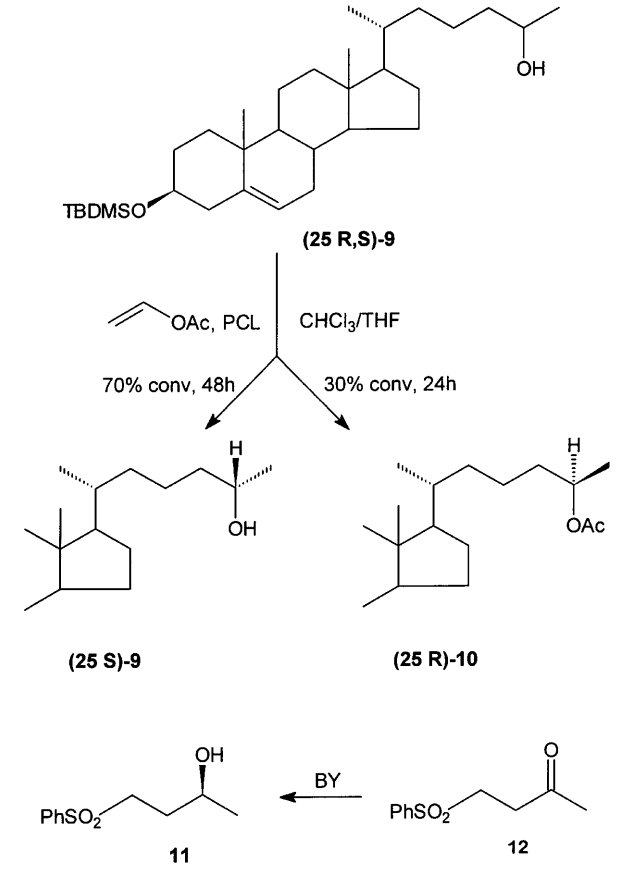
The enzymatic acetylation of secondary hydroxy groups in a steroid side chain was also studied. In fact, 24-hydroxycholesterol, (24*R*,*S*)-**7** did not react under the usual conditions, and we assume that the isopropyl residue hinders the enzymatic acetylation. A similar negative result was obtained with the phenylsulfonyl alcohol **8** (Scheme 3).

For this reason, the 27-*nor*-(25*R*,*S*)-25-hydroxy-cholesterol **9** was prepared and subjected to the reaction with lipase and vinyl acetate in chloroform [20]. After 24 h, 30% of the 25-acetate **10** was formed, whereas a 70% conversion to **10** was reached in 48 h. The enzymatically prepared 25-alcohol **9** and the corresponding acetate **10** were obtained from two separate reactions in epimerically pure state as shown by the 500 MHz ^{1}H NMR spectra of their 25-MTPA esters. The configurations of the



7 8

Scheme 3



Scheme 4

enzymatic products were assigned by comparison with the resonances of a sample of a 25*S*-alcohol (**9**) synthesized from enantiomerically pure (*S*)-4-phenylsulfonyl-2-butanol (**11**), in turn prepared by baker's yeast-mediated reduction of 4-phenylsulfonyl-2-butanone (**12**) [20] (Scheme 4).

The results obtained from the steroidal alcohol **9** confirm that the 2-methyl-1-alkanols react faster than a secondary alcohol and that the stereochemical outcome of the enzymatic acylation of the alcohol **9** is in agreement with the configurational

preference of the enzyme when a secondary alcohol is the substrate [21]. It should also be noted that the resolution of (25*R*,*S*)-26 acetate **4** and (25*R*,*S*)-25-acetate **10** by transesterification with methanol in *tert*-butyl methyl ether did not proceed satisfactorily. Finally, the results presented here show the first examples of enantioselective control of the lipase-catalyzed acylation of hydroxy groups present in steroid side chains and offer an additional chemoenzymatic access to the preparation of stereochemically pure sterogenic centers at these positions.

Experimental

Lipase-catalyzed transesterification; general procedure

A solution of 1 mmol of the steroid alcohol and 0.32 cm^3 vinyl acetate (3.46 mmol) in 5.5 cm^3 of a suitable organic solvent was added to 14 mg of solid lipase (31.5 U/mg) under stirring at room temperature for the time required for the desired conversion to the acetate. The reaction progress was monitored by TLC and GLC analysis (Hewlett Packard, model 5890/II, HP-5 capillary column, 280°C).

^{1}H NMR analysis of the stereochemical course of the enzymatic reactions

The ^{1}H NMR spectra (500 MHz) were registered using a Bruker AM 500 spectrometer. For the alcohol **1** no derivatization was required, and the 20*R*- and 20*S*-22-CH_2OH resonances were found at 3.58–3.63 and 3.65–3.72 ppm, respectively. The regioselective acylation of compound **5** was monitored following the formation of the 26-acetate **6** (multiplet centered at 3.87 ppm) and the unchanged multiplet centered at 3.50 ppm (3β-hydroxy group). The (*R*)-*MTPA* esters were required for establishing the epimeric ratio of the acetylation of 26-hydroxycholesterol **3**. The 25*R*- and 25*S*-26-CH_2OH resonances appeared as double doublets between 4.00–4.08 and 4.18–4.25 ppm and a doublet at 4.13 ppm, respectively. In the case of the 25-alcohol **9**, the significant resonances of the (*R*)-*MTPA* esters were those of the C-26 methyl group (1.23 and 1.30 ppm for 25*R* and *S*, respectively).

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