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Lipase Catalyzed Regioselective Esterification of a Terminal Diol

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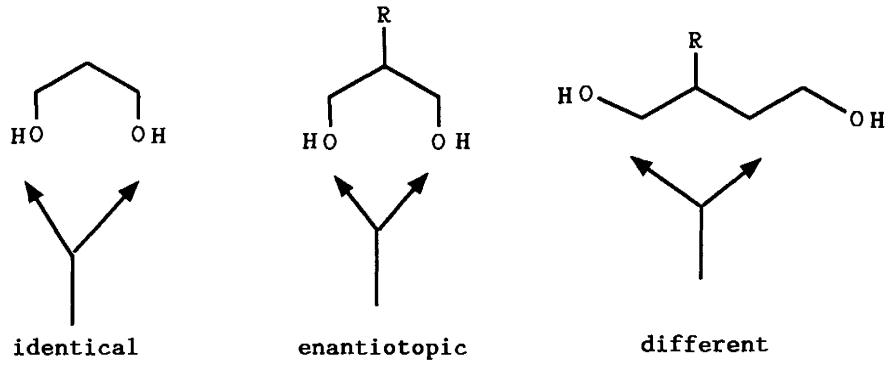
Lipase from *Candida cylindracea* catalyzes regioselective interesterification between an acyl donor and a terminal diol. Discrimination between primary hydroxyl groups in a dissymmetric molecule has not been previously described. Preparation of (*R*)-2-thiobenzyl-4-acetoxy-hydroxybutane **4** obtained in this way, allows the assignement of the (*R*) absolute configuration to 2-thiobenzyl-1,4-butandiol derivatives previously obtained in a yeast transformation.

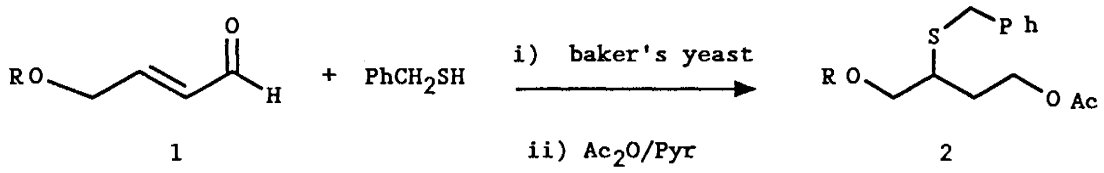
Hydrolytic enzymes, particularly lipases, have found widespread applications in organic synthesis due to low cost, wide versatility and easy use.[1] They have been used in resolution of racemic alcohols or esters, production of chiral compounds from prochiral precursors *via* selective hydrolysis or interesterification in acqueous or organic medium.[2] The regioselective capabilities of lipases have also been recognised and the use of these enzymes for solving problems of different alcoholic group recognition within the same molecule has been mainly applied to the selective hydrolysis/esterification of carbohydrates. In this field they have been exploited in two ways: a) selective functionalization of the



Scheme 1.

primary C-6 hydroxyl group,[3] b) partial discrimination between chemically

similar secondary ones.[4] In the field of fats and oils interesterification, the use of hydrolytic enzymes has been applied extensively and the ability of discriminating between the primary and secondary hydroxyl groups has been attributed to some microbial enzymes like *A. niger* and *P. fluorescens* lipases , whereas the enzymes from *C. cilindracea* and *G. candidum* do not show this kind of selectivity.[5] No examples have been reported so far in the regioselective hydrolysis/esterification of a non-identical non-enantiotopic primary hydroxyl group (Scheme 1). Yet the problem, of selective protection-deprotection of one of two primary hydroxyl groups, is one which is often encountered in organic synthesis. We wish to report the application of a lipase regioselective interesterification of a terminal diol. Recently,[6] we have reported that fermenting yeast-mediated reaction of benzyl mercaptan and unsaturated γ-alkoxyaldehydes **1**, gave addition products to which after acetylation, structure **2a** and **b** were assigned but whose absolute configuration was undetermined (Scheme 2).In order to



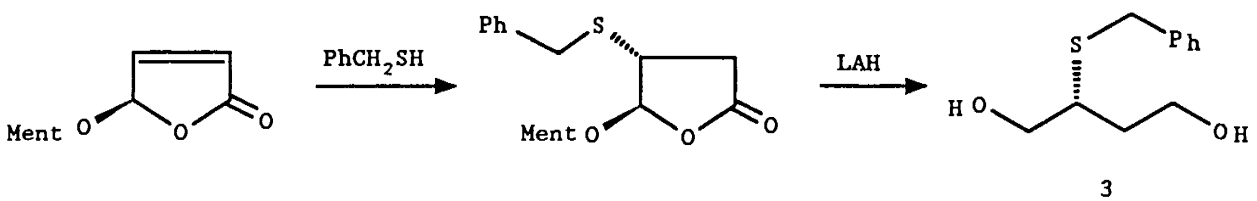
1 2

a = PhCO

b = $PhCH_2$

Scheme 2.

establish the stereostructure of the compounds obtained, the diol **3** was prepared in optically pure form according to a known procedure[7] as shown in Scheme 3. Transformation of the diol **3** in compound **2a**, requires regiose-

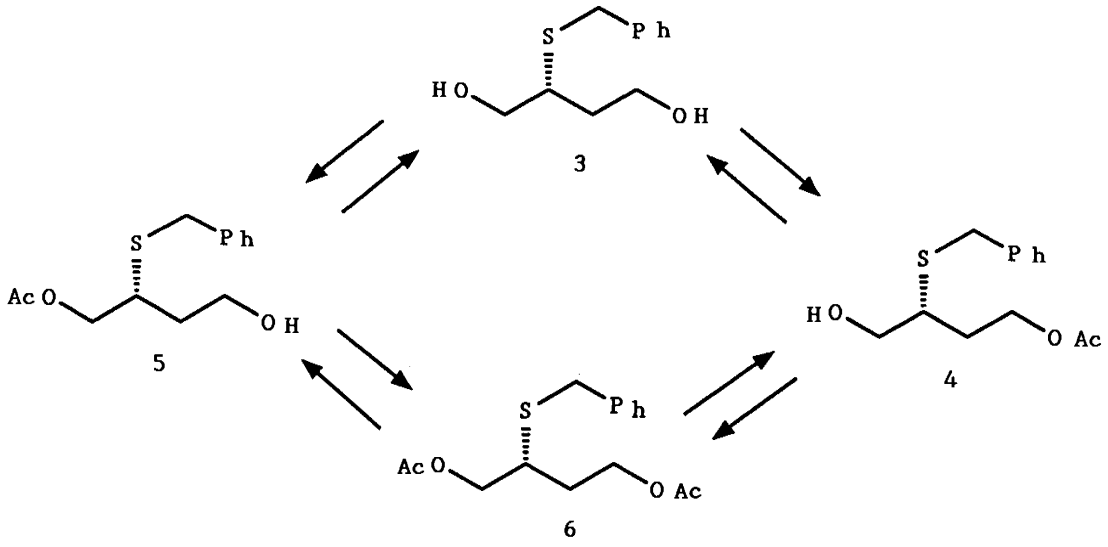


3

Ment = (-)-Menthyl

Scheme 3.

selective acetylation at position 4 of 3. Thus we explored the selectivity of lipases to avoid tedious separation of isomeric monoacetates and diacetate, as well as to obtain informations on the ability of these enzymes to discriminate between the two primary hydroxyl groups. We first prepared the diacetate 6 and tried to obtained 4 by partial hydrolysis, but with a number of different hydrolytic enzymes mixtures of the four compounds were obtained in all cases.[8] We then considered interesterification as a possible means to obtain 4. The diol 3 was dissolved in an acyl donor, and the solution diluted with hexane to form a biphasic system to which the enzyme was added, and the mixture stirred at temperatures between 0 and 30 °C. The reaction was followed by TLC and GC . When isopropenyl acetate was used as an acyl donor in the presence of *Candida cylindracea* lipase (Sigma type VII) in hexane at 0 °C, the monoacetate 4 was obtained contaminated with less then 10% 5 and no diacetate 6, when the reaction was stopped at 70% conversion.[9] After this point formation of the diacetate was prevalent while the relative amount of 5 was always below 10%.



Vinyl acetate and ethyl acetate proved to be less convenient acyl donors, the former for the lower selectivity and the latter for the much lower reaction rate. Other enzymes tested[8] were all much less adequate, showing lower or no selectivity at all. A preparate of *C. cylindracea* lipase on celite was equally effective as the free enzyme. When racemic 3 was used, the reaction had the same course giving racemic 4 as main product, thus suggesting that the recognition is due to steric reasons indipendent from chiral interactions. Compound 4 thus obtained was then

converted into the corresponding benzoate **2a**, $[\alpha]_D^{20}$ +53° (c 1, MeOH) thus allowing the assignement of the (*R*) absolute configuration[10] to the product obtained from baker's yeast reduction.[6] Although the regioselectivity observed in the present case was not as efficient as to avoid chromatographic purification of the desired product we believe that the regioselective properties of lipases should deserve further attention.

References

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8) The following commercial enzymatic powders were used both in hydrolysis and transesterifications: *C. cylindracea, M. javanicus, A. niger, P.fluorescens, C. lipolitica,* pig pancreatic lipase, pig liver esterase.
9) 100 mg of diol **3** was dissolved in 1 ml of isopropenyl acetate and dilute with 5 ml of hexane stirring at 0 °C. CCL was then added (50 mg Sigma type VII) and the mixture was analysed by GLC. After 2 h the enzyme was filtered off and the solvent was evaporated. After purification 60 mg of monoacetate **4** was obtained in a pure form. $[\alpha]_D^{20}$ (c 1, $CHCl_3$) : **3** +88.2°, **4** +70.2°, **6** +119.2°. Isomeric hydroxy acetates **4** and **5** could be easily identified by ^1H NMR.
10) Diol **3** was assigned the (**R**) absolute configuration in analogy to the compound obtained from thiophenol and the same precursor as in Scheme 3.

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