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IMMOBILIZED PORCINE LIVER ESTERASE: A CONVENIENT REAGENT FOR THE PREPARATION OF CHIRAL BUILDING BLOCKS [1].

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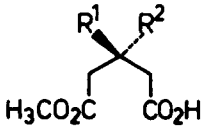
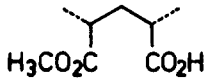
Summary : A simple method for the effective, covalent, immobilization of porcine liver esterase (PLE) is described, and the application of this reagent for the preparation of chiral building blocks on a 50 - 500 mmol scale is demonstrated .

The synthesis of enantiomerically pure, biologically active , compounds is one of the most important and rapidly expanding fields in organic chemistry. In view of present needs in this area both in academic and industrial research, the use of enzymes for the preparation of chiral building blocks is highly attractive and of immediate interest .

Hydrolytic enzymes are particularly easy to handle experimentally and an esterase from porcine liver (PLE , E.C. 3.1.1.1) has found extensive use in this regard for the enantioselective hydrolysis of prochiral and racemic esters , respectively . Many useful chiral building blocks like 1 (→(R)-/(S)- mevalonolactone)[2], 2 (→pimaricin fragments)[3],



1 ; R^1=OH, R^2=CH_3
2 ; R^1=OH, R^2= H
3 ; R^1=CH_3 , R^2=CH_2Ph
4 R^1=NHZ , R^2= H



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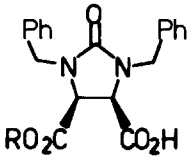

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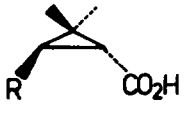
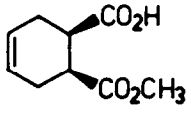


7 ; R = CH=CMe_2
8 ; R = $CH{=}CCl_2$
9 ; R = CO_2Me



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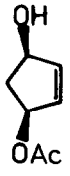


11

SCHEME

3 (→verrucarinic acid)[4] , 4 (→2-azetidinone [5] , negamycin [6]) , 5 [7] (→macrolides)[8] , 6 (→ (+)- biotin) [9] , 7 - 9 [10] (→pyrethroids)[11], 10 (→brefeldin A)[12] , 11 [13] (→ cyclopentanoid natural products)[14] have been prepared by this method (Scheme) .

To our best knowledge , all these published experiments with PLE have so far been carried out with the soluble enzyme . We found , however , that the application of PLE on a synthetic scale (50 - 500 mmol) is greatly facilitated by its immobilization for obvious economical and practical reasons :

a) the catalyst is reusable many times ;

b) it can be recovered and separated from the reaction mixture by simple filtration;

c) reactions can be terminated exactly after desired conversions and / or optical purities are achieved .

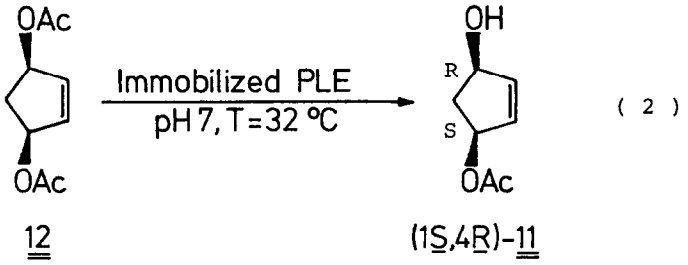
It is our feeling that an increased use of enzymes by organic chemists would be greatly stimulated by providing simple, experimental , procedures for the preparation and application of enzymatic " reagents " which can be handled and stored much like other laboratory chemicals . We report here a simple method for the covalent immobilization of PLE , which can be carried out , without any previous experience in the techniques of enzyme immobilization, with a commercially available support and in an effective working time of only a few minutes. The commercially available PLE is simply dialysed against phosphate buffer and then mixed with oxirane activated acrylic beads (Eupergit C , Röhm Pharma, D-6100 Darmstadt) to provide a covalent bond between the polymer matrix and the enzyme (eq. 1 , schematic).

(1)

The spacer unit between matrix and the protein largely minimizes interferences between the support and the enzyme . Indeed this method of immobilization only slightly reduces the activity of the enzyme .

The resulting reagent displays excellent specific activity (68% ! of the soluble en - zyme) and can be stored for several months in a fridge at 7 °C [15] . It was used during that period repeatedly many times for the preparation of 1 , 7 - 11 and others on a 50- 500 mmol scale and exclusively for all enzymatic resolutions , where the exact termination of reactions is particularly important .

The ease of preparation, its high activity and stability , makes this reagent especially attractive for large scale synthetic applications . As a representative example we describe here the preparation of 11 by enantioselective hydrolysis of 500 mmol of cis-1,4-diacetoxycyclopentene (12) in one day (14 h) (eq. 2) .



12 (1S,4R)-11 (2)

Further synthetic applications of this reagent , also in comparison with the use of the soluble enzyme in a membrane reactor , will be published soon.

EXPERIMENTAL

Immobilized PLE on Eupergit C : 60 mg (6 ml suspension in 3.2 M ($NH_4)_2SO_4$ - solution, 6000 units , standard n-BuOAc) of PLE (Boehringer) were transferred into a dialysis bag (Serva , 16 mm diameter , 30 ml final volume) with 10 ml of 1M phosphate buffer (pH 7.5) and left for 48h in 1000 ml of that buffer at 7 °C in a fridge without stirring . The contents of the dialysis bag was diluted with 20 ml buffer and simply mixed with 4 g of acrylic beads (Eupergit C , Röhm Pharma , D - 6100 Darmstadt , Germany) . After 24 h at R. T. the acrylic beads were filtered off (G 3) , washed once with 250 ml of buffer and stored in a buffer suspension (with 0.02 % NaN_3) at 7 °C. The specific activity of the preparation was determined with the standard (n-BuOAc) to be 68% of the soluble enzyme .

(-)-(1S,4R)-4-Hydroxy-2-cyclopentenylacetate (11) : 92 g (500 mmol) of 12 [13] were suspended in 0.1 M phosphate buffer (200 ml , pH 7 , T = 32 °C) and treated with 60 mg of immobilized PLE (4080 units = 6000 units of the soluble enzyme , see above) . The mixture was stirred mechanically (!) and the pH was kept constant during the hydrolysis by continous addition of 1 N NaOH - solution from an autoburette . After addition of 550 ml 1 N NaOH (14 h) the " reagent " was removed by filtration (G 3) and , after washing with buffer , stored for reuse . The remaining mixture was extracted continously with Et_2O (24h) to yield , after distillation , 61.8 g (87%) of crude 11 , b.p.$_{0.1}$ = 85 °C ; $[\alpha]_D^{20}$ = 49.7 ° (c 0.86 , $CHCl_3$) . Recrystallisation from Et_2O / PE (2 : 1) produced 42 g (68 % from crude 11) of optically pure ($\geqslant$98% e.e. by VPC of the diastereomeric " Mosher" esters) 11 , m.p. 49 - 50 °C , $[\alpha]_D^{20}$ - 66 ° (c 0.63 , $CHCl_3$) .

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15. The long term stability of the catalyst is presently studied in greater detail with a multitude of different substrates . It seems to be very high. The preparation survived e. g. a continous " endurance test " in a flow reactor (0. 5 M MeOAc in H_2O) for 200 h with only 30 % loss of activity .

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