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Hydrolases in organic synthesis: Preparation of enantiomerically pure compounds

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Abstract : Esterhydrolases (Esterases , Lipases) are highly (chemo-, regio- and enantio-) selective biocatalysts for the transformation of racemic and achiral substrates into enantiomerically pure compounds. Numerous examples for their application in the preparation of synthetically useful chiral auxiliaries and building blocks for flavour compounds, pheromones and several pharmaceuticals including ß-adrenergic blockers, antidepressants and ACE inhibitors are presented.

The close relationship between biological activity and absolute configuration both in natural products and synthetic materials (e.g. drugs , agrochemicals) has prompted considerable efforts regarding the synthesis of enantiomerically pure compounds. They are widely used as

- chiral building blocks for biologically active compounds,
- auxiliaries and stationary phases for analytical purposes or
- chiral ligands for the design of enantioselective catalysts

Next to other methods , e.g. classical resolutions *via* diastereoisomers, asymmetric and " chiral pool" syntheses enzymes - nature's chiral catalysts - have emerged as highly effective " reagents " for their preparation.
They display highly attractive properties for the solution of synthetic organic problems, some of which are summarized in Fig 1.

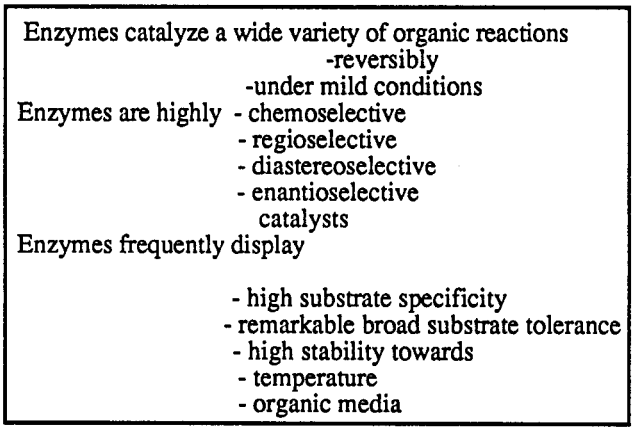


Fig. 1. Enzymes in Organic Synthesis-Attractive properties

By enzyme catalyzed differentiation of enantiomers, enantiotopic groups or faces in achiral substrates and enantiotopic groups in *meso* - substrates enantiomerically pure compounds can be prepared.Regioselective transformations of optically pure substrates , e.g. natural products allow the preparation of selectively functionalized enantiomers.

In view of their convenient accessibility, high stability, broad substrate tolerance and frequently high reaction selectivity esterhydrolases (esterases , lipases) are particularly well suited for synthetic applications. They catalyze both the hydrolysis and synthesis of esters by basically four different routes-

esterification,transesterification, reversible and irreversible acyltransfer,

all of which - in combination with the above mentioned stereoselectivities - can be used for the preparation of enantiomerically pure carboxylic acids , alcohols and esters. Using both approaches - enzymatic hydrolysis and esterification - in the following examples the use of these biocatalysts for the preparation of enantiomerically and/ or regioisomerically pure hydroxy compounds will be described.

Some years ago we discovered a commercially available lipase from *Pseudomonas sp.* (SAM - 2), which proved to be ideally suited for the highly selective resolution of numerous secondary alcohols- both by enzymatic hydrolysis and esterification (e.g. Fig 2) (ref. 1 ,2).

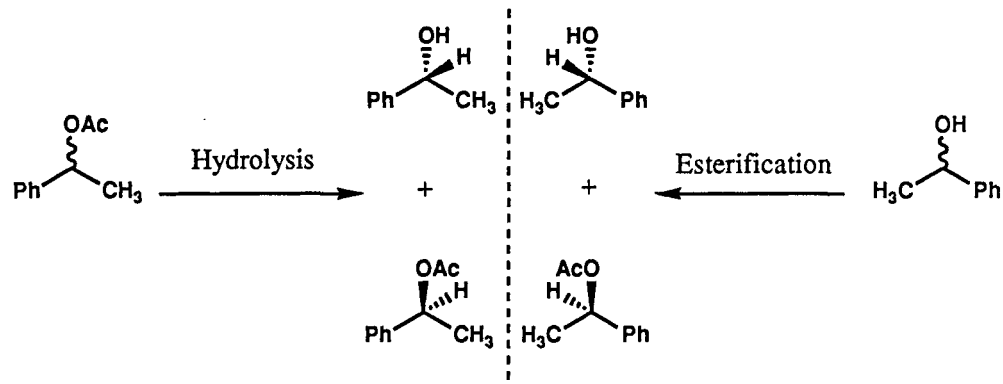


Fig. 2

A thorough study of its substrate dependence , the " chemical mapping " of its active site led to a qualitative model for this enzyme (Fig. 3) (ref. 3) soon allowing rather reliable predictions regarding

- substrate tolerance
- relative rate of transformation
- absolute configuration

resulting from potential target molecule.
Based on this knowledge numerous racemic and achiral substrates were transformed into a large number of enantiomerically pure alcohols , diols, cyanohydrins, chlorohydrins, hydroxycarboxylic acids, lactones, epoxides and many others (ref.4, 6 - 11).
The following , selected examples may serve to illustrate the synthetic usefulness of this particular enzymes underlining the synthetic usefulness of this class of biocatalysts.

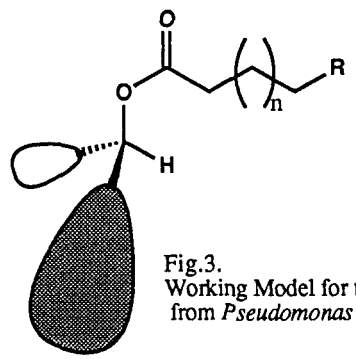


Fig.3.
Working Model for the Lipase
from *Pseudomonas sp* (SAM - 2)

Chiral auxiliaries and chiral ligands Using the lipase from Pseudomonas sp. (SAM - 2) and the methods of enantioselective enzymatic hydrolysis and irreversible acyltransfer numerous enantiomerically pure secondary alcohols were prepared, among them (*R*)- and (*S*)- 2-phenylethanol, (*R*)- and (*S*)-2-pyridylethanols as auxiliaries for diastereomeric resolutions, (*R,R*)- and (*S,S*)- 2-phenylcyclohexanols as substitutes for 8-Phenylmenthol and (*R,R*)- and (*S,S*) - 1,2-cyclopentanediols as ligands for chiral catalysts (Fig. 4).

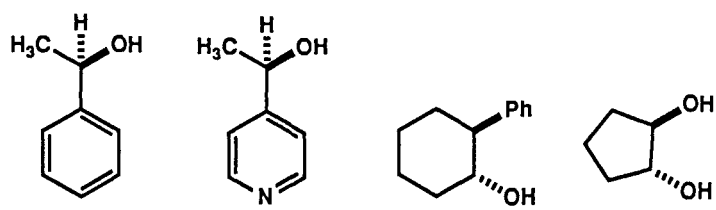


Fig. 4

β-Adrenergic blockers Based on earlier work by Watanabe et al. (ref. 5) numerous chlorohydrins, building blocks for a wide variety of ß-adrenergic blockers (e.g. Propranolol, Penbuterol etc) were prepared using enzyme mediated routes as outlined in Fig. 5 (ref. 6) .

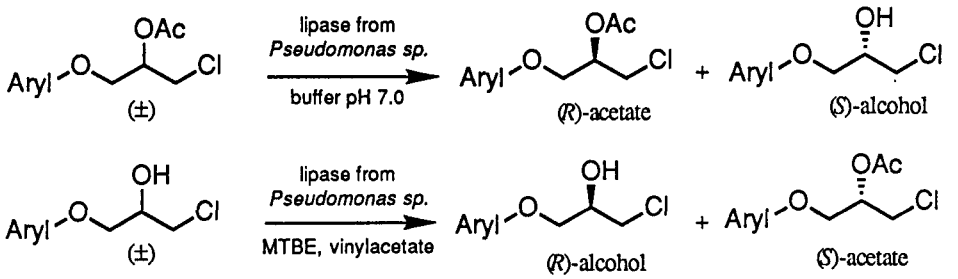


Fig. 5

Antidepressants A number of enantiomerically pure antidepressants, among them Tomoxetine , Nisoxetine and the highly selective serotonin re-uptake inhibitor (S) -Fluoxetine , proved to be highly attractive targets for synthetic organic chemists in recent years (Fig.6).Enantioselective epoxidations, reductions, hydroborations and others were described in the literature for the preparation of suitable chiral building blocks for these materials.

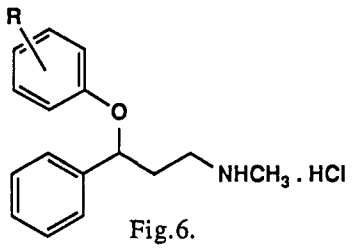


Fig.6.

R = 2- CH_3 Tomoxetine
R = 4- CF_3 Fluoxetine
R = 2- OCH_3 Nisoxetine

On the basis of our previous experience in the enzymatic preparation of enantiomerically pure benzylalcohols we recently developed a practically useful and highly efficient method for the lipase catalyzed resolution of the required (*R*) - and (*S*) - 3-chloro-1-phenyl-1-propanols (Fig 7) (ref. 7) .

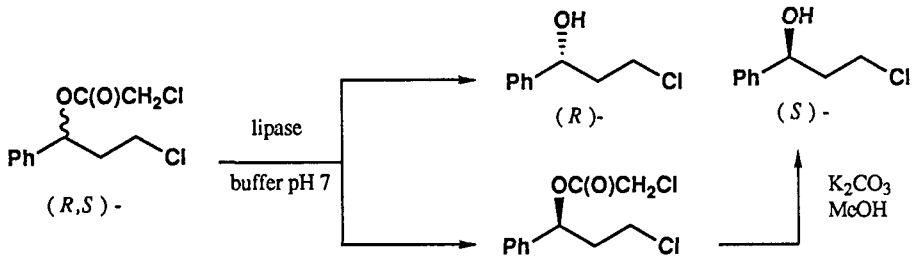


Fig.7 (*S*) -

By a combination of direct substitution and " Mitsonubu " inversion both molecules can be converted into either enantiomer of these antidepressants in an enantioconvergent manner (Fig. 8).

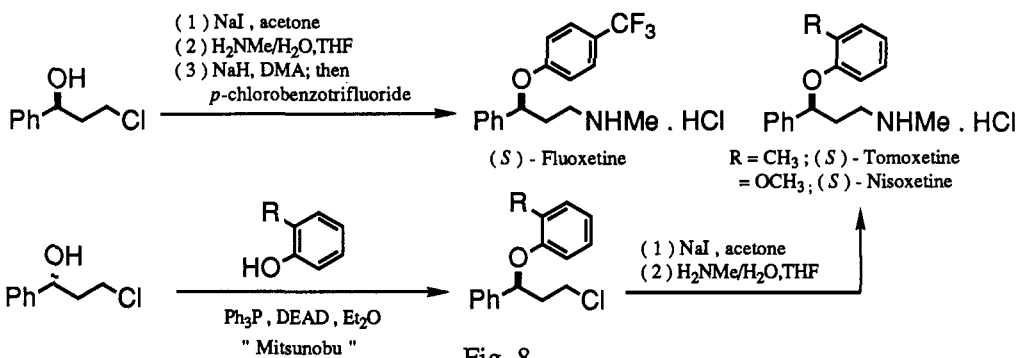


Fig. 8

The method also allows access to α-hydroxycarboxylic acids *via* esterhydrolase catalyzed resolution of the corresponding cyanohydrin acetates (Fig. 9). Acid catalyzed hydrolysis of 1-acetoxy-3-phenylpropionitrile

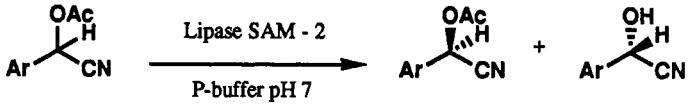


Fig. 9

proceeds without racemisation and leads to (*R*)-1-hydroxy-3-phenylpropionic acid, avaluable building block for numerous ACE inhibitors, e.g. Enalapril (Fig.10) (ref. 8) .

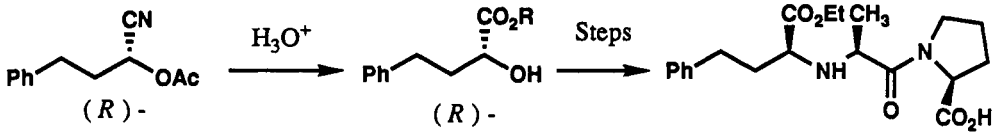


Fig. 10

This use of the lipase from *Pseudomonas sp.* (SAM-2) can further be extended also towards the preparation of several other hydroxycarboxylic acids.Based on the model described above, we were able to resolve a variety of these compounds using the corresponding t-butylesters in order to provide the steric bulk required to accomodate said model.As obvious from Fig. 11 , the *t*-butylesters of e.g. lactic acid derivatives closely resemble the structure of the acylated benzylalcohols described above in their preferred absolute configuration.

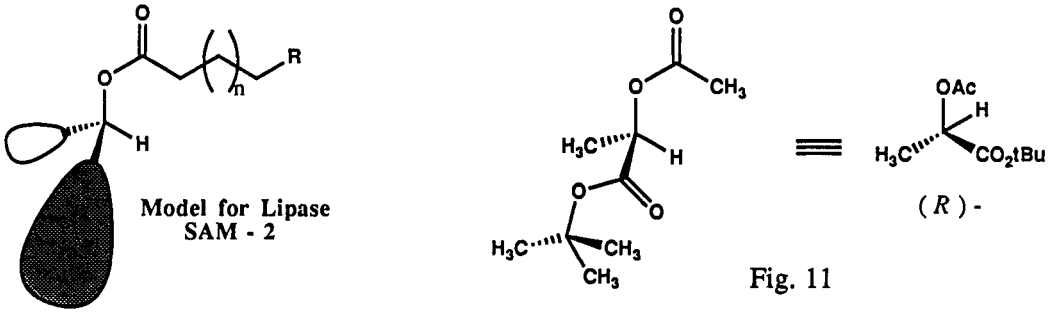
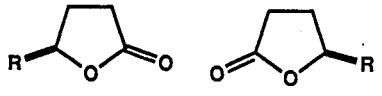


Fig. 11

In view of the high enantiomeric purities obtained eveen in the enzymatic resolution of the corresponding g-hydroxy carboxylic acids, we were able to provide access to a whole series of γ-lactones having high optical purities and which are displaying numerous biological activities as flavour constitutents, pheromones and deterrants (Fig 12) (ref. 9) .



(*S*) - (*R*) -

R = Me, Et, n-Pr, n-Bu , n-Pentyl,n-Hexyl

Fig. 12 .Biologically Active Lactones: Flavour Constitutents,Pheromones,Deterrants

None of the methods described above allow, however , the enzyme mediated preparation of simple enantiomerically pure alkanols or alkanediols with a carbon skeleton consisting of 3 - 4 C-atoms.By reflecting on the structural requirements for suitable substrates it is obvious that in small molecules the differences in steric bulk are just too little in order to allow a highly selective differentiation by the enzyme.We were able to overcome this problem recently by using what we call the " Trojan horse " approach (Fig.13) (ref. 10) .

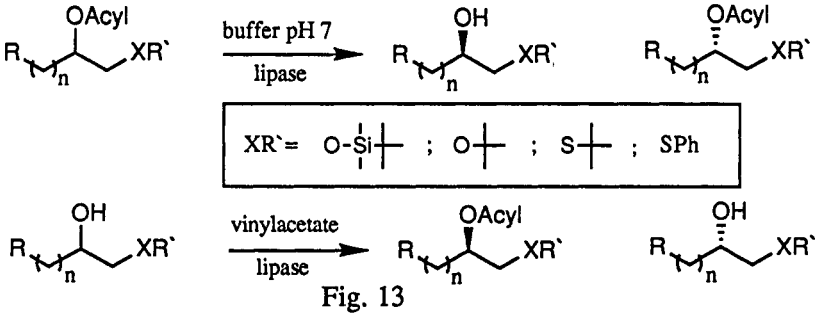


Fig. 13

By introducing sterically demanding groups into these small molecules, which are both bulky and functional, the enzyme is able to differentiate with very high selectivity between the two enantiomers allowing an efficient resolution of the antipodes. If these sterically demanding groups are chosen in a way which allows their convenient removal or further transformation the method can be employed for the synthesis of numerous highly useful molecules , e.g. building blocks for liquid crystals, pheromones and enantiomerically pure epoxides for many applications.(Fig. 14)

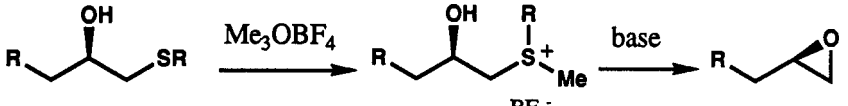


Fig. 14

While all methods aimed at the resolution of enantiomers with few exceptions lead to the desired enantiomer in less than 50 % yield, the differentiation of enantiotopic groups allows the conversion of achiral molecules into one desired enantiomer, at least in theory.
Enantiomerically pure compounds with glycerol substructures are among the most widely used chiral auxiliaries and / or building blocks in organic synthesis. They are usually prepared from the " chiral pool " using e.g. mannitol or l-serin as starting materials.If, however , the differentiation of the enantiotopic hydroxyl groups in glycerol could be achieved with high selectivity, achiral glycerol would be by far the cheapest and most convenient starting material for these molecules. Unfortunately, most experiments along these lines were unsuccessful .
We found, however , some time ago that. such a differentiation can indeed be achieved if a sterically demanding protection group is introduced (ref 11) . As shown in Fig.15 enzymatic hydrolysis and esterification can lead to both enantiomeric series of chiral building blocks in high optical and chemical yields. With the attach-ment of suitable functionalities versatile enantiomers with glycerol substructures are thus obtained.

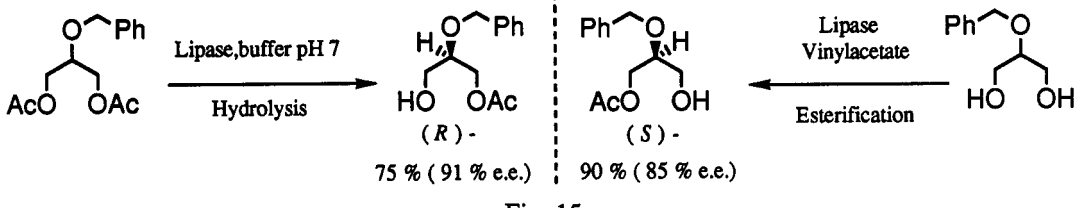
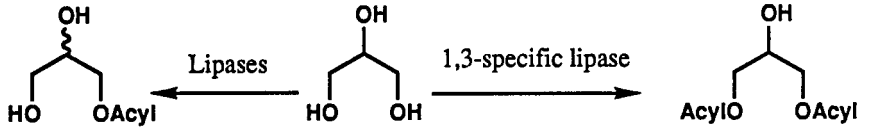


Fig. 15

The esterification of glycerol can , however , be controlled in a regioselective fashion. We recently discovered, that , under suitable conditions and at will , regioisomerically pure 1,3- diglycerides (ref. 12) or 1(3)-monoglycerides (ref. 13) can be obtained by direct esterification of glycerol in purely organic reaction media.(Fig.16).
The obtained molecules are extremely valuable biosurfactants with numerous applications in the food, cosmetic and pharmaceutical area.



1(3)-Monoglyceride 1,3-Diglyceride

Fig.16

The use of esterhydrolases is , however, not limited to the preparation of enantiomerically pure compounds from racemic and achiral substrtes. They can also be used very efficiently and with great preparative advantage for the regioselective functionalisation of enantiomerically pure natural products
Due to the improved bioavailability Isosorbide- 5 - nitrate is used with considerable advantage over Isosorbide-2,5-dinitrate for the treatment of angina pectoris. The preparation of this molecule requires the selective functionalisation of isosorbide prior to the introduction of the nitro group.We found that this can be achieved with high efficiency and complementary stereochemistry using suitable conditions and the highly selective lipase from *Pseudomonas sp.* (SAM - 2) (Fig 17) (ref. 14) .

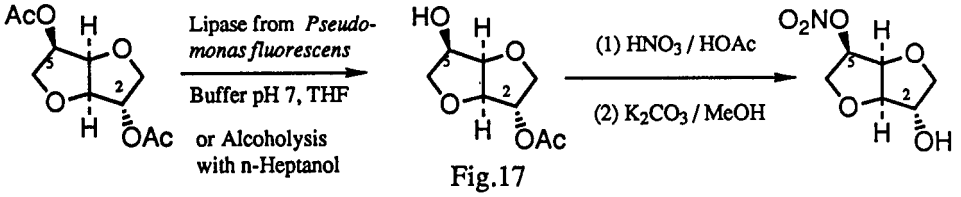


Fig.17

While the esterification of isosorbide exclusively leads to the 5-acyl derivative, by hydrolysis and alcoholysis of the 2,5-diacetate the 2-acyl derivative is obtained.Simple nitration of these molecules , followed by removal of the acyl groups leads quantitatively and without the requirement for any purification step to the desired enantiomerically pure pharmaceuticals.

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