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Enzyme assisted routes to bioactive molecules†

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Using the ability of lipases to distinguish between enantiomers and enantiotopic groups the syntheses of several classes of enantiomericaly pure compounds such as γ- and δ-lactones, deoxysugars including nucleosides, carba analogues of glycerides including carba analogues of phospholipids and *myo*-inositol phosphates are described.

Enzymes have emerged in recent years as highly efficient catalysts in organic synthesis. This is particularly true for ester hydrolases (esterases, lipases) many of which ideally combine the required high reaction selectivities with the synthetically so important broad substrate tolerance. Due to their ability to differentiate between (a) enantiomers, (b) enantiotopic groups attached to prochiral centers and (c) enantiotopic groups in *meso*-compounds they are highly suited for the preparation of enantiomerically pure carboxylic acids and alcohols of widely different structures. In the sections below—and based on the three different modes of substrate recognition—examples for the preparation of several classes of enantiomerically pure compounds ((98% ee) are described.

Lipase catalysed reactions

Based on the generally accepted mechanism of lipase catalyzed reactions four types of transformations can be identified covering the synthetically most useful applications of these enzymes (Fig. 1a-c): (i) ester hydrolysis and ester synthesis by direct esterification; (ii) ester synthesis *via* reversible acyl transfer and (iii) ester synthesis *via* irreversible acyl transfer.

From Fig. 1. it becomes also clear that these transformations can, in principle, be employed for the synthesis of both carboxylic acids and alcohols. In aqueous media, with a large excess of water present, ester hydrolysis clearly is the dominating reaction and enzymatic hydrolyses are next to the corresponding esterifications *via* irreversible acyltransfer, the most widely used synthetic transformations catalysed by lipases. Esterifications, regardless of the method employed, can only be achieved successfully under low water conditions. In contrast to direct esterifications, acyl transfer reactions do not involve any water and are thus preferable especially in cases where the intermediate acyl-enzyme can be prepared irreversibly. Vinyl and isopropenyl esters of various carboxylic acids have been most widely employed for this purpose next to anhydrides, oxime esters and vinyl carbonates. A summary of acyldonors employed for esterifications can be found in Fig. 2[1].

In the sections to follow enzymatic hydrolyses and esterifications using vinylesters as acyldonors are used exclusively and applied to the preparation of enantiomerically pure hydroxy compounds.

Lipases—modes of substrate recognition

As already mentioned in the introduction lipases display essentially three different modes of substrate recognition:(i) enantiomer differentiation, (ii) enantiotopos differentiation in achiral precursers carrying a prochiral center, and (iii) enantiotopos differentiation in achiral *meso*-compounds.

The fundamental differences as applied to the preparation of enantiomerically pure hydroxy-compounds are summarized schematically in Fig. 3 using the hydrolytic mode of transformation.

Resolutions

Starting with racemic substrates, lipase catalyzed kinetic resolutions can lead to enantiomerically pure diastereomers which can be separated by classical techniques. The quality of

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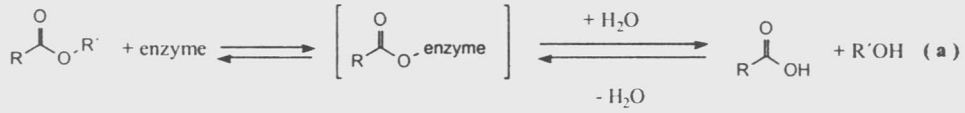
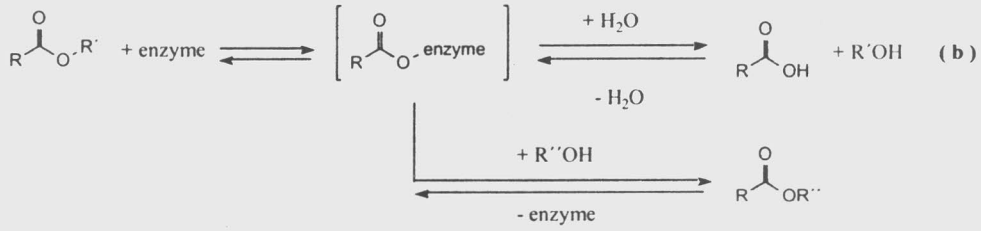
Esterhydrolases

Catalytic Transformations

Hydrolysis and Esterification



Esterification *via* Reversible Acyltransfer



Esterification *via* Irreversible Acyltransfer

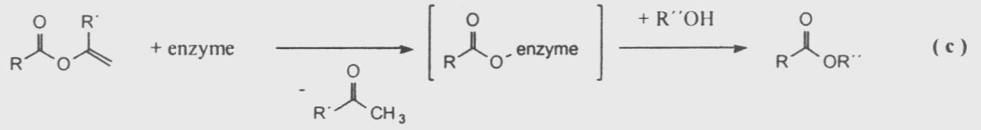


Figure 1—Lipase cartaalyzed reactions

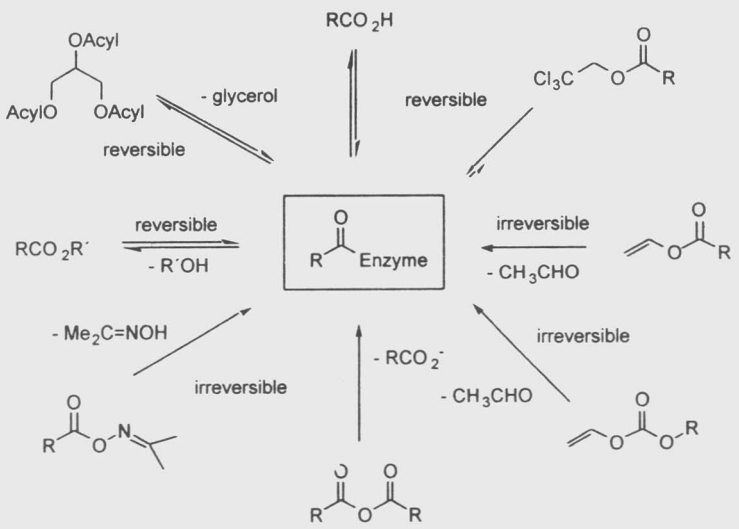


Figure 2—Acylodonors for lipase cartalyzed esterification

such resolutions depends both on the enantioselectivity of the lipase E and the achieved conversion with E values of >> 100 being required if both enantiomers are to be obtained in close to enantiomerically pure form.

Enantiotopos differentiation

In contrast to resolutions of racemates where inherent to the starting material only 50% of one particular enantiomer can be obtained, the differentiation of enantiotopic groups in achiral substrates can lead, at least in theory, to products with 100% optical and chemical yield. In the examples to follow we have chosen lipase catalyzed transformations which in nearly all cases achieved the desired high selectivities and thus produced throughout molecules in enantiomerically pure (≥98 % ee) form.

Resolutions

Enantiomerically pure, alkylsubstituted γ- and δ-lactones (Fig. 4) are ubiquitous in nature and display a wide variety of biological activities e.g. as aroma compounds or pheromones.

In spite of the importance of many of these molecules as aroma constituents there seems to exist no systematic study in which the relationship between organoleptic properties and their absolute configurations has been studied in detail. The reason for this situation probably resides in the fact that enantiomerically pure γ- and δ-lactones of both absolute configurations are not easily accessible in multigram quantities. Interestingly enough, although natural products, many of these flavor lactones are isolated from natural sources frequently not optically pure but as mixtures of enantiomers with one enantiomer usually dominating.

In view of a systematic study of organoleptic properties it was our goal to synthesize both enantiomeric series of these molecules in good Chemical yields and high (≥99% ee) enantiomeric purities.

Retrosynthetic analysis

Here exemplified for saturated and unsaturated 6-alkyl substituted δ-lactones, these molecules can be correlated retrosynthetically next to the

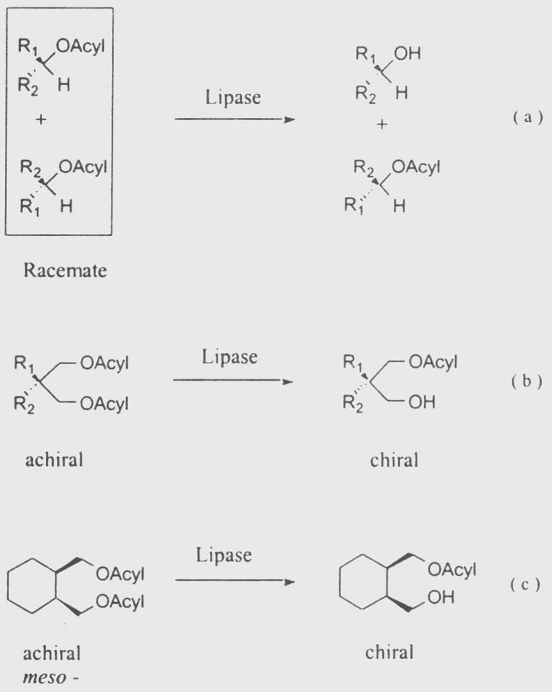


Figure 3—Lipases-modes of substrate recognition (schematic)

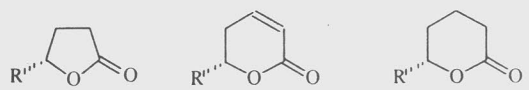


Figure 4—Enantiomerically pure, alkylsubstituted γ- and δ-lactones (only one absolute configuration is shown)

corresponding δ-hydroxycarboxylic acids to enantiomerically pure alkyloxiranes (Fig. 5). It can easily be envisaged that regioselective, nucleophilic ring opening of these chiral building blocks with C_3 or C_2-carbon units would lead to both series of our target molecules i. e. γ- and δ-lactones.

Enantiomerically pure alkyloxiranes

Based on our earlier work, resulting in the development of a working model for a lipase from *Pseudomonas species* (SAM II)[2] we were able to predict and then demonstrate that the lipase catalyzed resolutions of *t*-butyl-β-hydroxythio-ethers can be achieved with very high (≥98% ee) enantioselectivities (Fig. 6).

For this, racemic alkyloxiranes, conveniently accessible commercially or by simple epoxidation of the corresponding 1, alkenes, were converted into the respective β-hydroxythioethers *via*

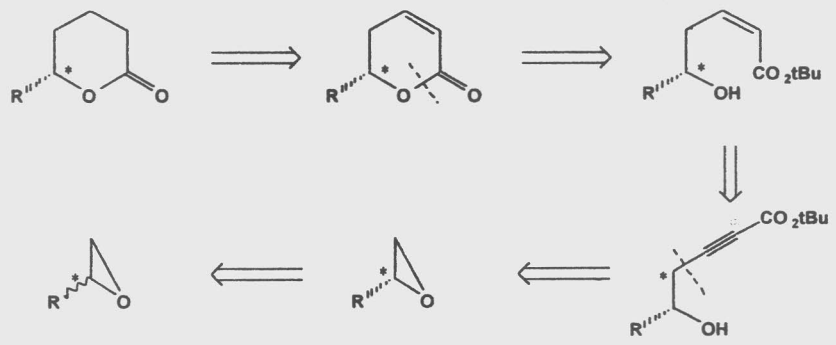


Figure 5—Retrosynthetic analysis of unsaturated and saturated δ-lactones

regioselective ring opening using ^{t}BuSH/NaH. Treatment of the thus obtained products with chloroacetic anhydride/pyridine in presence of *N*,*N*-dimethyl-4-aminopyridine (DMAP) led to the corresponding racemic chloroacetates which were then hydrolyzed in presence of the lipase from *Pseudomonas species* (SAM II) under *p*H-stat conditions. These kinetic resolutions proceeded with probably the highest enantioselectivities (E_{calc} >> 1000) ever recorded in our laboratory. All reactions came to a standstill after 50% conversion, the products were enantiomerically pure to the limits of detectability (GC on Cyclodex I/P). Due to the substantial differences in boiling points the resulting products (*R*)-alcohols and (*S*)-esters could be separated by simple distillation. Both series of products can be converted into the corresponding oxiranes by *S*-alkylation using Meerwein salt followed by treatment with base. The very high enantiomeric purities of the resulting (*R*)- and (*S*)-alkyloxiranes were confirmed by chromatography using a recently developed method (BGIT)[3].

δ-Lactones

The thus obtained building blocks were converted into the corresponding series of unsaturated and saturated δ-lactones following the procedure outlined in Fig. 7[4,5].

Regioselective, borontrifluoride assisted ring opening of the oxirane moiety with the carbanion derived from *t*-butylpropiolate led to the corresponding (*R*)- and (*S*)-*t*-butyl-5-hydroxy-2-alkinates. Partial hydrogenation in presence of Lindlar catalyst proceeded quantitatively leading to the *Z*-configurated (*R*)- and (*S*)-*t*-butyl-hydroxy-2-alkenates which were finally cyclisized to the desired unsaturated δ-lactones in presence of *p*-TsOH. Their hydrogenation in presence of Pd/C cleanly produced the saturated series of our target molecules. As confirmed by GC analysis on a chiral support (Lipodex E), the whole reaction sequence can be carried out without any measurable loss of enantiomeric purity. As a result, both enantiomeric series of these molecules are now available for studies aimed at determining the relationship between their sensoric properties and absolute configurations.

γ-Lactones

Alkylsubstituted γ-lactones display a wide variety of biological activities, i.e. as key aroma constituents in numerous fruits, as insect pheromones or as deterrents. It was tempting, therefore, to use the above building blocks—enantiomerically pure alkyloxiranes also for the synthesis of this series of natural products (Fig. 8)[5]. Based on earlier work along these lines by Mori[6] we decided to use diethylmalonate for the introduction of the required two carbon unit. Regioselective, boron trifluoride assisted ring opening of the oxirane function with the carbanion derived from diethylmalonate led to the corresponding malonates —synthetic equivalents for the required (*R*)- and (*S*)-4-hydroxycarboxylic esters—precursers for our target molecules. The conversion of these malonates *via* ester hydrolysis, decarboxylation and ring closure can be achieved in one step using an optimized method originally developed by Krapcho[7]. Heating in DMSO, NaCl to 150°C for *ca* 6h leads directly to the desired target molecules. The enantiomeric purities of the

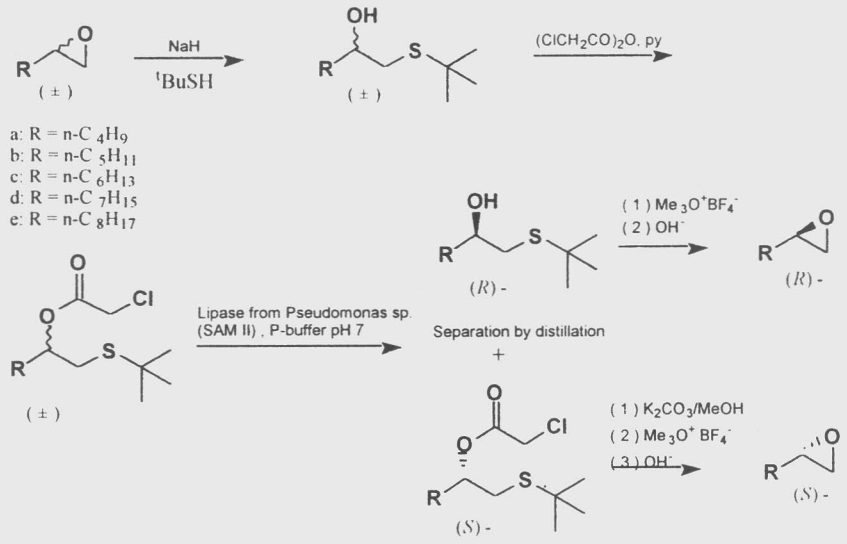


Figure 6—Enzyme assisted route to enantiomerically pure (*R*)- and (*S*)-alkyloxiranes

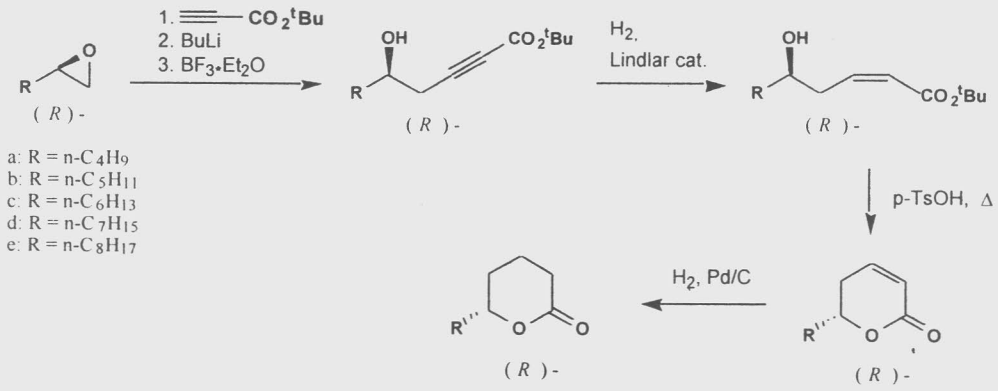


Figure 7—Synthesis of enantiomerically pure δ-lactones

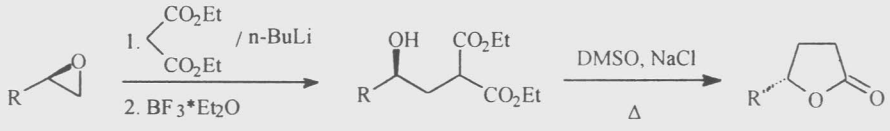


Figure 8—Synthesis of γ-lactones

thus obtained series of γ-lactones are high as long as the reaction temperature is carefully controlled[5].

Deoxisugars and nucleosides

Reflecting on the close structural relationship of the above described γ- and δ-lactones with pyranoses and furanoses (including nucleosides) it was tempting to extend the above described method also towards the synthesis of these classes of molecules. For this and in order to introduce the essential primary hydroxy groups selectively protected glycidol derivatives had to be prepared in optically pure form (Fig. 9).

Among the numerous protection groups tested (such as MOM, OMe, OBn, OAllyl), the allyl derivative proved to be the best choice. The β-hydroxythioethers derived thereof could be resolved with high enantioselectivity and

satisfactory reaction rates[5] (Fig. 10). Thus following the above described enzyme assisted route racemic allylglycidol was converted into the corresponding enantiomers (Fig. 10).

In complete analogy to the above described methodology (compare method for synthesis of δ-lactones) these building blocks were then transformed into the corresponding deoxisugar lactones[5] (Fig. 11).

Thus, using again *t*-butylpropiolate for the regioselective opening of the oxirane moiety the corresponding (*R*)- and (*S*)-*t*-butyl-5-hydroxy-2-alkinates were produced. The only critical point was the selective (Lindlar-catalyzed) hydrogenation of the triple bond in presence of the allyl protection group. This problem was solved successfully by adding an excess of 1-hexene, which served to protect the olefinic double bonds during this step.

The thus obtained allyl protected sugarlactone can due to its many functionalities, serve again as building block for numerous further selective transformations, here exemplified by (a) reduction, (b) epoxidation[8], (c) Michael addition[5] (Fig. 12).

It should be noted that the stereochemistry of these reactions is controlled by the substituent at C-5 leading to pure diastereomers in all cases. The synthetic potential of these molecules is presently further exploited in our laboratory.

Again in analogy to the examples described above (under synthesis of γ-lactones) enantiomerically pure (*R*)- and (*S*)-allylglycidol can be converted also into the correponding deoxysugar—γ-lactones with furanoid substructure (Fig. 13).

Regioselective ring opening of the oxirane function in (*R*)-allylglycidol with the anion of diethylmalonate led to the corresponding hydroxymalonate which was converted into the saturated, allyl protected sugar lactone using the previously described method of Krapcho[7] (Fig. 13).

Using classical chemistry[9] a double bond can be introduced into the furane ring thus allowing the synthesis of further derivatives, e.g. *via* Michael additions.

Clearly these furanoid systems are closely

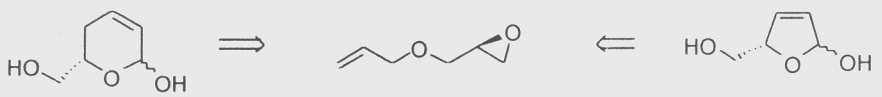


Figure 9—Retrosynthetic analysis of deoxypyranoses and -furanoses

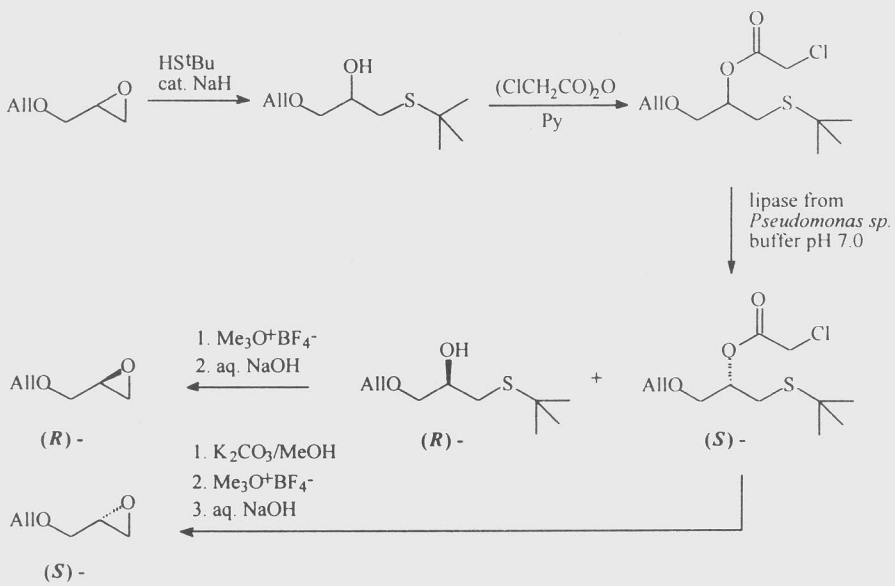


Figure 10—Enzyme assisted route to enantiomerically pure (*R*)- and (*S*)-allylglycidol

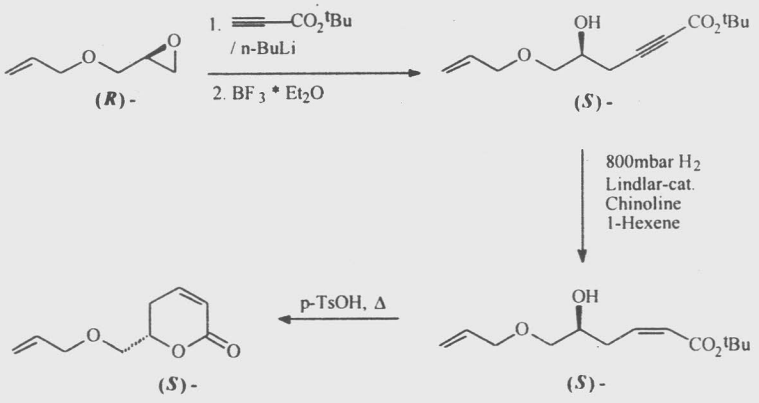


Figure 11—Enzyme assisted route to deoxisugar-δ-lactones

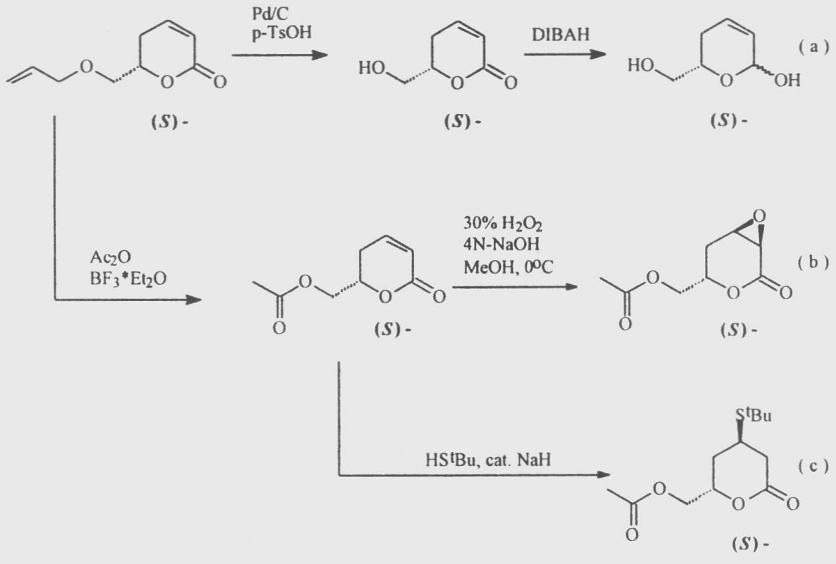


Figure 12—Deoxisugar-δ-lactones-selective transformations

related to deoxiriboses and thus well suited for the synthesis of various nucleosides[5]. For the simplest case this is illustrated in Fig. 14.

Reduction of the lactone group using DIBAH, followed by acylation leads to the corresponding diacetate of D-dideoxyribose as mixture of anomers. Using known chemistry these can be converted into the corresponding mixtures of nucleosides by reaction with the TMS derivative of thymine. Further experiments along these lines are presently carried out in our laboratory.

Enantiotopos differentiation — achiral 1,3-diols

The enantiotopos differentiation of the primary hydroxy groups in achiral diols carrying a prochiral center can lead—provided the reactions terminate after the (selective) conversion of only one functional grou—to enantiomerically pure or enriched monoesters of opposite absolute configurations resulting from the corresponding hydrolyses and esterifications (Fig. 15).

Carba analogues of glycerides

Based on our interest in intracellular signalling processes[10] we became increasingly engaged in the synthesis of molecules involved in the phosphatidyl inositol pathway such as enantiomerically pure structural analogues of 1,2(2,3)-*sn*-diglycerides and *myo*-inositol phosphates (see below), both types of molecules

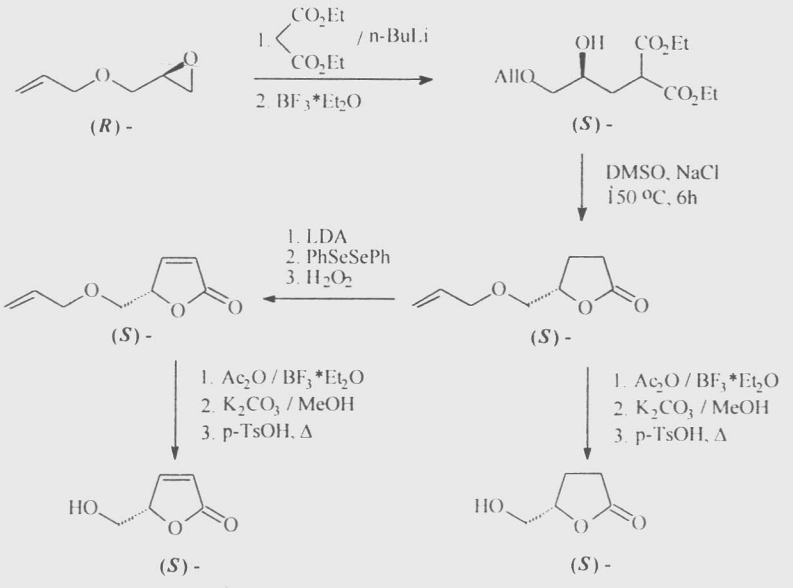


Figure 13—Enzyme assisted route to deoxisugar-γ-lactones

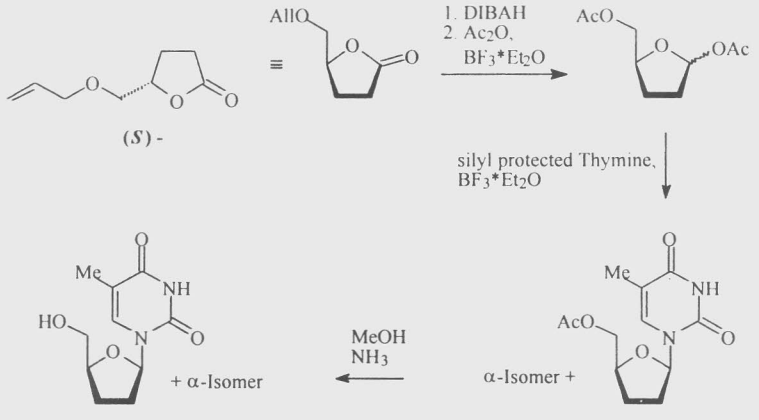


Figure 14—Deoxisugar lactones as building blocks for nucleosides

constituting important classes of second messengers.

Optically pure 1,2-*sn*- and 2,3-*sn*-diglycerides are notoriously unstable due to rapid acyl group migrations especially under protic conditions and at elevated temperatures causing immediate loss of optical purity (Fig. 16).

We therefore decided to explore enzyme assisted syntheses of carba-analogous triglycerides in which the sp^3-oxygens of the acyl moieties are replaced by sp^3-carbons in a systematic way[11a-c] (Fig. 17).

These structural analogues should, with the exception of hydrolytic cleavage, behave identical towards biological systems. In order to test this hypothesis first suitable synthetic routes to the corresponding C-analogues of triglycerides were developed — one of them is shown in Fig.18[11c].

Conversion of the chosen acid chloride with CH_2N_2 and HBr leads *via* the corresponding diazoketones to the desired α-bromoketone. Nucleophilic substitution with the anion of diethylmalonate leads to the required carbon backbone of the target molecule. In order to avoid intramolecular lactole formation of the ensuing 1,3-diol the carbonyl group was masked as *exo*-methylene function using a Wittig reaction before reducing the diester to the diol with $LiAlH_4$.

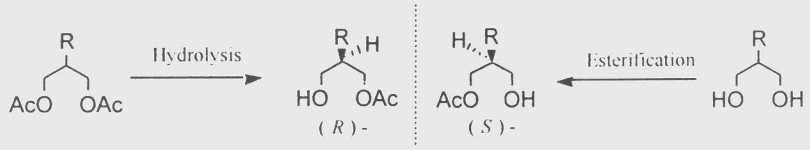


Figure 15—Enantiotopos differentiations in achiral 1,3-diols-stereochemical outcome

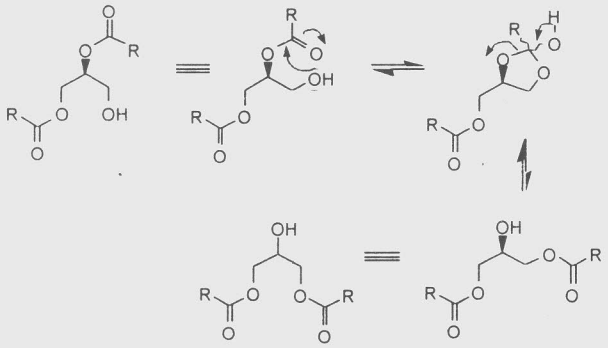


Figure 16—Acyl group migrations in 1,2(2,3)-*sn*-diglycerides

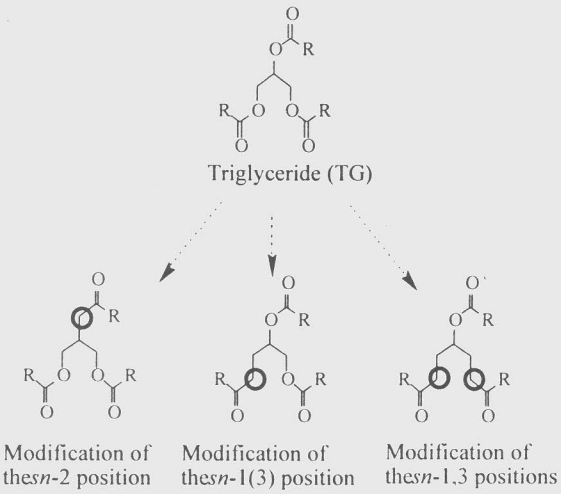


Figure 17—Carba analogues of triglycerides as mimics of natural lipids

Acylation of the diol and regeneration of the carbonyl function either by $RuCl_3/NaIO_4$ or O_3 leads to the desired target molecules.

Binding studies with lipases and lipase catalyzed hydrolyses as well as esterifications clearly demonstrated that native triglycerides and their C-analogues behave identically towards these biological systems[11a,b]. In order to provide C-analogues of 1,2(2,3)-*sn*-diglycerides in optically pure form the corresponding *exo*-methylene derivatives were hydrolyzed (or esterified) enantioselectively under the conditions of irreversible acyltransfer in the presence of a lipase from *Pseudomonas species* (Fig. 19)[11c].

The thus prepared C-analogues of 1,2(2,3)-*sn*-diglycerides, obtained in very high optical purities are not only interesting as potential second messengers, but can also be considered as highly useful synthetic building blocks for a new class of phospholipids including PAF-analogues and other molecules with this general backbone[11c] (Fig. 20).

Myo-inositol phosphates

Inositol phospholipids and their molecular constituents such as D-*myo*-inositol phosphates and 1,2-*sn*-diglycerides play an important role as second messengers in living cells with numerous functions as regulators and signal transducers[10,12]. Unfortunately, however, frequently *myo*-inositol phosphates are only accessible in minute amounts from scarce natural sources after laborious isolation and purification procedures.

Clearly, the elucidation of their biological role would be greatly facilitated if these molecules could be made available *via* facile synthetic routes. In this sense we embarked on the exploration of new synthetic routes toward these molecules with the aim of using enzymes for the introduction of chirality into the respective molecular backbone. *Myo*-inositol itself being derived from ubiquitous and abundantly available phytic acid is by far the most conveniently accessible and economical starting material.

It should be appreciated that *myo*-inositol is achiral while many *myo*-inositol phosphates are chiral and only biologically active in enantiomerically pure form. Consequently all known synthetic approaches are focussed towards the problem of converting this achiral molecule into enantiomerically pure building blocks. Thus the target was clear and well defined. For the

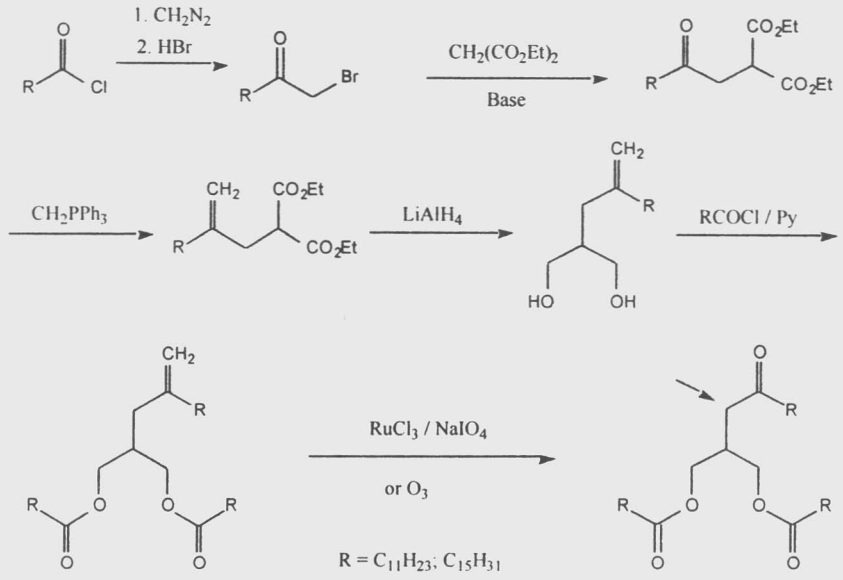


Figure 18—Synthesis of carba-analogous triglycerides

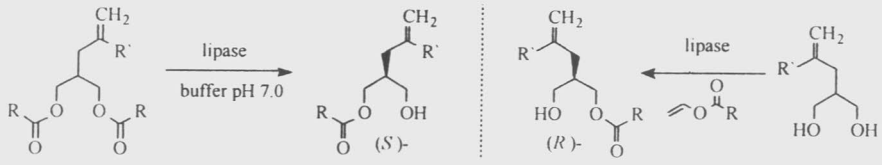


Figure 19—Enzymatic esterification and hydrolysis of carba-analogous triglycerides

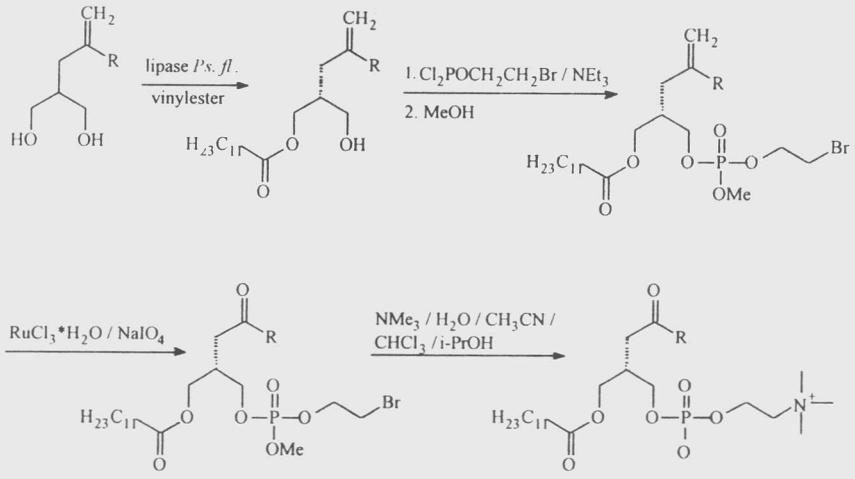


Figure 20—Carba analogues of phospholipids

synthesis of enantiomerically pure *myo*-inositol polyphosphates, starting from *myo*-inositol itself, a central building block was needed which was felt could be prepared by enantioselective lipase catalyzed esterification of a suitably protected 4,6-derivatised *myo*-inositol (Fig. 21).

The indicated derivatisation was required in order to: (a) obtain a *meso*-derivative with a

reduced number of hydroxy groups, (b) increase the solubility of the substrate in organic solvents.

After screening numerous esterhydrolases (lipases) for their ability of selectively converting substrates of this kind we found a lipoprotein lipase from the portfolio of Boehringer Mannheim† which was able to convert this substrate in one step into a single enantiomer (Fig. 22).

This reaction, in which only *one* out of *four* different hydroxy groups is selectively esterified, demonstrates once more the power of enzymatic methods as applied to organic synthesis[13a-c]. The absolute configuration of this building block was determined unambiguously by chemical correlation[14].

After this key step—the introduction of chirality—the obtained enantiomerically pure building blocks (Ar = Bz, Bn) can be converted further into numerous selectively protected *myo*-inositol derivatives which can be further

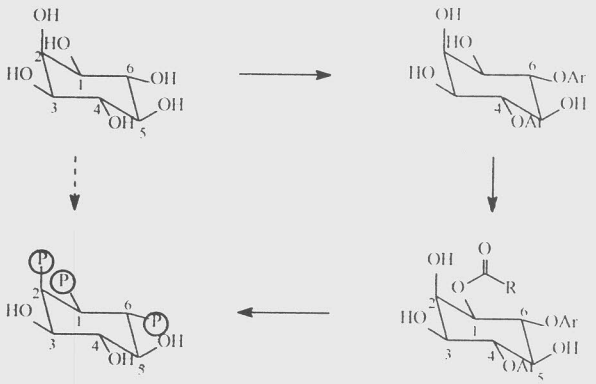


Figure 21—From *myo*-inositol to enantiomericaally pur epolyphosphates

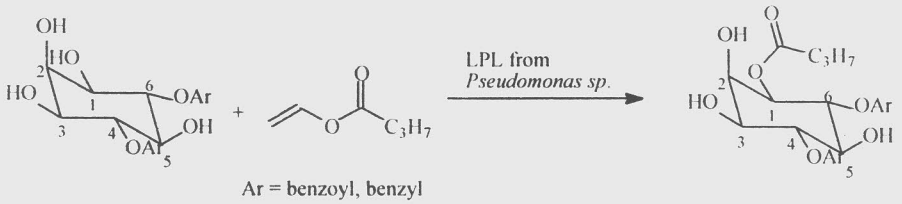


Figure 22—Differentiation of enantiotopic hydroxy groups in *myo*-inositol derivatives

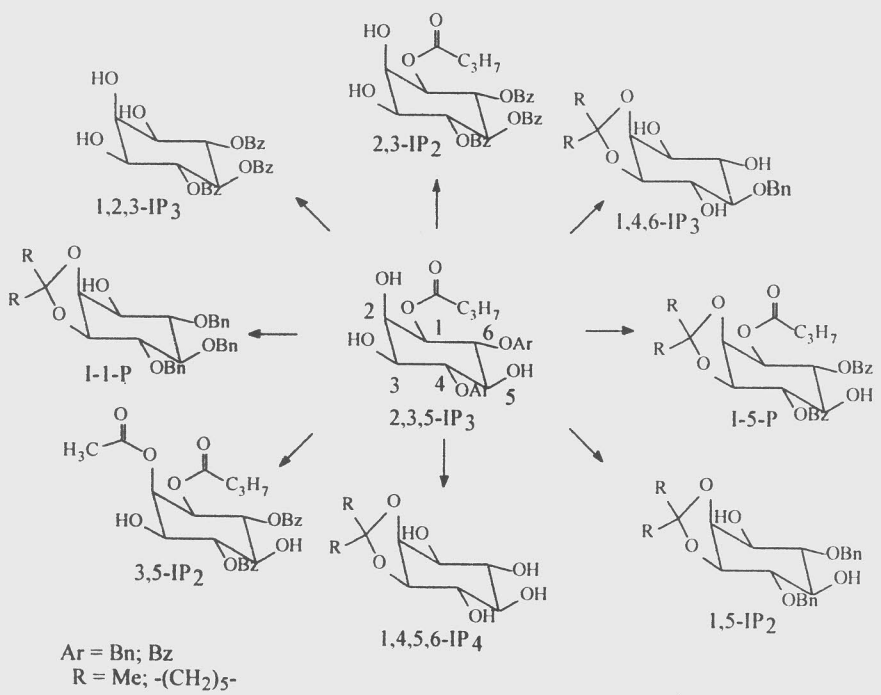


Figure 23—Selectively protected *myo*-inositol derivatives-building blocks for inositol phosphates

†Lipoprotein lipase from *Pseudomonas* species, Boehringer Mannheim GmbH, Penzberg, Germany

phophorylated leading to the corresponding *myo*-inositol phosphates (Fig. 23).

Using the preparation of D-*myo*-inositol-1-phosphate (I-1-P)[15] and D-*myo*-inositol-1,2,6-trisphosphate (1,2,6-IP_3)[15,16], a novel experimental drug, as examples two typical synthetic sequences are shown in Fig. 24 and Fig. 25.

Transketalisation with acetone dimethylketal followed by benzylation of the hydroxy group at C-5 under acidic conditions and saponification of the esterfunction at C-1 leads to a suitable building block for I-1-P. Phosphorylation of the free hydroxy group using *N,N*-dimethyl dibenzyl phosphoamidate in presence of tetrazole led to the trivalent phosphorous derivate. This in turn is oxidized to the required pentavalent phosphorylate using MCPBA or *t*-BuOOH. Removal of the benzyl groups by catalytic hydrogenation, addition of NaOH, followed by ion exchange chromatography leads to the chemically and optically pure *myo*-inositol-1-phosphate[15].

Another complete synthetic sequence, exemplified here by the synthesis of D-*myo*-inositol-1,2,6-trisphosphate (1,2,6-IP_3; PP56; a-Trinositol), a novel experimental drug, is outlined in Fig. 25[15,16].

Using the well known orthoester method our central building block is converted selectively into the corresponding monoacetate in which only the

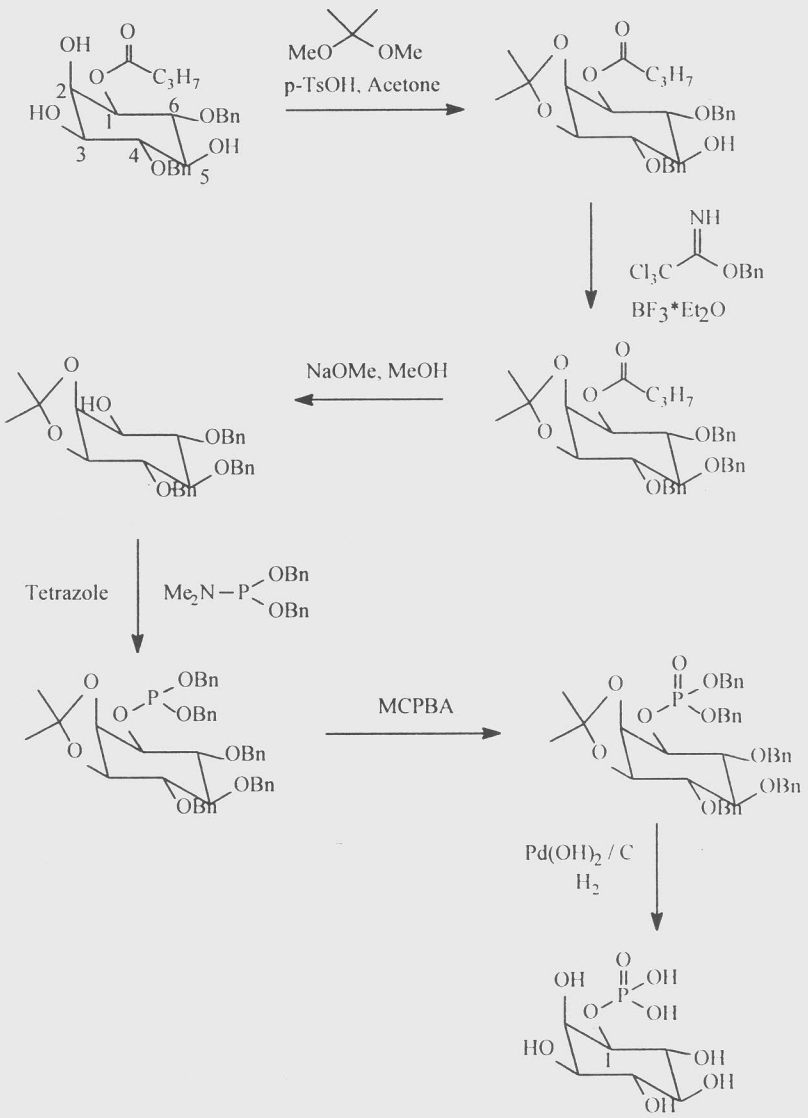


Figure 24—Synthesis of D-*myo*-inositol-1-phosphate (1-1-P)

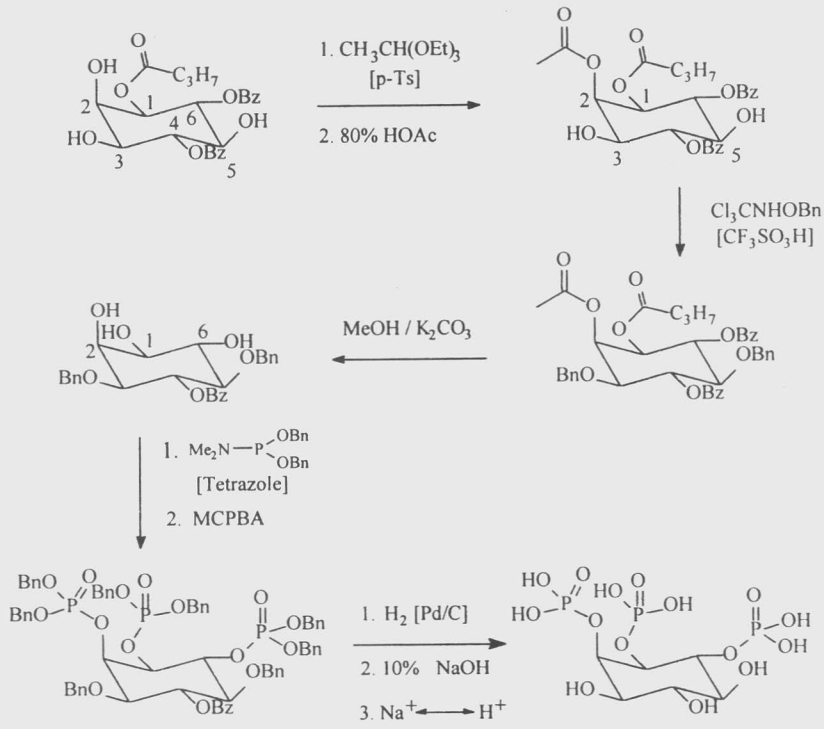


Figure 25—Enzyme assisted synthesis of D-*myo*-inositol-1,2,6-trisphosphate (1,2,6-IP_3)

axial hydroxy group in the 2-position becomes acylated. Benzylation of the equatorial hydroxy groups at C_3 and C_5 under acidic conditions leads to the fully protected *myo*-inositol derivative. We were extremely pleased to find that the following removal of the ester functions was highly regioselective indeed, resulting in the rapid formation of the free hydroxy groups in the desired positions 1,2 and 6. While it is easily understandable that in the base catalysed methanolysis the acetate and butyrate functions are removed rapidly and faster than the more stable benzoate groups, it was somewhat surprising to find that in the progress of the reaction only one of the benzoate groups, exclusively the one in position 6 is removed selectively. The obtained triol can be phosphorylated as described above. Deprotection of the resulting trisphosphate ester with H_2/Pd-C followed by saponification (NaOH, *p*H 11 -12) leads to 1,2,6-P_3 in nearly quantitative yield. All materials are obtained with very high isomeric purity as confirmed by ion exchange chromatography[13b].

Summary

Using the three major modes of substrate recognition as a guideline the use of esterhydrolases (lipases) for the preparation of a wide variety of structurally different, enantiomerically pure compounds is described. Once more it becomes clear that lipases are versatile and highly useful synthetic tools in the hands of the organic chemist.

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