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Chemoenzymatic synthesis of a novel LTD_4 antagonist

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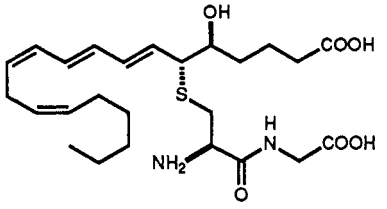
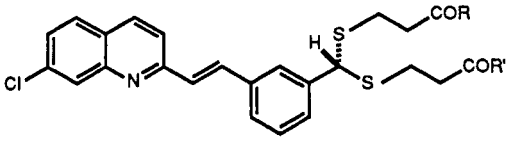
Abstract : An efficient four-step synthesis of the novel receptor antagonist (MK-0679) of leukotriene D_4 (LTD_4) will be described. The key steps are enzymatic hydrolysis of prochiral diester to the ester-acid in 98% enantiomeric excess followed by aluminum mediated amidation of the methyl ester which affords MK-0679 in high overall yield.

1. INTRODUCTION

Aryl and alkyl dithioacetals of mercaptopropionic acid derivatives are potent receptor antagonists of leukotriene D_4 (**1**) and are being developed as therapeutic agents for bronchial diseases[1]. One of the most promising clinical candidates is MK-679 (**2a**).



Leukotriene D_4 (1)



2a(*R*) : R=NMe_2, R'=OH (MK-0679)

2b(*S*) : R=OH, R'=NMe_2

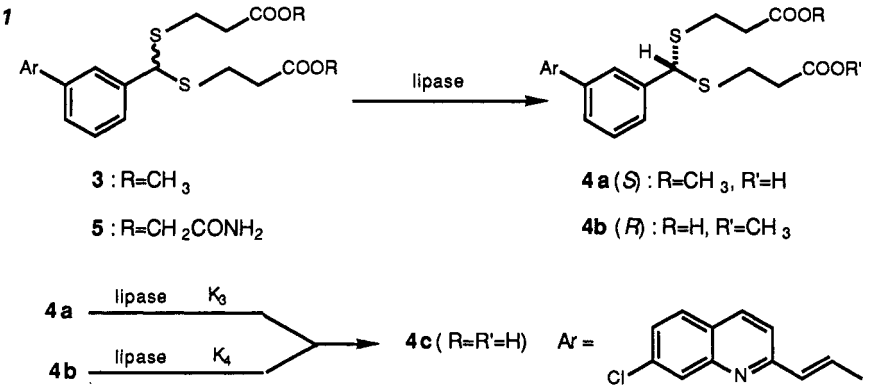
Use of hydrolytic enzymes to resolve racemic carboxylic esters and amides, or to stereospecifically hydrolyze prochiral or meso diesters, has become a powerful tool in organic synthesis[2]. However, in most cases, the compounds undergoing the enzymatic reaction have the prochiral or chiral center only one or two bonds away from the reacting carbonyl group, and often the ester groups are held in rigid, cyclic frameworks. Only a few examples have been reported in which the chiral/prochiral centers are three or more bonds from the reacting carbonyl center. It was found that increasing the length between the prochiral center and the ester group from two bonds to three bonds resulted in a lowering of the enantiomeric excess from 90% to 10% in the resulting half ester product[3]. Better success was achieved by Whitesides in the porcin pancreatic lipase hydrolysis of chiral epoxy esters[4]. Similarly, Klivanov used pig liver esterase to esterify alcohols having three bonds between the alcohol and the chiral center with ee's generally above 90%[5]. From the few examples reported of asymmetric hydrolysis of ester groups remote from the chiral/prochiral center, it is not clear whether enzymes are not suitable for these transformations or there are just few cases where they have been tried. Due to the similarity of the two thioalkyl side chains of **2**, attempts to resolve racemic compounds by classical methods, such as crystallization of diastereomeric salts or chromatographic separation of diastereomeric derivatives, proved difficult. Recently, the elegant chiral synthesis of both enantiomers has been reported which involve preparation of diastereomers early in the synthesis and separation of the diastereomers either by chromatography or by fractional crystallization, followed by conversion of each diastereomer into the appropriate enatiomer[6]. However, scale up of such a process would be difficult. In principle, a more straightforward route to the enantiomers is through resolution of amide-ester or selective hydrolysis of the prochiral diester (**3**). However, in

(3) the prochiral center is four bonds away from the reacting carbonyl group, plus the ester groups are on highly flexible chains which may not be optimal for selective fitting into a rigid enzyme active site. Despite these potential drawbacks to the enzymatic route, the rewards in terms of the synthetic simplicity, yield and cost prompted us to explore the enzymatic route to this drug candidate[7].

2. ENZYMATIC HYDROLYSIS OF PROCHIRAL DIESTER

Two enzymes, lipase from *Pseudomonas* species (Amano, Sigma) and from *Chromobacterium viscosum* (Sigma), were found to selectively hydrolyze the dimethyl ester (3), providing ester-acid (4a) in >98 % ee. The lipase from *Pseudomonas* species was also effective in hydrolyzing the (aminocarbonylmethyl) diester (5 R=CH_2CONH_2) with > 98% ee. The lipase from *Candida cylindracea* was sluggish toward the dimethyl

Scheme 1



Enzymatic Hydrolysis of Diester

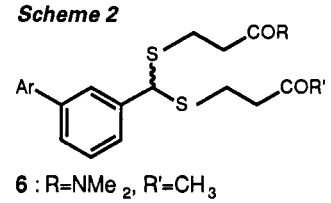
ester, but was effective in hydrolyzing the activated esters. However, the selectivity was poor and overhydrolysis to the diacid (4c) was a major side reaction. The diester (5) gave the best results with lipase from *C. cylindracea*, giving a 90% chemical yield and an 86% ee. The ester-acid product obtained in this case could be upgraded to 95% ee by two recrystallizations from i-PrOH. It is not clear why the activated ester give higher enantioselectivity than the methyl ester with *C. cylindracea*. One explanation is that the hydrogen-bonded-donating or-accepting ability of the activated ester moiety (a carbonyl) provides an additional handle for chiral binding. In several control experiments we found that no hydrolysis of diester (3) or ester-acid (4b) occurred in the absence of enzyme. The enantioselectivity was measured by preparation of the diastereomeric (R)- or (S)-1-(1-naphthyl)ethyl amides, which were analyzed by reversed-phase HPLC on a C8 column. When derivatized with (R)-(+)-1-(1-naphthyl)ethylamine, the R,R diastereomer elutes first, followed by the S,R diastereomer. The order of elution is reversed when the derivatization is done with the (S)-(-)-1-(1-naphthyl)ethylamine. As other control experiments, several synthetic mixtures of differing enantiomeric ratios were derivatized and analyzed to ensure that no chiral discrimination was taking place during the derivatization process. The diastereomeric mixture could also be distinguished by ^1H NMR with the dithioacetal methine singlet resonances separated by about 0.05 ppm. Important for these lipase-catalyzed reactions is the use of the nonionic surfactant Triton X-100. In the absence of the surfactant, the hydrolysis proceeded very slowly, presumably at the liquid-solid interface. The surfactant solubilizes the diester to a small extent and thereby increases the rate of hydrolysis. No hydrolysis occurred in the presence of cationic or anionic surfactants. Addition of organic solvents such as DMF, EtOH or 2-propanol decreased the rate of hydrolysis. The minor enantiomer formed in the first hydrolysis is a better substrate for the enzyme than the major enantiomer, so a kinetic resolution occurs (k4>k3 in Scheme 1). As confirmation of this effect, racemic ester-acid, when subjected to hydrolysis using *Pseudomonas* lipase, predominantly hydrolyzed the R enantiomer in a slow reaction, giving enrichment of the S enantiomer of the ester-acid.

3. ENZYMATIC RESOLUTION OF RACEMIC ESTER-AMIDE

The other approach to the preparation of the enantiomers was enzymatic resolution of racemic ester-amide (**6**).

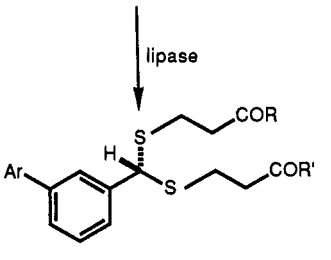
Of many commercially available enzymes tried, none gave good results with the methyl ester. The best result was a disappointing 20% ee with *C. cylindracea* (Sigma). On the basis of the literature precedent with ibuprofen enzymatic resolution[8], several activated esters were prepared and subjected to hydrolysis with *C. cylindracea*. Most of the activated esters gave results superior to those given by the methyl ester, with the CH_2CONEt_2 ester (**7**) providing an 85/15 enantiomeric ratio. In all cases, the product was enriched in the *R* enantiomer (**8**), which has the same absolute configuration as that obtained from hydrolysis of the prochiral diester (**3**). This indicates that both compounds are fitted into the active site in the same way. Because the acid-amide (**8**) crystallizes as a racemic compound instead of as a racemic mixture, the 85/15 ratio could not be significantly upgraded by crystallization. In light of the success with the prochiral diester route, further efforts to improve the resolution route were not made.

Scheme 2



6 : R=NMe_2, R'=CH_3

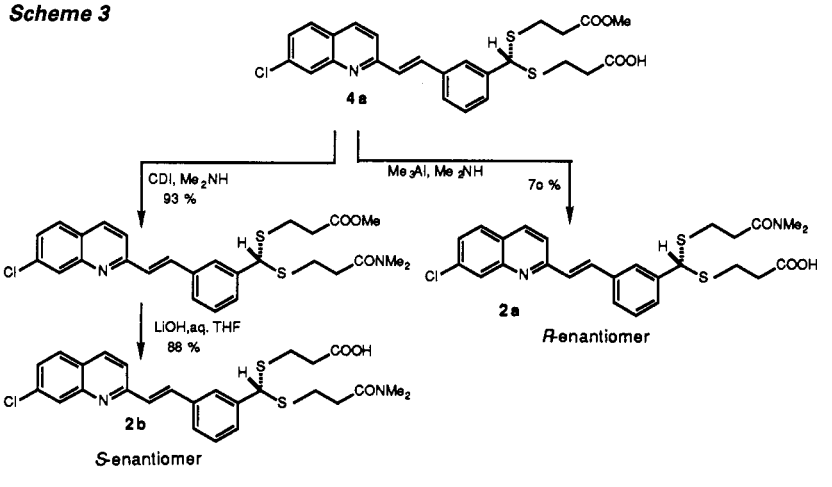
7 : R=NMe_2, R'=CH_2CONEt_2



8 : R=NMe_2, R'=OH

Enzymatic Hydrolysis of Ester-Amide

Scheme 3



Conversion of Ester-Acid to MK-0679

4. CONVERSION OF CHIRAL ESTER-ACID TO MK-679

One potential drawback to the enzymatic hydrolysis of a prochiral diester is that only one enantiomer is produced. Generally, if both enantiomers are desired, then another enzyme must be found that will selectively produce the opposite enantiomer. However, in this case, the chiral nonracemic acid-ester was readily converted to each enantiomer (**2a**) and (**2b**) by straightforward chemical modification of each arm of the compound. To make the R enantiomer (**2a**), ester-acid (**4a**) was treated with 2.5 equiv. of Me_3Al/Me_2NH_2Cl(Weinreb reagent) in 70 % yield after crystallization from isopropyl alcohol. Alternatively, reaction with a 10% solution of Me_2NH in toluene at 100°C for 6 hr provided **2a** in 50% yield after chromatography. The *S* enantiomer **2b** was prepared in overall 80% yield from ester-acid **4a** by activating the acid with carbonyldiimidazole and displacement with Me_2NH to give amide-ester, followed by hydrolysis with LiOH in aq. THF. No loss in chirality was observed on taking ester-acid **4a** to final products **2a** and **2b**. The absolute configurations are based on the work of Gauthier and co-workers in which an X-ray crystal structure of a synthetic diastereomeric intermediate was determined [6].

5. SUMMARY

Lipases are capable of selectively hydrolyzing prochiral diesters. With lipase from *Pseudomonas* sp., chemical yields and ee's were better with the substrate having four bonds between the prochiral center and the ester carbonyl than with the three-bond or five bond analogues, demonstrating that selectivity does not necessarily diminish as the distance between the chiral center and the reaction site increase. These results, taken along with those in the literature, indicate that the lipase from *Pseudomonas* sp. can hydrolyze a diverse range of ester substrates, both large and small. On the basis of this information, a possible model for the enzyme active site is one in which the active site contains many different binding pockets. In order to selectively hydrolyze the large prochiral diester (3), a large, chiral hydrophobic pocket must be available for binding. However, several other smaller, chiral hydrophobic pockets must also be present near the active site that can be used to selectively bind substrates of smaller size. Alternatively, the lipase may have more than one active site which may be used depending on the size and nature of the substrate. Another possibility is that the enzyme has enough conformational flexibility to accommodate different size structure in the same active site and binding pocket. In this work we have shown that lipases are capable of selectively hydrolyzing esters having remote chiral/prochiral centers and therefore are a very powerful tool for organic synthesis. Chiral syntheses which by conventional chemical methods would be exceedingly difficult are performed readily and inexpensively with commercially available enzymes.

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