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Enzymes and practical asymmetric synthesis

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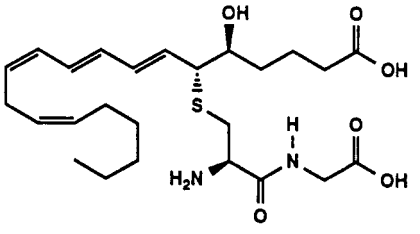
Abstract: A practical, chemoenzymatic, four-step synthesis of the LTD_4 antagonist MK-0679 is described. The key steps are enzymatic hydrolysis of the prochiral diester to the ester-acid in 99% enantiomeric excess followed by aluminum mediated amidation of the methyl ester to afford MK-0679 in high overall yield. This synthesis is superior to the synthesis of the racemate MK-0571.

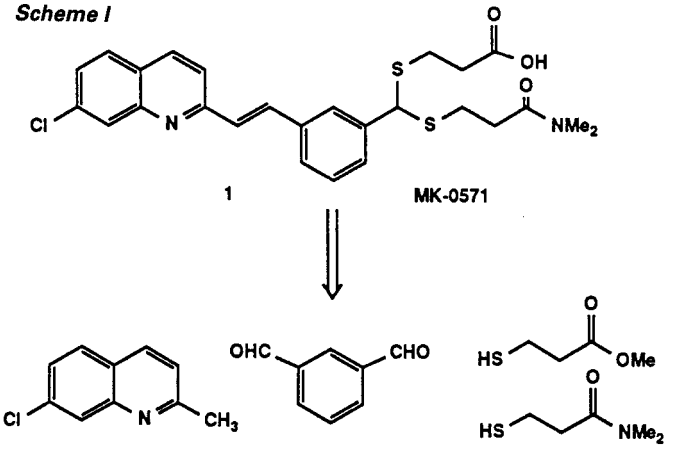
INTRODUCTION

Leukotrienes, products derived from arachidonic acid, are potentially active on smooth muscles and are responsible for respiratory and inflammatory diseases such as asthma and arthritis.[1] A novel approach for the treatment of asthma is through the use of an LTD_4 (Leukotriene D_4) antagonist.[2] MK-0571 (**1**) is a potent, orally active, specific LTD_4 antagonist.[3] This paper discusses the synthesis[4] of the racemate MK-0571 (**1**) as well as the chemoenzymatic synthesis[5] of the *R* enantiomer **2** (MK-0679) and the *S* enantiomer **3** (L-668,018).



LEUKOTRIENE D_4

Scheme I

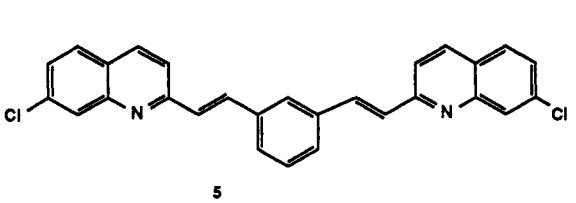


SYNTHESIS OF MK-0571

A retrosynthetic analysis of **1** is outlined in Scheme I. All the starting materials are readily available. There are two selectivity challenges in this approach, the first being the selective functionalization of 1,3-benzenedicarboxaldehyde and the second challenge being the construction of the unsymmetrical dithioacetal.

Synthesis of aldehyde 4

Aldehyde **4** is prepared by the condensation of 7-chloroquinaldine with 1.5 equivalents of 1,3-benzenecarboxaldehyde in the presence of 3.0 equivalents of acetic anhydride in xylene at reflux.[6] A



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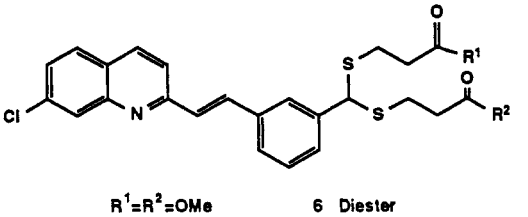
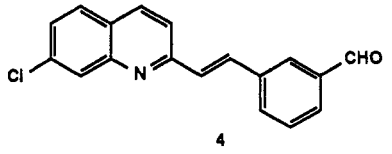


$R^1=R^2=OMe$ 6 Diester
$R^1=OMe, R^2=NMe_2$ 7 Ester-Amide
$R^1=R^2=NMe_2$ 8 Diamide



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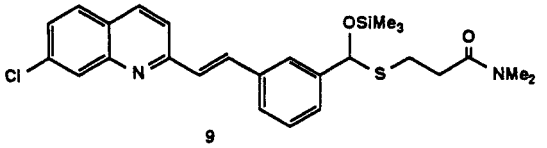


9

major byproduct, bis-adduct **5**, is produced to an extent of 20%. The crude product is digested in hot ethyl acetate and filtered to remove the insoluble bis-adduct **5**. The aldehyde **4** is crystallized from the filtrate in ≥98% purity and 65% yield.

Synthesis of unsymmetrical dithioacetal 7

Statistical Approach: Aldehyde **4** is treated with 1 equivalent of methyl 3-mercaptopropionate, 1 equivalent of N,N-dimethyl-3-mercaptopropionamide, and 3 equivalents of boron trifluoride etherate in anhydrous acetonitrile at 0 °C to obtain a 1 : 2 : 1 mixture of diester **6** : ester-amide **7** : diamide **8**. Pure unsymmetrical ester-amide **7** is obtained in 49% yield after silica gel chromatography and crystallization.

Selective Approach: In the above statistical approach, the maximum yield that can be obtained is only 50%. Hence, we pursued a more desirable selective method to prepare the unsymmetrical ester-amide **7**. Based on model studies, we envisaged the *O*-trimethylsilyl hemithioacetal **9** to be a suitable intermediate for this approach. Attempts to use Evans'[7] and Chan's[8] conditions to prepare **9** were unsuccessful due to competitive conjugate addition of the thiol to the unsaturated quinoline. However, a modification of Glass's procedure[9] was found to be suitable. Thus, treatment of aldehyde **4** with 1.06 equivalents of N,N-dimethyl-3-mercaptopropionamide and 2.0 equivalent of 1,1,1,3,3,3-hexamethyldisilazane in the presence of 0.1 equivalent of imidazole in anhydrous methylene chloride with a nitrogen sweep (to remove ammonia) afforded **9** with ≤ 5% unreacted aldehyde **4**.

Selective conversion of **9** to the desired ester-amide **7** is achieved using 1.1 equivalents of methyl-3-mercaptopropionate and 3.5 equivalents of boron trifluoride etherate in ethyl acetate at - 50 °C. The best conditions for this conversion were determined based on low temperature, *in situ* ^{1}H NMR studies. Under these conditions, a 1 : 12 : 3 mixture of diester **6** : ester-amide **7** : diamide **8** is obtained. The key ratio of ester-amide **7** : diester **6** is further improved to >200 : 1 by crystallizations. The presence of diamide **8** is not a serious concern as it gets removed as a neutral impurity after the hydrolysis step (see below). Thus we achieved a selective synthesis of the unsymmetrical dithioacetal **7** in 60% isolated yield.

Hydrolysis of ester-amide 7

Hydrolysis of **7** is carried out in tetrahydrofuran at 0 ± 2 °C using 1.05 equivalents of aqueous lithium hydroxide. After the removal of the diamide **8** impurity by extraction with ethyl acetate and acidification of the aqueous product solution, MK-0571 (**1**) is crystallized in 88% yield and ≥ 98% purity.

Thus, via the selective route, the synthesis of racemic LTD_4 antagonist MK-0571 (**1**) was achieved in four steps and in 34% overall yield.

CHEMOENZYMATIC SYNTHESIS OF THE ENANTIOMERS OF MK-0571

Having achieved an efficient synthesis of the racemate MK-0571 (**1**), we turned our attention to the preparation of its enantiomers **2** and **3**. Attempts to resolve **1** by crystallization or chromatographic separation of diastereomeric derivatives were not successful. Chemical synthesis involving the use of a chiral auxiliary and chromatographic separation of a 1 : 1 mixture of diastereomers early in the synthesis followed by conversion of each diastereomer into the appropriate enantiomer has been reported.[10] However, such a route would be impractical on a large scale. Hence, we explored[5] an enzymatic route to synthesize **2** and **3**.

Although the use of enzymes to effect difficult transformations has been recognized for many years, only recently have chemists begun to effectively harness the power of enzymes.[11] In most published examples, the reaction site is only one or two bonds away from the chiral or prochiral center. Only few successful cases with good ee's have been reported when the reaction center is greater than 2 bonds away.[12] Although the reaction center is four bonds away in the present case, we pursued our studies in an effort to identify a practical synthesis for **2** and **3**.

We examined two enzymatic approaches; (1) resolution by selective hydrolysis of esters of MK-0571 and (2) hydrolysis of prochiral diesters such as **6**.

Enzymatic resolution of racemic esters of MK-0571 (1)

With substantial quantities of MK-0571 (**1**) in hand by the route outlined above, we were prompted to study this resolution approach. Eight esters (methyl, allyl, CH_2COOEt, CH_2CONH_2, CH_2CONEt_2, CH_2COPh, CH_2CN, and CH_2CH_2OMe) of **1** were prepared and screened with several enzymes.[5] Lipase from *Candida cylindracea* (Sigma) gave some success with a few racemic esters of **1**. The best result was obtained with the CH_2CONEt_2 ester, which gave a 70% ee (enriched in the *R* isomer) with a 30% chemical yield. As this approach did not seem to lead to a practical synthesis of **2** and **3**, we concentrated our efforts on the hydrolysis of prochiral diesters.

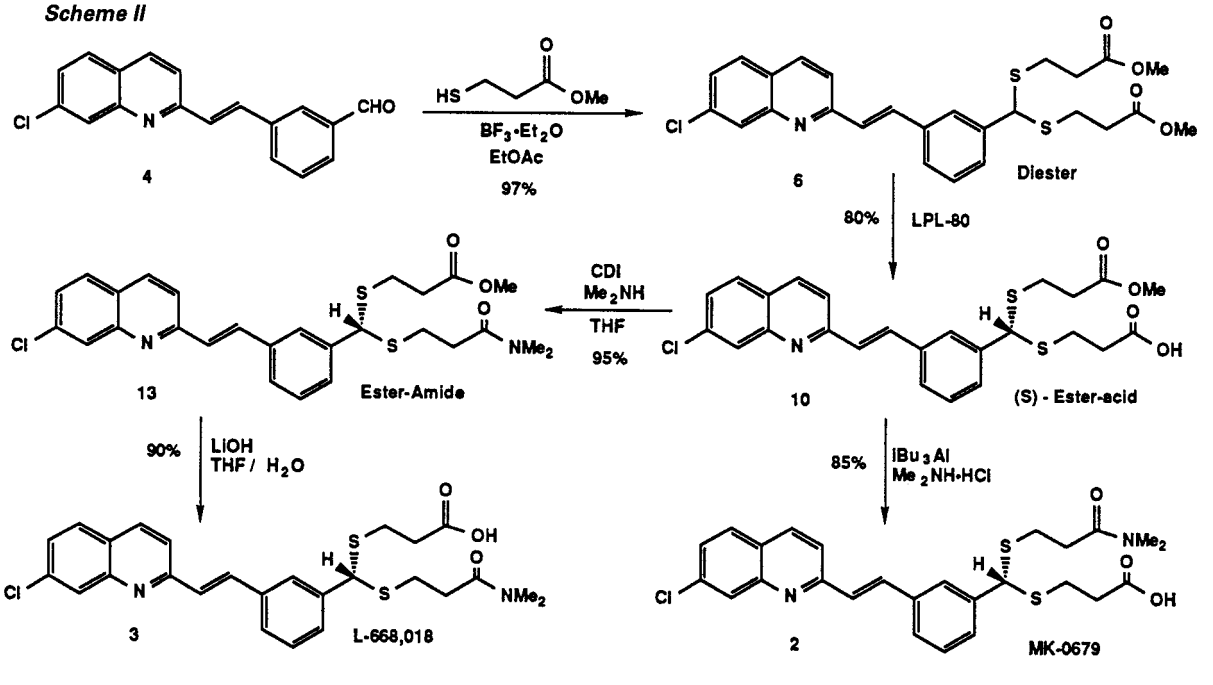
Enzymatic hydrolysis of prochiral diesters

Here again, we studied the hydrolysis of several prochiral diesters (methyl, CH_2CONEt_2, CH_2CH_2OMe and CH_2CONH_2) such as **6** with several enzymes (*Pseudomonas, Chromobacterium viscosum, C. cylindracea, Rhizopus javanicus, Rhizopus arrhizus*, pig liver esterase and pig pancreatic lipase).[5] Excellent results were obtained in the hydrolysis of the dimethyl ester **6** with *Pseudomonas* ; hence, this reaction was studied extensively to develop a practical, large scale synthesis of MK-0679 (**2**) and L-668,018 (**3**) as described below.

Synthesis of diester 6

A high yielding, straightforward synthesis of the symmetrical diester **6** was developed. Aldehyde **4** in ethyl acetate is treated with 2.05 equivalents of methyl 3-mercaptopropionate and 2.05 equivalents of boron trifluoride etherate at -10 to 0 °C. The reaction is complete in less than an hour. After workup, the ethyl acetate solution is concentrated, and the product **6** is directly crystallized by the addition of hexanes. Typically, the isolated yield of diester **6** is 97% (99.7 area% purity -- HPLC).

Scheme II



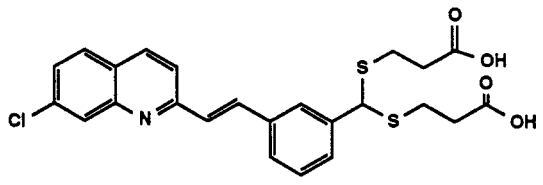
Synthesis of (*S*)-ester-acid 10

Enzymatic hydrolysis of the prochiral diester **6** to produce the (*S*)-ester-acid **10** in 80% yield with high enantioselectivity (≥ 99% ee) is central to our practical synthesis of the *R* enantiomer MK-0679 (**2**) and the *S* enantiomer L-668,018 (**3**) (SCHEME II). By using a prochiral substrate (**6**), the potential yield is 100% rather than the 50% maximum yield inherent in the enzymatic hydrolysis of a racemate. (*S*)-Ester-acid **10** offers the added advantage of serving as a common intermediate to synthesize both enantiomers of MK-0571; this has been achieved by selective modification of the two unsymmetrical arms of the dithioacetal unit.[5] The absolute configurations are based on the work of Gauthier et al.[10]

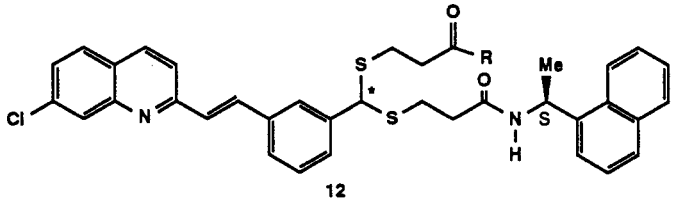
There are several key aspects of the enzymatic hydrolysis which deserve attention: (1) enzyme, (2) reaction conditions, (3) Triton X-100, and (4) chiral purity.

Enzyme: As mentioned earlier, based upon several screening experiments, the lipase enzyme from *Pseudomonas cepacia* (Amano Enzyme Co.) was identified to be suitable for the desired conversion. Four grades of this enzyme (P-30, PS-Conc, LPL-80, LPL-200), commercially available from Amano Enzyme Co., were evaluated. LPL-80 (PS-800)[13] and LPL-200 differ mainly in the activity; the former is more readily available. The performances of P-30, PS-conc, and LPL-80 are similar and all three have been successfully used. In the cases of P-30 and PS-Conc, the material had to be digested with the buffer and the insoluble additive removed by filtration prior to use. P-30 tends to produce higher levels of the over hydrolysis byproduct diacid **11** than PS-conc and LPL-80. Based on availability and reproducibility, LPL-80 has been mostly used. The L9518 lipase enzyme from *Pseudomonas* species (Sigma) is also very efficient in this hydrolysis.

Reaction Conditions: The enzymatic hydrolysis is typically carried out by suspending diester **6** (1 gram) in a solution of the surfactant (Triton X-100, 0.5 mL) and the enzyme (LPL-80, 20 to 40 mg) in 0.1M dipotassium hydrogen phosphate pH 7.5 buffer (30 mL) and then aging the mixture at 40 ± 2 °C. The reaction proceeds with about 95% conversion in about 40 hours. Kinetics of this heterogeneous enzymatic hydrolysis has been reported.[14] The amount of the byproduct diacid **11** formed is 2-3%. The reaction remains heterogeneous throughout. After cooling the reaction mixture to room temperature, the product is isolated by filtration. The crude product typically contains ~5% unreacted diester **6** and <0.3% of the diacid **11**. The product is purified by recrystallization and buffer extraction to get rid of diester **6** and diacid **11** impurities.



Diacid, 11



12

R = OMe or NMe_2

Triton X-100: The use of the nonionic surfactant Triton X-100 is important in this hydrolysis reaction. Triton X-100 increases the solubility of diester **6** in the reaction medium. With decreasing amounts of the surfactant, the reaction becomes slower and the amount of the diacid **11** produced increases. With increasing amount of the surfactant, the reaction is faster; but, unfortunately, the loss of ester-acid **10** in the filtrate is higher. As a compromise, 1.6% of Triton X-100 (0.5 mL in 30 mL of the buffer) is used.

Enantiomeric Purity: The enzymatic hydrolysis proceeds with high enantioselectivity. Typically, the ee of the crude isolated (*S*)-ester-acid **10** is 99 ± 0.2%. Enrichment of the *S* enantiomer has been achieved in three ways: (1) Crystallization of the product from the reaction mixture. The filtrate from the reaction has been found to contain more (3.7%) *R* isomer than the solid (0.6%). (2) Hydrolysis of the ester-acid to diacid **11**. Enzymatic hydrolysis of the (*R*)-ester-acid is faster than that of the *S* enantiomer.[5] (3) Recrystallization of crude (*S*)-ester-acid **10** using isopropyl acetate and hexanes.

Synthesis of the *R* enantiomer MK-0679 (2)

Conversion of the methyl ester group of (*S*)-ester-acid **10** to the dimethyl amide group of (*R*)-amide-acid[15] **2** is achieved in 2 : 1 tetrahydrofuran : toluene using triisobutylaluminum and dimethylamine hydrochloride. Due to its lower pyrophoricity, triisobutylaluminum is used for this conversion in place of the traditional trimethylaluminum.[16]

A solution (1M in toluene) of triisobutylaluminum (2.0 equivalents) is added to a 0 °C suspension of dimethylamine hydrochloride (2.1 equivalents) in dry tetrahydrofuran under nitrogen, maintaining the temperature below 10 °C. The suspension becomes a clear solution on aging (20-25 °C for 15 minutes then 50 °C for 2 hours). The aluminum-dimethyl amine reagent solution is cooled to 40 °C and a solution of (*S*)-ester-acid **10** (1.0 equivalent) in tetrahydrofuran is added. The cloudy mixture is aged at 40 °C and the reaction is usually complete in 15 - 17 hours. After workup and recrystallization, the product is obtained in 85% yield (>99 HPLC purity and >99% ee).

Synthesis of the *S* enantiomer L-668,018 (3)

As mentioned earlier, (*S*)-ester-acid **10** can also be readily converted into the (*S*)-amide-acid, L-668,018 (**3**), as outlined in SCHEME II. (*S*)-Ester-acid **10** is reacted with 3.0 eq. of carbonyldiimidazole in tetrahydrofuran at 0 °C. The activated acyl imidazolide thus formed is treated with excess dimethyl amine gas at 0 °C to form the ester-amide **13**. Hydrolysis of the ester-amide **13** using lithium hydroxide in tetrahydrofuran/water gave crystalline L-668,018 (**3**) in ~80% overall yield (99.0% HPLC purity and > 99% ee).

Enantiomeric purity assay

(*S*)-Ester-acid **10** and final products **2** and **3** are assayed for enantiomeric excess by HPLC assay of the corresponding (*R*)-(+)- or (*S*)-(-)-1-(1-naphthyl)ethylamide derivatives **12**.[5] The diastereomeric derivatives are also distinguishable by ^{1}H NMR spectra. In the case of the ester-acid derivatives, the methyl ester signals are cleanly resolved; the methyl protons of the ester group are seen as a singlet at 3.60 ppm for the minor diastereomer from the (*R*)-ester-acid and (*S*)-(-)-1-(1-naphthyl)ethylamine and at 3.62 ppm for the major diastereomer from the (*S*)-ester-acid and (*S*)-(-)-1-(1-naphthyl)ethylamine. In the amide-acid series, the NH protons of the naphthylethyl amide are easily resolved; the NH proton of the 1-(1-naphthyl)ethyl amide group is seen as a doublet (J = 8 Hz) at 6.7 ppm for the minor diastereomer from the (*S*)-amide-acid and (*R*)-(+)-1-(1-naphthyl)ethylamine and at 7.1 ppm for the major diastereomer from the (*R*)-amide-acid and (*R*)-(+)-1-(1-naphthyl)ethylamine.

SUMMARY

We have achieved a practical synthesis of the *R* enantiomer MK-0679 (**2**) and the *S* enantiomer L-668,018 (**3**) starting from the same chiral, nonracemic (*S*)-ester-acid **10** obtained by the enzymatic hydrolysis of the prochiral diester **6** in >99% enantiomeric purity. The enzymatic hydrolysis has been successfully carried out on multikilogram scale. The overall yield of the enantiomers is close to 45% whereas that of the racemate is 34%. The major selectivity problem in constructing the unsymmetrical dithioacetal unit in the synthesis of the racemate MK-0571 (**1**) has been readily solved in the enzymatic hydrolysis of **6** to produce **10**. Synthesis of pure enantiomers **2** and **3**, which by conventional chemical methods is difficult on a large scale, has been achieved readily and inexpensively with commercially available *Pseudomonas* lipase.

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