Tetrahedron: Asymmetry Vol. 4, No. 3, pp. 425-432, 1993
Printed in Great Britain

0957-4166/93 $6.00+.00

Resolution of Methyl *cis*-3-Chloromethyl-2-tetrahydrofurancarboxylate via Enzymatic Hydrolysis

Jan H. Udding, Jan Fraanje, Kees Goubitz, Henk Hiemstra* and W. Nico Speckamp*

Departments of Organic Chemistry and Crystallography, University of Amsterdam, Nieuwe Achtergracht 129, 1018 WS Amsterdam, The Netherlands

Bernard Kaptein, Hans E. Schoemaker and Johan Kamphuis

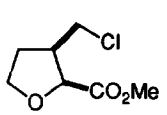
DSM Research, P. O. Box 18, 6160 MD Geleen, The Netherlands

(*Received in UK* 16 *December* 1992)

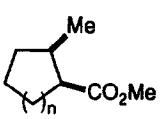
Abstract: Kinetic resolution via enzyme catalyzed hydrolysis under neutral conditions was successfully applied to racemic methyl *cis*-3-chloromethyl-2-tetrahydrofurancarboxylate (**1**). The highest selectivities were attained with the enzymes acylase-I (E = 51) and α-chymotrypsin (E = 28). The optically active methyl ester recovered in both cases was (+)-(2*S*,3*R*)-**1**. The hydrolysis product spontaneously cyclized under the reaction conditions to the bicyclic lactone (-)-(3a*R*,6a*R*)-**9**, which was obtained enantiopure after one recrystallization. The X-ray crystal structure analysis of (-)-**9** allowed the determination of its absolute configuration

INTRODUCTION

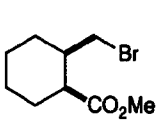
The application of enzymes in organic synthesis for gaining access to enantiomerically pure compounds is now well established.[1,2] Probably the most actively studied group of enzymes are the hydrolytic enzymes, which, operating without the need for coenzymes, have proved to be very useful. Pig liver esterase (PLE) is a particularly well-documented example showing broad substrate specificity and high stereoselectivity.[3]



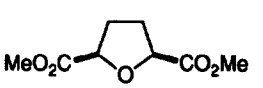
1



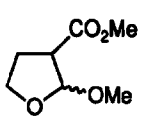
2



3



4



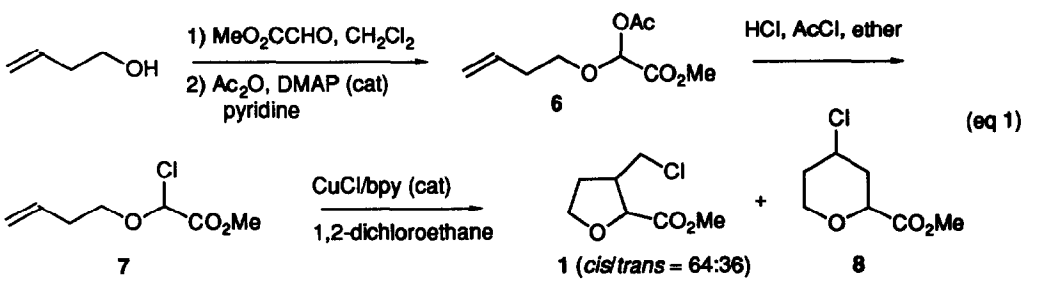
5

In the course of our work on cyclization reactions of α-alkoxyacetic ester radicals, a facile synthesis of 3-substituted 2-tetrahydrofurancarboxylic esters (e.g. **1**) was developed.[4,5] To enhance the utility of these products we then investigated the possible resolution of **1** by using enzymatic hydrolysis. Recently, several structurally related cyclic esters have been shown to be successfully resolved by hydrolytic enzymes. Thus,

carbocyclic esters **2** (n = 0 and 2) and **3** were hydrolyzed with high selectivity by using PLE.[6] Interestingly, hydrolysis of cyclopentane derivative **2** (n = 1) with PLE occurred with low selectivity.[6] Meso-2,5-tetrahydrofuranyl diester **4** and related diesters were monohydrolyzed with moderate selectivity by using PLE and porcine pancreatic lipase (PPL).[7] 3-Tetrahydrofurancarboxylate **5** was successfully resolved by using *Candida cylindracea* lipase.[8] Details of our study of the enzymatic hydrolysis of **1** are reported herein.

RESULTS

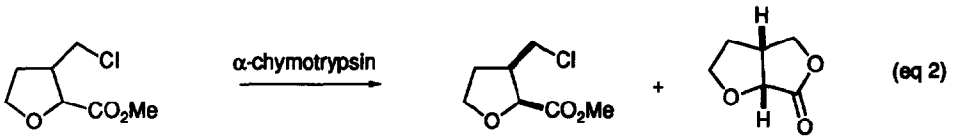
Racemic methyl 3-chloromethyl-2-tetrahydrofurancarboxylate (**1**) was prepared using the radical transfer cyclization method with copper(I) chloride/2,2'-bipyridine as the catalyst[5] (eq 1). The cyclization precursor **7** was made in three steps from commercially available 3-buten-1-ol in 63% overall yield. Reaction of this alcohol with methyl glyoxylate[9] gave an unstable hemiacetal, which was directly acetylated to **6** in the usual way. Conversion of **6** to chloride **7** was readily accomplished by treatment with excess of acetyl chloride and hydrogen chloride in ether. Cyclization of **7** with the copper(I) chloride/2,2'-bipyridine catalyst[5] gave methyl 3-chloromethyl-2-tetrahydrofurancarboxylate (**1**) in 75% yield (*cis*/*trans* = 64:36), along with a small amount of 6-*endo* cyclization product **8** (7%). While **8** was readily removed, complete chromatographic separation of the *cis*/*trans* isomers of **1** was difficult. The enzymatic hydrolyses of **1** were performed with material which contained at least 80% of the *cis*-isomer (see experimental). Only the kinetic resolution of *cis*-**1** will be considered. We suppose that the presence of some of the *trans*-isomer will not influence the course of the hydrolysis of the *cis*-isomer, although product analysis and purification were somewhat compromised.



Ten different enzymes were tested for their activity in the hydrolysis of **1**. In this screening, a 1:1 *cis*/*trans* mixture of racemic **1** (0.10 g, 0.56 mmol) in 0.01 M KH_2PO_4 buffer (5 mL) of pH 7 was treated with the different enzymes. A clear drop in the pH of the mixture (to pH 2-3) was noted in the case of α-chymotrypsin (Merck), lypozym (NOVO), acylase-I (A-2156, Sigma) and PPL (L-3126, Sigma),[10] indicating catalytic activity. These four enzymes were selected for further investigation.

The enzymatic hydrolyses of racemic **1** were carried out in a phosphate buffer at pH 7 at room temperature (eq 2). In the case of the acylase and lipase reactions, toluene was admixed to create a two-phase system. The pH was maintained at 7 by addition of 1 N aqueous NaOH from an autotitrator. After termination of the reaction by quenching with aqueous HCl, a mixture of optically active **1** and optically active lactone **9** was isolated by extraction at pH 2. Lactone **9** is formed after hydrolysis of the ester by spontaneous intramolecular substitution

of chloride in the *cis*-3-chloromethyl-2-tetrahydrofuran-carboxylate.[6] This lactone could be easily separated from ester **1** by using chromatography. From the *cis/trans* mixture of **1**, small amounts of optically active *cis*-ester **1** were obtained pure by using chromatography. The *trans*-isomer could not be obtained pure and will not be further considered.



racemic *cis*-**1** (+)-(2*S*,3*R*)-**1** (-)-(3a*R*,6a*R*)-**9** (eq 2)

The results of the enzymatic kinetic resolution of **1** with the different enzymes are summarized in Table I. The ee of *cis*-ester **1** was determined by using the chiral shift reagent $Eu(hfc)_3$ (see experimental). Lactone **9** was obtained as a solid. One single recrystallization of the lactone from the α-chymotrypsin reaction (entry 1) led to beautiful crystals (mp 55-56 °C, $[\alpha]_D$ -84.4 (*c* 0.6, $CHCl_3$), which appeared suitable for X-ray analysis (Figure 1). This X-ray study not only proved the bicyclic structure, but also allowed the assignment of the absolute configuration to (-)-**9** as being (3a*R*,6a*R*). Consequently, the recovered methyl ester from the α-chymotrypsin reaction (+)-*cis*-**1** must be assigned as (2*S*,3*R*).

Table I. Enzyme catalyzed hydrolysis of racemic **1**.

	racemic **1**			recovered *cis*-**1**			lactone **9**				
entry	*cis/trans* ratio	enzyme	base[a]	absolute config.	yield[b]	ee	absolute config.	yield[b]	optical purity	conversion[c]	E[d]
1	81:19	α-chymotrypsin	0.76	(+)-(*S*,*R*)	38%	86%	(-)-(*R*,*R*)	35%	82%	0.51	28
2	90:10	acylase-I	0.52	(+)-(*S*,*R*)	40%	>90%	(-)-(*R*,*R*)	12%	88%	0.51	51
3	81:19	PPL	0.69	(+)-(*S*,*R*)	34%	40%	(-)-(*R*,*R*)	10%	74%	0.35	10
4	>95: 5	lypozym	0.83	(-)-(*R*,*S*)	43%	56%	(+)-(*S*,*S*)	5%	72%	0.50	11

[a]Number of equiv. of base added. [b]Corrected for *trans*-ester (±)-**1**. [c]Calculated from the ee's of lactone **2** and remaining *cis*-ester **1**: conversion (*c*) = [ee(S)]/[ee(S)+ee(P)]. [d]This enantiomeric ratio E is calculated from the equation: E = ln {1-*c*[1+ee(P)]}/ln {1-*c*[1-ee(P)]} and provides a measure of the enzyme's ability to discriminate between the two enantiomers of the substrate.[11]

DISCUSSION

Table I shows that *cis*-ester **1** is a good substrate for all four enzymes. Enzymatic hydrolysis with α-chymotrypsin, acylase and porcine pancreatic lipase (PPL, entries 1-3) gave rise to the formation of (-)-lactone **9** and (+)-*cis*-ester **1**. On the other hand, the use of lypozym (entry 4) gave (+)-lactone **9** and (-)-*cis*-ester **1**. Thus, use of the appropriate enzyme allows access to either enantiomer of both ester and lactone. The results obtained from the hydrolysis of racemic **1** with α-chymotrypsin and acylase-I were satisfactory, with ee's of

both unchanged ester and lactone above 80% and enantiomeric ratio's of 28 and 51, respectively, indicating a moderate to good selectivity of the enzyme in discriminating between the two enantiomers of the substrate.

For α-chymotrypsin and PPL, yields of lactone **9** corrected for conversion were 69% and 42%. For lypozym and acylase-I the corrected yields were much lower, *i.e.* only 10% and 22%. The relatively high solubility of the lactone in water (as derived from chemical hydrolysis of **1**[12]) in combination with the possibility of complexation to the enzyme may account for these results. A similar solubility problem has been described for the enzyme-catalyzed hydrolysis of 2-methoxy-3-carbomethoxytetrahydrofuran.[8] The conversion based on the volume of base added is not in agreement with the chemical yields of ester and lactone. The actual uptake of base is higher than would be expected for hydrolysis (even if the hydrolysis of the minor *trans*-isomer is accounted for), especially in the case of lypozym. This extra base consumption may be explained as a result of the acidity of the enzyme solution itself; a solution of lypozym in water showed a pH of 5.

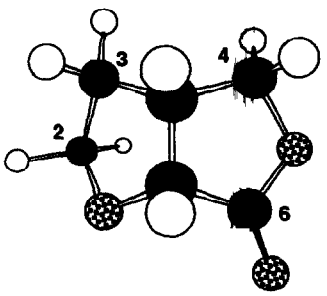


Figure 1 Chem3D™ perspective view of the crystal structure of lactone (-)-(3a*R*,6a*R*)-**9**.

In conclusion, enantioselective hydrolysis of racemic methyl *cis*-3-chloromethyl-2-tetrahydrofuran-carboxylate (**1**) by using four different enzymes provided access to optically active *cis*-substituted ester **1** and optically active bicyclic lactone **9**. The highest enantioselectivities were obtained with acylase-I and α-chymotrypsin. The use of lypozym led to an excess of the optical antipodes, but with lower selectivity. Application of the methodology described herein in natural product synthesis, e.g. avenaciolide and congeners[13], will be published in due course.[12]

EXPERIMENTAL

General information. Infrared (IR) spectra were obtained from chloroform solutions using a Perkin Elmer 298 spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were determined in $CDCl_3$ as solvent using a Bruker AC 200 (200 MHz) or a Bruker WM 250 (250 MHz) instrument. These machines were also used for the ^{13}C NMR (APT) spectra (50 MHz and 63 MHz) in $CDCl_3$ solutions. Chemical shifts are given in ppm downfield from tetramethylsilane. Mass spectra and accurate mass measurements were carried out using a VG Micromass ZAB-2HF instrument. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. R_f values were obtained by using thin layer chromatography (TLC) on silica gel-coated plastic sheets (Merck silica gel 60 F_{254}) with the indicated solvent (mixture). Chromatographic purification refers to flash chromatography[14] using Merck silica gel (230-400 mesh). Melting and boiling points are uncorrected. CH_2Cl_2 and $ClCH_2CH_2Cl$ were distilled from P_2O_5 and kept under an atmosphere of dry nitrogen. Dry Et_2O

was distilled under an atmosphere of dry nitrogen from sodium benzophenone ketyl prior to use. Reactions under a dry nitrogen atmosphere were performed in flame-dried glassware. Copper(I) chloride was purified according to a literature procedure[15]. α-Chymotrypsin was a product of Merck, acylase-I (A-2156) and porcine pancreatic lipase (L-3126) were products of Sigma, and lypozym was a product of NOVO.

Methyl *O*-(3-butenyl)-α-acetoxyglycolate (6). In a dry nitrogen atmosphere, freshly prepared methyl glyoxylate[9] (10.21 g, 0.121 mol) was added to a solution of 3-buten-1-ol (8.68 g, 0.121 mol) in 60 mL of dichloromethane. After being stirred for 3 days, the mixture was concentrated *in vacuo*. The residue was taken up in 100 mL of pyridine, and DMAP (catalytic amount) and acetic anhydride (17.1 mL, 0.181 mol) were added. After being stirred for 3 h, the mixture was concentrated *in vacuo*, treated with toluene, and again concentrated *in vacuo* (this procedure was repeated 3 times). The residue was distilled to give **6** (15.41 g, 76.3 mmol, 63%) as a colourless oil after distillation (62 °C/0.003 mm Hg). IR 3075, 1745, 1635, 1435 and 1365 cm^{-1}. ^1H NMR (200 MHz) δ 2.16 (s, 3 H, Ac), 2.39 (q, J = 6.8 Hz, 2 H, =CHCH_2), 3.80 (s, 3 H, OMe), 3.64-3.90 (m, 2 H, OCH_2), 5.03-5.15 (m, 2 H, $=CH_2$), 5.69-5.86 (m, 1 H, -CH=), 5.98 (s, 1 H, OCHO).

Methyl *O*-(3-butenyl)-α-chloroglycolate (7) Hydrogen chloride was passed through a solution of **6** (1.184 g, 5.86 mmol) in 12 mL of dry ether and 12 mL of freshly distilled acetyl chloride at 0 °C for 0.5 h. Evaporation of the volatiles gave chloride **7** (1.040 g, 5.84 mmol, 100%) as a slightly yellow oil. IR 3075, 2950, 1760, 1635, 1435 and 1295 cm^{-1}. ^1H NMR (200 MHz) δ 2.44 (qt, J_q = 6.7, J_t = 1.3 Hz, 2 H, =CHCH_2), 3.64 (dt, J_d = 9.5, J_t = 6.9 Hz, 1 H, OCH_2), 3.85 (s, 3 H, OMe), 4.02 (dt, J_d = 9.5, J_t = 6.9 Hz, 1 H, OCH_2), 5.06-5.18 (m, 2 H, $=CH_2$), 5.69-5.90 (m, 1 H, -CH=), 5.83 (s, 1 H, OCHCl). ^{13}C NMR (50 MHz) δ 32.8 (=CHCH_2), 52.9 (OMe), 69.5 (OCH_2), 88.2 (OCHCl), 117.2 ($=CH_2$), 133.4 (-CH=), 165.4 (C=O).

Copper(I) chloride catalyzed cyclization of 7. In a dry nitrogen atmosphere, 2,2'-bipyridine (2.85 g, 18.3 mmol) and copper(I) chloride (1.81 g, 18.3 mmol) were added to a solution of chloride **3** (10.84 g, 60.90 mmol) in 120 mL of 1,2-dichloroethane. The reddish brown solution was refluxed for 2 days. After evaporation of the solvent *in vacuo*, chromatography (EtOAc/hexanes = 1:6) gave as the first fraction a 83:17 mixture of the *cis* and *trans* isomer of **methyl 3-chloromethyl-2-tetrahydrofurancarboxylate (1)** as a colourless oil (5.42 g, 30.4 mmol, 50%), R_f = 0.30 and 0.25 (EtOAc/hexanes = 1:6). IR 2950, 2880, 1740, 1435, 1370 and 1290 cm^{-1}. Spectroscopic data for the *cis* isomer: ^1H NMR (200 MHz) δ 1.97 (dq, J_d = 12.7, J_q = 7.9 Hz, 1 H, H-4), 2.23 (ddt, J_d = 12.5, J_d = 7.6, J_t = 4.9 Hz, 1 H, H-4), 2.77-2.93 (m, 1 H, H-3), 3.39 (dd, J = 11.0, 8.6 Hz, 1 H, CH_2Cl), 3.59 (dd, J = 11.0, 6.0, Hz, 1 H, CH_2Cl), 3.75 (s, 3 H, OMe), 3.91 (q, J = 7.7 Hz, 1 H, H-5), 4.20 (dt, J_d = 4.7, J_t = 8.3 Hz, 1 H, H-5), 4.50 (d, J = 7.5 Hz, 1 H, H-2). ^{13}C NMR (63 MHz) δ 29.83 (C-4), 43.45 (CH_2Cl), 45.06 (C-3), 51.86 (OMe), 68.17 (C-5), 78.64 (C-2), 171.14 (C=O). Spectroscopic data for the *trans* isomer: ^1H NMR (200 MHz) δ 1.79-1.96 (m, 1 H, H-4), 2.12-2.20 (m, 1 H, H-4), 2.68-2.84 (m, 1 H, H-3), 3.59 (dd, J = 11.1, 6.7 Hz, 1 H, CH_2Cl), 3.71 (dd, J = 11.1, 6.0 Hz, CH_2Cl), 3.76 (s, 3 H, OMe), 3.98-4.05 (m, 2 H, H-5), 4.29 (*d*, J = 5.6 Hz, 1 H, H-2). ^{13}C NMR (63 MHz) δ 29.97 (C-4), 45.95 (CH_2Cl), 46.15 (C-3), 52.12 (OMe), 68.67 (C-5), 79.28 (C-2), 172.48 (C=O). MS (EI, 70 eV) 178 (M^+, 3), 177 (M^+-H, 33), 119 (M^+-CO_2Me, 100). Accurate mass for M^+-H 177.0326 (calcd for $C_7H_{10}O_3Cl$ 177.0318). The second fraction consisted of a 78:22 mixture of **1** and tetrahydropyranyl

ester **8** (3.43 g, 19.3 mmol, 32%). According to ^{1}H NMR, the *cis/trans* ratio for **1** in this fraction was 27:73.

General procedure for the enzyme catalyzed hydrolysis of ester 1. The following procedure is representative. The enzyme was added to a rapidly stirred suspension of **1** in 0.005 M KH_2PO_4 buffer of pH 7 at 21 °C. The pH was maintained at 7 by pH stat-controlled addition of 1 N aqueous NaOH. The reaction was allowed to proceed until the desired extent of hydrolysis, as determined by the volume of base added, had been achieved. The pH of the mixture was then adjusted to 2 by addition of 6 N HCl. EtOAc (30 mL) was added, and the mixture was filtered over silica. After separation of the organic layer, the aqueous phase was extracted with EtOAc (3 × 25 mL). The combined organic layers were dried (Na_2SO_4) and concentrated *in vacuo* to yield a mixture of ester **1** and lactone **9**. Separation of this mixture was accomplished by chromatography (EtOAc/hexanes = 1:1 to 2:1). The ee of *cis*-**1** was determined as follows. To a solution of racemic *cis*-ester **1** (ca. 5 mg) in $CDCl_3$ (0.5 mL) was added 60 μL of a 0.04 M solution of tris[3-(heptafluoropropyl hydroxymethylene)-(+)-camphorato] europium(III) [Eu(hfc)$_3$] (99%, Aldrich). The homodecoupled ^{1}H NMR spectrum of this mixture (double resonance, irridiation at 2.8 ppm, H-3) gave separation of the two singlets for H-2 of the two enantiomers (at 4.70 and 4.80 ppm). Integration of the these singlets showed a clear 1:1 ratio for racemic **1**. The ee of the optically active *cis*-esters **1** could be determined in this way. The optical purity of **9** was based on the $[\alpha]_D$ of enantiomerically pure lactone **9**, obtained via recrystallization (*vide infra*).

α-Chymotrypsin catalyzed hydrolysis of 1. Hydrolysis of racemic **1** (1.23 g, 6.91 mmol, *cis/trans* = 81:19) in 24 mL of the buffer with α-chymotrypsin (0.20 g) gave after a base consumption of 0.76 equiv (15 h) and chromatography (+)-(2*S*, 3*R*)-**1** (0.094 g, 8% yield, 86% ee). $[\alpha]_D$ +20.3 (*c* 1.5, $CHCl_3$). R_f 0.75 (EtOAc/hexanes = 1:1). The second fraction consisted of a 78:22 mixture of *cis*- and *trans*- ester **1** (0.362 g, 29%), R_f 0.75 and 0.70 (EtOAc/hexanes = 1:1). The third fraction consisted of lactone **9** (0.245 g, 28% yield, optical purity 82%), $[\alpha]_D$ -69 (*c* 1, $CHCl_3$). R_f 0.20 (EtOAc/hexanes = 1:1). Recrystallization from di-isopropyl ether gave enantiomerically pure **(-)-(3a*R*, 6a*R*)-tetrahydrofuro[3,4-*b*]furan-6(4*H*)-one** (**9**): mp 55-56 °C; $[\alpha]_D$ -84.4 (*c* 0.6, $CHCl_3$). IR ($CHCl_3$) 2980, 2910, 2870, 1775, 1475, 1445, 1375, 1225, 1170, 1080, 1015 and 970 cm^{-1}. ^{1}H NMR (200 MHz) δ 1.81-1.96 (m, 1 H, H-3), 2.18-2.36 (m, 1 H, H-3), 3.11-3.27 (m, 1 H, H-3a), 3.75-3.87 (m, 1 H, H-2), 3.96-4.07 (m, 1 H, H-2), 4.14 (dd, *J* = 9.8, 3.1 Hz, 1 H, H-4), 4.51 (dd, *J* = 7.6, 9.8 Hz, 1 H, H-4), 4.63 (d, J = 10.0 Hz, 1 H, H-6a). ^{13}C NMR (50 MHz) δ 32.87 (C-3), 38.50 (C-3a), 68.78 (C-2), 71.43 (C-4), 77.63 (C-6a), 175.20 (C=O). Anal. calcd. for $C_6H_8O_3$: C, 56.25; H, 6.29. Found: C, 56.18; H, 6.35.

Determination of the crystal structure and absolute configuration of (-)-9 by X-ray analysis.[16] Orthorhombic crystals, $P2_12_12_1$, *a* = 5.1494 (5), *b* = 10.4332 (6), *c* = 11.5129 (8) Å, *V* = 618.53(8) Å^3, *Z* = 4, ρ_{calcd} = 1.38 g/cm^{-3}, F(000) = 272. A crystal with dimensions 0.05 × 0.30 × 0.65 mm was used for data collection on an Enraf-Nonius CAD-4 diffractometer at room temperature with graphite-monochromated CuKα radiation (λ = 1.5418 Å, μ = 9.0 cm^{-1}) and ω-2θ scan. A total of 2461 reflections was measured within the range $-6 \le h \le 6$, $-12 \le k \le 12$, $-13 \le l \le 14$. Of these, 2357 were above the significance level of 2.5σ(I). The maximum value of sin(θ)/λ was 0.61 Å^{-1}. Two reference reflections [12(-3), 032] were measured hourly and showed no decrease during the 30 h collecting time. There were 1137 unique reflections; the rest of the observed reflections were used in the determination of the absolute configuration. Unit-cell

parameters were refined by a least-squares fitting procedure using 23 reflections with $82^\circ < 2\theta < 90^\circ$. Corrections for Lorentz and polarisation effects were applied. The structure was solved by Direct Methods. The positions of the hydrogen atoms were calculated. Full-matrix least-squares refinement on F, anisotropic for the non-hydrogen atoms and isotropic for the hydrogen atoms, with a fixed temperature factor of $U = 0.15 Å^2$, restraining the latter in such a way that the distance to their carrier remained constant at approximately 1.09 Å, converged to $R = 0.054$, $R_w = 0.064$, $(\Delta/\sigma)_{max} = 0.52$. A weighting scheme $w = (6.72 + F_{obs} + 0.0038*F_{obs}^2)^{-1}$ was used. An empirical absorption correction (DIFABS[17]) was applied, with coefficients in the range of 0.69-1.38. The secondary isotropic extinction coefficient[18,19] refined to G = 0.09(1). The absolute-structure parameter[20] refined to $X_{abs} = 0.07(6)$, thus indicating the correct enantiomorph. A final difference Fourier map revealed a residual electron density between -0.4 and 0.3 $eÅ^{-3}$. Scattering factors were taken from Cromer and Man[21]. All calculations were performed with XTAL[22].

Acylase-I catalyzed hydrolysis 1. Hydrolysis of racemic **1** (1.23 g, 6.91 mmol, *cis/trans* = 90:10) in 23 mL of the buffer with acylase-I (0.21 g) gave after a base consumption of 0.83 equiv (15 h), (+)-(2*S*, 3*R*)-**1** (0.372 g, 30% yield, >90% ee), $[\alpha]_D$ +23.4 (*c* 1.3, $CHCl_3$), a 65:35 mixture of *cis* and *trans* ester **1** (0.109 g, 9%), and lactone (-)-(3a*R*, 6a*R*)-**9** (0.092 g, 10% yield, optical purity 88%), $[\alpha]_D$ -74 (*c* 0.65, $CHCl_3$).

Porcine pancreatic lipase catalyzed hydrolysis of 1. Hydrolysis of racemic **1** (1.044 g, 5.86 mmol, *cis/trans* = 81:19) in 22 mL of the buffer and 7 mL of toluene with PPL (0.10 g) gave after a base consumption of 0.52 equiv (5 h), (+)-(2*S*, 3*R*)-**1** (0.050 g, 5% yield, 40% ee), $[\alpha]_D$ +9.51 (*c* 1.1, $CHCl_3$). The second fraction consisted of a 82:18 mixture of *cis* and *trans* ester **1** (0.282 g, 27% yield). The third fraction was (-)-(3a*R*, 6a*R*)-**9** (0.058 g, 8% yield, optical purity 74%), $[\alpha]_D$ -62.4 (*c* 1.85, $CHCl_3$).

Lypozym catalyzed hydrolysis of 1. Hydrolysis of racemic **1** (0.87 g, 4.90 mmol, *cis/trans* = >95:5) in 18 mL of the buffer and 5 mL of toluene with lypozym (1.0 g) gave after a base consumption of 0.69 equiv (16 h), (-)-(2*R*, 3*S*)-**1** (0.378 g, 43% yield, 56% ee), $[\alpha]_D$ -13.2 (*c* 10.4, $CHCl_3$) and (+)-(3a*S*, 6a*S*)-**9** (0.030 g, 5% yield, optical purity 72%), $[\alpha]_D$ +60.4 (*c* 1.2, $CHCl_3$).

ACKNOWLEDGEMENT

This investigation was supported by the Netherlands' Foundation for Chemical Research (SON) with financial aid from the Netherlands' Organization for Advancement of Pure Research (NWO).

REFERENCES AND NOTES

1. Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071.
2. Boland, W.; Frössl, C.; Lorenz, M. *Synthesis* **1991**, 1049.
3. (a) Ohno, M.; Otsuka, M. In *Organic Reactions*; Kende, A. S. Ed.; Wiley: New York, 1989; vol. 37, p. 1. (b) Jones, J. B. *Pure Appl. Chem.* **1990**, *62*, 1445. (c) Zhu, L.-M.; Tedford, C. *Tetrahedron* **1990**, *46*,

6587.

4. Lolkema, L. D. M.; Hiemstra, H.; Al Ghouch, A. A.; Speckamp, W. N. *Tetrahedron Lett.* **1991**, *32*, 1491.
5. (a) Udding, J. H.; Hiemstra, H.;Van Zanden, M. N. A.; Speckamp, W. N. *Tetrahedron Lett.* **1991**, *32*, 3123. (b) Udding, J. H.; Hiemstra, H.; Speckamp, W. N. *J. Chem. Soc., Perkin Trans. 2* **1992**, 1529.
6. Toone, E. J.; Jones, J. B. *Tetrahedron: Asymm.* **1991**, *2*, 207.
7. (a) Jones, J. B.; Hinks, R. S.; Hultin, P. G. *Can. J. Chem.* **1985**, *63*, 452. (b) Hultin, P. G.; Mueseler, F.-J.; Jones, J. B. *J. Org. Chem.* **1991**, *56*, 5375. (c) Estermann, H.; Prasad, K.; Shapiro, M. J.; Repic, O.; Hardtmann, G. E.; Bolsterli, J. J.; Walkinshaw, M. D. *Tetrahedron Lett.* **1990**, *31*, 445.
8. Franssen, M. C. R.; Boavida dos Santos, P. M. A. C.; Camacho Mondril, N. L. F. L.; De Groot, Æ. *Pure Appl. Chem.* **1992**, *64*, 1089.
9. Generated from the commercially available methyl hemiacetal of methyl glyoxylate through distillation from phosphorus pentoxide (cf. Hook, J. M. *Synth. Commun.* **1984**, *14*, 83).
10. Other enzymes screened included lipase A and lipase P (Amano), PLE (Amano), esterase E-3128 (Sigma) and lipases from *Chromobacterium viscosum* (Biocatalysts) and *Pseudomonas fluorescence* [L700] (GGU mbH).
11. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.
12. Udding, J. H.; Tuijp, C. J. M.; Hiemstra, H.; Van Zanden, M. N. A.; Speckamp, W. N., manuscript in preparation.
13. For a recent synthesis of avenaciolide, see: Burke, S. D.; Pacofsky, G. J.; Piscopio, A. D. *J. Org. Chem.* **1992**, *57*, 2228.
14. Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.
15. Keller, R. N.; Wycoff, H. D. *Inorg. Synth.* **1946**, *2*, 1
16. Lists of refined coordinates and e.s.d.'s were deposited at the Cambridge Crystallographic Data Centre.
17 Walker, N.; Stuart, D. *Acta Cryst.* **1983**, *A39*, 158.
18 Zachariasen, W. H *Acta Cryst.* **1967**, *A23*, 558.
19 Larson, A. C. The Inclusion of Secondary Extinction in Least-Squares Refinement of Crystal Structures. In *Crystallographic Computing*, Ahmed, F. R.; Hall, S. R.; Huber, C. P., Eds.; Munksgaard: Copenhagen, 1969; pp. 291-294.
20 Flack, H. D. *Acta Cryst.* **1983**, *A39*, 876.
21 Cromer, D. T.; Mann, J. B. *Acta Cryst.* **1968**, *A24*, 321.
22 Hall, S. R.; Stewart, J. M., Eds. *XTAL3.0 User's manual.* Universities of Western Australia and Maryland 1990.