Kinetic Resolutions and Enantioselective Transformations of 5-(Acyloxy)pyrrolinones Using *Candida antarctica* Lipase B: Synthetic and Structural Aspects

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Various 5-(acyloxy)pyrrolinones have been prepared in enantiomerically pure form by means of an enzymatic resolution or an asymmetric transformation. Either enantiomer is obtained using the same enzyme, *Candida antarctica* lipase B, by modification of the procedure from transesterification to esterification. *N*-Acyl-5-(acyloxy)pyrrolinones **1** (R^2 = acyl) are synthesized by applying this method with 100% yield and >99% ee. To rationalize the observed enantioselectivity and the substituent effects of these reactions both empirical models and molecular modeling studies have been used, and a qualitative agreement was found between the results from these studies and the experimental results.

1. Introduction

Lipases are among the most commonly used enzymes in organic synthesis because of their stability, availability, and acceptance of a broad range of substrates.[1] In nature they catalyze the hydrolysis of lipids to fatty acids and glycerol, which takes place at the water−lipid interface. During the past decade many examples of the hydrolysis of ester and amide moieties of unnatural substrates have been demonstrated.[2] Because most lipases are quite stable in organic solvents it is also possible to shift the hydrolysis−esterification equilibrium toward ester formation by irreversible transesterification of the hydroxy compound with, for example, vinyl acetate.[3] Since many of these reactions proceed with high stereoselectivity, lipases are very useful in the preparation of chiral building blocks,[4] which can be used for the synthesis of both natural and unnatural products.

Candida antarctica lipase B (CALB) is one of the most frequently employed lipases especially for (trans)esterification in organic solvents. It has been used successfully for enantioselective transformations of a variety of substrates. For example, many cyclic[5] and acyclic[6] secondary alcohols are resolved with high stereoselectivities (*E* values >100). The same lipase has also been used for the kinetic resolution of amines and thiols in organic solvents. *E* values of up to 100 have been obtained.[7]

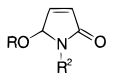

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Figure 1. R = H, alkyl, and acyl; R^2 = alkyl, acyl, and aryl.

Our interest is focused on chiral heterocycles, which can be used as building blocks for the synthesis of biologically active compounds. In particular we have concentrated on 3-pyrrolin-2-ones (Figure 1). These are versatile C4 synthons that can undergo several asymmetric transformations, leading, for example, to alkaloids[8] or unusual amino acids.[9] Classical methods to obtain these five-membered lactams in enantiomerically pure form require many steps.[10] A major aim of our research is to develop highly efficient routes to these chiral synthons by means of enzymatic resolution.

Recently some of us reported in a preliminary communication simple and efficient enzymatic methodology to obtain enantiomerically pure (acyloxy)pyrrolinones.[11] In this process both enantiomers of an (acyloxy)pyrroli-

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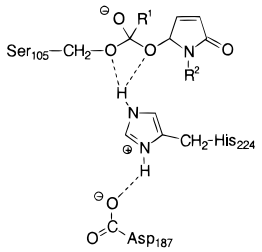
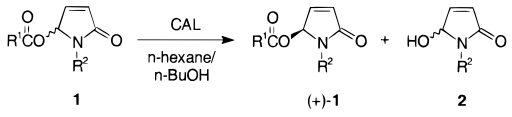
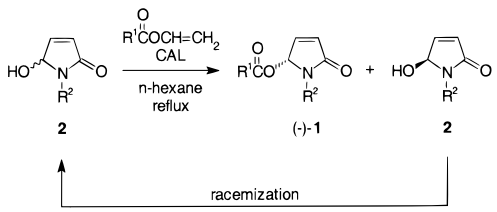


Figure 2. Schematic depiction of the reaction intermediate of CALB with a 5-(acyloxy)pyrrolinone as a substrate.

Scheme 1. Lipase-Catalyzed Transesterification (Kinetic Resolution) of Pyrrolinones



Scheme 2. . Lipase-Catalyzed Esterification (Enantioselective Transformation) of Pyrrolinones



none can be obtained with >99% ee using either an esterification (Scheme 1) or a transesterification (Scheme 2) catalyzed by CALB.

Like some other lipases *C. antarctica* B contains a Ser-His-Asp catalytic triad in its active site. Since the crystal structure of lipase B from the yeast *C. antarctica* is available,[12] several molecular modeling studies have been performed in an attempt to rationalize on a molecular level the selectivity of this enzyme in the discrimination of enantiomers.[13]

C. antarctica B shows a very high stereoselectivity, both in transesterification (kinetic resolution) and in esterification of many 5-(acyloxy)pyrrolinones (vide infra).[11] Figure 2 shows a schematic picture of reaction intermediate in the reaction center of the lipase with a 5-(acyloxy)pyrrolinone as a substrate (only the residues of the catalytic triad are shown). In most of the reactions *R*-enantiomers are strongly favored, with an enantiomeric ratio, $E > 200$ (for transesterifications involving kinetic resolution), or an enantiomeric excess, ee > 99% (for esterifications, i.e., asymmetric transformation). However, some pyrrolinones exhibit little enantioselectivity toward *C. antarctica*. For example pyrrolinone **1a** ($R^1 = CH_3$, $R^2 = COCH_3$) is obtained enantiomerically pure on esterification with vinyl acetate, whereas pyrrolinone **1f** ($R^1 = CH_3$, $R^2 = H$) is obtained as a racemate under the same conditions (Scheme 2).

In the present paper the enzymatic synthesis of a series of optically active pyrrolinones is addressed. Absolute configurations have been assigned, and both empirical models and molecular modeling studies have been used to rationalize the observed enantioselectivity of this process.

2. Methods

2.1. Enantioselective Enzymatic Synthesis. General Information. The conversions of the lipase-catalyzed (trans)-esterifications were measured on a 50 m × 0.53 mm HP-1 cross-linked methyl silicon gum column. Ee's were determined with a CP cyclodextrin-β-2,3,6-M-19 capillary column (Chrompack, diluted β-cyclodextrine phase, 50 m × 0.25 mm × 0.25 μm) (105−150 °C, for compounds **1b**,**g**,**j**), with a Lipodex C capillary column (Macherey-Nagel, heptakis(2,3,6-tri-*O*-pentyl)-β-cyclodextrine, 50 m × 0.25 mm) (90 °C, for compounds **1a**,**e**,**i**), with a Chiraldex B-TA capillary column (Astec, G9409-15, β-cyclodextrine trifluoroacetylated phase, 30 m × 0.25 mm × 0.125 μm) (140 °C, for compounds **1a**−**d**), or with a Chiraldex G-TA capillary column (Astec, G9807-08, γ-cyclodextrine trifluoroacetylated phase, 30 m × 0.25 mm × 0.125 μm) (160 °C, for compound **1h**). The compounds **2** were racemic as established with the same chiral GC column used for the corresponding compounds **1**. For all pyrrolinones **1** racemates and enantiomerically enriched samples were separated by chiral GC. Enantiomeric excesses are accurate to at least 0.5%. Merck silica gel 60 (230−400 mesh) was used for filtration and for flash chromatography. Immobilized *Candida antarctica* lipase B (Novozym 435) was obtained from NOVO Nordisk (Bagsvaerd, DK). The solvents were distilled and dried, if necessary, using standard methods. Reagents and starting materials were used as obtained from Acros Chimica, Aldrich, Fluka, or Merck, unless otherwise stated. Racemic compounds **1a**−**d**,**f** and **2a** were prepared as was previously reported.[11]

Acetic Acid 1-Methyl-5-oxo-2,5-dihydro-1*H*-pyrrol-2-yl Ester (1e). First 5-hydroxy-1-methyl-1,5-dihydropyrrol-2-one was synthesized by a slightly modified literature procedure.[14] A solution of *N*-methylpyrrole (5.00 g, 61.7 mmol) in 1 L of water was placed in a MeOH-cooled reaction vessel (0−5 °C). A stream of oxygen was introduced through a glass filter (P2) in the bottom of the vessel. A sheet of Kapton was used as a UV filter, and a high-pressure sodium lamp (Philips SON 150 W) served as the light source. The mixture was irradiated for 3.5 h, and 1.5 mL of a Methylene Blue solution (1 g/100 mL) was added slowly during the reaction time. The solvent was removed below 25 °C and the green oil was dissolved in ethyl acetate and filtered over silica gel. The dark green solution was stirred with activated carbon and filtered over silica gel again. The colorless solution was dried ($MgSO_4$) and concentrated under vacuum, yielding the nearly pure hydroxypyrrolinone as a white solid (3.60 g, 31.9 mmol, 52%), mp 78−80 °C: ^{1}H NMR ($CDCl_3$) δ 2.92 (s, 3H, NC*H*$_3$), 5.19 (br s, 1H, O*H*), 5.32 (s, 1H, OC*H*N), 6.05 (d, $J = 5.9$ Hz, 1H, CH=C*H*CO), 6.95 (d, $J = 5.9$ Hz, 1H, C*H*=CHCO); ^{13}C NMR ($CDCl_3$) δ 25.34 (q), 84.44 (d), 127.53 (d), 146.07 (d), 167.71 (s), 169.70 (s). A solution of the hydroxypyrrolinone (2.25 g, 19.9 mmol) and acetic anhydride (2.05 g, 20.1 mmol) in pyridine (35 mL) was stirred at room temperature for 18 h. The solution was concentrated in vacuo at room temperature. The resulting brown oil was filtered over silica gel and concentrated in vacuo. After Kugelrohr distillation (0.1 mmHg, 100 °C), **1e** (2.46 g, 15.9 mmol, 80% yield) was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.17 (s, 3H, OCOC*H*$_3$), 2.93 (s, 3H, NC*H*$_3$), 6.26 (d, $J = 6.0$ Hz, 1H, CH=C*H*CO), 6.44 (s, 1H, OC*H*N), 6.97 (dd, $J = 6.0$, 1.7 Hz, 1H, C*H*=CHCO); ^{13}C NMR ($CDCl_3$) δ 20.67 (q), 26.59 (q), 83.91 (d), 129.85 (d), 141.80 (d), 169.62 (s), 170.31 (s). HRMS: calcd for $C_7H_9NO_3$, *m*/*z* 155.058; found, *m*/*z* 155.058.

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Acetic Acid 1-Phenyl-5-oxo-2,5-dihydro-1*H*-pyrrol-2-yl Ester (1g). The procedure described above for **1e** was followed, starting from *N*-phenylpyrrole (3.95 g, 27.6 mmol) in 1.5 L of 1:1 water/THF. The brown solid product was recrystallized from toluene, yielding 5-hydroxy-1-phenyl-1,5-dihydro-pyrrol-2-one (0.95 g, 5.40 mmol, 20%) as a beige solid: ^{1}H NMR ($CDCl_3$) δ 3.09 (d, J = 10.0 Hz, 1H, OH), 5.98 (d, J = 10.0 Hz, 1H, OC*H*N), 6.20 (d, J = 6.1 Hz, 1H, CH=C*H*CO), 7.03 (dd, J = 6.1, 1.7 Hz, 1H, C*H*=CHCO), 7.15−7.67 (m, 5H) (^{1}H NMR corresponds to the literature NMR in acetone-d_6);[14c] ^{13}C NMR ($CDCl_3$) δ 83.89 (d), 121.02 (d), 125.01, (d), 129.01 (d), 136.49 (s), 144.82 (d), 168.35 (s). A solution of the hydroxypyrrolinone (0.15 g, 0.86 mmol) and acetic anhydride (0.18 g, 1.74 mmol) in pyridine (2 mL) was stirred at room temperature for 18 h. The solution was concentrated in vacuo at room temperature. The resulting brown oil was filtered over silica gel and concentrated in vacuo. After Kugelrohr distillation (0.001 mmHg, 100 °C), **1g** (0.16 g, 0.74 mmol, 86%) was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.01 (s, 3H, OCOC*H*$_3$), 6.33 (d, J = 7.0 Hz, 1H, CH=C*H*CO), 7.05 (s, 1H, OC*H*N), 7.06 (dd, J = 7.0, 1.8 Hz, 1H, C*H*=CHCO), 7.18−7.48 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 20.76 (q), 82.79 (d), 121.90 (d), 125.70 (d), 129.20 (d), 130.20 (d), 135.85 (s), 141.85 (d), 168.51 (s), 170.17 (s). HRMS: calcd for $C_{12}H_{11}NO_3$, m/z 217.075; found, m/z 217.074.

Acetic Acid 1-(2-(Methylsulfanyl)acetyl)-5-oxo-2,5-dihydro-1*H*-pyrrol-2-yl Ester (1h). First (methylthio)acetic anhydride was synthesized from (methylthio)acetic acid (3.00 mL, 34.5 mmol) and acetic anhydride (3.25 mL, 34.5 mmol). The mixture was refluxed for 4 h, and the reaction was forced to completion by distilling off the acetic acid. After Kugelrohr distillation (0.05 mmHg, 80−100 °C), (methylthio)acetic anhydride (2.81 g, 14.45 mmol, 84% yield) was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.13 (s, 3H), 3.12 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 15.88 (q), 36.17 (t), 165.02 (s). To a mixture of acetic acid 5-oxo-2,5-dihydro-1*H*-pyrrol-2-yl ester (**1f**) (1.00 g, 7.09 mmol), DMAP (0.09 g, 0.71 mmol), and pyridine (7 mL) was added slowly (methylthio)acetic anhydride (1.89 g, 10.6 mmol). After 1 night of stirring at room temperature the conversion was 58% (as judged by ^{1}H NMR). Water (300 mL) was added, and the solution was concentrated in vacuo, taking care that the temperature not exceeds 25 °C. Toluene (100 mL) was added, and the solution was concentrated in vacuo again. The brown solid was dissolved in dichloromethane, and the mixture was filtered over silica gel. The yellow solution was concentrated in vacuo. Purification by flash chromatography (silica gel, 7:1 hexane/EtOAc) afforded pure **1h** (0.87 g, 3.77 mmol, 53%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.04 (s, 3H), 2.08 (s, 3H), 3.71 (s, 2H), 6.21 (dd, J = 5.5, 0.6 Hz, 1H, CH=C*H*CO), 7.08−7.16 (m, 2H, OC*H*N and C*H*=CHCO); ^{13}C NMR ($CDCl_3$) δ 15.19 (q), 20.30 (q), 37.14 (t), 80.11 (d), 128.52 (d), 145.14 (d), 167.19 (s), 167.58 (s), 169.28 (s); MS (CI) for $C_9H_{11}NO_4S$ m/z 247 $(M + NH_4)^+$.

Acetic Acid 1-Acetyl-5-oxopyrrolidin-2-yl Ester (1i). A solution of acetic acid 1-acetyl-5-oxo-2,5-dihydro-1*H*-pyrrol-2-yl ester (**1a**) (0.16 g, 0.88 mmol) and 0.2 mL of triethylamine in 1 mL of ethyl acetate was hydrogenated (at normal pressure) in the presence of 10 mg of Pd/C (10%). After 1 night of stirring at room temperature the reaction was complete (as judged by ^{1}H NMR). The reaction mixture was filtered over Celite and washed with ethyl acetate. The solution was concentrated under vacuum to give almost pure **1i** in quantitative yield: ^{1}H NMR ($CDCl_3$) δ 2.12 (s, 3H, OCOC*H*$_3$), 2.05−2.19 (m, 1H), 2.31−2.45 (m, 1H), 2.54−2.70 (m, 1H), 2.58 (s, 3H, NCOC*H*$_3$), 2.82−2.94 (m, 1H), 6.85 (d, J = 5.5 Hz, 1H, OC*H*N); ^{13}C NMR ($CDCl_3$) δ 20.52 (q), 24.60 (q), 24.75 (t), 30.23 (t), 81.05 (d), 169.36 (s), 169.77 (s), 174.70 (s); MS (CI) for $C_8H_{11}NO_4$ m/z 203 $(M + NH_4)^+$.

Acetic Acid 1-Acetyl-3-methyl-5-oxo-2,5-dihydro-1*H*-pyrrol-2-yl Ester (1j). Diazomethane (ca. 12 mmol) could be obtained rapidly from *p*-tolylsulfonylmethylnitrosamide (4.00 g, 18.7 mmol of Diazald) according to a literature procedure[15] and was introduced, using a stream of nitrogen gas, into a solution of **1a** (0.37 g, 2.00 mmol) in 40 mL of dichloromethane at −10 °C. The mixture was stirred at −10 °C for 1 h and subsequently at room temperature for 1 night. The solvent was removed by passing air through the solution. The addition product of diazomethane was obtained pure as a white solid (0.44 g, 1.94 mmol, 97%), a mixture of two trans diastereomers: ^{1}H NMR ($CDCl_3$) δ 1.94 (s, 3H, OCOC*H*$_3$), 2.37 (s, 3H, NCOC*H*$_3$), 2.41−2.55 (m, 1H, C*H*CHCO), 4.65−4.74 (m, 1H, *H*CHN=N), 4.95−5.05 (m, 1H, HC*H*N=N), 5.69−5.72 (m, 1H, C*H*CO), 6.17 (s, 1H, OC*H*N); ^{13}C NMR ($CDCl_3$) δ 18.11 (q), 32.23 (q), 81.72 (t), 81.86 (d), 92.56 (d), 163.35 (s), 166.92 (s), 167.34 (s). The addition product (0.22 g, 0.97 mmol) was dissolved in 20 mL of toluene and heated at reflux for 1 night under a nitrogen atmosphere. After evaporation of the solvent a light yellow solid was obtained. Purification by flash chromatography (silica gel, 1:1 hexane/EtOAc) afforded pure **1j** (0.16 g, 0.81 mmol, 84%) as a white solid: ^{1}H NMR ($CDCl_3$) δ 1.99 (s, 3H), 2.10 (s, 3H, OCOC*H*$_3$), 2.47 (s, 3H, NCOC*H*$_3$), 5.88 (s, 1H, CH=C*H*CO), 7.05 (s, 1H, OC*H*N); ^{13}C NMR ($CDCl_3$) δ 13.54 (q), 20.29 (q), 24.25 (q), 80.50 (d), 123.27 (d), 158.20 (s), 168.28 (s), 168.72 (s), 169.41 (s); HRMS calcd for $C_9H_{11}NO_4$ m/z 197.069, found m/z 197.069.

Acetic Acid 5-Acetyl-6-oxo-3,3a,4,5,6,6a-hexahydropyrrolo[3,4-*c*]pyrazo-4-yl Ester (1k). Racemic acetic acid 1-acetyl-5-oxo-2,5-dihydro-1*H*-pyrrol-2-yl ester (**1a**) (0.18 g, 1.00 mmol) and cyclopentadiene (0.30 mL, 3.00 mmol) were dissolved in 3 mL of dry toluene, and the solution was stirred at 100 °C in a sealed flask for 6 h. After evaporation of the solvent a light yellow oil was obtained. Purification by flash chromatography (silica gel, 1: 3 hexane/EtOAc) afforded the pure Diels−Alder product **1k** (0.22 g, 0.89 mmol, 89%) as a white solid, mixture of two trans diastereomers: ^{1}H NMR ($CDCl_3$) δ 1.31−1.56 (m, 2H, C*H*$_2$), 1.97 and 1.98 (s, 3H, OCOC*H*$_3$), 2.32 and 2.33 (s, 3H, NCOC*H*$_3$), 2.56−2.62 (m, 1H, *C*HCO), 3.28−3.32 (m, 3H, C*H*CH$_2$C*H* and NCOC*H*), 6.02 (s, 1H, OC*H*N), 6.05−6.13 (m, 3H, C*H*=C*H*); ^{13}C NMR ($CDCl_3$) δ 20.58 (q), 24.84 (q), 42.90 (d), 44.90 (d), 49.84 (d), 82.64 (d), 133.88 (d), 135,48 (d), 169.48 (s), 170.00 (s), 175.90 (s). Anal. Calcd for $C_{13}H_{15}NO_4$: C, 62.64; H, 6.07; N, 5.62. Found: C, 62.84; H, 6.19; N, 5.73.

General Procedure for the Lipase-Catalyzed Transesterifications of Pyrrolinones. A typical procedure is as follows: 10 mg of immobilized enzyme (CALB) was added to a solution of 0.15 mmol of substrate and 8 mg of tridecane (internal standard) in 3 mL of hexane/*n*-BuOH (3:1). Dichloromethane was added until the solution was clear (0−0.3 mL). The suspension was stirred at room temperature for the time indicated (Table 2). During the reaction aliquots of 0.3 mL

Table 1. Atom Types and Partial Charges for the Lipase−Substrate 1a Reaction Intermediate[a]

atom name	atom type	charge
Cβ	CT	0.05
Hβ1, Hβ2	HA	0.10
Oγ	OE	−0.65
C7	CT	0.44
C8	CT	−0.07
H7, H8, H9	HA	0.12
O4	OC	−0.75
O3	OS	−0.65
C5	CT	−0.08
H3	HA	0.12
C3	CUA1	−0.20
H2	HA	0.17
C2	CUA1	−0.20
H1	HA	0.17
C1	C	0.60
O1	O	0.55
N1	NP	0.40

[a] CT = aliphatic carbon (tetrahedral), HA = aliphatic or aromatic hydrogen, OE = ether or acetal oxygen, OC = charged oxygen, OS = ester oxygen, CUA1 = carbon in double bond, first pair, C = carbonyl carbon, O = carbonyl oxygen, and NP = nitrogen in peptide, amide, or related group.

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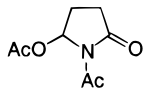
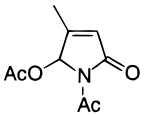
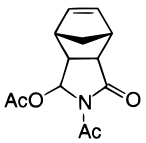
Table 2. Lipase[a]-Catalyzed Transesterification of Pyrrolinones As Illustrated in Scheme 1

product	R^1	R^2	time (h)	conversion[b] (%)	ee[c] (%) of (+)-**1**	E [d]
(+)-**1a**	CH_3	$COCH_3$	24	50	>99	>1500
(+)-**1b**	C_2H_5	COC_2H_5	72	50	>99	>1500
(+)-**1c**	CH_3	COC_2H_5	24	50	>99	>1500
(+)-**1d**	C_2H_5	$COCH_3$	24	50	>99	>1500
(+)-**1e**	CH_3	CH_3	48	67	>99	>14
(+)-**1f**	CH_3	H	20	100	nd	nd
(+)-**1g**	CH_3	C_6H_5	67	54	>99	>61
(+)-**1h**	CH_3	$COCH_2SCH_3$	23	51	99	211
(+)-**1i**			67	50	>99	>1500
(+)-**1j**			67	31	49	38
(+)-**1k**			67	0	nd	nd

[a] Commercial immobilized *Candida antarctica B* lipase (CALB) was used. [b] The conversion was determined by chiral GC using tridecane as an internal standard. [c] The ee was determined by chiral GC; >99% indicates that the other enantiomer could not be detected. [d] The E value was calculated from $E = \ln[(1 - c)(1 - ee)]/\ln[(1 - c)(1 + ee)]$. Nd denotes not determined.

were taken and diluted with 0.5 mL of acetone. This crude mixture was analyzed by GC for conversion and ee.

General Procedure for the Lipase-Catalyzed Esterifications of Pyrrolinones. A typical procedure is as follows: 20 mg of immobilized CAL was added to a solution of 20 mg of substrate and 10 mg of tridecane (internal standard) in 3 mL of hexane. To this mixture the appropriate vinyl ester (0.5 mL) was added. The mixture was heated at reflux. At given intervals aliquots of 0.3 mL were taken and diluted with 0.5 mL of acetone. This crude mixture was analyzed by GC for conversion and ee. For compounds **1l**−**p** the ee was determined after conversion to 1-acetyl-5-isopropoxy-3-pyrrolin-2-one.[16]

Acrylic Acid 1-Acetyl-5-oxo-2,5-dihydro-1*H*-pyrrol-2-yl Ester (1l). ^{1}H NMR ($CDCl_3$): δ 2.52 (s, 3H, NCOCH_3), 5.88−6.15 (m, 2H, CH_2=CH), 6.25 (d, J = 5.6 Hz, 1H, CH=CHCO), 6.41−6.50 (m, 1H, CH_2=CH), 7.16−7.22, m, 2H, OCHN and CH=CHCO). ^{13}C NMR ($CDCl_3$): δ 24.31 (q), 80.23 (d), 126.82 (d), 128.66 (d), 132.74 (t), 144.81 (d), 164.35 (s), 168.02 (s), 168.63 (s). MS (CI) for $C_9H_9NO_4$: m/z 213 $(M + NH_4)^+$.

But-2-enoic Acid 1-Acetyl-5-oxo-2,5-dihydro-1*H*-pyrrol-2-yl Ester (1m). ^{1}H NMR ($CDCl_3$): δ 1.87 (dd, J = 7.0, 1.7 Hz), 2.51 (s, 3H, NCOCH_3), 5.80 (dq, J = 1.7, 15.6 Hz, 1H, CHCO$_2$), 6.23 (dd, J = 5.8, 0.5 Hz, 1H, CH=CHCO), 7.03 (dq, J = 7.0, 15.6 Hz, 1H, CH_3CH), 7.14 (dd, J = 0.5, 2.0 Hz, 1H, OCHN), 7.18 (dd, J = 2.0, 5.8 Hz, 1H, CH=CHCO). ^{13}C NMR ($CDCl_3$): δ 17.90 (q), 24.33 (q), 80.01 (d), 120.99 (d), 128.45 (d), 145.14 (d), 147.23 (d), 147.23 (d), 164.58 (s), 168.13 (s), 168.64 (s). MS (CI) for $C_{10}H_{11}NO_4$: m/z 227 $(M + NH_4)^+$.

Decanoic Acid 1-Acetyl-5-oxo-2,5-dihydro-1*H*-pyrrol-2-yl Ester (1n). ^{1}H NMR ($CDCl_3$): δ 0.87 (t, J = 6.4 Hz, 3H), 1.26 (m, 12H), 1.56−1.66 (m, 2H), 2.35 (t, J = 7.5 Hz, 2H), 2,54 (s, 3H, NCOCH_3), 6.22 (dd, J = 0.5, 5.7 Hz, 1H, CH=CHCO), 7.11−7.18 (m, 2H, OCHN and CH=CHCO). ^{13}C NMR ($CDCl_3$): δ 13.70 (q), 24.09 (q), 22.34 (t), 24.35 (t), 28.66 (t), 28.76 (t), 28.96 (t), 29.10 (t), 31.56 (t), 33.78 (t), 80.00 (d), 128.35 (d), 144.98 (d), 168.07 (s), 168.65 (s), 172.16 (s). MS (CI) for $C_{16}H_{25}NO_4$: m/z 313 $(M + NH_4)^+$.

2,2-Dimethylpropionic Acid 1-Acetyl-5-oxo-2,5-dihydro-1*H*-pyrrol-2-yl Ester (1o). ^{1}H NMR ($CDCl_3$): δ 1.19 (s, 9H) 2.53 (s, 3H, NCOCH_3), 6.23 (dd, J = 0.5, 5.4 Hz, 1H, CH=CHCO), 7.08−7.17 (m, 2H, OCHN and CH=CHCO). ^{13}C NMR ($CDCl_3$): δ 22.00 (q), 24.39 (q), 78.03 (d), 126.14 (d), 142.63 (d), 165.72 (s), 166.03 (s), 174.51 (s). MS (CI) for $C_{11}H_{15}NO_4$: m/z 243 $(M + NH_4)^+$.

Benzoic Acid 1-Acetyl-5-oxo-2,5-dihydro-1*H*-pyrrol-2-yl Ester (1p). ^{1}H NMR ($CDCl_3$): δ 2.57 (s, 3H, NCOCH_3), 6.29 (dd, J = 0.5, 6.1 Hz, 1H, CH=CHCO), 7.30 (dd, J = 2.0, 6.1 Hz, 1H, CH=CHCO), 7.34−8.03 (m, 6H, OCHN and C_6H_5). ^{13}C NMR ($CDCl_3$): δ 24.37 (q), 80.76 (d), 128.37 (d), 128.57 (s, *i*-C), 128.71 (d), 129.80 (d), 133.57 (d), 145.03 (d), 165.05 (s), 168.16 (s), 168.65 (s). MS (CI) for $C_{13}H_{11}NO_4$: m/z 263 $(M + NH_4)^+$.

Palladium-Catalyzed Allylic Substitution of (Acyloxy)-pyrrolinones. (Acyloxy)pyrrolinones **1l**−**p** were converted to 1-acetyl-5-isopropoxy-3-pyrrolin-2-one by a palladium-catalyzed allylic substitution with 2-propanol in the presence of $Pd(CH_3CN)_2Cl_2$ (5 mol %) as was previously reported for 1-acetyl-5-acetoxy-3-pyrrolin-2-one (**1a**).[16]

2.2. Molecular Modeling of CALB−Pyrrolinone Interactions. General Procedures and Starting Structures. To provide an explanation at the molecular level for the observed differences in enantioselectivety between the various pyrrolinones, molecular modeling studies were carried out using QUANTA/CHARMm.[17] To calculate which enantiomer will be more easily converted, covalent (*R*)- and (*S*)-pyrrolinone−CALB reaction intermediates were constructed for a number of pyrrolinones, and the lowest energy conformation for each of these complexes was calculated through a systematic search.

The enzyme coordinates were taken from a 2.5 Å resolution X-ray structure of *C. antarctica* B cocrystallized with the detergent Tween 80 (obtained from the Brookhaven Protein Data Bank,[18] entry 1LBT).[12c] The protonated form of His 224 was used; coordinates for missing atoms were constructed by CHARMm. An initial energy minimization was carried out to remove internal strain by the adopted basis Newton Raphson (ABNR) algorithm until the root-mean-square (rms) energy gradient was less than 1 kcal (mol Å)$^{-1}$. A cutoff radius of 15 Å was used for the nonbonded interactions in combination with a distance dependent dielectric constant ($\epsilon = 4r$, with r in Å). Between 11 and 14 Å shift and switch smoothing functions were used for the electrostatic and van der Waals interactions, respectively. The rms deviation between the X-ray and the minimized structure for the Cα atoms was 0.05 Å.

Pyrrolinone coordinates were taken from the X-ray structure of (acyloxy)pyrrolinone **1a**.[19] Models of pyrrolinones **1c**,**g**,**l** were built on the basis of this structure. Standard atoms, bond lengths, and bond angles were used. For the partial point charges QUANTA's atom charge template method was used, and any residual charge was smoothed over carbon and nonpolar hydrogen atoms.

A lipase−pyrrolinone reaction intermediate model was constructed by adding the pyrrolinone to the lipase, removing the H atom on Ser-105 Oγ, and making a bond between Ser-105 Oγ and the carbonyl carbon (C7) of the pyrrolinone (Figure 3). The resulting tetrahedral carbon C7 and the oxygen atoms were assigned sp^3 hybridization. Partial atomic charges for this reaction intermediate were obtained from an AM1 calculation of a model system.[20] In Table 1 the atom types and partial charges for the reaction intermediate of substrate **1a** are

(16) Cuiper, A. D.; Kellogg, R. M.; Feringa, B. L. *Chem. Commun.* **1998**, 655−656.

(17) QUANTA/CHARMm, version 4.0, Molecular Simulations Inc., Burlington, MA.

(18) (a)Abola, E. E.; Bernstein, F. C.; Bryant, S. H.; Koezle, T. F.; Weng, J. Protein Data Bank. In *Crystallographic Databases Content, Software Systems, Scientific Applications*; Allen, F. H., Bergerhoff, G., Sievers, R., Eds.; Data Commission of the International Union of Crystallography: Bonn/Cambridge/Chester, 1987; pp 107−132. (b) Bernstein, F. C.; Koezle, T. F.; Williams, G. J. B.; Meyer, Jr., E. F.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. The Protein Data Bank: a Computer-based Archival file for Macromolecular Structures. *J. Mol. Biol.* **1977**, *112*, 535−542.

(19) The X-ray structure of **1a** will be published in *Acta Crystallogr. C*.

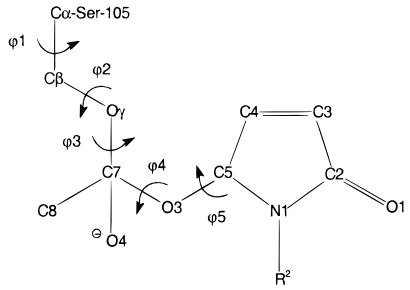


Figure 3. Atomic and torsional numbering of the lipase−substrate **1a** reaction intermediate model.

shown. For the reaction intermediates of other pyrrolinones similar partial charges were used. The OS-CT-NP and OS-CT-OC force field parameters are not available in the CHARMm force field (version 23); we copied them from the corresponding OE-CT-NP and OE-CT-OC bond angles.

Because a new asymmetric center (C7) is formed, each pyrrolinone enantiomer forms two diastereomeric reaction intermediates. Due to the presence of C5 and C7 stereocenters, for each pyrrolinone there are four diastereomers (C7(*S*) C5(*R*), C7(*S*) C5(*S*), C7(*R*) C5(*R*), and C7(*R*) C5(*S*)). To assess the energy difference between the reaction intermediates of the two enantiomers of a pyrrolinone (different stereochemistry at C5), the most favorable energy of all four diasteromeric reaction intermediates (with different stereochemistry at C7 too) has to be taken into account.

Systematic Conformational Search. To determine low-energy minima of the *C. antarctica* B−pyrrolinone reaction intermediates, a systematic conformational search protocol was employed. To that end the torsion angles around the five bonds shown in Figure 3 were varied systematically in each complex and the resulting conformations were energy minimized. The angles $\varphi 2$, $\varphi 3$, and $\varphi 4$ were varied from 0 to 360° with a grid size of 60°. The angles $\varphi 1$ and $\varphi 5$ were varied from 60 to 300° with a grid size of 120° (a smaller grid size did not reveal other minima). The resulting 1944 conformations were first subjected to 10 steps of steepest descent (SD) minimization whereby all atoms positions except those of the substrate and Ser-105 Oγ, Cβ, Hβ1, and Hβ2 were fixed. Subsequent minimization was only done if the (unconstrained) energy at that point was below a rather arbitrarily chosen energy threshold of 7000 kcal (mol)$^{-1}$. If so, another SD minimization was performed (at most 100 steps) until the rms energy gradient was less than or equal to 15.0 kcal (mol Å)$^{-1}$, followed by a maximum 1000 steps ABNR minimization until the energy gradient was less than or equal to 0.1 kcal (mol Å)$^{-1}$. The same settings for the nonbonded interactions were used as described above. After these energy minimizations the final energies were calculated without constraints using $\epsilon = r$ instead of $4r$.[21] For a single CALB−pyrrolinone reaction intermediate such a systematic search takes ca. 12 CPU h on an SGI workstation with an R10000 processor or 31 CPU h with an R5000 processor. The conformer with the lowest energy was selected for comparison with its diastereomers.

3. Results and Discussion

3.1. Enantioselective Enzymatic Synthesis. Several (acyloxy)pyrrolinones can be obtained enantiomerically pure via kinetic resolution from racemic (acyloxy)-pyrrolinones with *Candida antarctica* B by a simple and efficient method.[11] For example the (+)- or (*S*)-enantiomers[22] (+)-**1** of the pyrrolinones are obtained by an enzymatic transesterification at room temperature with *n*-butanol in an *n*-hexane/*n*-butanol (3:1) solvent mixture (Scheme 1). The results with a variety of pyrrolinones **1** are compiled in Table 2. Most products were obtained with high stereoselectivity. *N*-Alkyl and *N*-acyl substituents, variations in acyl substituents at C5, and substituents at C4 are tolerated.

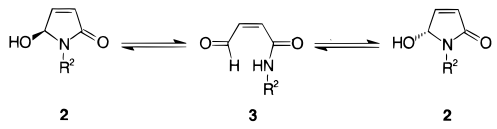
This kinetic resolution was completely enantioselective for the *N*-acyl-substituted (acyloxy)pyrrolinones **1a**−**d**,**i**. In these cases the remaining (*S*)-enantiomer was obtained enantiopure after 50% conversion and the reaction proceeded no further even on extended standing. The transesterification of **1a** was easily scaled up to 10 g[11] (and **1b** to 5 g) When the reaction of **1a** was performed at 69 °C, a considerable rate enhancement was observed, and after 1 h the conversion was 47% and the ee of the remaining substrate (*S*)-**1** 88%. This means that the reaction is still very selective ($E = 760$) even at elevated temperatures. The transesterification can also be performed with ethanol instead of *n*-butanol, but in this case the selectivity is lower. After 24 h at room temperature enantiomerically pure product (+)-**1a** is obtained, but the conversion is 54% commensurate with an enantiomeric ratio of >61. This observation is peculiar; a reviewer has suggested that perhaps ethanol relaxes the tertiary structure of the enzyme to a greater extent. Compound **1e** was converted to the corresponding hydroxypyrrolinone with much lower selectivity than found for the *N*-acylpyrrolinones. We were not able to determine the ee of compound **1f**, but the *E* value will probably be low compared to the other pyrrolinones, because both enantiomers were converted in a short time. If a phenyl group is placed on the nitrogen (compound **1g**), the stereoselectivity is higher than for **1e** with a methyl at that position, which might be due to the size of the substituent. Compared to the *N*-acyl-substituted pyrrolinones the selectivity is lower in the case of *N*-alkyl- or *N*-aryl-substituted pyrrolinones although still acceptable yields of product with ee > 99% can be obtained. Likely either the phenyl group is too large and/or the carbonyl group plays an important role in determining the enantioselectivity. This might also explain the higher selectivity for compound **1h**. In this case the substituent on nitrogen is large although it also contains a carbonyl moiety. Although a 4-methyl substituent on the pyrrolinone **1j** is tolerated, the reaction becomes much slower and less stereoselective compared to the unsubstituted analogue

(20) The structure of the substrate−protein complex was previously optimized with CHARMm. From this structure, residues in close contact (cutoff = 5 Å) with the substrate were selected (selected residues: Gly39, Thr40, Gly41, Thr42, Ser47, Trp104, Ser105, Gln106, Gly107, Asp134, Thr138, Val154, Gln157, Ile189, Val190, His224, Leu227, Leu278, Ala281, Ala282, Ile285). The structure was edited manually: all peptidic CO and NH at the edges of the selected residues were removed and the hydrogens were added in their place for completeness. Atomic charges for the entire system (307 atoms) were obtained at the AM1 semiemperical level (Dewar, M. J. S.; Zoebisch, E. G.; Healy, E. F.; Steward, J. P. P. *J. Am. Chem. Soc.* **1985**, *107*, 3902) as implemented in the program Gaussian94 (Fritsch, M. J.; Trucks, G. W.; Schlegel, H. B.; Gill, P. M. W.; Johnson, B. G.; Robb, M. A.; Cheeseman, J. R.; Keith, T. A.; Petersson, G. A.; Montgomery, J. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Ciolowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Repogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Steward, J. J. P.; Head-Gordon, M.; Gonzalez, C.; Pople, J. A. *GAUSSIAN 94, Revision E.2*; Gaussian, Inc.: Pittsburgh, PA, 1995).

(21) Grootenhuis, P. D. J.; Galen van, P. Correlation of Binding Affinities with Non-bonded Interaction Energies of Thrombin-Inhibitor Complexes. *Acta Crystallogr.* **1995**, *D51*, 560−566.

(22) The absolute configurations were determined by X-ray analysis of a pyrrolinone−iron carbonyl complex (for **1a**) and by a new CD method (for **1a**−**e**): Cuiper, A. D.; Brzostowska, M.; Gawronski, J. K.; Smeets, W. J. J.; Spek, A. L.; Hiemstra, H.; Kellogg, R. M.; Feringa, B. L. *J. Org. Chem.* **1999**, *64*, 2567−2570.

Scheme 3



2 **3** **2**

1a. Compound **1k** did not undergo detectable reaction, probably due to steric effects.

In all the transesterifications the hydroxypyrrolinone product **2** was obtained as a racemate (in about 50% yield) and can be used as a substrate in the reverse reaction with the same enzyme, the lipase-catalyzed esterification (Scheme 2). In this reaction the (−)- or (*R*)-enantiomers of the 5-acyloxy-2(5*H*)-pyrrolinones **1** are obtained enantiomerically pure. From the studies of the transesterification we observed that the hydroxypyrrolinone starting material **2** could be racemized under certain conditions, probably owing to ring opening at C5 (Scheme 3).

If the hydroxypyrrolinone can be racemized in situ, it is in principle possible to obtain an enantiomerically pure product (−)-**1** from the esterification in excess of 50% yield. When this reaction was performed with vinyl acetate at room temperature in several solvents and solvent mixtures (vinyl acetate, vinyl acetate/hexane, butyl acetate, butyl acetate/hexane, ethyl acetate, ethyl acetate/hexane, ethyl acetate/cyclohexane, ethyl acetate/toluene dichloromethane, dichloromethane/hexane, hexane, cyclohexane, toluene), the conversions were about 50% after 48 h, but after 7 d they were still 50−55% although in all cases the ee of the ester was >99%. This implies that the reaction is highly selective in all solvents examined but that there is almost no racemization at room temperature under the conditions used. However, when the reaction was performed in hexane at 40 °C the conversion was 99% after 72 h (ee >99%). The reaction rate could be enhanced drastically by further raising the reaction temperature to 69 °C. The product was obtained after 18 h with 100% conversion and >99% ee. It should be emphasized that the experimental procedure is extremely versatile. Esterification can be accomplished by heating the hydroxypyrrolinone **2** with the immobilized enzyme in hexane followed by filtration to remove the enzyme and removal of the solvent. Enantiopure product is obtained quantitatively. In this way it is possible to carry out a complete resolution by coupling the kinetic resolution to an asymmetric transformation (second-order asymmetric transformation or dynamic kinetic resolution[23]). Most of the pyrrolinones were obtained with complete conversion and ee's up to >99% (Table 3).

As with the transesterification the *N*-acyl-substituted (acyloxy)pyrrolinones **1a**−**d** were the most successful substrates. The enzymatic synthesis of **1a** was scaled up to 5 g. Compound **1e** was obtained with a very low ee. Because the enantioselectivity in the transesterification was also low, we expected a low ee that drops with the conversion because both enantiomers can be converted, only with a different rate. Furthermore the selectivity

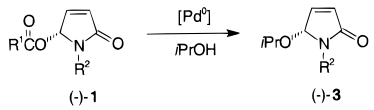
(23) (a) Morrison, J. D. In *Asymmetric Synthesis*; Academic Press: New York, 1983; Vol. 1, p 3. (b) Eliel, E. L.; Wilen, S. H. *Stereochemistry of Organic Compounds*; Wiley-Interscience: New York, 1994; p 414. (c) Ward, R. S. *Tetrahedron: Asymmetry* **1995**, *6*, 1475. (d) Brand, S. A.; Jones, M. F.; Rayner, C. M. *Tetrahedron Lett.* **1995**, *36*, 8493−8496. (e) Tan, D. S.; Günter, M. M.; Drueckhammer, D. G. *J. Am. Chem. Soc.* **1995**, *117*, 9093−9094. (f) Stürmer, R. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1173−1174.

Table 3. Lipase[a]-Catalyzed Esterification of Pyrrolinones As Illustrated in Scheme 2

product	R^1	R^2	time (h)	conversion[b] (%)	ee[c] (%) of (−)-**1**
(−)-**1a**	CH_3	$COCH_3$	18	100	>99
(−)-**1b**	C_2H_5	COC_2H_5	18	100	>99
(−)-**1c**	CH_3	COC_2H_5	18	100	>99
(−)-**1d**	C_2H_5	$COCH_3$	18	100	>99
(−)-**1e**	CH_3	CH_3	18	30	13
(−)-**1f**	CH_3	H	18	100	0[d]
(−)-**1l**	CH_2=CH	$COCH_3$	62[e]	96	>75[f]
(−)-**1m**	CH_3CH=CH	$COCH_3$	62[e]	98	>96[f]
(−)-**1n**	C_9H_{19}	$COCH_3$	62[e]	98	>94[f]
(−)-**1o**	*t*-Bu	$COCH_3$	62[e]	47	>95[f]
(−)-**1p**	C_6H_5	$COCH_3$	62[e]	19	>93[f]

[a] Commercial immobilized *Candida antarctica* B lipase (CALB) was used. [b] The conversion was determined by chiral GC using tridecane as an internal standard. [c] The ee was determined by chiral GC; >99% indicates that the other enantiomer could not be detected. [d] The optical rotation was $[\alpha]_D \sim 0.0$. [e] The reactions were performed in a hexane/dichloromethane solvent mixture. [f] The ee was determined after conversion to 1-acetyl-5-isopropoxy-3-pyrrolin-2-one[16] and is therefore a lower limit value of the ee.

Scheme 4



(-)-**1** (-)-**3**

decreases when this reaction is performed at 69 °C. When the reaction was performed at room temperature, the conversion was 60% after 24 h with an ee of 38%. Most remarkable is the fact that the reaction is much slower than with the other pyrrolinones and that the rate is higher at room temperature than at 69 °C. At present we have no explanation for this peculiar behavior of **1e**. Pyrrolinone **1f** was obtained as a racemate with 100% conversion within 18 h at 69 °C. Although we did not succeed in finding a method to determine the ee by chiral GC or HPLC, there is little chance of enantioselectivity because no optical rotation was detected. However, when the reaction was performed at room temperature, 37% conversion in 8 d was achieved, and the product showed an optical rotation of $[\alpha]_D = (+)\text{-}13°$ ($c = 1$, $CHCl_3$). The reaction is slow due to the poor solubility of the starting material. Note that for this substrate the (+)-enantiomer is formed in the esterification in contrast to the (−)-enantiomer that is obtained with the other pyrrolinones.

In addition to acetic and propionic esters **1a**−**f** the formation of a variety of other esters **1l**−**p** was examined. They were made as new enantiomerically enriched compounds in the lipase-catalyzed esterification, and we did not succeed in determining the ee by chiral GC or HPLC. Therefore these pyrrolinones were converted to a known compound for which the ee could be easily established. A palladium-catalyzed allylic substitution with 2-propanol in the presence of $Pd(CH_3CN)_2Cl_2$ (5 mol %) was used to convert the esters **1l**−**p** to 1-acetyl-5-isopropoxy-3-pyrrolin-2-one with retention of configuration (Scheme 4).[16]

Because it cannot be excluded that a slight loss of enantioselectivity has taken place, the ee's of compounds **1l**−**p** given in Table 3 are minimum values. From these values it is seen that these pyrrolinones were formed with high stereoselectivity in the enzymatic esterification. The reaction time was long compared to other pyrrolinones because hexane/dichloromethane was used as a solvent mixture (to follow the reactions in time by GC the

Figure 4. (a) Kazlauskas enantioselectivity rule for esters of secondary alcohols. The enantiomer shown reacts faster. (b) For pyrrolinones, the (*R*)-enantiomer shown reacts faster (or is the only reacting enantiomer: compounds **1a**−**d**,**l**), except for the case where R = R′ = H (compound **1f**).

starting material must be dissolved completely), which made the reflux temperature much lower. The enzymatic syntheses of **1o**,**p** were even slower.

If we compare the transesterification (Scheme 1) and the esterification (Scheme 2) of pyrrolinones, we can see that pyrrolinones that are converted to the corresponding hydroxypyrrolinones with high stereoselectivity in a transesterification can be obtained enantiomerically pure in an esterification at 69 °C (Tables 2 and 3). For pyrrolinones that show low to moderate selectivity in the transesterification, however, the ee's are very low in the related esterification at 69 °C.

To rationalize why some pyrrolinones are converted with higher enantioselectivity than others, we might apply the rule of Kazlauskas.[24] This empirical rule is an extension of Prelog's rule[25] and predicts which enantiomer of a secondary alcohol is converted faster by a lipase. The rule is based on the assumption that the enantioselectivity is proportional to the difference in size between the large (L) and the medium (M) substituent depicted in Figure 4a. For pyrrolinones the large group is the amide in the ring with its N-substituent. It should be noted that the absolute configuration of a number of pyrrolinones was unequivocally established by a new method[22] and the (−)-enantiomer always has the (*R*) configuration. The (*R*)- or (−)-enantiomer shown in Figure 4b is the faster reacting enantiomer. As can be seen from the results in Table 2, pyrrolinones with a substituent on nitrogen and no substituent at C4 (in Figure **4b**, R = H), thus having a large difference in size between L and M, are converted with high enantioselectivity. In contrast compounds with no substituent on N, like **1f** (R′ = H) or a methyl substituent like **1e** (R′ = CH_3), thus having a small difference in size between L and M, are converted with low or no enantioselectivity. If compounds **1a**,**j** are compared, it is seen that the introduction of a methyl substituent on C3 (in Figure **4b**, R = CH_3 compared with R = H and R′ = $COCH_3$) results in a dramatic decrease in enantioselectivity. This can be explained by the loss of difference in size between L and M.

3.2. Molecular Modeling of CALB−Pyrrolinone Interactions. As described in the Methods section, for four pyrrolinones reaction intermediates with CALB were constructed and the lowest energy for each of the four diastereomers per CALB−pyrrolinone complex was calculated. The results are summarized in Table 4, and in Figure 5 pictures are shown for the pyrrolinone **1a**−CALB complexes.

For pyrrolinones **1a**,**c**,**g** an (*R*)-configuration at C5 is favored, which implies that the (*R*)-enantiomer of the pyrrolinone will be converted more readily than the (*S*)-enantiomer. This is in good agreement with the experimentally observed enantioselectivities for these substrates (Tables 2 and 3). However, the quantitative comparison between these three pyrrolinones is not correct: in Table 2 it is seen that $E_{1a} \approx E_{1c} > E_{1g}$, which indicates that the energy difference between the best C5(*R*) and C5(*S*) diastereomers for **1g** should be smaller than for **1a**,**c**. This is not the case, so the calculated selectivity for compound **1g** is too high compared with the experimental results on the enantioselectivity of the reaction.

Table 4. Relative Energies of Diastereomeric Reaction Intermediates of 1a,c,g,l[a]

		ΔE_{min}			
substrate	R^2	**A**, C7(*S*) C5(*R*)	**B**, C7(*S*) C5(*S*)	**C**, C7(*R*) C5(*R*)	**D**, C7(*R*) C5(*S*)
1a	CH_3	0.00	3.31	14.56	16.04
1c	C_2H_5	0.00	5.68	14.26	15.49
1g	C_6H_5	0.00	10.96	15.10	16.99
1l	H	1.50	0.00	11.55	12.69

[a] The second-best conformations were always at least 3 kcal/mol higher in energy. The energies are given relative to the lowest energy for each pyrrolinone.

For compound **1l** the C5(*S*) enantiomer has the lowest energy, whereas the energy difference between the most favorable C5(*S*) and C5(*R*) diastereomers is relatively small. This implies a low enantioselectivity as well as a preference for the other enantiomer (the (*S*)-pyrrolinone). The low selectivity agrees with the experimental data for the esterification (Table 3). The small optical rotation found in a room-temperature reaction has a positive sign, in contrast to the sign of the optical rotation found for all other pyrrolinone esters. Therefore, here too our calculations agree well with the experimental data.

Our approach to calculate which enantiomer of a pyrrolinone will be more easily converted differs mainly in two aspects from published molecular modeling studies of *C. antarctica*−,[12c,13] *chymotrypsin*−,[26] and other lipase[27]−substrate interactions. First, in those studies the configurational space was not sampled systematically as we have done but in a manner which was influenced by the choice of the starting structure. Second, and more importantly, in other studies the (*S*)-configuration at C7 of the reaction intermediate has not been taken into account: the modeling is based on the (*R*)-configuration of the inhibitor−CALB crystal structure.[12c,18,28] We have studied both configurations at C7 and have concluded that the (*S*)-configuration is clearly more favorable for our pyrrolinone−CALB reaction intermediates (see Table 4). A possible explanation could lie in the nature of our substrates. Although the configuration at C7 is different from what is observed in the crystal structure, the overall conformations are rather similar. Both the most favorable CALB−**1a** reaction intermediate and the phosphate inhibitor−CALB complex are shown in Figure 6. Furthermore, the two diastereomers of the pyrrolinone **1a**−CALB complex that have an (*S*)-configuration at C7 show the previously reported[13] hydrogen bonds (with Gln-106, Thr-40, and His-224; see Figure 5) for a substrate−CALB complex, whereas not all of these can be found in the complexes with an (*R*)-configuration at C7.

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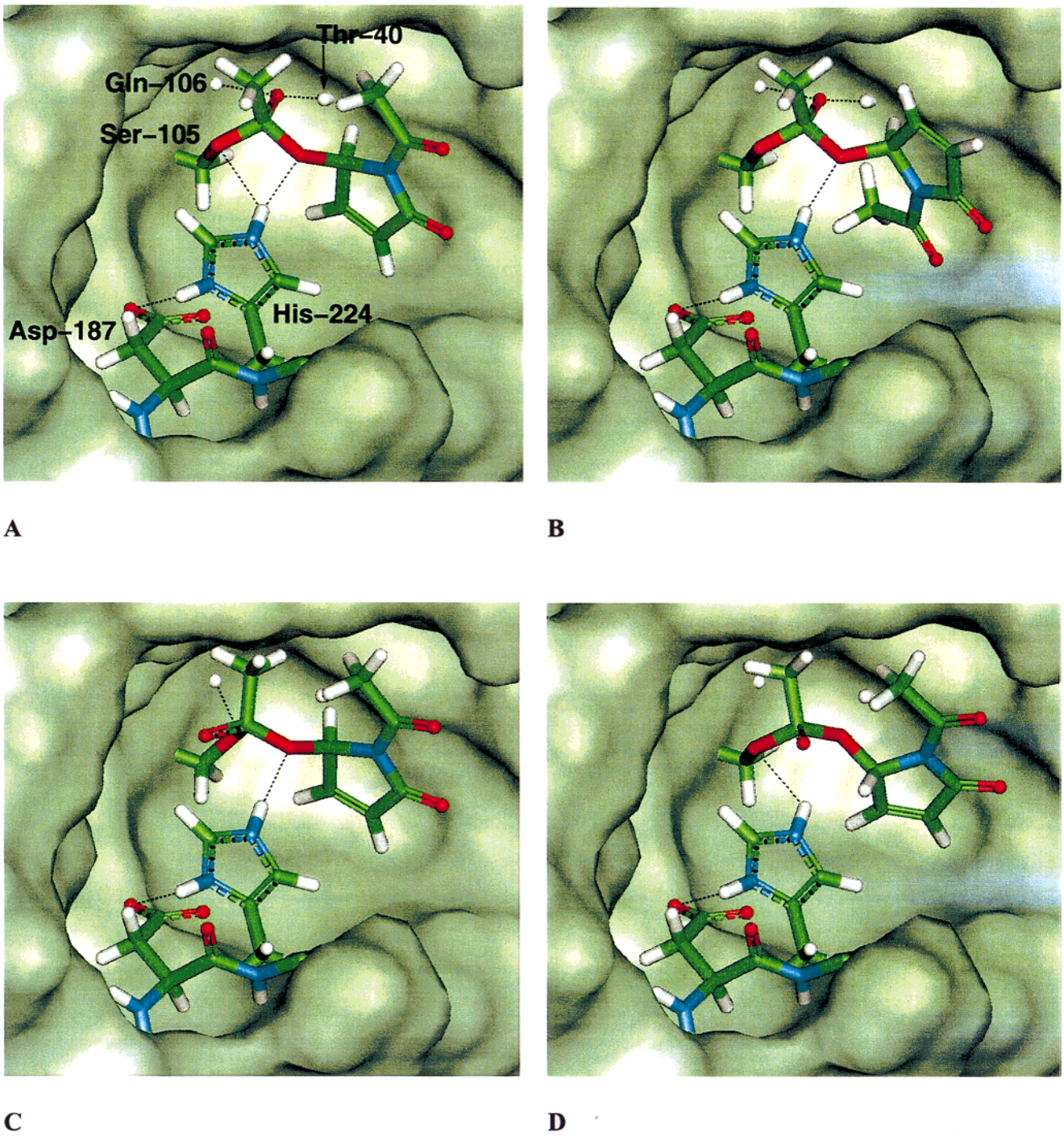

Figure 5. Lowest energy conformations of the diastereomeric pyrrolinone **1a**−CALB reaction intermediates: (A) C7(*S*) C5(*R*); (B) C7(*S*) C5(*S*); (C) C7(*R*) C5(*R*); (D) C7(*R*) C5(*S*).

Figure 5A,B can be used to interpret the preference for the (*R*)-enantiomer for substrates **1a**,**c**,**g** (the conformations and hydrogen bonds of all corresponding diastereomers of pyrrolinones **1a**,**c**,**g** are identical, and no hydrogen bonds were found between substituents on nitrogen and the enzyme). Although the same hydrogen bonds are formed, the ring of the (*S*)-enantiomer is oriented differently, with the substituent on nitrogen having unfavorable electrostatic interactions with His224 and Gly39, whereas in the case of an (*R*)-enantiomer this substituent points toward the protein surface and interacts favorably with Ser105, Thr40, and Asp134. This effect is even more dramatic for substrates **1c**,**g**, because of the enhanced size of the substituent on nitrogen. This corresponds to the finding of Orrenius et al.[13b] that the (*S*)-enantiomer of a secondary alcohol is forced to place its larger substituent in the stereospecificity pocket (mainly consisting of residues Trp104, Pro38, Gly39, Thr40, Thr42, Ser47, and Ala281). For substrate **1l** the conformations and hydrogen bonds of diastereomers **A**−**C** are almost identical to those of substrate **1a**. Only in diastereomer **B** can the pyrrolinone ring enter the pocket in the active site more deeply, because there is no substituent on nitrogen pointing in that direction. This

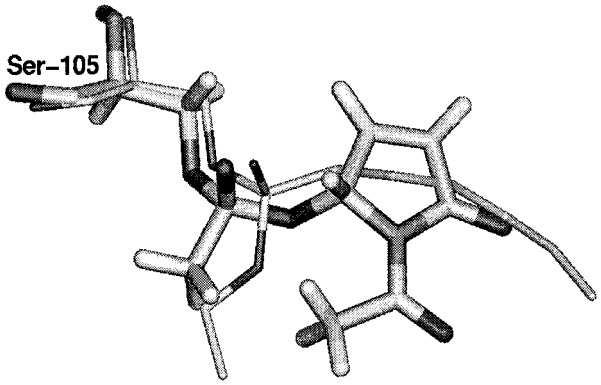

Figure 6. Conformations of lowest energy pyrrolinone **1a**−CALB reaction intermediate and the phosphate inhibitor−CALB complex.

may explain the small difference in energy between diastereomers **A** and **B** for pyrrolinone **1l**. Diastereomer **D** has its pyrrolinone ring also in that orientation (in contrast to the other pyrrolinones), and there again is only a rather small energy difference between diastereomers **C** and **D**.

Of course, the methodology we have used to explain the stereoselectivity of *C. antarctica* lipase B for the (−)- or (*R*)-enantiomers of (acyloxy)pyrrolinones can be improved. More atoms could be taken into account during the systematic search, dynamical instead of statical calculations can be done, water molecules can be included explicitly instead of implicitly (distance-dependent dielectric constant), etc. However, the methodology used here is fast, simple, and extensive, and systematic errors will cancel each other because energy differences between diastereomers are calculated. Therefore, it can be very useful in predicting which (new) compounds will be converted enantioselectively.

4. Conclusions

For most of the studied (acyloxy)pyrrolinones it is possible to obtain either enantiomer (with ee's up to >99%, in excellent yield) with the use of a single enzyme, simply by variation of procedure between transesterification and esterification.

A systematic conformational analysis has been used to give an explanation for the stereoselectivity of *C. antarctica* lipase B for the (*R*)-enantiomers of most (acyloxy)pyrrolinones. The size of the substituent on nitrogen plays an important role in the control of the enantioselectivity. A qualitative agreement was found between the results from empirical models and molecular modeling and the experimental results.

Although only an (*R*)-configuration in the reaction intermediates at C7 has been used in *C. antarctica* B−substrate reaction intermediates in modeling studies reported earlier, it is very likely that for the *C. antarctica* B−pyrrolinone reaction intermediates an (*S*)-configuration at C7 is preferred.

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