Schiff Bases of Amino Acid Esters as New Substrates for the Enantioselective Enzymatic Hydrolysis and Accompanied Asymmetric Transformations in Aqueous Organic Solvents[1,2]

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The enzyme (lipases and chymotrypsin)-catalyzed hydrolysis of Schiff bases derived from racemic amino acid esters and aromatic aldehydes has been investigated. The reactions were successfully carried out in different aqueous organic solvents at ambient temperature, but the aqueous acetonitrile (5.4% water content by volume) was the solvent of choice. The L-amino acid (ee 98%) precipitated out from the solution as the reaction progressed, and the liberated aldehyde and unhydrolyzed D-ester (ee 40−98%) remained in the solution. The range of substrates included amino acids having different types of side chains. The addition of an organic base (DABCO) into the solution resulted in the racemization of the remaining D-ester and the additional hydrolysis of the substrate, thus leading to the effective asymmetric transformation of the initial ester. Upto 87.5% of the initial racemate was converted into the L-enantiomer.

Introduction

Enzymes have been widely used for the resolution of racemic organic compounds.[3,4] The use of acylases derived from different sources for the resolution of α-amino acids is well documented and broadly employed both in chemical laboratories and industry.[5] Recently it has been shown from our laboratories and by other groups of workers that some proteases and lipases are good catalysts for the enantioselective hydrolysis of amino acid derivatives.[6] Another traditional proteolytic enzyme, chymotrypsin, was used to resolve hydrophobic N-protected amino acids esters and free amino acids esters,[7] but its application was limited in scope due partly to high instability of the free amino acids esters; after a week, more than 50% of the free amino acid ester was converted to diketopiperazine and other polycondensation products.[8]

We put ourselves a task of elaborating a new resolution procedure based on the use of chymotrypsin and lipases as catalysts and Schiff bases derived from aromatic aldehydes and racemic amino acids esters as substrates. The envisaged advantages of these substrates are the lability of the N-protective group, their increased solubility in organic and aqueous−organic solvents, and high α-C−H bond acidity of the amino acid moiety. The latter properties might be employed to bring about the racemization of the unhydrolyzed enantiomer and thus induce asymmetric transformation[9] of the initial racemic Schiff base during the course of hydrolysis. This paper reports a successful application of this approach to the resolution of Phe, substituted Phe, Ala, nor-Val, and α-Me-Phe. It has been shown that the asymmetric transformation of Phe could be carried out, and L-Phe was obtained in 87.5% yield from the racemic Schiff base derived from *p*-chlorobenzaldehyde and D,L-Phe ester when the chymotrypsin-catalyzed hydrolysis was carried out in the presence of DABCO in aqueous organic solvents.

Results and Discussion

The Schiff bases were prepared according to the literature procedures from the corresponding amino acid ester and the aldehyde.[10] The Schiff bases of 4-F-D,L-Phe, and 2-F-D,L-Phe were prepared by alkylation of the corresponding Schiff base with 4-fluoro- and 2-fluorobenzyl bromide, respectively. The Schiff bases of D,L-Ala, D,L-nor-Val, D,L-Phe, 4-F-D,L-Phe, 2-F-D,L-Phe, and D,L-α-Me-Phe ethyl esters with benzaldehyde were also prepared by direct alkylation of the Schiff bases derived from the corresponding aldehyde and the glycine ester[11] (Scheme 1). The Schiff bases of D,L-Ph-Gly-OEt, D,L-nor-Val-OEt, D,L-Trp-OEt, D,L-4-F-Phe-OEt, and D,L-2-F-Phe-OEt have been prepared for the first time.

The reaction protocol was a very simple one. Chymotrypsin (10^{-7}−10^{-6} mol) or a lipase was added as an insoluble powder to the aqueous organic solvent (1:19,

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(2) Part of the results have been presented at the 10th IUPAC International Conference on Organic Synthesis, Bangalore, India, December 11−16, 1994.

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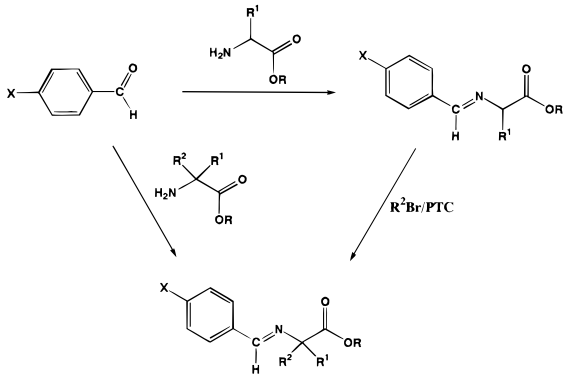
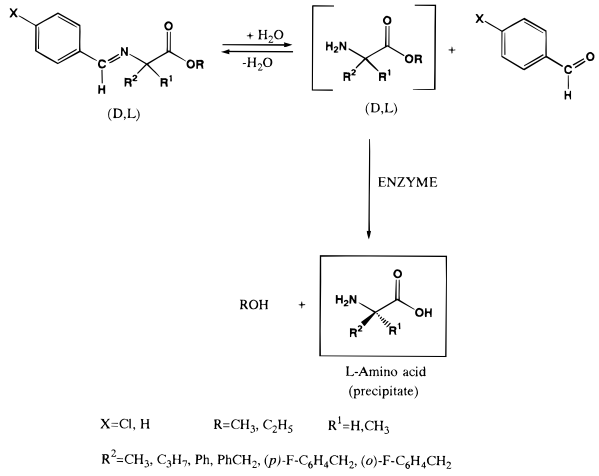
Scheme 1



X=Cl, H
R=CH_3, C_2H_5
R^1=H, CH_3
R^2=CH_3, C_3H_7, Ph, $PhCH_2$, (*p*)-F-$C_6H_4CH_2$, (*o*)-F-$C_6H_4CH_2$

Scheme 2



X=Cl, H R=CH_3, C_2H_5 R^1=H,CH_3
R^2=CH_3, C_3H_7, Ph, $PhCH_2$, (*p*)-F-$C_6H_4CH_2$, (*o*)-F-$C_6H_4CH_2$

Table 1. Hydrolysis of Schiff Bases of α-Amino Acids Ethyl Esters (see Scheme 1) Catalyzed by Chymotrypsin in Aqueous Organic Solvents at Ambient Temperature

						precipitate[a] L-amino acid		filtrate[a] D-amino acid[b]	
run	X	R^1	R^2	time (h)	aqueous solvent (1:19)	yield, %	*ee*, %	yield, %	*ee*, %
1	Cl	H	$PhCH_2$	24	t-BuOH	14	98	79	11
2	Cl	H	$PhCH_2$	65	t-BuOH	36.5	98	59	69
3	Cl	H	$PhCH_2$	260	t-BuOH	46.5	97	52	81
4	H	H	$PhCH_2$	30	$MeNO_2$	20	98	–	–
5	H	H	$PhCH_2$	24	MeCN	38	99	37	95
6	H	H	*p*-$FC_6H_4CH_2$	48	MeCN	37	99.5	40	95
7	H	H	*o*-$FC_6H_4CH_2$	24	MeCN	21	98.5	59	49
8	H	H	Me	108	MeCN	30	85	46	36
9	H	H	C_3H_7	208	MeCN	33	92	40	40
10	H	CH_3	$PhCH_2$	200	MeCN	25	99.5	50	29
11	H	H	Ph	240	MeCN	29	89	40.5	5.5
12	Cl	H	Ph	240	MeCN	36	5	37	2
13	H	H	PhCH(OH)	200	MeCN	0	–	100	0

[a] Chemical yields and enantiomeric analyses were performed by GLC; however, the isolated yields were only 2−3% less from those determined by GLC. [b] The D−amino acid was obtained by hydrolysis of the remaining Schiff's base by 6 N HCl.

8−10 mL) immediately followed by the substrate (10^{-2}−10^{-3} mol) with stirring at ambient temperature. The addition of any salts or buffer components into the reaction mixture was found to be unnecessary. The amount of water in the organic solvent was kept at a 20−30 fold molar excess over the substrate and it was enough for the hydrolysis to proceed. At the same time, the content of water in the organic solvent was not sufficient to dissolve the precipitated amino acid or chymotrypsin. As the reaction progressed, the amount of white precipitate in the reaction mixture increased. The chiral GLC and ^{1}H NMR spectral analysis of the precipitate and the filterate indicated that it was the free L-amino acid (ee 98%) which was insoluble, and derivatives of D-amino acid ester stayed in the solution. No side reaction products were detected by NMR either in solution or in the precipitate. The enantiomeric purity of the unhydrolyzed amino acid ester depended on the extent of the reaction, as illustrated by the data on the chymotrypsin-catalyzed hydrolysis of the Schiff base of phenylalanine ester (Table 1) in aqueous t-BuOH. Table 1 shows the chemical yields of the resolved amino acids as calculated, assuming that the theoretically possible yields of the enantiomers of amino acids were 50% for the L-enantiomer and 50% for the D-enantiomer.

The enantiomeric purities of the product amino acids were insensitive to the method of preparation of the Schiff bases. However, it was desirable to distill the aldimine substrate *in vacuo* before applying the resolution procedure. The use of the alkylation reaction mixture without prior distillation decreased the yields of both L- and D-amino acids.

Chymotrypsin retained its activity for several days in the aqueous organic solvents as was shown by addition of new portions of the fresh substrate after the initial one was completely hydrolyzed. The formation of the additional amino acid precipitate was detected even if the fresh substrate was added after 5 days since the beginning of the experiment.

The rate of the reaction depends on the type of the solvent used and was approximately proportional to the rate of dissociation of the initial Schiff base into the components (free ester and aldehyde), as measured by ^{1}H NMR spectroscopy under the experimental conditions without adding the enzyme. The results may be rationalized assuming that the real substrates were the free amino acids esters that were liberated from the Schiff bases by reaction with water (see Scheme 2) and the dissociation of the Schiff bases was the rate-limiting process. An equimolar mixture of D,L-phenylalanine ethyl ester and *p*-chlorobenzaldehyde (instead of the preformed Schiff base of the two), under similar conditions in the presence of added DABCO, yielded only 40.5% of L-phenylalanine. The reaction stopped after this conversion, and no additional L-phenylalanine could be obtained. The low yield might be due to the instability of the free amino acid ester and its tendency to rearrange into diketopiperazine kind of compounds.[8] Further, the dissociation of Schiff bases in the reaction mixture, which is the rate-limiting process, also controls the concentration of the free amino acid esters so that their rearrangement/cyclization is minimized.

Different solvents were successfully used for the enantioselective hydrolysis as illustrated by the results in Table 2. Aqueous acetonitrile was probably the solvent of choice providing faster reaction rates, better chemical yields, and enantiomeric purity of both the amino acid and the unhydrolyzed ester.

The Schiff bases of aromatic, as well as of aliphatic amino acid esters were utilized as substrates for the chymotrypsin-catalyzed hydrolysis, although the enzymatic hydrolysis of aliphatic amino acid esters proceeds 1 order of magnitude slower than that of the aromatic

Table 2. Hydrolysis of the Schiff's Base Derived from *p*-Chlorobenzaldehyde and D,L-Phe-OEt with Different Enzymes

enzyme	aqueous solvent (1:19)	time (h)	L-Phe[a] yield, %	L-Phe[a] *ee*, %	D-Phe[a] yield, %	D-Phe[a] *ee*, %
PPL	MeCN	70	29.5[b]	92	69[b]	14
PPL	Me_2CO	24	26.5[b]	99	23[b]	49
PPL	t-BuOH	70	41[b]	91	52[b]	16
PPL	MeCN	66	14	99.5	63	14
PPL	MeCN	120	18	99	23	49
PPL	t-BuOH	144	14	97	60	28
PPL	THF	120	19	98.5	75.5	11
Amano P	t-BuOH	96	43.5[b]	96	45.5	34
CCL	Me_2CO	144	18[b]	73	61[b]	6

[a] The enantiomeric purity and chemical yields were established by using chiral GLC; however, the isolated yields were only 2−3% less from those determined by GLC. [b] Determined by weighing the sample before purifying it on Sephadex G-25.

ones. The only Schiff base that failed to hydrolyze was the one derived from phenylserine ester; the possible explanation of the phenomenon might be the formation of the oxazolidine ring via the interaction of the C=N bond with the OH group of the substrate side chain. However, the compound (Schiff base) was stable under the experimental conditions, and no dissociation producing the amino acid ester (i.e. phenyl serine ethyl ester) took place.

Partial racemization of the remaining ester accompanied the resolution of the Schiff base derived from phenylglycine ester and benzaldehyde catalyzed by chymotrypsin on prolonged incubation (run 11, Table 1). A greater C−H bond acidity of phenylglycine relative to other α-amino acids might be the underlying reason for this observation.[9] A better electron-withdrawing aldehyde component *viz*. *p*-chlorobenzaldehyde under similar conditions brought about, in addition, the racemization of the L-amino acid itself (run 12, Table 1). The PPL, CCL, and Amano P-lipases were also used for the resolution purposes (Table 2), but the effectiveness of the process was not as good as in the case of chymotrypsin-catalyzed reactions, probably because the ethyl esters are not that good substrates for the lipases.[6]

We believe that the choice of appropriate reaction conditions and use of additional bases might induce selective racemization of the esters leaving the precipitated amino acids intact, since the acidities of the amino acid Schiff bases were, at least, 3 orders of magnitude smaller than the acidity of the corresponding ester derivatives and because the precipitation of the amino acid removes it from the reaction mixture and its recombination with the aromatic aldehyde gives insignificant amount of the corresponding Schiff base. For amino acids which are weak C−H acids and for short intervals of the reaction, the effect is almost negligible. The case of phenylglycine hydrolysis is an exception because the amino acid is a relatively strong C−H acid and significant racemization of the precipitated amino acid was observed (run 11, Table 1).

Keeping in view the acidities of Schiff bases and the fact that the enzymatic hydrolysis of the initial substrate takes place enantioselectively, we reasoned that the remaining unhydrolyzed substrate might undergo base-catalyzed racemization in the presence of the enantiomerically enriched amino acid without any loss of enantiomeric purity of the latter. This concept has been proved as we have successfully carried out the asymmetric transformation of the Schiff base derived from

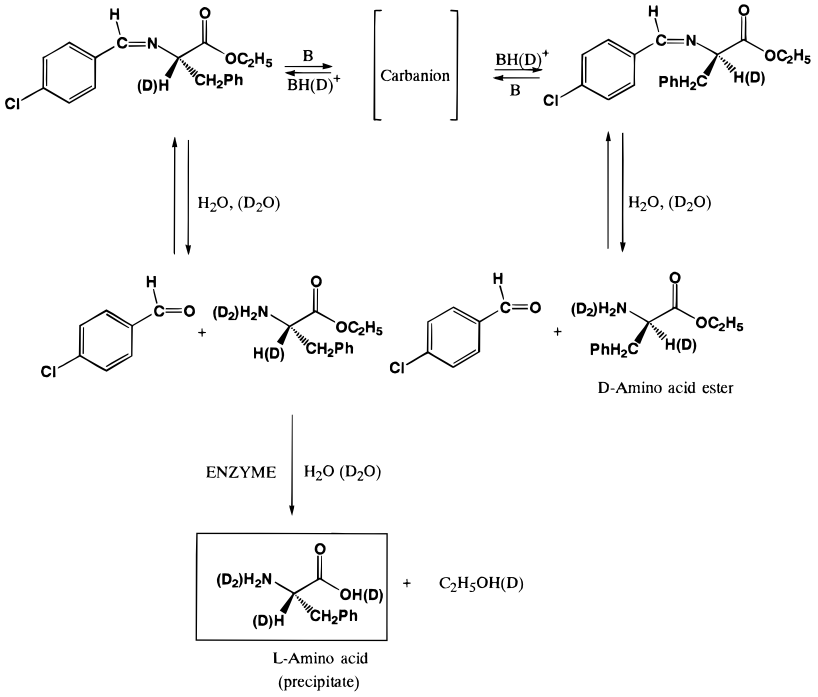
Table 3. Hydrolysis of Schiff's Base of D,L-Phe-OEt with *p*-Chlorobenzaldehyde (see Scheme 3) in Aqueous Organic Solvents at Ambient Temperature in the Presence of DABCO

run	enzyme	DABCO[a]	time (h)	aqueous solvent (1:19)	precipitate[b] L-amino acid yield, %	precipitate[b] L-amino acid *ee*, %	filtrate[b] D-amino acid yield, %	filtrate[b] D-amino acid *ee*, %[c]
1	chymotrypsin	5	24	MeCN	30	92.5	41.5	14
2	chymotrypsin	10	24	MeCN	59.5	87.5	14.5	3
3	chymotrypsin	5	96	Me_2CO	62	90	32	0
4	chymotrypsin	5	96	t-BuOH	58	99.5	30	4
5	chymotrypsin	10	96	t-BuOH	63	94	25	1
6	PPL	10[d]	240	Me_2CO	27	91.5	65	19
7	PPL	10[d]	240	MeCN	17	96	40	2
8	Amano P	10[d]	230	t-BuOH	45.5	92	–	–
9	chymotrypsin[e]	10	168	MeCN	78	86	13	3.5[g]
10	chymotrypsin[f]	10	96	MeCN	87.5	90	6.8	0.6[g]

[a] Mole percent in relation to the substrate. [b] The chemical yields and enantiomeric analyses were performed by GLC; however, the isolated yields were only 2−3% less from those determined by GLC. [c] The *ee* values are lower, probably because the racemic Schiff base (obtained after the attainment of optimum equilibrium state) is chemically hydrolyzed by 6 N HCl. [d] 1,8-Diaminooctane (10 mol %) in relation to the substrate was used in this case. [e] Reaction was run for 96 h without adding DABCO, and then enzyme was filtered off and filtrate concentrated *in vacuo*. The concentrated filtrate was subjected to hydrolysis with a fresh lot of α-chymotrypsin and DABCO (10 mol %), and the contents were stirred for 72 h. The yield is reported for the combined amino acid obtained in two lots. [f] Reaction was run for 48 h without adding DABCO, and then the precipitate was filtered off and the filtrate concentrated *in vacuo*; DABCO solution in MeCN was added to the residue and the mixture was kept for 48 h. Then a fresh portion of aqueous chymotrypsin solution was added, and the mixture was agitated for another 48 h. [g] *ee* of L-enantiomer.

racemic phenylalanine ethyl ester and *p*-chlorobenzaldehyde in the case of chymotrypsin-catalyzed hydrolysis. As can be seen from the results summarized in Table 3, more than 50% (up to 63%) of the initial racemic ester of phenylalanine was converted into L-phenylalanine when DABCO (5−10 mol % relative to the substrate) was added into the solution in an aqueous organic solvent. In fact the reactions simply stopped after these conversions (about 60%), and no change in the amount of the remaining ester was observed. One reason for this kind of behavior might be that the water from the hydration shell of the enzyme was consumed to hydrolyze the ester, the liberated ethanol went into the solution to shift equilibrium toward the initial ester, and the reaction stopped at some stage. In a water−methanol mixture, no reaction was observed which might serve as an argument to support the equilibrium hypothesis. It was also anticipated that over the period of time, DABCO might cause loss in the catalytic activity of the enzyme. In fact it was found that sequential hydrolysis carried out by addition of DABCO and a fresh lot of α-chymotrypsin to the D-Schiff base, obtained by evaporation of the filtrate of the reaction mixture instead of its addition in the beginning of the reaction, increased the total yield of the precipitated amino acid. The chemical yield and enantiomeric purity of the material could be significantly increased if the racemization of the remaining D-PheOEt was carried out in a separate stage. First, the resolution procedure was carried out as described above without adding any base. In a typical experiment, after 96 h the precipitated L-Phe was filtered, the filtrate was concentrated to remove ethanol, and the unreacted aldimine was again hydrolyzed under the same conditions with added DABCO and a fresh lot of α-chymotrypsin. The

Scheme 3



total isolated yield of L-Phe was as high as 78% in 86% ee (Table 3, run 9).

In an optimized experiment, the precipitate was filtered after 48 h to give L-Phe in 46.5% chemical yield and 98% ee; the solution was evaporated, a new portion of organic solvent (without water) and DABCO added to the residue, and the mixture kept at ambient temperature for 48 h to ensure complete racemization of the remaining Schiff base of D-PheOEt (as monitored by polarimetry). Then a fresh portion of chymotrypsin, dissolved in the required amount of water, was added to the solution, and the mixture was agitated at ambient temperature for 48 h after which time another 41% of L-Phe (83% ee) was filtered off. The filterate contained 6.8% of almost racemic Phe (0.6% ee in L-enantiomer). The data is summarized in Table 3 (run 10).

The increase in the concentration of DABCO enhanced the rate of the reaction. Aqueous MeCN, Me_2CO, and t-BuOH were used as solvents in the reaction successfully. In all the cases studied, the ester remaining in solution was either racemic or had an admixture of L-amino acid, which was extracted into the solution by DABCO. Similar trends were observed in the experiments conducted with PPL, which gives increase in the yields of the amino acids (Table 3). A 10% of 1,8-diaminooctane in the reaction mixture was also found to cause racemization, and Amano P-lipase in t-BuOH was used to affect the resolution.

We believe that the transformation might be considered as being a combination of three processes: (a) The chemical dissociation of the aldimine bond, giving some amount of free amino acid ester and aldehyde. (b) The enantioselective enzyme-catalyzed hydrolysis of the free L-amino acid ester accompanied by the precipitation of L-amino acid. (c) The base-induced racemization (*via* the intermediate carbanion) of the remaining racemic aldimine of D-amino acid ester, followed by the enzymatic hydrolysis *via* a and b (see Scheme 3).

To check if the additional amount of L-Phe came from the partial racemization of the D-ester, we performed the enzymatic hydrolysis in a mixture of D_2O/CD_3CN. The ^1H NMR spectral analysis of the precipitated L-amino acid showed that approximately 40% of its α-proton was substituted by deuterium. As could have been expected, even larger enrichment was detected (upto 60%) in the phenylalanine ester remaining in the solution, and this further supported our argument.

The effect of other bases, i.e. Et_3N, $(i\text{-}Pr)_2NH$, 1,8-diaminooctane, and 1,9-diaminononane on the selective racemization and asymmetric transformation of the Schiff base, derived from racemic phenylalanine ethyl ester and *p*-chlorobenzaldehyde or benzaldehyde in chymotrypsin-catalyzed hydrolysis has been studied. It was found that ee of the precipitated amino acid in all the cases was less than 92%.

In conclusion, the resolution method elaborated in this work relies on use of inexpensive chemicals and reagents and experimentally it is very simple. The asymmetric transformation, which can be carried out successfully, opens a new perspective in the synthesis of enantiomerically enriched amino acids.

Experimental Section

Gas chromatographic analyses were performed with a chiral glass capillary column (l = 41 m, i.d. = 0.21 mm) of diamide polysiloxane phase type "Chirasil-Val" (synthesized in the laboratory) and an FID detector. The carrier gas was helium, temp 147 °C, flow rate 1 mL/min, and inlet pressure was 1.6 bar. Amino acids were analyzed as *N*-trifluoroacetyl derivatives[12] of their isopropyl esters. Chymotrypsin from bovine pancreas, lyophilized powder, was obtained from Fluka. Porcine pancreatic lipase (PPL, type-II) and *Candida cylindracea* lipase (CCL, type-VII) were acquired from Sigma. Amano P-lipase was obtained from Amano, Japan.

Preparation of Schiff Bases. The Schiff bases of amino acid esters were synthesized according to the literature procedure in excellent yields starting from the corresponding amino acid ester and the corresponding benzaldehyde.[10]

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Schiff base of D,L-Phe-OEt with benzaldehyde: viscous colorless liquid, bp 182−84 °C (∼1 Torr), yield 47% (after distillation; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.90 (s, 1H), 7.10−7.70 (m, 10H), 4.10 (q, 2H), 3.85−4.05 (m, 1H), 3.05−3.40 (m, 2H), 1.20 (t, 3H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 172.1, 162.5, 129.7, 129.2, 128.1, 127.7, 126.6, 126.2, 66.0, 60.5, 45.3, 14.0.

Schiff base of D,L-Ph-Gly-OEt with benzaldehyde: colorless solid crystallized from hexane, mp 61−62 °C, bp 140−42 °C (2 Torr), yield 51% (after distillation); IR (KBr) 1730, 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.20 (s, 1H), 7.15−7.75 (m, 10H), 5.10 (s, 1H), 4.10 (q, 2H), 1.05 (t, 3H). Anal. Calcd for $C_{17}H_{17}NO_2$: C, 76.40; H, 6.37; N, 5.24. Found: C, 77.21; H, 6.42; N, 5.00.

Schiff base of D,L-nor-Val-OEt with benzaldehyde: colorless viscous oil, yield 81%; IR (KBr) 1740, 1645 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.15 (s, 1H), 7.25−7.70 (m, 5H), 4.05 (q, 2H), 3.85 (t, 1H), 1.85 (m, 2H), 1.15 (m, 2H), 1.05 (t, 3H), 0.90 (t, 3H). Anal. Calcd for $C_{14}H_{19}NO_2$: C, 72.10; H, 8.15; N, 6.01. Found: C, 71.85; H, 8.41; N, 5.88.

Schiff base of D,L-Trp-OEt with benzaldehyde: colorless solid crystallized from benzene, mp 129−30 °C, yield 57%; IR (KBr) 1735, 1635 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 8.25 (s, 1H, NH), 7.75 (s, 1H, CH═N), 6.70−7.65 (m, 5H, Ind), 4.05 (q, 2H, CH_2O), 3.95−4.20 (m, 1H, CH), 3.05−3.50 (m, 2H, CH_2-Ind), 1.10 (t, 3H, CH_3). Anal. Calcd for $C_{20}H_{20}N_2O_2$: C, 75.00; H, 6.25; N, 8.75. Found: C, 74.28; H, 6.25; N, 8.77.

General Procedure of Alkylation of Schiff Base of Gly-OEt with Substituted Benzyl Bromide.[11] A heterogenous mixture of Schiff base of Gly-OEt with benzaldehyde[10] (1.91 g, 10 mmol), tetrabutylammonium bromide (0.16 g, 0.5 mmol) in CH_2Cl_2 (5 mL), and 30% NaOH (2 mL) was stirred at 0 °C, and a solution of benzyl bromide (11 mmol) in CH_2Cl_2 (5 mL) was added dropwise during 1 h. The stirring was continued for 1 h more and then for 3 h at room temperature before the reaction mixture was filtered and solvent removed. The residue was extracted with CCl_4 (3 × 20 mL). The CCl_4 extracts were combined and washed with cold water (2 × 10 mL) and saturated aqueous NaCl (2 × 10 mL) and dried over Na_2SO_4. Solvent was evaporated and the product purified by distillation *in vacuo*.

Schiff base of D,L-4-F-Phe-OEt with benzaldehyde: colorless viscous oil, bp 183−85 °C (∼1 Torr), yield 48% (after distillation); IR (KBr) 1730, 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.85 (s, 1H, CH═N), 7.05−7.45 (m, 5H, Ph), 6.80 (m, 4H, C_6H_4), 3.85−4.0 (m, 1H, CH), 3.95 (q, 2H, CH_2O), 2.95−3.20 (m, 2H, CH_2Ph), 1.05 (t, 3H, CH_3). Anal. Calcd for $C_{18}H_{18}NO_2F$: C, 72.24; H, 6.02; N, 4.68; F, 6.35. Found: C, 72.42; H, 6.21; N, 4.30; F, 6.14.

Schiff base D,L-2-F-Phe-OEt with benzaldehyde: colorless viscous oil, bp 185−90 °C (∼1 Torr), yield 31% (after distillation); IR (KBr) 1730, 1630 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 7.85 (s, 1H, CH═N), 6.85−7.65 (m, 9H, aromatic protons), 4.05 (q, 2H, CH_2O), 3.70−3.85 (m, 1H, CH), 2.90−3.25 (m, 2H, CH_2Ph), 1.05 (t, 3H, CH_3). Anal. Calcd for $C_{18}H_{18}NO_2F$: C, 72.24; H, 6.02; N, 4.68; F, 6.35. Found: C, 72.51; H, 6.30; N, 4.25; F, 6.21.

Schiff bases of D,L-Ala; D,L-nor-Val and D,L-α-Me-Phe esters with benzaldehyde were also obtained by direct alkylation of the intermediate Schiff base derived from glycine ester and benzaldehyde[11] with corresponding aryl/alkyl bromide under phase transfer catalytic conditions.

Typical Procedure of the Enzymatic Hydrolysis with Lipase or Chymotrypsin. To the solution of the Schiff base derived from *p*-chlorobenzaldehyde and Phe-OEt (0.3 g, 10^{-3} mol) in MeCN (8.75 mL) was added PPL (0.3 g) or chymotrypsin (0.010 g, 4×10^{-7} mol) in 0.5 mL of H_2O with stirring, and the contents were stirred for several hours (see Table 1) at ambient temperature. The precipitate containing the L-amino acid and the enzyme was filtered and washed with 1% aqueous ammonia (twice), the solution was evaporated *in vacuo*, standard amino acid (L-Ile) solution was added to the residue, and the mixture was purified first on Sephadex G-25 (medium) with ammonia (1% solution; instead a 5% solution of chloroacetic acid was also used to separate the enzymes as precipitates from the solution) and then on a column of ion-exchange resin DOWEX-50W (H^+). The amino acids were eluted from the resin with 5% aqueous ammonia, the solution was evaporated, and the residue was analyzed without further purification as described above. For the preparation of D-amino acids, the MeCN filterate was evaporated *in vacuo* and hydrolyzed by stirring with 6 N HCl at 20 °C for 1 h, the mixture was extracted with toluene to remove the initial benzaldehyde. The water layer was refluxed for 5 h and evaporated. The residue containing D-amino acid was analyzed after purification on DOWEX-50W (H^+). Isolated yields of both L- and D-amino acids do not differ significantly (2−3% less) from those determined by GLC in the case of the α-chymotrypsin-catalyzed reaction (Table 1), and the purity of the amino acids (precipitate or in the solution) was checked from ^{1}H NMR spectra and TLC. Large scale experiments could easily be carried out using the same proportions of the reagents and solvents.

Asymmetric Transformation of D,L-Phe-OEt in the Presence of an Added Base during Enzymatic Hydrolysis of Its Schiff Base with *p*-Chlorobenzaldehyde. The reaction was carried out as described above, the only difference being that DABCO was added into the reaction mixture. The amino acid recovered from the organic filtrate as described above was separated from DABCO by ion-exchange chromatography on DOWEX-50 and analyzed (see Table 3).

Base-Catalyzed Deuterium Exchange of the α-Proton of Phenylalanine Ester during Enzymatic Hydrolysis of the Schiff Base with *p*-Chlorobenzaldehyde. The reaction was performed as described above, the only exception being that it was carried out in the mixture of CD_3CN/D_2O in the presence of DABCO (5%). As the reaction in the mixture was slower than usual, it took 87 h for 42% of L-Phe (81% ee) to precipitate. The workup of the reaction mixture was made in the usual manner. No internal standard was added to the amino acid. The products were analyzed by GLC on chiral column and by ^{1}H NMR. Approximately 40.4% of α-protons of L-Phe was substituted by deuterium, and 60.6% of deuterium was found in the D-Phe-OEt (10% *ee*) remaining in the solution.

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