Lipase Catalysis in the Optical Resolution of 2-Amino-1-phenylethanol Derivatives

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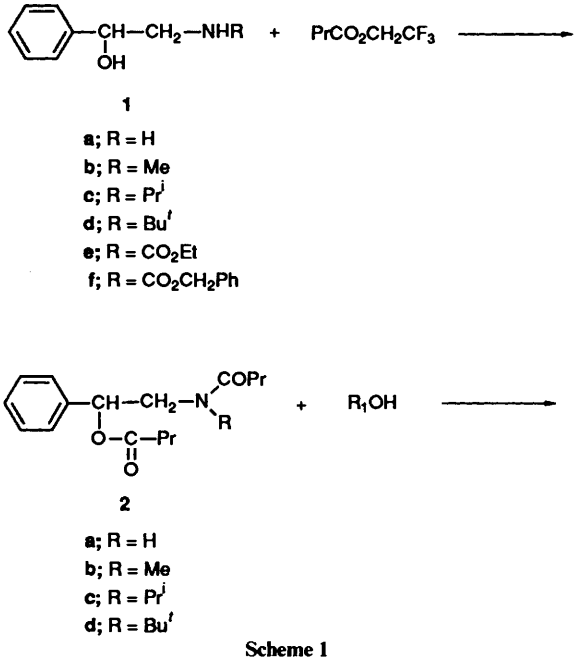
The lipase PS- and CCL-catalysed resolution of unsubstituted and *N*-alkyl substituted 2-amino-1-phenylethanols with 2,2,2-trifluoroethyl butyrate or with butyric anhydride and those of the corresponding diacylated 1,2-amino alcohols with 1-alcohols in organic media have been studied. The enzymatic deacylation of diacylated 2-amino-1-phenylethanol stops at approximately 50% conversion yielding the two enantiomers with an e.e. of the order of 100%. Enantioselectivity in the case of *N*-alkyl substituted compounds is only somewhat lower. Also the enzymatic acylation of 2-amino-1-phenylethanol shows high enantioselectivity when the protection of the NH_2 group with CO_2Et or CO_2CH_2Ph has been used. However, the direct acylation of 2-amino-1-phenylethanol or of its *N*-alkyl substituted derivatives are slow, the reactions stop before 40% conversion and nonenzymatic aminolysis is usually significant.

In the synthesis of compounds such as pharmaceuticals which are used for living systems, chirality is often of the utmost importance. For instance, many pharmaceutically important compounds are 1,2-amino alcohols the enantiomers of which may show remarkable differences in their biological effects. A fascinating method for the preparation of pure enantiomers is to use biocatalysts either through enzyme-catalysed synthesis of prochiral compounds or through enzymatic resolution of racemic mixtures. The use of organic solvents instead of water as a reaction medium has further increased the validity of this methodology.[1] Thus, using hydrolytic enzymes the optical resolution of some racemic 1,2-amino alcohols has been performed.[2–6] Lipase catalysis has also been used to resolve racemic synthons which can subsequently be used to prepare optically active 1,2-amino alcohols. Chiral cyanohydrins, the LAH (lithium aluminium hydride) reduction of which results in 1,2-aminoethanol derivatives, serve as a good example of such synthons.[7–12] The (*R*)- or (*S*)-oxynitrilase-catalysed production of optically active cyanohydrins by the addition of hydrogen cyanide to aldehydes is also possible.[13–18] Effenberger *et al.*[19] have performed the (*R*)-oxynitrilase-catalysed addition of hydrogen cyanide even to aliphatic ketones in organic solvents. Optically active α-chloro-, α-bromo-, α-nitro- and α-azido-hydrins are other potential precursors which can serve as starting materials for 1,2-amino alcohols. The baker's yeast-catalysed reduction of suitable ketones is a versatile, inexpensive enzymatic method to such precursors.[20,21]

Our purpose has been to resolve different 2-amino-1-phenylethanol derivatives (Scheme 1) either by the lipase-catalysed acylation of the secondary OH group in **1** with 2,2,2-trifluoroethyl butyrate and acid anhydrides or by the lipase-catalysed deacylation of the corresponding diacylated compounds **2** with primary alcohols in organic solvents. The literature data for the enzymatic preparation of optically active precursors of 2-amino-1-phenylethanols are also described.



Scheme 1

Results and Discussion

The enzymatic resolution of a racemic mixture is usually more successful when the reaction, leading to the pure enantiomers, can be directed to the asymmetric centre of the molecule. That is why, the specific *O*-acylation of the secondary OH group in **1** or the deacylation of the ester moiety in **2** is desired. In the deacylation reactions, there is no sign of reaction at the amide moiety of **2**. In the acylation reactions, however, the detection of the real acylation site is difficult if the NH_2 group is not properly protected. The reason for that is the fast spontaneous O → N migration of the acyl group in *O*-monoacylated 1,2-amino-alcohols.[22] In accordance with this, the acylations of **1a–c** catalysed by four different lipases in *tert*-pentyl alcohol resulted, in practice, in the formations of the corresponding *N*-acylated amide products. The O → N acyl migration as well as the possible enzymatic or nonenzymatic *N*-acylations become less favourable with increasing steric hindrance at the amino group. Accordingly, the acylations of **1d–f** lead to the formation of the *O*-acylated ester product only.

In our previous studies, lipase PS from *Pseudomonas cepacia* showed a profound chemoselectivity toward the *O*-acylation of 6-aminohexan-1-ol in *tert*-pentyl alcohol.[22] This enzyme is also known to display high activity toward secondary alcohols.[23] Because of the above reasons lipase PS was used as a catalyst throughout this work. The lipase from *Candida cylindracea* (CCL) is also a useful catalyst for the present purposes although the information concerning its chemoselectivity is somewhat obscure. On the other hand, independent

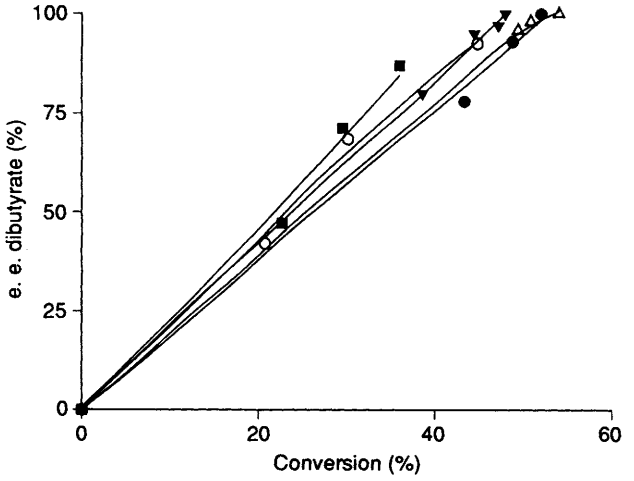


Fig. 1 The values of e.e. for the unchanged **2a** *vs.* the % conversion for the lipase PS-catalysed deacylation of (±)-**2a** with butan-1-ol in *tert*-pentyl alcohol (●), toluene (▼), dibutyl ether (△), THF (○) and acetonitrile (■)

of the size of the *N*-alkyl substituent in **1b–d** the CCL-catalysed acylations with 2,2,2-trifluoroethyl butyrate in toluene proceed at approximately the same rate, strongly suggesting the preferred *O*- over *N*-acylation. The initial formation of the ester products in these reactions can also be detected by the GLC (gas liquid chromatography)–FT-IR (Fourier-Transform Infrared) method.[22]

The results for the lipase-catalysed resolutions of racemic **1b–f** and **2** in toluene are shown in Table 1. In the case of the compounds **1a**, **e** and **f**, which are insoluble in toluene, *tert*-pentyl alcohol was used as a reaction medium. The resolution of **1a–d** by the direct acylation of the OH group is unsuccessful due to the slow rates of the enzymatic reactions and to the fact that these reactions stop before 40% conversion. Moreover, nonenzymatic aminolysis of 2,2,2-trifluoroethyl butyrate is significant especially with **1a** and **b**, leading to the nonstereospecific formation of the butyramide product. Consequently, the protection of the NH_2 group in 2-amino-1-phenylethanol with groups such as CO_2Et or CO_2CH_2Ph is necessary for a successful lipase-mediated resolution of a racemate. This result is in full accordance with the previous results obtained in the lipase-catalysed resolutions of racemic 2-aminopropan-1-ol and 2-aminobutan-1-ol.[3,4] Of the two lipases used only lipase PS is an efficient catalyst for the acylations of **1e** and **f** in *tert*-pentyl alcohol. The solvent is known to affect both catalytic efficiency and enantioselectivity of an enzyme.[24,25] This effect differs from one enzyme to another. Roughly, enzymatic activity should decrease with decreasing hydrophobicity of the solvent. Of the two enzymes, lipase PS works in a wide variety of organic solvents (Table 2), whereas there is a dramatic loss in CCL activity in the case of solvents more hydrophilic than toluene (log *P* 2.5).[26] The solvent is evidently the main reason for the observation that CCL does not work properly in the present acylations in *tert*-pentyl alcohol. If the re-use of an enzyme is important, 2,2,2-trifluoroethyl butyrate is preferred as the acylating agent because the butyric acid then formed leads to some enzyme inactivation. To avoid this the immobilization of the enzyme to some solid support is necessary.[23] It is also obvious from Table 1 that the enantioselectivity of lipase PS somewhat decreases when the protecting group in the amino alcohol is changed from CO_2Et of **1e** to CO_2CH_2Ph of **1f**.

The enzymes exhibit higher enantioselectivity in catalysing the deacylations of **2** than its acylation counterparts (Table 1). Thus, the deacylations of dibutyrylated **2a** by lipase PS and CCL in toluene tend to stop spontaneously at 50% conversion. At this stage and in a wide variety of organic solvents, lipase PS yields the two enantiomers, the remaining substrate (−)-**2a** and the resulting (+)-amide product, with practically 100% optical purity (Table 2, Fig. 1). On the other hand, the solvent has a notable effect on the time needed for the resolution, the most hydrophobic solvents, dibutyl ether and toluene, being the best. The solvent affects also the enzyme activity that remains for re-use, the loss of activity in the case of lipase PS being 20% in toluene but close to 50% in THF (tetrahydrofuran) or *tert*-pentyl alcohol. The loss of activity is negligible in subsequent uses, and the re-use does not effect the enantioselectivity of the enzyme. The reaction time is also dependent on the 1-alcohol used as a nucleophile so that the longer the alkyl chain of alcohol the faster the reaction (Table 2). Although lipase PS is relatively effective in the resolution of **2a**, it does not catalyse the deacylations of *N*-alkyl-substituted compounds **2b–d**. On the other hand, the kinetically controlled CCL-catalysed resolution of racemic **2b** and **c** with 1-alcohols are possible (Table 1). There is no reaction within 180 h when **2d** is used as a substrate.

To obtain the pure enantiomers of **1a** the basic hydrolyses of unchanged (−)-**2a**, (−)-**1e** and (−)-**1f** as well as of the corresponding (+)-deacylation or -acylation products, respectively, were performed. In this process, the levorotatory compounds result in (*R*)-(−)- and the dextrorotatory products in (*S*)-(+)-**1a** (Table 3). This conclusion is based on the literature data for the specific rotations of the (*R*)- and (*S*)-isomers.[15] The (*S*)-specificity generally observed for lipase PS and CCL catalysis also support this conclusion.

To summarize, the lipase PS-catalysed deacylation of racemic **2a** is a novel enzymatic approach to obtain simultaneously both enantiomers of the racemate with high yields of 80–90% (expecting that 50% yield corresponds to 100% yield) and with extremely high optical purity (e.e. ⩾95% for both enantiomers) (Tables 2 and 3). CCL catalysis makes this approach more complete, allowing the resolution of *N*-alkyl substituted amino alcohols **2b** and **c** (Table 1). The kinetically controlled, lipase PS-catalysed acylation of racemic **1e** or **f** is another possibility for the preparation of the enantiomers of **1a** (Tables 1 and 3). From the three enzymatic methods (resolution, condensation and reduction) shown in Table 3, the (*R*)- or (*S*)-oxynitrilase-catalysed addition of HCN to benzaldehyde followed by the LAH reduction of optically active mandelonitrile thus obtained is also a versatile method to (*R*)- or (*S*)-**1a**, but only one enantiomer is obtained with one enzyme. The baker's yeast-catalysed reduction of ketones, on the other hand, cannot compete with the above methods as to the yield or optical purity of the product.

Experimental

All the solvents used were of the highest analytical grade and were dried over molecular sieves (3 Å) before use. Lipase PS from *Pseudomonas cepacia* was purchased from Amano Pharmaceuticals and the lipase from *Candida cylindracea* (CCL) from Sigma Chemical. 2,2,2-Trifluoroethyl butyrate was prepared from butyryl chloride and 2,2,2-trifluoroethanol. 2-Amino-1-phenylethanol **1a** was a commercial product which was recrystallized from toluene before use. Compounds **1b–d** were prepared by the known method from styrene oxide and primary amine.[27] The reaction between **1a** and ethyl chloroformate in aqueous sodium carbonate produced compound **1e**.[3] Compound **1f** was prepared by the modified method from benzyl chloroformate using toluene as the reaction medium. The *N*-monoacylated compounds, which were used to follow the enzymatic acylation reactions, were prepared chemically using equivalent amounts of butyric anhydride and compounds **1a–d** and **2a–d** in toluene while the corresponding diacylated compounds were easily obtained by the same procedure in the presence of 4-dimethylaminopyridine (DMAP) as a catalyst.

Table 1 The lipase-catalysed transesterification of **1** and **2** with various reagents in toluene at 40 °C

Substrate	Reagent	Enzyme	t/h	Conversion (%)	Recovered (*R*)-**1** or -**2**			(*S*)-Product		
					Yield[a] (%)	e.e. (%)	$[\alpha]_D^{25}$	Yield[a] (%)	e.e. (%)	$[\alpha]_D^{25}$
1b	$PrCO_2CH_2CF_3$	CCL	600	31						
1c	$PrCO_2CH_2CF_3$	CCL	600	35						
1c	$PrCO_2CH_2CF_3$	Lipase PS	600	35						
1d	$PrCO_2CH_2CF_3$	CCL	600	25						
1e	$PrCO_2CH_2CF_3$	Lipase PS[b]	73	40	89	79[c]	−40[d]	65	⩾95[e]	+51[d]
1e	$(PrCO)_2O$	Lipase Ps[b]	44	50	60	94[c]	−49[d]	83	81[e]	+42[d]
1f	$PrCO_2CH_2CF_3$	Lipase Ps[b]	72	53	96	83[c]	−25[d]	98	65[f]	+33
2a	Butan-1-ol	Lipase PS	101	48	81	⩾95[g]	−51[h]	78	⩾95[i]	+71[j]
2a	Butan-1-ol	CCL	95	54	87	⩾95[g]	−53[h]	95	88[i]	+62[j]
2b	Hexan-1-ol	CCL	173	40	99	77[e]	−44[d]	33	93[c]	+90[d]
2c	Hexan-1-ol	CCL	234	38	98	26[k]	−16[d]	64	82[c]	+15[d]
2d	Butan-1-ol	CCL	180	0						

[a] Yield 100% at 50% conversion. [b] In *tert*-pentyl alcohol. [c] According to chiral GLC after the product is separated and subsequently derivatized with $(PrCO)_2O$. [d] (*c* 2 in $CHCl_3$). [e] According to chiral GLC. [f] By ^1H NMR in the presence of $Eu(hfc)_3$ after basic hydrolysis followed by the derivatization with acetic anhydride. [g] According to chiral HPLC. [h] (*c* 7 in $CHCl_3$). [i] According to chiral HPLC after the product is separated and subsequently derivatized with Ac_2O. [j] (*c* 5 in $CHCl_3$). [k] According to chiral HPLC after the product is separated and subsequently methanolysed in MeOH–MeONa system.

Table 2 The lipase PS-catalysed deacylation of **2a** with 1-alcohol (ROH) in different organic solvents at 40 °C

Solvent	ROH	t/h	Conversion (%)	Recovered (*R*)-**2a**			(*S*)-Product		
				Yield[a] (%)	e.e.[b] (%)	$[\alpha]_D^{25,c}$	Yield[a] (%)	e.e.[d] (%)	$[\alpha_D^{25,e}$
tert-Pentyl alcohol	Butan-1-ol	148	52	66	⩾95	−52	68	⩾95	+69
Toluene	Butan-1-ol	101	48	81	⩾95	−51	78	⩾95	+71
	Hexan-1-ol	89	48	92	⩾95	−50	85	⩾95	+75
	Octan-1-ol	44	48	82	⩾95	−51	87	⩾95	+73
Dibutyl ether	Butan-1-ol	116	54	79	⩾95	−54	90	⩾95	+69
	Hexan-1-ol	48	50	83	⩾95	−53	72	⩾95	+70
THF	Butan-1-ol	195	45	91	79	−40	89	⩾95	+71
Acetonitrile	Butan-1-ol	716	45	92	85	−44	86	⩾95	+71
Toluene[f]	Butan-1-ol	95	54	87	⩾95	−53	95	88	+62

[a] Yield 100% at 50% conversion. [b] According to chiral HPLC. [c] (*c* 7 in $CHCl_3$). [d] According to chiral HPLC obtained after the product is separated and subsequently derivatized with Ac_2O. [e] (*c* 5 in $CHCl_3$). [f] Enzyme CCL.

The progress of the reaction was followed by taking samples from the reaction mixture and by analysing them by GLC.[28] The disappearance of the starting esters and the formation of the reaction products were followed. A gas chromatograph was equipped with 25 m NB-30 capillary column and with the flame-ionization detector for this purpose. The enantiomeric excesses (e.e.) for the butyrylated reaction product of **1e** and for the unchanged **2b** were determined using GLC equipped with a 25 m Chirasil-L-Val column. The prior butyrylation of the corresponding secondary alcohols with butyryl chloride was necessary to obtain values of e.e. for unchanged (−)-**1e** and -**1f** and for the deacylated (+)-**2b**. HPLC equipped with a 25 cm Chiralcel OG column was used to obtain the values of e.e. in the case of unchanged (−)-**2a** and of the corresponding (+)-amide product which was first acetylated with acetic anhydride. The MeONa-catalysed methanolysis of the recovered (−)-**2c** followed by chiral HPLC and the KOH-catalysed hydrolysis of the butyrylated (+)-**1f** in aqueous methanol followed by the acetylation with acetic anhydride and ^1H NMR spectroscopy in the presence $Eu(hfc)_3$ produced the e.e. values for (−)-**2c** and (+)-**1f**, respectively. $[\alpha]_D$ Values are given in units of 10^{-1} deg cm^2 g^{-1}.

Enzymatic Acylation and Deacylation.—The procedure for the two reaction types was the same. As a typical example, a solution of racemic **2a** (50 cm^3; 0.1 mol dm^{-3}) in toluene was added to lipase PS (5 g). After sonication (10 s) butan-1-ol (915 mm^3; 0.2 mol dm^{-3} in the reaction mixture) was added to start the reaction. The reaction mixture was shaken at 40 °C until the reaction spontaneously stopped at 48% conversion within 5 d. The enzyme was filtered off and the solvent was evaporated. The unchanged **2a** and the amide product were separated by flash chromatography;[29] (−)-**2a** {81% of the theoretical yield; purity 98% by GLC; e.e. ⩾95% by HPLC; $[\alpha]_D^{25}$ −50.7 (*c* 7.21 in $CHCl_3$)} was first eluted with EtOAc–hexane (1:1). The subsequent elution with EtOAc–hexane (7:1) produced the corresponding deacylated (+)-amide (406 mg) [78% of the theoretical yield; purity 96% by GLC; $[\alpha]_D^{25}$ +70.7 (*c* 5.38 in $CHCl_3$)] with an e.e. ⩾95% according to its acetylated derivative. The value of e.e. ⩾95% (Tables 1-3) means that only one enantiomer of the unchanged (−)-substrate and of (+)-product is detected by chiral HPLC or GLC.

Basic Hydrolyses of Recovered (−)-**1e**, -**f** *and* -**2a** *and of the Corresponding* (+)-*Reaction Products.*—The resolution products were all hydrolysed by the same procedure except that the reaction times were varied depending on the compound hydrolysed. As a typical example; (−)-**2a** (528 mg, 1.90 mmol) was refluxed for 40 h in 1.9 mol dm^{-3} KOH (8 cm^3) in methanol–water solution (1:1). The hydrolysed product was extracted with chloroform. The organic extract was dried (Na_2SO_4) and subsequently evaporated to dryness to give of (*R*)-(−)-**1a** (260 mg, 1.90 mmol) {$[\alpha]_D^{23}$ −65 (*c* 2.6 in C_6H_6) $[\alpha]_D^{23}$ lit.,[15] −47}. The hydrolysis of the corresponding

Table 3 Production of (*R*)-(−)- and (*S*)-(+)-**1a** or their precursors PhCH(OR)X after enzymatic resolution or synthesis

Product							Isomer [e.e. (%); $[\alpha]_D^{25}$		
R	X	Solvent	Enzyme	Reaction type	*t*/h	Conversion* or yield (%)	Recovered substrate	Product	Ref.
H	NH_2	Toluene	Lipase PS	Deacylation	101	48*	(*R*) (⩾95; −65)[a]	(*S*) (⩾95; +66)[a]	
H	NH_2	*tert*-pentyl alcohol	Lipase PS	Acylation	104	45*	(*R*) (71; −52)[a]	(*S*) (96; +63)[a]	
H	NH_2	*tert*-pentyl alcohol	Lipase PS	Acylation	72	53*	(*R*) (72; −51)[a]	(*S*) (65; +44)[a]	
PrCO	CH_2Cl	Diisopropyl ether	Lipase PS	Acylation	24	52*	(*R*) (96; −51.4[b])	(*S*) (97; 66.2[c]	30
Ac	CN	Diisopropyl ether	Lipase PS	Acylation	1512	100*[d]		(*S*) (84; —)	8
H	CN	Diisopropyl ether	CCL	Deacylation	48	38*	(*S*) (—; +11.0[e]	(*R*) (77; +33.5[f])	7
PrCO	CN	THF	Lipase PS	Acylation	48	60*	(*R*) (80; +33[g])	(*S*) (63; −4.8[g])	31
H	CN	EtOH (30%)/W	*R*-oxynitrilase	Condensation	0.7	95		(*R*) (99; +40[h])	13
H	CN	Ethyl acetate	*R*-oxynitrilase	Condensation	2.5	95		(*R*) (99; −)	14
H	CN	Ethyl acetate	*R*-oxynitrilase	Condensation	2.5	90		(*R*) (99; +49[g])	15
H	CN	Diethyl ether	*R*-oxynitrilase	Condensation	10	72		(*R*) (92; +40.6[g])	16
H	CN	Water	*S*-oxynitrilase	Condensation	0.7	80		(*S*) (96; —)	17
H	CN	Diisopropyl ether	*S*-oxynitrilase	Condensation		91		(*S*) (97; —)	18
H	CH_2Cl	Water	Baker's yeast	Reduction	48	37		(*R*) (90; −43.3[b])	20
H	CH_2N_3	Water	Baker's yeast	Reduction		36		(*R*) (—; −62.7[i])	21
H	CH_2NH_2	Water	Baker's yeast	Reduction		6		(*R*) (—; −62.7[i])	21
H	CN	Water	Baker's yeast	Reduction	No reaction				21

[a] e.e. for (*R*)- and (*S*)-**1a** obtained after the basic hydrolysis of the enzymatic resolution products (see Table 1) followed by the derivatization with acetic anhydride using ^1H NMR in the presence of Eu(hfc)$_3$; $[\alpha]_D^{23}$ (*c* 2–4 in C_6H_6). [b] (*c* 2.0 in cyclohexane). [c] (*c* 1.0 in acetone). [d] *In situ* racemization of the (*R*)-isomer performed. [e] (*c* 15 in C_6H_6). [f] (*c* 7 in C_6H_6). [g] $[\alpha]_D^{23}$ (*c* 1 in $CHCl_3$). [h] (*c* 1 in $CHCl_3$). [i] (*c* 3.09 in $CHCl_3$).

deacylated (+)-amide product (384 mg, 1.85 mmol) resulted in (*S*)-(+)-**1a** (235 mg, 1.71 mmol) {$[\alpha]_D^{23}$ +66 (*c* 2.35 in C_6H_6) $[\alpha]_D^{23}$ lit.,[15] 49.4}. To determine the e.e. values, (−)- and (+)-**1a** were acetylated with acetic anhydride in pyridine using DMAP as a catalyst. The e.e. values ⩾95% were subsequently determined in the presence of Eu(hfc)$_3$ by ^1H NMR spectroscopy. This result indicates that the basic hydrolysis does not lead to the racemization of the enantiomer.

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