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Biocatalytic Ways to Optically Active 2-Amino-1-phenylethanols

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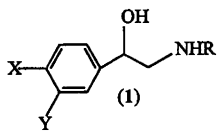
Optically active building blocks of 2-amino-1-ethanols, such as 2-amino-, 2-halo- and 2-azido-1-phenylethanols, 2-hydroxy carboxylic acid derivatives and aryl cyanohydrins, can be conveniently prepared by biocatalytic ways. Systems that do not need added cofactors are described. Whole cell catalysis is economical for asymmetric synthesis. Oxynitrilases produce (*R*)- and (*S*)-aryl cyanohydrins with high enantiomeric purities (in most cases ee > 90%) and chemical yields (usually over 80%). Baker's yeast preferentially works *pro*-(*R*)-selectively on ketones, but the chemical shift is usually far from theoretical. Kinetic resolution by lipases shows the widest spectrum of use, the enantiomeric purity over 95% and the chemical yield close to the theoretical 50% value being usually attained for the enantiomers of a racemic starting material. *In situ* methods enable the enhancement of the chemical yield of one enantiomer over the 50% limit.

Many 2-amino-1-phenylethanols (**1**; Scheme 1), including adrenaline (R = Me and X = Y = OH) as a fundamental compound, are important adrenergic drugs.[1] Structural modifications at the substituent R have mainly been used for the variation of their α- and β-agonist activities, the potency of a drug as a β-agonist increasing with the increasing size of the substituent. As to the synthesis of compounds **1**, there is a stereogenic centre with the secondary HO function at the aminoethanol chain of the molecule. Accordingly, the number of stereoisomers is two, the *R*-configuration in the final drug being usually preferred. The other enantiomer is often less active or may cause unpleasant side effects.



Scheme 1.

Enantioselective reduction of ketones, the use of chiral building blocks and diastereomeric resolution with L-tartaric acid derivatives are commonly used non-enzymatic methods for the preparation of optically active aminoethanol.[2–8] In addition to conventional catalysis, organic synthetic chemists are becoming more and more familiar with biocatalysis for the preparation of optically active compounds.[9] Mild reaction conditions connected with high stereoselectivity of many enzymes and the lack of similar transformations using traditional chemical means are reasons for turning to a biocatalytic reaction. This article describes some biocatalytic ways for the preparation of optically active building blocks (**2–7**; Scheme 2) for the synthesis of 2-amino-1-phenylethanols (**1**). In terms of economy and simplicity, biocatalysts which do not need added cofactors or extensive microbiological know-how are preferable. The biocatalytic methods reviewed include *enzymatic asymmetric synthesis* by (*R*)- and (*S*)-oxynitrilases (Scheme 3) and *enzymatic kinetic resolution* by lipases (Scheme 4). Moreover, the potential of baker's yeast (*Saccharomyces cerevisiae*) for the enantioselective reduction of prochiral ketones (Scheme 3) under fermentation conditions is briefly discussed.

Assuming that the enantiomeric purity of the product obtained both in enzymatic asymmetric synthesis and kinetic resolution is acceptable, certain advantages and disadvantages between the two methods still exist. Thus, the theoretical chemical yield of asymmetric synthesis is 100% while the yield in a highly enantioselective kinetic resolution never exceeds 50% as calculated based on the starting material. The advantage of asymmetric synthesis

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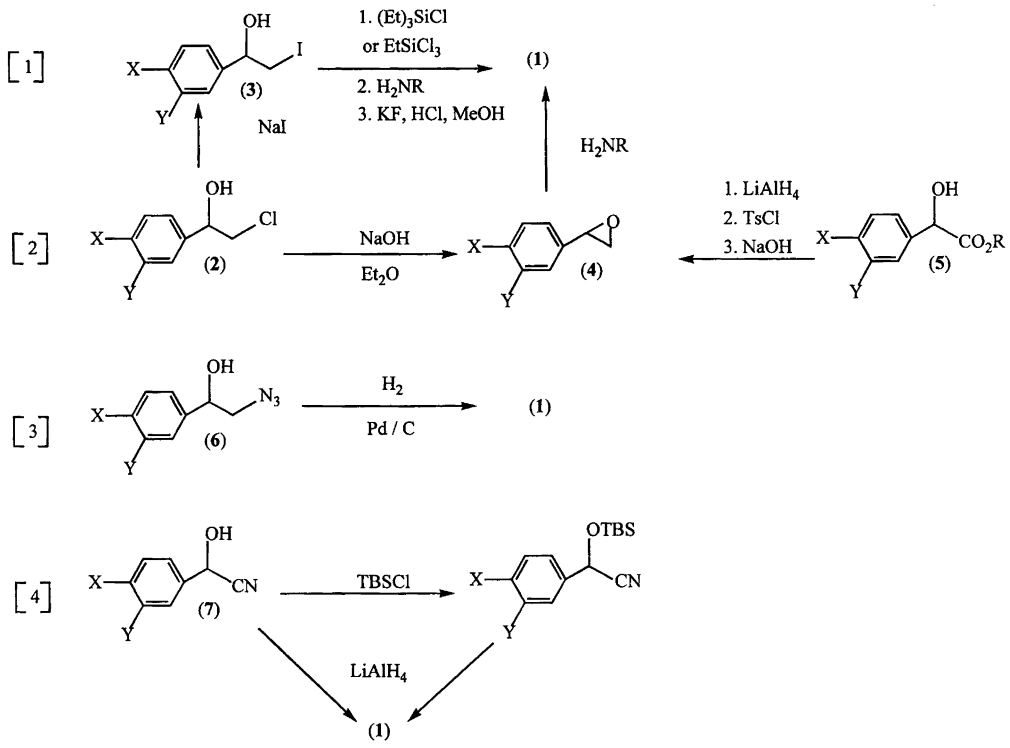
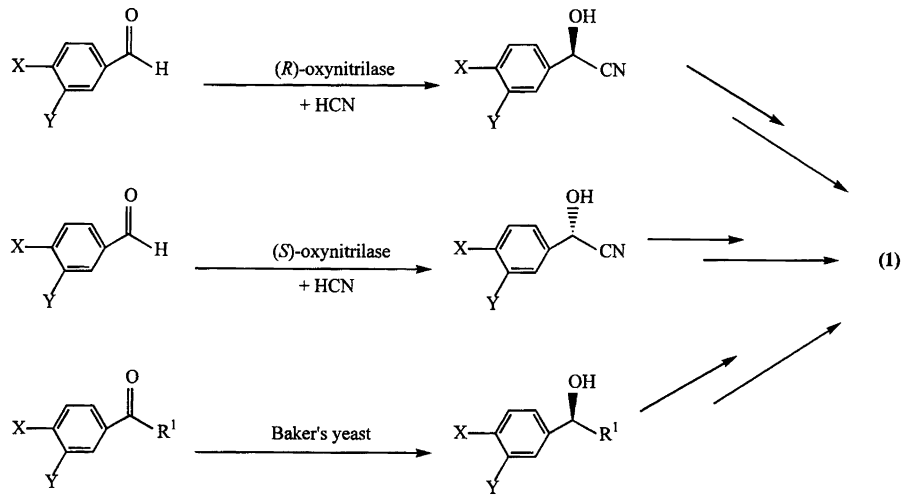


Scheme 2.

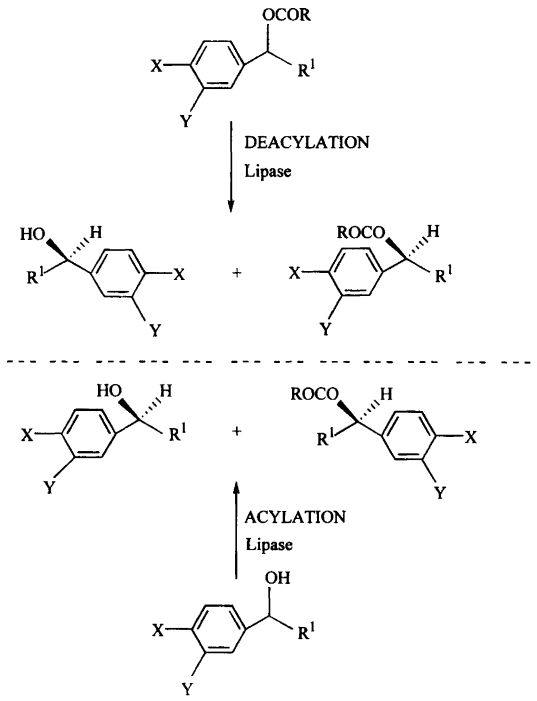
is reduced by the fact that partially purified, commercial enzymes, such as (*R*)- and (*S*)-oxynitrilases from almond and *Sorghum bicolor*, respectively, are rather expensive. Lipases, on the other hand, are relatively cheap catalysts which are readily commercially available and usually show high stability under synthetic conditions. One focus of this article is on ways in which the expense of enzymatic asymmetric synthesis is minimized on the one hand and the chemical yield increased of enzymatic kinetic resolution on the other.

Useful building blocks

2-Halo-(**2** and **3**) and 2-azido-(**6**) -1-phenylethanols, 2-hydroxycarboxylic acid derivatives (**5**) and aryl cyanohydrins (**7**) are potential building blocks of 2-amino-1-phenylethanols (Scheme 2). There are many possibilities for the transformation of an optically active building block into the corresponding amino ethanol derivative. In such conversions, the final product (**1**) must be obtained in the same high enantiomeric purity as the original building



Scheme 3.



Scheme 4.

block was prepared. Examples of some useful methods are shown in Scheme 2.

2-Halo-1-phenylethanols as chiral building blocks can be easily converted into aminoethanols with high chemical yields starting with the corresponding 2-iodo derivatives (**3**) (Scheme 2; eqn. [1]).[5] The reaction sequence includes the protection of the hydroxy group, the substitution of the halogen atom with a suitable amine (commonly with isopropylamine) and finally the deprotection to the corresponding compound (**1**). Terminal epoxides (**4**) provide another route for the transformation of halohydrins into compounds (**1**) (Scheme 2; eqn. [2]).[6,10] Epoxides (**4**) are also obtained from chiral 2-hydroxy carboxylic acids or esters (**5**).[11]

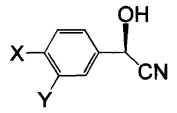
Cyanohydrins (**7**) as chiral building blocks are conveniently transformed into the corresponding aminoethanols using $LiAlH_4$ reduction (Scheme 2; eqn. [4]).[12–14] Free cyanohydrins can be directly reduced or they can be *O*-protected prior the procedure. Among the protective groups, *tert*-butyldimethylsilyl (TBS) and acyl groups are favoured because the compounds can be deprotected during the reduction.[12,15]

The preparation of aminoethanols (**1**) from azido analogues proceeds with catalytic hydrogenation (Scheme 2; eqn. [3]).[10,16] Finally, hydrolysis is usable in the case of acylated optically active 2-amino-1-phenylethanols.[17]

Oxynitrilases

Almond (EC 4.1.2.10) and *Sorghum* (EC 4.1.2.11) oxynitrilases effectively catalyse the C–C bond formation between a prochiral arenecarbaldehyde and hydrogen cyanide, leading to the formation of (*R*)- and (*S*)-cyanohydrins, respectively (Scheme 3).[18] As is shown in Tables 1 and 2 many non-natural arenecarbaldehydes are accepted as substrates. Polar effects of the substituents X and Y are clearly less important than steric effects on reactivity. The two oxynitrilases evidently catalyse the reactions stereospecifically; the low ee values sometimes observed are due to the chemical formation of a racemic

Table 1. (*R*)-Oxynitrilase-catalysed synthesis of cyanohydrins from the corresponding aldehydes.



X	Y	State of the enzyme	Solvent	*t*/h	Yield (%)	$ee^{(R)\text{-alcohol}}$ (%)	Ref.
H	NO_2	Purified[a]	AcOEt	–	89	89	13
Cl	H	Purified[a]	AcOEt	–	94	97	13
H	H	Purified[a]	AcOEt	2.5	95	99	21
		Purified	H_2O (pH 5.0)+Et_2O[b]	10	72	92	24
		Purified	H_2O (pH 5.4)+EtOH	1	99	86	21
		Extract	H_2O (pH 5.4)+EtOH	0.7	95	99	25, 26
		Meal	AcOEt	16	100	99	27
		Meal	Pr^i_2O	14	94	98	31
Me	H	Purified[a]	AcOEt	–	75	98.5	13
MeO	H	Extract	H_2O (pH 5.4)+EtOH	0.3	85	78	25, 26
		Meal	AcOEt	89	47	99	27
H	MeO	Meal	AcOEt	36	85	98	29
MeO	MeO	Meal	AcOEt	36	5	0	29
H	PhO	Purified[a]	AcOEt	192	99	98	21
		Purified	H_2O (pH 5.4)+EtOH	5	99	10.5	21
O O		Purified	H_2O (pH 5.0)+Et_2O[b]	14	35	90	24
		Extract	H_2O (pH 5.4)+EtOH	5	50	93	26

[a]On Avicel-cellulose. [b]HCN donor acetone cyanohydrin.

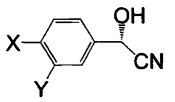
cyanohydrin proceeding parallel to the enzymatic biotransformation.

Solvent systems of a buffer [pH at around 5.4 and <4 for (*R*)- and (*S*)-oxynitrilases, respectively] with a water-miscible (homogeneous) or water-immiscible (two-phase) organic solvent as well as an apolar organic solvent saturated with a buffer are suitable media, the organic solvent allowing the use of reasonable substrate concentrations for oxynitrilase-catalysed synthesis.[13,19–31] A gram-scale synthesis of (*R*)-mandelonitrile using immobilized (*R*)-oxynitrilase from bitter almonds in aqueous ethanol (30% v/v; pH 5.5) was performed as early as some 30 years ago.[20] In aqueous solutions, however, the lability of non-natural cyanohydrins and the non-enzymatic formation of racemic compounds at the operational pH of (*R*)-oxynitrilase give rise to low yields and/or moderate enantiomeric purities (Table 1; entries 10, 15 and 17).[21,25,26] These drawbacks become negligible at lower pH values (Table 2; entries 5, 10, 11 and 13).[19] The stability of cyanohydrins in organic solvents and the discovery that the two oxynitrilases work in organic solvents saturated with a buffer have tremendously improved the synthetic usability of oxynitrilases. Of organic solvents, diisopropyl ether is among the best with respect to the ee values obtained and to the stability of the enzyme.[23]

Immobilization of the isolated enzyme on solid supports (Avicel cellulose, Celite, etc.) is often recommended for the stabilization and catalytic activity of an enzyme.[13,18c,21–23] Moreover, a crude extract from ground almonds in aqueous buffer and ground almond meal (emulsin) itself in organic solvents saturated with a buffer are useful catalysts.[25–29,31] Later, a simple method applying powdered, dechlorophylled shoots from *Sorghum bicolor* was developed in our laboratory.[30] The possibility to use almond meal and *Sorghum* shoots in the place of isolated oxynitrilases is an important discovery which reduces the expense of synthetic applications. Moreover, in the whole cell systems the plant material itself serves as a support, making immobilization unnecessary. The results in Tables 1 (entries 7, 8 and 11–13) and 2 (entries 7 and 8) clearly show that the unisolated enzymes in the whole cells produce cyanohydrins with the same efficiency and selectivity as the isolated enzymes. The preliminary results obtained with non-natural aldehydes as substrates strongly support this conclusion.[31]

In the oxynitrilase-catalysed synthesis, hydrogen cyanide can be introduced into the reaction mixture as a neat liquid or as an aqueous solution.[19–23] For the (*R*)-oxynitrilase-catalysed reactions in aqueous solutions, the production of hydrogen cyanide *in situ* using potassium cyanide/acetic acid buffer (pH 5.4) is to be preferred (Table 1; entries 6, 10 and 17).[25,26] For the reactions in organic solvents, hydrogen cyanide can be extracted from the acetate buffer which is used to saturate the solvent (Table 1; entries 7 and 11).[27] Acetone cyanohydrin is a convenient donor of hydrogen cyanide for the two-phasic buffer/diethyl or diisopropyl ether systems although in the case of (*S*)-oxynitrilase the formation of (*S*)-cyanohydrins becomes unreasonably slow (Table 2; entry 7).[24,28,30] This is evidently due to the stability of acetone cyanohydrin at pH < 4 and to the fact that the *Sorghum* oxynitrilase does not catalyse the decomposition of aliphatic ketone (nor aldehyde) cyanohydrins, a reaction which is catalysed by the almond enzyme.[24,28]

Table 2. (*S*)-Oxynitrilase-catalysed synthesis of cyanohydrins from the corresponding aldehydes.



X	Y	State of the enzyme	Solvent	*t*/h	Yield (%)	$ee^{(S)\text{-alcohol}}$ (%)	Ref.
H	CF_3	Purified[a]	Pr^i_2O	20	87	52	18(c)
Cl	H	Purified[a]	Pr^i_2O	48	87	54	18(c), 22
H	Br	Purified[a]	Pr^i_2O	18	94	92	18(c), 22
H	Cl	Purified[a]	Pr^i_2O	48	95	91	18(c), 22
H	H	Purified	H_2O (pH 3.25)	0.75	80	96	19
		Purified[a]	Pr^i_2O	3	91	97	18(c), 22
		Shoots	H_2O (pH 3.25)+Pr^i_2O[b]	284	75	81	30
		Shoots	H_2O (pH 3.75)+Pr^i_2O	30	91	97	31
Me	H	Purified[a]	Pr^i_2O	32	78	87	18(c), 22
H	Me	Purified	H_2O (pH 3.25)	1.5	80	96	19
HO	H	Purified	H_2O (pH 3.75)	0.4	87	99	19
		Purified[a]	Pr^i_2O	–	84	94	22
H	HO	Purified	H_2O (pH 3.20)	0.7	90	98	19
		Purified[a]	Pr^i_2O	24	97	91	18(c), 22
H	MeO	Purified[a]	Pr^i_2O	20	93	89	18(c), 22
H	PhO	Purified[a]	Pr^i_2O	144	93	96	18(c), 22

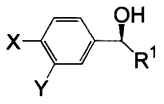
[a] On Avicel-cellulose. [b] HCN donor acetone cyanohydrin.

Baker's yeast

A great number of biocatalytic reductions (Scheme 3) for the preparation of chiral building blocks rely upon baker's yeast.[32] In this case, the benefit of the whole cell catalysis compared with purified oxidoreductases is the availability of the nicotinamide adenine dinucleotide cofactors (NADH and NADPH) inside the cheap cell material where they are recycled as the part of the overall metabolism of the cell. As a drawback, there are competing hydrogenases with reversed stereochemistries in the cell. Baker's yeast-catalysed reductions effected by treating a prochiral ketone with a fermenting sugar–water–yeast suspension in many cases affords chiral alcohols of high enantiomeric purity (Table 3; entries 1, 2, 9–12, 15 and 16).[17] Fermenting baker's yeast without added sugar in the presence of a large excess of yeast is also possible (Table 3; entries 3–8).[10,16,33,34] In the latter case, saccharides present in the cell are supposed to produce NADH or NADPH necessary for the reduction.[35] In fact, the reductions of halohydrins or azido analogues in a sucrose–water–yeast suspension have been reported to lead to lower chemical yields and negligible enantioselectivities, compared with the results shown in Table 3 (entries 3–8).[33]

Baker's yeast-catalysed reductions of phenyl ketones shown in Table 3 typically lead to the formation of (*R*)-alcohols, the enantiomeric purity of which varies from good to excellent. The chemical yield, on the other hand, seems to remain far from the theoretical expectation. The change of the substrate structure and reaction conditions as well as the immobilization of the yeast are methods which have been used to improve enantiomeric purity. Interestingly enough, recycling immobilized baker's yeast leads to the formation of the (*S*)-alcohol in the reduction of α-chloroacetophenone (Table 3; entry 6).[10,16] The importance of the substrate structure to enantioselectivity is clearly seen for the reductions of α-(acylamino)acetophenones (Table 3; entries 1, 2, 9, 15 and 16).

Table 3. Baker's yeast-catalysed synthesis of alcohols from the corresponding ketones.



X	Y	R^1	t/d	Yield (%)	ee$^{(R)\text{-alcohol}}$ (%)	Ref.
Br	H	$CH_2NHCOMe$[a]	21	77	94	17
Cl	H	$CH_2NHCOMe$[a]	21	70	90	17
H	H	CH_2F[b]	1	67	97	33
		CH_2Cl[b]	1	74	82	33
		CH_2Cl[b,c]	1	62	69	10, 16
		CH_2Cl[b,c]	1	78	97[d]	10, 16
		CH_2Br[b]	1	9	97	33
		CH_2N_3[b]	1	24	86	10, 16
		$CH_2NHCOMe$[a]	21	19	26	17
		$CH_2NHCOCF_3$[a]	21	23	6	17
		$CH_2NHCOPh$[a]	21	52	91	17
		CH_2NHCO_2Et[a]	21	40	63	17
		CO_2ME[e]	2	59	100	36
		$CONH_2$[e]	2	70	100	36
Me	H	$CH_2NHCOMe$[a]	21	25	43	17
MeO	H	$CH_2NHCOMe$[a]	21	71	99	17

[a]In the presence of water–sucrose–yeast. [b]In the presence of water–yeast. [c]Immobilized yeast on chrysotile. [d]Second use with immobilized yeast; ee for the (*S*)-alcohol produced. [e]In the presence of water–sucrose–mutant of *Saccharomyces cerevisiae*.

Lipases

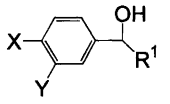
Conventional lipase-catalysed resolution

Lipase-catalysed asymmetric acyl transfers (Scheme 4), *deacylation* by hydrolysis or alcoholysis and *acylation* by irreversible acyl donors such as activated esters and acid anhydrides, are widely exploited methods for the resolution of racemic alcohols.[9,32c] The highly enantioselective resolution tends to stop at 50% conversion, one of the resolution products then appearing as an alcohol and the other as an ester, one of them being a new reaction product and the other a less reactive (or unreactive) counterpart. In the case of less enantioselective resolution, kinetic control usually affords one of the enantiomers with high enantiomeric purity, the other counterpart staying enantiomerically enriched. As a rule, the less reactive enantiomer can be prepared with a higher ee value than the new product.[37] The choice between lipase-catalysed acylation and deacylation determines which of the two products (an alcohol or ester) is less reactive (Scheme 4). The two resolution products are usually separated by appropriate physical means.

Lipase PS (*Pseudomonas cepacia* lipase) and CRL (*Candida rugosa* lipase, formerly *Candida cylindracea* lipase) are the most commonly used lipases for the resolution of the present building blocks and aminoethanols. The reactions are conveniently run in ethers, toluene or other organic solvents in the presence of a commercial lipase powder or of an enzyme which is adsorbed onto solid supports such as Celite. Moreover, the above lipases are activated when the adsorption is performed in the presence of sucrose.[38,39]

Typically, lipase PS allows the resolution of 2-halo- and 2-amino-1-phenylethanols, 2-hydroxycarboxylic acid derivatives and cyanohydrins with excellent (*S*)-selectivity (Tables 4–6).[17,29,40–47] For the acylation of bifunctional aminoethanols, the reaction directed to the secondary alcohol function at the stereogenic centre leads to the enantioselection.[42] *N*-Acyl, *N*-ethoxycarbonyl and *N*-benzyloxycarbonyl derivatives of 2-amino-1-phenylethanols have been successfully resolved (Table 4; entries 1, 3, 4, 6–9, 12, 13, 15, 16 and 18).[17,41,42] The prior protection of the amino group is not necessary, though, due to the fast simultaneous chemical and enzymatic *N*-acylation which is followed by the enantioselective *O*-acylation *in situ* (Table 4; entry 10).[42] Because of the *O*-selectivity of li-

Table 4. Lipase PS-catalysed acylation of alcohols in organic solvents.



X	Y	R^1	*t*/h	Conversion (%)	$ee^{(S)\text{-product}}$ (%)	$ee^{(R)\text{-alcohol}}$ (%)	Ref.
CF_3	H	$CH_2NHCOPr^a$	4	50	96	98	42
Br	H	CH_2Br^b	26	50	95	94	40
		$CH_2NHCOMe^c$	192	48	99	99	17
Cl	H	$CH_2NHCOMe^c$	168	44	99	99	17
H	H	CH_2Cl^b	17	52	92	97	40
		$CH_2NHCO_2Et^{d/c}$	44/240	50/45	81/96	94/85	41/17
		$CH_2NHCOMe^c$	27	43	98	82	17
		$CH_2NHCOPr^a$	3	50	>99	98	42
		$CH_2NHCOCF_3{}^c$	240	30	24	28	17
		$CH_2NH_2{}^e$	7	52	92	>99	42
		CO_2Me^d	30	54	87	98	45
Me	H	$CH_2NHCOMe^c$	96	27	95	25	17
		$CH_2NHCOPr^a$	6	50	98	99	42
MeO	H	CH_2Br^b	30	49	93	87	40
		$CH_2NHCOMe^c$	84	40	94	35	17
		$CH_2NHCOPr^a$	2	50	98	99	42
MeO	MeO	CH_2Cl^b	42	50	97	87	40
		$CH_2NHCOPr^a$	22	51	89	99	42

[a]Acyl donor propionic anhydride. [b]Acyl donor isopropenyl acetate; catalyst lipase P from *Pseudomonas fluorescens*. [c]Acyl donor vinyl acetate. [d]Acyl donor butyric anhydride. [e]Acyl donor acetic anhydride.

pase PS,[48] enzymatic *O*-acylation of an unprotected amino alcohol may well proceed at least to some extent but due to the fast $O \rightarrow N$ acyl migration and to the higher stability of an amide, the observed monoacylation product is an amide rather than the corresponding ester.

Deacylations of secondary alcohol esters by achiral alcohols often proceed slowly (Table 5). Although the lipase PS-catalysed alcoholysis of *N,O*-diacylated 2-amino-1-phenylethanol followed by the normal work-up and deprotection gives the two enantiomers simultaneously from the same resolution, the substituent effects of X and Y on reactivity lead to unacceptable rate retardations as is shown, e.g., for the important salbutamol precursor (Table 5; entry 22).[41,42] The cleavage of the amide bond is not catalysed by the lipase. Deacylations are recommended, however, when the less reactive enantiomer as an ester is preferred, e.g., when the alcohol itself is liable to racemization. That is the case for the resolution of cyanohydrins. As is shown in Table 5 (entries 2, 3, 11, 15, 18 and 20), lipase PS-catalysed alcoholyses in diisopropyl ether give the (*R*)-cyanohydrin proprionates almost enantiomerically pure when the reactions are conducted to somewhat over 50% conversion.[4] Structurally different cyanohydrin esters with the *R*-absolute configuration have also been prepared through deacylation by hydrolysis in the presence of various *Pseudomonas* lipases.[46,49]

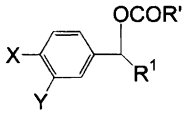
CRL-catalysed deacylations allow the preparation of acylated cyanohydrins and alkyl mandelates as the less reactive *S*-counterparts. Enantioselectivity strongly depends on the nature of the substituents X and Y. (Table 5; entries 2, 3, 10, 11, 15, 18 and 20).[43,47] For the other building blocks CRL shows the normal *S*-selectivity. The *O*-deacylation of *N,O*-diacylated *N*-alkyl aminoethanols also takes place (Table 5; entries 6 and 7) although the reaction is not catalysed by lipase PS.[41] Secondary alcohols are not acylated by CRL.

Enhancement of the chemical yield

Various *in situ* methods exist in order to turn the unwanted enantiomer of a racemic mixture into the desired one. In such a case the need to separate the resolved enantiomers is also overcome. Thus, the lability of aryl cyanohydrins, especially in the presence of a base, provides a tool for the transformation of a racemic mixture to one enantiomer as an ester. This tool has been exploited for the lipase PS-catalysed acylation of several *para*-substituted (*S*)-cyanohydrins in a one-pot synthesis starting with the corresponding aldehydes and using acetone cyanohydrin as the source of hydrogen cyanide (Table 6).[38,47] A base catalyst in this system causes the fast condensation of hydrogen cyanide with the aldehyde to form a racemic cyanohydrin, and the decomposition of the less reactive cyanohydrin enantiomer to its counterparts. Thus far, the method is restricted to the acylation of aromatic (*S*)-cyanohydrins because lipase PS seems to be the only lipase which tolerates the presence of a base.[47]

Another way to turn a racemic mixture into one enantiomer is inversion of configuration at the stereogenic centre of the other enantiomer. Accordingly, the lipase-catalysed kinetic resolution followed by the Mitsunobu esterification of the free alcohol with the carboxylic acid (RCO_2H) *in situ* should lead to the product which is chemically and configurationally identical with the origi-

Table 5. Lipase-catalysed deacylation of esters with 1-hexanol in organic solvents.



X	Y	R^1	R'	Lipase	*t*/h	Conversion (%)	ee$^{(S)\text{-product}}$ (%)	ee$^{(R)\text{-ester}}$ (%)	Ref.
CF_3	H	$CH_2NHCOPr$	Pr	Lipase PS	48	50	97	99	42
		CN	Et	Lipase PS/CRL	29/100	59/61		96/51[a]	47
Br	H	CN	Et	Lipase PS/CRL	32/32	62/62	–	98/77[a]	47
H	H	CH_2Cl[c]	H	CRL	48	40	29	–	51
		CH_2Cl[c]	Me	CRL	456	42	22	19	50
		$CH_2N(Me)COPr$	Pr	CRL	173	40	93	77	41
		$CH_2N(Pr^i)COPr$	Pr	CRL	234	38	82	26	41
		$CH_2NHCOPr$	Pr	Lipase PS	32	50	97	97	42
		$CH_2NHCOPr$	CH_2Cl	Lipase PS	3	50	96	>99	42
		CN	Me	Lipase PS/CRL	–/48	51/38	95/77[b]	97/–[a]	43/50
		CN	Et	Lipase PS/CRL	48/53	54/55	–/77[b]	>99/77[a]	47
		CO_2Bu[c]	H	CRL	27	39	75[b]	[a]	51
		CO_2Bu[c]	Me	CRL	168	45	92[b]	82[a]	50
Me	H	$CH_2NHCOPr$	Pr	Lipase PS	144	51	97	98	42
		CN	Et	Lipase PS/CRL	39/97	52/65	–	>99/>99[a]	47
MeO	H	$CH_2NHCOPr$	Pr	Lipase PS	144	49	94	96	42
		CN[d]	Me	Lipase PS	–	51	91	95	43
		CN	Et	Lipase PS/CRL	45/98	55/66	–	>99/>99[a]	47
MeO	MeO	CN[d]	Me	Lipase PS	–	44	>99	79	43
		CN	Et	Lipase PS/CRL	43/70	47/56	–	74/82[a]	47
		CN[d]	Me	Lipase PS	–	50	95	93	43
		$CH_2NHCOPr$	Pr	Lipase PS	300	48	98	92	42
PivO	H	CN[d]	Me	Lipase PS	–	51	96	>99	43
H	PivO	CN[d]	Me	Lipase PS	–	52	–	96	43
PivO	PivO	CN[d]	Me	Lipase PS	–	48	>99	91	43
PivO	$PivOCH_2$	CN[d]	Me	Lipase PS	–	50	95	95	[e]

[a]Absolute configuration (*S*). [b]Absolute configuration (*R*). [c]Deacylation with 1-butanol. [d]Deacylation with 1-propanol. [e]New result.

nal ester enantiomer in the resolved mixture (Table 4).[43] As a benefit, the method is valid both for enzymatic acylation and deacylation. For every resolution–inversion sequence, there is a maximal ee for the ester finally produced corresponding to the certain conversion of an enzymatic reaction.[43] When the enantiomeric ratio, *E*,[37] for the resolution is known the theoretical plots shown in Fig. 1 give values to this optimal termination conversion, *c*(termination), and the corresponding value of ee(final) for the final ester obtained after the Mitsunobu esterification through inversion.

The resolution–inversion method shows excellent validity when the *E* value is of the order of 100 or higher. In accordance with the above, the (*R*)-ester amide $C_6H_5CH(OCOPr)CH_2NH_2COPr$ with 100% isolated yield and 97% ee was prepared using lipase PS-catalysed deacylation ($E = 420$) in diisopropyl ether linked with the Mitsunobu esterification of the (*S*)-product with butyric acid. It can be concluded that the method is also usable in the other highly enantioselective acylations and deacylations of Tables 4–6; the structural features of the esterified alcohol would be expected to favour the Mitsunobu esterification by the S_N2 mechanism. Accordingly, when the (*S*)-alcohols that resulted from the lipase PS-catalysed deacylation of substituted mandelonitrile acetates (Table 5; entries 10, 17, 19, 21 and 23–26) were

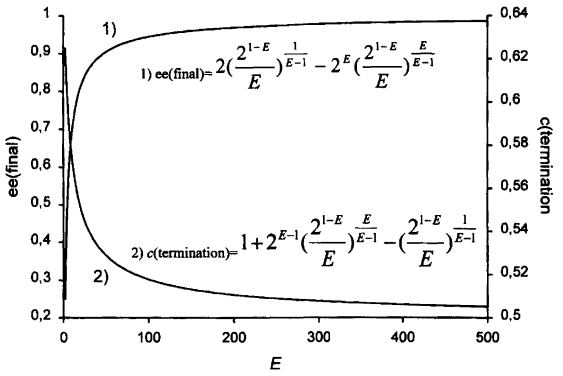
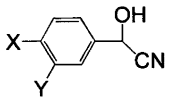
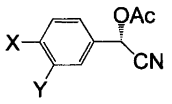


Fig. 1. The correlation between ee(final) (eqn. [1]) and *c*(termination) (eqn. [2]) vs. *E* for the resolution–Mitsunobu esterification procedure *in situ* in the resolution mixture.

Table 6. Lipase PS-catalysed acylation of cyanohydrins and one-pot synthesis of (*S*)-acetates from the corresponding aldehydes, isopropenyl acetate and acetone cyanohydrin in the presence of lipase PS and Amberlite IRA-904 resin in diisopropyl ether.





		Resolution			One-pot			
X	Y	*t*/h	Conversion (%)	ee$^{(S)\text{-ester}}$ (%)	*t*/h	Yield[a] (%)	ee$^{(S)\text{-ester}}$ (%)	Ref.
CF_3	H	24	44	93	47	76	73	47
Br	H	23	34	95	95	94	92	47
Cl	H	–	–	–	91	83	84	38
H	H	19	49	84	96	99	91	47
Me	H	25	42	89	168	99	94	47
MeO	H	42	49	84	165	90	92	47
H	MeO	144	50[b]	55	–	–	–	29
MeO	MeO	45	46	83	168	84	90	47
HO	MeO	216	44[c]	79	–	–	–	46
H	PhO	–	–	–	70	80	89	38
H*p*-F-PhO		–	–	–	72	92	87	38
F	PhO	–	–	–	72	88	91	38
⌒		136	48	95	156	81	91	[d]38

[a]Isolated yield based on to the starting aldehyde. [b]Acyl donor vinyl acetate; catalyst *Pseudomonas* sp. [c]Acyl donor vinyl acteta. [d]New result.

acetylated with acetic acid under the Mitsunobu conditions the theoretical and observed ee values of the (*R*)-acetates in the above order are 96 (93), 93 (63), >88 (61), 94 (78), >98 (97), 92 (63), >95 (81) and 95 (86)%, respectively.[43] Thus, the method is valid in the cases of mandelonitrile (X = H, Y = H) and the *para*-pivaloyl-substituted compound (X = PivO, Y = H). Electron-donating substituents favour unimolecular nucleophilic substitution leading to racemization. The *meta*-pivaloyl substituent seems to be responsible for the racemization of cyanohydrins with X = H, Y = PivO and X = Y = PivO.

Conclusions

The most frequently used biocatalytic routes applied to organic synthesis are micro-organisms, such as baker's yeast, and enzymes, such as lipases and oxynitrilases, which do not need added cofactors. There are often several types of optically active building block or precursor to a enantiomerically pure compound. There may also be several biocatalytic possibilities for the preparation of one building block. Most frequently, these possibilities should be seen as complementary rather than competitive. In this review, this is outlined for the biocatalytic pathways to optically active 2-amino-1-ethanols. Thus, a lipase-catalysed kinetic resolution and oxynitrilase-catalysed asymmetric synthesis provide an easy access to optically active aryl cyanohydrins although the substrate specificities vary from one enzyme to another. Chemical means, such as *in situ* racemization or the Mitsunobu esterification, modify the conventional kinetic resolution and by doing this make the method equal to asymmetric synthesis as regards the theoretical yield of one enantiomer. On the other hand, the poor availability and accordingly, the high price of (*S*)-oxynitrilase, in particular, can be avoided by using whole cell catalysis. The use of whole cell systems will be problematic though owing to possibly competitive enzymes in the cell. This is not a problem in the case of oxynitrilase catalysis but may be in the case of baker's yeast.

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