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Enantiomers of Ring-Substituted 2-Amino-1-phenylethanols by *Pseudomonas cepacia* Lipase

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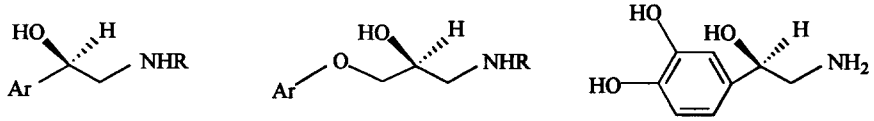
Abstract: The enantiomers of 2-amino-1-phenylethanols were obtained enantiomerically pure (ee > 95%) at 50% conversion by the *Pseudomonas cepacia* lipase-catalysed *O*-acylation of amino alcohols or *O*-deacylation of the corresponding *N,O*-diacylated compounds.

Many adrenergic drugs are 2-amino-1-arylethanol **1** or 1-amino-3-aryloxy-2-propanol **2** derivatives, both structures possessing a stereogenic centre with a secondary alcohol function (Scheme 1).[1] It is well-known that the two enantiomers of a chiral drug often display different biological activities. Consequently, the enantiomeric purity of such compounds is highly important, the (*R*)-enantiomers in the case of amino ethanol and the (*S*)-enantiomers in the case of amino-2-propanol type drugs being generally associated with the highest agonist or β-blocking activity in accordance with the structural similarity to the natural catecholamine, (*R*)-noradrenaline **3**. The opposite enantiomers are less potent or they may exert unpleasant side effects.



1 **2** **3**

Scheme 1

Biocatalytic approaches to optically active compounds are gaining more and more importance in organic synthesis.[2] In particular, lipase-catalysed asymmetric acyl transfers have been widely utilized in kinetic resolutions of racemic alcohols, amines and carboxylic acid esters in aqueous as well as in non-aqueous conditions. In the case of compounds **1** and **2** with R = H (or in the case of the corresponding ester-amides), there are two functional groups (HO and NH_2 or OCOR' and NHCOR) which, in principle, can be subjected to enzymatic acylation (or deacylation), and the reaction directed to the functional group at the stereogenic centre should result in the highest enantioselection. In spite of enzymatic chemoselectivity the monoacylation of compounds **1** and **2** evidently always leads to the formation of an amide product due to a favourable $O \rightarrow N$ acyl migration and to the higher stability of an amide product compared to the corresponding ester.[3] In the case of deacylation, a lipase works only at the ester bond, leaving the amide bond unreacted. The lipase-catalysed enantioselective acylation of racemic 2-amino-1-

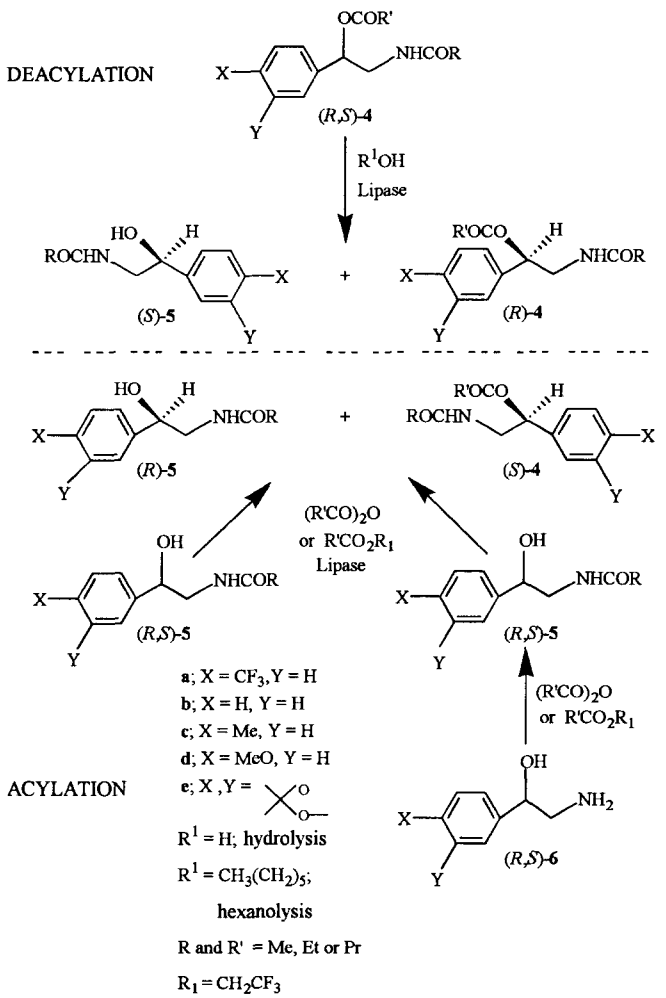
butanol and 1-amino-2-propanol has been described.[4] It seems, however, that the protection of the amino group is necessary in order to achieve effective enzymatic resolution.[5-8] Accordingly, the lipase from *Pseudomonas cepacia* (lipase PS from Amano Pharmaceuticals) enantioselectively acylated the secondary HO group of 2-amino-1-phenylethanol in the cases of *N*-acetyl, *N*-ethoxycarbonyl and *N*-benzyloxycarbonyl derivatives.[7,8] On the other hand, the lipase PS-catalysed deacylation of *N,O*-dibutyrylated 2-amino-1-phenylethanol with 1-alcohols gave the two enantiomers enantiomerically pure at 50% conversion after the normal work-up and deprotection of the functional groups.[7] In the above acylations and deacylations, long reaction times (usually 2-10 days to reach the optimal 50% conversion) limit the real synthetic usability.

In this work, the lipase PS-catalysed acylations of racemic *N*-acylated **5a-e** or free **6b** and **c** 2-amino-1-phenylethanols and deacylations of the corresponding *N,O*-diacylated counterparts **4a-e** were studied paying attention to the synthetic simplicity and economy as to time and number of synthetic steps (Scheme 2). Racemic amino ethanol derivatives were prepared according to the known procedures.[9] For the present resolutions, the low solubilities of ester-amides **4a-e**, amide alcohols **5a-e** and/or free amino alcohols **6a-e** restrict the number of usable solvents, toluene, toluene/THF or toluene/water being used throughout the work. The biotransformations in organic solvents were performed as previously described.[7] In the case of deacylation by hydrolysis, the reactions were performed in the two-phasic mixture of toluene [20% (v/v)] and phosphate buffer (0.01 M, pH 7.0), and the constant pH was maintained by adding NaOH (0.05 M) during the reaction. The enantiomeric purities were obtained by GLC equipped with a Chirasil-L-Val column where the two enantiomers of the resolution products [(*R*)- or (*S*)-amide **5a-e** and (*S*)- or (*R*)-ester-amide **4a-e**; Scheme 2] gave good base-line separations when the amide counterparts were first chemically derivatized with an appropriate acid anhydride in the presence of pyridine and 4-dimethylaminopyridine. The absolute configurations are according to the known (*S*)-selectivity of the enzyme[7,8] and to the separation order of the enantiomers in the chiral column. The results are shown in Table 1 and Figure 1.

1-Hexanol was used as a deacylating agent for the lipase PS-catalysed resolution of racemic **4a-e** in toluene. In order to enhance reactivity compared to the previous work, mainly three strategies were used. First, lipase PS was activated by adsorbing the enzyme on Celite in the presence of sucrose.[10] Accordingly, the hexanolysis of racemic butyrate **4b** (R=R'=Pr; R^1=C_6H_{13}) in the presence of lipase PS preparation[11] (0.1 g/ml) reached 50% conversion within 32 hours (Table 1, row 2) while 89 hours were needed in the case of commercial enzyme powder (0.1 g/ml) at 40 °C.[7] Optimization of the water content of the system is another generally used way in affecting reactivity. For that purpose, the effects of two salt hydrates ($Na_2HPO_4 \times 12H_2O$ and $Na_2SO_4 \times 10H_2O$) at the concentrations between 30-500 mg to 100 mg of the enzyme preparation or commercial enzyme powder were tested, sodium sulphate leading to highest rate enhancement in the last-mentioned case (Figure 1; open and black circles). For the catalysis by the enzyme preparation, the effect of salt hydrates (Figure 1; open and black squares) as well as that of dry salts on reactivity was negligible, indicating already appropriate water level of the catalyst. Rather, the slight rate retardation observed is due to the high amount of insoluble material in the reaction mixture. As the third possibility, the enantioselective hexanolysis of activated chlorocetate **4b** (R=Pr, R'=CH_2Cl; Table 1, row 3) needs only 3 hours to

reach 50% conversion, although one may want to avoid the use of halogenated esters. The results of Table 1 clearly show that the hexanolyses of racemic butyrates **4a-e** (R=R'=Pr) in the presence of the lipase PS preparation in toluene proceed with extremely high enantioselectivity. However, the substituent effects of X and Y on reactivity may lead to unbearable rate retardations as is shown especially for the important salbutamol precursor **4e**.

The lipase PS-catalysed deacylation of racemic butyrates **4a-d** (R=R'=Pr, R^1=H) by hydrolysis in the biphasic toluene/water system also proceeded with extremely high enantioselectivity (Table 1). Toluene in this system was used to dissolve the resolved counterparts (*R*)-**4a-d** and (*S*)-**5a-d** as well as racemic starting materials. Although the hydrolysis rates are considerably faster than the corresponding hexanolysis rates the disadvantage of the method is the solubility of the enzyme into water which makes the reuse of the enzyme difficult.



Scheme 2

Table 1. Lipase PS-catalysed resolution of amino alcohols by acyl transfers.

Method	Substrate	t/h	Conversion[h] /%	$ee_{substrate}(R)$ /%	$ee_{product}(S)$ /%
Hexanolysis[a]	**4a**	48	50	99	97
	4b	32	50	97	97
	4b[e]	3	50	>99[i]	96
	4c	144	51	98	97
	4d	144	49	96	94
	4e	300	48	92	98
Hydrolysis[b]	**4a**	34	50	96	97
	4b	23	50	>99[i]	>99[i]
	4c	40	50	>99[i]	98
	4d	64	50	98	97
Acylation[c]	**5a**	4	50	98	96
	5b	3	50	98	>99[i]
	5c	6	50	99	98
	5d	2	50	99	98
	5e	22	51	99	89
N-Protection and *O*-acylation *in situ*[d]	**6b**	6	51	>99[i]	97
	6b[f]	7	52	>99[i]	92
	6b[g]	48	49	93	96
	6c	10	50	>99[i]	96

[a] Enzyme preparation (0.3 g), substrate (0.1 M; R=R'=Pr) and hexanol (0.2 M) in toluene (3 ml) at 37 °C. [b] Substrate (1.8 mmol; R=R'=Pr) and lipase PS powder (0.6 g) in toluene (20% (v/v))/water (20 ml) at 25 °C. [c] Enzyme preparation (0.1 - 0.15 g), substrate (0.05 M; R=Pr) and propionic anhydride (0.05 M) in toluene/THF (3:1; 3 ml) at 47 °C. [d] Enzyme preparation (0.15 g), substrate (0.05 M) and butyric anhydride (0.2 M) in toluene/THF (3:1; 3 ml) at 47 °C; resolution products (*R*)-amide **5** and (*S*)-ester-amide **4**. [e] Substrate chloroacetate (R=Pr, R'=CH_2Cl). [f] Acylation with acetic anhydride (0.2 M). [g] Acylation with 2,2,2-trifluoroethyl butyrate (0.3 M). [h] From equation $c = ee_{substrate}/(ee_{substrate} + ee_{product})$. [i] No sign about the other enantiomer according to chiral GLC.

Preferring enzymatic resolutions in organic solvents, we finally relied on the enzymatic *O*-acylation of racemic amides **5a-e** in toluene/THF (3:1). Effective acyl donors are needed especially when sterically hindered secondary alcohols are resolved. Acid anhydrides are the most effective acyl donors whenever the enzyme tolerates the presence of the liberated acid and non-enzymatic *O*-acylation is not disturbing. Using propionic anhydride (R'=Et) as an acyl donor for the resolutions of *N*-acylated amino alcohols **5a-e** (R = Pr) in the presence of lipase PS preparation (33-50 mg/ml), the *O*-acylation smoothly proceeded to 50% conversion within less than a day, resulting

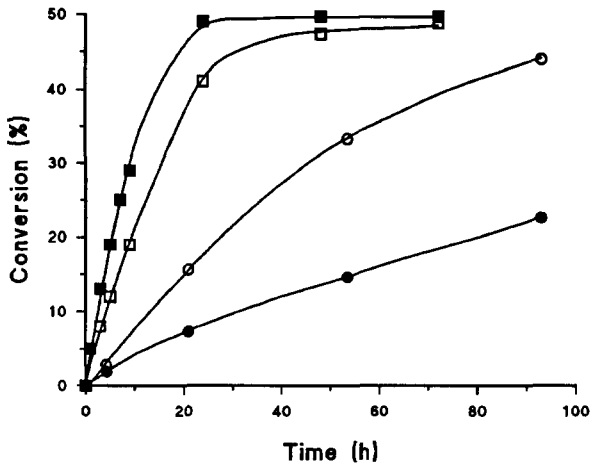


Figure 1. The formation of amide **5b** (R=Pr) with time for the hexanolysis of butyrate **4b** (R=R'=Pr) in toluene at 25 °C: (•) lipase PS powder (100 mg/ml) and (o) $Na_2SO_4 \times 10H_2O$ (300 mg/ml) added; (■) lipase PS preparation (100 mg/ml) and (□) $Na_2SO_4 \times 10H_2O$ (300 mg/ml) added.

in the two resolution products (*R*)-**5a-e** and (*S*)-**4a-d** enantiomerically pure (Table 1; ee 96-100%). The exception is ester-amide (*S*)-**4e** (R=Pr, R'=Et) with 89% ee only. The high enantioselectivities of the present acylations are in accordance with this observed for the slow acylations of amides **5b-c** (R = Me) with vinyl acetate.[8] Thus, the use of activated enzyme as a catalyst and acid anhydride as an acyl donor give synthetic value for the present method.

Trusting on the high enantioselectivity of enzymatic *O*-acylations, we wanted to reject the separate *N*-protection step for the resolution of amino alcohols. The *N*-acylation of amino ethanols **6a-e** with acid anhydrides in the presence of the lipase preparation rapidly proceeds to completion (usually within less than 5 minutes), enabling the use of unprotected amino ethanols as substrates (Table 1, *N*-protection and *O*-acylation *in situ*). On the other hand, activated esters, such as 2,2,2-trifluoroethyl butyrate (Table 1, row 18), as acyl donors result in the major enzymatic and minor non-enzymatic *N*-protection which again is followed by the highly selective *O*-acylation of the enantiomerically enriched amide. Enantioselectivity of the enzymatic *N*-acylation is negligible, however, as can be concluded from the ee value of only 50 % obtained for the less reactive **6b** after 95% of it was transformed to the corresponding amide **5b** (R=R'=Pr) under the experimental conditions of Table 1 (row 18). Moreover, it is worth mentioning that the enantiomeric purity of the less reactive amides (*R*)-**5a-e** tends to decrease at longer reaction times, the decrease being more significant at higher enzyme concentrations. This is due to the slow lipase-catalysed hydrolysis of ester amides (*S*)-**4a-e** to the corresponding amides (*S*)-**5a-e** by the water present in the enzyme preparation. Accordingly, the removal of the enzyme after the 50% conversion is reached is highly recommended.

In conclusion, the acylation of 2-amino-1-phenylethanols with acid anhydrides in the presence of activated

lipase PS (lipase and sucrose on Celite) in toluene/THF is a fast and economical method for the preparation of the two enantiomers [one as the less reactive (*R*)-amide and the other as the (*S*)-ester-amide] of the amino alcohol with high chemical (50% in theory) and optical (ee>95%) yields. Due to the effective *N*-protection *in situ* in the reaction mixture there is no need for a prior protection of the amino group. Activated esters work similarly, except that *in situ* *N*-acylations proceed slower and that the resolution times become unreasonably long. The resolution products can be easily separated by column chromatography.[7,8] Moreover, in the resolution mixture it is possible to increase the theoretical yield of the ester enantiomer from 50 to 100% by performing the Mitsunobu esterification of the amide enantiomer with R'COOH in one pot. We previously used this method for the preparation of butyrate (*R*)-**4b** (R=R'=Pr) with 100% isolated chemical yield and 97% ee starting with the lipase PS-catalysed propanolysis of racemic **4b**.[12] In the case of the present enzymatic acylations, the esterification of (*R*)-amides **5a-e**, on the other hand, should lead to the formation of the corresponding (*S*)-ester-amides **4a-e** (R=R'; Scheme 2).

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9. Compounds (*R,S*)-**6** were prepared from the corresponding aldehydes through cyanohydrin formation (Kanerva, L.T.; Rahiala, K.; Sundholm, O. *Biocatalysis* **1994**, *10*, 169) followed by the reduction with $LiAlH_4$ (Ziegler, T.; Hörsch B.; Effenberger F. *Synthesis* **1990**, 575). Amides (*R,S*)-**5** or ester-amides (*R,S*)-**4** were obtained from the corresponding amino alcohols and acid anhydrides in the presence of pyridine and 4-dimethylaminopyridine using stoichiometric amount or excess of acid anhydride, respectively (Ref. 7).

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11. The lipase (1 g) in Tris-HCl buffer (pH 7.8) was adsorbed on Celite (1 g) in the presence of sucrose (0.6 g).

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