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Enzymatic one-pot resolution of two nucleophiles: alcohol and amine

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Abstract

Enzymatic aminolysis of racemic secondary alcohol ester derivatives with racemic amines leads to the resolution, in one reaction, of the alcohol and the amine with very high enantioselectivity.

Lipases and esterases accept a wide range of substrates, which are usually converted with high enantioselectivity. Kinetic resolutions of primary and secondary alcohols in organic solvents are carried out, in most cases, through lipase-catalyzed transesterification and esterification reactions.[1–3] In order to shift the equilibrium inherent to these kinds of reactions towards product synthesis, activated esters such as vinyl acetate are routinely employed.[4] Taking into account that lipases do not have amidase activity,[5,6,†] resolution of alcohols by aminolysis of their corresponding ester derivatives constitutes an alternative to the use of activated esters. Moreover, this strategy opens the possibility to the one-pot resolution of both nucleophiles when a racemic amine is used. Optically active alcohols and amines are important classes of compounds. Thus, amines bearing an α stereogenic centre have been extensively employed as resolving agents,[8] chiral auxiliaries and intermediates in the synthesis of a wide range of biologically active molecules.[9] More recently, they have also been used for the induction of helicity in polymers.[10] On the other hand, enantiomerically pure secondary alcohols are useful chiral auxiliaries in organic chemistry both for analytical and synthetic applications.[11]

Despite the consequent advantages of carrying out two resolutions in a single process, this possibility has only been attempted using alcohols and a thiol[12] or a diol as nucleophiles.[13] In the former case, problems of reversibility led to enantiomeric excesses (ee) lower than for the corresponding single resolutions. However, this strategy has never been applied to amines to the best

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† The hydrolysis of *N*-acetylamines catalyzed by *Candida antarctica* lipase B in phosphate buffer has been described but >10 days of reaction were necessary to achieve a high conversion at 50°C.[7]

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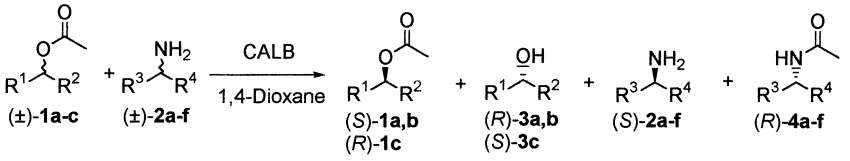
of our knowledge. Here we report for the first time the one-pot kinetic resolution of different secondary alcohols and amines bearing an α stereogenic centre.

All reactions (Table 1) were carried out using lipase B from *Candida antarctica* (CALB) as catalyst (500 mg), 1,4-dioxane as solvent (10 mL) at 30°C and 250 r.p.m. Enzyme and reaction conditions were selected on the basis of previous results obtained in the stereoselective amidation of racemic amines.[14] In all cases the corresponding acetyl derivative of the alcohol and the amine were in equimolar ratio (3.0 mmol). In order to avoid the competitive enzymatic hydrolysis of the ester, reagents and solvent were dried prior to use and nitrogen atmosphere was used. Moreover, 4 Å molecular sieves were added (350 mg) to ensure a low water activity in the reaction medium. When the reaction was stopped, the crude of the reaction consisted of a mixture of four compounds — ester, alcohol, amine and amide — but the isolation and purification of each one was easily accomplished by acidic extraction and subsequent column chromatography of the organic phase.‡ Absolute configurations for both nucleophiles were determined by comparison of the sign of the specific rotation of either substrate or product with the data published in the literature. Two conversion values were calculated for each aminolysis reaction: c_a obtained from ee values for

Table 1

Compounds **1a** and **3a**: R^1 = Ph, R^2 = Me; **1b** and **3b**: R^1 = *n*-hexyl, R^2 = Me; **1c** and **3c**: R^1 = Ph, R^2 = methoxymethyl; **2a** and **4a**: R^3 = Ph, R^4 = Me; **2b** and **4b**: R^3 = *n*-pentyl, R^4 = Me; **2c** and **4c**: R^3 = 1-naphthyl, R^4 = Me; **2d** and **4d**: R^3 = (3-trifluoromethyl)benzyl, R^4 = Me; **2e** and **4e**: R^3 = ethyl, R^4 = Me; **2f** and **4f**: R^3 = Ph, R^4 = *n*-propyl



				ee, %				ee, %			
Entry	Ester	Amine	t, h	**1**	**3**	c_e,[a] %	E_e[a]	**2**	**4**	c_a,[a] %	E_a[a]
1	**1a**	**2a**	17	37	>99	27	>200	36	99	27	>200
2	**1a**	**2b**	17	92	99	48	>200	93	97	49	>200
3	**1a**	**2c**	16	36	99	27	>200	41	>99	29	>200
4	**1a**	**2d**	13	59	>99	37	>200	53	98	35	168
5	**1b**	**2a**	16	70	>99	41	>200	64	99	39	>200
6	**1a**	**2e**	8	40	>99	29	>200	28	49	36	4
7	**1a**	**2f**	150	24	93	21	34	21	97	18	60
8	**1c**	**2a**	166	38	96	28	71	33	>99	25	>200

[a] See reference 15.

‡ Once the enzyme had been filtered off and washed with methylene chloride, the organic solution was extracted with 2N HCl and water. The crude resulting from the evaporation of the organic phase was purified by silica gel chromatography employing a hexane/ethyl acetate gradient. Evaporation of the aqueous phase yielded the remaining amine in its hydrochloride form. In all cases, ester and alcohol were isolated in >75% yield and amine and amide in >85% yield. Yields were calculated taking into account conversion values.

substrate (amine) and product (amide) and c_e obtained from ee of ester and alcohol.[15§] Coincidence in both conversion values was the criterion considered to confirm that side reactions had not taken place.[¶] Results obtained are summarized in Table 1.

First we examined the efficiency of the CALB-catalyzed aminolysis of (±)-1-(phenyl)ethyl alcohol employing its corresponding acetate (±)-**1a** as substrate. Different racemic amines such as (±)-1-(phenyl)ethylamine (±)-**2a**, (±)-2-heptylamine (±)-**2b**, (±)-1-[1-(naphthyl)]ethylamine (±)-**2c** and (±)-[1-(3-trifluoromethyl)phenyl]-2-propylamine (±)-**2d**[‖] (entries 1–4, respectively) were used as nucleophiles. In all cases the enzyme reacts faster with the *R* enantiomer of both ester and amine. Thus, we obtained the *S* esters and amines and the *R* alcohols and amides. Values of the enantiomeric ratio[15] obtained for the resolution of the alcohol with these four amines (E_e) were very high. On the other hand, the *E* values obtained for the amines (E_a) were excellent as well.

From the aminolysis of (±)-**2d** with (±)-**1a** (entry 4), acetamide (*R*)-**4d** is straightforwardly obtained. This amide **4d** constitutes an intermediate in the synthesis of fenfluramine,[16] a compound with biological properties as anorectic agent.[17] Due to the fact that both enantiomers of fenfluramine exhibit different biological properties, obtaining both *R*- and *S*-**4d** is always desirable. In our case, the remaining substrate (*S*)-**2d** can be transformed into (*S*)-**4d** thereby allowing the preparation of both enantiomers of fenfluramine easily.

Next, we used (±)-2-octyl acetate as substrate (±)-**1b** and (±)-1-(phenyl)ethylamine (±)-**2a** as nucleophile (entry 5). Again results show the same enantiopreference of CALB towards *R* enantiomers and high enantiomeric ratios for both alcohol and amine.

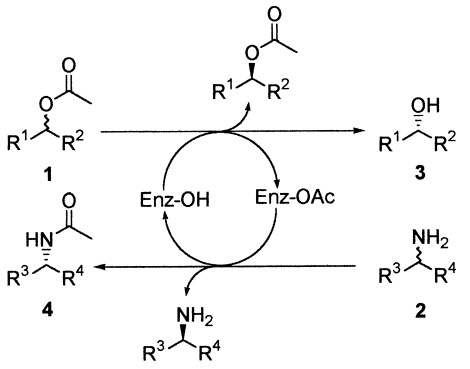
These results are in agreement with the general rule for the stereospecifity and efficiency of lipases in the resolution of secondary alcohols.[18] This rule is a consequence of the common structure of the active site of lipases.[19] This structure shows that the cavity of the active site, which hosts the leaving group and the nucleophile, is the same one. When nucleophiles are secondary alcohols, the higher difference in size between the substituents at the stereogenic centre, the greater *E* values will be obtained. In addition to this, when kinetic resolutions of secondary alcohols are accomplished with CALB, it has been shown that if the medium sized substituent at the stereogenic centre is larger than an ethyl group the enantiomeric ratio is significantly reduced.[20] Results obtained in the kinetic resolution of α-disubstituted primary amines with CALB show that these amines behave similarly to secondary alcohols.[12,14]

At this point, the question of the independence of both resolutions with regard to the enantioselectivity arose. The proposed mechanism for lipase catalysis (Scheme 1) shows that enantiodiscrimination of each substrate takes place in different steps. Thus, this mechanism supports the hypothesis that *E* values measured for each nucleophile of the pair do not depend at all on the nature of its counterpart. In order to shed light on this aspect we decided to carry out the resolution

§ Enantiomeric excesses of compounds **1a**, **3a**, **1c**, **3c**, **4a** and **4d** were determined by means of chiral HPLC using a Chiralcel OB-H column. Amines **2a** and **2d** were transformed into their corresponding acetamides (**4a** and **4d**) prior to ee analysis. The ee of ester **1b** was determined by chiral GC using a Cydex-B chiral capillary column. Alcohol **3b** was transformed into **1b** in order to determine its ee. The rest of the amines and amides were analyzed through chiral HPLC with a Chiralcel OD column: compounds **2b**, **4b**, **2e** and **4e** had to be transformed into their *N*-benzoylated derivatives prior to analysis, amides **4c** and **4f** were analyzed directly and amines **2c** and **2f** were previously transformed into their corresponding acetamides.

¶ Due to errors inherent in the measure of reagents and enantiomeric excesses, slight differences between c_e and c_a values were tolerated.

‖ Amine (±)-**2d** was prepared by reductive amination of 3-(trifluoromethyl)phenylacetone. Yield: 78%.



Scheme 1. Catalytic mechanism: acylation and deacylation steps

of acetate (±)-**1a** employing as nucleophiles (±)-2-butylamine (±)-**2e** and (±)-1-phenyl-1-butylamine (±)-**2f** (entries 6 and 7). In these cases and according to the aforementioned rule, amines (±)-**2e** and (±)-**2f** should be resolved with low E_a values. If the nature of the nucleophile does not affect the resolution of the ester, the E_e value measured for (±)-1-(phenyl)ethyl acetate (±)-**1a** should remain higher than 200.

Results obtained with 2-butylamine confirm this hypothesis (entry 6). While the enantiomeric ratio of the alcohol (E_e) remained higher than 200, the E_a value was 4.[**] When amine **2f** was the nucleophile (entry 7) the reaction rate dropped so much that the non-enzymatic aminolysis became significant under reaction conditions.[††] Thereby, E values obtained for the corresponding alcohol and amine underestimate the real ones and, consequently, they cannot be taken into account to confirm the independence of both resolutions. Despite this fact, amide (*R*)-**4f** was obtained with ee = 97%. The apparent enantiomeric ratio for the amine was 60. This apparent enantioselectivity is surprisingly greater than the one we expected. If we consider that a medium sized substituent larger than an ethyl group lowers the enantiomeric ratio of the reaction considerably, the enantiomeric ratio for amine **2f** should be similar to that obtained for analogous secondary alcohols bearing an *n*-propyl group as the medium sized substituent, such as (±)-4-decanol. When this alcohol is resolved by means of CALB-catalyzed transesterification, an E value of 10 is obtained.[20] We are currently investigating this fact with other secondary alcohols and α-disubstituted primary amines in simple and simultaneous resolutions of both nucleophiles.

Finally, we employed (±)-2-methoxy-1-(phenyl)ethyl acetate (±)-**1c** as substrate, an ester derived from an alcohol bearing a medium sized substituent larger than an ethyl group, and (±)-1-(phenyl)ethylamine (±)-**2a** (Table 1, entry 8).[‡‡] The reaction rate was again significantly lower than when esters derived from α-methyl substituted alcohols were used and the enantiomeric ratio obtained was moderate ($E_e = 71$). The E value measured for the amine ($E_a > 200$) does not differ

[**] Because of the low boiling point of amine **2e** (63°C) it was difficult to keep an equimolar amine/ester ratio under reaction conditions. This may account for the discrepancy in the conversion values measured for both nucleophiles (c_e and c_a). In addition to this, partial solubility of acetamide **4e** in both organic and aqueous phases made conventional extraction inefficient. Instead, the organic solution was submitted to continuous extraction over 12 h using methylene chloride as organic solvent.

[††] Analysis of control reaction showed that the non-enzymatic reaction took place with a contribution to the total conversion degree lower than 5%.

[‡‡] In this case, the nature of substituents at the stereogenic centre of ester **1c** leads to the opposite absolute configuration for the same stereochemistry.

from the one obtained when (±)-1-(phenyl)ethyl acetate (±)-**1a** was used as substrate (compare entries 1 and 8).

In conclusion, we have developed a simple and efficient procedure for the one-pot resolution of racemic secondary alcohols and analogous primary amines. The simplicity of the isolation procedure of the four products obtained in each reaction makes the methodology very attractive. The results obtained imply that this methodology can be applied successfully to other racemic alcohols and amines which show high *E* values in simple enzymatic catalyzed kinetic resolutions.

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