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Lipase catalyzed reactions of aliphatic and arylaliphatic carbonic acid esters

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Abstract

Symmetrical dialkyl carbonates and dibenzyl carbonates reacted with various nucleophiles in the presence of *Candida antarctica* lipase B in organic solvents. For example, reaction of dibutyl and dibenzyl carbonate with an alcohol gave a mixture of the mono- and disubstituted products. Aminolysis, however, afforded only the carbamates, without subsequent reaction to the ureum derivatives. The reaction rates were rather low compared with carboxylic esters; the reactivity increased in the order dimethyl < diethyl < dibutyl ≈ dibenzyl carbonate. Aminolysis of dibenzyl carbonate by chiral benzylamines gave the corresponding enantiomerically pure benzyloxycarbonyl amines, thus, providing a direct, phosgene-free route to the Z-protected amine enantiomer from the racemate. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Novozym 435; *Candida antarctica* lipase; Dibutyl carbonate; Dibenzyl carbonate; Resolution

1. Introduction

During the last decade, it has become clear that lipases (EC 3.1.1.3) efficiently catalyze a variety of reactions with non-natural acyl acceptors [1] such as alcohols, hydrogen peroxide, ammonia, amines, and oximes. The choice of the acyl donor, on the other hand, has generally been limited to simple carboxylic acid esters. Lipase-catalyzed transformations of the structurally closely related carbonic acid derivatives [2] has almost exclusively involved activated compounds such as vinyl [3], phenyl [4] or oxime [5] esters, pyrocarbonates [5] or mixed anhydrides [6], and only a few examples of lipase-catalyzed alcoholysis of dialkyl carbonates have been published [7–9].

We have found that Novozym 435 (*Candida antarctica* lipase B) catalyzes the alcoholysis and aminolysis of dibutyl and dibenzyl carbonate. These reactions are of potential interest because they provide a route to carbonic acid derivatives, which does not involve the use of phosgene. Because the benzyloxycarbonyl (Z) group in particular is a frequently used protecting group that can be cleaved by hydrogenolysis under very mild conditions, we have also explored the synthesis of enantiomerically pure Z-amines from racemic precursors [10].

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2. Experimental

2.1. Materials

Novozym 435 (immobilized *C. antarctica* lipase B) was a gift from Novo Nordisk, Denmark and was used as received. *Pseudomonas* lipoprotein lipase was a gift of Boehringer Mannheim. It was immobilized on Accurel EP 100 according to a published procedure [11]. Solvents and reagents except amines were dried on Zeolite CaA (Uetikon, activated at 400°C for 24 h before use). Amines were purchased from Aldrich and used as received.

Dibutyl carbonate:metallic sodium (0.23 g, 10 mmol) was dissolved in *n*-butanol (74.0 g, 1.0 mol) and dimethyl carbonate (88.5 g, 0.75 mol) was added. The mixture was slowly distilled and the fraction with a boiling point of 201–204°C was collected, yield 76.6 g (88%). Characterization by ^{1}H NMR ($CDCl_3$): δ 0.94 (t, 6H, $2\times CH_3$), 1.41 (sext, 4H, $2\times CH_2$), 1.66 (quint, 4H, $2\times CH_2$), 4.13 (t, 4H, $2\times CH_2$).

Dibenzyl carbonate:metallic sodium (0.23 g, 10 mmol) was dissolved in benzyl alcohol (108.0 g, 1.0 mol) and dimethyl carbonate (88.5 g, 0.75 mol) was added. The methanol and excess of dimethyl carbonate were removed by slow distillation at normal pressure. Subsequent distillation at reduced pressure afforded dibenzyl carbonate, yield 102.9 g, 85%, m.p. 29°C, b.p. 194 at 13 mbar. Characterization by ^{1}H NMR ($CDCl_3$): δ 5.16 (s, 4H, $2\times CH_2$), 7.4–7.3 (m, 10 H, $10\times CH$).

2.2. Analysis and equipment

GC analysis of the non-chiral products was performed on a Varian Star 3400 chromatograph, equipped with a 50 m $\times$ 0.53 mm CP-Sil 5CB column and a FID detector. The carrier gas was nitrogen at a flow of 3.0 ml/min. Temperature program: 50°C (5 min) to 280°C (15°C/min), the maximum temperature was held for 10 min. Conversion was measured against dibutyl ether as internal standard and was reproducible within 2% conversion.

Reversed phase HPLC analysis was performed using a Waters 8 $\times$ 100 mm 4 μ Novapak C18 Radial Pak cartridge contained in a Waters 8 $\times$ 10 compression unit, a Waters 510 pump, a Shodex RI SE-61 refractometer, and a SP 4270 integrator. The eluent was methanol/aqueous 0.1 M NaOAc/HOAc buffer pH 4.3 (65:35, v/v) at a flow of 1.0 ml/min. Conversion was measured against dibutyl ether as internal standard and was reproducible within 2% conversion.

The progress of the kinetic resolution experiments was monitored with reversed phase HPLC as described above. The enantiomeric excess was measured by chiral HPLC on a 4.6 $\times$ 25 mm 10 μ Chiralcel OD column with a Waters 510 pump, a Shimazu SPD-6A UV detector at 254 nm, and an SP 4270 Spectra-Physics integrator. 1,3-Dimethoxybenzene was used as internal standard. Hexane/isopropyl alcohol (90/10, v/v) was used as eluent for reactions with 1-phenylethyl amine, 1-aminoindane, and 1-amino-1,2,3,4-tetrahydronaphthalene. For 2-aminopentane and 2-aminooctane, hexane/isopropyl alcohol (99/1, v/v) was used as eluent. Samples of 50 μl were taken, dissolved in 1.0 ml of *tert*-butyl methyl ether and filtered over $MgSO_4$ to adsorb the unreacted amine.

^{1}H and ^{13}C NMR spectra were recorded using a 400 MHz Varian-VXR 400S spectrometer. Attempts to measure MS or GC–MS spectra from reaction mixtures using a variety of ionization techniques were not successful. Specific rotations were measured with a Perkin Elmer 241 polarimeter at 589 nm (Na-lamp).

2.3. Reactions

2.3.1. General procedure

The reactions of dibutyl carbonate were carried out on a 5.0-mmol scale. To 5.0 ml of a stock solution of 1.0 M dibutyl carbonate (174

g/l) and 100 g/l dibutyl ether (internal standard) in *tert*-butyl alcohol, 25 mmol of water, 10 mmol of an alcohol or 6.0 mmol of an amine was added. In the ammonolysis reaction, the same amount of dibutyl carbonate and internal standard were weighed in and 5.0 ml of a saturated solution of ammonia in *tert*-butyl alcohol (2.5 M, 12.5 mmol) was added. The solutions were shaken for 10 days with 100 mg of Novozym 435 at 40°C. At regular intervals, samples (50 μl) were taken and analyzed by GC. The products coeluted with chemically prepared samples on reversed phase HPLC as well as on GC.

The procedure for reactions of dibenzyl carbonate with achiral nucleophiles was similar. The stock solution consisted of 0.5 M dibenzyl carbonate (121 g/l) and 100 g/l dibutyl ether (internal standard). To 5.0 ml of this solution, 12.5 mmol of water, 5.0 mmol of an alcohol or 3.0 mmol of an amine was added. The ammonolysis reaction was performed with the same amount of dibenzyl carbonate and internal standard and 5.0 ml of a saturated solution of ammonia in *tert*-butyl alcohol (12.5 mmol). Novozym 435 was added (100 mg) and the mixtures were shaken for 10 days at 40°C, and samples (50 μl) were taken at regular intervals for GC analysis. The products coeluted with chemically prepared samples on reversed phase HPLC as well as on GC.

2.3.2. Initial rate experiments

The initial rate experiments were performed only with dibenzyl carbonate as acyl donor. A solution of 605 mg (2.5 mmol) dibenzyl carbonate, 200 mg 1,3-dimethoxybenzene, and 485 mg (4.0 mmol) 1-phenylethyl amine in 5.0 ml of organic solvent was shaken with 50 mg of Novozym 435 at 40°C. At regular intervals, samples (50 μl) were taken and analyzed by HPLC. The products coeluted with chemically prepared samples on reversed phase as well as on chiral HPLC. Response factors were determined with the chemically produced racemic compound. The initial rates were calculated from the linear part of the progress curves with an estimated error of ±5%.

2.3.3. Acylation of chiral amines

A solution of 1.210 g dibenzyl carbonate (5 mmol), 0.36 g 1,3-dimethoxybenzene (internal standard), and 8.0 mmol of the chiral amine in 10 ml hexane was shaken with 250 mg Novozym 435 at 40°C. Reactions with aliphatic amines were performed at room temperature. Conversions were measured by reversed phase HPLC and *ee* values by chiral straight phase HPLC.

All reactions were performed in duplo. From one reaction mixture, the precipitated product, if present, was filtered off and analyzed by chiral HPLC as well as ^{1}H and ^{13}C NMR. The duplo reaction mixture was worked up by addition of isopropyl alcohol until it was homogeneous and samples for HPLC were taken. Products were isolated by column chromatography over silica with 1:15 (v/v) ethyl acetate/petroleum ether for Z-2-aminopentane and Z-2-aminooctane and 1:10 (v/v) ethyl acetate/petroleum ether for the other products. The products coeluted with chemically prepared samples in all cases on chiral straight-phase and reversed-phase HPLC.

(−)-2-Benzyloxycarbonylaminopentane, ^{1}H NMR (400 MHz) ($CDCl_3$): δ 0.91 (t, 3H, CH_3), 1.13 (d, 3H, CH_3), 1.37 (m, 4H, CH_2), 3.72 (m, 1H, CH–NH), 4.52 (s, 1H, NH), 5.10 (s, 2H, O–CH_2), 7.3 (m, 5H, ring H). ^{13}C NMR (400 MHz) ($CDCl_3$): pentyl moiety, δ 13.922 (C_5), 19.150 (C_1), 21.229 (C_4), 39.377 (C_3), 46.926 (C_2). δ 66.471 (O–CH$_2$), ring: δ 128.047, 128.085, 128.518, 136.742. δ 155.786 (C═O). Specific rotation: $[\alpha]_D^{23} = -11.9°$ ethanol abs. $c = 1.0$.

(−)-2-Benzyloxycarbonylaminooctane, ^{1}H NMR (400 MHz) ($CDCl_3$): δ 0.87 (t, 3H, CH_3), 1.13 (d, 3H, CH_3), 1.26 (m, 6H, CH_2), 1.40 (m, 2H, CH_2), 3.70 (m, 1H, CH–NH), 4.52 (s, 1H, NH), 5.09 (s, 2H, O–CH_2), 7.3 (m, 5H, ring H). ^{13}C NMR (400 MHz) ($CDCl_3$): octyl moiety, δ 14.066 (C_8), 21.236 (C_1), 22.587 (C_7), 25.902 (C_6), 29.157 (C_5), 31.767 (C_4), 37.207 (C_3), 47.192 (C_2). δ 66.455 (O–

CH_2), ring: δ 128.047, 128.085, 128.510, 136.750. δ 155.778 (C═O). Specific rotation: $[\alpha]_D^{23} = -8.1°$ ethanol abs. $c = 1.0$.

(*R*)-*N*-Benzyloxycarbonyl-1-phenylethylamine, ^{1}H NMR (400 MHz) ($CDCl_3$): δ 1.47 (d, 3H, CH_3), 4.86 (qi, 1H, C*H*–NH), 5.07 (m, 3H, N*H*, O–CH_2), 7.3 (m, 10H, ring *H*). ^{13}C NMR (400 MHz) ($CDCl_3$): δ 22.458 (*C*H_3), 50.735 (NH–*C*H), 66.721 (O–*C*H_2), ring: δ 125.931, 127.327, 128.100, 128.495, 128.639, 136.492 (*C*–CH–NH), 143.495 (C–CH_2–O). δ 155.543 (C═O). Specific rotation: $[\alpha]_D^{23} = +45.0°$ ethanol abs. $c = 1.0$.

(+)-1-Benzyloxycarbonylaminoindane, ^{1}H NMR (400 MHz) ($CDCl_3$): δ 1.78 (m, 1H, CH_2-2), 2.56 (m, 1H, CH_2-2), 2.8 (br. m, 2H, CH_2-3), 5.0–5.3 (N*H*, C*H*–NH, CH_2–O), 7.0–7.3 (m, 9H, rings). ^{13}C NMR (400 MHz) ($CDCl_3$): δ 30.025 (*C*2), 34.240 (*C*3), 56.419 (*C*1), 66.711 (*C*H_2–O), aromatic rings: δ 123.981, 124.760, 126.704, 127.984, 128.094, 128.502. δ 136.523, 143.132 (*C*–CH–NH, *C*–CH_2–O). δ 156.176 (C═O). Specific rotation: $[\alpha]_D^{23} = +68.5°$ ethanol abs. $c = 1.0$.

(+)-1-Benzyloxycarbonylamino-1,2,3,4-tetrahydronaphthalene, ^{1}H NMR (400 MHz) ($CDCl_3$): δ 1.81 (m, 3H, CH_2–CH_2), 2.05 (1H, NH–CH–CH_2), 2.78 (m, 2H, Ph–CH_2), 4.8–5.2 (m, 4H, O–CH_2, N*H*, NH–C*H*), 7.0–7.4 (m, 9H, rings). ^{13}C NMR (400 MHz) ($CDCl_3$): δ 19.848 (*C*2), 29.180 (C3), 30.447 (C4), 49.316 (C1), 66.721 (O–*C*H_2), aromatic rings: δ 126.226, 127.053, 127.327, 128.100, 128.533, 128.662, 129.132. δ 136.590, 136.697, 137.425 (*C*–CH–NH, *C*–CH_2–O and *C*–CH_2–CH_2). δ 155.907 (C═O). Specific rotation: $[\alpha]_D^{23} = 46.0°$ ethanol abs. $c = 1.0$.

3. Results and discussion

A number of immobilized lipases and esterases were screened for activity in the alcoholysis of dibutyl carbonate by 1-propanol in *tert*-butyl alcohol. Only Novozym 435 (*C. antarctica* lipase B) showed a useful rate of reaction; with lipoprotein lipase from *Pseudomonas* the reaction was approximately 10 times slower. Other lipases and esterases showed no significant activity. Hence, Novozym 435 was used as catalyst in all further experiments.

The effect of the chain length of the alkyl groups in the dialkyl carbonate on its reactivity was assessed by subjecting dimethyl, diethyl, and dibutyl carbonate to Novozym 435 catalyzed hydrolysis. From the results (Table 1), it becomes clear that the reaction rate increases in the order methyl < ethyl < butyl. We ascribe this effect to the increasing hydrophobicity of the reactant. Moreover, inhibition of the lipase by the liberated alcohol, which would be more substantial with ethanol and particularly methanol, might also play a role. Dibenzyl carbonate (which eluded previous attempts at enzymatic reaction [8]) reacted more slowly than dibutyl carbonate. On the basis of these results, we selected dibutyl and dibenzyl carbonate for further study. The latter compound is of particular interest because its aminolysis would afford the highly interesting class of Z-protected amines as reaction product.

Dibutyl carbonate was subjected to Novozym 435 mediated hydrolysis, alcoholysis, and aminolysis. The general course of these reactions is depicted in Fig. 1. Alcoholysis of dibutyl carbonate with 1-propanol gave a statistical mixture of the starting compound and its two products (see Table 2). In contrast, isopropyl alcohol gave exclusively butyl isopropyl car-

Table 1
Effect of the alkyl chain length on the hydrolysis of dialkyl carbonates[a]

Carbonate	Conversion (%)
Dimethyl carbonate	13
Diethyl carbonate	48
Dibutyl carbonate	66
Dibenzyl carbonate[b]	56

[a]Reaction conditions: 5.0 mmol dialkyl carbonate (2.5 mmol dibenzylcarbonate), 5 mmol water and 50 mg Novozym 435 in 5 ml *tert*-butyl alcohol were shaken at 40°C for 48 h.

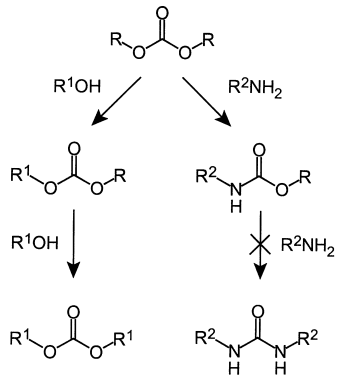

Fig. 1. Reactions of alkyl carbonates; R^1 = alkyl, arylalkyl; R^2 = H, alkyl, arylalkyl.

bonate; it would seem that the alcoholysis of the second butyl group is prevented by steric hindrance in this case.

Aminolysis, which is known to be irreversible due to the stability of the amide bond [12], did not proceed beyond the carbamate stage. In control experiments, dibutyl carbamate showed no reaction at all in hydrolysis, alcoholysis, and aminolysis under the same conditions as used with dibutyl carbonate, even after very long reaction times. Carbamates are known to be slow reacting substrates or even reversible inhibitors of lipases [13]. Dibutyl carbonate seems to react slower than carboxylic acid esters under comparable conditions. The Novozym 435 catalyzed ammoniolysis of ethyl octanoate was approximately four times faster than that of dibutyl carbonate [14].

Dibenzyl carbonate (see Table 3) reacted in much the same manner. The aminolysis of dibenzyl carbonate by simple amines such as 1-

Table 2
Transformations of dibutyl carbonate catalyzed by *C. antarctica* lipase[a]

Nucleophile	Reaction time and conversion (h/%)				
	24	48	96	168	240
Water	72	80	88	100	
1-Propanol	62	72	74	74[b]	
2-Propanol	39	52	60	66	69[c]
Ammonia	60	90	99	100	
1-Aminopropane	26	53	88	100	
2-Aminopropane	23	39	55	72	77
Benzylamine	44	70	98	100	

[a]Reaction conditions: 5.0 mmol dibutyl carbonate, nucleophile (25 mmol water, 10 mmol alcohol, 12.5 mmol ammonia or 6 mmol amine) and 100 mg Novozym 435 in 5 ml *tert*-butyl alcohol at 40°C.
[b]Diisopropyl carbonate (22%) and butyl propyl carbonate (52%).
[c]Only one product was formed: butyl isopropyl carbonate.

Table 3
Transformations of dibenzyl carbonate catalyzed by *C. antarctica* lipase[a]

Nucleophile	Reaction time and conversion (h/%)				
	24	48	96	168	240
Water	71	91	100		
1-Propanol	76	90	96[b]		
2-Propanol	52	70	79[c]		
Ammonia	98	100			
1-Aminopropane	61	91	100		
2-Aminopropane	33	44	66	76	89
Benzylamine	80	96	100		

[a]Reaction conditions: 2.5 mmol dibenzyl carbonate, nucleophile (12.5 mmol water, 5 mmol alcohol or 3 mmol amine) and 100 mg Novozym 435 in 5 ml *tert*-butyl alcohol at 40°C.
[b]Two products were formed: dipropyl carbonate (30%) and benzyl propyl carbonate (66%).
[c]Only one product was formed: benzyl isopropyl carbonate.

Table 4
Initial rate of benzyloxycarbonylation of 1-phenylethylamine in various solvents[a]

Solvent	Initial rate ($\mu mol \cdot h^{-1} \cdot g^{-1}$)	Conversion[b] (%, 168 h)
tert-Butyl alcohol	716	8
Hexane	402	16
Isooctane	396	16
tert-Butyl methyl ether	359	9
Cyclohexane	356	15
Diisopropyl ether	331	13
tert-Amyl alcohol	265	7
No solvent	244	14
Dioxane	198	4
Ether	158	6
Dimethoxyethane	147	3
Benzene	135	8
Toluene	133	8
Acetonitril	72	3
Tetrahydrofuran	65	3
Pyridine	22	0.4
Dichloromethane	16	1
N-Methylpyrolidone	8	0.4

[a]Reaction conditions: 2.5 mmol dibenzyl carbonate, 200 mg 1,3-dimethoxybenzene (internal standard), 4 mmol 1-phenylethylamine and 50 mg Novozym 435 in 5 ml solvent at 40°C.
[b]Percentage of amine acylated.

or 2-aminopropane gave high conversions at a moderate rate.

Because enzyme-catalyzed reactions are inherently enantioselective, the aminolysis of dibenzyl carbonate can be used as a one step synthesis of optically active Z-amines from the racemic amine. In order to develop a practical synthesis, we studied the effect of the solvent on the reaction of 1-phenylethyl amine and dibenzyl carbonate; initial rates are listed in Table 4. The high initial rate in *tert*-butyl alcohol suggested that it was a promising solvent but the reaction rate dropped dramatically in the course of the reaction. Higher conversions (Table 4) were reached in apolar solvents such as hexane and *iso*-octane; these solvents had the extra advantage that they dissolved the product only sparingly, which allowed its isolation by simple filtration in a number of cases. We note that the rates of lipase catalyzed reactions are generally higher in hydrophobic solvents than in water miscible ones [15,16].

The high initial rate that was measured in *tert*-butyl alcohol did not translate into a correspondingly high yield, as was noted above. To gain more insight the progress of the reactions in hexane and *tert*-butyl alcohol were monitored over time (see Fig. 2). It became apparent that in *tert*-butyl alcohol, the reaction soon abated and stagnated at approximately 8% conversion, for which product inhibition would seem the most likely cause. In contrast, when the reaction was carried out in hexane medium it continued at a steady rate. The product was very soluble in

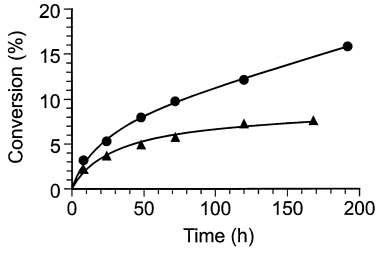

Fig. 2. The formation of Z-1-phenylethyl amine in hexane (●) and *tert*-butyl alcohol (▲). Reaction conditions: 605 mg dibenzyl carbonate (2.5 mmol), 485 mg 1-phenylethyl amine (4.0 mmol), 5 ml solvent and 50 mg Novozym 435 at 40°C.

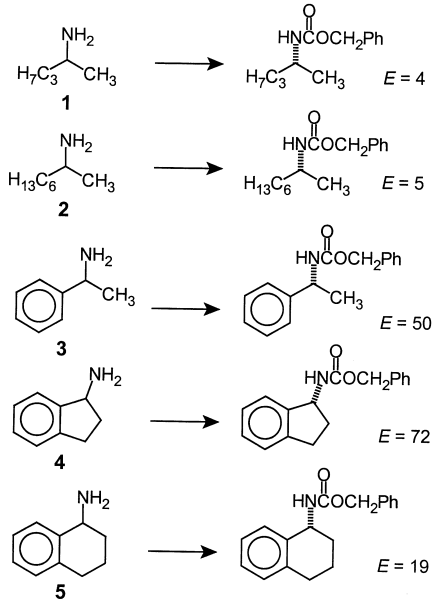

Fig. 3. Resolution of the chiral amines **1**–**5**. The configurational assignments are discussed in the text.

tert-butyl alcohol but precipitated from hexane before 2% conversion is reached.

We attempted the resolution of the chiral aliphatic and arylaliphatic amines **1**–**5** (see Fig. 3) via lipase-catalyzed benzyloxycarbonyl transfer. 2-aminopentane **1** and 2-aminooctane **2** reacted sluggishly in comparison with 1-phenylethylamine **3** and the enantiomeric ratio *E* [17] was only 2[2] at 40°C (data not shown). We tentatively conclude that the non-polar 2-alkylcarbamoyl moiety is an even more efficient lipase inhibitor. At 20°C, the reaction rate was still lower, but a modest enantioselectivity was achieved (Table 5).

The enantiomers of the benzylic amines **3**–**5** were efficiently discriminated by Novozym 435 with *E* factors ranging from 19 to 72. Because the reactions were carried out in hexane, the products precipitated from the reaction mixture which allowed for their isolation by filtration, making column chromatography unnecessary.

[2] The enantiomeric ratio was calculated from the conversion (c) and the optical purity of the product (ee_p) as follows [17]: $E = (\ln[1 - c(1 + ee_p)])/(\ln[1 - c(1 - ee_p)])$.

Table 5
Benzyloxycarbonylation of amines in hexane[a]

Amine	Conversion (%)	$ee_{product}$ (%)[b]	Sign of rotation	E
1	21	55	(−)	4
2	22	60	(−)	5
3	27	95	(+)	50 (*R*)
4	39	95	(+)	72
5	22	87	(+)	19

[a]Reaction conditions: 5 mmol dibenzyl carbonate (1.21 g), 0.36 g 1,3-dimethoxy benzene (internal standard), 8 mmol amine and 250 mg Novozym 435 in 10 ml hexane for 7 days at 40°C; aliphatic amines **1** and **2** for 12 days at room temperature.

[b]The reaction mixture was dissolved by addition of isopropyl alcohol and analyzed by chiral HPLC; see Section 2 for further details.

The precipitated product was enantiopure in all three cases but the yield was rather low due to the sluggish reaction. At 60°C, **3** reacted considerably faster (27% conversion in 96 h) but E decreased from 50 to 34.

From the report by Takayama et al. [10], it would seem that reaction at high concentrations in toluene results in a faster turnover as well as a better E. We did not attempt further optimisation, however.

In the case of benzyloxycarbonyl-1-phenylethyl amine, the absolute configuration was shown to be (*R*) by coelution with the chemically produced (*R*)-product on chiral HPLC. It is known that Novozym 435 has a strong tendency to catalyze acylation of (*R*)-enantiomers of chiral alcohols and amines [10,18–20] containing one hydrogen atom and a methyl group attached to the chiral center. Hence, we expect that all products have the (*R*)-configuration.

4. Conclusions

Novozym 435 catalyzes the hydrolysis, alcoholysis, aminolysis, and ammoniolysis of aliphatic and arylaliphatic carbonic acid esters. Aminolysis and ammoniolysis do not proceed beyond the carbamate stage.

Resolution and protection of chiral benzylamine derivatives was accomplished in one step by a Novozym 435 catalyzed reaction with dibenzyl carbonate, which provides a route that avoids the use of phosgene.

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References

[1] M.C. de Zoete, F. van Rantwijk, R.A. Sheldon, Catal. Today 22 (1994) 563.
[2] D.A. Abramowicz, C.R. Keese, Biotechnol. Bioeng. 33 (1987) 149.
[3] M. Pozo, V. Gotor, Tetrahedron 49 (1993) 4321.
[4] K. Kawashima, T. Hasegawa, Biotechnol. Lett. 14 (1992) 1135.
[5] E. Menendez, V. Gotor, Synthesis (1993) 72.
[6] E. Guibe-Jampel, M. Bassir, Tetrahedron Lett. 35 (1994) 421.
[7] D. Pioch, P. Lozano, J. Graille, Biotechnol. Lett. 13 (1991) 633.
[8] B. Orsat, P.B. Alper, W. Moree, C.P. Mak, C.H. Wong, J. Am. Chem. Soc. 118 (1996) 712.
[9] R.L. Rodney, J.L. Stagno, E.J. Beckman, A.J. Russell, Biotechnol. Bioeng. 62 (1999) 259.
[10] S. Takayama, S.T. Lee, S.-C. Hung, C.-H. Wong, Chem. Commun. (Cambridge) (1999) 127.
[11] S. Pedersen, P. Eigtved (Novo Nordisk) PCT Int. Appl. WO 90/15868 [Chem. Abstr. 114 (1991) 224537r].
[12] M.C. de Zoete, A.C. Kock-van Dalen, F. van Rantwijk, R.A. Sheldon, J. Mol. Catal. B: Enzym. 2 (1996) 19.
[13] D.S. Shin, D.M. Quinn, Biochemistry 31 (1992) 811.
[14] F. van Rantwijk, A.C. Kock-van Dalen, R.A. Sheldon, in: A. Ballasteros, F.J. Plou, J.L. Iborra, P. Halling (Eds.), Stability and Stabilisation of Enzymes, Progress in Biotechnology 15 Elsevier, Amsterdam, 1998, p. 447.
[15] J.B.A. van Tol, J.B. Odenthal, J.A. Jongejan, J.A. Duine, in: J. Tramper, M.H. Vermüe, H.H. Beeftink, U. von Stockar (Eds.), Biocatalysis in Non-Conventional Media, Progress in Biotechnology 8 Elsevier, Amsterdam, 1992, p. 229.

[16] Z. Yang, A.J. Russell, in: A.M.P. Koskinen, A.M. Klibanov (Eds.), Enzymatic Reactions in Organic Media, Blackie Academic and Professional, London, 1996, p. 43.
[17] C.-S. Chen, Y. Fujimoto, G. Girdaukas, C.J. Sih, J. Am. Chem. Soc. 104 (1982) 7294.
[18] C. Orrenius, N. Öhrner, D. Rotticci, A. Mattson, K. Hult, T. Norin, Tetrahedron: Asymmetry 6 (1995) 1217.
[19] M.T. Reetz, C. Dreisbach, Chimia 48 (1994) 570.
[20] N. Öhrner, C. Orrenius, A. Mattson, T. Norin, K. Hult, Enzyme Microb. Technol. 19 (1996) 328.