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LIPASE CATALYZED RESOLUTION OF CHIRAL ACIDS OR ALCOHOLS USING MIXED CARBOXYLIC-CARBONIC ANHYDRIDES

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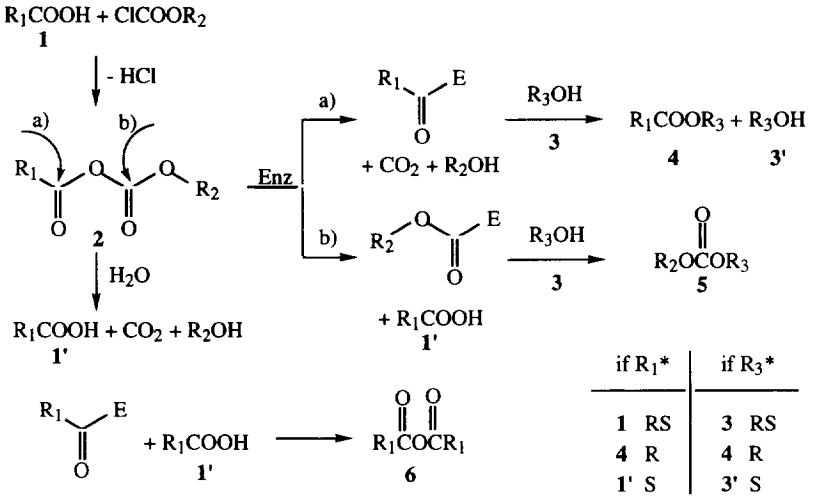
Abstract: Mixed carboxylic-carbonic anhydrides are efficient irreversible acyl transfer reagents for lipase catalyzed esterification in organic media, and can be used for the resolution of chiral carboxylic acids or alcohols.

Key words: Mixed carboxylic-carbonic anhydrides / lipase / enantiomeric resolutions.

In the recent years, chemo-enzymatic methodology has become a standard technique for the preparation of a variety of enantiomerically pure molecules[1]. Among them, lipases catalyzed acid and alcohol resolutions in anhydrous medium represent an important part of enzymatic transformations in organic chemistry. One of the major limitation of the process is their general reversibility which results in slow reaction rates and low selectivities[1a]. Using activated substrates such as vinyl[2a] or isopropenyl alkanoates[2b], oxime esters[2c] or symmetrical anhydrides[2d] may displace the equilibrium towards product formation. Unfortunately, some of these derivatives are not commercially available and must be synthesized from a conveniently choosen precursor, previously to the enzymatic step. Enol esters may be prepared from the corresponding acetates by mercury catalyzed transesterification[1a]. The oxime esters are usually prepared from the corresponding acyl chlorides and oxime. The selectivity of enzymatic catalysis of symmetrical anhydrides is acyl dependent. The reaction rates are almost the same as with enol esters but one half of the acyl group is lost in the process. Moreover, the above reaction may produce active by-products (acetaldehyde, acids) resulting in enzymatic deactivation[1a,3].

To avoid the difficult accessibility problems or partial loss of substrates and formation of active by-products, we describe here a convenient enzyme-catalyzed resolution method based on acyl-transfer between mixed carboxylic-carbonic anhydrides (MCCA) and alcohols (Scheme 1). The by-products of this reaction are CO_2 and alcohols which neither cause side reactions nor induce any enzyme modifications. Mixed carboxylic-carbonic anhydrides are readily prepared from acids and chlorocarbonates[4]. They are stable at low temperatures (ca. 4 °C) for several weeks and can be used without further purification. Moreover enantiomerically pure chiral MCCA are known to be resistant towards racemisation (regarding the stability of remaining substrate)[5]. Upon heating MCCA decompose to symmetric anhydrides. The MCCA method can be used for the resolution of alcohols and acids[6,7] as well, and in that latter respect may be considered as complementary to the above mentioned other methods.

Scheme 1

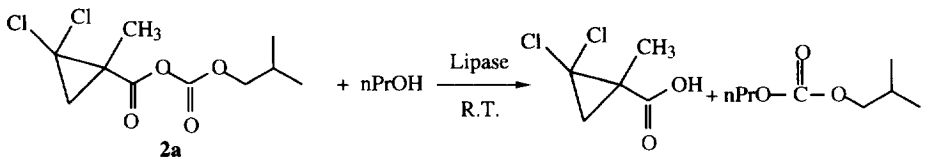


We have first studied the effects of the bulkiness and configuration of $\mathbf{R_1}$ group (1) and of the size of $\mathbf{R_2}$ alkyl group (2) on the competition of the acyl enzyme formation (**a/b** pathway).

(1) For enzymes such as Lipozyme (LM) or Novozym (SP435) and with "good" $\mathbf{R_1}$ acyl substrates, i.e. secondary groups of R configuration, or primary acyl group, the enzyme attacked preferentially according to pathway **a**. In the case of "poor" acyl substrates, i.e. substrates of S configuration, the enzyme attacked preferentially the carbonate-carbonyl group according to pathway **b** (it took place only for secondary or tertiary $\mathbf{R_1}$ groups). Lipase from porcine pancreas (PPL) attacked preferentially, inrespective of the R or S acyl configuration, the carbonate group rather than the secondary carbonyl.

(2) In the case of primary $\mathbf{R_2}$ (for instance R_2=iBu) the enzymatic resolution could not be exploited, indeed since the anhydride was cleaved both by pathways **a** and **b** (Table 1) so that the enantiomeric purity of the unreacted substrate remained low. Furthermore, the acid **1'** formed through pathway **b** might react with the acyl enzyme resulting from the cleavage by pathway **a** to give the symmetrical anhydride **6**. Nevertheless, the isobutyloxycarbonic-carboxylic anhydride method could be useful for resolution of more crowded acids such as tertiary ones. For instance, LM, *Pseudomonas cepacia* lipase (LP) and PPL attacked this mixed anhydride exclusively at the carbonyl group of the carbonate moiety leading to acid as the major product of reaction (Scheme 2 and Table 2). The formation of symmetrical anhydrides was not observed in that case.

Scheme 2



Scheme 3

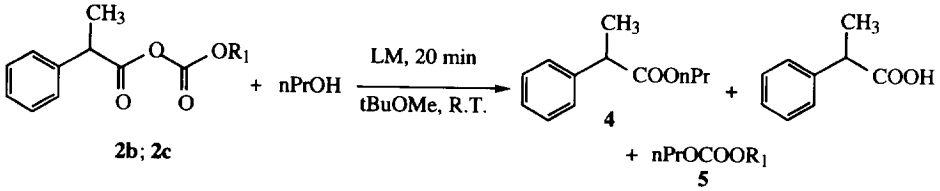


Table 1: a/b pathways competition in enzymatic resolutions of PPA using MCCA method.

Substrate configuration	2b $R^1 = CH_2CH(CH_3)_2$		2c $R^1 = CH(CH_3)_2$	
	% 4	% 5	% 4	% 5
R	38	32	21	< 1
S	13	47	2	3

Attack on the carbonate moiety might be diminished by introducing secondary alkoxy group. For instance, in the particular case of 2-phenyl propionic acid (PPA) derivatives and Lipozyme the ester/carbonate ratio was 2/3 for R_2= iBu and 10/1 for R_2= iPr. Therefore, for secondary acids and alcohols resolution with SP435 or LM, use of isopropyl-oxycarbonic-carboxylic anhydrides seems to be the best choice.

Table 2: Transesterification of MCCA from tertiary acid 2a

Lipase	t (h)	Acid (%)	Anhydride (%)
LM	46	100	0
PPL	40	65	35
LP	40	75	25

Acid esterification

From substituted 2-phenylpropionic MCCA and with Lipozyme as the catalyst, esters or acids could be obtained within good yields and enantiomeric excess. Attack at the carbonate-carbonyl occured only in a small extent (10% or less) leading to acid **1'** with the same configuration as those obtained from unreacted MCCA. The same reactions catalyzed by SP435 were less selective, a result which was in the line with previous works by Sinisterra and co-workers[8] who had obtained low enantiomeric ratio (E<3) in the esterification of α-substituted phenylpropionic acids with aliphatic alcohols. Primary alcohols reacted five times faster with MCCA than isopropanol when catalyzed by SP435[8] and 30 times faster when catalyzed by LM[9].

In the present work, we have used propanol, butanol and octanol as the nucleophiles. *Tert*-butylmethyl ether was the most convenient solvent, followed by diisopropyl ether, ethyl ether and hexane. The reactions were carried out at 20 °C, at which temperature ca. 1 h was required to obtain 50% conversion. The results obtained in terms of reaction time, substrate conversion (c), enantiomeric excess (e.e.) in residual MCCA or products and enantiomeric ratio E are reported in Table 3.

Scheme 4

Table 3: Chiral acid resolutions.

	R_1	R_2	R_3	Solvent	t (min)	c (%)	e.e. (%)	E
2c	Ph	Me	n-propyl	^{t}BuOMe	60	55	90 [a]	20
2c	Ph	Me	n-octyl	^{t}BuOMe	40	50	79 [a]	20
2d	p-^{s}Bu-Ph	Me	n-octyl	^{t}BuOMe	60	47	74 [a]	24
2e	Ph	MeO	n-butyl	^{t}BuOMe	15	28	33 [a]	17
2f	p-NO_2-Ph	Me	n-octyl	^{t}BuOMe	40	51	96 [b]	>100
2g	nPr	Me	n-octyl	Et_2O	20	47	80 [b]	19

[a] e.e. of residual substrate determined by GC of corresponding S- or R-methyl benzylamides and $[\alpha]_D$ of the acids were compared with the literature data[13]

[b] e.e. of product determined by NMR using Eu(tfc)$_3$

Alcohol esterification

Novozym SP435 has been, for the last years, the most recommended enzyme for alcohol esterification[10]. We describe here the acylation of chiral alcohols which have already been used as models in enzymatic resolutions, namely 1-phenyl ethanol **3a**, 1-phenyl propanol **3b**, cyclohexylmethyl carbinol **3c**.

SP435 catalyzed acylation of **3a** and **3c** have been studied by Hult[11] using ethyl octanoate as the acyl donor. The equilibrium was shifted in the desired direction through ethanol evaporation under reduced pressure. We found that the MCCA resolution was at least twice faster. E values were of the same order for **3a** (E>100) or better (E>100 vs E=70 by the Hult's method) for **3b**. 2-Hexanol resolution by SP435 with capronic acid as the acyl donor took 40 hours at 25 °C(Novo notice)[11]. The MCCA method thus appears particularly interesting for medium or long size acid chains. Furthermore, it offers excellent scaling up possibilities.

Scheme 5

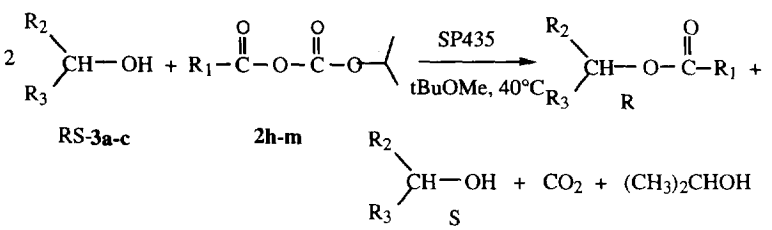


Table 4: Chiral alcohol resolutions.

	R_2	R_3	R_1		t (h)	c (%)	ee_S (%)[a]	ee_P (%)[b]	E
3a	phenyl	methyl	**2h**	ethyl	7	30	44	-	>100
3a	phenyl	methyl	**2i**	n-pentyl	4	45	80	>98	>100
3a	phenyl	methyl	**2j**	n-hexyl	1	42	71	>98	>100
3a	phenyl	methyl	**2k**	n-pentadecyl	2	43	75	-	>100
3b	cyclohexyl	methyl	**2l**	n-heptyl	3	49	94	95	>100
3b	cyclohexyl	methyl	**2m**	n-undecyl	3	48	89	-	>100
3c	phenyl	ethyl	**2l**	n-heptyl	6	44	73	-	60

[a] e.e. of residual alcohol (S) determined by GC analysis on a chiral column and $[\alpha]_D$ were compared with the literature data[13]

[b] e.e. of product (R) determined by ^{1}H NMR analysis using chiral shift reagents Eu(tfc)$_3$.

Experimental

General remarks: NMR spectra were recorded on a Bruker AC-250 spectrometer in $CDCl_3$ with TMS as the internal standard. Optical rotation measurements were recorded on a DiP-370 JASCO polarimeter. GC was performed on a Carlo Erba GC 8000 Fisons instrument fitted with a FID detector. Reactions were monitored by GLC on an OV1 column (15 m) and optical purity was controlled on a capillary chiral column Cydex B (25 m).

Novozym SP 435 (immobilized *Candida antarctica* lipase) and Lipozyme LM (immobilized *Mucor miehei* lipase) were kindly gifted by Novo Nordisk, *Pseudomonas cepacia* lipase (LP) was provided by Amano Co., and lipase from porcine pancreas (PPL) Type II was purchased from Sigma.

Racemic 2-phenylpropionic esters were prepared according to a literature method[12].

Preparation of mixed carboxylic-carbonic anhydrides (MCCA). General procedure: N-methylmorpholine (1 ml; 11 mmol) was added at 0 °C to a solution of carboxylic acid (10 mmol) and isopropyl-chlorocarbonate (11 ml; 11 mmol; 1M solution in toluene) in dry diethyl ether (20 ml). The reaction mixture was stirred for 30 min, followed by filtration. After washing the residue with dry diethyl ether, the filtrate was concentrated under vacuum. The MCCA thus obtained were stored in solution in ether at 4 °C and used without further purification.

2a: 0.99 (d, 6H); 1.55 (d, 1H); 1.65 (s, 3H); 2.04 (b, 1H); 2.36 (d, 1H); 4.08 (d, 1H).
2b: 0.94 (d, 6H); 1.57 (d, 3H); 1.98 (m, 1H); 3.85 (q, 1H); 4.00 (dd, 2H); 7.20-7.40 (m, 5H).
2c: 1.30 (d, 6H); 1.55 (d, 3H); 3.80 (q, 1H); 4.95 (m, 1H); 7.10-7.45 (m, 5H).
2d: 0.90 (t, 3H); 1.30 (d, 6H); 1.55 (d, 3H); 1.83 (m, 1H); 2.45 (d, 2H); 3.80 (q, 1H); 4.95 (m, 1H); 7.10-7.25 (dd, 4H).
2e: 0.85 (d, 6H); 1.20 (m, 2H); 1.55 (m, 2H); 3.40 (s, 3H); 4.10 (t, 2H); 4.75 (s, 1H); 7.30-7.50 (m, 5H).
2f: 1.35 (d, 6H); 1.61 (d, 3H); 3.95 (q, 1H); 5.00 (m, 1H); 7.50 (d, 2H); 8.12 (d, 2H).
2g: 0.95 (t, 3H); 1.20 (d, 3H); 1.40 (d, 6H); 1.50 (d, 2H); 1.70 (d, 2H); 2.02 (m, 1H); 5.00 (m, 1H);
2h: 1.20 (t, 3H); 1.35 (d, 6H); 2.50 (q, 2H); 5.00 (m, 1H).
2i: 0.86 (t, 3H); 1.25 (b, 6H); 1.35 (d, 6H); 1.68 (t, 2H); 5.00 (m, 1H).
2j: 0.86 (t, 3H); 1.25 (b, 8H); 1.35 (d. 6H); 1.68 (t, 2H); 5.00 (m, 1H).
2k: 0.87 (t, 3H); 1.23 (b, 26H); 1.32 (d, 6H); 1.64 (t, 2H); 4.80 (m, 1H).
2l: 0.87 (t, 3H); 1.30 (b, 10H); 1.35 (d, 6H); 1.68 (t, 2H); 5.00 (m, 1H).
2m: 0.87 (t, 3H); 1.30 (b, 18H); 1.35 (d, 6H); 1.68 (t, 2H); 5.00 (m, 1H).

Acid esterification: Lipozyme LM (200 mg) was stirred at R.T. with the alcohol (2 mmol) in *tert*-butylmethyl ether (2 ml) during 15 min, upon which MCCA (1 mmol) in solution in ether was added. The reactions were then conducted during the time periods and at the temperatures indicated in the Table 3. Reactions were stopped by filtering off the enzyme and filtrates were concentrated under vacuum. The conversions were determined from ^1H NMR spectra, while the ester / carbonate ratios were determined by GC analysis. Enantiomeric purities of unreacted MCCA were determined by GC after derivatisation with chiral phenylethyl amine.

Hydrolysis of MCCA catalyzed by H_2O_2: To the crude reactions mixtures from esterifications carried out on a 3-6 mmol scale in *tert.*-butylmethyl ether or diethyl ether (5 ml) was added H_2O_2 (20 ml, 10 % solution) at R.T. in an ultrasonic bath. The pH was adjusted at 8 and maintained at this value by continuous addition of 1M KOH. After decantation, the water fraction was acidified to pH 1-2 with HCl (1 M), extracted with ether and dried over Na_2SO_4. After concentration of both fractions under vacuum, the products were subjected to silica gel column chromatography with pentane-ether (9:1) as the eluent. Chemically pure esters and

acids were isolated with 80-90 % of yield. ^{1}H NMR spectra of esters[8] and GC and optical rotation values of acids[13] were in good agreement with the literature data.

Alcohol esterification: Novozym SP435 (300 mg) was stirred at 40 °C with 1-phenylethanol (1 mmol) in *tert*-butylmethyl ether (1 ml) during 10 min, upon which the MCCA solution (3.3 mmol) was added. The reactions were performed during periods of time as indicated in Table 4 and stopped by filtering off the enzyme. The filtrate was concentrated under vacuum. Enantiomeric purities and conversions of the crude reaction mixture were determined by chiral GC analysis. The pure esters and alcohols were then obtained and isolated in 90% or more yield after flash chromatography with hexane-ether (4:1) as the eluent. Enantiomeric purities of unreacted alcohols were determined by GC analysis on a chiral column and their optical rotation values were in good agreement with the literature data[13]. Enantiomeric purities of the formed esters were determined by ^{1}H NMR analysis using chiral shift reagents Eu(tfc)$_3$.

References

1a). Faber, K.; Riva, S.; *Synthesis*, **1992**, 895-910.
b). Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A.; *Chem.Rev.*, **1992**, *92*, 1071-1140.
c). Wong, C.-H.; Whitesides, G.M.; "Enzymes in Synthetic Organic Chemistry", Tetrahedron Organic Chemistry Series, Vol. 12, Pergamon 1994.
d). Wu, S.-H.; Guo, Z.-W.; Sih, C.J.; *J. Am. Chem. Soc.*, **1990**, *112*, 1990-1995.
e). Chen, C.-S.; Sih, C.J.; *Angew. Chem. Int. Ed. Engl.*, **1989**, *28*, 695-707.
2a). Degueil-Castaing, M.; De Jeso, B.; Drouillard, S.; Maillard, B.; *Tetrahedron Lett.*, **1987**, *28*, 953-954.
b). Wang, Y.-F.; Wong, C.-H.; *J. Org. Chem.*, **1988**, *53*, 3127-3129.
c). Ghogare, A.; Kumar, G.S.; *J. Chem. Soc. Chem. Commun.*, **1989**, 1533-1535.
d). Bianchi, D.; Cesti, P.; Battistel, E.; *J. Org. Chem.*, **1988**, *53*, 5531-5534.
3. Weber, H.K.; Stecher, H.; Faber, K.; *Biotechnol. Lett.*, **1995**, in the press.
4. Kim, S.; In Lee, J.; Chul Kim, Y.; *J. Org. Chem.*, **1985**, *50*, 560-565.
5. Jouin, P.; Castro, B.; Zeggaf, C.; Pantaloni, A.; Senet, J.P.; Lecolier, S.; Sennyey, G.; *Tetrahedron Lett.*, **1987**, *28*, 1661-1664.
6. Guibé-Jampel, E.; Bassir, M.; *Tetrahedron Lett.*, **1994**, *35*, 421-422.
7. Oumoch, S.; Paris-Sud University, June 1992, MSc-thesis of Organic Chemistry.
8. Arroyo, M.; Sinisterra, J.V.; *J. Org. Chem.*, **1994**, *59*, 4410-4417.
9. Miller, C.; Austin, H.; Posorske, L.; Gonzalez, J.; *J. Am. Oil Chem. Soc.*, **1988**, *165*, 927-931.
10. Morkeberg, A.; Novo Nordisk information sheets, 1993.
11. Öhrner, N.; Martinelle, M.; Mattson, A.; Norin, T.; Hult, K.; *Biotechnol. Lett.*, **1992**, *14*, 263-268.
12. Moreno, J.M.; Samoza, A.; Del Campo, C.; Llama, E.I.; Sinisterra, J.V.; *J. Mol. Catal. A Chemical*, **1995**, *95*, 179-192.
13. Aldrich Catalog for the alcohols **3a**,**3c**, and acids **1c**,**1d**,**1e**; Brown, H.C.; Cho, B.T.; Park, W.S.; *J. Org. Chem.*, **1988**, *53*, 1231-1238 for the alcohol **3b**; Folli, U.; Iarossi, D.; Montanari, F.; Torre, G.; *J. Chem. Soc.*, **1968**, 1317 for the acid **1f** and Engel, K.-H.; *Tetrahedron: Asymmetry*, **1991**, *2*, 165 for the acid **1g**.

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