Pergamon

Tetrahedron: *Asymmetry* 12 (2001) 2109–2119

TETRAHEDRON: *ASYMMETRY*

Resolution of racemic 3-aryloxy-1-nitrooxypropan-2-ols by lipase-catalyzed enantioselective acetylation

Beata Krystyna Pchelka,[a,b,*] André Loupy,[a] Jan Plenkiewicz,[b] Alain Petit[a] and Luis Blanco[c]

[a]*Laboratoire des Réactions Sélectives sur Supports (CNRS UMR 8615), Institut de Chimie Moléculaire d'Orsay, Bât. 410, Université de Paris-Sud, 91405 Orsay Cedex, France*

[b]*Faculty of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, Poland*

[c]*Laboratoire des Carbocycles (CNRS UMR 8615), Institut de Chimie Moléculaire d'Orsay, Bât. 420, Université de Paris-Sud, 91405 Orsay Cedex, France*

Received 30 May 2001; accepted 23 August 2001

Abstract—Both (*R*)- and (*S*)-enantiomers of 3-aryloxy-1-nitrooxypropan-2-ols (*R*)-(−)-**1**, (*S*)-(+)-**2** were prepared in high enantiomeric excess by lipase from *Pseudomonas cepacia* (Amano PS) or *Pseudomonas fluorescens* (Amano AK)-catalyzed acetylation of racemic alcohols **1a**–**g** with vinyl acetate in *n*-hexane at 4 or 22°C. The enantioselectivity of this transformation was dependent on the substitution pattern of the aryl ring with *E*-values ranging from 31 to 111.

1. Introduction

Chiral 1,3-disubstituted secondary propanols are known as useful building blocks in the synthesis of biologically active compounds such as β-blockers, antidepressant drugs and muscle relaxants.[1] A number of racemic mixtures of these compounds carrying various substituents has been resolved by an enzyme-catalyzed kinetic method. Most frequently[2] compounds substituted on the phenyl ring and with halogen, amino, hydroxy or carboxyl substituents on the propyl chain were investigated.

In continuation of our interest in the lipase-catalyzed resolutions of various 1,3-disubstituted secondary propanols[3,4] we focussed our attention on 3-aryloxy-1-nitrooxypropan-2-ols **1** which are expected to be potentially active cardiovascular agents similar to those from the large group of drugs termed nitrovasodilators.[5] At present, some nitrovasodilators such as nitroglycerin,[6] isosorbide dinitrate[7] and nicorandil[8] are clinically useful drugs (Fig. 1).

Organic nitrates and nitroglycerin (glyceryl trinitrate) have been mainstays of cardiovascular therapy[9] for many years and are of particular benefit in the treatment of angina pectoris attacks,[10] unstable angina[11] and early stages of acute myocardial infaction.[12] They have shown real efficacy in the presence of coronary atherosclerosis, hypercholesterolemia, and other associated vessel wall disorders involving endothelial dysfunction[13] and disordered vasodilatory capacity of the coronary arteries.[13,5]

Studies with isoidide dinitrate[14] and glyceryl-1-nitrate[15] (products of the metabolic denitration of nitroglycerin[16])

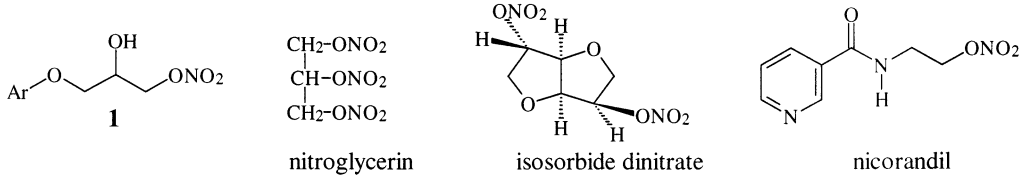

Figure 1.

* Corresponding author. Tel.: +33(0)1-69157256; fax: +33(0)1-69154679; e-mail: pbeata@icmo.u-psud.fr

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PII: S0957-4166(01)00378-0

have shown that organic nitrates may be subject to enantiospecific differences in their pharmacological effects. It was therefore of interest to prepared 3-aryloxy-1-nitrooxypropan-2-ols **1** in enantiomerically pure form for future characterization of their pharmacological properties in different in vitro and animal models.

The 3-aryloxy-1-nitrooxypropan-2-ols **1** are direct precursors of 3-aryloxy-1,2-propanediols **5** by hydrolysis[17] or hydrogenation[18] of the nitrate group of **1** (Fig. 2).

The 1,2-diol functionality is involved in various synthetic[19] and pharmaceutical[20] intermediates as well as in commercial medicines. For instance, enantiomerically pure 3-aryloxy-1,2-propanediols **5** are of interest as antifungal agents, e.g. Chlorphenesin (Ar = 4-Cl-C_6H_4-),[21] muscle relaxants such as Mephenesin (Ar = 2-CH_3-C_6H_4-),[20a,21a–c,22] and Guaifenesin (Ar = 2-CH_3O-C_6H_4-)[2a,21a–c,22b,23] as well as intermediates in the synthesis of β-blockers[20a,24] They can also be used as chiral ligands[25] in transition metal complexes, and as building blocks in the synthesis of crown ethers.[26] Several 1,2-propanediols after conversion into carbamates **6** were tested as muscle relaxants, analgesic agents and tranquilizers, e.g. chlorphenesin carbamate. It is well established that in the case of compounds **5** the desirable therapeutic activities belong mainly to the (*S*)-enantiomers.[21–23] In connection with the importance of this class of compounds, several methods have been reported for their synthesis.[27–35] The ready hydrolysis and hydrogenation of nitrooxy group suggests that preparation of optically active 3-aryloxy-1-nitrooxypropan-2-ols (±)-**1a–g** could provide an easy alternative route to the corresponding (*S*)- and (*R*)-1,2-diols **5a–g**.

Recently, our group reported some preliminary results[3] on the kinetic resolution of various ring-substituted racemic nitrooxy alcohols **1** by acetylation in the presence of lipase from *Pseudomonas fluorescens* (Amano AK). However, poor enantioselectivities ($E = 7$–17) were obtained, except for Ar = 3-CH_3-C_6H_4- ($E = 60$). As a part of our continuing interest, we undertook the present detailed study, investigating the influence of several parameters in an attempt to improve the enantioselectivity.

2. Results and discussion

2.1. Synthesis of 3-aryloxy-1-nitrooxypropan-2-ols (±)-1a–g

Various synthetic approaches have been tested to prepare the racemic 3-aryloxy-1-nitrooxypropan-2-ols (±)-**1**.[36] Among them, the ring-opening of appropriate arylglycidyl ethers **3a–g** with ammonium nitrate in the presence of ceric ammonium nitrate (CAN) in acetonitrile–water solution (6:1 v/v) has been found to be the most efficient one[37] (Scheme 1). The latter were previously prepared in high yields by Williamson reaction from the corresponding phenols **4a–g** and epichlorhydrin under solvent-free phase transfer catalysis conditions coupled with microwave (MW) activation.[38]

2.2. Kinetic resolution of (±)-1a–g by lipase-catalyzed transesterification

The conditions for the lipase-catalyzed acetylation of racemic nitrooxy alcohols (±)-**1** were optimized according to the conventional method described in the literature[39] (Scheme 2). The effects of lipase, solvent, acyl donor, temperature, additive (e.g. triethylamine, crown ethers and thiacrown ethers) and substituent on the aromatic ring on the enantioselectivity of enzymatic acetylation were evaluated on reactivity and selectivity by determination of the enantiomeric ratio (*E*).

The efficiency of different commercially available lipases to catalyze the acylation reaction was investigated. For this purpose, (±)-**1a**, taken as a model substrate, was allowed to react with 3 equiv. of vinyl acetate in the presence of different lipases in *tert*-butyl

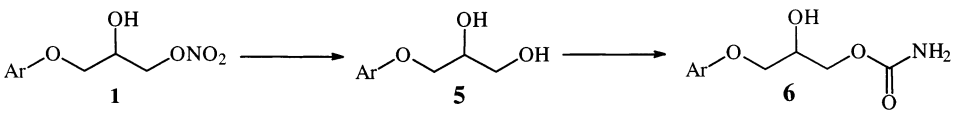
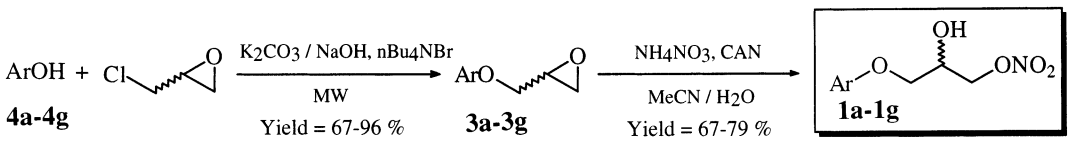
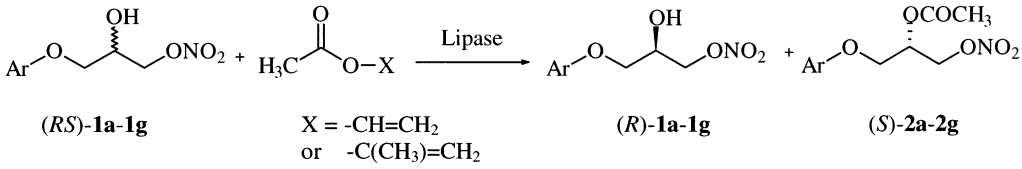


Figure 2.



Ar	a	b	c	d	e	f	g
	C_6H_5-	2-CH_3-C_6H_4-	3-CH_3-C_6H_4-	4-CH_3-C_6H_4-	2-CH_3O-C_6H_4-	4-Cl-C_6H_4-	2,6-Cl-C_6H_3-

Scheme 1.



Scheme 2.

methyl ether at 22°C. In control experiments, it was shown that the reaction did not proceed in the absence of enzyme. Generally, the transesterifications were stopped after 40–60% conversion. Removing the enzyme by filtration to give a mixture of enantiomerically enriched acetate **2a** and unreacted alcohol **1a**. The enantiomeric excesses of **1a** and **2a** were determined by HPLC using a chiral column. The absolute configurations of the products, alcohol **1a** and acetate **2a** were determined by comparison of their chiroptical and chromatographic properties with authentic samples, prepared by the method described in our previous paper.[3] The unreacted alcohol **1a** and its acetate **2a** had the (*R*)-(−) and (*S*)-(+) configuration, respectively. Additionally, the absolute configuration of unreacted alcohol **1a** was supported by correlation to authentic (*S*)-(+)-3-phenoxy-1,2-propanediol **5a**.[18,33a] This assignment agrees well with the Kazlauskas-rule.[39]

The results presented in Table 1 show that for most of the tested lipases the (*R*)-(−)-alcohol **1a** reacts slower than the (*S*)-(+) enantiomer and the reactions show poor to moderate enantioselectivities ($E=3$–20). Among the tested lipases, only *Pseudomonas cepacia* (Amano PS ($E=17$) and Amano PS immobilized on diatomite ($E=20$)) as well as *P. fluorescens* (Amano AK ($E=16$)) exhibited promising enantioselectivities for this substrate. Rather similar enantioselectivities ($E=10$–14) were obtained with lipase from *Burkholderia cepacia* formerly *P. cepacia* (Chirazyme® L-1, lyo. and immobilized Chirazyme® L-1, c.-f., lyo.) and lipase from *Candida antarctica*, fraction *B* (immobilized Chirazyme® L-2, c.-f., lyo., Chirazyme® L-2, c.-f., C2, lyo., Chirazyme® L-2, c.-f., C3, lyo. and Novozym® SP 435). The other examined lipases showed poor selectivities or reactivities.

We then investigated the proper choice of solvent. Its influence on the enantioselectivity in enzyme-catalyzed transesterifications is well documented, but for a new substrate type it is difficult to anticipate the best choice.[19a,41,42] The attempts were undertaken to find the correlation between enantioselectivity and physicochemical characteristics of the solvent such as hydrophobicity or dielectric constant.[43]

Table 1. Kinetic resolutions of (±)-**1a** by lipase-catalyzed acetylation with vinyl acetate in *tert*-butyl methyl ether at 22°C[a]

Lipases[d]	Amount of lipase (mg)	Time (h)	Conv. (%)[b]	Alcohol (*R*)-(−)-**1a** $e.e._s$ (%)[c]	Ester (*S*)-(+)-**2a** $e.e._p$ (%)[c]	E[b]
Amano AK [B]	**180**	**26**	**40**	**55**	**81**	**16**
Amano PS [A]	**180**	**28**	**43**	**61**	**80**	**17**
Amano PS on diatomite	**120**	**28**	**58**	**95**	**70**	**20**
Chirazyme® L-1, lyo.	5.6	9	48	61	66	10
Chirazyme® L-1, c.-f., lyo.	200	16	63	94	56	12
Novozym® SP 435	50	28	49	70	73	14
Chirazyme® L-2, c.-f., lyo.	50	18	34	38	75	10
Chirazyme® L-2, c.-f., C2, lyo.	50	27	32	36	76	11
Chirazyme® L-2, c.-f., C3, lyo.	50	22	35	42	77	12
Chirazyme® L-3, lyo.	200	312	30	Rac.	Rac.	1
Chirazyme® L-3, purified, lyo.	10	312	4	–	–	–
Chirazyme® L-5, lyo.	100	72	51	52	51	5
Chirazyme® L-6, lyo.	50	21	53	31	28	3
Chirazyme® L-7, lyo.	200	312	23	11	36	3
Chirazyme® L-8, lyo.	200	312	4	–	–	–
Chirazyme® L-10, lyo.	100	18	39	45	70	8
Chirazyme® L-12, lyo.	80	312	0	–	–	–

[a] Conditions: (±)-**1a** (213 mg, 1 mmol), vinyl acetate (258 mg, 3 mmol) and *tert*-butyl methyl ether (10 mL).

[b] Conversions and E-values were calculated from the enantiomeric excess of substrate **2** ($e.e._s$) and of product **3** ($e.e._p$) using the usual formula: $E=\ln[(1-e.e._s)(e.e._p/(e.e._s+e.e._p))]/\ln[(1+e.e._s)(e.e._p/(e.e._s+e.e._p))]$, $conv.=e.e._s/(e.e._s+e.e._p)$; according to Ref. 40.

[c] Determined by chiral HPLC analysis using Pharmachir 4 C (1/4×250 mm) column.

[d] Amano AK (*P. fluorescens*) [B]; Amano PS [A] and Amano PS immobilized on diatomite (*P. cepacia*); Chirazyme® L-1, lyo. and immobilized Chirazyme® L-1, c.-f., lyo. (*B. cepacia*, formerly *P. cepacia*); immobilized Chirazyme® L-2, c.-f., lyo., Chirazyme® L-2, c.-f., C2, lyo. and Chirazyme® L-2, c.-f., C3, lyo. and Novozym® SP 435 (*C. antarctica*, fraction *B*); Chirazyme® L-3, lyo. and Chirazyme® L-3, purified, lyo. (*C. rugosa*, formerly *C. cylindracea*); Chirazyme® L-5, lyo. (*C. antarctica*, fraction *A*); Chirazyme® L-6, lyo. (*Pseudomonas* species); Chirazyme® L-7, lyo. (porcine pancreas lipase); Chirazyme® L-8, lyo. (*Thermomyces* species, formerly *Humicola* species); Chirazyme® L-10, lyo. (*Alcaligines* species) and Chirazyme® L-12, lyo. (lipase from thermophilic microorganism).

Acetylations of (±)-**1a** with vinyl acetate at 22°C in the presence of lipase from *P. cepacia* (Amano PS) or *P. fluorescens* (Amano AK) were performed in organic solvents frequently used in this type of reaction. The main results are given in Table 2 and Fig. 3.

It is obvious from Table 2 that alteration of the solvent caused significant changes in the enantioselectivity and reaction rate in comparison with *tert*-butyl methyl ether as the standard solvent. Among the tested solvents, *n*-hexane gave the best result with regard to enantioselectivity ($E=57$) and reaction rate (17 h for 49% conversion). Enantioselectivities in toluene, dichloromethane as well as in solvent-free condition were similar in all cases ($E=41$, 42, 40 respectively) and slightly lower than in *n*-hexane. On the other hand, in toluene as well as in dichloromethane the reactions rates were significantly lower (46 h for 45% conversion and 120 h for 38% conversion, respectively) in comparison to *n*-hexane and solvent-free conditions. Fig. 3 shows the course of the conversion of (±)-**1a** in selected solvents and in solvent-free conditions with time. It is important to note that acetylation in solvent free-conditions was rather difficult to reproduce (reaction time ranging from 16–28 h for 31–35% conversion). In general, enzyme selectivity was good enough for practical use in all of the tested solvents ($E=24$–57) except *tert*-butyl methyl ether ($E=17$) and acetonitrile ($E=12$). Changing the enzyme from *P. cepacia* lipase (Amano PS) to *P. fluorescens* (Amano AK) in *n*-hexane (entries 3 and 4) did not alter the reaction rate considerably, but led to a noticeable decrease in the enantioselectivity (E decreased from 57 to 47). For reactions completed in toluene (entries 6 and 7) and *tert*-butyl methyl ether (entries 8 and 9) the reaction rates and enantioselectivities were similar for both lipases. Thus, we selected *n*-hexane as the solvent for further optimization of the reaction.

Next, the influence of the amount of *P. cepacia* lipase (Amano PS) on the reaction rate and enantioselectivity was investigated. The reaction of (±)-**1a** was carried out with vinyl acetate in *n*-hexane at 22°C. It appears clearly from Table 3 that use of enzyme in the amounts ranging from 50–180 mg/mmol of substrate (entries 1–3) results in good conversions (37–51%) in reasonable times (17–39 h) without significant changes in enantioselectivity ($E=48$–57). Further decreasing the amount of enzyme to 10–25 mg/mmol of substrate (entries 4 and 5) led to only a slight decrease of the enantioselectivity ($E=42$–43) but the reaction proceeded with slower rate (42–44% of conversion within 56–67 h).

The effect of the acyl donor structure on the enantioselectivity of lipase-catalyzed transesterification reaction

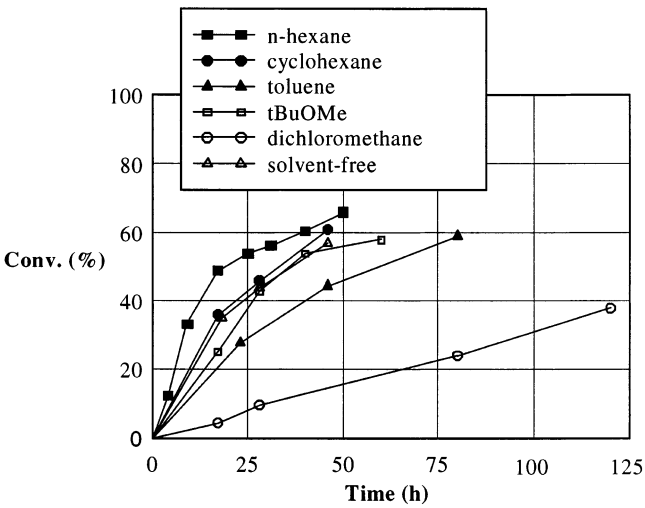


Figure 3. Conversion versus time for *P. cepacia* (Amano PS) [A] lipase-catalyzed transesterification of vinyl acetate with (±)-**1a** at 22°C in selected solvents.

Table 2. Transesterification of vinyl acetate with (±)-**1a** in various solvents[a]

Entry	Lipase[a]	Solvent	Log *P*	Time (h)	Conv.[b] (%)	Alcohol (*R*)-(−)-**1a** e.e.$_s$ (%)[c]	Ester (*S*)-(+)-**2a** e.e.$_p$ (%)[c]	E[b]
1	A	None	–	18	35	50	91	40
2	B	None	–	22	31	43	93	42
3	**A**	***n*-Hexane**	**3.5**	**17**	**49**	**87**	**90**	**57**
4	**B**	***n*-Hexane**	**3.5**	**22**	**41**	**64**	**92**	**47**
5	A	Cyclohexane	3.2	17	36	50	89	29
6	A	Toluene	2.5	46	45	72	90	41
7	B	Toluene	2.5	36	30	39	93	40
8	A	^{t}BuOMe	1.3	28	43	61	80	17
9	B	^{t}BuOMe	1.3	26	40	55	81	16
10	A	CH_2Cl_2	0.6	120	38	56	92	42
11	A	THF	0.49	120	25	30	91	29
12	A	Acetone	−0.23	120	33	44	88	24
13	A	Acetonitrile	−0.33	96	30	34	79	12
14	A	Dioxane	−1.1	120	27	33	91	30

[a] Conditions: (±)-**1a** (213 mg, 1 mmol), vinyl acetate (258 mg, 3 mmol), lipase from *P. cepacia* (Amano PS) [A] or *P. fluorescens* (Amano AK) [B] (180 mg) and solvent (10 mL) at 22°C.
[b] See Table 1.
[c] See Table 1.

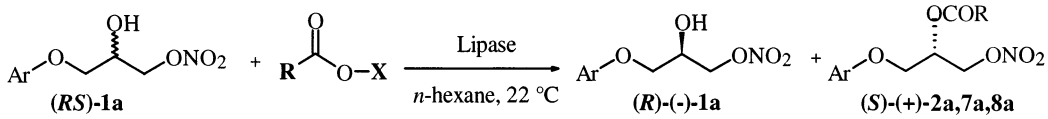
Table 3. Influence of the amount of *P. cepacia* lipase (Amano PS) [A] on the transesterification of vinyl acetate with (±)-**1a**[a]

Entry	Amount of lipase (mg)	Time (h)	Conv.[b] (%)	Alcohol (*R*)-(−)-**1a** e.e.$_s$ (%)[c]	Ester (*S*)-(+)-**2a** e.e.$_p$ (%)[c]	*E*[b]
1	180	17	49	87	90	57
2	90	29	37	55	94	53
3	50	39	51	92	87	48
4	25	56	42	66	91	43
5	10	67	44	71	90	42

[a] Conditions: (±)-**1a** (213 mg, 1 mmol), vinyl acetate (258 mg, 3 mmol), lipase from *P. cepacia* (Amano PS) [A] (10–180 mg) and *n*-hexane (10 mL) at 22°C.
[b] See Table 1.
[c] See Table 1.

has been well documented by Ema et al.[44] Generally, among the various types of acyl donors examined, enol esters are considered to be the most suitable for kinetic resolution by transesterification due to their high reactivity and reaction irreversibility.[45] Recently,[4] we found, that enol esters such as vinyl acetate and *iso*-propenyl acetate were appropriate acyl donors for the enzymatic acetylation of some azido alcohols. Thus, in order to enhance the enantioselectivity of lipase-catalyzed acetylation of (±)-**1a** in *n*-hexane at 22°C, four various enol esters were examined using *P. cepacia* (Amano PS) or *P. fluorescens* lipase (Amano AK) as catalysts (Scheme 3).

As shown in Table 4 and Fig. 4(a), the highest reaction rates and enantioselectivities ($E=57$ and 66 in 17 h for 49 and 47% conversions, respectively) were obtained when acetylations of (±)-**1a** catalyzed by *P. cepacia* lipase (Amano PS) were carried out with vinyl or *iso*-propenyl acetate. On the other hand, as can be seen from the conversion versus time curves in Fig. 4(a), changing the lipase from *P. cepacia* (Amano PS) to *P. fluorescens* (Amano AK) in the acetylation performed with *iso*-propenyl acetate considerably prolonged the reaction time (29 h for 24% conversion) making this approach synthetically useless in spite of the high enantiomeric ratio ($E=52$). Finally, it is interesting to note that lengthening the alkyl chain of the vinyl esters produces a significant decrease in the reaction rate (conversion ranging from 22 to 49% in 17–24 h) but only a marginal effect on the enantioselectivity of this reaction ($E=49$–57). Similar effects were also observed



Scheme 3.

Table 4. *P. cepacia* (Amano PS) [A] or *P. fluorescens* (Amano AK) [B] lipase-catalyzed acetylation of (±)-**1a** by use of various enol esters[a]

Enol ester	Lipase[a]	R	X	Time (h)	Conv.[b] (%)	Alcohol (*R*)-(−)-**1a** e.e.$_s$ (%)[c]	(*S*)-(+)-Ester e.e.$_p$ (%)[c]	*E*[b]
Vinyl acetate	A	CH_3	$CH=CH_2$	4	12	15	**2a**, 97	76
Vinyl acetate	A	CH_3	$CH=CH_2$	9	33	47	**2a**, 95	63
Vinyl acetate	**A**	$\mathbf{CH_3}$	$\mathbf{CH=CH_2}$	**17**	**49**	**87**	**2a**, **90**	**57**
Vinyl acetate	A	CH_3	$CH=CH_2$	25	54	98	**2a**, 84	53
Vinyl acetate	A	CH_3	$CH=CH_2$	31	56	99	**2a**, 77	39
Vinyl acetate	A	CH_3	$CH=CH_2$	40	60	99	**2a**, 65	23
Vinyl acetate	A	CH_3	$CH=CH_2$	50	66	99	**2a**, 50	14
Vinyl acetate	B	CH_3	$CH=CH_2$	22	41	64	**2a**, 92	47
Vinyl pentanoate	A	C_4H_9	$CH=CH_2$	28	22	27	**7a**, 95	51
Vinyl hexanoate	A	C_5H_{11}	$CH=CH_2$	42	39	59	**8a**, 94	49
***iso*-Propenyl acetate**	**A**	$\mathbf{CH_3}$	$\mathbf{C(CH_3)=CH_2}$	**17**	**47**	**98**	**2a**, **87**	**66**
iso-Propenyl acetate	B	CH_3	$C(CH_3)=CH_2$	29	24	30	**2a**, 95	52

[a] Conditions: (±)-**1a** (213 mg, 1 mmol), enol ester (258 mg, 3 mmol), lipase from *P. cepacia* (Amano PS) [A] or *P. fluorescens* (Amano AK) [B] (180 mg) and *n*-hexane (10 mL) at 22°C.
[b] See Table 1.
[c] See Table 1.

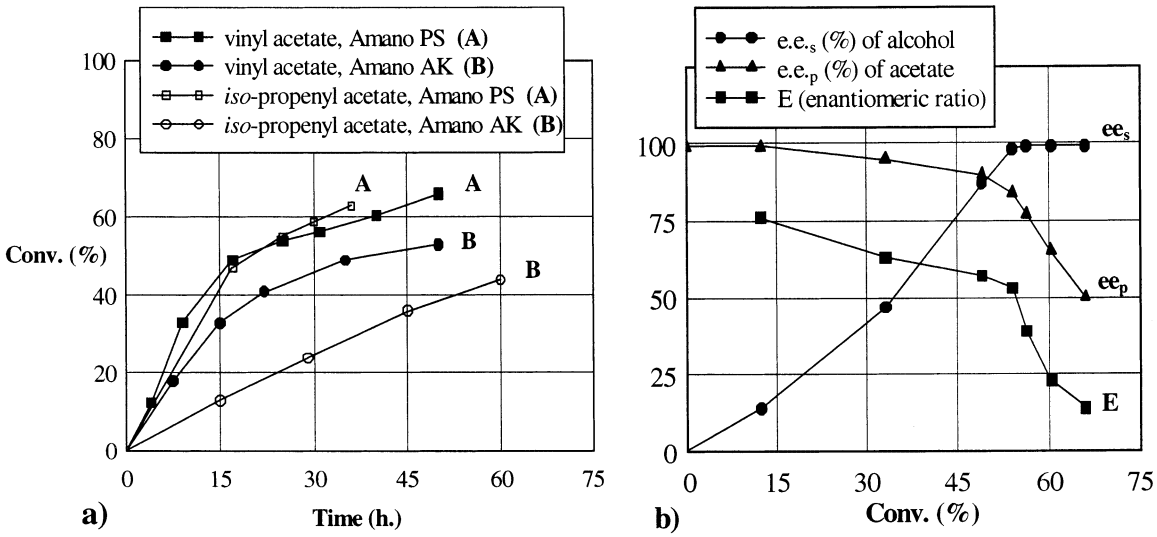


Figure 4. (a) Conversion versus time for Amano PS [A] and Amano AK [B] lipase-catalyzed acetylation of (±)-**1a** with selected enol esters; (b) dependance of enantiomeric purities of **1a** and **2a** on the conversion of (±)-**1a** in Amano PS lipase-catalyzed acetylation with vinyl acetate in *n*-hexane at 22°C.

in the kinetic resolution of some 1-azido-3-aryloxy-2-propanols[4] with lipase from *C. antarctica-B* (Novozym® SP 435).

In order to investigate whether the enantioselectivity of *P. cepacia* lipase-catalyzed acetylation of (±)-**1a** with vinyl acetate in *n*-hexane at 22°C changes during the reaction time we measured the enantiomeric purities of the products (*R*)-**1a** and (*S*)-**2a** over time. The dependence of the enantiomeric purities of the alcohol and acetate products on the conversion in this reaction is presented in Table 4 and Fig. 4(b).

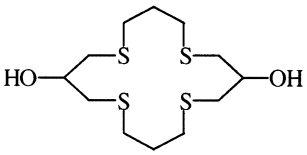
The results in Table 4 and Fig. 4(b) show that for this reaction *E* decreases over time. In fact, if the conversion of (±)-**1a** is increased from 12 to 54% the *E*-value decreased from 76 to 53. Further increase in the conversion induced significant decreases in the enantiomeric ratio for the reaction (*E* ranging from 39 to 14 for 56 and 66% conversions, respectively). As can be seen from the curves, the product (*S*)-**2a** is obtained in its highest yield and enantiomeric purities at 30–40% conversion (e.e. of **2a** = 95–98%). In the case of unreacted alcohol (*R*)-**1a** the enantiomeric purity remains low before 50% conversion and reaches its maximum (e.e. of **1a** = 95–99%) beyond the 50–60% conversion mark.

We recently found that use of some additives such as triethylamine,[46] crown[47] and thiacrown ethers[48] as well as TDA-1[47c] enhanced both enantioselectivity and reaction rate in transesterification with 1-azido-3-phenoxy-2-propanol catalyzed by Novozym® SP 435.[4] Therefore, in the next step, the influence of triethylamine, 15-crown-5 and 18-crown-6 ethers, 1,4,8,11-tetrathiacyclotetradecane (TTCTD), 1,5,9,13-tetrathiacyclohexadecane-3,11-diol (TTCHD-D), and tris-(3,6-dioxaheptyl)amine (TDA-1) were screened for the *P. cepacia* lipase-catalyzed acetylation of (±)-**1a** with vinyl acetate in *n*-hexane at 22°C. The main results are presented in Table 5.

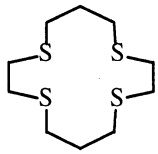
Table 5. Additive effects on the acetylation of (±)-**1a** with vinyl acetate using *P. cepacia* lipase (Amano PS) in *n*-hexane at 22°C[a]

Entry	Additive	Amount of additive (mg)	Time (h)	Conv. (%)[b]	Alcohol (*R*)-(−)-**1a** $e.e._s$ (%)[c]	Ester (*S*)-(+)-**2a** $e.e._p$ (%)[c]	E[b]
1	**None**	–	**17**	**49**	**87**	**90**	**57**
2	NEt_3	20	19	53	93	82	35
3	15-Crown-5	20	22	23	28	94	41
4	18-Crown-6	22	17	17	20	95	47
5	TDA-1[d]	14	19	24	30	94	43
6	TTCTD[d]	12	20	35	50	93	45
7	TTCHD-D[d]	12	20	38	57	94	49

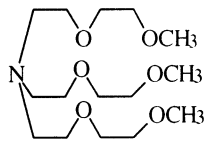
[a] Conditions: (±)-**1a** (213 mg, 1 mmol), vinyl acetate (258 mg, 3 mmol), lipase from *P. cepacia* (Amano PS) (180 mg), 14 mol% of triethylamine or 5 mol% additive and *n*-hexane (10 mL) at 22°C.
[b] See Table 1.
[c] See Table 1.
[d] TDA-1, tris-(3,6-dioxaheptyl)amine; TTCTD, 1,4,8,11-tetrathiacyclotetradecane; TTCHD-D, 1,5,9,13-tetrathiacyclohexadecane-3,11-diol.



TTCHD-D



TTCTD



TDA-1

It is obvious from Table 5 that the use of these additives in acetylation of (±)-**1a** has a negative effect on both the enantioselectivity and the rate of the reaction. Without additive the reaction proceeded with higher enantioselectivity ($E=57$) giving a good conversion in reasonable time (49% conversion in 17 h). The addition of triethylamine markedly decreased the enantiomeric ratio ($E=35$) without any change in reaction rate (53% conversion in 19 h). On the other hand, addition of crown ethers (15-5, 18-6) and thiacrown ethers (TTCTD, TTCHD-D) as well as TDA-1 significantly increased the reaction time (17–38% of conversion within 17–22 h) but has only a slight effect on the enantioselectivity of the reaction ($E=41$–49).

Sakai et al.[49] have recently reported that the enantioselectivity of *P. cepacia* lipase for substrates like azirine-2-methanol is significantly increased by lowering the temperature. Consequently, we have investigated the *P. cepacia* lipase-catalyzed acetylation of (±)-**1a** under the conditions described in Table 5 at temperatures ranging from 22 to −18°C (Table 6).

The results in Table 6 show that lipase from *P. cepacia* (Amano PS) remains active even at −18°C. Lowering of the temperature from 22 to −18°C enhances the lipase enantioselectivity (*E*-value increased from 57 at 22°C to 105 at −18°C) but decreases the reaction rate (27% conversion in 140 h at −18°C). An acceptable reaction time was obtained at −18°C by increasing the amount of lipase from 180 mg/mmol of substrate to 600 mg/mmol (entry 4). Reducing the temperature further to −30°C results in a significant decrease of the reaction rate (only 10% of conversion within 18 days). From these experiments it appears that temperatures between 4 and 22°C are good compromise for achieving the preparation of both (*R*)-**1a** and (*S*)-**2a** in terms of enantioselectivity and reaction time. Moreover at 22°C the enantiomeric ratio is also sufficiently high for practical use.

Finally, in order to investigate the influence of the substituent (Ar) at the aryl ring, 6 different racemic 3-aryloxy-1-nitrooxypropan-2-ols (±)-**1b–g** were used as substrates in a kinetic resolution by a lipase-catalyzed acetylation.

All acetylations of (±)-**1a–g** were carried out in *n*-hexane with vinyl acetate using *P. cepacia* (Amano PS) [A] lipase at 22 and 4°C. In the cases of racemic alcohols (±)-**1a**, (±)-**1d** and (±)-**1f**, lipase from *P. fluorescens* (Amano AK) [B] was used at 22°C. The results are collected in Table 7.

The enantiomeric excesses of the unreacted alcohol **1b–g** and the acetate product **2b–g** were determined by chiral HPLC analysis. To our knowledge, no data are available for the absolute configurations of **1b–g** and **2b–g** or their derivatives. For these compounds the absolute configurations were assigned by comparison of the sign of the specific rotation with the data for (*R*)-(−)-**1a** and (*S*)-(+)-**2a**. In all cases the unreacted alcohols **1b–g**, and their acetates **2b–g** had the (*R*)-(−) and (*S*)-(+) configurations, respectively.

It can be clearly seen from Table 7 that it is possible to run the acetylation of racemic alcohols (±)-**1a–g** with good enantioselectivities ($E=31$–111) whatever the substituents on the phenyl ring are. However, it is important to note that the substrates with *ortho*-substituents (**1b**, **1e** and **1f**) show significantly slower reaction rates and slightly lower enantiomeric excess.

3. Conclusion

In summary, we have presented a general method to realize enantioselective acetylations of 3-aryloxy-1-nitrooxypropan-2-ols **1a–g** whatever the aromatic substituents are. High enantioselectivities ($E=31$–111) were obtained using *P. cepacia* (Amano PS) or *P. fluorescens* (Amano AK) lipase and vinyl acetate in *n*-hexane at 22 or 4°C.

Table 6. Temperature influence in the *P. cepacia* lipase-catalyzed acetylation of (±)-**1a**[a]

Entry	Temp. (°C)	Amount of lipase (mg)	Time (h)	Conv. (%)[b]	Alcohol (*R*)-(−)-**1a** e.e.$_s$ (%)[c]	Ester (*S*)-(+)-**2a** e.e.$_p$ (%)[c]	*E*[b]
1	22	180	17	49	87	90	57
2	**4**	**180**	**65**	**49**	**90**	**93**	**94**
3	−18	180	140	27	36	97	105
4	**−18**	**600**	**68**	**48**	**87**	**94**	**96**

[a] Conditions: (±)-**1a** (213 mg, 1 mmol), vinyl acetate (258 mg, 3 mmol), lipase from *P. cepacia* (Amano PS) and *n*-hexane (10 mL).
[b] See Table 1.
[c] See Table 1.

Table 7. Transesterification of vinyl acetate with (±)-**1a**–**g** using *P. cepacia* (Amano PS) [A] lipase in *n*-hexane at 4 and 22°C[a]

Entry	Substrate (±)-**1**	Ar	Temp. (°C)	Enzyme	Time (h)	Conv. (%)[b]	Alcohol (*R*)-(−)-**1** e.e.$_s$ (%)[c]	Ester (*S*)-(+)-**2** e.e.$_p$ (%)[c]	E[b]
1	**a**	C_6H_5-	22	A	17	49	87	90	57
			4	**A**	**65**	**49**	**90**	93	94
			22	B	22	41	64	92	47
2	**b**	2-CH_3-C_6H_4-	22	A	48	50	89	88	46
			4	**A**	**98**	**53**	**98**	**87**	**65**
3	**c**	3-CH_3-C_6H_4-	22	A	24	50	90	91	66
			4	**A**	**79**	**52**	**99**	**91**	**111**
4	**d**	4-CH_3-C_6H_4-	22	A	21	51	93	88	53
			4	**A**	**74**	**51**	**96**	91	84
			22	B	28	55	98	80	41
5	**e**	2-CH_3O-C_6H_4-	22	A	52	53	95	83	39
			4	**A**	**103**	**46**	**80**	**93**	**68**
6	**f**	4-Cl-C_6H_4-	22	A	21	51	90	85	38
			4	**A**	**80**	**44**	**74**	93	62
			22	B	24	55	96	78	31
7	**g**	2,6-Cl-C_6H_3-	22	A	54	47	78	89	41
			4	**A**	**128**	**40**	**64**	**95**	**76**

[a] Conditions: (±)-**1a** (1 mmol), vinyl acetate (3 mmol, 258 mg), lipase from *P. cepacia* (Amano PS) [A] or *P. fluorescens* (Amano AK) [B] (180 mg) and *n*-hexane (10 mL) at 22 or 4°C.
[b] See Table 1.
[c] See Table 1.

4. Experimental

4.1. General

Lipases from *P. cepacia* (Amano PS, Amano PS immobilized on diatomite) and *P. fluorescens* (Amano AK) were purchased from Amano Pharmaceutical Co., Ltd (Nagoya, Japan). Novozym® SP 435 (immobilized *C. antarctica-B* lipase) was kindly gifted by Novo Nordisk (Bagsvaerd, Denmark). Chirazyme® Lipases & Esterases, Screening Set Industrial Enzymes 2 (Chirazyme® L-1, lyo. and Chirazyme® L-1, c.-f., lyo. (*B. cepacia*, formerly *P. cepacia*); immobilized Chirazyme® L-2, c.-f., lyo., Chirazyme® L-2, c.-f., C2, lyo. and Chirazyme® L-2, c.-f., C3, lyo. (*C. antarctica*, fraction *B*); Chirazyme® L-3, lyo. and Chirazyme® L-3, purified, lyo. (*Candida rugosa*, formerly *Candida cylindracea*); Chirazyme® L-5, lyo. (*C. antarctica*, fraction *A*); Chirazyme® L-6, lyo. (*Pseudomonas* species); Chirazyme® L-7, lyo. (porcine pancreas lipase); Chirazyme® L-8, lyo. (*Thermomyces* species, formerly *Humicola* species); Chirazyme® L-10, lyo. (*Alcaligines* species) and Chirazyme® L-12, lyo. (lipase from thermophilic microorganism)) was kindly gifted by Roche Molecular Biochemicals (Mannheim, Germany).

All the commercially available chemicals were obtained from Aldrich and Fluka. Solvents of analytical-grade quality were purchased from Lab Scan Ltd and Aldrich. The racemic nitrooxy alcohols **1a**–**g** were prepared by previously described methods.[3,36,37] The racemic acetates as well as the other esters were synthesized from the corresponding alcohols and acetyl chloride or acetic anhydride according to the usual procedures (e.g. 10 mmol of (±)-**1a**–**g**, 15 mmol of acetyl chloride, 15 mmol of pyridine in CH_2Cl_2 (30 mL) at 22°C).

4.2. Analytical methods

Microanalyses were performed by the Laboratoire Central de Microanalyse du CNRS, Gif sur Yvette, France. ^1H (250 or 400 MHz) and ^{13}C (50, 62.9 or 100.6 MHz) NMR spectra were recorded on Bruker AC-200, 250 or 400 spectrometer in $CDCl_3$ with TMS as the internal standard. Chemical shifts (δ) are given in ppm. Optical rotation measurements were recorded on a DiP-370 JASCO polarimeter. HPLC analyses were run on a Thermo-Separation Products P-100 instrument. Optical purities of unreacted nitrooxy alcohols (*R*)-**1a**–**g** and their acetates (*S*)-**2a**–**g** were controlled by HPLC analysis on a chiral column Pharmachir 4C (1/4×250 mm) and directly determined using racemic compounds as references. The conditions were: *n*-hexane: *iso*-propanol = 90:10 v/v, 70 bar, 254 nm, 22°C for **1a**–**c**, **1e** and **2a**–**c**, **2e**; *n*-hexane: *iso*-propanol = 95:5 v/v, 80 bar, 254 nm, 22°C for **1d**, **1f**, **1g**, and **2d**, **2f** and **2g**. The retention times (t_R/min) were as follows: **1a**: 13.06 (*S*), 20.93 (*R*); **2a**: 10.59 (*S*), 11.43 (*R*); **1b**: 15.03 (*S*), 26.09 (*R*); **2b**: 10.49 (*S*), 12.64 (*R*); **1c**: 14.28 (*S*), 19.99 (*R*); **2c**: 10.66 (*S*), 12.28 (*R*); **1d**: 16.01 (*S*), 21.93 (*R*); **2d**: 8.24 (*S*), 9.26 (*R*); **1e**: 11.55 (*S*), 12.93 (*R*); **2e**: 13.93 (*S*), 28.79 (*R*); **1f**: 17.09 (*S*), 17.97 (*R*); **2f**: 10.96 (*S*), 11.42 (*R*); **1g**: 12.78 (*S*), 14.09 (*R*); **2g**: 6.09 (*S*), 7.24 (*R*). Column chromatography was performed on Merck silica gel 60 (230–400 mesh). TLC was carried out using glass sheets pre-coated with silica gel 60 F_{254} prepared by Merck.

4.3. Typical acetylation procedure for racemic nitrooxy alcohols (±)-1a–g using *P. cepacia* (Amano PS) [A] or *P. fluorescens* (Amano AK) [B] lipase in *n*-hexane at 4 or 22°C

Vinyl acetate (3 mmol, 258 mg) and *P. cepacia* (Amano PS) [A] or *P. fluorescens* (Amano AK) [B] lipase (180 mg) were added to a solution of the racemic nitrooxy alcohol **1a–g** (1 mmol) in 10 mL of *n*-hexane. The mixture was stirred at 4 or 22°C and monitored by TLC. After the appropriate time (Table 7), the reaction was stopped by filtering off the solid enzyme and the solvent was evaporated under reduced pressure. The crude mixture of acetate (*S*)-(+)-**2a–g** and unreacted alcohol (*R*)-(−)-**1a–g** was separated by flash chromatography on silica gel with hexane–ethyl acetate (15:1 v/v) as the eluent. For all of unreacted alcohols (*R*)-**1a–g** and their acetates (*S*)-**2a–g** determination of optical purity was performed by means of chiral HPLC column chromatography (Pharmachir 4C (1/4×250 mm)). Optical rotation values in all cases were in accordance with the literature data.[3] ^{1}H NMR spectra of enantiomeric nitrooxy alcohols **1a–g** were identical with those of the racemic compounds (±)-**1a–g** as described in the literature[3] and their ^{13}C NMR data are as follows:

(*R*)-**1a**: ^{13}C NMR (62.9 MHz, $CDCl_3$, ppm): δ 67.03 (*C*H), 68.07 (*C*H_2O), 73.09 (*C*H_2ONO$_2$), 114.42, 121.65, 129.61, 157.87 (*C*-Ar).

(*R*)-**1b**: ^{13}C NMR (62.9 MHz, $CDCl_3$, ppm): δ 16.05 (*C*H_3-Ar), 67.07 (*C*H), 68.06 (*C*H_2O), 73.35 (*C*H_2ONO$_2$), 110.98, 121.25, 126.60, 126.88, 130.84, 155.81 (*C*-Ar).

(*R*)-**1c**: ^{13}C NMR (62.9 MHz, $CDCl_3$, ppm): δ 21.42 (*C*H_3-Ar), 66.98 (*C*H), 68.03 (*C*H_2O), 73.17 (*C*H_2ONO$_2$), 111.26, 115.24, 122.42, 129.31, 139.74, 157.89 (*C*-Ar).

(*R*)-**1d**: ^{13}C NMR (62.9 MHz, $CDCl_3$, ppm): δ 20.42 (*C*H_3-Ar), 67.02 (*C*H), 68.29 (*C*H_2O), 73.19 (*C*H_2ONO$_2$), 114.29, 130.02, 130.95, 155.80 (*C*-Ar).

(*R*)-**1e**: ^{13}C NMR (62.9 MHz, $CDCl_3$, ppm): δ 55.63 (*C*H_3O-Ar), 66.76 (*C*H), 70.47 (*C*H_2O), 73.06 (*C*H_2ONO$_2$), 111.87, 115.03, 121.01, 122.45, 147.39, 149.44 (*C*-Ar).

(*R*)-**1f**: ^{13}C NMR (50 MHz, $CDCl_3$, ppm): δ 66.92 (*C*H), 68.52 (*C*H_2O), 73.03 (*C*H_2ONO$_2$), 115.73, 126.51, 129.46, 156.53 (*C*-Ar).

(*R*)-**1g**: ^{13}C NMR (50 MHz, $CDCl_3$, ppm): δ 67.35 (*C*H), 72.87 (*C*H_2O), 73.08 (*C*H_2ONO$_2$), 125.70, 129.04, 150.19 (*C*-Ar).

^{1}H and ^{13}C NMR spectra, IR data as well as microanalyses of acetates **2a–g** are as follows:

(*S*)-**2a**: ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 2.12 (s, 3H, $COCH_3$), 4.15 (d, J=5.2 Hz, 2H, CH_2ONO_2), 4.69–4.91 (m, 2H, OCH_2), 5.39–5.50 (m, 1H, chiral CH), 6.88–6.95 (m, 2H, aromatic CH), 6.98–7.03 (m, 1H, aromatic CH), 7.27–7.34 (m, 2H, aromatic CH); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm): δ 20.80 (CO*C*H_3), 65.44 (*C*H_2O), 68.23 (*C*H), 70.53 (*C*H_2ONO$_2$), 114.47, 121.65, 129.61, 157.87 (*C*-Ar), 170.10 (C=O); IR (neat, cm^{-1}): 1630, 1240 (NO_2), 1710 (C=O). Anal. calcd for $C_{11}H_{13}NO_6$ (255): C, 51.76; H, 5.09; N, 5.49. Found: C, 51.82; H, 5.07; N, 5.33%.

(*S*)-**2b**: ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 2.13 (s, 3H, $COCH_3$), 2.23 (s, 3H, aromatic CH_3), 4.15 (d, J=5.2 Hz, 2H, CH_2ONO_2), 4.71–4.93 (m, 2H, OCH_2), 5.44–5.55 (m, 1H, chiral CH), 6.78–6.83 (m, 1H, aromatic CH), 6.89–6.95 (m, 1H, aromatic CH), 7.14–7.21 (m, 2H, aromatic CH); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm): δ 16.04 (*C*H_3-Ar), 20.75 (CO*C*H_3), 65.53 (*C*H_2O), 68.29 (*C*H), 70.62 (*C*H_2ONO$_2$), 110.88, 121.30, 126.78, 126.87, 130.88, 155.93 (*C*-Ar), 170.05 (C=O); IR (neat, cm^{-1}): 1630, 1245 (NO_2), 1710 (C=O). Anal. calcd for $C_{12}H_{15}NO_6$ (269): C, 53.53; H, 5.57; N, 5.20. Found: C, 53.48; H, 5.62; N, 5.17%.

(*S*)-**2c**: ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 2.13 (s, 3H, $COCH_3$), 2.30 (s, 3H, aromatic CH_3), 4.16 (d, J=5.18 Hz, 2H, CH_2ONO_2), 4.70–4.93 (m, 2H, OCH_2), 5.40–5.49 (m, 1H, chiral CH), 6.68–6.73 (m, 2H, aromatic CH), 6.75–6.85 (m, 1H, aromatic CH), 7.15–7.24 (m, 1H, aromatic CH); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm): δ 21.45 (*C*H_3–Ar), 20.74 (CO*C*H_3), 65.50 (*C*H_2O), 68.18 (*C*H), 70.46 (*C*H_2ONO$_2$), 111.32, 115.18, 122.54, 129.45, 139.89, 157.86 (*C*-Ar), 170.09 (C=O); IR (neat, cm^{-1}): 1630, 1230 (NO_2), 1720 (C=O). Anal. calcd for $C_{12}H_{15}NO_6$ (269): C, 53.53; H, 5.57; N, 5.20. Found: C, 53.59; H, 5.42; N, 5.11%.

(*S*)-**2d**: ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 2.12 (s, 3H, $COCH_3$), 2.30 (s, 3H, aromatic CH_3), 4.12 (d, J=5.2 Hz, 2H, CH_2ONO_2), 4.68–4.91 (m, 2H, OCH_2), 5.39–5.43 (m, 1H, chiral CH), 6.78–6.84 (m, 2H, aromatic CH), 7.08–7.13 (m, 2H, aromatic CH); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm): δ 20.40 (*C*H_3-Ar), 20.76 (CO*C*H_3), 65.64 (*C*H_2O), 68.24 (*C*H), 70.58 (*C*H_2ONO$_2$), 114.32, 129.99, 130.93, 155.79 (*C*-Ar), 170.07 (C=O); IR (neat, cm^{-1}): 1630, 1240 (NO_2), 1710 (C=O). Anal. calcd for $C_{12}H_{15}NO_6$ (269): C, 53.53; H, 5.57; N, 5.20. Found: C, 53.46; H, 5.39; N, 5.26%.

(*S*)-**2e**: ^{1}H NMR (400 MHz, $CDCl_3$, ppm): δ 2.10 (s, 3H, $COCH_3$), 3.84 (s, 3H, aromatic CH_3O), 4.18 (d, J=5.6 Hz, 2H, CH_2ONO_2), 4.71–4.93 (m, 2H, OCH_2), 5.38–5.48 (m, 1H, chiral CH), 6.78–7.04 (m, 4H, aromatic CH); ^{13}C NMR (100.6 MHz, $CDCl_3$, ppm): δ 20.79 (CO*C*H_3), 55.76 (*C*H_3O-Ar), 67.51 (*C*H_2O), 68.44 (*C*H), 70.67 (*C*H_2ONO$_2$), 112.24, 115.70, 120.87, 122.88, 147.49, 150.10 (*C*-Ar), 170.10 (C=O); IR (neat, cm^{-1}): 1630, 1235 (NO_2), 1718 (C=O). Anal. calcd for $C_{12}H_{15}NO_7$ (285): C, 50.52; H, 5.26; N, 4.91. Found: C, 50.48; H, 5.18; N, 4.79%.

(*S*)-**2f**: ^{1}H NMR (250 MHz, $CDCl_3$, ppm): δ 2.12 (s, 3H, $COCH_3$), 4.11 (d, J=5.08 Hz, 2H, CH_2ONO_2), 4.61–4.95 (m, 2H, OCH_2), 5.39–5.49 (m, 1H, chiral CH), 6.75–6.92 (m, 2H, aromatic CH), 7.21–7.32 (m, 2H, aromatic CH); ^{13}C NMR (50 MHz, $CDCl_3$, ppm): δ 20.71 (CO*C*H_3), 65.79 (*C*H_2O), 68.01 (*C*H), 70.34 (*C*H_2ONO$_2$), 115.75, 126.54, 129.44, 156.46 (*C*-Ar), 170.00 (C=O); IR (neat, cm^{-1}): 1630, 1255 (NO_2), 1710 (C=O). Anal. calcd for $C_{11}H_{12}ClNO_6$ (289.45): C, 45.60; H, 4.14; N, 4.83. Found: C, 45.51; H, 4.18; N, 4.72%.

(*S*)-**2g**: ^{1}H NMR (250 MHz, $CDCl_3$, ppm): δ 2.14 (s, 3H, $COCH_3$), 4.10–4.30 (m, 2H, CH_2ONO_2), 4.71–

5.09 (m, 2H, OCH_2), 5.39–5.50 (m, 1H, chiral CH), 6.98–7.09 (m, 1H, aromatic CH), 7.25–7.35 (m, 2H, aromatic CH); ^{13}C NMR (62.9 MHz, $CDCl_3$, ppm): δ 20.77 ($COCH_3$), 68.48 (CH), 70.06 (CH_2O), 70.51 (CH_2ONO_2), 125.76, 129.05, 150.20 (C-Ar), 170.09 (C=O); IR (neat, cm^{-1}): 1630, 1245 (NO_2), 1715 (C=O). Anal. calcd for $C_{11}H_{11}Cl_2NO_6$ (323.9): C, 40.75; H, 3.39; N, 4.32. Found: C, 40.65; H, 3.28; N, 4.19%.

Acknowledgements

We are grateful to Roche Molecular Biochemicals (Mannheim) for a kind gift of Chirazyme® Lipases & Esterases, Screening Set Industrial Enzymes 2. We also thank Amano Co. for a welcome supplying of *P. cepacia* lipase (Amano PS and Amano PS immobilized on diatomite) and Novo Nordisk for Novozym® SP 435.

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