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Lipase catalysed resolution of nitro aldol adducts

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Abstract—The kinetic resolution of a range of 1-nitro-2-alkanols by lipase-catalysed esterification using various lipases and succinic anhydride as an acyl donor has been studied. *E* values of up to 100 were obtained with Novozym 435 in the resolution of 1-nitro-2-pentanol with succinic anhydride in TBME. Acylation with succinic anhydride proved much more enantioselective than with vinyl acetate.

1. Introduction

The aldol reaction is one of the most important methods for C–C bond formation.[1] In the related nitro aldol condensation, the Henry reaction, the coupling of a nitro alkane to an aldehyde or ketone results in the corresponding β-nitro alcohol. Such chiral nitro alcohols are of interest as building blocks in organic synthesis as they can be converted into chiral β-hydroxy amines via reduction of the nitro group. Alternatively, further carbon–carbon bond formation on the α-carbon of the nitro group can lead to a wide variety of other useful intermediates.[2,3] The control of the stereochemistry is of importance for many synthetic purposes, especially for pharmaceutical and agricultural applications.

The products of Henry reactions are secondary alcohols; hence, they are suitable substrates for resolution by lipase catalysed enantioselective acylation (see Fig. 1). The lipase mediated kinetic resolution of secondary alcohols has been widely studied over the past 20 years[4] and has become a common synthetic and industrial methodology for producing chiral compounds as pure enantiomers.[5,6]

The choice of the acyl donor in such kinetic resolutions requires careful consideration because the transesterification equilibrium should be entirely on the side of the product.[4] The often-used vinyl esters, which react irreversibly because the liberated vinyl alcohol isomerises into acetaldehyde, satisfy this requirement.[4]

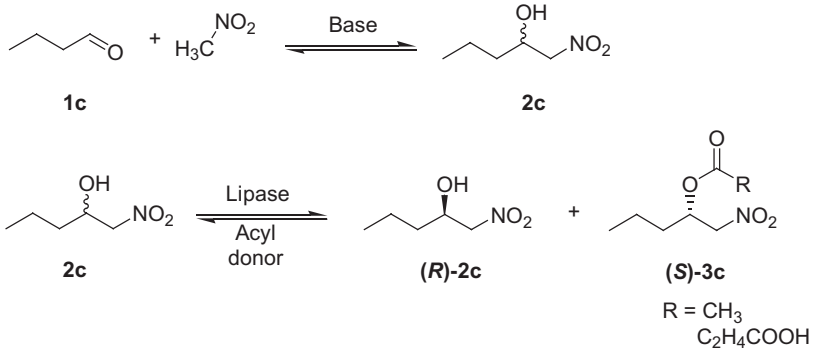

Figure 1. Nitro aldol synthesis of 1-nitro-2-pentanol followed by lipase catalysed resolution.

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Table 1. The performance of different lipases in the resolution of 1-nitro-2-pentanol[a]

Enzyme	Conversion (%, 24 h)	Ee_s (%)	Ee_p (%)	*E*
Novozym 435	54	92	93	82 (*S*)
CaLB CLEC	11	8	67	5 (*S*)
CaLB CLEA	7	0	5	1 (*S*)
CaLA SP 526	10	11	82	12 (*R*)
BcL (Amano 1)	45	34	46	4 (*R*)
BcL CLEC	54	59	37	4 (*R*)
CrL	3	2	14	1 (*R*)
CrL CLEC	8	1	3	1 (*S*)
ClL	Nr[b]	—	—	—
RmL SP 524	Nr	—	—	—

CaLB: *C. antarctica* lipase B; CaLA: *C. antarctica* lipase A; RmL: *R. miehei* lipase; CrL: *C. rugosa* lipase; ClL: *C. lipolytica* lipase, BcL: *B. cepacia* lipase.

[a] Reaction conditions: 1 mL diisopropyl ether, 100 mM 1-nitro-2-pentanol, 100 mM succinic anhydride, 25 mg enzyme or 5 mg CLEC or CLEA.

[b] No reaction.

The resolution of some β-nitro alcohols via transesterification with vinyl acetate as the acyl donor has been reported previously,[3,7,8] but the separation of the enantiomerically enriched ester and alcohol is often laborious. Acylation with a cyclic anhydride, which also reacts irreversibly, results in a half ester that can be readily extracted from the reaction mixture. This potential benefit for reaction work-up procedures has been reported for the resolution of several secondary alcohols.[9–14]

Herein we report the applicability of succinic anhydride as an acyl donor in the lipase-mediated resolution of a number of alkyl- and phenylalkyl substituted nitro alcohols. Furthermore the effects of the lipase and reaction medium will also be discussed.

2. Results and discussion

2.1. Resolution of nitro alcohols: lipases, acyl donors, solvents

The acylation of 1-nitro-2-pentanol **2c** (Fig. 1), which we selected as a suitable test reactant, was performed in the presence of a range of microbial lipases (see Table 1). The reaction proved fast and enantioselective when performed in the presence of Novozym 435, an immobilised preparation of *Candida antarctica* lipase B (CaLB). Two cross-linked preparations of CaLB, the cross-linked enzyme aggregate (CLEA) and the cross-linked enzyme crystal (ChiroCLEC™ CaB) were much less active, although comparable amounts of units were used,[†] with the enantioselectivity being low. In these hydrophilic particles, with a high density of active protein, the reaction is probably no longer kinetically controlled but limited by the rate of diffusion in and out of the particles. This could lead to lower conversion and enantioselectivity.

In the presence of CaLB, the (*S*)-enantiomer was preferentially converted, as predicted by the Kazlauskas rule.[15] Two preparations of *Burkholderia cepacia* lipase (Amano 1 and ChiroCLEC™ PC), in contrast, showed a slight preference for the (*R*)-enantiomer of the reactant. The activities of the other lipases tested were much lower and with the exception of *C. antarctica* lipase A (CaLA), the enantioselectivities were also poor.

In view of the above results, we selected Novozym 435 for our further investigations. We next compared the performance of the acyl donors vinyl acetate and succinic anhydride in the resolution of **2c**. These reactions were performed in a range of solvents because it is known that the outcome often critically depends on the nature of the solvent. We found (see Table 2) that the acylation with succinic anhydride took place with high enantioselectivity in *tert*-butyl methyl ether (TBME) or diisopropyl ether (DIPE) as the reaction medium. However when the reaction was performed in 1,2-dimethoxyethane (DME), it proceeded sluggishly and with low enantioselectivity.

Nitromethane and acetonitrile likewise proved to be unsuitable for this reaction. No adequate resolution of **2c** upon acylation with vinyl acetate could be obtained in any solvent.

2.2. Substrate specificity and enantiodiscrimination

A range of nitro alcohols, resulting from aldol condensation of aliphatic **1a–c** and aromatic aldehydes **1a–f** with nitromethane, was studied in the lipase-mediated resolution with succinic anhydride. The selectivity of Novozym 435 for these substrates followed the Kazlauskas rule[15] (see Fig. 2). This model intrinsically implies that the enantiodiscrimination of secondary alcohols by a lipase is dominated by steric interactions. The presence of a nitro group, which also interacts electrostatically, could affect the enantiodiscrimination.

Such effects would become visible when both enantiotopic groups are similar in size. We found, however, that the enantiomeric discrimination of **2b** (R = Et, see Fig. 3) was much lower that that of **2a** or **c** (R = Me or Pr, respectively, see Table 3).

From these results we can see that the enantiodiscrimination of the compound by CaLB is dominated by steric interactions and that electrostatic effects are at best minor. The longer alkyl **2c** and the phenyl alkyl substituted **2d** and **f** substrates all showed (*S*)-selectivity in accordance with the predictive model shown in Figure 2. Surprisingly, there was no reaction observed with 3-phenyl-1-nitro-2-propanol **2e** when succinic anhydride was used as the acyl donor. We have no plausible explanation for this observation.

[†] 1 unit will liberate 1 μmol of acetic acid per minute from triacetin.

Table 2. Resolution of 1-nitro-2-pentanol in the presence of CaLB; effect of the acyl donor and the solvent[a]

Solvent	Succinic anhydride				Vinyl acetate			
	Conversion (%)	Ee_s (%)	Ee_p (%)	E	Conversion (%)	Ee_s (%)	Ee_p (%)	E
$MeNO_2$	<1	0	1	—	3	1	45	3
ACN	8	2	100	2	Nd[b]	Nd	Nd	Nd
DME	4	3	76	7	37	38	52	5
TBME	42	70	95	100	46	41	35	3
DIPE	54	92	93	82	37	15	25	2

[a] Reaction conditions: 1 mL diisopropyl ether, 100 mM 1-nitro-2-pentanol, 100 mM acyl donor, 25 mg Novozym 435.
[b] Not determined.

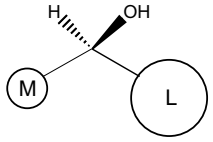

Figure 2. Steric model for the preferentially converted enantiomer of secondary alcohols by a lipase.

During the resolution of the aromatic nitro alcohols, some spontaneous elimination of carboxylic acid into the corresponding nitroalkene was observed. Elimination of acetic acid occurred more readily than that of succinic acid. No detectable alkene formation from the aliphatic nitroalcohols and their esters was observed under the reaction conditions.

Overall, much higher enantioselectivities were found in the resolution of 1-nitro-2-alkanols when succinic anhydride was used as the donor when compared with vinyl acetate.

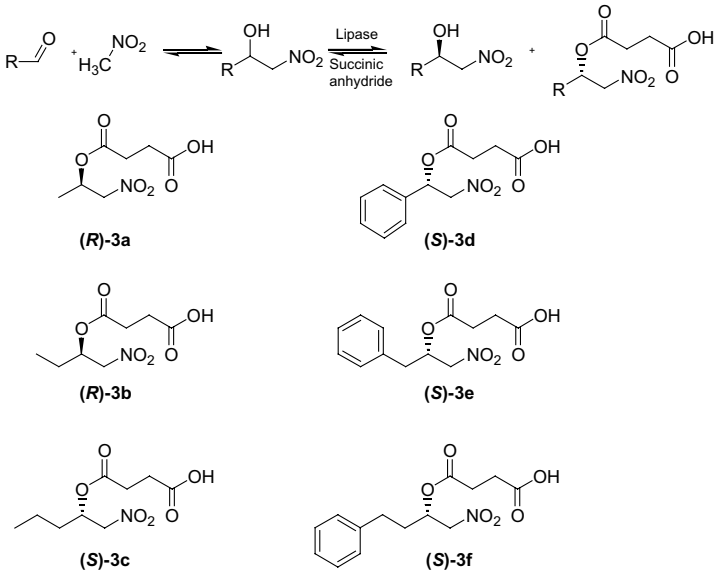

Figure 3. Preferentially formed enantiomers by Novozym 435 of 1-nitro-2-alkanols.

Table 3. Resolution of 1-nitro-2-alkanols with succinic anhydride and vinyl acetate in diisopropyl ether[a]

R	Succinic anhydride				Vinyl acetate			
	Conversion (%, 24 h)	Ee_s (%)	Ee_p (%)	E	Conversion (%, 24 h)	Ee_s (%)	Ee_p (%)	E
CH_3	39[b]	57	67	28 (*R*)	30	2	5	1 (*R*)
C_2H_5	47[b]	44	43	4 (*R*)	72	1	Nd[c]	1[d] (*R*)
C_3H_7	54	92	93	82 (*S*)	37	15	25	2 (*S*)
C_6H_5	4[e]	3	75	7 (*S*)	13[e]	13	100	20[d] (*S*)
$(C_6H_5)CH_2$	Nr	—	—	—	53[e]	34	Nd[c]	2[d] (*S*)
$(C_6H_5)C_2H_4$	42	71	97	96 (*S*)	56[e]	21	100	2[d] (*S*)

[a] Reaction conditions: 1 mL diisopropyl ether, 100 mM substrate, 100 mM succinic anhydride, 25 mg Novozym 435.
[b] Reaction finish after 5 h.
[c] Not determined.
[d] Calculated only with ee_s.
[e] Substrate and product degradation to the corresponding alkene.

3. Conclusions

Succinic anhydride, besides having potential benefits for reaction work-up, was shown to be an efficient acyl donor for the resolutions of β-nitro alcohols. Much higher *E* values can be achieved compared to other common acyl donors such as vinyl acetate. The best results, with regard to both rate and enantioselectivity, were observed in *tert*-butyl methyl ether and diisopropyl ether.

4. Experimental

4.1. Instruments

HPLC analyses were performed on a Chiralcel OD column with a flow rate of 0.6 mL/min and an eluent consisting of hexane–isopropyl alcohol–trifluoroacetic acid (95:5:0.1) for the aliphatic nitro alkanols and hexane–isopropyl alcohol–trifluoroacetic acid (80:20:0.1) for the aromatic nitro alkanols. Detection was performed with a Waters 486 UV detector at 215 nm.

4.2. Materials

All chemicals were of analytical purity and obtained from Sigma–Aldrich. The lipase from *Candida rugosa* was obtained from Sigma–Aldrich. *Candida lypolitica* lipase was bought from Fluka. Novozym 435 (*C. antarctica* lipase B on Lewatit E), SP524 (*Rhizomucor miehei* lipase), SP526 (*C. antarctica* lipase A) were kindly donated by Novozymes. Cross-linked enzyme crystals (CLECs) were donated by Altus Biologics; CaLB CLEA was donated by CLEA Technologies. The 1-nitro-2-alkanols were synthesised as described below.

4.3. General synthesis of aliphatic nitro alcohols 2a–c

Aldehydes **1a**–**c** (100 mmol) and KOH (1 mL, 1 M solution) were added to nitromethane (10 mL) at 0–5 °C. After 1 h, the mixture was brought to room temperature and left for another 2–4 h. The reactions were followed by TLC using ether–petroleum ether 3:2 as the eluent. Dichloromethane was then added and this mixture washed successively with 5% aqueous HCl and saturated aqueous $NaHCO_3$. The organic phase was dried over $MgSO_4$ and the solvent then evaporated. The crude product was purified by distillation, giving a colourless liquid.

4.3.1. 1-Nitro-2-propanol 2a. Isolated yield 46%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.3 (3H, d), 3.2 (1H, s), 4.4 (2H, m), 4.5 (1H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 19.8, 65.0, 81.6.

4.3.2. 1-Nitro-2-butanol 2b. Isolated yield 48%. ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 5.6, 26.9, 70.1, 80.5.

4.3.3. 1-Nitro-2-pentanol 2c. The reaction was carried out on 1 M scale. Isolated yield 69%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.0 (3H, t), 1.6 (2H, m), 3.0 (1H, s), 4.3 (1H, m), 4.5 (2H, m).

4.4. General synthesis of aromatic nitro alcohols 2d–f

Aldehydes **1d**–**f** (100 mmol) and KOH (1 mL, 1 M solution) were added to nitromethane (20 mL) at 0–5 °C. After 1 h, the mixture was brought to room temperature and then left to stir for another 2–4 h. The reactions were followed by TLC using ether–petroleum ether 1:1 as the eluent. The pH was adjusted to 7 and the excess of nitromethane evaporated. Ether was added and washed successively with acidic water, saturated $NaHCO_3$ solution and water. The organic phase was dried over $MgSO_4$ and the solvent evaporated to yield the crude product.

4.4.1. 2-Phenyl-1-nitro-2-ethanol 2d. The crude product was purified by distillation giving a yellow liquid. Isolated yield 57%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 3.2 (1H, s), 4.5 (2H, m), 5.4 (1H, q), 7.3 (5H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 70.9, 81.1, 125.9, 128.9, 129, 138.2.

4.4.2. 3-Phenyl-1-nitro-2-propanol 2e. The crude product was purified by distillation giving a yellow liquid. Isolated yield 23%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 2.8 (2H, m), 4.4 (2H, m), 4.5 (1H, q), 7.3 (5H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 40.4, 69.5, 79.7, 127.3, 128.9, 129.4, 135.9.

4.4.3. 4-Phenyl-1-nitro-2-butanol 2f. The product was recrystallised from diisopropyl ether resulting in a white solid. Isolated yield 56%. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.8 (2H, m), 2.6 (1H, d), 2.85 (2H, m), 4.35 (1H, m), 4.45 (2H, m), 7.3 (5H, m). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 31.3, 35.1, 67.7, 80.5, 126.3, 128.4, 128.6, 140.6.

4.5. Lipase catalysed resolutions

The lipase catalysed acylation reactions were performed in solvent (1 mL) at room temperature, with 100 mM nitro alcohol, an equivalent amount of acyl donor and 25 mg of the various enzyme preparations. When CLECs or CLEAs were used, 5 mg of enzyme was added instead. Except for the enzyme screening, all reactions were carried out with Novozym 435. Trimethoxybenzene (2.5 g/L) was used as the internal standard. The reactions were monitored by chiral HPLC.

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