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Direct Asymmetric Dynamic Kinetic Resolution by Combined Lipase Catalysis and Nitroaldol (Henry) Reaction

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Abstract: The asymmetric synthesis of β-nitroalkanol derivatives was simply achieved by a combined nitroaldol (Henry) reaction with lipase-catalyzed transesterification in high yield and enantiomeric purity (up to 92% and 99% *ee*) through a direct one-pot procedure.

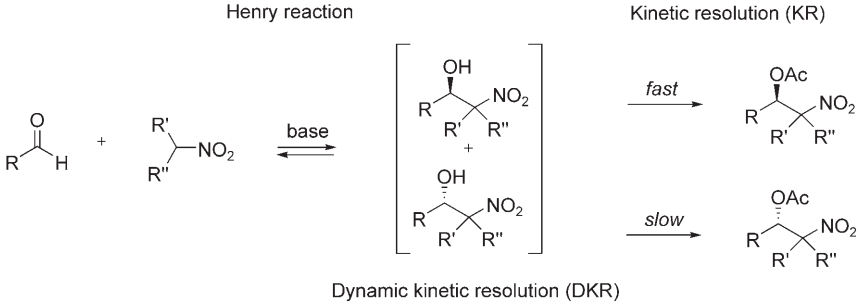
Keywords: dynamic kinetic resolution; enzyme catalysis; nitroaldol (Henry) reaction; secondary alcohols

Introduction

The nitroaldol, or Henry reaction, between a carbonyl group and a nitroalkane, constitutes a powerful method for C–C bond formation in organic chemistry.[1] Moreover, the diversity of further transformations of the nitroaldol adduct, for example oxidation, reduction and reductive denitration, provide efficient access to valuable functionalized structural motifs such as 1,2-amino alcohols common in chemical and biological expressions.[1,2] In recent years, control of the enantioselectivity of this reaction has attracted increasing interest. Most commonly, chiral metal complexes and other additives are used to obtain nitro alcohols with good to excellent optical purities.[3]

Nevertheless, the most common way in industry to achieve enantiomerically pure compounds is still by biotransformation processes due to economic efficiency as well as environmental impact.[4] For the last two decades, kinetic resolution (KR) by lipase catalysis of racemic secondary alcohol has proven to be a useful method for the synthesis of chiral molecules with high optical purity.[5] This approach, however, suffers from the limitation of attaining a reaction yield of maximally 50%. Fortunately, dynamic kinetic resolution (DKR), that is, the combination of racemization of the undesired enantiomer with *in situ* kinetic resolution (KR), is a powerful technique to address this problem.[6]

Recently, we developed a method for self-screening of dynamic combinatorial nitroaldol libraries through kinetically controlled lipase-mediated transesterification.[7] To explore the scope of the reaction, we herein report a novel asymmetric synthesis of β-nitroalkanol derivatives in good yield and high enantiomeric excess *via* a direct one-pot reaction combining a nitroaldol (Henry) reaction and kinetic resolution (KR), lipase-catalyzed transesterification, of the corresponding adduct at mild conditions (Scheme 1).



Scheme 1. General concept of one-pot reaction.

Results and Discussion

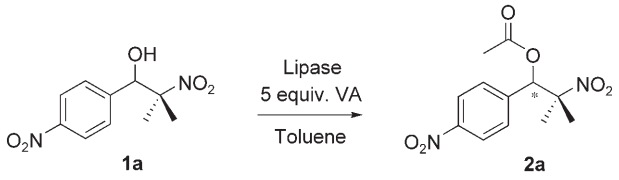
The nitroaldol reaction is thermodynamically controlled. It is very useful for most aldehydes but requires control of the equilibrium displacement to obtain useful yields. In the present study this is, however, used as an advantage and the equilibrium reaction is used as the racemization step of the undesired enantiomer (Scheme 1). Moreover, this reaction can be performed by many catalysts, for example, organic bases, inorganic bases, quaternary ammonium salts or using only ionic liquids as a solvent.[2,8] Therefore, several bases were initially screened to find optimized conditions for the nitroaldol reaction. Principally, the equilibration process should be rapid and stable and should not interact with the enzymatic reaction. In preliminary studies, the reactions were conducted in the presence of one equivalent each of nitroalkane, aldehyde and base, respectively. ^{1}H NMR spectroscopy was used to follow the reactions by comparing the signals of the aldehyde, 4-nitrobenzaldehyde (**4a**), and the nitro alcohol adduct (**1a**). Of the various bases initially tested, the majority was either showing low reactivity or was attacking the acyl donor. Triethylamine was finally chosen as catalyst for the reactions, yielding equilibration times of around three hours at room temperature.

In the kinetic resolution (KR) process, achieved by lipase-catalyzed transesterification, most enzymes used recognize broad ranges of substrates.[9] A size difference of the substituents at the secondary alcohol position of the substrates is, however, needed to obtain high enantiomeric excess.[9,10] In the present case, the size occupancies around the stereogenic position of the nitro alcohol adduct are similar to each other. Initially, a series of enzymes were screened using vinyl acetate (VA), commonly used for transesterification, as an acyl donor, and pure racemic 2-methyl-2-nitro-1-(4-nitrophenyl) propan-1-ol (**1a)** as an alcohol substrate. The reactions were followed for 24 h in dry toluene as a suitable solvent under argon atmosphere (Table 1).

From the enzymes tested, lipases from *Pseudomonas cepacia* (PS, PS-C I and PS-C II) and *Pseudomonas fluorescens* (PF) showed transesterification activity with high enantiosepecificity. In contrast, enzymes from different *Candida* species; *Candida antarctica* lipase B (Novozyme 435), and *Candida rugosa* (CRL), showed low or no reactivity with this substrate (entries 1 and 2, Table 1). However, the conversion yields using lipases from *Pseudomonas* sp. were relatively low, even though the amount of enzyme added was above the commonly used ratio (2.5 mg enzyme: 0.05 mmol of substrate).[9] A possible explanation for this effect is that the secondary alcohol position of the substrate is sterically congested with either two methyl groups or phenyl groups. Thus, the kinetic resolution process supposedly proceeds at a lower rate due to lower accessibility to the enzyme pocket and/or recognition of the right enantiomer. However, these experiments revealed high enantiospecificity of the transesterification product (over 90% *ee*, entries 4–6, Table 1), except for one biocatalyst, the non-immobilized *Pseudomonas cepacia* (PS), which exhibited no selectivity (entry 3, Table 1). Furthermore, the reaction conditions were optimized by initially increasing the amount of enzyme, up to 30 mg for 0.05 mmol of substrate. Only *Pseudomonas cepacia* lipase (PS-C I) exhibited a satisfactory enhancement of reactivity, 29% yield in 24 h and 99% *ee* (entry 7, Table 1). Attempts to change the reaction solvents by using TBME, DIPE, cyclohexane and hexane, all of which are compatible with the enzymes, did not lead to significant improvement. Increasing the temperature to 40 °C, resulted in almost complete kinetic resolution in 24 h (46% yield, entry 8, Table 1). Temperatures above 40 °C, however, resulted in decreasing enantiospecificity and also slow decomposition of the alcohol substrate.

After optimization of the kinetic resolution, we subsequently addressed the DKR process using PS-C I as enzyme and vinyl acetate as acylating agent. Due to the somewhat sluggish enzymatic reaction, the equilibrium of the nitroaldol reaction was forced to the product side to increase the concentration of the

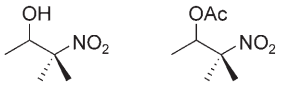
Table 1. Kinetic resolution of **1a** using various enzymes.[a]



Entry	Enzyme	Conversion [%][b]	*ee* [%][c]	E[d]
1	CALB	5	78	8
2	CRL[e]	0	0	0
3	PS	10	0	1
4	PS-C I	11	99	>200
5	PS-C II	10	90	21
6	PF	7	93	30
7[f]	PS-C I	29	99	>200
8[g]	PS-C I	46	99	>200

[a] Reactions were carried out with 0.05 mmol (12 mg) of *rac*-**1a** with 10 mg of enzyme and 5 equiv. of vinyl acetate (VA) in 0.3 mL of toluene at room temperature under an argon atmosphere for 24 h.
[b] Determined by ^{1}H NMR spectroscopy.
[c] Determined by HPLC analysis using OD column.
[d] Enantiomeric ratio
[e] Two different preparations were used with the same result: Sigma L1754/Fluka 62316.
[f] 30 mg of enzyme was used.
[g] 30 mg enzyme, the reaction was run at 40 °C for 24 h.

enzyme-substrate complex, the lipase-acyl donor-nitro alcohol intermediate. Thus, an excess of nitro compound was used together with the same amount of enzyme as in the KR process. In the first attempt, the reaction was examined with four equivalents of nitroalkane, two equivalents of base, one equivalent of 4-nitrobenzaldehyde, 90 mg of PS-C I and five equivalents of vinyl acetate in toluene at 40 °C for 2 days. Compound **2a** was isolated in 65% yield and 85% *ee* together with compounds **5** and **6** in high amounts. Compound **5** was obtained from the coupling between acetaldehyde, the transesterification by-product, and nitroalkane. Compound **5** then competed with compound **1a** in the enzymatic process yielding compound **6**.



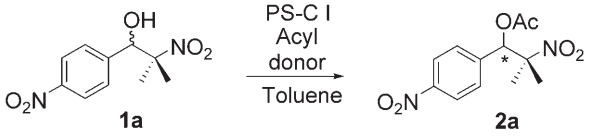
5 **6**

To address this problem, the effects from different acyl donors were subsequently screened. In these experiments, PS-C I lipase and racemic 2-methyl-2-nitro-1-(4-nitrophenyl) propan-1-ol (**1a**) were selected as biocatalyst and alcohol substrate, respectively (Table 2). As expected, the results showed low conversions in the KR process when isopropyl acetate and isopropenyl acetate were used as acyl donors (entries 1 and 2, Table 2). Better conversion was observed when ethoxyvinyl acetate was used. Unfortunately, this acylating agent cannot be use in the DKR process because of nitroalkane attack to the acyl position of ethoxyvinyl acetate yielding 3-methyl-3-nitrobutan-2-one and ethyl acetate. However, *p*-chlorophenyl acetate revealed satisfactory results similar to vinyl acetate (entry 4, Table 2). According to experience in the literature,[10] increasing the concentration of the enzyme-substrate complex using the acyl donor as solvent can lead to improved results. Surprisingly, this approach revealed no enhancement of the yield in the case of vinyl acetate, and no reaction for *p*-chlorophenyl acetate (entries 5 and 6, Table 2). Therefore, five equivalents of *p*-chlorophenyl acetate were used instead, even though this donor revealed slower conversion compared to vinyl acetate. No side reactions were observed in this case.

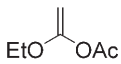
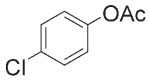
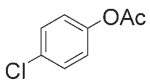
Finally, the one-pot DKR synthesis was carried out using an excess amount of 2-nitropropane together with *p*-nitrobenzaldehyde, in the presence of PS-C I and *p*-chlorophenyl acetate in toluene at 40 °C for 2 days without stirring. Compound **2a** was in this case isolated in 92% yield and 99% *ee* (entry 1, Table 3). The biocatalyst, *Pseudomonas cepacia* (PS-C I), was easily recovered by filtration, washed with organic solvent and dried under vacuum without loss of activity. This experiment demonstrated that the method is an alternative method for synthesizing nitroalkanol derivatives *via* a one-pot reaction in high yield and with high enantiospecificity. To our knowledge, DKR of nitro alcohol adducts has not been reported but the KR of similar substrates is known.[11] Therefore, this new one-pot synthesis was applied to a range of different aldehyde substrates (Table 3).

p-Cyanobenzaldehyde (**4b**) was used under the same conditions as for *p*-nitrobenzaldehyde but the results showed slightly lower enantiospecificity (Table 3, entry 2). In the case of benzaldehydes **4c–f**, five equivalents of 2-nitropropane were used. The results also showed high yields as well as enantiospecificities (entries 3–5, Table 3) except for 3,5-bis(trifluoromethyl)benzaldehyde (**4f**) where the enantiospecificity decreased to 80% *ee* (entry 6, Table 3). This effect may be due to the presence of two groups

Table 2. Kinetic resolution of **1a** using various acyl donors.[a]

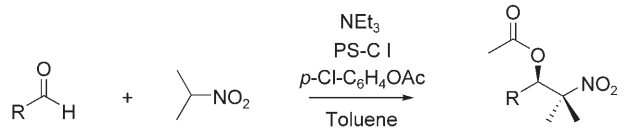


1a **2a**

Entry	Acyl donor	Conversion [%][b]	*ee* [%][c]	*E*[d]
1		10	98	110
2		7	95	42
3		24	98	134
4		40	99	>200
5		46	99	>200
6[e]		36	99	>200
7[f]		0	0	0

[a] Reactions were carried out with 0.05 mmol (12 mg) of *rac*-**1a** with 30 mg of enzyme and 5 equiv. of acyl donor in 0.3 mL of toluene at 40 °C under an argon atmosphere for 24 h.
[b] Determined by ^{1}H NMR spectroscopy.
[c] Determined by HPLC analysis using OD column.
[d] Enantiomeric ratio
[e] Vinyl acetate was used as a solvent.
[f] *p*-Chlorophenyl acetate was used as a solvent.

Table 3. One-pot combined nitroaldol (Henry) reaction and lipase-catalyzed transesterification.[a]



4a – m **3** **2a – m**

Entry	R	Product	Time [d]	Yield [%][b]	*ee* [%][c]
1	4-O_2N-C_6H_4	**2a**	2	90	99
2	4-NC-C_6H_4	**2b**	2	89	91
3[d]	4-F_3C-C_6H_4	**2c**	3	89	97
4[d]	3-O_2N-C_6H_4	**2d**	3	90	91
5[d]	3-NC-C_6H_4	**2e**	3	92	97
6[d]	3,5-bis(F_3C)-C_6H_3	**2f**	3	80	80
7[e]	4-F-C_6H_4	**2g**	4	85	98
8[e]	4-Cl-C_6H_4	**2h**	4	83	97
9[e]	4-Br-C_6H_4	**2i**	4	81	96
10[f]	C_6H_5	**2j**	4	79	91
11[f]	4-CH_3-C_6H_4	**2k**	4	35	93
12[f]	4-CH_3O-C_6H_4	**2l**	4	28	99
13[f]	2-Thiophene	**2m**	4	68	46

[a] *Reaction conditions:* 0.125 mmol benzaldehyde, 0.5 mmol **3**, 0.25 mmol TEA, 106 mg *p*-chlorophenyl acetate, 90 mg PS-CI, 0.25 mL toluene, 40 °C.
[b] Isolated yield.
[c] Determined by HPLC analysis using OD and OD-H column.
[d,e] 5 and 10 equivalents of 2-nitropropane and 2.5 and 5 equivalents of TEA were used, respectively.
[f] *Reaction conditions:* 0.125 mmol benzaldehyde, 1.25 mmol **3**, 0.625 mmol TEA, 106 mg *p*-chlorophenyl acetate, 120 mg PS-CI, 0.1 mL toluene, 40 °C.

of similar size next to the stereogenic center. To achieve a reasonable equilibration rate for less electron-withdrawing benzaldehydes, **4g–i**, the amount of nitropropane was increased up to ten equivalents. After a reaction time of 4 days, compounds **2g–i** were obtained in good yields and high enantiospecificities (entries 7–9, Table 3). Similar conditions as for benzaldehydes **4g–i** were also applied in the cases of unsubstituted benzaldehyde **4j**, benzaldehydes **4k–l** with electron-donating substituents, and heteroaromatic benzaldehyde **4m**. In 4 days reaction time, compound **2j** was obtained in high yield and high enantiospecificity (entry 10, Table 3). In contrast, benzaldehydes **4k** and **4l** resulted in rather low yields, as expected from the lower nitroaldol reaction rates, but retained high enantiospecificities (entries 11 and 12, Table 3). The heteroaromatic 2-thiophenecarboxzaldehyde (**4l**) resulted in a higher yield but considerably lower enantiospecificity of the β-nitroalkanol product within the same reaction time (entry 13, Table 3). In addition, several aliphatic aldehydes (butyraldehyde, isovalerylaldehyde, and octylaldehyde) were examined, however, all resulting in unsatisfactory enantiospecificities (*cf.* Supporting Information) Thus, diminishing the aldehyde ring size, or the use of aliphatic aldehydes, leads to decreasing enantiospecificities. On the other hand, this one-pot procedure results in high stereospecificities for all benzaldehydes tested, the reaction yields depending on the different substituents. The absolute configuration of the stereogenic position was assigned by a modified Mosher's method.[12] Compound **1a** (99% *ee*), left from the kinetic resolution by enzyme-lipase PS-CI, was subsequently treated with (−) and (+)-MTPACl in pyridine followed by purification gave the corresponding ester **7a** and **7a′**. Based on the $\Delta\delta$ [$\delta(-)-\delta(+)$] value, the (*R*)-configuration was established for compound **2a**. According to this experiment, we propose the (*R*)-configuration for compounds **2b–i**.

Conclusions

In summary, we have demonstrated for the first time that the asymmetric synthesis of β-nitroalkanol derivatives can be simply and efficiently achieved by a combined lipase-catalyzed transesterification and nitroaldol (Henry) reaction in a direct one-pot reaction. Simple tuning of the reaction conditions allows for the isolation of the desired products in good to excellent yield, together with high stereospecificity, in reasonable reaction times. Furthermore, easy handling of the chemicals employed and also recovery of biocatalysts makes this system suitable for upscaling.

Experimental Section

General Procedure for Dynamic Kinetic Resolution

In a typical experiment, a reaction mixture of 4-nitrobenzaldehyde (**4a**) (19 mg, 0.125 mmol), 2-nitropropane (**3**) (44 mg, 0.5 mmol), triethylamine (25 mg, 0.25 mmol), and 5 equivalents of *p*-chlorophenyl acetate (106 mg) in dry toluene (0.25 mL) were added to a sealed-cap vial (1.75 mL) containing *Pseudomonas cepacia* (PS-CI, immobilized on ceramic, Sigma–Aldrich, EC 3.1.1.3, 90 mg) together with ground molecular sieve 4 Å (20 mg). The reaction mixture was flushed with argon and stirred at 40 °C. After 2 days the mixture was cooled to room temperature, filtered and washed with CH_2Cl_2 (3×5 mL). The solvent was removed under vacuum and the crude product was purified by column chromatography [hexane:CH_2Cl_2 (1:4) to pure CH_2Cl_2] affording **2a** as a white amorphous; yield: 32 mg (90%); 99% *ee*.

Supporting Information

General synthetic methods and characterization data are available as Supporting Information.

Acknowledgements

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