# Switching from (*R*)- to (*S*)-selective chemoenzymatic DKR of amines involving sulfanyl radical-mediated racemization†

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Chemoenzymatic dynamic kinetic resolution (DKR) of amines involving sulfanyl radical-induced racemization happened to be the very first switchable DKR process allowing the synthesis of either (*R*)- or (*S*)-amides, in good yield and high enantiomeric excess, depending on the nature of the enzyme; the different steps of the development of (*S*)-selective DKR are discussed.

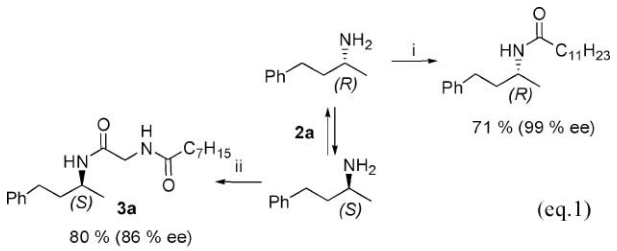
## Introduction

Owing to the prime importance of optically active amines in the synthesis of natural products and bioactive compounds for both academics and industrial chemists,[1,2] amines dynamic kinetic resolution (DKR) that allows the synthesis of optically pure amides from racemic amines has stimulated numerous strategies.[3,4] The chemoenzymatic process involving sulfanyl radical induced racemization of aliphatic amines is currently investigated in our group.[5,6] Until now, the DKR of amines has specifically been achieved with the thermostable polymer supported lipase B from *Candida antartica* (Novozyme 435).[4,6b] These processes lead to (*R*)-amides. Proteases have a catalytic triad that is nearly the mirror image of that of lipases[7] which formally enables to devise switchable chemoenzymatic DKR processes by changing the nature of the enzyme.

The compatibility between the amine resolution optimized with CAL-B and the sulfanyl radical promoted racemization enabled (*R*)-selective DKR to be achieved in good yield and high enantiomeric at 80 °C (Scheme 1).[6b] However, the route to (*S*)-selective DKR was not straightforward.

Most difficulties were inherent in protease characteristics. Proteases are far less selective and less stable than lipases in organic solvents.[7,8] The screening of various serine proteases led us to select the commercially available alkaline protease.[9,10] This subtilisin like protease, isolated from *Bacillus licheniformis*, was stabilized by coating.[8,11] The simultaneous screening of acyl donors led to the selection of an *N*-octanoylglycine derivative.[9]

Efficient racemization could be achieved at low temperature upon irradiation at 300 nm.[12] However, racemization was slowed down when it was carried out in KR solvent, *i.e.* 3-methyl-3-pentanol. A compromise was found by changing the nature of thiol and by adding a co-solvent.[13]




i) CAL-B, $C_{11}H_{23}CO_2Et$, AIBN, 80 °C, $MeCHSHCONEt_2$ (**1**). ii) a) Alkaline Protease, *n*-$C_7H_{15}CONHCH_2CO_2CH_2CF_3$, 3-Me-3-pentanol, b) $CF_3CH_2SH$, THF, hv (300 nm) c) Alkaline Protease, *n*-$C_7H_{15}CONHCH_2CO_2CH_2CF_3$

**Scheme 1** Preliminary results.

A serious drawback remained, that is, the low stability of alkaline protease under irradiation at 300 nm. An acceptable one-pot three-step procedure was devised that consisted in performing first a KR period in 3-methyl-3-pentanol, followed by addition of trifluoroethane thiol and THF while irradiating for 2 h, and after having stopped irradiation, a new cycle of enzymatic resolution was performed by adding fresh portions of enzyme and acyl donor (Scheme 1). These conditions allowed the preparation of (*S*)-amides in yields ranging from 58% to 80% with enantiomeric excesses ranging between 78% and 94%.[6c] However, the goal of a "real" DKR process was still not reached.

## Results and discussion

Fortunately, we found out that alkaline protease efficacy under irradiation was greatly improved when initiating the formation of sulfanyl radical at 350 nm in the presence of AIBN. Under these conditions, AIBN was photochemically decomposed and 2-cyanopropyl radicals initiated the S–H bond homolysis.

The most significant results of racemization tests performed under these new conditions on amine **2a** are reported in Table 1. The reproducibility of these racemizations was greatly improved in the presence of an aprotic co-solvent. Meanwhile, the use of *t*-butanol was reinvestigated, which was justified by the significant improvement of the enzyme enantioselectivity in the KR experiments that were performed simultaneously (Table 2).

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**Table 1** Racemization of amine **2a** upon irradiation for 2.5 h at 350 nm in the presence of AIBN (24–30 °C)

| Solvent[a] | **2a** ee (%) |
|---|---|
| 3-Me-3-pentanol | 38–76 |
| *t*-BuOH | 53–92 |
| *t*-BuOH/Toluene (2/1) | 22–28 |
| *t*-BuOH/THF (5/1) | 50–57 |

[a] AIBN was added in 3 portions (20 or 30 mol% each) every 45 min to a 0.1 M solution of **2a**.

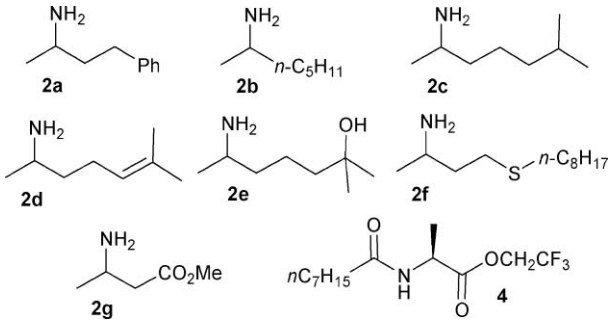



**Fig. 1** Structures of amines **2a–g** and acyl donor **4**.

The substantial increase in enantioselectivity gained by selecting *N*-acylalanine trifluoroethyl esters as acyl donors[10] led us to check if these donors were racemized by the same process as the amines, using trifluoroethane thiol. Indeed no racemization of *N*-octanoyl alanine trifluoroethyl ester (**4**) was detected.[14] According to G3B3-MP2 calculations, going from ethyl to trifluoroethyl esters lowers the C–H BDE at the captodative position by 5.7 kJ mol$^{-1}$ for the *N*-acetylglycine esters (354.5/348.8 kJ mol$^{-1}$), and by 8.9 kJ mol$^{-1}$ for the *N*-acetylalanine esters (355.2/346.3 kJ mol$^{-1}$). Going from *N*-acetylalanine trifluoroethyl ester to the corresponding *N*-*i*-propyl amide increases the C–H BDE by 14.6 kJ mol$^{-1}$. The variations in the series are significant. They suggest that the alanine derivatives are not poorer hydrogen atom donors than the glycine derivatives. In addition, in the case of alanine derived acyl donor (**4**) the *N*-*i*-propyl amide is even more difficult to racemize than the ester. This might explain why no trace of (*S*,*R*)-diastereomeric amide **5a** was detected in the reaction product.[15]

KR resolution with this acyl donor was reinvestigated in different solvent mixtures as reported in Table 2. This allowed us to define which mixtures would lead to both acceptable enantioselectivities and rates of reaction with amines **2a–g** (Fig. 1).

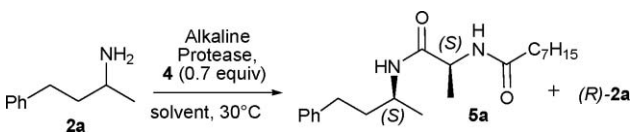
It must be underlined that *t*-butanol, that was once discarded as compared to 3-methyl-3-pentanol (for the sake of improving the rate of racemization when irradiating at 300 nm[6c]), happened to be acceptable for racemizations performed under irradiation at 350 nm. In addition, the results show that methyl-β-cyclodextrin could be as efficient or even superior to Brij® 56 when associated to α,β-D-glucopyranoside as coating agent. Good results were obtained for KR performed in the presence of the enzyme coated with α,β-D-glucopyranoside and methyl-β-cyclodextrin. A significant increase in *E* factor was registered when replacing 3-methyl-3-pentanol by *t*-butanol under these conditions (entries 1 to 3).

The use of a co-solvent appeared as a good compromise to run concomitantly, an efficient KR and an efficient racemization procedure.

Therefore, the chiral acyl donor **4** was tested in the DKR[17] process. These tests were first achieved on amine **2a**. The most significant results are given in Table 3. High ees were observed in entries 2–6. Although differences are tiny (all the combinations mentioned below are nearly equivalent) the highest yield in amide **5a** was recorded in entry 6, that is in 5/1 mixture of *t*-BuOH and THF.

All conditions were gathered to generalize the (*S*)-selective real DKR process to the series of aliphatic amines in Fig. 1. The results are reported in Table 4. The main limitation of the methodology

**Table 2** Alkaline protease-catalyzed KR of amines **2a–g** using **4** as the acyl donor

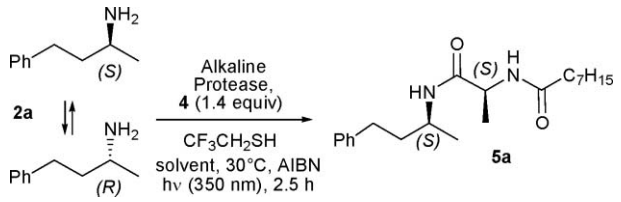



| | Amine | Solvent | t/h | **2** ee (yield)[c] | **5** de (yield) | *C*%[d] | *E*[e] |
|---|---|---|---|---|---|---|---|
| 1 | **2a** | 3-Me-3-pentanol[a] | 1.5 | 86.5 (–) | 94.3 (–) | 47.8 | 99 |
| 2 | **2a** | *t*-BuOH[a] | 2 | >99.5 (–) | 87 (–) | 53.3 | 86 |
| 3 | **2a** | *t*-BuOH[b] | 1 | 99 (48) | 93 (50) | 51.5 | 150 |
| 4 | **2b** | *t*-BuOH[b] | 1 | 97 (46) | 92 (45) | 51.3 | 101 |
| 5 | **2c** | *t*-BuOH[b] | 1.5 | 98 (44) | 98 (47) | 52.6 | 73 |
| 6 | **2d** | *t*-BuOH[b] | 1 | 98 (47) | 90 (52) | 52.1 | 88 |
| 7 | **2e** | *t*-BuOH[b] | 1.5 | 98 (40) | 80 (52) | 55.1 | 40 |
| 8 | **2f** | *t*-BuOH[b] | 1 | 92 (35) | 92 (50) | 50.0 | 79 |
| 9 | **2g** | *t*-BuOH[b] | 3 | 94 (28) | 82 (48) | 53.4 | 35 |
| 10 | **2a** | *t*-BuOH/toluene (2/1)[a] | 0.75 | 98 (–) | 96 (–) | 50.5 | 229 |
| 11 | **2a** | *t*-BuOH/toluene (5/1)[a] | 2 | >99.5 (–) | 90 (–) | 52.5 | 112 |
| 12 | **2a** | *t*-BuOH/THF (5/1)[b] | 1 | 94 (48) | 93 (51) | 50.2 | 101 |

[a] Enzyme coated with Brij® 56 and α,β-D-glucopyranoside (8/1/1, w/w/w). [b] Enzyme coated with α,β-D-glucopyranoside and Me-β-cyclodextrin (8/1/1, w/w/w). [c] Isolated after derivatization as a Boc-carbamate. [d] Calculated according to $C = ee_{amine}/(ee_{amine} + ee_{amide})$. [e] Enantioselectivity factors were calculated according to $E = \ln[(1 - C)(1 - ee_{amine})]/\ln[(1 - C)(1+ee_{amine})]$.[16]

**Table 3** Improvement of (*S*)-selective DKR of amine **2a**




| | Solvent | **5a** de (%) | Yield (%) |
|---|---|---|---|
| 1 | 3-Me-3-pentanol[a] | 70 | 81 |
| 2 | *t*-BuOH[a] | 94 | 68 |
| 3 | *t*-BuOH[b] | 94 | 70 |
| 4 | *t*-BuOH/Toluene[b] (2/1) | 94 | 72 |
| 5 | *t*-BuOH/Toluene[b] (5/1) | 94 | 76 |
| 6 | *t*-BuOH/THF [b] (5/1) | 93 | 78 |

[a] Enzyme coated with Brij® 56 and α,β-D-glucopyranoside (8/1/1, w/w/w). [b] Enzyme coated with α,β-D-glucopyranoside and Me-β-cyclodextrin (8/1/1, w/w/w).

**Table 4** (*S*)-Selective DKR of amines **2b–g**[a]

| Amine | Amide **5** de (%) | Yield (%) |
|---|---|---|
| **2b** | 92 | 68 |
| **2c** | 88 | 73 |
| **2d** | 90 | 66 |
| **2e** | 78 | 72 |
| **2f** | 88 | 68 |
| **2g** | 73 | 65 |

[a] Experimental conditions identical to Table 3 entry 6

is inherent in the rate of the KR that must not be too slow as compared to the rate of the radical racemization in order to limit competitive radical degradation processes. The DKR of amines for which the resolution process is too slow could not be achieved.[18]

## Conclusions

Reversible hydrogen atom abstraction from the stereogenic center adjacent to nitrogen atom by sulfanyl radical happened to be the very first racemization procedure allowing to achieve either (*R*)- or (*S*)-selective chemoenzymatic DKR of chiral aliphatic amines. Moreover, it must be emphasized that no metal was involved in the overall process. If (*R*)-selective DKR mediated with CAL-B lipase was rather easily devised, the achievement of (*S*)-selective DKR using alkaline protease was a real challenge. The stability of alkaline protease required racemization to be induced at room temperature photochemically(350 nm). This led us to change parameters such as the solvent (*t*-butanol/THF mixture) and the acyl donor (peptide mimetic **4**). Under these new experimental conditions, the synthesis of (*S*)-amides was achieved in good yield and high enantiomeric excess, through the chemoenzymatic DKR of a series of structurally related amines bearing various functional groups. Further progress in view of making the process viable for industrial applications will be reported in due course.

## Experimental

### Immobilization of alkaline protease

In a 100 mL flask, 60 mg of octyl α,β-D-glycopyranoside and 60 mg of methyl-β-cyclodextrin were diluted at 60 °C in 50 mL of buffer (Phosphate 0.1M, pH 7), this solution was then cooled to RT. The alkaline protease liquid extract (500 mg) from Valley Research was added to 25 mL of the previous solution. The mixture was rapidly frozen in liquid $N_2$ and lyophilized for 20 h to give 822 mg of enzymatic powder.

### General procedure for the kinetic resolution of amines

To a solution of acyl donor (**4**) (104 mg, 0.35 mmol) in *t*-BuOH (5 mL) was added coated alkaline protease (25 mg) and amine (**2**) (0.5 mmol). The resulting mixture was stirred at room temperature for 60 min. The amine ee was determined by GC after derivatization of an aliquot of the crude mixture in trifluoroacetamide with 1.5 equiv of *N*-methyl-bis-trifluoroacetamide. The enzyme was filtered off from the solution and washed with dichloromethane (10 mL). Then $Boc_2O$ (66 mg, 0.3 mmol) was added to the filtrate, and the mixture was stirred until complete consumption of the amine (TLC monitoring). The solvent was then evaporated and the crude mixture was purified on gel-silica (pentane–diethyl ether 0 to 10% to afford the Boc-amine, then dichloromethane–MeOH 0 to 1% to afford the amide).

### General Procedure for the dynamic kinetic resolution of amines

To a solution of *N*-octanoyl-L-alanine trifluoroethyl ester (**4**) (125 mg, 0.42 mmol) in *t*-BuOH/THF:5/1 (3 mL, 0.1 M) was added coated alkaline protease (15 mg) and the amine (0.3 mmol). The mixture was irradiated at 24–28 °C in a quartz tube (diameter 1.5 cm) in a Rayonet apparatus (RPR-200, 16 UV lamps Sylvania Blacklight 351 F15W/T5/BL350) for 2 h 30 min and AIBN (45 mg, 0.25 mmol) was added by portion every 45 min (3 × 15 mg). The enzyme was filtered off from the solution and washed with dichloromethane (5 mL). The solvent was then evaporated and the crude material was purified by flash column chromatography on silica gel (dichloromethane–methanol 0 to 5%) to give pure amide.

## Acknowledgements

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15 It must be underlined however, that the S–H BDE in trifluoroethane thiol is stronger than all C–H BDEs mentioned in the text. The direct comparison of the calculated values for BDEs of different types might be hazardous. C–H BDE in **4** : 346.3 kJ mol$^{-1}$; C–H BDE in *N*-acetylalanine *N*-*i*-propyl amide : 360.9 kJ mol$^{-1}$; trifluoroethane thiol S–H BDE: 365.6 kJ mol$^{-1}$ (at 298 K according to G3B3MP2 method).

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