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En Route to (*S*)-Selective Chemoenzymatic Dynamic Kinetic Resolution of Aliphatic Amines. One-Pot KR/Racemization/KR Sequence Leading to (*S*)-Amides

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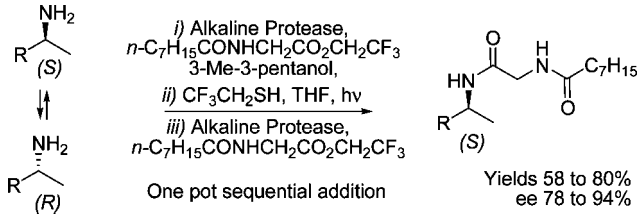
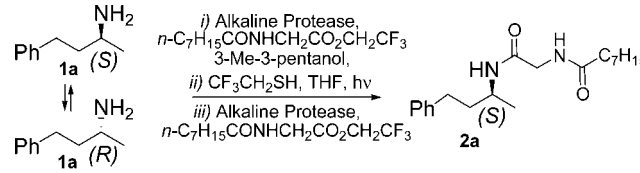


A one-pot sequential process, involving a radical racemization and an enzymatic resolution, provides access to (*S*)-amides, from racemic amines, with ee and yields ranging from 78 to 94% and 58 to 80%, respectively.

Dynamic kinetic resolution (DKR) of racemic amines enables their conversion into enantiomerically pure amides.[1] Our group[2] and others[3,4] have recently reported (*R*)-selective chemoenzymatic DKR of amines. In most cases, racemization was based on metal-catalyzed reversible dehydrogenation of the amine into the corresponding imine. Our approach was different since the racemization process relied on reversible hydrogen atom abstraction at the stereocenter (directly adjacent to the reactive amine moiety) by sulfanyl radical.[5,6] The known examples of (*S*)-selective chemoenzymatic DKRs involving hydrolases refer exclusively to alcohols.[7] We report herein chemoenzymatic conversion of aliphatic amines into the corresponding (*S*)-amides through a one-pot three-step sequence (Scheme 1).

SCHEME 1. One-Pot Sequential Procedure



Our aim was to devise a DKR process. To reach such an objective, several conditions had to be fulfilled. First of all, the racemization procedure had to be compatible with the kinetic resolution conditions. Besides the requirement of the highest possible enantioselectivity for the KR, it was also essential for the rate of racemization of the slow reacting amine enantiomer to be fast compared to its enzyme-mediated conversion into the corresponding amide.[8]

The synthesis of (*S*)-amides via kinetic resolution of a racemic mixture can be achieved with commercially available proteases.[9] The catalytic triad of the latter is the mirror image of that encountered in lipases.[10] We have shown recently that the use of carbamoyl glycine trifluoroacetates derived from octanoic acid, as acyl donors, enabled alkaline protease-catalyzed resolution of most aliphatic amines to proceed within 15 min to 6 h depending on the substrate, with good to high enantioselectivity.[11] This enzyme has less thermal stability than CAL-B. It was necessary to find out how to perform the radical racemization at a temperature that did not exceed 40 °C. This condition was fulfilled by generating sulfanyl radical via photolysis of

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TABLE 1. Racemization of (*S*)-1a with CF_3CH_2SH[a]

entry	solvent	time	ee % (yield %)[b]
1	THF	30 min	0 (70)[c]
2	toluene	30 min	0 (94)[c]
3	3-Me-3-pentanol	3−6 h	84−60 (nd)
4	THF/3-Me-3-pentanol (1:1)	1.5 h	14 (81)
5	THF/3-Me-3-pentanol (1:2)	1.5 h	14 (84)
6	*n*-C_6H_{14}/3-Me-3-pentanol (1:1)	1.5 h	25 (nd)
7	toluene/3-Me-3-pentanol (1:1)	1 h	18 (95)
8	toluene/3-Me-3-pentanol (2:1)	40 min	6 (79)

[a] Three milliliters of a 0.1 M solution of amine (0.3 mmol) was irradiated at 22−24 °C in the presence of 1.2 equiv of trifluoroethanethiol, in a quartz vessel, in a Rayonet apparatus.[6] [b] Isolated after derivatization as BOC-derivative unless otherwise stated. [c] Determined by ^{1}H NMR using pentamethylbenzene as internal standard after irradiating for 3 h, notwithstanding that racemization was completed in 30 min. This confirmed that no degradation interfered during the racemization process.

the corresponding thiol. In the presence of thioglycolic methyl ester, the racemization process was shown to be fast and efficient in benzene solution under these experimental conditions; moreover, it proceeded with little or no degradation of the amine via oxidative side reactions.[6]

Having in hand two separate performant processes, we had to test their compatibility. The kinetic resolution was not altered in the presence of thiol. However, the best solvent for KR (i.e., 3-methyl-3-pentanol) was a poor solvent regarding the photochemically induced racemization (slowed down with respect to toluene or THF). Moreover, thioglycolic derivatives were proven to be inefficient regarding racemization in the presence of the tertiary alcohol. This led us to use trifluoroethane thiol (Table 1), the S−H bond of which is slightly stronger than that of methylthioglycolate (or the corresponding amide) that was used in our previous studies.[2,6,12]

Although tertiary alcohols are not the best hydrogen bond donors among all alcohols, they might contribute to strengthen the α-C−H bond dissociation enthalpy of the solvated amine (Table 1, entry 3).[13]

Therefore, both the racemization process (Table 1) and the alkaline protease-mediated KR (Table 2) had to be reinvestigated in solvent mixtures involving the tertiary alcohol and hexane, toluene, or THF.

As exemplified in Table 1, racemization was quite efficient in solvent mixtures. It took less than 2 h in most of them (entries 4−8). For instance, monitoring the reaction showed that 30 min was needed to reach 14% ee in a 1:1 mixture of THF and 3-methyl-3-pentanol, whereas 60 min was needed to reach the same ee in a 1:2 mixture of the same solvents.

The mixtures of solvents giving satisfying results for racemization were concomitantly tested as solvents for the KR experiments. The results are given in Table 2.

Acceptable to good enantioselectivity factors were registered in all solvent mixtures. However, better results were obtained when 3-methyl-3-pentanol was associated with nonbasic, low polarity solvents such as hexane or toluene (entries 4−7). The highest *E* value (75), identical to that obtained in pure 3-methyl-3-pentanol, was observed in mixtures of hexane and 3-methyl-3-pentanol (entries 4 and 5).

TABLE 2. Kinetic Resolution of (±)-1a with Alkaline Protease[a]

entry	solvent	C% (time)	**1a** ee %	**2a** ee %	*E*
1	3-Me-3-pentanol	51.6 (3h)	96	90	75
2	THF/3-Me-3-pentanol (1:1)	46.7 (3h)	72	82	22
3	THF/3-Me-3-pentanol (1:2)	53.9 (3h)	96	82	40
4	*n*-C_6H_{14}/3-Me-3-pentanol (1:1)	52.6 (3h)	99	88	75
5	*n*-C_6H_{14}/3-Me-3-pentanol (1:2)	51.6 (3h)	96	90	75
6	toluene/3-Me-3-pentanol (1:1)	51.5 (2h)	94	88	58
7	toluene/3-Me-3-pentanol (1:2)	52.2 (3h)	96	88	61

[a] All reactions were performed at 18−24 °C on 1 mL of a 0.1 M solution (unless otherwise stated) of **1a** (0.1 mmol) with 5 mg of surfactant-coated alkaline protease (co-lyophilized in phosphate buffer 0.1 M pH 7.2 with Brij56 and α,β-D-glycopyranoside 8:1:1 w/w/w) and *n*-heptylCONHCH$_2$CO$_2$CH$_2$CF$_3$ (1 equiv).

Regarding the association of both processes to achieve the DKR experiments, one of the main difficulties arose from the rate of the photochemical transformation of the thiol into the corresponding disulfide. Racemization efficiency is closely related to the rate of oxidation of the thiol into the corresponding disulfide.[6] Trifluoroethane thiol is acidic, and trifluoroethyl sulfanyl radical is easy to reduce.[14] This favors the formation of disulfide radical anion, by increasing the concentration in thiolate anion in the reaction medium.[6] This side reaction slows the racemization. Even though the photolysis of the disulfide generates sulfanyl radicals, after complete consumption of the thiol, the concentration of hydrogen atom donor in the reaction medium becomes extremely low (the thiol being only regenerated through hydrogen abstraction from the amine or from the cosolvent).

The concentrations of trifluoroethane thiol and its disulfide were monitored by ^{19}F NMR during racemization experiments. The thiol/disulfide ratio dropped approximately from 100:0 to 75:25 within 1 h in most solvents. Further irradiation further decreased this ratio. As an example, in a 1:2 mixture of THF and 3-Me-3-pentanol, the thiol/disulfide ratio that was very close to 70:30 after irradiating for 60 min became 50:50 after 90 min.

Subtilisins contain no disulfide bridges that could interfere with the radical process.[15] This was a significant advantage anticipated from associating resolution by a subtilisin-like enzyme to light-induced racemization. Nevertheless, the major problem still arose from enzyme irradiation. It has been reported that subtilisin BPN′ was inactivated under laser flash photolysis.[16] The major damages were assigned to the formation of tryptophanyl and tyrosinyl radicals. Indeed, we observed that after irradiation the enzyme activity was reduced with respect to the acylation of **1a**.

In order to circumvent the encountered obstacles to perform a real DKR process, a one-pot sequential procedure was devised. A first KR period in pure 3-methyl-3-pentanol was followed by photochemically induced racemization for 2 h, after addition of trifluoroethane thiol and THF (DKR period). The irradiation was stopped, and KR was started again for

(12) BDE values calculated at 298 K according to the G3B3 methodology are 363.2, 362.7, and 364.3 kJ mol^{-1} for methyl thioglycolate, *N*,*N*-dimethyl thioglycolic amide, and trifluoroethane thiol, respectively. The variation in the series is tiny, but the trend can be considered as significant.

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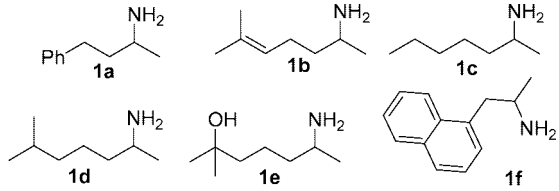

FIGURE 1. Amines structures.

TABLE 3. Kinetic Resolution of Amines 1a−f with Alkaline Protease[a]

entry	amine	C% (time)	amine ee %	amide ee %	*E*
1	**1a**	51.1 (1.5 h)	94	90	67
2	**1b**	53.6 (2 h, 40 min)	95	82	38
3	**1c**	53 (1.5 h)	96	85	48
4	**1d**	53 (1.5 h)	97	86	55
5	**1e**	53.8 (1.5 h)	98	84	52
6	**1f**	45.5 (2.5 h)	80	96	115

[a] All reactions were performed at 18−24 °C on 1 mL of a 0.3 M solution of amine (0.3 mmol) in 3-Me-3-pentanol with 15 mg of surfactant-coated alkaline protease (co-lyophilized in phosphate buffer 0.1 M pH 7.2 with Brij56 and α,β-D-glycopyranoside 8:1:1 w/w/w) and *n*-heptCONHCH$_2$CO$_2$CH$_2$CF$_3$ (0.7 equiv).

one night after adding a second portion of acyl donor (0.7 equiv) and enzyme (Scheme 1).

Selecting 3-methyl-3-pentanol as the solvent for the very first KR period was justified by the high enantioselectivity observed in this solvent for most amines in the series shown in Figure 1 (Table 3). Amide's enantiomeric excesses ranged from 82 to 96%, and enantioselectivity factor ranged from 38 to 115.

The choice of the 1:2 mixture of THF and 3-methyl-3-pentanol offered a good compromise for the second and third period. It was justified by the rate of racemization in this medium; all the more because in the presence of nonbasic low polarity solvents such as hexane or toluene racemization was slowed down after the first KR period. The reasons for this slowing down are not clear yet, but the amount of trifluoroethanol released by the KR might be responsible for additional hydrogen bonding interaction with the amine.

Enantiomeric excesses of the starting amine and the amide that were monitored at each stage of the process and the overall yield in isolated amide are reported in Table 4 for the series of amines given in Figure 1.

Yields exceeding 70% together with ee superior to 80% were observed in most cases except for amines **1f**, for which the data are rather close to those of a simple KR experiment.

In summary, performing successively in the same pot (i) alkaline protease-catalyzed KR using *N*-octanoyl glycine trifluoroethyl ester as the acyl donor, (ii) light-induced radical racemization mediated with trifluoroethane thiol in the presence of the enzyme and the residual acyl donor, (iii) a second KR (after stopping irradiation and adding a second portion of both the enzyme and the acyl donor) led to (*S*)-amides in yields ranging from 58 to 80%. The final enantiomeric excesses ranged between 78 and 94%. Other enzymes and acyl donors are currently under investigation in order to improve both the enantioselectivity and the rate of the KR. Further progress en route to effective DKR will be reported in due course.

TABLE 4. Conversion of Amines 1a−f into (*S*)-Amides 2a−f with Alkaline Protease (One-Pot Sequential Process)[a]

entry	amine	amine ee % after the first KR	amide ee % after the first KR	amine ee % after racemization	amide isolated yield % (ee %)
1	**1a**	94	90	15	80 (86)
2	**1b**	80	82	17	65 (78)
3	**1c**	96	92	8	65 (85)
4	**1d**	97	90	30	70 (86)
5	**1e**	100	82	24	70 (80)
6	**1f**	80	96	9	58 (94)

[a] All reactions were performed at 22−24 °C with alkaline protease (co-lyophilized in phosphate buffer 0.1 M pH 7.2 with Brij56 and α,β-D-glycopyranoside 8:1:1 w/w/w).

Experimental Section

Immobilization of Alkaline Protease. [11] Alkaline protease (120 mg) from Valley Research was dissolved in a solution of *n*-octyl α,β-D-glycopyranoside (15 mg) and Brij56 (polyethylene glycol hexadecyl ether, 15 mg) in a phosphate buffer (pH 7.2, unless otherwise stated, 6 mL), and the mixture was rapidly frozen in liquid N_2 and lyophilized for 24 h.

General Procedure for One-Pot Kinetic Resolution/Racemization/Kinetic Resolution Sequence. Amine (0.3 mmol) was added to a solution of *N*-octanoylglycine trifluoroethyl ester (60 mg, 0.21 mmol) and coated alkaline protease (15 mg) in 3-methyl-3-pentanol (1 mL). The resulting mixture was stirred at room temperature for 1.5−3 h depending on the substrate (see Table 3). The amine ee was determined by GC after derivatization in trifluoroacetamide of an aliquot of the crude mixture with *N*-methylbistrifluoroacetamide (1.5 equiv). The mixture was diluted with 3-methyl-3-pentanol (1 mL) and THF (1 mL), and trifluoroethane thiol (12.3 mg, 0.18 mmol, 1.2 equiv with respect to the remaining amine, assuming 50% conversion for the KR) was added. The mixture was irradiated at 22−24 °C in a quartz tube in a Rayonet apparatus (RPR-200, 16 UV lamps RPR-3000) for 2 h. The amine ee was determined by GC. *N*-Octanoylglycine trifluoroethyl ester (60 mg, 0.21 mmol) and coated alkaline protease (15 mg) were then added. The resulting mixture was stirred at room temperature for 15 h. The enzyme was filtered out from the solution and washed with 5 mL of dichloromethane. The combined organic phases were then evaporated, and the crude material was purified by flash chromatography on silica gel (dichloromethane/methanol; gradient from 0 to 5%) to isolate the amide.

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Supporting Information Available: Experimental procedures and full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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