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Enzyme-catalyzed kinetic resolution of *N*-Boc-*trans*-3-hydroxy-4-phenylpyrrolidine

Research Article

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Abstract: The first enzyme-catalyzed kinetic resolution of *tert*-butyl-3-hydroxy-4-phenylpyrrolidine-1-carboxylate is presented. Enzyme, solvent and temperature optimization resulted in a new resolution method with E = 40 enantioselectivity. The acetate derivative of the (+)-(3*S*,4*R*) enantiomer formed while the (-)-(3R,4S) isomer remained intact. Very good enantioselectivities (E > 200) were achieved in the enzyme-catalyzed alcoholysis of the racemic acetate in *i*-propanol and *t*-butanol where the (+)-(3*S*,4*R*) enantiomer was prepared in pure form (ee > 99.7%). Absolute configuration of the (-)-(3*R*,4*S*)-enantiomer was determined by single crystal X-ray diffraction method.

Keywords: *Enzyme-catalyzed resolution • Lipase enzymes • 3-hydroxy-4-phenylpyrrolidine • Enzymatic hydrolysis • Enantioselective alcoholysis*

1. Introduction

Substituted pyrrolidines are common structural subunits found in a variety of natural [1] and synthetic [2] bioactive compounds. Depending on the substitution pattern and functionality, pyrrolidines have been shown to be effective antibacterial [3], antiviral and anticancer agents [4], protein kinase C inhibitors [5], *etc*. In addition, enantiomerically pure pyrrolidines are known as useful chiral auxiliaries [6,7]. Hence, preparation of enantiopure polysubstituted pyrrolidine derivatives is quite a challenging subject.

A number of stereoselective methods for the synthesis of 2,5-disubstituted pyrrolidines have been reported [8], but general and effective methods for the enantioselective synthesis of 3,4-disubstituted pyrrolidines are noticeably rare. Recently, *tert*-butyl-3-hydroxy-4-phenylpyrrolidine-1-carboxylate (*N*-Boc-*trans*-3-hydroxy-4-phenylpyrrolidine), **1**) was described as an intermediate of a potential neurokinin-1 (NK1) receptor antagonist, which could be effective for treatment of emesis, depression and anxiety [9]. However, the enantiomers of **1** could only be separated by high performance liquid chromatography using chiral stationary phase containing columns [9,10].

Since the enantiomers of **1** are of particular interest, a search for improved methods for their preparation is warranted. There are numerous publications dealing with enzymatic resolution of secondary alcohols [11-13] in the presence of lipases. Compound **1** contains

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a hydroxylic group connected to one of the asymmetric centers; therefore, we used it as a function for resolution *via* enzyme-catalyzed transesterification.

2. Experimental procedure

2.1. General

All commercial starting materials were purchased from Sigma-Aldrich Hungary Ltd. and Merck Hungary Ltd. and were used without further purification.

Routine ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on a BRUKER AV 300 or DRX 500 spectrometer. Proton and carbon chemical shifts are reported in ppm relative to tetramethylsilane (dTMS = 0.00 ppm) or to the solvent ($dCDCl_3$ = 77.00 ppm), respectively. IR spectra were recorded on an appliance type PERKIN ELMER 1600 with a Fourier Transformer. Data are given in cm^{-1}. Specific rotation of the optically active samples were determined on a PERKIN ELMER 245 MC polarimeter using sodium lamp (589 nm).

Alufolien Kieselgel 60 F254 (Merck) plates were used for TLC investigations and the spots were visualized with uv light and/or an aqueous solution of $(NH_4)_6Mo_7O_{24}$, $Ce(SO_4)_2$ and sulfuric acid. Flash chromatography was applied for the separation and purification of the products using normal phase RediFlash Rf columns. Eluents are given for each experiment.

GC analysis were performed on an Agilent 4890 D equipment using ALPHA DEXTM 120 (30 m, 0.25 mm/0.25 mm) capillary column at 190°C.

HRMS analyses were performed on a LTQ FT Ultra (Thermo Fisher Scientific, Bremen, Germany) system. The ionization method was ESI and operated in positive ion mode. For CID, experiment helium was used as the collision gas, and normalized collision energy (expressed in percentage) was used to bring about fragmentation. The protonated molecular ion peaks were fragmented by CID at a normalized collision energy of 35%. The samples were solved in methanol. Data acquisition and analysis were accomplished with Xcalibur software version 2.0 (Thermo Fisher Scientific).

2.2. Preparation of rac-1 and rac-5

N-Boc-pyrroline (3) [10]

A 65/35 mixture of pyrroline and pyrrolidine (25.0 g, 0.235 mol) was dissolved in dichloromethane (500 mL). Di-*tert*-butyl-dicarbonate (75.8 g, 0.347 mol) was also dissolved in dichloromethane (250 mL) and the two precooled solutions were mixed under continuous cooling at 0-5°C. The reaction mixture was stirred for two hours at ambient temperature then aqueous hydrochloric acid solution (1 molar, 200 mL) was poured into it and the phases were separated. The organic phase was washed with brine (3×80 mL), dried over sodium sulfate, and it was concentrated in vacuum. The residue contained the crude *N*-Boc-pyrrolidine (**3**, 59.0 g). It was used in the next step without any purification.

^{1}H NMR (500 MHz, $CDCl_3$), d_H (ppm): 5.77 (d, 2H, J = 17.0 Hz), 4.11 (d, 4H, J = 22.5 Hz), 1.48 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) d_C (ppm): 154.3, 125.9, 125.8, 79.3, 53.1, 52.8 28.5. IR (film) n_{max} (cm^{-1}): 2978, 1742, 1699, 1341, 1317.

N-Boc-3,4-epoxypyrrolidine (4) [10]

A dichloromethane solution (15 mL) of the crude *N*-Boc-pyrroline (**3**, 2.0 g, 11.6 mmol) was cooled down to 0°C and the dichloromethane solution (45 mL) of *m*-chloroperbenzoic acid (70% content, 5.73 g, 23.2 mmol) was added into it within 2.5 hours. The reaction mixture was stirred at ambient temperature for 4 days,it was cooled down again to 0°C, and the precipitated *m*-chlorobenzoic acid was filtered off. The filtrate was washed with saturated aqueous sodium thiosulfate solution (100 mL, then 3×30 mL), with saturated aqueous sodium hydrogencarbonate solution (60 mL) and with saturated potassium carbonate solution (3×50 mL) until the peroxide test became negative (Merckoquant 1.10011.0001 peroxide test). The solution was washed with brine (40 mL) and dried over sodium sulfate before concentration in vacuum. The crude product (**4**, 2.45 g) was purified by column chromatography (eluent: hexane/ethyl acetate=4/1 then 2/1) to yield pure **4** (1.30 g, 72%).

^{1}H NMR (500 MHz, $CDCl_3$) d_H (ppm): 3.77 (dd, 2H, J_1 = 36.5, J_2 = 13.0 Hz), 3.66 (d, 2H, J = 5.0 Hz), 3.31 (dd, 2H, J_1 = 13.0, J_2 = 5.0 Hz), 1.44 (s, 9H). ^{13}C NMR (125 MHz, $CDCl_3$) d_C (ppm): 154.8, 79.8, 55.6, 55.1, 47.3, 46.9, 28.4. IR (film) n_{max} (cm^{-1}): 3051, 2976, 2876, 1698, 1423, 1389, 1338, 1174, 1116, 1027, 964.

Racemic N-Boc-3-hydroxy-4-phenylpyrrolidine (1) [10,11]

Compound **4** (1.40 g, 7.56 mmol) was dissolved in dry tetrahydrofuran (20 mL) under nitrogen atmosphere, cuprous iodide (0.108 g, 0.567 mmol) was added into it and the solution was cooled to 0°C. Tetrahydrofuran solution of phenylmagnesium chloride (2 mol L^{-1} solution, 4.35 mL, 8.69 mmol), was added dropwise and the mixture was stirred at 0°C for two hours then it was allowed to warm up to ambient temperature and stirred for overnight. The dark reaction mixture was poured into ice water and aqueous hydrochloric acid (1.5 molar solution, 3.8 mL) was added into it followed by the addition of diethyl ether (38 mL). The organic phase was separated and the aqueous phase was washed with diethyl ether (3×30 mL). The tetrahydrofuran content of the aqueous solution was distilled off in vacuum then it

was extracted with ethyl acetate (2×38 mL). This organic solution was dried over sodium sulfate and concentrated in vacuum to yield the crude product (**1**, 2.35 g). The pure product was obtained in 88% yield (1.75 g) after column chromatography (eluent hexane/ethyl acetate=4/1). Mp: 72-74°C (from hexane/ethyl acetate).

^{1}H NMR (300 MHz, $CDCl_3$) d_H (ppm): 7.33 (m, 2H), 7.25 (m, 3H), 4.28 (q, 1H, J = 6.0 Hz), 3.83 (m, 1H), 3.71 (m, 1H), 3.49 (m, 1H), 3.25 (m, 2H), 2.91 (br, 1H), 1.47 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) dC (ppm): 154.5, 139.3, 128.8, 127.4, 127.2, 79.7, 75.9, 55.3, 52.1, 51.4, 28.5. IR (film) n_{max} (cm^{-1}): 3420, 2975, 1678, 1422, 1134, 770, 699, 585.

Racemic N-Boc-3-acetoxy-4-phenylpyrrolidine (5)

Compound **1** (0.200 g, 0.698 mmol) was dissolved in dry pyridine (3 mL) under nitrogen atmosphere and the solution was cooled down to -10°C. Acetic anhydride 0.400 mL, 4.20 mmol) was added and the reaction mixture was stirred at -10°C for two hours and at 25°C for one day. Then it was poured into a mixture of ice (about 5 g), aqueous sulfuric acid (1 mol L^{-1}, 30 mL) and diethyl ether (20 mL). The phases were separated and the aqueous phase was extracted with diethyl ether (3×20 mL), then the ethereal solution was washed with brine (20 mL), dried over sodium sulfate and concentrated in vacuum. The crude product (0.240 g, yellowish oil) was purified by column chromatography (eluent hexane/ethyl acetate=4/1) to yield pure **5** (0.225 g, 97%).

^{1}H NMR (300 MHz, $CDCl_3$) dH (ppm): 7.31 (m, 5H), 5.15 (m, 1H), 3.79 (m, 3H), 3.46 (m, 1H), 3.36 (m, 1H), 2.08 (s, 3H), 1.50 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$) dC (ppm): 170.8, 154.5, 139.1, 129.1, 127.6, 127.4, 80.1, 77.5, 51.3, 49.9, 48.3, 28.7, 21.3. IR (film) n_{max} (cm^{-1}): 2972, 1737, 1699, 1403, 701. The adduct of protonated molecular ion peak [2M+H]+ can be detected at m/z 611. HRMS: 611.33281 ($C_{34}H_{47}O_8N_2$; calc. 611.33269; delta=0.2ppm). ESI-MS-MS (611.33@cid35) (rel. int. %, element composition): 555(2, [2M+H]-C_4H_8); 511(2, [2M+H]-$C_5H_8O_2$); 306(70, $C_{17}H_{24}NO_4$); 250(100, [2M+H]-M-C_4H_8); 206(5, [2M+H]-M-$C_5H_8O_2$); 190(18, $C_{11}H_{12}O_2N$).

2.3. Kinetic resolution of N-Boc-3-hydroxy-4-phenylpyrrolidine (rac-1)

Small-scale kinetic resolution of rac-1

Racemic *N*-Boc-3-hydroxy-4-phenylpyrrolidine (**1**, 10 mg, 38 µmol) was dissolved in the mixture of the solvent (0.5 mL) and vinyl acetate (0.2 mL, 2.2 mmol) and the selected enzyme (20 mg) was added into it. The reaction mixture was shaked at 30°C and samples were withdrawn time to time. Samples were analysed by gas chromatography.

Kinetic resolution of rac-1 under optimized conditions

Racemic *N*-Boc-3-hydroxy-4-phenylpyrrolidine (**1**, 0.80 g, 3.04 mmol) was dissolved in the mixture of methyl *tert*-butyl ether (40.0 mL) and vinyl acetate (15.0 mL, 163 mmol), and Novozym 435 enzyme (1.60 g) was added into it. The reaction mixture was stirred at 45°C and samples were withdrawn time to time. Samples were analysed by gas chromatography. When the half of the starting material consumed (at 54% conversion), the enzyme was filtered off, washed with acetone (3×5 mL) and the filtrate was concentrated in vacuum. The residue (1.06 g) contained the formed (+)-(3*S*,4*R*)-**5** ester and the unreacted (-)-(3*R*,4*S*)-**1**. These compounds were separated from their mixture by flash chromatography (eluent hexane/ethyl acetate = 1/0 → 3/2). Thus pure (-)-(3*R*,4*S*)-**1** (0.377 g, 94% yield, calculated to the half of the starting racemate) and (+)-(3*S*,4*R*)-**5** (0.542 g, 103% yield, calculated to the half of the starting racemate) were isolated. Retention times in GC, specific rotation powers and ee values of the products are given below.

(-)-(3*R*,4*S*)-**1**: $t_{R(GC)}$=97.7 min (for comparison (+)-**1**: $t_{R(GC)}$=98.3 min); $[\alpha]_D^{20}$ = -35.5 (c=1.94; $CHCl_3$); ee=97.3%.

(+)-(3*S*,4*R*)-**5**: $t_{R(GC)}$=69.0 min. $[\alpha]_D^{20}$ = +16.1 (c=2.33; $CHCl_3$); ee=78.0%.

2.4. Hydrolysis/alcoholysis of optically active N-Boc-3-acetoxy-4-phenylpyrrolidine ((+)-(3S,4R)-5)

Hydrolysis of (+)-(3S,4R)-5

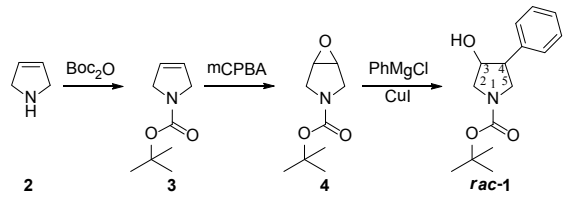
Aqueous sodium hydroxide (10% solution, 1.0 mL) was added to the solution of (+)-(3*S*,4*R*)-**5** (0.20 g, ee = 72.6%) in methanol (5.0 mL) and the mixture was stirred for 72 hours. Then it was diluted with water (20 mL) and extracted with ethyl acetate (4×10 mL). The ethyl acetate solution was dried over sodium sulfate and concentrated in vacuum to yield (+)-(3*S*,4*R*)-**1** (0.15 g, 87%, ee=72.6% (GC)).

Enzyme catalyzed alcoholysis of rac-5. General procedure

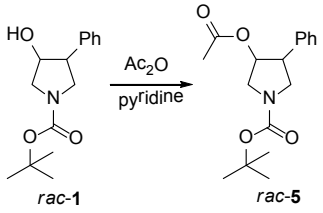
Racemic **5** (0.010 g, 0.033 mmol) was dissolved in the corresponding solvent (see in Table 4, 0.30 mL) and Novozym 435 (0.040 g) was added into it.Then the mixture was shaked for 8 days. Compositions of the reaction mixtures were determined by GC. The results are summarized in Tables 4 and 5.

Enzyme catalyzed hydrolysis of rac-5. General procedure

Racemic **5** (0.010 g, 0.033 mmol) was dissolved in the corresponding solvent (see in Table 4, 0.12 mL) and



Scheme 1. Synthesis of *rac*-**1**.



Scheme 2. Synthesis of *rac*-**5**.

a phosphate buffer (pH=7.0, 0.12 mL) then Novozym 435 (0.040 g) was added into it. The mixture was shaken at room temperature for 8 days. The mixture was filtered, diluted with distilled water (1.0 mL) and extracted with ethyl acetate (4×2.5 mL). Compositions of the ethyl acetate solutions were determined by GC. The results are summarized in Table 4.

2.5. Crystal structure determination

Single crystals of (-)-(3*R*,4*S*)-*tert*-butyl-3-hydroxy-4-phenylpyrrolidine-1-carboxylate ((-)-**1**) were obtained from ethyl acetate, mp= 88-90 °C.

Crystal data: $C_{15}H_{21}NO_3$, Fwt.: 263.33, colourless, bladed, size: 0.75×0.25×0.01 mm, monoclinic, space group P 21, a = 9.0787(6) Å, b = 5.8006(4) Å, c = 14.1226(9)Å, α = 90.00°, β = 94.054(3)°, γ = 90.00°, V = 741.86(9) Å^3, T = 295(2) K, Z= 2, F(000) = 284, Dx = 1.179 Mg m^{-3}, μ = 0.660 mm^{-1}.

A crystal was mounted on a loop. Cell parameters were determined by least-squares using 8392 (7.64≤θ≤71.82°) reflections.

Intensity data were collected on a Rigaku R-AXIS-RAPID diffractometer (graphite monochromator Cu-Kα radiation, λ = 1.54187 Å) at 295(2) K in the range 7.69≤θ≤58.91. A total of 8814 reflections were collected of which 2043 were unique [R(int) =0.0243, R(σ) =0.0224]; intensities of 1826 reflections were greater than 2σ(*I*). Completeness to θ = 0.972.

A numerical absorption correction was applied to the data (the minimum and maximum transmission factors were 0.715 and 0.973).

The structure was solved by direct methods [17]. Anisotropic full-matrix least-squares refinement [17] on F^2 for all non-hydrogen atoms yielded R_1 = 0.0328 and wR^2 = 0.0800 for 1332 [*I*>2s(*I*)] and R_1 = 0.0364 and wR^2 = 0.0823 for all (2043) intensity data, (number of parameters = 179, goodness-of-fit = 1.033, the maximum and mean shift/esd is 0.000 and 0.000). The maximum and minimum residual electron density in the final difference map was 0.120 and -0.099e.Å^{-3}. Absolute configuration crystal data: Friedel Pair Coverage = 95%, Flack parameter: 0.2(2), Hooft parameter: 0.19(9).

CCDC 921454 contains the supplementary crystallographic data for this Sructure. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* www.ccdc.cam.ac.uk/data_request/cif.

3. Results and discussion

3.1. Synthesis of *rac*-1 and *rac*-5

The starting material was prepared according to the literature method [10] from pyrroline (**2**) *via* Boc protection (intermediate **3**), epoxidation (intermediate **4**) and copper catalyzed phenylmagnesium bromide addition (Scheme 1). It is worth mentioning that commercial pyrroline (**2**) contained pyrrolidine, which can easily be separated from the main product after the epoxidation step. The epoxidation step provided *cis*-**4** and addition of the Grignard reagent was completely diastereoselective, thus racemic *trans*-**1** has formed in the reaction as sole product, according to the results of gas chromatographic and spectroscopic investigations.

The racemic acetate of **1** was prepared in good yield using acetic anhydride as acylating agent (**5**, Scheme 2). Ester **5** was used as a standard for analytical measurements and as a starting material of enzyme-catalyzed enantioselective alcoholysis and hydrolysis experiments.

3.2. Enzyme catalyzed kinetic resolution of *rac*-1

Enantioselectivity of the enzyme-catalyzed transesterification of racemic **1** was screened with a wide selection of enzymes [AK, A, F-AP15, M and PS lipases from Amano; Novozym 435, Liposime TL IM and CAL-A from Novozymes; porcine liver esterase (liver acetone powder), *Candida cylindracea* lipase and *Pseudomonas fluorescens* lipases from Fluka; papain, *Candida cylindracae* (Type VII, powder) and porcine pancreatic lipases(Type II, crude powder) from Sigma and BUTE-3 lipase (crude, precipitated with acetone), isolated at our university from *thermophilic filamentous* fungi [12]. Vinyl acetate was used as an acylating reagent and the transesterification was carried out in tetrahydrofuran in the test reactions (Scheme 3).

Gas chromatographic analyses performed on a chiral stationary phase containing capillary column

Table 1. Solvent dependent kinetic resolution of rac-1 in the presence BUTE-3 enzyme.

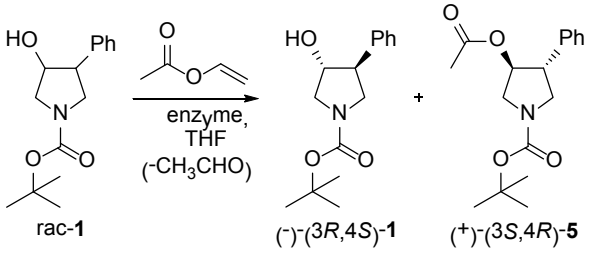
Solvent	Acylating agent[a]	Conversion (%)[b]	Ee of (3R,4S)-1 (%)[c]	E[d]
Tetrahydrofuran	vinyl acetate	16	10.0	4
Methyl *tert*-butyl ether	vinyl acetate	65	94.4	10
Diisopropyl ether	vinyl acetate	69	>99.0	14
Toluene	vinyl acetate	54	75.3	10
Hexane	vinyl acetate	68	96.4	9
Hexane/tetrahydrofuran=2/1	vinyl acetate	48	50.5	6
Acetone	vinyl acetate	22	16.7	5
Ethyl acetate	vinyl acetate	46	53.0	8
Ethyl acetate	ethyl acetate	8	5.1	4

[a] *Details of the small scale experiments are given in the experimental part.*
[b] *Conversions were determined after 72 hours reactions by GC analysis (details are given in the experimental section) and calculated to the whole amount of rac-1 as follows c: conversion, x_1 and x_5: mol% of 1 and 5.*
[c] *Ee values were determined by GC on chiral stationary phase containing capillary column.*
[d] *Enantioselectivity (E) of the resolution was calculated according to the literature standards [14,16]*

Table 2. Solvent dependent kinetic resolution of *rac*-**1** in the presence Novozym 435 enzyme[a].

Solvent	Conversion (%)[b]	Ee of (3R,4S)-1 (%)[c]	E[d]
Tetrahydrofuran	35	42.6	12
Methyl tert-butyl ether	60	94.3	15
Diisopropyl ether	59	91.1	13
Toluene	42	51.6	10
Hexane	60	89.3	11
Hexane/ tetrahydrofuran=2/1	44	60.2	13
Acetone	35	38.1	9
Ethyl acetate	55	82.8	13

[a] *Details of the small scale experiments are given in the experimental part.*
[b] *Conversions were determined after 72 hours reactions by GC analysis. Calculation is explained in the footnote of Table 1.*
[c] *Ee values were determined by GC on chiral stationary phase containing capillary column.*
[d] *Calculation method is given in Table 1 foot-note d.*



Scheme 3. Enzyme-catalyzed kinetic resolution of *rac*-**1**.

helped us in monitoring the enzymatic reactions. The starting racemate was completely pure *trans*-**1**, and we did not find any isomerization product (*cis*-**1** or *cis*-**5** isomer) during the resolution processes according to the GC investigations.

Consequently, the diastereoisomeric excess (de) was 100% in each case. Comparison of the yields of the produced acetate **5** and the residual alcohol **1** and ee values of **1** lead us to conclude that the Novozyme 435 and BUTE-3 were the most active and enantioselective catalysts. Therefore, further optimization experiments were carried out with these two enzymes.

Catalytic activities and enantioselectivities of the enzymes strongly depend on the solvent therefore a series of experiments were carried out with the above mentioned two enzymes in different solvents and solvent mixtures. Each reaction was accomplished at 30°C, under the same conditions (for details, see the experimental section). Samples were withdrawn time to time and the conversion (acetate formation) together with the ee values of the residual alcohol **1** were determined by gas chromatography. The results are summarized in Tables 1 and 2.

We achieved the best enantioselectivity in diisopropyl ether with BUTE-3 lipase. In this reaction, the unreacted (3*R*,4*S*)-**1** alcohol could be isolated in practically enantiopure form (31% yield) after a 72 hour reaction. Comparison of the results obtained in ethyl acetate/vinyl acetate mixture and pure ethyl acetate, demonstrate the importance of the quasy irreversible transesterification (with vinyl acetate). Much lower yield and enantioselectivity could be achieved during transesterification in pure ethyl acetate [13].

In the presence of Novozym 435, the kinetic resolution was more efficient in almost every solvent. The best enantioselectivity was observed in methyl *tert*-

Table 3. Temperature dependence of the conversion and enantioselectivity in kinetic resolution of **1**.

Temperature (°C)	Conversion (%)[a]	E
30	46	15
40	55	27
45	55	27
50	58	28
55	58	19

[a] *Conversions were determined after 72 hours reactions by GC analysis.*

Table 4. Novozyme 435 catalysed deacetylation of *rac*-5 in different solvents[a]. Results were obtained after 8 days reaction at room temperature.

Solvent	Conversion[b] (%)	Ee of 1[c] (%)	E[d]
Ethanol	8	98	90
***n*-Propanol**	0.6	92	24
***i*-Propanol**	36	99.7	>200
***n*-Butanol**	10	>99.7	>200
***t*-Butanol**	45	>99.7	>200
n-Heptanol	2	>99.7	>200
***n*-Octanol**	2	>99.7	>200
***n*-Butanol/buffer=1/1**	0.2	>99.	>200
***t*-Butanol/buffer=1/1**	1.2	75	7.1
Acetonitrile/buffer=1/1	0.6	45	2.6
Buffer	34	3.8	1.1

[a] *Details of the small scale experiments are given in the experimental part.*
[b] *Conversions and enantiomeric compositions were determined by GC. Conversion was calculated as follows:*
(c: conversion, x_1, x_2: mol% of 1 and 5, respectively).
[c] *Ee values were determined by GC on chiral stationary phase containing capillary column.*
[d] *Enantioselectivity of the resolution was calculated according to the literature standards [14,16]*

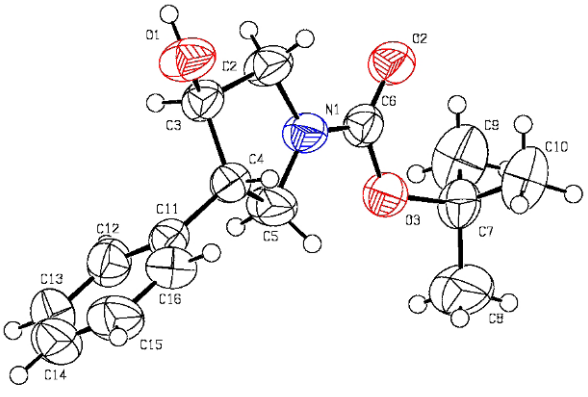


Figure 1. Ortep style diagram [17] of (-)-(3*R*,4*S*)-**1** in the single crystal.

butyl ether (E=15). The higher enantioselectivity of this enzyme let us to produce pure (3*R*,4*S*)-**1** with better yield than in the previous resolution.

Temperature dependence of the enzyme activity and enantioselectivity was also investigated in the presence of Novozyme 435 enzyme. In these experiments, methyl *tert*-butyl ether was used as a solvent and vinyl acetate was the acylating agent. Yields of (3*S*,4*R*)-**5** were determined after 24 hours reactions at different temperatures between 30°C and 55°C. The results are summarized in Table 3.

The best enantioselectivity was achieved in the 40-50°C range and, of course, in a much faster reaction (24 hours) than it was at 30°C. Scale up of the enzyme catalyzed reaction at 45°C resulted in further enhancement of the enantioselectivity (E=40). It is probably due to the slightly different conditions (stirring instead of shaking). It means that, in larger scale, under optimum conditions, (3*S*,4*R*)-**1** could be prepared in good yield (94%, calculated to half of racemic **1**) and high ee (97.3%) in methyl *tert*-butyl ether (E=40). It is probably due to the slightly different conditions (stirring instead of shaking). It means that, in larger scale under optimum conditions, (3*R*,4*S*)-**1** could be prepared in good yield (47%) and high *ee* (97.3%) in methyl *tert*-butyl ether.

3.3. Determination of the absolute configuration of (-)-1

In order to determine the absolute configuration of (-)-**1**, single crystal X-ray diffraction studies were accomplished. The molecular structure of the pure (-)-**1** enantiomer in the crystals is shown in Fig. 1. From this structure, one can conclude that the absolute configuration of the (-)-**1** enantiomer is (3*R*,4*S*)-**1**.

3.4. Hydrolysis/alcoholysis of *rac*-5

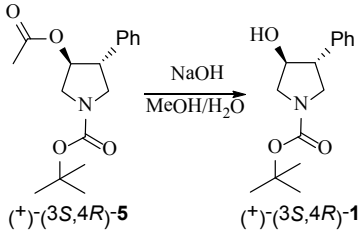
The (+)-(3*S*,4*R*)-**1** isomer was prepared first by hydrolysis of the optically active (+)-(3*S*,4*R*)-**5** ester (Scheme 4). However, the enantiomeric purity of (+)-(3*S*,4*R*)-**1** strongly depends on the conversion of the enzyme catalyzed resolution. Therefore, enzyme-catalyzed alcoholysis of (3*S*,4*R*>3*R*,4*S*)-**5** enantiomeric mixture was also studied with the hope of preparation (+)-(3*S*,4*R*)-**1** in high ee. According to the literature examples [15], lipase enzymes usually prefer the hydrolysis or alcoholysis of the faster formed ester enantiomer ((3*S*,4*R*)-**5** isomer in our case).

The rate of the enzyme-catalyzed alcoholysis and hydrolysis was one order of magnitude smaller than the rate of the acylation reactions. However, the enantioselectivities were much higher (Table 4). The best results were achieved in *i*-propanol and *t*-butanol.

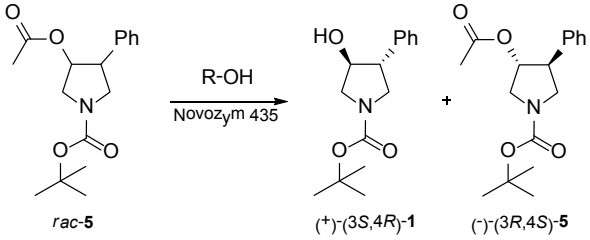
Table 5. Temperature dependence of the conversion and enantioselectivity of the Novozyme 435 catalyzed alcoholysis of *rac*-**5**[a].

Temperature (°C)	Conversion in *i*-PrOH (%)[b]	E[c] in *i*-PrOH	Conversion in *t*-BuOH (%)[b]	E[c] in *t*-BuOH
30	15	>200	42	>200
40	31	>200	45	>200
50	34	>200	46	>200
60	38	>200	47	>200

[a] *Details of the small scale experiments are given in the experimental part.*
[b] *Conversions were determined after 3 days reactions by GC analysis. Calculation is explained in Table 1.*
[c] *Calculation method is given in Table 4, footnote 4.*



Scheme 4. Hydrolysis of (+)-(3*S*,4*R*)-**5** with sodium hydroxide.



Scheme 5. Alcoholysis/hydrolysis of *rac*-**5**. (R-OH: ethanol, *n*-propanol, *i*-propanol, *n*-butanol, *t*-butanol, *n*-heptanol, *n*-octanol, water. Ee data are shown in Table 4).

The enantioselectivities were very high (E > 200) in both cases and practically pure (+)-(3S,4R)-1 (ee > 99.7%) could be isolated from the reaction mixture after 8 days of reaction at 30°C.

Presence of aqueous buffer caused significant decreasing of both the reaction rate and the enantioselectivity in alcohol/buffer mixtures and the hydrolysis was not enantioselective in pure aqueous buffer solution (Table 4). In order to enhance the rate of the alcoholysis, experiments were carried out at higher temperatures in i-propanol and t-butanol, too. The results are summarized in Table 5.

Experimental data show that the enantioselectivity remains very high at elevated temperature while the rate of the reaction increases. Thus, 38% and 47% conversion could be achieved in i-propanol and t-butanol after 70 hours, respectively. Consequently, *t*-butanol seems to be the best reactant and solvent for enzyme-catalyzed resolution of *rac*-**5**.

4. Conclusions

It has been found that Novzyme 435 and BUTE-3 lipases are suitable catalysts for the kinetic resolution of racemic *tert*-butyl-3-hydroxy-4-phenylpyrrolidine-1-carboxylate (**1**). The enzymes initiate the acetylation of (+)-(3*S,4R)*-1 while (-)-(3*R,4S)*-**1** remains intact. The best separation could be achieved with Novozyme 435 using vinyl acetate in methyl tert-butyl ether at 50°C. Under these conditions, the enantioselectivity E = 40 and (-)-(3*S,4R)*-**1** could be isolated in 94% yield (ee>97.3%).

Absolute configuration of the (-)-(3*R*,4*S*)-**1** enantiomer was determined by single crystal Xray diffraction method because such a measurement was missing from the literature until now.

High enantioselectivities could be achieved during the alcoholysis of the racemic acetate (*rac*-**5**). The Novozyme 435 catalyzed reaction was the most selective and the fastest in *i*-propanol and *t*-butanol (E > 200). Even though the reactions were quite slow at 30°C, 47% conversion could be achieved within 70 hours at 60°C, while the enantioselectivity remained high (E > 200). Thus practically enantiopure (+)-(3*R*,4*S*)-**1** could be prepared (*ee* = 99.7%).

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