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Enzymatic Preparation of Chiral 3-(Hydroxymethyl)piperidine Derivatives

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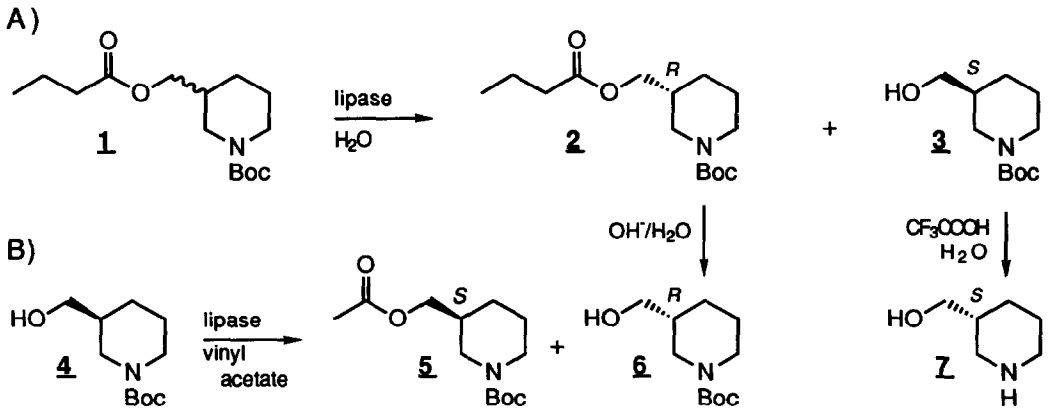
Abstract: t-Butyl (R)-3-(hydroxymethyl)-1-piperidinecarboxylate was prepared with lipase P in up to 98 % ee by means of enantioselective esterification of the racemic alcohol as well as by enantioselective hydrolysis of the corresponding butyryl ester and subsequent chemical hydrolysis of the retained (R)-ester. A work-up procedure feasible on the kg-scale is described.

t-Butyl (R)-3-(hydroxymethyl)-1-piperidinecarboxylate **6** is a key intermediate in the synthesis of a series of very potent low-molecular weight thrombin inhibitors[1]. Two well established enzymatic approaches aiming at this chiral intermediate have been evaluated, namely the enantioselective hydrolysis of the corresponding racemic ester and the enantioselective esterification of the racemic alcohol in organic solvent (Scheme 1).

Our first goal was the kinetic resolution of racemic O-acylated 3-(hydroxymethyl)-piperidines by means of *enantioselective hydrolysis*. Since many esterolytic enzymes do not accept water-soluble or charged substrates, the amine was masked by a Boc protecting group (cf. also Nieduzak & Carr[2]). Butyryl ester **1** was selected as substrate for screening with numerous commercial lipases and esterases. Lipase P from *Pseudomonas fluorescens* (now available as lipase PS) clearly showed the best results, a phenomenon also encountered with other stereoselective resolutions of esters of *primary* alcohols [3-7]. The enantiomeric ratio[8] (E) of the reaction was around 40 and could not be improved by employing a lower temperature (5 °C), a shorter acyl moiety (propionate) or by partially purifying[5] the commercial lipase (results not shown). Thus, an appropriate conversion degree[8] had to be chosen in order to generate the reaction products **2** and **3** in acceptable enantiomeric excess: at 42 % conversion **3** was obtained in 91 % ee and above 53 % conversion **2** in > 94 % ee[9].

Scheme 1: Enzymatic routes to t-butyl (R)-3-(hydroxymethyl)-1-piperidine-carboxylate **6** by enantioselective hydrolysis (A) and esterification (B).



Chemical hydrolysis of **2** led to (R)-alcohol **6**[10]. Recrystallization of **6** afforded only modest enrichment of the enantiomeric purity: a sample of 95 % ee recrystallized from hexane gained some 1 % ee.

A simple procedure for the separation of the reaction products **2** and **3**, also feasible on a larger scale, has been worked out. Partial separation was attained by selective crystallization of **3** from the product mixture dissolved in hexane; final purification of **3** was achieved by filtering the mother liquor on silicagel 60[11]. The present large-scale procedure was successfully carried out on a multi-kg scale with an initial substrate concentration of 6 %, yielding **6** in 97 % ee.

On a larger scale work-up would be greatly facilitated if the separation of the reaction products **2** and **3** could be achieved by means of extraction. Therefore, attempts had been made to make alcohol **3** water-soluble by succinylation. The reaction with succinic anhydride, however, was slow or, if accelerated (DMAP), led to racemization.

The absolute configuration was determined by chemical correlation of **3** to (S)-3-(hydroxymethyl)piperidine **7**[12]. The specific rotations were in excellent agreement, but, owing to their low absolute value, the configuration was additionally confirmed by x-ray structure analysis of a derivative of **6** (results will be reported elsewhere).

The inverse reaction, the *enantioselective esterification* of the racemic alcohol **4** using lipase P adsorbed on diatomaceous earth[13] was tested in a series of anhydrous organic solvents. Starting from **4** in 1 % concentration and vinyl acetate as acyl donor in 0.5 mass equivalents the remaining R-alcohol **6** was provided in high enantiomeric excess after ca. 60 % conversion. Best results were obtained in non-polar solvents: n-hexane (**6** in >99 % ee at 60 % conversion, E = 35), toluene (>98 % ee at 57 % conversion, E = 29) and c-hexane (98 % ee at 59 % conversion, E = 23). The reaction in n-hexane was also effectively carried out employing the theoretical amount of vinyl acetate for 55-60 % conversion (0.55-0.60 mol eq.)[14]. At higher substrate concentration (5 %) most of alcohol **4** remained undissolved and the enantiomeric

excess of retained (R)-alcohol **6** was markedly impaired. Thus, for preparation of larger amounts of **6** the hydrolytic approach, which allows much higher substrate concentrations (10 %) and gives a slightly higher E-value, was preferred.

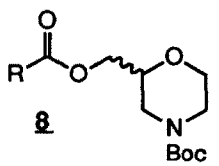
The (R)-3-(hydroxymethyl)morpholine **9** is also of considerable interest as a drug intermediate[1]. Intriguingly, however, the replacement of the methylene group in 4-position of the piperidine ring of **1** by an oxa group (leading to butyryl ester substrate **8a**) caused a dramatic reduction in the enantiomeric ratio of lipase P from ca. 40 down to 4 (Table 1). Moreover, hydrolysis of the phenylacetyl ester **8b** and phenoxyacetyl ester **8c** with various commercial penicillinacylases and proteases showed only poor enantioselectivitiy. The best results obtained are depicted in Table 1.

Table 1: Enantioselective enzymatic hydrolysis[a] of morpholine compounds **8a-c**.

			alcohol **9** formed		
substrate	enzyme	% conversion	% ee	conf.[b]	E
8a[c]	Lipase P	34	50	S*	4
8b	Prozyme 6	47	55	S*	5
8b	Alcalase 2.0 T	45	46	S*	4
8c[d]	Protease N	46	11	R*	1

a: 50 mg of enzyme dissolved in 26 ml of 0.1 M NaCl, 4 mM sodium phosphate buffer, pH 7.5, containing 200 µmol of **8**. b: Tentative configuration, based on the assumption that lipase P does not alter its stereochemical preference upon replacing the methylene group in 4-position of substrate **1** by an oxa group. c: 100 mg substrate and 6 mg of enzyme were employed. d: the solid substrate was dissolved in 0.77 ml of CCl_4 prior to application.

An attempt to extend the enzymatic esterification approach as outlined in Scheme 1B from alcohol **4** to the corresponding monoprotected 3-(aminomethyl)piperidine **10** using lipase P as a catalyst was unsuccessful. Also the acetylation experiments with a second monoprotected 3-(aminomethyl)piperidine derivative, the cyclic secondary amine **11**, failed: all of the various commercial enzymes tested yielded nearly racemic products. Similarly poor ee-values were observed by Asensio et al[15] with N-acetylation of the unprotected 3-(hydroxymethyl)piperidine and by Toone & Jones[16] with ester hydrolysis of N-acetyl-3-(acetoxymethyl)piperidine.



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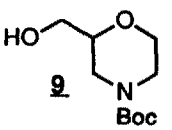

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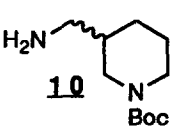

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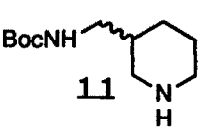

11

R =
a: $CH_3CH_2CH_2$-
b: $PhCH_2$-
c: $PhOCH_2$-

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[9] **Hydrolysis of t-butyl (R,S)-3-[(butyryloxy)methyl]-1-piperidine-carboxylate 1**[17]: Racemic ester **1** (116.6 g, 408 mmol) was emulsified in 0.1 M NaCl (2.0 l) and 0.1 M sodium phosphate buffer pH 7.0 (80 ml) under vigorous stirring. Lipase P-30 (1.0 g) was added and the pH kept constant at 7.0 by addition of 2.0 N NaOH (pH-stat). After 42 % conversion (after 15 h) the reaction mixture was extracted with CH_2Cl_2 (3x500 ml). The combined organic phases were dried ($MgSO_4$) and evaporated. The residue (103 g) was chromatographed on silicagel 60 [900 g; elution with hexane/ ethyl acetate 4:1 (4 l), 1:1 (2 l) and 1:2 (2 l)] yielding **3** (36.6 g, 170 mmol; 42 %) as white crystals: $[\alpha]_{365}$ = + 53.5 (1 % in EtOH); 91 % ee[18]; > 99 % (GLC). Recovered ester **2** (65.7 g, 230 mmol, 56 %) was subjected under analoguous conditions to further enzymatic hydrolysis up to a final conversion degree of 56 % (0.1 M NaCl (1.15 l), 0.1 M phosphate buffer (45 ml), lipase (0.5 g), 2.0 N NaOH (28.4 ml), 40 h). Analogous work-up afforded **2** (49.5 g, 173 mmol; 42 %) as a colourless oil: >98 % ee[18], >99 % (GLC); ^{1}H-NMR ($CDCl_3$): 0.95 (t, 3H, $-CH_3$), 1.11-1.93 (stack, 16H), 2.30 (t, 2H, $-COCH_2-$), 2.50 - 2.88 (broad s, 2H, $-NCH_{ax}-$) and 3.84 - 4.06 (stack, 4H, $-NCH_{eq}-$ and $-COOCH_2-$); IR (neat): 1738 (ester), 1695 (N-C=O), 1242 and 1178 (ester); MS (thermospray): 286 ($M+H^+$).

[10] **t-Butyl (R)-(hydroxymethyl)-1-piperidinecarboxylate 6**: (R)-ester 2[9] (49.4 g, 173 mmol) was dissolved in EtOH (250 ml) and hydrolyzed with 2 N NaOH (88 ml, 176 mmol). After stirring overnight the solution was evaporated, the residue taken up in CH_2Cl_2 (200 ml) and washed with water (3x200 ml). The organic phase was concentrated and chromatographed on silicagel 60 (500 g, hexane/EtOAc 2:1) to yield 6 (33.7 g, 157 mmol, 91 %) as white crystals: m.p. 91 - 92 °C; $[\alpha]_{365}$ = -60.7 (1 % in EtOH); > 98 % ee; > 99 % (GLC); ^{1}H-NMR ($CDCl_3$): 1.46 (s, 9H, Boc) overlapped by 1.19-1.87 (stack, 5H,-C(3)HC(4)H_2C(5)H_2-), 3.06 (broad s, 2H, -NCH_{ax}-), 3.51 (t, 2H, -CH_2OH) and 3.71 (broad s, 2H, -NCH_{eq}-); IR (KBr): 3463 (-OH), 1672 (N-C=O), 1270 (ester), 1080 (-OH); MS: 215 (M).

[11] **Large-scale preparation of t-butyl (R)-3-[hydroxymethyl]-1-piperidinecarboxylate 6**: Ester 1 (1.33 kg, 4.66 mol) was emulsified in 0.1 M NaCl (24 l) and 0.1 M sodium phosphate buffer pH 7.0 (1.06 l) by vigorous stirring. Lipase P-30 (13.3 g) was added and the pH maintained at 7.0 by addition of 1.0 N NaOH. After consumption of 2.58 kg NaOH solution (~ 53 % conversion, after 28 h) the reaction mixture was extracted with CH_2Cl_2 (2x10 l). The combined organic phases were dried ($MgSO_4$) and evaporated. The residue (1.09 kg) was dissolved in n-hexane (3.25 l) and the (S)-alcohol 3 started to crystallize. After stirring for one day the suspension was cooled to 0 °C and stirred for another two days. The crystals were filtered off and the filtrate evaporated to give crude (R)-ester 2 (585 g) which then was purified from residual 3 by filtration on silicagel 60 {3 kg, 0.040-0.063 mm, equilibrated in n-hexane, elution with hexane/ethyl acetate 4:1 (6 l) and 3:1 (8 l)}. (R) - Ester 2 (512 g, 1.79 mol; 38 %) was obtained as slightly yellowish oil: $[\alpha]_{365}$ = -57.5 [1% in EtOH], $[\alpha]_D$ = -17.1 [1% in EtOH]; 94 % ee; 96 % (GLC). 2 (511 g, 1.79 mol) was dissolved in EtOH (2.5 l). After adding 2 N NaOH (1.0 l, 2.0 mol) the solution was stirred at r.t. for 15 h. The solution was concentrated to a volume of ca.1.5 liter and adjusted to pH 7.0 with 25 % hydrochloric acid. The solution was adjusted with water to a volume of 1.8 liter and extracted with ethyl acetate (2x1 l). The combined organic phases were washed with water (2x1 l), dried ($MgSO_4$) and evaporated. The residue was pulverized and dried *in vacuo* to yield (R)-alcohol 6 (362 g, 1.68 mol, 94 %): $[\alpha]_{365}$ = -55.6 (1 % in EtOH); 94 % ee; 98 % (GLC);

[12] **Determination of the absolute configuration**: (S)-3 (1.0 g, 4.6 mmol, 91 % ee) was incubated in trifluoroacetic acid (5 ml) at 0°C for 45 min. The solvent was evaporated and the residue dissolved in MeOH (10 ml). Sodium hydrogencarbonate (2.5 g) was added slowly in portions and the resulting suspension filtered. The filtrate was passed through Dowex 1-X2 (2 g; OH^--form, elution with 0.1 N NaOH). After repeated extraction (CH_2Cl_2) of the eluate the extract was Kugelrohr-distilled to give white crystals (0.15 g, 1.3 mmol, 28 %) which were identified as (S)-3-(hydroxymethyl)piperidine 7 by ^{1}H-NMR, MS and optical rotation: $[\alpha]_D$ = - 6.8 (3.5 % in pyridine). In Bettoni et al.[19] a maximal specific rotation of + 6.7° is reported for the (R)-isomer.

[13] **Preparation of the catalyst for transesterification**: Lipase P-30 was adsorbed onto diatomaceous earth similar to Tombo et al.[20]: The commercial enzyme (6.0 g) was dissolved/ suspended in 0.05 M sodium phosphate buffer, pH 7.5 (72 ml) and the pH readjusted to 7.5 (0.1 N NaOH). After cooling to 4 °C Hyflo Super Cel (24 g) was added. Acetone of -20 °C (108 ml) was added under efficient stirring

within 25 min and the suspension stirred for further 30 min at 4 °C. The suspension was filtered and the filter cake dried *in vacuo* to yield 30.4 g of catalyst. The preparation contained 3 % (w/w) water (according to KF-determination) and was stored at 4 °C.

[14] **Preparation of t-butyl (R)-3-[hydroxymethyl]-1-piperidine-carboxylate 6 by transesterification:** Racemic alcohol 4 (250 mg, 1.16 mmol) and vinyl acetate (60 µl, 0.56 mol eq.) were dissolved in n-hexane (25 ml) with warming. Enzyme preparation[13] (25 mg) was added and the suspension gently agitated. After 22 h the enzyme was filtered off and the filtrate concentrated. Chromatography on silica gel 60 yielded 6 (84 mg, 0.39 mmol, 34 %) in > 99 % (GLC) and 95 % ee; $[\alpha]_{365} = -36.2$ (1 % in $CHCl_3$), $[\alpha]_D = -11.1$ (1 % in $CHCl_3$).

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[17] **General remarks:** Compound 1 and 4 was kindly supplied by Dr. G. Schmid, 9-11 by Dr. K. Hilpert. Esters 8a-c were synthesized from rac-9 according to standard methods. Lipase P-30 was purchased from Amano Pharmaceutical Co., Nagoya, Jpn. Reagents were from Fluka or Merck and used as received. The pH-stat was a MemoTitrator DL 40 RC (Mettler). ^{1}H-NMR spectra were recorded on a Bruker AC 250 (250 MHz) and mass spectra on a TSP 46 (Finnigan MAT) for 3 or an MS9 Spectrometer (direct inlet, 250 °C) with VG ZAB electronic console and Finnigan MAT data system SS 300 for 2. Optical rotations were measured at 20 °C on a Perkin Elmer 241 Polarimeter.

[18] **Determination of the enantiomeric excess:** 3 and 6 were converted to the trifluoroacetate and the enantiomers separated on a chiral GLC-phase (permethylated β-cyclodextrin (β-CD-M), 25 m capillary column, 110 - 200 °C with 1 °C/min). The enantiomeric excess of 2 was determined via hydrolysis to 6. The morpholine 9 was directly applied on a β-CD-M capillary column (25 m, 110-160 °C with 1 °C/min). The enantioselectivity of the enzymatic acetylation of primary amine 10 was measured by determining the enantiomeric excess of its acetamide product (25 m β-CD-M, 150-200 °C with 1 °C/min). The secondary amine 11 was propionylated prior to analysis (β-CD-M, 50 m capillary column, 170-210 °C with 1 °C/min).

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