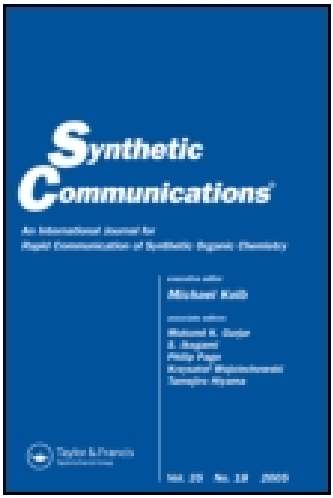

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ENZYMATIC METHOD OF PREPARATION OF OPTICALLLY ACTIVE *trans*-2-AMINO CYCLOHEXANOL DERIVATIVES

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Abstract. Supported Lipase Amano PS-D catalyzes the resolution of (±)-*trans*-2-tert butoxycarbonyl amino cyclohexanol by a selective acylation reaction. Using the supported enzyme gave a much faster reaction compared to existing methodology on similar substrates. A variety of acylating agents were investigated, with vinyl acetate providing the most practical and convenient procedure.

Among the recent examples of the use of enzymes in organic chemistry, the optical resolution of racemic compounds represents a valid alternative to classical procedures.

Much attention has been paid to the enzymatic resolution of racemic amino alcohols, probably due to their importance as chiral building blocks. In particular cyclic 2-amino alcohols such as (±)-*trans* -2-amino cyclohexanol have been the subject of great interest [1].

In the course of our work, the preparation of a sample of homochiral (1S,2S)-1-hydroxy-2-aminocyclohexane was initially attempted from the corresponding tartrate salt according to a literature precedent [2].

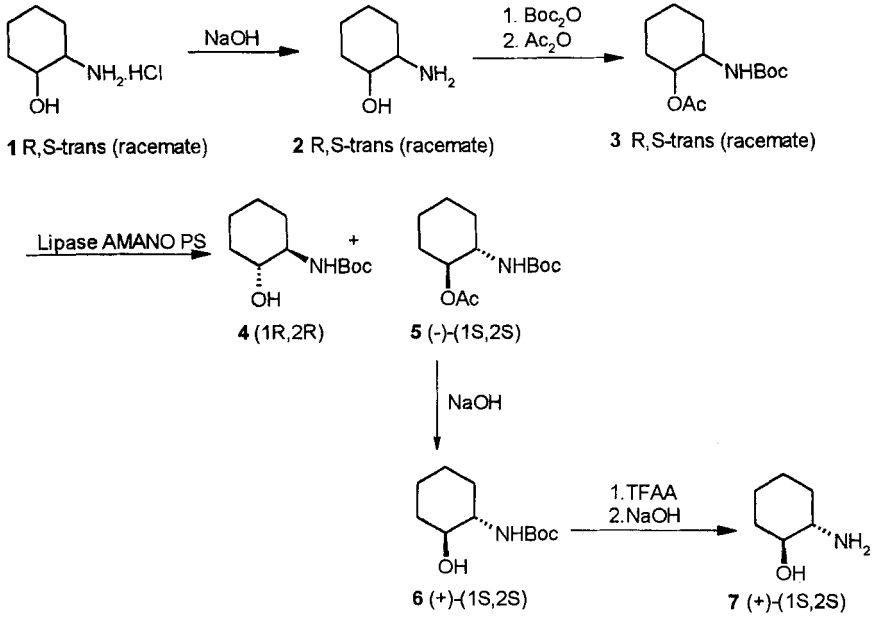
Although this method [2] has been successfully applied to related aminoalcohols, (e.g. the N-methyl analogue) when it was applied to the free amino counterpart it gave unsatisfactory results, prompting us into investigating alternative procedures.

Even though there are a number of approaches to the resolution of trans-1-hydroxy-2-aminocyclohexane [3], we needed a very simple and robust method to be eventually scaled up for industrial preparations.

The enzymatic resolution of these aminoalcohols is scarcely reported. [4,5]

We first tried the enzymatic hydrolysis of **3** using lipase Amano PS [6], (Scheme 1).

Scheme 1. Synthesis of the optically active (1S,2S)-trans-2-aminocyclohexanol (7) *via* lipase catalysed hydrolysis



After the reaction was completed a mixture of hydrolyzed product **4** and remaining acetate **5** was purified by column chromatography on silica gel (44%th yield of **5**). Consequentely, the O,N-protections were removed from **5** to afford **7** in very good yield and high ee (95%).

This method, although valid, was a long stepwise procedure, particularly because our preferred enantiomer was the unreacted one.

A direct acylation , hopefully of the unwanted isomer, would be much more practical.

It has previously been shown that N-protected -2-amino alkan-1-ols can be resolved through enzymatic transesterification reaction.[7]

In order to further speed up the process, a one-step procedure was investigated using unprotected **2** as starting material.

This procedure (N-acylation followed by an enantioselective O-acylation) has been reported in some cases[8,9], although other authors describe the O-acylation as a reversible step, leading at the end of the reaction only to the N-acyl derivatives[10].

We initially reacted compound **2** with acetic anhydride (4eq) as acyl donor, in the presence of the supported enzyme (Pseudomonas cepacea lipase Amano PS-D, 50%w/w) in Toluene/THF 3/1. An immediate conversion into the corresponding racemic N-acetyl derivative was observed by tlc, and subsequently confirmed by NMR analysis after work-up and chromatographic purification. However, on extended reaction times (6 hrs), only traces of the fully acetylated derivative were detected. The N-Acetyl derivative (2mmol) isolated from the previous reaction mixture, was further reacted with acetic anhydride (2ml, 9eq.) in ethyl acetate (4ml) in the presence of the enzyme (50%w/w). After stirring at 20°C for 30 hrs, only 45mg (9% conversion) of the corresponding N,O-diacetyl derivative were isolated by chromatography on silica. In order to verify its optical purity hydrolysis to 1-hydroxy-2-aminocyclohexane was attempted, without success.

Finally, we applied this reaction to racemic N-Boc aminocyclohexanol **8**, since the series of intermediates obtained by using the route of Scheme 1, were already known and characterised (in particular intermediate **6**-(1S,2S)).

To our knowledge, this compound had never been used as substrate for such a resolution. (Scheme 2)

By using the supported enzyme (PLS) in neat vinyl acetate, we achieved a much faster reaction (50% conversion in 6hrs) and obtained the desired (1S,2S) enantiomer **7** in

Scheme 2. Synthesis of the optically active (1S,2S)-trans-2-aminocyclohexanol (7) *via* lipase catalyzed acylation

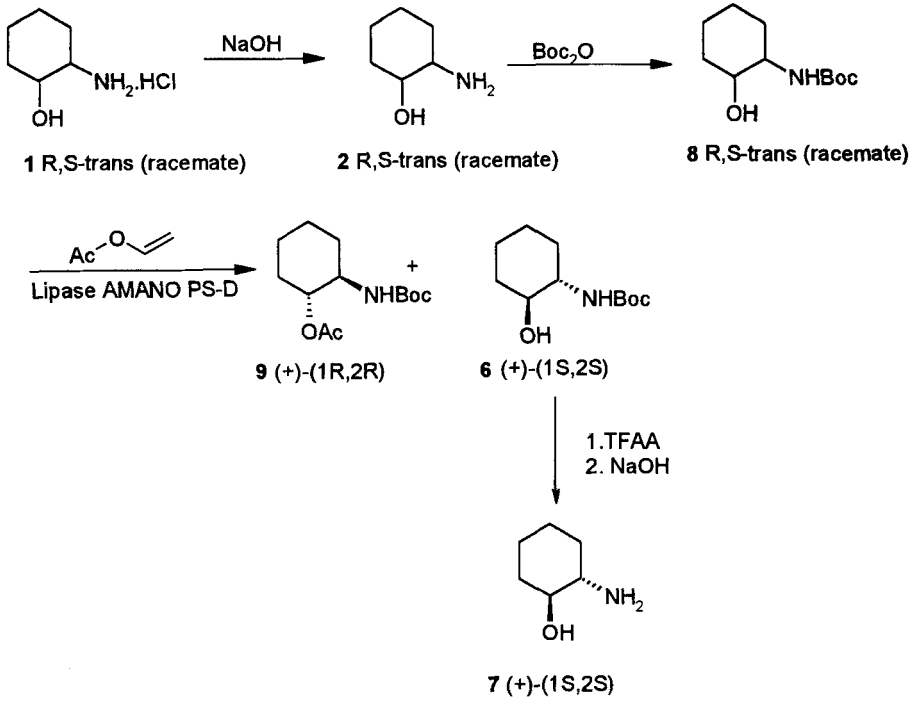


Table 1. Summary of the data on lipase catalysed acylation (Scheme 2)

6-(1S,2S) yield (%) [a]	6-(1S,2S) [b] conv. (%)	9-(1R,2R) [c] $[\alpha]_D$	6-(1S,2S) [d] $[\alpha]_D$	7-(1S,2S) e.e.(%) [e]	E [f]
46	50	+22.3° (*c* 0.8, MeOH)	+23.6° (*c* 0.5, MeOH))	92	79

[a]After flash chromatography. [b]Determined by TLC. [c]Value for opposite enantiomer: 5-(1S,2S) from hydrolysis (scheme 1) $[\alpha]_D$: -24.3° (*c* 1.1, $CHCl_3$). [d]Lit. ref.,[3c] $[\alpha]_D$: +26.5° (*c* 0.4, MeOH). [e]Determined by Chiral HPLC. [f]Lit. *J.Am.Chem.Soc.*, **1982,** *104*, 7294-7299

excellent ee (92%), which is considerably greater than previously reported in the literature [5] (49%ee).

In summary we have reported a very simple and efficient method for the enzymatic resolution of trans-2-amino cyclohexanol, in particular, this method allows the staightforward isolation of the desired (1S,2S) enantiomer.

It is worth noting that by running the reaction in neat vinyl acetate, complete conversion was achieved in a few hours, compared to several days using previous reported conditions [5].

The enzyme was filtered off and used again without significant loss of activity. Future work will focus on the effect of the N-protection on the kinetic of the reaction.

Experimental Section

In the Preparations and Examples,unless otherwise stated:

Melting points (m.p.) were determined on a Büchi m.p. apparatus and are uncorrected.

All temperatures refer to °C. Infrared spectra were measured on a Bruker IFS 48 spectrometer in chloroform-d1 solutions. Proton Magnetic Resonance (^{1}H-NMR) spectra were recorded on a Varian Unity 400 at 400MHz. Chemical shifts are reported in ppm downfield

from Me_4Si as an internal standard, and are assigned as singlets (s), doublets (d), doublet of doublets (dd) or multiplets (m).

Column chromatography was carried out over silica gel 60 (Merck AG Darmstadt, Germany). Mass spectra were recorded on a VG-4triple quadrupole Fison instrument in FAB mode. Elemental analyses were performed by our own analytical group on Carlo Erba elemental analyzer. Optical rotations were determined at 20°C with a Jasco DIP 360 Instrument (l=10 cm, cell volume=1ml, λ=589 nm).

Solutions were dried over anhydrous sodium sulphate.

Methylene chloride was redistilled over calcium hydride; tetrahydrofuran was redistilled over sodium; ethyl ether was redistilled over sodium and ethyl acetate was dried over activated molecular sieves.

Lipase Amano PS and Amano PS-D were a kind gift from Amano Pharmaceutical Co .

(±) trans-1-Hydroxy-2-tert-butoxycarbonylamino-cyclohexane (8)

Intermediate **8** was easily prepared from commercially available (±)-*trans*-2-amino cyclohexanol hydrochloride according to a literature example [6]. m.p. 94-96°C;

^{1}H-NMR ($CDCl_3$) δ= 1.28 (m, 4H), 1.44 (s, 9H), 1.70 (m, 2H), 2.00 (m, 2H), 3.28 (m, 3H), 4.52 (bs, 1H); IR (Nujol) 3298-3381 cm^{-1} (ν OH + NH), 1676 cm^{-1} (νc=o), Analysis calc'd for $C_{11}H_{21}NO_3$: C, 61.35; H, 9.85; N, 6.51; found: C, 61.20; H, 9.92; N, 6.49.

***(1S,2S)-1-Hydroxy-2-tert-butoxycarbonylamino-cyclohexane (6), (1R,2R)-1-acetoxy-2-tert-butoxycarbonylamino -cyclohexane* (9)**

Supported lipase Amano PS-D (65 mg, 50%w/w) was added to a solution of (±) trans-N-tert-butoxycarbonyl aminocyclohexanol (**8**, 107.65 mg, 0.5 mmol) in vinyl acetate (2mL). The

mixture was stirred at 35°C for 6h and then at 20 °C overnight. The enzyme was filtered off, and the vinyl acetate evaporated. The crude was chromatographed on Silica using Petroleun/Diethyl ether 8/2 as eluent to give (+) (1R,2R)-1-acetoxy-2- tert-Butoxycarbonyl amino-cyclohexane (**9**, 61mg, 47%th). m.p. 100-102°C; spectroscopic data in agreement with data reported for its enantiomer **5** obtained from hydrolysis reaction [6] (Scheme 1): ^{1}H-NMR ($CDCl_3$) δ= 1.18-1.47 (m, 4H), 1.44 (s, 9H), 1.69-1.76 (m, 2H), 1.97-2.07 (m, 2H), 2.06 (s, 3H), 3.56 (m, 1H), 4.50-4.60 (m, 2H); IR (Nujol) 3371 cm^{-1} (ν NH), 1691-1728 cm^{-1} (νc=o); MS *m/z* 258 $(MH)^+$; $[\alpha]_D$: +22.3° (*c* 0.8, methanol); Analysis calc'd for $C_{13}H_{23}NO_4$: C, 60.66; H, 9.03; N, 5.44; found: C, 60.66; H, 8.87; N, 5.32.

By using petroleum/diethyl ether 6/4 as eluant unreacted (+) (1S,2S)-1-hydroxy-2-tert-butoxycarbonylamino-cyclohexane (**6**, 50 mg, 46%th) was recovered. m.p. 108-110°C;

^{1}H-NMR ($CDCl_3$) δ= 1.36-1.70 (m, 4H), 1.45 (s, 9H), 1.70 (m, 2H), 1.96-2.04 (m, 2H), 3.23-3.28 (m, 1H+2H), 4.52 (bs, 1H); IR (Nujol) 1676 cm^{-1} (νc=o); MS *m/z* 216 $(MH)^+$; $[\alpha]_D$: +23.6° (*c* 0.5, methanol), literature value [3c] for **6**-(1S,2S) $[\alpha]_D$: +26.5° (*c* 0.4, methanol); Analysis calc'd for $C_{11}H_{21}NO_3$: C, 61.35; H, 9.85; N, 6.51; found: C, 61.44; H, 10.00; N, 6.49.

(1S,2S)-1-Hydroxy-2amino-cyclohexane (7)

To a solution of (1*S*,2*S*)-1-hydroxy-2- (*t*-butoxycarbonylamino)-cyclohexane (**6**, 45mg, 0.2mmol) in dry dichloromethane (2 mL), trifluoroacetic acid (0.4mL, 5mmol) was added in one portion. The mixture was stirred at 20°C for 1.5h and than evaporated in vacuo. To a suspension of the residue (70mg) in water (0.5mL), a 32%w/w NaOH solution (0.04mL, 0.4mmol) was added. After stirring at 20°C for 1h, the mixture was extracted with dichloromethane (3x2mL). The extract was dried over Na_2SO_4 and evaporated in vacuo to give the title compound as a white solid (7, 10mg, 2 steps 42%th). m.p. 90-91°C;

^{1}H-NMR (DMSO-d_6) δ= 0.9-1.2 (m, 4H), 1.44 (bs, 2H), 1.54 (m, 2H), 1.69 (m, 2H), 2.23 (m, 1H), 2.88 (m, 1H), 4.50 (bs, 1H); MS *m/z* 115 $(M)^+$; $[\alpha]_D$: +40,4° (*c* 0.85, methanol), literature value [3a] for 7-(1S,2S) $[\alpha]_D$: +48.2° (*c* 1.0, methanol).

The enantiomeric excess of 7 (ee = 92%) was determined by HPLC using a chiral column after conversion into its *m*-toluoyl derivative with *m*-toluoyl chloride.

Column: CHIRALPAK AD 25cmx4.6mm; Mobile phase: hexane/isopropanol=95/5.

The retention times of (1R,2R) and (1S,2S) isomers were 18 and 24.5min. respectively at a flow rate of 1.5ml/min.

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