Pergamon

Tetrahedron: Asymmetry, Vol. 7, No. 8, pp. 2247-2250, 1996

0957-4166/96 $15.00 + 0.00

PII: S0957-4166(96)00279-0

Efficient procedures for the large-scale preparation of (*1S,2S*)-*trans*-2-methoxycyclohexanol, a key chiral intermediate in the synthesis of tricyclic β-lactam antibiotics

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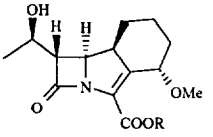
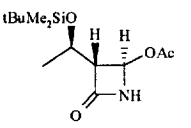
Abstract: Enzymatic and chemical resolution methods suitable for preparation of multi-kg quantities of (+)-(*1S,2S*)-2-methoxycyclohexanol from (+/-) *trans*-2-methoxycyclohexanol have been developed. The enzymatic resolution was found to offer a particularly simple process affording the resolved product in good yield and excellent enantiomeric excess and has now been operated consistently on a manufacturing scale.

Introduction

The Trinems **1** and **2** are members of a new class of totally synthetic β-lactam antibiotics, bearing the novel feature of a tricyclic skeleton, discovered recently by Glaxo Wellcome S.p.A, Italy.[1] Compounds **1** and **2**, currently in full clinical development at Glaxo Wellcome plc, are prepared *via* a multistep synthesis starting from the commercially available acetoxyazetidinone **3** and the chiral alcohol **4**.



1 R = Na
2 R = $CH(CH_3)OC(O)OC_6H_{11}$



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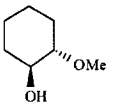

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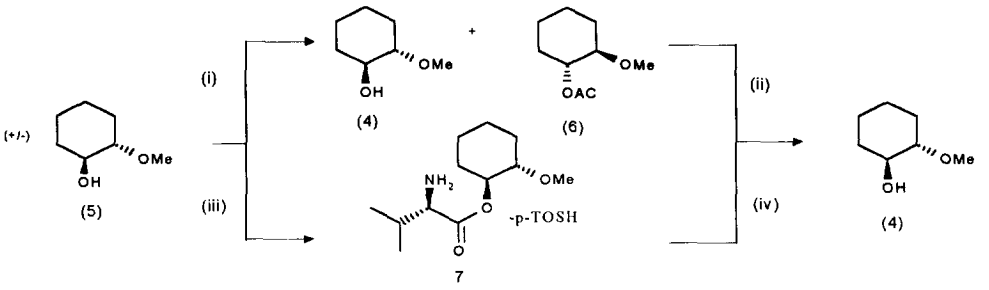
Figure 1

An efficient synthesis of the chiral alcohol **4** was therefore a prerequisite for the large-scale preparation of compounds **1** and **2**. We report here two routes to compound **4** from the racemate; firstly a lipase mediated transesterification of the unwanted (*1R,2R*) enantiomer followed by separation of the required alcohol **4** from the acylated species **6** by a simple partition method, and secondly the synthesis of diastereomeric L-valine ester derivatives followed by separation of the required diastereomer **7** by fractional crystallisation and hydrolysis back to the alcohol **4** (Scheme 1).

Results and Discussion

Enzymatic resolution: Lipases are well known for their ability to perform transesterification reactions in organic solvent systems in the presence of a suitable activated acyl donor such as a vinylic ester.[2] It has previously been shown that the racemic acetate of *trans*-2-methoxycyclohexanol may be hydrolysed to yield (-)-(*1R,2R*)-2-methoxycyclohexanol in high enantiomeric excess using SAM-II lipase from *Pseudomonas* spp.[3], or pig liver acetone powder.[4] Similarly the racemic butanoate of *trans*-2-methoxycyclohexanol was hydrolyzed by lipases from *Pseudomonas* and *Candida* spp. to yield the (-)-enantiomer of *trans*-2-methoxycyclohexanol.[5] Since the present work required the (+)-(*1S,2S*) enantiomer **4**, we considered the enantioselective acylation of the racemic alcohol **5** to provide a more direct route to the target compound without the need for subsequent ester hydrolysis.



(i) Lipase Et_3N, C_6H_{12}, vinyl accetate (ii) aqueous extraction (iii) L-Valine, p-TOSH, toluene, reflux, fractional crystallisation (iv) NaOH

Scheme 1

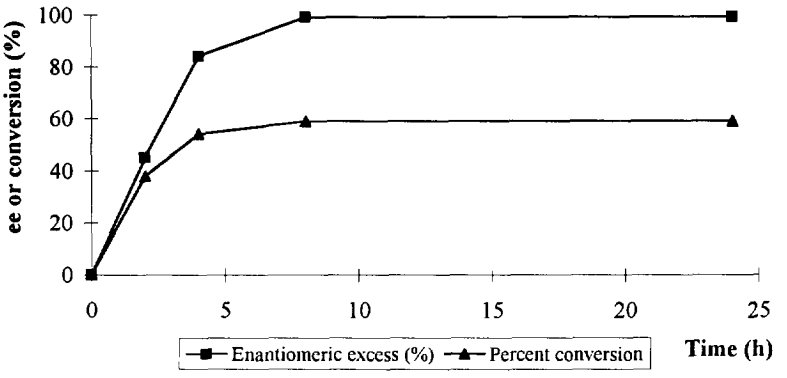
Several commercially-available lipases were screened for their ability to acylate racemic *trans*-2-methoxycyclohexanol **5** enantioselectively (scheme 1). Reactions were carried out in cyclohexane using vinyl acetate as acyl donor, and were monitored by chiral GC and HPLC.[6] The highest enantiomeric excess (ee) values were obtained using enzymes from *Candida antarctica* and *Pseudomonas fluorescens*. The *Candida antarctica* enzyme was commercially available in an immobilised form[7] and was particularly suitable as it exhibited excellent stability compared to free enzymes tested. The immobilised enzyme retained over half its initial activity after nine cycles of use. Studies with free *Pseudomonas fluorescens* lipase (Biocatalysts Ltd) demonstrated that activity decreased by 75 % over 4 cycles of use. However this enzyme could be immobilised onto celite powder to give preparations with stability and activity equivalent to the commercially-available immobilised *Candida antarctica* preparation.

Optimisation of the process showed changes in reaction solvent to be the most significant parameter. Of those solvents studied (cyclohexane, n-hexane, acetonitrile, THF, dichloromethane) cyclohexane supported the fastest bioconversion. No reaction was observed when dichloromethane was used. Acetaldehyde, resulting from transesterification of vinyl acetate, caused inhibition in this reaction. This was reversible however, since acetaldehyde-treated enzyme could be restored to its initial activity by washing with cyclohexane and recycling under standard reaction conditions. Reaction kinetics were very similar when isopropenyl acetate was used in place of vinyl acetate.

The optimised reaction conditions employed the *Candida antarctica* lipase as bio-catalyst (37.4 g/L). Vinyl acetate was utilised as acyl donor at a level of 1.7 M; substrate concentration was 1.4 M. A small quantity of triethylamine (0.16 M) was included to neutralise any acetic acid liberated by hydrolysis of vinyl acetate. Under these reaction conditions an ee of >98% was achieved in 6-8h. The conversion of racemic methoxycyclohexanol (**5**) after this time was *ca.* 55% by GC. A typical reaction profile is illustrated in fig. 2.
Cyclohexane was a particularly suitable solvent both for the bioconversion and, due to its very low polarity, for the selective extraction of the required alcohol **4** into water at the end of a reaction cycle. The desired alcohol **4** was then isolated in high ee (typically >99%ee) and in 36%th yield by extraction into ethyl acetate followed by evaporation of organic solvent.

L-valine resolution: The resolution of optically active alcohols by esterification with L-amino acids in the presence of *p*-toluenesulphonic acid has been reported.[8] The formation of crystalline ester derivatives and the ability to optimise the efficiency of the resolution either by varying the amino acid, or by using alternative acids made this approach very attractive to us for providing a large scale resolution of the racemic *trans*-2-methoxycyclohexanol **5.** In practice, we have found that a remarkably simple and efficient resolution could be achieved by heating the racemic alcohol **4** and L-valine in toluene at reflux in the presence of *p*-toluenesulphonic acid (1.3 molar equiv). The desired (+)-L-valine ester **7** was obtained directly in good yield (typically > 27%th) and high diastereomeric excess (d.e; 96% by PMR) on filtration of the cooled reaction mixture. Treatment of the (+)-L-valine ester **7** in a mixture of *t*-butylmethyl ether and aqueous sodium hydroxide under phase-transfer conditions then gave the chiral alcohol **4** in 95%th recovery.

Figure 2 Reaction profile for lipase-mediated resolution of (+-)-trans-2-methoxycyclohexanol



In conclusion, both chemical and enzymatic methods have been developed for the resolution of (±)-*trans*-2-methoxycyclohexanol. Whilst both processes are simple to operate and afford material of good optical purity, the enzymatic procedure is advantageous both economically and environmentally and is the current method of choice for production of the chiral alcohol **4** on a multi-kg scale.

Acknowledgements: We would like to thank Peter Faulkner for assistance with pilot plant operations, and Chris Drake for development of the chiral GC assay for (+-)-*trans*-2-methoxycyclohexanol.

Experimental

Materials Lipases were obtained from Biocatalysts Ltd, Novo-Nordisk, Fluka, Amano or Rohm- Pharma. Analytical Reagent and HPLC grade solvents were used throughout and were obtained from Rathburn Chemicals Ltd. All chemicals used in this work were Analytical Reagent grade and were obtained from BDH or Fisons.

General procedures: Melting points were determined on a Electrothermal digital melting point apparatus and are uncorrected. Microanalyses were performed using Leco CHNS-932. Optical rotations were measured with an Optical Activity Ltd polarimeter using a 1ml capacity cell (path length =10cm) for CH_2Cl_2 solutions. Spectra were recorded using the following instruments: ^{1}H NMR , Varian Unity 400MHz spectrometer in DMSO-d_6, chemical shifts are reported in δ units downfield from $(CH_3)_4Si$. Mass Spec. , Hewlett Packard 5989A'MS-Engine'. Infra-red , Nicolet 20 SXC FT-IR spectrometer. Solvents were evaporated on a rotary evaporator.

Methods: *Immobilisation of P. fluorescens lipase:* Lipase (1 g) was dispersed in phosphate buffer (0.01 M $NH_4H_2PO_4$ in water, pH 7; 40 ml) then added to adsorbent (10 g). The slurry was left at room temperature with occasional mixing for 1 h. Butan-1-ol (40 ml) was added, and the mixture reduced *in vacuo* at 37°C to a moist powder. Cyclohexane (200 ml) was added and rotary evaporation continued for 30 min. at 37°C.

Preparation of (1S,2S)-2-methoxycyclohexanol 4 by enzymatic transesterification:

Immobilised lipase (10 g) was added to a solution of (±)-*trans*-2- methoxycyclohexanol (50 g), vinyl acetate (42 ml) and triethylamine (6 ml) in cyclohexane (167 ml). The mixture was stirred magnetically at room temperature. The extent of resolution was determined either by HPLC or GC analysis of reaction mixtures. Reactions were terminated when the enantiomeric excess was > 98% (GC). Reaction mixture was harvested by filtration under slight vacuum (number 4 sinter funnel). The enzyme filter cake was washed through with reaction solvent (*ca* 0.1 reaction vol.) and then the enzyme recovered and stored in a sealed container at 4°C for use in future cycles. The filtrate was extracted vigorously with water (4 x 1/2 vol.) and the extracts were combined. Residual methoxycyclohexanol acetate which had partitioned into water was removed by back-extraction with reaction solvent (2 x 1/4 vol.). The aqueous layer was taken, sodium chloride added to give 5 M concentration, and the solution was extracted with ethyl acetate (2 x 1/2 vol.). The ethyl acetate extracts

were combined and reduced *in vacuo* at 30°C to yield the required alcohol **4** as a clear or pale yellow oil (yield 34-36% w/w corrected), $[\alpha]_D^{20}$ +69.5 (C =2, CH_2Cl_2), Lit.[2] $[\alpha]_D^{20}$ +69.5 (C =2, CH_2Cl_2), 99% ee by chiral GC.

Preparation of **4** *by resolution of the L-valine ester p-toluenesulphonate ester*: A mixture of L-valine (5 g, 43 mmol), *trans*-2-methoxycyclohexanol **5** (7.2 g, 55 mmol) and p-toluenesulphonic acid monohydrate (10.6 g, 56 mmol) in toluene (60 ml) was heated under reflux for 18 h. The resultant solution was allowed to cool to room temperature then stirred at room at 0 to 5 for 1 h then filtered to give the (+)-L-valine ester as a white solid (6.08 g, 27%th), m.p 247.5°C, ^{1}H NMR (DMSO-d_6) δ 1.01 (dd, 6H, J=7Hz), 1.13-1.48 (m, 4H), 1.66 (d, 2H, J=8.7Hz), 1.88 (m, 1H), 2.08-2.23 (m, 2H), 2.31 (s, 3H), 3.18 (m, 1H), 3.25 (s, 3H), 3.97 (s,1H), 4.78 (m, 1H, J =4.2Hz), 7.13 (d, 2H J =7.9Hz), 7.49 (d, 2H, J=8.0Hz), 8.27 (s,3H); IR (nujol) 2952, 2854, 1750, 1633, 1542, 1462; m/z M^++1= 230; Anal. calcd. for $C_{19}H_{31}NO_6S$: C, 56.8; H, 7.8; N, 3.5; S, 8.0. Found: C, 57.0; H, 7.9; N, 3.5; S, 8.0.

A suspension of the (+)-L-valine *p*-toluenesulphonate ester **7** (25 g, 62 mmol) and *t*-butylammonium hydrogen sulphate (1g, 6.2 mmol) in *t*-butylmethyl ether (100 ml) and sodium hydroxide solution (3.3 M) (150 ml) was stirred under reflux for 18 h then cooled to room temperature. The organic phase was collected and the aqueous phase extracted with t-butylmethyl ether (3 x 50 ml). The combined organic extract was washed with brine (3 x 50 ml) and concentrated *in vacuo* to give *trans*-(*1S,2S*)-2- methoxycyclohexanol **4** as a colourless oil (7.7g, 95%th),b.p 66°C (9 mbar); $[\alpha]_D^{20}$ +69 (C =2, CH_2Cl_2), Lit.[2] $[\alpha]_D^{20}$ +69.5 (C =2, CH_2Cl_2), 96% ee by chiral GC analysis.

HPLC analysis of trans-2-methoxycyclohexanol and trans-2-methoxycyclohexanol acetate: HPLC hardware consisted of a Constametric III pump (LDC Analytical, Florida, USA) with a Spectromonitor III variable wavelength UV-detector (LDC) and a Chiramonitor (ACS Ltd, Cambridge, UK) connected in series. Analysis was performed using a Spherisorb 5 μm C_6 column (15 cm x 0.46 cm). A linear gradient from 6 to 30 % v/v acetonitrile in water in 10 minutes was employed. The flow rate was 2 ml/min.

Sample preparation Reaction mixture (100 μl) was centrifuged (13,000 g; 1 min.), supernatant (50 μl) was taken and volatile organic solvent was blown off using a compressed air line. The oily residue was dissolved in water (180 μl) and 100 μl of this was injected onto the column.

The enantiomeric excess was monitored by measuring the ratio of optical rotation to UV absorbance, by reference to a calibration curve (data not shown). *Trans*-2-methoxycyclohexanol eluted after 3.2 min and *trans*-2-methoxycyclohexanol acetate after 9.2 min.

Chiral GC analysis of trans-2-methoxycyclohexanol: GC analysis was performed using a Perkin Elmer model 8420 capillary gas chromatograph. Separation of the (*1S,2S*) and (*1R,2R*) enantiomers of *trans*-2-methoxycyclohexanol was achieved using a 20 m Chiraldex B-PH column running isothermally at 70°C.

Sample preparation Reaction mixture (100 μl) was centrifuged at 13,000 g for 1 min. Supernatant (25 μl) was pipetted into a reactivial. Ethyl acetate (175 μl) was added followed by trifluoroacetic anhydride (50 μl) . The capped reactivial was heated to 70°C for 15 min. After cooling, 20 μl was diluted to 1 ml (ethyl acetate) then 1 μl analysed by GC. (*1R,2R*)-2-methoxycyclohexanol eluted after 6.4 min and (*1S,2S*)-2-methoxycyclohexanol after 7.0 min.

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6) The HPLC separation was non-chiral but employed a Chiramonitor as well as a UV detector; hence the ee could be estimated directly by analysing the reaction mixture and measuring the ratio of optical rotation to UV absorbance at 195 nm (data not shown).

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(*Received in UK* 24 *April* 1996; *accepted* 11 *June* 1996)