Pergamon

Tetrahedron Letters, Vol. 36, No. 7, pp. 1113-1116, 1995
Elsevier Science Ltd
Printed in Great Britain
0040-4039/95 $9.50+0.00

0040-4039(94)02408-1

Synthesis of Homochiral L-(S)-*tert*-Leucine *via* a Lipase Catalysed Dynamic Resolution Process

Nicholas J. Turner* and James R. Winterman

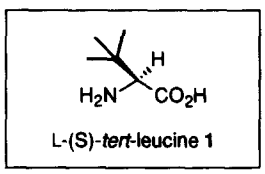
Department of Chemistry, University of Exeter, Stocker Road, Exeter EX4 4QD, UK.

Raymond McCague, Julian S. Parratt, and Stephen J.C. Taylor

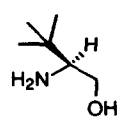
Chiroscience Ltd., Cambridge Science Park, Milton Road, Cambridge CB4 4WE, UK.

Abstract: Treatment of (±)-2-phenyl-4-*tert*-butyloxazolin-5(4*H*)-one **8** with Lipozyme® (*Mucor miehei*) in toluene containing *n*-butanol and a catalytic amount of triethylamine resulted in a 94% yield of (S)-*N*-benzoyl *tert*-leucine butyl ester **9** (99.5% e.e.). Subsequent two step hydrolysis (Alcalase® followed by 6N HCl, reflux) yielded homochiral L-(S)-*tert*-leucine **1**.

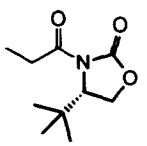
L-(S)-*tert*-leucine **1** is a non-proteinogenic chiral α-amino acid that has found widespread use as both a chiral auxillary[1] and increasingly as a component of potentially therapeutic pseudopeptides.[2] In addition the derivative L-(S)-*tert*-leucinol **2** has been widely used in a range of different chiral auxillaries and ligands (e.g. **3-5**) (**Scheme 1**).[3] The presence of the sterically demanding *tert*-butyl group presents unique problems for the preparation of **1** in homochiral form. Amongst a number of methods reported for the synthesis of **1**[4], the system devised and operated by Degussa AG is particularly elegant employing an enzyme catalysed reductive amination of 3,3-dimethyl-2-oxobutyric acid.[5] Although this process has been successfully operated on a large scale, it suffers from the drawback of needing to recycle the nicotinamide cofactor NADH. We therefore sought to devise a new and efficient method for the preparation of (S)-**1**.



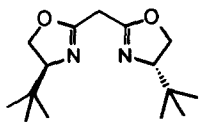
L-(S)-*tert*-leucine **1**



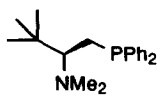
2



3



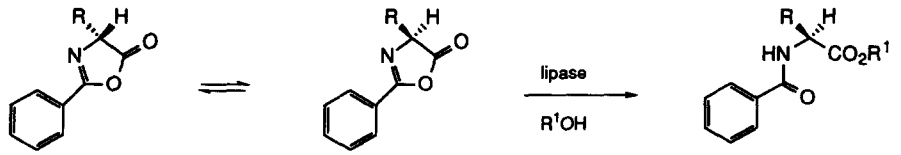
4



5

Scheme 1

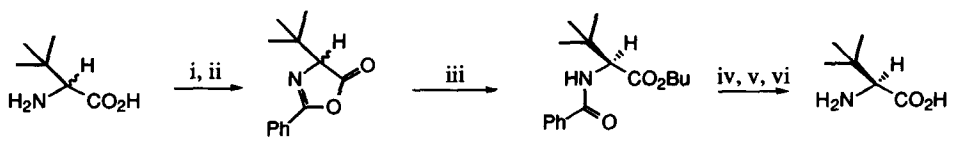
Both Bevinikatti[6] and Sih[7] have reported the enzyme catalysed ring opening of 2-phenyl-4-alkyl oxazolin-5(4*H*)-ones **6** using either water or simple alcohols as the nucleophiles (**Scheme 2**). This reaction has a number of attractive features (i) the substrate oxazolin-5-ones can be easily prepared *via* cyclodehydration of the corresponding *N*-benzoyl amino acids, (ii) owing to the relatively low pKa (~8.9)[8] of the C-4 proton the unreactive enantiomer of **6** undergoes facile epimerisation to the reactive enantiomer resulting in a dynamic resolution process (>50% yield) and (iii) the *N*-benzoyl group of the product can be hydrolytically cleaved without any attendant racemisation.



(R)-6 (S)-6 (S)-7

Scheme 2

Examination of Bevinikatti's work[6], using alcohols as the nucleophile, revealed that the oxazolin-5(4*H*)-one derived from *N*-benzoyl-DL-alanine (**6**, R = Me) was converted to the corresponding ester **7** but with modest enantiomeric excess (57% e.e.) after 45% conversion. Under similar conditions (Lipozyme®-*Mucor miehei*, toluene, 2 equiv. of ^{n}BuOH, Et_3N) we found that the phenylalanine derived compound 2-phenyl-4-benzyl oxazolin-5(4*H*)-one (**6**, R = Bn) yielded the butyl ester **7** ($R^1 = {}^{n}Bu$) in 69% yield and 73% e.e. Omission of the Et_3N from the reaction resulted in a significant decrease in both the yield (59%) and the enantiomeric excess (55%) of the product. Upon using the 4-*tert*-butyl derivative **8** the reaction was found to differ markedly as described below (**Scheme 3**).



(±)-1 8 9 L-(S)-1

Scheme 3: i) PhCOCl, NaOH, (80%); ii) Ac_2O (95%); iii) Lipozyme®, toluene, ^{n}BuOH (2 eq.), Et_3N (25 mol%) (94%); iv) Alcalase®, pH 8.0; v) 6N HCl, reflux; vi) Amberlite IRA-67 (free base).

2-Phenyl-4-*tert*-butyloxazolin-5(4*H*)-one **8** was prepared in good yield from DL-*tert*-leucine **1** *via* *N*-benzoylation (PhCOCl, Schotten-Baumann conditions, 80%) followed by dehydration (Ac_2O, 95%). Treatment of **8** with Lipozyme® in dry toluene containing *n*-butanol (2 equivalents) and triethylamine (25 mol %) resulted in conversion to *N*-benzoyl-L-*tert*-leucine butyl ester **9** (94% yield, 99.5% e.e.) (**Scheme 3**). Hydrolysis of **9** under standard conditions (6N HCl, reflux, 24 h) yielded L-(S)-*tert*-leucine **1** in 80% yield after purification. However, the enantiomeric excess of **1** was found to be only 73%, presumably due to partial reformation of the oxazolin-5(4*H*)-one **8** during the hydrolysis step. This racemisation could

be easily prevented by initial hydrolysis of the butyl ester (Alcalase®- *Bacillus licheniformis*, pH 8.0) followed by debenzoylation as before to give L-(S)-**1** (99.5% e.e.) (see ref. 9 for experimental detail).

In order to shed light on the factors influencing this reaction we systematically investigated a number of important parameters (**Table 1**). These reactions were carried out using lower ratios of enzyme:substrate and hence proceeded more slowly. Remarkably, despite the long reaction times (up to 13 days) there was no evidence for any competing background, non-enzyme catalysed, alcoholysis of the oxazolinone **8** as evidenced by the high enantiomeric excess of the product **9** in certain cases (entries 1 and 3).

Table 1: Effect of various parameters on the conversion of **8** to **9**.

entry	alcohol	Et_3N	yield/%	time/days	e.e./%
1	BuOH	yes	67	13	99.5
2	BuOH	no	44	15	79.9
3	EtOH	yes	56	11	97.1
4	EtOH	no	59	11	68.0
5	MeOH	yes	47	11	80.1
6	MeOH	no	30	11	38.6

The reaction was found to be sensitive to both the presence of a catalytic amount of triethylamine and also the nature of the nucleophilic alcohol. Although we have not yet obtained direct evidence to justify the following proposals we believe that at this stage they provide an adequate explanation for the observations. We believe that the immobilised lipase is slowly converted, in the presence of the substrate **8**, into an isozyme that has lower enantioselectivity, but that also this isozyme can be inhibited by triethylamine. Thus the effect of adding triethylamine is always to improve the enantiomeric excess of the product. Support for this proposal was derived from the large scale biotransformation of **8** in which a higher substrate concentration ensured that the reaction proceeded faster and yielded the butyl ester **9** in 97% e.e without the need for triethylamine.[10] The increase in e.e. with variation of the alcohol (BuOH>EtOH>MeOH) is presumably due to greater selectivity in the deacylation of the enzyme bound intermediate.

In summary, an efficient method has been developed for the conversion of DL-*tert*-leucine to homochiral L-*tert*-leucine **1**, employing an enzyme catalysed dynamic resolution in the key step. The control of the enantioselectivity of the enzymic resolution by the addition of triethylamine suggests that this may be a useful, simple, strategy for improving the enantiomeric excess of related enzymic transformations. We are currently exploring the application of this method to the synthesis of other sterically hindered α-amino acids.

Acknowledgement: We are grateful to the EPSRC for the provision of a CASE studentship (JRW).

References and Notes:

1. Behnen, W.; Dauelsberg, C.H.; Wallbaum, S.; Martens, J. *Synth. Commun.*, 1992, **22**, 2143; Hashimoto, S.I.; Koga, K. *Chem. Pharm. Bull.*, 1979, **27**, 2760.
2. Fauchere, J.-L; Petermann, C. *Helv. Chim. Acta*, 1980, **63**, 824.
3. For leading references see: (*oxazolidinone* **3**) McKennon, M.J.; Meyers, A.I.; Dranz, K.; Schwarm, M. *J. Org. Chem.*, 1993, 58, 3569; Evans, D.A.; Anderson, J.C.; Taylor, M.K. *Tetrahedron Lett.*, 1993, **34**, 5563: (*bis-oxazoline* **4**) Lowenthal, R.E.; Masamune, S. *Tetrahedron Lett.*, 1991, **32**, 7373; Corey, E.J.; Imai, N.; Zang, H.Y. *J. Am. Chem. Soc.*, 1991, **113**, 728: (*phosphine* **5**) Hayashi, T.; Konishi, M.; Fukushima, M.; Kanehira, K.; Hioki, T.; Kullisha, M. *J. Org. Chem.*, 1983, **48**, 2195.
4. Miyazawa, T.; Takashima, K.; Mitsuda, Y.; Yamada, T.; Kuwata, S.; Watanabe, H. *Bull. Chem. Soc. Jpn.*, 1979, **52**, 1539; Steglich, W.; Frauendorfer, E.; Weygand, F. *Chem. Ber.*, 1971, **104**, 687; Viret, J.; Patzelt, H.; Collet, A. *Tetrahedron Lett.*, 1986, **27**, 5865; Kunz, H.; Pfrengle, W.; Sager, W. *Tetrahedron Lett.*, 1989, **30**, 4109; Corey, E.J.; Link, J.O. *J. Am. Chem. Soc.*, 1992, **114**, 1906; Inaba, T.; Kozono, I.; Fujita, M.; Ogura, K. *Bull. Chem. Soc. Jpn.*, 1992, **65**, 2359; Groth, U.; Huhn, T.; Porsch, B.; Schmeck, C.; Schöllkopf, U. *Liebigs Ann. Chem.*, 1993, 715.
5. Schmidt, E.; Bossow, B.; Wichmann, R.; Wandrey, C. *Kem. Ind.*, 1986, **35**, 71.
6. Bevinakatti, H.S.; Newadkar, R.V.; Banerji, A.A. *J. Chem. Soc. Chem. Commun.*, 1990, 1091; Bevinakatti, H.S.; Banerji, A.A.; Newadkar, R.V.; Mokashi, A.A. *Tetrahedron: Asymmetry*, 1992, **3**, 1505.
7. Gu, R.-L.; Lee, I.S.; Sih, C.J. *Tetrahedron Lett.*, 1992, **33**, 1953; Crich, J..; Brieva, R.; Marquart, P.; Gu, R.-L.; Flemming, S.; Sih, C.J. *J. Org. Chem.*, 1993, **58**, 3252.
8. de Jersey, J.; Zerner, B. *Biochemistry*, 1969, **8**, 1967.
9. *Preparation of L-(S)-*tert*-leucine*: To a solution of 2-phenyl-4-*tert*-butyloxazoline-5(4*H*)-one **8** (1 g, 4.60 mmol) in toluene (80 ml) was added Lipozyme® (10 g), *n*-butanol (9 mmol, 2 eq.), and Et_3N (150 µl). The reaction mixture was shaken in an orbital incubator at 30 °C and 220 r.p.m. for 5 days after which time the immobilised enzyme was removed by filtration and the solvent removed by evaporation under reduced pressure. The crude material was purified by column chromatography on silica [eluent; petrol (40/60):ethyl acetate = 4:1] to yield *N*-benzoyl-L-*tert*-leucine butyl ester **9** (1.26 g, 94%, 99.5% e.e.). A sample of the butyl ester **9** (1g, 3.4 mmol) was dissolved in a mixture of propan-2-ol (8 ml) and potassium phosphate buffer (72 ml, pH 8, 0.1M). To this solution was added Alcalase® (4 ml) and the reaction stirred for 2d. The reaction mixture was basified (2M NaOH) to pH 10 and extracted with ethyl acetate (3 x 50ml). The organic phase was dried and evaporated to yield 416 mg of unreacted starting material. The aqueous phase was acidified to pH 2 (2M HCl) and extracted with ethyl acetate (3 x 100 ml). The organic fractions were combined, washed with brine (50 ml), dried and concentrated to give *N*-benzoyl-L-*tert*-leucine (530 mg, 59%) which was used directly in the next step. *N*-Benzoyl-L-*tert*-leucine (450 mg, 1.9 mmol) was dissolved in propan-2-ol (3 ml) and hydrochloric acid (6M, 8 ml) and heated at 70 °C for 15h after which the solvent was removed by evaporation under reduced pressure to give an off-white solid. The residue was dissolved in water (5 ml) and stirred with Amberlite IRA-67 resin (1 g, free base form) for 15h. The resin was removed by filtration and the solution evaporated under reduced pressure to give L-(S)-*tert*-leucine **1** as white solid (184 mg, 1.4 mmol, 74%; 99.5% e.e.). [E.e. assay for *tert*-leucine; Sumichiral OA-5000 (15 cm x 4.6 mm), eluant 2 mM $CuSO_4$:MeOH = 85:15, flow rate 1 ml min^{-1}, detection; UV λ = 254 nm; retention times L-**1** = 13 min, D-**1** = 23 min.
10. For scale up of the biotransformation, a solution of the oxazolin-5(4*H*)-one **8** in *iso*-octane (200g per litre) at 50 °C was treated with *n*-butanol (1.5 equiv.) and Lipozyme® (equal wt. to **8**). After 24h, the product ester **9** was formed at 96% conversion in 97% e.e. Under these conditions, the higher concentration of the oxazolin-5(4*H*)-one **8** appears to overcome the requirement for the triethylamine.

(*Received in UK* 8 *November* 1994; *revised* 7 *December* 1994; *accepted* 9 *December* 1994)