A Convenient Chemoenzymatic Synthesis of (*R*)- and (*S*)-(Chloromethyl)oxirane

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(*R*)- and (*S*)-1-Chloro-3-tosyloxypropan-2-ol have been prepared by biocatalysed enantioselective esterification in hexane, and in turn could be readily converted into optically active (chloromethyl)oxirane in high yield.

Optically active (chloromethyl)oxirane (epichlorohydrin) (**1**) serves as a versatile C_3-synthon in the synthesis of an array of important molecules.[1] As an enantioselective synthesis of compound (**1**) is lacking in the synthetic literature, a general

R *S*

Structure of (*R*)- and (*S*)-(Chloromethyl)oxirane.

access to both antipodes of the epoxide is to employ D-mannitol-based chemistry through a multi-step synthesis.[2] Recently, several other strategies have been used to attempt to gain direct entry to enantiomerically active (**1**), such as the preparation of optically active chloropropanols *via* chemical[3] or enzymatic[4] resolution of the corresponding racemates, biocatalysed asymmetric epoxidation,[5] *etc.* Nevertheless, these procedures generally gave low chemical yields as a result of poor stereochemical selectivity or low catalytic efficiency. As part of our research interest in natural product synthesis entailing a chiron approach, we herein report an efficient chemoenzymatic route which allowed us to prepare both enantiomers of epichlorohydrin (**1**) in suitable quantities.

Results and Discussion

The synthesis of (*R*)- and (*S*)-(**1**) from D-mannitol[2] *via* 1-chloro-3-tosyloxypropan-2-ol (**2**) prompted us to examine the preparation of the optically active intermediate (**2**) *via* an enzymatic route. (±)-(**2**) was readily obtained by reaction of epichlorohydrin with stoicheiometric amounts of toluene-*p*-sulphonic acid (PTSA). In view of the unique stereoselectivity of lipolytic catalysis, the butyryl ester of the monoalcohol (**2**), (±)-(**3**), was subjected to enantiospecific hydrolysis by commercial lipases. Among a variety of enzymes tested, the crude lipase from *Pseudomonas* sp. (Amano P-30)† exhibited good catalytic activity and selectivity (Scheme 1A). However, in a parallel experiment, when butyrate (±)-(**3**) was exposed to a highly purified lipase preparation from the same micro-organism (Amano LPL-80), (*S*)-(**2**) was obtained in higher enantiomeric purity (ee 0.97 at 50% conversion)‡ This finding implied that low levels of competing hydrolytic enzyme(s) possessing the opposite stereochemical preference were present in the crude enzyme P-30. Evidence from gel electrophoretic analysis of both enzyme preparations reinforced this conclusion.

Nevertheless, in light of the potential advantages of lipase catalysis in non-aqueous media,[6] we turned our attention to examining lipase-mediated transacylation reactions using lipase P-30. Schemes 1B and 1C thus summarise the results of the enzymatic transesterification in organic media, which entailed two different strategies.

As compared with the hydrolytic counterpart, enzymatic deacylation of butyrate (±)-(**3**) by butan-1-ol resulted in a marked improvement in the optical yield (Scheme B). Both (*S*)-(**2**) and (*R*)-(**3**) were obtained with satisfactory optical purity (ee 0.97). It is evident that the competing enzyme(s) in the crude enzyme powder was (were) virtually inactive in a water-restricted environment, whereas the lipase remained active. Hence, the crude lipase-mediated deacylation in hexane appears to be more enantioselective than does the corresponding hydrolytic reaction in water. This simple strategy may prove to be of practical use in enantioselectivity enhancement in reactions mediated by crude lipases. In addition, the acylation of the alcohol (**2**) by lipase P-30 with isopropenyl acetate as the acyl donor also provided an efficient means for the resolution of the racemic alcohol (**2**) (Scheme C). Enol esters such as isopropenyl acetate[9] and vinyl acetate[10] have favourably been used as the acyl donors in lipase-mediated esterifications due to their unique ability to shift the equilibrium in a favourable direction. In principle, enzymatic deacylation and esterification proceed *via* different transition states for the chirality-determining steps.[6c] However, despite this difference, both reactions exhibited nearly the same degree of enantioselectivity. Furthermore, it is noteworthy that the presence of trace amounts of water in the organic medium is necessary for the catalytic transacylations described here, which is in line with the general concept of essential water in lipase catalysis.[6a] A significant difference was noted between the reactions taking place in anhydrous hexane and in water-saturated hexane.

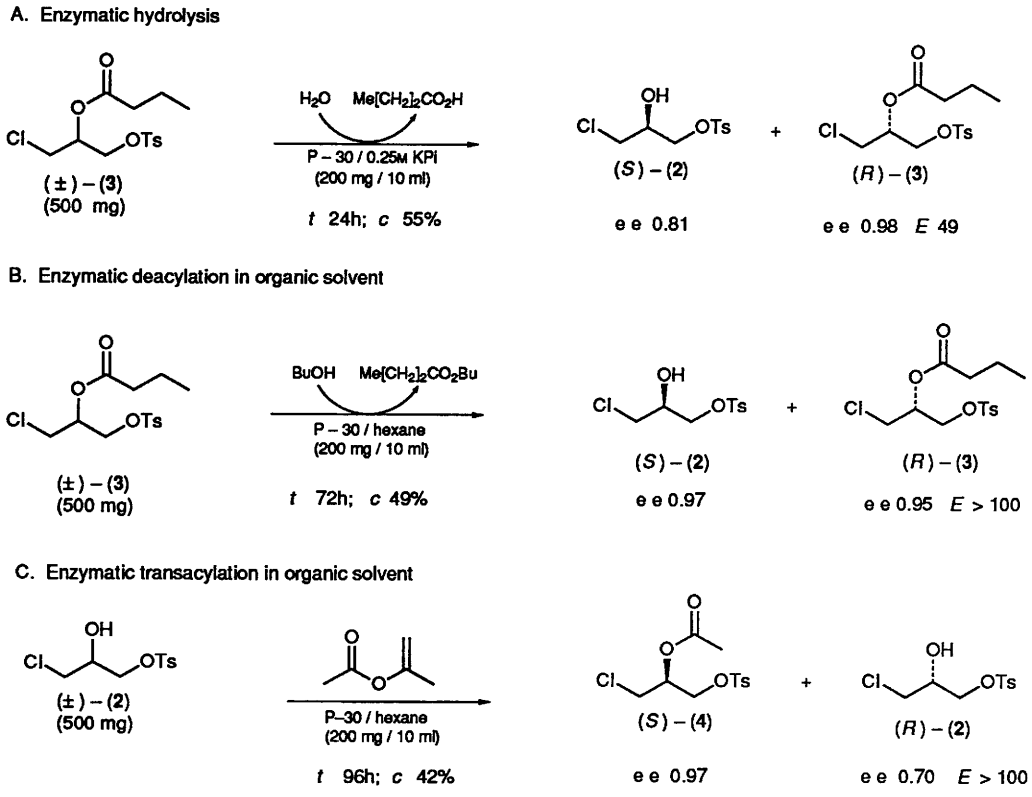
The enantiomerically active alcohol (*S*)-(**2**) obtained could be readily converted into the epoxide (*S*)-(**1**) in good yield by treatment with sodium ethylene glycolate (disodium ethane-1,2-diolate), followed by distillation *in vacuo*.[2] The remaining butyryl ester (*R*)-(**3**) was subjected to mild alkaline hydrolysis (K_2CO_3, MeOH, −5 °C) to afford the alcohol (*R*)-(**2**) in 90% yield, which was then accordingly transformed to the chiral epoxide (*R*)-(**1**). The enantiomeric excess of antipodal epoxide (**1**) thus prepared was confirmed to be >0.95 by ^{1}H NMR spectroscopy in the presence of a chiral shift reagent. In a bench-scale experiment, which started with butyrate (±)-(**3**) (20 g) and lipase P-30 (8 g), the overall yields for (*S*)- and (*R*)-epichlorhydrin were 74 and 68%, respectively.

Experimental

^{1}H NMR spectroscopy was carried out on a Varian EM-390 spectrometer for solutions in deuteriochloroform with tetra-

† The micro-organism was tentatively classified as *Pseudomonas fluorescens* (Amano Co.).

‡ The result obtained here is consistent with that reported in S. Hamaguchi, T. Ohashi, and K. Watanabe, *Agric. Biol. Chem.*, 1986, **50**, 375.



Scheme.

methylsilane as the internal standard. Optical rotations were determined with a Rudolph Autopol III polarimeter for solutions in the indicated solvent. High-pressure liquid chromatography was performed using a Model 501 pump (Water Associates) equipped with a Rheodyne injector and a Model 481 UV/Vis detector (Water Associates). Protein assays were performed by the Coomassie blue dye-binding method[9] using protein assay reagent (Pierce Chemical Co.) with bovine serum albumin (Sigma Chemical Co.) as the standard. Polyacrylamide disc gel electrophoresis was carried out according to the procedure described by Davis.[10] Reaction temperature was regulated by a Fisher Isotemp Model 9000 circulator. Lipase P-30 (crude *Pseudomonas* sp. lipase; 27.5 mg protein–g^{-1} solid) and LPL-80 (purified *P.* sp. lipase) were purchased from Amano Enzyme Co. All other chemicals and solvents were purchased from commercial sources.

(±)-1-*Chloro*-3-*tosyloxypropan*-2-*ol* (**2**).—Racemic (chloromethyl)oxirane (±)-(**1**) (50 g, 0.324 mol) was added dropwise to CH_2Cl_2 (240 cm^3) containing PTSA monohydrate (57 g, 0.3 mol) cooled in an ice-bath. The resulting mixture was stirred under reflux for 2 h. The solution was then washed successively with equal volumes of saturated aq. sodium hydrogen carbonate and brine. The organic layer was dried over sodium sulphate, and evaporated under reduced pressure to afford oily compound (**2**) (73.3 g, 91% yield), which was used directly in further reactions without purification; δ_H (90 MHz) 2.5 (3 H, s), 3.4 (1 H, br s), 3.5–3.8 (2 H, m), 3.9–4.4 (3 H, m), 7.35 (2 H, d, *J* 9 Hz), and 7.8 (2 H, d, *J* 9 Hz).

(±)-2-*Butyryloxy*-1-*chloro*-3-*tosyloxypropane* (**3**). Butyryl chloride (15.7 g, 0.147 mol) was added dropwise to ice-cooled CH_2Cl_2 (250 cm^3) containing the alcohol (**2**) (31.5 g, 0.127 mol) and triethylamine (15 g, 0.147 mol). The resulting mixture was stirred at room temperature for 3 h, and when then washed successively with equal volumes of saturated aq. sodium hydrogen carbonate, 1 M-HCl, and brine. The organic layer was dried over sodium sulphate, and evaporated under reduced pressure. Purification of the crude residue over a silica gel column hexane–ethyl acetate, 10:1 yielded the butyrate ester (**3**) (36.6 g, 90%), δ_H(90 MHz) 0.93 (3 H, t, *J* 6 Hz), 1.35–1.88 (2 H, m), 2.15 (2 H, t, *J* 7.5 Hz), 2.45 (3 H, s), 3.55 (2 H, d, *J* 6 Hz), 4.15 (2 H, d, *J* 6 Hz), 4.9–5.2 (1 H, m), 7.25 (2 H, d, *J* 9 Hz), and 7.65 (2 H, d, *J* 9 Hz).

Enantioselective Hydrolysis of Butyrate (±)-(**3**) *by Lipase P-30*.—The reaction mixture contained diester (±)-(**3**) (500 mg, 1.57 mmol) and crude lipase P-30 (200 mg; *ca.* 5.5 mg protein) in 0.25M-potassium phosphate buffer (10 cm^3; pH 7.0). The vigorously stirred mixture was incubated at 25 °C and the progress of the reaction was monitored by silica gel (hexane–ethyl acetate, 3:1) TLC analysis. After 24 h, the reaction was quenched by extraction of the mixture with an equal volume of ethyl acetate (×3). The combined extracts were dried over sodium sulphate, and concentrated to dryness. The residue was chromatographed over a silica gel column (hexane–ethyl acetate, 10:1 to 1:1) to afford the alcohol (*S*)-(**2**) (132 mg), $[\alpha]_D^{25}$ −3.0° (*c* 1.2 in $CHCl_3$) (ee 0.81) and the butyryl ester (hexane–ethyl acetate, 1:1) (*R*)-(**3**) (160 mg), $[\alpha]_D^{25}$ −5.7° (*c* 1.2 in $CHCl_3$) (ee 0.98). The remaining ester (*R*)-(**3**) (50 mg) was further treated with methanol (2.5 cm^3) in the presence of potassium carbonate (2.5 mg) at −5 °C until all the starting material disappeared (*ca.* 20 min). The solution was evaporated under reduced pressure and the residue was purified by silica gel chromatography to yield the alcohol (*R*)-(**2**) (35 mg, 90%).

Enantioselective Deacylation of Butyrate (±)-(**3**) *in Hexane by Lipase P-30*.—Lipase P-30 (8 g crude powder; 220 mg protein) was added to a suspension of butyrate (±)-(**3**) (20 g, 63 mmol) and butan-1-ol (100 cm^3) in hexane (300 cm^3) which was saturated with doubly distilled water. The resulting suspension

was stirred at 25 °C for 72 h, and was filtered to remove the protein powder, which still retained good activity. The organic solvent was concentrated to dryness under reduced pressure, and the residue was chromatographed over a silica gel column (hexane–ethyl acetate, 10:1 to 1:1) to give the alcohol (*S*)-(**2**) (7.3 g, 94%), $[\alpha]_D^{25}$ −3.6° (*c* 1.8 in $CHCl_3$) (ee 0.97) and the butyryl ester (*R*)-(**3**) (9.2 g, 93%), $[\alpha]_D^{25}$ −5.7° (*c* 1.0 in $CHCl_3$) (ee 0.95). The remaining ester (*R*)-(**3**) was subjected to the same hydrolytic treatment as described above to yield the alcohol (*R*)-(**2**).

Enantioselective Esterification of the Alcohol (±)-(**2**) *by Lipase P-30*.—To a suspension of compound (±)-(**2**) (500 mg, 2 mmol) and isopropenyl acetate (2.5 cm^3) in water-saturated hexane (7.5 cm^3) was added lipase P-30 (200 mg; 5.5 mg protein). The reaction mixture was incubated and stirred at 25 °C for 96 h. The suspension was treated as previously described to yield the acetate (*S*)-(**4**) (210 mg), $[\alpha]_D^{25}$ +6.70° (*c* 0.91 in $CHCl_3$) (ee 0.96) and the alcohol (*R*)-(**2**) (260 mg), $[\alpha]_D^{25}$ +2.5° (*c* 1.0 in $CHCl_3$) (ee 0.70).

(R)- *and* (S)-*Epichlorohydrin*.—The conversion of the hydroxy tosylate (**2**) to the epichlorohydrins (**1**) was achieved according to the procedure described by McClure and co-workers.[2] A solution of sodium ethylene glycolate, prepared from sodium (0.8 g) and ethylene glycol (20 cm^3), was added to a solution of the alcohol (**2**) (5.4 g) in dry ethylene glycol (20 cm^3). The mixture was stirred at room temperature for 15 min, and the resulting epichlorohydrin was distilled from the solution under reduced pressure, and trapped in a solid CO_2–acetone-bath. The chemical yield ranged from 75–85%; $[\alpha]_D^{25}$ (*R*)-(**1**) −32.5° (*c* 1.2 in MeOH); (*S*)-(**1**) +33.2° (*c* 1.1 in MeOH), which are consistent with the reported data.[2]

Enantiomeric Purity Determination.—The alcohol (**2**) was treated with (*S*)-(−)-2-methoxy-2-(trifluoromethyl)phenylacetyl (MPTA) chloride to form the corresponding (−)-MPTA ester. The MTPA derivatives were analysed by HPLC using a silica gel column (4.6 mm × 25 cm) with hexane–diethyl ether (4:1) as the mobile phase at a flow rate of 2 cm^3 min^{-1}. The retention times for the (−)-MTPA esters of the alcohol (**2**) were: *S*-isomer: 10 min; *R*-isomer: 11 min 20 s. The enantiomeric purity of epichlorohydrin (**1**) was confirmed by NMR analysis in the presence of tris-[(±)-3-(heptafluoropropyl(hydroxy)methylenecamphorato]europium(III) [$Eu(hfc)_3$].[2] Addition of 0.1–0.3 mol equiv. of the chiral shift reagent per mole of epichlorohydrin gave rise to a 10–30 Hz separation of the enantiomeric signals corresponding to the terminal epoxide protons.

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References

1 J. Jurczak, S. Pikul, and T. Bauer, *Tetrahedron*, 1986, **42**, 447.
2 J. J. Baldwin, A. W. Raab, K. Mensler, B. H. Arison, and D. E. McClure, *J. Org. Chem.*, 1978, **43**, 4876.
3 M. K. Ellis, B. T. Golding, and W. P. Watson, *J. Chem. Soc., Chem. Commun.*, 1984, 1600.
4 S. Iriuchijima, A. Keiyu, and N. Kojima, *Agric. Biol. Chem.*, 1982, **46**, 1593.
5 A. Q. H. Habets-Crutzen, S. J. N. Carlier, J. A. M. de Bont, D. Wistuba, V. Schurig, S. Hartmans, and J. Tramper, *Enzyme Microb. Technol.*, 1985, **7**, 17; C. A. G. Weijers, C. G. van Ginkel, and J. A. M. de Bont, *ibid.*, 1988, **10**, 214.
6 (*a*) A. M. Klibanov, *CHEMTECH*, 1986, 354; (*b*) M. W. Empie and A. Gross, *Annu. Rep. Med. Chem.*, 1988, **23**, 305; (*c*) C.-S. Chen and C. J. Sih, *Angew. Chem., Int. Ed. Engl.*, 1989, **28**, 695.
7 M. Degueil-Castaing, B. de Jose, S. Drouillard, and B. Maillard, *Tetrahedron Lett.*, 1987, **28**, 952.
8 J. Hiratake, M. Inagaki, T. Nishioka, and J. Oda, *J. Org. Chem.*, 1988, **53**, 6130.
9 M. Bradford, *Anal. Biochem.*, 1976, **72**, 248.
10 B. J. Davis, *Ann. N. Y. Acad. Sci.*, 1964, **121**, 404.

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