Chemo-Enzymatic Enantioconvergent Synthesis of C_4-Building Blocks Containing a Fully Substituted Chiral Carbon Center using Bacterial Epoxide Hydrolases

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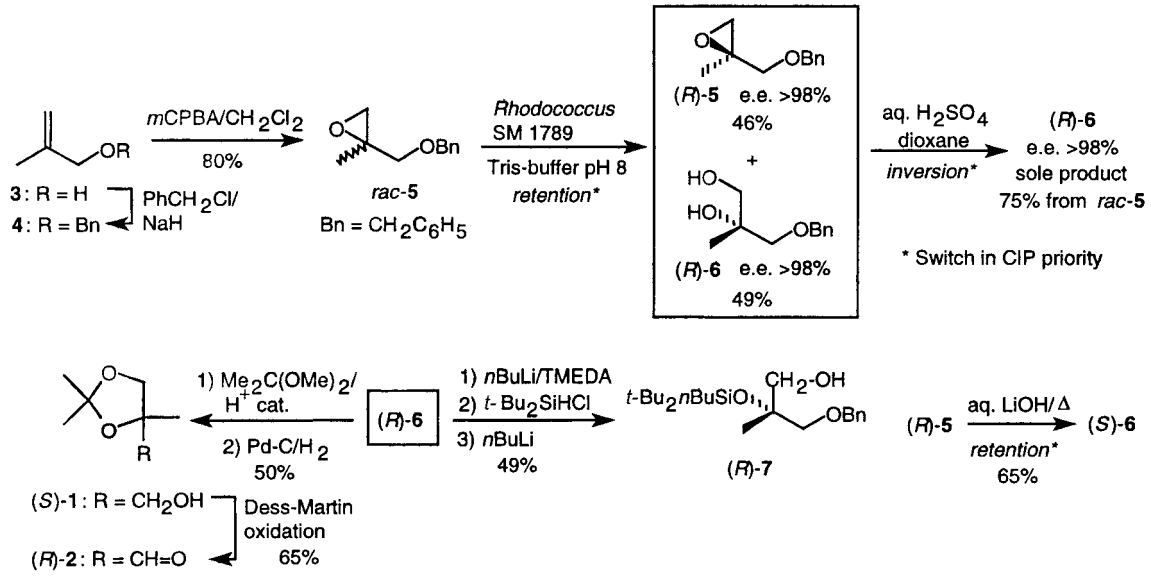
Abstract: A highly efficient chemo-enzymatic asymmetric synthesis of chiral C_4-building blocks containing a fully substituted carbon center is reported. The key transformation consists of a deracemization based on an enantioconvergent asymmetric hydrolysis of an epoxide using combined bio- and chemo-catalysis leading to a single enantiomeric product in >98% e.e. A simple switch of steps leads to kinetic resolution giving access to products of opposite configuration.

Key words: biotransformation, epoxide hydrolase, deracemization, C_4-building block, enantioconvergent

During the last few years, microbial epoxide hydrolases have proven to be powerful catalysts for controlling the stereochemistry of epoxides and their corresponding 1,2-diol derivatives.[2] Enzymes from bacterial sources are of special interest since they seem to be the only biocatalysts that selectively hydrolyze rather bulky oxiranes bearing a fully substituted chiral carbon center.[3] Bacterial epoxide hydrolases are cofactor-independent and are available in sufficient quantities by fermentation without the need for enzyme induction. The preparative applicability of bacterial epoxide hydrolases (usually employed as whole lyophilized cells) has been demonstrated on a range of synthetically useful substrates: In particular, 2,2-disubstituted epoxides and their corresponding *vic*-diols were obtained in excellent ee's.[3] In order to overcome the inherent drawbacks of kinetic resolution, deracemization methods have been developed which transform all of the racemic starting material into a single enantiomeric product.[4]

In this letter, we wish to report a simple and cheap chemoenzymatic protocol to produce both enantiomers of acetonides **1** and **2** on a preparative scale with high selectivity. The latter C_4−units have been used in the asymmetric synthesis of numerous natural products.[5-9] In addition, several structurally related chiral building blocks were synthesized.

From previous studies it was known that oxiranes bearing polar functional groups (e.g. -OH) in the side chain were not accepted by bacterial epoxide hydrolases.[10] However, suitable protection (e.g. with an ether moiety) rendered these compounds more lipophilic and thus made them acceptable for the enzymes.[10] From these considerations, benzyloxy oxirane (±)-**5** (readily prepared from methallyl alcohol[10], Scheme 1) was the substrate of choice. As previously reported,[11,12] the latter could be hydrolyzed on an analytical scale by using whole cells of *Rhodococcus* NCIMB 11216. Upon detailed investigation on a larger scale, however, *Rhodococcus* SM 1789 proved to be



Scheme 1

superior for preparative-scale transformations in view of reaction rate, enantioselectivity and reproducibility.[13]

Enzymatic hydrolysis of (±)-**5** was shown to proceed via attack at the less substituted carbon atom[2b] and, as a consequence, the biotransformation took place with *retention* of configuration at the stereogenic center to afford a 1:1 mixture of epoxide (*R*)-**5** and diol (*R*)-**6**. Note that the apparent *inversion* [(*S*)-**5** → (*R*)-**6**] of the biohydrolysis is only due to a switch in the CIP-priority and not due to inversion at the stereogenic center. The scale-up of this reaction was optimized using *Rhodococcus* SM 1789 as follows: (i) In order to keep the reaction on a manageable size and to improve recovery rates, the total volume of buffer (Tris, 50 mM, pH 8) was considerably decreased relative to the amount of epoxide and biocatalyst as compared to analytical-scale reactions.[10] (ii) The reaction temperature was elevated to enhance the rate of biohydrolysis. From previous studies it was known that (even for epoxide hydrolases), epoxides exert a certain inhibition at elevated concentrations[12] going in hand with some deactivation at prolonged reaction times. The biohydrolysis came to a standstill at 50% conversion indicating high enantioselectivity (E >100). Thus, (±)-**5** could be efficiently resolved to give (*R*)-**5**[14] and (*R*)-**6** within 80 hours.[15a] The optical purity of both the remaining epoxide (*R*)-**5** and the corresponding product diol (*R*)-**6** was found to be high (>98%)[10] and the total recovery of materials was optimal (>85%) when the reaction mixture was continuously extracted with CH_2Cl_2.

Deracemization of (±)-**5** was achieved by employing a protocol based on sequential bio- and chemo-hydrolysis.[4] Thus, the crude mixture of (*R*)-**5** and (*R*)-**6** resulting from biohydrolysis was directly treated with cat. 93% aq. H_2SO_4 in dioxane[15b] to effect hydrolysis of (*R*)-**5** with complete inversion. Thus, diol (*R*)-**6** was isolated as the sole product in 75% chemical yield and >95% e.e. Protection of the *vic*-diol as the acetonide [2,2-dimethoxypropane, cat. ion exchange resin IR 120 (H^+-form)] followed by hydrogenolysis (Pd/C 10%, H_2, 1 bar) of the benzyl ether afforded the desired enantioenriched synthon (*S*)-**1**[17] in 34% overall yield and >98% e.e. starting from methallyl alcohol. Finally, carbaldehyde (*R*)-**2**[17] was obtained without racemization via Dess-Martin oxidation[18] following a previously described procedure.

In order to broaden the applicability of this method, access to the opposite enantiomeric series [i.e. (*R*)-**1** and (*S*)-**2**] was achieved by a modification of the reaction sequence: Thus, treatment of (*R*)-**5** with aq. LiOH effected hydrolysis with complete *retention* to furnish diol (*S*)-**6**.

Direct oxidation of the primary alcohol in either enantiomer of diol **6** would give the corresponding α-hydroxyaldehyde, a useful intermediate for the preparation of α-substituted α-hydroxy carboxylic acids.[19] To our disappointment, none of several oxidation methods attempted proved to be successful;[20] whereas Dess-Martin or Swern conditions mainly led to condensation, chromium-based oxidants, such as PDC or PCC gave rearranged products.[20b] On the other hand, a recently reported one-pot protection-oxidation sequence[21] worked rather well: Thus, when (*R*)-**6** was subsequently treated with 1 equiv. of *n*-BuLi, di-*tert*-butylchlorosilane and another equiv. of *n*-BuLi[21] in the presence of *N,N,N',N'*-tetramethylene-diamine (TMEDA), silyl derivative (*R*)-**7**[17a] was isolated in good yield (49%) and without racemization.[22] The potential of silyloxy derivatives (*R*)-**7**, which could be oxidized using PDC[20c] to the corresponding aldehyde,[23] as chiral intermediate is apparent, since both hydroxyl moieties are differently protected which makes sequential chemical modifications at each hydroxyl group possible.

Table Chemical and Optical Yields.

Compound	Abs. Config.[a]	Yield[b] (%)	e.e.[c] (%)
1	*S*	34	95
2	*R*	20	95
5	*R*	34	99
6	*R*	60	99
6	*S*	22	98
7	*R*	28	93

[a] Determined by correlation with authentic material, see also reference 10.
[b] Isolated yields calculated from methallyl alcohol **3**.
[c] Determined by GC on a chiral stationary phase.[10,15e]

In conclusion, our synthesis of building blocks **1** and **2** shows the following advantages: (i) The method starts from cheap starting material and (ii) it gives access to both stereo-isomers in high optical purity. (iii) Aldehyde (*R*)-**2** may be obtained via deracemization, i.e. no 'unwanted' enantiomer occurs as by-product. In addition, a useful silylated synthon (*R*)-**7** was prepared. In the latter compound, the *tert*- and *prim*-hydroxyl group are differently protected, which allows highly flexible synthetic transformations. The application of these chiral synthons for the asymmetric synthesis of a physiologically active sesquiterpenoid compound is currently in progress.

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(13) Biocatalyst preparation:
Rhodococcus ruber SM 1789 from the culture collection of the Institute of Biotechnology (Graz University of Technology) was grown in baffled shake-flask cultures on the following medium: yeast extract (10.0 gL^{-1}), peptone (10.0 gL^{-1}), glucose (10.0 gL^{-1}), NaCl (2.0 gL^{-1}), $MgSO_4.7H_2O$ (0.147 gL^{-1}), NaH_2PO_4 (1.3 gL^{-1}), K_2HPO_4 (4.4 gL^{-1}). Cells were harvested at the late exponential growth phase by centrifugation (3000 × g), washed with Tris/HCl-buffer (0.05 M, pH 8.0) and lyophilized. The cells could be stored at 4 °C for several months without a noticeable loss of activity. For experimental details see ref. 12
(14) For example, epoxide **5** was used for the preparation of α-methylamino acids. See: Lakner, F. J.; Hager, L. P. *Tetrahedron: Asymmetry* **1997**, *8*, 3547 and references cited therein.
(15) For the synthesis of (±)-**5** see ref. 10.
a) Biohydrolysis of (±)-**5**. Epoxide (±)-**5** (1.2 g, 6.73 mmol) was added to a suspension of rehydrated lyophilized whole cells of *Rhodococcus* SM 1789 (2.00 g) in Tris-buffer (30 mL, 0.05 M, pH 8). The mixture was agitated on a rotary shaker at 30° C and 130 rpm. After about 80 h, the reaction ceased at 50% conversion and the mixture was continuously extracted with CH_2Cl_2 (100 mL, 20 h). The organic layer was washed with brine (50 mL), dried (Na_2SO_4) and evaporated to furnish a slightly orange oil (1.3 g). This residue can be directly deracemized (see b). Alternatively, (*R*)-**5** and (*R*)-**6** can be separated by flash chromatography (Merck silica gel 60, petroleum ether/EtOAc 2:1) to afford 552 mg (3.1 mmol, 46%, ee = 99%) (*R*)-**5** and 647 mg (3.3 mmol, 49%, ee = 99%) *(R)*-**6**. The products were analyzed by GC on a chiral stationary phase as described in reference 10.
b) Deracemization of (±)-**5**. The mixture of (*R*)-**5** and (*R*)-**6** obtained from the biohydrolysis (1.3 g) was dissolved in dioxane (225 mL), and 93% (v/v) aq. H_2SO_4 (18 mL) was added dropwise at 15 °C with stirring. After 15 min at r.t. the acid was neutralized with sat. aq. $NaHCO_3$. EtOAc (300 mL) was added, and the resulting biphasic mixture was vigorously stirred for an additional 30 min. The aq. layer was extracted with EtOAc (3 × 100 mL), the combined organic layers were dried (Na_2SO_4) and the solvents were evaporated. Flash chromatography of the oily residue (Merck silica gel 60, petroleum ether/EtOAc 2:1) afforded (*R*)-**6** as white crystals in 0.70 g (60%) yield and 98% ee. Spectroscopic, physical and optical data were in full agreement with those previously reported: Tanner, D.; Somfai, P. *Tetrahedron* **1986**, *42*, 5985.
c) Hydrolysis of (*R*)-5 to give (*S*)-6: To a stirred solution of *(R)*-**5** (150 mg, 0.84 mmol) in H_2O (10 mL) and THF (3 mL) LiOH (1.3 g, 54.3 mmol) was added and the mixture was refluxed for 30 h. The reaction was quenched by addition of H_2O (50 mL) and sat. aq. NH_4Cl (10 mL). Products were extracted with EtOAc (5 × 50 mL), the organic phase was dried (Na_2SO_4) and evaporated to give (*S*)-**6** (82 mg, 50%). No racemization occurred.
d) Preparation of 1 and 2 from 6: 261 mg of (*R*)-**6** (1.33 mmol, ee = 92%) were stirred in dimethoxypropane (5 mL, 40.7 mmol) under acidic conditions for 2 h (50 mg Amberlite IR 120, H^+ form). After filtration and evaporation, abs. ethanol (5 mL) and Pd/C (10%, 5% w:w) were added. The solution was stirred under an atmosphere of H_2 to furnish (after filtration and evaporation) (*S*)-**1** (111 mg, 0.76 mmol, 57%, e.e. = 94%).
(*S*)-**1** (45 mg, 0.31 mmol, ee = 97%) was stirred in CH_2Cl_2 (1.5 mL). To this solution Dess-Martin oxidant (periodinane, 0.25 g, 0.59 mmol)[18] in CH_2Cl_2 (mL) was added. The solution was diluted with ether (7 mL) and sat. $NaHCO_3$ solution (5 mL). The organic layer was dried (Na_2SO_4) and evaporated to yield (*R*)-**2** (29 mg, 0.20 mmol, 65%, ee = 98%).
e) Enantiomeric composition: Determined with Varian 3800 gas chromatograph equipped with FID, using a CP-Chirasil-DEX CB column (25m × 0.32mm, 0.25µm film, H_2). **1**: isotherm 55 °C, 14.5 psi, 6.39 min (*S*) and 6.71 min (*R*); **2**: isotherm 90 °C, 14.5 psi, 4.00 min (*S*) and 4.26 min (*R*).
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(22) **(*R*)-3-Benzyloxy-2-[*n*-butyl(di-*tert*butyl)silyloxy]-2-methyl-1-propanol (7):** ^1H NMR (360 MHz, $CDCl_3$): δ = 0.73-1.02 (m, 22H), 1.28-1.33 (m, 8H), 3.38 -3.61 (m, 4H), 3.49-3.59 (q, J = 12 Hz, 2H), 7.27-7.38 (m, 5H); ^{13}C NMR (90 MHz, $CDCl_3$): δ = 28.71, 76.72, 77.08, 77.43, 127.59, 127.68, 128.42; elemental analysis [calcd. (found)]: C: 69.84% (69.96), H: 10.71% (10.73). The enantiomeric composition of *(R)*-**7** was determined via HPLC using a Chiralpak AD column (4.6mm × 240mm, heptane/*i*-propanol 98/2, 0.6ml/min): *(S)*-**7** 10.39 min, *(R)*-**7** 11.28 min.
(23) **3-Benzyloxy-2-[*n*-butyl(di-*tert*butyl)silyloxy]-2-methyl-1-propanal:** ^1H NMR (360 MHz, $CDCl_3$): δ = 0.77-1.27 (m, 30H), 3.42-3.52 (m, 2H), 5.23 (s, 3H), 7.20-7.27 (m, 5H), 9.64 (s, 1H).

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