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An enzyme-triggered enantio-convergent cascade-reaction

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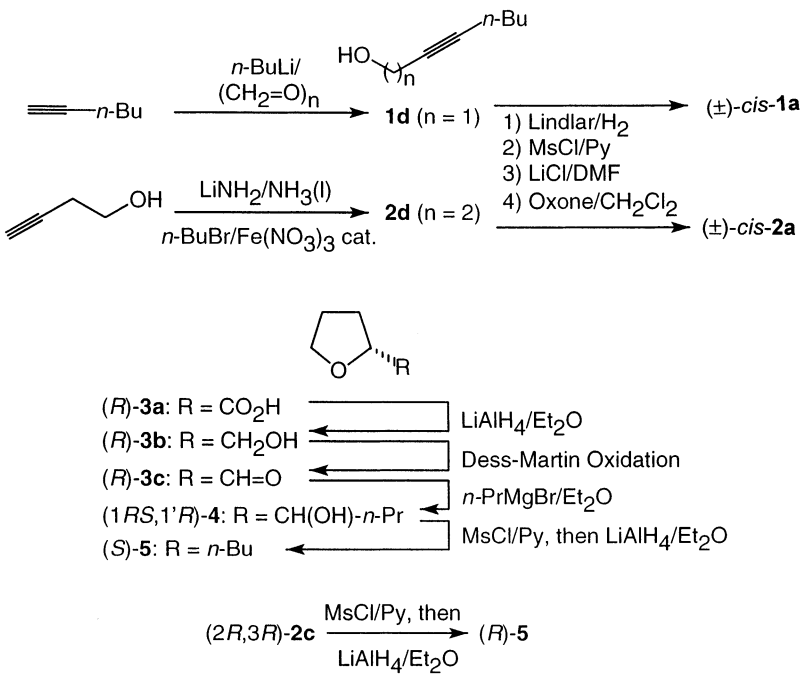
Abstract—The biocatalytic hydrolysis of the (±)-2,3-disubstituted *cis*-chloroalkyl epoxides **1a** and **2a** using resting cells of *Rhodococcus* sp. did not give the corresponding chloroalkyl *vic*-diols **1b**, and **2b**, respectively, but furnished the rearranged products (2*R*,3*R*)-**1c** and (2*R*,3*R*)-**2c** in high e.e. as the sole products via an enzyme-triggered enantio-convergent cascade-reaction.

We recently reported the asymmetric bio-hydrolysis of (±)-*cis*-2,3-dialkyl oxiranes by bacterial epoxide hydrolases, which led to the formation of the corresponding *vic*-diols in high enantiomeric purity.[1] The remarkable feature of this biotransformation is the fact that it did not follow via a kinetic resolution pathway, but proceeded in an enantio-convergent fashion. Thus, a single enantiomeric *vic*-diol was formed as the sole product in 100% theoretical yield. In view of the considerably improved economic balance, such 'deracemization' processes have recently gained considerable attention.[2–6]

In order to extend the preparative applicability of this method, we decided to investigate synthetically more useful substrates bearing various functional groups, which would allow further synthetic transformations.



Scheme 1. Synthesis of substrates and reference material for determination of absolute configuration.

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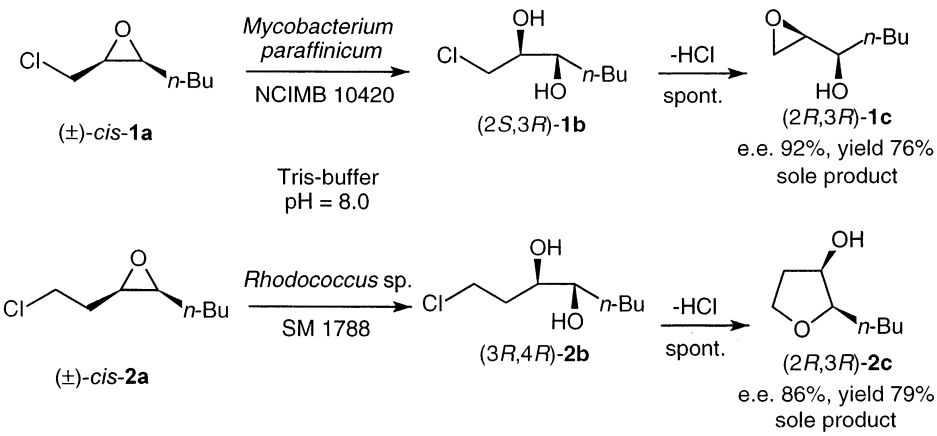
Guided by our previous experience that bacterial epoxide hydrolases generally do not accept substrates bearing polar functional groups (such as OH, N_3, etc.),[7] we turned our attention to haloalkyl oxiranes. Substrates (±)-*cis*-**1a** and **2a** were synthesized using standard methodology, as outlined in Scheme 1. Hydroxymethylation of 1-hexyne (*n*-BuLi/paraformaldehyde) and alkylation of 3-butyn-1-ol (*n*-BuBr/$LiNH_2$/NH_3(l)/$Fe(NO_3)_3$ cat.) gave acetylene derivatives **1d** and **2d**, respectively, which were stereoselectively hydrogenated (H_2/Lindlar cat.) to give the corresponding *cis*-alkenes. Nucleophilic exchange of the hydroxyl group by chloride (MsCl/Py, then LiCl/DMF) and epoxidation of the olefin moiety using oxone furnished (±)-*cis*-**1a** and (±)-*cis*-**2a** in good overall yields of 43 and 39%, respectively.

Both substrates were well accepted by epoxide hydrolase(s) from *Rhodococcus* SM 1788 (*rac*-*cis*-**2a**) and *Mycobacterium paraffinicum* NCIMB 10420 ((±)-*cis*-**1a**), employed as resting whole cells in Tris buffer (pH 8.0).[8] Much to our surprise, the biotransformation did not simply lead to the expected *vic*-diols **1b** and **2b** (Scheme 2), but revealed a more complex picture in that the final products turned out to be hydroxy-epoxide **1c** and tetrahydrofuran derivative **2c**.[9] The structure of products was determined on the basis of high resolution mass spectra and ^1H and ^{13}C NMR spectra. The relative configuration of **1c** was confirmed by comparison of the specific rotation to that in the literature, the *cis*-configuration of **2c** was elucidated by NOE experiments in NMR.[10] From each of the substrates only a single product was formed in high enantiomeric purity, i.e. 92% e.e. for **1c** and 86% e.e. for **2c**.[11] The absolute configuration of the latter was determined as follows: the specific rotation of **1c** matched the literature value[12] confirming the (2*R*,3*R*)-configuration. Compound **2c** was transformed into **5** by removal of the 3-hydroxyl group (MsCl/Py, then $LiAlH_4$/Et_2O) without affecting the chiral centre in position 2. The absolute configuration of the latter material was confirmed as (*R*)- by co-injection on GC[11] using independently synthesized material as a reference. A sample of (*S*)-**5** was obtained from commercially available (*R*)-**3a** via a four-step sequence depicted in Scheme 1.

This biotransformation has several unusual features:

1. Both epoxides **1a** and **2a** were hydrolyzed in an enantio-convergent fashion, i.e. both enantiomers were converted with opposite regioselectivity to give the single stereomeric vicinal diols **1b** and **2b**, respectively.[1,13]
2. The formation of the final products can be explained by an intramolecular cyclization of haloalkyl diols **1b** and **2b**, which are initially formed during the biohydrolysis. This latter reaction shows some resemblance to a Payne-type rearrangement.[14]
3. Depending on the length of the haloalkyl substituent, the relative reaction rate of hydrolysis versus cyclization varies to a significant extent: the rate of epoxide formation to form **1c** is in the same order of magnitude as the biohydrolysis. As a consequence, a certain amount of chloromethyl diol **1b** can be detected during the reaction. In contrast **2b** is formed only in minute amounts, since cyclization forming **2c** is considerably faster than biohydrolysis of **2a**. In both cases, ring-closure follows an *exo-tet* pattern.[15] The large difference in the relative rate of cyclization can be explained by energetic considerations, taking the large difference in ring-strain of **1c** versus **2c** into account.
4. Since the enantiomeric composition of haloalkyl diols **1b** and **2b** is identical to that of the corresponding rearrangement products. The involvement of an enzyme in the latter transformation can be excluded, i.e. the cyclization is of a spontaneous nature. However, the involvement of a halohydrin epoxidase in the transformation of **1b** to **1c** may be envisaged.[16]
5. The overall two-step sequence represents an enzyme-triggered enantio-convergent cascade-reaction.[17,18]

The remarkable synthetic potential of this biotransformation is evident, as a single (diastereomerically pure) chiral building block possessing two contiguous chiral centres is formed with high enantiomeric excess and in quantitative yield from easily available starting materials. The full scope and limitations of this method are currently under investigation.



Scheme 2. Enantio-convergent hydrolysis of haloalkyl epoxides followed by spontaneous ring-closure.

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8. SM refers to the culture collection of the Institute of Biotechnology, Graz University of Technology. Racemic epoxides *rac-cis*-**1a** (0.6 g, 4.05 mmol) and **2a** (0.6 g, 3.69 mmol) were hydrolyzed using rehydrated lyophilized microbial cells (1 g) in Tris buffer (20 mL, 0.05 M, pH 8.0) on a rotary shaker (120 rpm) at 30°C. When the starting material was consumed (after 98 or 120 h, respectively, as judged by TLC and GLC), the mixture was extracted with EtOAc. The organic layers were dried (Na_2SO_4) and evaporated. The residue was flash chromatographed (petrol ether/EtOAc, 10/1) to give (2*R*,3*R*)-**1c** (92% e.e., 76% yield) and (2*R*,3*R*)-**2c** (86% e.e., 79% yield).
9. (2*R*,3*R*)-**1c**: ^{1}H NMR ($CDCl_3$, 200 MHz) $\delta=0.91$ (t, $J=6.9$ Hz, 3H), 1.33–1.39 (m, 4H), 1.56–1.65 (m, 2H), 2.70–2.73 (m, 1H), 2.82 (dd, $J=4.6$ and 4.6 Hz, 1H), 2.98 (dd, $J=4.9$ and 4.9 Hz, 1H), 3.47 (dd, $J=7.1$ and 14.1 Hz, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) $\delta=14.0$, 22.7, 27.4, 34.2, 45.2, 55.4, 71.7; High resolution MS calcd for $C_7H_{14}O_2$ $(M+H)^+$: 129.091, found 129.088. (2*R*,3*R*)-**2c**: ^{1}H NMR ($CDCl_3$, 500 MHz) $\delta=0.90$ (t, $J=7.2$ Hz, 3H, -C$\underline{H}_3$), 1.33–1.37 (m, 4H, 2×CH_2), 1.60–1.64 (m, 2H, CH_2), 1.92–1.94 (m, 2H, CH_2), 2.17–2.19 (s, 1H, OH), 3.53 (dt, $J=3.1$ and 7.0 Hz, 1H, -C$\underline{H}$-OH), 3.73 (dt, $J=4.8$ and 9.2 Hz, 1H, -C$\underline{H}_{2AB}$-O-), 4.01 (dd, $J=16$ and 16 Hz, 1H, -C$\underline{H}_{2AB}$-O-), 4.18–4.20 (m, 1H, O-C$\underline{H}$-*n*-Bu); ^{13}C NMR ($CDCl_3$, 90 MHz) $\delta=14.1$ (4′-C), 22.8 (3′-C), 28.2 (2′-C), 33.3 (1′-C), 35.2 (4-C), 66.2 (5-C), 76.3 (3-C), 86.5 (2-C); High resolution MS calcd for $C_8H_{16}O_2$ $(M+H)^+$ 144.115, found 144.116.
10. Both compounds were diastereomerically pure.
11. Enantiomeric purities were analyzed on a Varian 3800 gas chromatograph equipped with FID. CP-Chirasil-DEX CB column (25 m×0.32 mm, 0.25 μm film), **1b**: 10 psi H_2, iso 125°C, 9.24 min (2*R*,3*S*) and 10.13 min (2*S*,3*R*); **1c**: 10 psi H_2, iso 80°C, 14.73 min (2*S*,3*S*) and 16.87 min (2*R*,3*R*); **5**: 10 psi H_2, iso 45°C, 14.54 min (2*R*) and 15.25 min (2*S*). Astec Chiraldex B-TA capillary column (30 m×0.25 mm), **2b**: 10 psi H_2, iso 120°C, 36.35 min (2*R*,3*R*) and 37.98 min (2*S*,3*S*); **2c**: 10 psi H_2, iso 95°C, 15.37 min (2*S*,3*S*) and 16.19 min (2*R*,3*R*).
12. (2*R*,3*R*)-**1c**: The optical rotation $\{[\alpha]_D^{20}=-3.1$ ($c=0.25$, CH_2Cl_2, e.e. = 92%)} is in agreement with the reported value $\{[\alpha]_D^{20}=-3.2$ ($c=1.3$, CH_2Cl_2, e.e. = 94%)}; see: Vanhessche, K. P. M.; Wang, Z.-M.; Sharpless, B. K. *Tetrahedron Lett.* **1994**, *35*, 3469–3472. (2*R*,3*R*)-**2c**: $[\alpha]_D^{20}=-24.4$ ($c=0.51$, EtOH, e.e. >99%). The latter sample was obtained from an experiment stopped at low conversion (ca. 30%).
13. The ratio of the reaction rates of enantiomers was calculated to be 8.6 and 1.9 for **1a** and **2a**, respectively. Details will be published in a full paper.
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