Pergamon

Tetrahedron Letters 43 (2002) 6763–6766

TETRAHEDRON LETTERS

Selective enzymatic epoxidation of dienes: generation of functional enantiomerically enriched diene monoepoxy monomers

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Received 20 June 2002; revised 12 July 2002; accepted 19 July 2002

Abstract—Enantiomerically enriched diene monoepoxides were selectively synthesized using oxidases from *Pseudomonas* sp. and chloroperoxidase from *Caldariomyces fumago*. These monoepoxides are useful monomers for generating functional chiral polymeric materials.

Enantiomerically enriched synthetic polymers are of special interest due to their potential applications as chromatographic supports for the separation of enantiomers.[1] Enantiomerically enriched polyacrylamide and polymethacrylate gels have been used to resolve racemic drugs on a preparative scale.[1,2] Selective polymerization of enantiomerically enriched monomers having two polymerizable groups may afford structurally well-defined functional polymeric materials. Enantiomerically enriched diene monoepoxides, such as divinylbenzene monoepoxide can be promising monomers in the synthesis of various optically active polymers having structurally defined groups in either the side chain or the main chain. However, there are few reports on the preparation of enantiomerically enriched/pure diene monoepoxides from the diene precursors. Biocatalysis seems to be a straightforward approach for the generation of this type of optically active functional monomer.

The oxidase systems from the bacteria *Pseudomonas* sp., such as xylene oxygenases catalyze the epoxidation of styrene to styrene oxide with high enantioselectivity.[3] Chloroperoxidase (CPO) is a versatile and efficient biocatalyst that catalyzes a variety of reactions, particularly asymmetric epoxidation and hydroxylation.[4] Herein, we have investigated the selective epoxidation of aryl dienes catalyzed by oxidases from *Pseudomonas putida* and the epoxidation of unsaturated acrylate derivatives catalyzed by CPO.

Commercially available divinylbenzene **1**, a mixture of isomers[5] was first used as a model substrate for the investigation of selective epoxidation catalyzed by oxidases from *P. putida*. The oxidases were produced and directly used as an *n*-octane-based two-phase fermentation system, which allowed us to reduce substrate and product toxicity to the cells and accumulate the products in the organic phase. In a typical enzymatic-epoxidation reaction, divinylbenzene (**1**, 0.25 g) in *n*-octane (20 mL) was added into 1 L of a culture medium of *P. putida*[6] and the progress of the reaction was monitored by TLC and GC analysis. After 72 h of incubation, the culture was extracted with ether, the organic layer was separated, dried and evaporated to afford the crude product, which was purified by flash-column chromatography using a pentane–ether mixture to afford the pure epoxy products **2** and **3** in about 30% yield (based on the individual isomers in the mixture). For *para*- and *meta*-**1**, the oxidases from *P. putida* demonstrated two types of selectivities: (i) the reaction stopped at the monoepoxide stage, hardly any diepoxide and other products were detected or isolated from the reaction medium as shown in Scheme 1; (ii) the epoxidation reactions showed excellent enantioselectivities (ee 95% for *para*-**2**, ee 94% for *meta*-**3**).[7] Very surprisingly, *ortho*-**1** is not a substrate for the oxidases from *P. putida*. These epoxidation reactions were also carried out with pure *para*- and *meta*-divinylbenzenes

Keywords: enzymatic epoxidation; *Pseudomonas putida*; *Caldariomyces fumago*; chloroperoxidase; diene monoepoxide; enantioselective.

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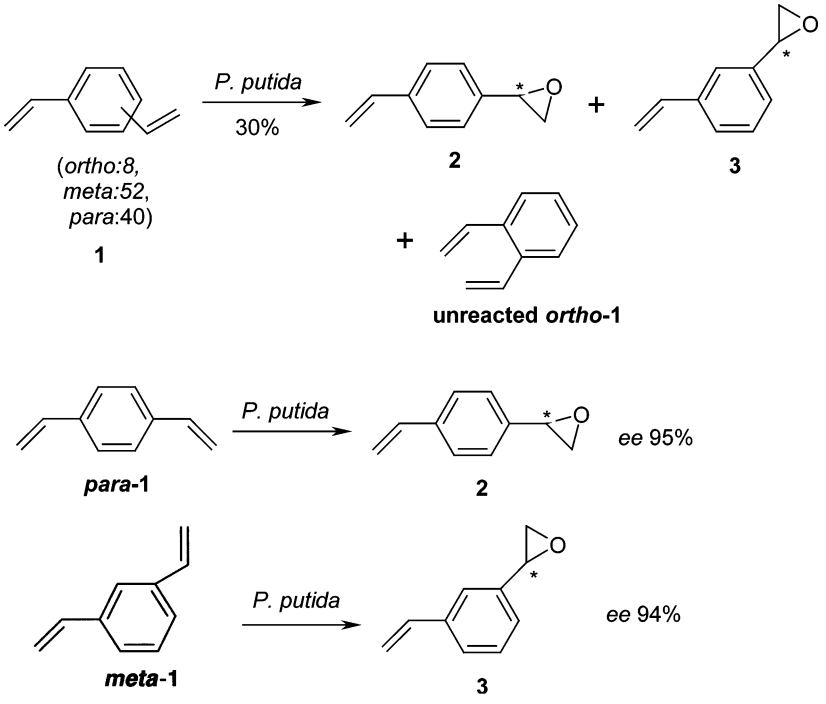
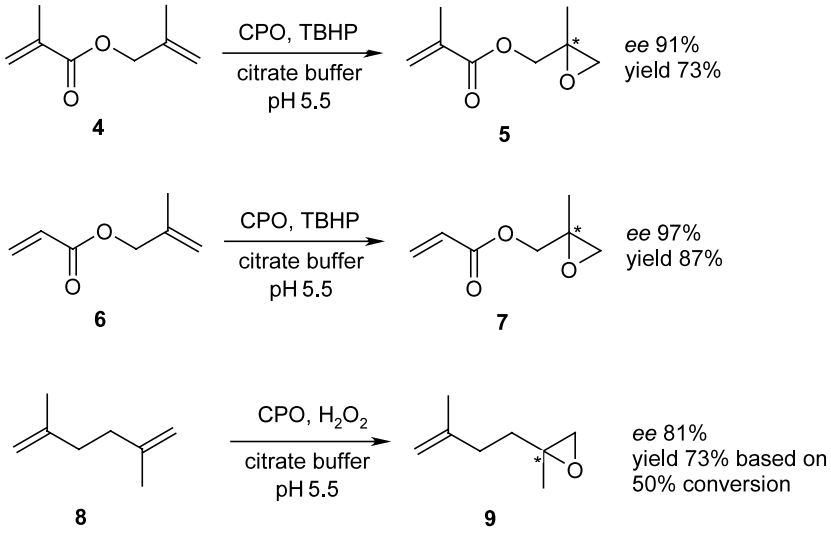
0040-4039/02/$ - see front matter
PII: S0040-4039(02)01519-8

as substrates.[8] In these cases, the oxidases gave very similar enantioselectivities for the *para*- and *meta*-divinylbenzene monoepoxides (ee 95% for *para*-isomer, ee 94% for *meta*-isomer), and the mono-epoxides were also detected as the unique products by TLC and GC analysis (38% and 25% yields for *para*- and *meta*-isomers, respectively). The physical and spectral data of divinylbenzene monoepoxides **2** and **3** were found to be identical with the data reported in the literature.[9] In contrast to *para*- and *meta*-divinylbenzenes, *para*- and *meta*-allylstyrenes[8] are not substrates for the oxidases from *P. putida* or *P. oleovorans*, which suggested that these enzymes showed very high substrate specificities.

In a typical CPO[6] catalyzed epoxidation reaction, methacrylate **4**,[10] acrylate **6**[10] or dimethylhexadiene **8**[10] (0.3 mmol, in each case) was stirred with *t*-BuOOH (0.60 mmol) or H_2O_2 (3%, 1.2 equiv.) in 2 ml of 10 mM sodium citrate buffer (pH 5.5) and 200 μL acetone. CPO (2.5 mg, 0.06 μmol) was added and the reaction mixture was stirred at room temperature for 2 h, after which Na_2SO_3 was added and the mixture was extracted twice with ether. The combined organic portions were dried over $MgSO_4$, the ether removed and the crude product was purified by flash-column chromatography using dichloromethane as eluting solvent to afford the pure epoxides **5**, **7** and **9** in 73–87% yields



Scheme 1. Selective epoxidation of aryl dienes by oxidases from *P. putida*.



Scheme 2. Selective epoxidation of dienes by CPO from *C. fumago*.

and 81–97% enantiomeric excesses (Scheme 2). The methacrylate **4** was a good substrate, which showed two types of selectivity: (i) only the isolated double bond was epoxidized to produce monoepoxide **5** [11] in 73% yield, and the conjugated α,β-unsaturated bond of the methacrylic acid moiety was untouched as shown in Scheme 2, (ii) the enantioselectivity was high (ee 91%). It suggested that conjugated terminal olefins might have a low effect on the inhibition of CPO activity compared to other aliphatic terminal alkenes to give an inactive derivative in which the active heme site is *N*-alkylated.[12] Indeed, acrylate **6** was an excellent substrate for CPO epoxidation and selectively afforded the monoepoxide **7**[11] (Scheme 2) in high yield and excellent enantioselectivity (87% yield and 97% *ee*). This is complementary to the epoxidation of the α,β-unsaturated double bond in enones using synzymes, viz polyleucine where the epoxidation takes place exclusively at the α,β-unsaturated double bond.[13] We further propose that CPO-catalyzed epoxidation should produce only monoepoxides from symmetrical dienes. This indeed was the case; when dimethylhexadiene **8** was used as a model substrate, biocatalytic epoxidation afforded exclusively the monoepoxide **9**[14] as a unique product (Scheme 2). The yield and ee values were moderate (73% yield based on 50% conversion, 81% ee). No diepoxide and other oxidized products were detected by GC analysis. The physical and spectral data of monoepoxides **5**, **7** and **9** were found to be identical with the data reported in the literature.[11,14]

In contrast to selective epoxidation reactions catalyzed by the oxidases from *P. putida* and CPO from *Caldariomyces fumago*, chemical epoxidation of divinylbenzene **1**, esters **4** and **6**, and diene **8** using MCPBA, produced a mixture of monoepoxides and diepoxides without any stereoselectivity. These results further demonstrate that the enzymatic epoxidation of dienes is a more powerful method for the production of optically active diene monoepoxides.

Enantiomerically enriched epoxy aryl monomers **2** and **3** can be chemically polymerized either through the vinyl group or oxide functional groups, which may generate functional polymers containing the chiral groups on the side chains and main chains, respectively. Well-defined enantiomerically enriched methacrylate and acrylate epoxy polymers can be generated via atom transfer radical polymerization (ATRP) or peroxidase-catalyzed polymerization of compounds **5** and **7**, respectively.[15]

In conclusion, this work demonstrates that enzymatic epoxidations are efficient methods for the preparation of chiral diene monoepoxides from dienes with high enantioselectivity. As the oxidases from *P. putida* and CPO from *C. fumago* are readily available, their use in the synthesis of other optically active epoxide monomers, which are difficult to obtain by chemical methods might be a promising approach. Furthermore, directed evolution of xylene oxidase from *P. putida* and CPO from *C. fumago* would provide an alternative to improve enzymatic activity and broaden the substrate specificity

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5. Divinylbenzene, a mixture of *meta*-, *para*- and *ortho*-isomers in the ratio 52:40:8 was obtained from Aldrich Chemical Co., USA.
6. *P. putida* ATCC and *P. oleovorans* ATCC 29347 were purchased from ATCC (VA, USA). *P. putida* and *P. oleovorans* were grown at 30°C on a rotary shaker (rpm 220) in the following media, respectively. **Media A:** $(NH_4)_2HPO_4$, 3.0 g; KH_2PO_4, 1.2 g; NaCl, 5.0 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; yeast extract 0.5 g; sodium benzoate (filter-sterilized), 3.0 g; distilled water, 1.0 L. Autoclaving was conducted at 120°C for 15 min with all ingredients except sodium benzoate. **Media B:** $(NH_4)_2HPO_4$, 10.0 g; K_2HPO_4, 5.0 g; Na_2SO_4, 0.5 g; distilled water 1 L. The mineral medium was autoclaved at 120°C for 15 min and at the time of culture, filter-sterilized octane (2.5% v/v) was added to the medium. CPO from *Caldariomyces fumago* was obtained as a 20 mg/ml phosphate buffer (0.1 M, pH 4.5) from Chirazyme (Urbana, IL).
7. The enantiomeric excess (ee) values of chiral epoxides were determined by HPLC analysis on a Chiralcel OJ column (25×0.46 cm) using hexane/*iso*-propanol as eluent with a flow rate of 1.0 mL/min or by GC analysis on an ALPHA DEX capillary column (30 m×0.32 mm).
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and 3.0 Hz, 2H, *meta* and *para*), 3.15 (dd, $J=8.0$ and 6.0 Hz, 2H, *meta* and *para*) and 2.81 (dd, $J=6.0$ and 3.0 Hz, 2H, *meta* and *para*).

10. The esters **4** and **6** were prepared by acylation of methyl allyl alcohol with the respective acyl chlorides. 2,5-Dimethyl-hexa-1,5-diene **8** was prepared by coupling of 2-methylallyl iodide using sodium.
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