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Enantiodivergent, Biocatalytic Routes to Both Taxol Side Chain Antipodes

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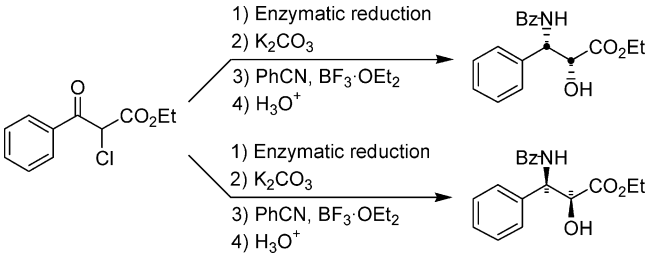
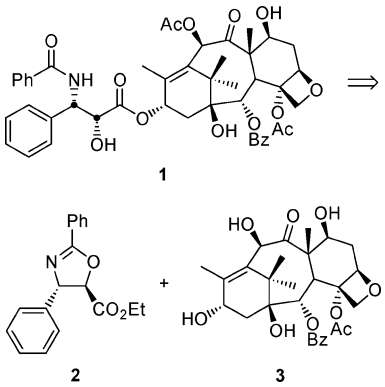


Two enantiocomplementary bakers' yeast enzymes reduced an α-chloro-β-keto ester to yield precursors for both enantiomers of the *N*-benzoyl phenylisoserine Taxol side chain. After base-mediated ring closure of the chlorohydrin enantiomers, the epoxides were converted directly to the oxazoline form of the target molecules using a Ritter reaction with benzonitrile. These were hydrolyzed to the ethyl ester form of the Taxol side chain enantiomers under acidic conditions. This brief and atom-efficient route to both target enantiomers demonstrates both the synthetic utility of individual yeast reductases and the power of genomic strategies in making these catalysts available.

Taxol **1** has emerged as the drug of choice for treating certain types of ovarian and breast cancers because it blocks microtubule disassembly (Scheme 1).[1,2] Because the quantities supplied by isolation from the natural source proved insufficient to meet commercial demand, routes for its semi-synthesis from a terpene core such as 10-deacetylbaccatin III **3** have been developed.[3,4] Most biological activity resides in the natural (2*R*,3*S*)-diastereomer of the *N*-benzoyl-phenyisoserine side chain, and its small size, dense functionality, and medical importance have made this molecule an important test-bed for methods in asymmetric synthesis.[5−17] Earlier, we explored a biocatalytic approach to a β-lactam derivative of the Taxol side chain in which whole bakers' yeast cells were used to kinetically resolve a ketone intermediate by asymmetric reduction.[18] In addition to a maximal 50% yield, this approach suffered from limited stereoselectivity in the crucial biocatalytic reduction, even when engineered cells were employed.[19] Since that time, we have systematically examined reductases encoded by the bakers' yeast genome.[20] In nearly all cases, individual yeast reductases afforded much higher stereoselectivities than whole cells of the native organism.[21] Our recent discovery that some yeast reductases show high diastereo- and enantioselectivity toward a variety of α-chloro-β-keto esters[22] suggested that the alcohols resulting from such dynamic kinetic resolutions might be useful glycidic ester precursors that could be converted to the Taxol side chain. Moreover, given the diversity of yeast reductases, we were intrigued by the possibility that both target enantiomers could be synthesized from a common precursor. This indeed proved to be the case.

SCHEME 1



We selected oxazoline **2** as a key intermediate for our synthesis since both the oxygen and nitrogen moieties are protected in this derivative, allowing its coupling to

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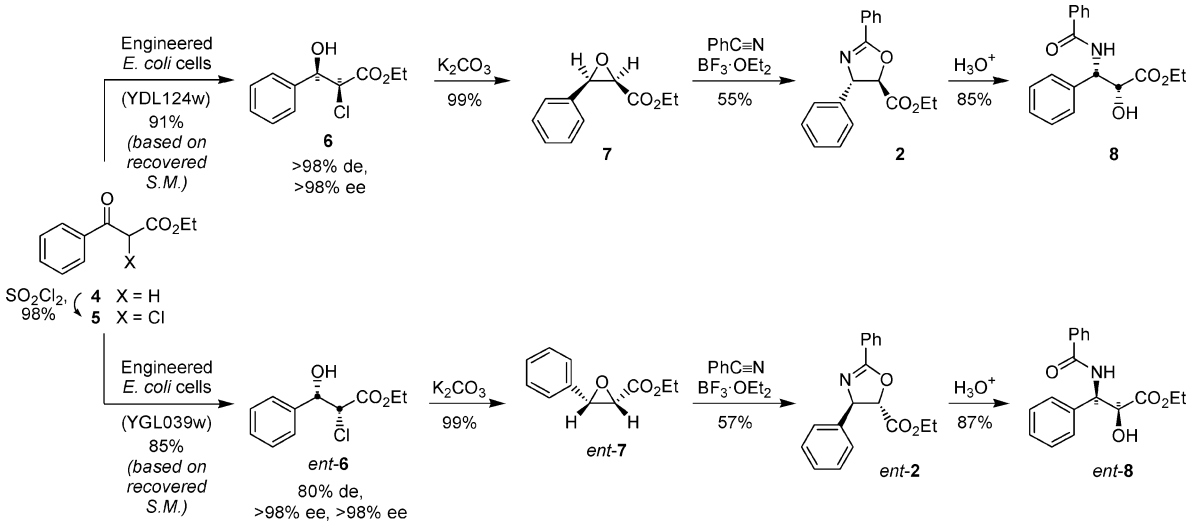
SCHEME 2



the terpene core prior to acid-catalyzed hydrolysis to yield the final target **1** (Scheme 1).[23] We planned to form glycidic esters **7** and *ent*-**7** by ring closure of the corresponding *syn*-chlorohydrin enantiomers, the products of enantiodivergent enzymatic reductions (Scheme 2). While facile enolization allows reductions of **5** to proceed by dynamic kinetic resolution, previous efforts to use chiral hydrogenation catalysts[24] or baker's yeast cells[25,26] were met with disappointingly low stereoselectivities. We hoped that individual yeast reductases might be more suitable. We also hoped that a Ritter reaction might allow one-step conversion of **7** and its enantiomer directly to the corresponding *trans*-oxazolines that are direct precursors of the final targets.[27,28] This would eliminate the need for lengthier sequences from epoxide **7** to **8**, some of which involve azide intermediates.[3,5,6,9,15]

α-Chloro-β-keto ester **5** was prepared by treating commercially available **4** with sulfuryl chloride.[29] We examined its NADPH-mediated reduction by 19 isolated *Saccharomyces cerevisiae* reductases expressed as fusion proteins with glutathione *S*-transferase.[22] Two reductions were successful: aldose reductase YDL124w produced *syn*-(2*S*,3*R*)-**6** as the only detectable product ($[\alpha]_D$ −3.0°, *c* 0.70, $CHCl_3$; lit.[25] $[\alpha]_D$ −3.0°, *c* 1.7, $CHCl_3$), whereas short chain dehydrogenase YGL039w afforded a 9:1 mixture of *syn*-(2*R*,3*S*)- and *anti*-(2*S*,3*S*)-alcohols. By comparison, reduction of **5** with whole baker's yeast cells yielded a mixture of all four stereosiomeric chlorohydrins in which the desired *syn*-(2*S*,3*R*)-product made up only half of the total yield.[25,26] Clearly, the use of individual yeast reductases allowed for a much cleaner conversion. Moreover, the ability to prepare both **6** and *ent*-**6** opened the door to synthesizing both the natural Taxol side chain as well as its enantiomer.

The use of an NADPH-regenerating system was convenient for small-scale reductions of **5**; however, larger-scale bioconversions were carried out with whole *Escherichia coli* cells that overexpressed one of the two key *S. cerevisiae* reductases (as GST fusion proteins). Glucose fed batch conditions with nondividing cells were employed[30] with the pH maintained at 5.6 to avoid spontaneous substrate decomposition under neutral or basic conditions. The ketone substrate was toxic to the cells at levels above 1 mM. We therefore added a nonpolar resin to the reaction mixture (XAD-4) to act as a reservoir for both substrate and product and used a slow-feeding strategy.[31] We observed reductive dechlorination of **5** during the early phases of the bioconversions. A similar side reaction has been observed by others,[32,33] and the extent of this dechlorination varied widely from batch to batch. Under optimized conditions, <5% of the total added substrate was lost to spontaneous decomposition and reductive dechlorination, which allowed isolation of ≥1.33 g/L of optically pure **6** from 1 L of fermentations. We did not attempt to improve the volumetric productivity of this process further since it was sufficient for laboratory-scale synthesis. Unreacted starting material was easily removed by column chromatography, and reported yields were corrected for any recovered **5**.

Conversion of **6** to *cis*-epoxide **7** proceeded smoothly according to the method of Azerad.[26] The cis stereochemistry and absolute configuration of **7** were confirmed by the $J_{2,3}$ value of 4.6 Hz and the optical rotation value ($[\alpha]_D$ +24°, *c* 1.5, $CHCl_3$; lit.[26] $[\alpha]_D$ +25°, *c* 1.1, $CHCl_3$), respectively. A Ritter reaction employing benzonitrile and a stoichiometric amount of $BF_3{\cdot}OEt_2$ converted **7** directly to a 5:1 mixture of *trans*- and *cis*-oxazolines. Systematic variation in reaction conditions (temperature, solvent,

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Lewis acid) failed to improve this ratio. Fortunately, pure *trans*-oxazoline **2** could be isolated from the crude mixture by column chromatography in 55% yield. This was hydrolyzed under mildly acidic conditions to yield the natural enantiomer of the Taxol side chain ethyl ester **8**. Its *syn*-configuration was evident from the ^{1}H NMR spectrum, particularly by the chemical shift (5.76 ppm) and $J_{2,3}$ value (1.8 Hz) of the C_3 proton (in the case of the *trans*-analogue, this signal lies 0.16 ppm upfield and the corresponding $J_{2,3}$ value is 3.5 Hz).[34] The optical purity of **8** was assessed by ^{1}H NMR after derivatization with (*S*)-α-methoxy-α-phenylacetic acid.

A complementary route was used to prepare the antipode of the Taxol side chain. In this case, an *E. coli* strain overexpressing the yeast YGL039w short chain dehydrogenase was used for the bioconversion. Because the C_2-epimeric chlorohydrins resulting from this reaction were inseparable by column chromatography, the mixture was subjected to mild base-mediated ring closure. The desired *cis*-(2*S*,3*S*)-epoxide *ent*-**7** was isolated from the crude product mixture in 66% overall yield from **5**. Its stereochemistry and absolute configuration were confirmed by proton coupling and optical rotation values. This material was carried on to *ent*-**2** and *ent*-**8** in the same manner described above.

The ability to produce directly either enantiomer of the chlorohydrin *cis*-epoxide precursor was a direct consequence of the highly *syn*-selective biocatalysts used to reduce **5** and our success in identifying individual yeast reductases with the required stereoselectivities. These results further underscore the power of genome-wide expression libraries for solving synthetic problems. In addition, the observation that two enzymes within the same organism possessed complementary enantioselectivities re-emphasizes the importance of screening individual enzymes, rather than whole cells where such enzymes would compete with one another.[21]

Using the Ritter reaction to install the nitrogen has significant safety advantages over earlier methods that require azide opening of epoxide **7**. Our approach also eliminated the need for a separate conversion to the oxazoline, thereby enhancing the brevity and atom economy of our synthesis. In fact, only the chlorine atom was lost in the conversion of **5** to the target compounds.

It should be noted that the Taxol side chain is only one member of a large family of α-hydroxy-β-amino acids that are key substructures in pharmaceutically active compounds, and our chemoenzymatic strategy may also be useful for other members of this family. Our success in the present case bodes well for such future applications.

Experimental Section

(2*S*,3*R*)-Ethyl 2-Chloro-3-hydroxy-3-phenylpropionate 6. A 45-mL portion of LB medium supplemented with 30 μg/mL kanamycin was inoculated with a single colony of *E. coli* BL21-(DE3)(pIK8). After being shaken overnight at 37 °C, 40 mL of this preculture was added to 4 L of the same medium containing 4 g/L glucose in a New Brunswick M19 fermenter. The culture was grown for 2 h at 37 °C with a stir rate of 800 rpm and an air flow of 0.5 vessel volumes per min (vvm) until it reached an optical density at 600 nm of 0.6. The cell suspension was cooled to 28 °C over 15 min, then GST fusion protein expression was induced by adding isopropylthio-β-D-galactoside to a final concentration of 0.1 mM. After being stirred for an additional 6 h under these conditions, the cells were collected by centrifugation (6000*g* for 10 min at 4 °C). These could be stored at 4 °C for later use, if desired. Approximately 25 g (wet weight) of cells was resuspended in 1 L of 10 mM KP_i (pH = 5.6) containing 4 g/L glucose in a Braun Biostat B fermenter vessel (2 L total size) containing 0.5 g of XAD-4 resin. The temperature, pH, and dissolved oxygen level were maintained at 30 °C, 5.6 (3 M NaOH titrant), and 75% saturation (fixed air flow of 0.25 vvm and variable stirring rate), respectively. Aliquots of neat **5** (0.2 mL) were added approximately every hour over a total of 12 h to a final concentration of 6 mM. Glucose was added portionwise after 3 and 6 h to maintain a glucose concentration of approximately 4 g/L. Consumption of both **5** and glucose slowed significantly after 8 h. After 24 h, the entire reaction mixture was gently extracted with CH_2Cl_2 (2 × 300 mL) to avoid an emulsion, using a procedure described in detail elsewhere.[22] The organic layer was dried with $MgSO_4$, concentrated in vacuo, and purified by flash chromatography (cyclohexane/Et_2O 85:15) to afford 1.3 g of **6** as a colorless oil (91% yield). $[\alpha]_D$ −3.0°, *c* 0.68, $CHCl_3$; lit.[33] $[\alpha]_D$ −3.0°, *c* 1.7, $CHCl_3$. Anal. Calcd for $C_{11}H_{13}O_3Cl$: C, 57.78; H, 5.73. Found: C, 57.92; H, 5.86. Spectral data matched those reported in the literature.[35]

Epoxides 7 and *ent*-7. To a solution of chlorohydrin **6** (1.3 g, 5.7 mmol) in DMF (28 mL) was added K_2CO_3 (2.2 g, 17 mmol, 3 equiv) and water (525 μL). After being stirred for 5 h at room temperature, the mixture was diluted with water (75 mL) and extracted with ether (3 × 75 mL). The combined organics were washed with water to remove residual DMF (6 × 5 mL), dried with $MgSO_4$, and concentrated in vacuo to yield 1.1 g of **7** as a colorless oil (99% yield). No further purification was necessary. $[\alpha]_D$ +24°, *c* 1.5, $CHCl_3$; lit.[26] $[\alpha]_D$ +25°, *c* 1.1, $CHCl_3$. Anal. Calcd for $C_{11}H_{12}O_3$: C, 68.74; H, 6.29. Found: C, 68.34; H, 6.57. Spectral data matched those reported in the literature.[26] Crude *ent*-**7** was prepared in the same way and subjected to silica gel chromatography to remove a small amount of *trans*-epoxide, affording 0.86 g of *ent*-**7** as a colorless oil (66% overall yield from **5**). $[\alpha]_D$ −29°, *c* 2.9, $CHCl_3$.

Oxazolines 2 and *ent*-2. A solution of glycidic ester **7** (0.50 g, 2.6 mmol) in benzonitrile (4 mL) was cooled to 0 °C under argon atmosphere, then $BF_3{\cdot}OEt_2$ (330 μL, 2.6 mmol, 1.0 equiv) was added over 10 min. The ice bath was removed, and the reaction was stirred for 3 h before aqueous saturated $NaHCO_3$ (4 mL) was added. After being stirred for an additional 2 h, the reaction mixture was diluted with water (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The combined organics were dried with $MgSO_4$, concentrated in vacuo, and purified by silica gel chromatography (cyclohexane/ether 85:15) to afford 0.42 g of **2** as a colorless oil (55% yield). **2**: $[\alpha]_D$ +11°, *c* 1.1, $CHCl_3$. *ent*-**2**: $[\alpha]_D$ −12°, *c* 1.7, $CHCl_3$. Spectral data for both **2** and *ent*-**2** matched those reported in the literature for the racemic compound.[36,37]

Taxol Side Chain Ethyl Esters 8 and *ent*-8. A solution of oxazoline **2** (0.32 g, 1.1 mmol) in 0.5 M HCl (2.5 mL) and EtOH (7 mL) was heated at reflux for 6 h. The solvent was removed under reduced pressure, and then the residue was dissolved in CH_2Cl_2 (10 mL), washed with water (2 × 7.5 mL), dried with $MgSO_4$, and concentrated under reduced pressure. The residue was purified by silica gel chromatography (hexanes/Et_2O 85:15) to afford 0.30 g of **8** (85% yield) as a white solid, mp 162−163 °C, lit.[38] mp 164−165 °C. $[\alpha]_D$ −12 °, *c* 2.0, $CHCl_3$; ^{1}H NMR ($CDCl_3$) δ 1.30 (t, 3H, *J* = 7.1), 3.30 (d, 1H, *J* = 3.9), 4.30 (m,

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2H), 4.62 (dd, 1H, J = 3.9, 1.9), 5.76 (dd, 1H, J = 9.0, 1.9), 6.99 (br d, 1H, J = 9.0), 7.30−7.55 (m, 8H), 7.77 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 14.3/14.4, 55.0, 60.6/62.9, 73.5, 127.1, 127.3, 128.1, 128.8, 128.9, 132.0, 134.4, 139.0, 167.1, 173.1;[39] IR (KBr): ν (cm^{-1}) 3416, 1718, 1638, 1529; HRMS (FAB) m/z calcd for $C_{18}H_{19}NO_4 \cdot Na^+$ 336.1206, found 336.1209. *ent*-**8**: $[\alpha]_D$ +12°, c 1.0, $CHCl_3$. The optical purities of **8** and *ent*-**8** were determined by ^{1}H NMR following derivatization with (*S*)-α-methoxy-α-phenyl acetic acid (carried out in the NMR tube with 1.0 equiv of (*S*)-MPA and 1.5 equiv of DCC and 0.5 equiv of DMAP in $CDCl_3$). The sample of *ent*-**8** showed only a single enantiomer within the limits of instrumental detection; that of **8** showed ca. 5% contamination by its enantiomer.

(39) Both the ^{1}H and ^{13}C NMR spectra showed evidence for two ethyl group conformers.

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Supporting Information Available: General experimental information, details for the preparation of *ent*-**6**, spectral data for intemediates, and NMR optical purity determination for **8** and *ent*-**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

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