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Bacillus subtilis epoxide hydrolase-catalyzed preparation of enantiopure 2-methylpropane-1,2,3-triol monobenzyl ether and its application to expeditious synthesis of (*R*)-bicalutamide

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Abstract—Expeditious synthesis of (*R*)-bicalutamide (**1**), a synthetic antiandrogen, from enantiopure 2-methylpropane-1,2,3-triol monobenzyl ether (**4**) was achieved. An engineered *Bacillus subtilis* epoxide hydrolase worked enantioselectively on the racemic epoxide (**7**) to provide the above starting material in highly enantiomerically enriched state.

Being a potent antiandrogen of a non-steroidal structure, bicalutamide [Casodex®, (**1**)][1] has been used in drug therapy to treat prostate cancer (Fig. 1). While the clinically prescribed entity is a racemic mixture,[1,2] its (*R*)-isomer was deduced to be an active principle from the following experimental evidences:[3] the (*R*)-isomer of **1** exhibited higher affinity to androgen receptors[4] and was less susceptible to metabolic degradation compared to the antipodal (*S*)-isomer.[5]

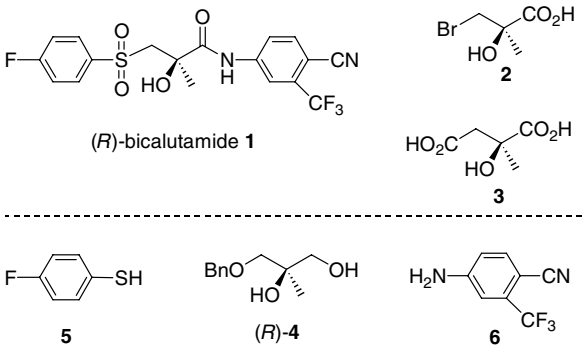
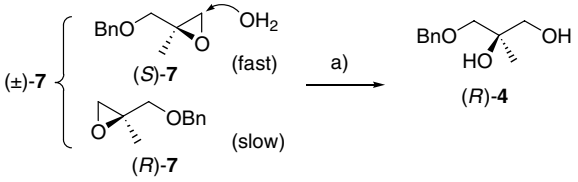


Figure 1.

So far, (*R*)-**1** and analogs thereof were assembled using two kinds of enantiomerically enriched 2-methyl-2-hydroxypropanoic acid derivatives: (1) (*R*)-3-bromo-2-hydroxy-2-methylpropanoic acid (**2**) prepared via asymmetric bromolactonization effected under the influence of D-proline as chiral auxiliary;[6–9] (2) (*S*)-citramalic acid (**3**) obtained by resolution.[10] Once its latent symmetry was recognized with **1**, terminally differentiated 2-methylpropane-1,2,3-triol, (*R*)-**4**, might well serve the synthesis of (*R*)-**1** providing that thiophenol (**5**) and aniline (**6**) could be installed at the proper ends of (*R*)-**4** (Fig. 1).

Preparation of an enantiomerically enriched form of **4** has been known by epoxide hydrolase (EH)-catalyzed enantioselective hydrolysis[11] of easily accessible racemic epoxide **7**.[12] While diverse catalytic activities and stereochemical courses have been reported,[13] we chose an



Scheme 1. Reagents and conditions: (a) *B. subtilis* epoxide hydrolase, 30 °C, 7 days, conv. 52%; (*R*)-**4**: 46%, 79.0% ee; (*R*)-**7**: 37%, 100% ee.

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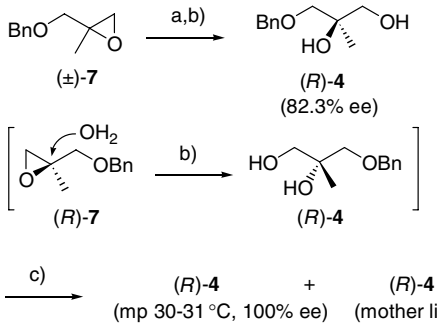
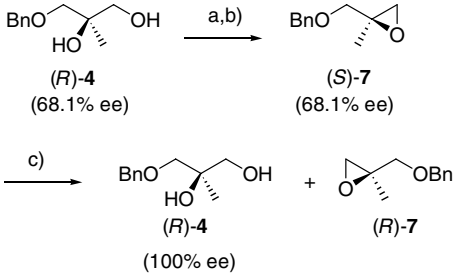


Scheme 2. Reagents and conditions: (a) *B. subtilis* epoxide hydrolase, 30 °C, 7 days, conv. 53%; (b) dil H_2SO_4, room temperature; (c) recrystallization from Et_2O at −30 °C, (*R*)-**4** as crystalline solid: 43%, 100% ee; as mother liquor, 40%, 68.1% ee.



Scheme 3. Reagents and conditions: (a) TsCl, pyridine; (b) K_2CO_3, MeOH, 94%; (c) *B. subtilis* epoxide hydrolase, 30 °C, 2 days, conv. 82%; (*R*)-**4**: 82%, 100% ee; (*R*)-**7**: 18%, 68.1% ee.

engineered enzyme, with high catalytic activity and availability in quantity, from an origin of *Bacillus subtilis* (BSEH).[14] When harvested cells of the engineered *B. subtilis* were incubated with (±)-**7** at 30 °C for a week, (*S*)-selective hydrolysis proceeded in 52% conversion to give (*R*)-**4** of 79.0% ee and unconsumed (*R*)-**7** of 100% ee in 46% and 37% isolated yield, respectively (Scheme 1).[15] For this BSEH-mediated kinetic resolution of (±)-**7**, the *E* value[16] was estimated to be as high as 73.

Now that BSEH had proven to be efficacious in resolving (±)-**7** kinetically to (*R*)-epoxide (**7**) and its antipodal diol (**4**) via (*S*)-selective hydrolysis on a preparative scale, attention was turned to defining the conditions to obtain only the hydrolysate (*R*)-**4** from (±)-**7** in a stereoconvergent manner[17] (Scheme 2). The above-mentioned cells of *B. subtilis* were incubated with (±)-**7** in 53% conversion. The resulting mixture of (*R*)-**4** and (*R*)-**7** as a whole was treated with dilute H_2SO_4,[18] whereby (*R*)-**4** underwent acid-catalyzed hydrolysis with stereochemical inversion at its quaternary stereogenic center[19,20] to afford (*R*)-**4** of 82.3% ee in 83% overall yield (Scheme 2). This was further crystallized from Et_2O at −30 °C, and enantiomerically pure (*R*)-**4** was obtained as a solid in 43% yield (52% recovery).[21]

The mother liquor (68.1% ee) in the previous crystallization procedure still contained the (*R*)-enantiomer (ca. 84% of the mixture). It was then attempted to reuse the (*R*)-**4**, recovered with a moderate enantiomeric purity, by converting it back to (*S*)-epoxide (**7**) and subjecting the latter to the BSEH-catalyzed kinetic resolution again (Scheme 3). Then, diol (*R*)-**4** was derived to enantiomerically enriched (*S*)-**7** (68.1% ee) in two conventional steps (94%).

Under the kinetically resolving conditions, pursuing high ee of the digested products (more reactive enantiomers) is always somewhat more difficult than of the unaffected substrates (less reactive enantiomers), even with high enantioselectivity. As the desired (*R*)-**4** is derived from the more reactive enantiomer (*S*)-**7**, termination of the reaction at the proper conversion is very important. We then simulated the relationship between conversion and ees of the digested product **4** and unaffected recovery **7** under a certain mathematical model,[16] and Figure 2 predicted ca. 80% conversion as the critical point.

The progress of the actual enzymatic reaction was monitored occasionally by HPLC. After 2 days, we stopped the reaction at 82% conversion, and enantiomerically pure (*R*)-**4** in 82% and (*R*)-**7** of 68.1% ee in 18% were obtained (Scheme 3). In this event, two interesting observations were noted. When starting with (*S*)-**7** of 68.1% ee, the BSEH-catalyzed hydrolysis proceeded with slightly higher enantioselectivity than the value of 73 that had been estimated for the hydrolysis of (±)-**7**. In addition, the reaction proceeded substantially faster with (*S*)-enriched **7**. This acceleration phenomenon should be ascribed to less amounts of (*R*)-**7** which, possessing a K_m value similar to that of (*S*)-**7**, must have worked as a competitive inhibitor against the BSEH.

The combined total yield of enantiomerically pure (*R*)-**4** as described in Schemes 2 and 3 was 74% based on the original starting material, (±)-**7**. With enantiomerically pure (*R*)-**4** being secured in quantity, effort was directed toward its conversion to (*R*)-bicalutamide (**1**) (Scheme 4). Selective oxidation of the diol was performed with TEMPO-mediated oxidation to give **8** (97%),[22] by avoiding any reagents possibly causing the undesired glycol cleavage through a cyclic intermediate by metallic oxidants.[23] For the next amide bond formation between

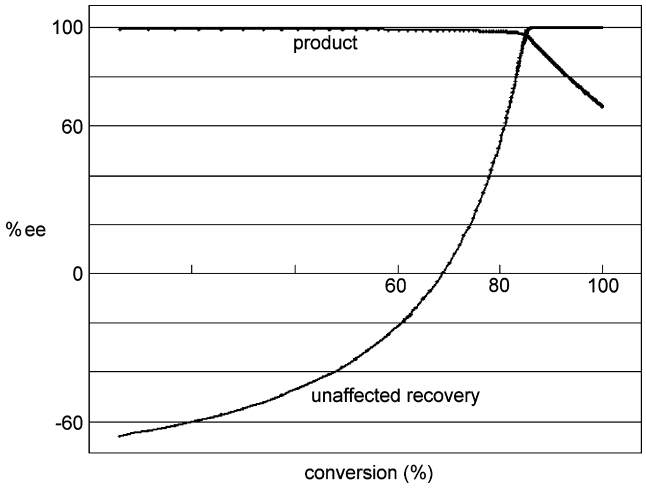


Figure 2. Simulation for the progress of *B. subtilis* epoxide hydrolase-catalyzed hydrolysis of (*S*)-**7** ($E = 73$, ee0 = 68.1%).