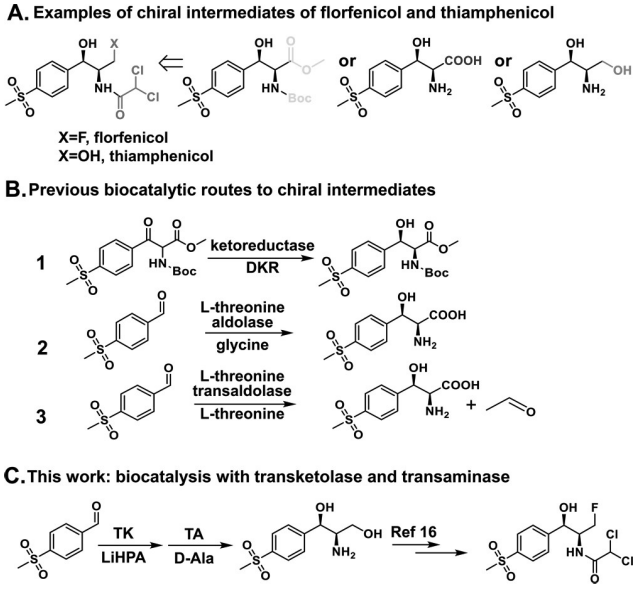


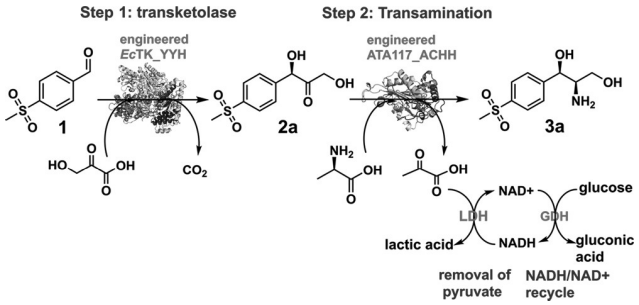
Figure 1. Synthesis of chiral intermediates of florfenicol and thiamphenicol. (A) Examples of chiral intermediates of florfenicol and thiamphenicol. (B) Previous synthetic methods using enzymes. (C) Our enzymatic route based on transketolase and transaminase. The aminodiol intermediate could be converted to the final florfenicol with fewer chemical steps than the reported intermediate.[16]

ment of protection and deprotection steps,[17−21] enzymatic catalysis could be a valuable alternative route for the chemical synthesis of the chiral florfenicol intermediate. A ketoreductase was reported to catalyze a stereoselective (2*S*,3*R*)-α-amino-β-hydroxyl ester formation from the corresponding α-amino-β-keto ester mediated by dynamic reductive kinetic resolution. In this approach, the majority of the product must be assembled in advance, with the enzyme mostly serving to set the stereochemistry at the end (Figure 1B, entry 1).[16] Besides, L-threonine aldolase (LTA) from *Pseudomonas putida* was reported to catalyze chiral L-threo-*p*-methylsulfonyl phenylserine (L-*threo*-MSPS) formation, whereas the diastereoselectivity of the reaction was relatively low, with 53% diastereomeric excess (de) (Figure 1B, entry 2).[22] The Zhu group performed enzyme engineering of an LTA from *Pseudomonas* sp. and obtained a mutant that catalyzed the formation of L-*threo*-MSPS with 71% de (Figure 1B, entry 2).[23] The Wu group also carried out enzyme engineering of an LTA from *Bacillus nealsonii* and obtained a mutant that catalyzed the formation of L-*threo*-MSPS with >99% de[24] (Figure 1B, entry 2). Recently, the Lin group reported an L-threonine transaldolase (LTTA) from *Pseudomonas* sp. Through conditional optimization, L-*threo*-MSPS was obtained with 94.6% de catalyzed by PsLTTA (Figure 1B, entry 3).[25,26] In general, L-*threo*-MSPS could be biosynthesized with good stereoselectivity using LTA or LTTA. To eventually be converted to florfenicol, L-*threo*-MSPS needs to be converted first to (1*R*,2*R*)-*p*-methylsulfonyl phenylserinol (**3a**), which requests two chemical steps comprising esterification and reduction.[27] In order to establish a simplified synthetic route of florfenicol, we attempted to achieve the biobased production of **3a**.

Recently, transketolases (TKs) have emerged as attractive biocatalysts for biotransformation of aliphatic and aromatic aldehydes to various chemicals.[28−36] TK is a thiamine pyrophosphate (ThDP)-dependent enzyme that catalyzes α-hydroxyketone formation by stereoselectively transferring a ketol unit from polyhydroxylated ketose phosphates as a "natural" donor to an aldehyde acceptor.[37] Several successful applications of TK have already been demonstrated for the production of α-hydroxyketones.[32−36] α-Hydroxyketones are of particular value as fine chemicals because of their utility as building blocks for the production of larger molecules, particularly for construction of heterocycles required in pharmaceuticals.[32] Akin to TKs, transaminases (TAs) have also attracted considerable interest in biocatalysis, both as an individual biocatalyst and as a part of multienzyme cascades for the synthesis of various amines.[38−42] TA catalyzes the transfer of an amino group between an amine donor (different amino acids and amines) and an amine acceptor (a ketone or aldehyde). Of particular interest are ω-TAs that do not require the presence of a carboxylic group in substrates, thereby accepting a large variety of carbonyl substrates.[38,39]

Here, we present an enzymatic one-pot two-step reaction for the synthesis of a stereoselective aminodiol intermediate, (1*R*,2*R*)-*p*-methylsulfonyl phenylserinol (**3a**), from readily available achiral 4-(methylsulfonyl)benzaldehyde (**1**) using a TK and a ω-TA (Figure 1C and Scheme 1). **1** is the raw material for the industrial production of florfenicol,[7] suggesting that our biocatalytic route has potential for industrial applications. However, there existed three challenges in this biocatalytic cascade reaction. First, TK family members are predominantly *S*-selective.[34] For aromatic aldehyde substrates, only one work reported an (*R*)-selective TK mutant that catalyzed the hydroxyketone formation toward benzaldehyde with 82% ee, but with a low conversion (10%).[36] Second, the hydroxyketone intermediate **2** formed by TK undergoes rapid racemization,[34] resulting in a decrease of optical purity. Third, **1** probably serves as a substrate for TK as well as for ω-TA, and ω-TA may display a higher chemical reactivity toward aldehyde **1** relative to ketone **2**.[43,44] As a consequence, most of **1** would be converted to the benzylamine side-product in the one-pot reaction. For addressing the first challenge, we reversed the enantioselectivity of TK from (*S*) (93% ee) to (*R*) (95% ee) via structure-guided enzyme engineering. For solving the second problem, we inverted the stereopreference of ω-TA toward chiral hydroxyketone intermediate **2** ($E(S)$ = 9 to $E(R)$ = 12). For addressing the third challenge, we reversed the aldehyde/ketone substrate selectivity of ω-TA. Combining the engineered TK and TA, the aminodiol intermediate (**3a**) of florfenicol was accessible with good yield (76%) and excellent stereoselectivity (96% de and >99% ee).

Scheme 1. One-Pot Two-Step Cascade Reaction to (1*R*,2*R*)-*p*-Methylsulfonyl Phenylserinol 3a, from the Industrial Raw Material 4-(Methylsulfonyl)-benzaldehyde 1



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