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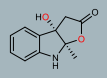
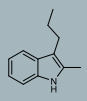
B



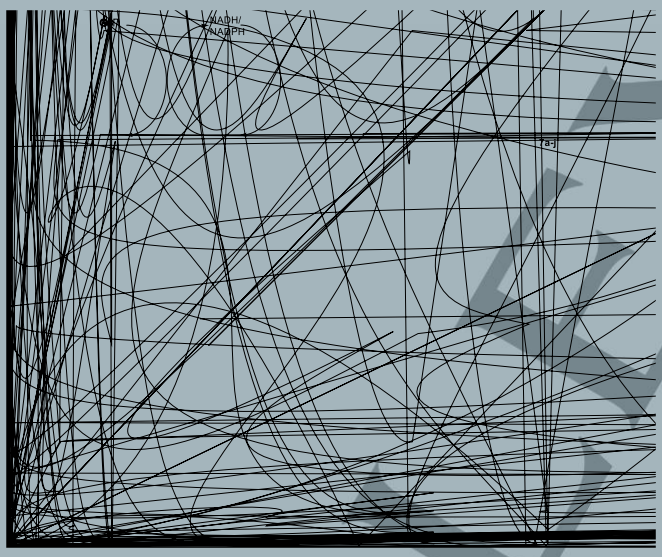
by-product (



Scheme 1. Structures, biosynthetic pathway and enzymatic reactions associated with TsrE activity. Atom ^{18}O in panels B and C is indicated in red. (A) TSR and specific PTMs for processing L-Trp to the QA moiety within the side-ring system. (B) Proposed mechanism for the formation of by-product **3**. (C) Selective hydroxylation of 2-methyl-3-propyl-indole to a (3*S*) product.

reduced dihydronicotinamide adenine dinucleotide (phosphate) (NAD(P)H) in the presence of the flavin reductase Fre.[9b,10] In addition to **2**, careful analysis of the TsrE-catalyzed transformation revealed a minor compound, **3**, which proved to be a shunt product bearing a distinct dearomatized 3a-hydroxy oxofuroindoline scaffold. Following this unexpected observation, in this st

model substrate 2-methyl-tryptophol (**7a**) was transformed to 8a-methyl-3a-hydroxyfuroindoline, **8a**, but not completely (i.e., 55% conversion observed over a 3-hr reaction period). Kinetic analysis under **RC-I** revealed a k_{cat} value of 6.6 ± 0.1 min^{-1}, approximately 3-fold lower than that in the conversion of **6**, indicating that reduced $FADH_2$, which was rapidly produced given the high catalytic efficiency of the flavin reductase Fre, might not be completely used for **8a** production and instead tend to be oxidized by O_2 to FAD and H_2O_2. Consistently, either time elongation or increase in NADPH supply (e.g., 3:1 for the ratio of NADPH to the substrate **7a**) in the reaction mixture did not cause conversion improvement. H_2O_2 appears to have little affect on TsrE activity under **RC-I**, because the addition of catalase (e.g., 0.1U/ml or 1U/ml) did not improve the conversion rate. To alleviate the inconsistency in reduced $FADH_2$ production and TsrE-catalyzed oxygenation, we utilized glucose 6-phosphate dehydrogenase (G6PD), which provides NAD(P)H *in situ* by converting glucose 6-phosphate (G6P) to 6-phosphogluconolactone, for sustainable $FADH_2$ supply in the presence of Fre. Consequently, the two-enzyme system composed of G6PD and Fre was optimized to provide the reducing power matching TsrE activity for **7a** transformation, leading to a reaction condition (**RC-II**) giving > 99% conversion and 99% *ee* for **8a** production over a 2-hr reaction period (**Scheme 3**).

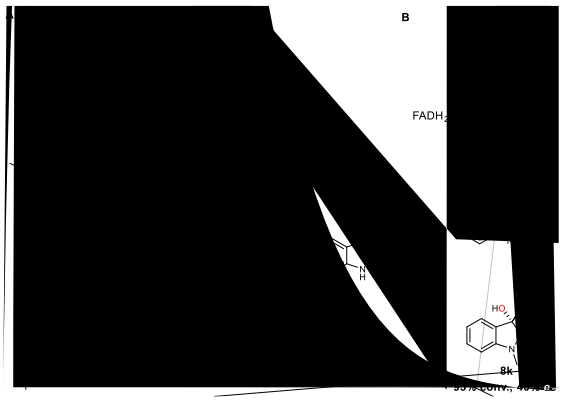


Scheme 3. Enantioselective synthesis of 3a-hydroxyfuroindolines by TsrE.

We then utilized different tryptophol derivatives (i.e., **7b-d** and **7g**) to test TsrE activity under **RC-II**. Over a 2-3 hr period, these substrates were nearly completely converted to individual 8a-methyl-3a-hydroxyfuroindolines (i.e., **8b-d** and **8g**, > 95% conversion); however, compared to the conversion of **7a**, the enantioselectivity of this enzyme appeared to be decreased (i.e., with the *ee* values of 97%, 87%, 75% and 44% for **8b**, **8c, 8d** and **8g**, respectively). Analysis of the control reactions proceeding in the absence of TsrE revealed background products. Likely, overproduced $FADH_2$ can react with O_2, leading to an excess of FAD-4a-OOH that could mediate non-enzymatic oxidative dearomatization in a diastereoselective manner. To further balance $FADH_2$ production with TsrE-catalyzed oxygenation, for which the catalytic efficiency might be decreased by indole substitution, we reduced exogenous FAD usage. Remarkably, under **RC-III**, where the ratio between FAD and the enzyme was adjusted to 1:1, the conversions of **7b-d** and **7g** by TsrE provided expected products **8b-d** and **8g** with comparable conversion rates (i.e., > 95% for each reaction) and improved *ee* values (i.e., 98%, 90%, 97% and 85% for **8b**, **8c, 8d** and **8g**, respectively). This further optimized condition was thus applied to the dearomatizations of additional 2-methyl-tryptophol derivatives (i.e., **7e**, **7h**, **7j**, **7i**,**7f** and **7k**) to evaluate the selectivity of TsrE catalysis in substrate.

With the exceptions of **7e** and **7f** that cannot be converted, TsrE activity is sufficient for (nearly) completely converting the other 2-methyl-tryptophol substrates (i.e., **7h**, **7j**, and **7i**) into related 3-hydroxy-furoindoline products (i.e., **8h**, **8i** and **8j**); however, its stereoselectivity varies in a substrate-dependent manner (i.e., 36-67% *ee* values). Based on the observation that TsrE tolerates C5 substitutions by F, Cl and CH_3 but does not accept CH_3O substitution at the same position, the size of a substituent group, instead of its electronic property, likely plays a key role in substrate tolerance. In addition, the change in fluorination position from C5 to C6 and C7 decreases the enantioselectivity of this enzyme, likely due to the FAD-4a-OOH-associated, competitive oxidative dearomatization as aforementioned. Similar results were observed by changes in chlorination position. This nature is different from that observed above in the conversions of 2-methyl-indole-3-acetic acids, where either CH_3O substitution at C5 or fluorination at C7 has little effect on the enantioselectivity of TsrE, indicating that the terminal carboxylate group shared between **6, 6a-d** and the native substrate **1** is of importance to substrate binding at the active site of the enzyme. Without this group, as shown in **7a-j**, TsrE catalysis appears to be sensitive to substitution changes in the indole part of the substrate. As observed in the conversions of indole-3-acetic acids, C2-subsitution is necessary for TsrE activity, because oxidative indole dearomatization failed to occur by using the demethylated derivative **7f** as the substrate. At this position, enantioselectivity tends to decease with the extension of the length of substituent groups.

The examination of N1 substitution was conducted by converting **7k** to provide insights into the oxygenation mechanism of TsrE catalysis (**Scheme 4**). TsrE-catalyzed oxygenation could facilitate indole dearomatization through two routes. **Route a** depends on the selective hydroxylation at C3 to produce intermediate **9**, which can result from deprotonation of the nitrogen hydrogen to drive double bond shift and thus increase C3 nucleophilicity for the attack to the peroxyl group of FAD-4a-OOH. In contrast, **Route b** requires deprotonation of the oxid



Scheme 4. Investigation into the catalytic mechanism of TsrE. (A) Two possible routes to furoindolines. (B) Conversion of **7k** into **8k**.

Finally, we tested the scale-up feasibility of TsrE-catalyzed oxidative dearomatization using the model substrates **6** and **7a**. In a 16-fold enlarged, 400 mL reaction mixture, TsrE completely transformed substrate **6** (75.6 mg and 0.4 mmol) or **7a** (70.1 mg and 0.4 mmol) into product **3** or **8a** over a 1.5-2.0 hr period at room temperature, with an *ee* value and a yield comparable with those obtained in a 25 mL reaction mixture. These enzymatic transformations were conducted under revised **RC-III**, where the only change is using glucose dehydrogenase and glucose to replace expensive G6PD and G6P for NADH supply.

In conclusion, following the unexpected TsrE-catalyzed shunt reaction during investigations into the formation of the QA moiety installed the side ring system of the thiopeptide antibiotic thiostrepton, we developed an effective enzymatic approach for the oxidative dearomatization of indoles in the asymmetric synthesis of a variety of furoindolines with a vicinal quaternary carbon stereogenic center. This approach, which relies on the 2,3-epoxidation of 2-methyl-indole-3-acetic acid or 2-methyl-tryptophol in a highly stereoselective manner and subsequent epoxide opening under mild reaction conditions, complements chemical methods for oxidative indole dearomatization and thus enriches the toolbox in the asymmetric synthesis of natural products possessing a furoindoline skeleton.[11,12]

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Keywords: l - dependent monooxygenase Thiostrepton Quinaldic acid

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Table of Contents

An enzymatic approach for oxidative indole dearomatization is reported. TsrE, a flavin-dependent monooxygenase involved in the biosynthesis of bicyclic thiopeptide antibiotic thiostrepton, catalyzes 2,3-epoxidation and subsequent epoxide opening during the conversion of 2-methyl-indole-3-acetic acid or 2-methyl-tryptophol to furoindoline, with up to > 99% conversion and > 99% *ee* under mild reaction conditions.