

Total Synthesis of Tambromycin by Combining Chemocatalytic and Biocatalytic C–H Functionalization

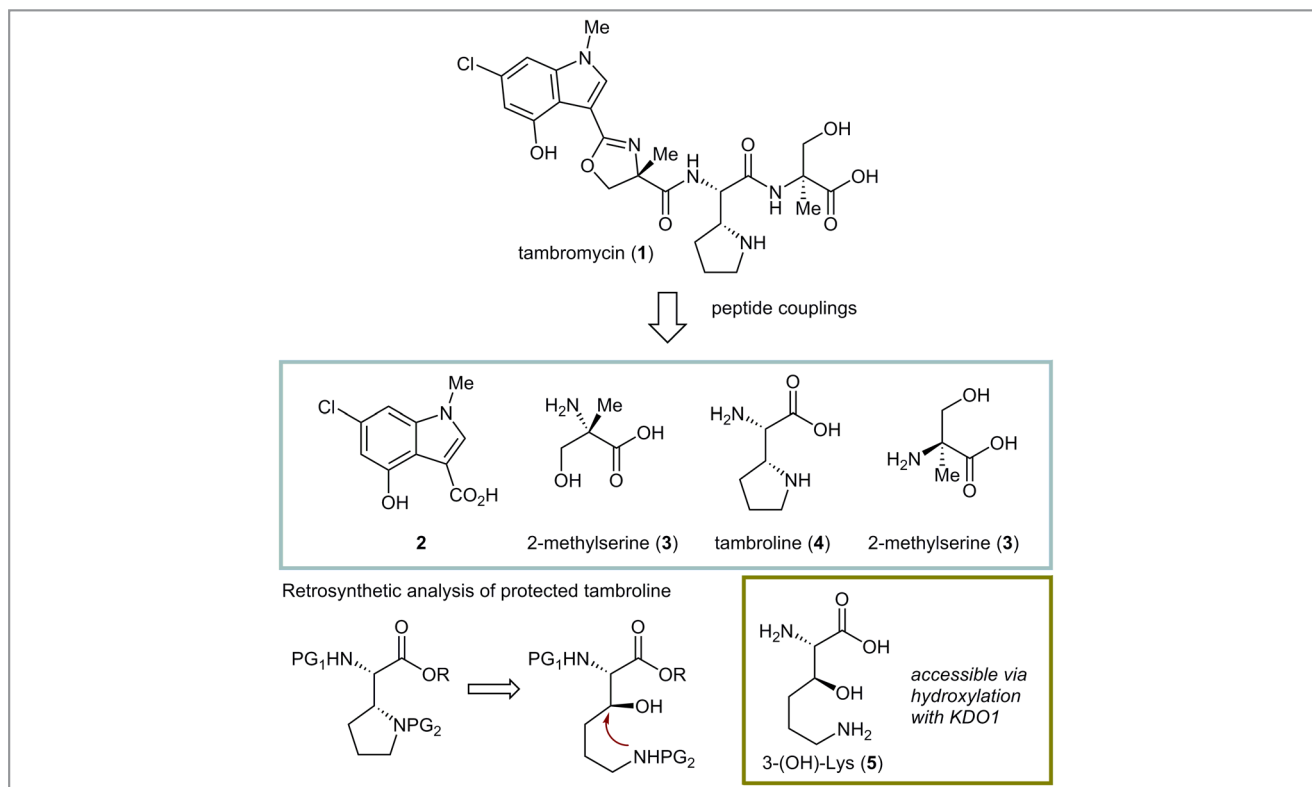
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Enzymes are capable of highly selective and specific synthetic transformations, but the potential of biocatalysis in total synthesis is far from being fully exploited.

The lab of Professor Hans Renata at The Scripps Research Institute (USA) is broadly interested in developing novel synthetic approaches to bioactive natural products by leveraging contemporary technology in enzyme catalysis. “We are particularly focused on the use of enzymatic hydroxylation as an enabling platform to simplify routes to complex scaffolds,” said Professor Renata. He continued: “In this regard, we chose to work with iron- and α -ketoglutarate-dependent dioxygenases (Fe/ α KGs) as their utility in organic synthesis has been relatively underexplored so far. Prior to this work, we were able to complete an efficient formal synthesis of manzacidin C by employing a selective hydroxylation of an L-leucine deriva-

tive as our key step (*J. Am. Chem. Soc.* **2018**, *140*, 1165–1169). To build on this success, we sought other synthetic targets wherein a similar synthetic logic could be applied.”

The group was first drawn to tambromycin due to its structural complexity and promising preliminary bioactivity. Notably, this natural product contains a highly unusual amino acid monomer, tambroline, which would provide a useful testbed for the use of biocatalytic retrosynthetic disconnection (Scheme 1). “In our minds, the best and most direct route to tambroline was via scission of the C3–N bond,” remarked Professor Renata. He explained: “While one could envision the use of Hofmann–Löffler–Freitag reaction on protected L-lysine to construct this bond, in our hands, the reaction failed to provide any desired product. Applying the principles of biocatalytic retrosynthesis, we realized that the pyrrolidine ring

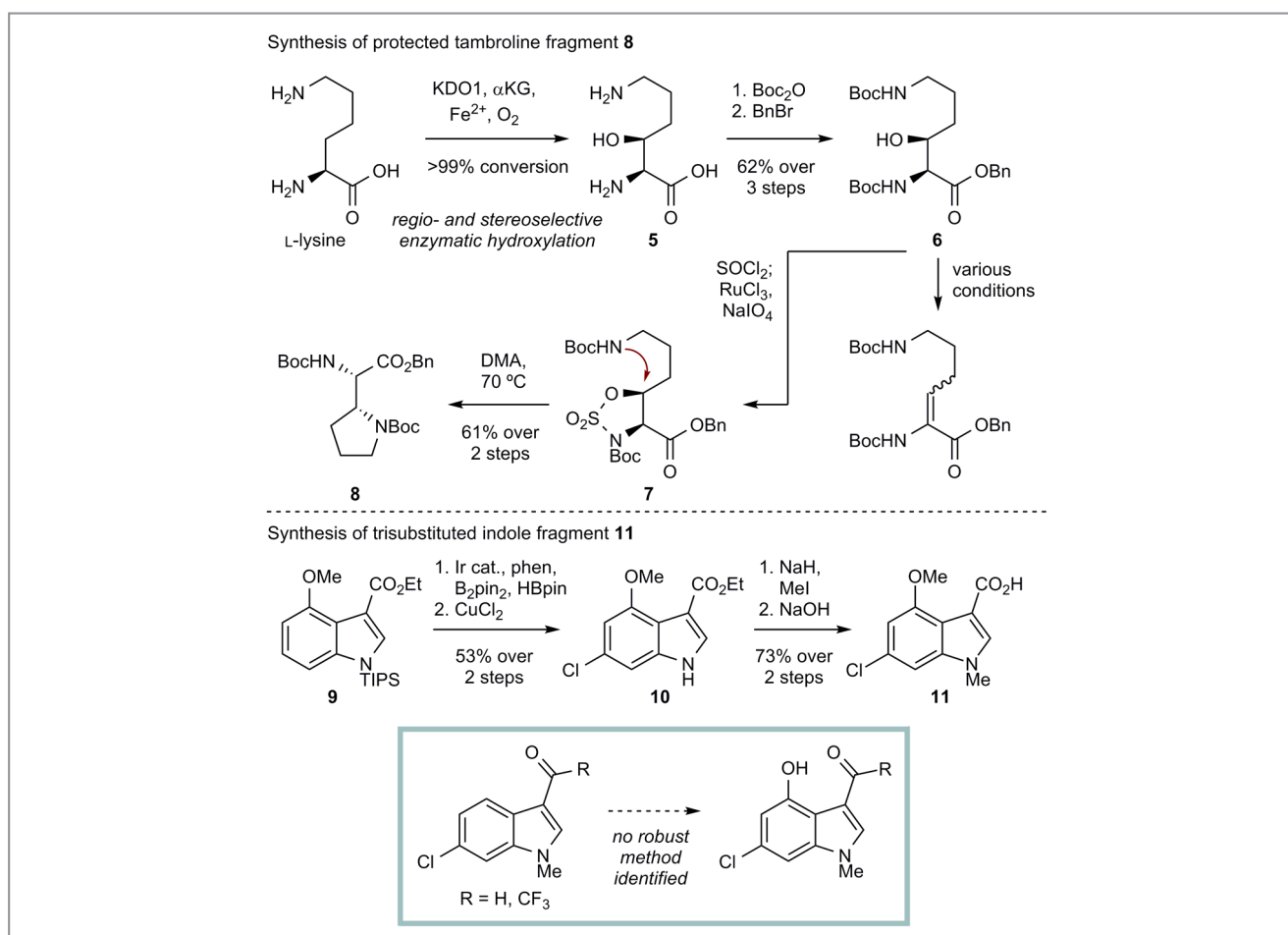


Scheme 1 Retrosynthetic analysis of tambromycin and tambroline

could be constructed via a nucleophilic substitution reaction on 3-OH-Lys, which in turn is a known product of lysine hydroxylation with an Fe/αKG, KDO1.” As the hydroxylation step would constitute the first step of their synthesis, Professor Renata and his co-workers needed to be able to conduct this transformation on multi-gram scale with good conversion and yield. At the outset, this was not a given, as prior reaction with KDO1 was only done on a scale of less than 100 mg. “We eventually found that while KDO1 has good catalytic activity, it suffers from poor soluble heterologous expression in *E. coli* even after codon optimization,” explained Professor Renata. “Fortunately, co-expression of the chaperones GroES/GroEL was able to improve the soluble expression of KDO1 significantly, and equipped with ample quantities of the enzyme, we were able to solve the initial material throughput issue in the preparation of the tambroline fragment.”

The pyrrolidine construction from **6** was the next major hurdle in the synthesis (Scheme 2, top). “The use of standard S_N2 or Mitsunobu conditions would lead to facile E1cB elimination to the dehydrolysine derivative,” explained Professor Renata. The group found that the formation of sulfamidate **7** was essential to prevent the E1cB competitive pathway. However, as revealed by Professor Renata, choice of solvent was also crucial, as the reaction kinetics of the cyclization in dimethylacetamide (DMA) were far superior to those in toluene. Indeed, conducting the reaction in the latter solvent required nearly three days of reflux to see full conversion from starting material.

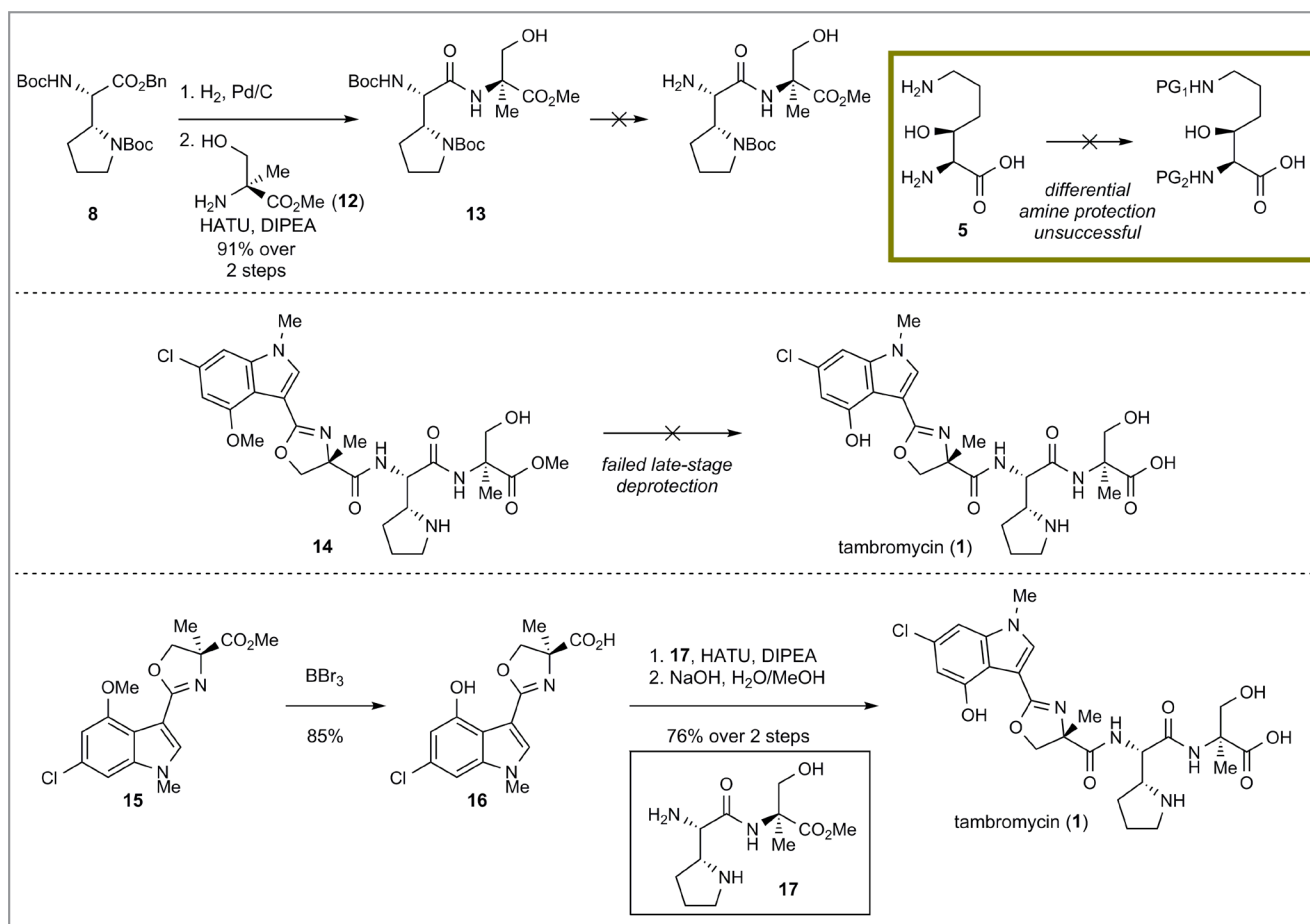
Professor Renata explained that the indole fragment was particularly interesting to tackle. “Despite the bounty of indole syntheses, the 3,4,6-trisubstituted indole motif still poses a significant synthetic challenge,” he said. “Not unlike



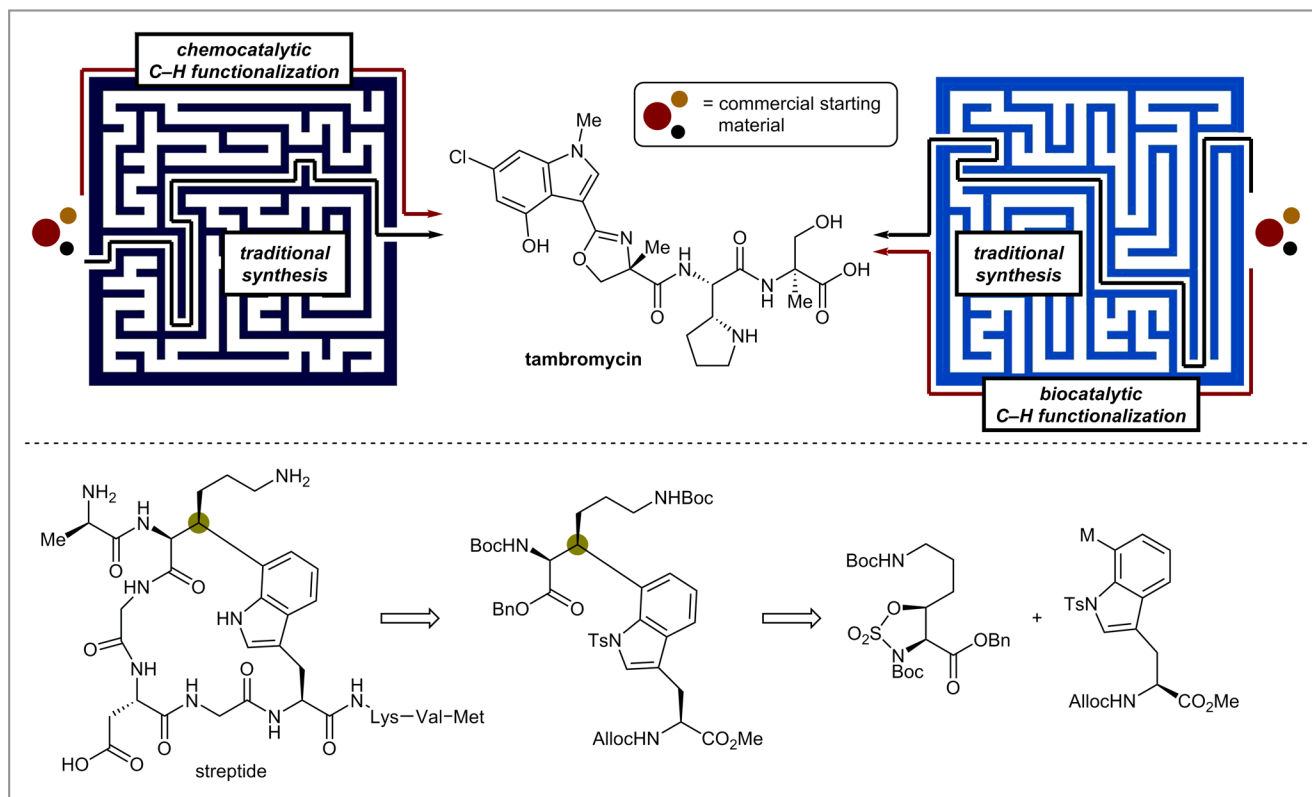
Scheme 2 Top: Preparation of protected tambroline (**8**) via enzymatic hydroxylation of L-lysine. Bottom: Preparation of key indole fragment **11** via regioselective C6 borylation of indole **9**.

the tambroline fragment, such indole is also a known structure, having previously been synthesized via Hemetsberger or Batcho–Leimgruber ring synthesis. However, none of the published routes was deemed to be particularly efficient.” Professor Renata continued: “We envisioned a ring substitution approach from a commercially available disubstituted indole would offer a more streamlined access to **11** (Scheme 2, bottom). After several failed attempts to effect C4 functionalization on various 6-chloroindole derivatives, we elected to focus on selective C6 functionalization of 3,4-disubstituted indole (e.g., **9**).” He added: “We were cognizant of recent C–H functionalization literature from Hartwig and Baran which showed that C6 borylation of indole derivatives could be effected through judicious combination of iridium catalyst, ligand and borylating reagent. To our delight, this chemistry could be performed on indole **9** on gram-scale with good yield, thereby addressing the preparation of the key indole fragment.”

At this stage, all the requisite building blocks had been prepared. However, their final assembly was not without its challenges (Scheme 3). The group had prepared the tambroline fragment as the bis-Boc derivative and initially sought methods for regioselective Boc deprotection to avoid any regioselectivity issues in subsequent coupling. However, no suitable methods could be identified. In parallel, the authors also went back to the very beginning of the synthesis to explore the possibility of effecting differential amine protections on **5** by employing an established copper-chelation method, but this also turned out to be problematic, likely due to the possibility of multiple metal chelation modes by **5**. Professor Renata explained: “Faced with these failures, we elected for a global Boc deprotection, reasoning that the local steric environments of the two free amines in the product would be different enough to allow for selective peptide coupling. Similarly, the indole fragment was prepared as the methyl ether derivative and we had hoped to keep this protecting



Scheme 3 Top: Failed attempts at effecting selective deprotection of **13** and differential protection of **5**. Middle: Unsuccessful attempt at late-stage deprotection of key intermediate **14**. Bottom: Final synthetic route to tambromycin (**1**).



Scheme 4 Top: Incorporation of biocatalytic and chemocatalytic C–H functionalization logic in the total synthesis of tambromycin. Bottom: Potential future application of biocatalytic C–H functionalization logic in the total synthesis of streptide.

group until the end of the synthesis. However, demethylation of the fully assembled tambromycin proved to be extremely difficult. Thus, we chose to remove the methyl ether group earlier in the synthesis. Of course, all of these complications made the key fragment coupling step rather daunting as both fragments **16** and **17** contained various free N–H and O–H groups. Gratifyingly, this step turned out to be highly selective as only our desired product was observed at the end of the reaction.”

“Our work highlights the use of new biocatalytic and chemocatalytic methods towards the total synthesis of a non-ribosomal peptide natural product (Scheme 4),” said Professor Renata. He concluded: “In the broader sense, we hope that this work will be the tip of the iceberg for the application of enzymatic C–H functionalization logic in total syntheses. For decades, synthetic organic chemists have developed countless methodologies for C–H functionalizations. But it is now prudent to include enzymatic transformations into our retrosynthetic designs and further applications of this strategy are being pursued very actively in our lab.”

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working towards the chemoenzymatic total syntheses of bioactive natural products.

Xiao Zhang received his B.S. in chemistry from Lanzhou University (P. R. of China) in 2009. After a brief stint at Wuxi AppTec (P. R. of China), he joined the lab of Prof. Guangxin Liang at Nankai University (P. R. of China) in 2010. He obtained his Ph.D. in 2015, completing the total syntheses of (–)-isatisine A and (–)-roseophilin. In 2016, he joined the lab of Prof. Hans Renata at The Scripps Research Institute (Florida, USA) where he is now

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2016. His current research focuses on the development of novel approaches to bioactive natural products through strategic application of biocatalytic methods.

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