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Total Synthesis of Tambromycin Enabled by Indole C−H Functionalization

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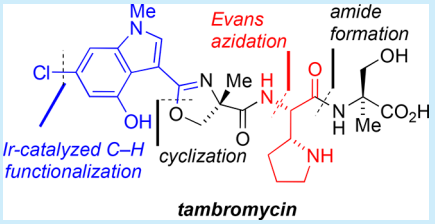
Supporting Information

ABSTRACT: The total synthesis of tambromycin (**1**), a recently isolated tetrapeptide, is reported. This unusual natural product possesses a highly modified tryptophan-derived indole fragment fused to an α-methylserine-derived oxazoline ring, and a unique noncanonical amino acid residue named tambroline (**11**). A convergent synthesis of tambromycin was achieved by a 13-step route that leveraged recent developments in the field of C−H functionalization to prepare the complex indole fragment, as well as an efficient synthesis of tambroline that featured a diastereoselective amination of homoproline.



Natural products have served as sources of structurally variable and biologically potent drugs and drug leads for decades.[1] Among the numerous classes of known natural products, peptides produced by nonribosomal peptide synthetases (NRPS) have found widespread application as potent antibiotics, immunosuppressants, and antitumor drugs.[2] Due to the diverse enzymatic machinery responsible for their biosynthesis, nonribosomal peptides often contain unusual noncanonical amino acids, resulting in highly modified and distinctive structural features.[3] In 2015, as part of an ongoing effort to discover novel natural products, Shin-ya and co-workers reported the structural determination of a natural product they catalogued as JBIR-126 (**1**), which was isolated from a culture of *Streptomyces* sp. NBRC 111228 derived from soil of a pineapple culture on Iriomote Island in the Okinawa Prefecture, Japan (Figure 1).[4] Shortly thereafter in early 2016, our research groups independently reported the discovery of the same molecule (i.e., **1**, which we named tambromycin)[5] within several species of *Streptomyces* through a correlative natural product discovery platform named metabologenomics.[6] JBIR-126/tambromycin (**1**), hereafter referred to as tambromycin, was shown to be a highly modified tetrapeptide that was structurally related to the previously reported peptide natural products, JBIR-34 (**2**) and JBIR-35 (**3**). Tambromycin (**1**) possessed the same characteristic indole substructure and oxazoline moiety as JBIR-35 (**3**), but contained a novel pyrrolidine-containing amino acid (named tambroline for *two*-*amino*-*beta*-homo*proline*) coupled to a C-terminal α-methylserine residue. The halogenation and hydroxylation pattern of the indole ring system within this family of compounds is reminiscent of those found within breitfussin B (**4**),[7] aspidostomide G (**5**),[8] and inducamide C (**6**),[9] while the tambroline substructure is unique to tambromycin (**1**).

Furthermore, although connectivity at the indole 3-position with oxazole motifs has been observed previously in natural products, to the best of our knowledge, the exact connectivity present within **1**, **2**, and **3** had not been observed in any known natural products.[10] A combination of feeding studies using isotopically labeled amino acid precursors and inspection of tambromycin's biosynthetic gene cluster revealed that the indole nucleus was derived from oxidative modification of tryptophan and that tambroline was biosynthesized by a

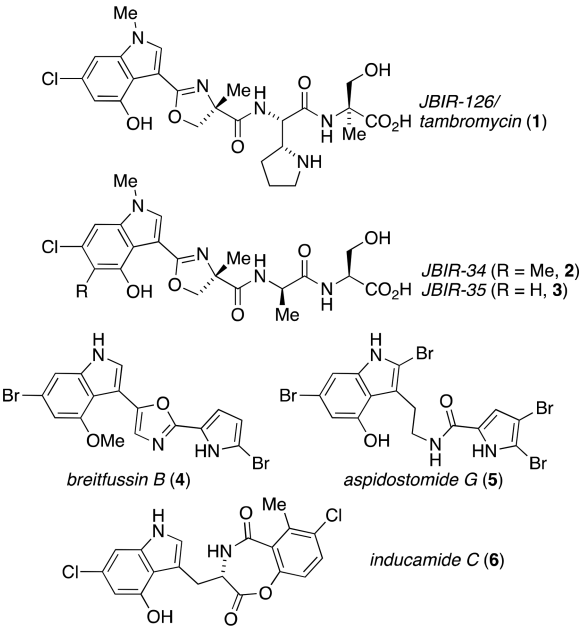


Figure 1. Structure of tambromycin (**1**) and related peptide-derived natural products possessing modified indoles.

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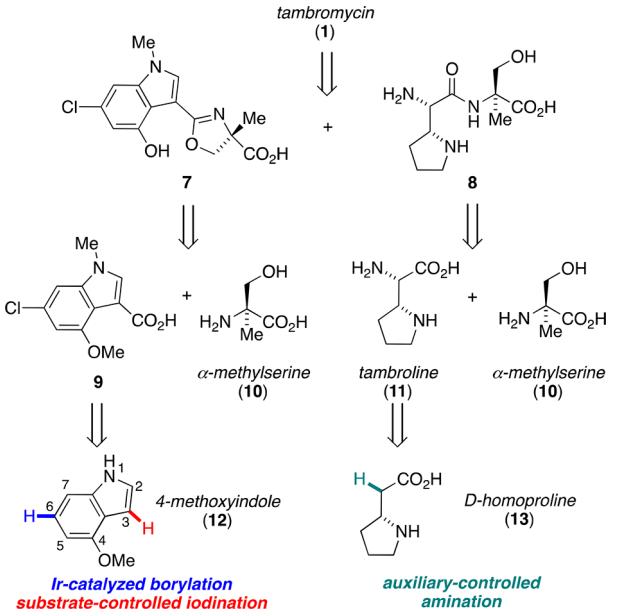
dehydrogenative cyclization of lysine.[5] Similarly, alanine was shown to be the starting precursor for both the oxazoline moiety and the C-terminal α-methylserine subunit. Preliminary biological evaluation of tambromycin indicates modest antiproliferative activity against B- and T-cell lines, important to leukemia and lymphoma, respectively.[4,5]

The combination of its unique structure, unusual biosynthesis, and preliminary biological results make tambromycin (**1**) an attractive target for chemical synthesis, prompting us to initiate a project aimed at securing synthetic **1**.[11] Retrosynthetic analysis (Scheme 1) of tambromycin (**1**) leads to disconnection

Scheme 1. Convergent Synthesis Plan for Tambromycin (1)

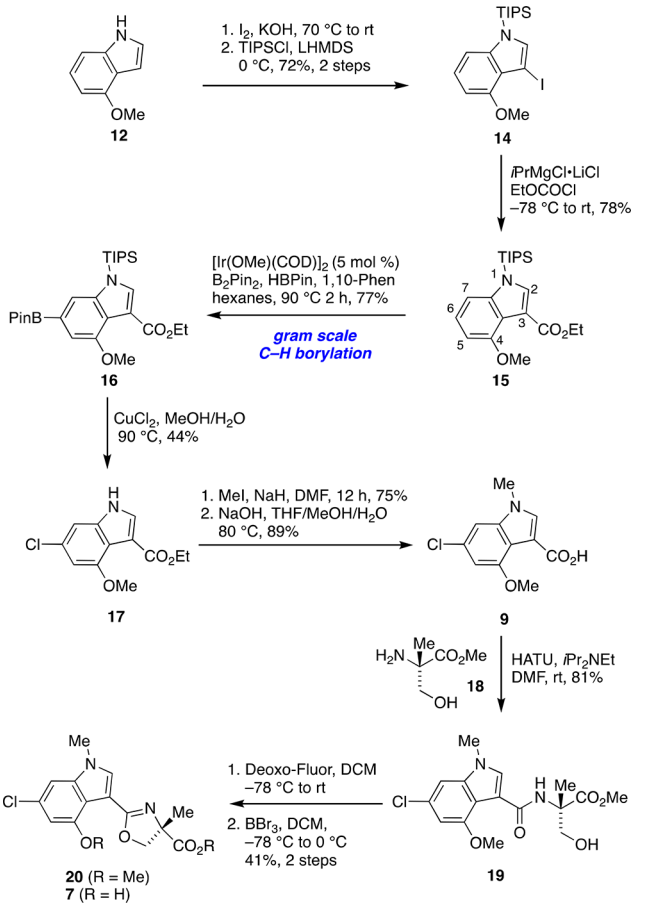


of the central amide bond linking the oxazoline subunit (i.e., acid **7**) to the tambroline terminus (i.e., amine **8**). Further disconnection of these fragments logically leads back to functionalized indole acid **9**, two α-methylserine residues (**10**) and tambroline (**11**). With α-methylserine available commercially, the key challenge for synthesizing **1** would lie in developing an efficient route to access the challenging 1,3,4,6-substituted indole **9** and establishing a practical stereoselective route to tambroline (**11**). We viewed the indole subtarget **9** as an opportunity to apply some recent advances in C−H borylation,[12] and were particularly drawn to the Ir-catalyzed method for regioselective C6-borylation of indoles reported by Baran and co-workers in 2015.[13,14] Thus, 4-methoxyindole (**12**) would serve as the starting material for indole acid **9**. The tambroline building block would be accessed by an auxiliary-controlled stereoselective α-amination of D-homoproline (**13**).[15]

Our synthesis of the requisite indole containing fragment **9** commenced with a regioselective substrate-controlled iodination of 4-methoxyindole (**12**) with molecular iodine (Scheme 2).

The intermediate 3-iodoindole proved unstable and was, therefore, converted directly to the chromatographically stable *N*-triisopropylsilyl derivative **14** upon treatment with TIPSCl (72% over two steps). While aiding purification, this silylation was also a necessary step for the planned Ir-catalyzed borylation (see below). Magnesium−halogen exchange using *i*-PrMgCl·

Scheme 2. Synthesis of the Functionalized Indole Fragment 7[a]



[a][Ir(OMe)(COD)]$_2$ = (1,5 cyclooctadiene)(methoxy)iridium(I) dimer, B_2Pin_2 = bis(pinacolato)diboron, HBPin = pinacolborane.

LiCl under Knochel's conditions[16] and subsequent quenching of the intermediate Grignard reagent with ethyl chloroformate delivered 3-carboxy intermediate **15** in 78% yield, setting the stage for the crucial Ir-catalyzed transformation.[17] In their total synthesis of the indole-containing natural products verruculogen and fumitremorgin A, Baran and co-workers developed a powerful ligand-controlled borylation of indoles that relied upon the combined actions of catalytic [Ir(OMe)(COD)]$_2$ and 1,10-phenanthroline (1,10-Phen) to induce borylation of a protected tryptophan derivative with 8:1 regioselectivity for C6 over C5.[13] They found that use of a TIPS group on the indole nitrogen was necessary in order to provide steric hindrance that would disfavor competitive borylation at C2 and C7. In light of these findings, we postulated TIPS-indole **15** should be an excellent substrate for this borylation chemistry, with the expectation that the 4-methoxy substituent would further direct borylation to the desired C6 position. Indeed, full consumption of starting material **15** was observed within 2 h using 5 mol % of [Ir(OMe)(COD)]$_2$ to deliver the desired boronate **16** as a single regioisomer. The reaction proved to be highly efficient and scalable, delivering the product in 77% yield on a 4.85 g scale, providing sufficient flux of material for our synthesis, while highlighting the power of modern methods for complex C−H functionalization in the context of total synthesis.[18]

While the borylation step was straightforward, conversion of the boronate group within **16** to the requisite chlorine

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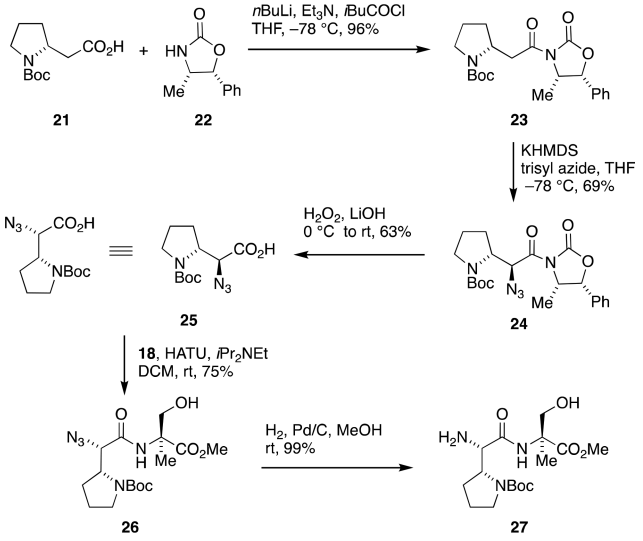
substituent proved more problematic. Exposure of **16** to copper(II) chloride in a 1:1 mixture of methanol and water at 90 °C led to successful chlorination,[19] but was complicated by isolation issues resulting from partial hydrolysis of the TIPS group and formation of a purple copper−indole complex that was observed to develop as the reaction progressed. Initial attempts to liberate the desired product from the reaction mixture using aqueous acid or base washes led to poor isolated yields of **17** as the major product. Viewing the partial hydrolysis of the TIPS group as an opportunity to streamline the synthesis, we optimized the timing of the reaction to ensure complete silyl cleavage prior to workup. Subsequent rigorous extraction of the reaction mixture with a 0.1 M EDTA solution allowed the effective removal of copper salts and ultimately enabled indole **17** to be isolated in a 44% yield on a 2 g scale. *N*-Methylation and hydrolysis of the ethyl ester within **17** delivered the first of the desired building blocks (i.e., **9**) in good yield over two steps (67% yield). We next explored the coupling of acid **9** to α-L-methyl-serine methyl ester (**18**) as a prelude to installing the necessary oxazoline group. We desired to elicit this amide bond formation without the need to first protect the free primary alcohol within **18**, and to this end, a number of reagents and conditions were investigated to find those favoring amide formation over the undesired esterification. Ultimately, we found that using 1-hydroxy-7-azabenzotriazole (HATU) with sonication cleanly produced linear dipeptide **19** in excellent yield (81%) with no observed formation of the corresponding ester (not shown).[20] Initial attempts at forming the oxazoline ring focused on first mesylating the α-L-methyl-serine residue, but cyclization of this intermediate proved inefficient. Instead, we found that exposure of **19** to [bis(2-methoxyethyl)amino]sulfur trifluoride (Deoxo-Fluor) directly generated desired oxazoline **20** in good yield (68%).[21] Finally, treating **20** with rigorously redistilled boron tribromide induced concomitant cleavage of both the methyl ether and methyl ester to cleanly deliver key fragment **7** in good yield (61%).

During structural elucidation of tambromycin (**1**) our laboratory prepared all four stereoisomers of tambroline (**11**) from either L- or D-proline in order to confirm its absolute and relative configuration.[5] This route, which was based on a report from Hanessian and co-workers,[22] relied on a substrate-controlled reaction of *N*-Cbz-D-homoproline ethyl ester to install the α-amino group. Unfortunately, direct amination of *N*-Cbz-D-homoproline yielded the incorrect relative stereochemistry, necessitating a protocol involving initial hydroxylation, followed by a Mitsunobu inversion using diphenylphosphoryl azide. This route was sufficient to establish the structure of tambroline within tambromycin (i.e., **11**), but we viewed it as somewhat inefficient in terms of completing a planned total synthesis of **1**. Instead, we devised a more effective route that commenced from commercially available *N*-Boc-D-homoproline (**21**), as shown in Scheme 3.

Seeking to overturn the inherent substrate selectivity of **21** toward electrophiles, we first synthesized Evans auxiliary derivative **23** by acylation with oxazolidinone **22**. Kim and co-workers reported that reaction of the related piperidine analog of **23** proceeded with high levels of stereocontrol that were fully controlled by the auxiliary.[23] We were pleased to find that under carefully optimized conditions using potassium hexamethyldisilazane (KHMDS) in conjunction with triisopropylbenzenesulfonyl azide (trisyl azide) that imide **24** was produced in 69% yield and as a single stereoisomer.[15] The

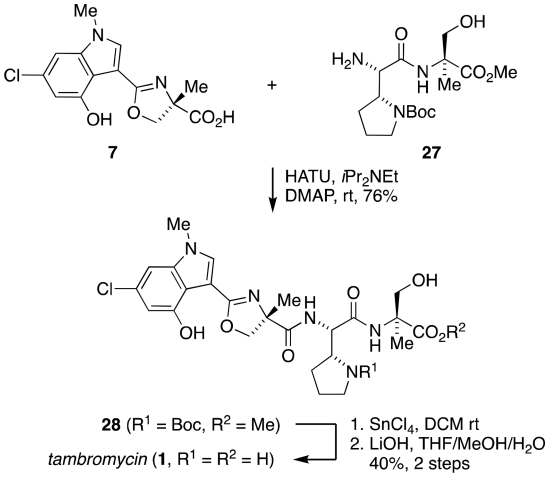
Scheme 3. Synthesis of the Tambroline Dipeptide Fragment 27



chiral auxiliary was removed without any epimerization using lithium hydrogen peroxide to afford free acid **25**.[24] Completion of tambroline dipeptide fragment **27** (a protected derivative of **8** in Scheme 1) was accomplished by HATU-mediated amide bond coupling to α-L-methyl-serine methyl ester (**18**), followed by mild azide reduction using Pd/C and hydrogen (74% yield over two steps).

With the two key dipeptide fragments **7** and **27** in hand, we established the complete framework of tambromycin by another HATU-mediated amide coupling reaction that delivered differentially protected derivative **28** in 76% yield (Scheme 4). Removal of the pyrrolidine *N*-Boc group

Scheme 4. Final Fragment Coupling and Completion of the Tambroline (1) Total Synthesis



presented itself as a late-stage obstacle because of the acid sensitivity of the oxazoline ring within **28**, which was readily hydrolyzed to a linear peptide when exposed to the standard conditions for the Boc group removal (e.g., TFA or HCl). Ultimately, we found that careful treatment of **28** with anhydrous tin(IV) chloride in dichloromethane for 10 min removed the Boc group cleanly and in high yield.[25] Finally, the C-terminal methyl ester was cleaved using LiOH to deliver

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tambromycin (**1**), which was isolated in 48% yield after purification using reversed phase C18 flash chromatography. A comparison of the spectral properties of synthetic material to the published spectra of natural isolates was initially hampered by an observed pH dependence in the organization and shift of the resonances. In both reported spectra of isolated, natural tambromycin a strong formic acid peak is present.[4,5] We postulated that the observed incongruity between our initial spectra and the published work resulted from a difference in protonation state. Indeed, upon the addition of a small amount of formic acid, the collected NMR spectra shifted to tightly align with that of the natural material (see Supporting Information for a tabulated comparison of chemical shifts). Analysis of the synthetic material by high resolution tandem mass spectrometry, infrared spectroscopy, and optical rotation further supported the identity of the sample, thereby establishing the completion of the total synthesis. Overall, tambromycin (**1**) was accessed in 13 steps (longest linear sequence) from 4-methyoxy indole (**12**) with a combined yield of 1.3%.

In conclusion, a concise total synthesis of the unusual tetrapeptide natural product, tambromycin (**1**), has been achieved. The route features a scalable, completely regioselective iridium-catalyzed C−H borylation allowing straightforward installation of the indole C6 chlorine substituent, as well as a diastereoselective amination to efficiently produce the novel amino acid, tambroline (**11**). Access to synthetic tambromycin (**1**) will facilitate future exploration of its biological activity and mechanism of action and enable investigations into structure−activity relationships among the related family of peptide natural products.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b00700.

Full experimental details and characterization data (NMR, MS, IR and optical rotation) for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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