Tubulysin Synthesis Featuring Stereoselective Catalysis and Highly Convergent Multicomponent Assembly

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ABSTRACT: A concise and modular total synthesis of the highly potent N14-desacetoxytubulysin H (1) has been accomplished in 18 steps in an overall yield of up to 30%. Our work highlights the complexity-augmenting and route-shortening power of diastereoselective multicomponent reaction (MCR) as well as the role of bulky ligands to perfectly control both the regioselective and diastereoselective synthesis of tubuphenylalanine in just two steps. The total synthesis not only provides an operationally simple and step economy but will also stimulate major advances in the development of new tubulysin analogues.

The Ugi four-component (U-4CR)1 and the Passerini three-component (P-3CR)2 are the lynchpin in modern multicomponent reaction organic chemistry. As “mix and go” reactions, multiple bonds are formed in one-pot reactions with minimal waste, ecce a blueprint of green chemistry.3 These truly versatile reactions have found extensive applications in drug discovery, natural products, pharmaceuticals, polymers, and complex macrocycles.4 Despite the massive benefits of U-4CR and P-3CR, they normally gave racemic products via the newly formed stereo center which results in tedious separations of the active enantiopure product for medicinal chemistry applications.5 To overcome the stereochemo limitation of multicomponent reactions, control is necessary.6 Lewis acids7 and chiral phosphoric acid catalysts8 recently emerged as promising catalysts to control the new chiral center formed in these multicomponent reactions. Thus, enantioselective multicomponent reactions open an exciting opportunity for the synthetic and medicinal chemists to easily access molecular complexity and diversity.9

The natural product family of tubulysins, since their discovery by Höfte in 2000 from a myxobacterial fermentation broth, has experienced impressive progress, with respect to understanding biology and drug development.10 Tubulysins exhibit extraordinary potent cytotoxicities against cancer cells exerted through tubulin binding.11 Strikingly, tubulysins are 20-fold to 1000-fold more potent than the epothilones, vinblastine, and taxol as cell growth inhibitors and thus they were promising lead compounds for the development of new anticancer drugs.12 However, it rapidly turned out that the therapeutic window for single agent use is too small for any human application. Recently, tubulysins as payloads, folic acid conjugates, or antibody drug conjugates (ADCs) showed high clinical promises (Figure 1).13 However, the large-scale fermentation of tubulysins is still a poorly solved challenge.

Figure 1. Importance and evolution of tubulysins for medicine and total synthesis (TS) to date.

Thus, they are exciting targets for total synthesis in several laboratories around the world.14 Our laboratory15 and the Zanda group16 disclosed the first total synthesis of tubulysin U and V; Ellman described the first total synthesis of Tubulysin D72 and N-methyl tubulysins.75 Since those reports, several...
approach and proceeds through sequential coupling of four amino acid fragments such as D-N-methyl piperacillic acid (Mep), isoleucine (Ile), tubulvaline (Tuv), and the tubupe-nylalanine (Tup) as logical precursors. Most of the current synthetic routes suffer from severe drawbacks. First, the syntheses of Tuv and Tup were only accomplished through extensive functional group manipulations and chiral separations. Next, the sequential difficult coupling of sterically hindered Mep, Ile and Tup amino acids was challenging. These costly and labor-intensive routes have hampered large-scale synthesis. We have previously shown the use of the multicomponent reaction tactic in our first total synthesis of tubulysin U and V, and also the Wessjohann and Kazmaier extensive functional group manipulations and chiral separations of Tuv and Tup were only accomplished through multicomponent reaction tactic in our synthesis. We have previously shown the use of the costly and labor-intensive routes have hampered large-scale synthesis.21 Based on these findings, the Ellman group,22 the Nicolau group,23 and the Wipf group24 have extensively screened various Lewis acids in P-3CR (see the SI) for the preparation of the building blocks on a multigram scale.

**RESULTS AND DISCUSSION**

With the desired three components in our hand, we initiated studies to probe various P-3CR parameters, such as solvent, temperature, and concentration, to obtain the desired diastereomer 5a (see the SI) at the best yield and selectivity. The first optimization revealed that excellent yield (90%) of the P-3CR product was obtained as a 1:1 mixture of diastereomers 5a and 5b at 1 M concentration at room temperature for 48 h. At 0.1 M concentration, we observed a slight improvement in the diastereoselectivity, but with a reduced yield of 50%. These studies showed that temperature, solvent, and concentration have great impact on the P-3CR. However, complete stereocontrol of the newly formed asymmetric carbon is challenging to tune, based on only those reaction parameters. Taking advantage of the chiral and bulky nature of our designed components, we hypothesized that the judicious choice of a catalyst or additive might help to improve the diastereoselectivity. Inspired by the work of Tan on chiral phosphoric acid (CPA)-catalyzed enantioselective MCRs,25 we initially examined various promising chiral phosphoric acid catalysts. After screening readily available catalysts, CPA 5 (see the SI) provided excellent 95:5 dr, albeit in very low yields (38%). This might be due to the fact that highly acidic phosphoric acids considerably cleave the side-chain trityl group, resulting in a series of side reactions (see the SI). We then speculated that basic ligands could protect the cleavage of the trityl group. Thus, P-3CR was conducted in the presence of 20 mol % pyridine and 10 mol % CPA 2. However, in this case, we obtained mostly hydroxylated product (P-2CR) with a 95:5 diastereomeric ratio (70%; see the SI). The Banfi group26 and our group2 showed that chiral components yielded enantioselective diastereoselective P-3CR, in the presence of achiral Lewis acids.25 Thus, we extensively screened various Lewis acids in P-3CR (see the SI) and we were delighted to find that 10 mol % ZnBr₂ gave moderate diastereoselectivity (80:20) without diminishing the yield (70%).

Our previous studies on the screening of Lewis acids along with ligands in enantioselective P-3CR revealed that Lewis acids, along with supporting bulky chiral ligands, may completely lock the other plane of the isocyanide attack, thereby leading to high diastereoselectivity.27 Based on this hypothesis, we examined several chiral ligands in association with ZnBr₂ and the results are summarized in Scheme 1. Interestingly, when ligand L1 was used as the chiral ligand together with ZnBr₂, the P-3CR pathway afforded 5a as the major product formation with high diastereoselectivity (92:8) in acceptable yield (71%). Other tested ligands gave moderate yields and diastereoselectivities (see Scheme 1).

The stereo chemical outcome of the P-3CR could be rationalized by the probable transition state (TS), as shown in Scheme 2. Coordination of the ZnBr₂ by the aldehyde carbonyl and the urethane protecting group carbonyl results in a Cram-chelated complex. The isocyanide attack from the less-hindered si face of the carbonyl group in a Cram chelate...
We set out to investigate the regioselective ring opening of 5a by a Grignard reagent. 27 However, the direct addition of 7a to the anhydride 5a gave a mixture of regioisomers 8a and 8b in equal amounts (see the SI). To improve the selective formation of 8a, various copper catalysts were examined (see the SI) in THF at −78 °C. To our delight, bulky t-BuXPhos along with CuI resulted in exclusive formation of 8a in >99:1 regioselectivity in 70% yield. The regioselective outcome can be rationalized by the steric encumbrance imparted by the bulky copper complex, effectively shielding the carbonyl group next to the methyl group of 7. The enantiopure keto acid 8a was then subjected to a modified ruthenium-catalyzed reductive amination protocol to afford 9 in good yield (81%) and excellent diastereoselectivity (>99:1). 28 A one-pot synthesis of 7 to 9 was also conducted; in this case, the yield obtained was quite low (43%).

With the key building blocks in our hand, we focused on the final assembly of 1. We first installed the thiazole via post-translational modification of 5a through one-pot two step strategy using TiCl4-mediated cyclodehydration of Cys(Trt) amide, followed by MnO2 oxidation to afford 10 in excellent yield without racemization (see the SI). 29 The resulting thiazole 10 was then subjected to Fmoc deprotection and then acylation under mild conditions to give hydroxy tetrapeptide analogue 11 in excellent yield (93%). This type of Passerini reaction—amine deprotection—acyl migration (PADAM) was first described by Banfi 30 and independently by Semple 31 and others. 32 However, its practical utility and superiority over other methods has never been disclosed in the sterically hindered amide formation. The resulting tetrapeptide 11 was then hydrolyzed to afford 12 in a yield of 98%. Synthesis of 13 was accomplished by activation of 12 as the pentafluorophenyl ester-mediated coupling of 9 and 12 gave 13 in 87% overall yield. Furthermore, acetylation of hydroxyl group was produced 14 in 88% yield. Finally, the late stage N-methylation of the N-terminus was accomplished by the catalytic hydrogenation with paraformaldehyde, affording 1 in good yield (90%; see Scheme 4). 33 The synthesized analogue 1 is equivalent to reported in the literature in all spectroscopic aspects (see the SI). 35

We evaluated the biological activity in HCT-116 cancer cells (Figure 3). Notably, the inhibition by N14-desacetoxytubulysin H 1 was most prominent in HCT-116 cells, but was less effective at sub-μM concentrations in HeLa cells.

In conclusion, the total synthesis of N14-desacetoxytubulysin H was accomplished in 18 steps with an overall yield of 30%. To the best of our knowledge, this is the shortest and most convergent total synthesis of 1. Key features of our strategy included (a) a one-pot unprecedented highly diastereoselective P-3CR, and (b) a two-step diastereoselective synthesis of Tup and (c) productive construction of the tripeptide unit Mep-Ile-Tuv, which is common and essential unit in all tubulysins. We anticipate that the practicality, scalability, and conciseness of our strategy should have implications to access a variety of other analogues of tubulysins, which are the focus of our future work. 34

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**Scheme 1.** Diastereoselective Passerini Three-Component Reaction for the Synthesis of 5a

**Scheme 2.** Predicted Model for Diastereoselective Synthesis of 5a

| Entry[c,b] | Ligand | Yield of 5a [%][c] | Dr 5a:5b [A:Z] |
|-----------|--------|-------------------|----------------|
| 1         | none   | 70                | 80:20          |
| 2         | L1     | 71                | 92:8           |
| 3         | L2     | 68                | 70:30          |
| 4         | L3     | 60                | 85:15          |
| 5         | L4     | 63                | 76:24          |
| 6         | L5     | 70                | 72:28          |
| 7         | L6     | 55                | 69:39          |

*a Screening of solvents, concentration and other catalysts is described in detail in the Supporting Information (SI).*  
*b Equimolar amounts of all three components were added to a solution containing ZnBr2 and corresponding ligand in CH2Cl2 at 0 °C; after 5 min, the reaction mixture was stirred at room temperature. *c Yield of 5a was determined after chromatographic purification. *d Diastereomeric ratio was determined by HPLC analysis of the crude reaction mixture. *e Confirmation of the stereochemistry is determined by applying 5a to the total synthesis of 1.  

**Scheme 3.** Diastereoselective Synthesis of Tup 9

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Scheme 4. End Game of the Total Synthesis of 1

Reagents and conditions: (a) step 1: 1 M TiCl4 in CH2Cl2 (3.0 equiv), 0 °C, 48 h; step 2: activated MnO2 (10 equiv) 70 °C, 3 h. (b) step 1: diethylamine (30 equiv), CH3CN, 0 °C to rt, 24 h. (c) 2 M LiOH, THF: H2O: MeOH, 6 h, room temperature (rt). (d) step 1: DIC, pentadenturopenol, 0 °C, CH2Cl2, 3 h. (e) CH2Cl2, 0 °C, CH2Cl2, 24 h; step 2: CH2Cl2, DIPEA, rt, 12 h. (f) H2, Pd/C, 37% aqueous formaldehyde, CH2Cl2, 0 °C to rt, 24 h. (f) H2, Pd/C, 37% aqueous formaldehyde, MeOH, rt, 24 h.

Figure 3. Dose-dependent inhibition of cell growth by N14-desacetoxytubulysin H (1) in HCT-116 cells.

ASSOCIATED CONTENT

# Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.0c01718.

Detailed experimental procedures for all compounds, optimization studies for the synthesis of 5a and 8a, chiral SFC-HPLC analysis of key building blocks, all spectral data, and full characterization (PDF)

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Notes

The authors declare no competing financial interest.

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