Letter

Tubulysin Synthesis Featuring Stereoselective Catalysis and Highly Convergent Multicomponent Assembly

Thimmalapura M. Vishwanatha, Ben Giepmans, Sayed K. Goda, and Alexander Dömling*

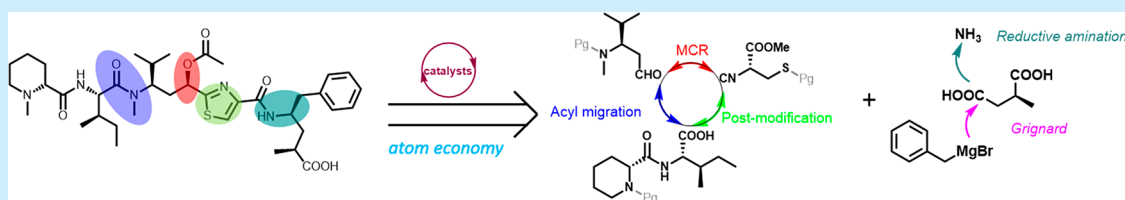Cite This: *Org. Lett.* 2020, 22, 5396−5400

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ABSTRACT: A concise and modular total synthesis of the highly potent N^{14}-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 stereochemical limitation of multicomponent reactions, control is necessary.[6] Lewis acids[7] and chiral phosphoric acid catalysts[8] 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 Höfle in 2000 from a myxobaterial 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.
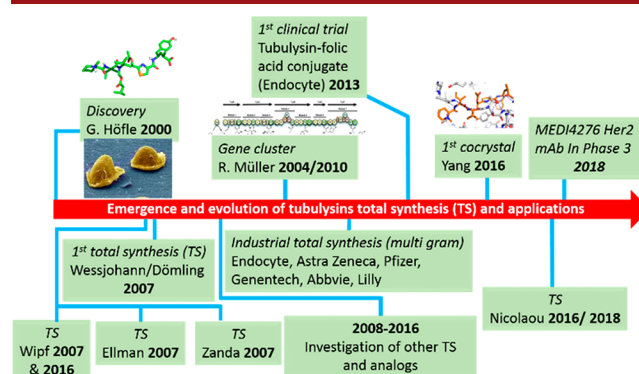

Figure 1. Importance and evolution of tubulysins for medicine and total synthesis (TS) to date.

Thus, they are exciting targets for total synthesis in several laboratories around the world.[14] Our laboratory[15] and the Zanda group[16] disclosed the first total synthesis of tubulysin U and V; Ellman described the first total synthesis of Tubulysin D[17a] and *N*-methyl tubulysins.[17b,c] Since those reports, several

modifications on tubulysins and their total synthesis have been well-documented.[18]

All the classical total synthesis of the nonribosomal peptidic structure of tubulysins **A** (Figure 2) involves a multistep
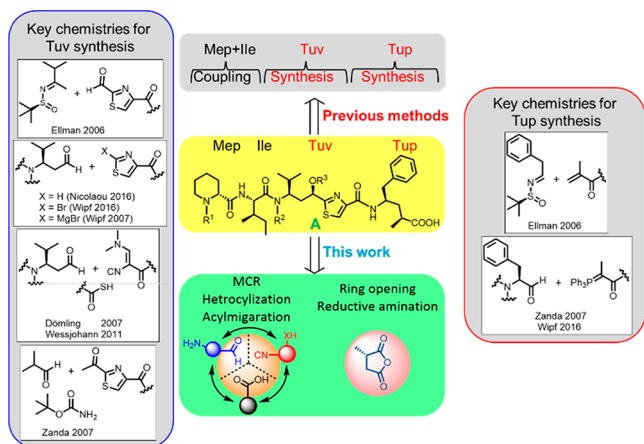

Figure 2. Retro synthesis of tubulysins **A** and most representative synthetic methods developed for Tuv and Tup synthesis.

approach and proceeds through sequential coupling of four amino acid fragments such as D-N-methyl pipecolic acid (Mep), isoleucine (Ile), tubuvaline (Tuv), and the tubuphenylalanine (Tup) as logical precursors.[19] 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 Tuv 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,[15] and also the Wessjohann and Kazmaier groups used multicomponent reactions for their synthesis of, however, unnatural tubulysin derivatives ("Tubugis") through the combination of P-3CR and U-4CR.[20]

However, from a biological activity point of view, N^{14}-desacetoxytubulysin H showed high potency, with increased hydrolytical stability.[21] Based on these findings, the Ellman group,[17c] the Nicolaou group,[21a] and the Wipf group[21b] have developed synthetic routes to access **1** and its analogues. While total synthesis landmark achievements, most reported approaches generally suffer from a high synthetic step count and low overall yields. For example, the tubulysin-linker part of the ADC MEDI4276 was reportedly synthesized by a more-than-40-step synthesis.[22] To attenuate the current demand for tubulysins in ADC therapies,[23] we targeted a general and stereoselective synthetic method to access tubulysins in just few steps and overall high yield. There are two important obstacles in the chemistry and biology of tubulysins natural products. The unnatural amino acids (Tup and Tuv) and the two contiguous stereocenters are ubiquitous; the N^{14} substituent on the Tuv has a great influence on the biological activities. Therefore, our synthetic plan was designed to rapidly and controllably assemble the core structure while, at the same time, maximizing the possibilities of peripheral modifications. This communication documents our high yielding short and convergent synthesis of **1** in just 18 steps from commercially available very simple building blocks. Based on our
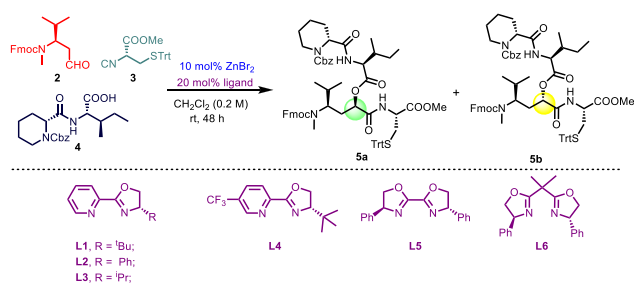
retrosynthetic analysis, we envisioned that the intermediates **2**, **3**, and **4** bearing orthogonal protecting groups could serve as advanced intermediates for our divergent multicomponent reaction synthesis of **1**. We thus initially designed efficient routes to synthesize the building blocks on a multigram scale (see the Supporting Information (SI) for the preparation of the building blocks **2**−**4**).[24]

■ 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,[8a] 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 group[7d,e] and our group[7a] showed that chiral components yielded enantioselective and 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 % $ZnBr_2$ 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.[7a,26] Based on this hypothesis, we examined serval chiral ligands in association with $ZnBr_2$, and the results are summarized in Scheme 1. Interestingly, when ligand **L1** was used as the chiral ligand together with $ZnBr_2$, 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 $ZnBr_2$ 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
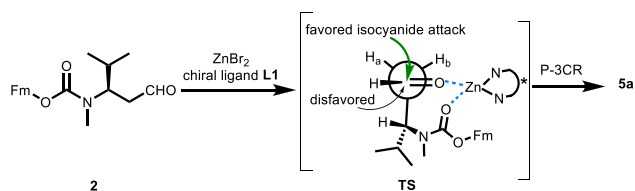
Scheme 1. Diastereoselective Passerini Three-Component Reaction for the Synthesis of 5a

Entry[a, b]	Ligand	Yield of 5a [%][c]	dr 5a:5b [d, e]
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 ZnBr$_2$ and corresponding ligand in CH$_2$Cl$_2$ 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.
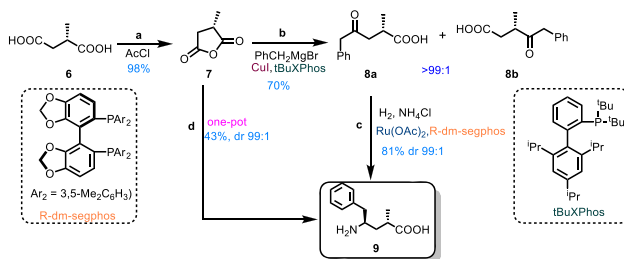
Scheme 2. Predicted Model for Diastereoselective Synthesis of 5a

complex favors the formation of 5a as the major product (see Scheme 2).

Next, we turned our attention to the development of an innovative synthesis of Tup (Scheme 3). We figured that commercially available and affordable S-(−)-methyl succinic acid 6 would provide a versatile component via its anhydride 7. We set out to investigate the regioselective ring opening of 7 by a Grignard reagent.[27] However, the direct addition of Grignard reagent to the anhydride 7 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

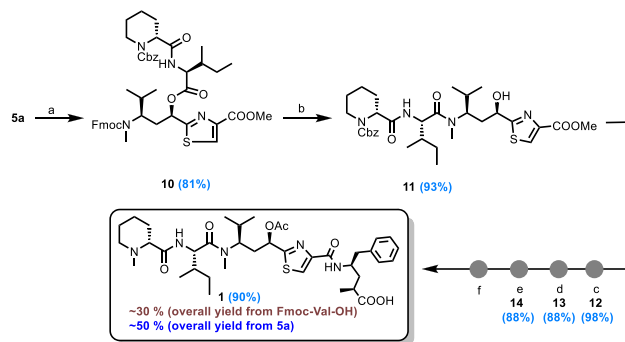**Scheme 3. Diastereoselective Synthesis of Tup 9***

*Reaction conditions: (a) AcCl (3 equiv), 100 °C, 2 h; (b) CuI (10 mol %), *t*BuXPhos (20 mol %), 2 M PhCH$_2$MgBr in THF (0.9 equiv), −78 °C, 6 h; (c) H$_2$ (55 bar), NH$_4$Cl (3.0 equiv), Ru(OAc)$_2$, *R*-dm-SEGPHOS, trifluoroethanol (TFE), 12 h; and (d) conditions of reactions (b) and (c) in one pot.

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 TiCl$_4$-mediated cyclodehydration of Cys(Trt) amide, followed by MnO$_2$ 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 acyl migration 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 N^{14}-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 N^{14}-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]

Scheme 4. End Game of the Total Synthesis of 1*

*Reagents and conditions: (a) step 1:1 M TiCl$_4$ in CH$_2$Cl$_2$ (3.0 equiv), 0 °C, 48 h; step 2: activated MnO$_2$, (10 equiv) 70 °C, 3 h. (b) step 1: diethylamine (30 equiv), CH$_3$CN, 0 °C, 2 h; step 2: CH$_2$Cl$_2$, DIPEA, 50 °C, 24 h. (c) 2 M LiOH, THF: H$_2$O: MeOH, 6 h, room temperature (rt). (d) step 1: DIC, pentafluorophenol, 0 °C, CH$_2$Cl$_2$, step 2:9 (3.0 equiv), DMF, DIPEA, rt, 12 h. (e) Ac$_2$O, pyridine, CH$_2$Cl$_2$, 0 °C to rt, 24 h. (f) H$_2$, Pd/C, 37% aqueous formaldehyde, MeOH, rt, 24 h.
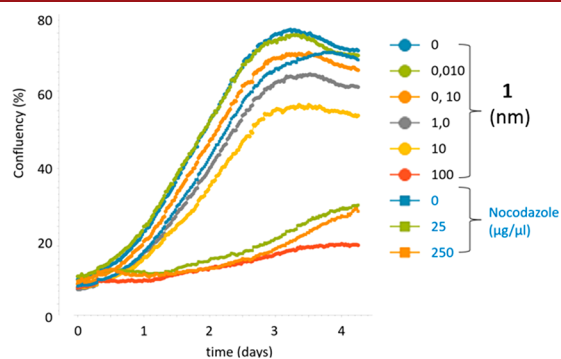
Figure 3. Dose-dependent inhibition of cell growth by N[14]-desacetoxytubulysin H (1) in HCT-116 cells.

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)

AUTHOR INFORMATION

Corresponding Author

Alexander Dömling — Department of Drug Design, University of Groningen, 9700 AD Groningen, The Netherlands; orcid.org/0000-0002-9923-8873; Email: a.s.s.domling@rug.nl

Authors

Thimmalapura M. Vishwanatha — Department of Drug Design, University of Groningen, 9700 AD Groningen, The Netherlands; orcid.org/0000-0002-0671-8908

Ben Giepmans — University Medical College Groningen, 9700 AD Groningen, The Netherlands; orcid.org/0000-0001-5105-5915

Sayed K. Goda — Faculty of Science, Chemistry Department, Cairo University, Giza, Egypt

Notes

The authors declare no competing financial interest.

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