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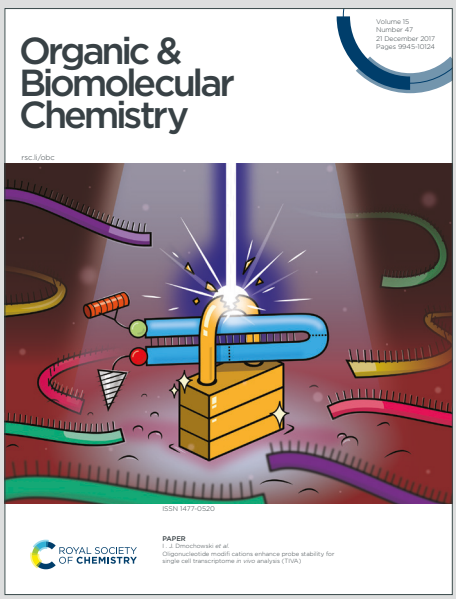

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COMMUNICATION

Total Synthesis of Tubulysin U and N^{14}-Desacetoxytubulysin H

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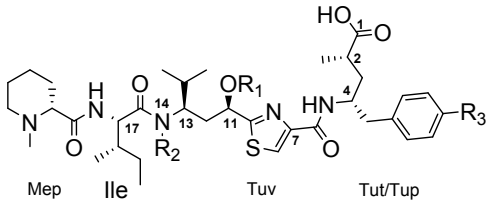
A concise and efficient procedure for the total synthesis of tubulysin U and N^{14}-desacetoxytubulysin H has been developed with high stereoselectivity on gram scale. This synthesis features an elegant cascade one-pot process to install the challenging thiazole moiety and the employment of stereoselective reductions and a serious of high-yield mild reactions to ensure the requisite stereochemistry, reaction scale, yield and to avoid the vexing epimerization occurring during peptide formation.

Tubulysins (Figure 1) are a family of architecturally complex tetrapeptides isolated from the myxobacteria *Archangium gephyra* and *Angiococus disciformis*.[1] The unique molecular architecture of tubulysins comprises four uncommon amino acid fragments, N-methylpipecolic acid (Mep), isoleucine (Ile), tubuvaline (Tuv), and tubuphenylalanine (Tup) or Tubutyrosine (Tut). Biologically, tubulysins exhibit extraordinary anticancer activities and many members of this family surpass the well-known chemotherapeutic agents like taxol, epothilones and vinblastine by a factor of 20–1000 with respect to growth inhibition potential.[2] Furthermore, tubulysins have been drawing attention as potential payloads for antibody-drug conjugates (ADCS).[3a,b,c] Structure-activity relationship studies of tubulysin derivatives indicated that the N,O-acetal moiety at R_2 position is not essential for picomolar cytotoxicity and further structural simplifications at this position were accessiable without abolishing their biological activity.[3d] Tubulysins U (**1**) and the unnatural N^{14}-desacetoxytubulysin H (**2**) are representative for the basic structures of the most potent members in this family. Tubulysin U (**1**) was found to exhibit extremely potent antiproliferative activity in 1A9 ovarian cancer cells (IC_{50} = 0.65 nM), MCF-7 breast cancer cells (IC_{50} = 0.4 nM) and for in vitro inhibition of tubulin polymerization (IC_{50} = 1.9 μM).[4] Furthermore, N^{14}-Desacetoxytubulysin H (2) exhibits consistently potent activities against leukemia, non-small-cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cell lines with IC_{50} values between 0.074 and 0.548 nM.[5] All of these features rendered tubulysins U (**1**) and N^{14}-desacetoxytubulysin H (**2**) as excellent anticancer drug candidates.



Tubulysin	R_1	R_2	R_3
A	Ac	$CH_2OC(O)CH_2CH(CH_3)_2$	OH
E	Ac	$CH_2OC(O)CH_2CH_2CH_3$	H
F	Ac	$CH_2OC(O)CH_2CH_3$	H
U (**1**)	Ac	H	H
V	H	H	H
(**2**)	Ac	Me	H

Figure 1 Representative members of tubulysins.

In recent years, tubulysin U (**1**)[4,6-11] and N^{14}-desacetoxytubulysin H (**2**)[5,12-15] have garnered considerable attention and many novel synthetic strategies have been developed for these two molecules. Each of these works employed novel strategies or methodologies to ensure the synthetic efficacy. However, the highly congested stereochemical complexity and several chemically sensitive moieties of **1** and **2** coupled with the vexing epimerization occurring during peptide formation[6,16] posed a particular synthetic challenge and impeded multigram-scale access to

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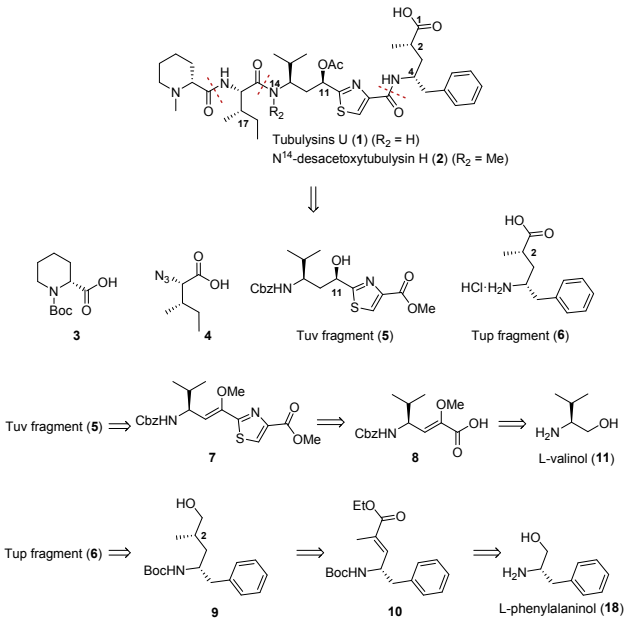
these compounds. An abundant synthetic supply remains necessary for further biological evaluation of tubulysins and their analogues, which prompted us to develop a more efficient route for these appealing molecules. As part of our long-term efforts on marine peptides,[17-20] we present herein a concise and efficient total synthesis of tubulysin U (**1**) and N^{14}-desacetoxytubulysin H (**2**).



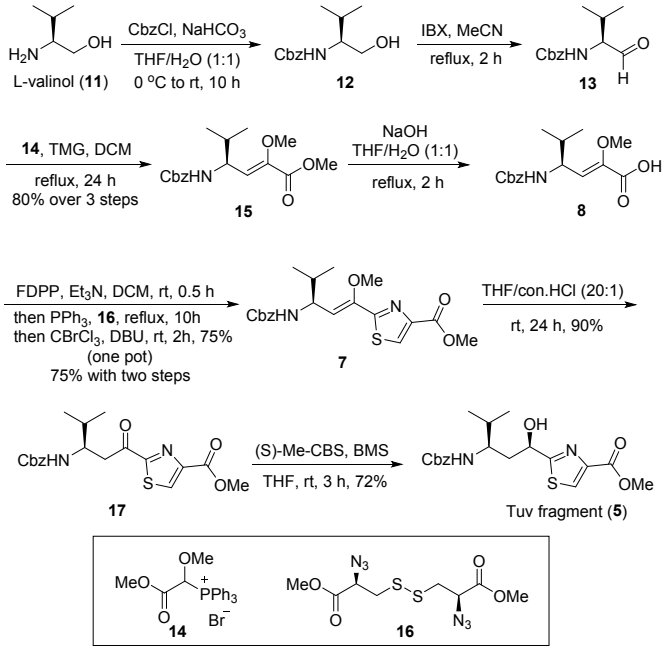
Scheme 1 Retrosynthetic analysis of **1** and **2**

The retrosynthetic analysis was depicted in Scheme 1. Initial disconnection of amide traced the origins of **1** and **2** to four segments: easily available known coupounds **3**[21], **4**[22], Tuv fragment (**5**), and Tup fragment (**6**). We envisaged that the C4 stereocenter on Tuv fragment (**5**) could be installed through a CBS reduction of ketone, which traced the origin of **5** to methyl enol ether **7**. The challenging thiazole moiety could be prepared from β-azido disulfide with the carboxylic acid **8** via a cascade one-pot process[23], and compound **8** could be prepared from the commercially available L-valinol **11**. Tup fragment (**6**) could be prepared through oxidation of alcohol **9**, and C2 stereocenter of **9** could be induced by reduction of α,β-unsaturated ester **10**, which could be easily prepared from the commercially available L-phenylalaninol (**18**).

Our synthetic strategy for the Tuv fragment (**5**) commenced with L-valinol (**11**) (Scheme 2). Protection of the amino group with CbzCl group led to alcohol **12**.[24] Oxidation of **12** with IBX in refluxing MeCN resulted in the aldehyde **13**. Without purification, aldehyde **13** was subjected to a wittig olefination with phosphonium reagent **14**[25] to provide unsaturated ester **15** in 80% yield over three steps. Hydrolysis of the methyl ester with sodium hydroxide in refluxing THF/H_2O system delivered acid **8**.

With the acid **8** in hand, we next turned our attention to the synthesis of thiazole **7**. The installation of the configurationally and chemically sensitive thiazole fragment has proved challenging in the synthesis of tubulysins. Although a lot of methods have been reported to address this vexing issue,[7,9,16,26,27] most of these methods suffer from one or more limitations such as tedious operations, multistep reactions, the use of strong dehydrating reagents and low overall yield, which impeded efficient access to tubulysins. Recently, Du and co-workers reported a novel method for the synthesis of 2,4-disubstituted thiazoles.[23] This cascade transformation consists of disulfide cleavage, thiocarbonylation, phosphine-promoted intramolecular Staudinger/aza-Wittig reaction and dehydrogenation to form the corresponding thiazoles, which promoted us to further explore this methods in the synthesis of tubulysins. Much to our delight, after numerous unsuccessful trials, the disired thiazole **7** was successfully synthesized in a cascade one-pot process under mild condition with high yield by utilizing a modified procedure of Du et al[23].

Then acid-promoted hydrolysis of **7** afforded thiazolyl ketone **17** in 90% yield. Reduction of thiazolyl ketone **17** with $BH_3{\cdot}SMe_2$ in the presence of (S)-Me-CBS at room temperature produced Tuv fragment (**5**) in 72% yield as a single diastereoisomer. As such, the key Tuv fragment (**5**) was synthesized from the cheap L-valinol (**11**) on gram scale in 7 steps with 38.9% overall yield and excellent stereoselectivity.



Scheme 2 Synthesis of Tuv fragment (**5**)

With the key Tuv fragment (**5**) in hand, the synthesis of Tup fragment (**6**) was then explored. The α,β-unsaturated eater **10** was prepared in a good overall yield by employing the three-step protection/oxidation/wittig olefination procedure similar to that of compound **15**. Chemoselective reduction of the double bond of **10** was performed by using $NaBH_4/NiCl_2$ in MeOH,[28] and the hydrogenation product **22** was then hydrolysized with sodium hydroxide in refluxing THF/H_2O system, after which the generating acid was further reduced through the mixed anhydride method to give a separable mixture of alcohol **9** and **9'** (anti:syn = 3:1), as reported by Wipf er al.[27] The desired major isomer **9** could be easily separated by flash chromatography with 60% overall yield over three steps and the spectral data for **9** and **9'** were consistent

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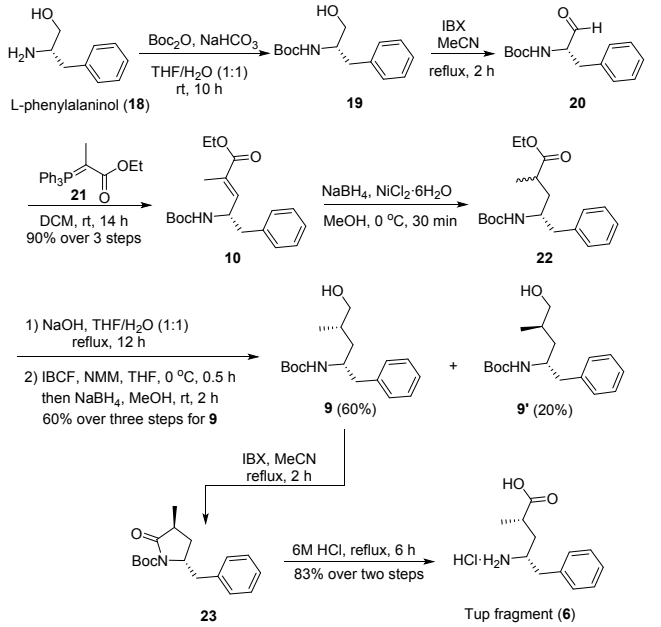
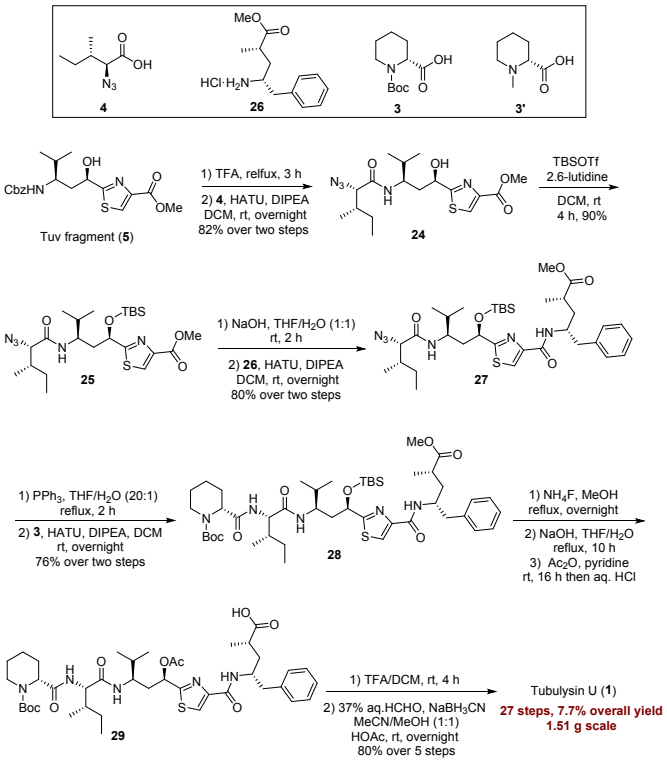
with earlier studies[27]. A two-step oxidation/cyclization procedure was performed as a tandem one-pot process when treating with IBX in refluxing MeCN, and then acidic hydrolysis of the resulting N-Boc-pyrrolidinone **23** delivered the enantiomerically pure Tup fragment (**6**) in 83% yield over two steps, which was then esterified to yield its carboxylate form H-Tup-OMe·HCl (**26**)[29] for subsequent couplings. At this stage, the Tup fragment (**6**) was successfully prepared from the cheap L-phenylalaninol (**18**) on gram scale in 8 steps with 44.8% overall yield.



Scheme 3 Synthesis of Tup fragment (**6**)

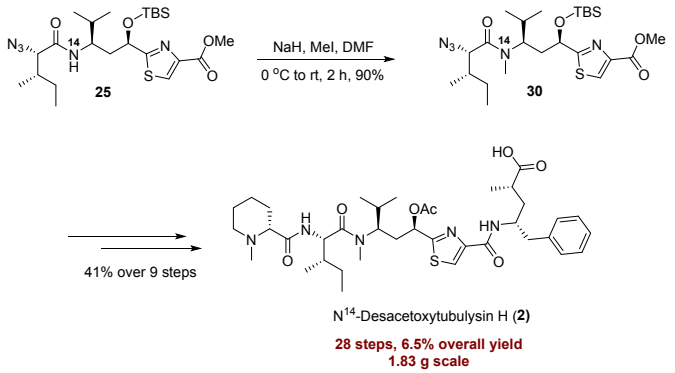
With the efficient access to key Tuv fragment (**5**) and Tup fragment (**6**) realized, we next turned our attention to the completion of the total synthesis of tubulysins U (**1**) (Scheme 4). Initially, we attempted to remove the Cbz group of **5** by Pd/C hydrogenolysis, but no reaction occurred under this condition. Gratifyingly, when **5** was heated at reflux in neat trifluoroacetic acid (TFA) for 3 h, the Cbz group was cleaved successfully to give the corresponding amine, which was then condensed with **4**[30] by using HATU in the presence of Hünig's base to generate dipeptide **24** in 82% yield over two steps. Treatment of **24** with TBSOTf in the presence of 2,6-lutidine resulted in the corresponding silyl ether **25** in 90% yield. Then saponification of **25** followed by coupling with H-Tup-OMe·HCl (**26**)[29] afforded azide **27** in 80% yield over two steps. Then a Staudinger reduction of azide **27** with PPh_3 in THF-H_2O system was performed followed by a condensation of the resulting amine with N-Boc-D-pipecolinic acid **3**[21] to afford tetrapeptide **28** in 76% overall yield for the two steps, which was easily separated by flash column chromatography on silica gel in EtOAc/Hex (1:4). Interestingly, introduction of the methyl group at this step turned out to be fruitless: when the amine was coupled with N-methyl-D-pipecolinic acid **3'**[6] under the same condition, the reaction was sluggish and unclean, and the product was very difficult to purify by silica gel chromatography. Therefore we attempted to induce the methyl group at final stage.

After removal of the TBS groups and saponification of the methyl ester, the resulting hydroxyl group then underwent acetylation with Ac_2O in pyridine to deliver acid **29**. Finally, removal of the Boc group and the methyl group was then induced by treating with 37% aqueous formaldehyde and sodium cyanoborohydride to afford the natural product tubulysins U (**1**) in 80% yield over five steps. Tubulysins U (**1**) was subsequently purified by a simple silica gel column chromatography method that did not result in noticeable epimerization. The spectral data for synthetic tubulysins U (**1**) (^{1}H, ^{13}C NMR and HRMS) were identical to those previously reported. [7,9,10] The optical rotation of our product **1** ($[\alpha]_D^{25}$ -10.5, c 0.46, MeOH) corresponded well with the literature value[10] (lit. ($[\alpha]_D^{25}$ -11.7, c 0.21, MeOH).



Scheme 4 Total synthesis of tubulysins U (**1**)

As shown in Scheme 5, treatment of **25** with NaH and MeI in DMF resulted in the formation of N^{14}-methylated product **30** in 90% yield, as reported by Ellman et al.[31] Using a similar synthesis route described above, N^{14}-desacetoxytubulysin H (**2**) was prepared successfully. The detailed procedures, see Electronic Supporting Information. The spectral data for synthetic **2** (^{1}H, ^{13}C NMR and HRMS) were identical to those previously reported.[5,12-15,31a] The optical rotation of our product **2** ($[\alpha]_D^{25}$ -14.4, c 0.26, MeOH) corresponded well with the literature value[31a] (lit. ($[\alpha]_D^{25}$ -19.2, c 0.9, MeOH).



Scheme 5 Total synthesis of N^{14}-desacetoxytubulysin H (**2**)

It should be noted that, the present route turned out to be rather practical, and we developed an effcient and cost effective large-scale process for the total synthesis of tubulysin U (**1**) and N^{14}-desacetoxytubulysin H (**2**) with high stereoselectivity by employing the mild reactions and cheap reagents.

Conclusions

In summary, we established a convenient and scalable procedure for the total synthesis of tubulysin U (**1**) (27 steps with 7.7% overall yield, 1.51 g scale) and N^{14}-Desacetoxytubulysin H (**2**) (28 steps with 6.5% overall yield, 1.83 g scale). Some of the key features of our synthesis include: 1) the challenging thiazole segment was elegantly installed via a cascade one-pot process under mild condition, 2) stereoselective reductions and a serious of high-yield mild reactions were employed to ensure the requisite stereochemistry, reaction scale, yield and to avoid the vexing epimerization occurring during peptide formation. The present route made a significant progress in the stereoselectivity, production scale, reagent cost and reaction yield. On the basis of the current work, the diversity-oriented syntheses and the biological investigation of tubulysin derivatives is ongoing in our laboratory.

Conflicts of interest

There are no conflicts to declare.

Acknowledgments

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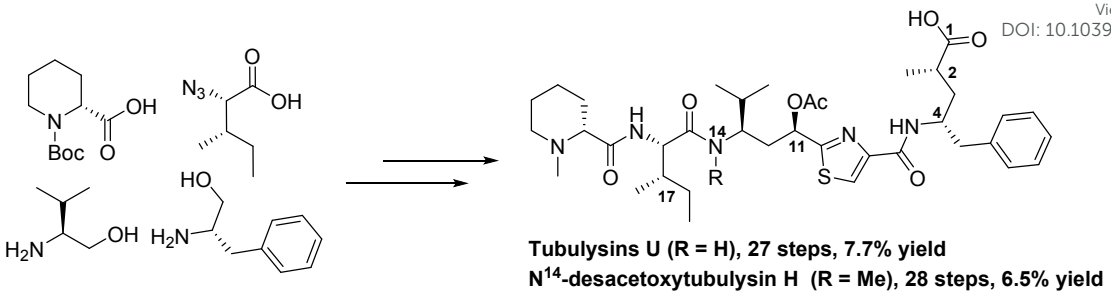

Tubulysins U (R = H), 27 steps, 7.7% yield
N^{14}-desacetoxytubulysin H (R = Me), 28 steps, 6.5% yield

- **gram-scale synthesis**
- **high stereoselectivity and reaction yield**
- **mild reaction conditions and cheap reagents**
- **a concise and practical synthetic protocol for tubulysins**