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Total Synthesis of N^{14}-Desacetoxytubulysin H

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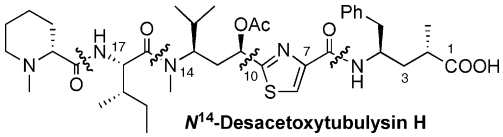
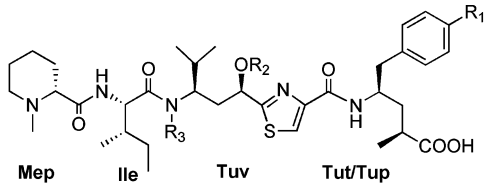
ABSTRACT



N^{14}-Desacetoxytubulysin H

The N^{14}-desacetoxy analogue of tubulysin H was prepared in 20 steps and 2.1% overall yield. Our strategy features a thiazole anion addition to assemble the tubuvaline residue at the C(10)–C(11) bond, as well as acylations at N^{6}, N^{14}, and N^{17}. This iterative coupling approach, as well as the removal of the labile *N,O*-acetal at N^{14}, enables the synthesis of analogues for detailed studies of structure–activity relationships in this family of potent tubulin disrupters.

Tubulysins are part of a family of antimitotic agents isolated in 1996 from the myxobacterial strains *Archangium gephyra* and *Angiococcus disciformis* (Figure 1).[1] They possess potent cell growth inhibitory activity that exceeds that of epothilones, vinblastine, and taxol. Therefore, they represent attractive leads for the development of new anticancer agents. Due to the limited amount and structural diversity generated in the fermentation process, a total synthesis of tubulysins is highly desirable. Structurally, these compounds are related to several short peptide derivatives, including the marine natural products dolastatins[2] and the anticancer drug LU 103793.[3] A dipeptide segment comprised of hydrophobic *N*-terminal amino acids, *N*-methylpipecolic acid (Mep), and isoleucine (Ile) is followed by the thiazole-containing residue tubuvaline (Tuv), and terminated by tubuphenylalanine (Tup) or tubutyrosine (Tut). The unusual *N,O*-acetal-containing tubulysins A−I have IC_{50} values of 0.3−7 ng/mL against the human



Tubulysin A: R_1 = OH, R_2 = Ac, R_3 = $CH_2OC(O)CH_2CH(CH_3)_2$
B: R_1 = OH, R_2 = Ac, R_3 = $CH_2OC(O)CH_2CH_2CH_3$
C: R_1 = OH, R_2 = Ac, R_3 = $CH_2OC(O)CH_2CH_3$
D: R_1 = H, R_2 = Ac, R_3 = $CH_2OC(O)CH_2CH(CH_3)_2$
E: R_1 = H, R_2 = Ac, R_3 = $CH_2OC(O)CH_2CH_2CH_3$
F: R_1 = H, R_2 = Ac, R_3 = $CH_2OC(O)CH_2CH_3$
G: R_1 = OH, R_2 = Ac, R_3 = $CH_2OC(O)CH_2{=}C(CH_3)_2$
H: R_1 = H, R_2 = Ac, R_3 = $CH_2OC(O)CH_3$
I: R_1 = OH, R_2 = Ac, R_3 = $CH_2OC(O)CH_3$

U: R_1 = H, R_2 = Ac, R_3 = H
V: R_1 = H, R_2 = H, R_3 = H
Z: R_1 = OH, R_2 = H, R_3 = H

Figure 1. Structures of tubulysins. A−I are reported in the Höfle et al. isolation papers,[1] and U, V, and Z appear in the work of Dömling et al.[4a]

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cervix carcinoma, multidrug resistant cell line KB-V1.[1b] Similar to dolastatin, hemiasterlin, and HTI-286,[5a] tubulysins inhibit vinblastine binding to tubulin, disrupt tubulin polymerization, and are likely to bind at the peptide site of the vinca domain of β-tubulin.[5b,c]

Because of their distinct structure and high potency, tubulysins have attracted considerable synthetic interest.[4] Very recently Dömling and co-workers synthesized stereoisomers of tubulysins U and V using a three-component condensation approach,[4a] Ellman and co-workers prepared tubulysin D featuring *tert*-butanesulfinamide chemistry,[4b] and Zanda and co-workers reported a scalable synthesis of tubulysins U and V.[4c] We hypothesized that the conversion of the labile *N*,*O*-acetal functionality in tubulysins A−I to a simple *N*-alkyl group would largely conserve the exceptional activity but increase the bioavailability of these analogues. As a proof of principle for this theory, we embarked on a synthesis of N^{14}-desacetoxytubulysin H (**1**). Retrosynthetically, we initially considered disconnecting **1** at all amide bonds and constructing the thiazole ring through a cyclodehydration process (Figure 2).[4f,6a−d] However, disconnection of the C(10)−C(11) bond offered a more convergent fragment assembly strategy. Since pathway B was also more attractive for structure−activity studies involving replacements of the thiazole ring, we decided to pursue this approach, which was mainly precedented in the use of 2-thiazolyllithium reagents as aldehyde synthons for chain extensions by Dondoni et al.[6e−f]

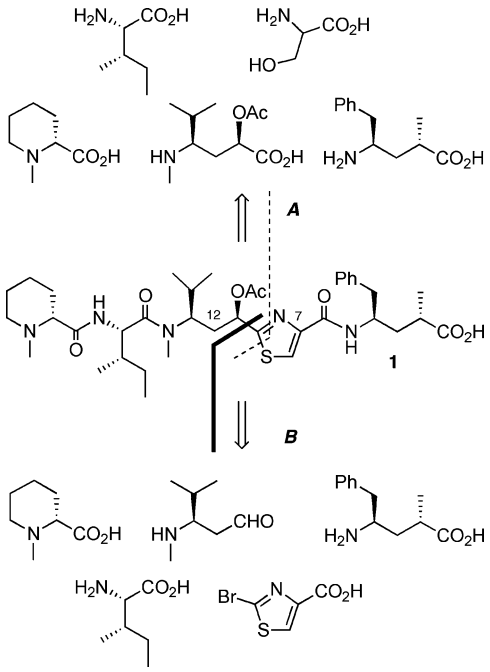
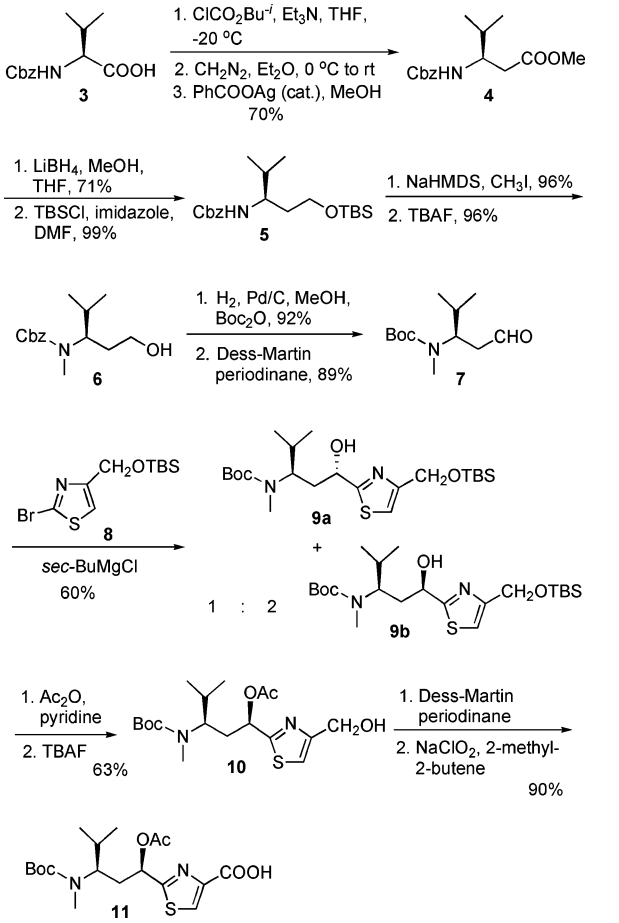


Figure 2. Alternative retrosynthetic analysis and building blocks for N^{14}-desacetoxytubulysin H (**1**) based on carbon−heteroatom bond formation (pathway A) or carbon−carbon bond formation (pathway B).

Previously, we prepared an advanced Tuv-Tup derivative,[4f] but were unable to efficiently remove the Cbz-protecting group by various methods including hydrogenation over Pd/C.[6g]

A Boc-protecting strategy was thus desired, and we attempted to construct the *syn*-alcohol **9b** by the addition of the thiazole anion from **8** to aldehyde **7** (Scheme 1). The requisite aldehyde **7** was prepared from Cbz-Val-OH **3** by chain homologation to ester **4**, reduction, *N*-methylation, and alcohol → aldehyde oxidation protocols. The thiazole **8**[7a] was generated in multigram quantities from thiourea in 30% yield

Scheme 1. Synthesis of *N*-Boc-*N*-methyl Tubuvaline **11**



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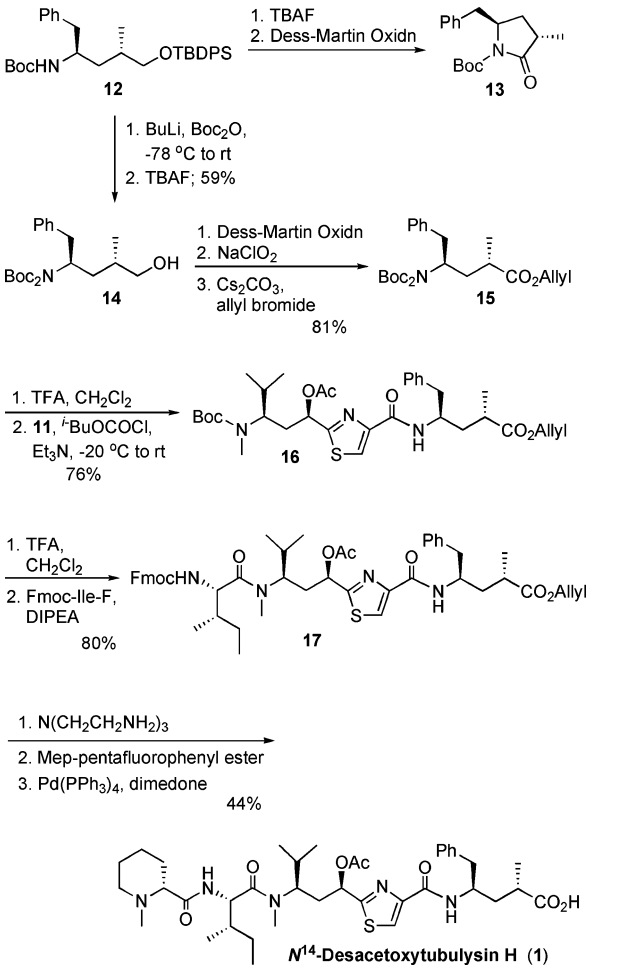
over a four-step sequence. Our initial attempt to generate the thiazole anion in the form of 2-(trimethylsilyl)thiazole[6e] was unsuccessful, and the corresponding lithium anion[7b] required carefully controlled reaction conditions. Alternatively, the thiazole Grignard reagent[8] generated by exchange with *sec*-butylmagnesium chloride led to a clean 1,2-addition to aldehyde **7**, forming the separable epimers **9a** and **9b** in a 1:2 ratio in 60% yield. The configuration at the newly formed stereogenic carbon was determined by a double derivatization method with α-methoxyphenylacetic acid (MPA).[9a] The desired major isomer **9b** was acylated, deprotected, and oxidized by a two-step sequence[9b−d] to give Tuv derivative **11**.

The synthesis of the Tup derivative **14** started from diastereomerically pure **12** (Scheme 2), which had previously

Scheme 2. Synthesis of **1**



been prepared during our synthesis of tubulysin segments.[4f] When **12** was deprotected with TBAF and oxidized, the *N*-Boc-pyrrolidinone **13** was obtained, and we were unable to further utilize **13** in the synthesis. Therefore, a double protection of the amino group was necessary. Alcohol **14** was smoothly oxidized and allylated to give ester **15**, and the subsequent *N*-deprotection with trifluoroacetic acid and coupling with the mixed anhydride of **11** afforded dipeptide **16**. Removal of the Boc-group from the methylated *N*-terminus of **16** followed by condensation with either Boc-Ile-OH or Fmoc-Ile-OH, using various coupling agents, including DEPBT,[10] BEP,[11] HATU,[12] TBTU,[13] PyBop,[14] and BOP-Cl,[15] always resulted in incomplete conversion and low yield due to the congested steric environment and the reduced reactivity of the *N*-methylated amine.[16] After extensive experimentation, we found that the acyl fluoride Fmoc-Ile-F[17] was superior for this difficult coupling, and **17** was obtained in 80% yield. Removal of the Fmoc-protective group and coupling with the pentafluorophenyl ester of Mep[4b] gave the desired tetrapeptide intermediate. Finally, the allyl ester was efficiently removed by $Pd(PPh_3)_4$ in the presence of dimedone[18] as an allyl cation scavenger to give target molecule **1** after HPLC purification in 44% overall yield for the three steps.

In summary, we have developed an asymmetric total synthesis of N^{14}-desacetoxytubulysin H in 20 steps and 2.1% yield for the longest linear sequence. The combination of readily available α-amino acids as building blocks and a convergent strategy should allow for the synthesis of a range of stereoisomers and structurally modified tubulysins. Our disconnection of the tubulysin scaffold at the C(10)−C(11) bond is unique among the previously reported synthetic approaches.[4] The biological evaluation of **1** and additional SAR studies will be reported in due course.

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Supporting Information Available: Experimental procedures and spectral data for all new compounds, including copies of ^1H and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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