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Synthesis and biological evaluation of tubulysin D analogs related to stereoisomers of tubuvaline

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ABSTRACT

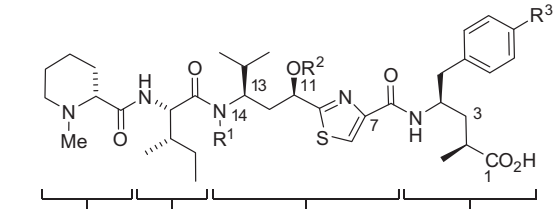
The synthesis and biological evaluation of stereoisomers in tubulysin D are described. The stereoselective synthesis of all possible stereoisomers of C-11 and C-13 positions in tubulysin D was achieved by employing 1′-*epi*-Tuv-Me, 3′-*epi*-Tuv-Me, and *ent*-Tuv-Me and their biological properties were evaluated. It is clear that the stereochemistries of the C-11 and C-13 positions in tubulysin D have no practical impact on the inhibition of tubulin polymerization but play a role in the potent antiproliferative activities.

Microtubules are composed of tubulin molecules, which are heterodimers of two closely related globular polypeptides, α-tubulin and β-tubulin. The microtubule is certified as one of the targets for effective chemotherapy of cancers.[1] The anticancer agents that act on microtubules can be classified into two major categories according to their mechanism of action.[2] One category consists of microtubule-stabilizing agents such as paclitaxel,[3] epothilones,[4] and discodermolide.[5] The other category includes microtubule destabilizing agents such as vinblastine and vincristine, which inhibit microtubule polymerization.[6]

The tubulysin A (**1a**) and D (**1b**), isolated from the two different species of myxobacteria, *Archangium gephyra* and *Angiococcus disciformis*, respectively, are novel tetrapeptides that display potent antitumor activity by inhibiting tubulin polymerization.[7–10] In particular, the antiproliferative activities of tubulysin D (**1b**), the most potent derivative among the tubulysins shown in Figure 1, have been reported to exceed those of vinblastine.[8,11]

Tubulysins **1a** and **1b** are composed of four amino acid fragments, *N*-methyl-D-pipecolic acid (D-Mep), L-isoleucine (L-Ile), tubuvaline (Tuv), and tubuphenylalanine (Tup)/tubutyrosine (Tut). The structural features of **1a** and **1b** would indicate the presence of unusual amino acids Tup (Tut) and Tuv, which has a labile *N*,*O*-acetal side chain at the *N*-14 position.[8,11] The tubulysins have received much attention in recent years as lead compounds for the development of new anticancer agents due to their remarkable biological activities and unique structural characteristics.[12]

To date, much effort has been devoted to the synthesis and biological evaluation of natural tubulysins and their analogs.[13–23] Among them, WZY-III-69A (**2**),[17] **3**,[18] and pretubulysin (**4**)[23] displayed nanomolar cell growth inhibition (Fig. 2). For potent cell growth inhibition, it appears that (i) the labile *N*,*O*-acetal at the



tubulysins	R^1	R^2	R^3
A (**1a**)	$CH_2OCOCH_2CH(CH_3)_2$	Ac	OH
D (**1b**)	$CH_2OCOCH_2CH(CH_3)_2$	Ac	H
U (**1c**)	H	Ac	H
V (**1d**)	H	H	H

Figure 1. Structures of tubulysins and their components.

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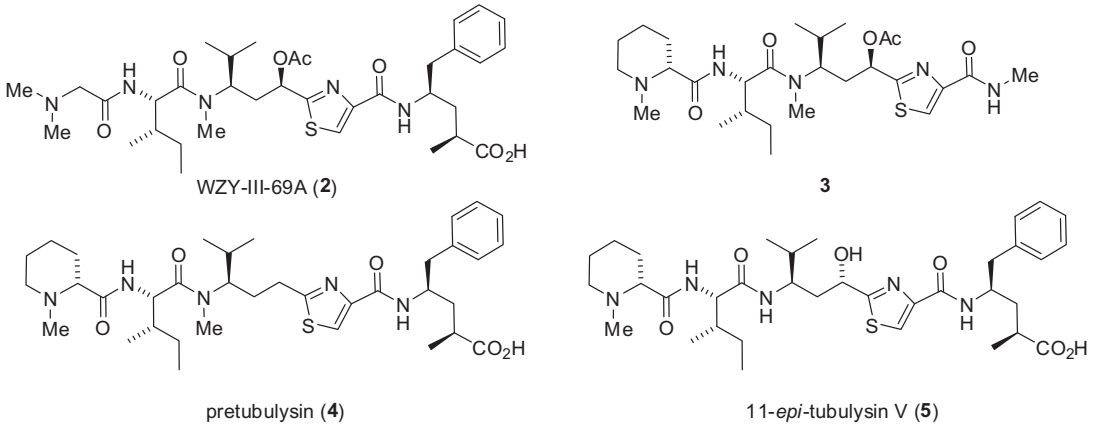
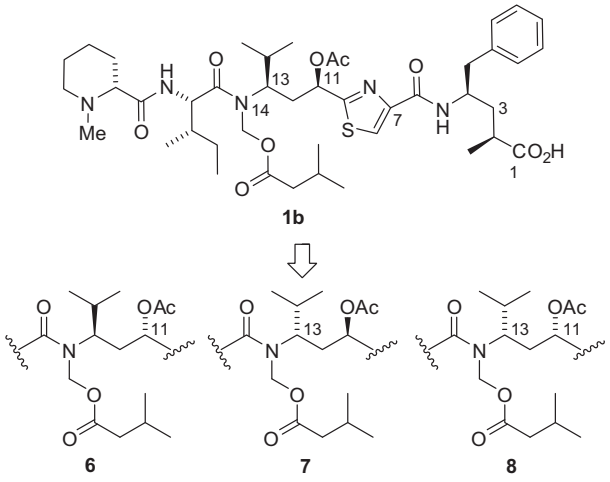
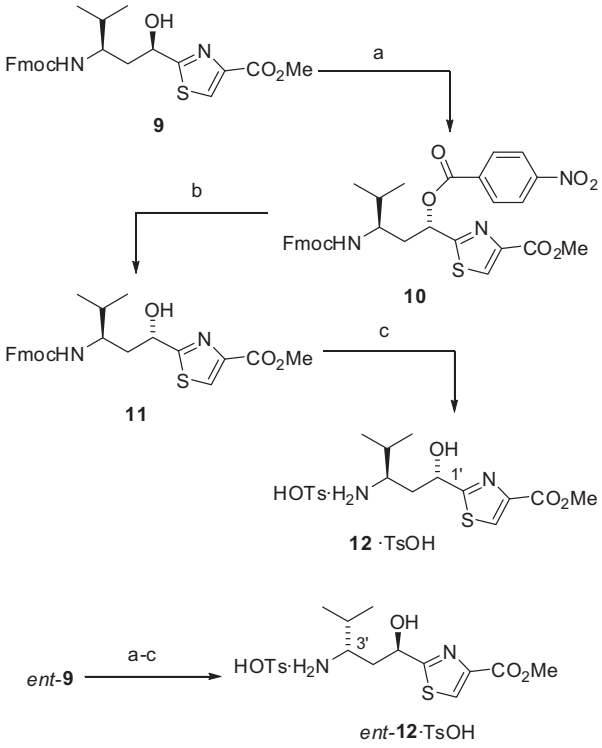


Figure 2.



Scheme 1.

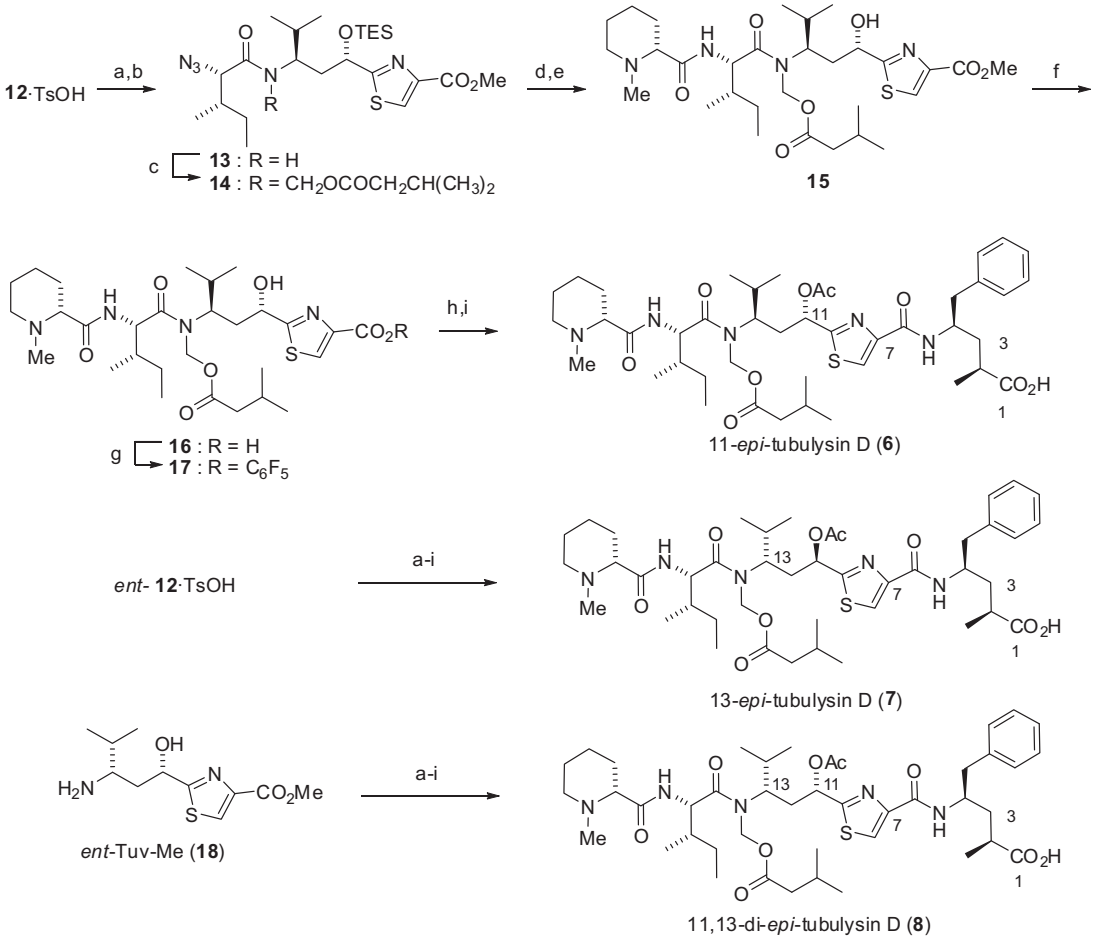


Scheme 2. Reagents and conditions: (a) *p*-NO_2-C_6H_4-CO_2H, DEAD, PPh_3, THF, 93% (for **10**), 91% (for *ent*-**10**); (b) NaN_3, MeOH, quant (for **11**), quant (for *ent*-**11**); (c) Et_2NH, MeCN then TsOH, MeCN, 83% (for **12**·TsOH), 93% (for *ent*-**12**·TsOH).

N-14 position would not be essential,[17] (ii) the D-Mep of the N-terminal amino acid could be replace with another amino acid such as *N*-methylsarcosine,[17] (iii) the Tup of the C-terminal amino acid would be able to be converted into a simple structure, (iv) the acetoxy group in Tuv is perhaps not necessary. Although it has been reported that the antiproliferative activities of 11-*epi*-tubulysin V (**5**) shows less activity than tubulysin V (**1d**), there is no remarkable difference between **5** and **1d** on regarding the activity of tubulin inhibition.[21] On the other hand, we have also reported a synthesis of tubulysin D (**1b**), *ent*-D (*ent*-**1b**), U (**1c**), and V (**1d**), and their preliminary biological activities against HEp-2 cell (human epidermoid carcinoma of the larynx) in vitro.[24] However, the details of the relationship between the two stereochemistries of Tuv and their biological properties remain unclear. We have therefore envisioned the stereoselective synthesis of all possible stereoisomers of Tuv in tubulysin D (**1b**), and have strived to synthesize 11-*epi*-tubulysin D (**6**), 13-*epi*-tubulysin D (**7**), and 11,13-di-*epi*-tubulysin D (**8**) (Scheme 1). We report herein the synthesis and biological evaluation, including inhibition of tubulin polymerization and antiproliferative activity, of the tubulysin D (**1b**) and its analogs.

The syntheses of 11-*epi*-tubulysin D (**6**) and 13-*epi*-tubulysin D (**7**) require 1′-*epi*-Tuv-Me (**12**) and 3′-*epi*-Tuv-Me (*ent*-**12**), respectively (Scheme 2). Mitsunobu reactions[25,26] of *N*-Fmoc-Tuv-Me (**9**) and *ent*-*N*-Fmoc-Tuv-Me (*ent*-**9**)[24] were employed for the preparation of **12** and *ent*-**12**, respectively. Thus, secondary alcohol **9**, on treatment with *p*-nitrobenzoic acid in the presence of diethyl azodicarboxylate (DEAD) and triphenylphosphine, underwent a Mitsunobu inversion to give **10** in 93% yield. Mild and selective cleavage of the *p*-nitrobenzoic ester in **10** was accomplished by using excess sodium azide in methanol to give 1′-*epi*-*N*-Fmoc-Tuv-Me (**11**) in excellent yield.[27] Removal of the 9-fluorenylmethyloxycarbonyl (Fmoc) group of **11** by treatment with diethylamine and subsequent treatment with *p*-toluenesulphonic acid afforded 1′-*epi*-Tuv-Me·tosylate (**12**·TsOH), which is the corresponding fragment in **6**. The 3′-*epi*-Tuv-Me (*ent*-**12**), which contains amino acids requisite for the synthesis of **7**, was prepared from *ent*-**9**[24] in the same synthetic sequence described above for the preparation of **12**.

With the desired 1′-*epi*-Tuv-Me (**12**) and 3′-*epi*-Tuv-Me (*ent*-**12**) in hand, the stage was set for the synthesis of *epi*-tubulysin D. To synthesized *epi*-tubulysin Ds from **12** and *ent*-**12**, we adopted a re-



Scheme 3. Reagents and conditions: (a) N_3-Ile-Cl, DIPEA, CH_2Cl_2, 97% (for **6**), 91% (for **7**), 88% (for **8**); (b) TESOTf, 2,6-lutidine, CH_2Cl_2, quant (for **6**), quant (for **7**), quant (for **8**); (c) KHMDS, $ClCH_2OCOCH_2CH(CH_3)_2$, THF, 71% (for **6**), 84% (for **7**), 88% (for **8**); (d) D-Mep, PFP, EtOAc, then 10% Pd-C, H_2, 60% (for **6**), 40% (for **7**), 76% (for **8**); (e) AcOH-THF-H_2O, 89% (for **6**), 78% (for **7**), 83% (for **8**); (f) Me_3SnOH, $Cl(CH_2)_2Cl$, 47% (for **6**), 57% (for **7**), 41% (for **8**); (g) PFP, DIC, CH_2Cl_2; (h) DIPEA, Tup·HCl, DMF, 64% (for **6**), 68% (for **7**), 45% (for **8**); (i) Ac_2O, pyridine then 1,4-dioxane-H_2O, 71% (for **6**), 90% (for **7**), 94% (for **8**).

ported synthetic sequence.[14] The condensation reaction of **12** with N_3-Ile-Cl followed by protection of the secondary alcohol with a triethylsilyl group, provided **13** (Scheme 3).

N-Alkylation of the amide nitrogen in **13** was executed by treatment with KHMDS and chloromethyl isobutyl carbonate to afford **14**. The coupling reaction of azide dipeptide **14** with D-Mep under hydrogenation conditions followed by the deprotection of triethylsilyl ether gave tripeptide **15**. Selective cleavage of the methyl ester in **15** was conducted by treatment with Me_3SnOH in 1,2-dichloroethane at 60 °C to provide the desired acid **16** in 47% yield along with undesired cleavage of the *N*-*O*-acetal function (48%). The activated ester **17** prepared from **16** with pentafluorophenol (PEP) with *N*,*N*′-diisopropylcarbodiimide (DIC) was condensed with Tup to afford deacetyl-11-*epi*-tubulysin D. Finally, the synthesis of 11-*epi*-tubulysin D (**6**) was achieved by acetylation of the secondary hydroxyl group. The stereoselective synthesis of 13-*epi*-tubulysin D (**7**) and 11,13-di-*epi*-tubulysin D (**8**) was accomplished in the same manner described above starting from *ent*-**12** and *ent*-Tuv-Me (**18**), respectively.

The synthesized tubulysin D analogs (**6**–**8**) were evaluated for the inhibition of tubulin polymerization using purified porcine brain tubulin, and assayed for in vitro cytotoxicity against four human cancer cell lines, including HL60 (human blood premyelocytic leukemia), HCT116 (human colon cancer), MCF7 (human breast adenocarcinoma), and A549 (human lung adenocarcinoma epithelial). The biological data are presented in Table 1. The inhibitory activity values for tubulin polymerization of 11-*epi*-tubulysin D (**6**) and 13-*epi*-tubulysin D (**7**) were comparable to those of tubulysin D (**1b**) and vinblastine, which are known to be capable of inhibiting tubulin polymerization [IC_{50} (μM): 1.4 for **6**, 1.7 for **7**, 1.7 for **1b**, and 1.6 for vinblastine], whereas IC_{50} (6.3 μM) of 11,13-di-*epi*-tubulysin D (**8**) showed rather weaker activity than the other ana-

Table 1
Biological activity of tublysin D and its analogs

Compound	Inhibition of Tubulin polymerization (IC_{50}, μM)	Antiproliferative activity (IC_{50}, nM)			
		HL60	HCT116	MCF7	A549
6	1.4	3.1	13	280	43
7	1.7	98	920	240	1200
8	6.3	3600	>10,000	>10,000	>10,000
1b	1.7	0.0047	0.0031	0.67	0.013
Vinblastine	1.6	1.0	27	0.79	17

logs. It should be noted that changing one of the stereochemistries at the C-11 and C-13 positions in **1b** does not influence inhibition activity of tubulin polymerization. However, inversion of both configurations at the C-11 and C-13 positions in **1b** resulted in a decrease in the inhibitory activity.

The antiproliferative activities of **6** were dramatically decreased compared with **1b** in a tested human cancer cell line. Compound **7**, which was a stereoisomer at the C-13 position in **1b**, also showed a pronounced decline in antiproliferative activities. When compared to **7**, compound **6** showed similar activity in MCF7 cells, whereas it exhibited potent activities in other cells. Compound **8**, bearing an inverted configuration at the two stereocenters in **1b**, exhibited much less activity than the other compounds in this assay. Our results indicate that the two stereochemistries of Tuv in tubulysin D (**1b**) play a major role in the potent antiproliferative activities. The inverted configuration at either the C-11 or C-13 position impairs the antiproliferative activities. Interestingly, these results regarding the antiproliferative activity do not correlate well with the inhibition of tubulin polymerization. In particular, the *epi*-tubulysin Ds (**6** and **7**) were found to possess tubulin polymerization inhibitory activity similar to that of tubulysin D (**1b**), whereas the di-*epi*-tubulysin D (**8**) exhibited much less activity than **1b**. One possibility is that an inverted configuration at either the C-11 or C-13 position in **1b** might induce the change in the cell membrane permeability.[19]

In summary, we have succeeded in carrying out stereoselective synthesis of all possible stereoisomers of Tuv in tubulysin D (**1b**), and have clarified the relationship between two stereochemistries of Tuv in **1b** as well as the biological properties. It has been demonstrated that an inverted configuration at either the C-11 or C-13 position in **1b** does not have a practical impact on the inhibition of tubulin polymerization but dose play a role in the potent antiproliferative activities. Further investigation related to biological studies of these and other tubulysin analogs is currently under way and will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.118.

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