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Stereoselective synthesis of tubuvaline methyl ester and tubuphenylalanine, components of tubulysins, tubulin polymerization inhibitors

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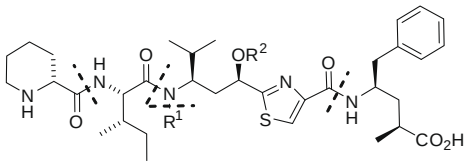
ABSTRACT

Synthetic studies of two components of tubulysins, tubulin polymerization inhibitors are described. The highly stereoselective synthesis of tubuvaline methyl ester (**2**) was accomplished by 1,3-dipolar cycloaddition of nitrone D-**6** and acrylic acid derivatives **7** as a key step. The synthesis of tubuphenylalanine (**3**) was conducted by an aldol reaction of a boron enolate of (*S*)-4-isopropyl-3-propionyl-2-oxazolidinone (**13**) with aldehyde **14**, readily prepared from phenylalanine, followed by Barton deoxygenation under radical conditions.

Tubulysins (**1a–d**), which are tetrapeptide derivatives, were first isolated from the myxobacterial strains *Archangium gephyra* and *Angiococous disciformis*, and they possess potent cell growth inhibitory activity exceeding that of both vinblastine and dolastatin10 (Fig. 1).[1] Tubulysins inhibit tubulin polymerization[2] and also possess antiangiogenic properties.[3] Since tubulysins are expected to become excellent lead compounds for the development of new anticancer agents that would inhibit tubulin polymerization, they have attracted considerable attention as synthetic target molecules. To date, the total syntheses of tubulysin D (**1a**), N^{14}-deacetoxytubulysin H (**1b**), U (**1c**), V (**1d**), and synthetic approaches have been reported.[4–7] The chemical degradation of tubulysin D (**1a**), the most active compound among tubulysins, and a comparison of the resulting amino acids with the corresponding natural and unnatural amino acids revealed that **1a** is composed of four amino acid fragments, *N*-methyl-D-pipecolic acid (D-Mep), L-isoleucine (L-Ile), tubuvaline (Tuv), and tubuphenylalanine (Tup) (Fig. 1).[1,8] Among these four amino acids, the stereoselective syntheses of two unusual amino acids, Tuv and Tup, would be essential for an effective synthesis of tubulysins. We report herein an efficient method for the stereoselective synthesis of Tuv featuring the 1,3-dipolar cycloaddition of a nitrone, as well as a method for stereoselective synthesis of Tup using an aldol reaction followed by the Barton deoxygenation.

Our synthetic plan for the synthesis of tubuvaline methyl ester (Tuv-Me, **2**) and Tup (**3**) is depicted in Scheme 1. The 1,3-amino alcohol structure in **2** can be made available by the reductive cleavage of the *N–O* bond of isoxazolidine **I**, and a thiazole ring can be constructed by the cyclization of the cysteine moiety of **I**. Compound **I** can be synthesized by the regio- and stereoselective 1,3-dipolar cycloaddition of the appropriate nitrone **III** and acrylic acid derivative **IV**, leading to **II**, followed by the removal of the chiral auxiliary and condensation with the cysteine derivative. The stereoselective synthesis of Tup (**3**) can be carried out by utilizing the Evans aldol reaction[9] of aldehyde **VI**, readily prepared from phenylalanine, followed by the Barton deoxygenation of **V** under radical conditions.[10]



N-methyl-D-pipecolic acid (D-Mep) | L-isoleucine (L-Ile) | **tubuvaline (Tuv)** | **tubuphenylalanine (Tup)**

	R^1	R^2
tubulysin D (**1a**)	$CH_2OCOCH_2CH(CH_3)_2$	$COCH_3$
H (**1b**)	CH_2OCOCH_3	$COCH_3$
U (**1c**)	H	$COCH_3$
V (**1d**)	H	H

Figure 1.

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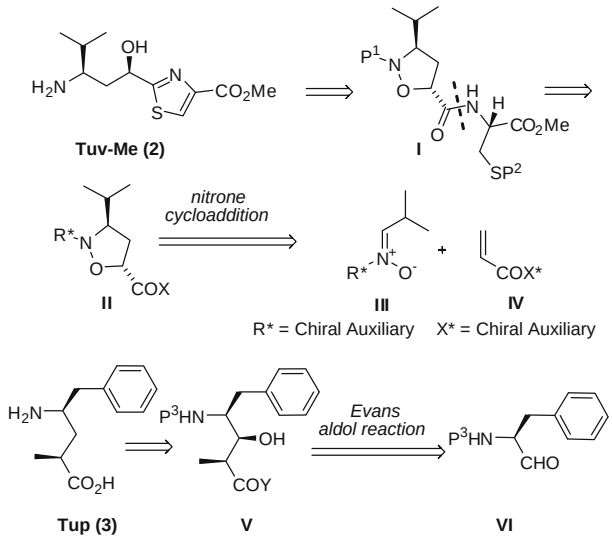
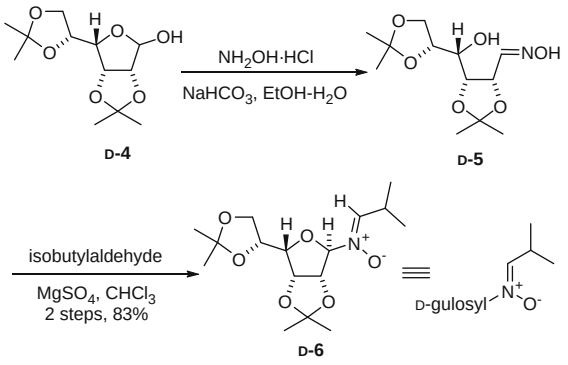


Scheme 1.



Scheme 2.

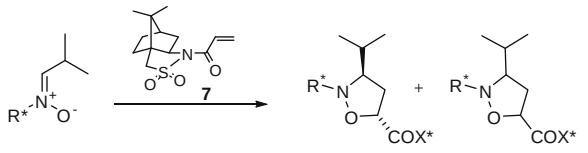
In the synthesis of Tuv-Me (**2**), the mild and selective removal of each chiral auxiliary (R^* or X^*) in cycloadduct **II** is essential. It was also expected that double asymmetric induction would improve the stereoselectivity of the 1,3-dipolar cycloaddition.[11] Taking this into account, we chose a gulose-derived auxiliary as R^* for nitrone **III** (see nitrone D-**6** in Scheme 2)[12,13] and Oppolzer's camphor sultam as the X^* of acrylate **IV** (see *N*-acryloyl sultam **7** in Table 1).

Our synthesis commenced with the preparation of nitrone D-**6** from 2,3:5,6-*O*-diisopropylidene-D-gulofuranose (D-**4**)[12,13] (Scheme 2). Treatment of D-**4** with hydroxylamine hydrochloride in the presence of sodium hydrogen carbonate gave oxime D-**5** as a 1:1 mixture of *E* and *Z* isomers, which, without separation, was reacted with isobutylaldehyde to afford nitrone D-**6** in 83% yield from D-**4**. In a similar manner, nitrone L-**6** was prepared from 2,3:5,6-*O*-diisopropylidene-L-gulofuranose (L-**4**) in 90% yield (2 steps).

Next, we attempted the crucial cycloaddition of nitrone D-**6** with (2*R*)-*N*-(acryloyl)bornane-10,2-sultams (**7**)[14] (Table 1). The exposure of nitrone D-**6** to **7** in refluxing toluene led to cycloaddition, to give (3*R*,5*R*)-isoxazolidine **8**[15] as the major product (76%) along with other isomers **9** (24%, mixture of isomers)[16] (run 1). Cycloadduct **8** was readily separated from the other isomers by column chromatography. The stereochemistry of **8** was precisely determined by X-ray crystallography (Fig. 2), which revealed that **8** had the correct stereochemistry for the synthesis of Tuv-Me (**2**).[17] Use of a protic polar solvent (EtOH) slightly improved stereoselectivity (run 2). At a rather low temperature (40 °C), this cycloaddition proceeded to exhibit higher selectivity (runs 3 and 4). Among reactions in several solvents (runs 5–8), the reaction in refluxing CH_2Cl_2 gave the best results, and cycloadduct **8** (85%) was obtained (run 8). On the other hand, the reaction of L-**6** derived from L-**4** with **7** in refluxing CH_2Cl_2 yielded a mixture of four isomers of cycloadduct. The yield of cycloadduct **8′**[15] which exhibited the correct stereochemistry for **2** (Fig. 2)[17] was diminished to 19% yield.[18] These results clearly demonstrated that the combination of nitrone D-**6** and alkene **7** represented a matched pair, and that of L-**6** and **7** was a mismatched pair.

As cycloadduct **8** showed the correct stereochemistry, an elaboration of adduct **8** was carried out to obtain Tuv-Me (**2**) (Scheme 3). Treatment of **8** with lithium hydroxide at room temperature followed by treatment with perchloric acid subsequently gave [(3*R*)-3-isopropylisoxazoline-5-yl]carboxylic acid, the nitrogen of which was protected by an Fmoc group to afford acid **10** in 86% yield from **8**. Next, **10** was condensed with L-*S*-tritylcysteine methyl ester[19] under usual conditions, yielding the fully protected cysteine-containing dipeptide **11**. When dipeptide **11** was exposed to bis(triphenyl)oxodiphosphonium trifluoromethanesulfonate,[20] which

Table 1
1,3-Dipolar cycloaddition of the chiral nitrone D-**6** or L-**6** with **7**



D-**6**: R* = D-gulosyl
L-**6**: R* = L-gulosyl

8: R* = D-gulosyl **9**: R* = D-gulosyl
8′: R* = L-gulosyl **9′**: R* = L-gulosyl

Run	**6**	Solvents	Temp (°C)	Time (h)	Yield (%)	Diastereomer ratio[a] **8** or **8′**:**9** or **9′**
1	D-**6**	Toluene	110	1	Quant	76:24
2	D-**6**	EtOH	78	6	Quant	78:22
3	D-**6**	Toluene	40	24	Quant	83:17
4	D-**6**	EtOH	40	48	Quant	81:19
5	D-**6**	THF	40	24	Quant	80:20
6	D-**6**	CH_3CN	40	48	Quant	79:21
7	D-**6**	Neat	40	48	Quant	82:18
8	D-**6**	CH_2Cl_2	40	48	Quant	85:15
9	L-**6**	CH_2Cl_2	40	48	83	23:77[b]

[a] Diastereomer ratios of **8** to **9** were determined by the ^1H NMR spectra of the mixtures.
[b] This sample **9′** contained three isomers detected by HPLC.

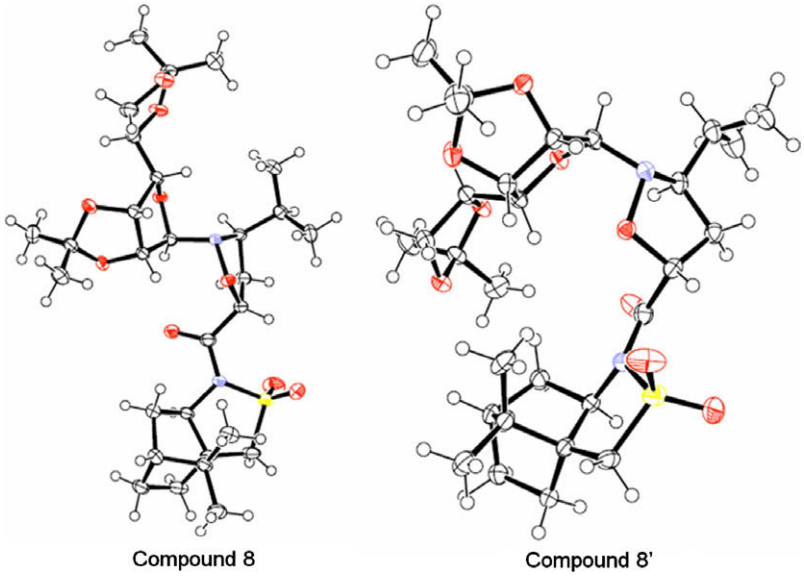
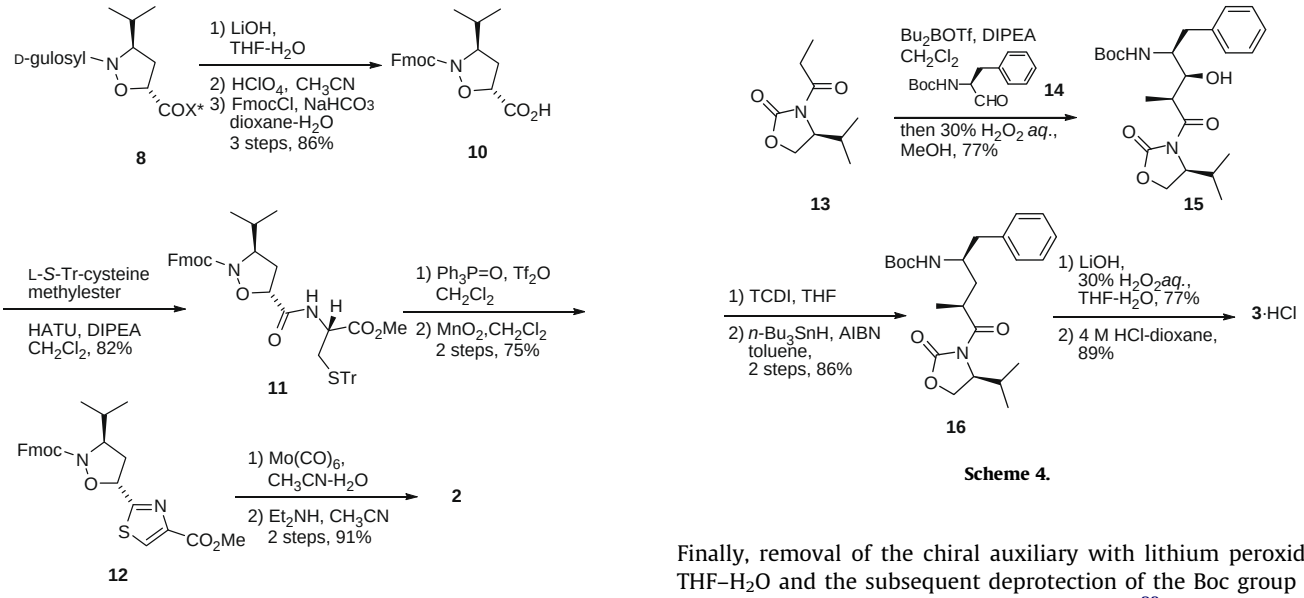


Figure 2. ORTEP drawings for **8** and **8′**.



Scheme 3.

Scheme 4.

had been prepared from triphenylphosphinoxide and triflic anhydride, cyclodehydration proceeded smoothly to the construction of a thiazoline moiety, which was oxidized by activated manganese dioxide to provide thiazole **12** in 75% yield (2 steps). Finally, reductive cleavage of the *N–O* bond of **12** by heating the sample with molybdenum hexacarbonyl in acetonitrile-water (10:1),[21] and the subsequent removal of the Fmoc group by treatment with diethylamine afforded Tuv-Me (**2**)[15] in 91% yield (2 steps).

The synthesis of Tup (**3**) was initiated by the stereoselective aldol reaction of aldehyde **14** with the (*Z*)-boron enolate of (*S*)-4-isopropyl-3-propionyl-2-oxazolidinone (**13**), yielding adduct **15** in 77% yield (Scheme 4).[9,22,23] Removal of the secondary hydroxyl group of **15** was then carried out according to the Barton–McCombie procedure.[10] Thus, exposure of **15** to 1,1′-thiocarbonyldiimidazole (TCDI) followed by treatment with Bu_3SnH in the presence of a catalytic amount of 2,2′-azobis(2-methylpropionitrile) (AIBN) gave the deoxygenated product **16** in 86% yield (2 steps). Finally, removal of the chiral auxiliary with lithium peroxide in $THF–H_2O$ and the subsequent deprotection of the Boc group successfully produced Tup·hydrochloride (**3**·HCl).[23]

In summary, we explored a novel synthetic route to yield tubuvaline methyl ester (**2**) and tubuphenylalanine hydrochloride (**3**·HCl). These two synthetic approaches are now available for producing tubulysin analogues required for the investigation of structure–activity relationships. The syntheses of natural tubulysins and novel tubulysins analogues that cannot be derived from natural resources are under investigation.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.04.046.

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15. For experimental procedure of **8** and spectral data of tubuvaline methyl ester (**2**), see Supplementary data.
16. The stereochemistries of two isomers were not determined.
17. The crystallographic data were collected on a CCD detector. The crystal structure was solved by direct methods SHELXS-97 and refined by full-matrix least-squares SHELXL-97 (Sheldrick, G.M. *Acta Crystallogr., Sect. A* 64, **2008**, 112–122). All non-hydrogen atoms were refined anisotropically. All hydrogen atoms were included as their calculated positions. Crystallographic data for **8**: $C_{29}H_{46}N_2O_9S$, $M = 598.74$, monoclinic, $a = 10.853(1)$, $b = 11.583(1)$, $c = 12.862(1)$ Å, $\beta = 109.902(1)°$, $V = 1520.4(3)$ Å^3, $T = 120$ K, space group $P2_1$, $Z = 2$, $D_c = 1.308$ Mg m^{-3}, μ(MoKα) = 0.161 mm^{-1}, *GOF* on $F^2 = 1.038$, $R_1 = 0.0331$, $wR_2 = 0.0790$ (all data), Flack parameter = −0.04(5), CCDC-719636. Crystallographic data for **8′**: $C_{29}H_{46}N_2O_9S$, $M = 598.74$, monoclinic, $a = 10.468(1)$, $b = 21.676(3)$, $c = 13.561(2)$ Å, $\beta = 93.987(1)°$, $V = 3069.4(7)$ Å^3, $T = 120$ K, space group $P2_1$, $Z = 4$, $D_c = 1.296$ Mg m^{-3}, μ(MoKα) = 0.160 mm^{-1}, *GOF* on $F^2 = 1.037$, $R_1 = 0.0468$, $wR_2 = 0.1114$ (all data). Flack parameter = 0.05(6), CCDC-719635. Two independent molecules are included an asymmetric unit of the crystal.
18. The separation of the four isomers was performed by HPLC with a chiral column to give pure samples of **8′**. Separation conditions were as follows: Daicel Chiralpak IA, ϕ 2.0 cm × 25 cm; hexane/2-propanol/EtOH = 70:15:15; flow rate 10 mL/min: HPLC analysis—Daicel Chiralpak IA, ϕ 0.46 cm × 25 cm; hexane/2-propanol/EtOH = 70:15:15, flow rate 0.5 mL/min; t_R 11.3 min (**8′**), 13.0, 15.5, and 19.7 min (other isomer **9′**). The stereochemistry of **8′** was determined by X-ray crystallography (Fig. 2).
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23. For experimental procedure of **15** and **16**, and spectral data of tubuphenylalanine hydrochloride (**3**·HCl), see Supplementary data.