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Total Syntheses of Tubulysins

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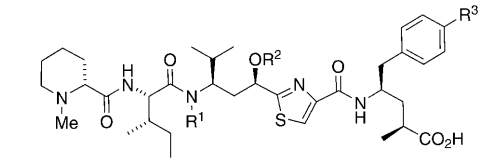
Abstract: The total syntheses of tetrapeptides tubulysins D (**1b**), U (**1c**), and V (**1d**), which are potent tubulin polymerization inhibitors, are described. The synthesis of Tuv (**2**), an unusual amino acid constituent of tubulysins, includes an 1,3-dipolar cycloaddition reaction of chiral nitrone D-**6** derived from D-gulose with *N*-acryloyl camphor sultam (−)-**9** employing the double asymmetric induction, whereas the synthesis of Tup (**20**), another unusual amino acid, involves a stereoselective Evans aldol reaction of (*Z*)-boron enolate generated from (*S*)-4-isopropyl-3-propionyl-2-oxazolidinone with *N*-protected phenylalaninal and a subsequent Barton deoxygenation protocol. We accomplished the total syntheses of tubulysins U (**1c**) and V (**1d**) by using these methodologies, in which the isoxazolidine ring was used as the effective protective group for γ-amido alcohol functionality. Furthermore, to understand the structure-activity relationship of tubulysins, we synthesized tubulysin D (**1b**) and cyclo-tubulysin D (**1e**) from **2**-Me and **20**, and *ent*-tubulysin D (*ent*-**1d**) from *ent*-**2**-Me and *ent*-**20**, respectively. The preliminary results regarding their biological activities are also reported.

Keywords: amino acids · antitumor agents · stereoselectivity · total synthesis · tubulysins

Introduction

Tubulysins A (**1a**) and D (**1b**), isolated from two species of myxobacteria, *Archangium gephyra* and *Angiococcus disciformis*, respectively, are tetrapeptides that are each composed of four amino acid fragments, *N*-methyl-D-pipecolic acid (D-Mep), L-isoleucine (L-Ile), tubuvaline (Tuv), and tubuphenylalanine (Tup)/tubutyrosine (Tut) (Scheme 1).[1,2] The linearly connected structures of tubulysins were determined by detailed two-dimensional NMR spectroscopic analyses and chemical degradation of tubulysin D (**1b**) by acidic hydrolysis.[3] Further X-ray diffraction of tubulysin A (**1a**) established the absolute configurations of seven stereocenters.[2] The common structural characteristics of tubulysins would include the existence of unusual amino acids, Tuv and Tup/Tut, as well as the *N*,*O*-acetal side chain at the 14-



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
cyclo-tubulysin D (**1e**)	$-CH_2-$		H

Scheme 1. Structures of tubulysins and their components.

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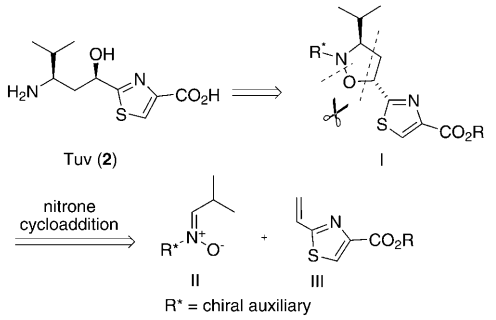
N-positions of **1a** and **1b**.[4] The tubulysins display potent antitumor activity by inhibiting tubulin polymerization and by antiangiogenic factors.[5,6] It is worth noting that the inhibition activity of tubulysin D (**1b**), the most potent derivative, exceeds that of vinblastine, which is used as an antitumor agent in clinical practice.[2,6] Due to their remarkable biological activity and unique structural characteristics, including unusual amino acids, such as Tuv and Tup, tubulysins have attracted considerable attention as synthetic target molecules as well as leads for the development of new anticancer agents, and hence total syntheses and synthetic studies have already been reported.[4b,7–12] However, only a few synthetic methods adaptable to analogue synthesis have been explored, probably due to the lack of stereoselectivity or efficiency for the syntheses of Tuv and Tup.[11] We have recently developed an efficient method for the synthesis of Tuv featuring the stereoselective 1,3-dipolar cycloaddition of a nitrone derived from D-gulose with *N*-acryloyl camphor sultam as well as a synthetic method for Tup by employing a stereoselective aldol reaction followed by Barton deoxygenation.[13] We describe herein efficient total syntheses of tubulysins U (**1c**), V (**1d**), and D (**1b**) by using the above methodologies. Furthermore, as a first step toward understanding the structure-activity relationship of tubulysins, we synthesized *ent*-tubulysin D (*ent*-**1b**) and cyclo-tubulysin D (**1e**), which was reported to be obtained from **1b** by treatment with dilute HCl.[3] Preliminary results regarding their biological activities are also reported.

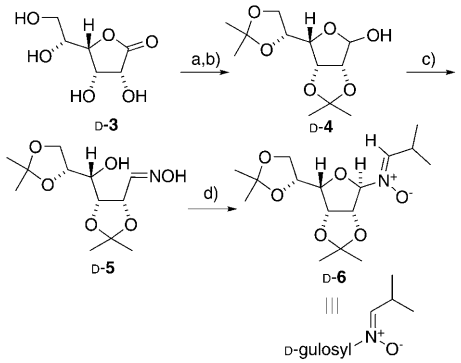
Results and Discussion

Synthesis of tubuvaline (Tuv): Our retrosynthetic analysis (Scheme 2) for Tuv (**2**) pivoted on the use of an 1,3-dipolar



Scheme 2. Synthetic strategy for Tuv (**2**).

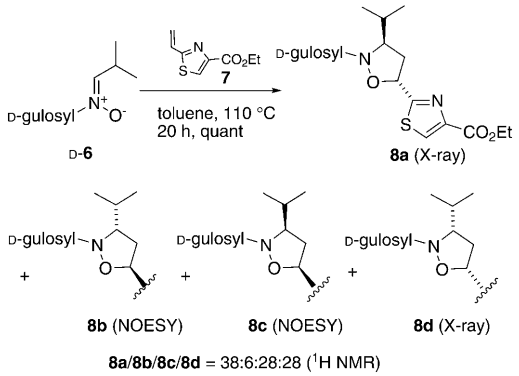
cycloaddition (1,3-DC) of nitrone.[14] Thus, isoxazoline **I**, which may be available by means of an 1,3-DC of nitrone **II** with 2-vinylthiazole **III**, would afford Tuv (**2**) by reductive cleavage of the N–O bond. To realize this plan, it would be very important to choose an appropriate chiral auxiliary R* in **II**, which would need 1) to be available in both enantiomeric forms and 2) to be removed under conditions other than hydrogenolysis due to the existence of the secondary alcohol at the benzylic position in Tuv (**2**). Our choice for the chiral nitrone **II** was gulose-derived nitrone **6** (Scheme 3). Both enantiomers of gulonic acid γ-lactone (**3**) are commercially available and the gulosyl group can be removed under acidic conditions.



Scheme 3. Preparation of chiral nitrone D-**6**: a) $CuSO_4$, conc. H_2SO_4, acetone, 88%; b) DIBAL-H, THF, 98%; c) $NH_2OH{\cdot}HCl$, $NaHCO_3$, EtOH/H_2O; d) isobutyraldehyde, $MgSO_4$, $CHCl_3$, 84% (2 steps). DIBAL-H = diisobutylaluminium hydride.

Our synthetic studies began with the preparation of chiral nitrone D-**6** from commercially available D-gulonic acid γ-lactone (D-**3**) (Scheme 3). Four hydroxyl groups of lactone D-**3** were protected by diacetonide formation followed by the reduction of carbonyl groups with DIBAL-H, leading to lactol D-**4**.[15] The reaction of D-**4** with hydroxylamine hydrochloride in the presence of sodium hydrogen carbonate yielded the corresponding oxime D-**5** as an 1:1 mixture of *E* and *Z* isomers. Without separation of isomers, D-**5** was converted to nitrone D-**6** by condensation with isobutylaldehyde in a satisfactory yield. These successive transformations from D-**3** to D-**6** were conducted on a multigram scale without chromatographic purification. In a similar manner, nitrone L-**6** was prepared from L-gulonic acid γ-lactone (L-**3**).

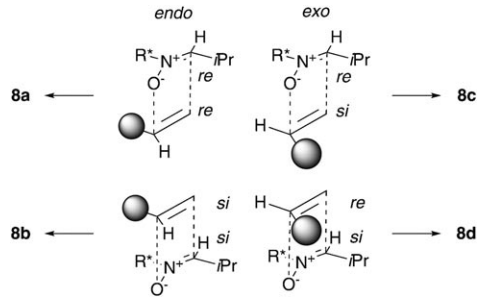
We next examined the crucial 1,3-DC reaction of chiral nitrone D-**6** with vinyl thiazole **7**[16] (Scheme 4). Nitrone D-**6**, on treatment with **7** in refluxing toluene, underwent an 1,3-DC reaction to give a mixture of cycloadducts **8a–d** (**8a**/**8b**/



Scheme 4. 1,3-DC reaction of chiral nitrone D-**6** with vinyl thiazole **7**.

8c/**8d** 38:6:28:28, as determined by ^{1}H NMR spectroscopy). After separation of the mixture, the stereochemistries of **8a** and **8d** were determined by their X-ray crystallographic data, and the stereochemistries of **8b** and **8c** were deducted by their NOESY correlations and the structures **8a** and **8d**.[17] The stereochemistry of cycloadduct **8a** corresponded to that of Tuv.

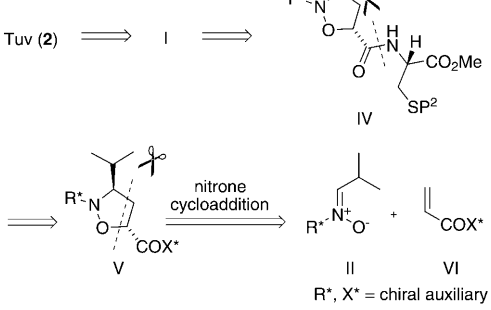
To explain the stereoselection in the 1,3-DC reaction of D-**6** with **7**, we proposed a plausible transition state from these results (Scheme 5). Although nitrone D-**6** exhibits



Scheme 5. Plausible transition states for cycloaddition of D-**6** and **7**.

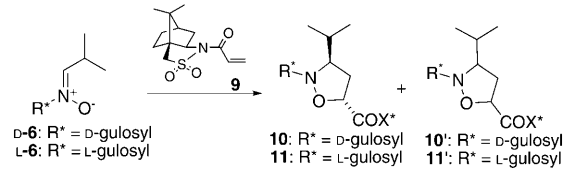
facial selectivity to some extent (**8a**+**8c**/**8b**+**8d** ca. 2:1), *endo*/*exo* selectivity was not observed. Various reaction conditions (solvents, temperature, and additives) were tested to improve stereoselectivity, but they were all unsuccessful.[18] Additionally, separation of cycloadduct **8a** containing the correct stereochemistry for **2** from stereoisomers **8b–d** required several chromatographic purifications. It was, therefore, necessary to explore a more stereoselective 1,3-DC to produce a cycloadduct containing the correct stereochemistry for **2**.

To gain the desired stereoselectivity, the dipolarophile has to react from the *re* face. The low stereoselectivity of the cycloaddition prompted us to consider the use of a dipolarophile with independent facial selectivity. We anticipated obtaining the desired stereochemistry by double asymmetric induction of an 1,3-DC reaction of chiral nitrone **II** (R*= gulosyl) with acrylate **VI** containing the appropriate chiral auxiliary (X*) (Scheme 6). The carbonyl functionality in isoxazoline **V** may provide a good foothold for the construction of the thiazole fragment of **2**. The key compound **I** can be obtained by condensation of **V** with a cysteine derivative, followed by cyclization of the resulting **IV**. Taking into account both availability and crystallinity, we adopted Oppolzer's camphor sultam as the chiral auxiliary X* of acrylate **VI** (see (−)-*N*-acryloyl sultam **9** in Table 1).[19a]



Scheme 6. Revised retrosynthetic plan for Tuv.

Table 1. 1,3-DC of chiral nitrone D-**6** or L-**6** with (−)-**9**.



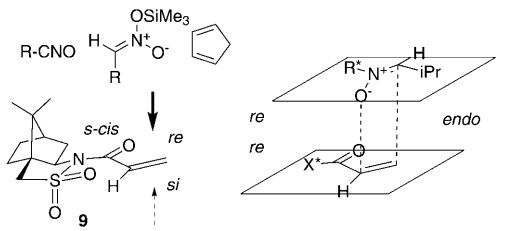
Entry	**6**	Solvent	T [°C]	t [h]	Yield [%]	Diastereomeric ratio[a] **10** or **11**/**10′** or **11′**
1	D	toluene	110	1	quant.	76:24
2	D	EtOH	78	6	quant.	78:22
3	D	toluene	40	24	quant.	83:17
4	D	EtOH	40	48	quant.	81:19
5	D	THF	40	24	quant.	80:20
6	D	CH_3CN	40	48	quant.	79:21
7	D	neat	40	48	quant.	82:18
8	D	CH_2Cl_2	40	48	quant.	85:15
9	L	CH_2Cl_2	40	48	83	23:77[b]

[a] Unless otherwise noted, diastereomeric ratios of **10** to **10′** were determined by the ^{1}H NMR spectra of the mixtures. [b] This sample contained three isomers detected by HPLC.

Heating D-**6** with (−)-**9**[19] in refluxing toluene gave the desired *endo*-cycloadduct **10** in 76% yield along with another stereoisomer **10′** (24%, mixture of isomers) (Table 1, entry 1). The stereochemistry of **10** was precisely determined by X-ray crystallography, which revealed that **10** had the correct stereochemistry for the synthesis of Tuv (**2**).[13,17] To improve the stereoselectivity, various reaction solvents and temperatures were examined next (Table 1, entries 2–8), and the use of refluxing dichloromethane was found to optimize conditions for providing adduct **10** (entry 8). Thus, the reaction of D-**6** with **9** in refluxing dichloromethane for 48 h afforded adduct **10** as pure crystals in 85% isolated yield. It should be noted that the cycloaddition can be conducted on a 60 g-scale without any chromatographic separation (see the Experimental Section).

In contrast, the 1,3-DC reaction of L-**6**, instead of D-**6**, with (−)-**9** gave a mixture of four isomers of cycloadducts (Table 1, entry 9). The separation of mixtures by HPLC revealed that the yield of adduct **11** bearing the correct stereochemistry for Tuv was only 19%.[17] These results clearly showed that the combination of nitrone D-**6** and (−)-**9** represents a matched pair, whereas that of L-**6** and (−)-**9** is a mismatched pair.

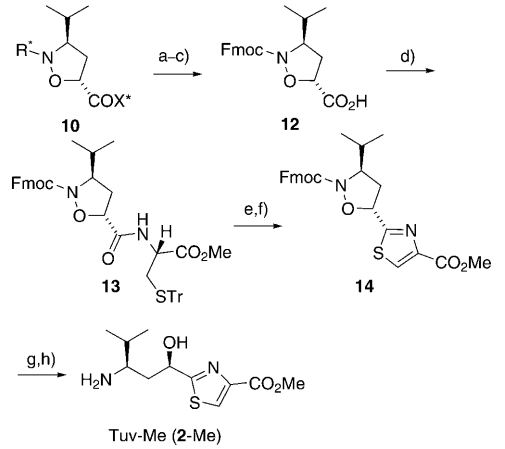
The high stereoselectivity of the 1,3-DC reaction in nitrone D-**6** and (−)-**9** can be explained by considering their transition states (Scheme 7). It is generally accepted that dipoles, such as nitrile oxide, silyl nitronates, and cyclopropanediene, attack the *re* face of (−)-*N*-acryloyl sultam **9**, which



Scheme 7. Facial selectivity of **9** and plausible transition state providing adduct **10**.

has predominantly the (*s*)-*cis* conformation.[19b] Chiral nitrone D-**6** also tends to react with a *re* face. Thus, the combination of D-**6** and **9** caused double asymmetric induction to provide cycloadduct **10** in high yield.

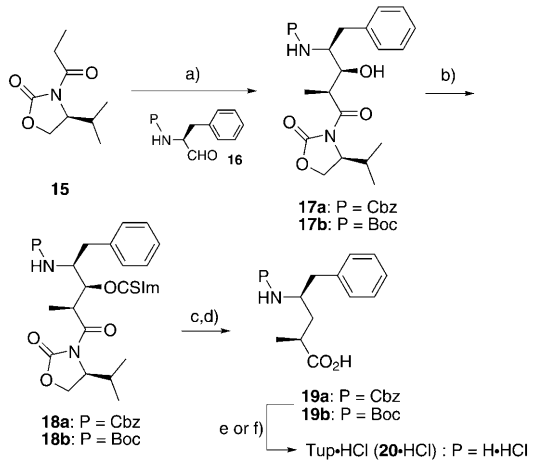
Since cycloadduct **10** already has the correct stereocenters regarded as those in Tuv, we turned our attention to the synthesis of **2** from adduct **10** (Scheme 8). Stepwise removal of



Scheme 8. Synthesis of Tuv-Me (**2**-Me): a) LiOH, THF/H_2O, 89%; b) $HClO_4$ aq., CH_3CN; c) Fmoc-Cl, $NaHCO_3$, dioxane/H_2O, 99% (2 steps); d) L-(*S*)-Tr-cysteine methyl ester, HATU, DIPEA, CH_2Cl_2, 82%; e) $Ph_3P{=}O$, Tf_2O, CH_2Cl_2; f) MnO_2, CH_2Cl_2, 75% (2 steps); g) $Mo(CO)_6$, CH_3CN/H_2O, 89%, h) Et_2NH, CH_3CN, quant. DIPEA = *N*,*N*-Diisopropylethylamine; Fmoc = 9-fluorenylmethoxycarbonyl; HATU = (7-azabenzotriazol-1-yl)tetramethyluronium hexafluorophosphate.

the two chiral auxiliaries in **10** was conducted by employing lithium hydroxide followed by perchloric acid to give (3*R*,5*R*)-3-isopropylisoxazolidine-5-carboxylic acid, of which the nitrogen atom was protected by a Fmoc group to afford carboxylic acid **12** in 88% yield from **10**. The acid **12** was coupled with L-(*S*)-tritylcysteine methyl ester[21] in the presence of HATU and DIPEA, yielding the fully protected cysteine-containing dipeptide **13**. When dipeptide **13** was exposed to bis(triphenyl)oxodiphosphonium trifluoromethanesulfonate,[22] prepared from triphenylphosphin oxide and triflic anhydride, cyclodehydration proceeded smoothly to construct a thiazoline ring, which was then oxidized with activated manganese dioxide to provide thiazole **14**, the so-called *N*,*O*-dehydroTuv derivative, in 75% yield (two steps). Finally, reductive cleavage of the N–O bond of **14** by heating with molybdenum hexacarbonyl in acetonitrile/water (10:1),[23] and the subsequent removal of the Fmoc group by exposure to diethylamine afforded Tuv-Me (**2**-Me) in 91% yield (two steps).

Synthesis of Tup: The stereoselective synthesis of Tup (**20**) was initiated by the Evans aldol reaction of the (*Z*)-boron enolate of (*S*)-4-isopropyl-3-propionyl-2-oxazolidinone (**15**) with *N*-protected L-phenylalaninal **16** affording aldol **17** with high selectivity and good yield (Scheme 9).[24–26] In these re-

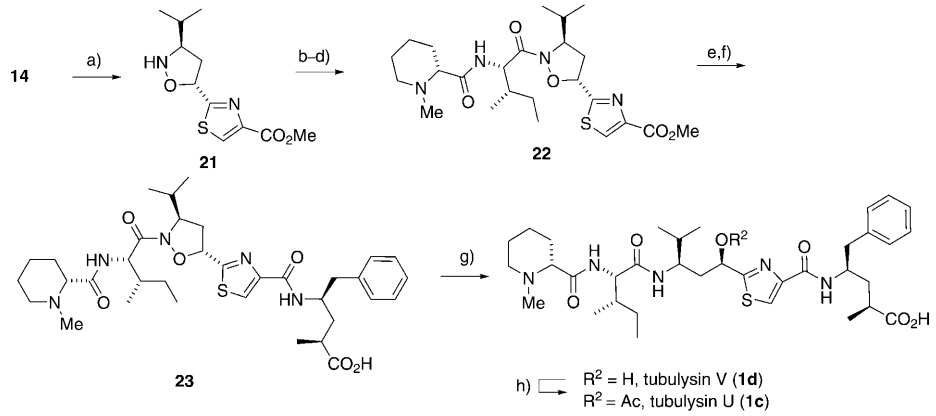


Scheme 9. Synthesis of Tup (**20**): a) Bu_2BOTf, DIPEA, **16**, CH_2Cl_2, then 30% H_2O_2 aq., MeOH, 92% (for **17a**), 77% (for **17b**); b) TCDI, THF, 95% (for **18a**), quant. (for **18b**); c) Bu_3SnH, AIBN, toluene; d) LiOH, 30% H_2O_2 aq., THF/H_2O, 70% (from **18a**), 74% (from **18b**); e) H_2, 10% Pd/C, 4 M HCl/dioxane, THF, 95% (from **19a**), f) 4 M HCl/dioxane, CH_2Cl_2 (from **19b**). AIBN = azobisisobutyronitrile; Boc = *tert*-butoxycarbonyl; Cbz = carbobenzyloxy; TCDI = 1,1′-thiocarbonyldiimidazole.

actions, the diastereomer of **17** was not detected. Leitheiser's group reported that, when *N*-(*tert*-butoxycarbonyl)-D-phenylalaninal was used instead of **16** (P = Boc), the yield of the aldol adduct was poor.[25] So it can be seen that this aldol reaction is again controlled by double asymmetric induction. Next, the secondary hydroxyl group of aldol **17** was removed by the Barton–McCombie procedure.[27] Thus, **17** was converted to thiocarbamate **18** by treatment with TCDI. Subsequent treatment with Bu_3SnH in the presence of a catalytic amount of AIBN and removal of the chiral auxiliary with lithium peroxide provided the *N*-protected Tup (**19**). It should be noted that in the homolytic deoxygenation step, tin residues were effectively removed by using KF-silica.[28] Finally, deprotection of the *N*-protecting group of **19** furnished Tup•HCl (**20**•HCl).[7] With regard to total yield in this synthetic sequence, the Cbz group was slightly better than the Boc group for protecting the nitrogen atom. This synthesis of Tup (**20**) is more stereoselective than reported syntheses to date; hence, Tup (**20**) was readily available in multigram quantities.

Syntheses of tubulysins

Syntheses of tubulysins U and V: With efficient approaches to the two unusual amino acids in tubulysins in hand, we aimed to synthesize tubulysins U (**1c**) and V (**1d**) (Scheme 10). In this synthesis, the isoxazoline ring played an important role as a protecting group for the amine and the hydroxyl groups. Deprotection of the Fmoc group in thiazole **14**, which possessed the correct stereochemistry for tubulysins, by treatment with diethylamine provided *N,O*-dehydroTuv **21** in 84% yield. The condensation reaction of **21** with Boc-L-Ile proceeded smoothly under the usual conditions to afford the dipeptide in 96% yield. Removal of the Boc group in the L-Ile moiety under acidic conditions, followed by coupling with D-Mep,[7] by using DECP and DIPEA,[28] gave tripeptide **22**. Saponification of the methyl ester in **22** and the subsequent condensation with Tup (**20**) afforded tetrapeptide **23**. Synthesis of tubulysin V (**1d**) was accomplished by reductive cleavage of the N–O bond to generate a γ-amido alcohol functionality with molybdenum hexacarbonyl in refluxing acetonitrile/water.[29] Finally, acetylation of the secondary alcohol in the Tuv moiety furnished tubulysin U (**1c**) in 91% yield.

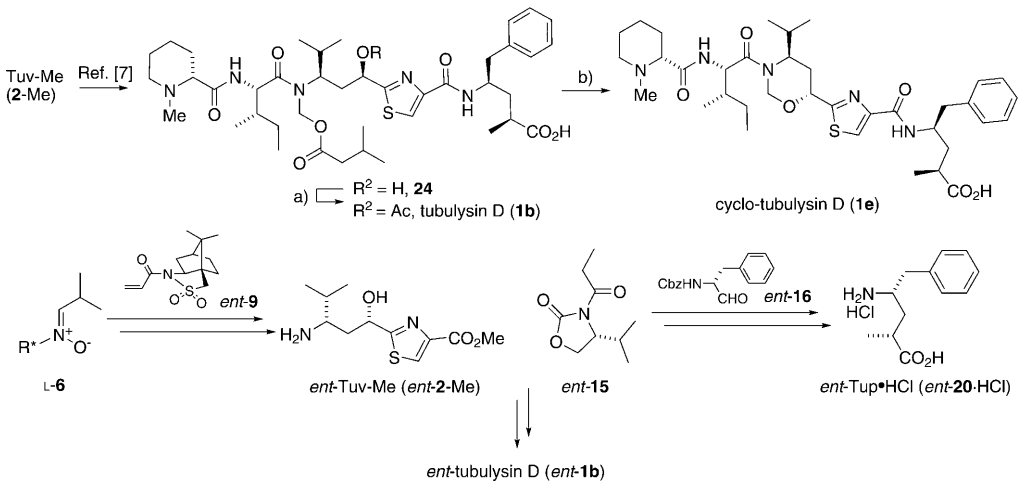


Scheme 10. Synthesis of tubulysins U (**1c**) and V (**1d**): a) Et_2NH, CH_3CN, 84%; b) Boc-L-Ile, HATU, DIPEA, CH_2Cl_2, 96%; c) TFA, CH_2Cl_2, quant.; d) D-Mep, DECP, DIPEA, DMF, 85%; e) LiOH, THF/H_2O, 96%; f) PFP, DIC, CH_2Cl_2 then **20** HCl, DIPEA, DMF, 87%; g) $[Mo(CO)_6]$, CH_3CN/H_2O, 71%; h) Ac_2O, pyridine then H_2O/dioxane, 91%. DECP = diethyl cyanophosphonate; TFA = trifluoroacetic acid.

Syntheses of tubulysin D, cyclotubulysin D, and *ent*-tubulysin D: To synthesize tubulysin D (**1b**) from Tuv-Me (**2**-Me) and Tup (**20**), we adopted a reported synthetic sequence (see Scheme 11 and the Supporting information).[7] Thus, deacetyl tubulysin D (**24**) was converted to tubulysin D (**1b**) by acetylation of the secondary alcohol on Tuv. Furthermore, treatment of **24** with 1 M HCl aq. at 60 °C afforded cyclo-tubulysin D (**1e**) with excellent yield via an intermediate *N*-acyl methylene-iminium ion.[3]

We next synthesized the *ent*-tubulysin D (*ent*-**1b**) to compare its biological activity with that of **1b**. All of the synthetic steps depicted in Schemes 3, 9, and 10 were repeated by starting from the enantiomers. Thus, *ent*-Tuv-Me (*ent*-**2**-Me) was synthesized from L-**6** and *ent*-**9**, whereas *ent*-Tup•HCl (*ent*-**20**•HCl) was prepared from *ent*-**15** and *ent*-**16**. Total synthesis of *ent*-tublysin D (*ent*-**1b**) from *ent*-**2**-Me and *ent*-**20** was achieved.

Biological activities: Biological studies were carried out with synthesized samples of tubulysins U (**1c**), V (**1d**), and D



Scheme 11. Synthesis of tubulysin D (**1b**), cyclo-tubulysin D (**1e**), and *ent*-tubulysin D (*ent*-**1b**): a) Ac_2O, pyridine then H_2O/dioxane, 93%; b) 1 M HCl aq., THF, 97%.

(**1b**), cyclo-tubulysin D (**1e**), and *ent*-tubulysin D (*ent*-**1b**). The antiproliferative activity against HEp-2 cells (human epidermoid carcinoma of the larynx) in vitro was evaluated by using vinblastine as a positive control. The antiproliferative activity of **1b** in HEp-2 cells was shown to be more potent than that of vinblastine; thus the IC_{50} [$ng\,mL^{-1}$] values were 0.21 and 7.0 for vinblastine. The activities of tubulysin U (**1c**) and cyclo-tubulysin D (**1e**) showed the same strength of activity as that of vinblastine (IC_{50} [$ng\,mL^{-1}$]: 1.4 for **1c** and 13.0 for **1e**). On the other hand, **1d** and *ent*-**1b** exhibited much weaker activities (IC_{50} [$ng\,mL^{-1}$]: 730 for **1d** and 520 for *ent*-**1b**). It is noteworthy that *ent*-**1b** is about 2000-fold less active than **1b**, which is the most active compound in tubulysins, in this antiproliferative assay. Here we clarified the antiproliferative activity of **1e** and *ent*-**1b** for the first time.

Conclusion

We established efficient and concise routes to two unusual amino acids, Tuv (**2**) and Tup (**20**), in tubulysins and synthesized tubulysins U (**1c**), V (**1d**), and D (**1b**), cyclo-tubulysin D (**1e**), and *ent*-tubulysin D (*ent*-**1d**). The synthesis of **2**-Me features a stereoselective 1,3-DC reaction of D-**6** with (−)-**9**, whereas synthesis of **20** was conducted by employing a stereoselective aldol reaction and Barton deoxygenation. These stereoselective and scalable methods enabled us to synthesize sufficient quantities of tubulysins for a biological assay. Thus, the total syntheses of **1c** and **1d** were accomplished, in which the isoxazoline ring played an important role as a protecting group for the γ-amido alcohol functionality. For the biological assay, **1b** was also synthesized from Tuv-Me (**2**-Me) and Tup (**20**). The assay clarified the antiproliferative activities of the synthesized tubulysins, especially **1e** and *ent*-**1b**, for the first time. Further biological evaluation, including that of the synthetic tubulysins and their analogues, is currently underway, and the results will be reported in due course.

Experimental Section

General information: All melting points are uncorrected. ^{1}H NMR spectra were measured with a 300 or 400 MHz spectrometer. Measurements of ^{13}C NMR spectra were measured with a 75 or 100 MHz spectrometer. The chemical shifts are expressed in parts per million (δ value) downfield from tetramethylsilane, by using tetramethylsilane ($\delta=0$) and/or residual solvents, such as chloroform ($\delta=7.26$), as an internal standard. Splitting patterns are indicated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad peak). Unless otherwise noted, all the experiments were carried out by using anhydrous solvents under an atmosphere of argon. Throughout this study, Merck precoated TLC plates (Silica gel 60 F_{254}, 0.25 mm) were used for TLC analysis, and all the spots were visualized by using UV light followed by coloring with phosphomolybdic acid or anisaldehyde. Silica gel 60N (40–50 μm, neutral; Kanto Chemical Co., Inc., Tokyo, Japan) and Wakogel 100C18 (63–212 μm, Wako Chemical Co., Inc., Tokyo, Japan) were used for the flash column chromatography. Analytical and preparative HPLC was carried out by using an apparatus equipped with a Hitachi L-7405 UV-detector, a Hitachi L-7100 HPLC pump, a Hitachi D-7500 chromato integrator, a GL Sciences UV702 UV/Vis detector, a GL Sciences PU716 HPLC pump, and a CO705 column oven.

1,3-DC reaction of chiral nitrone D-6 with (2*R*)-*N*-(acryloyl)bornane-10,2-sultams (9): A mixture of D-**6** (45.5 g 138 mmol) and (2*R*)-*N*-(acryloyl)bornane-10,2-sultams (**9**) (33.8 g, 126 mmol) in CH_2Cl_2 (250 mL) was heated under reflux for 48 h. After concentration, the residue was recrystallized from EtOH (300 mL) to give **10** (61.2 g 82% from **9**) as a colorless solid. Flash chromatography (silica gel, hexane/AcOEt 7:3) of the mother liquor gave further **10** (1.13 g, 2%) as a colorless solid. An analytical sample of **10** (colorless crystal) was obtained by further recrystallization from hexane/AcOEt. M.p. 190–192 °C; $[\alpha]_D^{26}=-111$ ($c=1.00$ in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.93$ (d, $J=6.7$ Hz, 3H), 0.95 (d, $J=6.7$ Hz, 3H), 0.98 (s, 3H), 1.19 (s, 3H), 1.26 (s, 3H), 1.32–1.46 (m, 2H), 1.38 (s, 3H), 1.41 (s, 3H), 1.43 (s, 3H), 1.60–1.70 (m, 1H), 1.82–1.98 (m, 3H), 2.08 (dd, $J=8.0$, 14.0 Hz, 1H), 2.22–2.32 (m, 1H), 2.45 (ddd, $J=1.8$, 7.9, 12.8 Hz, 1H), 2.65 (td, $J=7.4$, 13.5 Hz, 1H), 3.44 (d, $J=14.1$ Hz, 1H), 3.47–3.52 (m, 1H), 3.53 (d, $J=14.1$ Hz, 1H), 3.73 (dd, $J=6.7$, 8.6 Hz, 1H), 3.91 (dd, $J=4.9$, 8.0 Hz, 1H), 4.08 (dd, $J=3.6$, 8,7 Hz, 1H), 4.19 (dd, $J=6.7$, 8.6 Hz, 1H), 4.34 (td, $J=6.7$, 8.6 Hz, 1H), 4.64–4.68 (m, 2H), 4.99 (d, $J=6.1$ Hz, 1H), 5.00 ppm (br t, $J=7.9$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=19.7$, 19.9, 20.1, 20.9, 24.9, 25.3, 26.1, 26.5, 26.6, 30.5, 32.5, 32.9, 38.0, 44.7, 47.8, 48.7, 53.1, 65.6, 66.0, 68.3, 75.7, 77.9, 80.3, 83.97, 84.0, 96.6, 109.6, 112.4, 170.6 ppm; IR (ATR): $\tilde{\nu}=1702$, 2958 cm^{-1}; MS (ESI+): *m*/*z*: 599 $[M+H]^+$; HRMS (ESI+): *m*/*z*: calcd for $C_{29}H_{47}N_2O_9S$: 599.3002 $[M+H]^+$; found: 599.3007; elemental analysis calcd (%) for $C_{29}H_{46}N_2O_9S$: C 58.17, H 7.74, N 4.68; found: C 58.06, H 7.59, N 4.57; the stereochemistry of **10** was determined by X-ray crystallography.[13,17]

Compound *ent*-**10** (54.3 g, 84%) was prepared from L-**6** (39.5 g, 120 mmol) with (2*S*)-*N*-(acryloyl)bornane-10,2-sultams (*ent*-**9**) (29.3 g, 109 mmol) in the same manner as described for **10**. Colorless crystal; m.p. 190–191 °C; $[\alpha]_D^{23}=+117$ ($c=1.02$ in $CHCl_3$); MS (ESI+): *m*/*z*: 599 $[M+H]^+$; HRMS (ESI+): *m*/*z*: calcd for $C_{29}H_{47}N_2O_9S$: 599.3002 $[M+H]^+$; found: 599.3005; elemental analysis calcd (%) for $C_{29}H_{46}N_2O_9S$: C 58.17, H 7.74, N 4.68; found: C 58.01, H 7.79, N 4.64; ^{1}H and ^{13}C NMR and IR spectra of this sample were identical to those described for **10**.

(3*R*,5*R*)-2-[(9*H*-Fluoren-9-yl)methoxycarbonyl]-3-isopropylisoxazolidine-5-carboxylic acid (12) and its enantiomer (*ent*-12): A solution of LiOH (12.6 g, 526 mmol) in H_2O (130 mL) at 4 °C was added to a solution of **10** (63.0 g, 105 mmol) in THF (260 mL) and MeOH (130 mL), and the mixture was stirred at the same temperature for 1 h. After concentration, the mixture was poured into H_2O (300 mL) and the pH of the mixture was adjusted to pH 9 with 10% H_2SO_4 aq. The mixture was extracted with AcOEt, then the aqueous layer was adjusted to pH 3 with 10% H_2SO_4 aq. The mixture was extracted with AcOEt, the organic extract was washed with brine, dried over Na_2SO_4, filtered, and then concentrated in vacuo. The residue was triturated with hexane to give (3*R*,5*R*)-2-{(3*aR*,4*R*,6*S*,6*aR*)-6-[(*R*)-2,2-dimethyl-1,3-dioxolan-4-yl]-2,2-dimethyltetrahydrofuro[3,4-*d*,1,3]dioxol-4-yl}-3-isopropylisoxazolidine-5-carboxylic acid (40.2 g 89%) as a colorless solid. This material was used for the next step without further purification. To a solution of this material (5.17 g, 12.9 mmol) in MeCN (64 mL) was added 60% $HClO_4$ aq. (4.31 mL, 25.7 mmol) at 4 °C, and the mixture was stirred at room temperature for 1 h. After concentration, the residue was dissolved in 1,4-dioxane (32 mL), and then a suspension of $NaHCO_3$ (10.8 g, 129 mmol) in H_2O (32 mL) and Fmoc-Cl (4.00 g, 15.4 mmol) was added at 4 °C. The mixture was stirred at room temperature for 21 h. After concentration, the residue was diluted with H_2O and Et_2O and the whole was washed with Et_2O. The aqueous layer was adjusted to pH 3 with 10% H_2SO_4 aq. The mixture was extracted with AcOEt, the organic extract was washed with brine, dried over Na_2SO_4, filtered, and then concentrated in vacuo. The residue was triturated with hexane to give **12** (4.85 g, 99%) as a colorless amorphous solid. $[\alpha]_D^{25}=+57.3$ ($c=0.50$ in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.69$ (d, $J=6.1$ Hz, 3H), 0.71 (d, $J=6.7$ Hz, 3H), 1.53 (oct, $J=6.1$ Hz, 1H), 1.92–2.06 (m, 1H), 2.34 (ddd, $J=3.1$, 9.2, 12.8 Hz, 1H), 3.24–3.40 (m, 1H), 4.17 (br t, $J=4.9$ Hz, 1H), 4.50–4.62 (m, 2H), 4.77 (dd, $J=4.9$, 10.4 Hz, 1H), 7.26–7.34 (m, 2H), 7.39 (dt, $J=2.4$, 7.3 Hz,

2H), 7.50 (d, $J=7.4$ Hz, 1H), 7.53 (d, $J=7.4$ Hz, 1H), 7.71 (d, $J=7.4$ Hz, 1H); 7.73 ppm (d, $J=7.4$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=18.2$, 18.8, 30.8, 34.9, 47.3, 66.1, 67.9, 78.6, 119.85, 119.90, 124.6, 124.8, 127.3, 127.5, 127.8, 128.0, 141.3, 141.4, 142.8, 143.4, 160.2, 173.0 ppm; IR (ATR): $\tilde{\nu}=1708$, 2960 cm^{-1}; MS (CI+): m/z: 382 $[M+H]^+$; HRMS (CI+): m/z: calcd for $C_{22}H_{24}NO_5$: 382.1654 $[M+H]^+$; found: 382.1665. Compound *ent*-**12** (31.9 g, 95%) was prepared from *ent*-**10** (54.2 g, 90.5 mmol) in the same manner as described for **12**. Colorless amorphous solid; $[\alpha]_D^{23}=-58.0$ ($c=0.50$ in MeOH); MS (CI+): m/z: 382 $[M+H]^+$; HRMS (CI+): m/z: calcd for $C_{22}H_{24}NO_5$: 382.1654 $[M+H]^+$; found: 382.1680; ^{1}H and ^{13}C NMR and IR spectra of this sample were identical to those described for **12**.

Methyl (*R*)-2-[(3*R*,5*R*)-2-[(9*H*-fluoren-9-yl)methoxycarbonyl]-3-isopropylisoxazolidine-5-carboxamido]-3-(triphenylmethylthio)propionate (13) and its enantiomer (*ent*-13): iPr$_2$NEt (0.27 mL, 1.56 mmol) and HATU (324 mg, 0.85 mmol) were added to a solution of **12** (270 mg, 0.71 mmol) and L-(*S*)-Tr-cysteine methyl ester hydrochloride[21] (441 mg, 1.07 mmol) in CH_2Cl_2 (7 mL) at 4°C and the mixture was stirred at the same temperature for 30 min. The mixture was allowed to warm to room temperature and stirring was continued for 24 h. The mixture was poured into saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2, dried over Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (silica gel, hexane/AcOEt 1:2) of the residue gave **13** (431 mg, 82%) as colorless amorphous solid. $[\alpha]_D^{25}=+21.4$ ($c=0.50$ in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.87$ (d, $J=6.7$ Hz, 6H), 1.82 (oct, $J=6.7$ Hz, 1H), 2.40–2.53 (m, 3H), 2.73 (dd, $J=7.3$, 12.2 Hz, 1H), 3.61 (s, 3H), 3.82 (brq, $J=6.7$ Hz, 1H), 4.27 (t, $J=6.7$ Hz, 1H), 4.32–4.42 (m, 1H), 4.40–4.52 (m, 2H), 4.62 (dd, $J=6.1$, 7.3 Hz, 1H), 7.10–7.15 (m, 3H), 7.15–7.24 (m, 6H), 7.26–7.35 (m, 8H), 7.39 (t, $J=7.6$ Hz, 2H), 7.56 (d, $J=7.3$ Hz, 1H), 7.60 (d, $J=7.3$ Hz, 1H), 7.76 (d, $J=7.3$ Hz, 2H), 8.14 ppm (d, $J=7.9$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=17.6$, 19.0, 30.9, 33.5, 34.7, 46.9, 51.2, 52.5, 64.3, 67.2, 68.4, 79.7, 119.89, 119.93, 124.9, 125.1, 126.8, 127.09, 127.12, 127.7, 127.8, 127.9, 129.5, 141.3, 141.4, 143.4, 143.9, 144.2, 157.8, 170.3, 170.7 ppm; IR (ATR): $\tilde{\nu}=1682$, 1714, 1736 cm^{-1}; MS (ESI+): m/z: 741 $[M+H]^+$; HRMS (ESI+): m/z: calcd for $C_{45}H_{45}N_2O_6S$: 741.2998 $[M+H]^+$; found: 741.3002.

Compound *ent*-**13** (48.0 g, 89%) was prepared from *ent*-**12** (27.9 g, 73.1 mmol) and D-(*S*)-Tr-cysteine methyl ester hydrochloride (45.4 g, 110 mmol) in the same manner as described for **13**. Colorless amorphous solid; $[\alpha]_D^{25}=-27.1$ ($c=0.50$ in MeOH); MS (ESI+): m/z: 758 $[M+NH_4]^+$; HRMS (ESI+): m/z: calcd for $C_{45}H_{48}N_3O_6S$: 758.3264 $[M+NH_4]^+$; found: 758.3269; ^{1}H and ^{13}C NMR and IR spectra of this sample were identical with those described for **13**.

***N*-Fmoc-*N*,*O*-dehydrotubuvaline methyl ester (14) and its enantiomer (*ent*-14)**: Tf_2O (20.3 mL, 121 mmol) was added to a solution of $Ph_3P{=}O$ (42.6 g, 57.5 mmol) in CH_2Cl_2 (400 mL) at −10°C and the mixture was stirred at the same temperature for 1 h. A solution of **13** (42.6 g, 57.5 mmol) in CH_2Cl_2 (174 mL) was added to the reaction mixture at −10°C and the mixture was stirred at room temperature for 6 h. The mixture was added to saturated aqueous $NaHCO_3$ solution at 4°C and was extracted with CH_2Cl_2. The organic extract was washed with brine, dried over Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (silica gel, hexane/AcOEt 3:2) of the residue gave the corresponding thiazolidine derivative as a yellow amorphous solid. MnO_2 (58.0 g, 667 mmol) was added to a solution of this material in CH_2Cl_2 (450 mL) and the mixture was stirred at room temperature for 24 h. The mixture was filtrated through a pad of Celite and the filtrate was concentrated in vacuo. Flash chromatography (silica gel, hexane/AcOEt 3:2) of the residue gave **14** (20.5 g, 75%) as a colorless amorphous solid. $[\alpha]_D^{24}=+12.5$ ($c=0.50$ in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.92$ (d, $J=7.3$ Hz, 6H), 1.89 (oct, $J=7.3$ Hz, 1H), 2.60 (ddd, $J=5.5$, 7.9, 13.3 Hz, 1H), 2.79 (ddd, $J=4.2$, 8.5, 13.3 Hz, 1H), 3.88 (s, 3H), 4.07 (dt, $J=5.5$, 7.3 Hz, 1H), 4.16 (t, $J=6.1$ Hz, 1H), 4.34 (dd, $J=6.7$, 10.9 Hz, 1H), 4.50 (dd, $J=6.7$, 10.3 Hz, 1H), 5.51 (dd, $J=4.2$, 7.9 Hz, 1H), 7.23–7.33 (m, 2H), 7.37 (t, $J=8.6$ Hz, 1H), 7.39 (t, $J=7.9$ Hz, 1H), 7.56 (d, $J=7.9$ Hz, 1H), 7.59 (d, $J=7.3$ Hz, 1H), 7.73 (d, $J=7.4$ Hz, 1H), 7.75 (d, $J=7.9$ Hz, 1H), 8.09 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=17.6$, 19.1, 31.4, 36.6, 47.0, 52.3, 64.5, 67.6, 79.1, 119.83, 119.85, 125.0, 127.1, 127.7, 128.9, 141.24, 141.26, 143.4, 143.6, 146.6, 157.2, 161.4, 170.1 ppm; IR (ATR): $\tilde{\nu}=1717$, 2957 cm^{-1}; MS (ESI+): m/z: 479 $[M+H]^+$; HRMS (ESI+): m/z: calcd for $C_{26}H_{27}N_2O_5S$: 479.1641 $[M+H]^+$; found: 479.1647.

Compound *ent*-**14** (2.61 g, 64%) was prepared from *ent*-**13** (6.33 g, 8.54 mmol) in the same manner as described for **14**. Colorless amorphous solid; $[\alpha]_D^{25}=-13.0$ ($c=0.51$ in MeOH); MS (ESI+): m/z: 479 $[M+H]^+$; HRMS (ESI+): m/z: calcd for $C_{26}H_{27}N_2O_5S$: 479.1641 $[M+H]^+$; found: 479.1638. ^{1}H and ^{13}C NMR and IR spectra of this sample were identical with those described for **14**.

***N*-Fmoc-tubuvaline methyl ester (*N*-Fmoc-Tuv-Me) and its enantiomer (*ent*-*N*-Fmoc-Tuv-Me)**: $[Mo(CO)_6]$ (82.8 mg, 0.31 mmol) was added to a solution of **14** (100 mg, 0.21 mmol) in MeCN (2 mL) and H_2O (0.2 mL) and the mixture was stirred at 70°C for 16 h. After concentration, the residue was diluted with AcOEt (5 mL) and a 10% aq. solution of citric acid (5 mL). $NaIO_4$ was added to the mixture until the aqueous layer became clear, and the whole was extracted with AcOEt. The organic extract was washed with a 10% aq. solution of NaS_2O_3 and brine, dried over Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (silica gel, hexane/AcOEt 3:2) of the residue gave *N*-Fmoc-Tuv-Me (91.1 mg, 91%) as a colorless solid. An analytical sample of this material (colorless crystal) was obtained by recrystallization from hexane/AcOEt. M.p. 127–128°C; $[\alpha]_D^{25}=-19.8$ ($c=0.50$ in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=0.93$ (d, $J=6.7$ Hz, 3H), 0.94 (d, $J=6.7$ Hz, 3H), 1.67–1.83 (m, 2H), 2.09 (ddd, $J=2.4$, 11.7, 14.1 Hz, 1H), 3.69–3.79 (m, 1H), 3.95 (s, 3H), 4.19 (t, $J=6.1$ Hz, 1H), 4.46 (dd, $J=6.1$, 10.4 Hz, 1H), 4.60–4.72 (m, 3H), 4.78 (d, $J=4.9$ Hz, 1H), 7.24–7.30 (m, 2H), 7.34 (dd, $J=1.2$, 7.3 Hz, 1H), 7.40 (brt, $J=7.3$ Hz, 1H), 7.55–7.61 (m, 1H), 7.75 (d, $J=7.3$ Hz, 1H), 8.15 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=18.3$, 19.4, 32.2, 41.2, 47.5, 52.4, 53.1, 66.6, 68.8, 120.0, 120.1, 124.7, 124.9, 127.0, 127.1, 127.6, 127.69, 127.74, 141.37, 141.41, 143.3, 143.7, 146.5, 158.2, 162.0, 176.4 ppm; IR (ATR): $\tilde{\nu}=1523$, 1715, 2953, 3342 cm^{-1}; MS (ESI+): m/z: 481 $[M+H]^+$; HRMS (ESI+): m/z: calcd for $C_{26}H_{29}N_2O_5S$: 481.1797 $[M+H]^+$; found: 481.1793; elemental analysis calcd (%) for $C_{26}H_{28}N_2O_5S$: C 64.98, H 5.87, N 5.83; found: C 64.66, H 5.86, N 5.77.

Compound *ent*-*N*-Fmoc-Tuv-Me (13.3 g, 86%) was prepared from *ent*-**14** (15.5 g, 32.4 mmol) in the same manner as described for *N*-Fmoc-Tuv-Me. Colorless crystal; m.p. 128–130°C; $[\alpha]_D^{25}=+17.5$ ($c=0.50$ in MeOH); MS (ESI+): m/z: 481 $[M+H]^+$; HRMS (ESI+): m/z: calcd for $C_{26}H_{29}N_2O_5S$: 481.1797 $[M+H]^+$; found: 481.1793; elemental analysis calcd (%) for $C_{26}H_{28}N_2O_5S$: C 64.98, H 5.87, N 5.83; found: C 64.69, H 5.82, N 5.75; ^{1}H and ^{13}C NMR and IR spectra of this sample were identical with those described for *N*-Fmoc-Tuv-Me.

Tubuvalin methyl ester (2-Me) and its enantiomer (*ent*-2-Me): Et_2NH (0.65 mL, 6.24 mmol) was added to a solution of *N*-Fmoc-Tuv-Me (100 mg, 0.21 mmol) in MeCN (0.7 mL) and the mixture was stirred at room temperature for 2 h. After concentration, flash chromatography (silica gel, CH_2Cl_2/MeOH/NH_4OH 50:5:1) of the residue gave **2**-Me (54.3 mg, quant) as a colorless solid. An analytical sample of **2**-Me (colorless solid) was obtained by recrystallization from EtOH. M.p. 158–160°C; $[\alpha]_D^{26}=+73.2$ ($c=0.50$ in DMSO); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=0.78$ (d, $J=6.1$ Hz, 3H), 0.81 (d, $J=6.1$ Hz, 3H), 1.42–1.54 (m, 1H), 1.64–1.72 (m, 2H), 2.54–2.62 (m, 1H), 3.80 (s, 3H), 5.07 (brt, $J=6.1$ Hz, 1H), 8.42 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=17.6$, 18.8, 34.0, 40.9, 51.9, 52.5, 69.0, 128.6, 145.7, 161.4, 180.0 ppm. IR (ATR): $\tilde{\nu}=1704$, 2950, 3346 cm^{-1}; MS (ESI+): m/z: 259 $[M+H]^+$; HRMS (ESI+): m/z: calcd for $C_{11}H_{19}N_2O_3S$: 259.1116 $[M+H]^+$; found: 259.1120; elemental analysis calcd (%) for $C_{11}H_{18}N_2O_3S{\cdot}0.1\,H_2O$: C 50.79, H 7.05, N 10.77; found: C 50.66, H 6.81, N 10.67.

Compound *ent*-Tuv-Me (*ent*-**2**-Me, 2.45 g, 95%) was prepared from *ent*-*N*-Fmoc-Tuv-Me (4.81 g, 10.0 mmol) in the same manner as described for **2**-Me. Colorless solid; m.p. 168–169°C; $[\alpha]_D^{24}=-67.1$ ($c=0.50$ in DMSO); MS (ESI+): m/z: 259 $[M+H]^+$; HRMS (ESI+): m/z: calcd for $C_{11}H_{19}N_2O_3S$: 259.1116 $[M+H]^+$; found: 259.1114; elemental analysis calcd (%) for $C_{11}H_{18}N_2O_3S$: C 51.14, H 7.02, N 10.84; found: C 51.06, H 6.95, N 10.80; ^{1}H and ^{13}C NMR and IR spectra of this sample were identical with those described for **2**-Me.

Benzyl (2*S*,3*S*,4*S*)-3-hydroxy-5-[(*S*)-4-isopropyl-2-oxooxazolidin-3-yl]-4-methyl-5-oxo-1-phenylpentan-2-ylcarbamate (17a) and its enantiomer

(*ent*-17a): Bu_2BOTf (1 M in CH_2Cl_2, 46.2 mL, 46.2 mmol) and iPr_2NEt (8.78 mL, 50.4 mmol) were added to a solution of (*S*)-4-isopropyl-3-propionyloxazolidin-2-one (**15**) (7.78 g, 42.0 mmol) in CH_2Cl_2 (44 mL) at 4 °C and the mixture was stirred at the same temperature for 45 min. After cooling to −78 °C, a solution of *N*-(benzyloxycarbonyl)-L-phenylalaninal **16a**[26a] (13.1 g, 46.2 mmol) in CH_2Cl_2 (40 mL) was added at the same temperature and the solution was allowed to slowly warm to room temperature for 17 h. The mixture was poured into 80 mL of potassium phosphate buffer (pH 7.0) and the mixture was extracted with CH_2Cl_2. The organic extract was washed with brine, dried over Na_2SO_4, filtered, and then concentrated in vacuo. The residue was dissolved in MeOH (330 mL) and cooled to 4 °C, then 30% H_2O_2 aqueous solution (99 mL) was added slowly and the mixture was stirred at the same temperature for 4 h. After addition of H_2O (100 mL), the mixture was concentrated in vacuo to remove MeOH. The resulting aqueous solution was extracted with AcOEt and the organic extract was washed successively with 5% $NaHCO_3$ solution and brine, dried over Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (silica gel, hexane/AcOEt 2:1) of the residue gave **17a** as a colorless amorphous solid (18.2 g, 92%). $[\alpha]_D^{23}=+24.9$ ($c=0.50$ in MeOH); ^1H NMR (400 MHz, $CDCl_3$): $\delta=0.87$ (d, $J=7.3$ Hz, 3H), 0.91 (d, $J=6.7$ Hz, 3H), 1.31 (d, $J=6.7$ Hz, 3H), 2.06–2.14 (m, 1H), 2.20–2.30 (m, 1H), 2.88 (d, $J=7.3$ Hz, 2H), 3.84–3.96 (m, 2H), 3.95 (br q, $J=7.6$ Hz, 1H), 4.15 (dd, $J=1.8$, 8.6 Hz, 1H), 4.36 (t, $J=8.6$ Hz, 1H), 4.46 (ddd, $J=2.4$, 6.7, 7.9 Hz, 1H), 4.94 (d, $J=12.2$ Hz, 1H), 4.99 (br s, 1H), 5.02 (d, $J=11.6$ Hz, 1H), 7.16–7.24 (m, 3H), 7.24–7.36 ppm (m, 7H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=15.0$, 15.2, 17.9, 29.1, 38.8, 40.8, 53.7, 58.5, 63.9, 66.9, 73.4, 126.5, 127.97, 128.0, 128.46, 128.53, 129.1, 136.4, 137.8, 154.0, 156.3, 175.8 ppm; IR (ATR): $\tilde{\nu}=1514$, 1689, 1774 cm^{-1}; MS (ESI+): *m/z*: 469 $[M+H]^+$; HRMS (ESI+): *m/z*: calcd for $C_{26}H_{33}N_2O_6$: 469.2339 $[M+H]^+$; found: 469.2343.

Compound *ent*-**17a** (14.8 g, 89%) was prepared from (*R*)-4-isopropyl-3-propionyloxazolidin-2-one (*ent*-**15**) (6.53 g, 35.3 mmol) and *N*-(benzyloxycarbonyl)-D-phenylalaninal (11.0 g, 38.8 mmol) in the same manner as described for **17a**. Colorless amorphous solid; $[\alpha]_D^{24}=-21.8$ ($c=0.50$ in MeOH); MS (ESI+): *m/z*: 469 $[M+H]^+$; HRMS (ESI+): *m/z*: calcd for $C_{26}H_{33}N_2O_6$: 469.2339 $[M+H]^+$; found: 469.2336; ^1H and ^{13}C NMR and IR spectra of this sample were identical with those described for **17a**.

***O*-(2*S*,3*S*,4*S*)-1-[(*S*)-4-Isopropyl-2-oxooxazolidin-3-yl]-2-methyl-1-oxo-5-phenyl-4-(phosphinylideneamino)pentan-3-yl 1*H*-imidazole-1-carbothioate (18a) and its enantiomer (*ent*-18a)**: TCDI (1.71 g, 9.58 mmol) was added to a solution of **17a** (1.50 g, 3.19 mmol) in THF (30 mL) and the mixture was heated under reflux for 18 h. After consumption of **17a** by monitoring on TLC, the mixture was concentrated in vacuo. Flash chromatography (silica gel, hexane/AcOEt 3:1) of the residue gave **18a** (1.75 g, 95%.) as a yellow amorphous solid. $[\alpha]_D^{25}=-37.6$ ($c=0.50$ in MeOH); ^1H NMR (400 MHz, $CDCl_3$): $\delta=0.90$ (d, $J=6.7$ Hz, 3H), 0.93 (d, $J=6.7$ Hz, 3H), 1.31 (d, $J=7.3$ Hz, 3H), 2.22–2.34 (m, 1H), 2.56 (dd, $J=10.4$, 14.7 Hz, 1H), 3.06 (dd, $J=4.3$, 14.7 Hz, 1H), 4.19 (dd, $J=3.0$, 9.2 Hz, 1H), 4.32–4.44 (m, 2H), 4.44–4.56 (m, 2H), 4.80 (br d, $J=9.8$ Hz, 1H), 4.87 (d, $J=12.2$ Hz, 1H), 4.99 (d, $J=12.2$ Hz, 1H), 6.38 (dd, $J=2.4$, 9.8 Hz, 1H), 7.06–7.34 (m, 10H), 7.64 (br s, 1H), 8.39 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=14.5$, 15.4, 17.9, 29.3, 38.3, 39.6, 53.2, 58.6, 64.3, 67.2, 85.5, 117.8, 126.8, 127.9, 128.2, 128.5, 128.6, 128.8, 131.2, 135.9, 136.4, 137.2, 154.1, 156.6, 173.8, 184.3 ppm; IR (ATR): $\tilde{\nu}=1220$, 1385, 1693, 1777 cm^{-1}; MS (ESI+): *m/z*: 579 $[M+H]^+$; HRMS (ESI+): *m/z*: calcd for $C_{30}H_{35}N_4O_6S$: 579.2277 $[M+H]^+$; found: 579.2270.

Compound *ent*-**18a** (16.3 g, 90%) was prepared from *ent*-**17a** (14.7 g, 31.4 mmol) in the same manner as described for **18a**. Yellow amorphous solid; $[\alpha]_D^{25}=+44.0$ ($c=0.50$ in MeOH); MS (ESI+): *m/z*: 579 $[M+H]^+$; HRMS (ESI+): *m/z*: calcd for $C_{30}H_{35}N_4O_6S$: 579.2277 $[M+H]^+$; found: 579.2279; ^1H and ^{13}C NMR and IR spectra of this sample were identical with those described for **18a**.

***N*-Cbz-tubuphenylalanine (19a) and its enatiomer (*ent*-19a)**: nBu_3SnH (1.61 mL, 5.98 mmol) and AIBN (3.2 mg, 29.9 μM) were added to a solution of **18a** (1.73 g, 2.99 mmol) in toluene (30 mL) and the mixture was heated under reflux for 30 min. After consumption of **18a** by monitoring on TLC, the mixture was concentrated in vacuo. Flash chromatography (silica gel containing 10% w/w KF,[30] hexane/AcOEt 4:1) of the residue gave benzyl (2*R*,4*S*)-5-[(*S*)-4-isopropyl-2-oxooxazolidin-3-yl]-4-methyl-5-oxo-1-phenylpentan-2-ylcarbamate (1.08 g, 80%) as a colorless oil. $[\alpha]_D^{26}=+64.9$ ($c=0.50$ in MeOH); ^1H NMR (400 MHz, $CDCl_3$): $\delta=0.88$ (d, $J=6.7$ Hz, 3H), 0.91 (d, $J=6.7$ Hz, 3H), 1.20 (d, $J=6.7$ Hz, 3H), 1.27 (ddd, $J=3.1$, 11.6, 14.1 Hz, 1H), 2.12 (ddd, $J=3.1$, 11.6, 14.1 Hz, 1H), 2.21–2.33 (m, 1H), 2.68 (dd, $J=7.3$, 14.1 Hz, 1H), 2.83 (dd, $J=6.3$, 14.1 Hz, 1H), 3.84–4.02 (m, 2H), 4.15 (dd, $J=2.4$, 8.6 Hz, 1H), 4.36 (t, $J=8.6$ Hz, 1H), 4.44 (br d, $J=10.4$ Hz, 1H), 4.45–4.53 (m, 1H), 4.94 (d, $J=12.2$ Hz, 1H), 5.01 (d, $J=12.2$ Hz, 1H), 7.12–7.18 (m, 2H), 7.18–7.24 (m, 1H), 7.24–7.36 ppm (m, 7H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=15.3$, 17.9, 19.0, 29.2, 34.1, 39.3, 41.9, 49.6, 58.6, 63.9, 66.8, 126.5, 128.0, 128.1, 128.5, 129.3, 136.5, 137.4, 154.2, 156.4, 176.8 ppm; IR (ATR): $\tilde{\nu}=1202$, 1264, 1385, 1686, 1770 cm^{-1}; MS (ESI+): *m/z*: 453 $[M+H]^+$; HRMS (ESI+): *m/z*: calcd for $C_{26}H_{33}N_2O_5$: 453.2390 $[M+H]^+$; found: 453.2384.

An aqueous solution of LiOH (1.11 g, 46.4 mol) and 30% H_2O_2 (4.26 mL, 139 mmol) were added to a solution of the deoxygenated compound obtained above (10.5 g, 23.2 mmol) in THF (180 mL) and H_2O (60 mL) at 4 °C and the mixture was stirred at the same temperature for 3 h. The reaction mixture was poured into a 1.5 M aqueous solution of Na_2SO_3 and was adjusted to pH 3 with 2 M HCl aq. at 4 °C. The resulting aqueous solution was extracted with CH_2Cl_2 and the organic extract was washed brine, dried over Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (silica gel, hexane/AcOEt 2:3) of the residue gave **19a** (6.90 g, 87%) as a colorless solid. An analytical sample of **19a** (colorless solid) was obtained by recrystallization from hexane/AcOEt. M.p. 88–89 °C; $[\alpha]_D^{26}=+21.5$ ($c=0.50$ in $CHCl_3$); ^1H NMR (400 MHz, [D$_6$]DMSO): $\delta=1.04$ (d, $J=6.7$ Hz, 3H), 1.35 (ddd, $J=5.5$, 9.2, 13.5 Hz, 1H), 1.74 (ddd, $J=4.3$, 9.8, 14.1 Hz, 1H), 2.35–2.47 (m, 1H), 2.59–2.70 (m, 1H), 2.63 (d, $J=6.7$ Hz, 1H), 3.62–3.76 (m, 1H), 4.92 (d, $J=12.8$ Hz, 1H), 4.98 (d, $J=12.2$ Hz, 1H), 7.10–7.20 (m, 4H), 7.20–7.36 (m, 6H), 12.0 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=18.0$, 35.9, 38.0, 41.0, 50.7, 64.8, 125.9, 127.4, 127.6, 128.0, 128.3, 129.1, 137.4, 138.7, 155.6, 177.0 ppm; IR (ATR): $\tilde{\nu}=1263$, 1536, 1687, 3322 cm^{-1}; MS (ESI+): *m/z*: 342 $[M+H]^+$; HRMS (ESI$^+$): *m/z*: calcd for $C_{20}H_{24}NO_4$: 342.1705 $[M+H]^+$; found: 342.1705; elemental analysis calcd (%) for $C_{20}H_{23}NO_4$: C 70.36, H 6.79, N 4.10; found: C 70.29, H 6.74, N 4.08.

Compound *ent*-**19a** (6.96 g, 74% from *ent*-**18a**) was prepared from *ent*-**18a** (15.9 g, 27.5 mmol) in the same manner as described for **19a**. Colorless solid; m.p. 88–89 °C; $[\alpha]_D^{28}=-16.8$ ($c=0.50$ in $CHCl_3$); MS (ESI+): *m/z*: 342 $[M+H]^+$; HRMS (ESI+): *m/z*: calcd for $C_{20}H_{24}NO_4$: 342.1705 $[M+H]^+$; found: 342.1698; elemental analysis calcd (%) for $C_{20}H_{23}NO_4$: C 70.36, H 6.79, N 4.10; found: C 70.24, H 6.82, N 4.08; ^1H and ^{13}C NMR and IR spectra of this sample were identical with those described for **19a**.

Tubuphenylalanine hydrochloride (20·HCl) and its enantiomer (*ent*-20·HCl): 10% Pd/C (200 mg) and 4 M HCl/dioxane solution (14.6 mL, 58.6 mmol) were added to a solution of **19a** (2.00 g, 5.86 mmol) in THF (15 mL) and the mixture was stirred at room temperature under a hydrogen atmosphere for 5 h. The mixture was filtrated through a pad of Celite and then concentrated in vacuo. The residue was triturated with Et_2O to give **20**·HCl salt (1.35 g, 95%) as a colorless solid. M.p. 140–141 °C; $[\alpha]_D^{24}=+6.4$ ($c=1.00$ in MeOH) (lit.:[7] $[\alpha]_D^{25}=+3.2$ ($c=1.00$ in MeOH)); ^1H NMR (400 MHz, D_2O, internal standard [D$_4$]3-(trimethylsilyl)propionic acid sodium salt, [D$_4$]TSP): $\delta=1.19$ (d, $J=6.7$ Hz, 3H), 1.74 (td, $J=6.7$, 14.7 Hz, 1H), 2.04 (ddd, $J=6.1$, 8.6, 14.7 Hz, 1H), 2.70 (td, $J=6.7$, 8.6 Hz, 1H), 2.92 (dd, $J=7.9$, 14.1 Hz, 1H), 3.04 (dd, $J=6.7$, 14.1 Hz, 1H), 3.61 (br quin, $J=6.7$ Hz, 1H), 7.29–7.46 ppm (m, 5H); ^{13}C NMR (100 MHz, D_2O, internal standard [D$_4$]TSP): $\delta=19.7$, 38.4, 38.8, 41.3, 54.2, 130.5, 132.1, 132.4, 138.4, 183.1 ppm; IR (ATR): $\tilde{\nu}=1580$, 1711, 2965 cm^{-1}; MS (CI+): *m/z*: 208 $[M+H]^+$; HRMS (CI+): *m/z*: calcd for $C_{12}H_{18}NO_2$: 208.1338 $[M+H]^+$; found: 208.1361; elemental analysis calcd (%) for $C_{12}H_{17}NO_2$·HCl·0.25 H_2O: C 58.06, H 7.51, N 5.64; found: C 57.92, H 7.30, N 5.52.

ent-Tubuphenylalanine hydrochloride (*ent*-**20**·HCl, 1.27 g, 89%) was prepared from *ent*-**19a** (2.0 g, 5.86 mmol) in the same manner as described for **20**·HCl. Colorless solid; m.p. 143–144 °C; $[\alpha]_D^{25}=-4.8$ ($c=1.00$ in MeOH); MS (ESI−): *m/z*: 206 $[M-H]^-$; HRMS (ESI−): *m/z*: calcd for $C_{12}H_{16}NO_2$: 206.1181 $[M-H]^-$; found: 206.1189; elemental analysis calcd

(%) for $C_{12}H_{17}NO_2$·HCl·0.25 H_2O: C 58.06, H 7.51, N 5.64; found: C 57.96, H 7.23, N 5.60; ^{1}H and ^{13}C NMR and IR spectra of this sample were identical with those described for **20**·HCl.

***N,O*-Dehydrotubuvaline methyl ester (21)**: Et_2NH (10.5 mL, 101 mmol) was added to a solution of **14** (1.62 g, 3.38 mmol) in MeCN (11 mL) at 4 °C and the mixture was stirred at room temperature for 2 h. After concentration, Et_2O (20 mL) and 1 M HCl aq. (20 mL) were added to the residue and the mixture was stirred at room temperature for 15 min. After separation, the aqueous layer was washed with benzene and the pH adjusted to 9 with $NaHCO_3$. The resulting aqueous solution was extracted with AcOEt, and the organic extract was washed brine, dried over Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (silica gel, hexane/AcOEt 2:3) of the residue gave **21** (729 mg, 84 %) as a colorless solid. An analytical sample of **21** (colorless solid) was obtained by recrystallization from *i*Pr_2O. M.p. 117–118 °C; $[\alpha]_D^{24} = +9.6$ ($c = 1.05$ in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.98$ (d, $J = 6.7$ Hz, 3H), 1.04 (d, $J = 6.7$ Hz, 3H), 1.68 (broct, $J = 6.8$ Hz, 1H), 2.28–2.51 (brs, 1H), 2.58–2.76 (brs, 1H), 3.18 (brq, $J = 7.6$ Hz, 1H), 3.95 (s, 3H), 5.44 (dd, $J =$ 3.1, 9.2 Hz, 1H), 5.45–5.80 (brs, 1H), 8.17 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 20.0$, 20.6, 31.1, 52.5, 66.5, 80.3, 128.1, 147.1, 161.8, 173.9 ppm; IR (ATR): $\tilde{\nu} = 1238$, 1710 cm^{-1}; MS (EI+): *m/z*: 256 $[M]^+$; HRMS (EI+): *m/z*: calcd for $C_{11}H_{16}N_2O_3S$: 256.0882 $[M]^+$; found: 256.0911; elemental analysis calcd for $C_{11}H_{16}N_2O_3S$: C 51.54, H 6.29, N 10.93; found: C 51.19, H 6.12, N 10.87.

***N*-Methyl-D-pipecorinyl-L-isoleucinyl-*N,O*-dehydrotubuvaline methyl ester (22)**: HATU (7.79 g, 20.5 mmol) and *i*Pr_2NEt (3.57 mL, 20.5 mmol) were added to a solution of **21** (3.50 g, 13.6 mmol) and Boc-L-Ile (4.74 g, 20.5 mmol) in CH_2Cl_2 (68 mL) at 4 °C and the mixture was stirred at the same temperature for 30 min. The resulting mixture was allowed to warm to room temperature and stirring was continued for 74 h. The reaction mixture was poured into saturated aqueous NH_4Cl solution. After separation, the aqueous layer was extracted with AcOEt. The combined organic extracts were washed with brine, dried over Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (silica gel, hexane/AcOEt 3:2) of the residue gave *N*-Boc-L-isoleucinyl-*N,O*-dehydrotubuvaline methyl ester (6.16 g, 96 %) as a colorless oil. $[\alpha]_D^{24} = +39.5$ ($c = 0.50$ in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.74$–0.88 (m, 6H), 0.96 (d, $J =$ 7.3 Hz, 3H), 0.98 (d, $J = 7.3$ Hz, 3H), 1.00–1.12 (m, 1H), 1.41 (brs, 9H), 1.40–1.62 (m, 2H), 2.28–2.46 (m, 1H), 2.66–2.80 (m, 2H), 3.95 (s, 3H), 4.38–4.62 (m, 2H), 4.96–5.08 (m, 1H), 5.60 (t, $J = 6.7$ Hz, 1H), 8.21 ppm (brs, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 11.2$, 15.7, 16.6, 19.1, 23.8, 28.2, 29.7, 35.2, 36.9, 52.6, 55.7, 62.2, 78.3, 79.5, 128.7, 146.9, 155.6, 161.4, 168.1, 168.7 ppm; IR (ATR): $\tilde{\nu} = 1164$, 1215, 1242, 1455, 1498, 1641, 1711, 2964 cm^{-1}; MS (ESI+): *m/z*: 470 $[M+H]^+$; HRMS (ESI+): *m/z*: calcd for $C_{22}H_{36}N_3O_6S$: 470.2325 $[M+H]^+$; found: 470.2327.

TFA (11 mL) was added to a solution of this material (6.04 g, 12.9 mmol) in CH_2Cl_2 (53 mL) at 4 °C and the mixture was stirred at the same temperature for 1 h. After this time, the mixture was allowed to warm to room temperature. After further stirring for 1.5 h, the mixture was concentrated and the residue was partitioned between CH_2Cl_2 and saturated aqueous $NaHCO_3$ solution. The organic extract was dried over Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (silica gel, AcOEt/MeOH 15:1) of the residue gave L-isoleucinyl-*N,O*-dehydrotubuvaline methyl ester (4.74 g, quant.) as a colorless oil. $[\alpha]_D^{25} = +57.3$ ($c = 0.51$ in MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.80$ (d, $J = 6.7$ Hz, 3H), 0.81 (t, $J = 6.7$ Hz, 3H), 0.97 (d, $J = 7.4$ Hz, 3H), 0.99 (d, $J = 7.3$ Hz, 3H), 1.02–1.16 (m, 1H), 1.48–1.60 (m, 2H), 1.60–1.66 (m, 2H), 2.26–2.42 (m, 1H), 2.71 (brt, $J = 6.7$ Hz, 2H), 3.40–3.62 (brs, 1H), 3.96 (s, 3H), 4.47 (q, $J = 6.1$ Hz, 1H), 5.59 (t, $J = 6.7$ Hz, 1H), 8.23 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 11.2$, 16.2, 16.8, 19.1, 23.5, 30.1, 35.3, 37.0, 52.6, 56.9, 62.0, 78.3, 128.6, 147.0, 161.4, 168.5, 173.0 ppm; IR (ATR): *m/z*: 1214, 1242, 1467, 1638, 1729, 2961 cm^{-1}; MS (ESI+): *m/z*: 370 $[M+H]^+$; HRMS (ESI+): *m/z*: calcd for $C_{17}H_{28}N_3O_4S$: 370.1801 $[M+H]^+$; found: 370.1801.

iPr_2NEt (3.77 mL, 21.7 mmol) and DECP (2.74 mL, 16.2 mmol) were added to a solution of this material (4.0 g, 10.8 mmol) and D-Mep[7] (1.86 g 13.0 mmol) in DMF (54 mL) at 4 °C and the mixture was stirred at the same temperature for 30 min. The resulting mixture was allowed to warm to room temperature and stirring was continued for 18 h. After concentration, the residue was poured into CH_2Cl_2 and saturated aqueous $NaHCO_3$ solution and was extracted with CH_2Cl_2, dried over Na_2SO_4, filtered, and then concentrated in vacuo. Flash chromatography (silica gel, AcOEt/MeOH 7:1) of the residue gave **22** (4.54 g, 85 %) as a colorless solid. An analytical sample of **22** was obtained by trituration with *i*Pr_2O. M.p. 114–115 °C; $[\alpha]_D^{25} = +77.4$ ($c = 0.50$ in MeOH); ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80 °C): $\delta = 0.58$ (brd, $J = 6.7$ Hz, 3H), 0.68 (brt, $J = 7.3$ Hz, 3H), 0.90 (brd, $J = 6.7$ Hz, 3H), 0.91 (brd, $J = 6.7$ Hz, 3H), 1.08–1.24 (m, 1H), 1.28–1.66 (m, 7H), 1.97 (brtd, $J = 3.1$, 11.6 Hz, 1H), 2.09 (brs, 3H), 2.08–2.18 (m, 1H), 2.45 (dd, $J = 3.1$, 10.4 Hz, 1H), 2.60–2.70 (m, 1H), 2.76–2.86 (m, 2H), 3.83 (s, 3H), 4.40–4.56 (m, 2H), 5.59 (dd, $J = 4.3$, 7.4 Hz, 1H), 7.24 (brd, $J = 9.8$ Hz, 1H), 8.55 ppm (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 24 °C; mixture of rotamers): $\delta = 10.6$, 15.3, 16.6, 18.6, 22.8, 23.3, 24.8, 29.6, 29.9, 33.5, 34.9, 43.8, 52.1, 52.4, 54.7, 60.8, 68.4, 77.1, 131.3, 145.6, 161.0, 167.9, 168.1, 172.3 ppm; IR (ATR): $\tilde{\nu} = 1212$, 1449, 1536, 1623, 1682, 1734, 2937, 3293 cm^{-1}; MS (ESI+): *m/z*: 495 $[M+H]^+$; HRMS (ESI+): *m/z*: calcd for $C_{24}H_{39}N_4O_5S$: 495.2641 $[M+H]^+$; found: 495.2648; elemental analysis calcd (%) for $C_{24}H_{38}N_4O_5S$: C 58.28, H 7.74, N 11.33; found: C 58.07, H 7.68, N 11.22.

***N*-Methyl-D-pipecorinyl-L-isoleucinyl-*N,O*-dehydrotubuvalinyl-tubuphenylalanine (23)**: LiOH (26.3 mg, 1.10 mmol) was added to a solution of **22** (495 mg, 1.00 mmol) in THF (3.3 mL) and H_2O (1.7 mL) at 4 °C and the mixture was stirred at room temperature for 16 h. After concentration, the residue was poured into H_2O, and the pH of the mixture was adjusted to pH 4 with HCl aq. (1.00 mol L^{-1}) and then concentrated in vacuo. Flash chromatography (silica gel, CH_2Cl_2/MeOH/NH_4OH 20:5:1) of the residue gave *N*-methyl-D-pipecorinyl-L-isoleucinyl-*N,O*-dehydrotubuvaline (462 mg, 96 %) as a colorless solid. M.p. 122–124 °C; $[\alpha]_D^{26} = +26.0$ ($c = 0.51$ in MeOH); ^{1}H NMR (400 MHz, [D$_6$]DMSO, 80 °C): $\delta =$ 0.59 (brd, $J = 6.7$ Hz, 3H), 0.69 (brt, $J = 7.3$ Hz, 3H), 0.90 (brd, $J =$ 6.1 Hz, 3H), 0.94 (brd, $J = 6.7$ Hz, 3H), 0.96–1.00 (m, 1H), 1.10–1.60 (m, 7H), 1.63 (brd, $J = 11.0$ Hz, 2H), 1.99 (td, $J = 2.4$, 11.0 Hz, 1H), 2.11 (brs, 3H), 2.10–2.18 (m, 1H), 2.45–2.55 (m, 1H), 2.60–2.70 (m, 1H), 2.74–2.90 (m, 2H), 4.40–4.50 (m, 1H), 4.50–4.60 (m, 1H), 5.72 (dd, $J = 4.9$, 7.9 Hz, 1H), 7.20–7.40 ppm (m, 1H), 8.34 (s, 1H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta = 10.7$, 15.3, 16.6, 18.6, 22.7, 23.3, 24.7, 29.5, 29.9, 33.5, 34.9, 43.7, 52.4, 54.7, 60.8, 68.2, 77.3, 128.9, 149.6, 162.4, 166.7, 168.0, 172.0 ppm; IR (ATR): $\tilde{\nu} = 1275$, 1361, 1459, 1591, 1638, 2961, 3221 cm^{-1}; MS (ESI+): *m/z*: 481 $[M+H]^+$; HRMS (ESI+): *m/z*: calcd for $C_{23}H_{37}N_4O_5S$: 481.2485 $[M+H]^+$; found: 481.2483; elemental analysis calcd (%) for $C_{23}H_{36}N_4O_5S$·1.75 H_2O: C 53.94, H 7.77, N 10.94; found: C 53.90, H 7.50, N 11.20.

1,3-Diisopropylcarbodiimide (42.0 μL, 0.27 mmol) was added to a solution of carboxylic acid (118 mg, 0.25 mmol) prepared above and pentafluorophenol (67.8 mg, 0.37 mmol) in CH_2Cl_2 (1.3 mL) at 4 °C and the mixture was stirred at room temperature for 24 h. After concentration, AcOEt (2 mL) was added and the resulting mixture was filtered and the filtrate was concentrated in vacuo to give the crude pentafluorophenyl ester, which was used without further purification. *i*Pr_2NEt (0.21 mL, 1.23 mmol) and **20**·HCl (120 mg, 0.49 mmol) were added to a solution of the crude pentafluorophenyl ester in DMF (1 mL) at 4 °C and the mixture was stirred at the same temperature for 30 min. The resulting mixture was allowed to warm to room temperature and stirring was continued for 24 h. After concentration, the residue was poured into H_2O and was extracted with CH_2Cl_2, dried over Na_2SO_4, filtered, and then concentrated in vacuo. Preparative TLC of the residue on silica gel (CH_2Cl_2/MeOH 12:1) gave **23** (143 mg, 87 %) as a colorless amorphous solid. An analytical sample of **23** (colorless powder) was obtained by freeze-drying a solution of **23** in MeCN/H_2O. M.p. 82–84 °C; $[\alpha]_D^{26} = +51.2$ ($c = 0.50$ in MeOH); ^{1}H NMR (400 MHz, CD_3OD): $\delta = 0.45$ (brs, 3H), 0.66 (brs, 3H), 0.90–1.10 (m, 8H), 1.15 (brd, $J = 7.3$ Hz, 3H), 1.24–1.50 (m, 3H), 1.50–1.75 (m, 4H), 1.75–1.90 (m, 2H), 1.96–2.04 (m, 1H), 2.22–2.46 (m, 5H), 2.46–2.58 (m, 1H), 2.60–2.68 (m, 1H), 2.82–3.00 (m, 3H), 3.00–3.15 (m, 2H), 4.30–4.45 (m, 1H), 4.55–4.65 (m, 1H), 4.60–4.75 (m, 1H), 5.72 (dd, $J = 3.1$, 7.9 Hz, 1H), 7.15–7.20 (m, 1H), 7.20–7.30 (m, 4H), 8.21 ppm (brs, 1H); ^{13}C NMR (100 MHz, CD_3OD): $\delta = 11.4$, 16.0, 17.0, 18.7, 19.1, 23.7, 25.0, 25.5, 31.1, 31.6, 34.5, 36.5, 38.8, 39.2, 41.9, 44.1, 50.9, 55.4, 56.5, 63.0, 69.9, 79.1, 127.0, 127.3, 129.4, 130.5, 139.6, 151.2, 162.4, 168.6, 169.8,

173.6, 181.4 ppm; IR (ATR): $\tilde{\nu}$ = 1453, 1492, 1539, 1647, 2961 cm^{-1}; MS (ESI+): *m/z*: 670 $[M+H]^+$; HRMS (ESI+): *m/z*: calcd for $C_{35}H_{52}N_5O_6S$: 670.3638 $[M+H]^+$; found: 670.3642; elemental analysis calcd (%) for $C_{35}H_{51}N_5O_6S{\cdot}H_2O$: C 61.11, H 7.77, N 10.18; found: C 60.94, H 7.59, N 10.10.

Tubulysin V (1d): $[Mo(CO)_6]$ (31.7 mg, 0.12 mmol) was added to a solution of **23** (67.0 mg, 0.10 mmol) in MeCN (1 mL) and H_2O (0.1 mL) and the mixture was stirred at 90 °C for 1 h. After concentration, preparative TLC (CH_2Cl_2/MeOH 12:1) and subsequent reverse-phase chromatography (H_2O/CH_3CN 2:1) of the residue gave the tubulysin V (**1k**) (48.2 mg, 72 %) as a colorless solid. M.p. 118–120 °C; $[\alpha]_D^{27} = -40.4$ (c = 0.50 in H_2O); ^{1}H NMR (400 MHz, CD_3OD): δ = 0.91(t, J=7.3 Hz, 3H), 0.95 (d, J=6.7 Hz, 3H), 0.96 (d, J=6.7 Hz, 3H), 0.99 (d, J=6.7 Hz, 3H), 1.15 (br d, J=6.7 Hz, 3H), 1.17–1.26 (m, 1H), 1.32–1.42 (m, 1H), 1.52–1.92 (m, 10H), 1.96–2.06 (m, 1H), 2.08–2.18 (m, 1H), 2.33 (s, 3H), 2.34–2.42 (m, 1H), 2.46–2.60 (m, 1H), 2.93 (d, J=6.7 Hz, 2H), 2.98 (dd, J=3.1, 11.6 Hz, 2H), 3.04–3.12 (m, 1H), 4.04–4.12 (m, 1H), 4.20 (d, J=8.6 Hz, 1H), 4.28–4.40 (m, 1H), 4.79 (dd, J=2.4, 10.4 Hz, 1H), 7.10–7.20 (m, 1H), 7.20–7.35 (m, 4H), 8.02 ppm (br s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 11.0, 16.2, 18.8, 19.0, 19.5, 23.6, 25.5, 26.1, 31.1, 33.8., 37.5, 39.1, 39.2, 40.8, 41.7, 44.1, 51.0, 52.7, 56.4, 59.8, 69.71, 69.79, 124.2, 127.4, 129.3, 130.5, 139.6, 151.0, 163.1, 173.6, 173.9, 179.3, 182.3 ppm; IR (ATR): $\tilde{\nu}$ = 1495, 1539, 1646, 2960, cm^{-1}; MS (ESI+): *m/z*: 672 $[M+H]^+$; HRMS (ESI+): *m/z*: calcd for $C_{35}H_{54}N_5O_6S$: 672.3795 $[M+H]^+$; found: 672.3791; elemental analysis calcd (%) for $C_{35}H_{53}N_5O_6S{\cdot}0.75\,H_2O$: C 61.33, H 8.01, N 10.22; found: C 61.35, H 7.84, N 10.18.

Tubulysin U (1c): Ac_2O (0.17 mL, 1.75 mmol) was added to a solution of **1k** (147 mg, 0.22 mmol) in pyridine (2.1 mL) at 4 °C and the mixture was stirred at room temperature for 10 h. To the reaction mixture was added 1,4-dioxane (3.8 mL) and H_2O (3.8 mL) at 4 °C and the mixture was further stirred at room temperature for 11 h and then concentrated in vacuo. Flash chromatography (silica gel, CH_2Cl_2/MeOH 9:1) of the residue gave tubulysin U (**1c**) (143 mg, 92 %) as a colorless powder. An analytical sample of **1c** (colorless powder) was obtained by trituration with iPr_2O. M.p. 102–103 °C; $[\alpha]_D^{23} = -11.7$ (c = 0.21 in MeOH) (lit.:[8a] $[\alpha]_D^{23} = -1.42$ (c = 1.50 in MeOH)); ^{1}H NMR (400 MHz, CD_3OD): δ = 0.92 (t, J= 7.3 Hz, 3H), 0.95–0.96 (m, 6H), 0.99 (d, J=6.7 Hz, 3H), 1.15 (d, J= 6.7 Hz, 3H), 1.16–1.26 (m, 1H), 1.34–1.48 (m, 1H), 1.52–2.04 (m, 10H), 2.06–2.14 (m, 1H), 2.15 (s, 3H), 2.18–2.28 (m, 1H), 2.41, (s, 3H), 2.40–2.50 (m, 1H), 2.50–2.60 (m, 1H), 2.92 (dd, J=1.8, 6.7 Hz, 2H), 3.04–3.16 (m, 2H), 3.92–4.02 (m, 1H), 4.21 (d, J=8.6 Hz, 1H), 4.30–4.40 (m, 1H), 5.90 (dd, J=2.4, 10.4 Hz, 1H), 7.10–7.20 (m, 1H), 7.20–7.26 (m, 4H), 8.07 ppm (br s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 11.1, 16.2, 18.5, 18.9, 19.5, 20.7, 23.5, 25.4, 25.9, 31.0, 33.8, 37.6, 38.0, 38.9, 39.0, 41.7, 44.0, 51.1, 52.0, 56.3, 59.7, 69.6, 71.3, 124.9, 127.4, 129.3, 130.5, 139.7, 151.0, 162.7, 171.73, 171.76, 173.3, 173.8, 182.0 ppm; IR (ATR): $\tilde{\nu}$ = 1220, 1495, 1540, 1650, 1744, 2961 cm^{-1}; MS (ESI+): *m/z*: 714 $[M+H]^+$; HRMS (ESI+): *m/z*: calcd for $C_{37}H_{56}N_5O_7S$: 714.3900 $[M+H]^+$; found: 714.3896; elemental analysis calcd (%) for $C_{37}H_{55}N_5O_7S{\cdot}H_2O$: C 60.71, H 7.69, N 9.44; found: C 60.56, H 7.69, N 9.44.

Cyclo-tubulysin D (1e): A mixture of deacetyl-tubulysin D (**24**) (23.4 mg, 29.8 μM) and 1 M HCl aq. (2 mL) in THF (2 mL) was stirred at 60 °C for 24 h. After concentration, the residue was poured into CH_2Cl_2/MeOH (10:1, 10 mL) and phosphate buffer (pH 7.4, 10 mL). The mixture was extracted with CH_2Cl_2/MeOH (10:1) and the organic extract was dried over Na_2SO_4, filtered, and then concentrated in vacuo. Purification by preparative TLC (CH_2Cl_2/MeOH/NH_4OH 20:5:1) of the residue gave **5** (19.8 mg, 97 %) as a colorless amorphous solid. This material appeared to exist as an 84:16 mixture of rotamers in CD_3OD. $[\alpha]_D^{29} = +49.1$ (c = 0.34 in MeOH); ^{1}H NMR (400 MHz, CD_3OD): δ = 0.85 (d, J=6.7 Hz, 3H), 0.94 (t, J=7.3 Hz, 3H), 1.01 (d, J=6.7 Hz, 3H), 1.12 (d, J=6.7 Hz, 3H), 1.15 (d, J=6.7 Hz, 3H), 1.15–1.42 (m, 3H), 1.50–1.76 (m, 5H), 1.76–1.90 (m, 3H), 1.94–2.10 (m, 2H), 2.24–2.60 (m, 4H), 2.33 (s, 3H), 2.90 (br d, J=7.3 Hz, 3H), 3.08 (br d, J=11.6 Hz, 1H), 4.25 (m, 1H×14/100), 4.30–4.40 (m, 1H), 4.45 (m, 1H×86/100), 4.57 (d, J=10.4 Hz, 1H×14/100), 4.75 (d, J=7.9 Hz, 1H), 5.02 (d, J=11.6 Hz, 1H×86/100), 5.33 (dd, J= 1.8, 11.6 Hz, 1H), 5.76 (d, J=11.6 Hz, 1H×86/100), 5.76 (d, J=9.8 Hz, 1H×14/100), 7.10–7.18 (m, 1H), 7.20–7.26 (m, 4H), 8.06 ppm (s, 1H); ^{13}C NMR (100 MHz, CD_3OD): δ = 11.4, 16.4, 18.7, 19.5, 20.4, 23.8, 25.6, 25.8, 27.3, 31.1, 34.5, 37.8, 38.7, 39.3, 42.1, 44.1, 50.9, 54.7, 55.8, 56.5, 69.8, 74.0, 74.1, 124.7, 127.3, 129.3, 130.5, 139.6, 150.8, 162.88, 162.91, 172.9, 173.1, 174.1, 181.3 ppm; IR (ATR): $\tilde{\nu}$ = 1542, 1649, 2963 cm^{-1}; MS (ESI+): *m/z*: 684 $[M+H]^+$; HRMS (ESI+): *m/z*: calcd for $C_{36}H_{54}N_5O_6S$: 684.3795 $[M+H]^+$; found: 684.3793; elemental analysis calcd (%) for $C_{36}H_{53}N_5O_6S{\cdot}H_2O$: C 61.60, H 7.90, N 9.98; found: C 61.62, H 7.83, N 9.59.

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[16] Prepared as described in the Supporting Information.
[17] Crystallographic data: CCDC-760558 (**8a**), -760559 (**8d**), -719636 (**10**), and -719635 (**11**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
[18] A variety of solvents (EtOH, MeCN, THF, CH_2Cl_2, H_2O, DMF, acetone, hexane, AcOEt, CF_3CH_2OH, neat, etc.) and additives ($MgBr_2$, MS (4 Å), etc.) were tested in the 1,3-DC reaction of nitrone D-**6** with vinyl thiazole **7** at various temperatures (25, 60, 100 °C).
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[30] Our synthetic **1d** was a free base. Reported **1d** in references [8a] and [8b] were TFA and HCl salts, respectively.

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