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Toward Tubulysin: Gram-Scale Synthesis of Tubuvaline-Tubuphenylalanine Fragment

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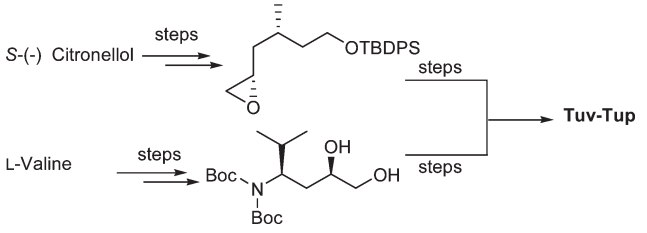
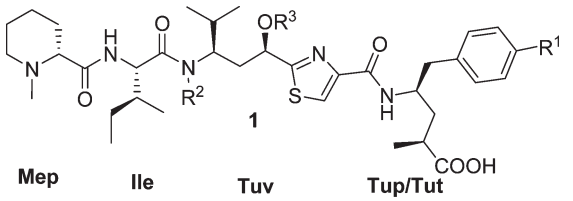


A practical and stereoselective synthesis of the tubuvaline-tubuphenylalanine (Tuv-Tup) fragment of tubulysin is achieved involving the opening of aziridine, crucial MacMillan α-hydroxylation on both fragments, and an epoxide-opening reaction.

Tubulysin is a peptide natural product in clinical development with potent anticancer activity against the P-glyco protein-expressing KBV_1 cell line.[1] This scarce natural product with very limited supplies from fermentation of *Angiococcus disciformis* An d48 (with an yield of 0.25−1 mg/L) binds to the peptide-binding site located near the vinca alkaloid binding site of β-tubulin (Figure 1).[1−3] The aryl-hydroxylated tubulysin A has shown interesting antiangiogenic effects.[4] This exotic profile triggered synthetic and medicinal chemists to initiate program toward its synthesis.



Mep = *N*- methyl pipecolic acid
Ile = isoleucin
Tuv = tubuvaline
Tup/Tut = tubuphenylalanine/tubutyrosine

Tubulysin	A	R^1 = OH	R^2 = CH_2OOC-isobutyl	R^3 = Ac	**1a**
	B	R^1 = OH	R^2 = CH_2OOC-n-propyl	R^3 = Ac	**1b**
	C	R^1 = OH	R^2 = CH_2OOC-ethyl	R^3 = Ac	**1c**
	D	R^1 = H	R^2 = CH_2OOC-isobutyl	R^3 = Ac	**1d**
	U	R^1 = H	R^2 = H	R^3 = Ac	**1e**
	V	R^1 = H	R^2 = H	R^3 = H	**1f**

FIGURE 1. Structures of tubulysins.

While few efforts have resulted in meaningful total syntheses,[5] others have led to new strategies for partial syntheses.[6]

More recently, simpler analogues of tubulysin have been reported to retain a potent level of cytotoxicity.[7] The potent analogues in this report were devoid of the Mep part of the molecule, indicating that the requirement of this part is "not so critical". Our continued interest in synthesis of peptide natural products and unusual amino acids[8] prompted us to take up the synthesis of tubulysin D, which culminated in the synthesis of the essential core Tuv-Tup portion, the results being disclosed herein. Our objective has been to achieve gram quantities of the core Tuv-Tup of the natural product so that new analogues could be designed. The classical retrosynthesis of this tetra peptide produced two fragments Mep-Ile-OMe **2**, *N*-boc-Tuv-Tup-OMe **3** (Scheme 1).

The synthesis of tubuphenylalanine (Tup) commenced from epoxysilyl ether **4** obtained from (−)-citronellol in six steps as reported by us recently[9] (Scheme 2). This epoxide **4** was treated with the Grignard reagent derived from bromobenzene to provide the single regioisomer **7** in over 94% yield. The secondary alcohol was silylated using TBSCl and imidazole to disilyl ether **8** followed by mono-desilylation of TBDPS resulted in **8a**. This was converted in a two-step process to olefinic compound **9** via iodo derivative followed by 1,2-elimination.

The desilylation in **9** was achieved using TBAF to realize alcohol **10** which was converted to the azido compound **11** via Mitsunobu type inversion process (TPP, DIAD, and DPPA).[10]

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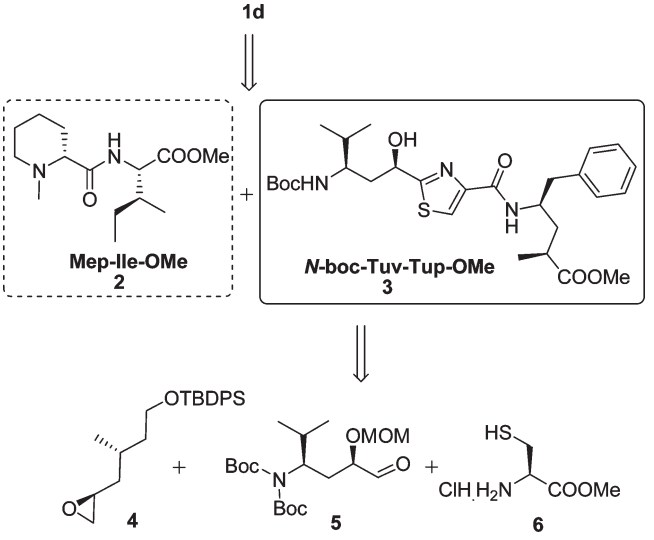
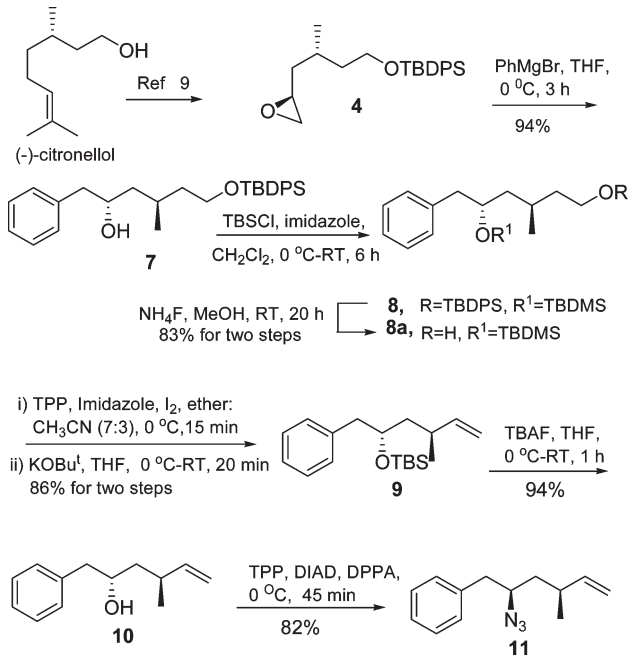
SCHEME 1. Retrosynthetic Analysis of Tubulysin

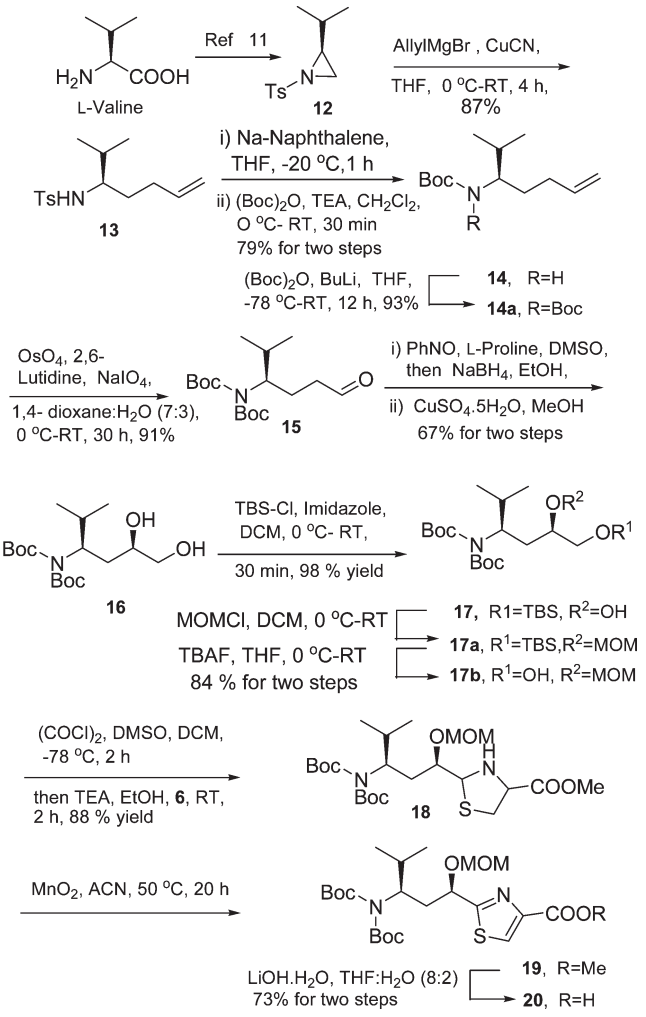


SCHEME 2



The other key fragment Tuv is synthesized on gram scale starting from L-valine and L-cysteine (Scheme 3). The L-valine was converted to the aziridine **12** by a known procedure.[11] The aziridine **12** was opened regioselectively with allylmagnesium bromide using CuCN as a catalyst to furnish **13**,[12] which on exposure to Na-naphthalene realized

SCHEME 3



the free amine. This on treatment with $(Boc)_2O$ in dichloromethane and TEA provided mono Boc derivative **14** which on further reaction with BuLi and $(Boc)_2O$ produced diboc derivative **14a** in 93% yield. The oxidative cleavage of olefin functionalily in **14a** was realized with OsO_4, 2,6 lutidine, and $NaIO_4$ furnished aldehyde **15** amicable for α-hydroxylation.

The aldehyde **15** was subjected to the crucial MacMillan α-hydroxylation[13] using nitrosobenzene and 40 mol % of L-proline in DMSO, followed by rapid reduction with sodium borohydride to furnish the unstable anilinoxy compound which was further treated with 30 mol % of $CuSO_4$ in methanol at room temperature to cleave the O−N bond providing the diol **16** with high enantio- and diastereoselectivity (67% yield, 98% de). This resulting 1,2-diol **16** on treatment with TBSCl, and imidazole was monosilylated to afford **17** in 98% yield. On further reaction with MOMCl and DIPEA in dichloromethane, this provided

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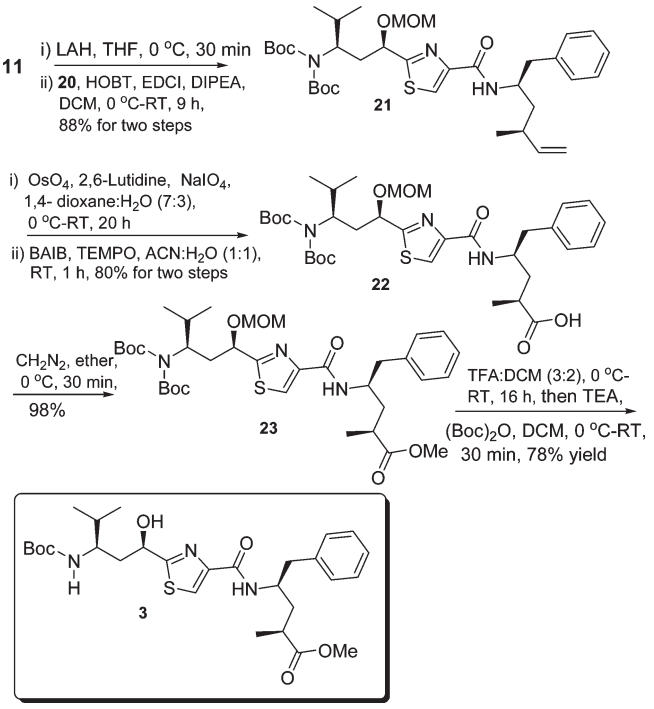
SCHEME 4



the diprotected compound **17a** which on desilylation resulted the free alcohol **17b** in over 84% yield. This free alcohol in a one-pot reaction was oxidized to aldehyde (unstable) which without isolation was condensed with the methyl ester of L-cysteine salt **6** using Swern oxidation conditions to afford the thiazolidine **18** in 88% yield .[14,15]

The key fragment (Tuv) **20** was obtained in over 73% yield for the two-step reaction from **18** via the oxidation with MnO_2[14] followed by the saponification using $LiOH \cdot H_2O$.

The azido group in **11** was subjected to $LiAlH_4$ reaction followed by coupling with acid **20** using EDCI/HOBT conditions to deliver the dipeptide olefinic compound **21** without any detectable loss of stereochemical purity and with very satisfactory yields (Scheme 4). This olefinic compound **21** was subjected to OsO_4 and 2,6-lutidine conditions in a single step to give the aldehyde, which without purification on reaction with bis(acetoxy)iodobenze and TEMPO provided the triprotected Tup-Tuv-OH **22** in over 80% yield. The acid functionality was converted to the methyl ester of the triprotected Tup-Tuv **23** in 98% yield. Finally, the reaction with TFA/CH_2Cl_2 followed by treatment with TEA and $(Boc)_2O$ afforded the desired *N*-Boc-Tuv-Tup-OMe **3** in 78% yield with desirable functionality for further elaborations and analogues.

Coincidentally, this fragment is also the late-stage intermediate reported by Zanda and co-workers[5b] which has indistinguishable spectroscopic data. In summary, we have developed an effective gram-scale synthesis of the essential core tubuvaline-tubuphenylalanine (Tuv-Tup) fragment. Further work toward *N*-terminal modified analogues of tubulysin and biological evaluation of the analogues is in progress.

Experimental Section

(2*S*,4*R*)-6-(*tert*-Butyldiphenylsilyloxy)-4-methyl-1-phenylhexan-2-ol (7). Magnesium turnings (1.36 g, 56.6 mmol) were placed in a 250 mL, two-neck, round-bottomed flask equipped with a reflux condenser and suspended in 80 mL of dry THF under nitrogen atmosphere. Bromobenzene (8.94 g, 6.0 mL, 56.6 mmol) was added slowly, and the suspension was vigorously stirred at room temperature by maintaining the cooled condenser for 1 h. The generated Grignard reagent was slowly added to epoxide **4** (7.0 g, 19.0 mmol) dissolved in dry THF (30 mL) at 0 °C using a cannula under N_2 atmosphere. The reaction mixture was stirred at the same temperature for 3 h. After completion of the reaction (monitored by TLC), the reaction was quenched with satd NH_4Cl solution (25 mL). The reaction mixture was diluted with EtOAc (100 mL), and the organic layer was separated. The organic layer was washed with water (75 mL) and brine (60 mL), dried over anhydrous Na_2SO_4 (5.0 g), and evaporated in vacuo. The crude product was chromatographed over silica gel (4% EtOAc/petroleum ether) to afford pure sigle regioisomer **7** (7.97 g, 94%) as a colorless gummy liquid: $[\alpha]^{20}_D = +0.5$ (*c* 0.5, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ_H (ppm) 7.70−7.64 (m, 4H), 7.46−7.17 (m, 11H), 3.94−3.84 (m, 1H), 3.71 (dt, $J = 2.2$, 6.7 Hz, 2H), 2.77 (dd, $J = 4.3$, 13.5 Hz, 1H), 2.63 (dd, $J = 8.3$, 13.5 Hz, 1H), 1.94−1.80 (m, 1H), 1.69−1.37 (m, 3H), 1.30−1.20 (m, 1H), 1.04 (s, 9H), 0.86 (d, $J = 6.6$ Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C (ppm) 138.6, 134.0, 129.5, 129.4, 128.5, 127.5, 126.3, 70.3, 62.0, 44.6, 44.2, 40.2, 26.8, 26.2, 19.4, 19.1; IR (neat) ν_{max} 3422, 3067, 2929, 2858, 1462, 1427, 1108, 823, 739, 701, 612, 504 cm^{-1}; MS (APCI) *m*/*z* 447 (100) $[M + H]^+$; HRMS (ESI) $[M + H]^+$ $C_{29}H_{39}O_2Si$ calcd 447.2714, found 447.2734.

Di- *tert*-butyl ((3*R*,5*R*)-5,6-Dihydroxy-2-methylhexan-3-yl)carbamate (16). (a) To a 250 mL, two-neck, round-bottomed flask equipped with a magnetic stir bar was charged with L-proline (0.98 g, 8.5 mmol), and DMSO (15 mL) was added at room temperature under nitrogen atmosphere. After the suspension was stirred for 10 min, nitrosobenzene (2.29 g, 21.4 mmol) was added in one portion at which time the solution became green. Aldehyde **15** (14.1 g, 42.8 mmol) in DMSO (25 mL) was added in one portion to the above greenish suspension and stirring continued at room temperature until the reaction was determined to be complete (the change of color of the green color solution to a yellow homogeneous solution was observed by TLC). The reaction mixture was then transferred to a suspension of $NaBH_4$ (2.43 g, 63.9 mmol) in ethanol (50 mL) at 0 °C. After 20 min of stirring, the reaction mixture was treated with saturated aqueous $NaHCO_3$ (50 mL) and extracted with dichloromethane (3 × 100 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude compound was subjected to flash chromatography using silica gel (15% EtOAc/petroleum ether) to afford an unstable anilinoxy compound (7.4 g, 85%) which was used immediately for the next reaction.

(b) To a solution of the above anilinoxy compound (7.4 g, 16.8 mmol) in methanol (60 mL), was added $CuSO_4$ (1.26 g, 5.0 mmol). The reaction mixture was stirred at room temperature overnight and then quenched with a cold saturated NH_4Cl solution (10 mL). The mixture was filtered on a Celite pad and washed thoroughly with ethyl acetate (60 mL), and the complete solvent was removed under reduced pressure. The compound was extracted with ethyl acetate (3 × 60 mL). The combined organic layers were washed with brine (40 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo.

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The residue was then purified by silica gel chromatography (5% EtOAc/petroleum ether) to afford diol **16** (4.63 g, 79% yield, 98% de) as a brownish gummy liquid: $[\alpha]^{30}_{D} = -26.4$ (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H (ppm) 3.80−3.44 (m, 4H), 2.28−2.17(m, 1H), 1.89−1.63 (m, 2H), 1.49 (s, 18H), 0.93 (d, J = 6.6 Hz, 6H); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C (ppm) 154.3, 82.6, 68.9, 66.6, 61.8, 33.6, 31.0, 27.9, 20.7, 20.2.; IR (KBr) ν_{max} 3430, 2973, 2930, 2361, 2335, 1736, 1693, 1460, 1368, 1247, 1165, 1127, 942, 854, 769, 669, 579 cm^{-1}; MS (ESI) m/z 348 (100) $[M + H]^+$; HRMS (ESI) $[M + Na]^+$ $C_{17}H_{33}NNaO_6$ calcd 370.2200, found 370.2204.

(2*S*,4*R*)-Methyl 4-(2-((1*R*,3*R*)-3-(*tert*-Butoxycarbonylamino)-1-hydroxy-4-methylpentyl)thiazole-4-carboxamido)-2-methyl-5-phenylpentanoate (3). The stirred solution of dipeptide ester **23** (3.0 g, 4.3 mmol) in dry CH_2Cl_2 (10 mL) at 0 °C under nitrogen atmosphere was treated with CF_3COOH (15 mL). The reaction mixture was warmed to room temperature and stirred for 16 h. The solvent and TFA were removed under reduced pressure. The resulted salt was dissolved in dichloromethane (30 mL), cooled to 0 °C, and treated with triethylamine (1.25 mL, 8.6 mmol). After the mixture was stirred for 10 min, $(Boc)_2O$ (1.0 g, 4.7 mmol) was added dropwise. The reaction was maintained at room temperature for 30 min. The solvent was evaporated on a rotary evaporator, and the crude compound was chromatographed over SiO_2 (35% EtOAc/petroleum ether) to afford the pure *N*-Boc-protected dipeptide compound **3** (1.85 g, 78%) as a colorless gummy liquid: $[\alpha]^{23}_{D} = +16.1$ (*c* 0.7, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H (ppm) 8.02 (s, 1H), 7.30−7.14 (m, 5H), 7.08 (d, J = 9.3 Hz, 1H), 5.20 (br s, 1H), 4.87 (br d, J = 10.1 Hz, 1H), 4.59 (d, J = 9.4 Hz, 1H), 4.46−4.33 (m, 1H), 3.80−3.68 (m, 1H), 3.63 (s, 3H), 2.98−2.83 (m, 2H), 2.65−2.54 (m, 1H), 2.09−1.90 (m, 2H), 1.87−1.73 (m, 2H), 1.66−1.53 (m, 1H), 1.46 (s, 9H), 1.15 (d, J = 7.0 Hz, 3H), 1.01 (d, J = 6.7 Hz, 3H), 1.00 (d, J = 6.7 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ_C (ppm) 176.6, 175.2, 160.7, 157.9, 149.6, 137.4, 129.6, 128.3, 126.4, 122.9, 80.5, 68.7, 52.2, 51.7, 48.0, 41.5, 41.0, 37.7, 36.4, 32.2, 28.3, 19.3, 18.3, 17.8; IR (KBr) ν_{max} 3364, 2970, 2915, 1742, 1703, 1650, 1536, 1483, 1460, 1376, 1269, 1227, 1105, 1086, 947, 761, 685, 670 cm^{-1}; MS (ESI) m/z 538 (100) $[M + H]^+$; HRMS (ESI) $[M + H]^+$ $C_{30}H_{40}N_3O_4S$ calcd 538.2734, found 538.2749.

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Supporting Information Available: Experimental procedures, melting points, specific rotation, and spectral data (^{1}H NMR, ^{13}C NMR, IR, MS, HRMS) and copies of ^{1}H NMR and ^{13}C NMR for all compounds described in the paper. This material is available free of charge via the Internet at http://pubs.acs.org.