Synthetic Communications

An International Journal for Rapid Communication of Synthetic Organic Chemistry

ISSN: (Print) (Online) Journal homepage: https://www.tandfonline.com/loi/lsyc20

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To cite this article: Ramesh B. Reddy , Vijay M , Mena Asha Krishnan & Venkatesh Chelvam (2020): Synthesis of tubuvaline (Tuv) fragment of tubulysin via diastereoselective dihydroxylation of homoallylamine, Synthetic Communications, DOI: 10.1080/00397911.2020.1855355

To link to this article: https://doi.org/10.1080/00397911.2020.1855355

SYNTHETIC COMMUNICATIONS®
https://doi.org/10.1080/00397911.2020.1855355

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Synthesis of tubuvaline (Tuv) fragment of tubulysin via diastereoselective dihydroxylation of homoallylamine

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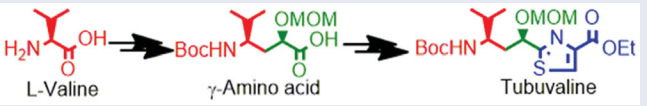
ABSTRACT

Tubulysins are natural anticancer molecules that directly bind and inhibit tubulin polymerization in actively dividing cells leading to apoptosis and cell death. Structurally, tubulysins are linear tetrapeptides, constituted by a natural amino acid (Ile) and three non-canonical amino acids (Mep, Tuv, and Tup). Herein, we report a convenient strategy for the practical synthesis of tubuvaline fragment of tubulysin natural products. In this approach, we describe the regioselective ring opening of a chiral aziridine **7** with vinyl Grignard reagent to obtain (R)-tert-butyl(2-methylhex-5-en-3-yl)(tosyl)carbamate **8** which was further subjected to Sharpless asymmetric dihydroxylation with AD-mix-β resulting in the formation of (2 R,4R)-4-((tert-butoxycarbonyl)amino)-2-(methoxymethoxy)-5-methylhexanoic acid **3** that was finally transformed to tubuvaline by heterocyclization.

GRAPHICAL ABSTRACT



ARTICLE HISTORY
Received 12 October 2020

KEYWORDS
γ-Amino acid; Sharpless dihydroxylation; tubuvaline; tubulysin

Introduction

Mitotic spindle microtubules, an ideal target for anticancer drugs play an essential role in suppressing tubulin dynamics and inhibit cell proliferation leading to apoptosis.[1] Most of the FDA approved drugs (vinca alkaloids and paclitaxel) for cancer treatment show multidrug resistance resulting in urgency for the discovery of new anticancer compounds. Recently, tubulysin family of natural products was demonstrated to show noncompetitive inhibition during tubulin protein polymerization and is found to be more efficacious compared to other tubulin-targeted anticancer drugs.[2] Superior biological activities and limited natural source of tubulysins have attracted synthetic chemists to introduce novel and sustainable methods toward the preparation of various fragments, total synthesis and their analogs.[3–6]

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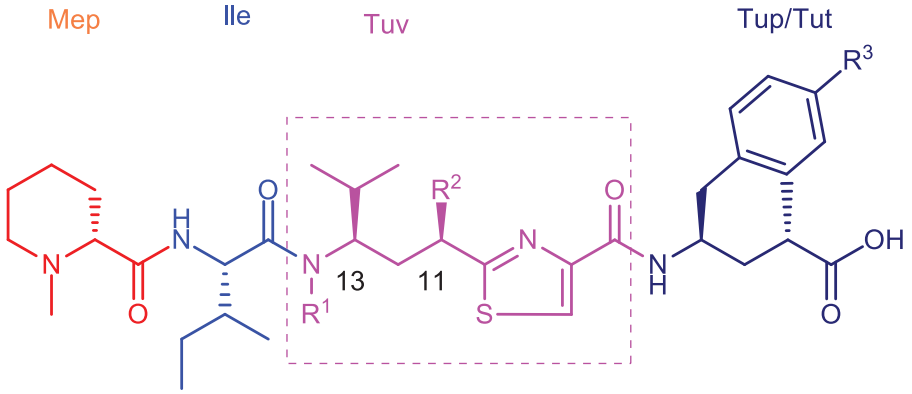


Tubulysin	R^1	R^2	R^3
A	$CH_2OCOCH_2CH(CH_3)_2$	OAc	OH
B	$CH_2OCOCH_2CH_2CH_3$	OAc	OH
D	$CH_2OCOCH_2CH(CH_3)_2$	OAc	H
U	H	OAc	H
V	H	H	H

Figure 1. Structure of tubulysin family of antimitotic agents.

Sasse et al. first isolated tubulysins from myxobacteria culture broth as cytotoxic tetrapeptides and found to be distantly related to dolastatins.[7] Later, Müller et al. discovered the biosynthetic gene cluster which is responsible for the biosynthesis of tubulysins.[8,9] Interestingly, tubulysin was found to possess higher growth inhibition activity compared to other known microtubule inhibitors such as epothilones, vinca alkaloids, and taxanes by a factor of 20–1000 fold.[10] Tubulysin inhibits microtubule formation through binding to the tubulin heterodimer, ultimately leading to cell death.[11] The exceptional biological activities and unique structural characteristics of tubulysins justify the fast-growing interest to discover novel chemosynthetic methods to prepare more potent new anticancer agents with IC_{50} values in the low nanomolar range.

Inspection of the tubulysin structure revealed that it can be disconnected into four amino acid fragments: N-methyl-D-pipecolinic acid derived from L-lysine[9] [(Mep)-non proteogenic amino acid], L-isoleucine [(Ile)-the only natural amino acid], and two uncommon amino acids: tubuvaline [(Tuv)-containing a thiazole moiety and is biosynthesized from L-valine] and tubuphenyl alanine or tubutyrosine which is homologous of phenylalanine or tyrosine aminoacids, respectively (Figure 1). Among these four amino acids, the most complex component of the tubulysins is the thiazole containing amino acid core called tubuvaline (Tuv). Tuv, the residue rich in functional groups than other fragments, was previously prepared through C11-hydroxy group installation directly[12] or reduction of a ketone functional group at C-11 using CBS reagent.[13,14] The C-11 alcohol can also be introduced stereoselectively by hydroxylation using the methods of Davis[15] and MacMillan.[16] Although several methods have been reported to date (Figure 2), a simple and large-scale synthesis of thiazole fragment of tubulysin remains elusive. In continuation of our research on the preparation of various fragments and

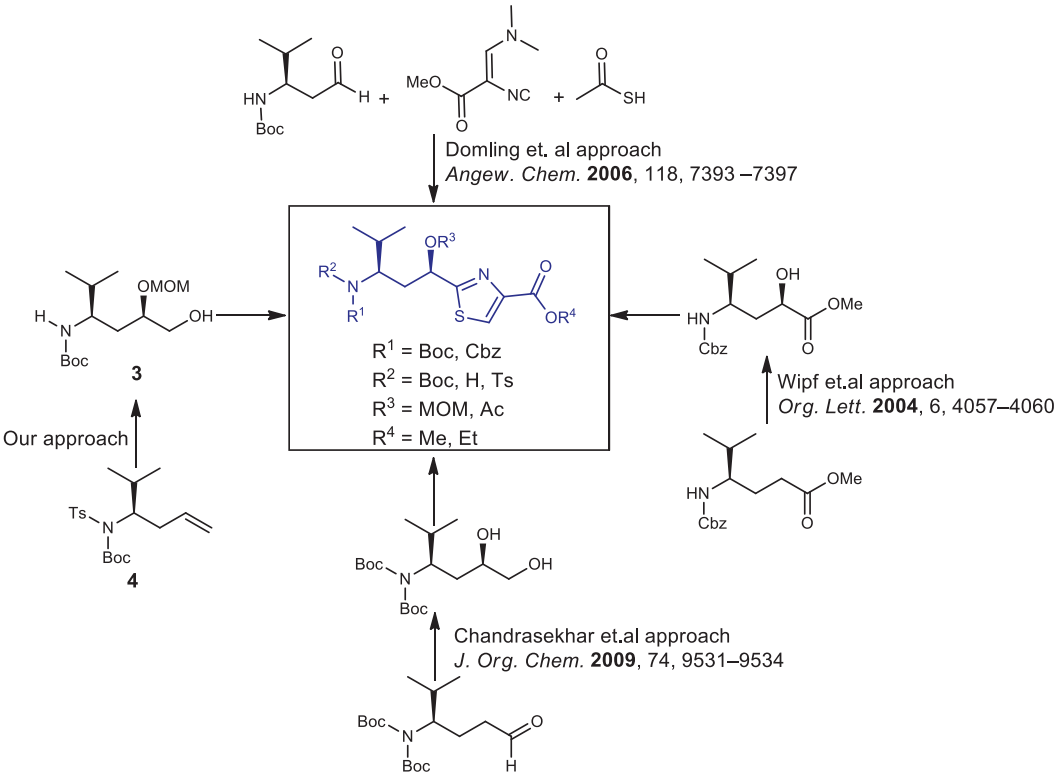
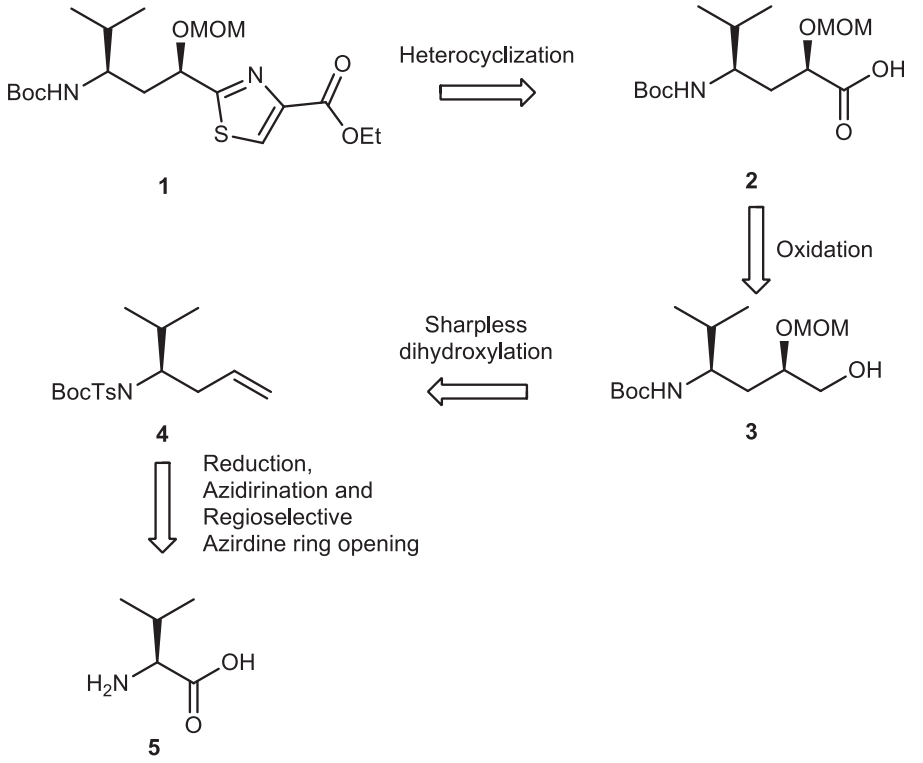


Figure 2. Synthetic approaches of tubuvaline fragment.

their analogs[17,18] of tubulysin family of natural products, herein, we report a novel and high yielding synthetic route for the synthesis of tubuvaline (Tuv) **1** fragment via the Grignard reagent ring opening of substituted aziridine **7** followed by asymmetric Sharpless dihydroxylation of homoallylamine **4**.

Structure-activity relationship studies of tubulysins have revealed that these linear tetrapeptides are moderately tolerant of structural modifications in the tubuvaline fragment. Inversion of C-11 hydroxy group configuration reduces activity, and hydrolysis of the C-11 acetoxy moiety led to reduced biological activity.[13,19] Oxidation of alcohol to a ketone at C-11 position also reduces the activity.[20] Inversion of C-13 isopropyl group configuration decreases the activity,[21–23] and the isopropyl group substituent at C-13 position appears to be intolerant to chemical modifications. Only a modest loss of activity was observed when the acetyl group was replaced with a methyl or MOM ether functionality.[24]

The retrosynthetic analysis of tubuvaline (Tuv) is illustrated in Scheme 1. We plan to synthesize tubuvaline from a key intermediate, N-Boc-methoxymethyl-α-hydroxy γ-amino acid **2**, which could, in turn, be obtained by the oxidation of N-Boc-O-methoxymethyl-1,2,4-amino alcohol **3**. Compound **3** could be prepared by the Sharpless asymmetric dihydroxylation of homoallylamine **4**, which can be obtained from commercially available L-valine by reduction, aziridine ring formation, and regioselective ring opening of aziridine ring using vinyl Grignard reagent (Scheme 1).



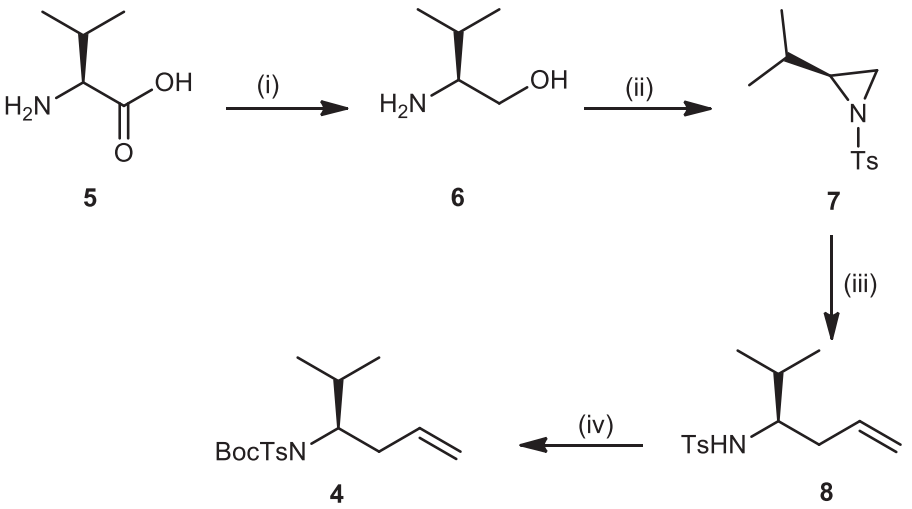
Scheme 1. Retrosynthetic analysis of tubuvaline **1** (Tuv).

Enantiomerically pure γ-amino acids are considered essential building blocks for the synthesis of substituted pyrrolidines, natural products and peptidomimetics. These γ-amino acid units are also found in molecules showing several biological activities, including antibacterial, anti-neoplastic, and anticancer activities.[25,26] In this perspective, we have developed an efficient and novel method for the preparation of desired N-Boc-methoxymethyl-α-hydroxy-γ-amino acid **2** by a two-carbon homologation, asymmetric dihydroxylation, and oxidation (Schemes 2–4).

The Sharpless asymmetric dihydroxylation (AD) of olefins, an inspiring innovation, provides one of the most elegant methods for the preparation of chiral vicinal diols.[27–29] The asymmetric dihydroxylation reaction offers many diversities that could be applied to synthesize biologically important molecules. Chiral vicinal diols are key intermediates in the synthesis of many bioactive molecules. The Sharpless asymmetric dihydroxylation (AD) of terminal olefins, is one of the most reliable reactions to date for preparation of such intermediates with high stereoselectivity.[30]

Results and discussion

We began our synthesis starting from commercially available natural amino acid L-valine to furnish the required homoallyl amine **4** for dihydroxylation. In the first step, L-valine **5** was converted to L-valinol **6** by performing reduction of acid functionality with



Scheme 2. Synthesis of N-tosyl-Boc protected homoallylamine, **4**. Reagents and conditions (i) $NaBH_4$, I_2, THF, reflux, 20 h, 89% (ii) TsCl, NEt_3, ACN, 0 °C to rt, 1 h, 80% (iii) Vinylmagnesium bromide, Cu(I)CN, THF, 0 °C to rt, 2 h, 70% (iv) Boc_2O, DMAP, CH_2Cl_2, rt, 2 h, 87%

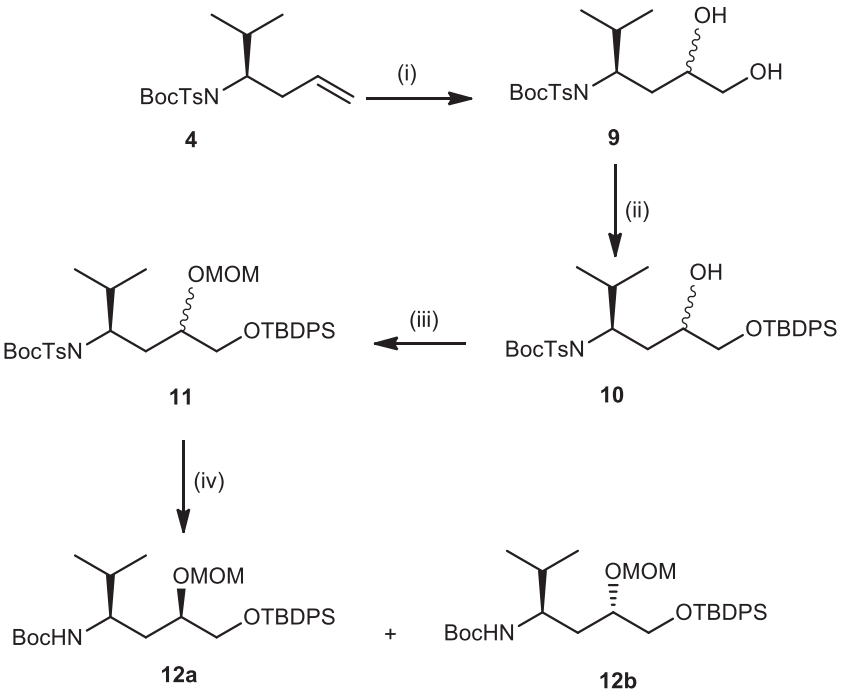
in situ generation of BH_3 from $NaBH_4$, I_2 under refluxing tetrahydrofuran. Without further purification, L-valinol **6** was treated with TsCl in presence of a base, Et_3N to obtain N-Ts-aziridine **7**[31–34] in 85% isolated yield, which was subsequently subjected to regioselective ring opening using vinyl magnesium bromide, Cu(I)CN in tetrahydrofuran to provide N-tosylamino alkene **8** in 70% yield. N-tosylamino alkene **8** was protected using Boc_2O in the presence of DMAP in CH_2Cl_2 to obtain N-tosyl-N-Boc protected homoallylamine **4** in 87% yield (Scheme 2).

The homoallylamine **4** was subjected to a crucial Sharpless asymmetric dihydroxylation using AD-mix-β, $CH_3SO_2NH_2$ and *t*BuOH/H_2O (1:1) to afford 1,2-diols **9** as a mixture of diastereomers in the ratio 2:1 (from ^{1}H NMR) in 88% yield. The diastereomers of the resulting 1,2-diols **9** were, at this time, inseparable, and they were thus carried through the synthesis until detosylation of **11**–**12a** as described below: 1,2-diol **9** was subjected to selective primary alcohol protection using TBDPSCl and imidazole in THF to afford **10** in 88% yield. Furthermore, the secondary hydroxy group of **10** was protected as methoxymethyl ether using MOMOCl and DIPEA to obtain **11** in 80% yield, which on detosylation resulted in the formation of N-Boc amides **12a** and **12b**. At this point, the desired diastereomer **12a** was readily separable from the undesired diastereomer **12b** by column chromatography (Scheme 3).

The separated diastereomer **12a** was desilylated using tetrabutylammonium fluoride to afford N-Boc-O-methoxymethyl-1,2,4-amino alcohol **3** in 97% yield. Oxidation of primary alcohol **3** was carried out using pyridinium dichromate (PDC) in DMF to obtain N-Boc-γ-lactam **13** in 85% yield.

Further hydrolysis of the five-membered lactam ring of **13** using LiOH in the presence of H_2O_2 furnished N-Boc-methoxymethyl-α-hydroxy-γ-amino acid **2**, a key precursor for the synthesis of tubuvaline (Scheme 4).

Treatment of γ-amino acid **2** with ammonium chloride under amide bond-formation conditions in the presence of coupling agent (HBTU) and DIPEA in CH_2Cl_2 provided



Scheme 3. Synthesis of N-Boc-α-hydroxymethoxymethyl-γ-amino-silylether intermediates **12a** and **12b**. Reagents and conditions (i) AD-mix-β, *t*-BuOH:H_2O (1:1), 0 °C, 12 h, 88% *dr* = 2:1 (non-separable) (ii) TBDPSCl, imidazole, CH_2Cl_2, 0 °C to rt, 2 h, 88% (non-separable) (iii) MOMCl, DIPEA, CH_2Cl_2, 0 °C to rt, 12 h, 80% (non-separable) (iv) Mg, MeOH, Sonication, rt, 3 h, 84%, dr = 2:1 (separable).

α-substituted N-Boc-γ-aminocarboxamide **14** in 95% yield. Intermediate amide **14** was then transformed[35] into thioamide **15** by treatment with Lawesson's reagent in THF at room temperature, which was further transformed to N-Boc protected tubuvaline ethyl-ester **1** (Scheme 5) in an excellent yield of 83%.

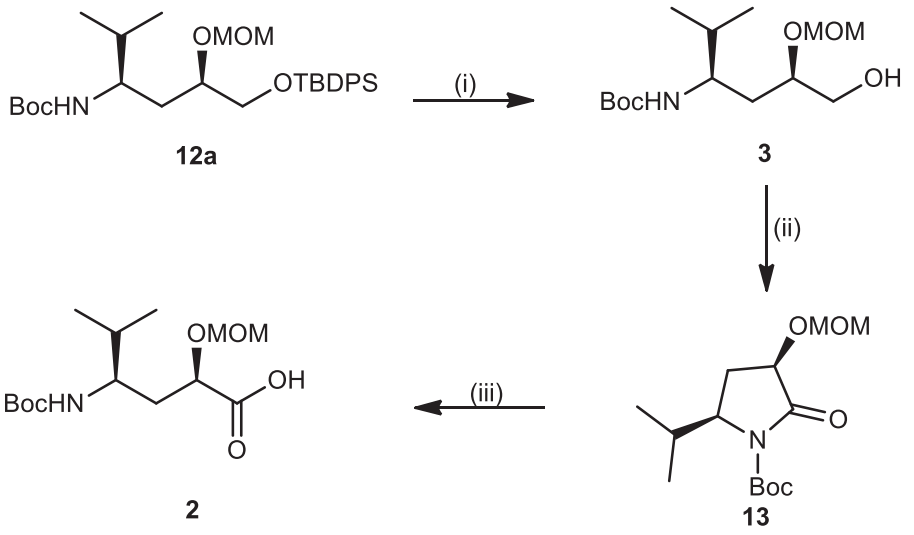
Conclusion

In summary, the chemistry described herein reports an asymmetric approach for the synthesis of tubuvaline ester **1** (Tuv) fragment of tubulysin based on a heterocyclization strategy involving Sharpless asymmetric dihydroxylation and regioselective aziridine ring opening reaction as key steps. The synthetic design is suitable for scale-up synthesis of this highly desirable and complex fragment of tubulysin in a simple and elegant way. Total syntheses of new tubulysin derivatives, containing this fragment and *in vitro* evaluation of their cytotoxicity in drug-resistant cancer cell lines is currently underway in our laboratory and will be reported in due course.

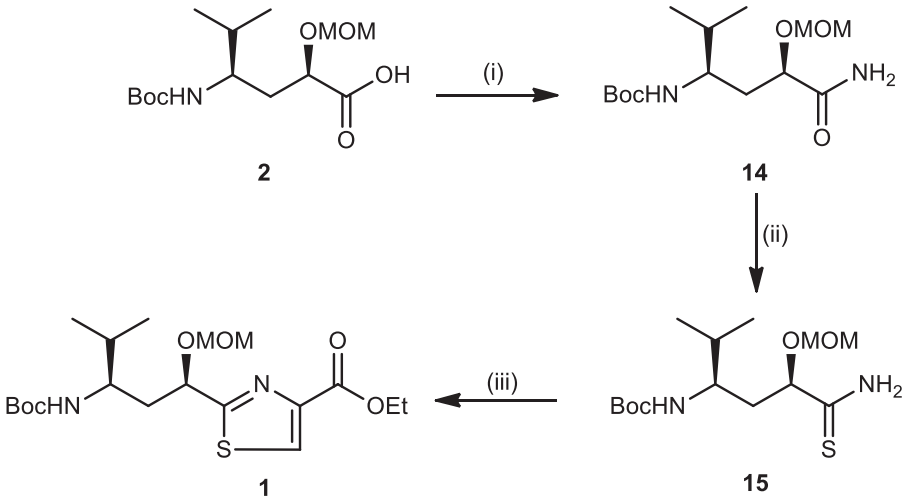
Experimental section

General

All reactions were performed in oven-dried glassware under the inert atmosphere with magnetic stirring. Air and moisture-sensitive liquids and solutions were transferred via



Scheme 4. Synthesis of N-Boc γ-substituted α-hydroxy γ-amino acid (**2**). Reagents and conditions (i) TBAF, THF, 0 °C to rt, 2 h, 97% (ii) DMF, PDC, rt, 24 h (iii) LiOH, H_2O_2, THF:H_2O (2:1), rt, 12 h, 66% (overall two steps).



Scheme 5. Synthesis of tubuvaline ester **1** (Tuv). Reagents and conditions (i) HOBt, HBTU, DIPEA, NH_4Cl, CH_2Cl_2 0 °C to rt, 4 h, 95% (ii) Lawesson's reagent, THF, 0 °C to rt, 12 h, 60% (iii) Ethyl bromopyruvate, EtOH, 65 °C, 1 h, 83%.

glass syringes. TLC was performed on 0.25 mm Merck TLC silica gel 60 F_{254} plates and visualized under UV light (254 nm) or by staining with bromocresol green, ninhydrin, $KMnO_4$. Flash chromatography was performed on 230–400 mesh silica gel. All reagents were purchased from commercial suppliers and used without further purification unless otherwise stated. Solvents were distilled using suitable drying agents (CaH_2 or Na wire, Mg turnings) under the nitrogen atmosphere. ^{1}H, ^{13}C-NMR spectra are recorded on Avance III 400 MHz Ascend Bruker. Chemical shifts are expressed in ppm relative to TMS (^{1}H, 0 ppm) or solvent signals: $CDCl_3$ (^{1}H, 7.26 ppm; ^{13}C, 77.26 ppm) coupling

constants are expressed in Hz. High resolution mass spectra electrospray ionization (HRMS-ESI) performed on Bruker Daltonik LC/MS spectrometer. FT-IR spectra of samples dissolved in CH_2Cl_2 were recorded using Fourier Transform Infrared-Attenuated Total reflection (FTIR-ATR) Spectrometer, Bruker (Tensor-27) over a range of 500–4000 cm^{-1}.

Synthesis of (R)-4-Methyl-N-(2-methylhex-5-en-3-yl)benzenesulfonamide (8)

A 250 mL two-neck round-bottom flask was charged with catalytic amount of CuCN (150 mg, 1.67 mmol) under an inert atmosphere. *N*-Tosylaziridine **7** (4.0 g, 16.74 mmol) dissolved in dry THF (50 mL) was added to CuCN at 0 °C and stirred under inert atmosphere. Vinyl magnesium bromide (20 mL, 20.0 mmol, 1.0 M in THF) was added dropwise to the reaction mixture over a period of 20 minutes with stirring. The reaction mixture was allowed to warm to room temperature and further stirred for 2 h. After the consumption of *N*-tosylaziridine **7**, as confirmed by TLC, the reaction mixture was quenched with saturated NH_4Cl (30 mL) solution and further diluted with EtOAc (100 mL). The aqueous layer was extracted with EtOAc (3 × 75 mL) and the combined organic extracts were washed with brine (100 mL) and dried over anhydrous Na_2SO_4. The organic layer was filtered, evaporated under reduced pressure, and the crude residue was purified over silica gel column chromatography (98:2 hexane/EtOAc) to afford the alkene **8** (3.13 g, 70%) as white solid. m.p. 64–66 °C. TLC: R_f 0.15 (92:8, hexane/EtOAc). IR (CH_2Cl_2): 2963 (N–H), 2924, 2842 (=C–H), 2842 (C–H), 1656, 1619 (C=C) 1459 (C–H), 1262, 1158 (S=O), 698 (=C–H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): $\delta = 7.68$ (d, $J = 8.4$ Hz, 2H), 7.21 (d, $J = 8.4$ Hz, 2H), 5.46–5.35 (m, 1H), 4.90–4.86 (m, 1H), 4.86 (d, $J = 12.0$ Hz, 1H), 4.61 (dd, $J = 12.4$, 2.8 Hz, 1H), 3.05–2.99 (m, 1H), 2.35 (s, 3H), 1.99 (dd, $J = 6.4$ Hz, 2H), 1.73–1.65 (m, 1H), 0.75 (d, 6.8 Hz, 6H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta = 143.1$, 138.3, 133.8, 129.5, 127.2, 118.3, 58.7, 36.1, 30.8, 21.5, 18.6, 17.8 ppm. HRMS (ESI) *m/z* $[M + Na]^+$ calcd. for $C_{14}H_{21}NO_2SNa$ 290.1185, found 290.1195.

Synthesis of (R)-tert-butyl (2-methylhex-5-en-3-yl)(tosyl)carbamate (4)

An oven dried 250 mL round-bottom flask was charged with a solution of alkene **8** (2.0 g, 7.48 mmol) in dry CH_2Cl_2 (50 mL). DMAP (183.2 mg, 1.50 mmol) was added in single portion to the reaction mixture and Boc_2O (3.3 g, 15.0 mmol) was added using syringe. The reaction mixture was stirred at room temperature for 2 h. After the completion of reaction, as conformed by TLC, CH_2Cl_2 was evaporated under reduced pressure and the residue was purified over silica gel column chromatography using 99:1 hexane/EtOAc mixture as eluent to afford **4** (2.39 g, 87%) as a white solid. m.p. 60–65 °C. TLC: R_f 0.29 (95:5, hexane/EtOAc). IR (CH_2Cl_2): 3080 (=C–H), 2968, (C–H), 1720 (C=O), 1639, 1597 (C=C) 1466 (C–H), 1359, 1154 (S=O), 721 (=C–H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): $\delta = 7.79$ (d, $J = 7.2$ Hz, 2H), 7.19 (d, $J = 7.2$ Hz, 2H), 5.73–5.63 (m, 1H), 4.96 (d, $J = 18.4$, 1.2 Hz, 1H), 4.88 (dd, $J = 11.2$, 1.2 Hz, 1H), 4.08–3.99 (m, 1H), 2.63–2.55 (m, 1H), 2.51–2.44 (m, 1H), 2.35 (s, 3H), 2.23–2.13 (m, 1H), 1.32 (s, 9H), 0.95 (d, $J = 6.4$ Hz, 3H), 0.89 (d, $J = 6.4$ Hz, 3H) ppm. ^{13}C NMR

(100 MHz, $CDCl_3$, 25 °C): $\delta = 150.9$, 143.8, 137.3, 136.2, 129.1, 128.7, 117.4, 83.8, 65.9, 36.2, 31.7, 28.1, 21.6, 21.1, 20.9 ppm. HRMS (ESI) *m/z* $[M + Na]^+$ calcd. for $C_{19}H_{29}NO_4SNa$ 390.1710, found 390.1718.

Synthesis of tert-butyl ((3R)-5,6-dihydroxy-2-methylhexan-3-yl)(tosyl)carbamate (9)

In an oven-dried, 50 mL, single-necked, round-bottom flask equipped with a magnetic stirring bar, rubber septum, and nitrogen balloon was placed the alkene (1.0 g, 2.70 mmol) in t-BuOH:H_2O (12 mL, 1:1) and methanesulfonamide (259 mg, 2.72 mmol) was added. The reaction mixture was cooled to 0 °C and $K_3Fe(CN)_6$ (2.687 g, 8.163 mmol), $(DHQD)_2PHAL$ (29.6 mg, 0.038 mmol), K_2OSO_4 (3.98 mg, 0.010 mmol), K_2CO_3 (1.13 g, 8.16 mmol) was added. The resulting mixture was stirred at 0 °C for 12 h. After the consumption of alkene **4**, as confirmed by TLC, the reaction mixture was quenched with solid Na_2SO_3 (5.0 g) and extracted with ethyl acetate (3 × 75 ml). The organic extracts were washed with NaOH (1 M, 20 mL), water and brine, dried over anhydrous Na_2SO_4. The organic layer was filtered, evaporated under reduced pressure, and the crude residue was purified over silica gel column chromatography (70:30 hexane/EtOAc) to afford the dihydroxy compound **9** (957 mg, combined yield 88%, dr = 2:1) as colorless liquid. TLC: R_f 0.14 (50:50, hexane/EtOAc). IR (CH_2Cl_2): 3444 (O–H), 3068 (=C–H), 2973 (C–H), 1726 (C=O), 1627, 1602 (C=C) 1458 (C–H), 1346, 1151 (S=O), 727 (=C–H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): $\delta = 7.92$ (d, $J = 7.6$ Hz, 2H), 7.33 (d, $J = 7.6$ Hz, 2H), 4.16–4.09 (m, 1H), 3.97–3.88 (m, 1H), 3.74–3.63 (m, 1H), 3.57–3.46 (m, 1H), 2.46 (s, 3H), 2.30 (brs, 1H), 2.19 (brs, 1H), 2.10–2.04 (m, 1H), 1.99–1.75 (m, 2H), 1.44 (s, 6H), 1.39* (s, 3H), 0.99–1.05 (m, 3H), 0.95–0.88 (m, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta = 150.9$, 144.5, 144.2*, 136.9, 136.3*, 129.1*, 129.0, 128.8, 84.5*, 84.3, 71.2, 68.7*, 66.8, 66.5*, 63.4, 43.3*, 36.3*, 35.1, 32.0*, 31.1, 28.0, 27.9*, 21.6, 21.5*, 21.3, 21.1, 20.9*, 20.8* ppm. HRMS (ESI) *m/z* $[M + Na]^+$ calcd. for $C_{19}H_{31}NO_6SNa$ 424.1764, found 424.1765. (*peaks from minor diastereoisomer)

Synthesis of tert-butyl ((3R,5R or 3R,5S)-6-((tert-butyldiphenylsilyl)oxy)-5-(methoxymethoxy)-2-methylhexan-3-yl)carbamate (12a and 12)

The N-Boc *N*-tosyl protected amine **11** (0.4 g, 0.59 mmol) was dissolved in dry MeOH (10 mL) in a 50 mL round-bottom flask under an inert atmosphere and then Mg (140 mg, 5.85 mmol) was added. The reaction mixture was submitted to sonic bath for 3 h at room temperature. After the consumption of **11**, as confirmed by TLC, the reaction mixture was concentrated under vacuum. The Reaction residue was quenched with sat. NH_4Cl solution (20 mL) and extracted with EtOAc (3 × 75 ml). The organic extracts were dried over anhydrous Na_2SO_4. The organic layer was filtered, evaporated under reduced pressure, and the crude residue was purified over silica gel column chromatography (90:10 hexane/EtOAc) to afford **12a** and **12b** as colorless liquid and colorless sticky liquid respectively (261 mg, combined yield 84%).

12a (major diastereoisomer) TLC: R_f 0.26 (90:10, hexane/EtOAc); IR (CH_2Cl_2): 3073 (=C–H), 3041 (N–H) 2958, 2922 (C–H), 1704 (C=O), 1510, 1469 (C=C), 1382, 1366

(C–H), 1116 (C–O), 691 (=C–H) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): $\delta = 7.70–7.62$ (m, 4H), 7.44–7.34 (m, 6H), 4.65 (AB system, $J_{AB} = 7.8$ Hz, 2H), 4.41 (d, $J = 8.8$ Hz, 1H), 3.77–3.69 (m, 1H), 3.67–3.55 (m, 3H), 3.30 (s, 3H), 1.81–1.68 (m, 1H), 1.66–1.48 (m, 2H), 1.44 (s, 9H), 1.06 (s, 9H), 0.87 (s, 6H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta = 155.8$, 135.7, 133.5, 129.7, 127.7, 97.2, 78.8, 76.1, 66.5, 55.7, 52.2, 34.9, 32.7, 28.4, 26.9, 19.3, 18.8, 17.9 ppm. HRMS (ESI) *m/z* $[M + Na]^+$ calcd. for $C_{30}H_{47}NO_5SiNa$ 552.3116, found 552.3122.

12b (minor diastereoisomer) TLC: R_f 0.25 (90:10, hexane/EtOAc); ^1H NMR (400 MHz, $CDCl_3$, 25 °C): $\delta = 7.64–7.57$ (m, 4H), 7.44–7.34 (m, 6H), 4.57 (d, $J = 6.68$ Hz, 1H), 4.65 (AB system, $J_{AB} = 6.60$ Hz, 2H), 3.71–3.63 (m, 1H), 3.63–3.55 (m, 2H), 3.54–3.48 (m, 1H), 3.22 (s, 3H), 1.84–1.73 (m, 2H), 1.49–1.38 (m, 1H), 1.34 (s, 9H), 0.98 (s, 9H), 0.82 (d, $J = 6.76$ Hz, 3H), 0.78 (d, $J = 6.76$ Hz, 3H).

Synthesis of ethyl-2-((5R,7R)-7-isopropyl-11,11-dimethyl-9-oxo-2,4,10-trioxa-8-azadodecan-5-yl)thiazole-4-carboxylate (1)

Lawesson's reagent (66.2 mg, 0.17 mmol) was added to a solution of N-Boc-γ-aminocarboxamide **14** (50 mg, 0.17 mmol) in dry THF (5 mL) in a round bottom flask (50 mL) at room temperature. The resulting solution was further stirred for 12 h at the same temperature under nitrogen atmosphere. After the complete consumption of **14**, the reaction mixture was quenched by addition of saturated aq. $NaHCO_3$ (20 mL) at room temperature and the aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic layers was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. The crude residue was purified over silica gel column chromatography using 80:20 hexane/EtOAc mixture as eluent to afford thioamide **15** (32 mg, 60%) as a light-yellow liquid and used immediately for thiazole ring formation in the next step. TLC: Rf 0.53 (30:70, hexane/EtOAc). A round-bottom flask (25 mL) was charged with activated 3 Å molecular sieves (100 mg), magnetic bead and dry EtOH (5 mL) under an inert atmosphere. N-Boc-γ-aminothioamide **15** (18 mg, 0.07 mmol) and ethyl bromopyruvate (0.02 mL, 0.10 mmol) was added to the reaction mixture at room temperature. The reaction mixture was heated at 65 °C for 1 h. After the consumption of thioamide **15**, as confirmed by TLC, molecular sieves were filtered through Buchner funnel, solvent evaporated under reduced pressure and the crude residue was purified over silica gel column chromatography (70:30 hexane/EtOAc) to afford 2-alkyl substituted thiazole ethyl ester **1** (25 mg, 83%) as colorless liquid. TLC: Rf 0.38 (70:30, hexane/EtOAc). IR (CH_2Cl_2): 2968, 2927 (C–H), 1781, 1745 (C=O), 1632 (C = N), 1474, 1366 (C–H), 1172 (C–O), 1162 (S–O), 1029 (C–O), 860 (=C–H), 701 (C–H) cm-1. ^1H NMR (400 MHz, $CDCl_3$, 25 °C): $\delta = 8.12$ (s, 1H), 5.09 (br d, $J = 7.2$ Hz, 1H), 4.71 (d, $J = 6.8$ Hz, 1H), 4.69 (d, $J = 6.8$ Hz, 1H), 4.42 (br d, $J = 6.8$ Hz, 1H), 4.31 (q, $J = 6.8$ Hz, 2H), 3.76–3.69 (m, 1H), 3.40 (s, 3H), 1.93–1.80 (m, 1H), 1.65–1.56 (m, 2H), 1.36 (s, 9H), 1.25 (t, $J = 6.8$ Hz, 3H), 0.89 (d, $J = 7.1$ Hz, 3H), 0.87 (d, $J = 7.1$ Hz, 3H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta = 174.7$, 161.4, 155.2, 147.1, 127.6, 95.8, 61.4, 56.3, 51.9, 38.1, 31.7, 29.5, 28.3, 18.7, 17.2, 14.3 ppm. HRMS (ESI) *m/z* $[M + Na]^+$ calcd. for $C_{19}H_{32}N_2O_6SNa$ 439.1873, found 439.1907.

Methods and materials of all the synthesized compounds, full experimental procedures, spectroscopic data (**2**, **3, 6, 7, 10–11** and **14**), copies of ^{1}H NMR and ^{13}C NMR (**1–4**, **6–11, 12a**, **12b** and **14**) have been included in the supplementary data. This material can be found via the Supplementary Information section of this article's webpage.

Acknowledgments

We thank Sophisticated Instrumentation Centre (SIC), IIT Indore for compound characterization facilities.

Disclosure statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

The authors are thankful to Ministry of Human Resource Development (MHRD), Government of India, Indian Institute of Technology (IIT) Indore, India, for research student's fellowship. We would like to extend our sincere gratitude to Science and Engineering Research Board, Department of Science and Technology, Govt. of India for providing funding under the grant [number EMR/2015/001764].

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