Electronic Supplementary Information

Synthesis of the Deacetoxytubuvaline Fragment of Pretubulysin and its Lipophilic Analogues for Enhanced Permeability in Cancer Cell Lines

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Experimental Section

General methods. All reactions were performed in oven-dried glassware under an inert atmosphere with magnetic stirring. Air and moisture-sensitive liquids and solutions were transferred via glass syringes. TLC was performed on 0.25 mm Merck TLC silica gel 60 F254 plates and visualized under UV light (254 nm) or by staining with bromocresol green, ninhydrin, KMnO4. Flash chromatography was performed using 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 (CaH2 or Na wire, Mg turnings) under the nitrogen atmosphere. 1H and 13C NMR spectra are recorded on Avance III 400 MHz Ascend Bruker. Chemical shifts are expressed in ppm relative to TMS (1H, 0 ppm) or solvent signals: CDCl3 (1H, 7.26 ppm; 13C, 77.26 ppm) coupling constants are expressed in Hz. High resolution mass spectra were recorded by electrospray ionization (HRMS-ESI) technique using Bruker Daltonik LC/MS spectrometer. FT-IR spectra 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 (S)-2-Isopropyl-1-tosylaziridine (4) 22

L-valine (10.0 g, 85.36 mmol) was dissolved in dry THF (100 mL) in a 250 mL round-bottom flask under an inert atmosphere. The reaction mixture was cooled to 0 °C, and solid NaBH₄ (8.07 g, 213.4 mmol) was added to the reaction mixture in a single portion with stirring. A solution of iodine (37.90 g, 149.38 mmol) in dry THF (50 mL) was added to the reaction mixture dropwise using dropping funnel over a period of 1 h. The reaction mixture was further heated to reflux on a preheated oil bath overnight. The reaction mixture was cooled to room temperature and methanol (50 mL) was slowly added until a clear solution is obtained. Solvent was evaporated under reduced pressure and the resulting white paste was dissolved in 2M NaOH (50 mL). The mixture was stirred at room temperature for 8 h. The aqueous phase was extracted with CH₂Cl₂ (100 x 3 mL) and the combined organic layers was dried over anhydrous Na₂SO₄, filtered, and concentrated under reduced pressure to afford L-valinol (7.50 g, 91%) as a colorless liquid, which was used in the next step without further purification.

4 Å molecular sieves (2.00 g) were flame dried in a 100 mL two neck round bottom flask under reduced pressure for 15 min or more until there is no sign of the appearance of water droplets or moisture in the flask. The heat dried molecular sieves were cooled to room temperature under inert atmosphere. CH₃CN (50 mL), magnetic bar and L-valinol (5.00 g, 48.47 mmol) was charged to this flask. The reaction mixture was briefly cooled to 0 °C and Et₃N (19.9 mL, 145.41 mmol) and tosyl chloride (18.5 g, 96.94 mmol) were added
sequentially via syringe to the reaction mixture. The reaction mixture was warmed to room temperature and further stirred for 1 h. After complete consumption of L-valinol, as confirmed by TLC, acetonitrile was evaporated under reduced pressure and the residue was dissolved in EtOAc (50 mL). The resultant precipitate and molecular sieves were filtered using Buchner funnel and washed with EtOAc (3 × 50 mL). The organic solvent was concentrated under reduced pressure and the crude mixture was purified by silica gel column chromatography (99:1 hexane/EtOAc) to afford 4 (9.00 g, 78%) as a white solid. m.p. 87–90 °C. TLC: Rf 0.30 (92:8, hexane/EtOAc). [α]D23 = +10.6 (c 0.1, CH2Cl2). IR (CH2Cl2): 3023 (≡C –H), 2922 (C –H), 1593, 1479, 1445 (C=C), 1317, 1157 (S=O), 720 (≡C –H) cm⁻¹. 1H NMR (400 MHz, CDCl3, 25 °C): δ = 7.76 (d, J = 7.6 Hz, 2H), 7.26 (d, J = 7.6 Hz, 2H), 2.54 (d, J = 6.8 Hz, 1H), 2.47–2.40 (m, 1H), 2.37 (s, 3H), 2.03 (d, J = 2.8, Hz, 1H), 1.38–1.30 (m, 1H), 0.83 (d, J = 6.4 Hz, 3H), 0.71 (d, J = 6.4 Hz, 3H) ppm. 13C NMR (100 MHz, CDCl3, 25 °C): δ = 144.4, 135.1, 129.5, 128.0, 46.2, 32.7, 30.1, 21.6, 19.5, 19.0 ppm. HRMS (ESI) m/z [M+Na]+ calcd. for C12H17NO2S 262.0872, found 262.0885.

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

A 250 mL two neck round-bottom flask was charged with a catalytic amount of CuCN (0.262 g, 2.928 mmol) under an inert atmosphere. N-Tosylaziridine 4 (7.00 g, 29.28 mmol) dissolved in dry THF (50 mL) was added to CuCN at 0 °C and stirred under inert atmosphere. Vinyl magnesium bromide (58.5 mL, 58.5 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 4, as confirmed by TLC, the reaction mixture was quenched with saturated NH4Cl (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 Na2SO4. 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 3 (5.50 g, 72%) as white solid. m.p. 64–66 °C. TLC: Rf 0.28 (92:8, hexane/EtOAc). [α]D22 = −50.8 (c 0.1, CH2Cl2). IR (CH2Cl2): 3283 (N –H), 3065, 3025 (=C –H), 2924 (C=C), 1643, 1602 (C=C) 1485–1445 (C –H), 1320, 1154 (S=O), 703 (=C –H) cm⁻¹. 1H NMR (400 MHz, CDCl3, 25 °C): δ = 7.68 (d, J = 8.4 Hz, 2H), 7.21 (d, J = 8.4 Hz, 2H), 5.46–5.36 (m, 1H), 4.88–4.83 (m, 2H), 4.61 (brs, 1H), 3.05–2.99 (m, 1H), 2.35 (s, 3H), 1.99 (t, J = 6.8, 6.4 Hz, 2H), 1.73–1.65 (m, 1H), 0.75 (d, 6.8 Hz, 6H) ppm. 13C NMR (100 MHz, CDCl3, 25 °C): δ = 143.1, 138.2, 133.8, 129.5, 127.1, 118.3, 58.7, 36.1, 30.8, 21.5, 18.5, 17.8 ppm. HRMS (ESI) m/z [M+Na]+ calcd. for C14H21NO2S 290.1185, found 290.1195.
Synthesis of (R)-tert-Butyl (2-methylhex-5-en-3-yl)(tosyl)carbamate (5)

An oven dried 250 mL round-bottom flask was charged with a solution of alkene 3 (5.83 g, 21.8 mmol) in dry CH₂Cl₂ (50 mL). DMAP (0.532 g, 4.36 mmol) was added in single portion to the reaction mixture and Boc₂O (9.50 g, 43.6 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, CH₂Cl₂ 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 5 (8.00 g, 87%) as a white solid. m.p. 60–65 °C. TLC: Rᵣ 0.35 (95:5, hexane/EtOAc). [α]D²³⁴ = −171.2 (c 0.1, CH₂Cl₂). IR (CH₂Cl₂): 3054, 3023 (=C –H), 2921 (C–H), 1700 (C=O), 1642, 1612 (C=C) 1485–1445 (C–H), 1320, 1151 (S=O), 703 (=C–H) cm⁻¹. ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 7.79 (d, J = 8.0 Hz, 2H), 7.19 (d, J = 8.0 Hz, 2H), 5.73–5.63 (m, 1H), 4.96 (d, J = 17.2 Hz, 1H), 4.88 (d, J = 10.0 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. ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 150.8, 143.8, 137.3, 136.1, 129.1, 128.6, 117.3, 83.8, 65.8, 36.2, 31.6, 28.0, 21.5, 21.1, 20.9 ppm. HRMS (ESI) m/z [M+Na]⁺ calcd. for C₁₉H₂₉NO₄S 390.1710, found 390.1732.

Synthesis of (R)-tert-Butyl (6-hydroxy-2-methylhexan-3-yl)(tosyl)carbamate (6)

9-BBN (29.3 ml, 14.698 mmol, 0.5 M in THF) was added to a solution of alkene 5 (2.00 g, 7.47 mmol) in dry THF (20 mL) in a round bottom flask (100 mL) at room temperature. The resulting solution was further stirred for 12 h at the same temperature under nitrogen atmosphere. 2M NaOH (13.0 mL, 26.14) and 30% H₂O₂ (10.4 ml, 104 mmol) were added at 0 °C to the reaction mixture and stirring was continued for 12 h at room temperature. The reaction mixture was quenched by adding saturated aq. NaCl (30 mL) at room temperature and the aqueous layer was extracted with EtOAc (3 × 100 mL). The combined organic layers was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude residue was purified over silica gel column chromatography using 70:30 hexane/EtOAc mixture as eluent to afford 6 (1.86 g, 93%) as a colorless liquid. TLC: Rᵣ 0.30 (70:30, hexane/EtOAc). [α]D²³⁶ = +14.7 (c 0.1, CH₂Cl₂). IR (CH₂Cl₂): 3367 (O–H), 3061, 3030 (=C–H), 2930 (C–H), 1642, 1639 (C=C) 1486–1461 (C–H), 1321, 1151 (S=O), 703 (=C–H) cm⁻¹. ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 7.88 (d, J = 8.0 Hz, 2H), 7.29 (d, J = 8.0 Hz, 2H), 4.02 (td, J = 10.0, 4.0 Hz, 1H), 3.70–3.63 (m, 2H), 2.43 (s, 3H), 2.29–2.14 (m, 1H), 2.02–1.92 (m, 1H), 1.89–1.79 (m, 1H), 1.67–1.57 (m, 2H), 1.41 (s, 9H), 1.31 (brs, 1H), 1.01 (d, J = 6.8 Hz, 3H), 0.88 (d, J = 6.8 Hz, 3H) ppm. ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 151.1, 144.0, 137.1, 129.1, 128.8, 83.97, 66.3, 62.7, 31.8, 30.6, 28.0, 27.9, 21.6, 21.2, 21.0 ppm. HRMS (ESI) m/z [M+Na]⁺ calcd. for C₁₉H₃₁NO₅S 408.1815, found 408.1740.
Synthesis of \((R)-4-(N-(\text{tert}-\text{Butoxycarbonyl})-4\text{-methylphenylsulfonamido})-5\text{-methylhexanoic acid (7)}\)

Dry DMF (20 mL) was added to 1,4-amino alcohol 6 (2.00 g, 5.18 mmol) taken in a 100 mL round-bottom flask charged with a magnetic bead and the mixture was stirred at room temperature. Pyridinium dichromate (9.70 g, 25.93 mmol) was added to above reaction mixture in one portion and stirred for 24 h at the same temperature. After the complete consumption of 1,4-amino alcohol 6, the reaction mixture was quenched with cold water (20 mL). The aqueous layer was extracted with EtOAc (3 × 100 mL) and the combined organic layers was washed with cold brine (3 × 50 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The residue was purified over silica gel using column chromatography (60:40 hexane/EtOAc), to afford 7 (1.70 g, 85 %) as colorless liquid. TLC: \(R_f\) 0.14 (30:70, hexane/EtOAc). \([\alpha]^{23.8}_{D} = +79.6\) (c 0.1, CH₂Cl₂). IR (CH₂Cl₂): 3751 (O–H), 3061, 3026 (=C–H), 2860 (C–H), 1719, 1610 (C=O), 1486–1475 (C–H), 1346, 1141 (S=O), 745 (C–H) cm⁻¹. \(^1\)H NMR (400 MHz, CDCl₃, 25 °C): \(\delta = 7.88\) (d, \(J = 7.6\) Hz, 2H), 7.29 (d, \(J = 7.6\) Hz, 2H), 4.08–3.93 (m, 1H), 2.49–2.39 (m, 5H), 2.31–2.15 (m, 2H), 2.14–2.04 (m, 1H), 1.42 (s, 9H), 1.03 (d, \(J = 6.4\) Hz, 3H), 0.86 (d, \(J = 6.4\) Hz, 3H) ppm. \(^{13}\)C NMR (100 MHz, CDCl₃, 25 °C): \(\delta = 178.8, 144.2, 136.8, 129.1, 128.9, 126.4, 84.2, 65.6, 31.6, 31.5, 28.0, 26.3, 21.6, 21.1, 20.1\) ppm. HRMS (ESI) m/z \([M+Na]^+\) calcd. for C₁₉H₂₉NO₆S 422.1608, found 422.1625.

Synthesis of \((R)-4-(\text{tert}-\text{Butoxycarbonyl}α\text{-amino})-5\text{-methylhexanoic acid (2)}\)

In a 100 mL round-bottom flask charged with magnetic bead, Mg turnings (0.600 g, 25 mmol) and NH₄Cl (0.534 g, 10 mmol), dry methanol (5 mL) was added under an inert atmosphere with stirring. A solution of N-Boc-N-tosyl-γ-amino acid 7 (1.00 g) in dry methanol (5mL) was added to the above suspension and stirred at room temperature for 10 minutes. The reaction mixture was further refluxed at 70 °C for 2 h. After the complete consumption of 7, methanol was concentrated under reduced pressure and the crude reaction mixture was purified over silica gel column chromatography (50:50 hexane/EtOAc) to afford 2 (498 mg, 81%) as pale yellow liquid. TLC: \(R_f\) 0.14 (20:80, hexane/EtOAc). \([\alpha]^{22.2}_{D} = +10.2\) (c 0.1, CH₂Cl₂). IR (CH₂Cl₂): 3745 (O–H), 2968 (C–H), 1704, 1596 (C=O), 1435, 1420, 735 (C–H) cm⁻¹. \(^1\)H NMR (400 MHz, CDCl₃): \(\delta = 4.38\) (d, \(J = 9.6\) Hz, 1H), 3.46–3.35 (m, 1H), 2.43–2.35 (m, 2H), 1.93–1.79 (m, 1H), 1.75–1.65 (m, 1H), 1.62–1.51 (m, 1H), 1.44 (s, 9H), 0.90 (t, \(J = 7.2\) Hz, 6H) ppm. \(^{13}\)C NMR (100 MHz, CDCl₃, 25 °C): \(\delta = 178.2, 156.3, 79.4, 55.2, 32.5, 31.31, 28.3, 27.8, 19.0, 17.7\) ppm. HRMS (ESI) m/z [M+Na]⁺ calcd. for C₁₂H₂₃NO₄ 268.1519, found 268.1519.
Synthesis of (S)-Methyl 2-((R)-4-((tert-butoxycarbonyl)amino)-5-methylhexanamido)-3-hydroxypropanoate (8)

A 50 mL round-bottom flask was charged with N-Boc-γ-aminoacid 2 (0.200 g, 0.81 mmol), magnetic bead and dry CH₂Cl₂ (10 mL) under an inert atmosphere. L-serine methyl ester (0.189 g, 1.21 mmol), HOBT (0.164 g, 1.215 mmol), EDC (0.232 g, 1.215 mmol) were added as solid and DIPEA (0.35 mL, 2.02 mmol) using syringe to the reaction mixture at 0 °C. The reaction mixture was warmed to room temperature and further stirred for 12 h. After the consumption of N-Boc-γ-aminoacid 2, as confirmed by TLC, the reaction mixture was quenched with saturated NH₄Cl (10 mL) solution and further diluted with CH₂Cl₂ (20 mL). The aqueous layer was extracted with CH₂Cl₂ (3 × 25 mL) and the combined organic extracts was washed with brine (50 mL) and dried over anhydrous Na₂SO₄. The organic layer was filtered, evaporated under reduced pressure and the crude residue was purified over silica gel column chromatography (60:40 hexane/EtOAc) to afford 8 (170 mg, 73%) as yellow solid. m.p. 131–136 °C. TLC: Rₜ 0.14 (20:80, hexane/EtOAc). [α]D²⁴ = −5.8 (c 0.1, CH₂Cl₂). IR (CH₂Cl₂): 3382 (O –H), 2978 (C–H), 1721, 1600 (C=O), 1470, 1455, 755 (C–H) cm⁻¹. ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 7.16 (d, J = 6.8 Hz, 1H), 4.61–4.58 (m, 1H), 4.54 (d, J = 10.0 Hz, 1H), 4.0 (dd, J = 11.2, 3.6 Hz, 1H), 3.93 (dd, J = 11.2, 3.2 Hz, 1H), 3.78 (s, 3H), 3.58–3.51 (m, 1H), 3.32 (brs, 1H), 2.32 (t, J = 6.8 Hz, 2H), 1.91–1.83 (m, 1H), 1.76–1.70 (m, 1H), 1.66–1.58 (m, 1H), 1.44 (s, 9H), 0.92 (d, J = 6.8 Hz, 3H), 0.90 (d, J = 6.8 Hz, 3H) ppm. ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 173.4, 170.8, 156.8, 79.7, 63.1, 55.1(2C*), 52.6, 33.3, 32.5, 28.6, 28.4, 19.1, 17.7 ppm. HRMS (ESI) m/z [M+Na]⁺ calcd. for C₁₆H₃₀N₂O₆ 369.1996, found 369.1987.

*higher intensity carbon

Synthesis of (R)-tert-Butyl (6-amino-2-methyl-6-oxohexan-3-yl)carbamate (9)

A 50 mL round-bottom flask was charged with N-Boc-γ-aminoacid 2 (0.200 g, 0.81 mmol), magnetic bead and dry CH₂Cl₂ (10 mL) under an inert atmosphere. Ammonium chloride (0.076 g, 1.75 mmol), HOBT (0.137 g, 1.01 mmol), HBTU (0.383 g, 1.01 mmol) were added as solid and DIPEA (0.50 mL, 2.85 mmol) using syringe to the reaction mixture at 0 °C. The reaction mixture was warmed to room temperature and further stirred for 4 h. After the consumption of N-Boc-γ-aminoacid 2, as confirmed by TLC, the reaction mixture was quenched with saturated NH₄Cl (10 mL) solution and further diluted with CH₂Cl₂ (20 mL). The aqueous layer was extracted with CH₂Cl₂ (3 × 25 mL) and the combined organic extracts was washed with brine (50 mL) and dried over anhydrous Na₂SO₄. The organic layer was filtered, evaporated under reduced pressure and the crude residue was purified over silica gel column chromatography (50:50 hexane/EtOAc) to afford
N-Boc-γ-aminocarboxamide 9 (190 mg, 95%) as white solid. m.p. 115–120 °C TLC: \( R_f \) 0.14 (20:80, hexane/EtOAc). \([\alpha]_{D}^{24.1}\) = −93.4 (c 0.1, CH\(_2\)Cl\(_2\)). IR (CH\(_2\)Cl\(_2\)): 3442 (N –H), 2976 (C –H), 1700, 1590 (C=O), 1465, 1453, 753 (C–H) cm\(^{-1}\). \(^1\)H NMR (400 MHz, CDCl\(_3\), 25 °C): \( \delta \) = 6.34 (brs, 1H), 5.44 (brs, 1H), 4.41 (d, \( J = 9.2 \) Hz, 1H), 3.54–3.38 (m, 1H), 2.26 (t, \( J = 6.4 \) Hz, 2H), 1.91–1.82 (m, 1H), 1.73–1.65 (m, 1H), 1.62–1.53 (m, 1H), 1.44 (s, 9H), 0.91 (t, \( J = 8.8 \) Hz, 6H) ppm. 13C NMR (100 MHz, CDCl\(_3\), 25 °C): \( \delta \) = 175.6, 156.8, 79.4, 55.2, 32.9, 32.6, 29.2, 28.4, 19.2, 17.7 ppm. HRMS (ESI) m/z [M+Na]+ calcd. for C\(_{12}\)H\(_{24}\)N\(_2\)O\(_3\) 267.1679, found 267.1683.

Synthesis of \((R)-\text{tert-Butyl (6-amino-2-methyl-6-thioxohexan-3-yl)carbamate (10)}\)

Lawesson’s reagent (0.165 g, 0.41 mmol) was added to a solution of N-Boc-γ-aminocarboxamide 9 (0.100 g, 0.41 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 9, the reaction mixture was quenched by adding saturated aq. NaHCO\(_3\) (30 mL) at room temperature and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers was dried over anhydrous Na\(_2\)SO\(_4\), filtered and concentrated under reduced pressure. The crude residue was purified over silica gel column chromatography using 70:30 hexane/EtOAc mixture as eluent to afford 10 (95.0 mg, 89%) as a colorless liquid. TLC: \( R_f \) 0.25 (70:30, hexane/EtOAc). \([\alpha]_{D}^{24.3}\) = +12.4 (c 0.1, CH\(_2\)Cl\(_2\)) IR (CH\(_2\)Cl\(_2\)): 3332 (N –H), 2976 (C–H), 1675 (C=O), 1440, 1430 (C–H), 1360, 1165 (S=O), 765 (C–H) cm\(^{-1}\). \(^1\)H NMR (400 MHz, CDCl\(_3\), 25 °C): \( \delta \) = 8.79 (brs, 1H), 7.52 (brs, 1H), 4.45 (d, \( J = 10 \) Hz, 1H), 3.46–3.40 (m, 1H), 2.85–2.79 (m, 1H), 2.67–2.60 (m, 1H), 2.06–1.97 (m, 1H), 1.74–1.64 (m, 2H), 1.45 (s, 9H), 1.86 (d, \( J = 6.8 \) Hz, 3H), 0.90 (d, \( J = 6.8 \) Hz, 3H) ppm. 13C NMR (100 MHz, CDCl\(_3\), 25 °C): \( \delta \) = 210.5, 157.6, 80.0, 54.4, 41.9, 33.4, 32.4, 28.36, 19.4, 17.6 ppm. HRMS (ESI) m/z [M+Na]+ calcd. for C\(_{12}\)H\(_{24}\)N\(_2\)O\(_3\)S 283.1451, found 283.1430.

Synthesis of \((R)-\text{Ethyl 2-(3-(((tert-butoxycarbonyl)amino)-4-methylpentyl)thiazole-4-carboxylate (11)}\)

A 25 mL round-bottom flask was charged with activated 3 Å molecular sieves (0.200 g), magnetic bead and dry EtOH (5 mL) under an inert atmosphere. N-Boc-γ-aminothioamide 10 (0.070 g, 0.26 mmol) and ethyl bromopyruvate (0.05 mL, 0.40 mmol) was added to the reaction mixture at room temperature. The reaction mixture was allowed to warm to 65 °C for 1 h. After the consumption of thioamide 10, 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 (80:20 hexane/EtOAc) to afford 2-alkyl substituted thiazole ester 11 (80.0 mg, 86%) as colorless liquid. TLC: \( R_f \) 0.43 (70:30, hexane/EtOAc). \([\alpha]_{D}^{24.3}\) = +11.7 (c 0.1,
CH$_2$Cl$_2$). IR (CH$_2$Cl$_2$): 2964, 2922 (C–H), 1721, 1695 (C=O), 1680 (C=N), 1482, 1392, 1365 (C–H), 1171 (C–O), 869 (=C–H), 770, 710 (C–H) cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$, 25 °C): $\delta = 8.04$ (s, 1H), 4.45–4.39 (m, 3H), 3.56–3.51 (m, 1H), 3.21–3.13 (m, 1H), 3.10–3.02 (m, 1H), 2.06–1.98 (m, 1H), 1.82–1.73 (m, 2H), 1.44 (s, 9H), 1.40 (t, $J = 7.2$ Hz, 3H), 0.92 (d, $J = 7.2$ Hz, 3H), 0.90 (d, $J = 7.2$ Hz, 3H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$, 25 °C): $\delta = 171.6, 161.4, 156.0, 146.9, 126.9, 79.1, 61.4, 55.3, 33.1, 32.5, 30.8, 28.4, 19.1, 17.7, 14.4$ ppm. HRMS (ESI) m/z [M+Na]$^+$ calcd. for C$_{17}$H$_{28}$N$_2$O$_4$S 379.1662, found 379.1692.

**Synthesis of (2S,3S)-Methyl 2-((R)-4-((tert-butoxycarbonyl)amino)-5-methylhexanamido)-3-hydroxybutanoate (12)**

A 50 mL round-bottom flask was charged with N-Boc-$\gamma$-aminoacid 2 (0.300 g, 1.22 mmol), magnetic bead and dry CH$_2$Cl$_2$ (10 mL) under an inert atmosphere. L-threonine methyl ester (0.310 g, 1.83 mmol), HOBT (0.247 g, 1.83 mmol), EDC (0.350 g, 1.83 mmol) were added as solid and DIPEA (0.53 mL, 2.5 mmol) using syringe to the reaction mixture at 0 °C. The reaction mixture was warmed to room temperature and further stirred for 12 h. After the consumption of N-Boc-$\gamma$-aminoacid 2, as confirmed by TLC, the reaction mixture was quenched with saturated NH$_4$Cl (10 mL) solution and further diluted with CH$_2$Cl$_2$ (20 mL). The aqueous layer was extracted with CH$_2$Cl$_2$ (3 × 25 mL) and the combined organic extracts was washed with brine (50 mL) and dried over anhydrous Na$_2$SO$_4$. The organic layer was filtered, evaporated under reduced pressure, and the crude residue was purified over silica gel column chromatography (60:40 hexane/EtOAc) to afford dipeptide 12 (300 mg, 70%) as colorless liquid. TLC: $R_f$ 0.23 (40:60, hexane/EtOAc). $[\alpha]_{D}^{24.3}$ = +15.2 ($c$ 0.1, CH$_2$Cl$_2$). IR (CH$_2$Cl$_2$): 3756 (O–H), 3445 (N–H), 2976 (C–H), 1740, 1670 (C=O), 1460, 1450 (C–H), 1172 (C–O), 750 (C–H) cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$, 25 °C): $\delta = 7.16$ (d, $J = 8.0$ Hz, 1H), 4.53–4.48 (m, 2H), 4.36–4.33 (m, 1H), 3.77 (s, 3H), 3.66–3.58 (m, 1H), 2.33 (t, $J = 7.6$ Hz, 2H), 1.93–1.85 (m, 1H), 1.76–1.68 (m, 2H), 1.62–1.54 (m, 1H), 1.44 (s, 9H), 1.25 (d, $J = 6.4$ Hz, 3H), 0.93 (d, $J = 6.8$ Hz, 3H), 0.91 (d, $J = 6.8$ Hz, 3H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$, 25 °C): $\delta = 173.7, 171.4, 156.9, 79.6, 67.9, 57.8, 55.1, 52.4, 33.3, 32.5, 30.8, 28.4, 19.1, 17.7$ ppm. HRMS (ESI) m/z [M+Na]$^+$ calcd. for C$_{17}$H$_{32}$N$_2$O$_6$ 383.2153, found 383.2158.

**Synthesis of (S)-Methyl 2-((R)-4-((tert-butoxycarbonyl)amino)-5-methylhexanamido)-3-oxobutanoate (13)**

Dess-Martin periodinane reagent (0.305 g, 0.72 mmol) was added at 22 °C to a stirred solution of dipeptide 12 (0.200 g, 0.55 mmol) in dry CH$_2$Cl$_2$ (5 mL) in a round bottom flask (50 mL). The reaction mixture was warmed to room temperature and further stirred for 4 h. After complete consumption of 12, as confirmed by TLC, the reaction mixture was quenched by addition of
saturated Na$_2$S$_2$O$_3$ (2 mL) and saturated NaHCO$_3$ (2 mL). The resulting reaction mixture was extracted with CH$_2$Cl$_2$ (3 × 50 mL). The combined organic layers was dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The crude residue was purified over silica gel column chromatography using 70:30 hexane/EtOAc mixture as eluent to afford ketoamide ester 13 (160 mg, 82%) as a yellow liquid. TLC: $R_f$ 0.43 (60:40, hexane/EtOAc). [$\alpha$]$D_{24}^{4,4}$ = +17.4 (c 0.1, CH$_2$Cl$_2$). IR (CH$_2$Cl$_2$): 3440 (N–H), 2976 (C–H), 1740, 1725 1670 (C=O), 1465, 1453 (C–H), 1172 (C–O), 745 (C–H) cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$, 25 °C): $\delta$ = 7.06 (brs, 1H), 5.22 (brs, 1H), 4.38 (s, 1H), 3.77 (s, 3H), 3.50–3.35 (m, 1H), 2.36–2.23 (m, 5H), 1.88–1.75 (m, 1H), 1.72–1.61 (m, 1H), 1.60–1.48 (m, 1H), 1.40 (s, 9H), 0.89–0.82 (m, 6H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$, 25 °C): $\delta$ = 198.6, 173.0, 166.6, 156.4, 79.2, 63.2, 55.2, 53.2, 33.0, 28.5, 28.4, 28.1, 19.0 ppm. Diagnostic signals of minor rotamer $^{13}$C NMR (100 MHz, CDCl$_3$, 25 °C): $\delta$ = 198.5, 172.9, 166.5, 141.0, 63.1, 51.1, 32.9, 28.4, 28.0, 17.8 ppm. HRMS (ESI) m/z [M+Na]$^+$ calcd. for C$_{17}$H$_{30}$N$_2$O$_6$ 381.1996, found 381.1985.

Synthesis of (R)-Methyl 2-(3-((tert-butoxycarbonyl)amino)-4-methylpentyl)-5-methylthiazole-4-carboxylate (14)

Lawesson’s reagent (0.225 g, 0.55 mmol) was added to a solution of ketoamide ester 13 (0.100 g, 0.28 mmol) in dry THF (5 mL) in a round bottom flask (25 mL) at room temperature. The resulting solution was refluxed for 6 h under nitrogen atmosphere. After the complete consumption of ketoamide ester 13, the reaction mixture was cooled to room temperature, quenched by addition of saturated aq. NaHCO$_3$ (30 mL) and the aqueous layer was extracted with EtOAc (3 × 50 mL). The combined organic layers was dried over anhydrous Na$_2$SO$_4$, filtered and concentrated under reduced pressure. The crude residue was purified over silica gel column chromatography using 85:15 hexane/EtOAc mixture as eluent to afford 14 (70.0 mg, 70%) as a yellow liquid. TLC: $R_f$ 0.52 (70:30, hexane/EtOAc). [$\alpha$]$D_{24}^{4,4}$ = −26.2 (c 0.1, CH$_2$Cl$_2$). IR (CH$_2$Cl$_2$): 2968, 2930 (C–H), 1728, 1696 (C=O), 1680 (C=N), 1485, 1395 (C–H), 1170 (C–O), 1162 (S–O), 865 (=C–H), 772, 720 (C–H) cm$^{-1}$. $^1$H NMR (400 MHz, CDCl$_3$, 25 °C): $\delta$ = 4.33 (d, $J$ = 9.6 Hz, 1H), 3.92 (s, 3H), 3.55–3.50 (m, 1H), 3.11–3.03 (m, 1H), 3.0–2.92 (m, 1H), 2.73 (s, 3H), 2.0–1.91 (m, 1H), 1.76–1.68 (m, 2H), 1.44 (s, 9H), 0.91 (d, $J$ = 6.8 Hz, 3H), 0.89 (d, $J$ = 6.8 Hz, 3H) ppm. $^{13}$C NMR (100 MHz, CDCl$_3$, 25 °C): $\delta$ = 166.8, 162.9, 155.9, 144.7, 140.4, 79.1, 55.3, 52.0, 33.1, 32.5, 30.5, 28.4, 19.1, 17.7, 13.1 ppm. HRMS (ESI) m/z [M+Na]$^+$ calcd. for C$_{17}$H$_{30}$N$_2$O$_6$S 379.1662, found 379.1675.

Synthesis of (R)-methyl 2-(3-((tert-butoxycarbonyl)(methyl)amino)-4-methylpentyl)-5-methylthiazole-4-carboxylate (15)

Dry DMF (2 mL) was added to 14 (0.070 g, 0.196 mmol) taken in a 10 mL round bottom flask charged with a magnetic bead. MeI (0.05 mL, 0.786 mmol) was added to above reaction mixture
and cooled to 0 °C. NaH (0.012 g, 0.49 mmol) was added portion wise over a period of 15 min and the reaction was stirred at 25 °C under N₂ atmosphere for 12 h. After the complete consumption of 14, the reaction mixture was quenched with a saturated solution of NH₄Cl (10 mL). The aqueous layer was extracted with EtOAc (3 × 25 mL) and the combined organic layers was washed with cold brine (3 × 10 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure and the crude residue was purified over silica gel column chromatography (80:20 hexane/EtOAc) to afford 5-methylthiazole analogue 15 (58.0 mg, 80%) as colorless liquid. TLC: Rₜ 0.25 (70:30, hexane/EtOAc). [α]D²⁴ = −15.8 (c 0.1, CH₂Cl₂). IR (CH₂Cl₂): 2961, 2925 (C-H), 1725, 1694 (C=O), 1683 (C=N), 1482, 1390 (C–H), 1170 (C–O), 1160 (S–O), 866 (=C–H), 774, 721 (C–H) cm⁻¹.¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 3.93 (s, 3H, rotamer 1), 3.92 (s, 3H, rotamer 2), 3.87–3.81 (m, 1H, rotamer 1), 3.69–3.65 (m, 1H, rotamer 2), 2.89–2.85 (m, 4H, rotamers 1+2), 2.74 (s, 3H, rotamer 1), 2.73 (s, 3H, rotamer 2), 2.70 (s, 3H, rotamer 1), 2.64 (s, 3H, rotamer 2), 2.14–2.05 (m, 2H, rotamers 1+2), 1.82–1.73 (m, 4H, rotamers 1+2), 1.46 (s, 9H, rotamer 1), 1.43 (s, 9H, rotamer 2), 0.95 (t, J = 6.5 Hz, 6H, rotamer 1), 0.85 (d, J = 7.0 Hz, 6H, rotamer 2) ppm. ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 167.0, 163.0, 156.5, 144.6, 140.4, 79.5, 60.3, 52.0, 30.5, 30.0, 28.4, 20.2, 20.1, 19.9, 13.0 ppm. Diagnostic signals of minor rotamer ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 166.6, 162.9, 144.5, 140.2, 79.2, 19.6 ppm. HRMS (ESI) m/z [M+H]+ calcd. for C₁₈H₃₀N₂O₄S 371.1999, found 371.1984. **Synthesis of (R)-Methyl 2-(3-((tert-butoxycarbonyl)(methyl)amino)-4-methylpentyl)-5-methyloxazole-4-carboxylate (16)**

**Step 1:** Dry THF (5 mL) was added to a round bottom flask (50 mL) charged with triphenylphosphine (0.110 g, 0.4 mmol) and iodine (0.050 g, 0.4 mmol) under nitrogen atmosphere and the reaction mixture was cooled to −40 °C. Et₃N (0.054 ml, 0.4 mmol) was added to the flask via glass syringe, followed by the addition of ketoamide ester 13 (0.100 g, 0.28 mmol). The reaction mixture was further stirred for 3 h, at the same temperature. After the complete consumption of 13, the reaction mixture was warmed to room temperature, diluted with milli-Q water (5 mL) and extracted with CH₂Cl₂ (3 × 25 mL). The combined organic layers was washed with brine (10 mL) and saturated aq. Sodiumthiosulfate (5 mL), then dried over anhyd. Na₂SO₄, filtered and concentrated under reduced pressure. The crude residue was used in the next step without further purification. TLC: Rₜ 0.33 (70:30, hexane/EtOAc).

**Step 2:** Dry DMF (2 mL) was added to the crude intermediate (0.080 g, 0.23 mmol) in a round bottom flask (10 mL) and charged with a magnetic bead. MeI (0.05 mL, 0.92 mmol) was added to above reaction mixture, cooled to 0 °C and NaH (0.013 g, 0.58 mmol) was added portion wise over a period of 15 min. The reaction mixture was stirred at 25 °C under inert atmosphere for 12 h. After
the complete consumption of intermediate, the reaction mixture was quenched with saturated solution of NH₄Cl (10 mL). The aqueous layer was extracted with EtOAc (3 × 25 mL) and the combined organic layers was washed with cold brine (3 × 10 mL). The organic layer was dried over anhydrous Na₂SO₄, filtered and concentrated under reduced pressure. The crude residue was purified over silica gel column chromatography (80:20 hexane/EtOAc) to afford 5-methyloxazole 16 (65 mg, 80%) as colorless liquid. TLC: Rf 0.25 (70:30, hexane/EtOAc). [α]D²⁴ = +50.4 (c 0.1, CH₂Cl₂). IR (CH₂Cl₂): 2966, 29320 (C–H), 1730, 1692 (C=O), 1681 (C=N), 1484, 1392 (C–H), 1175, 1165 (C–O), 864 (=C–H), 772, 722 (C–H) cm⁻¹. ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 3.89 (s, 6H, rotamers 1+2), 3.83–3.71 (m, 2H, rotamers 1+2), 2.67 (s, 3H, rotamer 1), 2.63 (s, 3H, rotamer 2), 2.59 (s, 6H, rotamers 1+2), 2.13–2.05 (m, 2H, rotamers 1+2), 1.90–1.82 (m, 2H, rotamers 1+2), 1.80–1.71 (m, 6H, rotamers 1+2), 1.46 (s, 9H, rotamer 1), 1.43 (s, 9H, rotamer 2), 0.96 (t, J = 6.4 Hz, 6H, rotamer 1), 0.85 (t, J = 6.8 Hz, 6H, rotamer 2) ppm. ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 162.7, 156.6, 156.4, 156.0, 126.8, 79.5, 67.9, 51.9, 31.6, 30.6, 28.4, 27.0, 25.2, 20.2, 19.9, 14.0 ppm. Diagnostic signals of minor rotamer ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 162.5, 79.2, 51.8, 30.9, 30.5, 26.97, 25.1, 20.0, 19.6, 11.8 ppm. HRMS (ESI) m/z [M+Na]⁺ calcd. for C₁₈H₃₀N₂O₅ 377.2052, found 377.2049.
$^1$H & $^{13}$C NMR spectra of (S)-2-Isopropyl-1-tosylaziridine (4)
$^1$H & $^{13}$C NMR spectra of (R)-4-Methyl-N-(2-methylhex-5-en-3-yl) benzenesulfonamide (3)
$^1$H & $^{13}$C NMR spectra of (R)-tert-Butyl (2-methylhex-5-en-3-yl)(tosyl)carbamate (5)

![NMR Spectra Image]

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$^1$H & $^{13}$C NMR spectra of (R)-tert-Butyl (6-hydroxy-2-methylhexan-3-yl)(tosyl)carbamate (6)
$^1$H & $^{13}$C NMR spectra of (R)-4-(N-(tert-Butoxycarbonyl)-4-methylphenylsulfonamido)-5-methylhexanoic acid (7)
$^1$H & $^{13}$C NMR spectra of (R)-4-((tert-Butoxycarbonyl)amino)-5-methylhexanoic acid (2)
$^1$H & $^{13}$C NMR spectra of (S)-Methyl 2-((R)-4-((tert-butoxycarbonyl)amino)-5-methylhexanamido)-3-hydroxypropanoate (8)
$^1$H & $^{13}$C NMR spectra of (R)-tert-Butyl (6-amino-2-methyl-6-oxohexan-3-yl)carbamate (9)
$^1$H & $^{13}$C NMR spectra of (R)-tert-Butyl (6-amino-2-methyl-6-thioxohexan-3-yl)carbamate (10)
$^{1} \text{H} \ & \ ^{13}\text{C} \ \text{NMR} \ \text{spectra} \ \text{of} \ \text{(R)-Ethyl} \ \text{2-} \ (3-\text{(tert-butoxycarbonyl)amino}-4\text{-methylpentyl)thiazole-4-carboxylate} \ (11)$
\^{1}H & \^{13}C NMR spectra of (R)-Methyl 2-(3-((tert-butoxycarbonyl)(methyl)amino)-4-methylpentyl)thiazole-4-carboxylate (1)

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$^1$H & $^{13}$C NMR spectra of (2S,3S)-Methyl 2-((R)-4-((tert-butoxycarbonyl)amino)-5-methylhexanamido)-3-hydroxybutanoate (12)
$^1$H & $^{13}$C NMR spectra of (S)-Methyl 2-((R)-4-((tert-butoxycarbonyl)amino)-5-methylhexanamido)-3-oxobutanoate (13)
$^1$H & $^{13}$C NMR of (R)-Methyl 2-(3-((tert-butoxycarbonyl)amino)-4-methylpentyl)-5-methylthiazole-4-carboxylate (14)
$^1$H & $^{13}$C NMR spectra of (R)-Methyl 2-(3-((tert-butoxycarbonyl)(methyl)amino)-4-methylpentyl)-5-methylthiazole-4-carboxylate (15)
$^{1}$H & $^{13}$C NMR spectra of (R)-Methyl 2-(3-((tert-butoxycarbonyl)(methyl)amino)-4-methylpentyl)-5-methylloxazole-4-carboxylate (16)