Stereoselective Synthesis of (*R*)-3-Methylthalidomide by Piperidin-2-one Ring Assembly Approach

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ABSTRACT A simple and stereoselective synthesis of 3-methylthalidomide, a configurationally stable thalidomide analog, is presented. Herein we describe the synthesis of (*R*)-3-methylthalidomide starting from (*S*)-alanine by piperidin-2-one ring assembly approach in high yield and enantiomeric purity without using a chiral auxiliary or reagent. Starting from (*R*)-alanine, the corresponding (*S*)-3-methylthalidomide can be prepared using the same methodology. *Chirality 27:619–624, 2015*

KEY WORDS: thalidomide; teratogenic; tumor necrosis factor-α; oxazolidinone; enantioretentive alkylation

Thalidomide (**1**) was marketed in racemic form as a sedative drug, but it was later withdrawn from the market after the emergence of its serious teratogenic effects leading to limb defects in newborns. Initially, thalidomide was given in racemic form and therefore it was thought that teratogenicity was associated with (*S*)-thalidomide only.[1] Later studies have shown that both the enantiomers of thalidomide are interconvertible to one another at physiological pH in biophase because of the presence of an exchangeable acidic proton at the asymmetric carbon (Fig. 1). Therefore, it was inferred that attribution of a biological response associated with a particular enantiomer is difficult.[2–4] Literature reports reveal a basis of thalidomide teratogenicity but the precise mechanism is still unclear.[5]

In the last decades thalidomide has shown a resurgence for its clinical potential towards the cure of various diseases because of its regulatory activity of tumor necrosis factor-alpha (TNF-α) release, a phenomenon associated with diseases such as rheumatoid arthritis, Crohn's disease, leprosy, AIDS, and cancers.[6–16] It is therefore desirable to develop pure enantiomers of configurationally stable thalidomide analogs to prevent racemization at physiological pH and to provide better insight on their enantiodependent biological activity.

MATERIALS AND METHODS

General Experimental

All the chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MO). The alkylation and hydroxylation reactions were carried out in freshly distilled anhydrous tetrahydrofuran (THF). Progress of the reaction was monitored by thin-layer chromatography (TLC) on ready-made silica gel plates (Merck, Darmstadt, Germany; UV active). The plates were either developed under iodine vapors or seen directly under UV-light (254 nm). Wherever required, compounds were also detected by spraying the TLC plates with 2N HBr/AcOH followed by 0.2 % ninhydrin solution in acetone and heating the plates at ~110°C in a hot air oven for 45 min. Column chromatography was performed over silica gel (60–120 mesh size). ^{1}H spectra were recorded at 300 MHz or 400 MHz and ^{13}C spectra were recorded at 75 MHz or 100 MHz using a Bruker (Billerica, MA) DRX-300 or Bruker DRX-400 spectrophotometer, respectively, and reported in parts per million (ppm) on the δ scale relative to tetramethylsilane as an internal standard. Coupling constants (J) are reported in Hz.. Melting points are reported uncorrected and were determined on melting point apparatus containing silicon oil. IR spectra were recorded using Agilent (Palo Alto, CA) Cary 630 FTIR-spectrophotometer. Mass spectra were obtained using JEOL (Tokyo, Japan) SX-102 (ESI), Agilent 6520 Q-TOF (ESI-HRMS) instruments. Optical rotations were measured on an AUTOPOL III digital polarimeter.

Preparative Procedure and Characterization

(2*S*, 4*S*)-Benzyl-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (3). *Z*-(*S*)-alanine was prepared initially by the treatment of (*S*)-alanine with benzyl chloroformate using the standard procedure.[17] Properly dried *Z*-(*S*)-alanine (22.3 g, 100 mmol) and benzaldehyde dimethyl acetal (14.4 mL, 95.9 mmol) in diethyl ether (500 mL) was cooled to –78°C followed by dropwise addition of $BF_3{\cdot}Et_2O$ (60.2 mL, 489 mmol) under nitrogen atmosphere. The reaction mixture was then allowed to warm to room temperature and stirred till completion of the reaction as monitored by TLC. The reaction mixture was treated with saturated aqueous $NaHCO_3$ at 0°C, initially in small portions until strong bubbling ceased, and then the mixture was extracted with Et_2O (3 × 150 mL). The combined organic phase was washed with brine and dried over anhydrous sodium sulfate. The solvent was removed under vacuum to obtain the residue, which was a mixture of *cis* and *trans* isomers (~90:10) on TLC. The predominant *cis* isomer was obtained by purification of the crude product by silica gel column chromatography using ethyl acetate-hexane (1:4) as eluent. The pure fractions were pooled and after removal of the solvent the residue was recrystallized from diethyl ether/hexane to yield compound **3** (18.66 g, 60% yield) as a colorless white crystalline solid in >98% diastereomeric purity as evident by NMR-spectroscopy; mp 53–54°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.25-7.40 (m, 10H, Ar*H*), 6.64 (s, 1H, C_6H_5CH), 5.11-5.21 (m, 2H, $C_6H_5CH_2$), 4.44-4.51 (m, 1H, CH_3CH), 1.56 (d, *J* = 6.9 Hz, 3H, CH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 172.5, 153.4, 136.9,135.4, 129.8, 128.8, 128.6, 128.5, 128.1, 126.3,89.1, 67.9, 52.1, 18.2; FTIR (KBr): υ = 3352, 1800, 1717, 763 cm^{-1}. HRMS (ESI-TOF): m/z calcd for $C_{15}H_{22}NO_5$ [M+H]: 312.1230, found: 312.1229.

(2*S*,4*R*)-benzyl 4-allyl-4-methyl-5-oxo-2-phenyloxazolidine-3-carboxylate (4). A solution of compound **3** (7.78 g, 25 mmol) and allyl bromide (2.6 mL, 30 mmol) in freshly dried THF (30 mL) was cooled to –78°C under stirring. To this solution, LHMDS (30 mL, 1M in THF) was added at –78°C under nitrogen atmosphere. The reaction mixture was further stirred at –78°C for 3 h. Then the reaction mixture was quenched with saturated ammonium chloride and extracted with ethyl acetate (3 × 150 mL). The organic layer was washed with brine, dried over sodium sulfate, and concentrated at reduced pressure. The crude product obtained was purified by column chromatography using ethyl

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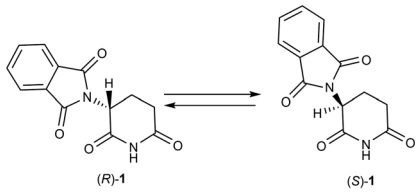


Fig. 1. Interconversion of thalidomide enantiomers at physiological pH.

acetate-hexane (1:5) to obtain compound **4** (7.02 g, 80%) as a colorless oil. $[\alpha]_D^{21}$ –63.4° (*c* = 0.73, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$, δ): (major rotamer) 7.20-7.41 (m, 9H, Ar*H*), 6.87 (d *J* = 6.3 Hz, 1H, Ar*H*), 6.28 (s, 1H, C_6H_5CH), 5.61-5.69 (m, 1H, CH=CH_2), 5.10-5.26 (m, 2H, CH=CH_2), 4.91-4.97 (m, 2H, $C_6H_5CH_2$), 3.24-3.30 (m, 1H, CCH_aH_b), 2.52 (dd, *J* = 13.7, 6.4 Hz, 1H, CCH_aH_b), 1.81(s, 3H, CH_3); (minor rotamer) 7.20-7.41 (m, 9H, Ar*H*), 6.87 (d *J* = 6.3 Hz, 1H, Ar*H*), 6.35 (s, 1H, C_6H_5CH), 5.61-5.69 (m, 1H, CH=CH_2), 5.10-5.26 (m, 2H, CH=CH_2), 4.91-4.97 (m, 2H, $C_6H_5CH_2$) 2.89-2.91 (m, 1H, CCH_aH_b), 2.52 (dd, *J* = 13.7, 6.4 Hz, 1H, CCH_aH_b), 1.72(s, 3H, CH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ): (major rotamer only), 174.2, 151.7, 137.1, 135.3, 131.0, 129.9, 128.7, 126.8, 121.4, 89.5, 67.3, 63.2, 39.5, 23.7; FTIR (neat) *v* = 3535, 1795, 1711, 1216 cm^{-1}. HRMS (ESI-TOF): m/z calcd for $C_{15}H_{22}NO_5$ [M+H]: 352.1543, found: 352.1549.

(*R*)-Methyl 2-(benzyloxycarbonylamino)-2-methylpent-4-enoate (5). Aqueous NaOH (1N, 20 mL) was added to the stirred solution of compound **4** (3.52 g, 10 mmol) in methanol (100 mL) and the reaction mixture was stirred at room temperature for 5–6 h. The solvent was removed under reduced pressure and the resulting residue was acidified with citric acid up to pH 3. This mixture was extracted with ethyl acetate (3 × 50 mL) and the combined organic layer was washed with brine, dried over sodium sulfate, and concentrated under vacuum. The crude product thus obtained was dissolved in dimethylformamide (DMF) (50 mL) and potassium carbonate (1.38 g, 10 mmol) was added followed by the addition of methyl iodide (684 μL, 11 mmol) at 0°C. The reaction mixture was stirred for several hours till completion of the reaction as indicated by TLC. After completion of the reaction, DMF was evaporated under reduced pressure and the residue was suspended in water (30 mL) and the aqueous layer was extracted with ethyl acetate (3 × 30 mL). The combined organic phase was washed with brine and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure and the crude product obtained was purified by column chromatography using ethyl acetate-hexane (1:4) to give compound **5** (2.5 g, 90%) as light yellow oil. $[\alpha]_D^{22}$ –15.5° (*c* = 0.67, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.25–7.34 (m, 5H, Ar*H*), 5.50–5.71 (m, 2H, CH=CH_2 and N*H*COO), 5.06–5.12 (m, 4H, CH=CH_2 and $C_6H_5CH_2$), 3.73 (s, 3H, $COOCH_3$), 2.76–2.78 (m, 1H, CCH_aH_b), 2.54–2.61 (dd, *J* = 13.9, 7.3 Hz, 1H, CCH_aH_b), 1.58 (s, 3H, CCH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 174.2, 154.7, 136.5, 132.1, 128.5, 128.1, 128.0, 119.6, 66.5, 59.5, 52.6, 41.4, 23.2; FTIR (neat) *v* = 3415, 3033, 2943, 1725 cm^{-1}; HRMS (ESI-TOF): m/z calcd for $C_{15}H_{20}NO_4$ [M+H]: 278.1387, found: 278.1372.

(*R*)-Methyl- 2-(benzyloxycarbonylamino)-5-hydroxy-2-methylpentanoate (6). To the stirred solution of compound **5** (2.5 g, 9 mmol) in dry THF (50 mL) was added 9-BBN (0.5M in THF, 36 mL) under nitrogen atmosphere at room temperature. The reaction mixture was stirred for 20 h, then water (9 mL) was carefully added, followed by addition of H_2O_2 (35% in water, 36 mL) and sodium acetate solution (20% in water, 60 mL). After 1 h of vigorous stirring, the two layers were separated and the aqueous phase was extracted with ethyl acetate (3 × 100 mL). The combined organic phase was washed with brine, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The crude product obtained was purified by column chromatography using methanol-chloroform (1:20) to give compound **6** (1.86 g, 70%) as a colorless oil. $[\alpha]_D^{23}$ –36.2° (*c* = 0.71, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.35 (m, 5H, Ar*H*), 5.73 (bs, 1H, N*H*COO), 5.07 (s, 2H, $C_6H_5CH_2$), 3.74 (s, 3H, $COOCH_3$), 3.58-3.62 (t, *J* = 6.6 Hz, 2H, CH_2CH_2OH), 2.08-2.20 (m, 1H, $CCH_aH_bCH_2$), 1.85-1.97 (m, 1H, $CCH_aH_bCH_2$), 1.58(s, 3H, CCH_3), 1.37-1.46 (m, 2H, $CH_2CH_2CH_2$); ^{13}C NMR (75 MHz, $CDCl_3$, δ) 174.7, 154.8, 136.4, 128.5, 128.1, 128.0, 66.5, 62.2, 59.7, 52.7, 33.4, 27.1, 23.4; FTIR (neat) *v* = 3414, 3015, 2949, 1720 cm^{-1}; HRMS (ESI-TOF): m/z calcd for $C_{15}H_{22}NO_5$ [M+H]: 296.1492, found: 296.1494.

(*R*)-Methyl-5-azido-2-(benzyloxycarbonylamino)-2-methylpentanoate (7). To the stirred solution of compound **6** (1.86 g, 6.3 mmol) in THF (50 mL) was added Et_3N (2.5 mL, 18 mmol) followed by mesyl chloride (1.4 mL, 18 mmol) at 0°C. The reaction mixture was stirred at 0°C for 15 min then saturated sodium bicarbonate (30 mL) was added and the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic layer was further washed with brine, dried over anhydrous sodium sulfate, and evaporated under reduced pressure. The crude product obtained was dissolved in DMF (35 mL) and sodium azide (780 mg, 12 mmol) was added to it. The reaction mixture was stirred for 5 h at 80°C. After completion of the reaction DMF was evaporated and the residue obtained was suspended in water (30 mL), then the aqueous layer was extracted with ethyl acetate (3 × 50 mL). The combined organic phase was washed with brine, dried over anhydrous sodium sulfate, and solvent was concentrated under reduced pressure to obtaine the crude product, which was purified by column chromatography using ethyl acetate-hexane (1:10) to give compound **7** (1.49 g, 74%) as a colorless oil. $[\alpha]_D^{21}$ –67.0° (*c* = 0.10, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.33-7.37 (m, 5H, Ar*H*), 5.71 (bs, 1H, N*H*COO), 5.1 (s, 2H, $C_6H_5CH_2$), 3.77 (s, 3H, $COOCH_3$), 3.21-3.29 (m, 2H, $CH_2CH_2N_3$), 1.88-2.26 (m, 2H, $CCH_aH_bCH_2$), 1.60 (s, 3H, CCH_3), 1.33-1.43 (m, 2H, $CH_2CH_2CH_2$); ^{13}C NMR (75 MHz,$CDCl_3$, δ): 174.5, 154.5, 136.4, 128.5, 128.2, 128.0, 66.5, 52.9, 59.8, 51.0, 33.6, 23.8, 23.7; FTIR (neat) *v* = 3431, 2953, 2097, 1712, 1655 cm^{-1}; HRMS (ESI-TOF): m/z calcd for $C_{15}H_{22}N_4O_4$[M+H]: 321.1557, found: 321.1564.

(*R*)-Benzyl 3-methyl-2-oxopiperidin-3-ylcarbamate (8). To the stirred solution of compound **7** (1.28 g, 4 mmol) in moist THF (50 mL) was added Ph_3P (1.3 g, 5 mmol). The reaction mixture was stirred at 50°C for 12 h. After completion of the reaction, THF was evaporated and the residue obtained was partitioned between 200 mL of a (1:1) water–ethyl acetate mixture. The organic layer was separated and the aqueous layer was further extracted with (2 × 100 mL) ethyl acetate. The combined organic layer was washed with brine, dried over anhydrous sodium sulfate, and evaporated under reduced pressure to obtain the crude product, which was purified by column chromatography using ethyl acetate-hexane (1:10) as eluent to obtain compound **8** (860 mg, 82%) as a white solid; mp 125–126°C; $[\alpha]_D^{23}$ -14.3° (*c* = 0.31, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$ δ): 7.29-7.35 (m, 5H, Ar*H*), 6.10 (bs, 1H, N*H*CO), 5.60 (bs, 1H, N*H*COO), 5.12 (dd, *J* = 16.5, 12.3 Hz, 2H, $C_6H_5CH_2$), 3.27-3.41 (m, 2H, CH_2NHCO), 2.31-2.42 (m, 1H, CCH_aH_b), 2.09-2.14 (m, 1H, CCH_aH_b), 1.77-1.91 (m, 2H, $CH_2CH_2CH_2$), 1.49 (s, 3H, CCH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ): 174.5, 155.2, 136.5, 128.5, 128.0, 127.9, 66.4, 55.6, 42.2, 33.2, 25.1, 20.3; FTIR (KBr) *v* = 3352, 3020, 1704, 1506 cm^{-1}; HRMS (ESI-TOF): m/z calcd for $C_{14}H_{19}N_2O_3$[M+H] : 263.1390, found: 263.1369.

(*R*)-2-(3-Methyl-2-oxopiperidin-3-yl)isoindoline-1,3-dione (9). To the solution of compound **8** (700 mg, 2.67 mmol) in methanol (30 mL) was added Pd/C (10%, 150 mg), then this reaction mixture was subjected to hydrogenation at 30 psi for 2 h. The catalyst was removed by filtration and the filtrate was evaporated under reduced pressure to obtain amine as a gummy residue. The residue was taken in 1,4-dioxane (30 mL) and to this stirred solution was added DIPEA (0.7 mL, 4 mmol) followed by addition of phthalic anhydride (445 mg, 3 mmol) and the reaction mixture was refluxed for 12 h. After completion of the reaction 1,4-dioxane was evaporated under reduced pressure and the crude material thus obtained was directly purified without workup by column chromatography using ethyl acetate-hexane (1:3) as eluent to obtain compound **9** (582 mg, 85 %) as a white solid; mp 141–142°C; $[\alpha]_D^{24}$ + 4.3° (*c* = 0.32, 1,4-dioxane); ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.69-7.78 (m, 4H, Ar*H*) 5.77 (bs, 1H, N*H*CO), 3.38-3.58 (m, 2H, CH_2NHCO), 2.32-2.40 (m, 1H, CCH_aH_b), 1.89-2.17 (m, 6H, CCH_aH_b, $CH_2CH_2CH_2$ and CCH_3); ^{13}C NMR (75 MHz, $CDCl_3$, δ) 172.6, 168.7, 134.0, 131.9, 123.1, 60.0, 42.4, 34.8, 23.5, 20.1; FTIR (KBr) *v* = 3420, 2941, 1708, 1663 cm^{-1}; HRMS (ESI-TOF): m/z calcd for $C_{15}H_{20}NO_4$ [M+H]: 259.1077, found: 259.1067.

(*R*)-3-Methylthalidomide (10). A mixture of H_5IO_6 (1.37 g, 6 mmol) and CrO_3 (20 mg, 0.20 mol) in acetonitrile (30 mL) was stirred at room temperature for 30 min, then acetic anhydride (570 μL, 6 mmol) was added. The reaction mixture was cooled to 0°C and compound **9** (259 mg, 1 mmol) was added in one portion and the reaction mixture was further stirred for 30 min at room temperature. After completion of the reaction, ice-water (15–20 mL) was added and the mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layer was washed with saturated $NaHCO_3$ solution, saturated $Na_2S_2O_3$ solution, and finally with brine. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure. The crude product was filtered through silica gel column using ethyl acetate–hexane (1:4) as eluent to obtain (*R*)-3-methylthalidomide **10** (260 mg, 95%) as a white crystalline solid; mp 249–250°C; $[\alpha]_D^{23}$ -35.6° (*c* = 0.25, 1,4-dioxane) (lit. -36.9°, c = 1, 1,4-dioxane)[18]; ^{1}H NMR (300 MHz, DMSO-d_6, δ) 11.03 (bs, 1H, CON*H*CO), 7.92 (s, 4H, Ar*H*), 2.60-2.78 (m, 3H, NHCOCH_2 and CCH_aH$_b$), 2.10-2.15 (m, 1H, CCH$_a$$H_b$), 1.96 (s, 3H, CCH$_3$); ^{13}C NMR (75 MHz, DMSO-d_6, δ): 172.7, 172.6, 168.4, 135.2, 131.5, 123.5, 59.3, 29.6, 29.1, 21.5; FTIR (KBr) ν = 3366, 2928, 1715 cm^{-1}; HRMS (ESI-TOF): m/z calcd for $C_{15}H_{20}NO_4$[M+H]: 273.0870, found: 273.0869.

Tert-butyl (*S*)-3-(benzyloxy)-1-((*R*)-3-methyl-2-oxopiperidin-3-ylamino)-1-oxopropan-2-ylcarbamate (11a). To the solution of compound **8** (262 mg, 1 mmol) in methanol (20 mL) was added Pd/C (10%, 50 mg), then this reaction mixture was subjected to hydrogenation at 30 psi for 2 h. The catalyst was removed by filtration and the filtrate was evaporated under reduced pressure to obtain amine. A solution of amine, thus obtained, in DMF (5 mL) was added to the prestirred mixture of *N*-Boc-*O*-benzyl-L-Serine (296 mg, 1 mmol), EDCI.HCl (230 mg, 1.2 mmol), and HOBt (168 mg, 1.2 mmol) in dry DCM at 0°C under inert atmosphere followed by addition of DIEA (0.2 mL, 1.2 mmol). The reaction mixture was further stirred for 12 h at room temperature. After completion of the reaction DCM was evaporated under reduced pressure and water (15–20 mL) was added. The aqueous mixture was extracted with ethyl acetate (3 × 30 mL). The combined organic layer was washed with 1N HCl, 10% aqueous $NaHCO_3$ solution and finally with brine. The organic phase was dried over anhydrous sodium sulfate and the solvent was removed under reduced pressure to obtain the crude product, which was purified by column chromatography using methanol-chloroform (1:20) as eluent to give compound **11a** as a gummy residue (290 mg, 72%); ^{1}H NMR (400 MHz, $CDCl_3$ δ): 7.34-7.27 (m, 5H, Ar*H*), 7.17 (bs, 1H, CON*H*) 5.80 (bs, 1H, CON*H*), 5,42 (bs, 1H, OCON*H*), 4.58-4.53 (m, 2H, $C_6H_5CH_2$), 4.26 (m, 1H, α-C*H*), 3.89-3.86 (m, 1H, α-CHCH_aH$_b$O), 3.60-3.56 (m, 1H, α-CHCH$_a$$H_b$O), 3.44-3.25 (m, 2H, COCH_2CH$_2$) 2.32-2.28 (m, 1H, CH$_2$CH_aH$_b$CH$_2$), 2.14-2.10 (m, 1H, CH$_2$CH_aH$_b$C), 1.88-1.82 (m, 2H, CH$_2$CH$_a$$H_bCH_a$$H_b$C), 1.44 (s, 3H, CC$H_3$), 1.43 (s, 9H C(C$H_3$)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 173.7, 169.9, 154.4, 137.6, 128.4, 127.9, 76.0, 73.5, 70.0, 55.9, 42.4, 32.8, 28.3, 24.8, 20.3; HRMS (ESI-TOF): m/z calcd for $C_{21}H_{32}N_3O_5$[M+H] : 406.2336, found: 406.2347.

(*S*)-Benzyl 3-(tert-butoxycarbonylamino)-4-((*R*)-3-methyl-2-oxopiperidin-3-ylamino)-4-oxobutanoate (11b). The method for synthesis was the same as reported for compound **11a,** while the amino acid used was *N*-Boc-L-Aspartic acid 4-benzyl ester (323 mg, 1 mmol). Yield (282 mg, 65%) as a gummy residue: ^{1}H NMR (400 MHz, $CDCl_3$ δ): 7.37-7.31 (m, 5H, Ar*H*), 7.16 (bs, 1H, CON*H*), 5.80 (bs, 1H, CON*H*), 5,65 (bs, 1H, OCON*H*), 5.19-5.13 (m, 2H, $C_6H_5CH_2$), 4.53 (m, 1H, α-C*H*), 3.48-3.28 (m, 2H, COCH_2CH$_2$), 3.06-3.01 (m, 1H, α-CHCH_aH$_b$CO), 2.78-2.72 (m, 1H, α-CHCH$_a$$H_b$CO), 2.34-2.26 (m, 1H, CH$_2$CH_aH$_b$CH$_2$), 2.14-2.06 (m, 1H, CH$_2$CH_aH$_b$C), 1.92-1.85 (m, 2H, CH$_2$CH$_a$$H_bCH_a$$H_b$C), 1.75 (s, 3H, CC$H_3$), 1.46 (s, 9H C(C$H_3$)$_3$); HRMS (ESI-TOF): m/z calcd for $C_{22}H_{32}N_3O_6$[M+H] : 434.2286, found: 434.2295.

(*R*)-Benzyl 3-methyl-2,6-dioxopiperidin-3-ylcarbamate (12). A solution of amide **8** (2.33 g, 8.9 mmol) in 40 mL of ethyl acetate was added to the mixture of $RuCl_3.xH_2O$ (190 mg, 0.9 mmol) and 10% aqueous $NaIO_4$ (90 mL). The biphasic reaction mixture was vigorously stirred at 40°C in a sealed flask until completion as indicated by TLC. After completion of the reaction, the two layers were separated and the aqueous layer was extracted with two 50 mL portions of ethyl acetate. The combined organic layer was washed with a saturated solution of $Na_2S_2O_3$, followed by brine and the organic layer was dried over anhydrous sodium sulfate. The solution was filtered and evaporated under reduced pressure to obtain the crude product, which was purified by column chromatography using ethyl acetate–dichloromethane (20:80) as eluent to obtain compound **12** (1.96 g, 80%) as a white solid.:^{1}H NMR (400 MHz, $CDCl_3$ δ): 8.03 (bs,1H, CON*H*CO), 7.29-7.38 (m, 5H, Ar*H*) 5.55 (bs, 1H, N*H*COO), 5.09 (dd, *J* = 11.8, 2.9 Hz, 2H, $C_6H_5CH_2$), 2.80-2.55 (m, 3H, CCH$_a$$H_bCH_2$CONH), 2.33-2.28 (m, 1H, CCH_aH$_b$), 1.56 (s, 3H, CCH_3); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 174.0, 171.2, 154.9, 136.0, 128.6, 128.3, 128.1, 66.9, 55.4, 29.5, 29.0, 22.5; HRMS (ESI-TOF): m/z calcd for $C_{14}H_{17}N_2O_4$[M+H] : 277.1182, found: 277.1183.

***(S)*-Tert-butyl 2-((*R*)-3-methyl-2,6-dioxopiperidin-3-ylcarbamoyl)pyrrolidine-1-carboxylate (13a).** The method for synthesis was the same as reported for compound **11a,** while the starting amine was derived from hydrogenolysis of compound **12** and the amino acid used was Boc-L-proline (215 mg, 1 mmol). Yield (230 mg, 68%) as a gummy residue; ^{1}H NMR (400 MHz, $CDCl_3$ δ): 7.95 (bs,1H, CON*H*CO), 4.27-4.19 (m, 1H, α-C*H*), 3.45-3.35 (m, 2H, NCH_2), 2.75-2.59 (m, 3H, CCH_aH$_b$CH$_2$ & COCH_2), 2.31-2.14 (m, 2H, CHCH_2CH$_2$), 1.90-1.88 (m, 3H, CCH$_a$$H_bCH_2$ & CH$_2$CH_2CH$_2$), 1.66 (s, 3H, CCH_3), 1.48 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (100 MHz, $CDCl_3$, δ): 173.4, 171.5, 155.8,80.7, 59.8, 55.2, 47.1, 29.4, 28.8, 28.3, 27.8, 24.5, 22.4; HRMS (ESI-TOF): m/z calcd for $C_{16}H_{26}N_3O_5$[M +H] : 340.1865, found: 340.1867.

Benzyl (*S*)-1-((*R*)-3-methyl-2,6-dioxopiperidin-3-ylamino)-1-oxo-3-phenylpropan-2-ylcarbamate (13b). The method for synthesis was the same as reported for compound **13a,** while the amino acid used was Cbz-L-phenylalanine (300 mg, 1 mmol). Yield (308 mg, 73%) as a gummy residue,; ^{1}H NMR (400 MHz, $CDCl_3$ δ): 8.30 (bs, 1H, CON*H*CO), 7.36-7.24 (m, 10H, Ar*H*), 6.39 (bs, 1H, CON*H*), 5.34-5.47 (m, 2H, $C_6H_5CH_2$), 4.48-4.39 (m, 1H, α-C*H*), 3.11-3.00 (m, 2H, $C_6H_5CH_2$CH), 2.67-2.52 (m, 2H, COCH_2CH$_2$), 2.36-2.18 (m, 2H, CHCH_2CH$_2$), 1.44 (s, 3H, CCH_3); HRMS (ESI-TOF): m/z calcd for $C_{23}H_{26}N_3O_5$[M+H] : 424.1867, found: 424.1865.

RESULTS AND DISCUSSION

As it has been well established that both enantiomers of thalidomide have different biological activities and are interconvertable at physiological pH, a number of approaches have been employed to develop configurationally stable thalidomide analogs (Fig. 2). These molecules will be important pharmacological tools for the study of enantiodependent pharmacological effects including the teratogenicity of thalidomides as well as development of configurationally stable analogs of thalidomide with better activity and selectivity. Conventional synthetic approaches include the exchange of C3 proton of thalidomide with an alkyl group,[19,20] fluoroalkyl group,[21] deuterium atom,[22] fluorine atom,[23] or introduction of

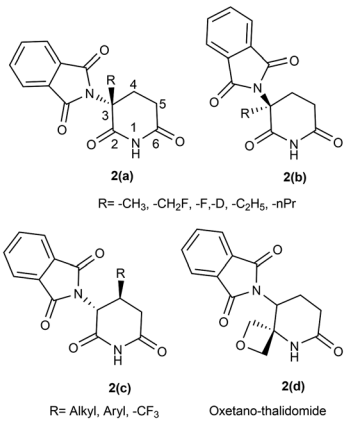


Fig. 2. Configurationally stable thalidomide analogs.

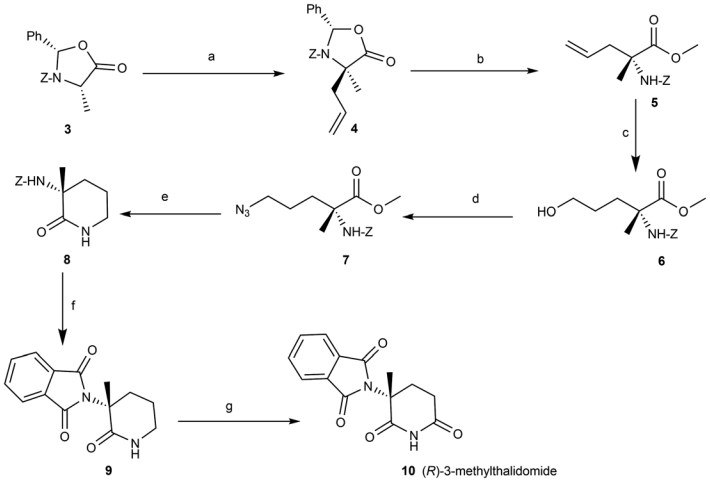
an alkyl group at C4[24] to prepare nonracemizable structural variants.

3-Methylthalidomide enantiomers have been extensively studied and were found to have better TNF-α inhibition activity when compare to thalidomide.[18,25] It has also been reported that regulation of TNF-α production by thalidomide analogs is specific to cell type and the inducer. It is well known that only (*S*)-3-methylthalidomide increases TNF-α production in the 12-*O*-tetradecanoylphorbol 13 acetate/human leukemia HL-60 assay system, while the (*R*)-isomer is more active than the (*S*)-isomer of 3-methylthalidomides in the okadaic acid/HL-60 assay system.[26–28]

The methodology for the stereoselective synthesis of 3-methylthalidomide enantiomers is not yet explored and the major limitation associated with previously reported methods is the formation of a racemic mixture which requires chiral purification to obtain the particular enantiomer of 3-methylthalidomide.[18,20] The biological activity including teratogenicity of 3-methylthalidomide derivatives are reported to exhibit enantiodependent bidirectional regulatory effects on TNF-α production.[26] Therefore, efforts were made to develop stereoselective synthesis of nonracemizable 4-trifluoromethyl substituted and 2-oxetano thalidomides (Fig. 2) in the recent literature.[29,30] Furthermore, the stereoselective synthesis of a structurally similar analog, (*S*)-3-trifluoromethyl thalidomide, is reported by Rh-catalyzed direct enantioselective alkynylation of α-ketiminoesters.[31] These configurationally stable thalidomide analogs will be of great help for the development of highly potent biologically active molecules, including TNF-α inhibitors.

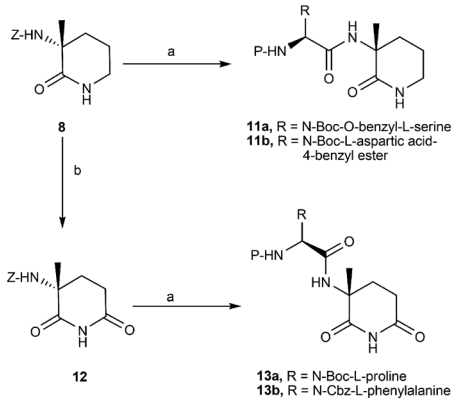
Herein we report the stereoselective synthesis of (*R*)-3-methylthalidomide enantiomer, as illustrated in Scheme 1. The key intermediate oxazolidinone **3** was synthesized via the improved procedure for the synthesis of Karady's oxazolidinone reported by Cheng et al.[32] The reaction products yielded predominantly *cis*-isomer (>90%) and both the *cis* and *trans* isomers are easily separable by silica gel chromatography. The diastereomeric purity of *cis* oxazolidinone **3** was further improved to >98% by crystallization from diethyl ether–hexane. The NMR-spectroscopy data exactly matched data reported by Kapadia et al.[33] for the same compound. Enantioretentive alkylation of **3** using LHMDS and allyl bromide resulted in the formation of compound **4**.[34] Since it has been reported that the addition of an electrophile to the preformed enolate leads to poor yield of the alkylated product, presumably due to the slow alkylation rate of oxazolidinone **3**, LHMDS was added slowly to a mixture of allyl bromide and oxazolidinone **3** to improve the yield of the alkylated product **4**.[33,35] The alkylated oxazolidinone **4** exists as Cbz-rotamers in a ratio of 2.5:1, as evident by NMR spectroscopy.[35] Compound **4** was subsequently hydrolyzed with aq. NaOH and then converted to methyl ester **5**. Terminal hydroxylation of **5** was carried out with 9-BBN to obtain primary alcohol **6** using a standard protocol.[36] Mesylation of compound **6** followed by reaction with sodium azide afforded azide **7**. When azide **7** was subjected to the Staudinger reduction, the expected amine product was not isolated, but cyclized in situ to the amide product **8**. Removal of the Cbz-protecting group was accomplished by catalytic hydrogenation, followed by reaction of the resulting amine product with phthalic anhydride to yield amide **9**. Oxidation of amide **9** with periodic acid yielded (*R*)-3-methylthalidomide **10**.[37]

Furthermore, we extended our synthetic methodology to the synthesis of some amino acid conjugates of 3-amino-3-methylpiperidin-2-one (**11a** and **11b**) and 3-amino-3-methylpiperidin-2,6-dione (**13a** and **13c**), which are structurally close to the compounds reported in the patent literature,[38] with reported biological activity as shown in Scheme 2. The *N*-Cbz protection of amide **8** was removed by hydrogenation followed by coupling with *N*-Boc-*O*-benzyl-L-serine and *N*-Boc-L-aspartic acid 4-benzyl ester in the presence of EDCI. HCl/HOBt to provide amino acid conjugates **11a** and **11b,** respectively, the cyclic amide variants. Furthermore, compound **8** was successfully oxidized to the corresponding imide derivative **12** using ruthenium catalyzed oxidation in water-ethyl acetate biphasic medium using excess sodium metaperiodate. Other oxidizing reagents, viz., Dess-Martin periodinane, *m*-CPBA, chromium trioxide/periodic acid did not give satisfactory results. The *N*-Cbz-3-methylpiperidin-2,6-dione **12** was subjected to hydrogenation and the resultant



Reagents and condition: (a) LHMDS, allyl bromide, THF, -78 °C, 3 h, 80%; (**b**) (i) NaOH, Methanol/water 9:1 (v/v), 5 h (ii) K_2CO_3, methyl iodide, DMF, rt, 2 h, 90%; (**c**) 9-BBN, THF, rt, 20 h, 70%; (**d**) (i) MsCl, Et_3N, THF, 0 °C, 1/2 h (ii) NaN_3, DMF, 80 °C, 5 h, 74 % after 2 steps; (**e**) Ph_3P, moist THF, 50 °C, 6 h, 82 %; (**f**) (i) H_2, Pd/C, rt, 2h (ii) Phthalic anhydride, DIPEA, 1,4-dioxane, 120 °C, 12h, 85%; (**g**) H_5IO_6, Ac_2O, CrO_3, CH_3CN, 0 °C, 95%.

Scheme 1. Stereoselective Synthesis of (*R*)-3-methylthalidomide.



Scheme 2. Synthesis of amino acid conjugates of (*R*)-3-amino-3-methylpiperidin-2-one and (*R*)-3-amino-3-methylpiperidin-2,6-dione.

amine was coupled with *N*-Boc-L-proline and *N*-Cbz-L-phenylalanine to afford amino acid conjugates **13a** and **13b,** respectively.

CONCLUSION

In summary, we have demonstrated a highly efficient and enantioselective method for the synthesis of (*R*)-3-methylthalidomide. In the present report synthesis is reported starting from (*S*)-alanine, but starting from the (*R*)-alanine corresponding enantiomer, (*S*)-3-methylthalidomide, could be obtained. This methodology may also be applicable for the synthesis of a particular enantiomer of other stable thalidomide analogs. After screening several reagents and conditions previously reported for oxidation of amide to imide, we concluded that $RuCl_3.xH_2O$/sodium metaperiodate in water-ethyl acetate a biphasic system gave the best yield and was also found compatible with Boc- and Cbz- amine protecting groups. We also synthesized amino acid conjugates of 3-amino-3-methylpiperidin-2-one and 3-amino-3-methylpiperidin-2,6-dione. Enantiopure 3-methylthalidomides and its amino acid conjugates are configurationally stable molecules and are very useful for the study of teratogenicity associated with a particular thalidomide enantiomer as well as they may provide enantiopure thalidomide derivatives useful for the development of drugs for different diseases. An efficient, enantioselective, and scalable synthesis is therefore very essential for the preparation of these interesting molecules. The present method is advantageous over the previously known procedures as it neither involves chiral separation nor the use of any chiral auxiliary or reagent for the synthesis of configurationally stable 3-methylthalidomides enantiomers.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

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