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Chemoenzymatic synthesis of (5*S*)- and (5*R*)-hydroxymethyl-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one: a precursor of thiolactomycin and determination of its absolute configuration☆

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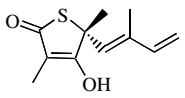
Abstract—A convenient enantioselective synthesis of (5*S*)- and (5*R*)-hydroxymethyl-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one, a key intermediate in the synthesis of thiolactomycin has been carried out by a *Carica papaya* lipase-mediated resolution protocol to provide (*R*)-**2** in a 94% ee and its enantiomer (*S*)-**9** in a 98% ee. The absolute configuration at the C-5 position has been determined by Mosher's method.

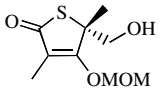
1. Introduction

Naturally occurring (5*R*)-thiolactomycin **1** (TLM) is a thiolactone antibiotic initially isolated from *Nocardia* sp. found in a Japanese soil sample.[2] It exhibits broad-spectrum antibiotic activity in vitro against Gram +ve and Gram −ve bacteria,[3] *Mycobacterium tuberculosis*,[4] the malarial parasite, *Plasmodium falciparum*[5a–e] and African trypanosomes.[5a,d] TLM inhibits bacterial and plant type II fatty acid synthase (FAS II) but not mammalian or yeast type I fatty acid synthase (FAS I).[6] In *Escherichia coli*, TLM inhibits both β-ketoacyl-ACP synthase I–III and acetyl coenzyme A (CoA):ACP transacylase activities in in vivo and in vitro conditions.[7] Thiolactomycin's favourable physical and pharmacokinetic properties, that is, low molecular weight, high water solubility, appropriate lipophilicity and low toxicity profile in mice[8] made it an attractive lead molecule for tuberculosis.[9]

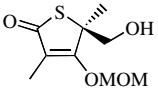
Based on TLM's unique selectivity and inherent potential for analogues of TLM to be potent therapeutic agents, we have recently synthesized a new class of thiolactomycin based analogues, among which some analogues have shown promising activity against *M. tuberculosis* cultures.[10] There are only a few synthetic strategies that have been developed for the synthesis of thiolactomycin. Wang and Salvino reported the first total synthesis of racemic thiolactomycin in lower yields.[11] Later Thomas et al. developed an asymmetric synthesis of (5*S*)-thiolactomycin[12] and Townsend and co-workers synthesized (5*R*)-thiolactomycin from (2*R*)-alanine.[13] Recently, Ohata et al. also developed an asymmetric synthesis of naturally occurring thiolactomycin and its 3-dimethyl derivative.[14] In view of our interest in the development of chemoenzymatic methodologies, it was considered of interest to develop a chemoenzymatic route for the preparation of optically active (5*R*)-hydroxymethyl-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one **2** and its enantiomer, which is a key intermediate for the synthesis of thiolactomycin. Herein, we report a chemoenzymatic procedure for the synthesis of (5*R*)-**2** and (5*S*)-**2** starting from methyl propionylacetate and involving a *Carica papaya* lipase-mediated resolution protocol.



(*R*)-**1**



(*R*)-**2**



(*S*)-**2**

☆ See Ref. 1.

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2. Results and discussion

In the present synthetic scheme methyl propionylacetate **3** is employed as a starting material. The methylation of β-keto ester **3** using CH_3I and anhydrous K_2CO_3 affords the corresponding methylated β-keto ester **4**.[15] Selective bromination of compound **4** with Br_2 in $CHCl_3$ yields γ-brominated product **5**,[16] which is followed by thioacetylation and cyclization to provide thiolactone **7** in moderate to good yields (Scheme 1).[10]

Compound (±)-5-hydroxymethyl-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one **2** was prepared from thiolactone **7** via a two-step procedure.[17] The primary hydroxyl group of compound **2** has been acetylated with acetic anhydride in DMAP to provide the desired (±)-5-methylacetate-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one **9** in good yields (Scheme 2). In view of our interest in enzyme-mediated kinetic resolution of chiral building blocks,[18] we performed the resolution of (±)-**2** and (±)-**9** by employing different lipases.

2.1. Lipase screening and enzyme concentration

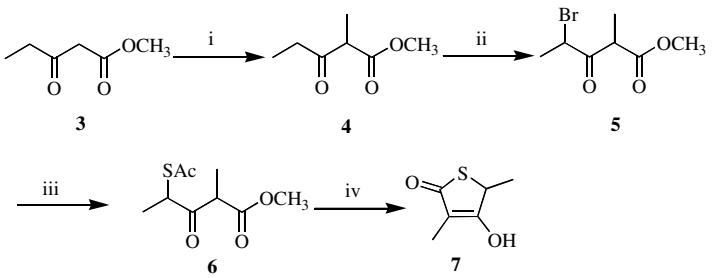
The selection of a suitable lipase is an important aspect for developing an efficient resolution protocol. Eleven commercially available lipases from different sources have been screened for this lipase-mediated alcoholysis of racemic thiolactone acetate (±)-**9** in diisopropyl ether and *n*-butanol. Lipases from *Pseudomonas cepacia* (PS) and their immobilized forms lipase PS-C (immobilized on ceramic particles) and lipase PS-D (immobilized on diatomaceous earth), *Candida antarctica*, *Candida cylindracea*, AK-20 *Pseudomonas fluorescens* (PFL) and lipase from *Porcine pancrease* (PPL) did not show any significant conversions, even after prolonged reaction times. The lipases *Mucor miehei* (lipozyme) and *Candida rugosa* (CRL) gave moderate results, when 4 equiv (w/w) of lipases have been used. Both of these lipases (*Mucor miehei* and *Candida rugosa*) provide thiolactone acetate (*S*)-**9** in 56% and (*R*)-**9** in a 48% ee and the corresponding hydroxymethyl thiolactone in moderate enantioselectivities, that is, (*R*)-**2** in 68% and (*S*)-**2** in 61%, respectively. However, *Carica papaya* lipase-mediated alcoholysis of racemic thiolactone acetate (±)-**9** has shown interesting results with good conversions and high enantioselectivities. The lipase-mediated alcoholysis of compound (±)-**9** by employing *Carica papaya* (2 w/w equiv) in diisopropyl ether, *n*-butanol at 40 °C gave thiolactone acetate (*S*)-**9** in a 98% ee and the corresponding hydroxy methyl thiolactone (*R*)-**2** in a 94% ee. It was observed that lipase-mediated alcoholysis of compound (±)-**9** with 1 equiv (w/w) of *Carica papaya* gave thiolactone acetate (*S*)-**9** in a 89% ee and the corresponding alcohol (*R*)-**2** in a 79% ee in 2 days. The results obtained during the present investigation are summarized in Table 1. On the basis of the results obtained from the screening of various lipases, it was observed that *Carica papaya* lipase with 2 equiv (w/w) provided high conversions with an excellent enantioselectivity for this alcoholysis process. Therefore, based on these preliminary results, *Carica papaya* lipase was selected for further studies.

2.2. Effect of alcohol

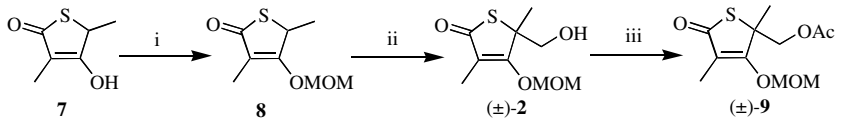
Lipase-mediated alcoholysis of (±)-5-methylacetate-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one **9** was attempted by employing two different alcohols, namely *n*-butanol and 2-propanol with *Carica papaya* 2 equiv (w/w). *Carica papaya* lipase in *n*-butanol gave better results where as this alcoholysis process in 2-propanol required 36 h to provide alcohol (*R*)-**2** in a 67% ee and acetate (*S*)-**9** in a 72% ee as shown in Table 1. Therefore, *n*-butanol was selected as a suitable alcohol for this resolution process.

2.3. Lipase-mediated transesterification

The lipase-mediated transesterification of (±)-5-hydroxymethyl-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one **2** was attempted with the above-mentioned 11 lipases employing isopropenyl acetate. The transesterification of compound (±)-**2** by employing *Carica papaya* 2 equiv (w/w) in isopropenyl acetate provides alcohol (*S*)-**2** in a 59% ee and the corresponding acetate (*R*)-**9** in a 72% ee. The immobilized forms of lipases from *Pseudomonas cepacia*, that is, PS-C and PS-D gives moderate enantioselectivities of acetate (*R*)-**9** in 81% and 69%, respectively, whereas the corresponding alcohols (*S*)-**2** were obtained in low enantioselectivities. The lipases from *Mucor miehei* and *Candida rugosa* provided thiolactone acetate (*R*)-**9** in 39% and (*S*)-**9** in 43% ee and the corresponding hydroxymethyl thiolactone in low enantioselectivities, that is, (*S*)-**2** in 27% and (*R*)-**2** in 28%. Other lipases did not show any significant conversion even after prolonged reactions time. Therefore, the lipase-mediated transesterification process



Scheme 1. Reagents and conditions: (i) anhydrous K_2CO_3, CH_3I, dry THF, reflux; (ii) Br_2, $CHCl_3$, rt; (iii) AcSH, Et_3N, CH_2Cl_2, rt; (iv) KOH, H_2O–EtOH, rt.



Scheme 2. Reagents and conditions: (i) MOMCl, *N*,*N*-diisopropylethylamine, dry CH_2Cl_2, rt; (ii) LDA, $(HCHO)_n$, dry THF, −78 °C; (iii) Ac_2O, DMAP, dry CH_2Cl_2, rt.

Table 1. Enzymatic alcoholysis of compound (±)-**9** by employing different lipases and alcohols[a]

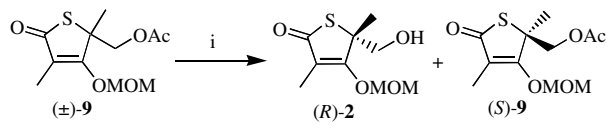
Lipase	Equiv (w/w)	Alcohol	Time (h)[b]	ee (%)[c]		Conversion (%)
				Alcohol-**2** (Config.)	Acetate-**9** (Config.)	
Carica papaya	2	*n*-Butanol	18	94 (*R*)	98 (*S*)	51
Carica papaya	1	*n*-Butanol	48	79 (*R*)	89 (*S*)	53
Mucor miehei	4	*n*-Butanol	38	68 (*R*)	56 (*S*)	45
Candida rugosa	4	*n*-Butanol	40	61 (*S*)	48 (*R*)	44
Carica papaya	2	2-Propanol	36	67 (*R*)	72 (*S*)	52

[a] Conditions: **9** (1 mmol), diisopropyl ether (10 mL), alcohol (10 mmol) at 40 °C.
[b] Time taken for alcoholysis.
[c] Enantiomeric excess of alcohol **2** and acetate **9** is determined by chiral HPLC analysis employing Daicel Chiralcel AS-H column (0.46 × 25 cm); eluent: hexane–isopropanol = 90:10; flow rate: 0.5 mL/min; UV detection: 254 nm.

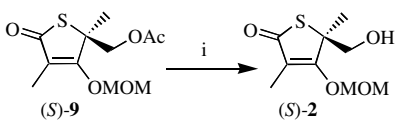
was not considered as a practical route for achieving good enantiopurities for these required intermediates.

Thus, lipase-catalyzed alcoholysis of (±)-5-methylacetate-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one **9** was carried out by employing *Carica papaya* lipase 2 equiv (w/w) and *n*-butanol in diisopropyl ether at 40 °C. The reaction progress was monitored on chiral HPLC employing a Chiralcel AS-H column. In order to obtain good enantioselectivities and yields, the reaction was stopped when it reached about 50% conversion. The pure products are isolated by silica gel column chromatography to obtain acetate (*S*)-**9** in a 98% ee and a 48% yield, while the corresponding alcohol (*R*)-**2** in a 94% ee and a 46% yield (Scheme 3).

Deacetylation of (*S*)-**9** was carried out under different reaction conditions by using anhydrous K_2CO_3 in methanol and with LiOH, to give a complex mixture of products. The successful deacetylation of (*S*)-**9** was carried out by using *Candida rugosa* lipase in *n*-butanol for 5 days to provide (*S*)-**2** in a 98% ee and with a 90% yield (Scheme 4).



Scheme 3. Reagents and conditions: (i) *Carica papaya* lipase, *n*-butanol, diisopropyl ether, 40 °C.



Scheme 4. Reagents and conditions: (i) lipase *Candiada rugosa*, *n*-butanol, diisopropyl ether, 40 °C.

2.4. Determination of the absolute configuration by Mosher's method

Over the course of our study, the absolute configuration of both the alcohols of 5-hydroxymethyl-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one **2** was determined by ^{1}H NMR spectra of their esters with α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) and correlated with the proposed model for 9-anthrylmethoxyacetic acid (9-AMA) by Riguera et al.[19] The enantiomerically enriched both 5-hydroxymethyl-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one **2** alcohols were derivatized to their corresponding (*R*)- and (*S*)-MTPA esters by the condensation with (*R*)- and (*S*)-MTPA. The alcohol obtained during the lipase-mediated alcoholysis was designated as alcohol-**I** while the other alcohol, which was obtained by *Candida rugosa* lipase-mediated deacetylation procedure, was designated as alcohol-**II**.

The condensation of (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) with alcohol-**I** by employing DCC gave (*R*)-MTPA ester-**I** and (*S*)-MTPA ester-**I**. Based on the chemical shift differences in the ^{1}H NMR spectra of (*R*)-MTPA ester-**I** and (*S*)-MTPA ester-**I**, a proposed model was demonstrated. According to Riguera's proposed empirical conformational model, the substitutent (shifted to higher field in the (*R*)-MTPA ester), placed above the plane ($\Delta\delta^{RS} < 0$) and the substitutent shifted to higher field in the (*S*)-MTPA ester is placed below the plane ($\Delta\delta^{RS} > 0$). Based on the ^{1}H NMR spectra of (*R*)-MTPA ester-**I 10** and (*S*)-MTPA ester-**I 11** of alcohol-**I**, which was obtained by lipase-mediated alcoholysis possessing an (*R*)-configuration at the asymmetric centre, and proposed model is as shown in Figure 1.

The chemical shift differences in ^{1}H NMR spectra of (*R*)-MTPA ester-**II 12** and (*S*)-MTPA ester-**II 13** indicated that alcohol-**II**, which was obtained by *Candida rugosa* lipase-mediated deacetylation procedure possesses the (*S*)-config-

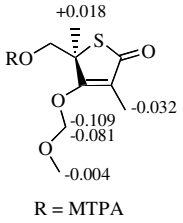


Figure 1. $\Delta\delta^{RS}$ values for MTPA derivatives of alcohol-**I**.

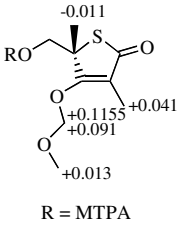


Figure 2. $\Delta\delta^{RS}$ values for MTPA derivatives of alcohol-**II**.

uration at the asymmetric centre, and the proposed model is shown in Figure 2.

3. Conclusion

In conclusion, a facile lipase-mediated resolution protocol to obtain 5-hydroxymethyl-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one, a key intermediate in the synthesis of thiolactomycin, has been achieved. The *Carica papaya* lipase-mediated alcoholysis has provided a good enantiomeric excess for alcohol (*R*)-**2** in a 94% ee as well as the corresponding acetate (*S*)-**9** in a 98% ee. The deacetylation process has been carried out by employing *Candida rugosa* lipase to provide the corresponding (5*S*)-alcohol under extremely mild reaction conditions. Furthermore, the absolute configuration of both the alcohols has been determined by the application of Mosher's method. In this connection the application of the MTPA ester method has been extended for the determination of absolute configuration of primary alcohols. Both enantiomers have been synthesized in a high enantiomeric excess, which will assist researchers to design and synthesize novel analogues in the desired stereochemical forms.

4. Experimental

4.1. Material and methods

Enzymatic reactions were carried out on 'New Brunswick-shaker' at 220 rpm. Infrared spectra of neat samples are reported in wave numbers (cm^{-1}). ^1H NMR was recorded as solutions in $CDCl_3$ and chemical shifts are reported in parts per million (PPM, δ) on a 200 MHz instrument. Coupling constants are reported in Hertz (Hz). LSIMS mass spectra were recorded on Autospec M. with 7 kV acceleration voltage and 25 kV gun voltage. HPLC analysis was performed on an instrument, which consisted of a Shimadzu LC-10AT system controller, SPD-10A fixed wavelength UV monitor as the detector. Specific rotations were recorded on SEPA-300 Horiba high sensitive polarimeter, fixed with sodium lamp of wavelength 589 nm.

4.2. Chemicals and enzymes

Methyl propionylacetate, (*R*)- and (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid, thioacetic acid, bromine, *n*-BuLi, diisopropyl amine, *N*,*N*-diisopropylethylamine, sodium hydroxide and solvents were obtained commercially and used without purification. *Pseudomonas cepacia* lipase immobilized on ceramic particles (PS-C) and *Pseudomonas cepacia* lipase immobilized on diatomaceous earth (PS-D) were purchased from Amano (Nagoya, Japan), *Carica papaya* lipase was purchased from Sigma–Aldrich.

4.2.1. Methyl-2-methyl-3-oxopentanoate 4. To a solution of methyl propionylacetate **3** (5.2 g, 40 mmol) in dry THF (70 mL) was added anhydrous K_2CO_3 (16.6 g, 120 mmol) under an N_2 atmosphere. The mixture was then refluxed for 3 h after which it turned to a pale yellow colour. The resulting mixture was cooled to 0 °C and CH_3I (3 mL, 48 mmol) was added dropwise and stirred for 6–8 h. The reaction was filtered through a Celite pad and the collected filtrate concentrated under reduced pressure to obtain an oily residue, which was purified by silica gel column chromatography to obtain 5.3 g of pure methylated β-ketoester **4**. Yield: 92%; IR (neat): 2995, 2910, 1746, 1710 cm^{-1}; ^1H NMR (200 MHz; $CDCl_3$) δ 1.03 (3H, t, $J = 7.03$ Hz), 1.30 (3H, d, $J = 7.03$ Hz), 2.4–2.6 (2H, m), 3.5 (1H, q, $J = 7.03$ Hz), 3.75 (3H, s); EIMS (*m*/*z*): 144 (M^+); Anal. Calcd for $C_7H_{12}O_3$: C, 58.32; H, 8.39. Found: C, 58.24; H, 8.29.

4.2.2. Methyl-4-bromo-2-methyl-3-oxopentanoate 5. To a solution of compound **4** (5.0 g, 34.7 mmol) in $CHCl_3$ (60 mL), Br_2 (1.78 mL, 34.7 mmol) in $CHCl_3$ (20 mL) was added at 0 °C and the reaction mixture was stirred at room temperature for 10 h. A stream of air was then passed through the solution for 1 h. After drying over anhydrous Na_2SO_4, the solvent was removed in vacuo and subjected to silica gel column chromatography to yield 4.8 g of pure γ-bromo β-ketoester **5**. Yield: 61.7%; IR (neat): 2990, 2920, 1760, 1730 cm^{-1}; ^1H NMR (200 MHz; $CDCl_3$) δ 1.29 (3H, d, $J = 7.03$ Hz), 1.76 (3H, d, $J = 7.03$ Hz), 3.75 (3H, s), 4.06 (1H, q, $J = 7.03$ Hz), 4.89 (1H, q, $J = 7.03$ Hz); FABMS (*m*/*z*): 224 (M^++2); Anal. Calcd for $C_7H_{11}BrO_3$: C, 37.69; H, 4.97. Found: C, 37.59; H, 4.91.

4.2.3. Methyl-4-(acetylsulfanyl)-2-methyl-3-oxopentanoate 6. To a stirred solution of thioacetic acid (1.57 mL, 22 mmol) in 30 mL of dry CH_2Cl_2 was added triethylamine (3.3 mL, 24 mmol) under an N_2 atmosphere and left for 20 min. The reaction mixture was then cooled to 0 °C and compound **5** (4.5 g, 20 mmol) added in 10 mL of dry CH_2Cl_2 dropwise over a period of 30 min. The resulting mixture was stirred at room temperature for 6 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2 (2×10 mL). The combined organic layers were dried over anhydrous Na_2SO_4. The solvent was removed in vacuo and the residue subjected it for silica gel column

chromatography to afford 4 g of pure **6**. Yield: 91%; IR (neat): 2980, 1750, 1715 cm^{-1}; ^1H NMR (200 MHz; $CDCl_3$) δ 1.38 (3H, d, $J = 7.03$ Hz), 1.41 (3H, d, $J = 7.03$ Hz), 2.4 (3H, m), 3.75 (3H, s), 3.8 (1H, m), 4.3 (1H, m); FABMS (*m*/*z*): 219 (M^++1); Anal. Calcd for $C_9H_{14}O_4S$: C, 49.53; H, 6.46. Found: C, 49.46; H, 6.39.

4.2.4. 3,5-Dimethyl-4-hydroxy-5*H*-thiophen-2-one 7. Potassium hydroxide (2 g, 35.7 mmol) in water (10 mL) was added dropwise to a stirred solution of thioacetate **6** (3.9 g, 17.8 mmol) in ethanol (20 mL) at an ambient temperature. The resulting solution was stirred at room temperature for 3 h. Ethanol was then evaporated and water (10 mL) added. The aqueous layer was washed with diethyl ether (2 × 10 mL) and acidified to pH 1 with the addition of 2 M HCl (10 mL). The aqueous layer was extracted with ethyl acetate. The organic layers were washed with brine, dried over anhydrous Na_2SO_4 and the solvent removed in vacuo. The crude residue was purified by flash column chromatography to obtain 2.3 g of **7** as a white solid. Mp 130–132 °C (lit.[11] mp 128–130 °C); Yield: 89%; IR (neat): 3200, 2994, 1650, 1610 cm^{-1}; ^1H NMR (200 MHz; DMSO-d_6) δ 1.52 (3H, d, $J = 7.03$ Hz), 1.7 (3H, s), 4.1 (1H, q, $J = 7.03$ Hz); EIMS (*m*/*z*): 144 (M^+); Anal. Calcd for $C_6H_8O_2S$: C, 49.98; H, 5.59. Found: C, 49.89; H, 5.46.

4.2.5. 3,5-Dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one 8. To a solution of thiolactone **7** (2.2 g, 15.27 mmol) in dry CH_2Cl_2 (20 mL) was added *N*,*N*-diisopropylethylamine (3.2 mL, 18.33 mmol) at room temperature. To this solution MOMCl (1.4 mL, 18.33 mmol) was added dropwise at 0 °C. The reaction mixture was stirred at 0 °C for 2–3 h, quenched with water and concentrated. The aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered and concentrated. The crude mixture was chromatographed on silica gel to give 2.64 g of pure compound **8**. Yield: 83%; IR (neat): 2964, 1720, 1670 cm^{-1}; ^1H NMR (200 MHz; CDCl3) δ 1.59 (3H, d, $J = 6.6$ Hz), 1.83 (3H, s), 3.51 (3H, s), 4.23 (1H, q, $J = 7.2$ Hz), 5.12 (1H, d, $J = 6.6$ Hz), 5.30 (1H, d, $J = 6.6$ Hz); EIMS (*m*/*z*): 188 (M^+); Anal. Calcd for $C_8H_{12}O_3S$: C, 51.05; H, 6.43. Found: C, 50.91; H, 6.40.

4.2.6. (±)-5-Hydroxymethyl-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one 2. To a solution of diisopropyl amine (2.3 mL, 15.95 mmmol) in dry THF (65 mL) was added *n*-BuLi (9.9 mL, 15.95 mmmol) at −78 °C dropwise for 10–20 min. The reaction mixture was warmed to 0 °C and stirred at that temperature for 30 min. The solution turned to a yellow colour indicating the generation of LDA. To this solution was added MOM protected thiolactone **8** (2.5 g, 13.29 mmol) in dry THF (10 mL) dropwise at −78 °C. After 30 min, 2.5 g of *para*-formaldehyde at the same temperature and the reaction mixture were slowly warmed to room temperature and left for 12 h. The reaction mixture was quenched with a saturated solution of NH_4Cl and extracted with ethyl acetate. The organic layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The crude mixture was chromatographed on silica gel to give pure 2.6 g of compound (±)-**2**. Yield: 89%; IR (neat): 3426, 2971, 1744, 1617 cm^{-1}; ^1H NMR (200 MHz; $CDCl_3$) δ 1.59 (3H, s), 1.89 (3H, s), 3.56 (3H, s), 3.65 (1H, d, $J = 11.12$ Hz), 3.86 (1H, d, $J = 11.54$ Hz), 5.28 (1H, d, $J = 5.98$ Hz), 5.34 (1H, d, $J = 5.98$ Hz); ^{13}C NMR (50 MHz, $CDCl_3$) 9.28, 22.04, 57.05, 59.72, 66.73, 72.21, 96.19, 111.27, 175.53, 195.28; FABMS (*m*/*z*): 219 (M^++1); Anal. Calcd for $C_9H_{14}O_4S$: C, 49.53; H, 6.46. Found: C, 49.46; H, 6.41.

4.2.7. (±)-5-Methylacetate-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one 9. To a solution of compound **2** (2.5 g, 11.46 mmol) in dry CH_2Cl_2 (20 mL), Et_3N (5.6 mL, 40.13 mmol) and a pinch of DMAP were added. The reaction mixture was then cooled to 0 °C, acetic anhydride (2.7 mL, 28.66 mmol) added dropwise and the reaction mixture warmed to room temperature and left for 1 h. The reaction mixture was quenched with water and extracted with CH_2Cl_2. The organic layers were combined, washed with brine and dried over anhydrous Na_2SO_4. The organic layer was evaporated under reduced pressure to give crude oil, which was purified by column chromatography to give pure 2.8 g of compound (±)-**9**. Yield: 94%; IR (neat): 2935, 1747, 1678, 1630 cm^{-1}; ^1H NMR (200 MHz; $CDCl_3$) δ 1.64 (3H, s), 1.94 (3H, s), 2.06 (3H, s), 3.53 (3H, s), 4.15 (1H, d, $J = 10.57$ Hz), 4.40 (1H, d, $J = 10.57$ Hz), 5.24–5.31 (2H, m); ^{13}C NMR (50 MHz, $CDCl_3$) 9.40, 20.81, 22.83, 56.14, 57.29, 66.32, 67.82, 96.18, 113.76, 170.50, 174.39, 194.94; ESIMS (*m*/*z*): 261 (M^++1). Anal. Calcd for $C_{11}H_{16}O_5S$: C, 50.76; H, 6.20. Found: C, 50.67; H, 6.12.

4.3. General procedure for lipase-mediated alcoholysis

To a solution of 5-methylacetate-3,5-dimethyl-4-methoxymethoxy-5*H*-thiophen-2-one **9** (2.8 g, 10.76 mmol) in diisopropyl ether (80 mL) were added *n*-butanol (9.9 mL, 107.6 mmol) and lipase *Carica papaya* [5.6 g, 2 equiv (w/w)]. The suspension was shaken at 220 rpm at 40 °C. The reaction monitored on chiral HPLC analysis, reached 50% conversion after 18 h. The reaction mixture was filtered and the solvent was evaporated. The residue was chromatographed on silica gel. The enantiopure products were analyzed by chiral HPLC and compared with the corresponding racemic products. Thiolactone acetate (*S*)-**9** 1.34 g was obtained. Yield: 48%; 98% ee, determined by HPLC analysis using Chiralcel AS-H column (hexane–isopropanol, 90:10) with 0.5 mL/min flow rate (t_{major} = 29.06, t_{minor} = 27.79 min); $[\alpha]_D^{25} = -27.9$ (*c* 1.03, $CHCl_3$) and 1.28 g of the corresponding alcohol (*R*)-**2**. Yield: 46%, 94% ee; determined by the HPLC analysis using Chiralcel AS-H column (hexane–isopropanol, 90:10) with 0.5 mL/min flow rate (t_{major} = 56.13, t_{minor} = 35.93 min); $[\alpha]_D^{25} = -17.73$ (*c* 1.02, $CHCl_3$).

4.3.1. (5*S*)-Hydroxymethyl-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one 2. To a solution of (5*S*)-methylacetate-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one **9** (1.3 g, 5 mmol) in diisopropyl ether (25 mL) were added *n*-butanol (4.5 mL, 50 mmol) and lipase *Candida rugosa* [2.6 g, 2 equiv (w/w)]. The suspension was shaken at 220 rpm at 40 °C. The reaction was monitored on TLC

and was completed in 5 days. The reaction mixture was then filtered and then the solvent evaporated. The residue was chromatographed on silica gel to give 0.98 g of pure (*S*)-**2**. Yield. 90%; 98% ee; determined by the HPLC analysis using Chiralcel AS-H column (hexane–isopropanol, 90:10) with 0.5 mL/min flow rate ($t_{major} = 32.86$, $t_{minor} = 54.21$ min); $[\alpha]_D^{25} = +15.8$ (*c* 1.0, $CHCl_3$).

4.4. General procedure for the preparation of MTPA esters of 5-hydroxymethyl-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one

To a solution of 5-hydroxymethyl-3,5-dimethyl-4-(methoxymethoxy)-5*H*-thiophen-2-one **2** (1 mmol) in dichloromethane (10 mL) were added a pinch of DMAP, α-methoxy-α-(trifluoromethyl)phenylacetic acid (1 mmol) and DCC (1.05 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 2–3 h, filtered, evaporated, to give the crude product, which on purification by column chromatography gave oily liquids as products.

4.4.1. [(5*R*)-4-(Methoxymethoxy)-3,5-dimethyl-5*H*-thiophen-2-one]methyl (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 10. Prepared from the above-mentioned DCC coupling procedure. Yield: 69%; $[\alpha]_D^{25} = +21.9$ (*c* 1.55, $CHCl_3$); ^1H NMR (500 MHz; $CDCl_3$) δ 1.653 (3H, s), 1.848 (3H, s), 3.446 (3H, s), 3.510 (3H, s), 4.436 (1H, d, $J = 7.2$ Hz), 4.598 (1H, d, $J = 7.2$ Hz), 5.096 (1H, d, $J = 6.6$ Hz), 5.186 (1H, d, $J = 6.3$ Hz), 7.37–7.395 (3H, m), 7.491–7.502 (2H, m); FABMS (*m*/*z*): 435 (M^++1).

4.4.2. [(5*R*)-4-(Methoxymethoxy)-3,5-dimethyl-5*H*-thiophen-2-one]methyl (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 11. Prepared from the above-mentioned DCC coupling procedure. Yield: 72%; $[\alpha]_D^{25} = -7.9$ (*c* 1.95, $CHCl_3$); ^1H NMR (500 MHz; $CDCl_3$) δ 1.635 (3H, s), 1.880 (3H, s), 3.489 (3H, s), 3.514 (3H, s), 4.431 (1H, d, $J = 7.2$ Hz), 4.661 (1H, d, $J = 7.2$ Hz), 5.205 (1H, d, $J = 6.6$ Hz), 5.267 (1H, d, $J = 6.3$ Hz), 7.376–7.401 (3H, m), 7.486–7.499 (2H, m); FABMS (*m*/*z*): 435 (M^++1).

4.4.3. [(5*S*)-4-(Methoxymethoxy)-3,5-dimethyl-5*H*-thiophen-2-one]methyl (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 12. Prepared from the above-mentioned DCC coupling procedure. Yield: 63%; $[\alpha]_D^{25} = +11.4$ (*c* 1.08, $CHCl_3$); ^1H NMR (500 MHz; $CDCl_3$) δ 1.640 (3H, s), 1.888 (3H, s), 3.493 (3H, s), 3.520 (3H, s), 4.4325 (1H, d, $J = 10.80$ Hz), 4.6685 (1H, d, $J = 10.80$ Hz), 5.213 (d, 1H, $J = 6.3$ Hz), 5.2755 (1H, d, $J = 6.3$ Hz), 7.392–7.411 (3H, m), 7.481–7.501 (2H, m); FABMS (*m*/*z*): 435 (M^++1).

4.4.4. [(5*S*)-4-(Methoxymethoxy)-3,5-dimethyl-5*H*-thiophen-2-one]methyl (2*S*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate 13. Prepared from the above-mentioned DCC coupling procedure. Yield: 75%; $[\alpha]_D^{25} = -24.9$ (*c* 2.45, $CHCl_3$); ^1H NMR (500 MHz; $CDCl_3$) δ 1.651 (s, 3H), 1.847 (3H, s), 3.438 (3H, s), 3.507 (3H, s), 4.435 (1H, d, $J = 10.80$ Hz), 4.602 (1H, d, $J = 11.70$ Hz), 5.0975 (1H, d, $J = 6.3$ Hz), 5.1845 (1H, d, $J = 6.3$ Hz), 7.379–7.384 (3H, m), 7.393–7.479 (2H, m); FABMS (*m*/*z*): 435 (M^++1).

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