Tetrahedron: Asymmetry Vol. 3, No. 1, pp. 107-114, 1992
Printed in Great Britain

0957-4166/92 $3.00+.00
Pergamon Press plc

MICROBIAL APPROACH TO CHIRAL 2-THIAZOLYL γ- AND δ-LACTONES

Giancarlo Fantin, Marco Fogagnolo, Alessandro Medici, Paola Pedrini*, and Silvia Poli

Dipartimento di Chimica, Università di Ferrara, Via Borsari 46, I-44100 Ferrara, Italy

Fausto Gardini and M. Elisabetta Guerzoni*

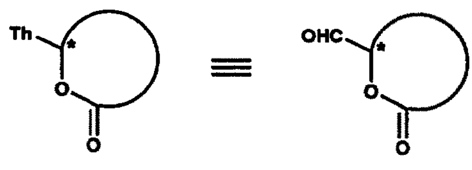
Dipartimento di Protezione e Valorizzazione Agroalimentare (DPVA), Sezione Chimica e Tecnologia degli Alimenti, Università di Bologna, Via S. Giacomo 7, I-40136 Bologna, Italy

(*Received* 25 *November* 1991)

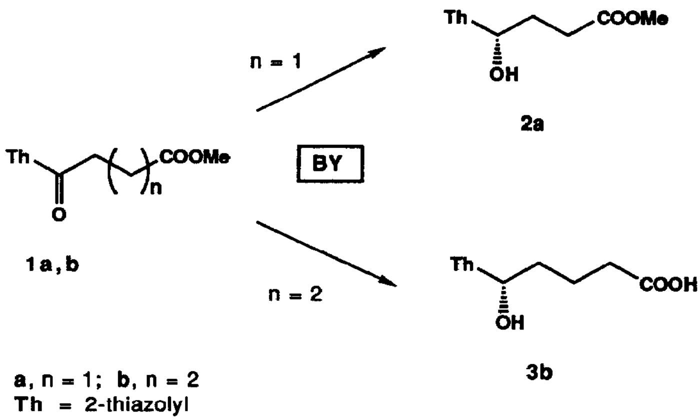
Abstract: The synthesis of chiral 2-thiazolyl γ- and δ-lactones **6** and **7** via microbial reduction of the appropiate keto esters to the homochiral hydroxy esters followed by chemical lactonization is described. Some attempts of enzymatic lactonization of the racemic hydroxy esters or resolution of racemic lactones are also reported.

Lactone derivatives are useful intermediates in the synthesis of natural products. Most of them are chiral and their physiological activity often depends on the absolute configuration.[1] In recent years, many optically active lactones have been the targets of an increasing number of attempts to synthesize them. Their synthesis in chiral forms depends, in cases of some γ- and δ-lactones, on enzymatic[2] or microbial[3] reduction of the appropriate keto acids followed by chemical lactonization and recently lipase-catalyzed asymmetric resolution of lactones, via hydrolysis, in aqueous solutions[4] or in anhydrous organic solvents[5] has been reported.

Given the importance of this class of compounds, in this paper we describe the synthesis of chiral γ- and δ-lactones bearing the thiazole ring respectively in C5 or C6 position. This heterocycle is an excellent latent formyl group equivalent since it associates the properties of stability toward hydrolysis, oxidation and reduction[6] with the ability of yielding the aldehydic group under conditions that do not affect other functional groups and chiral centers.[7]

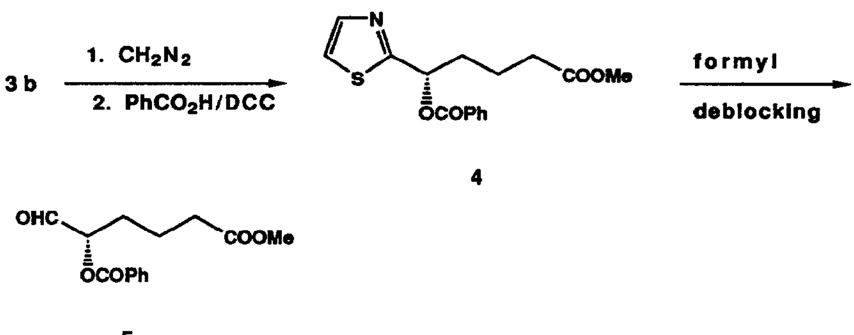


It is well documented that microbial reduction of α- and β-keto esters by baker's yeast (BY) affords chiral hydroxy esters while the same reduction with γ- and δ-keto esters or acids produces the corresponding chiral γ- and δ-lactones.[8] On the basis of this data, 2-thiazolyl γ- and δ-keto esters **1a** and **1b**, prepared from 2-trimethylsilylthiazole and the proper monomethyl ester acyl chloride[9], are initially treated with BY (Scheme 1) since it is readily available and easily manipulated.



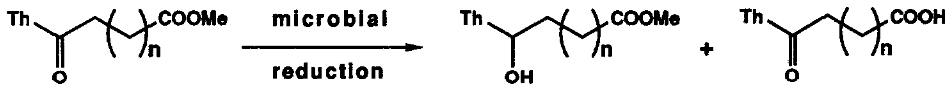
Scheme 1

The reactions were carried out through the incubation with BY at 30° C for 24 h. In both cases the prochiral carbonyl group is reduced in good yield and high enantiomeric excess producing the S-enantiomer (≥ 95%)[10] and no lactonization was found. Moreover the ester function of **1b** was hydrolyzed to acid. The absolute S-configuration of the δ-hydroxy acid **3b** is assigned by its transformation into methyl 5-(S)-benzoyloxy-5-formylpentanoate 5[11] (Scheme 2).



Scheme 2

Treatment of **3b** with diazomethane followed by reaction with benzoic acid and dicyclohexylcarbodiimmide gives the 5-(2-thiazolyl)-5-benzoyloxy methyl pentanoate **4**. Formyl deblocking of the thiazole ring produces the aldehyde ester **5** (overall yield 65%). In order to obtain also the R-enantiomer, which is not available by reduction with BY, and since the baker's yeast reductions are not always reproducible, we used a series of yeast and mould strains selected on the basis of the results obtained in a previous screening[12] (Scheme 3).



1a,b

Scheme 3

The yeast or mould culture were grown in the presence of small amounts of the substrate in order to induce or activate the production of particular enzymes during the growth phase. The substrate was added to a growing culture in a concentrated solution of a relatively non toxic solvent such as ethanol and the incubation was prolonged for 48 h at 30° C. No lactonization, but reduction, in some cases associated with hydrolysis, was found. Moreover the δ-hydroxy acid **3b** was never detected. The results of the reduction with selected yeast and mould strains are summarized in the Table.

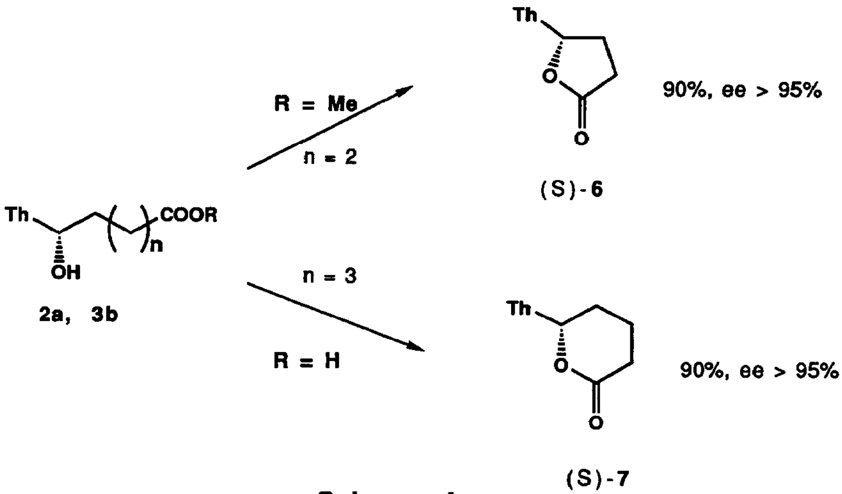
Table. Microbial Reduction of the Oxo-esters **1a** and **1b**.

Microorganism[a]	**2a** (%)[b]	ee (abs.conf.)[c]	**2b** (%)[b]	ee (abs. conf.)[c]
Trichoderma sp.	40	10 (S)	40 [d]	28 (R)
Rhizopus microsporus	63[e]	>95 (S)	96 [e]	>95 (S)
Saccharomyces cerevisiae RM9 (var. *capensis*)	9[d]	--	98	12 (S)
Saccharomyces cerevisiae ML30 (var. *chevalieri*)	91[e]	52 (R)	91[e]	48(S)
Saccharomyces cerevisiae ML38 (var. *steineri*)	13	6 (R)	27[d]	50(S)
Saccharomyces cerevisiae ML27 (var. *chevalieri*)	90	40 (R)	87	42 (S)
Saccharomyces cerevisiae BG9	20[e]	>95 (R)	35[e]	> 95 (R)
Saccharomyces cerevisiae MUT 207	5[d]	--	6[d]	60 (S)

[a] The yeast and mould culture belong to DPVA collection. [b] Yields are determined by GLC unless otherwise stated. [c] Determined by GLC by comparison with the racemic compound; absolute configuration in parenthesis. [d] The corresponding acid is present together with or without the reduction product. [e] The reactions were carried out in preparative scale.

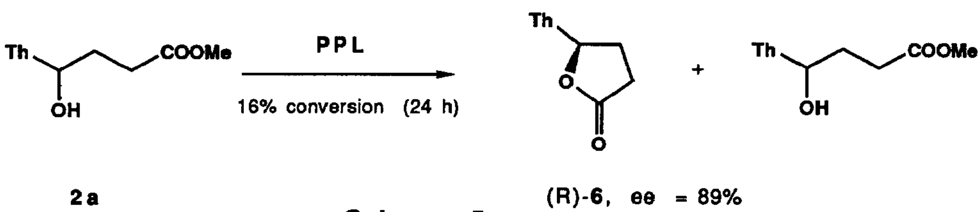
The most significant results are those with *Rhizopus microsporus* and *Saccharomyces cerevisiae* BG9. Both **1a** and **1b** are reduced in satisfactory yields and high enantiomeric excesses. The prevalence of the S-enantiomer (ee ≥ 95%) and that of the R-enantiomer (ee ≥ 95%) was obtained respectively with *Rhizopus microsporus* and *Sacch. cerevisiae* BG9. The other yeast and mould strains showed lower enantiomeric excesses and, moreover, the reduction of **1a** and **1b** afforded the opposite enantiomers.

Chemical lactonization of the homochiral 2-thiazolyl γ-hydroxy ester **2a** with TosOH-H_2O and of the homochiral 2-thiazolyl δ-hydroxy acid **3b** with DCC affords in high yields and enantiomeric excesses the homochiral 2-thiazolyl γ- and δ-lactones **6** and **7** respectively (Scheme 4).



Scheme 4

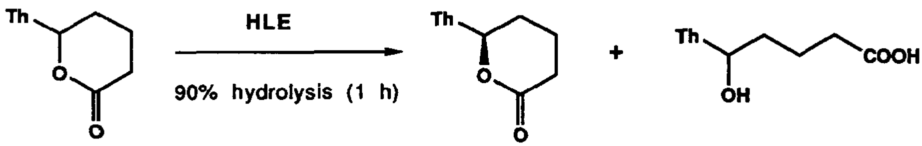
Though these results are rather satisfactory, we also checked the possibility of achieving chiral lactones via enzymatic lactonization of the racemic hydroxy esters **2a** and **2b**. The porcine pancreatic lipase (PPL) was chosen because it is stable, inexpensive and known to catalyze the lactonization of γ-hydroxy esters in organic solvents.[13] These reactions were carried out with a suspension of PPL in ether: the γ-hydroxy ester **2a** was converted into the corresponding (R)-γ-lactone **6** (16%, ee = 89%) (Scheme 5) while the δ-hydroxy ester **2b** was hydrolyzed to the acid **3b**.



Scheme 5

Since the enzymatic lactonization of the δ-hydroxy ester **2b** was uneffective, the direct enzymatic resolution of the racemic 2-thiazolyl δ-lactone **7** was achieved by using horse liver acetone powder (HLE) in phosphate buffer (Scheme 6). Thus (R)-**7** was obtained in 10% yield (ee 98%) after 90 % hydrolysis (1 h).

In conclusion, in the synthesis of chiral 2-thiazolyl γ- and δ-lactones the microbial reduction of the corresponding γ- and δ-keto esters, followed by chemical lactonization, is more efficient than enzymatic lactonization or resolution of racemic lactones.



7 (R)-7, ee = 98%

Scheme 6

Experimental

^{1}H NMR spectra were obtained on 80 MHz WP80 Bruker and on 300 MHz Gemini 300 Varian spectrometers. Chemical shifts were given in parts per million from Me_4Si as internal standard. IR spectra were recorded on a Perkin Elmer Model 297 grating spectrometer. Elemental analyses were performed on a 1106 Microanalyzer (Carlo Erba). Optical rotations were measured on a Perkin Elmer Model 241 polarimeter. Gas chromatographic analyses were performed on a Carlo Erba Fractovap 2450 T. PPL and HLAP are commercially available from Sigma.

Synthesis of the keto esters 1a and 1b. General procedure. A solution of the appropriate acyl chloride (succinic acid monomethyl ester chloride for **1a** and glutaric acid monomethyl ester chloride for **1b**) (2 mmol) in dry toluene (30 mL) was added to a stirred solution of 2-(trimethylsilyl)thiazole[9] (0.157 g, 1 mmol) in the same solvent (20 mL) under N_2. After 24 h, the reaction mixture was treated with saturated aq. $NaHCO_3$ and stirring was continued for a further 30 min. The organic layer was dried over anhydrous Na_2SO_4 and the solvent removed under vacuum. The residue was chromatographed on silica gel column. Elution with cyclohexane-ethyl acetate 7:3 afforded the title keto esters **1a** (0.18 g, 92%) and **1b** (0.17 g, 81%).

Methyl 4-oxo-4-(2-thiazolyl)butanoate (**1a**)[9]: oil; IR (film) 1740, 1695 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 2.8 (t, 2 H, *J* = 7 Hz), 3.53 (t, 2 H, *J* = 7 Hz), 3.72 (s, 3 H), 7.71 (d, 1 H, *J* = 3 Hz), 8.05 (d, 1 H, *J* = 3 Hz).

Methyl 5-oxo-5-(2-thiazolyl)pentanoate (**1b**): oil; IR (film) 1720, 1675 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.1 (m, 2 H, *J* = 7.7 Hz), 2.45 (t, 2 H, *J* = 7.7 Hz), 3.23 (t, 2 H, *J* = 7.68), 3.65 (s, 3 H), 7.67 (d, 1 H, *J* = 3 Hz), 7.98 (d, 1 H, *J* = 3 Hz). Anal Calcd for $C_9H_{11}NO_3S$: C, 50.70; H, 5.20; N, 6.57. Found: C, 50.76; H, 5.15; N, 6.51.

Reduction of the keto esters 1a and 1b with Baker's yeast. General procedure. To a slurry of fermenting Baker's yeast (10 g of yeast and 11 g of glucose in 50 mL of tap water) was added the selected keto ester (1 mmol) dissolved in ethanol (2 mL). The mixture was vigorously stirred at 30° C for 24 h, then filtered through a Celite pad and extracted with diethyl ether (30 X 20 mL). The extracts were dried over anhydrous Na_2SO_4 and evaporated under vacuum. The residue was chromatographed on silica gel column. Elution with cyclohexane-ethyl acetate 7:3 afforded **2a** (0.15g, 75%, ee ≥ 95%) and **3b** (0.12 g, 60%, ee ≥ 95%) respectively.

(-)-(S) Methyl 4-hydroxy-4-(2-thiazolyl)butanoate (**2a**) showed the following: oil; $[\alpha]_D$ = -22.1 (c 4.8, $CHCl_3$); IR (film) 3350, 1745 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.12-2.75 (m, 4 H), 3.72 (s, 3 H), 4.65 (br, 1 H), 5.1 (m, 1 H), 7.33 (d, 1 H, *J* = 3 Hz), 7.75 (d, 1 H, *J* = 3 Hz).

(-)-(S) Methyl 5-hydroxy-5-(2-thiazolyl)pentanoic acid (**3b**): oil; $[\alpha]_D$ = -20.8 (c 3, $CHCl_3$); IR ($CHCl_3$) 1695 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.7-2.05 (m, 4 H), 2.38 (t, 2 H, *J* = 7.2 Hz), 4.98 (dd, 1 H, *J* = 4.8 and 8 Hz), 7.55 (d, 1 H, *J* = 3.3 Hz), 7.75 (d, 1 H, *J* = 3.3 Hz. Anal Calcd for $C_8H_{11}NO_3S$: C, 47.76; H, 5.51; N, 6.96. Found: C, 47;85; H, 5;48; N, 6.91.

Methyl 5-(S)-(benzoyloxy)-5-formylpentanoate (5)[11] **from 3b.** To a solution of **3b** (0.2 g, 1 mmol) in diethyl ether (40 mL) was added a solution of CH_2N_2 (3 mmol) in the same solvent. After 30 min, the solvent was evaporated in vacuo and the crude methyl ester (0.2 g, 94%) was obtained [^1H NMR (80 MHz, $CDCl_3$) δ 1.9 (m, 4 H), 2.4 (t, 2 H), 3.67 (s, 3 H), 5.0 (m, 1 H), 7.27 (d, 1H, *J* = 3.2 Hz), 7.65 (d, 1 H, *J* = 3.2 Hz)], and used without further purification in the second step. A solution of the crude hydroxy ester (0.2 g), benzoic acid (0.12 g, dicyclohexylcarbodiimide (0.21g) and 4-dimethylaminopyridine (0.01 g) in diethyl ether (50 mL) was stirred at room temperature for 24 h according to the literature procedure.[14] The precipitate was filtered off, the solution was evaporated in vacuo and the residue chromatographed on silica column. Elution with petroleum ether-ethyl acetate 7:3 gave the benzoyl derivative **4** (0.23 g, 72%): oil; IR (film) 3100, 2960, 1730, 1270 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.85 (m, 2 H), 2.35 (m, 4 H), 3.65 (s, 3 H), 6.37 (t, 1 H, *J* = 6.2 Hz), 7.27 (d, 1 H, *J* = 3.4 Hz), 7.5 (m, 3 H), 7.75 (d, 1 H, *J* = 3.4 Hz), 8.05 (m, 2 H).

The thiazole ring of compound **4** (O.23 g) was transformed into formyl group according to the literature procedure[7] consisting of: i) N-methylation with methyl iodide to give the corresponding N-thiazolium salt; ii) reduction of the N-thiazolium salt with sodium borohydride to afford the thiazoline; iii) hydrolysis of the thiazoline with $HgCl_2$ in acetonitrile/water 4:1 to the corresponding aldehyde **5** (0.12 g, overall yield 65%) with physical and spectroscopic characteristics identical to published values: $[\alpha]_D$ = - 31.2 (c 2.5, $CHCl_3$) or -40 (neat) [lit[11] $[\alpha]_D$ = -33.3 (c 2.5, $CHCl_3$) or - 41.8 (neat)].

Microbial reduction of keto esters 1a and 1b. General procedure. To a yeast or mould culture (200 mL),[15] grown for 48 h in the presence of small amounts of the selected substrate (0.25 mL)[16], a further 1 mL of the substrate solution[16] was added and the incubation was prolonged for a further 48 h at 30° C. The suspension was removed by centrifugation, the mixture was extracted with diethyl ether and dried over anhydrous Na_2SO_4. The reduction products were analyzed by GLC on a chiral column.[17]

Chromatography of the reaction mixture (silica gel, petroleum ether-ethyl acetate 7:3) gave the corresponding hydroxy esters **2a** and **2b** (see Table).

(-)-(S) Methyl 5-hydroxy-5-(2-thiazolyl)pentanoate (**2b**): oil; $[\alpha]_D$ = -19.8 (c 7.6, $CHCl_3$); IR (film) 3400, 1720 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.75-2.1 (m, 4 H), 2.4 (t, 2 H), 3.5 (br, 1 H), 3.68 (s, 3 H), 5.03 (m, 1 H), 7.31 (d, 1 H, *J* = 3.2 Hz), 7.72 (d, 1 H, *J* = 3.2 Hz). Anal Calcd for $C_8H_{13}NO_3S$: C, 47.29; H, 6.45; N, 6.89. Found: C, 47.36; H, 6.49; N, 6.85.

Chemical lactonization of (-)-(S) 2a. A trace amount of p-TsOH monohydrate was added to a solution of (-)-S-**2a** (0.1 g) in C_6H_6 (30 mL) and the mixture was stirred and heated under reflux for 1 h. After cooling, it was diluted with ether (60 mL). The solution was washed with $NaHCO_3$ aqueous and brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was chromatographed on silica gel column (petroleum ether 7:3 as eluent) to give 0.1 g of the (S)-γ-lactone **6** (ee ≥ 95% from GLC)[18]: oil; $[\alpha]_D$ = 11.2 (c 3.2, $CHCl_3$); IR (film) 1775 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.65 (m, 4 H), 5.8 (m, 1 H), 7.42 (d, 1 H, *J* = 3.3 Hz), 7.83 (d, 1 H, *J* = 3.3 Hz); mass spectrum m/e 169 (M^+), 139, 105, 86. Anal Calcd for $C_7H_7NO_2S$: C, 49.71; H, 4.17; N, 8.28. Found: C, 49.25; H, 4.21; N, 8.35.

Chemical lactonization of (-)-(S)-3b. To a stirring solution of **3b** (0.5 g, 2.48 mmol) in THF (50 mL) was added dicyclohexylcarbodiimmide (0.51 g, 2.48 mmol) and 4-dimethylaminopyridine (0.05 mmol). After 12 h the precipitate was filtered off, the solvent was removed in vacuo, the residue was dissolved in diethyl ether and washed with a saturated solution of $NaHCO_3$. The organic layer was dried over anhydrous Na_2SO_4 and, after removing the solvent, the residue was chromatographed on silica gel column (petroleum ether 7:3 as eluent) to give 0.4 g (90%) of the (S)-δ-lactone **7** (ee> 95% from GLC)[9]: oil; $[\alpha]_D$ = -9.1 (c 0.7, $CHCl_3$); IR (film) 1740 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 2.0 (m, 2 H), 2.13 (m, 1 H), 2.45 (m, 1 H), 2.68 (m, 2 H), 5.72 (dd, 1 H, *J* = 4 and 9 Hz), 7.38 (d, 1 H, *J* = 3.3 Hz), 7.82 (d, 1 H, *J* = 3.3 Hz). Anal Calcd for $C_8H_9NO_2S$: C, 52.46; H, 4.95; N, 7.65. Found: C, 52.37; H, 4.98; N, 7.71.

PPL (porcine pancreatic lipase) catalyzed lactonization of the hydroxy ester 2a. The powdered commercial preparation of PPL (1 g) was added to a solution of **2a** (0.5 mmol) in dry ether (10 mL) and the suspension was vigorously stirred. Aliquots were withdrawn periodically and their GLC chromatogram on chiral column were obtained. A 16% conversion experiment (24 h) afforded (R)-γ-lactone **6** (ee = 89%).

HLE (horse liver acetone powder) enzymatic resolution of the racemic δ-lactone 7. The commercial HLAP (0.1 g) was added to a solution of the racemic **7** (0.1 g) in 5 mL of phosphate buffer (pH 7.2). Aliquots were withdrawn periodically, acidified, extracted with ether and treated with CH_2N_2. Their GLC chromatogram on chiral column showed that the 90% hydrolysis experiment (1 h) afforded the (R)-δ-lactone **7** (ee = 98%).

References and Notes

1. Silverstein, R. M. *Semiochemistry, Flavors, and Pheromones*, Proceedings ACS Symposium; Acree, T. E., Ed.; W. de Gruyter and Co.:Berlin, 1985; p 121.
2. Ng, G. S. Y.; Yuan, L. C.; Jakovac, I. J.; Jones, J. B. *Tetrahedron* **1984**, 40, 1235.
3. Seebach, D.; Eberie, M. *Synthesis* **1986**, 37. Morinchi, F.; Muroi, H.; Hiroshi, A. *Chem. Lett.* **1987**, 1141. Manzocchi, A.; Rosangela, C.; Fiecchi, A.; Santaniello, E. *J. Chem. Soc. Perkin I* **1987**, 2753.
4. Blanco, L.; Guibe-Jampel, E.; Roseeau, G. *Tetrahedron Lett.* **1988**, 29, 1915.
5. Gutman, A. L.; Zuobi, K.; Bravdo, T. *J.Org. Chem.* **1990**, 55, 3546.
6. Metzger, A. I. *The Chemistry of Heterocyclic Compounds. Thiazole and its Derivatives*; Wiley: New York, 1979; vol 34.
7. Dondoni, A.; Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P. *J. Org. Chem.* **1989**, 54, 693.
8. Csuk, R.; Glanzer, B. I. *Chem. Rev.* **1991**, 91, 49 and references cited therein.
9. Dondoni, A.; Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P. *J. Org. Chem.* **1988**, 53, 1748.
10. Enantiomeric excesses are determined by GLC on chiral column by comparison with the racemic compound.
11. Han, C.-Q.; Di Tullio, D.; Wang, Y.-F.; Sih, C. J. *J. Org. Chem.* **1986**, 51, 1253. Corey, E. J.; Marfat, A.; Goto, G.; Brion, F. *J. Am. Chem. Soc.* **1980**, 102, 7984.
12. Fantin, G.; Fogagnolo, M.; Medici, A.; Pedrini, P.; Poli, S.; Gardini, F.; Guerzoni, M. E. *Tetrahedron Asymmetry* **1991**, 2, 243.
13. Gutman, A. L.; Zuobi, K.; Bravdo, T. *J. Org. Chem.* **1990**, 55, 3546.
14. Ziegler, F. E.; Berger, G. D. *Synth. Comm.* **1979**, 9, 539.
15. A synthetic culture medium contained for 1 L of water: glucose (50 g), $(NH_4)_2SO_4$ (5 g), KH_2PO_4 (2 g), $CaCl_2$ (0.25 g), $MgSO_4.7H_2O$ (0.25 g), inositol (25 mg), H_3BO_3 (1 mg), $ZnSO_4$ (1 mg), $MnCl_2$ (1 mg), $FeCl_2$ (0.5 mg), $CuSO_4$ (0.1 mg), KI (0.1 mg), tiamine (0.3 g), biotine (0.025 mg), calcium panthothenate (0.3 mg), pyridoxine (0.3 mg) and nicotinic acid (0.3 mg), is inoculated with a spore suspension at grown at 30° C.
16. The solution is prepared dissolving 0.4 g of the selected keto ester in 2 mL of ethanol.
17. Enantiomer separation on Megadex 1 column (25 m X 0.32 mm) containing permethylated β-cyclodextrine in OV 1701 from Mega s.n.c.: carrier gas: helium (1 atm); temp: 100-200° C. Retention time in min (after silylation): **2a** (3° C/min) 21.3 and 21.4; **2b** (5° C/min) 19.08 and 19.17.
18. Retention time (in min) of lactones: **6** (3° C/min) 25.65 and 26.21; **7** (2.5° C/min) 30.69 and 31.09.