Pergamon

Tetrahedron: *Asymmetry* 11 (2000) 1353–1366

TETRAHEDRON: *ASYMMETRY*

Chemoenzymatic synthesis of optically active 4-methyl-tetrahydro-5-oxo-2-furancarboxylic acids and esters

Sara Drioli, Cristina Forzato, Patrizia Nitti, Giuliana Pitacco and Ennio Valentin*

Dipartimento di Scienze Chimiche, Università di Trieste, via Licio Giorgieri 1, I-34127, Trieste, Italy

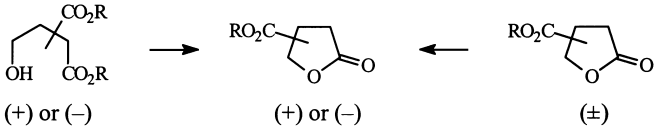
Received 21 January 2000; accepted 7 February 2000

Abstract

Enantiomerically pure 4-methyl-tetrahydro-5-oxo-2-furancarboxylic acids and esters are prepared by enzymatic resolution of the chiral racemic esters. Their stereochemistry as well as their absolute configurations have been established by chemical correlation. The influence of the alkoxycarbonyl group at C-2 and that of the methyl group at C-4 on the sign of the Cotton effect in their CD spectra have been investigated. Formation of enantiomerically pure hydroxydiesters, precursors of the above-mentioned γ-lactones, by baker's yeast reduction of the corresponding ketodiesters was unsatisfactory.

1. Introduction

The synthesis of enantiomerically pure γ-lactones bearing either a carboxy group or an alkoxycarbonyl group on the ring can be accomplished in many ways,[1] among which the cyclization reactions of the appropriate optically active functionalized hydroxydiesters and the enzymatic kinetic resolution of the desired chiral racemic lactonic esters should be mentioned[1b,d] (Scheme 1).



Scheme 1.

In particular, enantiomerically pure tetrahydro-5-oxo-2-furancarboxylic esters were obtained by enzymatic resolution of their racemates.[1b] Their α-methyl derivatives, namely the *trans*- and *cis*-4-methyl-tetrahydro-5-oxo-2-furancarboxylic esters **1** and **2** (Fig. 1), are precursors of (+)- and (–)-marmelo lactones A and B,[2] two monoterpenes isolated by Tsuneya et al.[3] from the fruit of a quince (*Cydonia oblonga* MILL, marmelo) as the main flavour components.

* Corresponding author. Tel: +39-040-6763917; fax: +39-040-6763903; e-mail: valentin@dsch.univ.trieste.it

0957-4166/00/$ - see front matter
PII: S0957-4166(00)00057-4

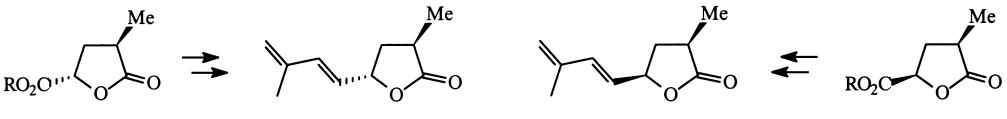


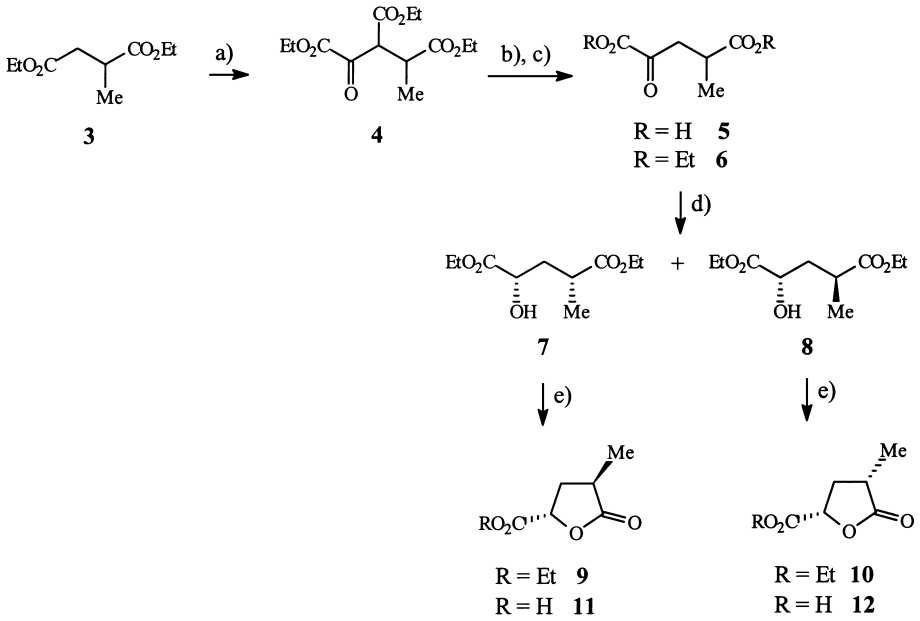
Figure 1. Structures of marmelo lactones A and B

In principle, the *trans*- and *cis*-lactones **1** and **2** could be prepared by direct α-methylation of the commercially available enantiomerically pure tetrahydro-5-oxo-2-furancarboxylic acids. However, this reaction is known to proceed predominantly in the *anti* mode, at least on the γ-alkoxymethyl analogues,[4] furnishing the *trans*-diastereomer preferentially. In the present paper, we propose the synthesis of a few derivatives of **1** and **2** with good enantiomeric excesses, by the use of enzymes in the enantiodifferentiating step. An alternative approach was also explored, namely the bioreduction with raw baker's yeast of the ketodiester precursor of the desired lactones.

2. Results and discussion

2.1. Synthesis of the substrates

The condensation of diethyl methylbutanedioate **3** with ethyl oxalate, acid hydrolysis and decarboxylation of the triester **4**, followed by esterification with ethanol of the resulting diacid **5**[5] (Scheme 2), furnished diethyl 4-methyl-2-oxopentanedioate **6**.[6,7] Its chemical reduction led to the corresponding hydroxydiesters **7** and **8** as a 1:1 mixture of *syn*- and *anti*-diastereomers. This assignment was made after cyclization of **7** and **8** into the respective γ-lactones **9** and **10**, which

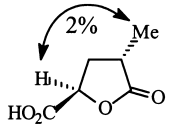


Scheme 2. (a) EtO^-Na^+, EtOH, diethyloxalate; (b) 4N HCl, 100°C; (c) EtOH, C_6H_6, PTSA, 80°C; (d) H_2, 5% Pd(C), EtOAc; (e) C_6H_6, PTSA, 80°C

were correctly attributed the *trans* and *cis* stereochemistries, respectively, as will be shown later. The reduction of the ketodiester **6** was carried out under heterogeneous catalysis because the use of sodium borohydride produced mixtures of products, as a consequence of the simultaneous partial reduction of the ester groups.[1n]

2.2. Determination of the geometry of lactones *9* and *10*

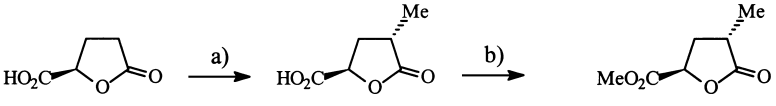
In order to assign the correct stereochemistry to lactones **9** and **10** and hence to their precursors **7** and **8**, NOE difference measurements were performed on both diastereomers, as well as on their acid derivatives **11** and **12**. Unfortunately, the only significant result (Fig. 2) was not considered conclusive proof, owing to the low value of the NOE effect observed.



(–)-**11**

Figure 2. DIFNOE measurements on lactone (–)-**11**

To produce further evidence, the commercially available lactone (*R*)-(–)-tetrahydro-5-oxo-2-furancarboxylic acid **13** was α-methylated following the procedure of Grieco and Miyashita.[8] The resulting lactonic acid (–)-**11** was isolated as a single diastereomer and purified as its methyl ester (–)-**14** (Scheme 3). Although the yield of the product (–)-**11** was very poor (10%), no trace of its *cis*-diastereomer **12** was detected of the ^{1}H NMR spectrum of the crude reaction mixture. Since the α-methylation of γ-lactones is known to be stereocontrolled, at least for the γ-alkoxymethyl γ-lactones,[4] the reaction product (–)-**11** and hence its ethyl ester (–)-**9** were assigned the *trans* configuration.



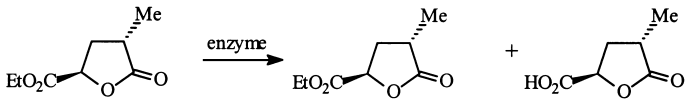
(–)-**13** (–)-**11** (–)-**14**

Scheme 3. (a) CH_3I, LDA, HMPA, THF, –78°C; (b) CH_2N_2

2.3. Enzymatic resolution of the lactonic esters (±)-*9* and (±)-*10*

Of the various enzymes checked, the best result for the enzymatic resolution of the *trans*-diastereomer (±)-**9** was obtained with α-chymotrypsin (α-CT), which showed the highest enantiomeric ratio E[9] (Table 1). At low conversion value (20%) the hydrolysis product (–)-**11** was isolated with 82% e.e. and 12% yield, while at high conversion (78%) the lactonic ester (+)-**9** was obtained with 98% e.e. and 21% yield. While porcine pancreatic lipase (PPL) and proteinase from *Bacillus subtilis* showed the same enantiopreference as α-CT, lipase PS (*Pseudomonas fluorescens*) and *Pseudomonas fluorescens* lipase (PFL) showed opposite enantiopreferences. However, in all cases the enantioselectivity was very poor. Other enzymes such as porcine lipase acetone powder (PLAP), lipase AY (*Candida rugosa*), lipase AP12 (*Aspergillus niger*) and *Mucor miehei* lipase (MML) were also effective in hydrolyzing the substrate, although with no enantioselectivity.

Table 1
Hydrolysis of ethyl *trans*-4-methyl-tetrahydro-5-oxo-2-furancarboxylate (±)-**9**

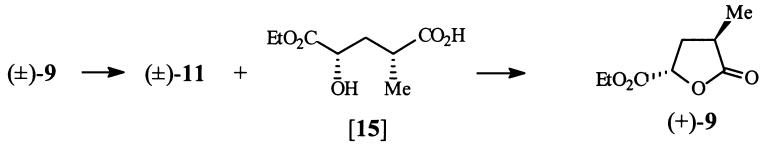


(±)-**9** (+) or (–)-**9** (+) or (–)-**11**

Enzyme	E	conv. (%)	Ester	Acid
α-Chymotrypsin (α-CT)[a]	12	20	(+)-**9** (20% e.e., 70% c.y.)	(–)-**11** (82% e.e., 12% c.y.)
		78	(+)-**9** (98% e.e., 21% c.y.)	(–)-**11** (26% e.e., 60% c.y.)
Lipase PS (*Pseudomonas fluorescens*) [b]	9	20	(–)-**9** (19 % e.e.)	(+)-**11** (76 % e.e.)
Porcine pancreatic lipase Type II (PPL)[c]	7	22	(+)-**9** (20 % e.e.)	(–)-**11** (69 % e.e.)
Pseudomonas fluorescens lipase (PFL)[d]	5	31	(–)-**9** (25 % e.e.)	(+)-**11** (56 % e.e.)
Proteinase from *Bacillus subtilis* [e]	3	18	(+)-**9** (10 % e.e.)	(–)-**11** (46 % e.e.)

[a]) 0.30 g (1.7 mmol) of (±)-**9**, 35 mg α-CT in 24 ml of phosphate buffer; [b]) 0.05 g (0.3 mmol) of (±)-**9**, 0.1 g of lipase PS in 4 ml of phosphate buffer; [c]) 0.20 g (1.1 mmol) of (±)-**9**, 171 mg PPL in 4 ml of phosphate buffer;[d]) 0.12 g (0.7 mmol) of (±)-**9**, 21 mg of PFL in 3 ml of phosphate buffer; [e]) 0.10 g (0.6 mmol) of (±)-**9**, 5 mg of proteinase from *Bacillus subtilis* in 12 ml of phosphate buffer.

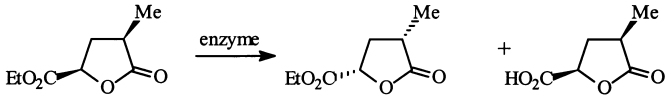
A particular reactivity was observed for horse liver esterase (HLAP), which hydrolyzed both the ethoxycarbonyl group and the lactone group, only the latter enantioselectively. In fact, at about 20% conversion, extraction of the reaction mixture (pH 7.4) afforded the lactonic ester (–)-**9** with 19% e.e. Acidification of the mother liquors to pH 1, followed by extraction, afforded about a 1:1 mixture of the racemic lactonic acid (±)-**11**, analyzed as its methyl ester by chiral HRGC, and the lactonic ethyl ester (+)-**9** having 78% e.e. Therefore, it is evident that this latter compound was formed by an enantioselective enzymatic ring opening of (±)-**9** and subsequent cyclization of the hydroxyacid **15**, not isolated, under acidic conditions (Scheme 4).



Scheme 4.

The most significant results of the enzymatic resolution of (±)-**10** are listed in Table 2. Using PPL, the lactonic acid (+)-**12** was isolated with 92% e.e. and 15% yield at low conversion values, while at high conversion values the unreacted lactonic ester (–)-**10** was obtained with 99% e.e. and 30% yield. The same enantiopreference was observed for α-CT and for proteinase from *Bacillus subtilis*, although with lower enantiomeric excesses. Other enzymes such as lipase AY, lipase PS, PFL and MML were also able to bring about the hydrolysis of the ethoxycarbonyl group, although with no enantioselectivity.

Table 2
Hydrolysis of ethyl *cis*-4-methyl-tetrahydro-5-oxo-2-furancarboxylate (±)-**10**



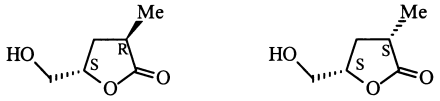
(±)-**10** (+) or (–)-**10** (+) or (–)-**12**

Enzyme	E	conv. (%)	Ester	Acid
Porcine pancreatic lipase Type II (PPL)[a]	35	30	(–)-**10** (40 % e.e., 67 % c.y.)	(+)-**12** (92 % e.e., 15 % c.y.)
		65	(–)-**10** (99 % e.e., 30 % c.y.)	(+)-**12** (40 % e.e., 55 % c.y.)
α-Chymotrypsin (α-CT)[b]	13	28	(–)-**10** (34 % e.e.)	(+)-**12** (82 % e.e.)
Proteinase from *Bacillus subtilis*[c]	7	22	(–)-**10** (10 % e.e.)	(+)-**12** (73 % e.e.)

[a]) 0.30 g (1.7 mmol) of (±)-**10**, 140 mg PPL in 24 ml of phosphate buffer. [b]) 0.15 g (0.8 mmol) of (±)-**10**, 17 mg α-CT in 3 ml of phosphate buffer. [c]) 0.10 g (0.6 mmol) of (±)-**10**, 5 mg of proteinase from *Bacillus subtilis* in 12 ml of phosphate buffer.

2.4. Determination of the absolute configuration of the γ-lactones (+)-9 and (–)-10

The absolute configuration of the lactones (+)-**9** and (–)-**10** was determined by reduction of their ethoxycarbonyl group with sodium borohydride at room temperature to give the corresponding lactonic alcohols (+)-**16** and (+)-**17**, respectively, whose absolute configurations are known (Fig. 3).[4a] As a consequence, the absolute configuration of the dextrorotatory ethyl *trans*-4-methyl-tetrahydro-5-oxo-2-furancarboxylate (+)-**9** is 2*S*,4*R* and that of the laevorotatory *cis*-diastereomer (–)-**10** is 2*S*,4*S*.



(+)-**16** (+)-**17**

Figure 3. Structures of lactones (+)-**16** and (+)-**17**

Incidentally, in this manner the assignment of the *trans–cis* relative stereochemistries was also confirmed.

2.5. Baker's yeast reduction of the ketodiester (±)-6

Reduction of the ketodiester (±)-**6** with baker's yeast was performed in water, after a thermal pretreatment of the yeast at 50°C for some period, as indicated in Table 3.

Table 3
Reduction of the ketodiester (±)-**6** with raw baker's yeast in water[a]

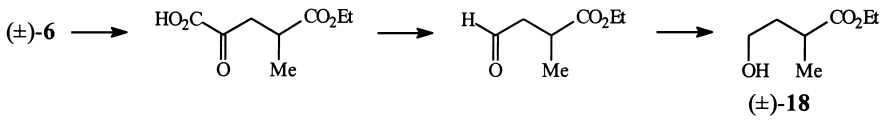
		7			**8**			**18**
Preincub. time (h)	React. time (h)	Rel. yield (%)	E.e. (%)[b]	Abs. config.[b])	Rel. yield (%)	E.e. (%)[b]	Abs. config.[b])	Rel. yield (%)
0.5	2	30	11	(2S,4R)	23	58	(2S,4S)	47
	72	25	25	(2R,4S)	18	55	(2S,4S)	57
1.0	6	30	6	(2S,4R)	15	78	(2S,4S)	55
	72	19	13	(2R,4S)	16	80	(2S,4S)	65

[a]) Conditions used: 4.6 mmoles of substrate, 46 g of b.y. in water (92 ml), room temp.

[b]) Determined after lactonization by chiral HRGC (γ-CDX).

In fact, without this preincubation, the only product formed was the hydroxyester **18**. This was formed by hydrolysis and decarboxylation of the ethoxycarbonyl group close to the carbonyl group, followed by reduction of the resulting formyl group, as already found for diethyl 2-oxopentanedioate[1b]

(Scheme 5). Formation of the hydroxyester **18**, which was not enantioselective, was minimized but not prevented when baker's yeast was preincubated at 50°C.



Scheme 5.

As shown in Table 3, baker's yeast reduction was by no means enantioselective or diastereoselective, as mixtures of *syn*- and *anti*-alcohols **7** and **8**, having low enantiomeric excesses, were always found. Interestingly, prolonged reaction times favoured the inversion of configuration of both stereocentres in the *syn*-hydroxydiester **7**. From an analysis of the variation of the relative percentages of the four stereoisomers and that of the racemic decomposition product it can be deduced that the oxidative process which initiates the decomposition of the hydroxydiesters occurred preferentially on the (2*S*,4*R*) and (2*S*,4*S*) diastereomers. However, since the enantiomeric excess of the *anti*-hydroxydiester **8** remained constant, the oxidative process must be counterbalanced by the reductive process occurring on the resulting ketodiester **6**, owing to the fact that reductases were still active.

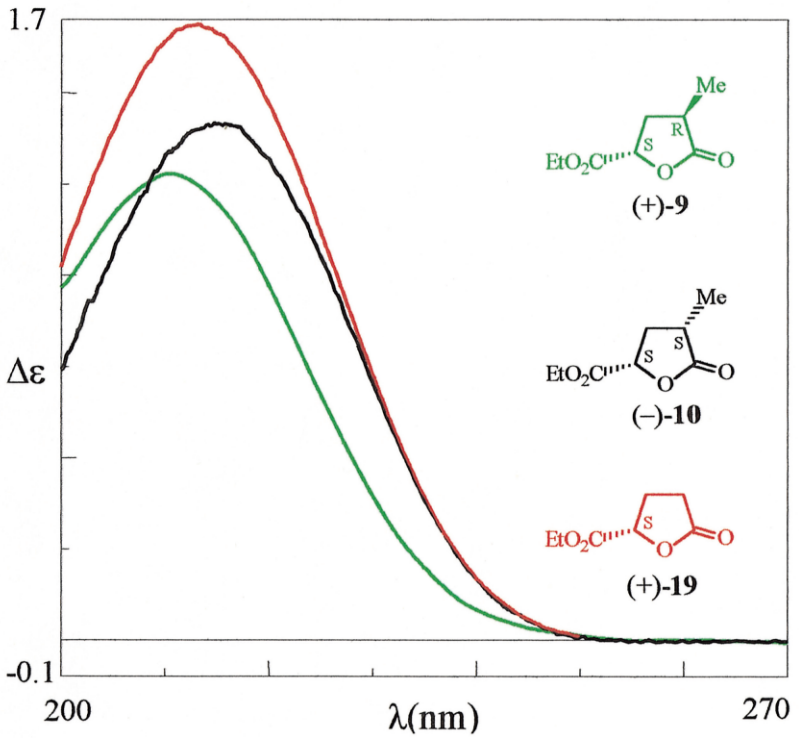


Figure 4. CD spectra of lactones (+)-**9**, (–)-**10** and (+)-**19**

2.6. Analysis of the CD spectra

The CD spectra of the γ-lactones (+)-**9** and (–)-**10**, bearing the ethoxycarbonyl group at the γ-position, are shown in Fig. 4, while those of the γ-lactones bearing the hydroxymethyl group at the same position, (+)-**16** and (+)-**17**, are shown in Fig. 5. Both figures also include, for a comparison, the respective lactones bearing no substituent at the α-position, namely ethyl (*S*)-(+)-tetrahydro-5-oxo-2-furancarboxylate **19** in Fig. 4 and (*S*)-(+)-5-hydroxymethyl-4,5-dihydro-2(3*H*)-furanone **20** in Fig. 5.

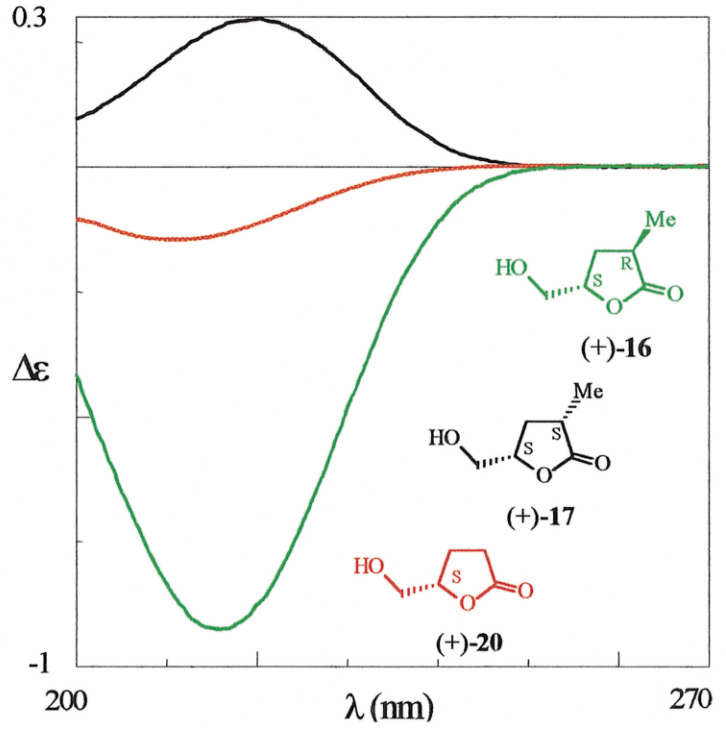


Figure 5. CD spectra of lactones (+)-**16**, (+)-**17** and (+)-**20**

The synthesis of (*S*)-(+)-**19**[1b] from (*S*)-(+)-**13** was achieved by the procedure used for the racemization-free peptide coupling,[10] to avoid undesired side reactions occurring by direct esterification.[1n] By treatment with sodium borohydride (*S*)-(+)-**19** afforded the corresponding hydroxymethyl-substituted γ-lactone (*S*)-(+)-**20**.

The γ-ethoxycarbonyl γ-lactones (+)-**9**, (–)-**10** and (+)-**19**, having the same absolute configuration of the γ-carbon atom, showed a positive Cotton effect associated with the n→π* transition of about the same magnitude. This effect is ascribed to the sole ethoxycarbonyl group at the γ-position, as already underlined by Matsumoto et al.,[11] whereas the configuration of the α-carbon atom has only minor, if any, effect on the sign of the Cotton effect. In fact, when the ethoxycarbonyl group was reduced to the hydroxymethyl group, as in (+)-**16**, (+)-**17** and (+)-**20**, the Cotton effect depends only on the configuration of C-α, i.e. on the orientation of the methyl group, in accordance with Okuda's rule.[12,13] In fact the hydroxymethyl-substituted γ-lactone **20** showed a weak negative Cotton effect, which was enhanced by the consignate effect of the methyl

group in (+)-**16**, whereas it was overwhelmed by the strong opposite effect of the same substituent in (+)-**17**.

3. Conclusions

It should be underlined that of the two diastereomeric 4-methyl-tetrahydro-5-oxo-2-furancarboxylic acids **11** and **12** only the *trans*-isomer could be prepared enantiomerically pure from the commercially available (*R*)-(+)- and (*S*)-(–)-tetrahydro-5-oxo-2-furancarboxylic acid. The chemoenzymatic procedure proposed in the present paper allows the synthesis of both enantiomers of both diastereomers with good enantiomeric excesses and satisfactory yields. Their CD spectra showed the relevance of the presence of a carboxy group at the γ-position of a γ-lactone ring.

4. Experimental

4.1. General

IR spectra were recorded in $CHCl_3$, unless otherwise stated, on a Jasco FT/IR-200 spectrometer. ^1H and ^{13}C NMR spectra were run on a Jeol EX-400 spectrometer (400 MHz for proton and 100.4 MHz for carbon) using deuteriochloroform as a solvent and tetramethylsilane as an internal standard. Coupling constants are given in hertz. Optical rotations were determined on a Perkin–Elmer Model 241 polarimeter. CD spectra were obtained on a Jasco J-710 spectropolarimeter for methanol solutions. GLC analyses were performed on a Carlo Erba GC 8000 and on a Shimadzu GC-14B instrument, the capillary columns being EC-WAX, 30 m×0.32 mm, OV1701, 25 m×0.32 mm (carrier gas He 40 kPa, split 1:50, 10 min at 100°C, 3°C/min, 200°C), Chiraldex™ type G-TA, trifluoroacetyl γ-cyclodextrin 40 m×0.25 mm (carrier gas He 180 kPa, split 1:100, 10 min at 100°C, 3°C/min, 150°C) or DMePe β-cyclodextrin 25 m×0.26 mm (carrier gas He 110 kPa, split 1:50, 10 min at 100°C, 3°C/min, 150°C). Mass spectra were run in the electron impact mode (20 eV and 70 eV) on a VG 7070 spectrometer. Enzymatic hydrolyses were performed using a pH-stat controller PHM290 Radiometer, Copenhagen. TLCs were performed on Whatman K6F silica gel plates (eluant: light petroleum:ethyl acetate, 7:3). Flash chromatography was run on a silica gel 230–400 mesh ASTM (Kieselgel 60, Merck). Light petroleum refers to the fraction with b.p. 40–70°C and ether to diethyl ether.

α-Chymotrypsin (α-CT, 51.8 U/mg), *Pseudomonas fluorescens* lipase (PFL, 42.5 U/mg), proteinase from *Bacillus subtilis* (13.1 U/mg) and *Mucor Miehei* lipase (Lipozyme) were purchased from Fluka; porcine pancreatic lipase Type II (PPL) and pig liver acetone powder (PLAP) were purchased from Sigma; lipase AY (*Candida rugosa*), lipase PS (*Pseudomonas fluorescens*) and lipase AP12 (*Aspergillus niger*) were purchased from Amano.

4.2. Synthesis of the substrates

4.2.1. Diethyl 3-ethoxycarbonyl-4-methyl-2-oxopentanedioate **4**[6]

The reaction between diethyl methylbutanedioate **3** and ethyl oxalate was carried out in accordance with the literature.[6] The product **4**, obtained as a 2:3 mixture of *syn*- and *anti*-diastereomers,

was purified by distillation, b.p. 125–128°C (0.7 torr); 28% yield; IR (film), ν, cm^{-1}: 1800 (sh), 1739 (COO), 1250 (C–O); ^1H NMR, δ, ppm: 4.44 (0.4H, d, $J=7.3$ Hz, H-3), 4.37 (0.6H, d, $J=8.4$ Hz, H-3), 4.27 (2H, 2 q, OCH_2CH_3), 4.11 (4H, m, 2 OCH_2CH_3), 3.17 (1H, 2 dq, $CHCH_3$), 1.30, 1.29 (3H, 2 t, OCH_2CH_3), 1.18 (9H, m, 2 OCH_2CH_3, CH_3); ^{13}C NMR, δ, ppm, for the *syn*-isomer: 187.4 (s), 173.1 (s), 167.2 (s), 159.8 (s), 61.6 (2 t), 60.8 (t), 55.5 (d), 38.3 (d), 14.0 (q), 13.66 (q), 13.63 (2 q); ^{13}C NMR, δ, ppm, for the *anti*-isomer: 187.9 (s), 173.4 (s), 167.0 (s), 160.1 (s), 62.6 (2 t), 60.9 (t), 55.9 (d), 38.2 (d), 14.5 (q), 13.7 (q), 13.63 (2 q).

4.2.2. Diethyl 4-methyl-2-oxopentanedioate **6**[7]

Acidic treatment of the triester **4** under reflux furnished the diacid **5**,[5] which was esterified with ethanol under reflux in benzene with *p*-toluene sulfonic acid (PTSA), to give the ketodiester **6**. Its IR, ^1H NMR and MS spectra were in accordance with those reported in the literature.[7] ^{13}C NMR, δ, ppm: 192.1 (s), 174.6 (s), 160.2 (s), 62.1 (t), 60.4 (t), 42.0 (t), 34.4 (d), 16.6 (q), 13.7 (q), 13.6 (q).

4.3. Synthesis of the hydroxydiesters 7 and **8**

The ketodiester **6** (2.0 g, 9.3 mmol) in ethyl acetate (100 ml) was hydrogenated at room temperature and atmospheric pressure (H_2/5% Pd/C, 0.4 g). After 20 h the suspension was filtered on Celite and a 1:1 mixture of compounds **7** and **8** was obtained in quantitative yield. Attempts to separate the diastereomeric mixture by flash chromatography resulted in the isolation of mixtures of **7** and **8** rich in each component. The spectroscopic data for the two diastereomers are given separately.

4.3.1. Diethyl (2R*,4S*)-2-hydroxy-4-methylpentanedioate 7

IR (film), ν, cm^{-1}: 3500 (OH), 1740 (COO), 1250, 1180 (C–O); ^1H NMR, δ, ppm: 4.25 (2H, q, OCH_2CH_3), 4.22 (1H, m, H-2), 4.16 (2H, dq, OCH_2CH_3), 2.95 (1H, d, $J=5.5$ Hz, OH), 2.75 (1H, m, H-4), 2.23 (1H, ddd, $J=13.7$, 9.7, 3.8 Hz, H-3), 1.66 (1H, ddd, $J=13.7$, 9.1, 4.4 Hz, H-3), 1.31, 1.27 (6H, 2 t, OCH_2CH_3), 1.21 (3H, d, $J=7.0$ Hz, CH_3); ^{13}C NMR, δ, ppm: 176.2 (s), 174.9 (s), 68.8 (d), 61.8 (t), 60.5 (t), 38.0 (t), 35.7 (d), 17.8 (q), 14.2 (2 q); MS, m/z: 99 (100).

4.3.2. Diethyl (2R*,4R*)-2-hydroxy-4-methylpentanedioate **8**

IR (film), ν, cm^{-1}: 3500 (OH), 1740 (COO), 1250, 1180 (C–O); ^1H NMR, δ, ppm: 4.24 (2H, dq, OCH_2CH_3), 4.20 (1H, m, H-2), 4.13 (2H, q, OCH_2CH_3), 2.85 (1H, d, $J=6.2$ Hz, OH), 2.75 (1H, m, H-4), 2.04 (1H, ddd, $J=13.9$, 9.5, 6.6 Hz, H-3), 1.85 (1H, ddd, $J=13.9$, 7.7, 3.8 Hz, H-3), 1.31, 1.25 (6H, 2 t, OCH_2CH_3), 1.23 (3H, d, $J=7.3$ Hz, CH_3); ^{13}C NMR, δ, ppm: 176.3 (s), 174.9 (s), 68.6 (d), 61.8 (t), 60.5 (t), 37.7 (t), 35.9 (d), 16.7 (q), 14.1 (2 q); MS, m/z: 99 (100).

4.4. Synthesis of lactones **9** and **10**

The mixture of **7** and **8** (2.0 g, 9.2 mmol) was refluxed in benzene in the presence of PTSA for 2 h. The solution was washed with a saturated solution of $NaHCO_3$ and the organic phase dried on anhydrous Na_2SO_4. After evaporation of the solvent, the lactones **9** and **10** were separated by flash chromatography (eluant: light petroleum:ethyl acetate, in gradients from 100:0 to 80:20). The *trans*-lactone **9** was obtained in 31% yield (0.50 g, 2.9 mmol) and the *cis*-lactone **10** was obtained in 28% yield (0.44 g, 2.6 mmol).

4.4.1. Ethyl trans*-4-methyl-tetrahydro-5-oxo-2-furancarboxylate* ***9***

Oil; IR (film), ν, cm^{-1}: 1770 (COO), 1736 (COOEt); ^1H NMR, δ, ppm: 4.88 (1H, dd, $J_1=2.2$, $J_2=9.2$, H-2), 4.26 (2H, q, $J=7.1$, OCH_2CH_3), 2.74 (1H, m, H-4), 2.55 (1H, ddd, $J_1=2.2$, $J_2=8.8$, $J_3=13.2$, H-3), 2.21 (1H, ddd, $J_1=9.2$, $J_2=11.2$, $J_3=13.2$, H-3), 1.32 (3H, d, $J=7.0$, CH_3), 1.30 (3H, t, $J=7.1$, CH_2CH_3); ^{13}C NMR, δ, ppm: 178.8 (s), 169.5 (s), 73.7 (d), 62.0 (t), 34.4 (t), 34.2 (d), 15.2 (q), 14.0 (q); MS, *m*/*z*: 172 ($M^{+\cdot}$, 1), 99 (100), 71 (39), 43 (52).

4.4.2. Ethyl cis*-4-methyl-tetrahydro-5-oxo-2-furancarboxylate* ***10***

Oil; IR (film), ν, cm^{-1}: 1770 (COO), 1736 (COOEt); ^1H NMR, δ, ppm: 4.80 (1H, dd, $J_1=7.1$, $J_2=9.3$, H-2), 4.28 (2H, q, $J=7.1$, OCH_2CH_3), 2.75 (2H, m, H-3, H-4), 1.95 (1H, ddd, $J_1=J_2=9.2$, $J_3=12.5$, H-3), 1.32 (3H, t, $J=7.1$, CH_2CH_3), 1.32 (3H, d, $J=7.0$, CH_3); ^{13}C NMR, δ, ppm: 178.0 (s), 169.5 (s), 74.0 (d), 61.9 (t), 34.4 (d), 34.0 (t), 15.4 (q), 14.0 (q); MS, *m*/*z*: 172 ($M^{+\cdot}$, 1), 99 (100), 71 (38), 43 (38).

4.5. General procedure for enzymatic hydrolyses of lactones ***9*** *and* ***10***

The lactonic ester was reacted with the enzyme in 0.1 M phosphate buffer (pH 7.4) under stirring. The pH value was continuously adjusted with 1N NaOH by the use of a pH-stat.

At the desired conversion value, the unreacted lactonic ester was extracted from the aqueous phase with diethyl ether (four times). From the mother liquors, acidified to pH 1 with 2N HCl, the lactonic acid was extracted with diethyl ether (four times). When the enzyme opened the heterocyclic ring to the corresponding hydroxy hemiester, this organic phase also contained the lactonic ester formed by cyclization of this latter compound. The organic phase was then dried on anhydrous Na_2SO_4.

The enantiomeric excesses of the lactonic esters and acids were determined by chiral HRGC after esterification of the lactonic acids with CH_2N_2.

*4.5.1. Ethyl (2*S*,4*R*)-(+)-*trans*-4-methyl-tetrahydro-5-oxo-2-furancarboxylate* ***9***

The lactonic ester was isolated with 98% e.e., $[\alpha]_D^{25}$ +41.0 (*c* 0.10, MeOH); CD: $\Delta\varepsilon_{211}$ +1.3 (MeOH).

*4.5.2. Ethyl (2*S*,4*S*)-(−)-*cis*-4-methyl-tetrahydro-5-oxo-2-furancarboxylate* ***10***

The lactonic ester was isolated with 99% e.e., $[\alpha]_D^{25}$ −3.9 (*c* 0.18, MeOH); CD: $\Delta\varepsilon_{216}$ +1.4 (MeOH).

*4.5.3. (2*R*,4*S*)-(−)-*trans*-4-Methyl-tetrahydro-5-oxo-2-furancarboxylic acid* ***11****[2b]*

IR, ν, cm^{-1}: 3680, 3610, 3450 (OH), 1770 (COO), 1740 (COOH); ^1H NMR, δ, ppm: 9.00 (1H, s, COOH), 4.94 (1H, dd, $J_1=2.3$, $J_2=9.3$, H-2), 2.80 (1H, m, H-4), 2.62 (1H, ddd, $J_1=2.3$, $J_2=8.7$, $J_3=13.0$, H-3), 2.25 (1H, ddd, $J_1=9.3$, $J_2=10.6$, $J_3=13.0$, H-3), 1.29 (3H, d, $J=6.9$, CH_3); ^{13}C NMR, δ, ppm: 179.7 (s), 173.9 (s), 73.6 (d), 34.0 (t), 32.6 (d), 15.0 (q); MS, *m*/*z*: 145 (MH^+, 35), 99 (100), 71 (62); 82% e.e., $[\alpha]_D^{25}$ −18.9 (*c* 0.65, MeOH); CD: $\Delta\varepsilon_{209}$ −0.7 (MeOH).

*4.5.4. (2*R*,4*R*)-(+)-*cis*-4-Methyl-tetrahydro-5-oxo-2-furancarboxylic acid* ***12****[2b]*

IR, ν, cm^{-1}: 3680, 3610, 3450 (OH), 1770 (COO), 1740 (COOH); ^1H NMR, δ, ppm: 9.12 (1H, s, COOH), 4.87 (1H, dd, $J_1=7.1$, $J_2=9.2$, H-2), 2.81 (2H, m, H-3, H-4), 2.00 (1H, ddd, $J_1=J_2=9.2$, $J_3=11.7$, H-3), 1.32 (3H, d, $J=6.8$, CH_3); ^{13}C NMR, δ, ppm: 178.8 (s), 173.1 (s), 73.7 (d), 34.6

(d), 33.8 (t), 15.2 (q); MS, *m/z*: 145 (MH^+, 100), 99 (63), 71 (69); 92% e.e., $[\alpha]_D^{25}$ +3.2 (*c* 0.95, MeOH); CD: $\Delta\varepsilon_{215}$ –1.1 (MeOH).

4.6. Methylation of (R)-(–)-tetrahydro-5-oxo-2-furancarboxylic acid **13**

The lactone of (*R*)-(–)-**13** (0.8 g, 6.2 mmol) was methylated according to the literature.[8] The crude reaction mixture was treated with CH_2N_2 and purified by flash chromatography by which the lactone (–)-**14** (0.1 g, 0.6 mmol, 10% yield) was obtained.

4.6.1. Methyl (2R,4S)-(–)-trans-4-methyl-tetrahydro-5-oxo-2-furancarboxylate **14**

IR, ν, cm^{-1}: 1770 (COO), 1736 (COO); ^1H NMR, δ, ppm: 4.92 (1H, dd, $J_1=2.2$, $J_2=9.2$, H-2), 3.81 (3H, s, OCH_3), 2.74 (1H, m, H-4), 2.56 (1H, ddd, $J_1=2.2$, $J_2=8.8$, $J_3=13.2$, H-3), 2.22 (1H, ddd, $J_1=9.2$, $J_2=11.0$, $J_3=13.2$, H-3), 1.29 (3H, d, $J=7.0$, CH_3); ^{13}C NMR, δ, ppm: 178.7 (s), 170.5 (s), 73.5 (d), 52.7 (q), 34.1 (t), 32.4 (d), 15.1 (q); >99% e.e., $[\alpha]_D^{25}$ –40.0 (*c* 0.13, MeOH); CD: $\Delta\varepsilon_{210}$ –1.3 (MeOH).

4.7. Reduction of the γ-lactones **9** and **10** with $NaBH_4$

4.7.1. (3R,5S)-(+)-trans-5-Hydroxymethyl-3-methyl-4,5-dihydro-2(3H)-furanone **16**

The *trans*-lactonic ester (+)-**9** (0.150 g, 0.9 mmol) was reduced with $NaBH_4$ following the procedure reported in the literature[1n] to give the corresponding hydroxymethyl lactone **16**. Its IR, ^1H NMR and ^{13}C NMR spectra were in accordance with those reported in the literature.[4a] Its enantiomeric excess was 98%; $[\alpha]_D^{25}$ +29.7 (*c* 0.38, EtOH) [lit.[4a] $[\alpha]_D^{25}$ +36.2 (*c* 0.3, EtOH)]; CD: $\Delta\varepsilon_{216}$ –0.9 (EtOH), $[\Theta]_{216}$ -3.0×10^3 [lit.[14] $[\Theta]_{218}$ -2.2×10^3].

4.7.2. (3S,5S)-(+)-cis-5-Hydroxymethyl-3-methyl-4,5-dihydro-2(3H)-furanone **17**

The *cis*-lactonic ester (–)-**10** (0.150 g, 0.9 mmol) was reduced with $NaBH_4$ according to the literature.[1n] Its IR and ^1H NMR spectra were in accordance with those reported in the literature.[4a] ^{13}C NMR, δ, ppm: 179.8 (s), 78.8 (d), 63.4 (t), 35.5 (d), 31.5 (t), 15.0 (q); 94% e.e., $[\alpha]_D^{25}$ +17.2 (*c* 0.9, EtOH) [lit.[4a] $[\alpha]_D^{25}$ +22.5 (*c* 0.3, EtOH)]; CD: $\Delta\varepsilon_{220}$ +0.3 (EtOH), $[\Theta]_{220}$ $+1.0\times10^3$ [lit.[14] $[\Theta]_{222}$ $+0.2\times10^3$].

4.8. Reductions of the ketodiester **6** with baker's yeast

A suspension of baker's yeast (46.0 g) in tap water (92 ml) was preincubated for different periods (Table 3). The ketodiester **6** (1.0 g, 4.6 mmol) was then added and the mixture stirred at room temperature for 72 h.

The course of the reduction was monitored by HRGC and ^1H NMR. At the end of the reaction, brine was added and the broth was extracted with ethyl ether. The organic phase was dried and evaporated and the products were separated by flash chromatography (eluant: light petroleum:ethyl acetate, in gradients from 95:5 to 80:20) to yield a mixture of the hydroxydiesters **7** and **8** (0.1 g, 0.46 mmol, 10% yield), and ethyl 2-hydroxy-4-methylbutanedioate **18** (0.08 g, 0.5 mmol, 12% yield).[15]

The mixture of the hydroxydiesters **7** and **8** was refluxed in benzene and PTSA for 30 min, then the solution was washed with a saturated solution of $NaHCO_3$ and dried on anhydrous Na_2SO_4. Evaporation of the solvent gave the two lactones **9** and **10**, respectively, which were separated by flash chromatography and analyzed by chiral HRGC.

4.8.1. Ethyl 4-hydroxy-2-methylbutanedioate ***18***[15]

IR (film), ν, cm^{-1}: 3420 (OH), 1740, 1720 (COO), 1180 (C–O); ^1H NMR, δ, ppm: 4.14 (2H, q, OCH_2CH_3), 3.69 (2H, m, 2 H-4), 2.62 (1H, ddq, $J=8.2$, 7.1, 5.8 Hz, H-2), 1.93 (1H, m, H-3), 1.70 (1H, m, H-3), 1.65 (1H, bs, OH), 1.26 (3H, t, OCH_2CH_3), 1.20 (3H, d, $J=7.1$ Hz, CH_3); ^{13}C NMR, δ, ppm: 176.8 (s), 60.7 (t), 60.4 (t), 36.5 (d), 36.3 (t), 17.2 (q), 14.2 (q).

4.9. Synthesis of model compounds ***19*** *and* ***20*** *for the analysis of CD spectra*

*4.9.1. Ethyl (*S*)-(+)-tetrahydro-5-oxo-2-furancarboxylate* ***19***

Esterification of compound (*S*)-(+)-**13** was carried out following the procedure used for peptide coupling.[10] Compound (*S*)-(+)-**13** (Aldrich) (0.13 g, 1.0 mmol) was dissolved in CH_2Cl_2 (2 ml). 1-Hydroxybenzotriazole (0.135 g, 1.0 mmol) and *N*-ethyl-*N*′-(3-(dimethylamino)propyl)carbodiimide hydrochloride (EDC) (0.23 g, 1.2 mmol) were added under magnetic stirring. After a few minutes, abs. EtOH (0.1 ml) and Et_3N (0.17 ml, 1.2 mmol) were added. After 15 h the solvent was evaporated, then the crude reaction mixture was dissolved in 100 ml of ethyl acetate and washed twice with 40 ml of a solution of 0.5 M citric acid, H_2O, 5% $NaHCO_3$, H_2O and dried on Na_2SO_4. Evaporation of the solvent gave 0.1 g (0.63 mmol, 63% yield) of (*S*)-(+)-**19**. All spectroscopic data were in accordance with the literature;[1b,n,16] $[\alpha]_D^{25}$ +13.6 (*c* 0.56, EtOH) [lit.[1b] $[\alpha]_D^{25}$ +13.3 (*c* 0.56, EtOH), lit.[1n] $[\alpha]_D^{25}$ +15.1 (*c* 0.6, EtOH), lit.[16] $[\alpha]_D^{25}$ +11.3 (*c* 10, EtOH)]; CD: $\Delta\varepsilon_{213}$ +1.7 (MeOH).

*4.9.2. (*S*)-(+)-5-Hydroxymethyl-4,5-dihydro-2(3*H*)-furanone* ***20***

Lactone **20** was prepared according to the literature.[1n] All spectroscopic data were in accordance with the literature;[1n] $[\alpha]_D^{25}$ +28.0 (*c* 0.3, EtOH) [lit.[1n] $[\alpha]_D^{20}$ +29.6 (*c* 0.4, EtOH)]; CD: $\Delta\varepsilon_{211}$ –0.1 (EtOH), $[\Theta]_{211}$ -0.5×10^3 [lit.[14] $[\Theta]_{210}$ -0.7×10^3].

Acknowledgements

The authors thank the MURST, CNR (Rome) and the University of Trieste for financial support.

References

1. (a) Donohoe, T. J.; Stevenson, C. A.; Helliwell, M.; Irshad, R.; Ladduwahetty, T. *Tetrahedron: Asymmetry* **1999**, *10*, 1315–1322; (b) Drioli, S.; Nitti, P.; Pitacco, G.; Tossut, L.; Valentin, E. *Tetrahedron: Asymmetry* **1999**, *10*, 2713–2728; (c) Chandrasekharam, M.; Rai-Shung, L. *J. Org. Chem.* **1998**, *63*, 9122–9124; (d) Drioli, S.; Felluga, F.; Forzato, C.; Nitti, P.; Pitacco, G.; Valentin, E. *J. Org. Chem.* **1998**, *63*, 2385–2388; (e) Ghattak, A.; Sarkar, S.; Ghosh, S. *Tetrahedron* **1997**, *53*, 17 335–17 342; (f) Vaupel, A.; Knochel, P. *J. Org. Chem.* **1996**, *61*, 5743–5753; (g) Sibi, M. P.; Deshpande, P. K.; La Loggia, A. J. *Synlett*. **1996**, 343–345; (h) Maiti, G.; Roy, S. C. *J. Chem. Soc., Perkin Trans. 1* **1996**, 403–404; (i) Zhu, G.; Lu, X. *Tetrahedron: Asymmetry* **1995**, *6*, 885–892; (j) Mawson, S. D.; Weavers, R. T. *Tetrahedron* **1995**, *51*, 11 257–11 270; (k) Honda, T.; Kimura, N. *J. Chem. Soc., Chem. Commun.* **1994**, 77–78; (l) de Azavedo, M. B. M.; Murta, M. M.; Greene, A. E. *J. Org. Chem.* **1992**, *57*, 4567–4569; (m) Mori, K.; Yamane, K. *Tetrahedron* **1982**, *38*, 2919–2921; (n) Ravid, U.; Silverstein, R. M.; Smith, L. R. *Tetrahedron* **1978**, *34*, 1449–1452; (o) Hussain, S. A. M. T.; Ollis, W. D.; Smith, C.; Stoddard, J. F. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1480–1492.
2. (a) Nishida, Y.; Ohrui, H.; Meguro, H. *Agric. Biol. Chem.* **1983**, *47*, 2123–2125; (b) Nishida, Y.; Ohrui, H.; Meguro, H. *Agric. Biol. Chem.* **1984**, *48*, 1211–1216.

3. Tsuneya, T.; Ishihara, M.; Shiota, H.; Shiga, M. *Agric. Biol. Chem.* **1980**, *44*, 957–958.
4. (a) Herdeis, C.; Lütsch, K. *Tetrahedron: Asymmetry* **1993**, *4*, 121–131; (b) Wilson, L. J.; Liotta, D. C. *J. Org. Chem.* **1992**, *57*, 1948–1950; (c) Tomioka, K.; Cho, Y.-S.; Sato, F.; Koga, K. *J. Org. Chem.* **1988**, *53*, 4094–4098; (d) Hanessian, S.; Murray, P. J.; Sahoo, S. P. *Tetrahedron Lett.* **1985**, *26*, 5623–5626.
5. Echalier, F.; Constant, O.; Bolte, J. *J. Org. Chem.* **1993**, *58*, 2747–2750.
6. Blaise, E. E.; Gault, H. *Bull. Soc. Chim. Fr.* **1911**, 458–464.
7. Dowd, P.; Choi, S.-C.; Duah, F. *Tetrahedron* **1988**, *44*, 2137–2148.
8. Grieco, P. A.; Miyashita, M. *J. Org. Chem.* **1974**, *39*, 120–122.
9. Chen, C.-S.; Fujimoto, Y.; Girdaukauas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
10. (a) Carpino, L. A. *J. Am. Chem. Soc.*, **1993**, *115*, 4397–4398; (b) König, W.; Geiger, R. *Chem. Ber.* **1970** *103*, 788–798.
11. Matsumoto, T.; Takahashi, M.; Kashihara, Y. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 3329–3336.
12. Okuda, T.; Harigaya, S.; Kiyomoto, A. *Chem. Pharm. Bull.* **1964**, *12*, 504–506.
13. Forzato, C.; Nitti, P.; Pitacco, G. *Tetrahedron: Asymmetry* **1997**, *8*, 4101–4110.
14. Nishida, Y.; Ohrui, H.; Meguro, H. *Croatica Chim. Acta* **1989**, *62(2B)*, 377–390.
15. (a) Kochinashvili, M. V.; Kuramshin, E. M.; Kotlyar, S. A.; Zlotskii, S. S.; Rakhmankulov, D. L. *Zh. Vses. Khim. O-va. im. D. I. Mendeleeva* **1986**, *31*, 596–597; *Chem. Abstr.* **1987**, *107*, 216892t; (b) Sakykh-Zade, S. I.; Rzaeva, A. S. *Azerb. Khim. Zh.* **1968**, *6*, 97–102; *Chem. Abstr.* **1969**, *71*, 90750n; (c) Boguslavskaya, L. S.; Razuvaev, G. A. *Zh. Obshch. Khim.* **1963**, *33*, 2021–2027; *Chem. Abstr.* **1966**, *64*, 586a.
16. Doolittle, R. E.; Tumlinson, J. H.; Proveaux, A. T.; Heat, R. R. *J. Chem. Ecol.* **1980**, *6*, 473–485.