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Biocatalytical Transformations. IV. Enantioselective Enzymatic Hydrolyses of Building Blocks for the Synthesis of Carbocyclic Nucleosides.[1]

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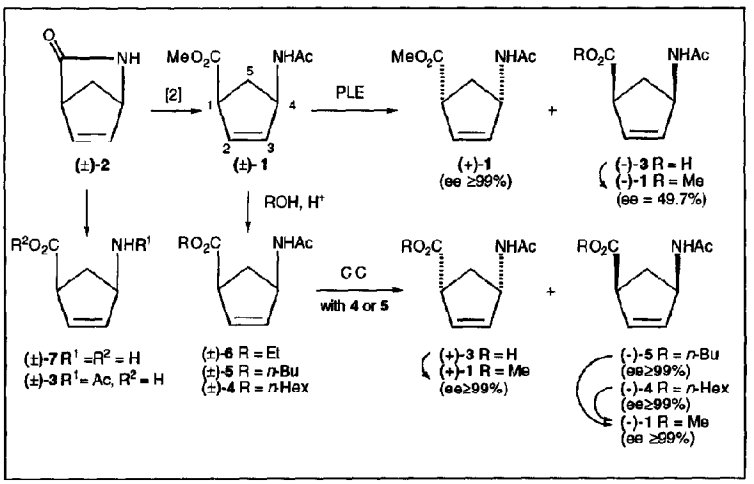
Abstract: Enantiomerically pure alkyl (1*S*, 4*R*)- and (1*R*, 4*S*)-4-acetamido-cyclopent-2-ene-carboxylates are obtained from their corresponding racemates by hydrolysis with PLE or the lipase from *Candida cylindracea*.

Introduction.- Carbocyclic nucleosides have gained much interest within the last years.[2, 3] In a project dealing with the synthesis of inhibitors of the S-adenosylmethionine synthetase we became interested in this class of compounds. As a starting point for our own syntheses we have chosen aristeromycin,[4] the carbocyclic analogue of adenosine. The synthesis should allow substantial amounts of aristeromycin and suitable precursors to be obtained. Since chiral pool approaches starting either from L-ribonolactone[5] or D-glucose[6] allow the synthesis of only one enantiomer in an economical way, a strategy was selected which included a separation of the respective enantiomers at an early stage of the synthetic scheme by means of an enzymatic process. Although somewhat more lengthy as compared to an approach starting from bicyclo[2.2.1]hepta-2,5-diene-2,3-dicarboxylic acid dimethyl ester[7] a scheme similar to that outlined by *Sicsic et al.*[8] was selected.

Results and Discussion.- The key intermediate in this approach is (±) methyl (1*SR*, 4*RS*) 4-acetamido-cyclopent-2-ene-1-carboxylate (**1**) which can be obtained in large quantities by the reaction of cyclopentadiene with tosyl [9] or mesyl [10] cyanide leading to the lactam, 2-azabicyclo[2.2.1]hept-5-en-3-one, (**2**) which is consecutively hydrolysed, esterified and acetylated to yield **1**. It was claimed[8] that a kinetic resolution of this material can be achieved by enantioselective enzymatic hydrolysis using porcine liver esterase PLE. Unfortunately, in our hands repetition of this procedure[8] did not yield material of the reported enantiomeric excess for the acid (-)-**3**. Our finding of an ee of 49% for (-)-**1** obtained from (-)-**3** by re-esterification with diazomethane is in excellent agreement with data reported recently for the enzymatic resolution of some N-substituted racemic methyl 4-amino-cyclopent-2-ene-carboxylate derivatives.[11, 12] Therefore the decision to study the enzymatic hydrolysis of different alkyl (1*SR*, 4*RS*) 4-acetamido-cyclopent-2-ene-1-carboxylates in more detail was taken. As an alternative enantiospecific and enantiocomplementary hydrolysis reactions of the

lactam **2** have been suggested,[13] but these routes have the need of using either whole cell preparations of microbial strains (*Rhodococcus equi* (NCIB 40213) or *Pseudomonas solanacearum* (NCIB 40249)) or of treating (±)-**2** with special γ-lactamases (*Pseudomonas fluorescens* or *Aureobacterium sp.*).[13, 14]

Enzymatic hydrolysis experiments of (±)-**1** in pH-stat equipment maintaining the pH constant by addition of 0.1 N NaOH were carried out using pronase (*Streptomyces griseus*, Boehringer-Mannheim, 25°C, pH 7.5, 8.0, 8.5, 9.0, respectively), lipase CC (*Candida cylindracea*, Boehringer Mannheim, 25°C, pH 7.0), lipase A6 (*Aspergillus niger*, 25°C, pH 7.0; 37°C, pH 7.0), lipase N (*Rhizopus sp.*, Amano, 25°C, pH 7.0), porcine pancreatic lipase PPL (Boehringer-Mannheim, 37°C, pH 7.0), lipase B (*Candida antarctica* recombinant with *Aspergillus oryzae*, immobilized on a macroporous acrylic resin, Novo, 37°C, pH 8.0), acetylcholine esterase (bovine brain, Sigma, 25°C, pH 8.0), acetylcholine esterase (*Electrophorus electricus*, Boehringer-Mannheim, 37°C, pH 8.0), subtilisine (*Bacillus licheniformis*, Fluka, 25°C, pH 8.0), thermolysine (*Bacillus thermoproteolyticus*, Fluka, 37°C, pH 7.0), and an esterase from rabbit liver (Sigma, 30°C, pH 7.0); but none of these enzymes showed any (CC, N, subtilisine, thermolysine) or unspecific hydrolysis of (±)-**1**. However, hydrolysis with PLE at 37°C at a pH of 7.5 throughout the duration of the reaction afforded unchanged ester (+)-**1** with an specific rotation $[\alpha]_D^{20}$ +86.6 in chloroform. Comparison of the sign and magnitude of the specific rotation of (+)-1 to that described in the literature[15] for an authentic sample of (-)-**1** suggested our (+)-**1** to be enantiomerically pure. As unambiguous proof of its enantiomeric purity a HPLC analysis of (+)-**1** using a β-cyclodextrine column (Fig. 1) was performed. (-)-1 was obtained after removal of (+)1 by exhaustive extraction with ethyl acetate from the reaction mixture followed by the re-esterification using an ether solution of diazomethane; (-)-**1** showed in chloroform a specific rotation $[\alpha]_D^{20}$ -42.7 corresponding to an enantiomeric excess of 49%. This is in excellent agreement with the data obtained from the HPLC analysis. Thus our results for the reaction of (±)-**1** with PLE are in striking contrast to previous findings.[8]



(±)-**1** represents a suitable precursor for the synthesis of a variety of carbocyclic nucleosides. Due to the finding that both enantiomers of some carbocyclic nucleosides can display potent biological activity[16]. This substrate was chosen for further development.[17]

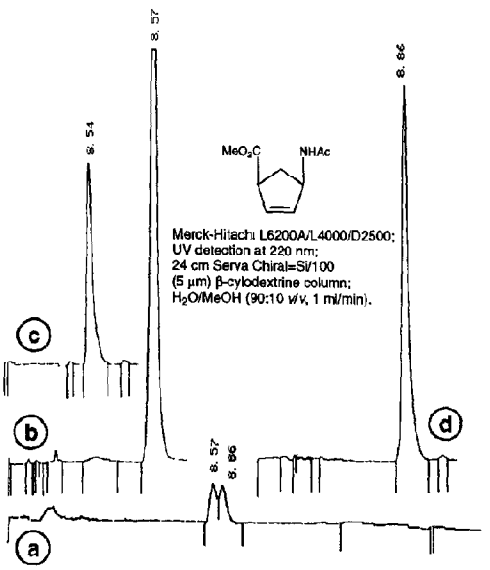


Fig. 1: HPLC investigations: a) (±)-**1**, retention time of 8.57 min for (+)-1, retention time of 8.66 min for (-)-**1**; b) (+)-**1** from the hydrolysis of (±)-**1** by PLE (after 73.5% conversion); c) (+)-1 from the hydrolysis of (±)-**4** by lipase CC (50% conversion, work up of the aq. phase followed by re-esterification with CH_2N_2); d) (-)-**1** from hydrolysis of (±)-**4** by lipase CC (50% conversion, work up of the organic phase followed by a hydrolysis of (-)-**4** with NaOH and re-esterification with CH_2N_2).

Consequently, (±)-**1** was transesterified with *n*-butanol or *n*-hexanol and the corresponding racemic alkyl-4-acetamido-cyclopent-2-ene-1-carboxylates (±)-**5** and (±)-**4** were obtained in 75% and 74% yield, respectively. The ethyl derivative (±)-**6** was obtained by *Brenner*-esterification[18] and acetylation of racemic (±)-**7** in 71% yield.

Under a variety of conditions enzymatic hydrolysis of (±)-**6** gave only 50-60% ee's for unhydrolyzed ester and/or acid whereas (±)-**5** as well as (±)-**4** afforded upon hydrolysis with the lipase from *Candida cylindracea* (-)-**5** (42.5% yield) and (-)-**4** (44% yield) in enantiomerically pure form, respectively. From the corresponding aqueous phases (+)-**1** was obtained after re-esterification with diazomethane. Similarly (-)-**1** is accessible by saponifaction of crude (-)-**5** or of (-)-**4** with 2 N NaOH followed by a re-esterification with diazomethane. Thus, enzymatic hydrolysis of either (±)-**5** or (±)-**4** conveniently gives access to both enantiomers of **1**.

Experimental

The melting points are uncorrected (*Reichert* hot stage microscope), optical rotations were obtained using a Perkin Elmer 243B polarimeter (1 cm micro-cell), NMR spectra (internal Me_4Si) were recorded using either a Bruker AM250 or a Varian XL300 instrument (δ given in ppm, J in Hz), IR spectra (film or KBr-pellet) on a Perkin-Elmer 298 instrument, MS-spectra were taken on a Varian-112S instrument; for elemental analysis a Foss-Heraeus Vario EL instrument was used. TLC was performed on silica gel (Merck 5554, detection either by dipping into a solution containing 10% sulfuric acid (400 *ml*), ammonium molybdate (20 g) and cerium(IV) sulfate (20 mg) followed by heating to 150°C or by dipping into a solution of ninhydrin followed by heating to 100°C. Alternatively, the TLC plate was allowed to stand in chlorine atmosphere (developed from $KMnO_4$/aqueous hydrochloric acid) for approx. 5 min, then heated to 100°C and sprayed with a freshly prepared mixture (1:1 *v/v*) of a 0.2 M pyridine solution of 1-phenyl-3-methylpyrazolone-(5) and an aqueous 1 N potassium cyanide solution. Moderate warming of the TLC plate afforded red/blue coloured spots. HPLC was performed on a Merck-Hitachi L6200A/L4000/D2500 instrument (UV detection at 220 nm) using a 24 cm Serva Chiral=Si/100 (5 µm) β-cylodextrine column and H_2O/MeOH (90:10 *v/v*) as the solvent (1 *ml*/min); the pH-stat equipment was obtained from Büchi.

(±)-Methyl (1 *SR*, 4 *RS*)-4-acetamido-cyclopent-2-ene-1-carboxylate (±)-1:- A solution of lactam (±)-**2** (11.5 g, 0.11 mol) in 5% aqueous hydrochloric acid (450 *ml*) was stirred at room temperature for 24 h. The pH was then adjusted to 1 by careful addition of 6 N sodium hydroxide solution, the solvent was evaporated and toluene (4 x 50 *ml*) and methanol (2 x 50 *ml*) was distilled off the residue. The semicrystalline material was dissolved in abs. methanol (190 *ml*) and heated under reflux for 18 h. After cooling to room temperature the sodium chloride was filtered off and washed with methanol. The filtrate and washings were combined, the solvent was evaporated under reduced pressure and the residue was dissolved in dry pyridine (90 *ml*). The solution was cooled to 0°C and acetic anhydride (52 *ml*) was slowly added at this temperature. After completion of the addition the mixture was allowed to warm to room temperature and stirred for another hour. Then the solvent was evaporated *in vacuo* and toluene (5 x 50 *ml*) was distilled off the residue. A dichloromethane solution (200 *ml*) of this material was washed with a saturated solution of bicarbonate (3 x 10 *ml*) and brine (10 *ml*), dried over magnesium sulfate, the solvent was removed under reduced pressure and the remaining solid subjected to column chromatography (silica gel, hexane/ethyl acetate 1:1) to yield (±)**1** (16.72 g, 86%); mp: 62-63°C, lit.: [9] 66-67°C.

(-)-Methyl (1*R*, 4*S*)-4-acetamido-cyclopent-2-ene-1-carboxylate (-)-1:

a) (From (-)-5: **(-)-5** (400 mg, 1.78 mmol) was suspended in 2 N NaOH (25 *ml*) and the mixture was stirred for several hours. After neutralisation with diluted hydrochloric acid the solvent was evaporated under reduced pressure. The residue was suspended in dry methanol (50 *ml*) and an ether solution of diazomethane was added. Usual work up afforded **(-)-1** (302 mg, 93%), mp: 83-85°C, $[\alpha]_D^{20}$ -86.4 (*c*, 0.8 $CHCl_3$), ee ≥ 99%; lit.[15] mp: 89.5-90.5°C, $[\alpha]_D^{20}$ -84.4 (*c*, 1 MeOH).

b) From (-)-4: Starting from **(-)-4** (450 mg, 1.78 mmol) following the procedure given for the synthesis of **(-)-1** from **(-)-5** **(-)-1** (300 mg, 92%) was obtained; mp: 83-85°C, $[\alpha]_D^{20}$ -86.5 (*c*, 0.9 $CHCl_3$), ee ≥ 99%.

(+)-Methyl (1*S*, 4*R*)-4-acetamido-cyclopent-2-ene-1-carboxylate (+)1:

a) From (±)-1 by hydrolysis with PLE: A suspension of (±)-1 (1.0 g, 5.46 mmol) and PLE (10 µl, content 30 mg/ml) was stirred in phosphate buffer (pH 7.0, 30 *ml*, 37°C); the pH was kept constant in the pH-stat equipment by the addition of 0.1 N NaOH (added 40.09 *ml*, corresponding to 73.5% conversion). The reaction mixture was extracted with ethyl acetate (3 x 100 *ml*), the organic layer was dried (sodium sulfate) and the solvent was removed *in vacuo* to afford (+)-1 (217 mg, 22%), $[\alpha]_D^{20}$ +86.6 (*c* , 1.3 $CHCl_3$), ee ≥ 99%, mp: 87-88°C, R_F (EtOAc/MeOH 5:1) 0.71; IR (KBr): 3300*s*, 3065*w*, 3000*w*, 2960*w*, 2895*w*, 1735*m*, 1630*s*, 1540*s*, 1440*m*, 1375*m*, 1340*w*, 1320*m*, 1295*m*, 1270*m*, 1260*m*, 1250*m*, 1215*s*, 1125*m*, 1080*s*, 1050*m*, 1025*m*, 1010*m*; ^{1}H-NMR (250 MHz, $CDCl_3$): 6.18 (*br s*, 1 H, NH), 5.80 (*m*, 2 H, H-C(2), H-C(3)), 4.95 (*dt*, *J* = 3.4, 8.5, 1 H, H-C(4)), 3 66 (*s*, 3 H, OMe), 3.50 (*dd*, *J* = 3.8, 8.5, 1 H, H-C(1)), 2.45 (*dt*, *J* = 8.5, 13.9, 1 H, H_A-C(5)), 1.87 (*s*, 3 H, NHAc), 1.80 (*dt*, *J* = 3.8, 13.9, 1 H, H_B-C(5)); ^{13}C-NMR (62.89 MHz, $CDCl_3$): 175.48 (*s*, CO), 169.15 (*s*, CO), 134.65 (*d*, C(3)), 131.60 (*d*, C(2)), 54.17 (*d*, C(4)), 52.28 (*q*, OMe), 49.19 (*d*, C(1)), 34.29 (*t*, C(5)), 23.43 (*q*, $COCH_3$); MS (FAB, glycerine): 185 (M+2, 2%), 184 (M+1, 100%); MS (FAB, glycerine + LiCl): 191 (M+1+Li, 11%), 190 (M+Li, 100%); Anal. calcd. for $C_9H_{13}NO_3$ (183.21): C, 59 00; H, 7.15; N, 7.65; found: C, 59.00; H, 7.16; N, 7.65.

The aqueous phase was evaporated. Treatment of the residue dissolved in methanol (50 *ml*) with an excess of an ether solution of diazomethane afforded after usual work up (*vide infra*) **(-)-1** (590 mg, 59%) $[\alpha]_D^{20}$ -43.0 (*c*, 1.1 $CHCl_3$), ee = 49.7%

b) From (±)-4 by hydrolysis with the lipase from Candida cylindracea*:* A suspension of **(±)-4** (1.0 g, 3.95 mmol) and the lipase (*Candida cylindracea*, 1 mg, 30 U/mg) in phosphate buffer (pH 7.0, 125 *ml*) was stirred at 37°C in the pH-stat equipment. After addition of a total amount of 19.8 *ml* of 0.1 N NaOH (corresponds to 50% conversion) the reaction mixture was extracted with ethyl acetate (3 x 100 *ml*). The aqueous layer was concentrated at reduced pressure and the remaining residue suspended in methanol (50*ml*). An excess of an etherical solution of diazomethane was added, the mixture was stirred for an additional 20 min and a few drops of acetic acid were added in order to destroy the excess of the diazomethane. The filtrate was evaporated and water (10 *ml*) and ethyl acetate (100 *ml*) were added. The two phase system was stirred for 10 min, the aqueous layer extracted with ethyl acetate (3 x 50 *ml*) and the combined organic phases were dried over sodium sulfate. The solvent was evaporated and **(+)-1** (325 mg, 45%) was obtained; mp: 85-86°C , $[\alpha]_D^{20}$ +86.5 (*c*, 1.1 $CHCl_3$).

(±)-Hexyl (1 *SR*, 4 *RS*)-4-acetamido-cyclopent-2-ene-carboxylate (±)-4:- (±)-4 (2.59 g, 75%) was prepared as described for **(±)-5** using **(±)-1** (2.5 g, 13.65 mmol), 1-hexanol (40 *ml*) and conc. sulfuric acid (0.25 *ml*); mp: 59-61°C.

(-)-Hexyl (1 *R*, 4 *S*)-4-acetamido-cyclopent-2-ene-carboxylate (-)-4:- A suspension of **(±)-4** (1.0 g, 3.95 mmol) and the lipase (*Candida cylindracea*, 1 mg, 30 U/mg) in phosphate buffer (pH 7.0, 125 *ml*) was stirred at 37°C in the pH-stat equipment. After addition of a total amount of 19.8 *ml* of 0.1 N NaOH (corresponds to

50% conversion) the reaction mixture was extracted with ethyl acetate (3 x 100 *ml*). The combined ethyl acetate extracts were dried over sodium sulfate. The solvent was evaporated and **(-)-4** was obtained (438 mg, 44%), mp: 74-76°C; $[\alpha]_D^{20}$ -67.4 (*c*, 0.93 $CHCl_3$); R_F 0.86 (EtOAc/MeOH 5:1); IR (KBr): 3000*s*, 3060*w*, 3300*m*, 3060*m*, 3000*m*, 2960*w*, 2940*w*, 2930*w*, 2860*w*, 1720*s*, 1625*s*, 1540*s*, 1510*w*, 1370*w*, 1320*w*, 1300*w*, 1270*m*, 1255*w*, 1240*m*, 1225*m*, 1120*m*, 1075*m*, 1050*w*, 1020*m*, 1010*m*; ^{1}H-NMR(300, $CDCl_3$): 6.15 (*br s*, 1 H, NH), 5.89 (*m*, 2 H, H-C(2), H-C(3)), 5.11 (*dt*, *J* = 3.2, 8.4, 1 H, H-C(4)), 4.10 (*t*, *J* = 6.8, 2 H, OCH_2), 3.50 (*dd*, *J* = 3.4, 8.4, 1 H, H-C(1)), 2.44 (*dt*, *J* = 8.5, 13.9, 1 H, H_A-C(5)), 1.96 (*s*, 3 H, NHAc), 1.88 (*dt*, *J* = 3.2, 14.1, 1 H, H_B-C(5)), 1.65 (*m*, 2 H, CH_2), 1.30 (*m*, 6 H, 3 x CH_2), 0.90 (*t*, *J* = 6.8, 3 H, Me); ^{13}C-NMR (75.43 MHz, $CDCl_3$): 174.73 (*s*, CO), 168.89 (*s*, CO), 134.39 (*d*, C(3)), 131.54 (*d*, C(2)), 65.27 (*t*, OCH_2), 54.18 (*d*, C(4)), 49.44 (*d*, C(1)), 34.27 (*t*, C(5)), 31.36, 28.53, 25.51 (each *t*, CH_2 of hexyl), 23.39 (*q*, $COCH_3$), 22.50 (*t*, CH_2 of hexyl), 13.96 (*q*, CH_3 of hexyl); MS (ei, 80 eV, 75°C): 253 (6.1%), 210 (5.2%), 168 (3.2%), 152 (2.4%), 124 (100%), 82 (82.4%); Anal. calcd. for: $C_{14}H_{23}NO_3$ (253.34): C, 66.37; H, 9.25; N, 5.53; found: C, 66.13; H, 9.03; N, 5.48.

The enantiomeric excess was determined ee≥99% by saponification of an analytical sample with NaOH, re-esterification with diazomethane followed by HPLC analysis that gave only one signal and no indication for the presence of the (+) enantiomer.

(±)-Butyl (1 *SR*, 4 *RS*)-4-acetamido-cyclopent-2-ene-1-carboxylate (±)-5:- (±)-1 (10.0 g, 54.58 mmol) was suspended in dry 1-butanol (150 *ml*) and conc. sulfuric acid (1.0 *ml*) was added dropwise under vigorous stirring. The mixture was then heated under reflux for 18 h. The methanol formed during the reaction was distilled off continuously by use of a distillation head attached onto the top of a warm (70°C) *Vigreux* column. The solvent was evaporated *in vacuo* and the residue purified by column chromatography (hexane/ethyl acetate 1:1) to yield **(±)-5** (9.14 g, 74%); mp 49-50°C.

(-)-Butyl (1 *R*, 4 *S*)-4-acetamido-cyclopent-2-ene-1-carboxylate (-)-5: A suspension of **(±)-5** (1.5 g, 6.66 mmol) and the lipase from *Candida cylindracea* (1 mg, 30 U/mg) in phosphate buffer (pH 7, 125 *ml*) was stirred at 37°C in the pH stat equipment until a total of 33.3 *ml* of 0.1 N NaOH was added (corresponds to 50% conversion). Work up as described for the synthesis of **(-)-4** afforded **(-)-5** (638 mg, 42.5%); mp: 69.5-70.5°C, $[\alpha]_D^{20}$ -68.7 (*c*,1.1 $CHCl_3$); R_F: 0.79 (EtOAc/MeOH 5:1); IR (KBr): 3300*s*, 3060*w*, 2900*m*, 2940*m*, 2880*m*, 1720*s*, 1630*s*, 1540*s*, 1480*m*, 1450*m*, 12370*m*, 1320*m*, 1300*m*, 1270*m*, 1250*m*, 1225*s*, 1120*m*, 1075*m*, 1010*m*; ^{1}H-NMR: (300 MHz, $CDCl_3$): 6.03 (*br s*, 1 H, NH), 5.89 (*m*, 2 H, H-C(2), H-C(3)), 5.09 (*dt*, *J* = 3.5, 8.5, 1 H, H-C(4)), 4.11 (*t*, *J* = 6.5, 2 H, OCH_2), 3.49 (*dd*, *J* = 3.5, 8.5, 1 H, H-C(1)), 2.44 (*dt*, *J* = 8.5, 14.0, 1 H, H_A-C(5)), 1.96 (*s*, 3 H, NHAc), 1.88 (*dt*, *J* = 4.0, 14.0, 1 H, H_B-C(5)), 1.64 (*m*, 2 H, CH_2), 1.40 (*m*, 2 H, CH_2), 0.95 (*t*, *J* = 6 5, 3 H, Me); ^{13}C-NMR (62.89 MHz, $CDCl_3$): 174.98 (*s*, CO), 169.11 (*s*, CO), 134.58 (*d*, C(3)), 131.72 (*d*, C(2)), 65.04 (*t*, OCH_2), 54.23 (*d*, C(4)), 49.47 (*d*, C(1)), 34.28 (*t*, C(5)), 30.62 (*t*, CH_2 of butyl), 23.38 (*q*, COMe), 19.09 (t, CH_2 of butyl), 13.65 (*q*, Me of butyl); MS (ei, 80 eV, 70°C): 225 (2.9%), 182 (3.3%), 152 (2.8%), 124 (73.3%), 82 (100%); Anal. calcd. for $C_{12}H_{19}NO_3$ (225.29): C, 63.98, H, 8.50; N, 6.22; found: C, 64.20; H, 8.44; N, 6.23.

The ee was determined ≥99% by saponification of an analytical sample followed by HPLC analysis; this material showed in the HPLC investigation no traces of the corresponding (+) enantiomer **(+)-1**

(±)-Ethyl (1 *SR*, 4 *RS*)-4-acetamido-cyclopent-2-ene-carboxylate (±)-6.- Abs. ethanol (5.4 *ml*) was cooled to 0°C and freshly distilled thionyl chloride (1.1 *ml*) was slowly added under stirring. The stirring was continued for an additional 90 min, the crude acid (±)-7 (2.5 g, 15.28 mmol) was added and the suspension was stirred at room temperature overnight. The solvent was removed and the residue suspended in abs. pyridine (20 *ml*) and at 0°C acetic anhydride (5 *ml*) was added dropwise. After completion of the addition the mixture was allowed to warm to room temperature, stirring was continued for 2 h, then the solvents were removed, toluene (3 x 50 *ml*) was distilled off and the dichloromethane solution (100 *ml*) of the residue was washed with a solution of bicarbonate (3 x 10 *ml*) and brine (10 *ml*) and dried over magnesium sulfate. The solvent was evaporated under reduced pressure and the residue purified by column chromatography (silica, hexane/ethyl acetate 1:1) to afford (±)-6 (2.18 g, 72%); mp: 52.5-53.5°C; R_F: 0.74 (EtOAc/MeOH 5:1); IR (KBr): 3300*s*, 3060*m*, 2990*m*, 2970*w*, 2930*w*, 2905*m*, 2880*w*, 1720*s*, 1630*s*, 1540*s*, 1480*w*, 1450*m*, 1370*m*, 1320*m*, 1300*m*, 1270*m*, 1255*m*, 1240*s*, 1225*s*, 1125*m*, 1110*w*, 1080*m*, 1050*m*, 1035*m*, 1010*m*; ^{1}H-NMR (300 MHz, $CDCl_3$): 6.08 (*br s*, 1 H, NH), 5.90 (*m*, 2 H, H-C(2), 1 H, H-C(3)), 5.07 (*dt*, *J* = 3.5, 8.5, 1 H, H-C(4)), 4.16 (*q*, *J* = 7.2, 2 H, OCH_2), 3.48 (*dd*, *J* = 3.9, 8.4, 1 H, H-C(1)), 2.45 (*dt*, *J* = 8.5, 14.0, 1 H, H_A-C(5)), 1.96 (*s*, 3 H, NHAc), 1.89 (*m*, 1 H, H_B-C(5)), 1.29 (*t*, *J* = 7.2, 3 H, Me); ^{13}C-NMR (62.89 MHz, $CDCl_3$): 175.02 (*s*, CO), 169.15 (*s*, CO), 134.63 (*d*, C(3)), 131.77 (*d*, C(2)), 61.20 (*t*, OCH_2), 54.24 (*d*, C(4)), 49.50 (*d*, C(1)), 34.33 (*t*, C(5)), 23 46 (*q*, $COCH_3$), 14.20 (*q*, CH_3); MS (ei, 80eV, 56°C): 197 (4.2%), 154 (4.0%), 152 (4.4%), 138 (1.1%), 126 (10.6%), 124 (7.8%), 82 (100%); Anal calcd. for: $C_{10}H_{15}NO_3$ (197.24): C, 60.90; H, 7.67; N, 7.10; found: C, 60.82; H, 7.64; N, 7.07.

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12 Comparison of our data and of ref. 11 with those provided by ref. 8 suggest that the enantiomeric excess of the reported ester [8] was only ca 49% and not 87%[8] as claimed.

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14 Both enantiomers of lactame **2** are now commercially available: Chiros Ltd., Cambridge, ca £8/g.

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