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Chemoenzymatic synthesis of optically active α-methylene-γ-carboxy-γ-lactams and γ-lactones

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ABSTRACT

Three α-methylene-γ-carbomethoxy-γ-butyrolactams (methyl α-methylene-pyroglutamates) **11**, **12** and **13**, differing in the substitution at the heterocyclic nitrogen, as well as the structurally related γ-lactones **14** and **15** were synthesised and resolved enzymatically by hydrolysis of their ester function, mediated by commercially available hydrolytic enzymes. In particular, the α-chymotrypsin proved to be active to all the substrates examined, displaying a different degree of activity and enantioselectivity, this latter increasing significantly towards the substrate with an aromatic substituent at the nitrogen.

1. Introduction

α-*exo*-Methylene-γ-butyrolactone and α-*exo*-methylene-γ-butyrolactam[1,2] rings are key structural units in many natural bioactive compounds (Fig. 1). The former system occurs mainly in sesquiterpene lactones,[1d,3] for example, **1** and **2**, a large class of natural compounds found almost exclusively in the family of *Compositae*, which display strong cytotoxic, antiinflammatory, phytotoxic and antimicrobial properties.[4] Similar activities are exhibited by methylenolactocin **3** and protolichesterinic acid **4**, both of which belong to the class of paraconic acids.[1a,b]

The bioactivity[4b,5] of these compounds towards many biological targets has been ascribed to its *exo* C=C double bond, as demonstrated by the complete loss of biological activity[6] after reduction of the *exo* double bond or its isomerisation to the *endo* position.

However, the application of these compounds for pharmaceutical purposes has been severely limited by their high toxicity exhibited in vitro.[1d,7]

The isosteric α-methylene-γ-lactam analogues are much less present in nature than in the parent lactones. Examples are pukeleimid E **5**,[8] isolated from cyanobacteria *Lyngbyamajuscula*, and two imidazole alkaloids anantin **6** and isoanantin **7**[9] found in the leaf tissue of *Cynometra*.

However, these compounds have received considerable attention as a consequence of their proven biological properties,[2b] associated with minor toxicity, when compared with the lactone analogues.[10]

As a result of their biological relevance, synthetic routes to these two classes of compounds have been explored, with particular attention to their asymmetric version accessing chiral non-racemic derivatives.

In addition to the classical α-methylenation of γ-lactones[11] and lactams,[12] the use of nitrocompounds,[13] as well as Baylis–Hillmann chemistry,[14] the method of most general applicability, leading to a large variety of differently substituted derivatives, is the addition of allylmetal compounds, in particular allyl boron,[15] -zinc[2b,10,16] and -indium[17] reagents to aldehydes[15a,c,d,f,g,17a–c,17e] for the construction of the α-methylenated γ-lactone skeleton, and to imines[10,15a–c,15e–g,16b–e,17d] and oximes,[18] for the nitrogen analogue.

To the best of our knowledge, the enzymatic kinetic resolution of a γ-lactam containing the α-*exo*-methylene functionality is so far unexplored in the literature. As to the lactonic analogues, no other examples have been described after the work of our group published in 2000[19] on the PPL mediated resolution of ethyl 2-methyl-4-methylene-tetrahydro-5-oxo-2-furancarboxylate **24**.

2. Results and discussion

In the frame of our research, focused on the asymmetric synthesis of chiral compounds of biological interest by means of biotransformation methods, we herein report the synthesis and resolution of three α-methylene-γ-carbomethoxy-γ-butyrolactams (methyl α-methylene-pyroglutamates) **12**, **13** and **14**, differing in the substitution at the heterocyclic nitrogen, as well as the structurally related γ-lactones **15** and **16** (Scheme 2).

Incidentally, α-methylene pyroglutamic acid[12a] is a cyclic analogue of 4-methylene glutamic acid **10** (Scheme 1) which is in turn a natural compound found in a variety of plants,[20] and exhibiting a potent central nervous system inhibitory action,[21] due to activation of NMDA (*N*-methyl D-aspartate) receptors.[22]

2.1. Synthesis of the racemic substrates

As a common starting material for the synthesis of the heterocyclic racemic substrates, we synthesised the dimethyl ester of

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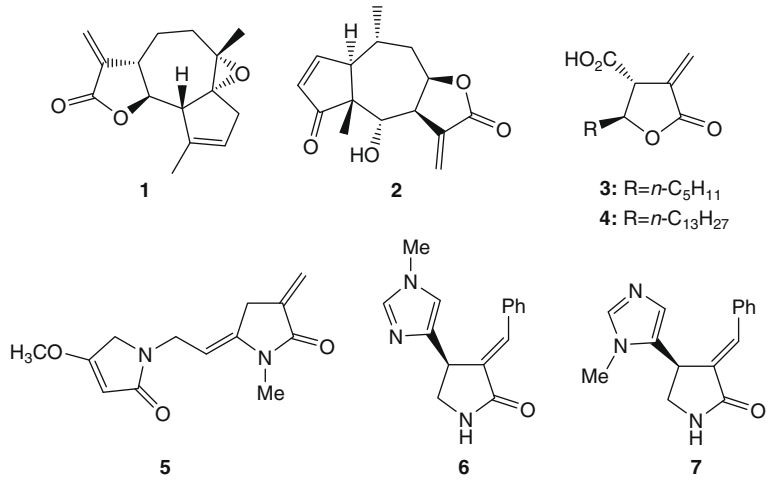
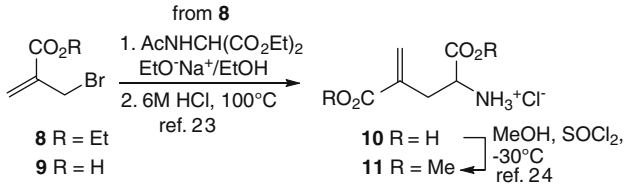


Figure 1. Selected examples of naturally occurring α-methylene-γ-lactones and lactams: arglabin **1**, helenalin **2**, methylenolactocin **3**, protolichesterinic acid **4**, pukeleimide E **5**, anantine **6**, isoanantine **7**.

4-methylene-glutamic acid in its hydrochloride salt form **11** (Scheme 1). Its preparation was carried out by a literature, three-step procedure.[23,24]

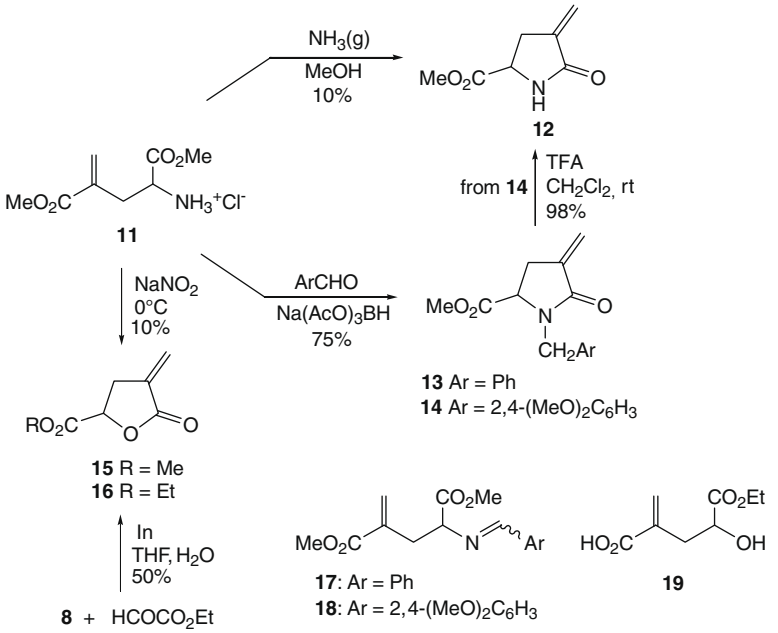


Scheme 1.

The transformations of the parent molecule **11** into the compounds of interest **12**, **13**, **14** and **15** occurred with yields ranging from 10% to 75% (Scheme 2). In particular, cyclisation of **11** in refluxing methanol saturated with ammonia, gave **12** in very poor yield.[25]

On the contrary, a convenient route was found for the conversion of **11** into the N-substituted compounds **13** and **14**. Reductive amination of benzaldehyde and 2,4-dimethoxybenzaldehyde respectively with **11** as the amine partner furnished the desired lactams in 75% yield. The reactions were performed in THF at room temperature, in the presence of an equimolar amount of triethylamine, using $Na(AcO)_3BH$ as the reducing agent.[26] The reaction of benzaldehyde proceeded smoothly to imine **17** (Scheme 2), which was not reduced in situ, as expected, but isolated as a pure compound. Its reduction, followed by spontaneous cyclisation to the target compound **13** took place in MeOH and $Na(AcO)_3BH$. On the contrary, imine **18**, derived from 2,4-dimethoxybenzaldehyde, was not isolated, but only detected by ^1H NMR in the reaction mixture after 2 h, and underwent complete reduction/cyclisation in situ to the desired lactam **14**.

It is important to note that the reductive amination of 2,4-dimethoxybenzaldehyde with amine **11** offers an alternative, efficient route to α-methylenepyroglutamate **12**. In fact the benzylic pro-



Scheme 2.

tecting group at the nitrogen atom could be easily removed from **14** by treatment with TFA at room temperature, to give **12** in quantitative yield.

On the contrary, transformation of **11** into the corresponding γ-lactone **15** by nitrosation, following the procedure described for the cyclisation of L-glutamic acid,[11b] gave very small amounts of the desired product, which formed in an admixture with unidentified compounds.

Yields in the lactonic product increased to an acceptable value (50%) by the indium-promoted allylation of ethylglyoxylate with 2-(bromomethyl)acrylic acid **9** (Scheme 2),[19] followed by cyclisation of the hydroxy hemiester intermediate **19**, to give lactone **16**.

2.2. Enzymatic resolution

The resolution of compounds **12**–**15** was accomplished by enzymatic hydrolysis of their respective racemic esters using α-chymotrypsin, while PPL was the preferred enzyme for the hydrolysis of lactone **16**.

The choice of α-chymotrypsin for the resolution of the three γ-carbomethoxy-α-methylene-γ-lactams **12**–**14** was based on the already known ability of this enzyme to hydrolyse the *laevo* enantiomer of unsubstituted methyl pyroglutamate and some other 5-substituted derivatives.[27]

The results found are summarised in Table 1, which lists the ee values of the isolated lactam and lactone acids **20**–**23**, those of their recovered unreacted esters **12**–**15** and yields. After determining the enantiomeric ratio *E*[28] at approximately 50% conversion, resolutions were run up to the conversions indicated in the table in order to isolate the carboxylic acid products and the unreacted ester in the highest possible ee.

As expected, enzymatic hydrolyses of heterocycles bearing no benzyl-type substituent at position 1 of the ring were poorly enantioselective (Table 1, entries 1, 6 and 7), as indicated by the low values of the enantiomeric ratios *E*. Enzymatic hydrolysis of **12** (entry 1) gave the corresponding carboxylic acid (*R*)-(−)-**20** with low ee, while the corresponding unreacted ester (*S*)-(+)-**12**[18,29] had 72% ee. Furthermore, this reaction was difficult as far as work-up is concerned. As a consequence of the high water solubility of the ester, its separation from the acid had to be performed by column chromatography. Due to the low enantioselectivity observed and the disadvantageous work-up, we did not investigate this reaction any further.

The insertion of a benzyl-type substituent at the heterocyclic nitrogen, in addition to increasing the overall yield of the racemic synthesis, markedly improved the enantioselectivity of the enzymatic resolution. In fact, α-chymotrypsin hydrolysed both lactams **13** and **14** with very high stereoselectivity (*E* >100 in both cases), thus allowing the obtainment of the carboxylic acids (*R*)-(−)-**21** and (*R*)-(−)-**22** with excellent enantiomeric excesses and recovery of their respective unreacted esters (*S*)-(+)-**13** and (*S*)-(+)-**14** as enantiomerically pure forms (Table 1, entries 2 and 3). This result is in accordance with the well-known affinity of α-Ct for substrates carrying a hydrophobic aromatic substituent near the reaction centre.[27,31]

A further important consequence was the opportunity to obtain lactam (*S*)-(+)-**12** in good yield and high enantiomeric excess, via acidic removal of the dimethoxybenzyl protecting group from (*S*)-(+)-**14**, as seen in Scheme 2. This indirect route gave (*S*)-(+)-**12** with >99% ee and 21% overall yield, starting from (±)-**11**. Moreover, a stereochemical correlation could be established between (*S*)-(+)-**14**, so far unknown in the literature, and (*S*)-(+)-**12**, whose absolute configuration had already been reported.[18,29] All γ-lactam esters isolated from the enzymatic hydrolyses, namely (*S*)-(+)-**12**, (*S*)-(+)-**13** and (*S*)-(+)-**14**, exhibited the same positive Cotton effect at approximately the same wavelength in their CD spectra in MeOH, (Δ_{233} +1.4; Δ_{239} +1.1; Δ_{240} +1.3, respectively), which allowed the configurational assignment to also be made for the unknown N-benzylsubstituted derivative (*S*)-(+)-**13**.

As for the enzymatic resolutions of lactones (±)-**15** and (±)-**16**, with α-chymotrypsin they occurred with unsatisfactory enantioselectivity. This had already been observed for the α-chymotrypsin mediated hydrolysis of the homologous γ-carboethoxy-α-methylene-γ-valerolactone **24** (Fig. 2),[19] as well as for the α-unsubstituted derivative **25**.[27]

However, the enantioselectivity of the reaction increased to a satisfactory degree using PPL, especially when acetone was added to the reaction medium as the cosolvent (*E* = 19). In this latter case, acid (*R*)-(−)-**23** was obtained in a 81% ee, at low conversion, while the unreacted ester (*S*)-(+)-**16** could be isolated as an enantiomeri-

Table 1
Enzymatic hydrolysis of substrates **12**–**16**[a]

Entry	Substrate	Enzyme	*E*	Conv. values (time)	(*R*)-(−)-Acid, ee %[b,c] (yield %)[d]	Conv. values (time)	(*S*)-(+)-Ester ee %[b] (yield %)[d]
1	(±)-**12** X = NH, R = Me	α-Ct	7	57[e] (3 h)	**20** 55 (16)[f]	57[e] (3 h)	**12** 72[b] (28)[f]
2	(±)-**13** X = NBn, R = Me	α-Ct	>200	35 (6 h)	**21** 99 (25)	64 (18 h)	**13** >99 (30)
3	(±)-**13** X = NBn, R = Me	α-Ct	>200	52 (8 h)	**21** 97 (38)	52 (8 h)	**13** 98 (39)
4	(±)-**14** X = N-DMBn, R = Me	α-Ct	140	28 (2 h)	**22** 98[c,g] (22)	65 (14 h)	**14** >99[g] (27)
5	(±)-**14** X = N-DMBn, R = Me	α-Ct	140	51 (4 h)	**22** 95 (37)	54 (4 h)	**14** 96 (41)
6	(±)-**15** X = O, R = Me	α-Ct	4	53 (15 min)	**23** 44 (32)	80 (2 h)	**15** 79 (16)
7	(±)-**16** X = O, R = Et	PPL in 10% acetone/buffer	19	20 (15 min)	**23** 81[h] (13)	71 (30 min)	**16** 99 (22)

[a] Reaction conditions: 1.0 g substrate, 0.1 g enzyme, 0.1 M phosphate buffer at pH 7.4 (5 ml/mmol), room temperature.
[b] Determined by HRGC (β-cyclodextrin).
[c] After esterification with MeOH, Me_3SiCl.[30]
[d] Yield in isolated product.
[e] Resolution at low conversion values was not carried out, because of work-up problems (see text).
[f] After chromatographic separation.
[g] After removal of the protecting group with TFA.
[h] After esterification with EtOH, Me_3SiCl.[30]

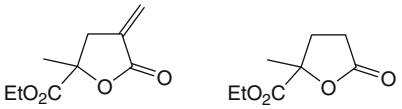


Figure 2.

cally pure compound at 71% conversion, with a 22% yield in the isolated product. In analogy to what was observed in the case of **24**,[19] other hydrolases tested (esterases and lipases) proved inactive or less enantioselective than PPL, while acetone was found to be the only co-solvent with any effect on the stereoselectivity of the resolution.

The absolute configuration was assigned to (+)-**16** by comparison with the literature data.[11b] In fact, compound (*S*)-(+)-**16** had been already described as being derived from L-glutamic acid,[11b] by a nitrosation/cyclisation reaction, which is known to occur with retention of configuration, followed by direct α-methylenation. Further confirmation of the stereochemical assignment came from the CD spectrum of (*S*)-(+)-**16** which showed a positive Cotton effect at 220 nm, (CD: Δ_{221} +1.1 (MeOH)) as reported for the spectrum of the (*S*)-enantiomer of lactone **24**, [CD: Δ_{226} +3.2 (MeOH)].[19]

Interestingly, this result suggests that the enantiopreference of PPL towards the substrate **16** is opposite with respect to the case of **24**. In fact, in its hydrolysis with lactone **24**, PPL showed an (*S*)-enantiopreference,[19] whereas with lactone **16**, it exhibited an (*R*)-enantiopreference, with the methyl group at C-2 in **24** playing an important role in the approach of the molecule to the active site of the enzyme.

3. Conclusion

In conclusion, the first example of a kinetic resolution of α-*exo*-methylene-γ-lactams was achieved. The preferred enzyme was α-chymotrypsin when a benzyl-protecting group was present at the nitrogen atom, thus reflecting the high tendency of this enzyme to interact with substrates bearing aromatic groups near the reaction centre.

4. Experimental part

4.1. General

IR spectra were recorded on an AVATAR 320 FT/IR spectrometer. ^{1}H NMR spectra were run on a Jeol EX-400 (400 MHz), ^{13}C NMR on a JEOL 270 (67.5 MHz for carbon) using deuterochloroform as a solvent and tetramethylsilane as an internal standard, unless otherwise stated. Optical rotations were determined on a Perkin–Elmer Model 241 polarimeter, at 25 °C. CD spectra were recorded on a JASCO J-710 spectropolarimeter. SI-MS were run on a Bruker Esquire 4000 instrument. Enzymatic hydrolyses were performed using a pH-stat Controller PHM290 Radiometer, Copenhagen. Chiral High Resolution GLC analyses were run on a Shimadzu GC-14B instrument, the capillary columns being Chiraldex™ type G-TA, γ-cyclodextrin (40 m × 0.25 mm) (carrier gas helium, 180 KPa, split 1:100), or DiMePe β-cyclodextrin (25 m × 0.25 mm) (carrier gas He, 110 Kpa, split 1:50); TLC's were performed on Polygram® Sil G/UV$_{254}$ silica gel pre-coated plastic sheets (eluent: light petroleum/ethyl acetate). Flash chromatography was run on silica gel, 230–400 mesh ASTM (Kieselgel 60, Merck), using mixtures of light petroleum 40–70 °C and ethyl acetate as the eluent. α-Chymotrypsin was purchased from Fluka, PPL (Porcine Pancreatic Lipase) from Sigma.

4.2. Synthesis of the substrates

4.2.1. Ethyl 2-(bromomethyl)acrylate 8

Compound **8** was synthesised as described in the literature.[32]

4.2.2. (±)-4-Methyleneglutamic acid, hydrochloride 10

Compound **10** was synthesised as described in the literature[23] yield 96%. Mp 179–180 °C; IR (Nujol) 3405, 1737, 1678, 1626, 1597 cm^{-1}; ^{1}H NMR (D_2O) δ 6.19 (s, 1H, =CH), 5.73 (s, 1H, =CH), 4.06 (dd, *J* 8.0, 6.1, 1H, H-2), 2.79 (dd, *J* 14.6, 6.1, 1H, H-3), 2.64 (dd, *J* 14.6, 8.0, 1H, H-3); ^{13}C NMR (D_2O) δ 172.0, 170.3, 134.3, 133.0, 53.0, 33.3; ESI-MS *m*/*z* 160.0 [M$^+$].

4.2.3. Dimethyl (±)-4-methyleneglutamate, hydrochloride 11

Esterification of **10** was run as described in the literature[24] to give **11** quantitatively. IR (film) 3391, 3006, 1750, 1720; ^{1}H NMR δ 6.46 (s, 1H, =CH), 5.97 (s, 1H, =CH), 4.38 (dd, *J* 7.5, 6.3, 1H, H-2), 3.84, 3.80 (2s, 6H, OCH_3), 3.07 (dd, *J* 14.5, 6.3, 1H, H-3), 2.93 (dd, *J* 14.5, 7.5, 1H, H-3). ^{13}C NMR δ 172.8, 171.2, 135.9, 135.4, 56.3, 55.5, 54.8, 35.2; IR (Nujol) 1633, 1594 cm^{-1}; ESI-MS *m*/*z* 188.0 [M$^+$], 210.0 [M+Na$^+$].

4.2.4. Methyl (±)-4-methylene 5-oxo-pyrrolidin-2-carboxylate 12[25]

A methanolic solution (20 ml) of **11** (1.20 g, 7.70 mmol) was saturated with $NH_{3(g)}$, then refluxed for 4 h. Evaporation of the solvent left an oily residue which was chromatographed on column (eluent: ethyl acetate) to give **12** as a white solid, mp 94–96 °C (10%), HRGC (β-CDX) t_R = 34.18 and 41.68 min (150 °C isoth). IR (film) 1728, 1698, 1659, cm^{-1}; ^{1}H NMR δ 6.55 (s, 1H, NH), 6.06 (t, *J* 2.4, 1H, =CH), 5.43 (br s, 1H, =CH), 4.29 (dd, *J* 9.2, 4.3, 1H, H-2), 3.79 (s, 3H, OCH_3), 3.19 (tdd, *J* 17.3, 9.2, 2.4, 1H, H-3), 2.98 (tdd, *J* 17.3, 4.4, 2.4, 1H, H-3); ^{13}C NMR δ 172.4, 170.9, 137.4, 117.0, 52.4, 30.1; ESI-MS *m*/*z* 156.0 [M+H$^+$], 178.0 [M+Na$^+$].

4.2.5. Methyl (±)-1-(methylphenyl)-4-methylene-5-oxo-pyrrolidin-2-carboxylate 13

A mixture of **11** (1.05 g, 4.80 mmol) and benzaldehyde (freshly distiled, 0.45 g, 4.2 mmol) in THF was added of Et_3N (0.25 ml, 5.1 mmol) and sodium triacetoxyborohydride, $Na(AcO)_3BH$ (1.12 g, 4.8 mmol). The mixture was left to stir overnight at room temperature, then washed with $NaHCO_3$ (aq, 5% w/v), water and brine. The organic phase was dried and evaporated to give 0.31 g of dimethyl *N*-benzal-4-methyleneglutamate **17** as an essentially pure compound.

IR (film) 2952, 1739, 1641 cm^{-1}; ^{1}H NMR δ 8.21 (s, 1H, CH=N), 7.75 (m, 2H, ArH), 7.42 (m, 3H, ArH), 6.22 (s, 1H, =CH), 5.63 (s, 1H, =CH), 4.26 (dd, *J* 8.3, 5.7, 1H, H-2), 3.76, 3.75 (2s, 6H, CO_2CH_3), 3.12 (dd, *J* 13.8, 5.7, 1H, H-3), 2.82 (dd, *J* 13.8, 8.3, 1H, H-3); ^{13}C NMR δ 171.7, 167.1, 164.1, 135.8, 135.4, 131.2, 129.1, 128.6, 128.5, 71.7, 52.2, 51.9, 36.2; ESI-MS (*m*/*z*) 276.1 [MH$^+$].

Imine **17** (0.6 g, 2.2 mmol) was dissolved in MeOH and a further amount of $Na(AcOEt)_3BH$ (0.32 g, 1.5 mmol) added. After 12 h stirring, the solvent was removed, the residue dissolved in diethyl ether and washed with a saturated solution of $NaHCO_3$ The organic phase was evaporated to dryness leaving a residue which was chromatographed on a column (eluent: petroleum ether/ethyl acetate, gradient) to give compound **13** (0.4 g, 75% yield); HRGC (β-CDX) t_R = 156.1 and 159.4 (150 °C isoth). IR (film) 3030, 1743, 1697, 1662, 754.1, 703 cm^{-1}; ^{1}H NMR δ 7.29 (m, 3H, Ph), 7.21 (d, 2H, Ph), 6.11 (t, *J* 2.7, 1H, =CH), 5.42 (t, *J* 2.2 Hz, 1H, =CH), 5.14 (d, *J* 14.8, 1H, CH_2Ph), 4.12 (d, *J* 14.8, 1H, CH_2Ph), 4.03 (dd, *J* 9.3, 3.3, H-2), 3.68 (s, 3H, OCH_3), 3.00 (tdd, *J* 17.2, 9.3, 2.7, 1H, H-3), 2.77 (tdd, *J* 17.2, 3.3, 2.7, 1H, H-3); ^{13}C NMR δ 171.6, 167.9, 136.9 135.5, 128.8, 128.7, 127.8, 117.0, 55.7, 52.4, 46.0, 29.0; ESI-MS *m*/

z 246.0 [M+H$^+$], 268.0 [M+Na$^+$]. Anal. Calcd for $C_{14}H_{15}NO_3$: C, 68.56; H, 6.16; N, 5.71. Found: C, 68.66; H, 6.10; N, 5.78.

4.2.6. Methyl (±)-1-(2,4-dimethoxyphenyl)methyl-4-methylene-5-oxo-2-pyrrolidinecarboxylate 15

Compound **11** (1.05 g, 4.8 mmol) was reacted with 2,4-dimethoxybenzaldehyde (0.69 g, 4.2 mmol) by the above-described procedure, to give the lactam **14**, 75% yield after purification on a column (eluent: petroleum ether/ethyl acetate gradient). Oil, IR (film) 3000, 1746, 1695, 1663, 835, 807 cm^{-1} ^{1}H NMR δ 7.20 (1H, d, ArH), 6.45 (m, 2H, ArH), 6.04 (t, *J* 2.8, 1H, =CH), 5.34 (t, *J* 2.3 Hz, 1H, =CH), 4.94 (d, *J* 14.5, 1H, CH_2Ar), 4.22 (d, *J* 14.5, 1H, CH_2Ar), 4.07 (dd, *J* 9.5, 3.4, 1H, H-2), 3.79, 3.76, (2s, 6H, OCH_3), 3.72 (3H, s, OCH_3), 2.96 (tdd, *J* 17.3, 9.5, 2.8, 1H, H-3), 2.68 (tdd, *J* 17.3, 3.4, 2.3, 1H, H-3); ^{13}C NMR δ 172.5, 168.2, 161.1, 159.1, 137.7, 132.2, 116.5, 104.6, 98.4, 56.2, 55.5, 55.4, 52.4, 40.4, 29.4 ppm; ESI-MS *m*/*z* 328.1 [M+Na$^+$]. Anal. Calcd for $C_{16}H_{19}NO_5$: C, 62.94; H, 6.27; N, 4.59. Found: C, 62.82; H, 6.20; N, 4.73.

Lactam **14** was stirred in TFA for 12 h. Evaporation of the solvent under reduced pressure gave a residue which was purified on a short SiO_2 column using ethyl acetate as the eluent, to give **12** in a quantitative yield.

4.2.7. Ethyl 4-methylene-tetrahydro-5-oxo-2-furancarboxylate 16

2-(Bromoethyl)acrylic acid **9** (1.68 g, 10 mmol), ethyl glyoxylate (10 mmol, 50% solution in toluene) and indium powder (1.12 g, 10 mmol) were mixed in a 1:1 mixture of THF and H_2O (10 ml). An exothermic reaction took place, with the formation of a turbid suspension from which a metallic indium conglomerate separated. Stirring was continued until the disappearance of the starting material (TLC, ethyl acetate/petroleum ether 1:4), then ethyl acetate was added and the suspension was centrifuged to separate the white precipitate formed. The organic phase was dried over anhydrous Na_2SO_4 to give, after evaporation, a colourless oil containing essentially pure 2-hydroxy-4-carboethoxy-1-pentenoic acid **19** (8.6 g 78% yield); IR (film) cm^{-1} 3600–2950, 480, 1740, 1633; ^{1}H NMR δ 6.30 (s, 1H, C=CH), 5.60, (br, 2H), 5.74 (s, 1H, C=CH), 4.40 (dd, *J* 7.8, 4.4, H-2), 4.14 (q, 2H, O*C*H_2CH$_3$), 2.86 (dd, *J* 14.3, 4.4, 1H, H-3), 2.65 (dd, *J* 14.2, 7.8, 1H, H-3), 1.22 (t, 3H, C*H*$_3$CH$_2$O); ^{13}C NMR (67.5 MHz) δ 174.6, 167.5, 135.4, 128.8, 69.5, 60.5, 37.2; ESI-MS *m*/*z* 211.0 [M+Na$^+$].

Refluxing the crude hydroxy hemiester **19** in toluene in the presence of a catalytic amount of *p*-toluensulfonic acid led to compound **16** in 70% overall yield after flash chromatography. HRGC (β-CDX) t_R = 9.42 and 9.72 (150 °C isoth.); IR (film) 1774, 1744, 1667 cm^{-1}; ^{1}H NMR δ 6.29 (t, *J* 2.9, 1H, =CH), 5.70 (t, *J* 2.5, 1H, =CH), 4.99 (dd, *J* 9.5, 2.9, 1H, H-2), 4.21 (q, 2H, O*C*H_2CH$_3$), 3.25 (tdd, *J* 17.3, 9.5, 2.9, 1H, H-3), 2.99 (tdd, *J* 17.3, 2.5, 1H, H-3), 1.25 (t, 3H, OCH$_2$C*H*$_3$).^{13}C NMR δ 169.6, 169.0, 131.6, 123.4, 72.8, 62.1, 31.2, 14.0; ESI-MS *m*/*z* 193.0 [M+Na$^+$]. Anal. Calcd for $C_8H_{10}O_4$: C, 56.47; H, 5.92; O, 37.61. Found: C, 56.6; H, 5.9; O, 37.5.

4.3. General procedure for enzymatic hydrolyses

A suspension of the racemic substrates **12**–**15** (0.2 g), in 20 ml of 0.1 N KH_2PO_4/Na_3PO_4 buffer (pH 7.4) was hydrolysed with α-chymotrypsin (100 mg/mmol substrate), at room temperature under vigorous stirring. Hydrolysis of **16** was also performed with PPL (100 mg/mmol substrate) in a 10% acetone/phosphate buffer solution. The pH was kept at the initial value by the continuous addition of 1 M NaOH. At the desired conversion value, the unreacted ester was extracted from the suspension with ethyl acetate (3 × 10 ml) at pH 7.8 (5% $NaHCO_3$) using a centrifuge for the separation of the layers. For the isolation of the acids **20**, **21**, **22** and **23** the aqueous layer was acidified to pH 2 with 2 M HCl and extracted with $CHCl_3$ (3 × 10 ml). In the case of enzymatic hydrolysis of **12**, a chromatographic separation was necessary to separate the ester from the carboxylic acid. Determination of the enantiomeric excesses of the acidic products was made after esterification with methanol and trimethylchlorosilane.[30]

4.3.1. Methyl (*S*)-(+)-4-methylene 5-oxo-pyrrolidin-2-carboxylate 12

Compound **12** was obtained in a 28% yield (purification by flash chromatography, eluent: ethyl acetate) by stopping the enzymatic hydrolysis at 57% conversion, 72% ee, $[\alpha]_D^{25}$ = +8.6 (*c* 1.3, AcOEt), lit.[18] $[\alpha]_D^{25}$ = +15.6 (*c* 0.34, AcOEt), Δ_{208} −0.4, Δ_{233} +1.4 (MeOH).

4.3.2. (*R*)-(−)-4-Methylene-5-oxo-pyrrolidine-2-carboxylic acid 20

Compound **20** was obtained in 16% yield (purification by flash chromatography, eluent: ethyl acetate) by stopping the enzymatic hydrolysis at 57% conversion, 55% ee; mp >200 °C (dec.); ^{1}H NMR (D_2O) δ 5.97 (t, *J* 2.8, 1H, =CH), 5.64 (t, *J* 2.5, 1H, =CH), 4.30 (dd, *J* 9.4, 4.2, 1H, H-2), 3.33 (tdd, *J* 17.7, 9.4, 2.8, 1H, H-3), 2.85 (tdd, *J* 17.7, 4.2, 2.5, 1H, H-3); ^{13}C NMR (D_2O) δ 179.9, 172.9, 138.6, 117.1, 55.2, 31.2; IR (Nujol) 3284, 1702, 1678, 1650, 1633, 1584 cm^{-1}; ESI-MS *m*/*z* 139.9 [M+H$^+$].

4.3.3. Methyl (*S*)-(+)-1-(methylphenyl)-4-methylene-5-oxo-pyrrolidin-2-carboxylate 13

Compound **13** was obtained in a 30% yield at 64% conversion with >99% ee $[\alpha]_D^{25}$ = +36 (*c* 0.95, AcOEt), Δ_{213} −0.4 (MeOH), Δ_{239} +1.1 (MeOH).

4.3.4. (*R*)-(−)-1-(Methylphenyl)-4-methylene-5-oxo-pyrrolidin-2-carboxylic acid 21

Compound **21** was obtained in 25% yield, at 42% conversion, 99% ee, $[\alpha]_D^{25}$ = −127.3 (*c* 1.0, MeOH); ^{1}H NMR δ 8.40 (br, 1H, COOH), 7.25 (5H, Ph), 6.10 (t, *J* 2.4, 1H, =CH), 5.44 (br s, 1H, =CH), 5.26 (d, *J* 14.8, 1H, CH_2Ph), 4.06 (d, *J* 14.8, 1H, CH_2Ph), 4.04 (1H, dd, *J* 9.7, 3.6, 1H, H-2), 3.01 (m, 1H, H-3), 2.83 (m, 1H, H-3); ^{13}C NMR δ 174.3, 168.4, 136.7, 135.1, 129.2, 129.0, 128.3, 118.1, 55.7, 46.2, 29.1; IR (Nujol) 1741, 1673, 741, 697 cm^{-1}; ESI-MS *m*/*z* 232.0 [M+H$^+$], 254.0 [M+Na$^+$]. Anal. Calcd for $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.68; H 5.50; N, 6.04.

4.3.5. Methyl (*S*)-(+)-1-[methyl(2,4-dimethoxyphenyl)]-4-methylene-5-oxo-pyrrolidin-2-carboxylate 14

Compound **14** was obtained at 70% conversion in a 21% yield, with >99% ee $[\alpha]_D^{25}$ = +88.0 (*c* 1, AcOEt); Δ_{221} −0.5; Δ_{240} +1.3; Δ_{279} +0.4 (MeOH).

4.3.6. (*R*)-(−)-1-[Methyl(2,4-dimethoxyphenyl)]-4-methylene-5-oxo-pyrrolidin-2-carboxylic acid 22

Compound **22** was obtained in 22% yield, at 28% conversion, 98% ee, $[\alpha]_D^{25}$ = −177.1 (*c* 1.35, MeOH); mp 157–159 °C; IR (film) 3006, 1742–1614, 933.2–754.6 cm^{-1}; ^{1}H NMR δ 7.22 (d, 1H, ArH), 6.43 (m, 2H, ArH), 6.07 (br s, 1H, =CH), 5.38 (br s, 1H, =CH), 4.99 (d, *J* 14.4, 1H, CH_2Ar,), 4.29 (d, *J* 14.4, 1H, CH_2Ar), 4.15 (dd, *J* 9.8, 3.2, 1H, H-2), 3.78, 3.76 (2s, 6H, OCH_3), 3.03 (dd, *J* 17.2, 9.6, 1H, H-2), 2.79 (bd, *J* 17.2, 1H, H-3); ^{13}C NMR δ 175.8, 168.8, 161.3, 159.1, 137.5, 132.5, 117.2, 116.2, 104.7, 98.5, 59.1, 55.6, 55.3, 40.7, 29.3; ESI-MS *m*/*z* (negative-ion polarity) 290.0 [M−1]. Anal. Calcd for $C_{15}H_{17}NO_5$: C, 61.85; H, 5.88; N, 4.81. Found: C, 61.63; H, 5.80; N, 4.78.

4.3.7. Ethyl (*S*)-(+)-4-methylene-tetrahydro-5-oxo-2-furancarboxylate 16

Compound **16** was isolated from the hydrolysis carried out with PPL, using 10% acetone/phosphate buffer as the solvent, 99% ee at

71% conversion $[\alpha]_D^{25} = +19.5$ (*c* 0.45, EtOH); (lit.[11b] $[\alpha]_D^{25} = +12.7$ (*c* 2.2, EtOH), Δ_{221} +1.1 (MeOH).

4.3.8. (*R*)-(−)-4-Methylene-tetrahydro-5-oxo-2-furancarboxylic acid 23

Compound **23** was isolated at 20% conversion, 81% ee, $[\alpha]_D^{25} = -11.7$ (*c* 2.5, EtOH) [lit.[11b]+15.5 (*c* 2.1, EtOH) for the enantiopure (*S*)-enantiomer]. IR (film) cm^{-1} 3200 (br), 1775, 1736; ^1H NMR δ 6.33 (t, *J* 2.9, 1H, =CH), 5.78 (t, *J* 2.5, 1H, =CH), 5.02 (dd, *J* 9.6, 4.8, 1H, H-2), 3.35 (tdd, *J* 17.5, 9.6, 2.9, 1H, H-3), 3.08 (tdd, *J* 17.5, 4.8, 2.5, 1H, H-3); ^{13}C NMR δ 174.1, 169.2, 131.3, 124.5, 72.3, 31.2; ESI-MS *m*/*z* (negative-ion polarity) 141.0 [M−1].

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References

1. (a) Bandichhor, R.; Nosse, B.; Reiser, O. *Top. Curr. Chem.* **2005**, *243*, 43–72; (b) Forzato, C.; Nitti, P.; Pitacco, G.; Valentin, E.. In *Targets in Heterocyclic Systems, Chemistry and Properties*; Attanasi, O., Spinelli, D., Eds.; S.C.I.: Roma, 1999; Vol. 3, pp 93–115; (c) Petragnani, N.; Ferraz, H. M. C.; Silva, G. V. J. *Synthesis* **1986**, 157–183; (d) Hoffmann, H. M. R.; Rabe, J. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 94–110; (e) Grieco, P. A. *Synthesis* **1975**, 67–82.
2. (a) Ikuta, H.; Shirota, S.; Kobayashi, Y.; Yamagishi, K.; Yamada, K.; Yamatsu, I.; Katayama, K. *J. Med. Chem.* **1987**, *30*, 1995–1998; (b) Belaud, C.; Roussakis, C.; Letourneux, Y.; El Alami, N.; Villiéras, J. *Synth. Commun.* **1985**, *15*, 1233–1243; (c) Konet, M. J. *J. Pharm. Sci.* **1979**, *68*, 350–353.
3. Yoshioka, H.; Mabry, T. J.; Timmermann, B. N. *Sesquiterpene Lactones*; University of Tokio: Tokio, 1973.
4. (a) Torres, F.; Quintana, J.; Cabrera, J.; Loro, J. F.; León, F.; Bermejo, J.; Estévez, F. *Cancer Lett.* **2008**, *269*, 139–147; (b) González, A. G.; Silva, M. H.; Padrón, J. I.; León, F.; Reyes, E.; Álvarez-Mon, M.; Pivel, J. P.; Quintana, J.; Estévez, F.; Bermejo, J. *J. Med. Chem.* **2002**, *45*, 2358–2361.
5. (a) Heilmann, J.; Wasescha, M. R.; Schmidt, T. J. *Bioorg. Med. Chem.* **2001**, *9*, 2189–2194; (b) Patel, B. P.; Waddel, T. G.; Pagni, R. M. *Fitoterapia* **2001**, *72*, 511–515; (c) Dirsch, V. M.; Stuppner, H.; Ellmerer-Muller, E. P.; Vollmar, A. M. *Bioorg. Med. Chem.* **2000**, *8*, 2747–2753; (d) Kupchan, S. M.; Fessler, D. C.; Eakin, M. A.; Thomas, A. M. *J. Med. Chem.* **1971**, *14*, 1147–1152; (e) Kupchan, S. M.; Fessler, D. C.; Eakin, M. A.; Giacobbe, T. J. *Science* **1970**, *168*, 376–378.
6. He, G.; Matsuura, H.; Yoshihara, T. *Phytochemistry* **2004**, *65*, 2803–2807.
7. Howie, G. A.; Stamos, I. K.; Cassady, J. M. *J. Med. Chem.* **1976**, *19*, 309–313.
8. Cardellinall, J. H.; Moore, R. E. *Tetrahedron Lett.* **1979**, *20*, 2007–2010.
9. Naito, T.; Honda, M.; Miyata, O.; Ninomiya, I. *Chem. Pharm. Bull.* **1993**, *41*, 217–219.
10. Dembélé, Y. A.; Belaud, C.; Villiéras, J. *Tetrahedron: Asymmetry* **1992**, *3*, 511–514.
11. (a) Sass, D. C.; de Oliveira, K. T.; Constantino, M. G. *Tetrahedron Lett.* **2008**, *49*, 5770–5772; (b) Tonari, H.; Yonemoto, K. *J. Oleo Sci.* **2002**, *51*, 259–264; (c) Drioli, S.; Felluga, F.; Forzato, C.; Pitacco, G.; Valentin, E. *J.Chem. Soc., Perkin Trans. 1* **2000**, *16*, 2839–2842.
12. (a) Dieterich, P.; Young, D. W. *Org. Biomol. Chem.* **2006**, *4*, 1492–1496; (b) Moody, C. M.; Young, D. W. *J. Chem. Soc., Perkin Trans. 1* **1997**, 3519–3530; (c) Ezquerra, J.; Pedregal, C.; Micó, I.; Nájera, C. *Tetrahedron: Asymmetry* **1994**, *5*, 921–926.
13. (a) Krawczyk, H.; Albrecht, L.; Wojciechowski, J.; Wolf, W. M.; Krajewska, U.; Rozalski, M. *Tetrahedron* **2008**, *42*, 6307–6314; (b) Béji, F.; Lebreton, J.; Villiéras, J.; Amri, H. *Tetrahedron* **2001**, *57*, 9959–9962; (c) Janecki, T.; Blaszcyk, E.; Studzian, K.; Janecka, A.; Krajewska, U.; Rozalski, M. *J. Med. Chem.* **2005**, *48*, 3516–3521; (d) Ballini, R.; Bosica, G.; Livi, D. *Synthesis* **2001**, *10*, 1519–1522; (e) Ballini, R.; Marcantoni, E.; Perella, S. *J .Org. Chem.* **1999**, *64*, 2954–2957.
14. (a) Lee, K. Y.; Lee, H. S.; Kim, J. N. *Tetrahedron Lett.* **2007**, *48*, 2007–2011; (b) Le Lamer, A. C.; Gouault, N.; David, M. *J. Comb. Chem.* **2006**, *8*, 643–645; (c) Kabalka, G. W.; Venkataiah, B.; Chen, C. *Tetrahedron Lett.* **2006**, *47*, 4187–4189.
15. (a) Elford, T. G.; Ulaczyk-Lesanko, A.; De Pascale, G.; Wright, G. D.; Hall, D. G. *J. Comb. Chem.* **2009**, *11*, 155–168; (b) Elford, T. G.; Hall, D. *Tetrahedron Lett.* **2008**, *49*, 6995–6998; (c) Chataigner, I.; Zammattio, F.; Lebreton, J.; Villiéras, J. *Tetrahedron* **2008**, *64*, 2441–2455; (d) Yu, S. H.; Ferguson, M. J.; McDonald, R.; Hall, D. G. *J. Am. Chem. Soc.* **2005**, *127*, 12808–12809; (e) Chataigner, I.; Zammattio, F.; Lebreton, J.; Villiéras, J. *Synlett* **1998**, 275–276; (f) Nyzam, V.; Belaud, C.; Zammattio, F.; Villiéras, J. *Bull. Soc. Chim. Fr.* **1997**, *134*, 583–592; (g) Chataigner, I.; Lebreton, J.; Zammattio, F.; Villiéras, J. *Tetrahedron Lett.* **1997**, *38*, 3719–3722.
16. (a) Petrini, M.; Profeta, R.; Righi, P. *J. Org. Chem.* **2002**, *67*, 4530–4535; (b) Nyzam, V.; Belaud, C.; Zammattio, F.; Villiéras, J. *Tetrahedron: Asymmetry* **1996**, *7*, 1835–1843; (c) Dembélé, Y. A.; Belaud, C.; Hitchcock, P.; Villiéras, J. *Tetrahedron: Asymmetry* **1992**, *3*, 351–354; (d) El Alami, N.; Belaud, C.; Villiéras, J. *Synth. Commun.* **1988**, *18*, 2073–2081; (e) El Alami, N.; Belaud, C.; Villiéras, J. *J. Organomet. Chem.* **1988**, *388*, 1–9.
17. (a) Kim, K. H.; Lee, H. S.; Kim, S. H.; Lee, K. Y.; Lee, J. -E.; Kim, J. N. *Bull. Korean Chem. Soc.* **2009**, *30*, 1012–1020; (b) Paquette, L. A. *Synthesis* **2003**, *5*, 765–774; (c) Chouhury, P. K.; Foubelo, F.; Yus, M. *Tetrahedron* **1999**, *55*, 10779–10788; (d) Choudhury, P. K.; Foubelo, F.; Yus, M. *J. Org. Chem.* **1999**, *64*, 3376–3378; (e) Li, C. J.; Chan, T. H. *Tetrahedron Lett.* **1991**, *32*, 7017–7020.
18. Hanessian, S.; Yang, R.-Y. *Tetrahedron Lett.* **1996**, *37*, 5273–5276.
19. Pitacco, G.; Sessanta o Santi, A.; Valentin, E. *Tetrahedron: Asymmetry* **2000**, *11*, 3263–3267.
20. Meier, L. K.; Sørensen, H. *Phytochemistry* **1979**, *18*, 1173–1175.
21. Done, J.; Fowden, L. *Biochem. J.* **1952**, *51*, 451–458.
22. Ouerfelli, O.; Ishida, M.; Shinozaki, H.; Nakanishi, K.; Ohfune, Y. *Synlett* **1993**, 409–410.
23. (a) Hellmann, H.; Lingens, F. *Chem. Ber.* **1956**, 77–81; (b) Cocolas, G. H.; Hartung, W. H. *Chem. Ber.* **1957**, 5203–5205.
24. Rosowsky, A.; Bader, H.; Freisheim, J. H. *J. Med. Chem.* **1991**, *34*, 203–208.
25. Baldwin, J. E.; Cutting, J.; Dupont, W.; Kruse, L.; Silberman, L.; Thomas, R. C. *J. Chem. Soc., Chem. Commun.* **1976**, 736–738.
26. Abdel-Magid, A. F.; Carson, K. G.; Harris, B. D.; Maryanoff, C. A.; Shah, R. D. *J. Org. Chem.* **1996**, *61*, 3849–3862.
27. Gillan, T.; Mor, G.; Pepper, F. W.; Cohen, S. G. *Bioorg. Chem.* **1977**, *6*, 329–340.
28. Chen, C.-S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
29. Panday, S. K.; Griffart-Brunet, D.; Langlois, N. *Tetrahedron Lett.* **1994**, *35*, 6673–6675.
30. Brook, M. A.; Chan, T. H. *Synthesis* **1983**, 201–203.
31. Felluga, F.; Fermeglia, M.; Ferrone, M.; Pricl, S.; Pitacco, G.; Valentin, E. *Tetrahedron: Asymmetry* **2002**, *13*, 475–489.
32. Villiéras, J.; Rambaud, M. *Synthesis* **1982**, 924–926.