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A facile chemoenzymatic approach to chiral non-racemic β-alkyl-γ-amino acids and 2-alkylsuccinic acids. A concise synthesis of (*S*)-(+)-Pregabalin

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Abstract—Both enantiomerically pure antipodes of GABA analogues were prepared as hydrochloride salts, by enzymatic kinetic resolution of their precursors ethyl 2-(nitromethyl)alkanoates. These latter compounds can be easily transformed into enantiomerically pure 2-alkylsuccinic acids by a Nef reaction followed by oxidation. Interestingly, this reaction was particularly easy for the neopentyl derivative (*S*)-(+)-**7d**, which underwent conversion into its corresponding succinic acid derivative (*S*)-(−)-**8d** in buffered solution. The absolute configurations of the main compounds of interest involved are given, together with their CD spectra.

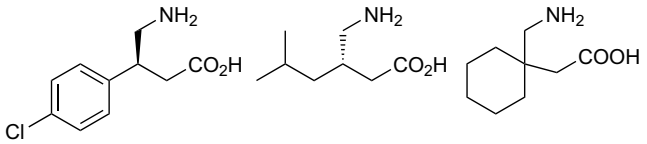
1. Introduction

γ-Aminobutyric acid (GABA) is the most important Central Nervous System (CNS) inhibitory neurotransmitter.[1] The disfunctioning of the central GABA system is associated with important neurological diseases such as epilepsy, Huntington's and Parkinson's diseases, and other psychiatric disorders, such as anxiety and peripheral neuropathic pain.[2]

The direct administration of GABA is, however, not an efficient therapy as, due to its high hydrophilicity, it very inefficiently crosses the blood-brain barrier (BBB).[3]

Many attempts have been made to produce GABAergic drugs and prodrugs. Structure–activity relationships of unnatural GABA analogues, and in particular those bearing a β-aryl-[4] or β-alkyl[5–7] substituent, have been the subject of extensive investigations, some of which have found pharmacological applications for a range of CNS disorders.[5] Due to their lipophilicity, these are able to cross the BBB, to inhibit GABA-aminotransferase (GABA-AT), the enzyme from which GABA is degraded.

Depending on the type of substituent at the β-carbon atom, these compounds show different activities. For instance, Baclofen[4,8] **1** (3-(4-chlorophenyl)-4-aminobutyric acid) is a selective agonist at the $GABA_B$ receptor, and is used in therapy (Kemstro®, Lioresal®, Baclon®) as a muscle relaxant and antispastic agent (Fig. 1). Pregabalin[9] **2** (3-aminomethyl-5-methylhexanoic acid), sold as Lyrica®, has anticonvulsant, anxiolytic-like and analgesic properties, even more potent than those of Gabapentin **3**,[5,10] an achiral β,β-disubstituted GABA analogue, commercialized as Neurontin® for the treatment of several cerebral diseases, and which is the only drug specifically licensed for the treatment of peripheral neuropatic pain. Both these latter compounds show very few, if any, toxic side effects.



(*R*)-(−)-Baclofen **1** (*S*)-(+)-Pregabalin **2** Gabapentin **3**

Figure 1.

The chirality of these compounds plays an important role. For instance, the biological activity of Baclofen is known to reside only in the (*R*)-(−)-enantiomer, while Pregabalin

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is active in its (*S*)-(+)-enantiomer. In spite of this, these are still commercialized in their racemic form.

The interest in a synthetic, stereoselective methodology for this class of compounds is justified by the increasing demand for enantiomerically pure drugs from the pharmaceutical industry not only because of the higher specificity exhibited by a pure enantiomer in its activity, but also because of the health and environmental risks often associated with the use of racemic mixtures, in which the inactive enantiomer may present dangerous side effects.[11]

Furthermore, enantiomerically pure γ-amino acids are present in the structure of natural compound showing antitumour activity,[12] and are also known to form γ-peptidomimetics, which have been reported[13] to be stable in a helicoidal conformation, both in solution and in the solid state, even for oligopeptides consisting of as few as four residues. This makes these systems potentially useful in the development of new therapeutic agents.

For all these reasons, the enantioselective synthesis of new chiral linear and cyclic γ-aminoacids, including β-alkyl,[6] β-aryl,[4] β-heteroaryl[14] and β,β′-dialkyl[15] substituted systems, has been the subject of many publications, recently collected in an extensive review[16] covering the literature from 1997 to 2006 and reporting data on the biological activity and practical applications of these compounds.

Since then, new synthetic approaches to (−)-Baclofen **1**[17] and (+)-Pregabalin **2**[18,19] have appeared in the literature, thus showing the interest in this topic.

In 2005 our research group published[8a] the chemoenzymatic synthesis of both enantiomers of Baclofen and β-phenyl GABA, a tranquillizer and mood elevator.

Herein, we report a convenient synthesis of a series of β-alkyl GABAs, including the pharmacologically relevant (*S*)-(+)-Pregabalin **2**, all exhibiting excellent enantiomeric excesses, by an efficient procedure, involving the use of hydrolytic enzymes in the enantiodifferentiating step.

2. Results and discussion

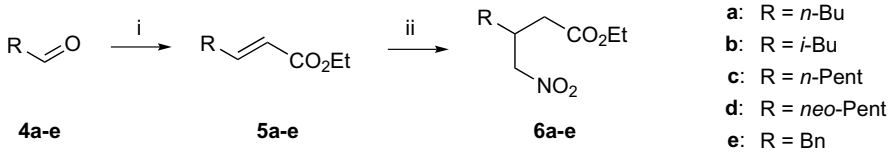
2.1. Synthesis and resolution of the γ-nitro ester substrates

Racemic γ-nitro esters (±)-**6a**–**e** were synthesized by the conjugate addition of nitromethane to a series of α,β-unsaturated esters **5a**–**e**, mediated by DBU, as reported in the literature,[20] while the Michael acceptor esters **5a**–**e** were prepared by Horner–Wadsworth–Emmons homologation of the corresponding aldehydes **4a**–**e**[21] (Scheme 1).

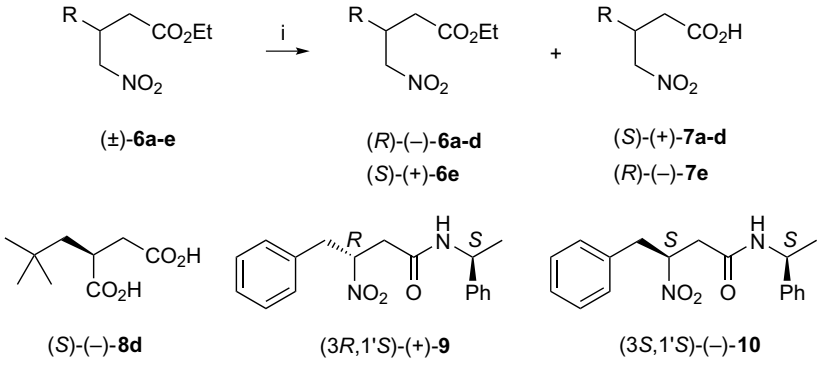
After an accurate screening of commercially available enzymes, the γ-nitro esters (±)-**6a**–**d** were found to be hydrolyzed enantioselectively by Novozyme 435 (Candida Antarctica B), in a buffer solution at pH 7.4, at room temperature, while (±)-**6e** could only be efficiently hydrolyzed by α-chymotrypsin under the same conditions. The resulting products were the corresponding γ-nitro acids (+)-**7a**–**d** and (−)-**7e** (Scheme 2).

The results are summarized in Table 1, reporting the enantiomeric excesses of the recovered unreacted substrates (*R*)-(−)-**6a**–**d**, (*S*)-(+)-**6e** and those of the acidic products (*S*)-(+)-**7a**–**d**, (*R*)-(−)-**7e**, at different conversion values.

Hydrolyses of (±)-**6a**–**c** proceeded with good enantioselectivity, as indicated by the value of their respective enantiomeric ratios.[22] The acidic compounds (+)-**7a**–**c** could be isolated in high enantiomeric excesses by stopping the



Scheme 1. Reagents and conditions: (i) triethyl phosphonoacetate, *tert*-BuOK, refluxing THF, 20 min; (ii) CH_3NO_2, DBU, rt, overnight.



Scheme 2. Reagents and conditions: (i) enzyme, buffer, pH 7.4, rt.

Table 1. Enzymatic resolution of the γ-nitro esters (±)-**6a**–**e**

Substrate	Enzyme	*E*	Unreacted γ-nitro ester			γ-Nitro acid		
			Ester (yield, %)[a]	Conv., % (time, h)	ee,[b] %	Acid (yield, %)[a]	Conv., % (time, h)	ee, %
6a	Novozym 435[c]	17	(−)-**6a** (21)	76 (3)	>99	(+)-**7a** (18)	18 (1)	87[d]
6b	Novozym 435[c]	44	(−)-**6b** (30)	64 (5)	>99	(+)-**7b** (21)	25 (2)	92[d]
6c	Novozym 435[c]	46	(−)-**6c** (32)	63 (6)	>99	(+)-**7c** (23)	29 (2)	94[d]
6d	Novozym 435[c]	6	(−)-**6d** (22)	75 (60)	95	(+)-**7d** (18)	23 (23)	65[d]
6e	α-Chymotrypsin[e]	36	(+)-**6e** (60)	36 (72)	42	(−)-**7e** (18)	21 (15)	94[f]

[a] Yields in isolated products.
[b] Enantiomeric excesses determined by chiral HRGC.
[c] Reaction conditions: 1.0 g substrate, 0.18 g enzyme, 0.1 phosphate buffer at pH 7.4 (5 mL/mmol), room temperature.
[d] Enantiomeric excesses determined by chiral HRGC after esterification of the carboxy group.
[e] Reaction conditions: 1.0 g substrate, 0.5 g enzyme, 0.1 phosphate buffer at pH 7.4 (5 mL/mmol), room temperature.
[f] Enantiomeric excess determined by ^{1}H NMR after coupling the carboxylic functions with (*S*)-(−)-1-phenylethylamine, followed by integration of the corresponding nitromethylene proton signals (see text and Section 4).

hydrolyses at around 20% conversion, while the corresponding nitro esters (−)-**6a**–**c** were recovered in enantiomerically pure form (99.9% ee) at approximately 70% conversion value.

In the hydrolysis of (±)-**6d**, owing to the low enantiomeric ratio, carboxylic acid (+)-**7d** was obtained in 65% ee by stopping the reaction at a low conversion, while the corresponding γ-nitro ester (−)-**6d** was isolated with 95% ee at 75% conversion. Furthermore, (+)-**7d** underwent a transformation into the corresponding succinic acid derivative (*S*)-(−)-**8d** (Scheme 2) in a few hours. The same transformation was found to occur when the γ-nitro acid (+)-**7d** was left in a buffer for a period of time. As a result, it was not possible to carry out the hydrolysis at high conversion values because the resulting nitro acid (+)-**7d** quantitatively converted to (−)-**8d**, as a result of the Nef reaction and subsequent oxidation.[23] Interestingly, the Nef reaction was not observed with the other γ-nitro acids **7a**–**c** and **7e**, nor with the γ-nitro esters **6a**–**e**. It left the stereocentre untouched, as the resulting product (*S*)-(−)-**8d** was optically active. This different reactivity could be ascribed to the highly bulky neopentyl group, which would enhance the energy of the molecule, sterically interacting with the nitro group.

As previously anticipated, the γ-nitro ester (±)-**6e** was hydrolyzed by α-chymotrypsin. This is known to be the enzyme of choice for substrates bearing a hydrophobic, aromatic substituent close to the reaction centre.[24] Accordingly, very high enantioselectivities had already been observed by us in the hydrolysis of β-aryl-γ-nitro esters.[8a] In the present case, the hydrolysis was very slow, reaching 21% conversion in 15 h. At this conversion value, the laevorotatory γ-nitro acid (*R*)-(−)-**7e** with 94% ee was isolated. However, after having reached 21% conversion, the hydrolysis rate decreased dramatically, and after 72 h, at 36% conversion, the reaction did not proceed any further. The recovered γ-nitro ester (*S*)-(+)-**6e**, having 42% ee, was subjected to repeated enzymatic hydrolyses to improve its ee up to 62% (8% yield from the racemate).

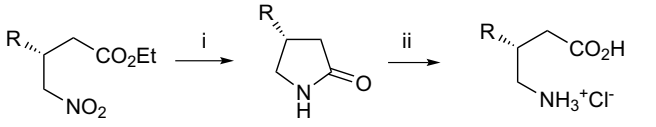
The enantiomeric excesses of both the γ-nitro esters and the γ-nitro acids were determined by chiral HRGC, the latter after esterification with ethanol in the presence of trimethylsilyl chloride,[25] with the exception of (+)-**7e**, which was determined by ^{1}H NMR analysis of the corresponding diastereomeric amides (3*R*,1′*S*)-**9** and (3*S*,1′*S*)-**10** (Scheme 2), obtained by reaction with (*S*)-(−)-1-phenylethylamine. In fact, integration of the relative nitromethylene proton signals allowed an evaluation of the enantiomeric excess to be made.

2.2. Transformations of optically active γ-nitro esters and γ-nitro acids

The enantiomerically pure γ-nitro esters (−)-**6a**–**e**, obtained by the chemoenzymatic procedure described above [enantiomerically pure (−)-**6e** was prepared from (−)-**7e** by esterification], are the direct precursors of the target β-alkyl GABA derivatives. In fact the reduction of the nitro group, carried out with hydrogen, in the presence of Ra-Ni as a catalyst, under atmospheric pressure, gave the pyrrolidin-2-one derivatives (+)-**11a**,**b**,**c**,**e** and (−)-**11d**, which were transformed into the hydrochloride salts of the GABA analogues (−)-**12a**,**c**,**d**,**e**, by hydrolysis in refluxing 6 N HCl (Scheme 3).

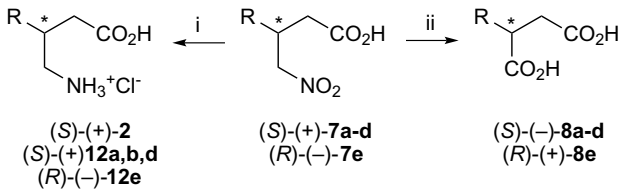
Enantiomers (*S*)-(+)-**2** and (*S*)-(+)-**12a**,**b**,**d**, were obtained when the reduction of the nitro group was carried out directly on the γ-nitro acids (+)-**7a**–**d** and (−)-**7e** (Scheme 4, left-hand side).

Moreover, the Nef reaction occurring on (*S*)-(+)-**7d** with the formation of the corresponding β-neopentylsuccinic acid (*S*)-(−)-**8d**, which to the best of our knowledge is unknown in the literature, prompted us to convert the optically active γ-nitro esters and γ-nitro acids into the



(*R*)-(−)-**6a-e** (*R*)-(+)-**11a,b,c** (*R*)-(−)-**11d,e** (*R*)-(−)-**12a,c,d,e**

Scheme 3. Reagents and conditions: (i) Ra-Ni, H_2 1 atm, EtOH/AcOEt, rt; (ii) refluxing 6 M HCl, overnight.



Scheme 4. Reagents and conditions: (i) Ra-Ni, H_2, 1 atm, EtOH/HCl, rt, overnight; (ii) refluxing AcOH/HCl 2 h (spontaneous in the case of **8d**).

corresponding 2-alkylsuccinic acids **8a**,**b**,**c**,**e**. These compounds have been reported in their enantiomerically pure forms since 1960[26] and their chiroptical and conformational properties have been studied extensively.[27] These are synthetic intermediates used for obtaining other important homochiral building blocks such as β-lactams, β-and γ-lactones. These are also important chiral subunits of pseudopeptides, which have been proven to be effective inhibitors of various zinc-enzymes,[28] and to have different biological activities. For instance, pseudopeptide Actinonin,[29] which possesses the α-pentylsuccinoyl moiety, displays an antibiotic activity in vitro against gram-positive and gram-negative bacteria. (*R*)-(+)-2-Benzylsuccinic **8e** acid has been shown to be an inhibitor of carboxypeptidase A[30] and thermolysin,[31] while its enantiomer (*S*)-(−)-**8e** has been recently used as a key synthon for the novel hypoglycemic agent KAD-1229.[32]

Different synthetic methodologies, directed towards the synthesis of chiral non-racemic 2-alkylsuccinic acids have been developed, most of which have been accomplished by asymmetric hydrogenation of substituted itaconic acid derivatives,[33] by resolution with chiral amines,[34] or by enzymes.[35] A number of patents have also appeared on the 2-benzyl substituted derivative **8e**, thus underlining the industrial interest for these latter compounds.[36]

2.3. Determination of the absolute configuration of the resolved compounds

In order to assign the absolute configuration to the resolved nitro esters (−)-**6a**–**d** and (+)-**6e**, and consequently to their respective nitro acids (+)-**7a**–**d** and (−)-**7e**, as well as to all the compounds derived from their transformations, chiroptical and chemical correlation methods were used.

The absolute configuration of (*S*)-(+)-5-methyl-3-(nitromethyl)hexanoic acid **7c**,[19b] directly correlated with (*S*)-(+)-Pregabalin **2**,[9] was assigned by comparison with the data reported in the literature.[19b] Consequently, the opposite (*R*)-configuration was assigned to the γ-nitro ester (−)-**6c**, recovered from the enzymatic hydrolysis. This compound is the precursor of (*R*)-(+)-4-isobutyl-γ-lactam **11c**,[37] which is also a compound described in the literature.

The specific rotation value observed for (+)-**2**, derived from (*S*)-(+)-**7c**, was in full agreement with the assignment.

The CD spectra of the other enantiomerically pure lactam derivatives (+)-**11a**,**b**,**d** were superimposable with those of

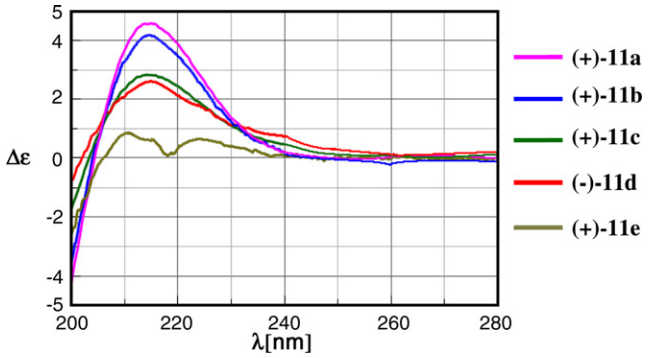


Figure 2. CD spectra of the optically active γ-lactams **11a**–**e**.

(+)-**11c**, all showing the same positive Cotton effect associated with the n–π* transition (215 nm) of the lactam group (Fig. 2). Consequently, the (*R*)-configuration was also assigned to (+)-**11a**,**b**,**d** and to their γ-nitro ester precursors (−)-**6a**,**b**,**d**, while the opposite (*S*)-configuration was attributed to the γ-nitro acids (+)-**7a**,**b**,**d** and therefore to the γ-aminoacids (+)-**12a**,**b**,**d** derived from them by the reduction of the nitro group.

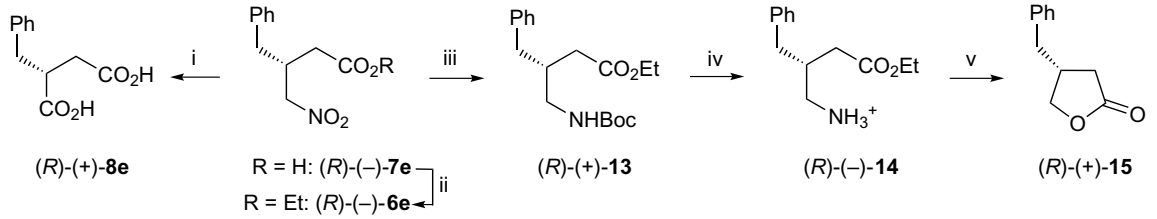
Further evidence supporting this assignment comes from the comparison of the specific rotation values of succinic acids (−)-**8a**,**b**,**c** derived from acidic treatment of the nitro acids (+)-**7a**,**b**,**c**, with those reported in the literature.[38,45]

β-Benzyl-γ-lactam (−)-**11e**, obtained from the nitro acid (−)-**7e** via esterification, reduction of the nitro group and cyclization, is already known in the literature in its optically active form. However, a comparison of the specific rotation values was not useful, because Enders[39] reported the (*S*)-absolute configuration for the dextrorotatory enantiomer, whereas Wee[40] recently assigned the opposite (*R*)-configuration to the same dextrorotatory antipode.

Since the CD spectrum of (−)-**11e** was not comparable with the spectra of the other γ-lactams (−)-**11a**–**d** because of the presence of an aromatic ring, we determined the absolute configuration of the β-benzyl-γ-nitro acid (−)-**7e** (94% ee), by chemical correlation, as described in Scheme 5.

A sample of (−)-**7e** was esterified into (−)-**6e**; this latter compound was reduced with hydrogen over Ra-Ni, in the presence of an equimolar amount of di-*tert*-butyl dicarbonate, affording the *N*-Boc-protected amino ester (*R*)-(+)-**13**. Deprotection of the amino group with 2 M HCl gave the free amino ester (*R*)-(−)-**14**, isolated as its hydrochloride salt. Its nitrosation furnished directly the known β-benzyl-γ-lactone (*R*)-(+)-**15**,[41] thus allowing the (*R*)-configuration to be assigned to the starting γ-nitro acid (−)-**7e**.

This assignment, which is in accordance with that proposed by Enders,[39] was confirmed by the correlation established between (−)-**7e** and the already described succinic acid derivative (*R*)-(+)-**8e**.[35,42]



Scheme 5. Reagents and conditions: (i) HCl/AcOH, Δ; (ii) EtOH,$(CH_3)_3SiCl$; (iii) H_2, Ra-Ni, 1 atm, Boc_2O, EtOH; (iv) 2 M HCl; (v) 1 M $NaNO_2$, H_2O.

3. Conclusion

In conclusion, an easy and short enantioselective synthesis of a series of potentially useful β-susbstituted GABA analogues has been developed, starting from the readily available racemic γ-nitro ester precursors **6**, through their enzymatic kinetic resolution, in yields ranging from 10% to 24% in isolated products. It is important to note that the application of this procedure to the substrate carrying the isobutyl group, allowed us to obtain therapeutically useful compound (*S*)-(+)-Pregabalin **2**, as well as its enantiomer. This procedure gives also access to optically active 2-alkylsuccinic acids. A study of the absolute configuration of all these compounds has also been presented.

4. Experimental

4.1. General

IR spectra were recorded on a Jasco FT-IR 200 spectrometer. ^1H NMR and ^{13}C NMR spectra were run on a Jeol EX-400 (400 MHz for proton, 100.1 MHz for carbon), using deuteriochloroform as the solvent and tetramethylsilane as the internal standard. Optical rotations were determined on a Perkin Elmer Model 241 polarimeter, at 25 °C. MS spectra were performed on an ion trap Finnigan MAT95XP spectrometer (70 eV). HRMS were run on a Finnigan MAT95XP spectrometer. ESI-MS were run on a Bruker Esquire 4000 instrument. Enzymatic hydrolyses were performed using a pH-stat Controller PHM290 Radiometer, Copenhagen. Chiral HRGC 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 He, 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 the mixtures of light petroleum 40–70 °C and ethyl acetate as the eluent.

Novozym 345 (CalB) was purchased from Novo Nordisk Bioindustrial A/S, Denmark. α-Chymotrypsin was purchased from Fluka.

4.2. General procedure for the synthesis of the α,β-unsaturated esters 5a–e[43]

To a solution of the appropriate aldehydes **4a**–**e** (36 mmol) and triethyl phosphonoacetate (30 mmol) in anhydrous THF, *t*-BuOK (30 mmol) was added portionwise and the mixture was heated at gentle reflux for 20 min. After cooling to rt, the organic solution was washed with 5% HCl, then with brine, dried over Na_2SO_4 and evaporated to dryness. The oily residue was distilled in vacuo.

4.2.1. Ethyl 2-heptenoate 5a.[20] Compound **5a** was prepared from pentanal **4a**, and obtained as a colourless oil, 70% yield, after distillation, bp 55 °C (0.5 mm Hg). IR (neat) 1723 (CO_2Et), 1655 (C=C) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 6.96 (dt, $J_{trans} = 15.6$, $^3J = 7.1$ Hz, 1H, H-3), 5.80 (dt, $J_{trans} = 15.6$, $^4J = 1.0$ Hz, 1H, H-2), 4.17 (q, 2H, CH_3CH_2O), 2.20 (m, 2H, H-4), 1.4 (m, 4H), 1.18 (t, 3H, CH_3CH_2O), 0.9 (t, 3H, CH_3CH_2). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 166.8 (s, C-1), 149.4 (d, C-3), 121.2 (d, C-2), 60.05 (t, CH_2O), 31.8 (t), 30.8 (t), 22.1 (t), 14.2 (q), 13.8 (q). HRMS (EI) calcd for $C_9H_{16}O_2$ (M^+) 156.1150, found 156.1140. ESI-MS 157.1 ($M+H^+$), 169.0 ($M+Na^+$). MS, *m*/*z* 156 ($M^{+\cdot}$, 41%), 141 (28), 127 (16), 113 (18), 83 (100).

4.2.2. Ethyl 5-methyl-2-hexenoate 5b.[20] Compound **5b** was prepared from 3-methylbutanal **4b**, and obtained as a colourless oil, 80% yield, after distillation, bp 62 °C (0.08 mmHg). IR (neat) 1723 (CO_2Et), 1655 (C=C) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 6.94 (dt, $J_{trans} = 15.2$, $^3J = 7.3$, 1H, H-3), 5.77 (dt, $J_{trans} = 15.2$, $^4J = 1.3$ Hz, 1H, H-2), 4.15 (q, 2H, CH_3CH_2O), 2.06 (m, 2H, H-4), 1.73 (quint, $J = 6.6$ Hz, 1H, H-5), 1.24 (t, 3H, CH_3CH_2O), 0.89 (d, $J = 6.6$ Hz, 6H, $(CH_3)_2CH$). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 166.6 (s, C-1), 148.2 (d, C-3), 122.2 (d, C-2), 61.8 (t, CH_2O), 41.4 (t, C-4), 27.7 (d, C-5), 22.3 (2q, $(CH_3)_2CH$), 14.2 (q). HRMS (EI) calcd for $C_9H_{16}O_2$ (M^+) 156.1150, found: 156.1135. ESI-MS 179.0 ($M+Na^+$). MS, *m*/*z* 156.0 (11%), 141 (28), 113 (100), 111 (10).

4.2.3. Ethyl 2-octenoate 5c. Compound **5c** was prepared from hexanal **4c**, and obtained as a colourless oil, 81% yield, after distillation, bp 55 °C (0.03 mmHg). IR (neat), 1723 (CO_2Et), 1655 (C=C) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 6.94 (dt, $J_{trans} = 15.3$, $^3J = 7.3$, 1H, H-3), 5.77 (dt, $J_{trans} = 15.3$, $^4J = 0.8$ Hz, 1H, H-2), 4.16 (q, 2H, CH_3CH_2O), 2.16 (m, 2H, H-4), 1.42 (quint, $J = 7.3$ Hz, 2H), 1.28 (m and t, 7H, CH_3CH_2O and CH_2), 0.92 (t, 3H, CH_3CH_2). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 166.7 (s, CO), 149.4 (d, C-3), 121.2 (d, C-2), 60.1 (t, CH_2O), 32.1 (t, C-4), 31.3 (t), 27.7 (t), 22.4 (t), 14.3 (q), 14.1 (q). HRMS (EI) calcd for $C_{10}H_{18}O_2$ (M^+) 170.1307, found 170.1328. ESI-MS 171.1 ($M+H^+$), 193.0 ($M+Na^+$). MS, *m*/*z* 170 ($M^{+\cdot}$, 12%), 155 (24), 141 (41), 127 (26), 113 (18), 97 (100).

4.2.4. Ethyl 5,5-dimethyl-2-hexenoate 5d. Compound **5d** was prepared from 3,3-dimethylbutanal **4d**, and obtained as a colourless oil, 85% yield, after distillation, bp 60 °C (0.05 mmHg). IR (neat), 1721 (CO_2Et), 1653 (C=C) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 6.96 (dt, $J_{trans} = 15.2$, $^3J = 7.2$ Hz, 1H, H-3), 5.77 (dt, $J_{trans} = 15.2$, $^4J = 1.3$ Hz, 1H, H-2), 4.15 (q, 2H, CH_3CH_2O), 2.04 (dd, $^3J = 7.2$, $^4J = 1.3$ Hz, 2H, H-4), 1.24 (t, 3H, CH_3CH_2O), 0.92 (s, 9H, *t*-Bu). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 166.5 (CO), 146.8 (C-3), 123.2 (d, C-2), 60.1 (t, CH_2O), 46.6 (t, C-4), 29.3 (s, *t*-Bu), 14.2 (q, CH_3CH_2). ESI-MS 171.1 ($M+H^+$), 193.0 ($M+Na^+$). HRMS (EI) calcd for $C_{10}H_{18}O_2$ (M^+) 170.1307, found 170.1299. MS, *m/z* 170 ($M^{+\cdot}$, 32%), 155 (24), 141 (76), 97 (100).

4.2.5. Ethyl 4-phenyl-2-butenoate 5e. Compound **5e** was prepared from phenylethanal **4e**, and obtained as a colourless oil, 78% yield, after distillation, bp 83 °C (0.1 mmHg). IR (neat), 3062, 3028, 1603, 1496, 1453, 751, 699 (Ph), 1717 (CO_2Et), 1653 (C=C) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.31 (t, 2H, Ph), 7.24 (t, 1H, Ph), 7.17 (d, 2H, Ph), 7.10 (dt, $J_{trans} = 15.7$, $^3J = 7.0$ Hz, 1H, H-3), 5.81 (dt, $J_{trans} = 15.7$, $^4J = 1.5$ Hz, 1H, H-2), 4.17 (q, 2H, CH_3CH_2O), 3.52 (dd, $^3J_{3,4} = 7.0$, $^4J_{2,4} = 1.5$ Hz, 2H, H-4), 1.27 (t, 3H, CH_3CH_2O). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 166.4 (s), 147.2 (d, C-3), 137.6 (s), 128.7, 128.6, 126.6 (d, Ph), 122.3 (d, C-2), 60.2 (t), 38.4 (t), 14.1 (q). ESI-MS 191.0 (MH^+). HRMS (EI) calcd for $C_{12}H_{14}O_2$ (M^+) 190.0994, found 190.0980. MS, *m/z* 190 ($M^{+\cdot}$, 32%), 175 (24), 144 (56), 117 (86), 115 (100).

4.3. General procedure for the synthesis of racemic γ-nitro esters 6a–e[20]

To a solution of the α,β-unsaturated ester (100 mmol) in nitromethane (25 mL), DBU (100 mmol) was added dropwise and the solution was left stirring overnight at room temperature. The nitromethane in excess was removed in vacuo, after which the residue was dissolved in diethyl ether and washed with 5% HCl; then the organic phase was dried over Na_2SO_4 and evaporated to give a dark brown oil which was purified by distillation or chromatographed on SiO_2 (eluent: light petroleum/ethyl acetate 99:1).

4.3.1. Ethyl (±)-3-nitromethylheptanoate 6a. Colourless oil, 72% yield, bp 100 °C (0.2 mmHg) [lit.[20] 124–126 °C (1.25 mmHg)]. IR (neat) 1732 (CO_2Et), 1552, 1378 (NO_2) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 4.51 (dd, $J_1 = 11.9$, $J_2 = 6.2$ Hz, 1H, CH_2NO_2), 4.43 (dd, $J_1 = 11.9$, $J_2 = 5.9$ Hz, 1H, CH_2NO_2), 4.14 (q, 2H, CH_3CH_2O), 2.62 (sept, $J = 6.6$ Hz, 1 H, H-3), 2.43 (apparent d, 2H, H-2), 1.41 (m, 2H), 1.33 (m, 4H), 1.25 (t, 3H, CH_3CH_2O), 0.90 (t, 3H, CH_3CH_2). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 171.5 (s), 78.5 (t, CH_2NO_2), 60.6 (t, CH_2O), 35.9 (t, C-2), 34.1 (d, C-3), 31.0 (t), 28.4 (t), 22.4 (t), 14.1 (q), 13.7 (q). HRMS (EI) calcd for $C_{10}H_{18}O_2$ ($M-HNO_2$) 170.1307, found 170.1312. ESI-MS 240.0 ($M+Na^+$). MS, *m/z* 217 ($M^{+\cdot}$, 12%), 170 ($M-HNO_2$, 81), 169 (100), 155 (63).

4.3.2. Ethyl (±)-5-methyl-3-nitromethylhexanoate 6b. Colourless oil, 75% yield, bp 110 °C (0.09 mmHg) [lit.[20] 103–104 °C (0.75 mmHg)]. IR (neat) 1731 (CO_2Et), 1550, 1370 (NO_2) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 4.49 (dd, $J_1 = 13.1$, $J_2 = 7.6$ Hz, 1H, CH_2NO_2), 4.42 (dd, $J_1 = 13.1$, $J_2 = 5.9$ Hz, 1H, CH_2NO_2), 4.14 (q, 2H, CH_3CH_2O); 2.67 (sept, $J = 6.6$ Hz, 1H, H-3), 2.41 (apparent d, 2H, H-2), 1.65 (sept, $J = 7.0$ Hz, $CH(CH_3)_2$), 1.24 (t + m, 5H, CH_3CH_2O and H-4), 0.92, 0.90 (2d, $J = 7.3$ Hz, 6H, $(CH_3)_2CH$). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 171.4 (s), 78.7 (t, CH_2NO_2), 60.7 (t, CH_2O), 40.5 (t), 36.0 (t), 32.1 (d, C-3), 25.0 (d, C-5), 22.5 (q), 22.2 (q), 14.1 (q). HRMS (EI) calcd for $C_{10}H_{18}O_2$ ($M-HNO_2$)$^{+\cdot}$ 170.1384, found 170.1397. MS-EI 188 (M−29, 100%), 170 ($M-HNO_2$)$^{+\cdot}$, 82), 160 (42), 144 (78), 114 (70), 72 (90), 46 (58).

4.3.3. Ethyl (±)-3-nitromethyloctanoate 6c. Colourless oil, 69% yield, bp 110 °C (0.1 mmHg). IR (neat) 1733 (CO_2Et), 1552, 1378 (NO_2) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 4.49 (dd, $J_1 = 12.1$, $J_2 = 6.2$ Hz, 1H, CH_2NO_2), 4.42 (dd, $J_1 = 12.1$, $J_2 = 7.2$ Hz, 1H, CH_2NO_2), 4.13 (q, 2H, CH_3CH_2O), 2.60 (sept, $J = 6.6$ Hz, 1H, H-3), 2.41 (apparent d, 2H, H-2), 1.34 (m, 2H), 1.33–1.24 (m + t, 9H, $CH_2 + CH_3CH_2O$), 0.85 (t, 3H, CH_3CH_2). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 171.5 (s), 78.5 (t, CH_2NO_2), 60.7 (t, CH_2O), 35.8 (t, C-2), 34.2 (C-3, d), 31.5 (t), 31.3 (t), 26.0 (t), 22.4 (t), 14.1 (q), 13.9 (q). ESI-MS 208.0 (M+1). HRMS (EI) calcd for $C_{11}H_{21}O_2$ ($M-NO_2$) 185.1542, found 185.1530. MS, *m/z* 231 (M+, 40%), 216 (12), 185 ($M-NO_2$, 27), 169 (41), 139 (34), 111 (45), 71 (100).

4.3.4. Ethyl (±)-5,5-dimethyl-3-nitromethylhexanoate (6d). Colourless oil, 65% yield, after flash chromatography (eluent: light petroleum/ethyl acetate 98:2). IR (neat) 1733 (CO_2Et), 1552, 1379 (NO_2) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 4.49 (dd, $J_1 = 12.4$, $J_2 = 7.0$ Hz, 1H, CH_2NO_2), 4.25 (dd, $J_1 = 12.4$, $J_2 = 5.9$ Hz, 1H, CH_2NO_2), 4.12 (q, 2H, CH_3CH_2O), 2.64 (sept, $J = 6.0$ Hz, 1H, H-3), 2.45 (apparent d, 2H, H-2), 1.29 (part AB of an ABX system, $J_{AB} = 12.4$, $J_{AX} = 5.1$, $J_{BX} = 4.7$ Hz, 2H, H-4), 1.25 (t, 3H, CH_3CH_2O), 0.92 (s, 9H, *t*-Bu). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 171.4 (s, CO_2Et), 79.8 (t, CH_2NO_2), 60.7 (t, CH_2O), 44.6 (t), 38.0 (t), 30.9 (s), 30.8 (d, C-3), 29.4 (q, *t*-Bu), 14.1 (q, CH_3CH_2). ESI-MS 254.0 ($M+Na^+$). HRMS (EI) calcd for $C_{11}H_{21}NO_4$ ($M^{+\cdot}$) 231.1471, found 231.1490. MS, *m/z* 231 ($M^{+\cdot}$, 36%), 197 (82), 184 ($M-HNO_2$, 71), 169 (100), 154 (65).

4.3.5. Ethyl (±)-3-nitromethyl-4-phenylbutanoate 6e. Colourless oil, 70% yield, after distillation, bp 125 °C (0.2 mmHg). IR (neat) 3064, 3029, 1603, 1496, 750, 702 (Ph), 1733 (CO_2Et), 1552, 1379 (NO_2) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 2H, Ph), 7.25 (t, 1H, Ph), 7.19 (d, 2H, Ph), 4.47 (dd, $J_1 = 12.8$, $J_2 = 6.6$ Hz, 1H, CH_2NO_2), 4.42 (dd, $J_1 = 12.8$, $J_2 = 5.8$, 1H, CH_2NO_2), 4.15 (q, CH_3CH_2O), 2.92 (sept, $J = 6.6$ Hz, 1H, H-3), 2.75 (apparent d, 2H, CH_2Ph), 2.43 (part AB of an ABX system, $J_{AB} = 16.8$, $J_{AX} = 11.4$, $J_{BX} = 7.0$ Hz, 2H, H-2), 1.26 (t, 3H, CH_3CH_2O). ^{13}C NMR (100.1 MHz, $CDCl_3$), δ 171.4 (s, CO), 137.7 (s, Ph), 129.3, 128.8, 127.0 (d, Ph), 77.5 (t, CH_2NO_2), 60.9 (t, CH_2O), 37.5 (t, C-2), 36.1 (d, C-3), 35.4 (t, CH_2Ph), 14.2 (q). HRMS (EI) calcd for $C_{13}H_{17}NO_4$ ($M^{+\cdot}$) 251.1158, found 251.1140. ESI-MS 252.1 (MH^+), 274.0 ($M+Na^+$). MS, *m/z* 251 ($M^{+\cdot}$, 12%),

204 (M−HNO_2, 10), 145 (81), 129 (63), 117 (100), 115 (65), 105 (18), 91 (69).

4.4. General procedure for enzymatic hydrolyses

A suspension of the appropriate γ-nitro esters **6a**–**d** (1.0 g), in 0.1 M KH_2PO_4/Na_3PO_4 buffer (pH 7.4) was hydrolyzed with Novozym 435 (0.18 g), at room temperature under vigorous stirring. The pH was kept at the initial value by the continuous addition of 1 M NaOH. At the desired conversion value, the unreacted γ-nitro ester was extracted from the suspension with ethyl acetate (3 × 10 mL) using a centrifuge for the separation of the layers. For the isolation of the γ-nitro acids **7a**–**d**, the aqueous layer was acidified to pH 2 with 2 M HCl and extracted with $CHCl_3$ (3 × 10 mL).

In the case of the nitro ester **6e**, hydrolysis was performed with α-chymotrypsin (0.12 g/mmol substrate), following the above procedure.

4.4.1. Ethyl (*R*)-(−)-3-nitromethylheptanoate 6a. Compound **6a** was obtained in 99.9% ee by stopping the enzymatic hydrolysis at 76% conversion (21% yield), $[\alpha]_D = -7.0$ (*c* 0.35, $CHCl_3$).

4.4.2. (*S*)-(+)-3-Nitromethylheptanoic acid 7a. Compound **7a** was obtained in 87% ee by stopping the enzymatic hydrolysis at 18% conversion, (18% yield), $[\alpha]_D = +9.2$ (*c* 0.5, $CHCl_3$). IR (neat) 3400–2400 (br, OH), 1711 (CO), 1551, 1381 (NO_2) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 4.46 (dd, $J_1 = 12.2$, $J_2 = 6.2$ Hz, 1H, CH_2NO_2), 4.39 (dd, $J_1 = 12.2$, $J_2 = 5.8$ Hz, 1H, CH_2NO_2), 2.61 (sept, $J =$ 6.2 Hz, 1H, H-3), 2.53 (apparent d, 2H, H-2), 1.50–1.20 (m, 6H), 0.87 (t, 3H, CH_3CH_2). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 177.2 (s, CO), 78.3 (t, CH_2NO_2), 35.4 (t, C-2), 33.7 (d, C-3), 30.8 (t), 28.3 (t), 22.3 (t), 22.1 (t), 13.6 (q). HRMS (EI) calcd for $C_8H_{15}NO_4$ ($M^{+\cdot}$) 189.0001, found: 189.0009. ESI-MS 212.0 ($M+Na^+$). MS, *m*/*z* 190 ($MH^{+\cdot}$, 10%), 171 ($M^{+\cdot}-H_2O$, 41), 160 (100), 146 (13), 142 [M−HNO_2, 28].

4.4.3. Ethyl (*R*)-(−)-5-methyl-3-nitromethylhexanoate 6b. Compound **6b** was obtained with >99% ee by stopping the enzymatic hydrolysis at 64% conversion (30% yield), $[\alpha]_D = -6.5$ (*c* 0.95, $CHCl_3$).

4.4.4. (*S*)-(+)-5-Methyl-3-nitromethylhexanoic acid 7b. Compound **7b** was obtained with 92% ee by stopping the enzymatic hydrolysis at 25% conversion (21% yield), $[\alpha]_D = +6.0$ (*c* 1.0, MeOH) [lit.[19b] $= +6.3$ (*c* 0.7, MeOH)], $[\alpha]_D = +8.9$ (*c* 0.7, $CHCl_3$). IR (neat) 3500–2500 (br, OH), 1710 (CO), 1550, 1380 (NO_2) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 4.50 (dd, $J_1 = 12.4$, $J_2 = 6.6$ Hz, 1H, CH_2NO_2), 4.44 (dd, $J_1 = 12.4$, $J_2 = 5.9$ Hz, 1H, CH_2NO_2), 2.67 (sept, $J = 6.5$ Hz, 1H, H-3), 2.49 (apparent d, 2H, H-2), 1.66 (sept, $J = 7.0$ Hz, 1H, $CH(CH_3)_2$), 1.28 (m, 2H, H-4), 0.93, 0.91 (2d, $J = 7.0$, $(CH_3)_2CH$). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 177.6 (s, CO), 78.4 (t, CH_2NO_2), 40.3 (t), 35.6 (t), 31.7 (d, C-3), 24.9 (d, C-5), 22.3 (q), 22.0 (q). HRMS (EI) calcd for $C_8H_{15}NO_4$ ($M^{+\cdot}$) 189.0001, found 189.0009. ESI-MS 212.0 ($M+Na^+$). MS, *m*/*z* 189 ($M^{+\cdot}$, 12%), 144 (17), 142 (M−HNO_2, 10), 118 (100), 72 (61), 46 (70).

4.4.5. Ethyl (*R*)-(−)-3-nitromethyloctanoate 6c. Compound **6c** was obtained with >99% ee by stopping the enzymatic hydrolysis at 63% conversion (32% yield), $[\alpha]_D = -8.8$ (*c* 0.25, $CHCl_3$).

4.4.6. (*S*)-(+)-3-Nitromethyloctanoic acid 7c. Compound **7c** was obtained with 94% ee by stopping the enzymatic hydrolysis at 29% conversion (23% yield), $[\alpha]_D = +11.4$ (*c* 0.5, $CHCl_3$). IR (neat) 3400–2400 (br, OH), 1710 (CO), 1552, 1381 (NO_2) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 4.48 (dd, $J_1 = 12.1$, $J_2 = 6.2$ Hz, 1H, CH_2NO_2), 4.39 (dd, $J_1 = 12.1$, $J_2 = 5.8$ Hz, 1H, CH_2NO_2), 2.61 (sept, $J =$ 6.1 Hz, 1H, H-3), 2.51 (apparent d, 2H, H-2), 1.49–1.20 (m, 8H), 0.85 (t, 3H, CH_3CH_2). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 177.2 (s, CO), 78.3 (t, CH_2NO_2), 35.5 (t, C-2), 33.9 (d, C-3), 31.4 (t), 31.2 (t), 26.0 (t), 22.3 (t), 13.9 (q). HRMS (EI) calcd for $C_9H_{17}NO_4$ ($M^{+\cdot}$) 203.1158, found 203.1152. ESI-MS 226 [$M+Na^+$]. MS, *m*/*z* 203 ($M^{+\cdot}$, 1%), 195 ($M^{+\cdot}-H_2O$, 11), 188 (43), 176 (100), 156 (M−HNO_2, 10), 146, (13).

4.4.7. Ethyl (*R*)-(−)-5,5-dimethyl-3-nitromethylhexanoate 6d. Compound **6d** was obtained with 95% ee by stopping the enzymatic hydrolysis at 75% conversion (22% yield), $[\alpha]_D = -6.8$ (0.5, $CHCl_3$).

4.4.8. (*S*)-(+)-5,5-Dimethyl-3-nitromethylhexanoic acid 7d. Compound **7d** was obtained with 65% ee by stopping the enzymatic hydrolysis at 23% conversion (18% yield), $[\alpha]_D = +4.0$ (*c* 0.1, $CHCl_3$). IR (neat) 3450–3200 (br, COOH), 1712 (COOH), 1551, 1378 (NO_2) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 4.43 (part AB of an ABX system, $J_{AB} = 12.2$, $J_{AX} = 6.3$, $J_{BX} = 5.7$ Hz, 2H, CH_2NO_2), 2.62 (sept, $J = 6.1$ Hz, 1H, H-3), 2.51 (apparent d, 2H, H-2), 1.27 (apparent d, 2H, H-4), 0.95 (s, 9H, *t*-Bu). HRMS (EI) calcd for $C_9H_{17}NO_4$ ($M^{+\cdot}$) 203.1158, found 203.1160. ESI-MS 204.0 (MH^+), 226.1 ($M+Na^+$). MS, *m*/*z* 189 ($M^{+\cdot}$, 12%), 144 (17), 142 (M−HNO_2, 10), 118 (100), 72 (61), 46 (70). When standing at room temperature for 24 h, this compound completely converted into the succinic acid derivative (*S*)-(−)-**8d**. This latter compound was also found in the acidic fraction, at 75% conversion.

4.4.9. Ethyl (*S*)-(+)-3-nitromethyl-4-phenylbutanoate 6e. Compound **6e** was obtained with 42% ee $[\alpha]_D = +3.3$ (*c* 0.5, $CHCl_3$). The enzymatic hydrolysis stopped spontaneously at 36% conversion.

A sample of the opposite enantiomer (*R*)-(−)-**6e** with 94% ee was obtained by the esterification of the nitro acid (*R*)-(−)-**7e** isolated at 21% conversion (18% yield), $[\alpha]_D = -6.1$ (*c* 0.75, $CHCl_3$).

4.4.10. (*R*)-(−)-3-Nitromethyl-4-phenylbutanoic acid 7e. Compound **7e** was obtained with 94% ee by stopping the enzymatic hydrolysis at 21% conversion (18% yield), $[\alpha]_D = -11.0$ (*c* 0.7, $CHCl_3$). IR (neat) 3700–2500 (br, COOH), 3028, 1603, 1496, 1454, 750, 702 (Ph), 1711 (COOH), 1551, 1381 (NO_2) cm^{-1}. ^1H NMR (400 MHz,

$CDCl_3$) δ 9.9 (br, 1H, COOH), 7.24 (m, 2H), 7.20 (t, 1H), 7.12 (d, 2H, Ph), 4.38 (part AB of an ABX system, $J_{AB} = 12.8$ Hz, 2H, CH_2NO_2), 2.84 (sept, $J = 6.6$ Hz, 1H, H-3), 2.74 (apparent d, 2H, CH_2Ph), 2.51 (apparent d, 2H, H-2). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 177.4 (s), 137.3 (s), 129.2 (d), 128.8 (d), 127.0 (d), 77.4 (t), 37.3 (t), 35.7 (d), 34.9 (t). HRMS (EI) calcd for $C_{11}H_{13}NO_4$ ($M^{+\cdot}$) 223.0845, found: 223.0960. ESI-MS 246.0 (M+Na^+). MS, *m*/*z* 223 ($M^{+\cdot}$, 11%), 205 (M−H_2O, 18), 176 ($M^{+\cdot}$−HNO_2, 100), 146 (71), 132 (65), 118 (12), 91 (69).

4.5. Determination of the enantiomeric excess of compound (−)-7e

To a suspension of the acid (*R*)-(−)-**7e** (0.05 g, 0.22 mmol) in 5 mL of CH_2Cl_2, 1-hydroxybenzotriazole (HOBt), (0.034 g, 0.22 mmol), then EDC·HCl (0.043 g, 0.22 mmol) were added in succession under stirring. After 10 min at room temperature, (*S*)-(+)-1-phenylethylamine (0.05 g, 0.4 mmol) was added and the solution stirred for a further 2 h. The solution was washed with 1 M HCl, and after drying the organic phase, the solvent was evaporated to give the corresponding amide (+)-**9** in a quantitative yield. Mp 112–114 °C. $[\alpha]_D = +24.9$ (*c* 0.22, $CHCl_3$). IR, cm^{-1} (Nujol): 3310 (NH), 3066 (Ph), 1640 (CONH), 1546, 1378 (NO_2), 1496 (Ph) cm^{-1}. ^1H NMR (400 MHz, C_6D_6) δ 7.24 (m, 5H, ArH), 5.25, 5.38 (quint, 1H, CHPh), 5.11 (br s, 1H, NH), 4.09 (part AB of an ABX system, $J_{AX} = 5.9$, $J_{BX} = 5.2$, $J_{AB} = 12.1$ Hz, 2H, CH_2NO_2), 2.82 (quint, 1H, H-3), 2.59 (part AB of an ABX system, $J_{AX} = 8.4$, $J_{BX} = 7.0$, $J_{AB} = 13.9$ Hz, 2H, H-2), 1.80 (apparent d, 2H, CH_2Ph), 1.26 (d, 3H, $J = 7.0$ Hz, CH_3CHPh). ^{13}C NMR (100.1 MHz, C_6D_6) δ 168.8 (s), 143.7 (s), 138.7 (s), 129.6–126.4 (6d), 77.8 (t), 48.8 (d), 37.3 (t), 36.7 (d), 36.6 (t), 21.5 (d). ESI-MS 339.1 (M+H^+), 362.2 (M+Na^+), 378.6 (M+K^+).

When the coupling reaction was carried out on a racemic mixture of the nitro acid **7e**, a 1:1 mixture of the corresponding diastereomeric amides **9** and **10** was obtained. From the ^1H NMR of the mixture (400 MHz, C_6D_6), the following signals of (−)-**10** could be identified: δ 4.18 (dd, part A of an ABX system, $J_{AB} = 12.1$, $J_{AX} = 5.9$ Hz, 1 H, CH_2NO_2), 4.02 (dd, part B of an ABX system, $J_{AB} = 12.1$, $J_{BX} = 5.1$ Hz, 1H, CH_2NO_2), 2.59 (part AB of an ABX system, $J_{AB} = 13.9$, $J_{AX} = 8.4$, $J_{BX} = 7.0$ Hz, 2H, H-2), 1.22 (d, $J = 7.0$ Hz, 3H, CH_3CHPh).

The integration of the nitromethylene proton signals of the mixture (+)-**9** and (−)-**10**, derived from the sample of (*R*)-(−)-**7e** isolated from the enzymatic hydrolysis, furnished the diastereomeric excess of (+)-**9** and hence the enantiomeric excess of (−)-**7e**, which was 94%.

4.6. General procedure for the transformation of the nitro esters (−)-6a–e to the corresponding γ-lactams (+)-11a–e

The nitro esters (−)-**6a**–**d** (2.0 mmol) were dissolved in a 1:1 ethyl acetate/ethanol solution (10 mL), and Ra-Ni (Aldrich) was added. The mixture was hydrogenated at atmospheric pressure until the disappearance of the starting material (TLC, eluent: ethyl acetate). The catalyst was filtered off on a pad of Celite and the solvent was removed in vacuo. The residue was dissolved in toluene and the solution was heated at reflux to assure complete cyclization. After the removal of the solvent, the residue was chromatographed on column (eluent: ethyl acetate), to give the pure γ-lactams (+)-**11a**–**d**. A sample of (+)-**11e** having 98% ee was obtained from the γ-nitro acid (−)-**7e**, (94% ee), isolated from the hydrolysis at 21% conversion, by esterification in EtOH and $(CH_3)_3SiCl$,[25] and subsequent reduction of the nitro group.

4.6.1. (*R*)-(+)-4-Butyl-2-pyrrolidinone 11a. Compound **11a** was obtained as a colourless oil, in 70% yield, after purification by flash chromatography, $[\alpha]_D = +4.0$ (*c* 0.25, $CHCl_3$). IR (neat) 3229 (NH), 1697 (CO) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 3.49 (dd, $J_1 = 9.1$, $J_2 = 6.6$ Hz, 1H, H-5), 3.01 (dd, $J_1 = 9.2$, $J_2 =$ d 7.2, 1H H-5), 2.46 (m, 1H, H-4), 2.02 (m, 2H, H-3 and H-4), 1.45 (m, 2H), 1.23 (m, 4H), 0.86 (t, CH_3CH_2). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 178.7 (s), 48.2 (t), 36.9 (t), 35.00 (t), 34.4 (d), 29.7 (t), 22.7 (t), 14.1 (q). MS-ESI 142 (MH^+), 164 (M+Na^+). MS, *m*/*z* 141 ($M^{+\cdot}$, 26%), 126 (13), 111 (23), 98 (18), 84 (73), 56 (100).

4.6.2. (*R*)-(+)-4-Isobutyl-2-pyrrolidinone 11b. Compound **11b** was isolated in 81% yield, $[\alpha]_D = +2.1$ (*c* 0.5, $CHCl_3$) [lit.[37] +2.1 (*c* 1.08, $CHCl_3$)]. Spectroscopic and analytical data are fully in agreement with the literature.[37]

4.6.3. (*R*)-(+)-4-Pentyl-2-pyrrolidinone 11c. Compound **11c** was obtained as a colourless oil in 72% yield, after purification by column chromatography, $[\alpha]_D = +3.1$ (*c* 0.85, $CHCl_3$). IR (neat) 3225 (NH), 1698 (CO) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 3.49 (dd, $J_1 = 9.1$, $J_2 = 6.6$ Hz, 1H, H-5), 3.01 (dd, $J_1 = 9.2$, $J_2 = 7.2$ Hz, 1H H-5), 2.46 (m, 1H, H-4), 2.02 (m, 2H, H-3 and H-4), 1.45 (m, 2H), 1.23 (m, 4H), 0.86 (t, CH_3CH$_2$). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 178.5 (s), 48.1 (t), 36.7 (t), 35.0 (d), 34.6 (t), 31.7 (t), 27.1 (t), 22.5 (t), 14.0 (q). IR (neat) 3233 (NH), 1698 (CO) cm^{-1}. ESI-MS 156.1 (MH^+), 178.0 (M+Na^+). MS, *m*/*z* 155 ($M^{+\cdot}$, 34%), 140 (100), 126 (28), 109 (84), 95 (55), 84 (100), 71 (67), 57 (94).

4.6.4. (*R*)-(−)-4-Neopentyl-2-pyrrolidinone 11d. Compound **11d** was isolated as a white solid in 76% yield, mp 73–75 °C, $[\alpha]_D = -7.2$ (*c* 0.75, $CHCl_3$). IR (Nujol) 3195 (NH), 1703 (CO) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.02 (s, 1H, NH), 3.45 (dd, $J_1 = 8.8$, $J_2 = 8.4$ Hz, 1H, H-5), 2.96 (dd, $J_1 = 9.1$, $J_2 = 8.8$ Hz, 1H, H-5), 2.49 (sept, $J = 6.6$ Hz, 1H, H-4), 2.38 (dd, $J_1 = 8.8$, $J_2 = 16.5$ Hz, 1H, H-3), 1.96 (dd, $J_1 = 9.5$, $J_2 = 16.5$ Hz, 1H, H-3), 1.36 (d, $J = 5.9$ Hz, *t*-BuCH_2), 0.87 (s, 9H, *t*-Bu). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 178.5 (s), 49.7 (t), 48.9 (t), 32.1 (d), 30.8 (s), 29.8 (q). MS-ESI 178 (M+Na^+), 156 (M+H^+). MS, *m*/*z* 155 ($M^{+\cdot}$, 24%), 140 (10), 125 (28), 109 (54), 95 (55), 86 (100).

4.6.5. (*R*)-(−)-4-Benzyl-2-pyrrolidinone[39] 11e. Compound **11e** was obtained by the esterification of the nitro acid (*R*)-(−)-**7e** having 94% ee, followed by the reduction of the nitro group and cyclization, 69% yield, mp 102–103 °C, $[\alpha]_D = -5.1$ (*c* 0.5, MeOH), {lit.[39] for the (*S*)-enan-

tiomer: +5.4 (*c* 1.0, MeOH), 95% ee}. IR (Nujol) 3224 (NH), 1697 (CO), 1601, 1494, 1455 (Ph) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.29 (m, 2H), 7.22 (t, 1H), 7.15 (d, 2H, Ph), 6.5 (br, 1H, NH), 3.40 (dd, $J_1 = 9.1$, $J_2 = 6.3$ Hz, 1H, H-5), 3.11 (dd, $J_1 = 9.1$, $J_2 = 5.1$ Hz, 1H, H-5), 2.82–2.72 (m, 3H, H-4 and CH_2Ph), 2.41 (dd, $J_1 = 16.5$, $J_2 = 8.0$ Hz, 1H, H-3), 2.12 (dd, $J_1 = 16.5$, $J_2 = 6.6$ Hz, 1H, H-3). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 178.1 (s), 139.2 (s), 128.7 (2d), 128.5 (2d), 126.4 (d), 47.4 (t), 40.3 (t), 36.3 (t), 36.25 (d). MS-ESI 176.1 ($M+H^+$), 198.0 ($M+Na^+$). MS, *m*/*z*: 175 ($M^{+\cdot}$, 48%), 147 (31), 117 (38), 91 (100), 88 (46), 84 (73), 71 (18), 65 (23), 57 (26).

4.7. Transformation of the nitro acids 7a–e into the corresponding γ-amino acid hydrochlorides 12a–e

The γ-amino acids **12a**–**e** were obtained as hydrochlorides by the hydrogenation of the nitro acids (*S*)-(+)-**7a**–**d** and (*R*)-(−)-**7e** under the conditions described above. The opposite enantiomers were obtained by the hydrolysis of the corresponding enantiomerically pure γ-lactams **11a**–**e** in refluxing 6 N HCl for 12 h.

4.7.1. (*S*)-(+)-3-Aminomethylheptanoic acid hydrochloride 12a. $[\alpha]_D = +4.4$ (*c* 1, H_2O), ee 87% The opposite enantiomer (*R*)-(−)-**12a**, derived from (+)-**11a**, >99% ee, had $[\alpha]_D = -5.0$ (*c* 0.45, H_2O); ^1H NMR (400 MHz, D_2O) δ 2.98 (apparent d, part AB of an ABX system, 2 H, $CH_2NH_3^+$), 2.44 (part AB of an ABX system, $J_{AB} = 11.5$, $J_{AX} = 9.5$, $J_{BX} = 4.9$ Hz, 2H, CH_2COOH), 2.12 (bsept, 1H, H-3), 1.45–1.24 (m, 6H), 0.82 (br t, 3H, H-7). ^{13}C NMR (100.1 MHz, D_2O) δ 177.5 (s), 43.6 (t, C-2), 37.3 (t, $CH_2NH_3^+$), 33.7 (d, C-3), 31.2 (t), 28.3 (t), 22.7 (t), 14.0 (q). ESI-MS 183.0 ($M+Na^+$), 160.1 (MH^+).

4.7.2. (*S*)-(+)-3-Aminomethyl-5-methylhexanoic acid hydrochloride (Pregabalin hydrochloride) 2. $[\alpha]_D = +9.9$ (*c* 1, H_2O), ee 92% The opposite enantiomer (*R*)-(−)-**2**, derived from (+)-**11b**, >99% ee, had $[\alpha]_D = -10.5$ (*c* 1, H_2O).

Spectroscopic and analytical data are in full agreement with the literature.[9f]

4.7.3. (*S*)-(+)-3-Aminomethyloctanoic acid hydrochloride 12c. $[\alpha]_D = +4.1$ (*c* 0.8, H_2O), ee 94%: The opposite enantiomer (*R*)-(−)-**12c**, derived from (+)-**11c,** >99% ee, had $[\alpha]_D = -3.3$ (*c* 1, H_2O); ^1H NMR (400 MHz, D_2O) δ 2.97 (part AB of an ABX system, $J_{AB} = 12.6$, $J_{AX} = 6.6$, $J_{BX} = 5.8$ Hz, 2H, $CH_2NH_3^+$), 2.31 (part AB of an ABX system, $J_{AB} = 15.3$, $J_{AX} = 6.6$, $J_{BX} = 5.9$ Hz, 2 H, H-2), 2.09 (br m, 1H, H-3), 1.36–1.21 (m, 8H), 0.83 (t, 3H, CH_3CH_2). ^{13}C NMR (100.1 MHz, D_2O) δ 177.1 (s), 43.2 (t, CH_2COOH), 36.9 (t, $CH_2NH_3^+$), 33.4 (d, C-3), 31.4 (t), 31.2 (t), 25.5 (t), 22.0 (t), 13.9 (q). ESI-MS 197.1 ($M+Na^+$), 175.2 (MH^+).

4.7.4. (*S*)-(+)-3-Aminomethyl-5,5-dimethylhexanoic acid hydrochloride 12d. $[\alpha]_D = +6.7$ (*c* 0.4, H_2O); The opposite enantiomer (*R*)-(−)-**12d**, derived from (−)-**11d,** 95% ee, had $[\alpha]_D = -9.7$ (*c* 1, H_2O); mp 121–122 °C. ^1H NMR (400 MHz, D_2O) δ 3.03 (dq, part AB of an ABX, $J_{AB} = 13.2$ Hz, 2H, $CH_2NH_3^+$), 2.52 (apparent d, 2 H, H-2), 2.21 (sept, $J = 5.9$ Hz, 1 H, H-3), 1.30 (apparent d, 2 H, H-4), 0.91 (s, 9H, *t*-Bu). ^{13}C NMR (100.1 MHz, D_2O) δ 176.7 (s), 44.7 (t), 44.5 (t), 38.4 (d), 30.2 (t), 29.9 (s), 28.9 (q). ESI-MS 197.0 ($M+Na^+$), 175.1 (MH^+).

4.7.5. (*R*)-(−)-3-Aminomethyl-4-phenylbutanoic acid hydrochloride 12e. $[\alpha]_D = -6.4$ (*c* 1.45, H_2O), 94% ee {lit.[40] +6.25 (*c* 1.2 MeOH)], mp 187–90 °C. [lit.[40] 169–169.6 °C (*c* 1.2 MeOH)}. ^1H NMR (400 MHz, D_2O) δ 7.40 (m, 2H, Ph), 7.31 (m, 3H, Ph), 3.08 (apparent d, 2H, $CH_2NH_3^+$), 2.85 (dd, part A of an ABX system, $J_{AB} = 13.6$, $J_{AX} = 5.9$ Hz, 1H, CH_2Ph), 2.68 (dd, part B of an ABX system, $J_{AB} = 13.6$, $J_{BX} = 8.0$ Hz1H, CH_2Ph), 2.52 (sept, $J = 6.6$ Hz, 1H, H-3), 2.46 (d, $J = 5.9$ Hz, 2H, H-2). ^{13}C NMR (100.1 MHz, D_2O) δ 177.0 (s), 139.7 (s), 130.2 (d), 129.7 (d), 127.7 (d), 43.8 (t), 38.7 (t), 36.8 (d), 36.4 (t). ESI-MS: 216.0 ($M+Na^+$), 194.1 (MH^+).

4.8. Transformation of the nitro acids 7a,b,c,e into the corresponding succinic acids 8a,b,c,e

A solution of the nitro acids **7a**,**b**,**c**,**e** in 2:1 HCl/AcOH was refluxed for 2 h to give, after evaporation of the solvents, the correponding succinic acids **8a**,**b**,**c**,**e**.

4.8.1. (*S*)-(−)-2-Butylbutanedioic acid 8a. White solid, mp 83–84 °C [lit.[44] 85–86 °C], ee 87%, $[\alpha]_D = -24$ (*c* 0.85, EtOH), [lit.[45] −22.5 (*c* 5, EtOH)]. IR (Nujol) 3150, 3000 (br, COOH), 1712 (COOH) cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 2.94 (m, 1H, H-2), 2.82 (part A of an ABX system, $J_{AB} = 15.5$, $J_{AX} = 5.9$ Hz, 1H, H-3), 2.62 (part B of an ABX system, $J_{AB} = 15.5$, $J_{BX} = 5.1$ Hz, 1H, H-3), 1.79, 1.73 (2 sept, 2H, (CH_2CH_2CH)), 1.50 (m, 4H, $(CH_2)_2$), 1.09 (br t, 3H, CH_3). ^{13}C NMR (100.1 MHz, CD_3OD) δ 178.9 (s), 175.7 (s), 42.4 (d, C-2), 36.7 (t), 32.6 (t, C-3), 30.1 (t), 23.5, (t), 14.2 (q). HRMS (EI) calcd for $C_8H_{14}O_4$ ($M^{+\cdot}$) 174.0892, found 174.0898. ESI-MS 173.0 (M−1).

4.8.2. (*S*)-(−)-2-(2-Methylpropyl)butanedioic acid 8b. Semisolid material, 94% ee, $[\alpha]_D = -24.0$ (*c* 1.0, EtOH) [lit.[38] +26.2 (*c* 1.01, EtOH) for (*R*)-(+)-**8b**]. IR (cm^{-1}) 3400–2500 (very br, COOH), 1712 (C=O) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 10.50 (br, 1H, COOH), 2.87 (m, 1H, H-2), 2.69 (dd, part A of an ABX, $J_{AB} = 17.2$, $J_{AX} = 10.6$ Hz, 1H, H-3), 2.49 (dd, part B of an ABX system, $J_{AB} = 17.2$, $J_{BX} = 4.0$ Hz, 1 H, H-3), 1.63 (m, 2H, CH_2CH), 1.32 (sept, $J = 6.6$ Hz, 1H, $CH(CH_3)_2$), 0.94, 0.91 (2 d, $J = 6.2$Hz, 6H, $(CH_3)_2$). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 181.8 (s), 178.3 (s), 40.8 (d), 39.2 (t), 35.9 (t), 25.6 (d), 22.3 (q), 22.25 (q). HRMS (EI) calcd for $C_8H_{14}O_4$ ($M^{+\cdot}$) 174.0892, found 174.0888. ESI MS 173.0 (M-H) (negative ion polarity), 197.0 [$M+Na^+$], 214.0 ($MH^+ + K^+$). MS, *m*/*z* 174 ($M^{+\cdot}$, 12%), 156 ($M-H_2O$, 6), 131 (21), 128 (100), 57 (16).

4.8.3. (*S*)-(−)-2-Pentylbutanedioic acid 8c. White solid, mp 85–87 °C, 92% ee, $[\alpha]_D = -24$ (*c* 1.1, MeOH). IR (Nujol), 3340–2890 (br, COOH), 1710 (CO) cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 2.95 (m, 1H, H-2), 2.81 (part A of an ABX system, $J_{AB} = 15.5$, $J_{AX} = 5.9$ Hz, 1H, H-3), 2.61 (part B of an ABX system, $J_{AB} = 15.5$, $J_{BX} = 5.1$ Hz,

1H, H-3), 1.78, 1.71 (2 sept, 2H, (CH_2CH_2CH), 1.50 (m, 6H, ($CH_2)_3$), 1.09 (br t, 3H, CH_3). ^{13}C NMR (100.1 MHz, CD_3OD) δ 179.0 (s), 175.9 (s), 42.5 (d), 36.8 (t), 32.9 (t), 32.6 (t), 27.5 (t), 23.4 (t) 14.3 (q). HRMS (EI) calcd for $C_9H_{16}O_4$ ($M^{+\cdot}$) 188.1049, found 188.1040. ESI-MS 186.9 [M–1], negative ion polarity.

4.8.4. (*S*)-(−)-2-(2,2-Dimethyl)propylbutanedioic acid 8d. Compound **8d** was formed by the spontaneous transformation of (*S*)-(+)-**7d** in phosphate buffer, at rt, within a few hours. White solid, mp 135–136 °C, 62% ee, $[\alpha]_D = -12.0$ (*c* 0.45, $CHCl_3$). IR (Nujol) 3150, 3000 (br, COOH), 1708 (COOH) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 2.84 (m, 1H, H-2), 2.71 (dd, part A of an ABX system, $J_{AB} = 16.8$, $J_{AX} = 9.9$ Hz, 1H, H-3), 2.54 (dd, part B of an ABX system, $J_{AB} = 16.8$, $J_{BX} = 4.4$ Hz, 1H, H-3), 1.78 (dd, part A of an ABX system, $J_{AB} = 15.3$, $J_{AX} = 6.6$ Hz, 1H, *t*-Bu-CH_2), 1.30 (dd, part B of an ABX system, $J_{AB} = 15.3$, $J_{BX} = 4.8$ Hz, *t*-Bu-CH_2), 0.94 (s, 9H, *t*-Bu). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 182.2 (s), 177.9 (s), 45.5 (t), 38.3 (t), 37.7 (d), 31.0 (s), 29.3 (q). HRMS (EI) calcd for $C_9H_{16}O_4$ ($M^{+\cdot}$) 188.1049, found 188.1060. ESI-MS 211.0 ($M+Na^+$). MS, *m*/*z* 188 ($M^{+\cdot}$, 8%), 173 (23), 170 ($M^{+\cdot}-H_2O$, 6], 142 (100), 117 (54), 97 (73).

4.8.5. (*R*)-(+)-2-(Phenylmethyl)butanedioic acid 8e.[35a] Mp 166–167 °C [lit.[35a] 162–163°C], 94% ee, $[\alpha]_D = +24.0$ (*c* 3.1, AcOEt) [lit.[35a] +27.0 (*c* 1.5, AcOEt)]. IR (nujol) 3420 (br, OH) 1787, 1708 (CO_2H), 1450, 1400, 940, 760, 705 (Ph) cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 7.44 (t, 2H, ArH), 7.38 (m, 3H, ArH), 3.18 and 3.11 (2m, 2H, H-3 and H-2), 2.97 (m, 1H, H-3), 2.74 (part A of an ABX system, $J_{AB} = 15.4$, $J_{AX} = 5.1$ Hz, 1H, CH_APh), 2.54 (part B of an ABX system, $J_{AB} = 15.4$, $J_{BX} = 6.0$ Hz, 1H, CH_BPh). ^{13}C NMR (100.1 MHz, CD_3OD) δ 175.5 (s), 174.1 (s), 139.7 (s), 130.0 (d), 129.4 (d), 127.6 (d), 44.3 (d), 38.5 (t), 35.6 (t). HRMS (EI) calcd for $C_{11}H_{12}O_4$ ($M^{+\cdot}$) 208.0736, found 208.0740. ESI-MS 206.9 $(M-H)^-$, 163.0 $(M-COOH)^-$. MS, *m*/*z* 208 ($M^{+\cdot}$, 6%), 190 ($M-H_2O$, 4], 162 (100), 149 (22), 147 (36), 145 (56), 131 (61), 117 (63), 103 (21), 91 (49).

4.9. Conversion of (*R*)-(−)-3-nitromethyl-4-phenylbutanic acid 6e into (*R*)-(+)-4-benzyl-2-tetrahydrofuran-2-one 15

4.9.1. Ethyl (*R*)-(+)-3-benzyl-4-(*N*-*tert*-butoxycarbonyl)aminomethylbutanoate 13. A sample of (*R*)-(−)-**7e** (0.112 g, 0.5 mmol) with 94% ee was esterified into (*R*)-(−)-**6e** in EtOH/$(CH_3)_3SiCl$ as described above, then hydrogenated over Ra-Ni in EtOH in the presence of Boc_2O (0.120 g, 0.55 mmol) for 12 h. After filtration on a pad of Celite and evaporation of the solvent, pure N-protected amino ester (*R*)-(+)-**13** (0.160 g, 100% yield) was obtained as a colourless oil, $[\alpha]_D = +2.7$ (*c* 1, MeOH). IR 3369 (NH), 1718, 1715 (CO_2Et and Boc), 1515 (NH) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 7.23 (m, 2H, Ph), 7.15 (m, 3H, Ph), 4.74 (br s, 1H, NH), 4.05 (q, 2H, CH_2O), 3.15, 3.06 (2 br m, 2H, CH_2NHBoc), 2.64 (dd, part A of an ABX system, $J_{AB} = 13.9$, $J_{AX} = 7.0$ Hz, 1H, CH_2Ph), 2.57 (dd, part B of an ABX system, $J_{AB} = 13.9$, $J_{BX} = 6.5$ Hz, 1H, CH_2Ph), 2.32 (sept, $J = 6.6$ Hz, 1H, H-3), 2.24 (apparent d, 2H, H-2). ^{13}C NMR (100.1 MHz, $CDCl_3$) δ 172.9 (s) 156.0 (s), 139.3 (s), 129.1 (d), 128.4 (d), 126.2 (d), 79.2 (s), 60.4 (t), 43.9 (t) 38.4 (t), 37.8 (d), 36.5 (t), 28.4 (q), 14.2 (q). ESI-MS 344.2 ($M+Na^+$), 360.1 ($M+K^+$).

4.9.2. Ethyl (*R*)-(−)-3-benzyl-4-aminomethylbutanoate hydrochloride 14. The *N*-Boc protected amino ester (*R*)-(+)-**13** (0.160 g, 0.5 mmol) was treated with 2 M HCl (5 mL) for 30 min at rt. The evaporation to dryness gave (*R*)-(−)-**14** as a semisolid material, which was used in the next step without any purification; $[\alpha]_D = -2.8$ (*c* 0.6, MeOH). ^1H NMR (400 MHz, CD_3OD) δ 7.20 (m, 5H, Ph), 3.91 (q, 2H, CH_2O), 2.82 (m, 2H), 2.69, 2.42 (2m, 2H, CH_2Ph), 2.31, 2.21 (2 m, 3H, H-2 and H-3). ^{13}C NMR (100.1 MHz, CD_3OD) δ 173.8 (s) 139.0 (s), 129.9 (d), 129.3 (d), 127.3 (d), 61.6 (t), 43.6 (t, $CH_2NH_3^+$); 38.4 (t), 36.2 (d), 36.1 (t), 35.8 (t), 14.2 (q). ESI-MS 222.1 (M^+).

4.9.3. (*R*)-(+)-4-Benzyl-2-tetrahydrofuran-2-one 15.[41] A 1 M aqueous solution of $NaNO_2$ (0.75 mL, 0.75 mmol) was added dropwise to a solution of (*R*)-(−)-**14** (0.5 mmol), in H_2O, at 0 °C, under vigorous stirring. The solution was stirred at rt overnight, then extracted with diethyl ether. Evaporation of the solvent furnished an oily residue which was chromatographed on silica gel (petroleum ether/ethyl acetate 9:1), to give pure β-benzyl-γ-butyrolactone (*R*)-(+)-**15**. Spectroscopic and analytical data are in accordance with the literature.[41] $[\alpha]_D = +7.2$ (*c* 1, $CHCl_3$) [lit.[41] {$[\alpha]_D = -7.3$ (*c* 1, $CHCl_3$, for the (*S*)-enantiomer, 94% ee)}.

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