Synthesis of all four diastereoisomers of 4-(carboxymethyl)proline, a conformationally constrained analogue of 2-aminoadipic acid

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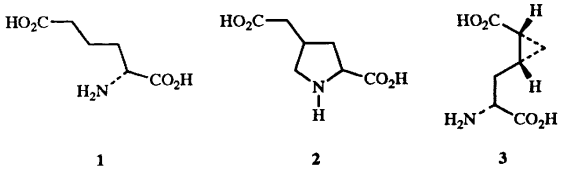
The dirhodium(II) tetraacetate catalysed reaction of ethyl diazoacetate with 2,3-dihydropyrrole-2,2-dicarboxylate **5** afforded the useful 2-azabicyclo[3.1.0]hexane derivative **6**. Its conversion into the proline-γ-acetic acid equivalent **9** as well as into the four isomers constituting the 4-(carboxymethyl)proline **13** (**16a**–**19a**) whose absolute configuration was established by an alternative asymmetric synthesis of two of them is described. Preliminary data concerning the affinity of compounds **16a**–**19a** for the NMDA site of the NMDA receptor complex are also reported.

As part of on-going studies directed to the synthesis and biological evaluation of conformationally restricted amino acid analogues,[1] we report here the preparation of compounds **16a**–**19a**, the four possible isomers of the non-proteinogenic amino acid 4-(carboxymethyl)proline **2**, which incorporate the (2*R*)- and (2*S*)-aminoadipic acid moieties [(2*R*)- and (2*S*)-AA]. The (2*R*)-isomers **16a** and **17a**, in particular, are partially constrained analogues of (2*R*)-AA **1**, a selective competitive antagonist for the NMDA receptor site of the NMDA receptor complex.[2] Previous attempts to reduce the conformational mobility of **1** in order to achieve energetically improved interactions with the NMDA receptor site have already been reported. Interestingly, (2*R*,4*R*,5*R*)-2-amino-4,5-methanoadipic acid **3**, a compound of this class, has been shown to be a selective

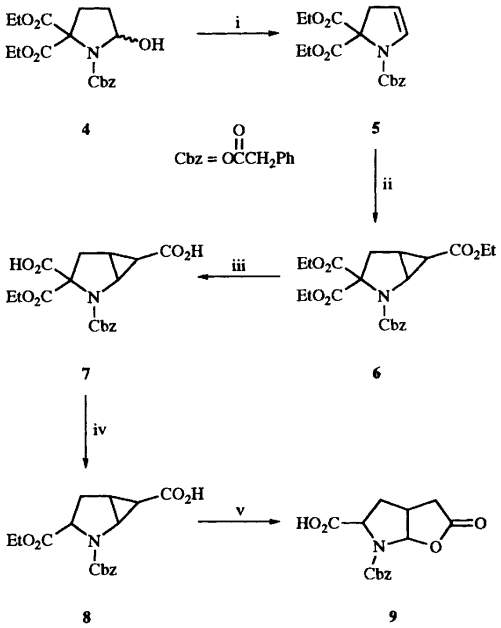


1 **2** **3**

NMDA receptor site partial agonist, endowed with promising biological properties.[3]

For the preparation of compounds **16a**–**19a** our attention was focused on the utilization of the 2-azabicyclo[3.1.0]hexane-3,3,6-tricarboxylate **6**, prepared as outlined in Scheme 1.[4] Condensation of diethyl *N*-Cbz-aminomalonate with acrolein in a benzene solution of sodium ethoxide gave the known diethyl 1-Cbz-5-hydroxypyrrolidine-2,2-dicarboxylate **4**[5] (82% yield), the dehydration of which with P_2O_5 in refluxing benzene for 2 h furnished the corresponding diethyl 1-Cbz-2,3-dihydropyrrole-2,2-dicarboxylate **5** in 35% yield. Dirhodium(II) tetraacetate catalysed decomposition of ethyl diazoacetate in the presence of **5** (CH_2Cl_2, RT, 12 h) afforded the 2-azabicyclo[3.1.0]hexane derivative **6** as a *ca.* 1:1 mixture of *exo* and *endo* forms in 46% yield.[6]

The utilization of **6** for the preparation of the synthetically useful lactone **9**, containing a γ-imino carbonyl system in masked form, has been the object of a preliminary communication.[4] This conversion was achieved by submitting **6** to partial hydrolysis (0.5 mol dm^{-3} NaOH, H_2O–MeOH, RT, 5 h, 87% yield) followed by monodecarboxylation of the resulting

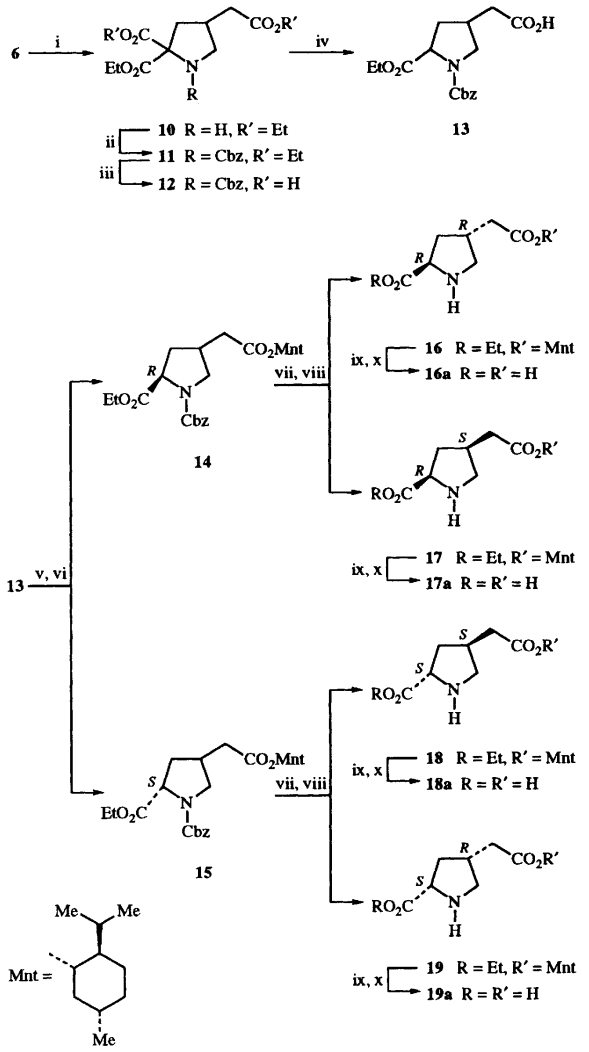


4 **5** **6** **7** **8** **9**

Scheme 1 *Reagents and conditions:* i, P_2O_5, C_6H_6, reflux (35%); ii, EDA, $Rh_2(OAc)_4$, CH_2Cl_2, RT (46%); iii, 0.5 mol dm^{-3} NaOH (H_2O–MeOH), RT (87%); iv, PhMe, reflux (75%); v, 6 mol dm^{-3} HCl (H_2O–dioxane, 5:1), RT (87%)

dicarboxylate **7** (PhMe, reflux, 12 h) and, finally, by acidic treatment (6 mol dm^{-3} HCl, RT, 72 h, 87% yield) of the resulting cyclopropyl derivative **8**.

We report now a new synthetic transformation of **6** which allows the entry into the title compounds. Indeed, treatment of **6** with hydrogen in the presence of 10% Pd–C in MeOH at room temperature for 1.5 h leads to reductive cleavage of the cyclopropyl moiety with the consequent formation of **10** (98% yield), which was sequentially submitted to Cbz protection, partial saponification (0.5 mol dm^{-3} NaOH, H_2O–MeOH, RT, 5 h) and thermal monodecarboxylation (PhMe, 15 h) of the dicarboxylic acid **12** to give the desired 4-(carboxymethyl)-proline derivative **13** with an overall yield of 62.5% starting from



Scheme 2 *Reagents and conditions:* i, H_2, 10% Pd–C, MeOH (98%); ii, CbzCl, $NaHCO_3$ (97%); iii, 0.5 mol dm^{-3} NaOH (H_2O–MeOH), RT (90%); iv, PhMe, reflux (73%); v, (+)-menthol, DCC, DMAP, CH_2Cl_2–DMF (1:1), 76 h; vi, MPC (**14**: 63%, **15**: 6.7%); vii, H_2, 10% Pd–C, MeOH; viii, MPC (**16**: 42%, **17**: 32%, **18**: 38%, **19**: 24%); ix, 6 mol dm^{-3} HCl, reflux, 14 h; x, Dowex, 50 × 2–200 (**16a**: 86%, **17a**: 98%, **18a**: 84%, **19a**: 83%)

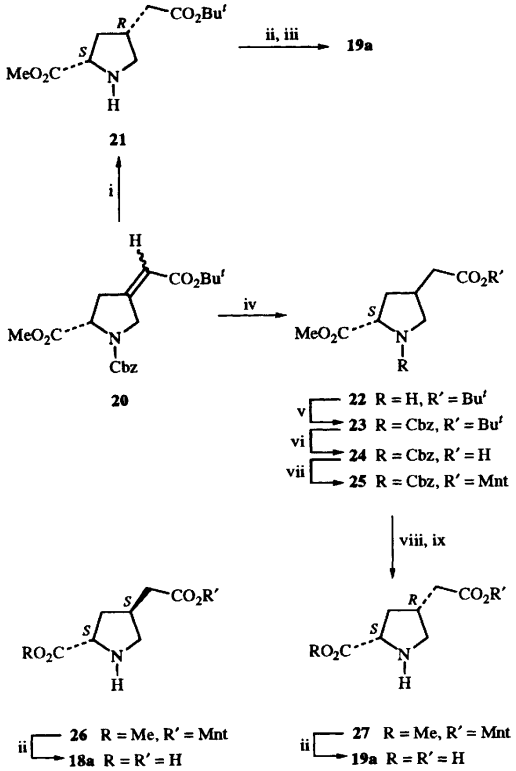
Table 1 Selected physicochemical properties of the four isomers **16a**–**19a**

	Mp (°C)	$[\alpha]_D^{22}$
16a	224–225	+66.8
17a	232–233	+34.2
18a	228–229	−66.5
19a	234–235	−33.4

6. HPLC analysis of **13** showed that it was a mixture of all the four possible isomers, whose resolution required the preparation of the corresponding (+)-menthyl esters,[7] as shown in Scheme 2. Thus, esterification of **13** with (+)-menthol in CH_2Cl_2–DMF (1:1) in the presence of DCC and DMAP for 76 h afforded the corresponding (2*R*) and (2*S*) diastereoisomeric compounds **14** and **15**, respectively, which were separated by medium pressure chromatography (MPC) in 63 and 7% yield, respectively. The (2*R*) derivative **14** was then submitted to catalytic hydrogenolysis (10% Pd–C, MeOH, RT, 2.5 h) followed by MPC to afford the two esters (2*R*,4*R*)-**16** and (2*R*,4*S*)-**17** (30 and 24% yield, respectively). Analogously, the (2*S*) derivative **15** afforded the two esters (2*S*,4*S*)-**18** and (2*S*,4*R*)-**19** in 38 and 25% yield, respectively.

Finally, each of the four isomeric esters **16**–**19** was submitted to acidic hydrolysis (6 mol dm^{-3}, HCl, reflux, 12 h) followed by cation exchange resin chromatography eluting with 10% pyridine in water. In this way, (+)-(2*R*,4*R*)-4-(carboxymethyl)-proline **16a**, (+)-(2*R*,4*S*)-4-(carboxymethyl)proline **17a**, (−)-(2*S*,4*S*)-4-(carboxymethyl)proline **18a** and (−)-(2*S*,4*R*)-4-(carboxymethyl)proline **19a** were obtained in 86, 96, 87 and 91% yield, respectively.[8] Relevant physicochemical properties and ^{13}C NMR data of the four isomers **16a**–**19a** are reported in Tables 1 and 2, respectively.

The assignment of the absolute configurations to the four isomers **16a**–**19a** was made possible by the enantiomerically pure compound (EPC) synthesis of two of them (Scheme 3).

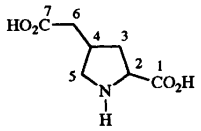


Scheme 3 *Reagents and conditions:* i, H_2, PtO_2, AcOH, 50 psi (55%); ii, 6 mol dm^{-3} HCl, 14 h, reflux; iii, Dowex 50 × 2–200 (**19a**: 70%, **18a**: 88%); iv, H_2, 10% Pd–C, AcOH, 40 psi (98%); v, CbzCl, $NaHCO_3$ (99%); vi, CF_3CO_2H, CH_2Cl_2, 6 h, RT (72.5%); vii, (+)-menthol, DCC, DMAP, CH_2Cl_2–DMF (1:1), 76 h (78%); viii, H_2, 10% Pd–C, MeOH; ix, MPC (**26**: 18%, **27**: 26.5%)

Thus, Peterson condensation of the protected *S*-4-oxoproline with *tert*-butyl α-trimethylsilylacetate afforded the corresponding (2*S*)-diester **20**[9] (34% yield) which was submitted to catalytic hydrogenation in the presence of PtO_2 in acetic acid (50 psi,† 10 h) to give stereospecifically the known (2*S*,4*R*)-diester **21** in 55% yield.[9] Acidic hydrolysis of **21** followed by cation exchange resin chromatography eluting with 10% pyridine in water afforded (−)-(2*S*,4*R*)-4-(carboxymethyl)-proline **19a** (70% yield), identical with that already reported in the literature.[8]

† 1 psi = 6.89 kPa.

Table 2 ^{13}C NMR data of the four isomers **16a–19a**



	16a	17a	18a	19a
C-1	176.44	177.17	177.03	177.84
C-2	60.79	61.97	61.55	62.06
C-3	34.39	35.46	35.15	35.71
C-4	33.39	35.34	34.13	35.46
C-5	50.14	50.78	50.90	50.85
C-6	36.43	37.26	37.06	37.83
C-7	174.24	174.82	174.93	174.97

Table 3 Displacement of NMDA specific L-[^{3}H]glutamate binding to rat cortical membranes by the four isomers **16a–19a**

Compound	IC_{50} (μmol dm^{-1})[a]
L-Glu	0.05 ± 0.005
NMDA	0.31 ± 0.04
16a	202 ± 43
17a	8.0 ± 2.0
18a	8.1 ± 1.4
19a	1.7 ± 0.65

[a] IC_{50} values were calculated from inhibition curves based on 4–6 different concentrations of the compounds using the ALLFIT computer program.[12] Values are the mean ± SEM of 3–4 separate determinations.

Conversely, catalytic hydrogenation of **20** with 10% Pd–C in acetic acid (40 psi, 12 h) afforded **22** as an inseparable mixture of the corresponding (2*S*,4*R*)- and (2*S*,4*S*)-diesters which was resolved *via* the formation of the corresponding (+)-menthyl esters. Thus, Cbz protection of **22** under mildly basic conditions followed by acidic hydrolysis (CF_3CO_2H, CH_2Cl_2, RT, 6 h) of the *N*-Cbz ester **23** afforded the corresponding acid **24** (72.5% yield) which was esterified with (+)-menthol to give a mixture of the corresponding diastereoisomeric esters **25** (56% yield). Removal of the *N*-Cbz group from **25** followed by MPC afforded the two diesters (2*S*,4*S*)-**26** and (2*S*,4*R*)-**27** in 18 and 26.5% yield, respectively, which were then submitted to acidic hydrolysis (6 mol dm^{-3} HCl) and cation exchange resin chromatography (10% pyridine in water) to yield the enantiomerically pure (−)-(2*S*,4*S*)-4-(carboxymethyl)proline **18a** {$[\alpha]_D^{22}$ −66.5 (*c* 0.5, H_2O)} and (−)-(2*S*,4*R*)-4-(carboxymethyl)proline **19a** {$[\alpha]_D^{22}$ −33.4 (*c* 0.35, H_2O)} in 88 and 91% yield, respectively. Since the two remaining isomers **16a** and **17a** are enantiomers of **18a** and **19a**, respectively, the examination of their optical rotations (see Table 1) made the assignment of their absolute configuration straightforward.

Biological results

A preliminary evaluation of the affinity of the four isomers of 4-(carboxymethyl)proline **16a–19a** at the NMDA site of the NMDA receptor complex is reported in Table 3. As previously shown,[10,11] the NMDA-sensitive L-[^{3}H]Glu binding to rat cortical membranes is almost exclusively due to the interaction of the ligand with the NMDA receptor site. The results clearly indicate that none of these compounds met the goal of a potent NMDA ligand. However, the higher binding potency of the two *cis*-4-(carboxymethyl)prolines **17a** and **19a** in comparison with the *trans* compounds **16a** and **18a**, can be explained with the suitable geometry of the former compounds which fits the pharmacophoric requirements for the interaction with the NMDA site of the NMDA receptor complex.[1a] In particular, the *cis*-*S*-proline derivative **19a** exhibited an IC_{50} value approximately five times lower than that reported for the *cis*-*R*-proline derivative **17a** (see Table 3).

In conclusion, we report here the preparation of the 2-azabicyclo[3.1.0]hexane derivative **6**, a versatile synthetic intermediate, and its exploitation for the preparation of the proline-γ-acetic acid equivalent **9** and for the synthesis of the four possible stereoisomers of 4-(carboxymethyl)proline, a conformationally constrained 2-aminoadipic acid analogue. The compounds **16a–19a** have been submitted to binding studies in order to test their affinity to the NMDA site of the NMDA receptor complex; full biological characterization is in progress and the results will be reported in due time.

Experimental

Mps were determined on a Kofler micro-hot-stage apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 1320 spectrometer. ^1H and ^{13}C NMR spectra were taken on a Bruker AC 200 spectrometer and the chemical shifts are reported on the δ scale relative to tetramethylsilane. Specific rotations were recorded on a Jasco Dip-360 digital polarimeter and are given in units of 10^{-1} deg cm^2 g^{-1}. HPLC was carried out on a Waters Delta Prep 3000 system equipped with a 484 UV-Visible detector and a computerized acquisition data system (Baseline 810™). Analytical reverse-phase HPLC was performed on a Beckman RP-C18 Ultrasphere (25 cm, 4.6 mm, 5 μm spherical silica, 80 Å pore). Gas chromatographic analyses were performed on a Hewlett-Packard HP 5890-II system [column and conditions: Supelco SP™ −2250, 30 m, 0.25 mm ID, 0.20 μm f.t., 190(5′)/290 °C, 10 °C/min] equipped with a flame ionization detector and a HP 3394A recorder-integrator. Combustion analyses were performed on a 1102 Automatic Analyzer, Carlo Erba (Italy). Flash chromatography was performed on Merck silica gel (0.040–0.063 mm). Medium pressure chromatography (MPC) was performed on Merck LiChroprep Si 60 (0.040–0.063 mm, lobar columns). Ether refers to diethyl ether. 10% Pyridine refers to 10% pyridine in water.

Diethyl 1-benzyloxycarbonyl-5-hydroxypyrrolidine-2,2-dicarboxylate 4

Sodium ethoxide (10.2 g, 0.15 mol) was added to a stirred solution of diethyl *N*-benzyloxycarbonylaminomalonate (30.0 g, 97 mmol) in anhydrous benzene (200 cm^3). After 15 min, acrylaldehyde (5.6 g, 100 mmol) was added dropwise to this mixture over 20 min and stirring was then continued for 12 h. Evaporation of the solvent gave a residue (33 g) which was submitted to flash chromatography, eluting with light petroleum–ether (1:1) to afford the title compound as a pale yellow oil (29.0 g, 82%); δ_H(200 MHz; $CDCl_3$) 1.00–1.40 (6 H, 2 m, 2 × CH_2CH_3), 1.80–2.10 (2 H, m, 4-H_2), 2.35–2.75 (2 H, m, 3-H_2), 3.40 (1 H, br s, OH), 3.90–4.30 (4 H, 2 m, 2 × CH_2CH_3), 5.05 (2 H, s, CH_2Ph), 5.55–5.70 (1 H, br s, 5-H) and 7.20 (5 H, m, ArH).

Diethyl 1-benzyloxycarbonyl-2,3-dihydropyrrole-2,2-dicarboxylate 5

Phosphorus pentoxide (9.0 g, 81 mmol) was added to a solution of the pyrrolidine **4** (29.0 g, 79 mmol) in anhydrous benzene (200 cm^3) and the resulting mixture was refluxed for 2 h. Filtration of the reaction mixture and evaporation of the solvent gave a residue (11 g) which was submitted to flash chromatography, eluting with light petroleum–ether (4:6), to give the title compound (9.62 g, 35%); δ_H(200 MHz; $CDCl_3$)

1.10 and 1.25 (6 H, 2 t, J 7.5, 2 × CH_2CH_3), 3.20 (2 H, d, J 15, 3-H_2), 4.00 and 4.25 (4 H, 2 q, J 7.5, 2 × CH_2CH_3), 4.90 (1 H, d, J 15, 4-H), 5.12 (2 H, 2 s, CH_2Ph), 6.60 (1 H, d, J 30, 5-H) and 7.30 (5 H, m, ArH).

Triethyl 2-benzyloxycarbonyl-2-azabicyclo[3.1.0]hexane-3,3,6-tricarboxylate 6

A solution of ethyl diazoacetate (13.12 g, 115 mmol) in anhydrous dichloromethane (250 cm^3) was added dropwise over 12 h to a magnetically stirred solution of the olefin **5** (9.62 g, 28 mmol) in anhydrous dichloromethane (100 cm^3) containing $Rh_2(OAc)_4$ (1.34 g, 3 mmol) under argon at room temperature. Evaporation of the solvent gave a residue (6.9 g) which was submitted to flash chromatography, eluting with light petroleum–ether (1:1), to give the title compound as a pale yellow oil (5.6 g, 46%); δ_H(200 MHz; $CDCl_3$) 1.30 (9 H, m, 3 × CH_2CH_3), 1.90 and 2.20 (2 H, 2 m, 3-H_2), 2.75 (1 H, m, 4-H), 2.95 (1 H, 2 d, J 7, $CHCO_2Et$), 3.95–4.35 (7 H, m, 3 × CH_2CH_3 and 5-H), 5.15 (2 H, 2 s, CH_2Ph) and 7.30 (5 H, m, ArH).

2-Benzyloxycarbonyl-3-ethoxycarbonyl-2-azabicyclo-[3.1.0]hexane-3,6-dicarboxylic acid 7

A solution of the triester **6** (0.320 g, 0.74 mmol) in aqueous sodium hydroxide (0.5 mol dm^{-3}; water–methanol 1.4:1) was magnetically stirred for 5 h at room temperature. After controlled ($t \leqslant 30$ °C) evaporation of the methanol, the reaction mixture was diluted with water (15 cm^3), acidified with 6 mol dm^{-3} HCl to pH 4–5 and extracted with chloroform (3 × 10 cm^3). The combined extracts were dried (Na_2SO_4) and evaporated and flash filtration of the residue on silica gel yielded the title compound (0.244 g, 87%); δ_H(200 MHz; $CDCl_3$) 1.20–1.35 (3 H, m, CH_2CH_3), 2.20–2.55 (3 H, m, 3-H_2 and 4-H), 3.45 (1 H, m, $CHCO_2H$), 3.95 (1 H, m, 5-H), 4.25 (2 H, m, CH_2CH_3), 5.30 (2 H, s, PhCH_2), 7.35 (5 H, m, ArH) and 9.70 (2 H, br s, 2 × CO_2H).

2-Benzyloxycarbonyl-3-ethoxycarbonyl-2-azabicyclo-[3.1.0]hexane-6-carboxylic acid 8

A solution of the dicarboxylic acid **7** (0.244 g, 0.64 mmol) in toluene (20 cm^3) was heated at reflux for 12 h. Evaporation of the solvent followed by flash filtration of the residue on silica gel yielded the title compound (0.160 g, 75%); δ_H(200 MHz; $CDCl_3$) 1.20 (3 H, m, CH_2CH_3), 2.10–2.60 (3 H, m, 3-H_2 and 4-H), 2.70 (1 H, m, $CHCO_2H$), 4.10 (3 H, m, CH_2CH_3 and 5-H), 4.20 and 4.60 (1 H, 2 m, 2-H), 5.15 (2 H, m, PhCH_2), 7.30 (5 H, m, ArH) and 8.00–9.50 (1 H, br s, CO_2H); δ_C(50.32 MHz; $CDCl_3$) 24.53, 25.58, 26.79, 29.62, 30.16, 31.90, 45.53, 46.11, 59.63, 60.58, 61.46, 67.66, 128.07, 128.45, 136.14, 154.42, 155.21, 170.97, 172.34, 174.94 and 175.55.

8-Benzyloxycarbonyl-3-oxo-8-aza-2-oxabicyclo[3.3.0]octane-7-carboxylic acid 9

A solution of compound **8** (0.160 g, 0.48 mmol) in 6 mol dm^{-3} HCl (18 cm^3, water–dioxane 5:1) was magnetically stirred for 72 h at room temperature. The reaction mixture was then extracted with dichloromethane (3 × 15 cm^3) and the combined organic phases were dried (Na_2SO_4). Evaporation of the solvent gave a solid which was recrystallized from dichloromethane–hexane to give the *title compound* (0.127 g, 87%), mp 97–99 °C; purity was 99% by HPLC (Found: C, 59.15; H, 5.0; N, 4.55. $C_{15}H_{15}NO_6$ requires C, 59.01; H, 4.95; N, 4.59%); ν_{max}($CHCl_3$)/cm^{-1} 1781 and 1717; δ_H(500 MHz; $CDCl_3$) 1.90–2.10 and 2.30–2.50 (2 H, 2 m, 6-H_2), 2.55 (1 H, m, 5-H), 2.75 and 3.10 (2 H, 2 m, 4-H_2), 4.55 (1 H, m, 7-H), 5.20 (2 H, s, CH_2Ph), 6.10 (1 H, 4 d, J 5.3, 1-H), 6.90 (1 H, br s, CO_2H) and 7.30 (5 H, br s, ArH); δ_C(50.32 MHz; $CDCl_3$) 33.61, 34.14, 34.78, 34.93, 36.58, 37.69, 59.67, 60.29, 68.10, 91.68, 92.00, 92.58, 127.75, 127.88, 128.00, 128.26, 128.55, 135.47, 154.00, 174.39 and 175.54.

Diethyl 4-[(ethoxycarbonyl)methyl]pyrrolidine-2,2-dicarboxylate 10

Hydrogen was bubbled for 1.5 h into a magnetically stirred suspension of compound **6** (5.6 g, 13 mmol) in methanol (100 cm^3) containing 10% Pd–C (1.7 g) at room temperature. Filtration of the mixture through Celite and evaporation of the filtrate gave the title compound as a yellow solid (3.86 g, 98%), mp 80–81 °C; purity was 99% by GC analysis; δ_H(200 MHz; $CDCl_3$) 1.25 (3 H, t, J 7, $CH_2CO_2CH_2CH_3$), 1.33 (6 H, t, 2 × CH_2CH_3), 2.30 and 2.90 (2 H, 2 m, 3-H_2), 2.55 (2 H, m, CH_2CO_2Et), 2.85 (1 H, m, 4-H), 3.32 and 3.88 (2 H, 2 m, 5-H_2), 4.12 (2 H, q, J 7, $CH_2CO_2CH_2CH_3$), 4.35 (4 H, m, 2 × CH_2CH_3).

Diethyl 1-benzyloxycarbonyl-4-[(ethoxycarbonyl)methyl]-pyrrolidine-2,2-dicarboxylate 11

Benzyl chloromethanoate (1.9 cm^3, 13.4 mmol) was added dropwise in 10 min under vigorous magnetic stirring to cold (0 °C) saturated aqueous $NaHCO_3$ (70 cm^3) containing the pyrrolidine **10** (3.86 g, 12.8 mmol). Stirring was continued for 12 h at room temperature after which the reaction mixture was extracted with ethyl acetate (3 × 20 cm^3). The combined organic phases were dried (Na_2SO_4) and evaporated and the residue upon flash filtration on silica gel afforded the title compound as an oil (5.43 g, 97%); δ_H(200 MHz; $CDCl_3$) 1.00–1.30 (9 H, m, 3 × CH_2CH_3), 2.15 and 2.78 (2 H, 2 m, 3-H_2), 2.40 (2 H, m, CH_2CO_2Et), 2.58 (1 H, m, 4-H), 3.23 and 3.90 (2 H, 2 m, 5-H_2), 4.00–4.30 (6 H, m, 3 × CH_2CH_3), 5.05–5.15 (2 H, m, CH_2Ph) and 7.20–7.40 (5 H, m, ArH).

1-Benzyloxycarbonyl-4-carboxymethyl-2-ethoxycarbonyl-pyrrolidine-2-carboxylic acid 12

The triester **11** (5.43 g, 12.5 mmol) was added to a solution of NaOH (4.57 g, 114 mmol) in water–methanol (200 cm^3; 6:4) and the resulting mixture was stirred for 5 h at room temperature. The methanol was carefully removed ($t \leqslant 30$ °C) and the resulting aqueous solution was acidified (to pH 4–5) with 6 mol dm^{-3} HCl and then extracted with chloroform (3 × 60 cm^3). The combined organic phases were dried (Na_2SO_4) and evaporated and flash filtration of the residue on silica gel afforded the title compound as an oil (4.25 g, 90%); δ_H(200 MHz; $CDCl_3$) 1.20 (3 H, t, J 6, CH_2CH_3), 2.20–2.80 (5 H, m, 3-H_2, 4-H and CH_2CO_2H), 3.20 and 3.90 (2 H, 2 m, 5-H_2), 4.20 (2 H, q, J 6, CH_2CH_3), 5.10 (2 H, s, CH_2Ph), 7.20 (5 H, m, ArH) and 9.10 (2 H, br s, 2 × CO_2H).

Ethyl 1-benzyloxycarbonyl-4-(carboxymethyl)pyrrolidine-2-carboxylate 13

A solution of compound **12** (4.25 g, 11.2 mmol) in toluene (120 cm^3) was refluxed for 15 h. After evaporation of the solvent, the residue (3 g) was submitted to flash chromatography, eluting with chloroform–methanol (99:1), to afford the title compound (2.76 g, 73%); δ_H(200 MHz; $CDCl_3$) 1.10–1.40 (3 H, m, CH_2CH_3), 1.60–2.30 (2 H, m, 3-H_2), 2.30–2.50 (2 H, m, CH_2CO_2H), 2.70 (1 H, m, 4-H), 3.20 and 3.90 (2 H, 2 m, 5-H_2), 4.00–4.50 (3 H, m, 2-H and CH_2CH_3), 5.15 (2 H, m, CH_2Ph) and 7.30 (5 H, m, ArH).

Ethyl (2*R*)-1-benzyloxycarbonyl-4-[(menthyloxycarbonyl)-methyl]pyrrolidine-2-carboxylate 14 and ethyl (2*S*)-1-benzyloxycarbonyl-4-[(menthyloxycarbonyl)methyl]pyrrolidine-2-carboxylate 15

(+)-Menthol (1.53 g, 9.8 mmol) was added to a solution of the carboxylic acid **13** (2.76 g, 8.2 mmol) in dichloromethane–DMF (300 cm^3, 1:1) containing DCC (2.0 g, 9.7 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) (0.1 g, 0.82 mmol) and the resulting mixture was stirred for 76 h at room temperature. After evaporation of the solvent, the residue was taken up in ethyl

acetate (150 cm^3) and washed with water (2 × 50 cm^3). The organic layer was dried (Na_2SO_4) and evaporated to give a residue (3.5 g) which was submitted to MPC, eluting with light petroleum–ethyl acetate (6:4), to afford the (2*R*) derivative **14** (2.45 g, 63%); δ_H(200 MHz; $CDCl_3$) 1.25 and 1.70 (21 H, 2 m, CH_2CH_3 and menthyl H), 1.95 (2 H, m, 3-H_2), 2.50 (2 H, m, CH_2CO_2), 2.70 (1 H, m, 4-H), 3.10 and 3.85 (2 H, 2 m, 5-H_2), 3.65 (1 H, m, CO_2CH), 4.00–4.40 (3 H, m, 2-H and CH_2CH_3), 5.05–5.15 (2 H, m, CH_2Ph) and 7.30 (5 H, m, ArH). Further elution with the same solvents gave a mixture of **14** and **15** (0.3 g). Continued elution with the same solvents (7:3) gave the pure (2*S*) derivative **15** (0.26 g, 6.7%); δ_H(200 MHz; $CDCl_3$) 1.30 and 1.70 (21 H, 2 m, CH_2CH_3 and menthylH), 1.95 (2 H, m, 3-H_2), 2.55 (2 H, m, CH_2CO_2), 2.70 (1 H, m, 4-H), 3.15 and 3.90 (3 H, 2 m, 5-H_2 and 2-H), 3.70 (1 H, m, CO_2CH), 4.40 (2 H, m, CH_2CH_3), 5.10–5.20 (2 H, m, CH_2Ph) and 7.30 (5 H, m, ArH).

Ethyl (2*R*,4*R*)-4-[(menthyloxycarbonyl)methyl]pyrrolidine-2-carboxylate 16 and (2*R*,4*S*)-4-[(menthyloxycarbonyl)methyl]-pyrrolidine-2-carboxylate 17

Hydrogen was bubbled for 2.5 h into a magnetically stirred suspension of compound **14** (2.45 g, 5.16 mmol) in methanol (200 cm^3) containing 10% Pd–C (0.250 g) at room temperature. Filtration of the mixture through Celite and evaporation of the filtrate gave a residue (1.8 g) which was submitted to MPC eluting with chloroform–methanol (99:1) to afford the (2*R*,4*R*) derivative **16** (0.74 g, 42%), mp 65–66 °C; purity was 99% by GC analysis; δ_H(200 MHz; $CDCl_3$) 1.20 and 1.75 (21 H, 2 m, CH_2CH_3 and menthylH), 1.90 and 2.10 (2 H, 2 m, 3-H_2), 2.50 (3 H, m, 4-H and CH_2CO_2), 3.00 (1 H, m, NH), 3.25 and 3.60 (2 H, 2 m, 5-H_2), 3.75 (1 H, m, CO_2CH), 3.85 (1 H, m, 2-H) and 4.15 (2 H, q, *J* 7.5, CH_2CH_3). Further elution with the same solvents gave a mixture of **16** and **17** (0.43 g). Continued elution with the same solvents gave the pure (2*R*,4*S*) derivative **17** (0.57 g, 32%), mp 122–123 °C; purity was 99% by GC analysis; δ_H(200 MHz; $CDCl_3$) 1.25 and 1.75 (21 H, 2 m, CH_2CH_3 and menthylH), 1.95 and 2.50 (2 H, 2 m, 3-H_2), 2.55 (2 H, m, CH_2CO_2), 2.70 (1 H, m, 4-H), 3.20 and 3.65 (2 H, 2 m, 5-H_2), 3.70 (1 H, m, NH), 3.80–4.00 (2 H, m, CO_2CH and 2-H) and 4.20 (2 H, q, *J* 7.5, CH_2CH_3).

Ethyl (2*S*,4*S*)-4-[(menthyloxycarbonyl)methyl]pyrrolidine-2-carboxylate 18 and (2*S*,4*R*)-4-[(menthyloxycarbonyl)methyl]-pyrrolidine-2-carboxylate 19

Hydrogen was bubbled for 1 h into a magnetically stirred suspension of compound **15** (0.26 g, 0.55 mmol) in methanol (50 cm^3) containing 10% Pd–C (0.026 g) at room temperature. Filtration of the mixture through Celite and evaporation of the filtrate gave a residue (0.2 g) which was submitted to MPC eluting with chloroform–methanol (99:1) to afford the (2*S*,4*S*) derivative **18** (0.070 g, 38%), mp 195–196 °C; purity was controlled by GC analysis (99%); δ_H(200 MHz; $CDCl_3$) 1.15 and 1.70 (21 H, 2 m, CH_2CH_3 and menthylH), 1.90 and 2.15 (2 H, 2 m, 3-H_2), 2.50 (3 H, m, 4-H and CH_2CO_2), 3.15 (1 H, m, NH), 3.25 and 3.55 (2 H, 2 m, 5-H_2), 3.65 (1 H, m, CO_2CH) and 3.85 (3 H, m, 2-H and CH_2CH_3). Further elution with the same solvents gave a mixture of **18** and **19** (0.065 g). Continued elution with the same solvents gave the title compound **19** (0.045 g, 24%), mp 100–101 °C; purity was controlled by GC analysis (99%); δ_H(200 MHz; $CDCl_3$) 1.20 and 1.70 (21 H, 2 m, CH_2CH_3 and menthylH), 1.95 and 2.35 (2 H, 2 m, 3-H_2), 2.50 (3 H, m, 4-H and CH_2CO_2), 2.80 (1 H, m, NH), 3.20 and 3.55 (2 H, 2 m, 5-H_2), 3.70 (1 H, m, CO_2CH) and 3.85 (3 H, m, 2-H and CH_2CH_3).

(2*R*,4*R*)-4-(Carboxymethyl)pyrrolidine-2-carboxylic acid 16a

A magnetically stirred suspension of **16** (0.160 g, 0.47 mmol) in 6 mol dm^{-3} HCl (8 cm^3) was refluxed for 12 h. After cooling, the reaction mixture was extracted with chloroform (5 cm^3) and then neutralized with 10% aqueous NH_4OH and concentrated under reduced pressure. The residue was diluted with water (5 cm^3) and submitted to cation exchange resin chromatography, eluting with 10% pyridine to afford the title compound (0.070 g, 86%), mp 224–225 °C (Found: C, 48.35; H, 6.6; N, 8.15. $C_7H_{11}NO_4$ requires C, 48.55; H, 6.40; N, 8.09%); $[\alpha]_D^{22}$ +66.8 (*c* 0.5, H_2O); δ_H(200 MHz; D_2O) 1.80–2.00 (1 H, m, 3-Ha), 2.12–2.30 (1 H, m, 3-Hb), 2.32–2.60 (3 H, m, 4-H and CH_2CO_2H), 2.80–2.95 (1 H, m, 5-Ha), 3.50–3.60 (1 H, m, 5-Hb) and 4.00–4.10 (1 H, m, 2-H); δ_C(50.32 MHz; D_2O + MeOH) 176.44, 174.24, 60.79, 50.14, 36.43, 34.39 and 33.39.

(2*R*,4*S*)-4-(Carboxymethyl)pyrrolidine-2-carboxylic acid 17a

A magnetically stirred suspension of **17** (0.100 g, 0.29 mmol) in 6 mol dm^{-3} HCl (5 cm^3) was refluxed for 12 h. After cooling, the reaction mixture was extracted with chloroform (5 cm^3) and then neutralized with 10% aqueous NH_4OH and concentrated under reduced pressure. The residue was diluted with water (5 cm^3) and submitted to cation exchange resin chromatography, eluting with 10% pyridine to afford the title compound (0.050 g, 98%), mp 232–233 °C (Found: C, 48.6; H, 6.55; N, 8.1. $C_7H_{11}NO_4$ requires C, 48.55; H, 6.40; N, 8.09%); $[\alpha]_D^{22}$ +34.2 (*c* 0.5, H_2O); δ_H(200 MHz; D_2O) 1.50–1.70 (1 H, m, 3-Ha), 2.38–2.70 (4 H, m, CH_2CO_2H, 3-Hb, 4-H), 2.85–3.00 (1 H, m, 5-Ha), 3.40–3.60 (1 H, m, 5-Hb) and 3.95–4.08 (1 H, m, 2-H); δ_C(50.32 MHz; D_2O + MeOH 99:1) 177.17, 174.82, 61.97, 50.78, 37.26, 35.46 and 35.34.

(2*S*,4*S*)-4-(Carboxymethyl)pyrrolidine-2-carboxylic acid 18a

A magnetically stirred suspension of **18** (0.070 g, 0.20 mmol) in 6 mol dm^{-3} HCl (3.5 cm^3) was refluxed for 12 h. After cooling, the reaction mixture was extracted with chloroform (5 cm^3) and then neutralized with 10% aqueous NH_4OH and concentrated under reduced pressure. The residue was diluted with water (5 cm^3) and submitted to cation exchange resin chromatography, eluting with 10% pyridine to afford the title compound (0.030 g, 84%), mp 228–229 °C (Found: C, 48.45; H, 6.6; N, 8.15. $C_7H_{11}NO_4$ requires C, 48.55; H, 6.40; N, 8.09%); $[\alpha]_D^{22}$ −66.5 (*c* 0.5, H_2O) {lit.,[8] $[\alpha]_D^{24}$ −69 (*c* 0.5, H_2O)}; δ_H(200 MHz; D_2O) 1.80–2.00 (1 H, m, 3-Ha), 2.12–2.30 (1 H, m, 3-Hb), 2.32–2.60 (3 H, m, CH_2CO_2H and 4-H), 2.80–2.95 (1 H, m, 5-Ha), 3.50–3.60 (1 H, m, 5-Hb) and 4.00–4.10 (1 H, m, 2-H); δ_C(50.32 MHz; D_2O + MeOH 99:1) 177.03, 174.93, 61.55, 50.90, 37.06, 35.15 and 34.13.

(2*S*,4*R*)-4-(Carboxymethyl)pyrrolidine-2-carboxylic acid 19a

A magnetically stirred suspension of **19** (0.045 g, 0.132 mmol) in 6 mol dm^{-3} HCl (3 cm^3) was refluxed for 12 h. After cooling, the reaction mixture was extracted with chloroform (5 cm^3) and then neutralized with 10% NH_4OH and concentrated under reduced pressure. The residue was diluted with water (5 cm^3) and submitted to cation exchange resin chromatography, eluting with 10% pyridine to afford the title compound (0.019 g, 83%), mp 234–235 °C (Found: C, 48.5; H, 6.4; N, 8.1. $C_7H_{11}NO_4$ requires C, 48.55; H, 6.40; N, 8.09%); $[\alpha]_D^{22}$ −33.4 (*c* 0.35, H_2O); δ_H(200 MHz; D_2O) 1.50–1.70 (1 H, m, 3-Ha), 2.20–2.68 (4 H, m, CH_2CO_2H, 3-Hb and 4-H), 2.85–3.00 (1 H, m, 5-Ha), 3.40–3.50 (1 H, m, 5-Hb) and 3.95–4.08 (1 H, m, 2-H); δ_C(50.32 MHz; D_2O + MeOH 99:1) 177.84, 174.97, 62.06, 50.85, 37.83, 35.71 and 35.46.

Methyl (2*S*)-1-benzyloxycarbonyl-4-[(*tert*-butoxycarbonyl)-methylidene]pyrrolidine-2-carboxylate 20

tert-Butyl (trimethylsilyl)acetate (1.634 g, 8.7 mmol) was added dropwise in 5 min *via* a syringe pump to a cold (−78 °C) solution of lithium diisopropylamide [prepared from addition of butyllithium in hexane (2.5 mol dm^{-3} solution, 3.5 cm^3) to a

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