Stereospecific synthesis of naturally-occurring 4-alkylideneglutamic acids, 4-alkylglutamates and 4-alkylprolines[1]

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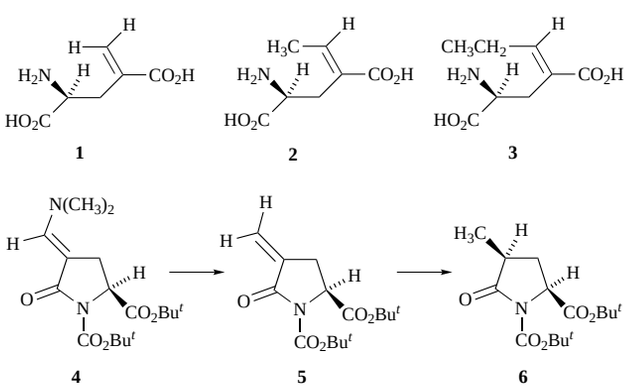
The enaminone 4, prepared from (2*S*)-pyroglutamic acid, has been found to react in an apparent 1,4-manner with DIBAL and Grignard reagents to afford a variety of alkylidene derivatives 8 which, except for the vinyl derivatives 8e, are formed only as the (*E*)-isomers. Three of these have been converted to the 4-alkylideneglutamic acids, 1, 2 and 3, which are identical to known natural products, the synthesis confirming 2 and 3 as the *E*-isomers. Catalytic reduction of the 4-alkylidenepyroglutamate derivatives 8 is stereospecific and affords an effective route to (2*S*,4*S*)-4-alkylglutamic acids and (2*S*,4*S*)-4-alkylprolines. Cuprate addition to the enone 5 affords access to the (2*S*,4*R*)-epimer 15 and carbene addition allows cyclopropylglutamic acids 32 and 33 to be prepared.

In addition to the amino acids found in proteins, nearly one thousand naturally occurring non-proteinogenic amino acids have now been discovered.[2,3] There has recently been enormous interest in these natural products and in unnatural synthetic amino acids[4] for their enzyme inhibitory and anti-metabolite properties and their ability to impart protease resistance and to induce conformation when incorporated into proteins. Our use of such compounds in proteins has allowed us to probe the three dimensional structure of an enzyme.[5] Substituted glutamic acids are of particular interest because of their interaction with glutamate receptors in the central nervous system (CNS) and their involvement in many other biological processes.[6] Proline and its analogues are constituent amino acids in antibiotics,[7–10] and are of interest in angiotensin converting enzyme inhibitors[11–13] and in providing conformational constraint in proteins.[14] We now report a method of synthesis of optically pure (*E*)-4-alkylidenepyroglutamate derivatives which is general and which we have used to gain access to three naturally-occurring non-proteinogenic 4-alkylideneglutamic acids, among other products. The synthesis has also been adapted to provide a general and stereospecific synthesis of (2*S*,4*S*)-4-alkylglutamic acid and (2*S*,4*S*)-4-alkylproline derivatives and to provide a further stereoselective route to the (2*S*,4*R*)-epimers and to spiro cyclopropyl glutamic acid derivatives.

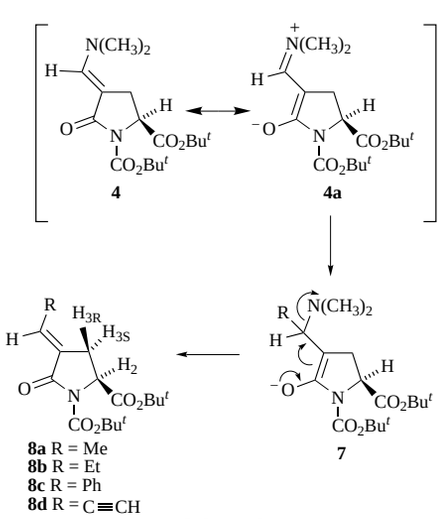
4-Methyleneglutamic acid **1** was first isolated as a product of germinated peanuts[15] and was then found in a variety of other plants.[16–24] It has been shown to exhibit strong CNS inhibitory action,[25] and, in peptides, to inhibit the vitamin K mediated γ-carboxylation of glutamic acid residues which is important in the blood clotting process.[26] 4-Ethylideneglutamic acid **2** is also a widespread amino acid in plants,[19–22,24,27] and 4-propylideneglutamic acid **3** has been found in the fungus *Mycena pura*.[20] Synthesis of compounds **1** and **2** has previously been achieved by reaction of substituted aziridines with stabilised Wittig reagents followed by reaction of the ylide product with carbonyl compounds.[28]

Our interest in the stereospecific synthesis of amino acids[29] led us to consider 4-alkylidenepyroglutamic acids as possible synthetic intermediates since we had shown in our synthesis of stereospecifically deuteriated leucine[30] that the enaminone **4** and the enone **5** could be reduced stereospecifically to yield the *cis*-4-methylpyroglutamate derivative **6** as shown in Scheme 1. We therefore reasoned that a general synthesis of 4-alkylidenepyroglutamic acids might allow access to stereospecifically alkylated derivatives of glutamic acid and proline. In developing such a general synthesis we have prepared the three 4-alkylideneglutamic acid natural products **1**, **2** and **3**.



Scheme 1

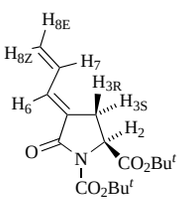
Our approach to 4-alkylidenepyroglutamic acid derivatives **8** was suggested by the fact that we had shown[30] that, in spite of its general tendency to react in a 1,2-fashion, DIBAL reduction of the enamine **4** led to the exomethylene derivative **5** by what appeared to be 1,4-addition followed by elimination. This suggested that the enaminone **4** might react directly with Grignard reagents to yield the alkylidene derivatives **8** as shown in Scheme 2 and that the reaction might be considered as a 1,2-



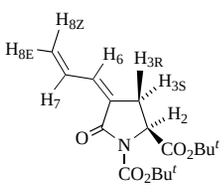
Scheme 2

addition to the resonance form **4a** of the vinylogous amide. A search of the literature showed that other vinylogous amides had been converted to alkylidene derivatives in this way.[31,32] The danger of 1,2-attack to give ring-opened products was real, however, since reaction of protected pyroglutamates with Grignard reagents has been shown to proceed with ring opening to yield chain elongated amino acids.[33,34] However, should 1,4-attack proceed as with other vinylogous amides,[31,32] the intermediate **7** might not undergo elimination until the magnesium salt was quenched and so ring opening would be prevented, even if excess Grignard reagent were used.

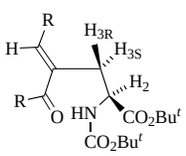
When the enaminone **4**[30] was reacted with 1.1 equiv. of methylmagnesium bromide in Et_2O at −78 °C, the (*E*)-ethylidene derivative **8a** was obtained in 40% yield. Increasing the amount of Grignard reagent to 2.5 equiv. caused the yield to increase to 71%. Only a very small amount of the methyl ketone **9a**, formed by ring opening, was obtained. In keeping with other work,[33,34] none of the alcohol which would result from attack of the Grignard reagent on the ketone **9a** was obtained and no reaction with the ester group was observed. The (*E*)-stereochemistry of the product **8a** was assigned on the basis of the observed nuclear Overhauser enhancement to H-3R when the methyl group of the ethylidene moiety † was irradiated, H-3R and H-3S being assigned on the basis of the presence of an NOE between H-2 and H-3S.



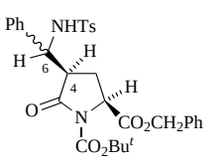
8e



8f



9a R = Me
9c R = Ph



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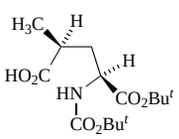

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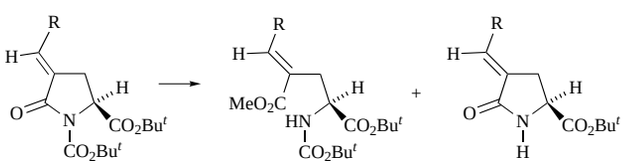
Reaction of the enaminone **4** with EtMgBr, PhMgBr and HC≡CMgBr gave the products **8b**, **8c** and **8d** in yields of 56, 78 and 59% respectively. (*E*)-Stereochemistry could be assigned to **8b** by observation of an NOE between the methylene of the ethyl group and H-3S, and to **8c** by an NOE between the aromatic protons and both H-3R and H-3S. The stereochemistry of the single isomer of **8d** was assigned by analogy, as no useful NOE could be observed. A small amount of the by-product **9c** was obtained in the reaction with phenylmagnesium bromide. When the enaminone **4** was treated with vinylmagnesium bromide, the major product, obtained in 47% yield, was evidently the (*E*)-isomer **8e**, since an NOE was observed between H_7 and both H-3R and H-3S. There was also a very small amount (1.6%) of the (*Z*)-isomer **8f** which exhibited an NOE between H-6 and H-3R.

The reaction proved to be general and remarkably stereospecific, the (*E*)-isomer being the preferred product in each case. It was felt that this was not necessarily due to initial attack of the Grignard reagent being stereospecific, since we showed in our work on imine addition[29b] that base catalysed elimination of both (6*R*)- and (6*S*)-isomers of the adduct **10** yielded the benzyl ester corresponding to the (*E*)-benzylidene derivative **8c** as the only isomer. Presumably in elimination of dimethylamine from the intermediate **7**, steric interaction of the alkyl group, R, and the imide carbonyl group must play an important role in determining the stereochemistry of the product.

Although reaction to yield the ring opened ketones **9** was not a problem in our synthesis, we were interested in the possibility of preparing these compounds in reasonable yields. The 4-ethylidene derivative **8a** was therefore reacted with MeMgBr at room temperature when the ketone **9a** was obtained in good yield. The fact that no 1,4-addition was observed in this reaction would suggest that the resonance form **4a** is important in achieving the original addition to the enaminone to yield the 4-alkylidenepyroglutamate derivative **8**. This might therefore suggest that the reaction be regarded as 1,2-attack on the iminium form **4a** rather than 1,4-attack on the enaminone form **4**.

Since 4-methyleneglutamic acid, 4-ethylideneglutamic acid and 4-propylideneglutamic acid were known to be natural products, a synthesis of these compounds required methods of ring opening of the pyroglutamates **8**. In previous studies, we had found that the saturated pyroglutamate **6** could be converted to the acid **11** by treatment with aqueous LiOH in THF,[30] but this was not successful with the less electrophilic carbonyl group in the unsaturated compounds **8**. When **5**,[30] **8a** and **8b** were reacted with LiOMe in THF at −40 °C, however, good yields of the esters **12h**, **12a** and **12b** were obtained as shown in Scheme 3. In addition to the ring opening reaction of



5, 8 → **12a** R = Me, **12b** R = Et, **12h** R = H + **13a** R = Me, **13b** R = Et

Scheme 3

the ethylidene and propylidene compounds, removal of the N-protecting group was observed and **13a** and **13b** were obtained in small amounts as by-products in these reactions. The protected esters **12** were hydrolysed to the amino acids **1**, **2** and **3** using 6 M HCl. The acid **2** exhibited an NOE at both H-3 protons when the methyl group of the ethylidene moiety was irradiated, and so the double bond had maintained the (*E*)-stereochemistry in both the ring opening reaction and the deprotection step.

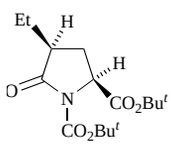
The sample of synthetic 4-methyleneglutamic acid **1**, $[\alpha]_D$ +13.2 (*c* 1, 3 M HCl), {lit.,[24] $[\alpha]_D$ +13.2 (*c* 2, 3 M HCl)} was spectroscopically identical to a sample obtained from *Lilium maximowiczii*, provided by Kasai *et al.*[24] The synthetic sample of 4-ethylideneglutamic acid **2**, $[\alpha]_D$ +27 (*c* 0.67, 3 M HCl) {lit.,[24] $[\alpha]_D$ +29.4 (*c* 1.4, 6 M HCl)} was identical in all respects with samples isolated from *Tulipa gesueriana*, provided by Kasai *et al.*,[24] and from *Mycena pura*, provided by Hatanaka and Katayama.[20] The sample of 4-propylideneglutamic acid **3** obtained synthetically was identical to a sample isolated from *Mycena pura*, provided by Hatanaka and Katayama,[20] and the synthesis defined the double bond geometry as (*E*).

Use of protected pyroglutamic acids is also attractive as a potential route to stereospecifically alkylated glutamic acid and proline derivatives, and reactions of the anion α to the amide carbonyl group of pyroglutamic acid have been investigated as possible routes to 4-substituted derivatives. Variable stereoselectivity in syntheses using highly modified derivatives[11,35,36] and in aldol condensations at C-4 of simple protected pyroglutamates[37,38] have so far made such an approach less useful, although we have achieved stereospecificity at C-4 in a high yielding aldol-type reaction using activated imines as the electrophiles and have also observed considerable stereoselectivity at the second asymmetric centre to be generated in this reac-

† For the purposes of ^1H and ^{13}C NMR spectral data the numbering system shown in compound **8e** below is used. The IUPAC nomenclature system, using a different numbering, is used to construct names however.

tion.[29] Direct alkylation of the α-anion of protected pyroglutamate derivatives has proved to be unrewarding as a stereospecific route to non-proteinogenic amino acids except in the case of benzylation.[38] Our discovery that the enone **5**, which is readily accessed from the corresponding protected pyroglutamic acid, can be catalytically hydrogenated from the less hindered side to yield the 4-methylpyroglutamate **6** with *cis*-stereospecificity[30] in spite of the reported lack of stereospecificity in reduction of 4-methyleneproline derivatives[10] suggested that catalytic reduction of 4-alkylidenepyroglutamic acid derivatives would allow a more general synthesis of (2*S*,4*S*)-4-alkylpyroglutamic acids to be developed. Access to the corresponding (2*S*,4*R*)-4-alkylpyroglutamates might be gained by conjugate addition of cuprates to the 4-alkylidenepyroglutamic acid derivatives since the thermodynamically more stable *trans* product at C_4 would be expected. If attack of the cuprate were from the less hindered face, then a second new chiral centre would be generated at C-6.

When the 4-ethylidene derivative **8b** was hydrogenated using 10% Pd–C in ethyl acetate, the product obtained in 99% yield was a single diastereoisomer. Overlap in the ^{1}H NMR spectra in various solvents, however, precluded easy assignment of stereochemistry. Although the (2*S*,4*S*)-product **14** was expected from



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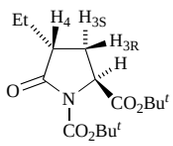

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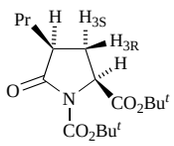
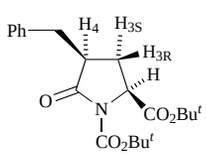


16

the reaction, this was proved when the (2*S*,4*R*)-isomer **15** was prepared and its stereochemistry assigned unambiguously as described below. The corresponding (2*S*,4*S*)-4-propylpyroglutamic acid derivative **16** was obtained as a single diastereoisomer by this route in 98% yield from the 4-propylidenepyroglutamate **8b**.

Hydrogenation of the (*E*)-benzylidene derivative **8c** using 10% Pd–C gave a 63% yield of a single diastereoisomer, the (2*S*,4*S*)-benzyl derivative **17**, which exhibited a clear NOE between H-3R and one of the benzyl protons and between H-2 and H-3S. This compound had possibilities for developing functionality on the side chain, and, when it was oxidised with ruthenium(III) chloride and sodium periodate, the acid **18** was



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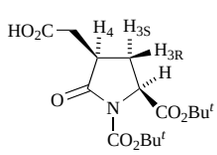

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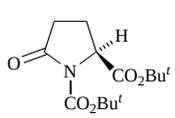

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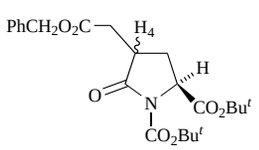

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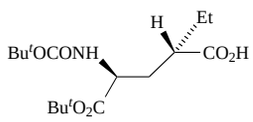
obtained in 54% yield. The stereochemistry of this product was confirmed as (2*S*,4*S*) by the observation of an NOE between H-3S and H-4 and between H-2 and H-3S. In connection with studies related to the synthesis of glutamate antagonists, we have alkylated *tert*-butyl *N*-*tert*-butoxycarbonylpyroglutamate **19** using benzyl bromoacetate and lithium hexamethyldisilazide (LHMDS) in THF.[39] As was subsequently confirmed on related compounds by other workers,[40,41] the reaction was non-stereospecific and led to a mixture of stereoisomers **20**. Separation and hydrogenolysis of the *cis*-isomer gave a sample of the acid **18**, identical spectroscopically with the compound prepared by oxidation of **17**.

To demonstrate that the synthetic *cis*-4-alkylpyroglutamic acid derivatives could be converted to the corresponding (2*S*,4*S*)-4-alkylglutamic acids, the ethyl derivative **14** was hydrolysed with aqueous LiOH in THF to give the acid **21** as a single diastereoisomer in 68% yield. This and the corresponding methyl derivative **11**[30] were then treated with 6 M HCl to yield the hydrochlorides of (2*S*,4*S*)-4-methylglutamic acid **22a** in 100% yield and (2*S*,4*S*)-4-ethylglutamic acid **22b** in 85% yield. The sample of **22a** was different from an authentic sample of the natural product supplied by Kasai *et al*. who isolated it from a variety of plant sources and designated it (2*S*,4*R*)-4-methylglutamic acid **23**.[24] The synthesis thus confirmed this assignment.



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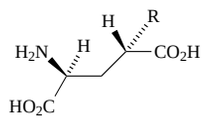
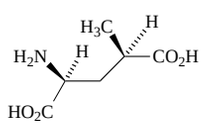
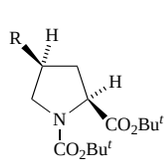


22a R = Me
22b R = Et

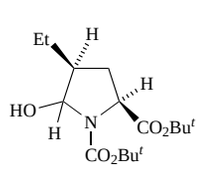


23

The 4-alkylpyroglutamic acid derivatives were now converted to the corresponding protected (2*S*,4*S*)-4-alkylprolines using borane–dimethyl sulfide in THF. The methyl derivative **24a** had already been prepared in this way in 65% yield,[5b] and the ethyl derivative **24b** was now prepared in 53% yield, and the 4-propyl derivative **24c** in 66% yield. The amino alcohol **25** was obtained as a single diastereoisomer as a by-product of reduction of the 4-ethylpyroglutamate derivative **14**. This would be the (2*S*,4*S*,5*S*)-isomer if, as expected, attack of the borane were from the less hindered side, but the complication of the ^{1}H NMR spectrum due to the conformational isomerism did not allow this to be verified. ^{1}H and ^{13}C NMR spectra of the protected prolines were complicated by conformational isomerism, although the ^{1}H NMR spectrum could be simplified by use of variable temperature techniques. Deprotection of these derivatives in 6 M HCl gave the desired (2*S*,4*S*)-4-alkylprolines **26a** and **26c** as the hydrochlorides in 94 and 89% yields respectively.



24a R = Me
24b R = Et
24c R = Pr



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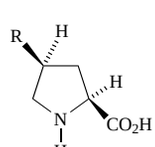


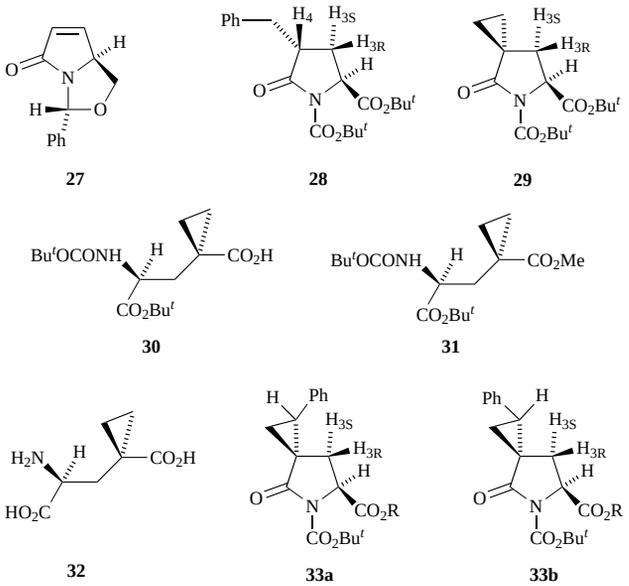
26a R = Me
26c R = Pr

cis-4-Methylproline **26a** has been isolated from natural sources but the reported $[\alpha]_D$ (−43 in 3 M HCl)[42] differed from that found for our synthetic product ($[\alpha]_D$ −19). The analytical and spectroscopic data were, however, in keeping with our structure, and an NOE between H-2 and H-3S and between the methyl and H-3R confirmed the *cis*-stereochemistry.

Having succeeded in using pyroglutamic acid for the synthesis of 4-alkylideneglutamic acids, 4-alkylglutamic acids and 4-alkylproline derivatives as single diastereoisomers, it was of interest to investigate the synthesis of the isomeric series of compounds which were epimeric at C-4. The enone **5** was therefore reacted with lithium dimethylcuprate in diethyl ether at

−78 °C. Although excellent control of stereochemistry had been reported in 3-alkylation of endocyclic enones such as **27** with alkyl cuprates,[36,43,44] we obtained a mixture of two diastereoisomers in 84% yield from the reaction of the exocyclic enone **5**. The mixture consisted of 20% of the (2*S*,4*S*)-isomer **14**, having an identical ^{1}H NMR spectrum as the product from hydrogenation of the enone **8a**, and 80% of the (2*S*,4*R*)-isomer **15**. The epimers were difficult to purify by flash chromatography but a pure sample of the (2*S*,4*R*)-isomer **15** was obtained, and its ^{1}H NMR spectrum in [^{2}H$_6$]benzene was well resolved. An NOE was observed between the protons H-3R and H-4 and between the protons H-3S and H-2 of this compound, confirming its stereochemistry. Thus, while our synthesis of the *cis*-isomer **14** was completely stereoselective, synthesis of the epimeric *trans*-isomer **15** was stereoselective to the extent of 4:1 in favour of the (2*S*,4*R*)-isomer. Reaction of the enone **5** with lithium diphenylcuprate gave a mixture of the *trans*-isomer **28** and the *cis*-isomer **17** in a ratio of 6:1.

In view of the current interest in cyclopropyl amino acids,[45] carbene addition to the enones **8** seemed to afford a facile route to spiro cyclopropyl glutamate derivatives. Reaction of the enone **5** with diazomethane and palladium acetate gave the crystalline product **29** in 67% yield. Ring opening to the acid **30** using LiOH–THF occurred in only 18% yield but LiOMe treatment gave the diester **31** in 72% yield. Treatment of this latter compound with 6 M HCl at reflux then yielded the amino acid **32** in 84% yield. An attempt was now made to see if addition of carbene to the enone **8c** would occur stereospecifically, creating two new chiral centres. Reaction of this compound with diazomethane and palladium acetate, however, yielded a mixture of the four possible isomers in a ratio of 11:8:9:2. Two of these compounds were isolated as white solids and were assigned the *trans* structures **33a** and **33b** from their spectroscopic properties.



Experimental

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations (given in units of 10^{-1} deg cm^2 g^{-1}) were measured on a Perkin-Elmer PE241 polarimeter, using a 1 dm path length micro cell. IR Spectra were recorded on a Perkin-Elmer 1720 Fourier transform instrument, and UV spectra on a Philips PU8720 UV/VIS scanning spectrophotometer. ^{1}H NMR spectra were recorded on Bruker WM 360 (360 MHz) and AMX 500 (500 MHz) Fourier transform instruments. *J* Values are given in Hz. ^{13}C NMR spectra (broad band ^{1}H decoupled) were recorded on Bruker WM 360 (90.6 MHz), AMX 500 (125.8 MHz) and AC-P 250 (62.9 MHz) Fourier transform instruments. Insensitive nuclei enhanced by polarisation transfer (INEPT) experiments were used to help assign ^{13}C NMR resonances where necessary. Residual solvent peaks were used as an internal reference in the NMR spectra. Mass spectra were recorded on Kratos MS80RF, MS50 and MS25 spectrometers and accurate mass measurements were recorded on Kratos MS80RF and V67070 spectrometers by Dr S. Chotai (Wellcome Research Laboratories) and a VG/Fisons AutoSpec instrument by Dr A. Abdul-Sada (Sussex). Microanalyses were performed by Mrs P Firmin (Wellcome Research Laboratories), and Miss K. Plowman and Miss M. Patel (Sussex). Thin layer chromatography was performed using Merck Kieselgel 60 F_{254} pre-coated silica gel plates of thickness 0.2 mm (ART 5554) and column chromatography was performed using Merck Kieselgel 60 (230–400 mesh—ART 9385).

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-ethylidenepyroglutamate 8a

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-dimethylaminomethylenepyroglutamate **4**[30] (1.00 g, 2.94 mmol) was dissolved in diethyl ether (60 ml) and cooled to −78 °C under argon. Methylmagnesium bromide (3.0 M in diethyl ether, 2.45 ml, 7.35 mmol) was added dropwise with stirring which was continued at −78 °C for 1 h. The mixture was allowed to warm to room temperature and stirring was continued for a further 3 h. The reaction was quenched with saturated aqueous ammonium chloride (60 ml) at room temperature, stirred overnight at room temperature and extracted with diethyl ether (3 × 100 ml). The combined organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford a pale yellow solid which was purified by column chromatography on silica gel using light petroleum (40–60 °C)–diethyl ether (1:1) as eluent to afford the major product as a white solid and the minor product as a colourless oil. The major product tert-*butyl* (2S)-N-tert-*butoxycarbonyl*-4-*ethylidenepyroglutamate* **8a** was recrystallised from diethyl ether–light petroleum (40–60 °C) to give a white crystalline solid (653 mg, 71%), mp 120 °C; $[\alpha]_D^{23}$ −16.0 (*c* 1, $CHCl_3$) (Found: C, 61.8; H, 8.1; N, 4.4. $C_{16}H_{25}NO_5$ requires C, 61.7; H, 8.0, N, 4.5%); *m*/*z* [+ve FAB (3-NBA)] 312 [M + H]$^+$; ν_{max}(KBr)/cm^{-1} 1774 ('imide'), 1739 (ester) and 1678 (C=C); λ_{max}(MeOH)/nm 236 (ε 24 500); δ_H(360 MHz, C^2HCl_3) 6.76 (1H, m, $J_{6,Me}$ 7.2, $J_{6,3S}$ $J_{6,3R}$ 2.6, H-6), 4.48 (1H, dd, $J_{2,3S}$ 10.3, $J_{2,3R}$ 3.4, H-2), 2.91 (1H, m, $J_{3S,3R}$ 17.3, $J_{3S,2}$ 10.3, $J_{3S,Me}$ 2, $J_{3S,6}$ 2.6, H-3S), 2.53 (1H, m, $J_{3R,3S}$ 17.3, $J_{3R,2}$ 3.4, $J_{3R,6}$ 2.6, $J_{3R,Me}$ 1.8, H-3R), 1.79 (3H, m, $J_{Me,6}$ 7.2, $J_{Me,3S}$ $J_{Me,3R}$ 1.8, CH_3), 1.50 [9H, s, $OC(CH_3)_3$] and 1.45 [9H, s, $OC(CH_3)_3$]; irradiation of the methyl resonance at δ 1.79 resulted in NOEs in the olefinic proton at δ 6.76 and in H-3R at δ 2.53; δ_C(125.8 MHz, C^2HCl_3) 170.3 (1-CO_2), 166.0 (CON), 149.9 (urethane), 133.8 (C-6), 129.6 (C-4), 83.0 [O*C*$(CH_3)_3$], 82.1 [O*C*$(CH_3)_3$], 56.3 (C-2), 27.8 [OC(*C*$H_3)_3$], 27.7 [OC(*C*$H_3)_3$], 25.4 (C-3) and 14.8 (CH_3). The minor product tert-*butyl* (2S)-2-tert-*butoxycarbonylamino*-4-*ethylidene*-5-*oxohexanoate* **9a** was an oil (39 mg, 4%), $[\alpha]_D^{21}$ −5.2 (*c* 1, $CHCl_3$); *m*/*z* [+ve FAB (3-NBA)] 328 [M + H]$^+$; ν_{max}(film)/cm^{-1} 3380 (br, NH), 1714 (ester) and 1669 (C=C); λ_{max}(MeOH)/nm 227 (ε 3200); δ_H(360 MHz, C^2HCl_3) 6.85 (1H, m, $J_{6,Me}$ 7.1, $J_{6,3S}$ $J_{6,3R}$ 2.8, H-6), 5.20 (1H, d, $J_{NH,2}$ 7.9, exchangeable, NH), 4.10 (1H, dd, $J_{2,3A}$ 14.4, $J_{2,NH}$ 8.3, H-2), 2.68 (2H, m, 2 × H-3), 2.30 (3H, s, $COCH_3$), 1.94 (3H, d, $J_{Me,6}$ 7.1, CH_3), 1.44 [9H, s, $OC(CH_3)_3$] and 1.39 [9H, s, OC(C*H*$_3)_3$]; δ_C(125.8 MHz, C^2HCl_3) 199.3 (5-CO), 171.3 (1-CO_2), 155.4 (urethane), 141.4 (C-6), 139.1 (C-4), 81.7 [O*C*$(CH_3)_3$], 79.3 [O*C*$(CH_3)_3$], 53.8 (C-2), 28.3 [OC(*C*$H_3)_3$], 28.0 [OC(*C*$H_3)_3$], 28.0 (C-3), 18.4 (CO*C*H_3) and 15.0 (CH_3).

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-propylidenepyroglutamate 8b

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-dimethylaminomethylenepyroglutamate **4**[30] (100 mg, 0.294 mmol) was dissolved in

diethyl ether (15 ml) and cooled to −78 °C under argon. Ethylmagnesium bromide (3.0 M in diethyl ether, 0.37 ml, 1.1 mmol) was added dropwise with stirring which was continued at −78 °C for 1 h. The mixture was allowed to warm to room temperature and was stirred for a further 3 h and quenched with saturated aqueous ammonium chloride (20 ml) at room temperature. The mixture was stirred overnight at room temperature and extracted with diethyl ether (3 × 25 ml). The organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford a pale yellow solid, which was purified by column chromatography on silica gel using light petroleum (40–60 °C)–diethyl ether (1 : 1) as eluent to afford tert-*butyl* (2S)-N-tert-*butoxycarbonyl*-4-*propylidenepyroglutamate* **8b**, which was recrystallised from diethyl ether–light petroleum as a white crystalline solid (53 mg, 56%), mp 69–70 °C; $[\alpha]_D^{28}$ −8.5 (*c* 1, $CHCl_3$) (Found: C, 62.8; H, 8.6; N, 4.1. $C_{17}H_{27}NO_5$ requires C, 62.8; H, 8.3; N, 4.3%); *m/z* [+ve FAB (3-NBA)] 326 $[M + H]^+$; ν_{max}(KBr)/cm^{-1} 1776 ('imide'), 1738 (ester) and 1675 (C=C); λ_{max}(MeOH)/nm 238 (ε 26 400); δ_H(360 MHz, C^2HCl_3) 6.66 (1H, m, $J_{6,7}$ 7.5, $J_{6,3S}$ 4.8, $J_{6,3R}$ 2.6, H-6), 4.45 (1H, dd, $J_{2,3S}$ 10.3, $J_{2,3R}$ 3.4, H-2), 2.89 (1H, m, $J_{3S,3R}$ 17.3, $J_{3S,2}$ 10.3, $J_{3S,6}$ 4.8, $J_{3S,7}$ 1.8, H-3S), 2.51 (1H, m, $J_{3R,3S}$ 17.3, $J_{3R,2}$ 3.4, $J_{3R,6}$ 2.6, H-3R), 2.11 (2H, q, $J_{7A,7B}$ 15.4, $J_{7,8}$ $J_{7,6}$ 7.5, 7-CH_2), 1.50 [9H, s, $OC(CH_3)_3$], 1.45 [9H, s, $OC(CH_3)_3$] and 1.02 (3H, t, *J* 7.5, CH_3); irradiation of the CH_3CH_2 absorption at δ 2.11 resulted in an NOE in H-3R at δ 2.51 and in H-3S at δ 2.89; δ_C(125.8 MHz, C^2HCl_3) 170.3 (1-CO_2), 166.2 (CON), 150.0 (urethane), 140.2 (C-6), 128.1 (C-4), 83.1 [$OC(CH_3)_3$], 82.1 [$OC(CH_3)_3$], 56.5 (C-2), 27.93 [$OC(CH_3)_3$], 27.86 [$OC(CH_3)_3$], 25.7 (C-3), 22.7 (C-7) and 12.6 (CH_3).

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-benzylidenepyroglutamate 8c

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-dimethylaminomethylenepyroglutamate **4**[30] (3.0 g, 8.82 mmol) was dissolved in diethyl ether (150 ml) and cooled to −78 °C under argon. A solution of phenylmagnesium bromide (3 M in diethyl ether, 3.53 ml, 10.6 mmol) was added dropwise with stirring which was continued at −78 °C for 1 h. The mixture was allowed to warm to room temperature, stirring was continued for a further 3 h and the reaction was quenched with saturated aqueous ammonium chloride (150 ml) at room temperature. The mixture was stirred overnight at room temperature and extracted with diethyl ether (3 × 100 ml). The organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford a pale yellow solid, which was purified by column chromatography on silica gel using chloroform–ethyl acetate (97 : 3) as eluent to afford the major product as a white solid and the minor product as a colourless oil. The major product was recrystallised from diethyl ether–chloroform to yield tert-*butyl* (2S)-N-tert-*butoxycarbonyl*-4-*benzylidenepyroglutamate* **8c** as a white crystalline solid (2.57 g, 78%), mp 158–159 °C; $[\alpha]_D^{25}$ +3.2 (*c* 0.5, $CHCl_3$); *m/z* (EI) found 373.18769; $C_{21}H_{27}NO_5$ requires 373.188923; *m/z* [+ve FAB (3-NBA)] 374 $[M + H]^+$; ν_{max}(KBr)/cm^{-1} 1775 ('imide'), 1737 (ester) and 1654 (C=C); λ_{max}(MeOH)/nm 290 (ε 30 000); δ_H(360 MHz, C^2HCl_3) 7.55 (1H, t, $J_{6,3S}$ $J_{6,3R}$ 2.8, H-6), 7.38 (5H, m, ArH), 4.59 (1H, dd, $J_{2,3S}$ 10.1, $J_{2,3R}$ 3.1, H-2), 3.35 (1H, m, $J_{3S,3R}$ 17.9, $J_{3S,2}$ 10.1, $J_{3S,6}$ 2.8, H-3S), 2.92 (1H, m, $J_{3R,3S}$ 17.9, $J_{3R,2}$ 3.1, $J_{3R,6}$ 2.8, H-3R), 1.55 [9H, s, $OC(CH_3)_3$] and 1.46 [9H, s, $OC(CH_3)_3$]; irradiation of the olefinic proton at δ 7.55 showed enhancement only to the aromatic protons but irradiation at H-3S at δ 3.35 gave an NOE in H-3R at δ 2.92, in H-2 at δ 4.59 and in the phenyl protons at δ 7.38; δ_C(125.8 MHz, C^2HCl_3) 165.7 (1-CO_2), 162.6 (CON), 145.4 (urethane), 130.5 (C-6), 130.2 (C-4), 125.6–122.8 (C_6H_5), 78.9 [$OC(CH_3)_3$], 77.9 [$OC(CH_3)_3$], 52.2 (C-2), 23.5 (C-3), 23.4 [$OC(CH_3)_3$] and 23.3 [$OC(CH_3)_3$]. The minor product tert-*butyl* (2S)-2-tert-*butoxycarbonylamino*-4-*benzylidene*-4-*benzoylbutanoate* **9c** (48 mg, 1.2%); *m/z* [+ve FAB (3-NBA)] 452 $[M + H]^+$; ν_{max}(film)/cm^{-1} 3372 (br, NH), 1713 (ester) and 1649 (C=C); δ_H(360 MHz, C^2HCl_3) 7.81–7.46 (10H, m, ArH), 7.41 (1H, s, H-6), 5.17 (1H, d, $J_{NH,2}$ 8.5, exch., NH), 4.48 (1H, dd, $J_{2,3A}$ 14.4, $J_{2,NH}$ 8.5, H-2), 3.16 (2H, m, H-3), 1.47 [9H, s, $OC(CH_3)_3$] and 1.30 [9H, s, $OC(CH_3)_3$]; δ_C(125.8 MHz, $C_6^2H_6$) 198.3 (5-CO), 171.3 (1-CO_2), 155.6 (urethane), 143.8 (C-6), 138.7 (C-4), 138.1–128.8 (C_6H_5), 81.6 [$OC(CH_3)_3$], 79.2 [$OC(CH_3)_3$], 54.7 (C-2), 31.1 (C-3), 28.3 [$OC(CH_3)_3$] and 27.8 [$OC(CH_3)_3$].

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-(prop-2-yn-1-ylidene)-pyroglutamate 8d

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-dimethylaminomethylenepyroglutamate **4**[30] (1.0 g, 2.94 mmol) was dissolved in diethyl ether (125 ml) and cooled to −78 °C under argon. Ethynylmagnesium bromide (0.5 M in diethyl ether, 17.6 ml, 8.8 mmol) was added dropwise with stirring which was continued at −78 °C for 1 h. The mixture was allowed to warm to room temperature, stirred for a further 5 h and quenched with saturated aqueous ammonium chloride (125 ml) at room temperature. After stirring overnight at room temperature, the mixture was extracted with diethyl ether (3 × 100 ml). The organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford tert-*butyl* (2S)-N-tert-*butoxycarbonyl*-4-(*prop*-2-*yn*-1-*ylidene*)*pyroglutamate* **8d**‡ as a pale brown oil, which was purified by column chromatography on silica gel using petroleum ether–diethyl ether (1 : 1) as eluent to give an orange oil (554 mg, 59%); *m/z* (EI) found 321.159908. $C_{17}H_{23}NO_5$ requires 321.157623; ν_{max}(film)/cm^{-1} 1794, 1703 ('imide') and 1743 (ester); λ_{max}(MeOH)/nm 263 (ε 11 000); δ_H(360 MHz, C^2HCl_3) 6.53 (1H, m, $J_{6,3S}$ 3.4, $J_{6,3R}$ $J_{6,8}$ 2.5, H-6), 4.52 (1H, dd, $J_{2,3S}$ 10.0, $J_{2,3R}$ 3.2, H-2), 3.54 (1H, m, $J_{8,6}$ 2.5, $J_{8,3S}$ 1.2, $J_{8,3R}$ 1.0, H-8), 3.11 (1H, m, $J_{3S,3R}$ 19.1, $J_{3S,2}$ 10.0, $J_{3S,6}$ 3.4, $J_{3S,8}$ 1.2, H-3S), 2.77 (1H, m, $J_{3R,3S}$ 19.1, $J_{3R,2}$ 3.2, $J_{3R,8}$ 1.0, H-3R), 1.53 [9H, s, $OC(CH_3)_3$] and 1.48 [9H, s, $OC(CH_3)_3$]; δ_C(125.8 MHz, C^2HCl_3) 169.7 (1-CO_2), 164.6 (CON), 149.7 (urethane), 142.3 (C-4), 114.0 (C-6), 89.2 (C-8), 83.8 [$OC(CH_3)_3$], 82.6 [$OC(CH_3)_3$], 79.6 (C-7), 56.4 (C-2), 27.92 [$OC(CH_3)_3$], 27.88 [$OC(CH_3)_3$] and 27.6 (C-3).

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-(prop-2-en-1-ylidene)-pyroglutamate 8e and 8f

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-dimethylaminomethylenepyroglutamate **4**[30] (4.0 g, 12 mmol) was dissolved in diethyl ether (500 ml) and cooled to −78 °C under argon. Vinylmagnesium bromide (1.0 M in diethyl ether, 35.3 ml, 35 mmol) was added dropwise with stirring which was continued at −78 °C for 1 h. The mixture was allowed to warm to room temperature, stirred for a further 3 h, quenched with saturated aqueous ammonium chloride (200 ml) at room temperature and stirred overnight at room temperature. It was extracted with diethyl ether (3 × 100 ml). The organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford a pale yellow foam, which was purified by column chromatography on silica gel using light petroleum (40–60 °C)–diethyl ether (1 : 1) as eluent to afford two white solids. The major product was recrystallised from diethyl ether–light petroleum (40–60 °C) to yield tert-*butyl* (2S)-N-tert-*butoxycarbonyl*-4-(*prop*-2-*en*-1-*ylidene*)*pyroglutamate* (*E*-isomer) **8e** as a white crystalline solid (1.79 g, 47%), mp 66–69 °C; $[\alpha]_D^{19}$ +1.4 (*c* 1, $CHCl_3$) (Found: C, 62.9; H, 7.7; N, 4.1. $C_{17}H_{25}NO_5$ requires C, 63.1; H, 7.7; N, 4.3%); *m/z* [+ve FAB (3-NBA–$CHCl_3$)] 268 $[M - C_4H_9 + 2H]^+$; ν_{max}(KBr)/cm^{-1} 1786, 1719 ('imide') and 1752 (ester); λ_{max}(MeOH)/nm 271 (ε 26 000); δ_H(360 MHz, C^2HCl_3) 7.07 (1H, m, $J_{6,7}$ 11.5, $J_{6,3S}$ $J_{6,3R}$ 2.7, H-6), 6.42 (1H, m, $J_{7,8A}$ 16.8, $J_{7,6}$ 11.4, $J_{7,8B}$ 10.1, H-7), 5.65 (1H, d, $J_{8A,7}$ 16.8, H-8A), 5.56 (1H, d, $J_{8B,7}$ 10.1, H-8B), 4.51 (1H, dd, $J_{2,3S}$ 10.1, $J_{2,3R}$ 3.4, H-2), 3.04 (1H, m, $J_{3S,3R}$ 17.9, $J_{3S,2}$ 10.1, $J_{3S,6}$ 2.7, H-3S),

‡ This compound is unstable on standing for prolonged periods at room temperature. We thank Dr D. H.-S. Poon for preparing a further sample of the compound for mass spectral analysis.

2.67 (1H, m, $J_{3R,3S}$ 17.9, $J_{3R,2}$ $J_{3R,6}$ 2.9, H-3R), 1.52 [9H, s, $OC(CH_3)_3$] and 1.46 [9H, s, $OC(CH_3)_3$]; irradiation of H-7 at δ 6.42 resulted in NOEs in H-6 at δ 7.07, in H-8B at δ 5.56, in H-3S at δ 3.04 and in H-3R at δ 2.67; δ_C(125.8 MHz, C^2HCl_3) 169.9 (1-CO_2), 166.3 (CON), 149.5 (urethane), 133.9 (C-6), 131.4 (C-7), 128.7 (C-4), 126.0 (C-8), 83.0 [$OC(CH_3)_3$], 82.0 [$OC(CH_3)_3$], 56.3 (C-2), 27.64 [$OC(CH_3)_3$], 27.60 [$OC(CH_3)_3$] and 25.7 (C-3); the minor product was tert-*butyl* (2S)-N-tert-*butoxycarbonyl*-4-(*prop*-2-*en*-1-*ylidene*)*pyroglutamate* (*Z*-isomer) **8f** (58 mg, 1.5%), mp 79–82 °C; *m/z* [+ve FAB (3-NBA–$CHCl_3$)] 168 [M − C_4H_9 − $CO_2C_4H_9$ + 3H]$^+$ and 122 [M − $(CO_2C_4H_9)_2$ + H]$^+$; ν_{max}(KBr)/cm^{-1} 1752, 1708 ('imide') and 1736 (ester); δ_H(360 MHz, C^2HCl_3) 7.82 (1H, m, $J_{7,8B}$ 17, $J_{7,6}$ 11.2, $J_{7,8A}$ 10, H-7), 6.42 (1H, m, $J_{6,7}$ 11.2, $J_{6,3S}$ 2.5, $J_{6,3R}$ 1.9, H-6), 5.41 (1H, d, $J_{8A,7}$ 10, H-8A), 5.40 (1H, d, $J_{8B,7}$ 17, H-8B), 4.44 (1H, dd, $J_{2,3S}$ 10.1, $J_{2,3R}$ 3.2, H-2), 3.04 (1H, m, $J_{3S,3R}$ 17.3, $J_{3S,2}$ 10.1, $J_{3S,6}$ 2.5, H-3S), 2.59 (1H, m, $J_{3R,3S}$ 17.3, H-3R), 1.51 [9H, s, $OC(CH_3)_3$] and 1.44 [9H, s, $OC(CH_3)_3$]; irradiation at H-6 at δ 6.42 resulted in NOEs in H-7 at δ 7.82, in H-8B at δ 5.40 and in H-3R at δ 2.59; δ_C(125.8 MHz, C^2HCl_3) 170.1 (1-CO_2), 165.5 (CON), 150 (urethane), 138.7 (C-6), 131.6 (C-7), 126.5 (C-4), 124.7 (C-8), 83.3 [$OC(CH_3)_3$], 82.2 [$OC(CH_3)_3$], 56.5 (C-2), 29.2 (C-3), 27.93 [$OC(CH_3)_3$] and 27.86 [$OC(CH_3)_3$].

tert-Butyl (2*S*)-2-*tert*-butoxycarbonylamino-4-ethylidene-5-oxohexanoate 9a

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-ethylidenepyroglutamate **8a** (100 mg, 0.322 mmol) was dissolved in diethyl ether (15 ml) and stirred under argon. Methylmagnesium bromide (3.0 M in diethyl ether, 0.54 ml, 1.61 mmol) was added dropwise with stirring which was continued at room temperature overnight. The mixture was quenched with saturated aqueous ammonium chloride (15 ml) at room temperature, stirred at room temperature for 6 h and extracted with diethyl ether (3 × 25 ml). The organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford a pale yellow oil (78 mg, 81%), which was purified by column chromatography on silica gel using light petroleum (40–60 °C)–diethyl ether (1:1) as eluent to afford tert-*butyl* (2S)-2-tert-*butoxycarbonylamino*-4-*ethylidene*-5-*oxohexanoate* **9a** as a colourless oil (37 mg, 35%) with a ^{1}H NMR spectrum identical to that of the methyl ketone **9a** synthesised as a by-product of the reaction between methylmagnesium bromide and the enaminone **4** above.

1-*tert*-Butyl 5-methyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methyleneglutamate 12h

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methylenepyroglutamate **5**[30] (100 mg, 0.34 mmol) was dissolved in tetrahydrofuran (1.7 ml) and cooled to −40 °C under argon. A 1.0 M solution of lithium methoxide in methanol (0.404 ml, 0.404 mmol) was added dropwise with stirring, which was continued at −40 °C for 30 min. The reaction mixture was quenched with saturated aqueous sodium chloride (10 ml) and ethyl acetate (10 ml) and allowed to warm to room temperature. The aqueous layer was extracted with ethyl acetate (10 ml) and the combined organic layers were dried (Na_2SO_4). The solvent was removed *in vacuo* to afford a pale yellow oil, which was purified by column chromatography on silica gel using petroleum (60–80 °C)–diethyl ether (2:1) as eluent to afford 1-tert-*butyl* 5-*methyl* (2S)-N-tert-*butoxycarbonyl*-4-*methyleneglutamate* **12h** as a white crystalline solid (66 mg, 60%), mp 81–84 °C; $[\alpha]_D^{23}$ +1.9 (*c* 1, $CHCl_3$) (Found: C, 58.2; H, 8.3; N, 4.2%. $C_{16}H_{27}NO_6$ requires C, 58.3; H, 8.2; N, 4.3%); *m/z* [+ve FAB (3-NBA)] 330 [M + H]$^+$; ν_{max}(KBr)/cm^{-1} 3346 (br, NH), 1709 (ester) and 1631 (C=C); δ_H(360 MHz, C^2HCl_3) 6.21 (1H, s, H-6A), 5.62 (1H, s, H-6B), 5.12 (1H, d, $J_{NH,2}$ 8.0, exchangeable, NH), 4.33 (1H, m, H-2), 3.74 (3H, s, OCH_3), 2.75 (1H, dd, $J_{3A,3B}$ 14.0, $J_{3A,2}$ 5.7, H-3A), 2.60 (1H, dd, $J_{3B,3A}$ 14.0, $J_{3B,2}$ 8.2, H-3B), 1.41 [9H, s, $OC(CH_3)_3$], 1.38 [9H, s, $OC(CH_3)_3$]; δ_C(125.8 MHz, C^2HCl_3) 171.0 (CO_2), 167.1 (CO_2), 155.1 (urethane), 136.0 (C-6), 128.1 (C-4), 82.1 [$O(CH_3)_3$], 79.6 [$OC(CH_3)_3$], 53.3 (C-2), 52.0 (OCH_3), 35.2 (C-3), 28.2 [$OC(CH_3)_3$] and 27.9 [$OC(CH_3)_3$].

1-*tert*-Butyl 5-methyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-ethylideneglutamate 12a

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-ethylidenepyroglutamate **8a** (75 mg, 0.241 mmol) was dissolved in tetrahydrofuran (1.2 ml) and cooled to −40 °C under argon. A 1.0 M solution of lithium methoxide in methanol (0.289 ml, 0.289 mmol) was added dropwise with stirring, and stirring was continued at −40 °C for 50 min. The mixture was quenched with saturated aqueous sodium chloride (10 ml) and ethyl acetate (10 ml) and allowed to warm to room temperature. The aqueous layer was separated and extracted with ethyl acetate (10 ml). The organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford a pale yellow oil, which was purified by column chromatography on silica gel using light petroleum (60–80 °C)–diethyl ether (2:1) as eluent to afford the major product as a colourless oil and the minor product as a white crystalline solid. The major product was 1-tert-*butyl* 5-*methyl* (2S)-N-tert-*butoxycarbonyl*-4-*ethylideneglutamate* **12a** (49 mg, 60%), $[\alpha]_D^{23}$ −0.75 (*c* 1.4, $CHCl_3$) (Found: C, 59.9; H, 8.6; N, 4.3. $C_{17}H_{29}NO_6$ requires C, 59.5; H, 8.5; N, 4.1%); *m/z* [+ve FAB (3-NBA)] 344 [M + H]$^+$; ν_{max}(film)/cm^{-1} 3377 (br, NH), 1718 (br, ester) and 1650 (C=C); δ_H(360 MHz, C^2HCl_3) 6.98 (1H, q, $J_{6,Me}$ 7.2, H-6), 5.18 (1H, d, $J_{NH,2}$ 8.1, exchangeable, NH), 4.25 (1H, m, H-2), 3.73 (3H, s, OCH_3), 2.71 (2H, m, $J_{3A,3B}$ 14.0, $J_{3A,2}$ 5.7, 2 × H-3), 1.84 (3H, d, $J_{Me,6}$ 7.2, CH_3), 1.43 [9H, s, $OC(CH_3)_3$] and 1.40 [9H, s, $O(CH_3)_3$]; δ_C(125.8 MHz, C^2HCl_3) 171.4 (CO_2), 168.0 (CO_2), 155.3 (urethane), 140.9 (C-6), 128.6 (C-4), 81.8 [$OC(CH_3)_3$], 79.5 [$OC(CH_3)_3$], 53.6 (C-2), 51.9 (OCH_3), 29.4 (C-3), 28.2 [$OC(CH_3)_3$], 27.9 [$OC(CH_3)_3$] and 14.6 (7-CH_3). The minor product was tert-*butyl* (2S)-4-*ethylidenepyroglutamate* **13a** (7 mg, 13%), mp 111–113 °C; $[\alpha]_D^{22}$ +41.1 (*c* 1, $CHCl_3$); *m/z* [+ve FAB (3-NBA)] 212 [M + H]$^+$; *m/z* (EI) found 211.12120. $C_{11}H_{17}NO_3$ requires 211.1208; ν_{max}(KBr)/cm^{-1} 3303 (br, NH), 1741 (ester) and 1654 (C=C); δ_H(360 MHz, C^2HCl_3) 6.54 (1H, m, $J_{6,Me}$ 7.1, $J_{6,3S}$ $J_{6,3R}$ 2.6, H-6), 6.39 (1H, s, exchangeable, NH), 4.16 (1H, dd, $J_{2,3S}$ 9.5, $J_{2,3R}$ 4.6, H-2), 3.02 (1H, m, $J_{3S,3R}$ 17.3, $J_{3S,2}$ 9.5, $J_{3S,6}$ 2.6, $J_{3S,Me}$ 1.9, H-3S), 2.80 (1H, m, $J_{3R,3S}$ 17.3, $J_{3R,2}$ 4.6, $J_{3R,6}$ 2.6, $J_{3R,Me}$ 1.9, H-3R), 1.78 (3H, m, $J_{Me,6}$ 7.1, $J_{Me,3S}$ $J_{Me,3R}$ 1.9, CH_3) and 1.47 [9H, s, $OC(CH_3)_3$]; δ_C(125.8 MHz, C^2HCl_3) 170.6 (1-CO_2), 130.2 (C-4), 128.2 (C-6), 81.6 [$OC(CH_3)_3$], 53.1 (C-2), 27.4 [$OC(CH_3)_3$], 27.3 (C-3) and 14.3 (CH_3).

1-*tert*-Butyl 5-methyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-propylideneglutamate 12b

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-propylidenepyroglutamate **8b** (582 mg, 1.79 mmol) was dissolved in tetrahydrofuran (9.0 ml) and cooled to −40 °C under argon. A 1.0 M solution of lithium methoxide in methanol (2.15 ml, 2.15 mmol) was added dropwise with stirring which was continued at −40 °C for 1 h. The reaction mixture was quenched with saturated aqueous sodium chloride (10 ml) and ethyl acetate (10 ml) and allowed to warm to room temperature. The aqueous layer was extracted with ethyl acetate (2 × 10 ml). The organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford a pale yellow oil, which was purified by column chromatography on silica gel using light petroleum (40–60 °C)–diethyl ether (2:1) as eluent to afford the major product as a colourless oil and the minor product as a white crystalline solid. The major product was 1-tert-*butyl* 5-*methyl* (2S)-N-tert-*butoxycarbonyl*-4-*propylideneglutamate* **12b** (324 mg, 51%), $[\alpha]_D^{25}$ −0.20 (*c* 2.0, $CHCl_3$) (Found: C, 60.1; H, 8.6; N, 4.0. $C_{18}H_{31}NO_6$ requires C, 60.5; H, 8.7; N, 3.9%); *m/z* [+ve FAB (3-NBA)] 358 [M + H]$^+$; ν_{max}(film)/cm^{-1} 3377 (br, NH), 1718 (ester) and 1648 (C=C); δ_H(500 MHz, C^2HCl_3) 6.79 (1H, t, $J_{6,7}$ 7.5, H-6), 5.14 (1H, d, $J_{NH,2}$ 8.3, exchangeable, NH), 4.18 (1H, m, $J_{2,NH}$ $J_{2,3B}$ 8.6, $J_{2,3A}$ 6.0, H-2), 3.66 (3H, s, OCH_3), 2.66 (1H, m, $J_{3A,3B}$ 13.8, $J_{3A,2}$ 6.0,

H-3A), 2.59 (1H, m, $J_{3B,3A}$ 13.8, $J_{3B,2}$ 8.7, H-3B), 2.17 (2H, quintet, $J_{7,6}$ $J_{7,8}$ 7.5, CH_2), 1.36 [9H, s, $OC(CH_3)_3$], 1.33 [9H, s, $OC(CH_3)_3$] and 0.98 (3H, t, $J_{8,7}$ 7.5, CH_3); δ_C(125.8 MHz, C^2HCl_3) 171.0 (CO_2), 167.7 (CO_2), 155.0 (urethane), 147.1 (C-6), 126.8 (C-4), 81.4 [$OC(CH_3)_3$], 79.0 [$OC(CH_3)_3$], 53.5 (C-2), 51.5 (OCH_3), 29.5 (C-3), 28.0 [$OC(CH_3)_3$], 27.7 [$OC(CH_3)_3$], 21.9 (C-7) and 12.9 (8-CH_3). The minor product was tert-*butyl* 4-*propylidenepyroglutamate* **13b** (55 mg, 14%), mp 65–67 °C; $[\alpha]_D^{24}$ +45 (*c* 1, $CHCl_3$); *m/z* [+ve FAB (3-NBA)] 226 $[M + H]^+$; ν_{max}(KBr)/cm^{-1} 3386 (br, NH), 1750 (ester), 1700 and 1678 (C=C); δ_H(360 MHz, C^2HCl_3) 7.27 (1H, s, exch., NH), 6.41 (1H, m, $J_{6,7}$ 7.4, $J_{6,3S}$ $J_{6,3R}$ *ca.* 2, H-6), 4.13 (1H, dd, $J_{2,3S}$ 9.3, $J_{2,3R}$ 4.5, H-2), 2.96 (1H, m, $J_{3S,3R}$ 17.3, $J_{3S,2}$ 9.3, $J_{3S,6}$ *ca.* 2, H-3S), 2.73 (1H, m, $J_{3R,3S}$ 17.3, $J_{3R,6}$ *ca.* 2, H-3R), 2.09 (2H, m, $J_{7,8}$ $J_{7,6}$ 7.5, CH_2), 1.42 [9H, s, $OC(CH_3)_3$] and 1.01 (3H, t, $J_{8,7}$ 7.5, CH_3); δ_C(125.8 MHz, C^2HCl_3) 170.9 (1-CO_2), 170.8 (CON), 135.7 (C-6), 128.5 (C-4), 82.2 [$OC(CH_3)_3$], 53.3 (C-2), 27.8 [$OC(CH_3)_3$], 27.7 (C-3), 22.5 (C-7) and 12.7 (CH_3).

(2*S*)-4-Methyleneglutamic acid hydrochloride 1

1-*tert*-Butyl 5-methyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methyleneglutamate **12h** (238 mg, 0.723 mmol) was heated to reflux for 2 h with 6 M aqueous hydrochloric acid (10 ml). The solvent was removed *in vacuo* to afford an off-white solid. Traces of residual hydrochloric acid were removed by azeotropic distillation with diethyl ether. The solid was recrystallised from ethanol–diethyl ether to yield (2S)-4-*methyleneglutamic acid hydrochloride* **1** as a white crystalline solid (106 mg, 81%), mp 162–164 °C; $[\alpha]_D^{23}$ +13.2 (*c* 1, 3 M HCl) (Found: C, 36.7; H, 5.1; N, 6.7. $C_6H_{10}NO_4Cl$ requires C, 36.8; H, 5.2; N, 7.2%); *m/z* [+ve FAB (glycerol–ethanol)] 160 [free amino acid + H]$^+$; ν_{max}(KBr)/cm^{-1} 3000–3500 (br, NH and OH), 1736, 1685 and 1638 (C=C); δ_H(500 MHz, 20% ^2HCl–^2H_2O) 6.49 (1H, s, H-6A), 6.08 (1H, s, H-6B), 4.42 (1H, dd, $J_{2,3B}$ 7.8, $J_{2,3A}$ 5.5, H-2), 3.11 (1H, dd, $J_{3A,3B}$ 14.7, $J_{3A,2}$ 5.5, H-3A) and 2.97 (1H, dd, $J_{3B,3A}$ 14.7, $J_{3B,2}$ 7.8, H-3B); δ_C(125.8 MHz, 20% ^2HCl–^2H_2O) 171.0 (CO_2), 169.9 (CO_2), 134.1 (6-CH_2), 133.3 (C-4), 52.8 (C-2) and 32.8 (C-3). The ^1H NMR spectrum was identical to that of a sample provided by Kasai *et al.*[24] and isolated from *L. maximowiczii*.

(2*S*)-4-Ethylideneglutamic acid hydrochloride 2

1-*tert*-Butyl 5-methyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-ethylideneglutamate **12a** (1.00 g, 2.92 mmol) was treated with 6 M aqueous hydrochloric acid (20 ml) and heated to reflux for 5 h. The solvent was removed *in vacuo* to afford an off-white solid. Traces of residual hydrochloric acid were removed by azeotropic distillation with diethyl ether to afford (2S)-4-*ethylideneglutamic acid hydrochloride* **2** as a white solid (470 mg, 77%), mp 181–184 °C; $[\alpha]_D^{18}$ +27 (*c* 0.67, 3 M HCl) (Found: C, 40.1; H, 5.7; N, 6.3. $C_7H_{12}NO_4Cl$ requires C, 40.1; H, 5.7; N, 6.7%); *m/z* [+ve FAB (glycerol–$CHCl_3$)] 174 [free amino acid + H]$^+$; ν_{max}(KBr)/cm^{-1} 2700–3300 (br, NH and OH), 1723 and 1672 (C=C); δ_H(360 MHz, $C^2H_3O^2H$) 7.17 (1H, q, $J_{6,7}$ 7.3, H-6), 4.08 (1H, dd, $J_{2,3B}$ 7.6, $J_{2,3A}$ 6.5, H-2), 2.98 (1H, dd, $J_{3A,3B}$ 14.3, $J_{3A,2}$ 6.5, H-3A), 2.83 (1H, dd, $J_{3B,3A}$ 14.3, $J_{3B,2}$ 7.6, H-3B) and 1.88 (3H, d, $J_{7,6}$ 7.2, CH_3); irradiation of the absorption due to the methyl group at δ 1.88 resulted in NOEs in the olefinic absorption at δ 7.17, in H_{3A} at δ 2.98, in H_{3B} at δ 2.83 and in H_2 at δ 4.08; δ_H(500 MHz, 20% ^2HCl–^2H_2O) 7.26 (1H, q, $J_{6,7}$ 7.3, H-6), 4.29 (1H, t, $J_{2,3B}$ $J_{2,3A}$ *ca.* 7.0, H-2), 3.06 (1H, dd, $J_{3A,3B}$ 14.8, $J_{3A,2}$ 6.5, H-3A), 3.01 (1H, dd, $J_{3B,3A}$ 14.8, $J_{3B,2}$ 7.5, H-3B) and 1.93 (3H, d, $J_{7,6}$ 7.3, CH_3); δ_C(125.8 MHz, $C^2H_3O^2H$) 171.7 (CO_2), 171.0 (CO_2), 144.7 (C-6), 128.8 (C-4), 53.9 (C-2), 29.1 (C-3) and 15.3 (CH_3). The spectra were identical with those of a sample isolated from *T. gesueriana* and supplied by Kasai *et al.*[24]

(2*S*)-4-Propylideneglutamic acid hydrochloride 3

1-*tert*-Butyl 5-methyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-propylideneglutamate **12b** (223 mg, 0.625 mmol) was heated to reflux with 6 M aqueous hydrochloric acid (5.0 ml) for 6 h. The solvent was removed *in vacuo* to afford (2S)-4-*propylideneglutamic acid hydrochloride* **3** as an off-white solid. Traces of residual hydrochloric acid were removed by azeotropic distillation with diethyl ether (118 mg, 85%); *m/z* [+ve FAB (glycerol–$CHCl_3$)] 188 [free amino acid + H]$^+$; *m/z* (EI) found 187.08349. $C_8H_{13}NO_4$ requires 187.0844; ν_{max}(KBr)/cm^{-1} 2900–3400 (br, NH and OH), 1736 (acid) and 1639 (C=C); δ_H(360 MHz, $C^2H_3O^2H$) 7.04 (1H, t, $J_{6,7}$ 7.6, H-6), 4.08 (1H, t, $J_{2,3B}$ $J_{2,3A}$ *ca.* 7, H-2), 2.95 (1H, dd, $J_{3A,3B}$ 14.3, $J_{3A,2}$ 6.7, H-3A), 2.81 (1H, dd, $J_{3B,3A}$ 14.3, $J_{3B,2}$ 7.5, H-3B), 2.27 (2H, quintet, $J_{7,6}$ $J_{7,8}$ 7.6, H-7) and 1.08 (3H, t, $J_{8,7}$ 7.5, CH_3); δ_H(500 MHz, 20% ^2HCl–^2H_2O) 4.28 (1H, t, $J_{2,3B}$ $J_{2,3A}$ 7.2, H-2), 3.01 (2H, m, $J_{3A,3B}$ 14.3, $J_{3,2}$ 7.3, 2 × H-3), 2.29 (2H, m, $J_{7,6}$ $J_{7,8}$ 7.6, 2 × H-7) and 1.06 (3H, t, $J_{8,7}$ 7.4, CH_3); the C-6 proton was masked by water at δ 7.18; δ_C(125.8 MHz, $C^2H_3O^2H$) 171.6 (CO_2), 171.1 (CO_2), 151.2 (C-6), 127.2 (C-4), 54.0 (C-2), 29.4 (C-3), 23.6 (C-7) and 13.8 (CH_3). The spectra were identical with those of a sample isolated from *M. pura* by Hatanaka and Katayama.[20]

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-ethylpyroglutamate 14

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-ethylidenepyroglutamate **8a** (300 mg, 0.965 mmol) was dissolved in ethyl acetate (10 ml) and 10% palladium on carbon (30 mg, 10% w/w) was added. The mixture was stirred under an atmosphere of hydrogen for four days at room temperature, filtered and the solvent was removed *in vacuo* to afford a colourless oil which was purified by column chromatography on silica gel using light petroleum (40–60 °C)–diethyl ether (1 : 1) as eluent to afford tert-*butyl* (2S,4S)-N-tert-*butoxycarbonyl*-4-*ethylpyroglutamate* **14** as a white crystalline solid (299 mg, 99%), mp 49–51 °C; $[\alpha]_D^{21}$ −32 (*c* 0.2, $CHCl_3$) (Found: C, 61.1; H, 8.4; N, 4.1. $C_{16}H_{27}NO_5$ requires C, 61.3; H, 8.6; N, 4.5%); *m/z* [+ve FAB (3-NBA–$CHCl_3$)] 314 $[M + H]^+$; ν_{max}(KBr)/cm^{-1} 1791 and 1727 ('imide'); δ_H(360 MHz, $C_6^2H_6$) 4.19 (1H, dd, $J_{2,3S}$ 8.6, $J_{2,3R}$ 6.7, H-2), 1.90–1.67 (3H, m, H-4 and H-3), 1.43 [9H, s, $OC(CH_3)_3$], 1.34 [9H, s, $OC(CH_3)_3$], 1.33 (2H, m, H-6) and 0.93 (3H, t, $J_{7,6}$ 7.4, CH_3); δ_H(360 MHz, C^2HCl_3) 4.37 (1H, m, $J_{2,3S}$ 8.8, $J_{2,3R}$ 6.2, H-2), 2.44 (2H, m, 2 × H-3), 1.89 (1H, m, H-4), 1.54 (2H, m, 6-CH_2), 1.50 [9H, s, $OC(CH_3)_3$], 1.47 [9H, s, $CO_2C(CH_3)_3$] and 0.96 (3H, t, $J_{7,6}$ 7.5, CH_3); δ_C(125.8 MHz, C^2HCl_3) 175.5 (CON), 170.6 (1-CO_2), 149.6 (urethane), 83.2 [$OC(CH_3)_3$], 82.0 [$OC(CH_3)_3$], 58.0 (C-2), 44.0 (C-4), 27.8 [$OC(CH_3)_3$], 27.8 [$OC(CH_3)_3$], 26.9 (C-3), 24.2 (C-6) and 11.4 (CH_3).

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-propylpyroglutamate 16

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-propylidenepyroglutamate **8b** (1.0 g, 3.1 mmol) was dissolved in ethyl acetate (20 ml) and 10% palladium on carbon (100 mg, 10% w/w) was added. The reaction was stirred under an atmosphere of hydrogen for three days at room temperature, filtered and the solvent was removed *in vacuo* to afford a white solid which was recrystallised from light petroleum (40–60 °C)–diethyl ether to afford tert-*butyl* (2S,4S)-N-tert-*butoxycarbonyl*-4-*propylpyroglutamate* **16** as a white crystalline solid (989 mg, 98%), mp 59–61 °C; $[\alpha]_D^{21}$ −35 (*c* 0.3, $CHCl_3$) (Found: C, 62.1; H, 8.9; N, 4.15. $C_{17}H_{29}NO_5$ requires C, 62.4; H, 8.9; N, 4.3%); *m/z* [+ve FAB (3-NBA–$CHCl_3$)] 328 $[M + H]^+$; ν_{max}(KBr)/cm^{-1} 1795, 1709 ('imide') and 1736 (ester); δ_H(500 MHz, $C_6^2H_6$) 4.20 (1H, dd, $J_{2,3S}$ 8.8, $J_{2,3R}$ 6.7, H-2), 1.91 (1H, m, $J_{3R,3S}$ 13.0, H-3R), 1.77 (1H, m, $J_{3S,3R}$ 13, $J_{3S,4}$ 9.2, $J_{3S,2}$ 8.8, H-3S), 1.71 (1H, m, H-6A), 1.45 [9H, s, $OC(CH_3)_3$], 1.36 [9H, s, $OC(CH_3)_3$], 1.34 (1H, m, H-4), 1.17 (1H, m, H-6B), 1.10 (2H, m, H-7) and 0.68 (3H, t, $J_{8,7}$ 7.3, CH_3); δ_H(360 MHz, C^2HCl_3) 4.27 (1H, m, H-2), 2.38 (2H, m, H-3), 1.71 (1H, m, H-4), 1.47 (2H, m, H-6), 1.38 [9H, s, $OC(CH_3)_3$], 1.36 [9H, s, $OC(CH_3)_3$], 1.27 (2H, m, H-7) and 0.79 (3H, t, $J_{8,7}$ 7.4, CH_3); δ_C(125.8 MHz, $C_6^2H_6$) 173.4 (CON), 170.9 (1-CO_2), 151.0 (urethane), 82.5 [$OC(CH_3)_3$], 81.2 [$OC(CH_3)_3$], 58.1 (C-2),

42.5 (C-4), 33.4 (C-6), 28.0 [OC(CH_3)$_3$], 27.8 [OC(CH_3)$_3$], 27.6 (C-3), 20.4 (C-7) and 13.8 (CH_3).

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-benzylpyroglutamate 17

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-benzylidenepyroglutamate **8c** (200 mg, 0.536 mmol) was dissolved in ethyl acetate (70 ml) and 10% palladium on carbon (20 mg, 10% w/w) was added. The reaction was stirred under an atmosphere of hydrogen for three days at room temperature, filtered and the solvent was removed *in vacuo* to afford a white solid (188 mg, 93%) which was purified by column chromatography on silica gel using light petroleum (40–60 °C)–diethyl ether (1:1) to afford tert-*butyl* (2S,4S)-N-tert-*butoxycarbonyl*-4-*benzylpyroglutamate* **17** as a white crystalline solid (127 mg, 63%), mp 130–131 °C; $[\alpha]_D^{22}$ +66 (*c* 0.2, $CHCl_3$) (Found: C, 66.7; H, 8.0; N, 3.5. $C_{21}H_{29}NO_5$ requires C, 67.2; H, 7.7; N, 3.7%); *m*/*z* [+ve FAB (3-NBA–$CHCl_3$)] 376 $[M + H]^+$; ν_{max}(KBr)/cm^{-1} 1756, 1715 (imide), 1736 (ester); δ_H(360 MHz, C^{2}HCl$_3$) 7.14–7.23 (5H, m, ArH), 4.37 (1H, dd, $J_{2,3S}$ 9.2, $J_{2,3R}$ 5.8, H-2), 3.30 (1H, dd, $J_{6A,6B}$ 13.8, $J_{6A,4}$ 4.0, H-6A), 2.83 (1H, m, $J_{4,6B}$ 10.9, $J_{4,3S}$ 9.4, $J_{4,3R}$ 6.8, $J_{4,6A}$ 4.0, H-4), 2.66 (1H, dd, $J_{6B,6A}$ 13.8, $J_{6B,4}$ 11.1, H-6B), 2.30 (1H, dd, $J_{3S,3R}$ 13.3, $J_{3S,2}$ $J_{3S,4}$ 9.4, H-3S), 1.67 (1H, m, $J_{3R,3S}$ 13.3, $J_{3R,2}$ 5.8, $J_{3R,4}$ 6.8, H-3R), 1.51 [9H, s, OC(CH$_3$)$_3$] and 1.48 [9H, s, OC(CH$_3$)$_3$]; irradiation of the absorption for H-2 at δ 4.37 gave an NOE in H-3S at δ 2.30; irradiation of H-6B at δ 2.66 gave NOEs in the absorption for H-6A at δ 3.30 and in H-3R at δ 1.67; δ_C(125.8 MHz, C^{2}HCl$_3$) 174.5 (CON), 170.6 (1-CO_2), 149.5 (N-CO_2), 138.6–126.6 (C_6H_5), 83.4 [O*C*(CH$_3$)$_3$], 82.2 [O*C*(CH$_3$)$_3$], 58.1 (C-2), 44.5 (C-4), 36.9 (C-3), 28.0 [OC(*C*H$_3$)$_3$], 27.9 [OC(*C*H$_3$)$_3$] and 26.5 (C-6).

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-carboxymethylpyroglutamate 18

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-benzylpyroglutamate **17** (1.0 g, 3.1 mmol) was dissolved in carbon tetrachloride (5 ml), acetonitrile (5 ml) and water (5 ml). Ruthenium trichloride hydrate (61 mg, 0.235 mmol) and sodium metaperiodate (5.02 g, 23.5 mmol) were added. The reaction mixture was stirred overnight at room temperature and partitioned between ethyl acetate (40 ml) and saturated aqueous sodium hydrogen carbonate (40 ml). The organic layer was washed with saturated aqueous sodium hydrogen carbonate (40 ml). The aqueous layers were carefully acidified to pH 4 at 0 °C with stirring by the dropwise addition of 10% aqueous citric acid. The aqueous layer was extracted with ethyl acetate (3 × 100 ml), the combined organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford a pale yellow solid, which was recrystallised from ethanol–diethyl ether to afford 1-tert-*butyl* (2S,4S)-N-tert-*butoxycarbonyl*-4-*carboxymethylpyroglutamate* **18** as a white crystalline solid (219 mg, 24%), mp 125–127 °C (Found: C, 55.8; H, 7.0; N, 4.0. $C_{16}H_{25}NO_7$ requires C, 55.95; H, 7.3; N, 4.1%); *m*/*z* [+ve FAB (3-NBA–$CHCl_3$)] 344 $[M + H]^+$; ν_{max}(KBr)/cm^{-1} 3405 (OH, acid), 1785 ('imide'), 1736 (ester); δ_H(360 MHz, C$_6$^{2}H$_6$) 4.10 (1H, t, $J_{2,3S}$ 9.2, $J_{2,3R}$ 5.8, H-2), 2.65 (1H, dd, $J_{6A,6B}$ 17.0, $J_{6A,4}$ 4.5, H-6A), 2.40 (1H, m, $J_{4,3S}$ 8.6, $J_{4,6B}$ 9.2, $J_{4,6A}$ 4.5, H-4), 2.10 (1H, dd, $J_{6B,6A}$ 17.0, $J_{6B,4}$ 9.2, H-6B), 1.95 (1H, m, $J_{3S,3R}$ 12.9, $J_{3S,2}$ 9.2, $J_{3S,4}$ 8.6, H-3S), 1.42 [9H, s, OC(CH$_3$)$_3$], 1.38 (1H, m, H-3R) and 1.35 [9H, s, OC(CH$_3$)$_3$]; irradiation of H-2 at δ 4.10 resulted in NOEs in H-3S at δ 1.95 and in H-4 at δ 2.40; irradiation at δ 1.95 of H-3S resulted in NOEs at δ 2.40 for H-4 and in H-2 at δ 4.10; δ_C(125.8 MHz, C^{2}HCl$_3$) 176.0 (7-CO_2), 173.9 (CON), 170.2 (1-CO_2), 149.2 (urethane), 83.8 [O*C*(CH$_3$)$_3$], 82.4 [O*C*(CH$_3$)$_3$], 58.0 (C-2), 39.0 (C-4), 35.2 (C-3), 27.76 [OC(*C*H$_3$)$_3$], 27.74 [OC(*C*H$_3$)$_3$] and 27.6 (C-6).

1-*tert*-Butyl hydrogen (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-ethylglutamate 21

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-ethylpyroglutamate **14** (271 mg, 0.865 mmol) was dissolved in tetrahydrofuran (3.0 ml) and 1 M aqueous lithium hydroxide (1.04 ml) was added dropwise at 0 °C with vigorous stirring over a period of 10 min. Stirring was continued for a further 15 min at 0 °C. Ethyl acetate (10 ml) and aqueous saturated sodium hydrogen carbonate (10 ml) were added and the organic layer was extracted with aqueous saturated sodium hydrogen carbonate (10 ml). The aqueous layers were carefully acidified to pH 5 at 0 °C with stirring by the dropwise addition of 10% aqueous citric acid. The aqueous layer was extracted with ethyl acetate (3 × 20 ml) and the organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford 1-tert-*butyl* hydrogen (2S,4S)-N-tert-*butoxycarbonyl*-4-*ethylglutamate* **21** as a white solid, which was recrystallised from light petroleum (60–80 °C)–diethyl ether (196 mg, 68%), mp 90–92 °C; $[\alpha]_D^{22}$ +0.9 (*c* 0.23, $CHCl_3$) (Found: C, 57.5; H, 8.7; N, 4.2. $C_{16}H_{29}NO_6$ requires C, 58.0; H, 8.8; N, 4.2%); *m*/*z* [+ve FAB (3-NBA)] 332 $[M + H]^+$; ν_{max}(KBr)/cm^{-1} 3350 (br, NH), 1734 (ester), 1707 (acid); δ_H(500 MHz, C^{2}H$_3$O^{2}H) 4.00 (1H, dd, $J_{2,3A}$ 8.9, $J_{2,3B}$ 6.4, H-2), 2.36 (1H, m, $J_{4,6}$ 7.4, H-4), 1.91 (1H, m, $J_{3A,3B}$ 16.4, $J_{3A,2}$ 8.9, $J_{3S,4}$ 7.4, H-3A), 1.85 (1H, dd, $J_{3B,3A}$ 16.4, $J_{3B,2}$ 6.4, H-3B), 1.61 (2H, m, $J_{6,7}$ $J_{6,4}$ 7.4, H-6), 1.46 [9H, s, OC(CH$_3$)$_3$], 1.43 [9H, s, OC(CH$_3$)$_3$] and 0.92 (3H, t, $J_{7,6}$ 7.4, H-7); δ_C(125.8 MHz, C^{2}H$_3$O^{2}H) 179.2 (CO_2), 173.6 (CO_2), 82.8 [O*C*(CH$_3$)$_3$], 80.6 [O*C*(CH$_3$)$_3$], 54.2 (C-2), 44.7 (C-4), 34.3 (C-3), 28.7 [OC(*C*H$_3$)$_3$], 28.2 [OC(*C*H$_3$)$_3$], 25.9 (C-6) and 11.6 (CH_3).

(2*S*,4*S*)-4-Methylglutamic acid hydrochloride 22a

1-*tert*-Butyl hydrogen (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-methylglutamate **11**[30] (100 mg, 0.315 mmol) was dissolved in 6 M aqueous hydrochloric acid (3 ml) and stirred at room temperature overnight. The solvent was removed *in vacuo* to afford an off-white solid which was recrystallised from ethanol–diethyl ether to yield (2S,4S)-4-*methylglutamic acid hydrochloride* **22a** as a white solid (62 mg, 100%), mp 148–153 °C (decomp.); $[\alpha]_D^{21}$ +25.35 (*c* 1, 1 M HCl) (Found: C, 36.5; H, 6.0; N, 6.8. $C_6H_{12}NO_4Cl$ requires C, 36.5; H, 6.1; N, 7.1%); *m*/*z* [+ve FAB (glycerol)] 162 [free amino acid + H]$^+$; ν_{max}(KBr)/cm^{-1} 3300 (br, NH and OH) and 1718 (acid); δ_H(500 MHz, 20% ^{2}HCl–^2H_2O) 4.23 (1H, t, $J_{2,3A}$ $J_{2,3B}$ *ca*. 7.0, H-2), 2.89 (1H, m, J_{4,CH_3} 7.1, H-4), 2.30 (1H, m, $J_{3A,3B}$ 14.5, $J_{3A,2}$ 6.5, $J_{3A,4}$ 9.8, H-3A), 2.15 (1H, m, $J_{3B,3A}$ 14.5, $J_{3B,2}$ 7.2, $J_{3R,4}$ 5.6, H-3B) and 1.28 (3H, d, $J_{CH_3,4}$ 7.1, CH_3); δ_C(125.8 MHz, 20% ^{2}HCl–^2H_2O) 181.6 (CO_2), 173.3 (CO_2), 53.6 (C-2), 38.2 (C-4), 35.2 (C-3) and 19.4 (CH_3).

(2*S*,4*S*)-4-Ethylglutamic acid hydrochloride 22b

1-*tert*-Butyl hydrogen (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-ethylglutamate **21** (576 mg, 1.74 mmol) was dissolved in 6 M aqueous hydrochloric acid (10 ml) and stirred at room temperature overnight. The solvent was removed *in vacuo* to afford an off-white solid which was recrystallised from ethanol–diethyl ether to yield (2S,4S)-4-*ethylglutamic acid hydrochloride* **22b** as a white solid (313 mg, 85%), mp 135–138 °C (decomp.); $[\alpha]_D^{24}$ +3.2 (*c* 1, 3 M HCl) (Found: C, 39.5; H, 6.6; N, 6.5. $C_7H_{14}NO_4Cl$ requires C, 39.7; H, 6.6; N, 6.6%); *m*/*z* [+ve FAB (glycerol)] 176 [free amino acid + H]$^+$; ν_{max}(KBr)/cm^{-1} 3305 (br, NH and OH) and 1708 (acid); δ_H(500 MHz, 20% ^{2}HCl–^2H_2O) 4.19 (1H, dd, $J_{2,3B}$ 8.0, $J_{2,3A}$ 6.0, H-2), 2.77 (1H, m, $J_{4,3A}$ 10.0, H-4), 2.30 (1H, m, $J_{3A,3B}$ 14.6, $J_{3A,2}$ 6.0, $J_{3A,4}$ 10.0, H-3A), 2.17 (1H, m, $J_{3B,3A}$ 14.6, $J_{3B,2}$ 8.0, $J_{3B,4}$ 4.8, H-3B), 1.69 (2H, quintet, $J_{6A,6B}$ 14.5, $J_{6,7}$ 7.5, H-6) and 0.93 (3H, t, $J_{7,6}$ 7.5, CH_3); δ_C(125.8 MHz, 20% ^{2}HCl–^2H_2O) 181.2 (CO_2), 173.3 (CO_2), 53.8 (C-2), 45.0 (C-4), 33.3 (C-3), 27.6 (C-6) and 12.9 (CH_3).

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-ethylprolinate 24b

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-ethylpyroglutamate **14** (500 mg, 1.60 mmol) was dissolved in tetrahydrofuran (50 ml), cooled to 0 °C under argon with stirring and borane·dimethyl sulfide (BMS) (2.0 M in tetrahydrofuran, 4 ml, 8 mmol) was added dropwise. The solution was allowed to warm to room temperature, stirred for four days, cooled to 0 °C and

quenched with methanol (10 ml). The solvent was removed *in vacuo* to afford a colourless oil, which was purified by column chromatography on silica gel using light petroleum (60–80 °C)–diethyl ether (3:1) to afford the major product as a colourless oil and the minor product as a white crystalline solid. The major product was tert-*butyl* (2S,4S)-N-tert-*butoxycarbonyl*-4-*ethylprolinate* **24b** (254 mg, 53%), $[\alpha]_D^{22}$ −49 (*c* 1.0, $CHCl_3$) (Found: C, 63.6; H, 9.8; N, 4.7. $C_{16}H_{29}NO_4$ requires C, 64.2; H, 9.7; N, 4.7%); *m/z* [+ve FAB (3-NBA–$CHCl_3$)] 300 $[M + H]^+$ (Found: 300.21536. $C_{16}H_{30}NO_4$ requires 300.21742); ν_{max}(film)/cm^{-1} 1745 (ester) and 1703 (urethane); δ_H(360 MHz, $C_6{}^2H_6$, complicated by conformational isomerism) 4.27 and 4.11 (1H, t, $J_{5A,5D}$ $J_{5A,4}$ 8.2, H-5A; t, $J_{5B,5C}$ $J_{5B,4}$ 8.1, H-5B), 3.81 and 3.55 (1H, dd, $J_{2A,3S}$ 10.0, $J_{2A,3R}$ 7.1, H-2A; dd, $J_{2B,3S}$ 9.9, $J_{2B,3R}$ 7.4, H-2B), 2.98 and 2.94 (1H, t, $J_{5D,5A}$ $J_{5D,4}$ 9.9, H-5D; t, $J_{5C,5B}$ $J_{5C,4}$ 9.9, H-5C), 1.99 (1H, m, H-3), 1.51 and 1.47 [9H, 2s, $OC(CH_3)_3$], 1.43 and 1.38 [9H, 2s, $OC(CH_3)_3$], 0.96 (2H, m, H-6) and 0.57 and 0.54 (3H, t, $J_{7,6}$ 7.4, CH_3; t, $J_{7,6}$ 7.4, CH_3); the *tert*-butyl singlets masked two absorptions for C-3 and two absorptions for C-4; δ_C(125.8 MHz, [$^2H_6$]-DMSO) 172.1 and 171.7 (1-CO_2), 153.4 and 153.0 (urethane), 80.3, 80.1, 78.8 and 78.7 [O*C*$(CH_3)_3$], 59.6 and 59.2 (C-2), 51.9 and 51.7 (C-5), 36.3 and 35.2 (C-4), 28.1 (C-3), 28.0 and 27.6 [OC(*C*$H_3)_3$], 25.3 (C-6) and 12.39 and 12.35 (CH_3). The minor product was tert-*butyl* (2S,4S)-N-tert-*butoxycarbonyl*-4-*ethyl*-5-*hydroxyprolinate* **25** (92 mg, 18%), $[\alpha]_D^{22}$ −29 (*c* 1.0, $CHCl_3$) (Found: C, 60.65; H, 9.1; N, 4.45. $C_{16}H_{29}NO_5$ requires C, 60.9; H, 9.2; N, 4.4%); *m/z* [+ve FAB (3-NBA–$CHCl_3$)] 298 $[M - OH]^+$; ν_{max}(KBr)/cm^{-1} 3461 (OH), 1749 (ester) and 1699 (urethane); δ_H(360 MHz, $C_6{}^2H_6$) 5.53 and 5.33 (1H, t, $J_{5A,4}$ $J_{5A,OH}$ 3.8, H-5A; t, $J_{5B,4}$ $J_{5B,OH}$ 5.1, H-5B), 4.23 and 4.04 (1H, t, $J_{2A,3S}$ $J_{2A,3R}$ 8.6, H-2A; dd, $J_{2B,3S}$ 9.7, $J_{2B,3R}$ 7.9, H-2B), 3.73 and 3.28 (1H, exchangeable d, $J_{OH,5A}$ 3.3, 5-OH_A; exchangeable d, $J_{OH,5B}$ 5.9, 5-OH_B), 1.94 and 1.75 (2H, m, 3A-CH_2; m, 3B-CH_2), 1.57 (1H, m, H-4), 1.48 and 1.44 [9H, s, $OC(CH_3)_3$, s, $OC(CH_3)_3$], 1.39 and 1.36 [9H, s, $OC(CH_3)_3$, s, $OC(CH_3)_3$], 1.27 (2H, m, H-6) and 0.76 (3H, t, $J_{7,6}$ 7.3, CH_3); δ_C(125.8 MHz, $C_6{}^2H_6$) 173.6 and 172.1 (1-CO_2), 154.0 and 153.5 (urethane), 82.5 and 82.4 (5-CHOH), 81.2 and 80.4 [O*C*$(CH_3)_3$], 80.1 and 80.0 [O*C*$(CH_3)_3$], 60.0 and 59.8 (C-2), 46.7 and 45.3 (C-4), 34.3 and 32.8 (C-3), 28.4 and 28.97 [OC(*C*$H_3)_3$], 28.92 [OC(*C*$H_3)_3$], 22.0 and 21.7 (C-6) and 12.4 (CH_3).

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-propylprolinate 24c

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-propylpyroglutamate **16** (500 mg, 1.53 mmol) was dissolved in tetrahydrofuran (40 ml), cooled to 0 °C under argon with stirring and borane·dimethyl sulfide (BMS) (2.0 M in tetrahydrofuran, 3.8 ml, 7.6 mmol) was added dropwise. The solution was allowed to warm to room temperature and stirred for eight days. The solution was then cooled to 0 °C and the reaction was quenched with methanol (10 ml). The solvent was removed *in vacuo* to afford a colourless oil, which was partitioned between diethyl ether (50 ml) and water (50 ml). The organic layer was washed with water (50 ml) and saturated aqueous sodium chloride (50 ml) and dried (Na_2SO_4). The solvent was removed *in vacuo* to afford a colourless oil (450 mg, 85%), which was purified by column chromatography on silica gel using light petroleum (40–60 °C)–diethyl ether (3:1) as eluent to afford tert-*butyl* (2S,4S)-N-tert-*butoxycarbonyl*-4-*propylprolinate* **24c** as a colourless oil (314 mg, 66%), $[\alpha]_D^{25}$ −52 (*c* 1.1, $CHCl_3$); *m/z* [+ve FAB (3-NBA–$CHCl_3$)] 314 $[M + H]^+$; *m/z* (EI) found 313.22728. $C_{17}H_{31}NO_4$ requires 313.2253; ν_{max}(film)/cm^{-1} 1746 (ester) and 1703 (urethane); δ_H(360 MHz, $C_6{}^2H_6$) 4.27 and 4.11 (1H, t, $J_{5A,5D}$ $J_{5A,4}$ 8.2, H-5A; t, $J_{5B,5C}$ $J_{5B,4}$ 8.2, H-5B), 3.81 and 3.55 (1H, dd, $J_{2A,3S}$ 10.4, $J_{2A,3R}$ 7.1, H-2A; dd, $J_{2B,3S}$ 10.0, $J_{2B,3R}$ 7.3, H-2B), 2.97 and 2.93 (1H, t, $J_{5C,5B}$ $J_{5C,4}$ 10.0, H-5C; t, $J_{5D,5A}$ $J_{5D,4}$ 9.9, H-5D), 2.00 (1H, m, $J_{3R,2}$ 7.1, H-3R), 1.52 and 1.48 [9H, 2s, $OC(CH_3)_3$], 1.44 and 1.39 [9H, 2s, $OC(CH_3)_3$], 0.91 (4H, m, H-6 and H-7) and 0.68 (3H, m, $J_{6,4}$ 6.6, CH_3); the *tert*-butyl singlets masked two absorptions for H-3S and two absorptions for H-4; δ_C(125.8 MHz, $C_6{}^2H_6$) 172.4 and 171.6 (1-CO_2), 154.0 and 153.5 (urethane), 80.1 and 80.0 [O*C*$(CH_3)_3$], 79.1 and 79.0 [O*C*$(CH_3)_3$], 60.4 and 60.0 (C-2), 52.7 and 52.6 (C-5), 38.4 and 37.6 (C-4), 37.5 and 36.3 (C-6), 35.2 and 35.1 (C-3), 28.5 and 28.0 [OC(*C*$H_3)_3$], 21.4 (CH_3) and 14.2 and 14.1 (CH_3).

(2*S*,4*S*)-4-Methylproline hydrochloride 26a

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-methylprolinate **24a**[5b] (300 mg, 1.05 mmol) was dissolved in 6 M aqueous hydrochloric acid (10 ml) and stirred at room temperature for five days. The solvent was removed *in vacuo* to afford an off-white solid which was recrystallised from ethanol–diethyl ether to yield (2S,4S)-4-*methylproline hydrochloride* **26a** as a white crystalline solid (163 mg, 94%), mp 165–166 °C; $[\alpha]_D^{24}$ −19 (*c* 0.1, 3 M HCl) (Found: C, 43.2; H, 7.3: N, 8.2. $C_6H_{12}NO_2Cl$ requires C, 43.5; H, 7.3; N, 8.5%); *m/z* [+ve FAB (glycerol–water)] 130 [free amino acid + H]$^+$; ν_{max}(KBr)/cm^{-1} 3375 (br, NH and OH) and 1742 (acid); δ_H(500 MHz, $C^2H_3O^2H$) 4.40 (1H, dd, $J_{2,3S}$ 9.4, $J_{2,3R}$ 8.2, H-2), 3.50 (1H, dd, $J_{5A,5B}$ 11.0, $J_{5A,4}$ 7.4, H-5A), 2.91 (1H, dd, $J_{5B,5A}$ 11.0, H-5B), 2.57 (1H, m, $J_{3R,3S}$ 12.7, $J_{3R,4}$ 7.5, H-3R), 2.51 (1H, m, H-4), 1.69 (1H, m, $J_{3S,3R}$ 12.7, $J_{3S,2}$ $J_{3S,4}$ *ca.* 9.8, H-3S) and 1.12 (3H, d, $J_{6,4}$ 6.5, CH_3); δ_C(125.8 MHz, $C^2H_3O^2H$) 171.4 (1-CO_2), 60.8 (C-2), 50.0 (C-5), 37.6 (C-4), 34.4 (C-3) and 16.9 (CH_3).

(2*S*,4*S*)-4-Propylproline hydrochloride 26c

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-propylprolinate **24c** (300 mg, 0.958 mmol) was dissolved in 6 M aqueous hydrochloric acid (10 ml) and stirred at room temperature for five days. The solvent was removed *in vacuo* to afford an off-white solid which was recrystallised from ethanol–diethyl ether to yield (2S,4S)-4-*propylproline hydrochloride* **26c** as a white crystalline solid (165 mg, 89%); *m/z* [+ve FAB (glycerol–MeOH)] 158 $[M - Cl]^+$; *m/z* (EI) found 157.1117. $C_8H_{15}NO_2$ requires 157.1103; δ_H(500 MHz, 20% ^2HCl–^2H_2O) 4.53 (1H, t, $J_{2,3B}$ $J_{2,3A}$ 7.5, H-2), 3.61 (1H, br t, H-5A), 3.07 (1H, br t, H-5B), 2.67 (1H, m, H-3A), 2.47 (1H, m, H-4), 1.80 (1H, m, H-3B), 1.45 (2H, m, H-6), 1.34 (2H, m, H-7) and 0.89 (3H, t, $J_{8,7}$ 6.7, CH_3); δ_C(125.8 MHz, 20% ^2HCl–^2H_2O) 173.3 (1-CO_2), 61.9 (C-2), 53.4 (C-5), 40.1 (C-4), 36.6 (CH_2), 35.8 (CH_2), 22.8 (C-7) and 15.8 (CH_3).

tert-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-4-ethylpyroglutamate 15

Methyllithium (1.4 M in diethyl ether, 4.33 ml, 6.06 mmol) was added to a stirred slurry of purified copper iodide (513 mg, 2.69 mmol) in diethyl ether (2 ml) under argon at −78 °C. The mixture was stirred for 1 h at −78 °C and *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methylenepyroglutamate **5**[30] (200 mg, 0.673 mmol) in diethyl ether (10 ml) was added. The solution was stirred for 2 h at −78 °C and the reaction was quenched by addition of saturated aqueous ammonium chloride (20 ml), allowed to warm to room temperature and stirred overnight at room temperature. The aqueous layer was extracted with diethyl ether (3 × 25 ml). The combined organic layers were washed with 10% aqueous citric acid (100 ml), saturated aqueous hydrogen carbonate (100 ml) and saturated aqueous sodium chloride (100 ml) and dried (Na_2SO_4). The solvent was removed *in vacuo* to afford a very pale yellow oil (178 mg, 84%) which contained the *trans*- and *cis*-isomers **15** and **14** in a ratio of 3:1. The oil was purified by column chromatography on silica gel using light petroleum (40–60 °C)–diethyl ether (1:1) as eluent. The *trans*-isomer tert-*butyl* (2S,4R)-N-tert-*butoxycarbonyl*-4-*ethylpyroglutamate* **15** was the major product as a white solid, mp 78–79 °C; *m/z* [+ve FAB (3-NBA–$CHCl_3$)] 314 $[M + H]^+$; *m/z* (EI) found 313.19048. $C_{16}H_{27}NO_5$ requires 313.1889; ν_{max}(KBr)/cm^{-1} 1791 ('imide'), 1727 (ester); δ_H(500 MHz, $C_6{}^2H_6$) 4.35 (1H, dd, $J_{2,3S}$ 9.6 $J_{2,3R}$ 1.5, H-2), 2.34 (1H, m, $J_{4,3S}$ 11.7, $J_{4,3R}$ 8.7, $J_{4,6A}$ 4.9, $J_{4,6B}$ 5.4, H-4), 1.77 (1H, m, $J_{3R,3S}$ 13.0, $J_{3R,4}$ 8.7, $J_{3R,2}$ 1.5, H-3R), 1.71 (1H, m, $J_{6A,6B}$ 13.8, $J_{6A,7}$ 7.5, $J_{6A,4}$ 4.9, H-6A), 1.44 [9H, s, $OC(CH_3)_3$], 1.34 [9H, s,

$OC(CH_3)_3$], 1.24 (1H, m, $J_{3S,3R}$ 13.0, $J_{3S,4}$ 11.7, $J_{3S,2}$ 9.6, H-3S), 1.11 (1H, m, $J_{6B,6A}$ 13.8, $J_{6B,7}$ 7.5, $J_{6B,4}$ 5.4, H-6A) and 0.61 (3H, t, $J_{7,6}$ 7.5, CH_3). Irradiation of the terminal methyl triplet at δ 0.61 resulted in an NOE in both adjacent methylene protons (δ 1.71 and 1.1), in H-4 at δ 2.34 and in each of the protons H-3 (δ 1.77 and 1.24); irradiation of H-3S at δ 1.24 resulted in NOEs in H-3R at δ 1.77, in H-2 at δ 4.35 and in the terminal methyl group at δ 0.61; irradiation of H-3R at δ 1.77 resulted in NOEs in H-3S at δ 1.24 and H-4 at δ 2.34; δ_H(360 MHz, C^2HCl_3) 4.42 (1H, dd, $J_{2,3S}$ 9.6, $J_{2,3R}$ 1.5, H-2), 2.52 (1H, m, $J_{4,3R}$ 8.7, $J_{4,3S}$ 4.6, H-4), 2.16 (1H, m, $J_{3R,3S}$ 13.1, $J_{3R,4}$ 8.7, $J_{3R,2}$ 1.5, H-3R), 1.91 (1H, m, $J_{3S,3R}$ 13.1, $J_{3S,2}$ 9.6, $J_{3S,4}$ 4.6, H-3S), 1.50 [9H, s, $OC(CH_3)_3$], 1.47 [9H, s, $OC(CH_3)_3$], 1.45 (2H, m, H-6) and 0.95 (3H, t, $J_{7,6}$ 7.5, CH_3); δ_C(125.8 MHz, C^2HCl_3) 175.1 (CON), 170.4 (1-CO_2), 149.4 (urethane), 83.1 [$OC(CH_3)_3$], 82.1 [$OC(CH_3)_3$], 57.7 (C-2), 42.9 (C-4), 28.0 (C-3), 27.8 [$OC(CH_3)_3$], 27.8 [$OC(CH_3)_3$], 23.3 (C-6) and 11.1 (CH_3). The minor product was tert-*butyl* (2S,4S)-N-tert-*butoxycarbonyl*-4-*ethylpyroglutamate* **14**, δ_H(360 MHz, $C_6^2H_6$) and δ_C(125.8 MHz, C^2HCl_3) NMR spectra identical with those of the sample prepared by the method described earlier.

tert-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-4-benzylpyroglutamate 28

Phenyllithium (1.8 M in diethyl ether, 8.42 ml, 15 mmol) was added to a stirred slurry of purified copper iodide (1.282 g, 6.73 mmol) in diethyl ether (10 ml) under argon at −78 °C. The mixture was stirred for 1 h at −78 °C and *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methylenepyroglutamate **5**[30] (500 mg, 1.68 mmol) in diethyl ether (30 ml) was added. The solution was stirred for 2 h at −78 °C and the reaction was quenched by addition of saturated aqueous ammonium chloride (40 ml) and allowed to warm to room temperature. After stirring overnight at room temperature the aqueous layer was extracted with diethyl ether (2 × 100 ml). The combined organic layers were washed with 10% aqueous citric acid (200 ml), saturated aqueous hydrogen carbonate (200 ml) and saturated aqueous sodium chloride (200 ml) and dried (Na_2SO_4). The solvent was removed *in vacuo* to afford a pale yellow oil. Column chromatography proved unsuccessful, with inadequate separation and poor recovery (587 mg, 93% crude). The *trans*- and *cis*-isomers **28** and **17** were present in a ratio of 6:1. The major product was tert-*butyl* (2S,4R)-N-tert-*butoxycarbonyl*-4-*benzylpyroglutamate* **28**, δ_H(360 MHz, C^2HCl_3) 7.62–6.83 (5H, m, ArH), 4.35 (1H, dd, $J_{2,3S}$ 8.4, $J_{2,3R}$ 2.9, H-2), 3.27 (1H, dd, H-6A), 2.93 (1H, m, H-4), 2.71 (1H, dd, H-6B), 1.99 (2H, m, H-3), 1.52 [9H, s, $OC(CH_3)_3$] and 1.45 [9H, s, $OC(CH_3)_3$]. The minor product was tert-*butyl* (2S,4S)-N-tert-*butoxycarbonyl*-4-*benzylpyroglutamate* **17** with δ_H(360 MHz, C^2H_3Cl) identical with that of the sample synthesised by hydrogenation.

Di-*tert*-butyl (6*S*)-4-oxo-5-azaspiro[2.4]heptane-5,6-dicarboxylate 29

Diazald (*N*-methyl-*N*-nitrosotoluene-*p*-sulfonamide) (20 g, 93 mmol) in diethyl ether (250 ml) was added dropwise to potassium hydroxide (10 g, 178 mmol) in 2-ethoxyethoxyethanol (20 ml), water (15 ml) and diethyl ether (10 ml) at 60 °C. The yellow ethereal solution of diazomethane generated by distillation was added dropwise to *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methylenepyroglutamate **5**[30] (4.550 g, 15 mmol) and palladium acetate (172 mg, 0.766 mmol) in diethyl ether (100 ml) at 0 °C. A black precipitate was observed. The mixture was allowed to warm to room temperature and stirred overnight at room temperature. The black precipitate was removed by filtration and the solvent was removed *in vacuo* to afford *di*-tert-*butyl* (6S)-4-*oxo*-5-*azaspiro*[2.4]*heptane*-5,6-*dicarboxylate* **29** as a white crystalline solid (3.186 g, 67%), mp 89–94 °C, $[a]_D^{19}$ −6.2 (*c* 1, $CHCl_3$) (Found: C, 62.2; H, 8.25; N, 4.4. $C_{16}H_{25}NO_5$ requires C, 61.7; H, 8.0; N, 4.5%); *m/z* [+ve FAB (3-NBA–$CHCl_3$)] 312 $[M + H]^+$; ν_{max}(KBr)/cm^{-1} 1782, 1701 ('imide') and 1741 (ester); δ_H(360 MHz, C^2HCl_3) 4.50 (1H, dd, $J_{2,3S}$ 9.9, $J_{2,3R}$ 2.9, H-2), 2.49 (1H, dd, $J_{3S,3R}$ 13.2, $J_{3S,2}$ 9.9, H-3R), 1.84 (1H, dd, $J_{3R,3S}$ 13.2, $J_{3R,2}$ 2.9, H-3R), 1.48 [9H, s, $OC(CH_3)_3$], 1.44 [9H, s, $OC(CH_3)_3$], 1.25 (1H, m, H-7A) and 1.18 (1H, m, H-7B); the *tert*-butyl singlets masked two of the cyclopropyl protons (H-6A and H-6B); δ_H(500 MHz, $C_6^2H_6$) 4.39 (1H, dd, $J_{2,3S}$ 9.8, $J_{2,3R}$ 2.9, H-2), 1.74 (1H, dd, $J_{3S,3R}$ 13.2, $J_{3S,2}$ 9.8, H-3S), 1.43 [9H, s, $OC(CH_3)_3$], 1.39 (1H, dd, $J_{3R,3S}$ 13.2, $J_{3R,2}$ 2.9, H-3R), 1.33 [9H, s, $OC(CH_3)_3$], 1.15 (1H, m, $J_{6A,6B}$ 10.2, $J_{6A,7A}$ 6.4, $J_{6A,7B}$ 3.8, 6-CH_A), 1.06 (1H, m, $J_{6B,6A}$ 10.2, $J_{6B,7B}$ 6.7, $J_{6B,7A}$ 3.4, H-6B), 1.29 (1H, m, $J_{7A,7B}$ 10.2, $J_{7A,6A}$ 6.4, $J_{7A,6B}$ 3.4, H-7A) and 0.21 (1H, m, $J_{7B,7A}$ 10.2, $J_{7B,6B}$ 6.7, $J_{7B,6A}$ 3.8, H-7B); δ_C(125.8 MHz, $C_6^2H_6$) 173.7 (CON), 170.8 (1-CO_2), 150.7 (urethane), 82.3 [$OC(CH_3)_3$], 81.2 [$OC(CH_3)_3$], 57.3 (C-2), 29.9 (C-3), 28.0 [$OC(CH_3)_3$], 27.8 [$OC(CH_3)_3$], 22.8 (C-4), 16.9 (C-6) and 13.2 (C-7).

1-[(2*S*)-3-*tert*-butoxy-2-(*tert*-butoxycarbonylamino)-3-oxopropyl]cyclopropane-1-carboxylic acid 30

Di-*tert*-butyl (6*S*)-4-oxo-5-azaspiro[2.4]heptane-5,6-dicarboxylate **29** (243 mg, 0.781 mmol) was dissolved in tetrahydrofuran (3.9 ml) and 1 M aqueous lithium hydroxide (0.94 ml) was added dropwise at 0 °C with vigorous stirring over a period of 5 min. Stirring was continued for a further 20 min at 0 °C. Ethyl acetate (10 ml) and saturated aqueous sodium hydrogen carbonate (10 ml) were added to the mixture and the organic layer was extracted with saturated aqueous sodium hydrogen carbonate (10 ml). The combined aqueous layers were carefully acidified to pH 5 at 0 °C with stirring by dropwise addition of ice cold 10% aqueous citric acid. The aqueous layer was extracted with ethyl acetate (3 × 40 ml) and the organic layers were dried (Na_2SO_4) and filtered. The solvent was removed *in vacuo* to afford 1-[(2S)-3-tert-*butoxy*-2-(tert-*butoxycarbonylamino*)-3-*oxopropyl*]*cyclopropane*-1-*carboxylic acid* **30** as a white solid (46 mg, 18%), mp 132–134 °C; $[a]_D^{18}$ −3.3 (*c* 0.3, $CHCl_3$) (Found: C, 58.2; H, 8.4; N, 4.1. $C_{16}H_{27}NO_6$ requires C, 58.3; H, 8.2; N, 4.25%); *m/z* [+ve FAB (3-NBA–$CHCl_3$)] 330 $[M + H]^+$; ν_{max}(KBr)/cm^{-1} 3350 (br, NH and OH), 1719 (acid) and 1703 (urethane); δ_H(500 MHz, $C_6^2H_6$) 5.34 (1H, d, $J_{NH,2}$ 8.5, exchangeable, NH), 4.76 (1H, m, $J_{2,3B}$ 9.2, $J_{2,NH}$ 8.5, $J_{2,3A}$ 5.6, H-2), 1.95 (1H, dd, $J_{3A,3B}$ 14.3, $J_{3A,2}$ 5.6, H-3A), 1.68 (1H, dd, $J_{3B,3A}$ 14.3, $J_{3B,2}$ 9.2, H-3B), 1.41 [9H, s, $OC(CH_3)_3$], 1.34 [9H, s, $OC(CH_3)_3$], 1.26 (1H, overlapping with *tert*-butyl singlet, H-6A), 1.18 (1H, m, $J_{6B,6A}$ 9.8, $J_{6B,7B}$ 6.5, $J_{6B,7A}$ 3.4, H-6B), 0.52 (1H, m, $J_{7A,7B}$ 10.2, $J_{7A,6A}$ 6.5, $J_{7A,6B}$ 3.4, H-7A) and 0.43 (1H, m, $J_{7B,7A}$ 10.2, $J_{7B,6B}$ 6.5, $J_{7B,6A}$ 3.7, H-7B); δ_C(125.8 MHz, $C_6^2H_6$) 181.0 (5-CO_2), 172.1 (1-CO_2), 155.5 (urethane), 81.3 [$OC(CH_3)_3$], 79.2 [$OC(CH_3)_3$], 53.8 (C-2), 36.5 (C-3), 28.4 [$OC(CH_3)_3$], 27.8 [$OC(CH_3)_3$], 21.0 (C-4), 16.9 (C-6) and 16.1 (C-7).

Methyl 1-[(2*S*)-3-*tert*-butoxy-2-(*tert*-butoxycarbonylamino)-3-oxopropyl]cyclopropanecarboxylate 31

Di-*tert*-butyl (6*S*)-4-oxo-5-azaspiro[2.4]heptane-5,6-dicarboxylate **29** (1.00 g, 3.22 mmol) was dissolved in tetrahydrofuran (16 ml) and cooled to −12 °C under argon. A 1.0 M solution of lithium methoxide in methanol (3.86 ml, 3.86 mmol) was added dropwise with stirring which was continued at −12 °C for 30 min. The mixture was quenched with saturated aqueous sodium chloride (20 ml) and diethyl ether (20 ml) and allowed to warm to room temperature. The aqueous layer was extracted with diethyl ether (20 ml). The combined organic layers were dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford a pale yellow oil, which was purified by column chromatography on silica gel using light petroleum (40–60 °C)–diethyl ether (2:1) as eluent to afford *methyl* 1-[(2S)-3-tert-*butoxy*-2-(tert-*butoxycarbonylamino*)-3-*oxopropyl*]*cyclopropanecarboxylate* **31** as a colourless oil (796 mg, 72%); $[a]_D^{18}$ +4.25 (*c* 1, $CHCl_3$); *m/z* [+ve FAB (3-NBA–$CHCl_3$)] 344 $[M + H]^+$; ν_{max}(film)/cm^{-1} 3377 (br, NH) and 1719 (acid); δ_H(500 MHz, $C_6^2H_6$) 5.46 (1H, d, $J_{NH,2}$

8.4, exchangeable, NH), 4.73 (1H, m, $J_{2,3B}$ 9.0, $J_{2,NH}$ 8.9, $J_{2,3A}$ 5.6, H-2), 3.28 (3H, s, OCH_3), 1.90 (1H, dd, $J_{3A,3B}$ 14.3, $J_{3A,2}$ 5.6, H-3A), 1.79 (1H, dd, $J_{3B,3A}$ 14.3, $J_{3B,2}$ 9.4, H-3B), 1.39 [9H, s, $OC(CH_3)_3$], 1.32 [9H, s, $OC(CH_3)_3$], 1.19 (1H, m, $J_{6A,6B}$ 9.9, $J_{6A,7A}$ 6.4, $J_{6A,7B}$ 3.4, H-6A), 1.13 (1H, m, $J_{6B,6A}$ 9.8, $J_{6B,7B}$ 6.3, $J_{6B,7A}$ 3.6, H-6B), 0.51 (1H, m, $J_{7A,7B}$ 9.6, $J_{7A,6A}$ 6.1, $J_{7A,6B}$ 3.8, H-7A) and 0.43 (1H, m, $J_{7B,7A}$ 9.7, $J_{7B,6B}$ 6.3, $J_{7B,6A}$ 3.4, H-7B); δ_C(125.8 MHz, $C_6{}^2H_6$) 174.7 (CO_2), 172.1 (CO_2), 155.5 (urethane), 81.1 [$OC(CH_3)_3$], 79.0 [$OC(CH_3)_3$], 54.0 (C-2), 51.5 (OCH_3), 37.0 (C-3), 28.4 [$OC(CH_3)_3$], 27.9 [$OC(CH_3)_3$], 21.1 (C-4), 16.3 (C-6) and 15.1 (C-7).

1-[(2*S*)-2-Amino-2-carboxyethyl]cyclopropanecarboxylic acid hydrochloride

Methyl 1-[(2*S*)-3-*tert*-butoxy-2-(*tert*-butoxycarbonylamino)-3-oxopropyl]cyclopropanecarboxylate **31** (661 mg, 1.93 mmol) was heated to reflux with 6 M aqueous hydrochloric acid (10 ml) for three hours. The solvent was removed *in vacuo* to afford an off-white solid. Traces of residual hydrochloric acid were removed by azeotropic distillation with diethyl ether and the residue was recrystallised from ethanol–diethyl ether to yield 1-[(2*S*)-2-*amino*-2-*carboxyethyl*]*cyclopropanecarboxylic acid hydrochloride* **32** as a white crystalline solid (340 mg, 84%), mp 57–61 °C; $[\alpha]_D^{22}$ +14.8 (*c* 1, 1 M HCl); *m*/*z* [+ve FAB (glycerol–dilute HCl)] 174 [free amino acid + H]$^+$ ([M + H]$^+$ found 174.07482. $C_7H_{11}NO_4$ + H requires 174.07663); ν_{max}(KBr)/cm^{-1} 2900–3600 (br, NH and OH) and 1733 (acid); δ_H(500 MHz, $C^2H_3O^2H$) 4.25 (1H, t, $J_{2,3B}$ $J_{2,3A}$ 6.9, H-2), 2.11 (2H, m, H-3), 1.37 (1H, m, $J_{6A,6B}$ 10.0, $J_{6A,7A}$ 6.3, $J_{6A,7B}$ 3.5, H-6A), 1.31 (1H, m, $J_{6B,6A}$ 10.0, $J_{6B,7B}$ 6.3, $J_{6B,7A}$ 3.4, H-6B), 0.95 (1H, m, $J_{7A,7B}$ 9.7, $J_{7A,6A}$ 6.3, $J_{7A,6B}$ 3.4, H-7A) and 0.88 (1H, m, $J_{7B,7A}$ 9.7, $J_{7B,6B}$ 6.3, $J_{7B,6A}$ 3.5, H-7B); δ_C(125.8 MHz, $C^2H_3O^2H$) 178.5 (CO_2), 171.8 (CO_2), 58.3 (C-2), 53.4 (C-3), 21.7 (C-4), 17.0 (C-6) and 16.5 (C-7).

Di-*tert*-butyl (6*S*)-1-phenyl-4-oxo-5-azaspiro[2.4]heptane-5,6-dicarboxylate 33

Diazald (20 g, 93 mmol) in diethyl ether (250 ml) was added dropwise to potassium hydroxide (10 g, 178 mmol) in 2-ethoxyethoxyethanol (20 ml), water (15 ml) and diethyl ether (10 ml) at 60 °C. The yellow ethereal solution of diazomethane generated by distillation was added dropwise to *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-benzylidenepyroglutamate **8c** (250 mg, 0.670 mmol) and palladium acetate (75 mg, 0.335 mmol) in diethyl ether (50 ml) at 0 °C. A black precipitate was observed. The mixture was allowed to warm to room temperature and stirred overnight at room temperature. The black precipitate was removed by filtration and the solvent was removed *in vacuo* to afford a colourless oil (257 mg, 99% crude) which was a mixture of diastereoisomers in the ratio 11 : 8 : 9 : 2 (*cis* : *trans* : *trans* : *cis*). The oil was purified by column chromatography on silica gel using light petroleum (30–40 °C)–diethyl ether (7 : 3) as eluent. Column chromatography afforded pure samples of the *trans*-isomers **33a** and **33b** as white solids and various fractions containing diastereoisomeric mixtures of the *trans*- and *cis*-isomers. The first *trans* product was *di*-tert-*butyl* (1S,3R,6S)-1-*phenyl*-4-*oxo*-5-*azaspiro*[2.4]*heptane*-5,6-*dicarboxylate* **33a** (28 mg, 11%), mp 80–83 °C; $[\alpha]_D^{22}$ +6.9 (*c* 0.77, $CHCl_3$) (Found: C, 68.1; H, 7.6; N, 3.5. $C_{22}H_{29}NO_5$ requires C, 68.2; H, 7.5; N, 3.6%); *m*/*z* [+ve FAB (3-NBA–$CHCl_3$)] 388 [M + H]$^+$; ν_{max}(KBr)/cm^{-1} 1782, 1719 ('imide') and 1736 (ester); δ_H(360 MHz, $C_6{}^2H_6$) 6.96 (3H, m, ArH), 6.78 (2H, m, ArH), 4.34 (1H, dd, $J_{2,3S}$ 10.5, $J_{2,3R}$ 2.1, H-2), 2.69 (1H, dd, $J_{7B,7A}$ 9.2, $J_{7B,6}$ 7.2, H-7B), 1.69 (1H, dd, $J_{7A,7B}$ 9.2, $J_{7A,6}$ 5.1, H-7A), 1.60 (1H, dd, $J_{3S,3R}$ 13.8, $J_{3S,2}$ 10.5, H-3S), 1.45 [9H, s, $OC(CH_3)_3$], 1.36 (1H, dd, $J_{3R,3S}$ 13.8, $J_{3R,2}$ 2.1, H-3R), 1.15 [9H, s, $OC(CH_3)_3$] and 0.73 (1H, dd, $J_{6,7B}$7.2, $J_{6,7A}$ 5.1, H-6); irradiation of the resonance for H-2 at δ 4.34 resulted in an NOE in H-3S at δ 1.60; irradiation of H-3S at δ 1.60 resulted in NOEs in H-2 at δ 4.34 and in H-6 at δ 0.73; irradiation of H-6 at δ 0.73 resulted in NOEs at H-7A (δ 1.69) and the aromatic protons (δ 6.78) and H-3S (δ 1.60); irradiation at δ 6.78 (ArH) resulted in NOEs at δ 2.69 (H-7B) and δ 0.73 (H-6); δ_C(125.8 MHz, $C_6{}^2H_6$) 172.9 (CON), 170.6 (1-CO_2), 150.7 (urethane), 136.7–126.7 (C_6H_5), 82.5 [$OC(CH_3)_3$], 81.2 [$OC(CH_3)_3$], 56.8 (C-2), 32.4 (C-6), 30.9 (C-4), 28.0 [$OC(CH_3)_3$], 27.7 [$OC(CH_3)_3$], 24.6 (C-3) and 17.1 (C-7). The second *trans* product was *di*-tert-*butyl* (1S,3R,6R)-1-*phenyl*-4-*oxo*-5-*azaspiro*[2.4]*heptane*-5,6-*dicarboxylate* **33b** (37 mg, 14%), *m*/*z* [+ve FAB (3-NBA–$CHCl_3$)] 388 [M + H]$^+$; δ_H(360 MHz, $C_6{}^2H_6$) 7.00 (3H, m, ArH), 6.65 (2H, m, ArH), 4.30 (1H, dd, $J_{2,3S}$ 10.0, $J_{2,3R}$ 3.2, H-2), 2.74 (1H, dd, $J_{7B,7A}$ 9.4, $J_{7B,6}$ 7.1, H-7B), 1.63 (1H, dd, $J_{7A,7B}$ 9.4, $J_{7A,6}$ 4.9, H-7A), 1.44 [9H, s, $OC(CH_3)_3$], 1.33 [9H, s, $OC(CH_3)_3$] and 0.91 (1H, dd, $J_{6,7B}$ 7.1, $J_{6,7A}$ 4.9, H-6); the *tert*-butyl singlets masked the absorptions for the H-3S and H-3R protons; δ_C(125.8 MHz, $C_6{}^2H_6$) 172.9 (CON), 170.9 (1-CO_2), 150.7 (urethane), 136.6–126.8 (C_6H_5), 82.5 [$OC(CH_3)_3$], 81.3 [$OC(CH_3)_3$], 57.2 (C-2), 30.6 (C-6), 29.9 (C-4), 28.0 [$OC(CH_3)_3$], 27.8 [$OC(CH_3)_3$], 26.6 (C-3) and 20.0 (C-7).

Acknowledgements

We thank the EPSRC for a studentship to (C. M. M.) and Dr J. A. Khan and Dr P. Dieterich for useful discussions. We thank Professor T. Kasai, Faculty of Agriculture, Hokkaido University, Japan for his kindness in providing us with samples of the compounds **1**, **2** and **23** and Professor S. Hatanaka, Department of Biology, University of Tokyo, Japan for his kindness in providing us with a sample of the compounds **2** and **3**.

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Paper 7/03489J
Received 20*th May* 1997
Accepted 31*st July* 1997