1 PERKIN

Use of a modified ring-switching strategy to synthesise the glutamate antagonist (2*S*)-2-amino-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)propionate and related compounds with two chiral centres [1]

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(2*S*)-2-Amino-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)propionic acid **8**, an isomer of the natural product willardiine **7**, was synthesised by treatment of the pyroglutamate urea **19** with mild base followed by deprotection in a two-step modification of our 'ring-switching' approach to the synthesis of glutamate antagonists. Use of this two-step strategy has allowed us to synthesise L-alanine derivatives, which are β-substituted by a reduced pyrimidinedione which contains a second chiral centre. In one case, the antagonist activity at metabotropic glutamate receptors of two diastereoisomers showed little difference.

Excitatory glutamate receptors in the central nervous system have been classified into various ionotropic and metabotropic sub-types and these have long been identified as targets for therapeutic intervention in a variety of illnesses, including Alzheimer's disease,[2] epilepsy[3] and ischaemia.[4] L-Alanine derivatives substituted at the β-carbon with a heterocyclic ring have been shown to be of particular interest in this context[5] and we recently devised a novel 'ring-switching' strategy to allow the versatile synthesis of homochiral compounds with structures typical of glutamate agonists and antagonists.[6] In this synthesis, shown in Scheme 1, reaction of a 4-formylpyroglutamate ester urethane **3** with a bisnucleophile gave rise, on deprotection, to a variety of homochiral heterocyclic amino acids such as **4**, **5** or **6**.

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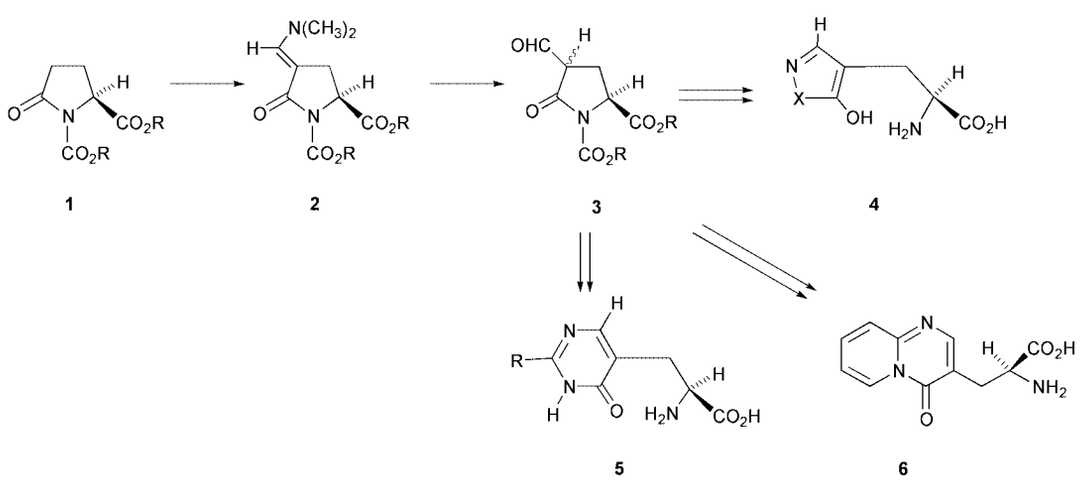
Results and discussion

The reported biological activity of the natural product willardiine **7**,[7,8] made the synthesis of isomeric pyrimidinediones of interest. (2*S*)-2-Amino-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)propionic acid **8** was erroneously reported in the secondary literature[9] as the structure of a naturally-occurring compound present in pea seedlings. We have prepared a series of pyrimidinones **5**[6] by reaction of an enol ether **9** with formamidine, benzamidine, acetamidine or guanidine followed by deprotection, but our attempts to prepare the pyrimidinedione **8** and related compounds by reacting the poorer nucleophiles urea and thiourea with the pyroglutamate aldehyde **3** proved ineffective.[6] An alternative synthetic approach to pyrimidinedione analogues of willardiine was therefore required.

We argued that, if a bond could be made between the carbon atom of the aldehyde group **3** and a nitrogen atom from the poor nucleophile, then the subsequent 'ring-switching' process might be possible. The aldehyde **11** was therefore prepared by hydrolysis of the enaminone **10**[6] as in Scheme 2. This was con-



Scheme 1

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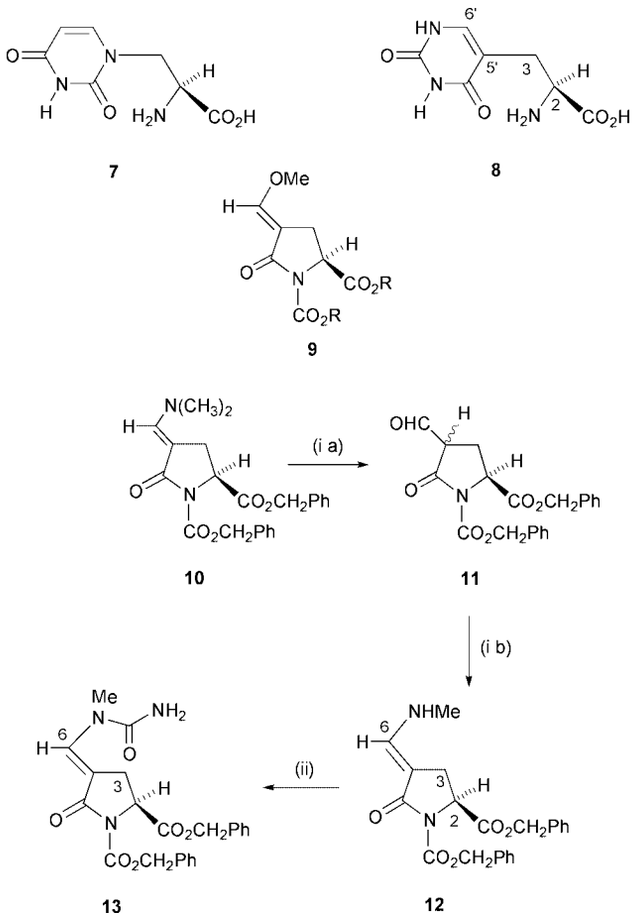
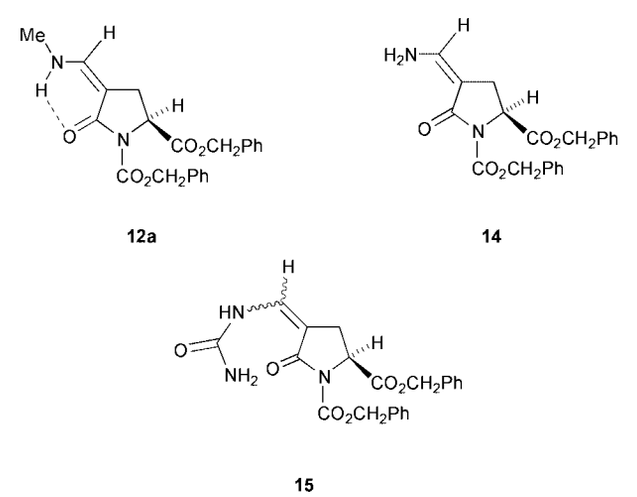


Scheme 2 (i) (a) HCl–MeOH; (b) $MeNH_2$–MeOH; 86%; (ii) $ClSO_2NCO$; CH_2Cl_2; 88%.

verted into the secondary enaminone **12** by treatment with excess methylamine at room temperature for 15 minutes. The ^1H NMR spectrum indicated that the enaminone occurred entirely as the *E*-isomer **12** in the crystalline state but that it formed a mixture of *E*- and *Z*-isomers on standing in solution. The signal for the NH proton at δ 7.75 ppm for the *Z*-isomer **12a** was over 3 ppm downfield from the corresponding proton (δ 4.42 ppm) in the *E*-isomer, consistent with the hydrogen bonding expected in the former isomer and NOE results were in keeping with the assignments. The enaminone **12** reacted with chlorosulfonyl isocyanate to afford the urea **13** in 88% yield. Attempts to cause this compound to undergo 'ring-switching' by thermolysis failed completely and heating at reflux in K_2CO_3–EtOH seemed merely to remove the protecting groups.

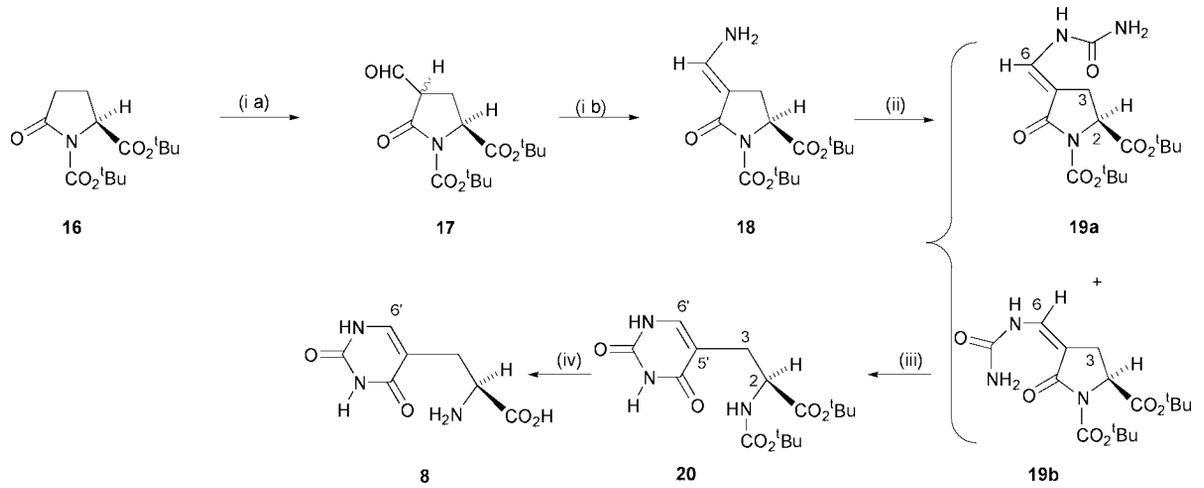


Because failure of the urea **13** to undergo 'ring-switching' might be due to it being fixed as the *E*-isomer, the primary enaminone **14** was prepared from the aldehyde **11** using ammonium acetate and acetic acid in benzene in the presence of 3 Å molecular sieves. This was converted into the corresponding urea **15** using chlorosulfonyl isocyanate. On treatment with base, the urea **15** should be capable of yielding the *Z*-isomer, required for cyclisation. It was therefore heated at reflux in K_2CO_3–EtOH but again deprotection appeared to be the only reaction.

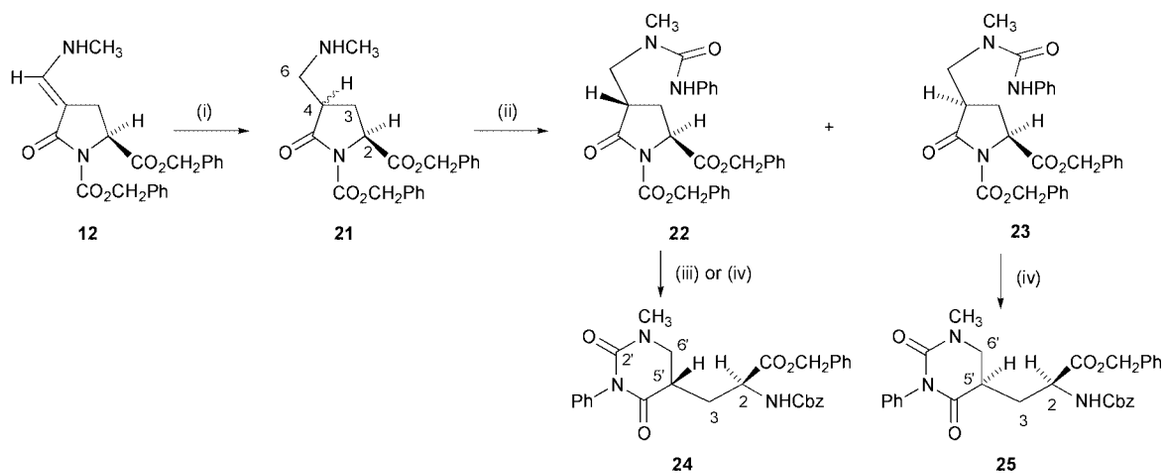
Since instability of the protecting groups to base seemed to be a problem, we prepared the *tert*-butoxycarbonyl *tert*-butyl ester **18** in 40% overall yield, as shown in Scheme 3, by first reacting the corresponding unfunctionalised diprotected pyroglutamate **16**[10] with lithium hexamethyldisilazide followed by treatment with methyl formate to yield the intermediate aldehyde **17** as an oil and then reacting this with ammonium acetate, acetic acid and 3 Å molecular sieves. The primary enaminone **18** had similar spectroscopic properties at room temperature, 60 and −50 °C to those exhibited by the analogous enaminone **14**, existing as the *E*-isomer shown in the crystalline form but equilibrating to a 1 : 1 mixture of geometrical isomers on standing in C^2HCl_3. Reaction with chlorosulfonyl isocyanate and flash chromatography of the product on silica gel gave a mixture of the *Z*-isomer **19b** in 18% yield and the *E*-isomer **19a** in 22% yield, the stereochemistry and structure of the individual isomers being indicated by NOE experiments. The NH signal in the ^1H NMR spectrum of the *Z*-isomer **19b** was 0.7 ppm downfield from that in the spectrum of the *E*-isomer **19a**, indicating hydrogen bonding in **19b**. The stereochemistry was confirmed by single crystal X-ray structure analysis of the *Z*-isomer **19b**.[11]

When the *E*-urea **19a** was heated at reflux in ethanol containing one equivalent of K_2CO_3, 'ring-switching' was finally accomplished and the protected pyrimidine-2,4-dione **20** was obtained in 57% yield. This reaction presumably involved the intermediacy of the *Z*-isomer **19b**, although this isomer was not observed when TLC was used to follow the reaction. Ring-switching to the pyrimidine-2,4-dione **20** was observed when the *Z*-urea **19b** was heated at reflux in potassium carbonate–ethanol. TLC analysis suggested that the *E*-isomer was produced concurrently with this process. Deprotection of the pyrimidine-2,4-dione **20** using hydrochloric acid gave the amino acid **8** in quantitative yield.

All of the compounds which we have prepared to date with potential for interaction with glutamate receptors have been α-amino acids of the L-series with but one asymmetric centre. It was of interest to see whether compounds containing a non-aromatic heterocyclic ring system with a second asymmetric centre might be biologically active and whether the biological activity might be related to the stereochemistry of the second asymmetric centre. We therefore reduced the secondary enaminone **12** with sodium cyanoborohydride in methanol at pH 3 to 4.6 using screened methyl orange and 1 M HCl to maintain pH as shown in Scheme 4. The diastereoisomeric amines **21** were obtained as an inseparable mixture which on reaction with phenyl isocyanate gave the corresponding ureas **22** and **23** in 90% yield. These were separated chromatographically and assigned stereochemistry using NOE experiments. The ratio of *trans* **22** to *cis* **23** isomers was 7 : 3. Although treatment of the major isomer **22** with sodium hydride in THF caused 'ring-switching' in 40% yield, this was accompanied by epimerisation to give a 1 : 1 mixture of **24** and **25** which could be separated chromatographically. Thermal rearrangement could be achieved without epimerisation, albeit in only 20% yield, but this allowed the stereochemistry of the products to be assigned. Deprotection of each of the diastereoisomers **24** and **25** by hydrogenolysis gave samples of the separate free amino acids **26** and **27** and these were found by Dr A. Batchelor of the Wellcome Foundation to be equally



Scheme 3 (i) (a) LiHMDS, HCO_2Me; (b) NH_4OAc–ACOH; 40%; (ii) $ClSO_2NCO$, CH_2Cl_2; **19a**, 22%, **19b**, 18%; (iii) Δ, K_2CO_3–EtOH; 57%; (iv) HCl, 94%.



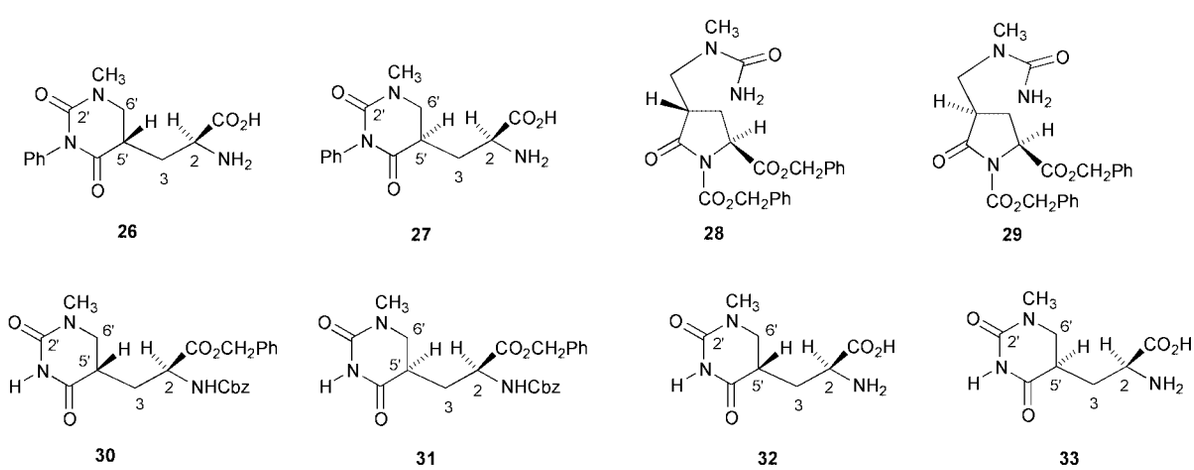
Scheme 4 (i) $Na(CN)BH_3$, pH 4; 99%; (ii) PhNCO–CH_2Cl_2; 90%; (iii) **22** to **24**, Δ; 20%; (iv) **22** + **23** to **24** + **25**, NaH–THF; 41%.

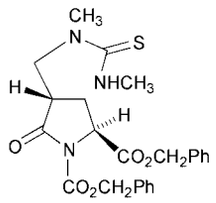
weakly antagonistic to the action of the metabotropic agonist *trans*-aminocyclopentanedicarboxylic acid (ACDP) in Purkinje rat cells using the method of East and Garthwaite.[12]

Reaction of the mixture of amines **21** with KCNO–HOAc gave the ureas **28** and **29**, which could be rearranged to the protected heterocyclic amino acids **30** and **31** respectively using NaH. These could be deprotected by hydrogenolysis, giving the amino acids **32** and **33**. Unfortunately none of the mixtures of diastereoisomers could be separated in this series.

Reaction of the amines **21** with methyl isothiocyanate in CH_2Cl_2 gave an inseparable 7 : 3 mixture of the diastereoisomeric thioureas **34** and **35** in 72% yield. Thermal rearrangement gave the 'ring-switched' products **36** and **37** in unchanged ratio and these were separated chromatographically. Single crystal X-ray structural analysis[11] confirmed that the major isomer was the (2*S*,4*S*)-isomer **36**. All attempts to deprotect the thioxopyrimidones to obtain the corresponding amino acids were unsuccessful.

Reduction of the primary enaminone **14** and reaction with KCNO–HOAc has also allowed us to access the diastereoisomeric mixture **38**. This underwent the 'ring-switching' reaction to give the epimers **39** on heating. Deprotection by hydrogenolysis gave the diastereoisomeric mixture **40** which could not be separated.





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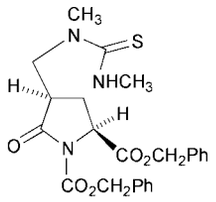

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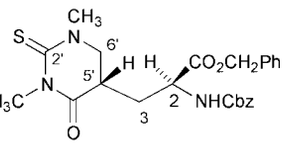

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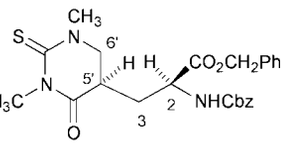

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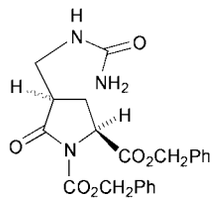

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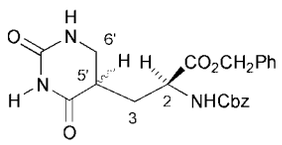

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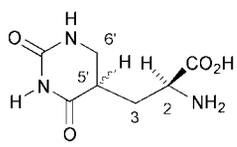

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We have extended our novel "ring-switching" methodology to allow the synthesis of compounds which are unavailable by our original methodology by virtue of the poor reactivity of the bisnucleophile. When the bond which would have required an intermolecular reaction is formed by an alternative route then the second intramolecular "ring-switching" step can be effected. Thus (2*S*)-2-amino-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)propionic acid **8**, and L-alanine derivatives, which are β-substituted by a reduced pyrimidinedione containing a second chiral centre have been prepared. In one case, the antagonist activity at metabotropic glutamate receptors of two diastereoisomers showed little difference.

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 recorded on a Perkin–Elmer PE241 polarimeter. UV spectra were recorded on a Phillips PU800 spectrometer and IR spectra on a Perkin-Elmer 1710 Fourier transform spectrometer. ^{1}H NMR spectra were recorded on Bruker WM360 (360 MHz) or AMX 500 (500 MHz) spectrometers. ^{13}C NMR spectra were recorded on a Bruker AMX 500 spectrometer (125.76 MHz) by Dr A. Avent. DEPT analysis was used in all ^{13}C NMR spectra to help assign signals. FAB mass spectra were recorded on a Kratos MS25 spectrometer by Mr A. Greenway. EI mass spectra and accurate mass determinations were recorded on a Kratos Concept spectrometer by Dr S. Chotai of the Wellcome Foundation. Elemental analyses were carried out by Ms M. Patel of the University of Sussex or by the staff of the Wellcome Foundation Physical Sciences Department. Flash column chromatography was carried out using Merck silica gel 60H (230–300 mesh).

Benzyl (2*S*,4*RS*)-*N*-benzyloxycarbonyl-4-formylpyroglutamate 11

Benzyl (2*S*)-*N*-benzyloxycarbonyl-4-dimethylaminomethylenepyroglutamate **10**[6] (500 mg, 1.22 mmol) and 1 M aqueous HCl (1.6 ml, 1.59 mmol) were dissolved in methanol and stirred for 30 min at room temperature. The solvent was removed *in vacuo* and the resultant oil was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate and the combined organic phases were washed with brine and dried ($MgSO_4$). The solvent was removed *in vacuo* to give a quantitative yield of *benzyl (2S,4RS)-N-benzyloxycarbonyl-4-formylpyroglutamate* **11** as a pale yellow oil. The product was homogeneous as judged by TLC and was employed immediately after preparation in subsequent reactions without further purification.

Benzyl (2*S*)-*N*-benzyloxycarbonyl-4-*N*-methylaminomethylenepyroglutamate 12

Benzyl (2*S*,4*RS*)-*N*-benzyloxycarbonyl-4-formylpyroglutamate **11** (9.88 g, 26 mmol) and methylamine (4.8 ml of a 25% aqueous solution, 32 mmol) were dissolved in methanol (32 ml) and left for 15 min at room temperature. The solvent was removed *in vacuo* and the resultant oil was allowed to stand for 14 h. Partial crystallisation occurred. Recrystallisation from ethanol yielded *benzyl (2S)-N-benzyloxycarbonyl-4-N-methylaminomethylenepyroglutamate* **12** as pale yellow crystals (8.77 g, 86%), mp 144–148 °C (Found: C, 67.0; H, 5.45; N, 7.0. $C_{22}H_{22}N_2O_5$ requires C, 67.0; H, 5.6; N, 7.1%); *m*/*z* (+ve FAB, 3-nitrobenzyl alcohol (3-NBA)) 395 ([M + H]$^+$) and 417 ([M + Na]$^+$); ν_{max} (KBr)/cm^{-1} 1748 (ester/imide) and 1624 (C=C); λ_{max}(MeOH)/nm 217 and 315 (log ε 3.93 and 3.86); *E-isomer* **12**: δ_H (360 MHz, C^{2}HCl$_3$, recorded immediately) 7.30 (10H, m, Ar*H*), 7.20 (1H, dt, $J_{6,NH}$ 13.5, $J_{6,3}$ 1.8, =C*H*N), 5.19 (2H, m, PhCH_2O), 5.09 (2H, m, PhCH_2O), 4.69 (1H, dd, $J_{2,3A}$ 10.7, $J_{2,3B}$ 3.6, H-2), 4.42 (1H, br, exch. m, N*H*Me), 2.95 (3H, d, $J_{Me,NH}$ 4.9, NCH_3), 2.85 (1H, m, H-3B) and 2.44 (1H, br d, $J_{3A,3B}$ 14.7, H-3A); *Z-isomer* **12a**: δ_H (360 MHz, C^{2}HCl$_3$ formed on standing in solution) 7.75 (1H, br m, exch. N*H*), 7.3 (10H, m, Ar*H*), 6.53 (1H, d, $J_{6,NH}$ 11, =C*H*N), 5.2 (2H, m, PhCH_2O), 5.1 (2H, m, PhCH_2O), 4.67 (1H, m, H-2), 2.93 (3H, d, $J_{Me,NH}$ 5.1, NCH_3), 2.93 (1H, m, H-3A) and 2.52 (1H, dd, $J_{3,2}$ 3.6, $J_{3A,3B}$ 14.8, H-3); irradiation of the broad N*H* multiplet at 4.42 (*E*-isomer) gave NOE at the peaks for H-3 at 2.85 (0.7%) and 2.44 ppm (1.7%); irradiation at the =CH doublet at 6.53 ppm (*Z*-isomer) gave NOE to NCH_3 at 2.93 (2%), NH at 7.75 (1.1%) and H-3 at 2.52 ppm (1.6%); δ_C (125.76 MHz, C^{2}HCl$_3$, *both isomers*) 171.4, 171.3, 168.8 and 167.8 (4 × *C*O), 151.8 (urethane), 149.1 (C-6), 145.36 (urethane), 135.5, 135.5 and 135.2 (3 × *ipso C*), 128.4, 128.4, 128.3, 128.2, 128.0, 128.0, 127.8 and 127.75 (8 × Ar*C*H), 92.5 and 89.3 (2 × C-4), 67.5, 67.4 and 66.9 (3 × Ar*C*H$_2$), 56.7 and 55.7 (2 × C-2), 35.0 and 34.6 (2 × N*C*H$_3$), and 25.9 and 24.5 (2 × C-3).

Benzyl (2*S*)-*N*-benzyloxycarbonyl-4-[(1-methylureido)-methylene]pyroglutamate 13

Benzyl (2*S*)-*N*-benzyloxycarbonyl-4-*N*-methylaminomethylenepyroglutamate **12** (725 mg, 1.84 mmol) was dissolved in dichloromethane (10 ml) and cooled to ice bath temperature with stirring and under an atmosphere of nitrogen. Chlorosulfonyl isocyanate (0.24 ml, 2.76 mmol) was added dropwise and stirring was continued for 100 min at ice bath temperature. Water (5 ml) was added causing an instant emulsion. The mixture was concentrated to a small volume *in vacuo*, diluted with water and extracted with ethyl acetate. The combined organic phases were washed with brine and dried ($MgSO_4$). The

solvent was removed *in vacuo* to yield a pale yellow oil which was purified by flash chromatography on silica gel, eluting with CH_2Cl_2–MeOH (94.5 : 5.5) to yield *benzyl (2S)-N-benzyloxycarbonyl-4-[(1-methylureido)methylene]pyroglutamate* **13** as a glassy solid; (0.708 g, 88%); $[\alpha]_D^{23}$ +11 (*c* 0.6, $CHCl_3$) (Found: C, 62.3; H, 5.3; N, 9.8. $C_{23}H_{23}N_3O_6$ requires C, 63.15; H, 5.3; N, 9.6%); *m/z* (+ve FAB, 3-NBA) 460 ([M + Na]$^+$) and 438 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1749 (imide) and 1710 (C=O); λ_{max}(MeOH)/nm 214 and 274 (log ε 5.07 and 3.96); δ_H (360 MHz, C^2HCl_3) 7.77 (1H, br s, =C*H*N), 7.4–7.25 (10H, m, Ar*H*), 5.76 (2H, exch. br s, NH_2), 5.16 (2H, m, PhCH_2O), 5.10 (2H, m, PhCH_2O), 4.69 (1H, dd, $J_{2,3A}$ 3.2, $J_{2,3B}$ 10.4, H-2), 3.24 (1H, ddd, $J_{3B,6}$ 2.0, $J_{3B,2}$ 10.4, $J_{3B,3A}$ 16.2, H-3B), 3.18 (3H, s, NCH_3) and 2.89 (1H, br d, $J_{3A,3B}$ 16.2, H-3A).

Benzyl (2*S*)-*N*-benzyloxycarbonyl-4-aminomethylene-pyroglutamate 14

Benzyl (2*S*,4*RS*)-*N*-benzyloxycarbonyl-4-formylpyroglutamate **11** (2.08 g, 5.49 mmol), acetic acid (0.275 ml), ammonium acetate (0.845 g, 11 mmol) and 3 Å molecular sieves (5 g) were added to benzene (15 ml) with stirring. The mixture was left for 18 h at room temperature and filtered through Celite. The residue was washed with ethyl acetate. The combined filtrate and washings were washed with 5% aqueous sodium hydrogen carbonate and brine and dried (Na_2SO_4). The solvent was removed *in vacuo* to yield a tan coloured oil. This was recrystallised from toluene to yield *benzyl (2S)-N-benzyloxycarbonyl-4-aminomethylenepyroglutamate* **14** as a white solid (1.43 g, 68%); mp 82–85 °C; $[\alpha]_D^{23}$ −33 (*c* 1, $CHCl_3$) (Found: C, 66.4; H, 5.2; N, 7.6. $C_{21}H_{20}N_2O_5$ requires C, 66.3; H, 5.3; N, 7.4%); *m/z* (+ve FAB, 3-NBA) 403 ([M + Na]$^+$) and 381 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1762 (C=O) and 1682; λ_{max}(MeOH)/nm 216 and 296 (log ε 4.04 and 4.14); δ_H (360 MHz, C^2HCl_3) 7.37–7.26 (11H, m, Ar*H* and =C*H*N), 5.20 (2H, m, PhCH_2O), 5.10 (2H, m, PhCH_2O), 4.72 (1H, dd, $J_{2,3A}$ 3.6, $J_{2,3B}$ 10.7, H-2), 4.52 (2H, br exch., NH_2), 2.84 (1H, ddd, $J_{3B,6}$ 2.0, $J_{3B,2}$ 10.7, $J_{3B,3A}$ 15.2, H-3B) and 2.43 (1H, ddd, $J_{3A,6}$ 1.9, $J_{3A,2}$ 3.6, $J_{3A,3B}$ 15.2, H-3A); irradiation at the exch. NH_2 at 4.52 ppm gave NOE to ArH at 7.34 (7.7%) and H-3 at 2.84 (0.7%) and 2.43 ppm (1.2%); on standing in solution, distinct peaks associated with the *Z*-isomer became evident at 6.67 (1H, t, $J_{6,NH}$ 10.6, =C*H*NH_2), 4.72 (1H, overlapping with H-2 of *Z*-isomer, H-2), 2.98 (1H, dd, $J_{3A,2}$ 10.8, $J_{3A,3B}$ 14.5, H-3A) and 2.53 (1H, dd, $J_{3B,2}$ 3.3, $J_{3B,3A}$ 14.5, H-3B); irradiation of the olefinic t at 6.67 ppm gave NOE to H-3 at 2.98 (0.5%) and 2.53 ppm (1.2%); when the spectrum was recorded at 332 K, the NH_2 group of the *Z*-isomer was evident as a broad hump at 6.16 ppm; irradiation of this peak caused the t for =CH at 6.67 ppm to collapse to a br s; δ_C (127.56 MHz, C^2HCl_3, both isomers) 171.43, 169.0, 168.1 and 151.9 (4 × CO), 144.6 and 141.1 (2 × C-6), 135.5 and 135.3 (2 × *ipso C*), 128.6, 128.5, 128.4, 128.2, 128.1 and 128.1 (6 × Ar*C*H), 96.0 and 92.4 (2 × C-4), 67.8, 67.7, 67.1 and 67.1 (4 × Ph*C*O), 56.8 and 55.8 (2 × C-2), and 25.9 and 24.0 (2 × C-3).

Benzyl (2*S*)-*N*-benzyloxycarbonyl-4-ureidomethylene-pyroglutamate 15

Benzyl (2*S*)-*N*-benzyloxycarbonyl-4-aminomethylenepyroglutamate **14** (191 mg, 0.503 mmol) was dissolved in dichloromethane (3 ml) and cooled to dry ice–industrial methylated spirits (IMS) bath temperature with stirring under an atmosphere of nitrogen. Chlorosulfonyl isocyanate (0.053 ml, 0.604 mmol) was added dropwise, stirring was continued for 10 min and the reaction was warmed to ice bath temperature. After standing for 1 h, the reaction was quenched by addition of 5% aqueous citric acid (5 ml), concentrated to a small volume *in vacuo*, diluted with water and extracted with ethyl acetate. The organic phase was separated and on standing a white precipitate formed. This was collected by filtration. Addition of petroleum ether (60–80 °C) to the filtrate yielded further precipitate. The combined solids were recrystallised from ethanol to yield *benzyl (2S)-N-benzyloxycarbonyl-4-ureidomethylenepyroglutamate* **15** as a white solid (0.142 g, 67%); mp 199–200 °C; $[\alpha]_D^{23}$ +17 (*c* 0.8, $CHCl_3$); *m/z* (EI) found: 423.14259. $C_{22}H_{21}N_3O_6$ requires 423.14304; *m/z* (+ve FAB, thioglycerol) 446 ([M + Na]$^+$) and 424 ([M + H]$^+$); $[\alpha]_D^{23}$ +17 (*c* 1.1, $CHCl_3$); ν_{max} (KBr)/cm^{-1} 3340 (NH), 1778 (imide), 1749 (ester) and 1713 (C=O); λ_{max}(MeOH)/nm 212 and 284 (log ε 5.12 and 4.38); δ_H (360 MHz, [$^2H_6$]DMSO) 9.24 (1H, exch. d, $J_{NH,6}$ 12.1, N*H*), 7.58 (1H, br d, $J_{6,NH}$ 12.1, =C*H*N), 7.38–7.28 (10H, m, Ar*H*), 6.49 (2H, br exch., NH_2), 5.17 (2H, m, PhCH_2-O), 5.12 (2H, m, PhCH_2O), 4.82 (1H, dd, $J_{2,3A}$ 3.05, $J_{2,3B}$ 10.7, H-2), 2.98 (1H, ddd, $J_{3B,6}$ 2.5, $J_{3B,2}$ 10.7, $J_{3B,3A}$ 16.5, H-3B) and 2.54 (1H, br d, $J_{3A,3B}$ 16.5, H-3A); addition of ^2H_2O caused the olefinic d at 7.58 ppm to collapse to a br s; δ_C (125.76 MHz, [$^2H_6$]DMSO) 171.1, 166.6 and 154.0 (3 × *C*O), 151.0 (urethane), 135.5 and 135.4 (2 × *ipso-C*), 132.2 (C-6), 128.4, 128.3, 128.2, 128.0, 128.0 and 127.5 (6 × Ar*C*H), 102.1 (C-4), 67.1 and 66.6 (2 × Ph*C*O), and 55.2 (C-2) and 24.3 (*C*-3).

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-aminomethylene-pyroglutamate 18

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate **16**[10] (5.33 g, 19 mmol) was dissolved in tetrahydrofuran (20 ml) and cooled to −78 °C, with stirring and under an atmosphere of nitrogen. Lithium hexamethyldisilazide (20.6 ml of a 1 M solution in THF, 26 mmol) was added dropwise over 2 min and stirring was continued for 55 min. Methyl formate (2.4 ml, 38 mmol) was added and stirring was continued for 25 min at −78 °C. The reaction was warmed to ice bath temperature, left for a further 30 min and quenched by addition of 10% aqueous citric acid (40 ml) with rapid stirring. The mixture was concentrated to a small volume *in vacuo* and extracted with ethyl acetate. The combined organic phases were dried ($MgSO_4$) and the solvent was removed *in vacuo* to yield a yellow oil, which was dissolved in benzene (45 ml) with ammonium acetate (3.08 g, 0.04 mol), acetic acid (1 ml) and 3 Å molecular sieves (13 g). The mixture was stirred vigorously for 18 h at room temperature and filtered through Celite. The solvent was removed *in vacuo* to yield a red oil which was purified by flash chromatography on silica gel, eluting with EtOAc–MeOH (96.8 : 3.2) followed by EtOAc–MeOH (95 : 5). *tert-Butyl (2S)-N-tert-butoxycarbonyl-4-aminomethylenepyroglutamate* **18** was isolated as a yellow oil (2.356 g, 40%); $[\alpha]_D^{23}$ −16.8 (*c* 1, $CHCl_3$); *m/z* (EI) found: 312.17030. $C_{16}H_{24}N_2O_5$ requires 312.16852; *m/z* (+ve FAB, thioglycerol + sodium) 335 ([M + Na]$^+$); ν_{max} (film)/cm^{-1} 1757 (C=O); λ_{max}(MeOH)/nm 212 and 294 (log ε 4.03 and 4.13); δ_H (500 MHz, C^2HCl_3) (*E*-isomer) 7.29 (1H, t, $J_{6,NH}$ 10.5, =C*H*N), 5.04 (2H, br exch. d, $J_{NH,6}$ 10.5, NH_2), 4.42 (1H, dd, $J_{2,3A}$ 3.7, $J_{2,3B}$ 10.8, H-2), 2.80 (1H, ddd, $J_{3B,6}$ 2.0, $J_{3B,2}$ 10.8, $J_{3B,3A}$ 15.3, H-3B), 2.34 (1H, ddd, $J_{3A,6}$ 1.7, $J_{3A,2}$ 3.7, $J_{3A,3B}$ 15.3, H-3A), 1.49 (9H, s, C(CH_3)$_3$) and 1.45 (9H, s, C(CH_3)$_3$); addition of ^2H_2O caused the olefinic t at 7.29 ppm to simplify to a br s; irradiation of the br d at 5.04 ppm gave NOE to =CH at 7.29 (1.6%) and to H-3 at 2.80 (1.4%) and 2.34 ppm (1.6%); peaks associated with the *Z*-isomer became apparent when the spectrum was recorded after the sample had been allowed to stand in solution; δ_H (500 MHz, C^2HCl_3) 6.67 (1H, t, $J_{6,NH}$ 10.7, =C*H*N), 4.37 (1H, dd, $J_{2,3B}$ 3.7, $J_{2,3A}$ 10.7, H-2), 2.88 (1H, ddd, $J_{3A,6}$ 1.2, $J_{3A,2}$ 10.7, $J_{3A,3B}$ 14.8, H-3A), 2.41 (1H, dd, $J_{3B,2}$ 3.7, $J_{3B,3A}$ 14.8, H-3B) and 1.45–1.42 (18H, singlets overlapping with those of the *E*-isomer, C(CH_3)$_3$); addition of ^2H_2O caused the t at 6.67 ppm to simplify to a br s; irradiation of the olefinic t at 6.67 ppm gave NOE to H-3 at 2.88 (0.5%) and 2.41 ppm (1.2%); when the spectrum was recorded at 332 K the NH_2 group was evident as a broad hump at 6.22 ppm; irradiation at this peak caused the olefinic t at 6.67 ppm to collapse to a br s; δ_C (127.56 MHz, C^2HCl_3 both isomers) 171.0, 170.9, 169.7 and 168.8 (4 × *C*O), 150.4 and 150.3 (2 × urethane), 143.5 and 140.3

(2 × C-6), 96.5 and 92.99 (2 × C-4), 82.2, 81.8 and 81.7 (3 × $C(CH_3)_3$), 57.4 and 56.5 (2 × C-2), 28.0 and 27.9 (2 × $C(CH_3)_3$), and 25.7 and 23.9 (2 × C-3).

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-ureidomethylene-pyroglutamate 19

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-aminomethylene-pyroglutamate **18** (1.103 g, 3.53 mmol) was dried by azeotropic removal of residual water with benzene, dissolved in dichloromethane (20 ml) and cooled to dry ice–IMS bath temperature with stirring under an atmosphere of argon. Chlorosulfonyl isocyanate (0.34 ml, 3.89 mmol) was added dropwise over 3 min. After stirring for a further 10 min, the solution was warmed to ice bath temperature, left for 30 min, and quenched by addition of saturated aqueous ammonium chloride (20 ml). The mixture was concentrated to a small volume *in vacuo* and extracted with ethyl acetate. The combined organic phases were filtered through Celite to break up the emulsion, washed with brine and dried ($MgSO_4$). The solvent was removed *in vacuo* to yield a yellow oil, which was purified by flash chromatography on silica gel, eluting with CH_2Cl_2–MeOH (89 : 11) for the first two products and with CH_2Cl_2–MeOH (85 : 15). Three components were isolated. *tert-Butyl (2S,Z)-N-tert-butoxycarbonyl-4-ureidomethylenepyroglutamate* **19b** (223 mg, 18%) which was recrystallised from ethanol, mp 183–185 °C; $[\alpha]_D^{23}$ −31.6 (*c* 0.6, MeOH) (Found: C, 54.3; H, 7.35; N, 11.8. $C_{16}H_{25}N_3O_6$ requires C, 54.1; H, 7.1; N, 11.8%); *m*/*z* (+ve FAB, 3-NBA) 733 ($[2M + Na]^+$), 711 ($[2M + H]^+$), 378 ($[M + Na]^+$) and 356 ($[M + H]^+$); ν_{max} (KBr)/cm^{-1} 3427 (NH), 3406, 1773 (imide), 1747 (ester) and 1720; λ_{max}(MeOH)/nm 204 and 299 (log ε 3.67 and 4.16); δ_H (360 MHz, [$^2H_6$]DMSO) 9.67 (1H, exch. d, $J_{NH,6}$ 11.6, N*H*), 7.24 (1H, dt, $J_{6,3B}$ 2, $J_{6,NH}$ 11.6, =CHN), 6.90 (2H, br exch. NH_2), 4.45 (1H, dd, $J_{2,3A}$ 3.1, $J_{2,3B}$ 10.6, H-2), 2.97 (1H, ddd, $J_{3B,6}$ 2, $J_{3B,2}$ 10.6, $J_{3A,3B}$ 15.8, H-3B), 2.46 (obscured by residual DMSO peak, H-3A), 1.42 (9H, s, $C(CH_3)_3$) and 1.40 (9H, s, $C(CH_3)_3$); addition of ^2H_2O caused the olefinic dt at 7.24 ppm to collapse to a br s; irradiation at the olefinic dt at 7.24 ppm gave NOE to H-3A at 2.46 ppm (1.8%); irradiation at the NH exch. d at 9.67 ppm gave NOE at the br exch. at *ca.* 6.9 ppm (1.9%); δ_C (125.76 MHz, [$^2H_6$]DMSO) 170.5, 167.5 and 153.9 (3 × *C*O), 149.6 (urethane), 134.2 (C-6), 100.0 (C-4), 81.8 and 81.3 (2 × $C(CH_3)_3$), 56.7 (C-2), 27.6 and 27.5 (2 × $C(CH_3)_3$) and 25.1 (C-3). The structure of this compound was confirmed by single crystal X-ray diffraction analysis, reported in our preliminary communication,[1] and atomic coordinate data were lodged with the Cambridge Crystallography Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW at that time. They are available on request from the Director of the CCDC at the above address, quoting CCDC reference code QENMEB and CCDC number 153592.

tert-Butyl (2S,E)-N-tert-butoxycarbonyl-4-ureidomethylene-pyroglutamate **19a** (278 mg, 22%) was recrystallised from ethanol, mp 175–177 °C; $[\alpha]_D^{23}$ +13 (*c* 0.5, MeOH); *m*/*z* (EI) found: 355.17142. $C_{16}H_{25}N_3O_6$ requires 355.17434; *m*/*z* (+ve FAB, 3-NBA) 733 ($[2M + Na]^+$), 711 ($[2M + H]^+$), 378 ($[M + Na]^+$) and 356 ($[M + H]^+$); ν_{max} (KBr)/cm^{-1} 3455 (NH), 1771 (imide), 1732 (ester) and 1689 (C=O); λ_{max}(MeOH)/nm 282 (log ε 4.38); δ_H (360 MHz, [$^2H_6$]DMSO) 8.99 (1H, exch. d, $J_{NH,6}$ 12.1, N*H*), 7.53 (1H, dt, $J_{6,3}$ 2.2, $J_{6,NH}$ 12.1, =C*H*N), 6.40 (2H, br exch., NH_2), 4.51 (1H, dd, $J_{2,3A}$ 3.3, $J_{2,3B}$ 10.7, H-2), 2.92 (1H, ddd, $J_{3B,6}$ 2.4, $J_{3B,2}$ 10.7, $J_{3A,3B}$ 16.6, H-3B), 2.34 (1H, ddd, $J_{3A,6}$ 2.3, $J_{3A,2}$ 3.3, $J_{3A,3B}$ 16.6, H-3A), 1.42 (9H, s, $C(CH_3)_3$) and 1.40 (9H, s, $C(CH_3)_3$); addition of ^2H_2O caused the olefinic dt at 7.53 ppm to collapse to a t with $J_{6,3}$ 2.4; irradiation at the olefinic dt at 7.53 ppm changed the appearance of the NH peak at 8.99 ppm, and H-3 at 2.92 and 2.34 ppm; irradiation at the NH peak at 8.99 ppm gave NOE at the olefinic peak at 7.53 (3.1%), and H-3 at 2.92 (0.6%) and 2.34 ppm (0.9%); δ_C (125.76 MHz, [$^2H_6$]DMSO) 170.6, 166.9 and 154.1 (3 × *C*O), 149.2 (urethane), 131.4 (C-6), 102.7 (C-4), 81.5 and 81.3 (2 × $C(CH_3)_3$), 55.8 (C-2), 27.5 ($C(CH_3)_3$) and 24.2 (C-3). A further unidentified component was recovered (0.4 g).

tert-Butyl (2*S*)-2-*tert*-butoxycarbonylamino-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)propionate 20

tert-Butyl (2*S*,*E*)-*N*-*tert*-butoxycarbonyl-4-ureidomethylene-pyroglutamate **19a** (175 mg, 0.49 mmol) and potassium carbonate (69 mg, 0.49 mmol) were heated at reflux in ethanol (10 ml) for 20 h. The solution was filtered and the solvent was removed *in vacuo* to yield an oil which was purified by flash chromatography on silica gel, eluting with CH_2Cl_2–MeOH (92.5 : 7.5) to yield *tert-butyl (2S)-2-tert-butoxycarbonylamino-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)propionate* **20** as a white solid (100 mg, 57%). An analytical sample was recrystallised from ethanol and ethyl acetate; mp 184 °C (decomp.); *m*/*z* (EI) found: 355.17289. $C_{16}H_{25}N_3O_6$ requires 355.17434); *m*/*z* (+ve FAB, 3-NBA) 711 ($[2M + H]^+$) and 356 ($[M + H]^+$); ν_{max} (KBr)/cm^{-1} 3394 (NH), 1719 (C=O) and 1682 (C=O); λ_{max}(MeOH)/nm 210 and 263 (log ε 3.94 and 3.89); δ_H (500 MHz, [$^2H_6$]DMSO, 392 K) 10.54 (1H, br exch., N*H*), 10.29 (1H, br exch., N*H*), 7.13 (1H, d, $J_{6',NH}$ 4.9, H-6′), 6.38 (1H, exch. d, $J_{NH,2}$ 6.0, N*H*CO_2R), 4.07 (1H, m, H-2), 2.65 (1H, dd, $J_{3A,2}$ 5.5, $J_{3A,3B}$ 14.2, H-3A), 2.44 (1H, dd, $J_{3B,2}$ 8.9, $J_{3B,3A}$ 14.2, H-3B), 1.40 (9H, s, $C(CH_3)_3$) and 1.38 (9H, s, $C(CH_3)_3$).

(2*S*)-2-Amino-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-propionic acid 8

tert-Butyl (2*S*)-2-*tert*-butoxycarbonylamino-3-(2,4-dioxo-pyrimidin-5-yl)propionate **20** (33 mg, 0.09 mmol) was dissolved in concentrated aqueous hydrochloric acid (*ca.* 1 ml). After standing for 5 min at room temperature, the acid was removed *in vacuo* with gentle warming to yield *(2S)-2-amino-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)propionic acid hydrochloride* **8** as a white solid (20 mg, 94%); mp 245 °C (decomp.); $[\alpha]_D^{23}$ −11.6 (*c* 1.2, H_2O); *m*/*z* (EI) found: 199.05857. $C_7H_9N_3O_4$ requires 199.05931; *m*/*z* (+ve FAB, glycerol) 200 ($[M + H]^+$); ν_{max} (KBr)/cm^{-1} 3387, 3197 (NH, OH), 1724 (C=O) and 1670 (C=O); λ_{max}(H_2O)/nm 206 and 264 (log ε 3.85 and 3.72); δ_H (360 MHz, ^2H_2O) 7.30 (1H, br s, H-6′), 4.03 (1H, dd, $J_{2,3A}$ 5.4, $J_{2,3B}$ 7.2, H-2), 2.82 (1H, ddd, $J_{3A,4'}$ 0.6, $J_{3A,2}$ 5.4, $J_{3A,3B}$ 15.1, H-3A) and 2.66 (1H, ddd, $J_{3B,6'}$ 0.4, $J_{3B,2}$ 7.2, $J_{3B,3A}$ 15.1, H-3B); irradiation of the br s for H-6′ at 7.30 ppm changed the appearance of H-3 at 2.82 and 2.66 ppm; δ_C (125.76 MHz, ^2H_2O) 171.3, 167.0 and 153.2 (3 × *C*O), 142.9 (C-6′), 107.4 (C-5′), 52.5 (C-2) and 28.0 (C-3).

Benzyl(2*S*,4*RS*)-*N*-benzyloxycarbonyl-4-*N*-methylaminomethyl-pyroglutamate 21

Method A. Benzyl (2*S*)-*N*-benzyloxycarbonyl-4-methylaminomethylenepyroglutamate **12** (490 mg, 1.2 mmol), sodium cyanoborohydride (86 mg, 1.37 mmol) and screened methyl orange (2 drops) were dissolved in methanol (15 ml). 1 M Aqueous HCl (2.1 ml) was added dropwise with stirring at room temperature, at such a rate as to keep the pH at or above the neutral point of the indicator. The acid was consumed as the reduction commenced until the end point when the indicator stayed red. This required 25 min. After addition of acid was complete the solution was stirred for a further 40 min and concentrated to a small volume *in vacuo*. It was basified by cautious addition of saturated aqueous $NaHCO_3$ (15 ml) and extracted with ethyl acetate. The combined organic phases were washed with brine and dried ($MgSO_4$), and the solvent was removed *in vacuo* to yield *benzyl (2S,4RS)-N-benzyloxycarbonyl-4-N-methylaminomethylpyroglutamate* **21** as a colourless oil (485 mg, 98%). This compound was not characterised further but was used directly in the next step.

Method B. Sodium borohydride (2.88 g, 76.2 mmol) was cautiously added with stirring at room temperature to acetic acid (200 ml). After addition was complete, stirring was continued for 20 min. Benzyl (2*S*)-*N*-benzyloxycarbonyl-4-methylaminomethylenepyroglutamate **12** (5.0 g, 12.7 mmol) was added and the solution stirred for 25 min. Water (7 ml) was added and, once effervescence had slowed, the bulk of the solvent was removed *in vacuo* and the resultant oil was cautiously basified with saturated aqueous $NaHCO_3$ (*ca.* 70 ml) until a milky emulsion resulted. The mixture was extracted with ethyl acetate, further basified with 15% w/v aqueous NaOH (*ca.* 5 ml) to a pH of 9 and extracted with ethyl acetate. The combined organic phases were washed with saturated aqueous $NaHCO_3$ until no further effervescence was seen, and dried (Na_2SO_4). The solvent was removed *in vacuo* to yield *benzyl (2S,4RS)-N-benzyloxycarbonyl-4-N-methylaminomethylpyroglutamate* **21** as a pale yellow oil (5.022 g, 99%).

Benzyl (2*S*,4*RS*)-*N*-benzyloxycarbonyl-4-[(1-methyl-3-phenylureido)methyl]pyroglutamate 22 and 23

Benzyl (2*S*,4*RS*)-*N*-benzyloxycarbonyl-4-*N*-methylaminomethylpyroglutamate **21** (1.14 g, 2.9 mmol) and phenyl isocyanate (0.37 ml, 3.4 mmol) were dissolved in dichloromethane (10 ml). After standing for 15 h at room temperature the solvent was removed *in vacuo* and the resultant oil was purified by flash chromatography on silica gel, eluting with CH_2Cl_2–MeOH (97 : 3). *Benzyl (2S,4RS)-N-benzyloxycarbonyl-4-[1-methyl-3-phenylureido)methyl]pyroglutamate* **22** and **23** was isolated as a colourless oil (1.321 g, 90%). The diastereoisomers were separated by repeated flash chromatography on silica gel, eluting with EtOAc–petroleum ether (60–80 °C) (55 : 45). The major isomer, *benzyl (2S,4S)-N-benzyloxycarbonyl-4-[(1-methyl-3-phenylureido)methyl]pyroglutamate* **22**, was a colourless foam; $[\alpha]_D^{23}$ +13.5 (*c* 0.7, $CHCl_3$) (Found: C, 67.4; H, 5.8; N, 8.0. $C_{29}H_{29}N_3O_6$ requires C, 67.6; H, 5.7; N, 8.15%); *m*/*z* (+ve FAB 3-NBA) 516 ([M + H]$^+$) and 1031 ([2M + H]$^+$); ν_{max} (film)/cm^{-1} 1796 (imide), 1745 (ester) and 1657 (urea); λ_{max} (MeOH)/nm 217 and 234 (log ε 4.02 and 3.98); δ_H (360 MHz, C^2HCl_3) 7.35 (14H, m, Ar*H*), 7.03 (1H, m, Ar*H*), 6.93 (1H, br m, exch. N*H*Ar), 5.28 (2H, m, PhCH_2O), 5.14 (2H, m, PhCH_2O), 4.71 (1H, dd, $J_{2,3A}$ 2.1, $J_{2,3B}$ 8.1, H-2), 3.77 (1H, dd, $J_{6A,4}$ 4.7, $J_{6A,6B}$ 15.0, H-6A), 3.61 (1H, dd, $J_{6B,6A}$ 15.0, $J_{6B,4}$ 5.1, H-6B), 3.04 (3H, s, NCH_3), 2.94 (1H, m, H-4) and 2.29 (2H, m, H-3); δ_C (125.76 MHz, $C_6^2H_6$) 174.2, 171.0 and 156.0 (3 × *C*O), 151.2 (imide), 140.6, 135.7 and 135.6 (3 × *ipso C*), 129.0, 128.8, 128.7, 128.6, 128.5, 128.4, 122.7 and 119.9 (8 × Ar*C*H), 68.4 and 67.4 (2 × Ph*C*O), 57.2 (C-2), 48.1 (C-6), 42.8 (N*C*H$_3$), 35.7 (C-4) and 26.4 (C-3). The minor isomer, *benzyl (2S,4R)-N-benzyloxycarbonyl-4-[(1-methyl-3-phenylureido)methyl]pyroglutamate* **23**, was a colourless foam; $[\alpha]_D^{23}$ +8.2 (*c* 0.45, $CHCl_3$) (Found: C, 67.45; H, 5.7; N, 8.05. $C_{29}H_{29}N_3O_6$ requires C, 67.6; H, 5.7; N, 8.15%); *m*/*z* (+ve FAB 3-NBA) 538 ([M + Na]$^+$) and 516 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 1791 (imide), 1747 (ester) and 1658 (urea); λ_{max}(MeOH)/nm 217 and 234 (log ε 4.02 and 3.98); δ_H (360 MHz, C^2HCl_3) 7.27 (15H, br m, Ar*H*), 6.64 (1H, br exch. N*H*), 5.22 (2H, s, PhCH_2O), 4.99 (2H, m, PhCH_2O), 4.64 (1H, dd, $J_{2,3B}$ 6, $J_{2,3A}$ 9.5, H-2), 3.72 (2H, m, H-6), 3.04, (3H, s, NCH_3), 2.91 (1H, m, H-4), 2.55 (1H, dt, $J_{3A,2} = J_{3A,6}$ 9.5, $J_{3A,3B}$ 13.4, H-3A) and 2.05 (1H, dt, $J_{3B,2} = J_{3B,6}$ 6, $J_{3B,3A}$ 13.4, H-3B); irradiation of H-3A at 2.55 ppm resulted in NOE of H-3B at 2.05 (22.5%), H-4 at 2.91 (5.1%) and H-2 at 4.64 ppm (10.1%); irradiation at H-2 at 4.64 ppm resulted in NOE at peaks for H-3 at 2.55 (4.1%) and 2.91 ppm (0.8%).

Benzyl (2*S*,5′*S*)- and (2*S*,5′*R*)-2-benzyloxycarbonylamino-3-(1-methyl-2,4-dioxo-3-phenylhexahydropyrimidin-5-yl)propionate 24 and 25

Method A. Benzyl (2*S*,4*S*)-*N*-benzyloxycarbonyl-4-[(1-methyl-3-phenylureido)methyl]pyroglutamate **22** (440 mg, 0.85 mmol) was heated in an atmosphere of nitrogen to 130 °C for 111 h. Repeated flash chromatography on silica gel of the resultant oil, eluting with CH_2Cl_2–MeOH (96 : 4) and EtOAc–Et_2O (92 : 8) yielded *benzyl (2S,5′S)-2-benzyloxycarbonylamino-3-(1-methyl-2,4-dioxo-3-phenylhexahydropyrimidin-5-yl)propionate* **24** as a colourless foam (89 mg, 20%); $[\alpha]_D^{23}$ −17 (*c* 0.8, $CHCl_3$) (Found: C, 67.3; H, 5.6; N, 8.0. $C_{29}H_{29}N_3O_6$ requires C, 67.6; H, 5.7; N, 8.15%); *m*/*z* (+ve FAB, 3-NBA) 538 ([M + Na]$^+$) and 516 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 3064 (NH), 1723 (C=O) and 1679 (C=O); δ_H (360 MHz, C^2HCl_3) 7.08–7.04 (15H, m, ArH), 5.89 (1H, br exch. d, $J_{NH,2}$ 7.6, N*H*), 5.20 (2H, m, PhCH_2O), 5.12 (2H, m, PhCH_2O), 4.57 (1H, m, H-2), 3.28 (2H, m, H-6′), 2.98 (3H, s, NCH_3), 2.90 (1H, m, H-5′), 2.43 (1H, dt, $J_{3A,2}$ 6.5, $J_{3A,3B}$ 13.5, H-3A) and 1.96 (1H, m, H-3B); δ_C (125.76 MHz, C^2HCl_3) 171.7, 171.3, 156.0 and 153.1 (4 × *C*O), 135.9, 135.5 and 135.0 (3 × *ipso C*), 129.0, 128.6, 128.6, 128.5, 128.4, 128.3, 128.2 and 128.1 (8 × Ar*C*H), 67.4 and 67.1 (2 × Ph*C*O), 52.1 (C-2), 47.7 (C-6′), 37.7 (NCH$_3$), 35.7 (C-5′), and 30.1 (C-3).

Method B. Benzyl (2*S*,4*RS*)-*N*-benzyloxycarbonyl-4-[(1-methyl-3-phenylureido)methyl]pyroglutamate **22** and **23** (1.321 g, 2.5 mmol) and sodium hydride (123 mg of 60% dispersion in oil, 3 mmol) were mixed, with stirring, in tetrahydrofuran (10 ml) under nitrogen. After stirring for 2 h at room temperature the reaction was quenched by addition of 10% w/v aqueous citric acid (10 ml). The mixture was concentrated to a small volume *in vacuo*, diluted with water and extracted with ethyl acetate. The combined organic phases were washed with brine and dried ($MgSO_4$). The solvent was removed *in vacuo* to yield a yellow oil. This was purified by flash chromatography on silica gel, eluting with 10% ethyl acetate in diethyl ether, yielding fractions containing various ratios of the two diastereoisomers present. The overall yield was 41%. *Benzyl (2S,5′R)-2-benzyloxycarbonylamino-3-(1-methyl-2,4-dioxo-3-phenylhexahydropyrimidin-5-yl)propionate* **25** was isolated as a colourless foam (166 mg, 12.6%); $[\alpha]_D^{23}$ +2.3 (*c* 1.1, $CHCl_3$) (Found: C, 67.6; H, 5.65; N, 8.1. $C_{29}H_{29}N_3O_6$ requires C, 67.6; H, 5.7; N, 8.15%); *m*/*z* (+ve FAB, 3-NBA) 516 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 3064 (NH), 3034 (NH), 1723 (C=O) and 1679 (C=O); δ_H (360 MHz, C^2HCl_3) 7.1–7.45 (15H, m, Ar*H*), 5.69 (1H, exch. d, $J_{NH,2}$ 8.4, N*H*), 5.18 (2H, s, PhCH_2O), 5.13 (2H, m, PhCH_2O), 4.51 (1H, ddd, $J_{2,NH}$ 8.4, $J_{2,3B}$ 4.1, $J_{2,3A}$ 10.6, H-2), 3.63 (1H, dd, $J_{6'A,5'}$ 5.9, $J_{6'A,6'B}$ 12.6, H-6′A), 3.37 (1H, dd, $J_{6'B,5'}$ 10.9, $J_{6'B,6'A}$ 12.6, H-6′B), 3.07 (3H, s, NCH_3), 2.90 (1H, m, H-5′), 2.37 (1H, ddd, $J_{3A,5'}$ 4.7, $J_{3A,2}$ 10.6, $J_{3A,3B}$ 14.5, H-3A) and 2.03 (1H, ddd, $J_{3B,5'}$ 9.2, $J_{3B,2}$ 4.1, $J_{3B,3A}$ 14.5, H-3B).

(2*S*,5′*S*)-2-Amino-3-(1-methyl-2,4-dioxo-3-phenylhexahydropyrimidin-5-yl)propionic acid 26

Benzyl (2*S*,5′*S*)-2-benzyloxycarbonylamino-3-(1-methyl-3-phenyl-2,4-dioxohexahydropyrimidin-5-yl)propionate **24** (117 mg, 0.23 mmol) and 5% palladium on carbon (23 mg) were mixed in acetic acid (10 ml) and stirred vigorously in an atmosphere of hydrogen for 1 h. The catalyst was removed by filtration through Celite and the solvent was removed *in vacuo* to yield a colourless oil which solidified on standing. Residual acetic acid was removed by washing with a little methanol and drying (high vacuum, desiccator, over NaOH) to yield *(2S,5′S)-2-amino-3-(1-methyl-2,4-dioxo-3-phenylhexahydropyrimidin-5-yl)propionic* acid **26** as a white solid (62 mg, 94%), mp 178–180 °C; $[\alpha]_D^{23}$ −19.2 (*c* 0.5, H_2O) (Found: C, 54.8; H, 6.0; N, 13.6. $C_{14}H_{17}N_3O_4 \cdot H_2O$ requires C, 54.4; H, 6.2; N, 13.6%); *m*/*z* (+ve FAB, glycerol) 292 ([M + H]$^+$) and 583 ([2M + H]$^+$); ν_{max} (KBr)/cm^{-1} 3416 br (NH, OH) and 1712 (C=O); λ_{max}(MeOH)/nm 207 (log ε 4.01); δ_H (360 MHz, ^2H_2O) 7.32 (3H, m, Ar*H*), 7.02 (2H, m, Ar*H*), 3.78 (1H, t, $J_{2,3}$ 5.7, H-2), 3.44 (2H, m, H-6′), 3.01 (1H, m, H-5′), 2.89 (3H, s, NCH_3), 2.24 (1H, ddd, $J_{3A,2}$ 5.7, $J_{3A,5'}$ 7.5, $J_{3A,3B}$ 15.1, H-3A) and 1.88

(1H, dt, $J_{3B,2}$ 5.7, $J_{3B,3A}$ 15.1, H-3B). A bioassay[12] was performed by Dr A. Batchelor of the Wellcome Foundation. 100 μm of compound **26** elicited an 85% diminution in the response of 50 μm ACDP.

(2*S*,5′*R*)-2-Amino-3-(1-methyl-2,4-dioxo-3-phenylhexahydropyrimidin-5-yl)propionic acid 27

Benzyl (2*S*,3*R*)-2-benzyloxycarbonylamino-3-(1-methyl-2,4-dioxo-3-phenylhexahydropyrimidin-5-yl)propionate **25** (110 mg, 0.21 mmol) and 5% Pd on carbon (26 mg) were stirred vigorously in acetic acid (10 ml) in an atmosphere of hydrogen for 1h at room temperature. The catalyst was removed by filtration and the solvent was removed *in vacuo* to yield *(2S,5′R)-2-amino-(1-methyl-2,4-dioxo-3-phenylhexahydropyrimidin-5-yl)-propionic* acid **27** as a white solid (60 mg, 96%), mp 184–6 °C, $[\alpha]_D^{23}$ −8.3 (*c* 0.5, H_2O); *m*/*z* (EI) found: 291.12155. $C_{14}H_{17}N_3O_4$ requires 291.12191; *m*/*z* (+ve FAB, glycerol) 292 ($[M + H]^+$) and 583 ($[2M + H]^+$); ν_{max} (KBr)/cm^{-1} 3391(OH, NH) and 1714 (C=O); λ_{max}(MeOH)/nm 207 (log ε 3.98); δ_H (360 MHz, ^2H_2O) 7.32 (3H, m, Ar*H*), 7.02 (2H, m, Ar*H*), 3.69 (1H, dd, $J_{2,3B}$ 6.0, $J_{2,3A}$ 7.5, H-2), 3.47 (2H, m, H-6′), 3.17 (1H, m, H-5′), 2.89 (3H, s, NCH_3), 2.09 (1H, dt, $J_{3A,2}$ 7.5, $J_{3A,3B}$ 15.0, H-3A) and 1.89 (1H, ddd, $J_{3B,2}$ 6.0, $J_{3B,5'}$ 3.5, $J_{3B,3A}$ 15.0, H-3B). A bioassay[12] was performed by Dr A. Batchelor of the Wellcome Foundation. 100 μm of compound **27** elicited a 76% diminution in the response of 50 μm ACDP.

Benzyl (2*S*,4*RS*)-*N*-benzyloxycarbonyl-4-[(1-methylureido)-methyl]pyroglutamate 28 and 29

Benzyl (2*S*,4*RS*)-*N*-benzyloxycarbonyl-4-*N*-methylaminomethylpyroglutamate **21** (1.025 g, 2.6 mmol) and potassium cyanate (420 mg, 5.2 mmol) were dissolved with stirring in a mixture of methanol (19 ml) and acetic acid (1 ml). After standing at room temperature for 16 h, the solvent was removed *in vacuo* and the resultant oil was basified by cautious addition of saturated aqueous $NaHCO_3$ (20 ml). The mixture was extracted with ethyl acetate. The combined organic phases were washed with brine and dried ($MgSO_4$), and the solvent was removed *in vacuo* to yield a colourless oil, which was purified by flash chromatography on silica gel, eluting with CH_2Cl_2–MeOH (96 : 4), to yield *benzyl (2S,4RS)-N-benzyloxycarbonyl-4-[(1-methylureido)methyl]pyroglutamate* **28** and **29** as a white foam (974 mg, 86%) The compound was present as two diastereoisomers in a ratio of *ca.* 3 : 7 as indicated by integration in the ^1H NMR spectrum, and these could not be further separated; *m*/*z* (EI) Found: 439.17281. $C_{23}H_{25}N_3O_6$ requires 439.17434; ν_{max} (film)/cm^{-1} 1794 (imide), 1747 (ester) and 1657 (urea); δ_H (360 MHz, C^2HCl_3) 7.48–7.25 (10H, m, Ar*H*), 5.2 (2H, m, PhCH_2O), 5.12 (1.4H, s, PhCH_2O), 5.09 (0.6H, m, PhCH_2O), 4.98 (1.4H, br exch. NH_2), 4.86 (0.6H, br exch. NH_2), 4.68 (0.7H, t, $J_{2,3}$ 5.6, H-2), 4.61 (0.3H, dd, $J_{2,3A}$ 9.5, $J_{2,3B}$ 5.7, H-2), 3.57 (2H, m, H-6), 2.89 (1H, m, H-4), 2.87 (2.1H, s, NCH_3), 2.84 (0.9H, s, NCH_3), 2.49 (0.3H, dt, $J_{3A,2}$ 9.5, $J_{3A,3B}$ 13.7, H-3A), 2.24 (1.4H, m, H-3) and 1.97 (0.3H, ddd, $J_{3B,2}$ 5.7, $J_{3A,4}$ 7.0, $J_{3B,3A}$ 13.7, H-3B); δ_C (125.76 MHz, C^2HCl_3) 173.7, 171.0, 159.3 and 159.3 (5 × *C*O), 150.4 (urethane), 134.8, 134.7, 134.6 and 134.6 (4 × ipso *C*), 128.5, 128.4, 128.4, 128.3, 128.3, 128.3, 128.2, 128.1, 128.0, 128.0 and 127.9 (11 × Ar*C*), 68.2 and 67.3 (2 × PhCO), 57.2 and 56.8 (2 × C-2), 48.8 and 48.0 (2 × C-6), 42.4 and 41.8 (2 × N*C*H_3), 35.6 and 35.5 (C-4), 26.4 and 24.8 (2 × C-3).

Benzyl (2*S*,5′*RS*)-2-benzyloxycarbonylamino-3-(1-methyl-2,4-dioxohexahydropyrimidin-5-yl)propionate 30 and 31

Benzyl (2*S*,4*RS*)-*N*-benzyloxycarbonyl-4-[(1-methylureido)-methyl]pyroglutamate **28** and **29** (524 mg, 1.2 mmol) and sodium hydride (53 mg of a 60% dispersion in oil, 1.4 mmol) were stirred together in tetrahydrofuran (3 ml) for 30 min at room temperature. The reaction was quenched by addition of 10% w/v aqueous citric acid (10 ml) and the resultant mixture was concentrated to a small volume *in vacuo*, diluted with water (10 ml) and extracted with ethyl acetate. The combined organic phases were washed with brine and dried ($MgSO_4$), and the solvent was removed *in vacuo* to yield a pale yellow oil, which was purified by flash chromatography on silica gel, eluting with CH_2Cl_2–MeOH (96 : 4), to yield *benzyl (2S,5′RS)-2-benzyloxycarbonylamino-3-(1-methyl-2,4-dioxohexahydropyrimidin-5-yl)propionate* **30** and **31** as a colourless foam (472 mg, 90%) The two diastereoisomers were present in a ratio of *ca.* 3 : 7 as indicated by integration in the ^1H NMR spectrum, and could not be further separated; *m*/*z* (EI) Found: 439.17755 ($[M]^+$). $C_{23}H_{25}N_3O_6$ requires 439.17434); *m*/*z* (FAB, NBA) 440 ($[M + H]^+$); ν_{max} (film)/cm^{-1} 1702 (C=O); λ_{max}(MeOH)/nm 217 (log ε 3.53); δ_H (360 MHz, C^2HCl_3) 8.40 (1H, br exch. N*H*), 7.26–7.43 (10H, m, Ar*H*), 6.02 (0.7H, exch. d, $J_{NH,2}$ 8, N*H*), 5.87 (0.3H, exch. d, $J_{NH,2}$ 8.8, N*H*), 5.18 (2H, m, PhCH_2O), 5.09 (2H, m, PhCH_2O), 4.50 (0.7H, dt, $J_{2,NH}$ 8.0, $J_{2,3}$ 6.3, H-2), 4.43 (0.3H, ddd, $J_{2,NH}$ 8.8, $J_{2,3A}$ 4.0, $J_{2,3B}$ 10.8, H-2), 3.48 (0.6H, m, H-6′), 3.14 (1.4H, m, H-6′), 2.97 (0.9H, s, NCH_3), 2.89 (2.1H, s, NCH_3), 2.68 (2H, m, H-4), 2.36 (0.7H, dt, $J_{3B,2}$ 6.3, $J_{3B,3A}$ 14.5, H-3B), 2.3 (0.3H, m, H-3), 1.92 (0.3H, m, H-3) and 1.88 (0.7H, m, H-3A).

(2*S*,5′*RS*)-2-Amino-3-(1-methyl-2,4-dioxohexahydropyrimidin-5-yl)propionic acid hydrochloride 32 and 33

Benzyl (2*S*,5′*RS*)-2-benzyloxycarbonylamino-3-(1-methyl-2,4-dioxohexahydropyrimidin-5-yl)propionate **30** and **31** (346 mg, 0.79 mmol) and 10% palladium on carbon (40 mg) were stirred vigorously together in acetic acid (10 ml) in an atmosphere of hydrogen for 1 h at room temperature. The catalyst was removed by filtration through Celite and the solvent was removed *in vacuo* to yield a tan coloured oil. Trace acetic acid was removed by treatment with Dowex 1X8-400 anion exchange resin and elution with water. Elution with 2 M aqueous HCl and removal of excess acid *in vacuo* gave *(2S,5′RS)-2-amino-(1-methyl-2,4-dioxohexahydropyrimidin-5-yl)propionic acid hydrochloride* **32** and **33** (153 mg, 90%). The compound was present as two diastereoisomers in a ratio of *ca.* 3 : 7, which could not be separated; *m*/*z* (EI) Found: 215.08848. $C_8H_{13}N_3O_4$ requires 215.09061; *m*/*z* (+ve FAB, glycerol) 216 ($[M + H]^+$); δ_H (360 MHz, ^2H_2O) 4.06 (0.7H, t, $J_{2,3}$ 6.4, H-2), 4.03 (0.3H, m, H-2), 3.1–3.4 (2H, overlapping m, H-6′), 2.94 (0.3H, m, H-5′), 2.85 (0.7H, m, H-5′), 2.75 (3H, s, NCH_3), 2.23 (0.7H, dt, $J_{3A,2}$ 7.5, $J_{3A,3B}$ 14.4, H-3A), 2.02 (0.3H, m, H-3A), 1.92 (0.3H, m, H-3B) and 1.82 (0.7H, m, H-3B); δ_C (125.76 MHz, ^2H_2O, *both isomers*) 175.0, 174.7, 171.6, 171.4 and 154.6 (5 × *C*O), 51.7 and 51.3 (2 × C-2), 48.75 (C-6′), 37.53 and 36.62 (2 × C-5′), 34.86 and 34.76 (2 × *C*H_3), and 28.51 and 28.14 (2 × C-3).

Benzyl (2*S*,4*RS*)-*N*-benzyloxycarbonyl-4-[(1,3-dimethylthioureido)methyl]pyroglutamate 34 *and* 35

Benzyl (2*S*,4*RS*)-*N*-benzyloxycarbonyl-4-*N*-methylaminomethylpyroglutamate **21** (960 mg, 2.4 mmol) and methyl isothiocyanate (310 mg, 4.2 mmol) were dissolved in dichloromethane (15 ml). After standing for 6 h at room temperature the solvent was removed *in vacuo* and the resultant oil was purified by flash chromatography on silica gel, eluting with 4.5% methanol in dichloromethane to yield *benzyl (2S,4RS)-N-benzyloxycarbonyl-4-[(1,3-dimethylthioureido)methyl]pyroglutamate* **34** and **35** as a colourless foam (818 mg, 72%). The two diastereoisomers were present in a ratio of *ca.* 3 : 7 as indicated by integration in the ^1H NMR spectrum, and could not be further separated (Found: C, 61.1; H, 5.8, N, 8.8. $C_{24}H_{27}N_3O_5S$ requires C, 61.4; H, 5.8; N, 8.95%); *m*/*z* (+ve FAB, 3-NBA) 470 ($[M + H]^+$); δ_H (360 MHz, C^2HCl_3) 7.27–7.45 (10H, m, Ar*H*), 5.84 (0.7H, exch. br, N*H*), 5.66 (0.3H, exch. br, N*H*), 5.21

(2H, m, PhCH_2O), 5.13 (1.4H, s, PhCH_2O), 5.08 (0.6H, m, PhCH_2O), 4.70 (0.7H, dd, $J_{2,3B}$ 1.3, $J_{2,3A}$ 9.3, H-2), 4.63 (0.3H, dd, $J_{2,3B}$ 5.9, $J_{2,3A}$ 9.3, H-2), 4.32 (0.7H, dd, $J_{6A,4}$ 4.9, $J_{6A,6B}$ 14.9, H-6A), 4.31 (0.3H, dd, $J_{6A,4}$ 4.9, $J_{6A,6B}$ 14.9, H-6A), 3.9 (0.7H, dd, $J_{6A,4}$ 5.9, $J_{6B,6A}$ 14.9, H-6B), 3.4 (0.3H, dd, $J_{6B,6A}$ 14.9, $J_{6B,4}$ 6.8, H-6B), 3.07–3.36 (7H, m, H-4 and NCH_3), 2.59 (0.3H, dt, J 9.3, $J_{3A,3B}$ 13.6, H-3A), 2.4 (0.7H, ddd, $J_{3B,2}$ 1.3, $J_{3B,4}$ 8.8, $J_{3B,3A}$ 13.6, H-3A), 2.3 (0.7H, m, H-3B) and 2.03 (0.3H, m, H-3).

Benzyl (2*S*,5′*S*)- and (2*S*,5′*R*)-2-benzyloxycarbonylamino-3-(1,3-dimethyl-2-thioxo-4-oxohexahydropyrimidin-5-yl)propionate 36 and 37

Benzyl (2*S*,4*RS*)-*N*-benzyloxycarbonyl-4-[(1,3-dimethylthioureido)methyl]pyroglutamate **34** and **35** (546 mg, 1.1 mmol) were heated under an atmosphere of nitrogen to 110 °C for 46 h. The resultant oil was purified by flash chromatography on silica gel, eluting with petroleum ether (60–80 °C)–Et_2O (15 : 85) (410 mg, 75%). Partial separation of the diastereoisomers was achieved. Fractions containing pure samples of each diastereoisomer were further purified by recrystallisation from ethanol. The ratio of diastereoisomers in the crude product was unchanged from that of the precursor thioureas **34** and **35**, tentatively allowing us to assign the absolute configuration to each diastereoisomer. The major isomer, *benzyl (2S,5′R)-2-benzyloxycarbonylamino-3-(1,3-dimethyl-2-thioxo-4-oxohexahydropyrimidin-5-yl)propionate* **36**, was a solid, mp 130–131 °C; $[a]_D^{23}$ −24 (*c* 0.9, $CHCl_3$) (Found: C, 61.3; H, 5.8; N, 8.9. $C_{24}H_{27}N_3O_5S$ requires C, 61.4; H, 5.8; N, 8.95%); *m*/*z* (+ve FAB, 3-NBA) 470 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1747 (ester) and 1720 (urethane); λ_{max}(MeOH)/nm 215, 250 and 272 (log ε 3.97, 3.72 and 3.87); δ_H (360 MHz, C^{2}HCl$_3$) 7.30–7.47 (10H, m, Ar*H*), 5.62 (1H, exch. d, $J_{NH,2}$ 8.3, N*H*), 5.17 (2H, m, PhCH_2O), 5.10 (2H, s, PhCH_2O), 4.45 (1H, ddd, $J_{2,NH}$ 8.3, $J_{2,3A}$ 10, $J_{2,3B}$ 4.2, H-2), 3.79 (1H, dd, $J_{6'A,6'B}$ 12.8, $J_{6'A,5'}$ 5.4, H-6′A), 3.51 (3H, s, NCH_3), 3.48 (3H, s, NCH_3), 3.42 (1H, t, $J_{6'B,6'A}$, $J_{6'B,5'}$ 12.8, H-6′B), 2.73 (1H, m, H-5′), 2.31 (1H, ddd, $J_{3B,2}$ 4.2, $J_{3B,5'}$ 9.4, $J_{3B,3A}$ 13.9, H-3B) and 1.93 (1H, ddd, $J_{3A,5'}$ 4.3, $J_{3A,2}$ 10, $J_{3A,3B}$ 13.9, H-3A). The structure of this compound was confirmed by single crystal X-ray diffraction analysis, reported in our preliminary communication,[1] and atomic coordinate data were lodged with the Cambridge Crystallography Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW at that time. They are available on request from the Director of the CCDC at the above address, quoting CCDC reference code QENLUQ, CCDC number 153590.

The minor isomer, *benzyl (2S,5′S)-2-benzyloxycarbonylamino-3-(1,3-dimethyl-2-thioxo-4-oxohexahydropyrimidin-yl)-propionate* **37**, was a solid, mp 105–106 °C; $[a]_D^{23}$ −11.5 (*c* 1, $CHCl_3$) (Found: C, 61.2; H, 5.8; N, 8.8. $C_{24}H_{27}N_3O_5S$ requires C, 61.4; H, 5.8; N, 8.95%); *m*/*z* (+ve FAB, 3-NBA) 470 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 1747 (ester) and 1720 (urethane); λ_{max}(MeOH)/nm 216, 246 and 271 (log ε 4.02, 3.62 and 3.66); δ_H (360 MHz, C^{2}HCl$_3$) 7.27–7.41 (10H, m, Ar*H*), 5.74 (1H, exch. br d, $J_{NH,2}$ 8, N*H*), 5.19 (2H, m, PhCH_2O), 5.10 (2H, m, PhCH_2O), 4.52 (1H, ddd, $J_{2,NH}$ 8, $J_{2,3A}$ 9.8, $J_{2,3B}$ 6, H-2), 3.51 (3H, s, NCH_3), 3.38 (5H, m, NCH_3 and H-6′), 2.74 (1H, m, H-5′), 2.38 (1H, dt, $J_{3B,2}$ 6, $J_{3B,3A}$ 14.5, H-3B) and 1.86 (1H, m, H-3A); irradiation of the peak for H-2 at 4.52 ppm changed peaks for NH at 5.74 ppm and H-3 at 2.38 and 1.86 ppm; δ_C (125.76 MHz, C^{2}HCl$_3$) 180.8, 171.1 and 168.9 (3 × *C*O), 155.8 (urethane), 135.9 and 134.9 (2 × *ipso C*), 178.6, 128.4, 128.4, 128.1 and 128.0 (5 × Ar*C*), 67.4 and 67.0 (2 × Ar*C*H$_2$), 52.0 (C-2), 50.5 (C-6′), 43.8 (N*C*H$_3$) 36.9 (N*C*H$_3$), 34.3 (C-5′) and 30.2 (C-3).

Benzyl (2*S*)-*N*-benzyloxycarbonyl-4-ureidomethylpyroglutamate 38

Benzyl (2*S*)-*N*-benzyloxycarbonyl-4-aminomethylenepyroglutamate **14** (117 mg, 0.31 mmol), sodium cyanoborohydride (23 mg, 0.37 mmol) and screened methyl orange (1 drop) were dissolved in methanol (5 ml). 2 M Aqueous HCl (0.28 ml) was added dropwise with stirring at room temperature at such a rate as to keep the indicator at the neutral point until the indicator remained red following addition of acid. The solution was stirred for a further 2 min, and acetic acid (1.5 ml) and potassium cyanate (100 mg, 1.2 mmol) were added. Stirring was continued until the potassium cyanate had dissolved and the solution was allowed to stand at room temperature for a further 18 h. The solution was concentrated to a small volume *in vacuo*, diluted with saturated aqueous $NaHCO_3$ (10 ml) and extracted with ethyl acetate. The combined organic phases were washed with 10% aqueous citric acid. The combined aqueous acid washings were extracted with ethyl acetate. The combined organic phases were washed with brine and dried ($MgSO_4$), and the solvent was removed *in vacuo* to yield a colourless oil. This was purified by flash chromatography on silica gel, eluting with CH_2Cl_2–MeOH (94 : 6) to yield *benzyl (2S)-N-benzyloxycarbonyl-4-ureidomethylpyroglutamate* **38** as a colourless oil (75 mg, 57%) The compound was present as two diastereoisomers in a ratio of *ca.* 3 : 7 as indicated by integration in the ^{1}H NMR spectrum, and these could not be further separated; *m*/*z* (EI) Found: 425.16140. $C_{22}H_{23}N_3O_6$ requires 425.15869); *m*/*z* (+ve FAB, 3-NBA) 425 ([M]$^+$) and 851 ([2M + H]$^+$); ν_{max} (film)/cm^{-1} 1792 (imide), 1746 (ester) and 1658 (urea); δ_H (360 MHz, C^{2}HCl$_3$) 7.2–7.4 (10H, m, Ar*H*), 6.14 (0.7H, exch. t, $J_{NH,2}$ 5.7, N*H*), 5.88 (0.3H, exch. t, $J_{NH,2}$ 6.5, NH), 5.06–5.25 (4H, m, PhCH_2O), 4.95 (1.4H, s, exch. NH_2), 4.81 (0.6H, s, exch. NH_2), 4.63 (0.7H, dd, $J_{2,3A}$ 1.4, $J_{2,3B}$ 9.5, H-2), 4.58 (0.3H, dd, $J_{2,3A}$ 7.0, $J_{2,3B}$ 9, H-2), 3.56–3.38 (2H, m, H-6), 2.84–2.72 (1H, m, H-4), 2.54 (0.3H, dt, $J_{3B,2}$ 9, $J_{3B,3A}$ 13.6, H-3B), 2.30 (0.7H, ddd, $J_{3B,2}$ 9, $J_{3B,4}$ 12.0, $J_{3A,3B}$ 13.6, H-3A), 2.20 (0.7H, ddd, $J_{3A,2}$ 1.4, $J_{3A,4}$ 9.0, $J_{3A,3B}$ 13.6, H-3A) and 1.92 (0.3H, ddd, $J_{3B,2}$ 7, $J_{3B,4}$ 8.4, $J_{3A,3B}$ 13.6, H-3B); ^2H_2O exchange changed the appearance of the H-6 multiplets at 3.38–3.56 ppm; irradiation of the peaks for H-2 at 4.63 ppm showed a change in appearance of all the H-3 peaks upfield of 2.55 ppm.

Benzyl (2*S*,5′*RS*)-2-benzyloxycarbonylamino-3-(2,4-dioxohexahydropyrimidin-5-yl)propionate 39

Benzyl (2*S*)-*N*-benzyloxycarbonyl-4-ureidomethylpyroglutamate **38** (35 mg, 0.082 mmol) was heated in an atmosphere of argon at 122 °C for 41 h. The resultant oil was purified by flash chromatography on silica gel, eluting with CH_2Cl_2–MeOH (95 : 5), to yield *benzyl (2S,5′RS)-2-benzyloxycarbonylamino-3-(2,4-dioxohexahydropyrimidin-5-yl)propionate* **39** as a colourless oil (18 mg, 51%). No separation of the diastereoisomers could be achieved; *m*/*z* (EI) Found: 425.15617. $C_{22}H_{23}N_3O_6$ requires 425.15869; ν_{max} (film)/cm^{-1} 1718 (ester); δ_H (360 MHz, C^{2}HCl$_3$) 8.57 (1H, exch. s, N*H*), 7.22–7.55 (10H, m, Ar*H*), 6.33 (0.5H, br s, exch. N*H*), 6.25 (0.5H, br s, exch. N*H*), 6.01 (0.5H, d, exch. $J_{NH,2}$ 7.9, N*H*), 5.82 (0.5H, exch d, $J_{NH,2}$ 8.5, N*H*), 5.04–5.24 (4H, m, PhCH_2O), 4.53 (0.5H, m, H-2), 4.45 (0.5H, m, H-2), 3.50 (0.5H, m, H-6′), 3.22 (0.5H, m, H-6′), 3.12 (1H, m, H-6′), 2.64 (1H, m, H-5′), 2.41–2.27 (1H, m, H-3) and 1.96–1.80 (1H, m, H-3); ^2H_2O exchange simplifed the multiplets at 4.53, 4.45, 3.50, 3.22 and 3.12 ppm; irradiation of H-5′ at 2.64 ppm changed the appearance of all multiplets downfield of 3.5 ppm.

(2*S*,5′*RS*)-2-Amino-3-(2,4-dioxohexahydropyrimidin-5-yl)-propionic acid 40

Benzyl (2*S*,5′*RS*)-2-benzyloxycarbonylamino-3-(2,4-dioxohexahydropyrimidin-5-yl)propionate **39** (15 mg, 0.035 mmol) and 10% palladium on carbon (6 mg) were mixed in acetic acid (2 ml) and stirred vigorously in an atmosphere of hydrogen for 95 min at room temperature. The catalyst was removed by

filtration through Celite and the solvent was removed *in vacuo* to yield a colourless oil. This was triturated with methanol, and dried (high vacuum desiccator) to yield *(2S,5′RS)-2-amino-3-(2,4-dioxohexahydropyrimidin-5-yl)propionic acid* **40** as a white solid (4.8 mg, 68%); mp 200 °C (softens). No separation of the diastereoisomers was attempted; *m*/*z* (EI) Found: 201.06141. $C_7H_{11}N_3O_4$ requires 201.07496); *m*/*z* (+ve FAB, thioglycerol) 202 ($[M + H]^+$); ν_{max} (KBr)/cm^{-1} 3250 (NH and OH) and 1747 (C=O); δ_H (360 MHz, ^2H_2O) 3.73 (0.5H, t, $J_{2,3}$ 5.85, H-2), 3.64 (0.5H, dd, $J_{2,3A}$ 5.7, $J_{2,3B}$ 8.1, H-2), 3.3 (1H, m, H-6′), 3.04–3.12 (1H, m, H-6′), 2.79 (0.5H, m, H-5′), 2.64 (0.5H, m, H-5′), 2.13 (0.5H, m, H-3), 1.98 (0.5H, m, H-3) and 1.85–1.73 (1H, m, H-3); irradiation at the multiplet for H-5′ at 2.79 ppm showed a change in multiplets for H-4′ and H-3 at 3.30, 3.08, 1.98 and 1.84 ppm; irradiation at the multiplet for H-5′ at 2.64 ppm showed a change in multiplets for H-4′ and H-3 at 3.30, 3.08, 2.13 and 1.78 ppm; irradiation at the H-2 dd at 3.64 ppm showed a change in multiplets for H-3 at 1.98 and 1.84 ppm; irradiation at the t for H-2 at 3.73 ppm showed a change in the multiplets for H-3 at 2.13 and 1.78 ppm.

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