Synthesis of homochiral amino acid pyrazine and pyrrole analogues of glutamate antagonists

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Received (in Cambridge, UK) 30th November 2001, Accepted 16th January 2002
First published as an Advance Article on the web 7th February 2002

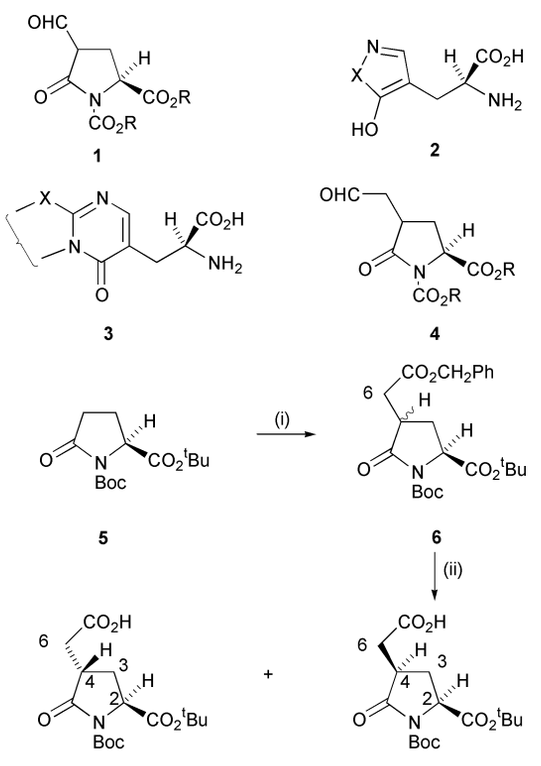
Use of the acid **7** and the aldehydes **23a** and **23b** in "ring switching" reactions with hydrazines has given β-(1-aminopyrrole)amino acids as kinetic products. The products from the reaction of the aldehyde have been converted into β-(pyrazine)amino acids by an equilibration–dehydration sequence. A variety of homochiral reduced heterocyclic amino acids containing two chiral centres has been prepared in this way. Some of the product amino acids undergo "reverse ring switching" to the corresponding pyroglutamic acid derivatives.

Introduction

Antagonists of excitatory glutamate receptors in the central nervous system have potential as drugs to treat a variety of illness, including Alzheimer's disease,[1] epilepsy[2] and ischaemia.[3] L-Alanine derivatives substituted at the β-carbon with a heterocyclic system are of particular interest in this context and we recently devised a novel "ring switching" strategy to allow for the versatile synthesis of homochiral compounds with structures typical of glutamate agonists and antagonists.[4] This involved synthesis of a protected pyroglutamic acid 4-aldehyde **1** or equivalent, which was then reacted with a bisnucleophile. 1,2-Bisnucleophiles gave compounds such as pyrazoles **2** (X = N) and oxazoles **2** (X = O) and 1,3-bisnucleophiles gave a variety of pyrimidine derivatives **3**. We have been able to adapt the method so that poor nucleophiles might be employed, by devising a two-step modification of the method.[5] The modified method also allowed us to access glutamate antagonists containing a second chiral centre.[5] Since it seemed that preparation of the homologous aldehyde **4** and use of 1,2-bisnucleophiles might allow us to extend our synthesis to obtain reduced pyrazine derivatives, we determined to investigate the use of homologues of our original aldehydes as electrophiles in our "ring switching" strategy.

Results and discussion

The pyroglutamate urethane ester **5** was therefore treated with LiHMDS followed by reaction with benzyl bromoacetate to afford an inseparable mixture of the diastereoisomers **6**, as shown in Scheme 1. This mixture was hydrogenolysed to yield the corresponding diastereoisomeric acids, **7** and **8**, in a yield of 37% and a 1 : 1 ratio. A small quantity of the *cis*-(2*S*,4*R*)-isomer **7** could be separated from this mixture by fractional crystallisation. This compound had identical ^{1}H- and ^{13}C-NMR spectra in $C^{2}HCl_3$ to those of a sample which we had previously prepared by oxidation of the 4-benzyl derivative **9**.[6] We were also able to isolate the pure *trans*-(2*S*,4*S*)-isomer **8** for characterisation but not in useful enough quantities for further investigation. In a large scale preparation, a small amount of the *N*-deprotected compound **10** was also obtained. We were unable to assign stereochemistry to this compound by NOE experiments but thermal deprotection of the *cis*-isomer **7** gave an identical compound, implying that this was the (2*S*,4*R*)-isomer shown.



Scheme 1 (i) (a) LiHMDS, (b) $BrCH_2CO_2CH_2Ph$; (ii) H_2/10% Pd–C (37% for (i) + (ii)).

We now sought conditions to convert the *cis*-acid **7** into the corresponding acyl hydrazide, which we hoped might spontaneously undergo our "ring switching" reaction to yield the pyridazine **11**. Coupling with hydrazine in the presence of *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) eventually gave a product in 78% yield as shown in Scheme 2. This seemed to have ^{1}H-NMR spectral

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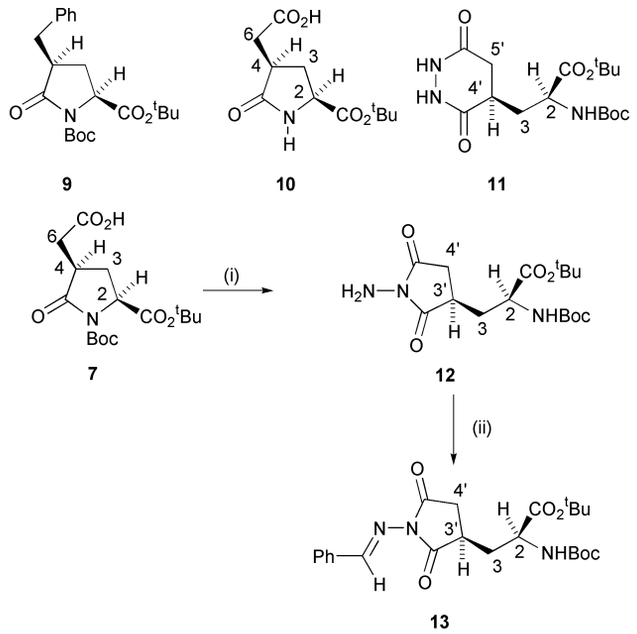
DOI: 10.1039/b110979k

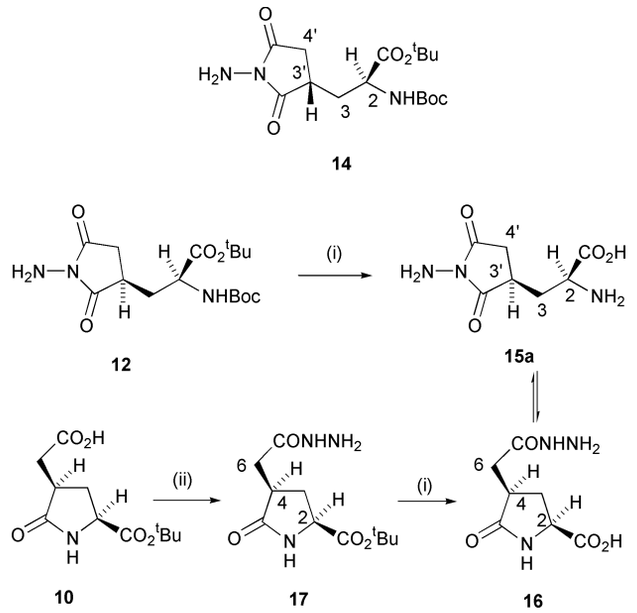


Scheme 2 (i) H_2NNH_2–TBTU (78%); (ii) PhCHO–HOAc–CH_2Cl_2 (69%).

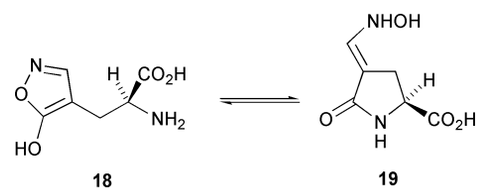
characteristics expected of the pyridazine **11**. The INEPT ^{15}N-NMR spectrum, however, did not show the three NH resonances expected of this compound but had one NH_2 and one NH resonance in keeping with the *N*-aminosuccinimide structure **12**. A carbonyl stretch at 1781 cm^{-1} in the infrared spectrum was indicative of the "imide"-type structure of **12** rather than the isomeric pyridazinedione structure **11**. We found that reaction of our product with benzaldehyde and acetic acid gave the adduct **13** as a single geometric isomer in 69% yield. Although we had hoped to obtain the pyridazine **11** from our reaction, a reasonable body of literature exists on the preparation of amino-imides and diacylhydrazides and indeed phthalic anhydride is known to react with hydrazine to give both the five membered amino-imide and the six membered diacylhydrazide.[7] Use of the diastereoisomeric mixture of acids **7** and **8** in this reaction gave a mixture of the amino-imides **12** and **14** from which the pure diastereoisomer **14** could be separated.

The diastereoisomers **12** and **14** of the amino-imide were separately deprotected using hydrochloric acid as shown in Scheme 3 to give compounds with spectra which were consistent with them being the expected free amino acids **15**. After samples of these compounds had been allowed to stand for some time, however, either neat or in solution, it seemed from the spectra that they were rearranging into one principal new product. The spectra suggested that the rearrangement was in fact a reverse "ring switch" to the pyroglutamic acid hydrazide **16**, a reaction similar to that noted with the oxazole **18**, synthesised in our laboratory[8] and isolated as a natural product.[9] Since we had a reasonable amount of the deprotected *cis*-acid **10**, we were in a position to complete an unequivocal synthesis of the rearrangement product **16**. We therefore converted this, as shown in Scheme 3, to the mixed anhydride with *iso*-butyl chloroformate and converted the product *in situ* to the acyl hydrazide **17** using hydrazine. The acyl hydrazide **17** was deprotected using hydrochloric acid to give an oil, the ^{1}H NMR spectrum of which, when recorded immediately, was identical to that of the compound **16** formed from the (2*S*,4*R*)-amino-imine **15a** on standing. When the sample was left for a significant time, a new component appeared with peaks corresponding to those in the ^{1}H-NMR spectrum of the compound **15a**.

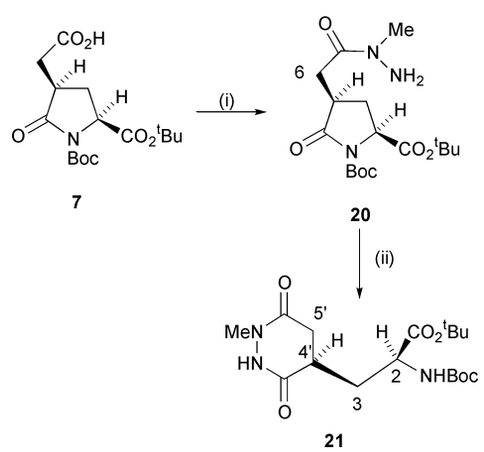
Since methylhydrazine is known to be acylated on the methyl substituted nitrogen,[10] it seemed that use of this reagent might allow us direct access to the pyridazine series of compounds.



Scheme 3 (i) HCl (quant.); (ii) (a) *iso*-butyl chloroformate (b) H_2NNH_2 (82%).



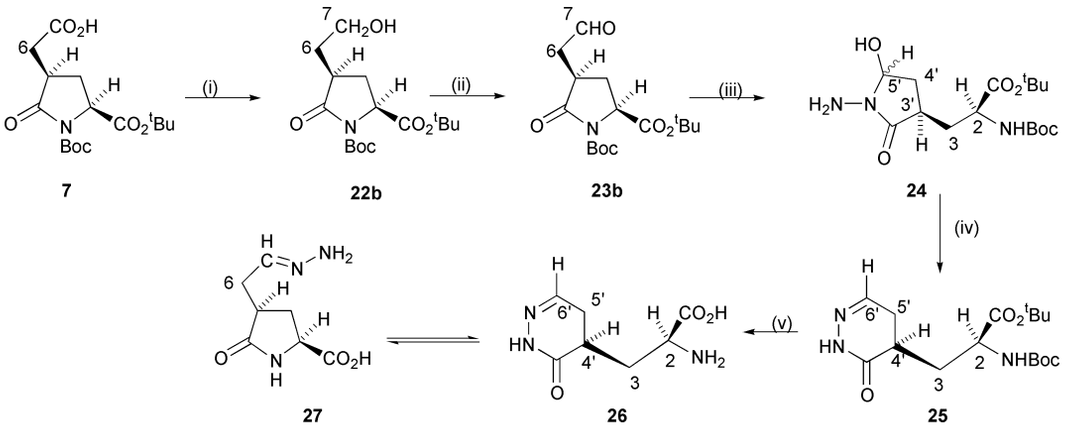
We therefore reacted the (2*S*,4*R*)-acid **7** with methylhydrazine and TBTU and obtained the methyl acylhydrazide **20** in 85% yield, as shown in Scheme 4. The fact that "ring switching" had



Scheme 4 MeNHNH$_2$–TBTU (85%); (ii) Δ/iPr_2NEt–CH_3CN (71%).

not occurred spontaneously was indicated by the fact that the product lacked an exchangeable NH doublet in the ^{1}H-NMR spectrum and had a carbonyl absorption in the infrared spectrum at 1789 cm^{-1}. Rearrangement of the hydrazide **20** to the pyridazine **21** was achieved in 71% yield by heating a solution of the compound at reflux in acetonitrile containing diisopropylethylamine. Attempts to deprotect the pyridazine **21** led to decomposition of the product.

Having achieved "ring switching" to give homochiral heterocyclic amino acids with biologically interesting structures using "ring switching reactions" of the acids **7** and **8** with bisnucleophiles, we now turned to our original interest in the homologous aldehyde **4**. In our initial studies, we first reduced the acid **7** to the alcohol **22b** using $H_3B{\cdot}SMe_2$ and then oxidised this to the aldehyde **23b**, as shown in Scheme 5, using oxalyl chloride

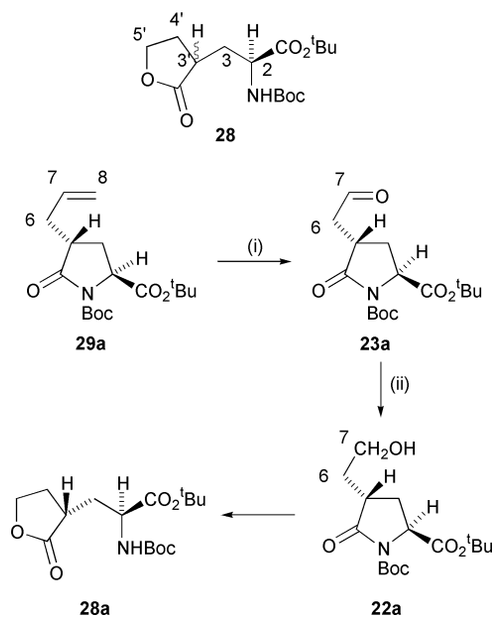


Scheme 5 (i) $H_3B{\cdot}SMe_2$; (ii) ClCOCOCl–Me_2SO; (iii) H_2NNH_2–MeOH [73% for steps (i) to (iii)]; (iv) Δ–HOAc–CH_3CN (64%); (v) F_3CCO_2H (quant.).

and dimethyl sulfoxide. The aldehyde **23b** was then used directly in our "ring switching" studies. Treatment of the aldehyde **23b** with excess of hydrazine in methanol gave a new compound in 73% overall yield from the acid **7**. The spectroscopic data for the product indicated that it was a mixture of the diastereoisomeric carbinolamines **24**. Thus it seemed that, on reaction of hydrazine with the aldehyde **23b**, "ring switching" to the carbinolamines **24** was faster than dehydration to the intermediate hydrazone, which might have undergone ring switching directly to the pyridazine **25**. The INEPT ^{15}N-NMR spectrum indicated an NH_2 and an NH group, consistent with the structure.

In the hope that the "ring switching" reaction might be reversible and that dehydration of the intermediate carbinolamine to a hydrazone might occur, thus leading irreversibly to the pyridazine **25**, we heated the carbinolamines **24** at reflux in acetonitrile containing acetic acid and 3 Å molecular sieves. This resulted in the slow formation of a new compound in 64% yield. The analytical and spectroscopic data for this compound were in accord with its having the pyridazine structure **25**. Deprotection of the pyridazine **25** using trifluoroacetic acid gave a compound with a ^{1}H-NMR spectrum, which was consistent with it being the desired amino acid **26** when recorded immediately. However, after standing for a significant time, changes became apparent in the spectrum which suggested that the "reverse ring switch" to the lactam **27** might have occurred.

In later studies when we attempted to prepare the *trans*-aldehyde **23a** using this method, attempted reduction of the mixed acids **7** + **8** to the alcohols **22** gave a compound which could not be oxidised to the aldehydes **23**. This product exhibited a N*H*CO_2R proton coupled to an α-CH proton in the ^{1}H NMR spectrum and the urethane carbon in the ^{13}C-NMR spectrum showed a characteristic shift from *ca.* 150 ppm, typical of a pyroglutamate type urethane, to 155.7 ppm, typical of a ring opened urethane. The product was therefore a mixture of the diastereoisomeric lactones **28** which would be formed by a "ring switching" reaction of the intermediate alcohols **22**. Because of the apparent unsuitability of the original method for preparation of the *trans*-aldehyde **23a**, the allylpyroglutamate ester urethanes **29a** and **29b**, which we had already prepared,[11] were chosen as an alternative starting point. Since the *trans*-(2*S*,4*R*)-isomer **29a** was the more abundant isomer and, since we had already prepared potential glutamate antagonists in the "*cis*-series", we first subjected the allylpyroglutamate ester urethane **29a** to ozonolysis followed by reductive quenching with triphenylphosphine as shown in Scheme 6. The product had the spectral properties of the desired aldehyde **23a**. To investigate the reactivity of the corresponding alcohol **22a**, we reduced the aldehyde **23a** with sodium cyanoborohydride in methanol at pH 4 and obtained the "ring switched" lactone **28a** in 76% yield. However, a ^{1}H-NMR spectrum recorded immediately after



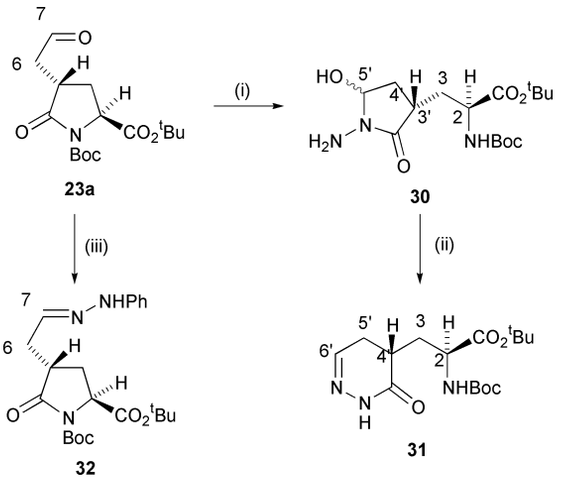
Scheme 6 (i) (a) O_3–CH_2Cl_2–−78 °C, (b) Ph_3P (93%); (ii) $NaBH_3CN$–MeOH pH 4 (76%).

purification indicated the presence of a mixture of the lactone **28a** and what was probably the alcohol **22a**. After *ca.* 4 hours the product had become solely the lactone **28a**.

Having the epimeric aldehyde **23a**, we proceeded to investigate the "ring switching" reactions that we had already carried out on aldehyde **23b**. The aldehyde **23a** was therefore reacted with hydrazine hydrate in methanol as shown in Scheme 7. Two products were obtained and these proved to be the carbinolamine **30** in 65% yield and the pyridazine **31** in 11% yield. Dehydrative rearrangement of the carbinolamine **30** by the method used for the epimer **24** resulted in the pyridazine **31** without loss of stereochemical integrity. When we prepared the *cis*-aldehyde **23b** by ozonolysis of the *cis*-4-allyl derivative **29b**, and it was treated with hydrazine hydrate in methanol, a mixture of the carbinolamine **25** and the pyrazine **26** was obtained, the compounds having identical spectra to those prepared previously.

In an attempt to see if the "ring switching" reaction could be extended to the use of aromatic hydrazines, the aldehyde **23a** was reacted with phenylhydrazine hydrochloride and sodium acetate in methanol. The sole product was the phenylhydrazone **32** in 98% yield.

We have therefore extended our ring switching strategy to the stereochemically discrete aldehydes **23** and the acids **7** and **8** which are homologous to the aldehyde **1** previously used. The acids have been reacted with hydrazine and *N*-methylhydrazine



Scheme 7 (i) H_2HNH_2–MeOH (65%); (ii) Δ–HOAc–CH_3CN (72%); (iii) $PhNHNH_2$·HCl–NaOAc–MeOH (98%).

to give L-alanine substituted in the β-position with succinimide and pyridazinedione respectively. The aldehydes gave five membered carbinolamine derivatives of L-alanine rather than the alternative six membered ring heterocyclic compounds. The five membered carbinolamine could be converted to the corresponding pyrazine using an equilibrium–dehydration sequence. The alcohols **22** were found to be prone to lactonisation. Some of the deprotected amino acids were found to undergo "reverse ring switching" reactions to yield pyroglutamic acid derivatives.

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 or an ATI Unicam UV2-100 Fourier transform scanning spectrophotometer. IR spectra were recorded on Perkin-Elmer 1710 and 1720 Fourier transform spectrometers. ^{1}H-NMR spectra were recorded on Bruker WM360 (360 MHz), DPX 300 (300 MHz), or AMX 500 (500 MHz) spectrometers. *J* values are given in Hz. ^{13}C-NMR spectra were recorded on Bruker AMX 500 (125.76 MHz) or DPX 300 (75.48 MHz) instruments and ^{15}N-spectra on a Bruker AMX 500 (50.7 MHz) instrument. DEPT analysis was used in all ^{13}C-NMR spectra to help assign signals. Low resolution mass spectra were recorded on Kratos MS25 and Kratos MS-80RF spectrometers by Dr A. Abdul Sada and Mr A. Greenway. E.I. mass spectra and some accurate mass measurements were recorded on a Kratos Concept spectrometer by Dr S. Chotai of the Wellcome Foundation and other accurate mass measurements by the EPSRC Central Mass Spectrometry Service at Swansea. Elemental analyses were carried out by the staff of the Wellcome Foundation Physical Sciences Department, or by Medac Ltd. Flash column chromatography was carried out using Merck silica gel 60H (230–300 mesh) or Fluka Silica Gel 60 (220–440 mesh).

tert-Butyl (2*S*,4*RS*)-*N*-*tert*-butoxycarbonyl-4-benzyloxycarbonylmethylpyroglutamate 6

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate **5** [12] (44.84 g, 0.157 mol) was dissolved in tetrahydrofuran (300 ml) and cooled to dry ice–IMS bath temperature, with stirring under an atmosphere of nitrogen. Lithium hexamethyldisilazide (1 M in tetrahydrofuran, 165 ml, 0.165 mol) was added dropwise by cannula, over 8 min, and stirring was continued for a further 55 min. Benzyl bromoacetate (27.5 ml, 0.172 mol) was added dropwise over 3 min. The temperature rose during this time to *ca.* −50 °C. Stirring was continued at *ca.* −70 °C for a further 25 min and the reaction was quenched by rapid addition of saturated aqueous ammonium chloride (500 ml). Vigorous stirring was continued and the reaction mixture was allowed to warm to room temperature. The organic layer was separated and the aqueous phase was extracted with ethyl acetate. The combined organic phases were washed with brine and dried ($MgSO_4$). The solvent was removed *in vacuo* to yield an impure tan-coloured oil (90 g); *m/z* (E.I.) Found 333.15853 ([M − $CO_2{}^tBu$]$^+$), $C_{18}H_{23}NO_5$ requires 333.157623; *m/z* (+ve FAB, thioglycerol + sodium) 456 ([M + Na]$^+$) and 356 ([M − $CO_2{}^tBu$ + Na]$^+$). The ^{1}H NMR spectrum was complex and no satisfactory eluant for purification by flash chromatography on silica gel was found. This material was hydrogenolysed without further purification.

Hydrogenolysis of *tert*-butyl (2*S*,4*RS*)-*N*-*tert*-butoxycarbonyl-4-benzyloxycarbonylmethylpyroglutamate 6

Crude *tert*-butyl (2*S*,4*RS*)-*N*-*tert*-butoxycarbonyl-4-benzyloxycarbonylmethylpyroglutamate **6** (90 g, 0.2 mol) from the above reaction was dissolved in ethanol (120 ml) and 10% palladium on carbon (8 g) was added under an atmosphere of nitrogen. The mixture was stirred vigorously under an atmosphere of hydrogen for 5 h at room temperature. The mixture was filtered through Celite and the solvent was removed *in vacuo* to yield a pale yellow oil which was dissolved in ethyl acetate and extracted with saturated aqueous sodium hydrogen carbonate (4 × 30 ml). The organic phase was washed with brine and dried ($MgSO_4$). The solvent was removed *in vacuo* to yield a tan coloured oil (25 g) which was discarded. The combined aqueous phases were acidified by cautious addition of solid citric acid, until no effervescence was seen on further addition, and the solution was extracted with ethyl acetate. The combined organic phases were washed with brine and dried ($MgSO_4$). The solvent was removed *in vacuo* to yield the product as a colourless oil (20 g, 37% overall from **5**). Recrystallisation from diethyl ether gave an initial crop of material (1.45 g, 3.8%) which proved to be *tert-butyl (2S,4R)-4-carboxymethylpyroglutamate* **10**. Flash chromatography on silica gel, eluting with CH_2Cl_2–MeOH–HOAc (94 : 3 : 3) yielded *tert-butyl (2S,4RS)-N-tert-butoxycarbonyl-4-carboxymethylpyroglutamate* as a colourless oil (16 g, 30% overall from **5**). Recrystallisation from diethyl ether gave variable mixtures of the two diastereoisomers present. The three products were isolated in pure form by repeated recrystallisation.

***tert*-Butyl (2*S*,4*R*)-4-carboxymethylpyroglutamate 10.** (1.45 g, 3.8%); mp 112–115 °C; $[\alpha]_D^{23}$ −15 (*c* 0.7, MeOH); (Found C, 54.1; H, 7.15; N, 5.6. $C_{11}H_{17}NO_5$ requires C, 54.3; H, 7.0; N, 5.8%); *m/z* (+ve FAB, 3-NBA) 487 ([2M + H]$^+$) and 244 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 3261 (OH, NH), 1727 (ester) and 1707 (acid); δ_H (360 MHz, [$^2H_6$]-DMSO) 10.31 (1H, exch., COO*H*), 8.06 (1H, exch., N*H*), 4.03 (1H, t, $J_{2,3}$ 7.7, H-2), 2.65–2.50 (3H, m, overlapping with DMSO residual solvent peak, H-6 and H-4), 2.21 (1H, m, H-3A), 1.62 (1H, m, H-3B) and 1.41 (9H, s, C(CH_3)$_3$).

***tert*-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-4-carboxymethylpyroglutamate 7.** mp 134–136 °C (lit[6] mp 125–127 °C); $[\alpha]_D^{23}$ −5.3 (*c* 2.52, $CHCl_3$); (Found C, 56.0; H, 7.45; N, 4.0. $C_{16}H_{25}NO_7$ requires C, 56.0; H, 7.3; N, 4.1%); *m/z* (+ve FAB, thioglycerol) 366 ([M + Na]$^+$) and 344 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 3416 (OH), 1783 (imide), 1737 (ester) and 1704 (acid); δ_H (360 MHz, C^2HCl_3) 4.42 (1H, dd, $J_{2,3A}$ 8.7, $J_{2,3B}$ 7.6, H-2), 2.99 (2H, m, H-4 and H-6A), 2.67 (1H, dt, $J_{3A,2}$ 8.7, $J_{3A,3B}$ 13.3, H-3A), 2.53 (1H, dd, $J_{6B,4}$ 10.7, $J_{6B,6A}$ 18.0, H-6B), 1.70 (1H, dt, $J_{3B,2}$ 7.6, $J_{3B,3A}$ 13.3, H-3B), 1.52 (9H, s, C(CH_3)$_3$) and 1.49 (9H, s, C(CH_3)$_3$); irradiation at H-6B at 2.53 ppm gave NOE to H-6A at δ 2.99 (19%) and H-3B at 1.70 ppm (1.7%); irradiation at H-2 at δ 4.42 ppm gave NOE to H-6A at δ 2.99 (1.2%) and H-3A

2.67 ppm (2.8%); δ_C (127.56 MHz, C^2HCl_3) 176.1, 173.7 and 170.2 (3 × *C*O), 148.0 (urethane), 83.8 and 82.5 (2 × O*C*(CH_3)$_3$), 58.1 (C-2), 39.1 (C-2), 35.3 (C-6), 27.86 and 27.85 (2 × C(*C*H_3)$_3$) and 27.73 (C-3).

***tert*-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-carboxymethylpyroglutamate 8.** mp 112–116 °C; $[\alpha]_D^{23}$ −17.3 (*c* 1, $CHCl_3$); (Found C, 56.1; H, 7.6; N, 4.05. $C_{16}H_{25}NO_7$ requires C, 56.0; H, 7.3; N, 4.1%); *m*/*z* (+ve FAB, 3-NBA) 366 ([M + Na]$^+$) and 344 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 2982 (OH), 1782 (imide), 1740 (ester) and 1713 (acid); δ_H (360 MHz, C^2HCl_3) 12.1 (1H, br exch., COO*H*), 4.48 (1H, dd, $J_{2,3A}$ 1.0, $J_{2,3B}$ 9.7, H-2), 3.01 (1H, m, H-4), 2.94 (1H, dd, $J_{6A,4}$ 4.3, $J_{6A,6B}$ 17.3, H-6A), 2.54 (1H, dd, $J_{6B,4}$ 8.3, $J_{6B,6A}$ 17.3, H-6B), 2.34 (1H, ddd, $J_{3A,2}$ 1.0, $J_{3A,4}$ 8.7, $J_{3A,3B}$ 13.2, H-3A), 2.07 (1H, ddd, $J_{3B,2}$ 9.7, $J_{3B,4}$ 11.9, $J_{3A,3B}$ 13.2, H-3B), 1.51 (9H, s, C(CH_3)$_3$) and 1.49 (9H, s, C(CH_3)$_3$).

***tert*-Butyl (2*S*,3′*R*)-3-(1-amino-2,5-dioxopyrrolidin-3-yl)-2-*tert*-butoxycarbonylaminopropionate 12**

tert-Butyl (2*S*, 4*R*)-*N*-*tert*-butoxycarbonyl-4-carboxymethylpyroglutamate **7** (258 mg, 0.75 mmol) and diisopropylethylamine (0.131 ml, 0.75 mmol) were dissolved in dimethylformamide (2 ml) at room temperature with stirring under an atmosphere of nitrogen. *O*-(Benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (254 mg, 0.79 mmol) was added, followed after 1 min by aqueous hydrazine hydrate (55%, 0.09 ml, 1.5 mmol), causing a noticeable rise in temperature. Stirring at room temperature was continued for 3 h, and the solvent was removed *in vacuo*. The resultant oily solid was dissolved in ethyl acetate and washed with 10% aqueous citric acid. The aqueous phase was separated 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 colourless oil containing some solid. This was dissolved in a minimum of dichloromethane and filtered twice to remove the solid. The solvent was removed *in vacuo* and the resultant oil was purified by flash chromatography on silica gel, eluting with CH_2Cl_2–MeOH (95 : 5). *tert-Butyl (2S,3′R)-3-(1-amino-2,5-dioxopyrrolidin-3-yl)-2-tert-butoxycarbonylaminopropionate* **12** was recovered as a colourless foam (209 mg, 78%); $[\alpha]_D^{23}$ + 6.8 (*c* 3, $CHCl_3$); (*m*/*z* (E.I.) Found 357.19053, $C_{16}H_{27}N_3O_6$ requires 357.18999); *m*/*z* (+ve FAB, 3-NBA) 380 ([M + Na]$^+$) and 358 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 3334 (NH), 1781 (imide) and 1707 (ester/imide); δ_H (360 MHz, C^2HCl_3) 5.22 (1H, exch d, $J_{NH,2}$ 8.4, N*H*), 4.30 (2H, br exch, NH_2), 4.22 (1H, m, H-2), 3.04 (1H, dd, $J_{4'A,3'}$ 8.8, $J_{4'A,4'B}$ 18.1, H-4′A), 2.88 (1H, m, H-3′), 2.50 (1H, dd, $J_{4'B,3'}$ 4.15,$J_{4'B,4'A}$ 18.1, H-4′B), 2.19 (1H, m, H-3A), 1.92 (1H, m, H-3B), 1.46 (9H, s, C(CH_3)$_3$) and 1.42 (9H, s, C(CH_3)$_3$); δ_C (127.56 MHz, C^2HCl_3) 176.4, 173.4 and 170.6 (3 × *C*O), 155.6 (urethane), 82.8 and 80.13 (2 × O*C*(CH_3)$_3$), 51.8 (C-2), 35.5 (C-3′), 35.0 and 33.1 (C-4′ and C-3), 28.2 and 27.9 ppm (2 × C(*C*H_3)$_3$); δ_N (50.7 M, C^2HCl_3) −296 (+1; −1 d, *J* 92, *N*H) and −323 ppm (+1; 0, −1 t, *J* 69.3, *N*H_2).

***tert*-Butyl (2*S*,3′*S*)-3-(1-amino-2,5-dioxopyrrolidin-3-yl)-2-*tert*-butoxycarbonylaminopropionate 14**

Since a pure sample of *tert*-butyl (2*S*, 4*S*)-*N*-*tert*-butoxycarbonyl-4-carboxymethylpyroglutamate **8** was not available, the mixture of diastereoisomers **7** + **8**, was employed in the coupling procedure with hydrazine described above. The product *tert*-butyl (2*S*,3*RS*)-3-(1-amino-2,5-dioxopyrrolidin-3-yl)-2-*tert*-butoxycarbonylaminopropionate was isolated and partially separated by flash chromatography on silica gel, eluting with Et_2O–EtOAc (7 : 3). Repeated flash chromatography on silica gel resulted in a low yield of a pure sample of *tert-butyl (2S,3′S)-3-(1-amino-2,5-dioxopyrrolidin-3-yl)-2-tert-butoxycarbonylaminopropionate* **14** as a colourless oil; $[\alpha]_D^{23}$ +11.5 (*c* 1, $CHCl_3$); *m*/*z* (E.I.) Found 301.127068 ([M − $CO_2{}^tBu$]$^+$), $C_{12}H_{18}N_3O_6$ requires 301.127386; *m*/*z* (E.I.) 358 ([M + H]$^+$) and 301 (M − $CO_2{}^tBu$]$^+$); ν_{max} (film)/cm^{-1} 3337 and 2980 (NH), 1785 (imide) and 1708 (ester/imide); δ_H (360 MHz, C^2HCl_3) 5.28 (1H, exch. d, $J_{NH,2}$ 7.5, N*H*), 4.28 (2H, br exch, NH_2), 4.28 (1H, m, H-2), 2.99 (1H, m, H-4), 2.88 (1H, dd, $J_{4'A,3'}$ 8.9, $J_{4'A,4'B}$ 18.0, H-4′A), 2.59 (1H, dd, $J_{4'B,4}$ 3.6, $J_{4'B,4'A}$ 18.0, H-4′B), 2.43 (1H, ddd, $J_{3A,3'}$ 4.5, $J_{3A,2}$ 5.75, $J_{3A,3B}$ 14.5, H-3A), 1.85 (1H, ddd, $J_{3B,2}$ 8.2, $J_{3B,3'}$ 8.75, $J_{3B,3A}$ 14.5, H-3B), 1.46 (9H, s, C(CH_3)$_3$) and 1.42 (9H, s, C(CH_3)$_3$); addition of ^2H_2O caused the multiplet for H-2 at 4.28 ppm to simplify to a dd, $J_{2,3A}$ 5.75, $J_{2,3B}$ 8.2; δ_C (127.56 MHz, C^2HCl_3) 176.4, 173.3 and 170.6 (3 × *C*O), 155.2 (urethane), 82.7 and 80.1 (2 × O*C*(CH_3)$_3$), 52.3 (C-2), 35.9 (C-3′), 34.0 and 32.6 (C-4′ and C-3), 28.2 and 27.9 (2 × C(*C*H_3)$_3$).

***tert*-Butyl (2*S*,3′*R*)-3-(1-benzylideneamino-2,5-dioxopyrrolidin-3-yl)-2-*tert*-butoxycarbonylaminopropionate 13**

tert-Butyl (2*S*, 3′*R*)-3-(1-amino-2,5-dioxopyrrolidin-3-yl)-2-*tert*-butoxycarbonylaminopropionate **12** (50 mg, 0.14 mmol), benzaldehyde (0.021 ml, 0.21 mmol) and acetic acid (0.016 ml, 0.28 mmol) were dissolved in dichloromethane (2 ml) and heated at reflux for 68 h under an atmosphere of nitrogen. Further benzaldehyde (0.021 ml, 0.21 mmol) and acetic acid (0.016 ml, 0.28 mmol) were added after 21 h at reflux. The solvent was removed *in vacuo* and the resultant oil was purified by flash chromatography on silica gel, eluting with CH_2Cl_2–MeOH (98 : 2). *tert-Butyl (2S,3′R)-3-(1-benzylideneamino-2,5-dioxopyrrolidin-3-yl)-2-tert-butoxycarbonylaminopropionate* **13** was isolated as an off-white solid (43 mg, 69%). An analytical sample was obtained by recrystallisation from diethyl ether as a white solid (23 mg, 37%); mp 121–122 °C; $[\alpha]_D^{23}$ −12.6 (*c* 1.5, $CHCl_3$); (Found C, 62.2; H, 7.05; N, 9.3. $C_{23}H_{31}N_3O_6$ requires C, 62.0; H, 7.0; N, 9.4%); *m*/*z* (+ve FAB, 3-NBA) 891 ([2M + H]$^+$) and 446 ([M + H]$^+$); λ_{max} (MeOH)/nm 221 and 271 (log ε 3.99 and 4.17); ν_{max} (KBr)/cm^{-1} 1780 (w, imide), 1718 (s, ester/imide) and 1694 (C=O); δ_H (360 MHz, C^2HCl_3) 9.13 (1H, s, PhC*H*=), 7.83 (2H, m, Ar*H*), 7.48 (3H, m, Ar*H*), 5.23 (1H, exch.d, $J_{NH,2}$ 8.3, N*H*), 4.28 (1H, m, H-2), 3.16 (1H, dd, $J_{4'A,3'}$ 9.0, $J_{4'A,4'B}$ 18.3, H-4′A), 2.97 (1H, m, H-3′), 2.63 (1H, dd, $J_{4'B,3'}$ 4.7, $J_{4'B,4'A}$ 18.3, H-4′B), 2.28 (1H, m, H-3A), 2.01 (1H, m, H-3B), 1.48 (9H, s, C(CH_3)$_3$) and 1.45 (9H, s, C(CH_3)$_3$); addition of ^2H_2O caused the multiplet for H-2 at 4.28 ppm to simplify to a dd $J_{2,3A}$ 2.8 $J_{2,3B}$ 10.2; δ_C (127.56 MHz, C^2HCl_3) 175.5, 172.3 and 170.6 (3 × CO), 162.1 (C=N), 155.7 (urethane), 132.9 (*ipso C*), 132.1, 128.7 and 128.6 (3 × ArC), 82.9 and 80.1 (2 × O*C*(CH_3)$_3$), 51.8 (C-2), 35.6 (C-3′), 35.5 and 33.6 (C-3 and C-4′), 28.2 and 27.9 (2 × C(*C*H_3)$_3$).

(2*S*,3′*R*)-3-(1-Amino-2,5-dioxopyrrolidin-3-yl)-2-aminopropionic acid hydrochloride 15a

tert-Butyl (2*S*,3′*R*)-3-(1-amino-2,5-dioxopyrrolidin-3-yl)-2-*tert*-butoxycarbonylaminopropionate **12** (66 mg, 0.18 mmol) was shaken with conc. hydrochloric acid (*ca.* 1 ml) at room temperature. Vigorous effervescence occurred as the deprotection proceeded and, when this had subsided, the acid was removed *in vacuo* with gentle warming to yield *(2S,3′R)-3-(1-amino-2,5-dioxopyrrolidin-3-yl)-2-aminopropionic acid hydrochloride* **15a** as a white solid (62 mg, hydrate, quantitative); mp 120–130 °C; $[\alpha]_D^{23}$ +8.4 (*c* 1.2, H_2O); *m*/*z* (E.I.) Found 201.07651, $C_7H_{11}N_3O_4$ requires 201.07496; *m*/*z* (E.I.) 202 ([M + H]$^+$); ν_{max} (film)/cm^{-1} 3200 (OH) and 1703 (ester/imide); δ_H (360 MHz, ^2H_2O) 4.02 (1H, t, $J_{2,3}$ 6.55, H-2), 2.98 (1H, m, H-4), 2.83 (1H, dd, $J_{4'A,3'}$ 8.8, $J_{4'A,4'B}$ 18.1, H-4′A), 2.34 (1H, dd, $J_{4'B,3'}$ 4.6, $J_{4'B,4'A}$ 18.1, H-4′B), 2.25 (1H, dt, $J_{3A,2\ +\ 3'}$ 6.95, $J_{3A,3B}$ 14.5, H-3A) and 1.96 (1H, ddd, $J_{3B,2}$ 6.1, $J_{3B,3'}$ 8.5, $J_{3B,3A}$ 14.5, H-3B); irradiation at the dd for H-2 at 4.02 ppm showed a change in appearance of the peaks for H-3 at 2.25 and 1.96 ppm; δ_C (127.56 MHz, ^2H_2O) 178.9, 176.8 and 170.8 (3 × *C*O), 50.7 (C-2), 34.4 (C-3′), 32.4 and 30.4 (C-3 and C-4′). The spectral properties changed on standing in solution.

(2*S*,3′*S*)-3-(1-Amino-2,5-dioxopyrrolidin-3-yl)-2-aminopropionic acid hydrochloride 15b

tert-Butyl (2*S*,3′*S*)-3-(1-amino-2,5-dioxopyrrolidin-3-yl)-2-*tert*-butoxycarbonylaminopropionate **14** (62 mg, 0.17 mmol) was shaken with conc. hydrochloric acid (*ca.* 1 ml) until effervescence had ceased. The acid was removed *in vacuo* with gentle warming to yield *(2S,3′S)-3-(1-amino-2,5-dioxopyrrolidin-3-yl)-2-aminopropionic acid hydrochloride* **15b** as a viscous oil (60 mg, hydrate, quantitative); $[\alpha]_D^{23}$ +25.9 (*c* 2.5, H_2O); *m*/*z* (E.I.) Found 201.07404, $C_7H_{11}N_3O_4$ requires 201.07496; *m*/*z* (+ve FAB, glycerol) 403 ($[2M + H]^+$) and 202 ($[M + H]^+$); ν_{max} (film)/cm^{-1} 3900–2900 (OH, NH) and 1703 (C=O); δ_H (360 MHz, ^2H_2O) 4.04 (1H, t, $J_{2,3}$ 7.0, H-2), 3.0 (1H, m, H-3′), 2.78 (1H, dd, $J_{4'A,3'}$ 8.9, $J_{4'A,4'B}$ 18.2, H-4′A), 2.31 (1H, dd, $J_{4'B,3'}$ 4.6, $J_{4'B,4'A}$ 18.2, H-4′B) and 2.05 (2H, m, H-3); irradiation at the triplet for H-2 at 4.04 ppm showed a change in the appearance of H-3 at 2.05 ppm; δ_C (127.56 MHz, ^2H_2O) 178.81, 176.5 and 170.8 (3 × *C*O), 50.90 (C-2), 35.87 (C-3′), 32.33 and 30.54 ppm (C-3 & C-4′). The spectral properties were seen to change on standing.

***tert*-Butyl (2*S*,4*R*)-4-hydrazinocarbonylmethylpyroglutamate 17**

tert-Butyl (2*S*,4*R*)-4-carboxymethylpyroglutamate **10** (107 mg, 0.44 mmol) and *N*-methylmorpholine (0.048 ml, 0.44 mmol) were dissolved in dimethylformamide (2 ml) with stirring and under an atmosphere of nitrogen. The solution was cooled to *ca.* −12 °C in an ice–ammonium chloride bath. *iso*-Butyl chloroformate (0.063 ml, 0.48 mmol) was added and the solution was stirred for 3 min. Aqueous hydrazine hydrate (55%, 0.051 ml, 0.88 mmol) was added, causing an instant thick precipitate. Stirring was continued for 30 min at *ca.* −12 °C. The solution was concentrated to a small volume *in vacuo* and purified by flash chromatography on silica gel, eluting with CH_2Cl_2: MeOH–NEt_3 (91 : 5 : 4). *tert-Butyl (2S,4R)-4-hydrazinocarbonylmethylpyroglutamate* **17** was isolated as a colourless oil, (93 mg, 82%); *m*/*z* (E.I.) Found 257.13789, $C_{11}H_{19}N_3O_4$ requires 257.13756; ν_{max} (film)/cm^{-1} 2981 (NH) and 1699 (C=O); δ_H (360 MHz, C^2HCl_3) 9.49 (1H, br d. exch. N*H*NH_2), 6.79 (1H, exch. N*H*CO), 4.13 (1H, t, $J_{2,3}$ 8.1, H-2), 3.07–2.63 (3H, m, H-3A, H-4 and H-6A), 2.35 (1H, dd, $J_{6B,4}$ 7.8, $J_{6B,6A}$ 15.1, H-6B), 1.88 (1H, m, H-3B) and 1.46 (9H, s, $C(CH_3)_3$). Irradiation at the t for H-2 at 4.13 ppm showed a change in appearance of the multiplet for H-3B at 1.88 ppm and to the complex multiplet at 3.07–2.63 ppm; residual triethylamine was evident in the spectrum.

(2*S*,4*R*)-4-(Hydrazinocarbonylmethyl)pyroglutamate 16

tert-Butyl 4-(hydrazinocarbonylmethyl)-(2*S*,4*R*)-pyroglutamate **17** (30 mg, 0.11 mmol) was shaken at room temperature with conc. hydrochloric acid (*ca.* 0.5 ml) until effervescence had subsided. The acid was removed *in vacuo* with gentle warming to yield *(2S,4R)-4-(hydrazinocarbonylmethyl)pyroglutamate* **16** as a colourless oil (25 mg, hydrate, quantitative); *m*/*z* (E.I.) Found 201.07597 ($[M]^+$), $C_7H_{11}N_3O_4$ requires 201.07496; *m*/*z* (+ve FAB, thioglycerol + sodium) 224 ($[M + Na]^+$), 202 ($M + H]^+$); δ_H (360 MHz, ^2H_2O) 4.19 (1H, t, $J_{2,3}$ 8.4, H-2), 2.82 (1H, m, H-4), 2.59 (1H, dt, $J_{3A,2}$ 8.4, $J_{3A,3B}$ 13.0, H-3A), 2.51 (1H, dd, $J_{6A,4}$ 5.1, $J_{6A,6B}$ 16.0, H-6A), 2.33 (1H, dd, $J_{6B,4}$ 7.7, $J_{6B,6A}$ 16.0, H-6B) and 1.70 (1H, dt, $J_{3B,2}$ 8.4, $J_{3B,3A}$ 13.0, H-3B); residual triethylamine was evident. The spectral properties were seen to change on standing.

***tert*-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-4-(*N*-methylhydrazinocarbonylmethyl)pyroglutamate 20**

tert-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-4-carboxymethylpyroglutamate **7** (535 mg, 1.56 mmol) and diisopropylethylamine (0.272 ml, 1.56 mmol) were dissolved in dimethylformamide (4 ml) with stirring under an atmosphere of argon. The solution was cooled to ice bath temperature and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (526 mg, 1.64 mmol) was added with stirring. Methylhydrazine (0.101 ml, 1.87 mmol) was added and stirring was continued for 24 h. The solution was concentrated *in vacuo* and the resultant oil was partitioned between 10% aqueous citric acid and ethyl acetate. The organic phase was separated and washed with 10% aqueous citric acid. The combined aqueous phases were extracted with ethyl acetate. The combined organic phases were washed with saturated aqueous sodium hydrogen carbonate. The combined basic washings were 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 colourless oil which was purified by flash chromatography on silica gel, eluting with CH_2Cl_2–MeOH (95 : 5) to yield *tert-butyl (2S,4R)-N-tert-butoxycarbonyl-4-(N-methylhydrazinocarbonylmethyl)pyroglutamate* **20** as a white solid; (491 mg, 85%), which was recrystallised from diethyl ether, mp 118–120 °C; $[\alpha]_D^{23}$ +17.2 (*c* 1.2, $CHCl_3$); (Found C, 54.7; H, 7.8; N, 11.1. $C_{17}H_{29}N_3O_6$ requires C, 55.0; H, 7.9; N, 11.3%); *m*/*z* (+ve FAB, 3-NBA) 394 ($[M + Na]^+$) and 372 ($[M + H]^+$); ν_{max} (film)/cm^{-1} 2926 (br NH), 1789 (imide), 1746 (ester) and 1643 (hydrazide); δ_H (360 MHz, C^2HCl_3) 4.38 (1H, m, H-2), 3.23 (1H, dd, $J_{6A,4}$ 3.9, $J_{6A,6B}$ 16.7, H-6A), 3.14 (3H, s, NCH_3), 3.00 (1H, m, H-3A), 2.71 (< 2H, m, H-6B and H-4), 1.60 (1H, m, H-3B), 1.41 (9H, s, $C(CH_3)_3$) and 1.40 (9H, s, $C(CH_3)_3$); irradiation at the dd for H-2 at 4.38 ppm showed a change in appearance of the multiplets for H-3 at 3.0 and 1.6 ppm.

***tert*-Butyl (2*S*,4′*R*)-2-(*tert*-butoxycarbonylamino)-3-(1-methyl-3,6-dioxohexahydropyridazin-4-yl)propionate 21**

tert-Butyl (2S,4R)-N-tert-butoxycarbonyl-4-(N-methylhydrazinocarbonylmethyl)pyroglutamate **20** (324 mg, 0.87 mmol) was dissolved in acetonitrile (15 ml) containing diisopropylethylamine (1 ml) and the solution was heated at reflux for 24 h under an atmosphere of nitrogen. The solvents were removed *in vacuo* and the resultant oil was purified by flash chromatography on silica gel, eluting with CH_2Cl_2–MeOH–AcOH (94 : 3 : 3) to yield *tert-butyl (2S,4′R)-2-(tert-butoxycarbonylamino)-3-(1-methyl-3,6-dioxohexahydropyridazin-4-yl)propionate* **21** as a pale yellow foam (231 mg, 71%); *m*/*z* (E.I.) Found 315.144268, $C_{13}H_{21}N_3O_6$ ($[M - C_4H_9 + H]^+$) requires 315.143036; *m*/*z* (+ve FAB, 3-NBA) 394 ($[M + Na]^+$) and 372 ($[M + H]^+$); λ_{max}(MeOH)/nm 205 and 243 (log ε 3.81 and 3.65); λ_{max}(MeOH, NaOH)/nm 208 and 263 (log ε 4.15 and 3.74) (reversible); ν_{max} (film)/cm^{-1} 3258 (NH), 2979, 1713 (ester) and 1658 (hydrazide); δ_H (360 MHz, C^2HCl_3) 10.1 (1H, br exch. N*H*), 5.31 (1H, exch. d, $J_{NH,2}$ 8.0, N*H*), 4.19 (1H, m, H-2), 3.23 (3H, s, NCH_3), 2.76 (2H, m, H-5′), 2.49 (1H, m, H-4′), 1.82 (2H, m, H-3), 1.46 (9H, s, $C(CH_3)_3$) and 1.45 (9H, s, $C(CH_3)_3$); addition of ^2H_2O caused the multiplet at 4.19 ppm to change in appearance.

***tert*-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-4-(2-hydroxyethyl)-pyroglutamate 22b**

tert-Butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-4-carboxymethylpyroglutamate **7** (2.068 g, 6.03 mmol) was dissolved in tetrahydrofuran (15 ml) and cooled to ice bath temperature, with stirring and under an atmosphere of nitrogen. Borane–dimethyl sulfide complex (2 M in tetrahydrofuran, 4 ml, 8 mmol) was added dropwise over 1 min. The mixture was allowed to warm to room temperature and left for a further 1.5 h. Methanol (20 ml) was added and stirring was continued for 2 min. The solvents were removed *in vacuo* to yield *tert-butyl (2S,4R)-N-tert-butoxycarbonyl-4-(2-hydroxyethyl)pyroglutamate* **22b** (2.1 g, 100%) as a white solid used directly in the next stage. An analytical sample was recrystallised from diethyl ether–petroleum ether (60 : 80); mp 80–82 °C; $[\alpha]_D^{23}$ −21.1 (*c* 1.4,

$CHCl_3$); (Found C, 58.2; H, 8.5; N, 4.2. $C_{16}H_{27}NO_6$ requires C, 58.3; H, 8.3; N, 4.25%); *m/z* (+ve FAB, thioglycerol + sodium) 352 ([M + Na]$^+$) and 330 ([M + H]$^+$); ν_{max} (KBr)/cm^{-1} 3295 (OH), 1777 (imide), 1739 (ester) and 1703 (imide); δ_H (360 MHz, C^2HCl_3) 4.39 (1H, dd, $J_{2,3B}$ 6.5, $J_{2,3A}$ 9.0, H-2), 3.79 (1H, m, H-7A), 3.69 (1H, m, H-7B), 2.73 (1H, m, H-4), 2.56 (1H, dt, $J_{3A,4}$ 9.0, $J_{3A,2}$ 13.1, H-3A), 2.03 (1H, m, H-6A), 1.66 (2H, m, H-6B and H-3B), 1.50 (9H, s, C(CH_3)$_3$) and 1.48 (9H, s, C(CH_3)$_3$); irradiation of the peak for H-2 at 4.39 ppm gave a change in appearance of the peaks for H-3 at 2.56 and 1.66 ppm; irradiation at the peaks for H-3A at 2.56 ppm showed a change in appearance of the peaks at 2.73 for H-4 and 1.66 ppm for H-3B; δ_C (127.56 MHz, C^2HCl_3) 176.03 and 170.42 (2 × *C*O), 149.27 (urethane), 83.49 and 82.16 (2 × O*C*(CH$_3$)$_3$), 60.36 (C-7), 58.29 (C-2), 40.35 (C-4), 34.07 (C-6), 27.97 and 27.80 ((2 × C(*C*H$_3$)$_3$) and 27.77 (C-3).

tert-Butyl (2*S*,3′*R*)-3-[(5*RS*)-1-amino-5-hydroxy-2-oxopyrrolidin-3-yl]-2-*tert*-butoxycarbonylaminopropionate 24

Oxalyl chloride (0.026 ml, 0.29 mmol) was dissolved in dichloromethane (0.8 ml) and the solution was cooled to *ca.* −60 °C in a dry ice–chloroform bath with stirring under an atmosphere of nitrogen. Dimethyl sulfoxide (0.042 ml, 0.59 mmol) was added by syringe/subaseal, and the solution was stirred for 3 min. A solution of *tert*-butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-4-(2-hydroxyethyl)pyroglutamate **22b** (89 mg, 0.27 mmol) in dichloromethane (0.5 ml) was added followed by dichloromethane (2 × 0.5 ml). Stirring was continued for a further 15 min and triethylamine (0.188 ml, 1.35 mmol) was added. Stirring at *ca.* −60 °C was continued for 5 min, the solution was allowed to warm to room temperature and water (2 ml) was added. Further water (10 ml) was added and the organic phase was separated. The aqueous phase was extracted with dichloromethane. 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 dissolved in methanol (2 ml) and hydrazine hydrate (0.083 ml, 0.97 mmol) was added. After standing for 30 min at room temperature the solvents were removed *in vacuo* and the resultant oil was purified by flash chromatography on silica gel, eluting with CH_2Cl_2–MeOH (92 : 8) to yield *tert-butyl (2S,3′R)-3-[(5RS)-1-amino-5-hydroxy-2-oxopyrrolidin-3-yl]-2-tert-butoxycarbonylaminopropionate* **24** as a colourless oil; (71 mg, 73%); *m/z* (E.I.) Found 359.20764, $C_{16}H_{29}N_3O_6$ requires 359.20564; ν_{max} (film)/cm^{-1} 1702 (ester/imide); δ_H (500 MHz, 368 K, [$^2H_6$]-DMSO, *mixed diastereoisomers*) 6.79 (1H, br d exch. O*H*), 5.85 (0.5H, exch. d, $J_{NH,2}$ 5.2, N*H*), 5.73 (0.5H, exch. d, $J_{NH,2}$ 5.5, N*H*), 4.92 (1H, m, H-6), 4.24 (2H, br exch. s, NH_2), 3.90 (1H, m, H-2), 2.57 (0.5H, m, H-4′A), 2.37 (0.5H, ddd, $J_{4'A,3'}$ 6.4, $J_{4',5'}$ 9.3, $J_{4'A,4'B}$ 15.8, H-4′A), 2.33 (0.5H, m, H-4′B), 2.02 (1H, m, H-3′), 1.92 (0.5H, ddd, $J_{3A,3'}$ 1.2, $J_{3A,2}$ 8.4, $J_{3A,3B}$ 13.1, H-3A), 1.78 (0.5H, ddd, $J_{3B,3'}$ 6.25, $J_{3B,2}$ 8.6, $J_{3B,3A}$ 13.1, H-3B), 1.67 (0.5H, ddd, $J_{3A,2}$ 4.7, $J_{3A,3'}$ 9.5, $J_{3A,3B}$ 14.2, H-3A), 1.54 (0.5H, ddd, $J_{3B,2}$ 4.5, $J_{3B,3'}$ 9.6, $J_{3B,3A}$14.2, H-3B), 1.4–1.39 (18H, overlapping singlets, C(CH_3)$_3$) and 1.35 (0.5H, m, H-4′B); δ_C (127.56 MHz, C^2HCl_3, *mixed diastereoisomers*) 175.23, 173.88, 171.51 and 171.48 (4 × *C*O), 155.69 (urethane), 82.55 and 82.42 (2 × C-5′), 82.08, 81.99 and 79.70 (3 × O*C*(CH$_3$)$_3$), 52.38 and 52.26 (2 × C-2), 36.08 and 35.38 (C-3′), 34.52, 32.98 and 32.43 (3 × C-4′ and C-3), 28.22, 27.91 and 27.85 (3 × C(*C*H$_3$)$_3$); δ_N (50.7 M, INEPT, C^2HCl_3) −294 (+1; −1 d, *J* 91.9, *N*H) and −323 (+1; 0; −1 br t, *J* 42, *N*H$_2$).

tert-Butyl (2*S*,4′*R*)-2-(*tert*-butoxycarbonylamino)-3-(3-oxo-2,3,4,5-tetrahydropyridazin-4-yl)propionate 25

tert-Butyl (2*S*,3′*R*)-3-[(5*RS*)-1-amino-5-hydroxy-2-oxopyrrolidin-3-yl]-2-*tert*-butoxycarbonylaminopropionate **24** (497 mg, 1.38 mmol) was dissolved in acetonitrile (10 ml). The solution was heated at reflux with 3 Å sieves (1 g) under nitrogen for 18 h and acetic acid (3 drops) was added. Heating at reflux was continued for a further 26 h and the solvents were removed *in vacuo*. The resultant oil was purified by flash chromatography on silica gel, eluting with CH_2Cl_2–MeOH (95 : 5), to yield *tert-butyl (2S,4′R)-2-(tert-butoxycarbonylamino)-3-(3-oxo-2,3,4,5-tetrahydropyridazin-4-yl)propionate* **25** as a white solid (300 mg, 64%), mp 142–144 °C; $[\alpha]_D^{23}$ + 47.2 (*c* 0.6, $CHCl_3$); (Found C, 56.3; H, 7.9; N, 12.2. $C_{16}H_{27}N_3O_5$ requires C, 56.3; H, 8.0; N, 12.3%); *m/z* (+ve FAB, 3-NBA) 342 ([M + H]$^+$) and 286 ([M − C_4H_9]$^+$); λ_{max} (MeOH)/nm 213 and 241 (log ε 4.16 and 3.79); ν_{max} (KBr)/cm^{-1} 3382 (NH) and 1715 (ester); δ_H (360 MHz, C^2HCl_3) 8.45 (1H, exch. s, N*H*), 7.18 (1H, t, $J_{6',5'}$ 3.0, H-6′), 5.17 (1H, exch. d, $J_{NH,2}$ 9, N*H*), 4.21 (1H, m, H-2), 2.90 (1H, m, H-5′A), 2.49 (1H, m, H-5′B), 2.31 (1H, ddd, $J_{3A,2}$ 2.4, $J_{3A,4'}$ 12.6, $J_{3A,3B}$ 14, H-3A), 2.27 (1H, m, H-4′), 1.82 (1H, ddd, $J_{3B,4'}$ 3.6, $J_{3B,2}$ 10.1, $J_{3B,3A}$ 14, H-3B), 1.46 (9H, s, C(CH_3)$_3$) and 1.44 (9H, s, C(CH_3)$_3$); addition of ^2H_2O caused the multiplet for H-2 at 4.21 ppm to change in appearance; δ_C (127.56 MHz, C^2HCl_3) 171.19 and 169.75 (2 × *C*O), 155.87 (urethane), 144.74 (N*C*H), 82.42 and 80.01 (2 × O*C*(CH$_3$)$_3$), 51.29 (C-2), 32.95 (C-5′), 32.34 (C-4′), 28.24 and 27.92 (2 × C(*C*H$_3$)$_3$) and 27.07 (C-3).

(2*S*,4′*R*)-2-Amino-3-(3-oxo-2,3,4,5-tetrahydropyridazin-4-yl)-propionic acid trifluoroacetate 26

tert-Butyl (2*S*,4′*R*)-2-*tert*-butoxycarbonylamino-3-(3-oxo-2,3,4,5-tetrahydropyridazin-4-yl)propionate **25** (45 mg, 0.13 mmol) was dissolved in a 1 : 1 mixture of trifluoroacetic acid and dichloromethane (2 ml). After standing for two hours at room temperature the solvents were removed *in vacuo* to yield *(2S,4′R)-2-amino-3-(3-oxo-2,3,4,5-tetrahydropyridazin-4-yl)-propionic acid trifluoroacetate* **26** as a pale yellow oil (42 mg). The ^{1}H-NMR spectrum was recorded immediately; δ_H (360 MHz, ^2H_2O) 7.01 (1H, br s, H-6′), 3.90 (1H, t, $J_{2,3}$ 6.72, H-2), 2.53 (1H, m, H-4′), 2.45 (1H, m, H-5′A), 2.13 (1H, m, H-5′B) and 1.87 (2H, m, H-3); irradiation at the t for H-2 at 3.90 ppm showed a change in the appearance of the m for H-3 at 1.87 ppm. When re-recorded soon after, it had undergone significant change.

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-(2-oxoethyl)pyroglutamate 23a

Oxygen was passed through a solution of *tert*-butyl (2*S*,4*R*)-*N*-*tert*-butoxycarbonyl-4-(prop-2-enyl)pyroglutamate **29a**[11] (385 mg, 1.18 mmol) in dichloromethane (10 ml) cooled to −78 °C for 20 min. Ozone was then passed through the solution for 15 min, during which time the solution turned blue. The reaction was quenched by adding triphenylphosphine (341 mg, 1.30 mmol) at −78 °C. The solution was allowed to warm slowly to room temperature. The solvent was removed *in vacuo* to yield a clear oil which was purified by flash column chromatography on silica gel using petroleum ether–ethyl acetate (2 : 1) as eluent. *tert-Butyl (2S,4S)-N-tert-butoxycarbonyl-4-(2-oxoethyl)pyroglutamate* **23a** was obtained as a white solid (358 mg, 93%), mp 119–121 °C; $[\alpha]_D^{20}$ −24.0 (*c* 1.00, CH_2Cl_2); Found C, 58.7; H, 7.7; N, 4.2. $C_{16}H_{25}NO_6$ requires C, 58.7; H, 7.7; N, 4.3%; *m/z* [+ve FAB (3-NBA)] 350 [M + Na]$^+$ and 328 [M + H]$^+$; ν_{max} (KBr)/cm^{-1} 2732 (CH=O), 1776 (imide), 1743 (ester), 1722 and 1702 (C=O); δ_H (300 MHz, C^2HCl_3) 9.69 (1H, s, C*H*=O), 4.36 (1H, d, $J_{2,3B}$ 9.8, H-2), 3.04–2.91 (2H, m, H-4 and H-6A), 2.50 (1H, dd, $J_{6B,4}$ 9.2, $J_{6B,6A}$ 19.6, H-6B), 2.25 (1H, dd, $J_{3A,4}$ 8.5, $J_{3A,3B}$ 13.2, H-3A), 1.83 (1H, ddd, $J_{3B,4}$ 3.1, $J_{3B,2}$ 9.8, $J_{3A,3B}$ 13.2, H-3B) and 1.40 and 1.38 (2 × 9H, 2 × s, 2 × C(CH_3)$_3$); δ_C (75.48 MHz, C^2HCl_3) 200.62 (*C*H=O), 175.62 (lactam), 171.45 (ester), 150.61 (urethane), 84.93 and 83.93 (2 × O*C*(CH$_3$)$_3$), 59.34 (C-2), 45.76 (C-6), 37.73 (C-4), 30.04 (C-3) and 29.32 and 29.29 (2 × C(*C*H$_3$)$_3$).

tert-Butyl (2*S*,3′*RS*)-2-(*tert*-butoxycarbonylamino)-3-(2-oxotetrahydrofuran-3-yl)propionate 28

tert-Butyl (2*S*,4*RS*)-*N*-*tert*-butoxycarbonyl-4-carboxymethylpyroglutamate (**7** + **8**) (100 mg, 0.29 mmol) was dissolved in THF (1 ml) and cooled to ice bath temperature, with stirring under nitrogen. Borane–dimethyl sulfide complex (2 M in tetrahydrofuran, 0.2 ml, 0.4 mmol) was added dropwise over 1 min. The mixture was allowed to warm to room temperature and left for a further 1.5 h. Methanol (1 ml) was added and, after stirring for 2 min, the solvents were removed *in vacuo* to yield *tert-butyl (2S,3RS)-2-(tert-butoxycarbonylamino)-3-(2-oxotetrahydrofuran-3-yl)propionate* **28** (88 mg, 92%) as a white solid. The spectra of the product were similar to those of the sample **28a** prepared below by reduction of the aldehyde **23a**.

tert-Butyl (2*S*,3′*S*)-2-(*tert*-butoxycarbonylamino)-3-(2-oxotetrahydrofuran-3-yl)propionate 28a

tert-Butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-(2-oxoethyl)pyroglutamate **23a** (108 mg, 0.33 mmol) and a trace of bromocresol green were dissolved in methanol (1.5 ml) and sodium cyanoborohydride (30 mg, 0.49 mmol) was added. The solution immediately turned deep blue and a 2 M solution of HCl in methanol was added dropwise with stirring to restore the yellow colour. After 2 h the solvents were removed *in vacuo* and the crude product was purified by column chromatography on silica gel using petroleum ether–ethyl acetate (1 : 2) as eluent. *tert-Butyl (2S,3′S)-2-(tert-butoxycarbonylamino)-3-(2-oxotetrahydrofuran-3-yl)propionate* **28a** was obtained as a white solid (83 mg, 76%), mp 75–76 °C; $[\alpha]_D^{36}$ −7.4 (*c* 1.00, CH_2Cl_2); Found C, 58.0; H, 8.2; N, 4.2. $C_{16}H_{27}NO_6$ requires C, 58.3; H, 8.3; N, 4.3%; *m/z* [+ve FAB (3-NBA)]: 352 $[M + Na]^+$ and 330 $[M + H]^+$; ν_{max} (KBr)/cm^{-1} 3348 (NH), 1781 (lactone), 1752 (ester) and 1714; δ_H (300 MHz, C^2HCl_3) 5.16 (1H, d, $J_{NH,2}$ 7.5, NH), 4.30 (1H, t, $J_{5'A,\ 4'}$ 8.4, H-5′A), 4.25–4.22 (1H, m, H-2), 4.20–4.07 (1H, m, H-5′B), 2.66–2.59 (1H, m, H-3′), 2.44–2.27 (2H, m, H-4′A and H-3A), 2.05–1.98 (1H, m, H-4′B), 1.78–1.68 (1H, m, H-3B) and 1.41 and 1.38 (2 × 9H, 2 × s, 2 × $C(CH_3)_3$); δ_C (C^2HCl_3, 75.48 MHz) 179.32 (lactone), 171.46 (ester), 155.73 (urethane), 82.94 and 80.40 (2 × $OC(CH_3)_3$), 67.01 (C-5′), 53.06 (C-2), 37.39 (C-3′), 33.83 (C-4′), 28.99 (C-3) and 28.70 and 28. 41 (2 × $C(CH_3)_3$).

Reaction of *tert*-butyl (2*S*,4*S*)-*N*-(*tert*-butoxycarbonyl)-4-(2-oxoethyl)pyroglutamate 23a with hydrazine hydrate

Hydrazine hydrate (64–65%) (8.6 mg, 0.17 mmol) was added to a solution of *tert*-butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-(2-oxoethyl)pyroglutamate **23a** (51 mg, 0.16 mmol) in methanol (1 ml) under nitrogen. The mixture was stirred at room temperature for 1 h and the solvent was removed *in vacuo* to yield a clear oil. Flash chromatography on silica gel using petroleum ether–ethyl acetate (1 : 3) as eluent gave *tert-butyl (2S,3′S)-3-[(5RS)-1-amino-5-hydroxy-2-oxopyrrolidin-3-yl]-2-tert-butoxycarbonylaminopropionate* **30**, and using CH_2Cl_2–MeOH (92 : 8) as eluent gave *tert-butyl (2S,4′S)-2-tert-butoxycarbonylamino-3-[3-oxo-2,3,4,5-tetrahydropyridazin-4-yl]propionate* **31**. *tert-Butyl (2S,3′S)-3-[(5RS)-1-amino-5-hydroxy-2-oxopyrrolidin-3-yl]-2-tert-butoxycarbonylaminopropionate* **30** was obtained as a clear oil (37 mg, 65%); *m/z* [+ve FAB (3-NBA)] 382 $[M + Na]^+$ and 360 $[M + H]^+$; ν_{max} (film)/cm^{-1} 3334 (OH), 3268 (NH), 1754, 1736, 1708 and 1688 (C=O); δ_H (300 MHz, C^2HCl_3) 5.64–5.59 (1H, m, N*H*Boc), 5.13 (1H, m, H-5′), 4.28–4.19 (1H, m, H-2), 2.76–2.72 (1H, m, H-3′), 2.58–2.47 (1H, m, H-4′A), 2.28–2.08 (2H, m, H-3), 1.96–1.82 (1H, m, H-4′B) and 1.40 and 1.37 (2 × 9H, 2 × s, 2 × $C(CH_3)_3$); δ_C (75.48 MHz, C^2HCl_3) 174.52 (lactam), 171.64 (ester), 155.80 (urethane), 129.44 and 128.63 (C-5′), 83.09, 80.69 and 80.23, 80.21 (2 × $OC(CH_3)_3$), 52.84 (C-2), 36.86 and 35.92 (C-3′), 34.38 (C-4′), 32.77 and 32.26 (C-3) and 28.77 and 28.42 (2 × $C(CH_3)_3$). *tert-Butyl (2S,4′S)-2-tert-butoxycarbonylamino-3-[3-oxo-2,3,4,5-tetrahydropyridazin-4-yl]propionate* **31** was obtained as a clear, colourless oil (6 mg, 11%); $[\alpha]_D^{33}$ −53.0 (*c* 1.00, CH_2Cl_2); λ_{max} (MeOH)/nm 240 (log ε 3.74); *m/z* [+ve FAB (PEGH/NOBA)] Found: 342.2022 $[M + H]^+$, $C_{16}H_{28}N_3O_5$ requires 342.2089 (2.0 ppm); *m/z* [+ve FAB (3-NBA)] 364 $[M + Na]^+$ and 342 $[M + H]^+$; ν_{max} (film)/cm^{-1} 3294 (NH), 1702 (br, C=O) and 1638 (C=N); δ_H (300 MHz, C^2HCl_3) 8.51 (1H, br s, NH), 7.12 (1H, br s, H-6′), 5.29 (1H, d, $J_{NH,2}$ 7.3, NHBoc), 4.18–4.16 (1H, m, H-2), 2.63–2.48 (2H, m, H-5′), 2.36–2.22 (2H, m, H-4′ and H-3A), 1.84–1.79 (1H, m, H-3B) and 1.40 and 1.37 (2 × 9H, 2 × s, 2 × $C(CH_3)_3$); δ_C (75.48 MHz, C^2HCl_3) 171.56 (C-3′), 170.13 (ester), 155.84 (urethane), 145.18 (C=N), 82.81 and 80.35 (2 × $OC(CH_3)_3$), 52.56 (C-2), 33.18 (C-4′), 32.37 (C-5′), 28.76 and 28.46 (2 × $C(CH_3)_3$) and 27.88 (C-3).

tert-Butyl (2*S*,4′*S*)-2-*tert*-butoxycarbonylamino-3-(3-oxo-2,3,4,5-tetrahydropyridazin-4-yl)propionate 31 by dehydration of the aminopyrrolidine 30

tert-Butyl (2*S*,3′*S*)-3-[(5*RS*)-1-amino-5-hydroxy-2-oxopyrrolidin-3-yl]-2-*tert*-butoxycarbonylaminopropionate **30** (33 mg, 0.09 mmol) was dissolved in acetonitrile (2 ml). The solution was heated at reflux in the presence of 3 Å molecular sieves (60 mg) and acetic acid (55 mg, 0.92 mmol) for 24 h. The solvent was removed *in vacuo* and the resultant oil was purified by flash chromatography on silica gel, eluting with petroleum ether and ethyl acetate (1 : 2), to yield *tert-butyl (2S,4′S)-2-tert-butoxycarbonylamino-3-(3-oxo-2,3,4,5-tetrahydropyridazin-4-yl)propionate* **31** (22 mg, 72%) as a colourless oil. Spectra were identical with those above.

tert-Butyl(2*S*,4*S*)-*N*-*tert*-butoxycarbonyl-4-(2-phenylhydrazonoethyl)pyroglutamate 32

Phenylhydrazine hydrochloride (25 mg, 0.18 mmol) and sodium acetate (20 mg, 0.24 mmol) were added to a solution of *tert*-butyl (2*S*, 4*S*)-*N*-*tert*-butoxycarbonyl-4-(2-oxoethyl)pyroglutamate **23a** (52 mg, 0.16 mmol) in methanol (1 ml) under nitrogen. The mixture was stirred at room temperature for 90 min and dichloromethane (10 ml) was added. The organic layer was washed with water and brine and dried (Na_2SO_4). The solvent was removed *in vacuo* to yield *tert-butyl (2S,4S)-N-tert-butoxycarbonyl-4-(2-phenylhydrazonoethyl)pyroglutamate* **32** as a dark-red oil (65 mg, 98%); $[\alpha]_D^{32}$ −21.8 (*c* 1.00, CH_2Cl_2); *m/z* [+ve FAB (PEGH/NOBA)]: Found: 441.2207 $[M + H + Na]^+$, $C_{22}H_{32}N_3O_5Na$ requires 441.2240 (7.5 ppm); *m/z* [+ve FAB (3-NBA)]: 441 $[M + H + Na]^+$ and 418 $[M + H]^+$; λ_{max} (MeOH)/nm 275 (log ε 3.87); ν_{max} (film)/cm^{-1} 3308 (NH), 1786 (imide), 1741 (ester) and 1655 (C=N); δ_H (300 MHz, C^2HCl_3) 7.19–6.72 (5 H, m, ArH), 7.04 (1 H, t, $J_{7,6}$ 4.4, H-7), 4.39 (1H, dd, $J_{2,3A}$ 0.9, $J_{2,3B}$ 9.6, H-2), 2.96 (1H, ddt, $J_{4,3A}$ 3.4, $J_{4,3B}$ 3.5, $J_{4,6A}$ 4.4, $J_{4,6B}$ 7.1, H-4), 2.76 (1H, dt, $J_{6A,7}$, $J_{6A,4}$ 4.4, $J_{6A,6B}$ 16.5, H-6A), 2.37 (1H, ddd, $J_{6B,7}$ 4.4, $J_{6B,4}$ 7.1, $J_{6B,6A}$ 16.5, H-6B), 2.30 (1H, ddd, $J_{3A,2}$ 0.9, $J_{3A,4}$ 3.4, $J_{3A,3B}$ 11.9, H-3A), 2.01 (1H, ddd, $J_{3B,2}$ 9.6, $J_{3B,4}$ 3.5, $J_{3B,3A}$ 11.9, H-3B) and 1.44 and 1.43 (2 × 9H, 2 × s, 2 × $C(CH_3)_3$); δ_C (75.48 MHz, C^2HCl_3) 175.27 (lactam), 170.77 (ester), 149.80 (urethane), 137.06 (C-7), 145.47, 129.63, 120.12 and 112.76 (Ar), 83.79 and 82.73 (2 × $OC(CH_3)_3$), 58.37 (C-2), 40.02 (C-4), 32.95 (C-6), 29.01 (C-3) and 28.35 (2 × $C(CH_3)_3$).

Acknowledgements

We thank GlaxoWellcome and EPSRC for the award of a CASE studentship (to A. D.) and Dr A. Avent for NMR spectra.

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