Synthesis of agonists and antagonists for central glutamate receptors by a novel 'ring switching' strategy[1]

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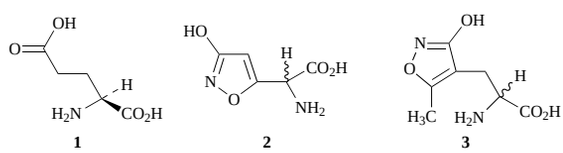
A novel and versatile ring switching strategy has been developed for the synthesis of compounds with structural features consistent with activity at glutamate receptors. A variety of homochiral L-alanine derivatives substituted at the β-carbon atom with planar five and six-membered heteroaromatic rings have been prepared in a one- or two-pot reaction using this strategy and some of the products have been shown to have biological activity at central glutamate receptors.

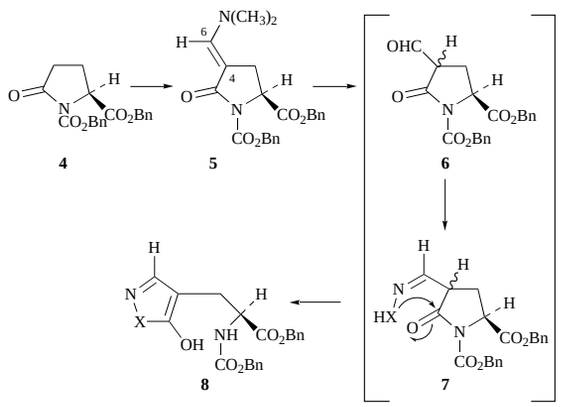
Since the excitatory action of L-glutamic acid **1** on single neurones in the central nervous system was first demonstrated by Curtis *et al.* in 1959,[2] a number of structurally related amino acids have been tested as agonists and antagonists. This led to the realisation that there are several different sub-types of glutamate receptors. The implication of these receptors in memory processes and Alzheimer's disease[3] and the potential of excitatory amino acid antagonists in anti-epileptic therapy[4] and in limiting the damage caused following a stroke[5] has initiated great interest in the field.

The discovery that ibotenic acid **2**, the active component of the psychotropic fly agaric mushroom *Amanita muscaria* Fr., had potent neuroexcitatory properties led to the synthesis of a number of heterocyclic analogues of glutamic acid[6–12] and the compound (*RS*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)-propanoic acid (AMPA, **3**) was prepared.[6] The 3-hydroxyisoxazole moiety in these compounds acts as a bioisostere of the γ-carboxy group of (*S*)-glutamic acid **1** and, whereas ibotenic acid **2** seems to activate the NMDA sub-type of glutamate receptor, AMPA **3** is a potent agonist at a different sub-type of glutamate receptor. The syntheses of the heterocyclic analogues were lengthy and non-versatile and led to racemic products. When the enantiomers were separated, it was found that there was pronounced receptor stereoselectivity, with compounds of the L-configuration often being the more active.[13–15] Synthesis of conformationally restricted analogues has led to some understanding of the spatial requirements of the receptors.[16]



1 **2** **3**

Because of the lack of versatility and the large number of steps in the existing syntheses of AMPA agonists and antagonists and the fact that these syntheses led to racemates, we have now devised a more versatile synthesis leading to homochiral protected AMPA analogues in a one- or two-pot process. In this synthesis we have converted benzyl *N*-benzyloxycarbonyl-(2*S*)-pyroglutamate **4**[17] into the enaminone **5** using *tert*-butoxybis(dimethylamino)methane following the method of Danishefsky *et al.*[18] We have shown that this and related compounds can be hydrolysed to the aldehyde **6** under carefully controlled conditions[19,20] and we reasoned that, if this were allowed to react *in situ* with a suitable α-nucleophile, then the resultant intermediate **7** might undergo intramolecular nucleophilic attack at the ring carbonyl group as in Scheme 1 to yield a homochiral compound **8** with a heterocyclic moiety at the β-carbon atom of an alanine moiety. Such a compound would have the structural features required of a glutamate agonist or



Scheme 1

antagonist. We have termed this reaction, where a heterocyclic ring is generated at the expense of the pyroglutamate ring, a 'ring switching' process.[1]

The enaminone **5** was, therefore, hydrolysed to the aldehyde **6** in methanol and then treated *in situ* either directly at pH 1 or buffered at pH 5 with an appropriate nucleophile. When the reaction was carried out at pH 5 and room temperature using methylhydrazine, TLC initially showed the presence of two new compounds but one was converted into the other within 15 h. The final product was purified by chromatography to give a gum, $C_{22}H_{23}N_3O_5$, λ_{max} 254 nm, which showed loss of the characteristic imide absorption at 1760 cm^{-1} present in the IR spectrum of the starting material. This and the ^1H NMR spectrum in [$^2H_6$]-DMSO which showed an additional aromatic singlet at δ 7.07 and a clear $-NHCHCH_2-$ system at 7.90 (NH, exchangeable), 4.18 (α-CH, q) and 2.74 and 2.58 (β-CH_2) suggested the structure **9b** and the other data were in accord with this assignment. Acetylation of the product using acetic anhydride and DMAP in acetonitrile at room temperature gave the acetate, $C_{24}H_{25}N_3O_6$ in 50% yield. This showed a considerable shift in the UV spectrum to higher wavelength (λ_{max} 332 nm) and a 13% NOE for the aromatic singlet at δ 8.17 in the ^1H NMR spectrum when the acetyl methyl singlet at δ 2.31 was irradiated. This indicated that acetylation had occurred at nitrogen in the pyrazolone tautomer to yield the fully conjugated compound **10b**.

When the aldehyde **6** was prepared *in situ* and allowed to react with hydrazine hydrate at pH 5, the reaction took a similar course to that above. An intermediate compound was seen on TLC and a final product, $C_{21}H_{21}N_3O_5$, was obtained with similar UV characteristics to those of compound **9b**. The chemical shift for the new one-proton aromatic singlet at δ 7.17 in [$^2H_6$]-DMSO was also similar to that in the former compound and so, although the scope for tautomerism is greater in this com-

pound, we prefer the structure **9a** rather than the alternative structure **11**. When this compound was acetylated, however, the monoacetate had a significantly different UV spectrum (λ_{max} 290 nm) to that of the acetate **10b** which suggested structure **12**. This was supported by the fact that no NOE was observed between the acetyl methyl group and the aromatic proton.

Reaction of the aldehyde **6** with phenylhydrazine at pH 5 gave the expected product **9c** but when *p*-nitrophenylhydrazine was used, the reaction was pH dependent, giving the expected pyrazole **9d** at pH 5 but yielding the *p*-nitrophenylhydrazone **13d** as a mixture of stereoisomers when the reaction was conducted at pH 1. Acetylation of the pyrazole **9c** gave two unstable products which could not fully be characterised. When the corresponding *p*-nitrophenyl derivative **9d** was acetylated, however, then the two products, although unstable, were obtained in a sufficiently pure state for the spectra to suggest that the major isomer was the pyrazolone **10d** and the minor isomer was the enol acetate **14**.

Reaction of the aldehyde with 2,4-dinitrophenylhydrazine gave only a diastereoisomeric mixture of 2,4-dinitrophenylhydrazones **13e** and it was not possible to obtain a ring-switched product when this hydrazine was used.

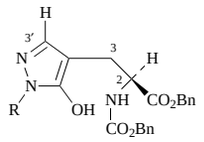
These results indicated that the reactions had proceeded *via* the hydrazones **7** (X = NR) which could then undergo a 'ring switching' reaction to the pyrazolones **9** if the second nitrogen atom was sufficiently nucleophilic. Although all of the products had optical rotations, we were able to check that the chiral centre at C-2 of the protected glutamate derivative **8** remained intact by repeating the reaction with hydrazine hydrate using ^{2}HCl and MeO^{2}H. Although the major product in this case was the acetal **15** which had evidently incorporated deuterium at C-4, the pyrazolone **9a** obtained contained no non-exchangeable deuterium as evidenced by mass spectrometry and ^{1}H and ^{2}H NMR spectroscopy.

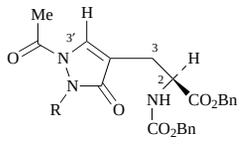
When the aldehyde **6** was prepared and allowed to react *in situ* with hydroxylamine at pH 5, an unstable product was obtained which had spectra consistent with the isoxazole structure **16**. Although there was no sign of an intermediate oxime in this case, reaction with *O*-benzylhydroxylamine gave the *O*-benzyl derivative **17** as a mixture of 4*R*, 4*S* and *syn* and *anti* isomers. Acetylation of the isoxazole **16** led to a stable acetate which appeared to be the enol acetate **18**, with ν_{max} 1771 cm^{-1} and no amide absorption in the IR spectrum.

Subsequent to our synthesis of these compounds, the antifungal antibiotic TAN-950 was isolated from culture filtrates of *Streptomyces platensis* A-136.[21] This was shown to be the isoxazole **19** and was present in equilibrium with the stereoisomeric pyroglutamic acid 4-oximes. It exhibited affinity for excitatory amino acid receptors in the rat brain.[21]

Having developed a versatile route to potential glutamate agonists and antagonists, we now investigated deprotection of the compounds **9a** and **9b** by hydrogenolysis. Initial attempts to hydrogenolyse **9a** using 10% palladium-on-charcoal in methanol gave a mixture of the expected product **20a** and the partially deprotected acid **21** and so we conducted the subsequent reactions in glacial acetic acid using Adam's catalyst. The resultant amino acids **20a** and **20b** both demonstrated an inhibitory effect on ibotenate stimulated phosphoinositide response, although they were two-fold less potent than the selective inhibitor L-AP3.[22] The amino acid **20a** [IC_{50} = 300 ± 30 μm (n = 3)] was slightly more potent than **20b** [IC_{50} = 380 ± 86 μm (n = 3)]. The inhibitory effects were not reversed by higher agonist concentration.

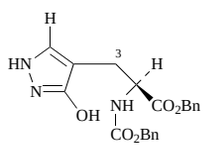
The reported activity of willardiine **22** at glutamate receptors[23,24] suggested that extension of our synthesis to include L-alanine derivatives substituted in the β-position by six-membered ring heteroaromatic compounds might yield biologically interesting compounds. We therefore treated the poorer nucleophiles urea, thiourea and guanidine directly with the aldehyde **6**, prepared *in situ*. This method proved ineffective and so an alternative approach was sought. Using the *tert*-butyl ester of the *tert*-butoxycarbonyl enaminone **23**,[25] careful acid hydrolysis gave the aldehyde **24** which, on treatment with diazomethane, yielded the enol ether **25** in 74% overall yield. Attempts to form useful 'ring-switched' products by allowing this to react with urea, thiourea or guanidine failed but, when heated with acetamidine hydrochloride and K_2CO_3 in ethanol at reflux, it gave the 2-methylpyrimidin-4-one **26b** in 87% yield. The ^{1}H NMR spectrum showed the expected exchangeable NH absorption coupled to the α-amino acid proton, a feature which was not present in the spectrum of the starting material, and the absence of an imide absorption at 1759 cm^{-1} in the IR spectrum which was present in the starting material. The UV spectrum was consistent with the behaviour expected of a 2,5-dialkylpyrimidin-4-one,[26] exhibiting λ_{max} 276 nm, reversibly shifting to λ_{max} 263 nm on addition of acid. The ^{1}H NMR spectrum also appeared to show the presence of a second compound (*ca.* 2:8 by integration) in spite of its sharp melting point, analytical purity and apparent chromatographic homogeneity. The spectrum associated with the minor component at room temperature in [$^{2}H_6$]-DMSO coalesced with that of the major component at 80 °C in this solvent, suggesting the presence of two conformational isomers. When either the aldehyde **24** or the enaminone **23** was used in this synthesis, the pyrimidinone **26b** was obtained but in lesser yield than in the reaction with the enol ether **25**.





9a R = H
b R = Me
c R = Ph
d R = *p*-$NO_2C_6H_4$

10a R = H
b R = Me
d R = *p*-$NO_2C_6H_4$



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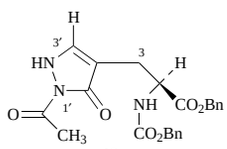

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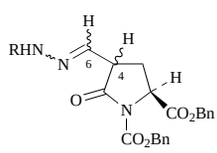
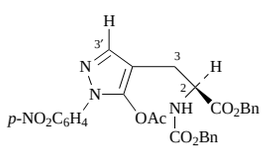


13d R = *p*-$NO_2C_6H_4$
e R = 2,4-$(NO_2)_2C_6H_3$



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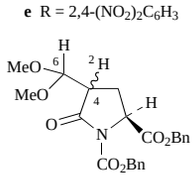

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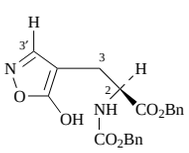

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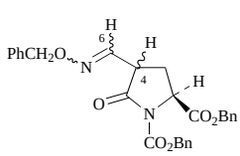

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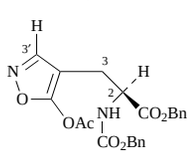

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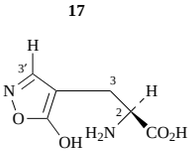

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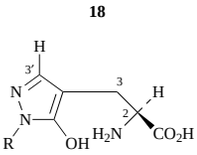
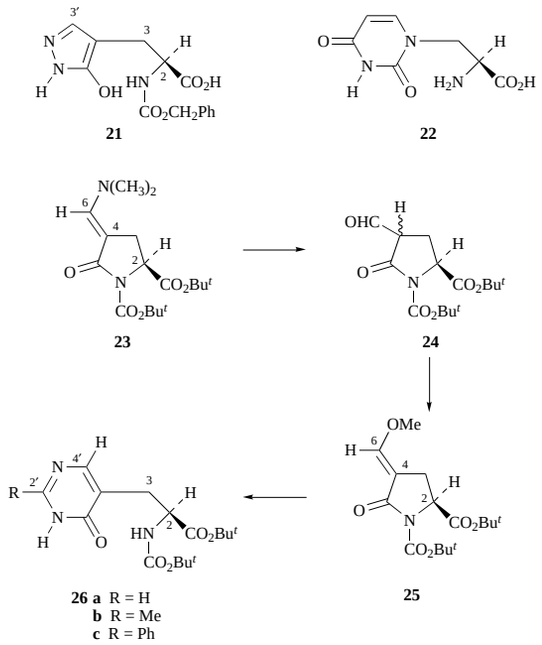


20a R = H
b R = Me



Scheme 2

Reaction of the enol ether **25** with benzamidine hydrochloride and with formamidine acetate, under the conditions used in the reaction of the enol ether with acetamidine hydrochloride above, gave the pyrimidinones **26c** (55%) and **26a** (76%). Both compounds had spectra in keeping with those of the methyl analogue **26b**, including the variable-temperature ^{1}H NMR spectral effect ascribed to conformational isomerism. All three pyrimidinones were cleanly deprotected by treatment with concentrated hydrochloric acid at room temperature, giving the hygroscopic amino acid hydrochlorides **27b**, **27c** and **27a**. Compound **27a** was tested for activity and found to be a glutamate agonist.[27,28]

The enol ether **25** did not react with guanidine hydrochloride and potassium carbonate in ethanol at reflux but, when heated with guanidine carbonate in ethanol at reflux, it gave a mixture of two compounds. One of these proved to be the desired 2-aminopyrimidinone **28** (39%). This showed the variable-temperature ^{1}H NMR spectral effects exhibited by related compounds in the series and was deprotected using concentrated hydrochloric acid at room temperature to yield the amino acid hydrochloride **29**, which was a weak antagonist to ACPD at the metabotropic receptor.[27,28] Attempts to prepare the 2-methoxypyrimidinone **30** by treating the enol ether **25** with *O*-methylisourea seemed to be partly successful from the ^{1}H NMR spectrum of the crude product but we were unable to purify the product fully.

In an attempt to extend the synthesis to polycyclic aromatic analogues of glutamate agonists and antagonists, the enol ether **25** was heated with 2-aminopyridine in ethanol at reflux but no reaction was observed. When the aldehyde **24**, prepared independently by treating *tert*-butyl 1-*tert*-butoxycarbonylpyroglutamate with lithium hexamethyldisilazide in THF followed by addition of methyl formate, was allowed to react with 2-aminopyridine in dioxane at reflux, a product was obtained in 68% yield. This still retained the imide absorption due to the *N*-*tert*-butoxycarbonylpyroglutamate system in the IR spectrum and microanalytical and spectral data indicated that it was the enaminone **31** (λ_{max} 287 and 335 nm). This compound was heated with potassium carbonate in ethanol at reflux to yield the desired pyrido[1,2-*a*]pyrimidine **32** (λ_{max} 242 and 339 nm) in 55% yield. Deprotection with concentrated hydrochloric acid at room temperature gave the hydrochloride of the amino acid **33**, in quantitative yield.

In an attempt to obtain the fused ring system **35**, we first heated the 4-formylpyroglutamate **24** with aminotetrazole in dioxane at reflux and obtained the enaminone **34** [mp 183 °C (decomp.); λ_{max} 297 nm] in 70% yield. A number of attempts were made to convert this compound into the pyridotetrazole **35** by the ring-switching reaction but none were successful.

Experimental

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. IR Spectra were recorded on a Perkin-Elmer 1720 Fourier transform instrument and UV spectra in methanol on Philips PU8720 and PU800 spectrophotometers or on a Perkin-Elmer PE 330 instrument. ^{1}H NMR Spectra were recorded on Bruker WM360 (360 MHz), AMX 500 (500 MHz) or AC-200 (200 MHz) Fourier transform instruments. *J* Values are given in Hz. ^{13}C NMR Spectra were recorded on Bruker AMX 500 (125.76 MHz), WM360 (90.6 MHz), AC-200 (50.3 MHz) or Bruker 250 (62.9 MHz) spectrometers. INEPT Experiments were used to help assign ^{13}C resonances where necessary. The residual solvent peak was used as reference for all NMR spectra. Low resolution mass spectra were recorded on Kratos MS80, MS25 or MS50 instruments. Accurate mass measurements were recorded on a VG7070 instrument by Dr S. Chotai (Wellcome Research Laboratories) and microanalyses were performed by Mrs K. Plowman and Miss M. Patel at the University of Sussex and by Mrs P. Firmin at Wellcome Research Laboratories. Optical rotations were measured on a Perkin-Elmer PE241 polarimeter using a 1 dm path length micro cell; they are recorded as 10^{-1} deg cm^2 g^{-1}. Thin layer chromatography was carried out using Merck Kieselgel 60 F_{254} pre-coated silica gel plates of thickness 0.2 mm (ART 5554 and ART 5714) and column chromatography was performed using Merck Kieselgel 60 (230–400 mesh, ART 9385) unless otherwise stated.

Benzyl (2*S*)-1-benzyloxycarbonyl-4-dimethylaminomethylene-pyroglutamate 5

This compound was prepared by the following adaptation of the method of Danishefsky *et al.*[18] Benzyl (2*S*)-1-benzyloxycarbonylpyroglutamate **4**[17] (40.0 g, 0.113 mol) was dissolved in dimethoxyethane (350 ml) and *tert*-butoxybis(dimethylamino)methane (Bredereck's reagent) (29.6 g, 0.170 mol) was added to the solution. The reaction mixture was heated at a constant temperature of 75 °C for 14 h after which the solvent was removed *in vacuo* to afford a brown oil which was crystallised from toluene–light petroleum (bp 60–80 °C) as a light yellow solid (41.1 g, 89%); mp 92–94 °C (lit.,[18] 92–93 °C); $[\alpha]_D^{26}$ −29.9 (*c* 0.81, $CHCl_3$) [lit.,[18] $[\alpha]_D$ −32.8 (*c* 1.4, $CHCl_3$)] (Found: C, 67.2; H, 6.0; N, 6.8. $C_{23}H_{24}N_2O_5$ requires C, 67.6; H, 5.9; N, 6.9%); *m*/*z* [+ve FAB (NBA)] 409 ($[M + H]^+$); ν_{max}(KBr)/cm^{-1} 1760 (imide); λ_{max}(MeOH)/nm 313 (ε 29 200); δ_H(360 MHz, C^2HCl_3) 7.33 (10 H, m, ArH), 7.17 (1 H, t, $J_{6,3A\text{-}6,3B}$ 1.6, C*H*NMe_2), 5.21 (2 H, AB, J_{AB} 12.5, PhCH_2O), 5.11 (2 H, AB, J_{AB} 12.1, PhCH_2O), 4.66 (1 H, d × d, $J_{2,3S}$ 10.6, $J_{2,3R}$ 3.6, H-2), 3.26 (1 H, m, $J_{3S,2}$ 10.6, $J_{3S,3R}$ 14.6, H-3*S*), 3.01 [6 H, s, $N(CH_3)_2$] and 2.90 (1 H, m, $J_{3R,2}$ 3.6, $J_{3R,3S}$ 14.6, H-3*R*).

Benzyl (2*S*)-2-benzyloxycarbonylamino-3-(1-methyl-5-hydroxypyrazol-4-yl)propionate 9b

Compound **5** (100 mg, 0.24 mmol) was dissolved in methanol (10 ml) and 1 M aqueous hydrochloric acid was added dropwise to the solution until its UV spectrum showed the reaction to be complete (*ca.* 1.5 equiv., 1 M aqueous HCl was required). Methylhydrazine (13 μl, 0.24 mmol) was added at room temperature to the reaction mixture which was then stirred for a further 30 h. After this the solvent was removed *in vacuo* and the residue was dissolved in ethyl acetate. The solution was washed consecutively with saturated aqueous sodium hydrogen carbonate, water and brine and then dried (Na_2SO_4). The solvent was removed *in vacuo* to yield a yellow oil (75 mg; 75% overall). This was chromatographed on silica gel with gradient elution from ethyl acetate to methanol–ethyl acetate (1:4) to give the title compound **9b** as a colourless gum, which failed to crystallise (35 mg, 35%); $[\alpha]_D^{24}$ −8.3 (*c* 0.35, $CHCl_3$) (Found: C, 63.1; H, 5.6; N, 9.9. $C_{22}H_{23}N_3O_5{\cdot}0.5H_2O$ requires C, 63.2; H, 5.7; N, 10.05%) [Found: *m*/*z* (EI), 409.1634 ($[M]^+$). $C_{22}H_{23}N_3O_5$ requires 409.1632]; ν_{max}(CHCl$_3$)/cm^{-1} 1740sh and 1719 (urethane and ester) and 1580 (C=N); λ_{max}(MeOH, pH 7)/nm 254 (ε 7500); λ_{max}(MeOH, pH 12)/nm 244 (ε 7300); λ_{max}-(MeOH, pH 2)/nm 233 (ε 6370); δ_H(360 MHz, $[^2H_6]$-DMSO) 7.90 (1 H, br exch. s, NH), 7.32 (10 H, s, ArH), 7.07 (1 H, s, H-3′), 5.08 and 5.01 (4 H, 2 × s, 2 × PhCH_2), 4.18 (1 H, br q, $J_{2,NH} = J_{2,3A} = J_{2,3B}$ 7, H-2), 3.40 (3 H, s, N–CH_3), 2.74 (1 H, d × d, $J_{3B,3A}$ 14.6, $J_{3B,2}$ 5.7, H-3B) and 2.58 (1 H, d × d, $J_{3A,3B}$ 14.6, $J_{3A,2}$ 8.4, H-3A); δ_C(50.3 MHz, $[^2H_6]$-DMSO) 171.82 (ester), 155.96 (urethane), 153.5 (br) (C-5′), 136.69 and 135.90 (2 × *ipso* C), 136.54 (C-3′), 128.36–127.66 (aromatic), 97.57 (C-4′), 65.88 and 65.46 (2 × PhCH_2), 55.01 (C-2), 32.32 (N–CH_3) and 24.76 (C-3).

Benzyl (2*S*)-2-benzyloxycarbonylamino-3-(2-acetyl-1-methyl-5-oxopyrazol-4-yl)propionate 10b

The pyrazole **9b** (130 mg, 0.32 mmol) was dissolved in dry acetonitrile (6 ml) with 4-dimethylaminopyridine (4 mg, 0.032 mmol) at room temperature under nitrogen. After the mixture had been stirred for 20 min, acetic anhydride (30 μl, 0.32 mmol) was added dropwise to it and stirring was continued for 15 h at room temperature. The solvent was then removed *in vacuo* to yield a pale brown oil (114 mg, 80%) which was chromatographed on silica gel using hexane–ethyl acetate (1:4) as eluent. The major product, the title compound **10b**, was obtained as a pale yellow oil (71 mg, 50%); $[\alpha]_D^{28}$ −1.3 (*c* 0.15, $CHCl_3$) (Found: C, 64.1; H, 5.65; N, 9.0. $C_{24}H_{25}N_3O_6$ requires C, 63.9; H, 5.5; N, 9.3%) [Found: *m*/*z* (EI) 451.1740 ($[M]^+$). $C_{24}H_{25}N_3O_6$ requires 451.1737]; ν_{max}(film)/cm^{-1} 3320 (NH), 1740sh and 1720 (urethane and ester) and 1657 (amide and pyrazolone); λ_{max}(MeOH, pH 7)/nm 281 and 332 (ε 9130 and 1280); λ_{max}-(MeOH, pH 12)/nm 243 and 330 (ε 5050 and 1330); λ_{max}-(MeOH, pH 2)/nm 281 and 332 (ε 12 400 and 1630); δ_H(200 MHz, $[^2H_6]$-DMSO) 8.17 (1 H, s, H-3′), 7.84 (1 H, br d, $J_{NH,2}$ 7.7, NH), 7.33 (10 H, s, ArH), 5.10 and 5.03 (4 H, 2 × s, 2 × PhCH_2), 4.41 (1 H, q, $J_{2,3B} = J_{2,3A} = J_{2,NH} = 7$, H-2), 3.40 (3 H, s, N–$CH_3$), 2.68 (2 H, m, overlapping, $J_{3B,3A}$ 14.1, $J_{3B,2} = J_{3A,2} = 7$, 3-CH_2) and 2.31 (3 H, s, CH_3CO). Irradiation of CH_3CO (δ 2.31) produced a 13% enhancement in H-3′ (δ 8.17). Irradiation of H-3′ gave an enhancement of 4% in CH_3CO; δ_C(50.3 MHz, $[^2H_6]$-DMSO) 171.85 (ester), 167.20 (C-5′), 164.86 (amide), 156.50 (urethane), 137.04 and 136.01 (2 × *ipso* C), 136.76 (C-3′), 128.94–128.07 (aromatic), 110.35 (C-4′), 66.83 and 66.24 (2 × PhCH_2), 53.19 (C-2), 34.00 (N–CH_3), 25.17 (C-3) and 21.84 (N–COCH_3).

Benzyl (2*S*)-2-benzyloxycarbonylamino-3-(5-hydroxypyrazol-4-yl)propionate 9a

Compound **5** (250 mg, 0.61 mmol) was dissolved in methanol (10 ml) and 1 M aqueous hydrochloric acid was added dropwise to the solution until its UV spectrum showed reaction to be complete (*ca.* 1.5 equiv. was required). Hydrazine hydrate (30 μl, 0.62 mmol; 98%) was then added to the mixture at room temperature, giving a deep yellow colouration. After being stirred at room temperature for 18 h, the solvent was removed *in vacuo* and the residue was partitioned between ethyl acetate and water. The organic phase was washed consecutively with saturated aqueous sodium hydrogen carbonate, water and brine and then dried (Na_2SO_4). The solvent was removed *in vacuo* to afford a yellow foam (80% yield). This was chromatographed on silica gel using gradient elution from light petroleum (bp 60–80 °C)–ethyl acetate (1:9) to pure ethyl acetate. The title compound **9a** was obtained as a colourless gum which could not be crystallised (71 mg, 30%); $[\alpha]_D^{28}$ −11.0 (*c* 0.39, $CHCl_3$) (Found: C, 62.1; H, 5.2; N, 10.2. $C_{21}H_{21}N_3O_5{\cdot}0.5H_2O$ requires C, 62.4; H, 5.45; N, 10.4%) [Found: *m*/*z* (EI) 395.1479 ($[M]^+$). $C_{21}H_{21}N_3O_5$ requires 395.1476]; ν_{max}(CHCl$_3$)/cm^{-1} 3324 (NH, OH), 1713 (urethane and ester) and 1605 (C=N); λ_{max}(MeOH, pH 7)/nm 225sh and 250 (ε 3800 and 2480); λ_{max}(MeOH, pH 12)/nm 240 (ε 4540); λ_{max}(MeOH, pH 2)/nm 231 (ε 4400); δ_H(360 MHz, $[^2H_6]$-DMSO) 11–10 (2 H, br exch. s, NH/OH), 7.67 (1 H, br exch. d, $J_{NH,2}$ 7.6, NH), 7.32 (10 H, s, 2 × *Ph*CH_2), 7.17 (1 H, s, H-3′), 5.10 and 5.00 (4 H, 2 × s, 2 × PhCH_2), 4.20 (1 H, d × t, $J_{2,NH} = J_{2,3A} = 8.5$, $J_{2,3B}$ 5.7, H-2), 2.76 (1 H, d × d, $J_{3B,3A}$ 14.6, $J_{3B,2}$ 5.7, H-3B) and 2.57 (1 H, d × d, $J_{3A,3B}$ 14.6, $J_{3A,2}$ 8.5, H-3A); δ_C(50.3 MHz, $[^2H_6]$-DMSO) 171.83 (ester), 159.28 (C-5′), 155.90 (urethane), 136.61 and 135.86 (2 × *ipso*), 129.25 (C-3′), 128.09–127.48 (aromatic), 98.36 (C-4′), 65.83 and 65.42 (2 × PhCH_2), 54.71 (C-2) and 24.29 (C-3).

Benzyl (2*S*)-2-benzyloxycarbonylamino-3-(1-acetylpyrazol-4-yl)propionate 12

Compound **9a** (170 mg, 0.43 mmol) was dissolved in dry acetonitrile (6 ml) and 4-dimethylaminopyridine (5.0 mg, 0.04 mmol) was added to the solution at room temperature under nitrogen. After the mixture had been stirred for 5 min, acetic anhydride (40 μl, 0.43 mmol) was added dropwise to it and stirring continued at room temperature for 14 h. The fine white suspended solid was filtered off and washed with cold acetonitrile. Upon recrystallisation from ethyl acetate it yielded the title compound **12** as white microcrystals (90 mg, 50%); mp 149–150 °C; $[\alpha]_D^{25}$ +0.3 (*c* 0.325, $CHCl_3$) (Found: C, 63.1; H, 5.2; N, 9.5. $C_{23}H_{23}N_3O_6$ requires C, 63.2; H, 5.3; N, 9.6%) [Found: *m*/*z* (EI) 437.1629 ($[M]^+$). $C_{23}H_{23}N_3O_6$ requires 437.1627]; ν_{max}(KBr)/cm^{-1} 3331 (NH, OH), 1734 and 1717 (urethane and ester), 1692 (amide) and 1627 (pyrazol-5-one); λ_{max}(MeOH, pH 7)/nm 270 and 290 (ε 2450 and 2080); λ_{max}(MeOH, pH 12)/nm 238 and 297 (ε 1460 and 1900); λ_{max}(MeOH, pH 2)/nm 231 and 264 (ε 1560 and 790); λ_{max}(MeOH, pH 7)/nm 225sh and 293 (ε 2090 and 3770); λ_{max}(MeOH, pH 12)/nm 239 and 296 (ε 3800

and 2060); λ_{max}(MeOH, pH 2)/nm 232 and 264 (ε 2720 and 4190); δ_H(360 MHz, [$^2H_6$]-DMSO) 11.27 (2 H, br exch. s, NH), 7.94 (1 H, s, H-3′), 7.86 (1 H, d, $J_{NH,2}$ 8, NH), 7.31 (10 H, m, ArH), 5.10 and 5.0 (4 H, 2 × s, 2 × PhCH_2), 4.27 (1 H, br d × t, $J_{2,3A}$ 9.5, $J_{2,3B}$ 5.6, $J_{2,NH}$ 8, H-2), 2.78 (1 H, d × d, $J_{3A,3B}$ 14.5, $J_{3B,2}$ 5.6, H-3B), 2.69 (1 H, d × d, $J_{3A,3B}$ 14.5, $J_{3A,2}$ 9.5, H-3A) and 2.42 (3 H, s, CH_3CO); when the CH_3CO protons (δ 2.42) were irradiated, no enhancement was detected in H-3′ (δ 7.94) and when H-3′ was irradiated, no enhancement was observed in the CH_3CO signal; δ_C(62.9 MHz, [$^2H_6$]-DMSO) 171.45 (ester), 167.50 (N–COCH$_3$), 163.01 (C-5′), 156.01 (urethane), 136.82 and 135.77 (2 × *ipso* C), 128.37–127.61 (aromatic), 127.15 (C-3′), 109.64 (C-4′), 66.1 and 65.5 (2 × PhCH_2), 53.42 (C-2), 24.07 (C-3) and 21.13 (N–COCH_3).

Benzyl (2*S*)-2-benzyloxycarbonylamino-3-(5-hydroxy-1-phenylpyrazol-4-yl)propionate 9c

Compound **5** (150 mg, 0.37 mmol) was dissolved in methanol (10 ml) and 1 M aqueous hydrochloric acid was added dropwise to the solution until its UV spectrum showed the reaction to be complete (*ca.* 1.5 equiv. was required). Phenylhydrazine hydrochloride (54 mg, 0.37 mmol) and sodium acetate (50 mg, 0.60 mmol) were added to the reaction mixture which was then stirred for 3 days at room temperature. After this the solvent was removed *in vacuo* and the residue was dissolved in chloroform. The solution was washed twice with water and once with brine and dried (Na_2SO_4). The solvent was removed *in vacuo* to yield a brown foam (140 mg, 80% overall) which was chromatographed on silica gel using ethyl acetate–dichloromethane (3:7). The major product, the title compound **9c**, was isolated as a solid and was recrystallised from ethyl acetate–hexane to give off-white crystals (70 mg, 40%); mp 126–128 °C; $[a]_D^{26}$ +2.0 (*c* 0.35, $CHCl_3$) (Found: C, 68.5; H, 5.3; N, 8.6. $C_{27}H_{25}N_3O_5$ requires C, 68.8; H, 5.3; N, 8.9%); *m/z* [+ve FAB (3-NBA)] 494 ([M + Na]$^+$) and 472 ([M + H]$^+$); ν_{max}($CHCl_3$)/cm^{-1} 1740sh and 1719 (urethane and ester); λ_{max}(MeOH, pH 7)/nm 247 and 275sh (ε 6960 and 2830); δ_H(360 MHz, [$^2H_6$]-DMSO) 11.0 (1 H, br exch. s, OH), 7.93 (1 H, br exch. s, NH), 7.70–7.20 (16 H, m, ArH), 5.10 and 5.02 (4 H, 2 × s, 2 × PhCH_2), 4.27 (1 H, br q, *J ca.* 7, H-2), 2.83 (1 H, d × d, $J_{3B,3A}$ 14.6, $J_{3B,2}$ 5.6, H-3B) and 2.68 (1 H, d × d, $J_{3A,3B}$ 14.6, $J_{3A,2}$ 9.0, H-3A); δ_C(50.3 MHz, [$^2H_6$]-DMSO) 171.31 (ester), 156.60 (urethane), 139.56, 137.21 and 136.17 (3 × *ipso* C), 138.0–121.0 (aromatics), 100.0 (C-4′), 66.62 and 66.11 (2 × PhCH_2), 54.90 (C-2) and 25.08 (C-3).

Benzyl (2*S*,4*RS*)-1-benzyloxycarbonyl-4-(*p*-nitrophenyl)-hydrazonomethylpyroglutamate 13d

Compound **5** (200 mg, 0.49 mmol) was dissolved in methanol (10 ml) and 1 M aqueous hydrochloric acid was added dropwise to the solution until its UV spectrum showed reaction to be complete (*ca.* 1.5 equiv. was required). 4-Nitrophenylhydrazine (75 mg, 0.49 mmol) was added to the reaction mixture which was then stirred for 4 h at room temperature. The resulting yellow solid was filtered off, washed with cold methanol and recrystallised from methanol to afford yellow needles (120 mg, 50%); mp 134–135 °C; $[a]_D^{28}$ 0.0 (*c* 0.31, $CHCl_3$) (Found: C, 62.8; H, 4.5; N, 10.8. $C_{27}H_{24}N_4O_7$ requires C, 62.8; H, 4.65; N, 10.85%); *m/z* [+ve FAB (3-NBA)] 517 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3295 (NH), 1790 and 1734 (urethane and ester) and 1595 (C=N); λ_{max}(MeOH, pH 7)/nm 376 (ε 10 500); λ_{max}(MeOH, pH 12)/nm 340 (ε 7990); λ_{max}(MeOH, pH 2)/nm 324 (ε 6000); δ_H(360 MHz, C^{2}HCl$_3$) 8.94 (1 H, exch. s, NH), 8.06 (2 H, d, $J_{3',2'}$ 9.1, ArH), 7.40 (1 H, d, $J_{6,4}$ 4.2, H-6), 7.32 (10 H, m, ArH), 6.97 (2 H, d, $J_{2',3'}$ 9.1, ArH), 5.20 and 5.16 (4 H, 2 × s, 2 × PhCH_2), 4.79 (1 H, d, $J_{2,3S}$ 8.7, H-2), 3.68 (1 H, d × d × d, $J_{4,3S}$ 10.0, $J_{4,3R}$ 9, $J_{4,6}$ 4.2, H-4), 2.72 (1 H, d × t, $J_{3S,3R}$ 13.4, $J_{3S,2} = J_{3S,4}$ 10, H-3*S*) and 2.38 (1 H, d × d, $J_{3R,3S}$ 13.4, $J_{3R,4}$ 9, H-3*R*). Irradiation of H-2 (δ 4.79) produced an enhancement of 4% in H-3*S* (δ 2.72). Irradiation of H-4 (δ 3.68) produced an enhancement of 2.5% in H-3*R* (δ 2.38).

Benzyl (2*S*)-2-benzyloxycarbonylamino-3-[5-hydroxy-1-(*p*-nitrophenyl)pyrazol-4-yl]propionate 9d

Compound **5** (300 mg, 0.74 mmol) was dissolved in methanol (10 ml) and 1 M aqueous hydrochloric acid was added dropwise to the solution until its UV spectrum showed reaction to be complete (*ca.* 1.5 equiv. was required). 4-Nitrophenylhydrazine (112 mg, 0.74 mmol) and sodium acetate (100 mg, 1.2 mmol) were added at room temperature to the mixture which was then stirred for 18 h at room temperature. The resulting brown solid was filtered off, washed with cold methanol and recrystallised from methanol to yield pale brown needles (138 mg, 36%); mp 175–177 °C; $[a]_D^{24}$ +11.5 (*c* 0.357, $CHCl_3$) (Found: C, 62.5; H, 4.4; N, 10.7. $C_{27}H_{24}N_4O_7$ requires C, 62.8; H, 4.65; N, 10.85%); *m/z* [+ve FAB (3-NBA, xenon)] 517 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3322 (NH), 1738 (urethane and ester), 1683 and 1637 (pyrazolone); λ_{max}(MeOH, pH 7)/nm 327 (ε 7800); λ_{max}(MeOH, pH 12)/nm 338 (ε 9340); λ_{max}(MeOH, pH 2)/nm 318 (ε 7300); δ_H(360 MHz, [$^2H_6$]-DMSO) 11.8 (2 H, br, NH_2), 8.32 (2 H, d, *J* 9.3, ArH), 8.06 (2 H, d, *J* 9.3, ArH), 7.88 (1 H, exch. d, $J_{NH,2}$ 7.9, NH), 7.55 (1 H, s, H-3′), 7.31 (10 H, m, aromatic), 5.11 (2 H, s, PhCH_2), 5.02 (2 H, AB, PhCH_2), 4.29 (1 H, d × d × d, $J_{2,3A}$ 9.1, $J_{2,NH}$ 7.9, $J_{2,3R}$ 5.8, H-2), 2.84 (1 H, d × d, $J_{3B,3A}$ 14.7, $J_{3B,2}$ 5.8, H-3B) and 2.69 (1 H, d × d, $J_{3A,3B}$ 14.7, $J_{3A,2}$ 9.1, H-3A); δ_C(62.9 MHz, [$^2H_6$]-DMSO) 171.82 (ester), 156.04 (urethane), 143.6, 142.13, 136.90 and 135.85 (4 × *ipso* C), 128.4–119.29 (aromatic), ~100.0 (C-4′), 66.10 and 65.56 (2 × PhCH_2), 54.25 (C-2) and 24.41 (C-3).

Benzyl (2*S*)-2-benzyloxycarbonylamino-3-[2-acetyl-1-(*p*-nitrophenyl)-5-oxopyrazol-4-yl]propionate 10d

The pyrazole **9d** (77 mg, 0.15 mmol) was dissolved in dry acetonitrile (4 ml) and 4-dimethylaminopyridine (3 mg, 0.02 mmol) was added to the solution at room temperature, under nitrogen. After the mixture had been stirred for 10 min, acetic anhydride (14 μl, 0.15 mmol) was added dropwise to it and stirring continued for 14 h at room temperature. Further acetic anhydride (5 μl, 0.05 mmol) was added to the mixture and, after a total of 20 h, the solvent was removed *in vacuo*. The resulting brown residue (85 mg) was chromatographed on silica gel using gradient elution from ethyl acetate–dichloromethane (1:9) to ethyl acetate–dichloromethane (2:3). A minor product was eluted as a gum using ethyl acetate–dichloromethane (1:9), followed by a more polar major product, eluted using ethyl acetate–dichloromethane (1:4). The minor product benzyl (2*S*)-2-benzyloxycarbonylamino-3-[5-acetoxy-1-(*p*-nitrophenyl)pyrazol-4-yl]propionate **14** (10 mg, 12%); *m/z* (EI) 516 ([M − CH_3CO]$^+$); δ_H(360 MHz, C^{2}HCl$_3$) 8.3 (2 H, d, *J* 9.0, ArH), 7.69 (2 H, d, *J* 9.2, ArH), 7.34 (11 H, m, ArH), 5.48 (1 H, exch. d, $J_{NH,2}$ 7.3, NH), 5.19 (2 H, AB, J_{AB} 12.1, PhCH_2), 5.12 (2 H, AB, J_{AB} 12.2, PhCH_2), 4.63 (1 H, q, *J ca.* 5.8, H-2), 2.93 (2 H, m, overlapping AB systems, $J_{3B,3A}$ 14.9, $J_{3B,2} = J_{3A,2}$ 5.8, 2 × H-3) and 2.19 (3 H, s, N–COCH$_3$). The major product was the title compound **10d** (45 mg, 54%); *m/z* [+ve FAB (3-NBA)] 559 [M + H]$^+$; ν_{max}($CHCl_3$)/cm^{-1} 1720 (urethane and ester) and 1656 (amide and pyrazolone); λ_{max}(MeOH, pH 7)/nm 285 and 312 (ε 15 700 and 14 400); λ_{max}(MeOH, pH 12)/nm 337 (ε 18 560); λ_{max}(MeOH, pH 2)/nm 318 (ε 15 000); δ_H(360 MHz, C^{2}HCl$_3$) 8.26 (2 H, d, *J* 9.2, ArH), 7.76 (1 H, s, H-3′), 7.34 (12 H, m, ArH), 6.01 (1 H, exch. d, $J_{NH,2}$ 7.7, NH), 5.17 (2 H, AB, J_{AB} 11.9, PhCH_2), 5.09 (2 H, AB, J_{AB} 12.2, PhCH_2), 4.65 (1 H, br q, *J ca.* 7, H-2), 2.96 (1 H, d × d, $J_{3B,3A}$ 15.5, $J_{3B,2}$ 5.1, H-3B), 2.85 (1 H, d × d, $J_{3A,3B}$ 15.5, $J_{3A,2}$ 7, H-3A) and 2.21 (3 H, s, N–COCH$_3$). Irradiation of H-3′ (δ 7.76) produced an enhancement of 10% in COCH$_3$ (δ 2.21). Irradiation of COCH$_3$, produced an enhancement of 15% in H-3′; δ_C(62.9 MHz, [$^2H_6$]-DMSO) 171.21 (ester), 166.0 (N–*C*OCH$_3$), 164.52 (C-5′), 155.95 (urethane), 144.46, 142.65, 141.24, 136.76 and 135.70 (4 × *ipso* C and C-3′), 128.37–122.49 (aromatic), 109.87 (C-4′), 66.28 and 65.63 (2 × PhCH$_2$), 52.47 (C-2), 24.76 (C-3) and 21.43 (N–*C*OCH$_3$).

Benzyl (2*S*,4*RS*)-1-benzyloxycarbonyl-4-(2,4-dinitrophenyl)-hydrazonomethylpyroglutamate 13e

Compound **5** (100 mg, 0.24 mmol) was dissolved in methanol (10 ml) and 1 M aqueous hydrochloric acid was added dropwise to the solution until its UV spectrum showed reaction to be complete (*ca.* 1.5 equiv. was required). 2,4-Dinitrophenylhydrazine (76 mg, 0.35 mmol) and sodium acetate (20 mg, 0.24 mmol) were added to the reaction mixture which was then stirred for 5 h at room temperature. The resulting yellow solid was filtered off, washed with cold methanol and recrystallised from ethanol to afford yellow needles. ^{1}H NMR Spectroscopy showed, by integration of the H-4 resonances, the presence of a 2:1 mixture of diastereoisomers. These isomers could not be separated by further recrystallisation (93 mg, 70%), mp 126–128 °C; $[\alpha]_D^{28}$ +5.8 (*c* 0.33, $CHCl_3$) (Found: C, 57.7; H, 4.1; N, 12.3. $C_{27}H_{23}N_5O_9$ requires C, 57.75; H, 4.1; N, 12.5%); *m*/*z* [+ve FAB (3-NBA)] 584 ([M + Na]$^+$) and 562 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 1796 and 1742 (urethane and ester); λ_{max}(MeOH)/nm 348 (ε 8060); δ_H(360 MHz, C^{2}HCl$_3$, major isomer) 11.10 (1 H, exch. s, NH), 9.11 (1 H, d, *J* 2.5, ArH), 8.30 (1 H, d × d, *J* 9.5, 2.5, ArH), 7.85 (1 H, d, *J* 9.5, ArH), 7.66 (1 H, d, $J_{6,4}$ 4.3, H-6), 7.50–7.20 (10 H, m, ArH), 5.24 and 5.18 (4 H, 2 × s, PhCH_2), 4.82 (1 H, d × d, $J_{2,3S}$ 9.6, $J_{2,3R}$ 1.2, H-2), 3.81 (1 H, d × d × d, $J_{4,3S}$ 11.7, $J_{4,3R}$ 8.6, $J_{4,6}$ 4.3, H-4), 2.74 (1 H, m, overlapping with minor isomer, H-3*S*) and 2.45 (1 H, d × d × d, $J_{3R,3S}$ 13.6, $J_{3R,4}$ 8.6, $J_{3R,2}$ 1.2, H-3*R*); δ_H(360 MHz, C^{2}HCl$_3$, minor isomer) 11.3 (1 H, exch. s, NH), 9.09 (1 H, d, *J* 2.5, ArH), 8.22 (1 H, d × d, *J* 9.5, 2.5, ArH), 7.76 (1 H, d, *J* 9.5, ArH), 7.54 (1 H, d, $J_{6,4}$ 4.2, H-6), 7.50–7.20 (10 H, m, ArH), 5.23 (2 H, s, PhCH_2), 5.05 (2 H, AB, J_{AB} 12.3, PhCH_2), 4.79 (1 H, d × d, $J_{2,3S}$ 9.1, $J_{2,3R}$ 5.5, H-2), 3.71 (1 H, d × t, $J_{4,3S} = J_{4,3R}$ 6.0, $J_{4,6}$ 4.2, H-4), 2.74 (1 H, m, overlapping with major isomer, H-3*S*) and 2.59 (1 H, d × t, $J_{3R,3S}$ 11.9, *J ca.* 6, H-3*R*).

Assessment of stereochemical integrity during the ring-switching process

Compound **5** (204 mg, 0.5 mmol) was dissolved in methan-[^{2}H$_1$]ol (8 ml) and hydrolysed by the dropwise addition of 20% ^{2}HCl in ^{2}H$_2$O. Complete hydrolysis was confirmed by UV spectroscopy after which hydrazine hydrate (22 μl, 0.45 mmol) was added to the solution; it was then stirred for 15 h at room temperature. TLC was indicative of the presence of a substantial amount of unchanged aldehyde **6**. Sodium acetate (250 mg, 3.0 mmol) was added to buffer the reaction mixture to pH 5–6 and, after 3 h, the solvent was removed *in vacuo* and the residue was partitioned between deuterium oxide and deuteriochloroform. The layers were separated and the organic phase was washed to neutrality with deuterium oxide and dried (Na_2SO_4) and then the solvent was removed *in vacuo* to yield a viscous yellow oil (190 mg, 90%) which was chromatographed on silica gel using a gradient elution from hexane–ethyl acetate (1:9) to methanol–ethyl acetate (1:9). The major product was eluted immediately, after which the more polar minor product was eluted as a broad band. The ^{1}H NMR spectrum of the minor product (40 mg, 20%) was identical with that of compound **11** obtained in the analogous reaction in MeOH, and ^{2}H NMR spectroscopy confirmed that deuterium had not been incorporated. The major product (70 mg, 33%) was shown by integration of the 2-C*H* resonances in the ^{1}H NMR spectrum to be a 3:1 mixture of diastereoisomers of the acetal **15**; $[\alpha]_D^{23}$ −45.8 (*c* 0.6, MeOH) [Found: *m*/*z* (EI) 428.1689 ([M]$^+$). $C_{23}H_{24}NO_7{}^2H$ requires 428.1685]; ν_{max}($CHCl_3$)/cm^{-1} 1796, 1750 and 1730 (urethane and ester); δ_H(360 MHz, [^{2}H$_6$]-DMSO, major diastereoisomer) 7.36 (10 H, m, ArH), 5.19 (2 H, AB, J_{AB} 10.0, PhCH_2), 5.14 (2 H, s, PhCH_2), 4.78 (1 H, d × d, $J_{2,3S}$ 10, $J_{2,3R}$ 2.2, H-2), 4.63 (1 H, s, H-6), 3.34 and 3.33 [6 H, 2 × s, C(OCH_3)$_2$], 2.44 (1 H, d × d, $J_{3S,3R}$ 13.4, $J_{3S,2}$ 10.0, H-3*S*) and 2.01 (1 H, d × d, $J_{3R,3S}$ 13.4, $J_{3R,2}$ 2.2, H-3*R*); δ_H(360 MHz, [^{2}H$_6$]-DMSO, minor diastereoisomer) 7.36 (10 H, m, ArH), 5.19 and 5.14 (4 H, 2 × s, 2 × PhCH_2), 4.73 (1 H, d × d, $J_{2,3S}$ 9.8, $J_{2,3R}$ 6.3, H-2), 4.55 (1 H, s, H-6), 3.30 and 3.26 [6 H, 2 × s, C(OCH_3)$_2$], 2.41 (1 H, d × d, $J_{3S,3R}$ 13.0, $J_{3S,2}$ 9.8, H-3*S*) and 2.01 (1 H, overlapped by major isomer, H-3*R*). The ^{2}H NMR spectrum (38.4 MHz) showed a deuterium signal at δ 3.0. The ^{1}H decoupled ^{13}C NMR spectrum showed the C-4 signal as a pair of triplets at δ 46.8 and 47.6.

Benzyl (2*S*)-2-benzyloxycarbonylamino-3-(5-hydroxyisoxazol-4-yl)propionate 16

Benzyl (2*S*,4*RS*)-*N*-benzyloxycarbonyl-4-formylpyroglutamate **6** was prepared as above by hydrolysis of the enaminone **5** (204 mg, 0.5 mmol). Hydroxylamine hydrochloride (35 mg, 0.51 mmol) was added to the methanolic solution of crude aldehyde **6** at room temperature, followed by sodium acetate (17.0 mg, 0.2 mmol), added to buffer the mixture to pH 5–6. The mixture was stirred at room temperature for 15 h after which the solvent was removed *in vacuo*. The residue was partitioned between chloroform and water and the organic phase was separated and washed to neutrality with water and brine and then dried ($MgSO_4$). The solvent was removed *in vacuo* to afford a pale yellow gum (160 mg, 81%); *m*/*z* (EI) 396 ([M]$^+$); *m*/*z* [FAB] 419 ([M + Na]$^+$); ν_{max}(film)/cm^{-1} 1790w and 1720s (ester); δ_H(360 MHz, C^{2}HCl$_3$, unstable with time) 7.47 (*ca.* 1 H, s, H-3′), 7.3 (*ca.* 10 H, ArH), 6.03 (*ca.* 1 H, d, *J* 8, NH), 5.1 (*ca.* 4 H, m, 2 × PhCH_2), 4.5 (*ca.* 1 H, m, H-2) and 2.7 (*ca.* 2 H, m, H-3).

Benzyl (2*S*)-2-benzyloxycarbonylamino-3-(5-acetoxyisoxazol-4-yl)propionate 18

The crude reaction product from the above reaction (70 mg, 0.18 mmol) was dissolved in dry acetonitrile (3 ml) with 4-dimethylaminopyridine (2.5 mg, 0.02 mmol) at room temperature under nitrogen. After being stirred for 10 min, the reaction mixture was treated with acetic anhydride (17 μl, 0.18 mmol), added dropwise and then stirred at room temperature for 2 h. The solvent was removed *in vacuo* to yield an orange gum (70 mg, 88%) which was chromatographed on silica gel using ethyl acetate in dichloromethane (1:9) to afford a colourless oil which could not be crystallised (35 mg, 45%); $[\alpha]_D^{29}$ +8.6 (*c* 0.233, $CHCl_3$); *m*/*z* [+ve FAB (3-NBA)] 461 ([M + Na]$^+$) and 439 ([M + H]$^+$); ν_{max}($CHCl_3$)/cm^{-1} 1771 (enol acetate) and 1724 (urethane and ester); λ_{max}(MeOH, pH 7)/nm 285 (ε 10 680); λ_{max}(MeOH, pH 12)/nm 256 (ε 7300); λ_{max}(MeOH, pH 2)/nm 263 (ε 6860); δ_H(200 MHz, C^{2}HCl$_3$) 8.08 (1 H, s, H-3′), 7.35 (10 H, m, ArH), 5.67 (1 H, br exch. d, $J_{NH,2}$ 6, NH), 5.17 and 5.11 (4 H, 2 × s, 2 × PhCH_2), 4.61 (1 H, br q, *J ca.* 6, H-2), 2.89 (1 H, d × d, $J_{3B,3A}$ 15, $J_{3B,2}$ 5.5, H-3B), 2.79 (1 H, d × d, $J_{3A,3B}$ 15, $J_{3A,2}$ 6.1, H-3A) and 2.35 (3 H, s, CH_3CO); irradiation of 3′-CH (δ 2.35) led to no detectable enhancement in CH_3CO (δ 2.35) and similarly, irradiation of CH_3CO led to no detectable enhancement in 3′-CH; δ_C(50.3 MHz, [^{2}H$_6$]-DMSO) 171.46 (ester), 167.94 (O–*C*OCH$_3$), 156.51 (urethane), 142.78 and 142.67 (C-3′ and C-5′), 137.26 and 136.20 (2 × *ipso* C), 128.91–128.21 (aromatic), 103.82 (C-4′), 66.89 and 66.19 (2 × Ph*C*H$_2$), 52.91 (C-2), 24.57 (C-3) and 20.98 (O–CO*C*H$_3$).

Benzyl (2*S*,4*RS*)-1-benzyloxycarbonyl-4-benzyloxyimino-methylpyroglutamate 17

Compound **6** was generated *in situ* by hydrolysis of the enaminone **5** (102 mg, 0.25 mmol) as described above. *O*-Benzylhydroxylamine hydrochloride (40 mg, 0.25 mmol) in methanol (1 ml) was added to it at pH 1 and the mixture was stirred at room temperature for 20 h. After this the solvent was removed *in vacuo* and the residue was partitioned between chloroform (3 ml) and saturated aqueous sodium hydrogen carbonate (2 ml). The organic phase was separated and washed with water and brine and then dried (Na_2SO_4). The solvent was removed *in vacuo* to afford a yellow oil (98%), $[\alpha]_D^{26}$ −11.9 (*c* 0.547, $CHCl_3$). This was further purified by chromatography on silica gel using dichloromethane as eluent, to give a pale yellow gum (75 mg,

62%) (Found: C, 68.8; H, 5.3; N, 5.5. $C_{28}H_{26}N_2O_6$ requires C, 69.1; H, 5.35; N, 5.8%); *m/z* [+ve FAB (thioglycerol)] 487 ([M + H]$^+$) and 509 ([M + Na]$^+$); ν_{max}(CHCl$_3$)/cm^{-1} 1799 (imide) and 1752 (ester); δ_H(360 MHz, C^{2}HCl$_3$) 7.57 (d, $J_{6,4}$ 4.9, H-6 *syn*, *trans*), 7.52 (d, $J_{6,4}$ 5.0, H-6 *syn*, *cis*), 7.34 (10 H, m, ArH), 6.90 (d, $J_{6,4}$ 5.1, H-6 *anti*, *trans*), 6.88 (d, $J_{6,4}$ 5.3, H-6 *anti*, *cis*) (the four peaks for H-6 integrated as a total of 1 H), 5.23–5.03 (4 H, m, 2 × PhCH_2), 4.73–4.67 (1 H, m, H-2), 3.88, 3.56 and 3.48 (1 H, 3 × m, H-4), 2.75, 2.54, 2.35, 2.29, 2.21 and 1.95 (1 H, 6 × m, 3-CH$_2$).

(2*S*)-2-Amino-3-(5-hydroxypyrazol-4-yl)propionic acid 20a and (2*S*)-2-benzyloxycarbonylamino-3-(5-hydroxypyrazol-4-yl)-propionic acid 21

Method A. Compound **9a** (200 mg, 0.50 mmol) was dissolved in methanol–water (9:1; 5 ml) containing 10% palladium-on-charcoal (20 mg). The system was purged with hydrogen and stirred vigorously under an atmosphere of hydrogen, at room temperature, for 3 days. After this time, the reaction was filtered through Celite and the catalyst was washed with methanol. Removal of the solvent from the combined filtrate and washings *in vacuo* afforded a brown oil which was dissolved in water and the solution washed twice with chloroform. The aqueous phase was lyophilised to a fine solid (70 mg), the ^{1}H NMR spectrum of which showed two major products to be present. These were separated by preparative reverse phase HPLC [ZORBAX C8 column (21.2 mm × 25 cm)] using water as eluent. Both products were obtained as hygroscopic lyophilates. The title acid **20a** (18 mg, 20%); $[\alpha]_D^{24}$ −37.7 (*c* 0.48, H$_2$O) [Found: *m/z* (EI) 171.0643 ([M]$^+$). $C_6H_9N_3O_3$ requires 171.0641]; ν_{max}(KBr)/cm^{-1} 3600–2800br (NH, OH) and 1607br (CO$_2$H); λ_{max}(MeOH, pH 7)/nm 231 and 247 (ε 3700 and 3800); λ_{max}(MeOH, pH 12)/nm 238 (ε 5690); λ_{max}(MeOH, pH 2)/nm 231 (ε 5130); δ_H(360 MHz, [^{2}H$_6$]-DMSO) 9.7–8.7 (3 H, br, exch. s, NH, OH), 7.15 (1 H, s, H-3′), 3.38 (1 H, d × d, $J_{2,3A}$ 6, $J_{2,3B}$ 4.3, H-2), 2.68 (2 H, overlapping AB system, $J_{3B,3A}$ 15.3, $J_{3A,2}$ 6, $J_{3B,2}$ 4.3, 3-CH$_2$); δ_C(62.9 MHz [^{2}H$_6$]-DMSO) 171.4 (C–*C*O$_2$H), 159.11 (C-5′), 130.85 (C-3′), 98.24 (C-4′), 54.62 (C-2) and 24.79 (C-3). The second product proved to be the title acid **21** (15 mg, 10%); [Found: *m/z* (EI) 287.0906 ([M − H$_2$O]$^+$). $C_{14}H_{13}N_3O_4$ requires 287.0904]; *m/z* [+ve FAB (thioglycerol)] 328 ([M + Na]$^+$) and 306 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 1721 (urethane) and 1622 (acid); λ_{max}(MeOH, pH 7)/nm 225sh and 250sh (ε 1500 and 1000); λ_{max}(MeOH, pH 12)/nm 240 (ε 3470); λ_{max}(MeOH, pH 2)/nm 230 (ε 3450); δ_H(360 MHz, [^{2}H$_6$]-DMSO) 12.0–11.0 (variable integration, br, exch. s, OH, NH), 7.41 (1 H, d, $J_{NH,2}$ 8.1, NH), 7.32 (5 H, m, ArH), 7.18 (1 H, s, H-3′), 4.99 (1 H, AB, J_{AB} 12.4, PhCH_2), 4.03 (1 H, d × t, $J_{2,NH} = J_{2,3A}$ = 9.1, $J_{2,3B}$ 4.8, H-2), 2.72 (1 H, d × d, $J_{3B,3A}$ 15, $J_{3B,2}$ 4.8, H-3B) and 2.54 (1 H, d × d, $J_{3A,3B}$ 15, $J_{3A,2}$ 9.1, H-3A); δ_C(62.9 MHz, [^{2}H$_6$]-DMSO) 173.55 (C–*C*O$_2$), 159.14 (C-5′), 155.90 (urethane), 137.01 (*ipso* C), 129.03 (C-3′), 128.33–127.60 (aromatic), 98.99 (C-4′), 65.29 (Ph*C*H$_2$), 54.62 (C-2) and 24.40 (C-3).

Method B. Platinum(IV) oxide monohydrate (Adam's catalyst) (25 mg) was added to glacial acetic acid (5 ml) and activated under an atmosphere of hydrogen. After *ca.* 5 min compound **9a** (210 mg, 0.53 mmol) was added to the reaction mixture in such a way as to maintain the hydrogen atmosphere. The reaction was vigorously stirred at room temperature for 22 h after which it was filtered through Celite to remove the catalyst and the solvent was removed *in vacuo*. The residue was dissolved in water and the aqueous solution was washed twice with chloroform. The aqueous phase was lyophilised to constant weight (58 mg, 65%). The product, the acid **20a**, had spectra identical with those obtained for the major product using method A.

(2*S*)-2-Amino-3-(1-methyl-5-hydroxypyrazol-4-yl)propionic acid 20b

Platinum(IV) oxide monohydrate (Adam's catalyst) (25 mg) was added to glacial acetic acid (5 ml) and activated under an atmosphere of hydrogen. After *ca.* 5 min compound **9b** (204 mg, 0.5 mmol) was added to the mixture as a solution in glacial acetic acid (2 ml). The reaction mixture was vigorously stirred at room temperature under hydrogen for 22 h after which it was filtered through Celite and the solvent was removed *in vacuo* to afford a brown gum. This was dissolved in water and the solution washed twice with ethyl acetate. The aqueous phase was lyophilised repeatedly until all of the acetic acid had been removed. This gave the title acid **20b** as a hygroscopic pink foam (60 mg, 70%); $[\alpha]_D^{25}$ −55.5 (*c* 0.51, CHCl$_3$) [Found: *m/z* (EI) 185.0800 ([M]$^+$). $C_7H_{11}N_3O_3$ requires 185.0799]; ν_{max}(KBr)/cm^{-1} 3600–2600br (OH, NH) and 1597 (CO of amino acid); λ_{max}(MeOH, pH 7)/nm 252 (ε 5400); λ_{max}(MeOH, pH 12)/nm 243 (ε 6220); λ_{max}(MeOH, pH 2)/nm 234 (ε 5000); δ_H(360 MHz, [^{2}H$_6$]-DMSO) 7.10 (1 H, s, H-3′), 3.39 (1 H, t, partially obscured by N–Me, $J_{2,3A} = J_{2,3B}$ = 5.5, H-2), 3.37 (3 H, s, N–CH$_3$), 2.70 (1 H, d × d, $J_{3A,3B}$ 15.5, $J_{3A,2}$ 5.5, H-3A) and 2.59 (1 H, d × d, $J_{3B,3A}$ 15.5, $J_{3B,2}$ 5, H-3B); δ_C(62.9 MHz, [^{2}H$_6$]-DMSO) 171.82 (CO$_2$H), 152.53 (C-5′), 137.42 (C-3′), 94.37 (C-4′), 54.71 (C-2), 32.89 (N–CH$_3$) and 25.76 (C-3).

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonyl-4-methoxymethylene-pyroglutamate 25

tert-Butyl (2*S*)-1-*tert*-butoxycarbonyl-4-*N*-dimethylamino-methylenepyroglutamate **23**[25] (10 g, 0.0294 mol) and screened Methyl Orange (2 drops) were dissolved in methanol (100 ml) with stirring. Aqueous 1 M hydrochloric acid (*ca.* 32 ml required) was added dropwise to the mixture at room temperature at such a rate as to keep the pH of the solution at or above the neutral point of the indicator. This took 40 min. The solution was then concentrated to a small volume *in vacuo*, diluted with water (15 ml) and extracted with ethyl acetate (3 × 30 ml). The combined organic phases were washed with brine, dried (MgSO$_4$) and the solvent was removed *in vacuo* to yield a yellow oil which was dissolved in a mixture of dichloromethane (10 ml) and diethyl ether (20 ml). The solution was cooled to ice-bath temperature with stirring. Diazomethane was prepared by dropwise addition of Diazald (21.4 g, 0.1 mol) in diethyl ether (30 ml) into ethanol (15 ml) containing potassium hydroxide (6 g) with stirring and heating to 60 °C to allow its distillation into the solution. The reaction mixture was left for 18 h at −18 °C after which the solvent and remaining diazomethane were removed by a stream of nitrogen. The resultant yellow solid was recrystallised from ethyl acetate–light petroleum (bp 60–80 °C) to yield the title compound **25** as fine needles (5.74 g, 60%), mp 96–99 °C. The mother liquors were concentrated to a small volume *in vacuo* and purified by chromatography on silica gel, eluting with dichloromethane–methanol (96:4) to yield further product (1.4 g, 14%). An analytical sample was recrystallised from ethyl acetate–light petroleum (bp 60–80%); mp 98–99 °C; $[\alpha]_D^{23}$ −17.7 (*c* 1.1, CHCl$_3$) (Found: C, 58.7; H, 8.0; N, 4.3. $C_{16}H_{25}NO_6$ requires C, 58.7; H, 7.7; N, 4.3%); *m/z* (+ve FAB, 3-NBA) 677 ([2M + Na]$^+$), 350 ([M + Na]$^+$) and 328 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 1759 (urethane), 1747 (ester), 1698 and 1674 (urethane); λ_{max}(MeOH, pH 7)/nm 263 (log ε 4.02); δ_H(360 MHz, C^{2}HCl$_3$) 7.21 (1 H, t, $J_{6,3}$ 2.5, H-6), 4.45 (1 H, d × d, $J_{2,3A}$ 3.6, $J_{2,3B}$ 10.5, H-2), 3.84 (3 H, s, OCH$_3$), 2.88 (1 H, d × d × d, $J_{3B,6}$ 2.5, $J_{3B,2}$ 10.5, $J_{3B,3A}$ 17, H-3B), 2.52 (1 H, $J_{3A,6}$ 2.5, $J_{3A,2}$ 3.6, $J_{3A,3B}$ 17, H-3A), 1.51 [9 H, s, C(CH$_3$)$_3$] and 1.47 [9 H, s, C(CH$_3$)$_3$]; δ_C(125.76 MHz, C^{2}HCl$_3$) 170.31 and 167.61 (2 × CO), 155.16 (HCO), 149.72 (urethane), 106.42 (C-4), 82.71 and 81.85 [2 × *C*(CH$_3$)$_3$], 61.68 (OCH$_3$), 56.61 (C-2), 27.83 and 27.77 [2 × C(*C*H$_3$)$_3$] and 23.68 (C-3).

tert-Butyl (2*S*)-2-*tert*-butoxycarbonylamino-3-(2-methyl-4-oxo-pyrimidin-5-yl)propionate 26b

Compound **25** (1.057 g, 3.23 mmol), acetamidine hydrochloride

(1.23 g, 13 mmol) and potassium carbonate (1.78 g, 13 mmol) were heated in ethanol (30 ml) at reflux for 70 h. The solvent was removed *in vacuo* and the resultant oil was partitioned between 5% aqueous citric acid (30 ml) and dichloromethane (20 ml). The aqueous phase was separated and extracted with dichloromethane (2 × 20 ml). The combined organic phases were washed with brine, dried ($MgSO_4$) and the solvent was removed *in vacuo* to yield a pale yellow oil which solidified when stored. This was purified by chromatography on silica gel, eluting with ethyl acetate–acetic acid (98:2) to give the title compound **26b** as a white solid (0.99 g, 87%). An analytical sample was recrystallised [toluene–light petroleum (bp 60–80 °C)], mp 128–130 °C; $[\alpha]_D^{23}$ −39.5 (*c* 0.5, $CHCl_3$) (Found: C, 58.0; H, 7.6; N, 11.7. $C_{17}H_{27}N_3O_5$ requires C, 57.8; H, 7.7; N, 11.9%); *m*/*z* [+ve FAB, 3-NBA] 707 ($[2M + H]^+$) and 354 ($[M + H]^+$); ν_{max}(KBr)/cm^{-1} 1710 (ester) and 1693 (urethane); λ_{max}(MeOH)/nm 226 and 276 (log ε 3.77 and 3.72); λ_{max}(MeOH, pH 1)/nm 230 and 263 (log ε 3.84 and 3.66); δ_H(360 MHz, C^2HCl_3) 11.5 (1 H, br exch., NH), 7.81 (1 H, s, H-4′), 6.05 (1 H, exch. d, $J_{NH,2}$ 7.7, NH), 4.41 (1 H, m, H-2), 2.93 (1 H, d × d, $J_{3A,2}$ 3.7, $J_{3A,3B}$ 14, H-3A), 2.76 (1 H, d × d, $J_{3B,2}$ 8.1, $J_{3B,3A}$ 14, H-3B), 2.47 (3 H, s, CH_3), 1.39 [9 H, s, $C(CH_3)_3$] and 1.36 [9 H, s, $C(CH_3)_3$]; addition of ^2H_2O caused the multiplet at δ 4.41 to simplify. A minor component was evident in the ^1H NMR spectrum (~20% by integration); distinct peaks associated with this occurred at δ 6.2 (br exch. d) and 4.25 (br m) but when the spectrum was recorded in [$^2H_6$]-DMSO at 352 K, peaks associated with the minor component were seen to have coalesced with those of the major component; δ_C(125.76 MHz, C^2HCl_3) 170.71 and 165.76 (2 × CO), 158.36 (C-2′), 155.38 (urethane), 154.99 (C-4′), 120.95 (C-5′), 81.59 and 79.48 [2 × *C*$(CH_3)_3$], 54.03 (C-2), 30.52 (C-3), 28.28 and 28.00 [2 × C(*C*$H_3)_3$] and 21.45 (CH_3).

tert-Butyl (2*S*)-2-*tert*-butoxycarbonylamino-3-(2-phenyl-4-oxopyrimidin 5-yl)propionate 26c

Compound **25** (1.113 g, 3.403 mmol), benzamidine hydrochloride monohydrate (2.13 g, 13.6 mmol) and potassium carbonate (0.94 g, 6.8 mmol) were heated in ethanol (30 ml) at reflux for 44 h. The solvent was removed *in vacuo* and the resultant oil was partitioned between ethyl acetate (20 ml) and water (20 ml). The aqueous phase was separated and washed with ethyl acetate (2 × 20 ml). The combined organic phases were washed with brine, dried ($MgSO_4$) and the solvent was removed *in vacuo* to yield a yellow oil which solidified on storage. This was recrystallised from ethanol to yield the title compound **26c** as a white solid (0.2 g, 14%). The mother liquors were further purified, by chromatography on silica gel, eluting with dichloromethane–methanol (95:5). Recrystallisation of the product obtained from ethanol gave further white solid (0.583 g, 41%; total yield 55%), mp 188–189 °C; $[\alpha]_D^{23}$ +14.5 (*c* 1.3, $CHCl_3$) (Found: C, 63.2; H, 7.3; N, 10.2. $C_{22}H_{29}N_3O_5$ requires C, 63.6; H, 7.0; N, 10.1%); *m*/*z* (+ve FAB, 3-NBA) 831 ($[2M + H]^+$), 438 ($[M + Na]^+$) and 416 ($[M + H]^+$); ν_{max}(KBr)/cm^{-1} 1725 (ester) and 1698 (urethane); λ_{max}(MeOH, pH 7)/nm 246 and 299 (log ε 3.81 and 3.89); λ_{max}(MeOH, pH 1)/nm 253 and 281 (log ε 3.88 and 3.94); δ_H(360 MHz, C^2HCl_3) 12.5 (1 H, exch. br s, N*H*), 8.26 (2 H, m, ArH), 8.03 (1 H, s, H-4′), 7.52 (3 H, m, ArH), 5.96 (1 H, exch. d, $J_{NH,2}$ 7.5, NH), 4.50 (1 H, m, H-2), 3.02 (1 H, d × d, $J_{3A,2}$ 4.6 and $J_{3A,3B}$ 14, H-3A), 2.85 (1 H, d × d, $J_{3B,2}$ 8.6 and 14, H-3B), 1.44 [9 H, s, $C(CH_3)_3$] and 1.33 [9 H, s, $C(CH_3)_3$]; δ_C(125.76 MHz, C^2HCl_3) 170.87 and 165.61 (2 × CO), 156.61 (C-2′), 155.34 (urethane), 131.91 (aromatic), 131.80 (*ipso*-C), 129.05 and 127.69 (aromatic), 121.83 (C-5′), 81.91 and 79.46 [*C*$(CH_3)_3$], 54.24 (C-2), 30.68 (C-3), 28.18 and 28.00 [2 × C(*C*$H_3)_3$].

tert-Butyl (2*S*)-2-*tert*-butoxycarbonylamino-3-(4-oxopyrimidin-5-yl)propionate 26a

Compound **25** (0.94 g, 2.87 mmol), formamidine acetate (1.2 g, 11.5 mmol) and potassium carbonate (0.79 g, 0.57 mmol) were heated in ethanol (30 ml) at reflux for 70 h. The solvent was removed *in vacuo* and the resultant oil was partitioned between ethyl acetate (20 ml) and water (20 ml). The aqueous phase was separated and washed with ethyl acetate (2 × 20 ml). The combined organic phases were washed with 5% aqueous citric acid and brine, dried ($MgSO_4$) and the solvent was removed *in vacuo* to yield a tan coloured oil. This was purified by chromatography on silica gel, eluting with dichloromethane–methanol (91:9) to yield the title compound **26a** as a tan coloured foam (0.743 g, 76%); $[\alpha]_D^{23}$ −19.7 (*c* 2.6, $CHCl_3$) [Found: *m*/*z* (EI) 339.180 96. $C_{16}H_{25}N_3O_5$ requires 339.179 42]; ν_{max}(film)/cm^{-1} 1712 (ester) and 1672 (urethane); λ_{max}(MeOH, pH 7)/nm 224 and 273 (log ε 3.65 and 3.57); λ_{max}(MeOH, pH 12)/nm 235 and 273 (log ε 3.71 and 3.57); λ_{max}(MeOH, pH 1)/nm 229 and 266 (log ε 3.71 and 2.52); δ_H(360 MHz, [$^2H_6$]-DMSO) 12.55 (1 H, br exch., NH), 8.08 (1 H, s, CH), 7.74 (1 H, s, CH), 7.12 (1 H, exch. d, $J_{NH,2}$ 8, NH), 4.01 (1 H, m, H-2), 2.76 (1 H, d × d, $J_{3A,2}$ 5.5, $J_{3A,3B}$ 13.6, H-3A), 2.51 (partly obscured by residual DMSO peak, *ca.* 1 H, d × d, $J_{3B,2}$ 9.25, $J_{3B,3A}$ 13.6, H-3B) and 1.33 [18 H, overlapping singlets, $C(CH_3)_3$]; on addition of ^2H_2O the multiplet at δ 4.01 simplified. A minor component was evident in the ^1H NMR spectrum (~18% by integration); the only distinct peak associated with this was an exchangeable doublet at δ 6.77 (*J* 7.2); when the spectrum was recorded at 332 K this peak was seen to have coalesced with the major exchangeable doublet at δ 7.12; δ_C(125.76 MHz, C^2HCl_3) 170.76 and 164.28 (2 × CO), 155.28 (urethane), 154.07 (C-2′), 147.79 (C-4′), 124.90 (C-5′), 82.14 and 79.63 [2 × *C*$(CH_3)_3$], 53.39 (C-2), 30.97 (C-3), 28.25 and 27.98 [2 × C(*C*$H_3)_3$].

(2*S*)-2-Amino-3-(2-methyl-4-oxopyrimidin-5-yl)propionic acid dihydrochloride 27b

Compound **26b** (0.171 g, 0.48 mmol) was suspended in conc. hydrochloric acid (1.5 ml) and stirred at room temperature for 1.5 h, during which time there was dissolution of the suspended solid accompanied by slow effervescence. The solvent was removed *in vacuo* with gentle warming, to yield the title compound **27b** as a hygroscopic glass (0.15 g, >100%); $[\alpha]_D^{23}$ +54 (*c* 1, H_2O); *m*/*z* (+ve FAB, thioglycerol + sodium) 242 ($[M + 2Na]^+$), 220 ($[M + Na]^+$) and 198 ($[M + H]^+$) [Found: *m*/*z* (EI) 197.081 17 ($[M]^+$). $C_8H_{11}N_3O_3$ requires 197.080 04]; ν_{max}(KBr)/cm^{-1} 3541 (OH, NH) and 1662 (C=O); λ_{max}(MeOH)/nm 227 and 275 (log ε 3.75 and 3.75); λ_{max}(MeOH, pH 1)/nm 228 and 264 (log ε 3.84 and 3.68); λ_{max}(MeOH, pH 12)/nm 235 and 275 (log ε 3.82 and 3.75); δ_H(360 MHz, ^2H_2O) 7.53 (1 H, s, H-4′), 3.72 (1 H, d × d, $J_{2,3A}$ 4.5, $J_{2,3B}$ 7.3, H-2), 2.76 (1 H, d × d, $J_{3A,2}$ 4.5, $J_{3A,3B}$ 14.9, H-3A), 2.59 (1 H, d × d, $J_{3B,2}$ 7.3, $J_{3B,3A}$ 14.9, H-3B) and 2.11 (3 H, s, CH_3); δ_C(125.76 MHz, ^2H_2O) 172.92 (CO_2H), 167.34 (C=O), 160.45 (C-2′), 149.51 (C-4′), 118.89 (C-5′), 53.84 (C-2), 28.93 (C-3) and 20.09 (CH_3).

(2*S*)-2-Amino-3-(2-phenyl-4-oxopyrimidin-5-yl)propionic acid hydrochloride 27c

Compound **26c** (0.117 g, 0.28 mmol) was dissolved in conc. hydrochloric acid (1.5 ml). After 1 h at room temperature the solvent was removed *in vacuo* with gentle warming to yield the title compound **27c** as a hygroscopic glass (0.15 g, >100%); $[\alpha]_D^{23}$ +0.5 (*c* 0.6, 2 M HCl) [Found: *m*/*z* (EI) 259.086 40 ($[M]^+$). $C_{13}H_{13}N_3O_3$ requires 259.095 69]; ν_{max}(KBr)/cm^{-1} 3012 (br, OH, NH) and 1740 (C=O); λ_{max}(MeOH, pH 7)/nm 243 and 287 (log ε 4.06 and 4.01); λ_{max}(MeOH, pH 12)/nm 235 and 294 (log ε 4.20 and 3.91); δ_H(360 MHz, ^2H_2O) 7.90 (1 H, s, H-4′), 7.65 (2 H, m, ArH), 7.59 (1 H, m, ArH), 7.45 (2 H, m, ArH), 4.21 (1 H, t, *J ca.* 6.3, H-2), 3.01 (1 H, d × d, $J_{3A,2}$ 6.3, $J_{3A,3B}$ 14.7, H-3A) and 2.92 (1 H, d × d, $J_{3B,2}$ 7.0, $J_{3B,3A}$ 14.7, H-3B); δ_C(125.76 MHz, ^2H_2O) 170.44 (CO_2H), 163.02 (C=O), 159.98 (C-2′), 142.94 (C-4′), 134.82 (aromatic), 129.55 and 128.33 (2 × aromatic), 125.59 (*ipso*-C), 121.23 (C-5′), 51.16 (C-2) and 28.03 (C-3).

(2*S*)-2-Amino-3-(4-oxopyrimidin-5-yl)propionic acid hydrochloride 27a

Compound **26a** (0.743 g, 2.19 mmol) was dissolved in conc. hydrochloric acid (3 ml). After 1.5 h at room temperature the solvent was removed *in vacuo* to yield the title compound **27a** as a glassy hygroscopic solid (0.65 g, >100%), crystals of which were obtained by slow precipitation from water–ethanol; mp (slowly softens) >163 °C; $[\alpha]_D^{23}$ −15.8 (*c* 1, H_2O) (Found: C, 30.8; H, 4.6; N, 15.6. $C_7H_9N_3O_3{\cdot}2HCl{\cdot}H_2O$ requires C, 30.7; H, 4.8; N, 15.3%); *m*/*z* (+ve FAB, glycerol) 393 ([2M + Na]$^+$), 367 ([2M + H]$^+$) and 184 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 3100 (br, NH, OH), 1737 (C=O) and 1709 (acid); λ_{max}(MeOH, pH 7)/nm 226 and 272 (log ε 3.64 and 3.56); λ_{max}(MeOH, pH 12)/nm 236 and 274 (log ε 3.28 and 3.58); λ_{max}(MeOH, pH 1)/nm 228 and 267 (log ε 3.68 and 3.51); δ_H(360 MHz, ^2H_2O) 8.69 (1 H, s, H-2′), 7.85 (1 H, s, H-4′), 4.15 (1 H, t, *J* 6.55, H-2), 2.99 (1 H, d × d, $J_{3A,2}$ 6.5, $J_{3A,3B}$ 14.8, H-3A) and 2.88 (1 H, d × d, $J_{3B,2}$ 6.5, $J_{3B,3A}$ 14.8, H-3B); δ_C(125.76 MHz, ^2H_2O) 171.18 (CO_2H), 163.14 (CO), 151.26 (C-2′), 144.25 (C-4′), 124.19 (C-5′), 51.82 (C-2) and 28.93 (C-3).

***tert*-Butyl (2*S*)-2-*tert*-butoxycarbonylamino-3-(2-amino-4-oxopyrimidin-5-yl)propionate 28**

Compound **25** (1.2 g, 3.67 mmol) and guanidine carbonate (1.98 g, 11 mmol) were heated in ethanol (20 ml) at reflux for 18 h. The solvent was removed *in vacuo* and the resultant oil was partitioned between ethyl acetate (20 ml) and water (20 ml). The aqueous phase was separated and washed with ethyl acetate (2 × 20 ml). The combined organic phases were washed with brine, dried ($MgSO_4$) and the solvent was removed *in vacuo* to yield a tan coloured oil. This was purified by chromatography on silica gel, eluting with dichloromethane–methanol–acetic acid (92:6:2) to yield the title compound **28** as a tan coloured foam (0.504 g, 39%); $[\alpha]_D^{23}$ −74 (*c* 2.4, $CHCl_3$); *m*/*z* (+ve FAB, 3-NBA) 710 ([2M + 2H]$^+$) and 355 ([M + H]$^+$) [Found: *m*/*z* (EI) 253.130 13 ([M − Boc]$^+$). $C_{11}H_{17}N_4O_3$ requires 253.130 07]; ν_{max}(film)/cm^{-1} 1710 (ester) and 1670 (urethane); λ_{max}(MeOH, pH 7)/nm 229 and 290 (log ε 3.65 and 3.73); λ_{max}(MeOH, pH 1)/nm 229 and 263 (log ε 3.65 and 3.70); λ_{max}(MeOH, pH 12)/nm 235 and 280 (log ε 3.68 and 3.72); δ_H(360 MHz, [$^2H_6$]-DMSO) 10.15 (1 H, br exch., NH), 7.35 (1 H, s, H-4′), 7.10 (1 H, exch. d, *J* 7.6, NH), 6.45 (2 H, br exch., NH_2), 4.00 (1 H, m, H-2), 2.59 (1 H, d × d, $J_{3A,2}$ 5.1, $J_{3A,3B}$ 13.7, H-3A), 2.38 (1 H, d × d, $J_{3B,2}$ 8.6, $J_{3B,3A}$ 13.7, H-3B) and 1.33–1.32 [18 H, overlapping singlets, $C(CH_3)_3$]. A minor component was evident in the ^{1}H NMR spectrum, the only distinct peak being an exchangeable doublet at δ 6.75; when the spectrum was recorded at 333 K this peak coalesced with the major exchangeable doublet at δ 7.10.

(2*S*)-2-Amino-3-(2-amino-4-oxopyrimidin-5-yl)propionic acid dihydrochloride 29

Compound **28** (0.50 g, 1.41 mmol) was dissolved in conc. hydrochloric acid (5 ml) and the solution was left for 2 h at room temperature. The solvent was then removed *in vacuo* with gentle warming to yield the title compound **29** as an off-white solid (0.45 g, >100%); mp 210 °C (decomp.); $[\alpha]_D^{23}$ −2.9 (*c* 1.8, H_2O) [Found: *m*/*z* (EI) 198.076 74 ([M]$^+$). $C_7H_{10}N_4O_3$ requires 198.075 29]; ν_{max}(film)/cm^{-1} 3006 (br, NH, OH), 1740 (C=O) and 1690; λ_{max}(MeOH, pH 7)/nm 220 and 263 (log ε 3.89 and 3.67); λ_{max}(MeOH, pH 1)/nm 224 and 262 (log ε 3.60 and 3.66); λ_{max}(MeOH, pH 12)/nm 220, 234 and 282 (log ε 3.66, 3.67 and 3.69); δ_H(360 MHz, ^2H_2O) 7.40 (1 H, s, H-4′), 4.05 (1 H, t, *J ca.* 6.4, H-2), 2.83 (1 H, d × d, $J_{3A,2}$ 6, $J_{3A,3B}$ 15, H-3A) and 2.70 (1 H, d × d, $J_{3B,2}$ 7.0, $J_{3B,3A}$ 15.0, H-3B); δ_C(125.76 MHz, ^2H_2O) 170.25 (CO_2H), 161.99 (C=O), 151.42 (C-2′), 140.19 (C-4′), 110.75 (C-5′), 51.22 (C-2) and 26.96 (C-3).

***tert*-Butyl (2*S*)-1-*tert*-butoxycarbonyl-4-(2-pyridinyl)aminomethylenepyroglutamate 31**

tert-Butyl 1-*tert*-butoxycarbonylpyroglutamate[25] (3.43 g, 12 mmol) was dissolved in tetrahydrofuran (20 ml) and cooled to solid CO_2–acetone bath temperature with stirring and under an atmosphere of nitrogen. Lithium hexamethyldisilazide (1 M solution in THF; 13.2 ml, 13.2 mmol) was added dropwise to the mixture and stirring was continued for 1 h; methyl formate (1.48 ml, 24 mmol) was then added to the mixture. The solution was stirred for 5 min at the temperature of the solid CO_2 bath, after which it was warmed to ice-bath temperature and stirred for a further 100 min. The reaction was quenched by addition of 5% aqueous citric acid (15 ml) to the mixture, after which it was concentrated to a small volume *in vacuo* and extracted with ethyl acetate (3 × 15 ml). The combined organic phases were washed with 5% aqueous citric acid (10 ml) and brine, dried ($MgSO_4$) and the solvent was removed *in vacuo* to yield a tan coloured foam which was dissolved in dioxane (20 ml). 2-Aminopyridine (5.31 g, 56 mmol) was added to the solution which was then heated at reflux for 4 h. After this the solvent was removed *in vacuo* and the resultant oil was partitioned between ethyl acetate (30 ml) and water (30 ml). The aqueous phase was separated and extracted with ethyl acetate (2 × 20 ml). The combined organic phases were washed with brine, dried (Na_2SO_4) and the solvent was removed *in vacuo* to yield an oil which partially crystallised when stored. A first crop of the title compound **31** was obtained by precipitation from ethyl acetate (0.652 g). The remaining solution was diluted with chloroform and washed first with 5% aqueous citric acid (4 × 15 ml) until the washings remained acidic and then with brine. It was then dried ($MgSO_4$) and the solvent was removed *in vacuo* to yield a red solid which was purified by recrystallisation from ethyl acetate (2.972 g, 68% combined yield); mp 186–188 °C (decomp.); $[\alpha]_D^{23}$ +0.5 (*c* 0.7, $CHCl_3$) (Found: C, 61.3; H, 6.9; N, 10.5. $C_{20}H_{27}N_3O_5$ requires C, 61.7; H, 7.0; N, 10.8%); *m*/*z* (+ve FAB, thioglycerol) 412 ([M + Na]$^+$) and 390 ([M + H]$^+$); ν_{max}(KBr)/cm^{-1} 1762 (imide) and 1637; λ_{max}(MeOH, pH 7)/nm 287 and 335 (log ε 4.19 and 4.40); λ_{max}(MeOH, pH 1)/nm 280 and 342 (log ε 4.22 and 4.37); λ_{max}(MeOH, pH 12)/nm 287 and 335 (log ε 4.17 and 4.48); δ_H(360 MHz, C^2HCl_3) 8.21 (1 H, m, ArH), 8.02 (1 H, d × t, $J_{6,3}$ 2.3, $J_{6,NH}$ 13.4, H-6), 7.62 (1 H, m, ArH), 6.91 (2 H, m, ArH), 6.84 (1 H, exch. d, $J_{NH,6}$ 13.4, NH), 4.56 (1 H, d × d, $J_{2,3B}$ 3.4, $J_{2,3A}$ 10.5, H-2), 2.98 (1 H, d × d × d, $J_{3,6}$ 2.3, $J_{3A,2}$ 10.5, $J_{3A,3B}$ 16.1, H-3A), 2.55 (1 H, d × d × d, $J_{3,6}$ 2.3, $J_{3B,2}$ 3.4, $J_{3B,3A}$ 16.1, H-3B), 1.52 [9 H, s, $C(CH_3)_3$] and 1.47 [9 H, s, $C(CH_3)_3$]; addition of ^2H_2O caused the d × t at δ 8.02 to collapse to a broadened undefined triplet; δ_C(125.76 MHz, C^2HCl_3) 170.45 and 167.69 (2 × CO), 152.60 (C=N), 150.05 (urethane), 148.50 (C-6′), 138.47 (C-6), 132.02, 117.74 and 108.16 (3 × aromatic), 102.53 (C-4), 82.72 and 82.06 [2 × *C*(CH_3)$_3$], 56.57 (C-2), 27.98, 27.88 [2 × C(*C*H_3)$_3$] and 24.43 (C-3).

***tert*-Butyl (2*S*)-2-*tert*-butoxycarbonylamino-3-(4-oxopyrido-[1,2-*a*]pyrimidin-3-yl)propionate 32**

Compound **31** (0.277 g, 0.71 mmol) and potassium carbonate (0.2 g, 1.45 mmol) were heated in ethanol (10 ml) at reflux for 2 h after which the solvent was removed *in vacuo*. The resultant mixture was partitioned between ethyl acetate (15 ml) and 10% aqueous citric acid (10 ml). The aqueous phase was separated and extracted with ethyl acetate (15 ml). The combined organic phases were washed with brine, dried ($MgSO_4$) and the solvent was removed *in vacuo* to yield a yellow oil which was purified by chromatography on silica gel, eluting with dichloromethane–methanol (96.5:3.5). Residual starting material contaminated a number of fractions but pure product was obtained as a yellow solid (0.154 g, 55%). An analytical sample was further purified by recrystallisation from diethyl ether–light petroleum ether (bp 60–80 °C) to yield the title compound **32** as a pale yellow solid, mp 78–90 °C; $[\alpha]_D^{23}$ −39.2 (*c* 0.5, $CHCl_3$); *m*/*z* (+ve FAB, 3-NBA) 779 ([2M + H]$^+$) and 390 ([M + H]$^+$) [Found: *m*/*z* (EI) 389.195 01 ([M]$^+$). $C_{20}H_{27}N_3O_5$ requires 389.195 07]; ν_{max}(KBr)/

cm^{-1} 1713 (ester) and 1673 (urethane); λ_{max}(MeOH, pH 7)/nm 242 and 339 (log ε 4.03 and 4.10); λ_{max}(MeOH, pH 1)/nm 239 and 322 (log ε 3.92 and 4.03); λ_{max}(MeOH, pH 12)/nm 242 and 339 (log ε 4.04 and 4.08); δ_H(360 MHz, C^2HCl_3) 9.07 (1 H, d, *J* 7.0, ArH), 8.21 (1 H, s, H-2′), 7.71 (1 H, t, *J* 7.2, ArH), 7.63 (1 H, d, *J* 8.9, ArH), 7.16 (1 H, t, *J* 6.75, ArH), 5.77 (1 H, exch. d, $J_{NH,2}$ 7.55, NH), 4.49 (1 H, m, H-2), 3.19 (1 H, d × d, $J_{3A,2}$ 4.6, $J_{3A,3B}$ 13.8, H-3A), 2.98 (1 H, d × d, $J_{3B,2}$ 8.1, $J_{3B,3A}$ 13.8, H-3B), 1.43 [9 H, s, $C(CH_3)_3$] and 1.36 [9 H, s, $C(CH_3)_3$]; addition of ^2H_2O caused the multiplet at δ 4.49 to simplify to a d × d, $J_{2,3A}$ 4.6, $J_{2,3B}$ 8; δ_C(125.76 MHz, C^2HCl_3) 170.89 (CO), 158.66 (C=N), 154.10 (C-2′), 150.66 (urethane), 135.49, 127.22, 126.34, 115.64 and 113.44 (5 × aromatic), 81.92 and 79.45 [2 × $C(CH_3)_3$], 54.14 (C-2), 31.39 (C-3), 28.21 and 27.97 [2 × $C(CH_3)_3$].

(2*S*)-2-Amino-3-(4-oxopyrido[1,2-*a*]pyrimidin-3-yl)propionic acid hydrochloride 33

Compound **32** (30 mg, 0.08 mmol) was dissolved in conc. hydrochloric acid (*ca.* 1 ml) at room temperature. When effervescence had ceased (*ca.* 30 s) the solvent was removed *in vacuo* to yield the title compound **33** as a glassy solid (28 mg, >100%); $[\alpha]_D^{23}$ −3.9 (*c* 1, H_2O) [Found: *m*/*z* (EI) 233.079 49 ($[M]^+$). $C_{11}H_{11}N_3O_3$ requires 233.080 04]; λ_{max}(MeOH, pH 7)/nm 242 and 348 (log ε 3.78 and 3.83); λ_{max}(MeOH, pH 1)/nm 239 and 320 (log ε 3.64 and 3.79); λ_{max}(MeOH, pH 12)/nm 243 and 339 (log ε 3.82 and 3.84); δ_H(360 MHz, ^2H_2O) 9.03 (1 H, d, *J* 6.9, ArH), 8.27 (1 H, d × d, *J* 7.4 and 8.6, ArH), 8.17 (1 H, s, H-2′), 7.80 (1 H, d × d, *J* 1.2 and 8.6, ArH), 7.61 (1 H, t, *J* 7.1, ArH), 4.21 (1 H, t, *J ca.* 6.5, H-2), 3.17 (1 H, d × d, $J_{3A,2}$ 6.5, $J_{3A,3B}$ 14.9, H-3A) and 3.05 (1 H, d × d, $J_{3B,2}$ 6.6, $J_{3B,3A}$ 14.9, H-3B); irradiation of the doublet at δ 9.03 showed a change in the appearance of the triplet at δ 7.61; irradiation at the d × d at δ 8.27 showed a change in appearance of the d × d at δ 7.80 and the triplet at δ 7.61; δ_C(125.76 MHz, ^2H_2O) 171.57 and 157.74 (2 × CO), 147.21 (NC=N), 145.45, 143.21, 129.65, 121.13, 118.84 and 111.25 (aromatic), 52.41 (C-2) and 29.36 (C-3).

tert-Butyl (2*S*)-1-*tert*-butoxycarbonyl-4-tetrazol-5-ylamino-methylenepyroglutamate 34

Compound **23** (0.65 g, 1.91 mmol) and screened Methyl Orange (1 drop) were dissolved in methanol (10 ml) with stirring at room temperature. 1 M Aqueous hydrochloric acid (*ca.* 3.2 ml required) was added dropwise to the mixture at such a rate as to keep the pH of the solution at or above the neutral point of the indicator and until the indicator remained red. The solution was concentrated to a small volume *in vacuo*, diluted with water (10 ml) and extracted with ethyl acetate (3 × 15 ml). The combined organic layers were washed with brine, dried ($MgSO_4$) and the solvent was removed *in vacuo* to yield a yellow oil which was dissolved in a mixture of ethanol (8 ml) and water (2 ml) with aminotetrazole monohydrate (0.395 g, 3.8 mmol) and heated at reflux for 1 h 40 min. Potassium carbonate (0.53 g, 3.8 mmol) was added to the mixture and heating at reflux was resumed for a further 4 h. The solvent was removed *in vacuo* and the resultant oily solid was partitioned between ethyl acetate (30 ml) and water (30 ml). The aqueous phase was separated and acidified by addition of acetic acid (5 ml) causing an instant precipitate which was filtered off, washed with water and dried *in vacuo* to yield the title compound **34** as a white solid (0.512 g, 70%). A sample was recrystallised from ethanol; mp 183 °C (decomp.); *m*/*z* (+ve FAB, 3-NBA) 783 ($[2M + Na]^+$), 403 ($[M + Na]^+$) and 381 ($[M + H]^+$); ν_{max}(KBr)/cm^{-1} 1773 (imide) and 1694 (urethane); λ_{max}(MeOH, pH 7)/nm 221 and 297 (log ε 3.76 and 4.48); no change on addition of acid; λ_{max}(MeOH, pH 12)/nm 310 (log ε 4.57); δ_H(360 MHz, [$^2H_6$]-DMSO) 15.9 (1 H, br exch., NH), 10.48 (1 H, exch. d, $J_{NH,6}$ 12.1, NH), 7.58 (1 H, d × t, $J_{6,3}$ *ca.* 2.3, $J_{6,NH}$ 12.1, H-6), 4.53 (1 H, d × d, $J_{2,3B}$ 2.8, $J_{2,3A}$ 10.7, H-2), 2.99 (1 H, d × d × d, $J_{3A,6}$ 2.4, $J_{3A,2}$ 10.8, $J_{3A.3B}$ 17.15, H-3A), 2.60 (1 H, d × t, $J_{3B,2;3B,6}$ 2.8, $J_{3B,3A}$ 17.15, H-3B) and 1.40 [18 H, overlapping singlets, $C(CH_3)_3$]; addition of ^2H_2O caused the peak at δ 7.58 to simplify to a triplet with *J* 2.3; δ_C(125.76 MHz, [$^2H_6$]-DMSO) 170.86 and 167.14 (2 × CO), 156.58 (C=N), 149.38 (urethane), 132.52 (C-6), 105.30 (C-4), 82.01 and 81.68 [2 × $C(CH_3)_3$], 56.24 (C-2), 27.69 and 24.58 [2 × $C(CH_3)_3$].

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