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Versatile Synthesis of Inhibitors of late Enzymes in the Bacterial Pathway to Lysine

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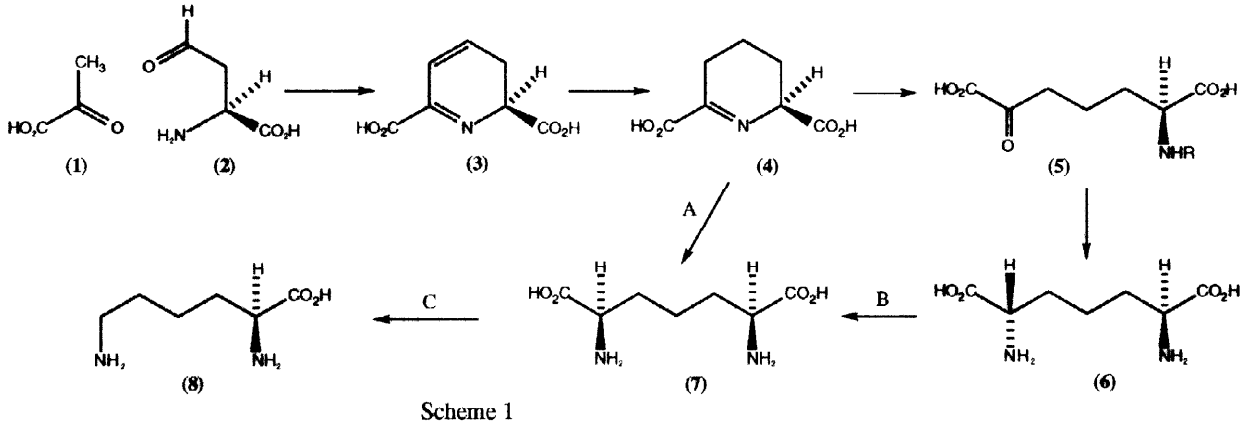
Abstract : Modification of our 'ring switching' reaction has allowed us to develop a versatile synthesis of potential inhibitors of the late enzymes in the bacterial pathway to lysine. The methodology is applicable to solid phase combinatorial synthesis. © 1999 Elsevier Science Ltd. All rights reserved.

There are two distinct biosynthetic pathways to the essential amino acid lysine (**8**), one in fungi and euglenoids and the other in bacteria.[1] Neither of these operates in man where lysine is one of the nine essential dietary amino acids. The bacterial pathway involves synthesis of *meso*-α,ω-diaminopimelate (**7**)[2,3] and it has long been recognised as a potential target for the development of antibacterial drugs since enzymes are specific to bacteria and not man. Lysine and diaminopimelate are also essential in bacterial cell wall cross-linking, a major target for intervention by antibacterial drugs. The pathway is shown in Scheme 1 and initially involves condensation of pyruvate (**1**) with aspartate semialdehyde (**2**).



Scheme 1

Vederas[4] has suggested that inhibitors of *meso*-DAP epimerase (EC 5.1.1.7), the enzyme B in Scheme 1 which catalyses conversion of L,L-diaminopimelate (**6**) to the corresponding *meso*-D,L-epimer (**7**), and of DAP dehydrogenase (EC1.4.1.16), the enzyme A above on an alternative pathway in some bacteria, will be useful antibacterial drugs. The epimerase is present in both Gram positive and Gram negative organisms and a two base mechanism involving a planar transition state (**9**) has been suggested. This is shown in Scheme 2 and planar compounds such as (**14**) are obvious candidates as transition state inhibitors for the process. The three dimensional structure of the dehydrogenase as a complex with heterocyclic inhibitors has recently been published.[5]

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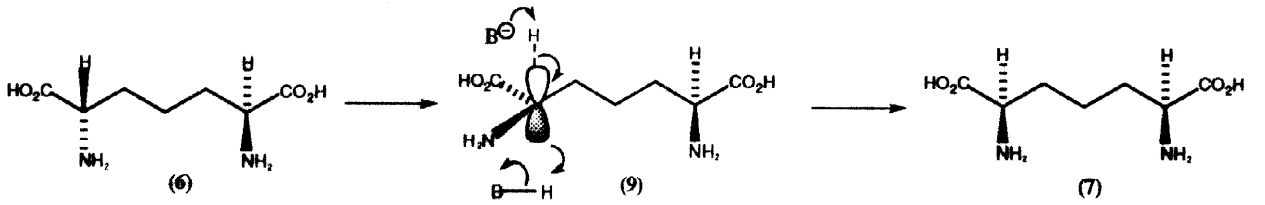
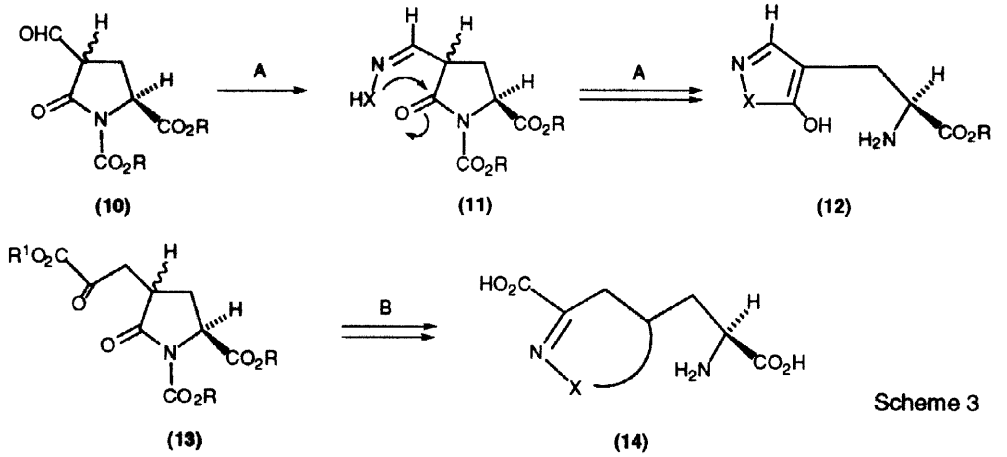


Scheme 2

Our recent development of a versatile synthesis of glutamate agonists and antagonists, using a strategy in which a pyroglutamate system is "switched" with a heterocyclic system (Scheme 3A),[6] has afforded useful entry to an important class of drugs. It seemed that simple modification of the pyroglutamate precursor (**10**) to a carboxyl derivative (**13**) might expand this useful and versatile methodology and allow the preparation of libraries of inhibitors (**14**) of DAP epimerase and other late enzymes in the bacterial pathway to lysine (Scheme 3B).

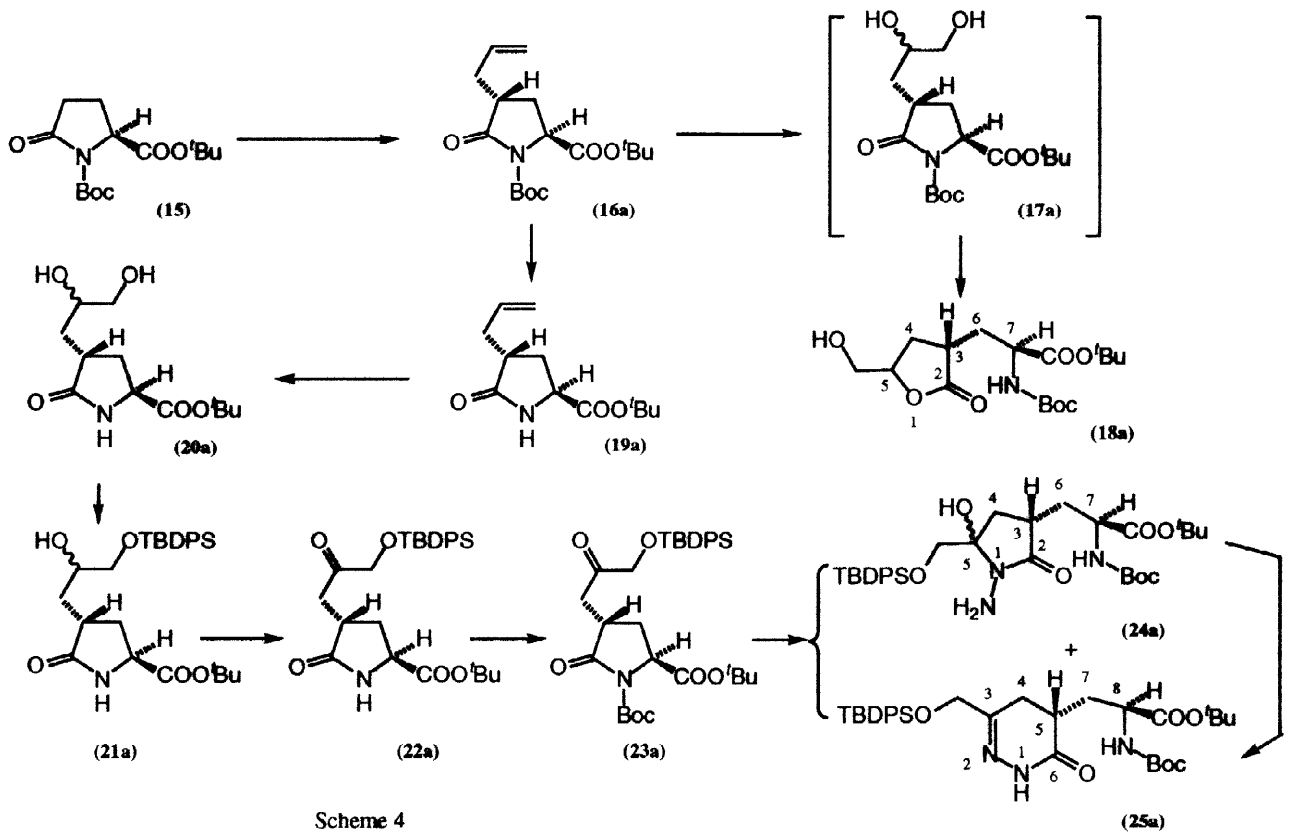


Scheme 3

The simplest approach to precursors (**13**) would be by direct alkylation of the protected pyroglutamate (**15**) with a bromopyruvate ester, but we were unable to effect this reaction, in spite of the well known alkylation of protected pyroglutamate anions with bromoacetate esters.[7,8]

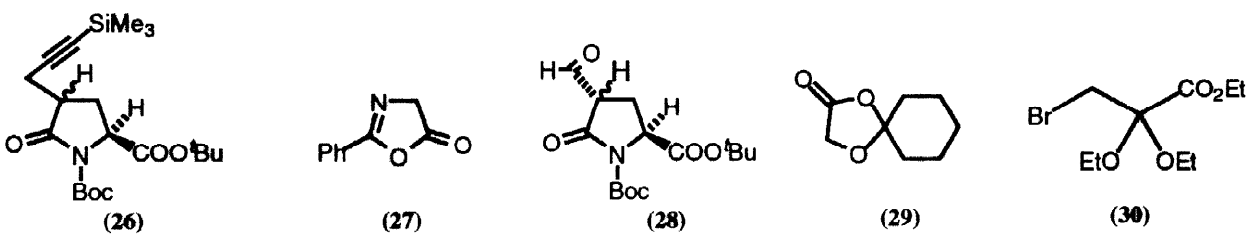
We therefore reacted the pyroglutamate (**15**) with LiHMDS and allyl bromide at -78 °C as shown in Scheme 4 to obtain the *trans* alkylated product (**16a**) and its *cis* epimer (**16b**) in a ratio of 3 : 2. These were separated, the stereochemistry was confirmed by nOe measurements, and an attempt was made to prepare the diols (**17**) from them. Reaction with $OsO_4/K_3Fe(CN)_6$ gave an excellent yield of a product which was evidently not the diol (**17a**). A 2D COSY spectrum indicated coupling between an NH proton and the α-proton and an intact CH_2OH group, and a γ-lactone was indicated by the infrared spectrum. This suggested that the lactone (**18a**) had been formed by premature "ring switching" of the diol (**17**). To prevent this, we selectively deprotected the Boc urethane in (**16a**) to obtain (**19a**) with the less electrophilic pyroglutamate amide group. This was then dihydroxylated to give the diol (**20a**) in good yield. Protection of the primary alcohol in (**20a**) gave the ether (**21a**) which could then be oxidised to the ketone (**22a**). Reinstatement of the urethane gave the product (**23a**), which on treatment with hydrazine, underwent "ring switching" to yield a mixture of two "ring switched" products with a characteristic CH-NHBoc grouping. One proved to be the carbinolamine (**24a**) with spectra similar to the *cis* isomer (**24b**) and the other was the pyridazine (**25a**). The products were in the ratio of 4: 1 ratio. The five membered carbinolamine rearranged on acid catalysed dehydration to yield the pyridazine as the sole product in 42 % yield.

The reaction sequence in Scheme 4 could also be effected on the *cis* series, giving the *cis* isomers (**18b**) and (**19b**) to (**25b**). The structure of the five membered carbinolamine was finally assigned on the basis of the ^{15}N NMR spectrum which showed one NH and one NH_2 group.

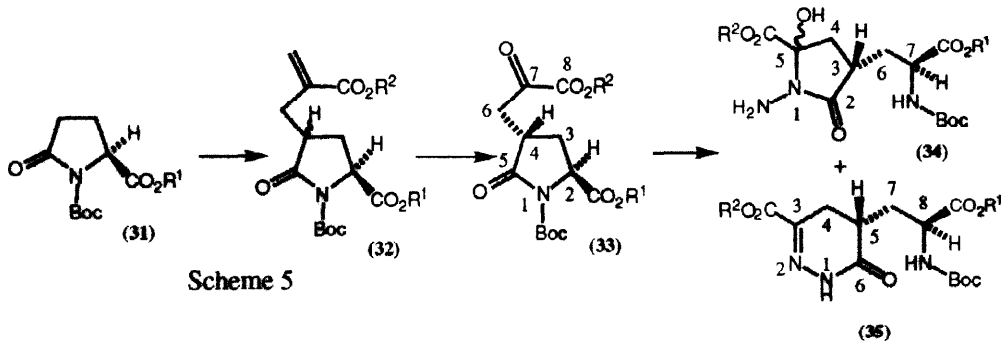


Scheme 4

We had therefore achieved the required "ring switching" reaction, albeit by a route which required wasteful deprotection/reprotection steps to prevent premature "ring switching". Further, the protected primary alcohol function still required deprotection/oxidation to yield the desired inhibitors (**14**). We therefore investigated a series of more direct approaches to the inhibitors. We were able to prepare the *cis*- and *trans*- acetylenes (**26**) and verify the stereochemistry by nOe measurements but could not oxidise these to the pyruvates using the method of Bulman Page and Rosenthal.[9] Reactions of the aldehyde (**28**) with either the azlactone (**27**) or the lactone (**29**) were also unsuccessful as was use of protected bromopyruvates such as compound (**30**).

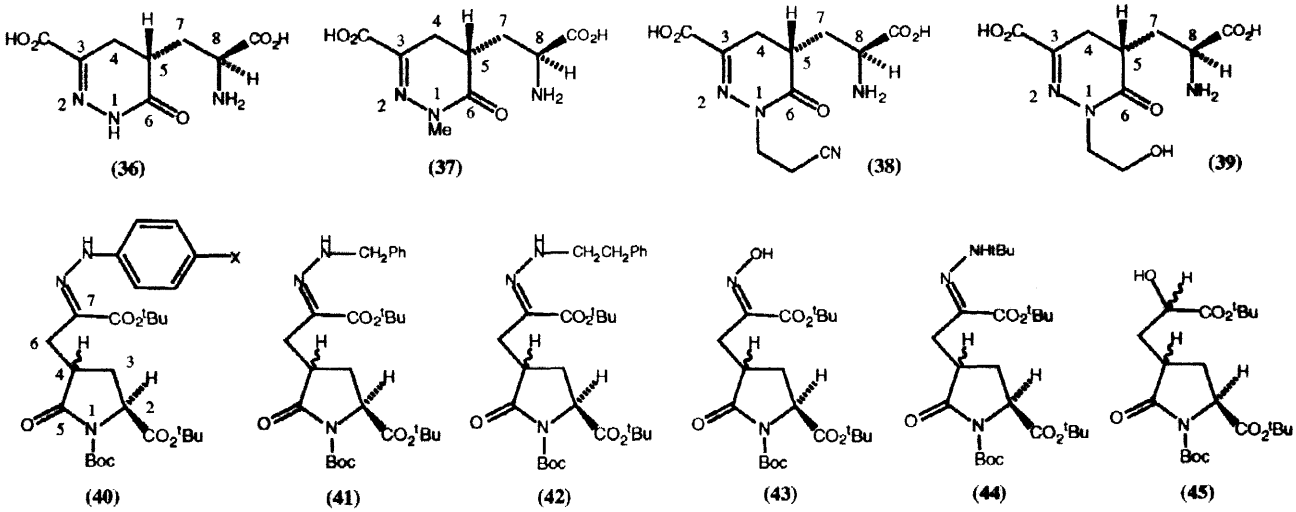


Eventually, we were able to achieve our goal by the method outlined in Scheme 5 below. This involved use of methyl bromomethylacrylate to obtain the *trans* : *cis* isomers (**32**, $R^1 = PhCH_2$, $R^2 = Me$) in a ratio of 8 : 1. The protection was chosen so that intermediates might be selectively deprotected for attachment to Wang resin to access libraries by solid phase combinatorial synthesis. The diastereoisomers were separated and ozonised to yield the *trans*-compound (**33a**, $R^1 = PhCH_2$, $R^2 = Me$). Reaction with hydrazine then led to a successful "ring switching" reaction yielding a 12 : 1 mixture of the carbinolamine (**34**, $R^1 = PhCH_2$, $R^2 = Me$) and the pyrazine (**35**, $R^1 = PhCH_2$, $R^2 = Me$). The carbinolamine was converted to the pyridazine in an acid catalysed process but some epimerisation occurred at the γ-carbon atom.

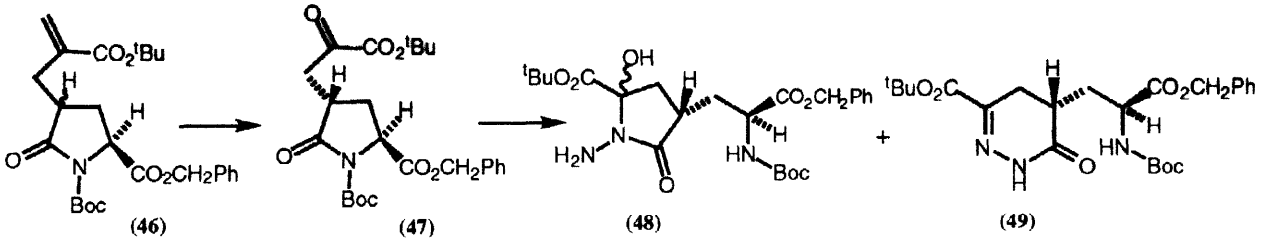


Scheme 5

tert-Butyl 2-bromomethylacrylate was prepared by a modification of the method of Haynes[10] and this was used in a similar reaction sequence to that described in Scheme 5 above to prepare (**33**, $R^1 = R^2 = {}^tBu$). Reaction with hydrazine gave a 9 : 1 ratio of the carbinolamine (**34**, $R^1 = R^2 = {}^tBu$) to the pyrazoline (**35**, $R^1 = R^2 = {}^tBu$). Dehydration of the carbinolamine using acetic acid and 3 Å molecular sieves gave the pyridazine (**35**, $R^1 = R^2 = {}^tBu$) with epimerisation at C-5 which was deprotected to the free amino acid inhibitor (**36**) and its epimer. A library of (**37**), (**38**) and (**39**) was also prepared by using the appropriate hydrazines in these reactions. The "ring switching" reaction could not be achieved in several instances and the aromatic hydrazones (**40**, X = H) and (**40**, X= OMe), (**41**), (**42**) were obtained but could not be reacted further. The aliphatic hydrazone (**44**), the oxime (**43**) and the alcohol (**45**) were also prepared but again could not be converted into the desired heterocyclic compounds.



In an attempt to investigate solid phase combinatorial chemistry, the benzyl ester (**47**) was prepared and reaction with hydrazine gave a 9 : 1 mixture of the carbinolamine (**48**) and the pyridazine (**49**). A variety of dehydrating conditions were applied to the carbinolamine (**48**), converting it to the pyridazine (**49**) in varying yields and with varying degrees of epimerisation at the γ-carbon atom as shown in Table 1 below. It was noticeable that the presence of a conjugating ester function improved the yield of the dehydrative rearrangement into the pyridazine (**49**).

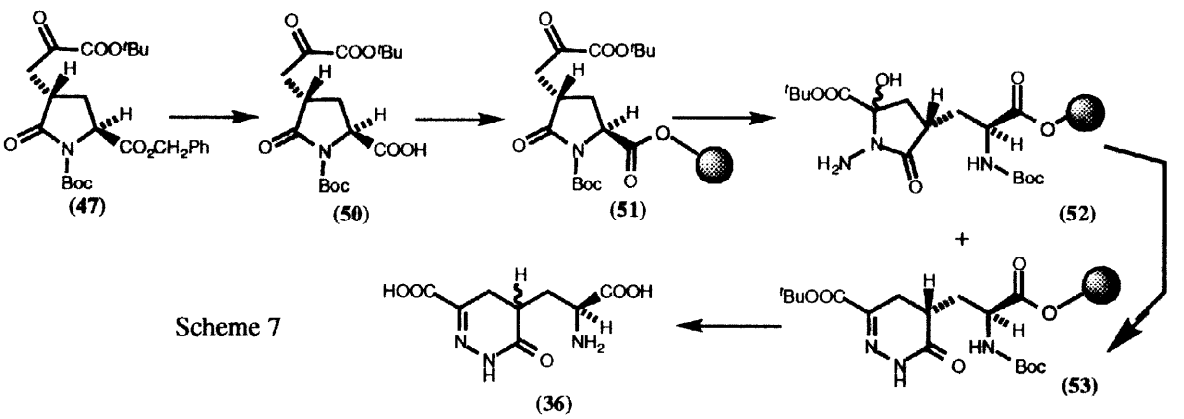


Scheme 6

Table 1 : Conversion of the carbinolamine (48) to the pyridazine (49)				
reagent	3Å molecular sieves/ AcOH. CH_3CN at reflux	3Å molecular sieves/ TsOH. CH_3CN at reflux	3Å molecular sieves/ 2,4,6-trimethylbenzenesulfonic acid. CH_3CN at reflux	$HC(OMe)_3$ 60 °C
yield	85%	67%	65%	97%
retention of stereochemistry	75%	86%	92%	60%

Use of deuteriated carbinolamine indicated that epimerisation was accompanied by deuteriation at the γ-carbon atom in these reactions from the absorption in the ^2H NMR spectrum at δ 2.3 ppm.

For solid phase combinatorial chemistry, hydrogenolysis of the ester (**47**) gave the acid (**50**) which was attached to Wang resin using EDCI, DMAP and dichloromethane. Reaction of the resin bound compound with hydrazine hydrate in methanol gave a resin bound mixture of the carbinolamine (**53**) and the pyridazine (**52**) as determined by MALDI-tof mass spectrometry in the presence of TFA. This also indicated that the carbinolamine could be converted to the pyrazine on resin, using *para*-toluenesulfonic acid in acetonitrile at 80 °C. Deprotection and removal from the resin was achieved using trifluoroacetic acid in dichloromethane giving the target potential inhibitor (**36**) and thus indicated that our versatile methodology is applicable to solid phase combinatorial methods.



Scheme 7

Experimental

M ps were determined on a Kofler hot-stage and are uncorrected. Optical rotations (in units of 10^{-1} deg cm^2 g^{-1}) were measured on a Perkin-Elmer PE241 polarimeter using a 1 dm pathlength cell. IR spectra were recorded on a Perkin-Elmer 1720 Fourier-transform (FT) instrument. Microanalyses were carried out by Medac Ltd. Low resolution mass spectra were recorded by Dr. A. Abdul Sada on a Kratos MS-80RF and MS25 double focusing spectrometers and MALDI-tof spectra by Mr C. R. Kowalczyk on a Micromass TofSpec E using a few beads of resin in the presence of TFA. Accurate mass measurements were obtained from the EPSRC Central Mass Spectrometry Service, Swansea. UV spectra were recorded on a ATI Unicam UV2-100 FT scanning spectrophotometer and ^1H-NMR and COSY spectra on a Bruker DPX 300 FT instrument (300 MHz). NOE and ^{15}N spectra (150.7 MHz, INEPT) were recorded by Dr. A. G. Avent on a Bruker AMX 500 FT instrument and ^{13}C-NMR spectra (^1H decoupled) on a Bruker DPX 300 (75.48 MHz) FT instrument. DEPT experiments were used to help assign ^{13}C resonances where necessary. ^2H-NMR spectra were recorded on a Bruker AC-P 250 (38.4 MHz) FT instrument by Mr. C. M. Dadswell. J values are given in Hz. Tlc was carried out on Merck Kieselgel 60 F254 precoated silica gel plates thickness 0.25 mm (ART 5719). Column chromatography was performed using Merck Kieselgel 60 (230-400 mesh-ART 9385), Sorbsil C60 40/60 A and Fluka Silica Gel 60 (220-440 mesh). Petroleum ether refers throughout to a fraction of alkanes, bp 60 - 80 °C.

***tert*-Butyl (*2S, 4S*)-N-*tert*-butoxycarbonyl-4-(prop-2-enyl)-pyroglutamate (16a)** and ***tert*-Butyl (*2S, 4R*)-N-*tert*-butoxycarbonyl-4-(prop-2-enyl)-pyroglutamate (16b).**

Lithium hexamethyldisilazide (1 M in THF, 50.9 ml, 50.9 mmol) was added to a stirred solution of *tert*-butyl (2S)-N-*tert*-butoxycarbonylpyroglutamate (**15**)[11] (13.2 g, 46.26 mmol) in THF (100 ml) at -78 °C under nitrogen. The mixture was stirred at -78 °C for 1h and allyl bromide (4.8 ml, 55.51 mmol) was added. Stirring was continued for 2h at -78 °C and saturated aqueous NH_4Cl (120 ml) was added. The mixture was extracted with diethyl ether (3x100 ml). The organic phases were dried (Na_2SO_4) and the solvents were removed *in vacuo*. The product was isolated as a brown oil. Purification and separation of the two stereoisomers was achieved by column chromatography using petroleum ether / ethyl acetate (4:1) as eluent. *tert-Butyl (2S, 4S)-N-tert-butoxycarbonyl-4-(prop-2-enyl)-pyroglutamate* (**16a**) (6.573 g, 44%) was obtained as white crystals, m.p. 64-66 °C; $[\alpha]_D^{19.5}$ -35.5 (*c* 0.76, CH_2Cl_2); Found: C, 62.4; H, 8.4; N, 4.5. $C_{17}H_{27}NO_5$ requires C, 62.7; H, 8.4; N, 4.3%; *m/z* [EI]: 325 $[M]^+$; ν_{max} (KBr) / cm^{-1} 1781, 1745 and 1709 (C=O) and 1646 (C=C); δ_H (C^2HCl_3) 5.72-5.69 (1H, *m*, H-7), 5.03 (1H, *d*, J_{trans} 17, H-8), 5.01 (1H, *d*, J_{cis} 10, H-8), 4.36 (1H, *dd*, $J_{2,3R}$ 1.4, $J_{2,3S}$ 9.4, H-2), 2.67-2.53 (2H, *m*, H-4 and H-6A), 2.15-2.03 (2H, *m*, H-6B and H-3R), 1.92 (1H, *ddd*, $J_{3S,2}$ 9.4, $J_{3S,4}$ 11.3, $J_{3S,3R}$ 13.2, H-3S) and 1.44 and 1.41 (2x9H, 2x*s*, $2xC(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 172.76 (lactam)), 168.51 (ester), 147.56 (urethane) 132.61 (C-7), 115.84 (C-8), 81.45 and 80.43 (2x $O\underline{C}(CH_3)_3$), 55.92 (C-2), 39.26 (C-4), 32.66 (C-6), 26.07 (C-3) and 26.01 ($OC(\underline{C}H_3)_3$). Irradiation of H-2 at δ 4.36 led to an nOe in H-3S at δ 1.92 which on irradiation gave nOe in the H-6 protons at δ 2.55 and 2.11. *tert-Butyl (2S, 4R)-N-tert-butoxycarbonyl-4-(prop-2-enyl)-pyroglutamate* (**16b**) (4.432 g, 29.5%) was obtained as a colourless oil; $[\alpha]_D^{21.5}$ -12.8 (*c* 3.13, CH_2Cl_2); Found: C, 62.5; H, 8.4; N, 4.6. $C_{17}H_{27}NO_5$ requires C, 62.7; H, 8.4; N, 4.3%; *m/z* [EI]: 325 $[M]^+$; ν_{max} (Nujol) / cm^{-1} 3080 (C=C), 1792, 1745 and 1724 (C=O) and 1641 (C=C); δ_H (C^2HCl_3) 5.70-5.62 (1H, *m*, H-7), 5.05-4.98 (2H, *m*, H-8), 4.36 (1H, *dd*, $J_{2,3R}$ 5.6, $J_{2,3S}$ 9.3, H-2), 2.62-2.53 (2H, *m*, H-4 and H-6A), 2.40 (1H, *ddd*, J 4.2, $J_{2,3S}$ 9.3, $J_{3S,3R}$ 13.4, H-3S), 2.20-2.15 (1H, *m*, H-6B), 1.71-1.63 (1H, *m*, H-3R) and 1.46 and 1.44 (2x9H, 2x*s*, 2x $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 175.32 (C=O (lactam)), 170.99 (ester), 149.84 (urethane), 135.24 (C-7), 118.03 (C-8), 83.82 and 82.55 (2x $O\underline{C}(CH_3)_3$), 58.49 (C-2), 42.49 (C-4), 35.81 (C-6), 28.31 ($2xOC(\underline{C}H_3)_3$) and 26.70 (C-3). Irradiation of H-2 at δ 4.35 gave an nOe in H-3S at δ 2.39 whereas irradiation of H-3R at δ 1.68 gave an nOe in H-6 at δ 2.57 and δ 2.17.

***tert*-Butyl (*3S, 5RS, 7S*)-[(2-oxo-5-hydroxymethyltetrahydrofuran-3-yl)methyl]-N-*tert*-butoxycarbonylglycinate (18a).**

tert-Butyl (*2S, 4S*)-N-*tert*-butoxycarbonyl-4-(prop-2-enyl)-pyroglutamate (**16a**) (566 mg, 1.74 mmol) was dissolved in *t*-BuOH (20 ml) and H_2O (20 ml) with stirring. $K_3Fe(CN)_6$ (5.690 g; 17.28 mmol) and K_2CO_3 (2.387 g; 17.27 mmol) were added. After stirring at room temperature for 10 min, OsO_4 (2.5 wt.% in *t*-BuOH) (1.1 ml; 0.087 mmol) was added and after 18 h at room temperature, sodium sulfite was added until the yellow colour disappeared. The mixture was extracted with ether (3x50 ml). The organic extracts were dried ($MgSO_4$) and the solvent was removed *in vacuo*. *tert-Butyl (3S, 5RS, 7S)-[(2-oxo-5-hydroxymethyl-tetrahydro furan-3-yl)methyl]-N-tert-butoxycarbonylglycinate* (**18a**) was isolated as an oil, which became a white solid after 5 h at 40 °C *in vacuo* (599 mg, 96%); m.p. 42-45 °C; *m/z* [CI]: Found: 360.2022 $[M+H]^+$, $C_{17}H_{30}NO_7$ requires 360.2021; *m/z* [+ve FAB (3-NBA)] 382 $[M+Na]^+$, 360 ($[M+H]^+$); ν_{max} (KBr) / cm^{-1} 3386 (N-H and O-H), 1768 and 1714 (C=O); δ_H (C^2HCl_3) 5.24 (1H, *d*, *J* 8.0, -NH), 4.61-4.58 (1/2H, *m*, H-5), 4.53-4.48 (1/2H, *m*, H-5), 4.27-4.26 (1H, *m*, H-7), 3.87 (1H, *dddd*, J_1 2.1, J_2 12.7, J_3 2.9, J_4 12.2, $HOC\underline{H}_2$), 3.67-3.62 (1H, *m*, $HOC\underline{H}_2$), 2.98-2.91 (1/2H, *m*, H-3), 2.87-2.80 (1/2H, *m*, H-3), 2.56 (1H, *br.s*, -OH), 2.42-2.34 (2H, *m*, H-4), 1.99-1.78 (2H, *m*, H-6) and 1.47 and 1.44 (2x9H, 2x*s*, 2x $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 178.56 (lactone), 171.46 (ester), 156.01 (urethane), 83.01 (2x $O\underline{C}(CH_3)_3$), 79.45 and 78.85 (C-5), 64.92 and 63.84 ($HOCH_2$), 52.81 (C-7), 34.51 and 33.86 (C-4), 31.61 (C-3), 29.80 (C-6), 28.80 and 28.72 (2x $OC(\underline{C}H_3)_3$).

***tert*-Butyl (*2S, 4S*)-4-(prop-2-enyl)-pyroglutamate (19a).**

tert-Butyl (*2S, 4S*)-N-*tert*-butoxycarbonyl-4-(prop-2-enyl)-pyroglutamate (**16a**) (3.440g, 10.57 mmol) was dissolved in 1 M HCl in EtOAc (150 ml, prepared by passing dry HCl into dry EtOAc and diluting, after titration, to 1 M with additional EtOAc). The mixture was stirred at room temperature until loss of starting material was

shown by tlc (23 h). The solvent was removed in the cold to yield *tert-butyl (2S, 4S)-4-(prop-2-enyl)-pyroglutamate* **(19a)** as a yellow oil (2.381 g, 100%); $[\alpha]_D^{24}$ +4.7 (*c* 1.00, CH_2Cl_2); *m/z* [+ve FAB (PEGH/NOBA)]: Found: 226.145065 $[M+H]^+$, $C_{12}H_{20}NO_3$ requires 226.144319; *m/z* [+ve FAB (3-NBA)] 226 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3371 (N-H), 1735 and 1707 (C=O); δ_H ($C^2H_3O^2H$) 5.73-5.64 (1H, *m*, H-7), 5.05-4.96 (2H, *m*, H-8), 3.97 (1H, *dd*, $J_{2,3A}$ 2.6, $J_{2,3B}$ 8.8, H-2), 2.47-2.37 (2H, *m*, H-4 and H-6), 2.17-2.02 (3H, *m*, H-6 and H-3) and 1.38 (9H, *s*, $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 179.69 (lactam), 171.65 (ester), 135.30 (C-7), 117.71 (C-8), 82.60 ($O\underline{C}(CH_3)_3$), 54.80 (C-2), 39.69 (C-4), 35.28 (C-6), 30.77 (C-3) and 27.28 ($OC(\underline{C}H_3)_3$).

***tert*-Butyl (*2S, 4S, 7RS*)-4-(2,3-dihydroxypropyl)-pyroglutamate (20a).**
tert-Butyl (*2S, 4S*)-4-(prop-2-enyl)-pyroglutamate **(19a)** (5.328 g, 23.65 mmol) was dissolved in 1:1 *t*-BuOH/ water (150 ml) under nitrogen at room temperature. To this solution $K_3Fe(CN)_6$ (77.87 g, 0.237 mmol), K_2CO_3 (32.686 g, 0.237 mmol) and OsO_4 were added as a solution in *t*-BuOH (2,5 wt%, 14.8 ml, 300.6 mg OsO_4, 1.18 mmol). After stirring 15h at room temperature, sodium sulfite was added until the colour changed from orange to brown. The mixture was extracted with diethyl ether (3x300 ml). The combined organic extracts were dried ($MgSO_4$) and the solvent was removed *in vacuo* to yield *tert-butyl (2S, 4S, 7RS)-4-(2,3-dihydroxypropyl)-pyroglutamate* **(20a)** as a yellow oil (5.30 g, 87%); *m/z* [CI]: Found: 260.1498 $[M+H]^+$, $C_{12}H_{22}NO_5$ requires 260.1497; *m/z* [+ve FAB (3-NBA)] 282 $[M+Na]^+$, 260 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3371 (NH), 1735 and 1686 (C=O); δ_H (C^2HCl_3) 6.57 (1/2H, *d*, *J* 9.3, NH), 6.45 (1/2H, *d*, *J* 9.1, NH), 4.03 (1H, *d*, *J* 9.0, H-2), 3.89-3.85 (1/2H, *m*, H-7), 3.78-3.75 (1/2H, *m*, H-7), 3.55 (1H, *dd*, $J_{8A,7}$ 3.3, $J_{8A,8B}$ 11.1, H-8A), 3.42 (1H, *dd*, $J_{8B,7}$ 6.3, $J_{8A,8B}$ 11.1, H-8B), 2.72-2.59 (1H, *m*, H-4), 2.45-2.34 (1H, *m*, H-3A), 2.17-1.98 (1H, *m*, H-3B), 1.80-1.49 (2H, *m*, H-6) and 1.41 (9H, *s*, $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 181.78 and 180.56 (lactam)), 171.51 and 180.84 (ester), 83.04 and 82.89 ($O\underline{C}(CH_3)_3$), 71.59 and 70.60 (C-7), 67.22 and 66.70 (C-8), 55.44 and 55.16 (C-2), 39.10 and 37.30 (C-4), 35.16 and 34.86 (C-6), 32.79 and 32.67 (C-3) and 28.36 ($OC(\underline{C}H_3)_3$).

***t*-Butyl (*2S,4S,7RS*)-4-(2-hydroxy-3-O-*tert*-butyldiphenylsilyloxypropyl)-pyroglutamate (21a)**
tert-Butyl (*2S, 4S, 7RS*)-4-(2,3-dihydroxypropyl)-pyroglutamate **(20a)** (350 mg, 1.35 mmol) was dissolved in CH_2Cl_2 (25 ml) under nitrogen. The solution was cooled to 0 °C and 4-dimethylaminopyridine (33 mg, 0.27 mmol), triethylamine (0.47 ml, 3.37 mmol) and further CH_2Cl_2 (5 ml) were added. After stirring for 15 min. at 0 °C, *tert*-butylchlorodiphenylsilane (0.42 ml, 1.62 mmol) was added and the solution was stirred at room temperature for 44 h. 0.05 N Aqueous HCl (6 ml) was added and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (3x10 ml). The organic layers were combined, washed with water (15 ml) and dried (Na_2SO_4). The solvent was removed *in vacuo* to afford a brown oil, which was purified by column chromatography on silica gel using petroleum ether / ethyl acetate (1:1) as eluent, to yield the *product* **(21a)** as a yellow oil (350 mg, 63%); *m/z* [+ve FAB (PEGH/NOBA)]: Found: 498.266144 $[M+H]^+$, $C_{28}H_{40}NO_5Si$ requires 498.267577; *m/z* [+ve FAB (3-NBA)] 536 $[M+K]^+$, 520 $[M+Na]^+$, 498 ($[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3252 (NH), 1736 and 1701 (C=O) and 1590 (C=C); δ_H (C^2HCl_3) 7.59-7.55 (4H, *m*, ArH), 7.38-7.22 (6H, *m*, ArH), 6.31 (1/2H, *s*, NH), 6.17 (1/2H, *s*, NH), 4.03-3.95 (1H, *m*, H-2), 3.87-3.80 (1/2H, *m*, H-7), 3.73-3.67 (1/2H, *m*, H-7), 3.59-3.43 (2H, *m*, H-8), 3.18 (1H, *br.s*, -OH), 2.65-2.52 (1H, *m*, H-4), 2.39 (1H, *ddd*, $J_{3A,2}$ 2.4, $J_{3A,4}$ 8.8, $J_{3A,3B}$ 12.2, H-3A), 2.17-1.96 (1H, *m*, H-3B), 1.83-1.48 (2H, *m*, H-6), 1.40 (9/2H, *s*, $OC(C\underline{H}_3)_3$), 1.38 (9/2H, *s*, $OC(C\underline{H}_3)_3$), 0.98 (9/2H, *s*, $SiC(C\underline{H}_3)_3$) and 0.97(9/2H, *s*, $Si\ C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 180.24 and 179.78 (lactam)), 170.73 and 170.57 (ester), 135.09-125.39 (Ar), 82.09 and 81.93 ($O\underline{C}(CH_3)_3$), 70.35 and 70.14 (C-7), 67.45 and 66.93 (C-8), 54.32 and 54.18 (C-2), 37.59 and 36.67 (C-4), 34.24 and 33.42 (C-6), 32.00 and 31.66 (C-3), 27.50 and 26.41 ($OC(\underline{C}H_3)_3$), 26.37 and 26.19 ($SiC(\underline{C}H_3)_3$) and 18.79 ($Si\underline{C}(CH_3)_3$).

***tert*-Butyl (*2S, 4S*)-4-(2-oxo-3-O-*tert*-butyldiphenylsilyloxypropyl)-pyroglutamate (22a).**
Solid tetrapropylammonium perruthenate (TPAP, 12.7 mg, 0.036 mmol) was added in one portion to a stirred mixture of *tert*-butyl (*2S, 4S, 7RS*)-4-(2-hydroxy-3-O-*tert*-butyldiphenylsilyloxypropyl)-pyroglutamate **(21a)** (360 mg, 0.72 mmol), 4-methylmorpholine-N-oxide (127 mg, 1.08mmol) and powdered 4Å molecular sieves (360 mg) in CH_2Cl_2 (3.5 ml) at room temperature under nitrogen. After 3h the reaction mixture was filtered through a short path of silica, eluting with CH_2Cl_2 and ethyl acetate. The filtrate was evaporated to yield an oily

residue, which was purified by column chromatography on silica gel using petroleum ether / ethyl acetate (3:2) as eluent to give *the ketone* **(22a)** as a clear, yellow oil (251 mg, 70%); $[\alpha]_D$ - 9.8 (*c* 1.00, CH_2Cl_2); *m/z* [+ve FAB (PEGH/NOBA)]: Found: 496.249947 $[M+H]^+$, $C_{28}H_{38}NO_5Si$ requires 496.251927; *m/z* [+ve FAB (3-NBA)] 518 $[M+Na]^+$, 496 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3233 (NH), 1737 and 1708 (C=O) and 1590 (C=C); δ_H (C^2HCl_3) 7.58-7.55 (4H, *m*, ArH), 7.40-7.29 (6H, *m*, ArH), 6.17 (1H, *br.s*, NH), 4.13 (2H, *s*, H-8), 3.98 (1H, *dd*, $J_{2,3A}$ 2.4, $J_{2,3B}$ 9.3, H-2), 3.03 (1H, *dd*, $J_{6B,4}$ 3.2, $J_{6A,6B}$ 18.4, H-6), 2.77 (1H, *ddddd*, $J_{4,6A}$ 3.2, $J_{4,6B}$ $J_{4,3A}$ $J_{4,3B}$ $J_{4,6B}$ 9.1, H-4), 2.58 (1H, *dd*, $J_{6B,4}$ 9.2, $J_{6A,6B}$ 18.4, H-6B), 2.43 (1H, *ddd*, $J_{3A,2}$ 2.4, $J_{3A,4}$ 9.1 $J_{3A,3B}$ 13.2, H-3A), 1.90 (1H, *ddd*, $J_{3B,4}$ 9.1, $J_{3B,2}$ 9.3, $J_{3B,3A}$ 13.2, H-3B), 1.40 (9H, *s*, $OC(C\underline{H}_3)_3$) and 1.02 (9H, *s*, $SiC(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 207.45 (ketone), 178.16 (lactam)), 170.56 (ester), 134.84-127.23 (Ar), 81.74 ($O\underline{C}(CH_3)_3$), 69.06 (C-8), 53.71 (C-2), 39.07 (C-6), 34.19 (C-4), 30.80 (C-3), 27.29 ($OC(\underline{C}H_3)_3$), 26.08 ($SiC(\underline{C}H_3)_3$) and 18.53 ($Si\underline{C}(CH_3)_3$).

***tert*-Butyl (*2S, 4S*)-N-*tert*-butoxycarbonyl-4-(2-oxo-3-O-*tert*-butyldiphenylsilyloxypropyl)-pyroglutamate (23a).**

A mixture of *tert*-butyl (*2S, 4S*)-4-(2-oxo-3-O-*tert*-butyldiphenylsilyloxypropyl)-pyroglutamate **(22a)** (72 mg, 0.145 mmol), 4-dimethylaminopyridine (5 mg, 0.041 mmol) and triethylamine (22 mg, 0.22 mmol) in dioxane (2.5 ml) was heated under nitrogen to 85 °C. Di-*tert*-butyl dicarbonate ($(Boc)_2O$) (793 mg, 3.63 mmol) in dioxane (0.5 ml) was added dropwise over 25 minutes. At this time the reaction was complete as shown by tlc. and was allowed to cool to room temperature. The solvent was removed *in vacuo* to yield a brown oil which was purified by column chromatography on silica gel using petroleum ether / ethyl acetate (7:1) as eluent to afford the product **(23a)** as a yellow oil (67 mg, 77%); $[\alpha]_D^{31}$ -14.7 (*c* 1.00, CH_2Cl_2); *m/z* [+ve FAB (3-NBA)] 618 $[M+Na]^+$; ν_{max} (Nujol) / cm^{-1} 1794, 1741 and 1724 (C=O) and 1590 (C=C); δ_H (C^2HCl_3) 7.58-7.55 (4H, *m*, ArH), 7.40-7.29 (6H, *m*, ArH), 4.36 (1H, *dd*, $J_{2,3B}$ 1.0, $J_{2,3A}$ 9.0, H-2), 4.12 (2H, *s*, H-8), 3.12 (1H, *dd*, $J_{6A,4}$ 3.3, $J_{6A,6B}$ 18.8, H-6A), 2.94 (1H, *m*, $J_{4,6A}$ 3.3, $J_{4,3A}$ 6.2, $J_{4,6B}$, 8.8 $J_{4,3B}$ 11.8, H-4), 2.60 (1H, *dd*, $J_{6B,4}$ 8.8, $J_{6B,6A}$ 18.8, H-6B), 2.24 (1H, *dd*, $J_{3A,2}$ 9.0, $J_{3A,3B}$ 13.1, H-3A), 1.78 (1H, *ddd*, $J_{3B,2}$ 1.1, $J_{3B,4}$ 11.8, $J_{3B,3A}$ 13.1, H-3B), 1.44 and 1.42 (2x9H, 2x*s*, $2xOC(C\underline{H}_3)_3$) and 1.02 (9H, *s*, $SiC(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 206.79 (ketone), 173.59 (C=O (lactam)), 169.09 (ester), 148.24 (urethane), 134.45-126.86 (Ar), 82.31 and 81.32 (2x $O\underline{C}(CH_3)_3$), 68.63 (C-8), 56.89 (C-2), 38.40 (C-6), 35.92 (C-4), 27.61 (C-3), 26.87 and 26.85 ($2xOC(\underline{C}H_3)_3$), 25.68 ($SiC(\underline{C}H_3)_3$) and 18.11 ($Si\underline{C}(CH_3)_3$).

***tert*-Butyl (*3S,5RS,7S*)-[(1-amino-2-oxo-5-hydroxy-5-O-*tert*-butyldiphenylsilyloxymethyl pyrrolidin-3-yl)methyl]-N-*tert*-butoxycarbonyl-glycinate (24a)** and ***tert*-Butyl (*5S, 8S*)-[(3-O-*tert*-butyldiphenylsilyloxymethyl-4,5-dihydro-1*H*-6-oxopyridazine-5-yl)methyl]-N-*tert*-butoxycarbonyl-glycinate (25a).**

Hydrazine hydrate (N_2H_4: 64-65%) (19 mg, 0.24 mmol) was added to a solution of *tert*-butyl (*2S, 4S*)-N-*tert*-butoxycarbonyl-4-(2-oxo-3-O-*tert*-butyldiphenylsilyloxypropyl)-pyroglutamate **(23a)** (62 mg, 0.10 mmol) in methanol (1 ml) under nitrogen. After stirring at room temperature for 3h, the solvent was removed *in vacuo* to yield a white foam. Purification of the two products was achieved by flash column chromatography on silica gel using petroleum ether / ethyl acetate (1:1) as eluent. *tert-Butyl (3S,5RS,7S)-[(1-amino-2-oxo-5-hydroxy-5-O-tert-butyldiphenylsilyloxymethylpyrrolidin-3-yl) methyl]-N-tert-butoxycarbonyl-glycinate* **(24a)** was obtained as a white foam (40 mg, 61%); *m/z* [+ve FAB (PEGH/NOBA)]: Found: 610.335442 $[MH-H_2O]^+$, $C_{33}H_{48}N_3O_6Si$ requires 610.331240; *m/z* [+ve FAB (3-NBA)]: 651 $[M+Na]^+$, 628 $[M+H]^+$, 610 $[M-H_2O]^+$; ν_{max} (film) / cm^{-1} 3341 (OH and NH), and 1736 (C=O); δ_H (C^2HCl_3) 7.52-7.42 (4H, *m*, ArH), 7.27-7.16 (6H, *m*, ArH), 5.60 (1/2H, *d*, *J* 8.0, NHBoc), 5.7 (1/2H, *d*, *J* 9.0, NHBoc), 4.22-4.11 (1H, *m*, H-7), 3.69-3.40 (2H, *m*, $TBDPSOC\underline{H}_2$), 2.61-2.46 (1H, *m*, H-3), 2.18-1.90 (1H, *m*, H-6A), 1.85-1.36 (3H, *m*, H-4 and H-6B), 1.28, 1.25 (2x9H, 2x*s*, $2xOC(C\underline{H}_3)_3$) and 0.86 (9H, *s*, $SiC(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 176.99 and 175.62 (lactam)), 172.40 (ester), 157.21 (urethane), 136.67-128.96 (Ar), 90.39 ($O\underline{C}(CH_3)_3$), 83.18 and 83.17 ($2xOC(\underline{C}H_3)_3$), 81.48 (C-5), 61.52 ($TBDPSO\underline{C}H_2$), 52.73 (C-7), 37.63 (C-3), 34.50 (C-4), 29.50 and 29.09 ($2xOC(\underline{C}H_3)_3$), 27.97 ($SiC(\underline{C}H_3)_3$), 20.31 (C-6) and 18.23 ($Si\underline{C}(CH_3)_3$). *tert-Butyl (5S, 8S)-[(3-O-tert-butyldiphenylsilyloxymethyl-4,5-dihydro-1H-6-oxopyridazine-5-yl)methyl]-N-tert-butoxycarbonyl-glycinate* **(25a)** was obtained as a

colourless oil (10 mg, 16%); $[\alpha]_D^{25}$ -17.0 (*c* 0.80, CH_2Cl_2); *m/z* [+ve FAB (PEGH / NOBA)]: Found: 610.329312 $[M+H]^+$, $C_{33}H_{48}N_3O_6Si$ requires 610.331240 ; *m/z* [+ve FAB (3-NBA)] 632 $[M+Na]^+$, 610 $[M]^+$; ν_{max} (Nujol) / cm^{-1} 3342 (N-H), 3072 (=C-H) and 1718 (C=O); δ_H (C^2HCl_3) 8.17 (1H, *s*, =N-NH-), 7.59-7.31 (10H, *m*, ArH), 5.35 (1H, *d*, *J* 7.3, NHBoc), 4.21-4.17 (3H, *m*, H-8 and $TBDPSOCH_2$), 2.71 (1H, *dd*, J_1 6.1, J_2 16.0, H-5), 2.47-2.18 (3H, *m*, H-4 and H-7A), 1.83-1.77 (1H, *m*, H-7B), 1.39 and 1.36 (2x9H, 2x*s*, $2xOC(C\underline{H}_3)_3$) and 1.00 (9H, *s*, $SiC(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 171.61 (lactam)), 170.27 (ester), 155.86 (urethane), 154.85 (C=N), 135.98-128.28 (Ar), 82.54 ($2xO\underline{C}(CH_3)_3$), 66.28 ($TBDPSO\underline{C}H_2$), 52.70 (C-8), 33.12 (C-5), 32.29 (C-4), 28.73 and 28.43 ($2xOC(\underline{C}H_3)_3$), 27.61 (C-7), 27.23 ($SiC(\underline{C}H_3)_3$) and 19.66 ($Si\underline{C}(CH_3)_3$).

tert-Butyl (*5S, 8S*)-[(3-O-_tert_-butyldiphenylsilyloxymethyl-4,5-dihydro-1*H*-6-oxopyridazine-5-yl)methyl]-N-_tert_-butoxycarbonylglycinate (25a).

tert-Butyl (*3S, 5RS, 7S*)-[(1-amino-2-oxo-5-hydroxy-5-methyl-O-_tert_-butyldiphenylsilyloxymethylpyrrolidin-3-yl)methyl]-N-_tert_-butoxycarbonylglycinate (**24a**) (32 mg, 0.05 mmol) was dissolved in acetonitrile (1 ml). The solution was heated to reflux in the presence of 3Å molecular sieves (70 mg) and acetic acid (3 drops) for 2 days. The solvents were removed *in vacuo* and the resultant oil was purified by flash column chromatography on silica gel using petroleum ether / ethyl acetate (2:1) as eluent to yield the *pyridazine* (**25a**) as a clear, colourless oil (13 mg, 42 %). Spectra were identical with those of the sample above.

tert-Butyl (*2S, 4R*)-4-(prop-2-enyl)-pyroglutamate (19b).

tert-Butyl (*2S, 4R*)-N-_tert_-butoxycarbonyl-4-(prop-2-enyl)-pyroglutamate (**16b**) (500 mg, 1.54 mmol) was stirred at room temperature in a 1M solution of HCl in ethyl acetate (30 ml) under nitrogen. After 4h the reaction was complete. The solvent was removed *in vacuo* and the crude product was purified by column chromatography on silica gel using petroleum ether / ethyl acetate (1:1) as eluent to yield *tert-butyl (2S, 4R)-4-(prop-2-enyl)-pyroglutamate* (**19b**) as a clear, yellow oil (216 mg, 62%); $[\alpha]_D^{24}$ +17.7 (*c* 1.00, CH_2Cl_2); *m/z* [+ve FAB (PEGH/NOBA)]: Found: 226.143474 $[M+H]^+$, $C_{12}H_{20}NO_3$ requires 226.144319 ; *m/z* [+ve FAB (3-NBA)] 248 $[M+Na]^+$, 226 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3229 (NH), 1737 and 1707 (C=O); δ_H (C^2HCl_3) 6.46 (1H, *br.s*, -NH), 5.74-5.63 (1H, *m*, H-7), 5.05-4.98 (2H, *m*, H-8), 4.04-3.99 (1H, *m*, H-2), 2.56-2.44 (3H, *m*, H-4, H-6A and H-3B), 2.12-2.07 (1H, *m*, H-6B), 1.78-1.75 (1H, *m*, H-3A) and 1.41 (9H, *s*, $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 178.85 (lactam)), 171.30 (ester), 135.60 (C-7), 117.55 (C-8), 82.78 ($O\underline{C}(CH_3)_3$), 54.74 (C-2), 41.03 (C-4), 35.42 (C-6), 30.94 (C-3) and 28.37 ($OC(\underline{C}H_3)_3$).

tert-Butyl (*2S, 4R, 7RS*)-4-(2,3-dihydroxypropyl)-pyroglutamate (20b).

A mixture of *tert*-butyl (*2S, 4R*)-4-(prop-2-enyl)-pyroglutamate (**19b**) (1.202 g, 5.34 mmol), $K_3Fe(CN)_6$ (10.540 g, 32.01 mmol), K_2CO_3 (4.424 g, 32.01 mmol) and OsO_4 as a solution in *t*-BuOH (2.5 wt%, 1.5 ml, 30.4 mg, 0.12 mmol OsO_4) in 1:1 *t*-BuOH / H_2O (40 ml) was stirred at room temperature for 24 h. The reaction was quenched with sodium sulfite until the orange colour disappeared. The solvents were removed *in vacuo* and the resulting solid was extracted with diethyl ether using a soxhlet apparatus for 48 h. The solvent was removed *in vacuo* to yield *tert-butyl (2S, 4R, 7RS)-4-(2,3-dihydroxypropyl)-pyroglutamate* (**20b**) as a mixture of enantiomers as a colourless oil (1.230 g, 89%), which crystallised on standing; m.p. 63-67 °C; *m/z* [CI]: Found 260.1498 $[M+H]^+$, $C_{12}H_{22}NO_5$ requires 260.1497; *m/z* [+ve FAB (3-NBA)] 282 $[M+Na]^+$, 260 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3346 (NH and OH), 1737 and 1696 (C=O); δ_H (C^2HCl_3) 6.44 (1/2H, *br.s*, NH), 6.34 (1/2H, *br.s*, NH), 4.12-4.03 (1H, *m*, H-2), 3.89-3.86 (1/2 H, *m*, H-7), 3.77-3.74 (1/2 H, *m*, H-7), 3.59-3.53 (1H, *m*, H-8A), 3.49-3.39 (1H, *m*, H-8B), 2.70-2.56 (2H, *m*, H-4 and H-3A), 1.84-1.69 (2H, *m*, H-3B and H-6A), 1.62-1.51 (1H, *m*, H-6B) and 1.41 (9H, *s*, $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 180.71 and 180.33 (lactam), 171.322 and 170.89 (ester), 83.20 and 83.05 ($O\underline{C}(CH_3)_3$), 71.45 and 70.59 (C-7), 67.26 and 66.74 (C-8), 55.42 and 55.21 (C-2), 40.65 and 38.64 (C-4), 35.07 and 34.70 (C-6), 33.15 and 32.99 (C-3) and 28.37 and 28.18 ($OC(\underline{C}H_3)_3$).

_t_-Butyl (*2S,4R,7RS*)-4-(2-hydroxy-3-O-_t_-butyldiphenylsilyloxypropyl)-pyroglutamate (21b).

tert-Butyl (*2S, 4R, 7RS*)-4-(2,3-dihydroxypropyl)-pyroglutamate (**20b**) (63 mg, 0.24 mmol) was dissolved in CH_2Cl_2 (1 ml) with stirring under nitrogen. The solution was cooled to 0 °C and 4-dimethylaminopyridine

(DMAP, 3 mg, 0.024 mmol) and triethylamine (61.5 mg, 0.61 mmol) were added. After 15 min *tert*-butylchlorodiphenylsilane (TBDPSCl, 100 mg, 0.36 mmol) was added at 0 °C and the reaction mixture was stirred for 19 h at room temperature. The white precipitate was filtered off and the solvent was removed *in vacuo*. Purification of the crude product by column chromatography on silica gel using petroleum ether / ethyl acetate (2:3) as eluent afforded *tert-butyl (2S, 4R, 7RS)-4-(2-hydroxy-3-O-tert-butyl diphenylsilyloxypropyl)-pyroglutamate* **(21b)** as a colourless oil (91 mg, 75%) *m/z* [+ve FAB (PEGH/NOBA)]: Found: 498.266242 $[M+H]^+$, $C_{28}H_{40}NO_5Si$ requires 498.267577; *m/z* [+ve FAB (3-NBA)] 520 $[M+Na]^+$, 498 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3244 (NH and OH), 3071 and 3050 (=C-H), 1740 and 1697 (C=O) and 1590 (C=C); δ_H (C^2HCl_3) 7.59-7.57 (4H, *m*, ArH), 7.39-7.28 (6H, *m*, ArH), 6.26 (1/2 H, *s*, NH), 6.17 (1/2 H, *s*, NH), 4.09-3.98 (1H, *m*, H-2), 3.89-3.86 (1/2H, *m*, H-7), 3.72-3.65 (1/2H, *m*, H-7), 3.59-3.45 (2H, *m*, H-8), 2.68-2.51 (2H, *m*, H-4 and H-3A), 1.89-1.68 (2H, *m*, H-6A and H-3B), 1.64-1.47 (1H, *m*, H-6B), 1.41 (9/2H, *s*, $OC(C\underline{H}_3)_3$), 1.40 (9/2H, *s*, $OC(C\underline{H}_3)_3$), 0.99 (9/2H, *s*, $SiC(C\underline{H}_3)_3$) and 0.97 (9/2H, *s*, $SiC(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 179.71 (lactam), 170.77 and 170.54 (ester), 135.71-126.01 (Ar), 82.78 and 82.67 ($O\underline{C}(CH_3)_3$), 70.95 and 70.80 (C-7), 68.09 and 67.45 (C-8), 54.89 and 54.80 (C-2), 39.98 and 38.90 (C-4), 34.73 and 34.05 (C-6), 33.22 and 32.67 (C-3), 28.49 and 28.13 ($OC(\underline{C}H_3)_3$), 27.04 ($SiC(\underline{C}H_3)_3$) and 19.42 ($Si\underline{C}(CH_3)_3$).

***tert*-Butyl (*2S, 4R*)-4-(2-oxo-3-O-*tert*-butyldiphenylsilyloxypropyl)-pyroglutamate (22b).**
Solid tetrapropylammonium perruthenate(VII) (TPAP, 47 mg, 0.13 mmol) was added in one portion to a stirred mixture of *tert*-butyl (*2S, 4R, 7RS*)-4-(2-hydroxy-3-O-*tert*-butyldiphenylsilyloxypropyl)-pyroglutamate **(21b)** (1.326 g, 2.66 mmol), 4-methylmorpholine-N-oxide (468 mg, 4.00 mmol) and powdered molecular sieves (4 Å, 1.33 g) in CH_2Cl_2 (15 ml) at room temperature under nitrogen. The reaction mixture was stirred for 2 h, filtered through a short path of silica and purified by column chromatography on silica gel using petroleum ether / ethyl acetate (1:1) as eluent to afford *tert-butyl (2S, 4R)-4-(2-oxo-3-O-tert-butyldiphenylsilyloxypropyl)-pyroglutamate* **(22b)** as a yellow oil (1.255 g, 95%); $[\alpha]_D^{32}$ +9.4 (*c* 1.00, CH_2Cl_2); *m/z* [CI]: Found: 496.2519 $[M+H]^+$, $C_{28}H_{38}NO_5Si$ requires 496.2518; *m/z* [+ve FAB (3-NBA)] 518 $[M+Na]^+$, 496 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3212 (NH), 3072 and 3049 (=C-H), 1737 and 1718 (C=O) and 1590 (C=C); δ_H (C^2HCl_3) 7.58-7.55 (4H, *m*, ArH), 7.40-7.29 (6H, *m*, ArH), 6.20 (1H, *br.s*, NH), 4.13 (2H, *s*, H-8), 4.04 (1H, *t*, *J* 8.0, H-2), 3.11 (1H, *dd*, $J_{6A,4}$ 2.7, $J_{6A,6B}$ 18.7, H-6A), 2.82 (1H, *dq*, $J_{4,6A}$ 2.7, $J_{4,6B}$ $J_{4,3A}$ $J_{4,3B}$ 9.1, H-4), 2.69 (1H, *dd*, $J_{3A,2}$ 8.0, $J_{3A,3B}$ 12.7, H-3A), 2.53 (1H, *dd*, $J_{6B,4}$ 9.1, $J_{6B,6A}$ 18.7, H-6B), 1.51 (1H, *ddd*, $J_{2,3B}$ 8.0, $J_{3B,4}$ 9.1, $J_{3A,3B}$ 12.7, H-3B), 1.40 (9H, *s*, $OC(C\underline{H}_3)_3$) and 1.02 (9H, *s*, $SiC(C\underline{H}_3)_3$; δ_C (C^2HCl_3) 208.18 (ketone), 177.78 (lactam), 170.52 (ester), 135.50-127.85 (Ar), 82.47 ($O\underline{C}(CH_3)_3$), 69.67 (C-8), 54.33 (C-2), 40.06 (C-6), 36.41 (C-4), 32.20 (C-3), 27.92 ($OC(\underline{C}H_3)_3$), 26.71 ($SiC(\underline{C}H_3)_3$) and 19.17 ($Si\underline{C}(CH_3)_3$).

***tert*-Butyl (*2S, 4R*)-N-*tert*-butoxycarbonyl-4-(2-oxo-3-O-*tert*-butyldiphenylsilyloxypropyl)-pyroglutamate (23b).**
A mixture of *tert*-butyl (*2S, 4R*)-4-(2-oxo-3-O-*tert*-butyldiphenylsilyloxypropyl)-pyroglutamate **(22b)** (1.255 g, 2.53 mmol), 4-dimethylaminopyridine (DMAP) (62 mg, 0.51 mmol) and triethylamine (384 mg, 3.80 mmol) in dioxane (40 ml) was heated under nitrogen to 85 °C. Di-*tert*-butyl dicarbonate (5.526 g, 25.32 mmol) in dioxane (5 ml) was added dropwise over a period of 45 min. At this time the reaction was complete as shown by tlc. The reaction mixture was allowed to cool to room temperature. The solvent was removed *in vacuo* to yield a brown oil, which was purified by column chromatography on silica gel using petroleum ether / ethyl acetate (5:2) as eluent to afford *tert-butyl (2S, 4R)-N-tert-butoxycarbonyl-4-(2-oxo-3-O-tert-butyldiphenylsilyl-propyl)-pyroglutamate* **(23b)** as a yellow oil (920 mg, 61%); $[\alpha]_D^{31}$ -1.5 (*c* 1.00, CH_2Cl_2); *m/z* [+ve FAB (PEGH/NOBA)]: Found: 618.290557 $[M+Na]^+$, $C_{33}H_{45}NO_7NaSi$ requires 618.286301; *m/z* [+ve FAB (3-NBA)] 619 $[M+Na]^+$, 597 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 1793, 1743 and 1724 (C=O) and 1590 (C=C); δ_H (C^2HCl_3) 7.51-7.47 (4H, *m*, ArH), 7.33-7.23 (6H, *m*, ArH), 4.24 (1H, *dd*, $J_{2,3}$ 7.3, $J_{2,3}$ 8.5, H-2), 4.04 (2H, *s*, H-8), 3.08 (1H, *dd*, $J_{6A,4}$ 3.1, $J_{6A,6B}$ 18.8, H-6A), 2.87-2.80 (1H, *m*, H-4), 2.60-2.45 (2H, *m*, H-6B and H-3B), 1.44-1.39 (1H, *m*, H-3A), 1.37 and 1.32 (2x9H, 2x*s*, $2xOC(C\underline{H}_3)_3$) and 0.95 (9H, *s*, $SiC(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 208.28 (ketone), 175.09 (lactam), 170.81 (ester), 149.76 (urethane), 135.95-126.28 (Ar), 84.05 and

82.59 (2xO<u>C</u>(CH$_3$)$_3$), 70.12 (C-8), 58.62 (C-2), 40.94 (C-6), 38.19 (C-4), 28.70 (C-3), 28.31 and 27.83 (2xOC(<u>C</u>H$_3$)$_3$), 27.17 (SiC(<u>C</u>H$_3$)$_3$) and 19.61 (Si<u>C</u>(CH$_3$)$_3$).

***tert*-Butyl (*3R,5RS,7S*)-[(1-amino-2-oxo-5-hydroxy-5-O-*tert*-butyldiphenylsilyloxymethyl pyrrolidin-3-yl)methyl]-N-*tert*-butoxycarbonylglycinate (24b)** and ***tert*-Butyl (*5R, 8S*)-[(3-O-*tert*-butyldiphenylsilyloxymethyl-4,5-dihydro-1*H*-6-oxopyridazine-5-yl)methyl]-N-*tert*-butoxycarbonylglycinate (25b).**

Hydrazine hydrate (64-65%) (26 mg, 0.34 mmol) was added to a solution of *tert*-butyl (*2S, 4R*)-N-*tert*-butoxycarbonyl-4-(2-oxo-3-O-*tert*-butyldiphenylsilyloxypropyl)-pyroglutamate **(23b)** (100 mg, 0.17 mmol) in methanol (1.2 ml) under nitrogen. After stirring at room temperature for 3 h, the solvent was removed *in vacuo* to yield the crude product as an oil. Purification of the two products was achieved by flash column chromatography on silica gel using petroleum ether / ethyl acetate (1:1) as eluent. *tert-Butyl (3R,5RS,7S)-[(1-amino-2-oxo-5-hydroxy-5-O-tert-butyldiphenylsilyloxymethylpyrrolidin-3-yl)methyl]-N-tert-butoxycarbonyl glycinate* **(24b)** was obtained as a colourless oil (63 mg, 60%); *m/z* [+ve FAB (PEGH/NOBA)]: Found: 628.345300 [M+H]$^+$, $C_{33}H_{50}N_3O_7Si$ requires 628.341805; *m/z* [+ve FAB (3-NBA)]: 650 [M+Na]$^+$, 628 [M+H]$^+$ and 610 [M-H$_2$O]$^+$; ν_{max} (film) / cm^{-1} 3338 (OH and NH), 1735 and 1702 (C=O), 1618 and 1590 (C=C); δ_H (C^{2}HCl$_3$) 7.57-7.53 (4H, *m*, ArH), 7.40-7.30 (6H, *m*, ArH), 5.28-5.25 (1H, *m*, NHBoc), 4.15-4.08 (1H, *m*, H-7), 3.73-3.28 (4H, *m*, TBDPSOCH$_2$ and NH$_2$), 2.61-2.60 (1H, *m*, H-3), 2.30-1.56 (4H, *m*, H-6 and H-4), 1.37, 1.36 (2x9H, 2x*s*, 2xOC(C<u>H</u>$_3$)$_3$) and 0.95 (9H, *s*, SiC(C<u>H</u>$_3$)$_3$); δ_C (C^{2}HCl$_3$) 174.86 and 174.71 (lactam), 171.67 (ester), 155.88 (urethane), 135.62-127.90 (Ar), 89.61 and 89.30 (Si<u>C</u>(CH$_3$)$_3$), 82.21 and 80.61 (2xOC(<u>C</u>H$_3$)$_3$), 79.87 (C-5), 65.91 and 65.51 (TBDPSO<u>C</u>H$_2$), 52.36 (C-7), 36.40 (C-3), 35.51 and 35.19 (C-4), 28.69, 28.37, 28.08 and 28.00 (2xOC(<u>C</u>H$_3$)$_3$), 26.91 (SiC(<u>C</u>H$_3$)$_3$), 21.12 (C-6) and 19.23 and 19.15 (Si<u>C</u>(CH$_3$)$_3$); δ_N (INEPT, -20 °C C^{2}HCl$_3$) -295 (+1;-1, d, J 91.2, NH) and 330 (+1;0;-1, t, J 138, NH$_2$). *Benzyl (5R,8S)-[(3-O-tert-butyldiphenylsilyloxymethyl-4,5-dihydro-1H-6-oxopyridazine-5-yl)methyl]-N-tert-butoxycarbonyl-glycinate* **(25b)** was obtained as a colourless oil (7 mg, 7%), $[\alpha]_D^{19}$ +22.5 (*c* 1.00, CH_2Cl_2); *m/z* [+ve FAB (PEGH/NOBA)]: Found: 610.329018 [M+H]$^+$, $C_{33}H_{48}N_3O_6Si$ requires 610.331240; *m/z* [+ve FAB (3-NBA)] 632 [M+Na]$^+$, 610 [M]$^+$; ν_{max} (Nujol) / cm^{-1} 3299 (NH), 3072 and 3051 (=C-H), 1794, 1719 (C=O), 1648 (C=N) and 1590 (C=C); δ_H (C^{2}HCl$_3$) 8.38 (1H, *s*, =N-NH), 7.59-7.33 (10H, *m*, ArH), 5.18 (1H, *d, J* 8.5, NHBoc), 4.27-4.13 (3H, *m*, H-8 and TBDPSOCH$_2$), 2.87 (1H, *d, J* 10.7, H-4A), 2.31-2.13 (3H, *m*, H-4B, H-5 and H-7B), 1.83-1.77 (1H, *m*, H-7A, 1H), 1.38 and 1.35 (2x9H, 2x*s*, 2xOC(C<u>H</u>$_3$)$_3$) and 1.00 (9H, *s*, SiC(C<u>H</u>$_3$)$_3$); δ_C (C^{2}HCl$_3$) 171.72 (lactam), 170.24 (ester), 156.22 (urethane), 154.83 (C=N), 136.02-128.27 (Ar), 82.67 and 82.25 (2xO<u>C</u>(CH$_3$)$_3$), 66.31 (TBDPSO<u>C</u>H$_2$), 52.14 (C-8), 33.21 (C-4), 32.89 (C-5), 28.70 and 28.36 (2xOC(<u>C</u>H$_3$)$_3$), 27.39 (C-7), 27.23 (SiC(<u>C</u>H$_3$)$_3$) and 19.64 (Si<u>C</u>(CH$_3$)$_3$).

***tert*-Butyl (*5R, 8S*)-[(3-O-*tert*-butyldiphenylsilyloxymethyl-4,5-dihydro-1*H*-6-oxopyridazine-5-yl)methyl]-N-*tert*-butoxycarbonylglycinate (25b).**

tert-Butyl (3R, 5RS, 7S)-[(1-amino-2-oxo-5-hydroxy-5-O-*tert*-butyldiphenylsilyloxymethylpyrrolidin-3-yl)methyl]-N-*tert*-butoxycarbonylglycinate **(24b)** (60 mg, 0.095 mmol) was dissolved in acetonitrile (1 ml). The solution was heated to reflux in the presence of 3Å molecular sieves (120 mg) and acetic acid (4 drops) for 2 days. The solvents were removed *in vacuo* and the resultant oil was purified by flash column chromatography on silica gel using petroleum ether / ethyl acetate (2:1) as eluent to yield the *pyridazine* **(25b)** as a clear, colourless oil (32 mg, 55 %). Spectra were identical with those of the sample prepared above.

***tert*-Butyl (*2S,4S*)-N-*tert*-butoxycarbonyl-4-(3-trimethylsilyl-prop-2-ynyl)-pyroglutamate (26a)** and ***tert*-Butyl (*2S,4R*)-N-*tert*-butoxycarbonyl-4-(3-trimethylsilylprop-2-ynyl)-pyroglutamate (26b).**

Lithium hexamethyldisilazide (1 M in THF, 1.3 ml, 1.3 mmol) was added to a stirred solution of *tert*-butyl (*2S*)-N-*tert*-butoxycarbonyl-pyroglutamate **(15)** (340 mg, 1.19 mmol) in THF (4 ml) at -78 °C under nitrogen. The mixture was stirred at -78 °C for 1 h and 3-bromo-1-(trimethylsilyl)-1-propyne (0.20 ml, 1.43 mmol) was added. Stirring was continued for further 2 h at -78 °C and saturated aqueous NH_4Cl (7 ml) was added. The mixture

was extracted with diethyl ether (3x10 ml). The organic phases were dried (Na_2SO_4) and the solvents were removed *in vacuo* to give a yellow oil. Purification of the two stereoisomers by column chromatography using petroleum ether / ethyl acetate (5:1) as eluent gave the *trans* and *cis* epimers in *ca.* 20% yield each and the *bis*-alkylated product in 17% yield. *tert-Butyl (2S, 4S)-N-tert-butoxycarbonyl-4-(3-trimethylsilyl-prop-2-ynyl)-pyroglutamate* (**26a**) was obtained as white crystals (83 mg, 18%), m.p. 58-62 °C; $[\alpha]_D^{32}$ -32.8 (*c* 1.00, CH_2Cl_2); *m/z* [CI]: Found: 396.2206 $[M+H]^+$, $C_{20}H_{34}NO_5Si$ requires 396.2205; *m/z* [+ve FAB (3-NBA)] 418 $[M+Na]^+$, 396 $[M+H]^+$; ν_{max} (KBr) / cm^{-1} 2177 (alkyne), 1795, 1743 and 1723 (C=O); δ_H (C^2HCl_3) 4.47 (1H, *dd*, $J_{2,3A}$ 3.4, $J_{2,3B}$ 8.2, H-2), 2.83-2.77 (1H, *m*, H-4), 2.70 (1H, *dd*, $J_{6A,4}$ 4.5, $J_{6A,6B}$ 17.2, H-6A), 2.47 (1H, *dd*, $J_{6B,4}$ 8.0, $J_{6B,6A}$ 17.2, H-6B), 2.26-2.18 (2H, *m*, H-3S and H-3R), 1.52 and 1.49 (18H, 2x*s*, 2xOC(CH_3)$_3$) and 0.13 (9H, *s*, SiC(CH_3)$_3$); δ_C (C^2HCl_3) 173.44 (lactam), 170.27 (ester), 149.33 (urethane), 102.67 (C-7), 87.23 (C-8), 83.47 and 82.40 (2xO<u>C</u>(CH$_3$)$_3$), 57.83 (C-2), 40.84 (C-4), 27.93 (OC(<u>C</u>H$_3$)$_3$), 27.63 (C-6), 21.11 (C-3) and 0.00 (Si (<u>C</u>H$_3$)$_3$). *tert-Butyl (2S, 4R)-N-tert-butoxycarbonyl-4-(3-trimethylsilyl-prop-2-ynyl)-pyroglutamate* (**26b**) was obtained as white crystals (89 mg, 19%), m.p. 82-84 °C; $[\alpha]_D^{31}$ +26.5 (*c* 1.00, CH_2Cl_2); *m/z* [CI]: Found: 396.2206 $[M+H]^+$, $C_{20}H_{34}NO_5Si$ requires 396.2205; *m/z* [+ve FAB (3-NBA)] 418 $[M+Na]^+$, 395 $[M]^+$; ν_{max} (KBr) / cm^{-1} 2178 (alkyne), 1791 and 1741 (C=O); δ_H (C^2HCl_3) 4.47 (1H, *dd*, $J_{2,3R}$ 6.4, $J_{2,3S}$ 9.0, H-2), 2.77 (1H, dd, $J_{6A,6A}$ 4.4, $J_{A,6B}$ 16.7, H-6A) 2.69 (1H, *m*, H-4), 2.56 (1H, *ddd*, $J_{3S,4}$ 4.3, $J_{3S,2}$ 9.0, $J_{3S,3R}$ 13.5, H-3S), 2.44 (1H, *dd*, $J_{6B,4}$ 9.9, $J_{6B,6A}$ 16.7, H-6B), 1.95 (1H, *ddd*, $J_{3R,2}$ 6.4, $J_{3R,4}$ 7.4, $J_{3R,3S}$ 13.5, H-3R), 1.51 and 1.50 (18H, 2x*s*, 2xOC(C<u>H</u>$_3$)$_3$) and 0.14 (9H, *s*, SiC(C<u>H</u>$_3$)$_3$); δ_C (C^2HCl_3) 173.40 (lactam), 170.27 (ester), 149.43 (urethane), 102.99 (C-7), 86.96 (C-8), 83.64 and 82.30 (2xO<u>C</u>(CH$_3$)$_3$), 58.04 (C-2), 41.97 (C-4), 27.90 (OC(<u>C</u>H$_3$)$_3$), 26.80 (C-6), 21.68 (C-3) and 0.02 (Si(<u>C</u>H$_3$)$_3$). Irradiation of H-2 at δ 4.47 led to an nOe in H-3S at δ 2.55 and irradiation of H-3R at δ 1.95 caused nOe in H-6 at δ 2.72 and δ 2.43.

Benzyl (*2S, 4S*)-N-*tert*-butoxycarbonyl-4-(2-carboxymethyl-prop-2-enyl)-pyroglutamate (32a, R^1=PhCH$_2$, R^2=Me) and **Benzyl (*2S, 4RS*)-N-*tert*-butoxycarbonyl-4-(2-carboxymethyl-prop-2-enyl)-pyroglutamate (32b, R^1=PhCH$_2$, R^2=Me).**

Lithium hexamethyldisilazide (1 M in THF, 2.30 ml, 2.30 mmol) was added to a solution of benzyl (*2S*)-N-*tert*-butoxycarbonylpyroglutamate (**31, R^1=PhCH$_2$**)[11] (611 mg, 1.91 mmol) in THF (7 ml) stirred at -78 °C under nitrogen. The mixture was stirred at -78 °C for 1 h and methyl 2-(bromomethyl)acrylate (411 mg, 2.30 mmol) was added. Stirring was continued for further 2 h at -78 °C. The mixture was quenched with saturated aqueous ammonium chloride (15 ml) at -78 °C and extracted with diethyl ether (3x15 ml). The organic phases were dried (Na_2SO_4) and the solvent was removed *in vacuo*. The crude product was isolated as a yellow oil which was purified by column chromatography on silica gel using petroleum ether / ethyl acetate (2:1) as eluent. *Benzyl (2S, 4RS)-N-tert-butoxycarbonyl-4-(2-carboxymethyl-prop-2-enyl)-pyro glutamate* (**32, R^1=PhCH$_2$, R^2=Me**) was isolated in 54% yield. The major, *trans* isomer (2S, 4S) was partly isolated as one stereoisomer as a white solid. The minor *cis* stereoisomer (2S, 4R) could not be separated from the *trans* isomer. The ratio was *ca.* 3:1 in favour of the *trans* stereoisomer, *benzyl (2S, 4S)-N-tert-butoxycarbonyl-4-(2-carboxymethyl-prop-2-enyl)-pyroglutamate* (**32a, R^1=PhCH$_2$, R^2=Me**) m.p. 93-95 °C; $[\alpha]_D^{30}$ -11.4 (*c* 1.00, CH_2Cl_2); Found: C, 62.9; H, 6.5; N, 3.2. $C_{22}H_{27}NO_7$ requires C, 63.3; H, 6.5; N, 3.3%; *m/z* [EI]: 417 $[M+H]^+$; ν_{max} (KBr) / cm^{-1} 3037 (=C-H), 1779, 1737 and 1716 (C=O) and 1629 (C=C); δ_H (C^2HCl_3) 7.20 (5H, *s*, ArH), 6.09 (1H, *s*, olefinic), 5.48 (1H, *s*, olefinic), 5.04 (2H, *s*, CH_2Ph), 4.43 (1H, *dd*, $J_{2,3R}$ 1.0, $J_{2,SR}$ 9.6, H-2), 3.59 (3H, *s*, OCH_3), 2.86 (1H, *dd*, $J_{6A,4}$ 4.4, $J_{6A,6B}$ 14.3, H-6A), 2.75-2.69 (1H, *m*, H-4), 2.13 (1H, *dd*, $J_{6B,4}$ 19.5, $J_{6B,6A}$ 14.3, H-6B), 2.0 (1H, *dd*, $J_{3R,4}$ 8.6, $J_{3R,3S}$ 13.3, H-3R), 1.83 (1H, *ddd*, $J_{3S,2}$ 9.6, $J_{3S,4}$ 12.0, $J_{3S,3R}$ 13.3, H-3S) and 1.23 (9H, *s*, C(C<u>H</u>$_3$)$_3$); δ_C (C^2HCl_3) 172.33 (lactam), 169.27 (ester), 165.35 (ester), 147.62 (urethane) 135.42 (C-7), 133.32-126.79 (Ar), 126.06 (C-8), 81.45 (O<u>C</u>(CH$_3$)$_3$), 65.68 (CH_2Ph), 55.27 (C-2), 50.35 (OCH_3), 39.33 (C-4), 31.03 (C-6), 26.63 (C-3) and 26.09 (OC(<u>C</u>H$_3$)$_3$). δ_H for *benzyl (2S, 4R)-N-tert-butoxycarbonyl-4-(2-carboxymethyl-prop-2-enyl)-pyroglutamate* (**32b, R^1=PhCH$_2$, R^2=Me**) was obtained from the *cis* / *trans* mixture) δ_H (C^2HCl_3) 7.29 (5H, *s*, ArH), 6.12 (1H, *s*, olefinic), 5.39 (1H, *s*, olefinic), 5.12 (2H, *s*, CH_2Ph), 4.44 (1H, *dd*, $J_{2,3B}$ 6.6, $J_{2,AS}$ 9.0, H-2), 3.66 (3H, *s*, OCH_3), 2.84-2.76 (2H, *m*, H-6A and H-4), 2.35 (1H, *ddd*, $J_{3A,4}$ 4.4, $J_{3A,2}$ 9.0, $J_{3A,3B}$ 13.5, H-3A), 2.23 (1H, dd, $J_{6B,4}$ 9.8, $J_{6B,6A}$ 13.9, H-6B), 1.59 (1H, *ddd*, $J_{3B,4}$ 6.2, $J_{3B,2}$ 6.6, $J_{3A,3B}$ 13.5, H-3B) and 1.36 (9H, *s*, C(C<u>H</u>$_3$)$_3$).

The (*2S,4S*) Pyruvylpyroglutamate derivative (33, R^1=$PhCH_2$, R^2=Me).
A solution of benzyl (*2S, 4S*)-N-*tert*-butoxycarbonyl-4-(2-carboxymethyl-prop-2-enyl)-pyroglutamate (**32a, R^1=$PhCH_2$, R^2=Me**) (400 mg, 0.96 mmol) in dichloromethane (15 ml) was cooled to -78 °C and oxygen was passed for 20 min. Ozone was passed through the solution for 15 min, during which time it turned blue. The reaction was quenched by adding triphenylphosphine (277 mg, 1.05 mmol) at -78 °C. The solution was allowed to warm slowly to room temperature. The solvent was removed *in vacuo* and the crude product, isolated as an oil, was purified by flash column chromatography on silica gel using petroleum ether / ethyl acetate (1:1) as eluent to yield *the pyruvate derivative* (**33, R^1=$PhCH_2$, R^2=Me**) as an oil (338 mg, 84%); $[\alpha]_D^{32}$ -13.8 (*c* 1.00, CH_2Cl_2); Found: C, 59.9; H, 6.0; N, 3.2. $C_{21}H_{25}NO_8$ requires C, 60.1; H, 6.0; N, 3.3%; *m/z* [+ve FAB (3-NBA)] 442 $[M+Na]^+$ and 420 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 1791, 1742 and 1736 (C=O); δ_H (C^2HCl_3) 7.41 (5H, *s*, ArH), 5.26 (2H, *s*, CH_2Ph), 4.68 (1H, *d*, $J_{2,3B}$ 9.7, H-2), 3.92 (3H, *s*, OCH_3), 3.50 (1H, *dd*, $J_{6A,4}$ 3.8, $J_{6A,6B}$ 19.2, H-6A), 3.16 (1H, *dq*, $J_{4,6A}$ 3.8, $J_{4,6B}$ 8.3, $J_{4,3A}$ 8.6, $J_{4,3B}$ 12.0, H-4), 3.02 (1H, *dd*, $J_{6B,4}$ 8.3, $J_{6B,6A}$ 19.2, H-6B), 2.44 (1H, *dd*, $J_{3A,4}$ 8.6, $J_{3A,3B}$ 13.0, H-3A), 2.01 (1H, *ddd*, $J_{3B,2}$ 9.7, $J_{3B,4}$ 12.0, $J_{3A,3B}$ 13.0, H-3B) and 1.46 (9H, *s*, $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 191.89 (ketone), 173.97 (lactam), 171.17 (ester), 161.00 (ester), 149.49 (urethane), 135.37-128.93 (Ar), 84.30 ($O\underline{C}(CH_3)_3$), 67.95 (CH_2Ph), 57.67 (C-2), 53.63 (OCH_3), 40.42 (C-6), 37.73 (C-4), 28.65 (C-3) and 28.18 ($OC(\underline{C}H_3)_3$).

Benzyl (*3S, 5RS, 7S*)-[(1-amino-2-oxo-5-hydroxy-5-methoxycarbonylpyrrolidin-3-yl)methyl]-N-*tert*-butoxycarbonylglycinate (34, R^1=$PhCH_2$, R^2=Me) and Benzyl (*5S, 8S*)-[(3-methoxycarbonyl-4,5-dihydro-1*H*-6-oxopyridazine-5-yl)methyl]-N-*tert*-butoxycarbonyl glycinate (35, R^1=$PhCH_2$, R^2=Me).
Hydrazine hydrate (64-65%) (29 mg, 0.38 mmol) was added to a solution of the derivative (**33, R^1=$PhCH_2$, R^2=Me**) (144 mg, 0.34 mmol) in methanol (1.5 ml) under nitrogen. After stirring at room temperature for 1 h, the solvent was removed *in vacuo* to yield a white foam. Purification and separation of the two products was achieved on silica gel using petroleum ether / ethyl acetate (1:3) as eluent. *Benzyl (3S, 5RS, 7S)-[(1-amino-2-oxo-5-hydroxy-5-methoxycarbonylpyrrolidin-3-yl)methyl]-N-tert-butoxycarbonylglycinate* (**34, R^1=$PhCH_2$, R^2=Me**) (93 mg, 61%) was obtained as a white solid; m.p. 156-159 °C; Found: C, 55.9; H, 6.5; N, 9.0. $C_{21}H_{29}N_3O_8$ requires C, 55.9; H, 6.5; N, 9.3%; *m/z* [+ve FAB (3-NBA)]: 474 $[M+Na]^+$, 452 $[M+H]^+$; ν_{max} (KBr) / cm^{-1} 3334 (OH), 3268 (NH), 1754, 1736, 1708 and 1688 (C=O); δ_H (C^2HCl_3) 7.28 (5H, *s*, ArH), 5.74 (1H, *m*, NHBoc), 5.23-5.03 (2H, *m*, CH_2Ph), 4.36 (3H, *br.s*, H-7 and NH_2), 3.76 and 3.71 (2x1.5H, 2x*s*, 2xOCH_3), 2.77-2.72 and 2.63-2.58 (2x0.5H, 2x*m*, 2xH-3), 2.49-2.41 (0.5H, *m*, H-4), 2.32-2.17 (1.5H, *m*, H-4 and H-6A), 2.05-1.98 (0.5H, *m*, H-4), 1.89-1.74 (1.5H, *m*, H-4, H-6B), and 1.36 (9H, *s*, $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 174.42 (C-2), 172.33 (ester), 171.73 and 171.56 (ester), 155.84 (urethane), 135.66-126.26 (Ar), 88.84 and 88.45 (2x$O\underline{C}(CH_3)_3$), 80.16 (C-5), 67.67 (CH_2Ph), 54.38 and 54.26 (C-7), 52.58 (OCH_3), 36.62 (C-3), 36.16 (C-4), 34.22 (C-6) and 28.74 ($OC(\underline{C}H_3)_3$). *Benzyl (5S, 8S)-[(3-methoxycarbonyl-4,5-dihydro-1H-6-oxopyridazine-5-yl)methyl]-N-tert-butoxycarbonyl-glycinate* (**35, R^1=$PhCH_2$, R^2=Me**) was obtained as a white solid (11 mg, 7.5%); m.p. 125-132 °C; $[\alpha]_D^{36}$ -110.7 (*c* 1.00, CH_2Cl_2); λ_{max} (MeOH) / nm 268 (ε 6,662); Found: C, 57.8; H, 6.2; N, 9.3. $C_{21}H_{27}N_3O_7$ requires C, 58.2; H, 6.3; N, 9.7%; *m/z* [+ve FAB (3-NBA)] 456 $[M+Na]^+$, 434 $[M+H]^+$; ν_{max} (KBr) / cm^{-1} 3350 (NH), 1730, 1697 and 1678 (C=O) and 1617 (C=N); δ_H (C^2HCl_3) 9.16 (1H, *d*, *J* 6.2, =N-NH-), 7.28 (5H, *s*, ArH), 5.37 (1H, *d*, *J* 8.0, N$\underline{H}$Boc), 5.23-5.04 (2H, *m*, $C\underline{H}_2Ph$), 4.43-4.41 (1H, *m*, H-8), 3.82 (3H, *s*, OCH_3), 3.06 (1H, *d*, *J* 10.4, H-3A), 2.43-2.39 (2H, *m*, H-3B and H-5) 2.34 - 2.25 (1H, m, H-7A), 1.98-1.85 (1H, *m*, H-7B) and 1.35 (9H, *s*, $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 173.77 (C-6), 171.46 (ester), 165.16 (ester), 157.64 (urethane), 144.87 (C=N), 137.18-130.35 (Ar), 84.30 ($O\underline{C}(CH_3)_3$), 69.42 ($\underline{C}H_2Ph$), 55.00 (C-8), 53.68 ($O\underline{C}H_3$), 34.15 (C-5), 33.73 (C-4), 30.23 ($OC(\underline{C}H_3)_3$) and 28.26 (C-7).

Benzyl (*5RS, 8S*)-[(3-methoxycarbonyl-4,5-dihydro-1*H*-6-oxopyridazine-5-yl)methyl]-N-*tert*-butoxycarbonylglycinate (35, R^1=$PhCH_2$, R^2=Me).
Benzyl (*3S, 5RS, 7S*)-[(1-amino-2-oxo-5-hydroxy-5-methoxycarbonyl-pyrrolidin-3-yl)methyl]-N-*tert*-butoxycarbonylglycinate (**34, R^1=$PhCH_2$, R^2=Me**) (118 mg, 0.26 mmol) was dissolved in acetonitrile (2 ml). The solution was heated to reflux in the presence of 3Å molecular sieves (400 mg) and acetic acid (8 drops) for 2

days. The molecular sieves were filtered off, the solvents removed *in vacuo* and the resultant oil was purified by flash column chromatography on silica gel, eluting with a 1:1 mixture of petroleum ether and ethyl acetate, to yield the *pyridazine* (**35, R^1=PhCH$_2$, R^2=Me**) (90 mg, 79%) as a mixture of stereoisomers, as a white solid. The mixture of stereoisomers contained absorptions due to (**35, R^1=PhCH$_2$, R^2=Me**) above; δ_H (C^2HCl_3) 9.16 (1H, *d*, *J* 8.4, =N-NH-), 7.28 (5H, *s*, ArH), 5.42 (0.5H, *d*, *J* 7.7, NHBoc), 5.28 (0.5H, *d*, *J* 8.2, NHBoc), 5.18-5.03 (2H, *m*, CH_2Ph), 4.43-4.35 (1H, *m*, H-8), 3.81 (3H, *s*, OCH_3), 3.21 (0.5H, *d*, *J* 10.9, H-3), 3.06 (0.5H, *d*, *J* 10.4, H-4A), 2.48-2.38 (2H, *m*, H-4A and H-5); 2.34-2.25 (1H, *m*, H-7R), 1.84 (1H, *m*, H-7S) and 1.35 (9H, *s*, $C(C\underline{H}_3)_3$).

tert-Butyl 2-(bromomethyl)acrylate

3-Bromo-2-(bromomethyl)propionic acid (5.0 g, 20.33 mmol) was added to a suspension of dibromotriphenylphosphorane, prepared from Ph_3P (12.0 g, 45.75 mmol) and Br_2 (7.3 g, 45.75 mmol) in benzene (100 ml) containing hexamethylphosphoramide (HMPA, 9.1 g, 50.83 mmol) under nitrogen at room temperature. *tert*-Butanol (10 ml) was added after 24 h and the mixture was stirred for another 24 h. The mixture was diluted with diethyl ether (250 ml) and quenched with ice-cooled saturated aqueous hydrogen carbonate (200 ml). The organic layer was washed consecutively with aqueous sodium sulfite, dilute sulfuric acid (0.8 M), water and aqueous sodium hydrogen carbonate. It was dried (Na_2SO_4) and the solvent removed *in vacuo*. The white, crystalline by-product was filtered off and washed with ice-cold diethyl ether to yield *tert-butyl 2-(bromomethyl)acrylate* (3.4 g, 76%) as a clear, yellow oil. Spectra were identical with those in the literature.[10]

tert-Butyl (*2S, 4S*)-N-*tert*-butoxycarbonyl-4-(2-*tert*-butyl-oxycarbonyl-prop-2-enyl)-pyroglutamate (32, R^1=R^2=^{t}Bu).

Lithium hexamethyldisilazide (1 M in THF 10.0 ml, 10.0 mmol) was added to a solution of *tert*-butyl (*2S*)-N-*tert*-butoxycarbonylpyroglutamate (**31, R^1=^{t}Bu**) (2.58 g, 9.05 mmol) in THF (25 ml) stirred at -78 °C under nitrogen. The reaction was stirred at -78 °C for 1 h and *tert*-butyl 2-(bromomethyl)acrylate (3.00 g, 13.57 mmol) was added dropwise. Stirring was continued for further 2 h at -78 °C. The mixture was quenched with saturated aqueous NH_4Cl (80 ml) at -78 °C and extracted with diethyl ether (3x100 ml). The combined organic phases were dried (Na_2SO_4) and the solvents were removed *in vacuo*. The crude product was isolated as a yellow oil which was purified by column chromatography on silica gel using petroleum ether / ethyl acetate (2:1) as eluent. *tert-Butyl (2S, 4S)-N-tert-butoxycarbonyl-4-(2-tert-butyl-oxycarbonyl-prop-2-enyl)-pyroglutamate* (**32, R^1=R^2=^{t}Bu**) was obtained as a white solid (2.40 g, 62%); m.p. 114-117 °C; $[\alpha]_D^{24}$ -18.5 (*c* 1.00, CH_2Cl_2); Found: C, 61.8; H, 8.3; N, 3.3. $C_{22}H_{35}NO_7$ requires C, 62.1; H, 8.3; N, 3.3%; *m/z* [+ve FAB (3-NBA)] 448 $[M+Na]^+$, 426 $[M+H]^+$; ν_{max} (KBr) / cm^{-1} 1781, 1733 and 1701 (C=O) and 1632 (C=C); δ_H (C^2HCl_3) 6.00 (1H, *s*, olefinic), 5.40 (1H, *s*, olefinic), 4.26 (1H, *d*, *J* 9.4, H-2), 2.84-2.79 (1H, *m*, H-6A), 2.75-2.72 (1H, *m*, H-4), 2.09 (1H, *dd*, $J_{6B,4}$ 9.5, $J_{6B,6A}$ 13.5, H-6B), 1.99-1.91 (1H, *m*, H-3A), 1.83-1.72 (1H, *m*, H-3B) and 1.36, 1.33 and 1.32 (3x9H, 3x*s*, 3x $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 173.06 (lactam), 168.86 (ester), 164.53 (ester), 148.06 (urethane), 137.53 (C-7), 125.24 (C-8), 81.93, 80.94 and 79.68 (3x$O\underline{C}(CH_3)_3$), 56.34 (C-2), 39.67 (C-4), 31.36 (C-6), 27.13 (C-3) and 26.66-26.56 (3x$OC(\underline{C}H_3)_3$).

The (*2S,4S*) Pyruvylpyroglutamate derivative (33, R^1=R^2=^{t}Bu).

A solution of *tert*-butyl (*2S, 4S*)-N-*tert*-butoxycarbonyl-4-(2-carboxymethyl-prop-2-enyl)-pyroglutamate (**32, R^1=R^2=^{t}Bu**) (2.40 g, 5.64 mmol) in CH_2Cl_2 (20 ml) was cooled to -78 °C and oxygen was passed for 20 min. Ozone was passed through the solution for 15 min, during which time the solution turned blue. The reaction was quenched by adding triphenyl phosphine (1.63 g, 6.20 mmol) at -78 °C. The solution was allowed to warm slowly to room temperature. The solvent was removed *in vacuo* and the crude product, an oil, was purified by flash column chromatography on silica gel using petroleum ether/ ethyl acetate (2:1) as eluent. *The pyruvate* (**33, R^1=R^2=^{t}Bu**) was obtained as white crystals (2.11 g, 87%); mp 109-112 °C; $[\alpha]_D^{27}$ -17.3 (*c* 1.00, CH_2Cl_2); Found: C, 58.9; H, 7.7; N, 3.2. $C_{21}H_{33}NO_8$ requires C, 59.0; H, 7.8; N, 3.3%; *m/z* [+ve FAB (3-NBA)] 450 $[M+Na]^+$, 428 $[M+H]^+$; ν_{max} (KBr) / cm^{-1} 1782, 1732 and 1726 (C=O); δ_H (C^2HCl_3) 4.26 (1H, *d*, $J_{2,3B}$ 9.4, H-2), 3.27 (1H, *dd*, $J_{6A,4}$ 3.2, $J_{6A,6B}$ 19.2, H-6A), 2.91 (1H, *dq*, $J_{4,6A}$ 3.2, $J_{4,6B}$ $J_{4,3A}$ 8.7, $J_{4,3B}$ 11.8, H-4), 2.78 (1H,

dd, $J_{6B,4}$ 8.7, $J_{6B,6A}$ 19.2, H-6B), 2.20 (1H, *dd*, $J_{3A,4}$ 8.7, $J_{3A,3B}$ 12.9, H-3A), 1.78 (1H, *ddd*, $J_{3B,2}$ 9.4, $J_{3B,4}$ 11.8, $J_{3B,3A}$ 12.9, H-3B) and 1.38, 1.35 and 1.33 (3x9H, 3x*s*, 3x $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 193.41 (ketone), 174.41 (lactam), 170.43 (ester), 159.90 (ester), 149.61 (urethane), 84.88, 83.92 and 82.92 (3x$O\underline{C}(CH_3)_3$), 58.35 (C-2), 40.37 (C-6), 37.64 (C-4), 28.89 (C-3) and 28.32, 28.31 and 28.15 (3x$OC(\underline{C}H_3)_3$).

***tert*-Butyl (*3S, 5RS, 7S*)-[(1-amino-2-oxo-5-hydroxy-5-*tert*-butoxycarbonyl-pyrrolidin-3-yl)methyl]-N-*tert*-butoxycarbonyl-glycinate (34, R^1=R^2=^{t}Bu) and *tert*-Butyl (*5S, 8S*)-[(3-*tert*-butoxycarbonyl-4,5-dihydro-1*H*-6-oxopyridazine-5-yl)methyl]-N-*tert*-butoxycarbonyl-glycinate (35, R^1=R^2=^{t}Bu).**

Hydrazine hydrate (64-65%) (49 mg, 0.64 mmol) was added to a solution of the pyruvate (**33, R^1=R^2=^{t}Bu**) (250 mg, 0.59 mmol) in methanol (3 ml) under nitrogen. After stirring at room temperature for 2 h, the solvent was removed *in vacuo* to yield the crude product as a yellow oil. Purification and separation of the two products was achieved on silica gel using petroleum ether / ethyl acetate (1:1) as eluent. *tert-Butyl (3S, 5RS, 7S)-[(1-amino-2-oxo-5-hydroxy-5-tert-butoxycarbonyl-pyrrolidin-3-yl)methyl]-N-tert-butoxycarbonyl-glycinate* (**34, R^1=R^2=^{t}Bu**) was obtained as a white solid (197 mg, 68%); mp 156-159 °C; *m/z* [+ve FAB (PEGH/NOBA)]: Found: 460.267762 $[M+H]^+$, $C_{21}H_{38}N_3O_8$ requires 460.265891; *m/z* [+ve FAB (3-NBA)]: 482 $[M+Na]^+$, 460 $[M+H]^+$; ν_{max} (KBr) / cm^{-1} 3334 (OH), 3268 (NH), 1754, 1736, 1708 and 1688 (C=O); δ_H (C^2HCl_3) 5.54 (1H, *d*, *J* 7.7, NHBoc), 4.54 (1H, *br.s*, OH), 4.23 (1H, *m*, H-7), 3.86 (2H, *br.s*, NH_2), 2.64-2.62 (1H, *m*, H-3), 2.54-2.47 (1H, *m*, H-4), 2.32-2.24 (1H, *m*, H-6A), 1.83-1.69 (2H, *m*, H-6B and H-4), and 1.40-1.37 (3x9H, 3x*s*, 3x $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 174.52 (lactam), 171.64 (ester), 170.74 (ester), 155.84 (urethane), 88.81-82.59 (3x$O\underline{C}(CH_3)_3$), 80.16 (C-5), 53.27 (C-7), 37.47 (C-3), 36.24 (C-4), 35.10 (C-6) and 28.74, 28.42 and 28.20 (3x$OC(\underline{C}H_3)_3$). *tert-Butyl (5S, 8S)-[(3-tert-butoxycarbonyl-4,5-dihydro-1H-6-oxopyridazine-5-yl)methyl]-N-tert-butoxycarbonyl-glycinate* (**35, R^1=R^2=^{t}Bu**) was obtained as a colourless oil (20 mg, 8%); $[\alpha]_D^{21}$ -88.5 (*c* 1.00, CH_2Cl_2); λ_{max}(MeOH) / nm 269 (ε 7462); *m/z* [+ve FAB (PEGH/NOBA)]: Found: 442.256660 $[M+H]^+$, $C_{21}H_{36}N_3O_7$ requires 442.255326; *m/z* [+ve FAB (3-NBA)] 464 $[M+Na]^+$, 442 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3312 (NH), 1713 (C=O) and 1619 (C=N); δ_H (C^2HCl_3) 8.51 (1H, *s*, =N-NH), 5.07 (1H, *d*, *J* 7.9, NHBoc), 4.15-4.13 (1H, *m*, H-8), 3.01 (1H, *dd*, $J_{4A,5}$ 5.1, $J_{4A,4B}$ 15.6, H-4A), 2.45-2.39 (2H, *m*, H-4B and H-5), 2.27-2.14 (1H, *m*, H-7A), 1.98-1.85 (1H, *m*, H-7B) and 1.38, 1.30 and 1.26 (3x9H, 3x*s*, 3x$C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 171.48 (C-6), 169.96 (ester), 162.22 (ester), 156.32 (urethane), 144.28 (C=N), 83.63 - 82.88 (3x$O\underline{C}(CH_3)_3$), 52.52 (C-8), 32.93 (C-5), 32.36 (C-4), 28.72, 28.42 and 28.40 (3x$OC(\underline{C}H_3)_3$) and 26.66 (C-7).

***tert*-Butyl (*5RS, 8S*)-[(3-*tert*-butoxycarbonyl-4,5-dihydro-1*H*-6-oxopyridazine-5-yl)methyl]-N-*tert*-butoxycarbonylglycinate (35, R^1=R^2=^{t}Bu).**

tert-Butyl (*3S, 5RS, 7S*)-[(1-amino-2-oxo-5-hydroxy-5-*tert*-butoxycarbonylpyrrolidin-3-yl)methyl]-N-*tert*-butoxycarbonylglycinate (**34, R^1=R^2=^{t}Bu**) (197 mg, 0.43 mmol) was dissolved in acetonitrile (5 ml). The solution was heated to reflux in the presence of 3Å molecular sieves (400 mg) and acetic acid (514 mg, 8.6 mmol) for 2 days. The molecular sieves were filtered off, the solvent was removed *in vacuo* and the resultant oil was purified on silica gel, eluting with a 1:1 mixture of petroleum ether and ethyl acetate, to yield the *pyridazine* (**35, R^1=R^2=^{t}Bu**) (190 mg, 100 %) as a mixture of stereoisomers, as a colourless oil. Spectra were identical with those above.

(*5RS, 8S*)-[(3-carboxy-4,5-dihydro-1*H*-6-oxopyridazine-5-yl)methyl]-glycine (36).

6 N Aqueous HCl (2 ml) was added to *tert*-butyl (*5RS, 8S*)-[(3-*tert*-butoxycarbonyl-4,5-dihydro-1*H*-6-oxopyridazine-5-yl)methyl]-N-*tert*-butoxycarbonylglycinate (**35**) (220 mg, 0.50 mmol) dissolved in tetrahydrofuran (2 ml). The solution was stirred at room temperature for 3 h. The solvents were removed *in vacuo* to yield the *potential inhibitor* (**36**) as a white foam (115 mg, 100%); λ_{max} (H_2O) / nm 261 (ε 8071); *m/z* [+ve FAB (PEGH/NOBA)] Found 230.079260 $[M+H]^+$. $C_8H_{12}N_3O_5$ requires 230.077696; *m/z* [+ve FAB (glycerol)] 230 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3435-2800 (acid) 1720 and 1664 (C=O); δ_H (20% ^2HCl/ ^2H_2O) epimer 1 - 4.47 (1H, *t*, *J* 6.23, H-8), 3.30 -3.24 (1H,m), 3.01-2.96 (1H, *m*), 2.69 (1H, *t, J 16.2)*, 2.57 - 2.52 (1H, *m*), and 2.23 (1H, *t, J 6.1*); epimer 2 - 4.39 (1H, t, J 6.3, H-8), 3.68 (1H, dt, J 6.6, J 13.3), 3.08 - 3.06 (1H,

m), 2.42 - 2.36 (1H,m), 2.31 (1H, dt, J 5.2, J 10.1) and 2.20 (1H, t, J 5.9); δ_C (^2H_2O) 172.48 and 172.11 (C=O), 171.69 and 171.44 (C=O), 166.0 and 158.78 (C=O), 146.0 and 145.80 (C-3), 52.71 and 52.04 (C-8), 33.84 and 32.69 (C-5), 30.99 and 30.57 (C-4) and 29.47 and 27.31 (C-7).

tert-Butyl (*5S, 8S*)-[(1-methyl-3-_tert_-butoxycarbonyl-4,5-dihydro-6-oxopyridazine-5-yl)methyl]-N-_tert_-butoxycarbonylglycinate.

Methylhydrazine (35 mg, 0.75 mmol) was added to a solution of the pyruvate **(33, R^1=R^2=^{t}Bu)** (215 mg, 0.50 mmol) dissolved in methanol (2.5 ml) under nitrogen. After stirring at room temperature for 4 h, the solvent was removed *in vacuo* to yield the crude product as a yellow oil. Purification was achieved by flash column chromatography on silica gel using petroleum ether / ethyl acetate (5 : 2) as eluent. *tert-Butyl (5S, 8S)-[(1-methyl-3-tert-butoxycarbonyl-4,5-dihydro-1H-6-oxopyridazine-5-yl)methyl]-N-tert-butoxycarbonylglycinate* was obtained as a colourless oil (190 mg, 83%); $[\alpha]_D^{37}$ -135.1 (*c* 1.00, CH_2Cl_2); λ_{max} (MeOH) / nm 280 (ε 6706); *m/z* [+ve FAB (PEGNa/NOBA)]: Found: 456.269244 $[M+H]^+$, $C_{22}H_{38}N_3O_7$ requires 456.270976; *m/z* [+ve FAB (3-NBA)] 478 $[M+Na]^+$, 456 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3367 (NH), 1735 and 1718 (C=O) and 1618 (C=N); δ_H (C^2HCl_3) 5.23 (1H, *d, J* 7.9, NH-Boc), 4.22-4.20 (1H, *m*, H-8), 3.38 (3H, *s*, NCH_3), 3.04 (1H, *d, J* 10.8, H-4A), 2.49-2.40 (2H, *m*, H-4B and H-5), 2.29-2.24 (1H, *m*, H-7A), 1.80-1.77 (1H, *m*, H-7B) and 1.49, 1.40 and 1.36 (3x9H, 3x*s*, 3x $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 171.53 (C-6), 168.89 (ester), 162.14 (ester), 155.75 (urethane), 144.22 (C=N), 83.45, 82.70 and 80.25 ($3xO\underline{C}(CH_3)_3$), 52.54 (C-8), 38.07 ($-NCH_3$), 33.29 (C-5), 32.72 (C-4), 28.71, 28.41 and 28.36 ($3xOC(\underline{C}H_3)_3$) and 27.12 (C-7).

(*5S, 8S*)-[(1-methyl-3-carboxy-4,5-dihydro-6-oxopyridazine-5-yl)methyl]-glycine (37).

6 N Aqueous HCl (1.5 ml) was added to *tert*-butyl (*5S, 8S*)-[(1-methyl-3-*tert*-butoxycarbonyl-4,5-dihydro-1*H*-6-oxopyridazine-5-yl)methyl]-N-*tert*-butoxycarbonyl-glycinate (185 mg, 0.41 mmol) dissolved in THF (1.5 ml). The solution was stirred at room temperature for 3 h. The solvents were removed *in vacuo* to yield the *potential inhibitor* (**37**) as a white foam (100 mg, 100%); $[\alpha]_D^{28}$ -99.8 (*c* 1.00, H_2O); $\lambda_{max}(H_2O)$ / nm 273 (ε 3800); *m/z* [+ve FAB (PEGH/NOBA)]: Found: 244.093625 $[M+H]^+$, $C_9H_{14}N_3O_5$ requires 244.093346; *m/z* [+ve FAB (glycerol)] 244 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3435-2800 (acid) 1726 and 1652 (C=O); δ_H (^2H_2O) 4.21 (1H, *t, J* 6.2, H-8), 3.34 (3H, *s*, NCH_3), 3.14 (1H, *dd*, $J_{4A,5}$ 6.8, $J_{4A,3B}$ 17.1, H-4A), 2.81 (1H, *dq*, $J_{5,3A}$ 6.8, $J_{5,3B}$ $J_{5,7A}$, $J_{5,7B}$ 13.2, H-5), 2.59 (1H, *dd*, $J_{4B,5}$ 13.2, $J_{4B,3A}$ 17.1, H-4B), 2.42 (1H, ddd, $J_{7A,8}$ 6.2, $J_{7A,5}$ 7.5, $J_{7A,7B}$ 13.2, H-7A) and 2.01 (1H, *ddd*, $J_{7B,8}$ 6.2, $J_{7B,5}$ 7.5 , $J_{7B,7A}$ 13.2, H-7B); δ_C (^2H_2O) 171.65 (C-6), 169.85 (acid), 165.61 (acid), 145.15 (C=N), 49.14 (C-8), 37.23 (NCH_3), 32.52 (C-5), 30.30 (C-4) and 26.60 (C-7).

tert-Butyl (*5S, 8S*)-[(1-cyanoethyl-3-_tert_-butoxycarbonyl-4,5-dihydro-6-oxopyridazine-5-yl)methyl]-N-_tert_-butoxycarbonylglycinate

Cyanoethylhydrazine (160 mg, 1.88 mmol) was added to a solution of the pyruvate **(33, R^1=R^2=^{t}Bu)** (254 mg, 0.59 mmol) in methanol (3 ml) under nitrogen. After stirring at room temperature for 4 h, the solvent was removed *in vacuo* to yield the crude product as a yellow oil. Purification by flash column chromatography on silica gel using petroleum ether / ethyl acetate (1:2) as eluent gave *tert-butyl (5S, 8S)-[(1-cyanoethyl-3-tert-butoxycarbonyl-4,5-dihydro-1H-6-oxopyridazine-5-yl)methyl]-N-tert-butoxycarbonylglycinate* as a white foam (203 mg, 70%); $[\alpha]_D^{18}$ -93.3 (*c* 1.00, CH_2Cl_2); λ_{max} (MeOH) / nm 274 (ε 11,029); *m/z* [+ve FAB (PEGH/NOBA)]: Found: 495.280353 $[M+H]^+$, $C_{24}H_{39}N_4O_7$ requires 495.281875; *m/z* [+ve FAB (3-NBA)] 517 $[M+Na]^+$, 494 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3367 (NH), 2253 (CN), 1788 and 1711 (C=O) and 1618 (C=N); δ_H (C^2HCl_3) 5.21 (1H, *d, J* 7.8, N<u>H</u>Boc), 4.30-4.27 (1H, *m*, H-8), 4.23-4.08 (2H, *m*, $NC\underline{H}_2CH_2CN$), 3.13 (1H, *dd*, $J_{4A,5}$ 4.6, $J_{4A,4B}$ 15.2, H-4A), 2.76 (2H, *t, J* 7.0, $NCH_2C\underline{H}_2CN$), 2.62-2.51 (2H, *m*, H-4B and H-5), 2.38-2.29 (1H, *m*, H-7A), 1.88-1.83 (1H, *m*, H-7B) and 1.56, 1.47 and 1.43 (3x9H, 3x*s*, 3x $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 171.38 (C-6), 168.89 (ester), 161.77 (ester), 155.76 (urethane), 145.71 (C=N), 117.48 (CN), 83.76, 82.89 and 80.40 ($3xO\underline{C}(CH_3)_3$), 52.54 (C-8), 45.18 ($N\underline{C}H_2CH_2CN$), 33.37 (C-5), 32.71 (C-4), 28.71, 28.55 and 28.41 ($3xOC(\underline{C}H_3)_3$), 27.14 (C-7) and 17.20 ($NCH_2\underline{C}H_2CN$).

(5S, 8S)-[(1-cyanoethyl-3-carboxy-4,5-dihydro-6-oxopyridazine-5-yl)methyl]-glycine (38). 6 N Aqueous HCl (2 ml) was added to *tert*-butyl (5S, 8S)-[(1-cyanoethyl-3-*tert*-butoxycarbonyl-4,5-dihydro-6-oxopyridazine-5-yl)methyl]-N-*tert*-butoxycarbonylglycinate (203 mg, 0.41 mmol) dissolved in THF (2 ml). The solution was stirred at room temperature for 3 h. The solvents were removed *in vacuo* to yield the *potential inhibitor* (**38**) as a white foam (116 mg, 100%); $[\alpha]_D^{30}$ -75.6 (*c* 1.00, H_2O); λ_{max} (H_2O) / nm 269 (ε 5708); *m/z* [+ve FAB (glycerol)] 283 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3434-2800 (acid) 1727 and 1668 (C=O); δ_H (^2H_2O) 4.09-4.07 (1H, *m*, H-8), 3.95-3.84 (2H, *m*, $NC\underline{H}_2CH_2CN$), 3.02-2.94 (1H, *m*, H-4A), 2.68-2.66 (1H, *m*, H-5, 2.54-2.50 (2H, *m*, $NCH_2C\underline{H}_2CN$), 2.44-2.40 (1H, *m*, H-4B), 2.28-2.26 (1H, *m*, H-7A) and 1.88-1.86 (1H, *m*, H-7B); δ_C (C^2HCl_3) 171.32 (C-6), 169.36 (acid), 165.41 (acid), 145.43 (C=N), 117.48 (CN), 50.99 (C-8), 45.12 ($N\underline{C}H_2CH_2CN$), 33.96 (C-4), 32.55 (C-5), 30.17 (C-7) and 26.51 ($NCH_2\underline{C}H_2CN$).

***tert*-Butyl (5S, 8S)-[(1-hydroxyethyl-3-*tert*-butoxycarbonyl-4,5-dihydro-6-oxopyridazine-5-yl)methyl]-N-*tert*-butoxycarbonylglycinate**

Hydroxyethylhydrazine (55 mg, 0.72 mmol) was added to a solution of the pyruvate (**33, R^1=R^2=^{t}Bu**) (55 mg, 0.13 mmol) dissolved in methanol (1.5 ml) under nitrogen. After stirring at room temperature for 4 h, the solvent was removed *in vacuo* to yield the crude product as a yellow oil. Purification was achieved by flash column chromatography on silica gel using petroleum ether / ethyl acetate (1:2) as eluent. *tert-Butyl (5S, 8S)-[(1-hydroxyethyl-3-tert-butoxycarbonyl-4,5-dihydro-6-oxopyridazine-5-yl)methyl]-N-tert-butoxycarbonylglycinate* was obtained as a clear, colourless oil (30 mg, 48%); $[\alpha]_D^{26}$ -96.0 (*c* 1.00, CH_2Cl_2); λ_{max}(MeOH) / nm 280 (ε 8517); *m/z* [+ve FAB (PEGH/NOBA)]: Found: 486.282355 $[M+H]^+$, $C_{23}H_{40}N_3O_8$ requires 486.281541; *m/z* [+ve FAB (3-NBA)] 508 $[M+Na]^+$, 486 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3569 (OH), 3423 (NH), 1713 (C=O) and 1611 (C=N); δ_H (C^2HCl_3) 5.19 (1H, *d*, *J* 7.9, NHBoc), 4.23-4.21 (1H, *m*, H-8), 3.97-3.93 (2H, *m*, $NCH_2C\underline{H}_2OH$), 3.82-3.81 (2H, *m*, $NC\underline{H}_2CH_2OH$), 3.06 (1H, *dd*, $J_{4A,5}$ 5.3, $J_{24A,4B}$15.8, H-4A), 2.56-2.43 (2H, *m*, H-4B and H-5), 2.30-2.23 (1H, *m*, H-7A), 1.82-1.77 (1H, *m*, H-7B) and 1.48, 1.40 and 1.36 (3x9H, 3x*s*, 3x $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 171.49 (C-6), 169.36 (ester), 161.90 (ester), 155.61 (urethane), 144.49 (C=N), 83.53, 82.82 and 80.40 (3x$O\underline{C}(CH_3)_3$), 61.90 ($NCH_2\underline{C}H_2OH$), 52.55 (C-8), 51.78 ($N\underline{C}H_2CH_2OH$), 33.37 (C-5), 32.76 (C-4), 28.71, 28.41 and 28.34 (3x$OC(\underline{C}H_3)_3$) and 26.84 (C-7).

(5S, 8S)-[(1-hydroxyethyl-3-carboxy-4,5-dihydro-6-oxopyridazine-5-yl)methyl]-glycine (39). 6 N Aqueous HCl (1.5 ml) was added to *tert*-butyl (*5S, 8S*)-[(1-hydroxyethyl-3-*tert*-butoxycarbonyl-4,5-dihydro-6-oxopyridazine-5-yl)methyl]-N-*tert*-butoxycarbonylglycinate (140 mg, 0.29 mmol) dissolved in THF (1.5 ml). The solution was stirred at room temperature for 5 h. The solvents were removed *in vacuo* to yield the *potential inhibitor* (**39**) as a white foam (79 mg, 100%); $[\alpha]_D^{32}$ -70.5 (*c* 1.00, H_2O); λ_{max} (H_2O) / nm 271 (ε 4,614); *m/z* [+ve FAB (glycerol)] 274 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3434-2800 (acid), 1728 and 1669 (C=O); δ_H (^2H_2O) 4.23-4.21 (1H, *t*, *J* 6.3, H-8), 3.89-3.44 (4H, *m*, NCH_2CH_2OH), 3.06 (1H, *dd*, $J_{4A,5}$ 6.7, $J_{4B,4A}$ 17.3, H-4A), 2.73 (1H, *dq*, $J_{5,4A}$ $J_{5,7A}$ $J_{5,7B}$ 6.7, $J_{5,4B}$ 13.3, H-5), 2.52 (1H, *dd*, $J_{4B,5}$ 13.3, $J_{4B,4A}$ 17.1, H-4B), 2.34 (1H, *ddd*, $J_{7A,8}$ 6.3, $J_{7A,5}$ 6.7, $J_{7A,7B}$ 14.4, H-7A) and 1.91 (1H, *ddd*, $J_{7B,8}$ 6.3, $J_{7B,5}$ 6.7, $J_{7A,7B}$ 14.4, H-7B); δ_C (^2H_2O) 171.44 (C-6), 169.97 (acid), 163.55 (acid), 143.19 (C=N), 59.22 ($NCH_2\underline{C}H_2OH$), 52.55 (C-8), 51.06 ($N\underline{C}H_2CH_2OH$), 32.62 (C-5), 30.23 (C-4) and 26.54 (C-7).

The Pyroglutamatepyruvate phenylhydrazone derivative (40, X=H).

Phenylhydrazine hydrochloride (30 mg, 0.21 mmol) and sodium acetate (23 mg, 0.28 mmol) were added to a solution of the pyruvate (**33, R^1=R^2=^{t}Bu**) (80 mg, 0.19 mmol) dissolved in methanol (1.5 ml) under nitrogen. After stirring at room temperature for 1 h, the solvent was removed *in vacuo* to yield a brown oil. Purification was achieved by flash column chromatography on silica gel using petroleum ether/ ethyl acetate (3:1) as eluent. *The phenylhydrazone* (**40, X=H**) was obtained as the *syn* and *anti* isomers as a yellow oil (97 mg, 100%). The *syn* isomer was the thermodynamically more stable product and after 12 h the colour of the oil changed from yellow to red and the ^{1}H-NMR spectrum showed only one isomer, the *syn*, to be present; $[\alpha]_D^{35}$ -9.2 (*c* 1.00, CH_2Cl_2); λ_{max} (MeOH)/nm 341 (ε 3206); *m/z* [+ve FAB (PEGH/NOBA)]: Found: 517.281929 $[M]^+$, $C_{27}H_{39}N_3O_7$ requires 517.278801; and 518.285984 $[M+H]^+$, $C_{27}H_{40}N_3O_7$ requires 518.286626; *m/z* [+ve FAB (3-NBA)]:

540 $[M+Na]^+$, 517 $[M]^+$; ν_{max} (KBr) / cm^{-1} 3296 (N-H), 3056 (=C-H), 1790, 1741 and 1692 (C=O), 1649 (C=N) and 1604 (C=C); δ_H (C^2HCl_3) (*syn*) 12.09 (1H, *s*, =N-NH-Ph), 7.20–6.83 (5H, *m*, ArH), 4.26 (1H, *d*, *J* 9.6, H-2), 3.15-3.06 (2H, *m*, H-6 and H-4), 2.45-2.28 (2H, *m*, H-6 and H-3A), 1.98-1.94 (1H, *m*, H-3B), and 1.46-1.45 (3x9H, 3x*s*, 3x $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 175.62 (lactam), 170.94 (ester), 163.51 (ester), 149.98 (urethane), 143.98 (C-7), 129.70-113.69 (Ar), 83.66, 82.98 and 82.58 ($3xO\underline{C}(CH_3)_3$), 58.36 (C-2), 39.86 (C-4), 34.17 (C-6), 29.47 (C-3) and 28.57-28.36 ($3xOC(\underline{C}H_3)_3$). δ_H (C^2HCl_3) (*anti*, ^{1}H-nmr data from the *syn* / *anti* mixture) 9.53 (1H, *s*, =N-NH-Ph), 7.21–6.86 (5H, *m*, ArH), 4.37 (1H, *d*, *J* 9.0, H-2), 2.93-2.79 (3H, *m*, H-6 and H-4), 2.28 (1H, *dd*, $J_{3A,2}$ 9.0, $J_{3A,3B}$ 12.6, H-3A), 2.05-1.93 (1H, *m*, H-3B), and 1.49-1.40 (3x9H, 3x*s*, 3x*t*-Bu); δ_C (C^2HCl_3) 177.74 (lactam), 170.17 (ester), 165.27 (ester), 149.11 (urethane), 144.02 (C-7), 132.97-113.69 (Ar), 84.26, 83.14 and 81.48 ($3xO\underline{C}(CH_3)_3$), 58.49 (C-2), 41.42 (C-4), 29.39 (C-6), 28.61, 28.35 and 28.27 ($3xOC(\underline{C}H_3)_3$) and 24.40 (C-3).

The Pyroglutamatepyruvate phenylhydrazone derivative (40, X=OMe).

4-Methoxyphenylhydrazine hydrochloride (33 mg, 0.19 mmol) and sodium acetate (21 mg, 0.26 mmol) were added to a solution of the pyruvate (**33, R^1=R^2=^{t}Bu**) (74 mg, 0.17 mmol) in methanol (1 ml) under nitrogen. After stirring at room temperature for 4 h, the solvent was removed *in vacuo* to yield the crude product, which was purified by flash column chromatography on silica gel using petroleum ether / ethyl acetate (3:1) as eluent. *The hydrazone* **(40, X=OMe)** (72 mg, 78%) was obtained as a clear, yellow oil; $[\alpha]_D^{27}$ -8.0 (*c* 1.00, CH_2Cl_2); λ_{max} (MeOH)/nm 357 (ε 8487); *m/z* [+ve FAB (PEGNa/NOBA)]: Found: 547.284214 $[M]^+$, $C_{28}H_{41}N_3O_8$ requires 547.289366; *m/z* [+ve FAB (3-NBA)]: 570 $[M+Na]^+$, 547 $[M]^+$; ν_{max} (film) / cm^{-1} 3257 (NH), 3056 (=C-H), 1793, 1741 and 1720 (C=O), 1638 (C=N), 1594 (C=C); δ_H (C^2HCl_3) 6.95 (2H, *d*, *J* 9.0, ArH), 6.74 (2H, *d*, *J* 9.0, ArH), 4.39 (1H, *d*, *J* 8.7, H-2), 3.70 (3H, *s*, OCH_3), 3.14-3.03 (2H, *m*, H-6A and H-4), 2.40 (1H, *dd*, $J_{6B,4}$ 9.9, $J_{6B,6A}$ 16.5, H-6B), 2.28 (1H, dd, $J_{3A,2}$ 9.0, $J_{3A,3B}$ 13.2, H-3A), 2.02-1.95 (1H, *m*, H-3B), and 1.45-1.44 (3x9H, 3x*s*, 3x $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 175.66 (lactam), 170.97 (ester), 163.71 (ester), 155.24 (C-7), 150.01 (urethane), 137.96-114.82 (Ar), 83.63, 82.68 and 82.55 ($3xOC(\underline{C}H_3)_3$), 58.37 (C-2), 56.02 (OCH_3), 39.99 (C-4), 34.01 (C-6), 29.39 (C-3) and 28.60-28.37 ($3xOC(\underline{C}H_3)_3$).

The Pyroglutamatepyruvate hydrazone derivative (41).

Benzylhydrazine hydrochloride (23 mg, 0.12 mmol) and sodium acetate (27 mg, 0.32 mmol) were added to a solution of the pyruvate (**33, R^1=R^2=^{t}Bu**) (46 mg, 0.11 mmol) in methanol (1.2 ml) under nitrogen. After stirring at room temperature for 2 h, the solvent was removed *in vacuo* to yield an oil. Purification by flash column chromatography on silica gel using petroleum ether / ethyl acetate (3:1) as eluent afforded the hydrazone (**41**) as a clear, colourless oil (37 mg, 65%); $[\alpha]_D^{24}$ -28.5 (*c* 1.00, CH_2Cl_2); λ_{max} (MeOH) / nm 285 (ε 10,622); *m/z* [EI]: Found 531.2953 $[M]^+$, $C_{28}H_{41}N_3O_7$ requires 531.2945; ν_{max} (film) / cm^{-1} 3422 (N-H), 3065 (=C-H), 1790 and 1740 (C=O), 1628 (C=N) and 1599 (C=C); δ_H (C^2HCl_3) 7.26–7.13 (5H, *m*, ArH), 4.54 (1H, *d*, *J* 4.7, CH_2Ph), 4.34 (1H, *d*, *J* 8.4, H-2), 2.78-2.72 (2H, *m*, H-6 and H-4), 2.65-2.60 (1H, *m*, H-6), 2.14 (1H, *dd*, $J_{3A,2}$ 8.4, $J_{3A,3B}$ 13.3, H-3A), 1.97 (1H, *dd*, $J_{3B,4}$ 9.5, $J_{3B,3A}$ 13.3, H-3B) and 1.45, 1.43 and 1.39 (3x9H, 3x*s*, 3x $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 176.50 (lactam), 170.29 (ester), 164.87 (ester), 149.37 (urethane), 138.71 (C-7), 132.10-127.82 (Ar), 84.02, 82.98 and 81.31 ($3xO\underline{C}(CH_3)_3$), 58.36 (C-2), 55.82 (CH_2Ph), 40.24 (C-4), 29.33 (C-6), 28.61, 28.34 and 28.29 ($3xOC(\underline{C}H_3)_3$) and 24.57 (C-3).

The Pyroglutamatepyruvate hydrazone derivative (42).

Phenylethylhydrazine sulfate (27 mg, 0.12 mmol) and NaOAc (27 mg, 0.32 mmol) were added to a solution of the pyruvate (**33, R^1=R^2=^{t}Bu**) (45 mg, 0.11 mmol) in methanol (1.2 ml) under nitrogen. After stirring at room temperature for 2 h, the solvent was removed *in vacuo* to yield an oil. Purification by flash column chromatography on silica gel using petroleum ether / ethyl acetate (3:1) as eluent afforded *the hydrazone* (**42**) as a clear, colourless oil (40 mg, 70%); $[\alpha]_D^{21}$ -21.9 (*c* 1.00, CH_2Cl_2); λ_{max}(MeOH) / nm 285 (ε 13,396); *m/z* [+ve FAB (PEGNa/NOBA)]: Found: 546.317409 $[M+H]^+$, $C_{29}H_{44}N_3O_7$ requires 546.317926; *m/z* [+ve FAB (3-NBA)]: 568 $[M+Na]^+$, 546 $[M+H]^+$; ν_{max} (film) / cm^{-1} 3483 (NH), 3088, 3065 and 3028 (=C-H), 1792 and 1741 (C=O), 1638 (C=N) and 1604 (C=C); δ_H (C^2HCl_3) 7.24–6.97 (5H, *m*, ArH), 4.34 (1H, *d*, $J_{2,3A}$ 8.5, H-2),

3.63-3.56 (2H, *m*, $CH_2\underline{CH_2}Ph$), 2.85 (2H, *t*, *J* 7.6, $\underline{CH_2}CH_2Ph$), 2.74-2.66 (2H, *m*, H-6 and H-4), 2.60-2.55 (1H, *m*, H-6), 2.13 (1H, *dd*, $J_{3A,2}$ 8.5, $J_{3A,3B}$ 13.4, H-3A), 2.00-1.91 (1H, *m*, H-3B) and 1.45, 1.43 and 1.40 (3x9H, 3x*s*, 3x $C(\underline{CH_3})_3$); δ_C (C^2HCl_3) 175.26 (lactam), 168.91 (ester), 163.46 (ester), 147.95 (urethane), 137.99 (C-7), 130.06-124.87 (Ar), 82.64, 81.58 and 79.81 (3x$O\underline{C}(CH_3)_3$), 56.96 (C-2), 51.36 ($CH_2\underline{C}H_2Ph$), 38.84 (C-4), 35.22 ($\underline{C}H_2CH_2Ph$) 28.01 (C-6), 27.22, 26.95 and 26.89 (3x$OC(\underline{C}H_3)_3$) and 23.18 (C-3).

The Pyroglutamatepyruvate Oxime (43).

Hydroxylamine hydrochloride (9 mg, 0.12 mmol) and sodium acetate (14 mg, 0.17 mmol) were added to a solution of the pyruvate (**33**, $\mathbf{R^1=R^2=^tBu}$) (48 mg, 0.11 mmol) in methanol (1 ml) under nitrogen. After stirring at room temperature for 1 h, the solvent was removed *in vacuo* to yield a yellow oil which was purified by flash column chromatography on silica gel using petroleum ether / ethyl acetate (2:1) as eluent. *The oxime* (**43**) was obtained as a clear, colourless oil (40 mg, 82%); $[\alpha]_D^{30}$ -1.1 (*c* 1.00, CH_2Cl_2), λ_{max} (MeOH) / nm 215 (ε 10,116); *m/z* [+ve FAB (PEGNa/NOBA)]: Found: 465.223321 $[M+Na]^+$, $C_{21}H_{34}N_2O_8Na$ requires 465.221286; *m/z* [+ve FAB (3-NBA)]: 465 $[M+Na]^+$, 443 $[M+H]^+$; ν_{max} (film) / cm^{-1} 3338 (O-H) , 1791 and 1720 (C=O) and 1637 (C=N); δ_H (C^2HCl_3) 4.26 (1H, *dd*, $J_{2,3}$ 3.4, $J_{2,3}$ 7.4, H-2), 2.94-2.87 (2H, *m*, H-6A and H-4), 2.78 (1H, *dd*, $J_{6B,4}$ 11.5, $J_{6B,6A}$ 14.6, H-6B), 1.95-1.89 (2H, *m*, H-3A and H-3B) and 1.35, 1.33 and 1.29 (3x9H, 3x*s*, 3x $C(\underline{CH_3})_3$); δ_C (C^2HCl_3) 193.41 (ketone), 174.19 (lactam), 170.52 (ester), 162.50 (ester), 151.09 (C=N), 149.79 (urethane), 83.80, 83.47 and 82.80 (3x$O\underline{C}(CH_3)_3$), 58.09 (C-2), 39.30 (C-4), 28.91 (C-6), 28.36-28.32 (3x$OC(\underline{C}H_3)_3$) and 25.77 (C-3).

The Pyroglutamatepyruvate hydrazone (44).

tert-Butylhydrazine hydrochloride (39 mg, 0.31 mmol) and sodium acetate (200 mg, 2.48 mmol) were added to a solution of the pyruvate (**33**, $\mathbf{R^1=R^2=^tBu}$) (53 mg, 0.124 mmol) in methanol (1.2 ml) under nitrogen. After stirring at room temperature for 3 h the solvent was removed *in vacuo* to yield an oil. Purification by flash column chromatography on silica gel using petroleum ether / ethyl acetate (2:1) as eluent gave the *hydrazone* (**44**), a *syn* and *anti* mixture, as a clear, colourless oil (43 mg, 70%); $[\alpha]_D^{27}$ -20.8 (*c* 1.00, CH_2Cl_2); λ_{max} (MeOH) / nm 302 (ε 761); *m/z* [+ve FAB (PEGH/NOBA)]: Found: 498.316583 $[M+H]^+$, $C_{25}H_{44}N_3O_7$ requires 498.317926; *m/z* [+ve FAB (3-NBA)]: 520 $[M+Na]^+$, 489 $[M+H]^+$; ν_{max} (film) / cm^{-1} 3276 (NH), 1791 1742 (C=O) and 1638 (C=N); δ_H (C^2HCl_3) 9.80 (0.5H, *s*, NH), 6.71 (0.5H, *s*, NH), 4.28-4.24 (1H, 2x*dd*, J_1 1.3, J_2 9.7, $J_{1'}$ 1.1, $J_{2'}$ 9.4, H-2), 2.88-2.81 (1H, *m*, H-6), 2.68-2.53 (1H, *m*, H-4), 2.51-2.48 (0.5H, *m*, H-6A), 2.29-2.20 (0.5H, *m*, H-6B), 2.09-2.01 (1H, *m*, H-3A), 1.92-1.82 (1H, *m*, H-3B), 1.34-1.30 (6x4.5H, 6x*s*, 3xO $C(\underline{CH_3})_3$) and 1.08 and 1.03 (2x4.5H, 2x*s*, N*t*-Bu); δ_C (C^2HCl_3) 176.65 and 175.89 (lactam), 171.03 and 170.42 (ester), 165.20-163.44 (ester), 150.08 and 149.50 (urethane), 130.40 and 122.58 (C=N), 83.93-80.62 (3x$O\underline{C}(CH_3)_3$), 58.37 (C-2), 55.26 and 54.50 ($N\underline{C}(CH_3)_3$), 40.37 and 40.02 (C-4), 33.57 (C-6), 29.26-28.15 (3x$OC(\underline{C}H_3)_3$ and $N(\underline{C}H_3)_3$) and 24.23 (C-3).

The alcohol (45).

The pyruvate (**33**, $\mathbf{R^1=R^2=^tBu}$) (50 mg, 0.12 mmol) and a trace of bromocresol green were dissolved in methanol (1 ml) and sodium cyanoborohydride (8 mg, 0.13 mmol) was added. The solution immediately turned deep blue and a 2 N HCl-MeOH solution 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 (2:1) as eluent. *The alcohol* (**45**) was obtained as a clear colourless oil (34 mg, 66%); *m/z* [+ve FAB (PEGNa/NOBA)]: Found: 452.228517 $[M+Na]^+$, $C_{21}H_{35}NO_8Na$ requires 452.226037; *m/z* [+ve FAB (3-NBA)]: 452 $[M+Na]^+$, 430 $[M+H]^+$; ν_{max} (film) / cm^{-1} 3483 (OH), 1789 and 1740 (C=O); δ_H (C^2HCl_3) 4.45 (1H, *t*, *J* 9.6, H-2), 4.22 (0.5H, *d*, *J* 7.3, H-7), 4.07 (0.5H, *d*, *J* 9.0, H-7), 3.21 (0.5H, *br.s*, -OH), 3.16 (0.5H, *br.s*, -OH), 2.87-2.76 (1H, *m*, H-6), 2.43-2.22 (2H, *m*, H-4 and H-6), 2.12-2.00 (1H, *m*, H-3A), 1.81-1.71 (1H, *m*, H-3B) and 1.48, 1.45 and 1.44 (3x9H, 3x*s*, 3x $C(\underline{CH_3})_3$); δ_C (C^2HCl_3) 175.55 (lactam), 174.34 and 174.16 (ester), 170.62 and 170.54 (ester), 149.77 and 147.97 (urethane), 83.78-82.77 (3x$O\underline{C}(CH_3)_3$), 69.86 and 69.02 (C-7), 58.38 (C-2), 39.70 and 39.03 (C-4), 35.71 and 35.44 (C-6), 29.86 and 29.49 (C-3) and 28.41-28.33 (3x$OC(\underline{C}H_3)_3$).

Benzyl (*2S, 4S*)-N-*tert*-butoxycarbonyl-4-(2-*tert*-butoxycarbonyl-prop-2-enyl)-pyroglutamate (46a) and **Benzyl (*2S, 4RS*)-N-*tert*-butoxycarbonyl-4-(2-*tert*-butoxycarbonyl-prop-2-enyl)-pyroglutamate (46b).**

Lithium hexamethyldisilazide (1 M in THF, 1.88 ml, 1.88 mmol) was added to a solution of benzyl (*2S*)-N-*tert*-butoxycarbonylpyroglutamate (**31**, R^1=PhCH$_2$) (499 mg, 1.56 mmol) in THF (5 ml) stirred at -78 °C under nitrogen. The mixture was stirred at -78 °C for 1 h and *tert*-butyl 2-(bromomethyl)acrylate (380 mg, 1.72 mmol) was added. Stirring was continued for further 2 h at -78 °C and saturated aqueous ammonium chloride (15 ml) was added. The solution was extracted with diethyl ether (3x15 ml). The organic phases were dried (Na_2SO_4) and the solvent was removed *in vacuo*. The crude product was isolated as a yellow oil which was purified by column chromatography on silica gel using petroleum ether / ethyl acetate (4:1) as eluent. *Benzyl (2S, 4R/S)-N-tert-butoxycarbonyl-4-(2-tert-butoxycarbonyl-prop-2-enyl)-pyroglutamate* (**46**) was isolated in 53% yield. The major, *trans* isomer (2S/4S) was isolated as one stereoisomer as a white solid. The minor, *cis* stereoisomer (*2S/4R*) could not be separated from the *trans* isomer. The ratio was *ca.* 24:1 in favour of the *trans* stereoisomer. *Benzyl (2S, 4S)-N-tert-butoxycarbonyl-4-(2-carboxymethyl-prop-2-enyl)-pyroglutamate* (**46a**) m.p. 82-84 °C; $[\alpha]_D^{36}$ -15.2 (*c* 1.00, CH_2Cl_2); Found: C, 65.3; H, 7.3; N, 3.0. $C_{25}H_{33}NO_7$ requires C, 65.3; H, 7.2; N, 3.1%; *m/z* [+ve FAB (3-NBA)]: 482 $[M+Na]^+$, 460 $[M+H]^+$; ν_{max} (KBr) / cm^{-1} 3120, 3037 and 3005 (=C-H), 1778, 1736 and 1710 (C=O) and 1630 (C=C); δ_H (C^2HCl_3) 7.19 (5H, *s*, ArH), 5.98 (1H, *s*, olefinic), 5.37 (1H, *s*, olefinic), 5.04 (2H, *dd*, J_1 12.2, J_{AB} 15.6, CH_2Ph), 4.43 (1H, *d*, $J_{2,3A}$ 8.7, H-2), 2.82-2.68 (2H, *m*, H-6A and H-4), 2.11 (1H, *dd*, $J_{6B,4}$ 9.2, $J_{6A,6B}$ 14.0, H-6B), 1.99 (1H, *dd*, $J_{3A,2}$ 8.7, $J_{3A,3B}$ 12.9, H-3A), 1.87-1.80 (1H, *m*, H-3B) and 1.31 and 1.27 (2x9H, 2x*s*, 2x C(C$\underline{H}_3$)$_3$); δ_C (C^2HCl_3) 174.55 (lactam), 171.44 (ester), 166.29 (ester), 149.79 (urethane) 139.25 (C-7), 135.45-128.90 (Ar), 126.92 (C-8), 84.03 and 81.48 (2xO$\underline{C}$(CH$_3$)$_3$), 67.78 (CH_2Ph), 57.43 (C-2), 41.61 (C-4), 33.00 (C-6), 28.74 (C-3) and 28.44 and 28.22 (2xOC($\underline{C}$H$_3$)$_3$). *Benzyl (2S, 4R)-N-tert-butoxycarbonyl-4-(2-tert-butoxycarbonyl-prop-2-enyl)-pyroglutamate* (**46b**) (^{1}H-NMR data from the *cis / trans* mixture) δ_H (C^2HCl_3) 7.28 (5H, *s*, ArH), 6.06 (1H, *s*, olefinic), 5.45 (1H, *s*, olefinic), 5.18-5.08 (2H, *m*, CH_2Ph), 4.53-4.46 (1H, *m*, H-2), 2.90-2.85 (2H, *m*, H-6 and H-4), 2.40-2.29 (1H, *m*, H-6), 2.23-2.15 (1H, *m*, H-3A), 1.95-1.84 (1H, *m*, H-3B) and 1.39 and 1.36 (2x9H, 2x*s*, 2x C(C$\underline{H}_3$)$_3$).

The (*2S,4S*) Pyroglutamtepyruvate (47).

A solution of benzyl (*2S, 4S*)-*N-tert*-butoxycarbonyl-4-(2-carboxymethyl-prop-2-enyl)-pyroglutamate (**46a**) (1.635 g, 3.56 mmol) in CH_2Cl_2 (40 ml) was cooled to -78 °C and oxygen was passed through for 20 min. Ozone was then passed through the solution for 15 min, during which time it turned blue. The reaction was quenched by adding triphenylphosphine (1.027 g, 3.91 mmol) at -78 °C. The solution was allowed to warm slowly to room temperature. The solvent was removed *in vacuo* and the oil was purified by flash column chromatography on silica gel using petroleum ether / ethyl acetate (2:1) as eluent. *The pyruvate* (**47**) was obtained as a clear oil (1.607 g, 98%), which crystallised to give a white solid; m.p. 86-89 °C; $[\alpha]_D^{36}$ -20.3 (*c* 1.00, CH_2Cl_2); *m/z* [CI)]: Found: 462.2128 $[M+H]^+$, $C_{24}H_{32}NO_8$ requires 462.2127; *m/z* [+ve FAB (3-NBA)]: 484 $[M+Na]^+$, 462 $[M+H]^+$; ν_{max} (KBr) / cm^{-1} 1793, 1747 and 1724 (C=O); δ_H (C^2HCl_3) 7.29 (5H, *s*, ArH), 5.15 (2H, 2*xd*, J_{AB} 12.2, -CH_2Ph), 4.58 (1H, *d*, *J* 9.7, H-2), 3.35 (1H, *dd*, $J_{6A,4}$ 3.0, $J_{6A,6B}$ 19.3, H-6A), 3.03-2.96 (1H, *m*, H-4), 2.88 (1H, *dd*, $J_{6B,4}$ 8.6, $J_{6A,6B}$ 19.3, H-6B), 2.33 (1H, *dd*, $J_{2,3A}$ 8.8, $J_{3A,3B}$ 13.2, H-3A), 1.92-1.88 (1H, *m*, H-3B) and 1.47 and 1.35 (2x9H, 2x*s*, 2x C(C$\underline{H}_3$)$_3$); δ_C (C^2HCl_3) 192.67 (ketone), 173.56 (lactam), 170.65 (ester), 159.25 (ester), 148.96 (urethane), 134.78-128.31 (Ar), 84.36 and 83.65 (2x O$\underline{C}$(CH$_3$)$_3$), 67.32 (CH_2Ph), 57.06 (C-2), 39.75 (C-6), 37.01 (C-4), 28.02 (C-3) and 27.58 and 27.56 (2xOC($\underline{C}$H$_3$)$_3$).

Benzyl (*3S, 5RS, 7S*)-[(1-amino-2-oxo-5-hydroxy-5-*tert*-butyloxycarbonyl-pyrrolidin-3-yl)methyl]-N-*tert*-butoxycarbonylglycinate (48) and **Benzyl (*5S, 8S*)-[(3-*tert*-butoxycarbonyl-4,5-dihydro-1*H*-6-oxopyridazine-5-yl)methyl]-N-*tert*-butoxycarbonylglycinate (49).**

Hydrazine hydrate (64-65%) (45 mg, 0.58 mmol) was added to a solution of the pyruvate (**47**) (243 mg, 0.53 mmol) in methanol (3 ml) under nitrogen. After stirring at room temperature for 1 h, the solvent was removed *in vacuo* to yield the crude product as a white foam. Purification of the products was achieved on silica gel using petroleum ether / ethyl acetate (1:2) as eluent. *Benzyl (3S, 5RS, 7S)-[(1-amino-2-oxo-5-hydroxy-5-tert-*

butoxycarbonyl-pyrrolidin-3-yl)methyl]-N-tert-butoxycarbonylglycinate (**48**) was obtained as a white solid (214 mg, 82%); m.p. 137-139 °C; Found: C, 58.5; H, 7.2; N, 8.4. $C_{24}H_{35}N_3O_8$ requires C, 58.4; H, 7.2; N, 8.5%; *m/z* [+ve FAB (3-NBA)]: 516 $[M+Na]^+$, 494 $[M+H]^+$; ν_{max} (KBr) / cm^{-1} 3343 (NH), 3291 (OH), 3064 and 3035 (=C-H), 1742, 1705 and 1688 (C=O) and 1631 (C=C); δ_H (C^2HCl_3) 7.49 (5H, *s*, ArH), 6.05 (1H, *d*, *J* 7.9, NHBoc), 5.23-5.03 (2H, *2xd*, J_1 12.3, CH_2Ph), 4.77 and 4.74 (1H, 2xs, 2xOH), 4.65-4.63 (1H, *m*, H-7), 4.07 and 4.01 (2H, 2xs, NH_2), 2.91-2.75 (1H, *m*, H-3), 2.65-2.34 (2H, *m*, H-4 and H-6A), 2.25-2.00 (2H, *m*, H-4 and H6B) and 1.58 (2x9H, *s*, 2x $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 175.41 and 174.47 (C-2), 172.34 and 172.26 (ester), 170.57 (ester), 155.87 (urethane), 135.71-128.63 (Ar), 88.79, 88.39, 85.03 and 84.91 (2x$O\underline{C}(CH_3)_3$), 80.34 (C-5), 67.65 and 67.55 (-CH_2Ph), 52.84 (C-7), 37.15 and 35.57 (C-3), 36.46 and 36.16 (C-4), 34.79 and 34.55 (C-6) and 28.73 and 28.17 (2x$OC(\underline{C}H_3)_3$). *Benzyl (5S, 8S)-[(3-tert-butoxycarbonyl-4,5-dihydro-1H-6-oxopyridazine-5-yl)methyl]-N-tert-butoxycarbonyl glycinate* (**49**) was obtained as a colourless oil (21 mg, 8.4%); $[\alpha]_D^{32}$ -95.5 (*c* 1.00, CH_2Cl_2); λ_{max} (MeOH)/nm 267 (ε 5668); *m/z* [CI]: Found: 476.2397 $[M+H]^+$, $C_{24}H_{34}N_3O_7$ requires 476.2396; *m/z* [+ve FAB (3-NBA)] 498 $[M+Na]^+$, 476 $[M+H]^+$; ν_{max} (film) / cm^{-1} 3314 (NH), 1744 and 1713 (C=O) and 1620 (C=N); δ_H (C^2HCl_3) 8.67 (1H, *br.s*, =N-NH-), 7.31-7.22 (5H, *s*, ArH), 5.37 (1H, *d*, *J* 7.8, NHBoc), 5.11 (2H, *2xd*, J 12.2, CH_2Ph), 4.43-4.41 (1H, *m*, H-8), 3.00 (1H, *d*, *J* 10.6, H-4A), 2.38-2.24 (3H, *m*, H-4B, H-5 and H-7A), 1.89-1.87 (1H, *m*, H-7B) and 1.49 and 1.35 (2x9H, 2x*s*, 2x $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 172.22 (C-6), 169.91 (ester), 162.18 (ester), 155.88 (urethane), 145.12 (C=N), 135.65-126.26 (Ar), 83.61 and 80.54 (2x$O\underline{C}(CH_3)_3$), 67.70 (-CH_2Ph), 52.14 (C-8), 32.72 (C-5), 32.11 (C-4), 28.69 and 28.40 (2x$OC(\underline{C}H_3)_3$) and 26.87 (C-7).

Benzyl (*5RS, 8S*)-[(3-*tert*-butoxycarbonyl-4,5-dihydro-1*H*-6-oxopyridazine-5-yl)methyl]-N-*tert*-butoxycarbonylglycinate (49).

Benzyl (*3S, 5RS, 7S*)-[(1-amino-2-oxo-5-hydroxy-5-*tert*-butoxycarbonyl-pyrrolidin-3-yl)methyl]-N-*tert*-butoxycarbonylglycinate (**48**) (247 mg, 0.50 mmol) was dissolved in acetonitrile (3 ml). The solution was heated to reflux in the presence of 3Å molecular sieves (500 mg) and acetic acid (300 mg, 5.00 mmol) for 2 days. The molecular sieves were filtered off, the solvents removed *in vacuo* and the resultant oil was purified by flash column chromatography on silica gel, eluting with a 1:1 mixture of petroleum ether and ethyl acetate, to yield the *pyridazine* (**49**) as a mixture of stereoisomers, as a clear, colourless oil (230 mg, 97 %); (^{1}H-NMR data as a mixture of stereoisomers - other data see **49** ***trans*** above) δ_H (C^2HCl_3) 9.03 (1H, *br.s*, =N-NH-), 7.27 (5H, *s*, ArH), 5.43 (0.5H, *d*, *J* 7.4, NHBoc), 5.26 (0.5H, *d*, *J* 7.8, NHBoc), 5.18-5.03 (2H, *m*, -CH_2Ph), 4.63-4.06 (1H, *m*, H-8), 3.18 (0.5H, *d*, *J* 11.9, H-3), 3.00 (0.5H, *d*, *J* 10.8, H-3), 2.43-2.25 (3H, *m*, H-3, H-5 and H-7A), 1.89-1.87 (1H, *m*, H-7B) and 1.49 and 1.35 (2x9H, 2x*s*, 2x $C(C\underline{H}_3)_3$).

The (2S,4S) Pyruvylpyroglutamate (50).

The pyruvate (**47**) (1.75 g, 3.8 mmol) and 5% Pd on carbon catalyst (170 mg) were stirred together in ethyl acetate (20 ml) in an atmosphere of hydrogen for 30 h at room temperature. The catalyst was removed by filtration over Celite and the solvent was removed *in vacuo* to yield *the pyruvate* (**50**) as a colourless oil (1.41 g, 100%), which formed a white foam *in vacuo*; $[\alpha]_D^{23}$ -17.3 (*c* 1.00, CH_2Cl_2); Found: C, 54.3; H, 6.9; N, 3.6. $C_{17}H_{25}NO_8$ requires C, 54.9; H, 6.8; N, 3.8%; m/z [+ve FAB (PEGH/NOBA)] Found 394.145778 $[M+Na]^+$ $C_{17}H_{25}NO_8Na$ requires 394.147787; *m/z* [+ve FAB (3-NBA)]: 394 $[M+Na]^+$, 372 $[M+H]^+$; ν_{max} (Nujol) / cm^{-1} 3201-2800 (acid), 1793, 1735 and 1724 (C=O); δ_H (C^2HCl_3) 8.96 (1H, *br.s*, -COO$\underline{H}$), 4.60 (1H, *d*, *J* 9.7, H-2), 3.36 (1H, *dd*, $J_{6A,4}$ 3.0, $J_{6A,6B}$ 19.2, H-6A), 3.07-3.00 (1H, *m*, H-4), 2.93 (1H, *dd*, $J_{6B,4}$ 8.3, $J_{6A,6B}$ 19.2, H-6B), 2.41 (1H, *dd*, $J_{2,3A}$ 8.8, $J_{3A,3B}$ 13.2, H-3A), 2.04-1.93 (1H, *m*, H-3B) and 1.47 and 1.44 (2x9H, 2x*s*, 2x $C(C\underline{H}_3)_3$); δ_C (C^2HCl_3) 192.26 (ketone), 175.68 (lactam), 173.18 (acid), 158.85 (ester), 148.74 (urethane), 84.11 and 83.68 (2x$O\underline{C}(CH_3)_3$), 56.31 (C-2), 39.30 (C-6), 36.66 (C-4), 27.31 (C-3) and 27.19 (2x$OC(\underline{C}H_3)_3$).

Wang-Resin Bound Pyruvate (51).

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) (80 mg, 0.42 mmol) dissolved in dichloromethane (3 ml) was added at 0 °C to a solution of the pyruvate (**50**) (155 mg, 0.42 mmol) in CH_2Cl_2 (4 ml) under nitrogen. The mixture was stirred at 0 °C for 30 min. Wang-resin, which was washed with dimethyl formamide,

dichloromethane, methanol and allowed to swell for 40 min in dichloromethane was added, followed by DMAP and the reaction mixture was shaken for 2 h at room temperature under nitrogen. The resin was washed with dichloromethane and diethyl ether and dried *in vacuo*: *m/z* [MALDI-tof in TFA]: 216.2 [M-Boc-*t*-Bu]$^+$.

Wang-Resin Bound Products (52) and (53).
Wang-resin bound pyruvate (**51**) (0.14 mmol) was swollen in CH_2Cl_2 (1.5 ml) for 10 min, before methanol (1 ml) and hydrazine hydrate (35 mg, 0.7 mmol) were added under nitrogen. The reaction mixture was shaken for 4 h at room temperature. The resin was washed with CH_2Cl_2 and diethyl ether and dried *in vacuo*. **53**: *m/z* [MALDI-tof in TFA]: 229.99 [M-Boc-*t*-Bu]$^+$; **52**: *m/z* [MALDI-tof in TFA]: 247.97 [M-Boc-*t*-Bu]$^+$.

Wang-Resin Bound Product (53).
Wang-resin bound products (**52**) and (**53**) (0.14 mmol) were suspended in acetonitrile. The mixture was heated to reflux in the presence of pTSA (133 mg, 0.7 mmol) for 24 h. MALDI-tof analysis in the presence of TFA showed that the mass-peak relating to (**52**) had disappeared, but the peak for (**53**) was still present.

(5RS, 8S)-[(3-carboxy-4,5-dihydro-1*H*-6-oxopyridazine-5-yl)methyl]-glycine (36).
Wang-resin bound product (**53**) (0.14 mmol) was swollen in dichloromethane (1 ml) for 10 minutes, TFA (2 ml) was added and the reaction mixture was shaken for four hours at room temperature. The resin was filtered off and the solvents were removed *in vacuo*. The spectra of the product were identical with those of the sample prepared above by solution phase methods.

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