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Stereoselective Synthesis of Non-Proteinogenic Amino Acids[1]

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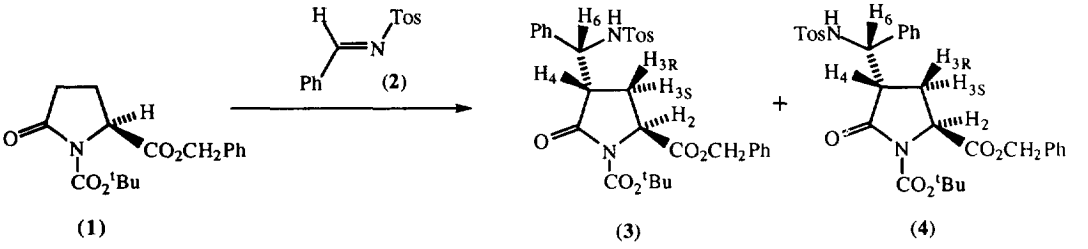
Abstract - The lactam enolate of the protected pyroglutamic acid ester (**1**) has been shown to react with activated imines to yield optically pure derivatives of non-proteinogenic amino acids. © 1997 Elsevier Science Ltd.

There has recently been considerable interest both in naturally-occurring non-proteinogenic amino acids[2,3] and in unnatural synthetic amino acids[4] for their antimetabolite properties and their ability to impart protease resistance and to induce conformation when incorporated into proteins. Our use of pyroglutamic acid for the stereospecific synthesis of such compounds was first manifest in an investigation of the reaction of activated imines with the protected amino acid (**1**)[1] and we now report these results in full. Since these early studies we have used the synthon to prepare stereospecifically labelled leucine[5] which we have used to assign the methyl groups of the leucine residues in the enzyme dihydrofolate reductase.[6] We have also developed versatile syntheses of glutamate agonists and antagonists[7] and a stereospecific approach to 4-alkylglutamates and 4-alkylprolines from it.[8] Other workers have examined the stereospecificity of aldol reactions at C-4 of protected pyroglutamate[9,10] and the outcome of alkylation reactions at this centre has been studied.[11-13]

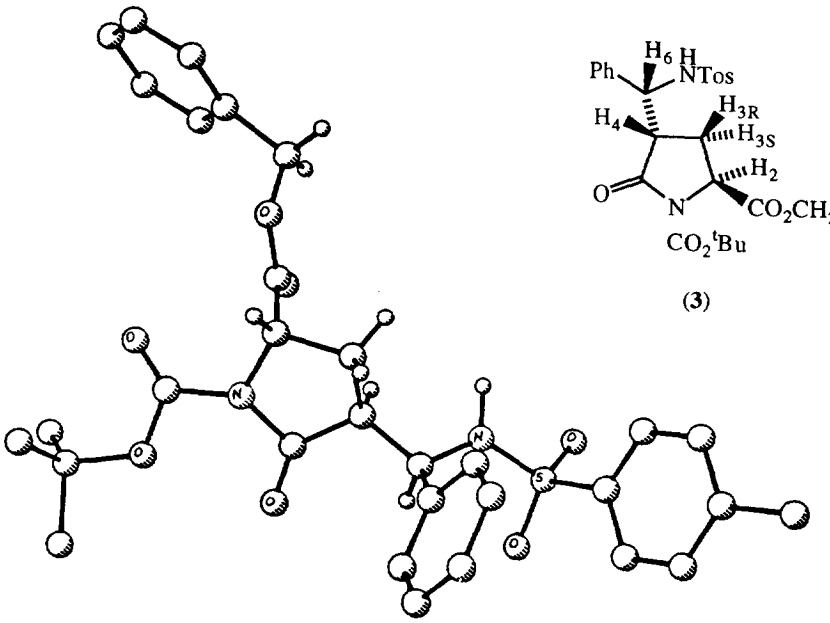
When the diprotected pyroglutamic acid derivative (**1**)[5] was reacted with lithium hexamethyldisilazide in tetrahydrofuran and the resultant anion was reacted with the activated imine (**2**)[14] for one hour at -78 °C, a mixture of two diastereoisomeric products was obtained in 87% yield in a ratio of 4:1 by ^{1}H-NMR spectroscopy as shown in Scheme 1.



Scheme 1

Chromatography and recrystallisation gave the pure major isomer (**3**) in 45% yield and an impure sample of the minor isomer (**4**). Since both isomers exhibited n.O.e.s between H-2 and H-3S and H-4 and

H-3R, the stereochemistry at C-4 was *trans* with respect to the centre C-2 in each case and so reaction had occurred from the less hindered face of the pyroglutamic acid moiety. The compounds were epimeric at C-6 although there was evidently a high degree of stereoselectivity at this centre in the reaction. The stereochemistry of the major isomer was assigned to be (2S,4S,6R) as shown in structure (**3**) by a single crystal X-ray structure determination, the result of which is shown in Figure 1 and so the minor isomer had the (2S,4S,6S) stereochemistry as in structure (**4**).

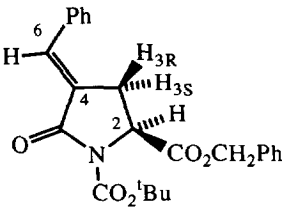


(**3**)

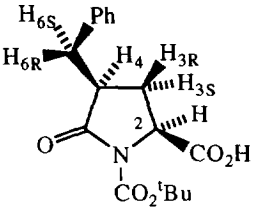
Figure 1 : X-ray crystal structure of the major product (**3**) from the reaction of the *trans* activated imine (**2**) with the anion of the protected pyroglutamic acid derivative (**1**).

It was noted that if the reaction of the protected pyroglutamate (**1**) with the imine (**2**) was allowed to warm to room temperature before quenching then two new products were obtained. These compounds were also produced when the compound (**3**) or a mixture of compounds (**3**) and (**4**) was treated with one equivalent of LiHMDS at -78 °C and warmed to room temperature. One compound was evidently *para*-toluenesulfonamide and the other was a solid with λ_{max} 286 nm (ε 30,900). This and the other spectra suggested that elimination had occurred to yield the conjugated enone (**5**). The low field shift (δ 7.56 ppm) of the vinylic proton was in keeping[15] with its assignment as the *(E)*-isomer (**5**) and this was confirmed by n.O.e. experiments. Irradiation at δ 7.56 ppm for the vinylic proton gave enhancements only in the neighbouring aromatic protons (δ 7.41 ppm) whereas irradiation at δ 3.35 ppm for H-3S produced enhancements in the aromatic protons but not in the vinyl proton. As only one isomer was obtained in this reaction, irrespective of the stereochemistry at C-6 in the starting material, the stereochemistry of the product must be determined by repulsion of the phenyl and carbonyl groups in the transition state leading to (**5**). Hydrogenation of the product (**5**) gave one diastereoisomeric product (**6**) in 80% yield. This was shown to be the *cis* product, expected for hydrogenation from the less hindered side of the molecule since irradiation at δ 2.39 ppm for H-3S led to enhancements of the resonances due

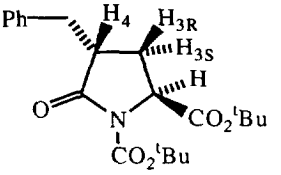
to H-4 at δ 2.89 ppm and to H-2 at δ 4.50 ppm. This was epimeric with the *trans*-ester (**7**) prepared by Baldwin[11] by alkylation of a suitably protected pyroglutamic acid derivative. When deuterium gas was substituted in the hydrogenation experiment, again one diastereoisomer (**6, H-4=H-6S=^{2}H**) was produced in 70% yield, the proton at δ 3.33 ppm for H-6S being much diminished. There was *ca.* 10% of the undeuteriated compound present.



(**5**)

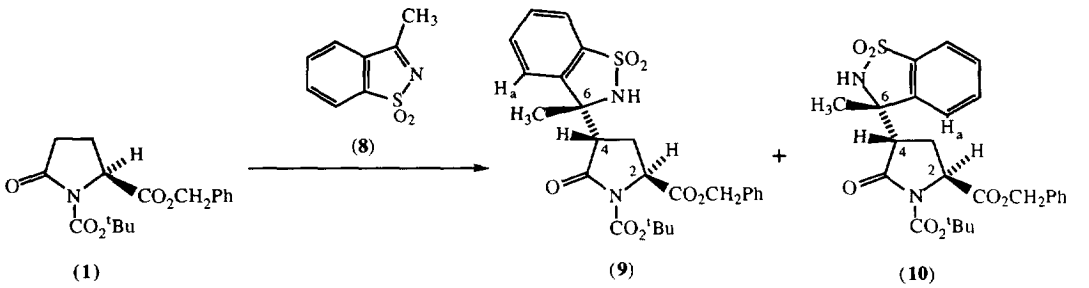


(**6**)



(**7**)

The high degree of stereoselectivity at both centres in this reaction was intriguing and, since the activated imine (**2**) was expected to have the *(E)*-geometry shown,[16] investigation of the fate of a *(Z)*-imine in this reaction was of interest. The cyclic imine (**8**) was therefore prepared[17] and reacted with the enolate of the pyroglutamic acid derivative (**1**) to yield two diastereoisomeric adducts (**9**) and (**10**) in a ratio of 5:2 respectively. The ^{1}H-NMR spectra suggested a *trans* relationship between the substituents at C-2 and C-4 in both products and this was borne out by n.O.e. studies since, in each case, H-3S was clearly identified by its n.O.e. when H-2 was irradiated but, when H-4 was irradiated, it was the proton H-3R which exhibited an n.O.e. in both isomers.



(**1**) (**8**) (**9**) (**10**)

Scheme 2

Interestingly it was also possible to identify the stereochemistry at the apparently conformationally more mobile centre C-6 in each isomer by NMR techniques since each isomer appeared to exist mainly in one major conformation in $C_6{}^2H_6$ solution. In the major isomer (**9**) the conformation shown in Figure 2 with the C-6 methyl group in the 6R configuration was indicated by n.O.e.s between H-4 at δ 3.10 ppm and *both* the N-H at δ 5.25 ppm *and* the methyl group attached to C-6 at δ 1.39 ppm. In the minor isomer (**10**), an n.O.e between H-4 at δ 2.91 ppm and the C-6 methyl group at δ 1.68 ppm indicated the conformation shown in Figure 2. The fact that an n.O.e. was observed between the aromatic doublet at δ 6.33 ppm and *both* H-3R at δ 1.23 ppm *and* H-4 at δ 2.91 ppm for the minor isomer, and that this was not apparent for the major isomer implied 6S stereochemistry in the minor isomer.

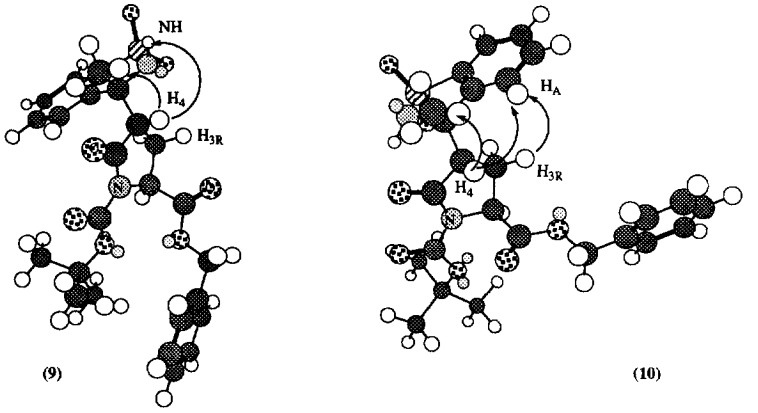


Figure 2. The conformations and stereochemistries at C-6 of the major (**9**) and minor (**10**) isomers from the reaction described in Scheme 2 as deduced by the n.O.e. experiments indicated.

These experiments indicated that the major isomer was the (2S,4S,6R) isomer and this was confirmed when the compound was hydrogenolysed to yield the free acid (**11**) which was converted to the dicyclohexylamine salt. The X-ray crystal structure of this salt is shown in Figure 3. The conformation adopted by the salt in the solid state is different from that deduced for the ester (**9**) in $C_6{}^2H_6$ solution since the C-6 methyl and C-4 hydrogen are in a *transoid* relationship in the solid salt whereas they appear to be in a *cisoid* in the ester in solution.

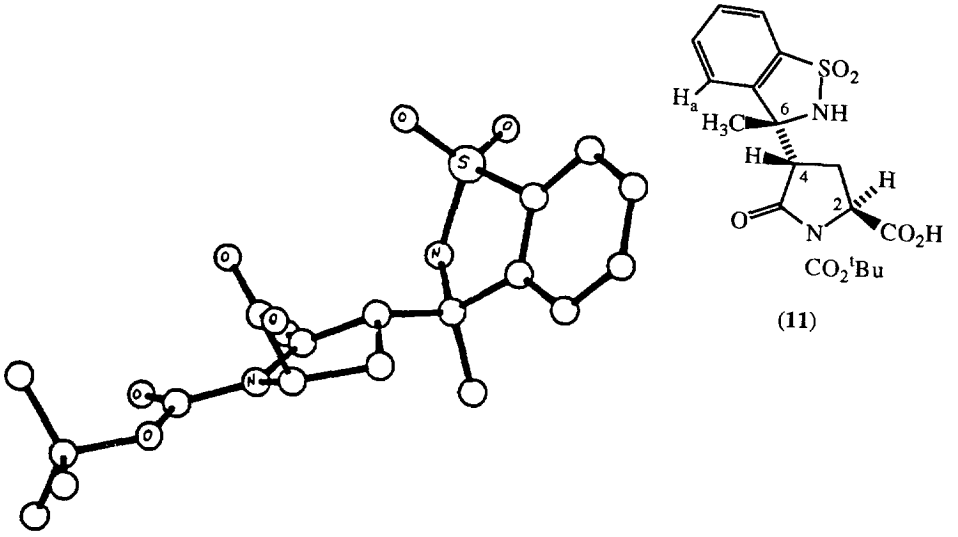
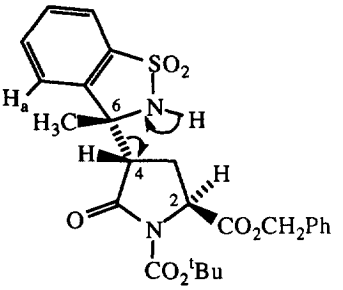


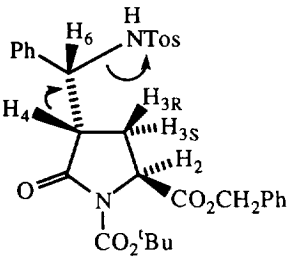
Figure 3. X-ray crystal structure of the anion of the dicyclohexylamine salt of the acid (**11**) derived from the major isomer (**9**) from the reaction shown in Scheme 2.

Since treatment of the adduct (**3**) with base had led to elimination of *para*-toluenesulfonamide to give the product (**5**), it was of interest to see how the cyclic adduct (**9**) would behave on similar treatment. In this case, reaction with lithium hexamethylsilazide resulted in a retro-aldol type reaction initiated by base catalysed removal

of the NH proton as in **(12)** below, rather than the elimination shown in **(13)** below initiated by removal of the proton H-4.



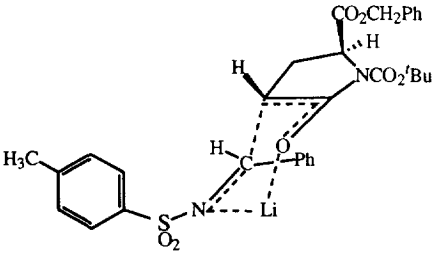
(12)



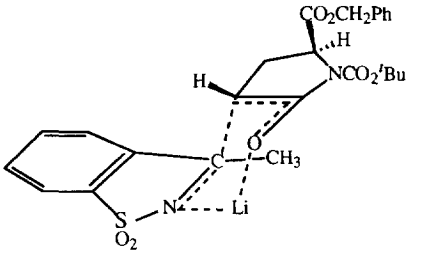
(13)

The 4α-stereoselectivity of the reactions with imines reported here contrasts with the subsequent report by Dikshit and Panday that the corresponding reaction of the lithium enolate of protected pyroglutamic esters with aromatic aldehydes[9] gave mixtures of 4α- and 4β-substituted products, with the 4α-adduct as the major product. Later studies by this group achieved 4α-selectivity by use of the titanium enolate[10] but no indication of the degree of stereoselectivity at C-6 was given.

The C-4 stereospecificity in these reactions follows from the expected steric control from C-2 of the pyroglutamic acid moiety, but the question of stereoselectivity at C-6 is less immediately apparent. If chair like transition states **(14)** and **(15)** are assumed for the two reactions,[16] then the kinetically favoured product from the reaction of the *(E)*-imine **(2)** would be the 6S isomer **(4)**. However the bulk of the phenyl group in **(14)** would suggest that the product of thermodynamic control is the 6R isomer **(3)** which is in fact the major product found in the reaction. The major isomer **(9)** from the reaction of the *(Z)*-imine **(8)** has 6R stereochemistry and this is the product expected for either kinetic or thermodynamic control.



(14)



(15)

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected and optical rotations (given in units of 10^{-1} deg cm^2 gm^{-1}) were measured on a Perkin Elmer PE241 polarimeter using a 1 dm path length microcell. IR spectra were recorded on a Perkin Elmer 1720 Fourier transform instrument and UV spectra on a Philips PU8720 spectrophotometer or a Perkin Elmer PE 330 instrument. ^{1}H-NMR spectra were recorded on Bruker WM360 (360 MHz) or AC-200 (200 MHz) Fourier transform instruments. J values are given in Hz.

The residual solvent peak was used as reference for all NMR spectra. Mass spectra were recorded on Kratos MS80, MS25, MS50 instruments and accurate mass-measurement on a VG7070 instrument by Dr. S. Chotai, Wellcome Research Laboratories, beckenham. Microanalyses were performed by Miss K. Plowman and Miss M. Patel at Sussex University and by Mrs. P. Firmin (Wellcome Research Laboratories). Thin layer chromatography was carried out using Merck Kieselgel 60 F_{254} pre-coated silica gel plates of thickness 0.2 mm (ART 5554 and ART 5714) and column chromatography was performed using Merck Kieselgel 60 (230 - 400 mesh — ART 9385).

*Benzyl (2S,4S,1'R)-N-*tert*-butoxycarbonyl-4-(1'-N-*para*-toluenesulfonamidobenzyl)pyroglutamate* **(3)** — *n*-Butyllithium (2.5M in hexane, 230 µl; 0.575 mmol) was added to a solution of hexamethyldisilazane (121 µl; 0.57 mmol) in dry tetrahydrofuran (5 ml) at -78 °C, under nitrogen. The mixture was stirred for 1 hour and a solution of benzyl N-*tert*-butoxycarbonyl-(2S)-pyroglutamate **(1)**[5] (160 mg; 0.50 mmol) in tetrahydrofuran (2 ml) was added. Stirring was continued at -78 °C and N-benzylidinetoluenesulfonamide **(2)**[14] (129 mg, 0.50 mmol) in tetrahydrofuran (5 ml) was added. The reaction was stirred for a further 1 hour at -78 °C and quenched with saturated aqueous ammonium chloride (3 ml). The mixture was allowed to warm to room temperature and diluted with diethyl ether / water (3 : 1; 16 ml). The organic layer was washed to neutrality with water and brine and dried (Na_2SO_4). Removal of the solvent *in vacuo* yielded a white foam (87 %), the ^{1}H-NMR spectrum of which showed the presence of a diastereoisomeric mixture in a ratio of 4:1 by integration of the resonances assigned to H-2; (Found C, 64.3; H, 5.8; N 4.8. $C_{31}H_{34}N_2O_7S$ requires C, 64.3: H, 5.9; N, 4.8%); m/z (+ve FAB, 3-NBA, xenon) 579 $[M+H]^+$; ν_{max} (film, cm^{-1}) 1788 and 1746 ("imide" and ester). The major isomer *benzyl (2S,4S,1'R)-N-*tert*-butoxycarbonyl-4-(1'-N-toluenesulfonylamidobenzyl)pyroglutamate* **(3)** was obtained pure by chromatography on silica gel, using chloroform / petroleum ether 60-80° (3:1) as eluant, and repeated recrystallisation from ethyl acetate / hexane (130 mg; 45%); m p 168-170 °C; $[\alpha]_D^{29}$ +19.5 (c 0.40, $CHCl_3$); δ_H (360 MHz, C^2HCl_3) 7.5-7.0 (14H, overlapping m, Ar*H*), 6.37 (1H, d, $J_{NH,6}$ 4.0, TosN*H*), 5.14 (2H, s, PhCH_2), 4.46 (1H, dxd, $J_{2,3S}$ 9.6, $J_{2,3R}$ 1.2, *H*-2), 4.26 (1H, dxd, $J_{6,4}$ 7.1, $J_{6,NH}$ 4.0, *H*-6), 3.02 (1H, m, *H*-4), 2.05 (1H, dxdxd, $J_{3S,3R}$ 13.5, $J_{3S,4}$ 12, $J_{3S,2}$ 9.6, *H*-3S), 1.78 (1H, dxdxd, $J_{3R,3S}$ 13.5, $J_{3R,4}$ 8.7, $J_{3R,2}$ 1.2, *H*-3R), and 1.39 (9H, s, $(CH_3)_3CO$). The 2,4-*trans*-stereochemistry was confirmed by n.O.e. experiments in C^2HCl_3 since irradiation at δ 4.46 ppm for H-2 caused a 6% enhancement in H-3S at δ 2.05 ppm and irradiation at δ 3.02 ppm for H-4 caused a 6% enhancement in H-3R at δ 1.78 ppm. The minor isomer *benzyl (2S,4S,1'S)-N-tert-butoxycarbonyl-4-(1'-N-toluenesulfonylamidobenzyl)pyroglutamate* **(4)** could not be obtained pure but the spectrum was obtained by subtraction, δ_H (360 MHz in C^2HCl_3) 7.5-7.0 (14H, m, Ar*H*), 6.78 (1H, d, $J_{NH,6}$ 9, TosN*H*), 5.20 (2H, AB, J_{AB} 11, PhCH_2), 4.61(1H, dxd, $J_{6,NH}$ 9.0, $J_{6,4}$ 4.7, *H*-6), 4.17 (1H, dxd, $J_{2,3S}$ 9.6, $J_{2,3R}$ 2.2, *H*-2), 3.21 (1H, m, *H*-4), 2.05 (1H, m, obscured by major isomer, *H*-3R), 1.90 (1H, m, *H*-3S), and 1.34 (9H, s, $(CH_3)_3CO$). N.O.e. experiments carried out on the crude mixture in C^2HCl_3 confirmed 2,4-*trans*-stereochemistry since irradiation at δ 4.17 ppm for H-2 caused a 2% enhancement of H-3S at δ 1.90 ppm and irradiation at H-4 at δ 3.21 ppm caused a 2% enhancement at H-3R at δ 2.05 ppm.

*X-ray data on benzyl (2S,4S,1'R)-N-*tert*-butoxycarbonyl-4-(1'-N-toluenesulfonylamidobenzyl)pyroglutamate* **(3)** — Crystals [Rigaku AFC5R diffractometer with graphite monochromated Cu Kα radiation and a 12 kW rotating anode generator] are monoclinic, $P2_1$ (No 4), a = 14.242 (4), b = 6.179 (2), c = 17.080 (4) Å, β = 98.47 (2)°, Z = 2, Dc = 1.29 gcm^{-3}, F(000) = 612, R = 0.084, wR = 0.082 ($w = 4Fo^2/\sigma^2(Fo^2)$) for 996

observed reflections (I>3σ(I)). The atomic coordinates and e.s.d.s are available on request from The Director, Cambridge Crystallography Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW (reference code VIRWOI).†

*Benzyl (E)-N-*tert*-butoxycarbonyl-4-benzylidene-(2S)-pyroglutamate* (**5**) — Lithium hexamethyldisilazide was prepared from *n*-butyllithium (2.3 M in hexane, 50 μl; 0.11 mmol) and hexamethyldisilazane (24 μl; 0.11 mmol) in dry tetrahydrofuran (1 ml) at -78 °C under nitrogen and a solution of benzyl (2S,4S,1'R) N-*tert*-butoxycarbonyl-4-(1'-N-para-toluenesulfonylamidobenzyl)pyroglutamate (58 mg; 0.1 mmol) in tetrahydrofuran (1 ml) was added. The mixture was left at -78 °C for 30 minutes, warmed gradually to room temperature (25 hours) and stirred for a further 1 hour at this temperature. The reaction was quenched with saturated aqueous ammonium chloride (3 ml), diethyl ether / water (3:1; 16 ml) was added and the organic layer was washed to neutrality with water and brine and dried (Na_2SO_4). Removal of the solvent *in vacuo* afforded a viscous yellow oil (quantitative yield) which was purified by chromatography on silica gel using gradient elution from 2.5% ethyl acetate in dichloromethane to 10% ethyl acetate in dichloromethane to give a colourless oil, which crystallised on standing for several days (28 mg; 69%). This was recrystallised from ethyl acetate / hexane to afford *benzyl (E)-N-*tert*-butoxycarbonyl-4-benzylidene-(2S)-pyroglutamate* (**5**) as white microcrystals; m p 98-100 °C; $[\alpha]_D^{27}$ +26.0 (c 0.43, $CHCl_3$); (Found C, 70.45; H, 6.0; N, 3.5%. $C_{24}H_{25}NO_5$ requires C, 70.75; H 6.1; N 3.4%); m/z [EI] 307 ([M-$CO_2{}^tBu$ + H]$^+$); ν_{max} (KBr) / cm^{-1} 1774 and 1745 ("imide" and ester) and 1645 (C=C); λ_{max} (MeOH) 286 (ε 30,900); δ_H (200 MHz, C^2HCl_3) 7.56 (1H, t, $J_{6,3R}$ $J_{6,3S}$ 2.9, *H*-6), 7.41 (5H, s, Ar*H*) and 7.31 (5H, s, Ar*H*), 5.18 (2H, AB, J_{AB} 12.1, PhCH_2), 4.76 (1H, dxd, $J_{2,3S}$ 10.1, $J_{2,3R}$ 3.3, *H*-2), 3.35 (1H, dxdxd, $J_{3S,3R}$ 17.8, $J_{3S,2}$ 10.1, $J_{3S,6}$ 2.9, *H*-3S), 2.97 (1H, dxt, $J_{3R,3S}$ 17.8, $J_{3R,2}$ $J_{3R,6}$ 3, *H*-3R) and 1.46 (9H, s, $(CH_3)_3CO$). The (*E*)-configuration about the olefinic bond was deduced by n.O.e. experiments in C^2HCl_3. Irradiation of the vinylic proton at δ 7.56 ppm produced enhancements in the neighbouring aromatic protons (δ 7.41 ppm) and no detectable effect in the 3-CH_2 protons. Irradiation of H-3S at δ 3.35 ppm produced enhancements in the same aromatic protons (δ 7.41 ppm) and no detectable effect in the vinylic proton at δ 7.56 ppm.

*(2S,4S)-N-*tert*-Butoxycarbonyl-4-benzylpyroglutamic acid* (**6**) — Benzyl (*E*)-N-*tert*-butoxycarbonyl-4-benzylidene-(2S)-pyroglutamate (**5**) (240 mg; 0.59 mmol) was dissolved in ethyl acetate (12 ml) and 10% palladium on carbon (30 mg) was added. The system was purged with hydrogen and the reaction was stirred vigorously at room temperature under an atmosphere of hydrogen for 15 hours. The solution was filtered through Celite and the solvent was removed *in vacuo* giving a clear oil which crystallised on standing. This was recrystallised from diethyl ether to afford *(2S,4S)-N-*tert*-butoxycarbonyl-4-benzylpyroglutamic acid* (**6**) as white microcrystals (110 mg; 58%) m p 135-138 °C; $[\alpha]_D^{27}$ +51.2 (c 0.25, MeOH); (Found C, 63.2; H, 6.6; N, 4.0. $C_{17}H_{21}NO_5$ requires C, 63.9; H, 6.6; N 4.4%); (m/z [EI] found: 319.1419. $C_{17}H_{21}NO_5$ ([M]$^+$) requires 319.1417); ν_{max} (KBr) / cm^{-1} 3171 (CO*OH*), 1773 and 1746 ("imide") and 1683 (CO_2H); δ_H (360 MHz, C^2HCl_3) 7.5 (1H, br. exch. s, CO_2H), 7.3 -7.14 (5H, m, Ar*H*), 4.50 (1H, dxd, $J_{2,3S}$ 8.7, $J_{2,3R}$ 7.2, *H*-2), 3.33 (1H, dxd, J_{AB} 13.9, $J_{6S,4}$ 3.9, *H*-6S), 2.89 (1H, m, *H*-4), 2.66 (1H, dxd, J_{AB} 13.9, $J_{6R,4}$ 10.8, *H*-6R), 2.39 (1H, dxt, $J_{3S,3R}$ 13.4, $J_{3S,4}$ $J_{3S,2}$ 9.0, *H*-3S), 1.79 (1H, dxt, $J_{3R,3S}$ 13.4, $J_{3R,2}$ $J_{3R,4}$ 7.2, *H*-3R) and 1.50 (9H, s, $(CH_3)_3CO$). The 2,4-*cis*-configuration was confirmed by n.O.e. experiments since irradiation of H-3S at δ 2.39 ppm enhanced both H-4 at δ 2.89 ppm by 7% and H-2 at δ 4.50 ppm by 15%.

*(2S,4R,6S)-[4,6-$^2H_2$] N-*tert*-butoxycarbonyl-4-benzylpyroglutamic acid* (**6, H_4 = H_6 = ^2H**) — Benzyl (*E*)-N-*tert*-butoxycarbonyl-4-benzylidene-(2S)-pyroglutamate (**5**) (203 mg; 0.5 mmol) was dissolved in ethyl acetate (10 ml) and 10% palladium on carbon (15 mg) was added. After purging the system with deuterium gas, the reaction was stirred vigorously at room temperature under an atmosphere of deuterium for 15 hours. The solution was filtered through Celite and the solvent was removed *in vacuo* to afford an off-white solid (70 %). Recrystallisation from ethyl acetate / hexane gave*(2S,4R,6S)-[4,6-$^2H_2$]-N-*tert*-butoxycarbonyl-4-benzylpyroglutamic acid* (**6, H_4 = H_6 = ^2H**) as white needles; (60 mg; 37 %); m p 133 - 136 °C; $[\alpha]_D^{23}$ + 43.8 (c 0.260, MeOH); (Found C, 64.0; H, 6.35; N, 4.8. $C_{17}H_{19}{}^2H_2NO_5$ requires C 63.5, H, 7.2; N, 4.35%); (m/z [EI] Found: 277.1656. $C_{16}H_{19}{}^2H_2NO_3$ [M - CO_2]$^+$ requires 277.1654); m/z [FAB (3-NBA)] 322 [M+H]$^+$; ν_{max} (KBr) / cm^{-1} 1773, 1745 ("imide") and 1683 (CO_2H); δ_H (360 MHz, C^2HCl_3) 7.30-7.14 (5H, m, Ar*H*), 5.97 (1H, brd. exch. s, CO_2H), 4.50 (1H, dxd, $J_{2,3S}$ 8.7, $J_{2,3R}$ 7.0, *H*-2), 2.64 (1H, s, *H*-6R), 2.38 (1H, dxd, $J_{3S,3R}$ 13.4, $J_{3S,2}$ 8.7, *H*-3S), 1.78 (1H, dxd, $J_{3R,3S}$ 13.4, $J_{3R,2}$ 7.0, *H*-3R) and 1.49 (9H, s, $(CH_3)_3CO$).

(2S,4S,6RS) imine adduct (**9**) — A solution of butyllithium (2.3 M in hexane; 2.0 ml; 4.6 mmol) was added to hexamethyldisilazane (970 µl; 4.6 mmol) in dry tetrahydrofuran (20 ml) at -78 °C under nitrogen. This was stirred for 1 hour and benzyl N-*tert*-butoxycarbonyl-(2S)-pyroglutamate (**1**) (1.28 g; 4.0 mmol) in tetrahydrofuran (10 ml) was added dropwise. The reaction was warmed gradually to -60 °C (1 hour) and then cooled to -78 °C prior to addition of 3-methyl-1,2-benzisothiazole-1,1-dioxide (**8**)[17] (725 mg; 4.0 mmol) in tetrahydrofuran (40 ml). The reaction mixture was maintained at -78 °C for 1 hour, warmed gradually to -50 °C over 1 hour and quenched with excess (+)-camphorsulfonic acid (4 g) in tetrahydrofuran (10 ml). The mixture was warmed to room temperature, diluted with diethyl ether (70 ml) and washed with saturated aqueous sodium bicarbonate, water and brine and dried (Na_2SO_4). Removal of the solvent *in vacuo* yielded a yellow foam (1.90 g; 95%), which the ^{1}H-NMR spectrum in [$^2H_6$]-benzene showed to be a 5:2 mixture of diastereoisomers by integration of the H-2 resonances. The diastereoisomers were separated by chromatography on silica gel using gradient elution from 6% ethyl acetate in dichloromethane to 30% ethyl acetate in dichloromethane. The major isomer (**9**) eluted from the column using between 6 and 10% ethyl acetate in dichloromethane as a gum (900 mg; 45%); $[\alpha]_D^{23}$ +4.7 (c 0.865, $CHCl_3$); (m/z [EI] found 400.1093. $C_{20}H_{20}N_2O_5S$ [M - Boc + H]$^+$ requires 400.1090); ν_{max} ($CHCl_3$) / cm^{-1} 3260 (NH), 1785 and 1748 ("imide"); δ_H (360 MHz, $C_6{}^2H_6$) 7.40-6.72 (9H, m, Ar*H*), 5.25 (1H, brd. exch. s, N*H*), 4.88 (2H, AB, J_{AB} 12.2, PhCH_2), 4.20 (1H, dxd, $J_{2,3S}$ 9.6, $J_{2,3R}$ 1.4, *H*-2), 3.10 (1H, dxd, $J_{4,3S}$ 12.0, $J_{4,3R}$ 8.6, *H*-4), 2.02 (1H, dxdxd, $J_{3R,3S}$ 13.6, $J_{3R,4}$ 8.6, $J_{3R,2}$ 1.4, *H*-3R), 1.67 (1H, dxdxd, $J_{3S,3R}$ 13.6, $J_{3S,4}$ 12.0, $J_{3S,2}$ 9.6, *H*-3S), 1.39 (3H, s, CH_3) and 1.31 (9H, s, $(CH_3)_3CO$). The results from n.O.e. experiments in $C_6{}^2H_6$ showed that the major isomer (**9**) had the 2,4-*trans*-geometry and (R)-configuration at C-6. Irradiation at H-2 at δ 4.20 ppm enhanced the signal for H-3S at δ 1.67 ppm by 2% and irradiation at H-4 at δ 3.10 ppm enhanced both the NH at δ 5.25 ppm and H-3R at δ 2.02 ppm. Irradiation at the methyl at δ 1.39 ppm enhanced the NH at δ 5.25 ppm and H-4 at δ 3.10 ppm. The more polar minor isomer (**10**) eluted from the column between 10 and 20% ethyl acetate in dichloromethane as a gum (330 mg; 17%); $[\alpha]_D^{23}$ -50.9 (c 0.333, $CHCl_3$); (m/z [EI] found 400.1093, $C_{20}H_{20}N_2O_5S$ [M - Boc + H]$^+$ requires 400.1090); m/z [+ve FAB, (3-NBA)] 523 [M + Na]$^+$; ν_{max} ($CHCl_3$) / cm^{-1} 3255 (NH), 1789, 1747 and 1720 (sh) (CO); δ_H (360 MHz, $C_6{}^2H_6$) 7.40-6.33 (9H, m, Ar*H*), 5.10 (1H, exch. s, N*H*), 4.88 (2H, AB, J_{AB} 12.2, PhCH_2), 4.30 (1H, dxd, $J_{2,3S}$ 9.7, $J_{2,3R}$ 1.5, *H*-2), 2.91 (1H, dxd, $J_{4,3S}$ 11.6, $J_{4,3R}$ 8.5, *H*-4), 2.01 (1H, dxdxd, $J_{3S,3R}$ 13.4,

$J_{3S,4}$ 11.6, $J_{3S,2}$ 9.7, *H*-3S), 1.68 (3H, s, CH_3), 1.37 (9H, s, $(CH_3)_3CO$) and 1.23 (1H, dxdxd, $J_{3R,3S}$ 13.4, $J_{3R,4}$ 8.5, $J_{3R,2}$ 1.5, *H*-3R). n.O.e. experiments in $C_6^2H_6$ showed that the minor isomer (**9**) had the 2,4-*trans*-geometry and (S)-configuration at C-6. Irradiation of H-2 at δ 4.30 ppm enhanced H-3S at δ 2.01 ppm by 4%; irradiation of H-4 at 2.91 enhanced H-3R at δ 1.23 ppm by 9% and the aromatic proton Ha at δ 6.33 ppm by 10%.

Dicyclohexylamine salt of acid (**11**) *of adduct* (**9**) — The adduct (**9**) (125 mg; 0.25 mmol) was dissolved in ethyl acetate containing 10% palladium on carbon. The system was purged with hydrogen and the reaction was stirred vigorously at room temperature, under an atmosphere of hydrogen. After 5 hours, the catalyst was filtered through Celite and washed with ethyl acetate. Removal of solvent *in vacuo* afforded the acid (**11**) as a colourless gum (100 mg; 97 %); δ_H (360 MHz, C^2HCl_3) 7.79, 7.60 and 7.41 (4H, 3xm, ArH), 6.47 (1H, exch. s., NH), 4.54 (H, dxd, $J_{2,3S}$ 9.3, $J_{2,3R}$ 1.0, H-2), 3.22 (1H, dxd, $J_{4,3S}$ 10.1, $J_{4,3R}$ 7.9, H-4), 2.30 - 2.10 (2H, overlapping multiplets, H-3), 1.75 (3H, s, CH_3) and 1.49 (9H, s, $(CH_3)_3CO$). The product (**11**) (95 mg; 0.23 mmol) was dissolved in tetrahydrofuran (2 ml) and treated dropwise with dicyclohexylamine (46 µl; 0.23 mmol) at room temperature. After complete addition, the reaction was stirred at room temperature for 10 minutes, by which time a white solid was apparent. The mixture was left to stand at 4 °C for 14 hours and the white plates were filtered off and recrystallised from methanol (72 mg; 53 %); m p 185-187 °C; $[\alpha]_D^{23}$ -17.6 (c 0.510, MeOH); (Found C, 60.4; H, 7.3; N, 6.8. $C_{30}H_{45}N_3O_7S$ requires C, 60.9; H, 7.6; N, 7.1%); ν_{max} (KBr) / cm^{-1} 3275 (NH), 2945 (NH), 1767 (" imide") and 1577 (CO_2^-).

X-ray data on Dicyclohexylamine salt of acid (**11**) *of adduct* (**9**) — Crystals [Enraf-Nonius CAD4 diffractometer with Mo-Kα radiation] are monoclinic, $P2_1$ (No 4), a = 11.945 (4), b = 10.442 (3), c = 13.244 (2) Å, β = 108.69 (2)°, Z = 2, Dc = 1.26 gcm^{-3}, F(000) = 636, R = 0.065, wR = 0.050 (w = 1/σ(F)) for 2027 observed reflections (I>σ(I)). The atomic coordinates and e.s.d.s are available on request from The Director, Cambridge Crystallography Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW (reference code VIRWUO).†

Treatment of Major Adduct Isomer (**9**) *with Lithium Hexamethyldisilazide* — Lithium hexamethyldisilazide was prepared as above from *n*-butyllithium (2.3 M in hexane, 250 µl; 0.57 mmol) and hexamethyldisilazane (120 µl; 0.57 mmol) in dry tetrahydrofuran (4 ml) at -78 °C under nitrogen. The major adduct isomer (**9**) (250 mg; 0.50 mmol) in dry tetrahydrofuran (4 ml) was added at this temperature, and the reaction was stirred for 30 min and warmed to room temperature over 2 hours. When t.l.c. indicated that all of the starting material had reacted (total 21 hours at room temperature), (+)-camphorsulfonic acid (excess) in tetrahydrofuran (2 ml) was added and the reaction mixture was diluted with diethyl ether (20 ml). The solution was washed with saturated aqueous sodium bicarbonate, water and brine and dried (Na_2SO_4). Removal of solvent *in vacuo* yielded a brown oil which was chromatographed on silica gel using a gradient from 7% ethyl acetate in dichloromethane to 20% ethyl acetate in dichloromethane. Further purification was achieved using preparative thin layer chromatography on silica gel with 5% ethyl acetate in dichloromethane as eluant. The major products were identified by ^{1}H-NMR spectroscopy, as benzyl N-*tert*-butoxycarbonyl-(2S)-pyroglutamate (**1**) (38% yield) and 3-methyl-1,2-benzisothiazole-1,1-dioxide (**8**) (40 % yield).

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† These data were lodged with the Cambridge Data Centre in 1991 and have the reference codes indicated.

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