Unusual stereoselectivity in the alkylation of pyroglutamate ester urethanes [1]

Jean-Damien Charrier, James E. S. Duffy, Peter B. Hitchcock and Douglas W. Young *

Sussex Centre for Biomolecular Design and Drug Development, CPES, University of Sussex, Falmer, Brighton, UK BN1 9QJ

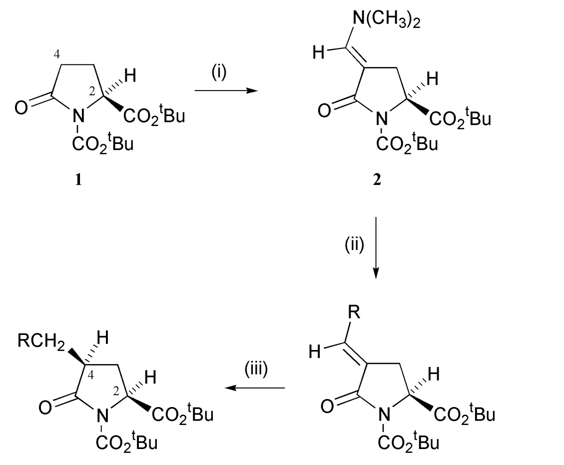
Received (in Cambridge, UK) 19th July 2001, Accepted 8th August 2001
First published as an Advance Article on the web 7th September 2001

Previous studies on the alkylation of pyroglutamate ester urethanes have led to a consensus that alkylation at C-4 occurs to give a mixture of diastereoisomers, the major isomer of which usually has the alkyl group *trans* to the ester group at C-2. We have now discovered that this generalisation is not invariably correct and that, although for S_N1-type electrophiles stereoselectivity is in fact *trans*, S_N2-type electrophiles can give the thermodynamically less stable *cis* compounds as the predominant products. Use of the bulky proton source 2,6-di-*tert*-butylphenol to quench these reactions yields the *cis* isomers as the only products in good yield, thus making direct alkylation of pyroglutamic acid derivatives a useful alternative to our hydrogenation approach to these synthons.

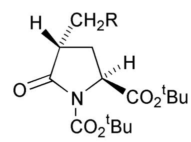
Protected pyroglutamic acid derivatives have been used as homochiral starting materials in the synthesis of a variety of interesting natural products. At first, derivatives in which the ester at C-2 had been converted to a protected alcohol were used,[2] presumably due to fears that the centre C-2 might prove configurationally unstable under some of the reaction conditions. However, subsequent work using the esters themselves has shown that these fears are largely groundless and reactions have been carried out using the anion at C-4 of protected pyroglutamate esters without loss of stereochemical integrity at C-2. However, 4-alkylation has rarely been fully diastereoselective,[3] except in the case of 4-benzylation in which alkylation gives only that diastereoisomer where the benzyl group is *trans* to the ester group at C-2.[4]

Results and discussion

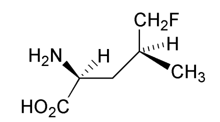
Our interest in discovering the alignment of the diastereotopic methyl groups in leucine residues in proteins,[5] led us to develop a synthesis of (2*S*,4*R*)-[5,5,5-$^2H_3$]leucine.[6] Methodology developed in this work was extended to provide a general method for stereospecific synthesis of 4-alkylpyroglutamic ester urethanes, which reliably gave the product in which the alkyl group was always *cis* to the ester at C-2.[7] In this method, shown in Scheme 1, the pyroglutamic ester urethane **1** was reacted with Bredereck's reagent to yield the enaminone **2**. On reaction with DIBAL-H or Grignard reagents[7] this gave a variety of alkylidene derivatives **3**. Catalytic reduction of these compounds then led to addition from the less hindered side of the molecule to afford the single diastereoisomer **4**, which could be converted into diastereoisomerically pure (2*S*,4*S*)-4-alkylglutamic acids and (2*S*,4*S*)-4-alkylprolines.[7] Cuprate addition to the enone **3** (R = H), although only 80% diastereoselective,[7] afforded access to the epimeric (2*S*,4*R*)-series of compounds from the product **5**.[7]



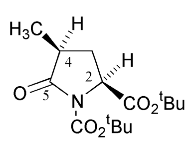
Scheme 1 (i) HC(O^{t}Bu)(NMe$_2$); (ii) (a) DIBAL-H ⟶ **3**, R = H; or (b) RMgBr ⟶ **3**, R = alkyl, aryl vinyl; (iii) H$_2$–Pd-C.



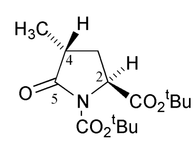
5



6



7

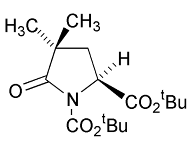


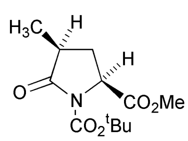
8

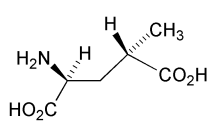
Use of ^{19}F NMR spectroscopy to study protein conformation has been under-exploited except in the case of fluoroaromatic amino acid residues. We have, therefore, adapted our general synthesis of 4-alkylpyroglutamate derivatives to provide stereochemically pure (2*S*,4*S*)-5-fluoroleucine **6**,[8,9] which we incorporated into the enzyme dihydrofolate reductase using biological methods.[9] Although useful quantities were obtained, we realised that if the potential of this fluorinated amino acid were to be exploited to the full in molecular recognition studies, then larger quantities would be required.

This meant that our synthesis would have to be improved. The stereoselective methylation in our synthesis involved two steps: reaction of the protected pyroglutamate **1** with the costly Bredereck's reagent to obtain the enaminone **2**, followed by treatment of this with hydrogen and large amounts of catalyst to give the *cis*-methyl compound **7** as the sole product by a process involving reduction, elimination and reduction. We therefore decided to reinvestigate direct alkylation of the protected pyroglutamic ester **1** as a way of preparing our key synthetic intermediate **7** or its *trans* epimer **8**. Reports of direct methylation of pyroglutamate ester urethanes were not promising as Baldwin *et al.*[4] had not been successful in attempting to methylate a pyroglutamate enolate using methyl iodide and Ezquerra *et al.*[3] obtained yields of less than 10% when this electrophile was used. However, when we treated the protected ester **1** with lithium hexamethyldisilazide in THF at −78 °C, followed by reaction with methyl iodide, we obtained a mixture of monoalkylated products in 67% yield together with 10% of the dialkylated compound **9**.

The monoalkylated products were present in a diastereoisomeric ratio of 5 : 1 and could be separated by chromatography. To our surprise, the major isomer was spectroscopically identical to the *cis* isomer **7**,[6] which we had prepared by the route outlined in Scheme 1. We had assigned stereochemistry to this product on the basis of NOE studies, and the coupling constants were in keeping with the considerable number of examples in the literature.[6] Further, the rationale of hydrogenation occurring from the less hindered side of the intermediate **3** (R = H) was in keeping with the result. Subsequently, Coudert *et al.*[10] confirmed our stereochemical assignments by preparing the methyl ester **10** from the corresponding enaminone using our method, and converting it to an authentic sample of (2*S*,4*S*)-4-methylglutamic acid **11**. This compound had previously been degraded to L-α-methylsuccinic acid,[11] the absolute configuration of which had been confirmed by the solution of the X-ray structure of ergoflavin by anomolous dispersion methods, L-α-methylsuccinic acid being a degradation product of this fungal metabolite.[12] Although these results seemed unassailable, the alkylations reported by Ezquerra *et al.*[3] had all given the *trans* isomer as the major product and so we confirmed our assignment by single crystal X-ray structure determination on the major isomer.†


9


10


11

Interestingly, Langlois and Rojas[13] had noted that treatment of the protected pyroglutamate **12** with LiHMDS followed by reaction with *tert*-butyl bromoacetate had given a 3 : 1 mixture in which the *cis* isomer **13** was the major product, although Ezquerra[3] reported a 2 : 1 ratio in favour of the *trans* isomer **14** when he treated the protected ester **1** with LiHMDS followed by reaction with ethyl bromoacetate. When we reacted our pyroglutamic ester urethane **1** with 1.5 equivalents of LiHMDS at −78 °C, followed by addition of 1.2 equivalents of *tert*-butyl bromoacetate at the same temperature, a mixture of the diastereoisomeric monoalkylated products **15** and **16** was obtained in 70% yield in a 4 : 1 ratio, together with a 16% yield of the 4,4-dialkyl derivative **17**. The major monoalkylated product **15** showed an NOE at H-3*S* (2.62 ppm) and at H-4 (2.93 ppm) when H-2 (4.39 ppm) was irradiated and an NOE at H-3*R* (1.65 ppm) when H-6 (2.36 ppm) was irradiated. The

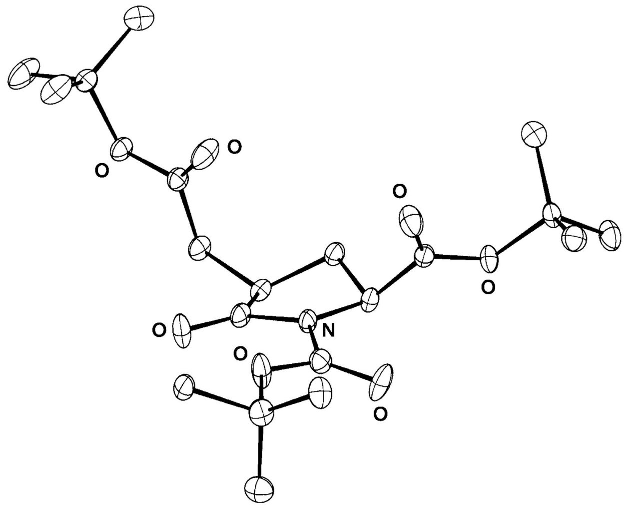
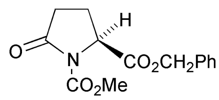
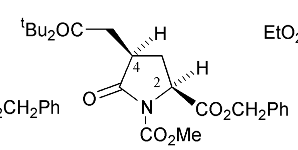
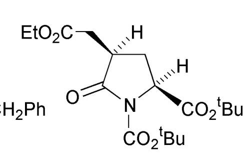
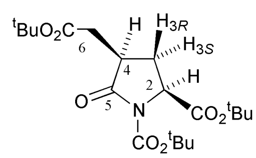


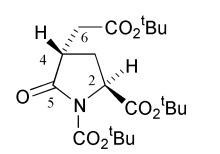
Fig. 1 X-Ray structure of the major isomer **15**, from alkylation of the ester urethane **1** with *tert*-butyl bromoacetate.

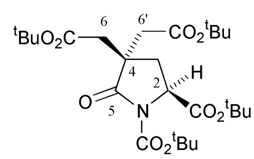

12


13


14


15

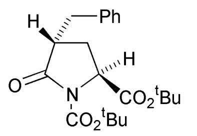

16


17

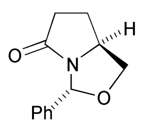
major isomer was therefore the *cis* isomer **15** and this was confirmed by a single crystal X-ray structure as shown in Fig. 1.

As improving the yield and diastereoselectivity of the methylation reaction had been our first objective, we next used methyl triflate as the electrophile in the reaction. This resulted in monoalkylation in increased yield (75%) and with no dialkylation. The *cis* : *trans* ratio of 5 : 1 was unaltered. The "contra-steric" *cis* stereoselectivity found in methylation and in alkylation using *tert*-butyl bromoacetate might be due to the fact that these electrophiles imply an S_N2 mechanism and so chelation of the leaving group *via* lithium with the ester at C-2 might account for *suprafacial* alkylation. Such an explanation would not apply to electrophiles which are more likely to operate in an S_N1 manner and when we used benzyl bromide in the reaction, the sole product observed was the *trans* product **18**, obtained in 72% yield. This result is in keeping with that observed by Baldwin *et al.*[4] When the methylation reaction using LiHMDS and methyl triflate was repeated in toluene as solvent, a 77% yield of the monoalkylated product was obtained with a *cis* : *trans* ratio of 17 : 1. Chelation therefore seems a reasonable explanation. Interestingly, Armstrong and DeMattei[14] noted that alkylation of the enolate of the bicyclic compound **19** with methyl iodide gave kinetic *endo* alkylation, and Meyers *et al.*[15] have explained such alkylation as a preference for attack by small electrophiles *anti* to a pseudopyrimidal lone pair on the amide nitrogen.

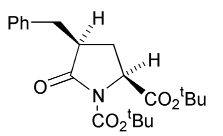
† The X-ray data for this compound were reported in our preliminary communication (ref. 1) and the atomic coordinates were lodged with the Cambridge Crystallography Data Centre, University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW. They are available on request from the Director of the CCDC at the above address, quoting CCDC reference code POYBAG.



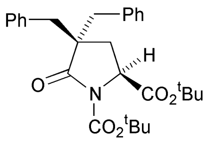
18



19



20



21

Alkylation of pyroglutamate ester urethanes at C-4 is not as simple a process as had been previously thought and it is evident from the variation between our results and those of Ezquerra, that subtle changes in reaction conditions can alter diastereoisomeric ratios considerably. However, although we had improved *cis* diastereoselectivity by using solvents of low relative permittivity, we had yet to achieve complete diastereoselectivity in these reactions except in the case of the *trans* specificity previously discovered by Baldwin *et al.* for the benzylation reaction.[4] We were finally able to achieve total *cis* diastereoselectivity by recourse to the hindered proton source 2,6-di-*tert*-butylphenol.[16,17] When the *trans*-methylpyroglutamate **8** was reacted with LiHMDS at −78 °C and quenched with 2,6-di-*tert*-butylphenol, then the sole product was the *cis* epimer **7**, obtained in 89% yield. Amending the conditions of the *trans*-specific benzylation reaction was now investigated and the pyroglutamate ester urethane **1** was treated with 1.15 equivalents of LiHMDS and 1.2 equivalents of benzyl bromide followed by addition of a further 1.3 equivalents of LiHMDS. 2,6-Di-*tert*-butylphenol was finally added and the sole monoalkylated product of the reaction, obtained in 63% yield was now the *cis* product **20** accompanied by *ca.* 9% of the dialkylated product **21**.

Experimental

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations (given in units of 10^{-1} deg cm^{-2} g^{-1}) were measured on a Perkin-Elmer PE241 polarimeter using a 1 dm path length micro cell. IR spectra were recorded on a Perkin-Elmer 1720 Fourier transform instrument. ^{1}H NMR spectra were recorded on Bruker DPX300 (300 MHz) and AMX500 (500 MHz) Fourier transform instruments. *J* values are given in Hz. ^{13}C NMR spectra (broad band ^{1}H decoupled) were recorded on a Bruker DPX300 (75.5 MHz) Fourier transform instrument. Distortionless enhancement polarisation transfer (DEPT) experiments were used to help assign ^{13}C NMR resonances. Either tetramethylsilane (0.00 ppm) or residual solvent peaks were used as internal references in the NMR spectra unless otherwise stated. Mass spectra were recorded on Kratos MS80F and MS25 double focusing spectrometers by Dr A. Abdul-Sada (Sussex). 3-NBA refers to 3-nitrobenzyl alcohol. Accurate mass measurements were recorded by the EPSRC National Mass Spectrometry Service, Swansea. Microanalyses were performed by Medac Ltd (Brunel). Column chromatography was performed using Fluka silica gel 60 (200–400 mesh ASTM). Petroleum ether refers to that fraction boiling between 60–80 °C.

Alkylation of *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate 1 with methyl iodide in THF

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate **1**[6] (5 g, 17.5 mmol) was dissolved in THF (50 ml) under nitrogen with stirring. The solution was cooled to −78 °C and LiHMDS (1.0 M in THF, 20.17 ml, 20.17 mmol) was added. After stirring for 1 h at −78 °C methyl iodide (1.31 ml, 21.05 mmol) was added dropwise and stirring was continued at −78 °C for a further 2 h. The reaction was quenched with saturated aqueous ammonium chloride and allowed to warm to room temperature. The crude product was extracted into ethyl acetate, the combined organic layers were washed with water and brine, and dried (Na_2SO_4). The solvent was removed *in vacuo* to give an orange oil, which was purified by column chromatography on silica gel using a gradient of EtOAc (5–25%) in petroleum ether as eluant. *tert*-Butyl (2*S*)-*N*-(*tert*-butoxycarbonyl)-4,4-dimethylpyroglutamate **9** eluted first as a yellow solid (0.55 g, 10%), mp 100–102 °C; $[\alpha]_D^{20}$ −26.24 (*c* 1.0, $CHCl_3$) (Found: C, 61.3; H, 8.7; N, 4.5. $C_{16}H_{27}NO_5$ requires C, 61.3; H, 8.7; N, 4.5%); *m*/*z* [+ FAB, NBA] 314 [M + H]$^+$; ν_{max} (KBr)/cm^{-1} 1785 (imide/urethane) and 1714 (ester); δ_H (300 MHz, C^2HCl_3) 1.21 (6H, s, 2 × CH_3), 1.48 [9H, s, C(CH_3)$_3$], 1.52 [9H, s, C(CH_3)$_3$], 1.92 (1H, dd, $J_{3R,3S}$ 13.3, $J_{3R,2}$ 4.3, H3*R*), 2.19 (1H, dd, $J_{3S,3R}$ 13.3, $J_{3S,2}$ 9.7, H-3*S*), 4.41 (1H, dd, $J_{2,3S}$ 9.7, $J_{2,3R}$ 4.3, H-2); δ_C (75.5 MHz, C^2HCl_3) 25.1 (*C*H$_3$), 25.7 (*C*H$_3$), 27.7 [C(*C*H$_3$)$_3$], 36.5 (C-3), 41.4 (C-4), 56.3 (C-2), 82.0 and 83.0 [2 × O*C*(CH$_3$)$_3$], 149.6 (urethane), 170.5 (ester) and 178.2 (C-5). *tert*-Butyl (2*S*,4*R*)-*N*-(*tert*-butoxycarbonyl)-4-methylpyroglutamate **8** was the second compound from the column, a white solid (0.63 g, 12%), mp 62–64 °C; $[\alpha]_D^{22}$ −16.9 (*c* 1.0, $CHCl_3$); *m*/*z* [+ FAB, NBA] 300 [M + H]$^+$; ν_{max} (film)/cm^{-1} 1793 (imide/urethane) and 1742 (ester); δ_H (300 MHz, C^2HCl_3) 1.21 (3H, d, *J* 7.0, CH_3), 1.48 [9H, s, C(CH_3)$_3$], 1.51 [9H, s, C(CH_3)$_3$], 1.89 (1H, m, H-3*R*), 2.24 (1H, ddd, $J_{3S,3R}$ 13.5, $J_{3S,4}$ 8.7, $J_{3S,2}$ 1, H-3*S*), 2.65 (1H, m, H-4), 4.42 (1H, d, $J_{2,3}$ 9.5, H-2); δ_C (75.5 MHz, C^2HCl_3) 15.0 (*C*H$_3$), 27.7 [C(*C*H$_3$)$_3$], 30.4 (C-3), 36.2 (C-4), 57.4 (C-2), 82.0 and 82.9 [2 × O*C*(CH$_3$)$_3$], 149.2 (urethane), 170.1 (ester) and 175.7 (C-5). Irradiation at the methyl resonance (1.21 ppm) caused enhancement of H-3*S* (2.24 ppm) and the same resonance was enhanced on irradiation at H-2 (4.42 ppm). The third compound from the column was *tert*-butyl (2*S*,4*S*)-*N*-(*tert*-butoxycarbonyl)-4-methylpyroglutamate **7** which was obtained as a white solid (2.88 g, 55%), mp 68–69 °C; $[\alpha]_D^{22}$ −44.8 (*c* 1.12, $CHCl_3$) [lit.[6b] mp 54–56 °C, $[\alpha]_D$ −6.1; lit.[18] mp 69–71 °C, $[\alpha]_D$ −44.8 (*c* 1.12, $CHCl_3$)]. ‡ The ^{1}H and ^{13}C NMR spectra of this compound were identical to those of a sample obtained using the method in Scheme 1[6] and NOE experiments were also in keeping with the structure. A single crystal X-ray structure for this compound has been reported in our preliminary communication[1] and lodged with the CCDC. †

Alkylation of *tert*-butyl (2*S*,4*S*)-*N*-*tert*-butoxycarbonylpyroglutamate 1 with *tert*-butyl bromoacetate

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate **1**[6] (0.200 g, 0.70 mmol) was dissolved in tetrahydrofuran (2 ml) under nitrogen with stirring. The solution was cooled to −78 °C and LiHMDS (1.0 M in tetrahydrofuran, 0.77 ml, 0.77 mmol) was added. After stirring for 1 h at −78 °C, *tert*-butyl bromoacetate (0.125 ml, 0.84 mmol) was added dropwise and stirring was continued at −78 °C for a further 2 h. The reaction was quenched with saturated aqueous ammonium chloride and allowed to warm to room temperature. The crude product was extracted into ethyl acetate, the combined organic layers were washed with water and saturated aqueous sodium chloride, and

‡ We reported[6] mp 54–56 °C, $[\alpha]_D$ −6.1 (*c* 1.12, $CHCl_3$) for a sample of the *cis* isomer **7** prepared as in Scheme 1. Subsequently[18] we found that a sample of this compound, prepared by the method of Scheme 1 had mp 69–71 °C, $[\alpha]_D$ −44.8 (*c* 1.12, $CHCl_3$). Both samples were prepared from samples of enaminone **2** with comparable $[\alpha]_D$ values and had identical spectra. We assume that our rotation in ref. 6 was quoted in error.

dried (Na_2SO_4). The solvent was removed *in vacuo* to give an orange oil, which was purified by column chromatography on silica gel using a gradient of ethyl acetate (5–25%) in petroleum ether as eluant.

*tert-Butyl (2S)-4,4-bis(tert-butoxycarbonylmethyl)-N-tert-butoxycarbonylpyroglutamate **17*** eluted first as a white crystalline solid (58 mg, 16%); mp 89.5–91.0 °C; $[\alpha]_D^{25}$ +16.5 (*c* 1.0, $CHCl_3$) (Found: C, 60.7; H, 8.6; N, 2.7. $C_{26}H_{43}NO_9$ requires C, 60.8; H, 8.4; N, 2.7%); ν_{max} (KBr)/cm^{-1} 1761 (imide/urethane) and 1723 (ester); δ_H (300 MHz, C^2HCl_3) 1.37, 1.39, 1.45 and 1.47 [4 × 9H, 4 × s, OC(CH_3)$_3$], 2.23 (1H, dd, $J_{3S,3R}$ 14.0, $J_{3S,2}$ 3.4, H-3*S*), 2.40 (1H, d, $J_{6A,6B}$ 16.1, H-6A), 2.49 (1H, dd, $J_{3R,3S}$ 14.0, $J_{3R,2}$ 11.0, H-3*R*), 2.59 (1H, d, $J_{6B,6A}$ 16.1, H-6B), 2.69 (1H, d, $J_{6'A,6'B}$ 17.0, H-6′A), 2.77 (1H, d, $J_{6'B,6'A}$ 17.0, H-6′B) and 4.45 (1 H, dd, $J_{2,3R}$ 11.0, $J_{2,3S}$ 3.4, H-2); δ_C (75.5 MHz, C^2HCl_3) 28.3 [2 × OC(CH$_3$)$_3$], 28.4 [2 × OC(CH$_3$)$_3$], 30.2 (C-3), 41.1 (C-6), 42.0 (C-6′), 45.7 (C-4), 57.3 (C-2), 81.8, 81.9, 82.5 and 83.7 [4 × OC(CH$_3$)$_3$], 149.7 (urethane), 169.9, 170.1 and 171.4 (esters) and 176.0 (C-5).

*tert-Butyl (2S,4S)-N-tert-butoxycarbonyl-4-tert-butoxycarbonylmethylpyroglutamate **16*** eluted second as a clear oil (39 mg, 14%), mp 81.0–82.0 °C; $[\alpha]_D^{25}$ −20.8 (*c* 1.0, $CHCl_3$); *m*/*z* [CI] Found: 400.2335. $[C_{26}H_{33}NO_7 + H]^+$ requires 400.2341; *m*/*z* [+ve FAB, (3-NBA)] 422 $[M + Na]^+$; ν_{max} (film)/cm^{-1} 1781 (imide/urethane) and 1733 (ester); δ_H (500 MHz, C^2HCl_3) 1.43 [9H, s, OC(CH_3)$_3$], 1.48 [9H, s, O(CH_3)$_3$], 1.50 [9H, s, OC(CH_3)$_3$], 2.03 (1H, ddd, $J_{3S,3R}$ 13.2, $J_{3S,4}$ 11.8, $J_{3S,2}$ 9.8, H-3*S*), 2.30 (1H, ddd, $J_{3S,2}$ 13.2, $J_{3R,4}$ 8.8, $J_{3R,2}$ 1.3, H-3*R*), 2.38 (1H, dd, $J_{6A,6B}$ 17.0, $J_{6A,4}$ 8.8, H-6A), 2.80 (1H, dd, $J_{6B,6A}$ 17.0, $J_{6B,4}$ 3.9, H-6B), 2.96 (1H, dddd, $J_{4,3S}$ 11.8, $J_{4,3R}$ 8.8, $J_{4,6A}$ 8.8, $J_{4,6B}$ 3.9, H-4) and 4.43 (1H, dd, $J_{2,3R}$ 1.3, $J_{2,3S}$ 9.8, H-2); δ_C (75.5 MHz, C^2HCl_3) 28.3 [OC(CH$_3$)$_3$], 28.5 [OC(CH$_3$)$_3$], 28.9 (C-3), 31.4 [OC(CH$_3$)$_3$], 36.3 (C-6), 38.8 (C-4), 58.2 (C-2), 81.6, 82.8 and 83.8 [3 × OC(CH$_3$)$_3$], 149.7 (urethane), 170.6 and 170.8 (2 × ester) and 174.5 (C-5). Selective irradiation of H-3*S* (2.03 ppm) led to a 24.2% enhancement in H-3*R* (2.30 ppm), a 2.8% enhancement in H-6A (2.38 ppm) and a 10.0% enhancement H-2 (4.43 ppm). Selective irradiation of H-6B (2.80 ppm) led to a 27.3% enhancement in H-6A (2.38 ppm) and a 6.0% enhancement in H-4 (2.96 ppm). Selective irradiation of H-2 (4.43 ppm) led to a 4.8% enhancement in H-3*S* (2.03 ppm).

*tert-Butyl (2S,4R)-N-tert-butoxycarbonyl-4-tert-butoxycarbonylmethylpyroglutamate **15*** eluted last as a white crystalline solid (156 mg, 56%), mp 114–119 °C; $[\alpha]_D^{25}$ +2.7 (*c* 1.0, $CHCl_3$) (Found: C, 59.9; H, 8.3; N, 3.4. $C_{20}H_{33}NO_7$ requires C, 60.1; H, 8.3; N, 3.5%); *m*/*z* [+ FAB (3-NBA)] 422 $[M + Na]^+$; ν_{max} (film)/cm^{-1} 1764 (imide), 1744 (ester) and 1715 (urethane); δ_H (500 MHz, C^2HCl_3) 1.43 [9H, s, OC(CH_3)$_3$], 1.47 [9H, s, O(CH_3)$_3$], 1.50 [9H, s, OC(CH_3)$_3$], 1.65 (1H, ddd, $J_{3R,3S}$ 13.4, $J_{3R,4}$ 8.1, $J_{3R,2}$ 6.8, H-3*R*), 2.36 (1H, dd, $J_{6A,6B}$ 16.6, $J_{6A,4}$ 3.8, H-6A), 2.62 (1H, ddd, $J_{3S,3R}$ 13.4, $J_{3S,4}$ 9.2, $J_{3S,2}$ 8.9, H-3*S*), 2.83 (1H, dd, $J_{6B,6A}$ 16.6, $J_{6B,4}$ 10.4, H-6B), 2.93 (1H, dddd, $J_{4,6B}$ 10.4, $J_{4,3S}$ 9.2, $J_{4,3R}$ 8.1, $J_{4,6A}$ 3.8, H-4) and 4.39 (1H, dd, $J_{2,3S}$ 8.9, $J_{2,3R}$ 6.8, H-2); δ_C (75.5 MHz, C^2HCl_3) 28.3 [3 × OC(CH$_3$)$_3$], 28.5 (C-3), 37.9 (C-6), 39.9 (C-4), 58.5 (C-2), 81.7, 82.7 and 84.0 [3 × OC(CH$_3$)$_3$], 149.8 (urethane), 170.7 and 171.0 (2 × ester) and 174.5 (C-5). Selective irradiation of H-6A (2.36 ppm) led to a 1.7% enhancement in H-3*R* (1.65 ppm), a 26.8% enhancement in H-6B (2.83 ppm) and a 1.0% enhancement in H-4 (2.93 ppm). Selective irradiation of H-2 (4.39 ppm) led to a 5.0% enhancement in H-3*S* (2.62 ppm) and a 1.8% enhancement in H-4 (2.93 ppm).

Crystal data—compound 15. $C_{20}H_{33}NO_7$, $M = 399.5$, monoclinic, space group $P2_1$, $a = 5.634(7)$, $b = 16.312(6)$, $c = 11.909(6)$ Å, $\beta = 94.20(9)°$, $V = 1092(2)$ Å^3, $Z = 2$, $D_{calc} = 1.22$ Mg m^{-3}, μ(Mo-Kα) = 0.09 mm^{-1}, $T = 173$ K. Nonius CAD4, 2196 total reflections measured; 1998 unique ($R_{int} = 0.061$). Refined on F^2 using SHELXL-93.[19] Final $R_1 = 0.060$ for 1768 reflections with $I > 2\sigma(I)$, $wR2 = 0.169$ for all reflections.§

Alkylation of *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate with methyl triflate in THF

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate **1**[6] (5 g, 17.5 mmol) was dissolved in THF (50 ml) under nitrogen with stirring. The solution was cooled to −78 °C and LiHMDS (1.0 M in THF, 20.17 ml, 20.17 mmol) was added. After stirring for 1 h at −78 °C, methyl triflate (2.38 ml, 21.05 mmol) was added dropwise and stirring was continued at −78 °C for a further 2 h. The reaction was quenched with saturated aqueous ammonium chloride and allowed to warm to room temperature. The crude product was extracted into ethyl acetate, and the combined organic layers were washed with water and brine, and dried (Na_2SO_4). The solvent was removed *in vacuo* to give an orange oil which was purified by column chromatography on silica gel using a gradient of ethyl acetate (5–25%) in petroleum ether as the eluant to give the *trans* isomer **8** (0.68 g, 13%), and the *cis* isomer **7** (3.25 g, 62%) with spectra identical to those of the compounds prepared above.

Alkylation of *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate with methyl triflate in toluene

tert-Butyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate **1**[6] (0.5 g, 1.75 mmol) was dissolved in toluene (5 ml) under nitrogen with stirring. The solution was cooled to −78 °C and LiHMDS (1.0 M in THF, 2.02 ml, 2.02 mmol) was added. After stirring for 1 h at −78 °C, methyl triflate (0.238 ml, 2.11 mmol) was added dropwise and stirring was continued at −78 °C for a further 2 h. The reaction was quenched with saturated aqueous ammonium chloride and allowed to warm to room temperature. The crude product was extracted into ethyl acetate, and the combined organic layers were washed with water and brine, and dried (Na_2SO_4). The solvent was removed *in vacuo* to give an orange oil. The crude product was purified by column chromatography on silica gel using a gradient of ethyl acetate (5–25%) in petroleum ether as eluant to give the *trans* isomer **8** (0.022 g, 4%) and the *cis* isomer **7** (0.382 g, 73%) with spectra identical to those of the compounds prepared above.

Epimerisation of *tert*-butyl (2*S*,4*R*)-*N*-(*tert*-butoxycarbonyl)-4-methylpyroglutamate

tert-Butyl (2*S*,4*R*)-*N*-(*tert*-butoxycarbonyl)-4-methylpyroglutamate **8** (0.175 g, 0.585 mmol) was dissolved in THF (5 ml) under nitrogen with stirring. The solution was cooled to −78 °C and LiHMDS (1.0 M in THF, 0.80 ml, 0.80 mmol) was added. After stirring for 1 h at −78 °C, 2,6-di-*tert*-butylphenol (0.25 g, 1.23 mmol) was added and stirring was continued at −78 °C for a further 30 min. Saturated aqueous ammonium chloride was added to the mixture and the reaction was allowed to warm to room temperature. The crude product was extracted into ethyl acetate and the combined organic layers were washed with water and brine, and dried (Na_2SO_4). The solvent was removed *in vacuo* to give an orange oil. The crude product was purified by column chromatography on silica gel using a gradient of ethyl acetate (5–25%) in petroleum ether as eluant to give the *cis* isomer **7** (0.156 g, 89%) as the sole product of the reaction.

Reaction of *tert*-butyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate with benzyl bromide

Method A—without 2,6-di-*tert*-butylphenol. *tert*-Butyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate **1**[6] (0.5 g, 1.75 mmol) was dissolved in THF (5 ml) under nitrogen with stirring. The

§ CCDC reference number 167665. See http://www.rsc.org/suppdata/p1/b1/b106451g/ for crystallographic files in .cif or other electronic format.

solution was cooled to −78 °C and LiHMDS (1.0 M in THF, 2.02 ml, 2.02 mmol) was added. After stirring for 1 h at −78 °C, benzyl bromide (0.25 ml, 2.1 mmol) was added dropwise and stirring was continued at −78 °C for a further 2 h. Saturated aqueous ammonium chloride was added and the mixture was allowed to warm to room temperature. The crude product was extracted into ethyl acetate and the combined organic layers were washed with water and brine, and dried (Na_2SO_4). The solvent was removed *in vacuo* to give an orange oil. The crude product was purified by column chromatography on silica gel using a gradient of ethyl acetate (5–25%) in petroleum ether as eluant to give *tert*-butyl (2*S*,4*R*)-*N*-(*tert*-butoxycarbonyl)-4-benzylpyroglutamate **18** (0.495 g, 72%), as white crystals, mp 125–126 °C; $[a]_D^{22}$ −52.6 (*c* 1.02, $CHCl_3$) [lit.[4] mp 125.5–126.5, $[a]_D^{22}$ −49.2 (*c* 0.9, $CHCl_3$)]. The spectra were in keeping with those reported in the literature.[4]

Method B—with 2,6-di-*tert*-butylphenol. *tert*-Butyl (2*S*)-*N*-*tert*-butoxycarbonylpyroglutamate **1**[6] (0.5 g, 1.75 mmol) was dissolved in THF (5 ml) under nitrogen with stirring. The solution was cooled to −78 °C and LiHMDS (1.0 M in THF, 2.02 ml, 2.02 mmol) was added. After stirring for 1 h at −78 °C, benzyl bromide (0.25 ml, 2.1 mmol) was added dropwise and stirring was continued at −78 °C for a further 2 h. LiHMDS (1.0 M in THF, 2.28 ml, 2.28 mmol) was added and the reaction was stirred for 1 h at −78 °C. 2,6-Di-*tert*-butylphenol (0.724 g, 3.51 mmol) was added and stirring was continued at −78 °C for a further 30 min. Saturated aqueous ammonium chloride was added and the reaction was allowed to warm to room temperature. The crude product was extracted into ethyl acetate and the combined organic layers were washed with water and brine, and dried (Na_2SO_4). The solvent was removed *in vacuo* to give an orange oil, which was purified by column chromatography on silica gel using a gradient of ethyl acetate (5–25%) in petroleum ether as eluant. *tert*-Butyl (2*S*)-*N*-(*tert*-butoxycarbonyl)-4,4-dibenzylpyroglutamate **21** eluted first as a yellow oil (0.073 g, 9%); $[a]_D^{22}$ −18.8 (*c* 0.47, $CHCl_3$) (Found [CI] 466.2593. $[C_{28}H_{35}NO_5 + H]^+$ requires 466.2592); *m*/*z* [+ FAB, NBA] 488 $[M + Na]^+$ and 466 $[M + H]^+$; ν_{max} (KBr)/cm^{-1} 1788 (imide/urethane), 1737 (ester) and 1723; δ_H (300 MHz, C^2HCl_3) 1.22 [9H, s, $C(CH_3)_3$], 1.39 [9H, s, $C(CH_3)_3$], 1.85 (1H, dd, $J_{3R,3S}$ 13.5, $J_{3R,2}$ 7.7, H-3*R*), 2.17 (1H, dd, $J_{3S,3R}$ 13.5, $J_{3S,2}$ 9.4, H-3*S*), 2.60 (1H, d, J_{AB} 13.1, 4-$CH_AH_BC_6H_5$), 2.75 (1H, d, J_{CD} 13.7, 4-$CH_CH_DC_6H_5$), 3.17 (1H, dd, $J_{2,3S}$ 9.4, $J_{2,3R}$ 7.7, H-2), 3.18 (1H, d, J_{AB} 13.1, 4-$CH_AH_BC_6H_5$), 3.32 (1H, d, J_{CD} 13.7, 4-$CH_CH_DC_6H_5$) and 7.16–7.31 (10H, m, ArH); δ_C (75.5 MHz, C^2HCl_3) 27.5 and 27.7 [2 × $C(CH_3)_3$], 28.1 (C-3), 42.8 (4-$CH_2C_6H_5$), 45.0 (4-$CH_2C_6H_5$), 52.1 (C-4), 56.8 (C-2), 81.5 and 82.9 [2 × $OC(CH_3)_3$], 126.7, 127.0, 128.3, 128.4, 129.7, 130.6, 136.0 and 136.6 (8 × ArC), 148.6 (urethane), 170.0 (ester) and 176.8 (C-5). Finally, *tert*-butyl (2*S*,4*S*)-*N*-(*tert*-butoxycarbonyl)-4-benzylpyroglutamate **20** eluted as white crystals (0.416 g, 63%), mp 127–129 °C; $[a]_D^{22}$ +50.8 (*c* 1.01, $CHCl_3$) [lit.[7c] mp 130–131 °C, $[a]_D^{22}$ +66 (*c* 0.2, $CHCl_3$)]. The spectra were identical with those of an authentic sample.[7c]

Acknowledgements

We thank the BBSRC/EPSRC for a fellowship (to J.-D. C.); the EPSRC for a studentship (to J. E. S. D), Dr A. Abdul Sada for low resolution mass spectra, the EPSRC National Mass Spectrometry Service, Swansea for accurate mass measurements and Dr A. G. Avent for 500 MHz NMR spectra.

References

1 Part of this work has been reported in preliminary form in J.-D. Charrier, J. E. S. Duffy, P. B. Hitchcock and D. W. Young, *Tetrahedron Lett.*, 1998, **39**, 2199.
2 See for example J. K. Thottathill, J. L. Moniot, R. H. Mueller, M. K. Y. Wong and T. P. Kissick, *J. Org. Chem.*, 1986, **51**, 3140.
3 J. Ezquerra, C. Pedregal, A. Rubio, B. Yruretagoyena, A. Escribano and F. Sanchez-Ferrando, *Tetrahedron*, 1993, **49**, 8665.
4 J. E. Baldwin, T. Miranda, M. Moloney and T. Hokelek, *Tetrahedron*, 1989, **45**, 7459.
5 G. Ostler, A. Soteriou, C. M. Moody, J. A. Khan, B. Birdsall, M. D. Carr, D. W. Young and J. Feeney, *FEBS Lett.*, 1993, **318**, 177.
6 (*a*) R. A. August, J. A. Khan, C. M. Moody and D. W. Young, *Tetrahedron Lett.*, 1992, **33**, 4617; (*b*) R. A. August, J. A. Khan, C. M. Moody and D. W. Young, *J. Chem. Soc., Perkin Trans. 1*, 1996, 507.
7 (*a*) C. M. Moody and D. W. Young, *Tetrahedron Lett.*, 1993, **34**, 4667; (*b*) C. M. Moody and D. W. Young, *Tetrahedron Lett.*, 1994, **35**, 7277; (*c*) C. M. Moody and D. W. Young, *J. Chem. Soc., Perkin Trans. 1*, 1997, 3519.
8 C. M. Moody, B. A. Starkmann and D. W. Young, *Tetrahedron Lett.*, 1994, **35**, 5485.
9 (*a*) J. Feeney, J. E. McCormick, C. J. Bauer, B. Birdsall, C. M. Moody, B. A. Starkmann, D. W. Young, P. Francis, R. H. Havlin, W. D. Arnold and E. Oldfield, *J. Am. Chem. Soc.*, 1996, **118**, 8700; and its accompanying supplementary material.
10 E. Coudert, F. Acher and R. Azerad, *Synthesis*, 1997, 863.
11 H. M. Kagan and A. Meister, *Biochemistry*, 1966, **5**, 2423.
12 A. T. McPhail, G. A. Sim, J. D. M. Asher, J. M. Robertson and J. V. Silverton, *J. Chem. Soc. (B)*, 1966, 18.
13 N. Langlois and A. Rojas, *Tetrahedron Lett.*, 1993, **34**, 2477 and corrigendum N. Langlois and A. Rojas, *Tetrahedron Lett.*, 1993, **34**, 6156.
14 R. W. Armstrong and J. A. DeMattei, *Tetrahedron Lett.*, 1991, **32**, 5749.
15 A. I. Meyers, M. A. Seefeld, B. A. Lefker, J. F. Blake and P. G. Willard, *J. Am. Chem. Soc.*, 1998, **120**, 7429.
16 S. Takano, W. Uchida, S. Hatakeyama and K. Ogasawara, *Chem. Lett.*, 1982, 733.
17 S. Hanessian, U. Reinhold and G. Gentile, *Angew. Chem., Int. Ed. Engl.*, 1997, **36**, 1881.
18 B. A. Starkmann, DPhil Thesis, University of Sussex, 1996.
19 SHELXL-93—Program for Crystal Structure Refinement G. M. Sheldrick, Institut für Anorganische Chemie der Universität, Tammanstrasse 4, D-3400 Göttingen, Germany, 1993.