Synthesis of Optically Pure 5-Aminopyrrolidin-2-one Formic Acid Salt: A Key Intermediate for the Incorporation of the Pyroglutamyl Geminal Diamino Analogue into *Retro-Inverso* Peptides

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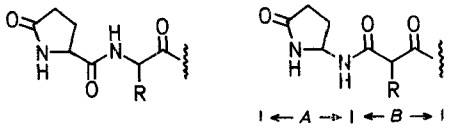
Pyroglutamate aminopeptidases of mammalian tissues are enzymes potentially capable of inactivating biologically active pyroglutamyl-peptides[1-7]. To prevent enzymatic breakdown and maintain a close structural relationship to the original molecules, modification of pyroglutamyl-peptide bonds by isosteric replacement of the CO—NH group by the NH—CO group is particularly promising[8-13].

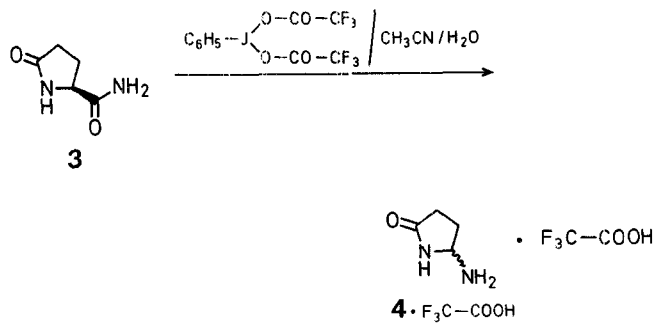
Reversal of the pyroglutamyl-peptide bonds in **1** is achieved by incorporation of the geminal diamino moiety *A* and the 2-alkylmalonyl residue *B* into the peptide skeleton to form the *retro-inverso* peptides **2**.



1 **2**

For the preparation of 'L'-5-aminopyrrolidin-2-one ('L'-**4**)[14], the key intermediate for the incorporation of unit *A* into peptide chains, a low-yield, multi-step procedure was reported[10-13]. The inherent instability of **4** requires the immediate coupling with 2-alkylmalonate monoesters after formation from *N,N'*-bis[benzyloxycarbonyl]-L-5-aminopyrrolidin-2-one ('L'-**6**) by heterogeneous hydrogenation[13].

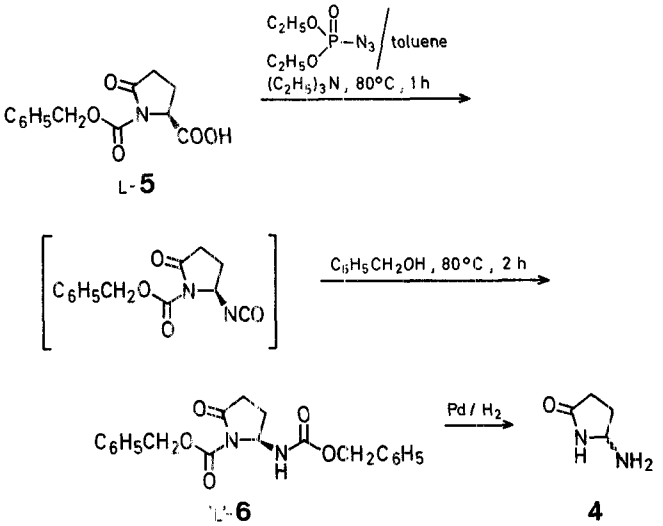
In an attempt to simplify the synthesis of 'L'-**4**, L-pyroglutamic acid amide (**3**) was reacted with bis[trifluoroacetoxy] phenyliodine (Scheme **A**) under the mild conditions reported for applications of this reagent[15-18]. After recrystallisation from ethyl acetate, analytically pure crystals of 5-aminopyrrolidin-2-one trifluoroacetic acid salt (**4** · F_3C—COOH) were obtained. However, in contrast to previous conversions mediated by bis[trifluoroacetoxy] phenyliodine, the reaction leads to complete racemisation. This was demonstrated by coupling of the product **4** · F_3C—COOH to Boc-L-Phe—OH, and subsequent H.P.L.C. analysis of the resultant pseudo-dipeptide. Analytical H.P.L.C. indicates the presence of 2 epimers, both of which have been fully characterised by microanalysis, H.R.M.S., ^{1}H-N.M.R., and I.R. spectra after separation by preparative H.P.L.C.



3 **4** · F_3C—COOH

Scheme **A**

Preparation of **4** by the sequence **5** → **6** → **4** (Scheme **B**) followed by coupling with Boc-L-Phe—OH yields a pseudo-dipeptide consisting of an identical epimeric mixture. Since the deprotection conditions are slightly different from those described[10], the catalytic hydrogenation has been repeated twice under strict adherence to the published procedure. Again, coupling of the hydrogenation product **4** to Boc-L-Phe—OH results a low yield of an epimeric pseudo-dipeptide mixture (~ 30:70). Since the coupling of Boc-L-Phe—OH with the 2 optical antipodes of **4** is likely to proceed with different rates, the epimeric ratio of the coupling product does not exactly reflect the extent of racemisation of **4**. However, our experiments indicate that the racemisation of the geminal diamine moiety occurs during deprotection of 'L'-**6** and is strongly dependent on the experimental conditions. To assess the effect of the hydrogenation time on the epimer ratio accurately, the conversion of 'L'-**6** to **4** in ethyl acetate/dioxan (30/70) has been stopped at regular intervals and the oily residue, obtained by removal of the solvents, immediately coupled to Boc-L-Phe—OH. From H.P.L.C. analysis, it follows that the conversion of 'L'-**6** to **4** is an exceedingly slow reaction (25% conversion after ~ 2 h) and, more important, that the amount of racemisation does not vary proportionally to the degree of conversion. The lowest epimeric ratios are observed at the early stage of the reaction. The finding that loss of optical activity of **4** is somewhat slower than the deprotection of 'L'-**6** suggests that high yields of optically pure **4** should be obtained by very rapid and efficient removal of the benzyloxycarbonyl group.



L-**5** 'L'-**6** **4**

Scheme **B**

In fact, the deprotection of both optical isomers of **6** under catalytic transfer hydrogenation conditions using freshly prepared platinum black catalyst and ammonium formate[21] yields in ~ 10 min excellent yields (≥ 95%) of stable 'L'- and 'D'-aminopyrrolidin-2-one formic acid salts ('L'- and 'D'-**4** · HCOOH), respectively, subsequent coupling of each optical isomer to Boc-L-Phe—OH furnishes pure epimers of the corresponding pseudo-dipeptides.

In conclusion, the method described here provides the only high yield synthesis of optically pure **4** · HCOOH and hence of *retro-inverso* peptides incorporating pyroglutamyl geminal diamino analogues of defined chirality.

Melting points were determined with a Büchi apparatus and are uncorrected. Optical rotations were recorded on a Perkin Elmer

141 M polarimeter. Thin layer cromatography was performed on precoated silica gel 60 sheets F-254 (Merck) using as solvents systems; BWA: *n*-butanol/water/acetic acid (4/1/1); CMA: chloroform/methanol/acetic acid (85:10:5). Spots were detected under a U.V. lamp at 254 nm or by spraying with ninhydrin after exposure of the plates to hydrochloric acid vapour. For analytical H.P.L.C. runs an apparatus consisting of a Series 3 B Perkin Elmer pump coupled to Perkin Elmer LC-75 UV monitor was used; the chromatograph was equipped with stainless-steel columns Lichrosorb RP-18, particle size 10 μ (Merck). Elution was achieved with acetonitrile (Lichrosolv, Merck)/water/trifluoroacetic acid (82/18/0.1) at a flow rate of 1 ml/min. For preparative H.P.L.C. a Miniprep (Jobin Yvon) liquid preparative chromatograph equipped with an M-45 Waters pump. Uvidec 100 II (Jasco) monitor, LKB 2210 2 channel recorder and Ultrorac LKB 2070 II fraction collector was used. The column was prepared by packing 35 g of Lichroprep RP-18, particle size 25–40 μ (Merck). I.R. spectra were recorded on a Perkin Elmer 700 I.R. spectrophotometer, ^{1}H-N.M.R. spectra were obtained on a Bruker WP-80 apparatus using TMS as an internal standard.

Pyroglutamyl Amide:
To a solution of pyroglutamic acid (Fluka; 3.06 g, 23.71 mmol) and 1-hydroxybenzotriazole ammonium salt (54.33 g, 28.46 mmol) in freshly distilled DMF (30 ml) cooled to 0 °C, dicyclohexylcarbodiimide (4.88 g, 23.71 mmol) is added portionswise with stirring. The mixture is stirred for 1 h at 0 °C, for 1 h at room temperature, and then filtered from dicyclohexyl urea. After removal of the solvent, the oily residue is triturated with ethyl acetate and the product collected by filtration, washed with ether, and dried under vacuum. The crude product is recrystallised from methanol/ether; yield: 2.56 g (84%); T.L.C. (BWA), R_f: 0.22; m.p. 166–168 °C; $[\alpha]_D^{25}$: −43.0° (*c* 2, water) [Ref.[18], m.p. 166–168 °C; $[\alpha]_D^{25}$: −42.2° (*c* 2, water)].

'D'- and 'L'-5-aminopyrrolidin-2-one Trifluoroacetic Acid Salt ('D'- and 'L'-4 · F_3C—COOH):
A stirred solution of pyroglutamyl amide (1.28 g, 10 mmol) in 3/2 acetonitrile/water (30 ml) is flushed with nitrogen and treated with bis[trifluoroacetoxy]phenyliodine (4.73 g, 1.1 mmol) in acetonitrile (5 ml). The reaction is allowed to proceed for 3 h under nitrogen, then the solvent is removed, and the residue solubilised in ethyl acetate. A white solid precipitates on standing. After filtration, the product is washed throughly with ether and dried under vacuum; yield: 0.67 g (30%); T.I.C. (BWA), R_f: 0.1; m.p. 125–127 °C.

***N*-(Butoxycarbonyl-L-phenylalanyl)-('D'- and 'L')-5-aminopyrrolidin-2-one:**
N-Methylmorpholine (0.097 ml, 0.8 mmol) is added to a solution of 'D' and 'L'-**4** · F_3C—COOH (0.180 g, 0.8 mmol), Boc-L-Phe—OH (0.233 g, 0.96 mmol), 1-hydroxybenzotriazole (0.132 g, 0.96 mmol), and dicyclohexylcarbodiimide (0.181 g, 0.88 mmol) in DMF (10 ml) maintained at 0 °C. After stirring for 1 h at 0 °C and 2 h at room temperature dicyclohexylurea is removed by filtration. Evaporation of the solvent furnishes a pale yellow solid which is dissolved in ethyl acetate and washed with 5% aqueous sodium hydrogen carbonate and saturated brine. After drying with anhydrous sodium sulfate, the crystalline product precipitates on standing at room temperature; yield: 0.22 g (79%); m.p. 167–168 °C; $[\alpha]_D^{25}$: +20.0° (*c*, 1, CH_3OH); T.L.C. (CMA) R_f: 0.3.

The epimeric mixture is separated by preparative H.P.L.C. using acetonitrile/water/trifluoroacetic acid (79/21/0.1) as eluent at a flow rate of 9 ml/min.

Epimer A; m.p. 122–123 °C; $[\alpha]_D^{25}$: −16.0° (*c* 0.5, CH_3OH)

Epimer B; m.p. 123–124 °C; $[\alpha]_D^{25}$: +57.5° (*c*, 0.5, CH_3OH).

Both epimers give identical microanalysis, M.S., I.R. and ^{1}H-N.M.R. spectra.

***N,N'*-Bis[benzyloxycarbonyl]-'L'-5-aminopyrrolydin-2-one ('L'-6):**
To a suspension of *N*-benzyloxycarbonyl-L-pyroglutamic acid (2 g, 7.59 mmol; Fluka) in freshly distilled dry toluene (10 ml), diphenylphosphoryl azide (1.64 ml, 7.59 mmol) is added under stirring and nitrogen. The mixture is heated to 80 °C under nitrogen, then triethylamine (1.05 ml, 7.59 mmol) in toluene (5 ml) is added dropwise during 2 h; to the clear solution benzyl alcohol (0.86 ml, 8.35 mmol) in toluene (5 ml) is added and the mixture allowed to warm to room temperature. The precipitate is collected by filtration, washed with cold toluene, and dried under vacuum. The crude product is recrystallised from ethyl acetate; yield: 2.54 (90%); T.L.C. (CMA) R_f: 0.55; m.p. 150–151 °C; $[\alpha]_D^{25}$: −36.7° (*c* 1, DMF) [Ref.[13], m.p. 147–150°; $[\alpha]_D^{25}$: −5.5° (*c* 1, CH_3OH)].

***N,N'*-Bis[benzyloxycarbonyl]-'D'-5-aminopyrrolidin-2-one ('D'-6):**
This compound is prepared in the same manner as described for the synthesis of 'L'-**6**; yield: 87%; m.p. 151–152 °C; $[\alpha]_D^{25}$: +36.0 °C (*c* 1, DMF); $[\alpha]_D^{40}$: +17° (*c* 1, CH_3OH).

'L'-5-Aminopyrrolidin-2-one Formic Acid Salt ('L'-4 · HCOOH):
To a solution of L-**6** (1 g, 2.71 mmol) in DMF (5 ml) ammonium formate (0.34 g, 10.84 mmol) in methanol (10 ml) and palladium sponge (50 mg) are added under stirring. After 10 min, the reaction is complete as indicated by T.L.C. analysis. The solvent is removed and the residue lyophilised twice from dioxan/water; yield: 0.360 g (95%); m.p. 106–107 °C; $[\alpha]_D^{25}$: +6.0° (*c* 1, DMF); T.L.C. (BWA), R_f: 0.1.

I.R. ^{1}H-N.M.R. and mass spectra are in agreement with the structure.

'D'-5-aminopyrrolidin-2-one Formic Acid Salt ('D'-4 · HCOOH):
This compound is prepared by following the procedure described for the synthesis of ('L'-**4** · HCOOH); yield: 97%; m.p. 106–107 °C; $[\alpha]_D^{25}$: −5.9° (*c* 1, DMF); T.L.C. (BWA) R_f: 0.1.

***N*-(Butoxycarbonyl-L-phenylalanyl)-'L'-5-aminopyrrolidin-2-one:**
L-**4** · HCOOH is coupled to Boc-L-Phe—OH with dicyclohexylcarbodiimide and 1-hydroxybenzotriazole by following the procedure described above for the synthesis of the epimeric pseudodipeptide mixture; yield: 75%; m.p. 123–124 °C; $[\alpha]_D^{25}$: +57.5° (*c* 0.5, CH_3OH).

In H.P.L.C. measurements the product showed the same R_f value as the second peak of the epimeric mixture.

***N*-(Butoxycarbonyl-'L'-phenylalanyl)-'D'-5-aminopyrrolidin-2-one:**
This compound is prepared in the same way as the 'L'-epimer; yield: 78%; m.p. 122–123 °C; $[\alpha]_D^{25}$: −16.0° (*c* 0.5, CH_3OH).

By H.P.L.C. comparision it was shown that the product is identical with the first eluted peak from the epimeric mixture.

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[14] The bracketed configuration notation i.e. 'D' or 'L' defines the configuration of the geminal residue which is related to the specified configuration of the transformed pyroglutamic acid residue.
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