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Pyroglutamic acid as a pseudoproline moiety: a facile method for its introduction into polypeptide chains

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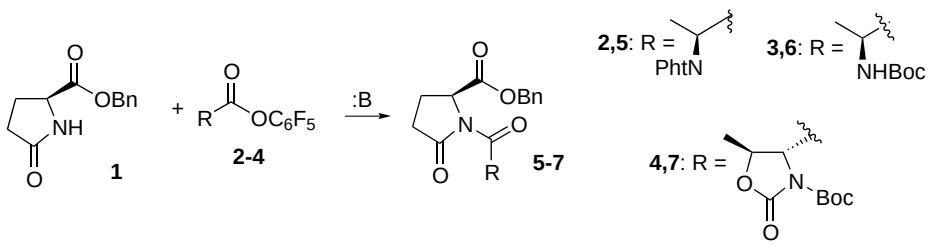
Abstract—The acylation of benzyl (*S*)-pyroglutamate is reported by reaction with the pentafluorophenyl ester of protected alanine and threonine. The ^{1}H NMR analysis of the acylated products shows that the α hydrogens of the protected amino acids are very deshielded. This effect is due to the presence of the carbonyl of the lactam ring and shows that the peptide bond is in the *trans* conformation. The deprotection of the amino acids is also reported. © 2001 Elsevier Science Ltd. All rights reserved.

The amino acyl-proline *cis*/*trans* isomerisation is a rate-limiting step of protein folding[1] and modulates the biological activity of peptides.[2] Therefore the possibility of controlling this isomerisation is very important in the design of peptidomimetics. For example, it has been demonstrated by ^{1}H NMR analysis that proline oligomers ($n=2$–4) contain nearly random distribution of *cis* and *trans* peptide bonds in $CDCl_3$,[3] while in D_2O the dimer ($n=2$) exists in a 35:65 *cis*/*trans* mixture and in the higher oligomers ($n=3$–5) the percentage of peptide bonds in the *trans* conformation remains approximately constant (90%).[4]

As a consequence, many proline surrogates have been designed for the study and control of conformational transitions in peptides and proteins.[5] For instance, many pseudoprolines (Ψ-Pro) have been obtained by reaction of amino acids with aldehydes and preferentially assume the *cis* conformation.[6]

In a previous paper we have described the synthesis of poly 4-carboxy 5-methyl oxazolidin-2-ones,[7] whose monomer belongs to the family of pseudoprolines, and we have suggested that the oligomerisation affords efficiently very rigid structures, which exist in a single stable conformer, where the Xaa_{i-1}–Xaa_i bond are exclusively in the *trans* conformation. The ^{1}H NMR spectra of the trimer and of the tetramer suggest that these molecules fold in ordered structures, where the C-4 hydrogen of a ring is always close to the carbonyl of the next ring. This phenomenon has been confirmed by semi-empirical calculations and can be ascribed to the presence of a carboxy group in the pseudoproline ring near the peptide bond.

In order to use cheap natural compounds which are able to form imidic bonds, we have checked the possibility of utilising pyroglutamic acid as a pseudoproline moiety. Although pyroglutamic acid is available in both configurations at low price, it is usually present in polypeptide chains only as N-terminal amino acid, owing to the low reactivity of the nitrogen.[8] Very few examples of acylation of the nitrogen are described,[9] and some oligopeptides containing *N*-acylated pyroglutamic acid have been isolated from the glandular secretion of *Litoria rubella*.[10]



Scheme 1. General procedure for the acylation of benzyl (*S*)-pyroglutamate **1**.

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We wish to describe here the results we have obtained in the acylation of benzyl (*S*)-pyroglutamate **1** with protected alanine and threonine, whose acidic moiety was activated as pentafluorophenyl ester.[11]

L-Alanine was transformed into Pht-L-Ala-OH (Pht = phthalimide) in quantitative yield by heating the amino acid and phthalic anhydride in the absence of solvent,[12] and Boc-L-Ala-OH was purchased. On the other hand L-threonine was transformed into (4*S*,5*R*)-*N*-benzyloxycarbonyl-4-carboxy 5-methyl oxazolidin-2-one by reaction with triphosgene and sodium hydroxide in water/dioxane[13] and subsequent protection of the nitrogen with $(Boc)_2O$ and DIEA/DMAP (DIEA = iPr_2EtN) in DMF. This compound can also be synthesized by rearrangement of the corresponding *N*-Boc aziridine: following this route a wide range of five-substituted 4-carboxy oxazolidin-2-ones can be obtained.[14]

Then the acid moieties were transformed into the corresponding pentafluorophenyl esters **2**–**4** by reaction with pentafluorophenyl trifuoroacetate and pyridine in dry DMF.[15] The coupling with benzyl pyroglutamate was performed by means of several bases and all the reactions were carried out under an inert atmosphere (Scheme 1 and Table 1).

As could be foreseen, the reactivity of the γ-lactam ring is very poor, compared with the oxazolidin-2-one ring,[7] whose nitrogen is far more basic, owing to the back donation of the oxygen adjacent to the carbonyl.

Thus in the reaction of benzyl pyroglutamate **1** with Pht-L-Ala-OC_6F_5 **2**, mild reaction conditions such as the use of DIEA and DIEA/DMAP (entries 1 and 2) as bases failed completely, and poor results were obtained with DBU (entry 3). In addition, by using Cs_2CO_3 (entry 4) a complex mixture was recovered with no required product. On the other hand, the formation of an anion by reaction with NaH or LiHMDS (entries 5 and 6) afforded the desired dipeptide **5** in good to high yield. The coupling with activated esters **3** and **4** proved to be even harder, so that DBU afforded no results at all (entries 7 and 10). Once again good to excellent results were obtained with NaH and with LiHMDS, which turned out to be the reagent of choice.

An interesting outcome was obtained from the analysis of the ^{1}H NMR spectra: compounds **5**, **6** and **7** show signals between 5 and 6 ppm[17] (Fig. 1), which correspond to the α-hydrogens of protected alanine and threonine and are very deshielded compared with Boc-L-Ala-L-Pro-OBn ($\delta = 4.46$ ppm).[18]

This outcome should be ascribed to the presence of the carbonyl of the γ-lactam which strongly deshields the hydrogen. Therefore H_α must be nearby the carbonyl, so that the peptide bond is in the *trans* conformation, as we had previously shown for oligomers of 4-carboxy oxazolidin-2-ones.[7] The ^{1}H NMR spectrum of compound **5** shows the presence of two stable conformers, both with a very deshielded quartet (5.66 and 5.85 ppm), which do not collapse even by recording the ^{1}H NMR spectrum at 120°C in DMSO-d_6.

Dipeptides **5**, **6** and **7** were deprotected, respectively, by reaction with hydrazine or with trifluoroacetic acid

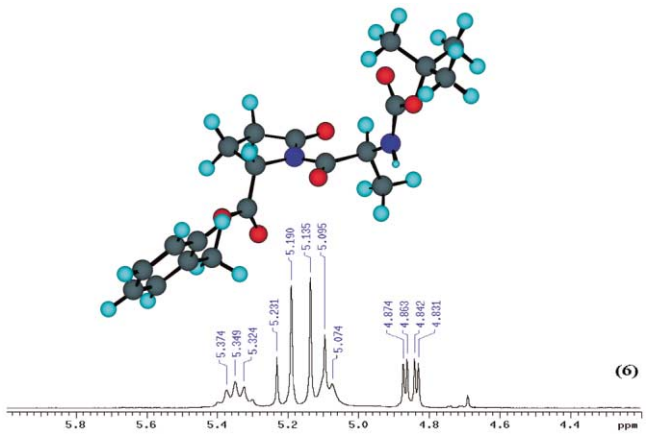
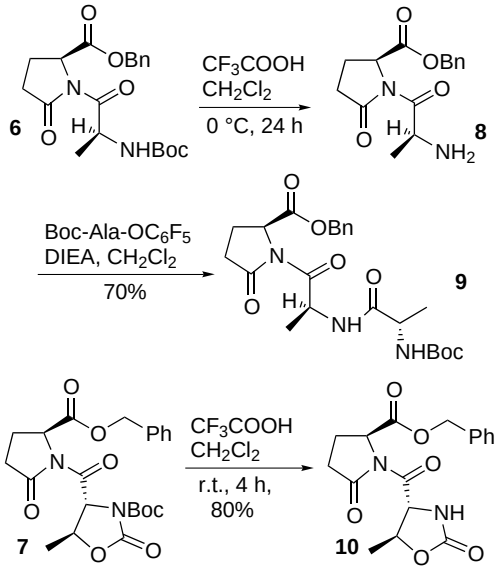


Figure 1. ^{1}H NMR (the areas regarding the α hydrogens are reported) and conformation of Boc-L-Ala-L-Pyr-OBn **6**. This conformation is in agreement both with ^{1}H NMR analysis and with AM1 calculations and accounts for the anomalous downfield chemical shift of H_α hydrogen of L-Ala.

Table 1. Reaction conditions and chemical yields for the acylation of benzyl (*S*)-pyroglutamate **1**

Entry	Acylating agent	Method[16]	Base (equiv.)	Solv.	React. time (h)	React. temp.	Yield (%)
1	**2**	A	DIEA (3)	DMF	16	Rt	–
2	**2**	A	DIEA (3), DMAP (0.25)	DMF	16	Rt	–
3	**2**	A	DBU (3)	DMF	16	Rt	44
4	**2**	A	Cs_2CO_3 (3)	DMF	60	Rt	–
5	**2**	C	NaH (1.2)	DMF	2.5	0°C–rt	77
6	**2**	B	LiHMDS (1.2)	THF	2.5	0°C–rt	95
7	**3**	A	DBU (3)	DMF	16	Rt	–
8	**3**	C	NaH (1.2)	DMF	2.5	0°C–rt	50
9	**3**	B	LiHMDS (1.2)	THF	2.5	0°C–rt	95
10	**4**	A	DBU (3)	DMF	16	Rt	–
11	**4**	C	NaH (1.2)	DMF	2.5	0°C–rt	71
12	**4**	B	LiHMDS (1.2)	THF	2.5	0°C–rt	77



Scheme 2. Deprotection and derivatization of the dipeptides **6** and **7**.

(Scheme 2). The deprotection of Pht-L-Ala-L-Pyr-OBn **5** with hydrazine failed completely and (*S*)-benzyl pyroglutamate **1** was obtained in quantitative yield. On the other hand, the deprotection of **6** and **7** with trifluoroacetic acid affords **8** and **10** in quantitative yield, which can be further derivatized in order to obtain polypeptide chains. So H_2N-L-Ala-L-Pyr-OBn **8** was derivatized by reaction with Boc-L-Ala-OC_6F_5 **2** in the presence of DIEA in methylene chloride and Boc-L-Ala-L-Ala-L-Pyr-OBn **9** was obtained in good yield.

In conclusion, we have shown that pyroglutamic acid can be easily introduced in a polypeptide chain and forces the newly formed peptide bond into the *trans* conformation.

Acknowledgements

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16. Typical experimental procedure: **Method A**: The pentafluorophenyl ester **2**, **3** or **4** (0.6 mmol) was added to a stirred solution of benzyl (*S*)-pyroglutamate **1** (0.5 mmol, 110 mg) and the base reported in Table 1 in dry DMF (1.5 mL) under a nitrogen atmosphere at 0°C. The mixture was stirred at room temperature for a variable period of time (see Table 1), then ethyl acetate (10 mL) was added, the mixture was washed with 1 M aqueous HCl (2×7 mL) and with an aqueous saturated solution of $NaHCO_3$ (1×10 mL). The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (cyclohexane/ethyl acetate, 8:2 as eluant). **Method B**: LiHMDS (2.4 mmol, 1 M sol. in THF, 2.4 mL) was added to a stirred solution of benzyl (*S*)-pyroglutamate **1** (2.0 mmol, 438 mg) in dry THF (7 mL) under a nitrogen atmosphere at 0°C. The mixture was stirred for 20 min at 0°C and 40 min at room temperature. A solution of pentafluorophenyl ester **2**, **3** or **4** (2.4 mmol) in dry THF (4 mL) was added dropwise at 0°C. The mixture was stirred for 20 min at 0°C and 3 h at room temperature. Water (10 mL) was added and the mixture was concentrated (rotary evaporator) to remove THF, the aqueous layer was extracted with ethyl acetate (3×10 mL), the combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (cyclohexane/ethyl acetate, 8:2 as eluant). **Method C**: NaH (0.6 mmol, 14 mg) was added to a stirred solution of benzyl (*S*)-pyroglutamate **1** (0.5 mmol, 110 mg) in dry THF (7 mL) under a nitrogen atmosphere at 0°C. The mixture was stirred for 20 min at 0°C and 20 min at room temperature. A solution of pentafluorophenyl ester **2**, **3** or **4** (0.6 mmol) in dry THF (2 mL) was added dropwise at 0°C. The mixture was stirred for 20 min at 0°C and 3 h at room temperature. Water (10 mL) was added and the mixture was concentrated (rotary evaporator) to remove THF, the aqueous layer was extracted with ethyl acetate (3×10 mL). The combined organic layers were dried over sodium sulfate and the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography (cyclohexane/ethyl acetate, 8:2 as eluant).
17. (**5**): low melting solid—$[\alpha]_D$ −80 (DCM, *c* 1.00).—IR (Nujol): $\nu = 1752$, 1713 cm^{-1}.—^1H NMR ($CDCl_3$) (mixture of conformers): $\delta = 1.73$ (d, 3H, $J = 7.4$ Hz, CH_3) and 1.86 (d, 3H, $J = 7.2$ Hz, CH_3), 2.00–2.19 (m, 1H, CH_2), 2.22–2.82 (m, 3H, CH_2), 4.79 (dd, 1H, $J = 1.8$, 9.0 Hz, CHN) and 4.83 (dd, 1H, $J = 3.0$, 9.2 Hz, CHN), 5.15 (AB, 2H, $J = 12.0$ Hz, CH_2Ph) and 5.22 (AB, 2H, $J = 12.2$ Hz, CH_2Ph), 5.76 (q, 1H, $J = 7.2$ Hz, CHN) and 5.95 (q, 1H, $J = 7.4$ Hz, CHN), 7.30–7.38 (m, 5H, Ph), 7.65–7.91 (m, 4H, Pht).—^{13}C NMR ($CDCl_3$) (mixture of conformers): $\delta = 14.7$ and 15.1, 21.6 and 21.7, 31.5, 50.2 and 50.4, 58.1 and 58.4, 67.2 and 67.4, 123.2, 128.0, 128.1, 128.2, 128.4, 128.5, 131.6, 131.7, 133.8 and 133.9, 134.8, 167.4 and 167.5, 170.0, 170.3 and 170.4, 173.6 and 173.7. (**6**): low melting solid—$[\alpha]_D$ −54 (DCM, *c* 1.05).—IR (Nujol): $\nu = 3397$, 1739, 1700 cm^{-1}.—^1H NMR ($CDCl_3$): $\delta = 1.30$ (d, 3H, $J = 6.9$ Hz, CH_3), 1.41 (s, 9H, *t*-Bu), 2.01–2.18 (m, 1H, CH_2), 2.30–2.45 (m, 1H, CH_2), 2.52–2.75 (m, 2H, CH_2), 4.85 (dd, 1H, $J = 3.3$, 9.6 Hz, CHN), 5.07 (d, 1H, $J = 6.9$ Hz, NH), 5.16 (AB, 2H, $J = 12.3$ Hz, CH_2Ph), 5.35 (dq, 1H, $J = 6.9$ Hz, CHN), 7.32–7.40 (m, 5H, Ph).—^{13}C NMR ($CDCl_3$): $\delta = 17.6$, 21.3, 28.2, 31.7, 49.7, 57.7, 67.4, 79.8, 128.2, 128.5, 134.8, 155.1, 170.4, 173.7, 174.5. (**7**): low melting solid—$[\alpha]_D$ −116 (DCM, *c* 1.00).—IR (Nujol): $\nu = 1825$, 1752, 1726, 1700 cm^{-1}.—^1H NMR ($CDCl_3$): $\delta = 1.52$ (s, 9H, *t*-Bu), 1.57 (d, 3H, $J = 6.6$ Hz, CH_3), 2.08–2.22 (m, 1H, CH_2), 2.32–2.78 (m, 3H, CH_2), 4.43 (dq, 1H, $J = 1.8$, 6.6 Hz, CHO), 4.90 (dd, 1H, $J = 3.0$, 9.6 Hz, CHN), 5.19 (AB, 2H, $J = 12.1$ Hz, CH_2Ph), 5.46 (d, 1H, $J = 1.8$ Hz, CHN), 7.30–7.42 (m, 5H, Ph).—^{13}C NMR ($CDCl_3$): $\delta = 21.0$, 22.0, 28.1, 31.5, 57.8, 62.7, 68.1, 72.7, 84.5, 128.5, 128.9, 134.7, 151.0, 168.6, 170.2, 175.2.
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