Tetrahedron Letters 51 (2010) 1333–1335

Contents lists available at ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Facile transformation of glutamic acid into proline residue inside a tripeptide backbone

Adriano Mollica [a], Azzurra Stefanucci [a], Federica Feliciani [a], Domenica Torino [b], Ivana Cacciatore [a], Francesco Pinnen [a], Gino Lucente [b,*]

[a] Dipartimento di Scienze del Farmaco, Università di Chieti-Pescara 'G. d'Annunzio', Via dei Vestini 31, 66100 Chieti, Italy
[b] Dipartimento di Chimica e Tecnologie del Farmaco, 'Sapienza', Università di Roma, P.le A. Moro 5, 00185 Roma, Italy

ARTICLE INFO

Article history:
Received 9 November 2009
Revised 22 December 2009
Accepted 6 January 2010
Available online 11 January 2010

Keywords:
δ-Lactam-constrained peptides
Glutamic acid
Peptides
Proline

ABSTRACT

In this Letter we present a simple reaction pathway which allows the conversion of the glutamic acid residue of a tripeptide into a proline residue. The reaction was performed by using Boc-Val-Glu-Phe-NH_2 as a starting material and is based on a NaH-induced intraresidue alkylation under reaction conditions analogous to that adopted during the Freidinger lactams formation.

Although the synthesis of usual linear peptides is at the present a straightforward procedure, several peptidic molecules of natural origin and their structurally modified analogues may still represent a synthetic challenge for the researchers. In particular several cyclic peptides and pseudopeptides from plants and marine organisms are still available only by extraction from the original crude material by adopting expensive and often low yielding procedures. The encountered synthetic difficulties, obviously strictly bound to the overall structures involved, can be enhanced by the presence of amino acid residues which, due to their unusual structure and reactivity, hamper the adoption of the usual coupling procedures.

Here we focused our attention on the proline residue, the only example among the DNA encoded amino acids, possessing a cyclic structure in which both the C^{α} atom and the amino group are included as critical elements. This structural feature confers to both proline and proline-containing peptides distinctive and relevant properties. In addition to some steric hindrance to acylation at the secondary amino group[1] and the inherent inability of the -Xaa-Pro- peptide bond to act as a H-bond donor, a well-known tendency to enhance the population of *cis*-amide isomers[2–6] and to induce folded backbone conformations[7–9] is found in proline-containing peptides. The latter property is largely capitalized during the synthesis of cyclopeptides in order to control the oligomers' formation.[10,11] However, the restriction of the number of the possible conformational structures, with enhancement of the folded forms, represents an undesirable effect when the elongation of linear chains is the synthetic target. In these cases the insertion of an amino acid with linear side chain, followed by its transformation into the desired proline residue, represents a useful strategy to improve the N-acylation reaction and to avoid or limit the probability of undesired intramolecular reactions.

Syntheses of proline have been frequently reported in the past[12–21] and the simplest and common routes are based on intramolecular displacement, by the α-NH_2, of a leaving group positioned on the δ-carbon atom of an amino acid residue side chain. Quite surprisingly this strategy has been only applied to the isolated residues and no data are so far available on its application to peptides. This specific subject has been previously treated by Freidinger in his classic paper on the synthesis of lactam-constrained dipeptides.[22] Here is clearly inferred that, in analogy with the finding concerning the synthesis of five-membered lactams by intramolecular displacement of the γ-positioned leaving group, the reaction performed on dipeptides containing a residue with a δ-positioned leaving group -namely homomethionine methyl sulfonium salt- should lead to six-membered δ-lactams. Thus, the alkylation of the amide nitrogen of the following residue in the peptide backbone, should be preferred to the intraresidue alkylation leading to the pyrrolidine ring of a proline residue.

By taking into account the above cited synthetic procedures leading to proline starting from the isolated residues of glutamic acid or equivalent synthons, as well as the above cited Freidinger's considerations on lactam-bridged dipeptides,[22] we wish to report here the results concerning the direct transformation of a tripeptide

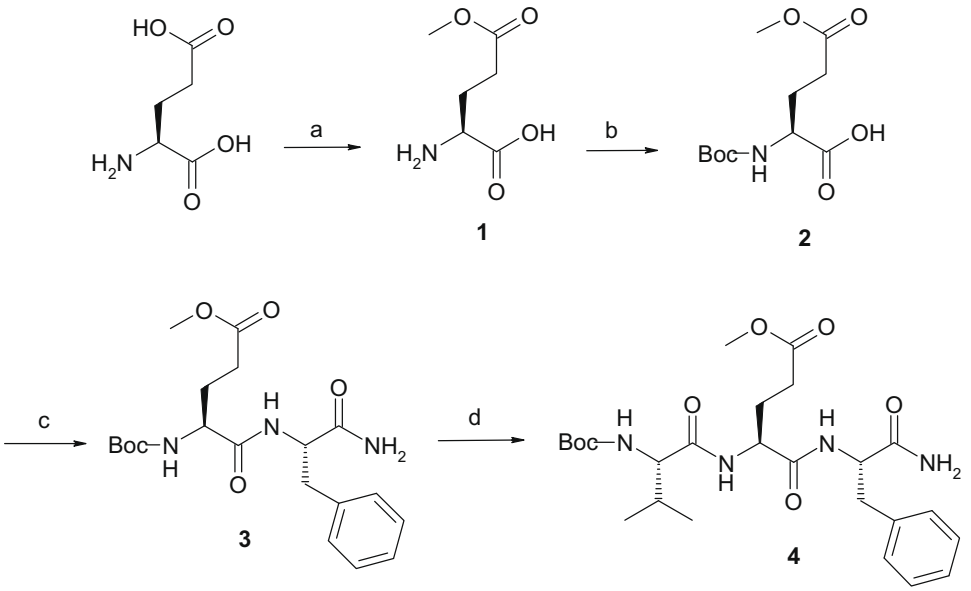
* Corresponding author. Tel.: +39 06 49913237; fax: +39 06 491491.
E-mail address: gino.lucente@uniroma1.it (G. Lucente).

0040-4039/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2010.01.008

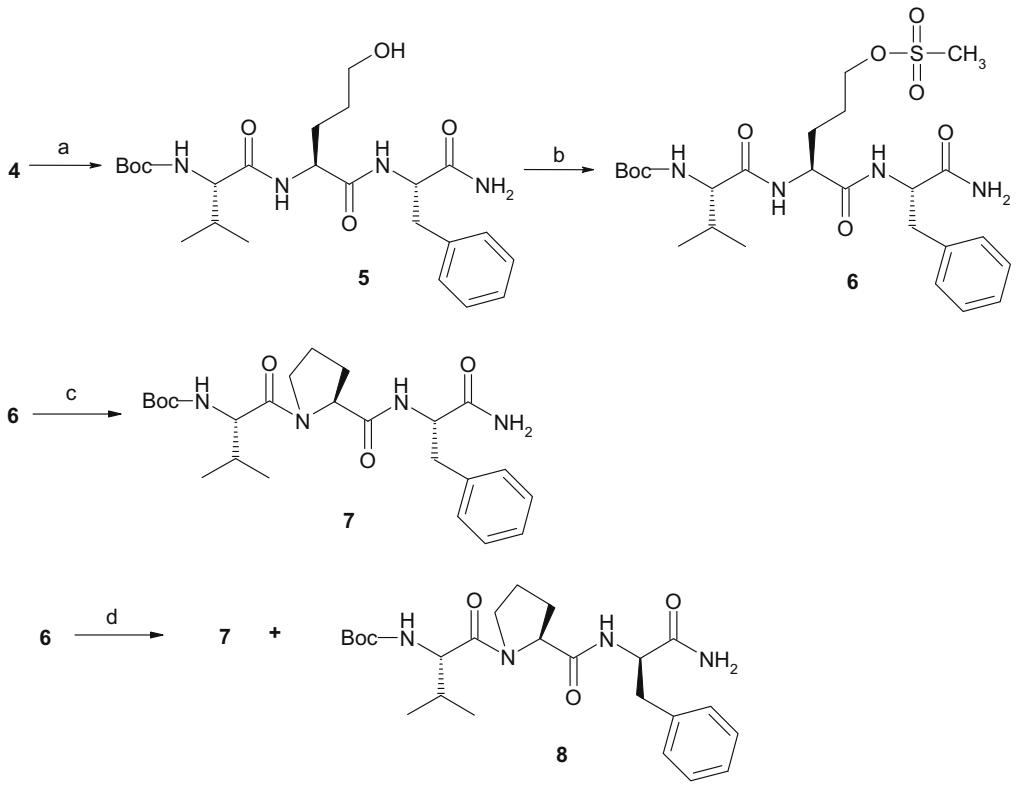
containing a central residue of glutamic acid, namely Boc-Val-Glu-Phe-NH_2 (**4**), into the corresponding proline-containing tripeptide Boc-Val-Pro-Phe-NH_2.

Tripeptide amide (**4**) was synthesized as delineated in Scheme 1. The starting H-Glu(OMe)-OH (**1**) was obtained by γ-selective and quantitative esterification of free glutamic acid.[9] *N*-Boc deprotection followed by coupling with H-Phe-NH_2 gave the dipeptide amide **3** which, after deprotection and coupling with Boc-Val-OH afforded the desired tripeptide amide **4**.

The strategy adopted in order to perform the cyclization reaction is illustrated in Scheme 2. Treatment of **4** with $NaBH_4$ afforded the pure alcohol derivative **5** which was then activated at the δ-hydroxy functional group by treatment with mesyl chloride. After purification of the mesylate **6** by silica gel chromatography the cyclization



Scheme 1. Synthesis of the precursor tripeptide amide **4**. Reagents and conditions: (a) H-Glu-OH, TMS-Cl, MeOH, rt, 10 min, 97%; (b) H-Glu(OMe), Boc_2O, Na_2CO_3, dioxane/H_2O, rt, 36 h, 87%; (c) H-Phe-NH_2, EDC·HCl, HOBt, NMM, DMF, rt, 14 h, 90%; (d) TFA/DCM, rt, 1.5 h, then Boc-Val-OH, EDC·HCl, HOBt, NMM, DMF, rt, 14 h, 80% (two steps).



Scheme 2. Activation strategy of glutamic acid side chain and structure of the cyclization products **7** and **8**. Reagents and conditions: (a) $NaBH_4$, THF, H_2O, rt, 7 h, 90%; (b) MsCl, TEA, THF, rt, 3 h, 56%; (c) NaH (4 equiv), THF, rt, 48 h, 90%; (d) NaH (16 equiv), THF, rt, 150 h, 90% (ca. 1/1 mixture of **7** and **8**).

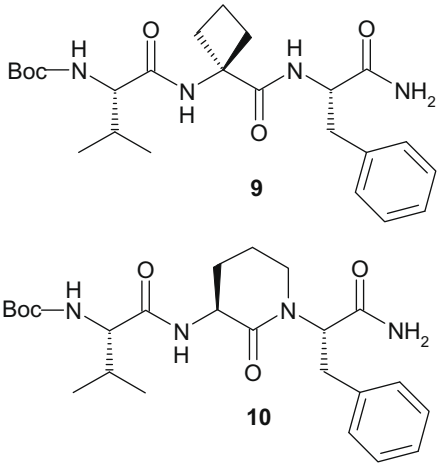


Figure 1. Structure of alternative cyclization products **9** and **10** cited in the text.

was induced by treatment with NaH (4 equiv) in anhydrous THF at room temperature for 48 h. A crude single product was isolated and purified by silica gel preparative layer chromatography (90% yield). Examination of the ^{1}H NMR spectrum in DMSO-d_6 on this compound revealed the presence, in addition to the urethane NH doublet (6.81 δ) of only one amide NH doublet (7.82 δ) clearly coupled with the Phe C$^{\alpha}$H, confirmed by 1D TOCSY NMR experiments. On the basis of these evidences the structure of Boc-Val-Pro-Phe-NH_2 (**7**) was assigned to the product and the assignment was confirmed by its independent synthesis, performed by following a synthetic pathway analogous to that reported in Scheme 1 for compound **4** and by using Boc-Pro-OH in the place of Boc-Glu(OMe)-OH.[23]

It should be noted that in the adopted cyclization conditions neither the α,α-disubstituted derivative **9** deriving from the intra-residue alkylation of the Glu C$^{\alpha}$-carbon atom,[17] nor the Freidinger δ-lactam **10**[22] (Fig. 1) was found. However, when the starting tripeptide mesylate **6** was treated with a higher excess of NaH (Scheme 2, route d) to induce the cyclization reaction, a mixture (about in equal parts) of the homochiral tripeptide **7** and its diastereomer was obtained. Isolation and preliminary examination of the ^{1}H NMR data of this new component suggested an epimerization at the phenylalanine chiral center of the tripeptide. By following this indication an authentic specimen of Boc-Val-Pro-DPhe-NH_2 (**8**) was synthesized starting from Boc-Pro-DPhe-NH_2 and by following the same procedures reported in Scheme 1 and 2. Tripeptide **8** resulted to be identical to that formed, together with **7**, when prolonged reaction time and higher excess of NaH were adopted in the cyclization reaction.

In summary, we have demonstrated that glutamic acid can be converted into the constrained five-membered ring of proline after its insertion in the peptide backbone and with maintenance of the starting stereochemistry. This new result, although at the present only applied to the *N*-Boc protected tripeptide amide **4** and still requiring generalization, appears of interest in the chemistry of peptides and should help to limit undesired cyclic or oligomeric products during the syntheses. In addition to this, the lack of involvement of the near backbone amide groups, in the cyclization reaction of the activated Glu side chain, adds useful information on the synthesis of lactam-constrained dipeptides[22] suggesting that, under the adopted reaction conditions, the formation of a five-membered ring prevails, as driving force of the reaction, on the concurrent expected cyclization leading to a six-membered lactam ring.

References and notes

1. Jones, J. In *The Chemical Synthesis of Peptides*; Rowlinson, J. S., Ed.; Clarendon Press: Oxford, 1994; pp 93–94.
2. MacArthur, M. W.; Thornton, J. M. *J. Mol. Biol.* **1991**, *218*, 397–412.
3. Shi, T.; Spain, S. M.; Rabenstein, D. L. *J. Am. Chem. Soc.* **2004**, *126*, 790–796.
4. Shi, T.; Spain, S. M.; Rabenstein, D. L. *Angew. Chem., Int. Ed.* **2006**, *45*, 1780–1783.
5. Che, Y.; Marshall, G. R. *Biopolymers* **2006**, *81*, 392–406.
6. Kang, Y. K. *J. Phys. Chem. B* **2006**, *110*, 21338–21348.
7. Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1–109.
8. Hayashi, T.; Asai, T.; Ogoshi, H. *Tetrahedron Lett.* **1997**, *38*, 3039–3042.
9. Baeza, J. L.; Gerona-Navarro, G.; Perez de Vega, M. J.; Garcia-Lopez, M. T.; González-Muñiz, R.; Martin-Martínez, M. *J. Org. Chem.* **2008**, *73*, 1704–1715.
10. Mutter, M. *J. Am. Chem. Soc.* **1977**, *99*, 8307–8314.
11. Rückle, T.; deLavallaz, P.; Keller, M.; Dumy, P.; Mutter, M. *Tetrahedron* **1999**, *55*, 11281–11288.
12. Pravda, Z.; Rudinger, J. *Collect. Czech. Chem. Commun.* **1955**, *20*, 1–8.
13. Monteiro, H. J. *Synthesis* **1974**, 137.
14. Lawson, P. J.; McCarthy, M. J.; Sargeson, A. M. *J. Am. Chem. Soc.* **1982**, *104*, 6710–6716.
15. Olsen, R. K.; Ramasamy, K.; Emery, T. *J. Org. Chem.* **1984**, *49*, 3527–3534.
16. Drauz, K.; Kleemann, A.; Martens, J.; Scherberich, P.; Effenberger, F. *J. Org. Chem.* **1986**, *51*, 3494–3498.
17. Yamaguchi, J.-i.; Ueki, M. *Chem. Lett.* **1996**, *8*, 621–622.
18. Córdova, A.; Reed, N. N.; Ashley, A. J.; Janda, K. D. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3119–3122.
19. Ma, Z.-s.; Li, P.; Chen, K.-x. *Xibei Daxue Xuebao* **2000**, *30*, 403–405.
20. Siebum, A. H. J.; Tsang, R. K. F.; van der Stehen, R.; Raap, J.; Lugtenburg, J. *Eur. J. Org. Chem.* **2004**, 4391–4396.
21. Jiang, S.; Li, P.; Lai, C. C.; Kelley, J. A.; Roller, P. P. *J. Org. Chem.* **2006**, *71*, 7307–7314.
22. Freidinger, R. M.; Schwenk Perlow, D.; Veber, D. F. *J. Org. Chem.* **1982**, *47*, 104–109.
23. Chemical and spectral data (^{1}H NMR, 300 MHz, TMS, DMSO-d_6,) for compounds **4**–**8**: *Compound* **4***:* δ = 0.76 (m, 6H, –CH(CH_3)$_2$), 1.35 (s, 9H, Boc), 1.71–1.91 (m, 3H, β-CH_2-Glu and –CH(CH_3)$_2$), 2.25 (m, 2H, γ-CH_2-Glu), 2.73–3.01 (m, 2H, β-CH_2-Phe), 3.54 (s, 3H, –OCH_3), 3.76 (m, 1H, α-CH-Val), 4.26 (m, 1H, α-CH-Glu), 4.41 (m, 1H, α-CH Phe), 6.82 (d, 1H, NH-Val), 7.07–7.41 (m, 7H, $CONH_2$ and aromatics), 7.83 (d, 1H, NH-Glu), 7.98 (d, 1H, NH-Phe).
Compound **5***:* δ = 0.75 (m, 6H, –CH(CH_3)$_2$), 1.35 (s, 9H, Boc), 1.43–1.60 (m, 4H, β-CH_2 and γ-CH_2-Glu), 1.89 (m, 1H, –CH(CH_3)$_2$), 2.77–2.97 (m, 2H, β-CH_2-Phe), 3.31 (m, 2H, –CH_2OH), 3.75 (m, 1H, α-CH-Val), 4.20 (m, 1H, α-CH-Glu), 4.41 (m, 1H, α-CH-Phe), 6.52 (s, 1H, –OH), 6.75 (d, 1H, NH-Val), 7.04–7.34 (m, 7H, $CONH_2$ and aromatics), 7.75 (d, 1H, NH-Glu), 7.94 (d, 1H, NH-Phe).
Compound **6***:* δ = 0.75 (m, 6H, –CH(CH_3)$_2$), 1.34 (s, 9H, Boc), 1.56–1.66 (m, 4H, β-CH_2 and γ-CH_2-Glu), 1.89 (m, 1H, –CH(CH_3)$_2$), 2.74–2.98 (m, 2H, β-CH_2-Phe), 3.13 (s, 3H, –SO_2CH_3), 3.73 (m, 1H, α-CH-Val), 4.12 (m, 2H, –CH_2O–), 4.28 (m, 1H, α-CH-Glu), 4.41 (m, 1H, α-CH-Phe), 6.80 (d, 1H, NH-Val), 7.05–7.41 (m, 7H, $CONH_2$ and aromatics), 7.82 (d, 1H, NH-Glu), 8.01 (d, 1H, NH-Phe).
Compound **7***:* δ = 0.87 (m, 6H, –CH(CH_3)$_2$), 1.34 (s, 9H, Boc), 1.72–1.96 (m, 5H, β-CH_2 and γ-CH_2-Pro and –CH(CH_3)$_2$), 2.75–3.02 (m, 2H, β-CH_2-Phe), 3.32–3.65 (m, 2H, δ-CH_2-Pro), 4.00 (m, 1H, α-CH-Val), 4.27 (m, 2H, α-CH-Phe and α-CH-Pro), 6.81 (d, 1H, NH-Val), 7.04–7.23 (m, 7H, $CONH_2$ and aromatics), 7.82 (d, 1H, NH-Phe). R_f 0.30 (Silica gel TLC; EtOAc). Anal. Calcd for $C_{24}H_{36}N_4O_5$: C, 62.59; H, 7.88; N, 12.16. Found: C, 62.54; H, 7.80; N, 12.20.
Compound **8***:* δ = 0.82 (m, 6H, –CH(CH_3)$_2$), 1.34 (s, 9H, Boc), 1.72–1.85 (m, 5H, β-CH_2 and γ-CH_2-Pro and –CH(CH_3)$_2$), 2.62–3.18 (m, 2H, β-CH_2-Phe), 3.38–3.68 (m, 2H, δ-CH_2-Pro), 3.90 (m, 1H, α-CH-Val), 4.20 (m, 1H, α-CH-Pro), 4.32 (m, 1H, α-CH-Phe), 6.82 (d, 1H, NH-Val), 7.13–7.28 (m, 7H, $CONH_2$ and aromatics), 8.36 (d, 1H, NH-Phe). R_f 0.35 (Silica gel TLC; EtOAc). Anal. Calcd for $C_{24}H_{36}N_4O_5$: C, 62.59; H, 7.88; N, 12.16. Found: C, 62.65; H, 7.95; N, 12.10.