Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 6000–6002

Expedient synthesis of *N*-*Z*-pyroglutamyl-amino acid derivatives

Alan R. Katritzky,[a,*] Parul Angrish,[a] Ekaterina Todadze[a] and Ion Ghiviriga[b]

[a]*Center for Heterocyclic Compounds, Department of Chemistry, University of Florida, Gainesville, FL 32611-7200, USA*
[b]*Department of Chemistry, University of Florida, USA*

Received 10 April 2007; revised 16 July 2007; accepted 17 July 2007
Available online 19 August 2007

Abstract—*N*-*Z*-Pyroglutamyl pseudopeptides **3a–c** are shown to be conveniently prepared from glutamyl-bis-Bt **1a** by cyclization of an N-terminal glutamic acid residue. Structures are supported by 2D NMR studies and by comparison with the same products prepared by direct coupling of the C-terminus activated N-pGlu **1b** and free amino acids **2a–c**.
© 2007 Elsevier Ltd. All rights reserved.

Cellular mechanisms use regulation at different stages of diverse functions. DNA to mRNA transcription control, mRNA to protein translation control, and further protein post-translation modification are important examples in the regulation of numerous cellular functions and activities.[1] Post-translational modification of a protein is one of the later steps in protein biosynthesis for many proteins. Post-translational modifications of amino acids extend the range of functions of a protein by attaching amino acids with other biochemically active functional groups such as acetate, phosphate, lipids or carbohydrates. Other post-translational changes affect an amino acid more directly as in citrullination or the formation of pyroglutamic acid (pGlu).[2–7]

The formation of pGlu occurs through cyclization of the amino terminal residue (glu or gln) to pGlu inside the cell prior to the activation of the completed protein.[1] In nature, simple and complex pseudopeptides participate in various biological processes. Much effort has been focused on the synthesis of pseudopeptides as agonists and antagonists in medical applications. The presence of pGlu is important for the biological activity of various proteins and peptides.[1,2,8–11] Thyrotropin-releasing hormone (TRH), L-pGlu-L-His-Pro-NH_2, is a tripeptide responsible for maintaining thyroid-stimulating hormone (TSH) levels in the anterior pituitary.[1] Pyroglutamic acid, the first residue of TRH, is responsible for at least half of the peptide's binding energy.[12,13] Further, *N*-benzylpyroglutamyl-L-phenylalanine derivatives are very late antigen-4 (VLA-4) antagonists designed to inhibit the vascular cell adhesion molecule (VCAM)/VLA-4 interaction and thus treat inflammatory diseases such as asthma.[14,15]

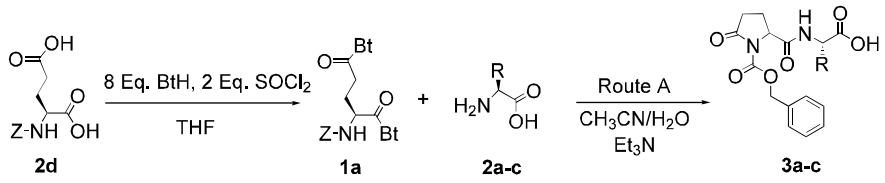
Now, we report a convenient one-pot preparation of *N*-*Z*-pyroglutamyl pseudopeptides derived from the cyclization of the amino terminal glutamic acid residue (Scheme 1, Route A). Thus, we show that peptide coupling of *N*-*Z*-Glu-diBt **1a**[16] with diverse L-amino acids **2a–c** (Ala, Phe and Val) in aqueous acetonitrile (CH_3CN/H_2O) in the presence of Et_3N for 1 h (Scheme 1)[16–18] followed by washing the crude products with 4 N HCl gave **3a–c** in 58–88% yields in high purity without chromatography (Table 1).

In the present paper the structures of products **3a–c** are rigorously proved by 2D NMR (Fig. 1). Thus for **3a**, the cross-peaks in the gHMBC spectrum between the methylene protons at 2.43, 2.37, 2.26, 1.86 and the carbon at 174.3 indicate that the carbon at 174.3 is bound to that at 31.5. Similar cross-peaks between the protons at 2.26, 1.86, 4.63 and the carbon at 171.4 indicate that this carbon is next to 59.4. The proton at 4.63 couples with the carbon at 174.3 which confirms the 5-oxoproline moiety. Other couplings confirm the presence of the second amino acid moiety (alanine) and the coupling of protons at 8.51, 4.17 with carbon 171.4 indicates that this amino acid is bound to proline. No coupling between 4.63 and 151.2 was seen, but the *Z* fragment must be connected to the nitrogen in proline because these are the only valences left unpaired.

In a previous paper from our group,[16] the product from the coupling reaction between *Z*-L-Glu-diBt **1a** (1 equiv)

Keywords: Protein; Protein biosynthesis; Functional groups; Acetate; Phosphate; Lipids; Carbohydrates; Citrullination; Pyroglutamic.
* Corresponding author. Tel.: +1 352 392 0554; fax: +1 352 392 9199; e-mail: katritzky@chem.ufl.edu

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.07.052



Scheme 1. Preparation of **3a–c** using Route A.

Table 1. Preparation of **3a–c** using Route A (Scheme 1) and Route B (Scheme 4)

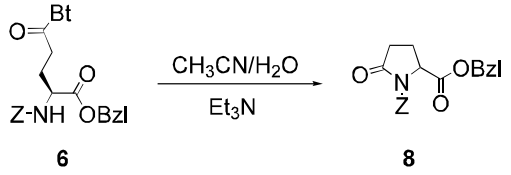
Entry	Amino acid	Product	Yield[a] (%)		Mp (°C)
			Route A	Route B	
1	L-Ala **2a**	*Z*-pGlu-Ala **3a**	58	55	205–207
2	L-Phe **2b**	*Z*-pGlu-Phe **3b**	88	81	173–174
3	L-Val **2c**	*Z*-pGlu-Val **3c**	71	70	154–156

[a] Isolated yield.

and phenylalanine **2b** (2 equiv) was reported as *Z*-L-Glu-L-Phe-OH (see structure **5t** in Ref. 16) We now know that this was erroneous, because of a regrettable mistake in the reading of 1D ^{1}H NMR and ^{13}C NMR. We also assigned an incorrect structure to *Z*-L-Glu-L-di-Val-OH (**5u** in Ref. 16) due to a similar error.

Recently, we reported the preparation of dipeptides using C-α- and C-γ-activated glutamic acid under varied conditions leading to the formation of products **5** and **7** as shown in Scheme 2.[17,18] However, we now find that when **6**[18] is stirred in aqueous acetonitrile in the presence of Et_3N (but with no amino acid added), **6** cyclized to form **8** (Scheme 3).

We also prepared **3a–c** by direct coupling (Route B) of *Z*-L-pGlu-Bt **1b** with diverse amino acids **2a–c** (Ala, Phe, and Val) (Scheme 4). Routes A (Scheme 1) and B (Scheme 4) gave comparable yields of **3a–c** (Table 1).



Scheme 3. Preparation of **8**.

We show that our methodology offers two fast and convenient routes (A, Scheme 1; B, Scheme 4) to prepare pGlu pseudopeptides in good yields and high purity as compared with literature methods. Preparations of pseudopeptides of type **3** from the corresponding esters or salts have been achieved using coupling reagents including (i) DCC/HOBt/Et_3N,[19] (ii) DEPC/Et_3N,[20] (iii) HBTU/DIEA,[14,15] (iv) EDC/HOBt/NEM.[12] Other methods are (v) active ester derived from 2,4,5-trichlorophenol[21] or ethyl chloroformate,[13] and (vi) a one-pot Ugi-4-center-3-component reaction both in solution phase[22] and under microwave irradiation.[23]

In conclusion, we have demonstrated the preference in glutamic acid derivatives of activated α-COOH over activated γ-COOH to undergo coupling thereby leading to the formation of pyroglutamic dipeptides. These results were further supported by the preferred reactivity route of activated γ-COOH of glutamic acid in the absence and presence of an amino acid with protected α-COOH.

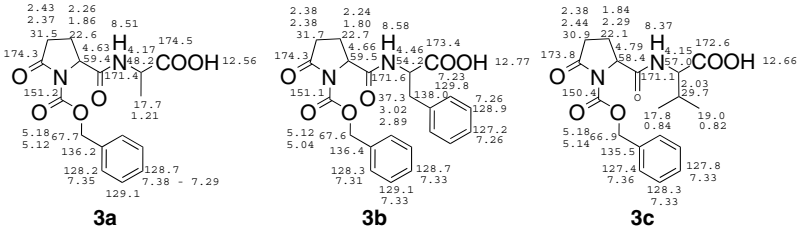
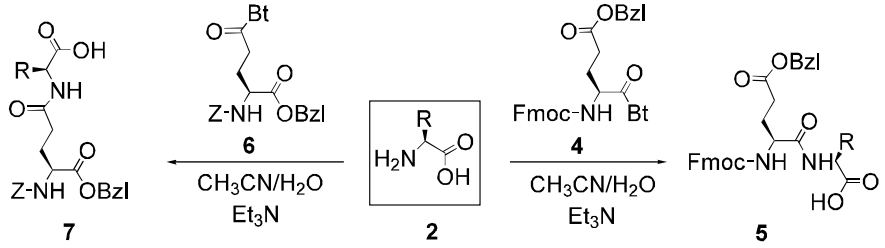
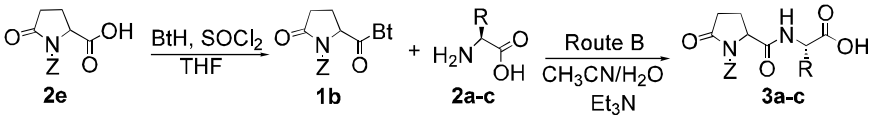


Figure 1. ^{1}H and ^{13}C chemical shifts in compounds **3a–c**, in DMSO-d_6 at 25 °C.



Scheme 2. Preparation of natural (**5**) and unnatural (**7**) dipeptides.



Scheme 4. Preparation of **3a**–**c** using Route B.

Supplementary data

Synthetic details and compound characterization data for **1b**, **3a**–**c**, and **8** is available free of charge via the Internet at http://www.elsevier.com. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.07.052.

References and notes

1. Abraham, G. N.; Podell, D. N. *Mol. Cell. Biochem.* **1981**, *38*, 181.
2. Gerona-Navarro, G.; Bonache, M. A.; Herranz, R.; Garcia-Lopez, M. T.; Gonzalez-Muniz, R. *J. Org. Chem.* **2001**, *66*, 3538.
3. Morgan, B. A.; Gainor, J. A. *Annu. Rep. Med. Chem.* **1989**, *24*, 243.
4. Toniolo, C. *Int. J. Pept. Protein Res.* **1990**, *35*, 287.
5. Giannis, A.; Kolter, T. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1244.
6. Cativiela, C.; Diaz-de-Villegas, M. D. *Tetrahedron: Asymmetry* **1998**, *9*, 3517.
7. Cativiela, C.; Diaz-de-Villegas, M. D. *Tetrahedron: Asymmetry* **2000**, *11*, 645.
8. Hanessian, S.; McNaughtonSmith, G.; Lombart, H. G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789.
9. Polyak, F.; Lubell, W. D. *J. Org. Chem.* **1998**, *63*, 5937.
10. Curran, T. P.; Marcaurelle, L. A.; O'Sullivan, K. M. *Org. Lett.* **1999**, *1*, 1225.
11. Gosselin, F.; Lubell, W. D. *J. Org. Chem.* **2000**, *65*, 2163.
12. Simpson, J. C.; Ho, C.; Shands, E. F. B.; Gershengorn, M. C.; Marshall, G. R.; Moeller, K. D. *Bioorg. Med. Chem.* **2002**, *10*, 291.
13. Sievertsson, H.; Chang, J. K.; Folkers, K. *J. Med. Chem.* **1972**, *15*, 8.
14. Chen, L.; Tilley, J. W.; Guthrie, R. W.; Mennona, F.; Huang, T. N.; Kaplan, G.; Trilles, R.; Miklowski, D.; Huby, N.; Schwinge, V.; Wolitzky, B.; Rowan, K. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 729.
15. Chen, L.; Tilley, J.; Trilles, R. V.; Yun, W. Y.; Fry, D.; Cook, C.; Rowan, K.; Schwinge, V.; Campbell, R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 137.
16. Katritzky, A. R.; Angrish, P.; Suzuki, K. *Synthesis* **2006**, 411.
17. Katritzky, A. R.; Todadze, E.; Cusido, J.; Angrish, P.; Shestopalov, A. A. *Chem. Biol. Drug Des.* **2006**, *68*, 37.
18. Katritzky, A. R.; Todadze, E.; Shestopalov, A. A.; Cusido, J.; Angrish, P. *Chem. Biol. Drug Des.* **2006**, *68*, 42.
19. Nizova, I. A.; Krasnov, V. P.; Levit, G. L.; Kodess, M. I. *Amino Acids* **2002**, *22*, 179.
20. Aoki, S.; Cao, L. W.; Matsui, K.; Rachmat, R.; Akiyama, S.; Kobayashi, M. *Tetrahedron* **2004**, *60*, 7053.
21. Jain, R.; Singh, J.; Perlman, J. H.; Gershengorn, M. C. *Bioorg. Med. Chem.* **2002**, *10*, 189.
22. Hanusch-Kompa, C.; Ugi, I. *Tetrahedron Lett.* **1998**, *39*, 2725.
23. Tye, H.; Whittaker, M. *Org. Biomol. Chem.* **2004**, *2*, 813.