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The Synthesis of β-Peptides Containing Guanidino Groups

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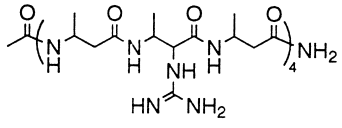
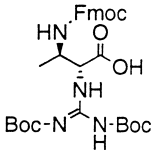
Abstract—The synthesis of the β-peptide **1** by the postsynthetic modification of the corresponding amino-containing peptide **3** is described. The potential of **1** to act as a template for the ligation of complementary negatively-charged peptides is discussed. © 2001 Published by Elsevier Science Ltd.

Oligomers of β-amino acids (β-peptides) fold into well-defined secondary structures including helices, sheets and turns that are analogous to the well-known secondary structures of proteins.[1–3] Some β-peptides composed of as few as six amino acids adopt a stable helical conformation designated as the L + 2, 3_1 or 14 helix which is characterized by a perfect three-residue geometric repeat with a 5 Å pitch.[4] This regular repeat should make it possible to design peptides in which selected side chains lie on a straight line parallel to the helix axis. Such molecules might act as templates for the ligation of 'complementary' β-peptides as do some α-peptides.[5] Here, we describe the synthesis of a positively charged peptide of this nature that could, in principle, facilitate the ligation of complementary negatively charged β-peptides (Fig. 1).

The guanidino group plays an important role in biologically active proteins, peptides and peptidomimetics,[6] particularly in arginine-containing peptides and proteins. Guanidine and its simple derivatives are strongly basic (the pK_a of the guanidinium ion is approximately 13.5), and are fully protonated under physiological conditions. Since positive charge of guanidino groups and their potential to form hydrogen bonds serve as a basis for specific interactions between biological molecules we decided to synthesize peptide **1** as a potential template.



1



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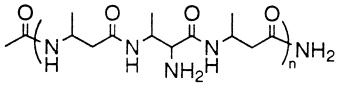
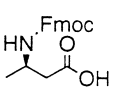


3 (n = 4), **4** (n = 6)



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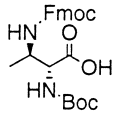

6

Attempts to use the β-amino acid derivative **2** with a protected guanidino group as a building block in β-peptide chain elongation failed. We surmise that the bulky Boc functionality used as a protecting group on the guanidino group of **2** may have prevented access of the required amino functionality to the activated carboxylate group. Peptide **1**, therefore, could only be obtained post-synthetically from peptide **3** by converting its amino moieties to guanidino groups. The syntheses of the β-peptide amides **3** and **4** were carried out

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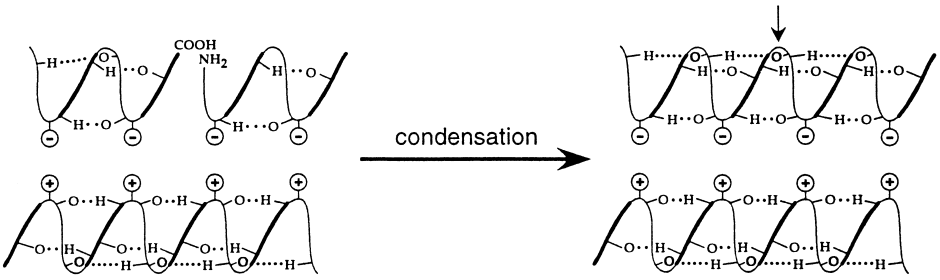
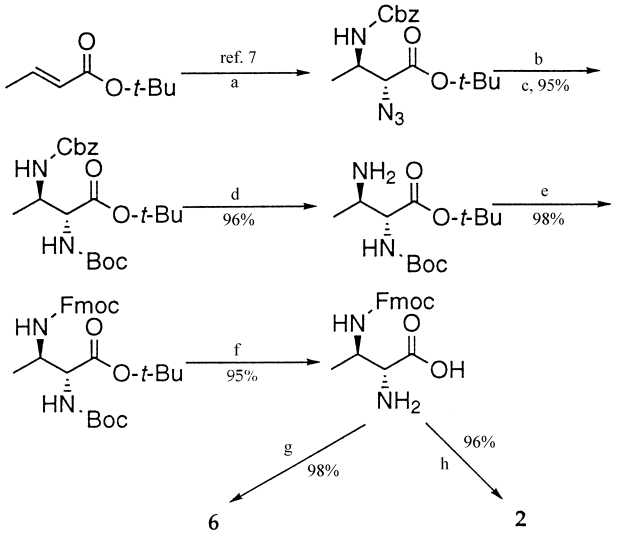


Figure 1. Schematic representation of proposed template-directed ligation. The positive charged β-peptide **1** serves as a template by aligning complementary negative charges on substrate peptides. The arrow indicates the position of the new peptide bond.

using Fmoc solid-phase peptide synthesis on Applied Biosystems' AM_{NH2} resin. β-Amino acid derivatives **5** and **6** were employed as building blocks. Compound **5** was synthesized by a published procedure[7] in 29% yield. Compound **6** was synthesized by an extension of a previously published synthesis[8] from one of our laboratories in an overall yield of 38% (Scheme 1). NMR and mass spectroscopy were used to characterize these compounds.[9]

In the course of the syntheses of peptides **3** and **4**, the first amino acid (**5**) was attached to the resin very efficiently. The next seven residues in the peptide sequences were incorporated using the standard protocol.[10] However, the coupling and deprotection efficiency decreased after the formation of the octapeptide, possibly due to the folding of the peptide in the swelling solution. To overcome this difficulty, in subsequent elongation steps we carried out two successive couplings using prolonged coupling times and also a prolonged deprotection time (standard coupling time 45–60 min and deprotection time 3 min; the coupling time employed in these steps was 120 min and the deprotection time was 6 min). The N-terminal β-amino groups of these peptides were acetylated after the completion of chain elongation. The peptides were cleaved from the resin using TFA, which simultaneously removed the Boc protecting groups. Crude peptides were obtained after cleavage with purities of 33% for **3**, and 27% for **4**, determined by RP-HPLC on a C18 column.[11] The purities of the purified peptides were above 95% for **3** and **4**. The presence of several peaks close to each other in front of the major peaks (product peaks) in the HPLC profiles indicates that the coupling reactions were incomplete. The purified peptides were water soluble. They were characterized by electron spray ionization MS (**3** MH^+ 1141, **3** MNa^+ 1163; **4** MH^+ 1681, and **4** MNa^+ 1703), and (**3** $M+TFA^-$ 1253; **4** $M+TFA^-$ 1793). All the measured masses agreed with theoretical values.



Scheme 1. (a) Ref 7; (b) Ph_3P (1.1 equiv), H_2O (5 equiv), THF; (c) $(Boc)_2O$ (2 equiv); (d) 10% Pd/C, MeOH; (e) Fmoc-Cl (1.1 equiv), DIEA (1.1 equiv), CH_2Cl_2; (f) TFA; (g) $(Boc)_2O$ (1.5 equiv), DIEA (3 equiv); (h) **8** (1.1 equiv), DIEA (3 equiv).

The circular dichroism spectra of **3** in pure water, pH 7 buffer, pH 12 buffer and methanol solution (0.28 mM) were recorded (Fig. 2) in an attempt to characterize its conformation. The spectrum in water has a minimum at 203 nm and a maximum at 186 nm, indicating the presence of a well-defined secondary structure. The blue-shift of the maximum and minimum relative to those reported for other β-peptides,[12] was possibly caused by the amino groups in the backbone chain, which are protonated under the conditions of the experiment. When the pH was increased, slight red shifts of the peaks were observed. These might arise from the deprotonation of the amino groups. The peak intensity decreased at high pH values, perhaps indicating a decrease in the percentage of the peptide that has the defined secondary structure. The same behavior was observed in methanol solution. The shape of the curve suggests that the peptide forms a 12/10/12 helix,[12] or a

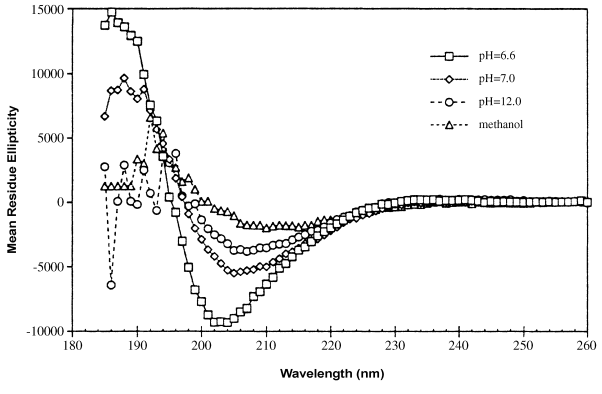


Figure 2. CD spectra of 0.28 mM β-peptide **3** in aqueous solution at different pHs or in methanol. The mean residue ellipticity is given in $deg.cm^2/dmol$.

hair-pin structure.[3] An NMR study designated to give detailed structural information is in progress.

In the guanidinylation reaction of resin-bound **3** (4-methylbenzhydrylamine resin), a number of reagents were explored. The most commonly used reagent **7**, 1*H*-pyrazole-carboxamidine, failed, but reagent **8**, *N*,*N*′-di-Boc-*N*″-triflylguanidine, was used successfully.[13,14] The guanidinylation of the amino groups was carried out in a 1 M solution of **8** in dichloromethane in the presence of at least a 4-fold excess of triethylamine. After 6 days, only two, on average, of the four amino groups in **3** were converted to guanidino groups. However, this intermediate product after cleavage from the resin with trifluoromethanesulfonic acid,[15] could be further guanidinylated in solution as described below to give the desired product.

A more efficient synthesis, entirely in homogeneous solution, was then developed. The guanidinylating reagent **8** is usually used in nonpolar solvents, such as dichloromethane. However, since **3** is insoluble in dichloromethane, methanol was chosen as the solvent. Peptide **3** (1 mg) was added to a mixture of 0.75 μL of diisopropylethylamine and 150 μL of a 0.5 M solution of **8** in methanol. The solution was allowed to stand for 6 days at 45 °C. Dialysis of the reaction mixture followed by RP-HPLC purification yielded peptide **1** in 31% yield (electron spray ionization MS, MH^+ 1309, $M+TFA^-$ 1421; theoretical values MH^+ 1309, $M+TFA^-$ 1421).

The methods described above should make available a variety of β-peptides containing 2-guanidino-3-aminobutanoic acid residues. They should prove useful in template-directed synthesis and perhaps as high affinity aptomers for proteins and other biomolecules.

Acknowledgements

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References and Notes

1. Krauthäuser, S.; Christianson, L. A.; Powell, D. R.; Gellman, S. H. *J. Am. Chem. Soc.* **1997**, *119*, 11719.
2. Seebach, D.; Overhand, M.; Kuhnle, F. N. M.; Martinon, B.; Oberer, L.; Hommel, V.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913.
3. Seebach, D.; Schreiber, J. V.; Abele, S.; Daura, X.; van Gunsteren, W. F. *Helv. Chim. Acta* **2000**, *83*, 34.
4. Seebach, D.; Matthews, J. L. *Chem. Commun.* **1997**, 2015.
5. Lee, D. H.; Granja, J. R.; Martinez, J. A.; Severin, K.; Ghadiri, M. R. *Nature* **1996**, *382*, 525.
6. Berlinck, R. G. S. *Nat. Prod. Rep.* **1996**, 377.
7. Davies, S. G.; Ichihara, O. *Tetrahedron: Asymmetry* **1991**, *2*, 183.
8. Han, H.; Yoon, J.; Janda, K. D. *J. Org. Chem.* **1998**, *63*, 2045.
9. Compound **6** $[\alpha]_D^{rt}$ +8.5° (methanol). ^1H NMR (400 MHz, $CDCl_3$) δ 1.23 (br. s, 3H), 1.46 (s, 9H), 4.21–4.47 (m, 5H), 5.67 (br. s, 1H), 7.28–7.32 (m, 2H), 7.39 (t, $J=7.2$ Hz, 2H), 7.58–7.60 (m, 2H), 7.75 (d, $J=7.2$ Hz, 2H). FABMS: MNa^+ 463. Theoretical value 463.
10. Beck-Sickinger, A. G.; Dürr, H.; Jung, G. *Peptide Res.* **1991**, *4*, 88.
11. HPLC condition: 0.1% TFA H_2O/acetonitrile gradient (0–40% acetonitrile in 30 min) at a flow rate of 1 mL/min.
12. Seebach, D.; Abele, S.; Gademan, K.; Guichard, G.; Hintermann, T.; Jaun, B.; Matthews, J. L.; Schreiber, J. V.; Oberer, L.; Hommel, V.; Widmer, H. *Helv. Chim. Acta* **1998**, *91*, 932.
13. Feichtinger, K.; Zaft, C.; Sings, H. L.; Goodman, M. *J. Org. Chem.* **1998**, *63*, 3804.
14. Feichtinger, K.; Sings, H. L.; Baker, T. J.; Matthews, K.; Goodman, M. *J. Org. Chem.* **1998**, *63*, 8432.
15. Thompson, P. E.; Keah, H. H.; Gomme, P. T.; Stanton, P. G.; Hearn, M. T. W. *Int. J. Pept. Prot. Res.* **1995**, *46*, 174.