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Solid-Phase Synthesis and CD Spectroscopic Investigations of Novel β-Peptides from L-Aspartic Acid and β-Amino-L-alanine

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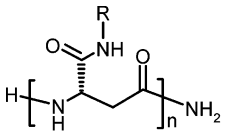
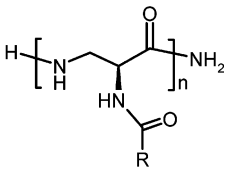
ABSTRACT



β³-peptide



β²-peptide

A solid-phase synthesis method for the preparation of novel β³- and β²-peptides derived from L-aspartic acid and β-amino-L-alanine, respectively, is described. The methodology allows independent buildup of the β-peptide backbone and the introduction of sequential side chain substitutions. Representative peptides from the two classes, an amino-substituted β³-hexapeptide and an acyl-substituted β²-hexapeptide, have been prepared, and their solution conformation is studied by circular dichroism (CD) spectroscopy.

β-Peptides are becoming increasingly important from biological and pharmaceutical stand points.[1] Although they can display similar biological activity as α-peptides, for instance, as a somatostatin receptor agonist β-peptide,[2] they are considerably more resilient to proteolysis and metabolism.[3] Furthermore, these molecules exhibit well-defined secondary structures such as helices, sheets, and turns in solution.[4] β-Peptides are made from β-amino acids that are subdivided into mainly three types, β³-, β²-, and β²,³-amino acids, depending upon the substitution of the backbone carbon. The solution conformation of β-peptides is dictated by the substitution pattern. In general, monosubstituted (β³- or β²-) peptides form a 12- or 14-helical structure and alternating β²/β³-peptides prefer a 10/12 helix. The secondary structure is enhanced by the presence of organic solvents such as methanol and 2,2,2-trifluoroethanol (TFE) or by introduction of other constraints such as cyclic ring systems, salt-bridge formation, or neutralization of the helix macrodipole.[5]

β-Peptides are accessible by solid-phase peptide synthesis. Parallel syntheses of these molecules have made it possible

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(1) (a) Koyack, M. J.; Cheng, R. P. *Methods Mol. Biol.* **2006**, *340*, 95−109. (b) Patch, J. A.; Barron, A. E. *Curr. Opin. Chem. Biol.* **2002**, *6*, 872−877. (c) English, E. P.; Chumanov, R. S.; Gellman, S. H.; Compton, T. *J. Biol. Chem.* **2006**, *281*, 2661−2667. (d) Kritzer, J. A.; Stephens, O. M.; Guarracino, D. A.; Reznik, S. K.; Schepartz, A. *Bioorg. Med. Chem.* **2005**, *13*, 11−16. (e) Potocky, T. B.; Menon, A. K.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 3686−3687. (f) Porter, E. A.; Weisblum, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2005**, *127*, 11516−11529. (g) Qiu, J. X.; Petersson, E. J.; Matthews, E. E.; Schepartz, A. *J. Am. Chem. Soc.* **2006**, *128*, 11338−11339.

(2) Gademann, K.; Kimmerlin, T.; Hoyer, D.; Seebach, D. *J. Med. Chem.* **2001**, *44*, 2460−2468.

(3) Gademann, K.; Hintermann, T.; Schreiber, J. V. *Curr. Med. Chem.* **1999**, *6*, 905−925.

(4) (a) Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. *Chem. Rev.* **2001**, *101*, 3219−3232. (b) Langenhan, J. M.; Guzei, I. A.; Gellman, S. H. *Angew. Chem., Int. Ed.* **2003**, *42*, 2402−2405. (c) Daura, X.; Gademann, K.; Schafer, H.; Jaun, B.; Seebach, D.; van Gunsteren, W. F. *J. Am. Chem. Soc.* **2001**, *123*, 2393−2404.

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to study their structure−activity relationship in a systematic manner.[1] Generally, β-peptides are synthesized using *N*-Fmoc-protected β-amino acid monomers.[6] These β-amino acids are usually made from corresponding α-amino acids. The synthesis of Fmoc-β-amino acids from Fmoc-α-amino acids is nontrivial, and this synthetic procedure allows access to only a limited number of Fmoc-β-amino acids. This also renders β-amino acids extremely expensive starting materials.

Adopting an approach similar to that of Farrera−Sinfreu,[7] which they applied for the synthesis of γ-peptides, we have developed a synthetic strategy to obtain two new families of β-peptides formed by L-aspartic acid and β-amino-L-alanine monomers (Figure 1). These monomers allow

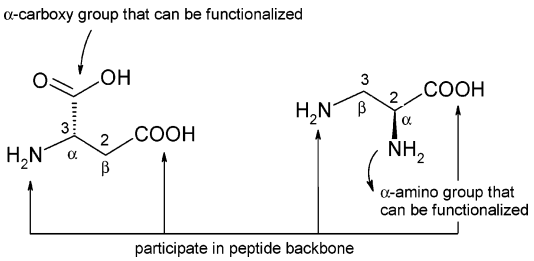


Figure 1. L-Aspartic acid (left) and β-amino-L-alanine (right) monomers used in this study for the synthesis of β^3- and β^2-peptides, respectively.

synthesis of β^3- and β^2-peptides with a wide variety of side chains. The α-carboxy group of L-aspartic acid and the α-amino group of β-amino-L-alanine (L-diaminopropionic acid, Dap) are left free for the introduction of different substituents (to obtain a heterooligomer) during the synthesis or of the same substituent as a final functionalization step (to obtain a homooligomer). The independent buildup of the backbone and side chain sequences leads to a very high level of synthetic versatility. Using this strategy, we report here the synthesis of representative β-peptides from both classes, namely, a β^3-hexapeptide (**1**, Figure 2) with different side chain substitutions and two β^2-hexapeptides, one with different side chain substitutions (**2**) and the other with no side chain substitution (**3**). Finally, CD spectroscopy is used to elucidate key structural features of the two new families of compounds in different solvent systems.

β^3-Hexapeptide (**1**) and β^2-hexapeptides (**2** and **3**) were synthesized utilizing a Fmoc/allyl combined solid-phase strategy (Scheme 1), where Fmoc protection was used to

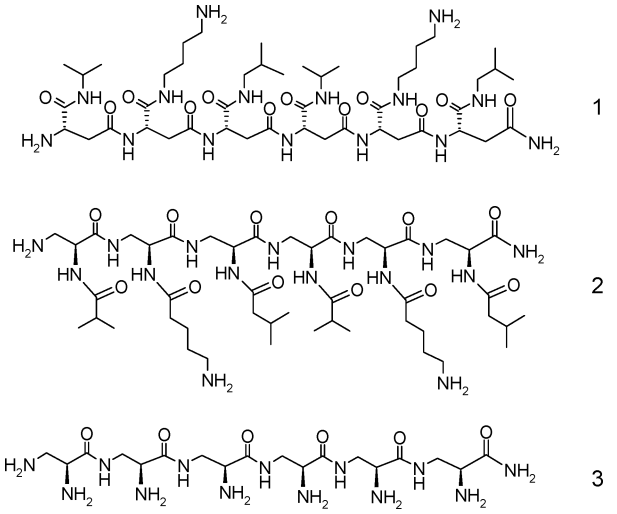


Figure 2. Structure of β^3- (**1**) and β^2-hexapeptides (**2**, **3**).

build the backbone of the peptide and allyl protection was used to introduce the side chain functionalities. The same strategy allows the synthesis of either heterooligomeric β-peptides, such as **1** and **2**, or homooligomeric β-peptides (e.g., **3**). In heterooligomeric β-peptides **1** and **2**, the different side chains were introduced after coupling of each monomer and deallylation of the side chain carboxy or amino group. Two of the side chains were chosen with a free amino group (lysine side chain mimic) to keep a balance between both the hydrophobic and the charged groups and to enhance solubility in an aqueous medium. In the case of β-peptide homooligomer **3**, the backbone was synthesized first, and all the side chain allyl protections were removed in the end.

The backbone of β^3-peptide **1** was prepared from orthogonally protected L-aspartic acid, N^{α}-Fmoc-L-aspartic acid α-allyl ester, using BOP with HOBt on rink amide MBHA resin (Scheme 1). The reaction was monitored by the ninhydrin test. The side chain was deallylated followed by the introduction of the corresponding amine using the same coupling reagents as mentioned above. To achieve complete deallylation of the side chain carboxyl, the reaction was optimized by varying (i) the amount of palladium catalyst used, (ii) the length of the reaction time, and (iii) the number of times the reaction was repeated (Table S1).[8] Treatment with 0.08 equiv of $Pd(PPh_3)_4$ and 8 equiv of $PhSiH_3$ for 35 min and repeating the reaction twice led to complete deallylation. During optimization, the reaction was monitored by test cleavage followed by analytical reversed-phase HPLC and mass spectrometric analysis.

β^2-Peptides **2** and **3** were prepared from orthogonally protected β-amino-L-alanine, N^{α}-Alloc-N^{β}-Fmoc-L-diaminopropionic acid (**4**), essentially following the same procedure as that described for β^3-peptide **1** (Scheme 1). However, N^{α}-Alloc-N^{β}-Fmoc-L-Dap is not commercially available. It was

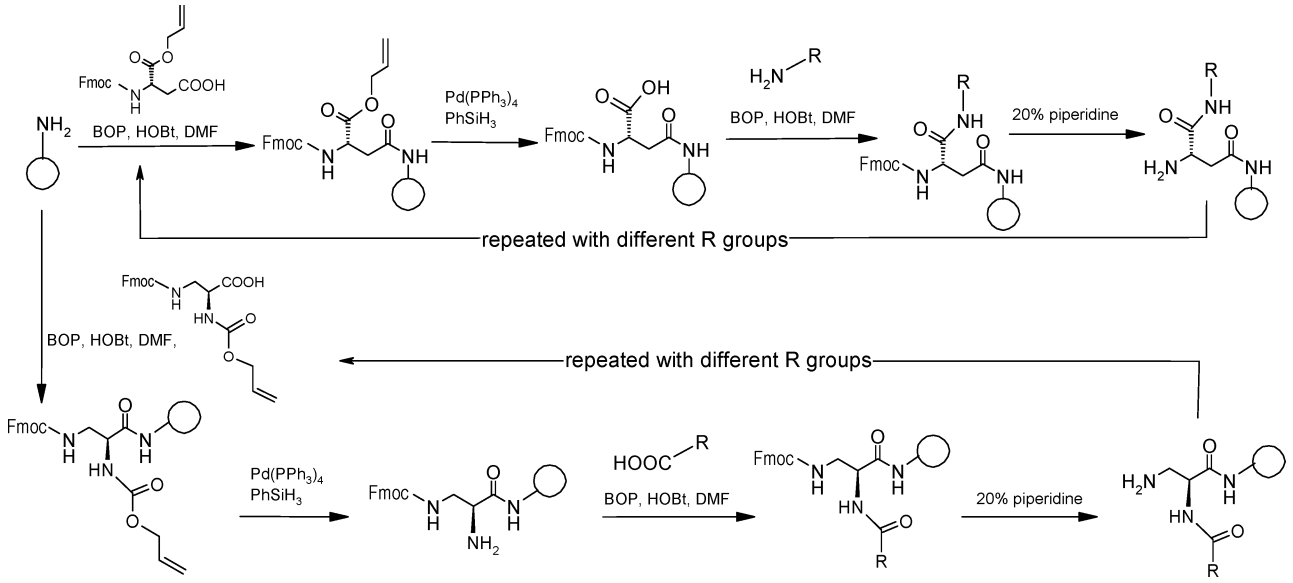
(5) (a) Appella, D. H.; Christianson, L. A.; Klein, D. A.; Powell, D. R.; Huang, X.; Barchi, J. J., Jr.; Gellman, S. H. *Nature* **1997**, *387*, 381−384. (b) Arvidsson, P. I.; Rueping, M.; Seebach, D. *Chem. Commun.* **2001**, 649−650. (c) Rueping, M.; Mahajan, Y. R.; Jaun, B.; Seebach, D. *Chemistry* **2004**, *10*, 1607−1615. (d) Hart, S. A.; Bahadoor, A. B.; Matthews, E. E.; Qiu, X. J.; Schepartz, A. *J. Am. Chem. Soc.* **2003**, *125*, 4022−4023.

(6) Seebach, D.; Overhand, M.; Kuhnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Amstutz, R.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913−941.

(7) Farrera-Sinfreu, J.; Zaccaro, L.; Vidal, D.; Salvatella, X.; Giralt, E.; Pons, M.; Albericio, F.; Royo, M. *J. Am. Chem. Soc.* **2004**, *126*, 6048−6057.

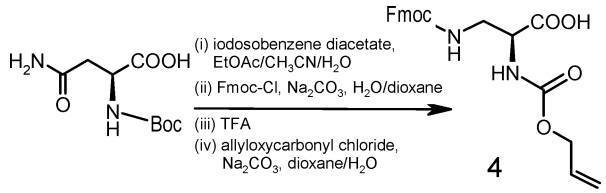
(8) See Supporting Information.

Scheme 1. General Pathway of Solid-Phase Synthesis of Amino-Substituted β^3-Peptides and Acyl-Substituted β^2-Peptides



synthesized in four steps starting with an α-amino acid, Boc-protected L-asparagine (Scheme 2). The first three steps of

Scheme 2. Solution-Phase Synthesis of Orthogonally Protected L-Diaminopropionic Acid



the synthesis yield N^{β}-Fmoc-L-alanine and have been reported by Daleat et al.[9] Following their procedure, in the first step, Boc-L-Asn was subjected to rearrangement, in the presence of iodosobenzene, yielding N^{α}-Boc-L-Dap. Fmoc protection of the β-amino group followed by replacement of the α-amino Boc with an allyloxycarbonyl (Alloc) group gave the orthogonally protected **4** in overall 54% yield.

At the end of the synthesis, and after the removal of the terminal Fmoc group, β-peptides (**1**−**3**) were cleaved from the resin using TFA/H_2O/silane at room temperature for 2 h. Crude peptides were purified using semipreparative reversed-phase HPLC prior to characterization by electrospray and/or MALDI-TOF mass spectrometry.

The ability of the β-peptides (**1**−**3**) to adopt an ordered secondary structure was evaluated by CD spectroscopy in four different solvents, namely, TFE, methanol, water, and phosphate buffer (pH 7.4). Peptides were studied at different concentrations (100−500 μM), and the CD spectra were found to be independent of concentration, suggesting that no changes in aggregation state occur in this concentration range. The CD spectra of **1** display a marked difference in organic solvents (TFE and methanol) and aqueous solvents (water and phosphate buffer). In TFE, there is a minimum at 214 nm ($\Theta = -20 \times 10^3$) and a maximum at 204 nm (Figure 3a). Similarly, in methanol there is a minimum at 214 nm; however, this peak is not present in aqueous solvents. Organic solvents such as TFE and methanol have been suggested to be conducive to secondary structure formation,[5c,10] and the CD spectra of **1** suggest a gain of some structural feature in organic solvents. The CD spectra of monosubstituted β^3-peptides typically display a negative minimum at 214−220 nm and a positive maximum near 200 nm that have been ascribed to 14-helical structure.[5] β-Peptide **1** is different from the reported β-peptides[4,5] as it contains an extra amide group in the side chain and the CD of this peptide measures the collective signal of all the amides, including those within the side chain making the spectrum difficult to interpret. The 1D NMR spectrum of **1** in TFE (Figure S1)[8] and the preliminary 2D NMR experiments (spectra not shown) and MD simulations (not shown here) suggest the presence of a helical structure and one major conformational isomer. In this context, it is of particular interest to note that Fernandez−Santin et al. observed a helical structure for a β^3-polypeptide, poly(α-isobutyl-L-aspartate), made by polymerization of α-isobutyl-L-aspartate based on X-ray diffraction and molecular modeling studies.[11] This also supports our conjecture regarding the helical nature of β^3-peptides from L-aspartic acid. However, for complete three-dimensional structure elucida-

(9) Delaet, N. G.; Robinson, L. A.; Wilson, D. M.; Sullivan, R. W.; Bradley, E. K.; Dankwardt, S. M.; Martin, R. L.; Van Wart, H. E.; Walker, K. A. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2101−2104.

(10) Abele, S.; Guichard, G.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 2141−2156.

(11) Fernandez-Santin, J. M.; Aymami, J.; Rodriguez-Galan, A.; Munoz-Guerra, S.; Subirana, J. A. *Nature* **1984**, *311*, 53−54.

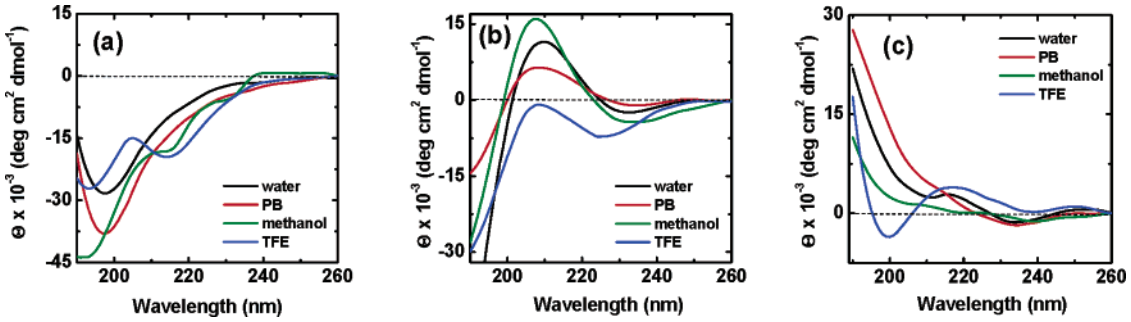


Figure 3. Circular dichroism spectra in water, phosphate buffer (1 mM, pH 7.4), methanol, and TFE at 25 °C for β-peptide hexamers (a) β^3-peptide **1**, (b) β^2-peptide **2**, and (c) β^2-peptide **3**.

tion of β^3-peptides such as **1**, detailed NMR and MD simulation studies are required.

The CD spectrum of β^2-hexapeptide **2** in TFE displays a minimum at 224 nm and a maximum at 208 nm (Figure 3b). In methanol, the broad minimum at ca. 230 nm is much less intense; however, at 208 nm, a strong positive maximum is observed. The intensity of peaks in water and phosphate buffer is reduced substantially. **2** seems to have some structural feature in TFE but not in other solvents. Furthermore, preliminary NMR studies of **2** in TFE suggest the presence of a single major conformational isomer. In general, β^2-peptides have been less explored and conformational studies of few β^2-peptides suggest that they form less-stable structures compared to their β^3 counterparts.[12,13] The CD spectrum of **2** in TFE appears similar to the CD spectra of monosubstituted β^2-peptides reported previously that form helical structures.[13,14] These spectra cannot be directly compared due to the presence of an additional side chain amide bond in **2** that also contributes to the spectrum. Therefore, high-resolution structures will be required to fully interpret the CD results, and these studies are currently underway. The β^2-hexapeptide **3** with free amino groups in the side chain seems to be essentially unstructured in all the solvents as no minima are observed between 210 and 230 nm (Figure 3c). This could be due to the interruption of the backbone hydrogen bonding by the side chain amino group and destabilization of the structure. The two classes of β-peptides described here are more polar than previously reported β-peptides with similar side chains due to the extra amide bond in the side chain. Furthermore, this extra amide bond provides more hydrogen-bonding capability and may perhaps lead to unprecedented secondary structures for β-peptides.

To summarize, we have developed a facile synthetic route for solid-phase synthesis of β^3- and β^2-peptides. These β-peptides allow grafting of a wide variety of side chains critical for bioactivity onto the β-peptide backbone. The CD spectra of these molecules (**1** and **2**) suggest the presence of a solvent-dependent secondary structure. They represent an interesting class of biomimetic oligomers that may find potential application in pharmaceutical sciences.

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Supporting Information Available: Experimental details and characterization of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Lelais, G.; Seebach, D. *Biopolymers* **2004**, *76*, 206−243.
(13) Seebach, D.; Abele, S.; Gademann, K.; Guichard, G.; Hintermann, T.; Jaun, B.; Mathews, J. L.; Schreiber, J. V. *Helv. Chim. Acta* **1998**, *81*, 932−982.
(14) Guichard, G.; Abele, S.; Seebach, D. *Helv. Chim. Acta* **1998**, *81*, 187−206.