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Solid-Phase Synthesis of β-Oligopeptides

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Abstract: Fmoc-*N*-Protected β-amino acids of (*S*)-configuration bearing the side chains of Ala, Val, Leu, and Phe in the 3- and 2-position have been prepared (**1c–4c** and **1d–4d**). Manual solid-phase synthesis (*ortho*-chlorotrityl-chloride resin) of the β-heptapeptides H-β³-HVal-β³-HAla-β³-HLeu-β³-HPhe-β³-HVal-β³-HAla-β³-HLeu-OH (**9**) and H-β²-HVal-β²-HAla-β²-HLeu-β²-HPhe-β²-HVal-β²-HAla-β²-HLeu-OH (**10**), and, for comparison, of the corresponding α-heptapeptide H-Val-Ala-Leu-Phe-Val-Ala-Leu-OH (**8**) was achieved under standard conditions (HPLC/NMR identification). With the β³-peptide **9**, the yield and purity of the crude product are similar to those of the α-peptide **8**, while the β²-peptide synthesis needs to be optimized. CD measurements show that the β-peptides have helical secondary structures in MeOH, while the α-peptide does not.

Introduction

A novel class of peptide analogues, the β-peptides (consisting entirely of β- rather than the proteinogenic α-amino acids), has received a lot of attention recently [1]. There are mainly two reasons for excitement: *i*) in spite of added degrees of conformational freedom, they form surprisingly stable helices and pleated-sheet sec-

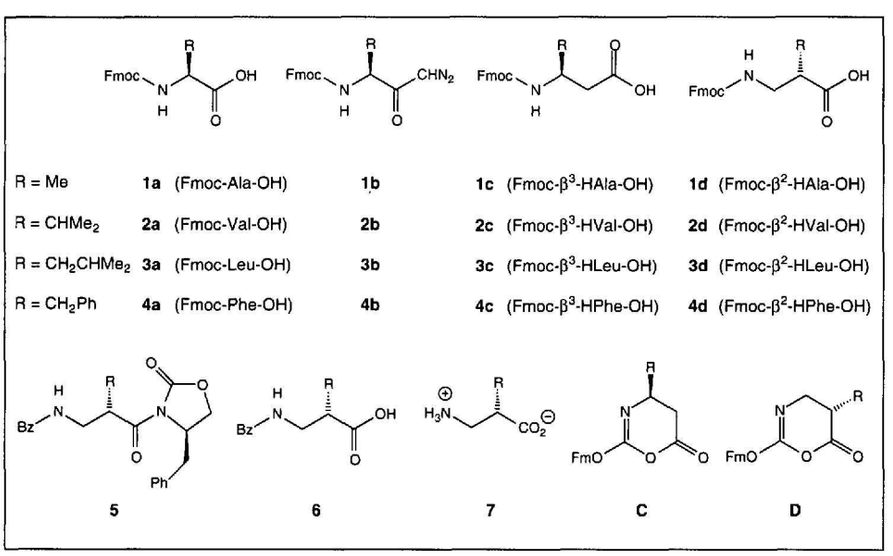
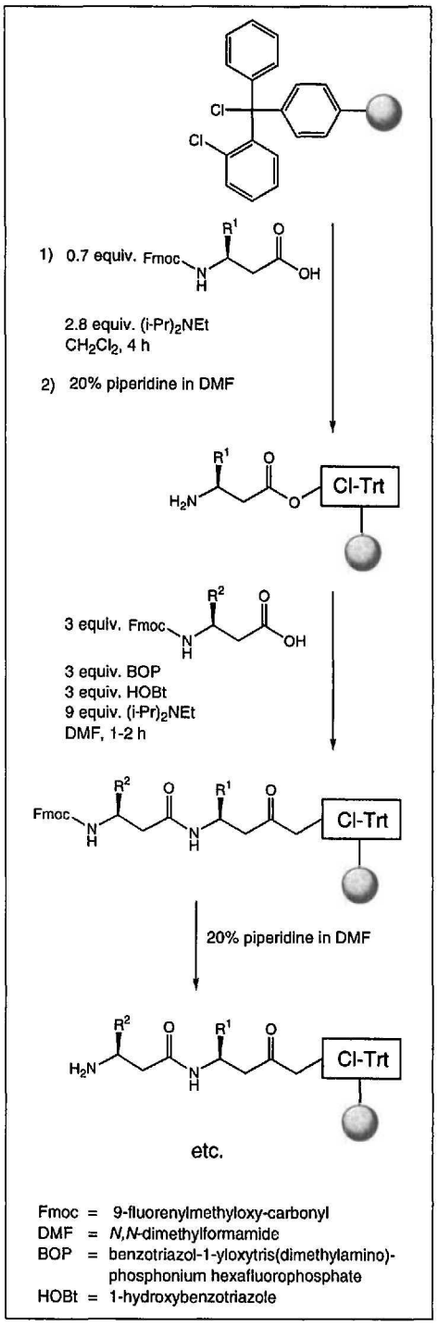


Fig. 1. *Building blocks for the synthesis of* β*-peptides.* The β²-amino acids **7** were *N*-protected by treatment with Fmoc-*N*-hydroxysuccinimide (H_2O/acetone, Na_2CO_3) to give **1d–4d**. The protected β²- and β³-amino acids were purified by flash chromatography (AcOEt/hexane/AcOH) before use in the coupling steps (*Scheme*).

Scheme. *Strategy Used for the Solid-Phase Synthesis of* β*-Heptapeptides as Shown for the* β³*-Derivatives* (for details see *Experimental Part*).



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ondary structures even when they contain as few as six β-amino-acid residues [2][3], and *ii*) they appear to be totally stable to the action of peptidases [4]. This latter property suggests that, given the necessary proteinogenic side chains for recognition by a receptor, β-peptides are candidates for drugs with high bioavailability. So far, β-peptides with common side chains in either the 2- and/or 3-position of the β-amino-acid residues have been prepared in solution, using *N*-Boc (= *tert*-butyloxycarbonyl) protection and EDC (= 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride)/HOBt coupling [2][3]. However, for the efficient discovery of active β-peptidic lead compounds, access by solid-phase and combinatorial synthesis is a prerequisite [5]. We have started work in this area, following two different strategies: *a*) conventional coupling of activated, separately prepared, β-amino acids (reported here) and *b*) acylation of the growing chain by ketene intermediates generated in the *Arndt-Eistert* homologation of α-amino acids (to be described separately [6][7]).

Solid-Phase Synthesis

We decided to use the Fmoc methodology which is well established in α-peptide solid-phase and automated synthesis. To this end, the commercially available Fmoc derivatives **1a–4a** of the amino acids alanine, valine, leucine, and phenylalanine (*Fig. 1*) were converted to the diazo ketones **1b–4b**. The subsequent *Wolff* rearrangement of these diazo ketones in aqueous THF gave the desired Fmoc-protected β^3-amino acids (*S*)-**1c**, (*R*)-**2c**, (*S*)-**3c**, and (*S*)-**4c**, as described previously for the Boc analogues [2][3]. The Fmoc-protected (*S*)-β^2-amino acids **1d–4d** with Ala, Val, Leu, and Phe side chains in the α-carbonyl position were obtained *via* the (*R*)-*N*-acyl-oxazolidinones **5** (hydrolysis, → **6** → **7**) (*Evans'* methodology) [8] (*Fig. 1*).

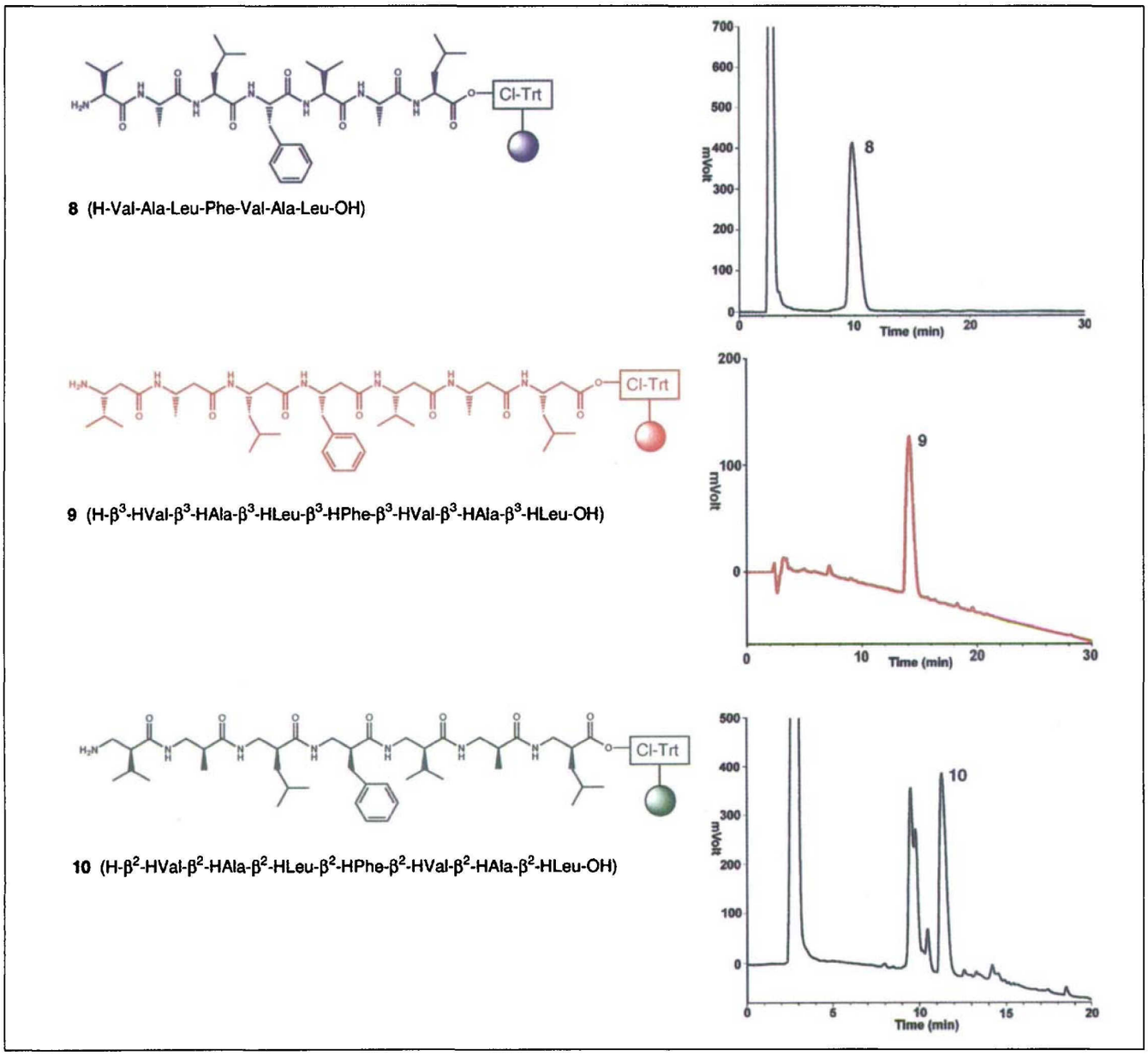

Fig. 2. *Comparison of HPLC profiles of the crude products obtained in the assembly of* **8–10** *under the conditions described in the* Scheme (for details see *Experimental Part*).

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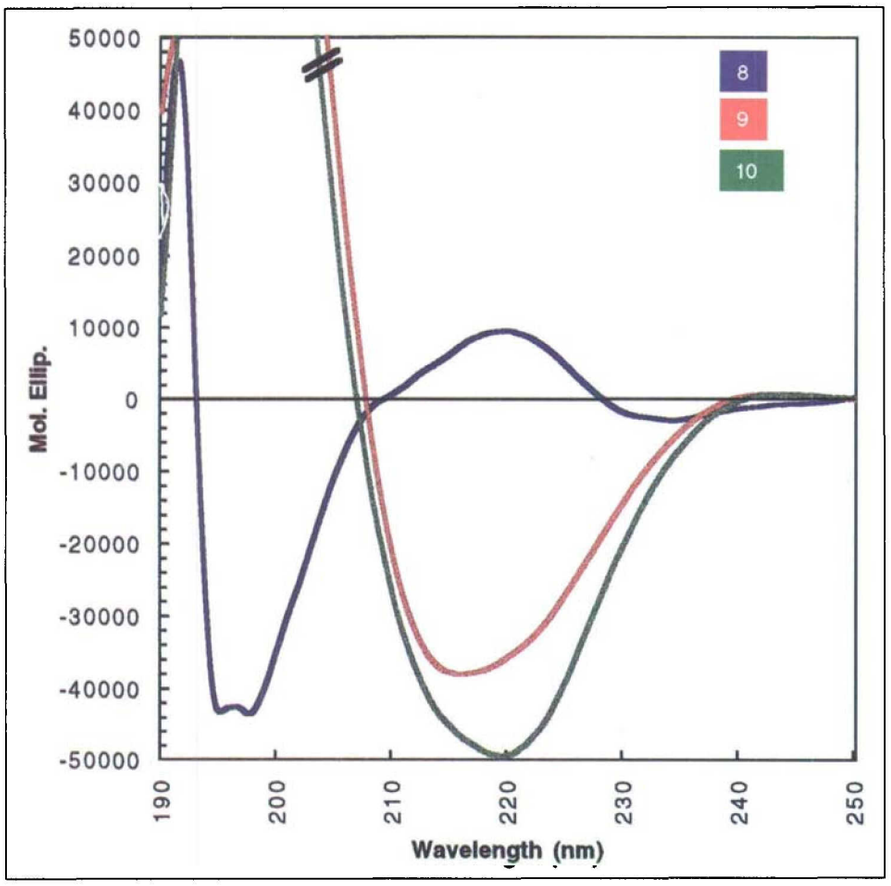

Fig. 3. *Overlay of the CD spectra of* **8–10** (as trifluoroacetate salts) *in MeOH*. The spectra were recorded between 190–250 nm using a *Jobin-Yvon-Mark-III* system. Peptide concentrations were 0.2 mM in MeOH. Molar ellipticity [Θ] in 10 deg cm^2 mol^{-1}.

The assembly of the β^3-amino acids is outlined in the *Scheme*: We employed the *ortho*-chlorotrityl-chloride resin [9], applied conventional conditions for the coupling and deprotecting steps, as well as for the final cleavage from the resin, and used a manual solid-phase apparatus [10]. For comparison, we first synthesized the α-heptapeptide **8**, and then the corresponding β^3-heptapeptide **9**. In *Fig. 2*, the analytical reverse-phase HPLC profiles of the two crude products are shown. After subsequent preparative HPLC, they were isolated in pure form and identified by CD, ^{1}H-NMR, and HPLC/electrospray MS analysis. As can be seen, the β^3-peptide (having a much longer retention time!) was formed in a purity very similar to that of the α-peptide. Much less satisfactory was our first attempt at the solid-phase synthesis of the isomeric β^2-heptapeptide **10**, assembled from the β^2-amino acids **1d–4d** under exactly the same conditions as the other two heptapeptides: the desired compound amounted only to *ca.* 40% of the crude product mixture after cleavage from the resin; **10** was separated by HPLC, isolated in pure form, and identified as mentioned above. We have not optimized these syntheses as yet, and we do not know the structures of the other components in the mixture from which we separated the β^2-peptide **10**. The problem could be caused by less efficient coupling of the β^2- as compared to the β^3-amino acids, or by partial racemization of the activated form in the first case (*cf.* **A** and **B**, possible candidates for the actual acylating reagent [6], *Fig. 1*).

CD Spectroscopy

As for α-peptides, optical measurements give reliable information about the presence of helical secondary structures of β-peptides [2–4]. Thus, a left-handed or (*M*)-3_1-helix of a β-peptide (with Me, i-Pr, i-Bu side chains) has a negative *Cotton* effect at *ca.* 216 nm.

We measured the CD spectra of the three pure peptides **8–10** (*Fig. 3*). As expected, there is no indication of a secondary structure for the α-hexapeptide in methanol solution, but the two β-peptides show intensive minima ($\Theta = -3.8 \times 10^4$ for **9** and -4.9×10^4 for **10**) at 216 and 220 nm, respectively (the somewhat longer wavelength observed for **10** may be caused by the benzene chromophore of β-HPhe).

Conclusion and Perspectives

We have demonstrated that β^3-peptides (and also β^2-peptides to a certain extent) can be efficiently prepared on solid support by *exactly* the same procedure as the conventional α-peptides. Thus, the β-heptapeptide **9** (68 mg, yield 89%, purity 94%) was obtained from 150 mg resin in *ca.* 35 working hours. This opens up the possibility of quick access to a wide variety of β-peptides carrying the side chains of natural (or unnatural) amino acids. NMR and CD analyses, and tests of physiological activity of β-peptides produced in this way will teach us more about the supramolecular interactions which govern the properties of α-peptides and natural proteins.

Experimental Part

Solid-Phase Synthesis

Esterification of **3c** and **3d** with the *ortho*-chlorotrityl-chloride resin was performed according to [9]. The resin (150 mg, 1.05 mmol Cl$^-$/g) was swelled in 2 ml of CH_2Cl_2 for 10 min. A soln. of **3c** or **3d** (41 mg, 112 µmol) in CH_2Cl_2 (2 ml) and $(i\text{-Pr})_2EtN$ (77 µl, 448 µmol) were then added successively and the suspension was mixed under Argon for 4 h. Subsequently, the resin was filtered and washed with CH_2Cl_2/MeOH/$(i\text{-Pr})_2$-EtN 17:2:1, (3 × 3 min), CH_2Cl_2 (3 × 3 min), DMF (2 × 3 min), CH_2Cl_2 (3 × 3 min), MeOH (2 × 3 min). The substitution of the resin determined on a 3-mg sample by measuring the absorbance of the dibenzofulvene-piperidine adduct at 300 nm, was 0.42 (74%) and 0.43 mmol/g (75%) with **3c**

and **3d**, respectively. The Fmoc group was removed using 20% piperidine in DMF (4 ml, 2 × 20 min) under Ar bubbling. The resin was then filtered and washed with DMF (6 × 3 min). For each coupling step, a soln. of the β^2- or β^3-amino acid (3 equiv.), BOP (3 equiv.) and HOBt (3 equiv.) in DMF (2 ml) and $(i\text{-Pr})_2EtN$ (9 equiv.) were added successively to the resin and the suspension was mixed for 1 h under Ar. Monitoring of the coupling reaction was performed with 2,4,6-trinitrobenzenesulfonic acid (TNBS) [11] instead of the classical ninhydrin test [12] (Fmoc-deprotected β^3-peptide resin fails to give a blue color with ninhydrin). In case of a positive TNBS test (indicating incomplete coupling), the suspension was allowed to react for a further 1 h. The resin was then filtered and washed with DMF (3 × 3 min) prior to the following Fmoc deprotection step. After the removal of the last Fmoc protecting group, the resin was washed with DMF (6 × 3 min), CH_2Cl_2 (3 × 3 min), Et_2O (3 × 3 min), and dried under h.v. for 3 h. Finally, the peptides were cleaved from the resin using 2% TFA (= trifluoroacetic acid) in CH_2Cl_2 (2 ml, 5 × 15 min) under Ar. The solvent was removed and the oily residues were triturated in ether to give the crude heptapeptides **9** (68 mg, 89%) and **10** (60 mg, 79%) as white solids.

HPLC Analysis and Purification

RP-HPLC analysis was performed on a *Macherey-Nagel* C_8 column/*Nucleosil 100-5* C_8 (250 × 4 mm) by using a mixture of 30% MeCN in H_2O (containing 0.1% CF_3COOH) as eluent for **8** or a linear gradient of A (0.1% CF_3COOH in H_2O) and B (MeCN) for **9** (40–90% B, 30 min) and **10** (30–90% B, 20 min) at a flow rate of 1 ml/min with UV detection at 220 nm. Crude products were purified by preparative RP-HPLC on a *Macherey-Nagel* C_8 column/*Nucleosil 100-5* C_8 (250 × 20 mm) using a mixture of MeCN/H_2O (containing 0.1% CF_3COOH) as eluent (30:70 for **8** and 40:60 for **9** and **10**) at a flow rate of 4 ml/min with UV detection at 214 nm and then lyophilized.

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