Pergamon

Tetrahedron Letters, Vol. 38, No. 35, pp. 6145-6148, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)01420-2

Solid Phase Synthesis of β-Peptides *via* Arndt-Eistert Homologation of Fmoc-Protected Amino Acid Diazoketones

Roger E. Marti*, Konrad H. Bleicher, and Kenneth W. Bair

Novartis Pharmaceuticals Corporation, Departments of Oncology and Metabolic & Cardiovascular Diseases
59 Route 10, East Hanover, N.J. 07936

Dedicated to Professor Dr. Ernst Bayer on the occasion of his 70th birthday and to Professor Dr. Dieter Seebach on the occasion of his 60th birthday.

Abstract. *The solid phase synthesis of β-peptides and β-amino acid containing peptides via Arndt-Eistert homologation of Fmoc-protected amino acid diazoketones is described. Syntheses were carried out on Wang, Rink and 2-chlorotrityl resins. Quantitative coupling efficiency gave highly pure compounds as proven by RP-HPLC and MS.* © 1997 Elsevier Science Ltd.

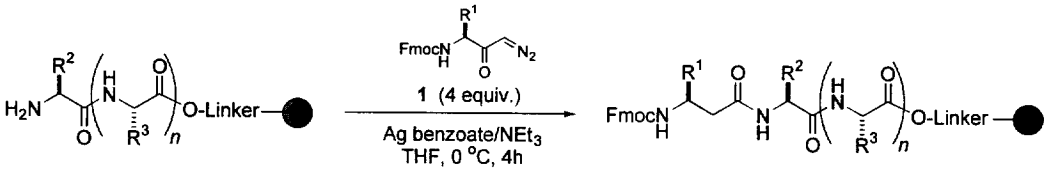
The growing understanding of protein-protein and protein-oligonucleotide interactions has resulted in the discovery of varying peptide sequence motifs that mediate these interactions in different biological processes.[1] These peptides, when validated, become valuable tools for drug discovery. Based on these sequences, medicinal chemists synthesize series of peptide analogs and peptide mimics to determine the structure-activity relationships.[2] Use of state-of-the-art solid phase techniques allows synthesis of peptides of varying sizes and complexity as well as peptide libraries.[3] Recently synthesis of the so called β-peptides, built from β-amino acids, was reported by Seebach[4] and Gellman.[5] In contrast to the corresponding α-amino acid peptides, this new class of peptides seems to possess surprisingly stable secondary structures, even for relatively short sequences. We now report a solid phase synthesis approach to β-peptides.

The synthesis of β-peptides has so far been achieved in solution by standard peptide coupling of the Boc-protected β-amino acids[4] or by Arndt-Eistert homologation of Boc-protected α-amino acid diazoketones with the free amine of β-peptides.[6] The β-amino acid building blocks were prepared from the corresponding α-amino acid by the Arndt-Eistert procedure in enantiomerically pure form in high yields.[4,6,7] Seebach et al. recently described the solid phase coupling of diazoketones to amino-modified oligonucleotides.[8] These results encouraged us to use this approach for the first semi-automated solid phase synthesis of β-amino acid containing α-peptides as well as the synthesis of β-peptides.

Initially, α-peptides were assembled on solid support by a peptide synthesizer employing a standard Fmoc-protocol up to the desired position of the β-amino acid.[9] After removal of the terminal Fmoc protecting group with piperidine, the resin **2** and a solution of the appropriate Fmoc-protected amino acid diazoketone **1** (Fmoc-Xaa-CHN_2, 4-fold excess) in dry THF were placed in a reaction flask (in all cases, we used crude

Fmoc-Xaa-CHN_2 **1**; any Fmoc-Xaa-OMe ester present did not interfere with the reaction).[10] The yellow suspension was cooled to 0 °C and a solution of silver benzoate (0.35 equivalent) in triethylamine was added and stirred in the dark for 4 hours (*Scheme 1*). The ketene resulting from silver catalyzed decomposition of the diazoketone and Wolff rearrangement is trapped by the free amine of the amino acid, forming the new amide bond. The brownish suspension was then transferred to a solid phase reaction vessel, and washed successively with DMF, a 5% solution of sodium diethyldithiocarbamate in DMF (to remove silver salts), DMF, and methylene chloride. In all cases, Kaiser ninhydrin test of the resin **3** gave a negative result.[11] Cleavage of a small sample of **3** with TFA/H_2O 95 : 5 and analysis by RP-HPLC showed also quantitative coupling. Synthesis of the peptides was then completed on the automated synthesizer. The peptides were cleaved from the resin with TFA/H_2O (95 : 5). The crude peptides were obtained in good yields (see Table in *Scheme 1*, calculated based on the initial loading of the commercially available preloaded Fmoc-Ile-Wang or H-Pro-2-chlorotrityl resin) and high purity as determined by RP-HPLC.[12] They were further purified by RP-HPLC and characterized by electrospray mass spectrometry.

Scheme 1: Incorporation of Single β-Amino Acid Residue in α-Peptides by Solid Phase Arndt-Eistert Homologation.



2 **3**

Linker = Wang or 2-Chlorotrityl

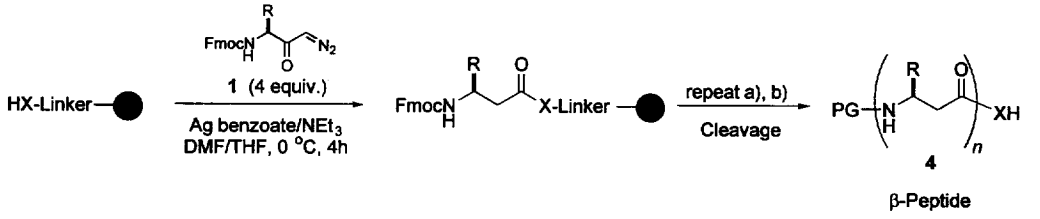
Final Peptide Sequence	Crude Yield (%)	Purity by RP-HPLC (215 nm)
Acetyl-Val-Glu-β-**HPhe**-Ile-OH	85	> 90 %
Acetyl-Val-β-**HGlu**-Phe-Ile-OH	95	> 90 %
Acetyl-Ser-Asp-β-**HLys**-Pro-OH	80	> 95 %

For the synthesis of all β-peptides we immobilized the first β-amino acid by reacting the Fmoc-Xaa-CHN_2 **1** under silver catalysis with Wang resin[13] or the Fmoc-deprotected Rink amide resin.[14] The Wang resin (initial loading 1.2 mmol/g) was treated with either one or two equivalents of the diazoketone, and gave, after capping with benzoyl chloride/pyridine, loadings of 0.45 and 0.65 mmol/g respectively as determined by photometric Fmoc determination (coupling yield 55% and 80% respectively). Rink amide resin showed after the same treatment (3 equivalents of Fmoc-Phe-CHN_2 **1**) by the ninhydrin test no more free amine (*Scheme 2*).

After deprotection of the Fmoc group by 20% piperidine in NMP and washing with NMP, the next amino acid diazoketone was coupled by the same procedure.[15] A 1 : 1 mixture of THF and DMF was used as solvent. Again, the Kaiser test showed no free amine. Further deprotections and Arndt-Eistert homologations provided up to a tetramer. After cleavage with TFA/H_2O (95 : 5), the solution was evaporated to a third of the volume, and treated with cold ether. The precipitated peptide was collected and washed once with ether, and

dried. The yields were good and the purities of these β-peptides **4a-e** were excellent (see Table in *Scheme 2* and RP-HPLC chromatogram for the tetramer **4e** in *Figure 1a*). All products are characterized by electrospray mass spectrometry (see Table in *Scheme 2*). For selected samples, we verified the correct structure by ^{1}H NMR spectrometry. The CD spectrum of the tetramer Fmoc-β-HTyr-β-HTyr-β-HVal-β-HPhe-NH_2 (**4e**) in water (ca. 0.15 mM) showed the characteristic pattern for the helical structure (see *Figure 1b*, for a detailed discussion of CD spectra of β-peptides see ref. [4a, b]).

Scheme 2: Synthesis of β-Peptides **4a-e** *via* Arndt-Eistert Homologation on Solid Support.



Conditions: a) 20% Piperidine in DMF. b) Fmoc-Xaa-CHN_2 **1**, Ag benzoate/NEt_3, THF/DMF, 4h, 0 °C.
Linker-X: Wang-O or Rink-NH. PG = H, Acetyl, or Fmoc

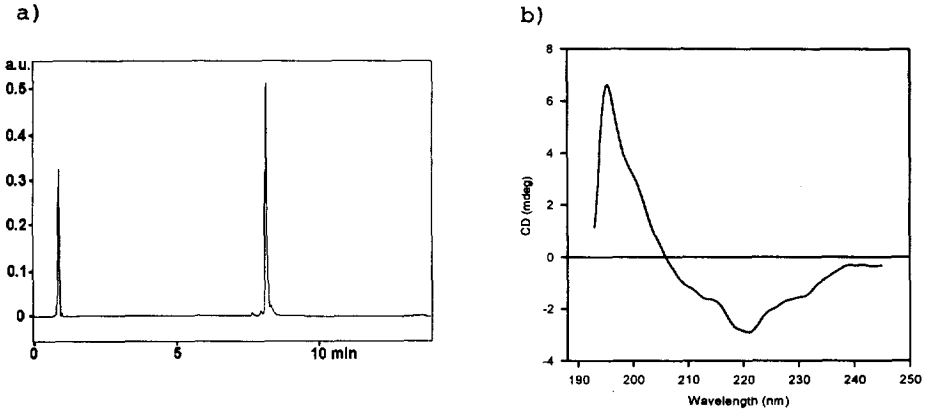
Final β-Peptide Sequence	Crude Yield (%)	Purity by RP-HPLC (215 nm)	MS (Electrospray)
4a Fmoc-β-HVal-β-HPhe-NH_2	80	80 %	514 (MH^+, 100%)
4b Fmoc-β-HTyr-β-HPhe-OH	65	75 %	579 (MH^+, 100%)
4c TFA · H-β-HTyr-β-HVal-β-HPhe-NH_2	75	75 %	469 (MH^+, 100%)
4d Acetyl-β-HLys-β-HTyr-β-HPhe-OH	65	80 %	541 (MH^+, 100%)
4e Fmoc-β-HTyr-β-HTyr-β-HVal-β-HPhe-NH_2	60	90 %	690 (MH^+, 100%)

In conclusion, we have demonstrated that the solid phase synthesis of β-peptides *via* the Arndt-Eistert homologation of Fmoc-protected amino acid diazoketones is a versatile and easy process. The starting materials, the Fmoc-Xaa-CHN_2 **1**, are easily prepared and need no further purification, which is an important advantage over solution phase synthesis. In principle, all appropriately protected α-amino acid could be used in this method. The procedure could be easily automated and would enable the generation of new β-peptides, the positional scanning of α-peptides with β-amino acids, and synthesis of combinatorial libraries.[16]

Acknowledgments

We thank the Novartis Postdoctoral Program, Dr. B. Yan for measurement of the CD spectra, and Dr. N. Chandramouli for critical reading of the manuscript.

Figure 1: a) RP-HPLC Chromatogram and b) CD Spectrum in Water (0.2 mM) of the β-Peptide Fmoc-β-HTyr-β-HTyr-β-HVal-β-HPhe-NH_2 (**4e**).



References and Notes

[1] For a recent example, see: Adams, P.D.; Sellers, W.R.; Sharma, S.K.; Wu, A.D.; Nalin, C.M.; Kaelin, W.G. *Mol. Cell. Biol.* **1996**, *16*, 6623-6633.

[2] (a) Giannis, A.; Kolter, T. *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 1244-1267. (b) Gante, J. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699-1720.

[3] (a) Bayer, E. *Angew. Chem. Int. Ed. Engl.* **1991**, 30, 113-129. (b) Jung, G.; Beck-Sickinger, A.G. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 367-383.

[4] (a) Seebach, D.; Overhand, M.; Kühnle, F.N.M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913-941. (b) Seebach, D.; Ciceri, P.E.; Overhand, M.; Jaun, B.; Rigo, D.; Oberer, L.; Hommel, U.; Amstutz, R.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 2043-2066. (c) Seebach, D; Matthews, J.L.; Meden, A.; Wessels, T.; Baerlocher, C.; McCusker, L.B. *Helv. Chim. Acta* **1997**, *80*, 173-182.

[5] Appella, D.H.; Christianson, L.A.; Karle, I.L.; Powell, D.R.; Gellman, S.H. *J. Am. Chem. Soc.* **1996**, *118*, 13071-13072.

[6] (a) Podlech, J.; Seebach, D. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 471-471. (b) Podlech, J.; Seebach, D. *Liebigs Ann.* **1995**, 1217-1228. (c) Guibourdenche, C.; Podlech, J.; Seebach, D. *Liebigs Ann.* **1996**, 1121-1129.

[7] For reviews, dealing with the synthesis of β-amino acids, see: (a) Juaristi, E.; Quintana, D.; Escalante, J. *Aldrichchim. Acta* **1994**, *27*, 3-11. (b) Cole, D.C. *Tetrahedron* **1994**, *50*, 9517-9582.

[8] Guibourdenche, C.; Seebach, D.; Natt, F. *Helv. Chim. Acta* **1997**, *80*, 1-13.

[9] The syntheses were performed on an Applied Biosystems Peptide Synthesizer ABI 433A using the Fastmoc™ Fmoc/OtBu protocol (HBTU/HOBt activation) on Fmoc-Ile-Wang resin or H-Pro-2-chlorotrityl resin.

[10] The Fmoc-amino acid diazoketones **1** were synthesized using isobutyl chloroformate/NMM activation and excess of CH_2N_2 in ether (CAUTION) starting from the Fmoc-protected α-amino acids. In all cases the crude diazoketones are obtained as yellow solids, which were used without further purification.

[11] (a) Kaiser, E.; Colescott, R.L.; Bossinger, C.D.; Cook, P.I *Anal. Biochem.* **1970**, *34*, 595-599. (b) Sarin, V.; Kent, S.; Tam, J.; Merrifield, B. *Anal. Biochem.* **1981**, *117*, 147-157.

[12] RP-HPLC was performed on a Deltapak C18 column (5 μ, 100 Å) with a gradient 10-100% acetonitrile in water (0.1% TFA) over 15 min with a diode array detector.

[13] Wang, S.-S. *J. Am. Chem. Soc.* **1973**, *95*, 1328-1333.

[14] Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787-3790.

[15] *Typical coupling procedure*: The resin (0.25 mmol) was suspended in DMF/THF 1:1 (20 ml/g resin), Fmoc-Xaa-CHN_2 **1** (1.0 mmol, 4 equiv.) was added, and the yellow suspension was cooled to 0 °C. A soln. of silver benzoate (20 mg, 0.35 equiv.) in NEt_3 (320 μl) was then added, and the suspension stirred for 4 h at 0 °C under the exclusion of light. The mixture was washed (N_2-bubbler) with DMF/CH_2Cl_2, a soln. of diethyldithio carbamic acid sodium salt in DMF (ca. 5%), DMF and CH_2Cl_2 and dried in vacuo.

[16] For recent reviews about combinatorial chemistry, see: (a) Balkenhohl, F.; von dem Bussche-Hünnefeld, C.; Lansky, A.; Zechel, C. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2288-2337. (b) Thompson, L.A.; Ellman, J.A. *Chem. Rev.* **1996**, *96*, 555-600. (c) Hermkens, P.H.H; Ottenheijm, H.C.J.; Rees, D.C. *Tetrahedron* **1997**, *53*, 5643-5678.

(*Received in USA* 28 *May* 1997; *revised* 3 *July* 1997; *accepted* 10 *July* 1997)