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CONVERSION OF N^G-URETHANE PROTECTED ARGININE TO ORNITHINE IN PEPTIDE SOLID PHASE SYNTHESIS

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Abstract: It is shown that Di-Adoc or Boc as guanidino protecting groups do not prevent the acylation and the subsequent conversion of arginine to ornithine in Fmoc solid phase peptide synthesis.

9-Fluorenylmethyloxycarbonyl (1) (Fmoc) as α-amino protecting group has been used successfully several times in solid phase peptide synthesis (2-5). Applying this technique to the synthesis of the 15 amino acid carboxyl terminus of human leucocyte interferon αF (6), we were faced with the challenge of introducing three arginine residues into sequence 1. Satisfactory protection of the guanidino function is still an unsolved problem. The most promising approach seemed to be to use a N^G-bis-adamantyloxycarbonyl (Adoc) arginine derivative (3). We report here a side reaction at the arginines which however restricts significantly the applicability of this method.

Fmoc-Glu(OBut)-OH was attached to the acid labile p-benzyloxybenzyl alcohol resin (7)(.30 meq./g). Amino acid side chains were protected, if necessary, with tert. butyl type groups (except Arg) and coupling was effected by preformed symmetrical anhydrides (2) except glutamine and arginine which were activated as hydroxybenzotriazolide derivatives (8).

$$\overset{R_1}{\text{Z-Phe}}\text{-}\overset{R_1}{\text{Ser}}\text{-Leu-}\overset{R_2}{\text{Ser}}\text{-Lys-Ile-Phe-Gln-}\overset{R_3}{\text{Glu}}\text{-}\overset{R_4}{\text{Arg}}\text{-Leu-}\overset{R_4}{\text{Arg}}\text{-}\overset{R_4}{\text{Arg}}\text{-Lys-}\overset{R_2}{\text{Glu}}\text{-}\overset{R_3}{\text{O}}\text{-CH}_2\text{- resin}$$

R_1 = -But R_2 = -Boc R_3 = -OBut R_4 = $-(\text{Adoc})_2$

1

The synthesis was performed with a semiautomatic Schwarz Bioresearch Synthesizer using essentially the same procedures as described in (2). The completeness of the coupling reactions were monitored by Ninhydrin tests (9) and the removed Fmoc residues were determined spectrophotometrically (2). To facilitate subsequent further specific processing of the ε-amino groups of the lysines we introduced the last amino acid as a benzyloxycarbonyl (Z) derivative.

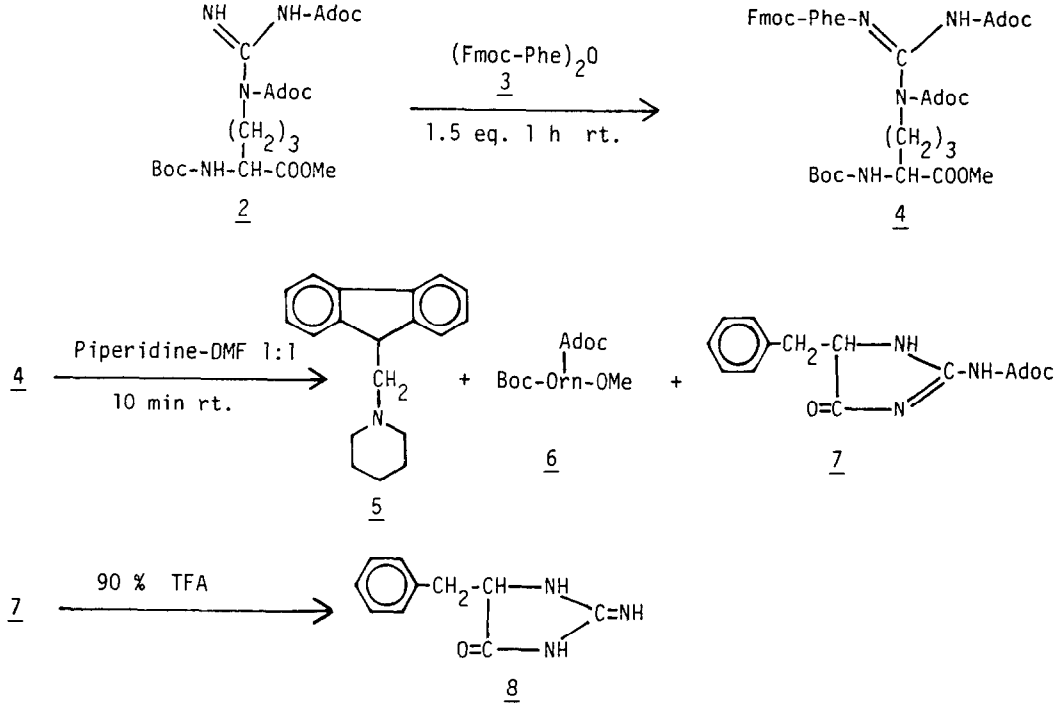
The resin and the side chain protecting groups were cleaved off by means of 55%

trifluoroacetic acid (TFA) in dichloromethane (2 hrs. rt) and the crude product was precipitated with ether (100% yield). Countercurrent distribution (n-butanol/AcoH/water 4:1:5) over 740 steps resulted in a broad distribution and indicated, as did tlc and hplc data, the presence of a rather heterogenous product. The main peak was collected and gave the following amino acid analysis: Lys & Orn 3.3 (2) Arg 1.6 (3) Ser 1.6 (2) Glu 3.3 (3) Ile 1.0 (1) Leu 2.1 (2) Phe 2.0 (2). The observed amino acid ratios suggest a partial conversion of arginine to ornithine which could be confirmed by gaschromatographic analysis of a derivatized hydrolysate (10): Lys/Orn 1.8/1.5. Further investigations by FAB mass-spectrometry allowed us to deduce the presence of two peptides with the following sequences (11):

Z-Phe-Ser-Leu-Ser-Lys-Ile-Phe-Gln-Glu-Orn-Leu-Orn-Arg-Lys-Glu-OH

Z-Phe-Ser-Leu-Ser-Lys-Ile-Phe-Gln-Glu-Arg-Leu-Orn-Arg-Lys-Glu-OH

Since Photaki (12) had described the conversion of N^G-aminoacyl-arginine to ornithine derivatives and 2-iminoimidazolidin-4-one we strongly suspected the occurrence of an acylation of the bis-Adoc-guanido residue during the coupling procedures and the subsequent formation of N-Adoc-ornithine with piperidine. The following set of model reactions confirmed this hypothesis (scheme 1).

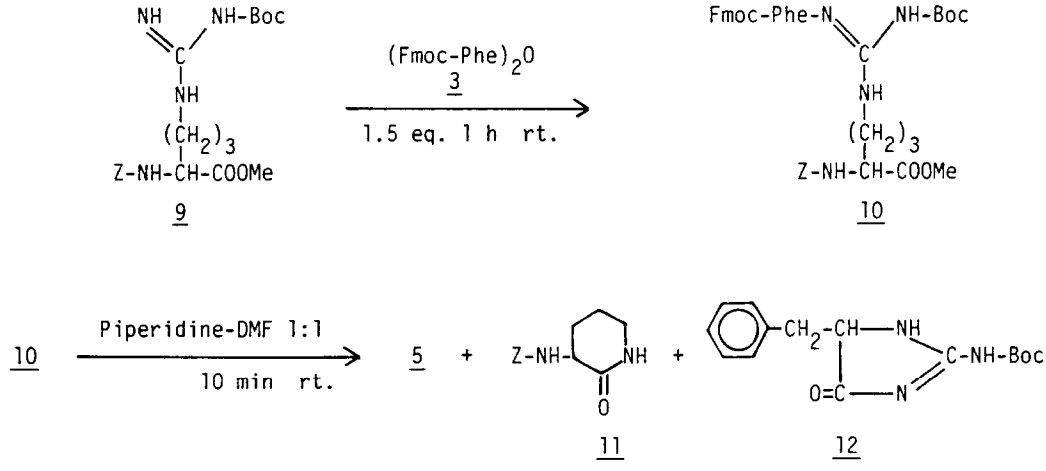


Scheme 1

2 (the positions of the Adoc groups were determined by ^{13}C and ^{1}H-NMR spectra (13)) was easily converted to 4 using conditions similar to those in the solid phase technique. 4 was isolated with 68% yield and its structure was confirmed by NMR. A short treatment of 4 with piperidine gave 5 (1), 6 and 7. 6 was treated with TFA and the resulting ornithine-methylester-ditrifluoroacetate was identified by tlc comparison with an authentic sample. The MS of 7 showed the expected mass peak (367) and after removing the Adoc group with TFA the crystalline 8 gave a correct elemental analysis (C,H,N).

Colombo (14) had reported the successful application of Fmoc-Arg(Boc)-OH in peptide solid phase synthesis. However, in another set of experiments we were able to show that the Boc group does not provide a more extensive protection than $(Adoc)_2$ against acylation of the guanidino function (scheme 2).

Acylation of 9 gave 10 (78% yield). Surprisingly no second Fmoc-Phe residue could be introduced, even when a large excess (10 eq.) of 3 was used. The field desorption MS of 10 showed the expected mass peak (791) and the ^{13}C and ^{1}H-NMR data were compatible with the structure of 10, essentially a ^{13}C signal at 40.5 ppm (instead of 44 ppm) confirmed the existence of the nonacylated N neighbored to the δ-C (13). Piperidine treatment gave the expected 5, 11 was proved to be the 3-Z-aminopiperidine-2-one by tlc comparison with an authentic sample prepared from Z-ornithine-methylester. 12 could be cleaved with TFA to the already identified 8.



Scheme 2

The presented data demonstrate clearly that Adoc or Boc and probably all urethane type protecting groups (15) do not effectively prevent guanidino acylation under conditions of peptide synthesis and the subsequent partial conversion of arginine to ornithine when strongly basic conditions have to be used to remove the α-amino protecting groups. Some Arg are less prone to guanidino acylations as in our case (Arg^{13}), probably

due to some specific tertiary structure of the growing peptide chain in the resin. Whether the recently developed multisubstituted benzenesulfonyl groups (16) will provide an adequate protection of the guanidino function remains to be shown.

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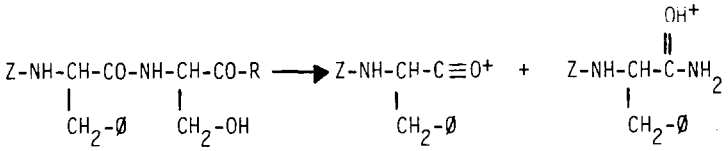
11. The bombardment was performed with Xe atoms at 8 KeV kinetic energy. Accelerating voltage was 8 KV on a ZAB-HF mass-spectrometer.

    Two molecular peaks could be observed: MH^+ = 2028 (one Orn) and MH^+ = 1986 (two Orn). Fragmentation occurs under formation of N-terminal ions as follows



    The following ions could be detected (m/z): 282, 369, 482, 569, 810, 957, 827, 974, 1102, 1231, 1345, 1387, 1500, 1572, 1614, 1728, 1770.

    Compare also M. Barber et al., Nature 293, 270 (1981).
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