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SYNTHESIS OF DIFFICULT PEPTIDE SEQUENCES: A COMPARISON OF FMOC-AND BOC-TECHNIQUE

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Abstract: In comparison with Fmoc-technique the BOC-technique with *in situ* neutralization proved advantageous for the synthesis of difficult peptides forming ß-sheet structures.

In view of the most serious problems of the stepwise solid phase peptide synthesis, namely chemical side-reactions and incomplete aminoacylations, BOC/benzyl and Fmoc/t-butyl strategy have been compared mainly with regard to chemical side-reactions[1]. It was recently described that a protonated peptide-polystyrene resin swells much more in polar solvents than the deprotonated one[2] and moreover, that an application of a protonated peptide-resin instead of the deprotonated one to the coupling step with an *in situ* neutralization during the coupling resulted for some examples in better coupling yields[3]. Both findings indicate that the application of a protonated peptide-resin with better swelling properties in polar solvents might be advantageous especially for syntheses of so-called "difficult peptides", such as the homooligo-peptides $(Val)_n$ and $(Ala)_n$ [4], which tend strongly to ß-sheet formation followed by shrinking of the peptide-resin and slow acylation rates.

To proof the idea we investigated the course of synthesis of $(Val)_n$ on 4-methylbenzhydrylamine-polystyrene (1% divinylbenzene) with a high capacity (1-1.2 mmol/g) to force the aggregation of growing peptide chains. The MBHA-linker allows the application of BOC- as well as of Fmoc-strategy. Starting with 100 mg of the neutralized resin, couplings were carried out with Fmoc-Val/TBTU[5] (1.0 M in DMF, 0.4 ml; 2 equiv. DIEA[5], for 20 min). After deblocking with piperidine/DMF (1/4 , 10 min) the coupling yield was determined by estimation of liberated dibenzofulvene-piperidine adduct (u.v. 301 nm). For the synthesis a drastic decrease of the coupling yield was observed at the fifth step (Fig. 1A,"Fmoc"). Under the same conditions but with ultrasonification[6] during the couplings we observed the same result (Fig.1A). To imitate the conditions of the BOC-strategy with *in situ* neutralization, but using Fmoc-Val for couplings to estimate coupling yields, the deprotected peptide-resin was washed with TFA[5] (15 sec) followed by DMF (3x) before coupling steps. By it, we observed a much higher resin swelling and high coupling yields (Fig. 1A,"TFA"). In the traditional BOC-strategy the TFA-treatment is followed by an separated neutralization step (10% DIEA/DMF, 2x 30 sec), but this resulted here in poor couplings (Fig. 1A). Couplings of Fmoc-Val in NMP/DMSO (4/1)[7] gave some better results than in DMF, but again a drastic decrease at the fifth step was found (Fig. 1B). The next coupling at 50 °C [8] did not show any improvement, but the seventh coupling after TFA-wash resulted in a high coupling yield, again (Fig. 1B).

For the synthesis of $(Ala)_n$ we used an analogous protocol, but a lower resin capacity (0.25 mmol/g),double

couplings (2x10 min) and 0.5 M Fmoc-Ala/TBTU/ 2 equ. DIEA in DCM/DMF (1/1). The effect of the TFA-wash on coupling yields is also significant (Fig. 2A), but between valine and alanine there is a substantial difference in view of the deblocking. For $(Val)_n$ the deblocking is always fast, but in the case of $(Ala)_n$ starting from n=6 the deblocking becomes very slow. It needs partially more than 100 min [9]. Finally, the ion-spray MS spectra (IO-MS)[10] of a crude $(Ala)_{10}$-NH_2 (MH^+: 728.41) synthesized using the described protocol with TFA-wash demonstrates the success of the method (Fig. 2B). Unfortunately, in contrast to others[9] by now we did not find proper RP-HPLC conditions for an analytical characterization.

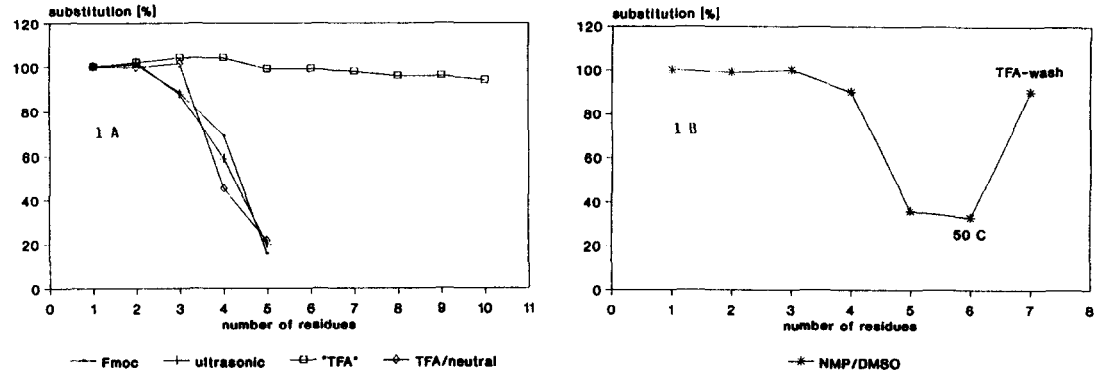

Figure 1: Influence of solvents, ultrasonification (35 kHz), elevated temperature and TFA-wash with in situ or separated neutralization on the synthesis of $(Val)_n$ on MBHA-resin

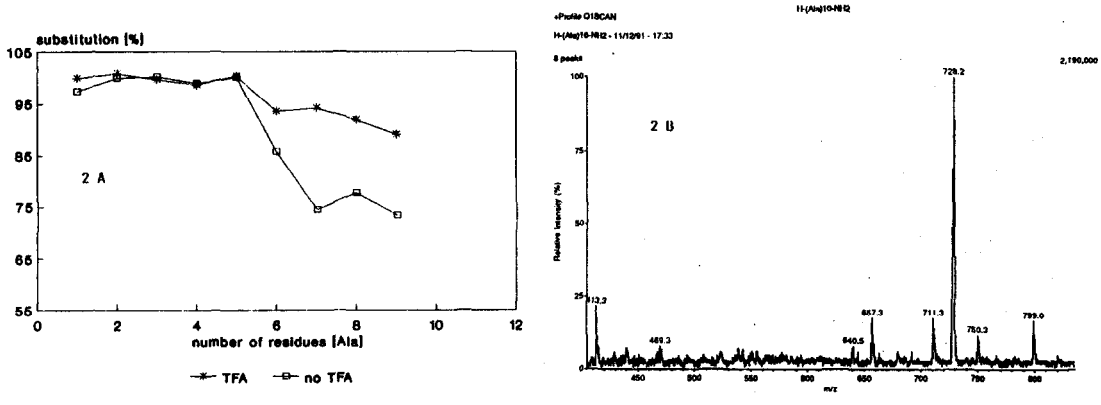

Figure 2: A) Course of the synthesis of $(Ala)_n$ on MBHA-polystyrene with and without TFA-wash; deblocking with piperidine/ DMSO (1/1) until constant u.v. at 301 nm; B) IS-MS of a crude $(Ala)_{10}$-NH_2

For a real comparison of BOC- and Fmoc-strategy to prepare difficult peptides we synthesized the antimicrobial peptide Magainin-II-NH_2 which has been described to form inaccessible structures for the acylation during its stepwise synthesis on solid support[11] and which was hard to be synthesized on polystyrene resins using Fmoc-strategy[12]. For the syntheses at a batchwise working synthesizer (SYNOSTAT P, Biotronic/Eppendorf, Germany) we used a protocol similar to the procedure developed in the group of Kent[3] on high-loaded MBHA-polystyrene (1-1.2 mmol/g): DMF; 2x2 min TFA; 4x DMF; 0.3 M BOC-aa/TBTU/2 DIEA/DMF for 13 min; 2x DMF (Fig. 3A). Using the same protocol we made a second synthesis with a separated neutralization step (5% DIEA/DMF) after TFA-deblocking (Fig. 3B) and a third synthesis but with Fmoc/t-butyl-strategy (Fig. 3C) and instead of TFA of course piperidine/DMF (1/1) for deblockings (2x 5 min). In the case of Fmoc-strategy the peptide-resin was treated with TFA for one hour before the final HF-cleavage (1 h , 0 °C, 5% anisole; the same for all three products). The comparison of crude products demonstrates clearly that the BOC-strategy with TFA-deblocking and *in situ* neutralization gave the best result under these conditions.

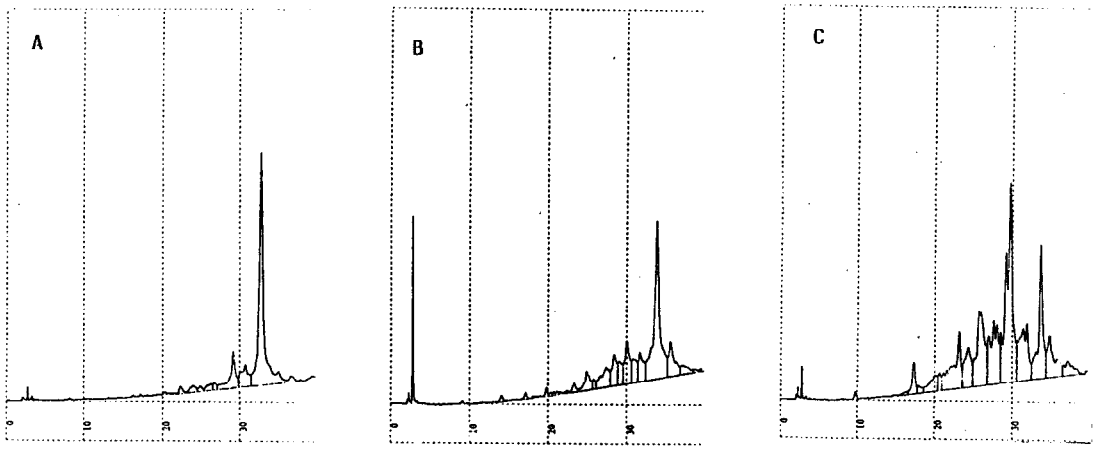

Figure 3: RP-HPLC profiles of crude Magainin-II-amide synthesized with BOC-strategy with *in situ* (A) or separated neutralization (B), and with Fmoc-strategy[13]

An explanation for the effect of TFA might be given by its property to destroy amide hydrogen bondings. After DMF-washes some TFA remains in the peptide-resin and keeps it in a swollen state for couplings. A separated neutralization removes traces of TFA and causes the shrinkage. For the Fmoc-strategy such conditions for breaking strong hydrogen bondings are missing. Futher studies will investigate whether TFA might be replaced by other, non-carbonic acids.

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5. abbreviations: TBTU benzotriazol-1-yl-1.1.3.3.-tetramethyluronium tetrafluoroborate, DIEA diisopropylethylamine, TFA trifluoroacetic acid.

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13. HPLC-conditions: LiChrosorb RP-8, 10 μm, 250x4 mm, 1 ml/ min, u.v. 220 nm, A: 0.07 M KH_2PO_4, pH 2.5, B: 50% of A/ 50% acetonitrile,20-80% B/40 min.

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