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PEPTIDE SYNTHESIS BY A COMBINATION OF SOLID-PHASE AND SOLUTION METHODS II[1)]

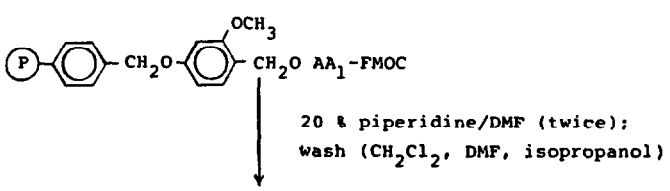
SYNTHESIS OF FULLY PROTECTED PEPTIDE FRAGMENTS ON 2-METHOXY-4-ALKOXY-BENZYL ALCOHOL RESIN

M. Mergler, R. Nyfeler, R. Tanner, J. Gosteli, P. Grogg
BACHEM AG, Hauptstrasse 144, 4416 Bubendorf/Switzerland

SUMMARY
The synthesis of fully protected peptide fragments by the Fmoc/ t-butyl-method on a new polymeric support is described. The fragments are obtained in good yields and high purity upon cleavage from the resin with 0.5 - 1 % trifluoroacetic acid in methylene chloride.

The conventional solution peptide synthesis may be cumbersome and time-consuming but it allows the purification of fully protected intermediary fragments. This is an advantage over the solid-phase peptide synthesis (SPPS), in which case the final product has to be singled out from a mixture of closely structured contaminants.
This led to many attempts at combining the ease of SPPS and the advantage of solution synthesis: synthesizing anchors which allow the preparation by cleavage of fully protected fragments. This cleavage is often accompanied by some racemization at the C-terminus, other side reactions, or proceeding in low yield.
The new 2-methoxy-4-alkoxy-benzyl alcohol resin[1)] allows the synthesis of fully t-butyl type protected peptides by the mild and well-established Fmoc-strategy. Synthesis of and low-racemization coupling to the resin have been described.[1)] The SPPS synthesis protocol does not differ from those used with Wang's resin (4-alkoxy-benzyl alcohol resin)[2)]. See scheme I.

Scheme I



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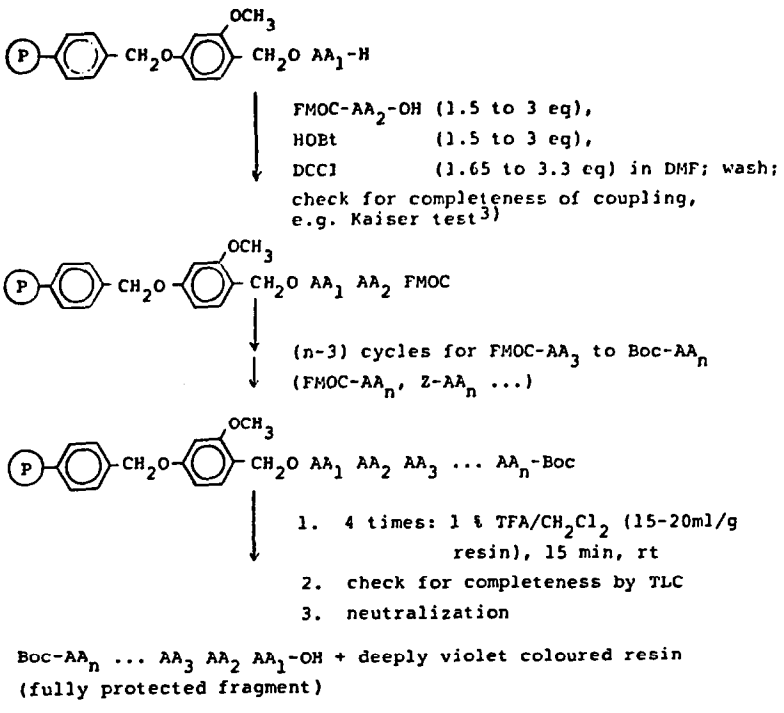

The feature of the new resin are the mild cleavage conditions: Even large fully protected peptide fragments can be cleaved in excellent yields.

Residual dicyclohexyl urea and polar solvents have to be washed out carefully prior to cleavage. Otherwise the urea would consume acid and contaminate the peptide.

During the cleavage the resin gradually turns red to deep violet in the absence of scavengers. At this point further treatments with acid will yield only minor amounts of product (TLC). 1 % TFA / CH_2Cl_2 appears to be a good solvent for protected peptide fragments, which may precipitate after neutralisation (usually with pyridine). Scavengers need not be added except in the case of Trp-containing peptides. Trp might be alkylated by the stable deep violet cation derived from the linker thereby causing a substantial decrease in yield. No problems so far have been experienced with Met and Tyr.

Many fragments of different chain-length have been synthesized and cleaved from the resin in high yield and purity. See Table I.

Some of them were directly used for fragment condensations. It suffices to check the purity of the fragments in different TLC systems. However, their structure should be ascertained by amino acid analysis or other appropriate method. Several syntheses were repeated to demonstrate their reproducibility.

Table I

Fragment	crude yield % (precipitated product)	purity % (HPLC[+])
Boc-Asp(OtBu)-Val-Pro-Lys(Boc)-Ser(tBu)-OH [Kassinin (1-5)]	90	78
Pyr-Thr(tBu)-Ser(tBu)-Phe-Thr(tBu)-Pro-OH[+++]	54	91
Pyr-Pro-Ser(tBu)-Lys(Boc)-Asp(OtBu)-Ala-OH [Eledoisin (1-6)]	66	83
Boc-Asp(OtBu)-Tyr(tBu)-Met-Gly-Trp-Met-Asp(OtBu)-OH (without scavenger!) [CCK-8 (1-7)]	47	88
Boc-His(Boc)-Lys(Boc)-Thr(tBu)-Asp(OtBu)-Ser(tBu)-Phe-Val-Gly-OH [Substance K (1-8)]	82	96
Pyr-Ala-Asp(OtBu)-Pro-Asn-Lys(Boc)-Phe-Tyr(tBu)-Gly-OH Physalaemin (1-9)	93	80 (after t-Butyl-cleavage)
Boc-Gly-Asp(OtBu)-Arg(Mtr)-Ala-Gly-Gln-Pro-Ala-OH [Malaria epitope][6)]	85	78
Boc-Ser(tBu)-Leu-Arg(Mtr)-Arg(Mtr)-Ser(tBu)-Ser(tBu)-Cys(Acm)-Phe-Gly-Gly-OH ANF (1-10)[+++]	75 (60[++])	74 (>98[++])
Z-Asn-Lys(Boc)-Phe-His(Boc)-Thr(tBu)-Phe-Pro-Gln-Thr(tBu)-Ala-Ile-Gly-OH [Human calcitonin (17-28)]	43[++]	95[++]
Boc-Arg(Mtr)-Ser(tBu)-Ser(tBu)-Cys(Acm)-Phe-Gly-Gly-Arg(Mtr)-Met-Asp(OtBu)-Arg(Mtr)-Ile-Gly-OH [ANF (4-16)]	77	91

[++] after counter current distribution (CCD)

[+] HPLC's were performed on a reversed phase column (ODS 2) with appropriate gradients of triethylammonium phosphate buffer (pH 2,3) and acetonitrile. The UV detection was at 220 nm.

[+++] coupling of dipeptide to avoid diketopiperazine formation (see text)

Racemization at the C-terminus of the head fragment is one of the main problems of fragment coupling. Therefore fragments with C-terminal Gly or Pro are employed whenever possible. The latter, however, causes another problem: Upon coupling of a second a Fmoc-amino acid to Pro-resin diketopiperazine formation in the subsequent removal of the Fmoc-group leads to substantial cleavage of dipeptide intermediate from the resin. This phenomenon has already been experienced and examined with Wang's resin [4]. The best way to prevent diketopiperazine formation is to couple Fmoc-dipeptide to C-terminal Pro-resin.

All fragments of table I were coupled in solution to form the corresponding peptides or peptide amides. Our preferred conventional coupling method is dicyclohexylcarbodiimide / hydroxybenzotriazole (hydroxysuccinimide). The methodology of fragment coupling in solution is excellently documented [5].

In our experience (see table I) the described combination proves to be a useful method for the synthesis of peptide amides avoiding the use of hydrogen fluoride. Furthermore, the resin can be used to synthesize peptides by the solid-phase technique which are unstable under the common (e.g. Wang's resin), more acidic cleavage conditions.

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