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# PREPARATION AND APPLICATION OF NEW ACID LABILE ANCHOR GROUPS FOR THE SYNTHESIS OF PEPTIDE AMIDES BY FMOC SOLID PHASE SYNTHESIS

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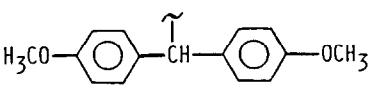
Abstract: The synthesis and application of new linkage agents for the preparation of peptide amides using a modified Fmoc strategy is described.

The use of the base labile 9-fluorenylmethyloxycarbonyl group[1] (Fmoc) for temporary N-alpha protection in solid phase peptide synthesis has gained an increasing interest during the last years. Whereas a variety of acid-labile anchor groups for the synthesis of peptide acids by the Fmoc-strategy are described, for the synthesis of peptide amides commonly [2] still the benzhydrylamine and methylbenzhydrylamineresins are used [3]. This anchor groups, however, require liquid hydrogenfluoride or trifluoromethanesulfonic acid for cleavage from the polymeric support. An alternative is the ammonolysis [4] of resinbound peptide benzylesters.

We now report a new anchorgroup [5] which is coupled as a fully characterized compound to the resin and releases the peptide amide upon treatment with trifluroacetic acid.

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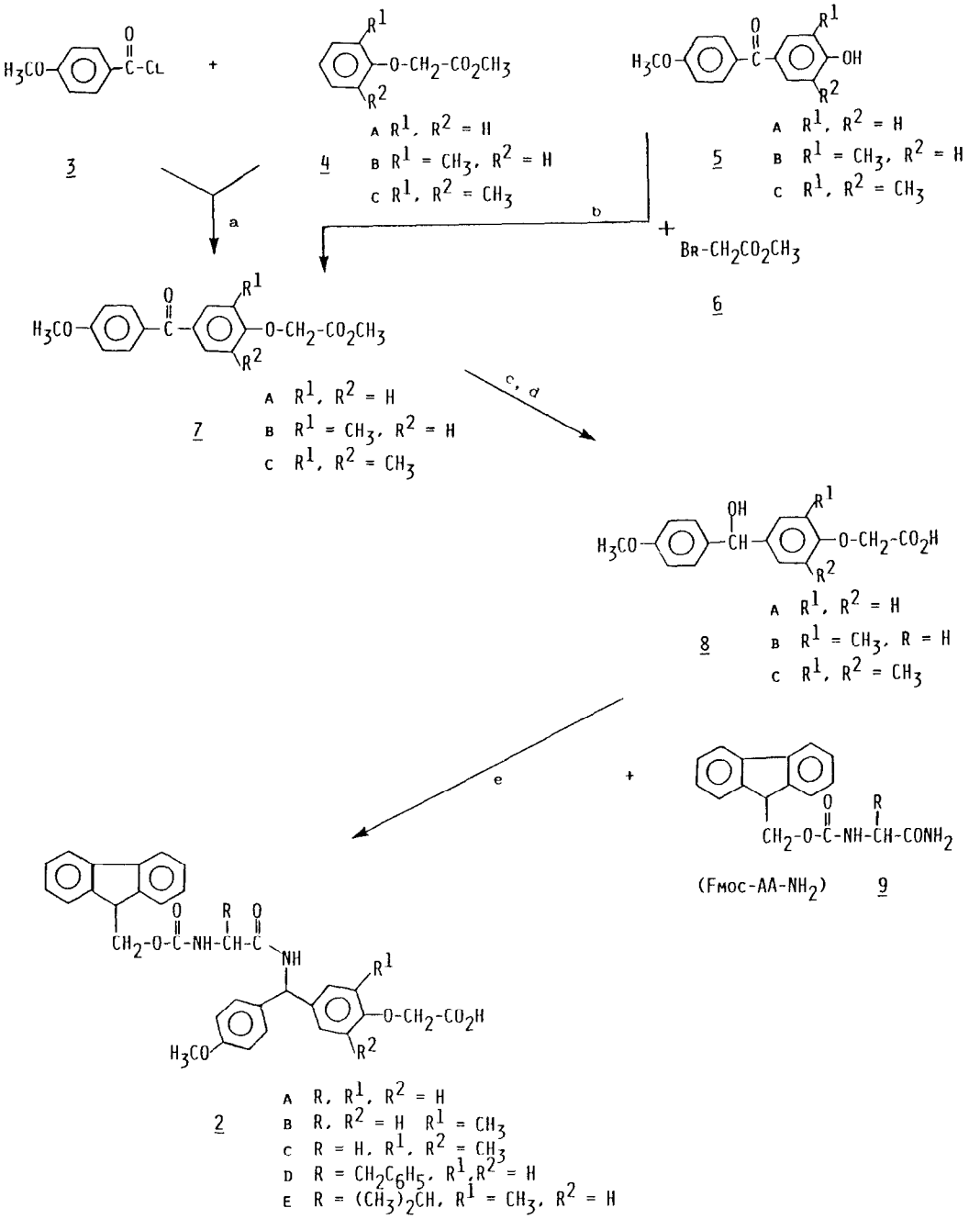
In the literature the 4,4'-dimethoxybenzhydryl group 1 (Mbh) [6] is described for the protection of amides which can be removed by TFA. Combination of this structure with a moiety which can be coupled to commercially available resins commonly used for solid phase peptide synthesis leads to linking agents of structure 2.

Scheme 1




(a) $AlCl_3$, 1.2-dichloroethane, 50°C ; (b) $K_2CO_3$, acetone, RT ; (c) NaOH, MeOH/$H_2O$, RT
(d) $NaBH_4$, MeOH, RT ; (e) AcOH ($H_2SO_4$ kat.)

The synthesis of these compounds is straight-forward as shown in scheme 1[7]. Key compound is the 4-(4'-methoxybenzoyl)-phenoxyacetic acid ester 7 which can be obtained by two different ways. Firstly by Friedel-Crafts acylation of the corresponding phenoxyacetic acid ester 4 with 4-methoxybenzoyl chloride 3 or secondly by reaction of alpha-halogenoacetic acid esters 6 with substituted 4-hydroxybenzophenons 5. Saponification of the ester 7 and subsequent reduction of the benzophenone carbonyl group gives the corresponding carbinol 8 [8]. This is treated in glacial acetic acid with the Fmoc-amino acid amide using a few drops of concentrated sulfuric acid as catalyst and molecular sieves as water trap.

Thus the anchor group 2 with the desired amino acid attached is obtained. Before it is coupled to the resin used for peptide synthesis, it can be fully characterized by spectroscopic and analytical methods.

The usefulness of the new linkers was demonstrated by solid phase synthesis of oxytocin 10 and LHRH 11.

H-Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-$NH_2$ 10

pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-$NH_2$ 11

For the synthesis of oxytocin, compound 2 was coupled to a resin having valine as an anchor group using DCC/HOBt. The peptide was synthesized using in situ prepared acid HOBt esters of Fmoc-Leu-OH, Fmoc-Pro-OH, Fmoc-Cys (StBu)-OH, Fmoc-Asn-OH, Fmoc-Gln-OH, Fmoc-Ile-OH and Fmoc-Tyr(tBu)-OH. Cleavage of the Fmoc-group was performed by 20% piperidine in DMF. When the synthesis was completed, the peptide amide was split off by TFA thioanisole/ethanedithiol 90/5/5. The crude peptide was precipitated in tert.butyl methyl ether, washed to remove the scavengers and dried. Then the cystein was deprotected by treatment with tri n-butylphosphine and oxidized to the disulfide with iodine in 60% acetic acid. After purification on sephadex LH20 pure oxytocin was obtained in 33% yield, which was identical to a authentic sample [9].

For the synthesis of LHRH, compound 2 was coupled to an aminomethyl resin and the peptide synthesized in a similar way as described for oxytocin. For the coupling reaction, however, HOObt esters were used instead. Fmoc-Arg (Mtr)-OH, Fmoc-His(Trt)-OH and pGlu-OH were activated in situ, all other amino acids were used as preformed active esters. After the synthesis was

finished, the peptide amide was cleaved from the resin as described above and the crude product purified by chromatography on sephadex G25. LHRH was obtained in 43% yield identical to an authentic sample [10].

## REFERENCES AND NOTES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5) When preparing the manuscript B. Penke and J. Rivier, J. Org. Chem. 52 1197 (1987) described the use of 2.4-dimethoxybenzhydrylamine resin.

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7) Every new compound was characterized by NMR and MS .

8) This compounds do not give single tlc spots or definite spectroscopic data as probably a mixtures of the carbinol andzwitterionic structures resulting from water elimination are isolated. The obtained product, however, reacts smoothly in the following coupling reaction with the Fmoc-amino acid amide.

9) Authentic oxytocin was purchased from Bachem .

10) Authentic LHRH was kindly provided by Dr. W. König .


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