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SOLID-PHASE SYNTHESIS OF PROTECTED PEPTIDE FRAGMENTS USING A TRIALKOXY-DIPHENYL-METHYLESTER RESIN.

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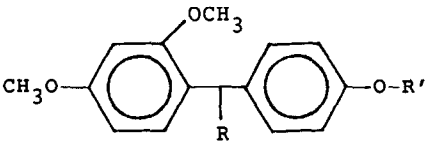
Summary: A trialkoxy-diphenyl-methylester and amide linkage for solid-phase peptide synthesis with Fmoc strategy is described. Protected peptide esters can be smoothly cleaved with weak acid, resulting in fragments with intact side-chain protection. Mild acidic cleavage of the corresponding peptide amide resins yields peptide amides.

Partially protected peptide fragments are useful in the synthesis of cyclic peptides, or as intermediates for assembling in solution or at a carrier to build up large sequences. There is a need for an efficient solid-phase synthesis method for the preparation of such fragments. Attempts have been made with the Fmoc solid-phase technique using an acid-sensitive link to a polystyrene carrier. Atherton et al.[1] described a dialkoxy-benzylester linkage allowing cleavage from the resin by treatment with 1 % TFA[2] in DCM. This carrier has proved useful for preparing certain protected sequences[3], but has considerable drawbacks when Tyr(But) and/or Lys(Boc) with their markedly acid-sensitive side-chain protecting groups are present[1].

In an attempt to further increase acid-lability, we synthesized 4-(2',4'-dimethoxyphenyl-hydroxymethyl)-phenoxymethyl-polystyrene (3). The application of its derivatives in the Fmoc solid-phase synthesis of a protected nonadecapeptide fragment and of a tetrapeptide amide and their mild acidolytic cleavage from the resin is demonstrated.

The convenient and fast preparation of the resin 3 proceeded as follows: Traces of water were carefully removed from the cesium salt of 2,4-dimethoxy-



1 R: =O, R': -H. 2 R: =O, R': -CH_2-polystyrene (1 % cross-linked) = -PS

3 R: -OH, R': -PS. 4 R: Fmoc-Gly-O-, R': -PS.

5 R: Fmoc-Asp(OBut)-Arg(Mtr)-Gly-Phe-Tyr(But)-Phe-Ser(But)-Arg(Mtr)-Pro-Ala-Ser(But)-Arg(Mtr)-Val-Ser(But)-Arg(Mtr)-Arg(Mtr)-Ser(But)-Arg(Mtr)-Gly-O-, R': -PS. 5a free acid of 5.

6 R: Fmoc-Pro-NH-, R': -PS.

7 R: Boc-Pro-Glu(OBut)-Ile-Pro-NH-, R': -PS. 7a Pro-Glu-Ile-Pro-NH_2.

8 R: Fmoc-NH-, R': -PS.

4'-hydroxy-benzophenone (1)[4] (26.1 g, 67 mmol) by co-evaporating with pyridine and DMF in order to suppress subsequent hydrolysis of the chloromethyl resin. Chloromethyl-polystyrene[5] (20.0 g, 13.4 mmol Cl) was added in 400 ml DMF and the mixture was shaken at r.t. for 20 h. After exhaustive washings of the resin with isopropanol, DMF, water, isopropanol and drying in vacuo, 22.0 g of ketone resin 2 was isolated. 7.07 g 2 (ca. 4 mmol) was refluxed with $LiBH_4$ (14,5 mmol) in 50 ml THF for 1 h. Longer reaction time significantly decreased functionalization owing to reduction of the alcohol. After addition of MeOH and acetone at 0-5°, the resin was washed with MeOH, 10^{-3} M HCl_{aq}, MeOH, and dried, yielding 6.8 g of the alcohol resin 3 (ca. 0.4 mmol/g). Esterification of Fmoc-Gly-OH (4 mmol) with the secondary alcohol 3 (2.00 g) occurred with unexpected ease in 20 ml DCE with DCCI (4.2 mmol), DMAP (0.2 mmol) and N-methyl-morpholine (1 mmol) at r.t. for 4 h. Unreacted OH-groups were acylated with benzoic anhydride (5 eq) in pyridine/DMA 1/4. The Fmoc content of the resin 4 (cleavage with 20 % piperidine in DMA) was 0.36 mmol/g. The amount of DMAP in the esterification procedure was optimized in order to minimize dipeptide formation via Fmoc removal, and we found less than 1 % Fmoc-Gly-Gly-OH (HPLC) in the cleavage product of the resin (0.1 % TFA in DCM, 2 min, r.t.). From partial hydrolysis of the chloromethyl resin,

when synthesizing 2, a certain amount of acid-stable Fmoc-Gly benzylester was expected to form during esterification. From Fmoc determination of the acid treated resin, this part was found to be less than 4 % of the totally bound Fmoc-Gly. Although this stably bound first amino acid will consume some reagent during peptide synthesis, it will obviously not affect the quality of the product. Other Fmoc-amino acids were also successfully esterified to the resin by the same method.

4 was extended to 5 by attaching the amino acids of fragment 23-41 of the insulin-like growth factor-2 with a non-optimized standard solid-phase coupling procedure (3 eq symmetrical anhydrides prepared in DCM with DCCI and coupled in DMA, 1 h, r.t., in the presence of 1 eq DIPEA, Fmoc-cleavage with 20 % piperidine in DMA, 10 min). Average coupling yields were 97 % according to Fmoc monitoring. Cleavage from the resin was performed with AcOH/DCM 1/9 for 1.5 h at r.t.[6]. After removal of the DCM *in vacuo* the solution was lyophilized. In separate experiments it was shown that these conditions do not cleave Lys(Boc) and Tyr(But) in detectable amounts (TLC). The peptide can also be cleaved from the resin by a 3 min treatment with 0.2 % TFA in DCM at r.t., neutralization of the TFA with 1 eq NEt_3 and extraction with DMF. The crude product was purified by countercurrent distribution (MeOH/water/ $CHCl_3$/ CCl_4 9/2/3/5 k<0.1). The pure (TLC, HPLC) protected fragment 5a (yield 23 % with respect to the first amino acid) was demonstrated to have the expected structure (FAB-MS). This fragment can be used as a building block in the synthesis of larger peptides.

In order to test the usefulness of the same linkage type for the preparation of peptide amides, 3 was reacted with Fmoc-Pro-NH_2 (3 eq) in the presence of benzenesulphonic acid (0.5 eq) in dioxane (50°, 20 h) to 6 (0.23 mmol/g), which was extended to sequence 7. Cleavage from the resin and of the two tert. butyl protecting-groups (TFA/DCM 1/1, r.t., 15 min) resulted in 80 % yield of crude 7a, which was identical with an authentic sample. The conditions of cleavage from resin 7 have not been optimized. A more convenient educt for preparing peptide amides is 8 (0.28 mmol/g), which was prepared from 3 and 9-fluorenylmethyl-carbamate (Fmoc-NH_2) by acid catalysis[7].

Our results demonstrate the new resin to be a greatly improved tool for the solid-phase synthesis of protected peptide fragments and of acid-sensitive

peptide amides. However, it should be noted that the acid-sensitivity of the new ester-linkage does not allow the use of coupling catalysts such as HOBt without buffering with DIPEA. We have, for example, found severe losses of the peptide chain from the resin with another of our standard coupling procedures using DCCI in the presence of 0.3 M HOBt in DMA at 50° ($t_{1/2}$ ca. 3 h for cleavage of Fmoc-Gly). We also recommend the addition of base when using symmetrical anhydrides or active esters for coupling.

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References and Notes.

1. E. Atherton, E. Brown, G. Priestley, R.C. Sheppard, and B.J. Williams, Proceedings of the Seventh American Peptide Symposium (1981), Pierce Chemical Company, Rockford IL, pp. 163-174.
2. **Uncommon abbreviations:** TFA = trifluoroacetic acid, DCM = dichloromethane, DIPEA = diisopropyl-ethylamine, DMA = dimethylacetamide, DCE = 1,2-dichloro-ethane, DMAP = 4-dimethylamino-pyridine, Mtr = 4-methoxy--2,3,6-trimethylbenzenesulfonyl-.
3. N.L. Johansen, P. Faarup, and B.F. Lundt, European Peptide Symposium, Porto Carras, Greece (1986), Abstracts pp. 75.
4. 1 was prepared in analogy to the synthesis of 2,4'-dihydroxy-4-methoxybenzophenone: S. Ray, P.K. Grover, and N. Anand, (1971) Indian Journal of Chemistry 9, 619-623 (4-hydroxy-benzoic acid 1 eq, resorcin-dimethyl ether 2 eq, $ZnCl_2$ 2.2 eq, $POCl_3$ 7.6 eq, 2h 60°, crystallization from MeOH). Fp. 135-7°. Elemental analysis, H-NMR- and IR-spectra were compatible with structure 1. The cesium salt was prepared by neutralizing 1 with 0.95 eq CsOH-hydrate in tert. butanol/water 1/1, lyophilization and recrystallization from tert. butanol.
5. Merrifield Polymer Fluka, 0.67 mmol Cl/g.
6. This cleavage procedure was first reported by Bernhard Riniker (personal communication).
7. This reaction with a hydroxy-xanthenyl-resin has also been described by Peter Sieber, Tetrahedron Lett. (1987) 28, 2107-2110.

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