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A New Route to Sequential Polypeptides Combining Solid Phase Synthesis and Solution Peptide Synthesis

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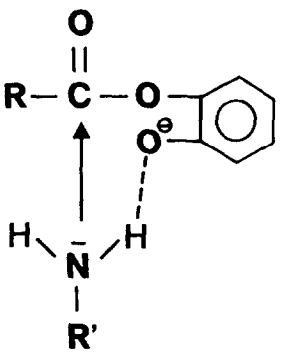
Abstract: *The cleavage of a resin-bound peptide from an oxime resin using the 2-phenacyloxyphenyl ester of an aminoacid provides a peptide with this aminoacid in C-terminal position. The inactive 2-phenacyloxyphenyl peptide ester thus obtained can then be converted into 2-hydroxyphenyl active ester. The polymerization of the repeat unit using this mode of activation gives sequential polypeptides of reasonable molecular weight and of high optical purity.*

Sequential polypeptides are polymers formed by the repeat of a short peptide motif. They proved valuable models of proteins and they still remain useful tools in a number of problems. The routine way of synthesis of these polypeptides is to synthesize first the repeat unit bearing an active ester in C-terminal position which is then polymerized. The most popular active esters are p-nitrophenyl, halogenophenyl and succinimidyl esters. The synthesis of the activated unit is generally performed in solution and therefore becomes sometimes difficult above 4 or 5 residues only. Another shortcoming of this strategy is the risk of racemisation of the C-terminal residue. When polypeptides of large repeat sequences are desired SPPS must be used. The peptide unit is cleaved from the resin without affecting the protecting groups. After purification the peptide unit is converted into its active ester form and polymerised. This way requires specifically cleavable peptide-resin linkages, for example super acid sensitive resins. It leads to an increased risk of racemisation since loss of optical purity may occur both in the course of the activation and polymerisation processes. A certain number of attempts avoiding the synthesis of a transient active ester have been reported [1,2,3] The peptide with free C- and N-termini is mixed with an effective coupling reagent (DPPA for example). Unfortunately this procedure does not furnish polymers of sufficient molecular weight in all cases [3] and in our hands sometimes failed.

For these reasons we sought a new method of preparation of the repeat unit taking advantage of SPPS and able to furnish directly after cleavage from the resin the peptide unit in a preactivated and thus easily activable ester form. Such a way avoids the drawbacks of an activation step of a free carboxyl group after cleavage from the resin (low yields and racemization). To our knowledge, the first

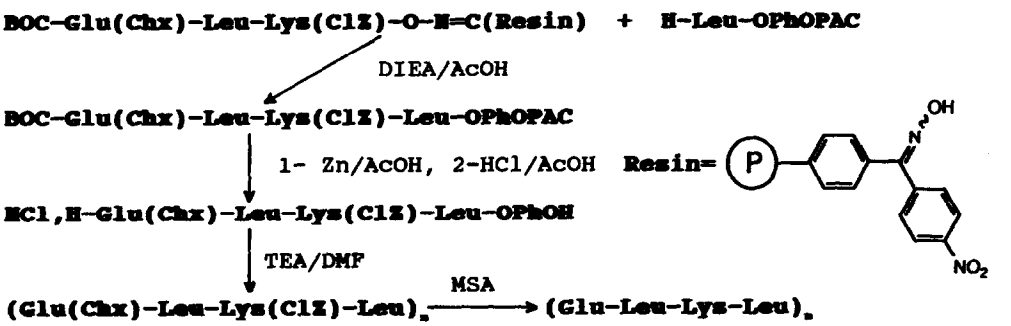
successful attempt achieving this aim has been reported by Siemens *et al.*[4]. These authors used oxime resins [5,6,7]„ from which the peptide was cleaved by the 4-(methylthio)-phenyl ester of the desired C-terminal aminoacid. Oxidation of the sulfide into sulfone furnished a peptide active ester. In the new method herein reported the peptide was synthesized using also an oxime resin. The cleavage of the peptide from the resin was carried out by a reaction involving the free amino group of the desired C-terminal aminoacid in a blocked and thus weakly reactive form of its 2-hydroxyphenyl ester (catechol ester). The reactivity of catechol esters, first proposed by Young *et al* [8,9] and Jones [10] twenty five years ago, is not due to an electron-withdrawing effect, since catechol is only weakly acidic (pK 9.85), but rather to an intramolecular base catalysis generally called anchimeric assistance. This mechanism involves the ionized form of the free hydroxy group, as shown in scheme 1.



***Scheme 1* : anchimeric assistance**

Although not extremely reactive, catechol esters were used with success to synthesize optically pure sequential polypeptides [10-14] and applied also to the synthesis of $(ALKEAAE)_{25}$ [15] by fragment condensation. The moderate activity of catechol esters is largely counterbalanced by the racemization-free polypeptides thus obtainable. Catechol esters can be nearly inactivated by masking the 2-hydroxy group. Two temporary protection for this purpose have been reported, the benzyl group [9,10] and the phenacyl group [11,12,13]. The second one has been used in the present work because it can be cleaved by a mild and specific reductive reaction with Zn in aqueous AcOH, which leaves intact all other usual protecting groups. BOC group remains also unaffected in this acidic medium as verified by TLC and optical rotation measurements. The 2-phenacyloxyphenyl esters of an aminoacid were found able to cleave a peptide from an oxime resin in the presence of acetic acid as catalyst. The resulting peptide ester remains perfectly stable in the operating conditions used as verified by IR spectroscopy. However, even in the presence of acetic acid, a 2-phenacyloxyphenyl ester of an aminoacid undergoes a slow

self-condensation reaction which competes with the cleavage reaction. At r.t., the initial rate constant of the self-condensation of HCl,H-Leu-OPhOPAC in chloroform and in the presence of DIEA (1 eq.) and of acetic acid (1 eq.) was estimated by UV and IR spectroscopy and found to be 6.10^{-2} $M^{-1}.min.^{-1}$, corresponding to a half-life of 166 min. for a 0.1M concentration. In the same conditions, the cleavage process from the resin is much faster.For exemple a 45 min. reaction time is sufficient to cleave a BOC-Leu-resin by H-Gly-OEt.



Strategy of synthesis of a polypeptide

Experimental: BOC-Glu(Chx)-Leu-Lys(2-ClZ)-Oxime-Resin was first synthesised using symmetrical anhydrides according to the procedure of Kaiser *et al* [5,6] The crude BOC-Glu(Chx)-Leu-Lys(2-ClZ)-Leu-OPhOPAC was then obtained by reacting the peptide-resin with an excess of HCl,H-Leu-OPhOPAC (2 eq.) in DCM in the presence of DIEA (2 eq.) and AcOH (2.25 eq.) for 2 days at r.t.. The IR spectrum of the resulting resin showed the presence of a little amount of peptide still attached. However a second treatment in the same conditions did not yield a significant additional quantity of peptide. The protected tetrapeptide thus obtained was easily purified by usual washings with slightly basic and acidic aqueous solutions and then by crystallisation from an isopropanol-DCM mixture. The removal of the phenacyl group was achieved by a reductive reaction with Zn in aqueous AcOH [12] and, after cleavage of BOC by HCl/AcOH, the polymerisation was carried out in DMF. In a typical experiment, 0.114 mmole of hydrochloride in a test tube was triturated with a glass rod with DMF (0.032 ml) and TEA (0.0318 ml, 2 eq.) until a homogeneous paste was obtained. After one day a further 0.032 ml DMF was added. The mixture was left in the darkness for 4 days, the polymer was then deprotected by acidolysis with MSA for 1h. at 20°C. Exhaustive dialysis against dilute HCl and water furnished the deprotected polymer in 35% yield refering to the monomer. Its average molecular weight was estimated by viscometry in TFA and found to be 16,200 (DPn=116) [16] This molecular weight is not much lower than those usually obtained by other routes such as p-nitrophenyl esters. As

an example, the same polymer synthesised by the p-nitrophenyl ester method showed an average molecular weight of 23,100.

Catechol esters have been demonstrated to avoid racemization. However, a loss of optical purity could occur during the cleavage of the peptide from the resin since this reaction is in fact an aminolysis. Kaiser *et al* [5], using the Young's test [17] did not find any racemization. We agree with this conclusion since in our hands the same result was obtained. Bz-Leu-Gly-OEt prepared by reacting Bz-Leu-oxime resin with H-Gly-OEt showed an optical rotation identical to that of the reference peptide : α =-34°(reference); –33.8° (found), c=3, EtOH.

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