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SOLID PHASE PEPTIDE SYNTHESIS: FLUORIDE ION RELEASE OF PEPTIDE FROM THE RESIN

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Summary. A linkage to anchor the growing peptide chain has been designed in order to allow swift release of the peptide product by $Bu_4N^+F^-$. Preparation of the linker (4) is described.

Solid Phase Peptide Synthesis (SPPS)[1] has four primary problems; namely (i) nature of the polymer, (ii) type of linkage between the polymer and the growing peptide chain, (iii) selection of protecting groups (side chain and Nα amino) and (iv) choice of activation method for the incoming protected α-amino acid. Once assembled the peptide must be cleaved from the linkage under controlled conditions compatible with side chain protection and which do not cause deleterious modification of the peptide. The most common linker is a benzyl ester type which may be subdivided into the two most common classes, (i) very stable and cleaved by HF[2] or CF_3SO_3H[3] and (ii) acid labile and cleaved by milder acid such as CF_3COOH.[4] In both of these cases the side chain protecting groups are generally cleaved concomitantly with the release of the peptide.

In order to add another dimension to the strategy of SPPS we sought to design a linker which would allow rapid release of the final peptide under mild conditions and permit retention of the side chain protection thus affording fully protected fragments. Our approach to the design of a new linker was inspired by the mechanism proposed by Carpino[5] for the rapid cleavage of the 2-(trimethylsilyl)ethoxycarbonyl (TEOC) group by fluoride ion. Barany[6] has recently devised a linker based upon this concept and we describe another linker which meets the criteria described above and is readily accessible. The basic thesis was that the system shown in Figure 1 would be susceptible to fluoride induced fragmentation to afford the Bu_4N^+ salt of the peptide leaving the bis-quinone methide attached to the resin which would tautomerise to the resin-bound cinnamide.

The target molecule (4) was synthesised by the route shown in Figure 2 which starts from the readily available crystalline methyl *p*-methyl-cinnamate (1).[+] Conversion of (1) to (2) by the method of Picard[7] presented difficulties with the initial use of HMPA as complexing agent for

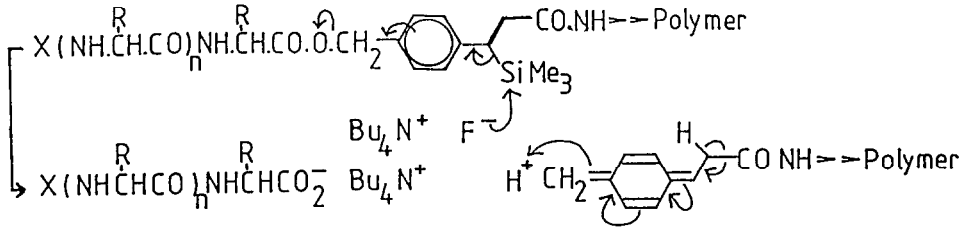
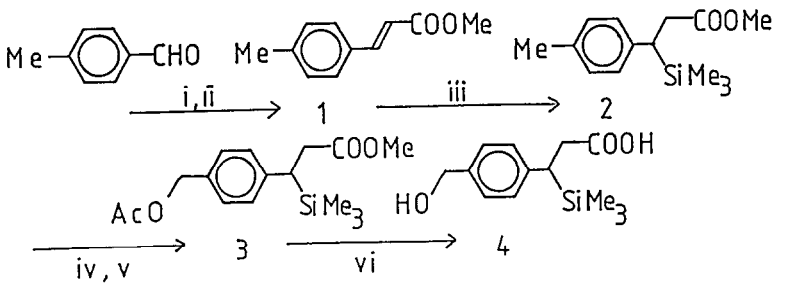
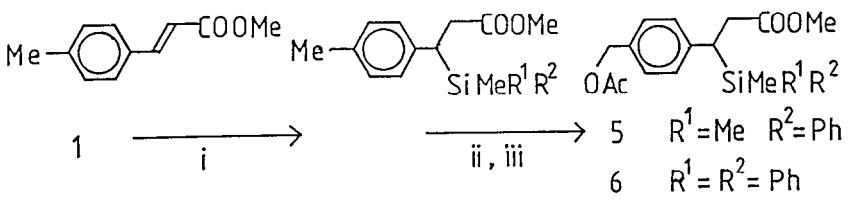


Figure 1



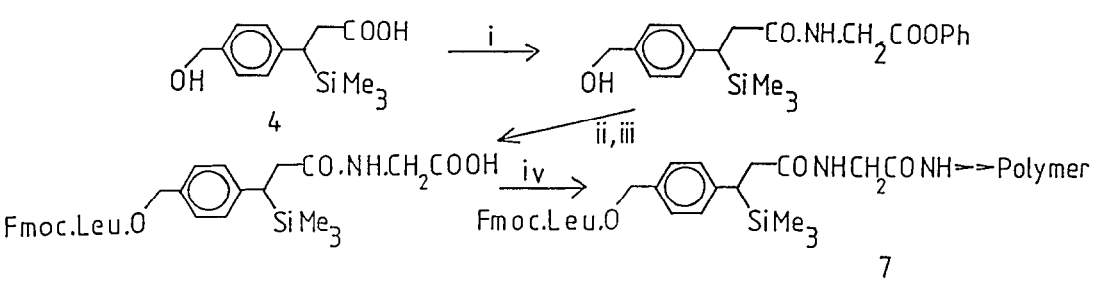
(i) $CH_2(COOH)_2$, piperidine; (ii) MeOH, $SOCl_2$;
(iii) Mg, Me_3SiCl, DMEU, THF; (iv) NBS, CCl_4, hν,
$(PhCOO)_2$; (v) KOAc, MeCN, 18-Crown-6 (0.04 equiv.);
(vi) NaOH aq, dioxan.

Figure 2



(i) RPhMeSiCl, Li/THF, CuI/THF; (ii) NBS, CCl_4, hν,
$(PhCO.O)_2$; (iii) KOAc, HOAc, Δ

Figure 3



(i) $H_2N.CH_2.COOPh$, DCCI; (ii) Fmoc.Leu.OH, DCCI, CH_2Cl_2/DMF,
DMAP (0.1 equiv.); (iii) NaOH aq, H_2O_2, dioxan/H_2O (pH 10.5);
(iv) DCCI, HOBt, aminomethylpolystyrene.

Figure 4

the $Me_3SiMgCl$ and also control of the exothermic reaction which resulted in yields varying between 0 and 80%. However using N,N-dimethyl ethylene urea (DMEU)[8] with careful temperature control between 55-60°C, the required conjugate addition product (2) was obtained as a distillable liquid. Bromination of (2) using N-bromosuccinimide followed by bromide displacement with potassium acetate, in the presence of 0.04% 18-Crown-6,[9] gave the acetoxy ester (3) which could be purified by distillation and subsequently hydrolysed to the desired crystalline linker reagent (4). An earlier, successful synthesis of (4) involved functionalisation of the methyl group in (1) followed by conjugate addition of $Me_3SiMgCl$ but this was much less efficient. Incorporation of a nitro substituent into the position ortho to the acetoxymethyl group in (3) was not possible in spite of much experimentation.

The syntheses of the analogues (5) and (6) were accomplished using the method of Fleming[10] for the introduction of the silyl group by reaction of the silyl cuprates derived from $Me_2PhSiCl$ and $MePh_2SiCl$ respectively upon the cinnamate ester (1) (Figure 3).

In order to test the feasibility of fluoride induced fragmentation of peptide esters of (4) indicated in Figure 1, we examined the rate of cleavage by fluoride ion and the relative acid stability of the series (3), (5) and (6). It was considered important to find out whether the Si moiety was participating in enhanced acid cleavage of these benzyl esters. Such a situation would result in the formation of the cinnamate (1), but in the event it was found that 6 equiv. HCl in methanol or 100% TFA did not result in cinnamate formation. The transesterification products expected from the benzyl esters were formed hence the linker (4) should not be used in conjunction with acid labile Nα protecting groups in SPPS, but is compatible with the use of base labile Nα-protection.[11] $Bu_4N^+F^-$ treatment of the esters (3), (5) and (6) showed interesting solvent and structure dependence. As expected the trimethylsilyl analogue (3) was cleaved fastest by fluoride ion in solvents ranging from CH_2Cl_2, CH_3CN to CH_3CONMe_2. In the latter the fragmentation of (3) was almost instantaneous whereas (5) and (6) were cleaved to the extents of 72% and 87% respectively after 1 hr. These results led us to decide upon (4) as the linker of choice.

Application of (4) to SPPS first involved conversion to the polystyrene-bound Fmoc.Leu ester (7) shown in Figure 4. Base induced deprotection of the Fmoc group followed by stepwise synthesis using 1.5 equiv. of Fmoc amino acids activatd by the phosphinic-carboxylic mixed anhydride method[12] produced the protected hexapeptide Dpp.Leu.Val.Gly.Phe.Ala.Leu bound to the polymer (the final residue being added as the Nα-diphenylphosphinamide).[13] Each coupling (30 min) employed 2 equiv. of the activated amino acid plus 2 additional equiv. of 2,6-lutidine. Cleavage of the peptide by the mechanism shown in Figure 1 was achieved by $Bu_4N^+F^-$ in DMF (2 equiv.) within 5 min. and Dpp.Leu.Val.Gly.Phe.Ala.Leu.OH was isolated in 62% yield after h.p.l.c. (Hypersil C_{18}MeCN/H_2O).

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References and Notes

+ All compounds quoted in the text were analytically pure with physical data consistent with the structures given.

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