A New Benzyl Ester Resin with Increased Stability during N^{α}-t-Butyloxycarbonyl Deblocking in Solid-phase Peptide Synthesis

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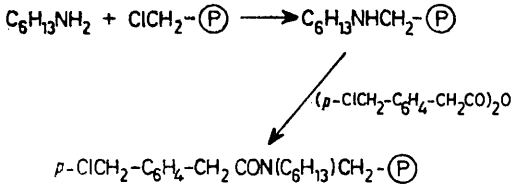
Summary A *p*-carbamoylmethylbenzyl ester linkage between Boc-phenylalanine and polystyrene resin has been prepared and shown to possess great stability toward trifluoroacetic acid and good reactivity toward liquid HF as demonstrated by the synthesis of a model peptide.

AN important problem in solid-phase peptide synthesis[1] is the acid lability of the standard *p*-alkylbenzyl ester linkage of the peptide to the polystyrene resin. In the synthesis of ribonuclease[2] it was estimated that 1·4% of the peptide chains were lost in each cycle during deprotection of the N^{α}-Boc group by reaction of the peptide resin with 50%

CF_3CO_2H in CH_2Cl_2. In addition to a significant reduction in yield, peptide chain cleavage could also result in the initiation of new peptide chains at the uncovered sites on the resin, and increase the difficulty in purifying the peptide product.



SCHEME

We have found that if the standard *p*-alkylbenzyl ester linkage is replaced by a *p*-carbamoylmethylbenzyl ester[3] linkage (see Scheme) the stability in CF_3CO_2H–CH_2Cl_2 is increased about fifty times. Thus, the reaction of chloromethyl-polystyrene with 30% n-hexylamine in CH_2Cl_2 for 7 days at 4 °C gave hexylamine resin. The amine content of the hexylamine resin was 95% of that expected from the original chlorine content as measured by the picric acid method.[4] The hexylamine resin was acylated by reaction with the preformed symmetrical anhydride[5] of *p*-chloromethylphenylacetic acid,[6] and the chloromethyl product was treated with the caesium salt[7] of Boc-phenylalanine. The stability of the resultant Boc-phenylalanine resin to CF_3CO_2H–CH_2Cl_2 and its reactivity toward liquid HF was measured in two ways.

In the first case, the amino-acid resin was converted into acetylphenylalanine resin. Treatment of this resin with 50% CF_3CO_2H in CH_2Cl_2 for 17 h gave 0·4% cleavage of Ac-Phe-OH (determined by hydrolysis of the filtrate with HCl and measurement of phenylalanine on an amino-acid analyser) which corresponds to 0·006% cleavage per 15 min deblocking cycle. Reaction of the acetylphenylalanine resin with liquid HF for 30 min at 0 °C gave 62% cleavage.

In the second case, the new resin was used to synthesize the model peptide H-Lys_5-Glu_3-Leu_2-Trp(Nps)-Phe-OH by the same procedure as previously reported for synthesis on the standard resin.[8] The filtrates from the deblocking procedure (55% CF_3CO_2H in CH_2Cl_2; 15 min) and subsequent washes were isolated, and spectral analysis for the Trp(Nps) unit indicated that an average of 0·01% peptide cleavage had occurred during the deblocking cycle. This is considerably less than the observed cleavage (0·4–1·1%) when the same peptide was synthesized on a resin which has the standard *p*-alkylbenzyl ester linkage to the peptide chain.[9] Reaction of the peptide resin with liquid HF under the same conditions as previously reported[8] (1 h; 0 °C) and purification of the crude peptide by chromatography on carboxymethyl-cellulose gave a 59% yield of the desired peptide.

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