Tetrahedron Letters No. 32, pp 3041 - 3042 0040-4039/79/0801-3041$02.00/0

STABILITY OF FLUORENYLMETHOXYCARBONYLAMINO GROUPS IN PEPTIDE SYNTHESIS. CLEAVAGE BY HYDROGENOLYSIS AND BY DIPOLAR APROTIC SOLVENTS

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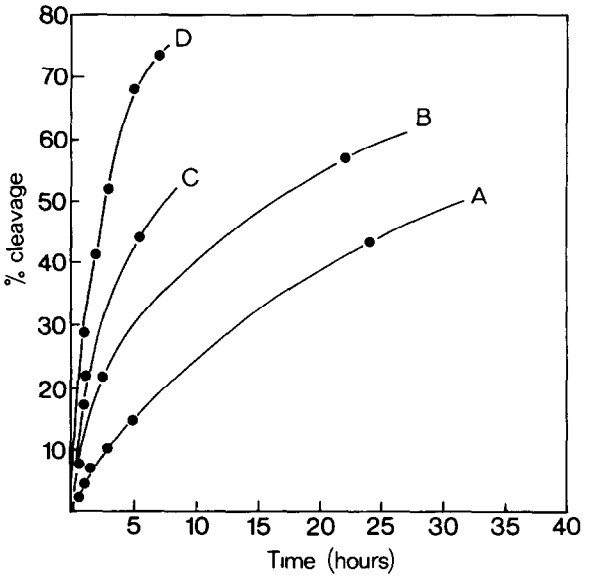
Summary: N-Fluorenylmethoxycarbonyl derivatives of amines are unexpectedly cleaved by catalytic hydrogenation with $t_{\frac{1}{2}}$ 3-33 h. under various conditions. They are also cleaved on standing in the solvents dimethylformamide, dimethylacetamide, and N-methylpyrrolidone, but much more slowly

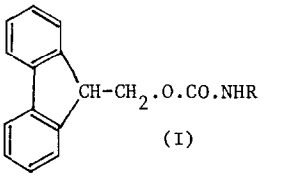
Base-labile 9-fluorenylmethoxycarbonyl (Fmoc) derivatives of amines (I) were introduced into peptide chemistry in 1970.[1] Recently they have assumed increasing importance following their application to solid phase synthesis[2-6] in which they present certain advantages over the customary t-butoxycarbonyl (Boc) derivatives. The recent successful hydrogenolysis of benzyl ester-linked, resin-bound peptides[7] and the reported stability of Fmoc-derivatives to hydrogenation[1] has prompted their use in solid phase fragment condensation strategies.[6] We now report observations on the stability of fluorenylmethoxycarbonyl groups which have important implications regarding this last application and in peptide synthesis generally.

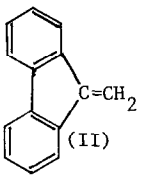
Recently we described[6] hydrogenation of the insoluble polydimethylacrylamide-bound nonapeptide derivative (III) in the presence of dimethylformamide containing palladium acetate. Hydrogenolysis of the benzyl ester linkage was efficient, but subsequently it was found that substantial cleavage of the Fmoc protecting group had occurred simultaneously. This is at variance with the results of Carpino and Han who reported[1] complete stability of Fmoc-aniline to hydrogenolysis. We have now repeated the preparation of this anilide and find that it is cleaved by hydrogenation in the presence of 10% w/w 10% Pd-C (Fluka) in 20% acetic acid-methanol with $t_{\frac{1}{2}} \sim 6$ h. Similar results were obtained using dimethylacetamide or ethyl acetate-ethanol (2:1) as solvent. From this last experiment, 9-methylfluorene (90%, m.p. 42-44°) was isolated and identified by nmr spectroscopy. A more detailed study of the hydrogenolysis reaction using Fmoc-glycine with product analysis by reversed phase hplc gave the results depicted in the figure.

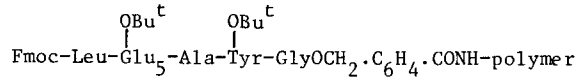
The mechanism of this hydrogenolysis reaction presents an interesting problem. On prolonged standing in dipolar aprotic solvents, fluorenylmethoxycarbonylamino derivatives undergo spontaneous decomposition with formation of dibenzofulvene (II), $\lambda_{max.}$ (MeOH) 246, 255 nm. Although there is presumably autocatalysis by liberated amine, the rates are very slow. In dimethylacetamide, dimethylformamide, and N-methylpyrrolidone, Fmoc-glycine decomposed in 7 days at room temperature to the extent of 1, 5, and 14% respectively (hplc). If hydrogenolysis proceeds simply by acceleration of this β-elimination reaction and saturation of (II), then the catalyst must additionally promote the elimination step. The spontaneous decomposition of Fmoc-glycine was not accelerated by prior hydrogenation of solvent dimethylformamide which could conceivably have liberated traces of dimethylamine.[6]

Alternatively, the unexpectedly facile hydrogenolysis reaction may be a consequence of the special resonance stabilisation properties of the fluorenyl system. In any event, caution should be exercised in the use of hydrogenation steps in procedures involving fluorenylmethoxycarbonyl protecting groups.









(III)

Figure. Hydrogenolysis in A, 20% AcOH-MeOH; B, in DMF; C, in DMF-diisopropylethylamine (2 eq.); D, in DMF with Pd-black prepared by in situ hydrogenolysis of dissolved $Pd(OAc)_2$ (solid phase conditions).

The spontaneous decomposition of Fmoc-derivatives is unlikely to be significant in peptide synthesis if prolonged contact with solvent is avoided. Thus one cycle of our polar solid phase system[8,9] requiring 1 - 2 h. corresponds to 0.03 - 0.06% decomposition in dimethylformamide and 0.006 - 0.012% in dimethylacetamide. The latter is our preferred solvent for other reasons.[9] Use of N-methylpyrrolidone is contra-indicated.

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(Received in UK 17 May 1979)