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THE SYNTHESIS OF PEPTIDES IN AQUEOUS MEDIUM. IV. A NOVEL PROTECTING GROUP FOR CYSTEINE

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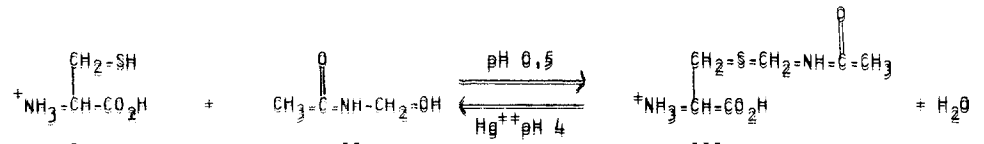
The usefulness of Leuchs' anhydrides in the synthesis of peptides in aqueous medium (1,2,3) prompted us to prepare S-acetamidomethyl cysteine (III). The solubility characteristics of this blocking group make it attractive for peptide synthesis in both anhydrous and aqueous media. Furthermore, this blocking group is stable under conditions which cause the removal of commonly employed, acid labile substituents. The new blocking group is stable, for example, to trifluoroacetic acid, HBr in acetic acid, HCl in ethanol and to acidic and alkaline aqueous solutions in the pH range 0-13 at 25°. It is also stable to anhydrous HF at 0°, conditions which have recently been found useful in the removal of most of the commonly used protecting groups, including benzyloxycarbonyl (4,5).

Conversely, one can remove the new blocking group in high yield with 2 equivalents of Hg (II) at pH 4 at room temperature, conditions which do not affect such groups as N-benzyloxycarbonyl, N-t-butyloxycarbonyl and S-benzyl. In this manner the acetamidomethyl function was removed from III. The resulting cysteine, after precipitation of mercuric ion with H_2S, was air oxidized to cystine in nearly quantitative yield. N-Glycyl-S-acetamidomethyl-L-cysteine was similarly converted to N,N'-diglycyl-L-cystine in ≅ 95% yield. The product was identical with an authentic specimen in its nmr and IR spectra.

S-Acetamidomethyl-L-cysteine hydrochloride (III) is prepared by treatment of L-cysteine (I) with acetamidomethanol (II) (6) in hydrochloric acid at pH 0.5 at 25°. This salt was crystallized



I II III

from methanol-ether and converted to the analytically pure free base ($[\alpha]_{589}^{25}$ = 42.5°, c=1, H_2O) on

successive treatment with Ag_2O and H_2S. The infrared spectrum showed a carbonyl peak at 1672 cm^{-1} (nujol) and the nmr spectrum at 60 MHz in D_2O-DCl showed: CH_3(s) 7.95τ(3); CH_2(m) 6.80τ(2); CH(m) 5.80τ(1); CH_2(s) 5.6τ(2). The N-t-butyloxycarbonyl derivative, mp 110-112°(d), $[\alpha]_{589}^{25}$ -35.5°, c=1, H_2O, prepared with t-butylazidoformate also gave elemental analyses, nmr and IR spectra consistent with the proposed structure. The N-hydroxysuccinimide ester, mp 106-8°, $[\alpha]_{589}^{25}$ 43.0°, c=1, $CHCl_3$, prepared in the usual manner (7) from N-t-butyloxycarbonyl-S-acetamidomethyl-L-cysteine was also fully characterized. We have found this ester useful for the introduction of cysteine in aqueous and anhydrous media. The resulting peptides, after removal of the N-blocking group, have been completely cleaved by leucine aminopeptidase and by aminopeptidase M (8). Furthermore, tlc has revealed none of the racemized peptide in cases where the D-L isomer was separable from the all L-peptide.

The stability of the S-acetamidomethyl group to acidic conditions permits its use in conjunction with the benzyloxycarbonyl group (ε-amino nitrogen of lysine) and the t-butyloxycarbonyl group (α-amino groups). It is also stable to the conditions of preparation of hydrazides and azides, permitting the coupling of peptide chains containing this group by the azide method. Thus, this blocking group has potential utility in the synthesis of peptides containing any of the twenty genetically coded amino acids.

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