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THE 1,1,1,3,3,3-HEXAFLUORO-2-(p-CHLOROPHENOXYMETHOXY)PROPYL (HF-PA) BLOCKING GROUP FOR THE IMIDAZOLE FUNCTION OF HISTIDINE AND ITS USE IN SYNTHESES OF THYROTROPIN RELEASING HORMONE

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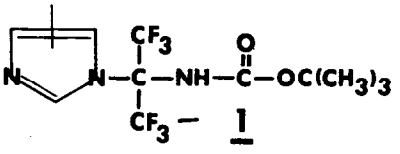
The incorporation of histidine, an amino acid important for the activity of a number of enzymes and hormones into synthetic peptides is not without difficulty. A blocking group able to diminish the basicity and nucleophilicity of the imidazole moiety permits the utility of the active ester procedure to be extended to histidine. Sakiyama[2] prepared the p-nitrophenyl ester of N^{α}-N^{im}-dicarbobenzyloxyhistidine and more recently active esters have been prepared from N^{im}-2,4-dinitrophenyl[3] and N^{im}-piperidinoxycarbonyl[4] derivatives. Weygand[5], et. al. developed the N^{im}-(2,2,2-trifluoro-1-acylaminoethyl-) blocking group which lowers the pKa of the imidazole and is acid labile as the O-t-butyl carbamate congener but did not report any active esters and could not derive any crystalline intermediates, presumably due to the introduction of a new asymmetric center. The corresponding derivative 1 based upon hexafluoroacetone rather than trifluoroacetaldehyde hydrolyzes spontaneously in the presence of water.[5] We wish to report the preparation of N^{α}-carbobenzyloxy-N^{im}-HF-PA-L-histidine methyl ester 2, a corresponding active ester, and



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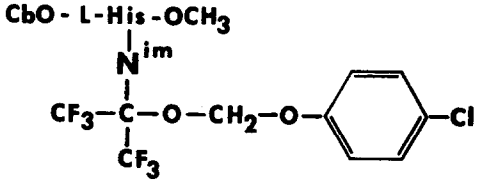

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its use in peptide synthesis. The proximity of the two trifluoromethyl groups lowers the reactivity of the imidazole permitting the preparation of relatively stable active esters and the acetal functionality confers acid lability.

Cbo-L-histidine methyl ester reacts with ammoniacal silver nitrate to form an insoluble silver imidazolate.[7] Reaction of this salt with hexafluoroacetone in tetrahydrofuran at room temperature followed by reaction with α,p-dichloroanisole[8] gives crystalline 2 (69% mp 56-58° from hexane/ethyl acetate). Previously reported attempts to alkylate carbinolamines from haloketones have been unsuccessful.[9] Saponification of the methyl ester in dioxane/aqueous NaOH proceeds in 89% yield without racemization[10] and the corresponding N-hydroxysuccinimide ester[11] 3 is prepared using dicyclohexylcarbodiimide, purified by extraction with 5% sodium bicarbonate and 0.5M citric acid, and isolated as an oil.[12] To demonstrate the potential of this active ester in peptide synthesis we have prepared thyrotropin releasing hormone (TRH, L-pyroglutamyl-L-histidinyl-L-prolineamide)[13,14] using a stepwise approach from the carboxy-terminal amino acid. Other syntheses of this tripeptide have been reported.[15-19]

The reaction of 3 with a 25% excess of L-proline amide in acetonitrile for 18 hr gives dipeptide 4 in 82% yield after chromatographic purification. Hydrogenolysis over palladium followed by reaction with N^{α}-t-Boc-γ-methyl-L-glutamate N-hydroxysuccinimide ester gives the blocked glutamylhistidinylproline tripeptide 5 in 38% yield (C, H, N, F, Cl analysis within 0.23% of calculated; amino acid analysis: his, 0.97, glu, 0.98; pro, 1.05). A competing side reaction is diketopiperazine formation (ca. 20%), known to be problematic with peptides terminating in proline.[20,21] Treatment of 5 with 2.4M HCl in 20% aqueous acetic acid at room temperature for 0.5 hr and subsequent treatment with ammonia (saturated) in anhydrous methanol to affect cyclization affords TRH in 63% yield over these last two steps.

In another approach, hydrogenolysis of 4 followed by condensation with one equivalent of t-Boc-pyroglutamic acid using the mixed anhydride procedure of Anderson[22] gives tripeptide 6 in 74% yield after silica gel chromatography. This product contains a trace of a Pauly positive impurity as detected by tlc. Pure tripeptide, obtained from thick layer chromatography was quantitatively converted to TRH hydrochloride with aqueous HCl in acetic acid (1:4 concentrated HCl:HOAc) at room temperature within 30 minutes. The product gives one spot on tlc (two solvent systems), gives a good amino acid analysis, exhibits a mass spectrum showing all the recorded peaks reported for TRH[23], and has a specific rotation of -50° (C = .65 in 6NHCl).

In conclusion the HF-PA blocking group allows the incorporation of histidine into peptides through high yield active ester couplings and is removed in high yield under relatively mild acidic conditions. It is stable to saponification and hydrogenolysis. In addition, all the blocked peptides were highly soluble in organic solvents.

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