CARBOXAMIDE PROTECTION OF GLUTAMINE AND ISOASPARAGINE AND SYNTHESIS OF AMINO ACID DERIVATIVES AS INTERMEDIATES IN PEPTIDE SYNTHESIS

By

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ABSTRACT

A study was carried out to ascertain the suitability of the cyano group as the protecting group for Asparagine and Glutamine side-chain amino groups. Some model compounds were synthesized viz, carbobenzoxy-β-cyano-ℓ-alanine; benzyl carbobenzoxy-β-cyano-ℓ-alanyl-alaninate, Z-β-cyano-ℓ-alanine methyl ester. The Z-group from these compounds was cleaved by using 5% Pd/C as catalyst at room temperature. The Z-group was completely cleaved and the cyano group was stable under the cleavage conditions. Thus the cyano group is a promising carboxamide protecting group for the side-chain amino groups of Asparagine and Glutamine during peptide synthesis. The cyano group was opened up as the imidate hydrochloride by the Pinner reaction. The derivatives, ethyl Z-alaninimidate hydrochloride; Z-β-methyl-imidate alanylalanine methyl ester hydrochloride; Z-β-ethyl alanylalanine methyl ester imidate hydrochloride; Z-β-benzyl-alanylalanine benzyl ester imidate hydrochloride were synthesized. Z-β-benzyl alanylalanine benzyl ester imidate hydrochloride was hydrogenated by hydrogen with 5% Pd/C at room temperature and atmospheric pressure to cleave the Z-group. The cyano group was opened as the acetamide and thioacetamide. This was an attempt to gain

entrance to amino and thio-analogues of asparagine and glutamine derivatives.

Valylglycine methyl ester was synthesized and converted to 2,5-diketo-3-isopropyl piperazine. This derivative was reacted with triethyloxoniumtetrafluoroborate to generate 2,5 diethoxy-3-isopropyl-3,6-dihydropyrazine. N-Butyl lithium was used to remove a proton from 6-position. The resulting electron-rich intermediate was reacted with some selected electrophiles, viz 2-(β-Chloroethyl) 1,3-dithiane; benzyl bromide; 2-(β-Iodoethyl) 1,3-dithiane; and ethyl ethoxymethylene malonate. The substituted pyrazine ring derivatives can be cleaved by acid hydrolysis to yeild optically active non-proteinogenic amino acid derivatives which can be used in the synthesis of biologically active peptides e.g. as enzyme inhibitors or pharmaceuticals.

Substitution of N-alkylated-5-chloro-2-one-pyrimidine derivatives at position 4 with amino acids would yield biologically interesting molecules. Thus, S-methyl-thiouronium sulphate was coupled with mucochloric acid to yield 5-chloro-2-methylthiopyrimidyl-4-carboxylic acid which was converted to its acid chloride by reaction with thionyl chloride. The acid chloride was the active coupling intermediate with carboxyl protected amino acid derivatives. The S-methyl

group of the resulting derivatives was converted to sulfone by m-chloro-perbenzoic acid. Base hydrolysis of the sulfones yield the corresponding pyrimidones. Crude Methyl N-(5-chloro-pyrimidine-2-one-4-yl-carbonyl) glycinate and crude Di-t-Butyl N-(5-chloro-pyrimidine-2-one-4-yl-carbonyl) glutamate were synthesized. An attempt was made to N-alkylate the former pyrimidine-2-one derivative with methyl and benzyl groups. Also an attempt was made to N-alkylate the latter pyrimidine-2-one derivative with methyl iodide via the DMF/K salt method.

Two potential carboxamide protecting group precursors viz, 4-methoxy-2-methylbenzylamine and 3-methylbenzyhydrylamine were synthesized. These groups were used to prepare the carboxamide protected derivatives viz, α-Benzyl tert butyloxycarbonyl-N^{CA}-4-methoxy-2-methylbenzyl-glutaminate; β-Benzyl tert-butyloxycarbonyl-N^{CA}-4-methoxy-2-methyl-benzyl-isoasparaginate; α-Benzyl tert-butyloxycarbonyl-N^{CA}-3-methylbenzhydrylglutaminate; and β-Benzyl tert-butyloxycarbonyl-N^{CA}-3-methylbenzhydrylisoasparaginate. These four derivatives were subjected to cleavage studies in trifluoroacetic acid-dichloromethane-anisole mixture (50 : 48 : 2). In addition, the former two derivatives were subjected to cleavage studies in borontrifluoride complex with acetic acid

(36%, $BF_3.2CH_3COOH$). It was found that in the former two derivatives the 4-methoxy-2-methylbenzyl group was stable to TFA-CH_2Cl_2- anisole treatment at room temperature for 24 hours. In the latter two derivatives the 3-methylbenzhydryl group was partially cleaved after 24 hours. When Boc-Gln(4-MeO, 2-MeBzl)-OBzl and Boc-Asn(4MeO, 2MeBzl)-β-OBzl were each treated with borontrifluoride complex with acetic acid at room temperature, it was found that the 4-methoxy-2-methylbenzyl group was completely removed from the former compound after five hours and from the latter compound only three hours. Thus, it was concluded that 4-methoxy-2-methylbenzyl is potentially a good carboxamide protecting group for the side-chain of Glutamine and Isoasparagine in peptide synthesis, while the 3-methylbenzhydryl is unsuitable.