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

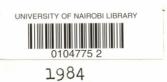
Ву

BHALENDU MANSHUKHLAL BHATT

BSc. (makerere), MSc. (Nairobi), NORAD dip. (Oslo), ADCS. (East Anglia).

UNIVERSITY OF NAIROD

A thesis submitted in fulfilment for the Degree of Doctor of Philosophy in the University of Nairobi.



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 Some model compounds were synthesized viz, carbobenzoxy-β-cyano-L-alanine; benzyl carbobenzoxy- β -cyano-L-alanyl-alaninate, $Z-\beta$ -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-\beta-ethyl alanylalanine methyl ester imidate hydrochloride; Z- β -benzyl-alanylalanine benzyl ester imidate hydrochloride were synthesized. Z-\beta-benzyl alanylalanine benzyl ester imidate hydrochloride was hydrogenated by hydrogen with 5% Pd/C at room temperature and atmospheric pressure to cleave the Zgroup. 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 triethyloxoniumtetra-fluoroborate 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 $2-(\beta-\text{Chloroethyl})$ 1,3-dithiane; benzyl bromide; $2-(\beta-\text{Chloroethyl})$ 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%, BF3.2CH3COOH). It was found that in the former two derivatives the 4-methoxy-2-methylbenzyl group was stable to TFA-CH2Cl2- 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)-\$-OBzl were each treated with borontrifluoride complex with acetic acid at room temperature, it was found that the 4methoxy-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 sidechain of Glutamine and Isoasparagine in peptide synthesis, while the 3-methylbenzhydryl is unsuitable.