Bull. Chem. Soc. Ethiop. **1998**, 12(1), 35-43.
Printed in Ethiopia
ISSN 1011-3294

APPLICATION OF TETRALINYLS AS CARBOXAMIDE PROTECTING GROUPS IN PEPTIDE SYNTHESIS

Peter M. Gitu, Amir O. Yusuf and Bhalendu M. Bhatt*

Department of Chemistry, University of Nairobi, P. O. Box 30197, Nairobi, Kenya

(Received November 19, 1997)

ABSTRACT. 1-Tetralinylamines were used as precursors to prepare the carboxamide protected derivatives of asparagine (lb-5b) and glutamine (la-5a). The carboxamide protected derivatives were then subjected to cleavage studies in trifluoroacetic acid-dichloromethane-anisole at 25 °C. All the glutamine carboxamide protecting groups were completely removed within 24 hours, however, only 5-methoxy-l,2,3,4-tetrahydro-1-naphthyl and 6-methoxy-1,2,3,4-tetrahydro-1-naphthyl groups of the asparagine derivatives were removed. Boron tristrifluoroacetate (BTFA)-trifluoroacetic acid (TFA) removed all the carboxamide protecting groups in glutamine and asparagine derivatives within 24 hours. These asparagine derivatives whose protecting groups were not removed by TFA were then used to synthesize the dipeptides and tripeptides.

INTRODUCTION

A number of biologically active peptides contain in their skeletons glutamine and/or asparagine amino acid residues and other amino acids having amide groups at the carboxyl end of the peptide. In synthesizing peptides containing glutamine and asparagine, it is important to protect carboxamide side chains of these residues. Without protection, the amide groups of asparagine and glutamine undergo the following side reactions during peptide synthesis [1, 2]:- (a) deamination to the corresponding acid, (b) formation of imides and subsequent hydrolysis, (c) formation of pyroglutamyl derivatives from glutaminyl peptides, and (d) dehydration to the cyano group.

In order to improve the synthesis of peptides with asparagine and glutamine residues, various carboxamide protecting groups have been studied. These include, *p*-methoxybenzyl [3], 2,4-dimethoxybenzyl [4], 4,4'-dimethoxybenzhydryl [5,6], 2,4,6-trimethoxybenzyl [7, 8], xanthenyl [9], 4-methoxy-2-methylbenzyl [10], 2,2,4,4-tetramethoxybenzhydryl [11], benzhydryl [12], and trityl [13]. Although these groups have been successfully used in preparing dipepetides, only benzyldryl group has been used in preparing longer peptides, where several amino-deprotecting and peptide bond formation steps have been successfully carried out in the presence of carboxamide groups fully protected with benzhydryl group.

A good protecting group prevents side reactions, racemization and increases solubility of the protected amide. Generally a good carboxamide protecting group should be stable in TFA/dichloromethane [14] and HCl/dioxane [15] (reagents commonly used for removal of amino protecting groups) and should be readily removed with strong cleavage reagents like BTFA [12, 16] and HF [17-19] which are used for complete removal of most of the protecting groups at the end of peptide synthesis. Optical activity [20] of the peptide or amino acid should be maintaned during the introduction and removal of t he protecting groups. The protecting group that is stable in TFA/dichloro-

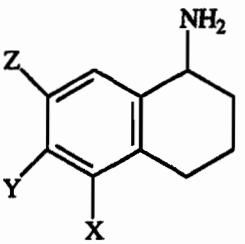
methane cleavage after 24 hours but readily cleaved by BTFA/TFA will be a potential carboxamide protecting group in peptide synthesis.

RESULTS AND DISCUSSION

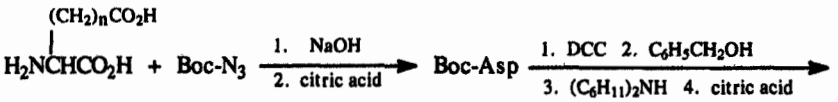
1-Tetralinylamines used as precursors to prepare the carboxamide derivatives of asparagine and glutamine are shown in Table 1:

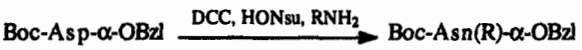
Table 1. Summary of 1-tetralinyl amines

Amines	Aromatic ring		
	X	Y	Z
1	H	H	H
2	OCH_3	H	H
3	H	OCH_3	H
4	H	H	OCH_3
5	CH_3	H	CH_3



Amines were reacted with Boc-Asp-α-OBzl in the presence of DCC to give Boc-Asn(R)-α-OBzl and Boc-Gln(R)-α-OBzl, respectively as shown in Scheme 1. The yields ranged from 40-89%.

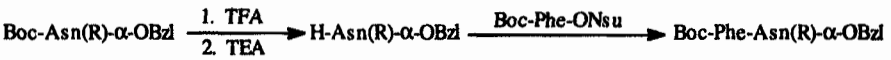




$n = 1$ asparatic acid or asparagine derivatives, $n = 2$ glutamic acid or glutamine derivatives.

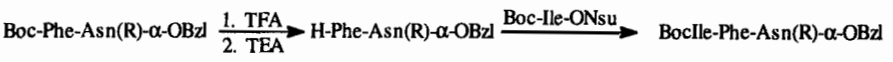
Scheme 1. Synthesis of carboxamide protected asparagine and glutamine derivatives.

The carboxamide protecting groups in asparagine derivatives **1b**, **4b**, **4c** and **5b** were found suitable as protecting groups [14]. These derivatives were used in synthesizing dipeptides **6a**, **6b**, **6c** and **6d**, respectively. This was done by deprotecting Boc group of the carboxamide derivatives followed by coupling, with N-hydroxysuccinimide ester of N-protected amino acid as shown in Scheme 2.



Scheme 2. Dipeptide synthesis from carboxamide protected derivative.

These dipeptides were used in synthesizing tripeptides **7a**, **7b** and **7c** as shown in Scheme 3. Dipeptide yields ranged from 76-90%, while tripeptides were 70-78%. The structures of the compounds were confirmed by standard spectroscopic methods and elemental analyses.

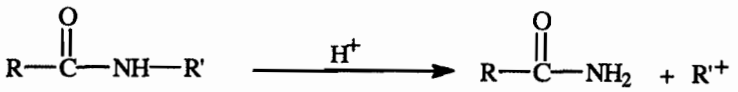


TEA = triethylamine, R = carboxamide protecting group in **1b**, **4b**, **4c** and **5b**

Scheme 3. Tripeptide synthesis from fully protected dipeptide

In order to improve peptide synthesis efficiency, one can easily make use of these protecting groups in solid-phase peptide synthesis (SPPS). This method eliminates all laborious purification at intermediate steps, and simple washing and filteration of the solid is substituted. When t-butyloxycarbonyl (Boc) is in combination with benzyl ester bond, one will be able to synthesize peptides by both DCC and active ester coupling. Boc group could be removed with a mild acid (TFA) with essentially no loss of the anchoring benzyl ester and the carboxamide protecting groups. HF will then be able to cleave Boc, carboxamide protecting group and also detach peptide from the resin. In the case when base labile fluorenylmethoxycarbonyl (Fmoc) is combined with t-butyl esters, TFA cleaves both t-butyl and ester linkage to the support. If at all tetralinyl carboxamide protecting groups can be utilized in this type of synthesis, then only the TFA labile carboxamide protecting groups will be promising.

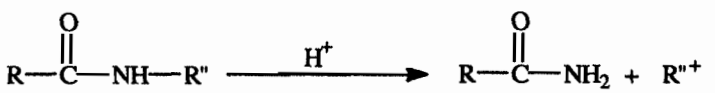
The ease with which the carboxamide protecting group is removed will in theory depend on the stabilization of the carbenium ion formed, by either inductive or resonance effect of the substituent group of the tetralinyl group. The unsubstituted 1-tetralinyl group will give a secondary carbenium ion which is stabilized by the delocalization process and the inductive effect of alkyl ring (Scheme 4).



R is glutamic or aspartic acid residue, R' is tetralinyl group.

Scheme 4. Cleavage of 1-tetralinyl group.

When an electron donating group such as methyl or methoxy group is attached on positions 5, 6, 7 or 8 of the 1-tetralinyl group, the carbenium ion formed is more stable than the unsubstituted 1-tetralinyl group. These groups would then be more readily removed under acidic medium than unsubstituted l-tetralinyl group. When the electron donating group is on position 6 or 8, the carbenium ion would be even more stabilized. Hence the cleavage would take place more readily. Thus after cleavage the delocalized structures will at one stage have the carbon bearing the positive charge attached to the electron donating group as shown in Scheme 5.



R is glutamic or aspartic acid residue, R" is tetralinyl group.

Scheme 5. Cleavage of 1-tetralinyl group containing a substituent at position 6.

Cleavage studies on the glutamine carboxamide protected derivatives showed that all the protecting groups were removed by TFA-dichloromethane-anisole at room temperature within 24 hours. With the asparagine carboxamide protected derivatives, only 5-methoxy-1,2,3,4-tetrahydro-1-naphthyl and 6-methoxy- 1 ,2,3,4-tetrahydro-1-naphthyl were completely removed by TFA-dichloromethane-anisole within 24 hours (Table 2). The former was partially cleaved in 24 hours while the latter was completely removed within 24 hours. The groups 1,2,3,4-tetrahydro-1-naphthyl, 7-methoxy-1,2,3,4-tetrahydro-l-naphthyl and 5,7-dimethyl-1,2,3,4-tetrahydro-1-naphthyl groups were completely stable in TFA-dichloromethane-anisole, but were completely removed by BTFA-TFA (Table 3). They were completely cleaved after 4, 3, and 3 hours, respectively. These three groups, are therefore potentially suitable for protecting carboxamide group of asparagine.

Table 2. TFA-dichloromethane-anisole (50:48:2 v/v) cleavage studies of the carboxamide protecting groups of asparagine and glutamine.

Compound	Extent of carboxamide protecting group removal after 24 hours
Boc-Gln(R_1)-α-OBzl (**1a**)	Partial
Boc-Asn(R_1)-β-OBzl (**1b**)	Not removed
Boc-Gln(R_2)-α-OBzl (**2a**)	Partial
Boc-Asn(R_2)-β-OBzl (**2b**)	Partial
Boc-Gln(R_3)-α-OBzl (**3a**)	Complete removal
Boc-Asn(R_3)-β-OBzl (**3b**)	Complete removal
Boc-Gln(R_4)-α-OBzl (**4a**)	Partial
Boc-Asn(R_4)-β-OBzl (**4c**)	Not removed
Boc-Gln(R_5)-α-OBzl (**5a**)	Partial
Boc-Asn(R_5)-β-OBzl (**5b**)	Not removed

R_1 = 1,2,3,4-tetrahydro-1-naphthyl; R_4 = 7-methoxy-1,2,3,4-tetrahydro-1-naphthyl; R_5 = 5,7-dimethyl-1,2,3,4-tetrahydro-1-naphthyl.

Table 3: BTFA studies of carboxamide protecting groups of asparagine.

Compound	Duration of complete removal of carboxamide protecting groups (hours)
Boc-Asn(R_1)-β-OBzl (**1b**)	4
Boc-Asn(R_4)-β-OBzl (**4b**)	3
Boc-Asn(R_5)-β-OBzl (**5b**)	3

R_2 = 5-methoxy-1,2,3,4-tetrahydro-1-naphthyl; R_3 = 6-methoxy-1,2,3,4-tetrahydro-1-naphathyl.

EXPERIMENTAL

Ascending thin-layer chromatograms (TLC) were run on silica gel G60 with solvent systems chloroform-ethyl acetate (3:1 v/v) (A) and chloroform-methanol-glacial acetic acid (85:10:5 v/v) (B). Spots were revealed with iodine vapour and ninhydrin in solution (0.2 g ninhydrin in 100 mL acetone). Melting points (uncorrected in °C) were determined

in capillary tubes in a Gallenkamp melting point aparatus. The peptides were hydrolyzed in 6 N hydrochloric acid at 110 °C for 16 h. Cleavage solvents were TFA-dichloromethane-anisole (50:48:2 v/v) and BTFA-TFA (mixing 0.5 mL TFA and 0.5 mL of 1.0 M BTFA in TFA). Fully protected glutamine and asparagine derivatives were prepared from α-benzyl t-butyloxycarbonyl-aspartate or α-benzyl t-butyloxycarbonyl glutamate and the corresponding 1,2,3,4-tetrahydro-1-naphthyl derivatives [21]. Nuclear magnetic resonance spectra (NMR) were recorded using Perkin-Elmer R12B (60 MHz). All signals were expressed in ppm using tetramethylsilane as an internal standard. The following abbreviations are used: singlet (s), doublet (d), multiplet (m), trifluoroacetic acid (TFA), t-butyloxycarbonyl (Boc), asparagine (Asn), glutamine (Gln), isoleucine (Ile), phenylalanine (Phe), boron tristrifluoroacetate (BTFA), N,N-dimethylformamide (DMF). Infrared spectra were recorded using Pye-Unicam SP3-300 spectrophotometer. They were calibrated against polystyrene film and are expressed in cm^{-1}. Elemental analysis in percentage were done by Ilse Beetz Microanalytisches Laboratorium 8640 Kronach West Germany.

TFA-dichloromethane-anisole cleavages. Small quantity of the carboxamide protected glutaminate or asparaginate derivative was placed in a vessel. TFA-dichloromethane-anisole (2 mL) was added and the mixture was stirred at room temperature for 24 h. Small sample was withdrawn from the reaction vessel and subjected to thin layer chromatography at the start and after 1, 3, 7 and 24 h. Ninhydrin spray was used for detection. Solvent system was B.

BTFA-TFA cleavages. Small quantity of the carboxamide protected derivative was dissolved in 0.5 mL of TFA and 0.5 mL of 1.0 M BTFA in TFA was added. The mixture was stirred and a small sample withdrawn after 1, 2, 3 and 4 h and subjected to the thin layer chromatography. Detection was with ninhydrin. Solvent system was B.

Carboxamide protected derivatives

*α-Benzyl t-butyloxycarbonyl-N^{CA}-1,2,3,4-tetrahydro-1-naphthylglutaminate (**1a**).* A stirred mixture of 0.5 g (1.5 mmol) of Boc-Glu-α-OBzl and 0.26 g (2.25 mmol) of N-hydro xysuccinimide in 3 mL of dichloromethane was cooled to -5 °C. To this mixture was added 0.34 g (1.65 mmol) of DCC in 5 mL of dichloromethane, and the mixture stirred at -5 °C for 50 min. A solution of 0.24 g (1.65 mmol) of 1-aminotetralin was added and the mixture was stirred for an additional 50 min and at room temperature for 24 h. Acetic acid (0.075 mL) was added, the mixture was stirred for 15 min, and the dicyclohexylurea (DCHU) was filtered off and washed with three 3 mL portions of dicholoromethane. The solvents were removed on a rotary evaporator in vacuo and the residue dissolved in 5 mL of dichloromethane. Some insoluble crystals were filtered off. Chloroform (7 mL) was added to the filtrate, the solution was washed with three 9 mL portions of 5% aqueous citric acid, three 12 mL portions of 5% aqueous sodium bicarbonate, and five 15 mL portions of deionised water. The organic layer was dried over anhydrous sodium sulphate and the solvents removed on a rotary evaporator in vacuo. The semi-solid was dissolved in 6 mL of hot ethyl acetate, cooled to room temperature and filtered off. To the filtrate, a solution 24 mL of petroleum ether (b.p. 40-60 °C) was added and the mixture kept at 0-5 °C overnight. The precipitate was filtered, washed with three 5 mL portions of petroleum ether (40-60 °C)-ethyl acetate (4:1) and dried in vacuo to give the product. Yield 0.31 g (45%); m.p. 82-83 °C;

TLC, solvent system A, R_f 0.8; IR (nujol) 3310, 1740, 1680, 1630 cm^{-1}; NMR ($CDCl_3$) 7.45, (s, 5H), 7.3 (m, 4H), 6.0 (s, 1H), 5.4 (s, 1H), 5.25 (s, 2H), 4.3 (s, 1H), 2.85 (m, 3H), 2.28 (m, 4H), 1.96 (m, 4H), 1.52 (s, 9H). Elemental analysis calculated for $C_{27}H_{34}N_2O_5$: C, 69.49; H, 7.35; N, 6.01. Found: C, 69.51; H, 7.38; N, 6.02.

*β-Benzyl t-butyloxycarbonyl-N^{CA}-1,2,3,4-tetrahydro-1-naphthylisoasparaginate (**1b**).* This compound was prepared from 0.485 g (1.5 mmol) of β-benzyl t-butyloxy carbonylaspartate and 0.24 g (1.65 mmol) of **1** in the same manner as described for **1a**: yield. 0.6 g (89%); m.p. 124-125 °C; TLC, solvent system A, R_f 0.93; IR (KBr) 3290, 1730, 1680, 1645 cm^{-1}; NMR ($CDCl_3$) 7.35 (s, 5H), 7.15 (m, 4H), 6.7 (s, 1H), 5.6 (s, 1H), 5.15 (s, 2H), 4.5 (s, 1H), 1.8 (m, 4H), 1.34 (s, 9H). Elemental analysis calculated for $C_{26}H_{32}O_5N_2$: C, 68.99; H, 7.13; N, 6.19. Found C, 70.02; H, 7.1; N, 6.10.

*α-Benzyl t-butyloxycarbonyl-N-^{CA}-5-methoxy-1,2,3,4-tetrahydro-1-naphthylglutaminate(**2a**).* This compound was prepared from 0.5 g (1.5 mmol) of α-benzyl t-butyloxy carbonylglutamate and 0.29 g of **2** in the same manner as described for compound **1a**: yield 0.54 g (73%); m.p. 104-105 °C; TLC, solvent system A, R_f 0.83; IR (nujol) 3320, 1740, 1690, 1635 cm^{-1}; NMR ($CDCl_3$) 7.33 (s, 5H), 6.95 (m, 3H), 5.9 (s, 1H), 5.2 (s, 1H), 5.17 (s, 2H), 4.25 (s, 1H), 3.8 (s, 3H), 1.82 (m, 4H), 1.4 (s, 9H). Elemental analysis calculated for $C_{28}H_{36}N_2O_6$: C, 67.71; H, 7.31; N, 5.64. Found: C, 67.75; H, 7.33; N, 5.61.

*β-Benzyl t-butyloxycarbonyl-N^{CA}-5-methoxy-1,2,3,4-tetrahydro-1-naphthylisoasparaginate (**2b**).* This was prepared from 0.485 g (1.5 mmol) of β-benzyl t-butyloxycarbonyl aspartate and 0.29 g (1.65 mmol) of **2** in the same manner as described for **1a**: yield 0.52 g (72%); m.p. 60-62 °C; TLC, solvent system A, R_f 0.93; IR (nujol) 3310, 1720, 1680, 1645 cm^{-1}; NMR ($CDCl_3$) 7.33 (s, 5H) 6.9 (m, 3H), 5.69 (s, 1H), 4.9 (s, 1H), 5.12 (s, 2H), 4.6 (s, 1H), 3.79 (s, 3H), 1.8 (m, 4H), 1.39 (s, 9H). Elemental analysis calculated for $C_{27}H_{34}N_2O_6$: C, 67.19; H, 7.11; N, 5.81. Found: C, 67.17; H, 7.13; N, 5.81.

*α-Benzyl t-butyloxycarbonyl-N^{CA}-6-methoxy-1,2,3,4-tetrahydro-1-naphthylglutaminate (**3a**).* This was prepared from 0.5 g (1.5 mmol) of α-benzyl t-butyloxycarbonylglutamate and 0.29 g (1.65 mmol) of **3** in the same manner as described for **1a**: yield 0.56 g (76%); m.p. 91-92 °C; TLC, solvent system A, R_f 0.67; IR (KBr) 3310, 1730, 1680, 1630 cm^{-1}; NMR ($CDCl_3$) 7.34 (s, 5H), 7.1 (m, 1H), 6.7 (m, 2H), 5.8 (s, 1H), 5.0 (s, 1H), 5.19 (s, 2H), 4.25 (s, 1H), 3.77 (s, 3H), 1.82 (m, 4H), 1.4 (s, 9H). Elemental analysis calculated for $C_{28}H_{36}O_6N_2$: C, 67.71; H, 7.31; N, 5.64. Found: C, 67.69; H, 7.33; N, 5.67.

*β-Benzyl t-butyloxycarbonyl-N^{CA}-6-methoxy-1,2,3,4-tetrahydro-1-naphthylisoaspara-ginate (**3b**).* This was prepared from 0.485 g (1.5 mmol) of β-benzyl t-butyloxycarbonyl aspartate and 0.29 g (1.65 mmol) of **3** in the same manner as described for **1a**: yield 0.56 g (78%); m.p. 75-76 °C; TLC, solvent system A, R_f 0.73; IR (KBr) 3320, 1730, 1680, 1630 cm^{-1}; NMR ($CDCl_3$) 7.36 (s, 5H), 7.1 (m, 1H), 6.62 (m, 2H), 5.85 (s, 1H), 5.05 (s, 1H), 5.2 (s, 2H), 4.45 (s, 1H), 3.77 (s, 3H), 1.8 (m, 4H), 1.4 (s, 9H). Elemental analysis calculated for $C_{27}H_{34}O_6N_2$: C, 67.19; H, 7.11; N, 5.81. Found: C, 67.21; H, 7.13; N, 5.81.

*α-Benzyl t-butyloxycarbonyl-N^{CA}-7-methoxy-1,2,3,4-tetrahydro-1-naphthylglutaminate (**4a**).* This was prepared from 0.5 g (1.5 mmol) of α -benzyl t-butyloxycarbonylglutamate and 0.29 g (1.65 mmol) of **4** in the same manner as described for **1a**: yield 0.44 g (60%); m.p.

145-146 °C; TLC, solvent system A, R_f 0.68; IR (KBr) 3320, 1720, 1680, 1620 cm^{-1}; NMR ($CDCl_3$) 7.32 (s, 5H), 6.8 (m, 3H), 5.75 (s, 1H), 4.95 (s, 1H), 5.17 (s, 2H), 4.3 (s, 1H), 3.75 (s, 3H), 1.8 (m, 4H), 1.4 (s, 9H). Elemental analysis calculated for $C_{28}H_{36}O_6N_2$: C, 67.71; H, 7.31; N, 5.64. Found: C, 67.70; H, 7.32; N, 5.66.

α -Benzyl t-butyloxycarbonyl-N^{CA}-7-methoxy-1,2,3,4-tetrahydro-1-naphthylasparagin- ate as prepared from 0.485 g (1.5 mmol) of α -benzyl t-butyloxycarbonylaspartate and 0.29 g (1.65 mmol) of **4** in the same manner as described for **1a**: yield 0.45 g (63%); m.p. 85-86°C; TLC, solvent system A, R_f 0.75; IR (KBr) 3310, 1730, 1680, 1630 cm^{-1}; NMR ($CDCl_3$) 7.34 (s, 5H), 6.78 (m, 3H), 5.83 (s, 1H), 5.1 (s, 1H), 5.19 (s, 2H), 4.55 (s, 1H), 3.74 (s, 3H), 1.82 (m, 4H), 1.4 (s, 9H). Elemental analysis calculated for $C_{27}H_{34}O_6N_2$: C, 67.19; H, 7.11; N, 5.81. Found: C, 67.22; H, 7.10, N, 5.79.

β-Benzyl t-butyloxycarbonyl-N^{CA}-7-methoxy-1,2,3,4-tetrahydro-1-naphthylisoasparaginate (4c). This was prepared from 0.485 g (1.5 mmol) of β -benzyl t-butyloxycarbonylaspartate and 0.29 g (1.65 mmol) of **4** in the same manner as described for **1a**: yield 0.4 g (56%); m.p. 136-137 °C; TLC, solvent system A, R_f 0.90; IR (KBr) 3310, 1720, 1670, 1635 cm^{-1}; NMR ($CDCl_3$) 7.38 (s, 5H), 6.85 (m, 3H), 5.65 (s, 1H), 5.0 (s, 1H), 5.2 (s, 2H), 4.55 (s, 1H), 3.81 (s, 3H), 1.85 (m, 4H), 1.46 (s, 9H). Elemental analysis calculated for $C_{27}H_{34}O_6N_2$: C, 67.19; H, 7.11; N, 5.81. Found: C, 67.20; H, 7.12, N, 5.80.

α-Benzyl t-butyloxycarbonyl-N^{CA}-5,7-dimethyl-1,2,3,4-tetrahydro-1-naphthylglutaminate (5a). This was prepared from 0.5 g (1.5 mmol) of α-benzyl t-butyloxycarbonyl glutamate and 0.29 g (1.65 mmol) of **5** in the same manner as described for **1a**: yield 0.4 g (54%); m.p. 110-111 °C; TLC, solvent system A, R_f 0.84; IR (nujol) 3285, 1720, 1670, 1620 cm^{-1}; NMR ($CDCl_3$) 7.3 (s, 5H), 6.87 (s, 2H), 5.9 (s, 1H), 5.3 (s, 1H), 5.13 (s, 2H), 4.3 (s, 1H) 2.2 (d, 10H), 1.8 (m, 4H), 1.38 (s, 9H). Elemental analysis calculated for $C_{29}H_{38}O_5N_2$: C, 70.41; H, 7.75; N, 5.67. Found: C, 70.39; H, 7.73; N, 5.69.

β-Benzyl t-butyloxycarbonyl-N^{CA}-5,7-dimethyl-1,2,3,4-tetrahydro-1-naphthylisoasparagin-ate (5b). This was prepared from 0.485 g (1.5 mmol) of β-benzyl t-butyloxycarbonyl aspartate and 0.29 g (1.65 mmol) of **5** in the same manner as described for **1a**: yield 0.29 g (40%); m.p. 143-144 °C; TLC, solvent system A, R_f 0.94; IR (nujol) 3300, 1714, 1672, 1633 cm^{-1}; NMR ($CDCl_3$) 7.36 (s, 5H) 6.95 (s, 2H), 6.6 (s, 1H), 5.6 (s, 1H), 5.19 (s, 2H), 4.5 (s, 1H), 2.27 (s, 3H), 2.2 (s, 3H), 1.8 (m, 4H), 1.41 (s, 9H). Elemental analysis calculated for $C_{28}H_{36}O_5N_2$: C, 69.96; H, 7.56; N, 5.83. Found: C, 69.99; H, 7.59; N, 5.85.

Dipeptides

Boc-Phe-Asn(1,2,3,4-tetrahydro-1-naphthyl)-β-OBzl (6a). A solution of Boc-Asn (R_1)-β-OBzl (0.4 g, 0.88 mmol) in 6 mL of TFA and 0.5 ml anisole was stirred for 25 min at room temperature. TFA was then removed on a rotary evaporator in vacuo at 25-30 °C. 4 mL of DMF was then added to the above solution and pH was adjusted to 7 with triethylamine. To this solution was added Boc-Phe-ONsu (0.32 g, 0.88 mmol) and solution was stirred for 14 h at room temperature. Ethyl acetate (20 mL) was added and the solution washed with three 20 mL portions of 5% aqueous citric acid, two 20 mL portions of 5% aqueous sodium bicarbonate and five 15 mL portions of deionised water. The organic portion was dried over anhydrous sodium sulphate. The solvent was removed on a rotary evaporator in vacuo: yield

0.4 g (76%); m.p. 151-152 °C; TLC, solvent system A, R_f 0.76; IR (nujol) 3280, 1720, 1680, 1625 cm^{-1}; NMR ($CDCl_3$) 7.36 (s, 5H), 7.2 (s, 5H), 7.1 (s, 4H), 5.12 (s, 2H), 3.05 (m, 2H), 1.8 (m, 4H), 1.22 (s, 9H). Elemental analysis calculated for $C_{35}H_{41}O_6N_3$: C, 70.08; H, 6.89; N, 7.01. Found: C, 70.11; H, 6.89; N, 7.03.

Boc-Phe-Asn (7-methoxy-1,2,3,4,-tetrahydro-1-naphthyl)-α-OBzl ***(6b)***. This was prepared from 0.39 g (0.81 mmol) of **4b** and 0.29 g (0.81 mmol) of Boc-Phe-ONsu as described for **6a**: yield 0.46 g (90.2%); m.p. 165-166 °C; TLC, solvent system A, R_f 0.59; IR (KBr) 3290, 1735, 1680, 1630 cm^{-1}; NMR ($CDCl_3$) 7.37 (s, 5H), 7.22 (s, 5H), 6.8 (m, 3H), 5.22 (s, 2H), 3.7 (s, 3H), 1.75 (m, 4H) 1.37 (s, 9H). Elemental analysis calculated for $C_{36}H_{43}O_7N_3$: C, 68.65; H, 6.89; N, 6.68. Found: C, 68.66; H, 6.87; N, 6.65.

Boc-Phe-Asn (7-methoxy-1,2,3,4,-tetrahydro-1-naphthyl)-β-OBzl ***(6c)***. This was prepared from 0.39 g (0.81 mmol) of **4c** and 0.29 g (0.81 mmol) of Boc-Phe-ONsu as described for **6a**: yield 0.44 g (86%); m.p. 164-165 °C; TLC, solvent system A, R_f 0.72; IR (KBr) 3290, 1720, 1680, 1630 cm^{-1}; NMR ($CDCl_3$) 7.34 (s, 5H), 7.19 (s, 5H), 6.8 (m, 3H), 5.12 (s, 2H), 3.69 (s, 3H), 3.0 (m, 2H), 1.75 (m, 4H), 1.18 (s, 9H). Elemental analysis calculated for $C_{36}H_{43}O_7N_3$: C, 68.65; H, 6.89; N, 6.68. Found: C, 68.66; H, 6.91; N, 6.69.

Boc-Phe-Asn (5,7-dimethyl-1,2,3,4,-tetrahydro-1-naphthyl)-β-OBzl ***(6d)***. This was prepared from 0.39 g (0.81 mmol) of **5b** and 0.29 g (0.81 mmol) of Boc-Phe-ONsu as described for **6a**: yield 0.4 g (79%); m.p. 149-150 °C; TLC, solvent system A, R_f 0.77; IR (nujol) 3300, 1730, 1685, 1635 cm^{-1}; NMR ($CDCl_3$) 7.38 (s, 5H), 7.22 (s, 5H), 6.87 (s, 2H), 5.16 (s, 2H), 3.1 (m, 2H), 2.21 (s, 6H), 1,83 (m, 4H) 1.23 (s, 9H). Elemental analysis calculated for $C_{37}H_{45}O_6N_3$: C, 70.78; H, 7.23; N, 6.70. Found: C, 70.76; H, 7.20; N, 6.72.

Tripeptides

Boc-Ile-Phe-Asn(1,2,3,4-tetrahydro-1-naphthyl)-β-OBzl ***(7a)***. A solution of Boc-Phe-Asn(1,2,3,4-tetrahydrol-1-naphthyl)-β-OBzl (0.15 g, 0.25 mmol) in 3 mL TFA and 0.25 mL of anisole was stirred at room temperature for 25 min. The solvent was removed on a rotary evaporator in vacuo at room temperature. The oil residue was treated twice with 10 mL portions of ethyl ether, and the ether was evaporated to dryness each time. The white solid was dissolved in 10 mL of dichloromethane and the pH was adjusted to 7 with triethylamine. Boc-Ile-ONsu (0.08 g, 0.25 mmol) was added, and the mixture was stirred for 35 min at room temperature. The mixture was filtered and the solvent removed in vacuo on a rotary evaporator at room temperature. The residue was dissolved in 4 mL ethyl acetate and 20 mL of petroleum ether (b.p. 40-60 °C) was added and placed in the freezer overnight. The precipitate was filtered off and dried in vacuo to give a white crystalline product: yield 0.14 g (78%); m.p. 203-204 °C; TLC, solvent system A, R_f 0.73; IR (KBr) 3260, 1725, 1680, 1625; NMR ($CDCl_3$) 7.33 (s, 5H), 7.15 (s, 5H), 7.08 (s, 4H), 5.12 (s, 2H), 3.27 (m, 2H), 1.8 (m, 4H), 1.35 (s, 9H), 0.75 (m, 6H). Elemental analysis calculated for $C_{41}H_{52}O_7N_4$: C, 69.06; H, 7.36; N, 7.86. Found: C, 69.08; H, 7.35; N, 7.84.

Boc-Ile-Phe-Asn(7-methoxy-1,2,3,4-tetrahydro-1-naphthyl)-β-OBzl ***(7b)***. This was prepared from 0.16 g (0.25 mmol) of **6c** and 0.08 g (0.25 mmol) of Boc-Ile-ONsu as described for **7a**: yield 0.15 g (78%); m.p. 214-216 °C; TLC, solvent system A, R_f 0.33; IR (KBr) 3270, 1730, 1680, 1630 cm^{-1}; NMR ($CDCl_3$) 7.36 (s, 5H), 7.23 (s, 5H), 6.75 (m, 3H), 5.2 (s, 2H),

3.76 (s, 3H), 1.8 (m, 4H), 1.44 (s, 9H), 0.9 (m, 6H). Elemental analysis calculated for $C_{42}H_{54}O_8N_4$: C, 67.89; H, 7.33; N, 7.54. Found: C, 67.91; H, 7.36; N, 7.54.

Boc-Ile-Phe-Asn(5,7-dimethyl-1,2,3,4-tetrahydro-1-naphthyl)-β-OBzl ***(7c)***. This was prepared from 0.16 g (0.25 mmol) of **6d** and 0.08 g (0.25 mmol) of Boc-Ile-ONsu as described for **7a**: yield 0.13 g (70%); m.p. 202-203 °C; TLC, solvent system A, R_f 0.70; IR (KBr) 3260, 1720, 1670, 1625 cm^{-1}; NMR ($CDCl_3$) 7.35 (s, 5H), 7.17 (s, 5H) 6.83 (s, 2H), 5.15 (s, 2H), 2.17 (s, 6H), 1.84 (m, 4H), 1.38 (s, 9H), 0.75 (m, 6H). Elemental analysis calculated for $C_{43}H_{56}O_7N_4$: C, 69.69; H, 7.62; N, 7.56. Found: C, 69.71; H, 7.60; N, 7.59.

ACKNOWLEDGEMENTS

The authors thank the German Government for giving A.O. Yusuf a DAAD Scholarship and also the Department of Chemistry, University of Nairobi, for its support.

REFERENCES

1. Ressler, C.; Ratzkin, H. *J. Org. Chem.* **1961**, 26, 3356.
2. Battersby, A.R.; Robinson, J.C. *J. Chem. Soc.* **1955**, 259.
3. Pietta, P.G.; Marshall, G.R. *Chem. Comm.* **1970**, 650.
4. Pietta, P.G.; Cavallo, P. *J. Org. Chem.* **1971**, 36, 3966.
5. Geiger, R.; Konig, W.; Jager, G.; Siedel, W. *Peptides*, **1968**, E. Bricas (Ed.), North Holland Publishing Co., Amsterdam, 1968, pp 98-103.
6. Konig, W.; Geiger, R. *Chem. Ber.* **1970**, 103, 204.
7. Weygand, F.; Steglich, W.; Bjarnason, J.; Akhar, R.; Khan, M. *Tetrahedron Lett. (London)* **1966**, 3483.
8. Weygand, F.; Steglich, W.; Bjarnason, J.; Akhar, R.; Chytil, N. *Chem. Ber.* **1968**, 101, 3623.
9. Shimonishi, Y.; Sakakibara, S.; Akabori, S. *Bull. Chem. Soc. Jpn.* **1962**, 35, 1966.
10. Bhatt, B.M. *M.Sc. Thesis*, The University of Nairobi, **1978**, pp 51-58.
11. Juhasz, A.; Bajusz, S. *Acta Chim. Acad. Sci. Hung.* **1979**, 102, 289.
12. Gitu, P.M. *Ph.D Dissertation,* The University of Arizona, **1974**.
13. Sieber, P.; Riniker, B. *Tetrahedron Lett.* **1991**, 32, 739.
14. Gutte, B.; Merrifield, R.B. *J. Biol. Chem.* **1971**, 246, 1922.
15. Stewart, J.M.; Wooley, D.W. *Nature* **1955**, 20, 619.
16. Pless, J.; Bauer, W. *Angew. Chem. Internat. Ed.* **1973**, 12, 147.
17. Hruby, V.J.; Muscio, F.A.; Brown, W.; Gitu, P.M. *Chemistry and Biology of Peptides*, Meienhofer (Ed.), Ann Arbor Science Publishers, Inc., Ann Arbor, **1972,** pp 331-333.
18. Sakakibara, E.; Shimonishi, Y. *Bull. Chem. Soc. Jpn.* **1965**, 38, 1412.
19. Sakakibara, E.; Shimonishi, Y.; Kishida, Y.; Okada, M.; Sugihara, H. *Bull. Chem. Soc. Jpn.* **1967**, 40, 2164.
20. Schroder, E.; Lubke, K. *The Peptides,* Vol.1, Academic Press Inc. New York; 1966.
21. Yusuf, A.O. *M.Sc. Thesis*, The University of Nairobi, 1988.