Indian Journal of Chemistry
Vol. 37B, November 1998, pp. 1109-1113

Synthesis of peptides mediated by AgCN

V V Suresh Babu* & K Gayathri
Department of Chemistry, Central College, Bangalore University, Bangalore 560 001, India

Received 8 December 1997; accepted (revised) 5 October 1998

The acylation reactions employing Fmoc-amino acid chlorides have been carried out in the presence of AgCN. There is no addition of any base. The coupling is fast and racemization free. The work up and the isolation of products are easy. Thus the synthesis of several dipeptides, a model tetrapeptide, Leu-Ala-Gly-Val and β-casomorphin (Tyr-Pro-Phe-Pro-Gly) are accomplished.

Although the use of acid chlorides in peptide synthesis has long been regarded as obsolete,[1,2] 9-fluorenylmethoxycarbonyl (Fmoc)-amino acid chlorides now appear to serve as efficient coupling reagents for peptide bond formation following the stepwise strategy[3-7]. Fmoc-amino acid chlorides possessing benzyl group have been found to be stable for long period and can be easily prepared. They are shown to be useful in a novel method of rapid, repetitive peptide synthesis. Acylation reactions employing them have to be carried out in the presence of a base. A biphasic system like 5% Na_2CO_3 or 10% $NaHCO_3/CH_2Cl_2$ has been used for coupling in the solution phase. Instead, an organic base like diisopropylethylamine, triethylamine, pyridine or *N*-methylmorpholine has been tried but this leads to the conversion of the acid chloride to the corresponding oxazolone formation. Consequently, the coupling was carried out using a base and 1-hydroxybenzotriazole (HOBt). Alternatively, the acylation reactions using acid chlorides can also be accomplished in presence of potassium salt of HOBt[8].

It was reported[9] that sterically hindered esters can be prepared from their corresponding acyl chlorides and alcohols using silver cyanide as a catalyst which is superior to the conventional acyl chloride-alcohol-pyridine method as regards yield and the rapidity of reaction. Rich *et al.*[10], employed AgCN as a catalyst for the preparation of the sterically hindered Fmoc-N-MeVal-OBu^t using Fmoc-N-MeVal-Cl and *t*-butyl alcohol. Freidinger *et al*[11]., used AgCN as a co-coupling agent for the introduction of dehydropiperazic acid during the solid phase synthesis of cyclichexapeptide oxytocin agonists.

The present paper describes the coupling of Fmoc-amino acid chlorides in the presence of AgCN in solution phase. The general procedure for coupling involves the treatment of equimolar quantities of an amino acid ester/peptide ester with Fmoc-amino acid chloride and AgCN. The reaction mixture was refluxed with stirring at 60° and the progress of the reaction was monitored by TLC. The reaction was complete within 10-15 min. No other addition of base was required during coupling. Thus several dipeptide esters containing simple as well as sterically hindered amino acids have been prepared. The HPLC profile of crude dipeptide ester, Fmoc-Ala-Val-OBzl is given in Figure 1. The acylation reactions in the presence of AgCN as catalyst was found to significantly enhance the rate of coupling. It may be due to the increase in the electrophilicity of acid chloride. This method appears promising for the synthesis of peptides possessing steric hinderance in the amino component (Table I).

The ^{1}H NMR[12] spectra of Tos-Phg-Ala-OMe [δ 3.50 (s, OCH_3)] and Tos-D-Phg-Ala-OMe [δ 3.61 (s, OCH_3)], prepared by this method, demonstrated that the coupling is free from racemization. Further this method is used for the synthesis of peptides for extending the chain from C-terminal to N-terminal by following the stepwise strategy. Thus the synthesis of the model tetrapeptide, Leu-Ala-Gly-Val and the opioid pentapeptide β-casomorphin (Tyr-Pro-Phe-Pro-Gly) have been accomplished (Scheme I). The protected tetrapeptide Fmoc-Leu-

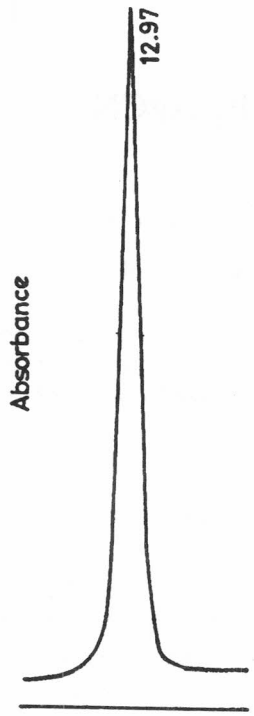


Figure 1—Analytical RP-HPLC of Fmoc-Ala-Val-OBzl; Deltapak C-18 column (3.9 mm × 300 mm was used); flowrate: 0.40 ml/min; UV monitoring at 220 nm; eluant: isocratic 65% methanol in water.

Ala-Gly-Val-OBzl and the protected pentapeptide β-casomorphin [Fmoc-Tyr(Bzl)-Pro-Phe-Pro-Gly-Obzl] were converted to free peptides by catalytic transfer hydrogenation (CTH) using Pd black/85% formic acid. The purity of the free peptides was checked by HPLC (Figures 2 and 3). An yield of 85% of the tetrapeptide and 50% of β-casomorphin was obtained.However, several attempts made to couple Fmoc-Sar-Cl with Sar-OMe/OBzl were unsuccessful. It resulted in less yield and pure crystalline peptides could not be isolated.

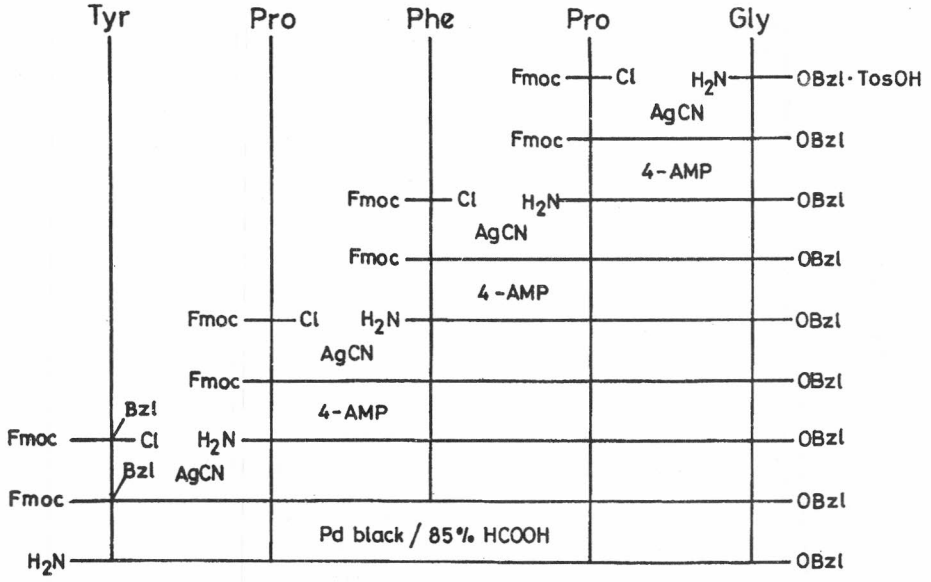
Experimental Section

All amino acids used except Gly and Sar, have the L-configuration. Melting points were determined using a Leitz-Wetzlar apparatus and are uncorrected. TLC analysis was carried out using the precoated silica gel G plates using i) $CHCl_3$: methanol : acetic acid :: 40:2:1; ii) $CHCl_3$: methanol :: 9:1; iii) *n*-butanol : acetic acid : water :: 4:1:1; iv) *n*-butanol : acetic acid : water :: 4:1:5 and the R_f values are designated as R_f A, R_f B, R_f C and R_f D respectively. Elemental analysis was carried out after drying the compounds for 24 hr at 50° *in vacuo*. Amino acid analysis was carried out with a Waters HPLC system using a PICO-TAG column (3.9 mm × 30 cm) after hydrolysing the

Table I—Protected dipeptide esters

Peptide *	Yield %	M.p. (°C)	R_f values		$[\alpha]^{25}_D$	Mol. formula	Found (Calc) %		
			R_fA	R_fB			C	H	N
Fmoc-Ala-Val-OBzl	80	112-15	0.56	0.77	+ 18.5 (c 1, CH_2Cl_2)	$C_{30}H_{32}N_2O_5$	72.10 (72.00)	6.3 (6.4)	5.70 (5.6)
Fmoc-Phe-Leu-OBzl	85	155-57	0.82	0.71	+ 24 (c 1, $CHCl_3$)	$C_{37}H_{38}N_2O_5$	75.12 (75.25)	6.51 (6.44)	4.66 (4.74)
Fmoc-Leu-Val-OBzl	75	125-28	0.78	0.63	+ 11.8 (c 0.5, $CHCl_3$)	$C_{33}H_{38}N_2O_5$	74.10 (73.06)	6.8 (7.0)	5.20 (5.16)
Tos-Phg-Ala-OCH_3	70	201-03	0.56	0.68	+ 60.0 (c 1, $CHCl_3$)	$C_{19}H_{22}N_2O_5S$	58.20 (58.46)	5.10 (5.64)	7.40 (7.1)
Tos-D-Phg-Ala-OCH_3	75	174-76	0.55	0.66	- 64.0 (c 1, $CHCl_3$)	$C_{19}H_{22}N_2O_5S$	58.20 (58.46)	5.10 (5.64)	7.40 (7.1)
Fmoc-N-Pgy-Sar-OBzl	60	142-45	0.45	0.68	+ 20.2 (c 1, $CHCl_3$)	$C_{33}H_{30}N_2O_5$	73.80 (74.15)	5.70 (5.61)	5.40 (5.2)
Fmoc-Ile-Sar-OBzl	65	Gum	0.50	0.72	-52.6 (c 0.5, $CHCl_3$)	$C_{31}H_{34}N_2O_5$	72.12 (72.33)	6.80 (6.61)	5.30 (5.44)
Fmoc-Sar-Ile-OBzl	60	Gum	0.47	0.70	- 42.4 (c 0.5, $CHCl_3$)	$C_{31}H_{34}N_2O_5$	72.12 (72.33)	6.70 (6.61)	5.20 (5.44)
Fmoc-Gly-Val-OBzl	80	184-86	0.52	0.68	- 20.9 (c 1, $CHCl_3$)	$C_{29}H_{30}N_2O_5$	76.20 (76.54)	6.10 (6.17)	6.20 (5.76)
Fmoc-Pro-Gly-OBzl	75	75	0.58	0.74	+ 27.7 (c 1, CH_2Cl_2)	$C_{29}H_{28}N_2O_5$	72.00 (71.9)	6.1 (5.9)	5.60 (5.7)

*Abbreviations used for amino acids are in accordance with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature published in 'Pure and Applied Chemistry', 40, **1974**, 314. Additional ones used are Sar, sarcosine; Phg, α-aminophenylacetic acid (α-phenylglycine); N-Pgy, N-phenylglycine.



Scheme I—Schematic representation of the synthesis of β-casomorphin using appropriate Fmoc-amino acid chloride/AgCN in toluene medium.

peptides using 6 *N* HCl at 110° for 24 hr. Optical rotations were measured with an automatic digital AA-10 polarimeter (Optical Activity, U.K.). The HPLC system (Waters) consisted of two pumps (model 501), 484 tunable UV detector and a 820 peak integration system.

Synthesis of peptides using Fmoc-amino acid chlorides/AgCN : General procedure. To a solution of amino acid ester/peptide acid ester (1 mmole) in toluene (3 mL) was added a solution of Fmoc-amino acid chloride (1 mmole) and AgCN (1 mmole) in toluene (3 mL) and the mixture was refluxed with stirring for 10-15 min at 60°C. The mixture was then washed thrice with 15 mL portions of 5% $NaHCO_3$, 5% HCl, water, saturated NaCl solution and dried over Na_2SO_4. The organic layer was evaporated *in vacuo* and recrystallisation of oily residue from suitable solvent gave the product in good yield and purity.

General procedure for deprotection of Fmoc-group using 4-aminomethylpiperidine (4-AMP). A solution of the Fmoc-N-protected peptide ester (0.75 mmole) in CH_2Cl_2 (5 mL) was treated with 4-AMP (5 mL). After 30 min (completion of deprotection was determined by TLC), CH_2Cl_2 (30 mL) was added and the solution was washed with phosphate buffer (*p*H 5.5, prepared from 90 g of $NaH_2PO_4.H_2O$ and 32.7 g of Na_2HPO_4 in 500 mL

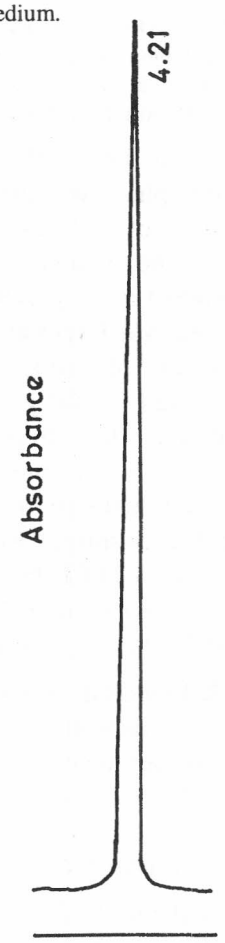


Figure 2—HPLC profile of Leu-Ala-Gla-Val [Waters C_{18} deltapak column (3.9 mm × 30 cm, 15 μ, spherical); flow rate: 0.8 ml/min; UV monitoring at 210 nm; eluant: 60% CH_3CN-H_2O (0.1% trifluoroacetic acid)].

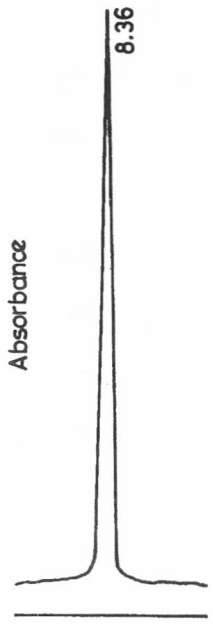


Figure 3—Analytical RP-HPLC of pure β-casomorphin: waters C-18 deltapak column (3.9 mm × 300 mm, 15μ); flow rate: 1ml/min; eluant A: 0.1% TFA in H_2O, B: 0.1% TFA in CH_3CN; linear gradient 15-40% B in 20 min.

of distilled water) and then with saturated NaCl solution. The organic phase was dried over anhyd. Na_2SO_4. Evaporation of solvent *in vacuo* and recrystallisation of the residue using suitable solvent gave the product in good yield.

Synthesis of Leu-Ala-Gly-Val : Fmoc-Gly-Val-OBzl 1. A mixture of valine benzyl ester *p*-toluenesulfonic acid salt (0.800 g, 2 mmole) in 6 mL of toluene and NEt_3 (few drops) was coupled with Fmoc-Gly-Cl (0.636 g, 2 mmole) and AgCN (0.266 g, 2 mmole) in toluene (6 mL) following the general procedure for coupling. Recrystallization of the peptide using CH_2Cl_2-hexane gave the product, yield 0.820 g (80%), m.p. 184-86°C; R_fA, 0.52; R_fB, 0.72; $[\alpha]^{25}_D$ -20.9° (c 1, $CHCl_3$).

Gly-Val-OBzl 2. Fmoc-Gly-Val-OBzl (1, 0.729 g, 1.5 mmole) was N-deprotected following the general procedure for deprotection of Fmoc group using 4-AMP (10 mL) to yield 0.340 g (86%) of the free peptide **2**, m.p. 202-205°C; R_fC, 0.50; R_fD, 0.38; $[\alpha]^{25}_D$ -12.8° (c 1, $CHCl_3$).

Fmoc-Ala-Gly-Val-OBzl 3. A solution of Fmoc-Ala-Cl (0.395 g, 1.2 mmole) and AgCN (0.160 g, 1.2 mmole) in toluene (4 mL) was coupled with peptide 3 (0.316 g, 1.2 mmole) in toluene (4 mL) to yield the peptide 3, yield 0.490 g (73%), m.p. 140-42°C; R_fA, 0.45 ; R_fB, 0.68; $[\alpha]^{25}_D$ + 33.50 (c 1, CH_2Cl_2).

Ala-Gly-Val-OBzl 4. Fmoc-Ala-Gly-Val-OBzl (3, 0.450 g, 0.9 mmole) was N-deprotected using 4-AMP (6 mL) to get the title peptide **4**, yield 0.220 g (81%), m.p. 105-108°C; R_fC, 0.33; R_fD, 0.25; $[\alpha]^{25}_D$ -26.40 (c 0.5, $CHCl_3$).

Fmoc-Leu-Ala-Gly-Val-OBzl 5. Peptide 5 (0.168 g, 0.5 mmole) in toluene (2 mL) was coupled with Fmoc-Leu-Cl (0.185 g, 0.5 mmole) and AgCN (0.101 mg, 0.76 mmole) in toluene (2 mL) to yield 0.220 g (66%) of the title peptide **5**, m.p. 130-32°C; R_fC, 0.43; R_fD, 0.75; $[\alpha]^{25}_D$ + 40° (c 1, $CHCl_3$).

Leu-Ala-Gly-Val 6. Fmoc and benzyl groups in peptide **5** were removed by CTH. To a solution of the protected peptide **5** (0.1 g, 1 mmole) in anhyd. methanol (0.5 mL), 85% HCOOH (1.0 mL) and palladium black (0.07 g) were added and stirred at room temperature for 3 hr. The completion of the reaction was monitored by TLC. The catalyst was filtered off and washed with hot methanol. The combined filtrate was evaporated *in vacuo* and the residue after trituration with ether to dissolve 9-methylfluorene, was precipitated using methanol-ether to obtain the peptide salt, Leu-Ala-Gly-Val. HCOOH, yield 0.06 g (85%). The peptide salt was then stirred with water (1 mL) and aq. $NaHCO_3$ (2%, 5 mL) for 20 min and extracted with EtOAc (3 × 40 mL). The organic layer was evaporated *in vacuo* to get the free tetrapeptide **6**, m.p. 142-43°C; $[\alpha]^{25}_D$ + 22.5° (c 1, ethanol), R_fC, 0.33; R_fD, 0.35 [Reported[13], m.p. 140-43°C; $[\alpha]^{25}_D$ + 22° (c 1, ethanol); R_fC, 0.32; R_fD, 0.36]. Anal. Found : C, 53.42; H, 8.2; N, 15.82. Calc. for $C_{16}H_{30}N_4O_5$ (358.72) : C, 53.57; H, 8.43; N, 15.69%. Amino acid analysis : (Calc.), Found : Leu (1) 0.98, Ala (1) 0.96, Gly (1) 0.96, Val (1) 1.01.

Synthesis of β-casomorphin : Fmoc-Pro-Gly-OBzl 7. A mixture of glycine benzyl ester *p*-toluene sulfonic acid salt (1.071 g, 3 mmole) in toluene (9 mL) and NEt_3 (few drops) was reacted with Fmoc-Pro-Cl (1.068 g, 3 mmole) and AgCN (0.399 g) in toluene (9 mL) to get the dipeptide **7**, yield 1.050 g (72%), m.p. 60-62°C; R_fA, 0.58; R_fB, 0.74; $[\alpha]^{25}_D$ + 27.7° (c 1, CH_2Cl_2).

Pro-Gly-OBzl (8). Following the general procedure for deprotection, compound **7** (0.900 g, 1.8 mmole) was N-deprotected using 4-AMP (12

mL) to yield 0.420 g (86%) of the title peptide **8**, m.p. 108-10°C; R_fA, 0.34; R_fB, 0.44; $[\alpha]^{25}_D$ - 60.5° (c 0.5, $CHCl_3$).

Fmoc-Phe-Pro-Gly-OBzl 9. A solution of Fmoc-Phe-Cl (0.630 g, 1.5 mmole) and AgCN (0.198 g, 1.5 mmole) in toluene (6 mL) was reacted with peptide **8** (0.414 g, 1.6 mmole) in toluene (6 mL) to yield the peptide **9**, yield 0.630 g (64%), m.p. 146-48°C; $[\alpha]^{25}_D$ - 41.2° (c 1, $CHCl_3$).

Phe-Pro-Gly-OBzl 10. The cleavage of Fmoc group from peptide **9** (0.471 g, 0.75 mmole) using 4-AMP (6 mL) led to 0.255 g (84%) of the peptide **10**, m.p. 188-90°C; R_fA, 0.27; R_fB, 0.34; $[\alpha]^{25}_D$ - 30.8° (c 1, $CHCl_3$).

Fmoc-Pro-Phe-Pro-Gly-OBzl 11. A solution of Fmoc-Pro-Cl (0.480 g, 1.35 mmole) and AgCN (0.198 g, 1.5 mmole) in toluene (6 mL) was reacted with pepide 10 (0.552 g, 1.35 mmole) in toluene (6 mL) to yield 0.780 g (79%) of peptide **11**, m.p. 120°C; R_fA, 0.59; R_fB, 0.72; $[\alpha]^{25}_D$ - 60.2 (c 1, $CHCl_3$).

Pro-Phe-Pro-Gly-OBzl 12. The cleavage of Fmoc-group from the peptide **11** (0.435 g, 0.6 mmole) by using 4-AMP (3 mL) led to 0.240 g (80%) of the peptide **12**, m.p. 175-77°C; R_fA, 0.29; R_fB, 0.38.

Fmoc-Tyr(Bzl)-Pro-Phe-Pro-Gly-OBzl 13. A solution of Fmoc-Tyr(Bzl)-Cl (0.16 g, 0.3 mmole) and AgCN (0.039 g) in toluene (3 mL) was reacted with peptide **12** (0.150 g, 0.3 mmole) in toluene (6 mL) to yield 0.213 g (72%) of the peptide **13**, m.p. 160-61°C; R_fA, 0.60; R_fB, 0.73; $[\alpha]^{25}_D$ - 80 (c 0.5, $CHCl_3$).

Tyr-Pro-Phe-Pro-Gly 14. The N-protected peptide ester **13** (0.200 g, 0.2 mmole) was subjected to CTH using palladium black (0.140 g) and 85% HCOOH (2 mL) in anhyd. MeOH (1 mL). After workup, the free pentapeptide Tyr-Pro-Phe-Pro-Gly was isolated, yield 0.600 g (50%), m.p. 151-53°; $[\alpha]^{25}_D$ - 48.5° (c 0.5, dimethylformamide), R_fC, 0.40 [(Reported[16] m.p. 152-54°; $[\alpha]^{25}_D$ - 48° (c 0.5, dimethylformamide); R_fC, 0.42]. Anal. Found: C, 62.69; H 6.02; N, 12.28. Calc. For $C_{30}H_{35}N_5O_7$ (578) : C, 62.34; H, 6.10; N, 12.17%. Amino acid analysis : (Calc.) Found : Gly(1) 1.00; Pro (2) 2.12; Phe (1) 0.98; Tyr(1) 0.97. The synthetic peptide exhibited the biological activities similar to that of the natural molecule.

Acknowledgement

This work is partly supported under UGC-DRS programme. We thank Prof. K M Sivanandaiah for useful discussions and Prof B S Seshadri, Chairman, Department of Chemistry, Central College, Bangalore, for providing facilities.

References

1 Jones J H, *The peptides,* Vol 1, edited by E Gross & J Meinehofer (Academic Press, New York), **1979**, 65.
2 Gutte B, Peptide synthesis, structures and applications (Academic Press, San Diego), **1995**.
3 Carpino L A, Cohen B J, Stephens K K, Sadat-Aalaee S, Tein J H & Langridge D C, *J Org Chem*, 51, **1986**, 3732.
4 Beyermann M, Bienert M, Niedrich H, Carpino L A & Sadat Aalaee D, *J Org Chem*, 55, **1990**, 721.
5 Carpino L A, Chao H G, Beyermann M & Bienert M, *J Org Chem*, 56, **1991**, 2635.
6 Sivanandaiah K M, Suresh Babu V V & Renukeshwar H C, *Int J Peptide Protein Res*, 39, **1992**, 201.
7 Sivanandaiah K M, Suresh Babu V V, Renukeshwar H C & Gangadhar B P, *Indian J Chem, 32B*, **1993**, 465.
8 Sivanandaiah K M, Suresh Babu V V & Shankaramma S C, *Int J Peptide Protein Res,* 44, **1994**, 24.
9 Takimoto S, Inanaga J, Kastuki T & Yamaguchi M, *Bull Chem Soc, Japan*, 46, **1976**, 2335.
10 Tung R D, Dhaon M K & Rich D H, *J Org Chem*, 51, **1986**, 3350.
11 Freidinger R M, Williams P D, Tung R D, Bock M G, Pettibone D J, Clineschmist B V, di Pardo R M, Erb J M, Garsky V M, Gould N P, Kaufman M J, Lundell G F, Perlow D S, Whitter W L & Veber D F, *J Med Chem*, 33, **1990**, 1845.
12 Carpino L A, *J Org Chem*, 53, **1988**, 875.
13 Chang C D, Felix A M, Jimenez M H & Meienhofer J, *Int J Peptide Protein Res*, 15, **1980**, 485.
14 Sivanandaiah K M, Suresh Babu V V, Renukeshwar H C & Gangadhar B P, *Indian J Chem*, 33B, **1994**, 618.
15 Sivanandaiah K M, Suresh Babu V V & Gangadhar B P, *Indian J Chem*, 33B, **1994**, 620.
16 Rangaraju N S, *Synthesis of peptides of biological interest (peptides related to substance P, enkephalins and β-casomorphin),* Ph.D. thesis, **1985**, Bangalore University, Bangalore.