Rapid and efficient synthesis of the pentapeptide of elastin protein and peptides containing highly hindered α,α-dialkyl amino acids employing Fmoc-amino acid chlorides under microwave irradiation in the solution phase

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Abstract

A rapid and efficient synthesis of peptides in solution employing Fmoc-amino acid chlorides under microwave irradiation is described. A comparison study of the microwave assisted method with those of conventional peptide synthesis using acid chlorides and various coupling additives has been performed. It has been found that, in general, the formation of a peptide bond, employing Fmoc-amino acid chloride and zinc dust or TBDMS-OBt under microwave irradiation is complete in 30-45 seconds with 90% yield of pure isolated peptide. Employing zinc dust as a coupling additive, the synthesis of several dipeptides, the pentapeptide fragment Fmoc-Val-Pro-Gly-Val-Gly-OBzl, of elastin and the difficult highly hindered couplings of α,α-dialkylamino acids are reported.

Keywords: Fmoc-amino acid chlorides, zinc dust, TBDMS-OBt, elastin-pentapeptide, α,α-dialkylamino acids

Introduction

Synthesis of peptides employing the shelf-stable 9-fluorenylmethoxycarbonyl (Fmoc) amino acid chlorides makes couplings of even very hindered or weakly nucleophilic systems possible where other methods give only poor results.[1,2] The trapping of the liberated hydrogen chloride during coupling has been accomplished by using a two-phase system with a mild inorganic base in the aqueous layer.[3] Improvements in the coupling in homogeneous system by the use of coupling additives like AgCN,[4,5] potassium salt of 1-hydroxybenzotriazole[6,7] and 1-hydroxy-7-aza-benzotriazole[8] along with Fmoc-amino acid chloride circumvented the formation of oxazol-5(4H)-one but also resulted in elimination of the premature deblocking of Fmoc group during

prolonged coupling reactions. 1-(*t*-Butyldimethylsilyloxy)benzotriazole (TBDMS-OBt)[9] has been found to be advantageous due to its solubility in CH_2Cl_2/$CHCl_3$ and the separation of the side product TBDMS-Cl is also simple. Zinc[10] and indium[11] mediated coupling is an alternative reaction pathway for the mediation of coupling by the acid chloride method. Some of the advancements led to complete coupling in about 30 min. The need for further acceleration of the synthesis of peptides with rapid, efficient and high yield coupling is addressed in this communication, demonstrating yet another utility of microwave irradiation in organic synthesis.[12-14]

The utility of microwave irradiation for the synthesis of several building blocks[15-19] useful in the synthesis of peptides and peptidomimetics like benzyl esters, conversion of Fmoc-/Boc-α-aminodiazoketones to β-amino acids by Wolff rearrangement, Fmoc-/Z-/Boc-α-amino acids to Fmoc-/Z-/Boc-5-oxazolidinones and Fmoc-α-amino acid azides to the corresponding isocyanates has been demonstrated by us. The solid phase peptide synthesis employing Fmoc-amino acids and symmetric anhydride or preformed benzotriazole esters or PyBOP for coupling under microwave irradiation enhancing the coupling efficiency has been reported.[20,21] Recently, the solid phase synthesis of *β*-peptides containing *trans*-2-aminocyclohexane carboxylic acid was realized efficiently by microwave irradiation. A comparison of microwave over conventional heating showed that, on treatment with microwave irradiation, the purity of peptide raised to 81%, whereas the conventional method resulted in only 21 % pure compound.[22] The synthesis of dipeptides containing Z-/Boc-α-aminoisobutyric acid (Aib) in solution employing PyBOP/HOBt and HBTU/HOBt was also explored.[23]

Results and Discussion

In this study, the effect of microwave irradiation on the formation of the peptide bond employing Fmoc-amino acid chlorides has been explored. The microwave reaction was carried out in a LG MS 194A microwave oven producing microwave radiation with a frequency of 2450 MHz. The microwave oven was a 1200 W oven and the reaction was specifically carried out at 60% of the total power output, which would correspond to an average power of 720 W. For the reactions carried out using a bi-phasic system, the reaction mixture was $CHCl_3$-10% $NaHCO_3$. The synthesis in a homogeneous system in the presence of several hydrogen chloride acceptors was also performed. In the case of the coupling additive TBDMS-OBt, a solution of Fmoc-amino acid chloride in CH_2Cl_2 and TBDMS-OBt was used. The zinc, bismuth and indium metal mediated synthesis was carried out using a suspension of metallic powder and Fmoc-amino acid chloride in CH_2Cl_2. The completion of the coupling was confirmed by TLC using the solvent systems a) chloroform: methanol: acetic acid (40:2:1) and b) ethyl acetate: *n*-hexane (35:65). A simple work-up of the reaction mixture and recrystallization using a suitable solvent gave the peptide. The duration of the coupling as well as the yield of Fmoc-Phe-Leu-OBzl using various coupling additives in both homogeneous and bi-phasic systems along with the reported data are

furnished in the **Table 1**. It has been found that the coupling mediated by zinc dust and TBDMS-OBt is complete within 30 to 45 sec. The isolated yield of the pure peptide was 90 %. The method was subjected to a racemization test and, as demonstrated by the ^{1}H NMR, and HPLC analysis of the diastereomeric dipeptides, Fmoc-L-Phe- and D-Phe-Ala-OMe, made by employing zinc dust as well as the TBDMS-OBt method, revealed that the method is completely free from racemization. Also, the scale-up of the synthesis of Fmoc-Phe-Leu-OBzl by this method using a 20 mmolar quantity resulted in isolation of 10.8 g (92 %) of the pure peptide. Employing a rapid deprotection condition using *tris*(2-aminoethyl)amine (TAEA), the present method was extended towards the synthesis of Fmoc-Val-Pro-Gly-Val-Gly-OBzl, [24] a repeating pentapeptide fragment of the protein elastin. Thus, all four of the couplings involved in its synthesis, namely the di, tri, tetra and peptapeptide couplings, have been accomplished under microwave irradiation. The final pure pentapeptide was obtained in 67% yield (**Table 2**). The ^{1}H NMR, HPLC (**Fig 2**) and mass spectrum (**Fig 3**) of the pentapeptide were found to be satisfactory.

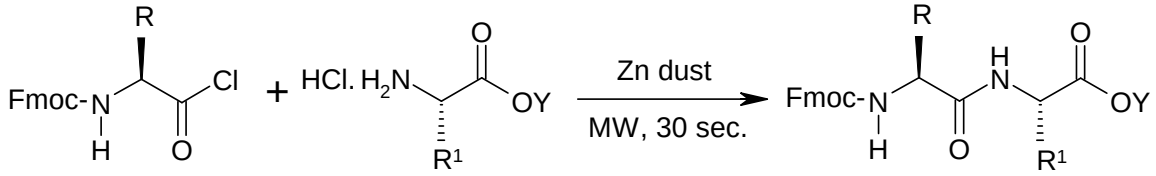

Figure 1. Synthesis of peptides employing zinc dust

Table 1. Synthesis of Fmoc-Phe-Leu-OBzl employing Fmoc-Phe-Cl under various conditions

Sl No	Coupling additives	Duration of Coupling		Yield (%)	
		r.t.	Microwave conditions	r.t.	Microwave conditions
1.	Zinc (preactivated)	15 min	40 sec	88	92
2.	TBDMS-OBt	30 min	45 sec	85	89
3.	10% NaHCO3-CHCl3	10 min	60 sec	70	60
4.	Bismuth (preactivated)	2 h	2 min	65	64
5.	Indium	9 h	3 min	60	40

Table 2. List of peptides made by the zinc dust mediated coupling microwave irradiation method

Sl. No.	Peptide*	M.P. (°C)	Yield (%)	Time (sec)	$[\alpha]^{25}_D$
1.	Fmoc-Phg-Phe-OMe	190-92	93	30	-22.5 (c 0.5, DMF)
2.	Fmoc-Phe-Ala-OMe	183-85	89	30	-18.0 (c1, $CHCl_3$)
3.	Fmoc-D-Phe-Ala-OMe	186-88	92	30	+18.3 (c1, $CHCl_3$)
4.	Fmoc-Ile-Pro-OMe	65-67	93	30	+19.6 (c1,$CHCl_3$)
5.	Fmoc-Gly-Phe-OMe	131-34	92	30	-16.0 (c1, MeOH)
6.	Fmoc-Met-Val-OMe	90-93	80	30	-12.3 (c1, EtOH)
7.	Fmoc-Tyr(Bzl)-Phe-OMe	171-73	91	35	+16.3 (c1, $CHCl_3$)
8.	Fmoc-Pro-Gly-OBzl	60-62	79	30	-27.7 (c1, $CHCl_3$)
9.	Fmoc-Leu-Val-OBzl	125-28	81	40	-11.8 (c0.5, $CHCl_3$)
10.	Fmoc-Phe-Leu-OBzl	155-58	88	35	-24.9 (c1, DMF)
11.	Fmoc-Val-Gly-OBzl	182-84	90	30	-32.2 (c1, $CHCl_3$)
12.	Fmoc-Gly-Val-Gly-OBzl	176-79	88	25	-30.4 (c1, $CHCl_3$)
13.	Fmoc-Pro-Gly-Val-Gly-OBzl	158-60	78	30	-24.2 (c1, $CHCl_3$)
14.	Fmoc-Val-Pro-Gly-Val-Gly-OBzl	93-95	67	30	-29.2 (c1, $CHCl_3$)

*^{1}H NMR of all the peptides obtained was satisfactory

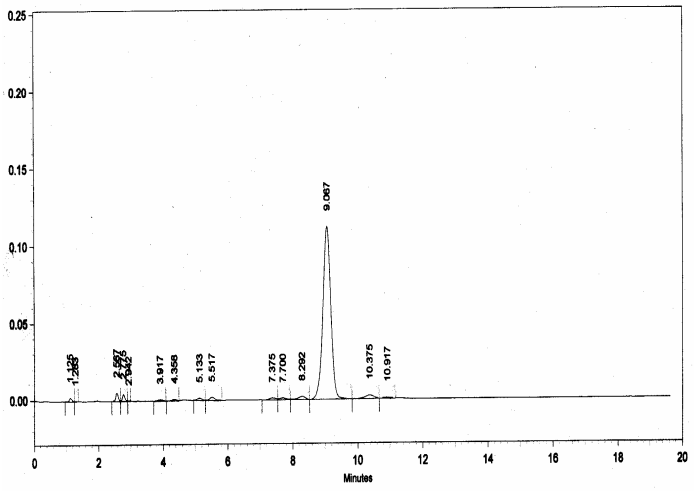

Figure 2. The HPLC of Fmoc-Val-Pro-Gly-Val-Gly-OBzl detection at 210 nm; eluents: acetonitrile (60 %) and water (40 %); flow rate of 1.0 mL/min.; R_t value : 9.06 min., purity >90%

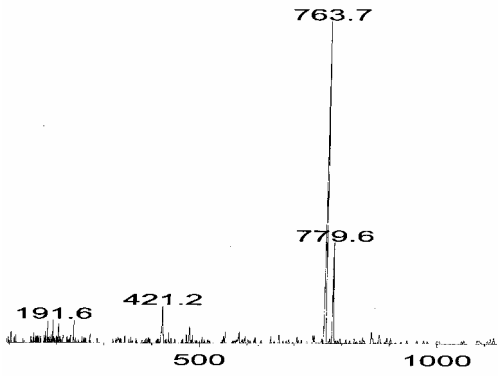

Figure 3. ES MS; $[M+ Na]^+$: 763.7, $[M+K]^+$: 779.6

Encouraged by these results, we have extended the similar reaction conditions for the incorporation of α,α -dialkylamino acids. The formation of a peptide bond between α,α-dialkylamino acids, both acyclic and cyclic, under normal conditions employing even efficient coupling agents like PyBOP, HBTU etc., needs several hours with poor yields. Even under microwave irradiation, the synthesis of Boc-/Z-Aib-Aib-OMe using HBTU/HOBt required 30 min. It is now found that the coupling of Fmoc-Aib-Cl to Aib-OBzl hydrochloride, mediated by activated zinc dust under microwave irradiation, has been found to be complete within 90 sec. with 84% yield. Similar results have been obtained in the coupling of several other peptides possessing cyclic as well as acyclic α,α -dialkylamino acids, and these are summarized in **Table 3**.

Table 3. List of dipeptides containing dialkyl amino acids synthesized employing Fmoc- α,α-dialkyl amino acid chloride and zinc dust under microwave irradiation

Sl. No.	Peptide*	M.P. (°C)	Yield (%)	Time (min)
1.	Fmoc-Aib-Aib-OBzl	131-32	84	1.5
2.	Fmoc-Ac_6c-Ac_6c-OMe	164-65	89	2.0
3.	Fmoc-Ac_6c-Ac_6c-Ac_6c-OMe	185-88	79	2.0
4.	Fmoc-Ac_7c-Ac_7c-OMe	207-09	80	2.5
5.	Fmoc-Deg-Deg-OMe	122-25	81	2.5
6.	Fmoc-Dpg-Dpg-OMe	128-29	89	2.0
7.	Fmoc-Dpg-Dpg-Dpg-OMe	151-22	90	2.5
8.	Fmoc-Dphg-Dphg-OMe	198-200	88	2.5
9.	Fmoc-Dphg-Aib-OBzl	170-73	90	2.5

- ^{1}H NMR and mass spectra of all the products were satisfactory
- Aib, 2-amino isobutyric acid; Dpg, diisopropyl glycine; Dphg, diphenyl glycine; Ac_6c, 1-amino cyclohexane carboxylic acic; Ac_7c, 1-amino cycloheptane carboxylic acid.

Conclusions

The synthesis of peptides employing Fmoc-amino acid chlorides using a mild microwave irradiation technique in the solution phase has been described. The method achieves the purpose of high speed and efficient coupling of peptides, including those of highly hindered α,α-dialkylamino acids. Thus, a facile method to produce peptides in high yield makes this yet another useful and attractive procedure employing the microwave technique in organic synthesis.

Experimental Section

General Procedures. All the solvents were freshly distilled prior to use. Melting points were determined by the capillary method and are uncorrected. TLC analysis was carried out on precoated silica gel-GF_{254} plates purchased from Merck containing 13% $CaSO_4$ as binding agent, using solvent systems a) chloroform : methanol : acetic acid (40:2:1) and b) ethyl acetate : *n*-hexane (35:65). IR spectra were recorded on a Nicolet model impact 400D FT-IR spectrometer. The HPLC analysis was carried on Shimadzu CLASS-VP V6.1 analytical HPLC using a Merck RP-18, 250 x 4.0 mm column, with detection at 215 nm. Optical rotations were determined using automatic AA-10 polarimeter (Optical Activity, U. K.). Elemental analyses were carried out on a Perkin-Elmer MODEL 240 analyzer. ^{1}H NMR spectra were recorded using a Bruker AMX 400 MHz spectrometer. Mass spectra were obtained using a Kratos PCKompact SEQ V1.2.2 spectrometer.

General procedure for the synthesis of peptides mediated by zinc dust under MW irradiation. A beaker containing a mixture of Fmoc-amino acid chloride (1 mmol), zinc dust (140 mg, 2 mmol) and amino acid ester hydrochloride (1.05 mmol) in DCM (10 mL) was irradiated with microwave (at P-60) for 30-40 sec. (1.5-2.5 min. in case of dialkyl amino acids). After completion of the reaction, the residue was diluted with $CHCl_3$ (25 mL), washed with 5% HCl (5 mL X 2), 10% $NaHCO_3$ (5 mL X 2), and water (5 mL X 2). The organic layer was dried over anhyd. Na_2SO_4 and evaporated under reduced pressure. Recrystallization of the resulting residue with DCM: *n*-hexane (1:3) gave the pure peptide.

General procedure for the deprotection of Fmoc-peptides using *tris* (2-aminoethyl)amine (TAEA). TAEA (5 mL) was added to a solution of Fmoc- protected peptide (1 mmol) in DCM (5 mL) and stirred for 20 min. After completion of the reaction, the solution was further diluted with DCM (30 mL), washed with phosphate buffer (5 mL X 3) and water (5 mL X 3). The organic layer was dried over Na_2SO_4 and taken for the coupling of next amino acid.

Racemization studies Following the general procedure for the synthesis of peptides mediated by zinc dust, a mixture of Fmoc-Phe-Cl/Fmoc-D-Phe-Cl (400 mg, 1 mmol), zinc dust (140 mg, 2 mmol) and HCl.H-Ala-OMe (145 mg, 1.05 mmol) in DCM (10 mL) resulted in Fmoc-Phe-Ala-OMe (420 mg, 89%) and Fmoc-*D*-Phe-Ala-OMe (440 mg, 92%) of the pure peptide. The HPLC analysis was carried on Shimadzu CLASS-VP V6.1 analytical HPLC using a Merck RP-18, 250

x 4.0 mm column, with detection at 215 nm. The eluent was acetonitrile (50 %) and water (50 %) with the flow rate of 1.0 mL/min.; R_t value for L, L diastereomer : 16.62 min.; R_t value for D,L diastereomer : 17.56 min.

Fmoc-Phg-Phe-OMe. White solid (93%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 2.96 (d, J= 5.7 Hz, 2H), 3.67 (s, 3H), 4.05 (m, 1H), 4.21 (t, J= 6.6Hz, 1H), 4.39 (d, J= 6.7 Hz, 2H), 4.72 (m, 1H), 5.3 (br s, 1H), 6.26 (br s, 1H), 6.97-7.69 (m, 18H). Anal. Calcd. for $C_{33}H_{30}N_2O_5$ (534.62): C, 74.14; H, 5.66; N, 5.24. Found: C, 74.02; H, 5.57; N, 5.14. ES MS: m/z =557.4 $[M + Na]^+$.

Fmoc-Phe-Ala-OMe. White solid (89%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 1.26 (d, J=7.1, 3H), 2.98 (d, J= 7.0 Hz, 2H), 3.63 (s, 3H), 4.12 (t, J= 6.8 Hz, 1H), 4.25-4.45 (m, 4H), 5.31 (br s, 1H), 6.26 (br s, 1H), 7.10- 7.70 (ArH, 13H). Anal. Calcd. for $C_{28}H_{28}N_2O_5$ (472.55): C, 71.17; H, 5.97; N, 5.93. Found: C, 71.09; H, 5.87; N, 5.90. ES MS: m/z =495.5 $[M + Na]^+$.

Fmoc-*D*-Phe-Ala-OMe. White solid (92%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 1.30 (d, J=7.1, 3H), 3.06 (d, J= 6.9 Hz, 2H), 3.71 (s, 3H), 4.15 (t, J= 6.8 Hz, 1H), 4.25-4.45 (m, 4H), 5.30 (br s, 1H), 6.26 (br s, 1H), 7.13- 7.70 (ArH, 13H). Anal. Calcd. for $C_{28}H_{28}N_2O_5$ (472.55): C, 71.17; H, 5.97; N, 5.93. Found: C, 71.15; H, 5.91; N, 5.92. ES MS: m/z =495.7 $[M + Na]^+$.

Fmoc-Ile-Pro-OMe. Colourless oil solidifies on standing (93%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 0.81 (m, 6H), 1.13 (m, 1H), 1.22-2.05 (m, 6H), 3.60 (m, 2H), 3.68 (s, 3H), 4.03 (m, 2H), 4.21 (t, J=6.6Hz, 1H), 4.44 (m, 2H), 5.91 (br s, 1H), 7.25-7.76 (m, 8H). Anal. Calcd. for $C_{27}H_{32}N_2O_5$ (464.57): C, 69.81; H, 6.94; N, 6.03. Found: C, 69.78; H, 6.91; N, 5.97. ES MS: m/z =487.8 $[M + Na]^+$.

Fmoc-Gly-Phe-OMe. White solid (92%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 2.87 (d, 2H, J=6.2 Hz), 3.69 (s, 3H), 4.03 (m, 3H), 4.18 (t, J=6.6 Hz, 1H), 4.40 (m, 2H), 5.96 (br s, 1H), 6.2 (br s, 1H), 7.1-7.76 (m, 13H). Anal. Calcd. for $C_{27}H_{26}N_2O_5$ (458.52): C, 70.73; H, 5.72; N, 6.11. Found: C, 70.69.09; H, 5.67; N, 6.08. ES MS: m/z =481.1 $[M + Na]^+$.

Fmoc-Met-Val-OMe. Yellowish residue slowly crystallizes to give solid (80%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 0.93 (2d, J= 6 Hz, 6H), 1.81 - 2.55 (m, 5H), 2.78 (s, 3H), 3.62 (s, 3H), 3.75 (m, 1H),4.05 (m, 1H), 4.21 (t, J=6.6, 1H), 4.50 (d, J= 6 Hz, 2H), 5.05 (d, J=8.0 Hz, 1H,), 6.10 (br, s, 1H), 7.26-7.7 (m, 8H). Anal. Calcd. for $C_{26}H_{32}N_2O_5S_1$ (484.62): C, 64.44; H, 6.66; N, 5.78. Found: C, 64.33; H, 6.61; N, 5.75. ES MS: m/z =507.3 $[M + Na]^+$.

Fmoc-Tyr(Bzl)-Phe-OMe. White solid (91%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 2.93 (m, 2H), 3.08 (m, 2H), 3.67 (s, 3H), 4.00-4.09 (m, 2H), 4.16 (t, J=6.6 Hz, 1H), 4.44 (d, J= 6.7 Hz, 2H), 5.16 (m, 2H), 5.71 (d, J= 8.6 Hz, 1H), 5.82 (m, J=8.8 Hz, 1H), 7.26-7.8 (m, 22H). Anal. Calcd. for $C_{41}H_{38}N_2O_6$ (654.77): C, 75.21; H, 5.85; N, 4.28. Found: C, 75.22; H, 5.88; N, 4.38. ES MS: m/z =677.9 $[M + Na]^+$.

Fmoc-Pro-Gly-OMe. Viscous liquid solidifies on standing to give solid (79%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 1.22-2.05 (m, 4H), 3.33 (d, 2H), 3.62 (s, 3H), 3.90 (m, 2H), 4.03 (m, 1H), 4.19 (t, J=6.6 Hz, 1H), 4.38 (m, 2H), 5.18 (br s, 1H), 7.26-7.8 (m, 8H). Anal. Calcd. for $C_{23}H_{24}N_2O_5$ (408.45): C, 67.3; H, 5.92; N, 6.85. Found: C, 69.11; H, 5.88; N, 6.75. ES MS: m/z =431.9 $[M + Na]^+$.

Fmoc-Leu-Val-OBzl. White solid (81%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 0.83-1.11 (m, 12H), 1.33 (m, 1H), 1.63 (m, 2H), 2.1 (m, 1H), 4.09 (m, 1H), 4.19 (t, J=6.5 Hz, 1H), 4.34 (m, 1H), 4.45 (d, J=6.6 Hz, 2H), 5.14 (m, 2H), 5.55 (d, J= 7.2 Hz, 1H), 6.2 (d, J=8.2 Hz, 1H), 7.26-7.8 (m, 13H). Anal. Calcd. for $C_{33}H_{38}N_2O_5$ (542.68): C, 73.04; H, 7.06; N, 5.17. Found: C, 72.95; H, 6.99; N, 5.06. ES MS: m/z =565.6 $[M + Na]^+$.

Fmoc-Phe-Leu-OBzl. White solid (88%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 0.91 (d, J=5.2 Hz, 6H), 1.33 (m, 1H), 1.63 (m, 2H), 2.87 (d, J=6.2 Hz, 2H), 3.96 (m, 1H), 4.05 (m, 1H), 4.20(t, J= 6.6 Hz, 1H), 4.54 (m, 2H), 5.13 (m, 2H), 5.5 (br s, 1H), 6.21 (br s, 1H), 7.26-7.8 (m, 18H). Anal. Calcd. for $C_{37}H_{38}N_2O_5$ (590.73): C, 75.23; H, 6.48; N, 4.74. Found: C, 75.19; H, 6.42; N, 4.70. ES MS: m/z =613.7 $[M + Na]^+$.

Synthesis of Fmoc-Val-Pro-Gly-Val-Gly-OBzl

Fmoc-Val-Gly-OBzl. White solid (90%), ^{1}H NMR δ (400 MHz, $CDCl_3$): δ 0.9-1.01 (2d, J= 6.1 Hz, 6H), 2.01 (m, 1H), 3.67 (d, 2H), 3.93 (m, 1H), 4.18 (t, J= 6.5 Hz, 1H), 4.48 (d, J= 6.1 Hz, 2H), 5.05 (m, 2H), 5.21 (br s, 1H), 5.66 (br s, 1H), 7.27-7.68 (m, 13H). Anal. Calcd. for $C_{29}H_{30}N_2O_5$ (486.57): C, 71.59; H, 6.21; N, 5.76. Found: C, 71.55; H, 6.19; N, 5.73. ES MS: m/z =509.3 $[M + Na]^+$.

Fmoc-Gly-Val-Gly-OBzl. White solid (88%), ^{1}H NMR δ (400 MHz, $CDCl_3$): δ 0.9-1.01 (2d, J= 6.6 Hz, 6H), 2.11 (m, 1H), 3.67 (m, 4H), 3.93 (m, 1H), 4.18 (t, J= 55 Hz, 1H), 4.45 (m, 2H), 5.07 (d, 2H), 5.21 (m, 2H), 6.2 (d, 1H), 7.21-7.75 (m, 13H). Anal. Calcd. for $C_{31}H_{33}N_3O_6$ (543.63): C, 68.49; H, 6.12; N, 7.73. Found: C, 68.55; H, 6.19; N, 7.79. ES MS: m/z =566.2 $[M + Na]^+$.

Fmoc-Pro-Gly-Val-Gly-OBzl. White solid (78%), ^{1}H NMR δ (400 MHz, $CDCl_3$): δ 0.9-1.01 (2d, J=6.7 Hz, 6H), 1.73-2.24 (m, 5H), 3.67 (m, 2H), 3.93 - 4.12 (m, 6H), 4.19 (t, J= 5.6 Hz, 1H), 4.45 (m, 2H), 5.07 (m, 2H), 5.11 (m, 1H), 5.19 (m, 1H), 6.00 (d, J=9.0 Hz, 1H), 7.21-7.75 (m, 13H). Anal. Calcd. for $C_{36}H_{40}N_4O_7$ (640.74): C, 67.48; H, 6.29; N, 8.74. Found: C, 67.20; H, 6.29; N, 8.73. ES MS: m/z =663.7 $[M + Na]^+$.

Fmoc-Val-Pro-Gly-Val-Gly-OBzl. Sowly crystallizes to result in white solid (67%), ^{1}H NMR δ (400 MHz, $CDCl_3$): δ 0.83-1.01 (m, 12H), 1.42-2.14 (m, 6H), 3.33(m, 2H), 3.63 (m, 2H), 3.93 (m, 2H), 4.12 (m, 1H), 4.21 (t, J= 8.7 Hz, 1H), 4.34 (m, 2H), 4.45 (d, J= 5.8 Hz, 2H), 5.07 (m, 2H), 5.11-5.19 (m, 2H), 5.37 (br s, 1H), 6.00 (d, J=9.0 Hz, 1H), 7.21-7.75 (m, 13H). Anal. Calcd. for $C_{41}H_{49}N_5O_8$ (739.98): C, 66.56; H, 6.68; N, 9.47. Found: C, 66.47; H, 6.57; N, 9.40. ES MS: m/z =763.7 $[M + Na]^+$.

Fmoc-Aib-Aib-OBzl. White solid (84%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 1.53 (s, 6H), 1.58 (s, 6H), 4.18 (t, J= 6.4 Hz, 1H), 4.44 (d, J= 6.3 Hz, 2H), 5.1 (s, 2H), 5.34 (d, J= 7.7 Hz, 1H), 6.16 (d, 8.8 Hz, 1H), 7.26-7.8 (m, 13H). Anal. Calcd. for $C_{30}H_{32}N_2O_5$ (500.60): C, 71.98; H, 6.44; N, 5.60. Found: C, 71.88; H, 6.37; N, 5.56. ES MS: m/z =523.9 $[M + Na]^+$.

Fmoc-Ac_6c-Ac_6c-OMe. White solid (89%), ^{1}H NMR δ (300 MHz, $CDCl_3$): 0.93-2.17 (m, 20H), 3.67 (s, 3H), 4.22 (t, J= 6.8 Hz, 1H), 4.40 (d, J= 6.9 Hz, 2H), 5.8 (br s, 1H), 6.3 (br s, 1H), 7.24-7.1 (m, 8H). Anal. Calcd. for $C_{29}H_{36}N_2O_5$ (492.61): C, 70.70; H, 7.36; N, 5.68. Found: C, 70.75; H, 7.39; N, 5.73. ES MS: m/z =516.1 $[M + Na]^+$.

Fmoc-Ac$_6$c-Ac$_6$c-Ac$_6$c-OMe. White solid (79%), ^{1}H NMR δ (300 MHz, $CDCl_3$): 0.93-2.27 (m, 30H), 3.7 (s, 3H), 4.19 (t, J=6.6 Hz, 1H), 4.3 (d, J= 6.5 Hz, 2H), 5.2 (br s, 1H), 5.6 (br s, 1H), 6.3 (br s, 1H), 7.23-7.82 (m, 8H). Anal. Calcd. for $C_{36}H_{47}N_3O_6$ (617.78): C, 69.99; H, 7.66; N, 6.8. Found: C, 70.08; H, 7.72; N, 6.71. ES MS: m/z =641.5 $[M + Na]^+$.

Fmoc-Ac$_7$c-Ac$_7$c-OMe. White solid (80%), ^{1}H NMR δ (300 MHz, $CDCl_3$): 0.76-2.31 (m, 24H), 3.7 (s, 3H), 4.1 (t, J= 6.5 Hz, 1H), 4.3 (m, 2H), 5.3 (br s, 1H), 6.1 (br s, 1H), 7.25-7.77 (m, 8H). Anal. Calcd. for $C_{31}H_{40}N_2O_5$ (520.63): C, 71.51; H, 7.73; N, 5.38. Found: C, 71.038; H, 7.65; N, 5.25. ES MS: m/z =543.9 $[M + Na]^+$.

Fmoc-Deg-Deg-OMe. White solid (81%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 0.76- 0.87(m, 12H), 1.79 (m, 8H), 3.67 (s, 3H), 4.18 (t, J=6.6Hz, 1H), 4.4 (d, J= 5.9 Hz, 2H), 5.26 (br s, 1H), 6.4 (br s, 1H), 7.27- 7.80 (m, 8H). Anal. Calcd. for $C_{28}H_{36}N_2O_5$ (481.16): C, 69.89; H, 7.65; N, 5.82. Found: C, 70.01; H, 7.72; N, 5.71. ES MS: m/z =504.7 $[M + Na]^+$.

Fmoc-Dpg-Dpg-OMe. White solid (89%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 0.82-0.94 (m, 12H), 0.99-1.13 (m, 8H), 1.18-1.32 (m, 8H), 3.68 (s, 3H), 4.18 (t, J= 6.4 Hz, 1H), 4.43 (d, J= 6.6 Hz, 2H), 4.5 (br s, 1H), 5.6 (br s, 1H), 7.28-7.8 (m, 8H). Anal. Calcd. for $C_{32}H_{44}N_2O_5$ (536.38): C, 71.59; H, 8.26; N, 5.22. Found: C, 79.66; H, 8.14; N, 5.14. ES MS: m/z =559.9 $[M + Na]^+$.

Fmoc-Dpg-Dpg-Dpg-OMe. White solid (90%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 0.82-0.94 (m, 18H), 0.99-1.13 (m, 12H), 1.18-1.32 (m, 12H), 3.68 (s, 3H), 4.18 (t, J= 6.4 Hz, 1H), 4.43 (d, J= 6.6 Hz, 2H), 5.5 (br s, 1H), 5.6 (br s, 1H), 6.1(s, 1H), 7.28-7.8 (m, 8H). Anal. Calcd. for $C_{40}H_{59}N_3O_6$ (677.52): C, 70.85; H, 8.77; N, 6.20. Found: C, 70.70; H, 8.72; N, 6.15. ES MS: m/z =700.9 $[M + Na]^+$.

Fmoc-Dphg-Dphg-OMe. White solid (88%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 3.78 (s, 3H), 4.18 (t, 1H, J=6.6 Hz), 4.49, (m, 2H), 5.18 (br s, 1H), 6.6 (br, s, 1H), 7.26-7.81 (m, 28H). Anal. Calcd. for $C_{44}H_{36}N_2O_5$ (672.77): C, 78.55; H, 5.39; N, 4.16. Found: C, 78.51; H, 5.32; N, 4.04. ES MS: m/z =696.3 $[M + Na]^+$.

Fmoc-Dphg-Aib-OBzl. White solid (90%), ^{1}H NMR δ (400 MHz, $CDCl_3$): 1.53 (s, 6H), 4.18 (t, J= 6.5 Hz, 1H), 4.45 (m, J= 6.6 Hz, 2H), 5.14 (s, 2H), 5.8 (br s, 1H), 6.3 (br, 1H), 7.2 - 7.81 (m, 23H). Anal. Calcd. for $C_{40}H_{36}N_2O_5$ (624.33): C, 76.88; H, 5.81; N, 4.48. Found: C, 76.69; H, 5.70; N, 4.33. ES MS: m/z =647.1 $[M + Na]^+$.

Acknowledgements

We thank CSIR, Govt. of India for financial support. One of the authors (RVRR) is grateful to the CSIR for award of a fellowship. The authors also thank Professor Fred Naider, City University of New York, USA for useful discussions.

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