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An efficient concomitant synthesis of *O*-succinimidyl-(9*H*-fluoren-9-yl methoxy carbonylamino)peptidyl carbamates and their application in the synthesis of oligo-α-peptidyl ureas

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An efficient method for the synthesis of *O*-succinimidyl (9*H*-fluorene-9-yl methoxycarbonylamine) peptidyl carbamates from the corresponding N^{α}-Fmoc peptidyl isocyanates through the concomitant Curtius rearrangement of N^{α}-Fmoc peptidyl acid azide and coupling with *N*-hydroxysuccinimide is described. The introduction of urea moiety at various positions in peptidyl backbone of VALVAL hexapeptide sequence has been carried out by the fragment coupling using peptidyl carbamates. All the oligo-α-peptidyl ureas are isolated as crystalline solids in 80-85% yield and have been fully characterized by ^{1}H and ^{13}C NMR and mass spectrometry.

Keywords: Oligo-α-ureas, Curtius rearrangement, peptide acid azides, isocyanates, peptidyl carbamates

Modification of peptide structures by incorporating the ureido unit in the backbone has been demonstrated to be useful in de nova design as well as in developing potent bioactive molecules. Thus, several families of unnatural biopolymers with urea backbone such as *N*,*N*′-linked oligoureas[1], *N*-alkyl-*N*,*N*-linked oligo-ureas[2], ureidopeptoids, oligourea-peptide hybrids and oligomeric cyclic ureas have been synthesized[3]. The insertion of urea moiety as nonpeptide linkage, due to structural similarities with polyamides, has lead to potent HIV protease inhibitors, CCK-B receptor antagonists and endothelin antagonists[4-6]. Its utility in aspartic acid protease[7], microbial alkaline proteinase[8-10] and γ-secretases is also well documented[11-14]. Peptidyl carbamates themselves exhibit good inhibitory activity towards porcine pancreatic elastase, trypsin, chymotrypsin[15] and human leukocyte elastase[16]. Rana and co-workers[17] have demonstrated that the oligourea version of Tat 48-57 is capable of suppressing HIV-1 gene expression *in vivo*. On the other hand, Nowick's group has developed oligourea based scaffolds to make artificial β-sheet structures[18]. Guichard's group has synthesized (*P*)2.5 helical oligourea [CF_3CO_2H-(HN-*β*-Tyr^{u}-*β*-Ala^{u}-*β*-Val^{u})$_2$-NH-*β*-Tyr^{u}] which is likely to be useful in *de novo* design of oligoureas with controlled shape and defined biological activity. They have also synthesized a C4- symmetric (*all-S*) cyclotetraurea of Ala which was demonstrated to form square shaped nanotubes. Further, a helix-forming oligourea nonamer H_2N-*β*-Tyr^{u}-*β*-Lys^{u}-*β*–Leu^{u}-*β*-Val^{u}-*β*-Phe^{u}-*β*-Lys^{u}-*β*-Val^{u}-*β*-Tyr^{u}-*β*-Ala^{u}-H] was also constructed[19-21].

Many of the earlier reports for the incorporation of a urea moiety in the designated position of *α*-peptidyl urea involves the synthesis of peptide isocyanates by the conversion of *α*-amino group of peptide ester hydrochloride salt to its isocyanate employing phosgene/triphosgene[22,23]. Alternatively, Chipens *et al*., have reported the synthesis of [1-Asn, 5-Val] angiotensin-II employing *Z*-protected peptide isocyanates[24]. A similar approach was used by Schwyzer *et al*., for the synthesis of the angiotensin analogs containing azahomotyrosine[25]. Kawasaki *et al*., made [Leu^{5}]enkephalin analogs containing a ureylene bond. At the end of the synthesis, the Z group was deprotected using catalytic hydrogenation which is not only cumbersome but also known to offer handling problems[26]. The authors' group demonstrated the synthesis of proteinogenic amino acid derived isocyanates from Fmoc-amino acid as well as peptide acids *via* acid azides through Curtius rearrangement[27-29]. Although such isocyanates have been isolated, they have been found to have limited shelf stability. Consequently, in the present study,

shelf stable peptidyl carbamates have been prepared and their utility in the synthesis of oligo-α-peptidyl ureas has been demonstrated through the synthesis of four ureido analogs of hexapeptide VALVAL hexapeptide.

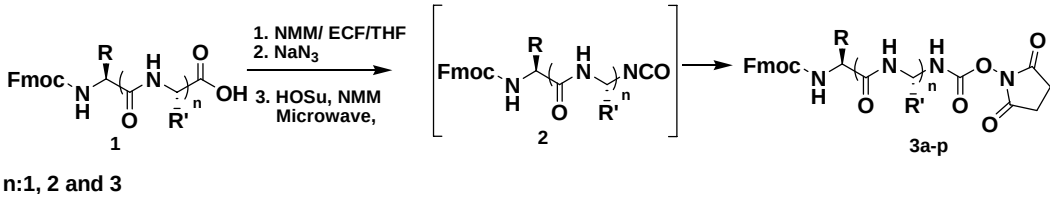
Results and Discussion

N^{α}-Fmoc-peptide acids were obtained by coupling Fmoc-amino acid with bis-TMS-amino acid through its mixed anhydride. They were then converted to the corresponding acid azides again through a mixed anhydride generated using ethyl chloroformate (ECF) in the presence of *N*-methylmorpholine (NMM) at –20°C and then reacted with sodium azide. A solution of toluene containing Fmoc-peptide azide, *N*-hydroxysuccinimde and NMM was refluxed at 65°C for 30 min. Alternatively, it was exposed to microwave irradiation for 2 min. This lead to the formation of isocyanate and its concomitant coupling with the succinimde to yield *O*-succinimidyl-(9*H*-fluoren-9-ylmethoxycarbonylamino)peptidyl carbamates **3a-p** in a single step. In most of the cases, the crude products separated out as solids. The isolated solid, after filtration, was recrystallised using DMF-CH_2Cl_2 to obtain the products in 75-90% as analytically pure ones (**Scheme I**, **Table I**). Their mass was in full agreement with the calculated molecular weights. During the course of the above reaction, an aliquot of the reaction-mixture was used to study IR analysis. It revealed the disappearance of peak at 2100 cm^{-1} and formation of another peak at around 1800 cm^{-1}. This clearly confirmed the conversion of azide to the corresponding carbamate. Thus the carbamate formation was rapid, clean and almost quantitative. The synthesis was also found to be free from racemization. This was evident by the ^1H NMR analysis of the adducts obtained by the coupling of Fmoc-Phe-Val-NHCOOSu with R-(+), S-(-) and racemic mixture of 1-phenylethylamine prepared in three different experiments using the present protocol. The methyl protons of 1-phenylethylamine urea adducts of Fmoc-Phe-Val-ψ(NH-CO-NH)-R-(+)-phenylethylamine (δ 1.31 and 1.29) and Fmoc-Phe-Val-ψ(NH-CO-NH)-S-(-)-phenylethylamine (δ 1.30 and 1.29) were observed as doublets, while the methyl protons of Fmoc-Phe-Val-ψ(NH-CO-NH)-R,S-(±)-1-phenylethylamine (δ 1.31, 1.30, 1.2 and 1.29) was observed as two doublets.

Further, the peptidyl carbamates **3a-p** were employed as reactive building blocks in the synthesis of oligoureas by the fragment coupling (**Scheme II** and **Table II**). They were coupled with amino acid esters as well as *N*,*O*-bis[trimethylsilyl]amino/peptide acid **4**. The coupling was complete within 15-25 min. All the peptidyl ureas **5a-l** separated out as solids. After their isolation, a single recrystalization using DMSO-water was sufficient to obtain them as analytically pure ones. N^{α}-Fmoc-peptidyl ureas **5a-l** made were obtained as stable crystalline solids in 85-95% yield.

Finally, the present protocol was successfully employed for the synthesis of four analogs of H-Val-Ala-Leu-Val-Ala-Leu-OH, **9-12**, each one containing one ureido bond at 2nd, 3rd, 4th and 5th positions respectively. They were made by the 2 + 4, 3 + 3, 4 + 2 and 5 + 1 fragment coupling. The Fmoc group from the final protected ureas was deprotected using diethylamine (DEA)/dichloromethane (DCM) (1:1) at RT in an hour. The strategy followed for the synthesis of **9** is outlined in **Scheme III**.

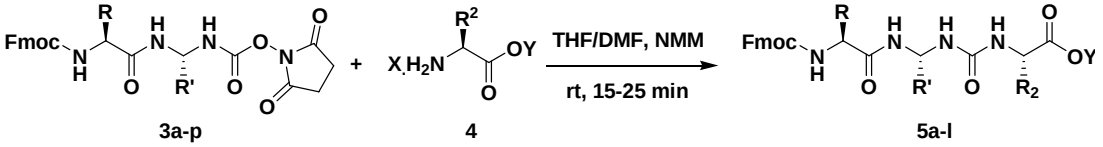
In summary, several di, tri, tetra and penta *O*-succinimidyl-(9*H*-fluoren-9-yl methoxy carbonylamino) peptidyl carbamates were prepared in high yields from their corresponding *N*-Fmoc-protected α-peptide acid azides through Curtius rearrangement followed by the concomitant coupling with *N*-hydroxysuccinimides and their utility in the synthesis of oligo-α-peptidyl ureas is demonstrated. Synthesis of ureido analogs of the hexapeptide VALVAL is carried out using the active succinimidyl carbamates. The NMR analysis of the synthesis of active carbamates and their conversion into ureas has revealed both the protocols to be epimerization free. The final deprotection of the Fmoc group was accomplished using a secondary amine under standard conditions to obtain the free oligo-α-peptidyl ureas in good yields.



Scheme I — Synthesis of peptidyl carbamates

Table I — Physical parameters of N^{α}-Fmoc-peptidyl carbamates

Entry	Compd	m.p. (°C)	Mass[a] (*m/z*) Calcd./Found	Yield (%)	IR (cm^{-1})	^{1}H NMR (δ, DMSO-d_6)
3a	Fmoc-Val-*g*Ala-COOSu	185-87	523.2/523.2	88	1760	0.97 (d, *J* = 5.3, 6H), 1.35 (d, *J* = 5.6, 3H), 2.52-2.75 (m, 5H), 4.21 (t, *J* = 6.2 , 1H), 4.46 (d, *J* =5.2, 2H), 4.93 (m, 1H), 5.39 (m, 1H), 7.15-7.80 (m, 8H)
3b	Fmoc-Phe-*g*Ala-COOSu	188-90	571.2/571.3	90	1755	1.27 (d, *J* = 5.4, 6H), 2.05 (m, 1H), 2.75-2.95 (m, 6H), 3.98 (m, 1H), 4.20 (t, *J* = 6.3, 1H), 4.51-4.62 (m, 3H), 5.37 (m, 1H), 7.10-8.00(m, 13H)
3c	Fmoc-Ser(OBzl)-*g*Val-COOSu	178-80	629.2/629.7	85	1760	0.97 (d, *J* = 5.3, 6H), 2.30 (m, 1H), 2.60-2.75 (m, 4H), 4.21 (m, 1H), 4.51 (m, 2H), 4.87 (s, 2H), 5.12 (m, 2H), 6.12 (br, 1H), 7.10-7.95 (m, 13H)
3d	Fmoc-Ile-*g*Gly-COOSu	103-05	523.2/523.5	82	1758	0.85-1.25 (m, 8H), 2.35 (m, 1H), 2.60-2.75 (m, 4H), 4.20 (t,1H), 4.35 (m, 1H), 4.45 (d, *J* = 5.6, 2H), 4.90 (m, 2H), 7.20-7.80 (m, 8H)
3e	Fmoc-Ala-*g*Ser(OBzl)-COOSu	190-92	601.2/601.3	85	1755	1.21 (d, 3H), 2.61-2.72 (m, 4H), 3.65-3.85 (m, 2H), 4.20 (t, 1H), 4.49 (d, *J* = 5.6, 2H), 4.70 (s, 2H), 4.82 (m, 1H), 5.52 (m, 1H), 6.20 (br, 1H), 7.10-8.05 (m, 13H)
3f	Fmoc-Leu-*g*Ala-COOSu	198-00	537.2/537.3	87	1760	0.91 (d, *J* = 5.3, 6H), 1.31 (d, *J* = 5.5, 3H), 1.55-1.67 (m, 3H), 2.68 (m, 4H), 4.19 (t, *J* = 6.7, 1H), 4.40 (m, 5H), 5.37 (m, 1H), 6.23 (br, 1H), 7.25-7.80 (m, 8H)
3g	Fmoc-Asp(OBzl)-*g*Phe-COOSu	182-84	705.2/705.7	85	1760	2.25 -3.10 (m, 4H), 2.62-2.75 (m, 4H), 4.20 (t, *J* = 5.9, 1H), 4.45 (d, *J* = 5.5, 2H), 5,75 (m, 1H), 6.40 (m, 1H), 7.10-8.00 (m, 13H)
3h	Fmoc-Leu-*g*Val-COOSu	190-92	565.2/565.7	84	1755	0.91-1.10 (m, 12H), 1.55-1.75 (m, 3H), 2.65-2.78 (m, 5H), 4.19 (t, *J* = 6.7, 1H), 4.47 (d, *J* = 5.5, 2H)5.20 (m, 1H), 6.45 (br, 1H), 7.20-7.80 (m, 8H)
3i	Fmoc-Asp(OBzl)-*g*Ile-COOSu	172-74	671.3/671.2	84	1758	0.95-1.20 (m, 6H), 1.75 (m, 1H), 2.65-2.90 (m, 6H), 4.19 (t, *J* = 6.5, 1H), 4.45 (d, *J* = 5.6, 2H), 4.85 (m, 1H), 5.25 (s, 2H), 6.40 (br, 1H), 7.10-8.05 (m, 13H)
3j	Fmoc-Glu(OBzl)-*g*Ala-COOSu	176-78	642.2/642.3	84	1751	1.21 (d, 5.7 3H), 1.95 (m, 2H), 2.60-2.78 (m, 6H), 4.20 (t, *J* = 6.6, 1H), 4.39 (m, 1H), 4.45 (d, *J* = 5.6, 2H), 5.57 (m, 1H), 6.62 (br, 1H), 7.10-8.00 (m, 13H)
3k	Fmoc-Ser(OBzl)-*g*Gly-COOSu	167-69	587.2/587.5	84	1760	2.65-2.71 (m, 4H), 3.80 (m, 2H), 4.21 (t, *J* = 6.5, 1H), 4.45-4.65 (m, 5H), 4.85 (m, 1H), 5.00 (m, 1H), 7.10-8.00 (m, 13H)
3l	Fmoc-Gly-*g*Phe-COOSu	195-97	557.2/557.6	84	1755	2.65-2.71 (m, 4H), 2.75-2.90 (m, 2H), 3.80 (m, 2H), 4.19 (t, *J* = 6.7, 1H), 4.45 (d, *J* = 5.7, 2H), 5.55 (m, 1H), 6.32 (br, 1H), 7.10-8,00 (m, 13H)
3m	Fmoc-Ser(^{t}Bu)-*g*Phe-COOSu	186-88	643.2/643.6	88	1755	1.32 (s, 9H), 2.50-2.72 (m, 6H), 3.50 (m, 2H), 2H), 4.20 (t, *J* = 6.5, 1H), 4.46 (d, *J* = 5.6, 2H)4.56 (m, 1H), 5.37 (m, 1H), 6.21(m, 1H), 7.21-7.90 (m, 8H)
3n	Fmoc-Val-Ala-*g*Leu-COOSu	187-89	636.2/636.8	88	1760	0.85-1.05 (m, 12H), 1.25-1.70 (m, 6H), 2.10 (m, 1H), 2.65-2.79 (m, 4H), 4.21 (t, *J* = 6.6, 1H), 4.45 (d, *J* = 5.6, 2H), 4.80 (m, 1H), 4.90 (br, 1H), 5.10 (m, 1H), 5.64 (m, 1H), 6.18 (m, 1H), 7.20-7.85 (m, 8H)
3o	Fmoc-Val-Ala-Leu-*g*Val-COOSu	-	735.2/735.5	82	1758	0.85-1.25(m, 21H), 1.55-1.70 (m, 3H), 2.35 (m, 1H), 2.65-2.79 (m, 5H), 4.20 (t, *J* = 6.6, 1H), 4.35 (m, 3H), 4.52-4.65 (br, 2H), 5.32 (m, 1H), 6.30 (br, 1H), 7.20-7.89 (m, 8H)
3p	Fmoc-Val-Ala-Leu-Val-*g*Ala-COOSu	-	806.2/806.9	80	1760	0.82-1.29 (m, 18H), 1.30-1.75 (m, 9H), 1.90 (m, 1H), 2.25 (m, 1H), 2.60-2.72 (m, 4H), 4.19 (t, *J* = 6.6, 1H), 4.32 (m, 3H), 4.55 (m, 2H), 4.95 (m, 1H), 5.58 (m, 1H), 6.12 (m, 1H), 5.91 (m, 1H), 6.54 (br, 1H), 7.20-7.89(m, 8H)



3a-p 4 5a-l

5 a-l: **X** = HCl or *p*-TsOH salt; **Y** = CH_3 or $CH_2C_6H_5$ group.

Scheme II — Synthesis of N^{α}-Fmoc-tripeptidylureas

Table II — Physical parameters of N^{α}-Fmoc-peptidyl ureas

Entry	R	R^1	R^2	Y	Yield (%)	m.p. °C	Mass[a] (*m/z*)
5a	$CH_2CH(CH_3)_2$	H	$CH_2C_6H_5$	CH_3	89	-	609.3
5b	$CH_2CH(CH_3)_2$	CH_3	$CH_2C_6H_5$	CH_3	90	147-49	623.4
5c	$CH_2C_6H_5$	CH_3	H	CH_3	80	205-07	533.3
5d	$CH(CH_3)C_2H_5$	H	CH_2OBzl	CH_3	78	160-62	639.3
5e	$CH_2COOBzl$	$CH(CH_3)C_2H_5$	$CH_2C_6H_5$	CH_3	82	173-75	757.4
5f	$CH_2COOBzl$	$CH_2C_6H_5$	H	CH_3	80	167-69	701.4
5g	H	$CH_2C_6H_5$	H	CH_3	94	173-75	553.4
5h	CH_2O^tBu	$CH_2C_6H_5$	$CH_2CH(CH_3)_2$	CH_3	90	180-81	695.4
5i	CH_2OBzl	H	$CH_2CH(CH_3)_2$	$CH_2C_6H_5$	89	175-76	715.3
5j	CH_2O^tBu	$CH_2C_6H_5$	CH_3	$CH_2C_6H_5$	89	156-57	729.4
5k	$CH_2COOBzl$	$CH(C_2H_5)CH_3$	CH_3	$CH_2C_6H_5$	89	156-57	757.4
5l	CH_2OBzl	H	CH_3	$CH_2C_6H_5$	89	156-57	673.3

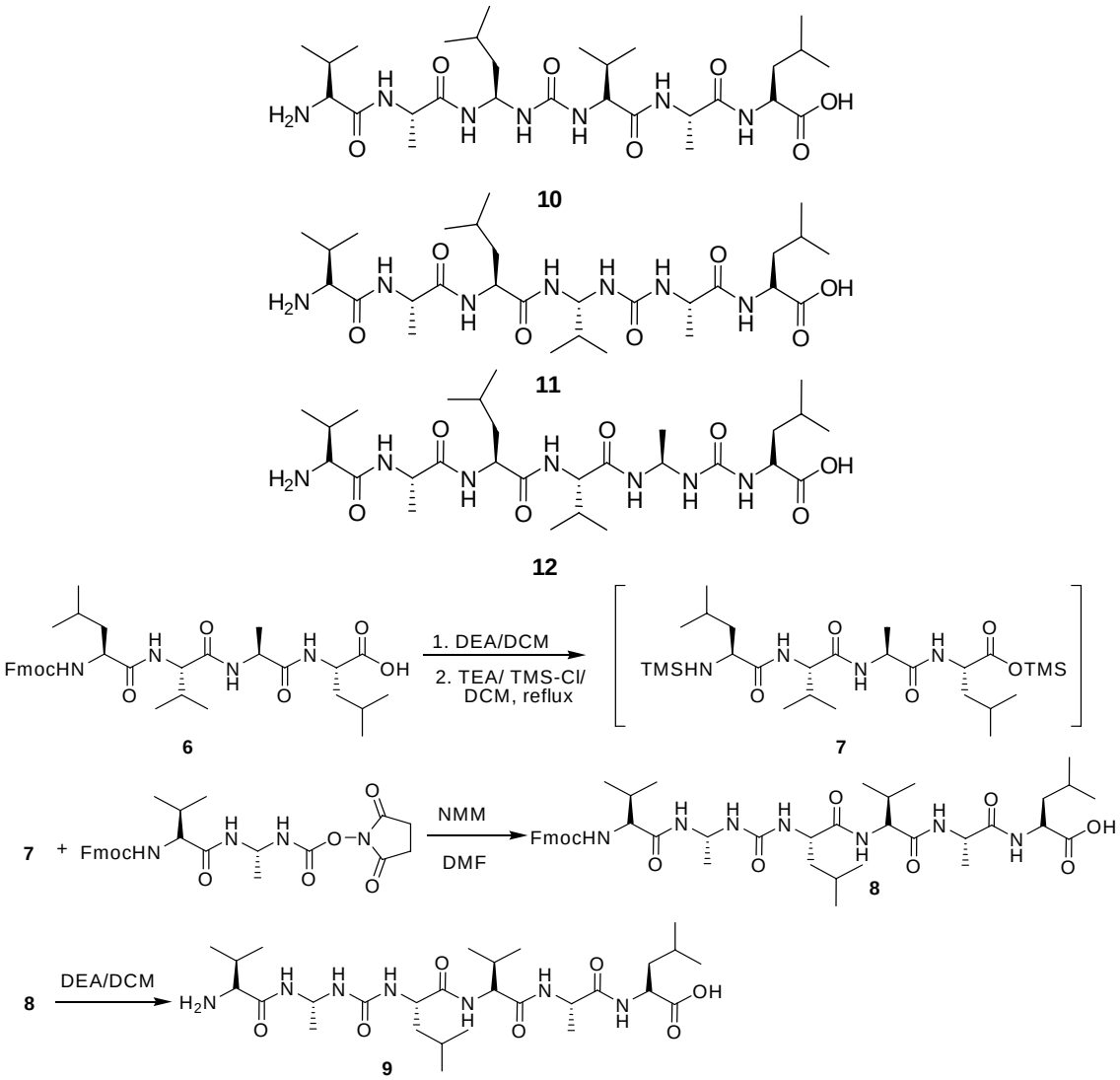
[a]MS (MALDI-TOF): *m/z* [M+Na]

Experimental Section

Melting points were determined using capillary method and are uncorrected. IR spectra were recorded on a Nicolet model impact 400D FT-IR spectrometer (KBr pellets, 3 cm^{-1} resolution). Specific rotations were recorded on Rudolf Research Autopol IV automatic polarimeter. Elemental analyses were carried out using Perkin-Elmer analyser and the samples were dried for 24 hr under vacuum before analysis. ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX 400 MHz spectrometer. Mass spectra were recorded on MALDI-TOF (KRATOS) and ESI-MS. All solvents were freshly distilled prior to use. Amino acid methyl ester hydrochlorides were prepared by using methanol and thionyl chloride. *p*-Toluene sulfonate salts of amino acid benzyl esters and *N*-Fmoc-peptide acid and azides were prepared by the procedures reported by us[30,31].

General procedure for the preparation of *O*-succinimidyl-(9*H*-fluoren-9-yl methoxycarbonylamino)-peptidyl carbamates, 3a-p

Fmoc-α-peptide acid (1 mmole) was dissolved in THF (5 mL) and cooled to –20°C. After addition of NMM (1.1 mmole) and ECF (1.1 mmole), it was stirred at -20°C for 15 min and allowed to warm to –5°C. It was then treated with an aqueous solution of NaN_3 (2.5 mmole in 2 mL of water) for 5 min. After concentration of the solvent under reduced pressure, the resulting residue was diluted with CH_2Cl_2 (15 mL), washed with 5% citric acid solution (3 × 5 mL), 5% sodium bicarbonate solution (3 × 5 mL), brine and dried over anhydrous Na_2SO_4. It was concentrated under reduced pressure to obtain the acyl azide which was used directly. The acyl azide was taken in toluene (5 mL) to which *N*-hydroxysuccinimide (1 mmole) and NMM (1.1 mmole) were added and refluxed at 65°C for 30 min. Alternatively the



Scheme III — Synthesis of H-Val-Ala- ψ(NH-CO-NH) -Leu-Val-Ala-Leu-OH 9

solution was exposed to microwave irradiation for 1-2 min. After the completion of the reaction, the carbamates **3a-p** precipitated from the toluene solution. The products were collected by filtration**,** washed with CH_2Cl_2, toluene and recrystallised from DMF/CH_2Cl_2 to afford the carbamates.

General procedure for the synthesis of N^{α}-Fmoc-oligopeptidyl ureas by the fragment condensation, 5a-l

To a stirred solution of amino acid methyl ester hydrochloride salt or amino acid benzyl ester *p*-toluene sulfonate (1.2 mmole) in 5 mL of DMF were successively added *O*-succinimidyl peptidyl carbamate **3a-p** (1 mmole) and NMM (1 mmole). After 10-30 min., the separated solid was filtered, washed with CH_2Cl_2/hexane and recrystallised using DMSO-water (7:3) to obtain peptidyl ureas.

Fmoc-Leu-Gly-*ψ*(NH-CO-NH)-Phe-OMe, 5a: IR (thin film): 1740, 1693, 1650 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 0.85-0.99 (m, 6H), 1.25 (m, 2H), 1.35-1.39 (m, 1H), 2.55 (d, 2H), 2.92 (m, 2H), 3.64 (s, 3H), 4.10 (m, 2H), 4.24-4.40 (m, 3H), 5.25 (m, 1H), 6.45 (m, 2H), 7.15-7.5 (m, 9H), 7.75 (d, 2H), 7.91 (d, 2H), 8.22 (d, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.2, 23.7, 24.9, 37.5, 40.3, 47.1, 52.0, 54.9, 61.7,

66.3, 67.7, 120.3, 125.7, 126.5, 127.3, 127.8, 128.4, 129.0, 137.5, 141.5, 144.0, 156.2, 169.0, 171.3, 174.4.

Fmoc-Leu-Ala-ψ(NH-CO-NH)-Phe-OMe, 5b: IR (thin film): 1735, 1693, 1655 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 0.85-0.99 (m, 6H), 1.17 (d, 3H), 1.35 (m, 2H), 1.41-1.60 (m, 1H), 2.95 (d, 2H), 3.15 (m, 2H), 3.61 (s, 3H), 3.95 (m, 1H), 4.2-4.45 (m, 3H), 5.3 (br, 1H), 6.5 (m, 2H), 7.15-7.5 (m, 9H), 7.75 (d, 2H), 7.9 (d, 2H), 8.15 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.5, 22.0, 23.7, 25.0, 37.9, 41.5, 47.6, 49.5, 52.3, 54.0, 65.3, 67.3, 120.1, 125.3, 126.7, 127.3, 127.9, 128.5, 129.3, 137.8, 141.5, 144.0, 156.7, 169.1, 172.1, 173.6.

Fmoc-Phe-Ala-ψ(NH-CO-NH)-Gly-OMe, 5c: IR (thin film): 1738, 1693, 1652 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.19 (d, 3H), 2.95 (d, 2H), 3.61 (s, 3H), 3.85-3.95 (m, 4H), 4.21-4.45 (m, 3H), 5.34 (d, 1H), 6.36 (d, 1H), 6.70 (d, 1H), 7.21-7.45 (m, 9H), 7.60-7.80 (m, 4H), 8.31 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.4, 37.5, 47.0, 49.7, 54.3, 64.0, 67.3, 120.0, 125.3, 126.9, 127.3, 127.9, 128.7, 129.3, 137.3, 141.0, 143.9, 156.0, 170.0, 171.8, 173.7.

Fmoc-Ile-Gly-ψ(NH-CO-NH)-Ser(Bzl)-OMe, 5d: IR (thin film): 1740, 1693, 1650 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 0.91 (m, 6H), 1.13 (m, 1H), 1.55 (m, 2H), 3.60 (s, 3H), 3.82-4.01 (m, 6H), 4.25-4.43 (m, 3H), 4.95 (s, 2H), 5.32 (d, 1H), 6.33 (m, 1H), 6.71 (s, 1H), 7.25-7.46 (m, 9H), 7.70-7.81 (m, 4H), 8.32 (d, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 11.7, 15.9, 25.6, 36.4, 37.5, 51.9, 57.3, 62.5, 65.3, 66.1, 67.5, 120.3, 125.1, 126.7, 127.1, 127.9, 128.9, 129.5, 137.8, 141.3, 143.9, 156.6, 170.1, 171.6, 173.4.

Fmoc-Asp(OBzl)-Ile-ψ(NH-CO-NH)-Phe-OMe, 5e: IR (thin film): 1742, 1693, 1655 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 0.92 (m, 6H), 1.13 (m, 1H), 1.55 (m, 2H), 2.55 (d, 2H), 2.94 (d, 2H), 3.61 (s, 3H), 3.85-4.05 (m, 3H), 4.21-4.42 (m, 3H), 4.90 (s, 2H), 5.31 (d, 1H), 6.33 (d, 1H), 6.75 (br,1H), 7.19-7.45 (m, 14H), 7.70 (d, 2H), 7.85 (d, 2H), 8.30 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 11.5, 15.9, 25.7, 36.1, 37.5, 47.7, 50.3, 55.3, 57.5, 65.3, 66.0, 67.5, 120.0, 121.3, 125.3, 126.9, 127.0, 127.6, 128.9, 129.5, 137.8, 141.6, 143.9, 156.3, 169.9, 172.3, 174.0, 176.1.

Fmoc-Asp(OBzl)-Phe-ψ(NH-CO-NH)-Gly-OMe, 5f: IR (thin film): 1745, 1693, 1652 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.55 (d, 2H), 2.87 (d, 2H), 3.60 (s, 3H), 3.81-4.05 (m, 4H), 4.20-4.41 (m, 3H), 5.00 (s, 2H), 5.34 (d, 1H), 6.36 (d, 1H), 6.72 (d, 1H), 7.15-7.45 (m, 14H), 7.70 (d, 2H), 7.92 (d, 2H), 8.31 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.0, 37.3, 47.3, 50.2, 54.3, 62.1, 64.4, 66.3, 67.9, 120.1, 125.3, 127.0, 127.6, 128.1, 128.6, 129.1, 137.6, 141.5, 144.2, 156.1, 170.3, 172.6, 174.3, 175.7.

Fmoc-Gly-Phe-ψ(NH-CO-NH)-Gly-OMe, 5g: IR (thin film): 1742, 1693, 1652 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.85 (d, 2H), 3.20 (m, 1H), 3.65 (s, 3H), 3.81-4.08 (m, 4H), 4.20-4.41 (m, 3H), 5.35 (m, 1H), 6.40-6.69 (br, 2H), 7.15-7.52 (m, 9H), 7.73 (d, 2H), 7.90 (d, 2H), 8.22 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 37.3, 47.3, 54.6, 60.6, 61.9, 62.3, 66.9, 119.9, 124.8, 126.6, 126.7, 128.0, 129.2, 137.5, 141.3, 143.8, 156.7, 169.8, 172.1, 173.5.

Fmoc-Ser(^{t}Bu)-Phe-ψ(NH-CO-NH)-Ile-OMe, 5h: IR (thin film): 1740, 1693, 1652 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 0.93 (6H, m), 1.15 (s, 10H), 1.55 (m, 2H), 2.86 (d, 2H), 3.64 (s, 3H), 3.80-4.01 (m, 5H), 4.22-4.43 (m, 3H), 5.29 (d, 1H), 6.44-6.56 (m, 2H), 7.17-7.50 (m, 9H), 7.70 (d, 2H), 7.97 (d, 2H), 8.35 (1H, m); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.1, 23.3, 24.9, 27.0, 37.6, 47.1, 51.8, 52.7, 54.5, 62.1, 66.4, 67.3, 73.8, 120.1, 125.2, 126.6, 127.2, 128.0, 128.5, 129.5, 138.0, 141.1, 144.2, 156.2, 169.7, 172.3, 174.4.

Fmoc-Ser(Bzl)-Gly-ψ(NH-CO-NH)-Leu-OBzl, 5i: IR (thin film): 1742, 1693, 1652 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 0.93 (d, 6H), 1.25 (m, 2H), 1.63 (m,1H), 3.32-3.90 (m, 6H), 4.22-4.45 (m, 3H), 4.95 (s, 2H), 5.25 (s, 2H), 5.45 (d, 1H), 6.54-6.75 (m, 2H), 7.15-7.55 (m, 14H), 7.71 (d, 2H), 7.95 (d, 2H), 8.25 (1H, m); ^{13}C NMR (100 MHz, DMSO-d_6): δ 22.1, 23.0, 24.6, 40.3, 46.9, 51.2, 52.8, 62.1, 64.5, 65.3, 66.7, 68.9, 119.7, 124.8, 126.7, 126.7, 127.3, 128.5, 129.2, 137.5, 138.3, 140.9, 143.6, 156.3, 170.1, 172.7, 175.4.

Fmoc-Ser(^{t}Bu)-Phe-ψ(NH-CO-NH)-Ala-OBzl, 5j: IR (thin film): 1740, 1693, 1650 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 1.15 (d, 3H), 1.25 (m, 9H), 2.92 (d, 2H), 3.31-3.9 (m, 5H), 5.10 (s, 2H), 5.35 (d, 1H), 6.31-6.50 (m, 2H), 7.24-7.55 (m, 14H), 7.80 (d, 2H), 7.95 (d, 2H), 8.33 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.2, 27.3, 37.5, 46.7, 50.1, 52.2, 54.5, 62.3, 64.2, 66.9, 73.7, 119.9, 124.8, 126.2, 126.7, 127.3, 127.8, 128.6, 129.2, 137.4, 140.9, 143.9, 156.8, 170.1, 172.3, 175.9.

Fmoc-Asp(OBzl)-Ile-ψ(NH-CO-NH)-Ala-OBzl, 5k: IR (thin film): 1742, 1693, 1655 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 0.91 (m, 6H), 1.11–1.25 (m, 4H), 1.55 (m, 2H), 2.55 (d, 2H), 3.55-3.90 (m, 3H), 4.15-4.35 (m, 3H), 5.00-5.21 (s, 4H), 5.65 (d, 1H), 6.54-6.75 (m, 2H), 7.10-7.55 (m, 14H), 7.75 (d, 2H), 7.90 (d, 2H), 8.35 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 11.3, 15.7, 17.2, 25.9, 36.0, 37.2, 47.3,

50.1, 51.2, 53.8, 62.1, 63.3, 66.9, 119.9, 124.8, 126.7, 126.9, 127.3, 127.8, 128.5, 129.2, 137.5, 140.9, 143.9, 156.7, 170.1, 173.4, 174.5, 176.0.

Fmoc-Ser(Bzl)-Gly-ψ(NH-CO-NH)-Ala-OBzl, 5l: IR (thin film): 1740, 1693, 1652 cm^{-1};^{1}H NMR (200 MHz, DMSO-d_6): δ 1.15 (d, 3H), 3.40-3.92 (m, 6H), 4.15-4.44 (m, 3H), 4.91 (s, 2H), 5.25 (s, 2H), 5.42 (d, 1H), 6.40-6.71 (m, 2H), 7.25-7.63 (m, 14H), 7.75 (d, 2H), 7.94 (d, 2H), 8.30 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 17.5, 47.3, 49.0, 52.2, 61.7, 62.3, 64.7, 67.1, 69.2, 120.2, 124.8, 126.5, 126.9, 127.3, 128.6, 129.2, 137.5, 140.9, 143.6, 156.3, 170.1, 172.1, 174.9.

Synthesis of [Val-Ala-Leu-Val-Ala-Leu] containing urea bond in different positions

Val-Ala-ψ(NH-CO-NH)-Leu-Val-Ala-Leu-OH, 9: To a stirred solution of **3a** (0.509 g, 1 mmole) in DMF (5 mL) was added freshly prepared *bis*-TMS-Leu-Val-Ala-Leu (from 0.497 g, 1.2 mmole of H_2N-Leu-Val-Ala-Leu-OH refluxed with 2.5 mmoles each of TEA and TMS-Cl in 10 mL of DCM) at RT. The resulting mixture was stirred for 30 min. After the completion of the reaction, the product was obtained by routine work-up. The crude Fmoc-Val-Ala-ψ(NH-CO-NH)-Leu-Val-Ala-Leu-OH was crystallized in DMSO-water. Yield: 0.650 g (79.0%); m.p., 203-05°C; MS (MALDI-TOF) *m/z* observed: 844.5 $[M+Na]^+$, 860.4 $[M+K]^+$. The suspension of Fmoc-hexapeptidylurea in 4 mL of DCM and 4 mL of DEA was stirred for an hr at RT. After the completion of the deprotection (by IR analysis), the solvent was concentrated under reduced pressure. The residue was recrystallized by adding ether. Yield: 0.385 g (64.1%); m.p. 156 – 158°C; IR (thin film): 1700, 1650 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.85- 1.25 (m, 24H), 1.39-1.54 (m, 10H), 1.85-1.96 (m, 2H), 2.05-2.21 (m, 2H), 3.57 (m, 1H), 4.12 (m, 1H), 4.90 (m,. 1H), 5.20 (m, 1H), 5-52-5.61 (m, 2H), 6.12 (br, 1H), 6.32 (m, 1H); ^{13}C NMR (200 MHz, DMSO- d_6) δ 10.9, 13.7, 21.4, 22.7, 24.2, 36.1, 39.0, 41.3, 50.4, 53.6, 115.3, 115.7, 116.2, 118.7, 124.8, 126.5, 127.9, 129.2, 130.4, 131.2, 154.9, 156.6, 158.0, 158.3, 169.8; MS (ESI-MS) Calcd. for $C_{28}H_{53}N_7O_7$: *m/z* 600.4 $[M+1]^+$. Found: 600.4 $[M+1]^+$.

Val-Ala-Leu-ψ(NH-CO-NH)-Val-Ala-Leu-OH, 10: To a stirred solution of **3n** (0.635.8 g, 1 mmole) in DMF (5 mL) was added freshly prepared *bis*-TMS-Val-Ala-Leu (from 0.362 g, 1.2 mmole of H_2N-Val-Ala-Leu-OH refluxed with 2.5 mmoles each of TEA and TMS-Cl in 10 mL of DCM) at RT. and stirred for 30 min. The crude Fmoc-Val-Ala-Leu-ψ(NH-CO-NH)-Val-Ala-Leu-OH was crystallized in DMSO-water. Yield: 0.640 g (77.8%); m.p. 214-16°C; MS (MALDI-TOF) *m/z* observed: 844.6 $[M+Na]^+$, 860.6 $[M+K]^+$. The suspension of Fmoc-hexapeptidylurea in 4 mL of DCM was treated with 4 mL of DEA for an hr at RT and the product was isolated after work-up. Yield: 0.375 g (62.5%); m.p. 156-58°C; IR (thin film): 1700, 1655 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.85-1.19 (m, 24H), 1.39-1.54 (m, 10H), 1.87-2.00 (m, 4H), 2.00-2.21 (m, 2H), 4.12 (m, 1H), 4.75 (m, 3H), 5.32-5.45 (m, 3H), 6.52 (m, 1H), 6.82 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.9, 13.7, 21.4, 22.7, 24.2, 36.1, 39.0, 41.3, 50.4, 53.6, 115.3, 115.7, 116.2, 118.7, 124.8, 126.5, 127.9, 129.2, 130.4, 131.2, 154.9, 156.6, 158.0, 158.3, 169.8; MS (ESI-MS) Calcd. for $C_{28}H_{53}N_7O_7$: *m/z* 600.4 $[M+1]^+$. Found: 600.5 $[M+1]^+$.

Val-Ala-Leu-Val-ψ(NH-CO-NH)-Ala-Leu-OH, 11: To a stirred solution of **3o** (0.735 g, 1 mmole) in DMF (5 mL) was added freshly prepared *bis*-TMS-Ala-Leu (from 0.243 g, 1.2 mmole of H_2N-Ala-Leu-OH refluxed with 2.5 mmoles each of TEA and TMS-Cl in 10 mL of DCM) at RT. and stirred for 30 min. The crude Fmoc-Val-Ala-Leu-Val-ψ(NH-CO-NH)-Ala-Leu-OH was crystallized in DMSO-water. Yield: 0.640 g (77.8%); m.p. 212-14°C; MS (MALDI-TOF) *m/z* observed: 844.5 $[M+Na]^+$, 860.5 $[M+K]^+$. The suspension of Fmoc-hexapeptidylurea in 4 mL of DCM was treated with 4 mL of DEA and the product was isolated after work-up. The resulting residue was triturated with ether. Yield: 0.380 g (63.3%); m.p. 156-58°C; IR (thin film): 1702, 1652 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 0.90- 1.15 (m, 24H), 1.29-1.40 (m, 10H), 1.79-1.90 (m, 2H), 1.99-2.35 (m, 3H), 3.57-3.64 (m, 3H), 4.20- 4.35 (m, 2H), 4.85 (m,. 2H), 5.21 (m, 2H), 5.52-5.61 (m, 1H), 6.27 (m, 1H), 6.39 (m, 1H), 7.12 (br, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.9, 13.7, 21.4, 22.7, 24.2, 36.1, 39.0, 41.3, 50.4, 53.6, 115.3, 115.7, 116.2, 118.7, 124.8, 126.5, 127.9, 129.2, 130.4, 131.2, 154.9, 156.6, 158.0, 158.3, 169.8; MS (ESI-MS) Calcd. for $C_{28}H_{53}N_7O_7$: *m/z* 600.4 $[M+1]^+$. Found: 600.4 $[M+1]^+$.

Val-Ala-Leu-Val-Ala-ψ(NH-CO-NH)-Leu-OH, 12: To a stirred solution of **3p** (0.806 g, 1 mmole) in DMF (5 mL) was added freshly prepared *bis*-TMS-Leu (from 0.158 g, 1.2 mmole of Leu refluxed with 2.5 mmoles each of TEA and TMS-Cl in 10 mL of DCM) at RT. and stirred for 30 min. The crude Fmoc-Val-Ala-Leu-Val-Ala-ψ(NH-CO-NH)-Leu-OH was crystallized in DMSO-water. Yield: 0.625 g (76.1%); m.p. 208-10°C; MS (MALDI-TOF) *m/z*

observed: 844.6 $[M+Na]^+$, 860.5 $[M+K]^+$. The suspension of Fmoc-hexapeptidylurea in 4 mL of DCM was treated with 4 mL of DEA and the product was isolated after work-up.The resulting residue was triturated with ether. Yield: 0.390 g (65.1%); m.p. 156-58°C; IR (thin film): 1699, 1650 cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 0.89-1.10 (m, 24H), 1.21-1.55 (m, 10H), 1.70-1.91 (m, 4H), 1.98-2.10 (m, 3H), 3.75 (m, 2H), 4.05 (m, 1H), 4.60 (m, 1H), 4.98 (m, 1H), 5.02-5.10 (m, 1H), 6.33 (br, 1H), 6.82 (br, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ 10.9, 13.7, 21.4, 22.7, 24.2, 36.1, 39.0, 41.3, 50.4, 53.6, 115.3, 115.7, 116.2, 118.7, 124.8, 126.5, 127.9, 129.2, 130.4, 131.2, 154.9, 156.6, 158.0, 158.3, 169.8; MS (ESI-MS) Calcd. for $C_{28}H_{53}N_7O_7$: *m/z* 600.4 $[M+1]^+$. Found: 600.5 $[M+1]^+$.

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