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Synthesis of ureidopeptides using pentafluorophenyl carbamates from N^{α}-Fmoc-peptide acids

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Highly active and shelf stable pentafluoro phenol derived Fmoc-peptidyl carbamates have been synthesized from corresponding Fmoc-peptidyl isocyanates. The utility of pentafluorophenyl carbamate intermediates has been demonstrated by the synthesis of tri, penta and hexapeptidyl ureas which are obtained in good yields. All the synthesised compounds have been characterized by ^{1}H NMR, ^{13}C NMR and mass spectroscopy. The coupling reaction using pentafluorophenyl carbamates to insert urea bond between α amino acids is fast, clean and high yielding.

Keywords: Curtius rearrangement, peptide isocyanate, pentafluorophenyl carbamate, peptidyl urea

Peptides play an important role in drug discovery as they can present various avenues by permutations of acidic, basic, hydrophilic, hydrophobic and aromatic side chains. Peptides are constituents of hormones and play important roles as neuro-transmitters and neuromodulators in organisms. As most of the biochemical changes are controlled by these molecules, one of the major approaches in drug design is to target and exploit the interactions of these molecules with other macromolecules. Problems arise from their rapid metabolism by proteolysis and their interaction with multiple receptors. The mammalian body presents many barriers to the entry of macro-molecules and thus peptides fall for poor absorption because they do not readily pass across biological membranes. There is a swift metabolism by pro-teolytic enzymes and rapid excretion takes place through liver and kidneys. These problems prevent peptides from being the most sought after molecules for drug design.

Among the backbone modified peptidomimetics[1], oligoureas are the most interesting molecules. As the urea bond resembles the amide bond, the molecules have many common but enhanced properties like bonds that are rigid, resistant towards proteolytic degradation and improved hydrogen bonding capacity due to the presence of both H-bond donor and acceptor in the same molecule[2-4]. The extensive demand for ureas has arisen due to its pronounced hydrogen bonding capacity which makes them more soluble and therefore, biologically more useful. Moreover, the urea incorporated peptides have shown good metabolic stability and better translocation across membranes. Oligoureas are capable of folding into well defined 3-D structures similar to those of natural peptides, which makes them ideal precursors for the synthesis of novel foldamers[5], β-sheets and hair pin turns[6,7]. These molecules have gained importance as novel therapeutic agents also. They act as antagonists for CCK-B receptors and endothelin[7]. They have also shown poitive results when used as antagonists for tat proteins, which bind with TAR (RNA) and this interaction is crucial for the pathogenesis of HIV1 proteases[8]. Oligourea analogues effectively interact with TAR (RNA) and inhibit HIV1 protease activity. Hence, they can be developed as anti-HIV therapeutics.

An important feature of the N^{α}-Fmoc-peptidyl isocyanates is that they are stable at RT and can be conveniently stored for several days at 4°C. On the other hand, the peptide ester isocyanates will undergo cyclization to form a cyclic hydantoin on storage (**Figure 1**) (Ref 9).

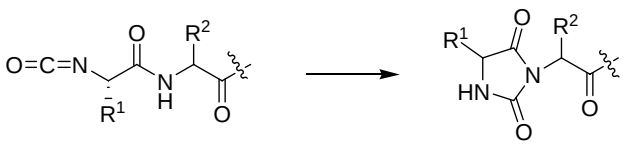


Figure 1 — Byproduct formation from peptidyl ester isocyanate

Results and Discussion

In the earlier procedures, the amino of the amino acid ester was converted to its isocyanate employing phosgene which was subsequently coupled to another amine to incorporate urea bond to obtain both symmetrical and unsymmetrical ureas[10]. The same method was followed for the synthesis of peptide ester isocyanates also (**Figure 2**). The idea of employing Curtius rearrangement for *N*-protected peptide to obtain isocyanate group on C termini has also been reported earlier. But for both the stability and synthetic convenience, β-amino acids were employed which were converted to the corresponding isocyanates *via* acid azides and the ureas were obtained after coupling with a peptide/amino acid ester. This led to the insertion of one extra carbon atom into the product[11]. The Curtius rearrangement has been employed for N^{α}-Fmoc-protected α-peptide acid azides which gave ureas after coupling with another amino acid ester[12]. In continuation of these studies, the isocyanates have been now converted into active N^{α}-Fmoc-peptidyl pentaflourophenylmethyl carbamates which also serve as starting materials for the synthesis of ureas. Because, such carbamates are more stable precursors and offer the advantage of long storage with higher shelf life than the corresponding isocyanates. Guichard *et al*., have reported application of succinamidyl carbamates in the synthesis of ureidopeptides[13].

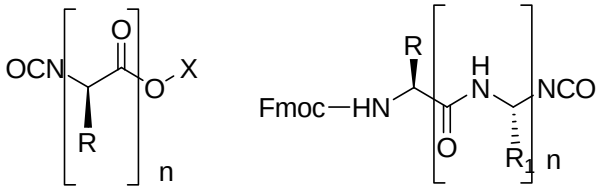
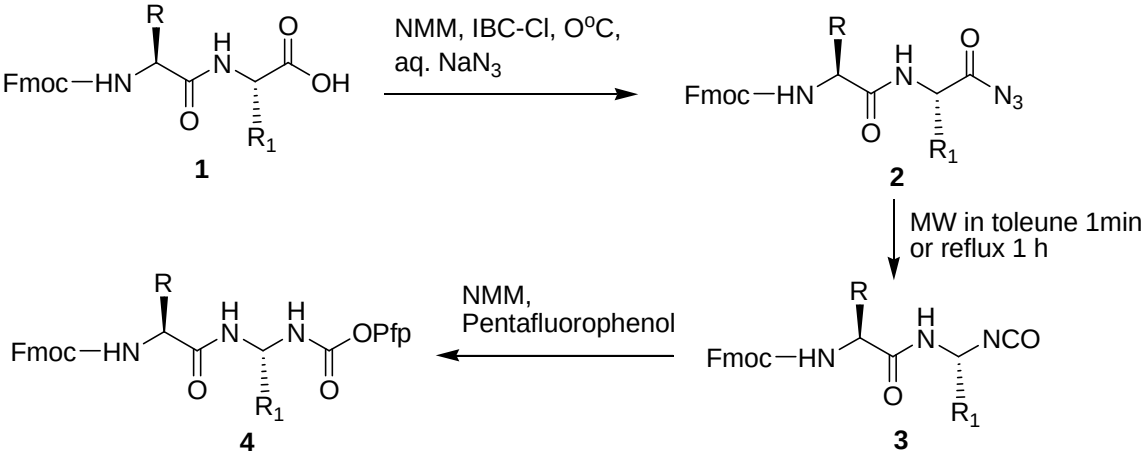


Figure 2 — N-terminal peptide isocyanate

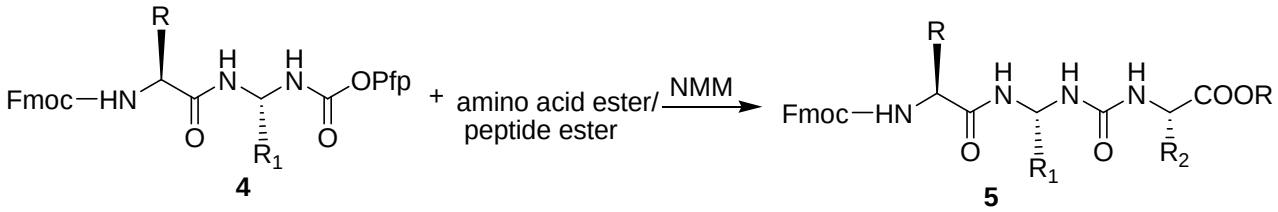
N^{α}-Fmoc-peptide acids **1** were prepared by employing the *bis*-TMS method[14]. N^{α}-Fmoc-amino acid was dissolved in THF and to it was added isobutyl chloroformate and NMM at 0°C. To this milieu was added freshly prepared *bis*-TMS-amino acid and stirred for 4 hr. The peptide acid **1** was converted to its acid azide **2** using mixed anhydride method. N^{α}-Fmoc-peptide acid **1** was dissolved in dry THF and treated with isobutyl chloroformate and NMM at 0°C to make a mixed anhydride. To this milieu was added aq. NaN_3 to obtain the corresponding acid azide **2** (Ref 15). The solvent was evaporated at low temperature under reduced pressure. The residue so obtained was dissolved in DCM and treated with 5% $NaHCO_3$ solution to remove any unreacted peptide acid present. The peptide acid azides were characterized by spectroscopic analysis including IR. The N^{α}-Fmoc-peptide acyl azide **2** was subsequently converted into the corresponding isocyanate **3** *via* Curtius rearrangement by exposure to microwave irradiation for about 1 min in toluene or refluxing in toluene for 1 hr. The progress of reaction was monitored by IR spectroscopy. The disappearance of acid azide peak at 2140 cm^{-1} and appearance of strong absorption peak at 2250 cm^{-1} in the IR spectrum indicated its formation. Toluene was evaporated and the residue obtained was dissolved in DCM. An equimolar quantity of pentafluorophenol and NMM were added to the reaction mixture and stirred for 30 min (**Scheme I**). The resulting N^{α}-Fmoc-peptidyl pentafluorophenyl carbamates **4** were recrystallized from ethyl acetate and hexane (2:8) and were obtained in excellent yield (**Table I**).



Scheme I — Synthesis of pentafluorophenyl (9*H*-fluoren-9-ylmethoxy carbonylamino) peptidyl carbamates

Table I — Pentafluorophenyl (9*H*-fluoren-9-ylmethoxycarbonylamino) peptidyl carbamates

Sl. No.	Compd	m.p. °C	Yield (%)	Mass (calc./obsd.)
1	Fmoc-Val-Ala-NHCOOPfp	162	95	614.5/614.3
2	Fmoc-Ala-Phe-NHCOOPfp	215	96	662.5/662.3
3	Fmoc-Gly-Val-NHCOOPfp	192	94	600.4/600.6
4	Fmoc-Phe-Leu-NHCOOPfp	211	98	704.6/704.5
5	Fmoc-Leu-Ala-NHCOOPfp	208	96	628.5/628.5
6	Fmoc-Phe-Pro-NHCOOPfp	133	97	688.6/688.1
7	Fmoc-Asp(OBzl)-Glu(OBzl)-NHCOOPfp	191	92	868.7/868.7
8	Fmoc-Leu-Val-NHCOOPfp	216	96	656.6/656.3
9	Fmoc-Val-Ala-Leu-NHCOOPfp	212	94	727.6/727.9
10	Fmoc-Tyr(O^{t}Bu)-Pro-NHCOOPfp	152	94	760.7/760.2



4 **5**

Scheme II — General scheme for the synthesis of peptidyl ureas

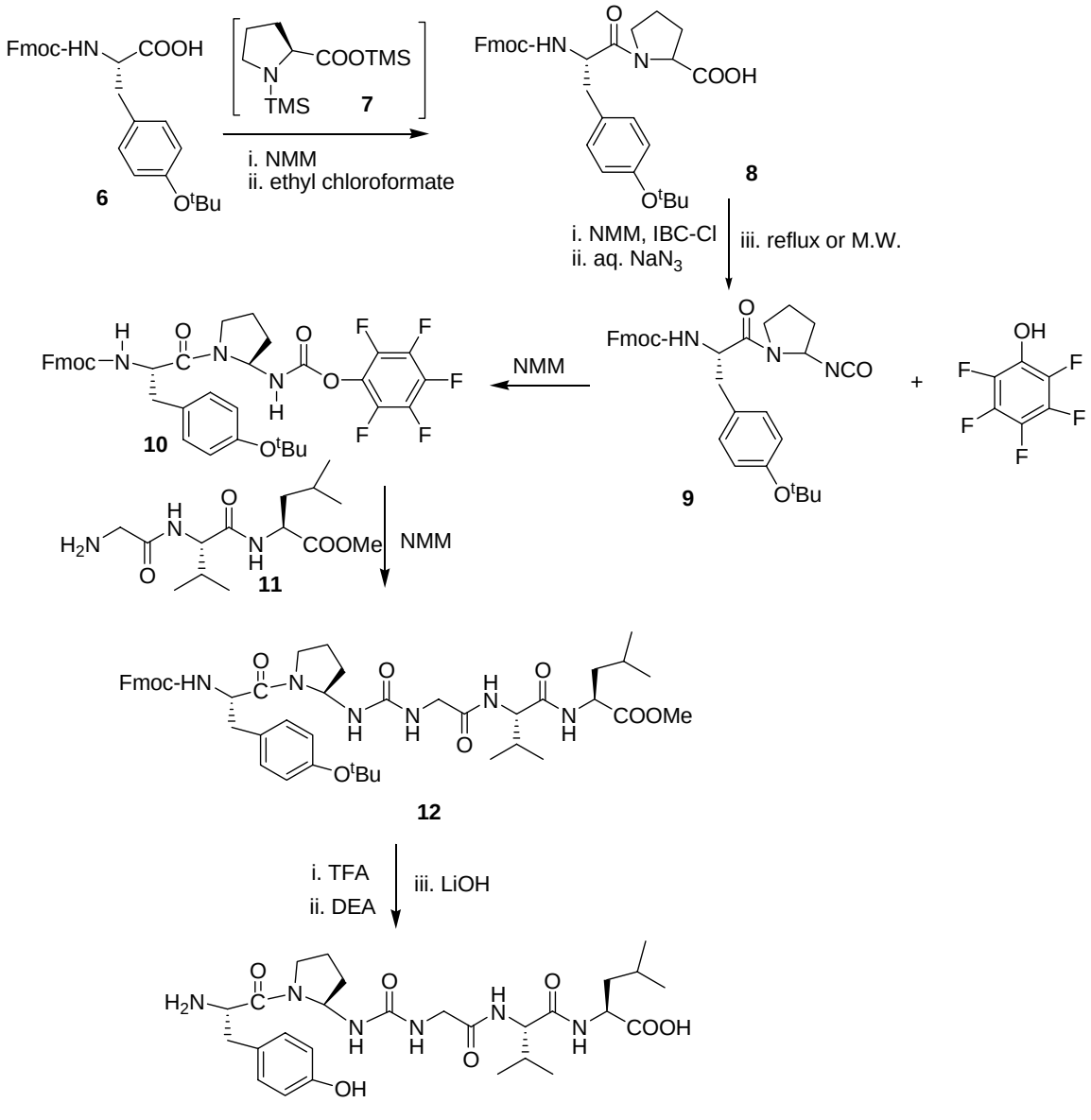
Table II — N^{α}-Fmoc-peptidyl ureas

Sl. No.	Compd	m.p. °C	Yield (%)	Mass (cald./obsd.)
1	Fmoc-Val-Ala-ψ(NH-CO-NH)-Leu-OMe	130	89	575.6/575.5
2	Fmoc-Gly-Val-ψ(NH-CO-NH)-Phe-OMe	175	94	595.6/595.7
3	Fmoc-Ala- Phe-ψ(NH-CO-NH)-Gly-OMe	142	88	567.5/567.6
4	Fmoc-Phe-Leu-ψ(NH-CO-NH)-Ala-OMe	170	89	623.7/623.7
5	Fmoc-Val-Ala-Leu-ψ(NH-CO-NH)-Val-Ala-Leu-OMe	206	90	858.0/858.4
6	Fmoc-Leu-Ala-ψ(NH-CO-NH)-Val-OMe	103	78	910.0/910.3
7	Fmoc-Tyr(O^{t}Bu)-Pro-ψ(NH-CO-NH)-Phe-Pro-Gly-OMe	193	82	725.8/725.8
8	Fmoc-Phe-Pro-ψ(NH-CO-NH)-Leu-OBzl	121	80	891.9/892.5
9	Fmoc-Asp(OBzl)-Glu(OBzl)-ψ(NH-CO-NH)-Val-OBzl	172	84	671.7/671.7
10	Fmoc-Gly-Val-ψ(NH-CO-NH)-Phe-OBzl	153	70	617.6/617.6

N^{α}-Fmoc-peptidyl pentafluorophenyl carbamates **4** were found to be completely stable at RT and can be stored for several months without any noticeable decomposition. The stable active pentafluorophenyl carbamates have been used for the synthesis of N^{α}-Fmoc-peptidyl urea esters **5**. In a typical procedure, N^{α}-Fmoc-peptidyl pentafluorophenyl carbamate **4** was dissolved in DMF: THF (1:1) at RT and methyl or benzyl ester of amino acid or peptide ester was added and stirred for about 4 hr to obtain N^{α}-Fmoc-protected peptidyl ureas **(Scheme II)**. Using this protocol, several N^{α}-Fmoc-tripeptidyl ureas esters have been prepared **(Table II)**.

Further useful application of the N^{α}-Fmoc-peptidyl pentafluorophenyl carbamates was demonstrated by the synthesis of ureido β-casomorphin (H-Tyr-Pro-ψ(NH-CO-NH)-Gly-Val-Leu-OH) by 2+3 fragment coupling (**Scheme III**) and ureido H-Val-Aal-Leu-ψ(NH-CO-NH)-Val-Ala-Leu-OH hexapeptide by 3+3 approach (**Scheme IV**).

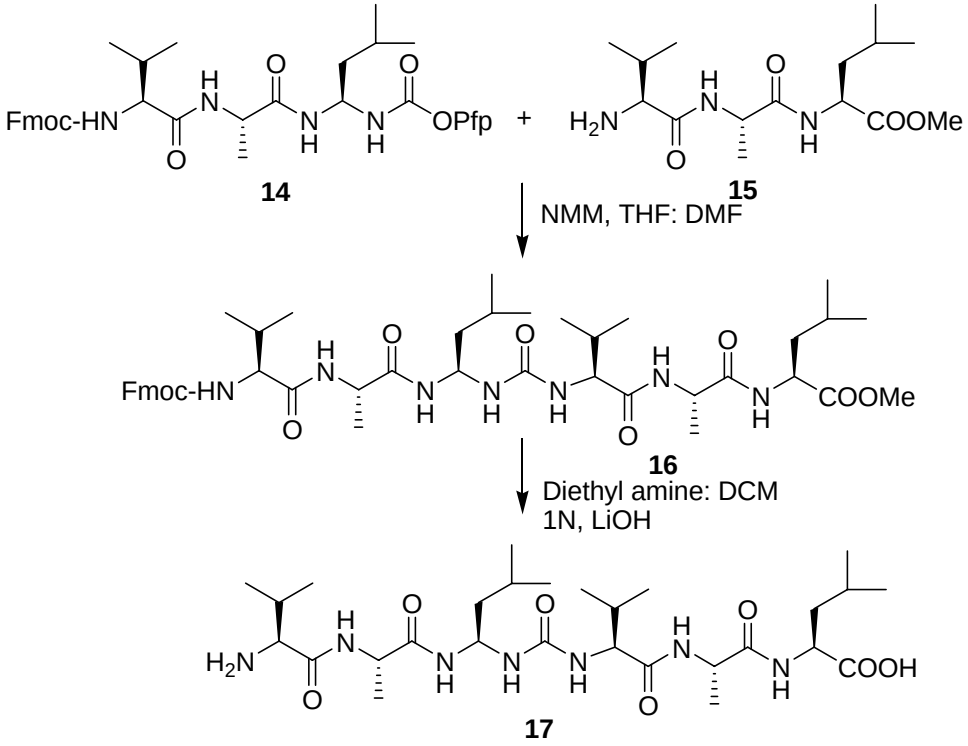
The N^{α}-Fmoc-Tyr(^{t}Bu)-Pro-OH **8** was prepared *via* mixed anhydride route by employing O,N-*bis*-



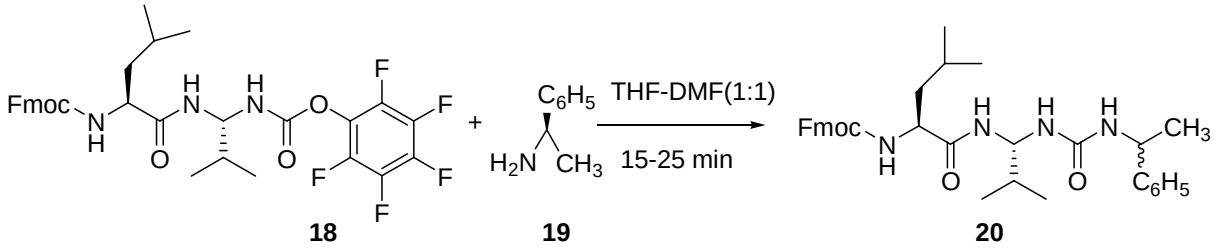
Scheme III — Synthesis of H-Tyr-Pro-ψ(NH-CO-NH)-Gly-Val-Leu-OH

trimethylsilylproline **7**. The dipeptide acid **8** was converted to acyl azide using mixed anhydride and aq. NaN_3 and then subjected to Curtius rearrangement. The resulting N^{α}-Fmoc-Tyr(tBu)-Pro-NCO **9** was trapped with pentafluorophenol in presence of an equimolar quantity of NMM at RT to afford the activated carbamate N^{α}-Fmoc-Tyr(^{t}Bu)-Pro-NHCOOPfp **10** as a crystalline solid. On the other hand, amino free Gly-Val-Leu-OMe **11** was prepared by step wise method. In a typical procedure, Fmoc-Val-OH was activated *via* mixed anhydride and coupled with Leu-OMe to obtain Fmoc-Val-Leu-OMe. From this dipeptide, Fmoc function was deprotected using diethyl amine (60% DEA in DCM). The deprotection, as monitored by TLC, was found to be complete in about 45 min. The free dipeptide ester (H-Val-Leu-OMe) was coupled to Fmoc-Gly-OH by using ethyl chloroformate in presence of NMM. The resultant Fmoc-Gly-Val-Leu-OMe was purified by column chromatography. After the deprotection of Fmoc functionality, **11** was coupled with N^{α}-Fmoc-Tyr(^{t}Bu)-Pro-NHCOOPfp **10**.

The coupling, when carried out at RT, was complete in about 4 hr. The routine workup of the reaction mixture gave the crude N^{α}-Fmoc-Tyr(^{t}Bu)-Pro-ψ(NH-CO-NH)-Gly-Val-Leu-OMe **12** (**Scheme II**).



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



Scheme V — Synthesis of peptidyl urea adducts

This was then purified by column chromatography. A similar strategy was followed for the synthesis of N^{α}-Fmoc-Val-Ala-Leu-ψ(NH-CO-NH)-Val-Ala-Leu-OMe **16** (**Scheme IV**) also. The deprotection of ^{t}butyl group from N^{α}-Fmoc-Tyr (O^{t}Bu)-Pro-ψ(NH-CO-NH)-Gly-Val-Leu-OMe **12** was carried out by treating with TFA. Excess of TFA was removed under reduced pressure. The deprotection reaction was quantitative. To the resulting residue, DEA was added to deprotect the Fmoc group completely. Finally, the peptide was hydrolysed to cleave the methyl ester group using 1N LiOH in methanol. The reaction mixture was concentrated under reduced pressure and the product purified by recrytallization from ethyl acetate and hexane. The prepared free peptidyl urea acid H-Tyr-Pro-ψ(NH-CO-NH)-Gly-Val-Leu-OH **13** was isoated and fully characterized after purification.

Racemization Study

The conversion of N^{α}-Fmoc-peptide acid azides to their *O*-pentafluorophenyl peptidyl carbamates *via* their isocyanates as well as coupling to amino acid esters was found to retain enantiomeric purity. This was confirmed by ^{1}H NMR analysis of the diastereomers of peptidyl urea adducts made by the coupling of Fmoc-Leu-Val-NHCOOPfp **18** with *R*-(+), *S*-(-) and racemic mixture of 1-phenylethyl-amine[16] **19** (**Scheme V**) in three separate experiments. The urea adducts **20a**, **20b** and **20c** were isolated as solids and their ^{1}H NMR analysis was carried out. The methyl protons of 1-phenylethylamine urea adducts **20b** (δ 1.31 and 1.29) and **20c** (δ 1.30 and 1.29) were observed as doublets, while the methyl protons of the urea adduct **20a** (δ 1.31, 1.30, 1.29) was observed as

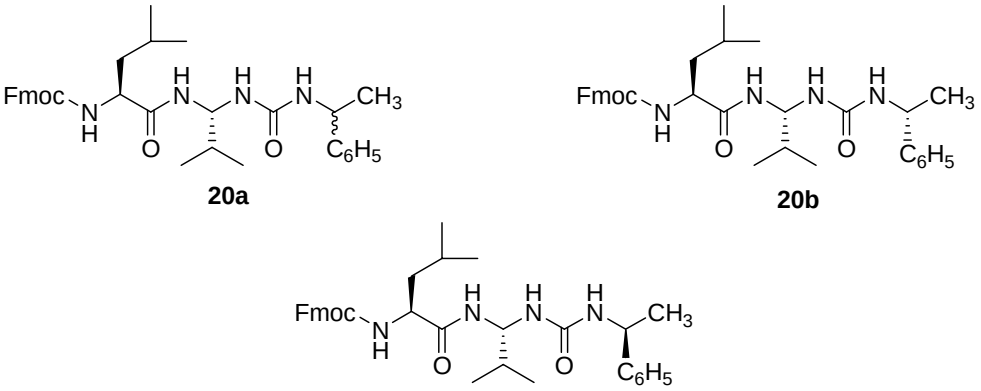


Figure 3 — Peptides for recemization studies

two doublets. Thus, the protocol employed in these studies is devoid of racemization (**Figure 3**).

Experimental Section

All chemicals were commercial products of the best grade available. Melting points were determined on a Buchi model 150 melting point apparatus in open capillaries and are uncorrected. IR spectra were recorded on a Nicolet model impact 400 D FT-IR spectrometer (KBr pellets, 3 cm^{-1} resolution). ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX 300 MHz spectrometer. The microwave reaction was carried out in a LG MS 194A microwave oven producing microwave radiation with a frequency of 2450 MHz.

General procedure for the synthesis of N^{α}-Fmoc-peptide acids, 1

A solution of N^{α}-Fmoc-amino acid/peptide acid (1 mmole) in dry THF (10 mL) was cooled to 0°C and added NMM (1 mmole) and isobutyl chloroformate (1 mmole). The resulting mixture was stirred for 5 min and the solution containing *bis*-trimethyl silyl amino acid in DCM was added directly in one lot. The stirring was continued till the completion of the reaction. The solvent was evaporated under reduced pressure and the residue was dissolved in 10% aq. Na_2CO_3 solution (10 mL) and washed with diethyl ether (2×10 mL). The aqueous layer was acidified using 5% HCl (10 % citric acid solution in case of amino acid containing side chain protecting groups such as Boc, ^{t}Bu). The precipitated solid was extracted into EtOAc (3×10 mL) and dried over anhyd. Na_2SO_4. The solvent was removed under reduced pressure and the residue was recrystallised using EtOAc and *n*-hexane to obtain analytically pure N^{α}–Fmoc-peptide acids.

General procedure for the synthesis of N^{α}-Fmoc-peptide acid azides, 2

To an ice-cold solution of N^{α}-Fmoc-peptide acid (1 mmole) in dry THF (5 mL) were added NMM (0.11 mL, 1 mmole) and isobutyl chloroformate (1.1 mmole) and the mixture was stirred at –10 °C for 5 min. The resulting reaction mixture was treated with aq. NaN_3 (0.098 g, 1.5 mmoles in 1 mL of water) and stirred for another 30 min. After the completion of the reaction, the organic layer was evaporated and the residue was dissolved in CH_2Cl_2 (30 mL). It was washed thrice with 10 mL portions of 5% HCl, 5% aq. $NaHCO_3$, brine and finally dried over anhyd. Na_2SO_4. The solvent was evaporated *in vacuo* and recrystalized using ethyl acetate and hexane (3:7).

General procedure for the synthesis of isocyanates of N^{α}-Fmoc-peptide acids, 3

N^{α}-Fmoc-peptide acid azide (1 mmole) was dissolved in toluene (10 mL) and exposed to microwave irradiation until the rearrangement was complete. The rearrangement was monitored by IR spectra by the complete disappearance of azide peak at 2110 cm^{-1} and a new strong absorption peak at 2250 cm^{-1}. The remaining toluene solution was evaporated and the resulting compound was used in the next step directly.

General procedure for the synthesis of pentafluorophenyl (9*H*-fluoren-9-ylmethoxy carbonyl-amino) peptidyl carbamates, 4

To a stirred solution of N^{α}-Fmoc-peptide isocyanate (1 mmole) in toluene (5 mL) was added pentafluoro phenol (1 mmole) and NMM (1.1 mmole) at RT. It was stirred till the completion of the reaction

(generally 1 hr). The separated solid was filtered and washed with DCM and toluene (1:1) to get the product. It was recrystallized from DMF-DCM.

General procedure for the synthesis of peptidyl ureas using carbamates, 5

To a stirred solution of amino acid ester/ peptide ester (1 mmole) in DMF (5 mL), NMM (2 mmole), carbamate (1 mmole) was added and stirred at RT till the completion of the reaction. The separated solid was filtered and recrystallized from ethyl acetate and hexane (2:1) to get the dipeptidyl ureas as crystalline white solids.

Fmoc-Val-Ala-NHCOOPfp: IR (thin film): 1756, 1700 cm^{-1}; NMR (DMSO-d_6): δ 0.92 (t, 6H), 1.18 (d, J = 6.2 Hz, 1H), 1.88 (m, 1H), 3.88 (m, 1H), 4.21 (t, J = 5.9 Hz, 1H), 4.41 (d, J = 5.9 Hz, 2H), 4.9 (m, 1H), 5.03 (d, J = 7.7 Hz, 1H), 7.25-7.76 (m, 8H); ^{13}C NMR (DMSO-d_6): δ 17.3, 22.2, 28.5, 47.0, 51.0, 59.2, 69. 4, 119.9, 124.0, 126.2, 127.2, 137.5, 139.1, 141.4, 143.8, 155.5, 156.3, 169.3. Anal. Calcd. for $C_{29}H_{26}F_5NaN_3O_5$, $[M+Na]^+$: 614.52. Found: 614.3.

Fmoc-Aal-Phe-NHCOOPfp: IR (thin film): 1745, 1702 cm^{-1}; NMR (DMSO-d_6): δ 1.32 (d, J = 6.5 Hz, 3H), 2.95 (d, J = 6.4 Hz, 2H), 3.81 (m, 1H), 4.10 (t, J=5.9 Hz, 1H), 4.30 (d, J = 5.9 Hz, 1H), 5.03 (d, J=7.7 Hz, 1H), 5.31 (m, 1H), 7.13-7.87 (m, 13H)); ^{13}C NMR (DMSO- d_6): δ 17.4, 46.4, 47.0, 52.9, 44.7, 69.4, 120.0, 124.1, 126.2, 127.2, 128.2, 136.4, 141.5, 148.7, 154.7, 169.2. Anal. Calcd. for $C_{33}H_{26}F_5NaN_3O_5$, $[M+Na]^+$: 662.56. Found: 662.3.

Fmoc-Gly-Val-NHCOOPfp: IR (thin film): 1750, 1698 cm^{-1}; NMR (DMSO- d_6): δ 0.93 (t, 6H), 1.84 (m, 1H), 3.90 (m, 1H), 4.21 (t, J = 6.6 Hz, 1H), 4.43 (m, 2H), 4.26 (br t, 2H), 7.25-7.76 (m, 8H); ^{13}C NMR (DMSO- d_6): δ 18.3, 24.2, 36.0, 43.7, 47.2, 52.3, 55.1, 69.4, 119.9, 120.8, 124.2, 126.1, 127.2, 128.8, 130.4, 137.4, 141.5, 148.7, 154.3, 170.4. Anal. Calcd. for $C_{28}H_{24}F_5NaN_3O_5$, $[M+Na]^+$: 600.49. Found: 600.6.

Fmoc-Phe-Leu-NHCOOPfp: IR (thin film): 1752, 1700 cm^{-1}; NMR (DMSO- d_6): δ 0.93 (d, J = 5.2 Hz, 6H), 1.33 (m, 2H), 1.63 (m, 1H), 2.87 (d, J = 6.2Hz, 2H), 4.19 (t, J = 6.6 Hz, 1H), 4.41, (m, 2H), 5.07 (d, J = 8.8, 1H), 7.27-7.41 (m, 13H); ^{13}C NMR (DMSO-d_6): δ 17.2, 21.4, 24.2, 36.0, 43.7, 47.2, 52.3, 55.1, 69.4, 119.9, 120.8, 124.2, 126.1, 127.2, 128.8, 130.4, 137.5, 141.5, 148.7, 154.3, 170.4. Anal. Calcd. for $C_{36}H_{32}F_5NaN_3O_5$, $[M+Na]^+$: 704.64. Found: 704.5.

Fmoc-Leu-Ala-NHCOOPfp: IR (thin film): 1740, 1698 cm^{-1}; NMR (DMSO- d_6): δ 0.80-0.88 (m, 6H), 1.17 (d, J = 7.13, 3H), 1.47-1.53 (m, 3H), 2.76 (s, 4H), 4.03 (m, 1H), 4.19 (t, J = 6.6 HZ, 1H), 4.41 (m, 2H), 5.07 (d, J = 8.6 Hz, 1H), 7.27-7.41 (m, 13H); ^{13}C NMR (DMSO- d_6): δ 22.2, 23.6, 23.7, 44.2, 47.1, 52.7, 52.8, 69.4, 119.9, 121.1, 121.3, 120.8, 124.1, 126.2, 127.2, 138.2, 137.5, 139.1, 141.5, 148.7, 155.2, 170.1. Anal. Calcd. for $C_{30}H_{28}F_5NaN_3O_5$, $[M+Na]^+$: 628.54. Found: 628.5.

Fmoc-Phe-Pro -NHCOOPfp: IR (thin film): 1738, 1708 cm^{-1}; NMR (DMSO- d_6): δ 1.62-2.07 (m, 4H), 2.72 (s, 4H), 2.87 (d, J = 6.2 Hz, 2H), 3.98 (m, 1H), 4.18 (t, J = 6.6 Hz, 1H), 4.40 (m, 2H), 5.96 (br s, 1H), 7.20-7.72 (m, 13H); ^{13}C NMR (DMSO- d_6): δ 18.1, 18.3,36.6, 47.1, 48.6, 54.6, 56.2, 70.1, 120.0, 122.0, 124.2, 126.2, 127.1, 128.4, 130.7, 137.9, 141.7, 148.9, 154.6, 176.7. Anal. Calcd. for $C_{35}H_{28}F_5NaN_3O_5$, $[M+Na]^+$: 688.603. Found: 688.1.

Fmoc-Asp(OBzl)-Glu(OBzl)-NHCOOPfp: IR (thin film): 1750, 1738, 1708 cm^{-1}; NMR (DMSO-d_6): δ 1.50-1.90 (m, 5H), 2.16-2.21 (t, 2H), 2.55 (br d, 2H), 4.04 (br s, 1H), 4.20 (t, J =6.6 Hz, 1H), 4.38 (d, J=6.5 Hz, 2H), 5.67 (d, J=8.4 Hz, 1H), 5.2 (m, 4H), 7.21-7.82 (m, 18H); ^{13}C NMR (DMSO- d_6): δ 16.5, 30.7, 33.5, 47.9, 53.6, 54.7, 63.9, 70.4, 121.0, 121.7, 121.8, 119.9, 126.2, 127.8, 128.2, 128.6, 128.7, 139.7, 141.6, 148.8, 154.9, 168.1, 169.3, 172.0. Anal. Calcd. for $C_{44}H_{36}F_5NaN_3O_9$, $[M+Na]^+$: 868.76. Found: 868.7.

Fmoc-Leu-Val-NHCOOPfp: IR (thin film): 1738, 1700 cm^{-1}; NMR (DMSO- d_6): δ 0.93 (m, 12 H), 1.34 (m, 2H), 1.62 (m, 1H), 1.84 (m, 1H), 3.82 (m, 1H), 4.21 (t, J=6.6 Hz, 1H), 4.43 (m, 2H), 5.03 (d, J=8.0 Hz, 1H), 5.08 (d, J=8.8 Hz, 1H) 7.27-7.82 (m, 8H); ^{13}C NMR (DMSO- d_6): δ 17.3, 23.7, 29.3, 44.2, 47.1, 53.9, 60.0, 70.7, 119.9, 121.0, 121.5, 121.7, 124.1, 126.3, 127.2, 136.82, 139.1, 148.8, 154.6, 155.0, 170.0. Anal. Calcd. for $C_{32}H_{32}F_5NaN_3O_5$, $[M+Na]^+$: 656.602. Found: 656.3.

Fmoc-Val-Ala-Leu-NHCOOPfp: IR (thin film): 1740, 1700 cm^{-1}; NMR (DMSO- d_6): δ 0.92 (t, 6H), 1.18 (d, J = 6.2 Hz, 1H), 1.88 (m, 1H), 3.88 (m, 1H), 4.21 (t, J = 5.9 Hz, 1H), 4.41 (d, J = 5.9 Hz, 2H), 4.9 (m, 1H), 5.03 (d, J = 7.7 Hz, 1H), 7.25-7.76 (m, 8H); ^{13}C NMR (DMSO- d_6): δ 17.0, 19.1, 21.6, 21.8, 28.5, 43.7, 47.4, 52.6, 53.5, 61.5, 70.0, 120.0, 124.1, 126.1, 127.2, 129.9, 136.9, 139.0, 148.9, 154.6, 155.5, 164.9, 174.0. Anal. Calcd. for $C_{35}H_{37}F_5NaN_4O_6$, $[M+Na]^+$: 727.68. Found: 727.9.

Fmoc-Tyr(O^{t}Bu)-Pro-NHCOOPfp: IR(thin film): 1740, 1702 cm^{-1}; NMR (DMSO-d_6): δ 1.22 (s, 9 H), 1.23-2.04 (m, 4H), 2.87 (d, J=6.2 Hz, 2H), 3.20-3.67 (m, 4H), 3.89 (m, 1H), 4.18 (t, J=6.6Hz, 1H), 4.40 (m, 2H), 5.96 (br s, 1H), 6.89-7.08 (m, 4H), 7.25-7.75 (m,

8H); ^{13}C NMR (DMSO-d_6): δ 17.9, 18.0, 29.8, 47.0, 48.7, 51.9, 56.8, 70.1, 78.4, 120.0, 123.5, 124.1, 125.4, 126.3, 127.4, 141.4, 142.7, 148.8, 153.2, 154.5, 170.0. Anal. Calcd. for $C_{39}H_{36}F_5NaN_3O_6$, $[M+Na]^+$: 760.709. Found: 760.2.

Fmoc-Val-Ala-*ψ*(NH-CO-NH)-Leu-OMe: IR (thin film): 1735, 1702 cm^{-1}; NMR (DMSO-d_6): δ 0.92-0.94 (m, 12H), 1.18 (d, *J*=6.2 Hz, 1H), 1.33 (m, 2H), 1.63 (m, 1H), 1.18 (d, *J*=6.2 Hz, 1H), 1.88 (m, 1H), 3.88 (m, 1H), 4.21 (t, *J*=5.9Hz, 1H), 4.41 (d, *J*=5.9 Hz, 2H), 4.9 (m, 1H), 5.03 (d, *J*=7.7Hz, 1H), 7.25-7.76 (m, 8H); ^{13}C NMR (DMSO-d_6): δ 16.8, 22.2, 23.6, 25.2, 28.5, 42.3, 47.1, 53.2, 56.0, 59.2, 70.4, 120.1, 123.9, 126.3, 127.2, 141.8, 154.1, 160.5, 167.6, 174.1. Anal. Calcd. for $C_{30}H_{40}NaN_4O_6$, $[M+Na]^+$: 575.65. Found: 575.5.

Fmoc-Gly-Val-*ψ*(NH-CO-NH)-Phe-OMe: IR (thin film): 1740, 1702 cm^{-1}; NMR (DMSO-d_6): δ 0.92 (t, 6H), 1.84 (m, 1H), 2.87 (d, *J*=6.2 Hz, 2H), 3.61(s, 3H), 3.85 (m, 2H), 3.94 (m, 1H), 4.21 (t, 1H), 4.41 (m, 2H), 5.00 (d, 1H), 6.41-6.50 (m, 2H), 7.25-7.81 (m, 13H); ^{13}C NMR (DMSO-d_6): δ 16.9, 30.9, 39.1, 47.2, 47.8, 54.5, 58.4, 64.1, 70.2, 119.9, 124.1, 126.3, 126.6, 127.4, 127.8, 128.3, 130.1, 137.1, 141.7, 148.8, 154.4, 172.9, 173.2. Anal. Calcd. for $C_{32}H_{36}N_4O_6$, $[M+Na]^+$: 595.64. Found: 595.7.

Fmoc-Ala-Phe-*ψ*(NH-CO-NH)-Gly-OMe: IR (thin film): 1735, 1700 cm^{-1}; NMR (DMSO-d_6): δ 1.17 (d, *J*=6.2 Hz, 3H), 2.88 (d, *J*=6.2 Hz, 2H), 3.60 (s, 3H), 3.85 (m, 2H), 3.95 (m, 1H), 4.22 (t, 1H), 4.41 (m, 2H), 5.02 (br d, 1H), 6.40-6.51 (m, 2H), 7.25-7.80 (m, 13H); ^{13}C NMR (DMSO-d_6): δ 19.4, 37. 2, 46.0, 46.2, 51.9, 53.4, 56.7, 69.5, 120.1, 124.4, 126.3, 127.2, 127.7, 128.4, 136.3, 141.5, 148.7, 154.7, 159.0, 166.8, 169.2. Anal. Calcd. for $C_{30}H_{32}N_4O_6$, $[M+Na]^+$: 567.59. Found: 567.6.

Fmoc-Phe-Leu-*ψ*(NH-CO-NH)-Ala-OMe: IR (thin film): 1735, 1700 cm^{-1}; NMR (DMSO-d_6): δ 1.15 (d, *J*=6.2 Hz, 3H), 0.92 (d, *J*=5.2 Hz, 6H), 1.34 (m, 2H), 2.87 (d, 2H), 3.62 (s, 3H), 3.81 (m, 1H), 4.20 (t, *J*=5.9 Hz, 1H), 4.41 (d, *J*=5.9 Hz, 2H), 5.03 (d, *J*=7.7 Hz, 1H), 7.25-7.80 (m, 13H); ^{13}C NMR (DMSO-d_6): δ 17.0, 21.5, 23.4, 36.5, 44.0, 46.9, 50.1, 52.2, 5.5, 55.7, 70.4, 120.1, 124.9, 126.3, 127.2, 129.1, 130.3, 138.2, 142.0, 148.7, 155.1, 160.2, 169.4, 170.0. Anal. Calcd. for $C_{34}H_{40}N_4O_6$, $[M+Na]^+$: 623.701. Found: 623.7.

Fmoc-Val-Ala-Leu-*ψ*(NH-CO-NH)-Val-Ala-Leu-OMe: IR (thin film): 1735, 1700 cm^{-1}; NMR (DMSO-d_6): δ 0.92-0.95 (m, 24H), 1.14-1.16 (m, 6H), 1.33 (m, 4H), 1.88 (m, 2H), 3.60 (s, 3H), 4.19 (t, 1H), 4.41 (m, 2H), 5.09 (br s, 1H), 6.41-6.51 (m, 3H), 7.26-7,72 (m, 8H); ^{13}C NMR (DMSO-d_6): δ 16.8, 16.9, 17.1, 20.1, 21.5, 23.4, 23.6, 26.0, 28.2, 30.3, 43.0, 47.1, 51.2, 52.6, 58.1, 61.4, 70.1, 120.1, 124.1, 126.2, 127.2, 141.5, 148.7, 158.2, 169.1, 172.1, 173.3, 176.4. Anal. Calcd. for $C_{44}H_{65}N_7O_9$, $[M+Na]^+$: 858.0253. Found: 858.4741.

Fmoc-Leu-Ala-*ψ*(NH-CO-NH)-Val-OMe: IR (thin film): 1735, 1702 cm^{-1}; NMR (DMSO-d_6): δ 0.92-0.94 (m, 12H), 1.18 (d, *J*=6.2 Hz, 1H), 1.33 (m, 2H), 1.63 (m, 1H), 1.18 (d, *J*=6.2 Hz, 1H), 1.88 (m, 1H), 3.88 (m, 1H), 4.21 (t, *J*=5.9Hz, 1H), 4.41 (d, *J*=5.9 Hz, 2H), 4.9 (m, 1H), 5.03 (d, *J*=7.7 Hz, 1H), 7.25-7.76 (m, 8H); ^{13}C NMR (DMSO-d_6): δ 16.8, 22.2, 23.6, 25.2, 28.5, 42.3, 47.1, 53.2, 56.0, 59.2, 70.4, 120.1, 123.9, 126.3, 127.2, 141.8, 154.1, 160.5, 167.6, 174.1. Anal. Calcd. for $C_{30}H_{40}NaN_4O_6$, $[M+Na]^+$: 575.65. Found: 575.5.

Fmoc-Tyr(O^{t}Bu)-Pro-*ψ*(NH-CO-NH)-Phe-Pro-Gly-OMe: IR (thin film): 1742, 1698 cm^{-1}; NMR (DMSO-d_6): δ 1.35 (s, 9H), 1.37-2.06 (m, 8H), 2.89 (m, 4H), 3.20-3.67 (m, 8H), 3.69 (s, 3H), 3.81 (m, 2H), 4.18 (t, *J*=6.6 Hz, 1H), 4.40 (m, 2H), 5.96 (br s, 1H), 7.26-7.81 (m, 17H); ^{13}C NMR (DMSO-d_6): δ 16.9, 17.8, 19.2, 27.7, 28.9, 29.6, 36.2, 38.7, 47.0, 48.1, 51.4, 52.1, 54.6, 56.6, 58.2, 70.0, 77.4, 120.0, 124.1, 124.3, 126.2, 126.7, 128.4, 130.0, 138.4, 141.5, 148.7, 152.4, 152.4, 159.7, 169.9, 170.0, 175.9. Anal. Calcd. for $C_{50}H_{58}N_6O_9$, $[M+Na]^+$: 910.02. Found: 910.3.

Fmoc-Phe-Pro-*ψ*(NH-CO-NH)-Leu-OBzl: IR (thin film): 1752, 1698 cm^{-1}; NMR (DMSO-d_6): δ 0.92 (d, 6H), 1.23 (m, 6H), 3.20-3.67 (m, 4H), 2.88 (m, 2H), 4.19 (t, *J*=6.6 Hz, 1H), 4.41 (m, 2H), 5.07 (br d, 1H), 5.11 (s, 2H), 7.27-7.81 (m, 18H); ^{13}C NMR (DMSO-d_6): δ 17.1, 17.9, 23.4, 25.1, 36.7, 41.1, 48.0, 53.1, 54.7, 56.2, 58.7, 70.4, 120.0, 124.1, 126.4, 127.2, 128.4, 130.2, 137.4, 141.5, 148.7, 155.0, 163.2, 173.2, 176.0. Anal. Calcd. for $C_{42}H_{46}N_4O_6$, $[M+Na]^+$: 725.83. Found: 725.8.

Fmoc-Asp(OBzl)-Glu(OBzl)-*ψ*(NH-CO-NH)-Val-OBzl: IR (thin film): 1750, 1700 cm^{-1}; NMR (DMSO-d_6): δ 2.27 (br d, 2H), 2.55 (br d, 2H), 3.15 (br d, 2H), 4.03 (br s, 1H), 4.20 (t, *J*=6.6 Hz, 1H), 4.38 (d, *J*=6.5 Hz, 2H), 5.10 (d, 2H), 6.61 (d, 1H), 7.27-7.75 (m, 23H); ^{13}C NMR (DMSO-d_6): δ 17.0, 17.5, 29.3, 34.16, 47.1, 55.7, 61.3, 63.9, 66.7, 70.1, 120.1, 127.8, 140.2, 141.5, 142.7, 143.2, 149.0, 159.1, 168.2, 169.0,

170.1, 171.2. Anal. Calcd. for $C_{50}H_{52}N_4O_{10}$, $[M+Na]^+$: 891.96. Found: 892.5.

Fmoc-Gly-Val-ψ(NH-CO-NH)-Phe-OBzl: IR (thin film): 1750, 1700 cm^{-1}; NMR (DMSO-d_6): δ 0.93 (t, 6H), 1.86 (m, 1H), 2.84 (d, 2H), 3.80 (m, 2H), 4.21 (t, *J*=6.6Hz, 1H), 4.43 (m, 2H), 5.03, (br d, 1H), 7.26-7.82 (m, 18H); ^{13}C NMR (DMSO-d_6): δ 20.1, 30.0, 40.1, 47.8, 60.0, 63.2, 66.1, 70.2, 120.0, 124.0, 126.2, 127.2, 128.2, 128.6, 130.1, 136.8, 140.4, 141.5, 148.7, 160.4, 172.7, 176.0. Anal. Calcd. for for $C_{38}H_{40}N_4O_6$, $[M+Na]^+$: 671.74. Found: 671.7.

Tyr-Pro-ψ(NH-CO-NH)-Gly-Val-Leu-OH: IR (thin film): 1642, 1255 cm^{-1}; NMR (DMSO-d_6): δ 1.35 (s, 9H), 1.37-2.06 (m, 8H), 2.89 (m, 4H), 3.20-3.67 (m, 8H), 3.69 (s, 3H), 3.81 (m, 2H), 5.96 (br s, 1H); ^{13}C NMR (DMSO-d_6): δ 16.9, 17.8, 19.2, 27.7, 28.9, 29.6, 36.2, 38.7, 47.0, 48.1, 51.4, 52.1, 54.6, 56.6, 58.2, 70.0, 77.4, 152.4, 159.7, 169.9, 170.0, 175.9. Anal. Calcd. for $C_{30}H_{38}N_6O_7$, $[M+Na]^+$: 617.65. Found: 617.6.

Val-Ala-Leu-ψ(NH-CO-NH)-Val-Ala-Leu-OH: IR (thin film): 1645, 1222 cm^{-1}; NMR (DMSO-d_6): δ 0.92-0.95 (m, 24H), 1.14-1.16 (m, 6H), 1.33 (m, 4H), 1.88 (m, 2H), 3.60 (s, 3H), 5.09 (br s, 1H), 6.41-6.51 (m, 3H); ^{13}C NMR (DMSO-d_6): δ 16.8, 16.9, 17.1, 20.1, 21.5, 23.4, 23.6, 26.0, 28.2, 30.3, 43.0, 47.1, 51.2, 52.6, 58.1, 61.4, 70.1, 158.2, 169.1, 172.1, 173.3, 176.4. Anal. Calcd. for $C_{28}H_{53}N_7O_7$, $[M+Na]^+$: 622.75. Found: 623.9.

Fmoc-Leu-Val-ψ(NH-CO-NH)-(R&S)-1-phenyl-ethylamine: IR (thin film): 1645, 1222 cm^{-1}; NMR (DMSO-d_6): δ 0.80-0.86 (m, 12H), 1.27 (m, 2H), 1.31 (t, 3H), 1.34-1.88 (m, 4H), 4.00 (t, 1H), 4.20 (d, 2H), 4.70 (br d, 1H), 4.96 (br d, 1H), 6.15 (s, 1H), 6.56 (s, 1H), 7.19-7.90 (m, 13H). Anal. Calcd. for $C_{34}H_{42}N_4O_4$, $[M+Na]^+$: 593.7181. Found: 593.3094.

Fmoc-Leu-Val-ψ(NH-CO-NH)-R-(+)-1-phenyl-ethylamine: IR (thin film): 1645, 1222 cm^{-1}; NMR (DMSO-d_6): δ 0.80-0.86 (m, 12H), 1.27 (m, 2H), 1.31 (t, 3H), 1.34-1.88 (m, 4H), 4.00 (t, 1H), 4.20 (d, 2H), 4.70 (br d, 1H), 4.96 (br d, 1H), 6.15 (s, 1H), 6.56 (s, 1H), 7.19-7.90 (m, 13H). Anal. Calcd. for $C_{34}H_{42}N_4O_4$, $[M+Na]^+$: 593.7181. Found: 593.3094.

Fmoc-Leu-Val-ψ(NH-CO-NH)-S-(-)-1-phenyl-ethylamine: IR (thin film): 1645, 1222 cm^{-1}; NMR (DMSO-d_6): δ 0.80-0.86 (m, 12H), 1.27 (m, 2H), 1.30 (t, 3H), 1.34-1.88 (m, 4H), 4.00 (t, 1H), 4.20 (d, 2H), 4.70 (br d, 1H), 4.96 (br d, 1H), 6.15 (s, 1H), 6.56 (s, 1H), 7.19-7.90 (m, 13H). Anal. Calcd. for $C_{34}H_{42}N_4O_4$, $[M+Na]^+$: 593.7181. Found: 593.3094.

Conclusions

A facile method for the synthesis of peptidyl ureas has been developed through pentaflourophenyl peptidyl carbamates which are shelf stable intermediates. The reactions are very high yielding without any racemiation. The method was successfully used for the synthesis of tri, penta and hexapeptidyl ureas. The use of pentafluorophenyl carbamates as key fragments in the coupling step results in the insertion of a ureido linkage ψ[NH-CO-NH] between two designated neighbouring α-amino acids. Thus, the peptidyl ureas with a urea bond in the designated position in place of a peptide bond can be obtained in a facile way. The entire protocol, which is devoid of the use of phosgene/triphosgene, is of particular interest with respect to the development of greener approaches for the synthesis of urea-peptide hybrid molecules.

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