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Preparation, Isolation, and Characterization of N^{α}-Fmoc-peptide Isocyanates: Solution Synthesis of Oligo-α-peptidyl Ureas

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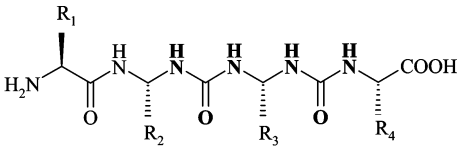

The N^{α}-Fmoc-peptide isocyanates **3a−q**, **4a−c**, and **5a−c** were prepared by the Curtius rearrangement of N^{α}-Fmoc-peptide acid azides in toluene under thermal, microwave, and ultrasonic conditions. All the N^{α}-Fmoc-oligo-peptide isocyanates made were isolated as stable crystalline solids with 71 to 94% yield and were fully characterized by ^{1}H NMR, ^{13}C NMR, and mass spectroscopy. Their utility for the synthesis of oligo-α-peptidyl ureas **7a−f** and **8a−c** by the divergent coupling approach was demonstrated. The coupling of N^{α}-Fmoc-dipeptide isocyanates with amino acid ester or with *N*,*O*-bis(trimethylsilyl)amino acids resulted in N^{α}-Fmoc-tripeptidyl urea ester and acids containing one each of peptide bond and urea bond. The divergent approach is extended to the synthesis of tetrapeptidyl ureas by the 2 + 2 strategy using bis-TMS−peptide acid as an amino component. To incorporate urea bonds in adjacent positions, N^{α}-Fmoc-peptidyl urea isocyanates **9a−d** were prepared and employed in the synthesis of three tetrapeptidyl ureas **10a−b** and **11** containing one peptide bond and two urea bonds in series from the *N*-terminal end. The protocol was then employed for the synthesis of five urea analogues **13−15**, **18**, and **21** of [Leu^{5}]enkephalin containing urea bonds at the 2, 3, 4 positions as well as at the 2, 4 and 2, 3, 4 positions. The analogue **2l** was made by the convergent synthesis by the N → C terminal chain extension. Finally, two urea analogues **22** and **23** of repeat units of bioelasto polymers, namely Val-Pro-Gly-Val-Gly-OH and Pro-Gly-Val-Gly-Val-OH, were synthesized incorporating the urea bond by the concomitant isocyanate generation and urea bond formation under thermal conditions.

Introduction

Peptides, sequence specific oligomers, are ubiquitous in living cells and have diverse roles. Each one of their roles is due to a unique three-dimensional structure. Nature, in this way, has refined the overall structure of bioactive peptides. However, the development of peptides as drug candidates needs to overcome several of their disadvantageous characteristics such as low oral bioavailability, poor metabolic stability in vivo, etc. Peptidomimetics, on the other hand, are often protease-resistant, and may reduce immunogenecity when compared to the naturally occurring α-peptide analogues and thus generate viable pharmaceutical therapies.[1,2]

Among several classes of pseudopeptides,[3] peptidyl urea peptidomimetics are one of the important classes of molecules with increasing applications both in de novo design of proteins as well as in developing lead molecules in drug therapy. Nowick, et al., developed methods for the preparation of artificial β-sheets which are mimics of proteins.[4] The molecular scaffold holds a number of peptide strands in proximity. The peptide ester isocyanates were employed successfully as building blocks for the introduction of peptide strands during the construction of artificial β-sheets.[5−8] Guichard, et al., have reported (p)2.5

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helical structure in solution for a heptapeptidyl urea bearing amino acids Tyr, Ala, and Val.[9] A high degree of self-complementarity that allows a self-assembly process resulting in C4-symmetry (all *S*) structure by a cyclictetraalanylurea [$-\beta$-HAla$^{u}-\beta$-HAla$^{u}-\beta$-HAla$^{u}-\beta$-HAla$^{u}-$] which possesses nanotubular structure was also demonstrated by X-ray analyis.[10] Some of the illustrative examples befitting the incorporation of urea moiety into native peptides to obtain peptidyl ureas as potential inhibitors are TAR-binding portion of the Tat protein,[11] γ-secretase,[12] aspartic acid protease,[13] HIV-1 proteases,[14,15] microbial alkaline proteinase inhibitors (MAPI),[16] and aspartic peptidases.[17] As anticipated by Nowick, et al.,[4] peptide isocyanates, being reactive with nucleophiles, are attractive building blocks for the development of new biomolecules through combinatorial chemistry and drug discovery efforts.

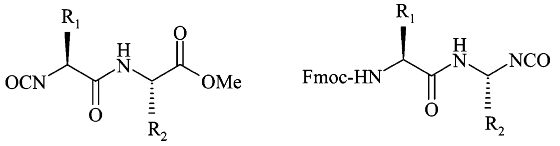

FIGURE 1. Peptide ester isocyanate and N^{α}-Fmoc-peptide isocyanate.

Peptide ester isocyanates (OCN$-$CHR$^1-$**CONH**$-$CHR$^2-$COOMe, Figure 1), prepared by the treatment of peptide ester hydrochloride salts with phosgene in toluene, in the presence of saturated aqueous NaHCO$_3$ under modified Schotten$-$Baumann conditions or with triphosgene [bis(trichloromethyl)-carbamate] in the presence of diisopropylethylamine (DIEA), were coupled with amino acid esters to obtain peptidyl ureas possessing one urea bond (MeO$_2$C$-$CHR$^3-$**NHCONH**$-$CHR$^2-$**CONH**$-$CHR$^1-$CO$_2$Me).[18] Peptide ester isocyanate generation is usually followed by trapping with an amino component. Unlike amino acid ester isocyanates, peptide ester isocyanates cannot be purified by Kugelrohr distillation. Instead, crude isocyanates are trapped, and the final products are purified. Similarly, several reagents such as *N*,*N*^{1}-carbonyldiimidazole (CDI),[19] 1,1^1-carbonylbisbenzotriazole,[20] and *p*-nitrophenyl carbamate,[21] have also been used for the synthesis of peptidyl ureas. The coupling of isocyanates derived from peptide ester with amino acid ester or peptide ester results in peptidyl ureas comprising carboxyl group at both the terminal ends.

Alternatively, organic synthesis also permits insertion of an amino group in place of α-carboxyl group that can then be converted to its isocyanate. This functional group transformation was accomplished by converting N^{α}-Boc-α-amino acid amide to nitrile followed by its reduction to amine.[22a] Alternatively, N^{α}-Fmoc-amino acid was converted to its β-amino alcohol which was transformed into an amine via azide.[22b] A similar protocol was employed by Burgess et al.[23] Consequently, the backbone in each repeating unit of oligourea peptidomimetic is generally extended by one carbon atom. Further, the mono-protected diamines were either converted to the corresponding isocyanates (using phosgene) or were treated with chlorofor-

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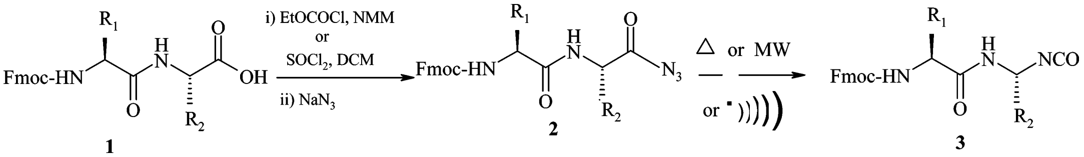
SCHEME 1. Synthesis of N^{α}-Fmoc-peptide Acid Azides and Their Isocyanates



mates (*p*-nitrophenyl chloroformate and 2,4,5-trichlorophenyl chloroformate) to obtain carbamates which serve as monomeric building blocks for the synthesis of peptidyl ureas.[21,24] Almost all of these routes give oligo-β-peptidyl ureas. Boeijen and Liskamp have described that this was for reasons of synthetic accessibility as well as product stability.[25]

The oxidative Hoffmann type rearrangement of Boc-/Z-α-amino acid/peptide acid amides using bis(trifluoroacetoxy)-iodobenzene (IBTFA) mediated by the isocyanate intermediate is another known approach for the synthesis of peptidyl ureas.[26] In addition to longer duration of reaction period, less yields,[27] and carboxamide formation in the case of Gln, Asn, etc., this protocol was found to be ineffective in Fmoc chemistry.[27] Sureshbabu, et al.,[28] isolated the isocyanates derived from N^{α}-Fmoc-amino acids including the ones with side chain protection possessing *tert*-butyl, benzyl, and Boc groups, as crystalline solids and were fully characterized. The removal of Boc or *Z* groups from protected gem-diamine systems[29,30] under normal acidic or basic conditions hampers the synthesis of oligo-α-peptidyl ureas by the C → N terminal stepwise chain extension.[31] However, N^{α}-Fmoc-peptide isocyanates comprising coded amino acids are hitherto unreported compounds which are demonstrated, in this paper, to be useful as key intermediates in the synthesis of oligo-α-peptidyl ureas both by the divergent as well as convergent N → C terminal extension strategies. The utility of the protocol is demonstrated by the synthesis of five urea analogues of [Leu^5]enkephalin and two analogues of bioelastic polymer repeat units Val-Pro-Gly-Val-Gly-OH and Pro-Gly-Val-Gly-Val-OH.

Results and Discussion

N^{α}-Fmoc-peptide Isocyanates. Fmoc-peptide acids **1a−q**[32,33] were converted to the corresponding acid azides by employing the standard procedures. As demonstrated by us in the case of N^{α}-Fmoc-amino acid azides,[34] all the N^{α}-Fmoc-peptide acid azides **2a−q** prepared were isolated as shelf-stable crystalline solids and fully characterized before use. They were then subjected to Curtius rearrangement (Scheme 1) by refluxing the toluene solution at 65 °C. The conversion, as monitored by TLC as well as by IR spectra, was found to be complete in about 45 min. Evaporation of toluene solution under reduced pressure resulted in the isolation of the isocyanates **3a−q** as solids. The crude N^{α}-Fmoc-peptide isocyanates were recrystallized using ethyl acetate (EtOAc) and *n*-hexane to obtain the pure crystalline compounds in 71 to 85% yields (Table 1). The tri- and tetrapeptide isocyanates Fmoc-Val-Ala-Leu-NCO **4a**, Fmoc-Tyr-(Bu^t)-Gly-Gly-NCO **4b**, Fmoc-Val-Pro-Gly-NCO **4c**, Fmoc-Gly-Phe-Leu-Val-NCO **5a**, Fmoc-Tyr(Bu^t)-Gly-Gly-Phe-NCO **5b**, and Fmoc-Pro-Gly-Val-Gly-NCO **5c** were also prepared starting from the corresponding peptide acid azides and isolated as analytically pure solids in 89 to 94% yields. The N^{α}-Fmoc-oligo-α-peptide isocyanates **3−5** were characterized by IR, ^{1}H, ^{13}C NMR, ES-MS, and MALDI-mass spectral analysis.

When stored for longer periods at 4 °C, the N^{α}-Fmoc-peptide isocyanates **3−5** did not undergo noticeable degradation or show any change in their ^{1}H and ^{13}C NMR spectral properties recorded after several weeks of their preparation. The N^{α}-Fmoc-peptide isocyanates containing the amino acids Ser, Thr, Tyr, Asp, Glu, Gln, His, Arg, bearing side chains protected by Bu^t, Boc, Trt, Pmc, etc., were made and isolated as pure compounds after a single recrystallization step. N^{α}-Fmoc-peptide acid azides as well as their isocyanates were found to be stable while carrying out HPLC analysis by an isocratic method using the mobile phase acetonitrile and water (60:40) during which the chromatographic run was initiated and completed in 20 min. Thus, purity as well as the progress of the reaction involving isocyanates synthesis can be qualitatively monitored using HPLC analysis.

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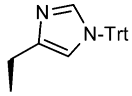
TABLE 1. ***N*$^{\alpha}$-Fmoc-peptide Isocyanates Fmoc-NH−CHR$_1$−CONH−CHR$_2$−NCO**

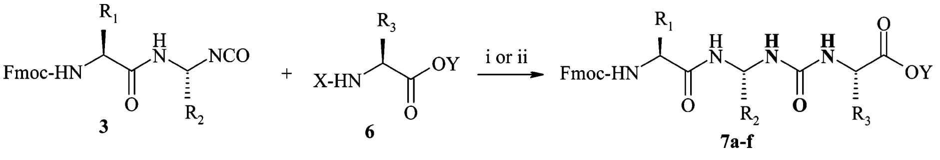
product	R$_1$	R$_2$	method^a	time	yield^b (%)	mp (°C)
3a	H	$CH_2C_6H_5$	A	30 min	83	208−209
			C	40 s	87	
			D	20 min	86	
3b	$CH(CH_3)_2$	CH_3	A	30 min	84	178−180
			C	40 s	92	
			D	25 min	91	
3c	$CH_2CH(CH_3)_2$	$CH(CH_3)_2$	A	30 min	85	193−194
			C	40 s	91	
			D	30 min	88	
3d	CH_3	$CH_2CH(CH_3)_2$	A	30 min	84	178−180
			C	40 s	88	
			D	30 min	91	
3e	$CH(CH_3)_2$	$CH_2C_6H_5$	A	30 min	81	198−199
			C	35 s	87	
			D	30 min	82	
3f	$CH(CH_3)_2$	C_6H_5	A	30 min	79	172−174
			C	45 s	82	
			D	30 min	80	
3g	$CH(CH_3)_2$	$N{-}(CH_2)_3{-}C$	A	30 min	79	168−169
			C	45 s	82	
			D	30 min	80	
3h	$CH(CH_3)CH_2CH_3$	$CH_2OCH_2C_6H_5$	A	40 min	85	168−170
			C	45 s	91	
			D	25 min	86	
3i	$CH_2COOC(CH_3)_3$	$CH_2CH(CH_3)_2$	A	35 min	84	148−149
			C	35 s	88	
			D	30 min	84	
3j	$CH_2CH_2CONH(Trt)$	$CH(CH_3)CH_2CH_3$	A	30 min	72	164−165
			C	35 s	74	
			D	30 min	74	
3k		$CH(CH_3)CH_2CH_3$	A	40 min	71	167−169
			C	45 s	72	
			D	25 min	71	
3l	$(CH_2)_3NCN(NHPmc)$	$CH_2CH(CH_3)_2$	A	35 min	82	182−183
			C	45 s	86	
			D	30 min	82	
3m	$CH(SCH_2C_6H_5)CH_3$	$CH(CH_3)_2$	A	40 min	79	154−155
			C	45 s	82	
			D	25 min	80	
3n	$CH_2OCH_2C_6H_5$	$CH_2C_6H_5$	A	35 min	82	188−189
			C	45 s	88	
			D	30 min	83	
3o	$CH_2OC(CH_3)_3$	$CH_2C_6H_5$	A	40 min	81	168−169
			C	30 s	86	
			D	25 min	80	
3p	$CH(CH_3)OC(CH_3)_3$	$CH_2CH_2COOCH_2C_6H_5$	A	45 min	76	139−140
			C	60 s	78	
			D	20 min	76	
3q	$CH_2C_6H_4OC(CH_3)_3$	H	A	30 min	88	123−125
			C	30 s	89	
			D	20 min	85	

[a] Method A, reflux in toluene; method B, microwave irradiation in toluene; method C, microwave irradiation using solid powder; method D, conversion by ultrasonication. Method B results are not shown in the table. [b] Isolated yield after crystallization.

The conversion of *N*$^{\alpha}$-Fmoc-peptide acid azides to their isocyanates was accomplished under microwave irradiation in an open vessel using both toluene solution as well as naked solid powder. The rearrangement was completed in about 30−60 s. In the later protocol, after the reaction, a single recrystallization has directly resulted in the isolation of the pure isocyanates. This simplified procedure avoids tedious workup such as evaporation of toluene. Both the yields and the purity of all the isocyanates made by this route were satisfactory. In addition, under the influence of the ultrasound, *N*$^{\alpha}$-Fmoc-peptide acid azides in toluene solution could also be converted to their corresponding isocyanates at ambient temperature in about 20−30 min. It is noteworthy that the mild conditions employed are compatible with the use of a number of protecting groups in the side chains of bifunctional amino acids.

***N*$^{\alpha}$-Fmoc-oligo-α-peptidyl Ureas.** *N*$^{\alpha}$-Fmoc-peptide isocyanates **3** were coupled with bis-TMS/tris-TMS amino acids to obtain *N*$^{\alpha}$-Fmoc-peptidyl urea acids (Scheme 2). The reaction was found to be complete in about 20−30 min at room temperature, and the routine workup of the reaction mixture gave the crude peptidyl urea acids which were purified by crystallization or using column chromatography whenever necessary. The 2 + 2 fragment coupling strategy has been adopted to couple **3e**, **3f**, and **3b** with appropriate bis-TMS−dipeptides to obtain the tetra-α-peptidyl ureas Fmoc-Val-Phe-*ψ*(NH−CO−NH)-Ala-Leu **8a**, Fmoc-Val-Phg-*ψ*(NH−CO−NH)-Val-Gly **8b**, and Fmoc-Val-Ala-*ψ*(NH−CO−NH)-Val-Gly **8c** as analytically pure compounds in 88−89% yield. Similarly, the coupling of the isocyanates **3** with amino acid esters as well as peptide esters led to peptidyl urea esters.

SCHEME 2. Synthesis of N^{α}-Fmoc-tripeptidyl Ureas[a]



[a] (i) (**6**) X = Y = TMS, (**7a−c**) Y = H; (ii) (**7d−f**) X = HCl or p-TsOH salt, Y = CH_3, $CH_2C_6H_5$ group. [**7a**: Fmoc-Val-Phe-ψ(NH−CO−NH)-Ala-OH. **7b**: Fmoc-Val-Pro-ψ(NH−CO−NH)-Gly-OH. **7c**: Fmoc-Ser(Bzl)-Phe-ψ(NH−CO−NH)-Ile-OH. **7d**: Fmoc-Gly-Phe-ψ(NH−CO−NH)-Gly-OCH_3. **7e**: Fmoc-Ser(Bu^t)-Phe-ψ(NH−CO−NH)-Leu-OCH_3. **7f**: Fmoc-Thr(Bu^t)-Gly-ψ((NH−CO−NH)-Leu-OBzl].

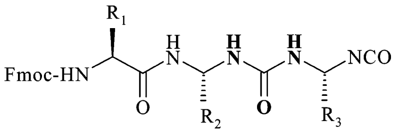

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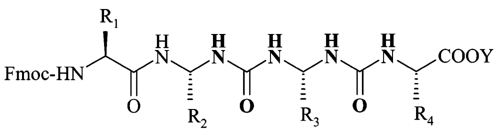
FIGURE 2. N^{α}-Fmoc-α-peptidyl urea isocyanates: **9a**, Fmoc-Ala-Leu-ψ(NH−CO−NH)-Val-NCO; **9b**, Fmoc-Pro-Val-ψ(NH−CO−NH)-Gly-NCO; **9c**, Fmoc-Leu-Phe-ψ(NH−CO−NH)-Ala-NCO; **9d**, Fmoc-Tyr(Bu^t)-Gly-ψ(NH−CO−NH)-Gly-NCO.


10

Compound	R₁	R₂	R₃	R₄	Y
10a	$CH_2CH(CH_3)_2$	$CH_2C_6H_5$	CH_3	CH_2OH	H
10b	$CH_2CH(CH_3)_2$	$CH_2C_6H_5$	CH_3	H	CH_3

FIGURE 3. N^{α}-Fmoc-α-tetrapeptidyl ureas containing urea bonds at 2nd and 3rd positions.

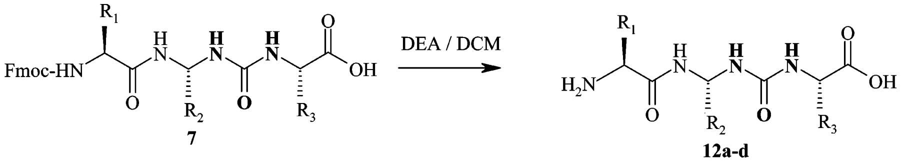
N^{α}-Fmoc-α-peptidyl Urea Isocyanates. For the synthesis of oligo-α-peptidyl ureas containing urea bonds in both adjacent positions as well as continuously, N^{α}-Fmoc-α-peptidyl urea isocyanates are the key intermediates (Figure 2). For this part of study, N^{α}-Fmoc-peptidyl urea acids **7** were converted to the corresponding isocyanates **9** via their azides. Thus, **9a−d** have been obtained in 89−95% yield and were found to be soluble as well as stable compounds. The isocyanate **9c** was then coupled with tris-TMS-Ser and Gly-OMe to obtain the tetrapeptidyl ureas **10a**,**b** containing two consecutive urea bonds in 90−94% yield (Figure 3). Then, **9c,** using 3 + 2 strategy, was coupled with tris-TMS-Val-Tyr to obtain the pentapeptidyl urea **11** in 88% yield.

N^{α}-Amino Free Oligo-α-peptidyl Ureas. The deprotection of Fmoc group from the N^{α}-Fmoc-peptidyl ureas was carried out employing the normal deprotection conditions. Thus, N^{α}-Fmoc-peptidyl urea on treatment with diethylamine (DEA, Scheme 3) at room temperature has resulted in complete deprotection of the Fmoc group in 20−25 min. And, all the resulting amino free peptidyl urea acids **12a−d** made have been isolated as crystalline solids and fully characterized.

Urea Analogues of [Leu^5]enkephalin. Employing N^{α}-Fmoc-peptide isocyanates, the synthesis of urea analogues of [Leu^5]-enkephalin containing urea bonds at the 2nd and 3rd as well as at 4th position was carried out by the fragment condensation strategy. The analogues synthesized were H-Tyr(Bu^t)-Gly-ψ-(NH−CO−NH)-Gly-Phe-Leu-OH **13**, H-Tyr(Bu^t)-Gly-Gly-ψ-(NH−CO−NH)-Phe-Leu-OH **14** and H-Tyr(Bu^t)-Gly-Gly-Phe-ψ(NH−CO−NH)-Leu-OH **15**. After the deprotection of the N^{α}-Fmoc group, the final compounds were isolated as analytically pure compounds in good yield. The divergent strategy was then extended for the synthesis of **18** possessing urea bonds at alternative positions. The analogue **21** possessing urea bonds in place of peptide bonds at positions 2, 3, and 4 were synthesized by the convergent strategy. The steps involved and the key intermediates as well as the protected and free final compounds isolated and characterized during the synthesis of **18** and **21** are given in the Schemes 4 and 5, respectively. The urea analogues were tested for opioid activity in the isolated GPI and MVD preparations. The insertion of the urea bond resulted in decrease or loss of the opioid activity in vitro when compared with the natural peptide in both the assays.

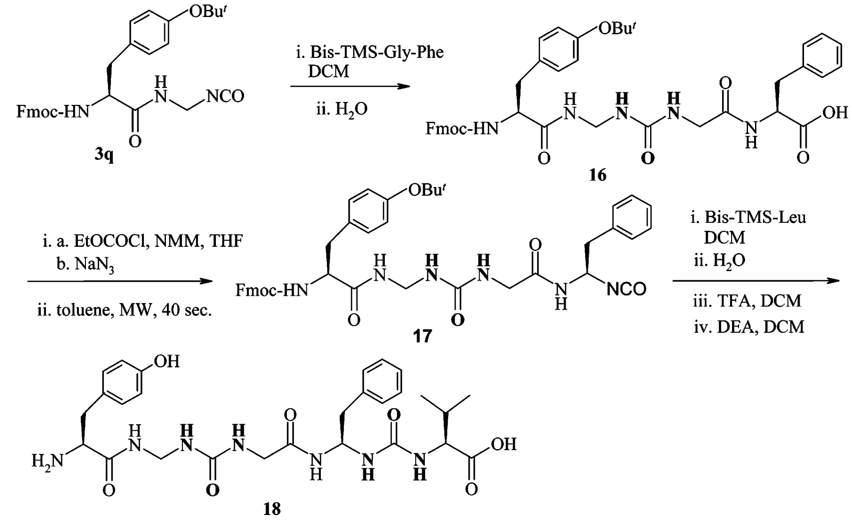
Urea Analogues of Repeat Units of Bioelasic Polymers: Concomitant Isocyanate Generation and Urea Bond Formation. Bioelastic materials are elastomeric polypeptides composed of repeating sequences having their origins found in the mammalian elastic protein, elastin. The polymers of the repeating sequences Val-Pro-Gly-Val-Gly-OH, Pro-Gly-Val-Gly-Val-OH, Gly-Val-Gly-Val-Pro-OH, Val-Gly-Val-Pro-Gly-OH, and Gly-Val-Pro-Gly-Val-OH carried out by polymerizing the pentamer permutations were cross linked into sheets, rods, and tubes.[35] Some of the polypentapeptides were found to be elastomeric capable of an elastic modulus similar to that of the natural elastic fibers.[36] As a part of our interest in such molecules, the urea analogues Val-Pro-Gly-ψ(NH−CO−NH)-Val-Gly-OH **22** and Pro-Gly-Val-Gly-ψ(NH−CO−NH)-Val-OH **23** (Figures 4 and 5) were prepared in the present study. In general, incorporation of urea moiety into the peptidyl urea peptidomimetics was accomplished initially by generating isocyanates from azides and then coupling them with an amino component. Since the isocyanates are reactive, we envisaged that both the generation and coupling steps could be carried

SCHEME 3. Deprotection of Fmoc-Group from N^{α}-Fmoc-peptidyl Ureas[a]

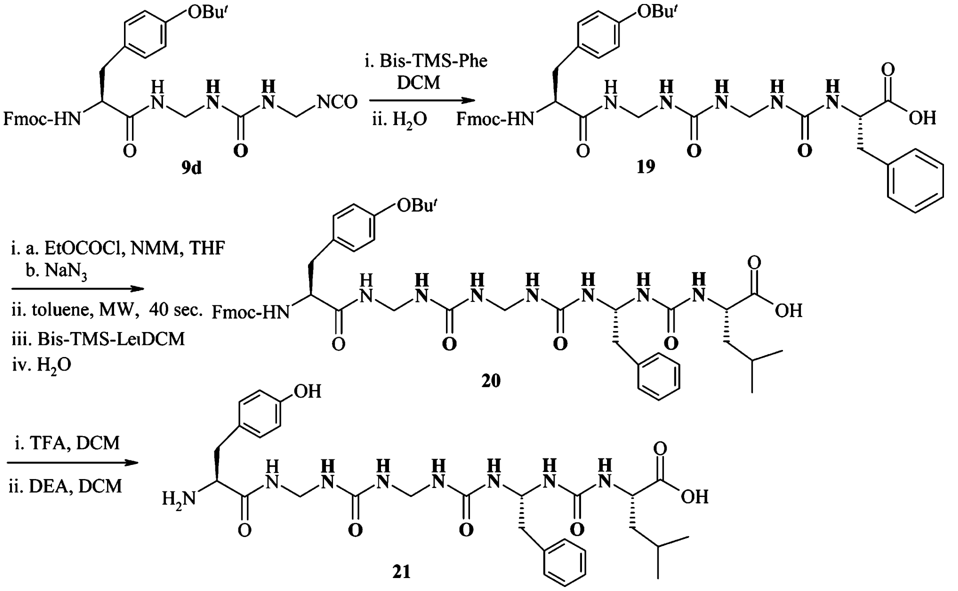


[a] **12a**: Val-Pro-ψ(NH−CO−NH)-Gly-OH. **12b**: Ser(Bzl)-Phe-ψ(NH−CO−NH)-Leu-OH. **12c**: Tyr(Bu^t)-Gly-ψ(NH−CO−NH)-Gly-OH. **12d**: Val-Phe-ψ(NH−CO−NH)-Ala-Leu-OH.

SCHEME 4. Synthesis of [Leu^5]enkephalin Containing Urea Bonds at 2nd and 4th Positions

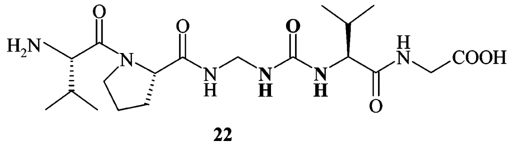


SCHEME 5. Synthesis of [Leu^5]enkephalin Possessing Three Urea Bonds



out in a single step. Thus the synthesis of **22** was carried out by the concomitant isocyanate generation starting from N^α-Fmoc-Val-Pro-Gly-N_3 in toluene and peptidyl urea formation under thermal conditions by coupling with bis-TMS-Val-Gly. The concomitant reaction resulted in pure pentapeptidyl urea after a routine workup and a single recrystallization. A similar approach was used for the synthesis of **23** also. The final free peptidyl ureas **22** and **23** were obtained, after the deprotection of Fmoc group, in 76−79% yield.

Test for Racemization. The conversion of N^α-Fmoc-peptide acid azides to its isocyanates was found to retain their enantiomeric purity. This was confirmed by ^{1}H NMR of the diastereomeric of peptidyl urea adducts **25** made by the coupling of Fmoc-Leu-Val-NCO with R−(+)-, S−(−)-, and a racemic mixture of 1-phenylethylamine in the presence of DIEA (Scheme 6). The urea adducts **25a**−**c** were isolated as solids. Their ^{1}H NMR analysis was carried out. The methyl protons of 1-phenylethylamine urea adducts **25a**,**b** were observed as doublets with the separation of 0.02 ppm, while the methyl protons of the urea adduct **25c** was observed as a triplet. On the basis of these observations, it was demonstrated that the present protocol does not lead to racemization of Fmoc-Leu-Val-NCO at levels detectable by ^{1}H NMR.



22

FIGURE 4. Bioelastic polymer fragment Val-Pro-Gly-ψ(NH−CO−NH)-Val-Gly-OH.

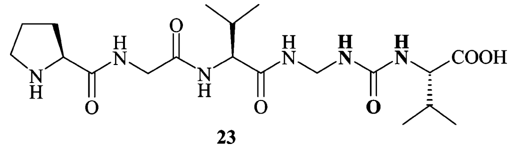


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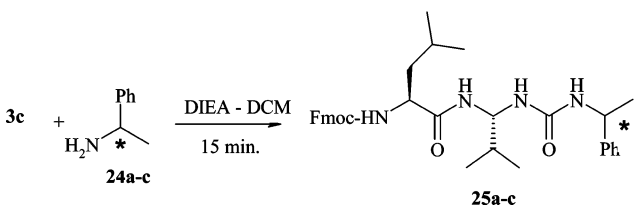
FIGURE 5. Bioelastic polymer fragment Pro-Gly-Val-Gly-ψ(NH−CO−NH)-Val-OH.

SCHEME 6. Preparation of Urea Adducts for Racemization Study[a]



[a] **25a**: Fmoc-Leu Val-ψ(NH−CO−NH)-(*R*)-phenylethylamine (*R* at ∗). **25b**: Fmoc-Leu-Val-ψ(NH−CO−NH)-(*S*)-phenylethylamine. **25c**: Fmoc-Leu-Val-ψ(NH−CO−NH)-1-phenylethylamine (*R* and *S* at ∗).

Conclusions

A simple and efficient method for the rapid synthesis of stable and crystalline *N*α-Fmoc-peptide isocyanates, including the one at ambient temperature, employing ultrasound was developed. The scale-up of the rearrangement up to 75 mmol was accomplished smoothly. The protocol is particularly noteworthy with regard to "chemistry of peptidomimetics: a green approach". The use of *N*α-Fmoc-peptidyl urea isocyanates allowed the incorporation of urea bonds in the adjacent positions as well as continuously. The *N*α-Fmoc- group from *N*α-Fmoc-peptidyl ureas was deprotected under normal deprotection conditions. All the *N*α-Fmoc-protected as well as free oligo-α-peptidyl ureas prepared resulted in good yield as well as purity.

The removal of Fmoc group under normal basic conditions from *N*α-Fmoc-oligo-α-peptidyl ureas possessing a urea bond in the place of a *N*-terminal peptide bond was not feasible. In such cases, catalytic hydrogenation or catalytic transfer hydrogenation techniques could be employed for deportection of the Fmoc group. The use of *N*α-Fmoc-peptide isocyanates as useful fragments in solid-phase fragment condensation (SPFC)[37] for the synthesis of bioelastic polymer fragment Val-Pro-Gly-ψ-(NH−CO−NH)-Val-Gly-OH will be published soon.

Experimental Section

Some representative experimental procedures for key intermediates and final compounds are furnished here. Complete experimental procedures and characterization data are available in the Supporting Information.

***N*α-Fmoc-peptide Acid Azides 2: General Procedure. Method A: Employing Mixed Anhydride Method.** *N*α-Fmoc-peptide acid (1 mmol) was dissolved in dry THF (5 mL) and cooled in an ice-salt bath. EtOCOCl (0.1 mL, 1 mmol) and NMM (0.11 mL, 1 mmol) were added to the above cooled solution and stirred for 10 min. while maintaining the temperature at −10 °C. The resulting reaction mixture was treated with aqueous NaN_3 (0.1 g, 1.5 mmol in 1 mL of water) at the same temperature. The reaction, as monitored by TLC as well as IR analysis, was complete in about 15 min. The THF solution was evaporated under reduced pressure at rt and the residue was taken in CH_2Cl_2 (15 mL). The CH_2Cl_2 layer was washed with cold water (2 × 10 mL), dried over anhydrous Na_2SO_4, and concentrated to half of the volume. *n*-Hexane (10 mL) was added to obtain pure *N*α-Fmoc-peptide acid azide as a crystalline solid.

Method B: Employing Acid Chloride Method. To a cold solution of *N*α-Fmoc-peptide acid chloride (1 mmol) in acetone (5 mL) was added aqueous NaN_3 (0.1 g, 1.5 mmol in 1 mL of water). The resulting reaction mixture was stirred for 15 min. at −10 °C. The separated solid was filtered and washed with cold water. The resulting solid was dissolved in CH_2Cl_2 (5 mL), dried over anhydrous Na_2SO_4, and crystallized using *n*-hexane.

Fmoc-Gly-Phe-N_3 2a. A 0.44 g (1 mmol) of Fmoc-Gly-Phe-OH was converted to its azide following the general procedure for *N*α-Fmoc-peptide acid azides **2** (method A). Yield 0.43 g (92%); mp 205−206 °C. IR: 2142 cm^{-1}. ^1H NMR (δ, $CDCl_3$): 2.9 (2H, d), 3.25 (2H, d), 4.2−4.45 (3H, m), 5.2 (1H, d), 6.3 (1H, s), 7.2−7.8 (13H, m).

Nα-Fmoc-peptide Isocyanates 3: General Procedure. Method A: Employing Conventional Method. A solution of *N*α-Fmoc-peptide acid azide (1 mmol) in toluene (5 mL) was heated at 65 °C under nitrogen. After the completion of the rearrangement, the solvent was removed under reduced pressure to obtain the crude isocyanate that was recrystallized using DCM and *n*-hexane.

Method B: Employing MW Irradiation. *N*α-Fmoc-peptide acid azide (1 mmol) was dissolved in toluene (5 mL) and exposed to microwave irradiation until the rearrangement was complete. The solvent was removed under reduced pressure to obtain the isocyanate that was recrystallized as described in the method A.

Method C: Employing MW Irradiation under Solvent Free Condition. Powdered *N*α-Fmoc-peptide acid azide (1 mmol) placed in a glass beaker was exposed to microwave irradiation until the rearrangement was complete. The resulting mass was recrystallized.

Method D: Employing Ultrasonic Waves. A solution of *N*α-Fmoc-peptide acid azide (1 mmol) in toluene (5 mL) was sonicated for 30 min, until the completion of the reaction. The solvent was removed under reduced pressure to obtain the crude isocyanate that was recrystallized using DCM and *n*-hexane.

Fmoc-Gln(Trt)-Ile-NCO 3j. An amount of 0.764 g (1 mmol) of Fmoc-Gln(Trt)-Ile-CON_3 **2j** was converted to its isocyanate following the general procedure for *N*α-Fmoc-peptide isocyanates **3** (method A). IR: 2252 cm^{-1}. ^1H NMR (δ, $CDCl_3$): 0.93 (6H, m), 1.21 (1H, m), 1.71 (2H, m), 2.12 (2H, t), 2.56 (2H, m), 3.71−4.48 (5H, m), 5.6 (1H, d), 6.2−6.45 (3H, m), 7.2−7.9 (23H, m). ^{13}C NMR (δ, $CDCl_3$): 11.3, 15.5, 23.0, 31.4, 34.8, 47.0, 54.7, 57.3, 66.7, 69.6, 119.9, 124.9, 125.8, 126.9, 127.6, 127.9, 128.1, 128.9, 141.1, 143.7, 145.3, 156.1, 158.2, 161.3. ES MS *m*/*z* observed, 722.2.

Fmoc-His(Trt)-Ile-NCO 3k. An amount of 0.757 g (1 mmol) of Fmoc-His(Trt)-Ile-CON_3 **2k** was converted to its isocyanate following the general procedure for *N*α-Fmoc-peptide isocyanates **3** (method C). IR: 2256 cm^{-1}. ^1H NMR (δ, $CDCl_3$): 0.92 (6H, m), 1.19 (1H, m), 1.71 (2H, m), 3.68−4.45 (10H, m), 5.6 (1H, d), 6.45 (1H, m), 7.2−8.4 (27H, m). ^{13}C NMR (δ, $CDCl_3$): 11.3, 15.5, 25.4, 35.8, 47.0, 57.3, 66.7, 119.9, 121.3, 124.9, 125.2, 126.3, 126.9, 127.6, 127.9, 128.1, 128.9, 132.4, 141.1, 143.7, 145.3, 156.1, 158.2, 161.3. ES MS *m*/*z* observed, 730.7.

Fmoc-Arg(Pmc)-Leu-NCO 3l. An amount of 0.80 g (1 mmol) of Fmoc-Arg(Pmc)-Leu-CON_3 **2l** was converted to its isocyanate following the general procedure for *N*α-Fmoc-peptide isocyanates **3** (method D). IR: 2247 cm^{-1}. ^1H NMR (δ, $CDCl_3$): 0.93 (6H, d), 1.33 (2H, m), 1.63 (1H, m), 3.77 (1H, m), 4.2 (1H, t), 4.4 (2H, m), 5.07 (1H, d), 6.58 (1H, m), 7.25−7.75 (8H, m). ^{13}C NMR (δ, $CDCl_3$): 22.0, 23.0, 24.6, 40.3, 47.2, 51.2, 66.6, 119.8, 124.8, 126.8, 126.9, 127.6, 141.2, 143.8, 156.7, 169.1. ES MS *m*/*z* observed, 773.8.

***N*α-Fmoc-peptidyl Urea Acids 7a−c: General Procedure.** To a solution of *N*α-Fmoc-peptide isocyanate **3** (1 mmol) in DCM (5 mL) at 0 °C was added freshly prepared bis-TMS− or tris-TMS−amino acid or peptide (from 1.2 mmol of amino acid or peptide refluxed with 2.5 mmol each of TEA and TMS−Cl in 10 mL of DCM or 4 mmol each of TEA and TMS−Cl in the case of tris-TMS derivatives) and stirring was continued for 15−30 min. After completion of the reaction, the solvent was evaporated under

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(35) (a) Sandberg, L. B.; Leslie, J. G.; Leach, C. T.; Torres, V. L.; Smith, A. R.; Smith, D. W. *Pathol. Biol.* **1985**, *33*, 266.

(36) (a) Urry, D. W. *Prog. Biophys. Mol. Biol.* **1992**, *57*, 23. (b) Urry, D. W. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 819. (c) Urry, D. W.; Gowda, D. C.; Harris, C.; Harris, R. D.; Cox, B. A. *J. Am. Chem. Soc.* **1992**, *33*, 84.

(37) For solid-phase fragment condensation see Benz, H. *Synthesis* **1994**, 337.

reduced pressure below 40 °C. The residue was dissolved in 10% Na$_2$CO$_3$ (20 mL) and washed with diethyl ether (20 mL × 2). The aqueous layer was acidified with dilute HCl until pH 2. The separated product was extracted with EtOAc (20 mL × 3). The water wash (20 mL × 3) was given to the combined organic layer and dried over anhydrous Na$_2$SO$_4$. The organic layer was concentrated to obtain the title compound. The peptidyl ureas were purified either by crystallization using DMF and water or by column chromatography using the solvent system MeOH and CHCl$_3$ (5% to 10% of MeOH in CHCl$_3$).

Fmoc-Ser(Bzl)-Phe-ψ(NH−CO−NH)-Ile-OH 7c. An amount of 0.561 g (1 mmol) of **3n** was coupled with freshly prepared bis-TMS-Ile (from 0.421 g, 1.2 mmol of Ile-OH in 10 mL of DCM) to obtain **7c** as a white solid. Yield 0.567 g (88%); mp 192−193 °C. ^{1}H NMR (δ, DMSO): 0.91 (6H, m), 1.13 (1H, m), 1.53 (2H, m), 2.84−2.92 (4H, m), 3.5 (2H, m), 3.82−4.46 (6H, m), 5.65 (1H, d), 6.5−6.8 (2H, m), 7.08−7.85 (18H, m), 8.35 (1H, m). ^{13}C NMR (δ, DMSO): 11.3, 15.5, 25.4, 35.8, 37.3, 37.4, 46.7, 51.8, 54.1, 57.3, 62.1, 66.5, 119.7, 124.8, 126.7, 126.7, 126.9, 127.3, 128.6, 128.6, 129.2, 129.3, 137.4, 137.5, 140.9, 143.6, 156.1, 157.2, 169.8, 175.2. ES MS *m/z* observed, 693.7.

N^{α}-Fmoc-peptidyl Urea Esters 7d−f: General Procedure. To an ice-cold mixture of amino acid or peptide ester hydrochloride or *p*-toluenesulfonate salt (1.1 mmol) in THF (5 mL) and NMM (1.21 mL, 1.1 mmol) was added a solution of N^{α}-Fmoc-peptide isocyanate (1 mmol) in DCM (5 mL), and the solutions was stirred for about 15−20 min. After the completion of the reaction, the solvent was removed under reduced pressure, and the residue was dissolved in EtOAc. The organic layer was washed with 10% Na$_2$CO$_3$ (10 mL) and water (10 mL × 3) and finally dried over anhydrous Na$_2$SO$_4$. The solvent was removed under reduced pressure to obtain the N^{α}-Fmoc-peptidyl urea esters. The peptidyl ureas were purified either by the crystallization using DMF-water or by column chromatography using the solvent system MeOH and CHCl$_3$ (2% to 10% of MeOH in CHCl$_3$).

Fmoc-Ser(Bu^t)-Phe-ψ(NH−CO−NH)-Leu-OMe 7e. An amount of 0.527 g (1 mmol) of **3o** was coupled with Leu-OMe.HCl (0.199 g, 1.1 mmol) to obtain **7e**. Yield 0.575 g (96%); mp 180−181 °C. ^{1}H NMR (δ, DMSO): 0.8−0.86 (6H, d), 1.1 (9H, s), 1.39 (2H, m), 1.41 (1H, m), 2.92 (2H, d), 3.3 (2H, m), 3.59 (3H, s), 3.99−4.41 (6H, m), 5.29 (1H, d), 6.4−6.56 (2H, m), 7.17−7.9 (13H, m), 8.35 (1H, m). ^{13}C NMR (δ, DMSO): 22.0, 23.1, 24.6, 27.6, 39.3, 40.9, 41.2, 41.4, 47.1, 51.1, 52.0, 56.0, 62.4, 66.2, 73.3, 120.5, 125.7, 126.6, 127.5, 128.1, 128.5, 129.7, 138.0, 141.1, 144.2, 144.3, 156.2, 156.6, 169.7, 174.4. ES MS *m/z* observed, 673.4.

Fmoc-Tyr(Bu^t)-Gly-ψ(NH−CO−NH)-Gly-NCO 9d. An amount of 0.588 g (1 mmol) of Fmoc-Tyr(Bu^t)-Gly-ψ(NH−CO−NH)-Gly-OH was converted to its isocyanate following the general procedure for **3**. Yield 0.556 g (95%); mp 148−149 °C. IR: 2256 cm^{-1}. ^{1}H NMR (δ, CDCl$_3$): 2.88 (2H, d), 3.84−4.46 (8H, m), 5.31 (1H, d), 6.36−6.85 (3H, m), 7.2−7.78 (12H, m). ^{13}C NMR (δ, CDCl$_3$): 36.7, 43.4, 43.5, 47.3, 54.4, 66.6, 119.8, 124.5, 125.2, 126.6, 127.3, 127.8, 129.6, 132.3, 141.3, 144.2, 154.5, 156.8. ES MS *m/z* observed, 586.7.

Fmoc-Leu-Phe-ψ(NH−CO−NH)-Ala-ψ(NH−CO−NH)-Val-Tyr-OH 11. An amount of 0.583 g (1 mmol) of Fmoc-Leu-Phe-ψ(NH−CO−NH)-Ala-NCO was coupled with tris-TMS-Val-Tyr (from 0.336 g, 1.2 mmol of Val-Tyr-OH in 10 mL of DCM) to obtain the title compound. It was further purified by column chromatography using the solvent system CHCl$_3$ /MeOH/AcOH (5% to 20% of MeOH in CHCl$_3$ containing 1% AcOH) to get the analytically pure compound. Yield 0.76 g (88%); mp 218−219 °C. ^{1}H NMR (δ, DMSO): 0.86−0.98 (12H, m), 1.16 (3H, d), 1.33 (2H, m), 1.63 (1H, m), 1.85 (1H, m), 2.85−2.9 (4H, m), 3.68−4.48 (8H, m), 5.26 (1H, s), 6.35−6.8 (4H, m), 7.08−7.85 (17H, m), 8.26 (1H, m), 8.45 (1H, m). ^{13}C NMR (δ, DMSO): 17.2, 18.6, 19.5, 22.0, 23.0, 24.6, 29.1, 36.5, 37.3, 40.3, 47.2, 48.9, 51.2, 54.1, 54.2, 58.5, 66.6, 120.0, 124.3, 125.0, 126.7, 127.0, 127.6, 128.4, 129.3, 129.7, 132.4, 137.6, 141.3, 143.9, 154.2, 154.2, 155.2, 156.4, 168.7, 169.6, 174.3. ES MS *m/z* observed, 865.1.

Deprotection of Fmoc Group from the N^{α}-Fmoc-peptidyl Urea Acids 12: General Procedure. A mixture of N^{α}-Fmoc-peptidyl urea (1 mmol) in DCM (5 mL) and DEA (5 mL) was stirred until the deprotection was complete. After the completion of the reaction, the solvent was removed under reduced pressure below 40 °C, and the residue was recrystallized using EtOAc and *n*-hexane to get the solid crystalline compounds in most of the cases. Free peptidyl ureas were purified by column chromatography using the solvent system CHCl$_3$/MeOH (5% to 15% of MeOH in CHCl$_3$).

Val-Pro-ψ(NH−CO−NH)-Gly-OH 12a. A suspension of 0.493 g (1 mmol) of **9c** in 5 mL of DCM was treated with 5 mL of DEA to obtain **12a**. Yield 0.235 g (87%); mp 146−148 °C. ^{1}H NMR (δ, DMSO): 0.95 (6H, d), 1.2−2.1 (5H, m), 3.2−3.85 (6H, m), 4.0 (1H, m), 6.25−6.75 (2H, m), 7.8 (1H, d), 8.34 (1H, m) 8.6 (1H, br s). ^{13}C NMR (δ, DMSO): 18.6, 19.5, 24.1, 28.6, 29.1, 47.3, 58.5, 60.8, 154.9, 158.3, 169.8. ES MS *m/z* observed, 286.4.

Tyr(Bu^t)-Gly-ψ(NH−CO−NH)-Gly-OH 12c. A suspension of 0.573 g (1 mmol) of Fmoc-Tyr(Bu^t)-Gly-ψ(NH−CO−NH)-Gly-OH in 5 mL of DCM was treated with 5 mL of DEA, and the reaction mixture was stirred until the deprotection was complete. After the completion of the reaction, the solvent was removed under reduced pressure below 40 °C, and the residue was recrystallized using EtOAc and *n*-hexane to get the solid crystalline compound. Free **12c** compounds were purified by column chromatography using the solvent system CHCl$_3$/MeOH (5% to 15% of MeOH in CHCl$_3$). Yield 0.315 g (90%); mp 134−135 °C. ^{1}H NMR (δ, DMSO): 1.22 (9H, s), 2.87 (2H, d), 3.6−3.75 (4H, m), 3.87 (1H, m), 6.46−6.9 (2H, m), 7.9−8.6 (3H, m). ^{13}C NMR (δ, DMSO): 28.7, 37.4, 52.1, 78.5, 124.3, 129.4, 132.4, 154.2, 155.4, 159.1, 170.3. ES MS *m/z* observed, 366.3.

Tyr-Gly-ψ(NH−CO−NH)-Gly-ψ(NH−CO−NH)-Phe-ψ(NH−CO−NH)-Leu-OH 21. A 0.556 g (0.95 mmol) of Fmoc-Tyr-Gly-ψ(NH−CO−NH)-Gly-NCO **9d** was coupled with bis-TMS-Phe (1.1 mmol) to obtain the Fmoc-Tyr(Bu^t)-Gly-ψ(NH−CO−NH)-Gly-ψ(NH−CO−NH)-Phe-OH **19**. Yield 0.634 g (89%); mp 184−187 °C. MS (MALDI-TOF) *m/z* observed: 772.8 [M + Na]$^+$, 789.0 [M + K]$^+$. An amount of 0.634 g (0.84 mmol) of **19** was converted to the corresponding isocyanate. Yield 0.574 g (91%); mp 184−187 °C. IR: 2252 cm^{-1}. ^{1}H NMR (δ, CDCl$_3$): 2.86−2.95 (4H, m), 3.84−4.52 (8H, m), 5.36 (1H, d), 6.22−6.98 (5H, m), 7.12−7.95 (17H, m). ^{13}C NMR (δ, CDCl$_3$): 36.7, 37.4, 43.3, 43.4, 47.3, 54.2, 54.4, 66.7, 119.9, 124.5, 125.1, 126.7, 126.8, 127.3, 128.7, 127.9, 129.3, 129.8, 132.3, 137.6, 141.4, 144.3, 154.5, 156.7. ES MS *m/z* observed, 748.7. A solution of 0.574 g (0.768 mmol) of Fmoc-Tyr(Bu^t)-Gly-ψ(NH−CO−NH)-Gly-ψ(NH−CO−NH)-Phe-NCO in 5 mL of DCM was coupled with bis-TMS-Leu (0.92 mmol in 10 mL of DCM) and after the routine workup, **20** was obtained. The recrystallization in methanol gave almost pure product, and it was used directly for the deprotection. Yield 0.533 g (79%); mp 186−187 °C. MS (MALDI-TOF) *m/z* observed: 901.0 [M + Na]$^+$, 917.2 [M + K]$^+$. After deprotection of Fmoc and Bu^t groups as described above for **13**, the free peptidyl urea **21** was obtained. It was purified by flash column chromatography using the solvent system CHCl$_3$/MeOH (5% to 15% of MeOH in CHCl$_3$) to obtain an analytically pure compound. Yield 0.298 g (82%); mp 113−114 °C. ^{1}H NMR (δ, DMSO): 1.02 (6H, m), 1.42 (2H, m), 1.56 (1H, m), 2.78−4.63 (11H, m), 6.46−8.75 (18H, m). ^{13}C NMR (δ, DMSO): 10.9, 13.8, 21.5, 22.9, 24.3, 36.1, 39.2, 41.3, 50.4, 53.8, 115.5, 115.9, 116.3, 118.9, 124.8, 126.5, 128.0, 129.3, 130.5, 131.4, 155.3, 156.8, 158.2, 158.6, 169.9. MS (MALDI-TOF) *m/z* observed: 623.3 [M + Na]$^+$, 639.3 [M + K]$^+$.

Test for Racemization. To a solution of N^{α}-Fmoc-peptide isocyanate **3c** (1 mmol) was added 1-phenylethylamine (0.121 g, 1 mmol) in the presence of DIEA (0.172 mL, 1 mmol). After the completion of the coupling, the separated solid was filtered and recrystallized using DMF and water (3:1), to obtain the urea adduct **25** as a solid crystalline powder.

Fmoc-Leu-Val-ψ(NH−CO−NH)-*R*-(+)-1-phenylethylamine 25a. Yield 92%; mp 206−207 °C. ^{1}H NMR (δ, DMSO): 0.79−0.90 (12H, m), 1.28−1.30 (3H, d), 1.36−1.92 (4H, m), 4.0 (1H, m), 4.2−4.3 (3H, m), 4.7 (1H, d), 4.9 (1H, d), 6.1 (1H, m), 6.6 (1H, m), 7.2−8.0 (14H, m). ES MS *m*/*z* observed, 573.4.

Fmoc-Leu-Val-ψ(NH−CO−NH)-*S*-(−)-1-phenylethylamine 25b. Yield 94%; mp 205−207 °C. ^{1}H NMR (δ, DMSO): 0.8−0.86 (12H, m), 1.27−1.29 (3H, d), 1.31−1.88 (4H, m), 3.98 (1H, m), 4.2−4.29 (3H, m), 4.7 (1H, d), 4.9 (1H, d), 6.1 (1H, m), 6.5 (1H, m), 7.2−8.0 (14H, m). ES MS *m*/*z* observed, 573.4.

Fmoc-Leu-Val-ψ(NH−CO−NH)-(*R*&*S*)-1-phenylethylamine 25c. Yield 89%; mp 208−209 °C. ^{1}H NMR (δ, DMSO): 0.81−0.90 (12H, m), 1.27−1.30 (3H, t), 1.34−1.88 (4H, m), 4.0 (1H, m), 4.21−4.29 (3H, m), 4.7 (1H, m), 4.9 (1H, m), 6.1 (1H, m), 6.5 (1H, m), 7.2−8.0 (14H, m). ES MS *m*/*z* observed, 573.4.

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Supporting Information Available: IR spectrum for **2o**, **2g**, and **3g**; ^{1}H NMR spectra for the compounds **3b**, **7a**,**e**, **22**, and **25a**−**c**; ^{13}C NMR spectra for the compounds **3b**, **7a**,**e**, and **14**; mass spectra for **3b**,**d**, **4c**, **7a**,**e**, **13**, **21**, **22a**, **23a**, and **25b** and the analytical HPLC data of **2e** and **3e**. This material is available free of charge via the Internet at http://pubs.acs.org.

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