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Synthesis of retro-inverso peptides employing isocyanates of N^{α}-Fmoc-amino acids/peptide acids catalyzed by DMAP☆

Rao Venkataramanarao and Vommina V. Sureshbabu*

Department of Studies in Chemistry, Central College Campus, Bangalore University, Dr. B.R. Ambedkar Veedhi, Bangalore 560 001, India

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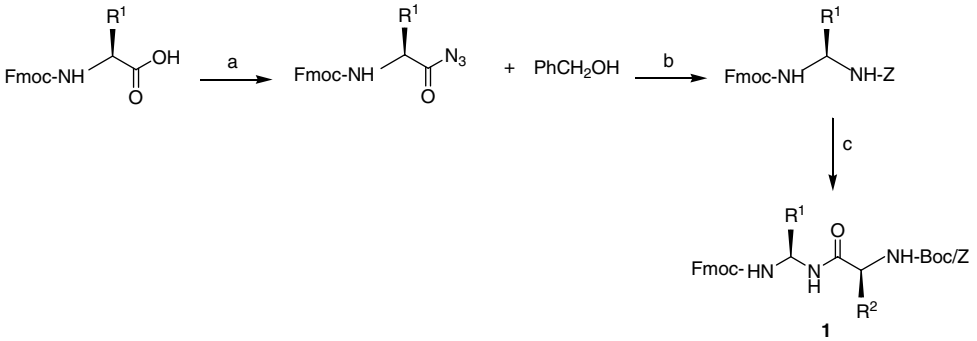
Abstract—The Goldschmidt–Wick type reaction between isocyanates of N^{α}-Fmoc-amino acids/peptide acids and N^{α}-Boc-/Z-/Bsmoc-amino acids catalyzed by DMAP leads to the incorporation of a reversed peptide bond. It was found to be a simple, efficient and clean reaction. All the retro-inverso peptides made were obtained as crystalline compounds in 70–92% yields.

The concept of retro-inverso peptides with free and blocked C- and N-termini has led to new molecules with improved biological activity based on conformational and topological properties.[1] Important classes of molecules studied include neurotransmitters, hormones, inhibitors of proteases and protein kinases, sweeteners, antimicrobial peptides, adhesion molecules, antigenic epitopes, immunomodulators and immunological probes.[2] A large number of molecules have been synthesized by two approaches.[3,4] The first protocol developed by Chorev et al. involves the preferential use of acetyl protecting groups rather than carbamates (specifically Boc or Z moieties) due to the stability of isocyanate, which avoids the displacement reactions.[5,6]

The more commonly used route involves a Hofmann rearrangement of N^{α}-protected amino acid amides using iodobenzene bis(trifluoroacetate) (IBTFA) as an oxidizing agent to obtain mono N-acetylated, *gem*-diaminoalkyl trifluoroacetates as key intermediates.[7–9] As concluded recently by Chorev, rigorous purification is required for building blocks; thus selection of protecting groups and the presence of reactive side-chains are key factors which cannot be overlooked.[3] Further, IBTFA oxidation is not compatible with Fmoc chemistry.[10] Acid azides[11,12] and isocyanates[13] derived from Fmoc protected natural amino acids can be prepared easily and can also be isolated, characterized and employed as key intermediates at ambient temperature in the syn-



Scheme 1. Reagents and conditions: (a) NMM, IBC-Cl, 0 °C, aq NaN_3, 30 min; (b) microwave irradiation for four 15 min intervals; (c) (i) Pd/C, H_2, (ii) Boc-/Z-amino acid, HBTU, DIEA.

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* Corresponding author. Tel.: +91 0822961339; e-mail: haricc b@rediffmail.com

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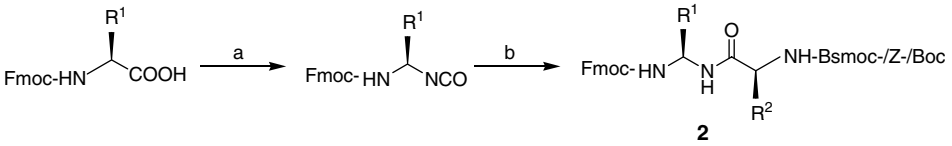
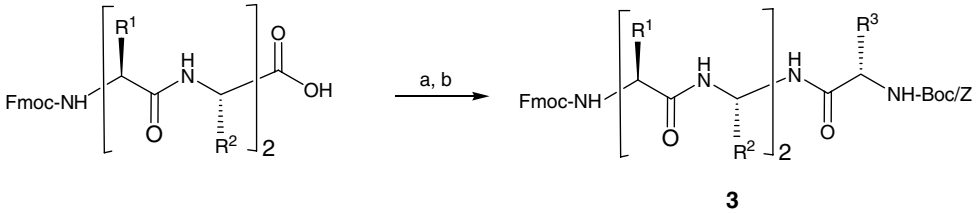


Scheme 2. Reagents and conditions: (a) NMM, IBC-Cl, 0 °C, aq NaN_3, 30 min, microwave irradiation in toluene, 1 min or reflux 1 h; (b) Boc-/Z-/Bsmoc-amino acid, cat. DMAP, temp 0 °C to rt.



Scheme 3. Reagents and conditions: (a) NMM, IBC-Cl, 0 °C, aq NaN_3, 30 min, microwave irradiation in toluene, 1 min or reflux for 1 h; (b) Boc-/Z-amino acid, cat. DMAP, temp 0 °C to rt.

Table 1. Retro-inverso peptides synthesized through the DMAP catalyzed method

Entry	Compound	Mp (°C)	Yield (%)	Mass	^{1}H NMR
a	Fmoc-gLeu-rPhe-Boc	174	90	594.7032	δ 0.93 (d, $J = 5.1$ Hz, 6H), 1.33 (m, 2H), 1.37 (s, 9H), 1.62 (m, 1H), 3.10 (d, $J = 6.2$ Hz, 2H), 3.77 (m, 1H), 3.96 (m, 1H), 4.19 (t, $J = 6.6$ Hz, 1H), 4.42 (d, $J = 5.9$ Hz, 2H), 5.08 (br s, 1H), 5.62 (br s, 1H), 6.20 (br s, 1H), 7.17–7.77 (m, 13H)
b	Fmoc-gAla-rLeu-Boc	190	92	518.2631	0.82 (m, $J = 5.2$ Hz, 6H), 1.21 (d, $J = 6.2$ Hz, 3H), 1.34 (s, 9H), 1.42 (m, 2H), 4.18 (t, $J = 6.6$ Hz, 1H), 4.21 (d, $J = 6.5$ Hz, 2H), 5.10 (d, $J = 8.8$ Hz, 1H), 6.10 (d, $J = 6.6$ Hz, 1H), 6.35 (d, $J = 9.2$ Hz, 1H), 7.31–7.87 (m, 8H)
c	Fmoc-gIle-rCys(Acm)-Boc	202	75	621.7504	0.91 (m, 6H), 1.33 (s, 9H), 1.41 (m, 1H), 1.56 (m, 2H), 2.30 (s, 3H), 3.11 (d, $J = 6.2$ Hz, 2H), 3.62 (m, 1H), 4.19 (t, $J = 6.6$ Hz, 1H), 4.42 (d, $J = 6.5$ Hz, 2H), 4.27 (m, 1H), 5.12 (br d, $J = 9.2$ Hz, 2H), 7.27–7.77 (m, 13H)
d	Fmoc-gLeu-rAsp(Bzl)-Boc	198	79	652.7466	0.82 (m, $J = 5.1$ Hz, 6H), 1.34 (s, 9H), 1.42 (m, 2H), 2.21–2.56 (m, 1H), 3.00–3.33 (m, 1H), 4.21 (t, $J = 6.6$ Hz, 1H), 4.42 (d, $J = 6.9$ Hz, 2H), 5.11 (m, 2H), 5.21 (d, $J = 8.8$ Hz, 1H), 6.20 (d, $J = 6.6$ Hz, 1H), 6.38 (d, $J = 9.2$ Hz, 1H), 7.24–7.87 (m, 13H)
e	Fmoc-gPhe-rVal-Bsmoc	172	89	702.7854	0.94 (t, $J = 13.2$ Hz, 6H), 1.89 (m, 1H), 3.19 (d, $J = 6.1$ Hz, 2H), 3.91 (br s, 1H), 4.19 (t, $J = 6.6$ Hz, 1H), 4.25 (br s, 1H), 4.42 (d, $J = 6.5$ Hz, 2H), 5.21 (s, 2H), 7.13–7.79 (m, 18H)
f	Fmoc-gAla-rPhe-Bsmoc	180	85	674.7295	1.30 (d, $J = 6.3$ Hz, 3H), 2.96 (d, $J = 6.0$ Hz, 2H), 3.33 (m, 1H), 3.88 (m, 1H), 4.19 (t, $J = 6.6$ Hz, 1H), 4.42 (d, $J = 6.9$ Hz, 2H), 5.21 (d, $J = 8.8$ Hz, 2H), 5.91 (br s, 1H), 6.71 (br s, 1H), 7.13–7.90 (m, 17H)
g	Fmoc-gAla-rVal-Boc	218	88	504.5642	0.94 (t, $J = 13.2$ Hz, 6H), 1.33 (s, 9H), 1.39 (d, $J = 6.4$ Hz, 3H), 1.89 (m, 1H), 3.87 (br s, 1H), 4.19 (t, $J = 6.6$ Hz, 1H), 4.26 (br s, 1H), 4.42 (d, $J = 6.5$ Hz, 2H), 7.39–7.78 (m, 8H)
h	Fmoc-gGly-rPhe-Boc	166	85	538.6246	1.33 (s, 9H), 3.21 (d, $J = 4.4$ Hz, 1H), 3.88 (br s, 1H), 4.19 (t, $J = 6.6$ Hz, 1H), 4.30 (br d, $J = 9.2$ Hz, 2H), 4.42 (d, $J = 6.5$ Hz, 2H), 7.13–7.79 (m, 13H)
i	Fmoc-gSer(tBu)-rLeu-Z	171	87	624.7435	0.92 (d, $J = 5.2$ Hz, 6H), 1.33 (m, 2H), 1.38 (s, 9H), 1.62 (m, 1H), 3.55 (br m, 2H), 3.91 (br s, 1H), 4.10 (m, 1H), 4.19 (t, $J = 6.6$ Hz, 1H), 4.42 (d, $J = 6.9$ Hz, 2H), 5.10 (s, 2H), 7.13–7.79 (m, 13H)
j	Fmoc-Phe-gAla-rLeu-Boc	110	88	665.7854	0.90 (d, $J = 5.4$ Hz, 6H), 1.33 (m, $J = 6.0$ Hz, 2H), 1.37 (s, 9H), 1.62 (m, 1H), 3.19 (d, $J = 6.2$ Hz, 2H), 3.88 (m, 1H), 4.10 (m, 1H), 4.19 (t, $J = 6.6$ Hz, 1H), 4.42 (d, $J = 6.9$ Hz, 2H), 7.13–7.79 (m, 13H)
k	Fmoc-Leu-gPhe-rPro-Boc	105	91	691.3472	0.88 (d, $J = 5.4$ Hz, 6H), 1.25 (m, 2H), 1.41 (s, 9H), 1.55 (m, 1H), 1.78 (m, 1H), 1.97 (m, 4H), 3.18 (d, $J = 6.2$ Hz, 2H), 3.35 (m, 2H), 3.88 (br s, 1H), 4.19 (t, $J = 6.6$ Hz, 1H), 4.42 (d, $J = 6.9$ Hz, 2H), 7.13–7.79 (m, 13H)
l	Fmoc-Val-gAla-rPhe-Z	123	89	685.7735	0.93 (t, $J = 13.2$ Hz, 6H), 1.39 (d, $J = 6.6$ Hz, 3H), 1.89 (m, 1H), 3.20 (m, 2H), 3.81 (br s, 1H), 4.19 (t, $J = 6.6$ Hz, 1H), 4.42 (d, $J = 6.9$ Hz, 2H), 5.12 (d, $J = 9.2$ Hz, 2H), 7.21–7.81 (m, 18H)
m	Fmoc-Ala-gLeu-rAib-Z	134	82	637.7371	0.92 (d, $J = 5.2$ Hz, 6H), 1.32 (m, $J = 6.4$ Hz, 2H), 1.39 (d, $J = 6.6$ Hz, 3H), 1.43 (s, 6H), 3.90 (m, 1H), 4.19 (t, $J = 6.6$ Hz, 1H), 4.42 (d, $J = 6.5$ Hz, 2H), 5.12 (d, $J = 9.2$ Hz, 2H), 7.21–7.81 (m, 13H)

thesis of peptides, peptidomimetics and various methyl carbamates.[14] This letter demonstrates the utility of isocyanates derived from Fmoc-α-amino acids for the synthesis of retro-inverso peptides by the Goldschmidt–Wick type reaction.

In the context of our ongoing interest in the incorporation of retro-inverso bonds in peptides, we initially envisaged a route involving the preparation of N,N′-bis-protected *gem*-diamines (Scheme 1). After deprotection of the Z group employing Pd/C, the coupling of Boc-/Z-amino acids with Fmoc–NH–CHR^1–NH_2 using HBTU was explored. In addition to the multi-step protocol, the alcoholysis of isocyanates has to be carried out under microwave irradiation for about 15 min. Recently, Gurtler et al. developed a Mg catalyst system for the reaction of aliphatic isocyanates and blocked isocyanates with carboxylic acids. However, our efforts to couple the isocyanate of Fmoc-Leu-OH with Boc-Phe-OH in the presence of Mg as a catalyst was unsuccessful.[15]

Later we found that a catalytic amount of DMAP[16,17] accelerates the coupling leading to the formation of a retro-inverso peptide bond. In a typical reaction, Fmoc-amino acid azides were prepared by generating a mixed anhydride of Fmoc-amino acid and then reaction with NaN_3. The resulting azide was dissolved in toluene and subjected to Curtius rearrangement.[18] After evaporation of toluene under reduced pressure, it was dissolved in DCM and a mixture of Boc-/Z-/Bsmoc-amino acid and a catalytic amount of DMAP were added at 0 °C and the mixture was stirred for 30 min, allowed to come to rt and stirring was continued for another 2 h. A simple work-up and recrystallization led to the isolation of product **2** in 70–92% yield (Scheme 2). The same methodology has also been applied to Fmoc-peptide acids which resulted in products **3** (Scheme 3).[19] The entire course of the reaction can be completed in about 4 h. All the retro-inverso peptides made were isolated by a single recrystallization (Table 1) and were fully characterized by ^{1}H NMR, ^{13}C NMR and mass spectroscopic measurements.

In conclusion, the Goldschmidt–Wick type reaction between isocyanates of Fmoc-amino acids and N^{α}-protected amino acids catalyzed by DMAP results in retro-inverso peptides. The protocol is simple, efficient and scale-up of the reaction up to 25 mmol per batch did not pose any problems. Thus, it is now demonstrated that the Fmoc group for N^{α}-protection during the synthesis of retro-inverso peptides permits the use of a urethane as a protecting group which can be easily deprotected, unlike an *N*-acetyl, and can also allow further extension of the peptide chain.

Acknowledgements

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18. *General procedure for the synthesis of isocyanates of N^{α}-Fmoc-amino acids/peptide acids*: To an ice-cold solution of N^{α}-Fmoc-amino acid or peptide acid (1 mmol) in dry THF (5 mL) were added *N*-methylmorpholine (NMM) (0.11 mL, 1 mmol) and IBC-Cl (0.135 mL, 1 mmol) 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 mmol in 1 mL) and stirred for another 30 min. After completion of the reaction, the organic layer was evaporated and the residue was dissolved in CH_2Cl_2 (30 mL), washed with 10 mL each of 5% HCl, 5% aq $NaHCO_3$ and brine and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo and the residue dissolved in toluene (10 mL) and heated at 90 °C under nitrogen. After 1 h, the solvent was removed under reduced pressure to give the isocyanate, which was used for the next step.
19. *General procedure for the synthesis of retro-inverso peptides*: To a mixture of N^{α}-Fmoc-amino acid/peptide isocyanate (1 mmol) and Boc-/Z-amino acid (1.2 mmol) in dry CH_2Cl_2 (20 mL) at 0 °C was added DMAP (0.3 mmol). After 30 min at 0 °C, stirring was continued at rt for another 2 h. The solvent was evaporated in vacuo and the residue was dissolved in ethyl acetate, washed with 10 mL each of 5% Na_2CO_3, 5% citric acid and brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the resulting residue crystallized using ethyl acetate/hexane (2:8).