A facile one step synthesis of *N*-2 substituted 3-phenyliminoisoindolinones from *N*-(2-carboxybenzoyl)-anthranilic acid and the design of reverse-turn mimetics

Pradyot Koley,[a] Arpita Dutta,[a] Michael G. B. Drew,[b] Sudeshna Kar,[a] and Animesh Pramanik[a,*]

[a]*Department of Chemistry, University of Calcutta, 92, A. P. C. Road, Kolkata-700 009, India*
[b]*School of Chemistry, The University of Reading, Whiteknights, Reading, UK RG6 6AD*
E-mail: animesh_in2001@yahoo.co.in

Abstract

Stirring of *N*-(2-carboxybenzoyl)anthranilic acid with anilines and amines such as *p*-toluidine, benzylamine, methyl esters of Leu, Phe, Ile and Val in presence of DCC produces *N*-2 substituted 3-phenyliminoisoindolinones in very good yields. Single crystal X-ray diffraction studies and solution phase NMR and CD studies reveal that the 3-phenyliminoisoindolinone moiety is a turn-inducing scaffold which should be useful for reverse-turn mimetics.

Keywords: *N*-(2-Carboxybenzoyl)anthranilic acid, 3-phenyliminoisoindolinones, amino acids, DCC mediated coupling, reverse-turn mimetics

Introduction

Generally 3-iminoisoindolinone derivatives are pigments with excellent color strength and suitable for pigmenting organic materials of high molecular weight, such as ethyl cellulose, acetyl cellulose, nitrocellulose, polyamide, polyester, natural resins and synthetic resins.[1] Further iminoisoindolinones are used to prepare nanosized organic pigments which are used effectively as additives for crystal growth during pigment synthesis.[2] 3-Iminoisoindolinone itself is found to form inclusion complexes with β- and γ-cyclodextrins.[3] Interestingly it has been observed that 3-iminoisoindolinone can bind to an enzyme possessing *N*-iminylamidase activity isolated from pig liver.[4] Although 3-iminoisoindolinone and its derivatives are important for diverse kinds of applications, there has been only a limited number of methods available in the literature for their synthesis. As for example iminoisoindolinone pigments are obtained by condensation of suitably substituted isoindolinones with diamines.[1,2] The synthesis of 3-phenyliminoisoindolinone is achieved by refluxing 1-amino-3-phenyliminoisoindoline with phthalonitrile.[5] In this report we

wish to present a general and efficient method for the synthesis of *N*-2 substituted 3-phenyliminoisoindolinones from easily prepared *N*-(2-carboxybenzoyl)anthranilic acid.

It is well established that reverse-turns play important roles in stabilizing tertiary structures, initiating folding and facilitating intermolecular recognition.[6] There have been increasing efforts to rationally design and synthesize biologically active non-peptidic analogues of peptide reverse turns.[7] Since enzymes like mammalian imidase recognizes 3-iminoisoindolinone as substrate for hydrolysis,[4] it will be quite pertinent to design reverse turns on a 3-iminoisoindolinone scaffold. They will provide an opportunity to explore the substrate specificity, chemoselectivity and the mechanism of enzymatic hydrolysis of compounds bearing the *N*-iminylamide functional group.

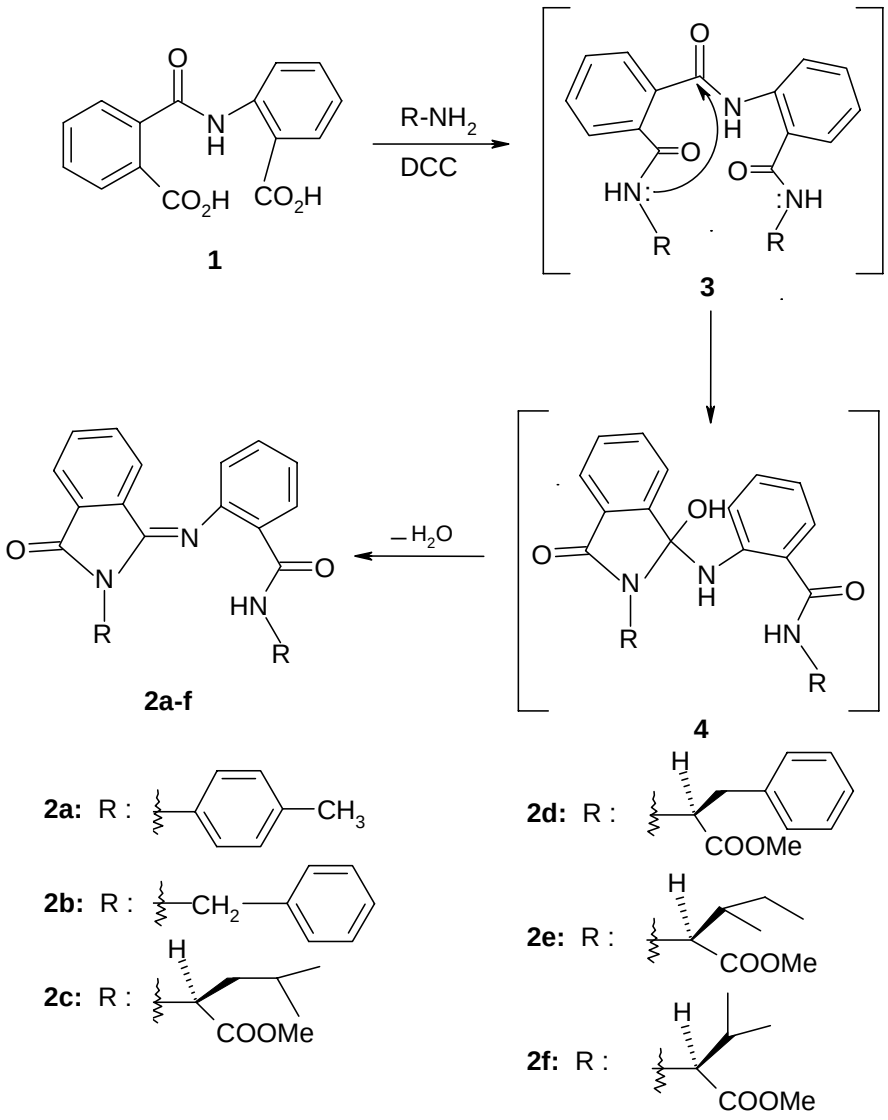
Results and Discussion

Initially we thought that *N*-(2-carboxybenzoyl)anthranilic acid **1**, the alkaline hydrolysis product of 2-phthalimidobenzoic acid,[8] could be a reverse-turn inducing scaffold. Therefore, we were trying to develop reverse-turn mimetics through DCC mediated coupling of **1** with various anilines, amines and amino acids. Surprisingly we observed that stirring of **1** with *p*-toluidine in the presence of DCC in DMF produces an unusual product *N*-2 substituted 3-phenyliminoisoindolinone **2a** in very good yield (Scheme 1, Table 1, Entry 1). Under similar conditions, **1** also produces **2b** by reaction with benzylamine (Entry 2). We have proposed a mechanism for this reaction. The intermediate **3** which is formed through the DCC mediated coupling of **1** with *p*-toluidine and benzylamine, undergoes intramolecular nucleophilic attack of the amide NH to the phthalamide CO to produce the isoindolinone based intermediate **4**. Subsequently dehydration produces the final compounds **2a** and **2b**. Previous reports show that 3-phenyliminoisoindolinone can be prepared by refluxing 1-amino-3-phenyliminoisoindoline with phthalonitrile.[5] Synthesis of compounds **2a** and **2b** by this method is not straightforward. Some synthetic optimization is necessary to achieve the target. The present method represents a novel one step reaction to derive *N*-2 substituted 3-phenyliminoisoindolinones such as **2a** and **2b** starting from **1**.

The formation **2a** and **2b** in the above method indicates that the natural L-α-amino acids would be good candidates for reaction with **1** in a similar fashion to provide a useful route to achieve reverse-turn mimetics on the basis of a 3-phenyliminoisoindolinone scaffold. In fact, the DCC mediated coupling of **1** with methyl esters of various amino acids such as Leu, Phe, Ile and Val produces 3-phenyliminoisoindolinones **2c-f** in very good yields (Scheme 1, Table 1, Entry 3-6). The structures of compounds **2a-f** were confirmed by their IR, ^{1}H NMR, and ^{13}C NMR spectra. The X-ray crystal structures of **2c** and **2d** further confirm the product formation.[9,10] The formation of **2a-f** through the coupling of **1** with *p*-toluidine, benzylamine and various amino acids demonstrates the synthetic potential and generality of the present method.

The DCC mediated reaction of **1** with anilines containing electron withdrawing groups such as methyl *m*-aminobenzoate and *p*-nitroaniline produces different products **5a** and **5b** (Scheme 2,

Table 1, Entry 7 and 8). Since the coupling of methyl *m*-aminobenzoate and *p*-nitroaniline with **1** is slow, the intermediate 2-phthalimidobenzoic acid **6** is formed in a higher rate than intermediate **3**. Finally coupling of **6** with anilines produces **5a** and **5b** (Scheme 2).

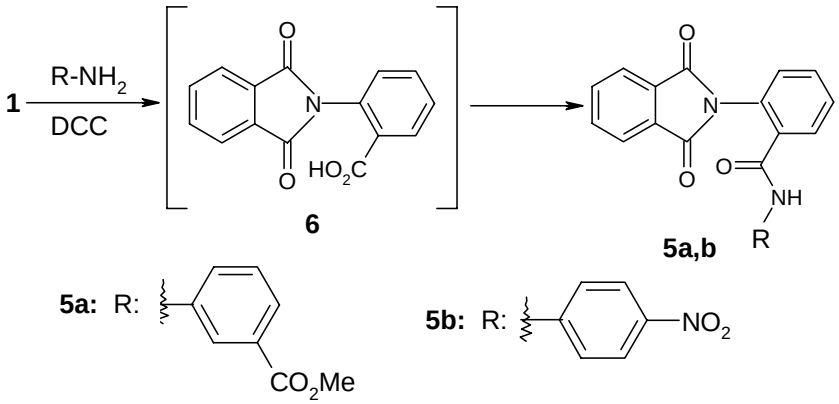


Scheme 1

Table 1. Formation of **2** and **5** from N-(2-carboxybenzoyl)anthranilic acid **1**

Entry	R-NH_2	Products	Yields[a](%)	Mp(^{0}C)[b]
1	*p*-toluidine	**2a**	85.0	280
2	benzylamine	**2b**	82.5	206
3	methyl ester of L-Leu	**2c**	90.2	148
4	methyl ester of L-Phe	**2d**	94.2	165
5	methyl ester of L-Ile	**2e**	87.3	146
6	methyl ester of L-Val	**2f**	83.0	149
7	methyl *m*-aminobenzoate	**5a**	80.6	238
8	*p*-nitroaniline	**5b**	70.5	182

[a]Yields refer to pure isolated products.
[b]MPs are uncorrected.



Scheme 2

Single crystal X-ray diffraction studies show that both **2c** and **2d** adopt turn structures around the central 3-phenyliminoisoindolinone scaffold (Figures 1 and 2). Although a previous report showed that 3-phenyliminoisoindolinone prefers the *anti* conformation in the solid state,[5] in **2c** and **2d** the centrally placed 3-phenyliminoisoindolinone moiety adopts the *syn* conformation. This helps **2c** and **2d** to attain a turn structure around the imino C=N bond. The torsion angle C(41)-N(51)-C(52)-N(10) is -5.2(2)^o at iminoisoindolinone moiety and N(10)-C9-C4-C3 = 7.1(2)^o at the anthranilic acid moiety are responsible for inducing a turn structure in **2c** (Table 2). The corresponding torsion angles values 0.9(4)^o and 3.0(4)^o in **2d** ensure a turn conformation. A β-turn is defined as a tetrapeptide sequence in which the α-carbon distance between first and fourth residue is less than 7 Å.[11] Crystal structure analysis reveals that the distance between the α-carbon atoms (between C(41) and C(1)) in **2c** and **2d** are 6.32 and 4.95 Å respectively, well within the limit for satisfying the criteria of β-turn mimetics (Figures 1, 2). The only major difference in the conformation of the backbone of **2c** and **2d** can be seen in the torsion angle C(9)-C(4)-C(3)-N(2) which is -145.3(2)^o in **2c** and -24.1(4)^o in **2d**. Actually this change in

torsion angle brings C(41) and C(1) closer in **2d** than that of in **2c**. Both **2c** and **2d** can not form any intramolecular hydrogen bond in the solid state. β-turns which do not contain hydrogen bonds are generally referred to as open turns.[12,13]

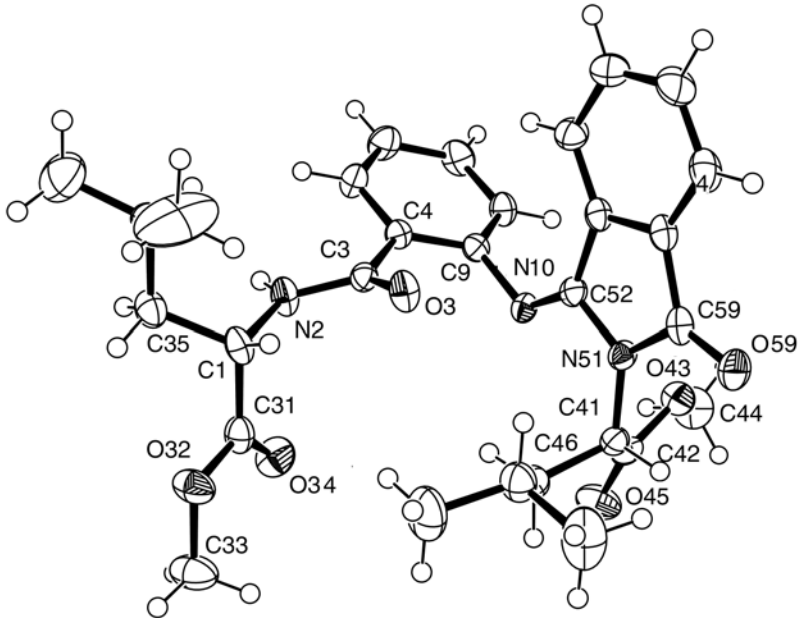


Figure 1. The ORTEP diagram of **2c** including atom numbering scheme. Thermal ellipsoids are shown at the level of 50% probability.

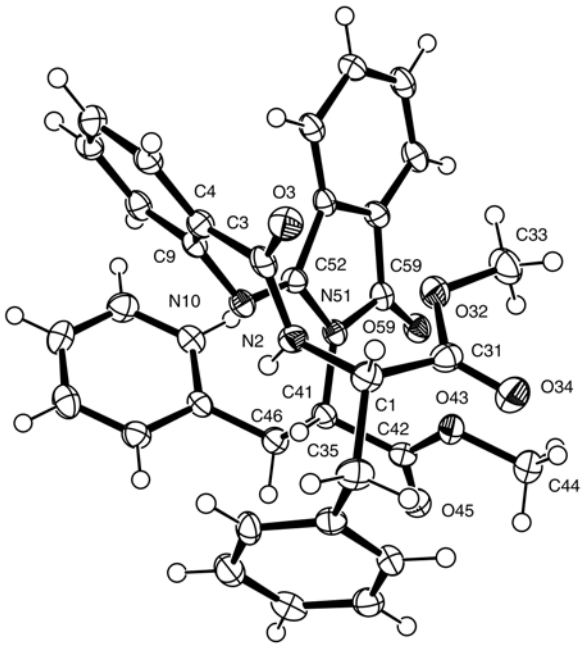


Figure 2. The ORTEP diagram of **2d** including atom numbering scheme. Thermal ellipsoids are shown at the level of 50% probability.

Table 2. Selected backbone torsion angles (°) of pseudopeptides **2c** and **2d** as determined by X-ray diffraction studies

	2c	**2d**
C(44)- C(43)-C(42)-C(41)	177.3(2)	177.4(2)
C(43)-C(42)-C(41)-N(51)	31.8(2)	51.3(8)
C(42)-C(41)-N(51)-C(52)	59.9(2)	-136.7(2)
C(41)-N(51)-C(52)-N(10)	-5.2(2)	0.9(4)
N(51)-C(52)-N(10)-C(9)	178.8(2)	-172.6(2)
C(52)-N(10)-C(9)-C(4)	-94.2(2)	-109.7(3)
N(10)-C(9)-C(4)-C(3)	7.1(2)	3.0(4)
C(9)-C(4)-C(3)-N(2)	-145.3(2)	-24.1(4)
C(4)-C(3)-N(2)-C(1)	176.0(2)	167.4(2)
C(3)-N(2)-C(1)-C(31)	-96.9(2)	-94.6(3)
N(2)-C(1)-C(31)-O(32)	-177.2(2)	19.0(3)
C(1)-C(31)-O(32)-C(33)	-178.5(2)	174.1(2)

In the ^{1}H NMR solvent titration it has been observed that by increasing the percentage of $(CD_3)_2SO$ in $CDCl_3$ from 0 to 8.1 % (v/v) the net changes in the chemical shift ($\Delta\delta$) values for Leu-NH in **2c** and Phe-NH in **2d** are negligible, 0.00, and 0.02 ppm respectively (Figure 3).[14]

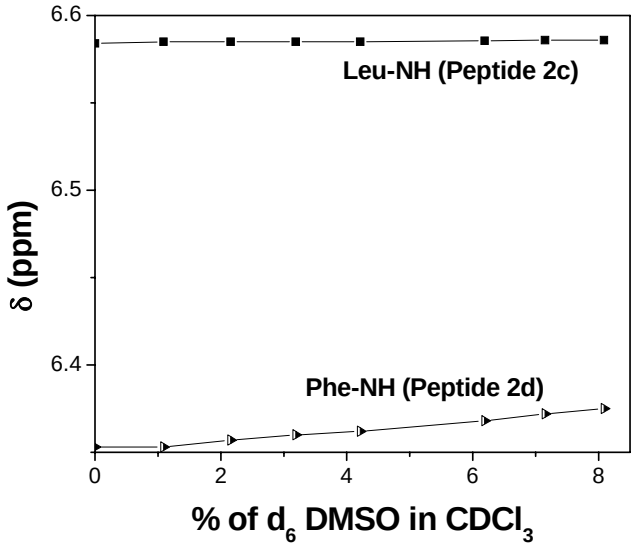


Figure 3. Plot of solvent dependence of NH chemical shifts (δ in ppm) of pseudopeptides **2c** and **2d** at varying concentration of DMSO-d_6 in $CDCl_3$.

The result indicates that in the solution phase Leu-NH of **2c** and Phe-NH of **2d** are strongly hydrogen bonded preferably with the C=O of the methyl ester group of the remaining amino acid within the same molecule to stabilize the 11-membered turn structures (Figure 4). Further the ability of **2c** and **2d** to adopt a turn structure in solution was also evaluated by CD spectroscopy (Figure 5). The spectra were measured in methanol (1.5 mM) and showed a similar behaviour: two negative minima, one at 207-210 nm and a second one at 230-235 nm and a positive maximum at 119-223 nm were displayed by both the pseudopeptides **2c** and **2d**. The CD pattern is found to be quite similar to that of reported β-hairpin mimetics containing diketopiperazine as turn inducing scaffold.[15] Therefore, the results of solvent dependent NMR titrations and CD spectroscopy favor the conclusion that both **2c** and **2d** are folded into turn structures in the solution phase.

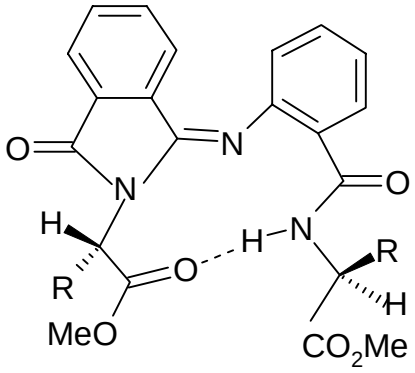


Figure 4. Schematic diagram of intramolecular hydrogen bond of **2c** and **2d** in the solution phase.

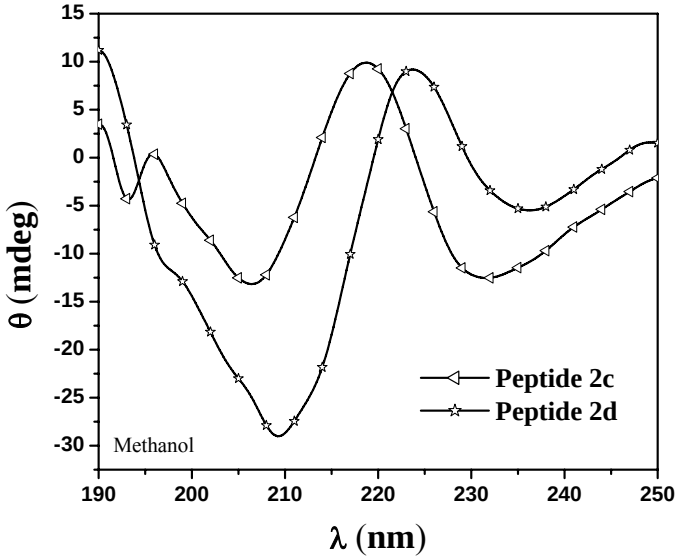


Figure 5. CD spectra of pseudopeptides **2c** and **2d** (1.5 mM in methanol).

Generally 3-iminoisoindolinone derivatives are color pigments.[1] They are also useful for preparing nanosized organic pigments.[2] In the present study **2a** and **2b** are found to be white. Interestingly all the compounds **2c-f** derived from the amino acids are found to be yellow in color. The photograph of yellow crystals of **2d** is presented in Figure 6.

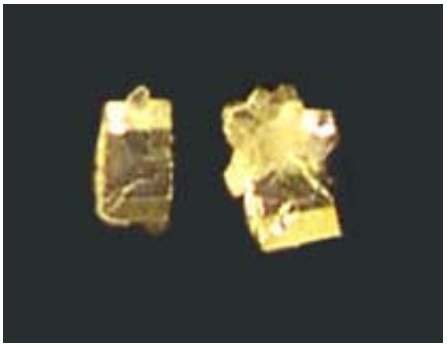


Figure 6. Photograph of the crystals of compound **2d**.

Conclusions and out look

In summary, we have found a general and efficient method for the preparation of *N*-2 substituted 3-phenyliminoisoindolinones by DCC mediated coupling of *N*-(2-carboxybenzoyl)anthranilic acid with activated anilines, amines and amino acids. The present method is a simple one-step reaction involving easily prepared starting materials. Single crystal X-ray diffraction studies and solution phase NMR and CD studies on two pseudopeptides reveal that 3-phenyliminoisoindolinone is a turn-inducing scaffold. Apart from developing reverse-turns this scaffold could be utilized in engineering the turn regions of β-hairpins and multiple antiparallel β-strands. The exploration of the substrate specificity, chemoselectivity and catalytic mechanism of hydrolysis of enzyme mammalian imidase[4] with respect to these turns and hairpins may provide more insights.

Experimental Section

General procedure for products 2a and 2b

(2.0 g, 7.02 mmol) of compound **1** was dissolved in dimethylformamide (DMF, 8 mL). Appropriate anilines and amines such as *p*-toluidine and benzylamine(42.12 mmol) were added to the former solution followed by addition of DCC (4.34 g, 21.06 mmol) in an ice-cold condition. The reaction mixture was stirred at room temperature for 1 day. The precipitated dicyclohexylurea (DCU) was filtered off. The filtrate was diluted with ethylacetate. The organic layer was washed with 1N HCl (3 x 30 mL), brine, 1M Na_2CO_3 solution (3 x 30 mL) and then

again with brine. The solvent was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*, giving a light yellow gum. The products were purified by column chromatography over silica gel (ethyl acetate-petroleum ether). The final compounds were fully characterized by 300 MHz ^{1}H NMR spectroscopy, 75MHz ^{13}C NMR spectroscopy, IR spectroscopy.

2a. Elemental analysis calcd for $C_{29}H_{23}N_3O_2$ (445.52) C, 78.18; H, 5.20; N, 9.43 %. Found: C, 78.05; H, 5.13; N, 9.32 %. IR (KBr): 3286, 1664, 1601, 1523, 1471 cm^{-1}. ^{1}H NMR 300 MHz ($CDCl_3$, δ ppm): 8.33 (ArH, 1H, dd, *J* = 8.1, 0.6 Hz), 7.99 (p-Toluidine NH, 1H, s), 7.80-7.71 (ArH, 2H, m), 7.59-7.51 (ArH, 2H, m), 7.35-7.26 (ArH, 5H, m), 7.15-7.01 (ArH, 6H, m), 2.29 (p-Toluidine (1) -CH_3, 3H, s), 2.25 (p-Toluidine (2) -CH_3, 3H, s); ^{13}C NMR 75 MHz ($CDCl_3$, δ ppm): 165.6, 162.0, 155.4, 147.2, 138.5, 135.3, 135.2, 135.1, 134.7, 134.6, 134.4, 134.2, 130.2, 130.0, 129.6 (2C), 129.3, 128.6 (2C), 127.4 (2C), 127.3 (2C), 126.1, 121.4, 120.4, 120.1, 21.1, 20.8.

2b. Elemental analysis calcd for $C_{29}H_{23}N_3O_2$ (445.52) C, 78.18; H, 5.20; N, 9.43 %. Found: C, 78.08; H, 5.09; N, 9.33 %. IR (KBr): 3353, 1656, 1596, 1532, 1473 cm^{-1}. ^{1}H NMR 300 MHz ($CDCl_3$, δ ppm): 8.36 (ArH, 1H, dd, *J* = 8.0, 1.2 Hz), 7.77-7.71 (ArH, 2H, m), 7.59-7.46 (ArH, 3H, m), 7.33-7.26 (ArH, 2H, m), 7.17-7.06 (ArH, 5H, m), 7.02-7.00 (ArH, 2H, m), 6.92 (ArH, 1H, d, *J* = 8.1 Hz), 6.87-6.84 (ArH, 2H, m), 6.39 (Benzylamine NH, 1H, t, *J* = 7.8 Hz), 5.65 (-CH_2(1), 1H, d, *J* = 15.3 Hz), 4.68 (-CH_2(1), 1H, d, *J* = 15.3 Hz), 4.53-4.46 (-CH_2(2), 1H, m), 4.28-4.23 (-CH_2(2), 1H, m); ^{13}C NMR 75 MHz ($CDCl_3$, δ ppm): 167.2, 162.1, 156.0, 147.0, 137.3, 136.5, 134.5, 134.4, 133.5, 130.3, 129.9, 129.3, 128.6(2C), 128.4(2C), 127.9, 127.8, 127.6(2C), 127.5, 127.4, 127.3(2C), 127.2, 127.1, 121.1, 48.9, 44.1.

General procedure for products 2c-f

(2.0 g, 7.02 mmol) of compound **1** was dissolved in dimethylformamide (DMF, 8mL). Methyl esters of various amino acids such as Leu, Phe, Ile and Val obtained from their hydrochloride (42.12 mmol) were added to the former solution followed by addition of DCC (4.34 g, 21.06 mmol) and HOBT (1.90 g, 14.04 mmol) in an ice-cold (solvent) solution.[16] The reaction mixture was stirred at room temperature for 1 day. The precipitated dicyclohexylurea (DCU) was filtered off. The filtrate was diluted with ethyl acetate. The organic layer was washed with 1N HCl (3x30mL), brine, 1M Na_2CO_3 solution (3x30mL) and then again with brine. The solvent was dried over anhydrous Na_2SO_4 and evaporated in *vacuo*, giving a light yellow gum. The products were purified by column chromatography over silica gel (ethylacetate-petroleum ether). The final compounds were fully characterized by 300 MHz ^{1}H NMR spectroscopy, 75MHz ^{13}C NMR spectroscopy, IR spectroscopy. Single crystals were grown from a mixture of ethyl acetate-petroleum ether (for **2c**) and chloroform-petroleum ether (for **2d**) by slow evaporation and were stable at room temp.

2c. Elemental analysis calcd for $C_{29}H_{35}N_3O_6$ (521.61) C, 66.78; H, 6.76; N, 8.05 %. Found: C, 66.64; H, 6.66; N, 7.97 %. $[\alpha]_D^{25}$ -6.36 (c = 0.10 g per 100 ml); IR (KBr): 3370, 1743, 1676, 1527 cm^{-1}; ^{1}H NMR 300 MHz ($CDCl_3$, δ ppm): 8.30 (ArH, 1H, d, *J* = 7.5 Hz), 7.87 (ArH, 1H, d, *J* = 7.5 Hz), 7.57 (ArH, 1H, t, *J* = 7.5 Hz), 7.44 (ArH, 2H, m), 7.32 (ArH, 2H, m), 6.80 (ArH,

1H, d, *J* = 7.5 Hz), 6.58 (Leu NH, 1H, d, *J* = 7.8 Hz), 5.29 (C^{α}H of Leu (1), 1H, m), 4.79 (C^{α}H of Leu(2), 1H, m), 3.75 (Leu (1) -OCH_3, 3H, s), 3.52 (Leu (2) -OCH_3, 3H, s), 1.61 (C^{β} Hs of Leu (1) and Leu (2), 4H, m), 1.04-0.97 (C^{γ} Hs of Leu (1) and Leu (2), 2H, m), 0.85-0.72 (C^{δ} Hs of Leu (1) and Leu (2), 12H, m); ^{13}C NMR 75 MHz ($CDCl_3$, δ ppm): 173.1, 170.7, 167.2, 165.7, 146.3, 133.3, 132.5 (2C), 132.2, 131.8 (2C), 131.4, 129.5, 125.6, 124.9, 123.8, 121.2, 52.6, 51.9, 51.6, 51.2, 40.8, 37.9, 25.3, 24.9, 23.1, 22.8, 22.7, 21.5.

2d. Elemental analysis calcd for $C_{35}H_{31}N_3O_6$ (589.64) C, 71.29; H, 5.30; N, 7.12 %. Found: C, 71.17; H, 5.14; N, 7.01 %. $[\alpha]_D^{25}$ -27.4 (c = 0.10 g per 100 ml); IR (KBr): 3322, 1737, 1650, 1529 cm^{-1}; ^{1}H NMR 300 MHz ($CDCl_3$, δ ppm): 8.11 (ArH, 1H, m), 7.81 (ArH, 1H, d, *J* = 7.5 Hz), 7.69 (ArH, 1H, d, *J* = 6.9 Hz), 7.52 (ArH, 2H, t, *J* = 7.5 Hz), 7.28-7.02 (ArH, 13H, m), 6.35 (Phe NH, 1H, d, *J* = 7.8 Hz), 5.32 (C^{α}H of Phe (1), 1H, m), 4.99 (C^{α}H of Phe (2), 1H, m), 3.76 (Phe (1) -OCH_3, 3H, s), 3.58 (Phe (2) -OCH_3, 3H, s), 3.21 (C^{β} Hs of Phe (1) and Phe (2), 4H, m); ^{13}C NMR 75 MHz ($CDCl_3$, δ ppm): 171.7, 169.7, 166.9, 165.5, 151.7, 145.9, 137.1, 136.6, 136.5, 133.0, 132.8, 132.4, 131.8, 131.5 (2C), 131.0 (2C), 129.3 (2C), 129.2, 128.6, 128.4 (2C), 128.2, 126.6, 125.5, 124.9, 123.6, 121.5, 53.9, 53.8, 52.5, 52.0, 37.2, 35.0.

2e. Elemental analysis calcd for $C_{29}H_{35}N_3O_6$ (521.61) C, 66.78; H, 6.76; N, 8.05 %. Found: C, 66.68; H, 6.64; N, 7.94 %. ^{1}H NMR 300 MHz ($CDCl_3$, δ ppm): 8.06 (ArH, 1H, d, *J* = 7.8 Hz), 7.85 (ArH, 1H, d, *J* = 7.5 Hz), 7.56 (ArH, 2H, m), 7.43 (ArH, 1H, m), 7.33 (ArH, 2H, m), 6.84 (ArH, 1H, d, *J* = 7.2 Hz), 6.59 (Ile NH, 1H, d, *J* = 7.8 Hz), 5.02 (C^{α}H of Ile (1), 1H, m), 4.67 (C^{α}H of Ile(2), 1H, m), 3.72 (Ile (1) -OCH_3, 3H, s), 3.58 (Ile (2) -OCH_3, 3H, s), 1.65-1.63 (C^{β} Hs of Ile (1) and Ile (2), 2H, m), 1.40-1.20 (C^{γ} Hs of Ile (1) and Ile (2), 10H, m), 0.95-0.88 (C^{δ} Hs of Ile (1) and Ile (2), 6H, m); ^{13}C NMR 75 MHz ($CDCl_3$, δ ppm): 173.2, 171.9, 167.3, 166.4, 153.1, 148.8, 134.2(2C), 133.4, 132.1, 131.4, 131.1, 129.9, 129.7, 129.1, 128.3, 123.6, 56.7, 55.9, 52.3, 51.9, 39.2, 37.8, 25.1, 24.8, 15.2, 14.1, 12.2, 11.4.

2f. Elmental analysis calcd for $C_{27}H_{31}N_3O_6$ (493.56) C, 65.71; H, 6.33; N, 8.51 %. Found: C, 65.58; H, 6.21; N, 8.44 %. ^{1}H NMR 300 MHz ($CDCl_3$, δ ppm): 8.30 (ArH, 1H, d, *J* = 7.5 Hz), 7.90 (ArH, 1H,d, *J* = 7.5 Hz), 7.80 (ArH, 2H, t, *J* = 7.8 Hz), 7.67-7.46 (ArH, 4H, m), 7.10 (Val NH, 1H, d, *J* = 7.8 Hz), 5.21 (C^{α}H of Val (1), 1H, m), 4.70 (C^{α}H of Val (2), 1H, m), 3.72 (Val (1) -OCH_3, 3H, s), 3.48 (Val (2) -OCH_3, 3H, s), 2.09-2.03 (C^{β} Hs of Val (1) and Val (2), 2H, m), 1.18-1.03 (C^{γ} Hs of Val (1) and Val (2), 12H, m); ^{13}C NMR 75 MHz ($CDCl_3$, δ ppm): 172.0, 167.4, 166.8, 161.3, 149.2, 143.4, 135.4, 134.6, 132.5, 131.8, 131.6, 129.8, 128.7, 124.7, 123.9, 120.3, 108.7, 57.4, 57.2, 52.1, 52.0, 31.2, 30.6, 18.7, 18.2, 17.7, 17.4.

General procedure for products 5a and 5b

5a and **5b** were produced following the similar procedure as that of **2a** and **2b** described previously. Here **1** was coupled with methyl *m*-aminobenzoate and *p*-nitroaniline in presence of DCC to produce **5a** and **5b** respectively (Table 1, Scheme 2).

5a. Elemental analysis calcd for $C_{23}H_{16}N_2O_5$ (400.39) C, 69.00; H, 4.03; N, 7.00 %. Found: C, 68.85; H, 3.90; N, 6.94 %. IR (KBr): 3345, 1715, 1602, 1531, 1488 cm^{-1}. ^{1}H NMR 300 MHz (DMSO-d_6, δ ppm): 10.75 (*m*-ABA NH, 1H, s); 8.25 (ArH, 1H, s); 7.91-7.78 (ArH, 6H, m); 7.70-

7.53 (ArH, 4H, m); 7.40 (ArH, 1H, t, *J* = 7.8 Hz); 3.78 (*m*-ABA -OCH_3, 3H, s); ^{13}C NMR 75 MHz (DMSO-d_6, δ ppm): 167.0 (2C), 166.1, 165.3, 139.5, 135.0 (2C), 133.7, 131.6, 131.5 (2C), 130.2 (2C), 130.1, 129.2, 129.1, 128.9, 124.6, 124.4, 123.7(2C), 120.6, 52.3.

5b. Elemental analysis calcd for $C_{21}H_{13}N_3O_5$ (387.35) C, 65.12; H, 3.38; N, 10.85 %. Found: C, 65.01; H, 3.45; N, 10.71 %. IR (KBr): 3337, 1708, 1496, 1459 cm^{-1}. ^{1}H NMR 300 MHz ($CDCl_3$, δ ppm): 8.18 (ArH, 1H, dd, *J* = 7.8, 1.5 Hz), 7.95-7.87 (ArH and *p*-NA NH, 4H,m), 7.80-7.75 (ArH, 3H, m), 7.70-7.46 (ArH, 2H, m), 7.58-7.34 (ArH, 3H, m); ^{13}C NMR 75 MHz ($CDCl_3$, δ ppm): 167.3 (2C), 166.4, 133.9 (2C), 132.6(2C), 132.1, 131.7(2C), 131.5, 130.0(2C), 129.0, 128.8, 126.6, 124.6, 123.4 (2C), 118.5, 109.8.

FT-IR spectroscopy

IR spectra was examined in Perkin Elmer – 782 model spectrophotometer.The solid- state FT-IR measurements were performed using the KBr disk technique.

NMR experiments

All ^{1}H NMR and ^{13}C NMR study was recorded on a Bruker Avance 300 model spectrometer operating at 300, 75 MHz respectively. The peptide concentration was in the range 1-10 mM in $CDCl_3$ and d_6-DMSO for ^{1}H NMR and 30 – 40 mM in $CDCl_3$ and d_6-DMSO for ^{13}C NMR. Solvent titration experiments were carried out at a concentration of 10mM in $CDCl_3$ with gradual addition of d_6-DMSO from 0-8.1 % v/v approximately.

Circular Dichroism Spectroscopy

A methanolic solution of peptide **2c** and **2d** (1.5 mM as final concentration) was used for obtaining the spectra. Far- UV CD measurement was recorded at 25^{o}C with a 0.5sec averaging time, a scan speed of 50 nm/min, using a JASCO spectropolarimeter (J 720 model) equipped with a 0.1 cm pathlength cuvette. The measurement was taken at 0.2nm wavelength interval, 2.0 nm spectral bandwidth and five sequential scans were recorded for the sample.

X-ray crystal analysis

2c. $C_{29}H_{35}N_3O_6$, M = 521.61, orthorhombic, Z = 4, spacegroup $P2_12_12_1$, a = 8.7113(2), b = 15.9102(6), c = 20.4083(7)Å, U = 2828.56(16)Å^3, dcalc = 1.225 gcm^{-3}.

2d. $C_{35}H_{31}N_3O_6$, M = 589.63, monoclinic, Z = 2, spacegroup $P2_1$, a = 9.0115(5), b = 16.6627(6), c = 10.4293(6)Å, β = 109.916(6)^o, U = 1427.36(13)Å^3, dcalc = 1.330 gcm^{-3}; 7820 and 5862 independent data for **2c** and **2d** respectively were collected with MoKα radiation at 150K using the Oxford Diffraction X-Calibur CCD System. The crystals were positioned at 50 mm from the CCD. 321 frames were measured with a counting time of 10s. Data analyses were carried out with the CrysAlis program.[9] The structures were solved using direct methods with the Shelxs97 program.[10] The non-hydrogen atoms were refined with anisotropic thermal parameters. The hydrogen atoms bonded to carbon were included in geometric positions and given thermal

parameters equivalent to 1.2 times those of the atom to which they were attached. The structures were refined on F^2 using Shelxl97 [10] to respectively R1 0.0440 and 0.0548; wR2 0.0987 and 0.1403 for 5103 and 4656 reflections with $I>2\sigma(I)$. Crystallographic data have been deposited at the Cambridge Crystallographic data Centre with reference number CCDC 699562 and 699563 for peptides **2c** and **2d** respectively.

Acknowledgements

PK thanks UGC, New Delhi for providing Project Fellowship. AD and SK would like to thank CSIR, New Delhi, India, for providing senior research fellowship. The financial assistance of UGC, New Delhi is acknowledged [Major Research Project, No.32-190/2006 (SR)]. We thank EPSRC and the University of Reading, UK for funds for Oxford Diffraction X-Calibur CCD diffractometer.

References and Notes

1. (a) Model, E. U. S. Patent 4,327,024, 1982. (b) Model, E. U. S. Patent 3,971,805, 1976. (c) Iqbal, A.; Herren, F.; Wallquist, O. *High Performance Pigments* (Ed. Smith, H. M.); Wiley-VCH, Germany, 2002; Ch. 15, pp. 231.
2. Bäbler, F. U. S. Patent 6,902,613 B2, 2005.
3. Kawamura, M.; Higashi, M. *Journal of Inclusion Phenomena and Macrocyclic Chemistry.* **2005**, *51*, 11.
4. Huang, C. Y.; Yang, Y. S. *Protein Expression and Purification*. **2005**, *40*, 203.
5. Zhang, Z.; Uth, S.; Sandman, D. J.; Foxman, B. M. *Journal of Physical Organic Chemistry.* **2004,** *17*, 769.
6. (a) Mattos, C.; Petsko, G. A.; Karplus, M. *J. Mol. Biol*. **1994**, *238*, 733; (b) Wilmont, C. M.; Thornton, J. M. *J. Mol. Biol*. **1988**, *203*, 221; (c) Kabsch, W., Sander, C. *Biopolymers* **1983**, *22*, 2577.
7. (a) Cluzeau, J.; Lubell, W. D. *Biopolymers* **2005**, *80*, 98. (b) Belvisi, L.; Colombo, L.; Manzoni, L.; Potenza, D.; Scolastico, C. *Synlett* **2004**, 1449; (c) Lelais, G.; Seebach, D. *Biopolymers* **2004**, *76*, 206; (d) Hanessian, S.; McNaughtonSmith, G.; Lombart, H. G.; Lubell, W. D. *Tetrahedron* **1997**, *53*, 12789; (d) Nagai, U.; Sato, K. *Tetrahedron Lett.* **1985**, *26*, 647; (e) Genin, M. J.; Mishra, R. K.; Johnson, R. L. *J. Med. Chem*. **1993**, *36*, 3481; (f) Genin, M. J.; Johnson, R. L. *J. Am. Chem. Soc*. **1992**, *114*, 8778; (g) James, G. L.; Goldstein, J. L.; Brown, M. S.; Rawson, T. E.; Somers, T. C.; McDowell, R. S.; Crowley, C. W.; Lucas, B. K.; Levinson, A. D.; Marsters, Jr. J. C. *Science* **1993**, *260*, 1937; (h) Saragovi, H. U.; Fitzpatrick, D.; Raktabutr, A.; Nakanishi, H.; Khan, M.; Greene, M. I. *Science* **1991**, *253*, 792; (i) Baca, M.; Alewood, P. F.; Kent, S. B. H. *Protein Sci.* **1993**, *2*, 1085; (j) Belvisi, L.;

Bernardi, A.; Manzoni, L.; Potenza, D.; Scolastico, C. *Eur. J. Org. Chem.* **2000**, 2563; (k) Belvisi, L.; Gennari, C.; Madder, A.; Mielgo, A.; Potenza, D.; Scolastico, C. *Eur. J. Org. Chem.* **2000**, 695; (l) Belvisi, L.; Gennari, C.; Mielgo, A.; Potenza, D.; Scolastico, C. *Eur. J. Org. Chem.* **1999**, 389; (m) Franz, A. H.; Gross, P. H.; Samoshin, V. V. *Arkivoc* **2008** (i), 271.

8. Wiklund, P.; Romero, I.; Bergman, J. *Org. Biomol. Chem.* **2003**, *1*, 3396.
9. *CrysAlis*, (**2006**) Oxford Diffraction Ltd., Abingdon, U.K.
10. Sheldrick, G. M. Shelxs97 and Shelxl97, Programs for Crystallographic solution and refinement, *Acta Cryst*. **2008**, *A64*, 112.
11. Lewis, P. N.; Momany, F. A.; Sheraga, H. A. *Biochim. Biophys. Acta*. **1973**, *303*, 211.
12. Dutt, A.; Drew, M. G. B.; Pramanik, A. *Tetrahedron* **2005**, *61*, 11163.
13. (a) Crisma, M.; Valle, G.; Toniolo, C.; Prasad, S.; Balaji Rao, R.; Balaram, P. *Biopolymers* **1995**, *35*, 1; (b) Bosch, R.; Jung, G.; Voges, K. P.; Winter, W. *Liebigs Ann. Chem.* **1984**, 1117; (c) Jung, G.; Bruckner, H.; Bosch, R.; Winter, W., Schaal, H.; Strale, J. *Liebigs Ann. Chem.* **1983**, 1608.
14. Dutt, A.; Dutta, A.; Mondal, R.; Spencer, E. C.; Howard, J. A. K.; Pramanik, A. *Tetrahedron* **2007**, *63*, 10282.
15. Ressurreicão, A. S. M.; Bordessa, A.; Civera, M.; Belvisi, L.; Gennari, C.; Piarulli, U. *J. Org. Chem*. **2008**, *73*, 652.
16. Bodansky, M.; Bodansky, A. *The Practice of Peptide Synthesis*; Springer-Verlag: New York, 1984; pp 1.