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Synthesis and conformation of proline containing tripeptides constrained with phenylalanine-like aziridine and dehydrophenylalanine residues

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Abstract—Tripeptides containing proline and analogues of phenylalanine lead to the formation of β-turn structures. The synthesis and β-turn properties of four such compounds are discussed. © 2002 Elsevier Science Ltd. All rights reserved.

Recent advances[1] in constrained peptidomimetics have opened avenues for the study of the biologically active conformations of many inhibitors and related biomolecules. HIV protease is uniquely specific[2] towards cleaving the phenylalanine–proline (Phe–Pro) bond and thus a study of pseudopeptide inhibitors containing the constrained Phe of the scissile Phe–Pro peptide bond may lead to an insight into the biologically active conformation of such species. We were inspired by the work of Martin et al.,[1d] that cyclopropanes may be used as structural constraints to reduce the flexibility of linear peptides which may lead to the bio-active conformation of such species. Apart from their work, the strategy of constraining the Phe of Phe–Pro scissile bond has not figured prominently in the literature. Contrary to Martin's design, we reasoned that incorporating the nitrogen of amide in a three-membered ring would lead to aziridines (Fig. 1) which apart from containing the constrained conformation of the phenylalanine side chain, would also provide a functional group (i.e. the aziridine) for binding through the aspartate groups at the active site (Fig. 2). It is also evident that the introduction of an aziridine moiety at this bio-active Phe–Pro peptide bond would lead to species that may possess high stereoelectronic complementarity resulting in better recognition by the protease. Also, these modifications will lead to aziridine containing peptides, which have been shown[3] to be mimics for the transition structure of *cis–trans* isomerization. Thus, a transition state mimic for the Phe–Pro *cis–trans* isomerization also promises to be a good model for studying the conformational requirement of the side chain of Phe in the transition state and the information gained from this may lead to the development of potent HIV protease inhibitors. It is also noteworthy that the presence of two constrained amino acids alpha to each other (namely L-proline and constrained phenylalanine) may lead to either a β-strand conformation or a turn conformation for the peptide backbone thereby rendering an additional element of recognition for the protease active site. On the basis of

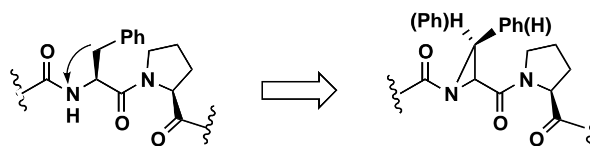

Figure 1.

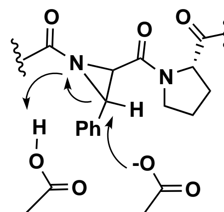

Figure 2.

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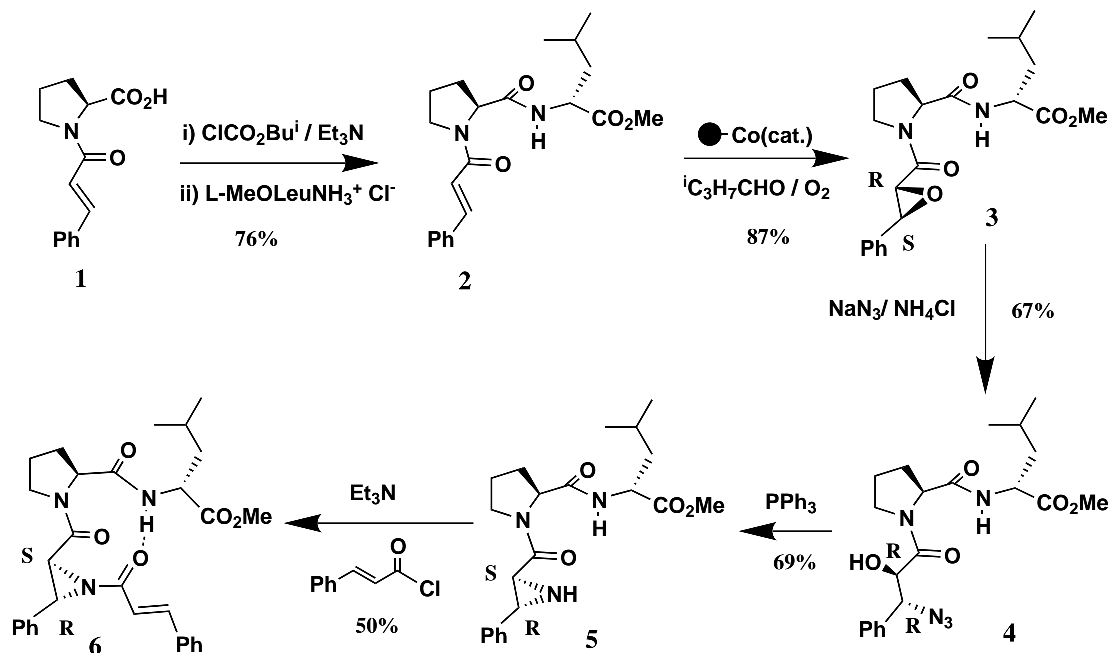
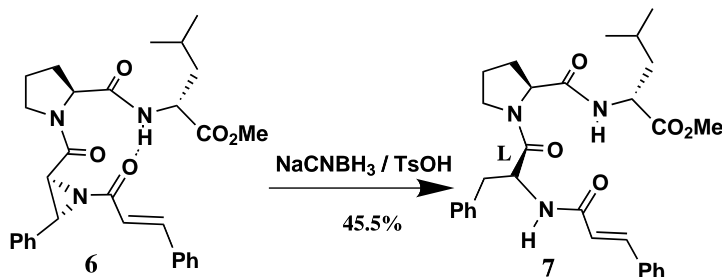
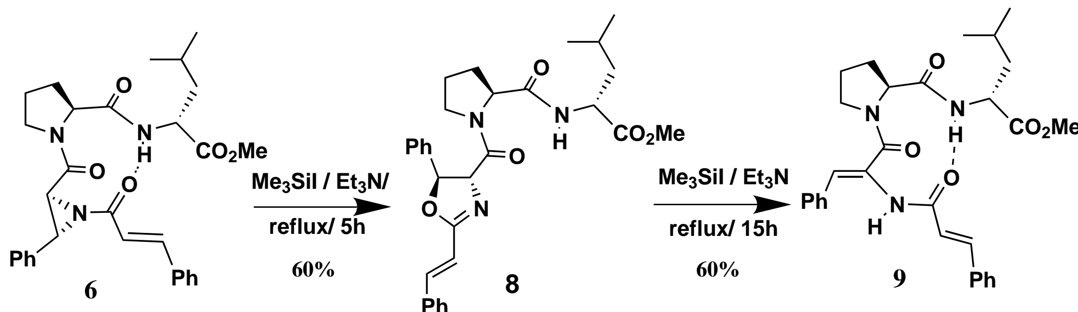
the above considerations, we reasoned that the conformationally constrained Phe–Pro containing peptides may possess the required bio-active conformation (i.e. a β-turn) and may function as irreversible competitive inhibitors of aspartyl proteases. The aziridine containing peptides were synthesized according to the following two protocols i.e. (1) epoxidation and aziridination and (2) kinetic resolution of a racemic aziridine. The first protocol was based on our earlier discovery[4] of an efficient diastereoselective aerobic epoxidation of the cinnamoyl L-proline peptide **2** using a polymer supported cobalt catalyst. The epoxide precursor **3** was prepared according to this procedure in high chemical and enantiomeric purity (Scheme 1). This was subjected to opening with sodium azide in a methanol:water solution in the presence of ammonium chloride to yield the azido alcohol as a mixture of regioisomers in which the benzyl azide **4** was found to be the predominant product (^{1}H NMR). To a solution of the azido alcohol **4** in dry acetonitrile was added triphenylphosphine at room temperature and the reaction mixture was heated under reflux. After completion of reaction, the solvent was removed and the contents were isolated by flash column chromatography to yield the required aziridine containing peptide **5** in good yield. The aziridine peptide **5** was optically pure as evidenced from HPLC and rotation ($[\alpha]_D^{25}$ −139.7). The absolute stereochemistry of the aziridine peptide **5** was assigned based on the correlation with the epoxide whose configuration had already been established.[4d] The aziridine **5** was converted to the corresponding acylaziridine **6**[5] on coupling with cinnamoyl chloride in the presence of triethylamine. The L-configuration of α-carbon of the aziridine **6** was confirmed by reducing it with $NaCNBH_3$/*p*-TsOH to the corresponding tripeptide **7** whose ^{1}H NMR and optical rotation were consistent with the authentic tripeptide[6] prepared from *N*-cinnamoyl L-Phe, L-Pro and L-Leu (Scheme 2). The acylaziridine **6** underwent smooth isomerization on treatment with Me_3SiI/Et_3N to the corresponding oxazoline **8**[7] which was transformed on heating in Et_3N/Me_3SiI to dehydrophenylalanine residue containing peptide **9**[7] (Scheme 3).



Scheme 1.

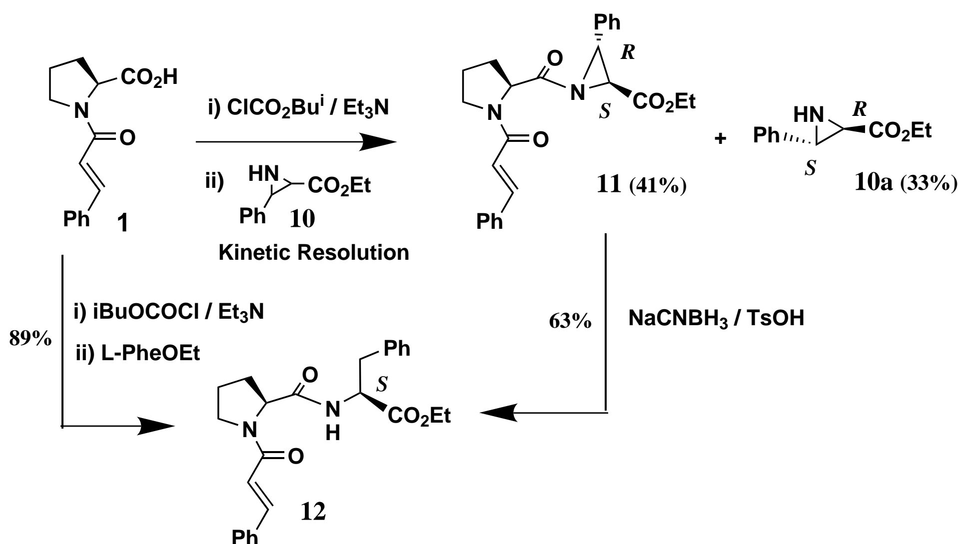


Scheme 2.

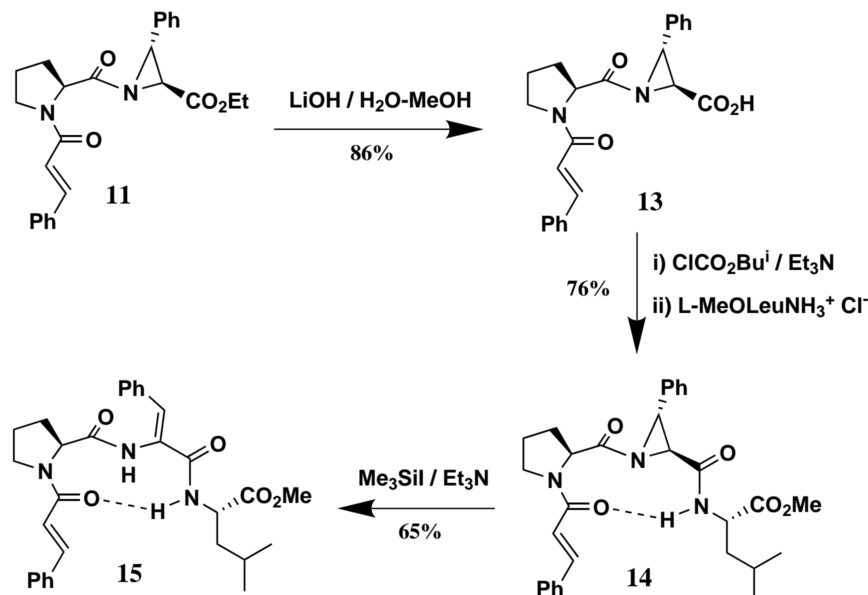


Scheme 3.

The second route to aziridine peptides was based on the kinetic resolution of the racemic aziridine **10** during peptide bond formation with cinnamoyl L-proline (Scheme 4). This protocol leads to the synthesis of the dipeptide **11** where the aziridine configuration is 2*S*,3*R*. The 2*S* configuration of the dipeptide **11** was confirmed by reducing it with $NaCNBH_3$/TsOH which gave the cinnamoyl L-proline-L-PheOEt dipeptide **12**. This dipeptide was compared with an authentic peptide prepared[6] from *N*-cinnamoyl L-Pro and L-PheOEt. The two peptides exhibited identical ^1H NMR spectra and similar optical rotation. Having ascertained the configuration of the aziridine in **11**, it was subjected to base hydrolysis to give the corresponding carboxylic acid **13** which was converted to the aziridine tripeptide **14** on coupling with L-LeuOMe. The tripeptide **14** was reacted with Me_3SiI/Et_3N to afford the corresponding dehydrophenylalanine derived peptide **15** (Scheme 5). The *Z*-geometry of the double bond in **15** was established by NOE studies. The amide NH NMR chemical shifts (δNH) for **6**, **9**, **14** and **15** in a non-hydrogen bonding solvent like $CDCl_3$ show that these molecules can form 10-membered ring C=O···NH hydrogen bonds analogous to those commonly observed[8] in β-turns. Table 1 contains δNH values measured at room temperature with 1 mM $CDCl_3$ solutions. It is noteworthy that only a single tertiary amide rotamer was detected for these peptides and it is clearly evident from the data in Table 1 that the Ha in **6** is internally hydrogen bonded (δNHa = 7.76 ppm). Similarly the Ha proton is involved in intramolecular bonding in **9** whereas the δNHd values (6.12 ppm) indicate that this amide proton is not internally hydrogen bonded to any significant extent. A similar pattern was observed in the aziridine peptide **14** where the δNHa (7.89 ppm) indicated that this proton was involved in intramolecular hydrogen bonding. Between the two-amide protons in **15**, only Ha (δ = 7.95 ppm) indicated that it is internally hydrogen bonded whereas the chemical shift for H_b (δ = 6.32 ppm) suggested that this proton does not experience much intramolecular hydrogen bonding. The chemical shift dependences of the NH groups as a function of increasing solvent polarity were measured and representative data are shown in Figs. 4 and 5. Nearly invariant chemical shifts for the Leu(NH) are observed for peptide **9** and **15** when it is dissolved in $CDCl_3$ with various concentration of DMSO-d_6.



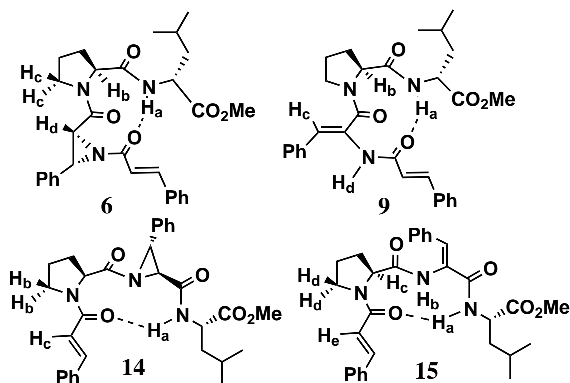
Scheme 4.



Scheme 5.

Interestingly, the evaluation of the ROSEY spectrum of these peptides reveals a number of critical NOEs. In particular the Pro(Cα-H) and the (NH) in **6**, **9**, **14** and **15** are close (2–2.5 Å) which clearly indicates that the solution phase conformations of these molecules are preorganized due to the presence of a β-turn. NOE studies on **6**, **9**, **14** and **15** clearly indicate that the Pro $C_\delta H$ and the aziridine H_d in **6** (Table 1) are in close proximity whereas in **14** the former protons are close to the vinylic α-proton of the cinnamoyl group. Similar studies on **9** and **15** also support the presence of a β-turn as indicated by the NOEs observed between H_a/H_b and H_c/H_d in **9** and between H_d/H_e in **15** (Table 1). It is noteworthy that the NOE between H_c and H_d in **9** also supports the configuration of the aziridine in **6**. Further, the minimum energy conformation (Fig. 3) of the aziridine peptides **6** and **14** also supports the presence of intramolecular hydrogen bond whose length falls well within the standard value observed in β-turns present in proteins.

Table 1. Amide proton chemical shifts in $CDCl_3$



Compound	δNHa	δNHb	δNHd
6	7.76	–	–
9	7.86		6.12
14	7.89	–	–
15	7.95	6.32	–

In conclusion, we have demonstrated that the aziridine residue confers a conformational restriction and the tripeptides derived from it and L-proline exhibit the formation of a β-turn structure in the solution phase. These tripeptides, which carry a constrained mimic of a Phe residue C- or N-linked to L-proline, are also good models for studying the mechanism of HIV protease, which specifically involves cleaving a Pro–Phe bond. Also, we have demonstrated that the aziridine peptides can be transformed stereoselectively to the corresponding dehydrophenylalanine containing tripeptides, which also show a strong preference for a β-turn formation in the solution phase.

Acknowledgements

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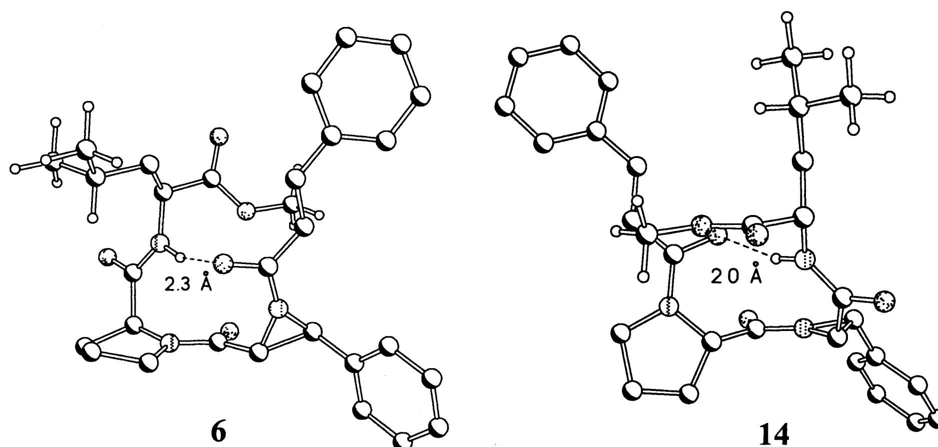

Figure 3. Minimum energy conformations of tripeptides **6** and **14**.

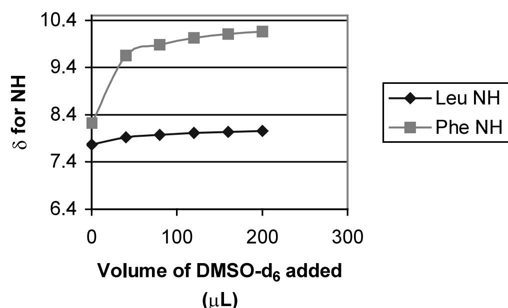

Figure 4. Shift of the NHs with the addition of DMSO-d_6 to the $CDCl_3$ solution of peptide **9**.

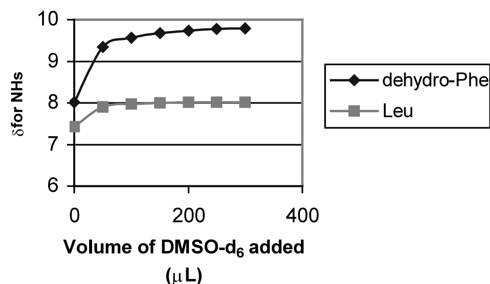

Figure 5. Shift of the NHs in $CDCl_3$ solution of peptide **15** with the addition of DMSO-d_6.

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5. ***Synthetic protocols***: Aziridine peptide **6**: To a stirring solution of *N*-cinnamoyl peptide **2** (3 mmol) in CH_3CN (25 mL) was added polyaniline supported[5] cobalt(II) salen (5 mg), and 2-methylpropanal (6 mmol) under an oxygen atmosphere. The resulting mixture was stirred for 12 h under an oxygen atmosphere and at this stage an additional amount of 2-methylpropanal (6 mmol) was added and the stirring continued until the epoxidation was complete (TLC). The usual aqueous work-up (3×10 mL saturated sodium bicarbonate solution) and drying followed by column chromatography (silica gel) afforded the epoxide **3** (87%) as the major diastereomer. To a solution of the oxirane **3** (398 mg, 1 mmol) in methanol (8 mL) were added NaN_3 (325 mg, 5 mmol) and NH_4Cl (118 mg, 2.2 mmol) followed by water (1 mL) and the reaction mixture was refluxed for 8 h. The solvent was removed and residue was taken in ethyl acetate (10 mL) and washed with water (2×15 mL) and the organic layer was dried (Na_2SO_4). Evaporation of the organic layer afforded a residue which was chromatographed over silica gel to yield the azido alcohol **4** (67%), $[\alpha]_D^{25}$ −71.1 (*c* 0.003, CH_2Cl_2). To a solution of the azido alcohol **4** (353 mg, 0.8 mmol) in acetonitrile (6.4 mL) was added triphenylphosphine (210 mg, 0.8 mmol) in portions, with vigorous stirring under ambient conditions. The reaction mixture was stirred for a further 0.5 h at ambient temperature and the solution set to reflux for 6 h. The solvent was evaporated under vacuum and the residue was subjected to flash chromatog-

raphy (silica gel: EtOAc/hexane) to yield aziridine peptide **5** (67%), $[\alpha]_D^{25}$ −139.7 (*c* 0.018, CH_2Cl_2). A solution of aziridine peptide **5** (200 mg, 0.52 mmol) and triethylamine (145 μL, 1.02 mmol) in DCM (5 mL) was cooled to 0°C and to it was added cinnamoyl chloride (86 mg, 0.52 mmol) in DCM (3 mL). The solution was warmed to room temperature and stirred for 6 h. The solvent was removed and the residue was taken in EtOAc (10 ml) and washed with saturated solution of $NaHCO_3$ (2×10 mL) and brine (10 mL). The organic phase was dried (Na_2SO_4) and concentrated to give a residue which was subjected to flash chromatography (silica gel: EtOAc/Pet ether) to yield the peptide **6** (50%), $[\alpha]_D^{25}$ −19.4 (*c* 0.5, $CHCl_3$). ^{1}**H NMR** (200 MHz, $CDCl_3$): δ 7.76 (d, *J*=7.0 Hz, NH), 7.68 (d, *J*=16.1 Hz, 1H), 7.52–7.29 (m, 10H), 7.03 (d, *J*=7.8 Hz, NH unbound), 6.62 (d, *J*=16.0 Hz, 0.5H), 6.56 (d, *J*=16.0 Hz, 0.5H), 4.71–4.45 (m, 2H), 4.26 (d, *J*=2.0 Hz, 0.5H), 4.07 (d, *J*=2.0 Hz, 0.5H), 3.80–3.60 (m, 5H), 3.36 (d, *J*=2.0 Hz, 0.5H), 3.29 (d, *J*=2.0 Hz, 0.5H), 2.60–1.50 (m, 7H), 1.00–0.84 (m, 6H). **Mass (*m*/*z*, CI)**: 518 (M^++1), 391, 345, 311, 257, 243, 148, 131, 107.

6. ***Synthetic protocols***: Compound **7**: To a solution of aziridine **6** (110 mg, 0.212 mmol) in dry THF (8 mL) $NaCNBH_3$ (27 mg, 0.43 mmol) and *p*-TsOH (5 mol%) were added and stirred at room temperature for 14–15 h. The solvent was removed and the residue was dissolved in ether. The organic layer was washed with brine (10 mL) followed by water (10 mL). Drying and evaporation of ether gave a residue, which was chromatographed over silica gel to afford the tripeptide **7** (45.5%). The ^{1}H NMR and specific rotation of **7** ($[\alpha]_D^{25}$ −37.9 (*c* 0.19, $CHCl_3$) were similar to the authentic tripeptide $[\alpha]_D^{25}$ −44.6 (*c* 0.19, $CHCl_3$) prepared from *N*-cinnamoyl L-Phe, L-Pro and L-Leu. ^{1}**H NMR** (200 MHz, $CDCl_3$): δ 8.07 (d, *J*=7.0 Hz, NH), 7.61 (d, *J*=16.1 Hz, 1H), 7.55–7.20 (m, 10H), 7.03 (d, *J*=7.3 Hz, NH), 6.39 (d, *J*=15.6 Hz, 1H), 5.11 (d, *J*=7.3 Hz, 1H), 4.62–4.43 (m, 2H), 3.75 (s, 3H), 3.70–3.50 (m, 2H), 3.10 (d, *J*=4.9 Hz, 2H), 2.38–2.20 (m, 1H), 2.00–1.80 (m, 2H), 1.80–1.50 (m, 4H), 1.10–0.80 (m, 6H). **Mass (*m*/*z*, CI)**: 520 (M^++1), 472, 374, 243, 182.

7. ***Synthetic protocols***: Compound **8** and **9**: To a stirred solution of trimethylsilyl chloride (43 μL, 337 μmol) in acetonitrile (10 mL) sodium iodide (50.7 mg, 338 μmol) was added under nitrogen and it was stirred at room temperature until the solution turned yellow. A solution of aziridine **6** (100 mg, 193 μmol) in acetonitrile (5 mL) was added to the above mixture and refluxed for 5 h. The reaction mixture was cooled down to room temperature and Et_3N (54 μL, 384 μmol) was added to it and was stirred at room temperature for further 6 h. The solvent and reagents were removed and the residue was purified by column chromatography (silica gel: EtOAc/Pet ether) to afford the oxazoline **8** (60%). This intermediate **8** (60 mg) in acetonitrile (7 mL) was mixed with Me_3SiCl (25 μL, 197 μmol), NaI (30 mg, 197 μmol) and refluxed for 15 h. The usual work up (as described for **8**) afforded the pure compound **9** (60%).
   Spectral data of 8: ^{1}**H NMR** (200 MHz, $CDCl_3$): δ 7.58–7.34 (m, 11H), 7.16 (d, *J*=7.8 Hz, NH), 6.70 (d, *J*=16.1 Hz, 1H), 6.14 (d, *J*=7.3 Hz, 1H), 4.79 (d, *J*=7.3 Hz, 1H), 4.62–4.10 (m, 2H), 3.72–3.43 (m, 5H), 2.40–2.20 (m, 1H), 2.13–1.91 (m, 3H), 1.74–1.54 (m, 3H), 0.96–0.84 (m, 6H). **Mass (*m*/*z*, CI):** 518 (M^++1), 431, 373, 345, 248, 209, 119.
   Spectral data of 9: ^{1}**H NMR** (200 MHz, $CDCl_3$): δ 7.86 (s, NH bound), 7.60 (d, *J*=15.6 Hz, 1H), 7.50–7.20 (m, 10H), 6.55 (d, *J*=15.6 Hz, 1H), 6.12 (s, 1H), 4.69–4.45 (m, 2H), 3.80–3.58 (m, 5H), 2.40–2.20 (m, 1H), 2.13–1.54 (m, 6H), 1.00–0.75 (m, 6H). **Mass (*m*/*z*, CI)**: 518 (M^++1), 372, 276, 243, 131.

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