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Synthesis of cyclic peptides as mimics for the constrained conformation of structural analogues of HIV protease inhibitors

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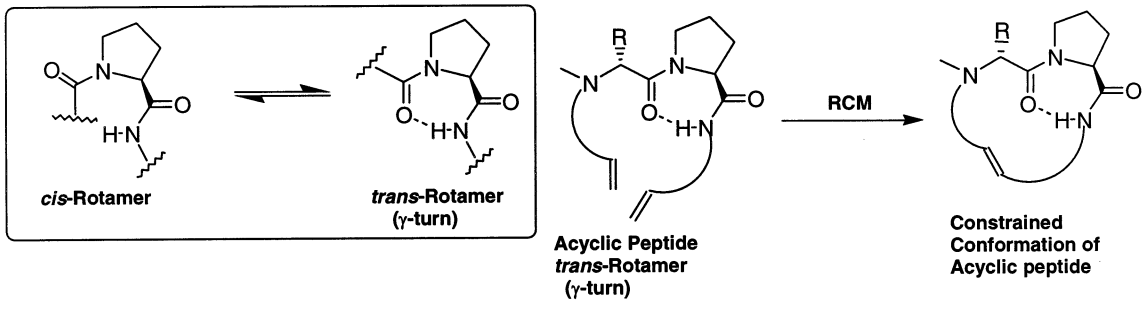
Abstract—A γ-turn induced cyclisation of tripeptides **4** can be performed in a ring-closing metathesis reaction using Grubb's catalyst to the corresponding cyclic peptides **5**. These cyclic peptides mimic the constrained conformation of structural analogues of potential HIV protease inhibitors.

Recent progress in the synthesis and screening of huge peptide libraries has focused attention on small peptides as important lead structures for the development[1] of potential therapeutic agents. The linear peptide fragments are flexible and exhibit numerous conformations in solution and even in the solid state. However, if one can restrict the conformational freedom of these linear peptides by introducing[2] some constraints in the structure, it may render a biologically active peptide more potent, more specific and orally active and this may give rise to species that are therapeutically useful. Thus, small cyclic peptides are of great interest for the elucidation of bioactive conformations due to their restricted conformational flexibility. In view of the importance of constrained conformations, there have been several attempts[3] to lock peptides into turn configurations and to synthesise molecules that might mimic a reverse turn in an otherwise normal peptide. Several types of turns are found in proteins and the type VI β-turn is a unique member of the beta-turn family because it is the only turn that involves an *s-cis* peptide bond. Type VI beta turns always contain a L-proline residue at the $i+2$ position, since peptides incorporating this amino acid are the only ones that can exist substantially in the *s-cis* conformation. The understanding of the conformation of the type VI beta turn is very crucial to the development of inhibitors of HIV protease.[4] This is mainly due to the specificity shown by the HIV protease for the selective cleavage of proline–phenylalanine/tyrosine amide bonds in the Matrix–Capsid domain of the gag-pol polyproteins. L-Proline containing peptides give rise to a β-turn conformation via the two rotamers that are obtained due to isomerisation around the proline amide bond. The *trans* rotamer can also adopt the γ-turn due to the appropriate positioning of carbonyl and the amide NH groups (Scheme 1).

In connection with our work on HIV protease inhibitors based on pyrrolidine containing α-hydroxy β-



Scheme 1.

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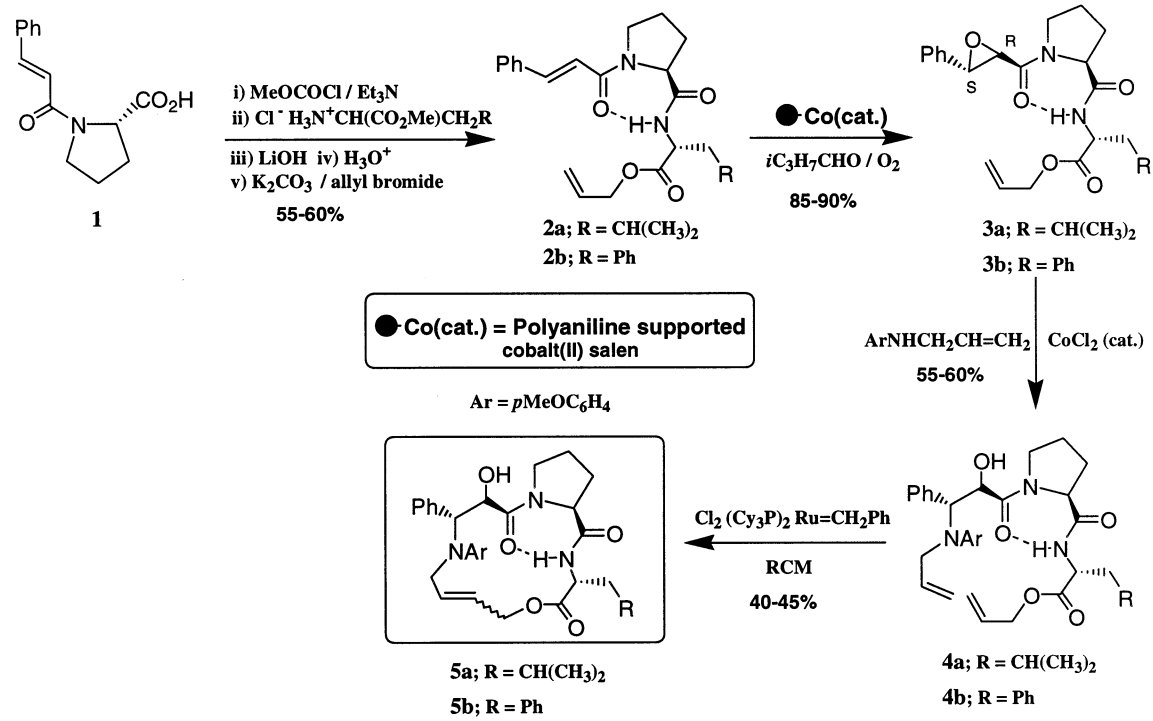
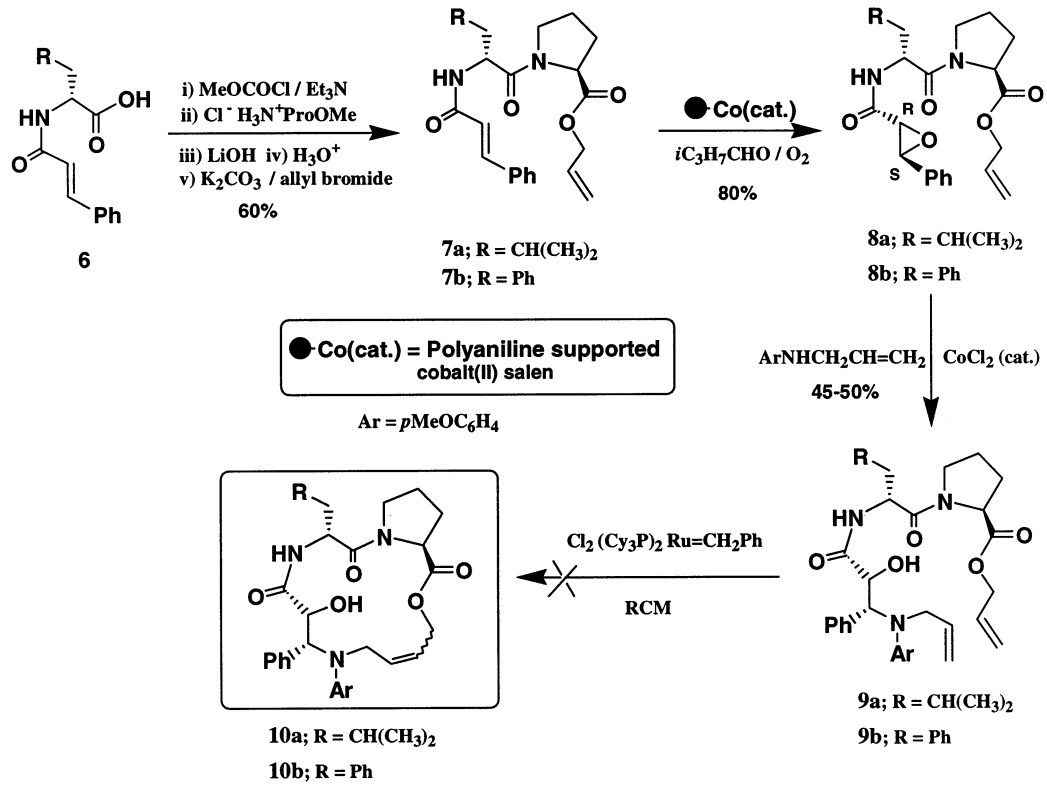
amino amide core structures,[4a] we reasoned that the latter structural unit can be incorporated into a constrained conformation by the cyclisation of tripeptides derived from such units. We also envisioned that the tripeptides derived from a sequence where L-proline is involved in a γ-turn would be ideal precursors for such cyclisations. We now show that tripeptides derived from *O*-allyl-Xaa-L-proline-β-phenylisoserine-*N*-allyl derivatives are preorganised due to a γ-turn and can be cyclised by ring-closing metathesis (Scheme 1) using Grubb's catalyst[3a] and the cyclisation is facile only when a turn-inducing L-proline residue[5] is in the middle of such a tripeptide. The tripeptide derivatives **4** were prepared by our polyaniline supported cobalt catalysed[6] aerobic epoxidation and its opening protocol as described earlier (Scheme 2). The peptide precursors were synthesised from *N*-cinnamoyl proline **1** by coupling with L-leucine or L-phenylalanine methyl ester and they were converted to the corresponding allyl ester **2a** and **2b**, respectively, by hydrolysis (LiOH) and esterification with allyl bromide.

These peptides showed an intramolecular hydrogen bond (γ-turn) in their proton NMR as indicated[2c] by the appearance of a low field signal at δ 7.43 ppm ($J = 8.28$) due to the amide proton. The peptides **2a** and **2b** were subjected to aerobic epoxidation in the presence of 2-methylpropanal and catalytic amount of polyaniline supported cobalt(II) salen to yield the corresponding epoxides **3a** and **3b**, respectively, as a single diastereomer in good yields. The absolute stereochemistry (2*R*,3*S*) for these epoxides was assigned based on the correlation studies as described in the preceding communication. The epoxides also exhibited the presence of intramolecular hydrogen bonding in their proton NMR at δ 6.99 ppm ($J = 8.8$ Hz). The opening of epoxides **3a** and **3b** were achieved with *N*-allyl anisidine in the presence of catalytic amount of cobalt(II) chloride to afford the corresponding tripeptide derivatives **4a** and **4b**, respectively, mainly as the *anti* diastereomers in 55–60% yields. We have shown[6] earlier that the cobalt-catalysed opening of cinnamoyl epoxide takes place by an SN^2 pathway leading to the *trans* diastereomer as the predominant product. The tripeptides **4a** and **4b** also showed the presence of intamolecular hydrogen bonding as indicated by the appearance of low field NMR signal at δ 7.43 ($J = 8$ Hz) due to the amide proton. The presence of intramolecular hydrogen bonding in all the three structures suggest that these molecules are preorganised due to the presence of a γ-turn, which may facilitate the cyclisation of **4** via ring-closing metathesis using Grubb's catalyst.[3a] This indeed was found to be the case; as subjecting **4a** and **4b** to heating[7] in the presence of 10 mol% of ruthenium alkylidene (Grubb's catalyst) in dichloromethane (0.6 mmol) for 15 hours yielded the cyclised peptides **5a** and **5b**, respectively, in 40–45% yields after column chromatography. These cyclic peptides were obtained as a mixture of *E*:*Z* (4:1) isomers as indicated[8] by NMR. The reaction mixture also consisted of some minor oligomeric products and ~20% of the tripeptides **4** were recovered unchanged. The proton NMR (δ 7.45 ppm, $J = 8.23$ Hz) of the cyclic peptides **5** revealed the presence[2] of the intramolecular hydrogen bonding which clearly suggests that a γ-turn is also present in the cyclic form and might have been responsible for the cyclisation. That the presence of a γ-turn may be responsible for such cyclisation is evident from the ring-closing studies on the acyclic tripeptide **9** containing L-proline at the terminal position of the residue



Scheme 2.



Scheme 3.

(Scheme 3). The tripeptides **9a**–**b**, which lack a γ-turn, were synthesised by the protocol defined for **4** in Scheme 2. Thus **6a**–**b** were converted to the corresponding *N*-cinnamoyl dipeptides **7a**–**b**, respectively, which were transformed to the corresponding epoxides **8a**–**b**. These epoxides were subjected to cobalt(II) chloride-catalysed opening by *N*-allyl anisidine as described earlier to afford the tripeptides **9a** and **9b**, respectively (Scheme 3). The tripeptides **9a**–**b** were subjected (0.06 mmol in dichloromethane) to ring-closing metathesis using Grubb's catalyst and it is interesting to note that the corresponding cyclic peptides **10a** or **10b** were not observed and the reaction mixture consisted of intractable oligomeric material apart from the unreacted **9** (20–30%). This study clearly suggests that absence of a γ-turn in **9** will not render these tripeptides preorganised for cyclisation under ring-closing metathesis conditions.

In conclusion we have synthesised the cyclic peptides consisting of Xaa-L-Proline-β-phenylisoserine tripeptides from the acyclic precursors having olefinic group at both ends via ring-closing metathesis using Grubb's catalyst. These cyclisations are controlled by the presence of a γ-turn in the acyclic precursor and the cyclic peptides thus obtained are mimics of the constrained conformation of the structural analogues of HIV protease inhibitors.

Acknowledgements

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7. **General procedure for the synthesis of tripeptides**: The *N*-cinnamoyl dipeptides **2** and **7** were synthesised by mixed anhydride coupling (MeOCOCl, Et_3N) of *N*-cinnamoyl amino acids with methylester hydrochlorides of the amino acids. Hydrolysis of the methyl ester (LiOH) followed by allylation with allyl bromide (K_2CO_3) gave **2** and **7**, respectively.

   General procedure for the synthesis of 4 and 8: To a stirring solution of *N*-cinnamoyl peptide **2** or **7** (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 hours under an oxygen atmosphere and at this stage an additional amount of 2-methylpropanal (6 mmol) was added and 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 **3a** or **3b** as a single diastereomer. The epoxide (2 mmol) was taken in acetonitrile and the *N*-allyl anisidine (2 mmol) and cobalt(II) chloride (5 mol%) were added to the reaction mixture followed by stirring for 12 hours at room temperature. On complete consumption of the epoxide (TLC), the solvent was removed under vacuum to yield a gummy material which was subjected to column chromatography (silica gel) to give compound **4** or **8** as semi-solid. These were subjected to HPLC analysis which showed them to be a mixture of diastereomers in which the *anti* isomer was found to be the major product. The diastereomers were further separated on silica gel (EtOAc/hexanes) to afford pure *anti* **4** or **8**.

   General procedure for ring-closure metathesis: To a stirring solution of ruthenium methylidene catalyst (Grubb's catalyst) (10 mol%) in dichloromethane (0.6 mM solution) under nitrogen, the diene **4** (2 mmol) was added and the mixture was refluxed for 10–12 hours. At this stage an additional ruthenium methylidine catalyst (10 mol%) was added and the mixture was further refluxed for 8 hours. The reaction mixture was treated with 0.1 mL of water and the solvent evaporated to yield a residue which was chromatographed over silica gel (30% EtOAc in hexane) to afford **5** (40–45%) as a gum.

8. **Spectral data for 5a**: ^{1}H NMR ($CDCl_3$) δ 7.40 (d, $J=7.4$ Hz, 1H), 7.32–7.20 (m, 10H), 7.01 ($J=8.8$ Hz, 2H), 6.84 (d, $J=8.8$ Hz, 2H), 5.91 (m, 1H), 5.62 (m, 1H), 5.31 (bd, $J=17.2$ Hz, 1H), 5.25 (d, $J=10.7$ Hz, 1H), 5.02 (d, $J=5.2$ Hz, 1H), 4.99 (bs, 1H), 4.75 (dd, $J=12$ and 4.8 Hz), 4.62 (m, 2H), 4.18 (dd, $J=11.9$ and 6 Hz, 1H), 3.77 (s, 3H), 3.60 (dd, $J=11.8$ and 5.2 Hz, 1H), 3.27 (dd, $J=8.4$ and 6 Hz, 1H), 2.87 (d, $J=7.6$ and 1H), 2.65 (d, $J=7.6$ and 1H), 1.86 (m, 1H), 1.75–1.60 (m, 3H). Mass (m/z): 549(M^+), 492, 386, 324, 267, 211, 196, 165, 155, 126. **5b**: ^{1}H NMR ($CDCl_3$) δ 7.40 (d, $J=7.4$ Hz, 1H), 7.20–7.32 (m, 10H), 7.00 ($J=8.8$ Hz, 2H), 6.84 (d, $J=8.8$ Hz, 2H), 5.91 (m, 1H), 5.31 (bd, $J=17.2$ Hz, 1H), 5.25 (d, $J=10.7$ Hz, 1H), 5.02 (d, $J=5.2$ Hz, 1H), 4.99 (bs, 1H), 4.75 (dd, $J=12$ and 4.8 Hz. 1H), 4.62 (m, 2H), 4.18 (dd, $J=11.9$ and 6 Hz, 1H), 3.77 (s, 3H), 3.60 (dd, $J=11.8$ and 5.2 Hz, 1H), 3.27 (dd, $J=8.4$ and 6 Hz, 1H), 2.87 (d, $J=7.8$ Hz, 1H), 1.86 (m, 1H), 1.60–1.75 (m, 3H). Mass (m/z): 584 (M^+), 527, 496, 466, 450, 406, 368, 342, 263, 212.