# Design, Synthesis, and Application of Peptide Secondary Structure Mimetics

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**Abstract**: The secondary structure peptidomimetic approach is a rational way to develop novel nonpeptide pharmaceutical agents based upon biologically significant proteinaceous leads. A part of this approach elaborated in this laboratory over the past ten years is reviewed along with the recent developments in this field.

## 1. INTRODUCTION

Secondary structure elements in proteins play a key role in molecular recognition events in the biological systems through their characteristic three-dimensional presentation of functional groups on their surfaces. Cytokine-receptor interaction [1] and many protein-DNA interactions [2] are mediated through α-helical, structure, many peptide ligand-receptor interactions and antigen-antibody interactions are mediated through reverse turns [3], and proteases [4], kinases [5-7], most SH2 domains [8], and MHC [9] recognize their substrates through β-strand structures. Most of these protein-protein interactions are initiated or mediated by a key local structure in the protein; therefore, small molecules bearing a similar local structural feature can effectively mimic the ligand binding function of a protein or peptide [10-15]. A successful peptidomimetic must be able to present the correct pharmacophoric residues in the proper three-dimensional space. Conformationally constrained analogs of such peptidomimetics pay a lower entropy cost upon binding to their receptor or enzymes. In principle, such peptidomimetics should have improved bioactivity and selectivity. In addition, the non-peptidic nature of mimetics can potentially improve the undesirable therapeutic characteristics of proteins or peptides, which include poor bioavailability, lack of oral activity, short duration of action, and potential antigenicity [16,17].

Recent advances in molecular biology, genomics, and proteomics continue to reveal an enormous amount of new information on the structural and functional roles of a wide array of proteinaceous species and provide enormous opportunities for new target validation efforts in drug discovery. Therefore, a methodology for the rapid and systematic preparation of secondary structure peptidomimetics, with complete control of their stereochemistry, would afford us the opportunity to dissect and investigate complex structure-function relationships of biologically significant peptides or proteins. Once the pharmacophore model is discerned, additional analogs can be designed, and further refinements can ultimately result in the generation of highly active and selective peptidomimetics.

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## 2. REVERSE TURN MIMETICS

Reverse turn structures are capable of participating in biological recognition events in either an active role, where the precise spatial orientation of pharmacophoric information at the i + 1 and i + 2 positions is critical [18], or in a more passive manner of properly positioning the two peptide chains as they enter and exit the reverse turn [19]. The majority of reverse turn mimetics systems have addressed one of these roles [20-22]; however, our strategies have from the outset attempted to confront both the situations.

β-Turns constitute tetrapeptide units which cause a reversal of the direction of the peptide chain. Formally, turns can be described by the distance from the Cα of the first residue to the Cα of the fourth residue (Fig. **1a**). When this distance is less than 7 Å and the tetrapeptide sequence is not in an α-helical region, it is considered a β-turn. Additionally, a three residue reverse turn (γ-turn) exists but is significantly less widely distributed (Fig. **1b**).

In the design of reverse turn mimetic systems, there are a number of concerns and criteria which need to be addressed. β-Turns, which comprise a rather diverse group of structures, are classified according to the φ and ψ angles of the i + 1 and i + 2 residues. In addition to a number of turn types (I, I', II, II', III, III', IV, V, Va, VIa, VIb, VII, and VIII) the Cα(i) to Cα(i+3) distance varies from 4 ~ 7 Å [23]. It appears that no one structure can accurately mimic this diversity of turns. The interaction of the amino acid side chains with their complementary receptor groups is the critical determinant of biological specificity. A successful peptidomimetics must appositely position the appropriate functional groups on a relatively rigid framework. Therefore, an idealized mimetic design should incorporate the ability to accurately display critical pharmacophoric information in the same manner in which it is presented in native reverse turns.

### 2.1 Monocyclic Reverse Turn Mimetics

#### *2.1.1* β-*Turn Mimetics*

A ten membered macrocyclic structure was chosen to mimic the β-turn structure which forms a pseudo ten membered ring through a 4 → 1 hydrogen bond. With this structure, the functional groups can be placed at the proper

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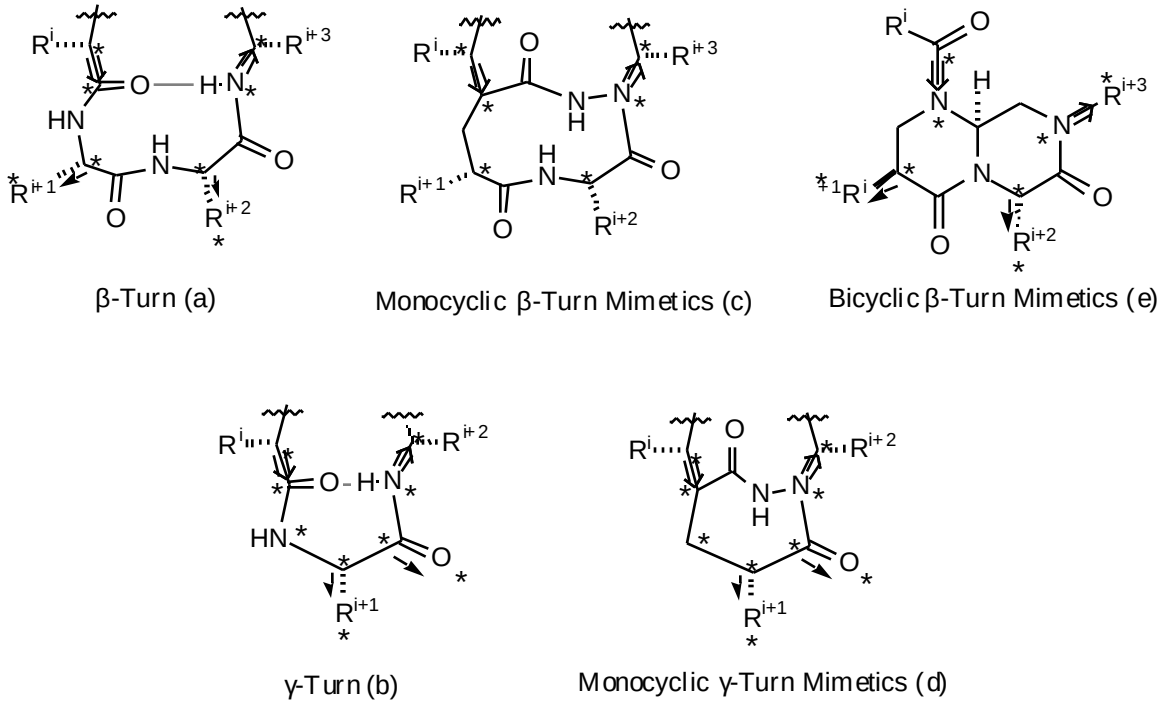


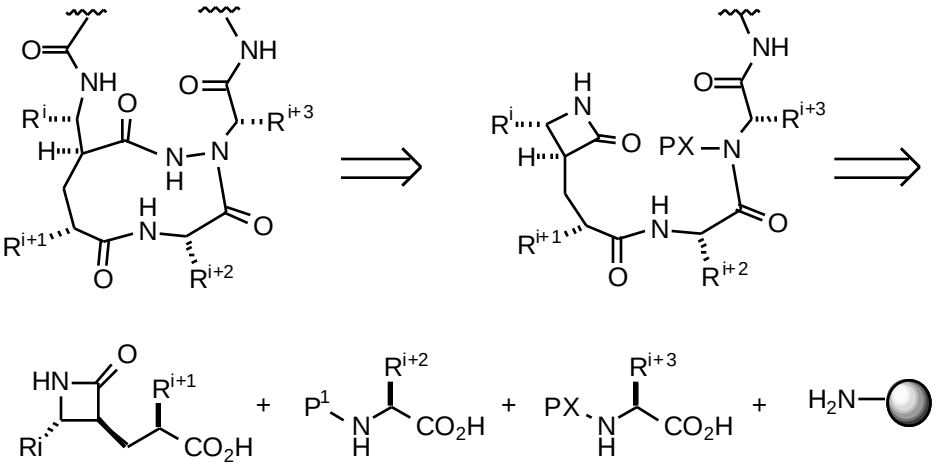

**Fig (1)**. The structure of β-turn, γ-turn, and their mimetics.

positions to mimic the i + 1 and i + 2 side chains of β-turn. In addition, the incoming and outgoing peptide chains can be accommodated (Fig. **1c**).

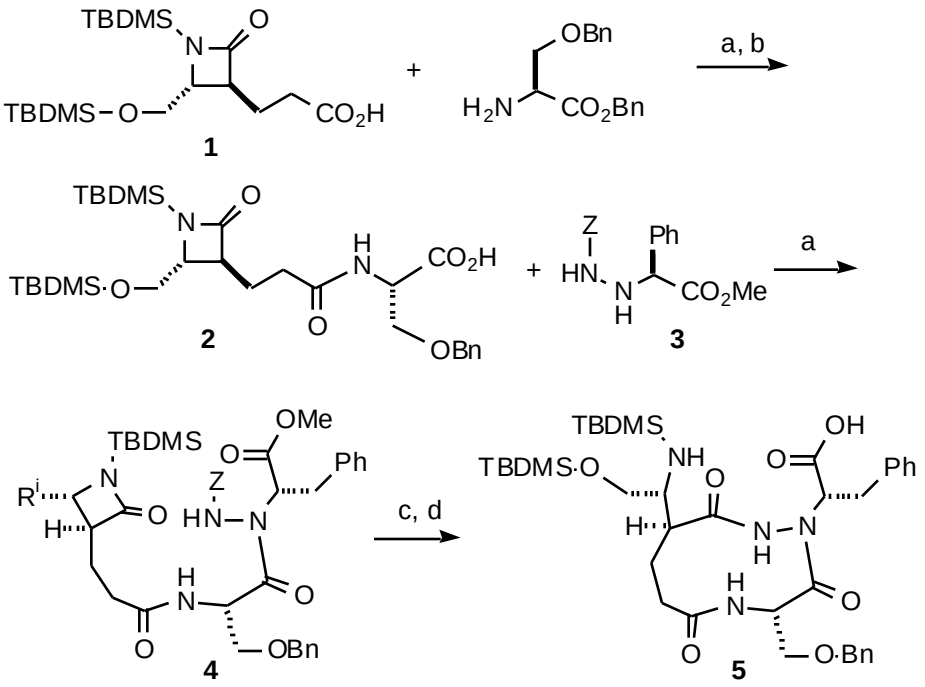
We have compared these monocyclic mimetics with idealized type I, I', II, II' β-turns. The idealized turns were generated by energy minimization of a tetrapeptide sequence consisting of either all alanines or alanines with one or two glycines depending on the steric requirements of the particular turn types with torsional constraints at the defined angles. The actual comparisons were made using six position root mean square (RMS) values at the four α carbons in the turn, the carbonyl carbon at the i position, and the amide nitrogen at the i + 3 position. The lowest energy conformer in the 10 membered ring system is an excellent mimic for an idealized type I' β-turn with a 6-atom RMS deviation (RMSD) of 0.22 Å [24].

The synthesis of the monocyclic mimetics involves the coupling of the first modular component piece to the amino terminus of a growing peptide chain (Scheme **1**). Coupling of the second component, removal of the protecting group and subsequent coupling of the third modular component provides the precursor of the mimetics. Macrocyclization reaction, employing the nucleophilic opening of the azetidinone by a hydrazino group, provides the β-turn mimetic. The synthesis allows for the introduction of natural or unnatural amino acid side chain functionality in either L or D configuration. Additionally, deletion of the second modular component provides access to γ-turn mimetics [25,26].

The monocyclic mimetic was prepared according to Scheme 2. Azetidinone **1** was prepared in six steps from dimethyl-D-aspartate according to the literature procedures



**Scheme 1**.




a) iBuOCOCl / NMM b) $H_2$, Pd/C, EtOAc (88%) c) $H_2$, Pd/C, MeOH
d) $K_2CO_3$ aq. MeOH (53%)

**Scheme 2.**

[18,27]. Mixed anhydride coupling of **1** to *O*-benzyl serine benzyl ester and subsequent hydrogenolytic cleavage of the benzyl ester group provided the corresponding acid **2** in 88% yield. Coupling of Z-protected hydrazinophenylalanine **3** to **2** proceeded smoothly to give the precursor **4**. After the hydrogenolytic removal of the protective groups, macrocyclization was carried out in basic media to afford the 10-membered ring β-turn mimetic **5** in 53% yield [28].

This monocyclic template was applied to explore structure-function relationships among molecules of the immunoglobulin gene superfamily. Immunoglobulins are constructed from a series of antiparallel β-pleated sheets connected by loops [29,30]. The specificity of these molecules is determined by the sequence and size of the canonical hypervariable complementarity determining regions (CDRs) [31,32]. The monoclonal antibody 87.92.6 (mAb 87.92.6) is an anti-idiotype antibody which binds to the cellular receptor of the type 3 reovirus. Sequence analysis revealed an intriguing homology between these two proteinaceous ligands [33]. In particular, a region within the CDR2 of the light chain of mAB 87.92.6 and the hemagglutinin of the type 3 reovirus exhibited strong primary sequence homology. The $V_L$ CDR 2 canonically exists in a reverse turn conformation [32,34]. Based on this, we incorporated the sequence Y, S, G, S, S. Importantly, the mimetic **6** displayed similar binding properties to the cellular reovirus receptor and to mAb 9BG5, and had the same inhibitory effect on cell proliferation as did the native antibody 87.92.6 [33]. We have also designed a mimetic of

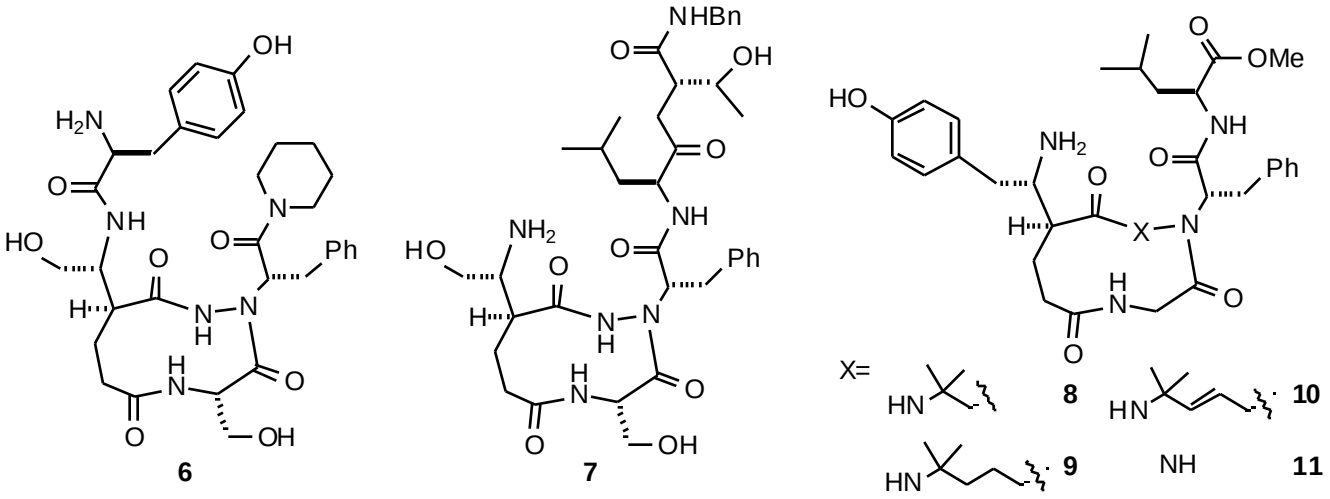



**Fig (2)**. Biologically active monocyclic β-turn mimetics.

the CDR-2 like region of human CD4. CD4 is a 55kD glycoprotein, primarily found on the cell surface of the helper class of T cells. It binds the Human Immunodeficiency Virus (HIV gp120) with high affinity (Kd = 1 ~ 4 nM), and is an important route of cellular entry for the virus. Extensive mutagenesis [35,36] and peptide mapping [37] experiments have shown that the region of amino acids 40-55 within the CDR2-like domain of CD4 is critical for gp120 binding. X-ray crystallographic analysis showed that residues $Gln^{40}$ through $Phe^{43}$ reside on a highly surface exposed β-turn connecting the C' and C" β-strands. Structure **7** was designed and synthesized as a monocyclic β-turn mimetic. This small molecule mimic abrogates the binding of HIV1(IIIB)gp120 to $CD4^+$ cells at low micromolar levels and reduces syncytium formation 50% at 250 μg/ml [28].

We have applied this template to discern the biologically active conformation of endogenous opioid peptides, enkephalin. The inherent mobility of the enkephalin framework and the existence of multiple receptor subtypes have hampered the assessment of its bioactive conformations. Several studies suggested reverse turn conformations as the biologically active conformation of the opioid peptides based upon X-ray crystallography [38] and spectroscopic studies [39]. In an effort to probe the receptor bound conformation of leucine enkephalin by incorporation of 4 → 1 β-turn prosthetic units, we have synthesized the family of nonpeptide mimetic structures depicted in Fig. **2**. Compounds **8**-**11** have been evaluated in an *in vitro* binding assay in an attempt to correlate structure function relationships. Compound **10** has an $IC_{50}$ value of 8 μM for μ opioid receptor [24].

### *2.1.2* γ-*Turn Mimetics*

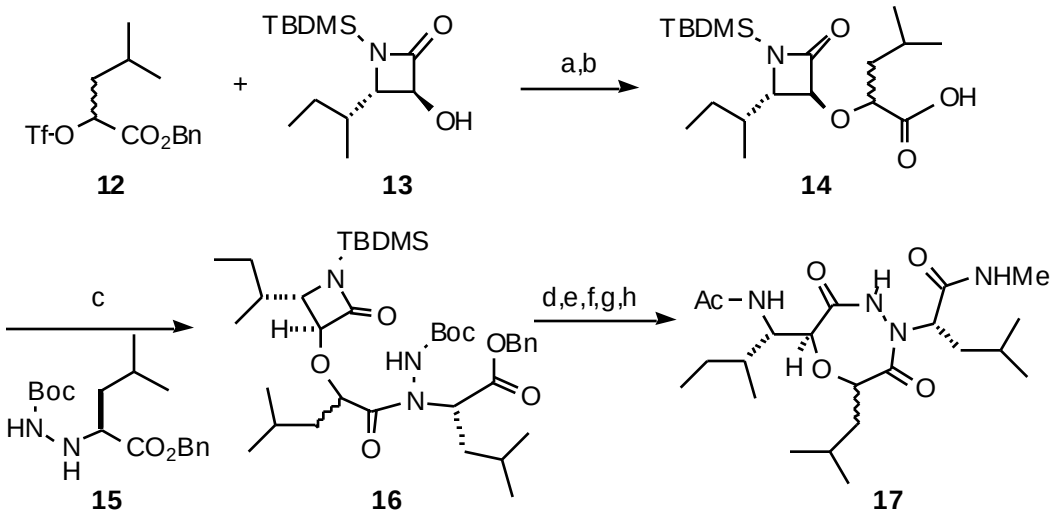
We have designed a 7 membered ring system to mimic a γ-turn in the same fashion as the monocyclic β-turn mimetics (Fig. **1d**). The structural comparison of this mimetic with an idealized γ-turn shows good structural agreement. The actual comparisons were made using nine position RMS values at the three α carbons in the turn, one β-carbon at the i + 1 position, the carbonyl carbon at the i and i + 1 positions, the amide nitrogen at the i + 1 and i + 2 positions, and the carbonyl oxygen at the i + 1 position (Fig. **1b** and **d**). The lowest energy conformer in the 7 member ring system is an excellent mimic for an idealized γ-turn with a 9-atom RMSD of 0.17 Å [26].

The synthetic strategy employed in the synthesis of the β-turn mimetics can be applied to the construction of the γ-turn mimetic. Leucine was derivatized to the corresponding hydroxyl ester, which was transformed into the corresponding triflate. This triflate **12** was coupled with the β-lactam by a substitution reaction with hydroxy β-lactam **13**. The ester was deprotected with Pearlman's catalyst and the corresponding acid **14** was converted to the acid fluoride. Treatment of the acid fluoride with hydrazino ester **15** yielded hydrazide **16**. The removal of the N-silyl group on hydrazide **16** with TBAF followed by Boc cleavage with TFA led to the seven-membered ring heterocycle. The resulting amino group was acetylated and the benzyl ester group was converted to the monomethyl amide to provide the desired γ-turn mimetic **17** [26].

A preliminary biological screen showed compound **17** displaying 10 % inhibition at 10 μM for Neureopeptide Y [26].

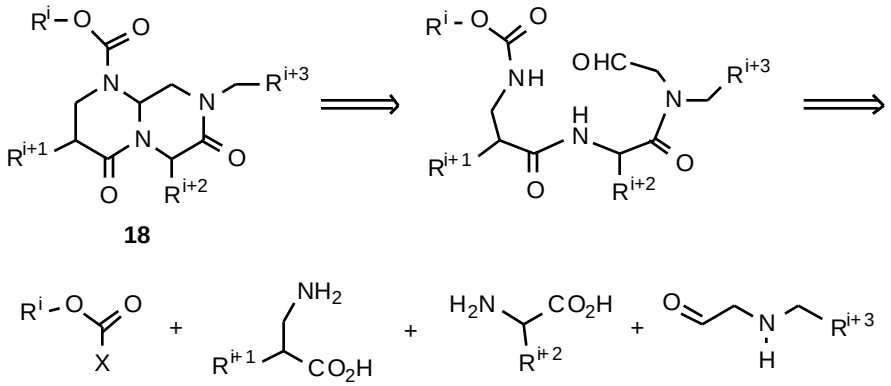
### 2.2 Bicyclic β-Turn Mimetics

A 6,6-bicyclic structure afforded more rigid analogs of β-turn mimetics than the previously described macrocyclic systems (Fig. **1e**). The additional conformational constraint would save the entropy required of a flexible molecule to preorganize for binding with target proteins, which can improve the potency and selectivity. The concept of the




(a) NaHMDS,18-crown-6, THF (44%) (b) $H_2$, $Pd(OH)_2/C$ (100%) (c) cyanuric fluoride, py, benzene (46%) (d) TBAF (e)TFA/anisole (f) $Ac_2O$, py (51%) (g) $H_2$, $Pd(OH)_2/C$ (h) EDCI, HOBT, $MeNH_2$ (70%)

**Scheme 3.**



**18**

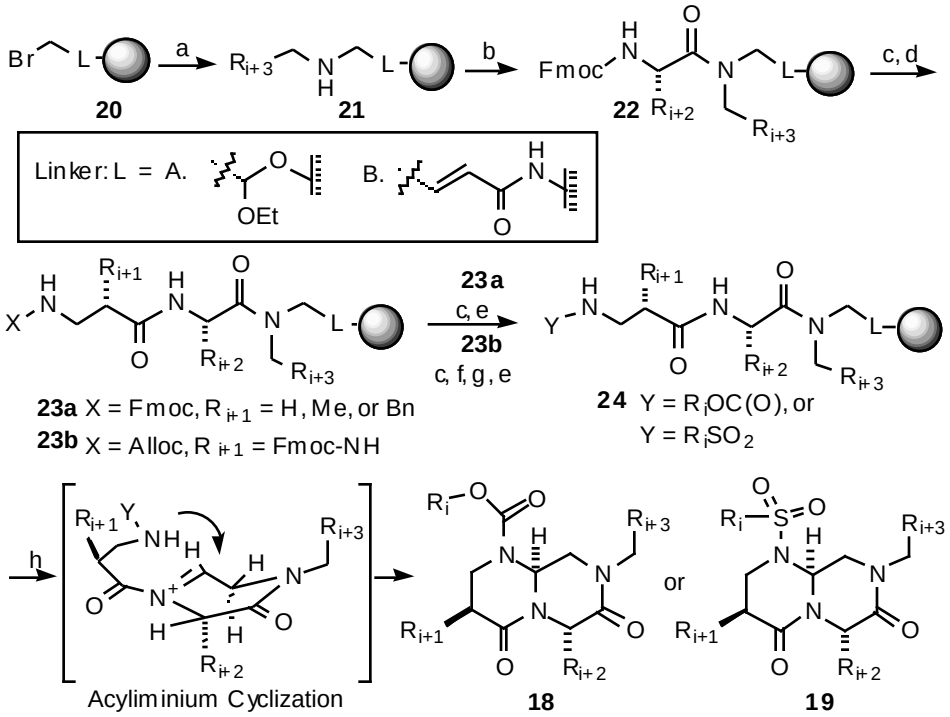
**Scheme 4.**

design is the same as the monocyclic β-turn in which the vectors of the functional groups at the i + 1 and i + 2 positions and the incoming and outgoing peptide chains adopt similar positions to the corresponding vectors of a β-turn (Fig. **1a** and **e**).

### *2.2.1 Tetrahydro-2H-pyrazino[1,2-a]pyrimidine-4,7-Diones*

Our Monte Carlo conformational analysis of the bicyclic templates, **18** and **19**, using the MMFF force field *in vacuo* as implemented in MacroModel, revealed a dominant pseudo-axial orientation of the $R_{i+2}$ for low energy conformers. All conformations with a Boltzmann population of more than 0.5% were compared with idealized β-turns [23] (type I, I', II, II', VIa and VIb) at seven positions. Good RMSD values (0.38-0.50Å) were obtained for a type I β-turn conformation [40].

The synthetic strategy to construct this template, 1,3,6,8-substituted tetrahydro-2H-pyrazino[1,2-a]pyrimidine-4,7-diones, is shown in Scheme 4. The key transformation involves the acid catalyzed tandem cyclization of a dipeptide aldehyde. The aldehyde can be obtained by standard peptide coupling reactions of the commercially available components. The four functional side chains can be introduced from the four components, alkyl *p*-nitrophenyl carbonates or alkyl haloformates, β-amino acids, α-amino acids, and *N*-alkyl aminoacetoaldehydes, which enhances the diversity of this β-turn mimetic.



(a) 2M primary amine in DMSO; (b) Fmoc-α-amino acid, DIC / HOAT in NMP; (c) 20% piperidine in DMF; (d) Fmoc-β-amino acid or Fmoc-Dpr(Alloc), DIC / HOBT; (e) $R_iOC(O)ONp$ or $R_iSO_2Cl$, DIEA; (f) $RCO_2H$, DIC / HOBT; (g) cat. $Pd(Ph_3P)_4$, $PhSiH_3$; (h) formic acid at r.t. or cat. $OsO_4$ / $NaIO_4$ then cat. TFA in $CH_2Cl_2$

**Scheme 5.**

**Table 1. β-Turn mimetics 18 and 19**

| | L | $R_i$ | $R_{i+1}$ | $R_{i+2}$ | $R_{i+3}$ | Yield[a] |
|---|---|---|---|---|---|---|
| 18a | A | Bn | H | Bn | *p*-Cl-Ph | 71 |
| 18b | A | Bn | Ac-NH | Me | H | 42 |
| 18c | A | Bn | Bz-NH | Me | H | 31 |
| 18d | A | Bn | Me | Me | H | 47 |
| 18e | A | *p*-OH-PhEt | H | Bn | *n*Bu | 36 |
| 18f | A | Bn | Bn | Bn | *n*Bu | 42 |
| 18g | B | Bn | H | Me | H | 33 |
| 18h | B | Bn | H | $t$BuO$_2$C-Et | H | 22 |
| 18i | A | *p*-OH-PhEt | H | PhEt | *n*Bu | 23 |
| 19a | A | Ph | H | Bn | c.Hexyl | 57 |
| 19b | A | Ph | Ac-NH | Me | H | 33 |
| 19c | A | *p*-Tolyl | H | Bn | *p*-Cl-Ph | 62 |

a. Isolated yield (%) for chromatographically purified compounds based on the initial loading of the solid support (ArgoGel$^{TM}$OH or $NH_2$).

The solid-phase generation of the intermediate was carried out by employing two types of linker, i.e., acetal type (A), or olefin type (B) (Scheme 5) [40]. Nucleophilic displacement of the bromide, **20**, with a number of primary amines gave the corresponding secondary amine, **21**, which was then coupled with the appropriate Fmoc-α-amino acids with HOAT/DIC in NMP. Treatment of **22** with 20% piperidine in DMF followed by coupling with Fmoc-β-amino acids or Fmoc-Dpr(Alloc)-OH afforded **23a** or **23b** respectively. To introduce functionality at the $i$ + 1 position of **23b**, the Fmoc group on the diaminopropionic acid (Dpr) residue was removed with 20% piperidine, and subsequently acylated with a carboxylic acid in the presence of DIC / HOBT. After deprotection of the Alloc group of **23b** with cat. $Pd(Ph_3P)_4/PhSiH_3$ or the Fmoc group of **23a** with 20% piperidine, the resin was treated with alkyl or aryl sulfonyl chlorides or alkyl *p*-nitrophenylcarbonates in the presence of DIEA to produce **24**. Cleavage from the acetal resin (Linker A) followed by stereoselective acyliminium cyclization was performed by treatment with formic acid at the room temperature. Cleavage of the olefin linkage (Linker B) was effected by oxidation with a catalytic amount of osmium

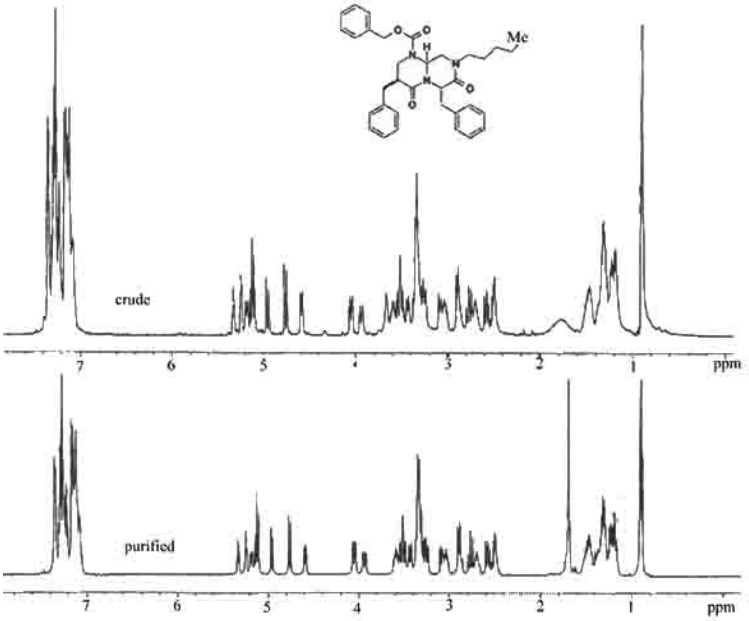
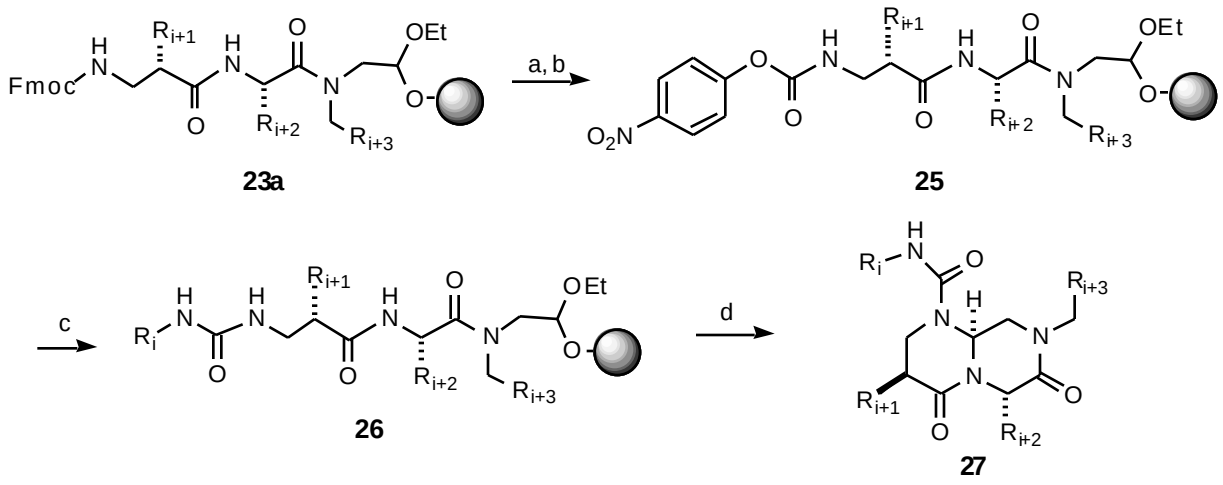



**Fig (3)**. NMR spectra of crude and purified **18f**.




(a) 20% piperidine in DMF. (b) ClC(O)ONp / DIEA. (c) $R_iNH_2$. (d) Formic acid at r.t.

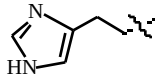
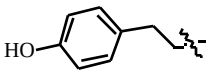
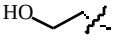
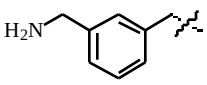
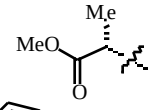
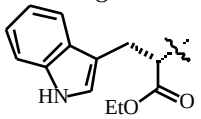
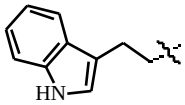
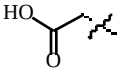
**Scheme 6**.

tetroxide and sodium periodate to liberate a mixture of aldehyde and hemiaminal. The product was then cyclized via an acyliminium intermediate with a catalytic amount of TFA in dichloromethane. This oxidation procedure for Linker B is mild enough to retain acid labile functional groups, such as a *t*-butyl ester. With this synthetic scheme, four functional side chains were introduced from four components: alkyl or aryl sulfonyl chloride or alkyl *p*-nitrophenylcarbonates ($R_i$), β-amino acid derivatives ($R_{i+1}$), α-amino acid derivatives ($R_{i+2}$), and primary amines ($R_{i+3}$). Alkyl *p*-nitrophenylcarbonates were generated *in situ* by the reaction of various alcohols with *p*-nitrophenyl chloroformate in the presence of 2,6-lutidine.

These readily available components and simple synthetic procedures facilitate the introduction of diversity (Table 1). HPLC analysis of the crude products with monitoring at 214 nm showed the bicyclic mimetics as the major product in all cases [40]. Fig. **3** shows the comparison of NMR spectra for crude and purified product at -20 °C. The only impurities observed in this case are polyethyleneglycol and formic acid residue.

With this synthetic scheme, diversity at the i position of the mimetic is limited to the commercially available sulfonyl chlorides or required the preparation of alkyl *p*-nitrophenyl carbonates in solution phase. Therefore, we have developed an efficient method to furnish diversity at the i position of our β-turn template compatible with automated solid-phase synthesis. The key modifications are utilization of a urea group for stereoselective tandem cyclization to produce the 1,6,8-substituted tetrahydro-2H-pyrazino[1,2-a]pyrimidine-4,7-dione **27** and the on-resin generation of the *p*-nitrophenyl carbamate species that can be derivatized to the corresponding urea by treatment with a variety of amines (Scheme 6) [41].

**Table 2. Preparation of Mimetics 27 ($R^{i+1}$, H; $R^{i+2}$, Me; $R^{i+3}$, H)**

| | $R^i$ | Yield (%) | | $R^i$ | Yield (%) |
|---|---|---|---|---|---|
| **27a** | Me | 76 | **27h** |  | 73 |
| **27b** | iBu | 50 | | | |
| **27c** | Bn | 48 | **27i** |  | 66 |
| **27d** |  | 58 | | | |
| **27e** |  | 45 | **27j** |  | 63 |
| **27f** |  | 68 | **27k** |  | 59 |
| **27g** |  | 26 | **27l** |  | 65 |

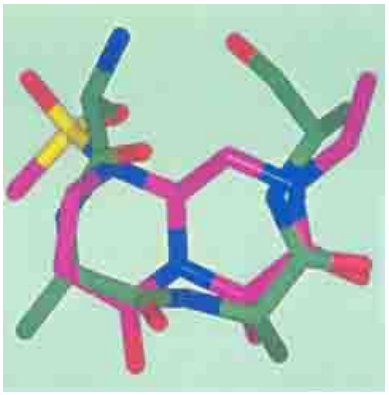
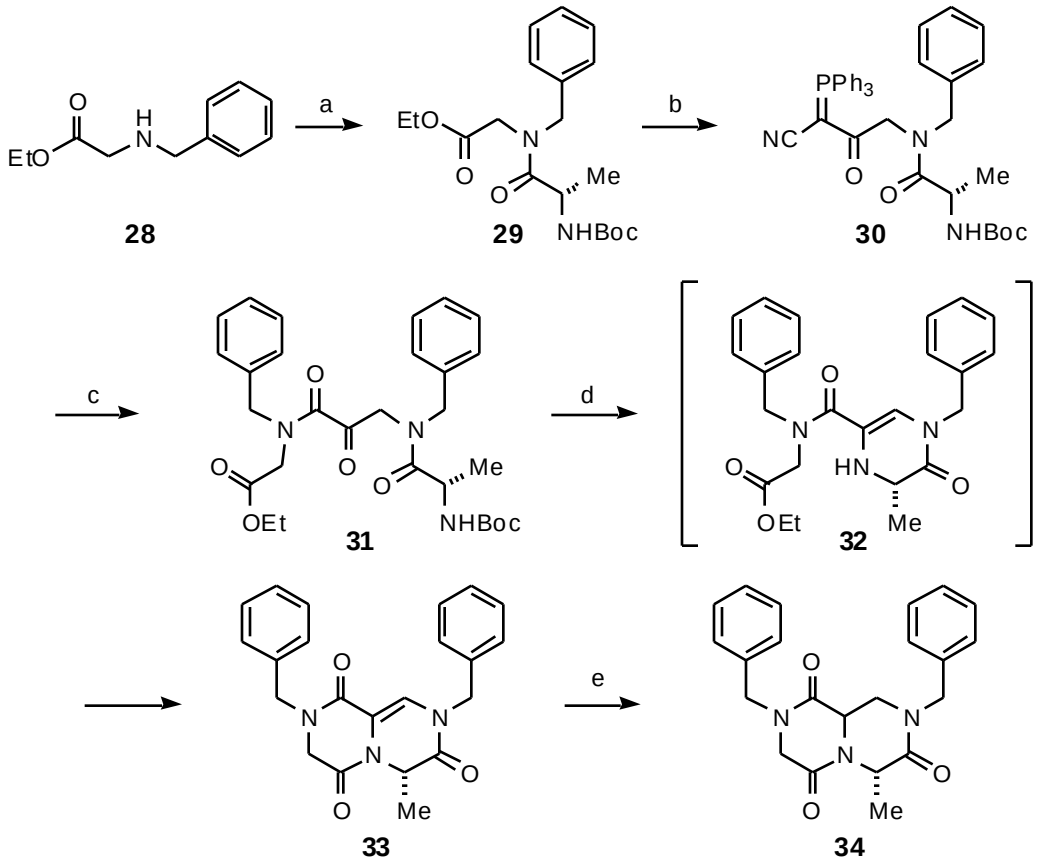


**Fig (4)**. Overlay of the X-ray structure (magenta) against type I β-turn (green). The core structure of the template **19a** is shown for clarity.

Precursor **23a** was prepared as outlined in Scheme 5. To introduce functionality at the *i* position, the Fmoc group was removed with 20% piperidine and subsequently treated with *p*-nitrophenyl chloroformate and DIEA in DCM/THF. The resulting *p*-nitrophenyl carbamate **25** was reacted with a variety of amines in DMF to give the corresponding urea derivatives **26**. Cleavage from the resin followed by stereoselective tandem cyclization was achieved by treatment with formic acid at room temperature. A wide range of amines can be applied to this derivatization of the *i* position as shown in Table 2.

Alkylamines were successfully applied with good yield and purity (**27a~c**). Ethanolamine gave the formylated product as the major product, which was subsequently hydrolyzed by sodium carbonate to afford the desired hydroxyl derivative (**27d**). α,α'-Diamino-*m*-xylene, piperidine, tryptamine, histamine, and tyramine gave the bicyclic products in good yields (**27e~i**). Amino acid derivatives, e.g., alanine methyl ester, tryptophan ethyl ester, and glycine t-butyl ester, gave the corresponding bicyclic scaffolds in good yields (**27j~l**) [41]. The bicyclic products **27** were observed as the major product by LC-MS and NMR analyses of the crude products except **27g**.

2D-NMR experiments indicate the formation of only a single diastereomer, in which the hydrogen at the ring junction is *trans* to the α−hydrogen at the *i* + 2 position. The stereochemistry was confirmed by X-ray crystallography for **19a** [40]. The RMSD values for the X-ray structure (Fig. **4**) of the mimetic with idealized β-turns are as follows; 0.55Å for type I, 1.35Å for type I', 0.92Å for type II, 1.06Å for type II', 1.07Å for type VIa, and 1.25Å for type VIb. It appears that mimetic **19a** best represents the most common type I β-turn actually observed in proteins [23]. In solution,




(a) Boc-Ala-OH, EDCI, HOBt, (100%) (b) i) LiOH, $H_2O$/THF (100%) ii) $Ph_3P$=CHCN, EDCI, DMAP, $CH_2Cl_2$ (71%) (c) i) $O_3$, $CH_2Cl_2$ -78C ii) N-Benzyl-Gly-OEt (48%) (d) TFA then sat. $NaHCO_3$ (77%) (e) $H_2$, $PtO_2$, MeOH (56%)

[Scheme 7]

($CDCl_3$, $CD_3OD$, or $d_6$-DMSO/$D_2O$) the same ring conformation as seen in the crystal structure predominates, as judged by the data from ROESY experiments for **19a**. In addition, the same patterns of ROE's for **18b**, **18f**, **19b** and **27a** suggest that the predominant ring conformation of **18b**, **18f**, **19b** and **27a** is the same as that of **19a**, in which the $R_{i+1}$ and $R_{i+2}$ take a pseudo-equatorial and a pseudo-axial orientation respectively [40,41].

This privileged template was applied to the preparation of mimetics of Leu-enkephalin (YGGFL) to identify a potent and selective ligand for opioid receptors (μ, δ, κ) and to

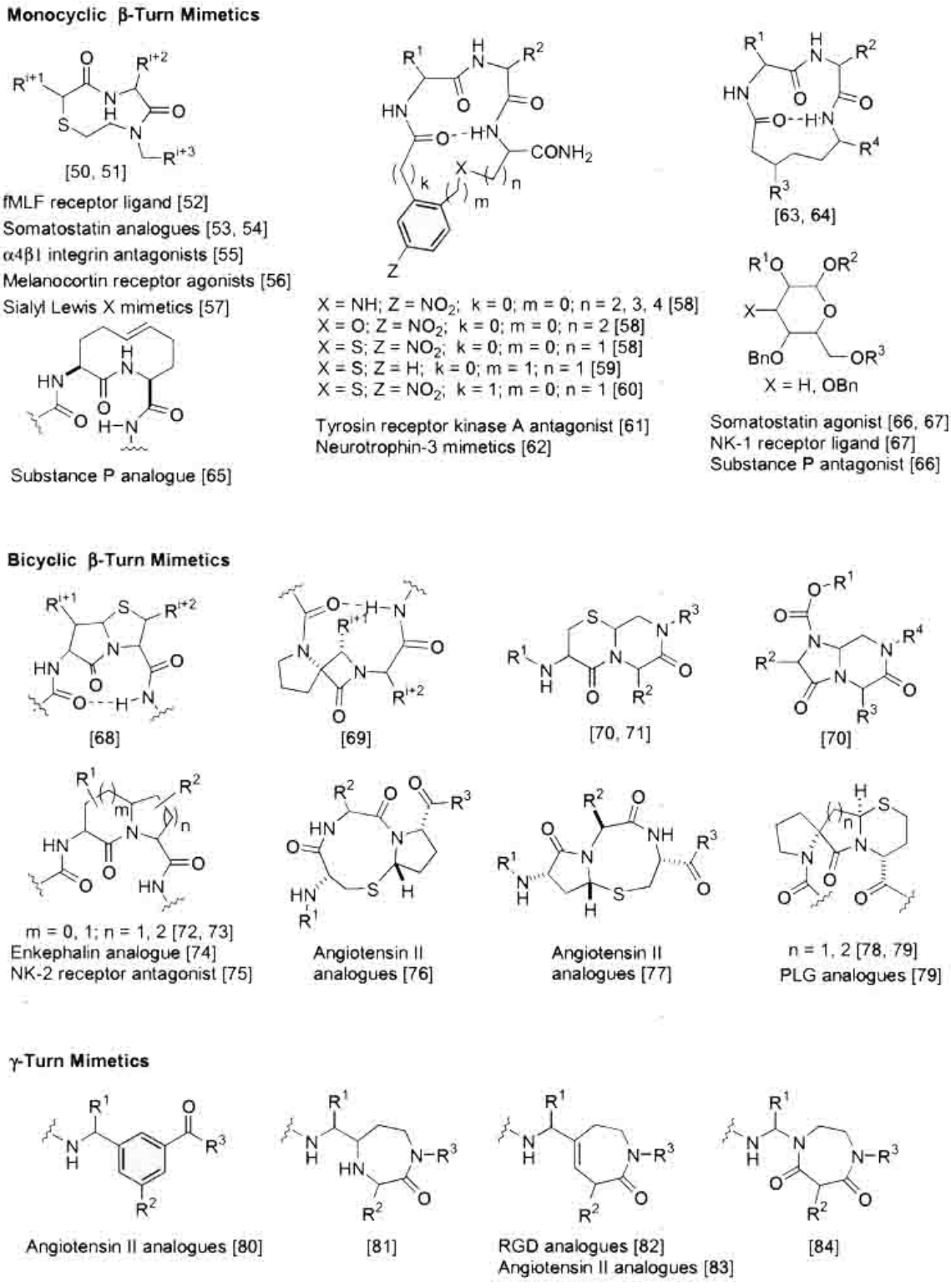



**Fig (5)**. Reverse turn scaffolds.

elucidate the structure-activity relationship of the ligand. A library of mimetics was evaluated for its inhibitory activity on the binding of radioligand ([$^3$H]naloxone) to the non-selective opioid receptor of rat cerebral cortex. The $IC_{50}$ values for selected compounds against the non-selective receptor were determined after the first screening. A couple of potent ligands (9 nM) were identified and one of them showed excellent μ-selectivity in human opioid receptors. Subsequently, we carried out *in vivo* evaluation of the analgesic effect of compound **18i**. The results of intravenous mouse hot plate analgesia assay showed that compound **18i** is acting as an agonist with a similar level of activity to morphine [42].

Libraries of the bicyclic β-turn mimetics based on the LDV sequence were also evaluated for the inhibitory activity of the α4β1 integrin(VLA-4)-Fibronectin-derived-CS-1 peptide interaction. The α4β1 integrin is involved in cell adhesion and migration events and known to bind to fibronectin and VCAM-1. The bioassay was based on the competition between the test compounds and the immobilized biotinylated CS-1 peptide in binding VLA-4 expressed on Ramos β-cells. Active compounds were identified with an $IC_{50}$ value of 0.7 μM [43].

#### *2.2.2 3,6,8-Trimethyl-tetrahydro-pyrazino[1,2-a]pyrazine-1,4,7-trione*

The preparation of an alternate 6,6-bicyclic mimetic **34** was carried out by solution phase chemistry. Acylation of *N*-benzyl-glycine ethyl ester **28** with Boc-alanine, EDCI and HOBt gave the corresponding amide **29** in quantitative yield. Hydrolysis of the ethyl ester and subsequent coupling of the carboxylic acid with cyanomethylenetriphenyl-phosphorane using EDCI and DMAP furnished **30** in 71%. Treatment of **30** with ozone, followed by coupling with secondary amino ester **28**, afforded the α-keto amide **31** in 40% yield. The tandem cyclization of **31** by the treatment with TFA gave **33** in 77% yield. The hydrogenation of **33** with Adam's catalyst afforded the trisubstituted tetrahydro-pyrazino[1,2-a]pyrazine-1,4,7-dione **34** in 56% [44]. Later this template was prepared using solid phase chemistry by the Proctor&Gamble group [45,46].

### 2.3. Recent Developments on ReverseTurn Mimetics

Recently, considerble progress has been made on designing and synthesizing reverse turn scaffolds with diversity and biological applications in mind. Notable reverse turn scaffolds along with their biological applications are listed in Figure **5**. It is noted that several comprehensive reviews on reverse turn mimetics have been reported previously [12,21,22,47-49].

## 3. β-STRAND MIMETICS

The β-strand conformation is a secondary structure present in many polypeptides. The β-strand conformation is nearly fully extended, with axial distances between adjacent amino acids of approximately 3.5 Å. A β-sheet conformation is stabilized by hydrogen bonds between NH and CO groups in different polypeptides strands. Additionally, the dipoles of the peptide bonds alternate along the strands which imparts intrinsic stability to this β-sheet. The adjacent strands in the secondary structure can run in the same direction (i.e., a parallel β-sheet) or in the opposite directions (i.e., an antiparallel β-sheet). Although the two forms differ slightly in dihedral angles, both are sterically favorable. The extended conformation of the β-strand results in the amino acid side chains protruding on alternating faces of the β-sheet. Due to the nature of the interaction, hydrogen bonding capability (donating and accepting) is a key force stabilizing this secondary structure. Therefore, the proper implementation of amide groups (NH and CO) and side chain functionality for adoption of a β-sheet structure within constrained peptide backbone structure of the mimetics are essential for successful β-strand mimetics. It is noted that a comprehensive review on β-strand mimetics has been reported in a preceding review article of this issue.

The rigid bicyclic structures **35**, **36**, **37** were chosen to mimic the backbone structure of the β-strand, which would place the hydrogen bonding donor and acceptor in the proper position for the interaction with an adjacent peptide or protein strand. In addition, other key functional groups can be introduced to the templates with proper three-dimensional orientations to closely approximate β-strand structure. Monte Carlo conformational searches were carried out on the β-strand templates **35** and **36** in a simulated water environment using MM2 force fields. Boltzmann weighed average RMSD values at the seven superimposable heavy atom positions were 0.4 and 0.5 Å for **35** and 0.5 and 0.5 Å for **36** against parallel and antiparallel β-strands, respectively [85]. The best conformers for the β-strand mimetic **37** were found within 1.2 kJ/mol of the global energy minimum. RMSD value from an ideal β-strand is 0.51 Å at the 7 atom positions for template **37** against an antiparallel β-strand [86].

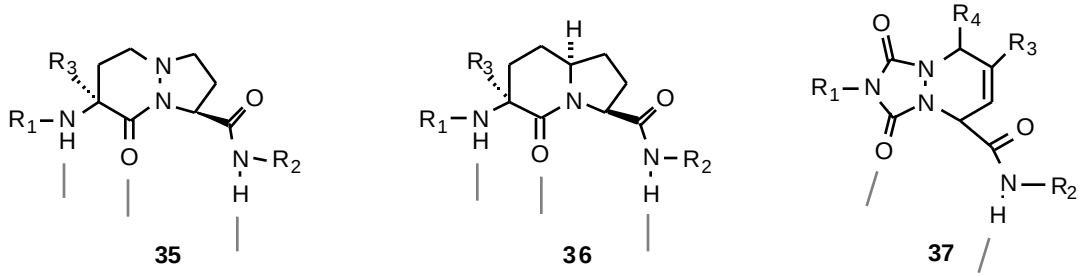
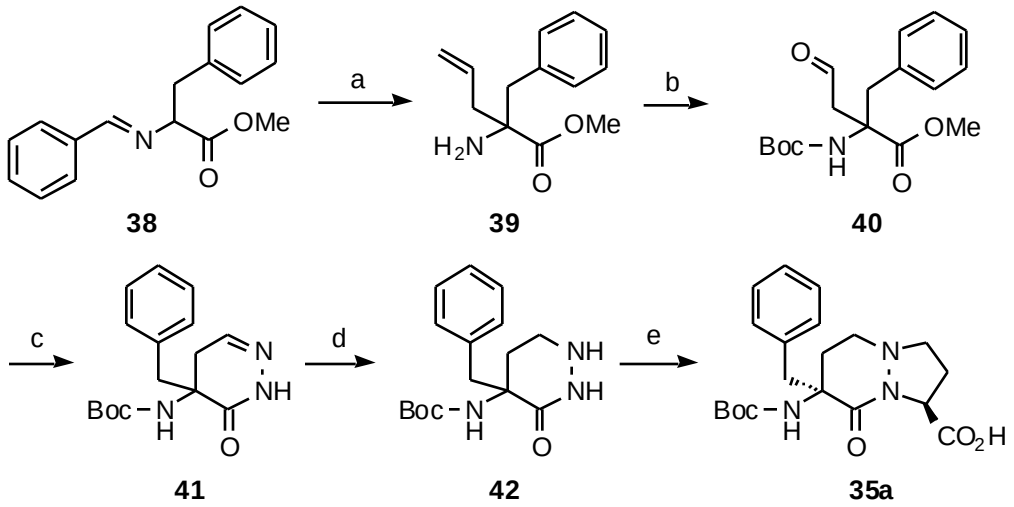



**Fig (6)**. Bicyclic β-strand mimetics.

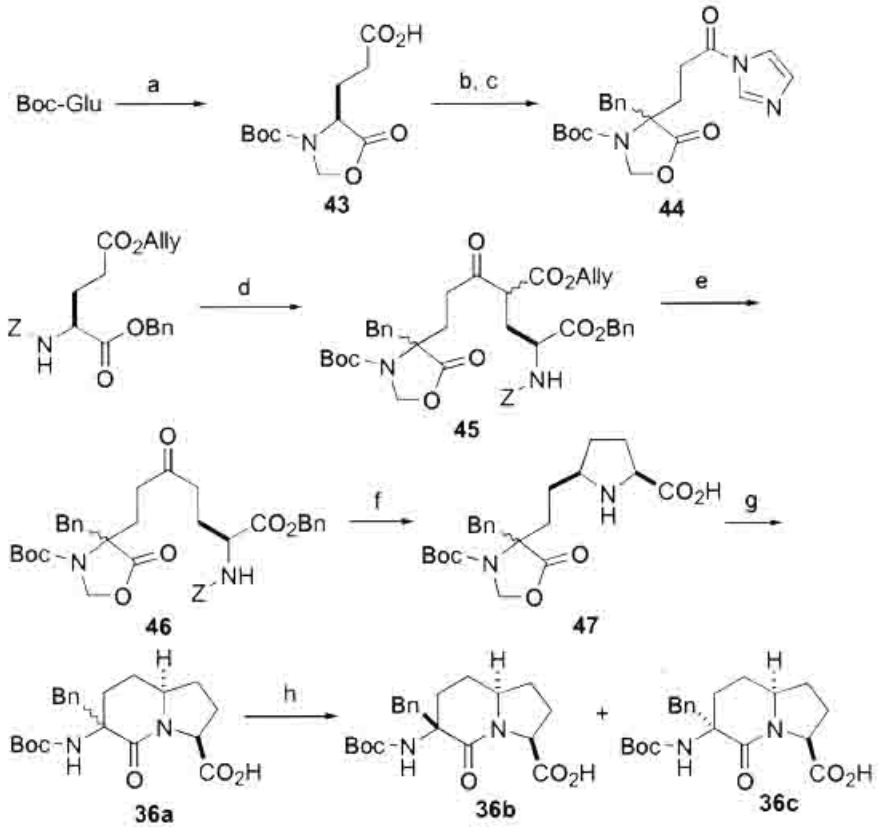



(a) i. LDA / Allyl bromide ii. 1N HCl (68%) (b) i. $Boc_2O$ / $NaHCO_3$aq. ii. $O_3$ (65%) (c) $H_2N$-$NH_2$ (76%) (d) $H_2$ / Pt (67%) (d) i. HCHO / Ethyl Acrylate ii.Separation iii. LiOH (14%)

**Scheme 8**.

### 3.1 Solution Phase Preparation and Application of Bicyclic β-Strand Mimetics

Template **35** was prepared in 8 steps from benzaldimine of phenylalanine methyl ester (Scheme 8) [85]. The alkylation of the ester with allyl bromide followed by the hydrolysis of the imine gave α-allylphenylalanine methyl ester, **39**. Protection of **39** as the *N*-Boc derivative and ozonolysis of the olefin, followed by reductive workup gave the aldehyde ester **40**. Treatment of the aldehyde ester with hydrazine afforded cyclic hydrazone **41** in good yield. Hydrogenation of **41** over Adam's catalyst yield cleanly the




a) Paraformaldehyde, cat. TsOH in DCE (78%) b) LHMDS, BnBr (98%) c) CDI (in situ) d) LHMDS then **44** (69%) e) $Pd(OAc)_2$ / $Ph_3P$ / $HCO_2H$ / TEA (73%) f) $H_2$ / cat. Pt and Pd/C (91%) g) 1N NaOHaq. in MeOH (92%) h) Flush chromatography (silica gel)

**Scheme 9**.

tetrahydropyridazinone **42**. The 1,3-dipolar cycloaddition of the azomethine imine, formed by treatment of **42** with formaldehyde, in the presence of a large excess of ethyl acrylate gave a mixture of diastereomeric and regioisomeric products from which the desired bicyclic template was isolated in 27% yield. Hydrolysis of the ester provided the Boc-protected mimetic **35a**.

Template **36** was prepared in 7 steps from Boc-Glu and Z-Glu-OBn in 36% overall yield (Scheme 9) [87]. A crossed Claisen condensation, reductive amination, and base-promoted lactam cyclization were employed as the key steps in a multi-gram scale synthesis. Treatment of Boc-Glu with paraformaldehyde and a catalytic amount of *p*-toluenesulfonic acid in refluxing DCE gave the corresponding oxazolidinone **43** in 78% yield. The benzyl derivative was synthesized in 98% yield via generation of the enolate with 2 equivalents of LHMDS in anhydrous THF, followed by the addition of benzyl bromide. Allyl esterification of Cbz-Glu-OBn was carried out by reacting the cesium salt of the carboxylic acid with allyl bromide in DMF to afford the corresponding allyl ester in quantitative yield. A crossed Claisen condensation was performed by the generation of Cbz-Glu(OAllyl)-OBn γ-enolate with 3 equivalents of LHMDS in THF at -78 ºC followed by reaction with the *N*-acyl imidazole derivative **44** generated *in situ* with 1,1'-carbonyldiimidazole in THF. The resulting allylic β-keto ester **45** was obtained in 69% yield. Palladium-catalyzed decarboxylation of **45** using Tsuji's procedure provided **46** in 81% yield. Hydrogenation of **46** in ethanol at 320 psi with a mixture of platinum oxide and palladium-carbon catalyst proceeded with the removal of the Cbz group, imine formation, and reduction of the imine to yield the delta-substituted proline **47** in 91% yield. Only the *cis*-isomer was observed due to hydrogenation proceeding from the less sterically hindered face of the imine. Base-promoted lactam cyclization was performed with 1N sodium hydroxide in methanol producing a mixture of **36b** and **36c** in 1:1 ratio in 92%. The complete diastereomeric separation of **36b** and **36c** was carried out by flash chromatography. The stereochemistry of the bicyclic templates was assigned by analysis of the ROESY experiment at -20 ºC [87].

Proteases recognize their substrates in extended conformation [4, 7]; therefore, the application of a β-strand template for the development of protease inhibitors is a reasonable choice to validate and evaluate the efficiency of β-strand mimicry. A serine protease, thrombin, was chosen as the target due to the importance of potential therapeutic applications and the well-documented structural information. The X-ray co-crystal structure analysis of the complex of thrombin and a thrombin inhibitor, PPACK, revealed the manner of the interaction between thrombin and PPACK (Fig. **7**) [88]. The β-strand backbone structure of thrombin interacts with the extended structure of PPACK through hydrogen bonding. The $Trp^{215}$ residue at the S3 pocket interacts with the phenyl group at the P3 position of PPACK through an aromatic-aromatic interaction. A guanidino group at the P1 position forms the critical acid base interaction with $Asp^{189}$ in the S1 specificity binding pocket and the chloromethylketone electrophile forms a covalent bond with $His^{57}$ which positions the tetrahedral negatively charged oxygen atom in the oxy-anion hole of the enzyme.

Templates **35** and **36** superimposed very well against the bound conformation of PPACK with a Boltzmann average RMSD value of 0.2 and 0.4 Å, respectively [85]. In principle, a thrombin inhibitor could be synthesized using these β-strand templates with the introduction of the proper pharmacophores. A hydrophobic group at the P3 position, a basic functional group at P1 position, and an electrophilic functionality were introduced to construct the proposed thrombin inhibitor. Mimetics **35a** and **36c** were coupled

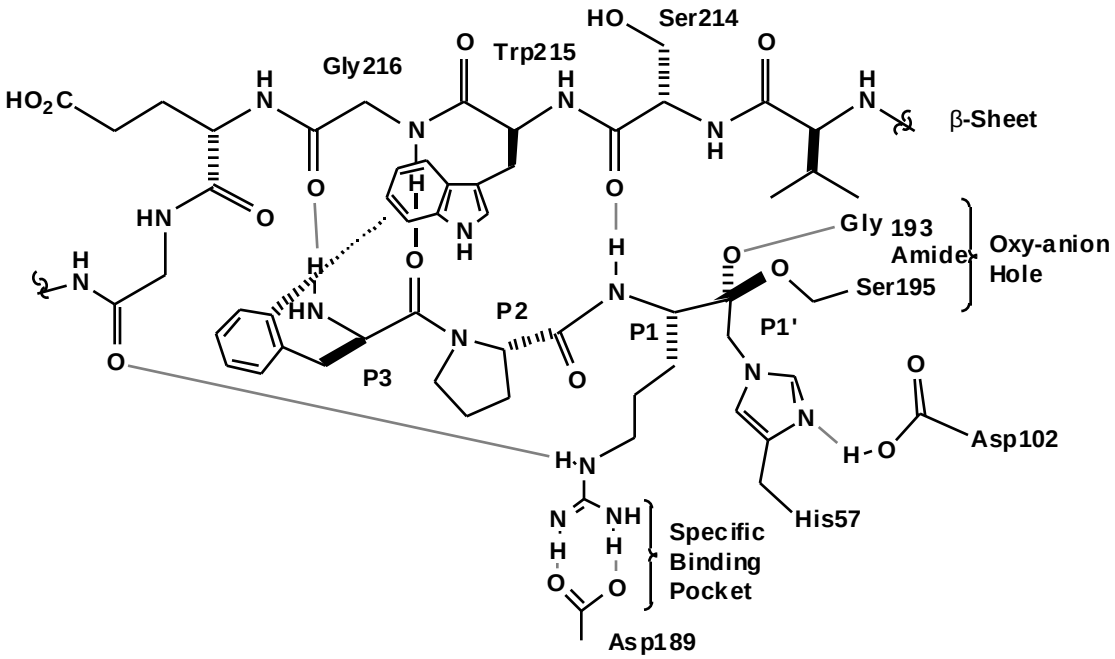
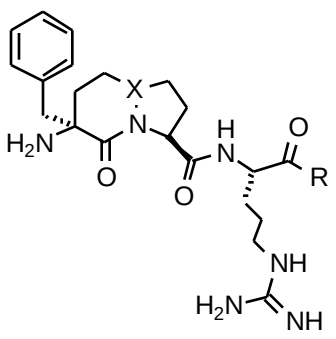
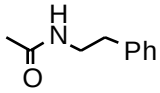
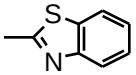
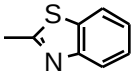



**Fig (7)**. PPAC bound to human thrombin active site based on the X-ray crystal structural analysis.

**Table 3. Inhibition of Thrombin**



| | X | R | $K_i$(nM) |
|---|---|---|---|
| **48** | N |  | 0.071 |
| **49** | N |  | 2.4 |
| **50** | N |  | 0.65 |
| **51** | CH |  | 0.85 |
| **52** | N |  | 1.2 * |

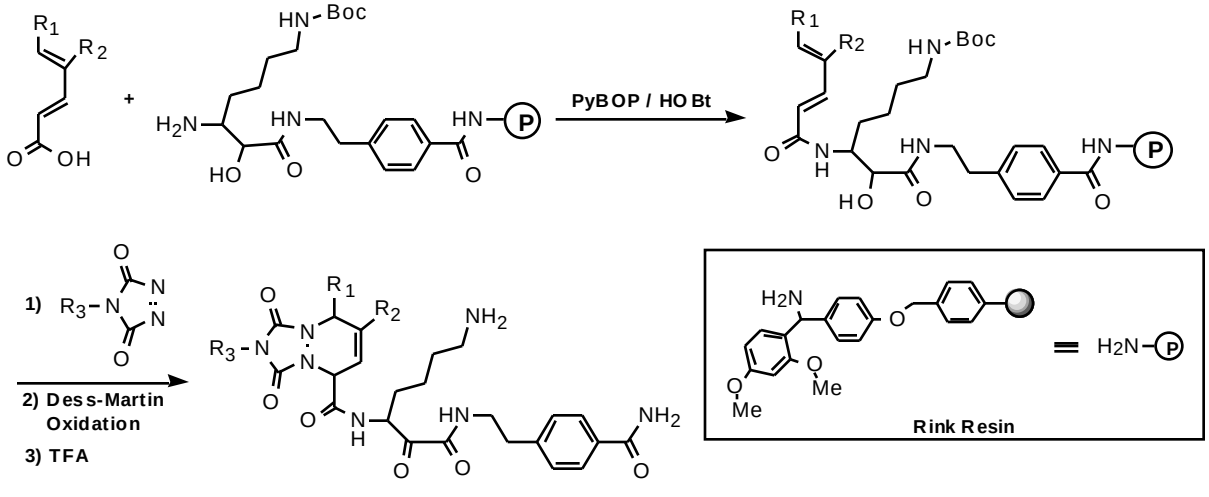
* $IC_{50}$ value is listed.

with Arg(Mtr)-α-ketothiazole derivatives, followed by removal of the Boc and Mtr groups to provide the electrophilic thrombin inhibitors. Excellent inhibitory activities ($k_i$ = 0.071 ~ 2.4 nM) were observed for the inhibitor as shown in Table 3 [85,87].

### 3.2 Solid Phase Synthesis and Application of Bicyclic β-Strand Mimetics

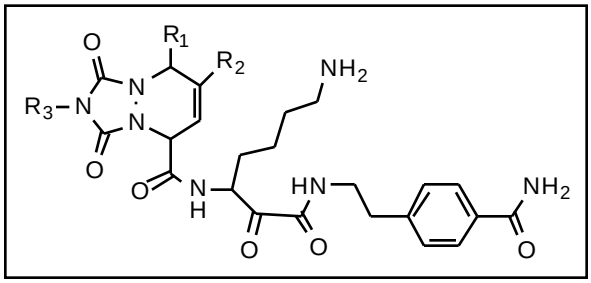
To facilitate the synthesis and enhance the diversity of our β-strand mimetics, we examined the utility of the Diels-Alder (D-A) cycloaddition reaction. This would allow for both a high degree of structural diversity in the synthesized templates as well as greater efficiency and reproducibility especially for the solid phase preparation of β-strand mimetics. To this end, we developed a solid phase synthesis of a variety of dienes to allow for the ready introduction of diversity to the template [89].

Scheme 10 illustrates the general approach that we implemented [86]. The attractive features of this approach are the ability to rapidly introduce several different diversity elements within the template as well as the ability to link the template to a variety of compounds. Among the most reactive dienophiles known for the D-A reaction are 1,2,4-triazolinediones. This dienophile is normally generated *in situ* by oxidation of the corresponding urazole. Although most of the accounts of 4-phenyl-1,2,4-triazoline-3,5-dione (PTAD) reacting with dienoic esters and amides have employed high temperature, we have found that 1,2,4-triazolinekiones generally undergo highly efficient D-A cycloadditions at room temperature. A typical synthetic sequence involving attachment of the linker, coupling of the dienoic acid, D-A cycloaddition and cleavage yields products which have consistently greater than 90% purity and high chemical yield [86]. The efficiency of this process allows for the rapid production of high numbers of diverse β-strand mimetics. A library of 1500 compounds was constructed and




**Scheme 10**.

**Table 4. Inhibition of Thrombin, Trypsin, Tryptase, and Kallikrein**



| R1 | R2 | R3 | Enzyme | $K_i$ (nM) |
|---|---|---|---|---|
| H | Me | $Ph_2CHCH_2$ | thrombin | 0.035 |
| H | H | $Ph_2CHCH_2$ | thrombin | 0.3 |
| $(Me)_2CHCH_2$ | H | $(Me)_2CH(CH_2)_2$ | trypsin | 0.43 |
| $(Me)_2CHCH_2$ | H | 4-MeOPh | trypsin | 0.28 |
| H | Me | $Ph_2CH$ | tryptase | 5.9 |
| $MeO_2C$ | $PhCH_2CH_2$ | 2,5-di-FPh$CH_2$ | kallikrein | 21 |

screened against several proteases and a number of potent and selective inhibitors were discovered. Some representative examples are listed in Table 4 [86].

## 4. CONCLUSION

Peptidomimetics are powerful tools for the study of molecular recognition in biological system. As such, they represent a critical step toward the rational design of low molecular weight nonpeptide pharmaceutical agents. We have focused here on our work with reverse turn mimetics and β-strand mimetics. Additionally, similar approaches are underway in our laboratory to develop mimetics of α-helical secondary structure motifs. Further work should provide important information for understanding the processes of molecular recognition and aid in the rational design of nonpeptide therapeutic agents from proteinaceous leads.

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