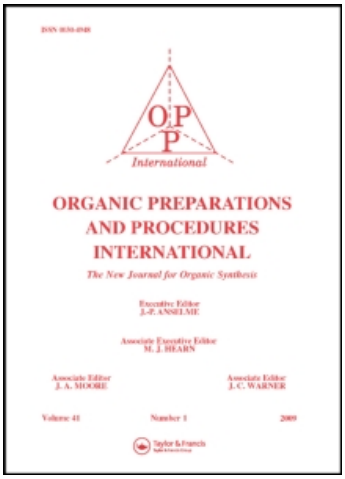

Organic Preparations and Procedures International

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t902189982

SMALL PEPTIDES WITH A β-HAIRPIN STRUCTURE

S. Miltschitzky[a]; B. Koenig[a]
[a] Department of Organic Chemistry, University of Regensburg, Regensburg, GERMANY

To cite this Article Miltschitzky, S. and Koenig, B.(2005) 'SMALL PEPTIDES WITH A β-HAIRPIN STRUCTURE', Organic Preparations and Procedures International, 37: 4, 307 — 336
To link to this Article: DOI: 10.1080/00304940509354968
URL: http://dx.doi.org/10.1080/00304940509354968

ORGANIC PREPARATIONS AND PROCEDURES INT., 37 (4), 307-336 (2005)

SMALL PEPTIDES WITH A β-HAIRPIN STRUCTURE

S. Miltschitzky and B. Koenig*

Department of Organic Chemistry, University of Regensburg
Universitaetsstrasse 31, 93051 Regensburg, GERMANY

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S. Miltschitzky and B. Koenig*

Department of Organic Chemistry, University of Regensburg
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INTRODUCTION

In recent years, β-hairpin peptides have been studied in detail. β-Turns are the most common element of non-repetitive structure recognized in proteins.[1] They consist of supersecondary structural elements in which two antiparallel adjacent peptide β-sheets are linked by a short loop. The loop size can vary; the majority of β-hairpins have loops of two to six residues,[2] the most common loop size being two. Their dihedral angles (*Table 1*) are used to classify them.[3]

Table 1. Most Common Hydrogen Bonded β-Turns in β-Hairpins and Their Dihedral Angels

Turn Type	β(i+1)	β(i+1)	β(i+2)	β(i+2)
Type I β	–60	–30	–90	0
Type I' β	60	30	90	0
Type I' β	60	30	90	0
Type II' β	60	–120	–90	0

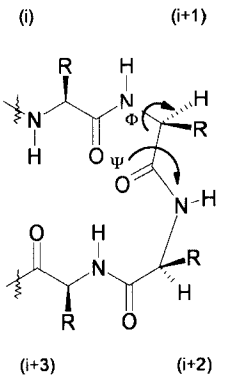
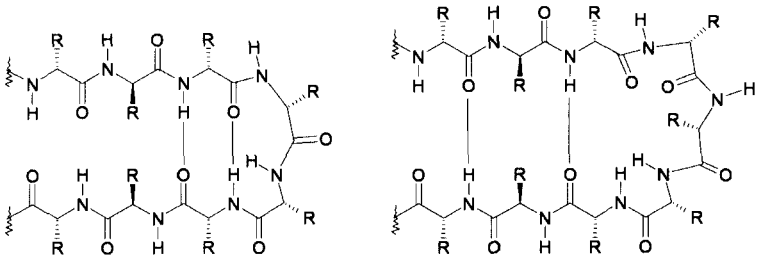


Illustration of the Four Residues in a β-Turn Demonstrating the Φ and Ψ Angles of the (i+ 1) Residue

Fig. 1

The two number nomenclature was introduced by *Sibanda and Thornton* using symbols of the form X:Y.[4] X is defined as the number of residues in the connecting segment if strand residues have at least one of their NH or CO main chain groups involved in the hydrogen bonding pattern. Y is defined as the number of residues in the connecting segment, when the strand residues have both their NH and CO main chain group involved. If both hydrogen bonds are formed, then X = Y. If only a single hydrogen bond is formed between the terminal NH of the first strand and the CO of the second strand, then Y = X + 2. In protein structures, 2:2 β-hairpins are most abundant, followed by 3:5 β-hairpins and 4:4 β-hairpins.[5]



2:2 β-hairpin 3:5 β-hairpin

Schematic representation of a 2:2 and a 3:5 β-hairpin structure in proteins. R = amino acid side chain

Fig. 2

To understand the formation of β-hairpins, it is necessary to analyze the energy contribution of the intrinsic secondary structure propensities of the different amino acids. In addition, interstrand side-chain to side-chain interactions in two structurally different regions of this secondary structure, the β-strand and the turn region have to be considered. This review will recapitulate the genesis of linear and cyclic, non-aggregating peptides and peptidomimetics that display β-sheet folding within this decade.[6] The following sections will focus on investigations that have studied the effect of turn-sequences, interstrand hydrogen bonding, side-chain interactions, and cyclization of the β-hairpin structure and its stability.

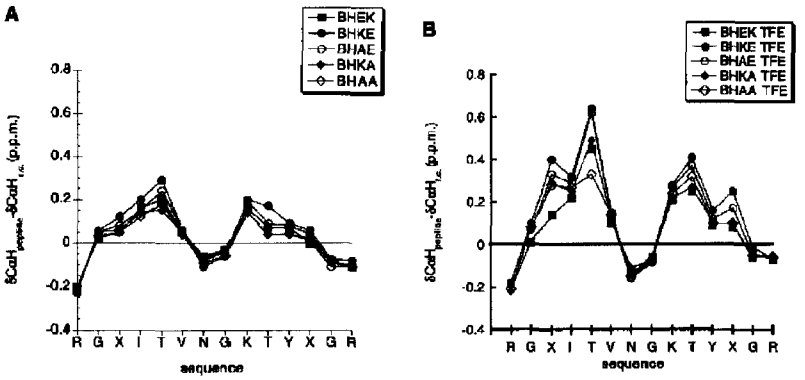
I. STABILIZED α-PEPTIDES WITH A β-HAIRPIN STRUCTURE AND THEIR BIOLOGICAL APPLICATIONS

Autonomously folding β-hairpins have emerged recently as vehicles for probing local interactions in β-sheet folding and assembly,[7] and as building blocks in protein design.[8] This section will focus on electrostatic and hydrophobic side-chain to side-chain interactions stabilizing the β-hairpin structure and leading to autonomous folding. It is generally believed, that salt bridges contribute significantly to the stability of the native state of the protein. An elongated version of the *de novo* designed β-hairpin peptide, BH8, has allowed *Ramirez-Alvarado et al.* to gain insight into the role of electrostatic interactions in β-hairpin stability.[9] A Lys-Glu electrostatic pair has been introduced by adding a residue at the beginning and at the *N*-terminal and *C*-

terminal strands of the β-hairpin structure, in both orientations. The two resulting peptides and controls having Ala residues at theses positions and different combinations of Ala with Lys, or Glu residues, were analyzed under different pH and ionic strength conditions. The investigated peptides had the following amino acid sequences:

BH8 (**1a**): Arg-Gly-Ile-Thr-Val-Asn-Gly-Lys-Thr-Tyr-Gly-Arg
BHKE (**1b**): Arg-Gly-**Lys**-Ile-Thr-Val-Asn-Gly-Lys-Thr-Tyr-**Glu**-Gly-Arg
BHEK (**1c**): Arg-Gly-**Glu**-Ile-Thr-Val-Asn-Gly-Lys-Thr-Tyr-**Lys**-Gly-Arg
BHKA (**1d**): Arg-Gly-**Lys**-Ile Thr-Val-Asn-Gly-Lys-Thr-Tyr-**Ala**-Gly-Arg
BHAE (**1e**): Arg-Gly-**Ala**-Ile-Thr-Val-Asn-Gly-Lys-Thr-Tyr-**Glu**-Gly-Arg
BHAA (**1f**): Arg-Gly-**Ala**-Ile-Thr-Val-Asn-Gly-Lys-Thr-Tyr-**Ala**-Gly-Arg.

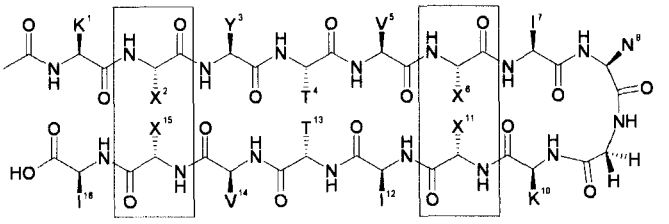
NMR analysis confirms that all the peptides adopt a β-hairpin structure in equilibrium with random-coil conformations in aqueous solution. The population ranking is BHKE > BHAE > BHEK > BHKA ≈ BHAA. In previous work, the authors have shown that a plateau of β-hairpin population was found at 40% TFE for the BH8.[10] The conformational shifts of the Cα protons in 40% TFE are larger than in aqueous solution indicating an increase of the structured population. As in aqueous solution, peptide BHKE is the one with the highest population, close to 100%. All of the peptides are more structured at higher pH values as shown by the downfield shift of the CαH of the two Thr residues.



CαH Conformational Shifts of the Different BH-peptides. The conformational shifts of the CαH protons are obtained by subtracting the random-coil values.[11] A: aqueous solution; B: 40% TFE

Fig. 3

Searle and co-workers, using a model β-hairpin system of 16 residues, investigated energetic contributions to the stability of Glu-Lys salt bridges.[12] The authors mutated two Ser-Lys interstrand pairs in **2a** to Glu-Lys salt bridges (**2b** and 2c) and examined the energetic effects of introducing two salt bridges simultaneously at these two positions (**2d**).



2a: X_2 = Lys, X_6 = Ser, X_{11} = Lys, X_{15} = Ser
2b: X_2 = Lys, X_6 = Glu, X_{11} = Lys, X_{15} = Ser
2b: X_2 = Lys, X_6 = Ser, X_{11} = Lys, X_{15} = Glu
2d: X_2 = Lys, X_6 = Glu, X_{11} = Lys, X_{15} = Glu

Schematic Representation of the β-Hairpin Peptides β1-β4. "X" indicates the sites of the mutated residues with boxes representing the position of the salt bridge

Fig. 4

The analytical data show clearly that the selective introduction of an ion-pairing interaction at either position enhances the stability of the folded state by increasing the magnitude of $\Delta\delta_{H\alpha}$ values. Individually, each interaction contributes 1.2-1.3 kJ/mol to stability; however, when introduced simultaneously, the contribution (-3.6 kJ/mol) is greater than the sum of the individual contributions.

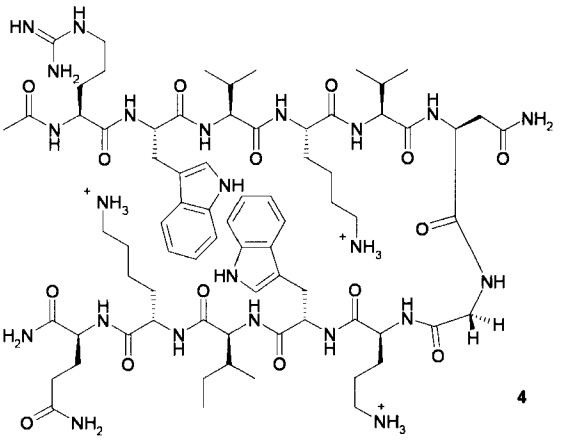
In addition to electrostatic interactions, hydrophobic interactions have been investigated. *Cochran* and co-workers used the stabilization of the β-hairpin conformation in short peptides by a tryptophan zipper (*trpzip*).[13] These trpzips are minimal units of β tertiary structure and have the thermodynamic properties of typical folded proteins.

Table 2. Amino Acid Sequences of the Trpzip Peptides

3a	trpzip1	Ser-Trp-Thr-Trp-Glu-Gly-Asn-Lys-Trp-Thr-Trp-Lys	Type II' turn
3b	trpzip2	Ser-Trp-Thr-Trp-Glu-Asn-Gly-Lys-Trp-Thr-Trp-Lys	Type I' turn
3c	trpzip3	Ser-Trp-Thr-Trp-Glu-^{D}Pro-Asn-Lys-Trp-Thr-Trp-Lys	Type II' turn

High-resolution NMR structures show the two cross-strand Trp pairs interdigitating in a zipper-like motif on the surface of the folded peptide. In each case, the peptides are highly water-soluble, well structured, and monomeric. Trpzip2 (**3b**) appears to be the most stable peptide due to the stronger promoting turn sequence Asn-Gly, despite previous conclusions that the D-Pro-Asn turn is more stabilizing than Asn-Gly.[14] Based on the results of *Cochran's* trpzip, a β-hairpin peptide, which functions as a molecular receptor for nucleotides in water, was synthesized by *Butterfield* and co-workers.[15] The diagonal Trp-Trp pair in the non-hydrogen-bonding sites of the β-hairpin peptide WKWK (**4**) provides a binding cleft for aromatic intercalation.

The ability of WKWK (**4**) to bind ATP in aqueous solution was investigated by fluorescence titration. A remarkable association constant for ATP in aqueous solvent of 5800 M^{-1} was determined from tryptophan emission quenching. Titration of ATP into peptide WKWK (**4**)



WKWK (**4**): Ac-Arg-Trp-Val-Lys-Val-Asn-Gly-Orn-Trp-Ile-Lys-Gln-NH_2

Structure of Peptide WKWK (4)

Fig. 5

produced significant upfield shifting of aromatic protons on both Trp residues as well as the adenine protons of ATP, indicating that the adenine base is interacting with both Trp residues of WKWK (**4**). The selectivity of nucleotide recognition by WKWK (**4**)[16] was investigated by proton NMR titrations and the affinity constants for WKWK binding to ATP, guanosine 5'-triphosphate (GTP), cytidine 5'-triphosphate (CTP), and thymidine 5'-triphosphate (dTTP) are summarized in *Table 3*.

Table 3. Affinity Constants for WKWK (**4**) Binding to Nucleotide Triphosphates (in 10 mM d_3-acetate buffer, 10 mM NaCl, pH 5.0 (uncorrected) at 298 K)

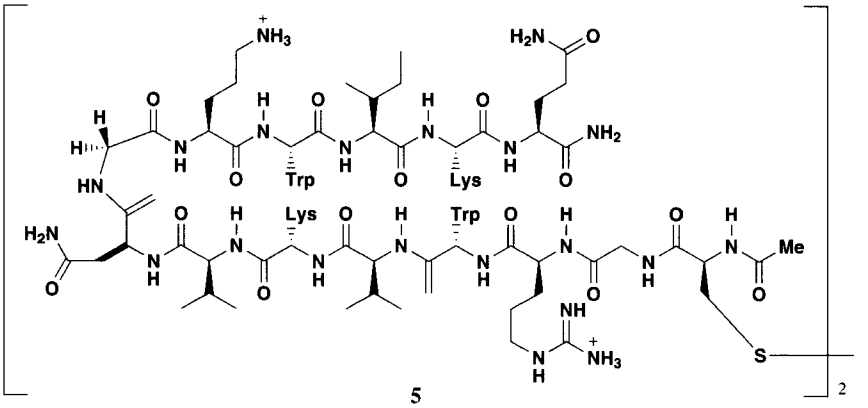
nucleotide	K_{assoc}, M^{-1}	K_d, mM	ΔG (error*), kcal/mol	Δ_0, ppm
ATP	700	1.4	–3.9 (0.1)	–0.12
GTP	2200	0.45	–4.6 (0.1)	–0.06
dTTP	3700	0.27	–4.9 (0.1)	–0.05
CTP	270	3.7	–3.3 (0.1)	–0.03

*Errors determined from the average deviation between 2 and 4 separate titration experiments

Peptide WKWK (**4**) demonstrates measurable selectivity of dTTP and GTP binding. The order of binding affinity follows dTTP > GTP > ATP > CTP, with differences in binding energies spanning as much as 1.6 kcal/mol. The two Lys residues of WKWK (**4**) on the same face of the hairpin allow a favorable electrostatic interaction with flavin mononucleotide (FMN).[17] Several analytical methods demonstrate that the flavin ring intercalates between the two Trp residues. The difference in binding affinities between FMN (K_{assoc} = 2200 M^{-1}) and riboflavin (K_{assoc} = 310 M^{-1}) indicates that electrostatic interactions of the FMN phosphate group contribute approxi-

mately –1 kcal/mol to the overall FMN binding. This value is in agreement with electrostatic contributions to ATP recognition by WKWK (**4**).

The interaction between proteins and single-stranded DNA (ssDNA) plays an important role in the regulation of critical biological processes such as the DNA replication and repair.[18] Due to the remarkable association constants of WKWK (**4**) to nucleotides, the authors expected the dimer $(WKWK)_2$ (**5**) as a potential receptor for ssDNA. A disulfide bond between *N*-terminal Cys side-chains dimerizes WKWK.



5

Structure of peptide $(WKWK)_2$ (5). The residues in bold create the nucleic acid binding site

Fig. 6

The structure of the $(WKWK)_2$ dimer (**5**) creates two nucleotide binding sites which are expected to be well-structured due to the highly populated β-hairpin conformation of WKWK. The recognition of a single-stranded pentanucleotide sequence 5'-d(AAAAA)-3' (dA_5) was investigated, followed by the determination of sequence selectivity between dA_5, dT_5, and dC_5. Finally, the binding of $(WKWK)_2$ (**5**) to an 11-residue single-stranded oligonucleotide and its corresponding dimer was investigated.

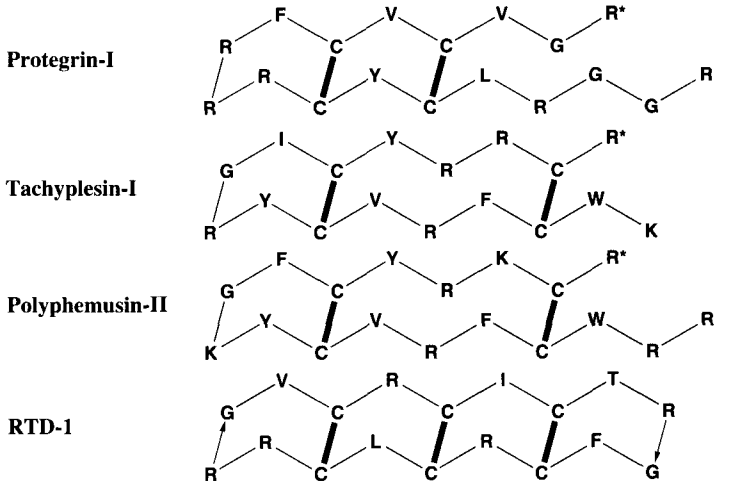
Table 4. Affinity Constants for $(WKWK)_2$ (**5**) with DNA Sequences in a 10 mM Sodium Phosphate Buffer, pH 7.0, at 298 K.

Entry	sequence	[NaCl], mM	K_a, M^{-1}	K_d, μM	ΔG (error*), kcal/mol
1	5'-AAAAA-3'	0	8×10^4	12	–6.7 (0.1)
2	5'-TTTTT-3'	0	3×10^4	30	–6.1 (0.1)
3	5'-CCCCC-3'	0	5×10^4	20	–6.4 (0.1)
4	5'-CCATCGCTACC-3' 5'-CCATCGCTACC-3'	100	3×10^5	3	–7.5 (0.1)
5	3'-GGTAGCGATGG-5'	100	2×10^5	5	–7.2 (0.1)

*Errors determined from the average deviation between 2 and 4 separate titration experiments

The modest selectivity for dA_5 is not surprising given the ssDNA-binding proteins interacting with their nucleotide target in a largely independent manner.[19] The stronger interaction with the 11-mer relative to the pentanucleotides can be attributed to an increased number of favorable contacts that are possible with the longer oligonucleotide.

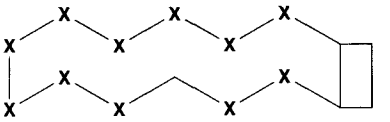
The growing problem of resistance to established antibiotics has stimulated intense interest in the development of novel antimicrobial agents with new modes of action. One emerging class of antibiotics is based on naturally occurring cationic peptides.[20] These include the disulfide-bridged β-hairpin and β-sheet peptides protegrins,[21] tachyplesins,[22] and defensins, which adopt a β-hairpin-like structure.[23]



Naturally Occurring β-Hairpin Antimicrobial Peptides. R* = C-terminal arginine amide.

Fig. 7

The naturally occurring protegrins and tachyplesins exert a significant haemolytic activity against human red blood cells, a key indicator of toxicity. Based on the natural peptides (*Fig. 3*), *Robinson et al.* synthesized new antimicrobial peptidomimetics that show potent and selective activity.[24]



β-Hairpin Mimetics. X = cationic and/or hydrophobic/aromatic amino acid residue, Temp. = template T1 or T2

Fig. 8

Screening of a library of 12-amino acid residue mimetics (*Fig. 4*), which contain a D-Pro-L-Pro template (T1) or a xanthene template (T2), revealed several members with activity against Gram-positive and Gram-negative bacteria, as well as the yeast *Candida albicans* (*Table 5*). The structure of the mimetics is different from the naturally occurring peptides that adopt a β-hairpin-like structure. While mimetic compound **6d** is largely unstructured in water despite the effect of the D-Pro-L-Pro template, it clearly adopts a β-hairpin conformation in the presence of

DPC micelles. A regular β-hairpin geometry is not present in compound **6g**, although a short stretch of 3_{10} helix is present.

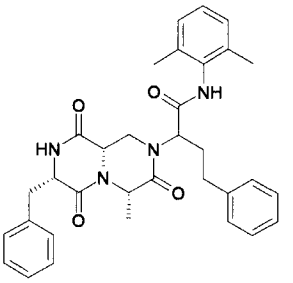
Table 5. Assay of Antibiotic and Haemolytic Activity.

Mimetic	Amino Acid Sequence*	MIC [μg ml^{-1}]				% Hemolysis
		S. aureus	*E. coli*	*P. aerug*	*C. albic*	
6a	LRLKYRRFKYRV-T1	25	12	25	12	27
6b	LRLQYRRFQYRV-T1	12	6	12	6	27
6c	LRLEYRRFEYRV-T1	100	100	>100	50	14
6d	LRLKKRRWKYRV-T1	12	12	6	12	1
6e	LRLKKRRWKYRV-T2	6	25	25	6	13
6f	LCLKKRRWKYCV-T1	25	6	25	12	3
6g	LRCKKRRWKCRV-T1	25	25	50	50	1
	Protegrin I	6	3	3	6	37
	Tachyplesin I	2	1	2	2	34

*Amino acids in the single letter code

II. TURN MIMICS FOR PEPTIDE HAIRPIN STRUCTURES

Peptides showing a turn conformation are of high relevance since, in many cases, the turn region is responsible for biological activity. Well known examples are somatostatin[25] and oxytocin.[26] Consequently, β-turns and β-turn mimetic adopting discrete conformations ("foldamers") have become target structures in medicinal research. This section will focus on turn mimetics and their ability to promote a turn structure. *Lubell et al.* pursued two strategies to generate peptide mimics: the first employs the use of bicyclics to constrain a dipeptide unit. The second uses the steric interactions of bulky ring substituents to influence the geometry.[27]

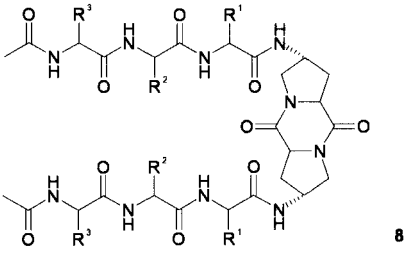


7

A Bicyclic Diketopiperazine β-Turn Mimetic

Fig. 9

Golebiowski and co-workers using the Ugi reaction as a key step describe the synthesis of a bicyclic diketopiperazine.[28] This compound overlaps well with a type II β-turn. In addition to their ability to induce β-turns, diketopiperazines are rigid backbones of peptide receptors.[29] Several receptors with two identical arms were prepared in order to examine their binding properties against a peptide library of 24,389 peptides.



8

Two-armed Diketopiperazine Receptors

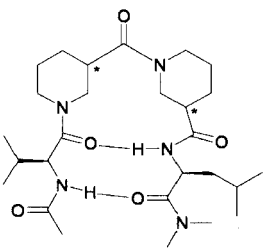
Fig. 10

The receptors were marked with the red azo dye Disperse Red 1 (DR1) which allows the monitoring of the peptide bonding. The following receptors were used:

DR mimetic 1 (**8a**)	Phe-Asn(Trt)-Tyr
DR mimetic 2 (**8b**)	Phe-Asn(Trt)-D-Tyr
DR mimetic 3 (**8c**)	Asn(Trt)-Phe-D-Tyr
DR mimetic 4 (**8d**)	Phe-Gln(Trt)-Tyr
DR mimetic 5 (**8e**)	Gln(Trt)-Phe-Tyr

The assays of DR mimetic 1 (**8a**) and DR mimetic 5 (**8e**) indicated a particularly high level of binding specificity since only 25 beads from all tested members of the library showed the red color of the receptor. This corresponds to a selectivity of one selected bead out of 5000. While **8a** exclusively selects peptides containing a D-His following two hydrophobic D-amino acids, **8e** only chooses peptides with an Asn following both a hydrophobic l- and D-amino acid. Affinity constants up to K_a = 1420±200 M^{-1} were observed.

Most of the synthetic foldamers have homogeneous backbones, *i. e.*, they are built from a single type of monomer. Oligomers of heterogeneous backbones are also important in conformational design. *Gellman* and co-workers reported an oligomer that adopts a β-hairpin shape. The loop is composed of β-amino acids while the strands consist of α-amino acids.[30] The loop segments made from dinipecotic acid (*Nip*) adopts a loop conformation in β-hairpins as shown in



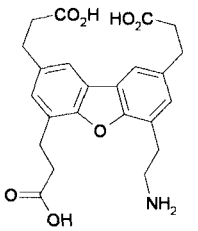
9a: *R*-NIP-*S*-NIP
9b: *S*-NIP-*R*-NIP
9c: *R*-NIP-*R*-NIP
9d: *S*-NIP-*S*-NIP

β-Turn Hairpins bearing a nipecotic acid loop segment

Fig. 11

previous studies.[31] The data of IR and NMR analyses indicate that the heterochiral loops (**9a**, **9b**) support hairpin formation while the homochiral loops (**9c**, **9d**) do not. A homochiral dinipecotic acid β-peptide does not allow the formation of a 12-membered ring hydrogen bond that is necessary for the hairpin folding.[32]

To help understand the folding and stability of peptides, a negatively charged dibenzofuran-based β-turn mimic was incorporated into the loop 1 of the PIN1 WW domain.[33] These three-stranded β-sheet domains are found in more than 200 multidomain proteins where loop 1 of PIN1 plays a critical role in binding of the phosphoserine (pS) residue in the YSPTpSPS peptide substrate.[34] The formation of the loop 1 is rate limiting for the folding of the PIN1 WW domain.[35]



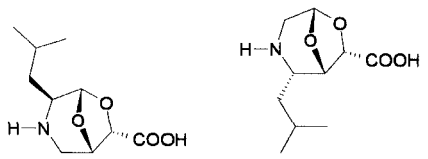
10

4-(2-Aminoethyl)-6-dibenzofuran Propionic Acid (10)

Fig. 12

The incorporation of the *bis*-anionic version of 4-(2-aminoethyl)-6-dibenzofuran propionic acid (**10**) as turn mimetic *in lieu* of the uncharged analogue resulted in enhanced solubility. The thermodynamic stability of the PIN WW domain is not perturbed significantly.

Dipeptide isosters derived from Leu and *meso*-tartraic acid derivatives (6-*endo*-BTL (**11a**) and 6-*endo*-BtL (**11b**)) were inserted in a small peptide.[36] The chair conformation of the six-membered ring locked in the bicyclic structure, in conjunction with the *endo* configuration at C6 gives the right shape for imposing a reverse β-turn.



6-*endo*-BTL (**11a**) 6-*endo*-BtL (**11b**)

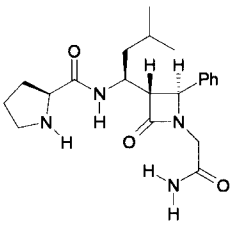
6-*endo*-BTL (11a) and 6-*endo*-BtL (11b)

Fig. 13

Both turn structures were incorporated into a tetrapeptide, giving Ac-Val-Ala-6-*endo*-BT(t)L-Val-Gly-OMe. The 6-*endo*-BTL peptide shows the turn structure as a minor conformer in a 1:3 ratio to an open reverse turn conformation, while the corresponding peptide shows a unique

turn conformation. However, the 6-*endo*-BTL peptide, having the Leu side-chain in axial position, is a better turn inducer since it can promote a tighter turn.

A turn mimic derived from PLG (prolyl-glycine amide) containing a β-lactam in the turn area was prepared by *Podlech et al.*[37] The substrate has a turn configuration even though no stabilizing central hydrogen bond is present. The so-called "open turn conformation"[38] is in fact stabilized by a hydrogen bond between the β-lactam carbonyl group and the neighboring NH moiety.



12

Targeted Peptidomimetic (12) bearing a β-lactam moiety

Fig. 14

The resulting six-membered twist-boat-like ring is presnt not only in the solid state but also in solution in $CDCl_3$. The stabilization is only possible because of the additional alkylidene group between incoming and leaving peptide strands.

Proline substitution has been widely used to search for turns[39] because Pro is frequently found at the i+1 position of a β-turn.[40] *Ishiguro et al.* have synthesized (2*S*,4*S*)- and (2*S*,4*R*)-4-(2'-guanidinoethyl)proline as a conformational restricted arginine.[41] These analogues were incorporated into mini atrial natruretic polypeptide (miniANP), which has an important turn-like conformation at Gly^6-Arg^7-Met^8-Asp^9.[42] The backbones of the arginine analogues fit the turn because of the bent backbone of proline. Although NMR could not determine the overall conformation of the analogues, in each case the distance constraints were consistent with and converged well into a type I β-turn. MiniANP is the smallest analogues of ANP-related peptides.[43] It contains 15 natural amino acids and binds selectively to natriuretic peptide receptor A (NPR-A). In biological activity measurements, the production of cGMP (*cyclic guanosine monophosphate*) in Chinese hamster ovary cells expressing NPR-A in response to peptides **13a-e** was measured.

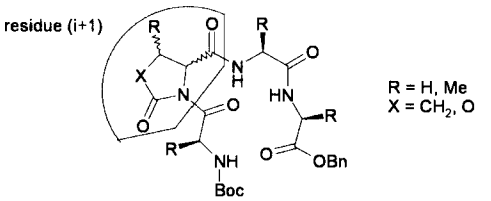
Table 6. Biological Activity of miniANP (**13a**) and Analogues (**13b-e**)

Peptide	Cmpd	Biological Activity EC_{50} (nmol)	Relative Biological Activity EC_{50} (analogue)/ EC_{50} (miniANP)
miniANP	**13a**	3.1 ± 0.5	1.0
[4*S*-GEPro^7]miniANP	**13b**	4.6 ± 0.4	1.5
[4*R*-GEPro^7]miniANP	**13c**	1.3 ± 0.6	0.4
[Pro^7]miniANP	**13d**	58.1 ± 17.2	18.7
[Ala^7]miniANP	**13e**	137.3 ± 11.3	44.3

The table shows that [4*S*-GEPro7]m**ini**ANP (13[b]) and [4R-GEPro7]m**ini**ANP (13c) are as potent as miniANP (13a) but of 19 times lower activity than [Pro7]miniANP (13d).

To develop a definitive approach towards the design of an I' turn nucleated β-hairpin, *Shamala and Balaram* investigated α-aminoisobutyric (Aib) Aib-D-Xxx nucleating segments.[44] The incorporation of a D-residue intends to favor the formation of a type I' β-turn, as in the analogue D-Pro-Gly segments.[45] The Aib-D-Ala segment was incorporated into a hexapeptide, giving Boc-Leu-Phe-Val-Aib-D-Ala-Leu-Phe-Val-OMe. This peptide adopts a β-hairpin structure with a type I' β-turn at the Aib-D-Ala segment.

Tomasini and co-workers described the synthesis and the conformational analysis of a small library of fully protected tetramers containing pseudoprolines.[46] The general structure of the tetramers and the pseudoprolines is shown in *Fig. 14*.



General Structure of the fully protected tetramers

Fig. 15

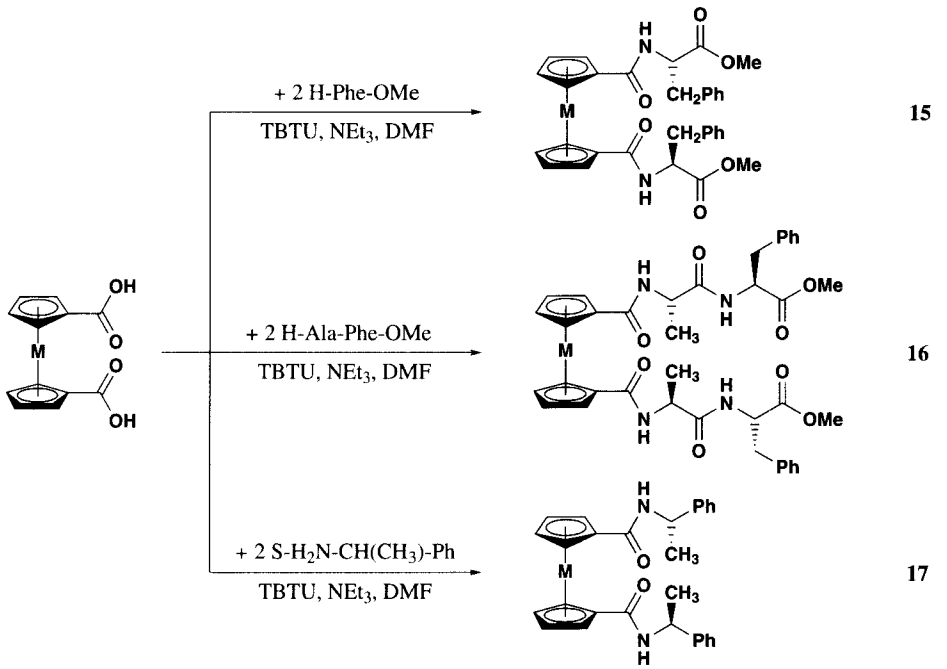
L-Pyroglutamic acid (L-pGlu), (4*S*,5*R*)-4-methyl-5-carboxybenzyloxazolidin-2-one (L-Oxd), or (4*R*,5*S*)-4-methyl-5-carboxybenzyloxazolidin-2-one (D-Oxd) are used in place of pseudoproline. *Table 7* summarizes the amino acid sequences of the investigated tetrapeptides.

Table 7. Amino Acid Sequences of the Investigated Tetrapeptides (**14a-f**)

Entry	Amino Acid Sequence
14a	Boc-Ala-L-pGlu-Gly-Ala-OBzl
14b	Boc-Val-L-pGlu-Gly-Ala-OBzl
14c	Boc-Val-L-pGlu-Aib-Ala-OBzl
14d	Boc-Val-L-Oxd-Gly-Ala-OBzl
14e	Boc-Val-D-Oxd-Gly-Ala-OBzl
14f	Boc-Val-D-Oxd-Aib-Ala-OBzl

Gly is widely found in natural reverse-turn structures while Aib tends to form helical structures (helycogenic).[47] The molecules containing D-Oxd showed a good propensity to form a β-hairpin conformation. Among them, Boc-Val-D-Oxd-Gly-Ala-OBzl (**14e**) had a preferential β-turn conformation in chloroform and a preferential γ-turn conformation in DMSO. Its epimer, Boc-Val-L-Oxd-Gly-Ala-OBzl (**14d**) is less able to assume ordered forms in solution.

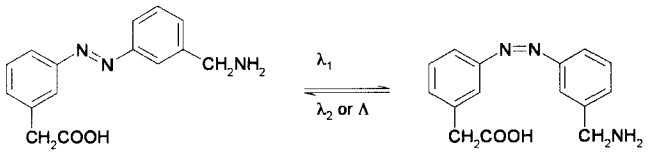
Metzler-Nolte and co-workers prepared di- and tetrapeptides bearing a metallocene backbone.[48] The metal was varied between Fe^{2+} and Co^{+} to change the overall charge of the constructs.



Synthesis of the Di- and Tetrapeptides with a metallocene backbone

Fig. 16

Evidence of strong interaction was found in **15** and **16**, while **17a** shows no intramolecular hydrogen bond. In the solid state, an intramolecular hydrogen bond is formed between the amide NH and the ferrocene CO in **15a**. If connecting atoms are counted, an 8-membered ring is formed. The situation for **16a** is different. Two intramolecular hydrogen bonds N_1–O_6 and N_3–O_2 are formed. Both define 11-membered rings between O (i) and N (i+3). This describes **15a** as a



Arg-Trp-Gln-Tyr-Val-X-Lys-Phe-Thr-Val-Gln-NH_2

GB1: X = D-Pro-Gly
mimetic: X = azobenzene

***cis-trans* Isomerization of Azobenzene and the amino acid sequence of the peptides**

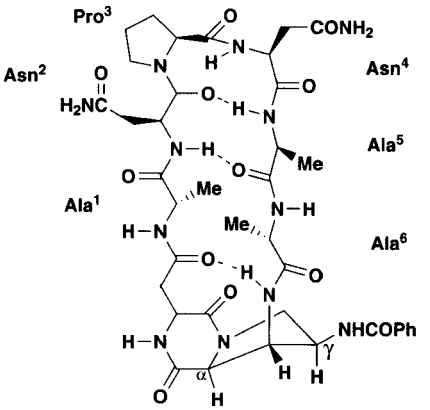
Fig. 17

γ-turn-like and **16a** as a β-turn-like structure. Similar results are found in chloroform solution for **16a** but **15a** shows now a NH_CO (ester) hydrogen bond. A comparison between uncharged ferrocene derivatives **15a** and 16**a** and positively charged cobaltocenium derivatives **15b** and **16b** shows that both pairs do presumably form very similar structures.

Photochromic compounds, which undergo large conformational changes when exposed to light, are also interesting in the field of turn mimetics. Azobenzenes are potentially well suited for this application. *Hilvert et al.* incorporated substituted azobenzenes in a 12-residue peptide, derived from GB1.[49] GB1 has been shown to adopt a hairpin structure in aqueous solution.[50]

Irradiation at the wavelength of the π→π* transition converts the *trans* (**18a**) into the *cis* (**18b**) isomer. The reverse process can be induced either thermally or by the irradiation at the wavelength of the π→π* transition. In its *cis* configuration, the *meta*-substituted azobenzene mimics the dipeptide D-Pro-Gly in nucleating a stable and monomeric hairpin structure. In contrast, the *trans* configurated peptide did not adopt a unique structure.

Short linear peptides are inherently flexible molecules, especially in aqueous solution, and thus are often poor mimetics of the secondary structures. To circumvent this folding-problem, much attention has been paid to the design of templates that constrain peptide chains into biologically relevant secondary structures such as cyclic peptides.[51] Linear synthetic peptides containing NPNA motifs repeated in tandem were evaluated in the late 1980s as potential malaria vaccine candidates.[52] Such linear peptides are flexible in water, but presumably adopt a folded conformation in the intact protein.[53] A bicyclic template, containing a diketopiperazine derived from Asp and (2*S*,3*R*,4*R*)-diaminoproline was incorporated in an acyclic peptide bearing the NPNA-motif.[54]

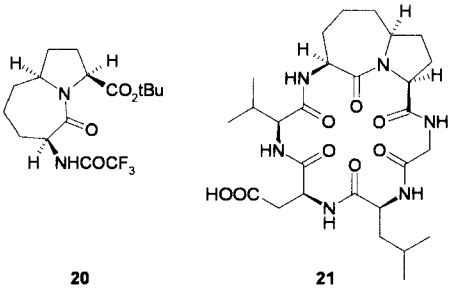


Structure of the NPNA-motif containing mimetic

Fig. 18

NMR spectra indicate a well-defined β-hairpin conformation for **19** in DMSO solution. NOEs and H/D-exchange rates show a well-populated 2:2 conformation with a type I β-turn in the Asn²-Pro³-Asn⁴-Ala⁵ (NPNA) motif in the tip of the hairpin loop.

Amino acid 7/5 bicyclic lactams are dipeptide surrogates in angiotensin-converting enzyme (ACE) inhibitors.[55] The 7/5 bicyclic lactam was used as external constraints for the GLDV motif by *Young* and co-workers.[56]



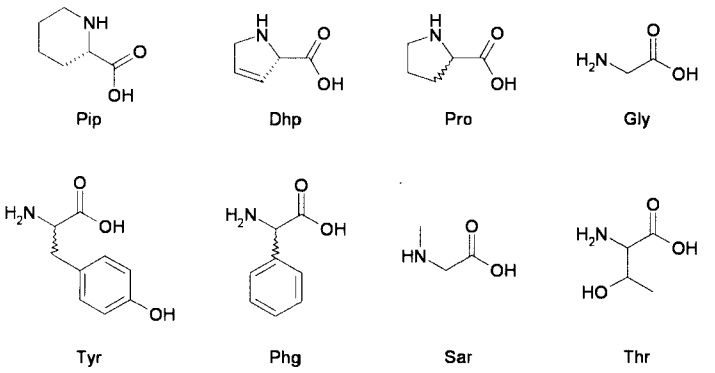
20 **21**

Structure of the 7/5 bicyclic lactam (20) and of the synthesized peptide (21)

Fig. 19

In agreement with X-ray crystal structure analysis and calculations, the 7/5 bicyclic lactam (**20**) was not a β-turn mimetic on its own. However, the high-resolution NMR spectroscopic data for the cyclic peptide (**21**) was consistent with a single backbone conformation, either type VI or type II' β-turn properties.

The effects of chirality and side-chain interactions on the formation of a type II' β-turn were studied by *Wishart et al.*[57] The appealing β-hairpin model is based on gramicidin S (*GS*). GS is a cyclic, amphiphatic decapeptide composed of two evenly spaced type II' β-turn connected by an antiparallel β-sheet.[58] For this study, a 14-residue cyclic analogue of GS bearing different amino acids in the i+1 and i+2 positions in both turns was selected.



Pip Dhp Pro Gly

Tyr Phg Sar Thr

Amino acids at position i^+ 1 and i^+ 2 in both turn 1 and turn 2

Fig. 20

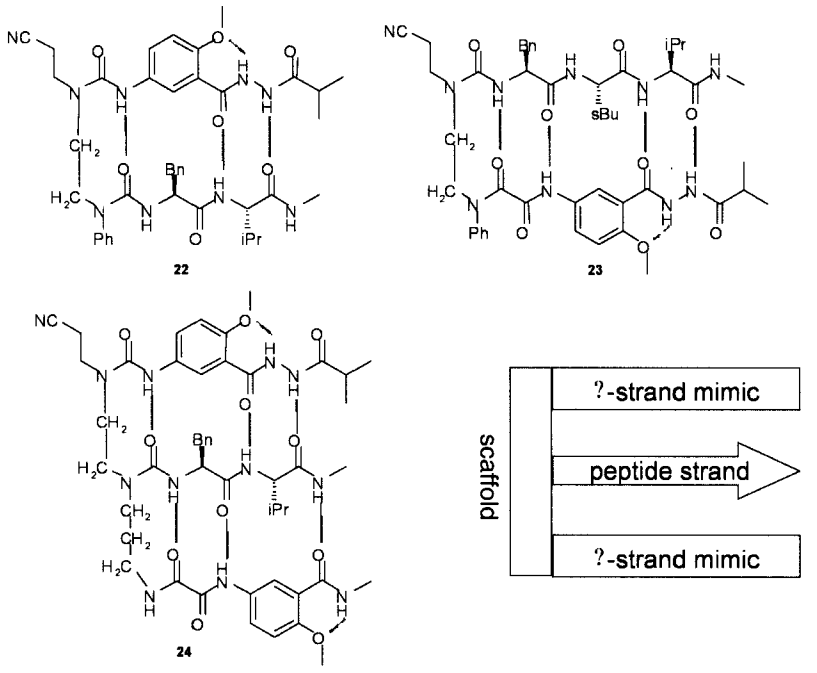
Heterochirality is an essential requirement for type II' β-turn conformation supporting Rose's "equatorial-axial rule".[59] Gly-Gly and Sar-Sar analogues are not able to adopt or stabilize a type II' β-turn. The content of β-sheet formation and proper side-chain interactions accounts for ≈ 10% type II' β-turn stabilization. Analogues with L-Pro at position i+2 and/or D-Pro at position i+1 have a predisposition to form a β-turn. Furthermore, D-Pro (i+1), pipecolic acid (*Pip*), and 3,4-dehydroproline (*Dhp*) act as excellent type II' β-turn promoters and lead to 20% type II' β-turn stability.

III. PEPTIDE HAIRSPIN STRUCTURE MIMETICS

β-Strands are usually found hydrogen-bonded at least in pairs, forming β-sheet structures in proteins. Isolated β-strands are not common. In the earlier sections, we focused on natural amino acids as (anti-)parallel strands connected by a β-turn. This section describes peptide mimetics incorporated into a hairpin structure.[60]

In immune defense, the major histocompatability complex (MHC) proteins selectively bind the extended β-strand conformation of peptides. The peptides evolve from intracellular process of viral, bacterial, and endogenous proteins.[61] This type of recognition has implications in leukemia, inflammatory, and neurological diseases.[62] A series of pathological processes is associated with the formation of a β-sheet structure and protein aggregation in the form of β-amyloid deposition. Alzheimer's disease as well as Creutzfeld-Jakob disease and BSE are connected with β-sheet aggregation.[63] For these reasons alone, small molecules that mimic β-strands are of great interest in medicinal applications.

Over the past decade, *Nowick et al.* investigated the β-sheet structure and developed useful peptidomimetic blocks that mimic protein β-proteins. The core is 5-amino-2-methoxybenzoic acid. This aromatic system contains an intramolecular hydrogen bond between the oxygen atom of the methoxy group and the amide hydrogen making the receptor more rigid.

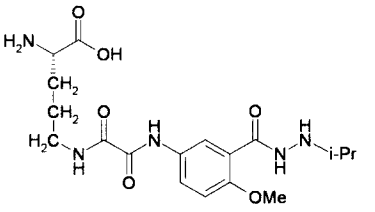


***Nowick's* double (22, 23) and triple stranded (24) artificial β-sheet structures**

Fig. 21

An artificial β-sheet with either β-strand mimics either along the upper or the lower edge forming a double stranded turn was described in the last decade.[64] In further studies, a triply templated artificial β-sheet is reported.

The NOE and coupling constant data of **24** indicate that the component peptide and the strand mimics adopt a β-sheet like conformation in chloroform. The folded structure is only observed in a non-competitive solvent such as chloroform. It is lost in methanol, a competitive solvent. The upper and lower strand mimics induces a β-sheet structure on an attached peptide strand.[65] The new amino acid, Orn (^{i}PrCO-Hao, **25**) consisting of an ornithine residue (Orn) with the β-strand-mimicking amino acid Hao, was introduced.[66]

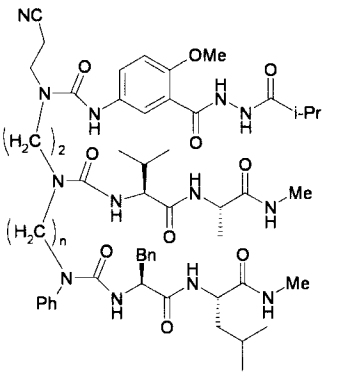


25

Structure of Orn(^{i}PrCO-Hao) (25)

Fig. 22

The Orn side chain allows the Hao oxalamide carbonyl group to form a 10-membered ring with the amino group of a connected peptide, forming a β-turn in a β-hairpin. The Orn(^{i}PrCO-Hao) amino acid (**25**) works as a splint that helps to enforce a β-sheet like structure. The triple stranded artificial β-sheet (**24**) depicted in *Fig. 21* shows both the upper and the lower sheet in an antiparallel binding motif. Replacing the lower artificial sheet by a α-peptide strand (Phe-Ile) leads to a combined parallel and anti-β-sheet (**26**).[67]

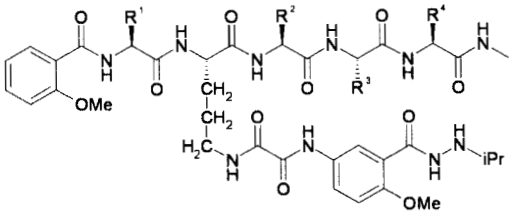


26a: n = 2
26b: n = 3

Triple-stranded β-sheets with mixed parallel and antiparallel β-sheets

Fig. 23

The Hao template forms a pattern of hydrogen bonds similar to that of an antiparallel β-sheet with the middle peptide strand while the middle peptide strand forms a pattern of hydrogen bonds similar to that of a parallel β-sheet with the bottom peptide. The β-strand mimic may be viewed as acting in conjunction with the triurea template to form a "corner bracket". This leads to a stabilized β-sheet structure (**26**) in both of the attached peptide strands. *Nowick's* artificial β-



27

Structure of the investigated molecules (27a-d)

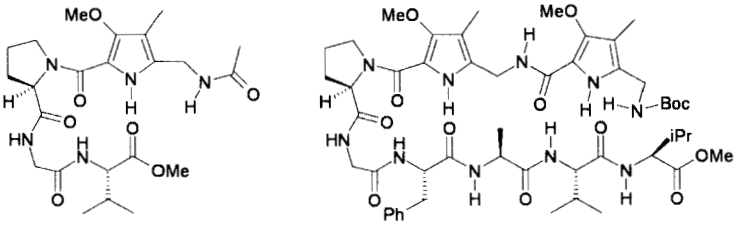
Fig. 24

strand mimetic was also used for the investigation of sequence-selective recognition of peptide strands across non-hydrogen-bonded rings.[68] Therefore, Orn(ⁱPrCO-Hao) (**25**) was attached to a peptide. Two residues, Thr and Val, were replaced in all possible orders at position R_1 and R_4. The residues R_2 (Phe) and R_3 (Ile) were not changed.

Table 8. Sequence of the Investigated Molecules (**27a-d**)

Entry	R^1	R^2	R^3	R^4
27a	Thr	Phe	Ile	Val
27b	Val	Phe	Ile	Thr
27c	Thr	Phe	Ile	Thr
27d	Val	Phe	Ile	Val

It was expected that Thr would preferentially pair with Thr and Val with Val, through self-complementary non-covalent interactions that occur frequently in the non-hydrogen-bonded rings of antiparallel β-sheets.[69] The investigation was carried out using ^{1}H-NMR spectroscopic studies. The analysis revealed a strong preference for Thr-Val (**27a**) and Val-Thr (**27b**) to form a heterodimer, while Thr-Thr (**27c**) and Val-Val (**27d**) remain as homodimers. These results confirm the hypothesis that pairing of like residues is preferred. *Koenig* and co-workers used



28 29

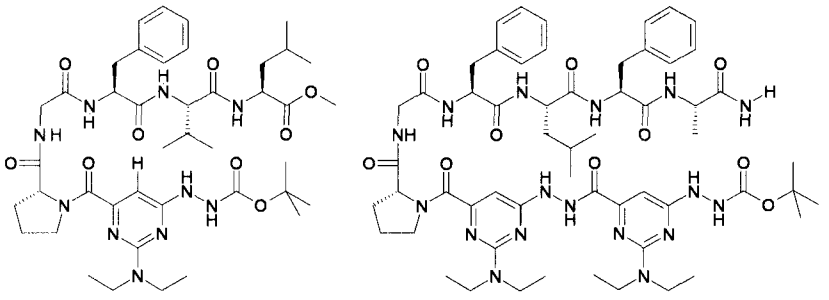
Monomer (28) and Dimer MOPAS receptors (29) connected to peptide strands

Fig. 25

structurally similar compounds. First, methoxypyrrole amino acids (MOPAS) and a peptide strand were connected by the D-Pro-Gly turn fragment.[70]

It was reported earlier that 5-(aminomethyl)pyrrole-2-carboxylic acid can be considered as a constrained surrogate of Gly-ΔAla.[71] The replacement of the methyl group by a methoxy

substituent also allows the formation of an intramolecular hydrogen bond between the hydrazine amide proton and the lone pair of the nitrogen of the pyrimidine ring. This should keep the pyrrole rings in one plane making the receptor more rigid. The turn structure was proven by ^{1}H-NMR investigation and by X-ray structure analysis of the monomer MOPAS turn **28**. In further studies, a pyrimidine ring is substituted for the pyrrole ring.[72] As shown by the systems of *Nowick*, a hydrazine unit is necessary for the right pattern of geometry of the pyrimidine hydrazine acids (PHA) to build up extended artificial β-strands. It also allows the formation of an intramolecular hydrogen bond to the lone pair of the nitrogen atom of the pyrimidine ring. This was confirmed by the analysis of temperature dependent ^{1}H-NMR spectra.



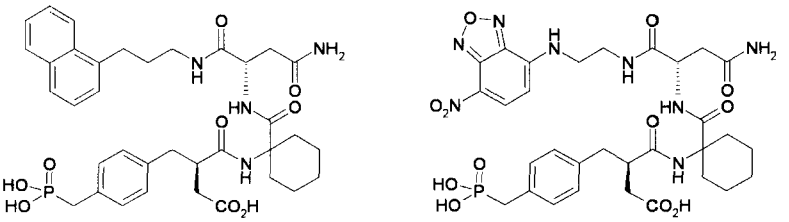
30 **31**

Monomer (30) and Dimer PHA receptors (31) connected to peptide strands

Fig. 26

The PHA receptors are not only UV active but show also emission around 420 nm in non-protic solvents after irradiation at 380 nm. The emission intensity decreased upon peptide binding indicating the binding event.

In this connection with heteroaromatic peptide mimetics, the intensive studies of sequence-selective DNA recognition have to be mentioned. Based on the natural dDNA binding peptides of distamycin[73] and netropsin,[74] *Dervan* and co-workers developed the art of polyamide technology, using *N*-methylpyrazole (*Py*) and *N*-methylimidazole (*Im*) covalently linked side-by-side and in a specific manner.[75] Although the work is not discussed in detailin this review, it is covered in recent reviews.[76]



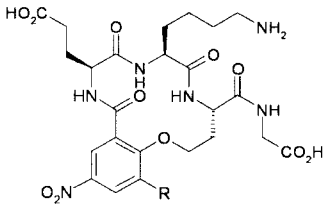
32 **33**

Structures of the parent compounds with a naphthylpropylamide ligand (left) and a nitrobenzoxadiazole ligand (NBD) (right)

Fig. 27

Fluorescence labeling has become also a general technique for studying the intermolecular accumulation and localization of exogenously administered materials.[77] Phosphotyrosyl (*pTyr*) mimetic-containing Grb2 SH2 domain binding antagonists are known as anticancer therapeutics typified by naphthylpropylamide analogues (**32**).[78] *Burke* and co-workers have used these results and modified a potential anticancer drug (**33**) to monitor cellular distribution studies of this class of inhibitors.[79] The environmentally sensitive nitrobenzoxadiazole (*NBD*) serves as fluorophore instead of the naphthyl function.

The binding affinities of the two compounds were determined using an ELISA-based competition assay. Although modeling studies suggest that the level of bonding interactions afforded by the NBD ring system may not be as great as those provided by the naphthyl ring, the binding affinities are similar.

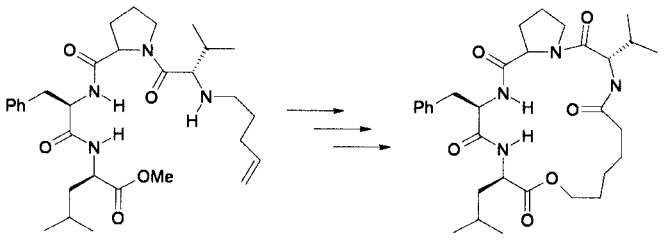


34a R = H (lead structure)
34b R = I
34c R = C?CPh
34d R = C_6H_4-4-OMe

Structure of the synthesized macrocycles (34a-d)

Fig. 28

Cyclic peptides and peptide mimetics are of great interest in medicinal applications because of their rigid shape. Starting from a combinatorial approach, *Burgess* and co-workers discovered a lead structure of a small macrocyclic molecule that strengthens the effect of nerve growth factor (NGF) *via* interactions with its high affinity transmembrane tyrosine kinase receptor (TrkA).[80] All the compounds consist of three natural amino acids (Glu-Lys-Ser) connected by 2-hydroxy-5-nitrobenzoic acid derivatives. However, there are conformational differences among them (**34a-d**), NMR experiments and molecular simulations indicate that all compounds can acquire a turn conformation, close to type I. The type VI β-turn involving a *cis* imide bond N-terminal to a L-Pro residue situated at the i+2 position plays a significant role in protein folding. It has a profound influence on the recognition process involving protein-ligand interaction.[81] *Iqbal et al.* synthesized a novel cis-Pro cyclic peptide (**36**) derived from the tetrapeptide *N*-cinnamoyl-Val-Pro-Phe-Leu-methyl ester (**35**) *via* ring closing metathesis adopting a type VI β-turn.[82]

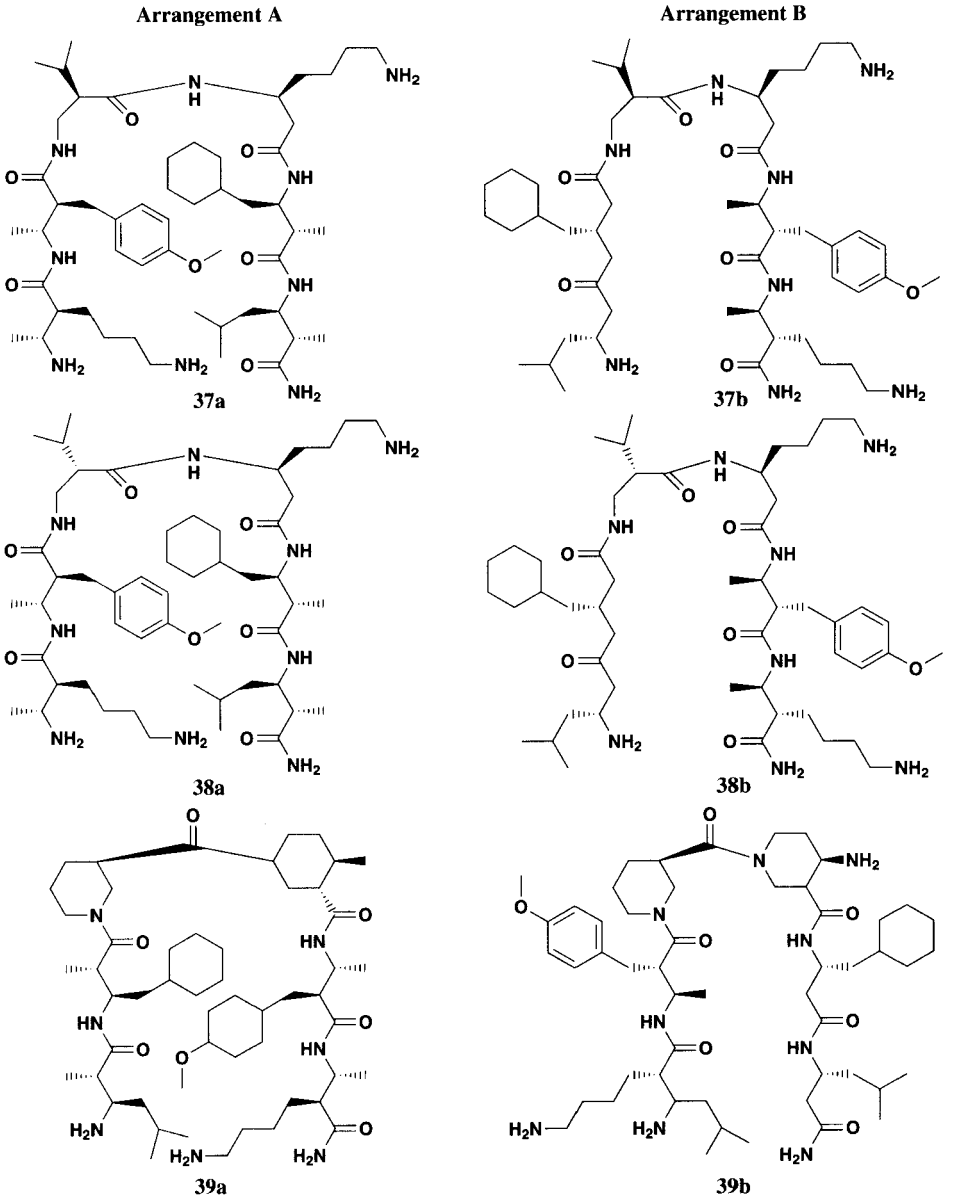


35 **36**

Synthesis of the desired cyclic peptide (36)

Fig. 29

The open peptide (**35**) exists in a 3_{10} helical structure.[83] The conformation of the cyclic peptide (**36**) depends on the solvent. NMR studies in solution indicate that the major conformer adopts a type VIa β-turn in chloroform, but a type VIb β-turn in DMSO. β-Peptide foldamers stabilize helical conformation in organic solvents.[84] Hairpin designs have been used to stabilize



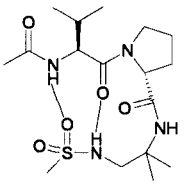
Six antiparallel hairpins with different reverse turn side chain arrangements

Fig. 30

antiparallel β-peptide sheets.[85] *Gellman* and co-workers report examples of these two concepts.[86] Two β-tetrapeptides were connected by (*R*)-nipecotic-acid-(3*S*,4*R*)-4-aminopiperidine-3-carboxylic acid (*Nip-APiC*) and both enantiomeric forms of β^2-valine-β^3-lysine.

The compounds differ in the arrangement of the side-chains. All the large side-chain groups are either oriented above (*arrangement A*) or below (*arrangement B*) the plane of the amide group. While each pair in the arrangements differs from the turn segment, the two peptides differ from one another in the juxtaposition of the side-chains. In methanol solution, **37** and **38** adopt at least a partial population of an expected hairpin conformation. The change from *S*- to *R*-configuration (**37a_37b**, **38a_38b**) has no influence on the hairpin population. The arrangements A and B are both tolerated in antiparallel β-peptide sheets. Finally, pairing of large side-chains on neighboring strands does not sterically disallow the hairpin formation. No distinct conformations were observed for **39a** and **39b**.

Non-peptidic β-strand complements could also provide a basis for disrupting protein-protein interactions that depend on the recognition of peptide segments in an extended conformation.[87] *Gellman* and co-workers introduced hydrogen bonding complementarity between a secondary sulfonamide and a α-amino acid residue.[88]



40

The two point hydrogen-bonded interaction between a secondary sulfonamide group and the C=O and N-H of a single peptide residue

Fig. 31

Compound **40** is the first molecule for which a double hydrogen-bonding pattern of the shown type has been characterized. Both, IR and NMR data confirm the turn structure.

IV. CONCLUSIONS

In summary, we report herein the recent publications in the field of small peptides adopting a hairpin conformation. These peptides are of great interest due to their biological activity and medicinal applications. Peptide mimetics based on β-turns are important, as many peptides are required to adopt such a conformation while effecting biological response. The article describes the different factors leading to stabilization or destabilization of the peptides' conformation and activity. In addition to natural peptides, we focus on mimetics incorporated either in the turn region, the peptide strand, or both of them. These pseudo amino acids consist mainly of (heteroaromatic) cycles, metallogenic centers, and inorganic units. The combination of naturally active peptides adopting one conformation as a lead structure and combinatorial chem-

istry as a tool is supposed to be a promising method resulting in new peptide mimetics. In the ongoing research of biological and medicinal active compounds mediating Alzheimer, AIDS, and cancer, these compounds might help understanding and treating the diseases.

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(Received April 9, 2005; in final form May 18, 2005)