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Peptides Containing β-Amino Acid Patterns: Challenges and Successes in Medicinal Chemistry

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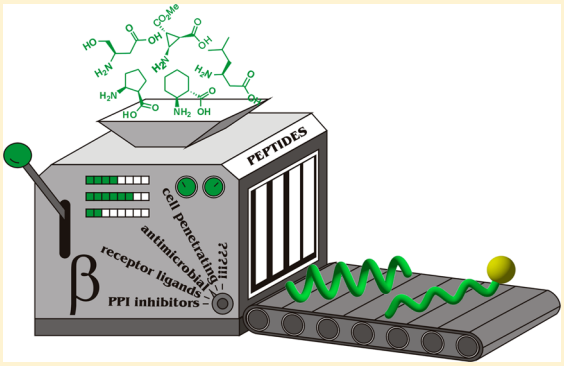
ABSTRACT: The construction of bioactive peptides using β-amino acid-containing sequence patterns is a very promising strategy to obtain analogues that exhibit properties of high interest for medicinal chemistry applications. β-Amino acids have been shown to modulate the conformation, dynamics, and proteolytic susceptibility of native peptides. They can be either combined with α-amino acids by following specific patterns, which results in backbone architectures with well-defined orientations of the side chain functional groups, or assembled in de novo-designed bioactive β- or α,β-peptidic sequences. Such peptides display various biological functions, including antimicrobial activity, inhibition of protein−protein interactions, agonism/antagonism of GPCR ligands, and anti-angiogenic activity.



INTRODUCTION

Precise control of the three-dimensional structure and properties of molecules is a major goal of modern medicinal chemistry. The appropriate spatial distribution of functional groups in a ligand is crucial for the effective interaction with a given molecular target, while physicochemical properties are responsible for the distribution of the ligand in a living system. Among numerous scaffolds used for the design of bioactive compounds, peptides and their analogues seem to be among the most widely explored.[1] Their compatibility with biological systems and their synthetic accessibility, including those with a structurally complex architecture, are major advantages. However, the development of effective drugs based on α-peptides is often limited due to the high conformational freedom of short fragments and low proteolytic stability in vivo. To overcome these drawbacks, modifications of native α-peptides have been developed, including variations of both the backbone and side chains. The construction of bioactive peptides containing β-amino acid units is a valuable method to effectively modulate structures, conformational preferences, and physicochemical properties.[2−5]

There are two major types of β-amino acid building blocks commonly used for bioactive peptides. The first type is based on homologues of natural α-amino acids that are extended by one methylene group incorporated adjacent to the carboxylate or amine functional groups (β^2- or β^3-analogue, respectively; Figure 1a).[6] The major advantage of this variation is the generally straightforward synthesis of such building blocks with various side chains (in particular, those analogous to natural ones).[6] The other type of β-amino acid is based on conformationally constrained monomers, most often through a cycloalkane ring (Figure 1b).[7] To obtain biologically active ligands, such building blocks ideally should stabilize a given conformation and confer functionality through groups typically present in the side chains of α-amino acids. While the diversification of β-amino acids by functional groups is synthetically challenging and has not yet been fully elaborated in peptide mimetics, the impact on the conformation imposed by them proved to be of great importance.[8] Such practical aspects have led to the design of two predominant types of peptides: homogeneous β-peptides (i.e., peptides containing exclusively β-amino acid units) with nonconstrained, linear, and constrained, cyclic building blocks and heterogeneous (also called hybrid) α,β-peptides containing constrained (cyclic) β-residues combined with α-residues bearing appropriate functional groups (Figure 1).[7,9]

The appropriate conformation of the peptide chain is crucial for its biological function. Structural investigations of β-amino acid-containing peptides with both homogeneous and heterogeneous backbones have shown their ability to form several types of helices, turns, and extended structures.[10] The most widely studied helical structure of β-peptides is the 14-helix, which was evidenced for all-β^3-peptides.[11] Moreover, 12-, 10-,

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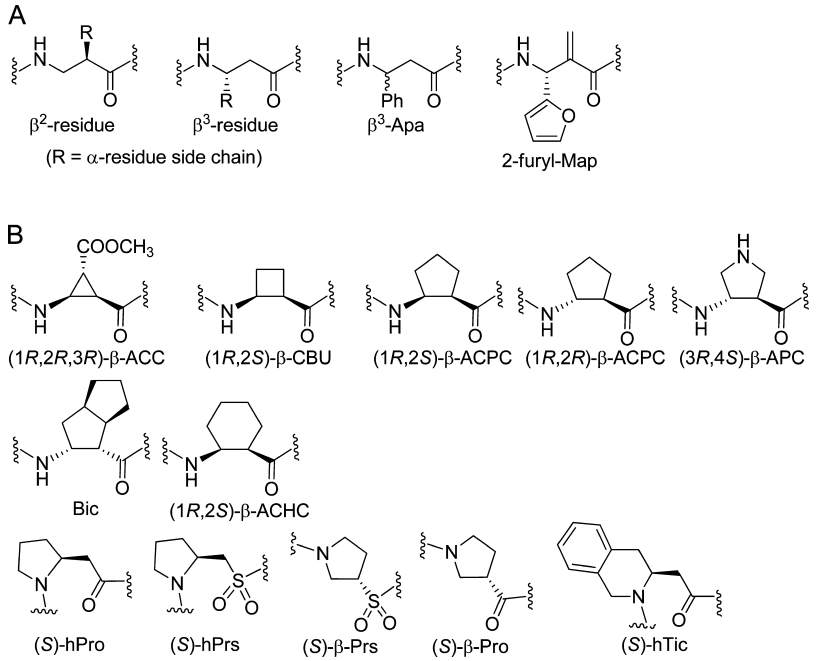

Figure 1. Linear (A) and cyclic (B) β-amino acids discussed in this Perspective (Apa, 3-amino-3-phenyl-propionic acid; Map, α-methylene-β-aminopropanoic acid; ACC, 2-aminocyclopropanecarboxylic acid; CBU, 2-aminocyclobutanecarboxylic acid; ACPC, 2-aminocyclopentanecarboxylic acid; APC, 4-aminopyrrolidine-3-carboxylic acid; Bic, 2-amino-octahydropentalene-1-carboxylic acid; ACHC, 2-aminocyclohexanecarboxylic acid; hPro- homoproline; hPrs, 2-pyrrolidinemethanesulfonic acid; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid).

8-, and 10/12-helices have been experimentally detected for β-peptides.[10] The number of conformations observed for α,β-peptides is even larger,[9,12] being dependent on the structure and stereochemistry of the β-units and the α,β-sequence pattern. In some cases, the type of secondary structure can be rationally deduced on the basis of the known tendency of single units to prefer dihedral angles of a specified magnitude and sign. For example, if the signs of the dihedral angles flanking an amide bond (φ and ψ) are the same, then a helical conformation should be observed (Figure 2).[13] This methodology, coined "stereochemical patterning", could substantially increase the chance for the accurate design of unexplored helical sequence patterns with stable conformations in solution, particularly for mixed α,β-peptides.[14]

The development of novel bioactive molecules using a structure-based approach requires the knowledge of the preferred conformation of a given sequence pattern.[2] Having a structurally well-defined, three-dimensional scaffold identified (e.g., an all-β^3-peptide adopting a 14-helix), it can be decorated with functional groups, which can interact favorably with the target once in appropriate spatial distribution. With building blocks that strongly impose conformational preferences, it can be assumed that the core structure is independent of the variation of side chain functional groups; thus, the design optimization can be focused on the type of functional groups introduced and their position in the sequence.

An alternative strategy is based on the reverse assumption, calling for the optimization of the scaffold structure (i.e., peptide backbone), while the side chains remain constant.[15] This approach, known as a sequence-based design, aims to identify the optimal position of the side chains in a heterogeneous sequence to achieve the perfect fit on the target.

The possibility to precisely control the shape of β-amino acid-containing peptides along with an appropriate spatial distribution of various functional groups opens up a wide

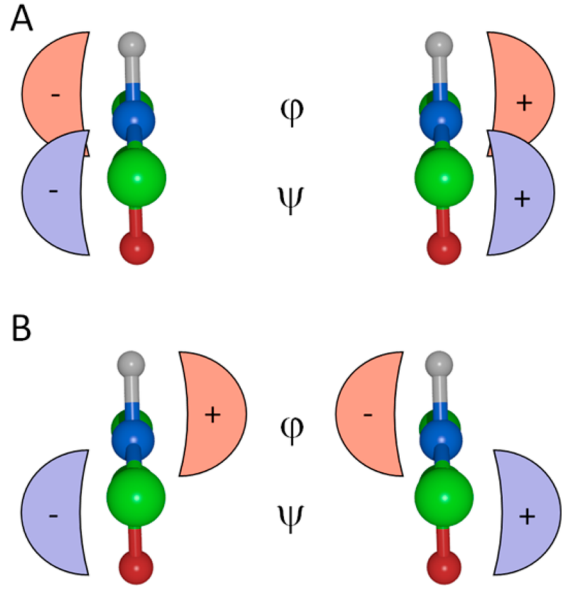

Figure 2. Conformational preferences of dihedral angles φ and ψ denoted by semicircles (light-red for φ and light-violet for ψ, with the sign of the dihedral angle included) around an amide bond (presented in ball-and-stick representation) leading to formation of helices (A) and strands (B). φ and ψ dihedral angles describe the rotation of the polypeptide chain around the N−C_α and C_α−C bonds, respectively. Stereochemical configuration pattern around the amide bonds determines φ and ψ dihedral angles and thereby the secondary structure.

variety of applications, particularly regarding medicinally relevant targets. In addition, compounds based on such scaffolds can exhibit several essential physicochemical properties. One of their most important features that is particularly relevant for drug development is the substantial increase in the

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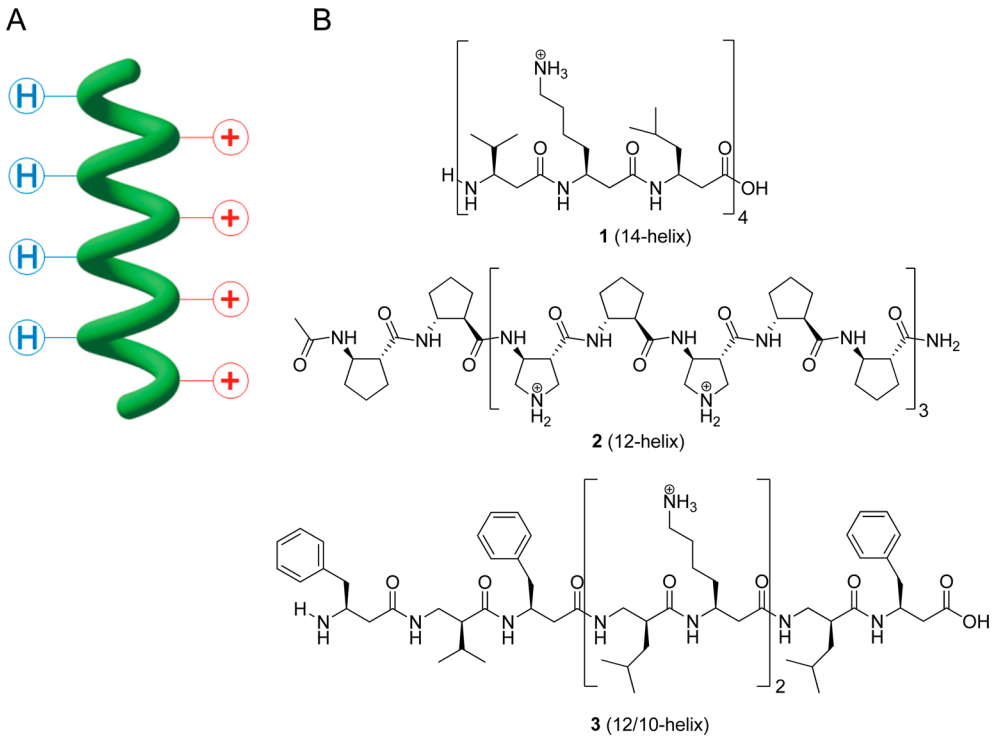

Figure 3. Basic concept of the construction ofantimicrobial peptides (H, hydrophobic group) (A) and examples of structures of β-peptides (B) forming various globally amphiphilic helices (the type of helix is given in parentheses).[26,27,30]

resistance to proteolysis compared to that of α-peptides.[16,17] Moreover, the molecular size of such peptide-based structures is significantly higher (1−5 kDa) than that of typical non-peptidergic compounds developed by a classical medicinal chemistry approach (0.3−0.7 kDa). This feature offers the possibility of binding to a large surface of the target, which is often necessary to effectively inhibit intermacromolecular interactions (e.g., protein−protein interactions).

In this Perspective, we present selected applications of β- and α,β-peptides, compound classes that are increasingly recognized for applications in medicinal chemistry. Helical β-peptides exhibiting amphiphilic character will be described first, followed by structurally more demanding targets, including protein−protein and receptor−ligand interactions. The potential of β- and α,β-peptides in drug design and development will be also discussed.

MEMBRANE-TARGETING PEPTIDES

Initial studies on the biological activity of β-amino acid-containing peptides focused on their interaction with biological membranes, and antimicrobial and cell-penetrating activities were evidenced.[18,19] The structural requirements for peptides targeting membranes are usually lower than those necessary for interaction with specific targets such as proteins or DNA because the molecular interaction relies on positively charged residues that bind to negatively charged elements of cell membranes following established concepts for α-peptides.

For most examples, the antimicrobial activity of peptides is related to their amphiphilic character.[20] Thus, a general design principle for such peptides calls for spatially separating positively charged side chains from lipophilic ones, regardless of the core structure of the peptide (Figure 3a). Such architecture is well-reflected in several naturally occurring host defense molecules, toxins, and antibiotics such as magainins,[21] bombolitins,[22] cecropins,[23] or mastoparans.[24] Following the precedent set by nature, many synthetic peptides derived from α-amino acids were generated this way rationally.[25] The underlying principles for such peptide design were successfully applied to β-peptides as well, starting with the pioneering work by DeGrado and co-workers,[26] who studied peptides containing the β^3-hVal/hLeu-β^3-hLys-β^3-hLeu motif (compound **1**, Figure 3b). These peptides showed antibacterial activity in the micromolar range; however, their hemolytic activity was also detected at a similar level. The specificity profile of such β^3-peptides could be later improved by changing the sequence to β^3-hAla-β^3-hLys-β^3-hLeu repeats, thus lowering the lipophilic character of the peptides.[27] Interestingly, similar peptides having $(\beta^3\text{-hAla-}\beta^3\text{-hLys-}\beta^3\text{-hPhe})_n$ sequences showed a poor profile with moderate antibacterial activity and high hemolytic activity.[28] With cyclic β-amino acids, Gellman and co-workers presented peptide **2** containing (3*R*,4*S*)-*trans*-4-aminopyrrolidine-3-carboxylic acid (APC, Figure 1) and (*R*,*R*)-*trans*-2-aminocyclopentanecarboxylic acid (ACPC, Figure 1), which also exhibited little hemolytic activity at the minimal inhibitory concentration (MIC) against various bacterial strains.[29]

Following these pioneering studies, many antimicrobial peptides were constructed using various β-amino acid units (e.g., peptides **3**−**6**, Figures 3b and 4). The analysis of those peptides further confirmed that an appropriate antimicrobial profile is the consequence of a delicate balance among lipophilicity, charge distribution, and structural flexibility. It was proven that the net charge of the peptide is highly important: negatively charged C-terminal carboxylates substantially decrease the activity compared with analogous amides.[31] The lipophilicity of peptides, deduced from the elution time on HPLC using a reverse-phase column, was found to reflect reasonably hemolytic activity but not antimicrobial activity.[16]

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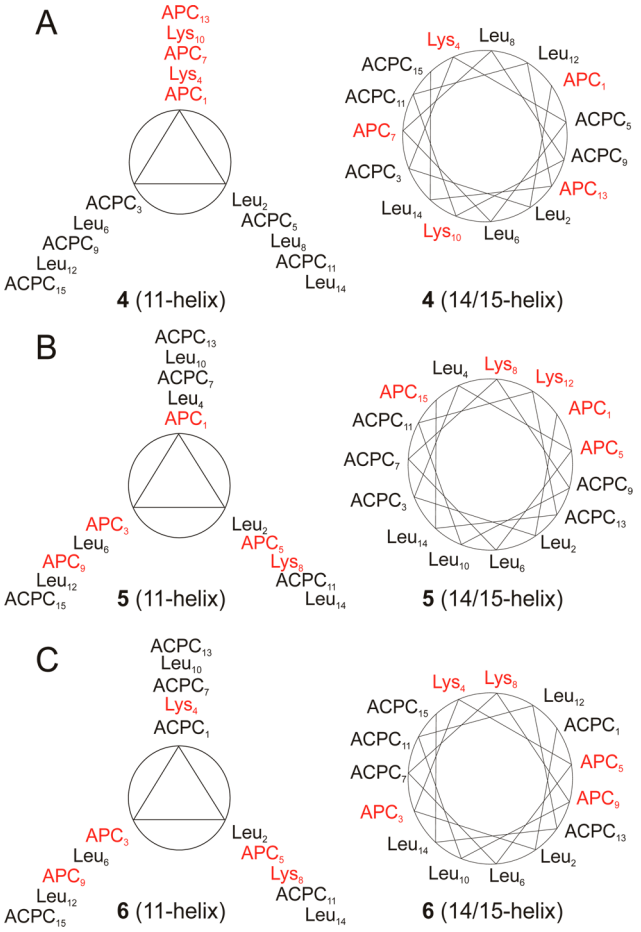

Figure 4. Helical wheel diagrams of antimicrobial α,β-peptides **4**–**6** shown for both 11-helical (left) and 14/15-helical (right) conformations. Examples of globally amphiphilic structures in the conformation of an 11-helix (A) and a 14/15-helix (B), as well as a scrambled peptide (C), are given.[16]

Furthermore, the screening of libraries of β-amino acid-containing peptides indicated that the limit of antibacterial activity (MIC in the range of 1 μg/mL) is similar to that found for α-peptides.[32] Finally, the content of helical conformation in aqueous solution does not correlate with antibacterial and hemolytic activities.[31]

Subsequently, the necessity of global amphiphilicity for antimicrobial activity was studied. Comparing α,β-peptides designed as globally amphiphilic in their 11-helix (peptide **4**) or 14/15-helix (peptide **5**) conformation with peptide **6**, which is not amphiphilic but has positively charged residues scrambled in any helical conformation possible (Figure 4), revealed that all three peptides exhibited high antimicrobial activity.[16,33] A possible explanation for this unexpected result could be that peptide **6** can still be amphiphilic but in a nonhelical conformation when interacting with the cell membrane. This theory was supported by the observation that only peptides with appropriate flexibility showed antimicrobial activity for scrambled sequences, while related peptides with rigid backbones were found to be inactive.[31] Moreover, studies on proteolytic stability of these α,β-peptides were done and indicated their high stability: peptide **4** was resistant to cleavage by trypsin or chymotrypsin (no proteolysis after 36 h of incubation was detected). It was slightly affected by Pronase: a small amount of cleavage products between ACPC9 and Lys10 was detected after 90 h.

Cell-penetrating peptides containing β-amino acid units were discovered simultaneously by Seebach and Gellman groups in 2002.[34,35] The first group described oligo-β^3-homoarginines (hexa- and heptamers), while the second group disclosed short cationic β-peptides analogous to certain fragments of the HIV transactivator of transcription (Tat) protein (β^3-analogue of YGRKKRRQRRR).[35] Both types of peptides contain 6–8 positively charged residues (mainly arginine), and they are able to carry a cargo such as covalently bound fluorescein inside cells (Figure 5). Recently, it has also been shown that oligo-β-proline can penetrate cell membranes.[36]

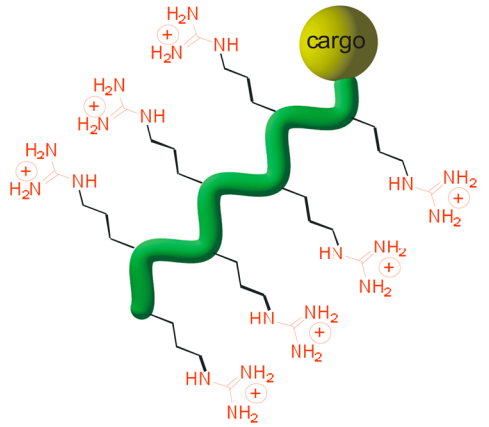

Figure 5. Schematic representation of arginine-based cell penetrating peptide with attached cargo molecule.

The mechanism of cell penetration is dependent on the sequence and conformation of the peptide and cell type. Confocal laser scanning microscopy and fluorescence quenching assays demonstrated that oligo-β^3-homoarginines can be transported into cells via a non-endocytotic mechanism.[37,38] More detailed studies using anionic lipid vesicles revealed that the mechanism of membrane penetration by this group of compounds follows a biphasic time course involving a fast, nonspecific, and relatively weak binding stage and a rate-limiting second step with stronger nonelectrostatic interactions and substantial membrane disruption.[39] Other studies showed that Tat-derived β-peptides and β^3-analogues of the $(VRR)_4$ peptide enter HeLa cells by endocytosis.[40] Subsequently, it was proven that enhanced penetration into cells can be achieved by formation of stable helical structures with arginine residues exposed to one side.[41,42]

A very interesting medicinal application was found in the course of evaluating the cell-penetrating ability of α- and β-oligoarginines in human erythrocytes. Healthy erythrocytes were unperturbed by these peptides, but those infected by *Plasmodium falciparum*, which is a parasite causing malaria, were penetrated.[43] The peptides studied also entered the cells of the parasites, opening up the possibility for selective delivery of antiparasitic drugs directly into *Plasmodium* cells, as shown for fosmidomycin in combination with octa-arginine.[44]

Amphiphilic helical β-peptides were also shown to exhibit antifungal activity in the micromolar range.[45] Importantly, analogous α-helix-forming host defense α-peptides were inactive toward the model pathogen *Candida albicans*. The two crucial parameters governing the antifungal activity of β-peptides were recognized to be hydrophobicity and helicity,[46]

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and moderate hydrophobicity enhanced by high levels of helicity was found to be optimal. It was indicated that this class of oligomers acts effectively on both planctonic cells and biofilms.[47] These peptides were also incorporated in multilayered polyelectrolyte thin films to inhibit the growth of *C. albicans* biofilms on surfaces (e.g., medical devices).[48]

Finally, peptides with cell-penetrating properties can be also antiviral.[49] It was shown for the α-peptide Tat fragment that it stops infection of herpes simplex virus type 1 (HSV-1). This property was also found for arginine-rich fully amphiphilic and helical β-peptides.[50]

MIMICRY OF PROTEIN FUNCTIONS

Protein−protein interfaces may be targeted by artificial structures aiming at molecular recognition and inhibition. Protein interactions occur through (i) solvent-exposed, flat surface patches, (ii) hot spots involving relatively few side chains that play important roles in the thermodynamic stabilization of the complex, or (iii) hot spots isolated from the solvent by the bulky interacting protein partners.[51] To address these features simultaneously, bulky and well-packed structures with rationally designed surface properties are needed. Such requirements can be met by artificial sequences with a tendency to fold into well-defined arrays. β-Amino acid patterns in peptidic chains have been shown to promote and control folding into well-defined secondary structures, while proteinogenic side chains can be largely retained with suitable building blocks.[5,10,11,52] These self-contained, medium-sized helix and sheet mimetics are excellent candidates to target protein surfaces, in contrast to a small molecule approach that cannot generally meet the geometrical requirements.[53] Another most welcome benefit is that 25−30% of β-amino acid incorporation into a peptide sequence increases the serum half-life by magnitudes due to their resistance to peptidases.[54,55]

The essential principles of molecular protein recognition have been well-established, and the steric, hydrophobic, and electrostatic features of native α-peptides and proteins can, in principle, be transferred to β-peptide foldamers. Nevertheless, finding and optimizing foldamer−protein interactions de novo remains a great challenge. Sequences containing non-proteinogenic amino acids cannot generally be synthesized via the ribosomal machinery using a genetic code; thus, searching for a protein that binds to unnatural foldamers cannot rely on evolutionary methods. To address this problem, design attempts can either build on top-down redesign and structurally mimicking known protein interfaces or on the bottom-up fragment-based de novo approach.

Mimicry of Regular Secondary Structures. Many protein−protein interactions occur through an α-helical interface, which is accommodated by a shallow cleft on the binding partner.[53,56,57] This arrangement is an ideal template to test the feasibility of mimicking helix−protein contacts with compact helical β-amino acid-containing sequences. There are also many known β-sheet protein interfaces, but the success in the mimicry of bioactive β-sheet-rich structures has been elusive.[58] The design of water-soluble, stand-alone β-sheet models with protein-like structural and functional features is an enduring challenge. In this section, regular β- and α,β-peptidic foldamer helices and sheets will be discussed that were top-down designed for protein recognition/inhibition.

hDM2−p53AD Interaction Inhibitors. A cleft on the surface of hDM2 (human oncogene product double minute 2) is recognized by a short helix motif of the p53 activation domain (p53AD),[59] making it a potential target for cancer therapy.[60] The well-characterized interface with a hot spot involves three residues on one helix face of p53AD: F19, W23, and L26.[61−63] This short helical recognition segment was mimicked by Schepartz and co-workers by using their β^3-peptide 14-helix platform that is designed to be soluble and stable in aqueous medium.[64] The β-14-helix has three faces, and the turns are separated by three residues. The β-H14 dodecamers constructed for hDM2 inhibition comprise a stabilizing salt bridge, a hydrophobic and the epitope face. The best sequence found, peptide **7** (also designated as β53-1) labeled with fluoresceine on its N-terminus, displayed a K_d value of 368 nM, which is at the same magnitude as that observed for the starting α-helical template (Figure 6).

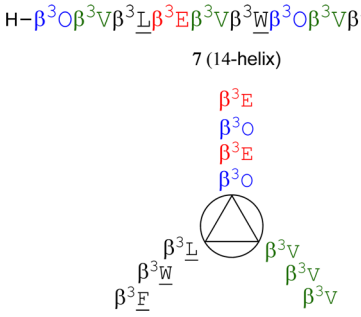

Figure 6. Sequence of β-peptide **7** (β53-1) and the helical wheel diagram showing spatial distribution of residues in the 14-helix. Residues crucial for interaction with the target are underlined. Positively charged, negatively charged, and hydrophobic residues, which stabilize the helix structure, are marked in blue, red, and green, respectively.

Moreover, peptide **7** could block the p53AD−hDM2 interaction (IC_{50} = 94.5 μM) by direct binding to hDM2. It has also been shown that 14-helical geometry is necessary for the activity. To improve the structural features and activity, a one-bead-one-peptide library method was developed, where rapid fluorescence-based screening was deployed.[65] Remarkably, although the optimization was carried out on a 14-helix face that was not involved in the recognition of hDM2, it yielded derivatives displaying an 8-fold increase in affinity and higher helix content.

The cell uptake and affinity of this β-peptide family was further improved by evaluating diether or hydrocarbon bridges as constraints, being aided by the X-ray structure available of the target protein.[66] Stapling together positions 4 and 7 improved affinity and cell uptake, whereas tethering positions 2 and 5 had a detrimental effect. Penetration characteristics were also better when a small cationic patch was introduced in the helices.[67] Such methodology of incorporation of positively charged residues (preferably arginine) in a biologically active sequence was assumed to be a general method of improving its cell permeability. The β-H14 scaffold combined with non-proteinogenic side chains such as the 3,4-dichlorophenyl moiety could extend the molecular recognition to protein hDMX.[68] A study conducted independently on similar β-peptides revealed the benefit of the β^3-(6-Cl)hTrp side chain instead of β^3-hTrp, which resulted in a ligand with low nanomolar affinity being accompanied by a considerably increased serum half-life due to the unnatural backbone.[69] These results clearly underline that the β^3-peptidic 14-helix together with the structure's stabilizing and cell-penetration-

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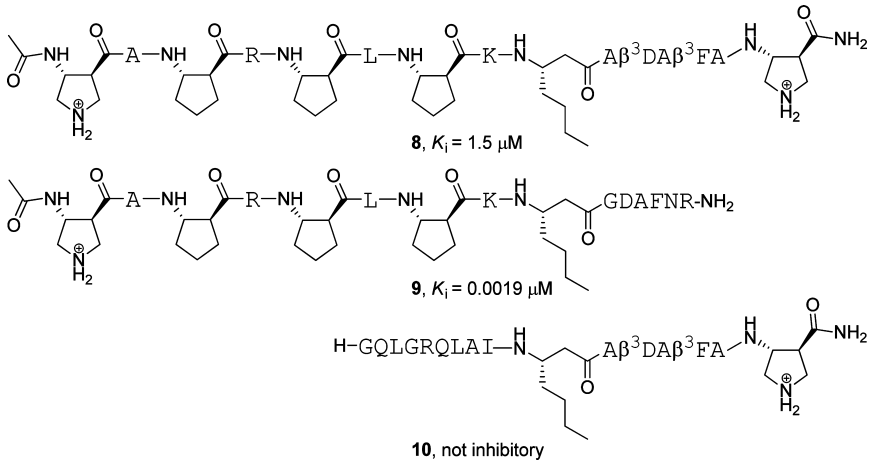

Figure 7. Sequences of α,β-peptide **8** and chimera $(\alpha,\beta + \alpha)$-peptides **9** and **10** exhibiting inhibitory properties against Bcl-x_L/Bak interaction.

improving design elements is a suitable platform for mimicking helical protein interfaces. Although small molecule inhibitors of hDM2 have been identified,[70] foldamers are promising protein targeting entities with their combination of affinity and pharmacokinetic properties (half-life and cell uptake) possibly unmatched by either protein therapeutics or small molecule drugs in general.

Bcl-2 Family: BH3 Domain Interaction Inhibitors. Anti-apoptotic Bcl-2 family proteins such as Bcl-x_L, Bcl-2, Bcl-w, Mcl-1, and A1 inhibit cell death by interacting with the Bcl-homology 3 (BH3) domain of pro-apoptotic family members. Overexpression of anti-apoptotic Bcl-2 proteins can block pro-apoptotic family members, thereby rescuing cancer cells from the cytotoxic effects of chemotherapy and radiation.[71] The key component at the interface is a 16-mer α-helix from the BH3 domain of Bak, which binds to a long hydrophobic groove on Bcl-x_L. There are six Bak residues in contact with the target, including four hydrophobic side chains, V74, L78, I81, and I84, filling the cleft.[72] Thus, the Bcl-x_L–Bak interaction offers a good model system to investigate the capability of β-peptidic helices to cover a contact surface larger than a minimal hot spot. In the initial studies, β-12- and β-14-helix scaffolds have been screened as possible templates to redesign the parent α-helix recognition segment, but the pure β-peptidic scaffolds proved to be unsuccessful.[73] This result pointed to the critical role of close geometrical matching between binding partners when the interaction surface area is large. In the same study, Gellman and co-workers proposed a strategy to overcome this problem based on the α,β-14/15-helix scaffold, which accumulated less geometrical deviation from the evolutionarily optimized parent α-helix than the pure β-peptidic helices.[73] The alternating α,β-peptide sequence (compound **8**, Figure 7) conformationally stabilized by five-membered-ring-containing β-amino acids was found to bind with a K_i value of 1.5 μM. While this demonstrated a successful design approach, it could not rival the K_i = 0.025 μM value for the parent Bak peptide, which was attributed to residual shape mismatch.

Chimera peptides were constructed in patterns $(\beta\alpha)_5\alpha_5$ and $\alpha_8(\alpha\beta)_4$ (peptides **9** and **10**, Figure 7) retaining the side-chain chemistry of the Bak peptide and the best α,β-peptide previously identified. The $(\beta\alpha)_5\alpha_5$ sequence was found to bind with a K_i value of 1.9 nM, indicating specific nanomolar affinity, while $\alpha_8(\alpha\beta)_4$ was inactive. The feasibility of constructing a complex protein epitope with the combination of different helix scaffolds was supported by sequence–affinity relationship studies using alanine and hydrophile scanning, and by a computational model of the foldamer/Bcl-x_L complex.[74] The binding of the most potent sequence to Bcl-w and Bcl-2 was proven, whereas no interaction was found for Mcl-1. It has also been shown that the designed helical ligands can induce cytochrome C release from mitochondria, leading to apoptosis.

These results and a pioneering X-ray study[75] revealed that two-thirds of the Bak peptide can be replaced with an α,β-motif, but the C-terminus is sensitive to the modifications, thus remaining proteolytically susceptible. To address this problem, an extensive study was performed to optimize the C-terminal segment including $\alpha \rightarrow \beta$ and $\alpha \rightarrow \alpha$ and $\alpha \rightarrow$ null modifications, where unnatural α-amino acids were utilized.[76] The mutations revealed that a 10-fold increase in affinity and a marked improvement in proteolytic stability can be achieved. These results strongly supported the hypothesis of geometry mismatch and located it to the C-terminus. The mimicry of the Bcl-2 family binding helices has been addressed in a sequence-based mutation approach. Building on the heptad-repeat principle of the globally amphipathic helices, $\alpha \rightarrow \beta^3$ mutations were introduced in an $\alpha\alpha\beta\alpha\alpha\alpha\beta$ pattern along the helix of the Puma BH3 domain.[77] The frame of the substitution sequence was shifted systematically along the helix to obtain β^3-residue stripes running along and around the helix (Figure 8). This approach yielded high-affinity analogues, with the allowed substitutions being at or in the proximity of the contact surface. Such properties of these analogues could be explained by the substitution pattern-dependent bulging propensities of the helices at the binding site.[15] It was also observed that the mutation pattern affected the selectivity regarding Bcl-x_L and Mcl-1.

Ac-EEQWAREIGAQLRRMADDLNAQYERR-NH_2
11, K_d (Bcl-x_L) < 1 nM, K_d (Mcl-1) < 2 nM

Ac-EEQβ^3WARβ^3EIGAβ^3QLRβ^3RMADβ^3DLNβ^3AQYEβ^3RR-NH_2
12, K_d (Bcl-x_L) = 2.2 nM, K_d (Mcl-1) = 110 nM

Ac-β^3EEQβ^3WAREβ^3IGAβ^3QLRRβ^3MADβ^3DLNAβ^3QYEβ^3RR-NH_2
13, K_d (Bcl-x_L) = 1 nM, K_d (Mcl-1) = 1100 nM

Figure 8. Sequence of the Puma BH3 peptide (**11**) and the most active α,β-peptide analogues (**12** and **13**).

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Although the substitution ratio was only in the range of 25−30%, significant enhancement of proteolytic stability was achieved. The $\alpha\alpha\beta$ and $\alpha\alpha\alpha\beta$ patterns, causing the β^3-residues to spiral around the helix periphery, have also been tested on the Bim BH3 domain. The latter motif successfully mimicked the helix geometry needed for tight binding and the biological response.[78]

Targeting the Transmembrane (TM) Domain of Integrin $\alpha_{IIb}\beta_3$. Molecular recognition of biological membrane protein using helical structures is a twofold challenge. First, the helix must insert into the hydrophobic interior in a TM orientation. Second, the scaffold must bear a recognition segment facilitating the interaction with the targeted protein. DeGrado and co-workers successfully addressed this problem and demonstrated the feasibility of their approach on the platelet integrin $\alpha_{IIb}\beta_3$,[79] where disruption of the interaction between TM helices from the α and β subunits with a designed α-peptide TM ligand led to integrin activation.[80] This methodology was successfully extended to β-peptides.[81] A sequence long enough to span through the membrane was functionalized with Trp and Lys residues at the termini for the desired TM orientation, and the side-chain sequence was in silico optimized against the GXXXG motif of the α_{IIb} helix. The calculations yielded the $(\beta$-hGly-X-X$)_3$ pattern for both the 14- and 12-helix scaffolds as an optimized recognition segment, and the designed sequence, referred to as β-CHAMP (Figure 9),

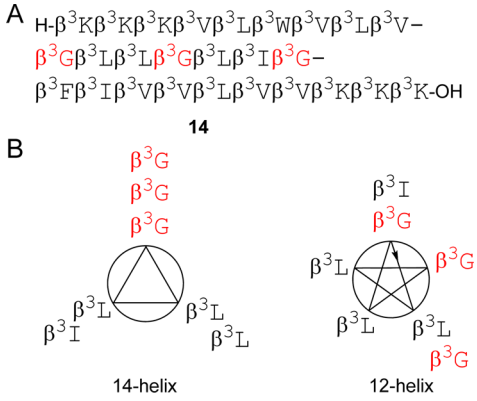

Figure 9. Sequence of the β-CHAMP peptide (**14**) (A) and helical wheel diagrams of its binding motif for 14- and 12-helical conformations (B). Glycine residues of the binding motif are marked in red.

was constructed accordingly. Analytical ultracentrifugation and CD measurements supported the binding of 12-helical β-CHAMP to α_{IIb}, and moreover, rupture force spectroscopy measurements and transmission electron microscopy images indicated that β-CHAMP peptide almost completely activated $\alpha_{IIb}\beta_3$ by disruption of interaction of TM domains. These results suggested that targeting natural TM helices can be tackled by an algorithmic approach and that β-peptide sequences are good candidates.

γ-Secretase Inhibitors. γ-Secretase is a membrane-embedded aspartic protease that processes amyloid precursor protein (APP) to obtain Aβ peptides.[82] Thus, inhibition of this enzyme could be an effective strategy against Alzheimer's disease. Interestingly, helical hydrophobic peptides, e.g., Aib-containing oligomers, were found to be effective modulators of the activity of γ-secretase.[83,84] Extending this strategy to β-peptides, it was found that oligomers of (1*S*,2*S*)-β-ACPC were highly active inhibitors of γ-secretase with a K_i = 5.2 nM for the dodecamer.[85] Further studies conducted on a series of analogues of Ac-$((1S,2S)$-β-ACPC$)_{12}$-NH_2, in which substitutions with linear β^3-amino acids were performed, pointed toward modifications of the parent sequence at position 3.[86] The most potent compound with β^3-hSer incorporated resulted in a 20-fold increase in inhibitory activity (IC_{50} = 0.40 nM).

HIV Fusion Protein gp41 Inhibitors. The fusion mechanism of HIV (class I) is common to several enveloped viruses such as those responsible for influenza, Ebola, and SARS.[87] The key feature in the process is the rearrangement of the protein gp41 from the trimeric prehairpin intermediate to the anti-parallel six-helix bundle.[88] Targeting this conversion by mimicking the components of complexes with stand-alone helices derived from the gp41 N-terminal heptad repeat (NHR) domain (peptide **15**) or C-terminal heptad repeat (CHR) domain (peptide **16**, Figure 10) has been investigated and applied as an anti-HIV approach.[89,90]

Ac-SGIVQQQNNLLRAIEAQQHLLQLTVWGIKQLQARIL-NH_2

15, NHR domain

Ac-WMEWDREINNYTSLIHSLIEESQNQQEKNEQELL-NH_2

16, CHR domain

Figure 10. Sequences of the N-terminal heptad repeat (NHR) domain (**15**) and C-terminal heptad repeat (CHR) domain (**16**) of HIV-1 gp41.

Despite the success of these sequences, the related pharmacokinetic/proteolytic properties are not satisfactory, and this initiated their top-down redesign using β-amino acid residues.

Schepartz and co-workers targeted a well-defined hydrophobic pocket formed by the trimeric NHR domain of gp41.[91] This is occupied by residues W628, W631, and I635 displayed over the surface of the CHR helix.[88] The β-peptidic 14-helix stabilized by a salt bridge was utilized as scaffold, and the WWI epitope was constructed in various arrangements on the binding face (β-WWI). Low micromolar binding affinity to the trimeric NHR domain model (IZN17) was measured for these sequences irrespective of the exact epitope display relative to the helix orientation. Cell fusion tests revealed EC_{50} values in the micromolar range. The central Trp residue is thought to be crucial in the epitope; accordingly, this side chain was optimized in the β-peptidic sequence.[92] It was found that *m*-trifluoromethylphenyl substitution of the indole moiety of Trp resulted in an improved biological response with micromolar EC_{50} values.

Gellman and co-workers applied the heptad-repeat approach described above to introduce $\alpha\alpha\beta\alpha\alpha\alpha\beta$ patterns into an optimized 38-residue variant of the CHR domain (peptide **17**, T-2635, Figure 11).[93] These replacements, yielding a β-residue stripe on the non-binding face of the helix, led to a dramatic decrease in affinity (peptide **18**). The latter finding again indicated the sensitivity of the system to the backbone geometry introduced by the β-residues that could be remedied by leaving the N-terminal 21-residue segment intact (peptide **19**). The binding affinities of **19** were tested against the protein gp41-5,[94] a designed model of the fusion helix bundle, and the chimera sequence displayed subnanomolar K_i values. It was speculated that the extra flexibility of the β^3-amino acid building blocks may cause an entropic loss on binding; therefore, Ala → ACPC and Arg → APC substitutions were carried out to make

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Ac-TTWEAWDRAIAEYAARIEALIRAAQEQQEKNEAALREL-NH_2

17, K_i < 0.2 nM

Ac-β³TTWEβ³AWDβ³RAIAβ³EYAβ³ARIEβ³ALIβ³RAAQβ³EQQβ³EKNEβ³AALβ³REL-NH_2

18, K_i = 3800 nM

Ac-TTWEAWDRAIAEYAARIEALIβ³RAAQβ³EQQβ³EKNEβ³AALβ³REL-NH_2

19, K_i < 0.2 nM

Ac-β³TTWEXWDZAIAβ³EYAXRIEXLIZAAQβ³EQQβ³EKNEXALZEL-NH_2

20, K_i = 9 nM

Figure 11. Sequences of pepetides tested against gp41-5 protein and their affinities to this protein (X = (1*S*,2*S*)-*β*-ACPC, Z = (3*S*,4*R*)-*β*-APC). *β*-Residues are marked in red.

the backbone more rigid. With this approach, *β*-mutations could be extended back to the N-terminus (peptide **20**), and the affinity (K_i) could be maintained in the low nanomolar range.

The same basic principle was followed when the helix conformation was successfully stabilized using dense complementary charge patterns along the helix surface.[55] The best sequences blocked HIV-1 infectivity with IC_{50} values comparable to those of the parent *α*-helix. In a further study, *β* → *α* reversion was performed in the N-terminal, middle, and C-terminal segments on the original *α*,*β*-analogue to screen the spatial pattern along the helix responsible for tight binding.[95] Substantial improvement of K_i was found for all reversed derivatives, indicating a broad distribution of contacts necessary for tight binding. The latter finding was supported also by the Ala-scan and X-ray results. Altogether, this study underlined the usefulness of the backbone geometry scan carried out using *β*-amino acid building blocks.

Anti-angiogenic *β*-Peptides. The design principles of *β*-sheet folding for natural *α*-peptidic sequences have been thoroughly studied,[96−102] and de novo structures have been reported to form *β*-hairpins,[103,104] three-stranded *β*-sheets,[105−111] and *β*-sandwiches.[112−114] Hairpins proved to be useful protein epitope mimetics with bioactive properties.[115] In a bottom-up approach, it has been shown that homologated amino acids can be utilized to construct short turns[116−119] and that cyclic *α*,*β*-peptides can serve as hairpin models.[120] Non-peptidic template and turn units have been combined with peptides to construct parallel and anti-parallel sheet structures with significant bioactivity.[121,122] Focusing on *β*-amino acid substitutions in two-stranded sheet models with a small hydrophobic core, Horne and co-workers found that these mutations destabilize the sheet structure in general, calling for a special substitution strategy to rescue the secondary structure.[123−125]

The biological relevance of the *β*-amino acid-induced unstructuring effect, however, remained an open question. Therefore, systematic probing of the effects of β^3-amino acid substitutions in water-soluble, biologically active, *β*-sheet-forming peptides was carried out.[126] The 33-mer peptide anginex (compound **21**, Figure 12), a designed *β*-sheet-forming peptide with anti-angiogenic and antitumor activities,[127−131] was chosen as a model system. Anginex forms a *β*-sandwich structure via association into dimers and tetramers at higher concentrations[105,132] as well as upon interaction with *n*-dodecylphosphocholine (DPC) micelles.[133] In the concentration range of in vitro studies, its prevailing conformation is a random coil,[105,134] but the three-stranded *β*-sheet-forming propensity is crucial for its anti-angiogenic activity.[134] It was

H-ANIKLSVQMKLFKRHLKWKIIVKLNDGRELSLDG-NH_2

21 (anginex), IC_{50} = 21.8 μM

H-ANIKLSVβ³QMKLFKRHLKWKIIVβ³KLNDGRβ³ELSLDG-NH_2

22, IC_{50} = 29.8 μM

H-ANIKLSVQβ³MKLFKRHLKWKIIβ³VKLNDGREβ³LSLDG-NH_2

23, IC_{50} = 44.2 μM

H-ANIKLSVQMKLβ³FKRHLKWβ³KIIVKLNDGRELSLβ³D-NH_2

24, IC_{50} = 29.7 μM

Figure 12. Sequences of anginex and its most active analogues containing β^3-amino acid residues. IC_{50} values of inhibition of the proliferation of the bEND.3 mouse microvascular endothelial cell line. *β*-Sheet regions are marked by yellow boxes.

hypothesized that, in the core of anginex, in-registry *α* → *β* substitutions will not fully destroy the *β*-sheet; thus, the biological activity of anginex could be retained. Eight foldameric analogues of anginex were synthesized with β^3-amino acid building blocks in the *β*-sheet region (e.g., peptides **22**−**24**, Figure 12).

The β^3-analogues displayed decreased folding propensities, whereas an interacting partner (DPC) could induce *β*-sheet formation in a very similar way to that of the parent anginex. The position of the in-registry β^3-residues relative to the *β*-sheet core determined the reduction in *β*-sheet content. The *α* → β^3 substitutions in the core region resulted in a greater destructuring effect than those at the edge. Temperature-dependent measurements revealed that the hydrophobic driving forces are scaled down in the *α*,*β*-peptidic analogues. This can be explained by the backbone curvature and angled side chain of the β^3-residues relative to the best plane of the *β*-sheet, which are possibly not fully compatible with the packing requirements of the hydrophobic interactions. Nevertheless, inducibility of the *β*-sheet for the *α*,*β*-peptidic anginex analogues was sufficient to diminish endothelial cell proliferation with IC_{50} values comparable with that of the parent anginex. Moreover, the general tendency for uncontrolled aggregation of the *β*-sheet proteins can be tamed using this approach, and insertion of the homologated residues improves the enzyme resistance and, consequently, the pharmacokinetic properties.

Topologically Complex Protein Mimetics. The regular folds at protein interfaces may be successfully targeted, but protein−protein interactions can be structurally more complex, and only a limited amount of geometrical information is often available for the interacting surface. In this section, we discuss *β*-amino acid-containing sequences where ligands contain more than one secondary structure or recognition element.

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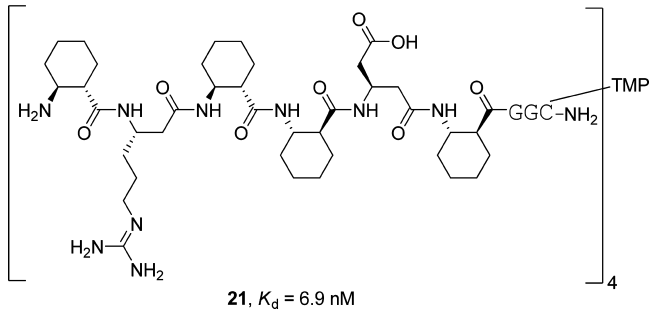
Vascular Endothelial Growth Factor Receptor (VEGFR) Mimetics. The interaction of VEGF with the cell-surface receptor tyrosine kinases VEGFR1 and VEGFR2 plays an important role in angiogenesis,[135] and inhibition of these interactions is effective in treating cancer.[136] The receptor-recognition surfaces on the VEGF homodimer can be targeted by therapeutic proteins (bevacizumab, ranibizumab, and aflibercept), and this system provides a good model to study the effects of β-amino acid substitution for cases in which the interface is not of regular topology. The phage display-optimized peptide v114 showed tight binding to the receptor recognition patch of $VEGF_{9-108}$.[137] A systematic β^3-scan on v114 revealed that the interaction is relatively insensitive to point β^3-mutations.[138] Interestingly, multiple replacements led to loss of affinity depending on the number of β-residues. Conversely, protease resistance improved. Sequences displaying the highest resistance against proteinase K were tested against HUVEC cell proliferation, and modest but still significant potency was observed.

Semisynthetic Interleukin-8 (IL-8) as a Chemokine Ligand. Human IL-8 is a pro-inflammatory chemokine that acts mainly on neutrophil granulocytes. IL-8 contains a central β-sheet motif, which is stabilized by an amphiphilic C-terminal helix through hydrophobic interactions. Although the direct interface is located at the N-terminus, the role of this helix in the biological function of hIL-8 is important but controversial.[139−141] Beck-Sickinger and co-workers tested the feasibility of chimera systems where the C-terminal helices are β-peptidic sequences and the N-terminal part is a recombinant protein.[142] The foldameric helices were attached using thioester ligation. A β-peptide H14 segment designed to have side chain orientations at the hydrophobic face very similar to those of the native sequence was able to stabilize the tertiary structure of the chimeric IL-8 derivative based on CD measurements. The tailored analogue chimera protein resulted in full activation of CXCR1 compared with hIL-8. These seminal results indicated that protein mimetics consisting of large artificial secondary structure elements can be functional.

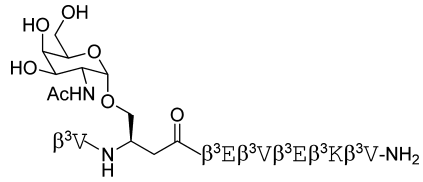
Aβ Oligomer Inhibitors. The presence of the Aβ oligomer species correlates with the severity of Alzheimer's disease (AD).[143−146] Soluble Aβ oligomers may contribute to learning and memory deficits in AD by inhibiting NMDA-receptor-dependent long-term potentiation (LTP), a cellular mechanism of learning and memory.[147−149] Peptides interacting with Aβ[150−156] and structural investigations of these interactions[157] have suggested that the Aβ(16−22) (KLVFFAE) segment offers a potential binding patch over the surface of the Aβ species. This region can participate in hydrophobic interactions in the core and potential salt bridges through the flanking K16 and E22 residues, a hypothesis that was adopted for the de novo design of the foldameric helices recognizing the Aβ(16−22) segment. High-resolution images of the potential interactions of Aβ are not known; therefore, a fragment-based bottom−up design was utilized.[158] To search for the recognition fragments, a small-sized library containing pure β-peptidic 14-, 12-, 10/12-, and 14/16-helices and the α,β-peptidic 9/12-helix type was synthesized and screened for weak binders using NMR. The best hit with micromolar affinity was a 14-helix scaffold with zwitterionic side chain pairs, and the interaction was found to be sequence-specific. To amplify the interaction over the periodic surface of Aβ oligomers, this helix was coupled to a tetravalent G0-PAMAM dendrimer (Figure 13). Low nanomolar binding between the foldamer conjugate and Aβ oligomer was observed in in vitro biophysical and biochemical assays. Ex vivo electrophysiological experiments revealed that the new material rescued the long-term potentiation from the toxic Aβ oligomers in mouse hippocampal slices at a submicromolar concentration. The results from the present study indicate that the bottom-up fragmentation approach is potentially feasible when the exact nature of the targeted protein interaction is not known.



21, K_d = 6.9 nM

Figure 13. Structure of foldamer−dendrimer conjugate **21** and its binding affinity to βA oligomers measured using ITC (TMP, tetramaleimidopropionyl-G0-PAMAM).

Lectin Ligands. Glycosylation is the most abundant post-translational modification of proteins and plays an important role in molecular recognition processes. Arvidsson and co-workers introduced an *O*-glycosyl moiety to β^3-peptides mainly using N-acetyl-galactosamine as the glycosyl donor.[160] Heptapeptides attaining a 14-helix were functionalized (Figure 14), and the binding to various lectins was tested using surface plasmon resonance.[160] Although the binding affinity did not improve, and selectivity did not appear toward specific lectins for the novel conjugates, indirect evidence supported that the β-peptidic moiety had direct contact with the targeted proteins. Moreover, saturation transfer difference NMR experiments directly confirmed this contact.[161] These results indicate the possibility of modulating carbohydrate−protein interactions using β-peptidic sequences.



26, K_d = 24.2 μM

Figure 14. Glycosylated β-peptide **22** interacting with lectins and its binding affinity to lectins from *Vicia villosa*.

■ RECEPTOR LIGANDS

The rational design of analogues of receptor-binding α-peptides is a challenging task because of the structural and functional prerequisites that a ligand must have to tightly bind and, if desired, activate the target receptor. Moreover, the so-called receptor-bound or biologically active conformation of a ligand (agonist or antagonist) is often unknown, making the design of bioactive analogues even more difficult. Thus, structure−activity relationship (SAR) studies with conformationally restricted analogues are very important not only to identify potent and selective receptor ligands but also to gain deeper insight into the mode of receptor binding and activation by the

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ligand, which is essential to construct a potential pharmacophore. During the last two decades, the application of α,β-peptides in receptor−ligand SAR studies has become increasingly frequent and has been accompanied by the development of various β-amino acid building blocks (Figure 1). The results concerning the β-residue-mediated control of ligand structure and function are highly encouraging; however, the identification of general rules both for the choice of the most suitable β-residues and their positioning is complicated by the fact that the interaction of a ligand with its own receptor is quite unique. Thus, the most common approach for the design of receptor-binding α,β-peptides is a sequence-based one. In general, the β-residue content of receptor-binding α,β-peptides is smaller than that tolerated by cell membrane- and protein interface-targeting peptides, a finding that underlines the more demanding structural features for the formation of a receptor−ligand complex.

Herein, selected receptor−ligand systems have been evaluated to show the suitability of β-residues for the replacement of α-residues in endogenous ligands of the G-protein-coupled receptor (GPCR) and integrin receptor families. The results, spread over almost 25 years, indicate that a very promising but still slowly growing research area has emerged that needs to be further strengthened by the synergistic work of synthetic, pharmaceutical, medicinal, and computational chemists as well as that of structural biologists.

Neuropeptide Y Receptor Ligands. The class A G-protein-coupled Y_n receptor (Y_nR, with n = 1, 2, 4, 5) subtypes are activated by the members of the neuropeptide Y (NPY) family, which include the 36-residue long, C-terminally amidated peptides NPY, peptide YY (PYY), and pancreatic polypeptide (PP).[162] The Y_nRs are interesting medicinal chemistry targets due to their role in the regulation of behavioral homeostasis and food intake.[163] The NMR structure of these ligands in a membrane-mimicking environment features a poorly defined, proline-rich N-terminal region and a well-defined C-terminal amphipathic helix that may extend to the C-terminal end, i.e., for NPY,[164] or until position 32, i.e., for PP due to the presence of a proline residue at position 34.[165] As the receptor-bound conformation of the C-terminal tetrapeptide is likely to be receptor-subtype-dependent, one of the approaches used to develop receptor-subtype-selective ligands has been based on the replacement of the native α-amino-acid residue at position 34 and/or 32 with β-amino-acid residues. For example, α,β-peptide analogues of the C-terminal NPY fragment 25−36 containing (1*R*,2*R*,3*R*)-β-ACC at position 34 or at both positions 32 and 34 have been found to be Y_1R-selective with low nanomolar affinity, in contrast to the micromolar affinity for Y_2R and Y_5R, whereas no binding data at Y_4R have yet been reported[166] (Figure 15). Unlike the CD spectrum of the non-substituted NPY fragment 25−36 in phosphate buffer at pH 7 with 30% TFE, which is characteristic of an α-helix conformation, the β-ACC-containing analogues show CD spectra that are reminiscent of a 3_{10}-helix CD spectrum, thus reflecting the β-ACC-induced shortening of the α-helical motif and its conversion into a 3_{10}-helix. Interestingly, the use of the enantiomer (1*S*,2*S*,3*S*)-β-ACC has led to complete loss of affinity for any Y_nR, probably due to a nonideal orientation of the substituted cyclopropane ring and/or the neighboring α-residues. Furthermore, the use of the *cis*-configured β-amino-cyclobutane or cyclopentane carboxylic acids, (1*R*,2*S*)-β-CBU or (1*R*,2*S*)-β-ACPC, has led to a change in the receptor-subtype selectivity from Y_1R to Y_4R[167] (Figure 15). The CD spectra of the β-CBU- or β-ACPC-containing Y_4R-selective ligands, which act as partial agonists in the nanomolar range, resemble those of the β-ACC-containing analogues, indicating that these cyclic β-amino acids are efficient α-helix breakers. Moreover, on the basis of the NMR structure of the NPY fragment 25−36 containing (1*R*,2*S*)-β-ACPC at position 34 in a membrane-mimicking environment, it can be deduced that this β-amino acid induces an extended conformation of the C-terminal tetrapeptide motif.

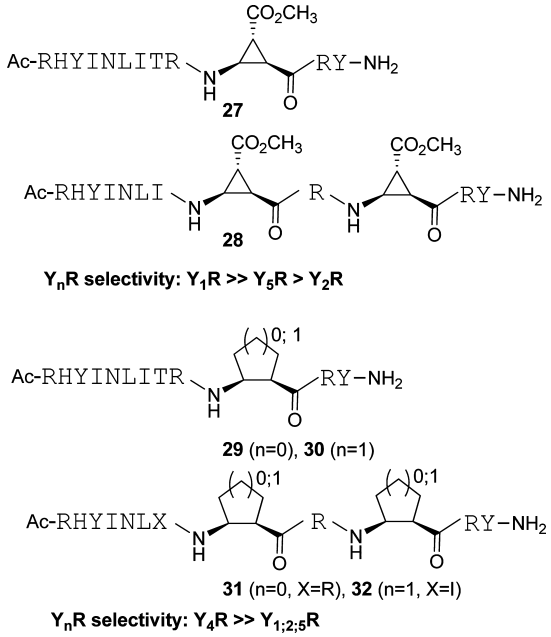

Figure 15. Effect of *cis*-configured β-amino-cyclopropane/butane/pentane carboxylic acids on the Y_nR selectivity of the C-terminal NPY fragment 25−36.[166,167]

Neurotensin Receptor Ligands. The class A G-protein-coupled neurotensin receptors (NTSR1 and NTSR2) are activated by the 13-residue-long peptide NTS and are involved in the regulation of analgesia, food intake, and cancer growth.[168] Because the C-terminal fragment NTS 8−13 is sufficient to efficiently activate the receptor, it has been used as a scaffold for the development of analogues with improved proteolytic stability.[169,170] For example, the analogues containing β^2-hArg at position 8 and/or β^3-hLeu at position 13 (Figure 16) show low nanomolar affinity for the NTSRs and high stability in human plasma in vitro (32 h for the monosubstituted analogue, **33**, with more than 7 days for the disubstituted one, **34**).[169,170] To investigate the in vivo properties of compounds **33** and **34**, the corresponding radiolabeled analogues containing the [^{68}Ga]-DOTA-β-Ala moiety at the N-terminus have been prepared. These radiopeptides are stable in human serum and plasma as well as in murine plasma.[170] In contrary, 10−15 min after injection into HT29 tumor-bearing nude mice, only a polar degradation product is detected in the animal blood. These results, together with a poorly specific biodistribution profile of the radiotracer (kidney > muscle, tumor > liver, lung), suggest that the peptides undergo degradation in the kidneys (and, probably, liver) and not in the blood. Thus, further enhancing both tumor tissue specificity and proteolytic/metabolic stability of the radiopeptides would help to overcome the problems encountered in vivo.

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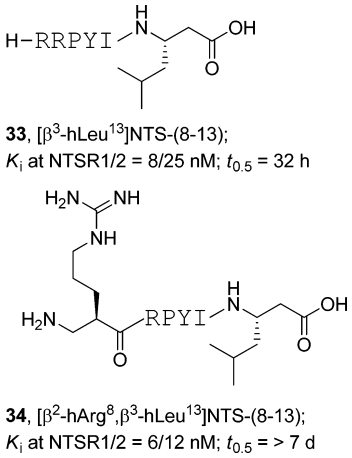

Figure 16. Analogues of the C-terminal neurotensin fragment 8–13 containing β-residues.[169,170]

Somatostatin Receptor Ligands. The class A G-protein-coupled somatostatin receptors (SST_nR, with n = 1–5) are activated by the cyclic tetradecapeptide SST and regulate the secretion of growth hormone, glucagon, insulin, and gastrin.[171] The functionally better characterized receptor subtypes are SST_2R and SST_5R, which are proposed to mediate the antiproliferative effect of SST on tumor growth.[172] An artificial analogue of SST, octreotide,[173] is clinically used for the treatment of pituitary tumors. The biologically active conformation of SST consists of a β-turn encompassing residues 6–9 (FWKT). A series of cyclic and linear β- and α/β-peptides is known that mimics the β-turn of SST (Figure 17).[174−177] Unfortunately, most of these analogues show affinity in the nanomolar range only for the SST_4R, whose function is not yet known. Compounds **39** and **40** have been shown to be stable in the presence of various peptidases (chymotrypsin, trypsin, Pronase, carboxypeptidase A, and proteinase K) for at least 2 days.[176]

GnRH Receptor Ligands. The class A G-protein-coupled gonadotropin-releasing hormone receptor (GnRHR) is activated by the 10-residue-long peptide hormone GnRH, which promotes the production of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). GnRH agonists and antagonists are used in the treatment of several sex hormone-dependent disorders, including prostate cancer and endometriosis, as well as in assisted reproduction.[178,179] The active conformation of GnRH is proposed to contain a reverse turn around Gly-6.[180] Among the different chemical constraints tested, one consists of the introduction of bicyclic *cis*-pentacin Bic(OH) in the place of Gly-6.[181] The NMR structure of the Bic-containing GnRH analogue in water confirms the presence of a Bic-induced open β-turn motif involving residues 5–8 (Figure 18). Importantly, the presence of Bic does not compromise the agonistic potency of the hormone (pIC_{50} of 7.7 for the Bic-containing analogue vs 8 for parental GnRH); however, the Bic-containing analogue is 100-fold less potent than buserelin that contains (*R*)-Ser(tBu) at position 6 and the substitution of the C-terminal glycylamide with ethylamide.

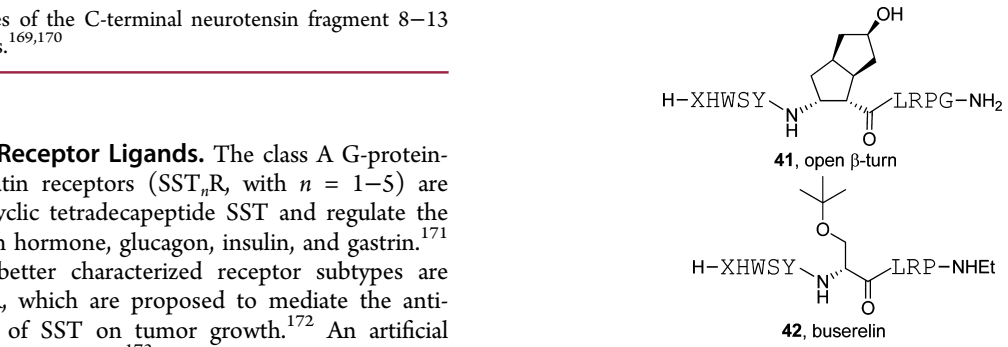

Figure 18. Induction of an open β-turn over residues 5–8 by replacement of Gly-6 with Bic(OH) in GnRH (X = pyroglutamic acid).[181]

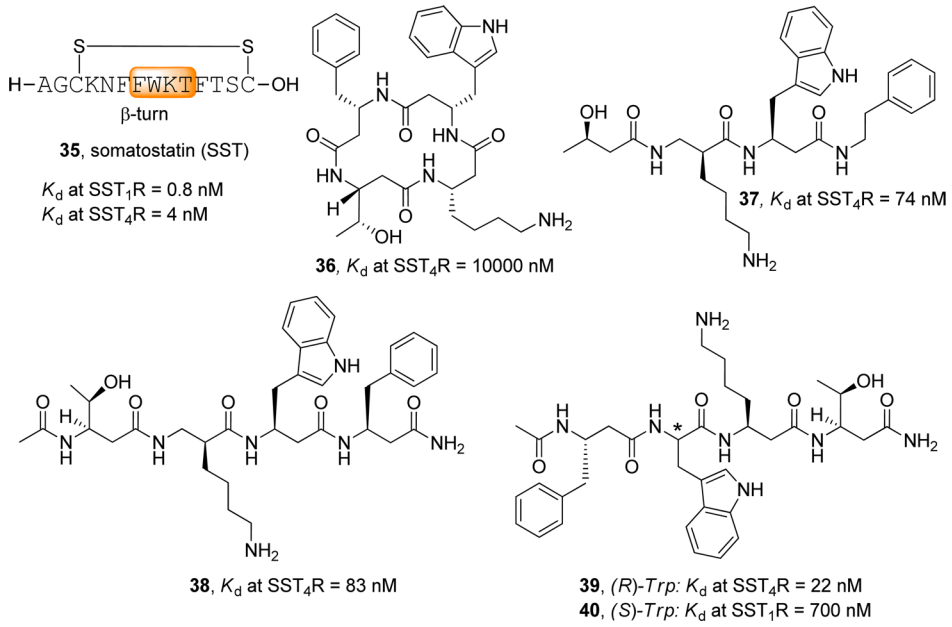

Figure 17. Cyclic and linear analogues of somatostatin (SST) containing β-residues.[176,177]

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Angiotensin Receptor Ligands. The class A G-protein-coupled angiotensin receptors (AT_nR, with n = 1, 2) are activated by angiotensin II (AT-II: H-DRVYIHPF-OH) and promote vasoconstriction (AT_1R) or vasodilatation (AT_2R) of blood vessels. These and related pathophysiological effects make the AT_nRs promising medicinal chemistry targets for the treatment of cardiovascular diseases.[182] Although AT-II binds both receptors with low nanomolar affinity (IC_{50} of 3.3/0.7 nM for AT_1R/AT_2R[183]), its action is primarily mediated by AT_1R. Although the receptor-bound structure of AT-II is not available, its NMR solution conformation in water,[184] as well as the solid structure of AT-II bound to a monoclonal antibody,[185] has been solved. In addition, NMR structures of AT-II constrained by a disulfide bond between positions 3 and 5 are also known.[186] All structures share a common U-shaped form of the central fragment 4−7 that is likely to be the biologically active conformation at AT_1R. However, the conformation of the ligand upon binding to AT_2R seems to be different, as suggested by the different binding behavior of a series of AT-II analogues obtained by a β^3-residue scan at all positions except position 6; indeed, this scan has revealed that the β^3-residue is tolerated in none of the tested positions with the exception of position 1 for AT_1R binding (IC_{50} between 130 nM and >1 μM), whereas it is tolerated for AT_2R binding (IC_{50} between 0.2 and 11 nM).[183] Unfortunately, no conformational data for these AT_2R-preferring ligands are available, a finding that would be helpful to provide insight into the most probable structure adopted by AT-II upon AT_2R binding.

With regard to proteolytic stability, the AT-II analogues show different stability in rat plasma, depending on the position of the β^3-residue; in particular, [β^3-hIle^5]-AT-II with a $t_{0.5}$ of 295 min was found to be much more stable than [β^3-hTyr^4]-AT-II ($t_{0.5}$ = 65 min), which is, in turn, more stable than AT-II ($t_{0.5}$ = 28 min).[183] The major degradation products of [β^3-hIle^5]-AT-II and [β^3-hTyr^4]-AT-II are the two shortened sequences lacking the N-terminal residue or both the N-terminal and C-terminal residues. Again, the degradation products containing β^3-hIle^5 are more stable than those containing β^3-hTyr^4.

Bombesin Receptor Subtype 3 Ligands. The class A G-protein-coupled bombesin receptor subtype 3 (BRS-3) is an orphan receptor that is suggested to play a role in energy balance and maintenance of blood pressure.[187] Whereas the 14-residue-long peptide amide bombesin (Bn) does not bind this receptor, its C-terminal fragment containing four substitutions, including β-Ala at position 11, [(*R*)-Tyr^6,β-Ala^{11},Phe^{13},Nle^{14}]-Bn-(6−14), shows subnanomolar affinity at BRS-3. However, Bn also binds two other bombesin receptor subtypes, namely, the gastrin-releasing peptide and neuromedin B receptors.[188] Interestingly, the replacement of β-Ala with (*R*)- or (*S*)-3-amino-3-phenyl-propionic acid (Apa) induces high BRS-3 selectivity over the other receptor subtypes. Molecular modeling of the BRS-3 ligands shows that the β^3-residue could mimic a β-bend conformation.[188]

Opioid Receptor Ligands. The class A G-protein-coupled opioid receptors (δ, κ, μ) mediate the analgesic action of opioid peptides.[189] Therefore, these receptors are very important medicinal chemistry targets for the development of painkillers.[190] Within the last two decades, much effort has been made for the design, synthesis, and characterization of analogues of endogenous opioid peptides with high receptor affinity and proteolytic stability. A consistent number of opioid-peptide analogues is represented by α,β-peptides containing one or two β-residues.[177,191−205] Selected examples are reported in the following paragraphs.

Morphiceptin is a tetrapeptide amide (H-YPFP-NH_2) with submicromolar potency at the μ-opioid receptor.[206] Analogues containing (1*S*,2*R*)-β-ACPC in the place of Pro-2 have been reported that show better activity than morphiceptin at the μ-opioid receptor.[203] By contrast, the incorporation of enantiomer (1*R*,2*S*)-β-ACPC leads to inactive analogues. This effect has been attributed to the unfavorable spatial arrangement of the two aromatic side chains; indeed, the distance between the Tyr and Phe side chains is 5−7 Å in the inactive analogues and 10−13 Å in the active ones. Additionally, regarding endomorphin-1 (H-YPWF-NH_2) and endomorphin-2 (H-YPFF-NH_2),[207] the introduction of (1*S*,2*R*)-β-ACPC or (1*S*,2*R*)-ACHC in the place of Pro-2 is well-tolerated at the μ-opioid receptor, whereas the introduction of the corresponding enantiomers leads to inactive compounds.[196,201] Structural studies on the active analogues in solution show their propensity to adopt turn conformations, suggesting that a compact rather than extended conformation is likely to be the biologically active one.[197,200] Furthermore, a β^3-residue scan of endomorphin-1 has shown that the introduction of (*S*)-hPro (but not (*R*)-hPro) in the place of Pro-2 is the best tolerated modification, with only a moderate loss of affinity and potency at the μ-opioid receptor.[205] This observation holds true also for endomorphin-2, for which it has been shown that not only (*S*)-hPro but also (*S*)-hPrs and (*S*)-βPrs are suitable surrogates of Pro-2.[198] In contrast to endomorphin-2, morphiceptin tolerates the (*R*)-enantiomer of hPro.[198] Interestingly, in the case of *cis*-β-ACPC, the (1*S*,2*R*)-enantiomer is tolerated, as mentioned above.[203] With respect to enzymatic resistance, the endomorphin-1/-2 analogues containing (1*S*,2*R*)-β-ACPC or (1*S*,2*R*)-ACHC in place of Pro-2 show remarkably long half-lives (>12 h in rat brain homogenate).[201] Also the presence of hPro leads to enhanced proteolytic stability; indeed, when [(*S*)-hPro^2]-endomorphin-1 was exposed to α-chymotrypsin (cleaving Trp-Phe) or aminopeptidase M (cleaving Pro-Trp), approximately 90% was still found to be intact after 3 h.[205]

The use of β-residues in the design of endomorphin-1 analogues has been further extended to the C-terminal residue Phe-4; for example, the α-methylene-β-aminopropanoic residue bearing the 2-furyl group at C-3 (Map) in the place of Phe-4 has led to an endomorphin-1 analogue with subnanomolar affinity at the μ-opioid receptor (K_i = 0.22 nM) and an half-life of about 86 min in mouse brain membrane homogenate, which is five times longer than that of endomorphin-1 ($t_{0.5}$ about 17 min).[195] Moreover, subnanomolar to picomolar affinity has been shown by analogues containing β-residues both at positions 2 and 4. For example, endomorphin-1 analogues containing (*R*)- or (*S*)-βPro-2 and Map-4 show subnanomolar affinities, with (*R*)-βPro-2 being preferred (compound **44**, Figure 19).[193] Moreover, the proteolytic stability of **44** in mouse brain membrane homogenate is much higher than that of endomorphin-1 ($t_{0.5}$ > 600 min against 17 min).[193]

Analogues of endomorphin-2 containing two β-residues at positions 3 and 4 have been recently reported; interestingly, (*R*)/(*S*)-β^2-hPhe and (*S*)-β^3-hPhe at position 3 seem to be favorable for the interaction with the δ- and μ-opioid receptors, respectively. For example, the two α,β-peptide analogues H-Tyr-Pro-(*R*)-β^2-hPhe-(*S*)-β^3-hPhe-NH_2 and H-Tyr-Pro-(*S*)-β^2-hPhe-(*S*)-hTic-NH_2 show δ-opioid receptor preference with an affinity of approximately 50 nM, whereas H-Tyr-Pro-(*S*)-β^3-hPhe-(*S*)-β^3-hPhe-NH_2 and H-Tyr-Pro-(*S*)-β^3-hPhe-(*S*)-hTic-

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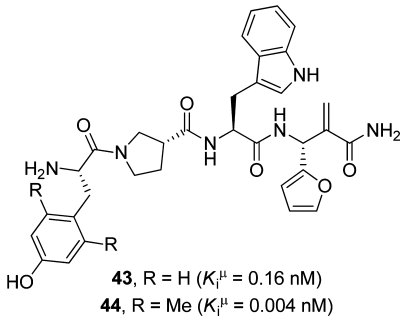

Figure 19. Analogues of endomorphin-1 containing β-residues at positions 2 and 4.[193]

NH_2 selectively bind the μ-opioid receptor with an affinity of about 20 nM.[191,202]

β-Peptide homologues of deltorphin I (H-YAFDVVG-NH_2), Leu-enkephalin (H-YGGFL-NH_2), and dermorphin (H-YAF-GYPS-NH_2) have been reported;[199] whereas the β-peptide homologues of Leu-enkephalin and dermorphin are not active, the β-peptide homologue of deltorphin I displays weak binding to the δ-opioid receptor (IC_{50} 640 nM). β-Residue monosubstitution is, however, better tolerated; for example, the deltorphin I analogue containing (*R*)-β^3-hAla in the place of Ala-2 shows nanomolar affinity at the δ-opioid receptor (IC_{50} 12 nM).[199]

GLP-1 Receptor Ligands. The class B G-protein-coupled glucagon-like peptide-1 receptor (GLP-1R) is activated by the 30-residue-long peptide hormone GLP-1 or its naturally occurring analogue exendin-4 from *Helodermantidae* venom and stimulates the secretion of insulin in a glucose-dependent manner.[159,208,209] Thus, GLP-1R plays a relevant role in the development of drugs for the treatment of diabetes mellitus type 2.[210] The crystal structure of GLP-1 bound to the extracellular domain of its receptor consists of an α-helix over residues 7−27 with a kink at position 16.[211] By contrast, the remaining N-terminal tail appears to be flexible. However, it is assumed that the conformation of this region may be similar to that found in the NMR structure of the 21-residue-long pituitary adenylate cyclase-activating polypeptide PACAP bound to its receptor, which is characterized by an extended N-terminal tripeptide followed by two consecutive type II′ and type I β-turns over residues 3−7.[212] The N-terminal region of GLP-1/exendin-4 is essential for receptor activation, whereas the well-defined C-terminal α-helix is likely to have a role in receptor binding. Therefore, an analogue of GLP-1/exendin-4 has been developed that contains the receptor-activating residues 1−9 connected via a PEG spacer with a 14-helix consisting of 10 β^3-residues (Figure 20). One face of the 14-helix reproduces the side chain triad AFW that is important for the peptide interaction with the receptor. Although this α,β-peptide construct is 10^6-fold less active than the endogenous ligand (micromolar vs picomolar activity), it should be considered that the native α-helix has been fully replaced by an artificial 14-helix.

Calcitonin Gene-Related Peptide Receptor Ligands. The class B G-protein-coupled calcitonin gene-related peptide receptor complex (CGRPR) is activated by the 37-residue-long peptide amide CGRP and induces vasodilatation.[214] A role of this peptide and its receptor has been proposed in the manifestation of migraine headache, making the development of CGRP antagonists of great interest.[215] The artificial antagonist [D^{31},P^{34},F^{35}]-CGRP-(27−37), which binds the receptor complex with subnanomolar affinity, adopts two β-turns around the two proline residues at positions 29 and 34 in its solution NMR conformation.[216] However, the replacement of proline with (1*R*,2*R*,3*R*)-β-ACC is tolerated only at position 29, suggesting that the two turns must be different in the biologically active conformation of the antagonist.[217]

Parathyroid Hormone 1 Receptor Ligands. The class B G-protein-coupled parathyroid hormone 1 receptor (PTH1-R) is activated by the 84-residue-long PTH and its related protein (PTHrP), it regulates calcium homeostasis and plays a key role in bone diseases, including osteoporosis.[218] Indeed, the N-terminal PTH fragment 1−34 is already being used as a drug to increase bone mineral density and strength.[219] Several NMR investigations have suggested that the biologically active conformation of PTH 1−34 consists of an N-terminal and C-

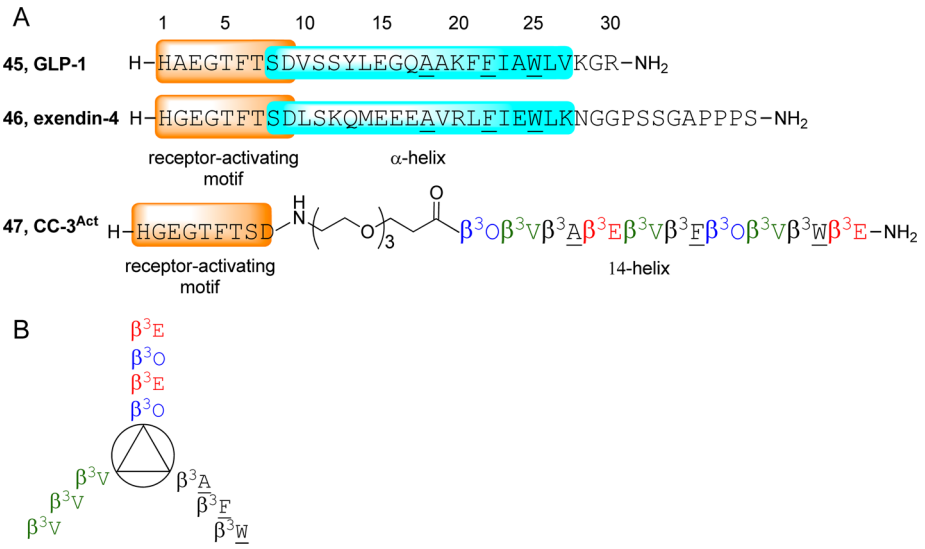

Figure 20. Replacement of the entire α-helix of GLP-1/exendin-4 by a β^3-residue-based 14-helix (β^3O = β^3-hOrn). The helical part of CC-3^{Act} peptide is also shown as a helical wheel diagram. The key residues for the receptor−helix interaction are underlined. Positively charged, negatively charged, and hydrophobic residues, which stabilize the helix structure, are marked in blue, red and green, respectively.[213]

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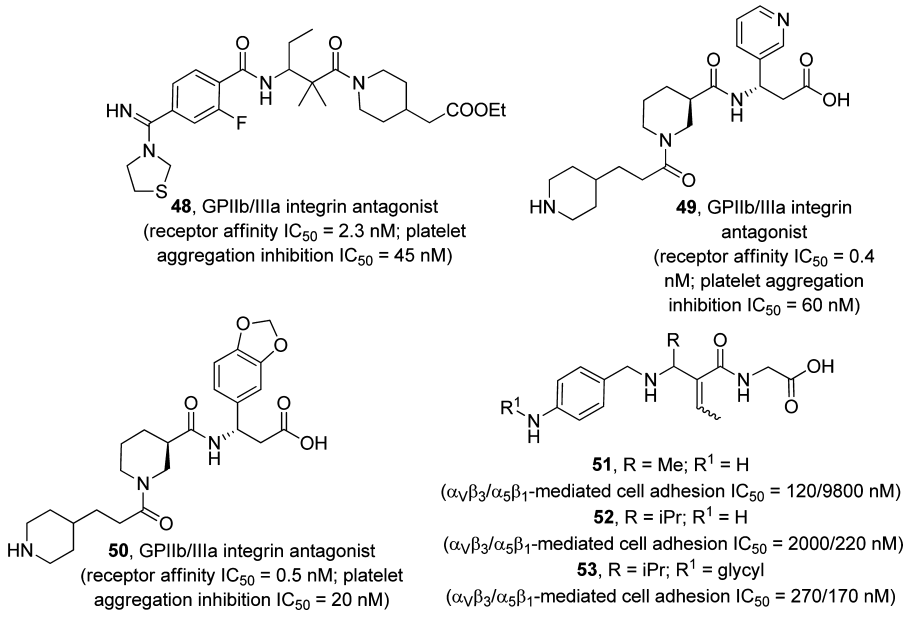

Figure 21. Linear RGD peptide mimetics containing β-residues.[224−226]

terminal α-helix with two hinges around positions 12 and 19. A series of analogues containing multiple amino-acid substitutions, including one or two β-residues in the region 11−13, shows that the agonistic activity is maintained with the following β-residue-based motifs: β-Ala at positions 12 and/or 13 and β^3-hLeu at position 11.[220] By contrast, β-Ala at position 11 is not tolerated. The analogues containing only one β-Ala show a better defined N-terminal helix than the analogue containing the β-Ala dyad. Moreover, the β-Ala dyad prevents the helix propagation beyond this residue, whereas the presence of one β-Ala does not seem to be sufficient to interrupt the helix elongation completely. Instead, the presence of β-Ala or β^3-hLeu at position 11 followed by the native glycine-12 seems to interrupt the N-terminal helix at glycine-12, implying that the β-residue is included in the helical motif.[220]

Integrin Receptor Ligands. Integrins are cell surface receptors for proteins of the extracellular matrix and plasma-borne adhesive proteins. Two important and well-investigated medicinal chemistry targets are the fibrinogen-specific GPIIb/IIIa integrin, which mediates platelet aggregation and is a target for anti-thrombotic agents,[221] and the vitronectin-specific $\alpha_V\beta_3$ integrin, which is involved in angiogenesis and is a target for anti-angiogenic, anti-restenotic, and anti-metastatic drugs.[222] Most integrin antagonists are based on peptide mimetics of the RGD motif. β-Residues have been used to replace Gly and/or Asp in linear RGD mimics (reviewed in refs 223 and 224). Some of them are shown in Figure 21. The oral bioavailability of the prodrug **48** has been investigated in guinea pigs:[225] after intraveneous administration, a biexponential decrease of its plasma concentration is observed, resulting in a half-life of about 110 min. However, the onset of the compound after oral administration (10 mg/kg) is very fast and reaches the maximum of anti-platelet activity already 30 min after administration.[225] The pharmacology of **49** and **50** has been evaluated in dogs with ex vivo experiments of platelet aggregation inhibition:[226] the half-lives in plasma after intraveneous/oral administration are about 85/114 min for **49** and 21/108 min for **50**. Instead, the duration of action after oral administration (3 mg/kg) is 360 min for **49** and 180 for **50**.[226]

Besides linear antagonists of the RGD motif, there are cyclic peptides that are mainly based on the $\alpha_V\beta_3$-selective ligand cyclo-(RGDfV).[227,228] β-Residues have been used to replace the (*R*)-configured residue; for example, the two enantiomers of β-ACC have led to two α,β-cyclic pentapeptides that inhibit the adhesion of tumor cells containing the $\alpha_V\beta_3$ receptor (WM115) and $\alpha_5\beta_1$ receptor (K562) in the nanomolar and micromolar ranges, respectively.[229] However, the (1*R*,2*R*,3*R*)-β-ACC-containing cyclic analogue shows higher potency than the (1*S*,2*S*,3*S*)-β-ACC-containing cyclic analogue at the $\alpha_V\beta_3$ receptor (20 nM vs 600 nM); this characteristic is likely to reflect the different conformation adopted by the cyclopeptides. Indeed, (1*R*,2*R*,3*R*)-β-ACC induces a pseudo-β-turn with the β-residue at position i + 1 and a γ-turn centered at Gly. Instead, (1*S*,2*S*,3*S*)-β-ACC induces a type III β-turn with Val at position i + 1 and an inverse-γ-turn centered at Asp.

SUMMARY AND OUTLOOK

β-Amino acid patterns as constituents in peptides to arrive at biologically active ligands have evolved as a promising tool in medicinal chemistry. Although single β-amino acid replacements in α-peptides have long been precedents both in nature and peptide mimetic designs, considering α,β- or β-peptides as substitutes for native α-peptide sequences has not been an obvious strategy to develop ligands with improved properties that can communicate with naturally occurring biomolecules. Moving from acyclic, proteinogenic α-amino acids to their β-homologues suggests that additional conformational flexibility should accompany such building blocks. The seminal work by Seebach and co-workers,[11] proving that the additional methylene group present in a β-amino acid actually reduces its conformational freedom, changed this perception, setting the stage for the development of oligomers consisting solely of acyclic β-amino acids with defined secondary structures. In parallel, cyclic β-amino acids were recognized by Gellman and co-workers[7] to be exceptional building blocks for the construction of oligomers that can fold into well-defined helical structures. The high stability of β-peptides against enzymatic degradation was recognized as a distinctive

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advantage compared with α-peptides; conversely, this property also indicated that interaction with naturally occurring proteins might be difficult. As a compromise, the combination of β-amino acids with proteinogenic α-amino acids has been successfully explored, demonstrating that regular patterns, such as α,β or α,α,β,β, also result in structurally well-defined entities. While biologically active peptides containing such patterns could be designed, the best results so far are obtained for their interactions with membranes, e.g., those resulting in antimicrobial properties. Interactions with receptors, which require more complex recognition patterns, remain challenging; however, during the past few years, several successful developments have shown the potential of peptides containing β-amino acid patterns for medicinal chemistry. Still, despite the plethora of new structures and, particularly, helices that have been discovered in the course of evaluating β-amino acid-containing foldamers, the exact mimicry of naturally occurring, common secondary structures has not been generally accomplished. For example, the 3_{10}-helix and α-helix, being abundant in biomolecules and constructed by α-amino acids, have found only few counterparts in which unnatural amino acids have been incorporated. Though, higher homologues of α-amino acids might very well have the potential to closely simulate such structures, as demonstrated by Balaram and co-workers[230] with the design of β,γ-peptide fragments in combination with α-amino acids or by Gellman and co-workers with peptides entirely consisting of β,γ-amino acid sequences.[231] The introduction of peptide sequence patterns containing higher homologues of α-amino acids clearly holds the promise to rival natural peptides and proteins with respect to functionality, with the added advantage of stability and a rational structure design, and might very well find direct applications for drug development in medicinal chemistry. A sufficient amount of data is now available, which supports the enhanced resistance of α,β-peptides against proteolytic enzymes when compared to α-peptides. However, the proteolytic stability may strongly vary with the position and number of the β-residues within the α,β-backbone. Obviously, the stability will increase with increasing the number of β- over α-residues. Accordingly, β-peptides have been shown to be essentially undestructable by mammalian proteases.[6] Despite very promising in vitro plasma stability data that are rapidly increasing, more sophisticated data on tissue distribution and metabolism are still limited. Nevertheless, the interesting pharmacological properties shown by some α,β-peptides reported above with regard to intraveneous and oral bioavailability certainly foresee a key role for this class of molecules in the expanding ensemble of future drug candidates.

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Notes

The authors declare no competing financial interest.

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Oliver Reiser studied chemistry at the universities of Hamburg, Jerusalem, and Los Angeles (UCLA) and received his Ph.D. in 1989 under the supervision of Professor A. de Meijere (University of Hamburg). After postdoctoral appointments at the IBM Research Center, San Jose, CA, USA (Dr. R. D. Miller) and Harvard University, Cambridge, MA, USA (Professor D. A. Evans), he started his independent career at the Universität Göttingen. In 1996, he moved to the Universität Stuttgart (Associate Professor); since 1997, he has been Professor of Organic Chemistry at Universität Regensburg. His research interests focus on stereoselective synthesis and catalysis toward natural products and analogues.

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ABBREVIATIONS USED

ACC, 2-aminocyclopropanecarboxylic acid; ACPC, 2-amino-cyclopentanecarboxylic acid; ACHC, 2-aminocyclohexanecarboxylic acid; Aib, α-aminoisobutyric acid; Apa, 3-amino-3-phenyl-propionic acid; APC, 4-aminopyrrolidine-3-carboxylic acid; Bic, 2-amino-octahydropentalene-1-carboxylic acid; CBU, 2-aminocyclobutanecarboxylic acid; DPC, *n*-dodecylphosphocholine; hPrs, 2-pyrrolidinemethanesulfonic acid; Map, α-methylene-β-aminopropanoic acid; MIC, minimal inhibitory concentration; Tat, trans-activator of transcription; Tic, 1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid; TM, trans-membrane

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