ACCOUNTS
of chemical research

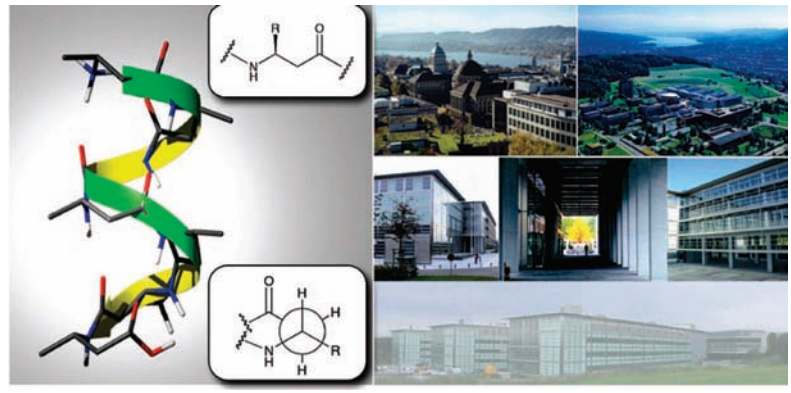
β-Peptidic Peptidomimetics

DIETER SEEBACH* AND JAMES GARDINER

Laboratorium für Organische Chemie, Departement Chemie und Angewandte Biowissenschaften, ETH Zürich, Wolfgang-Pauli-Strasse 10, CH-8093 Zürich, Switzerland

RECEIVED ON NOVEMBER 30, 2007

CONSPECTUS



For more than a decade now, a search for answers to the following two questions has taken us on a new and exciting journey into the world of β- and γ-peptides: What happens if the oxygen atoms in a 3_1-helix of a polymeric chain composed of (*R*)-3-hydroxybutanoic acid are replaced by NH units? What happens if one or two CH_2 groups are introduced into each amino acid building block in the chain of a peptide or protein, thereby providing homologues of the proteinogenic α-amino acids? Our journey has repeatedly thrown up surprises, continually expanding the potential of these classes of compound and deepening our understanding of the structures, properties, and multifaceted functions of the natural "models" to which they are related. β-Peptides differ from their natural counterparts, the α-peptides, by having CH_2 groups inserted into every amino acid residue, either between the C=O groups and the α-carbon atoms (β^3) or between the α-carbon and nitrogen atoms (β^2). The synthesis of these homologated proteinogenic amino acids and their assembly into β-peptides can be performed using known methods. Despite the increased number of possible conformers, the β-peptides form secondary structures (helices, turns, sheets) even when the chain lengths are as short as four residues. Furthermore, they are stable toward degrading and metabolizing enzymes in living organisms. Linear, helical, and hairpin-type structures of β-peptides can now be designed in such a way that they resemble the characteristic and activity-related structural features ("epitopes") of corresponding natural peptides or protein sections. This Account presents examples of β-peptidic compounds binding, as agonists or antagonists (inhibitors), to (i) major histocompatibility complex (MHC) proteins (immune response), (ii) the lipid-transport protein SR-B1 (cholesterol uptake from the small intestine), (iii) the core (1−60) of interleukin-8 (inflammation), (iv) the oncoprotein RDM2, (v) the HIVgp41 fusion protein, (vi) G-protein-coupled somatostatin hsst receptors, (vii) the TNF immune response receptor CD40 (apoptosis), and (viii) DNA. Short-chain β-peptides may be orally bioavailable and excreted from the body of mammals; long-chain β-peptides may require intravenous administration but will have longer half-lives of clearance. It has been said that an interesting field of research distinguishes itself in that the results always throw up new questions; in this sense, the structural and biological investigation of β-peptides has been a gold mine. We expect that these peptidic peptidomimetics will play an increasing role in biomedical research and drug development in the near future.

Published on the Web 06/26/2008 www.pubs.acs.org/acr
10.1021/ar700263g CCC: $40.75

1. Introduction

It is not the mountains that we conquer, but ourselves.

Sir Edmund Hillary (1919−2008)

β-Peptides consisting of homologated proteinogenic amino acids were first prepared and investigated in the mid-1990s.[1] Within less than ten years, they have evolved as a totally new class of unnatural peptidic oligomers with most surprising chemical and biological properties (Figure 1).[2]

1.1. Preparation of β-Amino Acids and Synthesis of β-Peptides. Prior to a discussion of the properties of β-peptides, a brief outline of their synthesis is appropriate. For quick access to the 21 homologated proteinogenic amino acids Fmoc-βhXaa(PG)-OH, with *N*-Fmoc protection and acid labile protection (PG) of functionalized side chains for solid phase peptide synthesis, a single, generally applicable method for each type of β-amino acid (Figure 1) was chosen by us. The β^3hXaa-type of β-amino acid was obtained by Arndt−Eistert homologation (Figure 2a);[3] apart from the histidine, cysteine, and selenocysteine derivatives,[4,5] all β^3-building blocks are now commercially available.[6] The $\beta^{2,3}$-amino acids were prepared from the β^3-derivatives by enolate alkylation[7] or from enolate esters by the Davies method (Figure 2b).[8] For the β^2-homoamino acids, the overall enantioselective Mannich reaction or benzyloxycarbonylmethylation/Curtius degradation was applied using the modified Evans auxiliary DIOZ (Figure 2c).[9] There are almost weekly reports in the literature about alternative

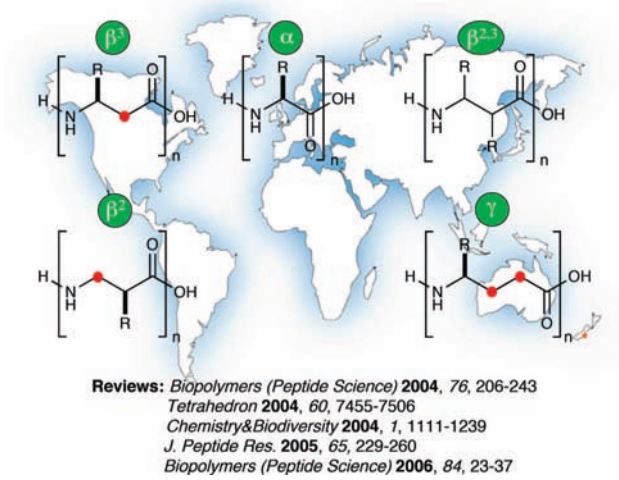


FIGURE 1. Three types of β-amino acids and β-peptides with proteinogenic side chains R: β^3 and β^2, insertion of a CH_2 group between the CO and the α-carbon and between the α-carbon and the nitrogen, respectively; $\beta^{2,3}$, β-amino acid with an additional proteinogenic side chain (for example, Me) in the α-position with like (*R*,*R* or *S*,*S*) or unlike (*R*,*S* or *S*,*R*) configuration. A γ^4 peptide results from insertion of two CH_2 groups into each α-amino acid residue.

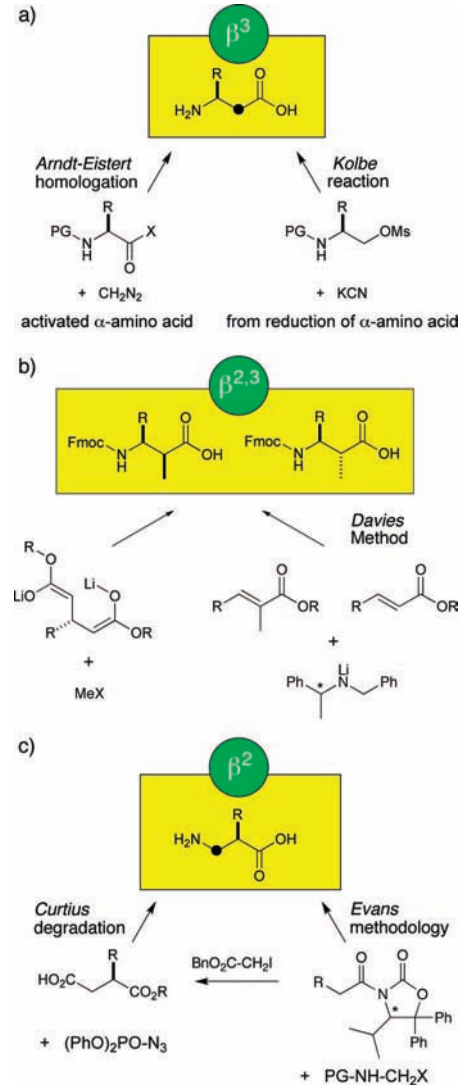


FIGURE 2. Generally applicable methods used by us for the preparation of the various types of β-amino acids with the 21 proteinogenic side chains R: (a) stereospecific classical homologation of α-amino acids (H-Xaa-OH) by insertion or addition of a one-carbon moiety to give β^3-amino acids (H-β^3hXaa-OH); (b) α-methylation of a β-amino acid derivative or overall enantioselective Michael addition of NH_2/H or NH_2/CH_3 to an acrylate ester for preparation of $\beta^{2,3}$-amino acids; (c) overall enantioselective aminomethylation of a "des-amino" acid, directly or through a succinic acid half-ester, to provide β^2-amino acids (H-β^2hXaa-OH).

methods for preparing β^2-amino acids, none of which has so far produced the 21 derivatives with proteinogenic side chains (with acid-labile protection of the functionalities), an F-moc group, and free carboxylic acid group, as required for solid-phase synthesis. A specialized review covering the literature up to 2004 has been published.[10]

The manual or machine coupling of the β-amino acid derivatives by the Fmoc-strategy on various resins had to be adapted for β-peptide synthesis but was otherwise straightfor-

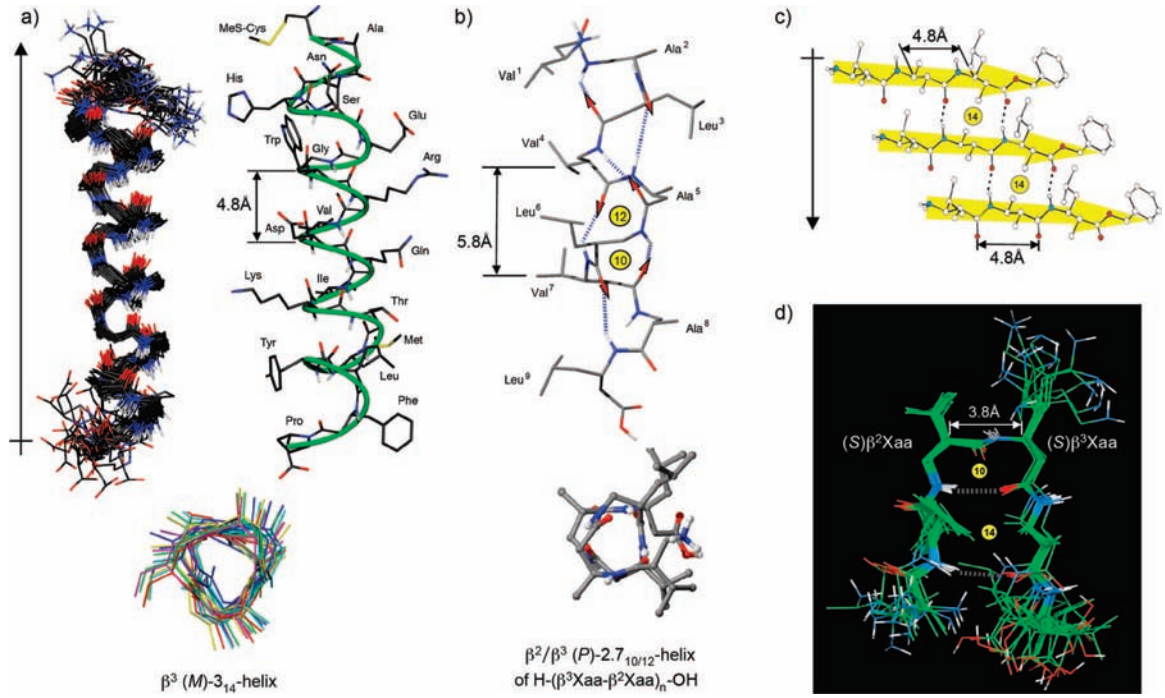


FIGURE 3. Helices, pleated sheets, and turns of β-peptides: (a) The 3_{14}-helix built of β^3hXaa (or β^2hXaa) residues with the side chains in almost parallel juxtaposition on the surface of the helix in *i* and (*i* + 3) positions at a distance of ca. 4.8 Å. The helicity is *M* and the helix has a macrodipole from C- to N-terminus (for comparison, the "α-helix" of R-$(Xaa)_n$-Y has *i* and (*i* + 4) side chains at a distance of 6.3 Å projecting at an angle of 40°, the helicity is *P*, and the macrodipole points from N- to C-terminus). (b) The (*P*)-β^3/β^2-12/10-helix without a macrodipole, with two different hydrogen-bonded rings, and with a more and a less "crowded" surface area. (c) The β-peptidic parallel pleated sheet. All substituents in the 3-position of the amino acid residues point in the same direction and incidentally are the same distance apart (4.8 Å) as the *i* and *i* + 3 side chains of the 3_{14}-helix (for comparison, in α-peptidic sheet structures, substituents alternately point in opposite directions with equidirectional distances of ca. 6.5 Å). (d) The β-peptidic turn (10-membered H-bonded ring, compare the 12/10-helix in panel b) with attached antiparallel sheet structure (compare panel c) form a hairpin turn (see also section 2.3). Figure reproduced from refs 2 and 31 by permission of *Verlag Helvetica Chimica Acta*.

ward;[2] in some cases, dimer-fragment coupling, rather than single amino acid coupling, turned out to be advantageous.[11]

1.2. Structural and Biological Properties of β-Peptides. β-Peptides fold to helices or hairpin-type structures, and they can be constructed such that they do not fold but are linear or assemble to pleated sheets (Figure 3).[1,2,12−15] In contrast to their natural α-peptidic counterparts, β-peptides form such secondary structures in protic solutions (MeOH, H_2O) with chain lengths as short as four residues and without restricted backbone rotation, as in oligomers containing 2-amino-cyclopentane- and -cyclohexane-carboxylic acid moieties.[16,17]

Furthermore, there are intriguing inherent differences between the helical, linear, sheet, and hairpin structures of the natural peptides and their β-peptidic analogs (Figure 3): (i) due to more constitutional (β^3, β^2, $\beta^{2,3}$) and configurational ((*R*), (*S*), like, unlike) variety of the building blocks there are more different secondary structures; (ii) the screw sense (*P* or *M*) of the helix from L-α-amino acids ((*P*)-3.6_{13}) and that from L-β^3-homoamino acids ((*M*)-3_{14}) is opposite and so is the direction of their macrodipole; (iii) a β-peptidic chain of β^2/β^3-segments folds to a most "unnatural" helix consisting of alternating 10- and 12-membered hydrogen-bonded rings; (iv) each layer of a β-peptidic sheet is polar, with all C═O bonds pointing in the same direction and all N−H bonds in the opposite direction; (v) the $A^{1,3}$-forbidden positions for non-hydrogen substituents in a hairpin turn of a β-peptide are different in the two antiparallel N- and C-terminal segments; (vi) while the geminally disubstituted α-amino-acid residue Aib is helix-inducing and sheet-breaking in the "α-world", $\beta^{2,2}$- and $\beta^{3,3}$-homo-Aib are both 3_{14}-helix- and sheet-breaking in the "β-world".

Besides the structural similarities and dissimilarities alluded to in the previous section, there is perhaps a more dramatic biochemical and biological difference between the α- and the β-peptidic compounds with proteinogenic side chains: it is suggested by the biological investigations carried out so far that β-peptides are stable against proteolytic, other hydrolytic,[18] and metabolizing enzymes in mammals,[19] insects,[20] plants,[20] and, with one exception,[21] even in microorganisms,[22] and only rarely has antibiotic or hemolytic activity been observed.[23]

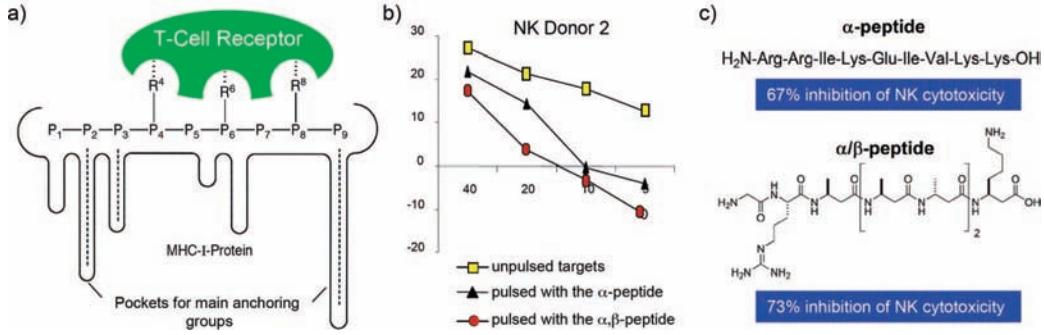


FIGURE 4. Inhibition of NK cytotoxicity by a β-hexapeptide with two terminal α-amino acid residues: (a) schematic picture of an MHC-protein-binding nonapeptide displayed on a cell surface and being "checked" by a T-cell; (b) reduction of NK killing rate in the presence of a natural α-peptide and of an α/β-peptide; (c) formulae and degree of NK inhibition by an α- and a β-peptidic compound. Figure reproduced from ref 2 by permission of *Verlag Helvetica Chimica Acta*.

Thus, living organisms do not seem to interact with the β-peptidic structures: bad news in a search for peptidomimetics!? In contrast, with increasing knowledge and understanding of the secondary structures of β-peptides, we were able to imitate α-peptidic secondary structures and thus mimic their biological functions without having to cope with their hydrolytic and metabolic instability.

2. Mimicking Peptide–Protein and Protein–Protein Interactions (PPIs) with β-Peptides

2.1. Linear Arrangements: Major Histocompatibility Complex (MHC) Protein Binding. Proteins encoded by the major histocompatibility complex bind short peptides and present them on the surface of vertebrate cells,[24–26] where T-cells are checking whether the presented peptide is "self" or "nonself"; when nonself, the corresponding cell is destroyed, for instance, by the natural killer (NK) cells. This mechanism is central to the immune system. The major contribution to the MHC-protein binding enthalpy is provided by peptide side chains near the termini, so-called anchoring groups, which fit in the protein's pockets (Figure 4).[27] The central section of the MHC-protein-binding peptides, a more or less linear conformation, is important for T-cell recognition. A group of MHC-protein-binding peptides, isolated from patients suffering from arthritis (an autoimmune disease), contain C- and N-terminal arginine and lysine side chains as anchoring groups. With a peptide consisting of six C-terminal β-amino acid residues of alternating (*S*)- and (*R*)-configuration (to prevent folding to a 3_{14}-helix and to a turn) and of two N-terminal α-amino acids, the inhibition of NK cytotoxicity of humanized pig cells in human blood was reduced to the same extent as by a natural α-nonapeptide (Figure 4).[28]

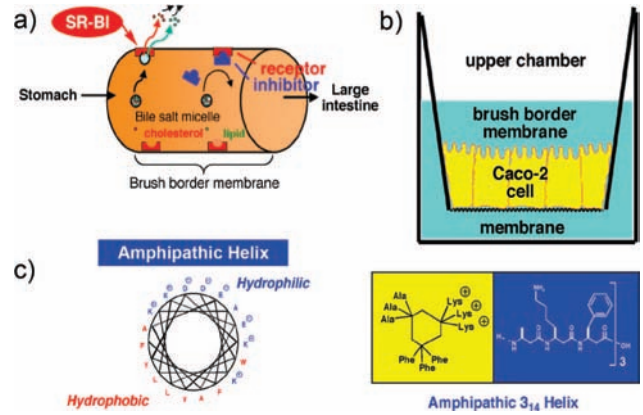


FIGURE 5. Inhibition of a lipid-transport protein by a β-nonapeptide capable of folding to a helical form with amphipathic character: (a) transport protein SR-B1 in the intestine wall; (b) CaCo-2 cell test for permeability through the brush-border membrane; (c) a typical α-peptidic 3.6_{13}-helix of amphipathic character and a simple β-peptidic mimic, reducing cholesterol transport through living CaCo-2 cells, in which the α-peptide is ineffective because of degradation by peptidases. Figure reproduced from ref 2 by permission of *Verlag Helvetica Chimica Acta*.

2.2. Mimicking PPIs Involving Helices. The first demonstration of a β-peptidic mimicry of an α-peptidic helix was the inhibition of the lipid-transport protein SR-B1, which transports, for instance, cholesterol from the small intestine into the lymph and blood system. It is inhibited by peptides and proteins folding to or containing amphipathic helices, which carry polar α-amino acid side chains on one face and nonpolar ones on the other face of their surface (see Figure 5). A β-nonapeptide, which in its 3_{14}-helical form has the hydrophobic β-amino acid side chains of alanine and phenylalanine on one side and the hydrophilic side chains of lysine on the other side, was shown to inhibit cholesterol transport by SR-B1 through the brush-border membrane (CaCo-2 cells).[29] In this case, it looks as if the only common property of the α- and β-peptidic inhibitors needs to be the amphipathic character of their helical forms.

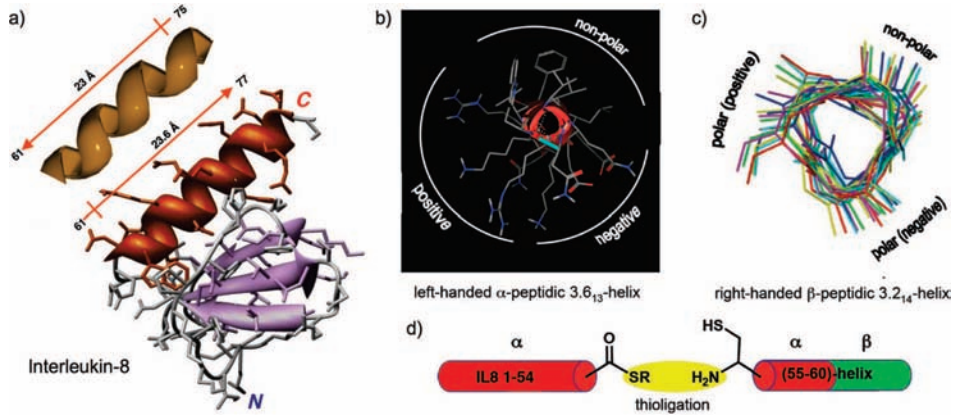


FIGURE 6. Replacing the entire α-peptidic 3.6$_{13}$-helix of the protein interleukin-8 (hIL-8) by a β-peptidic 3$_{14}$-helix with retention of activity: (a) The 16-residue long C-terminal helix section in hIL-8 is amphipathic (with basic and acidic polar side chains grouped on the polar surface). (b) The nonpolar surface of the helix is in contact with the other part of the 77-residue long protein, and its polar surface is exposed to the aqueous medium; replacement by a β^3-peptidic C-terminus leads to a functional chimeric protein. (c) Arrangement of nonpolar and polar side chains on the surface of the β-peptidic helix is shown (a β^3-icosapeptide in MeOH has the side chain distribution shown here[31]). (d) Attachment of the β-peptidic part to the remainder of the protein by thioligation is shown.

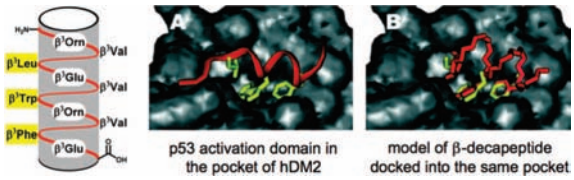


FIGURE 7. Modeled docking of an α- and a β-peptide helix into the pocket of a receptor protein. The β-decapeptide shown in its helical form is amphipathic (cf. Figure 5) and presents its leucine, tryptophan, and phenylalanine side chains in a similar arrangement (A) to the corresponding α-peptide (B).

Along the same lines, the entire C-terminal helical part (61−77) of interleukin-8 (hIL-8) was replaced by a β-peptidic section (61−75) imitating, in its helical conformation, the amphipathic character of the natural helix (Figure 6).[30] Despite the fact that the two helices have the opposite sense of chirality and a reversed direction of the macrodipole, the α/β-chimeric protein could be shown to have an identical efficacy and an only 10-fold reduced affinity (EC_{50}) for the CXCR1 receptor of hIL-8, as compared with the natural protein.

Inspection of α- and β-peptidic helix models shows that the relative positions of adjacent substituents are quite different $(i(i + 4)^{1/2}$ ca. 6 Å at an angle of *ca.* 40° in the 3.6$_{13}$-helix, and $i(i + 3)^{1/2}$ ca. 5 Å more or less exactly parallel in the 3$_{14}$-helix, cf. Figures 3 and 6).[31] Despite this distinct difference, it was possible to mimic with appropriately designed β-peptides the recognition epitopes of helical protein domains in protein−protein interactions, such as that between the activation domain of the human suppressor p53 and the oncoprotein hDM2 (Figure 7).[32,33] Also, an intramolecular interaction between the two protein domains of the HIVgp41 fusion protein, involving the so-called WWI (Trp628, Trp631, Ile635) epitope on a 3.6$_{13}$-helix, can be inhibited by a 3$_{14}$-helical β^3-decapeptide.[34,35] It is remarkable that the β-peptides used in these investigations may be considered amphipathic, with two polar "stripes" and one nonpolar "stripe" on the surface of a 3$_{14}$-helix (cf. Figure 6).

2.3. Imitating α-Peptidic Hairpin Turns and Their Interactions with Proteins. The most accurate and rational mimicking of an α-peptidic secondary structure by β- and also by γ-peptides[36] is possible with so-called "β"-turns. The peptidic dimer sequence −(*S*)-β^{2}hXaa-(*S*)-β^{3}hXaa− and its mirror image −(*R*)-β^{2}hXaa-(*R*)-β^{3}hXaa− favor folding with formation of a 10-membered hydrogen-bonded ring (Figure 8a,[14,37] compare the 12/10-helix in Figure 3). In this secondary-structural motif, the geometry of the (CHR-CO-NH-CHR) unit is superimposable with that of a corresponding α-peptidic so-called β_I-turn with a D-α-amino acid residue (Figure 8b). Therefore, a PPI involving recognition of the two side chains on the turn of one peptide/protein in the pocket of another can be mimicked by a β-peptide (cf. Figure 8c). This has been demonstrated with a large number of open-chain β-di-, β-tri-, and β-tetrapeptides, as well as cyclic β-tetrapeptides, that mimic octreotide (Sandostatin, Figure 9a), an analog of the peptide hormone somatostatin containing a hairpin turn (D-Trp-Lys, see also Figure 8).[37−41] The affinity of the β-tetrapeptide shown in Figure 9b was strikingly specific to one of the five human G-protein-coupled receptors (hsst4), which is present in highest concentration in brain tissue, and which is of unknown function. Note the dramatic difference between the constitutional isomers with (Figure 9b) and without (Figure 9c) the β^2/β^3-turn segment. A cyclic analog is shown in Figure 9d.[41] An open-chain "mixed" β^3,α,β^3,β^3-tetrapeptide was shown in an affinity investigation[40] and by ADME (absorption, distribu-

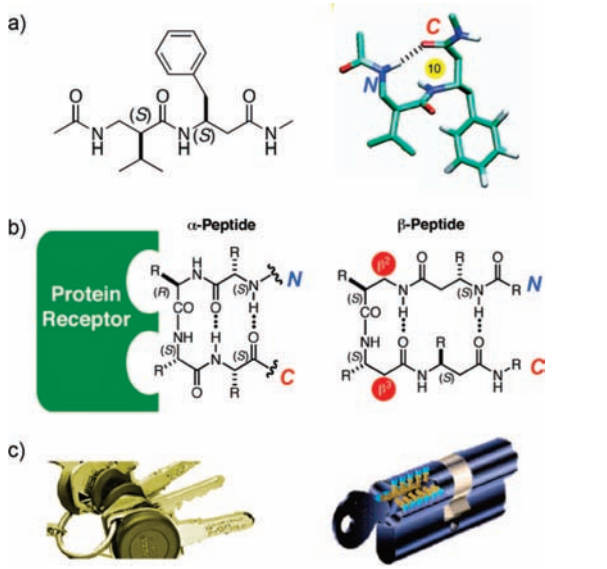


FIGURE 8. Mimicking α-peptidic hairpin turn structures and a turn-related peptide interaction: (a) (*S*,*S*)-β^2/β^3 segment forming a 10-membered hydrogen-bonded ring; (b) structural analogy of an (*R*,*S*)-α,α- and an (*S*,*S*)-β^2/β^3-turn with respect to the spatial arrangement of the attached side chains R; (c) when interaction of those side chains ("bit of a key") with pockets of a receptor protein ("lock") are important, the different structures of the attached antiparallel sheets may merely act as the "handle of the key".

tion, metabolism, excretion) investigation with rats to be a potent agonist of the somatostatin sst_4-receptors, to be orally bioavailable (25% in 15 min), to be metabolically stable, and to be completely excreted within 3 days (*vide infra*, Figure 10).[42] This and other ADME investigations with radioactively labeled β-peptides have uncovered the outstanding proteolytic and metabolic stability of these peptidomimetics, as well as the fact that their distribution in various tissues and organs of rats is highly structure-dependent (Figure 10).[19,42,43]

The successful mimicking of the somatostatin turn structure by short-chain ("small") β- and γ-peptides[36−42] can be considered as just a promising starting point. G-protein-coupled receptors (GPCRs), such as those for somatostatin, are ubiquitous in living organisms, and many are activated by peptides. The title of a recent review article[44] speaks for itself: "Over One Hundred Peptide-Activated G Protein-Coupled Receptors Recognize Ligands with Turn Structures". Note that targeting GPCRs with peptidomimetics does not require cell penetration!

2.4. Cyclic β-Tripeptide Derivatives with Carcinostatic Activity and as Scaffolds for the Mimicking of Protein−Protein Interactions. C_3-Symmetrical cyclic oligomers, such as enterobactin, play important roles in bacterial ion uptake and storage. Compounds of this type bind metal ions with high affinity and selectivity due to the unique structure of their

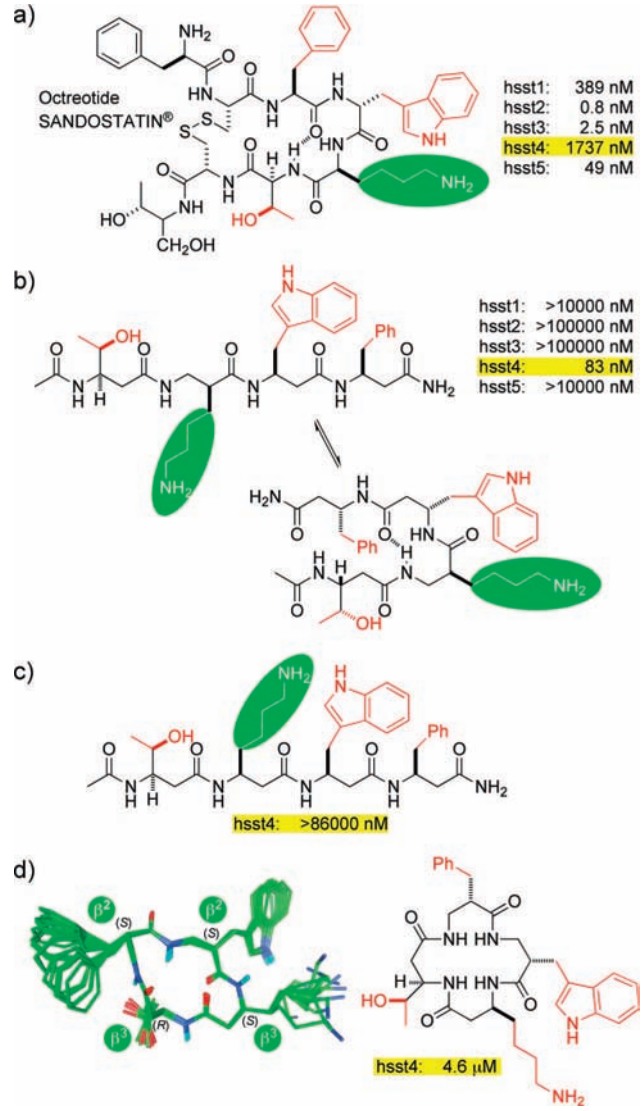


FIGURE 9. Affinities [nM] of various somatostatin-mimicking peptides for the human receptors hsst1−5: (a) Sandostatin, used for treatment of acromegalia and of certain colon cancers, with highest affinity for hsst2 (growth-hormone regulation) and lowest affinity for hsst4 (unknown function); the turn element contains (*R*)-tryptophan, stabilizing a β-turn; (b) a $\beta^3/\beta^2/\beta^3/\beta^3$-tetrapeptide folding to a turn and binding specifically to hsst4 (present in highest concentration in brain tissue); (c) the constitutional isomer consisting of *all* β^3-residues does not bind to hsst4; (d) a cyclic β-tetrapeptide built of two β^2- and two β^3-amino acid residues (NMR-solution structure shown) has moderate affinity for hsst4. Figure reproduced from ref 2 by permission of *Verlag Helvetica Chimica Acta*.

central macrocyclic core where the ester carbonyl groups all point upward and the metal ion is complexed by catachol units below the ring. Cyclic β-peptides resembling the core of enterobactin adopt similar structures, appearing to stack into indefinite tubes in the solid state and possessing a large dipole moment due to the unidirectional alignment of the C=O groups (Figure 11a).[45] Cyclic β-peptides of this type have been

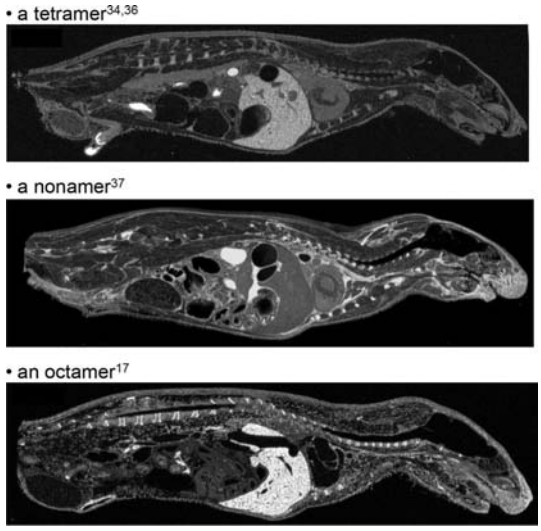


FIGURE 10. Structure-dependent and organ-specific distribution of three different ^{14}C-labeled β-peptides in rats. The white areas indicate the presence of the β-peptide in these autoradioluminograms of sagittal sections (top, kidney, bile, testes; center, kidney, cartilage, lymph nodes; bottom, liver, lymph nodes). The β-peptides are not degraded or metabolized and are more or less slowly excreted. The distribution among different organs shifts with time. Figure reproduced from refs 19 and 42 by permission of *Verlag Helvetica Chimica Acta*.

shown to have carcinostatic and antiproliferative activity against a range of human cancers including leukemia, CNS, renal, non-small-cell lung, ovarian, and breast cancer cell lines.[46]

Such β-peptidic macrocycles have also been used to mimic more complex processes. Multivalent ligand-induced oligomerization is a general and important process in initiating and controlling many cell-surface receptor-mediated biological processes with the activation of the receptors strongly relying on a stoichiometrically defined complex. Cyclic β-tripeptide derivatives have recently been used as key central scaffolds for mimicking the interactions of CD40L,[47,48] a C_3-symmetric *homotrimeric* ligand, with its receptor CD40,[49] an essential immune response glycoprotein and member of the tumor necrosis factor receptor (TNFR) superfamily (Figure 11b,c).

A general feature of such mimics is the cyclo-(β^3-hXaa) core, where the peptide backbone adopts a flat ring conformation with the side chains occupying equatorial positions along the ring's edge and radiating outward. Small, synthetic, C_3-symmetric molecules containing such β-peptidic cores interact significantly with CD40 (K_D = 2.4 nM) and induce high levels of apoptosis in lymphoma and leukemia cells. Ligands with central cores composed of α-amino acids (L and D) were found to be not as effective, and it is suggested that the diameter of the central β-peptidic ring (ca. 6−6.5 Å) is "just right" for presenting the side chains at the correct distance and location to

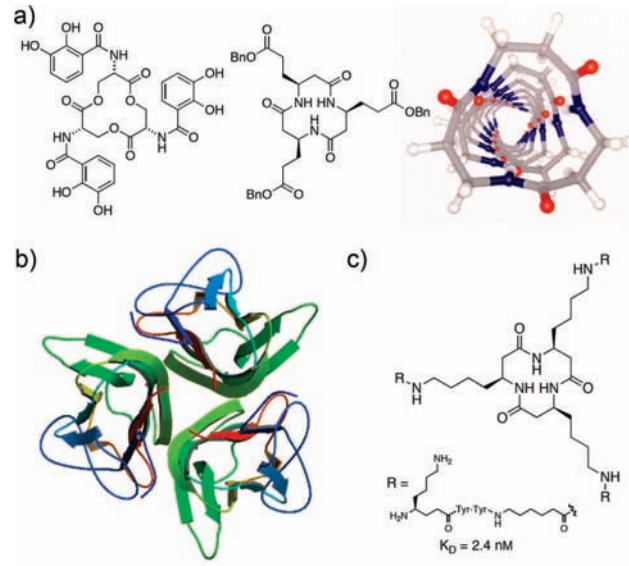


FIGURE 11. (a) Cyclic β-tripeptides, resembling the bacterial Fe chelator enterobactin, adopt indefinite tubes in the solid state due to unidirectional alignment of the C=O bonds and display antiproliferative activity against a range of cancer cell lines: cyclo-(β^3hGlu(OBn))$_3$—CNS (mean 10 μM), renal (mean 8 μM), non-small-cell lung (HOP-92 4 μM), ovarian (SK-OV 6 μM), breast (HS 5787 5 μM). Reproduced from refs 45 and 46 by permission of *Verlag Helvetica Chimica Acta*. (b) The 39 kDa CD40L (1aly in the RCSB Protein Data Bank) binds as a homotrimeric ligand to the TNF (tumor necrosis factor) immune response receptor CD40. (c) Small synthetic C_3-symmetrical ligands, containing a key β-tripeptidic core that allows the side chains to radiate out equatorially from the central scaffold, mimic CD40L binding and induce apoptosis in lymphoma and leukemia cells. Such synthetic ligands have also been shown to inhibit parasitemia in *Trypanosoma cruzi* infected mice.

induce a biological effect. Molecular modeling and X-ray studies indicate that the surface area buried upon complexation of CD40 and CD40L is ca. 850 Å^2, making the ability of such synthetic ligands to mimic the 39 kDa CD40L all the more remarkable. Recently, such molecules have been shown to inhibit parasitemia in *Trypanosoma cruzi* infected mice by binding to CD40 and eliciting an IL-12-mediated immune response.[50]

3. Interactions of β-Peptides with DNA and RNA

In an effort to mimic the binding portion of DNA-duplex-binding enzymes (proteins), which are known to generally fold to helical structures upon binding to the DNA ("induced fit") while being "unstructured" in the absence of DNA, we have prepared β-peptides in such a way that their 3_{14}-helical form would have asparagine and glutamine side chains in the central part and lysine side chains near the termini. This substitution pattern would allow the 3_{14}-helix to form hydrogen bonds with

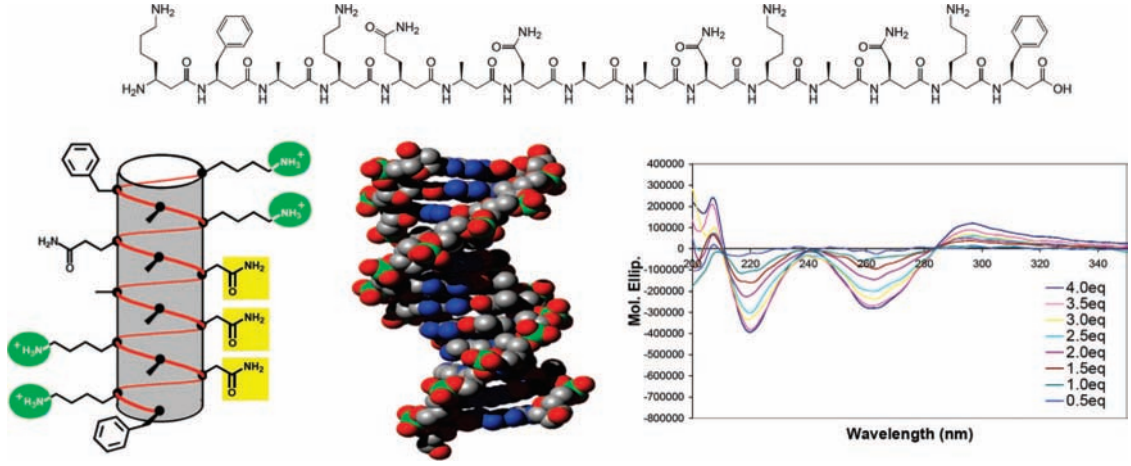


FIGURE 12. A β-pentadecapeptide designed for its helical form to bind to DNA duplexes and "titration" of the interaction with a 20-mer DNA duplex by circular dichroism spectroscopy. No information about the actual structure of the complex is available as yet. Figure reproduced from ref 51 by permission of *Verlag Helvetica Chimica Acta*.

the bases and have charge interactions with the backbone of DNA phosphate anions, just like the natural α-peptidic counterparts. As is evident from the circular dichroism titration curves in Figure 12, an appropriately designed β-pentadecapeptide interacts with the 20-mer DNA duplex containing the ATF/CREB-binding sequence for the protein GCN4 (involved in the regulation of cAMP).[51] A specific nanomolar interaction of a β-undecapeptide mimicking the HIV-tat protein in the binding of TAR-RNA (transcription activator responsive element) has also been reported.[52]

Structurally less well-defined interactions between polycationic β-peptides and DNA have been described as well by us[19,22,53] and by others.[54] The broadly demonstrated but poorly understood cell-penetrating ability of lysine- and arginine-rich peptide sequences allows organisms to carry cargoes through cell walls.[55–57] With simple oligolysines and oligoarginines (>8 residues), unnatural cargoes such as fullerene or magnetic particles can be transported into cells. If not proteolytically degraded, the positively charged peptides go all the way into eukaryotic nuclei and bind specifically to the nucleoli ("exposed" DNA). This result can also be obtained with the proteolytically stable peptides consisting of corresponding D-amino acids or of β-amino acid residues (Figure 13).

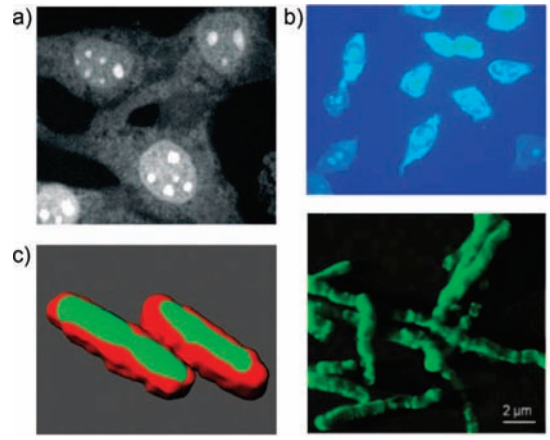


FIGURE 13. Fluorescence microscopy of fluorescein-labeled β-oligoarginines entering eukaryotic (a) mouse cells and (b) HeLa cells and (c) prokaryotic *Bacillus megaterium* cells. The metabolically stable β-oligoarginines bind preferentially to the nucleoli within the mammalian cell nuclei. In the bacterium, the entire interior "shines", compatible with the fact that there is no cell nucleus with the DNA being more or less evenly distributed throughout the cell. Figure reproduced from ref 2 by permission of *Verlag Helvetica Chimica Acta*.

4. Conclusion and Outlook

From the available data, there is no doubt that β-peptides carrying proteinogenic side chains can mimic their progenitors, the α-peptides. Helical mimics will be likely to consist of six or more β-amino acid residues, while turn- and hairpin-mimicking β-peptides might consist of as few as two β-amino acid moieties. As demonstrated with a β-peptidic tetrapeptide, there is a chance that the short-chain β-peptides are orally bioavailable and are excreted within a reasonably short half-life (see section 2.3), a prerequisite considered essential for a drug candidate by most medicinal chemists and clinical researchers. Proteolytic cleavage and metabolic processes do not seem to be an issue with β-peptides. Longer-chain β-peptides for which "pharmacokinetic" investigations have been carried out so far are neither orally bioavailable nor effectively excreted. In this respect, they resemble certain proteins, such as antibodies,[58,59] and peptides consisting of D-α-amino acid units.[60] Such compounds are actively investigated for application as drugs. We could foresee the development of β-peptides with

long-term activities, for instance, for the treatment of autoimmune diseases (see section 2.1).

The rational design and the stability of secondary structures of β- and γ-peptides will help to find candidates for biomedical application of "peptidic peptidomimetics".

We thank Novartis Pharma AG and the Swiss National Science Foundation for generously funding our research in the field of β- and γ-peptides. J.G. thanks the New Zealand Foundation for Research, Science and Technology for financial support (Grant SWSS0401, 2004−2007).

BIOGRAPHICAL INFORMATION

Dieter Seebach was born in Karlsruhe (Germany) in 1937. He completed his doctoral work at the University of Karlsruhe on small rings and peroxides under the supervision of R. Criegee (1964). After nearly two years of postdoctoral work at Harvard University with E. J. Corey, he qualified for habilitation (Karlsruhe 1969) with a paper based on sulfur- and selenium-stabilized carbanion and carbene derivatives. He was appointed to professorial positions first at the Justus Liebig University in Giessen in 1971 and subsequently (in 1977) at the Eidgenössische Technische Hochschule (ETH) in Zürich. He has been a visiting professor at numerous prestigious universities and is a member of the Deutsche Akademie der Naturforscher Leopoldina, the Swiss Academy of Technical Sciences (SATW), a corresponding member of the Akademie der Wissenschaften und Literatur in Mainz, and a foreign associate of the National Academy of Sciences U.S.A. He has received numerous awards, including the Havinga Medal (1985), Fluka Prize (reagent of the year 1987), ACS Award (1992) for Creative Work in Organic Synthesis, King Faisal Prize (1999), Chirality Medal (2002), Nagoya Medal (2002), Tetrahedron Prize (2003), and Noyori Prize (2004) and has been awarded an honorary doctorate by the University of Montpellier. His current research interests relate primarily to the development of new synthetic methods and the preparation, structural, and biological investigations of β-peptides.

James Gardiner was born in Oxford (England) in 1972. He studied chemistry at the University of Canterbury in Christchurch, New Zealand, and obtained his doctorate on the use of ring-closing metathesis for the synthesis of conformationally constrained amino acids and peptide mimics under the direction of Andrew Abell (2003). In 2004, he was awarded a New Zealand Foundation for Research Science and Technology (FRST) postdoctoral fellowship (2004−2007) to investigate structural and biological aspects of β-peptides with Prof. Dieter Seebach at ETH Zürich, Switzerland. In 2008, he returned to the southern hemisphere having been awarded an Australian Research Council (ARC) Link International fellowship.

FOOTNOTES

*To whom correspondence should be addressed. Phone: +41 44 632 2990. Fax: +41 44 632 1144. E-mail: seebach@org.chem.ethz.ch.

REFERENCES

1 Seebach, D.; Overhand, M.; Kühnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. β-Peptides: Synthesis by Arndt-Eistert Homologation with Concomitant Peptide Coupling. Structure Determination by NMR and CD Spectroscopy and by X-Ray Crystallography. Helical Secondary Structure of a β-Hexapeptide in Solution and its Stability towards Pepsin. *Helv. Chim. Acta* **1996**, *79*, 913–941.

2 (a) Seebach, D.; Beck, A. K.; Bierbaum, D. J. The World of β- and γ-Peptides Comprised of Homologated Proteinogenic Amino Acids and Other Components. *Chem. Biodiversity* **2004**, *1*, 1111–1239. (b) Seebach, D.; Beck, A. K.; Bierbaum, D. J. Die Welt der β- und γ-Peptide aus homologisierten proteinogenen Aminosäuren und anderen Bausteinen. *Chem. Biodiversity* **2004**, 1–133, original German text.

3 Kirmse, W. 100 Years of the Wolff Rearrangement. *Eur. J. Org. Chem.* **2002**, *2193*, 2256.

4 Lelais, G.; Micuch, P.; Josien-Lefebre, D.; Rossi, F.; Davies, S. G.; Seebach, D. Preparation of Protected β^2- and β^3-Homocysteine, β^2- and β^3-Homohistidine and β^2-homoserine for Solid-Phase Synthesis. *Helv. Chim. Acta* **2004**, *87*, 3131–3159.

5 Flögel, O.; Casi, G.; Hilvert, D.; Seebach, D. Preparation of the β^3-Homoselenocysteine Derivatives Fmoc-β^3hSec(PMB)-OH and Boc-β^3hSec(PMB)-OH for Solution and Solid-Phase-Peptide Synthesis and Selenoligation. *Helv. Chim. Acta* **2007**, *90*, 1651–1666.

6 We thank Fluka AG for discounted prices.

7 Seebach, D.; Abele, S.; Gademann, K.; Guichard, G.; Hintermann, T.; Jaun, B.; Mathews, J. L.; Schreiber, J. V.; Oberer, L.; Hommel, U.; Widmer, H. β^2- and β^3-Peptides with Proteinaceous Side Chains: Synthesis and Solution Structures of Constitutional Isomers, a Novel Helical Secondary Structure and the Influence of Solvation and Hydrophobic Interactions on Folding. *Helv. Chim. Acta* **1998**, *81*, 932–982.

8 Davies, S. G.; Garrido, N. M.; Kruchinin, D.; Ichihara, O.; Kotchie, L. J.; Price, P. D.; PriceMortimer, A. J.; Russell, A. J.; Smith, A. D. Homochiral Lithium Amides for the Asymmetric Synthesis of β-Amino Acids. *Tetrahedron: Asymmetry* **2006**, *17* (12), 1793–1811.

9 Seebach, D.; Schaeffer, L.; Gessier, F.; Bindschädler, P.; Jäger, C.; Josien, D.; Kopp, S.; Lelais, G.; Mahajan, Y.; Micuch, P.; Sebesta, R.; Schweizer, B. W. Enantioselective Preparation of 2-Aminomethyl Carboxylic Acid Derivatives: Solving the β^2-Amino Acid Problem with the Chiral Auxiliary 4-Isopropyl-5,5-diphenyloxazolidin-2-one (DIOZ). *Helv. Chim. Acta* **2003**, *86*, 1852–1861.

10 Lelais, G.; Seebach, D. β^2-Amino Acids - Syntheses, Occurance in Natural Products, and Components of β-Peptides. *Biopolymers* **2004**, *76*, 206–243.

11 Seebach, D.; Kimmerlin, T.; Sebesta, R.; Campo, M.; Beck, A. K. How We Drifted into Peptide Chemistry and Where We Have Arrived at. *Tetrahedron* **2004**, *60*, 7455–7506.

12 Rueping, M.; Schreiber, J. V.; Lelais, G.; Jaun, B.; Seebach, D. Mixed β^2/β^3-Hexapeptides and β^2/β^3-Nonapeptides Folding to (*P*)-Helices with Alternating Twelve- and Ten-Membered Hydrogen-Bonded Rings. *Helv. Chim. Acta* **2002**, *85*, 2577–2593.

13 Seebach, D.; Abele, S.; Gademann, K.; Jaun, B. Pleated Sheets and Turns of β-Peptides with Proteinogenic Side Chains. *Angew. Chem., Int. Ed.* **1999**, *38*, 1595–1597.

14 Lelais, G.; Seebach, D.; Jaun, B.; Mathad, R. I.; Flögel, O.; Rossi, F.; Campo, M.; Wortmann, A. β-Peptidic Secondary Structures Fortified and Enforced by Zn^{2+} Complexation - On the Way to β-Peptidic Zinc Fingers. *Helv. Chim. Acta* **2006**, *89*, 361–403.

15 Seebach, D.; Sifferlen, T.; Bierbaum, D. J.; Rueping, M.; Jaun, B.; Schweizer, B.; Schaefer, J.; Mehta, A. K.; O'Connor, R. D.; Meier, B. H.; Ernst, M.; Glättli, A. Isotopically Labelled and Unlabelled β-Peptides with Geminal Dimethyl Substitution in 2-Position of Each Residue: Synthesis and NMR Investigation in Solution and in the Solid State. *Helv. Chim. Acta* **2002**, *85*, 2877–2917.

16 Appella, D. H.; Christianson, L. A.; Karle, I. L.; Powell, D. R.; Gellman, S. H. β-Peptide Foldamers: Robust Helix Formation in a New Family of β-Amino Acid Oligomers. *J. Am. Chem. Soc.* **1996**, *118*, 13071–13072.

17 Cheng, R. P.; Gellman, S. H.; DeGrado, W. F. β-Peptides: From Structure to Function. *Chem. Rev.* **2001**, *101*, 3219–3232.

18 Disney, M. D.; Hook, D. F.; Namoto, K.; Seeberger, P. H.; Seebach, D. *N*-Linked Glycosylated β-Peptides Are Resistant to Degradation by Glycoamidase A. *Chem. Biodiversity* **2005**, *2*, 1624–1634.

19 Weiss, H. M.; Wirz, B.; Schweitzer, A.; Amstutz, R.; Rodriguez-Perez, M. I.; Andres, H.; Metz, Y.; Gardiner, J.; Seebach, D. ADME Investigations of Unnatural Peptides: Distribution of a ^{14}C-Labelled β^3-Octaarginine in Rats. *Chem. Biodiversity* **2007**, *4*, 1413–1437.

20 Lind, R.; Greenhow, D.; Perry, S.; Kimmerlin, T.; Seebach, D. Comparative Metabolism of α- and β-Peptides in the Insect *Heliothis virescens* and in Plant Cells of Black Mexican Sweet Maze. *Chem. Biodiversity* **2004**, *1*, 1391–1400.

21 Heck, T.; Limbach, M.; Geueke, B.; Zacharias, M.; Gardiner, J.; Kohler, H.-P.; Seebach, D. Enzymatic Degradation of β- and Mixed α,β-Oligopeptides. *Chem. Biodiversity* **2006**, *3*, 1325–1348.

22 Seebach, D.; Namoto, K.; Mahajan, Y. R.; Bindschädler, P.; Sustmann, R.; Kirsch, M.; Ryder, N. S.; Weiss, M.; Sauer, M.; Roth, C.; Werner, S.; Beer, H.-D.; Mundling, C.; Walde, P.; Voser, M. Chemical and Biological Investigations of β-Oligoarginines. *Chem. Biodiversity* **2004**, *1*, 65–97.

23 Arvidsson, P. I.; Ryder, N. S.; Weiss, H. M.; Hook, D. F.; Escalante, J.; Seebach, D. Exploring the Antibacterial and Hemolytic Activity or Shorter- and Longer-Chain β-α,β- and γ-Peptides, and of β-Peptides from β^2-3-Aza- and β^3-2-Methylidene-amino Acids Bearing Proteinogenic Side Chains - A Survey. *Chem. Biodiversity* **2005**, *2*, 401–420.

24 Baruj Benacerruf, Jean Dausset, and George C. Snell received the 1980 Nobel Prize in Physiology or Medicine for their discoveries concerning genetically determined structures on the cell surface that regulate immunological reactions, www.nobelprize.org.

25 Zinkernagel, R. M. Cellular Immune Recognition and the Biological Role of Major Transplantation Antigens (Nobel Lecture). *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 1938–1949.

26 Heemels, M. T.; Ploegh, H. L. Generation, Translocation, and Presentation of MHC Class 1 Restricted Peptides. *Annu. Rev. Biochem.* **1995**, *64*, 643–691.

27 Poenaru, S.; Lamas, J. R.; Folkers, G.; Lopez de Castro, J. A.; Seebach, D.; Rognan, D. Nonapeptide Analogues Containing (*R*)-3-Hydroxybutanoate and β-Homoalanine Oligomers: Synthesis and Binding Affinity to a Class I Major Histocompatibility Complex Protein. *J. Med. Chem.* **1999**, *42*, 2318–2331.

28 Seebach, J. D. et al., hitherto unpublished results, Dept. of Internal Medicine, Laboratory for Transplantation Immunology, University Hospital, Zürich, Switzerland.

29 Werder, M.; Hauser, H.; Abele, S.; Seebach, D. β-Peptides as Inhibitors of Small-Intestinal Cholesterol and Fat Absorption. *Helv. Chim. Acta* **1999**, *82*, 1774–1783.

30 David, R.; Günther, R.; Lühmann, T.; Seebach, D.; Hofmann, H.-J.; BeckSickinger, A. G. Artificial Chemokines - Combining Chemistry and Molecular Biology for the Elucidation of Function of Interleukin-8, hitherto unpublished results, Institute for Biochemistry, University Leipzig, Germany.

31 Seebach, D.; Mathad, R. I.; Kimmerlin, T.; Mahajan, Y. R.; Bindschädler, P.; Rueping, M.; Jaun, B. NMR-Solution Structures in MeOH of an α-Heptapeptide, of a β^3/β^2-Nonapeptide, and of an all-β^3-Icosapeptide Carrying the 20 Proteinogenic Side Chains. *Helv. Chim. Acta* **2005**, *88*, 1969–1982.

32 Kritzer, J.; Lear, J. D.; Hodsdon, M. E.; Schepartz, A. Helical β-Peptide Inhibitors of the p53-hDM2 Interaction. *J. Am. Chem. Soc.* **2004**, *126*, 9468–9469.

33 Kritzer, J. A.; Hodsdon, M. E.; Schepartz, A. Solution Structure of a β-Peptide Ligand for hDM2. *J. Am. Chem. Soc.* **2005**, *127*, 4118–4119.

34 Stephens, O.; Kim, S.; Welch, B. D.; Hodsdon, M. E.; Kay, M. S.; Schepartz, A. Inhibiting HIV Fusion with a β-Peptide Foldamer. *J. Am. Chem. Soc.* **2005**, *127*, 13126–13127.

35 Kritzer, J. S.; Stephens, O. M.; Guarracino, D. A.; Reznik, S. K.; Schepartz, A. β-Peptides as Inhibitors of Protein-Protein Interactions. *Bioorg. Med. Chem.* **2005**, *13*, 11–16.

36 Seebach, D.; Schaeffer, L.; Brenner, M.; Hoyer, D. Design and Synthesis of γ-Dipeptide Derivatives with Submicromolar Affinities for Human Somatostatin Receptors. *Angew. Chem., Int. Ed.* **2003**, *42*, 776–778.

37 Daura, X.; Gademann, K.; Schäfer, H.; Jaun, B.; Seebach, D.; van Gunsteren, W. F. The β-Peptide Hairpin in Solution: Conformational Study of a β-Hexapeptide in Methanol by NMR Spectroscopy and MD Simulation. *J. Am. Chem. Soc.* **2001**, *123*, 2393–2404.

38 Gademann, K.; Ernst, M.; Hoyer, D.; Seebach, D. Synthesis and Biological Evaluation of a Cyclo-β-tetrapeptide as a Somatostatin Analogue. *Angew. Chem., Int. Ed.* **1999**, *38*, 1223–1226.

39 Gademann, K.; Ernst, M.; Seebach, D.; Hoyer, D. The Cyclo-β-Tetrapeptide (β-HPhe-β-HThr-β-HLys-β-HTrp): Synthesis, NMR Structure in Methanol Solution, and Affinity for Human Somatostatin Receptors. *Helv. Chim. Acta* **2000**, *83*, 16–33.

40 Nunn, C.; Langenegger, M. R. D.; Schuepbach, E.; Kimmerlin, T.; Micuch, P.; Hurth, K.; Seebach, D.; Hoyer, D. β^2/β^3-Di- and α/β^3-Tetrapeptide Derivatives as Potent Agonists of Somatostain sst4 Receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **2003**, *367*, 95–103.

41 Seebach, D.; Dubost, E.; Mathad, R. I.; Jaun, B.; Limbach, M.; Löweneck, M.; Flögel, O.; Gardiner, J.; Capone, S.; Beck, A. K.; Hoyer, D.; Langenegger, D.; Widmer, H.,hitherto unpublished results, ETH Zürich and Novartis Pharma AG, Basel.

42 Wiegand, H.; Wirz, B.; Schweitzer, A.; Gross, G.; Rodriguez-Perez, M. I.; Andres, H.; Kimmerlin, T.; Rueping, M.; Seebach, D. Pharmacokinetic Investigation of a ^{14}C-Labelled β^3/α-Tetrapeptide in Rats. *Chem. Biodiversity* **2004**, *1*, 1812–1828.

43 Wiegand, H.; Wirz, B.; Schweitzer, A.; Camenisch, G. P.; Rodriguez Perez, M. I.; Gross, G.; Woessner, R.; Voges, R.; Arvidsson, P. I.; Frackenpohl, J.; Seebach, D. The Outstanding Metabolic Stability of a ^{14}C-Labelled β-Nonapeptide in Rats - *in vitro* and *in vivo* Pharmacokinetic Studies. *Biopharm. Drug Dispos.* **2002**, *23*, 251–262.

44 Tyndall, J. D. A.; Pfeiffer, B.; Abbenante, G.; Fairlie, D. P. Over One Hundred Peptide-Activated G Protein-Coupled Receptors Recognize Ligands with Turn Structure. *Chem. Rev.* **2005**, *105*, 793–826.

45 Gademann, K.; Seebach, D. Preparation and NMR Structure of the Cyclo-β-tripeptide $[\beta^3$-HGlu$]_3$ in Aqueous Solution: A New Class of Enterobactin-Type C_3-Symmetrical Ligands. *Helv. Chim. Acta* **1999**, *82*, 957–962.

46 Gademann, K.; Seebach, D. Synthesis of Cyclo-β-tripeptides and Their Biological in vitro Evaluation as Antiproliferatives Against the Growth of Human Cancer Cell Lines. *Helv. Chim. Acta* **2001**, *84*, 2924–2937.

47 Trouche, N.; Wieckowski, S.; Sun, W.; Chaloin, O.; Hoebeke, J.; Fournel, S.; Guichard, G. Small Multivalent Architectures Mimicking Homotrimers of the TNF Superfamily Member CD40L: Delineating the Relationship between Structure and Effector Function. *J. Am. Chem. Soc.* **2007**, *129*, 13480–13492, and references cited therein.

48 Fournel, S.; Wieckowski, S.; Sun, W.; Trouche, N.; Dumortier, H.; Bianco, A.; Chaloin, O.; Habib, M.; Peter, J.-C.; Schneider, P.; Vray, B.; Toes, R. E.; Offringa, R.; Melief, C. J. M.; Hoebeke, J.; Guichard, G. C_3-Symmetric Peptide Scaffolds are Functional Mimetics of Trimeric CD40L. *Nat. Chem. Biol.* **2005**, *1*, 377–382, and references cited therein.

49 van Kooten, C.; Banchereau, J. CD40 - CD40 Ligand. *J. Leukocyte Biol.* **2000**, *67*, 1–17.

50 Habib, M.; Rivas, M. N.; Chamekh, M.; Wieckowski, S.; Sun, W.; Bianco, A.; Trouche, N.; Chaloin, O.; Dumortier, H.; Goldman, M.; Guichard, G.; Fournel, S.; Vray, B. Small Molecule CD40 Ligand Mimetics Promote Control of Parasitemia and Enhance T Cells Producing IFN-γ During Experimental *Trypanosoma cruzi* Infection. *J. Immunol.* **2007**, *178*, 6700–6704.

51 Namoto, K.; Gardiner, J.; Kimmerlin, T.; Seebach, D. Investigation of the Interactions of β-Peptides with DNA Duplexes by Circular Dichroism Spectroscopy. *Helv. Chim. Acta* **2006**, *89*, 3087–3103.

52 Gelman, M. A.; Richter, S.; Cao, H.; Umezawa, N.; Gellman, S. H.; Rana, T. M. Selective Binding of TAR RNA by a Tat-Derived β-Peptide. *Org. Lett.* **2003**, *5*, 3563–3565.

53 Geueke, B.; Namoto, K.; Agarkova, I.; Perriard, J.-C.; Kohler, H.-P.; Seebach, D. Bacterial Cell Penetration by β^3-Oligoarginines: Indication for Passive Transfer through the Lipid Bilayer. *ChemBioChem* **2005**, *6*, 982–985.

54 Umezawa, N.; Gelman, M. A.; Haigis, M. C.; Raines, R. T.; Gellman, S. H. Translocation of a β-Peptide across Cell Membranes. *J. Am. Chem. Soc.* **2002**, *124*, 368–369.

55 Hitz, T.; Iten, R.; Gardiner, J.; Namoto, K.; Walde, P.; Seebach, D. Interaction of α- and β-Oligoarginine-Acids and Amides with Anionic Lipid Vesicles: A Mechanistic and Thermodynamic Study. *Biochemistry* **2006**, *45*, 5817–5829.

56 Shimanouchi, T.; Walde, P.; Gardiner, J.; Mahajan, Y. R.; Seebach, D.; Thomae, A.; Krämer, S. D.; Voser, M.; Kuboi, R. Permeation of a β-Heptapeptide Derivative Across Phospholipid Bilayers. *Biochim. Biophys. Acta: Biomembr.* **2007**, *1768*, 2726.

57 Langel, U. *Cell-Penetrating Peptides: Processes and Applications*; CRC Press: Boca Raton, FL, 2002.

58 Lonberg, N. Human Antibodies from Transgenic Animals. *Nat. Biotechnol.* **2005**, *23*, 1117–1125.

59 Chapman, A. P.; Antoniw, P.; Spitali, M.; West, S.; Stephens, S.; King, D. J. Therapeutic Antibody Fragments with Prolonged in vivo Half-Lives. *Nat. Biotechnol.* **1999**, *17*, 780–783.

60 Reddy, S. T.; Anantharamaiah, G. M.; Navab, M.; Hama, S.; Hough, G.; Grijalva, V.; Garber, D. W.; Datta, G.; Fogelman, A. M. Oral Amphipathic Peptides as Therapeutic Agents. *Expert Opin. Invest. Drugs* **2006**, *15*, 13–21.