From the Biopolymer PHB to Biological Investigations of Unnatural β- and γ-Peptides

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Abstract: An overview is given of the past and present activities of our group in the field of chemical biology. Thus, the polymer of 3-hydroxybutanoic acid (PHB), which is omnipresent in living organisms, triggered our search for a better analytical method for detecting PHB and our syntheses of oligomers of 3-hydroxyalkanoic acids (OHBs). Also, the regulation of DNA replication by poly(β-malic acid) (PMA) in certain eukaryotes inspired synthetic work on the corresponding cyclic and open-chain oligomers. With these oligomers we not only tested the mechanisms of depolymerases, but were able to study the properties and activities of well-characterized compounds (also with isotope and fluorescence labeling). The role of PHB as component of ion transport systems through phospholipid bilayers was unambiguously established, and models for the channel structure were proposed. Replacement of amino acids by 3-hydroxybutanoic acid residues in peptides and replacement of the chain-bound oxygens in OHB by NH paved our way into the world of β- and γ-peptides, the synthesis and physiological and pharmacological properties of which are being investigated. β-Peptides are stable to peptidases, have a long lifetime in mammalian serum and are rather resistant to environmental microbial degradation. The peptides consisting of homologated (β) or doubly homologated (γ) amino acids form stable secondary structures in solution (helices, turns, sheets) which can be used as scaffolds for peptide mimics, such as a β-tetrapeptide with affinity to a somatostatin receptor or a β-nonapeptide that inhibits intestinal lipid-transport protein (SR-BI) in Caco-2 cells. Certain β-peptides have antibacterial, antiproliferative and haemolytic properties. The lessons from studies of β- and γ-peptides teach us about the central role of natural α-peptidic proteins.

Keywords: Ion channels · MHC-binding ligands · Oligo- and poly(3-hydroxybutyrates) (OHB, PHB) · β-Peptides · Phospholipid bilayers · Somatostatin

Introduction

Originally, our group was interested in biological-chemical methods only for the preparation of chiral synthetic building blocks (yeast reduction [1], pig-liver esterase [2]). While studying (R)-3-hydroxybutanoate (HB), obtained by degradation of the biopolymer PHB, we learned about a fascinating class of chemically simple macromolecules (Fig. 1). Although, high-molecular-weight PHB had been well known as a microbial storage material (sPHB), a low-molecular-weight variety (ca. 150 residues) has now been recognized as being ubiquitous, albeit in small amounts, in all living organisms, and as being associated with Ca²⁺ ions, with polyphosphate, with membranes, and with proteins (complexing or cPHB) [3][4]. Another natural polyester, poly[β-(S)-malic acid] (PMA) has been found in numerous *fungi* (mold *fungi*, slime molds, and black yeasts); PMA is involved in regulation of the DNA-polymerase activity by binding to the enzyme (inhibition) and to the histones (displacement of DNA) (Scheme 1) [5]. Thus, we became interested in contributing chemical knowledge about these polyesters, with the goal of better understanding their physiological roles.

Investigations of Oligo- and Poly (β-Hydroxyalkanoates) (OHA, PHA)

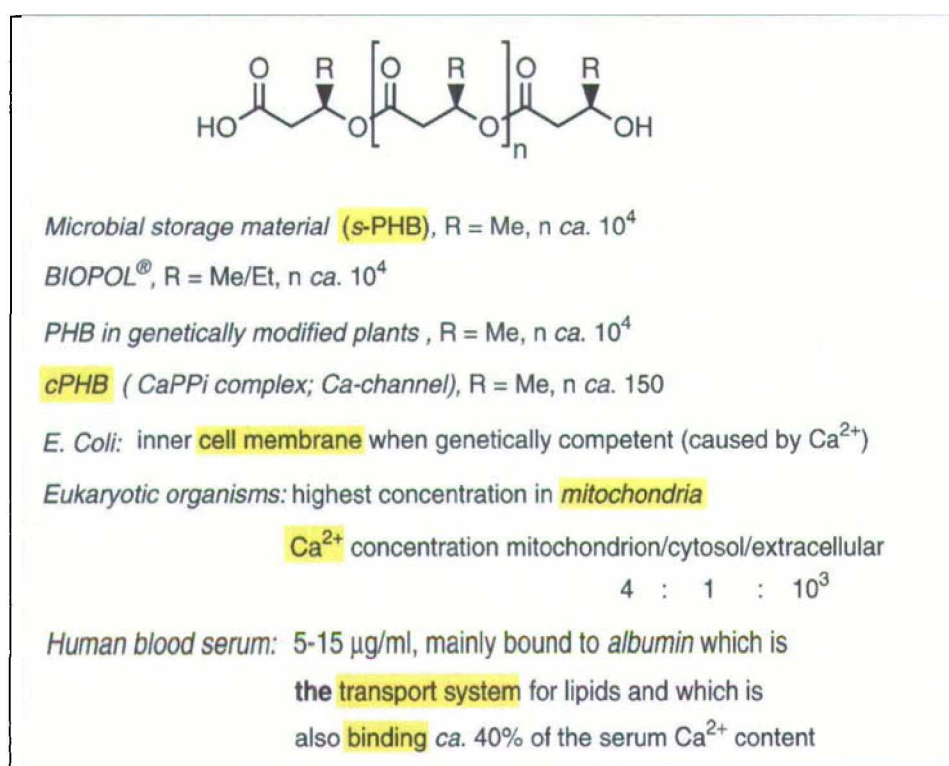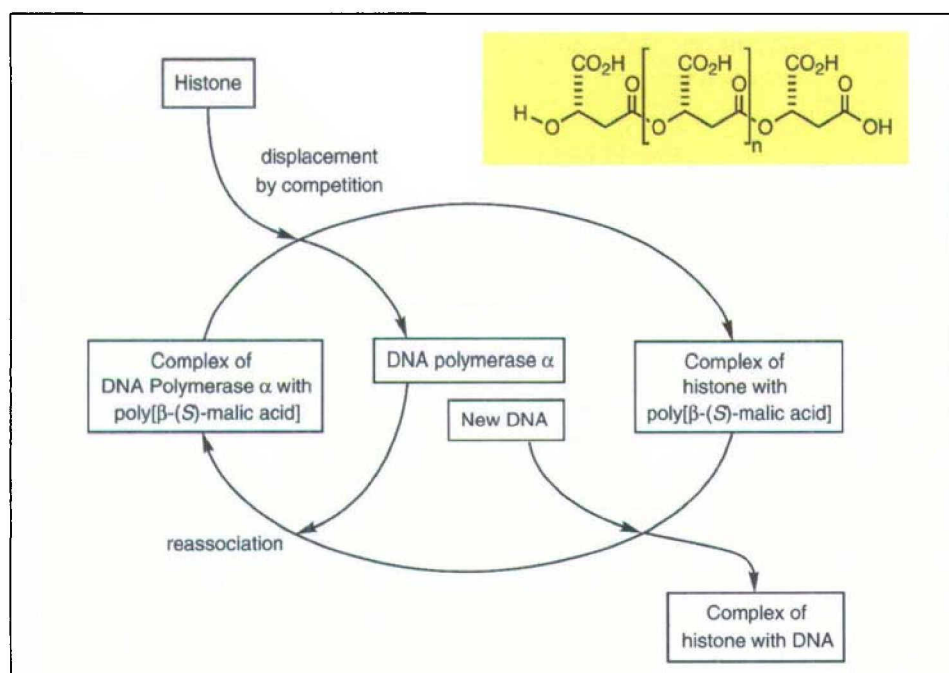NMR analysis of samples of eukaryotic origin, such as spinach, human albumin and heart tissue, led to the direct detection of PHB [6], and mild titanate-mediated degradation of PHB led to the discovery of 3-hydroxyvalerate (HV) in various organisms where previous methods of detection had analyzed only for HB (Scheme 2) [7]. The synthesis of oligo(3-hydroxybutanoates) (OHB, with up to 128 residues [4]), also with isotopic [9] and fluorescent [10] labels, and of analogs containing 3-hydroxybutyrates of (S) configuration [4] or other 3-hydroxyalkanoates [11] (Fig. 2) allowed us to study the structure of the polyester chain in solution and in phospholipid bilayers [4][9–12]. The oligomers were also used for preparing complexes with alkali and alkaline-earth salts, and to establish the corresponding crystal structures as models for ion channels [4]. OHBs of various chain lengths were tested in planar and spherical phospholipid bilayers for their ion transport abilities. In so-called patch-clamp experiments a voltage-driven ion transport (by a single-channel mechan-

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Microbial storage material (s-PHB), R = Me, n ca. 10^4

BIOPOL®, R = Me/Et, n ca. 10^4

PHB in genetically modified plants , R = Me, n ca. 10^4

cPHB (CaPPi complex; Ca-channel), R = Me, n ca. 150

E. Coli: inner cell membrane when genetically competent (caused by Ca^{2+})

Eukaryotic organisms: highest concentration in mitochondria

Ca^{2+} concentration mitochondrion/cytosol/extracellular

4 : 1 : 10^3

Human blood serum: 5-15 µg/ml, mainly bound to albumin which is

the transport system for lipids and which is

also binding ca. 40% of the serum Ca^{2+} content

Fig. 1. Polyhydroxy-butyrate and -valerate (PHB, PHV) are not only microbial storage materials, but have been found in numerous organisms and tissues, see also Scheme 2. They form CaPP$_i$ complexes and ion channels through phospholipid bilayers (cf. Fig. 3), and they are post-ribosomal appendages in proteins [3][4]. Their origin (biosynthesis?) in eukaryotic organisms is unknown.

Scheme 1. Poly[(S)-malic acid] (PMA) is found in concentrations of up to 150 (!) g/l in certain fungi. This anionic polyelectrolyte has been shown to be involved in the regulation of DNA polymerase and in displacing DNA (also an anionic polyelectrolyte) from the histones (like the polymerase, histones are proteins which may be considered a cationic polyelectrolytes!). The chain lengths range from ca. 50 to 500. PMA has been detected only in eukaryotic organisms to date. Pathways for its biosynthesis and degradation in the slime mold Physarum polycephalum have been elucidated [5].

ism) was detected [13], and in vesicles we found a concentration-driven passage of Ca^{2+} ions by a pore mechanism [14]. In both cases OHBs of chain lengths of 32 or more units were active, while shorter ones (8 or 16 HB residues) gave erratic effects. With a synthetic 128mer and Ca polyphosphate (ca. 60mer) we assembled a complex which, when incorporated into a phospholipid bilayer, gave a voltage/current slope (in a patch-clamp experiment, from +120 mV to –120 mV) and single-channel behavior identical to that of the corresponding complex extracted from the inner cell wall of genetically competent E. coli [15]. This result proves the identity of the microbial Ca-specific, non-proteinaceous channel beyond doubt! Three possible structures for the PHB channels are shown in Fig. 3 (models (b) and (c) are compatible with all the structural information we have about PHB [4]).

Along a totally different line of research, we have replaced amino acids in the variable region of nonapeptides binding to MHC class I protein (major histocompatibility proteins) by 3-hydroxybutanoyl residues (Fig. 4) [16]. Substitution of five α-amino-acid residues (15 chain atoms) by four HB units (16 chain atoms) led to compounds with a higher affinity than the nonapeptide used as a reference. A similar effect was observed with an analog in which five α-amino acid moieties had been replaced by two HB and two β-amino-acid residues (Fig. 4).

β- and γ-Peptides

Work on OHBs was also the starting point for our investigations of β-peptides. Cyclic [(R)-3-hydroxybutanoates] of ring sizes ranging from eight (dimer) all the way to 128 (32mer) have been prepared [4], including thiocarbonyl (C=S instead of C=O) and imino derivatives (C=NR instead of C=O) [17], as well as analogs built from malates. Many of these gave crystals, sometimes more than one form, suitable for X-ray structure analysis [17] [18]. All oligolides of (R)-3-hydroxy-butanoic acid containing more than five residues were found to be folded in these structures, with a characteristic motif consisting of a single right-handed helical turn built from three units with a pitch of 6 Å (Fig. 5a). A model of this helix revealed that the carbonyl oxygens and the chain-bound oxygens were at a distance (ca. 3.8 Å), suggesting to us that replacement of the chain oxygens by NH might lead to hydrogen bonding (Fig. 5b and c),
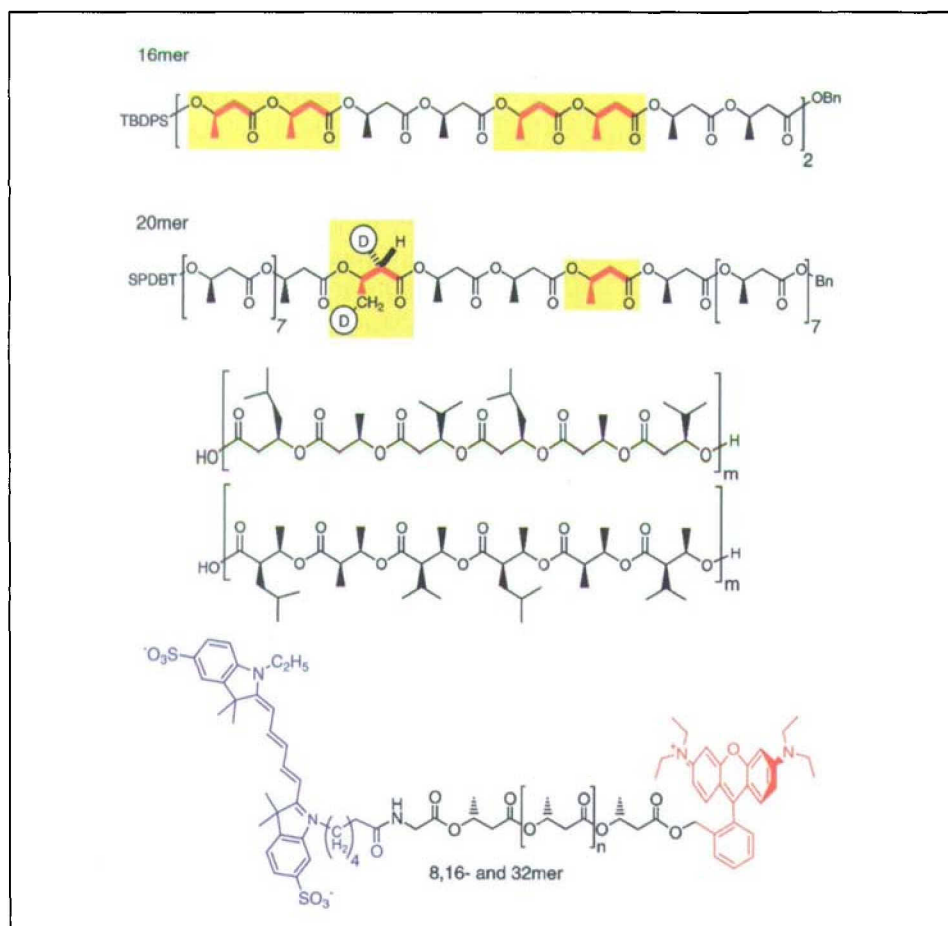
Fig. 2. Labeled oligomers of HB, containing up to 32 residues, have been prepared and investigated by NMR spectroscopy, FRET measurement and fluorescence microscopy in solution and in vesicles [4][9–12]. To this end, ^{2}H- and ^{13}C-isotopic labeling, substitution of the oligoester backbone, and attachment of one or two fluorescent groups were achieved by standard synthetic methods [9–11].
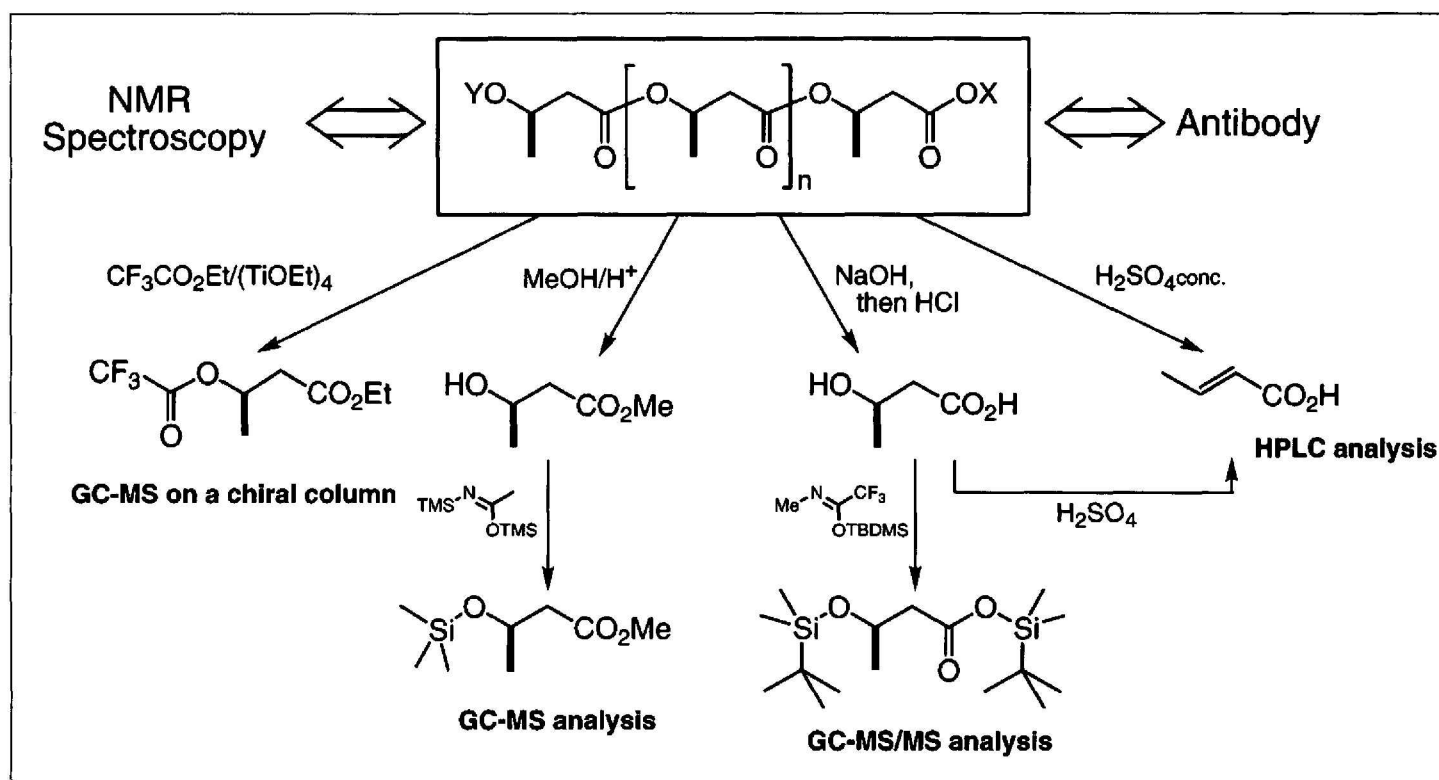
Scheme 2. Methods of PHB analysis are sensitive down to picogram/g material (antibody). The standard test involves treatment with H_2SO_4 (conc.) and detection of the crotonic acid formed (PHV cannot be detected by this analysis). NMR spectroscopy [6] allows for detection of $CHCl_3$-soluble PHB/PHV extractions from lyophilized samples (mol. weight *ca.* 10 000 Da). Transesterification with titanates and GC analysis on chiral columns can be used for determination of both HB and HV in the (*R*)- and (*S*)-forms [4][7][8].
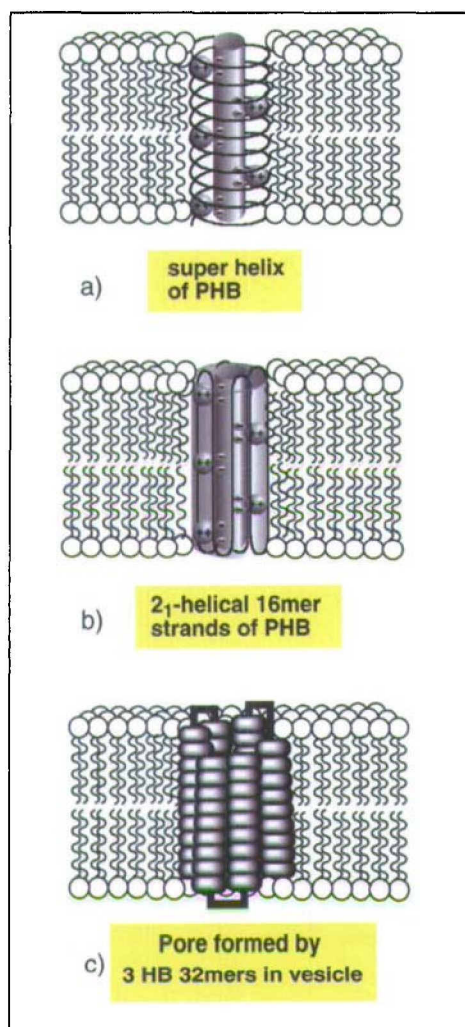
a) super helix of PHB

b) 2$_1$-helical 16mer strands of PHB

c) Pore formed by 3 HB 32mers in vesicle

Fig. 3. Three models of trans-membrane channels formed by OHB. Single-channel behavior was observed with OHBs and with OHB•CaPP$_i$ complexes in patch-clamp experiments (voltage-driven). A pore or ion-carrier mechanism was found with OHBs in vesicles (concentration-driven). a) A superhelix (14 HB units per turn) wrapping around the (likewise helical) CaPP$_i$. b) 2$_1$-Helical PHB strands arranged around a Ca^{2+} polyphosphate with eight passages through the phospholipid bilayer. c) A pore formed by three 32mers for passage of Ca^{2+} through POPC-bilayer vesicles [4] [13–15].

and thus to stabilization of the helix, so that it could be observed in solution [19].

The result of the O/NH replacement in the backbone of oligo(3-hydroxybutanoates) was not only taking a step away from the ester world, without hydrogen bonding, into the amido world with hydrogen bonding, but also from the well-known natural α-peptide world into a fascinating new, unnatural β-peptide world (we even had a glimpse at γ-peptides already). All we had to do was to insert a CH$_2$ group into each and every amino acid residue of a peptide (Fig. 6) [19–22]. Almost everything known about α-peptides and proteins is reversed when we go to β-peptides: they form stable helices in solution with only six residues; left-handed 3$_1$ (Fig. 5c) and right-handed 12/10 helices are observed, depending on the position of the side chains on the back-bone carbons [21]; they form a turn with only four residues [23b, c], and they assemble into highly insoluble pleated-sheet structures [24] – all by design [25]!

The biological and physiological properties of β- and γ-peptides can be considered from two points of view. On the one hand, nature's tools for cleaving α-peptidic bonds, the peptidases and proteinases, are unable to cleave a simple β-dipeptide bond (Fig. 7a), and blood-serum concentrations of simple water-soluble β-peptides in rats is constant over many hours (Fig. 7b). Even micro-organisms have difficulties living on small β-peptides as the sole carbon (and nitrogen) source (Fig. 7c) [26]. On the other hand, the design of β-peptidic sec-

Gln-Arg-Leu-Lys-Glu-Ala-Ala-Glu-Lys
Natural peptidic ligand

C_{50} = 10 μM

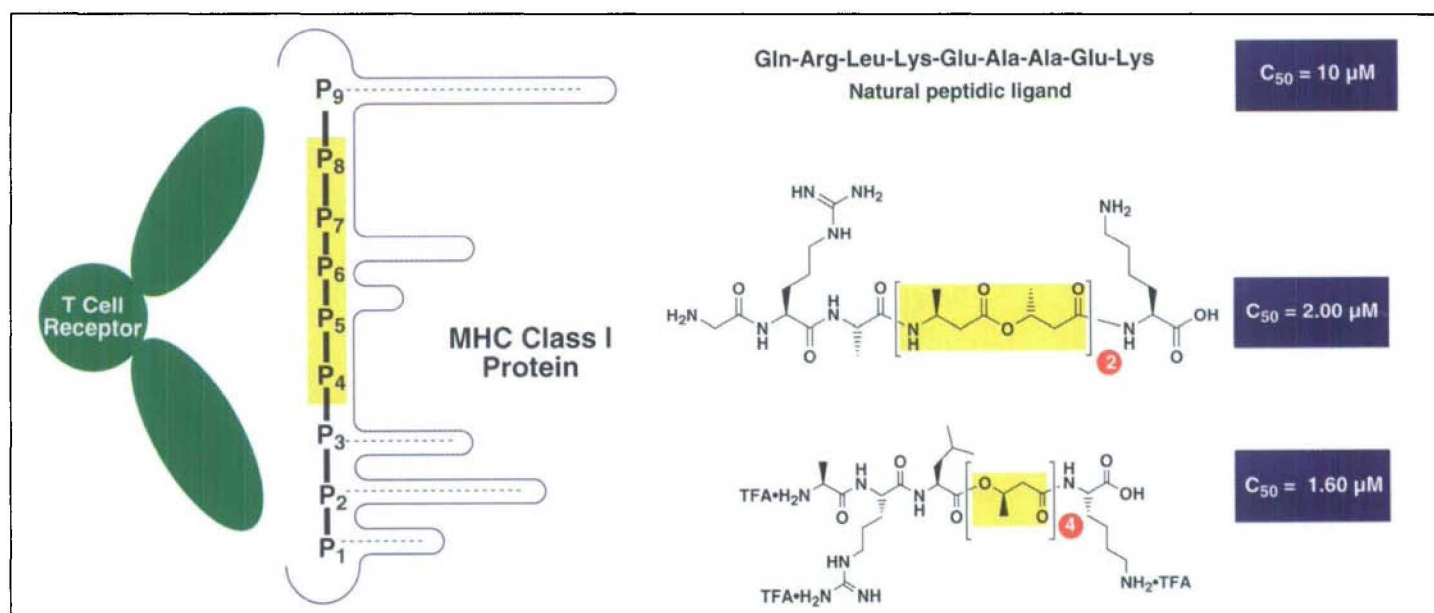C_{50} = 2.00 μM

C_{50} = 1.60 μM

Fig. 4. Synthetic incorporation of HB units into a peptide chain (four HB for five amino acid residues) gives 'Ligands' that bind to the major histocompatibility (MHC) HLA-B27 protein with affinities comparable to those found for a reference nonapeptide [16].
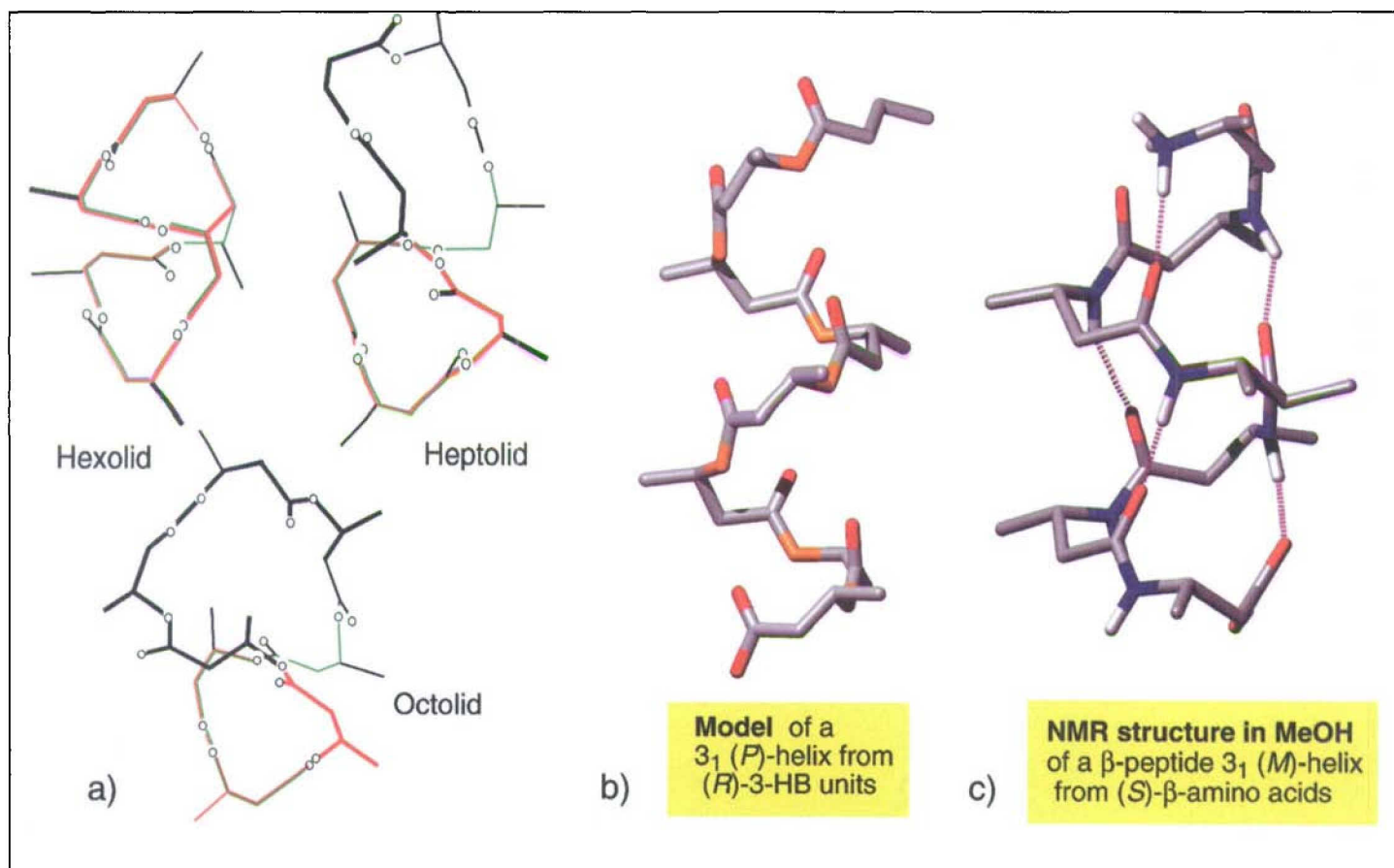
Fig. 5. Cyclic OHBs ('oligolides') fold with formation of right-handed single-pitch helix turns consisting of three HB units. a) The hexolide built from two such turns has the shape of a figure eight, whereas in the heptolide and octolide one such turn motif is present (crystal structures). b) Modelling of a 3_1 helix from the crystal data of HB and HV oligolides, with close proximity of C=O oxygen on HB unit i to chain oxygen of HB unit ($i + 3$). c) Helix derived from a β-amino-acid oligomer (replacement of the HB-chain oxygens by NH!) with hydrogen bonds from N-H of β-amino acid residue i to the C=O of residue ($i + 3$) [4][17–19].
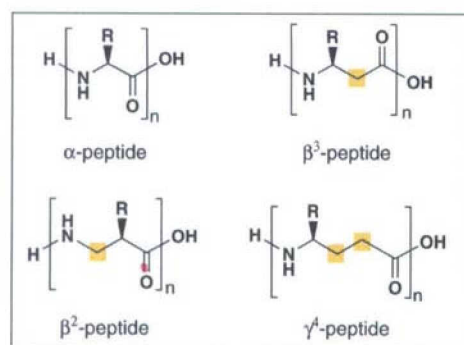

Fig. 6. Homologs of peptides, the β- and γ-peptides, are obtained by inserting one or two CH_2 groups between the carbonyl carbon and the stereogeneic center or between the stereogenic center and the NH group. γ-Peptides are designated γ^2, γ^3, or γ^4, depending upon which carbon bears side chains, $i.e.$ where two CH_2 groups have been inserted into the α-amino acid residues of a peptide. Of course, β-peptides can bear a second substituent ($\beta^{2,3}$) and γ-peptides even two additional substituents ($\gamma^{2,3}$, $\gamma^{2,4}$, $\gamma^{3,4}$, $\gamma^{2,3,4}$) on each amino acid moiety. Furthermore, β- and γ-peptides may have OH, SH, NHR functional groups attached directly to the back-bone carbons (unlike α-peptides!). Clearly, there are many more possible β- and γ-amino acids than natural α-amino acids, especially if we leave homologation strategies behind and allow imagination of the synthetic organic chemist free reign. – In our own work to date, we have retained the side chains present in the 20 proteinogenic amino acids. The synthetic methods we use involve 'real' insertions (Arndt-Eistert reaction or Wittig olefination/hydrogenation), or are modern versions of the Mannich reaction [20][22]

ondary structures allows for precise geometrical arrangement of functionalized side chains, and this in turn can lead to lock-key-type interaction between β-peptides and α-peptidic receptor proteins, as demonstrated by a nanomolar affinity of a designed β-tetrapeptide for one of the human somatostatin receptors (Fig. 8) [23]. Thus, there seems to be an orthogonality between the worlds of α- and β-peptides as far as backbone recognition and chemistry is concerned (for instance, the two types of peptides cannot form mixed ('chimeric') pleated sheets [21]

[24]). When correct placement of functionalized peptidic side chains in space is at stake, the β-peptidic backbone is as good as any peptidomimetic scaffold. This is also borne out by our attempts to mimic an amphipathic peptide helix for binding to the protein SR-BI and inhibiting its cholesterol- and lipid-transporting activity through the brush-border membrane (Fig. 9) [27]. A simple β-nonapeptide with a 3_{14}-helical secondary structure organizes lysine side chains on one face of the helix and hydrophobic alanine and phenylalanine side chains on the oth-

er. It has been shown to reduce cholesterol transport through living Caco-2 cells down to background levels at $ca.$ 0.36 mmolar concentration.

There are many more interesting, useful, and probably also dangerous physiological properties of the homologs of α-peptides, the β- and γ-peptides, to be discovered. Longer β-peptides, capable of forming helices of amphiphilic character, such as the one shown in Fig. 9, have been found to exhibit antimicrobial and also haemolytic properties [28]. Also, small cyclo-β-tripeptides have been

a) Substitution Pattern in Peptides Tested with these Peptidases

β^2 $\beta^{2,2,3}$ γ^2

β^3 $\beta^{2,3}$ γ^3

β^2, β^3 β^2-HPro γ^4

$\beta^{2,2}$ $\gamma^{2,3,4}$

pepsin (*pankreas*)
trypsin (*pankreas*)
carboxypeptidase A (*pankreas*)
elastase (*pankreas*)
chymotrypsin (*pankreas*)
leucine-aminopeptidase (*kidney*)

20s proteasome (human erythrocytes)
proteinase K (*T. album*)
pronase (*S. griseus*)
penicillin amidase (*E. coli*)
amidase (*P. aeruginosa*)
β-lactamase (*E. cloacae*)

b) Serum Concentration of β-Peptides in Rats

$\tau_{1/2}(\beta) = 3\ h$

$\tau_{1/2}(\beta) = 10\ h$
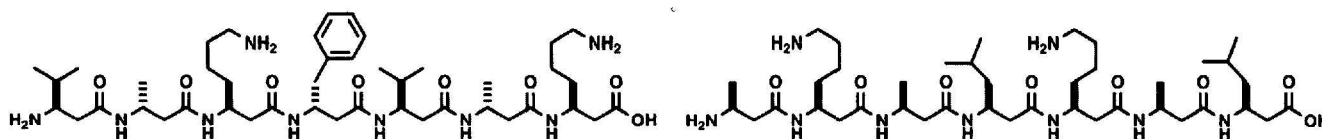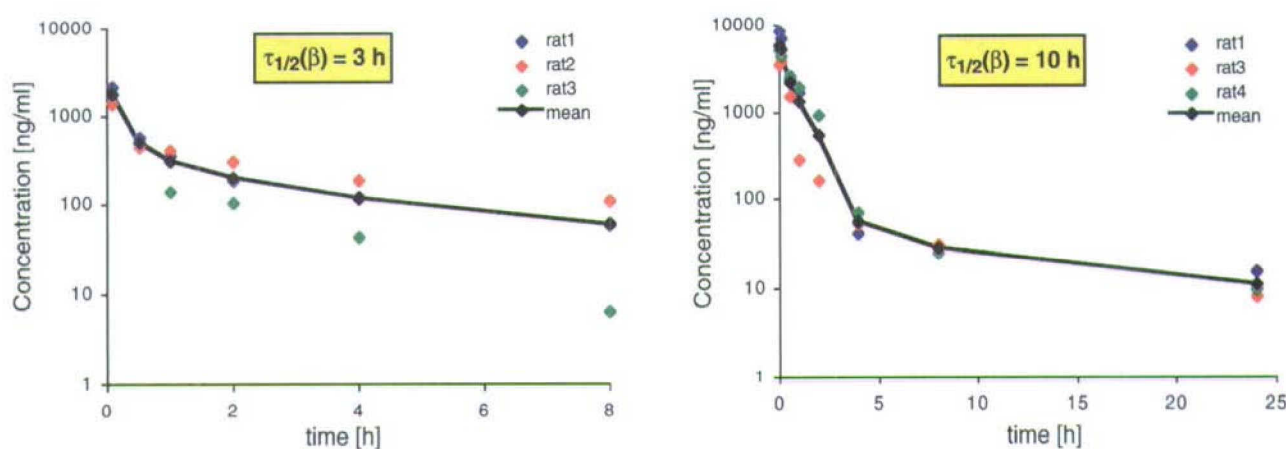
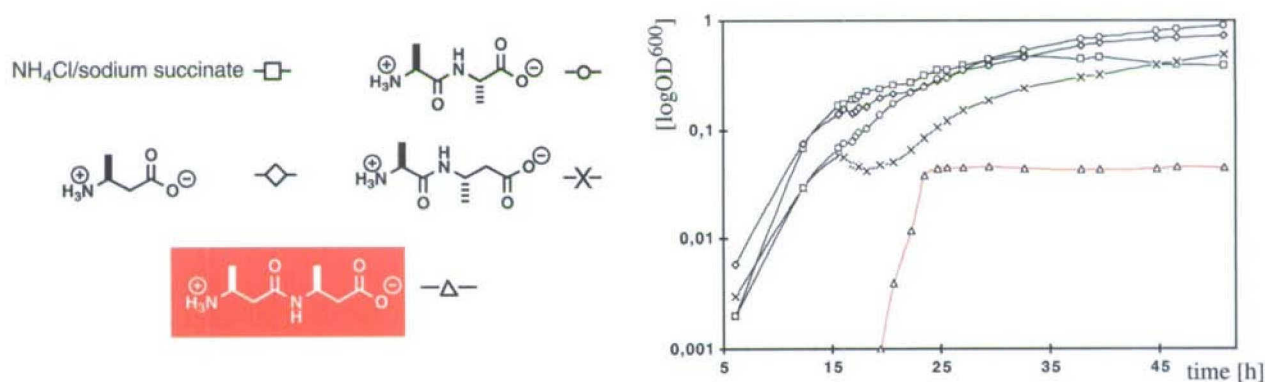## c) Substrates as Sole N- and C-source for Growth of *Pseudomonas aeruginosa*

Fig. 7. β-Peptides are stable against enzymatic and microbial degradation [26]. a) A large number of β-peptides (and also some γ-peptides) of various structural types and chain lengths (ranging from 2 to 18 residues) have been exposed to 12 proteases and peptidases (including some of the most active, such as the 20s proteasome) for 2 days, without any detectable cleavage [26c]. b) Intravenous injection of water-soluble β-heptapeptides into rats has led (after an initial decrease) to a constant serum concentration of these compounds for many hours [26a, b]. c) *Pseudomonas aeruginosa* (a soil bacterium) has been cultured on various amino acids and peptides; it grows on an α-dipeptide (H-Ala-Ala-OH) and on the β-amino acid H-β-HAla-OH, but there are 'difficulties' on a mixed α,β-dipeptide (H-Ala-β-HAla-OH) and great 'difficulties' on a β-dipeptide (H-β-HAla-β-HAla-OH). There is no growth at all on the β-tripeptide H-β-HVal-β-HAla-β-HLeu-OH, as sole carbon and nitrogen source [26b].
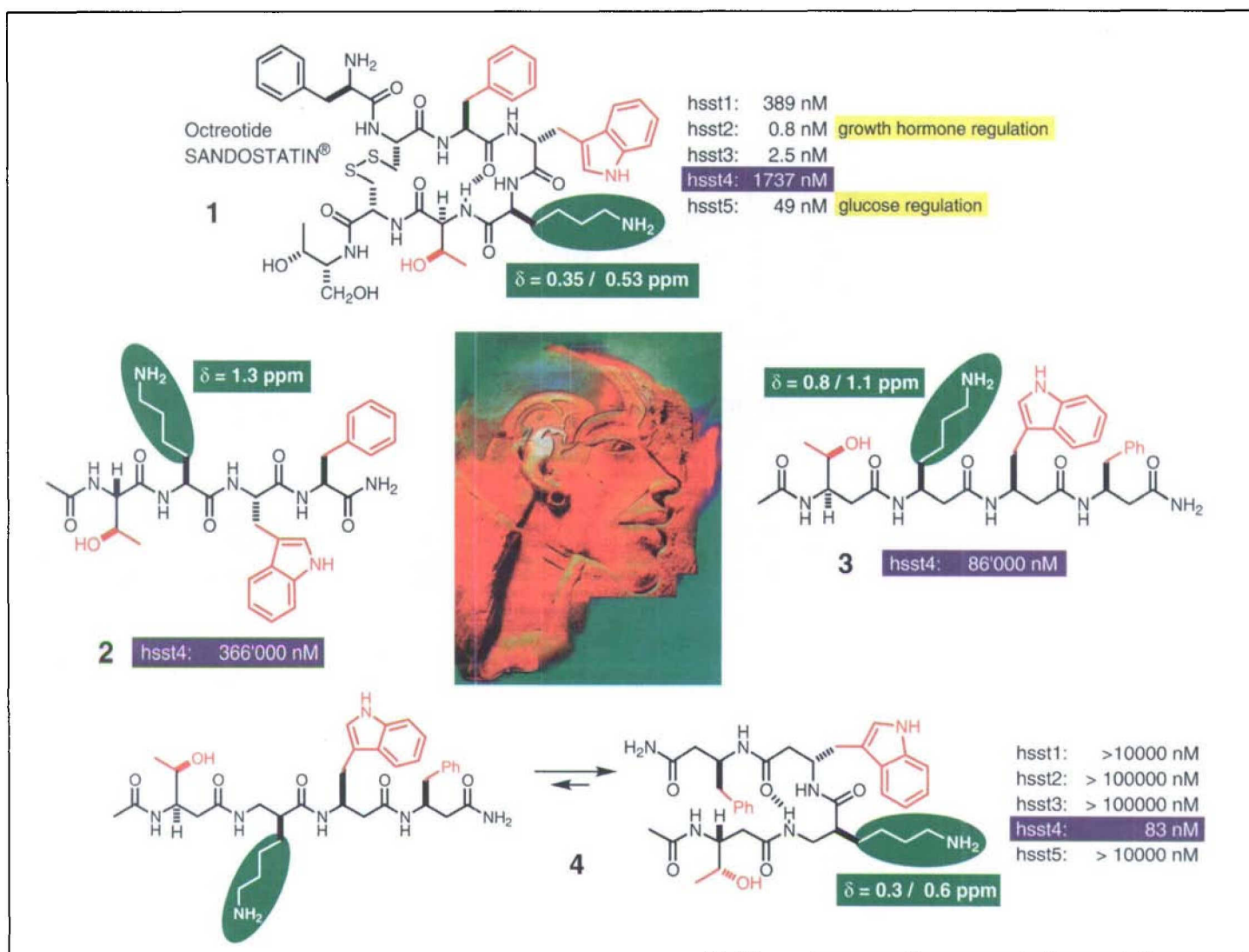
Fig. 8. Somatostatin mimics **1–4**, their binding constants to human receptors hsst 1–5, and NMR evidence for a turn structure. Somatostatin is an important peptide hormone (cyclic disulfide, containing 14 amino acids), and octreotide **1** is an analog with 8 amino acid residues (SANDOSTATIN®) that binds especially well to the receptor hsst 2 [23a]. The structural prerequisite for binding is a β-turn with the side chains of the amino acids Phe, Trp, Lys, Thr. A simple α-peptide **2** built of these four amino acids has essentially no affinity for hsst 4, and neither does the β-tetrapeptide **3**. Shift of one side chain of this β-peptide (β³-HLys in **3** to β²-HLys in **4**) causes a 1000-fold increase in affinity [23b]: the β²/β³-neighborhood of β-amino acids in β-peptides is known to generate a hair-pin-turn structure (10-membered hydrogen-bonded ring) [23b,c][24]. The shielding of lysine CH_2 groups by the proximal indole aromatic ring in the NMR spectrum is evidence for the turn structure (see chemical shifts on green background) and correlates with affinity. The ancient sculpture of an Egyptian head, reproduced in the center, is thought to show a man suffering from *acromegalia* (excessive growth of ears and chin), a desease now treated with SANDOSTATIN® [23d].

shown to posses antiproliferative (and cytotoxic) effects [29] (Fig. 10). Combinatorial syntheses of small β-peptides and high-throughput screening (HTS) is ongoing in pharmaceutical companies and will undoubtedly lead to the discovery of other interesting activities.

The most important aspect of the research on homologated peptides is that by discovering fundamental differences in their structures and properties we increase understanding of the natural peptides composed of the 20 natural α-amino-acid-building blocks.

Conclusion

Synthetic organic chemists skilled and up-to-date in the art of synthetic methodology can contribute to the advancement of biochemistry and biology. More organic chemists should move in this direction without abandoning their core business! It is our experience that simultaneous activities in both areas in the same research group can be extremely rewarding, and actually cross-fertilizing.

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[1] D. Seebach, M.A. Sutter, R.H. Weber, M.F. Züger, 'Yeast Reduction of Ethyl Acetoacetate: (S)-(+)-Ethyl 3-Hydroxy-butanoate (Butanoic acid, 3-hydroxy-, ethyl ester, (S))', *Org. Synth.* **1985**, *63*, 1, Collective Volume, **1990**, *VII*, 215; D. Seebach, S. Roggo, J. Zimmermann, in 'Stereochemistry of Organic and Bioorganic Transformations', Workshop Conferences Hoechst, Vol. 17, Eds. W. Bartmann, K.B. Sharpless, VCH, Weinheim, **1987**, p. 85; D. Seebach, S. Roggo, T. Maetzke, H. Braunschweiger, J. Cercus, M. Krieger, *Helv. Chim. Acta* **1987**, *70*, 1605.
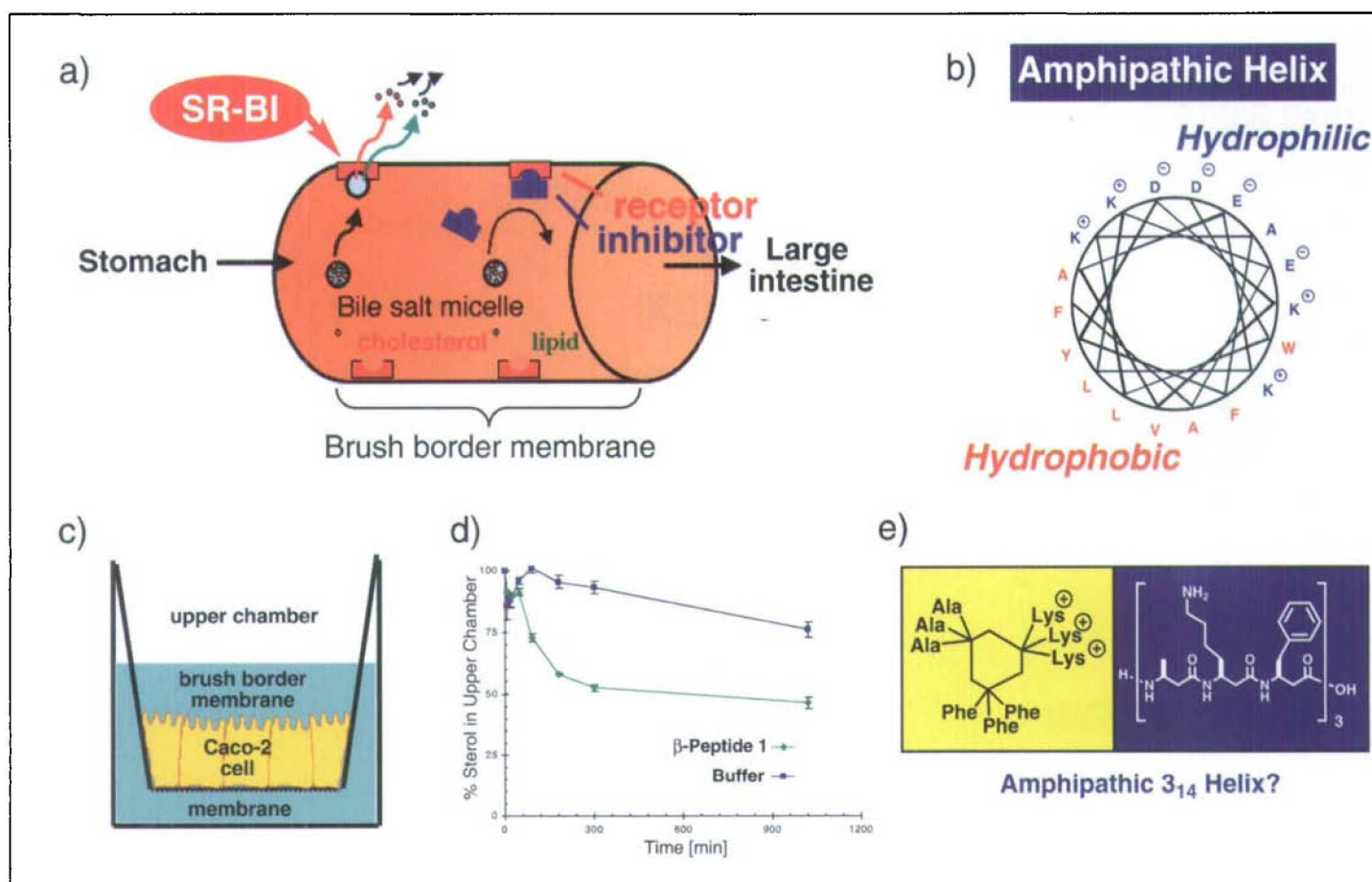
Fig. 9. Inhibition of an intestinal membrane-bound cholesterol- and lipid-transporting protein by a β-nonapeptide of amphiphilic character [27]. a) The transport protein SR-BI sitting in the brush-border membrane is inhibited by peptides and proteins which contain an amphipathic helix section (b). In the Caco-2 cell test (c), α-peptides are not effective because the living cells contain peptidases that cleave such peptides before they can reach the binding site of the SR-BI protein. d) The β-nonapeptide with Ala, Lys and Phe side chains, when folded as a 3_{14} helix, would have amphipathic character, as seen in the view down along the helix axis with the side chains protruding perpendicularly [30] (*cf.* the helical wheel presentation of an α-peptidic helix in b). e) Inhibition of sterol transport by the β-nonapeptide in the Caco-2 test (concentration 1.0 mg/ml).

[2] M. Eberle, M. Missbach, D. Seebach, 'Enantioselective Saponification with Pig Liver Esterase (PLE): (1*S*,2*S*,3*R*)-3-Hydroxy-2-Nitrocyclohexyl Acetate (1,3-Cyclohexanediol, 2-nitro-, 1-acetate, [1*S*-(1α,2β,3α)])', *Org. Synth.* **1990**, *69*, 19.

[3] R.N. Reusch, *Biochemistry* **1999**, *38*, 15666.

[4] H.-M. Müller, D. Seebach, 'Poly (hydroxyalkanoates): A Fifth Class of Physiologically Important Organic Biopolymers?', *Angew. Chem. Int. Ed. Engl.* **1993**, *32*, 477; *Angew. Chem.* **1993**, *105*, 483; D. Seebach, A. Brunner, B.M. Bachmann, T. Hoffmann, F.N.M. Kühnle, U.D. Lengweiler, 'Biopolymers and -oligomers of (*R*)-3-Hydroxyalkanoic Acids – Contributions of Synthetic Organic Chemists', *Ernst Schering Research Foundation* **1995**, *28*, 7; D. Seebach, M.G. Fritz, 'Detection, synthesis, structure, and function of oligo(3-hydroxyalkanoates): contributions by synthetic organic chemists', *Int. J. Biol. Macromol.* **1999**, *25*, 217.

[5] E. Holler, G. Achhammer, B. Angerer, B. Gantz, C. Hambach, H. Reisner, B. Seidel, C. Weber, C. Windisch, C. Braud, P. Guérin, M. Vert, *Eur. J. Biochem.* **1992**, *206*, 1; E. Holler, 'Poly(Malic Acid) from Natural Sources', in 'Handbook of Engi-

neering Polymeric Materials', Ed. N.P. Chereminisoff, Dekker, New York, **1997**, p. 93; K. Rathberger, H. Reisner, B. Willibald, H.-P. Molitoris, E. Holler, *Mycol. Res.* **1999**, *103*, 513; B. Gasslmaier, C.K. Krell, D. Seebach, E. Holler, *Eur. J. Biochem.* **2000**, *267*, 5101.

[6] D. Seebach, A. Brunner, H.M. Bürger, J. Schneider, R.N. Reusch, *Eur. J. Biochem.* **1994**, *224*, 317.

[7] M. Albert, B.M. Bachmann, T. Mäder, S. Ruckstuhl, D. Seebach, hitherto unpublished results, ETH Zürich, **1998-2001**.

[8] D. Elhottová, J. Tríska, H. Santrucková, J. Kveton, J. Santrucek, M. Simková, *Plant Soil* **1997**, *197*, 251; D. Elhottová, J. Tríska, S.O. Petersen, H. Santrucková, *Fresen. J. Anal. Chem.* **2000**, *367*, 157.

[9] P. Waser, Ph.D. thesis No. 13552, ETH Zürich, **2000**.

[10] M. Rueping, A. Dietrich, M. Sauer, D. Seebach, *Macromolecules*, **2001**, submitted.

[11] M. Albert, part of the projected Ph. D. thesis, ETH Zürich.

[12] C. Griesinger, H. Schwalbe, E. Duchardt, P. Waser, M. Rueping, D. Seebach, *Helv. Chim. Acta* **2001**, in preparation.

[13] D. Seebach, A. Brunner, H.M. Bürger, R.N. Reusch, L.L. Bramble, *Helv. Chim.*

Acta **1996**, *79*, 507.

[14] M. G. Fritz, P. Walde, D. Seebach, *Macromolecules* **1999**, *32*, 574.

[15] S. Das, U.D. Lengweiler, D. Seebach, R.N. Reusch, 'Proof for a nonproteinaceous calcium-selective channel in *Escherichia coli* by Total Synthesis from (*R*)-3-Hydroxybutanoic Acid and Inorganic Polyphosphate', *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 9075.

[16] S. Poenaru, J.R. Lamas, G. Folkers, J.A. López de Castro, D. Seebach, *J. Med. Chem.* **1999**, *42*, 22318.

[17] A. Brunner, F.N.M. Kühnle, D. Seebach, *Helv. Chim. Acta* **1996**, *79*, 319.

[18] D.A. Plattner, A. Brunner, M. Dobler, H.-M. Müller, W. Petter, P. Zbinden, D. Seebach, *Helv. Chim. Acta* **1993**, *76*, 2004; B.M. Bachmann, D. Seebach, *Helv. Chim. Acta* **1998**, *81*, 2430; C.M. Krell, D. Seebach, *Eur. J. Org. Chem.* **2000**, 1207.

[19] D. Seebach, J.L. Matthews, 'β-Peptides: a surprise at every turn', *Chem. Commun.* **1997**, 2015.

[20] J. Podlech, D. Seebach, *Liebigs Ann.* **1995**, 1217; J.L. Matthews, C. Braun, C. Guibourdenche, M. Overhand, D. Seebach, in 'Enantioselective Synthesis of β-Amino Acids', Chapter 5, Ed. E. Juaristi, Wiley-VCH, New York, **1997**, p. 105 –
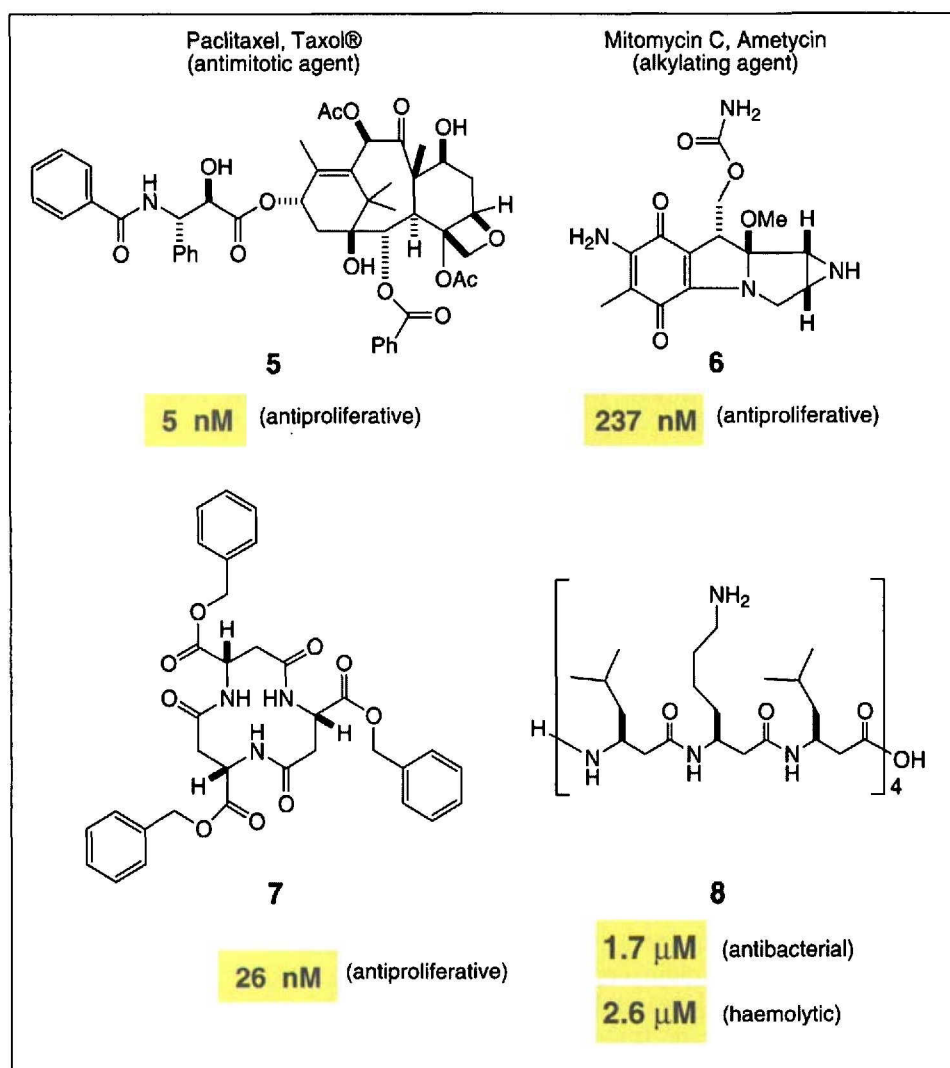
Fig. 10. Antiproliferative, cytotoxic, antimicrobial and haemolytic activities of β-peptides (7, 8), and comparison with two antiproliferative natural products (Taxol® 5 and Mitomycin 6). The concentrations given are antiproliferative activities (IG 50) of 5–7 against the leukemic cancer cell line HL-60(TB), antibacterial activity (IC 50 values) of 8 in cultures of K91 *E. coli*, and haemolytic activity (HD 50 values) of 8 towards human red-blood cells (RBC). The values are taken from tests of the NIH cancer institute for 5, 6, and for the cyclo-β-tripeptide derivative 7 [29][31], and from a paper by deGrado *et al.* for the β-dodecapeptide 8 [28]. A combination of antimicrobial and haemolytic activity has also been observed for β-peptides consisting of cationic cyclic β-amino acids (oligomer of (R,R)-*trans*-2-aminocyclopentanecarboxylic acid and (R,S)-*trans*-4-aminopyrrolidine-3-carboxylic acid) [32].

126; T. Hintermann, D. Seebach, *Synlett* 1997, 437; G. Guichard, S. Abele, D. Seebach, *Helv. Chim. Acta* 1998, *81*, 187; T. Hintermann, K. Gademann, B. Jaun, D. Seebach, *Helv. Chim. Acta* 1998, *81*, 983.

[21] D. Seebach, M. Overhand, F.N.M. Kühnle, B. Martinoni, L. Oberer, U. Hommel, H. Widmer *Helv. Chim. Acta* 1996, *79*, 913; D. Seebach, P.E. Ciceri, M. Overhand, B. Jaun, D. Rigo, L. Oberer, U. Hommel, R. Amstutz, H. Widmer, *Helv. Chim. Acta* 1996, *79*, 2043; D. Seebach, S. Abele, K. Gademann, G. Guichard, T. Hintermann, B. Jaun, J.L. Matthews, J.V. Schreiber, L. Oberer, U. Hommel, H. Widmer, *Helv. Chim. Acta* 1998, *81*, 932.

[22] T. Hintermann, D. Seebach, *Helv. Chim. Acta* 1998, *81*, 2093.

[23] a) G. Melacini, Q. Zhu, G. Osapay, M. Goodman, 'A Refined Model for the Somatostatin Pharmacophore: Conformational Analysis of Lanthionine-Sandostatin Analogs', *J. Med. Chem.* 1997, *40*, 2252; C. Scarpignato, in 'Octreotide: from Basic Science to Clinical Medicine', Ed. C. Scarpignato, Karger, Basel, 1996, *vol. 10*, pp. 54–72; b) K. Gademann, T. Kim-

merlin, D. Hoyer, D. Seebach, *J. Med. Chem.* 2001, in print; c) K. Gademann, X. Daura, W.F. van Gunsteren, D. Seebach, *J. Am. Chem. Soc.* 2001, *123*, 2393 d) Novartis Information sheet, 'Die Geschichte von Sandostatin®', September 2000.

[24] D. Seebach, S. Abele, K. Gademann, B. Jaun, *Angew. Chem.* 1999, *111*, 1700; *Angew. Chem. Int. Ed.* 1999, *38*, 1595; D. Seebach, J.V. Schreiber, S. Abele, S. Daura, W.F. van Gunsteren, *Helv. Chim. Acta* 2000, *83*, 34.

[25] For review articles see [19] and S.H. Gellman, 'Foldamers: A Manifesto', *Acc. Chem. Res.* 1998, *31*, 173; W.F. DeGrado, J.P. Schneider, Y. Hamuro, 'The twists and turns of β-peptides', *J. Pept. Res.* 1999, *54*, 206; K. Gademann, T. Hintermann, J.V. Schreiber, 'β-Peptides: Twisting and Turning', *Curr. Med. Chem.* 1999, *6*, 905.

[26] a) T. Hintermann, D. Seebach, *Chimia* 1997, *51*, 244; b) D. Seebach, S. Abele, J.V. Schreiber, B. Martinoni, A.K. Nussbaum, H. Schild, H. Schulz, H. Hennecke, R. Woessner, F. Bitsch, *Chimia* 1999, *52*, 734; c) J. Frackenpohl, P.I. Arvidsson,

J.V. Schreiber, D. Seebach, *ChemBioChem* 2001, in print.

[27] M. Werder, H. Hauser, S. Abele, D. Seebach, *Helv. Chim. Acta* 1999, *82*, 1774.

[28] Y. Hamuro, J.P. Schneider, W.F. DeGrado, *J. Am. Chem. Soc.* 1999, *121*, 12200.

[29] Developmental Therapeutics Program of the National Cancer Institute, USA; http://dtp.nci.nih.gov/.

[30] J.V. Schreiber, D. Seebach, *Helv. Chim. Acta* 2000, *83*, 3139; D. Seebach, P.I. Arvidsson, J. Frackenpohl, *Helv. Chim. Acta* 2001, *84*, 271.

[31] J.L. Matthews, K. Gademann, B. Jaun, D. Seebach, *J. Chem. Soc. Perkin Trans. 1*, 1998, 3331.

[32] E.A. Porter, X. Wang, H.-S. Lee, B. Weissblum, S.H. Gellman, *Nature (London)* 2000, *404*, 565; E.A. Porter, X. Wang, H.-S. Lee, B. Weissblum, S.H. Gellman, *Nature (London)* 2000, *405*, 298.