Modern Supramolecular Chemistry

Strategies for Macrocycle Synthesis

Edited by
François Diederich, Peter J. Stang, and Rik R. Tykwinski

WILEY-VCH Verlag GmbH & Co. KGaA

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Library of Congress Card No.: applied for

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library.

Bibliographic information published by the Deutsche Nationalbibliothek
Die Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available in the Internet at http://dnb.d-nb.de.

Typesetting Thomson Digital, India
Printing Strauss GmbH, Mörlenbach
Binding Litges & Dopf GmbH, Heppenheim

Printed in the Federal Republic of Germany
Printed on acid-free paper

ISBN: 978-3-527-31826-1

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Preface

The 1987 Nobel Prize in Chemistry was awarded to Donald J. Cram, Jean-Marie Lehn, and Charles J. Pedersen for "their development and use of molecules with structure-specific interactions of high selectivity". At this time, the award was bestowed for the synthesis of molecules that mimicked important biological processes – what Lehn deemed supramolecular chemistry. In the two decades that have followed, this field has expanded greatly, and supramolecular breakthroughs in organic synthesis, molecular electronics, and materials science have now been realized. It is interesting to note, however, that in many respects supramolecular chemistry has remained close to its roots. The pioneering efforts that merited the Nobel Prize were based primarily on the synthesis of macrocycles. These were the crown ethers, the cryptands, the cavitands, and other host molecules that ultimately provide a welcoming and selective environment for a particular guest species, whether it be a neutral molecule, a cation, or an anion.

Both historically and in the present day, supramolecular chemistry beautifully marries two scientific disciplines: organic synthesis and physical organic chemistry. It is here that the most modern aspects of this field of chemistry share the spotlight. No longer is the objective simply to mimic biological systems such as enzymes, but rather, today's supramolecular chemist is limited only by his or her imagination as to the role that a macrocycle might play in some well-orchestrated chemical scheme.

The goal of the present monograph is to tie together these seemingly diverse achievements under a common heading: the synthesis of macrocycles for use in supramolecular chemistry. To this end, the biological relevance of macrocycles continues to play a pivotal role, as illustrated by a chapter on macrocyclic peptides by Liskamp and co-workers. The emergence of ring-closing metathesis as a pre-eminent synthetic strategy for constructing naturally occurring macrocycles is described next by Prunet *et al*. Synthetic efforts toward macrocycles continue to be inspired by Nature, and these stories are recounted by de Mendoza and Ballester (hydrogen-bonding assembly), Isaacs and coworkers (curcurbiturils), Böhmer and Podoprygorina (tetra-urea calixarenes), and Sessler and coworkers (anion-binding macrocycles). From here, supramolecular chemistry melds into materials chemistry, where shape-persistent macrocycles (Tykwinski and Sadowy), three-dimensional

architectures (Dalcanale and Pirondini), molecular containers (Fujita and Yoshizawa), and rotaxanes (Wisner and Blight) share the stage. While it is not possible to review comprehensively each of the above topics, we believe this monograph provides expert insight and advice, covering both synthetic endeavors and applications of the resulting products. Most of all, however, we hope that these 10 chapters will instill the inspiration to further expand the boundaries of this captivating field of research.

This book results from the substantial efforts of a number of people. Most importantly, we appreciate the contributions of the authors. In this era of dwindling time and funding, their efforts and expertise have provided a monograph that is both interesting to read and a scientific resource. We express our gratitude to Ms. Annie Tykwinski for the original illustration that became the cover of this book, and we would like to thank Drs. Manfred Köhl and Andreas Sendtko at Wiley-VCH, as well as the production staff, for their aid in preparing this monograph.

November 2007

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1
Bioactive Macrocyclic Peptides and Peptide Mimics

Rob M.J. Liskamp, Dirk T.S. Rijkers, and Saskia E. Bakker

1.1
Introduction

The number of both naturally occurring and synthesized biologically active cyclic peptides, modified peptides, and peptide mimics is rapidly increasing. So far, many of these sometimes biologically very potent peptides have unfortunately unknown molecular targets or mechanisms of action. This certainly opens up very interesting and challenging research areas with respect to uncovering these targets and/or molecular mechanisms of biological activity, which – depending on the biological action – can be very promising, for example, for the development of new drugs. However, merely a review of most biologically active cyclic peptides – if this were possible at all – is not the aim of this chapter. Instead, we wish to focus on selected bioactive macrocyclic peptide systems with known molecular *peptide* or *protein* targets as well as details about their molecular interaction mechanism. Where possible, we would like to discuss the contribution of the *cyclic* nature of the peptides to the molecular mechanism of interaction and the ensuing biological activity. We will therefore not include cyclic peptides and mimics merely interacting with membrane lipids or cyclic peptides interacting with DNA or RNA. Each of these topics deserves a review on its own, especially in light of the increasing interest in membrane proteins and transcription activators/regulators.

The selection of cyclic peptides interacting with known molecular targets in this chapter is largely determined by their relevance in relation to possible treatments of diseases. In this respect, probably vancomycin and cyclosporin are the most well-known cyclic peptides containing modified amino acids, which have had a profound influence on the treatment of life-threatening diseases. The vancomycin-related antibiotics [1] are outstanding examples of cyclic peptide systems containing multiple knotted side chains by which almost absolute control over the shape of the molecule is achieved, leading to efficient binding of crucial fragments of the cell-wall precursor of disease-causing bacteria.

In the larger peptide antibiotic compounds comprising the class of lantibiotics, the shape of the molecule is determined by several cyclic peptides, including two annulated peptide rings, present within one molecule, giving the lantibiotic a unique way to interact with the target molecule lipid II and subsequent pore-forming capabilities in phospholipid membranes [2].

In an increasing number of examples, the diversity of functions and activities of proteins can be reflected in their smaller peptide counterparts, and this is especially prominent in RGD-containing cyclic peptides and peptide mimics derived from the corresponding RGD sequence in proteins. These are capable of interacting with a variety of integrin receptors ($\alpha_V\beta_3$ and $\alpha_{IIb}\beta_3$) involved in cellular adhesion and migration [3]. As a result, the RGD sequence is, for example, crucial in the construction of (cyclic) peptide compounds used for molecular imaging and treatment of diseases such as cancer and infections.

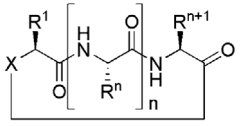
However, it is good to realize that many biologically relevant cyclic peptides are not derived from or do not correspond to particular peptide sequence(s) in a larger protein molecule. These cyclic peptides are produced by micro-organisms, and although they are macrocyclic structures, they are of course much smaller than proteins. In addition to vancomycin and the lantibiotics mentioned above, other important examples are cyclosporin A [4] and cyclotheonamide [5]. The former cyclic peptide is the widely used immunosuppressive drug while the latter is capable of specifically interacting with thrombin, a crucial serine protease in the blood clotting cascade. As a consequence, cyclotheonamide and chemically synthesized analogs are potentially important for modulating blood clotting.

In principle, there is no limitation to the nature of the biological process or cascade, which can be influenced by (cyclic) peptides and their derivatives, and it should be emphasized that cyclic peptides and mimics derived thereof could be powerful instruments in modulating signal transduction. This is apparent, for example, from the interaction of cyclic peptides with SH2-domains involved in, among others, allergy, cancer and other diseases.

At present many biologically extremely important cyclic peptides and derivatives such as octreotide/octreotate [6] fall outside the scope of this contribution, simply because their exact molecular interaction mechanism together with their biological target are still unknown [7], which certainly poses a challenge for future research directed toward unraveling their biomolecular mechanisms of action. In addition, in many cases the molecular events subsequent to the specific interaction with a receptor are still unknown, leaving many unanswered questions with respect to the ultimate biological effects.

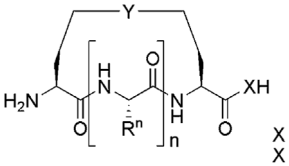
Cyclization of a linear peptide (Figure 1.1) endows the cyclic peptide first of all with a considerably reduced flexibility as compared to the parent peptide, which is (generally consisting of up to about ten amino acid residues) a very flexible molecule. Each amino acid residue contributes two single bonds to the conformational flexibility of the molecule, and generally speaking the only relatively rigid bond (*trans* and *trans/cis* in proline and *N*-alkylated amino acid-containing peptides) is the amide bond. After approximately ten to twenty amino acid residues, secondary structure elements (such as α-helices [8], turns, and β-strands) start to form. The reduced

"head to tail" cyclization



X = NH
X = O (depsipeptide)

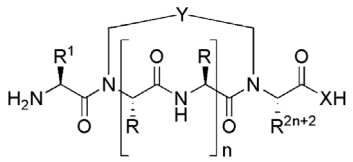
side chain to side chain cyclization



X = NH (peptide amide)
X = O (peptide acid)

Y = ~S-S~ (disulfide bridge)
Y = ~S~ (thio ether or sulfide bridge)
Y = ~NH-C(O)~ (lactam bridge)
Y = ~O-C(O)~ (depsipeptide; lactone bridge)
Y = ~CH=CH~ (alkene bridge)
Y = ~C≡C~ (alkyne bridge)

backbone to backbone cyclization



X = NH (peptide amide)
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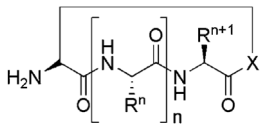
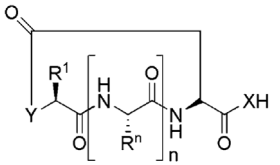
Y = ~S-S~ (disulfide bridge)
Y = ~NH-C(O)~ (lactam bridge)
Y = ~O-C(O)~ (depsipeptide; lactone bridge)
Y = ~CH=CH~ (alkene bridge)

side chain to C-terminus cyclization



X = NH
X = O (depsipeptide)

side chain to N-terminus cyclization



X = NH (peptide amide)
X = O (peptide acid)

Y = NH (lactam bridge)
Y = O (depsipeptide)

Figure 1.1 Different ways of peptide cyclization.

flexibility of the cyclic peptide derivative as compared to the open form is a distinct advantage for interaction with a potential molecular target. In general, provided that no significant enthalpy-entropy compensation takes place [9], a cyclized peptide which is less flexible and therefore more pre-organized, will display a higher affinity because it will lose less entropy upon interaction with its molecular target. As such, cyclization is also a universal first approach to increase the affinity of a peptide. An associated advantage of a cyclic peptide structure is the decreased sensitivity to proteolytic degradation, especially by exoproteases, which will be favorable for the half-life of the (cyclic) peptide and thereby its bioavailability.

1.2 Selected Cyclic Peptides

For this review we have selected the cyclic modified peptides mentioned above, i.e. vancomycin, nisin, cyclosporin, and cyclotheonamide, as well as two examples, i.e. RGD-containing cyclic peptides and SH2 domain binding cyclic peptides, which were inspired by proteins.

1.2.1 Vancomycin

Vancomycin (**1**, Figures 1.2 and 1.3) is a glycopeptide antibiotic with high activity against Gram-positive bacteria and is particularly renowned for its activity against the feared methicillin-resistant *Staphylococcus aureus* (MRSA) species [1b]. Vancomycin is produced by *Amycolatopsis orientalis*, a bacterium originally found in a soil

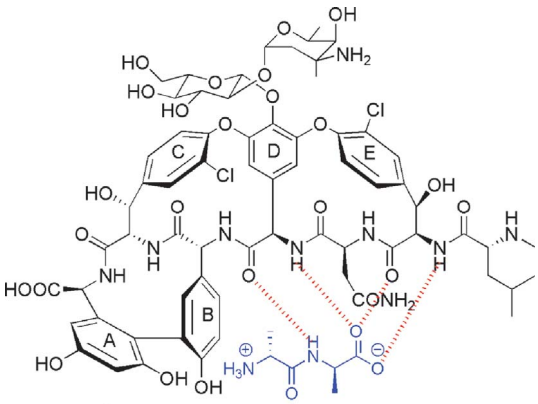


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Figure 1.2 Structure of vancomycin (**1**). The dipeptide ligand D-Ala-D-Ala is shown in blue and the hydrogen bonds are shown in red. In the case of the D-Ala-D-lactate ligand, one hydrogen bond is lost and replaced by a repulsion due to the free electron pairs on oxygen.

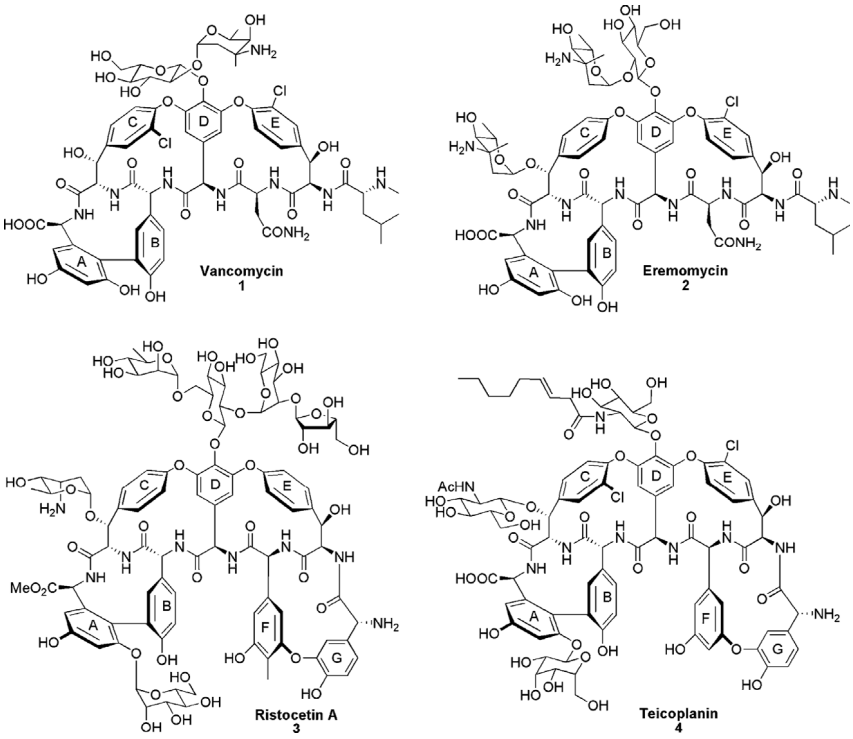

Figure 1.3 Chemical structures of glycopeptides antibiotics vancomycin (**1**) [1], eremomycin (**2**) [15a], ristocetin A (**3**) [15b], and teicoplanin (**4**) [15c].

sample from Borneo, Indonesia. The biosynthetic pathway and the total syntheses of vancomycin and related structures have been reviewed in a comprehensive account by Nicolaou and coworkers [1b]. Vancomycin is one of the representatives of the glycopeptide family [1]. The vancomycin aglycon consists of a heptapeptide with several non-proteinogenic amino acids. Residues 4, 5, and 7 are phenylglycine derivatives with different substitution patterns of the aromatic ring, while residues 2 and 6 are β-hydroxytyrosines with chlorine substituents in the *ortho* position. The side chains are involved in the formation of three macrocycles named after the component residues: residues 5 and 7 form the *A-B* biaryl system, while residues 2, 4, and 6 form the two bisaryl ether systems *C-D* and *D-E*, respectively (Figures 1.2 and 1.3). The sugar moieties are glucose and vancosamine [1b].

Vancomycin and other glycopeptides inhibit cell wall synthesis by non-covalent binding to the D-Ala-D-Ala peptide motif of the cell wall precursor lipid II [10]. Four hydrogen bonds are formed when vancomycin binds the D-Ala-D-Ala motif, and all of these involve the peptide backbone [1,10], as shown in Figure 1.2.

Dimerization of vancomycin and other glycopeptide antibiotics, as well as anchoring of glycopeptides in the bacterial cell membrane, contribute favorably to the

antibacterial effect [1]. Although eremomycin (**2**, Figure 1.3) has a lower affinity for model peptides *in vitro*, *in vivo* it is consistently more active than vancomycin. It seems that the actual activity of glycopeptide antibiotics depends on a balance between the dimerization constant and the affinity for model peptides *in vitro* [11]. The dimerization constant increases when ligand is added and *vice versa* [12], except in the case of ristocetin A [11] (**3**, Figure 1.3). Anchoring of the antibiotic to the bacterial cell wall by a lipophilic chain, as is the case with teicoplanin (**4**, Figure 1.3), also enhances antibacterial activity [13]. In both cases the enhanced activity can be explained by a decrease in entropy of binding, because the binding of the antibiotic to the target peptide becomes effectively intramolecular [1,12,13]. Cell wall synthesis may be inhibited because the enzymes involved in this can no longer bind to the peptide [10].

Resistance to vancomycin can follow three patterns [14]. In both VanA- and VanB-type resistance, the terminal D-alanine residue of the peptidoglycan cell wall precursor is replaced by a D-lactate. Because this changes the amide bond into an ester, a hydrogen bond donor is lost and repulsion between the carbonyl oxygen and lactate-ester oxygen is present, resulting in a thousand-fold loss of affinity (Figure 1.2). The VanA Enterococci (*E. faecium, E. faecalis*) are resistant to high concentrations of vancomycin and also clinically used teicoplanin (**4**), whereas VanB Enterococci are resistant to a wider range of vancomycin concentrations but remain susceptible to teicoplanin [14]. In VanC-type resistance (*E. gallinarum, E. casseliflavus*), the terminal amino acid D-alanine is replaced by D-serine, and loss of activity is probably due to steric hindrance from the serine side chain [1,14]. Knowing the molecular nature of resistance may be very important for the development of new antibiotics to fight resistant micro-organisms.

Although the total syntheses of vancomycin and derivatives can be heralded as top achievements in organic synthesis [1], they are of course not practical for obtaining these compounds for application purposes. Therefore, there has been considerable interest in the preparation of simplified mimics of vancomycin, having especially (part of) the binding cavity for D-Ala-D-Ala.

With respect to this, Ellman and coworkers [16], Zhu and coworkers [17], Arnusch and Pieters [18], and Liskamp and coworkers [19] have prepared (monocyclic) mimics of the *D-E* part of the cavity of these antibiotics *via* an intramolecular nucleophilic aromatic substitution [16–18] or a Sonogashira-based macrocyclization [19] (Figure 1.4). Recently, a bicyclic mimic of the *C-D-E* cavity, which was prepared by a Stille reaction followed by tandem ring-closing metathesis (**9**, Figure 1.4), was described by Liskamp and coworkers [20]. Considerable challenges lie ahead for the synthetic chemist in order to develop practical syntheses of mimics of vancomycin capable of binding not only D-Ala-D-Ala, but also cell wall parts of resistant bacteria, i.e. D-Ala-D-lactate.

1.2.2
Lantibiotic: Nisin

Nisin is a peptide-based antibiotic from the lantibiotic (*lant*hionine-containing an*tibiotic*) family [2]. It is produced by certain strains of *Lactococcus lactis* and is active

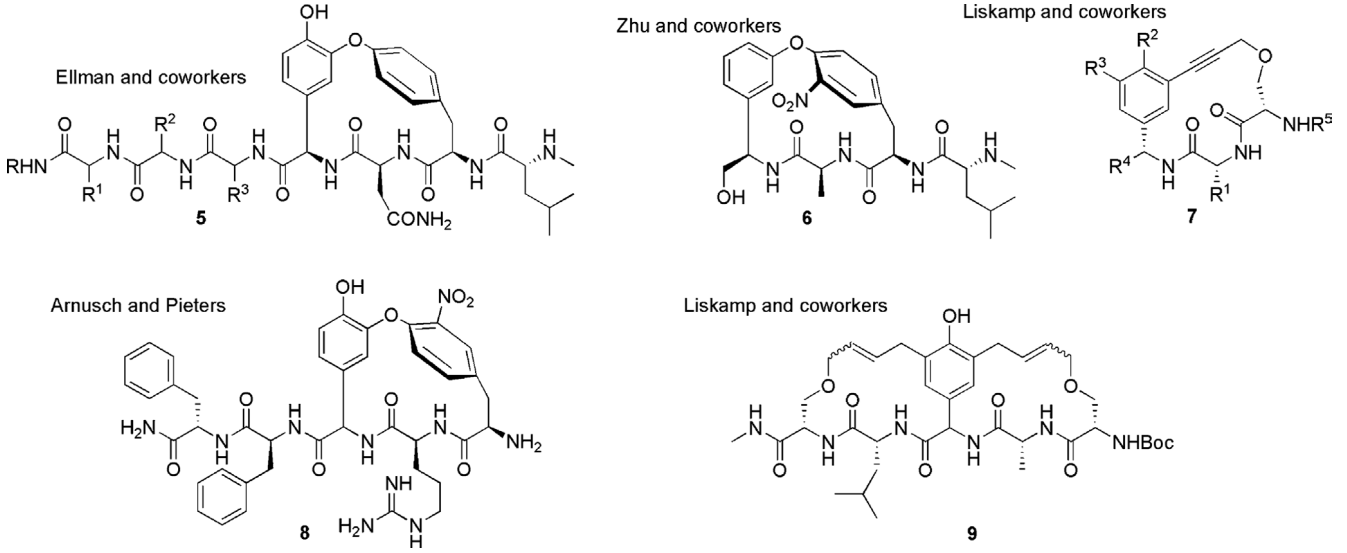

Figure 1.4 Structures of vancomycin mimics as described by Ellman and coworkers (**5**) [16], Zhu and coworkers (**6**) [17], Arnusch and Pieters (**8**) [18], and Liskamp and coworkers (**7**) [19] and (**9**) [20].

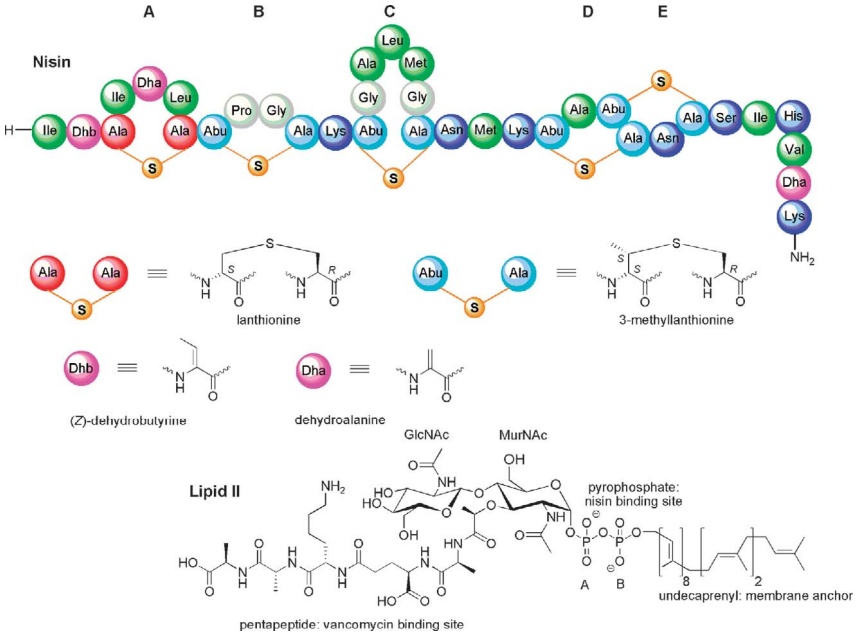

Figure 1.5 Representation of the structure of nisin. Hydrophobic amino acid residues are shown in green, polar residues in blue, and unsaturated amino acids in purple. The lanthionine ring is shown in red and the 3-methyllanthionine rings in turquoise. The structural formulas of lanthionine, 3-methyllanthionine, dehydroalanine, and (*Z*)-dehydrobutyrine are also shown. The chemical structure of lipid II is shown, and the binding sites of vancomycin and nisin are indicated.

against most Gram-positive bacteria. Its antimicrobial effect has been long known, and nisin has been used as a food preservative for over 30 years. Its structure [21] is characterized by several connected cyclic peptides, including an annulated cyclic peptide system, providing the peptide with a unique shape (or shapes) which could not have been achieved by the corresponding linear sequences (Figure 1.5). Like other antimicrobial peptides, e.g., magainin, nisin has a net positive charge and an amphiphatic character. In addition to the cyclic peptide structures that are so characteristic for the lantibiotics, it contains the unusual amino acids lanthionine and 3-methyllanthionine as well as the unsaturated amino acids dehydroalanine and (*Z*)-dehydrobutyrine (Figure 1.5).

In 1960, Ramseier [22] discovered that nisin causes leakage of intracellular molecules from cells. Later, it was shown that it disturbs the membrane potential and interferes with energy transduction [23]. In addition, it causes inhibition of biosynthesis of the cell wall processes by blocking the synthesis of peptidoglycans [24] and by binding to the precursor lipid II [25]. However, micromolar amounts of nisin are needed to permeate artificial membranes [26,27] or to inhibit cell wall synthesis *in vitro* [28], while the *in vivo* activity of nisin is in the nanomolar range. As vancomycin, which binds to the peptide motif in lipid II, inhibited the antibacterial activity and

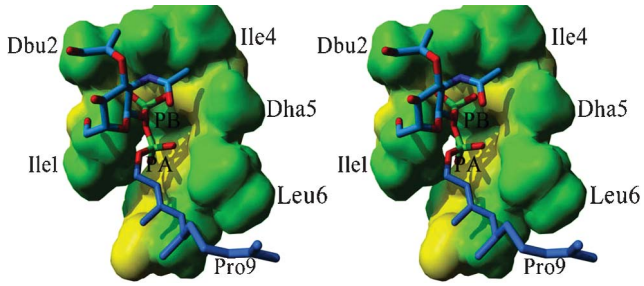

Figure 1.6 The pyrophosphate cage structure of nisin bound to lipid II. Side chains of nisin are depicted in green, backbone in yellow. Lipid II is depicted in blue [PDB entry code: 1WCO [32], molecular graphics created with YASARA (www.yasara.org) and PovRay (www.povray.org)].

membrane leakage by nisin in intact cells, Breukink *et al.* [26] concluded that lipid II is necessary for a high specific nisin activity and resulting pore formation. Studies with artificial membranes containing lipid II confirmed this conclusion [26].

The N-terminal fragment of nisin (1–12) has been shown to act as a nisin antagonist [29]. Mutation data have shown that changes in the N-terminal fragment reduced nisin activity [30]. NMR data showed that solvent accessibility of the A, B, and C rings of nisin decreased when it bound lipid II [31]. These data independently led to the conclusion that the N-terminal fragment of nisin, notably the ring structure A to C, is involved in lipid II binding. This was confirmed when the NMR structure of the nisin-lipid II complex was determined [32]. Rings A and B of nisin form a cage-like structure around the pyrophosphate moiety of lipid II. This structure shows that hydrogen bonds are formed between backbone amides of nisin and phosphate oxygens, while side chain interactions are of only minor importance. Only leucine residue six is conserved as a hydrophobic residue and interacts with the prenyl chain of lipid II (Figure 1.6).

In a model for pore formation, Hsu *et al.* proposed that after initial binding of nisin to the lipid II pyrophosphate, the C-ring embeds in the membrane, followed by a turn around the flexible hinge region and insertion of the C-terminus [31]. The size of these pores was calculated to be about 2 nm [33]. Studies with pyrene-labeled lipid II enabled Hasper *et al.* to calculate that a single nisin-lipid II pore consists of four lipid II molecules and eight nisin molecules (Figure 1.7). The pores are highly stable, even on addition of detergents that cause disruption of membranes [34].

Other lantibiotics that do not have the ability to form pores and indeed do not cause cell leakage have nevertheless still an impressive antibacterial effect *in vivo*. With respect to this, an alternative mechanism of antibacterial activity was recently described by which members of the lantibiotic family kill Gram-positive bacteria by binding lipid II and removing it from the cell division site (or septum) and thus block cell wall synthesis [35].

The cyclic peptide systems in nisin can be considered as side chain-cyclized peptide derivatives. However, they do not contain *disulfide* bridges, which is the most common

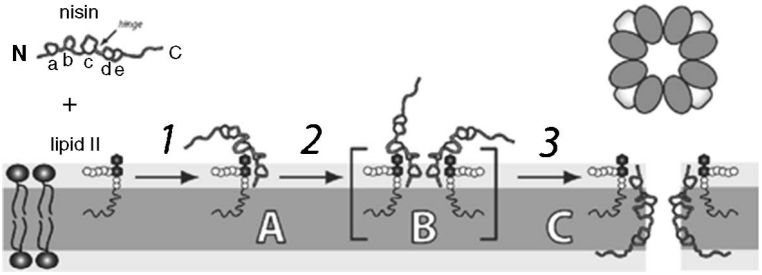

Figure 1.7 Interaction of nisin with phospholipid model membranes: mechanism of pore formation (reprinted with permission from Ref. [34]. Copyright 2007 © American Chemical Society).

way of generating side chain-cyclized peptides. In contrast they have *sulfide* or *thioether* bridges as part of their ring structures, which are introduced biosynthetically by post-translational modification. This rather unusual cyclization moiety enticed us to start investigating whether also "bridges" other than thioether bridges could be introduced as cyclization elements for obtaining mimics of the nisin ring structures [36]. Attention was directed towards designing and synthesizing alkene, alkyne, and alkane mimics of the thioether bridge, using ring-closing alkene and alkyne metathesis, sometimes followed by hydrogenation. Thus mimics of the A (**10**, **11**, and **14**), AB (**12**), C, and D/E (**16**) ring systems were prepared (Scheme 1.1). Apparently, the backbone structure of the D/E ring system has a predisposition towards a knotted ring structure, since it was possible to obtain an alkene mimic (**16**) directly in good yield (95%) from a double ring closing metathesis reaction of the tetra-allylglycine containing linear precursor (**15**) in a single reaction step [36d] (Scheme 1.1).

Based on the pyrophosphate cage structure of nisin bound to lipid II, a tricyclic mimic was designed and synthesized in which a lactam bridge connects the B-ring mimic with the A-ring mimic. The biological activity data are very promising and justify further improvement of the designed lipid II pyrophosphate binders. The inherent flexibility of a peptide, even when it is cyclized, challenges us to control its shape by alternative constraints to those already present.

1.2.3 Cyclosporin A

Cyclosporin was isolated from the fungus *Tolypocladium inflatum*. Its immunosuppressive activity was discovered in 1976 [4]. Although since then many analogs have been prepared and investigated, cyclosporin A (CsA, Sandimmune, **17**) remains the most effective cyclic peptide and is the major immunosuppressant drug to prevent graft rejection after transplant surgery.

CsA is a cyclic undecapeptide consisting completely of hydrophobic amino acids, as shown in Figure 1.8 [37]. Additional structural features are a threonine-derived butenyl-containing amino acid derivative as well as six *N*-methylated amino acid residues. In addition to reducing the proteolytic degradation rate and increasing the