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VOLUME E 22 d

SYNTHESIS OF PEPTIDES AND PEPTIDOMIMETICS

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Preface

As we approach the 100th anniversary of Emil Fischer's first synthesis of peptides, we bring together in a new presentation the wide variety of topics which demonstrate how far our field has grown and indicate the challenges that lie ahead. These four volumes cover the core of peptide and peptidomimetic chemistry and bring to peptide scientists, comprehensive and critical presentations in the *Houben–Weyl* tradition. Thus, the chapters are not encyclopedic, but rather focused on the most effective routes for peptide and peptidomimetic synthesis.

In 1974, Professor Erich Wünsch edited two *Houben–Weyl* volumes on the Synthesis of Peptides. That was a monumental undertaking and provided a resource widely used by chemists to prepare peptides and their derivatives with maximum attention to yield and purity of products. The current volumes continue these principles but encompass a much larger effort in the area of the organic chemistry of peptides and peptidomimetics. We as editors followed the *Houben–Weyl* concept of emphasizing protocols and reactions. Procedures for the often-used protections, deprotections, activations, and peptide and peptidomimetic bond formations remain the critical theme in all of the chapters. In addition, we make certain that there is a continuity that joins ongoing productivity in research to the accomplishments of the pioneers of our field. This format sets the stage for future scientists to undertake important research.

The founders of the field of peptide research paid great attention to methodology and isolation of products. The development of analytical and spectroscopic tools has enabled peptide scientists to create molecules of incredible variety and purity. Current researchers are in many ways mainly concerned with the interface between organic and biological chemistry. Thus, there is a strong emphasis on biologically interesting peptides and peptidomimetics in our volumes. However, the protocols and methodology covered in these volumes can also be applied to design novel materials, nanostructures, liquid crystals, and molecular sensors. Peptides and peptidomimetics represent structures applicable to broad vistas of molecular designs.

Recent developments in nucleic acids and protein sciences have allowed researchers to unravel the mechanisms of the biological action of many peptide ligands. As a consequence, many analogues and mimetics of natural ligands have been designed and synthesized. In these volumes we have paid substantial attention to these advances. With the exciting developments in genomics, proteomics, and pharmaconomics, the field of peptide and peptidomimetic research is expanding into new areas of molecular diversity. As a result, peptides remain a key component of the molecules of life.

The four volumes simultaneously represent a tribute to the pioneers in our field led by Emil Fischer and to the key figures who guided us more recently including Vincent du Vigneaud and Bruce Merrifield. We also acknowledge the hard work and creativity of all the scientists who have made substantial contributions to the chemistry of peptides and peptidomimetics. They are too numerous to name here. Nearly one hundred authors wrote chapters to create these volumes. Finally, the volumes are a challenge for future scientists to continue to make exciting discoveries as the new century unfolds.

The volumes are divided into sections covering a broad spectrum of reactions involved in peptide chemistry, synthesis of building blocks and target structures. Volume E 22a covers the protection of main-chain and side-chain functional groups, methods of peptide bond formation, and aspects of peptide synthesis in solution and on solid supports. Volume E 22b includes synthesis of large peptides and proteins. Specific methods are illustrated for the preparation of cysteine peptides, peptidomimetics, and conjugates such as glycopeptides, lipopeptides, phosphopeptides, and sulfated peptides. Cyclic peptides are extensively treated in this section. Volume E 22b concludes with a section on purification, analysis, and spec-

trometry of peptidic structures. Quality standards and comparative syntheses are also presented. Volume E 22c deals with peptidomimetic structures including side-chain-modified and main-chain-altered structures. In this volume, secondary structure inducing mimetics and scaffolds are treated. The synthesis of peptide nucleic acid (PNA) structures is also contained in this volume. The final volume of the series, E 22d deals with *de novo* designed peptide-like molecules including template-assisted formation of helices, sheets, and loops. In addition, coiled coils are covered. Reactive peptides are presented. These molecules are composed of peptidic structures containing a wide variety of reactive groups for biologically important targets. The series concludes with a chapter on peptide natural products which are illustrative of nature's formulation of biologically active peptides and peptidomimetics. These are points of departure for peptide chemists to design and synthesize analogues and mimetics.

This series on the synthetic aspects of peptides and peptidomimetics could not have come about without the dedication and commitment of my three coeditors, Arthur Felix, Luis Moroder, and Claudio Toniolo. Each brought a wealth of experience, insight, and knowledge to formulate the scope of the volumes and critically review each of the chapters. In addition, the coeditors and I wish to thank Dr. Guido F. Herrmann for his unflagging support throughout the project. The professional staff of Thieme, led by Dr. Fiona Shortt, have worked tirelessly to ensure that the volumes appear with great attention to detail consistent with the *Houben–Weyl* tradition.

As a final note, the success of this venture rests with you the scientists throughout the world. We will know if we have created a useful set of volumes by the attention paid to them. If we have succeeded, there can be no greater satisfaction.

Murray Goodman
Editor-in-Chief
San Diego, USA
April 2001

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Volume E 22d

Introduction to Volume E 22d

M. Goodman and A. Felix

This volume focuses on extensions of peptide chemistry into the novel areas of template-conjugated peptides, helical arrays of polypeptides, dendrimers, and C-terminally modified peptides. The volume concludes with an overview of the classic syntheses of representative peptide natural products.

The discussion of these fields commences with the template approach to peptide assemblies.[1] Many molecular families can serve as templates, including cyclic peptides and related structures (Section 13.1.1). Derivatives of tetrahydronaphthalene can function as β-turn inducers (Section 13.1.2). After the discussion of peptide-based templates, nonpeptide templates are covered (Section 13.1.3). These include porphyrins, pseudopeptides, macrocyclic compounds, calixarenes, cavitands, and cyclodextrins. These follow a major presentation dealing with template-assembled synthetic proteins (TASP) (Section 13.1.2).[2,3] The details and strategies for the preparation of these structures are covered including solid-phase syntheses and convergent syntheses in solution. A creative use of pseudoprolines and other constraining building blocks is described (Section 13.1.2.3.2) as is the use of ligation reactions (Section 13.1.2.4).

Applications of the TASP approach are then considered for such aspects as four-helical bundles (Section 13.1.2.4.2).[4] The details of structures and synthetic routes for the preparation of the bundles are described. Other chain assemblies and applications are discussed (Section 13.1.2.5) including metal-ion assisted self assemblies, disulfide linkages in the formation of four-helical bundles (Section 13.1.2.5.2), multiple antigenic peptide systems (Section 13.1.2.5.4), and templates constructed from a trialdehyde template by reductive amination to form a three-helical bundle (Section 13.1.2.5.5).[5]

Section 13.1 concludes with a consideration of some additional protein mimetic structures (Section 13.1.3). This part deals primarily with the development of a molecular tool kit that can lead to locked-in folds, bundles, and other molecular arrays (Section 13.1.3.1). An application leading to zinc finger mimetics is also presented (Section 13.1.3.1.2).[6]

Helical modes represent a most significant structural motif in biology. The α-helical coiled coil is nature's route for dimerizations.[7] Such molecules can interact with DNA and function as important components of many biological systems (Section 13.2). The synthesis of coiled coils in solution and by segment condensation is described (Sections 13.2.1.1 and 13.2.1.2). There follows a presentation of the structure and chemistries utilized in the formation of coiled coils (Section 13.2.2). Thiol and disulfide-bridge chemistries are employed (Sections 13.2.2.1.1, 13.2.2.2, and 13.2.2.3), as are lactam bridges (Section 13.2.3).[8]

The syntheses of heterostranded coiled coils are presented (Section 13.2.2.4), as are templates and conformationally defined peptide libraries[9] (Sections 13.2.4 and 13.2.4.1). Finally, Section 13.1 concludes with a presentation of the methods for characterization of the coiled coils including circular dichroism, microcalorimetry, size-exclusion chromatography, and analytical ultracentrifugation (Section 13.2.5).

The design and synthesis of cyclic β-sheet structures is also covered (Section 13.3).[10] Details of synthetic procedures to obtain the cyclic peptides are described (Section 13.3.1) as are considerations of racemization during synthesis and cyclization (Section 13.3.1.1). The remaining parts of this section deal with characterization of cyclic β-sheet structures (Section 13.3.2) including HPLC (Section 13.3.2.1) circular dichroism (Section 13.3.2.2), and NMR analysis (Section 13.3.3.2.3).[11,12]

Peptide dendrimers and related structures are covered in Section 14.1. They represent an important extension of peptide synthesis into novel structures.[13,14] Following the definition of dendrimers and the scope of their applications, strategies for the design and synthesis are described (Section 14.1.2). The routes include syntheses in solution (Section

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14.1.2.2.1) and solid-phase reactions (Section 14.1.2.2.2). Specific derivatizations involving chloroacetylation and thiolate dendrons are presented (Sections 14.1.2.2.3 and 14.1.2.2.4). Cores based on polyglutamic acid lead to dendrons containing multiple arrays of carboxy derivatives (Section 14.1.2.3).

Divergent and convergent strategies are outlined which are followed by an extensive description of the synthetic steps involved in both approaches (Section 14.1.3). These include the use of protected amino acids and stepwise syntheses in divergent routes (Sections 14.1.4.1 and 14.1.4.2). This is followed by descriptions of convergent methods[15,16] (Section 14.1.5). Ligation chemistry has recently been applied to the synthesis of polypeptides using unprotected segments in solid-phase syntheses (Section 14.1.5.2).[17] Specific reactions are elucidated covering topics such as thiol ligation (Section 14.1.5.3), thiol–thiol exchange reactions (Section 14.1.5.3.2), imine ligation (Section 14.1.5.4), and ligations based on oxime, hydrazone, and thiazolidine formation (Section 14.1.5.5).

This section concludes with a description of tandem synthetic strategies (Section 14.1.6) in which sequential reactions involve combinations of steps such as dendrimer formations combined with cyclic peptides (Section 14.1.6.1), chemoselective cyclization combined with divergent syntheses (Section 14.1.6.3), and many thiol-based reactions and cyclizations (Sections 14.1.6.4–14.1.6.7).[18]

Polymeric polypeptides have long been the focus of many research groups and are, therefore, covered in Section 14.2. Early on, these polymers were considered to be protein model structures. Discussions of homopolypeptides prepared by α-amino acid *N*-carboxy-anhydride (NCA) polymerizations are an important part of the volume (Sections 14.2, 14.2.1, and 14.2.1.1).[19,20] Copolypeptides are prepared by routes which lead to random or sequential copolymer structures (Sections 14.2.2 and 14.2.3).[21,22]

The remainder of Section 14 deals with triple helices and collagen mimetic structures (Sections 14.2.3.1 and 14.2.4). A description of routes is included which covers the synthesis of triple-helical structures using template (scaffolds) from a Lys-Lys dimer (Section 14.2.4.1), Glu-Glu dimer (Section 14.2.4.2), and appropriate Cys-Cys branches (Section 14.2.4.3). The syntheses were carried out using solid-phase techniques. A scaffold (template) using the Kemp triacid is also presented (Section 14.2.4.4). Lastly, a chemoselective ligation is presented which joins appropriately defined reaction sites (Section 14.2.4.5).[23]

Recent developments in chemical synthesis have provided important technological advances for the replacement of the C-terminal carboxylic acid group in peptides. These modifications are covered in Section 15. Several C-terminal peptide aldehydes occur naturally and many serve as protease inhibitors and have potential as therapeutic agents for a wide range of disorders.[24] C-Terminal aldehydes, usually prepared by the diisobutylaluminum hydride reduction of alkyl esters (Section 15.1.1), represent an important class of peptides as they have been used for the design of transition-state inhibitors for serine proteases.[25] Other methods for synthesizing C-terminal peptide aldehydes have been developed including oxidation of peptide alcohols and reduction of *N*-methoxy-*N*-methylamides (Weinreb amides). This latter method has been applied to the solid-phase synthesis of peptide aldehydes in good yield and optical purity.[26]

C-Terminal ketone derivatives of peptides have been used as effective inhibitors for a variety of proteases including serine, cysteine, and aspartyl proteases.[27] This class of peptides includes diazomethyl ketones (Section 15.1.2), halomethyl ketones (Section 15.1.3), and fluoromethyl ketones (Section 15.1.4). In general, the N^{α}-amino group and side chain must be protected. The diazomethyl ketones serve as good intermediates for conversion into chloromethyl and bromomethyl ketones. Fluoromethyl ketones, the most widely known class of peptide haloketones, can also be prepared from diazomethyl ketones or by halogen-exchange reactions. Other methods for the synthesis of fluoromethyl ketones are described in Section 15.1.4.

Peptide α-oxo acids, α-oxo esters, and α-oxoamides are also potent inhibitors of cysteine and serine proteases. Oxidation of peptide α-substituted carboxylic acid derivatives provides a general route to these compounds (Section 15.1.5). Peptide hydroxamic acids have been shown to be inhibitors of metalloproteinase and some have been reported to have antibiotic, anticarcinogenic, and antiviral activities. Peptide hydroxamic acids may be prepared by solution and solid-phase methods using a variety of resins (Section 15.1.6). α-Aminoboronic acids may be prepared by several routes and are reported to be inhibitors of aminopeptidases. Procedures have been developed for their incorporation into peptides (Section 15.1.7).

Peptides with C-terminal phosphonates, initially reported to have antibacterial properties, have also been found to possess inhibitory properties toward serine proteases.[28] The synthesis of peptide phosphonates (Section 15.1.8) usually requires protection of the phosphonic moiety as a diester, followed by selective deprotection in the final stage. The importance of peptide thiols (Section 15.1.9) is exemplified by captopril, an orally active angiotensin converting enzyme inhibitor used as a treatment for hypertension.[29] These peptide thiols are prepared by the reaction of sulfanylalkanoyl amino acids with α-amino esters followed by deprotection of carboxy and sulfanyl groups. Other peptide thiols have been reported to be inhibitors of zinc metalloproteases, collagenases, and aminopeptidases.

Peptide thioesters (Section 15.1.10) are generally prepared by coupling protected amino acids or peptides with thiols and are used for enzymatic hydrolysis. Peptide dithioesters, used to study the structures of endothiopeptides (Section 15.1.11), may be prepared by the reaction of peptide nitriles with thiols followed by thiolysis (Pinner reaction). Peptide vinyl sulfones (Section 15.1.12), inhibitors of various cysteine proteases, are prepared from N-protected C-terminal aldehydes with sulfonylphosphonates. Peptide nitriles (Section 15.1.13) prepared by dehydration of peptide amides, acylation of α-amino nitriles, or the reaction of Mannich adducts with alkali cyanides, are relatively weak inhibitors of serine proteases.

In Section 15, details are provided for the chemical modification of peptide C-terminal carboxylic acids. Many of these C-terminally modified peptides possess important biological activities and the wide range of experimental procedures may be applicable to the synthesis of novel peptide target structures.

The field of peptide synthesis is never far removed from the study of natural products, many of which exhibit peptide and peptidomimetic structures. It is fitting that this treatise be concluded with a section on the synthesis of key peptide-based natural products. Section 16 commences with the synthesis of bacitracin,[30] a cyclic peptide with antibiotic properties. The synthesis of this target structure is carried out in solution and by solid-phase chemistry. The molecule contains a lactam-bridged cyclic heptapeptide with a pendent tripeptide (Section 16.1.1). An elegant route to the synthesis of the thiazoline building block is included.

Bleomycin (Section 16.1.2) represents a group of glycopeptides with significant antitumor properties.[31] It is thought to act by degrading DNA selectively. Bleomycin is a complicated cyclic molecule of a peptidomimetic chain containing thiazoline β-hydroxy-L-histidine and an aminopyrimidine derivative. A disaccharide is conjugated to the hydroxy group of the β-hydroxy-L-histidine residue. The synthesis carried out in solution involves three components (Section 16.1.2). The C-terminal tetrapeptide is allowed to react with (−)-pyrimidoblamic acid while the disaccharide is linked to the hydroxyhistidine. All of these are then assembled to form bleomycin A_2.

Cyclosporin (Section 16.1.3) is an undecapeptide that acts as a potent immunosuppressant. Seven of its eleven residues are N-methylated. Its synthesis in solution was carried out by a series of segment condensations.[32,33] The didemnins (Section 16.1.4) are isolated from marine organisms known as tunicates. Their therapeutic potential lies in applications as antitumor, antiviral, and immunosuppressant agents. The didemnin family of natural products contains peptidomimetic residues including *N*,*O*-dimethyltyrosine–proline and leucine–2-(hydroxyisovaleryl)propionic acid and (4-hydroxy)-2,5-dimethyl-3-oxo-

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hexanoic acid. These segments are joined together in a well-designed strategy to prepare didemnin A.[34]

The microcystins (Section 16.1.5) are inhibitors of Ser-Thr phosphatases and are therefore part of the signaling processes. They are cyclic heptapeptides, containing key peptidomimetic residues. The synthesis of the segments for the preparation of microcystin LA is described in this section. The subunits are finally assembled to complete the synthesis of the product identical in all respects to naturally occurring microcystin LA. Following a retrosynthetic analysis, the preparation of segments containing the dehydropeptide and the methylated aspartic acid is described. Another segment includes the "Adda" residue followed by an iso-D-glutamic acid residue. The synthesis is accomplished according to the strategy shown in Scheme 12.[35]

Polymyxin B_1 (Section 16.1.6) is a cyclic heptapeptide with a pendent tripeptide segment that has topical antibiotic properties. The solid-phase synthesis is carried out with appropriate side-chain protections. The linear heptapeptide is removed from the resin and cyclized to yield the polymyxin B_1. The synthesis of the peptidomimetic (*S*)-6-methyloctanoic acid is also outlined.[36]

Vancomycin (Section 16.1.7) represents a major part of the presentation of Section 16. Vancomycin is a heptapeptide-like structure with interlocking macrocyclic ring systems linked to a disaccharide. Three representative syntheses of vancomycin aglycon are presented. They include a most impressive total synthesis from the Evans Group (Section 16.1.7.1),[37,38] the Nicolaou Group (Section 16.1.7.2),[39] and the Boger Group (Section 16.1.3).[40] Finally, the chapter is concluded with the Nicolaou total synthesis of vancomycin.[41]

The successful syntheses described above elucidate the strategies for the total synthesis of peptide-like natural products. These syntheses set the stage for challenges in peptide synthesis that will certainly arise from the world of genomics and proteonomics.

[1] Mutter, M.; Vuilleumier, S., *Angew. Chem.*, (1989) **101**, 551; *Angew. Chem. Int. Ed. Engl.*, (1989) **28**, 535.

[2] Mutter, M.; Tuchscherer, G., *Cell. Mol. Life Sci.*, (1997) **53**, 851.

[3] Mutter, M.; Tuchscherer, G.; Miller, C.; Altmann, K.-H.; Carey, R. I.; Wyss, D. F.; Labhardt, A. M.; Rivier, J. E., *J. Am. Chem. Soc.*, (1992) **114**, 1463.

[4] Dawson, P. E.; Kent, S. B. H., *J. Am. Chem. Soc.*, (1993) **115**, 7263.

[5] Tahmassabi, D. C.; Sasaki, T., *J. Org. Chem.*, (1998) **63**, 728.

[6] Tuchscherer, G.; Lehmann, C.; Mathieu, M., *Angew. Chem.*, (1998) **110**, 3160; *Angew. Chem. Int. Ed.*, (1998) **37**, 2990.

[7] Kohn, W. D.; Hodges, R. S., *Trends Biotechnol.*, (1998) **16**, 379.

[8] Houston, M. E., Jr.; Gannon, C. L.; Kay, C. M.; Hodges, R. S., *J. Pept. Sci.*, (1995) **1**, 274.

[9] Houghten, R. A.; Pinilla, C.; Blondelle, S. E.; Appel, J. R.; Dooley, C.; Cuervo, H. H., *Nature (London)*, (1991) **354**, 84.

[10] Rackovsky, S.; Scheraga, H. A., *Proc. Natl. Acad. Sci. U.S.A.*, (1980) **77**, 6965.

[11] Kondejewski, L. H.; Jelokhani-Niaraki, M.; Farmer, S. W.; Lix, B.; Kay, C. M.; Sykes, B. D.; Hancock, R. E. W.; Hodges, R. S., *J. Biol. Chem.*, (1999) **274**, 13181.

[12] Wishart, D. S.; Sykes, B. D.; Richards, F. M., *Biochemistry*, (1992) **31**, 1647.

[13] Tam, J. P., *Proc. Natl. Acad. Sci. U.S.A.*, (1988) **85**, 5409.

[14] Tam, J. P., *J. Immunol. Methods*, (1996) **196**, 17.

[15] Tam, J. P.; Spetzler, J. C.; Liu, C.-F.; Rao, C.; Tam, J. P., *J. Am. Chem. Soc.*, (1996) **118**, 307.

[16] Merrifield, R. B., *J. Am. Chem. Soc.*, (1963) **85**, 2149.

[17] Liu, C.-F.; Tam, J. P., *J. Am. Chem. Soc.*, (1994) **116**, 4149.

[18] Zhang, L.; Tam, J. P., *J. Am. Chem. Soc.*, (1997) **119**, 2363.

[19] Blout, E. R.; Karlson, R. H., *J. Am. Chem. Soc.*, (1956) **78**, 941.

[20] Goodman, M.; Hutchison, J., *J. Am. Chem. Soc.*, (1966) **88**, 3627.

[21] Scheraga, H. A., *Pure Appl. Chem.*, (1978) **50**, 315.

[22] Hodges, R. S.; Saund, A. K.; Chong, P. C. S.; St. Pierre, S. A.; Reid, R. E., *J. Biol. Chem.*, (1981) **256**, 1214.

[23] Tanaka, T.; Wada, Y.; Nakamura, H.; Doi, T.; Imanishi, T.; Kodama, T., *FEBS Lett.*, (1993) **334**, 272.

[24] Umezawa, H., *Pure Appl. Chem.*, (1973) **33**, 129.

[25] Thompson, R. C., *Biochemistry*, (1973) **12**, 47.

[26] Stewart, D. E.; Sarkar, A.; Wampler, J. E., *J. Mol. Biol.*, (1990) **254**, 253.

[27] Babine, R. E.; Bender, S. L., *Chem. Rev.*, (1997) **97**, 1359.
[28] Oleksyszyn, J.; Powers, J. C., *Methods Enzymol.*, (1994) **244**, 423.
[29] Cushman, D. W.; Cheung, H. S.; Sabo, E. F.; Ondetti, M. A., *Biochemistry*, (1977) **16**, 5484.
[30] Lee, J.; Griffin, J. H., Nicas, T., *J. Org. Chem.*, (1996) **61**, 3983.
[31] Boger, D. L.; Honda, T., *J. Am. Chem. Soc.*, (1994) **116**, 5647.
[32] Wenger, R. M., *Helv. Chim. Acta*, (1983) **66**, 2672.
[33] Wenger, R. M., *Helv. Chim. Acta*, (1984) **67**, 502.
[34] Li, W.-R.; Ewing, W. R.; Harris, B. D.; Joullie, M. M., *J. Am. Chem. Soc.*, (1990) **112**, 7659.
[35] Humphrey, J. M.; Aggen, J. B.; Chamberlin, A. R., *J. Am. Chem. Soc.*, (1996) **118**, 11759.
[36] Sharma, S. K.; Wu, A. D.; Chandramouli, N.; Fotsch, C.; Kardash, G.; Bair, K. W., *J. Pept. Res.*, (1999) **53**, 501.
[37] Evans, D. A.; Wood, M. R.; Trotter, B. W.; Richardson, T. I.; Barrow, J. C.; Katz, J. L., *Angew. Chem.*, (1998) **110**, 2864; *Angew. Chem. Int. Ed.*, (1998) **37**, 2700.
[38] Evans, D. A.; Dinsmore, C. J.; Watson, P. S.; Wood, M. R.; Richardson, T. I.; Trotter, B. W.; Katz, J. L., *Angew. Chem.*, (1998) **110**, 2868; *Angew. Chem. Int. Ed.*, (1998) **37**, 2704.
[39] Nicolaou K. C.; Koumbis, A. E.; Takayanagi, M.; Natarajan, S.; Jain, N. F.; Bando, T.; Li, H.; Hugnes, R., *Chem.–Eur J.*, (1999) **5**, 2622.
[40] Boger, D. L.; Miyazaki, S.; Kim, S. H.; Wu, J. H.; Castle, S. L.; Loiseleur, O.; Jin, Q. *J. Am. Chem. Soc.*, (1999) **121**, 10004.
[41] Nicolaou, K. C.; Mitchell, H. J.; Jain, N. F.; Bando, T.; Hughes, R.; Winssinger, N.; Natarajan, S.; Koumbis, A. E., *Chem.–Eur. J.*, (1999) **5**, 2648.

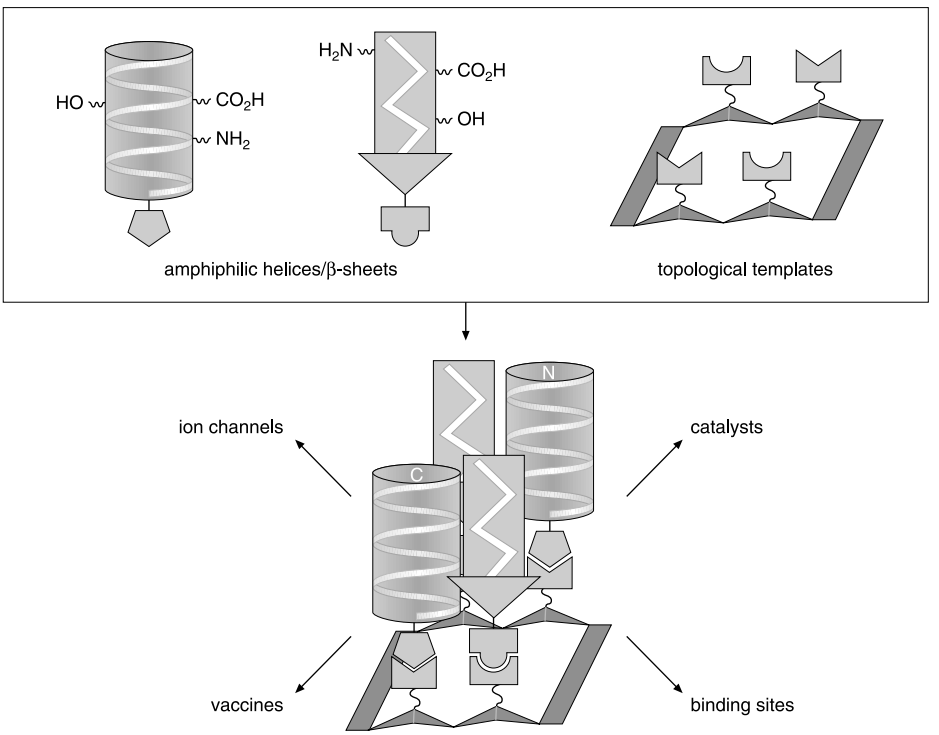
13 De Novo Peptide Structures

13.1 Template-Assembled Synthetic Proteins

G. Tuchscherer and M. Mutter

Protein design aims to mimic some of the structural and functional properties of native proteins.[1–6] The complexity of the folding mechanism, i.e. the pathway by which a linear polypeptide chain finds its unique three-dimensional structure, represents one of the most intriguing hurdles in this field of research. In order to bypass this well-known folding problem,[7–10] the construction of nonnative chain architectures with a high propensity for folding has been introduced (Scheme 1).[11–14] According to this concept, termed template-assembled synthetic proteins (TASP), topological templates[15–17] are used as built-in devices for directing covalently attached peptide blocks to a predetermined packing arrangement, resulting in branched chain architectures.

Scheme 1 The Concept of Template-Assembled Synthetic Proteins (TASP); Topological Templates Induce Folding of Covalently Attached Peptide Blocks into Predetermined Packing Arrangements[5,13,14]



The use of conformationally constrained molecules as templates by geometrically fixing the first amino acid in the proper orientation for helix or β-sheet initiation is one way to bypass the entropically unfavorable nucleation step in secondary structure formation. The amphiphilic character of such stabilized helical or β-sheet peptide blocks is the prerequisite for self-association in solution and the major driving force for formation of more complex packing topologies characteristic of proteins. As a key element, a topological template serves as a built-in device to induce and reinforce intramolecular interaction of the covalently attached amphipathic peptide blocks, thus leading to well-defined structural arrangements such as α-helical bundle or β-sheet TASP molecules. Typically, template molecules represent

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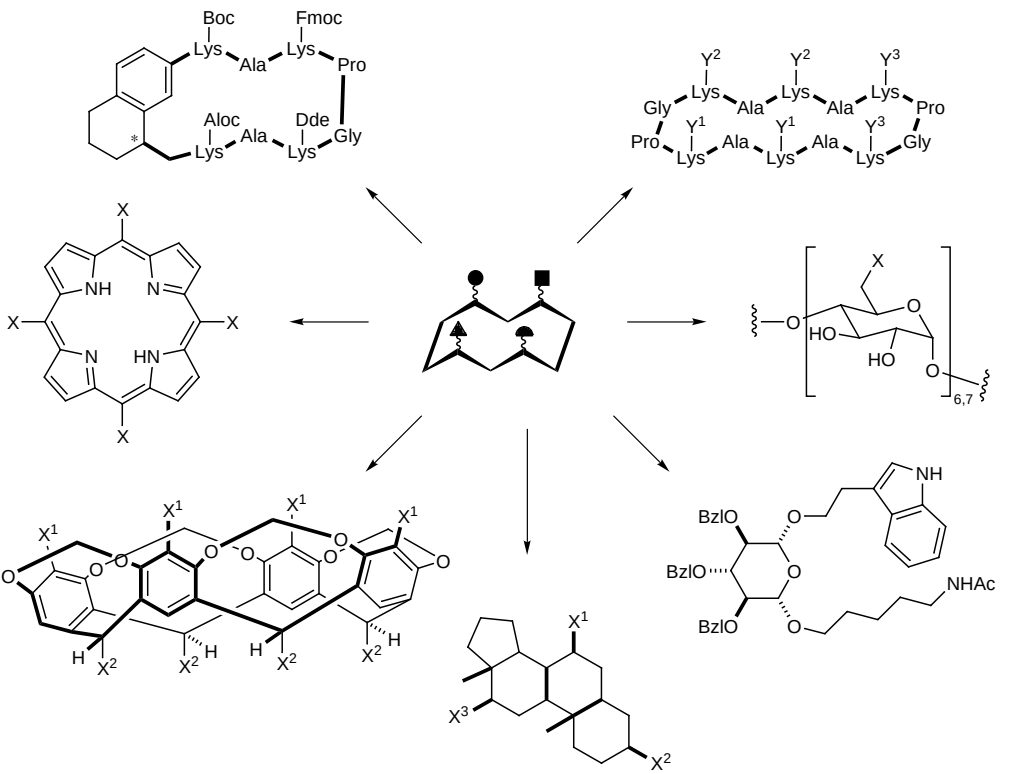
structural motifs such as constrained peptides, cyclodextrins, or polycyclic systems bearing selectivel addressable functional groups. As prototype template molecules in the TASP approach, cyclic decapeptides derived from the antibiotic gramicidin S, containing four lysines as attachment sites were used. As a second generation of this type of template, RAFT (regioselectively addressable functionalized template) molecules exhibit selectively addressable sites due to orthogonal protection techniques or unique chemical reactivity. Progress in the synthetic methodology for assembling peptides, i.e. chemoselective ligation methods[18–24] and orthogonal protection techniques, with special emphasis on progress in peptide synthesis and template design, allows some fundamental questions in protein assembly, structure, and function to be approached by designing protein mimetics of reduced structural and functional complexity.

13.1.1 Topological Templates

The use of topological templates (Scheme 2) to direct organic synthesis has a long-standing history[25–27] and becomes increasingly important as a versatile tool in peptide mimicry. Template molecules generally characterized as synthetic devices, that orient functional groups or structural units in well-defined spatial arrangements, are structural motifs such as constrained peptides,[14,17] cyclodextrins,[28] or polycyclic systems (e.g., porphyrins,[29,30] calixarenes,[31,32] etc.) bearing (selectively addressable) functional groups as attachment sites. The only requirements of these structural motifs is that they have an appropriate orientation of the attachment sites.

Scheme 2 Examples of Topological Templates: Cyclic Peptides,[14,17] Porphyrins,[29,30] Cyclodextrins,[28] Calixarenes[31,32]



13.1.1.1 Cyclic Peptides

In principle, any multifunctional molecule, e.g. peptides with conformational constraints, cyclic peptides, saccharides, polycyclic aromatic, or aliphatic systems with proper spatial arrangement of the attachment sites may serve as template molecules. In early studies of the TASP approach, linear oligopeptides with sequences derived from the cyclic antibiotic gramicidin S were used as templates. The crystal structure of gramicidin S[33] reveals a conformationally constrained cyclic molecule comprising two antiparallel β-sheet segments (Val-Orn-Leu) that are connected by two β-turn elements (D-Phe-Pro), thus offering the ideal structural geometry required for a TASP template. Molecular modeling studies[34] and X-ray analysis[147] on the cyclic decapeptides *c*[-Pro-Gly-Lys-Ala-Lys-Pro-Gly-Lys-Ala-Lys-] and decapeptides *c*[-D-Pro-Gly-Lys-Ala-Lys-D-Pro-Gly-Lys-Ala-Lys-] using gramicidin S as a modeling template, i.e. substituting the D-Phe-Pro turns by Pro-Gly or D-Pro-Gly and Val-Orn-Leu by Lys-Ala-Lys in gramicidin S, demonstrated that these molecules can adopt a low-energy conformation with the four lysine side chains directing to the same face and exhibiting the proper spacing for the construction, e.g. of a four-helix-bundle TASP molecule.[5]

13.1.1.1.1 Amide Bonds

For a prototype topological template, a linear decameric peptide template sequence is assembled following standard protocols for Fmoc-based peptide synthesis on solid support,[35] e.g. on a super acid labile polystyrene resin (SASRIN),[36] which yields a C-terminal carboxylic function for cyclization. In general, the protection for the ε-group of the four lysine side chains must be orthogonal to the base-labile N^{α}-Fmoc group and the acid-labile side-chain protection of other trifunctional amino acids to enable further functionalization of the template, e.g. attachment of functional groups or peptides. By using 2,4-diaminobutanoic acid, 1,3-diaminopropanoic acid, or a homocanaline derivative[22,37] instead of lysine, the length of the side-chain linkers can be varied and thus, the overall flexibility of the resulting TASP molecule tailored. Such cyclic peptides comprising four chemically identical attachment sites are prototype template molecules for the construction of, for example, four-helical bundle TASP molecules.

H-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-OH:[38]
The linear decapeptide was synthesized on Fmoc-Ala-SASRIN-resin (1.0 g, 0.67 mmol · g^{-1}) by repetitive deprotection/acylation steps. Fmoc cleavage was achieved by treating the peptide-resin with 20% piperidine/DMF (15 mL) for 5 min and a second time for 15 min. All amino acids were coupled for 45 min by using the Fmoc derivative (1.5 equiv), PyBOP (1.7 equiv), and DIPEA (5 equiv). After removal of the final N-terminal Fmoc group, the peptide-resin was treated several times (until the resin turned purple) with 1% TFA/CH_2Cl_2 (10 mL, 5 min each) and the filtrate immediately neutralized in an ice bath with an equal volume amount of 1.1% pyridine/CH_2Cl_2. After removal of the solvent, the residue was redissolved in a small amount of CH_2Cl_2 and the crude peptide precipitated with cold Et_2O. The precipitate was isolated by centrifugation, washed with cold Et_2O, and finally dried in vacuo; yield: 72%.

***c*[-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-Pro-Gly-Lys(Boc)-Ala-Lys(Boc)-]:**[38]
Cyclization of the peptide formed in the preceeding procedure (478 mg, 346 μmol) was performed in DMF (700 mL) at a concentration of 0.5 mM. DIPEA was added dropwise to adjust the pH to 8–9 and PyBOP (180 mg, 346 μmol) was added. The mixture was stirred for 1 h and the solvent removed in vacuo after completion of cyclization as monitored by analytical HPLC. The crude product was redissolved in EtOAc (100 mL), washed with 30% citric acid/H_2O (3 × 30 mL) and sat. NaCl (3 × 30 mL), and dried ($MgSO_4$). The organic phase was removed in vacuo, the residue redissolved in CH_2Cl_2, precipitated with cold Et_2O, and dried; yield: 655 mg (94%).

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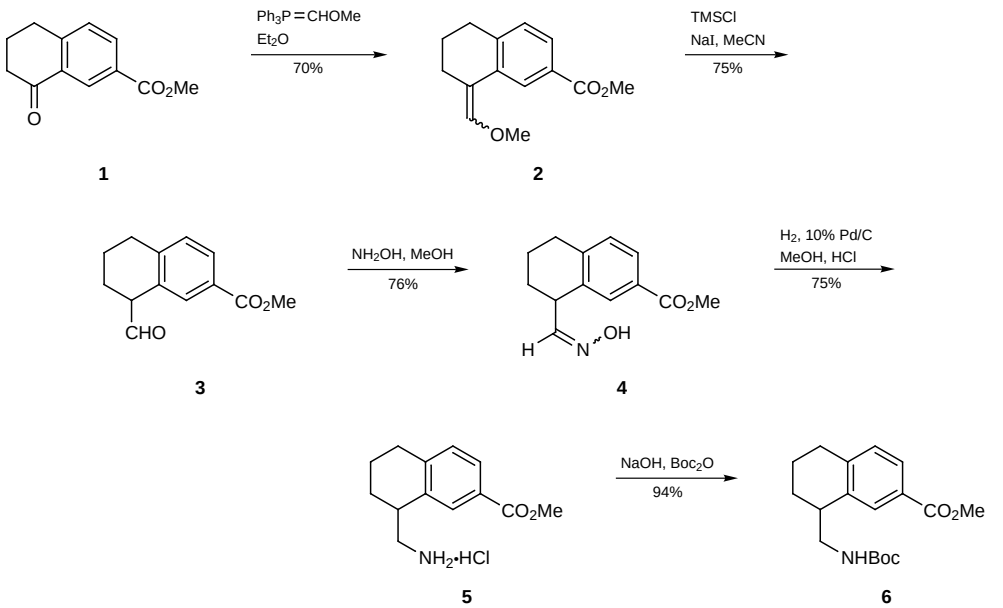
13.1.1.1.2 β-Turn Mimetic Containing Peptides

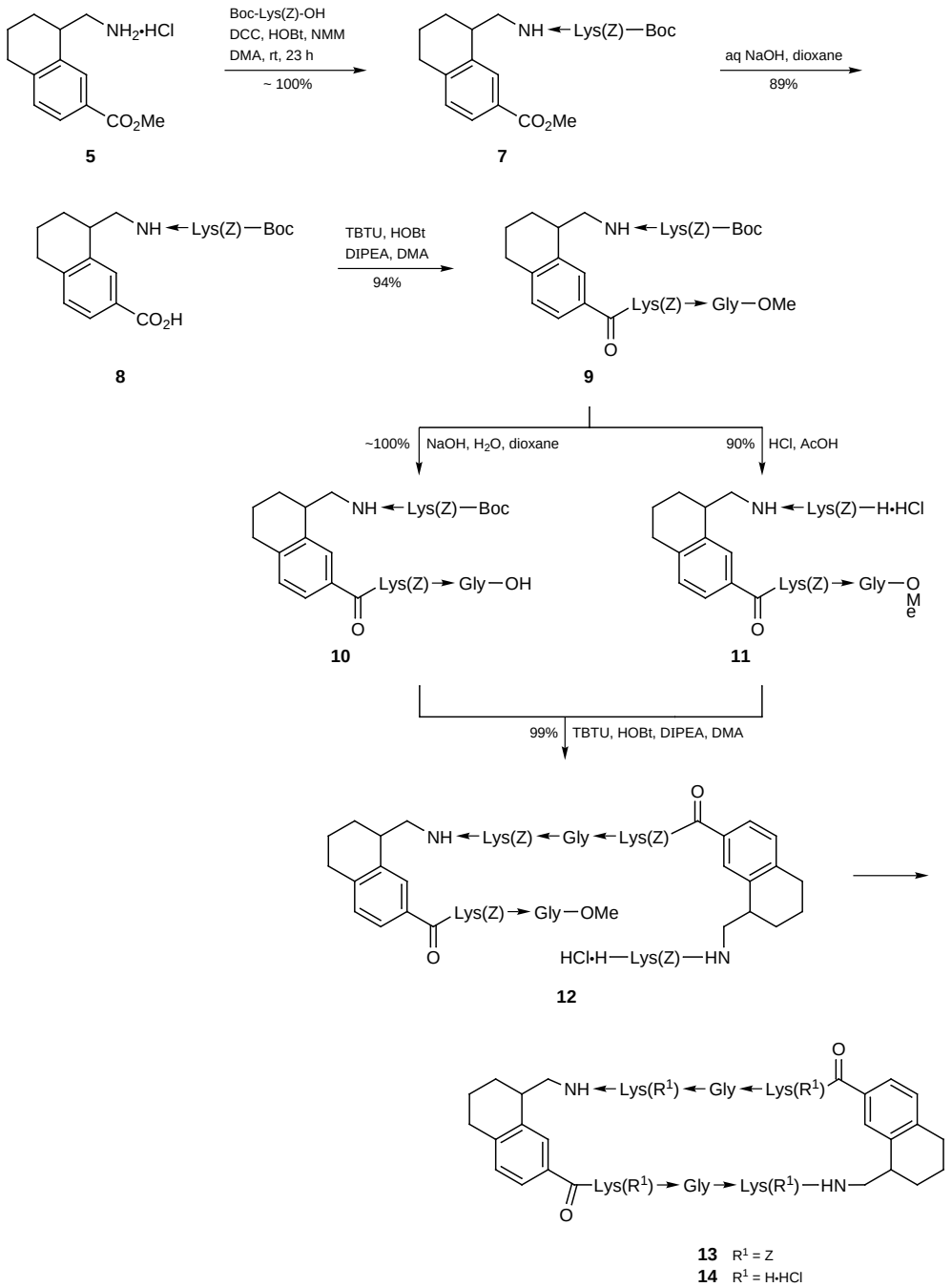
As a key feature of the TASP approach, the template is designed to direct and reinforce the folding of the covalently attached secondary structure elements in the predetermined tertiary structures (Scheme 1), e.g. a four α-helical bundle. The major purpose of artificial turn-inducing mimics is to constrain, when incorporated at the appropriate location, the peptide chain into a semi-rigid, defined, spatial arrangement.[39] 8-(Aminomethyl)-5,6,7,8-tetrahydro-2-naphthoic acid (Amhn) is designed to substitute for the central dipeptide unit of a reverse turn and is prepared in a five-step procedure starting from commercially available 4-phenylbutanoic acid[40] (Scheme 3).

Scheme 3 Synthesis of Methyl (*RS*)-8-[(*tert*-Butoxycarbonylamino)methyl]-5,6,7,8-tetrahydro-2-naphthoate[39]



For the synthesis of Amhn, the methyl ester **1** is first extended in a two-step process involving an isomeric mixture of the enol ethers **2** as intermediates to the aldehyde **3**. The corresponding oxime **4** is hydrogenated to give crystalline methyl ester hydrochloride **5** in its racemic form. For the incorporation into peptide chains, the mimic is transformed to the *N*-Boc **6** and *N*-Fmoc derivatives. For the methyl ester of racemic Boc-(*RS*)-Amhn **6**, a preparative chromatographic resolution procedure[41] on 3-methylbenzoyl cellulose beads[42] allows gram quantities of each enantiomer to be isolated in its optically pure form. The absolute configuration at the chiral center C8 was established by X-ray analysis of the (+)-camphor-10-sulfonate of the dextrorotatory amino ester with TFA; it was shown to be the *S*-enantiomer. The incorporation of the Amhn mimic into cyclic templates of the general type schematically shown in Scheme 2 in place of the dipeptide units involved in the turns, constrains the carriers to favorable conformations for the construction of TASP molecules. The cyclic template molecule shown in Scheme 4 features two identical, antiparallel tripeptide motifs connected at each end through the turn-inducing dipeptide mimic, thus forming templates suitable for the parallel attachment of four identical peptide fragments. All templates containing this type of turn mimetic are synthesized by classical methods in solution following a common synthetic pathway.

Scheme 4 Synthesis of Decamer Template Containing Two Amhn-β-Turn Mimetics[39]



A characteristic feature is the construction of the open-chain octapeptide intermediates **12** from two molecules of the tetrapeptides **9** that have been deblocked at their N-terminus (→ **11**) and at the C-terminus (→ **10**), respectively. In the synthesis of the octapeptide (*S*,*R*)-Amhn-**12**, one molecule of (*S*)-Amhn-**11** and of (*R*)-Amhn-**10**, each, is used. The tetrapeptide (*S*)-Amhn-**9** is prepared by a 2+2 condensation of Boc-Lys(Z)-(*S*)-Amhn-OH with HCl·H-Lys(Z)-Gly-OMe. The synthetic strategy uses N^{α}-Boc protection and DCC/HOBt

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activation throughout, except in the cyclization step where diphenyl phosphorazidate is used as activating agent. Hydrogenolysis (Pd/C in HCl/AcOH) of the Z-protecting groups affords the template **13** as tetrahydrochlorides, ready for use in TASP syntheses.

Synthesis of a Turn-Inducing Mimic:[39]

Methyl 8-Oxo-5,6,7,8-tetrahydro-2-naphthoate (1):
Esterification of 8-oxo-5,6,7,8-tetrahydro-2-naphthoic acid (285 g, 1.5 mol) was achieved by refluxing its soln in MeOH (2.8 L) containing concd H_2SO_4 (50 g). After 8 h, the solvent was removed and afforded crude, partially solid, dark-colored ester, which was decolorized in CH_2Cl_2/hexane on charcoal and further purified by crystallization and liquid chromatography of the mother liquor (Merck 60 silica gel, CH_2Cl_2/hexane 1:1); yield: 280.7 g (92%); mp 76–77 °C.

Methyl (*E*/*Z*)-8-(Methoxymethylene)-5,6,7,8-tetrahydro-2-naphthoate (2):
To a soln of the phosphorane prepared in situ from (methoxymethyl)triphenylphosphonium chloride (28.2 g, 82 mmol) in abs Et_2O (350 mL) and 1.6 M BuLi in hexane (47 mL, 75.2 mmol), a soln of of **1** (7.0 g, 34.3 mmol) in abs Et_2O (170 mL) was added dropwise at −30 °C. After another 30 min at −30 °C, the red mixture was stirred for 60 min at rt. Washing with 8% aq $NaHCO_3$ and concentration of the dried (Na_2CO_3) organic phase afforded an oily residue, which was submitted to flash chromatography (Merck 60 silica gel, 1.5 kg, toluene/EtOAc 9:1); yield: 5.6 g (70%); ratio (*E*/*Z*) 3:1 (by ^{1}H NMR); TLC (toluene/EtOAc 9:1) R_f 0.53 (single spot).

Methyl (*RS*)-8-Formyl-5,6,7,8-tetrahydro-2-naphthoate (3):
To a soln of **2** (3.0 g, 12.9 mmol) in 0.05 M NaI in MeCN (250 mL), TMSCl (1.56 mL, 12.9 mmol) was added dropwise at rt within 5 min under argon. After stirring for another 5 min at rt, the mixture was diluted with Et_2O and washed with 0.25 M $Na_2S_2O_3$. The aqueous phase was reextracted with Et_2O and the organic phase dried (Na_2SO_4) and the solvent removed to give 2.8 g of crude oil. Flash chromatography (Merck 60 silicia gel, 160 g, toluene/EtOAc 97:3) gave a yellowish oil; yield: 2.1 g (77%); TLC (toluene/EtOAc 9:1) R_f 0.42.

Methyl (*RS*,*E*/*Z*)-8-[(Hydroxyimino)methyl]-5,6,7,8-tetrahydro-2-naphthoate (4):
To a soln of **3** (6.4 g, 29.3 mmol) in MeOH (150 mL), 0.5 M NH_2OH/MeOH (60 mL) was added dropwise at rt over 10 min. After another hour at rt, the solvent was removed, the residue dissolved in CH_2Cl_2, and the organic phase washed with H_2O and sat. NaCl, dried (Na_2SO_4), and the solvent removed to give 6.93 g of slowly crystallizing oil. Chromatography (silica gel, 250 g, toluene/EtOAc 9:1) gave the product; yield: 5.2 g (76%); ratio (*E*/*Z*) 65:35 (by ^{1}H NMR); mp 84–87 °C; TLC (toluene/EtOAc 4:1) 2 spots, R_f 0.36 and 0.30.

Methyl (*RS*)-8-(Aminomethyl)-5,6,7,8-tetrahydro-2-naphthoate Hydrochloride (5):
A soln of **4** (1.50 g, 6.4 mmol) in MeOH (140 mL) containing excess HCl (added as 24% soln in MeOH, 2.8 mL) was hydrogenated, after addition of 5% Pd/C (330 mg), at 25 °C and at 1 atm H_2. After 20 h, the H_2 consumption stopped (92.5% of theoretical amount), the catalyst was filtered off, washed with MeOH, and the combined filtrates were concentrated to give white crystals. Crystallization (MeOH/Et_2O) afforded the product as white needles; total yield: 1.3 g (75%); mp 228–230 °C; TLC (AcOH/BuOH/H_2O 1:3:1) R_f 0.48.

Methyl (*RS*)-8-[(*tert*-Butoxycarbonylamino)methyl]-5,6,7,8-tetrahydro-2-naphthoate (6):
To a soln of **5** (25 g, 92.8 mmol) in dioxane (240 mL) and H_2O (120 mL), 2 M NaOH (50 mL) was added at 5 °C followed by Boc_2O (23.1 g, ca 106 mmol) in dioxane (70 mL). After several minutes of stirring at rt, the crystalline **6** started to separate. After a total of 2.5 h, it was extracted into Et_2O and the organic phase successively washed with 10% aq citric acid, 8% aq $NaHCO_3$, and brine, dried, and concentrated. The separating crystals were filtered off and washed (hexane/iPrOH 4:1). The filtrate was concentrated again and afforded, on treatment with hexane/iPrOH (4:1), a second crop of pure **6** as white needles; total yield: 28.8 g (97%); mp 151.0–152.1 °C; TLC (toluene/EtOAc 4:1) R_f 0.48.

Chromatographic Resolution of Racemic 6:
For the preparation of the chiral stationary phase (3-methylbenzoyl cellulose) and of the analytical HPLC columns, see ref[42]. The preparative column (glass column 5 cm i.d. × 75 cm; Büchi AG, Fläwil, Switzerland) was slurry-packed with a suspension of 3-methylbenzoyl-cellulose beads (550 g) in hexane/iPrOH (9:1). The glass column was topped with a column of the same dimension as a reservoir. After decantation of the material in the column, the reservoir was taken away and the stationary phase washed by pumping the eluent through the column equipped with an inlet plunger, at a flow rate of 60 mL · min^{-1}

until no more absorption was detected in UV at 254 nm. For analytical resolutions, a HPLC column (0.46 cm i.d. × 25 cm) was used with MeOH as mobile phase (separation and resolution factors, 1.49 and 2.06, respectively). For the preparative separation, a soln of racemate **6** (1 g) in hexane/iPrOH (6:4, 175 mL) was injected respectively and eluted with hexane/iPrOH 8:2 (flow rate 25 mL · min^{-1}, run time ca 6 h): (+)-(*S*)-**6** followed by (−)-(*R*)-**6**. (+)-(*S*)-**6**: mp 88.4–89.4 °C (Et_2O/pentane); $[\alpha]_D^{20}$ +2.4 ± 1. (−)-(*R*)-**6**: mp 87–88.9 °C (Et_2O/pentane); $[\alpha]_D^{20}$ −2.8 ± 1.

Synthesis of Template:[39]

Boc-Lys(Z)-Gly-OMe:
The dipeptide intermediate Boc-Lys(Z)-Gly-OMe was prepared from methyl glycinate hydrochloride (6.3 g, 50 mmol) and N^2-Boc-N^6-Z-L-lysine (19.0 g, 50 mmol) in THF (450 mL) using DCC (10.45 g, 1 equiv) and HOBt (8.45 g, 1 equiv) as condensing agent and NMM (5.5 mL, 1 equiv) as base. Usual workup after 20 h at rt and crystallization (EtOAc/Et_2O) gave fine, white needles; yield: 18.4 g (81%); mp 82.5–84 °C; TLC ($CHCl_3$/MeOH 9:1) R_f 0.60.

HCl · H-Lys(Z)-Gly-OMe:
Boc-Lys(Z)-Gly-OMe (17.0 g, 37.6 mmol) was stirred at rt for 10 min in 1.2 M HCl/AcOH (90 mL). The hydrochloride was precipitated with Et_2O (700 mL), filtered off, and washed with fresh Et_2O: white, crystalline powder; yield: 14.1 g (97%); mp 162–165.5 °C; TLC (AcOH/BuOH/H_2O 1:3:1) R_f 0.58.

Boc-Lys(Z)-(*S*)-Amhn-OMe (7):
Boc-Lys(Z)-(*S*)-Amhn-OMe was prepared from (*S*)-HCl · H-Amhn-OMe [(*S*)-**5**; 1.79 g, 7.0 mmol], as obtained from the Boc-Amhn-OMe [(*S*)-**6**] by the HCl/AcOH method, and from Boc-Lys(Z)-OH (2.66 g, 7.0 mmol) in DMA (30 mL) using DCC (1.45 g, 7.0 mmol) and HOBt (1.18 g, 7.0 mmol) as condensing agents and NMM (1.9 mL, ca. 9.1 mmol) as base. After 23 h at rt, DCU was filtered off, the filtrate concentrated, the residue dissolved in $CHCl_3$, the soln washed with 10% aq citric acid, 8% aq $NaHCO_3$, and brine, and concentrated. The crude product chromatographed (silica gel, 250 g, $CHCl_3$/MeOH 95:5) to give a white powder; yield: 4.09 g (ca. 100%); mp 153–155 °C; TLC ($CHCl_3$/MeOH 9:1) R_f 0.72.

Boc-Lys(Z)-(*S*)-Amhn-OH (8):
A soln of Boc-Lys(Z)-(*S*)-Amhn-OMe (3.8 g, 6.5 mmol) in dioxane (90 mL), H_2O (10 mL), and aq 1 M NaOH (7.0 mL) was stirred at rt. Another 30 mL of H_2O were added in 3 portions after 20, 40, and 60 min. After a total of 5 h, the soln was slightly diluted with H_2O, the remaining ester extracted with EtOAc (0.84 g of the ester was recovered), the alkaline, aqueous phase acidified with 0.5 M H_2SO_4, and the product taken into EtOAc. Drying (Na_2SO_4) and concentration afforded a white, amorphous powder (2.66 g). Hydrolysis of the recovered substrate under the same conditions gave another 0.67 g of Boc-Lys(Z)-(*S*)-Amhn-OH; total yield: 3.33 g (89%); TLC ($CHCl_3$/MeOH 9:1) R_f 0.38.

Boc-Lys(Z)-(*S*)-Amhn-Lys(Z)-Gly-OMe (9):
A soln of Boc-Lys(Z)-(*S*)-Amhn-OH (**8**; 3.0 g, 5.3 mmol), HCl · H-Lys(Z)-Gly-OMe (2.15 g, 5.5 mmol), TBTU (1.87 g, 1.1 equiv), HOBt (450 mg, 0.5 equiv), and DIPEA (2.0 mL, 2.2 equiv) in DMA (30 mL) was stirred at rt for 3.5 h. The soln was removed under high vacuum and the residue dissolved in EtOAc, washed with 10% aq citric acid, 8% aq $NaHCO_3$, and brine. After concentration, the residue was chromatographed (silica gel, 250 g, 3% MeOH/$CHCl_3$) to give a colorless, solid foam; yield: 4.48 g (94%); TLC ($CHCl_3$/MeOH 9:1) R_f 0.63.

Boc-Lys(Z)-(*S*)-Amhn-Lys(Z)-Gly-OH (10):
The methyl ester of **9** (1.72 g, 1.91 mmol) was hydrolyzed in dioxane (17 mL) and H_2O (17 mL) containing NaOH (2.9 mmol) at rt. After 1 h, the soln was diluted with H_2O (20 mL), the acid liberated with 0.5 M H_2SO_4 and extracted into EtOAc. Washing with brine, drying (Na_2SO_4), and concentration afforded a solid foam; yield: 1.70 g (~100%); TLC (AcOH/BuOH/H_2O 1:3:1) R_f 0.85.

HCl · H-Lys(Z)-(*S*)-Amhn-Lys(Z)-Gly-OMe (11):
To **9** (1.72 g, 1.91 mmol) was added 1.2 M dry HCl/AcOH (17 mL). The mixture was stirred at rt for 10 min, the clear soln cooled in an ice–water bath, and the product precipitated by adding Et_2O (750 mL). After stirring for 30 min in the cooling bath, the amorphous precipitate was sucked off and triturated, on the filter, with more Et_2O. A white powder of HCl · H-Lys(Z)-(*S*)-Amhn-Lys(Z)-Gly-OMe was thus obtained which was dried under high vacuum; yield: 1.44 g (90%); TLC (AcOH/BuOH/H_2O 1:3:1) R_f 0.69.

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Boc-Lys(Z)-(*S*)-Amhn-Lys(Z)-Gly-Lys(Z)-(*S*)-Amhn-Lys(Z)-Gly-OMe (12):
A soln of **11** (1.05 g, 1.25 mmol), **10** (1.11 g, 1.25 mmol), TBTU (0.49 g, ca. 1.13 equiv), HOBt (90 mg), and DIPEA (0.51 mL, 2.4 equiv) in DMA (18 mL) was stirred at rt for 3 h. The resulting soln was introduced into Et_2O (200 mL), the oily precipitate separated by decantation, triturated with several portions of Et_2O, and dissolved in $CHCl_3$/MeOH (4:1), and the soln removed. The solid foam was chromatographed (silica gel, 200 g, $CHCl_3$/MeOH 9:1) to give a colorless, solid foam; yield: 2.06 g (99%); TLC ($CHCl_3$/MeOH 9:1) R_f 0.36.

HCl·H-Lys(Z)-(*S*)-Amhn-Lys(Z)-Gly-Lys(Z)-(*S*)-Amhn-Lys(Z)-Gly-OH:
The peptide **12** (1.84 g, 1.10 mmol) was first hydrolyzed in dioxane (75 mL) and MeOH (37.5 mL) with aq 1 M NaOH (16.5 mL). H_2O (60 mL) was slowly added to the mixture while stirring at rt. Boc-Lys(Z)-(*S*)-Amhn-Lys(Z)-Gly-Lys(Z)-(*S*)-Amhn-Lys(Z)-Gly-OH was liberated after 1.5 h with 0.5 M H_2SO_4 and washed in EtOAc with H_2O and brine. Concentration afforded a colorless, solid foam; yield: 1.78 g (98%); TLC ($CHCl_3$/MeOH 4:1) R_f 0.38. Treatment of Boc-Lys(Z)-(*S*)-Amhn-Lys(Z)-Gly-Lys(Z)-(*S*)-Amhn-Lys(Z)-Gly-OH (1.73 g, 1.045 mmol) with 1.2 M HCl/AcOH (50 mL, rt, 10 min) and precipitation with Et_2O, after usual trituration of the precipitate with Et_2O and drying, gave the product as a white, amorphous powder; yield: 1.46 g (88%); TLC (AcOH/BuOH/H_2O 1:3:1) R_f 0.79.

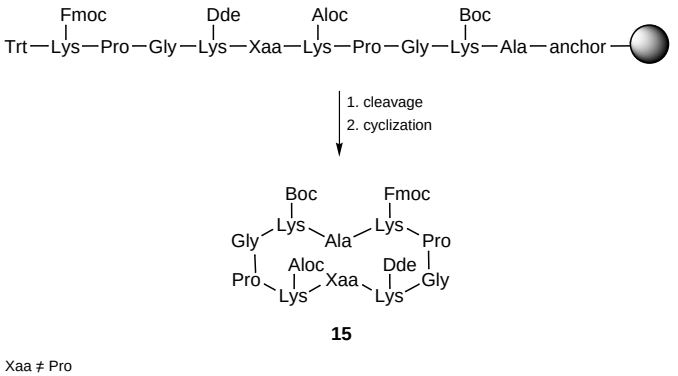
***c*[-(*S*)-Amhn-Lys(Z)-Gly-Lys(Z)-(*S*)-Amhn-Lys(Z)-Gly-Lys(Z)-] (13):**
A soln of HCl·H-Lys(Z)-(*S*)-Amhn-Lys(Z)-Gly-Lys(Z)-(*S*)-Amhn-Lys(Z)-Gly-OH (1.40 g, 0.879 mmol) in DMA (120 mL) containing DIPEA (350 µl, 2.3 equiv) and DPPA (210 µL, 1.1 equiv) was stirred at rt. After 4.5 h, another portion of DPPA (110 µL) and of DIPEA (90 µL) was added, and stirring was continued for a total 24 h. Concentration under high vacuum, trituration with Et_2O, and liquid chromatography (silica gel, 200 g, $CHCl_3$/MeOH 4:1) gave the product as a colorless, solid foam; yield: 1.03 g (76%); TLC ($CHCl_3$/MeOH 9:1) R_f 0.38.

***c*[-(*S*)-Amhn-Lys-Gly-Lys-(*S*)-Amhn-Lys-Gly-Lys-]·HCl (14):**
The cyclic peptide **13** (900 mg, 0.585 mmol) was hydrogenated in 90% AcOH/H_2O (77 mL) containing aq 1 M HCl (3.5 mL) over 10% Pd/C (0.9 g, 1 atm H_2, rt). Usual workup after 4.5 h and final concentration of the product soln in 0.1 M HCl (10 mL, repeated twice) afforded the product as a colorless, glassy oil; yield: 610 mg (91%); PD-MS (pos.):1002.1 $[M+H]^+$; calcd for $C_{52}H_{80}N_{12}O_8$ (base), M_r 1001.25.

13.1.1.2 Regioselectively Addressable Functionalized Template Molecules

For the construction of more complex TASP molecules that should more closely resemble natural proteins, template molecules with several selectively addressable sites are required. The preparation of cyclic decapeptide templates (e.g., **15**, Scheme 5) with appropriate multiple-orthogonally protected side chains is readily achieved by utilizing the flexibility of solid-phase methods for the synthesis of the linear precursors and the high efficiency and reproducibility of cyclization in solution.[17] The combined solid-phase and solution strategy offers an elegant approach for the synthesis of cyclic peptides as regioselectively addressable functionalized templates (RAFT) which contain trifunctional amino acids with orthogonally protected side chains or unique chemical reactivity. Linear peptides are assembled using standard Fmoc solid-phase chemistry with a highly acid-labile linker unit, the SASRIN handle.[36]

Scheme 5 Combined Solid-Phase and Solution Strategy for the Synthesis of Regioselectively Addressable Functionalized Templates (RAFT) with Four Orthogonally Protected Lysine Side Chains for Sequential Attachment of Four Different Peptide Sequences[17]



The combination of very mild coupling and deprotection protocols provides the flexibility needed in order to incorporate a variety of side-chain-protecting groups, cleavable by acid (Boc), base (Fmoc), Pd(II) (Aloc),[43] or nucleophiles (Dde).[44] Linear precursors of such templates with four orthogonally protected lysine residues are generally obtained in yields around 80% and cyclization proceeds quickly at high dilution almost quantitatively.

H-Lys(Fmoc)-Pro-Gly-Lys(Dde)-Ala-Lys(Boc)-Pro-Gly-Lys(Aloc)-Ala-OH:[17]
The linear protected decapeptide was assembled on N^{α}-Fmoc-Ala-SASRIN (1.92 g, 1.30 mmol) using a threefold excess of N^{α}-Fmoc-protected amino acids (3.9 mmol), preactivated by HOBt (0.53 g, 3.90 mmol) and DIC (0.49 g, 3.90 mmol) in DMF during 15 min. The active ester was coupled in the presence of DIPEA (0.67 mL, 3.90 mmol) in DMF during 40 min. The fourth orthogonal protecting group was incorporated by using Trt-Lys(Fmoc)-OH in the last coupling step. The completeness of each coupling was confirmed by the Kaiser test.[45] The N^{α}-Fmoc protecting groups were removed by treatment with 20% piperidine/DMF (3- and 15-min cycles); the completeness of each deprotection being verified by the UV absorption of the piperidine washings at 302 nm.[46] The protected peptide was then cleaved from the resin with 1% TFA in CH_2Cl_2 (15 mL, 6 cycles of 10 min) and was neutralized with 1% pyridine/CH_2Cl_2 (6×15 mL). The solvent was removed under reduced pressure to give a gum from which the title linear decapeptide was obtained by precipitation (CH_2Cl_2/Et_2O); yield: 1.63 g (83%).

***c*[-Lys(Fmoc)-Pro-Gly-Lys(Dde)-Ala-Lys(Boc)-Pro-Gly-Lys(Aloc)-Ala-] (15, Xaa = Ala):**[17]
A soln of H-Lys(Fmoc)-Pro-Gly-Lys(Dde)-Ala-Lys(Boc)-Pro-Gly-Lys(Aloc)-Ala-OH (0.73 g, 0.46 mmol) in DMF (800 mL) was treated at rt with a soln of DIPEA (0.3 mL, 1.75 mmol) in DMF (5 mL) over 10 min. The cyclization was complete after an additional 20 min, as determined by analytical RP-HPLC, and the soln was concentrated under reduced pressure. The residue was dissolved in CH_2Cl_2 and Et_2O was added to give the pure cyclic decapeptide as a white solid; yield: 0.55 g (76%).

13.1.1.2.1 Other Types of Regioselectively Addressable Functionalized Templates

Multifunctional cyclic peptides as RAFT molecules offer the advantage that potential attachment sites are oriented in defined spatial directions, thus offering ideal geometries for the construction of predetermined folding topologies. For example, by connecting two orthogonally protected $(Lys)_3$ tripeptide units in an antiparallel arrangement through two Pro-Gly type-II β-turns or alternatively, two D-Pro-Gly type-II′ β-turns, a hexalysine-comprising template is obtained. Conformational studies by NMR, restrained molecular dynamics, and CD spectroscopy indicate that this type of molecule can adopt a low energy conformation where four lysine side chains are disposed on one face of the template and two on the opposite side.[15,47] Such a two-domain RAFT molecule allows for the differentiation of the individual lysine side chains as attachment sites and thus a binary discrimination of the two faces of the template. This offers the possibility to combine effector and receptor properties

for references see p 64

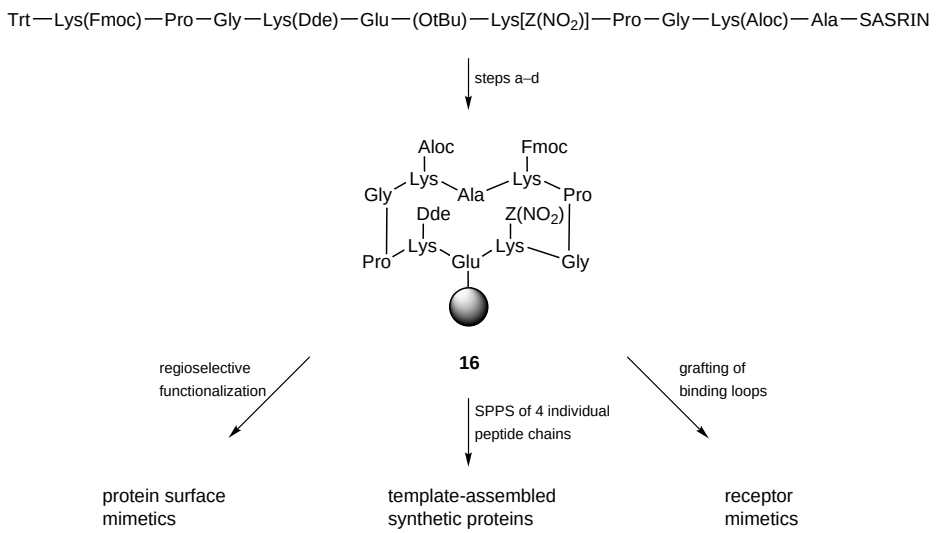
in one molecule at distinct sites[47] or to modulate the biological activity by changing the physicochemical features of TASP molecules upon ligation of non-peptide groups, e.g. lipids, carbohydrates, polyethylene glycols, steroids, or fluorescent markers for diagnostic purposes.[48] A very promising application of two-domain RAFT molecules lies in the construction of assemblies combining, for example, an ion-channel-forming four-α-helix bundle with a receptor ligand as a model for biosensor systems.[49–51] For example, the attachment of lipids on one side of the scaffold allows for the fixation of such chimeric TASP molecules to a gold surface, the opposite face of the template functionalized with four copies of, for example, an antigenic Ac-(Asn-Ala-Asn-Pro)$_3$-Gly-OH sequence positioned appropriately for antibody recognition.

H-Lys(Aloc)-Lys(Boc)-Pro-Gly-Lys(Boc)-Lys(Aloc)-Lys(Boc)-Pro-Gly-Lys(Boc)-OH:[49]
The linear decapeptide was synthesized on Fmoc-Lys(Boc)-SASRIN-resin (2.0 g, substitution: 0.6 mmol · g^{-1}) applying standard Fmoc strategy based on DIC/HOBt activation with DIPEA as base. After removal of the last Fmoc group, the peptide was cleaved from the resin and cyclized as described in Section 13.1.1.1.1; yield: 0.671 g (68%); ESI-MS: 1646.3 $[M+H]^+$, 823.8 $[M/2+H]^+$.

13.1.1.2.2 Polymer-Bound Regioselectively Addressable Functionalized Template Molecules

The availability of an increasing number of orthogonal protection techniques together with improved methodologies in solid-phase peptide synthesis allows for the construction of TASP molecules with increased structural complexity on solid supports[38,52] by exploiting the advantages characteristic for solid-phase strategies. As a key step, RAFT molecules are prepared by convergent methods and subsequently immobilized on solid supports (Scheme 6).[53] The resulting resin-bound template with up to four orthogonal amino protecting groups, i.e. **16** with Fmoc, Aloc, Dde, and Z(NO$_2$), gives access to the step-by-step synthesis of, for example, TASP molecules with four different helices, protein surface and receptor mimetics.

Scheme 6 Combined Solid-Phase and Solution Strategy: After Chain Assembly, the Linear Template Sequence Is Cleaved from the Resin, Cyclized in Solution and Re-immobilized on a Resin (Steps a-d); Orthogonal Protection Allows for the Construction of Protein Surface Mimetics, TASP Molecules, or Receptor Mimetics[52,53]



Due to the preferred β-sheet conformation of cyclic decapeptides of the general sequence *c*[-Xaa^1-Xaa^2-Xaa^1-Pro-Gly-]$_2$ as templates, the side chains of alternating amino acids are oriented above or below the plane of the template.[15,34,54] Consequently, the side chain of Xaa^2, e.g. the γ-carboxylic group of Glu, can serve as linker to fix the template molecule to the resin in a favorable orientation for subsequent solid-phase assembly of TASP molecules. The linear template sequence is assembled by standard SPPS[55] on the super-acid-sensitive SASRIN[36] resin using Trt-Lys(Fmoc) as the N-terminal amino acid. Treatment of the resin with dilute acid allows simultaneous release of the linear protected peptide and deprotection of the N-terminal moiety. After desalting by solid-phase extraction and lyophilization, the linear peptide is cyclized and the γ-carboxylic group of Glu deprotected selectively with 50% TFA in CH_2Cl_2 to yield the free carboxylic group in the presence of protected lysine side chains. Subsequently, the template is immobilized on the resin via amide bond formation. In order to prevent premature loss of the Fmoc protecting group due to extended exposure to strong base, collidine is preferred over DIPEA, and PyAOP as activating agent proves more efficient than PyBOP, HATU, or HBTU. Despite the sterically demanding template molecule, satisfactory substitution levels can be achieved independent of the physical and chemical nature of the polymer resin.[52,53,56–58] For example, the use of high-loaded polystyrene-based resins, e.g. H_2N-βAla-Xal-PSty (240 μmol · g^{-1}) or PEG-PS resins of lower capacity (118 μmol · g^{-1}), results in comparable substitution levels of the template fixed to the resin, on average 80–100 μmol · g^{-1}. More hydrophilic resins may play a pivotal role in applying chemoselective ligation methods[18–24] for the construction of highly diverse peptide structures. The orthogonality of Z(NO_2), Boc, Fmoc, Aloc, and Dde allows for sequential removal of the protecting groups and thus, selective stepwise functionalization of the four attachment sites according to general procedures used in solid-phase peptide synthesis.

H-Lys(Fmoc)-Pro-Gly-Lys[Z(NO_2)]-Glu(OtBu)-Lys(Dde)-Pro-Gly-Lys(Aloc)-Ala-OH:[52,53]
The assembly of the protected peptide was carried out starting from Fmoc-Ala-SASRIN (3 g, 0.47 mmol · g^{-1}). The resin was washed and swollen with CH_2Cl_2 (2 × 50 mL, 15 min) and DMF (2 × 50 mL, 15 min) and coupling reactions performed using N^{α}-protected amino acid (2 equiv) activated in situ with PyBOP (2 equiv) and DIPEA (4 equiv) in DMF (30 mL) for 30 min. After completion of the sequence the peptide was cleaved from the resin by repetitive treatment with 1% TFA in CH_2Cl_2 until the beads turned dark purple. Each filtrate was neutralized with pyridine/MeOH (1:4, 5 mL). The soln was concentrated under reduced pressure and precipitated (EtOAc/petroleum ether). The solid was dissolved in EtOAc and pyridinium salts were extracted with H_2O. The organic phase was dried (Na_2SO_4) and was then concentrated to dryness. Precipitation (CH_2Cl_2/Et_2O) afforded 2.75 g of a white solid which was further desalted by solid-phase extraction (Sep-Pak Vac cartridge, C18, 35 mL) and lyophilized to give the product linear peptide; yield: 2.35 g (93%).

***c*[-Lys(Fmoc)-Pro-Gly-Lys[Z(NO_2)]-Glu-Lys(Dde)-Pro-Gly-Lys(Aloc)-Ala-]:**[52,53]
H-Lys(Fmoc)-Pro-Gly-Lys[Z(NO_2)]-Glu(OtBu)-Lys(Dde)-Pro-Gly-Lys(Aloc)-Ala-OH (1.11 g, 0.60 mmol) was dissolved in DMF (600 mL) and the pH adjusted to 8–9 by addition of DIPEA. HATU (0.26 g, 1.1 equiv) was added and the soln was stirred at rt for 3 h. The solvent was removed under high vacuum and the residue was dissolved in TFA/CH_2Cl_2 (1:1, 70 mL) and allowed to stand at rt for 45 min. The soln was concentrated under reduced pressure and the residue triturated with Et_2O. Filtration afforded crude product; yield: 0.94 g; purity >90%. The crude product (780 mg, ~75%) was dissolved in MeCN/H_2O (1:1) and the pH raised to 7–8 by addition of collidine (180 μL). Solid-phase extraction (Sep-Pak Vac cartridge, C18, 15 mL) and lyophilization afforded the collidine salt of the title compound (750 mg) and was directly used for the immobilization reaction.

***c*[-Lys(Fmoc)-Pro-Gly-Lys[Z(NO_2)]-Glu(βAla-resin)-Lys(Dde)-Pro-Gly-Lys(Aloc)-Ala-] (16):**[52,53]
Fmoc-HN-XAL-PSty resin (1.0 g, 230 μequiv · g^{-1}) was washed and swollen with CH_2Cl_2 (2 × 20 mL, 15 min) and DMF (2 × 20 mL, 15 min) in a glass reaction vessel fitted with a sintered glass frit. The resin was treated with 20% piperidine/DMF (10 mL, 5 min and 10 min cycle). Fmoc-βAla-OH (0.47 g, 1.5 mmol) was coupled with PyBOP (0.78 g, 1.5 mmol) and DIPEA (0.5 mL, 3 mmol) in DMF (10 mL) for 30 min. Completeness of the reaction was tested by the TNBS test. After removal of the Fmoc group as described above, the resin was reacted with a soln of the cyclic peptide from the preceeding procedure (0.27 g, 0.150 mmol), PyAOP (0.12 g, 0.230 mmol), and collidine (0.3 mL, 2.3 mmol) in DMF (10 mL). After 13 h, free amines were quenched with pyrene-1-butanoic acid (0.43 g, 1.5 mmol), PyBOP (0.78 g,

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