CHIMIA 2001, 55, No. 4

Chimia 55 (2001) 302–305

ISSN 0009–4293

'Never Mind a Barrel'

Bodo Baumeister, Gopal Das, Naomi Sakai, and Stefan Matile*

Abstract: The general usefulness of rigid-rod molecules rather than β-sheets as molecular staves for the construction of artificial β-barrels with preserved structural and functional plasticity is described. Aspects of the structural plasticity of rigid-rod β-barrels are elaborated in more detail with respect to barrel truncation, elongation, contraction and expansion in water and/or bilayer membranes.

Keywords: Protein mimetics · Rigid-rod molecules · Supramolecular chemistry · Tertiary structure

1. Introduction

'Never mind a barrel' – Groucho Marx' admonition validly summarizes the apparent ease of designing one central protein tertiary structure, *i.e.* α-helix bundles [1], and the relative difficulty of constructing the other, *i.e.* β-barrels, from first principles [1–4]. By cleaning his teeth while hidden within a barrel offering deceiving advice, Groucho (near the beginning of *Monkey Business*) also provides a marvelous living illustration of the versatility of the confined interior of these *'cylindrical containers ... traditionally made of wooden staves ...'* (Oxford Dictionary, first option). The functional plasticity of their molecular counterparts, comprised of β-sheets instead of wooden staves, is even more intriguing, at least for chemists and biologists [2][4]. The more than one thousand biological β-barrels known today mediate a wide range of processes extending from molecular recognition to translocation and transformation [4].

The functional plasticity of β-barrels is, at least in part, a consequence of using β-stands as molecular staves. Namely, the rigidity of the scaffold and the opposite orientation of adjacent amino acid residues in β-sheets allows for precise positioning of more flexible loops and accurate tuning of the chemical nature of both the outer and inner barrel surface, respectively [2–4]. Among these properties, the easily varied but topologically precise placement of functional groups pointing toward the β-barrel interior is particularly intriguing in view of the increasingly recognized importance of confined space in chemistry and biology. Apparent difficulties in creating internal functionality with other supramolecular architecture (rosettes [5], spheres [6], nanotubes [7], and so on [8]), as well as the less accurate positioning of groups within the otherwise closely related *de novo* α-helix bundles [1][2] suggests that insufficient scientific significance does not account for the relatively moderate progress in designing β-barrels 'from scratch'. Comments on this situation (beyond that of Groucho Marx) emphasize more complex folding requirements that may result in unforeseen interactions during β-barrel formation that ultimately lead to irreversible peptide precipitation [1][3]. The development of novel routes to synthetic β-barrels that a) bypass troublesome protein folding (negative design, [3]) but b) maintain the structural and functional plasticity of β-strand staves (positive design) thus seems desirable. Here we summarize our recent efforts in this direction using rigid-rod β-barrels **1–8** with emphasis on their structural plasticity [9–14] (Fig. 1 and 2).

*Correspondence: Prof. Dr. S. Matile
Department of Organic Chemistry
University of Geneva
CH-1211 Geneva
Tel: +41 22 702 6085
Fax: +41 22 328 7396
E-Mail: stefan.matile@chiorg.unige.ch

2. Structural Plasticity of Rigid-Rod β-Barrels

The design of rigid-rod β-barrels is simple. Rigid *p*-oligophenyl rods serve as staves instead of the β-strands in biological β-barrels (Fig. 1). The choice of rigid-rod molecules as staves is crucial to introduce molecular preorganization (*i.e.* to minimize the inherent entropy penalty of supramolecular architecture and, therefore, the corresponding enthalpy requirements). Although never directly verified for rigid-rod β-barrels *per se*, clear-cut results reported for Lehn's supramolecular 'metallobarrels' constructed from bis(bipyridine) staves of different rigidity provide compelling experimental support for the central importance of preorganization by rigid-rod molecules [15].

The choice of *p*-oligophenyls as model rods is crucial with respect to negative design: the flexible arene-arene torsion angles ≠ 180° in *p*-oligophenyl staves induce the curvature needed for preferential formation of barrels instead of precipitating linear tapes [1][3][5]. With regard to positive design, that is introduction of the useful features responsible for the functional plasticity of biological β-barrels, all *p*-oligophenyl staves are equipped with short peptide strands. Rigid-rod β-barrel **1**, for example, is constructed from complementary anionic and cationic *p*-octiphenyl staves comprising tripeptide strands with the sequence ELE and KLK, respectively (ELE = -Glu-Leu-Glu- = -L-glutamate-L-leucine-L-glutamate-, KLK = -Lys-Leu-Lys- = -L-lysine-L-leucine-L-lysine-, compare Fig. 1). Programmed assembly in detergent-free water gives tetramer **1**, characterized by a hydrophilic outer surface decorated with 'EK-ion pairs' and a hydrophobic interior formed by leucine arrays [9].

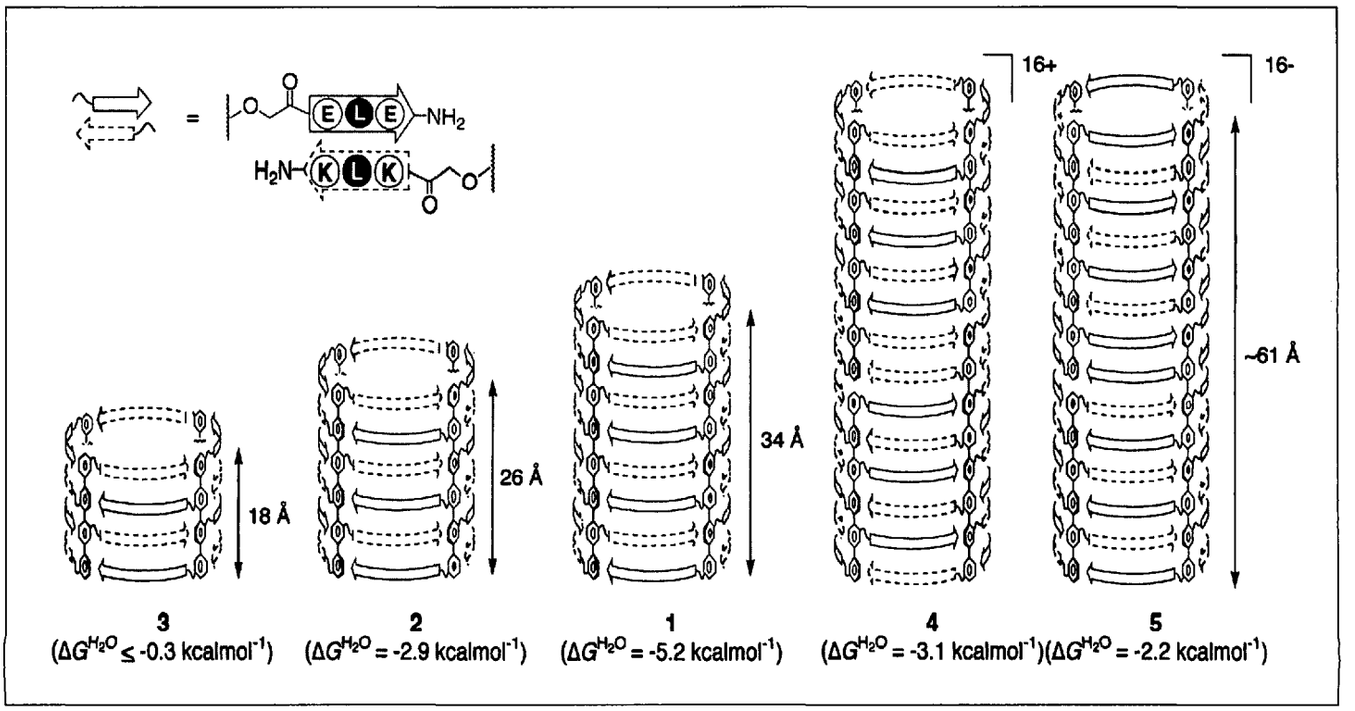

Fig 1. Structural plasticity of rigid-rod β-barrels with respect to barrel length. Amino acid residues (one letter abbreviations; E = L-glutamate, L = L-leucine, K = L-lysine) in β-strands (arrows pointing to C-terminus) on the external barrel surface are depicted black or gray on white, internal residues white on black or gray. Cationic peptide rods are in black, anionic peptide rods in gray, cationic β-strands are in black dotted lines, anionic β-strands in gray solid lines. ΔG^{H2O}-values refer to the barrel energy in denaturant-free water with respect to and calculated from denaturation experiments [10].

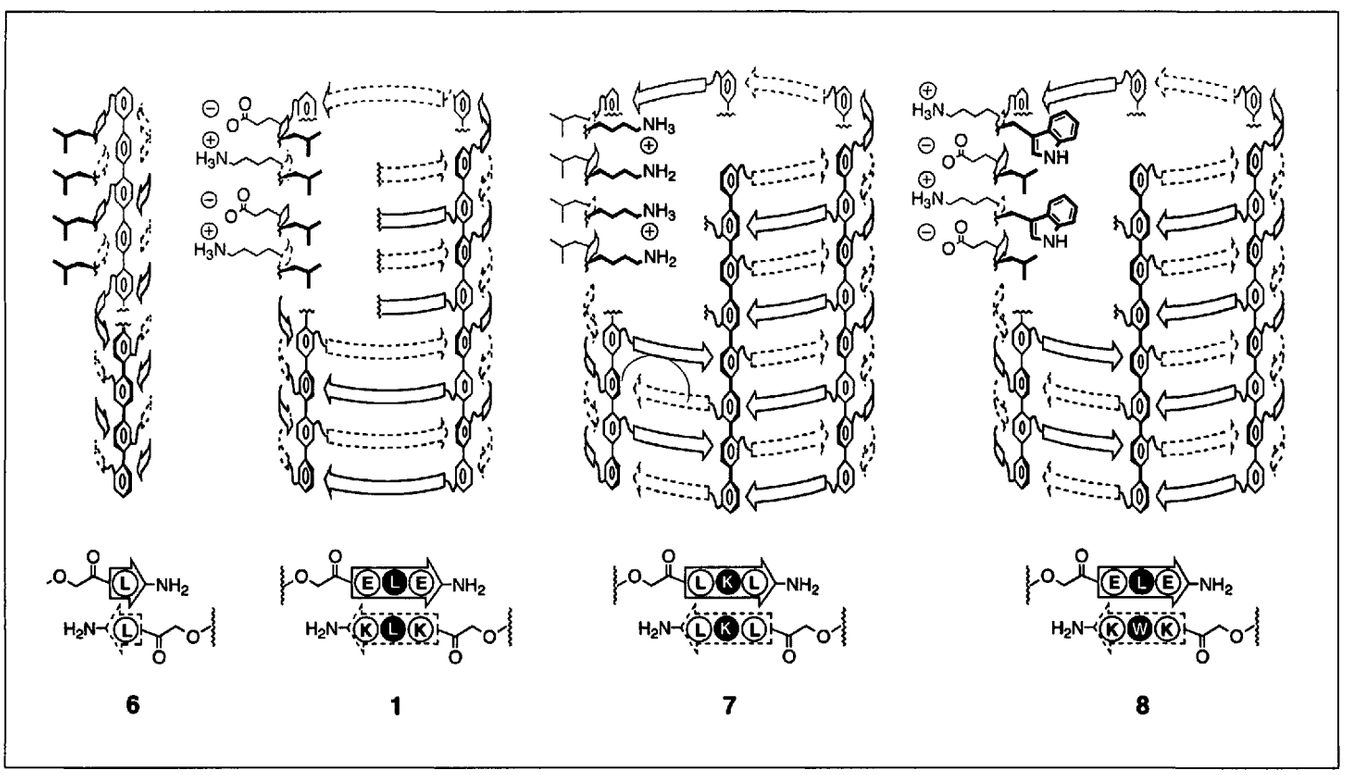

Fig. 2. Structural plasticity of rigid-rod β-barrels with respect to barrel diameter. Amino acid residues (one letter abbreviations; E = L-glutamate, L = L-leucine, K = L-lysine, W = tryptophan) in β-strands (arrows pointing to C-terminus) on the external barrel surface are depicted black on white, internal residues white on black. The upper left of each β-barrel is cut open to indicate chemical structure and orientation of amino acid residues in β-sheet conformation. Cationic β-strands in **1** and **8** are in dotted lines, anionic β-strands in **1** and **8** in solid lines. The precise degree of protonation of K in **7** is unknown [13][14].

2.1. *Tertiary Structure Determination*

'Suprastructure' determination proved challenging for rigid-rod β-barrels (but, of course, *not* for synthetic *p*-oligophenyl peptides in monomeric form). In fact, when our research program to investigate the usefulness of rigid-rod molecules in bioorganic chemistry was initiated, many experts in the field assessed a probability of success near 0% because the notoriously poor solubility of higher rods would prevent gaining insight about active suprastructures. These predictions were quite correct, not only for rigid-rod β-barrels [16]. With a few noteworthy exceptions, conventional methods for structure determination in organic chemistry turned out to be either poorly applicable due to the indeed often peculiar physical properties of higher *p*-oligophenyl rods, unrevealing because of their oligomeric nature, or both. In sharp contrast, however, it was confirmed that most biophysical methods devised for (often indirect) 'suprastructure' determination under biologically relevant conditions (often nanomolar concentrations) were directly applicable to rigid-rod β-barrels. For example, although mass spectrometry with rigid-rod β-barrels was possible [12], routine assessment of their molecular weights (*i.e.* aggregation number) under biologically relevant conditions by size exclusion chromatography against protein standards proved practical, reliable and thus preferable overall [9–11]. As with peptides and proteins, conformational studies by circular dichroism (CD) spectroscopy were informative [9–14], helping to determine β-barrel stoichiometry (*e.g.* Job-plots, [9–11]), the nature of intermolecular interactions (*e.g.* effects of pH and ionic strength, [9]), and β-barrel stability (*e.g.* denaturation with guanidinium chloride [10]). Assessment of the most characteristic aspect of β-barrels (*i.e.* their interior) is a challenge that is well recognized beyond rigid-rod β-barrels. In the case of rigid-rod β-barrels, it was possible to obtain experimental evidence for internal space in polar and nonpolar liquid and gas phase using complementary internal guests as reporter groups [9][12].

Whereas experimental support for the presence and the nature of the interior of molecular barrels in isotropic media is very difficult to obtain, the situation is the opposite for biological and rigid-rod β-barrels that span lipid bilayers. Because of the spatial compartmentalization provided by the surrounding membrane, the interior of the barrel can be accessed from two sides and internal processes can be detected rapidly, even at the single molecule level. (No surprise that the term 'barrel-stave motif' was originally introduced to describe ion channels!) This unique situation is attracting increasing scientific attention because of many possible applications extending from single molecule sensing to stochastic gene sequencing [17]. As with biological β-barrels, the anisotropy of biomembranes thus provides the most suitable environment for unambiguous characterization of space within rigid-rod β-barrels by dye leakage, blockage and planar bilayer conductance experiments. An important advantage of fluorescent rigid-rod staves is that the above experiments can be complemented with structural studies of unusually high precision using a simplified depth quenching technique [18].

2.2. *'Ideal' Barrel Length in Water and Lipid Bilayers*

p-Octiphenyl β-barrel **1** is a convenient starting point for an overview of the structural plasticity of rigid-rod β-barrels [9][10]. Structural studies along the lines discussed above indicated that the *p*-octiphenyl tetramer **1** forms by programmed assembly in detergent-free water as the major product (>80%, side product: hexamer). The presence of a hydrophobic β-barrel interior was visualized by encapsulated, planarized β-carotene templates. Tetramer **1** was stable over a wide range of pH as well as toward dilution and heat. Denaturation with guanidinium chloride revealed that the stability of rigid-rod β-barrel **1** in denaturant-free water is $\Delta G^{H_2O} = -5.2$ kcal/mol. This energy exceeds that reported for most peptide models and is within the range of biological proteins (Fig. 1). Considering that the intrinsic entropy penalty for supramolecular chemistry is included in these –5.2 kcal/mol [19], the stability of β-barrel **1** appears sufficiently remarkable to serve as a striking illustration of the importance of preorganization by rigid-rod molecules.

Interestingly, the 'ideal practical length' for rigid-rod β-barrels coincides with the ideal length for spanning bilayer membranes. Extensive studies on the interaction of *p*-oligophenyl guests to bilayer hosts (composed of egg yolk phosphatidylcholine) have shown that rods shorter than *p*-septiphenyls lack suprastructural organization in these membranes and that rods longer than *p*-octiphenyls do not bind at all [16]. Both trends are consistent with hydrophobic matching of rod length and membrane thickness as a prerequisite for transmembrane supramolecular architecture. In contrast to the situation in water, studies of the structural plasticity of rigid-rod β-barrels with respect to truncation and elongation in bilayer membranes were thus redundant.

2.3. *Truncated Barrels in Water*

The dependence of the stability of rigid-rod β-barrels on β-barrel length is substantial [10]. In sharp contrast to the superb stability of *p*-octiphenyl β-barrel **1**, that of truncated *p*-sexiphenyl β-barrel **2** is already in the range of most protein mimetics but clearly below that of biological proteins. *p*-Quarterphenyl β-barrel **3** forms only partially in neutral water at room temperature: a minimal length for the formation of rigid-rod β-barrels clearly exists. This strong dependence of β-barrel stability on β-barrel length is consistent with synergistic β-barrel formation (**1**: $\alpha = +1.4$). With an eye on the long-term goal of using rigid-rod β-barrels for solubilization of intact membrane proteins in detergent-free water, we are currently investigating truncated β-barrels **3** as potential 'living' receptors for hydrophobically matching templates.

2.4. *Elongated Barrels in Water*

Increasing β-barrel stability with β-barrel length makes efforts toward covalent elongation of *p*-octiphenyl β-barrel **1** seemingly attractive. However, although β-barrel stability increases with *p*-oligophenyl length, decreasing rod solubility is expected to complicate the synthesis of elongated peptide-rods and yield perhaps highly stable but presumably insoluble rigid-rod β-barrels.

Supramolecular rather than covalent elongation of β-barrels proved more practical [10]. A simple strategy focusing on supramolecular matching of mismatched rods was elaborated. Indeed, programmed assembly of *p*-octiphenyl peptide rods with complementary *p*-sexiphenyl peptide rods quantitatively affords rigid-rod β-barrels **4** and **5**. This finding is of interest because it shows that supramolecular matching of mismatched staves is more important than charge neutralization. Cation **4** and anion **5** comprise central cores that are dominated by external charged amino acid residues of the longer *p*-octiphenyl peptide rods. Interestingly, the reduction of β-barrel stability by supramolecular elongation remains in the acceptable range of truncated *p*-sexiphenyl β-barrel **2**. We are now trying to exploit the unusual central regions of elongated barrels for the creation

of internal active sites using mismatched rods with internal catalytic amino acid residues.

2.5. *Contracted Barrels in Lipid Bilayers*

β-Barrel contraction with respect to tetramer **1** is demonstrated in rigid-rod β-barrel **6** [12]. The design strategy is trivial: shorter side chains should yield a contracted β-barrel. According to molecular models, dimeric β-barrel **6** has hydrophobic leucine residues at the outer surface, practically no internal space, and lacks functional groups pointing to the interior. Nevertheless, contracted β-barrel **6** can be 'filled' with at least three cations in gas, liquid, and liquid crystalline phases. The latter property allowed efficient ion transport across bilayer membranes. Replacement of the four central leucines with methyl groups prevents spontaneous self-assembly of correspondingly contracted β-barrels. This finding underscores the importance of consecutive side chain interdigitation for the construction of rigid-rod β-barrels.

2.6. *Expanded Barrels in Lipid Bilayers*

β-Barrel expansion in bilayer membranes was achieved by exploiting internal electrostatic repulsion [13]. Namely, the lateral *p*-octiphenyl L-strands in contracted β-barrel **6** were replaced by LKL-strands in expanded β-barrel **7**. In β-sheet conformation, this design preserves the interactions between lipids and external leucines present in dimer **6** but adds multiple lysine residues pointing toward the β-barrel interior. Since the lysine amines are at least partially protonated at physiological pH, repulsion between these internal cations was expected to create and maintain internal space. By taking advantage of the (experimentally confirmed!) transmembrane orientation of the cationic interior of expanded β-barrel **7**, it was possible to characterize its chemical nature in detail. The findings are in full support of the designed β-barrel suprastructure. Remarkable stability, previously thought to be unique to biological β-barrels [20] illustrates once again the importance of preorganizing rigid-rod staves. Remarkably large inner diameters, well beyond that of the best characterized biological β-barrel [17], on the other hand, allow for unprecedented functional plasticity such as internal, nearly irreversible binding of topologically and electrostatically complementary oligonucleotide duplexes in transmembrane orientation and 'Watson-Crick'-conformation [14].

2.7. *Expanded Barrels in Water*

β-Barrel expansion in water was achieved by 'internal crowding' [5][11]. It was speculated that the replacement of every other internal leucine in rigid-rod β-barrel **1** with more bulky amino acid residues would hinder the formation of tetramers for steric reasons and produce hexamers that can, according to molecular models, accommodate larger internal residues without problems. For this reason, the leucine in the cationic KLK-rod in **1** was replaced by tryptophan. Programmed assembly of the new KWK-rod with the complementary ELE-rod in detergent-free water yields expanded rigid-rod β-barrels **8** in quantitative yield. Current work with expanded β-barrels in water (and bilayer membranes) focuses on the installation of internal catalytic residues to mediate molecular transformations within rigid-rod β-barrels.

3. Conclusion

This account summarizes recent results on the structural plasticity of rigid-rod β-barrels with regard to truncation, elongation, contraction, and expansion in water and bilayer membranes. For many chemists (including the authors), such tertiary structural studies of supramolecular synthetic oligomers may be unusual; for some, clearly unsatisfactory. Excellent reviews are available for readers who are interested in more general discussions of (tertiary) structure determination of supramolecules [5]. For the case of rigid-rod β-barrels, particularly at the beginning of our research program, it was important to realize that the relatively poor solubility and oligomeric nature of higher rigid-rod molecules *are compatible with the conditions of relevant biological and biomimetic function, without any restrictions.* To phrase this another way: if higher micro- or even millimolar concentrations are needed to determine function, biological and/or biomimetic relevance may be questionable. In fact, functional studies with rigid-rod β-barrels, including examples of molecular recognition (cations [12], carotenoids [9], oligonucleotide duplexes [13]), translocation (giant ion channels [12]), and transformation (esterases, unpublished results) that are often inaccessible *via* other routes, indicate the potential of extending the functional plasticity of biological β-barrels beyond peptide chemistry. With regard to the emphasis of this account, it may be further noted that *all* functional studies available today have been in superb agreement with (and therefore in indirect support of) the designed suprastructures. This summary will therefore hopefully serve as a design guideline for further extensions of the functional plasticity of synthetic β-barrels. Seemingly unlimited applicability of this approach in chemistry and biology is illustrated by pertinent examples of recombinant β-barrels and *de novo* α-helix bundles, and also by related 'barrel-stave' supramolecules constructed using oligonucleotides, coordination chemistry, and synthetic organic staves [2].

Acknowledgment

Present and past financial support of this research by the Swiss NSF (21-57059.99 and NRP 4047-057496), the NIH (GM56147), an award from Research Corporation, the donors of the Petroleum Research Fund, administered by the American Chemical Society, Suntory Institute for Bioorganic Research (SUNBOR Grant), Georgetown University and University of Geneva is gratefully acknowledged.

Received: February 9, 2001

[1] T. Sazaki, M. Lieberman, 'Protein Mimetics', in 'Comprehensive Supramolecular Chemistry, Vol. 4', Ed. Y. Murakami, Elsevier, Oxford, **1996**, 193.
[2] S. Matile, *Chem. Soc. Rev.* **2001**, *30, (Advance Article).*
[3] M.H. Hecht, *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 8729.
[4] N. Nagano, E.G. Hutchinson, J.M. Thornton, *Protein Sci.* **1999**, *8*, 2072, and references therein.
[5] G.M. Whitesides, E.E. Simanek, J.P. Mathias, C.T. Seto, D.N. Chin, M. Mammen, D.M. Gordon, *Acc. Chem. Res.* **1995**, *28*, 37.
[6] J. Rebek Jr., *Acc. Chem. Res.* **1999**, *32*, 278.
[7] J.D. Hartgerink, T.D. Clark, M.R. Ghadiri, *Chem. Eur. J.* **1998**, *4*,1367.
[8] G.F. Swiegers, T.J. Malefetse, *Chem. Rev.* **2000**, *100*, 3483.
[9] B. Baumeister, S. Matile, *Chem. Eur. J.* **2000**, *6*, 1739.
[10] G. Das, S. Matile, *Chirality* **2001**, *13*, 170.
[11] B. Baumeister, S. Matile, *Chem. Commun.* **2000**, 913.
[12] N. Sakai, N. Majumdar, S. Matile, *J. Am. Chem. Soc.* **1999**, *121*, 4294.
[13] B. Baumeister, N. Sakai, S. Matile, *Angew. Chem., Int. Ed.* **2000**, *39*, 1955.
[14] N. Sakai, B. Baumeister, S. Matile, *ChemBioChem* **2000**, *1*, 123.
[15] P.N.W. Baxter, J.-M. Lehn, G. Baum, D. Fenske, *Chem. Eur. J.*, **1999**, *5*, 102.
[16] S. Matile, *Chem. Rec.* **2001**, *1*, 162.
[17] H. Bayley, C.R. Martin, *Chem. Rev.* **2000**, *100*, 2575.
[18] N. Sakai, S. Matile, *Chem. Eur. J.* **2000**, *6*, 1731.
[19] L. Regan, W.F. DeGrado, *Science* **1988**, *241*, 976.
[20] M. Rouhi, *Chem. Eng. News* **2000**, *78(36)*, 43.