

# Design, Synthesis, and Application of a Trifluoromethylated Phenylalanine Analogue as a Label to Study Peptides by Solid-State $^{19}\text{F}$ NMR Spectroscopy\*\*

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$^{19}\text{F}$  NMR spectroscopy is a powerful technique to resolve the structure and dynamics of peptides and proteins. The large gyromagnetic ratio, the absence of a biological background, the high sensitivity of the chemical shift to the local environment, and the spin of  $\frac{1}{2}$  make fluorine an attractive nucleus for NMR spectroscopy.<sup>[1]</sup> Solid-state  $^{19}\text{F}$  NMR spectroscopy of macroscopically oriented samples, in particular, is used to determine the structure, alignment, and dynamics of membrane-bound peptides under quasi-native conditions.<sup>[2]</sup> To this aim, suitable fluorine-labeled amino acids have to be incorporated selectively into the polypeptide backbone. However, they have to meet several strict criteria: 1) they must be conformationally constrained to place the  $^{19}\text{F}$  reporter group in a well-defined position relative to the peptide backbone; 2) they must be compatible with standard procedures for solid-phase peptide synthesis (SPPS); 3) they must not perturb the native structure or function of the peptide. These criteria preclude the use of virtually all readily available fluorinated analogues of proteinogenic amino

acids,<sup>[3]</sup> as because of their conformational flexibility they do not fulfill criterion 1.

Recently, several trifluoromethyl-substituted compounds **1–4** have been designed and applied as labels for solid-state  $^{19}\text{F}$  NMR spectroscopy, specifically to replace either bulky aliphatic amino acids (**1**),<sup>[4]</sup> or proline (**2**),<sup>[5]</sup> or serine/threonine (**3**),<sup>[6]</sup> or  $\alpha$ -aminoisobutyric acid (**4**)<sup>[7]</sup> in membrane-active peptides. However, the aromatic phenylalanine analogues **5** and **6**, which have also been explored, showed significant disadvantages, as they racemized under standard Fmoc SPPS conditions (Fmoc = 9-fluorenylmethyloxycarbonyl) and thus did not meet criterion 3.<sup>[2a,c,8]</sup> Purification of the target peptides was problematic,<sup>[8]</sup> and in some cases even impossible.<sup>[9]</sup> Furthermore, analysis of the chemical shift anisotropy of the  $^{19}\text{F}$  atom in **5** had to take into account the side chain  $\chi_1$  angle and required complicated referencing.<sup>[10]</sup> We thus aimed to expand the scope of available labels and report herein the rational design, synthesis, and validation of a novel aromatic label for solid-state  $^{19}\text{F}$  NMR spectroscopy to replace phenylalanine.

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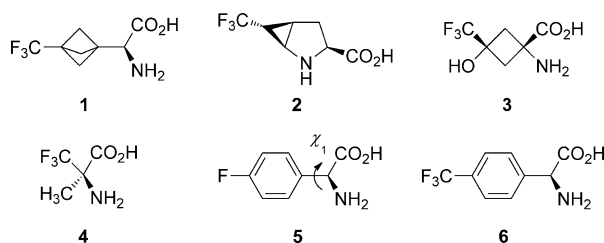
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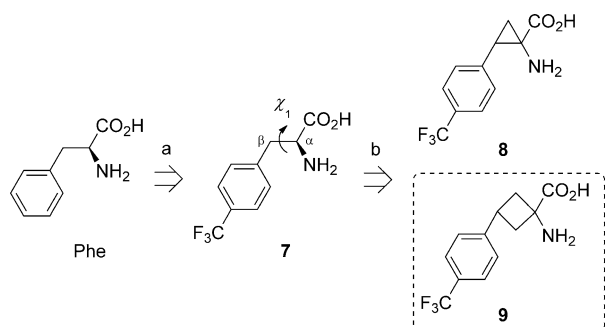
Supporting information for this article is available on the WWW under <http://dx.doi.org/10.1002/anie.201301344>.



In peptides and proteins, the aromatic ring of phenylalanine can participate in noncovalent inter- and intramolecular interactions with other aromatic residues ( $\pi$ - $\pi$  interaction),<sup>[11]</sup> with the positively charged side chains of arginine, lysine, and histidine (cation- $\pi$  interaction),<sup>[12]</sup> with negatively charged glutamic and aspartic acid (anion- $\pi$  interaction),<sup>[13]</sup> with the charged peptide termini, and with nucleic acids,<sup>[14]</sup> lipids,<sup>[15]</sup> and carbohydrates.<sup>[16]</sup> Phenylalanine-rich sequences are reported to assist other molecules crossing the blood-brain barrier,<sup>[17]</sup> to specifically bind the export proteins of *M. tuberculosis*,<sup>[18]</sup> and certain proline-rich peptide motifs,<sup>[19]</sup> to mediate homopeptide self-assembly in solution,<sup>[20]</sup> and to guide the assembly of transmembrane helices.<sup>[21]</sup> In membrane proteins phenylalanine is often found to be preferentially located near the hydrophobic-hydrophilic interface of the lipid bilayer.<sup>[22]</sup> Therefore, a specific label that can

appropriately replace phenylalanine residues in peptides will open new possibilities to study a broad range of important targets under biologically relevant conditions by using solid-state  $^{19}\text{F}$  NMR spectroscopy.

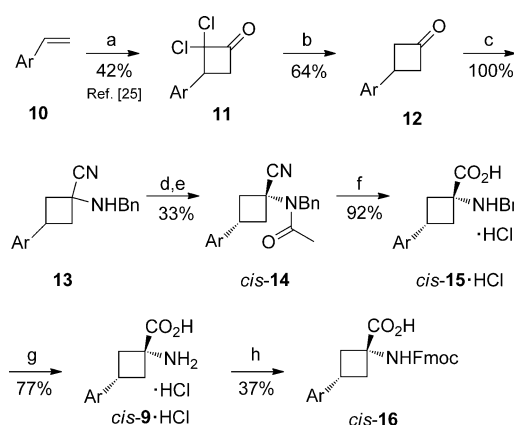
The principles of designing the target structure are summarized on Scheme 1. We chose a  $\text{CF}_3$  moiety as a reporter group in view of the simple spectral analysis that requires no referencing, and the advantageous relaxation



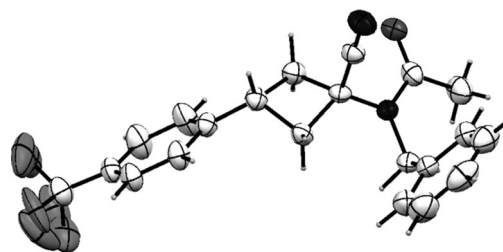
**Scheme 1.** Design of a novel  $^{19}\text{F}$  NMR label (**9**) to specifically replace phenylalanine in membrane-bound peptides: a) attachment of the  $\text{CF}_3$  reporter; b) restriction of the molecular mobility.

times of this rapidly rotating group.<sup>[8a,b]</sup> The  $\text{CF}_3$  substituent was placed in the *para* position of the phenyl group (**7**) (Scheme 1a) to maintain the symmetry of the rotationally unrestricted phenyl ring. However, to fulfill criterion 1, we had to prevent any further flexibility of the side chain in amino acid **7** by blocking the rotation around  $\chi_1$ . To achieve this aim, one could introduce the conformationally rigid cyclopropane (**8**) or restricted cyclobutane (**9**) moieties in place of the  $\text{C}_\alpha\text{--C}_\beta$  bond. In principle, both compounds **8** and **9** should be suitable for  $^{19}\text{F}$  NMR spectroscopy, but in our opinion amino acid **9** is more preferable, because a cyclopropane ring that is activated by electronegative substituents tends to be susceptible to nucleophilic attack.<sup>[23]</sup> Hence, amino acid **8** could decompose during SPPS. Moreover, in contrast to **8**, compound **9** is achiral and does not require the time-intensive and costly separation of enantiomers and/or asymmetric synthesis, which often limits the multigram preparation and subsequent application of chiral compounds.<sup>[24]</sup>

Synthesis of amino acid **9** commenced from the readily available styrene **10** (Scheme 2). Ketone **12** was obtained from compound **10** in two steps following a modified literature protocol.<sup>[25]</sup> A [2+2] cycloaddition with dichloroketene generated in situ from  $\text{Cl}_3\text{CCOCl}$  and  $\text{Cu}/\text{Zn}$ , gave cyclobutane **11**. Subsequent removal of the chlorine atoms in **11** with  $\text{Zn}$  in acetic acid afforded ketone **12**. Next, a Strecker reaction with benzylamine ( $\text{BnNH}_2$ ) and  $(\text{CH}_3)_3\text{SiCN}$  yielded aminonitrile **13** as a 4:1 mixture of stereoisomers. Our attempts to separate the compounds by column chromatography on silica gel led to decomposition of the product. Therefore, the amino function in **13** was protected with an acetyl group to produce, after recrystallization, amide *cis*-**14** as a single isomer. The stereoconfiguration of *cis*-**14** was determined by X-ray diffraction (Figure 1).<sup>[26]</sup> Acidic hydroly-



**Scheme 2.** Ar = 4- $\text{CF}_3\text{C}_6\text{H}_4$ . Reagents and conditions: a)  $\text{Cl}_3\text{CCOCl}$ ,  $\text{POCl}_3$ ,  $\text{Zn}/\text{Cu}$ ,  $\text{Et}_2\text{O}$ , reflux; b)  $\text{Zn}$ ,  $\text{AcOH}$ , reflux; c)  $(\text{CH}_3)_3\text{SiCN}$ ,  $\text{BnNH}_2$ ,  $\text{MeOH}$ , 0–25 °C; d)  $\text{CH}_3\text{COCl}$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , 0–25 °C; e) crystallization from *i*PrOH; f) 6 N  $\text{HCl}$ , reflux; g)  $\text{Pd}/\text{H}_2$ ,  $\text{MeOH}$ , 20 bar, 25 °C; h)  $(\text{CH}_3)_3\text{SiCl}$ ,  $\text{NEt}(\text{iPr})_2$ ,  $\text{Fmoc-Cl}$ ,  $\text{CH}_2\text{Cl}_2$ , 0–25 °C.



**Figure 1.** X-ray crystal structure of compound *cis*-**14**. Thermal ellipsoids are shown at 50% probability levels (see the Supporting Information for details). F: light grey (both equally populated positions observed for the  $\text{CF}_3$  group are shown), O: dark grey, N: black.

ysis of the amide and nitrile groups in *cis*-**14** gave acid *cis*-**15**· $\text{HCl}$  in 92% yield. Finally, the desired amino acid *cis*-**9**· $\text{HCl}$  was obtained from *cis*-**15**· $\text{HCl}$  by  $\text{Pd}$ -catalyzed hydrogenolysis of the  $\text{N}$ -benzyl bond.

$\text{N}$ -Fmoc protection of amino acid *cis*-**9**· $\text{HCl}$  with either  $\text{Fmoc-Cl}$  or  $\text{Fmoc-OSu}$  (9-fluorenylmethoxycarbonyl- $\text{N}$ -hydroxysuccinimide)<sup>[27]</sup> afforded, in less than 10% yield, the desired compound *cis*-**16** suitable for SPPS. Therefore, *cis*-**9**· $\text{HCl}$  was instead treated with  $(\text{CH}_3)_3\text{SiCl}/\text{Fmoc-Cl}$ ,<sup>[28]</sup> which gave the desired product in a much higher yield of 37%. It is worth mentioning that we have previously observed a similar behavior for amino acid **3** upon Fmoc protection.<sup>[6]</sup>

To evaluate the practical applicability of the obtained amino acid *cis*-**9** as a label in solid-state  $^{19}\text{F}$  NMR structure analysis, we incorporated it into the well-known antimicrobial peptide Magainin 2 (GIGKFLHSAKKFGKAFVGEIMNS) from *X. laevis*.<sup>[29]</sup> The three native phenylalanine residues were each replaced with **9** to produce three peptides: **17**, in which  $\text{Phe}^5$  has been replaced with *cis*-**9**, **18**, in which  $\text{Phe}^{12}$  has been replaced, and **19**, in which  $\text{Phe}^{16}$  has been replaced. Importantly, amino acid *cis*-**9** was readily incorporated into the polypeptide sequence by using a standard Fmoc SPPS protocol with TBTU as the coupling agent.<sup>[30]</sup> Neither degradation nor low reactivity of *cis*-**9** was observed. All

compounds **17–19** were obtained as single diastereomers and were stable during subsequent storage, thus fulfilling criterion 2.

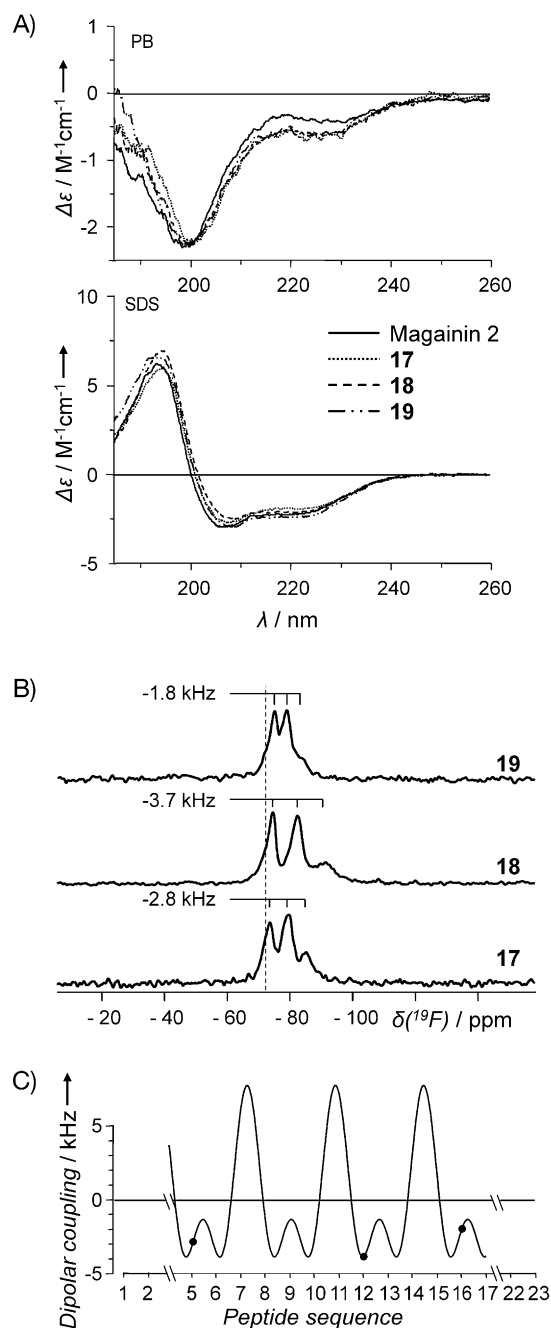
Magainin 2 is a representative antimicrobial peptide that changes from a random coil in aqueous solutions to an amphiphilic  $\alpha$ -helix in a membrane environment.<sup>[31]</sup> The  $^{19}\text{F}$ -labeled peptides **17–19** underwent the same conformational transition (Figure 2A) as the parent peptide. The biological activities of **17–19** were tested in bacterial-growth inhibition assays (see the Supporting Information for details). Like the parent peptide,<sup>[32]</sup> compounds **17–19** were active against Gram-negative *E. coli* and Gram-positive *S. aureus* strains with minimal growth inhibition concentrations in the micromolar range (5–30  $\mu\text{M}$ ). These results demonstrate that amino acid *cis*-**9** also satisfies fully criterion 3 with regard to an unperturbed peptide structure and function.

The labeled peptides were incorporated into macroscopically aligned bilayers of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) on solid supports, as previously described in detail.<sup>[10a]</sup> In these fully hydrated samples, Magainin 2 is known to form an amphipathic  $\alpha$ -helix and to align parallel to the membrane plane, that is, with a helix tilt angle of about  $90^\circ$  relative to the membrane normal.<sup>[33]</sup> The reconstituted samples of **17–19** were well behaved and no signs of aggregation were detected. Each peptide revealed a clear dipolar splitting, indicating a well-defined orientation of each  $\text{CF}_3$  reporter group (Figure 2B). The obtained splittings were fitted against the typical secondary structures: a continuous  $\alpha$ -helix and a  $\beta$ -strand. Even though more constraints from additional labels would be needed for a precise structural analysis including the dynamical behavior,<sup>[2,34]</sup> the  $^{19}\text{F}$  NMR data from **17–19** allows us to conclude that a) the conformation of Magainin 2 in DMPC is not a  $\beta$ -strand but an  $\alpha$ -helix, b) the  $\alpha$ -helix extends at least from position 5 to position 16, and c) it is consistent with a tilt angle of about  $90^\circ$  for a surface-bound helix. These results obtained here with the new  $^{19}\text{F}$ -label *cis*-**9** are in a full agreement with the known structural features of Magainin 2 determined independently by solid-state  $^{15}\text{N}$  NMR spectroscopy.<sup>[33]</sup>

In summary, we have synthesized the novel conformationally restricted trifluoromethyl-substituted  $\alpha$ -amino acid *cis*-1-amino-3-[4-(trifluoromethyl)phenyl]-cyclobutanecarboxylic acid (*cis*-**9**), designed as a label for the structure analysis of membrane-active peptides by solid-state  $^{19}\text{F}$  NMR spectroscopy. The compound was validated as a suitable substituent for phenylalanine by incorporating it into the membrane-active antimicrobial peptide Magainin 2. All criteria required for a  $^{19}\text{F}$  NMR label were fulfilled by amino acid *cis*-**9**, when it was used to replace either of the three native phenylalanine residues. Thus, a growing arsenal of available  $^{19}\text{F}$  labels can now be used to replace specific residues in peptides and study them by solid-state  $^{19}\text{F}$  NMR spectroscopy.

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**Figure 2.** Characterization of amino acid *cis*-**9**: A) Circular dichroism spectra of Magainin 2 and its  $^{19}\text{F}$ -labeled analogues **17–19** in phosphate buffer at pH 7 (PB, top) and in sodium dodecyl sulfate micelles (SDS, bottom). B) Solid-state  $^{19}\text{F}$  NMR spectra of **17–19**, each incorporated into oriented DMPC bilayers (peptide/lipid = 1/10 mol mol $^{-1}$ ,  $40^\circ\text{C}$ ). The observed values of the dipolar couplings are indicated above the signals, and the dashed line shows the isotropic chemical shift. C) Best-fit dipolar wave plot for a continuous  $\alpha$ -helix in the lipid bilayer.

- [1] a) J. T. Gerig, *Prog. Nucl. Magn. Reson. Spectrosc.* **1994**, 26, 293–370; b) M. A. Danielson, J. J. Falke, *Annu. Rev. Biophys. Biomol. Struct.* **1996**, 25, 163–195; c) A. S. Ulrich, *Prog. Nucl. Magn. Reson. Spectrosc.* **2005**, 46, 1–21; d) J. L. Kitevski-LeBlanc, R. S. Prosser, *Prog. Nucl. Magn. Reson. Spectrosc.* **2012**, 62, 1–33.

- [2] a) A. Ulrich, P. Wadhwani, U. H. N. Dürr, S. Afonin, R. W. Glaser, E. Strandberg, P. Tremouilhac, C. Sachse, M. Berditch-evskaia, S. L. Grage in *NMR spectroscopy of biological solids* (Ed.: A. Ramamoorthy), CRC, Boca Raton, FL, **2006**, pp. 215–236; b) S. L. Grage, S. Afonin, A. S. Ulrich, *Methods Mol. Biol.* **2010**, 618, 183–207; c) V. S. Kubyshev, I. V. Komarov, S. Afonin, P. K. Mykhailiuk, S. L. Grage, A. S. Ulrich in *Fluorine in Pharmaceutical and Medicinal Chemistry: From Biophysical Aspects to Clinical Applications* (Eds.: V. Gouverneur, K. Müller), Imperial College Press, London, **2012**, pp. 91–139; d) K. Koch, S. Afonin, M. Ieronimo, M. Berditsch, A. S. Ulrich, *Top. Curr. Chem.* **2012**, 306, 89–118.
- [3] a) V. P. Kukhar, V. A. Soloshonok, *Fluorine Containing Amino Acids: Synthesis and Properties*, Wiley, New York, **1995**; b) X.-L. Qiu, W.-D. Meng, F.-L. Qing, *Tetrahedron* **2004**, 60, 6711–6745; c) X.-L. Qiu, F.-L. Qing, *Eur. J. Org. Chem.* **2011**, 3261–3278.
- [4] a) P. K. Mikhailiuk, S. Afonin, A. N. Chernega, E. B. Rusanov, M. O. Platonov, G. G. Dubinina, M. Berditsch, A. S. Ulrich, I. V. Komarov, *Angew. Chem.* **2006**, 118, 5787–5789; *Angew. Chem. Int. Ed.* **2006**, 45, 5659–5661; b) S. Afonin, P. K. Mikhailiuk, I. V. Komarov, A. S. Ulrich, *J. Pept. Sci.* **2007**, 13, 614–623; c) P. K. Mykhailiuk, N. M. Voievoda, S. Afonin, A. S. Ulrich, I. V. Komarov, *J. Fluorine Chem.* **2010**, 131, 217–220; d) P. Wadhwani, E. Strandberg, N. Heidenreich, J. Bürck, S. Fanghanel, A. S. Ulrich, *J. Am. Chem. Soc.* **2012**, 134, 6512–6515.
- [5] P. K. Mykhailiuk, S. Afonin, G. V. Palamarchuk, O. V. Shishkin, A. S. Ulrich, I. V. Komarov, *Angew. Chem.* **2008**, 120, 5849–5851; *Angew. Chem. Int. Ed.* **2008**, 47, 5765–5767.
- [6] A. N. Tkachenko, P. K. Mykhailiuk, S. Afonin, D. S. Radchenko, V. S. Kubyshev, A. S. Ulrich, I. V. Komarov, *Angew. Chem.* **2013**, 125, 1526–1529; *Angew. Chem. Int. Ed.* **2013**, 52, 1486–1489.
- [7] D. Maisch, P. Wadhwani, S. Afonin, C. Böttcher, B. Koksche, A. S. Ulrich, *J. Am. Chem. Soc.* **2009**, 131, 15596–15597.
- [8] a) R. W. Glaser, C. Sachse, U. H. N. Dürr, P. Wadhwani, S. Afonin, E. Strandberg, A. S. Ulrich, *Biophys. J.* **2005**, 88, 3392–3397; b) R. W. Glaser, C. Sachse, U. H. N. Dürr, P. Wadhwani, A. S. Ulrich, *J. Magn. Reson.* **2004**, 168, 153–163; c) P. Wadhwani, J. Bürck, E. Strandberg, C. Mink, S. Afonin, A. S. Ulrich, *J. Am. Chem. Soc.* **2008**, 130, 16515–16517; d) S. Afonin, S. L. Grage, M. Ieronimo, P. Wadhwani, A. S. Ulrich, *J. Am. Chem. Soc.* **2008**, 130, 16512–16514.
- [9] A. Eisele, *Aggregationsverhalten von trojanischen Peptiden*, Diplomarbeit, Universität Karlsruhe, **2007**.
- [10] a) R. W. Glaser, A. S. Ulrich, *J. Magn. Reson.* **2003**, 164, 104–114; b) R. Ulrich, R. W. Glaser, A. S. Ulrich, *J. Magn. Reson.* **2003**, 164, 115–127.
- [11] a) J. Singh, J. M. Thornton, *FEBS Lett.* **1985**, 191, 1–6; b) G. B. McGaughey, M. Gagne, A. K. Rappe, *J. Biol. Chem.* **1998**, 273, 15458–15463; c) S. K. Burley, G. A. Petsko, *Science* **1985**, 229, 23–28; d) C. A. Hunter, J. Singh, J. M. Thornton, *J. Mol. Biol.* **1991**, 218, 837–846.
- [12] a) J. P. Gallivan, D. A. Dougherty, *Proc. Natl. Acad. Sci. USA* **1999**, 96, 9459–9464; b) D. A. Dougherty, *Science* **1996**, 271, 163–168; c) M. M. Gromiha, C. Santhosh, S. Ahmad, *Int. J. Biol. Macromol.* **2004**, 34, 203–211; d) P. B. Crowley, A. Golovin, *Proteins Struct. Funct. Bioinf.* **2005**, 59, 231–239.
- [13] B. L. Schottel, H. T. Chifotides, K. R. Dunbar, *Chem. Soc. Rev.* **2008**, 37, 68–83.
- [14] N. M. Luscombe, R. A. Laskowski, J. M. Thornton, *Nucleic Acids Res.* **2001**, 29, 2860–2874.
- [15] a) W. Zhang, E. Crocker, S. McLaughlin, S. O. Smith, *J. Biol. Chem.* **2003**, 278, 21459–21466; b) A. Gambhir, G. Hangyás-Mihályné, I. Zaitseva, D. S. Cafiso, J. Wang, D. Murray, S. N. Pentyala, S. O. Smith, S. McLaughlin, *Biophys. J.* **2004**, 86, 2188–2207.
- [16] J. L. Asensio, A. Ardá, F. J. Cañada, J. Jiménez-Barbero, *Acc. Chem. Res.* **2012**, 46, 946–954.
- [17] M. Malakoutikhah, R. Prades, M. Teixidó, E. Giralt, *J. Med. Chem.* **2010**, 53, 2354–2363.
- [18] K. Kumar, M. Tharad, S. Ganapathy, G. Ram, A. Narayan, J. A. Khan, R. Pratap, A. Ghosh, S. K. Samuchiwal, S. Kumar, K. Bhalla, D. Gupta, K. Natarajan, Y. Singh, A. Ranganathan, *PLoS One* **2009**, 4, e7615.
- [19] M. J. Macias, S. Wiesner, M. Sudol, *FEBS Lett.* **2002**, 513, 30–37.
- [20] E. Gazit, *Prion* **2007**, 1, 32–35.
- [21] N. Sal-Man, D. Gerber, I. Bloch, Y. Shai, *J. Biol. Chem.* **2007**, 282, 19753–19761.
- [22] L. Adamian, V. Nanda, W. DeGrado, J. Liang, *Proteins Struct. Funct. Bioinf.* **2005**, 59, 496–509.
- [23] a) M. Yu, B. L. Pagenkopf, *Tetrahedron* **2005**, 61, 321–347; b) H.-U. Reissig, R. Zimmer, *Chem. Rev.* **2003**, 103, 1151–1196; c) H.-U. Reissig, *Top. Curr. Chem.* **1988**, 144, 73–135.
- [24] For example, the practical application of chiral building blocks in medicinal chemistry strongly depends of the number of the chemical steps to synthesize them: a) R. A. Ward, J. G. Kettle, *J. Med. Chem.* **2011**, 54, 4670–4677; b) S. D. Roughley, A. M. Jordan, *J. Med. Chem.* **2011**, 54, 3451–3479.
- [25] K. S. Petersen, B. M. Stoltz, *Tetrahedron* **2011**, 67, 4352–4357.
- [26] CCDC 909069 *cis-14* contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via [www.ccdc.cam.ac.uk/data\\_request/cif](http://www.ccdc.cam.ac.uk/data_request/cif).
- [27] a) C. D. Chang, M. Waki, M. Ahmad, J. Meienhofer, E. O. Lundell, J. D. Haug, *Int. J. Peptide Prot. Res.* **1980**, 15, 59–66; b) R. C. de L. Milton, E. Becker, S. C. F. Milton, J. E. J. Baxter, J. F. Elsworth, *Int. J. Peptide Prot. Res.* **1987**, 30, 431–432; c) D. A. Wellings, E. Atherton, *Methods Enzymol.* **1997**, 289, 44–67.
- [28] D. R. Bolin, I. I. Sytwu, F. Humiec, J. Meienhofer, *Int. J. Peptide Prot. Res.* **1989**, 33, 353–359.
- [29] M. Zasloff, *Proc. Natl. Acad. Sci. USA* **1987**, 84, 5449–5453.
- [30] E. Atherton, R. C. Sheppard, *Solid Phase Peptide Synthesis*, University Press, Oxford, **1989**.
- [31] a) D. Marion, M. Zasloff, A. Bax, *FEBS Lett.* **1988**, 227, 21–26; b) H. C. Chen, J. H. Brown, J. L. Morell, C. M. Huang, *FEBS Lett.* **1988**, 236, 462–466; c) M. Jackson, H. H. Mantsch, J. H. Spencer, *Biochemistry* **1992**, 31, 7289–7293.
- [32] K. Matsuzaki, *Biochim. Biophys. Acta Rev. Biomembr.* **1998**, 1376, 391–400.
- [33] a) E. S. Salnikov, B. Bechinger, *Biophys. J.* **2011**, 100, 1473–1480; b) E. Strandberg, J. Zerweck, P. Wadhwani, A. S. Ulrich, *Biophys. J.* **2013**, 104, L9–L11.
- [34] S. Esteban-Martín, E. Strandberg, J. Salgado, A. S. Ulrich, *Biochim. Biophys. Acta Biomembr.* **2010**, 1798, 252–257.