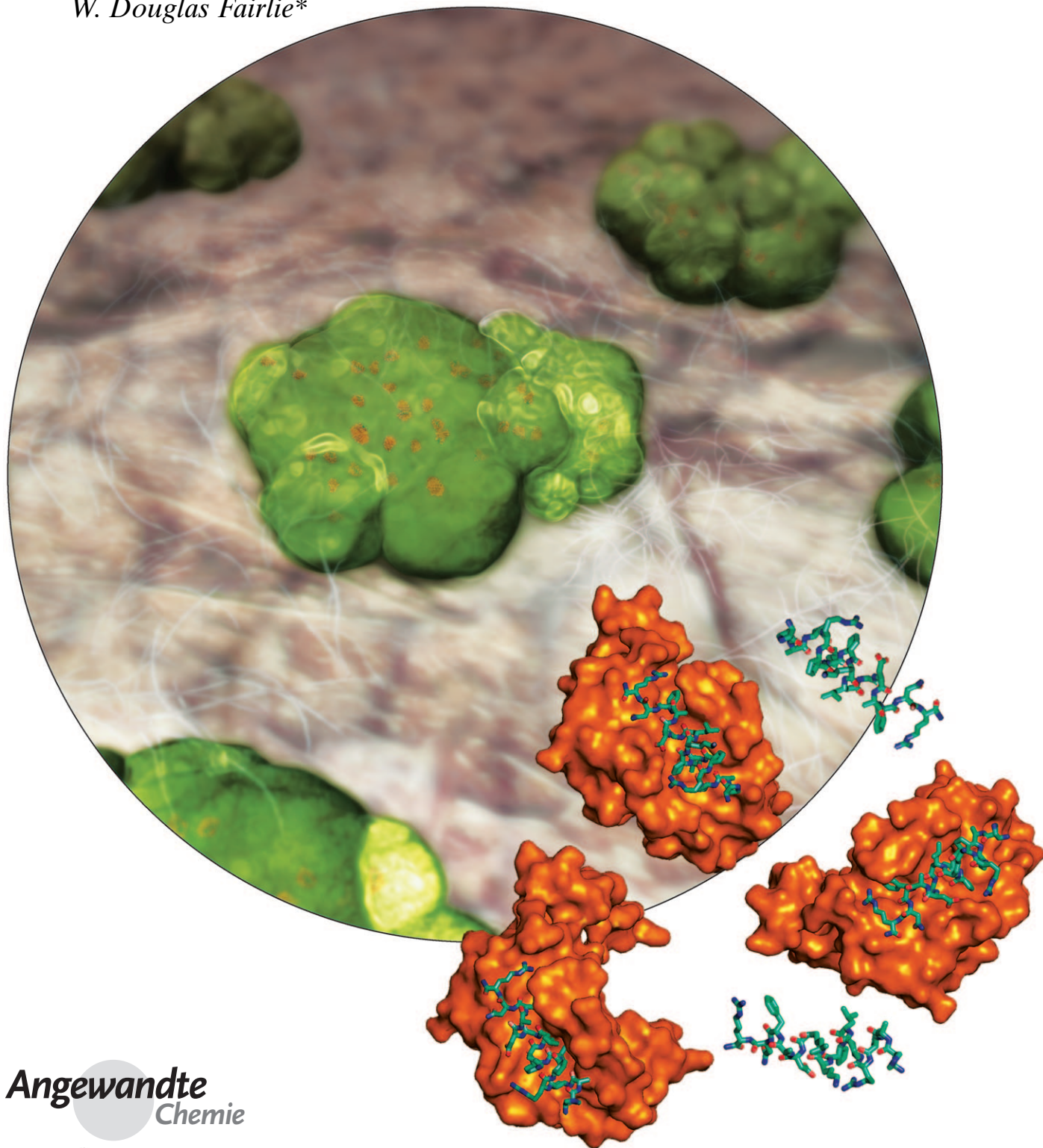# High-Resolution Structural Characterization of a Helical α/β-Peptide Foldamer Bound to the Anti-Apoptotic Protein Bcl-x$_L$**

Erinna F. Lee, Jack D. Sadowsky, Brian J. Smith, Peter E. Czabotar, Kimberly J. Peterson-Kaufman, Peter M. Colman, Samuel H. Gellman,* and W. Douglas Fairlie*

Development of potent small-molecule ($M_w < 500$) antagonists of protein–protein interactions is challenging because of the large surface areas (typically more than 500 Å^2) buried at such interfaces.[1] Unnatural oligomers with strong and predictable conformational propensities, referred to as foldamers, have shown potential as inhibitors of such interactions.[2] As with α-peptides and proteins, foldamers constitute scaffolds that can display diverse side chains in particular 3D arrangements, enabling mimicry of large protein-recognition surfaces. However, foldameric backbones are typically resistant to enzymatic degradation, which is an advantage relative to peptide- and protein-based inhibitors. Folding rules for oligomers containing β-amino acid residues exclusively (β-peptides[3]) or in combination with α-amino acid residues (e.g., α/β-peptides[4]), have been elucidated, and both have been used to block specific protein–protein interactions.[5–9]

Herein, we report the first high-resolution structure of a complex between an α/β-peptide foldamer and a protein partner, Bcl-x$_L$. Bcl-x$_L$ is a prosurvival member of the Bcl-2 family that inhibits apoptosis signaling.[10] As over-expression of pro-survival Bcl-2 proteins can lead to cancer, strategies that inhibit their action could lead to development of anti-cancer agents.

Anti-apoptotic Bcl-2 family members display a hydrophobic cleft that accommodates an α-helical segment from the Bcl-2 homology 3 (BH3) domain of a pro-apoptotic binding partner.[11] Peptide- and non-peptide-based scaffolds have been explored as BH3 domain mimics to antagonize Bcl-2 family interactions, including efforts to identify helical β- and α/β-peptide foldamer ligands for Bcl-x$_L$[5b,7,12] Our initial inhibitor designs focused on backbones composed entirely of β-amino acid residues or a 1:1 alternating α:β pattern, but we concluded that more complex patterns of α- and β-residues are necessary for effective BH3 domain mimicry. For example, the isomeric 15-residue α/β-peptides **1** and **2** (Figure 1a), which contain an N-terminal segment with alternating α:β residues and a C-terminal α segment, bind tightly to Bcl-x$_L$ ($K_i = 2$ nM for **1** and 5 nM for **2**).[7a] The first
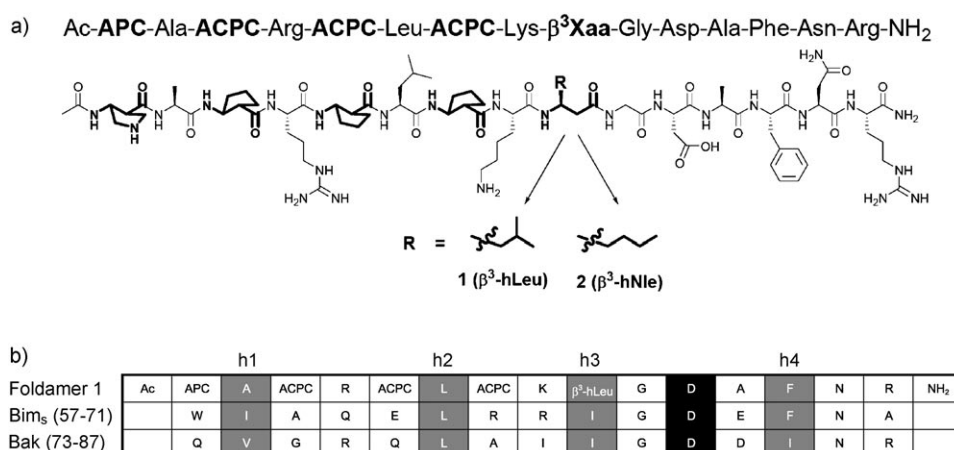

Figure 1. a) Structures of α/β-peptides **1** and **2**. β-Amino acid residues are shown in bold. b) Sequence alignment of BH3 domains from Bim and Bak with foldamer **1**. Gray: the four conserved hydrophobic residues of BH3 domains (h1–h4); black: the invariant Asp.

[*] J. D. Sadowsky,[+] K. J. Peterson-Kaufman, Prof. S. H. Gellman
Department of Chemistry, University of Wisconsin
1101 University Avenue, Madison, WI 53706 (USA)
Fax: (+1) 608-265-4534
E-mail: gellman@chem.wisc.edu

Dr. E. F. Lee,[+] Dr. B. J. Smith, Dr. P. E. Czabotar, Prof. P. M. Colman, Dr. W. D. Fairlie
Structural Biology Division
The Walter and Eliza Hall Institute of Medical Research
1G Royal Parade, Parkville, Victoria 3052 (Australia)
Fax: (+61) 3-9345-2686
E-mail: fairlie@wehi.edu.au

[+] These authors contributed equally.

four β residues in **1** and **2** have a five-membered ring constraint that promotes helical folding.[13] The fifth β residue is the only difference between **1** and **2** (β^3-hLeu in place of β^3-hNle, respectively). Several side chains in **1** and **2** (for example, those of α-Leu6 and α-Asp11) were intended to mimic side chains known to be important for binding of BH3 domains to Bcl-x$_L$ (e.g., Leu62 and Asp67 in Bim; Figure 1b).[14]

We co-crystallized α/β-peptide **1** with Bcl-x$_L$, and solved the structure to 2.3 Å resolution (protein database identifier: 3FDM; Supporting Information, Table S1). We used a "loop-deleted" form of human Bcl-x$_L$ (Δ27-82), which forms an α1 domain-swapped dimer, yet retains BH3 domain binding activity[15] (Supporting Information, Figure S1). The asymmetric unit contains three protein molecules (root-mean-square deviation in the superposition of Cα atoms over residues 83–197 of Bcl-x$_L$ molecule C versus A = 0.51 Å; B vs. A = 0.36 Å; of the foldamer alone, that is bound to Bcl-x$_L$ molecule C vs. A = 0.32 Å; B vs. A = 0.21 Å). The largest differences among A, B, and C are in the α3 helix of Bcl-x$_L$ (Supporting Information, Figure S2), which is best ordered in molecule A; therefore, we use A for the structural description below.

The structure shows that **1** adopts a helical conformation and is oriented within the BH3 recognition groove in a similar fashion to natural BH3 domains (Figure 2a,b; the Bim BH3 domain bound to Bcl-x_L is shown for comparison). The helix formed by Bcl-x_L-bound **1** features a regular pattern of backbone C=O(i)···H-N($i+4$) H-bonds over nearly the entire length of the oligomer (the helix is disrupted at the C terminus; Figure 2c). This H-bond pattern underlies the α-helix formed by pure α-residue peptides, and is characteristic also of the 14/15-helix, a secondary structure favored by 1:1 alternating α:β backbones in which β-residues have a five-membered ring constraint.[16]
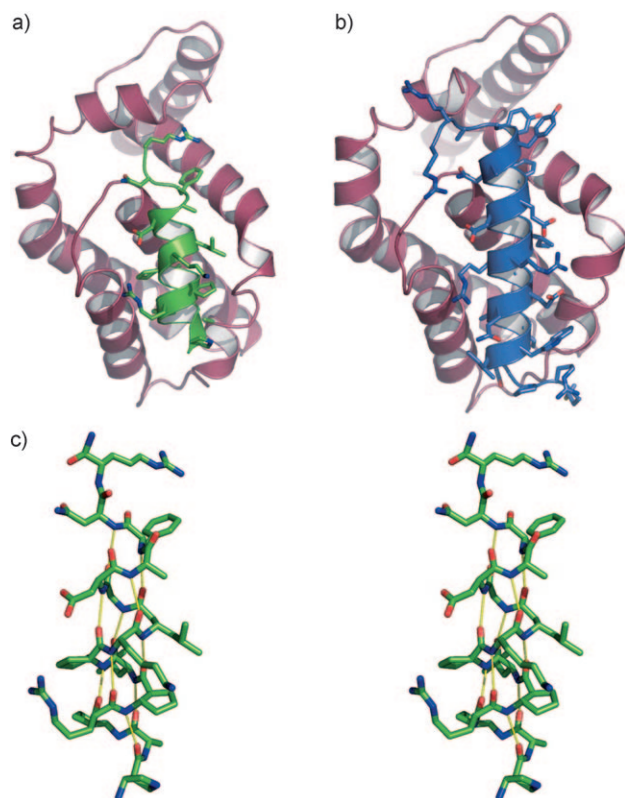

Figure 2. X-ray structures of human Bcl-x_L (magenta) complexed with a) foldamer **1** (green) or b) a 26-residue α peptide from human Bim (DMRPEIWIAQELRRIGDEFNAYYARR; blue). This structure (PDB ID: 3FDL) is essentially identical to 1PQ1, in which a murine Bim peptide was crystallized with murine Bcl-x_L (unpublished results).[19] c) Stereoview of foldamer **1**.

The canonical BH3 domain sequence includes four hydrophobic residues in an i, $i+4$, $i+7$, $i+11$ pattern, designated h1–h4 (Figure 1b), which project from one face of the amphipathic α helix into the hydrophobic groove of the pro-survival protein. One of the most important of these residues for binding to Bcl-2 family proteins is h2, which is conserved as leucine in all known BH3 domains from pro-apoptotic proteins.[14,17] In the complex with Bcl-x_L, the side chain of α-Leu6 in **1** occupies the h2 binding pocket, mimicking the side chain of the conserved BH3 domain leucine (Figure 3a). The h4 binding pocket on Bcl-x_L is filled by α-Phe13 in **1**; some natural BH3 domains, such as Bim^{BH3},
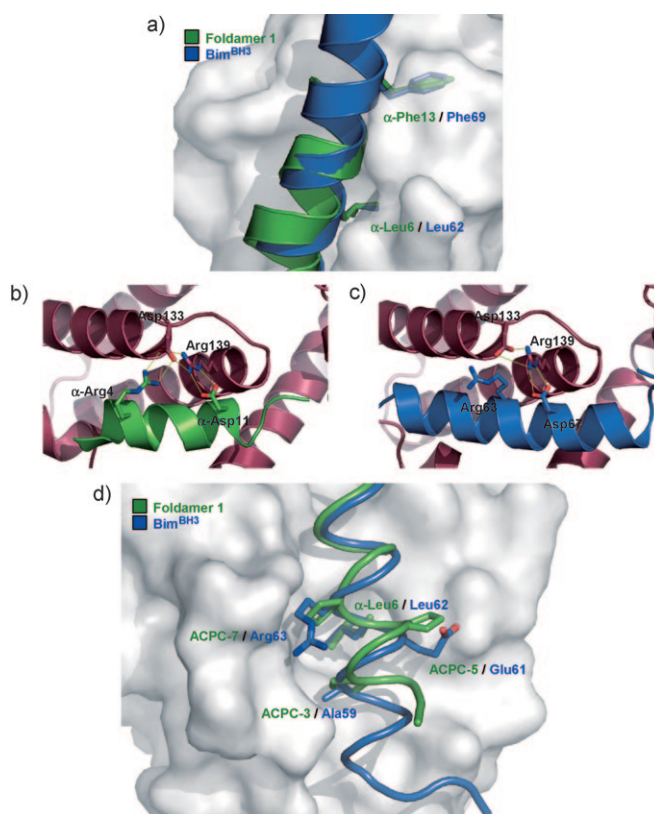

Figure 3. Detailed comparison of the foldamer/Bcl-x_L and Bim^{BH3}/Bcl-x_L interfaces. a) Overlay of key hydrophobic side chains on foldamer **1** (green) with analogous side chains on Bim^{BH3} (blue). b),c) Key electrostatic interactions in the foldamer/Bcl-x_L (b) and Bim^{BH3}/Bcl-x_L (c) interfaces (Bcl-x_L ribbon: magenta). d) ACPC residues of foldamer **1** overlaid with analogously positioned side chains on Bim^{BH3}.

have Phe at the analogous position. A critical salt bridge formed between the aspartate residue strictly conserved in all BH3 domains and the side chain of Arg139 of Bcl-x_L is observed also in the foldamer **1**/Bcl-x_L complex (Figure 3b,c). Interactions involving β residues include those of the *trans*-2-aminocyclopentanecarboxylic acid (ACPC) residues at positions 3 and 7 of **1**, which make contacts similar to those made by other BH3 domains (for example, the side chain of Ala59 and the propylene moiety of Arg63, respectively, in Bim^{BH3}) (Figure 3d).

In complexes of Bcl-x_L with BH3 domain peptides, a hydrophobic side chain on the BH3 domain (e.g., Ile58 in Bim^{BH3}) invariably occupies the h1 pocket on Bcl-x_L. In the complex of Bcl-x_L with **1**, by contrast, the h1 pocket is not formed, because Leu108 of Bcl-x_L occludes the site. This is but one of several significant changes in the shape of the BH3-recognition cleft of Bcl-x_L complexed with **1** relative to the shape of this cleft complexed with BH3 domain α-peptide ligands (e.g., Bim^{BH3}). Interestingly, these changes arise primarily from structural differences in the α3 helix segment of Bcl-x_L, which makes contact with the α/β segment of **1** (Supporting Information, Figure S2).

Specific aspects of foldamer–protein recognition observed in the crystal structure are consistent with previously described sequence/affinity relationships established

with modified α/β-peptide ligands,[7a] suggesting that the crystal structure faithfully reflects foldamer–protein recognition in solution. For example, Ala scanning results showed that side chain truncation at α-Leu6, α-Asp11, or α-Phe13 caused substantial decreases in foldamer binding.[7a] As discussed above, each of these residues in **1** makes a close contact with Bcl-x_L. The side chain of β³-hLeu at position 9 is largely solvent-exposed, which is consistent with the observation that replacing this residue with either β³-hNle or β³-hAla has little effect on affinity. However, moving this side chain one atom toward the C terminus (β³-hNle→β²-hNle) caused a large decrease in binding, indicating the importance of side-chain location at position 9. Indeed, the structure shows that the backbone methylene group of β³-hLeu is very close to Tyr101 of Bcl-x_L, which suggests low tolerance for a side chain at this position (i.e., a β² residue). Replacing a hydrophobic ACPC residue with the hydrophilic analogue APC was deleterious at position 3 or 7, but not position 5. The structure rationalizes these observations, because ACPC5 is the most solvent-exposed of these β residues in the complex.

The hypothesis that foldamer–protein interactions observed in the crystal structure mirror interactions in solution is further supported by the impact of the A142L mutation to Bcl-x_L on the binding of foldamer **2**. This mutation does not disrupt Bcl-x_L folding, but the modified side chain occupies more space within the BH3-recognition cleft, and the mutant protein displays diminished affinity for natural BH3 domains (Supporting Information, Figure S3).[18] We used fluorescence polarization to compare the binding affinities of wild-type Bcl-x_L and the A142L mutant for a fluorescein-labeled derivative of α/β-peptide **2** (Flu-Ahx-**2**). The mutant protein showed at least 13-fold weaker binding for Flu-Ahx-**2** relative to the wild-type protein, which is consistent with the crystal structure because α-Leu6 of **1** is in close contact with Ala142 of wild-type Bcl-x_L.

These results contribute significantly to our understanding of foldamer–protein recognition. The crystal structure of Bcl-x_L bound to **1** shows that this foldamer effectively mimics natural α-helical ligands. This finding validates our "chimeric" design strategy,[7c] as the N-terminal segment of **1** adopts the 14/15-helical conformation documented for simpler α/β-peptides,[16] whereas the C-terminal segment replicates the analogous segments of BH3-domain α-peptides bound to Bcl-x_L (e.g., the Bak BH3 domain).[17] The data suggest that the foldamer achieves high affinity in part by mimicking the three-dimensional display of the canonical side chains projected by natural BH3 domains. However, inspection of the foldamer–protein interface at atomic resolution indicates that β-residue contacts may also contribute significantly to foldamer affinity. These conclusions are supported by effects of mutations to the protein or to the foldamer on binding affinity. Overall, our results demonstrate the value of characterizing protein–foldamer interfaces at high-resolution. Elucidation of additional structures should enhance our ability to design foldamers that target specific protein surfaces.

[1] M. R. Arkin, J. A. Wells, *Nat. Rev. Drug Discovery* **2004**, *3*, 301.

[2] a) *Foldamers: Structure, Properties, and Applications* (Eds.: S. Hecht, I. Huc) Wiley-VCH, Weinheim, **2007**; b) S. H. Gellman, *Acc. Chem. Res.* **1998**, *31*, 173; c) C. M. Goodman, S. Choi, S. Shandler, W. F. DeGrado, *Nat. Chem. Biol.* **2007**, *3*, 252.

[3] R. P. Cheng, S. H. Gellman, W. F. DeGrado, *Chem. Rev.* **2001**, *101*, 3219.

[4] W. S. Horne, S. H. Gellman, *Acc. Chem. Res.* **2008**, *41*, 1399.

[5] a) E. P. English, R. S. Chumanov, S. H. Gellman, T. Compton, *J. Biol. Chem.* **2006**, *281*, 2661; b) W. S. Horne, M. D. Boersma, M. A. Windsor, S. H. Gellman, *Angew. Chem.* **2008**, *120*, 2895; *Angew. Chem. Int. Ed.* **2008**, *47*, 2853.

[6] J. A. Kritzer, J. D. Lear, M. E. Hodsdon, A. Schepartz, *J. Am. Chem. Soc.* **2004**, *126*, 9468.

[7] a) J. D. Sadowsky, W. D. Fairlie, E. B. Hadley, H. S. Lee, N. Umezawa, Z. Nikolovska-Coleska, S. Wang, D. C. Huang, Y. Tomita, S. H. Gellman, *J. Am. Chem. Soc.* **2007**, *129*, 139; b) J. D. Sadowsky, J. K. Murray, Y. Tomita, S. H. Gellman, *ChemBioChem* **2007**, *8*, 903; c) J. D. Sadowsky, M. A. Schmitt, H. S. Lee, N. Umezawa, S. Wang, Y. Tomita, S. H. Gellman, *J. Am. Chem. Soc.* **2005**, *127*, 11966.

[8] O. M. Stephens, S. Kim, B. D. Welch, M. E. Hodsdon, M. S. Kay, A. Schepartz, *J. Am. Chem. Soc.* **2005**, *127*, 13126.

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

[10] S. Cory, J. M. Adams, *Nat. Rev. Cancer* **2002**, *2*, 647.

[11] A. M. Petros, E. T. Olejniczak, S. W. Fesik, *Biochim. Biophys. Acta Mol. Cell Res.* **2004**, *1644*, 83.

[12] a) X. Fu, J. R. Apgar, A. E. Keating, *J. Mol. Biol.* **2007**, *371*, 1099; b) E. F. Lee, P. E. Czabotar, B. J. Smith, K. Deshayes, K. Zobel, P. M. Colman, W. D. Fairlie, *Cell Death Differ.* **2007**, *14*, 1711; c) G. Lessene, P. E. Czabotar, P. M. Colman, *Nat. Rev. Drug Discovery* **2008**, *7*, 989; d) L. D. Walensky, A. L. Kung, I. Escher, T. J. Malia, S. Barbuto, R. D. Wright, G. Wagner, G. L. Verdine, S. J. Korsmeyer, *Science* **2004**, *305*, 1466; e) O. Kutzki, H. S. Park, J. T. Ernst, B. P. Orner, H. Yin, A. D. Hamilton, *J. Am. Chem. Soc.* **2002**, *124*, 11838; f) T. Oltersdorf, S. W. Elmore, A. R. Shoemaker, R. C. Armstrong, D. J. Augeri, B. A. Belli, M. Bruncko, T. L. Deckwerth, J. Dinges, P. J. Hajduk, M. K. Joseph, S. Kitada, S. J. Korsmeyer, A. R. Kunzer, A. Letai, C. Li, M. J. Mitten, D. G. Nettesheim, S. Ng, P. M. Nimmer, J. M. O'Conner, A. Oleksijew, A. M. Petros, J. C. Reed, W. Shen, S. K. Tahir, C. B. Thompson, K. J. Tomaselli, B. L. Want, M. D. Wendt, H. C. Zhang, S. W. Fesik, S. H. Rosenberg, *Science* **2005**, *435*, 677.

[13] a) W. S. Horne, J. L. Price, S. H. Gellman, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 9151; b) M. A. Schmitt, S. H. Choi, I. A. Guzei, S. H. Gellman, *J. Am. Chem. Soc.* **2005**, *127*, 13130.

[14] E. F. Lee, P. E. Czabotar, M. F. van Delft, E. M. Michalak, M. J. Boyle, S. N. Willis, H. Puthalakath, P. Bouillet, P. M. Colman, D. C. Huang, W. D. Fairlie, *J. Cell Biol.* **2008**, *180*, 341.

[15] a) M. Kvansakul, H. Yang, W. D. Fairlie, P. E. Czabotar, S. F. Fischer, M. A. Perugini, D. C. Huang, P. M. Colman, *Cell Death Differ.* **2008**, *15*, 1564; b) A. Oberstein, P. D. Jeffrey, Y. Shi, *J. Biol. Chem.* **2007**, *282*, 13123.

[16] a) S. H. Choi, I. A. Guzei, S. H. Gellman, *J. Am. Chem. Soc.* **2007**, *129*, 13780; b) S. H. Choi, I. A. Guzei, L. C. Spencer, S. H. Gellman, *J. Am. Chem. Soc.* **2008**, *130*, 6544.

[17] M. Sattler, H. Liang, D. Nettesheim, R. P. Meadows, J. E. Harlan, M. Eberstadt, H. S. Yoon, S. B. Shuker, B. S. Chang, A. J. Minn, C. B. Thompson, S. W. Fesik, *Science* **1997**, *275*, 983.

[18] M. K. Manion, J. W. O'Neill, C. D. Giedt, K. M. Kim, K. Y. Zhang, D. M. Hockenbery, *J. Biol. Chem.* **2004**, *279*, 2159.

[19] X. Liu, S. Dai, Y. Zhu, P. Marrack, J. W. Kappler, *Immunity* **2003**, *19*, 341.