

Enhancing the Cell Permeability and Metabolic Stability of Peptidyl Drugs by Reversible Bicyclization

Ziqing Qian, Curran A. Rhodes, Lucas C. McCroskey, Jin Wen, George Appiah-Kubi, David J. Wang, Denis C. Guttridge, and Dehua Pei*

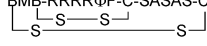
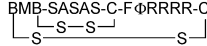
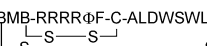
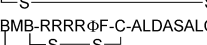
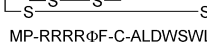
Abstract: Therapeutic applications of peptides are currently limited by their proteolytic instability and impermeability to the cell membrane. A general, reversible bicyclization strategy is now reported to increase both the proteolytic stability and cell permeability of peptidyl drugs. A peptide drug is fused with a short cell-penetrating motif and converted into a conformationally constrained bicyclic structure through the formation of a pair of disulfide bonds. The resulting bicyclic peptide has greatly enhanced proteolytic stability as well as cell-permeability. Once inside the cell, the disulfide bonds are reduced to produce a linear, biologically active peptide. This strategy was applied to generate a cell-permeable bicyclic peptidyl inhibitor against the NEMO-IKK interaction.

Compared to small-molecule drugs, peptides are highly selective and efficacious and, at the same time, relatively safe and well-tolerated. A particularly exciting application of peptides is the inhibition of protein–protein interactions (PPIs), which remain challenging targets for small molecules.^[1] Consequently, there is an increased interest in peptides in pharmaceutical research and development, and about 140 peptide therapeutics are currently being evaluated in clinical trials.^[2] However, peptides are inherently susceptible to proteolytic degradation. Additionally, peptides are generally impermeable to the cell membrane, largely limiting their applications to extracellular targets. Although *N*-methylation of the peptide backbone and formation of intramolecular hydrogen bonds have been shown to improve the proteolytic stability and membrane permeability of certain cyclic peptides,^[3,4] alternative strategies to increase both the metabolic stability and cell permeability of peptide drugs are clearly needed.

NF- κ B is a transcription factor that controls the expression of numerous gene products involved in immune, stress, inflammatory responses, cell proliferation, and apoptosis.^[5] Aberrant activation of NF- κ B signaling has been implicated

in a number of autoimmune diseases (for example, rheumatoid arthritis) and cancer (such as diffuse large B-cell lymphoma), among others.^[6] Canonical NF- κ B signaling is mediated by the interaction between the inhibitor of κ B (I κ B)-kinase (IKK) complex and regulatory protein NF- κ B essential modifier (NEMO).^[7] Binding to NEMO activates IKK, which in turn phosphorylates I κ B, promoting the proteasomal degradation of I κ B and release of active NF- κ B. Modulators targeting various steps of the NF- κ B signaling pathway have been reported, and some of them have progressed into the clinic.^[6,8] One attractive strategy for ameliorating the NF- κ B activity is to selectively disrupt the IKK-NEMO interaction. Previous studies generated a weak NEMO inhibitor ($K_D \approx 37 \mu\text{M}$), Antp-NBD (Table 1, peptide

Table 1: Sequences of peptides in this work.^[a]

Peptide ID	Sequence
1	RQIKIWFQNRMRKWKGG-TALDWSWLQTE
2	BMB-RRRR Φ F-C-SASAS-C-K 
3	BMB-SASAS-C-F Φ RRRR-C-K 
4	BMB-RRRR Φ F-C-ALDWSWLQ-C 
5	BMB-RRRR Φ F-C-ALDASALQ-C 
6	MP-RRRR Φ F-C-ALDWSWLQ 

[a] BMB = 3,5-bis(mercaptomethyl)benzoyl; Φ = L-2-naphthylalanine; MP = 3-mercaptopropionyl. See the Supporting Information, Figure S1 for detailed structures.

1), which consists of the 11-residue NEMO-binding domain (NBD) of IKK β covalently linked to a cell-penetrating peptide (CPP), Antp.^[9] Interestingly, Antp-NBD blocks the IKK activity stimulated by different pro-inflammatory stimuli, but does not affect the basal NF- κ B activity, thus providing a potentially safe and effective mechanism for reducing aberrant NF- κ B activity.^[9] In several pre-clinical studies, Antp-NBD demonstrated *in vivo* efficacy for treating Duchenne muscular dystrophy and large B-cell lymphoma in mouse and canine models.^[10] However, to achieve clinical utility, Antp-NBD would benefit significantly from improvements in its NEMO-binding affinity, metabolic stability, and cell permeability.

We previously reported cyclo(F Φ RRRRQ) (cF Φ R₄, where Φ is L-2-naphthylalanine) as a member of a novel class of cyclic CPPs.^[11] These CPPs bind directly to the membrane phospholipids, enter cells by endocytosis, and

[*] Dr. Z. Qian, C. A. Rhodes, L. C. McCroskey, J. Wen, G. Appiah-Kubi, Prof. Dr. D. Pei

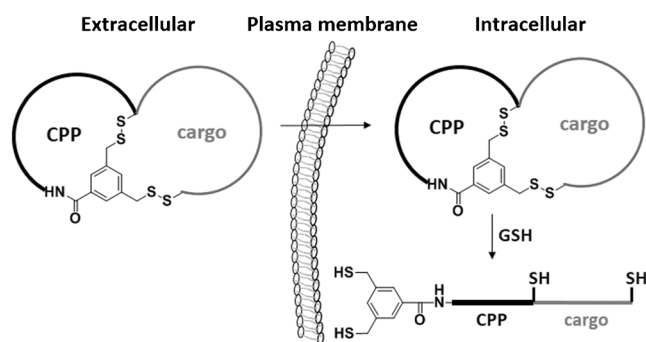
Department of Chemistry and Biochemistry
The Ohio State University
484 West 12th Avenue, Columbus, OH 43210 (USA)
E-mail: pei.3@osu.edu

Dr. D. J. Wang, Prof. Dr. D. C. Guttridge
Department of Molecular Virology, Immunology, and Medical Genetics, The Ohio State University
Columbus, OH 43210 (USA)

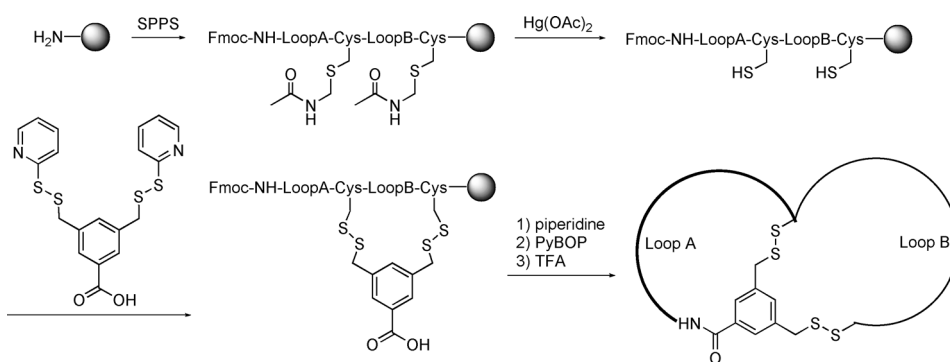
Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under:
<http://dx.doi.org/10.1002/anie.201610888>.

efficiently escape from the early endosome into the cytosol by inducing budding of small, unstable vesicles.^[11,12] With a cytosolic delivery efficiency (defined as the ratio of cytosolic over extracellular cargo concentration) of 20 %, cFΦR₄ is an order of magnitude more active than Tat, one of the most widely used CPPs.^[12] Most importantly, cFΦR₄ and other cyclic CPPs are capable of efficiently delivering a variety of cargo molecules including small molecules, peptides, and proteins into the cytosol of mammalian cells.^[11] For example, short peptidyl cargos were directly incorporated into the cFΦR₄ ring (endocyclic delivery) and the resulting cyclic peptides were cell-permeable.^[11–13] cFΦR₄ was also fused with 5.7 million different cyclic peptides to generate a library of cell-permeable bicyclic peptides (bicyclic delivery).^[14] However, many peptide ligands must be in their extended conformations to be biologically active and are not compatible with the above cyclization approaches. To this end, we recently developed a reversible cyclization strategy for intracellular delivery of linear peptidyl ligands, by fusing them with FΦR₄ and cyclizing the fusion peptides through a disulfide bond.^[15] Unfortunately, the previous approach is limited to relatively short peptides, as cyclization of longer peptides results in large rings, whose conformational flexibility limits the gains in metabolic stability and cell-permeability.^[11] Cyclization via an internal cysteine results in smaller rings and better cellular uptake, but leaves a portion of the peptidyl cargo in the linear form, which remains susceptible to proteolytic degradation. To overcome this limitation, we report here a reversible bicyclization strategy, which allows the entire CPP-cargo fusion to be converted into a bicyclic structure by the formation of a pair of disulfide bonds (Scheme 1). When outside the cell, the peptide exists as a highly constrained bicycle, which possesses enhanced cell permeability and proteolytic stability. Upon entering the cytosol, the disulfide bonds are reduced by the intracellular glutathione (GSH) to produce the linear, biologically active peptide. The bicyclic system permits the formation of a small CPP ring for optimal cellular uptake^[11] and a separate cargo ring to accommodate peptides of different lengths.

To test the validity of the reversible bicyclization strategy, we first designed two model peptides consisting of the CPP motif (RRRRΦF or FΦRRRR) and a mock cargo motif (SASAS) fused to its N- or C-terminus (Table 1, peptides **2** and **3**; see the Supporting Information, Figure S1 for detailed structures). Two cysteine residues were also incorporated into the sequences for later cyclization, one at the junction between the CPP and cargo motifs and one at the C-terminus. The linear peptides were synthesized by standard Fmoc solid-phase peptide synthesis (SPPS) chemistry on Rink amide resin (Scheme 2). The acetamidomethyl (Acm) groups on the



Scheme 1. A reversible peptide bicyclization strategy. GSH = glutathione.



Scheme 2. Solid-phase synthesis of disulfide-mediated bicyclic peptides.

two cysteine side chains were selectively removed by treatment with Hg(OAc)₂ and the exposed free thiols were then reacted on-resin with 3,5-bis((pyridin-2-yl)disulfanylmethyl)benzoic acid, which was readily prepared from commercially available starting materials (Supporting Information, Scheme S1). Formation of two disulfide bonds between the cysteine side chains and the 3,5-bis(mercaptomethyl)benzoic acid (BMB) scaffold resulted in cyclization of the peptide. Next, the N-terminal Fmoc group was removed by 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and the peptide was bicyclized by forming a lactam between the carboxyl group of BMB and the N-terminal amine (Scheme 2). BMB is ideally suited as the scaffold, because its structural symmetry ensures that a single bicyclic product is formed following the disulfide exchange reactions. Additionally, the rigidity of the scaffold prevents the formation of any intramolecular disulfide bond, simplifying both the synthesis of the scaffold and its reaction with the cysteine-containing peptides.

To monitor their cellular uptake, peptides **2** and **3** were labeled with fluorescein isothiocyanate (FITC) on the side chain of a C-terminal lysine. Flow cytometry analysis of HeLa cells treated with 5 μM peptides cFΦR₄, **2** and **3** for 2 h showed mean fluorescence intensity (MFI) values of 3020, 5180, and 4100, respectively (Figure 1a). Thus, bicyclic peptides **2** and **3** entered HeLa cells with 72 % and 36 % higher efficiencies, respectively, than cFΦR₄.

We next applied the reversible bicyclization strategy to generate a cell-permeable, biologically active peptidyl inhib-

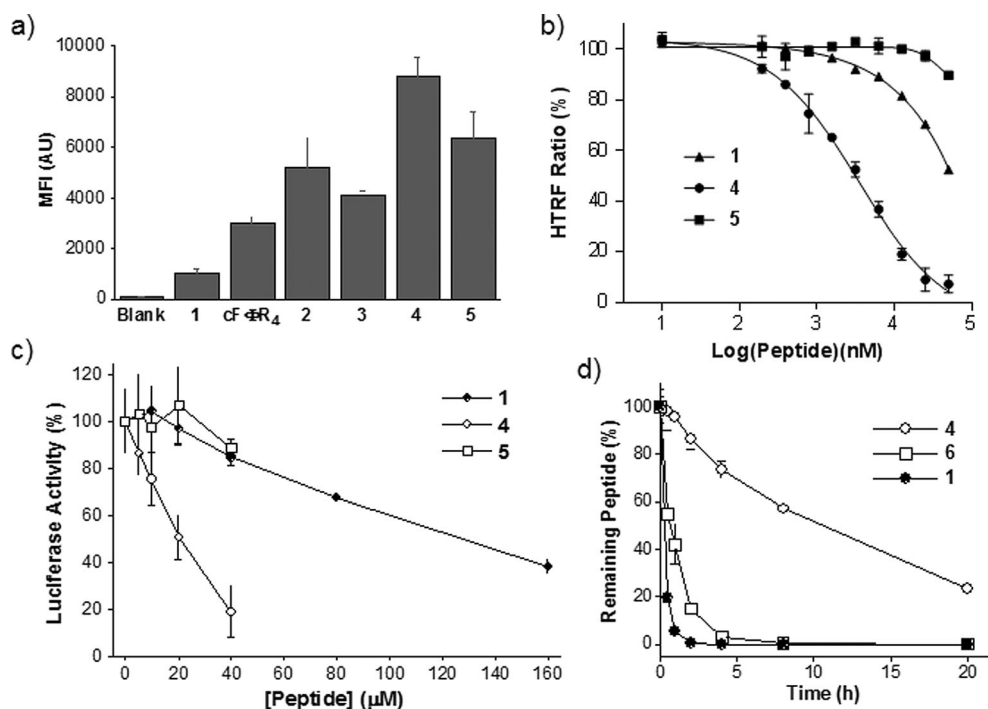


Figure 1. a) MFI of HeLa cells after 2 hour treatment with 5 μM FITC-labeled peptide cΦR₄ or 1–5, as determined by flow cytometry analysis. Blank = no peptide. b) Inhibition of the NEMO-IKKβ interaction by peptides 1, 4, and 5 as monitored by the HTRF assay. c) Dose-dependent inhibition of TNFα-induced activation of NF-κB signaling in HEK293 cells by peptides 1, 4, and 5. d) Comparison of the serum stability of peptides 1, 4, and 6. Data reported are the mean ± SD of three independent experiments.

itor against the NEMO-IKK interaction. Despite its *in vivo* efficacy, the linear Antp-NBD peptide has poor pharmacokinetics, which is due to rapid proteolytic degradation in serum ($t_{1/2} \approx 15$ min).^[10] We envisioned that conversion of Antp-NBD into a conformationally constrained bicyclic structure would substantially increase its proteolytic stability. The CPP motif RRRRΦF was fused to the N-terminus of NBD, TALDWSWLQT, and the N- and C-terminal threonine residues were replaced with two cysteines (Table 1, peptide 4; see the Supporting Information, Figure S2 for the detailed structure). The peptide fusion was bicyclized around the BMB scaffold by two disulfide bonds as described above, to give bicyclic peptide 4 as the predominant product (Supporting Information, Figure S3A). As a control, we also prepared peptide 5 (see the Supporting Information, Figure S2 for detailed structure), which is structurally similar to peptide 4 but contains two Ala residues in place of the two Trp residues. It was previously shown that replacement of the Trp residues with alanine largely abolished NEMO binding.^[9]

Peptides 4 and 5 were labeled with FITC at the side chain of a lysine added to their C-termini and their cellular entry was assessed by flow cytometry. Both peptides entered HeLa cells efficiently, exhibiting MFI values that were 3- and 2-fold higher than that of cΦR₄, respectively (Figure 1a). The NEMO-binding affinity of peptides 4 and 5 was determined using a homogenous time-resolved fluorescence (HTRF) assay.^[16] Briefly, in the presence of an anti-glutathione-S-transferase (GST) antibody labeled with a fluorescence donor (Tb) and streptavidin labeled with a fluorescence acceptor

(d2), binding of GST-NEMO to a biotinylated IKKβ fragment (amino acids 701–745)^[17] results in a resonance energy transfer. Addition of a NEMO inhibitor blocks the NEMO-IKKβ interaction and reduces the HTRF signal. In the presence of 5 mM tris(carboxylethyl)-phosphine (TCEP), which is expected to completely reduce the disulfide bonds in peptides 4 and 5, peptide 4 inhibited the NEMO-IKKβ interaction in a concentration-dependent manner, with a half-maximal inhibitory concentration (IC₅₀) value of 3.5 ± 0.2 μM (Figure 1b). Under the same conditions, Antp-NBD showed an IC₅₀ value of about 50 μM, in agreement with the previously reported binding affinity.^[16] As expected, up to 100 μM peptide 5 caused only minor inhibition of the

interaction. Since substitution of the two cysteine residues for threonine did not significantly change the NEMO binding affinity (Supporting Information, Figure S4), the enhanced NEMO binding of peptide 4 relative to Antp-NBD is likely caused by additional interactions between the phenylalanine of the CPP motif (RRRRΦF) and the NEMO protein surface. IKKβ contains a phenylalanine at the same position (Phe-734). The crystal structure of the NEMO-IKKβ complex shows that the side chain of Phe-734 inserts into a hydrophobic pocket on the NEMO surface.^[16] Thus, the phenylalanine in peptide 4 likely plays dual roles of cellular entry and NEMO binding.

The ability of the bicyclic peptides to modulate the NEMO-IKK interaction inside the cell was assessed by monitoring the TNFα-induced activation of NF-κB. HEK293 cells transfected with a luciferase reporter gene under the control of NF-κB were first treated with varying concentrations of a peptide for 2 h and then TNFα.^[9,10e] In the absence of any inhibitory peptide, treatment with 5 ng mL⁻¹ TNFα increased the luciferase activity from a basal level of 177 arbitrary units (AU) to 715 AU (data not shown). Peptide 4 reduced the TNFα-induced luciferase activity in a dose-dependent manner, with an IC₅₀ value of about 20 μM (Figure 1c). In contrast, the control peptide 5 had no significant effect on NF-κB signaling at 20 μM and resulted in about 10% inhibition at the highest concentration tested (40 μM). Consistent with the earlier report,^[9] Antp-NBD (peptide 1) also caused concentration-dependent inhibition, but showed an IC₅₀ value of 140 μM. The higher potency of

bicyclic peptide **4** relative to Antp-NBD in the cellular assay is likely the results of both improved cellular entry efficiency (Figure 1a) and greater NEMO-binding affinity (Figure 1b). In vitro treatment of bicyclic peptide **4** with 5 mM glutathione for 2 h completely reduced the disulfide bonds (Supporting Information, Figure S3b), suggesting that peptides **2–5** should undergo complete reduction upon cytosolic entry.

Finally, the proteolytic stability of peptide **4** and Antp-NBD was tested by incubating the peptides in human serum for varying lengths of time and the remaining amounts of intact peptides were quantitated by analytical HPLC. For comparison, we synthesized a control peptide (Table 1, peptide **6**), which has the same sequence as peptide **4** but only its CPP motif was cyclized. Peptide **6** also reduced the TNF α -induced luciferase activity in a dose-dependent manner, with an IC₅₀ value of about 40 μ M (Supporting Information, Figure S5). In agreement with the previous reports,^[10] Antp-NBD was rapidly degraded by human serum, with a half-life of about 15 min (Figure 1d). In contrast, bicyclic peptide **4** showed a half-life of about 10 h, and 23 % of the peptide remained intact after 20 h of incubation at 37 °C. The monocyclic control peptide **6** was also rapidly degraded (with a half-life of about 30 min), which is most likely due to proteolysis of the linear NBD sequence. We had previously shown that linear peptidyl cargos attached to the Gln side chain of cF Φ R₄ were rapidly degraded in human serum.^[15]

In conclusion, a simple method has been developed to efficiently deliver peptidyl ligands into mammalian cells, by fusing the peptide with a short CPP motif and reversibly cyclizing the fusion peptide through disulfide bonds. The resulting bicyclic peptide has greatly enhanced cellular uptake as well as proteolytic stability. This strategy should be applicable to delivering any linear peptides.

Acknowledgements

Financial support from the National Institutes of Health (GM110208) is gratefully acknowledged. We thank Dr. M. Pellegrini for the IKK β (701–745) plasmid and W. Lian and J. S. Stewart for technical assistance.

Conflict of interest

The authors declare no conflict of interest.

Keywords: bicyclization · cell-penetrating peptides · cyclic peptides · nemo inhibitors · protein–protein interactions

- [1] For excellent reviews on protein–protein interactions, see: a) J. Wells, C. McClendon, *Nature* **2007**, *450*, 1001; b) L. Nevala, E. Giralt, *Chem. Commun.* **2015**, *51*, 3302–3315; c) M. Pelay-Gimeno, A. Glas, O. Koch, T. N. Grossmann, *Angew. Chem. Int. Ed.* **2015**, *54*, 8896; *Angew. Chem.* **2015**, *127*, 9022.
- [2] For reviews on peptide therapeutics, see: a) D. J. Craik, D. P. Fairlie, S. Liras, D. Price, *Chem. Biol. Drug Des.* **2013**, *81*, 136;

- b) K. Fosgerau, T. Hoffmann, *Drug Discovery Today* **2015**, *20*, 122.
- [3] T. Rezaei, J. E. Bock, M. V. Zhou, C. Kalyanaraman, R. S. Lokey, M. P. Jacobson, *J. Am. Chem. Soc.* **2006**, *128*, 14073.
- [4] For selected examples on peptide *N*-methylation, see: a) J. Chatterjee, C. Gilon, A. Hoffman, H. Kessler, *Acc. Chem. Res.* **2008**, *41*, 1331; b) T. R. White, C. M. Renzelman, A. C. Rand, T. Rezaei, C. M. McEwen, V. M. Gelev, R. A. Turner, R. G. Linington, S. S. F. Leung, A. S. Kalgutkar, J. N. Bauman, Y. Z. Zhang, S. Liras, D. A. Price, A. M. Mathiowetz, M. P. Jacobson, R. S. Lokey, *Nat. Chem. Biol.* **2011**, *7*, 810.
- [5] A. Oeckinghaus, S. Ghosh, *Cold Spring Harbor Perspect. Biol.* **2009**, *1*, a000034.
- [6] a) V. Baud, M. Karin, *Nat. Rev. Drug Discovery* **2009**, *8*, 33; b) S.-C. Sun, J.-H. Chang, J. Jin, *Trends Immunol.* **2013**, *34*, 282; c) F. D. Herrington, R. J. Carmody, C. S. Goodyear, *J. Biomol. Screening* **2016**, *21*, 223; d) G. Cildir, K. C. Low, V. Tergaonkar, *Trends Mol. Med.* **2016**, *22*, 414.
- [7] a) S. Yamaoka, G. Courtois, C. Bessia, S. T. Whiteside, R. Weil, F. Agou, H. E. Kirk, R. J. Kay, A. Israel, *Cell* **1998**, *26*, 1231; b) D. M. Rothwarf, E. Zandi, G. Natoli, M. Karin, *Nature* **1998**, *395*, 297.
- [8] a) S. C. Gupta, C. Sundaram, S. Reuter, B. B. Aggarwal, *Biochim. Biophys. Acta Gene Regul. Mech.* **2010**, *1799*, 775; b) T. M. Herndon, A. Deisseroth, E. Kaminskas, R. C. Kane, K. M. Koti, M. D. Rothmann, B. Habtemariam, J. Bullock, J. D. Bray, J. Hawes, T. R. Palmby, J. Jee, W. Adams, H. Mahayni, J. Brown, A. Dorantes, R. Sridhara, A. T. Farrell, R. Pazdur, *Clin. Cancer Res.* **2013**, *19*, 4559.
- [9] M. J. May, F. D'Acquisto, L. A. Madge, J. Glockner, J. S. Pober, S. Ghosh, *Science* **2000**, *289*, 1550.
- [10] a) E. Jimi, K. Aoki, H. Saito, F. D'Acquisto, M. J. May, I. Nakamura, T. Sudo, T. Kojima, F. Okamoto, H. Fukushima, K. Okabe, K. Ohya, S. Ghosh, *Nat. Med.* **2004**, *10*, 617; b) S. Dai, T. Hirayama, S. Abbas, Y. Abu-Amer, *J. Biol. Chem.* **2004**, *279*, 37219; c) W. Shibata, S. Maeda, Y. Hikiba, A. Yanai, T. Ohmae, K. Sakamoto, H. Nakagawa, K. Ogura, M. Omata, *J. Immunol.* **2007**, *179*, 2681; d) S. H. Dave, J. S. Tilstra, K. Matsuoka, F. Li, T. Karrasch, J. K. Uno, A. R. Sepulveda, C. Jobin, A. S. Baldwin, P. D. Robbins, S. E. Plevy, *J. Immunol.* **2007**, *179*, 7852; e) A. Gaurnier-Hausser, R. Patel, A. S. Baldwin, M. J. May, N. J. Mason, *Clin. Cancer Res.* **2011**, *17*, 4661; f) J. M. Peterson, W. Kline, B. D. Canan, D. J. Ricca, B. Kaspar, D. A. Delfin, K. DiRienzo, P. R. Clemens, P. D. Robbins, A. S. Baldwin, P. Food, P. Kaumaya, M. Freitas, J. N. Kornegay, J. R. Mendell, J. A. Rafael-Fortney, D. C. Guttridge, P. M. Janssen, *Mol. Med.* **2011**, *17*, 508; g) D. A. Delfin, Y. Xu, J. M. Peterson, D. C. Guttridge, J. A. Rafael-Fortney, P. M. Janssen, *J. Transl. Med.* **2011**, *9*, 68; h) D. P. Reay, M. Yang, J. F. Watchko, M. Daood, T. L. O'Day, K. K. Rehman, D. C. Guttridge, P. D. Robbins, P. R. Clemens, *Neurobiol. Dis.* **2011**, *43*, 598; i) J. N. Kornegay, J. M. Peterson, D. J. Bogan, W. Kline, J. R. Bogan, J. L. Dow, Z. Fan, J. Wang, M. Ahn, H. Zhu, M. Styner, D. C. Guttridge, *Skeletal Muscle* **2014**, *4*, 18; j) G. Habineza Ndikuyeze, A. Gaunier-Hausser, R. Patel, A. S. Baldwin, M. J. May, P. Food, E. Krick, K. J. Propert, N. J. Masson, *PLoS One* **2014**, *9*, e95404.
- [11] a) Z. Qian, T. Liu, Y.-Y. Liu, R. Briesewitz, A. M. Barrios, S. M. Jhiang, D. Pei, *ACS Chem. Biol.* **2013**, *8*, 423; b) Z. Qian, J. R. LaRochelle, B. Jiang, W. Lian, R. L. Hard, N. G. Selner, R. Luechapanichkul, A. M. Barrios, D. Pei, *Biochemistry* **2014**, *53*, 4034.
- [12] Z. Qian, A. Martyna, R. L. Hard, J. Wang, G. Appiah-Kubi, C. Coss, M. A. Phelps, J. S. Rossman, D. Pei, *Biochemistry* **2016**, *55*, 2601.
- [13] P. Upadhyaya, Z. Qian, N. G. Selner, S. R. Clippinger, Z. Wu, R. Briesewitz, D. Pei, *Angew. Chem. Int. Ed.* **2015**, *54*, 7602; *Angew. Chem.* **2015**, *127*, 7712.



- [14] T. B. Trinh, P. Upadhyaya, Z. Qian, D. Pei, *ACS Comb. Sci.* **2016**, *18*, 75.
- [15] Z. Qian, X. Xu, J. F. Amacher, D. R. Madden, E. Cormet-Boyaka, D. Pei, *Angew. Chem. Int. Ed.* **2015**, *54*, 5874; *Angew. Chem.* **2015**, *127*, 5972.
- [16] a) M. Rushe, L. Silvian, S. Bixler, L. L. Chen, A. Cheung, S. Bowes, H. Cuervo, S. Berkowitz, T. Zheng, K. Guckian, M. Pellegrini, A. Lugovskoy, *Structure* **2008**, *16*, 798; b) Y. Gotoh, H. Nagata, H. Kase, M. Shimonishi, M. Ido, *Anal. Biochem.* **2010**, *405*, 19.
- [17] B. Gao, C. O. Audu, J. C. Cochran, D. F. Mierke, M. Pellegrini, *Biochemistry* **2014**, *53*, 6776.
- Manuscript received: November 7, 2016
Revised: December 1, 2016
Final Article published: ■ ■ ■ ■, ■ ■ ■ ■ ■
-

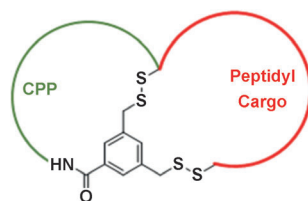
Communications



Drug Delivery

Z. Qian, C. A. Rhodes, L. C. McCroskey,
J. Wen, G. Appiah-Kubi, D. J. Wang,
D. C. Guttridge, D. Pei* — ■■■■—■■■■

Enhancing the Cell Permeability and
Metabolic Stability of Peptidyl Drugs by
Reversible Bicyclization



In the loop: Peptide bicyclization by a pair of disulfide bonds increases its proteolytic stability and cell permeability. This method also allows for regeneration of the functional linear peptide once inside the cytosol of the cell. CPP = cell-penetrating peptide.

