University of Groningen

Multicomponent Peptide Stapling as a Diversity-Driven Tool for the Development of Inhibitors of Protein-Protein Interactions

Ricardo, Manuel; Ali, Ameena; Plewka, Jacek; Surmiak, Ewa; Labuzek, Beata; Neochoritis, Constantinos; Atmaj, Jack; Skalniak, Lukasz; Zhang, Ran; Holak, Tad

Published in:
Angewandte Chemie (International ed. in English)

DOI:
[10.1002/anie.201916257](https://doi.org/10.1002/anie.201916257)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2020

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Ricardo, M., Ali, A., Plewka, J., Surmiak, E., Labuzek, B., Neochoritis, C., Atmaj, J., Skalniak, L., Zhang, R., Holak, T., Groves, M., Rivera, D., & Doemling, A. (2020). Multicomponent Peptide Stapling as a Diversity-Driven Tool for the Development of Inhibitors of Protein-Protein Interactions. *Angewandte Chemie (International ed. in English)*, 59(13), 5235-5241. <https://doi.org/10.1002/anie.201916257>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Stapled Peptides

International Edition: DOI: 10.1002/anie.201916257
German Edition: DOI: 10.1002/ange.201916257

Multicomponent Peptide Stapling as a Diversity-Driven Tool for the Development of Inhibitors of Protein–Protein Interactions

Manuel G. Ricardo⁺, Ameena M. Ali⁺, Jacek Plewka, Ewa Surmiak, Beata Labuzek, Constantinos G. Neochoritis, Jack Atmaj, Lukasz Skalniak, Ran Zhang, Tad A. Holak, Matthew Groves, Daniel G. Rivera,^{*} and Alexander Dömling^{*}

Abstract: Stapled peptides are chemical entities in-between biologics and small molecules, which have proven to be the solution to high affinity protein–protein interaction antagonism, while keeping control over pharmacological performance such as stability and membrane penetration. We demonstrate that the multicomponent reaction-based stapling is an effective strategy for the development of α -helical peptides with highly potent dual antagonistic action of MDM2 and MDMX binding p53. Such a potent inhibitory activity of p53-MDM2/X interactions was assessed by fluorescence polarization, microscale thermophoresis, and 2D NMR, while several cocrystal structures with MDM2 were obtained. This MCR stapling protocol proved efficient and versatile in terms of diversity generation at the staple, as evidenced by the incorporation of both *exo*- and *endo*-cyclic hydrophobic moieties at the side chain cross-linkers. The interaction of the Ugi-staple fragments with the target protein was demonstrated by crystallography.

Introduction

Peptide stapling is a technology in which the side chains of two amino acid residues, separated along the sequence, are cross-linked to render a conformationally constrained α -helical peptide.^[1–3] This synthetic approach does not only aim at the stabilization of such secondary structure and the increase of the binding affinity for a specific target,^[2,4,5] but it can also produce proteolytically stable^[6] and cell-permeable

peptides.^[1,7] An effective strategy for α -helix stabilization comprises the tethering of residues at either *i*, *i* + 4 or *i*, *i* + 7 positions in the peptide chain, so that the covalent bridge expands one or two turns of the α -helical peptide.^[1–5] While the original term “stapled peptides” was employed for hydrocarbon bridged α -helical peptides synthesized by ring-closing metathesis (RCM),^[1,2,8] several stapling techniques have been developed over the last decade.^[3,4,9,10] As a result, peptide stapling is now a mature technology^[11–16] that has been applied to target protein–protein interactions related to infectious diseases, cancer, neurological, endocrine, metabolic, and cardiovascular disorders, and a first example is tested in clinical trials.^[1,2,8,10,17–19]

As depicted in Scheme 1, besides RCM, one-component stapling techniques (that is, the peptide as the only component) currently include the traditional construction of lactam and disulfide bridges as well as others based on oxime and thioether bond formation^[3,20] or the Ugi cyclization between Lys and Asp/Glu side chains.^[21,22] On the other hand, there is an increasing interest in two-component stapling approaches (Scheme 1A), in which eventually biorthogonal processes, such as the click Cu^I-catalyzed alkyne–azide cycloaddition^[3,10,23] and dithiol (Cys) bis-alkylation,^[24] introduce a stapling linker capable to stabilize the α -helical structure, with the former one also enabling the functionalization of the staple moiety. This strategy could be potentially more efficient for the rapid optimization of the linker, since parallel syntheses and biological screening can be undertaken using a single peptide sequence and varying the length, flexibility, and hydrophobicity of the linker. Multi-component reactions (MCRs) are excellent diversity-generating tools and have recently emerged as powerful stapling tools capable to lock specific peptide conformations and simultaneously diversify the staple moiety by variation of *endo*- and *exo*-cyclic moieties during the multicomponent formation of the side chain cross-linker.^[20,21,25,26]

However, so far applications describing the utilization of a multicomponent stapling approach^[9] for the development of bioactive α -helical peptides are absent. Herein, we describe a diversity-driven peptide stapling approach for the discovery of potent, dual action p53-MDM2 and MDMX antagonists. The binding inhibition of the transcription factor p53 to MDM2 and MDMX is an efficient way for the p53 activation in tumor cells.^[27–29] The structure of the complex exhibits helical conformation,^[30] whereas this domain in free N-terminal domain of p53 is natively unfolded.^[31,32] For that reason, peptide stapling is nowadays recognized as an

[*] Dr. M. G. Ricardo,^[+] Prof. D. G. Rivera
Faculty of Chemistry, Center for Natural Product Research
University of Havana (Cuba)
E-mail: dgr@fq.uh.cu

Dr. A. M. Ali,^[+] Prof. C. G. Neochoritis, J. Atmaj, R. Zhang,
Prof. M. Groves, Prof. A. Dömling
Department of Pharmacy, Drug Design group
University of Groningen (The Netherlands)
E-mail: a.s.s.domling@rug.nl

Dr. J. Plewka, Dr. E. Surmiak, Dr. B. Labuzek, J. Atmaj, Dr. L. Skalniak,
Prof. T. A. Holak
Faculty of Chemistry, Jagiellonian University
Krakow (Poland)
Prof. C. G. Neochoritis
Department of Chemistry, University of Crete (Greece)

[+] These authors contributed equally to this work.

Supporting information and the ORCID identification number(s) for the author(s) of this article can be found under:
<https://doi.org/10.1002/anie.201916257>.

Scheme 1. A) One and two-component approaches based on different peptide stapling technologies. B) Novel multicomponent stapling strategy towards α -helical peptides described in this work.

effective approach to produce α -helical peptides mimicking the p53 transactivation domain.^[23,33–35]

By targeting the dual inhibition of p53-MDM2 and MDMX interactions as validated targets, we aim to demonstrate the potential of Ugi multicomponent macrocyclizations in the rapid discovery^[36] of peptide inhibitors of protein–protein interactions (PPIs).

Results and Discussion

Scheme 1 depicts the two different multicomponent macrocyclization variants devised for fine-tuning the nature and flexibility of the linker. Since the Ugi-4CR comprises the reaction of a carboxylic acid, usually a primary amine, an oxo compound, and an isocyanide,^[37,38] we focused on incorporating Asp or Glu at $i, i + 7$ residues of the p53 binding sequence, as these positions avoid engaging amino acids that are crucial for MDM2/X binding, that is, around the triad of Phe19, Trp23, and Leu26. As shown in Scheme 1B, two different classes of bifunctional building blocks were envisioned as counterparts of the peptide dicarboxylic acid, that is, diamines and diisocyanides, which upon double Ugi multicomponent macrocyclization^[39] would lead to α -helical structures, ideally pointing the critical “hot-spot” residues towards the same face. The difference between the dicarboxylic acid/diamine and the dicarboxylic acid/diisocyanide combinations does not only lie at the dissimilar length of the Ugi-4CR-derived moieties connecting the linker with the peptide chain, but also in the presence of two *exo*-cyclic fragments in the former one.

Scheme 2 depicts the implementation of such combinations with peptides **1** and **2**, which were previously produced by solid phase peptide synthesis (SPPS) and released from the resin (see Supporting Information). The sequence that is reported in Scheme 2 is not the original p53 protein sequence but a previously reported and optimized sequence.^[18] The design was focused on including two Ala at the C-terminus and changing the previously stapling residues by either two Asp or two Glu to enable the double Ugi macrocyclization. In addition, any remaining Glu was changed to Gln to avoid the participation in the Ugi reactions. Thus, in both cases, two Asp and Glu residues were placed at positions 4 and 11 of the

tetradecapeptide p53 sequence. Peptide **1**, bearing two Asp residues, was initially chosen for the double Ugi-MCR macrocyclization using 1.5 equiv each of *m*- and *p*-xylylene diisocyanides^[40] and 4.0 equiv of methylamine and paraformaldehyde as monofunctional components. Stapled peptides (SPs) **3** and **4** were produced in good macrocyclization yield—considering their complexity and the formation of 8 covalent bonds in one pot—by a protocol consisting of the slow addition with syringe pumps of solution of both the peptide and the diisocyanide each to a reaction mixture containing the preformed imine. Such *pseudo*-dilution conditions have proven successful in multicomponent macrocyclizations based on varied MCRs,^[24,25] and it constitutes a very effective way to avoid large solvent volumes at the same time, which typically avoids the formation of dimeric cyclic and acyclic byproducts.^[37] The same protocol was employed for the synthesis of SPs **7** and **8** from the linear **2**, also using methylamine as the simplest amine component.

On the other hand, the implementation of the dicarboxylic acid/diamine combination required a more elaborated experimental setup, as it had never been used before to macrocyclize bifunctional peptides.^[9] Since preformation of the imine is known to improve Ugi macrocyclization yields,^[37] we turned to initially stir the diamine component with paraformaldehyde to enable imine formation followed by slow addition of the diimine and the peptide dicarboxylic acid solutions to a stirring solution of cyclohexyl isocyanide. As before, satisfactory macrocyclization yields were obtained for SPs **5** and **6** after 72 h of reaction. These latter SPs include a flexible, aliphatic staple and *m*-xylylene linker, respectively, but both bear *exo*-cyclic cyclohexyl carboxamide groups positioned at the tertiary amides. The easy incorporation of such additional *exo*-cyclic fragments is one of the key and distinctive features of the Ugi multicomponent stapling, which might even become relevant in case these hydrophobic moieties participate in the binding process to the protein target. Scheme 2B shows the CD spectra of the linear and stapled peptides in 40% trifluoroethanol/phosphate buffer (PB), because the hydrophobic sequence of such peptides makes them insoluble in pure water or PB at 0.1 mM. Despite that the use of TFE in the solvent mixture is known to stimulate α -helical structures,^[41] the trend of increased helical

Scheme 2. A) Synthesis of stapled peptides by Ugi multicomponent macrocyclization using Asp and Glu-containing peptides. B) CD spectra of the stapled peptides as compared with the linear precursors. In parenthesis yields of isolated products.

propensity in the stapled peptides is clearly proven by CD. The relative percent of α -helicity for each peptide was estimated (as described in the Supporting Information) by the mean residue ellipticity at 222 nm, a value that is commonly downshifted for short peptides to 215–220 nm. Importantly, while the multicomponent macrocyclization led to a marked increment in the α -helical character of the stapled peptides derived from peptide 2 (having two Glu), this was not the case for stapled peptides 3 and 4 derived from 1. At this stage, it was intriguing to assess whether the higher α -helicity of peptides 5–8, as compared with 3 and 4, would correspond to a better inhibition of the p53-MDM2/X interactions.

Three orthogonal assays based on independent physico-chemical principles, fluorescence polarization (FP), microscale thermophoresis (MST), and 2D HSQC NMR were employed to test the *in vitro* activity of the SPs. FP allows for the determination of inhibitory constant IC_{50} , which are readily obtained, but hard to compare, as well as the inhibitory affinities (K_i), which should be comparable and consistent throughout used techniques. The K_i determination was performed based on the mass balance relationships as described.^[42] Thus, we found that all the stapled peptides exhibited very high activities for both MDM2 and MDMX in the nM range (Table 1). Peptides 3, 4, and 6 gave K_i in a range of 5–10 nM against MDM2 and 20–380 nM against MDMX.

However, SPs 5, 7, and 8 showed higher activity against MDM2 with a K_i of 2.2 nM, 1.5 nM and 2.0 nM, respectively. Furthermore, these peptides were also more active against MDMX with K_i of 11.8 nM, 7.1 nM and 12.7 nM, respectively, suggesting that they could serve as an excellent example of dual inhibition.^[43] Microscale thermophoresis (MST) was utilized to cross-validate the binding affinity of the tested peptides. This technique depends on the detection of the Temperature Related Intensity Change (TRIC), especially with the use of 2nd generation labeling dyes. This temperature-induced change in fluorescence of a target depends on the surrounding of the fluorophore and, therefore, is related to the concentration of a non-fluorescent ligand allowing the determination of the binding affinity.^[44,45] Assuming 1:1 interaction data, it was then fitted using the K_d model. All tested compounds showed an affinity towards MDM2 with K_d of a low nM range 3–13 nM with low to very low measurement errors and high goodness of fit (Table 1). Verifying the results from the FP assay, the stapled peptides 5, 7, and 8 showed the higher activity with a K_d of 4.5 nM, 4.8 nM and 2.9 nM, respectively. The efficiency of the stapling is now confirmed compared to the acyclic peptides (Table 1), as the binding affinities for the best stapled peptides are significantly better than for corresponding linear peptides. SPs 3 and 4 are at least twice as potent as parent peptide 1 for both MDM2 and MDMX. Similarly, SP 7 was twice as active as parent peptide

Table 1: Biophysical binding data of Ugi-SPs to MDM2/X.

Peptide	Structure	K_i (nM, FP)	K_d (nM, MST)
1		14.8 ± 0.2 (86 ± 17) ^[a]	21 ± 6
2		3.6 ± 0.1 (16 ± 3) ^[a]	20 ± 13
3		6.1 ± 0.1 (380 ± 169) ^[a]	13 ± 4
4		9.5 ± 0.1 (44 ± 2) ^[a]	9 ± 1
5		2.2 ± 0.1 (11.8 ± 0.2) ^[a]	4.5 ± 0.9
6		5.0 ± 0.1 (20.9 ± 0.4) ^[a]	9 ± 2
6, 52% conv. (32%), 79% helicity			
7		1.5 ± 0.1 (7.1 ± 0.1) ^[a]	4.8 ± 0.9
8		2.0 ± 0.1 (12.7 ± 0.2) ^[a]	2.9 ± 0.8

[a] Data in parentheses refers to the MDMX protein.

2 proving that the cyclization of the peptides does not only improve their stability, but also is essential for their affinity.

For binding confirmation, we performed an ^1H - ^{15}N HSQC of ^{15}N labeled MDM2 and MDMX protein with increasing amount of the representative peptide **8** as a third orthogonal assay to FP and MST.^[46–48] This method is based on monitoring of chemical shift changes in protein amide backbone resonances upon protein interaction with a small molecule and its advantage lays in the complete unambiguity of the results as there are no auxiliary molecules required, such as dyes (Figure 1). During the experiment with both proteins, significant changes in spectra were observed. Tight binding (slow chemical exchange) is present in multiple residues, which is indicated by doubling of corresponding cross-peaks

(Figure 1). Slow chemical exchange is characteristic for inhibitors with K_d lower than $1\ \mu\text{M}$. In such a case, when the compound is added to the protein with amount lower than the total concentration of the protein, the peaks originating from free and bound protein are seen separately on the spectra. Moreover, the peaks assignment of residues in MDM2 and MDMX proteins spectra is known, which allows to confirm the inhibitor binding.^[49,50] Especially important are the residues which lie near the binding pocket of the surface of MDM2/X proteins, for example, in the case of MDM2, the Leu82, which is placed deep inside the Trp23-p53 binding sub-pocket. Furthermore, multiple peaks that appear near the binding area disappeared from the spectrum, indicating strong binding (for example Gly58, Leu57, Met62, and Tyr60). For the MDMX analysis, the situation is much more complex than for the MDM2 protein. The main downfall in this case is the fact that approximately 30% of the MDMX residues are not seen in ^1H - ^{15}N HSQC spectra when the protein is not bound to a ligand.^[51] Upon binding, additional residues appear on the spectra, as it is also the case with our titration experiment of MDMX with the peptide **8**. The performed titration experiment of **8** with the MDMX, clearly shows strong binding between peptide and protein which is indicated by the doubling of several cross-peaks, especially those which are placed near the binding pocket, such as Gly35. Likewise, the other cross-peaks in near vicinity of the binding pocket which are changed in the spectra are Val26, Leu76, and Phe68.

To elucidate the binding mode of the SPs to their receptor, we crystallized three high affinity SPs with MDM2 (see Supporting Information). The three SPs **3** (PDB ID 6T2F), **7** (PDB ID 6T2E), and **8** (PDB ID 6T2D) were cocrystallized with MDM2 and the complex structures diffracted at high

resolution of 2.09, 2.24, and 1.80 Å, respectively. The overall SP sequence resembles the p53 peptide and especially the “hot-spot” triad Phe19, Trp23, and Leu26 is identical (Figure 2A). The bound peptide **8** forms a compact, short, two-turn α -helix (Figure 2B). Therefore, the overall binding is similar to the p53-MDM2 and also MDMX. When compared to the crystal structure of p53 helix in complex with the N-terminal domain of MDM2 (PDB ID 1YCR), all the peptides bind the MDM2 deep groove in a similar way to the WT-p53 peptide, by mimicking the conserved p53-derived MDM2 hot-spot interaction motif (Phe19, Trp23, and Leu26) into an identical orientation (Figure 2). A hydrogen bond was found between the indole NH of Trp23 (according to WT-p53 sequence numbering) in the stapled peptide helix and the

Figure 1. ^1H - ^{15}N HSQC titration experiment for the peptide **8** with ^{15}N labeled proteins MDM2 (A) and MDMX (B). A) MDM2 with the peptide **8** (PDB ID 6T2D), B) MDMX with the p53 (PDB ID 2MWY). Reference MDM2/X-red; MDM2/X:**8** ratio 4:1-blue; MDM2/X over titrated with **8**-green; Upper panel: residues which undergo the largest changes have been plotted on the crystal structure showing an interaction of the inhibitor with the p53-binding pocket. Cross-peaks which undergo slow chemical exchanges are marked with orange; cross-peaks which undergo other significant changes are marked in bright orange. Lower panel: Enlarged fragment of ^1H - ^{15}N spectrum showing cross peaks placed near binding pocket (especially L82 for MDM2 and G35 for MDMX proteins) and undergo slow chemical exchange (tight binding).

Figure 2. Ugi stapled peptides cocrystallized in MDM2. A) Sequence of peptides. B) Overall view of peptide **8** (stick presentation) bound to MDM2 (surface presentation). The linker is shown in blue and the α -helical peptide in cyan. The linker moiety interacting surface of MDM2 is shown in green. C–F) Close up view of some linker MDM2 interactions. C) Water mediated hydrogen bonding interaction between Gln59 and Glu-derived linker carbonyl. D) vdWals interaction of Met62 with the linker. E) Phe55 and the linker phenyl moiety in a T-shaped conformation.

carbonyl oxygen of Leu54 on MDM2 hydrophobic deep pocket. The staple is making multiple hydrophobic and

electrostatic contacts, partially mediated by water, to the MDM2 rim of Leu54, Ile103, Lys51, Phe55, Gln59, Gly58, and Met62 (Figure 2C–E). For example, the aromatic linker makes a T-shaped π -stacking interaction with Phe55 with a closest distance of 4 Å (Figure 2E). The ability of the staple in the stapled peptide to interact with the surface next to the major binding cleft is interesting; the “exploitation” of this surface by a MDM2 inhibitor was first observed with a beta-hairpin peptidomimetic of p53.^[52] MDM2 retains its native fold observed in other structures and undergoes only minor ligand-induced changes upon binding. For example, the Met62 side chain folds away from the p53 binding pocket to make room for the staple. The Tyr100 side chain is in the so-called “closed” conformation as in most other MDM2 structures. Overall the observed binding mode of the three cocrystallized peptides is similar to other described MDM2/X-SPs. The buried surface area (BSA) of both the peptide **8** and the p53 is given in Figure 3 (see also the Supporting Information).

Figure 3. The buried surface area (BSA) of both the peptide **8** and the p53.

We have implemented diversity-driven stapling approach for constraining short peptides into a p53-type bioactive conformation at the same time modulating the helical character, flexibility, and hydrophobicity of the staple moiety. By employing two different combinations of the Ugi MCR-macrocylation, we were able to vary not only the macrocycle ring size but also the number of amide bonds resulting at either *endo*- or *exo*-cyclic positions of the ring. Despite all compounds showed a high in vitro activity, such small differences arising from the variation of the macrocyclization strategy turned out to be crucial for a very potent inhibition effect. First of all, the peptides **1** and **2** have appeared to be less active than the macrocyclized ones as measured by both FP and MST (Table 1). Furthermore, using the same p53-based dicarboxylic acid peptide **1**, the diisocyanide/amine/aldehyde combination rendered SPs **3** and **4** which have the lowest helical character (Scheme 2) and proved to be less potent than the others (Table 1). Utilizing the acyclic peptide **2**, yielded the SPs **5–8** with the highest helical character (Scheme 2). It seems that the α -helical character is crucial for the affinity, especially if we compare the SPs **3**, **4** and **7**, **8**. Comparison of SPs **5** and **6** is also intriguing, as both of them derive from linear peptide **2** and from the same diamine/isocyanide/aldehyde combination, but the change in the flexibility of the linker from a flexible aliphatic one in **5** to the more rigid *m*-xylylene in **6** seems to be determinant for decreasing the activity from 2.3 nM to 5.0 nM, respectively

(Table 1). SP **7** has lower K_i and K_d than the corresponding peptide **6**. This is interesting, considering that both SPs **6** and **7** show a similar helical character and include the same *m*-xylylene linker. A key difference lies at the ring size and the presence of additional *exo*-cyclic cyclohexyl moieties in SP **6**, which, in this case, seems to be quite detrimental for the binding to MDM2. Moreover, the same amine/diisocyanide/aldehyde combination gave peptides **7** and **8** which are the most potent of the series as measured by all methods. The ring size, the α -helical character, ring flexibility, and substitution pattern on the ring (especially the exocyclic moieties) seem to influence the potency.

Conclusion

To the best of our knowledge, the dual activity reported for SPs **5**, **7**, and **8** is amongst the highest described for p53-MDM2 and MDMX inhibitors.^[43,53,54] A deeper insight was achieved with the analysis of the cocrystal structures, which demonstrates the participation of both the hydrophobic linker and the amide bonds of the Ugi-staple moiety in the binding process. It is worth mentioning that our method provides a high diversification of the staple. Therefore, it is not only the easy generation of staple diversity which makes this multi-component approach so promising, but also the rapid access to very complex structures created with formation of several covalent bonds in one synthetic operation. On the other hand, orthogonal protection methods could be needed if additional Asp or Glu residues used. As previously recognized by Spring^[23] and others,^[24] this stapling modality of using bifunctional peptides and linkers offers a better possibility for fine-tuning the activity and also the pharmacological properties.^[16] Our work demonstrates that the implementation of that concept in a multicomponent manner further increases the diversity elements to be varied and the possibilities to succeed in the discovery of PPI inhibitors. Because of the availability of varied MCRs, we foresee that the multicomponent stapling concept will become an enabling synthetic technology in the field of peptide pharmaceuticals.

Acknowledgements

We are grateful to the Leibniz Institute of Plant Biochemistry, Halle (Saale), for assistance in the MS and CD characterization of the staple peptides. This research has also been supported to (AD) by the National Institute of Health (NIH) (2R01GM097082-05), the European Lead Factory (IMI) under grant agreement number 115489, the Qatar National Research Foundation (NPRP6-065-3-012). Moreover, funding was received through ITN "Accelerated Early stage drug discovery" (AEGIS, grant agreement No 675555) and COFUND ALERT (grant agreement No 665250), Hartstichting (ESCAPE-HF, 2018B012) and KWF Kankerbestrijding grant (grant agreement No 10504). In addition, this research has been supported by grants UMO-2012/06/A/ST5/00224 (to T.A.H.) and UMO-2016/21/D/NZ7/00596 (to L.S.) from the National Science Centre, Poland.

Conflict of interest

The authors declare no conflict of interest.

Keywords: cancer · HSQC NMR · p53-MDM2/X · stapled peptides · Ugi reaction

How to cite: *Angew. Chem. Int. Ed.* **2020**, *59*, 5235–5241
Angew. Chem. **2020**, *132*, 5273–5279

- [1] G. L. Verdine, G. J. Hilinski in *Methods in Enzymology* (Eds.: K. D. Wittrup, G. L. Verdine), Academic Press, New York, **2012**, pp. 3–33.
- [2] L. D. Walensky, G. H. Bird, *J. Med. Chem.* **2014**, *57*, 6275–6288.
- [3] Y. H. Lau, P. de Andrade, Y. Wu, D. R. Spring, *Chem. Soc. Rev.* **2015**, *44*, 91–102.
- [4] T. A. Hill, N. E. Shepherd, F. Diness, D. P. Fairlie, *Angew. Chem. Int. Ed.* **2014**, *53*, 13020–13041; *Angew. Chem.* **2014**, *126*, 13234–13257.
- [5] M. Pelay-Gimeno, A. Glas, O. Koch, T. N. Grossmann, *Angew. Chem. Int. Ed.* **2015**, *54*, 8896–8927; *Angew. Chem.* **2015**, *127*, 9022–9054.
- [6] P.-Y. Yang, H. Zou, C. Lee, A. Muppidi, E. Chao, Q. Fu, X. Luo, D. Wang, P. G. Schultz, W. Shen, *J. Med. Chem.* **2018**, *61*, 3218–3223.
- [7] K. Sakagami, T. Masuda, K. Kawano, S. Futaki, *Mol. Pharm.* **2018**, *15*, 1332–1340.
- [8] P. M. Cromm, J. Spiegel, T. N. Grossmann, *ACS Chem. Biol.* **2015**, *10*, 1362–1375.
- [9] L. Reguera, D. G. Rivera, *Chem. Rev.* **2019**, <https://doi.org/acs.chemrev.8b00744>.
- [10] J. Iegre, J. S. Gaynor, N. S. Robertson, H. F. Sore, M. Hyvönen, D. R. Spring, *Adv. Ther.* **2018**, *1*, 1800052.
- [11] D. Wang, W. Liao, P. S. Arora, *Angew. Chem. Int. Ed.* **2005**, *44*, 6525–6529; *Angew. Chem.* **2005**, *117*, 6683–6687.
- [12] R. Fasan, R. L. A. Dias, K. Moehle, O. Zerbe, J. W. Vrijbloed, D. Obrecht, J. A. Robinson, *Angew. Chem. Int. Ed.* **2004**, *43*, 2109–2112; *Angew. Chem.* **2004**, *116*, 2161–2164.
- [13] S. A. Kawamoto, A. Coleska, X. Ran, H. Yi, C.-Y. Yang, S. Wang, *J. Med. Chem.* **2012**, *55*, 1137–1146.
- [14] C. E. Schafmeister, J. Po, G. L. Verdine, *J. Am. Chem. Soc.* **2000**, *122*, 5891–5892.
- [15] A. Muppidi, Z. Wang, X. Li, J. Chen, Q. Lin, *Chem. Commun.* **2011**, *47*, 9396.
- [16] J. M. Smith, J. R. Frost, R. Fasan, *Chem. Commun.* **2014**, *50*, 5027–5030.
- [17] A. M. Ali, J. Atmaj, N. Van Oosterwijk, M. R. Groves, A. Dömling, *Comput. Struct. Biotechnol. J.* **2019**, *17*, 263–281.
- [18] Y. S. Chang, B. Graves, V. Guerlavais, C. Tovar, K. Packman, K.-H. To, K. A. Olson, K. Kesavan, P. Gangurde, A. Mukherjee, et al., *Proc. Natl. Acad. Sci. USA* **2013**, *110*, E3445–E3454.
- [19] S. Baek, P. S. Kutchukian, G. L. Verdine, R. Huber, T. A. Holak, K. W. Lee, G. M. Popowicz, *J. Am. Chem. Soc.* **2012**, *134*, 103–106.
- [20] N. E. Shepherd, H. N. Hoang, G. Abbenante, D. P. Fairlie, *J. Am. Chem. Soc.* **2005**, *127*, 2974–2983.
- [21] A. V. Vasco, C. S. Pérez, F. E. Morales, H. E. Garay, D. Vasilev, J. A. Gavín, L. A. Wessjohann, D. G. Rivera, *J. Org. Chem.* **2015**, *80*, 6697–6707.
- [22] A. V. Vasco, Y. Méndez, A. Porzel, J. Balbach, L. A. Wessjohann, D. G. Rivera, *Bioconjugate Chem.* **2019**, *30*, 253–259.
- [23] Y. H. Lau, P. de Andrade, S.-T. Quah, M. Rossmann, L. Laraia, N. Sköld, T. J. Sum, P. J. E. Rowling, T. L. Joseph, C. Verma, et al., *Chem. Sci.* **2014**, *5*, 1804–1809.

- [24] L. Peraro, T. R. Siebert, J. A. Kritzer, *Methods Enzymol.* **2016**, 580, 303–332.
- [25] M. G. Ricardo, D. Llanes, L. A. Wessjohann, D. G. Rivera, *Angew. Chem. Int. Ed.* **2019**, 58, 2700–2704; *Angew. Chem.* **2019**, 131, 2726–2730.
- [26] M. G. Ricardo, F. E. Morales, H. Garay, O. Reyes, D. Vasilev, L. A. Wessjohann, D. G. Rivera, *Org. Biomol. Chem.* **2015**, 13, 438–446.
- [27] B. Hu, D. M. Gilkes, J. Chen, *Cancer Res.* **2007**, 67, 8810–8817.
- [28] D. P. Lane, *Nature* **1992**, 358, 15–16.
- [29] D. P. Lane, T. R. Hupp, *Drug Discovery Today* **2003**, 8, 347–355.
- [30] P. H. Kussie, S. Gorina, V. Marechal, B. Elenbaas, J. Moreau, A. J. Levine, N. P. Pavletich, *Science* **1996**, 274, 948–953.
- [31] R. Dawson, L. Müller, A. Dehner, C. Klein, H. Kessler, J. Buchner, *J. Mol. Biol.* **2003**, 332, 1131–1141.
- [32] C. G. Neochoritis, J. Atmaj, A. Twarda-Clapa, E. Surmiak, L. Skalniak, L.-M. Köhler, D. Muszak, K. Kurpiowska, J. Kalinowska-Thüscik, B. Beck, et al., *Eur. J. Med. Chem.* **2019**, 182, 111588.
- [33] F. Bernal, A. F. Tyler, S. J. Korsmeyer, L. D. Walensky, G. L. Verdine, *J. Am. Chem. Soc.* **2007**, 129, 2456–2457.
- [34] C. J. Brown, S. T. Quah, J. Jong, A. M. Goh, P. C. Chiam, K. H. Khoo, M. L. Choong, M. A. Lee, L. Yurlova, K. Zolghadr, et al., *ACS Chem. Biol.* **2013**, 8, 506–512.
- [35] Y. H. Lau, Y. Wu, M. Rossmann, B. X. Tan, P. de Andrade, Y. S. Tan, C. Verma, G. J. McKenzie, A. R. Venkitaraman, M. Hyvönen, et al., *Angew. Chem. Int. Ed.* **2015**, 54, 15410–15413; *Angew. Chem.* **2015**, 127, 15630–15633.
- [36] E. M. M. Abdelraheem, S. Shaabani, A. Dömling, *Drug Discovery Today Technol.* **2018**, 29, 11–17.
- [37] A. Dömling, I. Ugi, *Angew. Chem. Int. Ed.* **2000**, 39, 3168–3210; *Angew. Chem.* **2000**, 112, 3300–3344.
- [38] A. Dömling, W. Wang, K. Wang, *Chem. Rev.* **2012**, 112, 3083–3135.
- [39] L. A. Wessjohann, D. G. Rivera, O. E. Vercillo, *Chem. Rev.* **2009**, 109, 796–814.
- [40] D. G. Rivera, O. E. Vercillo, L. A. Wessjohann, *Org. Biomol. Chem.* **2008**, 6, 1787.
- [41] P. Luo, R. L. Baldwin, *Biochemistry* **1997**, 36, 8413–8421.
- [42] X. Huang, *J. Biomol. Screening* **2003**, 8, 34–38.
- [43] N. Estrada-Ortiz, C. G. Neochoritis, A. Dömling, *ChemMedChem* **2016**, 11, 757–772.
- [44] C. J. Wienken, P. Baaske, U. Rothbauer, D. Braun, S. Duhr, *Nat. Commun.* **2010**, 1, 100.
- [45] M. Jerabek-Willemsen, C. J. Wienken, D. Braun, P. Baaske, S. Duhr, *Assay Drug Dev. Technol.* **2011**, 9, 342–353.
- [46] R. Powers, *Expert Opin. Drug Discovery* **2009**, 4, 1077–1098.
- [47] S. B. Shuker, P. J. Hajduk, R. P. Meadows, S. W. Fesik, *Science* **1996**, 274, 1531–1534.
- [48] E. Barile, M. Pellecchia, *Chem. Rev.* **2014**, 114, 4749–4763.
- [49] R. Stoll, C. Renner, S. Hansen, S. Palme, C. Klein, A. Belling, W. Zeslawski, M. Kamionka, T. Rehm, P. Mühlhahn, et al., *Biochemistry* **2001**, 40, 336–344.
- [50] C. R. Grace, D. Ban, J. Min, A. Mayasundari, L. Min, K. E. Finch, L. Griffiths, N. Bharatham, D. Bashford, R. Kiplin Guy, et al., *J. Mol. Biol.* **2016**, 428, 1290–1303.
- [51] Y. Huang, S. Wolf, D. Koes, G. M. Popowicz, C. J. Camacho, T. A. Holak, A. Dömling, *ChemMedChem* **2012**, 7, 49–52.
- [52] R. Fasan, R. L. A. Dias, K. Moehle, O. Zerbe, D. Obrecht, P. R. E. Mittl, M. G. Grütter, J. A. Robinson, *ChemBioChem* **2006**, 7, 515–526.
- [53] L. Skalniak, E. Surmiak, T. A. Holak, *Expert Opin. Ther. Pat.* **2019**, 1–20.
- [54] C. Neochoritis, N. Estrada-Ortiz, K. Khoury, A. Dömling, *Annu. Rep. Med. Chem.* **2014**, 0, 167–187.

Manuscript received: December 18, 2019

Accepted manuscript online: January 15, 2020

Version of record online: February 6, 2020