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ChemPubSoc
Europe

DOI: 10.1002/cbic.201402374

Linear Aliphatic Dialkynes as Alternative Linkers for Double-Click Stapling of p53-Derived Peptides

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We investigated linear aliphatic dialkynes as a new structural class of *i*,*i*+7 linkers for the double-click stapling of p53-based peptides. The optimal combination of azido amino acids and dialkynyl linker length for MDM2 binding was determined. In a direct comparison between aliphatic and aromatic staple scaffolds, the aliphatic staples resulted in superior binding to MDM2 in vitro and superior p53-activating capability in cells when using a diazidopeptide derived from phage display. This work demonstrates that the nature of the staple scaffold is an important factor that can affect peptide bioactivity in cells.

Protein–protein interactions (PPIs) are involved in most cellular functions in living organisms, and aberrant PPIs are often linked to the onset of human diseases such as cancer.[1] One of the most important proteins in cancer biology is the tumour suppressor p53, which can trigger cell cycle arrest and apoptosis in cells with DNA damage or oxidative stress.[2] In healthy cells, the E3 ubiquitin ligase MDM2 suppresses the apoptotic function of p53 by inducing proteosomal degradation.[3] However, some cancer cell lines overexpress MDM2, thus leading to loss of p53 function and uncontrolled cell growth. Inhibiting the p53/MDM2 interaction is therefore seen as a promising therapeutic strategy in the treatment of such cancers.[4]

The p53/MDM2 interaction is mediated by the binding of a key α-helix of p53 to a corresponding hotspot at the N-terminal domain of MDM2.[5] Several different strategies have shown promise in the development of p53/MDM2 inhibitors, including peptidomimetic and helix-stabilising approaches.[6] One of the most extensively studied of these techniques is all-hydrocarbon peptide stapling, which involves the introduction of two olefinic amino acids into a peptide followed by ring-closing metathesis to reinforce an α-helical conformation.[7]

There is also an increasing number of alternative methods for creating constrained α-helical peptides by different macrocyclisation chemistries.[6a,8] These methods generate peptides with staple linkages that differ structurally and functionally from those generated by metathesis—an important consideration as the staple linkage can interact directly with the target protein.[9] To address the synthetic challenge of efficiently varying the staple component, we previously developed a divergent double-click strategy for generating *i*,*i*+7 stapled peptides,[10] after pioneering work by Torres et al. on an *i*,*i*+4 helix-dimer system.[11] The double-click strategy is one of several reported triazole-stapling techniques.[12] The most common approach involves single-click *i*,*i*+4 stapling.[13] Kawamoto et al. implemented two *i*,*i*+4 staples onto the same peptide by this technique,[8f] and Ingale and Dawson developed peptides with *i*,*i*+3 triazole-stapling.[14]

Starting from a single p53 diazidopeptide developed in our previous study,[10] different dialkynyl staple linkers were introduced under Cu^I catalysis to generate bis-triazole stapled peptides bearing different functionalities on the linker (Figure 1).

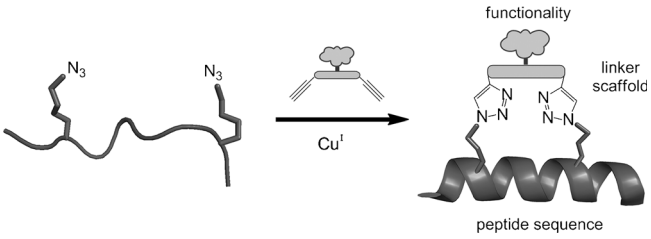

Figure 1. Double-click peptide stapling. Diazidopeptide is combined with a dialkynyl linker under Cu^I catalysis to give a *bis*-triazole stapled peptide. Previously, we appended linkers with different staple functionalities (top). We now vary the structure of the linker scaffold (middle), as well as the peptide sequence (bottom).

The ability of these peptides to inhibit MDM2 in cells was improved by simply changing the functionality appended to the staple linkage.

Our previously reported dialkynyl linkers **1**–**5** are based on a 1,3-dialkynylbenzene scaffold, with different functions at position 5 to vary the properties of the stapled peptide (Scheme 1 A).[10] As these dialkynyl linkers contain the same core aromatic staple structure, we were interested in finding new dialkynyl scaffolds that would allow us to explore the effect of structural changes in the linker. We therefore decided to investigate linear aliphatic dialkynes as a structurally distinct family of linkers for stapling (Scheme 1 B) with the aim of comparing their effectiveness to that of our previous aromatic dialkynyl linkers.

Starting with our previously reported diazidopeptide **SP0** (based on the wild-type $p53_{17-29}$ sequence), we introduced aliphatic dialkynyl linkers **6**–**8** under our optimised solution-

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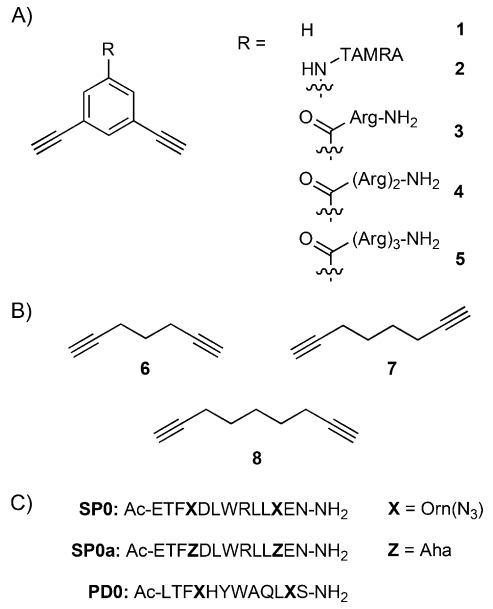
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cbic.201402374.



Scheme 1. A) Previously reported aromatic dialkynyl linkers **1**–**5**. B) Aliphatic dialkynyl linkers **6**–**8**. C) Linear diazidopeptides **SP0**, **SP0a** and **PD0**.

phase double-click conditions (copper sulfate, sodium ascorbate and THPTA ligand in a 1:1 mixture of *tert*-butanol and water) to generate the corresponding stapled peptides **SP6**–**SP8** (i.e., **SP0** stapled with linkers **6**–**8**). We also explored variations in the azido amino acid side-chain length by using the diazidopeptide **SP0a,**[15] which contains Aha (two methylene units on the side chain) in place of Orn(N_3) (three methylene units), thus generating the corresponding double-clicked stapled peptides **SP6a**–**SP8a** (i.e., **SP0a** stapled with linkers **6**–**8**). We were delighted to find that the stapling reactions with aliphatic linkers **6**–**8** proceeded efficiently to give the desired stapled peptides as the major product in all cases (Figure 2). Circular dichroism studies showed a high level of helicity for all six stapled peptides (Figure S4 in the Supporting Information), with linker **6** producing the most helical peptides **SP6** and **SP6a** (78 and 74%, respectively).

The binding of these six peptides to MDM2 was then tested in a competitive fluorescence polarisation assay (Table 1). As we have previously noted,[10] the unstapled peptide already

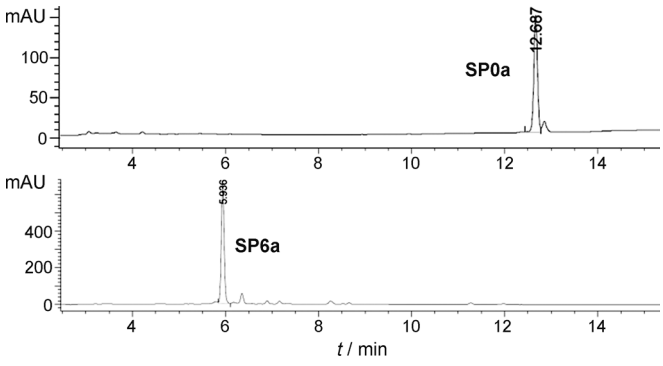


Figure 2. Example HPLC chromatograph of crude reaction mixture of **SP0a** stapled with **6** to form **SP6a** as the major product, as monitored by HPLC at 220 nm. See the Supporting Information for further examples.

Table 1. Binding affinities of peptides to MDM2 as determined by competitive fluorescence polarisation.

Peptide	Staple scaffold	MDM2 K_d [nM]
wt $p53_{17-29}$	none	820 ± 60
SP0	none	16 ± 1
SP1	aromatic	3.2 ± 0.4
SP6	aliphatic	2.6 ± 0.3
SP7	aliphatic	4.0 ± 0.4
SP8	aliphatic	3.1 ± 0.3
SP0a	none	19 ± 1
SP1a	aromatic	10.5 ± 0.8
SP6a	aliphatic	5.1 ± 0.6
SP7a	aliphatic	5.2 ± 0.6
SP8a	aliphatic	8.2 ± 0.8
PD0	none	36 ± 3
PD1	aromatic	14 ± 1
PD5	aromatic	67 ± 4
PD6	aliphatic	7.5 ± 0.7

displays fairly potent binding because the staple replaces the helix-breaking Pro27 residue of the wild-type sequence. All the resulting stapled peptides had higher affinity for MDM2 than either the wild-type or the corresponding unstapled peptide. Stapled peptide **SP6** (binding affinity 2.63 ± 0.30 nM) was the most potent p53 peptide in our assay (including our previously best peptide **SP1**, 3.21 ± 0.38 nM). Comparing the different azido amino acid side-chain lengths, we noted that **SP6**–**8** (three methylene series) consistently showed more potent binding than **SP6a**–**8a** (two methylene series). However, for each series, varying the length of the dialkynyl staples **6**–**8** did not greatly affect binding affinity. These comparisons indicate that the three methylene side-chain positions the two triazoles at a more favourable distance from the peptide backbone.

Despite the promising results in vitro, we did not expect **SP6**–**SP8** and **SP6a**–**SP8a** to be active in cells, as our previous studies (and work by Verdine)[7d] suggested that peptides based on the p53 wild-type sequence are inactive without an increase in the overall net positive charge. When tested in a cell-based gene reporter assay, there was indeed no activation of p53 with any of the six peptides at concentrations up to 100 μM (Figure 3). We previously overcame this issue by using the cationic-functionalised staple linker **5**, so we decided to try an alternative approach for achieving cellular activity by using the new linear peptide sequence **PD0**, a diazido version of the phage-display-derived sequence pDI first reported by Chen and co-workers.[16] This approach was inspired by the successful application of hydrocarbon stapling to this peptide sequence by Sawyer and co-workers.[7f]

As **SP6** gave the best results in our initial fluorescence polarisation assay, we chose linker **6** to staple onto **PD0**, thus giving stapled peptide **PD6**. Although this stapling only resulted in a modest improvement over **PD0** in terms of peptide helicity (by circular dichroism; Figure 4), importantly we observed a fivefold increase in binding affinity after stapling (Table 1). In the gene reporter assay, we were pleased to find that **PD6** induced dose-dependent activation of p53, whereas **PD0** did not (Figure 3). For comparison, we also synthesised **PD1,** which bears the aromatic staple linker **1**, and **PD5**, which has the cat-

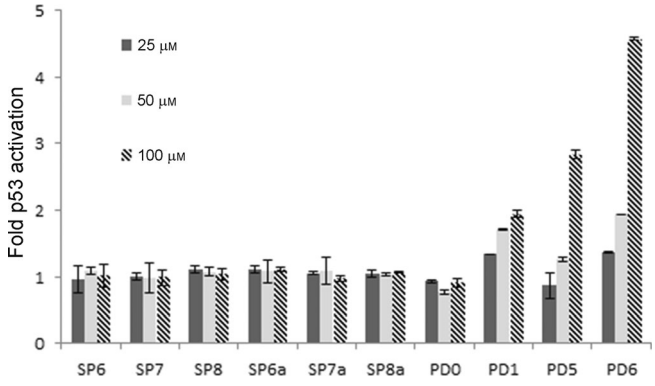


Figure 3. Activation of p53 by peptides in a cell-based gene reporter assay. Fold-activation is relative to 1% DMSO.

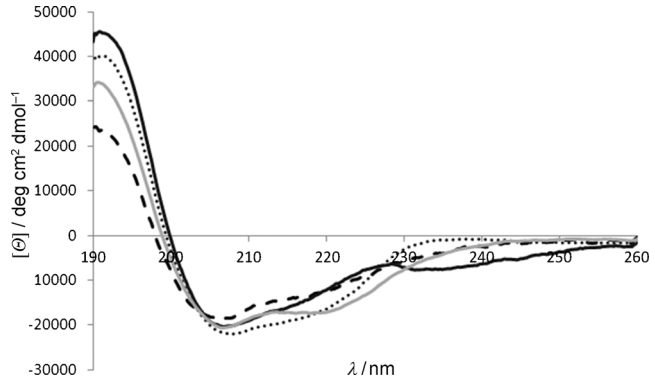


Figure 4. Circular dichroism spectra of stapled peptides with different stapling linkers based on a phage-derived diazidopeptide. -----: PD0 (34%), ——: PD1 (32%), ····: PD5 (44%), - - - - -: PD6 (49%). Estimated helicity is based on mean residue ellipticity at 222 nm.

ionic-functionalised aromatic linker **5**. **PD6** was superior to both **PD1** and **PD6** in both the in vitro binding assay (Table 1) and the in vivo gene reporter assay (Figure 3), thus demonstrating the impact of different linker scaffolds on peptide activity.

The improved in vitro binding affinity of **PD6** compared to **PD1** and **PD5** might arise from improved binding contacts of the staple with MDM2, facilitated by the improved flexibility of the aliphatic scaffold. We are currently working to obtain structural data to determine whether this is indeed the case. In the cellular studies, it appeared that an additional cationic charge is not always beneficial for peptide activity, given that **PD6** was superior to **PD5**. In light of our studies with stapling **SP0** and **PD0**, it appears that different rules apply to different peptide sequences when optimising cellular activity. Furthermore, the direct comparison between aromatic **PD1** and aliphatic **PD6** (differing only by the staple structure) demonstrates how the staple scaffold can affect the overall bioactive properties of a staple peptide.

In summary, we have established dialkynes **6**–**8** as a linkers for *i*,*i*+7 double-click stapling, with their aliphatic scaffolds complementing our previously developed aromatic series of linkers. After optimisation of the combination of linker and peptide sequence, we found that dialkyne **6** had MDM2 inhibitory activity in a cell-based assay, without relying on additional cationic tags for efficacy. We are now looking to systematically explore more combinations of linker structure/functionality and peptide sequence, in order to gain a better understanding of what factors are important for peptide cellular activity in general.

Experimental Section

Synthesis: Aromatic dialkynyl linkers **1** and **6** were synthesised as previously described.[10] Aliphatic dialkynyl linkers were purchased from Sigma–Aldrich.

Fmoc solid-phase peptide synthesis was carried out on a Liberty Automated Microwave Peptide Synthesiser; CEM, Matthews, NC) with Rink Amide MBHA resin LL (0.29–0.39 mmol g^{-1} loading; Merck Millipore). All peptide couplings were performed with Fmoc-protected amino acids (5 equiv) in DMF, HATU or HBTU (5 equiv) in DMF and DIPEA (10 equiv) in NMP (2 M). Arginine was coupled by using double couplings (15 min each) without microwave irradiation; other amino acids were coupled by single couplings (25 W, 75 °C, 15 min). Fmoc deprotection was achieved with piperidine (20% in DMF) at 75 °C (45 W, 3 min). N-terminal acetyl capping was carried out manually by treating resin swelled in dichloromethane with acetic anhydride (10 equiv) and *N*,*N*-diisopropylethylamine (10 equiv) for 45 min. Cleavage from the resin was achieved with triisopropylsilane (2.5%) and water (2.5%) in TFA for 2 h. The solvent was removed under a stream of nitrogen, and the residue was triturated with diethyl ether (3×5 mL) before HPLC purification.

HPLC analysis and purification: Analytical HPLC was run on a model 1260 Infinity chromatographer (Agilent Technologies) with a SUPELCOSIL ABZ+Plus column (150×4.6 mm, 3 μm) and linear gradient elution (solvent A: TFA (0.05% *v*/*v*) in water; solvent B: TFA (0.05% *v*/*v*) in acetonitrile); flow rate: 1 mL min^{-1} over 15 min). Semi-preparative HPLC was run on the Agilent 1260 Infinity with a SUPELCOSIL ABZ+Plus column (250×21.2 mm, 5 μm) and linear gradient elution (solvent A: TFA (0.1% *v*/*v*) in water; solvent B: TFA (0.1% *v*/*v*) in acetonitrile); flow rate: 20 mL min^{-1} over 20 min). HPLC was monitored by UV absorbance at 220 and 254 nm.

Double-click stapling:[10] A solution of diazido peptide (1 equiv; 1 mL mg^{-1}) and dialkynyl linker (1.1 equiv) in *tert*-butanol/water (1:1) was degassed with nitrogen for 15 min, followed by the addition of copper(II) sulfate pentahydrate (1 equiv), tris(3-hydroxypropyltriazolylmethyl)amine (1 equiv) and sodium ascorbate (3 equiv). After stirring under nitrogen at room temperature for 16 h, the reaction mixture was lyophilised and purified by HPLC to give the final stapled peptide.

Competitive fluorescence polarisation: Fluorescence polarisation and binding affinity calculations were carried out as previously described,[10] with a PHERAstar Plus plate reader (BMG Labtech, Ortenberg, Germany) and Prism software (GraphPad, La Jolla, CA). K_d values were calculated using a non-linear least-squares analysis fitting to the equations previously described for binding with receptor depletion,[7e] and are reported with standard errors. Experiments were conducted twice independently, each in triplicate. Accurate peptide concentrations were determined by amino acid analysis at the Peptide Nucleic Acid Chemistry Facility (Department of Biochemistry, University of Cambridge). For binding curves see the Supporting Information.

p53 reporter assay: Assay was carried out as previously described.[7e] T22 cells stably transfected with a p53-responsive β-galac-

tosidase reporter were obtained from Prof David Lane.[7e] Cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) with foetal bovine serum (FBS, 10%), penicillin (100 U mL^{-1}) and streptomycin (100 μg mL^{-1}). Cells were seeded (8000 cells per 100 μL well) for 24 h in a 96-well black-walled clear flat-bottom polystyrene plate (#655090; Greiner Bio One). The cells were then treated with peptide in triplicate for 18 h in DMEM with FBS (10%). β-galactosidase activity was quantified with a FluoReporter LacZ/Galactosidase Quantitation Kit (Life Technologies). Fluorescence was quantified on an Infinite 200 Pro plate reader (Tecan, Männedorf, Swizerland).

Circular dichroism: Circular dichroism spectra were obtained on a Chirascan CD spectrometer (Applied Photophysics Ltd., Leatherhead, UK) at 20 °C (1 mm path length, 260–190 nm, 0.2 nm s^{-1}, bandwidth 1.0 nm, response time 0.5 s). Each spectrum is the average of three scans. Peptides were dissolved in water/acetonitrile (85:15). Helicity was calculated as previously reported[17] by comparing the mean residue ellipticity at 222 nm (MRE_{222}) to the theoretical maximum as calculated by using the formula Eq. (1); n = number of amino acid residues] described by Forood et al.:[18]

$$MRE_{222} = -40\,000 \times (1-2.5/n) \quad (1)$$

Spectra were obtained at several concentrations (5–50 μM): no significant change in the shape of the spectrum was observed at different dilutions. Accurate peptide concentrations were determined by amino acid analysis at the Peptide Nucleic Acid Chemistry Facility.

Acknowledgements

*This work was supported by the European Union, Engineering and Physical Sciences Research Council, Biotechnology and Biological Sciences Research Council, Medical Research Council and Wellcome Trust. Y.H.L. acknowledges a scholarship from the Cambridge Trusts. P.D..A thanks the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). We thank Dr. Chris Brown and Prof. David Lane (p53lab, A*STAR Singapore) for providing the T22 cell line for the gene reporter assay, and Dr. Pamela Rowling and Dr. Laura Itzhaki (Department of Pharmacology, University of Cambridge) for providing MDM2 for fluorescence polarisation.*

Keywords: anticancer agents · click chemistry · macrocycles · peptides · stapling

[1] T. L. Nero, C. J. Morton, J. K. Holien, J. Wielens, M. W. Parker, *Nat. Rev. Cancer* **2014**, *14*, 248–262.
[2] a) A. J. Levine, M. Oren, *Nat. Rev. Cancer* **2009**, *9*, 749–758; b) S. Haupt, M. Berger, Z. Goldberg, Y. Haupt, *J. Cell Sci.* **2003**, *116*, 4077–4085.
[3] J. Piette, H. Neel, V. Maréchal, *Oncogene* **1997**, *15*, 1001–1010.
[4] a) C. J. Brown, C. F. Cheok, C. S. Verma, D. P. Lane, *Trends Pharmacol. Sci.* **2011**, *32*, 53–62; b) C. J. Brown, S. Lain, C. S. Verma, A. R. Fersht, D. P. Lane, *Nat. Rev. Cancer* **2009**, *9*, 862–873; c) P. Chène, *Nat. Rev. Cancer* **2003**, *3*, 102–109.
[5] P. H. Kussie, S. Gorina, V. Marechal, B. Elenbaas, J. Moreau, A. J. Levine, N. P. Pavletich, *Science* **1996**, *274*, 948–953.
[6] For recent reviews, see: a) V. Azzarito, K. Long, N. S. Murphy, A. J. Wilson, *Nat. Chem.* **2013**, *5*, 161–173; b) A. Grauer, B. König, *Eur. J. Org. Chem.* **2009**, 5099–5111; c) R. M. J. Liskamp, D. T. S. Rijkers, J. A. W. Kruijtzer, J. Kemmink, *ChemBioChem* **2011**, *12*, 1626–1653.
[7] a) C. E. Schafmeister, J. Po, G. L. Verdine, *J. Am. Chem. Soc.* **2000**, *122*, 5891–5895; b) H. E. Blackwell, R. H. Grubbs, *Angew. Chem. Int. Ed.* **1998**, *37*, 3281–3284; *Angew. Chem.* **1998**, *110*, 3469–3472; c) G. H. Bird, E. Gavathiotis, J. L. LaBelle, S. G. Katz, L. D. Walensky, *ACS Chem. Biol.* **2014**, *9*, 831–837. For hydrocarbon stapled peptide MDM2 inhibitors see: d) F. Bernal, A. F. Tyler, S. J. Korsmeyer, L. D. Walensky, G. L. Verdine, *J. Am. Chem. Soc.* **2007**, *129*, 2456–2457; e) 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, T. L. Joseph, C. S. Verma, D. P. Lane, *ACS Chem. Biol.* **2013**, *8*, 506–512; f) Y. S. Chang, B. Graves, V. Guerlavais, C. Tovar, K. Packman, K.-H. To, K. A. Olson, K. Kesavan, P. Gangurde, A. Mukherjee, T. Baker, K. Darlak, C. Elkin, Z. Filipovic, F. Z. Qureshi, H. Cai, P. Berry, E. Feyfant, X. E. Shi, J. Horstick, D. A. Annis, et al., *Proc. Natl. Acad. Sci. USA* **2013**, *110*, E3445–E3454.
[8] a) A.-M. Leduc, J. O. Trent, J. L. Wittliff, K. S. Bramlett, S. L. Briggs, N. Y. Chirgadze, Y. Wang, T. P. Burris, A. F. Spatola, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 11273–11278; b) R. S. Harrison, N. E. Shepherd, H. N. Hoang, G. Ruiz-Gómez, T. A. Hill, R. W. Driver, V. S. Desai, P. R. Young, G. Abbenante, D. P. Fairlie, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 11686–11691; c) M. M. Madden, C. I. Rivera Vera, W. Song, Q. Lin, *Chem. Commun.* **2009**, 5588–5590; d) M. M. Madden, A. Muppidi, Z. Li, X. Li, J. Chen, Q. Lin, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 1472–1475; e) A. Muppidi, Z. Wang, X. Li, J. Chen, Q. Lin, *Chem. Commun.* **2011**, *47*, 9396–9398; f) S. A. Kawamoto, A. Coleska, X. Ran, H. Yi, C.-Y. Yang, S. Wang, *J. Med. Chem.* **2012**, *55*, 1137–1146; g) H. Jo, N. Meinhardt, Y. Wu, S. Kulkarni, X. Hu, K. E. Low, P. L. Davies, W. F. DeGrado, D. C. Greenbaum, *J. Am. Chem. Soc.* **2012**, *134*, 17704–17713; h) K. Fujimoto, M. Kajino, M. Inouye, *Chem. Eur. J.* **2008**, *14*, 857–863; i) J. R. Kumita, O. S. Smart, G. A. Woolley, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 3803–3808; j) O. Torres, D. Yüksel, M. Bernardina, K. Kumar, D. Bong, *ChemBioChem* **2008**, *9*, 1701–1705; k) S. Kneissl, E. J. Loveridge, C. Williams, M. P. Crump, R. K. Allemann, *ChemBioChem* **2008**, *9*, 3046–3054; l) A. M. Spokoyny, Y. Zou, J. Ling, H. Yu, Y.-S. Lin, B. L. Pentelute, *J. Am. Chem. Soc.* **2013**, *135*, 5946–5949.
[9] a) T. L. Joseph, D. Lane, C. S. Verma, *Cell Cycle* **2010**, *9*, 4560–4568; b) C. Phillips, L. R. Roberts, M. Schade, R. Bazin, A. Bent, N. L. Davies, R. Moore, A. D. Pannifer, A. R. Pickford, S. H. Prior, C. M. Read, A. Scott, D. G. Brown, B. Xu, S. L. Irving, *J. Am. Chem. Soc.* **2011**, *133*, 9696–9699; c) 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; d) M. L. Stewart, E. Fire, A. E. Keating, L. D. Walensky, *Nat. Chem. Biol.* **2010**, *6*, 595–601.
[10] 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, M. Hyvönen, L. S. Itzhaki, A. R. Venkitaraman, C. J. Brown, D. P. Lane, D. R. Spring, *Chem. Sci.* **2014**, *5*, 1804–1809.
[11] O. Torres, D. Yüksel, M. Bernardina, K. Kumar, D. Bong, *ChemBioChem* **2008**, *9*, 1701–1705.
[12] D. S. Pedersen, A. Abell, *Eur. J. Org. Chem.* **2011**, 2399–2411.
[13] a) M. Scrima, A. Le Chavalier-Isaad, P. Rovero, A. M. Papini, M. Chorev, A. M. D'Ursi, *Eur. J. Org. Chem.* **2010**, 446–457; b) L. Frankiewicz, C. Betti, K. Guillemyn, D. Tourwé, Y. Jacquot, S. Ballet, *J. Pept. Sci.* **2013**, *19*, 423–432; c) A. D. de Araujo, H. N. Hoang, W. M. Kok, F. Diness, P. Gupta, T. A. Hill, R. W. Driver, D. A. Price, S. Liras, D. P. Fairlie, *Angew. Chem. Int. Ed.* **2014**, *53*, 6965–6969; *Angew. Chem.* **2014**, *126*, 7085–7089.
[14] S. Ingale, P. E. Dawson, *Org. Lett.* **2011**, *13*, 2822–2825.
[15] Y. H. Lau, P. de Andrade, N. Sköld, G. J. McKenzie, A. R. Venkitaraman, C. Verma, D. P. Lane, D. R. Spring, *Org. Biomol. Chem.* **2014**, *12*, 4074–4077.
[16] B. Hu, D. M. Gilkes, J. Chen, *Cancer Res.* **2007**, *67*, 8810–8817.
[17] G. H. Bird, F. Bernal, K. Pitter, L. D. Walensky, *Methods Enzymol.* **2008**, *446*, 369–386.
[18] B. Forood, J.-E. Feliciano, K. P. Nambiar, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 838–842.

Received: July 11, 2014
Published online on ■■ ■■, 0000

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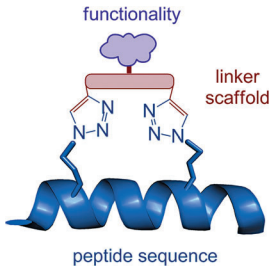
New scaffolds for stapling: A comparison of linear aliphatic dialkynes with aromatic dialkynes for the double-click stapling of p53 peptides demonstrates that the linker scaffold, along with the peptide sequence and staple functionality, can influence the biological activity of stapled peptides in a cellular context.



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