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Citation for published version:

Digital Object Identifier (DOI):
10.1021/ja910715u

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Peer reviewed version

Published In:
Journal of the American Chemical Society

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Bridged $\beta^3$-Peptide Inhibitors of p53-hDM2 Complexation—Correlation Between Affinity and Cell Permeability

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Abstract

$\beta$-peptides possess several features that are desirable in peptidomimetics; they are easily synthesized, fold into stable secondary structures in physiologic buffers, and resist proteolysis. They can also bind to a diverse array of proteins to inhibit their interactions with $\alpha$-helical ligands. $\beta$-peptides are not usually cell permeable, however, and this feature limits their utility as research tools and potential therapeutics. Appending an Arg sequence to a $\beta$-peptide improves uptake but adds considerable mass. We reported that embedding a small cationic patch within a PPII, $\alpha$- or $\beta$-peptide helix improves uptake without the addition of significant mass. Similarly, Verdine, Walensky, and others reported that insertion of a hydrocarbon bridge between the $i$ and $i+4$ positions of an $\alpha$-helix also increases cell uptake. Here we describe a series of $\beta$-peptides containing diether and hydrocarbon bridges and compare them on the basis of cell uptake and localization, affinities for hDM2, and 14-helix structure. Our results highlight the relative merits of cationic patch and hydrophobic bridge strategies for improving $\beta$-peptide uptake and identify a surprising correlation between uptake efficiency and hDM2 affinity.
Our studies began with an analysis of available x-ray\textsuperscript{39,40} and NMR structures\textsuperscript{13,41} of $\beta$-peptide 14-helices to identify those position pairs that would best tolerate an ether\textsuperscript{12,43} or hydrocarbon\textsuperscript{34} bridge. This analysis, supported by recent work of Perlmutter\textsuperscript{42} and Seebach\textsuperscript{44} suggested that a 21-atom bridge could be accommodated between most $i$ and $i+3$ positions of a 14-helix. To test this prediction, we synthesized an analog of $\beta$-peptide \(\beta\textsuperscript{3}-\text{L-Ser at positions 3 and 6 (2(3-6)\textsuperscript{s})} \) (Figure 1), and subjected it to on-resin ring-closing metathesis using bis(tricyclohexylphosphine)benzylidene ruthenium (IV) dichloride\textsuperscript{34} to generate \(2(3-6)\textsuperscript{i}\).\textsuperscript{45} The circular dichroism (CD) spectra of 2, 2(3-6)\textsuperscript{s} and 2(3-6)\textsuperscript{i} were identical (Figure S1), indicating that this 21-atom diether bridge is accommodated between positions 3 and 6. Introduction of the diether bridge did not significantly increase or decrease the extent of 14-helix structure as judged by CD.

In order to evaluate the relative uptake of bridged $\beta$-peptides in the context of a functional molecule of diverse sequence, we synthesized a series of variants of p53-hDM2 complexation (Figure 1). These variants contained either (O-allyl)\(-\text{L-Ser at positions 3 and 6 (25.O-s and 25.C-s, respectively) or 4 and 7 (47.O-s and 47.C-s, respectively). According to the CD spectra (Figure 2), all bridged $\beta$-peptides assumed a 14-helical structure and were modestly more helical than unbridged analogs (Figure S2).}

As a prelude to evaluating cell uptake and localization, we employed a direct fluorescence polarization assay to compare hydrocarbon and diether bridged $\beta$-peptides on the basis of affinity for hDM2\textsubscript{1-188} (Figure 2B). $\beta$-peptides containing a diether or hydrocarbon bridge between positions 4 and 7 bound hDM2\textsubscript{1-188} 2-fold better ($K_d = 53.9 \pm 22.7$ and 94.1 $\pm$ 18.4 nM, respectively) than the corresponding unbridged analogs ($K_d = 114 \pm 28$ and 253 $\pm$ 75 nM, respectively), in line with analogous comparisons in an $\alpha$-peptide context.\textsuperscript{35} By contrast, $\beta$-peptides containing a diether or hydrocarbon bridge between positions 2 and 5 bound hDM2\textsubscript{1-188} 4-8-fold worse ($K_d = 548 \pm 58$ and 546 $\pm$ 96 nM, respectively) than unbridged analogs ($K_d = 139 \pm 13$ and 68.1 $\pm$ 7.8 nM, respectively). In silico analysis suggests that the lower hDM2\textsubscript{1-188} affinity of $\beta$-peptides 25.C-s and 25.O-s results from steric hindrance between the hydrocarbon bridge and the hDM2 surface that is absent in the complex with peptides 47.C-s and 47.O-s (Figure 3, compare A and B).

We next set out to monitor the mammalian cell uptake and sub-cellular localization of diether- and hydrocarbon bridged $\beta$-peptides based on p53-12. Uptake was monitored using flow cytometry, whereas sub-cellular localization was assessed using confocal microscopy (Figure 4). $\beta$-peptides containing diether or hydrocarbon bridges between positions 4 and 7 were taken up significantly more efficiently (MCF = 8.21 $\pm$ 0.45 and 8.63 $\pm$ 0.77, respectively) than unbridged analogs (MCF = 3.23 $\pm$ 0.31 and 2.63 $\pm$ 0.32, respectively), irrespective of bridge structure. By contrast, $\beta$-peptides containing diether or hydrocarbon bridges between positions 2 and 5 were taken up poorly, irrespective of bridge structure, and behaved much like the unbridged analogs. In all cases, as judged by flow cytometry, the greatest uptake was observed with $\beta$-peptide p53-12SB3, which contains a cationic patch on one 14-helix face but no bridge of any kind (Figure 4AB).

The localization of bridged $\beta$-peptides upon cell uptake was explored in more detail using confocal microscopy. HeLa cells were treated with fluorescently labeled $\beta$-peptide (green) as well as Alexa Fluor® 647 labeled transferrin and Hoescht 33342 to visualize recycling endosomes\textsuperscript{36,47} (red) and nuclei (blue). $\beta$-peptides containing a diether or hydrocarbon bridge between positions 4 and 7 are distributed widely among Tf+ and Tf- endosomes, as well as nuclear and cytosolic compartments, whereas those containing the analogous bridge between positions 2 and 5 are not (Figure 3). Indeed, $\beta$-peptides containing a diether or hydrocarbon bridge between positions 2 and 5 are taken up more poorly than the unbridged analog (Figure
S4). These results highlight an intriguing correlation between hDM2 affinity and cell uptake; it is possible that the structural features that lower hDM2 affinity (Figure S3) also lower uptake efficiency. Indeed, it appears that for these β-peptides, an increase in 14-helix secondary structure does not necessarily confer increased cell uptake.26

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

This work was supported by the NIH (GM 74756), the National Foundation for Cancer Research, and a Marie Curie International Outgoing Fellowship within the 7th European Community Framework Programme (J.M.). A.D.B. is grateful to Bristol-Myers Squibb for a graduate research fellowship.

**References**


*J Am Chem Soc*. Author manuscript; available in PMC 2011 March 10.
45. See Supporting Information for details.
Figure 1.
Helical net representation of β-peptides studied herein. β3-homoamino acids are identified by the single-letter code used for the corresponding α-amino acid. Orn represents ornithine. Z represents 3-(S)-3-amino-4-(2-trifluoromethylphenyl)-butyric acid.
Figure 2.
CD analysis of β-peptides containing hydrocarbon or diether bridges between residues (A) 2 and 5 or (B) 4 and 7. Fluorescence polarization (FP) analysis of hDM2 binding by β-peptides containing (C) hydrocarbon or (D) diether bridges.
Figure 3.
Computational model of hDM2 (grey) in complex with (A) 25.C-s or (B) 47.C-s.\textsuperscript{45}
Figure 4.
HeLa cell uptake and localization of Flu-labeled β-peptides. (A,B) HeLa cells were incubated with 2 μM β-peptide for 4 h, treated with 0.25% trypsin for 10 min, washed with cold DMEM and PBS, and analyzed using flow cytometry. (C) Confocal microscopy of HeLa cells treated with 20 μM of the indicated β-peptide (green), 5 mg·mL⁻¹ Alexa Fluor 647-transferrin (red) and 150 nM Hoescht 33342 (blue).