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Bridged β^3-Peptide Inhibitors of p53-hDM2 Complexation: Correlation between Affinity and Cell Permeability

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β-peptides[1−4] possess several features that are desirable in peptidomimetics;[5,6] they are easily synthesized, fold into helices[1−3,7] in physiologic buffers,[8] and resist proteolysis.[9] They also bind in vitro to proteins such as hDM2,[10−14] hDMX,[10] gp41,[15,16] and others[17−19] and inhibit their interactions with α-helical ligands. β-peptides are usually not cell-permeable, however, and this feature limits their utility as research tools and potential therapeutics. Appending an Arg_8 sequence to a β-peptide can improve uptake[20,21] but adds considerable mass. We previously reported that embedding a small cationic patch within a PPII,[22] α-,[23] or β-peptide[11] helix improves uptake without the addition of significant mass.[24,25] Similarly, Verdine, Walensky, and others[26−33] reported that insertion of a hydrocarbon bridge (a "staple") between the i and $i + 4$ positions of an α-helix[34] increases uptake.[26,29,32,34−38] Here we describe a variety of β-peptides containing diether and hydrocarbon bridges and compare them on the basis of cell uptake and localization, affinity for hDM2, and 14-helix structure. Our results highlight the relative merits of the cationic-patch and hydrophobic-bridge strategies for improving β-peptide uptake and identify an unprecedented correlation between uptake efficiency and hDM2 affinity in vitro.

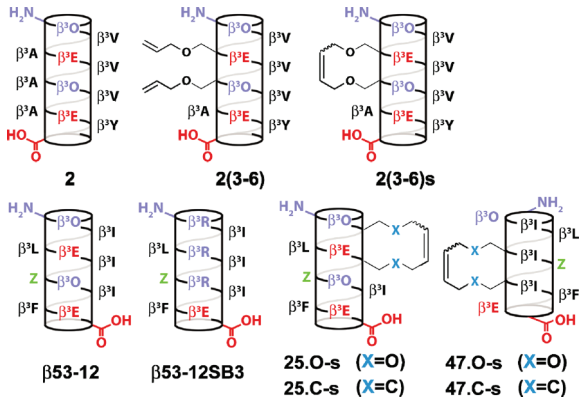

Figure 1. Helical net representations of β-peptides studied herein. β^3-homoamino acids are identified by the single-letter code used for the corresponding α-amino acid. O represents ornithine. Z represents 3-(*S*)-3-amino-4-(2-trifluoromethylphenyl)butyric acid.

Our studies began with an analysis of available X-ray[39,40] and NMR structures[13,41] of β-peptide 14-helices to identify those position pairs that would best tolerate an ether[42,43] or hydrocarbon[34] bridge. This analysis, supported by the recent work of Perlmutter[42] and Seebach,[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 analogue of β-peptide **2**[7] containing (*O*-allyl)-β^3-L-Ser at positions 3 and 6 [**2(3-6)**; Figure 1] and subjected it to on-resin ring-closing metathesis using bis(tricyclohexylphosphine)benzylideneruthenium(IV) dichloride[34] to generate **2(3-6)s**.[45] The circular dichroism (CD) spectra of **2**, **2(3-6)**, and **2(3-6)s** were identical (Figure S1 in the Supporting Information), 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.

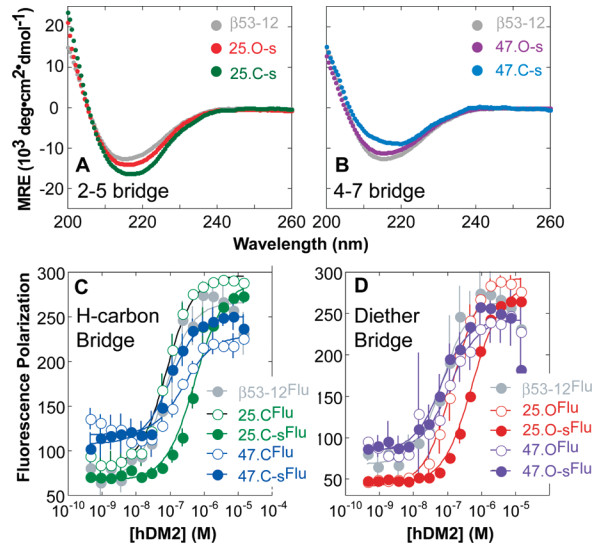

Figure 2. (A, B) CD analysis of β-peptides containing hydrocarbon or diether bridges between residues (A) 2 and 5 or (B) 4 and 7. (C, D) FP analysis of hDM2 binding by β-peptides containing (C) hydrocarbon or (D) diether bridges.

In order to evaluate the relative uptake of bridged β-peptides in the context of a functional molecule of diverse sequence, we synthesized a series of variants of ***β*53-12**,[10] an inhibitor of p53−hDM2 complexation (Figure 1). These variants contained either (*O*-allyl)-β^3-L-Ser (to generate a diether bridge) or (*S*)-3-aminooct-7-enoic acid (to generate a hydrocarbon bridge) at i and $i + 3$ positions 2 and 5 (**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 of the bridged β-peptides assumed a

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14-helical structure and were modestly more helical than the unbridged analogues (Figure S2 in the Supporting Information).

As a prelude to evaluating cell uptake and localization, we employed a direct fluorescence polarization (FP) assay to compare hydrocarbon- and diether-bridged β-peptides on the basis of affinity for hDM2$_{1-188}$ (Figure 2B). β-peptides containing a diether or hydrocarbon bridge between positions 4 and 7 bound hDM2$_{1-188}$ 2-fold better (K_d = 53.9 ± 22.7 and 94.1 ± 18.4 nM, respectively) than the corresponding unbridged analogues (K_d = 114 ± 28 and 253 ± 75 nM, respectively), in line with analogous comparisons in an α-peptide context.[35] In contrast, β-peptides containing a diether or hydrocarbon bridge between positions 2 and 5 bound hDM2$_{1-188}$ 4−8-fold worse (K_d = 548 ± 58 and 546 ± 96 nM, respectively) than the unbridged analogues (K_d = 139 ± 13 and 68.1 ± 7.8 nM, respectively). In silico analysis suggests that the lower hDM2$_{1-188}$ affinity of β-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).

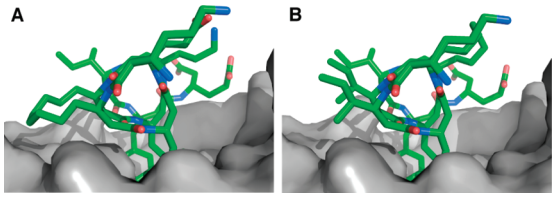

Figure 3. Computational models of hDM2 (gray) complexed with (A) **25.C-s** and (B) **47.C-s**.[45]

We next set out to monitor the mammalian cell uptake and subcellular localization of diether- and hydrocarbon-bridged β-peptides based on ***β*53-12**. Uptake was monitored using flow cytometry (Figure 4A,B), whereas subcellular localization was assessed using confocal microscopy (Figure 4C). β-peptides containing diether or hydrocarbon bridges between positions 4 and 7 were taken up significantly more efficiently [mean cellular fluorescence (MCF) = 8.21 ± 0.45 and 8.63 ± 0.77, respectively] than the unbridged analogues (MCF = 3.23 ± 0.31 and 2.63 ± 0.32, respectively), irrespective of bridge structure. In contrast, β-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 analogues. In all cases, as judged by flow cytometry, the greatest uptake was observed with β-peptide ***β*53-12SB3**, which contains a cationic patch on one 14-helix face but no bridge of any kind (Figure 4A,B).

The localization of bridged β-peptides upon cell uptake was explored in more detail using confocal microscopy. HeLa cells were treated with fluorescently labeled β-peptide (green) as well as Alexa Fluor 647-labeled transferrin and Hoescht 33342 to visualize recycling endosomes[46,47] (red) and nuclei (blue). β-peptides containing a diether or hydrocarbon bridge between positions 4 and 7 were 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 were not (Figure 4C). Indeed, β-peptides containing a diether or hydrocarbon bridge between positions 2 and 5 are taken up more poorly than the unbridged analogue (Figure S4 in the Supporting Information). These results highlight an intriguing correlation between hDM2 affinity and cell uptake; it is possible that the structural features that decrease the hDM2 affinity (Figure S3 in the Supporting Information) also decrease the 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]

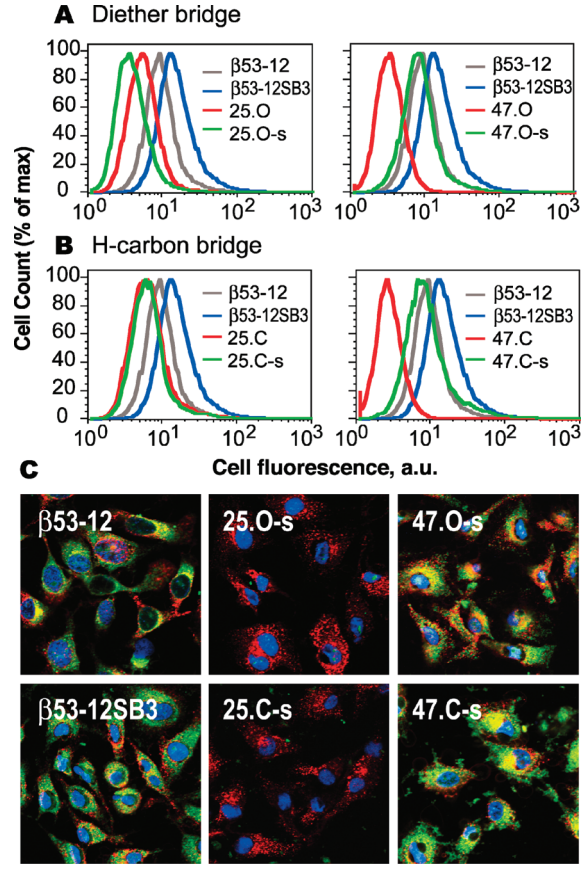

Figure 4. HeLa cell uptake and localization of Flu-labeled β-peptides. (A, B) HeLa cells were incubated with 2 μM β-peptide for 4 h, treated with 0.25% trypsin for 10 min, washed with cold DMEM and PBS, and analyzed using flow cytometry. (C) Confocal microscopy of HeLa cells treated with 20 μM β-peptide (green), 5 mg mL^{-1} Alexa Fluor 647-labeled transferrin (red), and 150 nM Hoescht 33342 (blue).

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Supporting Information Available: β-peptide synthesis, binding and cell uptake assays, confocal microscopy images, and complete ref 32. This material is available free of charge via the Internet at http://pubs.acs.org.

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