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Enhancing Cell Entry of Peptide Conjugates with Bicycle Formation Through TBMB Rigid Scaffold

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Introduction

Delivering therapeutic agents into cells has always been a major challenge. Usually, the conjugates decompose before entering the cells. Bicyclic peptides are promising agents and have some advantages. These peptides are usually not so long typically 9-20 amino acids and thanks to this small size these peptides have the potential to penetrate the cell membrane. Furthermore, the two rings may protect the construct from the enzymatic degradation, so bicyclic peptides remain intact. A sequence containing 3 cysteine residues can be cyclized via its thiol-groups in selective reaction with a small-molecule scaffold. This reaction may form three stable thio-ether bonds between the peptide and the scaffold, constraining the peptide into a bicyclic structure [1,2]. Another approach is the modification of the *N*-terminal amino-group with Dabcyl-group ((4–((4–(dimethylamino)phenyl)azo)benzoyl)) that can also enhance the cellular uptake of the peptides [3].

Results and Discussion

Our aim is to study the effect of bicycle formation on the internalization of different oligoarginines. For this oligoarginines with or without Dabcyl-group were synthesized and reacted with 1,3,5-tris(bromomethyl)benzene. Their labelling for cellular uptake studies was with 5(6)-carboxyfluorescein on the *C*-terminal Lys residue. The oligoarginines were synthesized by standard Fmoc/^{t}Bu strategy. The Dabcyl-group was coupled to the α-amino group of the peptides on the resin. The reaction between the peptides and 1,3,5-tris(bromomethyl)benzene as well as the fluorescent labelling took place in solution. The constructs were purified by RP-HPLC and characterized by analytical RP-HPLC and mass spectrometry (Table 1). The sequences were designed in a way that each loop contains equal number arginine residues.

In vitro cellular uptake was measured by flow cytometry experiments on cancerous cells. EBC-1 human cancer cells were treated with the solution of the conjugates for 90 min at RT. The fluorescence intensity of treated cells was measured by flow cytometry (Figure 1). The cyclization increased the cellular uptake of the Dabcyl containing octaarginine derivative in comparison with octaarginine. The cytotoxicity was also measured by flow cytometry (Figure 2). The live cells were always 70% or more indicating that these constructs have no cytotoxic effect.

Table 1 Chemical characteristics of conjugates.

Peptide sequence	*Code*	*Rt (min)*	$M_{calculated}$	$M_{measured}$
Dabcyl-CR_2CR_2CK-CF TBMB	D-CR4	14.8	1802.0	1801.8
Dabcyl-CR_3CR_3CK-CF TBMB	D-CR6	13.8	2114.4	2114.0
Dabcyl-CR_4CR_4CK-CF TBMB	D-CR8	13.5	2426.8	2426.2
Acyl-CR_2CR_2CK-CF TBMB	Ac-CR4	12.5	1592.8	1592.7
Acyl-CR_3CR_3CK-CF TBMB	Ac-CR6	11.4	1905.2	1904.9
Acyl-CR_4CR_4CK-CF TBMB	Ac-CR8	10.1	2217.6	2217.1

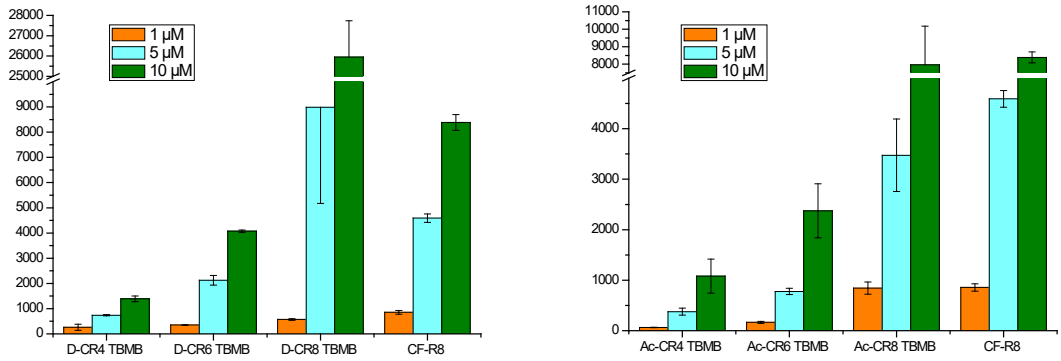

Fig. 1. The effect of bicycle formation on the internalization of oligoarginines with (left) and without Dabcyl-group (right) on EBC-1 cells at RT.

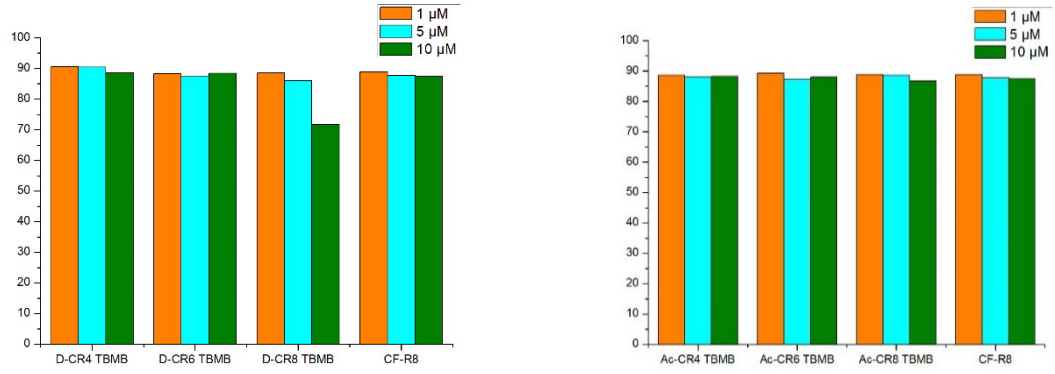

Fig. 2. Cytotoxicity measured by flow cytometry of oligoarginines with (left) and without Dabcyl-group (right).

These results indicate that the cyclization may enhance the internalization of oligoarginine. The bicyclic octaarginines; with or without Dabcyl-group had better or similar uptake than the linear one, respectively. These modifications enhance the internalization while they do not result in cytoxic effect.

Acknowledgments

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