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Faculty of Chemistry - Organic and Bioorganic Chemistry - OCIII

UNIVERSITÄT BIELEFELD

Synthesis and FRET-Based Cleavage Study of an $\alpha_V\beta_6$-Targeting Peptide Drug Conjugate

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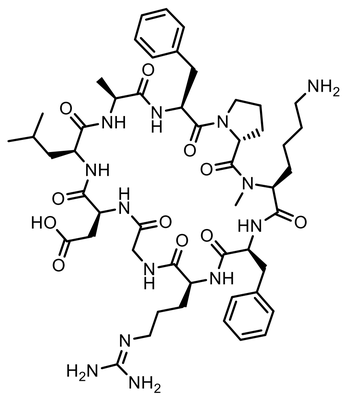
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$\alpha_V\beta_6$-Targeting Peptide

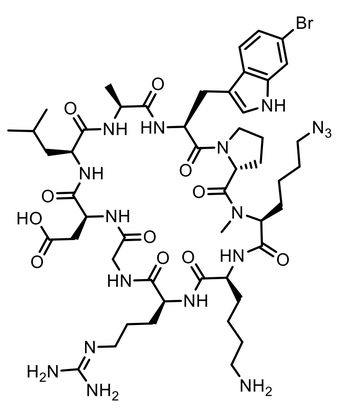
- Integrins are heterodimeric transmembrane receptors, whose functions can be exploited by tumor cells to support their survival.[1,2]
- Integrin $\alpha_V\beta_6$ is involved in breast and colon cancer. [3]
- RGD peptides can specifically bind to these integrins.[4]
- Incorporation of 6-Br-Trp at *i+3* relative to the RGD sequence improves the binding affinity.[5]

Maltsev *et al.*[6]



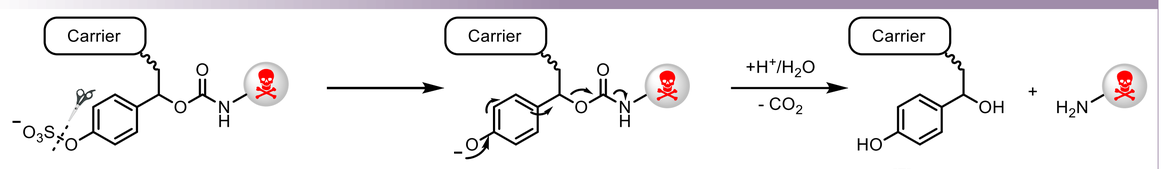
c(FRGDLAFp(*N*Me)K)
IC_{50} = 0.26 nM

This work



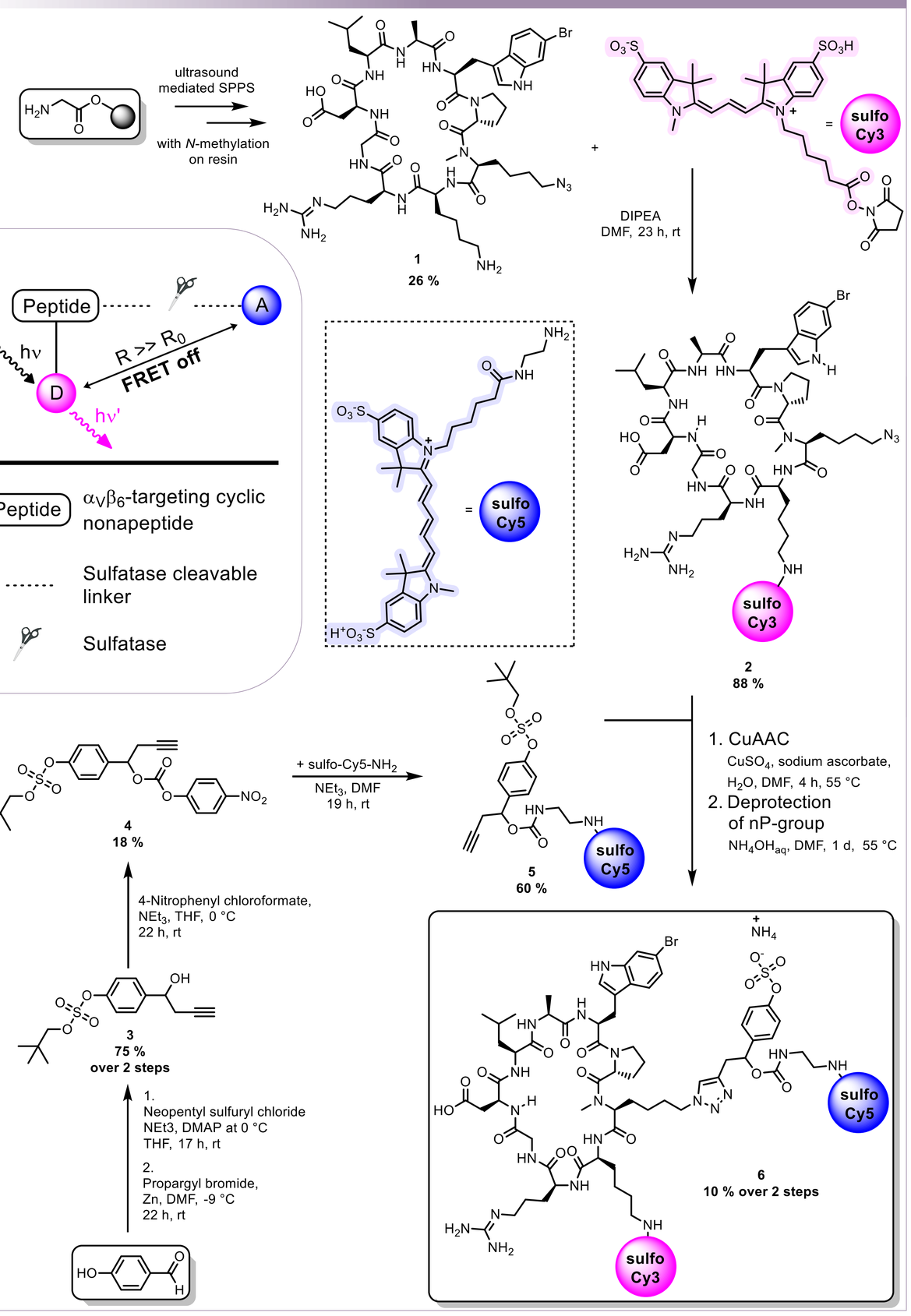
c(KRGDLA(6-Br)Wp(*N*Me)K(N_3))
IC_{50} = 0.12 ± 0.09 nM

Sulfatase Cleavable Linkers

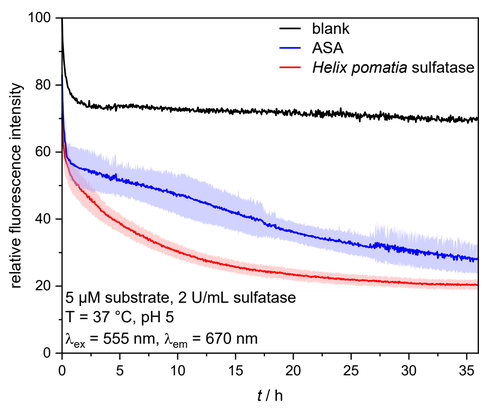


- Arylsulfatases (E.C.3.1.6.1.) catalyze the hydrolysis of aromatic sulfate esters.[7]
- It was found that sulfatase levels were twice as high in breast cancer tissue as in normal tissue.[7]
- Benefits of sulfatase cleavable linkers are stability in mouse and human plasma and their simple synthesis.[8]

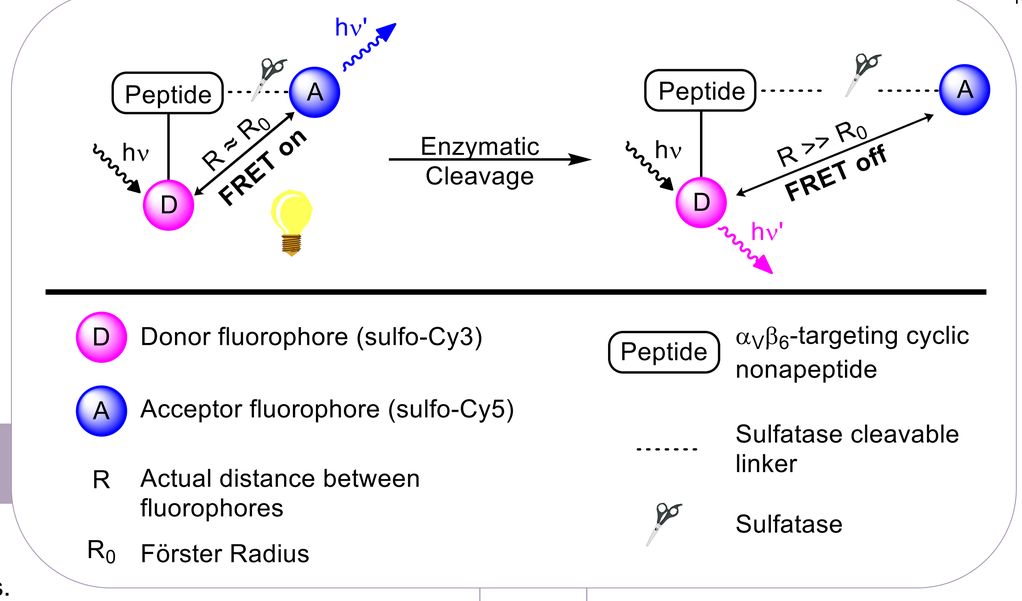
Synthesis



Cleavage Assay

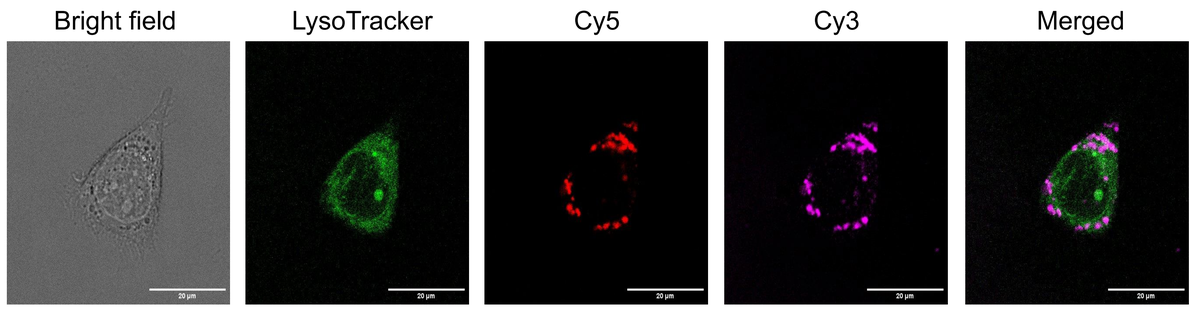


The cleavage of conjugate **6** can be detected via the decrease of acceptor fluorescence over time. It was carried out with two different sulfatases.



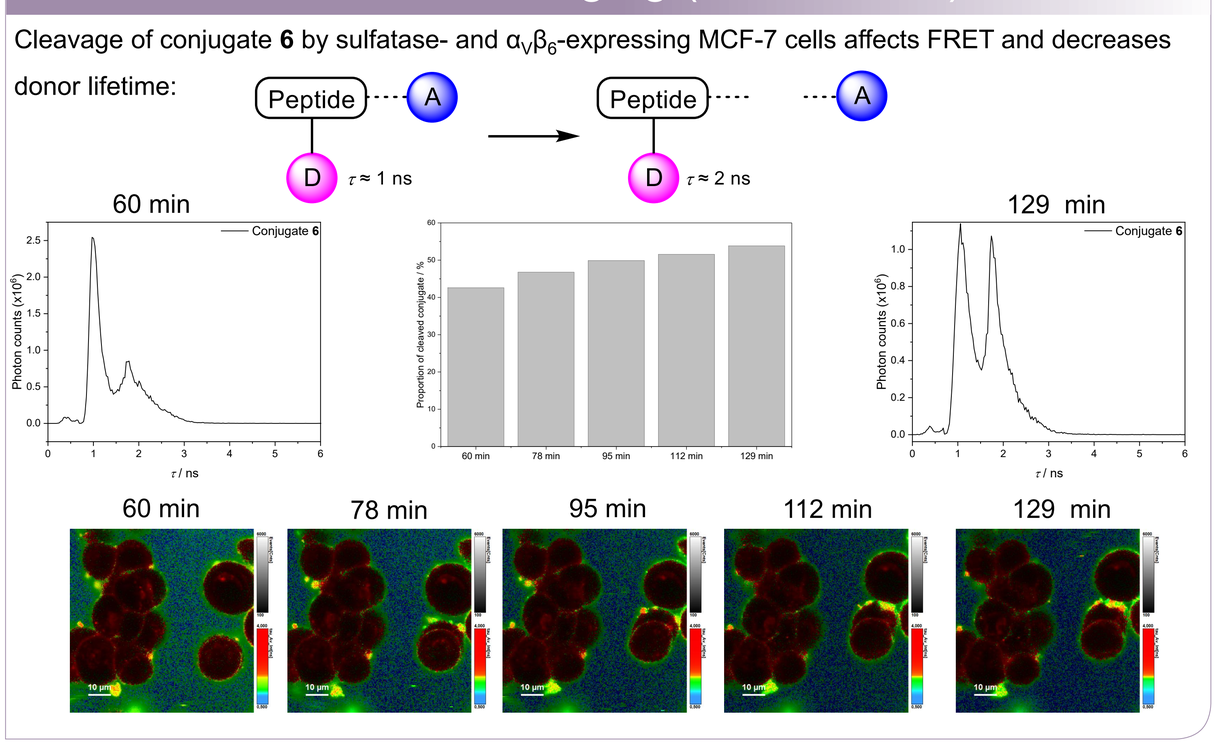
Internalization into MCF-7 cells

Conjugate **6** was incubated with $\alpha_V\beta_6$-expressing MCF-7 cells. LysoTracker Green was used to stain acidic cell compartments.



Fluorescence Lifetime Imaging (FLIM-FRET)

Cleavage of conjugate **6** by sulfatase- and $\alpha_V\beta_6$-expressing MCF-7 cells affects FRET and decreases donor lifetime:



Summary

- Conjugate **6** with sulfo-Cy3 as the FRET donor at the lysine side chain and sulfo-Cy5 as the FRET acceptor instead of a toxophore was synthesized.
- Both the human arylsulfatase A and the sulfatase from *Helix pomatia* cleave the conjugate **6**, which can be detected by the loss of FRET.
- Conjugate **6** is internalized by sulfatase- and $\alpha_V\beta_6$-expressing MCF-7 cells and its cleavage can be detected via FLIM-FRET.

References

[1] R. O. Hynes, *Cell* **2002**, *110*, 673. [2] J. Xiong, L. Yan, C. Zou, *et al. J. Hematol. Oncol.* **2021**, *14*, 177. [3] J. Paulus, N. Sewald, *J. Pept. Sci.* **2024**, *30*, e3561. [4] T. Inoue, P. G. Cavanaugh, P. A. Steck, N. Brünner, G. L. Nicolson, *J. Cell. Physiol.* **1993**, *156*, 212. [5] B. Nachtigall, C. Brinkmann, J. Langosch, A. Nieß, and N. Sewald, *Chem. – Eur. J.*, **2026**, e71559. [6] O. V. Maltsev, U. K. Marelli, T. G. Kapp et al., *Angew. Chem. Int. Ed.* **2016**, *55*, 1535. [7] S. Türkmen, P. Oner, F. Cinar, H. Koçak, G. Güvenen, H. Altun, Y. Eryavuz, *Cancer Letters* **2001**, *166*, 95. [8] J. D. Bargh, S. J. Walsh, A. Isidro-Llobet, S. Omarjee, J. S. Carroll, D. R. Spring, *Chem. Sci.* **2020**, *11*, 2375.

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