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Faculty of Chemistry - Organic and Bioorganic Chemistry - OCIII

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Synthesis of Halogenated Cyclic RGD-Peptides as Potent SMDC-ligands

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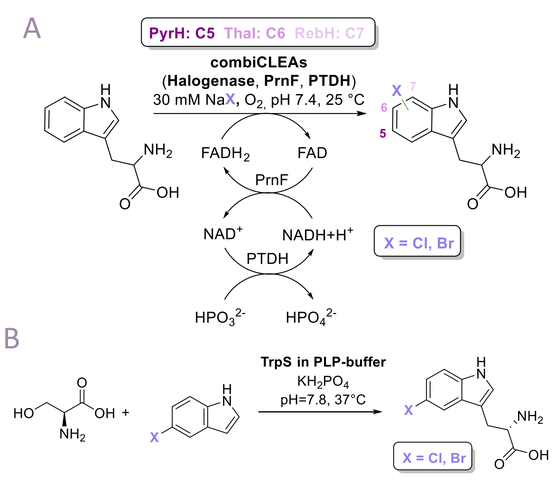
Selective Halogenation of Tryptophane

- FAD-dependent halogenases PyrH, Thal or RebH enable regioselective bromination or chlorination in C5, C6 or C7 position under mild reaction conditions
- Co-immobilization of the halogenase with cofactor regenerating enzymes enable production of halotryptophane on gram-scale.[1]
- Tryptophan synthase (TrpS) was used for the synthesis of 5-halotryptophan as the PyrH *combi*CLEAs (cross-linked enzyme aggregates) are less efficient.



Tab. 1: *Determined conversion to halogenated tryptophan with halogenases RebH, Thal or TrpS.*

	L-Trp [mM]	Br-Indol [mM]	Conv. RP-HPLC Br-Trp[%]
RebH	1	-	100
Thal	1	-	100
TrpS	-	1	100

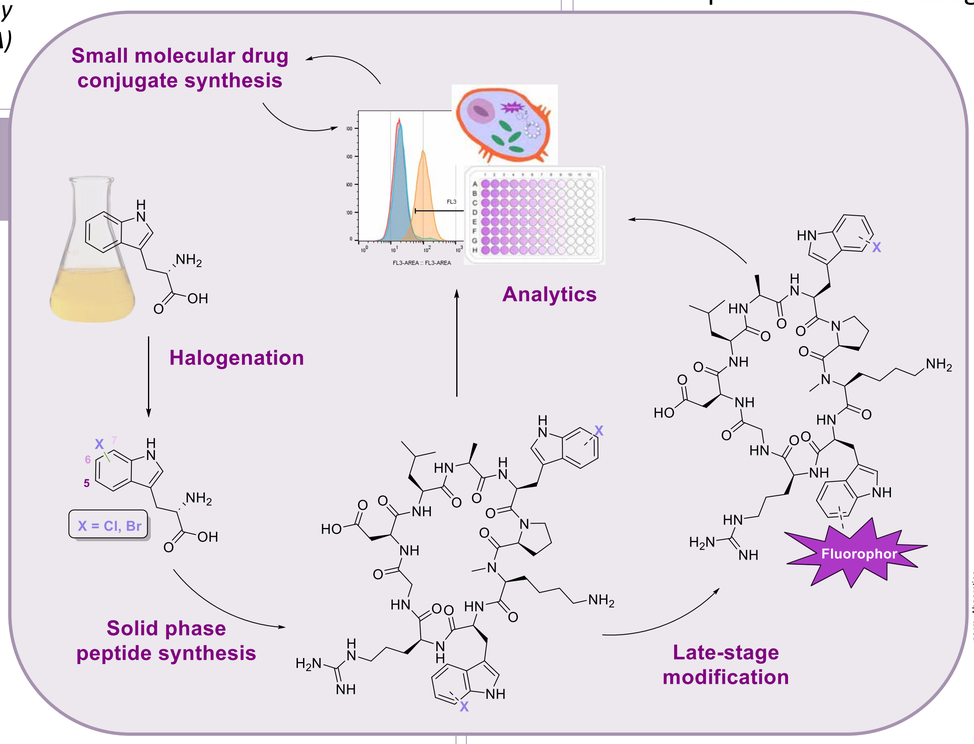
	L-Trp [mM]	Cl-Indol [mM]	Conv. RP-HPLC Cl-Trp[%]
RebH	1	-	100
Thal	1	-	100
TrpS (TmAzul)	-	1	100

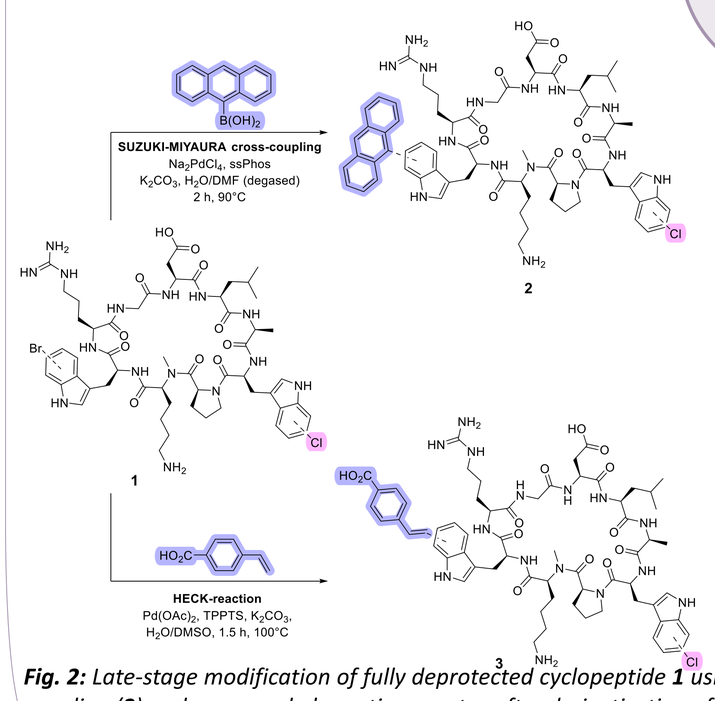
Fig. 1: *Biocatalytic synthesis of halogenated tryptophan by means of selective halogenation in the combiCLEAs process (**A**) or starting from halogenated indole and serine (**B**).*

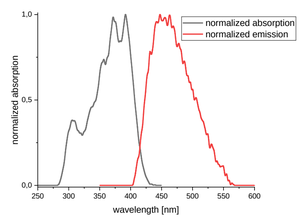
Late-Stage Modification

- Attachment of fluorescent residues to brominated indole function by selective C-C linkage such as by palladium-catalyzed HECK- or SUZUKI-MIYAURA cross-coupling[2,3]
- Investigation of the conversion via LC-ESI-MS
- Measurement of absorption spectra to prevent cell damage from overly energetic radiation in subsequent biological tests

➥ Identify fluorescent coupling reagents - extension of the π-system often leads to a desired redshift







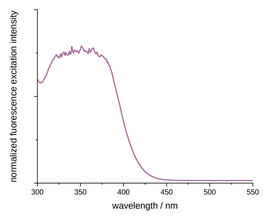


Fig. 2: *Late-stage modification of fully deprotected cyclopeptide **1** using Heck (**3**) or Suzuki-Miyaura cross-coupling (**2**) and measured absorption spectra after derivatization of one halogenated indole ring.*

Analysis of integrin-expression

- Screening of different cancer cell lines and culture conditions to increase and analyze the expression of individual integrins.
- Immunofluorescence imaging different $\alpha_V\beta_6$–integrins such α_V, $\alpha_V\beta_3$, $\alpha_V\beta_6$ or $\alpha_V\beta_8$ and investigation via flow cytometry or widefield deconvolution microscopy after fixation of the cells and labeling of the cell nucleus with DAPI

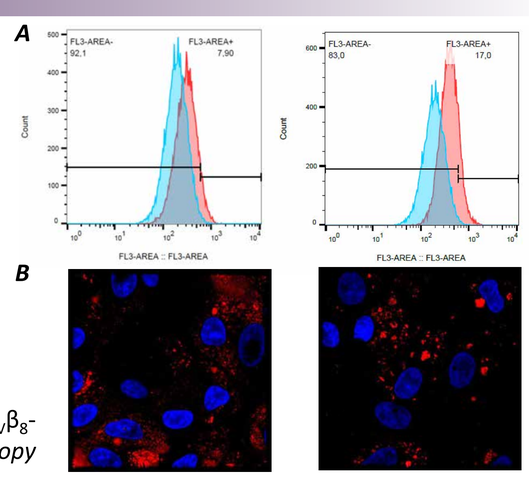


Fig. 3: A) *Flow cytometric measurement of* $\alpha_V\beta_6$- and $\alpha_V\beta_8$-expression in HN-cells. ***B)*** *Fluorescence spectroscopy*

Biological Evaluation of RGD-Peptides

- $\alpha_V\beta_6$ and $\alpha_V\beta_8$ integrin as proteins of interest, due to frequent expression in cells of viral and cancerogenic diseases
- Replacement of hydrophobic phenylalanine of the published nonapeptide c(DLAFp(NMe)KFRG) with halogenated tryptophan allows investigations due to its influence on bioactivity and increases affinity for $\alpha_V\beta_6$ and may improve selectivity toward $\alpha_V\beta_8$[4,5]
- Halogenated compounds are often essential for biological activity and, on the other hand, have a positive effect on metabolic stability, modulation of lipophilicity, hydrophobic interactions and pharmacological features
- N-Methylation on lysine increases affinity[4]

- **ELISA** (enzyme-linked immunosorbent assay) with isolated integrins to determine selective affinity towards individual integrins.

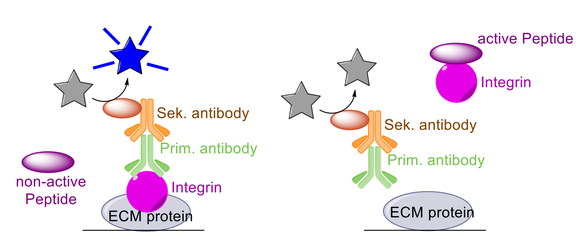


Fig.3: *Schematic representation of the ELISA assay for determination of IC_{50} values of peptides, left: EMC protein binds to the integrin.*

- Internalization investigation via **flow cytometry measurement** to ensure receptor-mediated cell uptake
- Compounds to be investigated must be fluorescently labeled in this respect

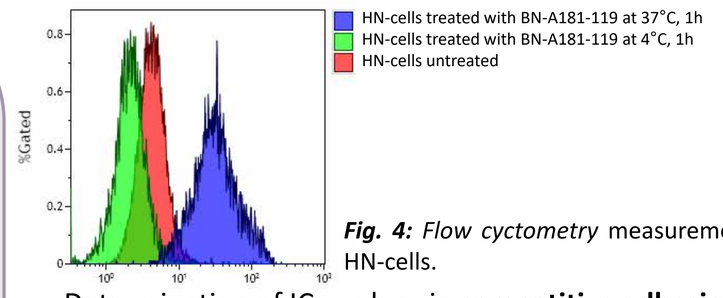


Fig. 4: *Flow cyctometry* measurement of treated HN-cells.

- Determination of IC_{50} values in **competitive adhesion assay**
- 96-well plate previously coated with the natural ligand TGF-ß1
- HN cells were seeded, which were cultivated on fibronectin
- Using Mn^{2+} (2mM) in buffer for integrin activation
- Cell lysis in DMSO, absorbance measurements at 570 nm

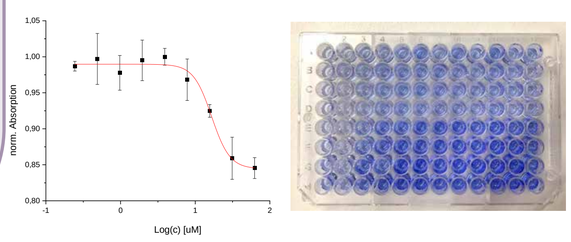


Fig. 5: *Flow cyctometry* measurement and evaluation using sigmoidal fit.

Peptide	IC_{50} $\alpha_v\beta_6$ [nM]	IC_{50} $\alpha_v\beta_8$ [nM]	IC_{50} $\alpha_v\beta_3$ [nM]	$\alpha_v\beta_8/\alpha_v\beta_6$ ratio	$\alpha_5\beta_1$ [nM]	$\alpha_{IIb}\beta_1$ [nM]	IC_{50} [nM] cell adhesion HN-cells
c(DLAFp(NMe)KFRG)	0.26[8]	23.6[8]	632[8]	91:1	72.9	$>10^4$	22.34±4.30
c(DLA(5-Br)Wp(NMe)KFRG)	0.083±0.03	14.0±0.8	242±67	169:1	0.82±0.85	$>10^4$	6.80±2.69
c(DLA(6-Br)Wp(NMe)KFRG)	0.036±0.01	39.7±3.4	247±132	1102:1	4.57±0.03	$>10^4$	4.24±1.24
c(DLA(7-Br)Wp(NMe)KFRG)	0.24±0.06	26.2±9.3	38±17	109:1	0.036±0.001	$>10^4$	3.75±0.77
c(DLA(6-(4-Vinyl-benzoic acid)Wp(NMe)KFRG	4.75±0.95	191.6±69.5		40:1			
c(DLA(5-(anthr-9-yl)Wp(NMe)KFRG)	18.11±0.38						
c(DLA(5-Cl)Wp(NMe)K(6-(anthr-9-yl)WRG	0.289± 0.02						
c(DLA(6-Cl)Wp(NMe)K(6-(anthr-9-yl)WRG	0.279± 0.05						

Conclusion

- Synthesis of high-affinity halogenated peptide ligands for selective targeting of $\alpha_V\beta_6$ integrin
- Halotryptophane-containing peptides exhibit higher affinity and selectivity than the known lead structure
- Late-stage modification by cross-coupling reactions like HECK- or SUZUKI-MIYAURA at the tryptophane of fully deprotected cyclopeptides containing different halogens
- Fluorogenic peptides allow Exzitation in the wavelength range above 350 nm and exhibit only a moderate reduction in IC_{50}value

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