https://doi.org/10.17952/37EPS.2024.P1210

Faculty of Chemistry - Organic and Bioorganic Chemistry - OCIII

UNIVERSITÄT BIELEFELD

Linkerology of Cryptophycin Drug Conjugates

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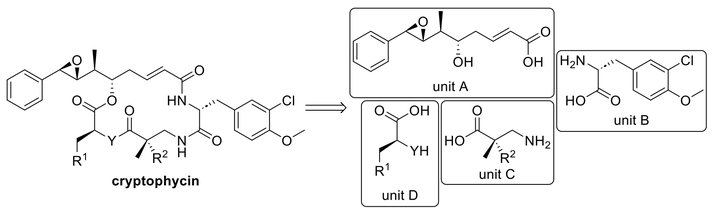
Introduction

- SMDCs consist of a cytotoxin connected to a small targeting ligand via a linker
- Payload is selectively delivered to the targeted cells through the homing device
- By utilizing cleavable linkers, the free payload is released at the target site
- Modified cryptophycin containing amino group was used as drug[1]
- Unit D is less exposed to the active site, making modifications in this unit promising[2]
- Cathepsin B cleavable linkers were attached using carbamate moiety
- Variation of peptide sequence in the linker for SAR study
- Folate receptor homing device was introduced via click reaction



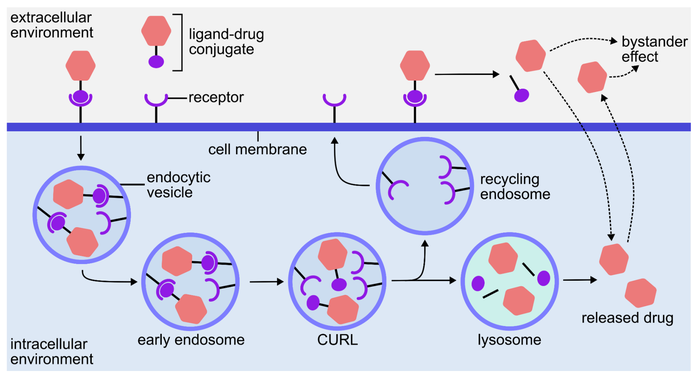


Figure 2: Pathways of drug conjugates that result in payload release, figure adapted from Low *et al.*[3] with modifications.

Homing Device

- Clickable folate ligand **8** adapted from vintafolide's homing device[4]
- Fluorophore conjugate **2** allows for FACS measurements
- >99% of KB-3-1 (FR+) cells were labelled, no labeling of HEK-293 (FR-) cells was detected
- Folic acid acts as inhibitor for labeling of KB-3-1 cells

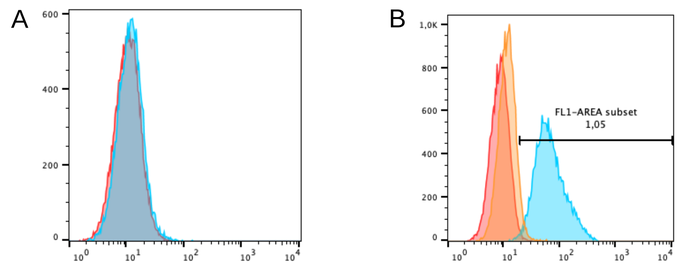
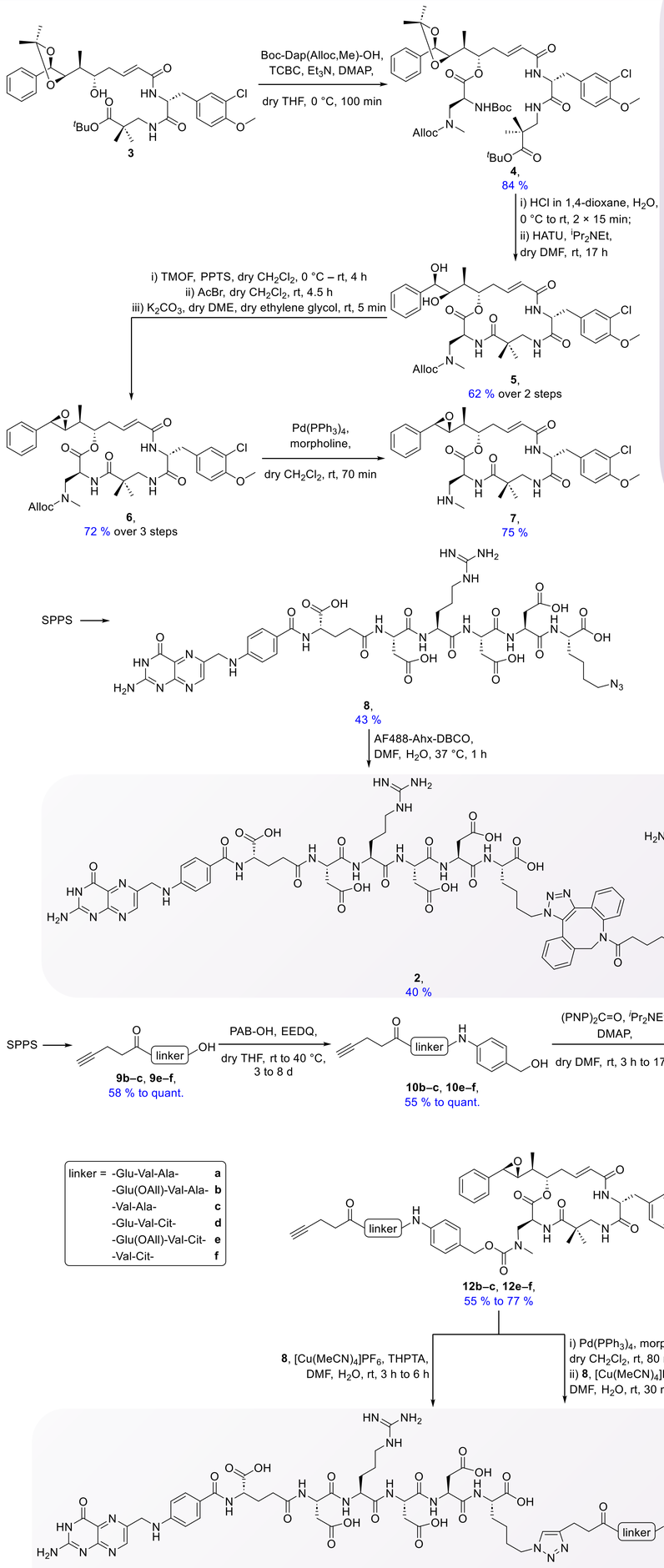


Figure 3: Flow cytometric analysis of conjugate **2** labeling FR- HEK297 (A) and FR+ KB-3-1 (B) cells. Unlabeled cells in red, cells incubated with **2** in blue, and cells incubated with **2** and 100 µm folic acid in orange.

Synthesis of Conjugates



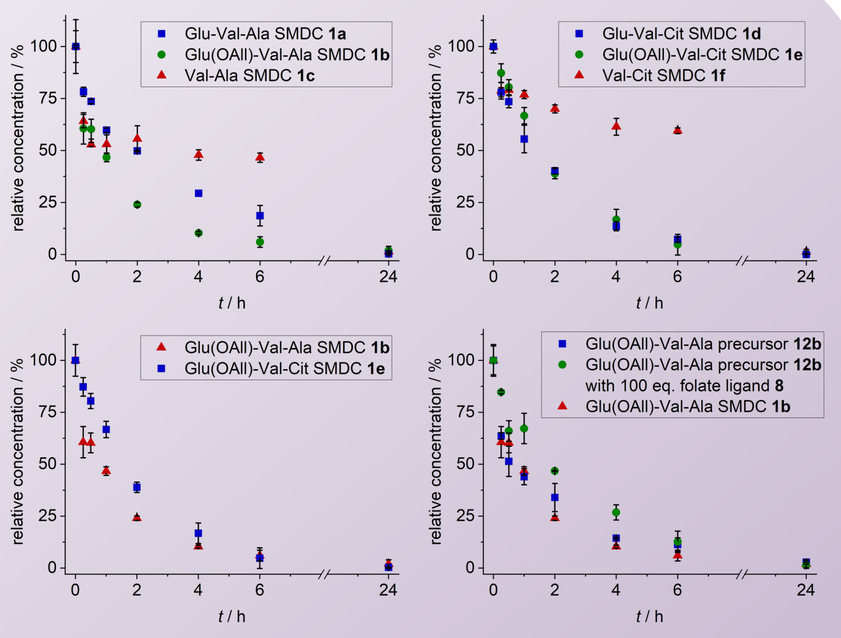
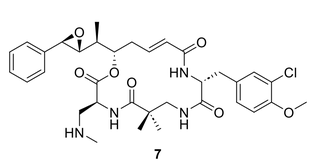
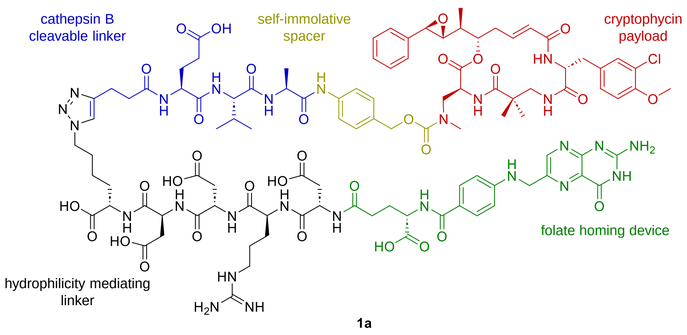


Figure 1: Cathepsin B cleavage assays of conjugates **1a–f**, conjugate precursor **12b**, and conjugate precursor **12b** with an excess of folate ligand **8**.

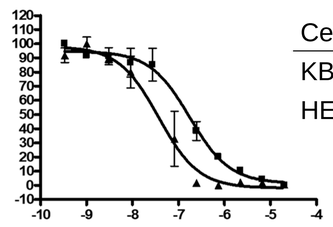
Cytotoxicity Data



7



1a



Cell Line	IC_{50} for **7**	IC_{50} for **1a**
KB-3-1 (FR+)	8.7 pM	155 nM
HEK 293 (FR-)	2.0 pM	32 nM

Targeting Index = 0.9

Figure 4: Dose-response curves of **1a** for cell viability of KB-3-1 (FR+) and HEK 293 (FR-) cells. Cell viability (%) plotted against logarithmic concentration (M^{-1}).

Cleavage Assays

- Substrate to enzyme ratio of 10:1, substrate concentration of 1 µM
- Glu(OAll)-Val-Ala SMDC **1b** is cleaved faster than Glu-Val-Ala SMDC **1a**
- Cit conjugates with glutamic acid (**1d**, **1e**) show similar cleavability
- Absence of glutamic acid (**1c**, **1f**) causes significant reduction in cleavability, in agreement with findings from Tsuchikama *et al.*[5]
- Only minor influence of the recognition sequence (Val-Ala or Val-Cit)
- Cleavability of precursor **12b** comparable to the cleavability of allyl protected SMDC **1b**
- Only minor influence of excess folate ligand **8** on the cleavability of precursor **12b**

Conclusion

- Construction of library of enzymatically cleavable SMDCs **1a–f** and fluorophore conjugate **2**
- Folate ligand **8** acts as excellent homing device for targeting the folate receptor
- SMDC **1a** exhibits low targeting index and cytotoxicity is reduced by more than four orders of magnitude compared to cryptophycin **7**, both presumably caused by slow cleavage of the linker
- SAR study of the linker and its cleavability demonstrates that neither folate ligand **8** nor the glutamic acid in P^3 position are causes for the slow cleavage
- Glu-Val-Ala conjugate **1a** strikes optimal balance between cleavage kinetics of the linker and hydrophilicity of the conjugate
- Further SAR studies needed to optimize payload release

For additional research regarding cryptophycins and corresponding conjugates, please visit Ninive Cati (P1-209), Dominic Seißenschmidt (P1-215), and Jona Voss (P1-212).

Further Info

References

[1] C. Dessin, T. Schachtsiek, N. G. Blanchard, N. Sewald, N. Janson, International Patent WO2022/175222, **2022**.
[2] A.-C. Abel, T. Mühlethaler, C. Dessin, T. Schachtsiek, B. Sammet, T. Sharpe, M. O. Steinmetz, N. Sewald, *J. Biol. Chem.* **2024**, *300*, 107363.
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[5] Y. Anami, C. M. Yamazaki, W. Xiong, X. Gui, N. Zhang, Z. An, K. Tsuchikama, *Nat. Commun.* **2018**, *9*, 2512.

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