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Faculty of Chemistry - Organic and Bioorganic Chemistry - OCIII

UNIVERSITÄT BIELEFELD

Click-to-Release-based Cryptophycin Drug Conjugates

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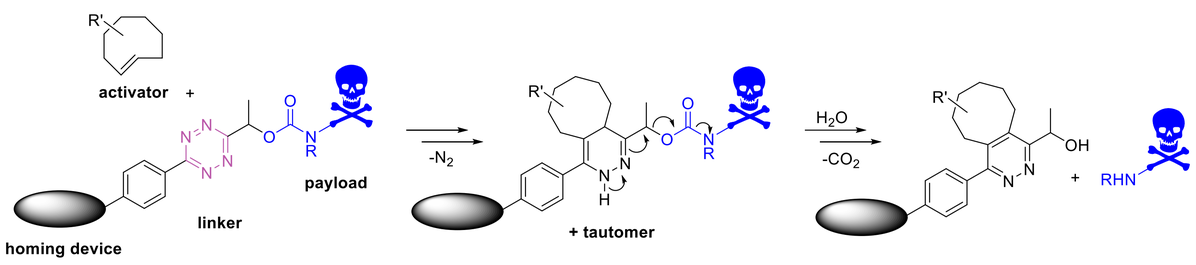
Click-to-Release

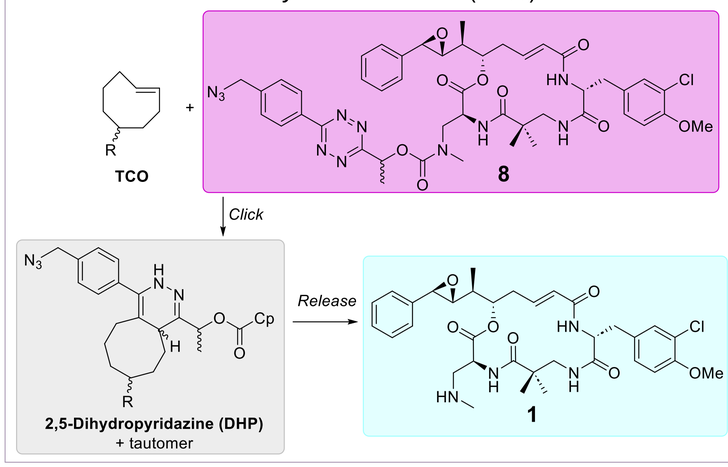
- Traceless bioconjugation method for release of tetrazine-carbamate-bound payloads[1]
- Release by deliberate administration of an activator (strained alkene, e.g. *trans*-cyclooctene (TCO))
- Established by Robillard *et al*. in 2013 with a proof of concept in 2016 (Doxorubicin release)[2]

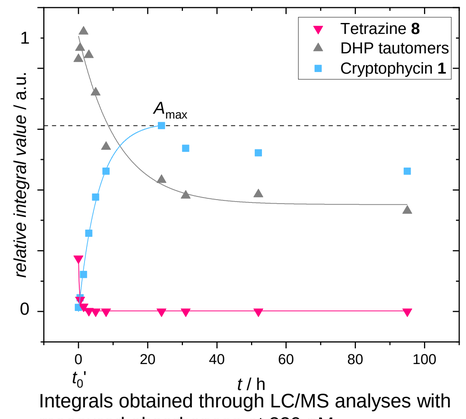


- Bioorthogonality through inverse electron-demand Diels-Alder reaction (iEDDA)
  - → High stability and selective formation of pyridazine-click-product
  - → Irreversibility through N_2 and concomitant CO_2 release
- Fast release kinetics (k_2 = 1 - 10^6 M^{-1})[3]
- Non-internalising antibodies as homing devices allow extracellular receptors as targets

in vitro Release

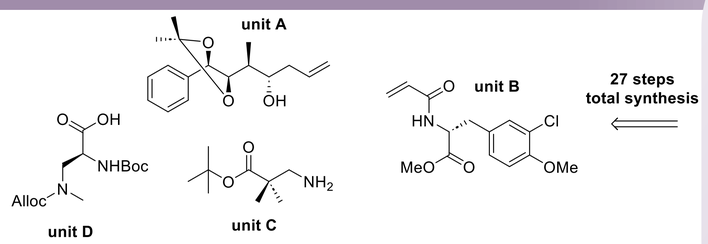
- In vitro study of release upon TCO addition (pH = 7.4, NH_4OAc buffer / MeCN, c_0 = 0.15 mM)
- Maximum "release yield" after 24 h (64%)



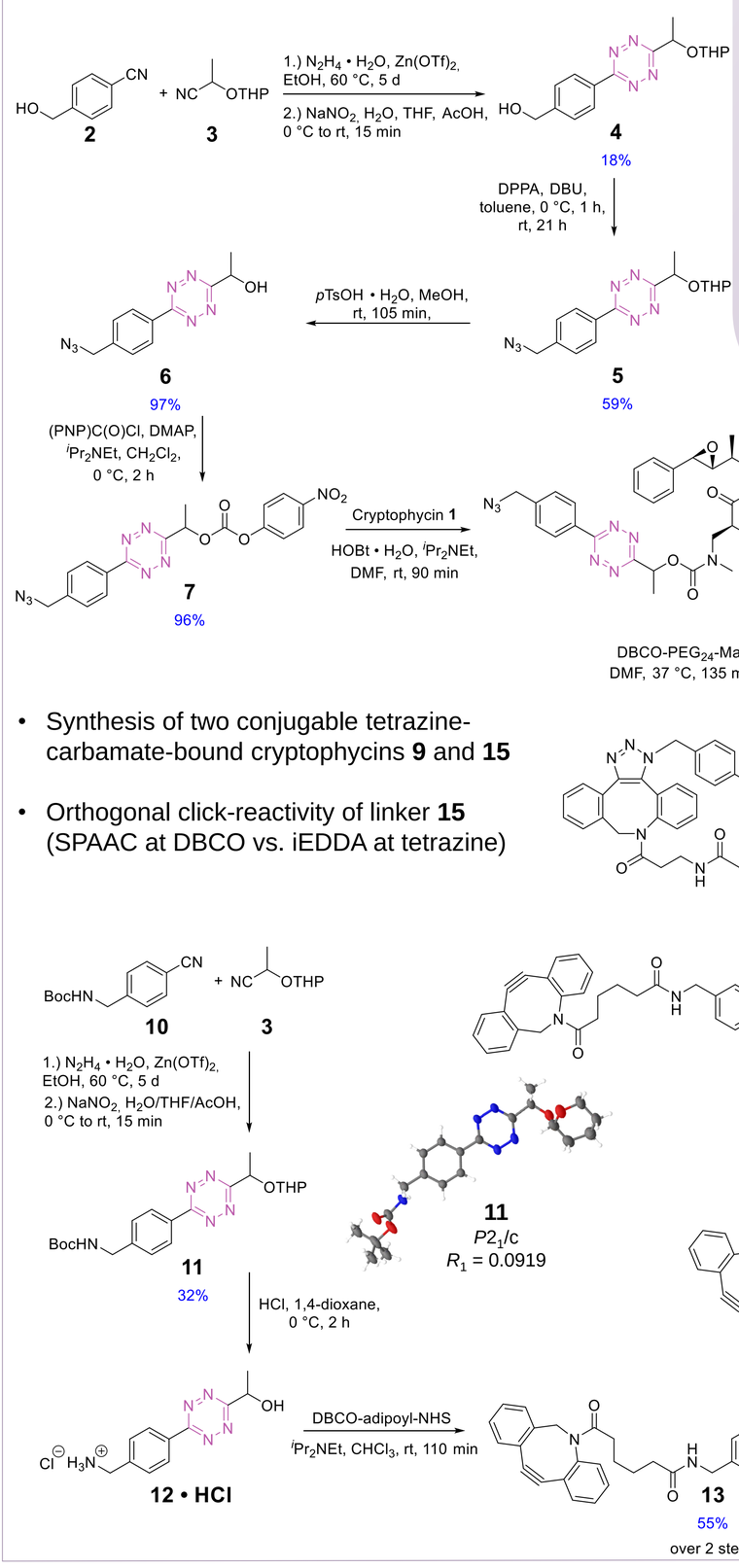


Integrals obtained through LC/MS analyses with measured absorbances at 220 nM.

Synthesis of Cryptophycin-Linkers

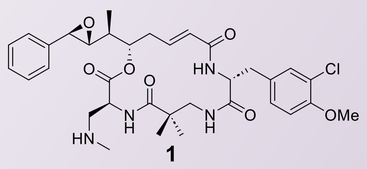


- Highly cytotoxic, usually in the pM range, even against multi-drug-resistant cell-lines[4]
- Modifications in unit D superior to retain high cytotoxicity[5]

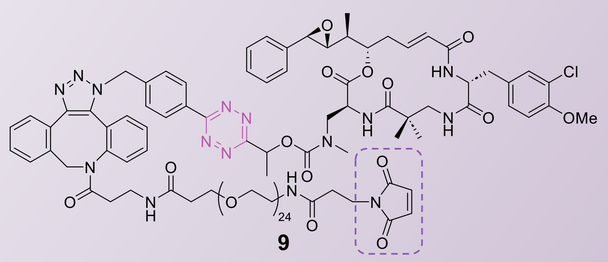


- Synthesis of two conjugable tetrazine-carbamate-bound cryptophycins **9** and **15**
- Orthogonal click-reactivity of linker **15** (SPAAC at DBCO vs. iEDDA at tetrazine)

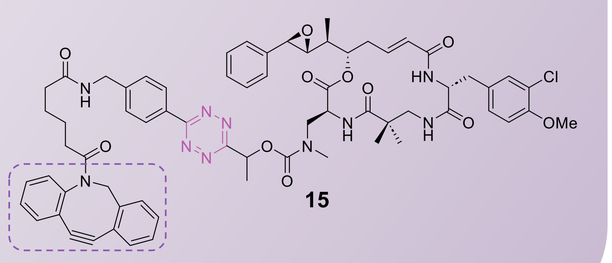
Payload: Cryptophycin-unit D [Dap(Me)]



IC_{50} [pM]	
KB-3-1	8.71
KB-V1	21•10^3 (MDR+)
A-549	21.7
A-431	160

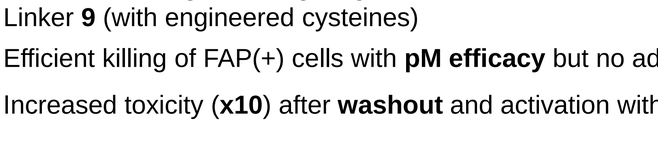


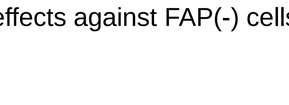
- Cysteine-addressable by Michael addition



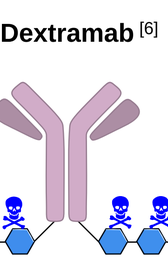
- Azide-addressable by SPAAC

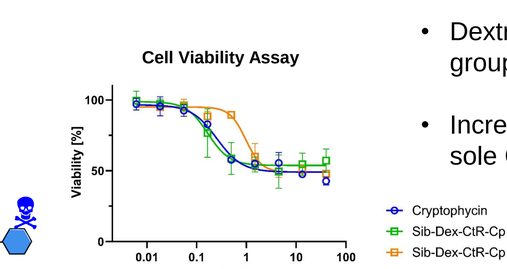
Antibody-Drug Conjugates

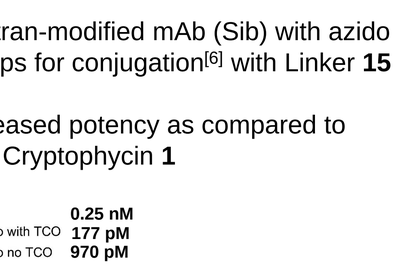




Trastuzumab-based ADCs

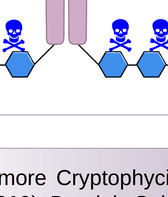


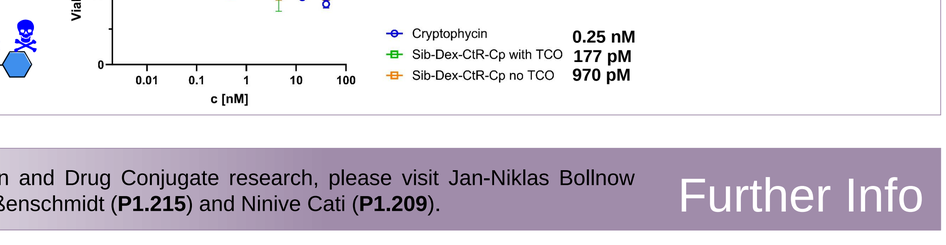




- Anti-Her2 mAb conjugated by Michael addition of Cryptophycin-Linker **9** to native cysteines
- Efficient cell-killing of medium and high Her2-expressing cell lines with **pM IC_{50}** values
- TCO activation only efficient for RT112 cells: proteolysis competing with internalization?

Sibrotuzumab-based ADCs





- Non-internalising mAb targeting cancer-associated fibroblasts (CAFs), conjugated to Cryptophycin-Linker **9** (with engineered cysteines)
- Efficient killing of FAP(+) cells with **pM efficacy** but no adverse effects against FAP(-) cells
- Increased toxicity (**x10**) after **washout** and activation with **TCO**

Dextramab [6]

- Dextran-modified mAb (Sib) with azido groups for conjugation[6] with Linker **15**
- Increased potency as compared to sole Cryptophycin **1**

Further Info

For more Cryptophycin and Drug Conjugate research, please visit Jan-Niklas Bollnow (**P1.210**), Dominic Seißenschmidt (**P1.215**) and Ninive Cati (**P1.209**).

Reference

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