Development of an amanitin-based antibody-drug conjugate via synthesis, derivatization, and functionalization of (2S,3R,4R)-dihydroxy-isoleucine derivatives

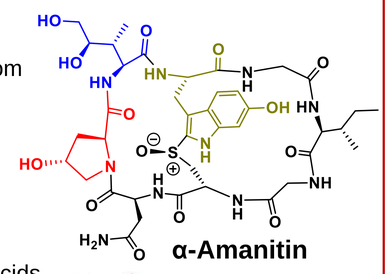
S. D. Chandra; S. Gunasekera; W. Nguyen; J. Froelich; A. Wong and **D. M. Perrin***

Department of Chemistry, University of British Columbia, 2036 Main Mall, Vancouver, BC, V6T 1Z1, Canada

Introduction

α-Amanitin

- Isolated from death cap mushroom ***Amanita phalloides***[1]
- Deadliest member of *Amatoxins*: LD_{50} ~ **0.1 mg/kg**[2]
- **Bicyclic, octapeptide** structure
- Oxidized **non-canonical** amino acids



- Strong, specific and allosteric inhibitor of **RNAP II** (K_i ~ 1-10 nM)
- Binds to the **bridge helix** of RNAP II (Fig 1)[3]
- Rate of **translocation** is significantly reduced

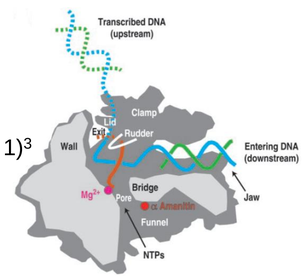


Fig 1: Cutaway view of pol II-transcribing complex showing the location of α-amanitin (red dot)[3]

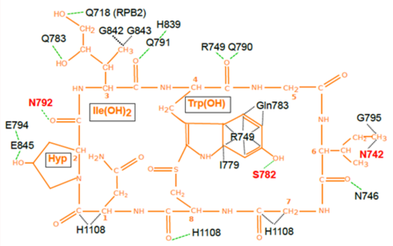


Fig 2: Schematic of the interactions. Green dashed lines Hydrogen-bonds[4]

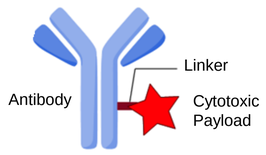


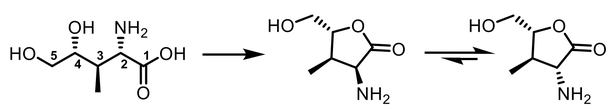
Fig 3: General ADC and its component

Application

- It can be used as **cytotoxic payload** in ADCs (Fig 3)
- It **augments** specificity, selectivity, potency and drug tolerability
- Most **FDA-approved** cytotoxic payloads need **active cell proliferation** to be **therapeutically effective**
- Amanitin exhibits its cytotoxicity irrespective of the **proliferation status** of tumor cells, hence eliminating **cancer relapse**

Challenges & objectives

- Biosynthetic fermentation **yield is low,** extraction is **expensive**
- **DHIle** presents **enormous synthetic** challenges



densely functionalized
defiant 1,2-anti/1,3-syn configuration
prone to lactonization

sterically congested
prone to epimerisation

Synthesis of number of DHIle derivatives

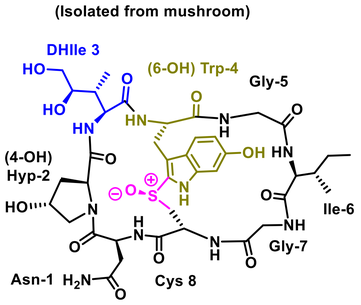
- **SAR** study of **DHIle**
- New **bioconjugation** handle

- Past amanitin based ADCs exhibit **intrinsic liver toxicity**
- Poor linker **stability** increases off **target toxicity**

Incorporation into amanitin core resulting in number of analogs

- Analog with **reduced** liver toxicity
- **Stable** conjugation handle

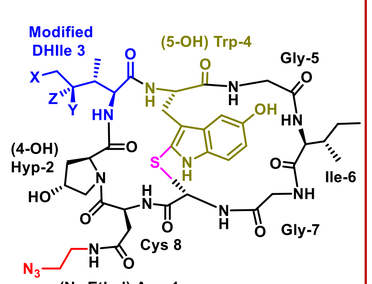
α-Amanitin
(Isolated from mushroom)



Non canonical amino acids ((4-OH) Hyp), (6-OH) Trp, DHIle

Key synthetic challenges:
(2*S*,3*R*,4*R*)-DHIle
(R)-sulfoxide
6-hydroxy-tryptathionine

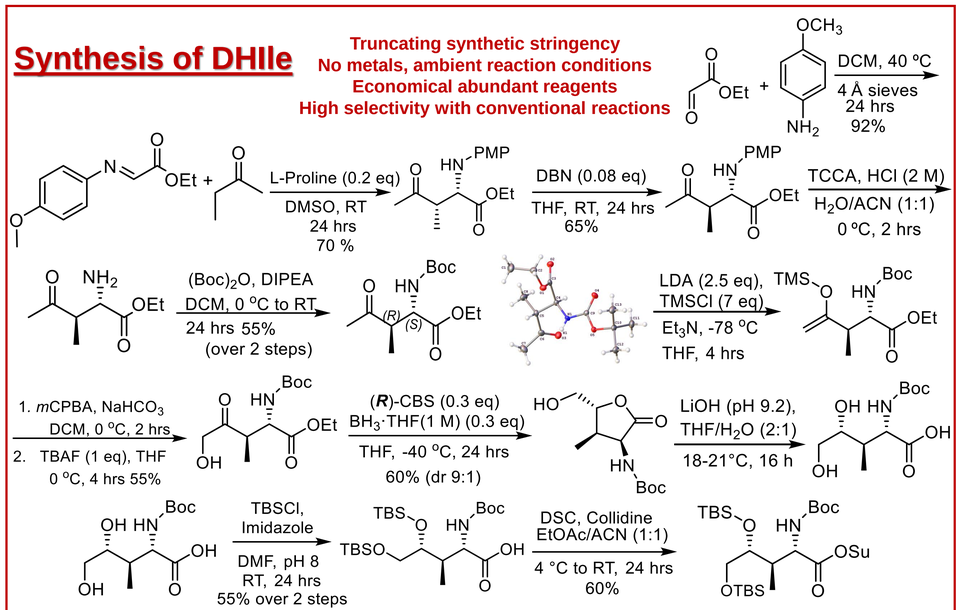
This Work
Synthetically trackable version
(derivatization of DHIle residue)



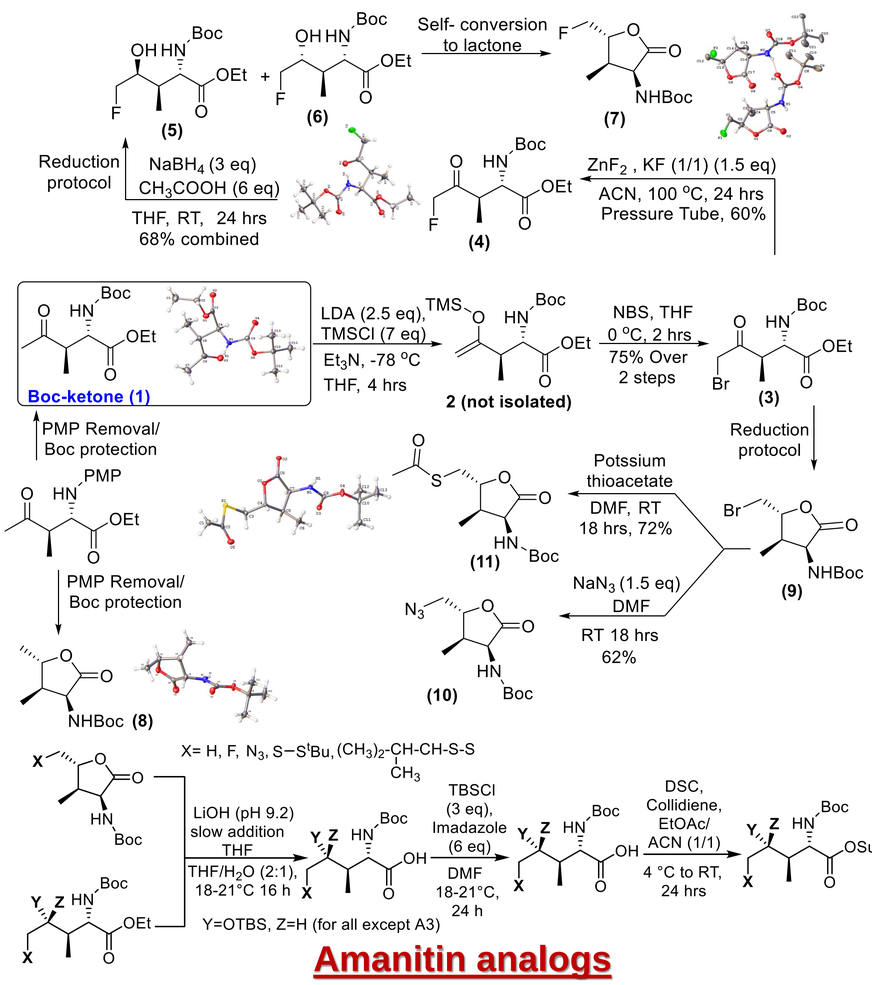
Functionalization of DHIle residue
N_3-ethyl Asn as bioconjugation handle
Commercially avaliable 5-OH-Trp
Synthetically avalibale thioether staple

Synthesis of DHIle

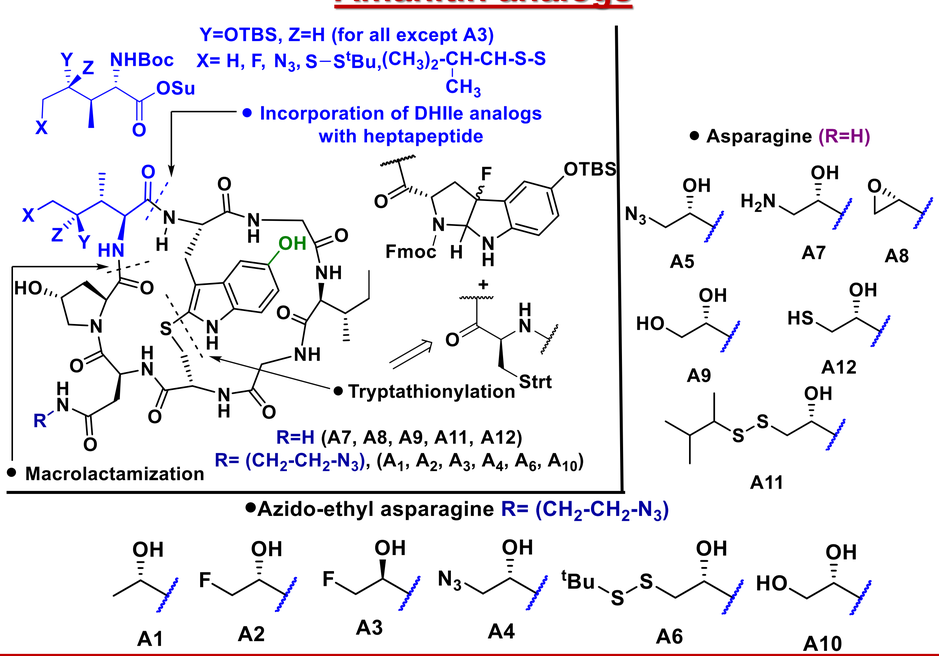
Truncating synthetic stringency
No metals, ambient reaction conditions
Economical abundant reagents
High selectivity with conventional reactions



Accessing new dihydroxy-isoleucine analogs



Amanitin analogs



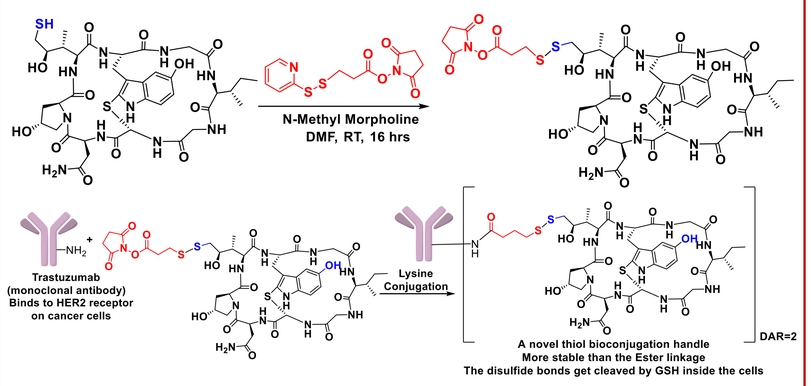
Cell viability assay

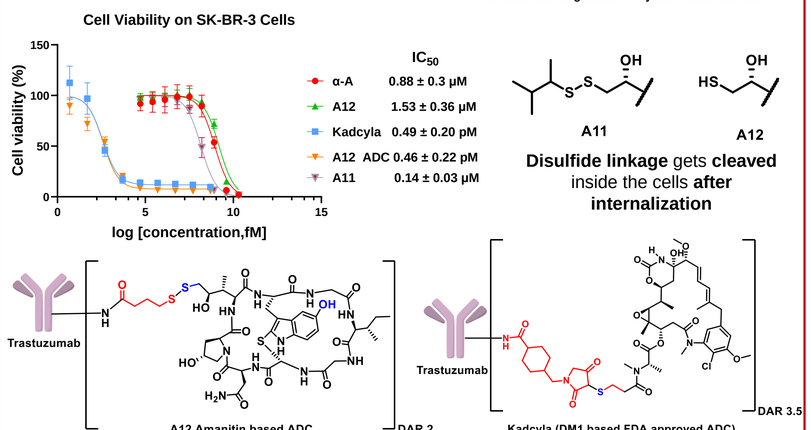
Analog	IC_{50}(μM) MTT Assay (Cell Line) HEK293 (Rel)	CHO (Rel)	HeLa (Rel)	HepG2 (Rel)	Pol II Assay IC_{50}(nRel)
α-A	0.57 (1)	0.34 (1)	2.54 (1)	2.30 (1)	7.9 (1)
A10	0.26 (0.93)	1.80 (3.33)	32.66 (12.56)	24.9 (21.65)	85 (10.6)
A1	1.94 (3.4)	6.88 (20.23)	35.12 (13.82)	37.45 (16.28)	166 (20.75)
A2	0.65 (1.14)	2.0 (5.88)	11.41 (4.49)	28.71 (12.48)	33 (4)
A3	>45 (N/A)	>45 (N/A)	>45 (N/A)	>45 (N/A)	897
A4	16.06 (28.17)	>45 (N/A)	>45 (N/A)	>45 (N/A)	218 (19.87)
A6	0.813 (2.9)	6.58 (12.16)	36.48 (14.03)	>45 (N/A)	TBD
A7	5.62 (11.24)	7.76 (35.27)	26.50 (10.43)	40.82 (14.52)	TBD
A8	5.75 (11.5)	31.92 (138)	25.52 (9.9)	14.52 (5.16)	TBD
A9	0.45 (0.78)	0.517 (2.48)	>12.5 (N/A)	>12.5 (N/A)	TBD
A11	0.90 (1.65)	1.80 (10)	13.62 (9.5)	10.9 (7.6)	TBD
A12	0.46 (0.84)	1.77 (12.6)	6.28 (3.7)	6.67 (5)	TBD

Translation into ADC

Stable carbamate linker
(Cleaves by Cathespin B)

Stable disulfide linker (Cleaves by disulfide exchange)





Conclusions & future directions

- **Facile, scalable** and **efficient** synthetic access of DHIle
- **Derivatization** and **functionalization** of DHIle residue
- First **SAR** study on DHIle and **amanitin analogs** are **bifunctional**
- **Selective** toxicity on **HEK293** and **CHO** Cells with reduced liver toxicity
- **Efficient**, **novel bioconjugation** handles and translation into **ADC**
- ADC exhibiting **equipotent activity** compared to **FDA approved** ADC

References

(1) T. J. Lindell *et al*, *Science* **1970**, *170*, 447-449; **(2)** G. Zanotti *et al*, *Res. Int. J. Peptide Protein* **1992**, *40*, 551-558; **(3)** R. D. Kornberg *et al*, *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 1218-1222; **(4)** P. Cramer *et al*, *J. Biol. Chem.* **2018,** *19,* 7189-7194.