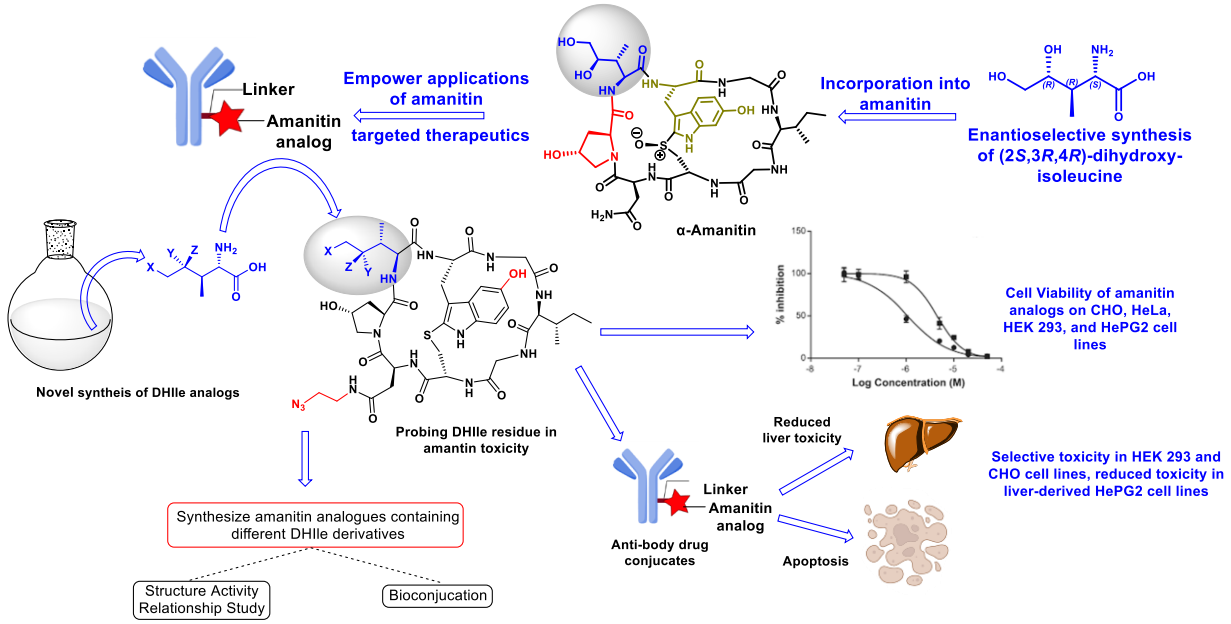
Development of an amanitin-based antibody-drug conjugate via synthesis, derivatization, and functionalization of (2S,3R,4R)-dihydroxy-isoleucine derivatives

S. D. Chandra; S. Gunasekera; A. Wong; W. Nguyen; J. Froelich; and D. M. Perrin[†]

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

α-Amanitin, a highly toxic bicyclic octapeptide extracted from death-cap mushroom (Amanita phalloides), is one of the deadliest natural compounds known, with an LD_{50} of 50–100 μg/kg.[1] It is a potent inhibitor of RNA polymerase II (K_i 1−10 nM),[2] an enzyme critical for cellular function and survival, thereby affecting both dividing and quiescent cells. Due to its stability, potency, and unique mechanism, it shows promise as a payload for antibody-drug conjugates (ADCs) in cancer therapy.[3] However, current extraction methods yield low amounts, underscoring the need for a synthetic production route.

The synthesis of di-hydroxy isoleucine (DHIle), a vital component, is particularly challenging. We successfully achieved a gram-scale synthesis of DHIle, streamlining the overall process and generating intermediates that were synthetically modified to produce 12 novel amanitin analogs. These analogs, compatible with solid-phase peptide synthesis, were utilized in SAR studies, revealing compounds with similar potency and selective toxicity toward HEK-293 cells. This work also introduced new bioconjugation handles for enhancing ADC applications with increased cytotoxicity and selectivity over liver-derived HepG2 cells, improving amanitin's therapeutic index for cancer treatment. This work led to the development of new ADCs with efficacy comparable to those currently available on the market.



References:

1. Wieland, T. The Toxic Peptides from Amanita Mushrooms. *Int. J. Pept. Protein Res.* **1983**, 22, 257.

2. Lindell, T. J.; Weinberg, F.; Morris, P. W.; Roeder, R. G.; Rutter, W. J. Specific Inhibition of Nuclear RNA Polymerase II by Alpha amanitin. *Science* **1970**, 170, 447.

3. Pahl, A.; Lutz, C.; Hechler, T. Amanitins and their development as a payload for antibody-drug conjugates. *Drug Discovery Today: Technol.* **2018**, 30, 85.