The bicyclic octapeptide α-amanitin is one of the deadliest toxins found in nature and is of interest as a therapeutic agent. However, the high hepatotoxicity of amanitin must be reduced, such as by increasing drug selectivity using prodrug strategies. Hypoxia is a key feature of cancerous tumours as their rapid proliferation outstrips the oxygen provided by surrounding vasculature. Nitroreductase enzymes catalyze the reduction of aromatic nitro moieties to their respective amines and are upregulated in hypoxic cells. We hypothesize that a nitrated amanitin analogue will be selectively activated by nitroreductases in hypoxic cancer cells.

We describe a late-stage nitration of the amanitin heptapeptide monocycle precursor towards the synthesis of nitrated amanitin prodrugs. Using nitrosaccharin as an alternative nitrating reagent, the nitration proceeded selectively, under mild conditions, and with tolerance to solid-phase peptide synthesis protecting groups. The bioreduction susceptibility of the heptapeptide was assessed through incubation with nitroreductase and easily detected via the distinctive absorbance profile of the nitrated tryptathionine. Cell viability assays will determine the toxicity of the nitrated amanitins under normoxic and hypoxic conditions.