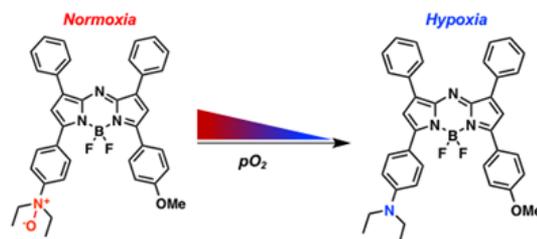


## Applications of Photoacoustic Imaging for Hypoxia Detection and Image-Guided Drug Delivery

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A key feature of solid tumors is their capacity for rapid proliferation. When tumor growth occurs at a faster rate than the formation of new tumor vasculature, oxygen diffusion into tumor tissue is significantly limited. The resulting state of reduced intratumoral oxygen levels is known as hypoxia. Hypoxia is a prevalent characteristic of many different tumor types and has been associated with aggressive phenotypes such as increased metastatic aptitude and resistance to chemotherapy. Hypoxia has also been exploited in prodrug strategies in which bioreductive chemotherapeutic agents are selectively activated in the absence of oxygen. The study of hypoxia and its effect on tumor biology and treatment prognosis is a critical research area that relies on the ability to effectively detect oxygen levels in tumor tissue. However, current techniques for hypoxia detection often involve invasive procedures or rely on indirect detection via hypoxia-induced molecular events. As an alternative to current strategies, we present the development of a photoacoustic imaging agent that enables non-invasive, real-time ratiometric imaging of hypoxia in cancer cells and tumor tissue. Furthermore, we demonstrate the application of photoacoustic imaging for the development of hypoxia-activated image guided drug delivery for cancer therapy.



## In Vitro Reconstitution and Substrate Specificity of the Biosynthesis of the Core Scaffold of the Thiopeptide Thiomuracin

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Thiopeptides are potent antibiotics that inhibit protein synthesis. They are made by a remarkable post-translational modification process that transforms a linear ribosomal peptide into a polycyclic structure. Herein, we report the in vitro biosynthesis of the core scaffold of the thiopeptide thiomuracin, the first of such example for this class of peptides. In vitro reconstitution of this well-orchestrated set of enzymatic reactions has allowed further determination of their underlying molecular details. We show that cyclodehydration precedes dehydration, and that dehydration is catalyzed by two proteins in a tRNA<sup>Glu</sup>-dependent manner to generate four alkenes. Then two of these alkenes undergo a formal [4+2] cycloaddition to form a tri-thiazole-substituted pyridine macrocycle. We show the order of thiazole and alkene formation, reveal the minimal structural changes necessary to render TbtA a substrate for dehydration, the parts of the TbtA peptide that are recognized by the various enzymes, and identify important residues of the enzyme TbtD for catalysis of a formal [4+2] cycloaddition process.