Indole is a widespread heterocycle found in many natural products. For instance, indigo dyes derived from indole have been applied in textile dyeing for thousands of years due to their outstanding spectral properties. Indigo (1) developed to a bulk chemical in the last century whereas its C6-bromination has never entered an industrial scale production.

Today biocatalysis offers a versatile methodology to address selectivity issues, e.g., arising from similarly reactive C–H moieties. Recent advancements on enzyme discovery, engineering as well as tremendous efforts on process development open up elaborate transformations that can be carried out under mild conditions, often with excellent selectivities. Immense progress has been achieved in enzyme-catalyzed C–H functionalization. Especially oxyfunctionalization is a paramount approach to activate C–H bonds by using biocatalysts that are capable of utilizing molecular oxygen. Manifold biocatalytic approaches in this rapidly evolving field, especially on the use of P450 enzymes, were extensively reviewed, giving a wide overview on the current state of the art. The first fermentative synthesis of indigo was reported by Ensley and later by Lee et al. using a dioxygenase for indole hydroxylation. Tryptophanase that originated from endogenous tryptophan catabolism was exploited to obtain indole. Heme-dependent monoxygenases (MOs) were later evolved towards C3-hydroxylation of indole.

Flitsch et al. used formation of indigo-derived pigments for detecting activity of MO mutants. Biocatalytic functionalization of unprotected indole was achieved using engineered myoglobin variants which catalyze non-native carbene transfer in whole cells. Besides using heme-dependent enzymes, also flavin-dependent MOs play a key role in oxyfunctionalization. Accordingly a flavin monoxygenase from Methylophaga sp. (mFMO) was established for the biotechnological production of indigo and indirubin. Nevertheless, the synthesis of valuable halogenated indigos has remained on analytical or small preparative scale, particularly due to the low efficiency of halogenases as a severe bottleneck. In more recent studies, Tischler and co-authors reported on the conversion of haloindoles using a small array of styrene MOs. One-pot synthesis of indigo either in bacteria or plant as the hosts was recently achieved: By introducing a tryptophan halogenase into the host strain along with a hydroxyase, production of indigos from tryptophan was feasible, omitting the need for costly substituted indole substrates through exploiting the cellular metabolism. However, product titers remained low and the isolation of the pigment from the cultivation broth can become a tedious procedure. Moreover, structural modifications that can be, for example by using biocatalysts.

**Abstract:** Indigoids represent natural product-based compounds applicable as organic semiconductors and photosensitive materials. Yet modified indigo derivatives are difficult to access by chemical synthesis. A biocatalytic approach applying several consecutive selective C–H functionalizations was developed that selectively provides access to various indigoids: Enzymatic halogenation of L-tryptophan followed by indole generation with tryptophanase yields 5-, 6- and 7-bromoindoles. Subsequent hydroxylation using a flavin monoxygenase furnishes dibromoindigo that is derivatized by acylation. This four-step one-pot cascade gives dibromoindigo in good isolated yields. Moreover, the halogen substituent allows for late-stage diversification by cross-coupling directly performed in the crude mixture, thus enabling synthesis of a small set of 6,6'-diarylindigo derivatives. This chemoenzymatic approach provides a modular platform towards novel indigoids with attractive spectral properties.

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