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Faculty of Chemistry - Organic and Bioorganic Chemistry - OCIII

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Biocatalytic Synthesis of Dihalogenated Tryptophan Derivatives

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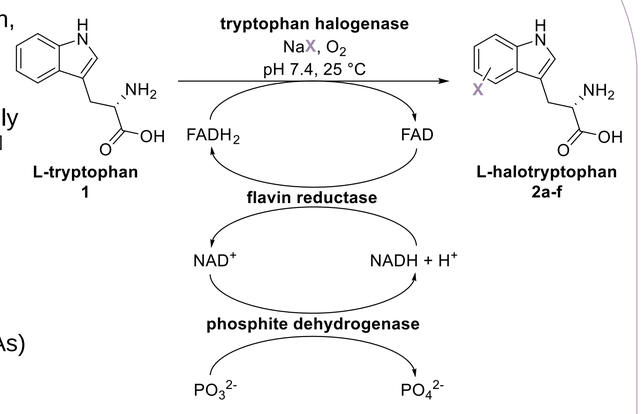
Flavin-Dependent Halogenases

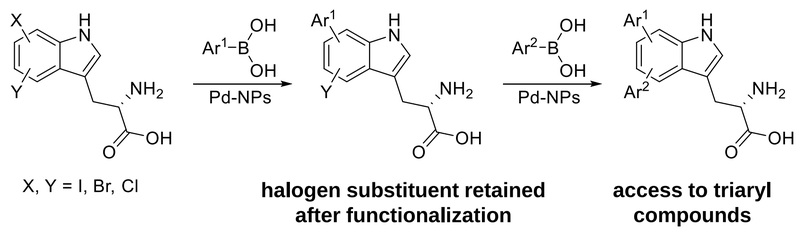
- **Flavin-dependent halogenases** (FDHs) offer an attractive strategy for the chlorination, bromination, and iodination of electron-rich arenes.
- They exhibit **remarkable regioselectivity** and simultaneously **obviate harsh conditions** typically found in conventional halogenation reactions.[1,2,3]
- Trp-FDHs can address even **electronically disfavored positions** on the indole moiety of tryptophan (**1**), otherwise not accessible by conventional halogenation.[1,2]
- **Cross-linking** of Trp-FDHs in tandem with an **enzymatic regeneration system** (→ combiCLEAs) allows for preparative synthesis of halotryptophans.[4]



- **Bromo substituent** can be functionalized via e.g. transition metal catalyzed cross-coupling reactions, giving access to a plethora of fine chemicals.[5,6]

Dihalogenation by Combining Trp-FDHs

- Cross-coupling of halotryptophans **removes the halogen substituent**, which could otherwise be useful in modulating the bioactivity or stability of target molecules.

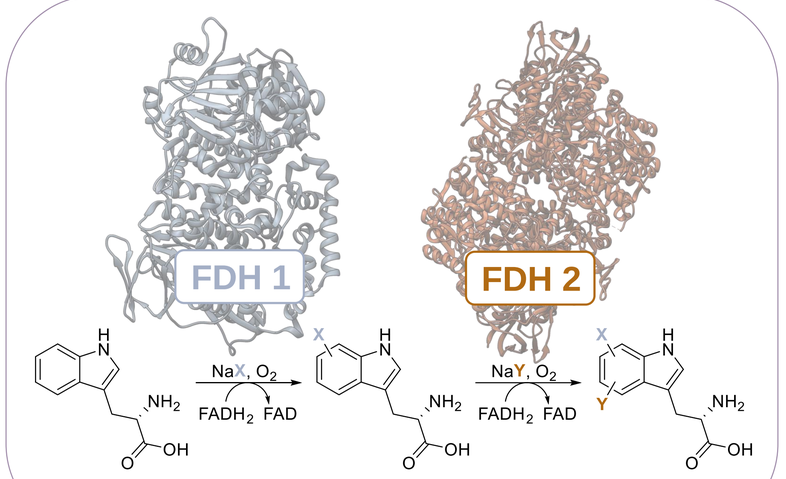


X, Y = I, Br, Cl

halogen substituent retained after functionalization

access to triaryl compounds

- Four representative Trp-FDHs were tested to identify viable combinations. The selection included Trp-5-halogenase **PyrH**, Trp-6-halogenase **Thal**, Trp-7-halogenase **RebH**, and Trp-5,7-dihalogenase **AetF** (**WT** and **S523A**).[7]
- Semi-preparative assays were conducted using the products of the respective other Trp-FDHs. **Specific activities** and **regioselectivities** were determined using HPLC(-MS) and NMR spectroscopy.



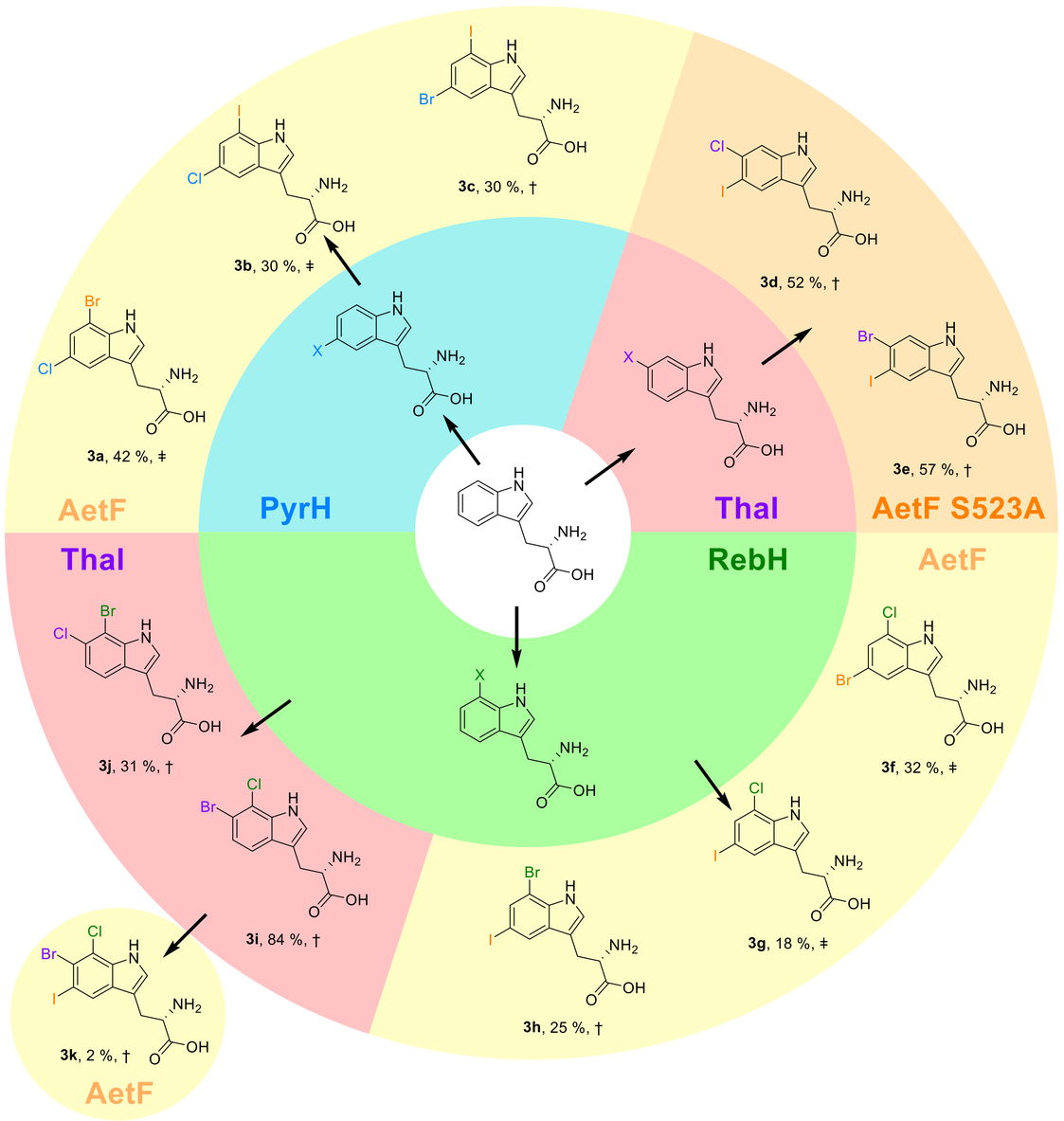
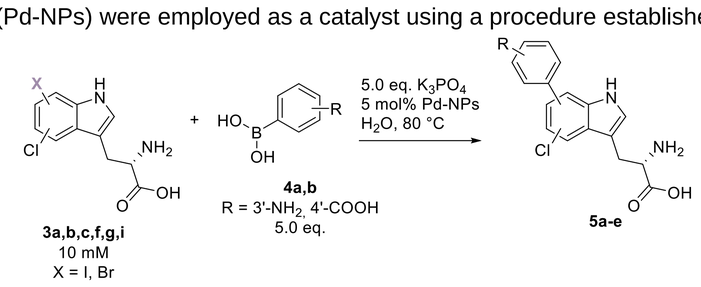


Figure 1: Obtained di- and trihalogenated tryptophan derivatives **3a-k**. Every colored section is marked with the halogenase used for the last halogenation step. † purification (RP-H/MPLC) was performed prior to the last halogenation step; ‡ no purification was performed between the last two halogenation steps. X = Cl, Br.

Orthogonal Cross-Coupling using SMC

- **Orthogonal addressability** of the halides in the dihalotryptophan derivatives **3a**, **3b**, **3f**, **3g** and **3i** was tested using **Suzuki-Miyaura cross-coupling** (SMC).
- **Pd-nanoparticles** (Pd-NPs) were employed as a catalyst using a procedure established by Dachwitz *et al.*[6]



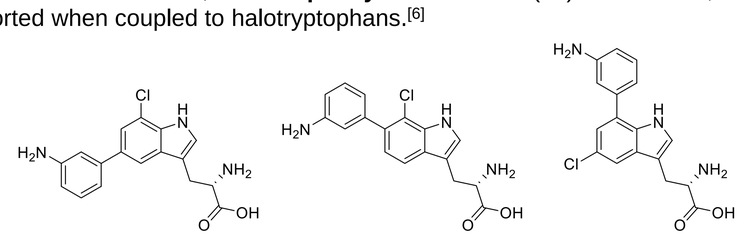
3a,b,c,f,g,i
10 mM
X = I, Br

4a,b
R = 3'-NH_2, 4'-COOH
5.0 eq.

5a-e

- To address the less reactive bromine substituent, **3-aminophenylboronic acid** (**4a**) was chosen, as good reactivity was previously reported when coupled to halotryptophans.[6]

X = Br, R = 3'-NH_2
→ Bromo-substituent addressable, orthogonality not always ideal



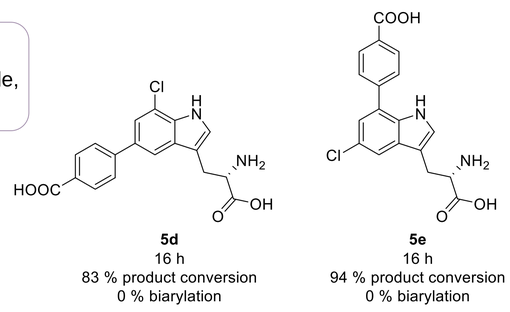
5a
23 h
65 % product conversion
35 % biarylation

5b
23 h
100 % product conversion
0 % biarylation

5c
23 h
69 % product conversion
5 % biarylation

- The more reactive **iodine substituent** promised better orthogonal addressability.
- **4-Carboxyphenylboronic acid** (**4b**) with more sluggish reactivity was chosen to further avoid side product formation.[6]

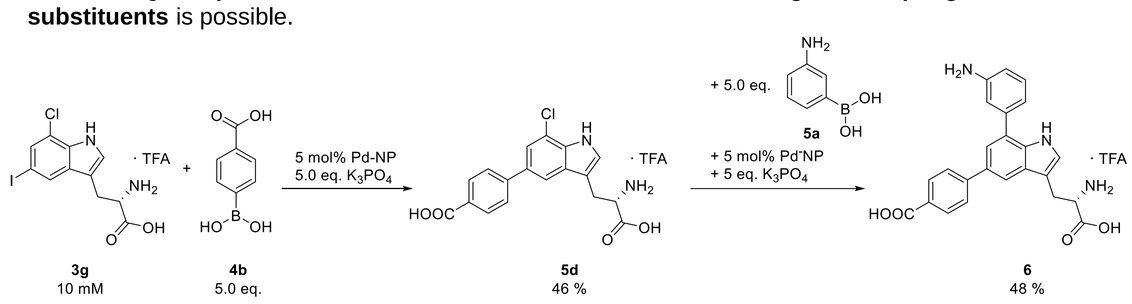
X = I, R = 4'-COOH
→ Iodo-substituent addressable, orthogonality given



5d
16 h
83 % product conversion
0 % biarylation

5e
16 h
94 % product conversion
0 % biarylation

- Based on the **biarylation** observed when coupling bromo-chloro-tryptophans (**3a,b,c**), **7-Cl-substituent** might be **addressable** using Pd-NPs and a highly reactive boronic acid.
- Good orthogonality of iodo- and chloro-substituents indicated, that **orthogonal coupling of both substituents** is possible.



3g
10 mM

4b
5.0 eq.

5d
46 %

6
48 %

both halogen substituents orthogonally addressable in SMC

Conclusion

- A selection of Trp-FDHs was successfully combined to iteratively introduce **orthogonal halogenation patterns** in L-tryptophan (**1**).
- Most halogenases retained their native regioselectivity. Due to the *ortho*-directing effect of the halogen substituents, **PyrH**-catalyzed halogenation of 7-halo-Trp (**2e,f**) and **RebH**-catalyzed halogenation of 5-Br-Trp (**2b**) proceeded with **C6-regioselectivity**.
- **5,6-**, **5,7-** and **6,7-dihalotryptophans** bearing **two different halogen substituents** were well accessible with the chosen panel of Trp-FDHs and could be isolated on a preparative scale.
- **AetF** proved to be especially valuable for this application, even allowing for the synthesis of a **trihalogenated** bromo-chloro-iodo-tryptophan **3k**.
- **Orthogonal addressability** of several dihalotryptophans was tested in SMC using Pd-NPs. While in Br-Cl-Trp only a preference for the bromo-substituents was found for some patterns, the respective Cl-I-Trp could be **selectively functionalized**.
- In 7-chloro-5-iodo-tryptophan (**3c**) **both halogen substituents** could be **orthogonally addressed** in two consecutive SMCs.

References

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