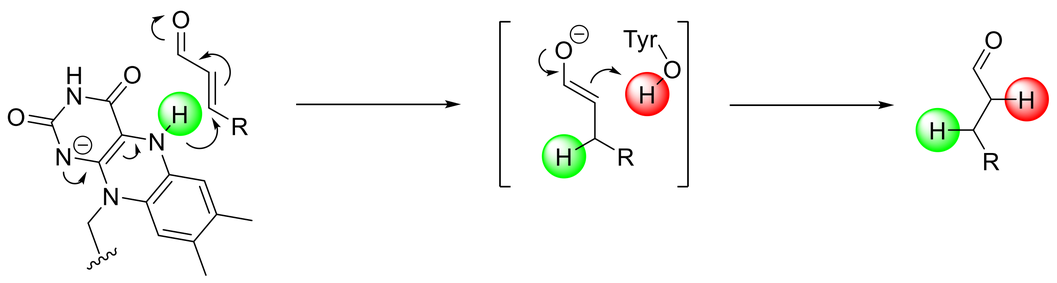
# Small ring synthesis via ene-reductase mediated reductive cyclization

*M. Friess [a], K. Heckenbichler [a], A. Schweiger [a], L. A. Brandner [a], A. Binter [b], M. Toplak [b], P. Macheroux [b], K. Gruber [b], and R. Breinbauer [a]*

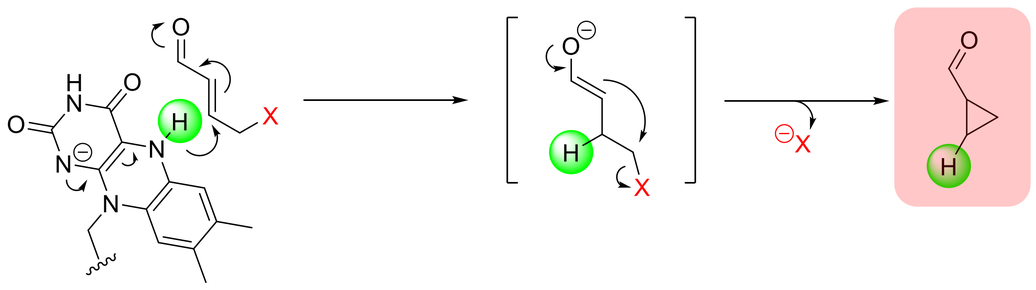
a) *Institute of Organic Chemistry, Graz University of Technology, Stremayrgasse 9, A-8010 Graz*
b) *Institute of Biochemistry, Graz University of Technology, Petersgasse 10-12, A-8010 Graz*
c) *Institute of Molecular Biosciences , University of Graz, Humboldtstraße 50, A-8010 Graz*

## Introduction

Ene-reductases are commonly known to reduce activated double bonds.[1] Mechanistically, a hydride from their reduced FMN cofactor is transferred to the electrophilic ß-carbon of the substrate. This hydride transfer induces the formation of an enolate intermediate. Protonation of this enolate intermediate leads to the canonical 1,4-reduction product (**Scheme 1a**).[2] Inspired by this mechanistic analysis, we envisioned that by providing substrates with additional electrophilic groups it could be possible to trap the enolate *via* an intramolecular substitution reaction, leading to a cyclization product. Such an ene-reductase mediated reductive cyclization is illustrated in **Scheme 1b**.



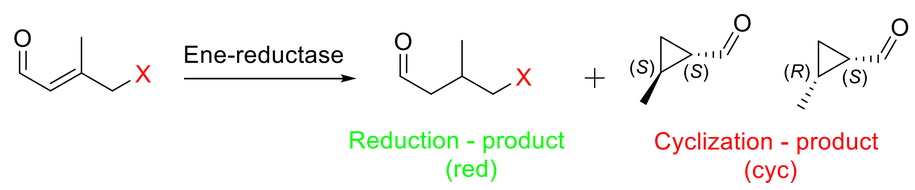

**Scheme 1a:** Natural 1,4-reduction catalyzed by ene-reductases.



**Scheme 1b:** Ene-reductase mediated reductive cyclization.

## Establishment of the reductive cyclization

We could demonstrate the concept of ene-reductase mediated reductive cyclizations with γ-halogenated enals.[3] OPR3 and YqjM in their respective wild type forms gave already considerable quantities of cyclization product (**Table 1**). Replacement of protic tyrosine residues within the active sites of these enzymes against non-protic amino acids gave variants with high selectivity for the reductive cyclization. This was especially the case for brominated substrates.




| Enzyme | X | Conv. [%] | Selectivity [red/cyc] | *de trans/cis* [%] | ee (*S,S*) [%] | ee (*R,S*) [%] |
|---|---|---|---|---|---|---|
| OPR3-WT | Cl | >99 | 57/43 | 68 | 87 | >99 |
| OPR3 Y190F | Cl | >99 | 19/81 | 57 | 61 | 54 |
| OPR3 Y190W | Cl | >99 | 22/78 | 64 | 31 | 24 |
| YqjM-WT | Cl | 95 | 69/31 | 67 | 52 | 56 |
| YqjM Y169F | Cl | >99 | 12/88 | 72 | -80 | -55 |
| OPR3-WT | Br | >99 | 13/87 | 14 | 92 | n.d. |
| OPR3 Y190F | Br | >99 | 1/99 | 58 | 22 | n.d. |
| OPR3 Y190W | Br | 70 | 1/99 | 13 | 39 | n.d. |
| YqjM-WT | Br | >99 | 35/65 | -54 | 81 | n.d. |
| YqjM Y169F | Br | >99 | 1/99 | -30 | -67 | n.d. |

**Table 1:** Conversion of γ-halogenated enals with ene-reductases.

*Conditions: 10 mM enzyme, 10 mM NADH, 1% v/v DMF, 50 mM NaPi at pH 7.5 and 150 mM NaCl.*

## Reason for selectivity enhancement

OPR3-Y190F is better suited to perform the reductive cyclization than its wild type counterpart, because this enzyme variant is less capable in protonating the enolate intermediate. Thus, the intended reductive cyclization pathway has the chance to become predominant within such enzyme variants. In **Figure 1** the active site of OPR3 is shown. The tyrosine residue selected as substitution target is highlighted.

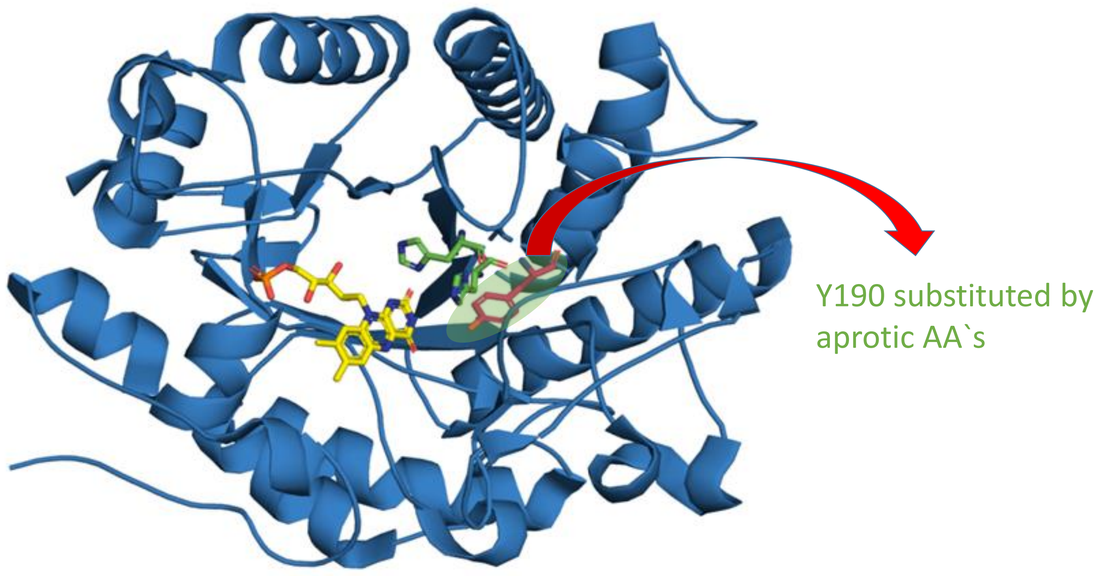
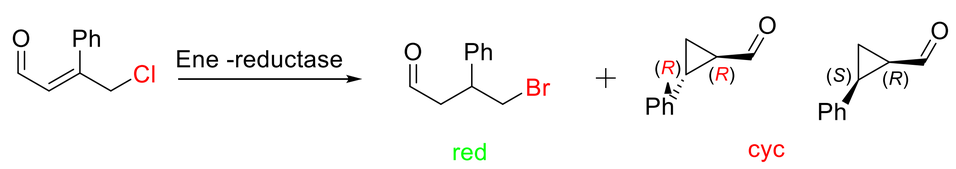



**Figure 1:** Active site of OPR3. The targeted substitution site (Y190) is highlighted. (Figure generated with Pymol by Karl Gruber).

## Substrate engineering

Increasing size and hydrophobicity of the substituent in ß-position proved to be beneficial for the obtained absolute stereoselectivity, which was found to be above 99% for all tested enzyme variants (**Table 2**).




| Enzyme | Conv. [%] | Selectivity [red/cyc] | *de trans/cis* [%] | *ee* (*R,R*) [%] | *ee* (*S,R*) [%] |
|---|---|---|---|---|---|
| OPR3-WT | >99 | 52/48 | 72 | **>99** | 54 |
| OPR3 Y190F | >99 | 25/75 | 71 | **>99** | -43 |
| OPR3 Y190W | 94 | 29/71 | 71 | **>99** | -51 |
| YqjM-WT | 81 | 28/72 | -30 | **>99** | -8 |
| YqjM Y169F | 89 | 5/95 | 94 | **>99** | -29 |

**Table 2:** Reductive cyclization of ß-phenyl substituted substrate.

## Conclusion

We could for the first time demonstrate that ene-reductases can be used to catalyze reductive cyclizations. With this reaction cyclopropylcarbaldehydes could be generated. The expansion of this ring forming biotransformation towards four- and five-membered rings is currently under investigation in our laboratory.

**References:**
[1] C. K. Winkler, G. Tasnadi, D. Clay, M. Hall, K. Faber, *J. Biotechnol.* **2012**, 162, 381-389.
[2] R. M. Kohli, V. Massey, *J. Org. Chem.* **1998**, 273, 32763-32770.
[3] K. Heckenbichler, A. Schweiger, L. A. Brandner, A. Binter, M. Toplak, P. Macheroux, R. Breinbauer, *Angew. Chem. Int. Ed.* **2018**, 57, 7240-7244.

**Acknowledgement:** We thank the Austrian Science Fund (FWF) for financial support *via* the *CATALOX* doc.fund project (DOC 46 doc.fund).