

## Electronic Supplementary Information

for the article

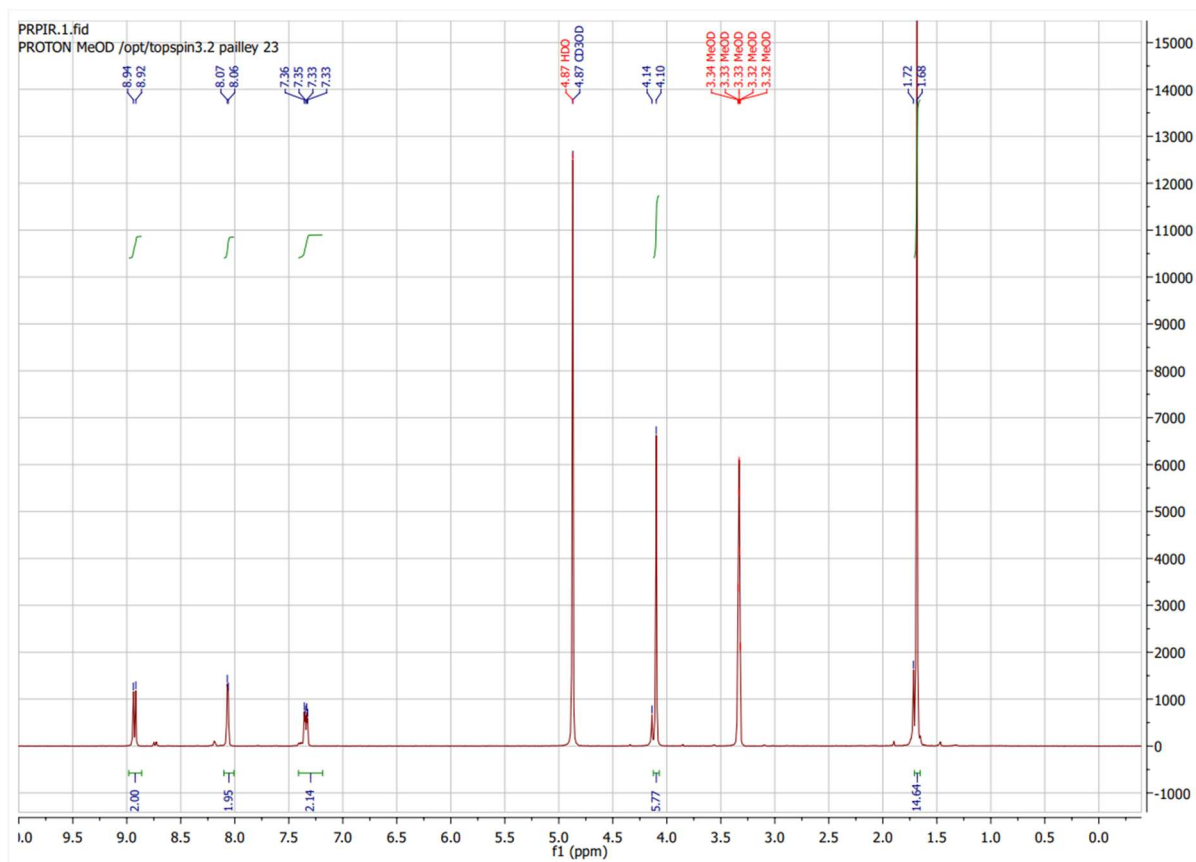
### Hybrid catalysis for enantioselective Baeyer-Villiger oxidation and epoxidation: A Cp\*Ir complex to fuel FMN and FAD reduction for flavoprotein monooxygenase modules.

Robert Röllig,<sup>\*a</sup> Caroline E. Paul,<sup>b</sup> Pierre Rousselot-Pailley,<sup>a</sup> Selin Kara,<sup>\*c,d</sup>  
Véronique Alphand<sup>\*a</sup>

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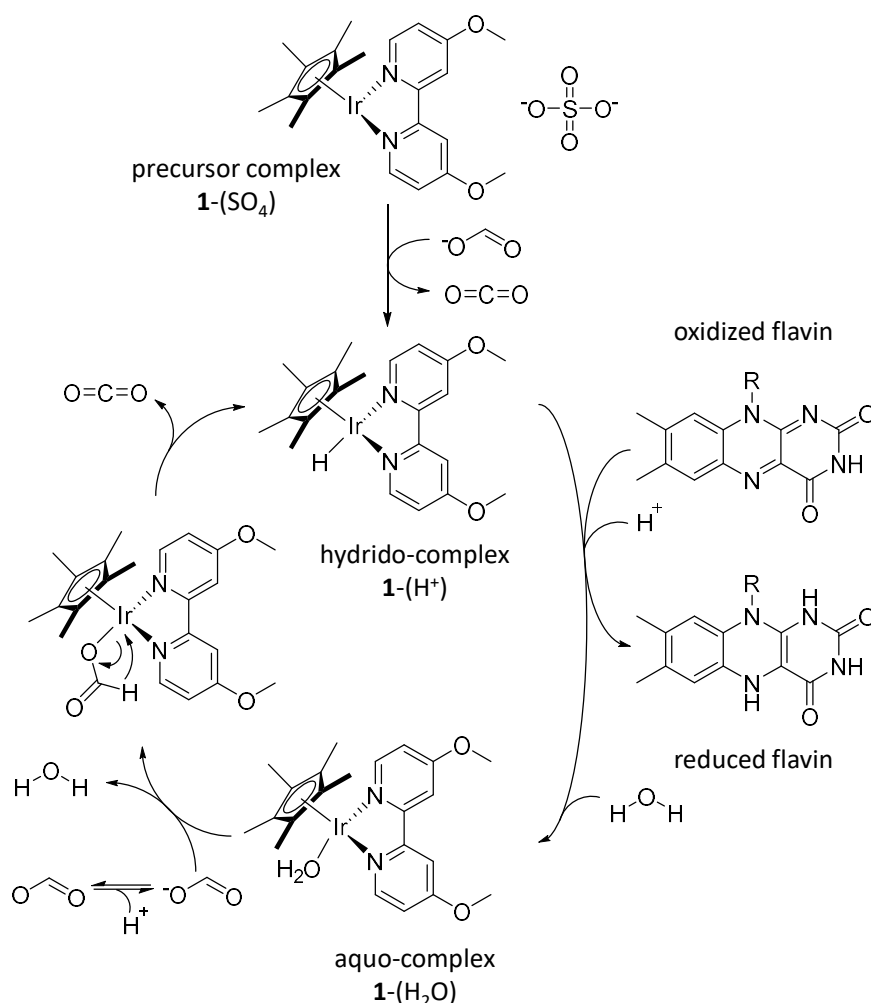
## Synthesis of $[\text{Cp}^*\text{Ir}(\text{bpy-OMe})\text{SO}_4]$



**Fig. S1**  $^1\text{H}$  NMR spectrum of the **1**-( $\text{SO}_4$ ). The NMR was performed at a 300 MHz field with deuterated methanol. The chemical shifts are expressed using the residual of the deuterated solvent as the reference.

$^1\text{H}$  NMR (300 Mhz, D<sub>3</sub>CO):  $\delta$  8.93 (d, 2H, 6 Hz), 8.07 (d, 2H, 3 Hz), 7,34 (dd, 2H, 3 Hz, 6 Hz), 4.10 (s, 6H), 1.70 (s, 15H)

## Mechanism of the activated complex



**Fig. S2** Proposed mechanism for the formation of the hydride species **1**-(H<sup>+</sup>) in the presence of formate ions from its **1**-(SO<sub>4</sub>) precursor as well as for the regioselective, catalytic reduction of flavins by **1**-(H<sup>+</sup>), the first electron mediator of the reaction system. After flavin reduction the reactive hydrido-complex **1**-(H<sup>+</sup>) is regenerated from the inactive aquo-precursor **1**-(H<sub>2</sub>O) by formic acid oxidation.

## Gas chromatography analyses

Gas chromatography (GC) analyses for substrate **2a** and the corresponding products **2b** and **2c** were performed with a FID detector and H<sub>2</sub> as the carrier gas. Injector and detector temperatures were set at 250 °C. Compounds assignments were determined by comparison with authentic samples. The two enantiomers of **2a** and the four lactone isomers **2b** and **2c** were separated using a 25 m x 0.25 mm CP-Chirasil Dex CB capillary column (Agilent Technologies). At an oven temperature of 120 °C, the retention times were 6.5 min for (-)-(1*S*,5*R*)-**2a**, 6.7 min for (+)-(1*R*,5*S*)-**2a**, 15.6 min for tridecane, 26.0 min for (+)-(1*R*,5*S*)-**2b**, 27.0 min for (+)-(1*S*,5*R*)-**2c**, 28.0 min for (-)-(1*S*,5*R*)-**2b**. Styrene, styrene oxide and their derivatives were separated in a 25 m x 0.25 mm β-Dex 120 column (Sigma-Aldrich). The parameters of GC analyses are reported **Table S1**.

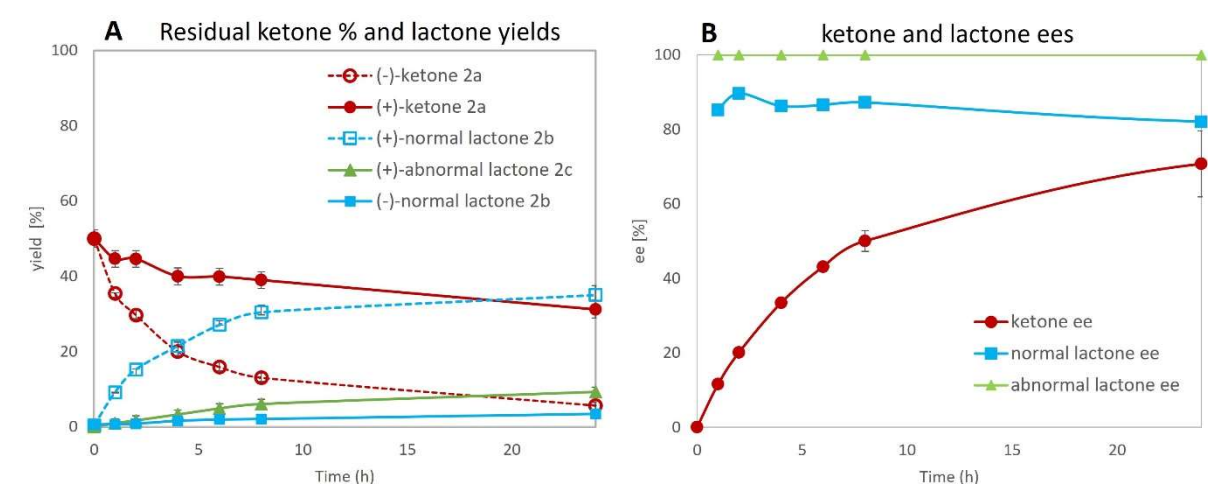
**Table S1.** Oven temperature and retention times (RT) of styrene and the derivatives.

oven temperature	substrates / RT [min]	products / RT [min]	RT [min] DMSO	RT [min] dodecane
100 °C	styrene <b>3a</b> 11.9	( <i>R</i> )-(+)-styrene oxide <b>3b</b> 39.2 ( <i>S</i> )-(-)-styrene oxide <b>3b</b> 40.6 phenyl ethylene glycol* 41.5	15.3	30.0
110 °C	<i>cis</i> - $\beta$ -methylstyrene <b>4a</b> 13.9	(2 <i>R</i> ,3 <i>S</i> )-2-methyl-3-phenyloxirane <b>4b</b> 32.2 phenylpropane-1,2-diol* 42.4	11.7	20.5
120 °C	<i>trans</i> - $\beta$ -methylstyrene <b>5a</b> 12.4	(2 <i>S</i> ,3 <i>S</i> )-2-methyl-3-phenyloxirane <b>5b</b> 21.9 phenylpropane-1,2-diol* 28.3	9.4	14.6
110 °C	4-fluorostyrene <b>6a</b> 9.6	( <i>S</i> )-4-fluorostyrene oxide <b>6b</b> 28.6 fluoro-phenylethylene glycol* 31.2	11.7	20.5

\* products from hydrolysis of the corresponding epoxides

## Enantioselective BV oxidation by 2,5-DKCMO





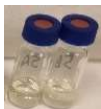
### Biotransformation of ketone **2a** with 100 mM of HCO<sub>2</sub>H and 100mM of Ir complex

**Fig. S3** Time course of ketone **2a** biotransformation by 2,5-DKCMO. **A** : % of each residual ketone **2a** enantiomers and yields of each lactone **2b** and **2c** enantiomers **B** : Enantiomeric excesses of ketone **2a** and regioisomeric lactones **2b** and **2c**

Experimental conditions: *rac*-**2a** [5 mM], lyophilized 2,5-DKCMO [312  $\mu\text{g mL}^{-1}$ ], catalase [400 – 1000 units  $\text{mL}^{-1}$ ], FMN [10  $\mu\text{M}$ ], 1-(SO<sub>4</sub>) [100  $\mu\text{M}$ ], formic acid [100mM], K<sub>2</sub>HPO<sub>4</sub> [0.225M] pH 6.7. Experiments in duplicates.

## pH effect

**Table S2.** Effect of pH and formic acid.

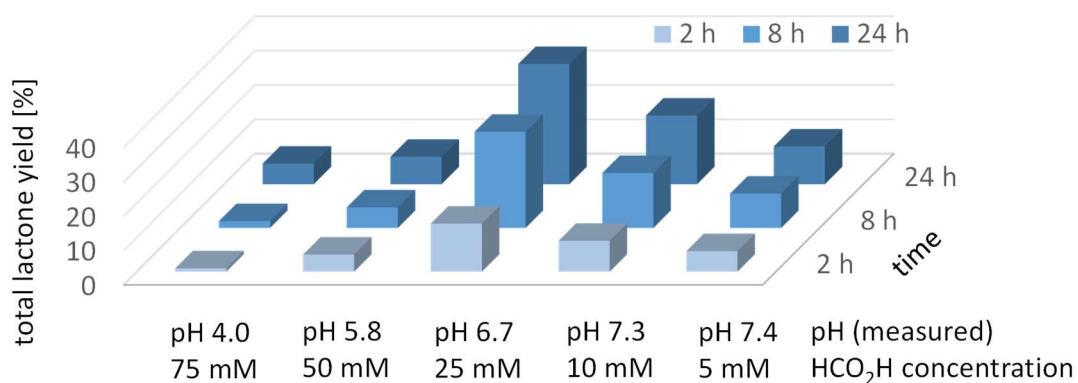
pH <sup>a</sup> HCOOH	7.4 5 mM		7.3 10 mM		6.7 25 mM		5.8 50 mM		4.0 75 mM	
	yield [%] <sup>b</sup>	ee [%] <sup>c</sup>	yield [%] <sup>b</sup>	ee [%] <sup>c</sup>	yield [%] <sup>b</sup>	ee [%] <sup>c</sup>	yield [%] <sup>b</sup>	ee [%] <sup>c</sup>	yield [%] <sup>b</sup>	ee [%] <sup>c</sup>
<b>2 h</b>	6 ± 2	5 ± 1	9 ± 0	8 ± 0	14 ± 2	13 ± 1	5 ± 2	4 ± 1	1 ± 0	0 ± 0
<b>4 h</b>	9 ± 2	6 ± 2	13 ± 1	11 ± 0	20 ± 1	20 ± 1	6 ± 2	5 ± 2	2 ± 0	0 ± 0
<b>8 h</b>	10 ± 2	8 ± 2	16 ± 1	15 ± 1	28 ± 0	29 ± 1	6 ± 1	5 ± 1	2 ± 0	0 ± 0
<b>24 h</b>	11 ± 2	10 ± 1	20 ± 0	20 ± 1	35 ± 1	39 ± 1	8 ± 2	5 ± 2	6 ± 1	0 ± 0
reaction at 24 h										

<sup>a</sup> initial pH after addition of various concentrations of formic acid (5 – 75 mM) to potassium phosphate-buffered reactions.

<sup>b</sup> yield of the total (normal **2b** + abnormal **2c**) lactones.

<sup>c</sup> enantiomeric excess of the ketone substrate **2a**.

*Experimental conditions:* various concentrations of formic acid [5 mM, 10 mM, 25 mM, 50 mM, 75 mM] in potassium phosphate [100 mM, initial pH 7.5], lyophilized 2,5-DKCMO [312 µg mL<sup>-1</sup>], catalase [400 – 1000 units mL<sup>-1</sup>], FMN [10 µM], **1**-(SO<sub>4</sub>) [100 µM], 5 mM *rac*-**2a** added in an ethanol solution (final ethanol concentration 5% (v/v)). Experiments in duplicates.



**Fig. S4** Time course of the reaction at various pH and formic acid concentrations (from **Table S2** data).

## Formic acid concentration effect

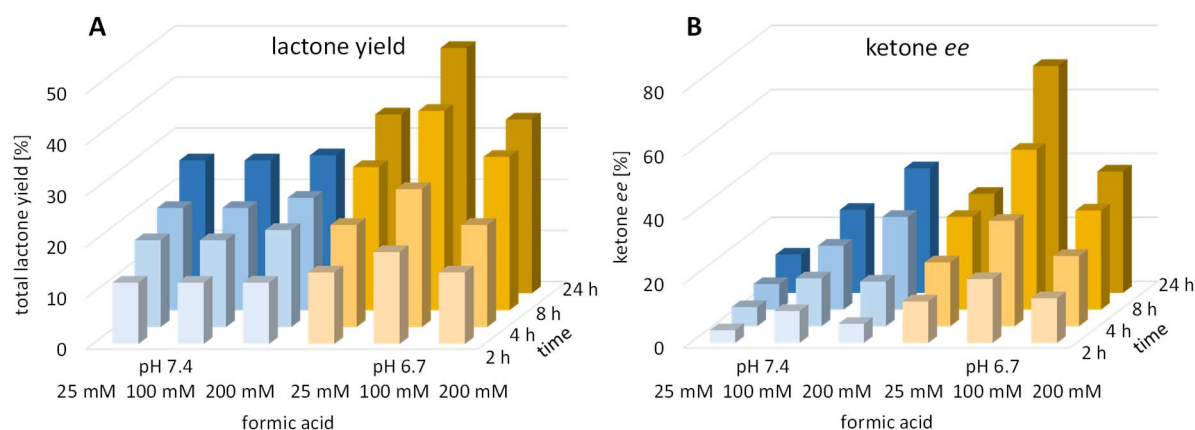
**Table S3.** Effect of the hydride donor concentration (HCO<sub>2</sub>H) at constant pH.

pH HCO <sub>2</sub> H	7.4		7.4		6.7		6.7	
	100 mM		200 mM		100 mM		200 mM	
	yield [%] <sup>a</sup>	ee [%] <sup>b</sup>	yield [%] <sup>a</sup>	ee [%] <sup>b</sup>	yield [%] <sup>a</sup>	ee [%] <sup>b</sup>	yield [%] <sup>a</sup>	ee [%] <sup>b</sup>
<b>2 h</b>	12 ± 1	10 ± 1	12 ± 1	9 ± 1	18 ± 0	20 ± 0	14 ± 1	14 ± 0
<b>4 h</b>	17 ± 1	15 ± 1	19 ± 1	14 ± 1	27 ± 0	33 ± 0	20 ± 0	22 ± 1
<b>8 h</b>	20 ± 1	20 ± 1	22 ± 1	18 ± 1	39 ± 1	50 ± 3	30 ± 1	31 ± 1
<b>24 h</b>	26 ± 1	26 ± 1	27 ± 1	23 ± 1	48 ± 4	71 ± 9	34 ± 1	38 ± 0

<sup>a</sup> yield of the total (normal **2b** and abnormal **2c**) lactones.

<sup>b</sup> enantiomeric excess of the ketone substrate **2a**.

*Experimental conditions:* At pH 7.4 – 100 mM, respectively 200 mM of formic acid was added to 0.5 M, respectively 1 M K<sub>2</sub>HPO<sub>4</sub> solution. At pH 6.7 – 100 mM, respectively 200 mM formic acid was added to 0.23 M, respectively 0.45 M K<sub>2</sub>HPO<sub>4</sub> solution. Lyophilized 2,5-DKCMO [312 μg mL<sup>-1</sup>], catalase [400 – 1000 units mL<sup>-1</sup>], FMN [10 μM], 1-(SO<sub>4</sub>) [100 μM], 5 mM *rac*-**2a** added in an ethanol solution (final ethanol concentration 5% v/v). Experiments in duplicates.



**Fig. S5** Time course of the reaction at pH 6.7 and 7.4, and various HCO<sub>2</sub>H concentrations (from **Table S3** and other data).

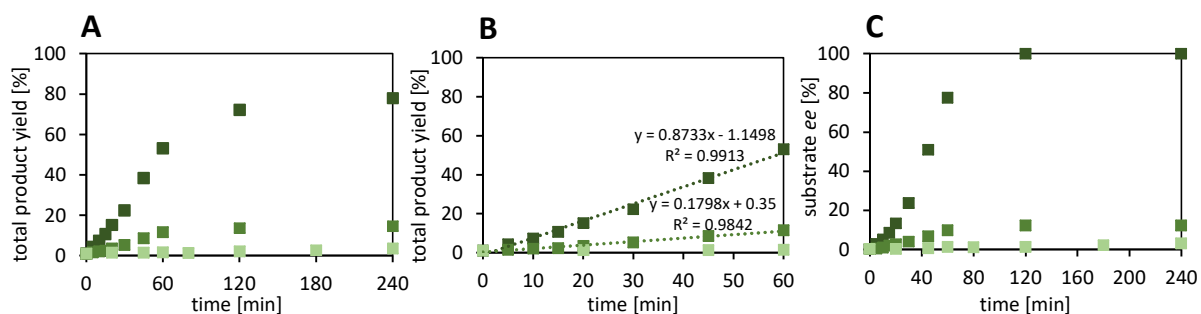
## Alternative hydride sources

**Table S4.** List of tested potential hydride sources and conditions.

Deep Eutectic Solvents	Carbonic acid/CO <sub>2</sub> (RIVER®, ALDI, Denmark)
Choline chloride/butanediol 1:4 v/v	in pure mineral water
Choline chloride/butanediol 1:4 v/v in potassium phosphate buffer [100 mM, pH 7.5] (1-to-10 v/v)	in 1 M solution (mineral water) of K <sub>2</sub> HPO <sub>4</sub> (1-to-10 v/v) (final K <sub>2</sub> HPO <sub>4</sub> concentration 0.1 M)
Choline chloride/ethylene glycol 1:2 v/v	
Choline chloride/ethylene glycol 1:2 v/v in potassium phosphate buffer [100 mM, pH 7.5] (1-to-10 v/v)	

*Experimental conditions* as described in **Table S2**.

### NADH as the hydride source



**Fig. S6** NADH-driven FMN reduction enhanced by the **1**-(H<sup>+</sup>)-mediated hydride transfer for the BV oxidation of *rac-2a* by 2,5-DKCMO. **A** Total yield of the lactone products over time. **B** Determination of the initial rates for 60 min. **C** ee of the ketone substrate. ■ oxygenase [312 μg mL<sup>-1</sup>] / **1**-(SO<sub>4</sub>) [100 μM], ■ oxygenase [62 μg mL<sup>-1</sup>] (five-fold less compared to ■ or ■) / **1**-(SO<sub>4</sub>) [100 μM], ■ oxygenase [312 μg mL<sup>-1</sup>] / no **1**-(SO<sub>4</sub>). *Experimental conditions:* NADH [25 mM], FMN [10 μM], catalase [400 - 1000 U mL<sup>-1</sup>], 0.5 mL potassium phosphate buffer [100 mM, pH 7.5]. Experiments in duplicates.