

# Biocatalyzed Sulfoxidation in Presence of Deep Eutectic Solvents

## 1. Experimental Procedures

### 1.1. Preparation of the Natural Deep Eutectic Solvents

Two components NADES were prepared by adding the ammonium salt (ChCl) and the hydrogen bond donors (Urea, Gly, EG and Xyl) at a molar ratio of 1:2 for Gly and EG, or 1:1 in the case of Urea and Xyl, in a beaker and incubated at 80 °C for 2 hours with intermediate stirring, until a colourless clear liquid was formed. The resulting DES was cooled down to room temperature and use without further purification. When obtaining Glu:Fru:H<sub>2</sub>O or ChCl:Glu:H<sub>2</sub>O, the same procedure was followed, mixing the three components in 1:1:6 or 5:2:5 molar ratio, respectively.

### 1.2. Study of the Ethyl Phenyl Sulfide Catalysed Sulfoxidation in Presence of Different NADESs.

Ethyl phenyl sulfide 1a (10 mM) was added to 1.0 mL Tris/HCl 50 mM pH 9.0/NADES mixture containing NADPH (0.2 mM), sodium phosphite (10 mM) and *m*FMO (1.0 μM). The reactions were stirred at 28 °C and 220 rpm for 24 h. Once finished, the reactions were extracted with EtOAc (3 × 0.5 mL) and dried onto Na<sub>2</sub>SO<sub>4</sub>. The samples were directly analysed by GC/MS and HPLC in order to determine, respectively, the level of conversion and the enantiomeric excess of chiral sulfoxide (S)-1b. Results for some of the NADESs tested are summarized in Table S1.

**Table S1.** Biocatalyzed oxidation of ethyl phenyl sulphide (1a) in buffer containing different NADESs catalysed by *m*FMO.

Entry	DES	% DES	Conv. (%) <sup>1</sup>	ee (%) <sup>2</sup>
1	Glu:Fru:H <sub>2</sub> O (1:1:6)	10	>40.5 ± 2.1	71.5 ± 2.1
2	Glu:Fru:H <sub>2</sub> O (1:1:6)	20	35.0 ± 1.4	67.0 ± 1.4
3	Glu:Fru:H <sub>2</sub> O (1:1:6)	40	10.5 ± 0.7	65.5 ± 0.7
4	ChCl:Glu:H <sub>2</sub> O (5:2:5)	10	37.5 ± 2.1	66.0 ± 1.4
5	ChCl:Glu:H <sub>2</sub> O (5:2:5)	20	12.0 ± 1.4	41.0 ± 1.4
6	ChCl:Xyl (1:1)	10	28.0 ± 1.4	61.5 ± 2.1
7	ChCl:Xyl (1:1)	20	5.5 ± 0.7	n.d.
8	ChCl:Urea (1:1)	10	8.5 ± 0.7	50.5 ± 0.7

<sup>1</sup> Conversion was determined by GC/MS. <sup>2</sup> Optical purity of (S)-1b was measured by HPLC. Average values of two or more experiments. n.d. not determined.

### 1.3. General Procedure for the *m*FMO-Catalysed Sulfoxidation of 1a at Different Substrate Concentrations.

Ethyl phenyl sulfide 1a (20-200 mM) was added to 1.0 mL Tris/HCl 50 mM pH 9.0/ 5 or 10% v/v ChCl:EG (1:1) or ChCl:Gly (1:1) mixture containing NADPH (0.2 mM), sodium phosphite (20-200 mM, 1.0 equiv.) and *m*FMO (1.0 μM). The reactions were stirred at 28°C and 220 rpm for the times established (24-96 hours). Once finished, the reactions were extracted with EtOAc (3 × 0.5 mL) and dried onto Na<sub>2</sub>SO<sub>4</sub>. The samples were directly analysed by GC/MS and HPLC in order to determine, respectively, the level of conversion and the enantiomeric excess of chiral sulfoxide (S)-1b.

### 1.4. Sulfoxidations Catalysed by *m*FMO in Buffer Tris/HCl 50 mM pH 9.0 Containing 10% v/v ChCl:Gly (1:1).

Prochiral sulphides 2-6a (100 mM) were dissolved in 1.0 mL of a mixture Tris/HCl 50 mM pH 9.0/ 10% v/v ChCl:Gly (1:1) containing NADPH (0.2 mM), sodium phosphite (100 mM) and *m*FMO (1.0 μM). The reactions were stirred at 28 °C and 220 rpm for 96 h. Once finished, the reactions were extracted with EtOAc (3 × 0.5 mL) and dried onto Na<sub>2</sub>SO<sub>4</sub>. The samples were directly analysed by GC/MS and HPLC in order to determine, respectively, the level of conversion and the enantiomeric excess of chiral sulfoxide (S)-2-6b.

## 2. GC Analyses

GC Analyses were performed on a HP-5MS cross-linked methyl siloxane column (30 m × 0.25 mm × 0.25 μm, 1.0 bar N<sub>2</sub>) and were used for the determination of the conversions and the amount of sulfoxides 1-6b (Table S2). For all the compounds, the following program was employed: 50 °C (5 min), 10 °C/min, 200 °C (3 min).

**Table S2.** Determination of conversions and amounts of sulfoxides and sulfones by employing GC.

Substrate	<i>t<sub>R</sub></i> (min) a	<i>t<sub>R</sub></i> (min) b
1	11.0	15.1
2	12.2	16.2
3	14.9	18.6
4	15.7	18.6
5	12.5	17.0
6	18.9	21.8

## 3. HPLC Analyses

For the determination of the enantiomeric excesses of compounds 1-12b (Table S3), the following column was employed: Chiralcel OD (0.46 cm × 25 cm) from Daicel.

**Table S3.** Determination of enantiomeric excesses by HPLC.

Substrate	T (°C)	Eluent <sup>a</sup>	Retention time [min]
1b	30	<i>n</i> -hexane-IPA 9:1	12.9 (R); 16.5 (S)
2b	30	<i>n</i> -hexane-IPA 95:5	10.2 (R); 12.0 (S)
3b	30	<i>n</i> -hexane-IPA 9:1	14.1 (R); 15.2 (S)
4b	30	<i>n</i> -hexane-IPA 9:1	24.0 (R); 28.5 (S)
5b	30	<i>n</i> -hexane-IPA 9:1	17.0 (R); 18.7 (S)
6b	30	<i>n</i> -hexane-IPA 95:5	26.1 (R); 29.0 (S)

<sup>a</sup> All the experiments were performed with isocratic eluent. Flow rate 1.0 mL min<sup>-1</sup>.