



HAL
open science

Repurposing myoglobin into a carbene transferase for a [2,3]-sigmatropic Sommelet-Hauser rearrangement

Manon Pujol, Lison Degeilh, Thibault Sauty de Chalon, Marius Réglier, A. Jalila Simaan, Christophe Decroos

► To cite this version:

Manon Pujol, Lison Degeilh, Thibault Sauty de Chalon, Marius Réglier, A. Jalila Simaan, et al.. Repurposing myoglobin into a carbene transferase for a [2,3]-sigmatropic Sommelet-Hauser rearrangement. *Journal of Inorganic Biochemistry*, 2024, 260, pp.112688. <10.1016/j.jinorgbio.2024.112688>. <hal-04668325>

HAL Id: hal-04668325

<https://hal.science/hal-04668325v1>

Submitted on 6 Aug 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire HAL, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Repurposing myoglobin into a carbene transferase for a [2,3]-sigmatropic Sommelet-Hauser rearrangement

Manon Pujol^a, Lison Degeilh^a, Thibault Sauty de Chalon^a, Marius Réglier^a, A. Jalila Simaan^a
and Christophe Decroos^{a,b,*}

^a Aix Marseille Univ, CNRS, Centrale Méditerranée, iSm2, Marseille, France

^b Université de Strasbourg, CNRS, INSERM, Institut de Génétique et de Biologie Moléculaire
et Cellulaire, Department of Integrated Structural Biology, Illkirch, France

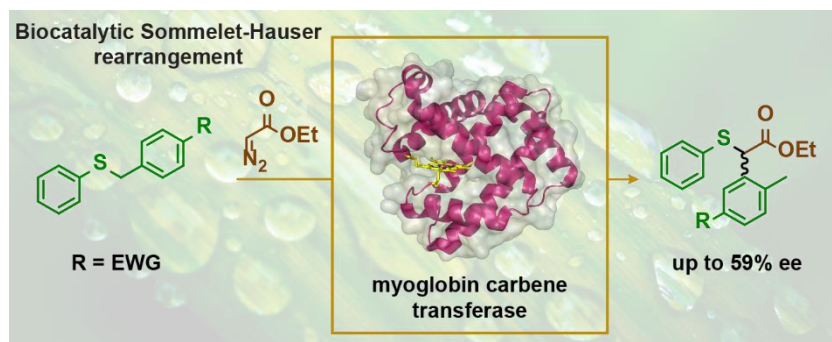
* Corresponding author: decroosc@igbmc.fr

Highlights:

- A myoglobin carbene transferase catalyzes a Sommelet-Hauser rearrangement.
- Benzyl thioethers bearing strong electron-withdrawing substituents are substrates.
- No reductant is needed to initiate the iron-carbene formation with ethyl diazoacetate.

Abstract: New-to-Nature biocatalysis has emerged as a promising tool in organic synthesis thanks to progress in protein engineering. Notably, heme proteins have been evolved into robust catalysts for carbene and nitrene transfers and related sigmatropic rearrangements. In this work, we report the first example of a [2,3]-sigmatropic Sommelet-Hauser rearrangement initiated by a carbene transfer of the sperm whale myoglobin mutant L29S,H64V,V68F that was previously reported to catalyze the mechanistically similar [2,3]-sigmatropic Doyle-Kirmse rearrangement. This repurposed heme enzyme catalyzes the Sommelet-Hauser rearrangement between ethyl diazoacetate and benzyl thioethers bearing strong electron-withdrawing substituents with good yields and enantiomeric excess. Optimized catalytic conditions in the absence of any reductant led to an increased asymmetric induction with up to 59% enantiomeric excess. This myoglobin mutant is therefore one of the few catalysts for the asymmetric Sommelet-Hauser rearrangement. This work broadens the scope of abiological reactions catalyzed by iron-carbene transferases with a new example of asymmetric sigmatropic rearrangement.

Graphical abstract:



Graphical abstract synopsis: We report the first example of a biocatalytic [2,3]-sigmatropic rearrangement initiated by a carbene transfer. A sperm whale myoglobin mutant catalyzes the reaction between ethyl diazoacetate and benzyl thioethers bearing strong electron-withdrawing substituents with good yields and up to 59% enantiomeric excess.

Keywords: artificial heme enzyme, sigmatropic rearrangement, carbene transfer, ylides, carbenoids

1. Introduction

Sigmatropic rearrangements are powerful tools in synthetic chemistry to build molecular complexity from readily accessible chemicals or reactive intermediates including onium ylides.^[1-3] They involve the selective reorganization of the molecule skeleton *via* the migration of a σ -bond across a π -system.^[4] Accordingly, they are often key-steps in the synthesis of high added-value molecules such as natural products or pharmaceuticals. Surprisingly, sigmatropic rearrangements remain rare transformations in Nature.^[5] Likewise, the synthetic potential of enzymes for such reactions is still underexplored with only a few reports so far.^[6] In these examples, the biocatalytic substrate functionalization leads to the formation of a reactive intermediate that further rearranges non-enzymatically. Alternatively, chemoenzymatic cascades involving a sigmatropic rearrangement have also been described.^[7] Nevertheless, such rearrangements are not initiated by the biochemical step within these cascades.

In the past decades, biocatalysts able to perform abiological reactions have emerged through the development of artificial and engineered enzymes.^[8,9] Catalytic antibodies were the first class of engineered enzymes to catalyze various sigmatropic rearrangements such as [3,3]-Cope and Claisen rearrangements, [2,3]-selenoxide elimination, or [2,3]-allylic sulfoxide-sulfonate rearrangement.^[10] Pioneering work from the group of Arnold further led to the development of a panel of cytochrome-derived catalysts for new-to-Nature carbene and nitrene transfers by directed evolution.^[9] Despite the variety of engineered abiological reactivities, biocatalytic sigmatropic rearrangements initiated by a carbene or a nitrene transfer are still limited to a few examples. Tyagi *et al.* reported the engineering of sperm whale myoglobin for the synthesis of homoallylic thioethers from allylic thioethers through a [2,3]-sigmatropic Doyle-Kirmse rearrangement of a sulfonium ylide intermediate (Figure 1).^[11] Following a similar approach, Prier *et al.* used a P411 nitrene transferase (derived from cytochrome P450 from *Bacillus megaterium*) for the synthesis of protected allylic amines *via* the [2,3]-rearrangement of

sulfimide obtained from allylic thioethers.^[12] More recently, Miller *et al.* evolved a P411 carbene transferase for the [1,2]-Stevens rearrangement of a reactive aziridium ylide intermediate allowing access to azetidines from aziridines.^[13]

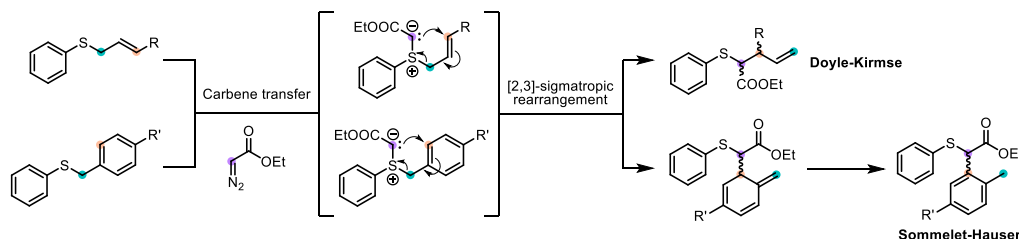


Figure 1. Mechanism of [2,3]-sigmatropic Doyle-Kirmse and Sommelet Hauser rearrangements from allyl and benzyl thioethers, respectively. Tyagi *et al.* reported the asymmetric biocatalytic Doyle-Kirmse of allyl thioethers with the L29S,H64V,V68F sperm whale myoglobin triple mutant.^[11] The Sommelet-Hauser rearrangement was described for benzyl thioethers by Xu *et al.* using hemin as catalyst^[16] and is investigated in this work with sperm whale myoglobin as catalyst.

Hemoprotein-based carbene and nitrene transferases rely on the broad reactivity of their heme prosthetic group.^[9,14] Accordingly, hemin itself^[15-19] and related iron-porphyrins^[14,20,21] have also been used as catalysts for carbene chemistry including a few sigmatropic rearrangements. Of particular interest, Xu *et al.* reported the hemin-catalyzed formation of benzyl sulfonium ylides leading to [1,2]-Stevens and/or [2,3]-Sommelet-Hauser products.^[16] Selectivity toward [2,3]-Sommelet-Hauser products was achieved under aqueous conditions with strong electron-donating para-substituents on the benzyl group. Given the mechanistic similarities between the [2,3]-sigmatropic Doyle-Kirmse and Sommelet-Hauser rearrangements^[22] (Figure 1), we hypothesized that an engineered heme-carbene transferase could also catalyze the latter transformation.

In this work, we investigated the ability of sperm whale myoglobin and the corresponding L29S,H64V,V68F mutant^[11] (named hereafter Mb(L29S,H64V,V68F)) to catalyze the [2,3]-

sigmatropic Sommelet-Hauser rearrangement initiated by a carbene transfer (Figure 1). Catalytic conditions were optimized to notably achieve good enantiomeric excess, which remains a challenge for this sigmatropic rearrangement. Overall, this study represents the first example of an asymmetric sigmatropic [2,3]-Sommelet-Hauser rearrangement catalyzed by a carbene transferase.

2. Experimental

2.1 Synthesis of substrates and authentic products

Chemicals and reagents were obtained from usual commercial suppliers (Merck Sigma-Aldrich, Fischer Scientific and VWR) at the highest commercial quality and were used without any further purification. Reactions were performed with magnetic stirring under air atmosphere unless otherwise stated. Reactions were monitored by TLC on silica plates using UV-light for visualization. Column chromatography was performed on silica gel 60. Evaporation of solvents was conducted under reduced pressure at temperatures less than 60°C. ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance III nanobay 400 spectrometer at the Plateforme Spectropole – Fédération des Sciences Chimiques de Marseille at 400 MHz and 100 MHz, respectively. Chemical shifts (δ) and coupling constants (J) are given in ppm and Hertz (Hz), respectively. The signal multiplicity is described according to the following abbreviations: s (singlet), bs (broad singlet), d (doublet), t (triplet), q (quadruplet) and m (multiplet). Chemical shifts are reported relative to the residual solvent as an internal standard (CDCl_3 : $\delta = 7.26$ ppm for ^1H and $\delta = 77.16$ ppm for ^{13}C – DMSO-d_6 : $\delta = 2.50$ ppm for ^1H and $\delta = 39.52$ ppm for ^{13}C).

General procedure A for the synthesis of benzyl phenyl sulfides / ether / amine. Benzyl bromide (2.0 mmol, 1 equiv.) was added to a solution of the nucleophile (thiophenol and 4-substituted thiophenols, 4-nitrophenol, *N*-methyl-4-nitroaniline; 2.4 mmol, 1.2 equiv.) and K_2CO_3 (2.88 mmol, 1.44 equiv.) in ethanol (10 mL) at room temperature, and the reaction was stirred overnight. The resulting mixture was concentrated under reduced pressure and dissolved in CH_2Cl_2 (20 mL). The organic phase was washed with water (20 mL), a saturated solution of NaHCO_3 (20 mL), brine (20 mL), dried over anhydrous MgSO_4 , and concentrated under reduced pressure. The residue was purified by column chromatography over silica gel to afford the expected product.

4-Nitro benzyl phenyl sulfide (1a): Prepared according to general procedure A. The product was purified by column chromatography with pentane/diethyl ether (95:5) as eluent to afford the expected product in pure form. Light yellow solid – Yield: 89% (438 mg) – Rf = 0.52 (cyclohexane/EtOAc, 9:1) – ^1H NMR (400 MHz, CDCl_3): δ (ppm) = 8.10 (d, J = 8.8 Hz, 2H), 7.36 (d, J = 8.8 Hz, 2H), 7.28-7.17 (m, 5H), 4.12 (s, 2H) – ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) = 146.9, 145.5, 134.6, 130.7, 129.5, 129.0, 127.1, 123.5, 38.7.

4-Cyano benzyl phenyl sulfide (1b): Prepared according to general procedure A. The product was purified by column chromatography with pentane/diethyl ether (10:0 to 9:1) as eluent to afford the expected product in pure form. White solid – Yield: 78% (350 mg) – Rf = 0.70 (cyclohexane/EtOAc, 7:3) – ^1H NMR (400 MHz, CDCl_3): δ (ppm) = 7.52 (d, 2H, J = 8.4 Hz), 7.32 (d, 2H, J = 8.4 Hz), 7.28-7.18 (m, 5H), 4.09 (s, 2H) – ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) = 142.6, 133.9, 131.4, 130.1, 128.6, 128.2, 126.3, 117.8, 110.2, 38.4.

Methyl 4-(phenylthiomethyl)benzoate (1c): Prepared according to general procedure A. Methanol was used as solvent instead of ethanol. The product was purified by column chromatography with pentane/diethyl ether (98:2) as eluent to afford the expected product in pure form. White solid – Yield: 89% (460 mg) – Rf = 0.56 (cyclohexane/EtOAc, 9:1) – ^1H NMR (400 MHz, CDCl_3): δ (ppm) = 7.94 (d, J = 8.4 Hz, 2H), 7.34-7.16 (m, 7H), 4.12 (s, 2H), 3.90 (s, 3H) – ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) = 167.0, 143.2, 135.5, 130.7, 129.9, 129.2, 129.1, 129.0, 127.0, 52.2, 39.3.

4-Chloro benzyl phenyl sulfide (1d): Prepared according to general procedure A. The product was purified by column chromatography with pentane as eluent to afford the expected product in pure form. White solid – Yield: 86% (406 mg) – Rf = 0.58 (cyclohexane) – ^1H NMR (400 MHz, CDCl_3): δ (ppm) = 7.33-7.20 (m, 9H), 4.09 (s, 2H) – ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) = 136.3, 135.8, 133.1, 130.5, 130.3, 129.1, 128.7, 126.8, 38.7.

Benzyl phenyl sulfide (1e): Prepared according to general procedure A (16.0 mmol scale). The product was purified by column chromatography with pentane/diethyl ether (100:0 to 94:6) as eluent to afford the expected product in pure form. White solid – Yield: 93% (3.0 g) – R_f = 0.69 (cyclohexane/EtOAc, 95:5) – ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 7.31-7.12 (m, 10H), 4.09 (s, 2H) – ¹³C NMR (100 MHz, CDCl₃): δ (ppm) = 137.6, 136.5, 130.0, 128.9, 128.9, 128.6, 127.3, 126.5, 39.2.

4-Methoxy benzyl phenyl sulfide (1f): Prepared according to general procedure A. The product was purified by column chromatography with pentane/diethyl ether (99:1) as eluent to afford the expected product in pure form. White solid – Yield: 89% (370 mg) – R_f = 0.86 (cyclohexane/EtOAc, 9:1) – ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 7.32-7.16 (m, 7H), 6.82 (d, *J* = 8.7 Hz, 2H), 4.08 (s, 2H), 3.79 (s, 3H) – ¹³C NMR (100 MHz, CDCl₃): δ (ppm) = 158.9, 136.7, 130.1, 130.0, 129.6, 129.0, 126.4, 114.1, 55.4, 38.7.

4-Nitro benzyl phenyl ether (1g): Prepared according to general procedure A. The product was purified by column chromatography with pentane/diethyl ether (98:2) as eluent to afford the expected product in pure form. Light yellow solid – Yield: 85% (390 mg) – R_f = 0.60 (cyclohexane/EtOAc, 7:3) – ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 8.25 (d, *J* = 8.8 Hz, 2H), 7.61 (d, *J* = 8.9 Hz, 2H), 7.34-7.29 (m, 2H), 7.02-6.94 (m, 3H), 5.18 (s, 2H) – ¹³C NMR (100 MHz, CDCl₃): δ (ppm) = 158.3, 147.7, 144.8, 129.8, 127.7, 124.0, 121.7, 115.0, 68.8.

N-methyl-N-(4-nitrobenzyl)-aniline (1h): Prepared according to general procedure A. The product was purified by column chromatography with pentane/diethyl ether (98:2) as eluent to afford the expected product in pure form. Orange-yellow oil – Yield: 85% (822 mg) – R_f = 0.61 (cyclohexane/EtOAc, 7:3) – ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 8.17 (d, *J* = 8.8 Hz, 2H), 7.42 (d, *J* = 8.8 Hz, 2H), 7.27-7.22 (m, 2H), 6.82-6.75 (m, 3H), 4.61 (s, 2H), 3.07 (s, 3H) – ¹³C

NMR (100 MHz, CDCl₃): δ (ppm) = 149.2, 147.3, 147.1, 129.5, 127.6, 124.0, 117.7, 112.8, 56.8, 39.1.

General procedure B for the Sommelet-Hauser rearrangement. This procedure was adapted from a previously described protocol.^[16] To a solution of hemin (0.025 mmol, 5.0 mmol%) and ascorbic acid (0.25 mmol, 0.5 equiv.) in ethanol (15 mL) was added 4-substituted-benzyl phenyl sulfide (0.5 mmol, 1 equiv.) under nitrogen, followed by ethyl diazoacetate (1.0 mmol, 2 equiv.) in one portion. The reaction was stirred overnight at 40°C under nitrogen. The resulting mixture was concentrated and the residue was dissolved in water (20 mL) and extracted with CH₂Cl₂ (3 x 20 mL). The organic phase was washed with brine (20 mL), dried over anhydrous MgSO₄ and concentrated under reduced pressure. The corresponding residue was purified by column chromatography over silica gel to afford the expected product.

Ethyl 2-(2-methyl-5-nitrophenyl)-2-(phenylthio)acetate (2a): Prepared according to general procedure B. The product was purified by column chromatography with pentane/diethyl ether (98:2 to 95:5) as eluent to afford the expected product in pure form. Slightly yellow oil – Yield: 58% (96 mg) – R_f = 0.38 (cyclohexane/EtOAc, 9 : 1) – ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 8.43 (d, *J* = 2.4 Hz, 1H), 8.01 (dd, *J* = 8.4, 2.5 Hz, 1H), 7.37 – 7.34 (m, 2H), 7.29 – 7.22 (m, 4H), 5.09 (s, 1H), 4.23 – 4.10 (m, 2H), 2.41 (s, 3H), 1.19 (t, *J* = 7.1 Hz, 3H) – ¹³C NMR (100 MHz, CDCl₃): δ (ppm) = 169.6, 146.8, 144.1, 136.2, 133.7, 132.7, 131.4, 129.3, 128.9, 124.1, 122.9, 62.3, 52.6, 19.9, 14.1.

Ethyl 2-(5-cyano-2-methylphenyl)-2-(phenylthio)acetate (2b): Prepared according to procedure B. The product was purified by column chromatography with pentane/diethyl ether (10:0 to 8:2) as eluent to afford the expected product in pure form. Colorless oil – Yield: 25% (39 mg) – R_f = 0.59 (cyclohexane/EtOAc, 7 : 3) – ¹H NMR (400 MHz, CDCl₃): δ (ppm) = 7.84 (d, *J* = 1.7 Hz, 1H), 7.43 (dd, *J* = 7.9, 1.8 Hz, 1H), 7.35 – 7.31 (m, 2H), 7.28 – 7.20 (m, 4H),

5.03 (s, 1H), 4.18 – 4.09 (m, 2H), 2.37 (s, 3H), 1.17 (t, $J = 7.1$ Hz, 3H) – ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) = 169.8, 142.1, 136.0, 133.6, 132.8, 132.5, 131.5, 131.4, 129.3, 128.8, 118.8, 110.6, 62.3, 52.3, 20.0, 14.1.

Ethyl 2-(2-methyl-5-(methoxycarbonyl)phenyl)-2-(phenylthio)acetate (2c): Prepared according to procedure B. The product was purified by column chromatography with pentane/diethyl ether (95:5 to 9:1) as eluent to afford the expected product (about 95% purity as estimated by NMR). Slightly yellow oil – Yield : 10% – $R_f = 0.56$ (cyclohexane/EtOAc, 7 : 3) – ^1H NMR (400 MHz, CDCl_3): δ (ppm) = 8.23 (d, $J = 1.8$ Hz, 1H), 7.86 (dd, $J = 8.0, 1.8$ Hz, 1H), 7.40-7.35 (m, 2H), 7.27-7.24 (m, 3H), 7.21 (d, $J = 7.9$ Hz, 1H), 5.11 (s, 1H), 4.20-4.10 (m, 2H), 3.89 (s, 3H), 2.38 (s, 3H), 1.17 (t, $J = 7.1$ Hz, 3H) – ^{13}C NMR (100 MHz, CDCl_3): δ (ppm) = 169.3, 165.9, 140.8, 133.8, 132.8, 132.1, 129.8, 128.9, 128.4, 128.1, 127.8, 127.3, 61.1, 51.9, 51.2, 18.9, 13.1.

2.2 Cloning and site directed mutagenesis

The synthetic codon-optimized DNA fragment (Life Technologies) coding for *Physeter macrocephalus* (sperm whale) myoglobin (UNIPROT P02185) with a C-terminal TEV protease cleavage site following by a His₆ tag (see supplementary material for nucleotide and amino acid sequences) was cloned into the pET-22b(+) vector (double-digested with NdeI and HindIII restriction enzymes) using the sequence- and ligation-independent cloning method.^[23] The corresponding pET-22b(+) Mb plasmids from positive clones were sequenced (GATC, Eurofins) to confirm the correct incorporation of the desired construct. L29S, H64V, and V68F mutations were introduced by site-directed mutagenesis on the pET-22b(+) Mb plasmid with corresponding primers (see supplementary material) and Q5 polymerase (NEB) according to the manufacturer's protocol. Successful incorporation of the desired mutations was confirmed by sequencing.

2.3 Protein expression and purification

pET-22b(+) Mb plasmids (encoding for WT sperm whale myoglobin or L29S-H64V-V68F mutant) were transformed into chemocompetent BL21(DE3)star *E. coli* cells. An overnight preculture (from a fresh transformant in LB medium supplemented with 100 $\mu\text{g}\cdot\text{mL}^{-1}$ ampicillin at 37°C and 200 rpm) was used to inoculate 5 x 200 mL cultures (1% v/v inoculum in TB medium supplemented with ampicillin 100 $\mu\text{g}\cdot\text{mL}^{-1}$) Cells were grown at 37°C (200 rpm) until OD_{600} reached 0.6 at which point protein expression was induced with IPTG (0.25 mM final concentration), aminolevulinic acid (0.3 mM final concentration) and $\text{Fe}(\text{SO}_4)_2(\text{NH}_4)_2\cdot 6\text{H}_2\text{O}$ (Mohr's salt, 50 μM final concentration). Cultures were further grown at 180 rpm and 25 °C for 20h. Cells were harvested by centrifugation at 4 °C (20 min at 6 000 g) and cell pellets stored at -20°C until further use. After thawing, cells were resuspended in running buffer (50 mM sodium phosphate (NaPi), 500 mM NaCl, 10 mM imidazole, pH = 8.0; 6 to 8 mL per gram of wet cell pellet) supplemented with a protease inhibitor (phenylmethanesulfonyl fluoride, 100 μM final concentration). Cells were lysed by sonication on ice (Bioblock Scientific (Vibracell) sonicator: 30% amplitude, 2 seconds on/8 seconds off for 12 min). Then, hemin (200 μM final concentration from a 20 mM stock solution in 10 mM NaOH) was added and cell debris was pelleted by centrifugation (30 min, 15 000 g). The supernatant was filtered over a 0.22- μm membrane and loaded at 4°C onto a Ni-NTA bed column pre-equilibrated with the running buffer. After washing with the running buffer, the enzyme was eluted with an increasing concentration (5 to 50%) of elution buffer (50 mM NaPi, 500 mM NaCl, 500 mM imidazole, pH = 8.0). Fractions containing purified enzyme as determined by SDS/PAGE were pooled and concentrated (5 kDa MWCO Vivaspin 20, Sartorius). After two successive buffer exchanges over a PD-10 column (GE Healthcare) with 50 mM potassium phosphate (KPi) (pH = 7.0) and final concentration, the proteins were stored at -80 °C. Myoglobin concentration was

determined using the extinction coefficient $\epsilon_{410} = 157\,000\text{ M}^{-1}\cdot\text{cm}^{-1}$.^[11] Protein yields were around 40 mg/L of culture for the wild-type myoglobin and the triple mutant.

2.4 Catalytic tests

Catalytic reactions were carried out under nitrogen atmosphere in a glovebox on a 300 μL scale. Buffer (15 μL from a 1 M stock solution – 50 mM final concentration) and enzyme (20 μM final concentration unless otherwise stated) were added to deoxygenated Milli-Q water in 1.5 mL vials in a glovebox. The mixture was left to deoxygenate for two hours. Substrates were then individually added (from a deoxygenated stock solution in THF, 5% (v/v) final concentration of organic co-solvent in catalytic tests) as well as reductant if required (0-10 mM from 20 mM or 200 mM deoxygenated stock solution). Reaction mixtures were stirred for 24 hours at 25°C and 200 rpm before being quenched. Depending on the analysis (yield or enantiomeric excess determination), different work-up (quenching) procedures were performed. For yield determination by HPLC analysis (see below), benzophenone or acetophenone (300 μL of a 2 mM solution stock in acetonitrile) was added as internal standard, followed by additional THF (600 μL). Solution mixtures were centrifugated (2 min, 14 000 g) and supernatants were analyzed by HPLC. For enantiomeric excess determination, reaction mixtures were extracted with ethyl acetate (1 x 900 μL). The organic layer was evaporated under reduced pressure before chiral HPLC analysis.

2.5 HPLC analysis

Substrate, products and by-products of catalytic reactions were identified by HPLC according to the retention time of corresponding standards.

Yield determination. High-performance liquid chromatography (HPLC) was carried out using an Alliance Waters 2695 HPLC with a 150/4 Nucleosil 100-5 C18 column fitted with a 4/3 Nucleosil 100-5 C18 guard column and a UV 996 detector. Product formation was quantified

by HPLC based on the calibration curve of the corresponding reference compound. The separation method (eluants, gradient program), compound retention times and calibration curves are given in the supplementary material.

Enantiomeric excess determination. Enantiomeric excess was determined by chiral HPLC with an Agilent 1260 Infinity unit (pump G1311B, autosampler G1329B, DAD G1315D) and Agilent OpenLAB Chemstation. Samples were dissolved in dichloromethane, injected on a chiral column, and detected with an UV detector at 254 nm and a circular dichroism detector at 254 nm. The separation method (type of column, eluants), compound retention times as well as significant chromatograms are given in the supplementary material.

2.6 Structure prediction and analysis

Structure predictions were performed with AlphaFold2^[24] on the online ColabFold notebook,^[25] including the relaxation of top 5 solutions using amber force fields. Active site cavity volumes for wild-type myoglobin and Mb(L29S,H64V,V68F) were calculated on the CASTp 3.0^[26] server using default parameters (Table S1, supplementary material).

3. Results and Discussion

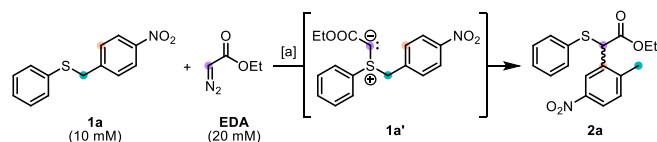
3.1 Myoglobin-catalyzed Sommelet-Hauser rearrangement

The [2,3]-sigmatropic Doyle-Kirmse and Sommelet-Hauser rearrangements of sulfonium ylides can be both initiated by a metallo-catalyzed carbene transfer.^[22] Trapping of the metallo-carbene species by a nucleophile (allyl thioether^[11] or benzyl thioether^[16] for the Doyle-Kirmse or Sommelet-Hauser rearrangement, respectively) leads to the formation of a sulfonium ylide. This reactive intermediate then undergoes a [2,3] sigmatropic rearrangement yielding the final product (Figure 1). Given the mechanistic similarities between the two rearrangements, we hypothesized that the myoglobin mutant Mb(L29S,H64V,V68F) optimized by Tyagi *et al.* as biocatalyst for the Doyle-Kirmse rearrangement^[11] could also be effective for its Sommelet-Hauser counterpart.

We first investigated Mb(L29S,H64V,V68F) for the Sommelet-Hauser rearrangement between *p*-nitrobenzyl phenyl thioether **1a** and ethyl diazoacetate (**EDA**) as reported by Xu *et al.* for hemin as catalyst.^[16] Under conditions similar to those used by the group of Fasan for the Doyle-Kirmse rearrangement,^[11] no trace of the Sommelet-Hauser product **2a** was observed (Table 1, entry 1). Substrate **1a** was stable in the presence of equimolar dithionite (10 mM) as performed in the assay but could be partially degraded with increasing dithionite/**1a** ratio. Product **2a** (3 mM) was however significantly degraded (over 95%) with 3.3 equivalent dithionite (10 mM). This reductant most likely reduces the nitro group of **1a** and **2a** into the corresponding anilines as already reported for other nitro aromatics,^[27] although further transformation cannot be excluded. Therefore, dithionite is not a suitable reducing agent for this reaction with **1a** as substrate, since **2a** can be degraded if formed. Interestingly, by using ascorbic acid instead, **2a** was formed with 59% yield and slight enantioinduction (Table 1, entry 2). Under identical conditions, wild-type myoglobin and hemin also catalyzed the reaction but with lower yields and no enantiomeric excess (Table 1, entry 3-4). These first results indicate

that myoglobin and its triple mutant are able to catalyze the Sommelet-Hauser rearrangement and that the protein scaffold seems important for enantioinduction as previously reported for the Doyle-Kirmse rearrangement.^[11]

Table 1. Myoglobin-catalyzed [2,3]-Sommelet-Hauser rearrangement between *p*-nitrobenzyl phenyl thioether and **EDA** via a reactive sulfonium ylide intermediate.^a



Entry	Catalyst	Reductant ^b	Yield (%) ^c	ee (%) ^c
1	Mb(L29S,H64V,V68F)	DT	0	-
2	Mb(L29S,H64V,V68F)	AA	59 ± 7	4 ± 1
3	Mb WT	AA	29 ± 8	0
4	Hemin	AA	40 ± 15	0

^a Reaction conditions: Catalyst (20 μM), reductant (10 mM), buffer (50 mM KPi, pH 8), N₂ atmosphere, 5% (v/v) THF, 25°C, 24 hours.

^b DT: dithionite, AA: ascorbic acid.

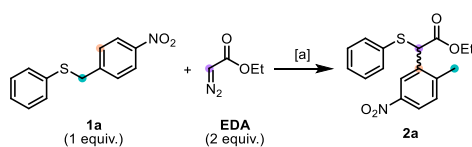
^c Experiments were done at least in triplicate.

3.2 Optimization of catalytic conditions

We then explored whether the catalytic performances of Mb(L29S,H64V,V68F) for the Sommelet-Hauser rearrangement could be improved with a particular focus on enantioinduction. Catalytic conditions were thus further optimized starting with reductant concentration. The limited decrease in yield (from 59 to 46%) in the absence of ascorbic acid

(Table 2, entry 1 and Figure S1, supplementary material) indicates that reductant is not strictly required. For carbene transfers, the reactive carbene intermediate is usually formed upon reaction of **EDA** with ferrous myoglobin obtained by reduction of the ferric species (generally with dithionite). However, Tyagi *et al.* suggested that ferric myoglobin could catalyze the Doyle-Kirmse rearrangement.^[11] as Fe(III) porphyrins for some reactions with diazoacetates.^[28] Similarly, this Sommelet-Hauser rearrangement seems to be initiated by reaction of the Fe(III) state of myoglobin with **EDA** although reduction to Fe(II) cannot be fully excluded. Indeed, the mild reductant **EDA**^[29] was previously reported to reduce *in situ* Fe(III) porphyrin catalysts into Fe(II) for carbene transfers.^[30] Strikingly, in the absence of ascorbic acid the enantiomeric excess increased to 16% (Table 2, entry 1 and Figure S1, supplementary material). We thus selected these conditions (no reductant) for further optimization.

Table 2. Reaction conditions optimization of the myoglobin-catalyzed [2,3]-Sommelet-Hauser rearrangement between *p*-nitrobenzyl phenyl thioether and **EDA**.^a



Entry	Catalyst	1a : EDA (mM)	Buffer (pH)	Yield (%) ^b	ee (%) ^b
1	Mb(L29S,H64V, V68F)	10:20	KPi (8.0)	46 ± 11	16 ± 1
2	Mb(L29S,H64V, V68F)	10:20	MES (6.5)	43 ± 8	28 ± 3
3	Mb(L29S,H64V, V68F)	1:2	KPi (8.0)	62 ± 11	35 ± 6
4	Mb(L29S,H64V, V68F)	1:2	MES (6.5)	73 ± 3	54 ± 4
5	Mb WT	1:2	MES (6.5)	59 ± 13	0

6	Hemin	1:2	MES (6.5)	30 ± 13	0
7	-	1:2	MES (6.5)	0	-

^a Reaction conditions: Catalyst (20 μM), Buffer (50 mM KPi or 2-(*N*-morpholino)ethane sulfonic acid (MES)), N₂ atmosphere, 5% (v/v) THF, 25°C, 24 hours.

^b Experiments were done at least in triplicate.

We investigated whether other parameters such as pH and reactant concentrations could also influence the efficiency of Mb(L29S,H64V,V68F) for the Sommelet-Hauser rearrangement. pH change had a negligible effect on Sommelet-Hauser reaction yield (Table 2, entry 2 and Figure S2, supplementary material). However, we observed a significant increase of the enantiomeric excess from 16 to 28% when decreasing the pH from 8.0 to 6.5 and no further evolution between 6.5 and 5.5 (Table 2, entry 2 and Figure S2, supplementary material). Finally, better yield (up to 73%) and enantiomeric excess (up to 54%) were obtained with lower reactant concentrations (Table 2, entries 3-4). Notably, diminishing **1a** and **EDA** concentrations down to 1 and 2 mM, respectively, resulted in a significant increase of the enantiomeric excess. The observed low enantioinduction at high reactant concentrations might be due to **2a** precipitation potentially leading to protein denaturation and/or to catalyst inactivation by carbene addition on the heme cofactor or on active site residues as previously observed.^[31,32] In the final reaction conditions (50 mM MES pH 6.5, 24 hour reaction 1 mM **1a** and 2 mM **EDA**), Mb(L29S,H64V,V68F) catalyzed the Sommelet-Hauser rearrangement between **1a** and **EDA** with 73% yield and 54% ee (Table 2, entry 4). Of note, wild-type myoglobin was more active than hemin under these conditions (Table 2, entries 5-6).

Asymmetric [2,3]-sigmatropic rearrangements of sulfonium ylides generated from metal carbenes remain challenging.^[22,33,34] Indeed, differentiation of the sulfur atom lone pairs upon reaction with the metallocarbene leading to the formation of a chiral sulfonium ylides is

intricate. Additionally, sulfonium ylides can dissociate from the metal,^[22,33,34] which facilitates their racemization. Accordingly, only two examples of asymmetric Sommelet-Hauser rearrangement were reported so far using either Ni(II)- or Cu(I)-based catalysts.^[35,36] In our case, the myoglobin scaffold around the iron-heme prosthetic group (Figure 2) is crucial for the stereocontrol of the Sommelet-Hauser rearrangement as evidenced by the lack of enantiomeric excess when using wild-type myoglobin as catalyst (Table 2, entry 5) This striking difference between wild-type myoglobin and Mb(L29S,H64V,V68F) was also reported for the Doyle-Kirmse rearrangement.^[11] The three mutations reshape the active site on the distal side of the heme prosthetic group (Figure 2). For instance, the active site volume is estimated to increase by 50 to 60 Å³ in Mb(L29S,H64V,V68F) as compared with wild-type myoglobin (Table S1, supplementary material). Notably, valine substitution of the distal histidine (H64) widens the opening of the distal heme pocket (Figure 2B and 2C) and enhances the hydrophobicity close to the heme as already reported.^[37] The L29S mutation increases the height of the active site cavity (Figure 2A). The side chain of F68 is oriented toward the upper back right of the pocket, which redesigns the shape of this side of the active site (Figure 2A). Overall, these mutations allow better substrate accessibility and reactivity at the heme iron center. We hypothesize that the size of the cavity is key in inducing a good enantiomeric excess for Mb(L29S,H64V,V68F). Due to steric hindrance at the entrance of the active site of wild-type myoglobin, the sulfonium ylide intermediate **1a'** most likely racemizes in solution after being formed, subsequently leading to racemic **2a**. On the contrary, the sigmatropic rearrangement of intermediate **1a'** is expected to occur within or close to the accessible and wider active site cavity of the triple mutant, which limits the intermediate racemization and thus induces enantioselectivity.

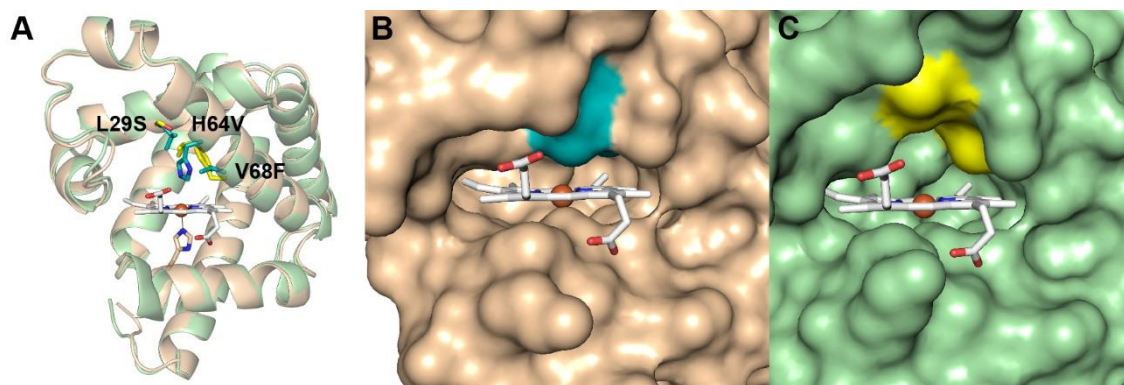


Figure 2. (A) Superimposition of wild-type myoglobin crystal structure (PDB 2MBW, colored in wheat and highlighted residues in teal) and predicted structure of Mb(L29S,H64V,V68F) (colored in light green and highlighted residues in yellow). (B) and (C) Side-by-side comparison of their active site cavity (same color code as in (A)).

3.3 Substrate scope

To study the scope of this reaction, we evaluated a set of substrates with different *para*-substituents on the benzyl aromatic ring (Table 3). Products **2a-c** were obtained from substrates **1a-c** with highly electron-withdrawing substituents in good yield (47 to 73%) and enantiomeric excess (31 to 55%) (Table 3, entries 1-3). On the contrary, no conversion was observed for substrates **1d-f** with weakly electron-withdrawing substituents (Table 3, entries 4-6). Therefore, the electronic effects of the *para*-substituent on the benzyl group play a crucial role in the reactivity as previously reported with hemin as catalyst.^[16] Products **2a-c** formed upon biocatalytic Sommelet-Hauser rearrangement with Mb(L29S,H64V,V68F) all exhibit a dextrorotatory major enantiomer, which suggests the same absolute configuration. As [1,2]- and [2,3]-sigmatropic rearrangements of sulfonium ylides often compete,^[16,38] we also analyzed if [1,2]-Stevens products could be formed under the reaction conditions. However, none was observed for all the tested substrates **1a-f**, highlighting the selectivity of this catalytic reaction toward the Sommelet-Hauser rearrangement. Oxonium and ammonium ylides are also prone to

6	OMe	2f	0	-	24 ± 8
			0 ^c	- ^c	(31 ± 2) ^c
7	NO ₂	2g	0	-	- ^e
8	NO ₂	2h	0	-	- ^e

^a Reaction conditions: Mb(L29S,H64V,V68F) (20 μM), MES (50 mM, pH 6.5), N₂ atmosphere, 5% (v/v) 5% (v/v) THF, 25°C, 24 hours.

^b Experiments were done at least in triplicate.

^c Same reaction conditions but with 100 mM NaCl.

^d Estimated yield by subtracting the yield of **3** from the conversion of **1c** as standard **2c** was not completely pure (see Experimental section).

^e Formation of the corresponding debenzoylation products was not studied.

No **EDA** dimerization products (diethyl fumarate or maleate) were formed over the course of the reaction. The only identified by-product is ethyl 2-(phenylthio)acetate **3**, the yield of which increases with the electron donating character of the substrate benzyl *para*-substituent (from 1.4% for R = NO₂ to *ca.* 30% for R = Cl, H, or OMe, Table 3, entries 1-6). Previous reports have shown that chloride ions favor the dealkylative interception of sigmatropic rearrangements.^[19,39] We thus performed the reaction in the presence of 100 mM NaCl to better understand the mechanism underlying the formation of **3**. The yield of **3** increased in the presence of chloride ions, which supports that debenzoylation leading to **3** is subsequent to a nucleophilic addition on the sulfonium ylide intermediate (Table 3, entry 1-6). In the absence of chloride ions, water can act as nucleophile as already proposed in the literature,^[18] accounting for the formation of debenzoylation product **3**. Of note, we observed a slightly better enantioinduction in the presence of NaCl despite a similar or lower yield for **2c** or **2a-b**,

respectively (Table 3, entries 1-3). This result might arise from a decreased solubility of the sulfonium ylide intermediate in solution and its subsequent rearrangement closer to or within the enzyme active site.

4. Conclusion

In conclusion, we provide the first evidence of a biocatalytic [2,3]-sigmatropic Sommelet-Hauser rearrangement initiated by a carbene transfer. We capitalized on the triple mutant Mb(L29S,H64V,V68F) reported to catalyze the [2,3]-sigmatropic Doyle-Kirmse rearrangement,^[11] the mechanism of which is close to that of Sommelet-Hauser. Namely, Mb(L29S,H64V,V68F) catalyzes the asymmetric Sommelet-Hauser rearrangement of benzyl phenylthioethers into the corresponding functionalized thioethers with yields up to 73% and enantiomeric excess up to 59%. Mb(L29S,H64V,V68F) is therefore one of the few catalysts for the asymmetric Sommelet-Hauser rearrangement and operates under greener conditions (*i.e.* in aqueous solution) contrary to others.^[35,36] As for hemin as catalyst,^[16] only benzyl phenylthioethers bearing high electron-withdrawing substituents on their benzyl group are substrates of myoglobin for this sigmatropic rearrangement. Strikingly, myoglobin-catalyzed Sommelet-Hauser rearrangement does not require the use of reductant to prime the reaction at its heme iron and exhibit different catalytic behaviors depending on reaction conditions, raising questions about its catalytic mechanism that could be further addressed. Overall, this work expands the toolbox of enzymes for the catalysis of abiological asymmetric reactions and provides the fourth example of sigmatropic rearrangements catalyzed by engineered carbene and nitrene transferases.^[11-13] Further engineering of the triple mutant Mb(L29S,H64V,V68F) or other hemoproteins such as cytochrome P450s by directed evolution could lead to robust biocatalysts with improved catalytic efficiency, asymmetric induction, and substrate scope for the [2,3]-sigmatropic Sommelet-Hauser rearrangement. Such biocatalysts might be useful tools for the synthesis of functionalized thioethers that are important pharmacophores found in pharmaceuticals and agrochemicals.^[40-42]

CRedit authorship contribution statement:

Manon Pujol: Methodology, Investigation, Validation, Writing – original draft. **Lison Degeilh:** Investigation. **Thibault Sauty de Chalon:** Investigation. **Marius Réglie:** Methodology, Supervision, Validation, Writing – review and editing. **A. Jalila Simaan:** Methodology, Supervision, Validation, Writing – review and editing. **Christophe Decroos:** Funding acquisition, conceptualization, Methodology, Supervision, Validation, Writing – review and editing

Declaration of competing interest:

The authors declare no conflict of interest.

Acknowledgements:

This project was supported by CNRS, Aix Marseille Université, and the Agence Nationale de la Recherche (Abiozyme, ANR-18-CE07-0003) including a doctoral fellowship to MP. Dr. Nicolas Vanthuylne, Marion Jean and Carla Deschodt (Plateforme de Chromatographie Chirale, iSm2) are gratefully acknowledged for enantiomeric excess determination by chiral chromatography.

Appendix A. Supplementary data:

Supplementary material to this article can be found online at <https://doi>

References

- [1] A. C. Jones, J. A. May, R. Sarpong and B. M. Stoltz, *Angew. Chem. Int. Ed.*, **2014**, *53*, 2556–2591.
- [2] J. B. Sweeney, *Chem. Soc. Rev.*, **2009**, *38*, 1027–1038.
- [3] Y. Liu, X. Liu and X. Feng, *Chem. Sci.*, **2022**, *13*, 12290–12308.
- [4] R. B. Woodward and R. Hoffmann, *J. Am. Chem. Soc.*, **1965**, *87*, 2511–2513.
- [5] a) A. Lee, J. D. Stewart, J. Clardy and B. Ganem, *Chem. Biol.*, **1995**, *2*, 195–203. b) L. W. Shipman, D. Li, C. A. Roessner, A. I. Scott and J. C. Sacchettini, *Structure*, **2001**, *9*, 587–596. c) D. E. Künzler, S. Sasso, M. Gamper, D. Hilvert and P. Kast, *J. Biol. Chem.*, **2005**, *280*, 32827–32834. d) C. Diederich, M. Leybold, M. Culka, H. Weber, R. Breinbauer, G. M. Ullmann and W. Blankenfeldt, *Sci. Rep.*, **2017**, *7*, 6272. e) C.-C. Chen, X. Hu, X. Tang, Y. Yang, T.-P. Ko, J. Gao, Y. Zheng, J.-W. Huang, Z. Yu, L. Li, S. Han, N. Cai, Y. Zhang, W. Liu and R.-T. Guo, *Angew. Chem. Int. Ed.*, **2018**, *57*, 15060–15064. f) S. A. Newmister, S. Li, M. Garcia-Borràs, J. N. Sanders, S. Yang, A. N. Lowell, F. Yu, J. L. Smith, R. M. Williams, K. N. Houk and D. H. Sherman, *Nat. Chem. Biol.*, **2018**, *14*, 345–351. g) Z. Liu, W. Li, P. Zhang, J. Fan, F. Zhang, C. Wang, S. Li, Y. Sun, S. Chen and W. Yin, *Acta Pharm. Sin. B*, **2021**, *11*, 3655–3664.
- [6] a) J. A. Latham, B. P. Branchaud, Y.-C. J. Chen and C. Walsh, *Chem. Commun.*, **1986**, 528–530. b) N. Thevenet, V. de la Sovera, M. A. Vila, N. Veiga, D. Gonzalez, G. Seoane and I. Carrera, *Org. Lett.*, **2015**, *17*, 684–687. c) V. de la Sovera, P. Garay, N. Thevenet, M. A. Macías, D. González, G. Seoane and I. Carrera, *Tetrahedron Lett.*, **2016**, *57*, 2484–2487. d) V. de la Sovera, S. Martínez, D. Umpiérrez, M. A. Vila, D. Gonzalez, G. Seoane, N. Veiga and I. Carrera, *Eur. J. Org. Chem.*, **2022**, 2022, e202101156.
- [7] a) D. Gonzalez, V. Schapiro, G. Seoane, T. Hudlicky and K. Abboud, *J. Org. Chem.*, **1997**, *62*, 1194–1195. b) P. Szcześniak, A. Październiak-Holewa, U. Klimczak and S. Stecko, *J.*

- Org. Chem.*, **2014**, *79*, 11700–11713. c) K. Panigrahi, G. A. Applegate, G. Malik and D. B. Berkowitz, *J. Am. Chem. Soc.*, **2015**, *137*, 3600–3609. d) B. Skrobo, N. E. Schlörer, J.-M. Neudörfl and J. Deska, *Chem. Eur. J.*, **2018**, *24*, 3209–3217. e) V. K. Tiwari, D. R. Powell, S. Broussy and D. B. Berkowitz, *J. Org. Chem.*, **2021**, *86*, 6494–6503.
- [8] F. Schwizer, Y. Okamoto, T. Heinisch, Y. Gu, M. M. Pellizzoni, V. Lebrun, R. Reuter, V. Köhler, J. C. Lewis and T. R. Ward, *Chem. Rev.*, **2018**, *118*, 142–231.
- [9] Y. Yang and F. H. Arnold, *Acc. Chem. Res.*, **2021**, *54*, 1209–1225.
- [10] a) D. Hilvert, S. H. Carpenter, K. D. Nared and M. T. Auditor, *Proc. Natl. Acad. Sci. U.S.A.*, **1988**, *85*, 4953–4955. b) A. C. Braisted and P. G. Schultz, *J. Am. Chem. Soc.*, **1994**, *116*, 2211–2212. c) Z. S. Zhou, N. Jiang and D. Hilvert, *J. Am. Chem. Soc.*, **1997**, *119*, 3623–3624. d) Z. S. Zhou, A. Flohr and D. Hilvert, *J. Org. Chem.*, **1999**, *64*, 8334–8341.
- [11] V. Tyagi, G. Sreenilayam, P. Bajaj, A. Tinoco and R. Fasan, *Angew. Chem. Int. Ed.*, **2016**, *55*, 13562–13566.
- [12] C. K. Prier, T. K. Hyster, C. C. Farwell, A. Huang and F. H. Arnold, *Angew. Chem. Int. Ed.*, **2016**, *55*, 4711–4715.
- [13] D. C. Miller, R. G. Lal, L. A. Marchetti and F. H. Arnold, *J. Am. Chem. Soc.*, **2022**, *144*, 4739–4745.
- [14] M. J. Weissenborn and R. M. Koenigs, *ChemCatChem*, **2020**, *12*, 2171–2179.
- [15] X. Xu, C. Li, Z. Tao and Y. Pan, *Adv. Synth. Catal.*, **2015**, *357*, 3341–3345.
- [16] X. Xu, C. Li, M. Xiong, Z. Tao and Y. Pan, *Chem. Commun.*, **2017**, *53*, 6219–6222.
- [17] X. Xu, C. Li, Z. Tao and Y. Pan, *Green Chem.*, **2017**, *19*, 1245–1249.
- [18] X. Yan, C. Li, X. Xu, Q. He, X. Zhao and Y. Pan, *Tetrahedron*, **2019**, *75*, 3081–3087.
- [19] X. Yan, C. Li, X. Xu, X. Zhao and Y. Pan, *Adv. Synth. Catal.*, **2020**, *362*, 2005–2011.
- [20] I. Aviv and Z. Gross, *Chem. Eur. J.*, **2008**, *14*, 3995–4005.
- [21] V. F. Batista, D. C. G. A. Pinto and A. M. S. Silva, *ACS Catal.*, **2020**, *10*, 10096–10116.

- [22] Y. Zhang and J. Wang, *Coord. Chem. Rev.*, **2010**, *254*, 941–953.
- [23] J.-Y. Jeong, H.-S. Yim, J.-Y. Ryu, H. S. Lee, J.-H. Lee, D.-S. Seen and S. G. Kang, *Appl. Environ. Microbiol.*, **2012**, *78*, 5440–5443.
- [24] J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Žídek, A. Potapenko, A. Bridgland, C. Meyer, S. A. A. Kohli, A. J. Ballard, A. Cowie, B. Romera-Paredes, S. Nikolov, R. Jain, J. Adler, T. Back, S. Petersen, D. Reiman, E. Clancy, M. Zielinski, M. Steinegger, M. Pacholska, T. Berghammer, S. Bodenstein, D. Silver, O. Vinyals, A. W. Senior, K. Kavukcuoglu, P. Kohli and D. Hassabis, *Nature*, **2021**, *596*, 583–589.
- [25] M. Mirdita, K. Schütze, Y. Moriwaki, L. Heo, S. Ovchinnikov and Martin Steinegger, *Nat. Methods*, **2022**, *19*, 679–682.
- [26] W. Tian, C. Chen, X. Lei, J. Zhao and J. Liang, *Nucleic Acids Res.*, **2018**, *46*, W363–W367.
- [27] R. Kaplánek and V. Krchnák, *Tetrahedron Lett.*, **2013**, *54*, 2600–2603.
- [28] a) L. K. Baumann, H. M. Mbuvi, G. Du and L.K. Woo, *Organometallics*, **2007**, *26*, 3995–4002 b) I. Aviv and Z. Gross, *Chem. Eur. J.*, **2008**, *14*, 3995–4005.
- [29] R. G. Salomon and J. K. Kochi, *J. Am. Chem. Soc.*, **1973**, *95*, 3300–3310.
- [30] a) J. R. Wolf, C. G. Hamaker, J.-P. Djukic, T. Kodadek and L. W. Woo, *J. Am. Chem. Soc.*, **1995**, *117*, 9194–9199. b) T.-S. Lai, F.-Y. Chan, P.-K. So, D.-L. Ma, K.-Y. Wong and C.-M. Che, *Dalton Trans.*, **2006**, 4845–4851. c) A. Rioz-Martínez, J. Oelerich, N. Ségaud and G. Roelfes. *Angew. Chem. Int. Ed. Engl.*, **2016**, *55*, 14136–14140.
- [31] H. Renata, R. D. Lewis, M. J. Sweredoski, A. Moradian, S. Hess, Z. J. Wang and F. H. Arnold, *J. Am. Chem. Soc.*, **2016**, *138*, 12527–12533.
- [32] M. W. Wolf, D. A. Vargas and N. Lehnert, *Inorg. Chem.*, **2017**, *56*, 5623–5635.
- [33] K. J. Hock and R. M. Koenigs, *Angew. Chem. Int. Ed.*, **2017**, *56*, 13566–13568.
- [34] S. Jana, Y. Guo and R. M. Koenigs, *Chem. Eur. J.*, **2021**, *27*, 1270 – 1281.

- [35] X. Lin, W. Yang, W. Yang, X. Liu and X. Feng, *Angew. Chem. Int. Ed.*, **2019**, 58, 13492–13498.
- [36] S.-S. Li and J. Wang, *J. Org. Chem.*, **2020**, 85, 12343–12358.
- [37] A. Tinoco, Y. Wei, J.-P. Bacik, D.M. Carminati, E.J. Moore, N. Ando, Y. Zhang and R. Fasan. *ACS Catal.*, **2019**, 9, 1514–1524.
- [38] V.N. Nair and U.K. Tambar, *Org. Biomol. Chem.*, **2022**, 20, 3427–3439.
- [39] C. Empel, K. J. Hock and R. M. Koenigs, *Chem. Commun.*, **2019**, 55, 338–341.
- [40] E. A. Ilardi, E. Vitaku and J. T. Njardarson, *J. Med. Chem.*, **2014**, 57, 7, 2832–2842.
- [41] H. Chen, W. Jiang, and Q. Zeng, *Chem. Rec.*, **2020**, 20, 1269–1296.
- [42] P. Li, Y. Yang, X. Wang and X. Wu, *J. Heterocyclic Chem.*, **2021**, 58, 1225–1251.