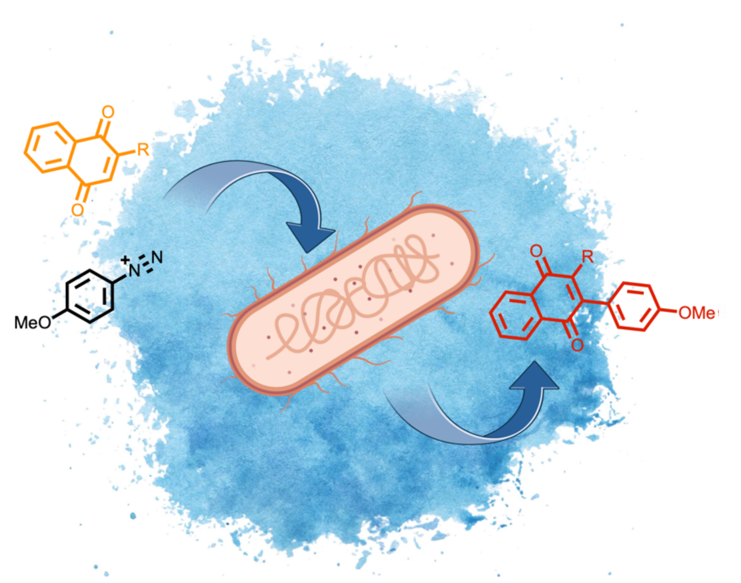
Master Dissertation



Bond-forming synthetic chemistry promoted by bacteria redox potential

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Santiago de Compostela
30th January, 2025

MASTER DISSERTATION

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Certify: this report attached, titled 'Bond-forming synthetic chemistry promoted by bacteria redox potential' and reported by David Montoto Pintos relates his work carried out under their direction and at their laboratories (CiQUS). Also, by considering this report as his master dissertation, they authorize its public defense at the University of Santiago de Compostela.

Santiago de Compostela, January 21st, 2025

Acknowledgements

Thanks to the directors of this Master dissertation, María Tomás Gamasa and José Luis Mascareñas Cid, for their invaluable help, support and dedication towards the development of this research work, my academic formation and my personal growth.

Thanks to all the members of the MetBioCat research group for their constant teaching and assistance. Especial thanks to Celia Mayer Mayer, Alba Casas Pais, Xulián Fernández González, Jesús Fernando Salgado Barca, Alejandra Vale Gómez, Álvaro Maza Barón and Adrián López Rivas for key contributions in the realization of this work.

Thanks to the CiQUS support staff for making possible to develop scientific research of the highest level in an environment of the best human quality. Special thanks to Arcadio Guerra Fandiño for HPLC-MS analysis, and María del Carmen Mosquera Losada and Paula Munín Cruz for NMR technical assistance.

Thanks to the ChemBioMat Master organizers and professors for giving me the opportunity for developing this research project while deepening into my scientific formation. This work is a continuation of my previous Bachelor's Degree in Chemistry and in Biology final projects, which were only possible with the help of Jesús Fernando Salgado Barca, Xulián Fernández González, Alba Casas Pais, Beatriz Orosa Puente, María Tomás Gamasa and José Luis Mascareñas Cid.

Figures were made with BioRender (www.biorender.com) and ChemDraw Professional (Revvity Signals Software, Inc., version 23.1.1).

Abbreviations

^{1}H-NMR	Proton nuclear magnetic resonance
^{13}C-NMR	Carbon-13 nuclear magnetic resonance
ATP	Adenosine triphosphate
ATRP	Atom transfer radical polymerization
CuAAC	Copper-catalyzed azide-alkyne cycloaddition
DMSO	Dimethyl sulfoxide
E. coli	*Escherichia coli*
EET	Extracellular electron transfer
EPR	Electron paramagnetic resonance
equiv.	Equivalent
ETC	Electron transport chain
HPLC	High-performance liquid chromatography
HPLC-MS	High-performance liquid chromatography coupled to mass spectrometry
L. lactis	*Lactococcus lactis*
LB	Lysogeny broth
LED	Light-emitting diode
Mtr	Metal-reducing
MPLC	Medium-pressure liquid chromatography
NAD^{+}	Nicotinamide adenine dinucleotide (oxidized form)
NADH	Nicotinamide adenine dinucleotide (reduced form)
NAD(P)H	Nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate (reduced form)
OD_{600}	Optical density measured at 600 nm
P. aeruginosa	*Pseudomonas aeruginosa*
PBS	Phosphate-saline buffer
RAFT	Reversible addition-fragmentation chain transfer
r.t.	Room temperature (approx. 21 ºC)
bipy	Bipyridine
SD	Standard deviation
S. enterica	*Salmonella enterica*

SET Single electron transfer

S. oneidensis *Shewanella oneidensis*

STC Small tetraheme cytochrome

TEMPO (2,2,6,6-Tetramethylpiperidin-1-yl)oxyl

UV Ultraviolet

UV-Vis Ultraviolet-visible

v/v Volume/volume

P values meaning:

ns not significative

* ($p<0.05$)

** ($p<0.01$)

*** ($p<0.001$)

**** ($p<0.0001$)

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Summary

The development of non-natural, synthetic reactions that interact with the metabolism of living systems is an emerging field at the interface of chemical and biological synthesis. These reactions would give access to new sustainable synthetic routes for molecules of interest, and have potential applications in biomedicine and biology. In this Master dissertation, we demonstrate that the redox metabolism of live bacteria can be harnessed to promote non-natural bond-forming radical reactions. We show that the electrogenic bacterium *Shewanella oneidensis* MR-1 and the model bacterium *Escherichia coli* DH5α can reduce aryl diazonium salts to promote the Meerwein arylation of naphthoquinones using electron transfer and radical chain mechanisms. We have also tested our methodology for the bacteria-promoted synthesis of benzothiophenes, opening the door to structures of biomedical interest.

This work was carried out in the Centro Singular de Química Biolóxica e Materiais Moleculares (CiQUS) of the University of Santiago de Compostela (USC) between September 2nd, 2024 and January 21st, 2025.

Manuscript of activities

1. Introduction

1.1. Interfacing abiotic reactions with the metabolism

1.1.1. Chemical and biological synthesis

Chemical and biological synthesis represent complementary strategies for synthetizing small molecules and polymers of interest. Chemical synthesis offers an ample array of reactions to produce all kind of molecules, but usually relies on fossil fuel-derived, non-renewable reagents. In contrast, biological synthesis employs enzymatic reactions present in the metabolism of living organisms to transform renewable feedstocks into high value chemicals, but its product scope is restricted by those biochemical reactions already present in Nature.[1,2] This constrain can be partly relieved by metabolic engineering, that enables to rationally design new metabolic pathways in heterologous hosts,[3] or by directed evolution of enzymes, that allows to incorporate non-natural reactivity.[4] Nonetheless, the chemical space accessible to biological systems remains limited when compared with that of chemical synthesis.

By interfacing chemical and biological synthesis, it may be possible to widen the scope of metabolic reactions with the more ample repertoire of chemical synthesis to access new sustainable synthetic routes to molecules of interest (Figure 1).[5] Additionally, these reactions may have biomedical applications —such as in new therapies and diagnostic imaging—, and in basic research in biology, providing valuable tools for interrogating and manipulating biological systems.[6]

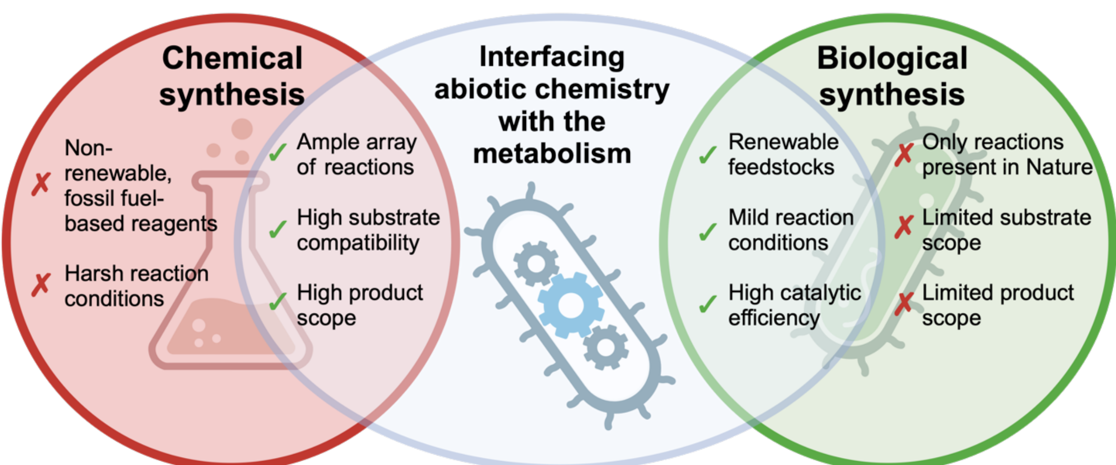


Figure 1. Interfacing chemical and biological synthesis.

1.1.2. Strategies for interfacing chemical and biological synthesis

Several strategies have been developed to incorporate new reactivity to biological systems. These may be based on enzymatic chemistry, or on abiotic, non-enzymatic reactions that interface with the metabolism.[1,5]

Naturally occurring enzymes can be modified through protein engineering to access non-natural reactions[7] by modifying their amino acid sequence either by rational design or directed evolution.[8] Another approach to extend the scope of enzymatic catalysts, especially towards new reaction mechanisms, is the design of artificial metalloenzymes that incorporate non-natural metal cofactors into protein scaffolds.[9]

On the other hand, it is possible to interface the metabolism with abiotic chemical reactions. Exogenous, abiotic catalysts may facilitate non-natural reactivity of natural metabolites and enable new synthetic pathways that would be inaccessible to metabolic transformations alone.[5] For instance, Fe(III) has been used as a biocompatible catalyst to bridge a native and a heterologous metabolic pathway in a *Lactococcus lactis* strain: Fe(III) catalyzes the oxidative decarboxylation of α-acetolactate (a metabolite that *L. lactis* produces from glucose) to diacetyl, that then can be reduced to (S,S)-2,3-butanediol by two successive heterologous reductases.[10]

Recent work is also starting to show that it is possible to use the intrinsic redox processes of living systems to promote abiotic reactions of non-natural reactants.[5]

1.2. Abiotic reactions promoted by biological redox processes

1.2.1. Metabolic redox processes

Living cells maintain an overall reducing redox potential as part of their homeostasis[11] that depends on the contribution of several low weight reducing biomolecules like NAD(P)H and glutathione. The redox state varies inside the different cellular organelles, and it also depends on the cell cycle stage, cellular lineage and tissue physiological state.[12] Furthermore, bacteria are known to generate a reducing redox potential in the medium during growth.[13] Eucaryotic cells possess the electron transport chain (ETC) in the inner membrane of mitochondria, the organelles responsible for cellular respiration. The ETC comprises a series of enzymatic complexes and coenzymes that transfer electrons from reducing biomolecules (NADH and succinate) to oxygen, while the released free energy is harnessed by the cell to generate ATP through oxidative phosphorylation.[14]

A similar mechanism is found in aerobic bacteria, that also perform aerobic cellular respiration. Moreover, some bacteria use anaerobic respiration, by employing respiratory

terminal electron acceptors different to oxygen. Those include inorganic oxidizing anions like NO_3^- and SO_4^{2-}, metallic cations like Fe(III), minerals, and organic molecules like fumarate.[15] Bacteria that perform anaerobic respiration exhibit extracellular electron transfer (EET) mechanisms for moving electrons through the microbial cell envelopes to external electron acceptors. These pathways have been well characterized in some model organisms, but the molecular mechanism by which the EET takes place is still not fully understood.[16]

Shewanella oneidensis MR-1 is one of the more studied bacteria that performs EET. It is a facultative anaerobic bacterium that can perform aerobic respiration, but in anaerobic conditions may reduce alternative electron acceptors, such as minerals containing Mn(III), Mn(IV) or Fe(III).[17] Since it does not uptake the reduced metal ions, it is considered a dissimilatory metal-reducing microorganism.[16] *S. oneidensis* performs EET to these acceptors through the Mtr (metal-reducing) pathway, that comprises a set of multi-heme *c*-cytochromes (CymA, Fcc_3, MtrA, MtrC, OmcA and STC) and porin-like MtrB (Figure 2). CymA, a cytoplasmic transmembrane protein, transfers electrons from the quinone pool of the cytoplasmic membrane to periplasmic Fcc_3 and STC. MtrA, MtrB, MtrC and OmcA form a transmembrane complex that is located in the outer membrane and in bacterial nanowires—micrometer-long extensions of the outer membrane and the periplasm.[18] MtrA faces the periplasm, where it oxidizes Fcc_3 and STC and transfers the electrons to MtrC and OmcA. These two *c*-cytochromes possess extracellular, solvent-exposed heme groups that are hypothesized to transfer electrons to minerals and other acceptors,[16] either directly or through bound flavin cofactors.[19] The species of the genus *Shewanella* can reduce an ample range of terminal electron acceptors, including metallic cations, inorganic ions and even organic molecules like fumarate, glycine[20] or DMSO,[21] which has been attributed to its high number of different *c*-type cytochromes (more than 40).[17]

EET is being explored as a promising green and sustainable tool for chemical synthesis, energy production, CO_2 fixation and bioremediation.[22]

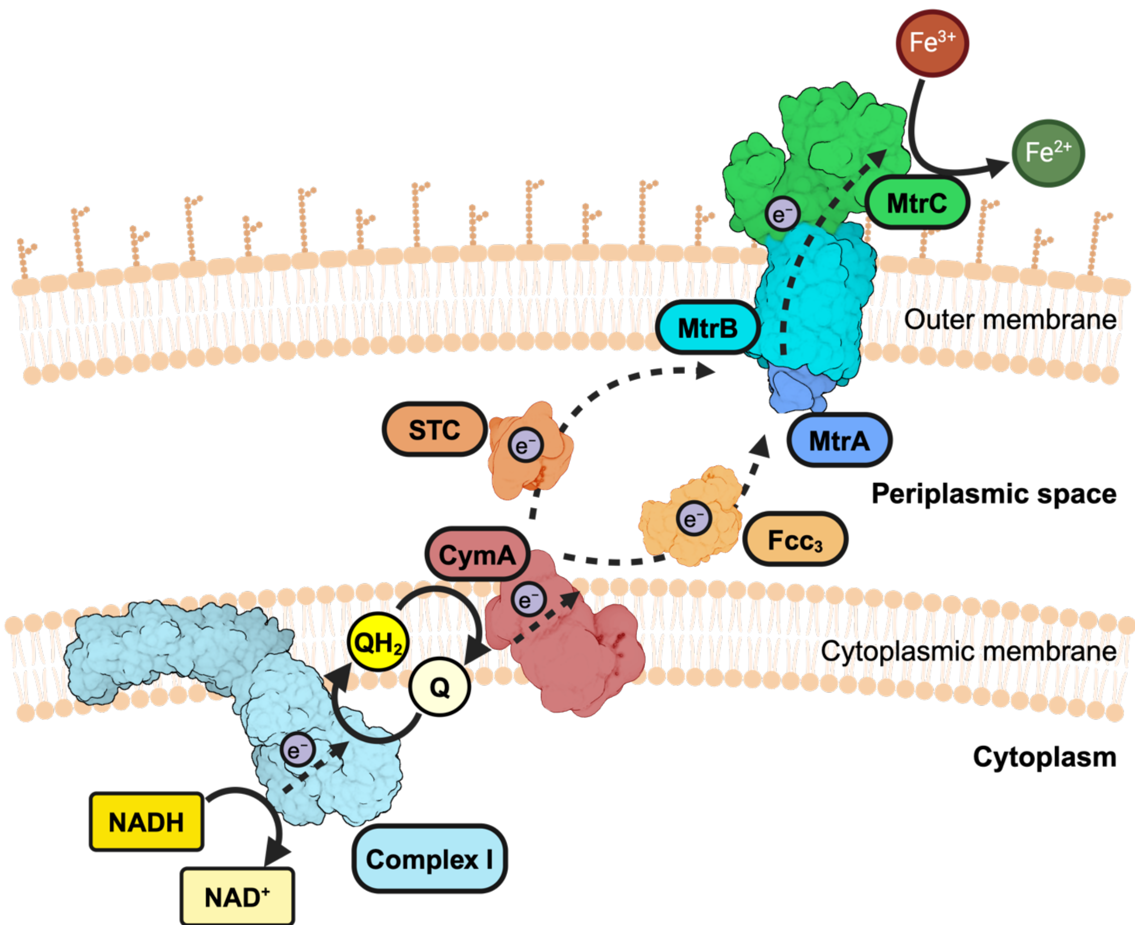


Figure 2. Metal reducing (Mtr) extracellular electron transfer (EET) pathway of Shewanella oneidensis *MR-1.*

1.2.2. Application of biological redox processes to abiotic reactions

This electron donating potential of living cells raises the question on whether it could be harnessed for promoting non-natural chemical reactions. Until now, cell-mediated electron transfer has been used mainly to trigger radical polymerization reactions.[5]

In a pioneering work, Alexander *et al.*[23] employed the reducing redox potential generated by bacteria in their growth medium to initiate a Cu-catalyzed atom transfer radical polymerization (ATRP) of acryloyl monomers. Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* were used to reduce Cu(II) to Cu(I) in anaerobic conditions, which in turn reacted with an initiator agent by abstracting a bromide atom to generate the propagating radical chain (Figure 3a). The same strategy was applied to promote a Cu-catalyzed azide-alkyne cycloaddition (CuAAC), the archetypical 'click' reaction,[24] to label polymers with fluorescent probes. In that case, bacteria were used to initiate polymerization of monomers that contained acetylenic groups, which were then labeled *in situ* with an azide-functionalized pro-fluorophore (Figure 3b). The Cu(I) necessary to catalyze the CuAAC was again generated by the intrinsic reducing redox potential of bacteria.

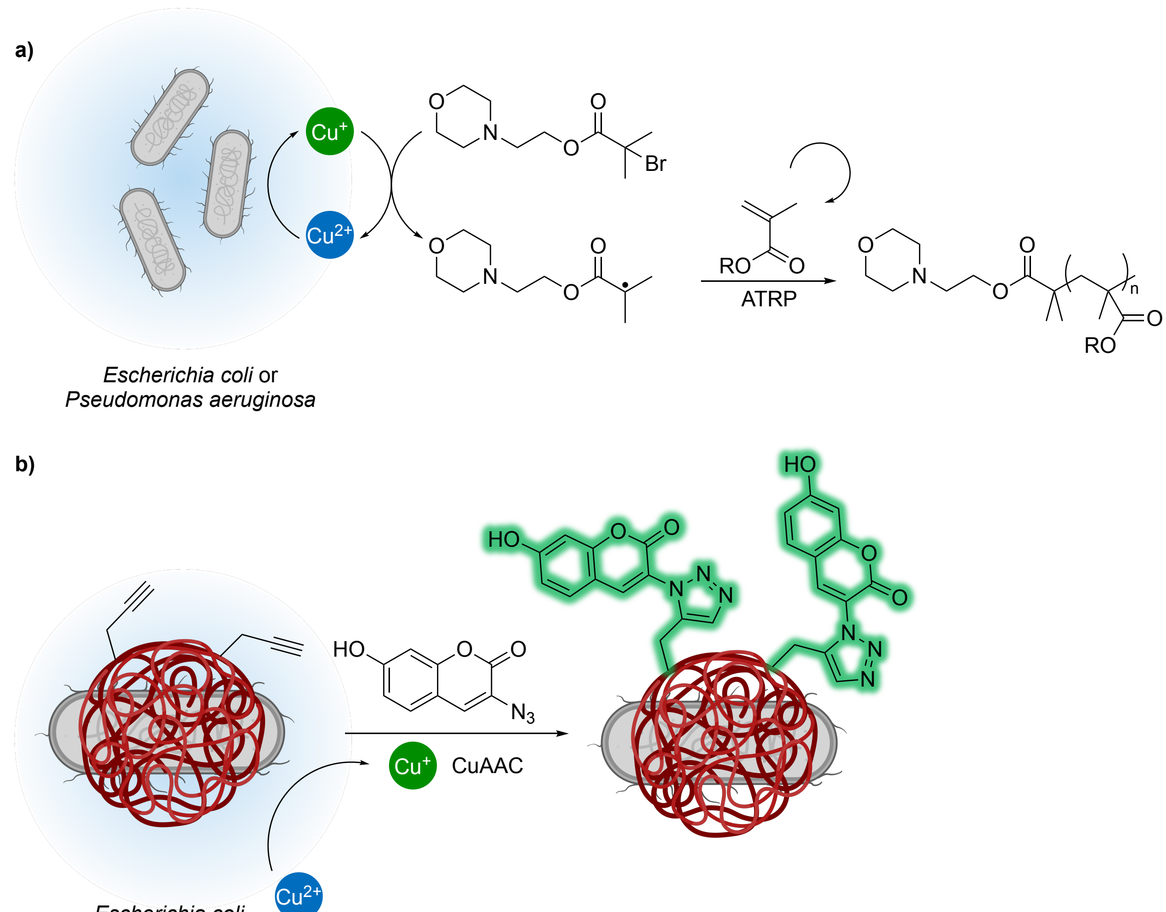


Figure 3. Cu(I)-mediated reactions promoted by bacteria redox potential.[23] (a) Cu-catalyzed atom transfer radical polymerization (ATRP). (b) Cu-catalyzed azide-alkyne cycloaddition (CuAAC).

Subsequently, Rawson *et al.*[25] reported Fe-mediated ATRP of acryloyl monomers initiated by *E. coli*, *Cupriavidus metallidurans* and *Clostridium sporogenes*. In anaerobic conditions, these bacteria reduced Fe(III) to Fe(II), which then generated the polymerization initiator. In this case, the authors demonstrated that living bacteria were needed for the reaction to proceed, and that the reaction conditions did not affect bacteria viability at least 24 h after performing the reaction.

The electroactive bacteria *Shewanella oneidensis* MR-1 has been employed successfully by Keitz *et al*.[26] to promote a similar Cu-, Fe- and Co-catalyzed ATRP of poly(ethylene glycol) methyl ether methacrylate monomers (Figure 4). The reaction only took place in presence of live, metabolically active bacteria under anaerobic conditions. Heat-killed bacteria did not show polymerization activity, and neither did controls performed with supernatant of *Shewanella* cultures, proving that secreted reducing factors, like flavins or glutathione, were not responsible for the polymerization. The role of the EET mechanism of *Shewanella* was demonstrated with knockout mutants for the outer membrane cytochromes MtrC and OmcA,

which showed significantly reduced polymerization activity. *E. coli*, that lacks EET mechanisms, showed reduced polymerization activity when compared with *Shewanella* in the same conditions. In a following study,[27] *Shewanella* was found to mediate ATRP under aerobic conditions, and it was shown that the bacteria first consumed dissolved oxygen by aerobic respiration, and then resumed anaerobic respiration, which in turn promoted polymerization.

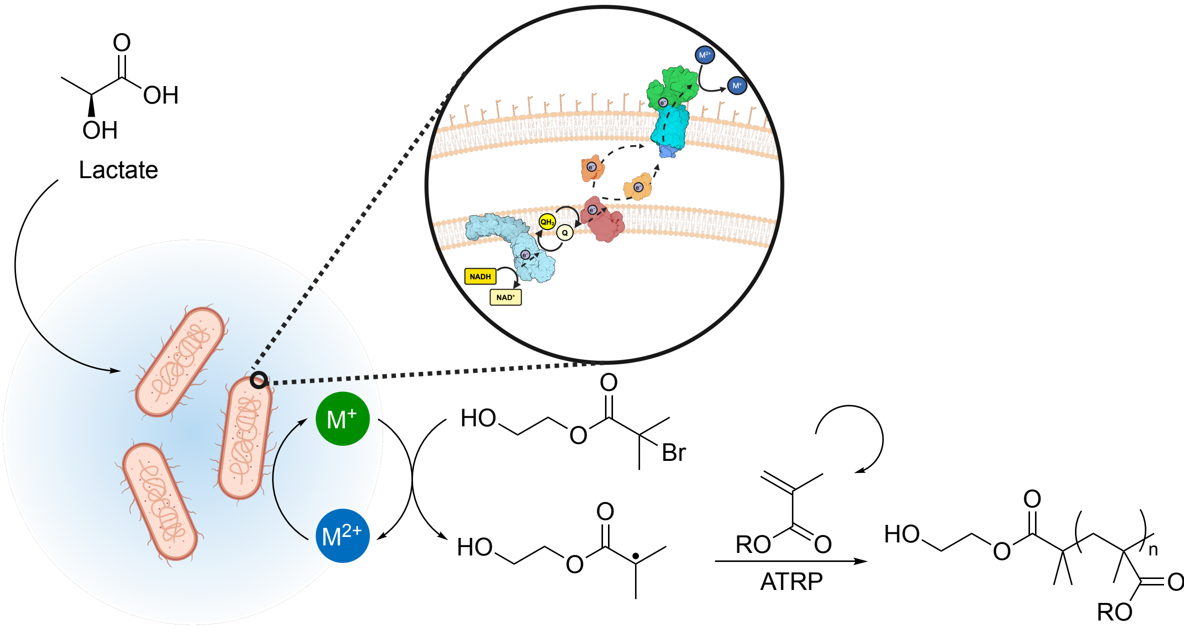


Figure 4. Metal-catalyzed atom transfer radical polymerization (ATRP) promoted by Shewanella oneidensis *MR-1, relying on extracellular electron transfer (EET).*[26]

Bacteria-promoted polymerization has also been performed in the absence of metals. Qiao *et al.*[28] employed as initiator an aryl diazonium salt that was reduced by bacteria cultures to give an aryl radical. This carbon radical initiated a reversible addition-fragmentation chain transfer (RAFT) polymerization of an oligo(ethylene glycol) methyl ether methacrylate (Figure 5). *E. coli* and *Salmonella enterica* serovar Typhimurium successfully performed RAFT in anaerobic, biocompatible conditions. Controls with the supernatant of bacteria cultures and with heat-killed *S. enterica* showed residual polymerization, but heat-killed *E. coli* were able to perform the polymerization in similar levels to those of live cells. The authors hypothesized that this may be because of differences in composition of the lipopolysaccharide layer of the bacteria: the employed *S. enterica* strain presented a highly charged O-antigen layer that could prevent the interaction of the diazonium salt with a remanent reducing membrane environment in heat-killed cells, while the selected *E. coli* strain lacked that feature. Experiments with *S. enterica* knockouts for glutathione production supported the role of

glutathione as a participant in the diazonium salt reduction, while a knockout for a key copper oxidase, that affords accumulation of Cu(I) in the medium, showed enhanced polymerization activity. This points to a complex combination of metabolic factors that may be sustaining the radical production.

Figure 5. Aryl diazonium salt-initiated reversible addition-fragmentation chain transfer (RAFT) polymerization promoted by bacteria redox potential.[28]

Very recently, S. Wang *et al.*[29] reported a catalyst-free, initiator-free biocompatible polymerization of zwitterionic methacrylate polymers promoted by *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*, and also the yeast *Candida albicans*. In this case, direct interaction of the zwitterionic monomers with the bacteria outer membrane was enough to initiate the radical polymerization. Live, whole bacteria were necessary for the reaction to proceed, and polymerization activity was found to be dependent on the redox potential of the cultures, as species that did not generate negative reducing potentials in solution, such as *Bacillus subtilis* and *Enterococcus faecalis*, did not show polymerization activity.

Bacteria-mediated electron transfer has rarely been applied to the synthesis of small molecules. As mentioned earlier, Alexander *et al.*[23] performed a CuAAC by harnessing the reducing power generated by *E. coli* to generate the Cu(I) catalyst. In a similar manner, Keitz *et al.*[30] reported a CuAAC promoted by EET of *S. oneidensis*. The reaction progressed under anaerobic conditions in presence of live bacteria, and the dependence on the Mtr EET pathway was demonstrated by the reduced reactivity of knockouts for MtrC, MtrA and CymA, which was recovered by inducible expression of the relevant genes.

ATRP has been also promoted by eucaryotic, mammalian cells. S. Wang *et al.*[31] reported the Cu(I)-promoted intracellular polymerization of N-hydroxyethylacrylamide in mammalian

cells. A Cu(II)-histidine complex was used as a source for Cu(I), which was continuously generated by reduction with endogenous glutathione. Cu(I) was generated in catalytic concentrations, which helped circumvent its toxicity. The reaction was shown to be selective towards cancer cells, which usually show glutathione overexpression,[32,33] and the polymerization was further promoted by addition of exogenous sodium ascorbate. The method was applied to the intracellular synthesis of a polymer which included Paclitaxel, a chemotherapy agent. The polymer showed a higher apoptosis-inducing effect when compared to that of the unpolymerized monomer, which was attributed to a higher intracellular retention of the polymerized drug. More recently, Z-F. Wang *et al.*[34] have reported a similar cancer-cell mediated, Cu(I)-promoted, extracellular polymerization of poly(ethylene glycol) methyl ether methacrylate monomer, as well as a cancer-cell promoted CuAAC click reaction.

1.3. Radical chemistry of aryl diazonium salts

1.3.1. General reactivity of aryl diazonium salts

Aryl diazonium salts are widely used reagents in organic chemistry.[35] They can be easily prepared from amines via diazotization.[36] They are far more stable than alkyl diazonium salts because of electronic delocalization between the nitrogen and the aromatic ring,[37] and though aryl diazonium chlorides are unstable,[35] other salts can be isolated as crystalline solids when using stabilizing counterions such as tetrafluoroborate[37] and tosylate.[38]

Aryl diazonium salts may react with nucleophiles that attack the diazo moiety to generate azo compounds, or they may lose N_2 via heterolytic cleavage to generate aryl cations, or via homolytic cleavage to give aryl radicals. The latter is a redox reaction that requires an electron transfer from a reductant (Scheme 1).[39] Aryl diazonium salts have gained interest as radical sources, especially under photocatalysis conditions, or in light-promoted, photocatalyst-free reactions.[35]

Scheme 1. General reactivity of aryl diazonium salts.

1.3.2. Reactivity of diazonium salts with biological reductants

Aryl diazonium salts can be reduced to aryl radicals by biological reductants like NADH and ascorbate in aqueous media.[40] They have thus been employed as radical sources for

synthetic, C–C bond forming reactions. Carrillo *et al.*[41] used ascorbic acid to promote the C–H arylation of arenes and heteroarenes with diazonium salts, generated *in situ* from the corresponding aniline (Scheme 2). The reaction took place at room temperature and was compatible with water.

Scheme 2. Ascorbic acid-initiated C–H arylation of (hetero)arenes with aryl diazonium salts generated in situ.[41]

The Hantzsch ester, a synthetic analogue of NADH, has been also used to initiate radical bond-forming reactions with aryl diazonium salts. McErlean *et al.*[42] employed the Hantzsch ester to promote C–H arylation of heteroarenes (Scheme 3a), and radical annulations with alkynes to give benzothiophenes (Scheme 3b) or phenanthrenes (Scheme 3c). The reactions took place at room temperature and open atmosphere, although compatibility with water was not demonstrated.

Scheme 3. Hantzsch ester-initiated synthetic reactions with aryl diazonium salts.[42]

2. Objectives

This Master dissertation aims to harness the bacteria redox metabolism to promote non-natural bond-forming synthetic radical reactions. Specifically, we aim: (1) to promote a Meerwein arylation (Scheme 4) by harnessing the redox metabolism of an electrogenic bacteria (*Shewanella oneidensis* MR-1) and a model bacteria (*Escherichia coli*); (2) to study the mechanisms of the reaction in presence of *S. oneidensis and E. coli*; (3) to expand the scope of bacteria-promoted, synthetic reactions based on aryl diazonium salts.

N_2BF_4 OMe + R O O — *S. oneidensis* or *E. coli* → R O O OMe

Scheme 4. Bacteria-promoted Meerwein arylation.

3. Planning of work

Task 1. Synthesis of precursors

Synthesis, purification and characterization of the starting materials and products for the studied reactions.

Task 2. Study of the Meerwein arylation promoted by biological reductants

The selected reaction will be explored, studying different biological reductants, solvent, time, concentration and reaction conditions. Besides, mechanistic studies will be performed. Finally, different Michael acceptors will be analyzed to determine which system is most suitable for translating to biological conditions.

Task 3. Study of a radical annulation promoted by biological reductants

To expand the scope of bacteria-promoted, synthetic reactions based on aryl diazonium salts, a radical annulation for the synthesis of benzothiophenes will be also tested. Optimization of solvent, reaction time and concentration will be performed.

Task 4. Evaluation of the bacteria-promoted Meerwein arylation

The ability of bacteria *S. oneidensis* MR-1 and *E. coli* to promote the selected Meerwein arylation will be investigated. The bacteria-promoted reaction will be studied at different conditions of bacteria concentration, oxygenation, and electron transport inhibitors, and in presence of heat-killed bacteria and bacteria-free supernatant.

Task 5. Evaluation of the bacteria-promoted radical annulation

The ability of bacteria *S. oneidensis* MR-1 and *E. coli* to promote the radical annulation for the synthesis of benzothiophenes will be studied at different conditions.

4. Discussion

4.1. Synthetic reactions of aryl diazonium salts promoted by biological reductants

4.1.1. Meerwein arylations promoted by biological reductants

Aryl diazonium salts are known to decompose to aryl radicals in presence of reducing biomolecules such as ascorbate (vitamin C) and NADH.[40] It has been demonstrated that the C–H arylation of electron poor alkenes with aryl diazonium salts, reaction known as the Meerwein arylation (Scheme 5), can be promoted by ascorbate[41] and the Hantzsch ester[42]—a NADH synthetic analogue.

Metal salt

$-N_2$, $-H^+$

Scheme 5. General representation of a Meerwein arylation.

On the other hand, the Meerwein arylation of 1,4-naphthoquinones has been reported under visible light photocatalysis in mild conditions (Scheme 6a), so we thought that it would be an adequate candidate to test the use of biological reductants (Scheme 6b).[43] Previous research in the group on this reaction was carried out by Dr. Joan Miguel Ávila and PhD students Xulián Fernández González and Jesús Fernando Salgado Barca.[44]

a) N_2BF_4 + … Eosyn Y and green LED or $[Ru(bipy)_3]^{2+}$ and blue LED; DMSO, 8 h, r.t.

b) N_2BF_4, OMe + … Biological reductant; DMSO/H_2O, 10 min, r.t.

Scheme 6. Meerwein arylation of naphthoquinones. (a) Photocatalyzed arylation.[43] (b) Arylation promoted by biological reductants (present work).

We studied the Meerwein arylation between *p*-methoxyaryldiazonium tetrafluoroborate (**1**) and 2-(4-methoxyphenyl)naphthalene-1,4-dione (**2a**) in presence of reducing biomolecules such as glutathione, NADH and ascorbate (Table 1). The diazonium salt, the naphthoquinone and the biological reductant were dissolved in DMSO under nitrogen atmosphere at a 10 mM concentration. 3 equiv. of the naphthoquinone were employed, since a high ratio of naphthoquinone / diazonium salt had been previously found to give better yields. NADH disodium salt and sodium ascorbate were used as sources of NADH and ascorbate, respectively. Preliminary water compatibility studies were also undertaken under these conditions. Controls were performed in absence of biological reductant. Yields after 2 h were determined by ^{1}H-NMR employing dibromomethane as internal standard.

Table 1. Screening of biological reductants for the Meerwein arylation.[a]

Entry	Biological reductant	Solvent	Yield of 3a/%
1	NADH	DMSO	**76**
2	NADH	DMSO/H_2O 1:1	**36**
3	Glutathione	DMSO	**53**
4	Glutathione	DMSO/H_2O 1:1	**12**
5	Ascorbate	DMSO	**33**
6	Ascorbate	DMSO/H_2O 1:1	**28**
7	-	DMSO	**<7**
8	-	DMSO/H_2O 1:1	**<7**

(a) Reaction conditions (unless otherwise noted): **1** (1 equiv., 10 mM), **2a** (3 equiv.), *biological reductant* (1 equiv.), *DMSO/H_2O*, 1 mL final volume, 2 h, r.t., inert atmosphere. Yields were determined by ^{1}H-NMR using dibromomethane as internal standard.

All three reductants were found to promote the reaction in DMSO (Table 1, entries 1, 3 and 5), with the highest yield being obtained with NADH (76%, entry 1). In all cases, immediate formation of bubbles was observed in the solution, which we attribute to the release

of nitrogen from the decomposition of the diazonium salt. The solution turned from orange to dark red in minutes, suggesting that the formation of the diarylated naphthoquinone **3a** was very fast. When water was added as cosolvent (DMSO/H_2O 1:1), a turbid orange suspension was formed, and yields decreased (Table 1, entries 2, 4 and 6). This was attributed to the low water solubility of naphthoquinone **2a**. It was apparent that poor solubility of the naphthoquinone and water compatibility of the reaction would be issues for translating the reaction to biologically relevant conditions with water as the main solvent. Importantly, in the absence of a reducing agent, trace amounts of the product were observed (Table 1, entries 7 and 8).

Since NADH was the biological reductant that had provided the highest yield, we selected it for a more thorough optimization and mechanistic study of the reaction in abiotic conditions, with the final aim of translating it to biological conditions.

Living systems are extremely complex milieus, so chemoselectivity is difficult to achieve when performing abiotic reactions in biological media. It is required that reagents do not present side reactions with biological components and react only with the desired reaction partners.[45] Chemoselectivity may be facilitated by fast reaction rates.[46] Evaluation of the NADH-promoted Meerwein arylation at different times showed fast kinetics (Figure 6). The reaction took place mainly in the first 10 minutes, with a yield of 64% after just 5 minutes. This was consistent with the fast color changes previously observed.

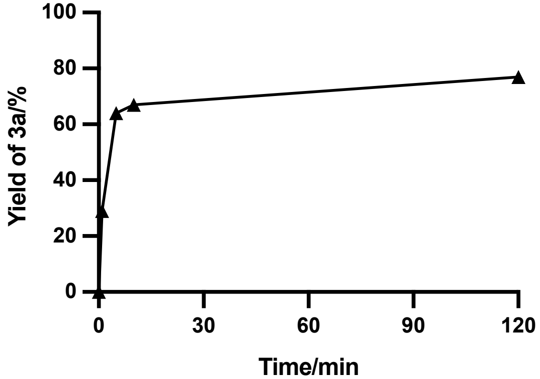


Figure 6. Time study of the Meerwein arylation promoted by NADH. Reaction conditions (unless otherwise noted): **1** (1 equiv., 10 mM), **2a** (3 equiv.), NADH (1 equiv.), 1 mL DMSO, r.t., inert atmosphere, *time*. Yields were determined by ^{1}H-NMR using dibromomethane as internal standard. See also Supplementary table 1.

Additional experiments were performed to evaluate the role of NADH in the reaction (Figure 7). Substoichiometric amounts of NADH (10 mol%) yielded 56% of product. This suggests a chain-reaction mechanism, in which NADH is needed only to initiate the reaction. Indeed, the addition of a second equivalent of diazonium salt after 1 h of reaction gave an

increase in yield from 0.53 equiv. to 0.76 equiv. of product **3a**. HPLC-MS analysis of a reaction crude with 1 equiv. of NADH showed that NADH had been reduced completely to NAD^+ after 1 h (Supplementary scheme 1a). This implies that the additional equivalent of diazonium salt was reduced not by NADH, but by radical intermediates that remained 'alive' in the reaction milieu.

Yields rose when the amount of NADH was increased up to 1 equiv., but were lower at higher quantities of NADH. HPLC-MS allowed for identifying a compound with mass compatible with that of the arylation of NAD^+ (Supplementary scheme 1b). Hence, a competitive reaction of NAD^+ with the diazonium salt would help to explain the decrease in yield when an excess of NADH is employed.

The influence of oxygen in the reaction was also studied (Figure 7). Deoxygenation of solvents gave higher yields when substoichiometric amounts of NADH were used. When 1 equiv. of NADH was used, almost no difference between the deoxygenated and non-deoxygenated reaction was observed, but the yield decreased when performing the reaction in open atmosphere. These results may be attributed to trapping of intermediate radicals by oxygen. Addition of TEMPO, a radical trapping agent, gave significant lower yields, supporting the radical mechanism (Figure 7). Unfortunately, HPLC-MS did not allow for identification of TEMPO adducts that would have provided information about reaction intermediates.

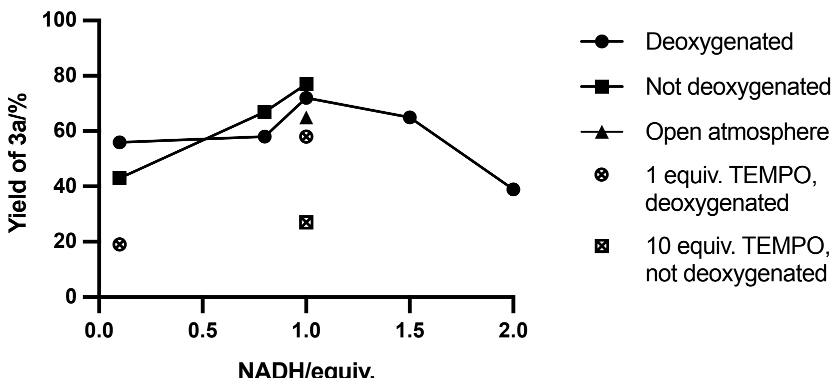


Figure 7. Mechanistic studies of the Meerwein arylation promoted by NADH. Reaction conditions (unless otherwise noted): **1** (1 equiv., 10 mM), **2a** (3 equiv.), *NADH*, 1 mL DMSO, 2 h, r.t. Yields were determined by ^{1}H-NMR using dibromomethane as internal standard. See also Supplementary table 2.

The arylation product of DMSO was detected when running the reaction either in DMSO or d6-DMSO (Supplementary scheme 1c). This was not unexpected, as the visible light-promoted methylsulfoxidation with DMSO of aryl diazonium salts has been already reported.[47]

Considering these results, we propose the following mechanism for the NADH-promoted reaction (Scheme 7). First, NADH reduces the aryl diazonium cation **I** to aryl radical **II** by single electron transfer (SET) through an outer sphere mechanism, as suggested by Yasui *et al.*[48] NAD• can reduce another aryl diazonium cation to give the oxidized form NAD^+. The aryl radical **II** is trapped by the naphthoquinone **2a** to yield radical **III**, that may propagate the reaction by reducing another molecule of diazonium cation, forming at the same time the carbocation **IV**. This species deprotonates to give the final product **3a**.

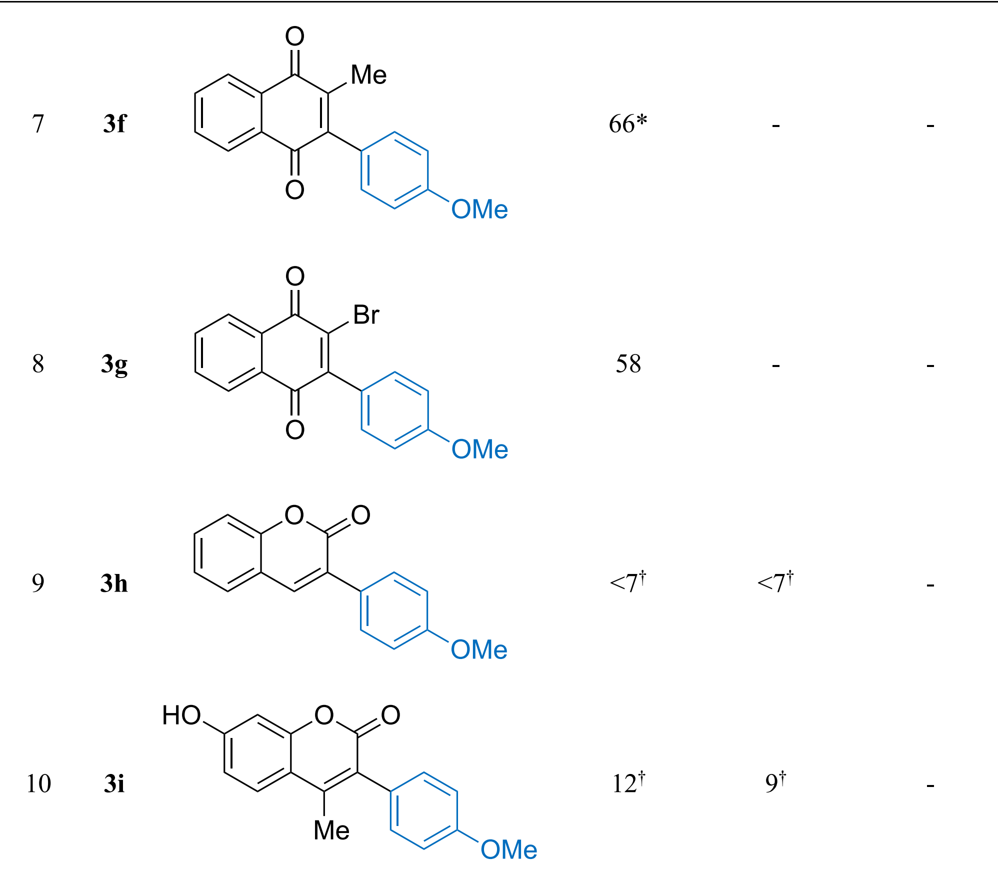
Scheme 7. Proposed mechanism for the Meerwein arylation promoted by NADH.

One of the main objectives of this project is the translation of the Meerwein arylation of naphthoquinones to biocompatible conditions. As seen before, when we performed the reaction in mixtures of DMSO/H_2O 1:1 with different biological reductants, yields decreased in comparison with the reaction in DMSO (Table 1), presumably due to water solubility issues. Indeed, naphthoquinone **2a** is insoluble in water in concentrations as low as 20 µM. Therefore, we explored the behavior of diverse radical acceptors in this NADH-promoted reaction under different proportions of water (Table 2). This study included not only 1,4-naphthoquinones, but also coumarins, since coumarin has been also reported as a suitable acceptor for the photocatalyzed Meerwein arylation.[43,49] The best yield in 80% water was obtained with the unsubstituted 1,4-naphthoquinone (to yield naphthoquinone **2a**). A small amount of 0.06 equiv. of the diarylated product **3a** also formed. The second-best yield was obtained for 2-hydroxy-(1,4-naphthoquinone) **2c** (product **3c**, 44%). Both 1,4-naphthoquinone and **2c**,

although not soluble in these conditions, are far more soluble than naphthoquinone **2a**. For the coumarins, very low yields were obtained in DMSO (products **3h–i**).

Table 2. Scope of the Meerwein arylation promoted by NADH of different 1,4-naphthoquinones and coumarins.[a]

Entry		**Product**	**Yield/%**		
			0% water	**50% water**	**80% water**
1	**2a**		70	65	60
2	**3a**		77	36	18
3	**3b**		69	35	16
4	**3c**		46	55	44
5	**3d**		43	35	26
6	**3e**		-	57	46



7	**3f**		66*	-	-
8	**3g**		58	-	-
9	**3h**		<7†	<7†	-
10	**3i**		12†	9†	-

(a) Reaction conditions (unless otherwise noted): **1** (1 equiv., 10 mM), *1,4-naphthoquinone, substituted naphthoquinone* ***2a–g*** or *coumarin* ***2h–i*** (3 equiv.), NADH (1 equiv.), *DMSO/*H_2O, 1 mL final volume, 2 h, r.t., inert atmosphere. Yields were determined by ^{1}H-NMR using dibromomethane as internal standard. (*) 4 h. (†) 1 h. Conditions marked with ‘-‘ were not studied.

To translate a reaction to biocompatible conditions, it is also necessary to work with very low concentrations of the reactants to prevent toxicity.[46] Hence, we also performed some of the reactions at a 1 mM concentration in water with 20% of DMSO as cosolvent (Figure 8, products **2a** and **3b–d**). We observed that yields were not affected by the dilution. In the case of the unsubstituted 1,4-naphthoquinone, a 64% yield of product **2a** was obtained, and 0.03 equiv. of the diarylated product **3a** were formed.

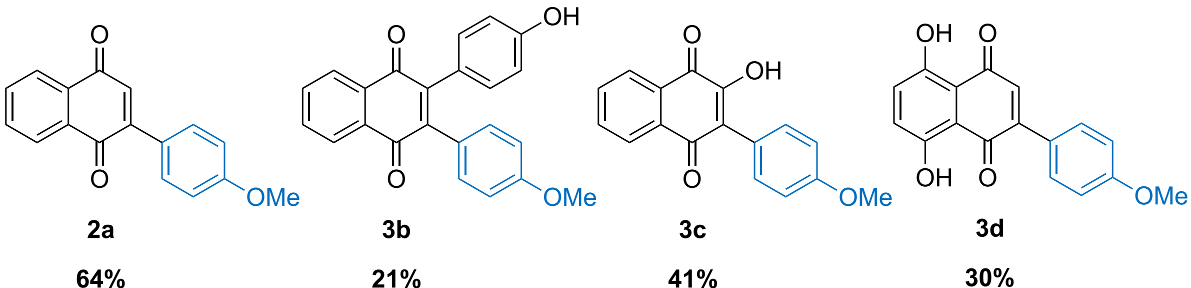


Figure 8. Yields for the Meerwein arylation promoted by NADH of different 1,4-naphthoquinones at 1 mM. Reaction conditions (unless otherwise noted): **1** (1 equiv., 1 mM), *1,4-naphthoquinone or substituted naphthoquinone* ***2b–c*** (3 equiv.), NADH (1 equiv.), DMSO/H_2O 2:8, 10 mL final volume, 2 h, r.t., inert atmosphere. Yields were determined by ^{1}H-NMR using dibromomethane as internal standard.

Finally, we performed the NADH-promoted arylation of 1,4-naphthoquinone at micromolar scale, and increasing the complexity of the aqueous media (Figure 9). The reactions were performed under inert atmosphere with deoxygenated solvents, and all reagents were added at a final concentration of 100 µM. DMSO was used as cosolvent, with a final concentration of 0.5% v/v. In water, we obtained 7% yield for the monoarylated product **2a** and 2% yield for the diarylated product **3a**. In PBS (phosphate-saline buffer, that is isotonic to most cells), yields were 18% and 7%, for **2a** and **3a** respectively. Negative controls performed in absence of NADH gave reduced yields of 0.9% for **2a** and <0.5% for **3a**. We also tested the reaction in LB (lysogeny broth), a typical undefined growth medium for bacteria, but found only trace amounts of the products, so it appears that components of the more complex, biomolecule-rich LB growth medium interfere with the reaction, at least at this low concentration.

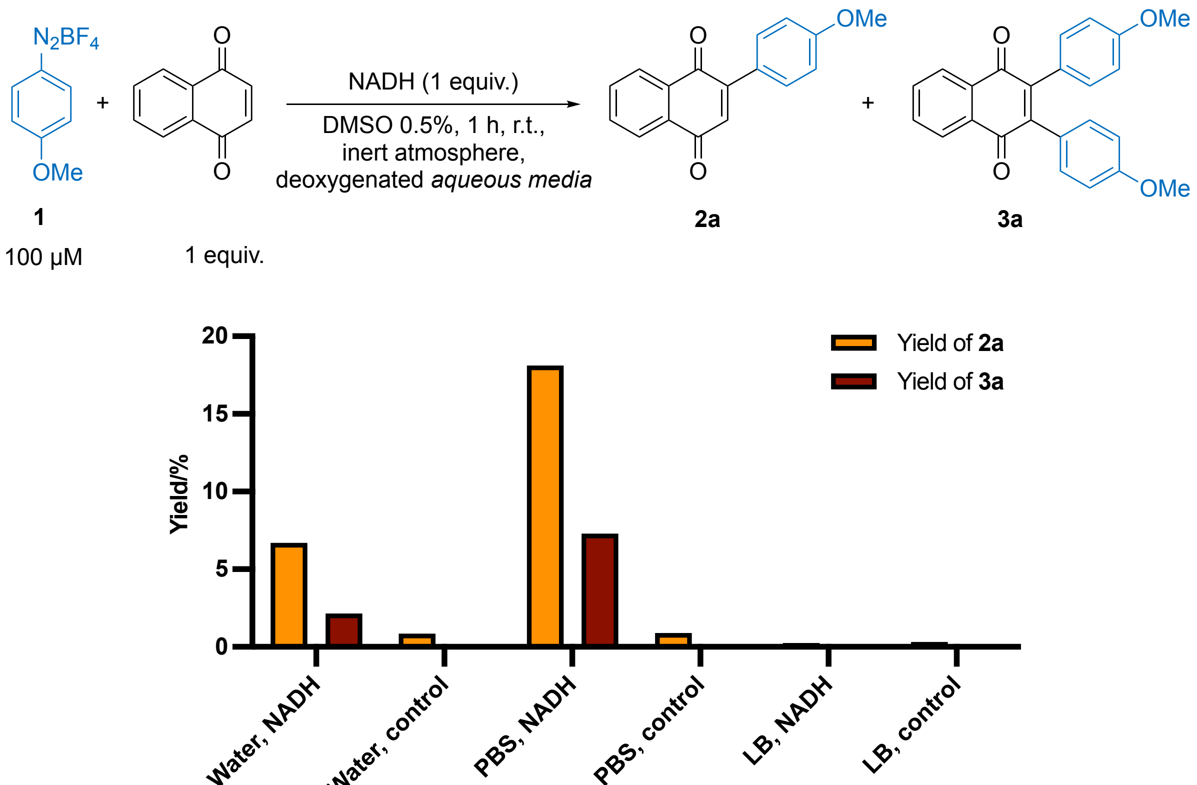


Figure 9. Arylation of 1,4-naphthoquinone promoted by NADH at micromolar concentration in different aqueous media. Reaction conditions (unless otherwise noted): **1** (1 equiv., 100 μM), 1,4-naphthoquinone (1 equiv.), *NADH (1 equiv.)*, 0.5% v/v DMSO, 2 mL deoxygenated *aqueous media*, 1 h, r.t., inert atmosphere. Yields were determined by HPLC-UV absorbance at 260 nm, using coumarin as internal standard. See also Supplementary table 3.

4.1.2. Synthesis of benzothiophenes from aryl diazonium salts promoted by biological reductants

With the aim of expanding the scope of synthetic reactions of aryl diazonium salts promoted by biological systems, we also evaluated a radical annulation to give a benzo[b]thiophene promoted by NADH (Table 3). We chose this system because many benzo[b]thiophene derivatives are bioactive molecules with pharmacological applications.[50]

The synthesis of benzothiophenes from aryl diazonium salts was first described with NaI, copper powder or $FeSO_4$ by Zanardi *et al.*[51] König *et al.*[52] performed the reaction with eosin Y photocatalyst and white LEDs, and McErlean *et al.*[42] reported the use of the Hantzsch ester to promote the reaction (Scheme 3b). With the knowledge achieved in the previous section, we initiated the study of the reaction of the *o*-methylthio-benzenediazonium salt **4** with phenyl acetylene (**5**) directly in mixtures of DMSO/water at 10 mM scale (Table 3). We tested different amounts of phenyl acetylene, as well as different proportions of water. We found that yields increased with higher amounts of phenyl acetylene. We also observed that

increasing amounts of water gave higher yields (81% yield with 80% water and 50 equiv. of phenyl acetylene, Table 3, entry 5). The reaction proved to be robust to dilution, maintaining a yield of 67% when lowering the concentration from 10 to 1 mM (Table 3, entry 6).

Table 3. Synthesis of benzothiophene ***6*** *promoted by NADH.[a]*

4 + **5** → NADH (1 equiv.), *DMSO/H$_2$O*, 2 h, r.t., inert atmosphere → **6**

Entry	[4]/mM	[5]/equiv.	Water/%	Yield of 6/%
1	10	10	0	**25**
2	10	30	0	**38**
3	10	10	50	**55**
4	10	10	80	**45**
5	10	50	80	**81**
6	1[b]	50	80	**67**

(a) Reaction conditions (unless otherwise noted): **4** (1 equiv.), **5**, NADH (1 equiv.), *DMSO/H$_2$O*, 1 mL final volume, 2 h, r.t., inert atmosphere. Yields were determined by ^{1}H-NMR using dibromomethane as internal standard. (b) 10 mL final volume.

We studied the reaction at different times at 10 mM, revealing it to be very fast, as we achieved a yield of 30% after just 1 minute, and 47% in 10 minutes (Figure 10).

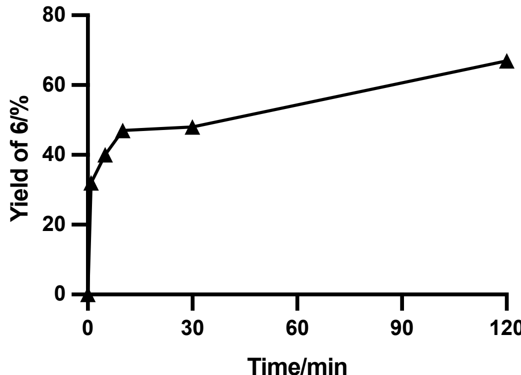


Figure 10. Time study of the synthesis of benzothiophene ***6*** *promoted by NADH.* Reaction conditions (unless otherwise noted): **4** (1 equiv., 10 mM), **5** (50 equiv.), NADH (1 equiv.), 1 mL DMSO/H$_2$O 2:8, r.t., inert atmosphere, *time*. Yields were determined by ^{1}H-NMR using dibromomethane as internal standard. See also Supplementary table 4.

Finally, we performed the reaction at micromolar scale in PBS, using a 100 µM concentration of diazonium salt **4** and NADH, and 500 µM of phenyl acetylene, with 0.5% v/v DMSO as cosolvent (Figure 11). The transformation was studied both under inert atmosphere with deoxygenated PBS, or in open atmosphere without deoxygenation, observing yields of 20% and 6%, respectively. The product was not detected in controls performed in absence of NADH.

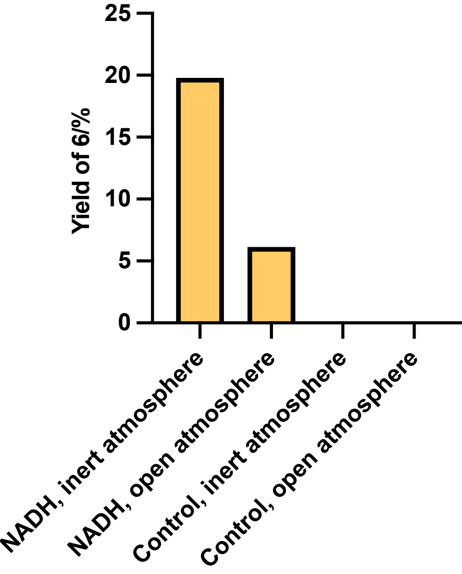


Figure 11. Synthesis of benzothiophene ***6*** *at micromolar concentration promoted by NADH.* Reaction conditions (unless otherwise noted): **4** (1 equiv., 100 µM), **5** (5 equiv.), *NADH (1 equiv.)*, 2 mL PBS, 0.5% v/v DMSO, 1 h, 30 °C. Yields were determined by HPLC-MS using caffeine as internal standard. See also Supplementary table 5.

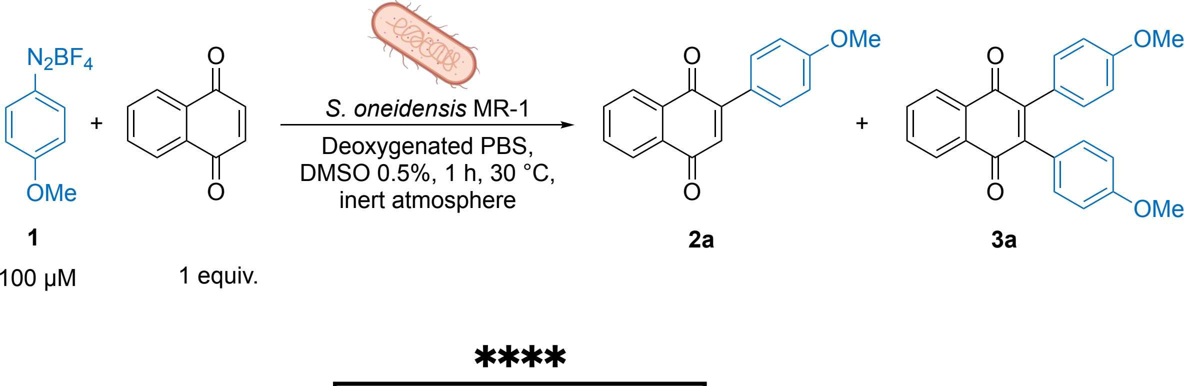
4.2. Synthetic reactions of aryl diazonium salts promoted by bacteria

4.2.1. Meerwein arylation of 1,4-naphthoquinones promoted by bacteria redox metabolism

Bacteria extracellular electron transfer (EET) is a biological process that may be harnessed to interface the natural metabolism with abiotic chemical reactions. One of the most studied bacteria that performs EET is *Shewanella oneidensis* MR-1, and its ability to reduce metal ions has already been employed to control metal-catalyzed abiotic reactions such as atom transfer radical polymerizations[26,27] and Cu-catalyzed azide-alkyne cycloadditions.[30] *S. oneidensis* can also use organic molecules as terminal electron acceptors. Hence, we hypothesized that this bacterium may reduce diazonium salts to the correspondent aryl radicals and, consequently, promote radical arylation reactions.

We first evaluated the arylation of 1,4-naphthoquinone with *p*-methoxyaryl diazonium tetrafluoroborate (**1**) promoted by *S. oneidensis* MR-1 (Figure 12). To perform the reaction, we mixed under nitrogen atmosphere 1 mL of a deoxygenated bacteria suspension in PBS

(adjusted to OD_{600}=2), 0.5 mL of a stock solution of the diazonium salt **1**, and 0.5 mL of a stock solution of 1,4-naphthoquinone, both in deoxygenated PBS and with DMSO as cosolvent. Final concentrations in the reaction mixture were 100 µM of the diazonium salt **1** and of 1,4-naphthoquinone, with 0.5% v/v of DMSO, and an OD_{600} of 1. The reaction mixture was incubated with shacking at 30 °C for 1 h. After that time, it had turned appreciably yellow, suggesting the formation of arylated naphthoquinones. The mixture was then frozen in liquid N_2 and lyophilized, and the solid residue was extracted with acetonitrile and analyzed by HPLC-MS to quantify the products. The average yield was 17% for the monoarylated product **2a** and 14% for the diarylated product **3a** (note that, due to the stoichiometry of the reaction, equal yields of products **2a** and **3a** represent half the quantity of product **3a**). In comparison, controls performed without bacteria gave background yields of 0.7% for **2a** and <0.5% for **3a**. We performed the reaction with a higher quantity of bacteria (OD_{600}=4), but no significant increase in the yields was observed. A preliminary evaluation of the toxicity of the reaction showed that bacteria did not retain viability after the reaction (Supplementary figure 2). Both diazonium salt **1** and naphthoquinone inhibited bacterial growth at 100 µM, but products **2a** and **3a** were not toxic at a concentration of 20 µM.



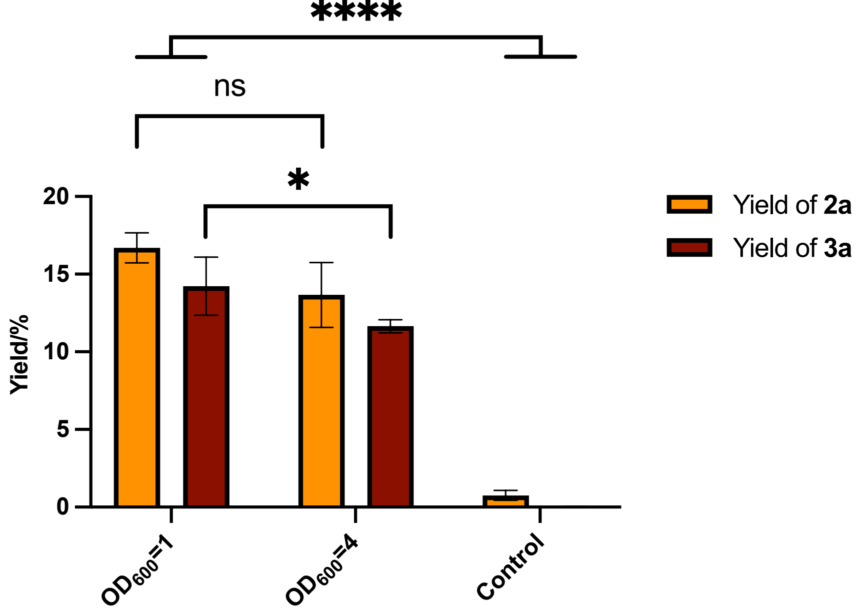


Figure 12. Arylation of 1,4-naphthoquinone promoted by S. oneidensis *MR-1.* Reaction conditions (unless otherwise noted): **1** (1 equiv., 100 µM), 1,4-naphthoquinone (1 equiv.), *S. oneidensis* MR-1, 2 mL PBS, 0.5% v/v DMSO, 1 h, 30 °C, inert atmosphere, deoxygenated aqueous media. Yields were determined by HPLC-UV absorbance at 260 nm, using coumarin as internal standard. For the reaction with bacteria, $n = 3$ replicates. For the control without bacteria, $n = 2$. Represented values are mean ± SD.

We then performed the reaction in aerobic conditions, and the yields decreased significatively, to 2% and <0.5% for **2a** and **3a**, respectively (Figure 13). This may be due to an intrinsic sensibility of the reaction to oxygen at low concentrations, since positive controls with NADH instead of bacteria also showed an important decrease in the yield when performing the reaction in oxygenated conditions, from 10% to 4% for **2a**, and from 8% to 1% for **3a**.

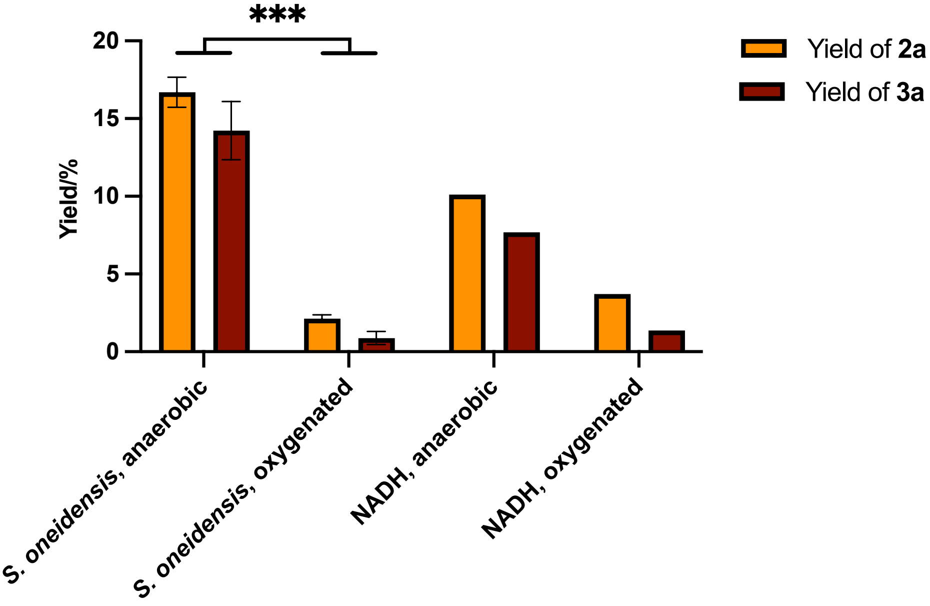


Figure 13. Arylation of 1,4-naphthoquinone promoted by S. oneidensis *MR-1 in anaerobic or oxygenated conditions*. Reaction conditions (unless otherwise noted): **1** (1 equiv., 100 µM), **1**,4-naphthoquinone (1 equiv.), *S. oneidensis* MR-1 (OD_{600}=1) or NADH (1 equiv.), 2 mL PBS, 0.5% v/v DMSO, 1 h, 30 °C. Yields were determined by HPLC-UV absorbance at 260 nm, using coumarin as internal standard. $n = 3$ replicates. Represented values are mean ± SD.

Now then, EET is not the only mechanism by which bacteria may donate electrons to promote abiotic reactions. In a more general manner, bacteria produce a reducing redox potential in their growth medium.[13] This redox potential has been already harnessed with diazonium salts to initiate radical polymerizations, in the case of bacteria that do not perform EET, like *E. coli*.[28] We thus carried out the reaction in presence of *E. coli* DH5α, both in anaerobic and oxygenated conditions (Figure 14). We found that *E. coli* also promotes the reaction in anaerobic conditions, while the yields lowered significatively in presence of oxygen.

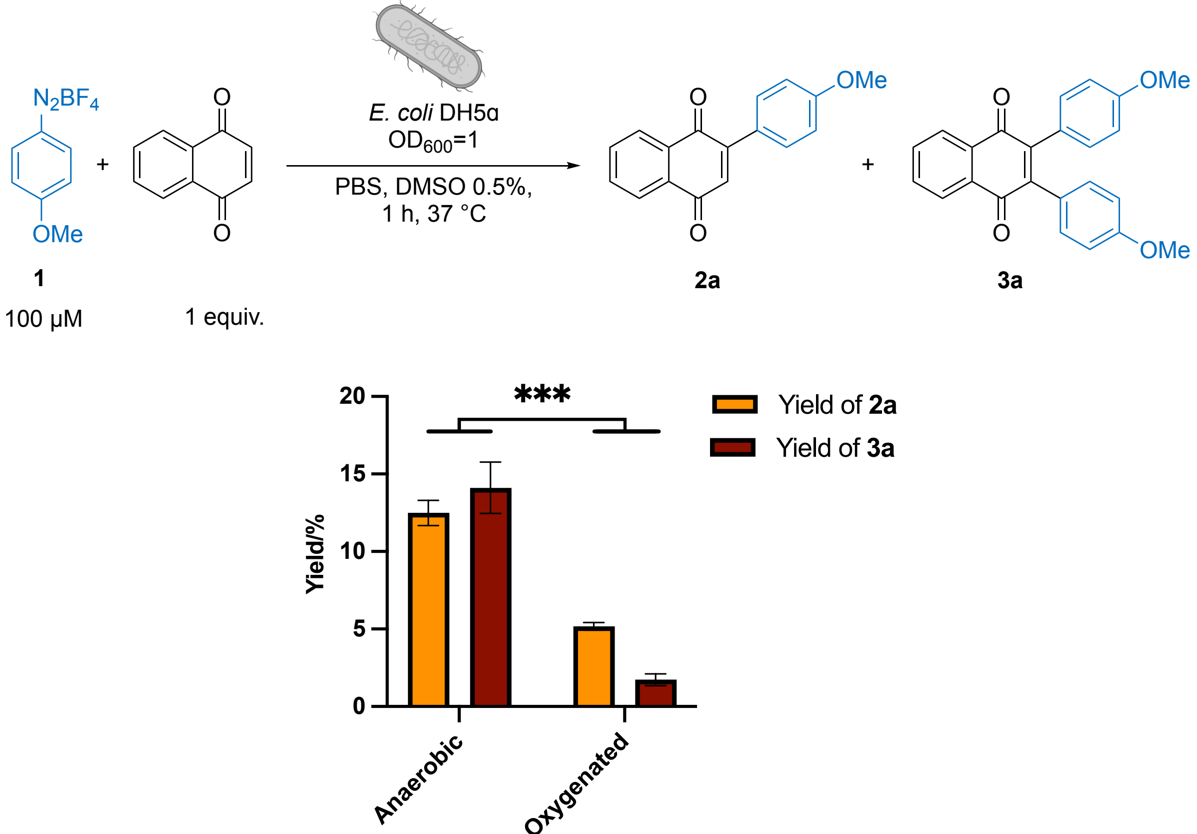


Figure 14. Arylation of 1,4-napthoquinone promoted by E. coli *DH5a in anaerobic or oxygenated conditions.* Reaction conditions (unless otherwise noted): **1** (1 equiv., 100 μM), 1,4-naphthoquinone (1 equiv.), *E. coli* DH5α (OD_{600}=1), 2 mL PBS, 0.5% v/v DMSO, 1 h, 37 °C. Yields were determined by HPLC-UV absorbance at 260 nm, using coumarin as internal standard. n = 3 replicates. Represented values are mean ± SD.

We then tried to identify the biological factors responsible for promoting the reaction. We wanted to assess whether live, metabolically active bacteria were needed for the reaction to proceed. Alternatively, bacteria can secrete reducing molecules that may promote the reaction: *E. coli* is known to secrete glutathione to the culture medium,[53,54] and *S. oneidensis* secretes flavins.[19]

For *S. oneidensis*, heat-killed bacteria failed to promote the reaction with significative yields (Figure 15). Supernatant after removing the bacteria also failed to promote the reaction. Thus, in the case of *S. oneidensis*, live, metabolically viable bacteria are needed for the reaction to proceed, but secreted reducing metabolites do not promote it significatively. These results agree with those reported in the literature for Cu-catalyzed reactions promoted by *S. oneidensis*.[26,30]

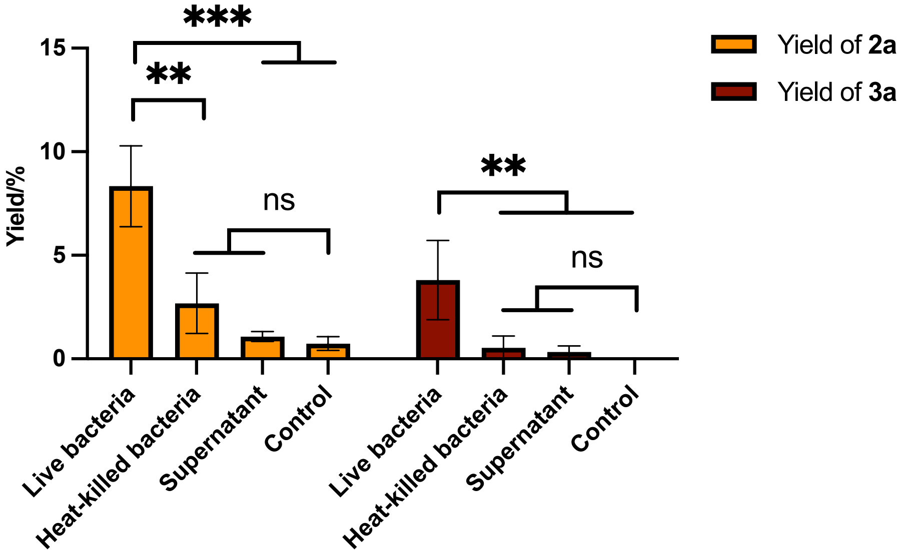


Figure 15. Controls for arylation of 1,4-naphthoquinone promoted by S. oneidensis *MR-1*. Reaction conditions (unless otherwise noted): **1** (1 equiv., 100 µM), 1,4-naphthoquinone (1 equiv.), *S. oneidensis* MR-1 (OD_{600}=1), 2 mL PBS, 0.5% v/v DMSO, 1 h, 30 °C. Yields were determined by HPLC-UV absorbance at 260 nm, using coumarin as internal standard. n = 3 replicates. Represented values are mean ± SD.

Additional studies are needed to determine the exact mechanism by which *S. oneidensis* promotes the reaction. To that end, we performed experiments with inhibitors of the electron transport, but all tested inhibitors interfered directly with the reaction (Supplementary figure 3). We intended to block the EET cytochromes with KCN, but this disrupted positive controls with NADH. We tested antimycin A, an inhibitor of complex III of the ETC (ubiquinone:cytochrome *c* oxidoreductase),[55] but found that this compound can promote the reaction without bacteria. We also intended to block reduction of quinone with rotenone at the level of complex I (NADH:ubiquinone oxidoreductase),[18] but it also promoted the reaction without bacteria.

Furthermore, we performed control experiments with heat-killed *E. coli* and supernatant from *E. coli* (Figure 16). In this case, we found that both heat-killed bacteria and supernatant promoted significatively the reaction, although with lower yields than those obtained with live bacteria. This behavior is opposite to that observed in *S. oneidensis*. The activity of heat-killed *E. coli* agrees with the published precedents. When using aryl diazonium salts to initiate bacteria-promoted polymerizations, Qiao *et al.*[28] reported significant polymerization activity for heat-killed *E. coli*. This was attributed to interaction of the diazonium salt with a remnant reducing redox environment in the proximity of the dead bacteria membrane, although the authors recognized the need for further research to confirm this effect. The activity of the

supernatant may be attributed to reducing factors secreted by the bacteria, such as glutathione.[53]

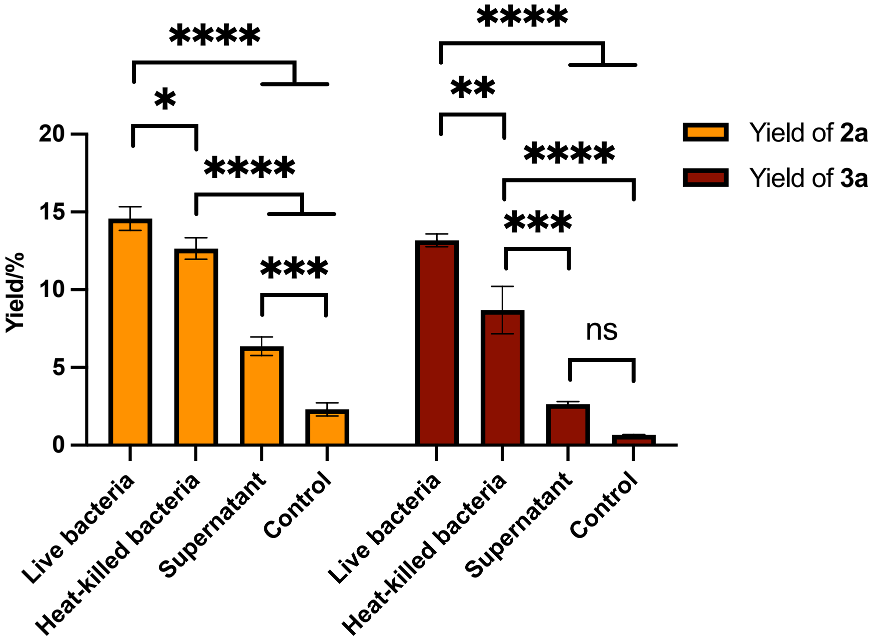


Figure 16. Controls for arylation of 1,4-naphthoquinone promoted by E. coli *DH5a*. Reaction conditions (unless otherwise noted): **1** (1 equiv., 100 µM), 1,4-naphthoquinone (1 equiv.), *E. coli* DH5α (OD_{600}=1), 2 mL PBS, 0.5% v/v DMSO, 1 h, 37 °C. Yields were determined by HPLC-UV absorbance at 260 nm, using coumarin as internal standard. n = 3 replicates. Represented values are mean ± SD.

As mentioned before, the monoarylated naphthoquinone **2a** is extremely insoluble in water, and shows a reduced reactivity in aqueous media when compared with the unsubstituted 1,4-naphthoquinone (Table 2, entries 1 and 2). Hence, we were surprised to obtain significant yields for the diarylated product **3a**, both in NADH-promoted reactions at 100 µM (Figure 9) and in the bacteria-promoted reactions. The yields for **2a** correspond to concentrations of around 10–15 µM. We thus tried the bacteria-promoted arylation of **2a** to give the diarylated product **3a**, employing a 20 µM concentration of **2a** and keeping a 100 µM concentration of the diazonium salt **1** (Figure 17). Although **2a** is not totally soluble at 20 µM in water, even with DMSO as cosolvent (0.5% v/v), we could achieve a yield of 7% for **3a**, proving that this reaction proceeds even at very low concentrations of the naphthoquinone.

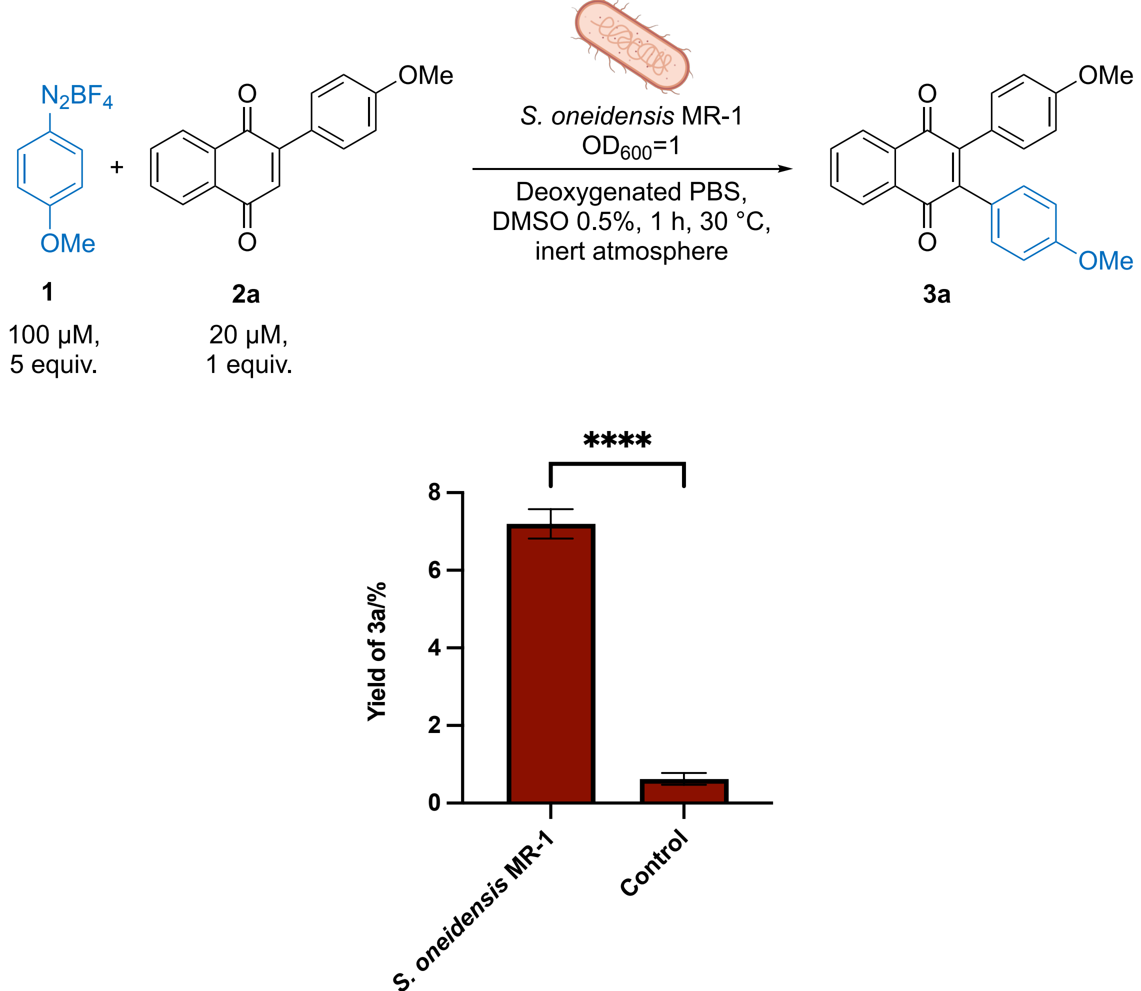


Figure 17. Arylation of naphthoquinone ***2a*** *promoted by* S. oneidensis *MR-1.* Reaction conditions (unless otherwise noted): **1** (5 equiv., 100 µM), **2a** (1 equiv., 20 µM), *S. oneidensis* MR-1 (OD_{600}=1), 2 mL PBS, 0.5% v/v DMSO, 1 h, 30 °C, inert atmosphere, deoxygenated aqueous medium. Yields were determined by HPLC-UV absorbance at 260 nm, using coumarin as internal standard. $n = 3$ replicates. Represented values are mean ± SD.

4.2.2. Synthesis of benzothiophenes from aryl diazonium salts promoted by bacteria redox metabolism

Having established that diazonium salts can be used to interface the metabolism of live bacteria with the Meerwein arylation of naphthoquinones, we tried to expand the scope of this strategy to other C–C bond-forming synthetic reactions based on diazonium salts. Therefore, we evaluated the radical annulation reaction of phenyl acetylene with diazonium salt **4** to give the benzo[b]thiophene **6** (Figure 18).

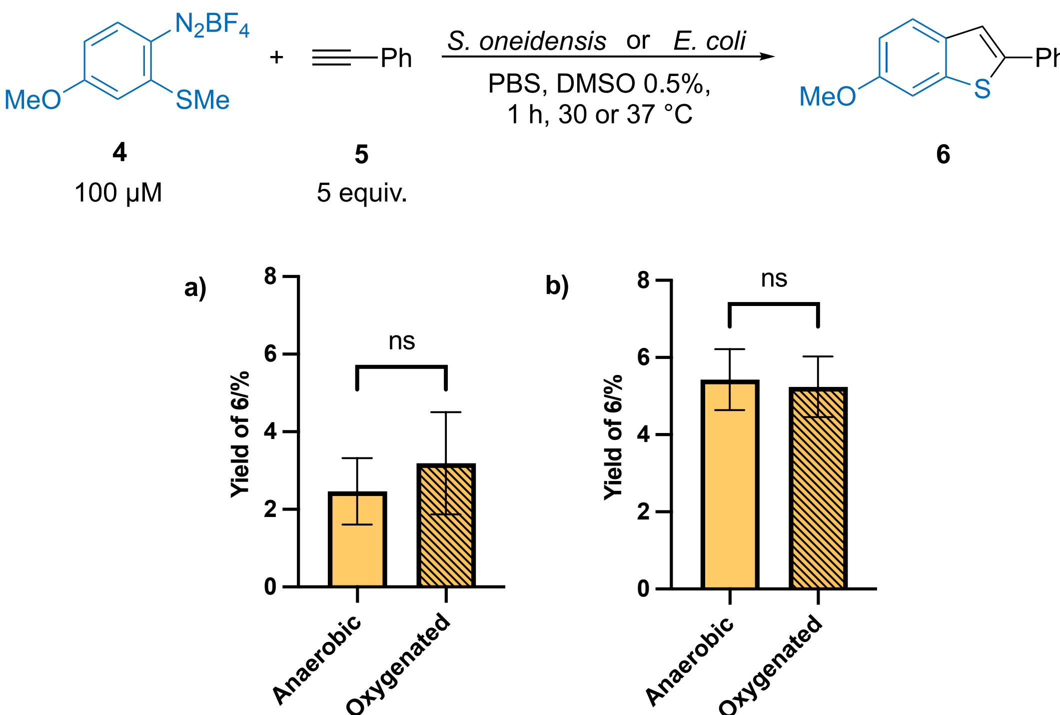


Figure 18. Synthesis of benzothiophene ***6*** *promoted by bacteria*. (a) *S. oneidensis* MR-1, 30 °C. (b) *E. coli* DH5α, 37 °C. Reaction conditions (unless otherwise noted): **4** (1 equiv., 100 μM), **5** (5 equiv.), *bacteria* (OD_{600}=1), 2 mL PBS, 0.5% v/v DMSO, 1 h. Yields were determined by HPLC-MS using caffeine as internal standard. $n = 3$ replicates. Represented values are mean ± SD.

Both *S. oneidensis* and *E. coli* were able to promote this reaction. In this case, we obtained a similar yield for the anaerobic and the oxygenated reaction. This is surprising, since the NADH-promoted reaction showed a reduced yield in presence of oxygen (from 20% to 6%, Figure 11). In any case, it should be noted that the yields for the bacteria-promoted reaction are very low, of less than 6% (corresponding to concentrations of less than 6 μM of the product in the reaction milieu), so an adequate quantification is difficult. Also, in contrast to the Meerwein arylation, this reaction shows no appreciable background activity in absence of bacteria or NADH (Figure 11). Additional studies are needed to clarify the mechanism of this reaction. In particular, the influence of the redox properties of different diazonium salts on the interaction with metabolic processes demands further study, as it may allow to devise reactions with greater selectivity.

4.3. Conclusions

In this Master dissertation, we have shown that the redox metabolism of live bacteria can be harnessed to promote non-natural, bond-forming synthetic chemical reactions by reduction of aryl diazonium salts to aryl radicals. We found that the electrogenic bacterium *Shewanella oneidensis* MR-1 and the model bacterium *Escherichia coli* DH5α can promote

the Meerwein arylation of naphthoquinones, and a radical annulation reaction for the synthesis of benzothiophenes. In abiotic conditions, biological reductants like NADH promote the Meerwein arylation by a radical chain mechanism. The reaction takes place in presence of live *S. oneidensis*, but further studies are needed to determine its mechanism. In the case of *E. coli*, both live and dead bacteria promote the reaction, and secreted reducing metabolites have been identified as one of the factors responsible for the reaction.

The possibility of hijacking the redox metabolism of microorganisms represents a new opportunity for interfacing chemical and biological synthesis and developing new sustainable synthetic strategies. It could also have application on biomedicine, for example for the *in situ* synthesis of antibiotics, and this strategy may be translated to eukaryotic organisms for localized *in situ* synthesis of drugs and probes in reductive milieus, like the microenvironments of many tumors. Tuning of the redox potential of the diazonium salt would allow for greater control and selectivity of the reaction. Additionally, alternatives to aryl diazonium salts as radical sources can be explored.

5. Schedule of practices and place of performance

This work was conducted in the Center for Research in Biological Chemistry and Molecular Materials of the University of Santiago de Compostela (CiQUS), Jenaro de la Fuente St., Santiago de Compostela, Spain. Experimental work was conducted at laboratories P3L4, NMR and Instrumental 3 of the CiQUS, as well as laboratory P0L2 of the CIBUS building.

Month	September				October					November				December				January				
Week	1	2	3	4	1	2	3	4	5	1	2	3	4	1	2	3	4	1	2	3	4	5
Task 1																						
Task 2																						
Task 3																						
Task 4																						
Task 5																						

Task 1	Synthesis of precursors
Task 2	Study of the Meerwein arylation promoted by biological reductants
Task 3	Study of a radical annulation promoted by biological reductants
Task 4	Evaluation of the bacteria-promoted Meerwein arylation
Task 5	Evaluation of the bacteria-promoted radical annulation

Figure 19. Schedule of practices.

Personal assessment

The experimental work carried out during this Master dissertation has benefited from the following theoretical knowledge acquired in the Master:

- Magnetic resonance: obtention and interpretation of ^{1}H- and ^{13}C-NMR spectra, quantification by NMR with internal standard. Interpreting EPR spectra.
- Spectroscopic and spectrometric techniques: obtaining and interpreting UV-Vis and MS spectra.
- Chemical and Cellular Biology: the concept of chemical biology and its relationship with chemical synthesis and cellular biology; the concepts of biocompatible and bioorthogonal chemistry.
- Experimental techniques in Molecular Biology and Biomedicine: techniques for microorganism culture.
- Catalysis: understanding and performing catalytical reactions for chemical synthesis.
- Chemical synthesis: understanding, planning and performing synthetical routes for the molecules employed in this work.
- Determination of reaction mechanisms: understanding mechanistic investigations and designing experiments to elucidate reaction mechanisms.

Technical skills acquired include: chemical synthesis techniques (work under inert atmosphere in a Schlenk line), purification techniques (flash column chromatography, MPLC), characterization and analytical techniques (NMR, HPLC-MS, UV-Vis spectroscopy, use of internal standards for NMR and HPLC UV-Vis quantification), microbiology techniques.

Bibliographic research allowed for deepening in the following topics: biocompatible and bioorthogonal chemistry, interfacing of chemical and biological synthesis, redox metabolism of eucaryotic and procaryotic cells, bacteria extracellular electron transfer, chemistry of aryl diazonium salts, redox chemistry of NADH.

Problems raised during the experimental work included:

- Synthesis of precursors: we could not successfully replicate some synthetic procedures found in the literature; specifically, synthesis of 2-(4-hydroxyphenyl)naphthalene-1,4-dione (molecule **2b**) proved challenging. This forced us to design alternative synthetic procedures.
- Translating reactions to biological conditions: reproducibility issues were raised when translating the studied reactions to low concentrations and biological conditions. The inherent variability of living systems was accounted for by

performing adequate replicates and statistical analysis. Also, oxygen was found to affect negatively the bacteria-promoted reaction. This was prevented with procedures that minimized oxygen exposure.

References

(1) Wallace, S., Balskus, E. P. Opportunities for Merging Chemical and Biological Synthesis. *Curr. Opin. Biotechnol.* **2014**, *30*, 1–8.

(2) Sadler, J. C. The Bipartisan Future of Synthetic Chemistry and Synthetic Biology. *ChemBioChem* **2020**, *21*, 3489–3491.

(3) Volk, M. J., Tran, V. G., Tan, S.-I., Mishra, S., Fatma, Z., Boob, A., Li, H., Xue, P., Martin, T. A., Zhao, H. Metabolic Engineering: Methodologies and Applications. *Chem. Rev.* **2023**, *123*, 5521–5570.

(4) Arnold, F. H. Directed Evolution: Bringing New Chemistry to Life. *Angew. Chem. Int. Ed.* **2018**, *57*, 4143–4148.

(5) Sadler, J. C., Dennis, J. A., Johnson, N. W., Wallace, S. Interfacing Non-Enzymatic Catalysis with Living Microorganisms. *RSC Chem. Biol.* **2021**, *2*, 1073–1083.

(6) Wu, J., Lin, J., Huang, P. Harnessing Abiotic Organic Chemistry in Living Systems for Biomedical Applications. *Chem. Soc. Rev.* **2023**, *52*, 3973–3990.

(7) Victorino da Silva Amatto, I., Gonsales da Rosa-Garzon, N., Antônio de Oliveira Simões, F., Santiago, F., Pereira da Silva Leite, N., Raspante Martins, J., Cabral, H. Enzyme Engineering and Its Industrial Applications. *Biotechnol. Appl. Biochem.* **2022**, *69*, 389–409.

(8) Kapoor, S., Rafiq, A., Sharma, S. Protein Engineering and Its Applications in Food Industry. *Crit. Rev. Food. Sci. Nutr.* **2017**, *57*, 2321–2329.

(9) Vornholt, T., Leiss-Maier, F., Jeong, W. J., Zeymer, C., Song, W. J., Roelfes, G., Ward, T. R. Artificial Metalloenzymes. *Nat. Rev. Methods Primers* **2024**, *4*, 78.

(10) Liu, J., Chan, S. H. J., Brock-Nannestad, T., Chen, J., Lee, S. Y., Solem, C., Jensen, P. R. Combining Metabolic Engineering and Biocompatible Chemistry for High-Yield Production of Homo-Diacetyl and Homo-(S,S)-2,3-Butanediol. *Metab. Eng.* **2016**, *36*, 57–67.

(11) Schafer, F. Q., Buettner, G. R. Redox Environment of the Cell as Viewed through the Redox State of the Glutathione Disulfide/Glutathione Couple. *Free Radic. Biol. Med.* **2001**, *30*, 1191–1212.

(12) Go, Y.-M., Jones, D. P. Redox Compartmentalization in Eukaryotic Cells. *Biochim. Biophys. Acta – Gen. Subj.* **2008**, *1780*, 1273–1290.

(13) Reichart, O., Szakmár, K., Jozwiak, Á., Felföldi, J., Baranyai, L. Redox Potential Measurement as a Rapid Method for Microbiological Testing and Its Validation for Coliform Determination. *Int. J. Food Microbiol.* **2007**, *114*, 143–148.

(14) Kang, J., Pervaiz, S. Mitochondria: Redox Metabolism and Dysfunction. *Biochem. Res. Int.* **2012**, *2012*, 896751.

(15) Madigan, M. T., Martinko, J. M., Bender, K. S., Buckley, D. H., Stahl, D. A., Brock, T. Brock Biology of Microorganisms (Pearson). 2014.

(16) Shi, L., Dong, H., Reguera, G., Beyenal, H., Lu, A., Liu, J., Yu, H.-Q., Fredrickson, J. K. Extracellular Electron Transfer Mechanisms between Microorganisms and Minerals. *Nat. Rev. Microbiol.* **2016**, *14*, 651–662.

(17) Nealson, K. H., Scott, J. Ecophysiology of the Genus Shewanella. In The Prokaryotes, Dworkin, M., Falkow, S., Rosenberg, S., Schleifer, K., Stackebrandt, E., eds. (Springer), 2006, pp. 1133–1151.

(18) Pirbadian, S., Barchinger, S. E., Leung, K. M., Byun, H. S., Jangir, Y., Bouhenni, R. A., Reed, S. B., Romine, M. F., Saffarini, D. A., Shi, L., Gorby, Y. A., Golbeck, J. H., El-Naggar, M. Y. Shewanella Oneidensis MR-1 Nanowires Are Outer Membrane and Periplasmic Extensions of the Extracellular Electron Transport Components. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111*, 12883–12888.

(19) Xu, S., Jangir, Y., El-Naggar, M. Y. Disentangling the Roles of Free and Cytochrome-Bound Flavins in Extracellular Electron Transport from Shewanella Oneidensis MR-1. *Electrochim. Acta* **2016**, *198*, 49–55.

(20) Myers, C. R., Nealson, K. H. Bacterial Manganese Reduction and Growth with Manganese Oxide as the Sole Electron Acceptor. *Science* **1988**, *240*, 1319–1321.

(21) Gralnick, J. A., Vali, H., Lies, D. P., Newman, D. K. Extracellular Respiration of Dimethyl Sulfoxide by *Shewanella Oneidensis* Strain MR-1. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 4669–4674.

(22) Zhang, J., Li, F., Liu, D., Liu, Q., Song, H. Engineering Extracellular Electron Transfer Pathways of Electroactive Microorganisms by Synthetic Biology for Energy and Chemicals Production. *Chem. Soc. Rev.* **2024**, *53*, 1375–1446.

(23) Magennis, E. P., Fernandez-Trillo, F., Sui, C., Spain, S. G., Bradshaw, D. J., Churchley, D., Mantovani, G., Winzer, K., Alexander, C. Bacteria-Instructed Synthesis of Polymers for Self-Selective Microbial Binding and Labelling. *Nat. Mater.* **2014**, *13*, 748–755.

(24) Kolb, H. C., Finn, M. G., Sharpless, K. B. Click Chemistry: Diverse Chemical Function from a Few Good Reactions. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021.

(25) Bennett, M. R., Gurnani, P., Hill, P. J., Alexander, C., Rawson, F. J. Iron-Catalysed Radical Polymerisation by Living Bacteria. *Angew. Chem. Int. Ed.* **2020**, *59*, 4750–4755.

(26) Fan, G., Dundas, C. M., Graham, A. J., Lynd, N. A., Keitz, B. K. Shewanella Oneidensis as a Living Electrode for Controlled Radical Polymerization. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115*, 4559–4564.

(27) Fan, G., Graham, A. J., Kolli, J., Lynd, N. A., Keitz, B. K. Aerobic Radical Polymerization Mediated by Microbial Metabolism. *Nat. Chem.* **2020**, *12*, 638–646.

(28) D. Nothling, M., Cao, H., G. McKenzie, T., M. Hocking, D., A. Strugnell, R., G. Qiao, G. Bacterial Redox Potential Powers Controlled Radical Polymerization. *J. Am. Chem. Soc.* **2021**, *143*, 286–293.

(29) Li, Y., Huang, Y., Gao, Z., Song, G., Lv, F., Bai, H., Wang, S. Living Cell-Mediated Catalyst-Free Spontaneous Polymerization of Zwitterionic Methacrylates for Preparation of Probiotic-Loaded Hydrogels. *Angew. Chem. Int. Ed.* **2024**, e202414400.

(30) Partipilo, G., Graham, A. J., Belardi, B., Keitz, B. K. Extracellular Electron Transfer Enables Cellular Control of Cu(I)-Catalyzed Alkyne–Azide Cycloaddition. *ACS Cent. Sci.* **2022**, *8*, 246–257.

(31) Shen, Q., Huang, Y., Zeng, Y., Zhang, E., Lv, F., Liu, L., Wang, S. Intracellular Radical Polymerization of Paclitaxel-Bearing Acrylamide for Self-Inflicted Apoptosis of Cancer Cells. *ACS Mater. Lett.* **2021**, *3*, 1307–1314.

(32) Gamcsik, M. P., Kasibhatla, M. S., Teeter, S. D., Colvin, O. M. Glutathione Levels in Human Tumors. *Biomarkers* **2012**, *17*, 671–691.

(33) Kalinina, E. V., Gavriliuk, L. A. Glutathione Synthesis in Cancer Cells. *Biochemistry (Moscow)* **2020**, *85*, 895–907.

(34) Chu, X., Dou, X., Yu, J., Zhou, J., Ma, D., Miao, M., Hu, S., Sun, K., Zhu, S., Liu, Q., Zhang, X., Jiang, Y., Wang, Z.-F. Synthesis of Polymers via Cancer Cell Metabolism-Mediated Controlled Radical Polymerization and Application in Engineering of Cell Surface. *Biomacromolecules* **2025**, *26*, 238–247.

(35) Scarpa de Souza, E. L., Oliveira, C. C. Selective Radical Transformations with Aryldiazonium Salts. *Eur. J. Org. Chem.* **2023**, *26*, 105–127.

(36) Schank, K. Preparation of Diazonium Groups. In Diazonium and Diazo Groups, Patai, S., ed. (John Wiley & Sons, Ltd.), 1978, Vol. 2, pp. 645–657.

(37) Bonin, H., Fouquet, E., Felpin, F.-X. Aryl Diazonium versus Iodonium Salts: Preparation, Applications and Mechanisms for the Suzuki-Miyaura Cross-Coupling Reaction. *Adv. Synth. Catal.* **2011**, *353*, 3063–3084.

(38) Bondarev, A. A., Naumov, E. V., Kassanova, A. Zh., Krasnokutskaya, E. A., Stankevich, K. S., Filimonov, V. D. First Study of the Thermal and Storage Stability of Arenediazonium Triflates Comparing to 4-Nitrobenzenediazonium Tosylate and Tetrafluoroborate by Calorimetric Methods. *Org. Process Res. Dev.* **2019**, *23*, 2405–2415.

(39) Galli, C. Radical Reactions of Arenediazonium Ions: An Easy Entry into the Chemistry of the Aryl Radical. *Chem. Rev.* **1988**, *88*, 765–792.

(40) Reszka, K. J., Chignell, C. F. One-Electron Reduction of Arenediazonium Compounds by Physiological Electron Donors Generates Aryl Radicals. An EPR and Spin Trapping Investigation. *Chem. Biol. Interact.* **1995**, *96*, 223–234.

(41) Crisóstomo, F. P., Martín, T., Carrillo, R. Ascorbic Acid as an Initiator for the Direct C-H Arylation of (Hetero)Arenes with Anilines Nitrosated in Situ. *Angew. Chem. Int. Ed.* **2014**, *53*, 2181–2185.

(42) Tatunashvili, E., Chan, B., Nashar, P. E., McErlean, C. S. P. σ-Bond Initiated Generation of Aryl Radicals from Aryl Diazonium Salts. *Org. Biomol. Chem.* **2020**, *18*, 1812–1819.

(43) Nagar, B., Dhar, B. B. Photochemical C–H Arylation of Napthoquinones Using Eosin Y. *ACS Omega* **2022**, *7*, 32615–32619.

(44) Fernández-González, X., Miguel-Ávila, J., Mascareñas, J. L., Tomás-Gamasa, M. Photocatalytic Arylations with Diazonium Salts in Aqueous and Biorelevant Media. *ChemCatChem* (in press).

(45) Sletten, E. M., Bertozzi, C. R. Bioorthogonal Chemistry: Fishing for Selectivity in a Sea of Functionality. *Angew. Chem. Int. Ed.* **2009**, *48*, 6974–6998.

(46) Lang, K., Chin, J. W. Bioorthogonal Reactions for Labeling Proteins. *ACS Chem. Biol.* **2014**, *9*, 16–20.

(47) Pramanik, M. M. D., Rastogi, N. Visible Light Catalyzed Methylsulfoxidation of (Het)Aryl Diazonium Salts Using DMSO. *Chem. Commun.* **2016**, *52*, 8557–8560.

(48) Yasui, S., Nakamura, K., Ohno, A. Reduction by a Model of NAD(P)H. 45. Mechanism for the Dediazoniation of Arenediazonium Salts Initiated by One-Electron Transfer from an NAD(P)H Model. *J. Org. Chem.* **1984**, *49*, 878–882.

(49) Schroll, P., Hari, D. P., König, B. Photocatalytic Arylation of Alkenes, Alkynes and Enones with Diazonium Salts. *ChemistryOpen* **2012**, *1*, 130–133.

(50) Keri, R. S., Chand, K., Budagumpi, S., Balappa Somappa, S., Patil, S. A., Nagaraja, B. M. An Overview of Benzo[b]Thiophene-Based Medicinal Chemistry. *Eur. J. Med. Chem.* **2017**, *138*, 1002–1033.

(51) Leardini, R., Pedulli, G. F., Tundo, A., Zanardi, G. Reaction Pathways for the Cyclization of Ortho-Thioalkyl and Ortho-Thioaryl Substituted Phenyl Radicals with Alkynes. Reaction of o-Methylthioarenediazonium Tetrafluoroborates with Alkynes to Give 2-Substituted

Benzo[b]Thiophenes. *J. Chem. Soc. Chem. Commun.* **1985**, *20*, 1390–1391.

(52) Hari, D. P., Hering, T., König, B. Visible Light Photocatalytic Synthesis of Benzothiophenes. *Org. Lett.* **2012**, *14*, 5334–5337.

(53) Smirnova, G., Muzyka, N., Oktyabrsky, O. Transmembrane Glutathione Cycling in Growing Escherichia Coli Cells. *Microbiol. Res.* **2012**, *167*, 166–172.

(54) Knoke, L. R., Zimmermann, J., Lupilov, N., Schneider, J. F., Celebi, B., Morgan, B., Leichert, L. I. The Role of Glutathione in Periplasmic Redox Homeostasis and Oxidative Protein Folding in Escherichia Coli. *Redox Biol.* **2023**, *64*, 102800.

(55) Rowe, A. R., Rajeev, P., Jain, A., Pirbadian, S., Okamoto, A., Gralnick, J. A., El-Naggar, M. Y., Nealson, K. H. Tracking Electron Uptake from a Cathode into *Shewanella* Cells: Implications for Energy Acquisition from Solid-Substrate Electron Donors. *mBio* **2018**, *9*, e0220317.

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Abbreviations

^{1}H-NMR	Proton nuclear magnetic resonance
^{13}C-NMR	Carbon-13 nuclear magnetic resonance
Abs	Absorbance
ANOVA	Analysis of variance
BCCM	Belgian Coordinated Collections of Microorganisms
br	Broad
cat. No.	Catalogue number
CFU	Colony formation unit
d	Doublet
DMSO	Dimethyl sulfoxide
E. coli	*Escherichia coli*
equiv.	Equivalent
ESI	Electrospray ionization
HPLC	High-performance liquid chromatography
HPLC-MS	High-performance liquid chromatography coupled to mass spectrometry
IS	Internal standard
LB	Lysogeny broth
LSD	Least significant difference
m	Multiplet
MPLC	Medium-pressure liquid chromatography
NADH	Nicotinamide adenine dinucleotide (reduced form)
n/d	Not detected
NMR	Nuclear magnetic resonance
OD_{600}	Optical density measured at 600 nm
PBS	Phosphate-saline buffer
ppm	Parts per million
R_F	Retardation factor
r.t.	Room temperature (approx. 21 ºC)
s	Singlet
SD	Standard deviation

S. oneidensis	*Shewanella oneidensis*
TLC	Thin layer chromatography
UV	Ultraviolet
UV-Vis	Ultraviolet-visible
v/v	Volume/volumen
vs.	Versus

1. Synthetic procedures

1.1. General information on synthetic procedures

Chemicals were acquired from Sigma Aldrich, Panreac, GE Healthcare, Alfa Aesar and Serva, and were used without further purification. The solvents used in reactions were of HPLC grade unless otherwise noted, and they were used without further purification.

Room temperature (r.t.) refers to approximately 21 °C.

TLC was performed in aluminum plates with silica gel Merck 60 F_{254} and visualized under UV (λ=254 nm).

Vacuum concentration was achieved by rotary evaporation on a Büchi R-210 rotary evaporator, with a V-850 vacuum regulator, V-700 vacuum pump and B-491 thermostatic bath, followed by evaporation under high vacuum.

Flash column chromatography was performed on Merck Geduran Si 60 (40–63 µm) silica gel. MPLC was performed on a Büchi Pure C-810 Flash MPLC.

Centrifugation before HPLC analysis was performed on an Eppendorf 5430 centrifuge.

NMR spectra were recorded on an Agilent VNMRS-300 spectrometer. Spectra were analyzed using MestreNova data processing software (www.mestrelab.com). Data are represented as follows: chemical shift (δ) in parts per million (ppm) downfield from tetramethylsilane, multiplicity (s = singlet, d = doublet, m = multiplet, br = broad), coupling constants (*J*) in Hertz (Hz). Spectra were calibrated to the residual solvent peak, when possible ($CDCl_3$ δ = 7.260 ppm. d6-DMSO δ = 2.500 ppm).

Analytical HPLC-MS was performed on a Fisher HPLC Thermo Ultimate 3000 coupled to a Bruker AmaZon SL mass spectrometer, using electrospray ionization (ESI) and a flow rate of 0.350 mL/min at r.t.

1.2. Synthesis of substrates and products

1.2.1. Synthesis of p-*methoxyphenyldiazonium tetrafluoroborate (**1**)*

Diazonium salt **1** was synthetized by the classical diazotization procedure.[1]

NH_2

HBF_4, $NaNO_2$

H_2O, 0 ºC, 1 h 15 min

N_2BF_4

OMe

OMe

1

p-Anisidine (1.0 g, 8.12 mmol, 1 equiv.) was suspended in 2.4 mL of water in a 25 mL round-bottom flask. Tetrafluoroboric acid (2.1 mL, 16.24 mmol, 2 equiv.) was added to give a black solution. The reaction was cooled to 0 ºC in a water-ice bath. A cold solution of sodium nitrite (1.12 g, 16.24 mmol, 2 equiv.) in 2.4 mL of water was added dropwise. The mixture turned dark green, and the formation of a grey solid was observed. After 1 h 15 min, the solid was isolated by filtration as a pearl grey powdery solid, washed with 10 mL of cold diethyl ether, redissolved in acetone, and recrystallized in 10 mL of diethyl ether to give pure **1** as a pearl grey powdery solid (1.2 g, 66% yield).

^{1}H-NMR (300 MHz, d6-DMSO) δ 8.61 (d, *J* = 9.0 Hz, 2H), 7.48 (d, *J* = 9.0 Hz, 2H), 4.04 (s, 3H).

NMR data in accordance with the literature.[2]

*1.2.2. Synthesis of 2-(4-methoxyphenyl)naphthalene-1,4-dione (**2a**)*

Procedure was adapted from literature.[3]

$Pd(PPh_3)_4$, Cs_2CO_3

THF, H_2O, 65 ºC, 24 h

2a

Tetrakis(triphenylphosphine)palladium(0) (28.9 mg, 0.025 mmol, 0.025 equiv.) was dissolved in 4.0 mL of anhydrous THF in a purged Schlenk tube under nitrogen atmosphere. 2-Bromonaphthalene-1,4-dione (237.5 mg, 1.0 mmol, 1 equiv.), cesium carbonate (488.7 mg, 1.5 mmol, 1.5 equiv.) and 4-methoxyphenylboronic acid (227.9 mg, 1.5 mmol, 1.5 equiv.) were successively added to the stirred solution under nitrogen flow. Then, 0.6 mL of water were added. The mixture was heated to 65 ºC until TLC (silica gel, *n*-hexane/ethyl acetate 9:1) showed complete consumption of the starting material (20 h). After cooling to ambient temperature, the mixture was diluted with water (15 mL) and extracted with ethyl acetate (3×10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The product was purified by flash chromatography on silica gel (*n*-hexane/ethyl acetate, gradient from 0% to 20% of ethyl acetate) as an orange solid (238.4 mg, 90% yield).

R_F=0.33 (*n*-hexane/ethyl acetate 9:1).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.19–8.05 (m, 2H), 7.78–7.70 (m, 2H), 7.60–7.53 (m, 2H), 7.04–6.95 (m, 3H), 3.86 (s, 3H).

NMR data in accordance with the literature.[4]

*1.2.3. Synthesis of 2-(4-hydroxyphenyl)naphthalene-1,4-dione (**2b**)*

Synthesis was achieved by the following procedure, which was adapted from the procedure employed for the synthesis of **2a**.[3]

$Pd(PPh_3)_4$, Cs_2CO_3
THF, H_2O, 65 ºC, 24 h

2b

Tetrakis(triphenylphosphine)palladium(0) (144.4 mg, 0.125 mmol, 0.025 equiv.) was dissolved in 20.0 mL of anhydrous THF in a purged Schlenk tube under nitrogen atmosphere. 2-Bromonaphthalene-1,4-dione (1 185.3 mg, 5.0 mmol, 1 equiv.), cesium carbonate (2 443.7 mg, 7.5 mmol, 1.5 equiv.), 3.0 mL of water and 4-hydroxyphenylboronic acid (1 034.5 mg, 7.5 mmol, 1.5 equiv.) were successively added to the stirred solution under nitrogen flow. The mixture was heated to 65 ºC for 24 h. At that time, TLC (silica gel, *n*-hexane/ethyl acetate 9:1) showed complete consumption of 2-bromonaphthalene-1,4-dione. After cooling to ambient temperature, the mixture was diluted with water (75 mL) and extracted with ethyl acetate (3×10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude was purified by flash chromatography on silica gel (*n*-hexane/ethyl acetate, gradient from 0% to 10% of ethyl acetate) followed by flash MPLC on silica gel (*n*-hexane/ethyl acetate, gradient from 0% to 20% of ethyl acetate) as a dark red solid (91.4 mg, 7% yield).

R_F=0.23 (*n*-hexane/ethyl acetate 9:1).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.26–8.03 (m, 2H), 7.86–7.68 (m, 2H), 7.54 (d, *J* = 8.2 Hz, 2H), 7.04 (s, 1H), 6.93 (d, *J* = 8.2 Hz, 2H), 5.22 (s, 1H).

NMR data in accordance with the literature.[5]

1.2.4. Synthesis of 4-methoxy-2-(methylthio)aniline

The synthesis of 4-methoxy-2-(methylthio)aniline was performed following the procedure reported by König *et al.*[6]

1. KOH, H_2O, 100 °C to r.t., 24 h
2. MeI, r.t., 1 h

A suspension of 6-methoxybenzo[d]thiazol-2-amine (2.703 g, 15.0 mmol, 1 equiv.) in 120 mL water with KOH (30 g, 534.0 mmol, 35.6 equiv.) was refluxed for 24 h at 100 °C. The suspension turned pale yellow. After cooling to r.t., MeI (0.934 mL, 15.0 mmol, 1 equiv.) was added. The suspension turned brown, and bubbling was observed. The reaction mixture was stirred for 1 h at r.t. and extracted with diethyl ether (3×5 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum as a dark brownish oil. The product was purified by flash column chromatography on silica gel (*n*-hexane/ethyl acetate, gradient from 0% to 10% of ethyl acetate) as a pale-yellow oil that solidified at −28 ºC (906.6 mg, 35.7% yield).

R_F=0.25 (*n*-hexane/ethyl acetate 9:1).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.00–6.83 (m, 1H), 6.74–6.58 (m, 2H), 3.94 (br, 2H), 3.73 (s, 3H), 2.36 (s, 3H).

NMR data in accordance with the literature.[6]

1.2.5. Synthesis of 4-*methoxy-2-(methylthio)benzenediazonium tetrafluoroborate (**4**)*

Diazonium salt **4** was synthetized following the procedure reported by König *et al.*[6]

NH2, MeO, SMe → HBF4, NaNO2 / H2O, 0 ºC, 1 h 15 min → N2BF4, MeO, SMe, **4**

4-Methoxy-2-(methylthio)aniline (203.1 mg, 1.2 mmol, 1 equiv.) was dissolved in a mixture of 0.5 mL of water and 0.4 mL of 50% tetrafluoroboric acid in water (3.2 mmol, 2.7 equiv.). The reaction mixture was cooled to 0 °C using an ice-water bath, and a sodium nitrite solution (165.5 mg, 2.4 mmol, 2 equiv.) in 0.4 mL of water was added dropwise to the mixture. A yellow precipitate formed. 1.0 mL of water was added. The resulting mixture was stirred for 40 min at 0–5 °C. Then, cold diethyl ether (5.0 mL) was added. A pale-yellow precipitate was formed, which was filtered and redissolved in minimum amount of acetone. 10 mL of cold diethyl ether were added to the solution to induce precipitation of the product. The precipitate was filtered again, washed with diethyl ether (3×5 mL) and dried under vacuum to obtain the desired product as pale-yellow solid (113.3 mg, 35.2% yield).

^{1}H-NMR (300 MHz, d6-DMSO): δ 8.58 (d, *J* = 7.8 Hz, 1H), 7.52–7.02 (m, 2H), 4.09 (s, 3H), 2.85 (s, 3H).

NMR data in accordance with the literature.[6]

*1.2.6. Synthesis of 2,3-bis(4-methoxyphenyl)naphthalene-1,4-dione (**3a**)*

Compound **3a** was synthesized for characterization following NADH-promoted Meerwein arylation of naphthoquinone **2a** with diazonium salt **1**.

N_2BF_4 OMe O O OMe O O OMe OMe
NADH 10 mol%
DMSO, r.t., 2 h,
inert atmosphere
1 **2a** **3a**
10 mM 3 equiv.

Diazonium salt **1** (22.2 mg, 0.10 mmol, 1 equiv.), nafotoquinone **2a** (79.3 mg, 0.3 mmol, 3 equiv.) and NADH disodium salt (7.1 mg, 0.010 mmol, 10 mol%) were introduced under nitrogen flow in a purged 50 mL Schlenk tube equipped with a stirring bar. The tube was then purged with three vacuum-nitrogen cycles. 10 mL of anhydrous DMSO were added to yield an orange solution. The solution was magnetically stirred for 2 h under nitrogen atmosphere. Bubbling was observed, and the solution turned dark red. Then, the solution was diluted with water (100 mL) and extracted with diethyl ether (3×10 mL). The combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude was purified by flash column chromatography on silica gel (*n*-hexane/ethyl acetate, 8:2) as a red solid (16.9 mg, 46%).

R_F=0.22 (*n*-hexane/ethyl acetate 9:1).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 8.23–8.14 (m, 2H), 7.81–7.72 (m, 2H), 7.11–6.98 (m, 4H), 6.86–6.72 (m, 4H), 3.78 (s, 6H).

NMR data in accordance with the literature.[4]

*1.2.7. Synthesis of 6-methoxy-2-phenylbenzo[b]thiophene (**6**)*

Compound **6** was synthesized for characterization following NADH-promoted radical annulation.

N_2BF_4 MeO SMe + Ph MeO S Ph
NADH 10 mol%
DMSO/H_2O 2:8,
2 h, r.t.,
inert atmosphere
4 **5** **6**
10 mM 50 equiv.

Diazonium salt **4** (26.8 mg, 0.10 mmol, 1 equiv.) was introduced under nitrogen flow in a purged 10 mL Schlenk tube equipped with a stirring bar. The tube was purged with three vacuum-nitrogen cycles. 2 mL of DMSO and phenyl acetylene **5** (549 µL, 5.0 mmol, 5 equiv.)

were added under nitrogen flow, and the solution was magnetically stirred. NADH disodium salt (7.1 mg, 0.010 mmol, 10 mol%) was added dissolved in 8.0 mL of water under nitrogen flow, and the mixture was magnetically stirred. After 2 h, the mixture was diluted with 20 mL of water and extracted with diethyl ether (3×5 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The crude was purified by flash column chromatography on silica gel (*n*-hexane/ethyl acetate, 95:5) as a light yellow solid (4.0 mg, 17%)

R_F=0.26 (*n*-hexane/ethyl acetate 95:5).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.73–7.61 (m, 3H), 7.56–7.36 (m, 5H), 6.99 (d, *J* = 8.6 Hz, 1H), 3.89 (s, 3H).

NMR data in accordance with the literature.[6]

1.3. Study of synthetic reactions of aryl diazonium salts promoted by biological reductants

1.3.1. General procedure for evaluation of Meerwein arylation of 1,4-naphthoquinones promoted by biological reductants at 10 mM in DMSO

N_2BF_4 / OMe **1** 10 mM + (naphthoquinone, R) 3 equiv. —*Biological reductant*, DMSO, r.t., inert atmosphere→ (2-aryl-naphthoquinone, R, OMe)

Diazonium salt **1** (2.2 mg, 0.01 mmol, 1 equiv.), naphthoquinone (0.03 mmol, 3 equiv.) and biological reductant (0.1–2 equiv.) were introduced under nitrogen flow in a purged 10 mL Schlenk tube equipped with a stirring bar. The tube was purged with three vacuum-nitrogen cycles. 1 mL of anhydrous DMSO was added, and the solution was magnetically stirred. After 1 min–4 h, the solution was diluted with 10 mL of water and extracted with diethyl ether (3×10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The obtained solid was dissolved in an adequate quantity of a solution of dibromomethane 10 mM in $CDCl_3$. Yield was determined by ^{1}H-NMR using dibromomethane as internal standard.

1.3.2. Representative procedure for evaluation of Meerwein arylation of 1,4-naphthoquinones promoted by NADH at 10 mM in DMSO

N_2BF_4 OMe **1** 10 mM + OMe **2a** 3 equiv. NADH (1 equiv.) DMSO, r.t., 2 h, inert atmosphere OMe OMe **3a**

Diazonium salt **1** (2.2 mg, 0.01 mmol, 1 equiv.), naphthoquinone **2a** (7.9 mg, 0.03 mmol, 3 equiv.) and NADH disodium salt (7.1 mg, 0.01 mmol, 1 equiv.) were introduced under nitrogen flow in a purged 10 mL Schlenk tube equipped with a stirring bar. The tube was purged with three vacuum-nitrogen cycles. 1 mL of anhydrous DMSO was added to yield an orange solution. The solution was magnetically stirred. Bubbling was observed, and the solution turned dark red in 10 min. After 2 h, the solution was diluted with 10 mL of water and extracted with diethyl ether (3×10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The obtained solid was dissolved in 1 mL of a solution of dibromomethane 10 mM in $CDCl_3$. A yield of 76% was determined by ^{1}H-NMR using dibromomethane as internal standard (1 equiv.) and the peak at 3.78 pm (3H, s) of the product **3a**.

1.3.3. General procedure for evaluation of Meerwein arylation of 1,4-naphthoquinones promoted by NADH at 10 mM in DMSO/H_2O

N_2BF_4 OMe **1** 10 mM + R 3 equiv. NADH (1 equiv.) DMSO/H_2O, r.t., inert atmosphere R OMe

Diazonium salt **1** (2.2 mg, 0.01 mmol, 1 equiv.) and naphthoquinone (0.03 mmol, 3 equiv.) were introduced under nitrogen flow in a purged 10 mL Schlenk tube equipped with a stirring bar. The tube was purged with three vacuum-nitrogen cycles. DMSO was added, and the solution was magnetically stirred. NADH disodium salt (7.1 mg, 0.01 mmol, 1 equiv.) was added dissolved in water. The mixture was magnetically stirred. After 1–2 h, the mixture was diluted with 10 mL of water and extracted with diethyl ether (3×10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum.

The obtained solid was dissolved in an adequate quantity of a solution of dibromomethane 10 mM in $CDCl_3$. Yield was determined by ^{1}H-NMR using dibromomethane as internal standard.

1.3.4. Representative procedure for evaluation of Meerwein arylation of 1,4-naphthoquinones promoted by NADH at 10 mM in DMSO/H_2O

N_2BF_4 OMe **1** 10 mM + O O 3 equiv. — NADH (1 equiv.) / DMSO/H_2O 2:8, r.t., 2 h, inert atmosphere → O O OMe **2a** + O O OMe OMe **3a**

Diazonium salt **1** (2.2 mg, 0.01 mmol, 1 equiv.) and 1,4-naphthoquinone (4.7 mg, 0.03 mmol, 3 equiv.) were introduced under nitrogen flow in a purged 10 mL Schlenk tube equipped with a stirring bar. The tube was purged with three vacuum-nitrogen cycles. 0.2 mL of DMSO were added to yield an orange solution. The solution was magnetically stirred. NADH disodium salt (7.1 mg, 0.01 mmol, 1 equiv.) was added dissolved in 0.8 mL of water. A yellowish-orange turbid mixture formed. The mixture was magnetically stirred. After 2 h, the mixture was diluted with 10 mL of water and extracted three times with diethyl ether. The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The obtained solid was dissolved in 1 mL of a solution of dibromomethane 10 mM in $CDCl_3$. Yields were determined by ^{1}H-NMR using dibromomethane as internal standard (1 equiv.). A yield of 60% was determined for product **2a** using the peak at 3.87 pm (3H, s), and a yield of 6% was determined for product **3a** using the peak at 3.78 pm (6H, s).

1.3.5. General procedure for evaluation of Meerwein arylation of 1,4-naphthoquinones promoted by NADH at 1 mM in DMSO/H_2O

N_2BF_4 OMe **1** 1 mM + (R-naphthoquinone) 3 equiv. → NADH (1 equiv.), DMSO/H_2O 2:8, r.t., inert atmosphere

Diazonium salt **1** (2.2 mg, 0.01 mmol, 1 equiv.) and naphthoquinone (0.03 mmol, 3 equiv.) were introduced under nitrogen flow in a purged 10 mL Schlenk tube equipped with a stirring bar. The tube was purged with three vacuum-nitrogen cycles. 2.0 mL of DMSO were added, and the solution was magnetically stirred. NADH disodium salt (7.1 mg, 0.01 mmol, 1 equiv.) was added dissolved in 8.0 mL of water. The mixture was magnetically stirred. After 1–2 h, the mixture was diluted with water and extracted with diethyl ether (3×10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The obtained solid was dissolved in an adequate quantity of a solution of dibromomethane 10 mM in $CDCl_3$. Yield was determined by ^{1}H-NMR using dibromomethane as internal standard.

1.3.6. Representative procedure for evaluation of Meerwein arylation of 1,4-naphthoquinones promoted by NADH at 1 mM in DMSO/H_2O

N_2BF_4 OMe **1** 1 mM + 3 equiv. → NADH (1 equiv.), DMSO/H_2O 2:8, r.t., 2 h, inert atmosphere → **2a** + **3a**

Diazonium salt **1** (2.2 mg, 0.01 mmol, 1 equiv.) and 1,4-naphthoquinone (4.7 mg, 0.03 mmol, 3 equiv.) were introduced under nitrogen flow in a purged 10 mL Schlenk tube equipped with a stirring bar. The tube was purged with three vacuum-nitrogen cycles. 2.0 mL of DMSO were added to yield an orange solution. The solution was magnetically stirred. NADH disodium salt (7.1 mg, 0.01 mmol, 1 equiv.) was added dissolved in 8.0 mL of water. A yellowish-orange turbid mixture formed. The mixture was magnetically stirred. After 2 h, the mixture was diluted with 10 mL of water and extracted with diethyl ether (3×10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The obtained solid was dissolved in 1 mL of a solution of dibromomethane 10 mM in $CDCl_3$. Yields were determined by ^{1}H-NMR using dibromomethane as internal standard (1 equiv.). A yield of 64% was determined for product **2a** using the peak at 3.87 pm (3H, s), and a yield of 3% was determined for product **3a** using the peak at 3.78 pm (6H, s).

1.3.7. General procedure for evaluation of Meerwein arylation of 1,4-naphthoquinones promoted by NADH at 100 µM

In a purged 15 mL Falcon tube, 0.5 mL of aqueous solvent, 0.5 mL of a stock solution of 1,4-naphthoquinone (400 µM in aqueous solvent with DMSO 1% v/v), 0.5 mL of a stock solution of diazonium salt **1** (400 µM in aqueous solvent with DMSO 1% v/v) and 0.5 mL of a stock solution of NADH disodium salt (400 µM in aqueous solvent) were added under nitrogen atmosphere. Aqueous solvent had been previously deoxygenated with nitrogen bubbling for 30 min. Final concentrations were 100 µM for **1**, 1,4-naphthoquinone and

NADH, and 0.5% v/v DMSO. The reaction mixture was then incubated for 1 h at r.t. with shaking at 200 rpm. The solution turned yellowish. After 1 h, the solution was frozen in liquid nitrogen and lyophilized. The solid residue was dissolved in 800 µL of acetonitrile and 200 µL of a 100 µM solution of coumarin in acetonitrile to achieve a final concentration of coumarin of 20 µM. The mixture was vortexed, scraped with a Pasteur pipette and centrifuged at 7 830 g for 3 min and the supernatant was collected, filtered with a 0.45 µm HPLC filter, and analyzed by HPLC-MS. The yield was determined by HPLC-UV absorbance at 260 nm, using coumarin as internal standard.

*1.3.8. General procedure for evaluation of synthesis of 6-methoxy-2-phenylbenzo[b]thiophene **6** promoted by NADH at 10 mM in DMSO*

N_2BF_4 MeO SMe + Ph → NADH (1 equiv.) / DMSO, 2 h, r.t., inert atmosphere → MeO S Ph

4 10 mM, **5**, **6**

Diazonium salt **4** (2.7 mg, 0.01 mmol, 1 equiv.) and NADH (7.1 mg, 0.01 mmol, 1 equiv.) were introduced under nitrogen flow in a purged 10 mL Schlenk tube equipped with a stirring bar. The tube was purged with three vacuum-nitrogen cycles. 1 mL of anhydrous DMSO and phenyl acetylene (**5**) were added under nitrogen flow, and the solution was magnetically stirred. After 2 h, the solution was diluted with 10 mL of water and extracted with diethyl ether (3×10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The obtained solid was dissolved in 500 µL of a solution of dibromomethane 10 mM in $CDCl_3$. Yield was determined by ^{1}H-NMR using dibromomethane as internal standard (1.5 equiv.) and the peak at 3.89 ppm (s, 3H) of the product **6**.

*1.3.9. Representative procedure for evaluation of synthesis of 6-methoxy-2-phenylbenzo[b]thiophene **6** promoted by NADH at 10 mM in DMSO*

N_2BF_4 MeO SMe + Ph → NADH (1 equiv.) / DMSO, 2 h, r.t., inert atmosphere → MeO S Ph

4 10 mM, **5** 30 equiv., **6**

Diazonium salt **4** (2.7 mg, 0.01 mmol, 1 equiv.) and NADH (7.1 mg, 0.01 mmol, 1 equiv.) were introduced under nitrogen flow in a purged 10 mL Schlenk tube equipped with

a stirring bar. The tube was purged with three vacuum-nitrogen cycles. 1 mL of anhydrous DMSO and phenyl acetylene (32.9 µL, 0.30 mmol, 3 equiv.) were added under nitrogen flow, and the solution was magnetically stirred. The resulting solution was yellow, but quickly turned orange. Bubbling was observed. After 2 h, the solution was diluted with 10 mL of water and extracted with diethyl ether (3×10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The obtained solid was dissolved in 500 µL of a solution of dibromomethane 10 mM in $CDCl_3$. A yield of 38% was determined by ^{1}H-NMR using dibromomethane as internal standard (1.5 equiv.) and the peak at 3.89 ppm (s, 3H) of the product **6**.

*1.3.10. General procedure for evaluation of synthesis of 6-methoxy-2-phenylbenzo[b]thiophene **6** promoted by NADH at 10 mM in DMSO/H_2O*

N_2BF_4
MeO SMe + ≡—Ph → NADH (1 equiv.) / DMSO/H_2O, 2 h, r.t., inert atmosphere → MeO S —Ph

4 (10 mM) **5** **6**

Diazonium salt **4** (2.7 mg, 0.01 mmol, 1 equiv.) was introduced under nitrogen flow in a purged 10 mL Schlenk tube equipped with a stirring bar. The tube was purged with three vacuum-nitrogen cycles. DMSO and phenyl acetylene were added under nitrogen flow, and the solution was magnetically stirred. NADH disodium salt (7.1 mg, 0.01 mmol, 1 equiv.) was added dissolved in water under nitrogen flow, and the mixture was magnetically stirred. After 2 h, the mixture was diluted with 10 mL of water and extracted with diethyl ether (3×10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The obtained solid was dissolved in 500 µL of a solution of dibromomethane 10 mM in $CDCl_3$. Yield was determined by ^{1}H-NMR using dibromomethane as internal standard (1.5 equiv.) and the peak at 3.89 ppm (s, 3H) of the product **6**.

*1.3.11. Representative procedure for evaluation of synthesis of 6-methoxy-2-phenylbenzo[b]thiophene **6** promoted by NADH at 10 mM in DMSO/H_2O*

N_2BF_4
MeO SMe + ≡—Ph → NADH (1 equiv.) / DMSO/H_2O 2:8, 2 h, r.t., inert atmosphere → MeO S —Ph

4 (10 mM) **5** (50 equiv.) **6**

Diazonium salt **4** (2.7 mg, 0.01 mmol, 1 equiv.) was introduced under nitrogen flow in a purged 10 mL Schlenk tube equipped with a stirring bar. The tube was purged with three vacuum-nitrogen cycles. 0.2 mL of DMSO and phenyl acetylene (54.9 µL, 0.50 mmol, 5 equiv.) were added under nitrogen flow, and the solution was magnetically stirred. NADH (7.1 mg, 0.01 mmol, 1 equiv.) was added dissolved in 0.8 mL of water, and the mixture was magnetically stirred. The resulting turbid mixture was yellow, but quickly turned orange. After 2 h, the mixture was diluted with 10 mL of water and extracted with diethyl ether (3×10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The obtained solid was dissolved in 500 µL of a solution of dibromomethane 10 mM in $CDCl_3$. A yield of 81% was determined by ^{1}H-NMR using dibromomethane as internal standard (1.5 equiv.) and the peak at 3.89 ppm (s, 3H) of the product **6**.

*1.3.12. Evaluation of synthesis of 6-methoxy-2-phenylbenzo[b]thiophene **6** promoted by NADH at 1 mM in DMSO/H₂O*

N_2BF_4 MeO SMe **4** 1 mM + Ph **5** 50 equiv. → NADH (1 equiv.), DMSO/H_2O 2:8, 2 h, r.t., inert atmosphere → MeO S Ph **6**

Diazonium salt **4** (2.7 mg, 0.01 mmol, 1 equiv.) was introduced under nitrogen flow in a purged 10 mL Schlenk tube equipped with a stirring bar. The tube was purged with three vacuum-nitrogen cycles. 2.0 mL of DMSO and phenyl acetylene (54.9 µL, 0.50 mmol, 5 equiv.) were added under nitrogen flow, and the solution was magnetically stirred. NADH (7.1 mg, 0.01 mmol, 1 equiv.) dissolved in 0.5 mL of water, and 7.5 mL of water were added. The resulting turbid mixture was yellow, but quickly turned orange. A dark orange solid was observed in suspension on the surface of the liquid. After 2 h, the solution was diluted with 5 mL of water and extracted with diethyl ether (3×10 mL). The combined organic layers were washed with brine, dried over $MgSO_4$ and concentrated under vacuum. The obtained solid was dissolved in 500 µL of a solution of dibromomethane 10 mM in $CDCl_3$. A yield of 67% was determined by ^{1}H-NMR using dibromomethane as internal standard (1.5 equiv.) and the peak at 3.89 ppm (s, 3H) of the product **6**.

*1.3.13. Evaluation of synthesis of 6-methoxy-2-phenylbenzo[b]thiophene **6** promoted by NADH at 100 µM under inert atmosphere*

N_2BF_4 MeO SMe + Ph → NADH (1 equiv.) / Deoxygenated PBS, DMSO 0.5%, 1 h, r.t., inert atmosphere → MeO S Ph

4 100 µM **5** 5 equiv. **6**

In a purged 15 mL Falcon tube, 0.5 mL of PBS, 0.5 mL of a stock solution of phenyl acetylene (2.0 mM in PBS with DMSO 1% v/v), 0.5 mL of a stock solution of diazonium salt **4** (400 µM in PBS with DMSO 1% v/v) and 0.5 mL of a stock solution of NADH disodium salt (400 µM in PBS) were added under nitrogen atmosphere. PBS had been previously deoxygenated with nitrogen bubbling for 30 min. Final concentrations were 100 µM for **4** and NADH, and 500 µM for phenyl acetylene, and 0.5% v/v DMSO. The reaction mixture was then incubated for 1 h at r.t. with shaking at 200 rpm. After 1 h, the solution was frozen in liquid nitrogen and lyophilized. The solid residue was dissolved in 800 µL of acetonitrile and 200 µL of a 100 µM solution of caffeine in acetonitrile to achieve a final concentration of caffeine of 20 µM. The mixture was vortexed, scraped with a Pasteur pipette and centrifuged at 7 830 g for 3 min and the supernatant was collected and filtered with a 0.45 µm HPLC filter. 500 µL of the filtrate were diluted with 500 µL of water and analyzed by HPLC-MS. The yield was determined by HPLC-MS using caffeine as internal standard.

*1.3.14. Evaluation of synthesis of 6-methoxy-2-phenylbenzo[b]thiophene **6** promoted by NADH at 100 µM in open atmosphere*

N_2BF_4 MeO SMe + Ph → NADH (1 equiv.) / PBS, DMSO 0.5%, 1 h, r.t., open atmosphere → MeO S Ph

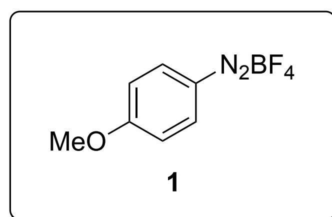
4 100 µM **5** 5 equiv. **6**

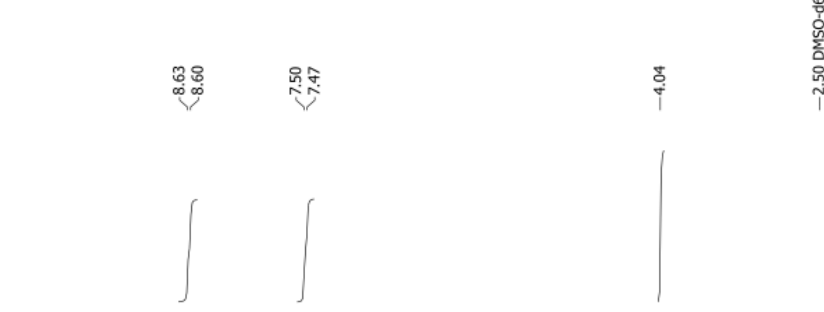
In a 15 mL Falcon tube, 1.5 mL of PBS, 0.5 mL of a stock solution of NADH disodium salt (400 µM in PBS), 5 µL of a stock solution of phenyl acetylene **5** (200 mM in DMSO) and 5 µL of a stock solution of diazonium salt **4** (40 mM in DMSO) were added. Final concentrations were 100 µM for **4** and NADH, and 500 µM for phenyl acetylene, and 0.5% v/v DMSO. The reaction mixture was then incubated for 1 h at r.t. with shaking at 200 rpm. After 1 h, the solution was frozen in liquid nitrogen and lyophilized. The solid residue was dissolved in 800 µL of acetonitrile and 200 µL of a 100 µM solution of caffeine in acetonitrile to achieve

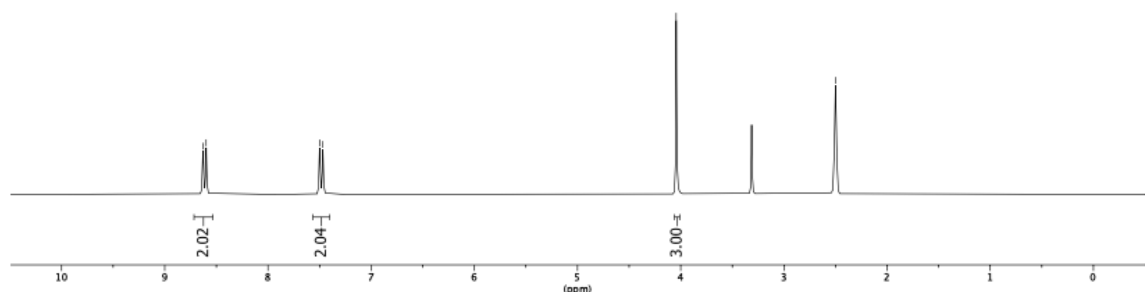
a final concentration of caffeine of 20 μM. The mixture was vortexed, scraped with a Pasteur pipette and centrifuged at 7 830 g for 3 min and the supernatant was collected and filtered with a 0.45 μm HPLC filter. 500 μL of the filtrate were diluted with 500 μL of water and analyzed by HPLC-MS. The yield was determined by HPLC-MS using caffeine as internal standard.

1.4. Selected NMR spectra

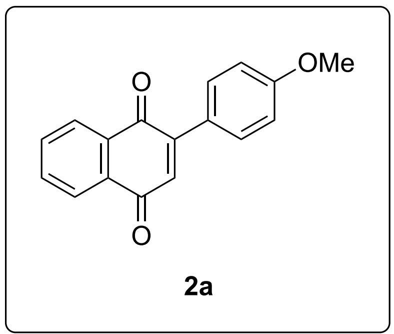
1.4.1. ^{1}H-NMR spectrum of p-*methoxyphenyldiazonium tetrafluoroborate (**1**)*

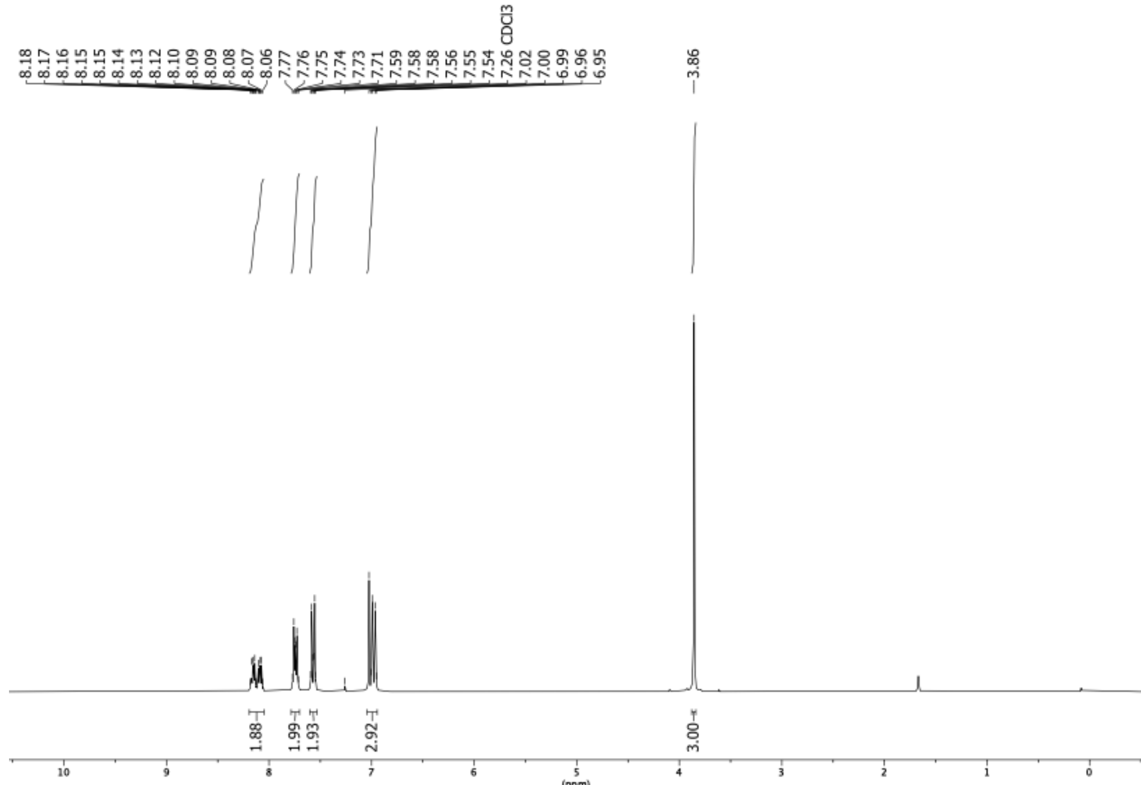




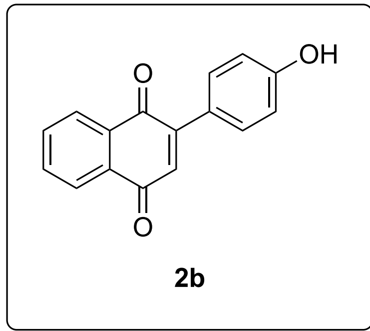


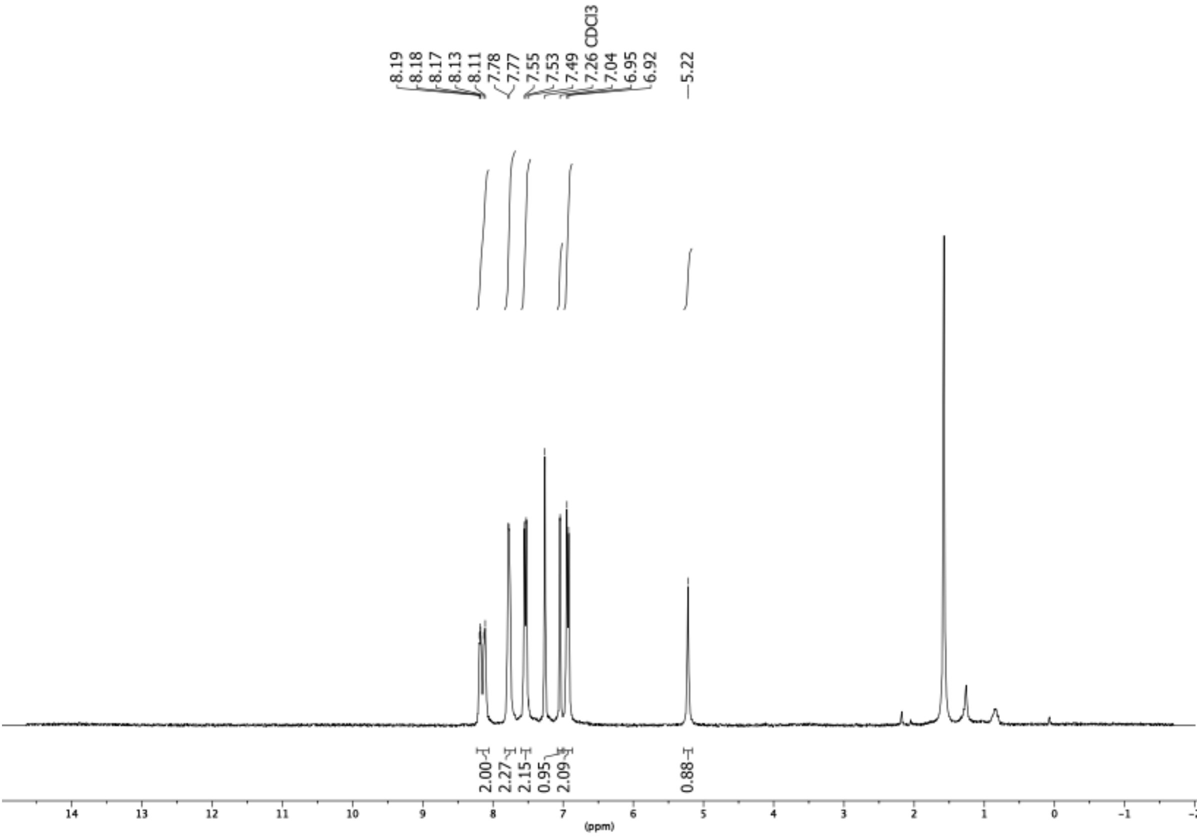
*1.4.2. ^{1}H-NMR spectrum of 2-(4-methoxyphenyl)naphthalene-1,4-dione (**2a**)*



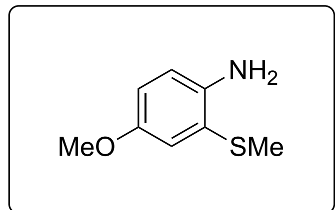


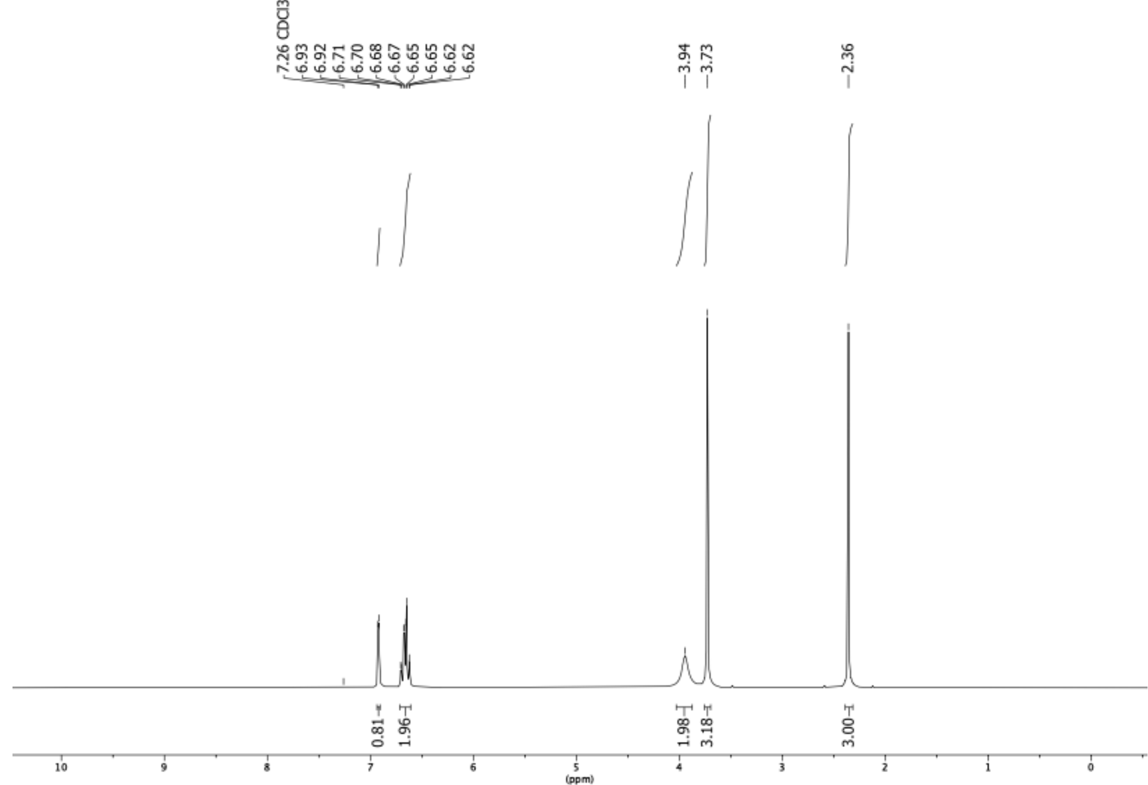
*1.4.3. ^{1}H-NMR spectrum of 2-(4-hydroxyphenyl)naphthalene-1,4-dione (**2b**)*



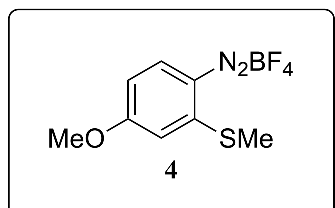


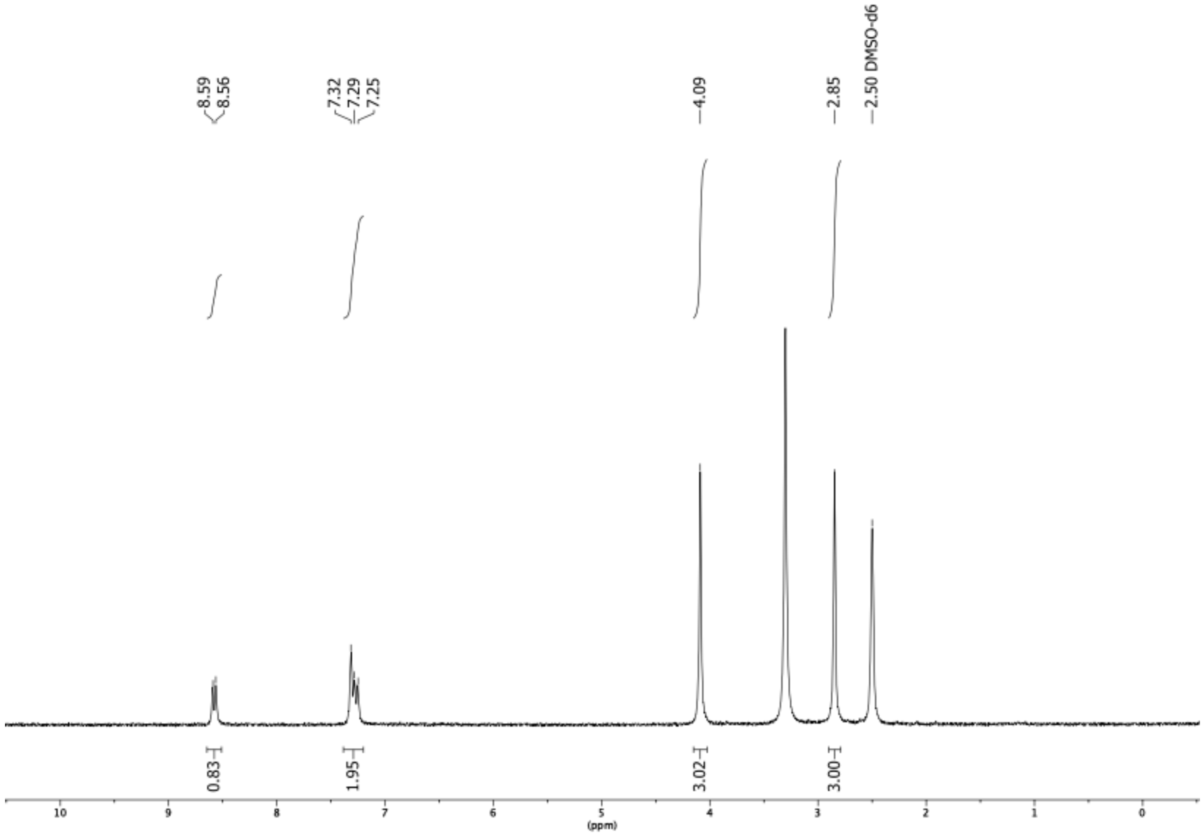
1.4.4. ^{1}H-NMR spectrum of 4-methoxy-2-(methylthio)aniline



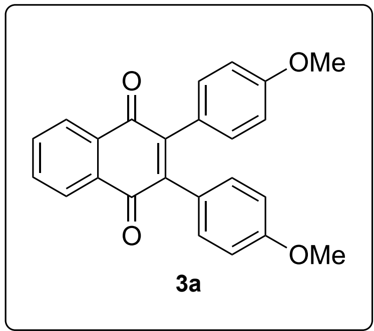


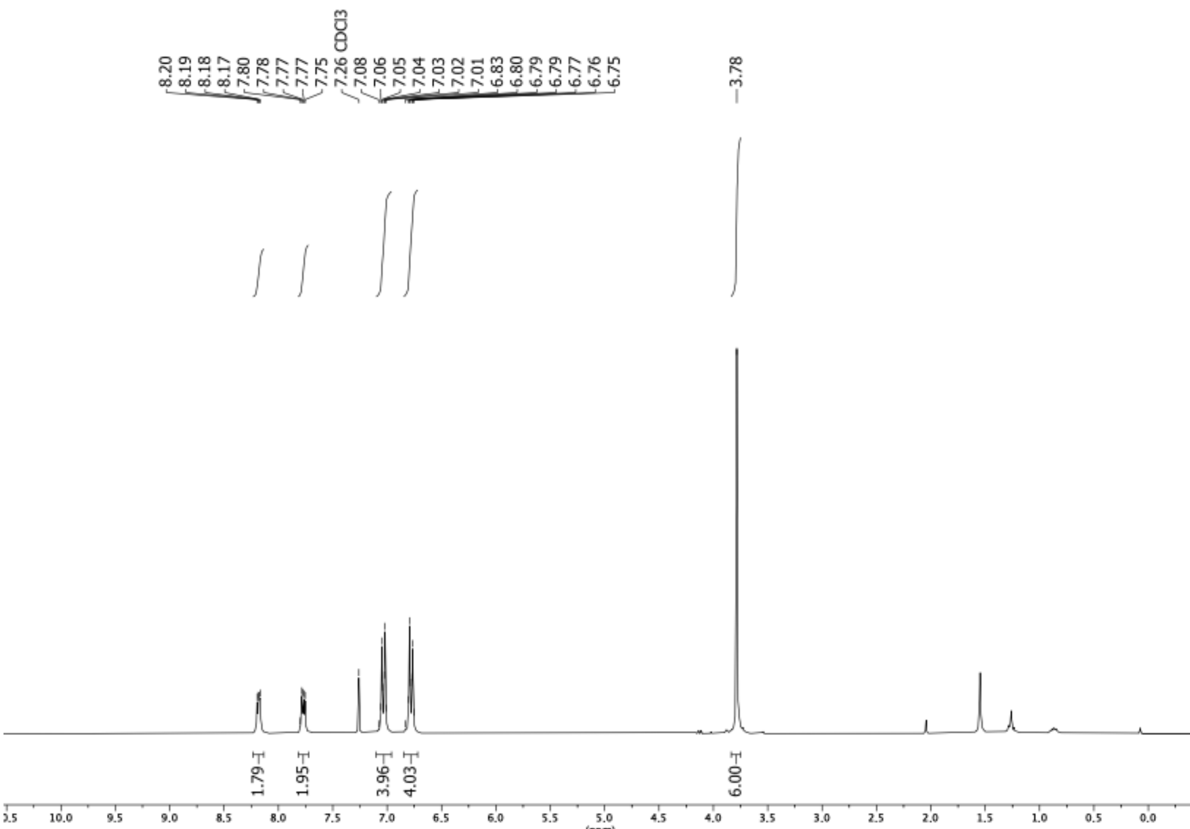
1.4.5. ^{1}H-NMR spectrum of 4-methoxy-2-(methylthio)benzenediazonium tetrafluoroborate (4)



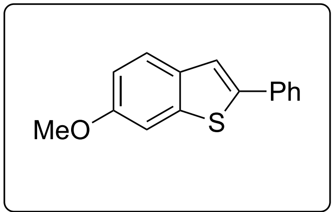


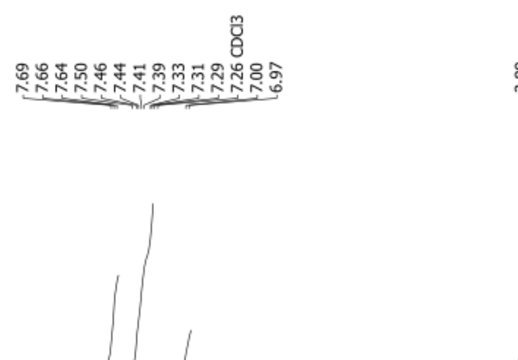
*1.4.6. ^{1}H-NMR spectrum of 2,3-bis(4-methoxyphenyl)naphthalene-1,4-dione (**3a**)*

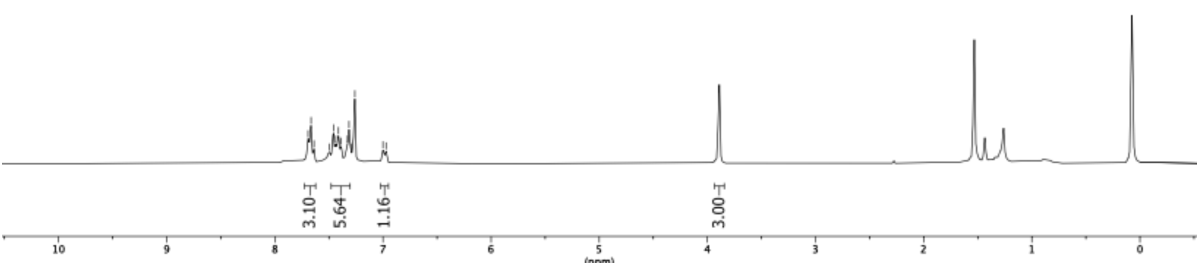




*1.4.7. ¹H-NMR spectrum of 6-methoxy-2-phenylbenzo[b]thiophene (**6**)*







1.5. Quantification by ¹H-NMR with internal standard

Determination of yields from non-purified crudes at 1–10 mM scale reactions was done by ¹H-NMR using dibromomethane (δ=4.95 ppm, s) as internal standard. The signals used for quantification are shown on Supplementary table 6. A representative spectrum for quantification by ¹H-NMR can be seen on Supplementary figure 1.

2. Reactions with bacteria

2.1. Bacterial strains and culture

Bacterial strains used were *Shewanella oneidensis* MR-1 LMG 19005 from BCCM (Belgian Coordinated Collections of Microorganisms) and *Escherichia coli* DH5α from Sigma.

Cultures were prepared from bacterial cultures in LB (Invitrogen, cat. No. 12780052) agar (Oxoid, cat. No. LP0011B) plates. Overnight cultures were grown by picking single colonies, inoculating in growth medium and incubating for 16 h with shaking at 200 rpm in anaerobic conditions. *S. oneidensis* MR1 was cultured in LB supplemented with 20 mM sodium lactate (Sigma, cat. No. L4263) and 40 mM sodium fumarate (Sigma, cat. No. F1506) at 30 °C. *E. coli* DH5α was cultured in LB medium at 37 °C. KCN (Sigma, cat. No. 207810), antimycin A from *Streptomyces* sp. (Sigma, cat. No. A8674) and rotenone (Sigma, cat. No. R8875) were used. An ONDA V-10 Plus spectrophotometer was used to measure optical density at 600 nm (OD_{600}). Centrifugations were performed in an Eppendorf 5810 R centrifuge. Culture media and PBS were autoclaved. Sodium fumarate solutions were sterilized using 0.22 μm nylon filters.

2.2. Procedures for reactions of aryl diazonium salts promoted by bacteria

2.2.1. Meerwein arylation of 1,4-naphthoquinone promoted by bacteria under anaerobic conditions

N_2BF_4 / OMe — **1** 100 μM + (O, O) 1 equiv.

S. oneidensis MR-1 or *E. coli* DH5α OD_{600}=1–4
Deoxygenated PBS, DMSO 0.5%, 1 h, 30 or 37 °C, inert atmosphere

→ **2a** + **3a**

14 mL of overnight bacterial culture were centrifuged at 3 900 rpm for 5 min, washed with 4 mL of PBS and resuspended in PBS to achieve an initial OD_{600} of 2–8. The suspension was deoxygenated with nitrogen bubbling for 30 min. In a purged 15 mL Falcon tube, the following was added under nitrogen atmosphere to yield a 2 mL reaction mixture in deoxygenated PBS: 1 mL of bacteria suspension, 0.5 mL of a stock solution of

1,4-naphthoquinone (400 µM in PBS with DMSO 1% v/v) and 0.5 mL of a stock solution of diazonium salt **1** (400 µM in PBS with DMSO 1% v/v). Final concentrations were 100 µM for both reagents and 0.5% v/v DMSO, with a bacteria OD_{600} of 1–4. The reaction mixture was incubated for 1 h at 30 °C (for *S. oneidensis* MR-1) or 37 °C (for *E. coli* DH5α) with shaking at 200 rpm in anaerobiosis. The mixture was then frozen in liquid nitrogen and lyophilized. The solid residue was dissolved in 800 µL of acetonitrile and 200 µL of a 100 µM solution of coumarin in acetonitrile to achieve a final concentration of coumarin of 20 µM. The mixture was vortexed, scraped with a Pasteur pipette and centrifuged at 7 830 g for 3 min and the supernatant was collected, filtered with a 0.45 µm HPLC filter, and analyzed by HPLC-MS. The yield was determined by HPLC UV-Vis absorbance at 260 nm, using coumarin as internal standard.

2.2.2. Meerwein arylation of 1,4-naphthoquinone promoted by bacteria under oxygenated conditions

N_2BF_4 / OMe — **1**, 100 µM + 1,4-naphthoquinone (1 equiv.)

S. oneidensis MR-1 or *E. coli* DH5α OD_{600}=1; PBS, DMSO 0.5%, 1 h, 30 or 37 °C, open atmosphere

→ **2a** + **3a**

14 mL of overnight bacterial culture were centrifuged at 3 900 rpm for 5 min, washed with 4 mL of PBS and resuspended in PBS to achieve an initial OD_{600} of 2. In a 15 mL Falcon tube, the following was added to yield a 2 mL reaction mixture in PBS: 1 mL of bacteria suspension, 1 mL of PBS, 5 µL of a stock solution of 1,4-naphthoquinone in DMSO (40 mM) and 5 µL of a stock solution of diazonium salt **1** in DMSO (40 mM). Final concentrations were 100 µM for both reagents and 0.5% v/v DMSO, with a bacteria OD_{600} of 1. The reaction mixture was incubated for 1 h at 30 °C (for *S. oneidensis* MR-1) or 37 °C (for *E. coli* DH5α) with shaking at 200 rpm. The mixture was then frozen in liquid nitrogen and lyophilized. The

solid residue was dissolved in 800 µL of acetonitrile and 200 µL of a 100 µM solution of coumarin in acetonitrile to achieve a final concentration of coumarin of 20 µM. The mixture was vortexed, scraped with a Pasteur pipette and centrifuged at 7 830 g for 3 min and the supernatant was collected, filtered with a 0.45 µm HPLC filter, and analyzed by HPLC-MS. The yield was determined by HPLC UV-Vis absorbance at 260 nm, using coumarin as internal standard.

*2.2.3. Meerwein arylation of 2-(4-methoxyphenyl)naphthalene-1,4-dione (**2a**) promoted by* S. oneidensis MR-1 *under anaerobic conditions*

N_2BF_4 OMe **1** 100 µM + **2a** 20 µM → *S. oneidensis* MR-1, OD_{600}=1; Deoxygenated PBS, DMSO 0.5%(v/v), 1 h, 30 °C, inert atmosphere → **3a**

14 mL of overnight bacterial culture were centrifuged at 3 900 rpm for 5 min, washed with 4 mL of PBS and resuspended in PBS to achieve an initial OD_{600} of 2. The suspension was deoxygenated with nitrogen bubbling for 30 min. In a purged 15 mL Falcon tube, the following was added under nitrogen atmosphere to yield a 2 mL reaction mixture in deoxygenated PBS: 1 mL of bacteria suspension, 0.5 mL of a stock solution of 2-(4-methoxyphenyl)naphthalene-1,4-dione **2a** (80 µM in PBS with DMSO 1% v/v) and 0.5 mL of a stock solution of diazonium salt **1** (400 µM in PBS with DMSO 1% v/v). Final concentrations were 20 µM for **2a**, 100 µM for **1** and 0.5% v/v DMSO, with a bacteria OD_{600} of 1. The reaction mixture was incubated for 1 h at 30 °C with shaking at 200 rpm in anaerobiosis. The mixture was then frozen in liquid nitrogen and lyophilized. The solid residue was dissolved in 800 µL of acetonitrile and 200 µL of a 100 µM solution of coumarin in acetonitrile to achieve a final concentration of coumarin of 20 µM. The mixture was vortexed, scraped with a Pasteur pipette and centrifuged at 7 830 g for 3 min and the supernatant was collected, filtered with a 0.45 µm HPLC filter, and analyzed by HPLC-MS. The yield was determined by HPLC UV-Vis absorbance at 260 nm, using coumarin as internal standard.

*2.2.4. Synthesis of 2-phenylbenzo[b]thiophene **6** promoted by bacteria under anaerobic conditions*

N_2BF_4 MeO SMe **4** 100 µM + ≡—Ph **5** 5 equiv. → *S. oneidensis* MR-1 or *E. coli* DH5α OD_{600}=1; Deoxygenated PBS, DMSO 0.5%, 1 h, 30 or 37 °C, inert atmosphere → MeO S **6**

14 mL of overnight bacterial culture were centrifuged at 3 900 rpm for 5 min, washed with 4 mL of PBS and resuspended in PBS to achieve an initial OD_{600} of 2. The suspension was deoxygenated with nitrogen bubbling for 30 min. In a purged 15 mL Falcon tube, the following was added under nitrogen atmosphere to yield a 2 mL reaction mixture in deoxygenated PBS: 1 mL of bacteria suspension, 0.5 mL of a stock solution of phenyl acetylene **5** (2 000 µM in PBS with DMSO 1% v/v) and 0.5 mL of a stock solution of diazonium salt **4** (400 µM in PBS with DMSO 1% v/v). Final concentrations were 100 µM for **4** and 500 µM for **5**, and 0.5% v/v DMSO, with a bacteria OD_{600} of 1. The reaction mixture was incubated for 1 h at 30 °C (for *S. oneidensis* MR-1) or 37 °C (for *E. coli* DH5α) with shaking at 200 rpm in anaerobiosis. The solution was then frozen in liquid nitrogen and lyophilized. The solid residue was dissolved in 800 µL of acetonitrile and 200 µL of a 100 µM solution of caffeine in acetonitrile to achieve a final concentration of caffeine of 20 µM. The mixture was vortexed, scraped with a Pasteur pipette and centrifuged at 7 830 g for 3 min and the supernatant was collected and filtered with a 0.45 µm HPLC filter. 500 µL of the filtrate were diluted with 500 µL of water and analyzed by HPLC-MS. The yield was determined by HPLC-MS using caffeine as internal standard.

*2.2.5. Synthesis of 2-phenylbenzo[b]thiophene **6** promoted by bacteria under oxygenated conditions*

N_2BF_4 MeO SMe **4** 100 µM + ≡—Ph **5** 5 equiv. → *S. oneidensis* MR-1 or *E. coli* DH5α OD_{600}=1; PBS, DMSO 0.5%, 1 h, 30 or 37 °C, open atmosphere → MeO S **6**

14 mL of overnight bacterial culture were centrifuged at 3 900 rpm for 5 min, washed with 4 mL of PBS and resuspended in PBS to achieve an initial OD_{600} of 2. In a 15 mL Falcon tube, the following was added to yield a 2 mL reaction mixture in PBS: 1 mL of bacteria

suspension, 1 mL of PBS, 5 µL of a stock solution of phenyl acetylene **5** in DMSO (200 mM) and 5 µL of a stock solution of diazonium salt **4** in DMSO (40 mM). Final concentrations were 100 µM for **4** and 500 µM for **5**, and 0.5% v/v DMSO, with a bacteria OD_{600} of 1. The reaction mixture was then incubated for 1 h at 30 °C (for *S. oneidensis* MR-1) or 37 °C (for *E. coli* DH5ɑ) with shaking at 200 rpm. The solution was then frozen in liquid nitrogen and lyophilized. The solid residue was dissolved in 800 µL of acetonitrile and 200 µL of a 100 µM solution of caffeine in acetonitrile to achieve a final concentration of caffeine of 20 µM. The mixture was vortexed, scraped with a Pasteur pipette and centrifuged at 7 830 g for 3 min and the supernatant was collected and filtered with a 0.45 µm HPLC filter. 500 µL of the filtrate were diluted with 500 µL of water and analyzed by HPLC-MS. The yield was determined by HPLC-MS using caffeine as internal standard.

2.3. Controls

2.3.1. Heat-killed bacteria

For the heat-killed bacteria, bacteria were resuspended in PBS to achieve an initial OD_{600} of 2 and incubated in a water bath at 65 °C for 1 h. 1.0 mL of this suspension were used to perform the reaction in place of bacteria suspension. 100 µL of the heat-killed sample were plated on a LB agar plate and incubated overnight at 30 °C (*S. oneidensis*) or 37 °C (*E. coli*) to ensure sterilization.

2.3.2. Supernatant

For the supernatant, bacteria were resuspended in PBS to achieve an initial OD_{600} of 2 and deoxygenated by nitrogen bubbling for 30 min. Bacteria were then incubated for 1 h at 30 °C (*S. oneidensis*) or 37 °C (*E. coli*), followed by centrifugation at 3 900 rpm for 10 min and filtration over a 0.22 µm nylon filter. 1.0 mL of the filtrated supernatant were used to perform the reaction in place of the bacteria suspension. 100 µL of the supernatant were plated on a LB agar plate and incubated overnight at 30 °C (*S. oneidensis*) or 37 °C (*E. coli*) to ensure sterilization.

2.3.3. Inhibitors of electron transport

In a purged 15 mL Falcon tube, the following were added under nitrogen atmosphere: 0.5 mL of a stock solution of 1,4-naphthoquinone (400 µM in PBS with DMSO 1% v/v), 0.5 mL of a stock solution of diazonium salt **1** (400 µM in PBS with DMSO 1% v/v), 0.5 mL of a stock solution of NADH disodium salt (400 µM in PBS) and one of the following: 0.5 mL of stock solution of KCN (40 mM in PBS), stock solution of antimycin A (400 µM in PBS

with DMSO 1% v/v), stock solution of rotenone (4 mM in PBS with DMSO 1% v/v) or PBS (control). In controls without NADH, 0.5 mL of PBS were added instead. PBS had been previously deoxygenated with nitrogen bubbling for 30 min. Final concentrations were 100 µM for **1**, 1,4-naphthoquinone and NADH, 10 mM for KCN, 100 µM for antimycin A, 1 mM for rotenone, and 0.5% v/v DMSO (KCN and control) or 0.75% v/v DMSO (antimycin A and rotenone). The reaction mixture was then incubated for 1 h at 30 °C with shaking at 200 rpm in inert atmosphere. After 1 h, the mixture was frozen in liquid nitrogen and lyophilized. The solid residue was dissolved in 800 µL of acetonitrile and 200 µL of a 100 µM solution of coumarin in acetonitrile to achieve a final concentration of coumarin of 20 µM. The mixture was vortexed, scraped with a Pasteur pipette and centrifuged at 7 830 g for 3 min and the supernatant was collected, filtered with a 0.45 µm HPLC filter, and analyzed by HPLC-MS. The yield was determined by HPLC-UV absorbance at 260 nm, using coumarin as internal standard.

2.4. Viability of bacteria

To quantify the viability of bacteria, counting of colony forming units (CFU) was undertaken by the method described by Miles and Misra.[7] After performing the reaction in anaerobic conditions for 2 h following procedure of Sections 2.2.1 or 2.2.3, 20 µL of the reaction mixture were serially diluted in sterile PBS (10^{-1} to 10^{-7}). Triplicates of each treatment were performed. Dilutions were seeded in LB agar plates by dropping 20 µL of the dilution in the agar. Three drops were seeded per dilution. Plates were incubated for 24 h at 30 °C and colonies were counted manually.

3. Quantification with HPLC-MS

3.1. Calibration curves of 2-(4-methoxyphenyl)naphthalene-1,4-dione (**2a**) and 2,3-bis(4-methoxyphenyl)naphthalene-1,4-dione (**3a**)

Stocks of naphthoquinones **2a** or **3a** at different concentrations (0.5–60 µM) in acetonitrile with a constant concentration of internal standard (IS = coumarin, 20 µM) were prepared and filtered with a 0.45 µm HPLC filter. For the calibration curve, the ratio of the peak area at the 260 nm HPLC-UV chromatogram of the product and of the internal standard was represented vs. the concentration (Supplementary figures 4 and 5).

3.2. Calibration curve of 2-phenylbenzo[b]thiophene (**6**)

This curve was constructed by Xulián Fernández González (PhD student, 2020–).

Stocks of 2-phenylbenzo[b]thiophene **6** at different concentrations (0.25–40 μM) in acetonitrile/H_2O 1:1 with a constant concentration of internal standard (IS = caffeine, 10 μM) were prepared and filtered with a 0.45 μm HPLC filter. For the calibration curve, the ratio of the peak areas of the product and the internal standard in the corresponding extracted-ion chromatograms was represented vs. the concentration (Supplementary figure 6).

4. Statistical analysis

Data in graphs are expressed as mean ± standard deviation. Statistical analysis was performed with GraphPad Prism Software (version 10.0). Significant differences were determined by two-tailed unpaired Student's *t* test or one-way analysis of variance (ANOVA) and *post hoc* Fisher least significant difference (LSD) test. Before performing ANOVA, homoscedasticity of the variance was determined by *F*-test, and normality of the residuals was determined by Kolmogorov-Smirnov test. P-values lower than 0.05 were considered statistically significative. P-values meaning: ns not significative, * ($p<0.05$), ** ($p<0.01$), *** ($p<0.001$) and **** ($p<0.0001$). Statistical analysis was performed with n=3 replicates unless otherwise noted.

For experiments involving bacteria, 'replicates' refers to experiments performed separately on the same day, using aliquots from the same bacteria preculture and reagents stock solutions, on different reaction vessels.

5. Supplementary tables

Supplementary table 1. Time study of the Meerwein arylation promoted by NADH.[a]

N_2BF_4 + ... NADH (1 equiv.), DMSO, r.t., innert atmosphere, *time*

1 10 mM; **2a** 3 equiv.; **3a**

Entry	Time	Yield/%
1	1	**29**
2	5	**64**
3	10	**67**
4	120	**77**

(a) Reaction conditions (unless otherwise noted): **1** (1 equiv., 10 mM), **2a** (3 equiv.), NADH (1 equiv.), 1 mL DMSO, r.t., inert atmosphere, *time*. Yields were determined by ^{1}H-NMR using dibromomethane as internal standard.

Supplementary table 2. Mechanistic study of the Meerwein arylation promoted by NADH.[a]

1 (10 mM) + 2a (3 equiv.) → (NADH, DMSO, 2 h, r.t.) → 3a

Entry	NADH/equiv.	Deoxygenation	Atmosphere	Yield/%
1	0.1	Yes	N_2	**56**
2	0.1	No	N_2	**43**
3	0.8	Yes	N_2	**58**
4	0.8	No	N_2	**67**
5	1.0	Yes	N_2	**72**
6	1.0	No	N_2	**77**
7	1.0	No	Open	**65**
8	1.5	Yes	N_2	**65**
9	2.0	Yes	N_2	**39**
10	0.1	Yes	N_2	**19**[b]
11	1.0	No	N_2	**58**[b]
12	1.0	No	N_2	**27**[c]

(a) Reaction conditions (unless otherwise noted): **1** (1 equiv., 10 mM), **2a** (3 equiv.), *NADH*, 1 mL DMSO, 2 h, r.t. Yields were determined by ^{1}H-NMR using dibromomethane as internal standard. (b) 1 equiv. of TEMPO. (c) 10 equiv. of TEMPO.

Supplementary table 3. Meerwein arylation of 1,4-naphthoquinone promoted by NADH at low concentrations in different aqueous media.[a]

N_2BF_4
OMe
1
100 µM
1 equiv.
NADH (1 equiv.)
DMSO 0.5%, 1 h, r.t.,
inert atmosphere,
deoxygenated *aqueous media*
OMe
2a
+
OMe
OMe
3a

Entry	Media	NADH/equiv.	Yield of 2a/%	Yield of 3a/%
1	Water	1	**6.7**	**2.1**
2	Water	0	**0.9**	**<0.5**
3	PBS	1	**18.1**	**7.3**
4	PBS	0	**0.9**	**<0.5**
5	LB	1	**<0.25**	**<0.5**
6	LB	0	**0.32**	**<0.5**

(a) Reaction conditions (unless otherwise noted): **1** (1 equiv., 100 µM), 1,4-naphthoquinone (1 equiv.), *NADH (1 equiv.)*, DMSO 0.5%, 2 mL deoxygenated *aqueous media*, 1 h, r.t., inert atmosphere. Yields were determined by HPLC-UV absorbance at 260 nm, using coumarin as internal standard.

Supplementary table 4. Time study of the synthesis of benzothiophene ***6*** *promoted by NADH.*[a]

4 (10 mM) + 5 (50 equiv.) → 6; NADH (1 equiv.), DMSO/H_2O 2:8, r.t., inert atmosphere, *time*

Entry	Time	Yield/%
1	1	**32**
2	5	**40**
3	10	**47**
4	30	**48**
5	120	**67**

(a) Reaction conditions (unless otherwise noted): **4** (1 equiv., 10 mM), **5** (50 equiv.), NADH (1 equiv.), 1 mL DMSO/H_2O 2:8, r.t., inert atmosphere, *time*. Yields were determined by ^{1}H-NMR using dibromomethane as internal standard.

Supplementary table 5. Synthesis of benzothiophene ***6*** *at micromolar concentration promoted by NADH.*[a]

4 (100 µM) + 5 (5 equiv.) → 6; *NADH (1 equiv.)*, PBS, DMSO 0.5%, 1 h, 30 °C

Entry	NADH/equiv.	Oxygenation conditions	Yield/%
1	1	Inert atmosphere, deoxygenated solvents	**20**
2	1	Inert atmosphere, deoxygenated solvents	**6**
3	0	Open atmosphere	**n/d**
4	0	Open atmosphere	**n/d**

(a) Reaction conditions (unless otherwise noted): **4** (1 equiv., 100 µM), **5** (5 equiv.), *NADH (1 equiv.)*, 2 mL PBS, 0.5% v/v DMSO, 1 h, 30 °C. Yields were determined by HPLC-MS using caffeine as internal standard. n/d: not detected.

Supplementary table 6. ^{1}H-NMR signals employed for quantification with internal standard.

Entry	Molecule	Structure	Signals/ppm (multiplicity, number of nuclei)
1	**2a**		3.87 (s, 3H)[4]
2	**3a**		3.78 (s, 6H), 6.78 (d, 2H)[4]
3	**3b**		3.78 (s, 3H)*
4	**3c**		3.86 (s, 3H)[8]
5	**3d**		3.88 (s, 3H)*
6	**3e**		3.80 (s, 3H)[5]

7	**3f**		3.86 (s, 3H)[4]
8	**3g**		3.88 (s, 3H)[4]
9	**3h**		3.85 (s, 3H)[9]
10	**3i**		3.79 (s, 3H)[10]
11	**6**		3.89 (s, 3H)[6]

*New compound.

6. Supplementary schemes

Supplementary scheme 1. Identification of subproducts of the Meerwein arylation promoted by NADH by HPLC-MS.[a]

a)

NAD^+
664 (M+1), 123 (nicotinamide+1)

c)

R=H 171 (M+1), 154 (M−CH_3)
R=D 174 (M+1), 157 (M−CD_3)[b]

b)

770 (M+1), 542 (M−nicotinamide−C_6H_4OMe),
229 (nicotinamide+C_6H_4OMe+1),
108 (C_6H_4OMe+1)

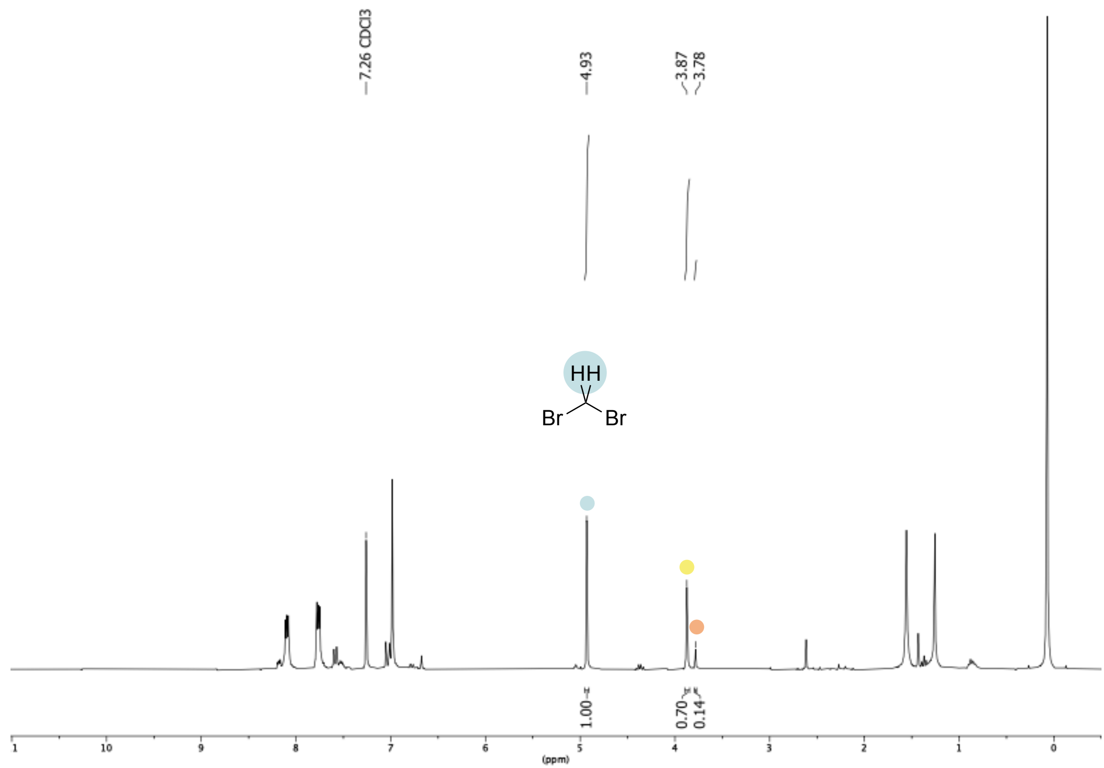
d)

243 (M+1)

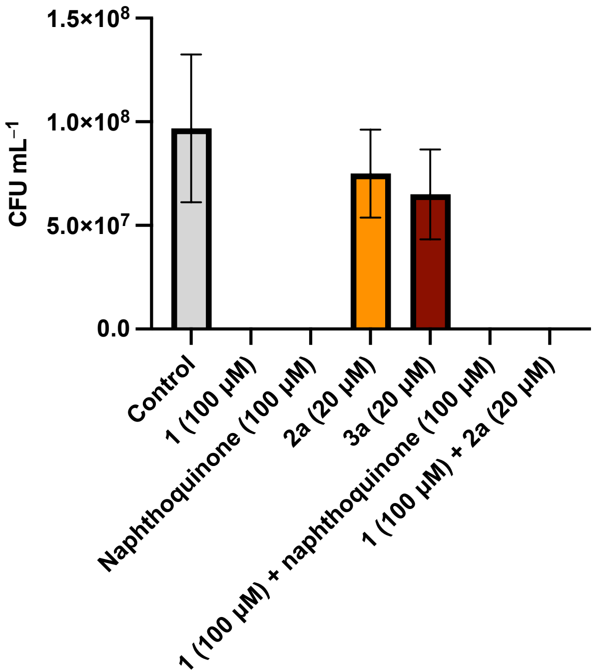
(a) Reaction conditions (unless otherwise noted): **1** (1 equiv., 10 mM), **2a** (3 equiv.), NADH (1 equiv.), 1 mL DMSO, 1 h, r.t., inert atmosphere. (b) With d6-DMSO instead of DMSO.

7. Supplementary figures

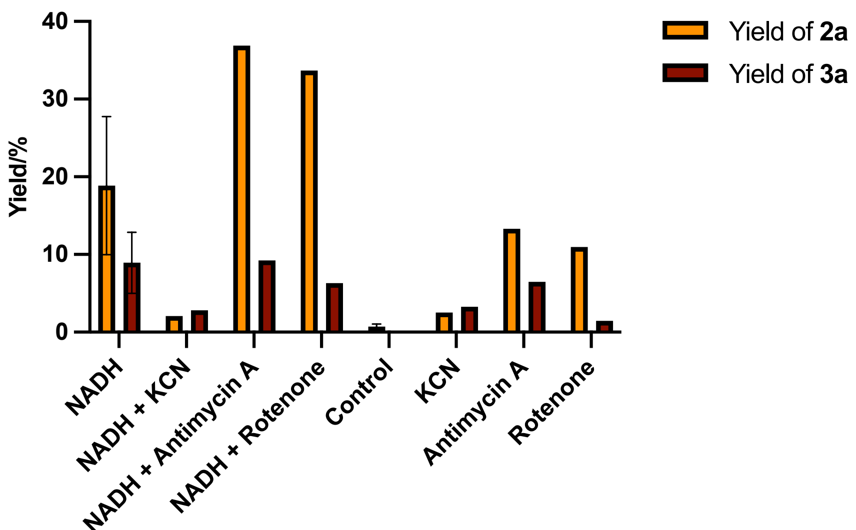
N_2BF_4
OMe
1
10 mM, 1 equiv.
+
3 equiv.
NADH (1 equiv.)
DMSO, r.t., 2 h,
inert atmosphere
OCH_3
2a
+
OCH_3
OCH_3
3a



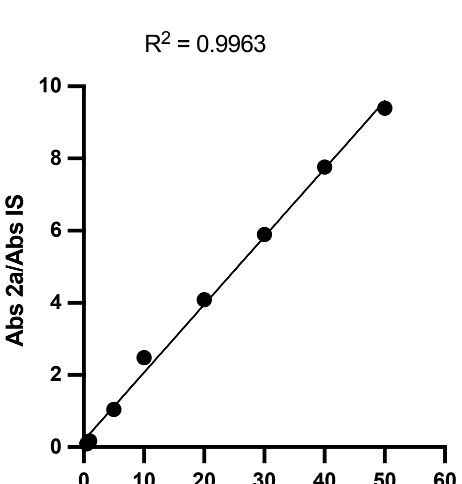
Supplementary figure 1. Example of quantification of the yield by ^{1}H-NMR with internal standard. Reaction conditions: **1** (1 equiv., 10 mM), 1,4-naphthoquinone (3 equiv.), NADH (1 equiv.), 2 mL DMSO, 2 h, r.t., inert atmosphere. Yields were determined by ^{1}H-NMR using dibromomethane as internal standard.



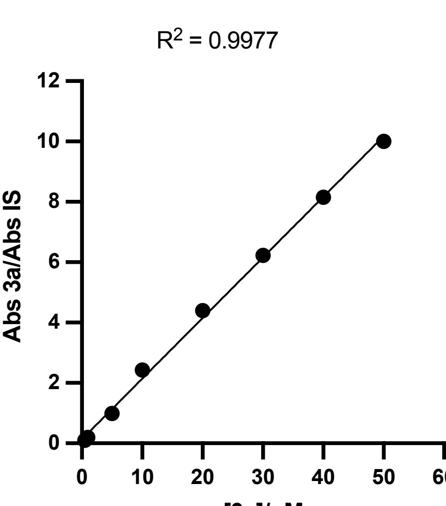
Supplementary figure 2. Viability of S. oneidensis *MR-1 under reaction conditions for the Meerwein arylation.* Reaction conditions (unless otherwise noted): *S. oneidensis* MR-1 (OD_{600}=1), 2 mL PBS, 0.5% v/v DMSO, 1 h, 30 °C. When present: **1** (1 equiv., 100 µM), 1,4-naphthoquinone (100 µM), **2a** (20 µM), **3a** (20 µM). CFU counting was performed following the method of Miles and Misra.[7] *n* = 3 replicates. Represented values are mean ± SD.



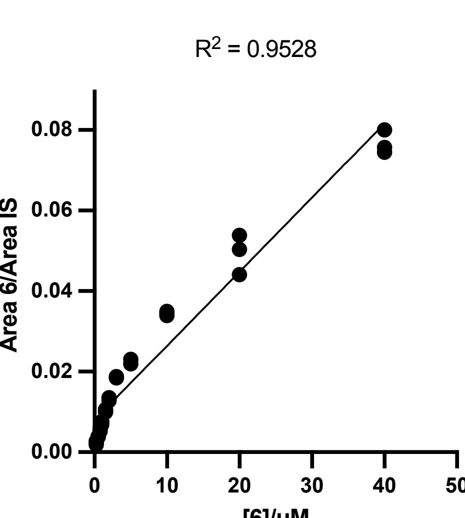
Supplementary figure 3. Evaluation of potential inhibitors of electron transfer in the arylation of 1,4-naphthoquinone promoted by NADH. Reaction conditions (unless otherwise noted): **1** (1 equiv., 100 µM), 1,4-naphthoquinone (1 equiv.), 2 mL PBS, 0.5% v/v DMSO, 1 h, 30 °C, inert atmosphere. When used: NADH (100 µM), KCN (10 mM), antimycin A (100 µM), rotenone (1 mM). For reactions with antimycin A and rotenone, 0.75% v/v DMSO was used. Yields were determined by HPLC-UV absorbance at 260 nm, using coumarin as internal standard. For NADH: *n* = 3 replicates. For control: *n* = 2 replicates. Represented values are mean ± SD.



*Supplementary figure 4. Calibration curve for molecule **2a**. IS = coumarin.*



*Supplementary figure 5. Calibration curve for molecule **3a**. IS = coumarin.*



*Supplementary figure 6. Calibration curve for molecule **6**. IS = caffeine.*

Supplementary references

(1) Bonin, H., Fouquet, E., Felpin, F.-X. Aryl Diazonium versus Iodonium Salts: Preparation, Applications and Mechanisms for the Suzuki-Miyaura Cross-Coupling Reaction. *Adv. Synth. Catal.* **2011**, *353*, 3063–3084.

(2) Govaerts, S., Nakamura, K., Constantin, T., Leonori, D. A Halogen-Atom Transfer (XAT)-Based Approach to Indole Synthesis Using Aryl Diazonium Salts and Alkyl Iodides. *Org. Lett.* **2022**, *24*, 7883–7887.

(3) Zhang, H., Wang, B., Xu, H., Li, F.-Y., Wang, J.-Y. Synthesis of Naphthodihydrofurans via an Iron(III)-Catalyzed Reduction Radical Cascade Reaction. *Org. Chem. Front.* **2021**, *8*, 6019–6025.

(4) Nagar, B., Dhar, B. B. Photochemical C–H Arylation of Napthoquinones Using Eosin Y. *ACS Omega* **2022**, *7*, 32615–32619.

(5) Molina, M. T., Navarro, C., Moreno, A., Csákÿ, A. G. Arylation of Benzo-Fused 1,4-Quinones by the Addition of Boronic Acids under Dicationic Pd(II)-Catalysis. *Org. Lett.* **2009**, *11*, 4938–4941.

(6) Hari, D. P., Hering, T., König, B. Visible Light Photocatalytic Synthesis of Benzothiophenes. *Org. Lett.* **2012**, *14*, 5334–5337.

(7) Miles, A. A., Misra, S. S., Irwin, J. O. The Estimation of the Bactericidal Power of the Blood. *Epidemiol. Infect.* **1938**, *38*, 732–749.

(8) Yu, D., Chen, X.-L., Ai, B.-R., Zhang, X.-M., Wang, J.-Y. Tetrabutylammonium Iodide Catalyzed Hydroxylation of Naphthoquinone Derivatives with Tert-Butyl Hydroperoxide as an Oxidant. *Tetrahedron Lett.* **2018**, *59*, 3620–3623.

(9) Lee, J.-W., List, B. Deracemization of α-Aryl Hydrocoumarins via Catalytic Asymmetric Protonation of Ketene Dithioacetals. *J. Am. Chem. Soc.* **2012**, *134*, 18245–18248.

(10) Schroll, P., Fehl, C., Dankesreiter, S., König, B. Photocatalytic Surface Patterning of Cellulose Using Diazonium Salts and Visible Light. *Org. Biomol. Chem.* **2013**, *11*, 6510.