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Biocatalytic site- and enantioselective oxidative dearomatization of phenols

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Abstract

Biocatalytic transformations employed by chemists are often restricted to simple functional group interconversions. In contrast, Nature has developed complexity-generating biocatalytic reactions within natural product pathways. These sophisticated catalysts are rarely employed by chemists as the substrate scope, selectivity and robustness of these catalysts are unknown. Our strategy to bridge the gap between the biosynthesis and synthetic chemistry communities leverages the diversity of catalysts available within natural product pathways. Starting from a suite of biosynthetic enzymes, catalysts with complementary substrate scope as well as selectivity can be identified. This strategy has been applied to the oxidative dearomatization of phenols, a chemical transformation that rapidly builds molecular complexity from simple starting materials and cannot be accomplished with high site- and enantioselectivity using existing catalytic methods. Using enzymes from biosynthetic pathways, we have successfully developed a method to produce *ortho*-quinol products with controlled site- and stereoselectivity. Further, we have capitalized on the scalability and robustness of this method in gram-scale reactions as well as multi-enzyme and chemoenzymatic cascades.

Graphical Abstract:

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Oxidative dearomatization of phenolic compounds is a powerful transformation for the synthesis of complex molecules, providing an avenue for simultaneously introducing stereochemical information and generating products that are primed for further reaction.¹ For example, chemical methods exist for the conversion of simple phenols to dearomatized products with concomitant formation of new C–C, C–N, C–halogen and C–O bonds.² A number of reagents for dearomatization to afford *ortho*-quinol products (see **2**, Fig. 1A),^{3–5} including I^{III}, I^V, Pb^{IV} and Cu^I, have been developed and leveraged for the chemical synthesis of a range of bioactive natural products (Fig. 1B).^{1,4,6–8} However, in addition to the requirement of stoichiometric amounts of these reagents, two major challenges associated with these chemical methods are site-selectivity and product stability. For example, highly substituted resorcinol substrates such as **3** are chemically oxidized to afford mixtures of isomers such as **4**, **5** and **6**, which can fragment to afford quinone products (see **11**, Fig. 1C).⁹ Furthermore, the desired quinol products are often difficult to use productively, as side reactions such as dimerization¹⁰ (**7**), rearomatization^{11,12} (**8**, **9** and **10**) and rearrangement¹³ are facile under the requisite reaction conditions. Moreover, the development of asymmetric catalytic versions of these oxidative dearomatizations has proven challenging, particularly for cases involving concomitant C–O bond formation (**1** to **2**, Fig. 1A).¹⁴ While high enantioselectivities have been achieved with stoichiometric amounts of chiral hypervalent iodine reagents, superstoichiometric chiral metal complexes⁴ and in cases where an intramolecular cyclization is possible,¹⁵ a highly enantioselective catalytic method has yet to be reported.

The limitations of traditional methods to achieve site- and enantioselective oxidative dearomatization led us to consider utilizing the tools employed by Nature to effect this transformation. Oxidative dearomatization has been proposed as the chirality-generating step in a number of natural product biosynthetic pathways. While enzymes capable of the dearomatization of arenes through dihydroxylation or oxidative ring cleavage have been known for decades, the proteins responsible for oxidative dearomatization of phenols to generate *ortho*-quinol products, such as **2**, have remained more elusive.¹⁶ Only recently have *in vivo* and *in vitro* studies identified enzymes responsible for mediating oxidative dearomatization of phenolic compounds to form *ortho*-quinol products in a handful of natural product pathways.¹⁷ We reasoned that these FAD-dependent monooxygenases represent ideal catalysts for oxidative dearomatization reactions, particularly since they require only molecular oxygen and a nicotinamide cofactor to enable catalysis under mild reaction conditions, with high site- and enantioselectivity.¹⁸

In order to harness the advantages offered by FAD-dependent monooxygenases, the challenges classically associated with biocatalysts, including limited substrate scope and the ability to achieve the desired selectivity, must be overcome.¹⁹ A common strategy in biocatalyst development involves: (1) first selecting a single enzyme; (2) evaluating its substrate scope, site- or enantioselectivity; and then (3) engineering the protein to operate on a different suite of compounds or with altered selectivity.²⁰ Given the diversity that Nature has evolved in the context of natural product biosynthetic pathways, we hypothesized that greater synthetic utility could be accessed by profiling the substrate promiscuity and selectivity of a number of different FAD-dependent monooxygenases capable of catalyzing the oxidative dearomatization of phenol and resorcinol substrates.

Our initial studies focused on a set of enzymes with orthogonal site- and stereoselectivities. We were guided by the work of the Cox,^{17,21} Tang²² and Watanabe²³ groups, which have each biochemically characterized FAD-dependent monooxygenases that mediate the oxidative dearomatization of resorcinol substrates to *ortho*-quinol products that are further elaborated into various classes of natural products by downstream biosynthetic enzymes. Cox and coworkers first demonstrated this transformation through *in vitro* characterization of a FAD-dependent monooxygenase, TropB, included in the gene cluster that encodes for the fungal tropolone natural product stipitatic acid (**16**). TropB catalyzes the site- and stereoselective oxidative dearomatization of resorcinol **14** to alcohol **15** (Fig. 2). Also in 2012, Tang identified a similar FAD-dependent monooxygenase from a silent *Aspergillus niger* ATCC 1015 gene cluster and demonstrated the role of this enzyme, AzaH, in the site-selective oxidative dearomatization of resorcinol substrate **17** to afford **18** with unknown configuration at the newly formed stereocenter. While TropB and AzaH operate with the same site-selectivity, a third enzyme in this class, SorbC, has evolved orthogonal site- and facial selectivity within the sorbicillactone A (**22**) pathway, oxidizing sorbicillin (**20**) to the C5-hydroxylation product **21**.²⁴

We began by synthesizing the native substrates for TropB, AzaH and SorbC (**14**, **17**, and **20**, respectively), which were subsequently used as positive controls for activity of each FAD-dependent monooxygenase (see supporting information for details). In parallel, the three proteins were heterologously expressed in *E. coli* and purified using standard Ni-affinity chromatography. Analytical-scale *in vitro* reactions with each enzyme and the corresponding native substrate confirmed productive catalysis. In the case of TropB, we observed complete consumption of the starting material in one hour, which corresponds to 1,000 turnovers of the catalyst (Table 1, entry 1). Partial conversions were seen in the analogous assays for AzaH and SorbC, with total turnover numbers (TTNs) of 725 and 816, respectively (entries 17 and 29).

With activity on the natural substrates benchmarked, we next assessed the reactivity of each monooxygenase on a panel of compounds designed to probe the steric and electronic requirements of a substrate for productive catalysis. As shown in Table 1, each enzyme demonstrated a unique footprint of substrate scope. For example, TropB can tolerate a *n*-butyl ketone in place of the C1 aldehyde present in the native substrate (entries 2 and 8–10), but a benzoyl substituent was not compatible (see Supplementary Fig. S5). In contrast, SorbC exhibited the highest TTNs on ketone substrates with butyl or pentyl substituents that

mirror the native SorbC substrate (**20**) (entries 8, 10, 26, 28–33). AzaH demonstrated the most flexibility in the carbonyl substituent, operating with TTNs exceeding 250 for a range of aldehyde and ketone substrates (entries 1, 4, 5, 11–13, 17–21, 27–28 and 30–33). Variation of the substitution pattern on the aryl ring was also tolerated. In the case of TropB, substrates lacking substituents at the C5 and C6 positions (entries 3 and 7) were converted to dearomatized products with TTNs approaching those observed with the native substrate, but AzaH and SorbC both required at least one substituent at either C5 or C6. The more sterically demanding biaryl and 6,7-bicyclic substrates were converted to dearomatized products by both TropB and AzaH (entries 11 and 6).

Since these transformations are anticipated to proceed through nucleophilic attack of the electron-rich phenol substrate onto the hydroperoxyflavin cofactor (**12**), we were interested in evaluating more electron-poor substrates with the three FAD-dependent monooxygenases. Interestingly, brominated substrates (entries 4 and 5) were dearomatized by TropB and AzaH. However, even the bromine-containing substrate that meets the steric preferences of SorbC showed no detectable conversion to oxidized product with SorbC, but was hydroxylated by AzaH (entry 21). AzaH was the only monooxygenase, of the three tested, with the ability to perform an oxidative dearomatization on even less electron-rich substrates such as a nitroresorcinol compound (entry 19) and monophenols (entries 20 and 22). These results prompted us to examine additional monophenol substrates (entries 23–25). AzaH catalyzed the oxidative dearomatization of brominated monophenols with low turn over numbers. Although the origin of this reactivity difference between AzaH and the other two monooxygenases is unclear, these results highlight the advantage of our approach of concurrently examining similar enzymes, which has allowed for the rapid identification of a promising general biocatalyst for the asymmetric transformation of phenols into *ortho*-quinols.

As a first step toward evaluating the selectivity and scalability of this oxidative dearomatization, substrates with the highest TTNs were selected for milligram-scale reactions. Based on the robust expression of these monooxygenases (>100 mg protein/1 L *E. coli* culture), purified protein was used in these reactions. An NADPH recycling system was employed to reduce cofactor cost.²⁵ As shown in Table 2, these conditions led to efficient transformation of substrates to dearomatized products with conversions mirroring those observed for initial analytical-scale reactions. In some cases with excellent conversions of starting material to product, the isolated yields do not mirror these conversions. In these instances, the mass balance can be accounted for as the product dimer arising through [4+2] cycloaddition, which occurs upon concentration of the isolated product. Characterization of products from these reactions provided information on the site- and stereoselectivity of each enzyme. Operating on their natural substrates, both TropB and AzaH selectively hydroxylate the C3 position (see **15** and **36**, Table 2). This site-selectivity is conserved across a panel of unnatural substrates (Tables 2A and B). Even in cases where the substrate bears a substituent at C5, the preference for C3 hydroxylation is maintained (see **28** and **35**). In contrast, SorbC demonstrates orthogonal site-selectivity, exclusively hydroxylating at C5 across the library of phenols (Table 2C).

With their native substrates, both TropB and SorbC deliver the expected product in >99% ee (Table 2). The configuration of the two stereocenters present in the native AzaH product **36** was previously unknown. Therefore, racemic **17** was synthesized and presented to AzaH. Both enantiomers of **17** were converted to azaphilone product **36** to afford a 1:1 mixture of diastereomers each formed in >99% ee. Without knowledge of the absolute configuration at C3 of azaphilone products from the *aza* gene cluster, we compared the products of TropB and AzaH with a common substrate **14**. Both enzymes gave rise to the same stereoisomer, providing evidence that AzaH installs a hydroxyl group to generate (*R*)-C3 products. Analysis of TropB and AzaH reactions with a range of unnatural substrates indicates that a high-level of stereoselectivity is maintained with product ee's typically >99%. These results can be compared favorably to the state-of-the-art copper-oxo-sparteine oxidant developed by Porco and coworkers, which, when used superstoichiometrically, delivers *o*-quinols from resorcinol precursors in up to 98% ee.⁴ SorbC displays altered facial selectivity producing (*S*)-C5 products (Table 2). Excellent stereoselectivity is observed in all cases examined, except when the C3 methyl group is replaced with an ethyl group or the carbonyl substituent is shortened to a butyl chain, eroding the stereoselectivity to deliver **40** and **43** in 94% and 95% ee, respectively. Notably, racemic **38** has been employed as a key intermediate in a number of natural product syntheses, however, to date no enantioselective method for accessing this class of *o*-quinols has been reported. The stereochemical outcome of the biotransformation is likely controlled by the pose the substrate adopts in the enzyme active site, wherein a specific face of the substrate is presented to the hydroperoxyflavin cofactor (**12**).

In contrast, isolation of racemic standards of these dearomatized products from traditional chemical methods employing IBX²⁶ or Pb(OAc)₄²⁷ proved challenging, often affording low yields of the desired product (listed in grey, Table 2), with the mass balance accounted for as isomers, quinol dimers arising through [4+2] cycloaddition, overoxidation and product decomposition pathways. Notably, a subset of the products generated enzymatically were not accessible using standard chemical methods, including nitroquinol **35** and **45**, thus precluding the determination of the enantioselectivity of the enzymatic transformation by comparison to racemic material. Additionally, products **31-33** were observed as a mixture of tautomers (see **33** and **34**) in a variety of solvents, which prohibited successful chiral separation and measurement of the enantiopurity of these compounds.

While enzymatic reactions are routinely carried out on sub-milligram quantities to characterize their biochemical function, the challenges associated with preparative-scale biocatalytic reactions can serve as a barrier to enzymes being embraced as tools for synthesis.¹⁹ Complications with enzyme supply, labor-intensive protein purification, substrate solubility in aqueous buffer, and cofactor expense can all be hurdles to preparative-scale biocatalysis. To move from milligram to gram-scale reactions and beyond, we envisioned transitioning from *in vitro* reactions, conducted with purified protein, to a scalable platform that would be readily accessible to synthetic chemists. Toward this goal, a series of experiments utilizing whole *E. coli* cells containing heterologously expressed protein in place of purified protein was undertaken. Whole cell reactions were sufficient for complete conversion of aldehyde **44** in 2 hours (Table 3, entry 1). Additionally, gram-scale

reactions on a given substrate could be carried with whole cells to give comparable conversions as observed on analytical scale. To enhance the accessibility of this method to traditional synthetic labs, wet whole cells were freeze-dried and stored as a lyophilized powder, which could be weighed on the benchtop and directly used in reactions without suffering a significant loss in activity compared to experiments with wet whole cells (entries 3–6). A number of excipients commonly employed to aid in the long-term stabilization of proteins were screened.²⁸ When lyophilized in the presence of 10 wt% sucrose or PEG 4 × 10³, full activity of the enzyme was retained and the freeze-dried catalyst could be stored in the freezer at –80 °C for at least six months without any impact on reactivity.

To rapidly build molecular complexity from enzymatically generated *ortho*-quinols, we sought to leverage the reactivity of these compounds in cascade reactions by performing subsequent *in situ* enzymatic or chemical transformations. We anticipated that various natural product scaffolds could be accessed in one-pot using biocatalytic dearomatization-initiated cascades. Toward this goal, we explored the synthesis of the tropolone (see **48**), azaphilone (see **51**) and sorbicillinoid (see **53**)²⁹ cores (Fig. 3). To access the tropolone natural product stipitatic aldehyde (**48**), TropB dearomatization of aldehyde **14** produced *ortho*-quinol **15**. Next, **15** was further hydroxylated by an alpha-ketoglutarate dependent non-heme iron enzyme, TropC, to afford 1,2-diol **46**. Diol **46** readily underwent ring expansion in the presence of TropC to afford seven-membered ring **47** which tautomerized to afford the tropolone, stipitatic aldehyde (**48**). Additionally, the first asymmetric synthesis of azaphilone natural product **51**³⁰ was achieved from methyl ketone **49**. Initial AzaH-mediated dearomatization delivered enol **50** which spontaneously cyclized to the natural azaphilone **51**, which could be isolated in 54% yield and >99% ee. Finally, urea sorbicillinoid **53** was generated directly from ketone **20** in a cascade that commenced with SorbC oxidation to deliver sorbicilinol (**21**). The addition of bisacylated urea **52** led to facile [4+2] cycloaddition. Addition of LiOH to the reaction completed the first synthesis of urea sorbicillinoid natural product **53** in 21% yield over three steps.

In sum, the results disclosed herein demonstrate the potential that FAD-dependent monooxygenases offer for site- and stereoselective oxidative dearomatization. The structural diversity available from various natural product pathways has provided a robust platform for developing a suite of biocatalysts with orthogonal selectivity and complementary substrate scope. The catalytic efficiency and exquisite stereoselectivity of these catalysts paired with the mild reaction conditions provide an excellent opportunity to apply these biocatalysts in complexity generating chemoenzymatic and multi-enzyme reaction cascades. This work provides an initial data set that will fuel the engineering of these catalysts and exploration of alternative reaction pathways.

Methods

Analytical-scale reactions:

Analytical-scale reactions were performed on 50 µL scale. Each reaction contained 25 µL 100 mM potassium phosphate buffer, pH 8.0, 2.5 mM substrate (2.5 µL of a 50 mM stock solution in DMSO), 2.5 µM FAD-dependent monooxygenase, 5 mM G6P (0.5 µL, 500 mM),

1 mM NADP⁺ (0.5 μ L, 100 mM), 1 U/mL G6P-DH (0.5 μ L, 100 U/mL), and Milli-Q water to a final volume of 50 μ L. The reaction was carried out at 30 °C for 1 h and quenched by addition of 75 μ L acetonitrile with 25 mM pentamethylbenzene as an internal standard. Precipitated biomolecules were pelleted by centrifugation (16,000 $\times$ g, 12 min). The supernatant was analyzed by UPLC-DAD and conversion obtained by comparison to calibration curves of the substrate.

Whole cell preparative-scale reactions:

Whole-cell enzymatic reactions were conducted on 1 g of substrate under the following conditions: 20 weight equivalents of wet cell pellet, 5 mM substrate, 10% (v/v) toluene, 0.1 mM NADP⁺, 0.1 U/mL G6PDH, and 10 mM G6P for NADPH regeneration in reaction buffer (50 mM potassium phosphate buffer, pH 8.0). The reaction mixture was added to a 1 L Erlenmeyer flask and incubated at 30 °C with shaking at 100 rpm. After 2 h, the reaction mixture was filtered through Celite, acidified to pH 2.0 and extracted with EtOAc (3 $\times$ 500 mL). The combined organic layers were dried over sodium sulfate, filtered and concentrated under reduced pressure. The resulting mixture was purified on silica gel (MeOH/AcOH/DCM, 1:1:10) to afford the *o*-quinol product.

Data availability.

Data supporting the findings of this study are available in the corresponding Supplementary Information file, or from the corresponding author upon request. The Supplementary Information file contains full details on the synthesis and characterization of compounds as well as the expression and purification of proteins employed in this work.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Roche SP & Porco JA Dearomatization Strategies in the Synthesis of Complex Natural Products. *Angew. Chem.-Int. Edit* 50, 4068–4093, doi:10.1002/anie.201006017 (2011).
2. Wu WT, Zhang LM & You SL Catalytic asymmetric dearomatization (CADA) reactions of phenol and aniline derivatives. *Chem. Soc. Rev* 45, 1570–1580, doi:10.1039/c5cs00356c (2016). [PubMed: 26796922]
3. Volp KA & Harned AM Chiral aryl iodide catalysts for the enantioselective synthesis of para-quinols. *Chem. Commun* 49, 3001–3003, doi:10.1039/c3cc00013c (2013).
4. Zhu JL, Grigoriadis NP, Lee JP & Porco JA Synthesis of the azaphilones using copper-mediated enantioselective oxidative dearomatization. *Journal of the American Chemical Society* 127, 9342–9343, doi:10.1021/ja052049g (2005). [PubMed: 15984841]

5. Bosset C et al. Asymmetric Hydroxylative Phenol Dearomatization Promoted by Chiral Binaphthyl and Biphenylic Iodanes. *Angew. Chem.-Int. Edit* 53, 9860–9864, doi:10.1002/anie.201403571 (2014).
6. Wang WX et al. Antibacterial Azaphilones from an Endophytic Fungus, *Colletotrichum* sp BS4. *J. Nat. Prod* 79, 704–710, doi:10.1021/acs.jnatprod.5b00436 (2016). [PubMed: 26905687]
7. Yang QL et al. Evolution of an oxidative dearomatization enabled total synthesis of vinigrol. *Org. Biomol. Chem* 12, 330–344, doi:10.1039/c3ob42191k (2014). [PubMed: 24258093]
8. Shiao HY, Hsieh HP & Liao CC First total syntheses of (+/-)-annuionone B and (+/-)-tanarifuranonol. *Org. Lett* 10, 449–452, doi:10.1021/ol7028178 (2008). [PubMed: 18173278]
9. Nicolaou KC et al. Biomimetic total synthesis of bisorbicillinol, bisorbibutenolide, trichodimerol, and designed analogues of the bisorbicillinoids. *J. Am. Chem. Soc* 122, 3071–3079, doi:10.1021/ja9942843 (2000).
10. Pettus LH, Van de Water RW & Pettus TRR Synthesis of (+/-)-epoxysorbicillinol using a novel cyclohexa-2,5-dienone with synthetic applications to other sorbicillin derivatives. *Org. Lett* 3, 905–908, doi:10.1021/ol0155438 (2001). [PubMed: 11263912]
11. Morrow GW & Schwind B SYNTHESIS OF PARA-TERPHENYL VIA REDUCTIVE DEOXYGENATION OF QUINOL DERIVATIVES. *Synth. Commun* 25, 269–276, doi:10.1080/00397919508010816 (1995).
12. Schultz AG & Antoulinakis EG Photochemical and acid-catalyzed rearrangements of 4-carbomethoxy-4-methyl-3-(trimethylsilyl)-2,5-cyclohexadien-1-one. *J. Org. Chem* 61, 4555–4559, doi:10.1021/jo952240g (1996). [PubMed: 11667379]
13. Dong SW, Zhu JL & Porco JA Enantioselective synthesis of bicyclo 2.2.2 octenones using a copper-mediated oxidative dearomatization/ 4+2 dimerization cascade. *J. Am. Chem. Soc* 130, 2738–2739, doi:10.1021/ja711018z (2008). [PubMed: 18266375]
14. Sun WS, Li GF, Hong L & Wang R Asymmetric dearomatization of phenols. *Org. Biomol. Chem* 14, 2164–2176, doi:10.1039/c5ob02526e (2016). [PubMed: 26740241]
15. Uyanik M, Yasui T & Ishihara K Hydrogen Bonding and Alcohol Effects in Asymmetric Hypervalent Iodine Catalysis: Enantioselective Oxidative Dearomatization of Phenols. *Angew. Chem.-Int. Edit* 52, 9215–9218, doi:10.1002/anie.201303559 (2013).
16. Ullrich R & Hofrichter M Enzymatic hydroxylation of aromatic compounds. *Cell. Mol. Life Sci* 64, 271–293, doi:10.1007/s00018-007-6362-1 (2007). [PubMed: 17221166]
17. Davison J et al. Genetic, molecular, and biochemical basis of fungal tropolone biosynthesis. *Proc. Natl. Acad. Sci. U. S. A* 109, 7642–7647, doi:10.1073/pnas.1201469109 (2012). [PubMed: 22508998]
18. van Berkel WJH, Kamerbeek NM & Fraaije MW Flavoprotein monooxygenases, a diverse class of oxidative biocatalysts. *J. Biotechnol* 124, 670–689, doi:10.1016/j.jbiotec.2006.03.044 (2006). [PubMed: 16712999]
19. Khalil AS & Collins JJ Synthetic biology: applications come of age. *Nat. Rev. Genet* 11, 367–379, doi:10.1038/nrg2775 (2010). [PubMed: 20395970]
20. Turner NJ Directed evolution drives the next generation of biocatalysts. *Nat. Chem. Biol* 5, 568–574, doi:10.1038/nchembio.203 (2009).
21. Abood A et al. Kinetic characterisation of the FAD dependent monooxygenase TropB and investigation of its biotransformation potential. *RSC Adv.* 5, 49987–49995, doi:10.1039/c5ra06693j (2015).
22. Zabala AO, Xu W, Chooi YH & Tang Y Characterization of a Silent Azaphilone Gene Cluster from *Aspergillus niger* ATCC 1015 Reveals a Hydroxylation-Mediated Pyran-Ring Formation. *Chem. Biol* 19, 1049–1059, doi:10.1016/j.chembiol.2012.07.004 (2012). [PubMed: 22921072]
23. Sato M et al. Combinatorial Generation of Chemical Diversity by Redox Enzymes in Chaetoviridin Biosynthesis. *Org. Lett* 18, 1446–1449, doi:10.1021/acs.orglett.6b00380 (2016). [PubMed: 26959241]
24. al Fahad A et al. Oxidative dearomatisation: the key step of sorbicillinoid biosynthesis. *Chem. Sci* 5, 523–527, doi:10.1039/c3sc52911h (2014). [PubMed: 25580210]

25. Chenault HK & Whitesides GM REGENERATION OF NICOTINAMIDE COFACTORS FOR USE IN ORGANIC-SYNTHESIS. *Appl. Biochem. Biotechnol* 14, 147–197, doi:10.1007/bf02798431 (1987). [PubMed: 3304160]
26. Zhu JL, Germain AR & Porco JA Synthesis of azaphilones and related molecules by employing cycloisomerization of o-alkynylbenzaldehydes. *Angew. Chem.-Int. Edit* 43, 1239–1243, doi: 10.1002/anie.200353037 (2004).
27. Barnes-Seeman D & Corey EJ A two-step total synthesis of the natural pentacycle trichodimerol, a novel inhibitor of TNF-alpha production. *Org. Lett* 1, 1503–1504, doi:10.1021/ol991070h (1999). [PubMed: 10825998]
28. Wessman P, Hakansson S, Leifer K & Rubino S Formulations for Freeze-drying of Bacteria and Their Influence on Cell Survival. *J. Vis. Exp*, 5, doi:10.3791/4058 (2013).
29. Cabrera GM et al. A sorbicillinoid urea from an intertidal *Paecilomyces marquandii*. *J. Nat. Prod* 69, 1806–1808, doi:10.1021/np060315d (2006). [PubMed: 17190466]
30. Zhang SP et al. Antiviral anthraquinones and azaphilones produced by an endophytic fungus *Nigrospora* sp from *Aconitum carmichaeli*. *Fitoterapia* 112, 85–89, doi:10.1016/j.fitote.2016.05.013 (2016). [PubMed: 27233986]

a, Oxidative dearomatization of phenolic substrates to afford *ortho*-quinol products and small molecule reagents employed for this transformation. **b**, Natural products accessible from *ortho*-quinol intermediates. **c**, Potential products afforded by conditions for resorcinol oxidative dearomatization including potential product isomers, dimers, and undesired products. **d**, biocatalytic oxidative dearomatization leveraging the diversity of a suite of flavin-dependent monooxygenases from natural product biosynthetic pathways, protein structure shown is a homology model of TropB based on the structure of PDB ID 2DKH using the Phyre2 server.

Figure 2 | Nature's tools for oxidative dearomatization of resorcinol compounds.

a. Secondary metabolite pathways containing FAD-dependent monooxygenases that mediate the oxidative dearomatization of resorcinol substrates with orthogonal selectivities including the biosynthetic pathways to stipitonic acid (**16**), azanigerone C (**19**) and sorbicillactone A (**22**). **b.** Generation of C4a-hydroperoxyflavin (**12**) from FAD (**23**) through NADPH reduction of FAD (**23**) to FADH₂ (**24**) and subsequent oxidation to **12**.

Figure 3 | One-pot cascades featuring biocatalytic oxidative dearomatization to access natural products.

a, Stipitatic aldehyde (**48**) synthesis from aldehyde **14** through a two-enzyme cascade. **b**, Synthesis of natural azaphilone **51** from methyl ketone **49**, **c**, and the first synthesis of the natural product, urea sorbicillinoid **53** through a chemoenzymatic sequence initiated by the SorbC-catalyzed oxidative dearomatization of **20**.

Table 1 |

Promiscuity profile substrate scope of FAD-dependent monooxygenases TropB, AzaH and SorbC.

Entry	Substrate	TropB Total Turnover Number	AzaH Total Turnover Number	SorbC Total Turnover Number	Entry	Substrate	TropB Total Turnover Number	AzaH Total Turnover Number	SorbC Total Turnover Number	Entry	Substrate	TropB Total Turnover Number	AzaH Total Turnover Number	SorbC Total Turnover Number
1		1000	296	0	12		377	385	371	23		0	49	0
2		1000	0	0	13		432	261	0	24		0	13	0
3		1000	0	0	14		793	0	0	25		0	28	0
4		1000	1000	0	15		100	0	0	26		0	16	68
5		1000	534	0	16		402	0	0	27		0	329	383
6		976	78	0	17		0	725	0	28		0	277	646
7		934	0	0	18		0	639	0	29		0	93	816
8		787	0	479	19		0	678	0	30		0	308	656
9		829	0	0	20		0	632	0	31		0	841	858
10		669	0	331	21		0	284	0	32		0	418	889
11		646	542	0	22		0	16	0	33		0	639	919

Values given are total turnover numbers (TTNs) of each enzyme/substrate pair. Complete conversion of substrate to dearomatized product: TTN = 1000 (dark green). No observed conversion of starting material is represented by 0 (light grey). Reaction conditions: 2.5 mM substrate, 2.5 μ M FAD-dependent monooxygenase, 1 mM NADP⁺, 5 mM glucose-6-phosphate (G6P), 1 U/mL glucose-6-phosphate dehydrogenase (G6PDH), 50 mM potassium phosphate buffer, pH 8.0, 30 °C, 1 h. TTN was assessed by quantifying the remaining substrate after 1 h by UPLC, where the area of the substrate peak was divided by the area of the internal standard and compared to a substrate standard curve prepared with the internal standard.

Table 2 |

Isolation and characterization of *ortho*-quinol products from reactions with (A) TropB, (B) AzaH and (C) SorbC.

25 26

TropB Products:

>99% conversion 56% isolated yield >99% ee IBX: 20% yield	82% conversion 39% isolated yield >99% ee IBX: 14% yield	38% conversion 24% isolated yield 98% ee IBX: 31% yield	54% conversion 26% isolated yield >99% ee IBX: 18% yield	87% conversion 24% isolated yield >99% ee IBX: 17% yield
84% conversion 68% isolated yield IBX: 11% yield	73% conversion 58% isolated yield IBX: 12% yield	>99% conversion 93% isolated yield IBX: 13% yield		

AzaH Products:

86% conversion 46% isolated yield >99% ee IBX: 20% yield	99% conversion 24% isolated yield IBX: 0% yield	33% conversion 19% isolated yield >99% ee IBX: 18% yield	>99% conversion 55% isolated yield 1:1 d.r.; >99% ee IBX: 44% yield	>99% conversion 82% isolated yield >99% ee IBX: 71% yield

SorbC Products:

82% conversion 43% isolated yield >99% ee Pb(OAc) ₄ : 20% yield	R = Me (39): 70% conversion 24% isolated yield >99% ee Pb(OAc) ₄ : 23% yield R = Et (40): 50% conversion 8% isolated yield 94% ee Pb(OAc) ₄ : 8% yield	77% conversion 63% isolated yield 99% ee Pb(OAc) ₄ : 10% yield	56% conversion 27% isolated yield >99% ee Pb(OAc) ₄ : 17% yield	64% conversion 22% isolated yield 95% ee Pb(OAc) ₄ : 18% yield

Reaction conditions: 2.5 mM substrate, 2.5 μ M FAD-dependent monooxygenase, 1 mM NADP⁺, 5 mM glucose-6-phosphate (G6P), 1 U/mL glucose-6-phosphate dehydrogenase (G6PDH), 50 mM potassium phosphate buffer, pH 8.0, 30 °C, 1 h.

Table 3 |

Whole cell TropB reactions with wet cells versus freeze-dried cells.

<div style="display: flex; align-items: center; justify-content: center;"> <div style="text-align: center;"> <p>44</p> </div> <div style="margin: 0 20px; text-align: center;"> $\xrightarrow[\text{NADP}^+, \text{G6P, G6PDH}]{\text{whole cell TropB, O}_2}$ KPi buffer 30 °C, 2 h </div> <div style="text-align: center;"> <p>45</p> </div> </div>			
entry	cell preparation	additive	% conversion
1	wet	none	>99%
2	wet	none	>99% ^a
3	lyophilized	none	98%
4	lyophilized	10 wt. % skim milk	83%
5	lyophilized	10 wt. % sucrose	>99%
6	lyophilized	PEG 4 × 10 ³	>99%

Reaction conditions: 2.5 mM substrate, 10 mg wet cell mass/mL, 1 mM NADP⁺, 5mM glucose-6-phosphate (G6P), 1 U/mL glucose-6-phosphate dehydrogenase (G6PDH), 50 mM potassium phosphate buffer, pH 8.0, 30 °C, 2 h. ^aReaction on 1 g **44**.