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Diverting non-haem iron catalysed aliphatic C–H hydroxylations towards desaturations

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Carboxylate-ligated, non-haem iron enzymes demonstrate the capacity for catalysing such remarkable processes as hydroxylations, chlorinations and desaturations of inert, aliphatic C–H bonds. A key to functional diversity is the enzymes' ability to divert fleeting radicals towards different types of functionalization using active site and/or substrate modifications. We report that a non-haem iron hydroxylase catalyst [Fe(PDP)] can also be diverted to catalytic, mixed hydroxylase/desaturase activity with aliphatic C–H bonds. Using a taxane-based radical trap that rearranges under Fe(PDP) oxidation to furnish a *nor*taxane skeleton, we provide the first direct evidence for a substrate radical using this class of stereoretentive hydroxylation catalysts. Hydroxylation and desaturation proceed by means of a short-lived radical that diverges in a substrate-dependent manner in the presence of carboxylic acids. The novel biomimetic reactivity displayed by this small molecule catalyst is harnessed to diversify natural product derivatives as well as interrogate their biosynthetic pathways.

Olefins are powerful intermediates in the synthesis of a diverse range of important compounds, from petrochemicals to pharmaceuticals. Because of this, the catalytic dehydrogenation of alkanes in a selective manner to give olefins is a reaction with tremendous potential value[1]. Breakthroughs in this area with small-molecule catalysts have occurred primarily in the realm of organometallic alkane activation, and make use of low valent, late transition metal catalysts (such as Ir, Rh) to promote C–H activation at the metal centre[2,3]. In particular, tandem systems in which alkane dehydrogenation is followed by secondary reactions such as alkene metathesis have led to promising methods for the synthesis of petroleum-based fuels[4]. A complementary approach is that found in biological systems where high valent, first-row transition metal catalysts effect C–H activation using reactive oxo ligands on the metal. This approach is inherently highly compatible with dense functionality and therefore holds potential for the synthesis and derivatization of natural products and medicinal compounds.

Nature has evolved a series of carboxylate-ligated non-haem iron enzymes that catalyse oxidative reactions of alkanes with remarkable selectivity and scope[5–7]. Relatively minor changes in the ligand environment of the high-energy metal oxidant [Fe(oxo)] are thought to control the fate of the substrate-derived carbon-centred radical generated subsequent to C–H activation, leading to a wide range of transformations, including dehydrogenation, hydroxylation and chlorination[8,9]. Additionally, a single enzyme is sometimes able to catalyse different classes of reactions depending on the chemical properties of the substrate. For example, clavaminate synthase 2 (CS2) (ref. 10), a non-haem iron hydroxylase, switches from hydroxylation to mixed hydroxylase/desaturase activity upon removal of the remote guanyl group to expose a primary amine on the proclavaminic substrate (Fig. 1a)[11]. In these enzymatic systems, it is hypothesized that substrate-dependent switches between hydroxylation and desaturation are due to either (i) the character of the carbon-centred radical and its tendency to undergo further oxidation to a carbocation or (ii) the orientation of the radical with respect to the reactive iron centre and the resulting favourable alignment of the adjacent C–H bond for abstraction (Fig. 1b)[8,12]. Using these postulates as a guide, one may envision achieving preparatively useful levels of mixed hydroxylase/desaturase activity with a small-molecule catalyst. The ability to predictably generate useful yields of several oxidized products from a common, complex starting material could streamline syntheses of natural product analogues with diverse physical properties and bioactivities.

Although numerous small-molecule non-haem iron hydroxylase catalysts have appeared in the literature, complexes capable of effecting desaturation are much less common. Of the few approaches reported, the majority require stoichiometric, preformed metal oxidants and/or the presence of excess substrate[13,14]. Moreover, mixed hydroxylase/desaturase reactivity outside enzymatic systems has previously only been observed for hydrocarbon substrates with relatively weak, activated C–H bonds, probably a result of a mechanistic requirement for a stabilized radical or cationic intermediate[15,16]. The presumed intermediates generated in these systems benefit from π-stabilization (that is, R is a heteroatom or π-system; Fig. 1b), leading to the proposal that this added stability allows desaturation to compete with the usual oxygenation reactivity. Such an approach to desaturation is clearly unsuitable for aliphatic C–H bonds due to the lack of adjacent stabilizing functional groups. Nevertheless, when faced with the challenge of aliphatic C–H desaturation, inspiration may be drawn from the second hypothesis described above (that is, the relative orientation of the substrate radical and metal centre promotes desaturation via a second hydrogen abstraction). If correct, it implies that desaturase activity caused by modification of the radical/catalyst orientation would not be limited to a relatively stabilized carbon-centred radical, and therefore might lead to a reaction with enhanced generality.

We recently disclosed a series of preparatively useful, site-selective aliphatic C–H oxidations of 2° and 3° C–H bonds using the non-haem iron catalyst Fe(PDP) **1** and H_2O_2 as terminal oxidant[17–19]. These studies allowed us to delineate the electronic, steric and stereoelectronic rules governing the reaction outcome. In particular, we have shown that sites that are proximal to electron-withdrawing groups are deactivated towards oxidation by the highly electrophilic metal oxidant thought to be generated from **1** and H_2O_2 (that is, Fe(oxo)), while sterically hindered C–H bonds

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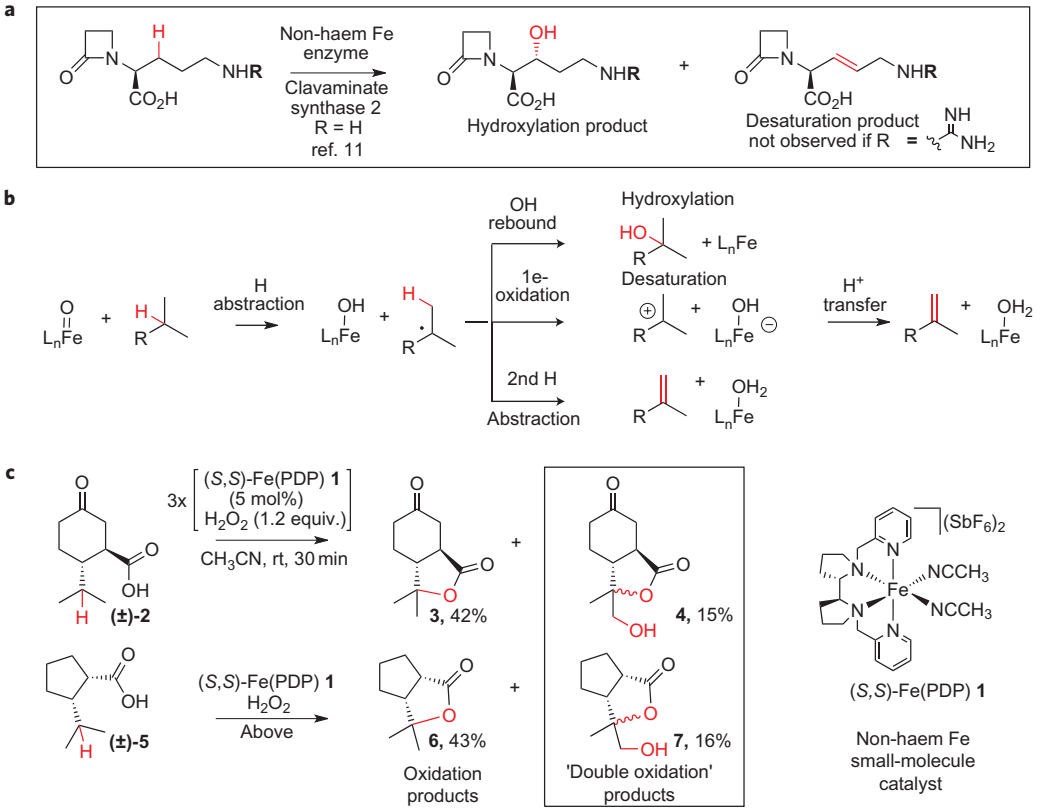

Figure 1 | Substrate-dependent mixed hydroxylase/desaturase reactivity of non-haem iron catalysts with aliphatic C–H bonds. a, Substrate-dependent switch from hydroxylase to mixed hydroxylase/desaturase activity with a non-haem iron enzyme. Clavaminate synthase 2, a hydroxylase, provides a mixture of hydroxylated and desaturated products when the terminal guanidine functionality of its standard substrate is removed. **b**, Postulated mechanisms for diverting hydroxylase to desaturase activity. **c**, Substrate-dependent switch from hydroxylase to mixed hydroxylase/desaturase activity with a small-molecule, non-haem iron catalyst. Hydroxylase catalyst Fe(PDP) **1** provides a mixture of hydroxylated products (lactones) and products resulting from a desaturation/epoxidation/lactonization sequence (hydroxylactones) with aliphatic C–H bonds in carboxylic acid substrates. For a description of the iterative addition reaction conditions (that is, 3 × [Fe(PDP), H_2O_2]), see Methods.

are shielded from the bulky oxidant. Moreover, we have validated the predictive nature of these rules in complex molecular settings. In the course of these studies, we found that acetic acid (AcOH) was a critical additive for enhancing reactivity while maintaining site-selectivity of the catalyst[20–22]. Moreover, when the carboxylic acid moiety was incorporated into the substrate, it showed the capacity for directing the site of oxidation to favour the formation of γ-butyrolactones (that is, five-membered ring lactones), presumably by coordinating to the Fe centre and effecting intramolecular C–H oxidation. While examining the capacity of the carboxylic acid directing group for overcoming the aforementioned electronic, steric and stereoelectronic biases in C–H oxidations with Fe(PDP) (M. A. Bigi and M. C. White, manuscript in preparation), we routinely observed the generation of novel 'double oxidation' products beside the expected lactone products.

As a result of our investigations into the origins of these products, we now present our discovery that Fe(PDP) catalyses substrate-dependent, dual hydroxylase/desaturase activity on aliphatic substrates containing carboxylic acids. Mechanistic studies suggest that, similar to enzymatic systems, both products arise from a common pathway via a very short-lived (lifetime $<1 \times 10^{-11}$ s) one-electron intermediate. This intermediate may diverge based on its orientation relative to the catalyst, a factor influenced by the intramolecular nature of the reaction. The first direct evidence for a carbon-centred substrate radical with this class of stereoretentive, iron hydroxylation catalysts is provided through a unique taxane-based radical trap that rearranges under Fe(PDP) oxidation conditions to furnish a *nor*taxane skeleton. Finally, the predictable mixed hydroxylase/desaturase activity of Fe(PDP) is proven capable of rapidly furnishing useful quantities of novel lactone and hydroxylactone compounds from a carboxylic acid-containing natural product derivative. The observation of mixed hydroxylase/desaturase products with aliphatic substrates, their substrate-dependent formation, and the rearrangement of the taxane skeleton to furnish the *nor*taxane skeleton all represent novel reactivity for a small-molecule oxidation catalyst.

Results

When a series of carboxylic acid substrates were evaluated for C–H hydroxylation reactivity under standard Fe(PDP)/H_2O_2 conditions, in addition to the anticipated butyrolactone products, unexpected hydroxylactone products were observed (Fig. 1c). In Fe(PDP)-catalysed hydroxylations, sites that are proximal to electron-withdrawing esters are deactivated towards oxidation. Moreover, 1° C–H bonds are thought to be inert to these conditions. Given these precedents, we hypothesized that the observed double-oxidation products did not arise from multiple C–H oxidation events but rather from olefins generated *in situ* by the Fe(PDP) catalyst via desaturation.

Under Fe(PDP)/H_2O_2 reaction conditions, desaturation products would be rapidly oxidized to epoxides[20], and subsequent intramolecular lactonization would generate the observed hydroxylactone products (Fig. 2). In accord with this proposal, exposure of alkene **10** to the standard Fe(PDP) reaction conditions afforded hydroxylactone **9** in 73% yield (Fig. 2a). The same double-oxidation product was furnished from C–H oxidation of analogous alkane **8**. For reactions that generate stereogenic centres at carbon,

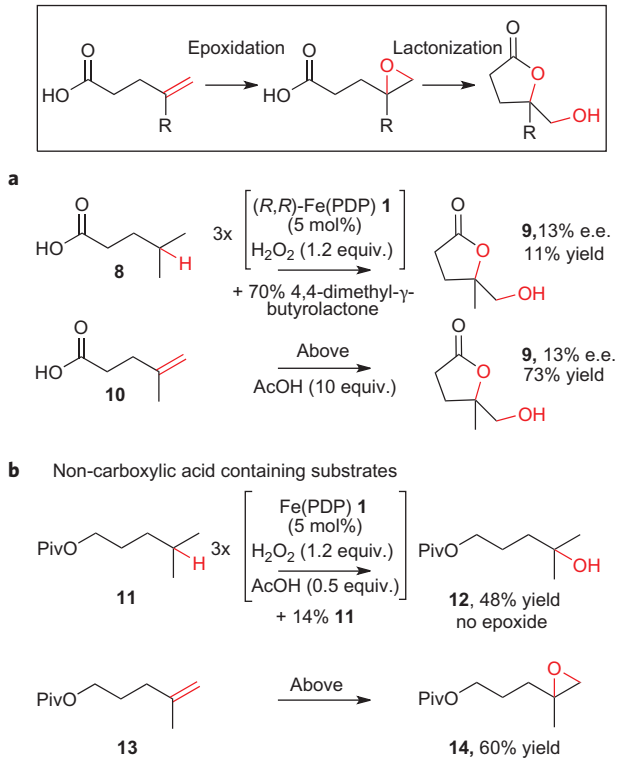

Figure 2 | Double-oxidation products arise from desaturation products (olefins) in a substrate-dependent manner. a, Oxidation of aliphatic carboxylic acid substrate **8** and analogous alkenoic acid **10** furnishes hydroxylactone **9** in identical enantiomeric excess (13% e.e.). The enantioenrichment of the hydroxylactone products generated using the other catalyst antipode, Fe(*S,S*-PDP), was equal and opposite (see Supplementary Information). **b**, Substrate **11**, lacking a carboxylic acid, does not afford detectable quantities of epoxide **14** (analysis done by gas chromatography and crude ^{1}H NMR analysis), the product expected if desaturation activity is operative in the intermolecular C–H oxidation reaction.

interrogation of the resulting stereochemistry is a sensitive mechanistic probe. Therefore, to further investigate the viability of olefin intermediates, we measured the enantioenrichment of the hydroxylactone products generated using the chiral Fe(PDP) catalyst from both alkanoic acid **8** and alkenoic acid **10**. Under conditions mimicking those of the catalytic aliphatic C–H oxidation, the stereochemical outcome with the olefin starting material was identical to that observed from alkane **8**: hydroxylactone **9** was generated with 13% e.e. favouring the same enantiomer. Collectively, these results demonstrate for the first time that a small-molecule non-haem Fe catalyst is capable of performing both hydroxylase and desaturase activity with unactivated, aliphatic C–H bonds.

Products arising from desaturation have not previously been observed in intermolecular Fe(PDP) C–H oxidations, suggesting that this novel, dual reactivity is substrate-dependent. In support of this, acylated alcohol **11**, lacking a carbonyl directing group, failed to form the epoxide product expected from a desaturation pathway (Fig. 2b). To evaluate if desaturation products arising from non-carboxylic acid-containing substrates are degraded more rapidly, and therefore not detectable under these conditions, alkene **13** was exposed to the C–H oxidation conditions. Catalyst Fe(PDP) **1**, like the closely related Fe(mep) epoxidation catalyst[20], is a capable promoter of epoxidations of unactivated alkenes with H_2O_2: Fe(PDP)-catalysed oxidation of alkene **13** furnished epoxide **14** in 60% yield. This result strongly supports the substrate-dependent nature of the mixed oxygenase/desaturase reactivity of Fe(PDP), which is highly reminiscent of that observed with non-haem iron enzymes (Fig. 1a).

We next sought to determine how olefin intermediates were generated during Fe(PDP)-catalysed oxidations. Guided by early mechanistic proposals in the enzymatic desaturation literature[8], we investigated the possibility that desaturation resulted from a simple hydroxylation/dehydration sequence. Carboxylate-directed Fe(PDP) C–H oxidations are thought to involve a high valent iron oxo carboxylate oxidant **15** (Fig. 3a)[22]. C–H oxidations from putative intermediate **15** may proceed by means of a concerted insertion pathway to directly furnish hydroxyacid intermediates, and, subsequently, such hydroxyacids could either lactonize or undergo dehydration to give olefins. To test the validity of the lactonization/dehydration proposal, we evaluated alkanoic acid **16** and a tentative oxidation intermediate, hydroxyacid **20**, under standard Fe(PDP) reaction conditions. Alkanoic acid **16**, in addition to the expected C–H oxidation product spirolactone **17** (28% yield), furnished desaturation products **18** and **19** in substantial combined yields (37%) (Fig. 3a). Exposure of **17** to the standard reaction conditions failed to provide more than trace quantities of **18** and **19** (3% total; see Supplementary Information). Moreover, when hydroxyacid **20** was exposed to identical conditions as **16**, only minor amounts of desaturation products were produced (6% total), which may arise through over-oxidation of lactone **17** or a minor dehydration pathway. We next evaluated oxidation of alkanoic acid **16** using an electrophilic oxidant, methyl(trifluoromethyl)dioxirane (TFDO), known to proceed via a concerted C–H oxidation pathway to directly furnish hydroxylated compounds[23]. Significantly, oxidation of **16** using TFDO also resulted in only minor amounts of desaturation products (5% total), closely matching levels observed under Fe(PDP) conditions when starting from hydroxyacid **20**. These results demonstrate that the desaturation activity observed with Fe(PDP) does not involve dehydration of a hydroxyacid intermediate. Moreover, the results suggest that a

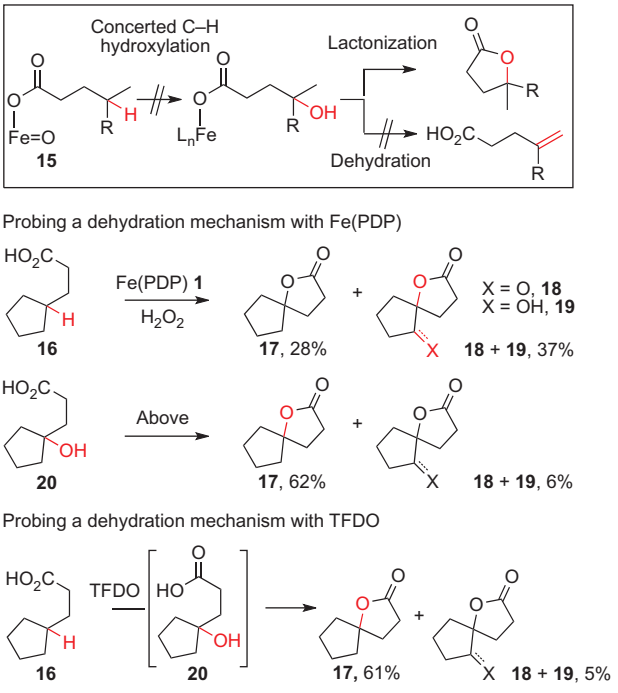

Figure 3 | Experiments probing a dehydration pathway in the formation of olefin intermediates. a, Although treatment of **16** with Fe(PDP) (**1**) affords a significant amount of double oxidation products **18** and **19**, only low levels of these products are observed on treatment of the hydroxyacid **20** under the same conditions. **b**, Oxidation using TFDO also only provides low levels of the double oxidation products. Taken together these reactions indicate that desaturation products in Fe(PDP) oxidations do not arise from a dehydration pathway.

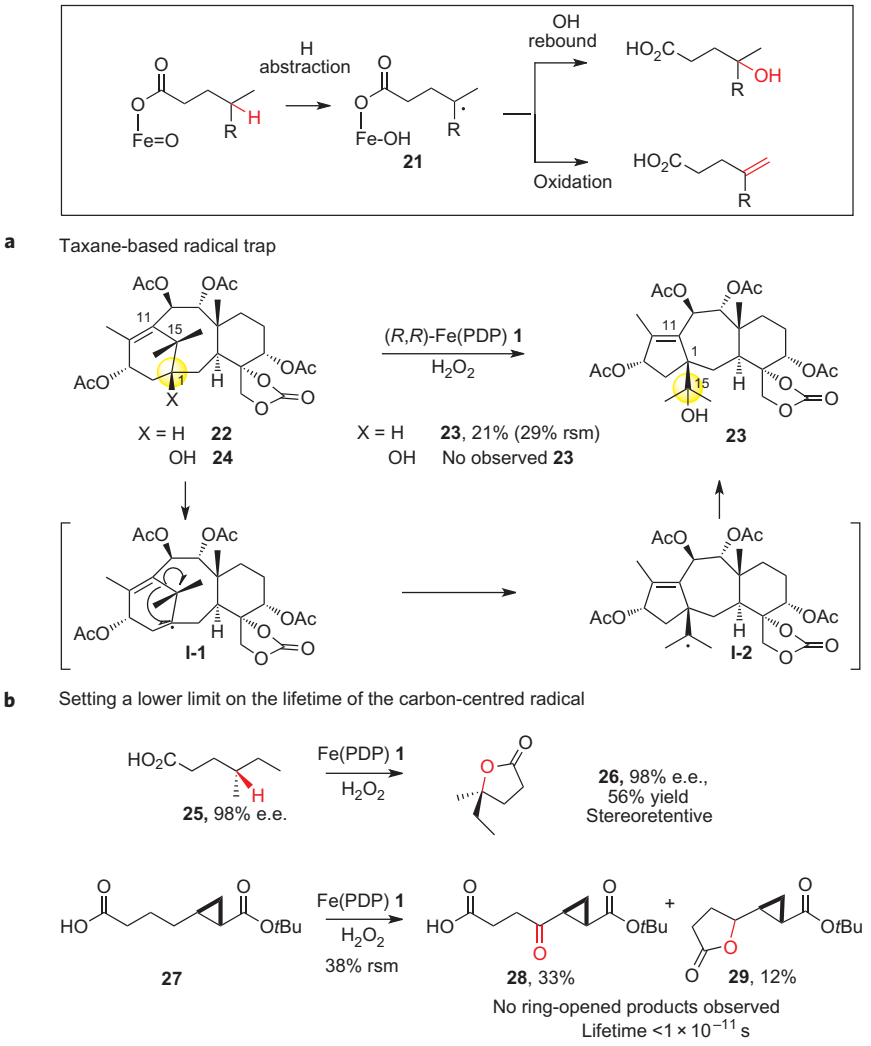

Figure 4 | Experiments probing the existence of a carbon-centred radical. Proposed mechanism for dual hydroxylase/desaturase activity in Fe(PDP) oxidation. Hydrogen abstraction by an Fe(oxo)carboxylate generates a carbon-centred radical that diverges to either hydroxyl rebound furnishing lactones or further oxidation generating desaturation products. ^{18}O labelling studies indicate that an iron peracid intermediate is not generated under the reaction conditions and that the C–H lactonization reaction occurs via initial hydroxylation, rather than direct radical rebound with its appended carboxylate (see Supplementary Information). **a**, Rearrangement of taxane **22** to *nor*taxane **23** provides evidence for a carbon-centred radical generated under Fe(PDP) conditions. **b**, The stereoretentive nature of 3° C–H lactonization and lack of ring-opened products originating from the cyclopropane probe **27** indicates that the carbon-centred radical is very short-lived ($<1 \times 10^{-11}$ s).

concerted C–H oxidation pathway is not involved in the mixed hydroxylase/desaturase activity observed with Fe(PDP).

So far, no definitive, direct evidence for carbon-centred radical intermediates in stereoretentive C–H oxidations with small-molecule non-haem iron complexes such as Fe(PDP) has been reported. This is in large part due to the fact that these complexes rapidly oxidize aromatic functionality, rendering them incompatible with Newcomb's hypersensitive aryl-substituted cyclopropane radical clocks (maximum ring-opening rate constant of 4×10^{11} s^{-1})[24]. Given the precedent for radical-induced rearrangements in the taxane skeleton via Barton–McCombie deoxygenation at C1 (ref. 25), we wondered if we could obtain direct evidence for a radical intermediate through oxidation of taxusin derivative **22** with Fe(PDP) (Fig. 4a). Gratifyingly, on oxidation of **22** with Fe(PDP), we observed 11(15 → 1)-*abeo*taxane **23** (*nor*taxane structure) as the major product (structure confirmed by X-ray crystallography; see Supplementary Information). We propose that rearrangement occurs via hydrogen abstraction (**I-1**), followed by a Wagner—Meerwein-type rearrangement (**I-2**), and finally hydroxyl rebound to form **23** (Fig. 4a). Significantly, exposure of independently prepared C1 hydroxylated compound **24** to these reaction conditions did not result in any rearranged products, ruling out a C–H hydroxylation/cationic rearrangement mechanism[26]. Notably, numerous biologically active *nor*taxanes are known in nature[27], although they are proposed to arise from an alternative cationic pathway during the cyclase phase of Taxol biosynthesis[28]. Given our results, and the fact that *nor*taxanes have been isolated from fungal P450 oxidations of related taxanes[29], an alternative biogenic hypothesis may be put forth: late-stage P450 mediated C–H oxidations at C1 during the oxidase phase of taxane biosynthesis generates a carbon-centred radical intermediate at C1 that may diverge either via OH rebound to provide an oxidized taxane (as seen in Taxol) or rearrangement followed by rebound to provide a *nor*taxane product.

Although we cannot exclude the possibility of two distinct pathways accounting for oxygenation and desaturation, we favour the simplest conclusion that Fe(PDP) C–H hydroxylations and desaturations uniformly proceed via a single pathway that diverges at a common carbon-centred radical intermediate. Based on the lack of involvement of hydroxyacid intermediates in the desaturation

process and the direct evidence for a carbon-centred radical intermediate in the intermolecular Fe(PDP) C–H hydroxylation, we propose that Fe(PDP) C–H oxidations proceed via a hydrogen abstraction pathway. Significantly, such a pathway is also consistent with what is proposed for analogous enzymatic systems (*vide supra*) and other small-molecule non-haem iron oxidation catalysts based on extensive spectroscopic investigations of the iron catalyst intermediates[30]. Under this mechanistic scenario, the high valent iron oxo carboxylate oxidant effects hydrogen abstraction to form a carbon-centred radical intermediate **21** that undergoes either hydroxyl rebound to give oxygenation products or a second oxidation to furnish olefin products (Fig. 4).

Perhaps the most intriguing question that this study raises centres around the mechanistic basis for the substrate-dependent switch between the hydroxylation and desaturation reaction pathways with Fe(PDP). As previously described for non-haem iron enzymatic systems, olefin formation may occur through additional hydrogen abstraction or through one-electron oxidation to a cationic intermediate, followed by deprotonation (Fig. 1b). A possible factor controlling the reaction outcome may be the differing ligand environments of the iron oxidant depending on the functionality present on the substrate. For example, the carboxylic acid-promoted desaturation activity with Fe(PDP) may arise if carboxylate ligation to the iron centre alters the rate of the –OH rebound step. A slower rebound would extend the lifetime of the carbon-centred radical and may promote an alternative pathway. To probe this mechanistic scenario, we sought to evaluate the lifetime of the carbon-centred radical in the intramolecular lactonization reactions. Intermolecular C–H hydroxylations with Fe(PDP) to furnish tertiary alcohol products have been shown to proceed with complete stereoretention, indicating a very rapid –OH rebound step (that is, the carbon-centred radical must have a lifetime shorter than 1×10^{-10} s, two orders of magnitude shorter than the lifetime required to observe epimerization: 1×10^{-8} to 1×10^{-9} s)[17,30,31]. We found that oxidation of enantioenriched alkanoic acid **25** (98% e.e.) also proceeded with complete retention of stereochemistry to furnish lactone **26** in 98% e.e. (Fig. 4b). These results suggest that the substrate-dependent desaturation pathway observed with carboxylic acids does not arise from an alkyl radical substantially longer-lived than that generated in the intermolecular system. Moreover, an ester-substituted cyclopropane probe **27** (ring-opening rate constant of $\sim 1 \times 10^{11}$ s^{-1})[32] containing a carboxylic acid directing group failed to generate detectable quantities of ring-opened products. The stereoretention of 3° C–H lactonization and the lack of ring-opened products generated from the cyclopropane radical probe experiment dictates that hydroxyl rebound with Fe(PDP) is extremely rapid; that is, it must occur with a rate constant faster than 1×10^{11} s^{-1}. The fact that mixed hydroxylase/desaturase activity can operate in such a narrow rate window with aliphatic substrates is novel for a small-molecule catalyst.

Collectively these results support the hypothesis that the intramolecular nature of the oxidation with carboxylic acid substrates has a strong impact on the orientation of the radical during the rebound step and promotes a dehydrogenation pathway. However, we cannot exclude the possibility that a carbonyl oxygen may be important for lowering the activation energy required for formation of a carbocationic intermediate through an as-yet undefined stabilizing interaction. Investigations into the substrate and catalyst requirements for promoting desaturation over hydroxylation are ongoing and are likely to illuminate these outstanding questions.

Although promiscuity in reaction pathways to generate intractable mixtures of oxidized compounds is not a desirable goal in synthesis, a small-molecule catalyst that mimics the 'selective promiscuity' seen in enzymatic systems could be highly enabling. We therefore set out to evaluate if Fe(PDP) oxidations of complex carboxylic acid-containing substrates would persist in furnishing both

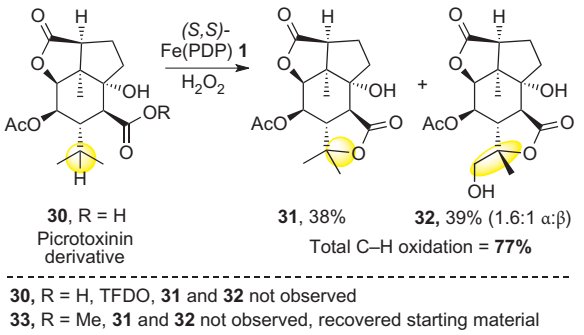

Figure 5 | Rapid diversification of carboxylic acid natural product analogues. Fe(PDP)-catalysed C–H oxidation of picrotoxinin carboxylic acid analogue **30** provides useful quantities of chemically diverse compounds lactone **31** and hydroxylactones **32**. Treatment of **30** with TFDO, an oxidant not capable of carboxylic acid coordination, failed to generate **31** or **32**. Moreover, Fe(PDP)-catalysed oxidation of the corresponding methyl ester **33** resulted in only recovered starting material.

hydroxylation and desaturation products and could do so in useful yields. Carboxylic acids and their corresponding esters are common motifs in many natural products and medicinally important compounds, providing ample opportunity for such late-stage diversification with Fe(PDP) **1**. Picrotoxinin, a naturally occurring sesquiterpenoid containing two lactones, is a potent GABA receptor antagonist that has become a nearly indispensible tool in neurochemistry[33]. Through a short synthetic sequence (see Supplementary Information), the bridgehead lactone was opened to reveal a latent carboxylic acid-containing compound **30**. Exposure of **30** to the standard reaction conditions produced novel lactone and hydroxylactone compounds with a combined oxidation yield of 77% (Fig. 5). Owing to large differences in their physical properties (for example, polarities and hydrogen bonding capacity), the two compounds can be readily separated using standard column chromatography to afford pure lactone **31** in 38% yield and hydroxylactones **32** in 39% yield. Significantly, treatment of **30** with TFDO, an electrophilic oxidant not thought capable of coordinating to the carboxylic acid directing group, did not furnish any lactone or hydroxylactone products. Furthermore, exposure of the corresponding methyl ester derivative **33** to standard Fe(PDP) conditions led only to recovery of unreacted starting material, a result likely due to the highly electronically deactivated and rigid structure of the picrotoxinin core.

Discussion

An intriguing parallel may be drawn between this work and the strategies by which biological systems, using a single ferrous active site, divert fleeting radical intermediates to a range of oxidized products. We have demonstrated that a small-molecule, non-haem iron hydroxylation catalyst is capable of effecting mixed hydroxylase/desaturase activity with aliphatic substrates containing carboxylic acids. Mechanistic studies suggest that the observed dual activity ensues from a short-lived alkyl radical ($<1 \times 10^{-11}$ s) that diverges in a substrate-dependent manner towards either hydroxyl functionalization to furnish lactone products or further oxidation to generate desaturation products. We provide the first direct evidence for a carbon-centred radical intermediate in non-haem small-molecule iron catalysis using a taxane-based radical probe **22** to furnish the rearranged *nor*taxane skeleton. Significantly, this suggests an alternative biogenic hypothesis for taxanes: a carbon-centred radical intermediate generated during the oxidase phase may diverge to undergo either direct hydroxylation or rearrangement/hydroxylation. Previously, such divergent reactivities with inert C–H bonds, operating in a narrow rate window from fleeting

carbon-centred substrate radicals, had been limited to enzymatic catalysis. Our results underscore that, due to mechanistic similarities with oxidation enzymes found in nature, the biomimetic small molecule Fe(PDP) catalyst has the capacity to interrogate natural product biosynthesis performed by such enzymes.

We postulate that the intramolecular nature of Fe(PDP) C–H activation with carboxylic acid substrates alters the orientation of the substrate radical during the hydroxyl rebound step and diverts reactivity towards a second hydrogen abstraction to furnish olefin intermediates. Knowledge of the subtle substrate/catalyst interactions required for promoting desaturation will aid in the development of new metal catalysts for aliphatic C–H desaturation. Additionally, we have demonstrated that this predictable dual hydroxylase/desaturase activity persists in complex natural product settings and directly furnishes novel, diverse compounds in useful yields. Identification of other common functionalities capable of catalyst coordination should expand the reaction scope and overall utility of this powerful transformation. We anticipate that the ability of this reaction, and others like it, to alter hydrocarbon cores and provide direct access to diverse structures will contribute to reinvigorating the use of natural products as a source of new therapeutics[34].

Methods

C–H oxidation of picrotoxinin derivative 30. Into a 40 ml borosilicate vial was added acid **30** (71.4 mg, 0.21 mmol, 1.0 equiv.), followed by 5 mol% Fe(*S*,*S*-PDP) **1** (10.3 mg, 0.011 mmol, 0.05 equiv.), 0.32 ml CH_3CN and a magnetic stir bar. While the resulting deep-red solution stirred, a solution of H_2O_2 (50 wt% in H_2O, 14.5 μl, 0.25 mmol, 1.2 equiv.) in 1.9 ml CH_3CN was added over a period of 1 min (dropwise addition for 45 s, followed by streamwise addition for 15 s), generating an amber-brown solution. Stirring followed for 10 min at ambient temperature, then a solution of 5 mol% **1** (10.3 mg, 0.011 mmol, 0.05 equiv.) in 0.2 ml CH_3CN was added in one burst. A second solution of H_2O_2 (50 wt% in H_2O, 14.5 μl, 0.25 mmol, 1.2 equiv.) in 1.9 ml CH_3CN was added as before and stirred for 10 min. Following this stirring period, a second solution of 5 mol% **1** (10.3 mg, 0.011 mmol, 0.05 equiv.) in 0.2 ml CH_3CN was added in one burst, followed by a third solution of H_2O_2 (50 wt% in H_2O, 14.5 μl, 0.25 mmol, 1.2 equiv.) in 1.9 ml CH_3CN. The reaction was stirred for a final 10 min and was analysed by thin layer chromatography (TLC). The crude reaction mixture was filtered through a short silica plug (100% EtOAc) and ^{1}H nuclear magnetic resonance (NMR) analysis indicated a mixture of hydroxylactone diastereomers (run 1, 1.6:1 d.r.; run 2, 1.5:1 d.r.; average, 1.6:1 d.r. (**32α**/**32β**, ^{1}H NMR, acetone-d_6). Flash chromatography with silica gel (gradient, 20% → 30% → 50% acetone/hexanes) was used to isolate the lactone product as white crystals (run 1: 26.1 mg, 0.077 mmol, 37% yield; run 2, 74.2 mg scale: 29.1 mg, 0.086 mmol, 39% yield; average: 38% yield), together with a mixture of hydroxylactones **32α** and **32β** (run 1: 29.6 mg, 0.084 mmol, 40% yield; run 2: 29.7 mg, 0.084 mmol, 38% yield; average: 39% yield). The hydroxylactone diastereomers could be separated by MPLC (gradient, 0 → 50% acetone/hexanes) to obtain pure samples for spectroscopic analysis.

Lactone [(+)-31]. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.10 (dd, J = 11.8, 3.8 Hz, 1H), 4.62 (d, J = 4.0 Hz, 1H), 2.80 (d, J = 15.0 Hz, 1H), 2.76 (d, J = 7.5 Hz, 1H), 2.68 (bs, 1H), 2.50 (dd, J = 14.5, 6.5 Hz, 1H), 2.30–2.35 (m, 1H), 2.19 (dd, J = 12.3, 5.8 Hz, 1H), 2.13 (s, 3H), 1.95 (dd, J = 13.3, 5.3 Hz, 1H), 1.59 (td, J = 13.5, 5.8 Hz, 1H), 1.53 (s, 3H), 1.35 (s, 3H), 1.34 (s, 3H); ^{13}C NMR: (125 MHz, $CDCl_3$) δ 178.5, 173.7, 170.0, 84.5, 84.2, 79.8, 70.8, 55.0, 54.1, 48.3, 44.0, 35.3, 28.7, 28.5, 20.7, 20.5, 19.1; IR (film, cm^{-1}): 3,489 (broad), 2,954, 2,922, 2,854, 1,780, 1,739 (2 peaks), 1,464, 1,377, 1,263, 1,240, 1,174, 1,120, 1,072, 1,036; HRMS (ESI) m/z calc'd for $C_{17}H_{23}O_7$ $[M+H]^+$: 339.1444, found 339.1448; $[\alpha]_D^{25} = +130.1^\circ$ (c = 1.22, $CHCl_3$).

Hydroxylactone [(+)-32α]. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.48 (dd, J = 12.0, 3.5 Hz, 1H), 4.66 (d, J = 4.0 Hz, 1H), 3.86 (d, J = 12.5 Hz, 1H), 3.72 (d, J = 12.5 Hz, 1H), 3.48 (d, 14.5 Hz, 1H), 2.75 (d, J = 7.5 Hz, 1H), 2.56 (dd, J = 14.5, 12.0 Hz, 1H), 2.30–2.38 (m, 1H), 2.19 (dd, J = 12.5, 6.0 Hz, 1H), 2.17 (s, 1H), 2.13 (s, 3H), 1.88 (dd, J = 13.3, 5.3 Hz, 1H), 1.58 (td, J = 13.5, 6.0 Hz, 1H), 1.43 (s, 3H), 1.35 (s, 3H); ^{13}C NMR: (125 MHz, $CDCl_3$) δ 178.7, 175.0, 170.2, 85.5, 84.4, 80.2, 70.9, 66.1, 55.2, 54.0, 48.7, 43.6, 34.6, 28.6, 24.3, 20.9, 19.2; IR (film, cm^{-1}): 3,473 (broad), 2,947, 2,929, 2,873, 1,763, 1,751, 1,462, 1,379, 1,329, 1,286, 1,236, 1,178, 1,119, 1,066, 1,038; HRMS (ESI) m/z calc'd for $C_{17}H_{22}O_8Na$ $[M+Na]^+$: 377.1212, found 377.1205; $[\alpha]_D^{25} = +132.7^\circ$ (c = 0.32, EtOH).

Hydroxylactone [(+)-32β]. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.11 (dd, J = 11.3, 3.8 Hz, 1H), 4.65 (d, J = 4.0 Hz, 1H), 3.78 (d, J = 12.5 Hz, 1H), 3.58 (d, J = 12.5 Hz, 1H), 2.88 (AB_q, $\Delta\nu$ = 34.9 Hz, J_{ab} = 15.0 Hz, 1H), 2.87 (AB_q, $\Delta\nu$ = 22.4 Hz, J_{ab} = 15.0 Hz, 1H), 2.77 (d, J = 7.5 Hz, 1H), 2.31–2.39 (m, 1H), 2.20 (dd, J = 12.5, 6.0 Hz, 1H), 2.13 (s, 3H), 1.94 (dd, J = 13.5, 6.0 Hz, 1H), 1.66 (bs, 2H); 1.64 (td, J = 13.5, 6.0 Hz, 1H), 1.37 (s, 3H), 1.32 (s, 3H); ^{13}C NMR: (125 MHz, $CDCl_3$) δ 178.4, 173.5, 170.0, 86.3, 84.1, 79.9, 70.7, 67.4, 55.1, 54.1, 47.9, 37.4, 35.3, 28.5, 20.8, 19.2, 15.9; IR (film, cm^{-1}): 3,464, 2,929, 2,872, 2,854, 1,765, 1,749, 1,462, 1,377, 1,329, 1,284, 1,236, 1,186, 1,115, 1,070, 1,036, 982; HRMS (ESI) m/z calc'd for $C_{17}H_{22}O_8Na$ $[M+Na]^+$: 377.1212, found 377.1220; $[\alpha]_D^{25} = +104.7^\circ$ (c = 0.47, EtOH).

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References

1. Dobereiner, G. E. & Crabtree, R. H. Dehydrogenation as a substrate-activating strategy in homogeneous transition-metal catalysis. *Chem. Rev.* **110,** 681–703 (2010).
2. Burk, M. J. & Crabtree, R. H. Selective catalytic dehydrogenation of alkanes to alkenes. *J. Am. Chem. Soc.* **109,** 8025–8032 (1987).
3. Liu, F., Pak, E. B., Singh, B., Jensen, C. M. & Goldman, A. S. Dehydrogenation of *n*-alkanes catalyzed by iridium 'pincer' complexes: regioselective formation of α-olefins. *J. Am. Chem. Soc.* **121,** 4086–4087 (1999).
4. Goldman, A. S. *et al.* Catalytic alkane metathesis by tandem alkane dehydrogenation–olefin metathesis. *Science* **312,** 257–261 (2006).
5. Wallar, B. J. & Lipscomb, J. D. Dioxygen activation by enzymes containing binuclear non-heme iron clusters. *Chem. Rev.* **96,** 2625–2657 (1996).
6. Costas, M., Mehn, M. P., Jensen, M. P. & Que, L. Jr. Dioxygen activation at mononuclear nonheme iron active sites: enzymes, models, and intermediates. *Chem. Rev.* **104,** 939–986 (2004).
7. Bruijnincx, P. C. A., van Koten, G. & Gebbink, R. J. M. K. Mononuclear non-heme iron enzymes with the 2-His-1-carboxylate facial triad: recent developments in enzymology and modeling studies. *Chem. Soc. Rev.* **37,** 2716–2744 (2008).
8. Buist, P. H. Fatty acid desaturases: selecting the dehydrogenation channel. *Nat. Prod. Rep.* **21,** 249–262 (2004).
9. Vaillancourt, F. H., Yeh, E., Vosburg, D. A., Garneau-Tsodikova, S. & Walsh, C. T. Nature's inventory of halogenation catalysts: oxidative strategies predominate. *Chem. Rev.* **106,** 3364–3378 (2006).
10. Salowe, S. P., Marsh, E. N. & Townsend, C. A. Purification and characterization of clavaminate synthase from *Streptomyces clavuligerus*: an unusual oxidative enzyme in natural product biosynthesis. *Biochemistry* **29,** 6499–6508 (1990).
11. Zhou, J. *et al.* Spectroscopic studies of substrate interactions with clavaminate synthase 2, a multifunctional α-KG-dependent non-heme iron enzyme: correlation with mechanisms and reactivities. *J. Am. Chem. Soc.* **123,** 7388–7398 (2001).
12. Kumar, D., de Visser, S. P. & Shaik, S. Oxygen economy of cytochrome p450: what is the origin of the mixed functionality as a dehydrogenase–oxidase enzyme compared with its normal function? *J. Am. Chem. Soc.* **126,** 5072–5073 (2004) and references therein.
13. Mukherjee, A., Martinho, M., Bominaar, E. L., Münck, E. & Que, L. Jr. Shape-selective interception by hydrocarbons of the O_2-derived oxidant of a biomimetic nonheme iron complex. *Angew. Chem. Int. Ed.* **48,** 1780–1783 (2009).
14. Wang, D., Farquhar, E. R., Stubna, A., Münck, E. & Que, L. Jr. A diiron(IV) complex that cleaves strong C–H and O–H bonds. *Nature Chem.* **1,** 145–150 (2009).
15. Kim, C., Dong, Y. & Que, L., Jr. Modeling nonheme diiron enzymes: hydrocarbon hydroxylation and desaturation by a high-valent Fe_2O_2 diamond core. *J. Am. Chem. Soc.* **119,** 3635–3636 (1997).
16. Hull, J. F. *et al.* Manganese catalysts for C–H activation: an experimental/theoretical study identifies the stereoelectronic factor that controls the switch between hydroxylation and desaturation pathways. *J. Am. Chem. Soc.* **132,** 7605–7616 (2010).
17. Chen, M. S. & White, M. C. A predictably selective aliphatic C–H oxidation reaction for complex molecule synthesis. *Science* **318,** 783–787 (2007).
18. Vermeulen, N. A., Chen, M. S. & White, M. C. The Fe(PDP)-catalyzed aliphatic C–H oxidation: a slow addition protocol. *Tetrahedron* **65,** 3078–3084 (2009).
19. Chen, M. S. & White, M. C. Combined effects on selectivity in Fe-catalyzed methylene oxidation. *Science* **327,** 566–571 (2010).
20. White, M. C., Doyle, A. G. & Jacobsen, E. N. A synthetically useful, self-assembling MMO mimic system for catalytic alkene epoxidation with aqueous H_2O_2. *J. Am. Chem. Soc.* **123,** 7194–7195 (2001).
21. Fujita, M. & Que, L. Jr. *In situ* formation of peracetic acid in iron-catalyzed epoxidations by hydrogen peroxide in the presence of acetic acid. *Adv. Synth. Catal.* **346,** 190–194 (2004).
22. Mas-Balleste, R. & Que, L. Jr. Iron-catalyzed olefin epoxidation in the presence of acetic acid: insights into the nature of the metal-based oxidant. *J. Am Chem. Soc.* **129,** 15964–15972 (2007).
23. Curci, R., D'Accolti, L. & Fusco, C. A novel approach to the efficient oxygenation of hydrocarbons under mild conditions. Superior oxo transfer selectivity using dioxiranes. *Acc. Chem. Res.* **39,** 1–9 (2006).
24. Newcomb, M. & Toy, P. H. Hypersensitive radical probes and the mechanisms of cytochrome P450-catalyzed hydroxylation reactions. *Acc. Chem. Res.* **33,** 449–455 (2000) and references therein.

25. Chen, S.-H., Huang, S., Gao, Q., Golik, J. & Farina, V. The chemistry of taxanes: skeletal rearrangements of baccatin derivatives via radical intermediates. *J. Org. Chem.* **59,** 1475–1484 (1994).
26. Horiguchi, T., Cheng, Q. & Oritani, T. Highly regio- and stereospecific hydroxylation of C-1 position of 2-deacetoxytaxinine J derivative with DMDO. *Tetrahedron Lett.* **41,** 3907–3910 (2000).
27. Baloglu, E. & Kingston, D. G. I. The taxane diterpenoids. *J. Nat. Prod.* **62,** 1448–1472 (1999).
28. Zhang, S. *et al.* Structure and stereochemistry of taxuchin A, a new 11(15→1) abeotaxane type diterpene from taxus chinensis. *Chem. Commun.* 1561–1562 (1994).
29. Hu, S., Sun, D.-A., Tian, X. & Fang, Q. Selective microbial hydroxylation and biological rearrangment of taxoids. *Tetrahedron Lett.* **38,** 2721–2724 (1997).
30. Chen, K. & Que, L. Jr. Stereospecific alkane hydroxylation by non-heme iron catalysts: mechanistic evidence for an Fe^{V}=O active species. *J. Am. Chem. Soc.* **123,** 6327–6337 (2001).
31. Bartlett, P. D., Pincock, R. E., Rolston, J. H., Schindel, W. G. & Singer, L. A. The stereochemistry of 9-decalyl free radicals. *J. Am. Chem. Soc.* **87,** 2590–2596 (1965).
32. Choi, S.-Y. & Newcomb, M. Picosecond radical kinetics. Alkoxycarbonyl accelerated cyclopropylcarbinyl radical ring openings. *Tetrahedron* **51,** 657–664 (1995).
33. Ticku, M. K. & Olsen, R. W. Picrotoxinin binding sites in brain, in *Brain Receptor Methodologies Part B Amino Acids. Peptides. Psychoactive drugs* (eds Marangos, P.J., Campbell, I.C. & Cohen, R.M.) 211–229 (Academic Press, 1984).
34. Bodner, M. J., Phelan, R. M., Freeman, M. F., Li, R. & Townsend, C. A. Non-heme iron oxygenases generate natural structural diversity in carbapenem antibiotics. *J. Am. Chem. Soc.* **132,** 12–13 (2010).

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Author contributions

M.A.B. and M.C.W. conceived and designed the experiments outlined in Figs 1– 3,4b, and M.A.B. performed these experiments. S.A.R. and M.C.W. conceived and designed the experiments outlined in Figs 4a,5 and S.A.R. performed these experiments. M.A.B and M.C.W co-wrote the paper, with assistance from S.A.R.

Additional information

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