ARTICLE

Received 29 Apr 2014 | Accepted 15 Sep 2014 | Published 22 Oct 2014

DOI: 10.1038/ncomms6278

Enzymatic aerobic ring rearrangement of optically active furylcarbinols

Daniel Thiel¹, Diana Doknić¹ & Jan Deska¹

Biogenic furans are currently discussed as highly attractive alternative feedstock in a post-fossil society; thus, also the creation of sustainable furan valorization pathways appears of great importance. Here an artificial Achmatowicz monooxygenase activity for the aerobic ring expansion of furans is achieved by the combination of commercial glucose oxidase as oxygen-activating biocatalyst and wild-type chloroperoxidase as oxygen-transfer mediator, providing a biological ready-to-use solution for this truly synthetic furan rearrangement. In concert with enzymatic transformations for the enantioselective preparation of optically active furylcarbinols, purely biocatalytic reaction cascades for the stereocontrolled construction of complex pyranones are obtained, exhibiting high functional group tolerance even to oxidation-sensitive moieties.

¹Department für Chemie, Universität zu Köln, Greinstrasse 4, 50939 Cologne, Germany. Correspondence and requests for materials should be addressed to J.D. (email: jan.deska@uni-koeln.de).

In the course of the inevitable medium- to long-term reorganization of our value chains from fossil resources towards a general supply on the basis of renewable raw materials, one of the great challenges in the near future will comprise the development of novel processes and the adaptation of existing synthetic strategies to those new requirements. Currently, biogenic furans, obtained through biorefinery of lignocellulose, are considered one of the most promising core structures among the plant-based, non-edible raw material platforms¹. While the energetic use of renewables has to be met with a certain scepticism not only from a chemist's perspective^{2,3}, the role of biomolecules as rich source of green synthetic building blocks is far less controversially discussed. In addition to defunctionalization routes from biogenic furans to structurally simple base chemicals^{4–6}, this heterocyclic moiety offers an interesting repertoire of unique rearrangement reactions, providing pathways for the generation of complex molecular architectures^{7–10}. The oxidative ring expansion of α -heterofunctionalized furans according to Achmatowicz represents a role model in this perspective¹⁰, opening up an elegant access to densely decorated pyran- (Fig. 1a) as well as piperidine derivatives¹¹. First mentioned already in 1971, various ways to substitute elemental bromine by other oxidative agents were investigated with the result that today a multitude of modern procedures for the selective furan cleavage are available^{12–16}. However, with the rapidly increasing number of applications of Achmatowicz's oxidation in complex synthetic scenarios, it remains to be noted that classical oxidants such as 3-chloroperbenzoic acid and *N*-bromosuccinimide still represent the means of choice^{17–19}.

In the light of a sustainable utilization of biogenic building blocks, the development of environmentally benign valorization pathways has to be regarded a paramount task, and in our opinion, biological catalysts have to be considered most suitable to fulfil these requirements. However, biocatalysis is hampered by one simple factor, that is, the lack of biosynthetic precedence for a variety of synthetically relevant transformations and the consequent lack of natural catalysts to promote the desired reactions. Thus, the identification of promiscuous activities by wild-type enzymes as well as the evolution of new reactivities with the aid of biotechnological and chemical tools are highly warranted^{20–22}.

In this report, we disclose an unprecedented, purely enzymatic protocol for the non-natural Achmatowicz-type rearrangement of furylcarbinols employing an oxygen activation cascade based on commercially available biocatalysts (Fig. 1b). Our experimental design rests on the initial finding of selective oxygen-transfer abilities of peroxidases in the oxidative ring expansion of substituted furfuryl alcohols. The linkage with a hydrogen peroxide-evolving system based on the bioreduction of aerial oxygen by cheap and ecologically benign biomolecules such as glucose renders a fully integrated, highly practicable and sustainable protocol allowing for the rearrangement not only of simple carbinols but likewise for a series of real-world substrates carrying synthetically versatile functional groups.

Results

Catalyst identification. Owing to the absence of Achmatowicz-like transformations in biosynthesis, studies on the furan metabolism in mammals served as foundation for our development of a synthetic enzyme-based protocol. Toxicokinetic studies on pulegone and menthofuran detoxification pathways by Nelson and co-workers²³ did not only identify human liver cytochrome P450 monooxygenases as catalysts involved in furan degradation, but also provided evidence for the formation of furanoepoxides, which is also expected in the chemical Achmatowicz oxidation by electrophilic oxygenation agents. Thus, as robust and relatively cheap functional mimics of cytochrome P450s²⁴, four haem-based peroxidases were evaluated on their potential to catalyse the selective oxidation of furylcarbinol *rac*-1a to give the ring-expanded product *rac*-2a. With gluconate as sole carbon-based side product, commercial glucose oxidase (*Aspergillus niger*, GOx) was chosen as a release system for hydrogen peroxide through the catalytic reduction of aerial oxygen, offering the benefits of simple product separation and an excellent ecological profile^{25,26}. In the absence of potential oxygen-transfer catalysts as well as in the presence of soybean peroxidase (Table 1, entry 1), no conversion was observed and *rac*-1a was recovered quantitatively after 2 days of incubation. In contrast, using bovine lactoperoxidase or horseradish peroxidase (Table 1, entries 2 and 3), slow consumption of the furylcarbinol was detected and in the latter case, traces of the Achmatowicz product *rac*-2a were isolated after 48 h. An entirely different picture was drawn implementing chloroperoxidase from *Caldariomyces fumago* (CPO) into the system²⁷. Sometimes referred to as peroxidase–cytochrome P450 functional hybrid, CPO shares structural properties with P450 enzymes such as a thiolate as axial haem ligand explaining a much more diverse reaction portfolio compared with other peroxidases including effective cytochrome-like oxygen-transfer processes²⁸. Within 2 h, full conversion of *rac*-1a was achieved and the desired pyranone *rac*-2a could be isolated in 51% yield (Table 1, entry 4). As pH variation did not result in any improvement of the catalytic system (Supplementary Fig. 1), we turned our attention to potentially beneficial effects of cosolvents (Supplementary Fig. 2). Here, polar additives (10% v/v) like dimethylsulfoxide, acetonitrile (Table 1, entries 5 and 6) or

Figure 1 | Oxidative ring expansion of furylcarbinols: historical precedent and modern interpretation. (a) Formation of 2,5-dimethoxy-2,5-dihydrofuran on oxidation with bromine in methanol is followed by acetal cleavage and ring expansion via an intermediate 4-ketoenal. (b) Schematic representation of the coupled biocatalytic Achmatowicz rearrangement combining aerobic reduction of oxygen by glucose to hydrogen peroxide with the peroxidase-mediated oxygen transfer onto the furan core.

acetone reduced the rates of oxidation. On the other hand, in two-phase systems based on hydrophobic solvents (toluene, heptane, ethyl acetate) the oxidative ring expansion did not take place at all. In strong contrast, in the presence of *tert*-butanol as cosolvent, not only the enzymes' activity was well conserved but more importantly, product *rac*-**2a** could be isolated in substantially higher yield of 82% (Table 1, entry 7). While the

addition of *t*BuOH had only marginal effect on the rate of furan consumption, the boost in yield can rather be attributed to considerably enhanced product stability with a half-life of pyranone **2a** of 40.7 h (± 3.7 h) as compared with 11.9 h (± 0.2 h) in the purely aqueous medium (Supplementary Fig. 3). In addition, we could prove that also other hydrogen peroxide-evolving systems such as L-leucine/L-amino-acid oxidase (*Crotalus atrox*) support the CPO-catalysed rearrangement; however, due to the significantly higher cost of the enzymes and lower reaction rates, these alternatives were not further pursued (Supplementary Fig. 4). Moreover, a single-enzyme approach was studied where hydrogen peroxide was slowly added by syringe pump to a buffered solution of *rac*-**1a** and CPO. In this case, full conversion was achieved after 2 h and *rac*-**2a** could be isolated in 67% yield (Table 1, entry 8).

Table 1 | Oxidase/peroxidase-mediated oxidation of *rac*-1-(2-furyl)ethanol.

Entry	Peroxidase	Additive*	Time (h)	Conversion (%) [†]
1	Soybean peroxidase	—	48	0
2	Lactoperoxidase (<i>B. taurus</i>)	—	48	12 (—) [‡]
3	Horseradish peroxidase	—	48	16 (<5)
4	Chloroperoxidase (<i>C. fumago</i>)	—	2	> 99 (51)
5	Chloroperoxidase (<i>C. fumago</i>)	DMSO	2	44
6	Chloroperoxidase (<i>C. fumago</i>)	MeCN	2	58
7	Chloroperoxidase (<i>C. fumago</i>)	<i>t</i> -BuOH	2	> 99 (82)
8 [§]	Chloroperoxidase (<i>C. fumago</i>)	<i>t</i> -BuOH	2	> 99 (67)

DMSO, dimethylsulphoxide.
 Reaction conditions: *rac*-**1a** (10 mM), D-glucose (D-Glc; 50 mM), glucose oxidase (3.6 μ M, 30 U), peroxidase (30 U), citrate buffer (3.0 ml, 100 mM, pH 5.5), 30 °C, 500 r.p.m.; conversions determined by gas chromatography using bromobenzene as standard.
[†]10 vol%.
[‡]Isolated yield (**2a**) in parentheses, 0.5 mmol scale.
[§]**2a** was not detected.
[§]Slow addition of aq. H₂O₂ instead of D-Glc/GOx.

Mechanistic considerations. With the identification of the GOx/chloroperoxidase couple as efficient system for the aerobic Achmatowicz oxidation, questions about the underlying functional principle arose. As the native functions of haloperoxidases include the oxidation of halides to hypohalites^{29,30}, the initial goal was to shed light on whether a halide/hypohalite mediator system qualified as final oxidizing species or rearrangement was induced through direct substrate–enzyme interaction and oxygen transfer from the prosthetic oxidized haem unit (compound I). First studies revealed that incubation of *rac*-**1a** in a buffered NaOCl solution in the absence of potential catalysts led indeed to oxidative ring expansion to give traces of pyranone *rac*-**2a**; however, reaction rates substantially lagged behind the GOx/CPO-catalysed transformation. Furthermore, considerable inhibition of the enzymatic rearrangement was observed in chloride-containing reaction media (half-maximal inhibitory concentration = 7.4 mM; Supplementary Fig. 5). Another obvious evidence for a direct interaction between substrate and chloroperoxidase resulted from the time-dependent stereochemical analysis of the GOx/CPO-mediated reaction of *rac*-**1a**.

Figure 2 | Indications for the direct peroxidase–furan interaction. (a) Conversion of racemic furylethanol by chloroperoxidase is accompanied by enrichment of the (*S*)-alcohol through kinetic resolution ($k_{\text{rel}} = 4.5$). (b) Incorporation of ¹⁸O by incubation under ¹⁸O₂-enriched argon atmosphere. (c) Coincubation of furylhydroperoxide and furylcarbinol in the absence of external oxidizing agents leads to selective formation of the hydroperoxide-derived product. The reaction was stopped at low conversion to deliberately overestimate **1a/1a**-d₃-scrambling as expected for a potential alcohol diffusion pathway.

At low conversion, a distinct kinetic resolution with an enantioselectivity of $E = 4.5$ was observed (Fig. 2a), implying at least the participation of a protein-bound oxidizing species. The strongest indication for the non-mediated furan oxidation through compound **1**, or alternatively through a hydroperoxoferric intermediate³¹, arose from experiments using isotopically labelled oxygen as terminal oxidant³². Ring expansion by the GOx/CPO system in an argon atmosphere enriched in heavy oxygen (20 vol% $^{18}\text{O}_2$) gave rise to the mono- ^{18}O -marked product *rac*-**2a**- ^{18}O in high isotopic yield (Fig. 2b). Moreover, mass spectrometric fragment analysis disclosed a low degree of regioselectivity in the oxygen transfer onto the furan core with a slight preference for the less-substituted position (Supplementary Fig. 6)³³. Determination of the kinetic parameters for the aerobic oxidation of *rac*-**1a** revealed an apparent turnover frequency of the peroxidase of $k_{\text{cat}} = 370.7 \text{ min}^{-1}$ ($\pm 9.3 \text{ min}^{-1}$) and a good affinity for this non-natural substrate of $K_M = 141 \mu\text{M}$ ($\pm 27 \mu\text{M}$) with a total turnover number of $\sim 121,400$ at 50 mM substrate concentration (Supplementary Fig. 7). In addition, the hypothesis of oxygen transfer from the oxidized prosthetic group was strongly supported by fundamental experiments on a redox-neutral enzymatic rearrangement. Oxygen-transfer-based redox-isomerization of hydroperoxide *rac*-**3a** by chloroperoxidase from *C. fumago* as sole catalyst did not only proceed smoothly, incubation with equimolar amounts of the deuterium-labelled alcohol *rac*-**1a**- d_3 yielded the Achmatowicz product **2a** alongside with only minor amounts of the CD_3 -marked pyranone **2a**- d_3 . While indicating that initial reduction of the hydroperoxide and reoxidation of the so-formed furylcarbinol take place in a coupled manner without diffusion as dominant pathway (Fig. 2c; Supplementary Fig. 8), this experiment also clearly refutes

working hypotheses about non-haem or ferric oxidizing species. Moreover, also the use of *t*-butyl hydroperoxide as external oxidizing agent proved to be effective ($k_{\text{cat}} = 98.8 \text{ min}^{-1}$ ($\pm 28.1 \text{ min}^{-1}$)) and led to the formation of **2a** from alcohol **1a** in the presence of chloroperoxidase, ruling out alternative reaction pathways such as formation and consequent reduction of endoperoxide intermediates. On the basis of these results, we propose that the biocatalytic Achmatowicz-type rearrangement by chloroperoxidase proceeds via oxygenation^{23,34} of the furan core through oxygen transfer from an oxoferryl porphyrin species and subsequent ring fission and recyclization to give the pyranone products (Supplementary Fig. 9).

Towards coupled multi-enzymatic processes. Even if a certain extent of stereoselectivity was observed in the oxidative transformation of *rac*-**1a**, the low degree of enantiodiscrimination would not allow for a preparatively reasonable kinetic resolution of this substrate. As kinetic resolutions exhibit the general flaw of yield limitation, our aim was to synthesize optically active furylcarbinols via preceding biotransformations to subsequently rearrange them on the basis of the artificial Achmatowicz monooxygenase system. The asymmetric reduction of the corresponding furylketones (**3a–k**) through alcohol dehydrogenases (ADHs) proved to be an efficient and convenient method^{35,36}. Two commercial ADHs in a substrate-coupled system with isopropanol as terminal reducing agent enabled the preparation of alcohol **1a** whereby, depending on the employed ADH, both (*R*)-**1a** and (*S*)-**1a** were obtained in nearly enantiopure form. The succeeding GOx/CPO-mediated aerobic rearrangement to the pyranones (*R*)-**2a** and (*S*)-**2a**, respectively, proceeded in good yield, with quantitative preservation of optical activity and

Table 2 | Biocatalytic Achmatowicz rearrangement of optically active furanols.

MOM, methoxymethyl.

Reaction conditions: D-glucose (Glc; 50 mM), furylcarbinol **1** (10 mM), GOx (3.6 μM , 0.5 kU), CPO (0.41 μM , 0.5 kU), citrate buffer (45 ml, 0.1 M, pH 5.5), *t*BuOH (5 ml), room temperature (RT), 500 r.p.m.; isolated yields after column chromatography.

*Prelog-selective ADH was used for the synthesis of (*S*)-**1a**.

†Alcohols **1c**, **1b** and **1g** were prepared in optically active form by lipase-catalysed kinetic resolution.

‡Absolute configuration established independently according to Prelog's rule (anti-Prelog-selective ADH) and Kazlauskas' rule in the acylative lipase-catalysed kinetic resolution⁴⁰. See Supplementary Methods for full experimental details.

Figure 3 | Combination of two glucose-dependent biotransformations: four enzymes—two steps—one pot. The reaction was performed in a sequential manner due to incompatible pH optima of the individual catalytic systems. See Supplementary Methods for full experimental details.

without perceptible matched/mismatched effects (Table 2). Employing the anti-Prelog-selective ADH, various ketones were reduced in high enantioselectivity and subsequently subjected to the aerobic biocatalytic rearrangement. Oxidative ring expansion of the enantiomerically enriched ethyl- and propyl-substituted alcohols **1b** and **1c** and even of the sterically encumbered isopropylcarbinol **1d** succeeded in good yield. Moreover, the CPO-catalysed transformation of the alcohols **1c** (95% ee) and **1d** (97% ee) was accompanied by further increase in optical purity of the pyranone products ($\geq 98\%$ ee). The extent of enantio-discrimination of CPO towards substrate **1d** was determined in an oxidative kinetic resolution with a relative rate of $k_{\text{rel}} = 9.6$. Hence, with regard to a broader substrate tolerance in both enantiomeric series, evolution of less-stereoselective chloroperoxidases appears desirable in the longer run. Apart from that, a high functional group tolerance of CPO could be illustrated in the rearrangement of the alcohols **1e–h**. Not only substrates such as chlorohydrin **1e** or β -hydroxyester **1f** smoothly rearranged in the presence of the GOx/CPO system to give the pyranones **2e** (64%) and **2f** (68%), respectively, more gratifyingly, even oxidation-sensitive elements like olefins (**2g**, 65%) and primary alcohols (**2h**, 31%) were well tolerated by the peroxidase. Solely, oxidation of the propargylic alcohol **1i** turned out problematic and the corresponding rearrangement product could not be isolated. With the use of core-substituted furylcarbinols, a structural limitation became apparent. While biocatalytic reduction of the 5-substituted furylketones gave rise to the enantiopure alcohols **1j** and **1k**, their oxidative ring expansion failed even with prolonged reaction time and substantially increased enzyme loadings.

Since both the asymmetric ketone reduction for the construction of enantioenriched furylcarbinols as well as their oxidative rearrangement could potentially be attained on the basis of glucose-dependent biotransformations, it seemed logical to strive after a direct linkage of both reactions to establish a multi-enzyme cascade for the synthesis of optically active Achmatowicz products. Essentially, pyranone (*R*)-**2a** could be detected in traces in a genuine four-enzyme/one-pot reaction; however, much more satisfying results were obtained by sequential addition of the individual catalysts and concurrent pH adjustment. Thus, after reaching nearly full conversion of the ketone **4a** by a glucose dehydrogenase (GDH)/ADH system, the reaction mixture was slightly acidified. With the addition of GOx and chloroperoxidase, fast and selective formation of the pyranone was observed (Supplementary Fig. 10) and (*R*)-**2a** was isolated in 47% yield and >99% ee (Fig. 3). In a triple catalytic system, where the GDH-mediated cofactor recycling was replaced by the previously employed dehydrogenation of isopropanol, the same one-pot principle provided (*R*)-**2a** in enantiopure form and 59% yield.

Diffusible redox mediators to overcome structural limitations. Although excellent selectivities, sustainable reaction conditions

Figure 4 | Aerobic Achmatowicz oxidation of 2,5-disubstituted furans.

On the basis of the enzyme cascade consisting of an oxidase and a lipase, peracetic acid is formed catalytically from the acetate buffer that can act as diffusible redox mediator in the oxidation of otherwise non-reactive furylcarbinols.

and high functional group tolerance of the presented Achmatowicz system leave no doubt that this novel application represents a valuable extension of the existing biocatalytic portfolio, our delight was marginally tempered by its limitation to mono-substituted furans. Directed evolution of the biocatalyst with regard to an extended substrate spectrum seems to be a logical next step to address this issue³⁷. From the chemist's perspective, however, also the reconstruction of the catalytic system's architecture by allowing for non-protein-bound diffusible oxygen-transfer mediators appeared as an attractive alternative to eliminate undesired substrate effects. In search of suitable redox mediators combining activity in the furan oxidation with the disability to oxidize secondary alcohols, our choice fell on peracetic acid. Perhydrolysis of carboxylic acid derivatives through lipases and utilization of the so-formed peracids in oxidation reactions represents a well-studied approach^{38,39}. In analogy to the oxidase/peroxidase couple, we aimed for the concatenation of the biocatalytic reduction of aerial oxygen with the activation of hydrogen peroxide by enzymatic peracetate formation. The combination of GOx with a lipase from *Candida antarctica* (type B, non-immobilized preparation) in acetate buffer at pH 4.5 proved to be a viable catalytic system for the selective aerobic oxidation of *rac*-**1j**, as the 2,6-dimethylated pyranone **2j** was produced in moderate yield (Fig. 4). Even if substantially prolonged reaction times and the unfavourable optimum pH with regard to configurational lability of the furylcarbinols (see Supplementary Fig. 11, for racemization under acidic conditions) unquestionably demand vernier adjustments of this biocatalytic Achmatowicz variant, the application of diffusible, yet selective mediators might mature to an auspicious and practical alternative to the direct oxidation by haem-based enzymes.

Discussion

Over the years, biocatalysis has made its way into synthetic organic chemistry; however, in most cases, the role of enzymatic transformations is limited to the supply of non-racemic small-molecule building blocks with only marginal-to-non-existing contribution to the creation of molecular complexity. Herein, we demonstrated the use of biocatalysts outside their natural setting, tackling a non-natural rearrangement reaction converting structurally simple and readily available furylcarbinols into high-value synthetic building blocks in a preparatively convenient and eco-friendly manner. Aiming for the valorization of biogenic furans, future investigations will expand towards biological cascades for the direct conversion of lignocellulose-derived furfurals by means of lyases as well as the construction of combined organo-/biocatalytic reaction sequences. With the recent advances in biotechnological process development, there is a true potential to design fermentation pathways implementing further optimized peroxidases or monooxygenases for selective

Achmatowicz-type rearrangements that would allow for integrated furan refinement up the ladder of molecular complexity also in a more technical context.

Methods

General methods. Commercially available reagents were used without further purification. $^{18}\text{O}_2$ (98 atom%) was purchased from Carbolution Chemicals GmbH. Catalysts and cofactors were obtained from: NADH, Carbolution Chemicals GmbH; chloroperoxidase from *C. fumago* (CPO), 21.9 kU ml $^{-1}$, Bio-Research Products; GOx type II (GOx), 17.3 U mg $^{-1}$, Sigma; ADH, recombinant from *Escherichia coli* (ADH 200), 17.6 U mg $^{-1}$, evocat GmbH; ADH, recombinant from *E. coli* (ADH 030), 18.8 U mg $^{-1}$, evocat GmbH; and glucose dehydrogenase, recombinant from *E. coli* (GDH 060), 214 U mg $^{-1}$, evocat GmbH. Presented data are average numbers of at least two replicate experiments. All products were purified by column chromatography over silica gel (Macherey-Nagel MN-Kieselgel 60 (40–60 μm , 240–400 mesh)). Analytical thin-layer chromatography was performed on Macherey-Nagel precoated silica gel plates (ALUGRAMM Sil G/UV254). ^1H - and ^{13}C -NMR spectra were recorded on a Bruker Avance 300 spectrometer at room temperature at 300 and 75 MHz, respectively. Chemical shifts are reported in parts per million (p.p.m.) relative to tetramethylsilane. Infrared spectra were recorded on a Shimadzu IRAffinity-1 Fourier transform infrared (FTIR) spectrometer, absorption bands were reported in wave numbers (cm $^{-1}$). Gas chromatography was performed on a Hewlett Packard HP 6890 Series GC System using a Macherey-Nagel FS-Lipodex A and Macherey-Nagel FS-Lipodex E column (25 m $\times$ 0.25 mm), N_2 , 1.0 ml min $^{-1}$; method A: 50 $^\circ\text{C}$ (1 min)/5 $^\circ\text{C}$ min $^{-1}$ (35 min)/120 $^\circ\text{C}$ (15 min); method B: 40 $^\circ\text{C}$ (5 min)/2 $^\circ\text{C}$ min $^{-1}$ (10 min)/5 $^\circ\text{C}$ min $^{-1}$ (50 min). High-performance liquid chromatography was performed on a Merck D-7000 with a Merck L-4500 PDA detector using an analytical Daicel Chiralpak column (250 $\times$ 4.6 mm). Optical rotations were measured on a Perkin-Elmer 343plus. For full experimental details, see Supplementary Methods.

Biocatalytic Achmatowicz rearrangement. In a 100-ml round bottom flask, furylcarbinol (**R**)-**1f** (99% ee, 10 mM, 0.5 mmol) was dissolved in a mixture of citrate buffer (45 ml, 100 mM, pH 5.5) and *t*-butanol (5 ml). After addition of chloroperoxidase (0.41 μM , 0.5 kU), GOx (3.6 μM , 0.5 kU) and D-glucose (50 mM), the solution was incubated (room temperature, 500 r.p.m.) for 120–180 min. L-Methionine (250 mg) was added and the reaction mixture was extracted with ethyl acetate (3 $\times$ 15 ml). The combined organic layers were dried over MgSO_4 , evaporated under reduced pressure and (2R)-6-hydroxy-2-(ethoxycarbonylmethyl)-2H-pyran-3(6H)-one (**2f**) was obtained after column chromatographic purification (SiO_2 , cyclohexane/ethyl acetate, 4/1) as colourless oil (68.1 mg, 0.34 mmol, 68%, $\alpha/\beta = 72/28$). [α_{D}^{20}]: +7.4 (c 1.00, CHCl_3), 99% ee. R_f (cyclohexane/ethyl acetate, 2/1): 0.19. α -**2f**: ^1H -NMR (300 MHz, CDCl_3): δ (p.p.m.) = 6.93 (dd, $^3J = 10.3$ Hz, $^3J = 3.5$ Hz, 1H), 6.17 (d, $^3J = 10.3$ Hz, 1H), 5.75–5.53 (m, 1H), 5.03 (dd, $^3J = 7.2$ Hz, $^3J = 4.0$ Hz, 1H), 3.00–2.98 (m, 1H), 2.85–2.68 (m, 1H), 1.30–1.25 (m, 3H); ^{13}C -NMR (75 MHz, CDCl_3): δ (p.p.m.) = 195.0, 171.0, 144.6, 127.1, 87.4, 70.7, 61.0, 35.3, 14.1. β -**2f** (selected signals): ^1H -NMR (300 MHz, CDCl_3): δ (p.p.m.) = 6.98 (dd, $^3J = 10.3$ Hz, $^3J = 1.5$ Hz, 1H), 6.13 (dd, $^3J = 10.3$ Hz, $^3J = 1.5$ Hz, 1H), 4.61–4.57 (m, 1H), 4.21–4.13 (m, 3H), 3.06–3.03 (m, 1H); ^{13}C -NMR (75 MHz, CDCl_3): δ (p.p.m.) = 194.4, 171.0, 148.4, 128.4, 90.9, 75.3, 67.4, 36.2, 14.1. FTIR (neat, ATR): ν (cm $^{-1}$) = 3420 (br), 2988 (w), 1732 (m), 1693 (m), 1375 (w), 1286 (w), 1178 (m), 1089 (m), 1020 (s), 931 (w), 846 (w), 759 (w).

Coupled one-pot reduction rearrangement. ADH (5.7 mg, 0.1 kU, ADH 200), glucose dehydrogenase (1.4 mg, 0.3 kU, GDH 060) and D-glucose (100 mM) were added to a solution of acetyl furan (**4a**, 18.5 mM, 110 mg) and NADH (1.8 mM) in phosphate buffer (54 ml, 50 mM, pH 7.0) and the mixture was incubated for 24 h (25 $^\circ\text{C}$, 500 r.p.m.). Citric acid was added to adjust the pH to 5.5. After further addition of *t*-butanol (5 ml), chloroperoxidase (0.35 μM , 0.5 kU), GOx (3.0 mM, 0.5 kU) and D-glucose (40 mM), the solution was incubated for 180 min (25 $^\circ\text{C}$, 500 r.p.m.). L-Methionine (250 mg) was added and the reaction mixture was extracted with ethyl acetate (3 $\times$ 15 ml). The combined organic layers were dried over MgSO_4 , evaporated under reduced pressure and the (2R)-6-hydroxy-2-methyl-2H-pyran-3(6H)-one (**2a**) was obtained after column chromatographic purification (SiO_2 , cyclohexane/ethyl acetate, 5/1) as colourless solid (60.1 mg, 0.47 mmol, 47%, $\alpha/\beta = 67/33$). [α_{D}^{20}]: –79.6 (c 0.50, CHCl_3), 99% ee. Melting point: 61 $^\circ\text{C}$. R_f (cyclohexane/ethyl acetate, 2/1): 0.25. α -**2a**: ^1H -NMR (300 MHz, CDCl_3): δ (p.p.m.) = 6.89 (dd, $^3J = 10.2$ Hz, $^3J = 3.2$ Hz, 1H), 6.10 (d, $^3J = 10.2$ Hz, 1H), 5.63 (m, 1H), 4.71 (q, $^3J = 6.7$ Hz, 1H), 3.53 (d, $^3J = 5.0$ Hz, 1H), 1.38 (d, $^3J = 6.7$ Hz, 3H); ^{13}C -NMR (75 MHz, CDCl_3): δ (p.p.m.) = 197.0, 144.5, 127.3, 87.7, 70.4, 15.3. β -**2a**: ^1H -NMR (300 MHz, CDCl_3): δ (p.p.m.) = 6.94 (d, $^3J = 10.2$ Hz, 1H), 6.15 (d, $^3J = 10.2$ Hz, 1H), 5.67 (d, $^3J = 7.2$ Hz, 1H), 4.23 (1H, m), 3.87 (d, $^3J = 7.2$ Hz, 1H), 1.45 (d, $^3J = 6.7$ Hz, 3H); ^{13}C -NMR (75 MHz, CDCl_3): δ (p.p.m.) = 196.5, 148.1, 128.6, 91.0, 75.3, 16.2. FT-IR (neat, ATR): ν (cm $^{-1}$) = 3294 (br), 3051 (w), 2987 (w), 1676 (s), 1435 (m), 1371 (m), 1334 (w), 1273 (w), 1232 (m), 1143 (m), 1109 (m), 1091 (m), 1031 (s), 937 (m), 900 (m), 808 (m), 690 (m).

References

- Ragauskas, A. J. *et al.* The path forward for biofuels and biomaterials. *Science* **311**, 484–489 (2006).
- Michel, H. The nonsense of biofuels. *Angew. Chem. Int. Ed.* **51**, 2516–2518 (2012).
- Fairley, P. Introduction: next generation biofuels. *Nature* **474**, S2–S4 (2011).
- Corma, A., Iborra, S. & Velty, A. Chemical routes for the transformation of biomass into chemicals. *Chem. Rev.* **107**, 2411–2502 (2007).
- Besson, M., Gallezot, P. & Pinel, C. Conversion of biomass into chemicals over metal catalysts. *Chem. Rev.* **114**, 1827–1870 (2014).
- Straathof, A. J. J. Transformation of biomass into commodity chemicals using enzymes and cells. *Chem. Rev.* **114**, 1871–1908 (2014).
- Hunsdiecker, H. Über das Verhalten der γ -Diketone. III. Mitteil.: Die Synthese des Jasmons. *Ber. dtsh. Chem. Ges.* **75**, 460–468 (1942).
- Piancatelli, G., Scettri, A. & Barbadoro, S. A useful preparation of 4-substituted 5-hydroxy-3-oxocyclopentene. *Tetrahedron Lett.* **39**, 3555–3558 (1976).
- Charbonnet, N., Riguet, E. & Bochet, C. G. Singlet-oxygen-induced rearrangement of furan derivatives. *Syn. Lett.* **15**, 2231–2233 (2011).
- Achmatowicz, Jr O., Bukowski, P., Szechner, B., Zwierchowska, Z. & Zamojski, A. Synthesis of methyl 2,3-dideoxy-DL-alk-2-enopyranosides from furan compounds: a general approach to the total synthesis of monosaccharides. *Tetrahedron* **27**, 1973–1996 (1971).
- Ciufolini, M. A. & Wood, C. Y. The aza-Achmatowicz rearrangement: a route to useful building blocks for N-containing structures. *Tetrahedron Lett.* **27**, 5085–5088 (1986).
- Montagnon, T., Noutsias, D., Alexopoulou, I., Tofi, M. & Vassilikogiannakis, G. Green oxidations of furans initiated by molecular oxygen that give key natural product motifs. *Org. Biomol. Chem.* **9**, 2031–2039 (2011).
- Ghosh, A. K. & Li, J. A stereoselective synthesis of (+)-herboxidiene/GEX1A. *Org. Lett.* **13**, 66–69 (2011).
- Cheng, K., Kelly, A. R., Kohn, R. A., Dweck, J. F. & Walsh, P. J. One-pot catalytic asymmetric synthesis of pyranones. *Org. Lett.* **11**, 2703–2706 (2009).
- Wahlen, J., Moens, B., De Vos, D. E., Alsters, P. L. & Jacobs, P. A. Titanium silicate 1 (TS-1) catalyzed oxidative transformation of furan derivatives with hydrogen peroxide. *Adv. Synth. Catal.* **346**, 333–338 (2004).
- Asta, C. *et al.* First enzymatic Achmatowicz reaction: selective laccase-catalyzed synthesis of 6-hydroxy-(2H)-pyran-3(6H)-ones and (2H)-pyran-2,5(6H)-diones. *RSC Adv.* **3**, 19259–19263 (2013).
- Nicolaou, K. C., Aversa, R. J., Jin, J. & Rivas, F. Synthesis of the ABCDEF ring system of maitotoxin. *J. Am. Chem. Soc.* **132**, 6855–6861 (2010).
- Burke, C. P., Haq, N. & Boger, D. L. Total synthesis, assignment of the relative and absolute stereochemistry, and structural reassignment of phostriecin (aka sultricin). *J. Am. Chem. Soc.* **132**, 2157–2159 (2010).
- Shimokawa, J., Harada, T., Yokoshima, S. & Fukuyama, T. Total synthesis of gelsemoxone. *J. Am. Chem. Soc.* **133**, 17634–17637 (2011).
- Bornscheuer, U. T. & Kazlauskas, R. J. Catalytic promiscuity in biocatalysis: using old enzymes to form new bonds and follow new pathways. *Angew. Chem. Int. Ed.* **43**, 6032–6040 (2004).
- Coelho, P. S., Brustad, E. M., Kannan, A. & Arnold, F. H. Olefin cyclopropanation via carbene transfer catalyzed by engineered cytochrome P450 enzymes. *Science* **339**, 307–310 (2013).
- Köhler, V. *et al.* Synthetic cascades are enabled by combining biocatalysts with artificial metalloenzymes. *Nat. Chem.* **5**, 93–99 (2013).
- Khoshdel-Bakht, S. C., Chen, W., Koenigs, L. L., Peter, R. M. & Nelson, S. D. Metabolism of (R)-(+)-pulegone and (R)-(+)-menthofuran by human liver cytochrome P-450s: evidence for formation of a furan epoxide. *Drug Metab. Dispos.* **27**, 574–580 (1999).
- van Rantwijk, F. & Sheldon, R. A. Selective oxygen transfer catalyzed by heme peroxidases: synthetic and mechanistic aspects. *Curr. Opin. Biotech.* **11**, 554–564 (2000).
- Wilson, R. & Turner, A. P. F. Glucose oxidase: an ideal enzyme. *Biosens. Bioelectron.* **7**, 165–185 (1992).
- Sheldon, R. A. Fundamentals of green chemistry: efficiency in reaction design. *Chem. Soc. Rev.* **41**, 1437–1451 (2012).
- Morris, D. R. & Hager, L. P. Chloroperoxidase: isolation and properties of the crystalline glycoprotein. *J. Biol. Chem.* **241**, 1763–1768 (1968).
- Sundaramoorthy, M., Turner, J. & Poulos, T. L. The crystal structure of chloroperoxidase: a heme peroxidase-cytochrome P450 functional hybrid. *Structure* **3**, 1367–1377 (1995).
- van Pée, K.-H. Microbial biosynthesis of halometabolites. *Arch. Microbiol.* **175**, 250–258 (2010).
- Manoj, K. M. & Hager, L. P. Chloroperoxidase, a Janus enzyme. *Biochemistry* **47**, 2997–3003 (2008).
- Kells, P. M., Ouellet, H., Santos-Aberturas, J., Aparicio, J. F. & Podust, L. M. Structure of cytochrome P450 PimD suggests epoxidation of the polyene mecolide pimaric occurs via a hydroperoxoferric intermediate. *Chem. Biol.* **17**, 841–851 (2010).
- Kuo, H. H. & Mauk, A. G. Indole peroxxygenase activity of indoleamine 2,3-dioxygenase. *Proc. Natl Acad. Sci. USA* **109**, 13966–13971 (2012).

33. Ho, T.-L. & Sapp, S. G. Sharpless epoxidation of 2-furancarbinols. Mass spectra of 6-hydroxy-3(2H)-pyranones. *Synth. Commun.* **13**, 207–211 (1983).
34. Ortiz de Montellano, P. R., Choe, Y. S., DePillis, G. & Catalano, C. E. Structure-mechanism relationships in hemoproteins: oxygenations catalyzed by chloroperoxidase and horseradish peroxidase. *J. Biol. Chem.* **262**, 11641–11646 (1987).
35. de Gonzalo, G., Lavandera, I., Faber, K. & Kroutil, W. Enzymatic reduction of ketones in micro-aqueous media catalyzed by ADH-A from *Rhodococcus ruber*. *Org. Lett.* **9**, 2163–2166 (2007).
36. Fleischer, T. & Pietruszka, J. Alcohol dehydrogenase-catalyzed synthesis of enantiomerically pure δ -lactones as versatile intermediates for natural product synthesis. *Adv. Synth. Catal.* **354**, 2521–2530 (2012).
37. Turner, N. J. Directed evolution drives the next generation of biocatalysts. *Nat. Chem. Biol.* **5**, 567–573 (2009).
38. Picard, M. *et al.* Metal-free bacterial haloperoxidases as unusual hydrolases: activation of H₂O₂ by the formation of peracetic acid. *Angew. Chem. Int. Ed.* **36**, 1196–1199 (1997).
39. Ríos, M. Y., Salazar, E. & Olivo, H. F. Baeyer-Villiger oxidation of substituted cyclohexanones via lipase-mediated perhydrolysis utilizing urea-hydrogen peroxide in ethyl acetate. *Green Chem.* **9**, 459–462 (2009).
40. Jing, Q. & Kazlauskas, R. J. Determination of absolute configurations of secondary alcohols using lipase-catalyzed kinetic resolutions. *Chirality* **20**, 724–735 (2008).

Acknowledgements

This research was financially supported by the Fonds der Chemischen Industrie (Liebig fellowship to J.D.) and the German Research Foundation (DFG: DE1599/4-1). Support by Bio-Research Products Inc. and Carbolution Chemicals GmbH is gratefully acknowledged.

Author contributions

D.T. and D.D. performed the experiments, compound characterization and data analysis and contributed to the experimental design. J.D. designed and supervised the project and wrote the manuscript.

Additional information

Supplementary Information accompanies this paper at <http://www.nature.com/naturecommunications>

Competing financial interests: The authors declare no competing financial interests.

Reprints and permission information is available online at <http://npg.nature.com/reprintsandpermissions/>

How to cite this article: Thiel, D. *et al.* Enzymatic aerobic ring rearrangement of optically active furylcarbinols. *Nat. Commun.* 5:5278 doi: 10.1038/ncomms6278 (2014).