Biocatalysis

NAD(P)H-Independent Asymmetric C–C Bond Reduction Catalyzed by Ene Reductases by Using Artificial Co-substrates as the Hydrogen Donor

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Abstract: To develop a nicotinamide-independent single flavoenzyme system for the asymmetric bioreduction of C=C bonds, four types of hydrogen donor, encompassing more than 50 candidates, were investigated. Six highly potent, cheap, and commercially available co-substrates were identified that (under the optimized conditions) resulted in conversions and enantioselectivities comparable with, or even superior to, those obtained with traditional two-enzyme nicotinamide adenine dinucleotide phosphate (NAD(P)H)-recycling systems.

Introduction

Flavin-dependent ene reductases from the "old yellow enzyme" (OYE) family have become frequently used for catalyzing the asymmetric reduction of activated C=C bonds. In recent years, these enzymes have been widely applied to the asymmetric synthesis of pharmaceutically relevant targets and industrial intermediates. Despite the excellent stereoselectivities often achieved and the possibility to control the stereochemical outcome of the bioreduction, the overall hydrogen transfer of the commonly employed coupled-enzyme system is rather complex (Scheme 1). After reduction of the substrate, the oxidized flavin cofactor is recycled by NAD(P)H. The latter has to be regenerated through a second redox cycle, requiring an additional dehydrogenase (such as formate, glucose, glucose-6-phosphate, alcohol, or phosphate dehydrogenase), and the corresponding natural co-substrate, which serves as the ultimate hydride source.

To find more economically advantageous systems, a variety of alternative flavin mononucleotide (FMN)-regeneration systems, such as direct, light-mediated recycling, have recently been developed, which still have to prove their viability in preparative-scale applications. In contrast to the coupled-enzyme method, the coupled-substrate system is appealingly simple because it requires only a single protein together with a suitable, cheap co-substrate, serving as the hydrogen donor for the direct recycling of the flavin cofactor. In this context, we have recently proposed a nicotinamide-independent system, which was developed from the flavoprotein-catalyzed disproportionation of conjugated enones—historically also termed “dismutase activity” or “aromatase activity” (Scheme 2). The desaturation of the co-substrate is thermodynamically unfavorable because it requires a strong external driving force for the breakage of C–H σ bonds, which are not energetically compensated for by the newly formed C=C π bond. However, during the dehydrogenation of cyclohex-2-enones, the newly formed dienone quickly tautomerizes to form the corresponding phenol, which provides a large energy gain of approximately –30 kcal mol⁻¹. Alternatively, elevated temperatures, artificial flavin cofactors with strongly elevated redox potentials, and synthetic nicotinamide analogues have been employed as the hydride source.

In addition to the typical ene reductase activities, OYEs also show NAD(P)H oxidase activity, in the course of which H₂O₂ is generated through oxidation of reduced FMNH₂ by molecular oxygen. Depending upon the type of substrate, hydrogen peroxide thus formed may cause spontaneous Weitl–Scheffer epoxidation of the activated C=C bond, which can be prevented by working under an inert atmosphere.

Scheme 1. Hydrogen-transfer pathways in the bioreduction of C=C bonds activated by an electron-withdrawing group (EWG): indirect hydrogen transfer from a natural hydrogen donor through nicotinamide catalyzed by a dehydrogenase (coupled-enzyme system); nicotinamide-independent direct hydrogen transfer from an artificial hydrogen donor catalyzed by a single ene reductase (coupled-substrate system).

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Although the nicotinamide-independent, coupled-substrate, hydrogen-transfer system could be successfully demonstrated, it suffered from incomplete conversion (<65%) due to the enzyme inhibition exerted by the co-product phenol, which forms a strong charge-transfer complex with FMN.\textsuperscript{15, 22–27} Although this drawback could be overcome by in situ co-product removal using solid-phase phenol scavengers, the macroscopic polymeric resins caused undesired racemization of chirally sensitive products, such as \(\alpha\)-substituted ketones (e.g., 1a).\textsuperscript{21} To develop a more robust and widely applicable coupled-substrate system, we initiated a search for “artificial” hydrogen donors that would form (quasi)aromatic, but non-inhibiting co-products.

**Results and Discussion**

For our screening of co-substrates, we chose 4-ketoisophorone (1a) as the substrate, which yields, upon bioreduction, chirally sensitive (\(R\))-levodione (1b). The latter is an important intermediate for the synthesis of carotenoids (Scheme 3).\textsuperscript{28} To account for the broad diversity of ene reductases, OYE1 from *Saccharomyces pastorianus*\textsuperscript{29, 30} and *XenA* from *Pseudomonas putida*\textsuperscript{31} were selected as representative candidates due to their distant sequence relationship (27\% identity, 55\% similarity). Both reductases displayed decidedly different activities in preliminary studies.\textsuperscript{21} Because activities have been shown to be strongly dependent on the pH of the reaction mixture, hydrogen donors were tested at pH 7.5 and 9. The hydrogen donors can be classified into four groups: type I: derivatives of cyclohex-2-enone, yielding phenols; type II: 1,2-, 1,3-, and 1,4-cyclohexanediones, furnishing hydroquinones; type III: N-, O-, and S-ketoheterocycles, forming heteroaromatics; and type IV: 1,3- and 1,4-cyclohexadiene derivatives, leading to nonphenolic co-products.

Surprisingly, co-substrates of all types served as hydrogen donors in the test reaction; several even proved to be superior to those previously described (1c, 34c).\textsuperscript{13} Furthermore, numerous trends could be delineated from the co-substrate screening (Scheme 4):

1. For type I donors, the molecular shape appears to be critical. The small co-substrate 1c was a poor hydrogen donor, whereas the larger analogue 13c gave conversions of up to 65\%; surprisingly, closely related structures 14c–17c were not accepted at all, nor were the 4-substituted derivatives 9c and 10c. Even more puzzling, compound 3c is a weak hydrogen donor (up to 14\% conversion by using XenA at pH 7.5), but 2c, lacking a distant para-methyl group, shows no activity. Large bicyclic structures 21c, 22c, and 24c–28c (but not 23c) acted favorably and proved to be active hydrogen donors. The tricyclic analogue 29c was apparently too bulky for this reaction.

2. In addition to steric constraints, electronic activation of the \(\alpha\)-carbon atom seems to play a major role, as demonstrated by both co-substrates bearing an additional electron-withdrawing acetyl group in the \(\alpha\)-position (30c and 31c) being accepted in the reaction. Likewise, compounds 18c and 19c were found to be weak hydrogen donors. Although 20c contains two electron-withdrawing substituents, steric restrictions seem to override the electronic activation. In contrast, enol ethers in the \(\alpha\)-position (4c, 7c, 8c), a \(\beta\)-enamine (5c), or a \(\beta\)-halo derivative (11c) were unsuitable for the reaction, although the \(\alpha\)-enol ether analogue (6c) was shown to be a weak donor. Type II derivatives lack a conjugated C=C bond, but
possess an enolizable carbonyl group, and hence are generally less suitable for this reaction and none of the tested co-substrates showed conversions of more than 20%. Interestingly, only 1,2-cyclohexanediones (32c, 33c) and 1,4-cyclohexanediones (34c, 35c) were accepted by the enzymes, whereas all of the 1,3-cyclohexanediones were inactive, regardless of their substitution pattern or the presence of electron-withdrawing groups (37c–42c).

3) Of the heterocyclic type III co-substrates, none of the six-membered-ring-containing substrates were accepted, including the dihydouracil derivatives (44c, 45c) and the well-known “Hantzsch ester” 43c, which has a structural resemblance to reduced nicotinamide and is widely applied as a hydride donor in organocatalytic C=C reduction reactions.[32, 33] In contrast, the majority of the five-membered heterocycles showed moderate to high activities; in particular, N-Boc-pyrolidinone (46c) and 2-methyltetrahydrofurane (48c) gave 78 and 82% yields of (R)-levodione (1b), respectively. However, low conversion was observed with the thiophenone bearing an additional activating nitrile moiety (49c; 8% by using XenA at pH 7.5). The surprising performance of five-membered ketoheterocycles as hydrogen donors can be attributed to two things: First, type IV hydrogen donors bear an electron-donating nitrogen or oxygen heteroatom in the γ-position, which facilitates the hydride departure from the β-carbon atom. Secondly, enzyme inhibition occurs due to formation of a charge-transfer complex between FMN and a phenolate anion,[34] which was shown in crystal structures of OYE1 in a complex with para-hydroxybenzaldehyde (Protein Data Bank (PDB), entry 1OYB)[35] and of the OYE1 mutant W116A in a complex with 2-methyl-5-(prop-1-en-2-yl)phenol (PDB, entries 4GBU and 4GXM). Clearly, the five-membered hydroxyheteroaromatics formed after hydrogen abstraction from 46c and 48c result in less favorable π interactions with FMN than phenols or hydroquinones.[24, 34, 36–38]

4) Not surprisingly, all co-substrates of type IV, lacking an electron-withdrawing group attached to the alkene moiety (51c, 52c), were inactive. Only compound 50c, bearing an activating group in the exo-position, gave a moderate conversion.

In summary, co-substrates from all four classes were active as hydrogen donors and their reverse (reduction) reaction was observed as a minor side reaction, if a side reaction occurred (<3% conversion). Steric hindrance plays an important role in the reaction with monocyclic cyclohexenones as the co-substrates, while bicyclic hexenones were more favorable in the reaction. Electronic activation through the presence of an additional electron-withdrawing group (such as an acetyl group) on Cα facilitates proton abstraction, whereas electron-donating

Scheme 4. Co-substrates used as hydrogen donors in the NAD(P)H-independent bioreduction of 4-ketoisophorone (1a) to form (R)-levodione (1b) by using OYE1 and XenA enzymes at pH 7.5 and pH 9 (Boc = tert-butoxycarbonyl).
groups at C8 support hydride departure to flavin. In contrast to six-membered heterocycles, five-membered rings were successful co-substrates. The presence of an activating carbonyl group is necessary for the acceptance of a co-substrate.

In the next step, the hydrogen donors that performed best in the co-substrate screening reactions were selected for further optimization studies by using a set of eight ene reductases, which have previously shown the highest acceptance of unnatural co-substrates (other than nicotinamide,[21] Table 1). Generally, all of the selected enzymes were able to accept the six co-substrates (13c, 24c, 25c, 30c, 46c, 48c) and showed up to 88% conversion (NCR with 46c) in the bioreduction of compound 1a. Among the enzymes, XenA exhibited the broadest co-substrate scope, with conversions of 59–78% with all hydrogen donors except 48c. Other favorable enzyme–co-substrate combinations were OYE1 and OYE2 with 25c (57 and 59% conversion at pH 9, respectively) and EBP1 with 48c (68% conversion). Ene reductases from thermophilic microorganisms showed good activities, yielding conversions of up to 88% (Crs with 1a [64 c]) in the bioreduction of hydrogen donor 1a. Other favorable enzyme–co-substrate combinations were OYE1 and OYE2 with 25c (57 and 59% conversion at pH 9, respectively) and EBP1 with 48c (68% conversion).

Table 1. Selection of the best hydrogen donors and ene reductases in the NAD(P)H-independent reduction of 4-ketoisophorone (1a) to form (R)-levodione (1b).[a]

| Co-substrate | pH | OYE1 | OYE2 | YgIM | XenA | NCR | EBP1 | GKOYE | Crs |
|--------------|----|------|------|------|------|-----|------|-------|-----|
| c. [%]       | 7.5| n.d. | n.d. | 53   | 73   | 47  | n.d. | 91    | 61  |
| ee (R)-1 b   | 7.5| n.d. | n.d. | 25   | 40   | 19  | n.d. | 18    | 80  |
| c. [%]       | 3  | 3    | 15   | 65   | 40   | 1   | 47   | 35    |
| ee (R)-1 b   | 9  | n.d. | n.d. | <10  | <10  | <10 | n.d. | 6     |
| ee (S)-1 b   | 9  | n.d. | n.d. | 22   | 85   | 70  | n.d. | 96    |
| c. [%]       | 7.5| n.d. | n.d. | 76   | 73   | 86  | n.d. | 77    |
| ee (R)-1 b   | 9  | 9    | 12   | 14   | 64   | 20  | 3    | 56    |
| c. [%]       | 19 | 19   | <10  | <10  | <10  |     |      |       |
| ee (R)-1 b   | 7.5| 2    | 2    | 2    | 13   | 7   | 7    | 1     |
| c. [%]       | 9  | 41   | 32   | 23   | 69   | 9   | 5    | 2     |
| ee (R)-1 b   | 7.5| 10   | 2    | 4    | 22   | 13  | 16   | 2     |
| c. [%]       | 9  | 57   | 59   | 33   | 59   | 8   | 5    | 1     |
| ee (R)-1 b   | 7.5| 13   | 16   | 30   | 45   | 72  | 4    | 17    |
| c. [%]       | 9  | 75   | 68   | 61   | 60   | 70  | n.d. | 65    |
| ee (R)-1 b   | 9  | 38   | 35   | 78   | 88   | 14  | 4    | 37    |
| c. [%]       | <10| <10  | <10  | <10  | <10  | <10 | <10  | 11    |

[a] Conversions of optimal enzyme–co-substrate combinations are highlighted in bold. Standard conditions: substrate 1a (10 mM), enzyme (100 μg ml⁻¹), co-substrate 13c, 24c, 25c, 30c, 46c, 48c (10 mM), OYE1 (Saccharomyces pastorianus), OYE2 (Saccharomyces cerevisiae), YgIM (Bacillus subtilis), NCR (nicotinamide-dependent cyclohexenone reductase; Zyymononas mobilis), Xenobiotic reductase XenA (Pseudomonas putida), EB1 (estrogen binding protein, Candida albicans), GKOYE (Geobacillus kaustophilus DSM 7283), Crs (chromate reductase, Thermus scotoductus SA-01); c.: conversion; ee := enantiomeric excess; n.d. := not determined; n.c. := no conversion.
Table 2. Nicotinamide-independent asymmetric bioreduction of activated alkenes 1a–6a by using selected artificial hydrogen donors, 24c, 25c, 30c, 46c, 48c (additional data are given in the Supporting Information).

| Substrate | Co-substrate [mM] | Enzyme [μg mL⁻¹] | pH | Conversion [%] | ee [%] |
|-----------|-------------------|-------------------|----|---------------|--------|
| 24c       | 10                | XenA              | 300 | 9             | 94     |
| 24c       | 50                | GS                | 100 | 9             | >99    |
| 25c       | 10                | XenA              | 300 | 9             | >99    |
| 25c       | 50                | GS                | 100 | 9             | <10(R) |
| 30c       | 10                | GKOYE             | 300 | 9             | 94     |
| 30c       | 50                | GKOYE             | 100 | 9             | 77(R)  |
| 46c       | 50                | NCR               | 300 | 7.5           | 93     |
| 46c       | 50                | XenA              | 100 | 9             | 94     |
| 46c       | 50                | NCR               | 100 | 9             | >99    |
| 48c       | 50                | EBP1              | 100 | 9             | >99    |

Further optimized with respect to enzyme loading and co-substrate concentration, which finally allowed conversions to reach completion (>99%) and also improved the enantioselectivities for (R)-1b (Table 2). To demonstrate the practical applicability of the optimized system, several types of substrate—enal 2a, enone 6a, α,β-unsaturated esters 4a and 5a, and the cyclic imide 3a—were tested (Table 2).

(S)-Citronellal (2b) was obtained from citral (2a) by using NCR with 46c as the hydrogen donor with quantitative conversion and excellent stereoselectivity (>99% ee). Likewise, compound 3a was reduced quantitatively by using XenA and CrS at elevated enzyme loading or in the presence of a five-fold excess of 24c as the hydrogen donor. With (E)-β-cyanoacrylic ester 4a, only enzymes NCR and EBP1 were active, and both gave similar conversions and stereoselectivities to the classic NAD(P)H system. Diester 5a and α-methylocyclohex-2-enone (6a) were quantitatively reduced with excellent stereoselectivities with various enzyme–co-substrate combinations. The ee value of 96% for 6b was caused by imperfect stereoselectivity and not due to racemization, as in case of 1b. The absolute configurations of products 1b–6b were determined as previously reported, and those of 13c and 48c were determined through co-injection on a GC with an independently synthesized reference material (see the Experimental Section for details). Aromatic co-products from the biotransformations were identified by co-injection on a GC with commercially available reference compounds 13d, 24d, 25d, and 30d and with independently synthesized reference materials 46d and 48d.

Conclusion

Four types of H-donor—encompassing more than 50 candidates consisting of cyclohex-2-enones, cyclohexanediones, 5- and 6-membered N-, O- and S-ketoheterocycles and dienes—were screened in the coupled-substrate, nicotinamide-independent bioreduction of C=C bonds by using flavin-dependent ene reductases. Six co-substrates were identified that (under optimized conditions) resulted in conversions and enantioselectivities comparable with, or even superior to, those obtained in the presence of an excess of nicotinamide cofactor or in combination with traditional NAD(P)H recycling. These results prove the practical applicability of the NAD(P)H-independent, single-enzyme, hydrogen-transfer system by using cheap (commercially available), artificial hydrogen donors. Although the in situ recycling of hydrogen donors is presently not feasible, the co-substrate costs for this reaction are modest.
Experimental Section

General

TLC plates were run on silica gel Merck 60 (F254). Silica gel 60 from Merck was also used for flash column chromatography. GC-MS analyses were performed on an HP 6890 Series GC system equipped with a 5973 mass selective detector and a 7683 Series injector using a (5 % phenyl)methylpolysiloxane capillary column (HP-5MS, 30 m x 0.25 mm, 0.25 μm film). GC-FID analyses were carried out on a Varian 3800 and on an Agilent 7890A by using H2 as the carrier gas (14.5 ps) NMR measurements were performed on a Bruker Avance III 300 MHz NMR spectrometer. Chemical shifts are reported relative to trimethylsilane (TMS, δ = 0.00 ppm) and coupling constants (J) are given in Hz.

General procedure for the nicotinamide-independent anaerobic enzymatic C–C reduction reaction

An aliquot of the isolated enzyme (OYE1, OYE2, CrS, EB1, NCR, XenA, YqjM, GKOYE; protein purity > 90%, protein content in reaction 100 μg/mL) was added to a screw-top glass vial (2 mL) containing a degassed buffer solution (0.8 mL, 50 mM, Tris(hydroxymethyl)amino methane-HCl (TrisHCl) buffer; pH 7.5 or pH 9), the substrate (1a–6a, 10 mM), and the hydrogen donor (1c–52c, 10 mM). The vial was flushed with argon, and sealed with a teflon-coated septum and a lid. The mixture was shaken for 24 h at 30 °C and 120 rpm by using an Infors Unitron shaker and the products were extracted with ethyl acetate (2×0.7 mL). The combined organic phase was dried over Na2SO4 and analyzed on a GC to determine the conversion and stereoselectivity. On a preparative scale, products could be easily separated from excess hydrogen donor and phenolic byproducts by simple silica gel filtration due to the large difference in Rf values.

Synthesis of α-(+)3,4-epoxycarene[46]

A solution of meta-chloroperoxybenzoic acid (1.037 g, 6.0 mmol in CHCl3 (12 mL)) was added dropwise to a stirred solution of (+)-carene (0.508 g, 3.7 mmol) in chloroform (6 mL) over a period of 75 min. The reaction was stirred for a further 40 min and then quenched with aqueous sodium bisulfite (40 %, 2 mL). The organic layer was separated, washed with saturated aqueous NaHCO3 (50 mL), and extracted three times with ethyl acetate (30 mL). The reaction mixture was then cooled to ambient temperature, poured into saturated aqueous NaHCO3, and 3-isopropyl-6-methylcyclohex-2-enone [((S)-3,4-epoxycarene (355 mg, 2.6 mmol) was dissolved in dichloromethane (10 mL) and cooled to −78 °C (N2/ETO). Trime-thysilyl triflate (TMSOTf; 44 mL) was added and the reaction was stirred for 3 h. Saturated aqueous NaHCO3 (15 mL) and brine (15 mL), dried with Na2SO4, and concentrated by evaporation of the solvent to give α-(+)-3,4-epoxycarene as a light yellow oil (0.561 g, 3.68 mmol).

Synthesis of (S)-3-isopropyl-6-methylcyclohex-2-enone ([S]-13 c)[47]

Crude α-(+)-3,4-epoxycarene (355 mg, 2.6 mmol) was dissolved in dichloromethane (10 mL) and cooled to −78 °C (N2/ETO). Trimethylsilyl triflate (TMSOTf; 44 mL) was added and the reaction was stirred for 3 h. Saturated aqueous NaHCO3 (5 mL) and diethyl ether (10 mL) were then added. The organic layer was separated, washed twice with brine (10 mL), dried with Na2SO4, and concentrated by evaporation of the solvent to give (S)-3-isopropyl-6-methylcyclohex-2-enone ([S]-13 c; 45 mg, 0.3 mmol, 12 %, 25 % ee). Spectroscopic data were in agreement with those of the commercially available reference compound rac-13 c.

Synthesis of methyl 5-methyl-4-oxotetrahydrofuran-3-carboxylate[48]

Methyl L-lactate (1.0 g, 9.8 mmol) was dissolved in diethyl ether (4 mL) and added to a cooled (−38 °C, N2/ETO) suspension of NaH (267 mg, 50 %, 5.6 mmol) in diethyl ether (6 mL). The mixture was allowed to warm to 0 °C and stirred for 20 min at this temperature. The solvent was evaporated and a solution of methyl acrylate (1 mL, 11.0 mmol) in DMSO (4 mL) was added to the residue. The reaction was stirred for 20 h at ambient temperature. The mixture was poured into cold, aqueous sulfuric acid (5 %) and extract ed three times with diethyl ether (40 mL). The organic layers were combined, washed with saturated aqueous NaHCO3 (20 mL) and brine (20 mL), dried over MgCO3, and concentrated by evaporation of the solvent. The residue was purified by column chromatography (hexane/ethyl acetate 20:1), which yielded methyl 5-methyl-4-oxotetrahydrofuran-3-carboxylate (990 mg, 6.26 mmol, 64%). TLC results were viewed by using a KMnO4 staining solution or UV254 (Rf = 0.34, hexane/ethyl acetate 2:1).

Synthesis of (R)-2-methyltetrahydrofuran-3(2H)-one ((R)-48 c)[48]

Methyl 5-methyl-4-oxotetrahydrofuran-3-carboxylate (200 mg, 1.3 mmol) was added to sulfuric acid (10 %, 5 mL) and the mixture was stirred for 3.5 h at 70 °C. The reaction mixture was then cooled to ambient temperature, poured into saturated aqueous NaHCO3, and extracted three times with ethyl acetate (30 mL). The organic layers were combined, washed with saturated aqueous NaHCO3, and brine (20 mL), dried with MgSO4, concentrated by evaporation of the solvent and purified by column chromatography (hexane/ethyl acetate, 5:1) to yield (R)-2-methyltetrahydrofuran-3(2H)-one ((R)-48 c). TLC results were viewed by using a KMnO4 staining solution (Rf = 0.36, hexane/ethyl acetate 2:1). Spectroscopic data were in agreement with those of the commercially available reference compound rac-48 c. 

Preparation of tert-butyl 3-oxo-2,3-dihydro-1H-pyrrole-1-carboxylate (46d)

An aliquot of isolated NCR (protein purity > 90 %, protein content in reaction 200 μg/mL) was added to 30 screw-top glass vials (2 mL) containing a degassed buffer solution (0.8 mL, 50 mM, TrisHCl buffer; pH 7.5 or pH 9), 4-ketoisophorone (2 mL) containing a degassed buffer solution (0.8 mL, 50 mM), tert-butyl 3-oxopyrrolidine-1-carboxylate (46c; 10 mm). The vials were flushed with argon, sealed with a teflon-coated septum and a lid. The mixtures were shaken for 24 h at 30 °C and 120 rpm by using an Infors Unitron shaker. After the transformation, all phases were collected and the products were extracted with ethyl acetate (2×30 mL). The combined organic phase was dried over Na2SO4, concentrated, and the product was purified by column chromatography (hexane/ethyl acetate, 5:1) to yield tert-butyl 3-oxo-2,3-dihydro-1H-pyrrole-1-carboxylate (46d; 10.5 mg). TLC results were viewed by using a KMnO4 staining solution (Rf = 0.65, hexane/ethyl acetate 2:1).

1H NMR (300 MHz, CDCl3); δ = 8.33 (d, J = 4.1 Hz, 2H), 5.65 (d, J = 4.2 Hz, 2H), 4.01 (s, 2 H), 4.01 (t, J = 4.1 Hz, 2H), 1.54 ppm (s, 9 H).

Synthesis of 2-methylfuran-3(2H)-one (48d)

2-Methyltetrahydrofuran-3(2H)-one (48c; 2 g, 20 mmol, 1.93 mL) was dissolved in dry THF (100 mL) and cooled to −80 °C (liquid N2/ETO) under an argon atmosphere. N,N-Diisopropylethylamine (7.78 g, 60 mmol, 10.4 mL) was then added over 10 min and the mixture was stirred for 10 min, followed by slow addition of trimethylsilyl trifluoromethanesulfonate (8.89 g, 40 mmol, 7.23 mL) over a further 10 min. The mixture was stirred and kept at between −60 °C and −80 °C for 90 min and then allowed to warm to room temperature over 90 min. The solution was then cooled to −60 °C, and N-bromosuccinimide (4 g in 50 mL of dry THF) was added, turning the yellow solution red. The mixture was stirred for 60 min
at this temperature and then the reaction was quenched by addition of water (100 mL) and dichloromethane (100 mL). The phases were separated and the aqueous phase was washed with dichloromethane (3 x 50 mL). The combined organic phases were dried over Na2SO4, concentrated, and the resulting oil was immediately purified by column chromatography (hexane/ethyl acetate, 10:1) to remove any residual base. This yielded 4-bromo-2-methyldihydropyran-3(2H)-one (1.35 g), which is unstable in concentrated form and thus was immediately used for the next step.

4-Bromo-2-methylthiodihydropyran-3(2H)-one (330 mg, 1.9 mmol) was dissolved in ethyl acetate (15 mL). LiBr (646 mg, 7.5 mmol) and Li2CO3 (562 mg, 7.5 mmol) were then added and the mixture was added to a G30 Anton Paar microwave reaction vessel. The reaction was heated for 5 min at 180 °C by using an Anton Paar Mono- wave 300 machine. The pH of the mixture was brought to 7 by using aqueous HCl (1%) and the phases were separated. The organic phase was dried over Na2SO4, concentrated, and purified by column chromatography (hexane/ethyl acetate, 10:1) to yield 2-methylthiodihydropyran-3(2H)-one (48 d, 15 mg). TLC: Rf = 0.32, hexane/ethyl acetate 6:1.

H NMR (300 MHz, CDCl3): δ = 8.21 (d, J = 2.4 Hz, 1 H), 5.68 (d, J = 2.5 Hz, 1 H), 4.45 (q, J = 7.2 Hz, 1 H), 1.48 ppm (d, J = 7.2 Hz, 3 H).

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[1] R. Stuermer, B. Bauer, M. Hall, K. Faber, Curr. Opin. Chem. Biol. 2007, 11, 203 – 213.
[2] H. S. Toogood, J. M. Gardiner, N. S. Scrutton, ChemCatChem 2010, 2, 892 – 914.
[3] C. K. Winkler, G. Tasnadi, D. Clay, M. Hall, K. Faber, J. Biotechnol. 2012, 162, 381 – 389.
[4] G. Oberdorfer, K. Gruber, K. Faber, M. Hall, Synlett 2012, 23, 1857 – 1864.
[5] K. Faber, Biotransformations in Organic Chemistry, 6th ed., Springer, Heidelberg, 2011, pp. 140 – 145.
[6] W. Wandrey, Chem. Rec. 2004, 4, 254 – 265.
[7] T. Matsuda, R. Yamanaka, K. Nakamura, Tetrahedron: Asymmetry 2009, 20, 513 – 537.
[8] M. M. Grau, J. C. van der Toorn, L. G. Otten, P. Macheraux, A. Taglieber, F. E. Zilly, W. J. H. van Berkel, Angew. Chem. Int. Ed. 2010, 49, 3934 – 3937.
[10] B. J. Brown, Z. Deng, P. A. Karplus, V. Massey, J. Biol. Chem. 2004, 279, 32753 – 32762.
[11] A. Frzyzowska, H. Toogood, M. Sakuma, J. M. Gardiner, G. M. Stephens, N. S. Scrutton, Adv. Synth. Catal. 2009, 351, 3279 – 3286.
[12] F. H. Dobbek, Nat. Rev. Mol. Cell. Biol. 2006, 7, 892 – 914.
[13] V. Massey, M. Stankovich, P. Hemmerich, Biochemistry 1998, 37, 14326 – 14336.