SAM-dependent enzyme-catalysed pericyclic reactions in natural product biosynthesis

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Pericyclic reactions—which proceed in a concerted fashion through a cyclic transition state—are among the most powerful synthetic transformations used to make multiple regioselective and stereoselective carbon–carbon bonds1. They have been widely applied to the synthesis of biologically active complex natural products containing contiguous stereogenic carbon centres2-6. Despite the prominence of pericyclic reactions in total synthesis, only three naturally existing enzymatic examples (the intramolecular Diels–Alder reaction7, and the Cope8 and the Claisen rearrangements9) have been characterized. Here we report a versatile S-adenosyl-L-methionine (SAM)-dependent enzyme, LepI, that can catalyse stereoselective dehydration followed by three pericyclic transformations: intramolecular Diels–Alder and hetero-Diels–Alder reactions via a single ambimodal transition state, and a retro-Claisen rearrangement. Together, these transformations lead to the formation of the dihydropyran core of the fungal natural product, leporin10. Combined in vitro enzymatic characterization and computational studies provide insight into how LepI regulates these bifurcating biosynthetic reaction pathways by using SAM as the cofactor. These pathways converge to the desired biosynthetic end product via the (SAM-dependent) retro-Claisen rearrangement catalysed by LepI. We expect that more pericyclic biosynthetic enzymatic transformations remain to be discovered in naturally occurring enzyme “toolbox”11. The new role of the versatile cofactor SAM is likely to be found in other examples of enzyme catalysis.

Naturally existing enzymatic pericyclic reactions are rare12-14. Indeed, only a handful of enzymes that can catalyse these reactions have been characterized over the past five decades (Fig. 1a)7-9,12-14, even though pericyclic reactions have been proposed as key transformations in the biosynthesis of many polycyclic natural products12,15,16. We sought an enzyme-catalysed inverse electron demand hetero-Diels–Alder (HDA) reaction17 that constructs heterocycles in natural products (Fig. 1a). The HDA reaction together as a key biotransformation yielding dihydropyran cores, which are prevalent structural features in natural products that include the cytotoxic leporin B (1) from Aspergillus species (Fig. 1b)10,15,16,18. The biomimetic synthesis of the dihydropyran core in leporin uses the E/Z geometric mixture of the unstable o-quinone methide19 intermediate 5 generated from the dehydration of alcohol 4. The uncatalysed process gives a mixture of the minor desired HDA adduct leporin C (2) and major other regio- and stereoisomeric intramolecular Diels–Alder (IMDA) and HDA adducts (Fig. 1c)10. It was therefore proposed that an enzyme must be encoded in the biosynthetic pathway of leporins to catalyse the HDA cycloaddition in a stereoselective fashion and to suppress the IMDA reaction to afford the dihydropyran core in 2 (Fig. 1c)14. The biosynthetic gene cluster of leporin B (1) in Aspergillus flavidus has been reported and genetically verified (Fig. 1c)10. However, no clear enzyme candidate that can catalyse the pericyclic reaction was apparent in the cluster. To identify the enzyme responsible for this biotransformation, we heterologously reconstituted the leporin B (1) biosynthetic pathway in Aspergillus nidulans (Fig. 1c, d)21. As shown in Fig. 1d, coexpression of the polyketide synthase—non-ribosomal peptide synthetase (PKS—NRPS) LepA, the partnering enoyl reductase (ER) LepG, and the ring-expansion P45022 LepH led to the biosynthesis of the ketone 3. Additional coexpression with the short chain dehydrogenase/reductase (SDR) LepF, which is hypothesized to reduce 3 to the alcohol 4, led to a mixture of HDA products including the desired dihydropyran 2 as a minor product and the diastereomer 9, as well as the spirocyclic IMDA products 6-8 (Fig. 1c, d). Among these products, 2 and 6 are proposed to be derived from the quinone methide (E)-5, while 7-9 are from (Z)-5 (Fig. 1c). These results are consistent with biomimetic synthetic observations, and indicate that in order to biosynthesize 2 as the desired pericyclic reaction product, enzymatic stereocontrol of dehydration of 4 to (E)-5, as well as control of the subsequent pericyclic reaction, are required.

The only remaining annotated enzyme in the gene cluster is LepI, which is predicted to be an O-methyltransferase (OMT) with a well-conserved SAM binding site even though no O-methylation step is required for leporin B (1) biosynthesis. When lepI was introduced into the A. nidulans strain that produced the various pericyclic products derived from reduction of 3, we were surprised to observe the exclusive production of 2 without any other products (Fig. 1d). Further addition of the P450 lepD yielded the final product 1, thereby completing heterologous pathway reconstitution (Fig. 1c, d). To first verify the function of SDR LepF, recombinant protein was expressed from Saccharomyces cerevisiae and assayed in the presence of 3 and NADPH, which yielded a single product 4 corresponding to the reduced compound (Extended Data Fig. 1). To obtain sufficient 4 for assay with LepI, we reduced 3 with NaBH4 which gave both 4 and diastereomer 4′ in a ratio of approximately 1:1 (Extended Data Figs 1, 2). Each isomer was isolated and immediately added to LepI expressed and purified from Escherichia coli. Both 4 and 4′ dehydrated spontaneously in the absence of LepI and afforded a mixture of IMDA (6-8) and HDA (2 and 9) products, with 2 being a very minor product (Fig. 2a). However, when LepI was added to 4, complete conversion to 2 was accomplished in the absence of any added cofactors (Fig. 2a). In contrast, addition of LepI to 4′ had only a small effect on product profile. The collective in vivo and in vitro data therefore point to LepI being solely responsible for formation of 2 starting from 4, which requires stereoselective dehydration to yield (E)-5 and subsequent HDA reaction to 2.

We performed a reaction time-course analysis using LepI with 4 to capture any possible reaction intermediates. We observed the (E)-5 derived IMDA product 6 at early time points, which subsequently converted to 2 when all the 4 was consumed (Fig. 2b, c). This result demonstrates that even in the presence of LepI, after the dehydration of 4 to (E)-5, both IMDA and HDA reactions could take place simultaneously.

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to give 6 and 2, respectively, hence enzymatic control of the HDA reaction is not absolute. More surprisingly, it suggests that LepI may further catalyse the retro-Claisen rearrangement of 6 to 2 (Fig. 2e, Extended Data Fig. 3), a pericyclic transformation that has not been observed in the enzymatic realm. To confirm this activity, we incubated LepI with 6, to give 7 and 8 in yields of 87 + 19% (Fig. 2e, Extended Data Fig. 5). LepI also displayed stereoselectivity towards 6, as other Diels–Alder products such as 7 and 8 were not converted to the corresponding dihydropyran products (Extended Data Fig. 4a), although 7 inhibited the conversion of 6 to 2 (Extended Data Fig. 4b). These results indicate that LepI catalyses the retro-Claisen rearrangement, in addition to the dehydration and the IMDA/HDA reactions (Fig. 2e).

Given that the SAM-binding motif (GXGXG) of LepI is strictly conserved with other methyltransferases, we next probed the involvement of SAM in the LepI-catalysed reactions. Because no exogenous SAM was added in the assays, we investigated if SAM copurified with LepI and remained bound. SAM was detected in the supernatant of LepI and remained bound. SAM was detected in the supernatant of LepI and remained bound. SAM was detected in the supernatant of LepI and remained bound.

The structures of natural products containing dihydropyran, which would be biosynthesized by HDA reaction. Variecolortide A is naturally racemic; the relative stereochemistry of epiprydione and leporin B are shown. c. The putative leporin biosynthetic gene cluster in A. flavus and the assignment of encoded genes and biosynthetic pathway of leporins. PKS–NRPS, polyketide synthase–non-ribosomal peptide synthetase; TF, transcription factor; MCT, monocarboxylate transporter; SDR, short-chain dehydrogenase/reductase; ER, enoylreductase; OMT, O-methyltransferase. The structures show the relative stereochemistry. d. Analysis of metabolites from the transformants of A. nidulans by HPLC. The peak at 12 min corresponds to the tetramic acid product that is biosynthesized by LepA (PKS–NRPS) and LepG (ER).
HPLC analysis showing the reactions catalysed by LepI.

(ii) LepI for 3 min. (i) 2-retro-Claisen rearrangement. The structures show the relative stereochemistry.

\[ \text{Ph} \quad \text{LepI} + 4 \quad \text{Ph} \quad \text{LepI} + 4 \]

Retention time (min)

\[ 0 \quad 2 \quad 4 \quad 6 \quad 8 \quad 10 \]

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Figure 2 | HPLC analysis showing the reactions catalysed by LepI.

a. Reaction analysis in vitro of 4 and 4′ with 3 μM LepI for 0.5 h. (i) 4 in buffer, (ii) 4 with 3 μM LepI, (iii) 4′ in buffer, (iv) 4′ with 3 μM LepI. *Indicates that 6 is overlapped with 4′. b. Time-course analysis of the conversion of 240 μM 4 to 2 in the presence of 300 nM LepI. c. Reaction analysis in vitro of 6 with 300 nM LepI for 3 min. (i) 6 in buffer, (ii) 6 with LepI. d. Kinetic analysis of LepI-catalysed retro-Claisen rearrangement. e. Scheme for putative LepI-catalysed retro-Claisen rearrangement. The structures show the relative stereochemistry.

Figure 3 | LepI-catalysed reactions are SAM-dependent.

a. Time-course analysis of the consumption of 240 μM 4 in the presence of 300 nM LepI with or without cofactors: SAM (100 μM), SAH (250 μM), sinefungin (100 μM). b. Time-course analysis of the conversion of 140 μM 6 to 2 in the presence of 300 nM LepI with or without cofactors: SAM (100 μM), SAH (250 μM), sinefungin (100 μM). c. Time-course analysis of the production of 2 and 6 from 240 μM 4 in the presence of 300 nM LepI with or without 250 μM SAH. d. Analysis of the relative production ratio of HDA adduct 2 and IMDA adduct 6 from 240 μM 4. In the case of non-enzymatic reaction, the reaction time is 10 min. In the case of LepI (300 nM)-catalysed reactions with or without SAH (250 μM), the reaction time is 4.0 min. Cont., LepI without cofactors. e. Structures of SAM, SAH and sinefungin.
IMDA adduct under non-enzymatic conditions is consistent with the shorter C–C bond (2.91 Å) compared to the C–O bond (3.34 Å) in TS-1 (Fig. 4b). We confirmed this conclusion by performing quasi-classical molecular dynamics simulations following the procedure described previously28 (see Supplementary Information); 50 trajectories gave the Diels–Alder adduct (39 trajectories) plus 11 recrossing trajectories for the transition state in the gas phase. Water catalysis should shift the trajectories more towards the HDA adduct as found experimentally (Fig. 3d). To change the outcome of this reaction, Lepl must control the post-ambimodal TS bifurcation dynamics while accelerating the reaction by lowering the activation barrier of TS-120. Electrostatic catalysis by either sulfonium or ammonium ions acting on the amide carbonyl in TS-1 decreases the C–O bond length (3.05–3.07 Å range) and increases C–C bond length (3.07–3.21 Å range), resulting in an increased preference for the HDA reaction (Fig. 4b, Extended Data Fig. 10)23. Therefore, Lepl shifts the post-ambimodal TS bifurcation towards the HDA adduct more effectively in the active site as compared to spontaneous reaction in water (Fig. 4b). Indeed, molecular dynamics calculations with the trimethylsulfonium ion as catalyst to serve as a simpler mimic of SAM gave a 34:7 ratio of HDA:Diels–Alder adducts, along with 9 recrossing trajectories. The sulfonium and ammonium interactions with 6 were also predicted to substantially lower the barrier (TS-2) of the retro-Claisen rearrangement of the IMDA adduct 6 to the final HDA product 2 by 2.4 or 3.4 kcal mol\(^{-1}\) (100–1,000-fold acceleration), which supports the catalytic role of the positively charged SAM (Fig. 4c, Extended Data Fig. 10).

Our results show that Lepl is a multifunctional SAM-dependent enzyme that catalyses (1) the stereoselective dehydration of 4 to (E)-5, (2) an ambimodal IMDA and HDA reaction of (E)-5 to 6 and 2, respectively, and (3) the first enzymatic example (to our knowledge) of retro-Claisen rearrangement of 6 to 2 (Fig. 4c). The enzyme can control the post-TS bifurcation dynamics by altering the energy surface such that the steepest downhill path from the TS leads to the catalysed product(s) (Fig. 4b). Although formation of the undesirable IMDA product cannot be completely suppressed owing to the ambimodal nature of the TS, Lepl relies on the additional retro-Claisen activity to convert 6 to the desired product 2. This represents a kinetic ‘by-product recycle’ process to overcome thermodynamic limitations and to arrive fully at the desired biosynthetic end product. Last, the discovery of a SAM-dependent enzyme provides additional evidence for the versatility and importance of SAM in metabolism.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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Supplementary Information is available in the online version of the paper.

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METHODS

Material, fungal strains and culture condition. *A. flavus* NRR1.357 was obtained from Agricultural Research Service Culture Collection (NRRL). *A. nidulans* FGSC A1145 was obtained from the Fungal Genetics Stock Center (http://www.fgsc.net/). *A. flavus* was maintained on PDA (potato dextrose agar, BD) for 3 days for sporulation or in liquid PDB medium (PDA medium without agar) for isolation of genomic DNA. *A. nidulans* was maintained on Czapek-Dox (CD) agar for sporulation or in liquid CD–ST medium for gene overexpression, compound production and RNA extraction (http://www.fgsc.net/).

General DNA manipulation technique. *E. coli* TOP10 and *E. coli* XL-1 were used for cloning, following standard recombinant DNA techniques. DNA restriction enzymes were used as recommended by the manufacturer (New England Biolabs, NEB). PCR was performed using Phusion High-Fidelity DNA Polymerase (NEB). The gene-specific primers are listed in Supplementary Information. PCR products were confirmed by DNA sequencing. *E. coli* BL21(DE3) (Novagen) was used as the *E. coli* host for protein expression. Saccharomyces cerevisiae strain BJ456-1ppA (MATs ara-52 his-3 Δ200 leu-211 trp1 pep4: HIS4; Prb1 Δ1.6r can1 GAL) was used as the yeast host for protein expression in vitro and in vivo homologous recombination to construct the *A. nidulans* overexpression plasmids.

Heterologous expression of lep in *A. nidulans*. *A. nidulans* A1145 was initially grown on CD agar plates containing 10 mM uracil, 0.5 μg ml−1 pyridoxine HCl and 2.5 μg ml−1 riboflavin at 30 °C for 5 days. Fresh spores of *A. nidulans* were inoculated into 25 ml liquid CD media (1 litre: 10 g glucose, 50 ml 20 mM L-glutamate, 50 ml 20 mM (NH₄)₂SO₄, 50 ml 30 mM KH₂PO₄, 50 ml 10 mM MgSO₄·7H₂O and 50 ml 10 mM KHCl) in 250-ml Erlenmeyer flasks and incubated at 30 °C and 250 r.p.m. for approximately 16 h. For the preparation of 20× nitrate salts, 120 g NaNO₃, 10.4 g KCl, 10.4 g MgSO₄·7H₂O, 30.4 g KH₂PO₄ were dissolved in 1 litre double distilled water. The 100 ml trace elements with pH 6.5 contains 1.2 M MgSO₄, 10.4 g KCl, 1.6 g CuSO₄·5H₂O and 0.11 g (NH₄)₂MoO₄·2H₂O. Mycelia were harvested by centrifugation at 3,500 r.p.m. for 10 min, and washed with 10 ml of 10% (v/v) acetic acid twice. The extract was concentrated and the residue was purified with reverse-phase column (Phenomenex Kinetex, C18, 1.7 μm) using positive- and negative-mode electrospray ionization with a linear gradient of 5−95% acetonitrile MeCN–H₂O with 0.5% formic acid in 15 min followed by 95% MeCN for 3 min with a flow rate of 0.3 ml min⁻¹. The result is shown in Extended Data Fig. 1.

Activity assay of Lep using the alcohol 4 as the substrate. Preparations of compound 4 and 4′ were performed as follows. The solution of compound 3 (3.5 mg, 10 μmol) in 0.5 ml EtOH was added NaBH₄ (4.0 mg, 100 μmol) at 0 °C and the mixture stirred at room temperature for 15 min. The reaction mixture was quenched with 0.5 ml 1 M HCl and the whole was extracted with 0.5 ml ethyl acetate twice. The extract was concentrated and the residue was purified with a semi-preparative reversed-phase column (Phenomenex Kinetics, C18, 5 μm, 100 Å, 10 × 250 mm). The separation conditions were a linear gradient of 40−95% acetonitrile MeCN–H₂O in 15 min followed by 95% MeCN for 3 min with a flow rate of 3.0 ml min⁻¹. The fractions containing 4 and 4′ were not concentrated and were used immediately as the substrate solution, because dehydration takes place during concentration.

Assays for Lep activity with 4 and 4′ in phosphate buffer (20 mM Na₂HPO₄, 50 mM NaCl, pH 8.0) were performed at the 50 μl scale with 3.0 μl MePep, at 30 °C for 0.5 h. Then the reaction was quenched with an equal volume of cold acetonitrile. Protein was precipitated and removed by centrifugation, and the supernatant analysed by LC–MS. LC–MS analyses were performed on a Shimadzu 2020 EV LC–MS with a reversed-phase column (Phenomenex Kinetics, C18, 1.7 μm, 100 Å, 10 × 2.1 mm) using positive- and negative-mode electrospray ionization with a linear gradient of 5−95% acetonitrile MeCN–H₂O with 0.5% formic acid in 15 min followed by 95% MeCN for 3 min with a flow rate of 0.3 ml min⁻¹. The result is shown in Extended Data Fig. 1.
50 mM NaCl, pH 8.0) was incubated with 30 μl LepI at 30°C for 12 h. Then reaction was quenched with 50 μl acetonitrile. Protein was precipitated and removed by centrifugation and the supernatant analysed by HPLC with a reversed-phase column (Phenomenex Kinetex, C18, 1.7 μm, 100 Å, 100 × 2.1 mm) with a linear gradient of 5–95% acetonitrile MeCN–H2O with 0.5% formic acid in 15 min followed by 95% MeCN for 3 min with a flow rate of 0.3 ml min
−1. The results are shown in Extended Data Fig. 4a.

**Evaluation of inhibitory activity of 6 against LepI-catalysed retro-Claisen rearrangement.** To a 50 μl solution containing 300 nM LepI in phosphate buffer (20 mM Na2HPO4, 50 mM NaCl, pH 8.0) was added DMSO or 7 (1–500 μM) dissolved in DMSO. Then reactions were initiated by the addition of 100 mM 6. After 10 min at 30°C, reactions were quenched by the addition of 50 μl of cold acetonitrile. Protein was precipitated and removed by centrifugation and the supernatant analysed by HPLC with a reversed-phase column (Phenomenex Luna, C18, 5 μm, 100 Å, 2.0 × 100 mm) with isocratic conditions (50% of H2O in CH3CN). Results were quantified by a standard curve of product 2. Final results were calculated as percentage of DMSO treated controls. Data fitting was performed using GraphPad Prism 6, and the IC50 value represents mean ± standard deviation (s.d.) of three independent replicates. This result is shown in Extended Data Fig. 4b.

**Activity assay and kinetic analysis of LepI using compound 6 as a substrate.** Assays for LepI activity with 6 in phosphate buffer (20 mM Na2HPO4, 50 mM NaCl, pH 8.0) were performed at the 50 μl scale with 300 nM LepI, 30°C for 3 min. Then the reaction was quenched with an equal volume of cold acetonitrile. Protein was precipitated and removed by centrifugation and the supernatant analysed by HPLC using a C18 column (Phenomenex Luna C18 (2) 5 μm, 2.0 × 100 mm) with isocratic conditions (50% of H2O in CH3CN). These results are shown in Extended Data Fig. 2c.

To determine the kinetics of LepI, the assays were performed at the 50 μl scale with 150 nM LepI and 3.0–300 μM 6 in phosphate buffer (20 mM Na2HPO4, 50 mM NaCl, pH 8.0) at 30°C for 3 min. The reactions were quenched by adding 50 μl acetonitrile. After centrifugation, supernatant was analysed by HPLC as mentioned above. Data fitting was performed using GraphPad Prism 6, and Km and kcat values represent mean ± standard deviation (s.d.) of three independent replicates. The result is shown in Fig. 2d.

**The confirmation of SAM presence in LepI and quantification of its efficiency.** 300 μM LepI in 20 μl storage buffer (50 mM Tris–HCl, 100 mM NaCl, 10% glycerol, pH 8.0) was denatured by adding 60 μl acetonitrile. 300 μM LepI in 50 μl storage buffer (50 mM Tris–HCl, 100 mM NaCl, 10% glycerol, pH 8.0) and 100 μM SAM in water was heated at 95°C for 10 min. Then, the solutions were centrifuged and the supernatants analysed by HPLC. The standards of SAM and MTA were also quantified by the standard curve of product 2. Three independent replicates. The results are shown in Extended Data Fig. 6.

**Computational methods.** The DFT calculations were performed with Gaussian 09.32 Geometry optimizations of all the minima and transition state structures were carried out at the B3LYP-D3 level of theory with the 6-311G(d,p) basis set. Vibrational frequencies were computed at the same level to verify that optimized structures were energy minima or transition states and to evaluate zero-point vibrational energies (ZPVE) and thermal corrections at 298 K. A quasi-harmonic correction was applied during the entropy calculation by setting all positive frequencies that are less than 100 cm
−1 to 100 cm
−1. Solvation energies were evaluated by a self-consistent reaction field (SCRF) using the CPCM model with a larger basis set, 6-311+G(d,p). Single point energy calculations were carried out at other levels of theory, such as M06-2X and wB97XD; systematic shifts in energies were observed, while the relative energetics remained the same. The results are shown in Fig. 4, Extended Data Fig. 10 and Supplementary Figs 11–17.

**Data availability.** The data that support the findings of this study are available within the paper and its Supplementary Information, or are available from the corresponding author upon reasonable request.

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Extended Data Figure 1 | LC–MS analysis of the *in vitro* reaction of 3 with LepF. The extracted ion chromatograms (EIC) under positive ionization are shown. The mass/charge (m/z) ratio of alcohols 4 and 4′ is 354 under positive ionization. Because the enzymatic activity of LepF is low and 4 is very unstable, we were not able to obtain enough 4 to use it as the substrate for *in vitro* reaction of LepF. Thus, we obtained 4 by reducing ketone 3 with NaBH₄. Since this reduction proceeds non-stereoselectively, 4 and diastereomer 4′ were formed. After the isolation of 4 and 4′ by HPLC, the fractions containing 4 and 4′ were not concentrated and were immediately used as the substrate. The stereochemistry of the secondary alcohol in 4 and 4′ was not determined.
Extended Data Figure 2 | HPLC analysis of the chemical reduction of 3 with NaBH₄. The reaction mixture containing 1 mM 3 and 10 mM NaBH₄ with EtOH (50 μl) was incubated at 0 °C for 1 min, then the reaction was quenched with water. After centrifugation, the supernatant was analysed by HPLC. The reduction of 3 gave the alcohol 4 and diastereomer 4’. The spontaneous dehydration of both alcohols resulted in the formation of HDA and IMDA products via the E/Z mixture of quinone methide 5. The isolated 4 and 4’ also readily dehydrated and converted to a mixture of the desired HDA (2) and the undesired HDA (9) and IMDA (6–8) products, showing the instability of these compounds. The structures (right) show the relative stereochemistry.
Extended Data Figure 3 | Reaction analysis of 6–9 under heating. 6 (dissolved in 5% DMSO with H₂O) was heated at 95 °C for 1 h. 7–9 (dissolved in 5% DMSO with H₂O) were heated at 95 °C for 10 h. 6 was completely converted to 2 via [3,3]-sigmatropic retro-Claisen rearrangement. This reaction is irreversible under these conditions. It should be noted that the conversion of 6 to 2 via cycloreversion can be ruled out, since 6 was completely converted to 2 without any other IMDA/HDA side products. No reactions occurred in the case of 7. 8 and 9 can be interconverted via Claisen rearrangement. In this case, retro-Claisen rearrangement (8 to 9) is preferable to forward Claisen rearrangement (9 to 8). The structures show the relative stereochemistry.
Extended Data Figure 4 | Analysis of the substrate specificity of LepI. 

a, *In vitro* reactions of other IMDA products 7–9 with 30 μM Lepl for 12 h. (i) 8 in buffer, (ii) 8 with Lepl, (iii) 7 in buffer, (iv) 7 with Lepl, (v) 9 in buffer, (vi) 9 with Lepl. The experimental details are described in Methods.

b, Elucidation of inhibitory activity of 7 on Lepl-catalysed retro-Claisen rearrangement of 6 to 2. The experimental details are described in Methods. The IC50 value is mean ± standard deviation (s.d.) of three independent experiments. The structures show the relative stereochemistry.
Extended Data Figure 5 | Time-course analysis of the Lepl-catalysed retro-Claisen rearrangement of 6 to 2. The experimental details are described in Methods. The data show one representative experiment from at least three independent replicates.
Extended Data Figure 6 | HPLC analysis showing that purified LepI retains SAM. SAM was detected in the supernatant of denatured (by acetonitrile) LepI. When LepI was denatured by heating the sample at 95 °C for 10 min, a single peak corresponding to 5′-deoxy-5′-(methylthio) adenosine (MTA), a major degradation product of SAM\(^ {25}\), was detected from the supernatant of boiled LepI. Since SAM to MTA conversion is nearly quantitative and an MTA standard curve can be readily constructed, we found that about 90% of LepI still retains SAM after purification. Shown are HPLC profiles of (i) LepI denatured by acetonitrile, (ii) LepI heated at 95 °C for 10 min, (iii) the authentic reference of SAM, (iv) SAM heated at 95 °C for 10 min, and (v) the authentic reference of MTA. The experimental details are described in Methods.
Extended Data Figure 7 | HPLC analysis of SAM-dependent LepI-catalysed reactions. a, Analysis of in vitro reaction of 240 μM 4 with 300 nM LepI at 30 °C for 5 min in the presence and absence of cofactors. The concentrations of SAH, SAM and sinefungin used in this experiment are 250 μM, 100 μM and 100 μM, respectively. The data show one representative experiment from at least three independent replicates.

b, Analysis of the in vitro reaction of 140 μM 6 with 300 nM LepI at 30 °C for 4 min in the presence and absence of cofactors. The concentrations of SAH, SAM and sinefungin used in this experiment are 250 μM, 100 μM and 100 μM, respectively. The data show one representative experiment from at least three independent replicates.
Extended Data Figure 8 | SAH is a competitive inhibitor of LepI retro-Claisen rearrangement. a, Dose-dependent inhibition of retro-Claisen rearrangement by SAH. b, Dose-dependent recovery of retro-Claisen rearrangement by SAM in the presence of 250μM SAH. The experimental details are described in Methods. Error bars, s.d. of three independent experiments.
Extended Data Figure 9 | Time-course analysis of the production of 2 divided by the sum of the production of 2 and 6. The substrate used in this study is alcohol 4. a. Lepl-catalysed reaction with or without SAH (250 μM). b. Non-enzymatic reaction. The initial production ratio (IMDA (6) versus HDA products (2)) between Lepl-catalysed (about 1:1 periselectivity) and non-catalysed reactions (about 94:6 periselectivity) are clearly different. These data support the suggestion that Lepl catalyses the competitive IMDA/HDA reactions by changing the product distribution resulting from IMDA versus HDA reactions.
Extended Data Figure 10 | Calculated free energies and bond distances. Data are shown for the ambimodal transition state (TS-1) and the transition state for the retro-Claisen rearrangement (TS-2), uncatalysed and with various catalysts, calculated with B3LYP-D3/6-311+G(d,p)//6-31G(d), CPCM water. Positions of the bonds are shown in the structures above.

|          | $\Delta G^\ddagger$ (kcal/mol) | CC1 (Å) | CC2 (Å) | CO (Å) | $\Delta G^\ddagger$ (kcal/mol) | CC (Å) | CO (Å) |
|----------|---------------------------------|---------|---------|--------|---------------------------------|---------|--------|
| Uncatalyzed | 12.9                            | 1.95    | 2.91    | 3.34   | 20.8                            | 2.68    | 2.50   |
| NH$_3$Me  | 15.2                            | 1.96    | 2.94    | 3.29   | 20.8                            | 2.64    | 2.50   |
| PhOH      | 9.9                             | 1.98    | 2.98    | 3.13   | 19.1                            | 2.57    | 2.51   |
| Me$_3$S$^+$| 11.1                            | 2.03    | 3.07    | 3.07   | 18.4                            | 2.61    | 2.76   |
| NH$_3^+$Me| 10.3                            | 2.11    | 3.21    | 3.05   | 17.4                            | 2.45    | 2.85   |