Supporting Information

**Diels–Alder Reactions During the Biosynthesis of Sorbicillinoids**

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General information

Reagents

Analytical grade chemicals and reagents were purchased from Sigma, Roth and Fischer. The solvents used for HPLC were analytical grade. General molecular biology procedures were performed as standard and molecular biology kits were used according to the manufacturer’s protocols. Analytical PCR was performed using OneTaq polymerase and preparative PCR for cloning procedures was performed using Q5 polymerase manufactured by NEB. Restriction endonucleases were also purchased from NEB.

Media

All media were prepared in deionised water and autoclaved at 126 °C for 20 min. See Table 1 for details.

Analytical LCMS

LCMS data were obtained with either (LCMS method 1): a Waters 2795HT HPLC using a Phenomenex Kinetex column (2.6μ,C18, 100 Å, 4.6 x 100mm) equipped with a Phenomenex Security Guard precolumn (Luna C5 300 Å) eluting at 1 mL min⁻¹, with a Waters 996 Diode Array detector between 210 and 600 nm and a Waters ZQ mass detector operating simultaneously in ES⁺ and ES⁻ modes between 100 and 1000 m/z; or (LCMS method 2): a Waters 2767 sample manager connected to Waters 2545 pumps and SFO, a Phenomenex Kinetex column (2.6μ,C18, 100 Å, 4.6 x 100 mm) equipped with a Phenomenex Security Guard precolumn (Luna C5 300 Å) eluted at 1.0 mL min⁻¹, with a waters 2998 Diode Array detector (210–600 nm) and Waters 2424 ELSD and Waters SQD-2 mass detector operating simultaneously in ES⁺ and ES⁻ modes between 100 and 1000 m/z. Solvents were: A, HPLC-grade H₂O containing 0.05% formic acid and B, HPLC-grade acetonitrile containing 0.045% formic acid. The gradient was run over 15 min starting at 10 % B and ramping to 90 % B within the first ten minutes, followed by two minutes at 90 % B and three minutes at 10 % A.

Preparative LCMS

Purification of all compounds was generally achieved using a Waters mass-directed autopurification system comprising of a Waters 2767 autosampler, Waters 2545 pump system, a Phenomenex Kinetex Axia column (5μ, C18, 100 Å, 21.2 × 250 mm) equipped with a Phenomenex Security Guard pre-column (Luna C5 300 Å) eluted at 20 mL min⁻¹ at ambient temperature. Solvents were used as above. The post-column flow was split (100:1) and the minority flow was made up with HPLC-grade MeOH + 0.045% formic acid to 1 mL min⁻¹ for simultaneous analysis by diode array (Waters 2998), evaporative light-scattering (Waters 2424) and electrospray ionisation mass spectrometry in positive and negative modes (Waters SQD-2). Detected peaks were collected into glass test tubes. Combined fractions were evaporated (vacuum centrifuge), weighed and residues dissolved directly in deuterated solvent for NMR.

HRMS and NMR

HRMS was obtained using a Waters Acquity Ultrasound performance UPLC system connected to a Q-TOF Premier mass spectrometer. NMR data were recorded using either a Bruker Ascend 400, a Bruker Ultrasound 500 or a Bruker Ascend 600 instrument each equipped with a cryo-cooled probe at 400/500/600 MHz (1H) and 125 MHz (13C) and are referenced relative to Me₄Si. 2D spectra (COSY, HSQC and HMBC) were recorded using standard parameters. Samples were dissolved in deuterated as solvents indicated in the respective NMR tables and figures.

Experimental Procedures

Fermentation and Extraction Protocols

A. oryzae: A spore suspension of transformants grown on selective agar plates (CZD/S, CZD/S1 or CZD/S1 w/o methionine) was inoculated into 100 mL DPY-medium in a 500 mL baffled flask and incubated for four days at 28 °C with 110 rpm shaking. Cultures were homogenized using a hand blender and cells were separated by filtration. The liquid supernatant was acidified with 2 M HCl to pH 3-4 and extracted twice with ethyl acetate (2 x 100 mL). Combined organic layers were dried over MgSO₄ and solvent was removed under reduced pressure. The organic residue was dissolved in methanol to a concentration of 10 mg/mL, filtered over glass wool and analysed by LCMS. For preparative LCMS cultures were grown in a scale between 1-2 L and the concentration of the organic extract
applied for purification was 50 mg/mL.

T. reesei: A spore solution of the wild type strain or transformants grown on PDB-agar plates or PDB-agar plates supplemented with hygromycin B (100 μg/mL), respectively, was inoculated into 100 mL ME-medium in a 500 mL flask and incubated for seven days at 28 °C with 180 rpm shaking. Cultures were homogenized using a hand blender and cells were separated by filtration. The liquid supernatant was acidified with 2 M HCl to pH 3-4 and extracted twice with ethyl acetate (2 x 100 mL). Combined organic layers were dried over MgSO4 and solvent was removed under reduced pressure. The organic residue was dissolved in methanol to a concentration of 10 mg/mL, filtered over glass wool and analysed by LCMS. For preparative LCMS cultures were grown in a scale between 1-2 L and the concentration of the organic extract applied for purification was 50 mg/mL

Construction of A. oryzae Expression Vectors

T. reesei total RNA was extracted using TRIzol reagent (Thermo Fischer Scientific) and subsequently converted to cDNA using the High-Capacity RNA-to-DNA™ kit (Thermo Fischer Scientific) according to the manufacturer’s instructions. The intron free cDNA was used for amplification of sorbicillinoids biosynthetic genes by PCR (See Table S2 for primers used in this study).

The tailoring genes sorC, sorD, sdr and p450 were cloned into any of the respective multigene expression vectors pTYGSarg/ade/met[11] via yeast homologous recombination under control of any of the constitutive promoters peno, padh or pgdphA (Table S3). The procedure was as followed: S. cerevisiae was streaked out on YPAD agar and incubated at 30 °C for 3-5 days. A single colony was transferred into 10 mL YPAD medium and incubated over night at 30 °C with 200 rpm shaking. This starter culture was added to 40 mL YPAD medium in a 250 mL flask and incubated for another 4.5 h at 30 °C with 200 rpm shaking. The culture was harvested by centrifugation at 3,000 g for 5 min. The pellet was washed with 25 mL ddH2O and centrifugation was repeated. The pellet was resuspended in 1 ddH2O, transferred into a 1.5 mL reaction tube and centrifuged at 18,000 g for 15 s. The pellet was resuspended in 400 μL ddH2O solution and aliquots of 50 μL were transferred into a separate 1.5 mL reaction tube. For each sample one aliquot was centrifuged at 18,000 g for 15 s and the pellet was dissolved in the transformation mixture consisting of 240 μL PEG solution (50 % (w/v) polyethylene glycol 3350),36 μL LIOAc (1 M), 50 μL denatured salmon testis DNA (2 mg/mL in TE buffer), 34 μL DNA master mix containing the linearized vector and desired inserted obtained by PCR in equimolar concentration. The PCR fragments contain each 30 bp overlap at both 5' and 3' with the cut sites of the vector fragments to facilitate homologous recombination. Cells were first incubated for 30 min at 30 °C, then for 40 min at 42 °C. Cells were pelleted by centrifugation at 18,000 g for 15 s and supernatant was removed. The pellet was resuspended in 500 μL ddH2O and 250 μL was spread on selective SM-Ura plates, which were incubated for four days at 30 °C. Construction plasmid DNA was extracted from yeast cells using a Zymoprep™ Yeast Plasmid Miniprep II kit (Zymo research, Orange, California, USA) and transformed into E. coli codB8 Survival cells by standard heat shock method for amplification. In same manner both PKS sorA and sorB were assembled in the entry vector pEYA which was subsequently used to transfer the target genes into any of the target multigene expression vectors pTYGSarg/ade/met by Gateway™ cloning.

Construction of T. reesei Knockout Vectors

All vectors were built using yeast homologous recombination as described above. For the construction of pEY-TR-sorA the gDNA of T. reesei QM6a was used as a template for amplifying the right and left fragments for sorA, including about 1.5 kb for each fragment. The hygR cassette was amplified from the plasmid pTH-GS-eGFP. The primers (Table S2) were designed to include 30 bps overlaps to be recombined in yeast with compatible overlaps in a cut pEYA plasmid. For the construction of pEY-TR-sdr, about 1 kb were amplified from the left and right ends of the sdr gene from the gDNA of T. reesei QM6a, together with the hygR cassette fragment to be recombined by yeast homologous recombination. Similarly, about 1 kb length were amplified from the P450 gene together with a hygR cassette to build the KO vector pEYA-TR-p450.

Transformation of A. oryzae NSAR1

Spore suspension collected from a fresh A. oryzae NSAR1 DPY plate (approximately 5 days) was used to inoculate 50 mL (250 mL flask) of GN liquid culture and incubated for 16 h (28 °C, 110 rpm). Cells were collected by filtration over sterile miracloth, washed with 0.8M NaCl and suspended in 10 mL of filter-sterilised A. oryzae NSAR1 protoplasting solution (10 mg/mL lysing enzyme from Trichoderma harzianum, Sigma-Aldrich, 0.8M NaCl, 10 mM CaCl2). The suspension was incubated for 4 h at ambient temperature with gentle shaking. Protoplasts were released by pipetting, collected by centrifugation (3000 x g, 5 min) and directly suspended in the required amount of fungal transformation solution I (10 mM CaCl2, 0.8M NaCl and 50 mM Tris-HCl at pH 7.5). Vector DNA (≥1 μg in 10 μL of ddH2O) was mixed with 100 μL protoplasts and incubated on ice for 5 min. One millilitre of fungal transformation solution II (10 mM CaCl2, 0.8M NaCl and 50 mM Tris-HCl at pH 7.5, 60% (w/v) PEG3350) was added and the mixture was incubated at ambient temperature for 20 min. Five millilitres of molten selective soft agar (CZD/S, CZD/S1 or CZD/S1 w/o methionine) was added and the mixture was poured over selective agar plates (CZD/S, CZD/S1 or CZD/S1 w/o methionine). Plates were incubated at 28 °C until colonies appeared, which were transferred to secondary plates of the respective selective agar. Vigorously growing colonies were transferred onto a third plate selective plate. For strains constructed in this study see Table S4.

Transformation of T. reesei QM6a

Spore suspension collected from a fresh T. reesei PD plate was used to inoculate 50 mL (250 mL flask) of GN liquid culture and incubated for 16 h (28 °C, 110 rpm). Cells were collected by filtration over sterile miracloth, washed with washing solution (1.2 M sorbitol, 10 mM Tris-HCl pH 7.5) and suspended in 10 mL of filter-sterilised T. reesei protoplasting solution (10 mg/mL lysing enzyme from Trichoderma harzianum, 5 mg/mL driselase, 1.2 M sorbitol, 100 mM potassium phosphate pH 5.6). The suspension was incubated for
2 h at 28 °C with gentle shaking. Protoplasts were released by pipetting, collected by centrifugation (3000 × g, 5 min) and directly suspended in the required amount of resuspension solution (1 M sorbitol, 10 mM Tris-HCl pH 7.5). Vector DNA (≥1 µg, in 10 µL of ddH₂O) was mixed with 200 µL protoplasts and 2 mL of transformation solution III (50mM CaCl₂, 10 mM Tris-HCl at pH 7.5, 25% (w/v) PEG6000). Samples were incubated on ice for 20 min followed by incubation at ambient temperature for 5 min. 4 mL of resuspension solution were added and aliquots of 200 µL were mixed with 20 ml molten, 50 °C warm PD agar containing 50 µg/mL hygromycin B, spread over cultivation plates and incubated at 28 °C for 3-5 days until colonies were visible. Colonies were picked from the transformation plates and selected for three rounds on PD agar plates supplemented with 100 µg/mL hygromycin B to obtain pure colonies for further analysis. Positive transformants were grown on ME medium flasks at 28 °C for 7 days at 110 rpm for sorbicillinoid production and analysis. For strains constructed in this study see Table S4.

Cloning, Expression and Purification of SorC

For expression of sorC in E. coli BL21 (DE3) the expression plasmid pET-28a-sorC (encoding for an N-terminal hexa-histidine tag) was built by restriction digest with NdeI and NotI, followed by ligation using T4 ligase. T. reesei cDNA was used as the DNA template (Figure S13). Transformation of competent cells was performed based on a standard heat shock protocol. A pre-culture was grown overnight in LB-medium containing50 µg/mL kanamycin at 37 °C with 200 rpm shaking. Each 1 mL of this seed culture was used to inoculate 100 mL 2TY-medium containing 50 µg/mL kanamycin. Cells were grown at 37 °C and 200 rpm until an OD₆₀₀ between 0.4-0.6 was reached. To induce protein expression Isopropyl-β-D-thiogalactopyranoside (IPTG) was added to a final concentration of 0.1 mM and cells were incubated for another 16 h at 16 °C and 200 rpm. Cells were harvested by centrifugation (3500 × g, 20 min) at 4 °C and resuspended in loading buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 10 mM imidazole, 10% glycerol (v/v)) and lysed by sonication.

Cell debris was removed from the total lysate by centrifugation (10,000 × g, 40 min, 4 °C). SorC containing a his₆-tag (52.8 kDa) was purified by Ni²⁺ affinity chromatography using a gravity column. 1 mL of Ni-NTA Agarose (Bioybm) was washed with loading buffer and added to each 15 mL supernatant obtained in the previous step. The mixture was incubated for 1 h at 8 °C on an agitator with gentle shaking to facilitate protein binding. A 15 mL CHROMABOND® column (Macherey-Nagel) was activated with 20 % ethanol and equilibrated with loading buffer. Ni-NTA with bound protein was collected by gravity and washed twice with each 15 mL loading buffer, followed by another wash step with loading buffer (supplemented with 50 mM imidazole). Protein was eluted in 2 mL fractions with elution buffer (loading buffer + 500 mM imidazole). The buffer was exchanged to 50 mM phosphate buffer pH 8 by ultrafiltration with a molecular weight cut-off of 30 kDa and protein concentration was determined based on extinction coefficient and molecular weight using a spectrophotometer. Purified protein was assessed by SDS-PAGE (Figure S14).

Cloning, Expression and Purification of SorD

First, the synthetic vector PET100D/TOPO-sorD was purchased from Thermofischer encoding for a codon optimized (Figure S15), intron-free sorD (65.0 kDa with N-terminal his₆-tag) for expression in E. coli (Figure S16A). Protein expression was performed as described above, varying the expression temperature between 12-37°C and the IPTG concentration between 0.1-1 mM. No overexpressed protein was observed (Figure S16B). The expression vector plasmid pET-28a-sorD (encoding for an N-terminal hexa-histidine tag) was constructed as described above, using NheI (5’) and NotI (3’). Expression temperatures between 12-37°C and IPTG concentrations between 0.1-1 mM were tested, but all resulted in insoluble protein. Autoinduction using TB-medium was also not successful for both plasmids. For a new construct the first 27 amino acids (possible signal sequence) of sorD were removed, still resulting in insoluble protein. Usage of E. coli Codon Plus cells also met with failure.

For expression of sorD in Saccharomyces cerevisiae W303B the expression plasmid pESC-ura-sorD was built by restriction digest/ligation procedure using Sall (5’) and KpnI (3’). Successful plasmid construction was confirmed by sequencing. Transformation of yeast was performed as described previously, with the 34 µL transformation mixture consisting only of the already assembled plasmid pESC-ura-sorD. Positive colonies were selected for two rounds on SM-ura agar and subsequently grown in glucose-ura medium (50 mL) for 2 days at 30 °C and 160 rpm. Cells were harvested by centrifugation, washed with ddH₂O and subsequently dissolved in 1 mL ddH₂O. 200 µL were used to inoculate 50 mL galactose-ura medium to induce protein expression. Cells were incubated for 16 h at 30 °C and 160 rpm. After washing with ddH₂O pelleted cells were dissolved in 1 mL PBS buffer (100 mM phosphate buffer pH 7.5, 8g/L NaCl) and lysed mechanically. The supernatant obtained after centrifugation was directly used for enzyme assays (assays were performed by adding 50 µL of the supernatant to the “standard” SorC assay) and was also analysed by SDS-PAGE. No putative target protein could be seen and the assays did not differ from any control.

Enzyme Assays with SorC

Enzymatic formation of 2a/2b: All analytical assays were performed on a 200 µL scale consisting of SorC (1 mg/mL) dissolved in 50 mM potassium phosphate buffer (pH 8), 10 mM NAD(P)H and 8 mM substrate 1a/1b (dissolved in acetone). Final concentration of acetone in the assay mixture was 5% (v/v), but the same results were obtained with 20% (v/v). Assays were incubated at ambient temperature for one hour with gentle shaking. 200 mL MeCN was added and precipitated enzyme was separated by centrifugation. Supernatant was directly subjected to LCMS analysis.

Enzymatic formation 10a/10b and 15a: Assays were performed as described above, but modified slightly by adding an excess of NAD(P)H (20 mM) and raising the incubation time to 16-24 hours.
**SUPPORTING INFORMATION**

**Enzymatic formation of bisorbicillinols 3a-c:** All analytical assays were performed on a 200 μL scale consisting of SorC (1 mg/mL) dissolved in 50 mM potassium phosphate buffer (pH 8), 10 mM NAD(P)H and 8 mM substrate 1a/1b (dissolved in acetone). Final concentration of acetone in the assay mixture was 20 % (v/v). Assays were incubated at ambient temperature for one hour with gentle shaking. Assays were extracted with 400 μL CH₂Cl₂ or CHCl₃ and the organic phase was removed by vacuum-centrifugation at 45 °C. Organic residue was dissolved in 100 μL MeOH and subjected to LCMS analysis. Formation of bisorbicillinols was also observed when the volume of acetone in the assay mixture was kept at 5% (v/v), indicating that the organic solvent inducing the dimerization is CH₂Cl₂/CHCl₃.

Enzymatic formation of spiroorbicillinols 6a/6b. Assays were performed as described for the formation of bisorbicillinols 3a-c, but adding an excess of scytalone 12 (20 mM) to the assay mixture.

**Feeding Experiments**

Cultures of the respective fungal transformants or the wt strain were inoculated into liquid cultures (50 mL DPY medium in 250 mL flask) and grown for two days at 28 °C with 110 rpm shaking. 2 mg of the respective compound (1a or 12) dissolved in DMSO were added and cultures were grown for another 16 h. Chemical extraction was performed as mentioned above.

**Synthesis of 15a**

6 mg of 15a were dissolved in 2 mL MeOH and a slight excess of TMS-diazomethane (2 M in diethyl ether) were added dropwise while stirring. The sample was incubated for 2 h, the solvent was evaporated and the sample was directly analysed by NMR.

**Biosynthetic Gene Cluster Analysis**

Cluster identification: Draft genome of *T. reesei* QM6a was obtained from NCBI (WGS: AAIL02) and putative gene clusters were predicted using the secondary metabolites analysis tool fungiSMASH. Among the 32 predicted gene clusters, the first shared 71 % homology with the sorbicillinoid biosynthetic gene cluster of *P. chrysogenum*. The respective cluster sequence was used as the query sequence for gene identification/protein prediction with FGEnESH. Subsequently conserved domain analysis was applied using BLASTp. In same manner the sorbicillinoid biosynthetic gene cluster was identified in the draft genome of *P. chrysogenum* (WGS: JMSF01).

Selection of candidate genes: Bioinformatic analysis of the sorbicillinoid biosynthetic gene cluster in *T. reesei* QM6a revealed three candidate genes that, based on their predicted function, could be involved in sorbicillinoid biosynthesis: a short FMO (sorD), a short-chain dehydrogenase/reductase (sdr) and a cytochrome P450 dependent monoxygenase (p450). Analysis of *T. reesei* cDNA showed that sorD and p450 were expressed under sorbicillinoid producing conditions, although the expression of p450 was weak. Although the SorD proteins from *T. reesei* (XP_006961562) and *P. chrysogenum* (XP_002567557) possess the same conserved domains, both proteins share only 18.3 % identity and 30.2 % similarity based on protein alignment using the software geneious® (standard parameters).

ARTEMIS analysis: The alignment tool tBLASTx was used to create a comparison file between the sorbicillinoid BGC of *T. reesei* and *P. chrysogenum*. This file was used for identification of homologous proteins between the two cluster using ARTEMIS. Results show that the following proteins are homologous: SorA, SorB, SorC, one MFS and one TF, but not SorD.

**Additional Results**

**Confirming Expression of sorD in +sorABD Transformants**

In order to confirm that sorD was truly expressed in +sorABD transformants their total RNA was extracted and converted to cDNA as described previously. Expression of sorD was confirmed by PCR (Figure S17). The expression plasmid pTYGSade-sorA was used as a positive control and the freshly isolated RNA was used as a negative control to exclude contamination with genomic DNA.

**Comparison of Sorbicillinoids Produced by +sorABCD Transformants Prior to Chemical Extraction**

Transformants were grown for four days in liquid DPY-medium at 28°C with 110 rpm shaking. Cultures were homogenized using a hand blender and cells were separated by filtration. Crude supernatant was directly subjected to LCMS-analysis. LCMS-analysis revealed presence of dimeric sorbicillinoids already prior to chemical extraction (Figure S10).
Sorbicillinol 2a was produced enzymatically by SorC using 1a as the substrate and extracted with ethyl acetate. Formation of 2a was confirmed by LCMS prior to incubation. 2a was incubated for 20 h in 50 mM phosphate (pH 8) buffer at ambient temperature either in presence or absence of NADPH. Upon longer incubation (16-24 h) epoxysorbicillins 10 and the reduced sorbicillinol 15a are formed from sorbicillinol 2 during in vitro assay with SorC when an excess of NADPH is present (Figure S12).
Table S1: Media used during this study.

| Media                  | Components                                                                 |
|------------------------|-----------------------------------------------------------------------------|
| 2TY medium             | 16 g/L tryptone, 10 g/L yeast extract, 5 g/L NaCl                            |
| CZD/S agar             | 35 g/L Czapek Dox broth, 182.2 g/L D-sorbitol, 1 g/L ammonium sulphate, 0.5 g/L adenine, 1.5 g/L-methionine, 15 g/L agar |
| CZD/S soft agar        | 35 g/L Czapek Dox broth, 182.2 g/L D-sorbitol, 1 g/L ammonium sulphate, 0.5 g/L adenine, 1.5 g/L-methionine, 8 g/L agar |
| CZD/S1 agar            | 35 g/L Czapek Dox broth, 182.2 g/L D-sorbitol, 1 g/L ammonium sulphate, 1.5 g/L-methionine, 15 g/L agar |
| CZD/S1 soft agar       | 35 g/L Czapek Dox broth, 182.2 g/L D-sorbitol, 1 g/L ammonium sulphate, 1.5 g/L-methionine, 8 g/L agar |
| CZD/S1 agar w/o methionine | 35 g/L Czapek Dox broth, 182.2 g/L D-sorbitol, 1 g/L ammonium sulphate, 15 g/L agar |
| CZD/S1 soft agar w/o methionine | 35 g/L Czapek Dox broth, 182.2 g/L D-sorbitol, 1 g/L ammonium sulphate, 8 g/L agar |
| DPY medium             | 20 g/L dextrine from potato starch, 10 g/L polypeptone, 5 g/L KH$_2$PO$_4$, 0.5 g/L MgSO$_4$· H$_2$O |
| Galactose-ura medium   | 6.7g/L yeast nitrogen base, 20g/L galactose monohydrate, 1.3g/L complete supplement mixture minus uracil |
| Glucose-ura medium     | 6.7g/L yeast nitrogen base, 20g/L glucose monohydrate, 1.3g/L complete supplement mixture minus uracil |
| GN medium              | 20 g/L D (+)-glucose monohydrate, 10 g/L nutrient broth No. 2 from oxoid (Thermo Scientific) |
| LB agar                | 5 g/L yeast extract, 10 g/L tryptone, 5 g/L NaCl, 15 g/L agar               |
| LB medium              | 5 g/L yeast extract, 10 g/L tryptone, 5 g/L NaCl                             |
| ME medium (Roth x923)  | 12.75 g/L malt extract, 0.78 g/L peptone from soy bean, 2.35 g/L glycerine, 2.75 g/L dextrin from potato starch |
| PD agar                | 24 g/L potato dextrose broth, 15 g/L agar                                   |
| PDB medium             | 24 g/L potato dextrose broth                                                |
| SM-ura agar            | 1.7 g/L yeast nitrogen base, 20 g/L D (+)-glucose monohydrate, 5 g/L ammonium sulphate, 0.77 g/L complete supplement mixture minus uracil (Q biogene), 25 g/L agar |
| SOB medium             | 2 g/L tryptone, 0.5 g/L yeast extract, 58.4 mg/L NaCl, 18.6 mg/L KCl       |
| SOC medium             | 937.5 mL/L SOB medium, 12.5 mL/L MgCl$_2$, 50 mL/L glucose (20 %) components were autoclaved separately and sterile filtrated after mixing |
| TB-medium              | 24 g/L yeast extract, 12 g/L tryptone, 4 mL/L glycerine,                   |
| YPAD agar              | 10 g/L yeast extract, 10 g/L tryptone, 0.3 g/L adenine, 20 g/L D (+)-glucose monohydrate, 15 g/L agar |
| YPAD medium            | 10 g/L yeast extract, 10 g/L tryptone, 0.3 g/L adenine, 20 g/L D (+)-glucose monohydrate |
Table S2: Primers used during this study.

| Primer name | Sequence (5’ to 3’) |
|-------------|---------------------|
| SorA P1     | GCCAACTTTGTACAAAAAAACGAGGCTCCGCACTGAAGCTGACGGCCCTCAA |
| SorA P3     | TTGCAAATGTCTACGTCGAGG |
| SorA P6     | CGAGATTAACAGGAGATGCTC |
| SorA P7     | TCCTGATATTGCCTGGCTT |
| SorA P9     | CCTGTACTCATGGCCTTAATGC |
| SorA P10    | ATCTGCATCTTTATCGGGGAAAT |
| SorB P1     | GCCAACTTTGTACAAAAAAGCAGGCTCCGCATGGCGGCCTCAAAGTACACG |
| SorB P2     | ATTAACACTAAACGCCAGGTCTTGGATATCGAGGTTCAAGATGGAGGCTT |
| SorB P3     | CCAATCTGATATCCCAAGTCAAGATGGAGGCGCAACAATCATCAC |
| SorB P4     | GTCAACCAAATCAGTACTGTAAG |
| SorB P5     | CTTAAGTTCACTATAGCTTGAGGCTGAGTTGAGTACGAATCACG |
| SorC F      | TTTTCTACACCCACAGTCAAAGATGGAGGAGCATGCAAGTCAATTGCTACACTCAC |
| SorC R      | TTTCTACACCCACAGTCAAAGATGGAGGAGCATGCAAGTCAATTGCTACACTCAC |
| SorC for ligation F | GAGAATTCCTGATAGAGGCGCAACAATCATCA |
| SorC for ligation R | TTTCTACACCCACAGTCAAAGATGGAGGAGCATGCAAGTCAATTGCTACACTCAC |
| SorD F      | CATGCCAACGCGGCTCTAAAGCTCTTACACCT |
| SorD R      | CATGCCAACGCGGCTCTAAAGCTCTTACACCT |
| SorD-ligation F | CATGCCAACGCGGCTCTAAAGCTCTTACACCT |
| SorD-ligation R | CATGCCAACGCGGCTCTAAAGCTCTTACACCT |
| SorD yeast F | GCGGCTGACATGGCTTAAGTCGAGG |
| SorD yeast R | CGTACGCTAGCTGGAAGGAGCATGCAAGTCAATTGCTACACTCAC |
| SorD-ligation F | CTAGCTAGCTACGCCCCCCCTTTTGTCCG |
| SorD-ligation R | CTAGCTAGCTACGCCCCCCCTTTTGTCCG |
| SorP450 F   | TTCTTTCAACACAAGATCCCAAAGTCAAAGATGTCAAACCTGGCCTTGGC |
| SorP450 R   | TTTCATTCTATGCGTTATGAACATGTTCCCTCTTTACGAGACTCGGCAAAGGC |
| SorP450 F2  | TCGTGTGAACCAGATGAGGAGCATGCAAGTCAATTGCTACACTCAC |
| SorP450 R4  | CGAATTCCTGATAGAGGAGCATGCAAGTCAATTGCTACACTCAC |
| Sor-SDR F   | ACAGCTTTCCCGGTGAGCAGACATACGGGTAATGCTTCTCCAGCCATAGG |
| Sor-SDR R   | ACAGCTTTCCCGGTGAGCAGACATACGGGTAATGCTTCTCCAGCCATAGG |
| Sor-SDR F2  | GCCAAATGGGTGTAAGAGGCGGAGTCAAGGCTGAGTTGACATGCAAGTCAATTGCTACACTCAC |
| Sor-SDR R2  | GCCAAATGGGTGTAAGAGGCGGAGTCAAGGCTGAGTTGACATGCAAGTCAATTGCTACACTCAC |
| TR-SorA-KO-P1F | AGGCCAACATTGCTCAAAAGACGAGGCTCCCGCATAATATATATAGGTAAG |
| TR-SorA-KO-P2R | CGGAAAGATCACAAGGAGATCCTCAGCGAGTCCAACAGGAGAAAGG |
| TR-SorA-KO-P3F | CCCAGGACTGTCCGAGGGCAAGGAATAGGGCAAGCAGGAAGCTCGCCG |
| TR-SorA-KO-P4R | AATGCCAAATTTTGTGCAAAAGACGAGGCTCCCGCATAATATATATAGGTAAG |
| HygR-KO-P1F | CATTGATGGGGATCCTCTAGTG |
| HygR-KO-P2R | TTTCTACACCCACAGTCAAAGATGGAGGAGCATGCAAGTCAATTGCTACACTCAC |
| TR-SR-FL-P1F | GCCAAACTTTGTACAAAAAAACGAGGCTCCGCACTGAAGCTGACGGCCCTCAA |
| TR-SR-FL-P2R | ACGATTTCTAGTGTAAGAGATCCTAAGGTCAATCTCATATCTCGAGGCTT |
| TR-SR-FR-P3F | GTCGGAGGCGGCAAAGGAATAGGGCAAGCAGGAAGCTCGCCG |
| TR-SR-FR-P4R | TGGGCAAATTTTGTGCAAAAGACGAGGCTCCCGCATAATATATATAGGTAAG |
| TR-P450-KO-P1F | ATGCCAACTTTGTACAAAAAAACGAGGCTCCGCACTGAAGCTGACGGCCCTCAA |
| TR-P450-KO-P2R | CGGAAAGATCACAAGGAGATCCTCAGCGAGTCCAACAGGAGAAAGG |
| TR-P450-KO-P3F | CCCAGGACTGTCCGAGGGCAAGGAATAGGGCAAGCAGGAAGCTCGCCG |
| TR-P450-KO-P4R | AATGCCAAATTTTGTGCAAAAGACGAGGCTCCCGCATAATATATATAGGTAAG |
| Sor-AKOp1-P1F | CTTGATGGGGATCCTCTAGTG |
| Sor-AKOp1-P2R | CGGAAAGATCACAAGGAGATCCTCAGCGAGTCCAACAGGAGAAAGG |
| Sor-AKOp2-P1F | CGGAAAGATCACAAGGAGATCCTCAGCGAGTCCAACAGGAGAAAGG |
| Sor-AKOp2-P2R | CGGAAAGATCACAAGGAGATCCTCAGCGAGTCCAACAGGAGAAAGG |
**Supporting Information**

| Plasmid                  | Features                                                                 |
|--------------------------|--------------------------------------------------------------------------|
| pESC-ura (AgilentTechnologies) | URA3 PGAL1 PGAL10 ampR 2µori ColE1                                      |
| pESC-ura-sorD (AgilentTechnologies) | sorD inserted into MCS2                                               |
| pET100/D-TOPO (Invitrogen) | kmpR pBR322 rop LacI pT7 His-Tag                                      |
| pET100/D-TOPO-sorD (Invitrogen) | encodes for sorD with codon-optimized sequence for expression in E.coli |
| pET28a (Novagen)          | kanR pUC ori 2µ ori URA3 ccdB                                         |
| pET28a-sorC               | sorC inserted by LR-recombination                                      |
| pET28a-sorD               | sorD inserted by LR-recombination                                      |
| pE-YA[1]                  | karR pUC ori 2µ ori URA3 ccdB                                         |
| pE-YA-sorA                | sorA inserted by LR-recombination                                      |
| pE-YA-sorB                | sorB inserted by LR-recombination                                      |
| pE-Y-TR-sorA              | hygR flanked by sorA fragments                                         |
| pE-Y-TR-sor"p450"[1]      | hygR flanked by sor"p450" fragments                                     |
| pE-Y-TR-sor"sdr"[1]       | hygR flanked by sor"sdr" fragments                                     |
| pTYGSargs/ade/met[1]      | PamyB Padh Peno PgdpA ampR ColE1 2µ ori URA3 ccdB argB/adeA/sC          |
| pTYGSad-sorA              | sorA under control of PamyB                                             |
| pTYGSad-sorD              | sorD under control of Padh                                             |
| pTYGSad-sorAD             | sorA under control of PamyB, sorD under control of Padh                |
| pTYGSad-sorB              | sorB under control of PamyB                                             |
| pTYGSargs-sorC            | sorC under control of Padh                                             |
| pTYGSargs-sorBC           | sorB under control of PamyB, sorC under control of Padh                |
| pTYGSmets-sor"p450"[1]    | Sor"p450" under control of Padh                                         |
| pTYGSmets-sor"sdr"[1]     | Sor"sdr" under control of PgdpA                                         |

Table S3: Plasmids used in this study.
### Table S4: Fungal strains used during this study.

| Fungal strain | genotype |
|---------------|----------|
| *A. oryzae* NSAR1 | argB, adeA, sC, niaD |
| *T. reesei* QM6a | wild type |
| *A. oryzae-sorA* | +sorA, sC, niaD |
| *A. oryzae-sorB* | +sorB, sC, niaD |
| *A. oryzae-sorAB* | +sorA, +sorB, sC, niaD |
| *A. oryzae-sorABC* | +sorA, +sorB, +sorC, sC, niaD |
| *A. oryzae-sorABCD* | +sorA, +sorB, +sorC, +sorD, sC, niaD |
| *A. oryzae-sorABD* | +sorA, +sorB, +sorD, sC, niaD |
| *A. oryzae-sorC* | +sorC, sC, niaD |
| *A. oryzae-sorD* | +sorD, sC, niaD |
| *A. oryzae-sorCD* | +sorC, +sorD, sC, niaD |
| *A. oryzae-sorABCDp450* | +sorA, +sorB, +sorC, +sorD, +sor"p450", niaD |
| *A. oryzae-sorABCDsdr* | +sorA, +sorB, +sorC, +sorD, +sor"sdr", niaD |
| *T. reesei* QM6a ∆tmus53[5] | deletion strain of human LIG4 homolog |
| *T. reesei* QM6a | ∆tmus53, ∆sorA, hygR |
| ∆tmus53∆sorA | ∆tmus53, ∆sor"p450", hygR |
| ∆tmus53∆sor"p450" | ∆tmus53, ∆sor"sdr", hygR |
| *T. reesei* QM6a | ∆tmus53, ∆sor"sdr", hygR |
| ∆tmus53∆sor"sdr" | ∆tmus53, ∆sor"sdr", hygR |
Sorbicillinoid Compounds Identified by Mass and UV Absorption Profiles

UV\textsubscript{max} (MeOH): 216 nm, 250 nm, 361 nm

UV-absorption, mass (identical) and fragmentation pattern (very similar) are matching spirosporicillinol B \textbf{6b} whose structure is fully characterized by NMR (see section NMR).

Figure S1: UV-absorption (top) and fragmentation pattern of \textbf{6a} in ES\textsuperscript{+} TIC (middle) and ES TIC (bottom).
UV$_{\text{max}}$ (MeOH): 230 nm, 295 nm, 310 nm, 363 nm

Compound was identified based on mass, retention time and UV-absorption$^6$

Figure S2: UV-absorption (top) and fragmentation pattern of 5 in ES$^+$ TIC (middle) and ES$^-$ TIC (bottom).
Compound was identified based on mass, retention time and UV-absorption[7]

Figure S3: UV-absorption (top) and fragmentation pattern of 11 in ES+ TIC (middle) and ES TIC (bottom).
Figure S4: UV-absorption (top) and fragmentation pattern in ES+ TIC (middle) and ES- TIC (bottom) for a putative sorbicillinoid related compound in Fig. 1A marked with * eluting at approx. 6.2 min.

Figure S5: UV-absorption (top) and fragmentation pattern in ES+ TIC (middle) and ES- TIC (bottom) for a putative sorbicillinoid related compound in Fig. 1A marked with * eluting at approx. 7.8 min.
**SUPPORTING INFORMATION**

**Figure S6:** UV-absorption (top) and fragmentation pattern of 2a in ES+ TIC (middle) and ES- TIC (bottom).

**UV**

\[ \lambda_{\text{max}} \text{ (MeOH): 292 nm} \]

Mass data and UV-absorption are in agreement with Cox et al.\(^8\)

**Chemical Formula:** C\(_{14}\)H\(_{18}\)O\(_4\)

**Exact Mass:** 248.1049

**Sorricillinol 2a**
2‴,3‴-dihydrobisvertinol 4b

Chemical Formula: C_{28}H_{30}O_{8}
Exact Mass: 500,2410

UV_{max} (MeOH): 224 nm, 269 nm, 306 nm, 379 nm
Compound was identified based on mass, retention time and UV-absorption\[7\]

Figure S7: UV-absorption (top) fragmentation pattern of 4b in ES+ TIC (middle) and ES- TIC (bottom).
2', 3'-dihydrosorbinol 2b

Chemical Formula: C_{14}H_{18}O_{4}
Exact Mass: 250,1205

$\text{UV}_{\text{max}}$ (MeOH): 220 nm, 272 nm, 315 nm
Mass data and UV-absorption are in agreement with Cox et al.$^8$

Figure S8: UV-absorption (top) and fragmentation pattern of 2b in ES+ TIC (middle) and ES- TIC (bottom).
SUPPORTING INFORMATION

reduced 2', 3'-dihydrosoorbicillinol 15b

Chemical Formula: C_{14}H_{20}O_{4}
Exact Mass: 252,1362

UV$_{\text{max}}$ (MeOH): 218 nm, 265 nm, 330 nm

Compound was identified based on mass, retention time and biosynthetic considerations.

Figure S9: UV-absorption (top) and fragmentation pattern of 15b in ES$^+$ TIC (middle) and ES$^-$ TIC (bottom).
**LCMS Chromatograms**

**Figure S10:** LCMS-chromatograms (ES TIC) of transformants expressing sorABCD before (A) or after (B) extraction. Dimeric sorbicillinoids are already formed prior to extraction with solvents.

**Figure S11:** LCMS analysis [DAD 210-600nm] of in vitro assays with SorC under different conditions. The control assay contained boiled enzyme; cs = co-solvent (v/v).
In Vitro Experimental Design and Results

Figure S1: Protein sequence of SorC based on T. reesei QM6a cDNA.

Figure S12: LCMS-chromatograms (ES TIC) of 1b incubated with SorC (top chromatogram) and of 1a incubated with SorC for a prolonged incubation time and an excess of NADPH (bottom chromatogram).
Supporting Information

Figure S14: SDS-PAGE of SorC during purification. M: marker, FT: flow through, W: wash, W50: wash (50 mM imidazole), E500: elution. 12% SDS gel run at 40 mA for 40 min.

Figure S15: Coding sequence of sorD from T. reesei QM6a based on cDNA sequencing.

Figure S16: A. map of the expression plasmid pET100-D-TOPO-sorD; B. SDS-PAGE of sorD-expression using the plasmid pET-100-D-TOPO-sorD in E. coli BL21. M: protein ladder; 1: pellet fraction (12 °C, 0.1 mM IPTG); 2: soluble fraction (12 °C, 0.1 mM IPTG); 3: pellet fraction (12 °C, 0.5 mM IPTG); 4: soluble fraction (12 °C, 0.5 mM IPTG); 5: pellet fraction (16 °C, 0.1 mM IPTG); 6: soluble fraction (16 °C, 0.1 mM IPTG); 7: pellet fraction (16 °C, 0.5 mM IPTG); 8: soluble fraction (16 °C, 0.5 mM IPTG). 12% SDS gel run at 40 mA for 40 min.
Figure S17: Confirmation of expression of sorD in +sorABD transformants by PCR using cDNA as template. M: DNA ladder marker; TF1: transformant 1; TF2: transformant 2; +: positive control using pTYGSade-sorD as template; -: negative control using freshly prepared RNA (from the respective TF tested) as template. 1% agarose gel run at 110 V for 20 min.
**In Vivo Experimental Design and Results**

(A) The strain QM6aΔTmus35, which contains the wild type gene cluster, like the parent strain QM6a, was transformed with bipartite fragments bearing the deletion cassette to replace the genes sorA, sdr and P450 with hygromycin B resistance gene. (B) PCRs were performed to test the successful integration of the deletion cassette using chromosomal DNA from ΔTmus35 (WT), ΔsorA, Δsdr and ΔP450 as templates and the indicated PCRs. Primers are shown by arrows and the primers sequences are found in Table S2. (C) LCMS analysis of the cultured strains ΔsorA, Δsdr and ΔP450 on ME medium showing the produced metabolites for each strain.

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**Figure S18. Deletion of SorA, Sdr and P450 genes in T. reesei QM6a.** (A) The strain QM6aΔTmus35, which contains the wild type gene cluster, like the parent strain QM6a, was transformed with bipartite fragments bearing the deletion cassette to replace the genes sorA, sdr and P450 with hygromycin B resistance gene. (B) PCRs were performed to test the successful integration of the deletion cassette using chromosomal DNA from ΔTmus35 (WT), ΔsorA, Δsdr and ΔP450 as templates and the indicated PCRs. Primers are shown by arrows and the primers sequences are found in Table S2. (C) LCMS analysis of the cultured strains ΔsorA, Δsdr and ΔP450 on ME medium showing the produced metabolites for each strain.
Figure S19. Heterologous co-expression of the cryptic P450 gene (top) chromatogram (DAD 210-600nm) or cryptic sdr gene (middle) with sorABCD. No differences in metabolite profile were observed compared to the sorABCD control strain (bottom).
**Compound Physical Data**

![Chemical Structure](image)

**sorbinillin 1a**

Chemical Formula: C_{14}H_{15}O_3  
Exact Mass: 232.1099

**UV\textsubscript{\text{max}} (MeOH):** 206 nm, 323 nm  
**HRMS (ESI) m/z (M-H):** calcd for C_{14}H_{15}O_3: 231.1021, found: 231.1022  

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**Figure S20:** UV-absorption (top) and fragmentation pattern of 1a in ES\textsuperscript{+} TIC (middle) and ES\textsuperscript{-} TIC (bottom).
Figure S21: HRMS data for 1a.

Table S5: Summarized NMR data for 1a recorded at 400 MHz in CDCl₃.

| Position | δC  | δH  | M  | J_H-H/Hz | HSQC | HMBC | H-H COSY |
|----------|-----|-----|----|----------|------|------|----------|
| 1        | 162.7 | 13.59 | s | - | - | - | 1, 2, 7 | - |
| 2        | 110.5 | - | s | - | - | - | - | - |
| 3        | 158.9 | - | - | - | - | - | - | - |
| 4        | 114.5 | - | - | - | - | - | - | - |
| 5        | 128.9 | 7.44 | s | - | CH | 1, 3, 8, 8 | - | - |
| 6        | 113.7 | - | - | - | - | - | - | - |
| 7        | 7.7 | 2.15 | s | - | CH₃ | 1, 2, 3 | - | - |
| 8        | 15.8 | 2.22 | s | - | CH₃ | 3, 4, 5 | 5 | - |
| 1'       | 192.7 | - | - | - | - | - | - | - |
| 2'       | 122.0 | 6.94 | d | 14.8 | CH | 1', 4' | 3', 6' | - |
| 3'       | 144.7 | 7.48 | dd | 10.2, 14.8 | CH | 1', 5' | 2', 6' | - |
| 4'       | 130.7 | 6.33 | m | - | CH | 2', 3', 6', 6' | - | - |
| 5'       | 141.3 | 6.29 | m | - | CH | 3', 6' | 6' | - |
| 6'       | 19.1 | 1.91 | d | 6.2 | CH₃ | 3', 4', 5', 2', 3', 4', 5' | - | - |
Figure S22: $^1$H-NMR spectrum for 1a recorded at 400 MHz in CDCl$_3$. 
Figure S2: $^{13}$C-NMR spectrum for 1a recorded at 125 MHz in CDCl$_3$. 
Figure S2: HSQC spectrum for 1a recorded at 400 MHz in CDCl$_3$. 
Figure S25: HMBC spectrum for 1a recorded at 400 MHz in CDC$_3$. 
Figure S26: $^1$H-$^1$H COSY spectrum for 1a recorded at 400 MHz in CDCl$_3$.

UV$_{\lambda_{\text{max}}}$ (MeOH): 215 nm, 283 nm, 332 nm

HRMS (ESI) $m/z$ (M-H)$^-$ calcd for C$_{14}$H$_{17}$O$_3$: 233.1178, found: 233.1177
Figure S27: UV-absorption (top) and fragmentation pattern of 1b in ES\(^+\) TIC (middle) and ES\(^-\) TIC (bottom).

Figure S28: HRMS data for 1b.
Table S6: Summarized NMR data for 1b recorded in CDCl₃.

| Position | δC  | δH  | M  | J_H-H/Hz | HSQC | HMBC H to C | H-H COSY |
|----------|-----|-----|----|----------|------|-------------|----------|
| 1        | 161.5 | 12.97 | s  | -        | -    | 1, 2, 3, 6  | -        |
| 2        | 110.4 | -    | -  | -        | -    | -           | -        |
| 3        | 158.7 | -    | -  | -        | -    | -           | -        |
| 4        | 114.5 | -    | -  | -        | -    | -           | -        |
| 5        | 129.3 | 7.39 | s  | -        | CH   | 1, 2, 3, 8  | 6, 8     |
| 6        | 113.1 | -    | -  | -        | -    | -           | -        |
| 7        | 7.6   | 2.16 | s  | -        | CH₃  | 1, 2, 3     | -        |
| 8        | 15.7  | 2.23 | s  | -        | CH₃  | 3, 4, 5     | -        |
| 1'       | 204.5 | -    | -  | -        | -    | -           | -        |
| 2'       | 38.0  | 2.99 | t  | 12.0     | CH₂  | 1', 3', 5', 3', 4' | 6' |
| 3'       | 27.7  | 2.43 | m  | -        | CH₂  | 1', 2', 4', 2', 4' | -        |
| 4'       | 126.3 | 5.53 | m  | -        | CH   | 3', 6'      | 3'       |
| 5'       | 129.7 | 5.53 | m  | -        | CH   | 3', 6'      | 6'       |
| 6'       | 18.0  | 1.68 | d  | 4.74     | CH₃  | 5', 4', 5'  | -        |

Figure S29: ¹H-NMR spectrum for 1b recorded at 400 MHz in CDCl₃.
Figure S30: $^{13}$C-NMR spectrum for 1b recorded at 125 MHz in CDCl$_3$. 
Figure S31: HSQC spectrum for 1b recorded at 400 MHz in CDCl₃.
Figure S32: HMBC spectrum for 1b recorded at 400 MHz in CDCl₃.
Figure S3: $^1$H-$^1$H COSY spectrum for 1b recorded at 400 MHz in CDCl$_3$.

UV$_\lambda_{max}$ (MeOH): 298 nm, 383 nm

HRMS (ESI) $m/z$ (M-H)$^-$ calcld for C$_{29}$H$_{31}$O$_8$: 495.2019, found: 495.2018
Figure S34: HRMS data for 3a.

Figure S35: UV-absorption (top) and fragmentation pattern of 3a in ES+ TIC (middle) and ES- TIC (bottom).
Table S7: Summarized NMR data for 3a recorded in CDCl₃.

| Position | δ_C | δ_H | M   | J_HH/Hz | HSQC | HMBC H to C | H-H COSY |
|----------|-----|-----|------|---------|------|-------------|----------|
| 1        | 41.5| 3.70| d    | 3.0     | CH   |              |          |
| 2        | 108.8| -   | -    | -       | -    |              |          |
| 3        | 194.8| -   | -    | -       | -    |              |          |
| 4        | 65.1 | -   | -    | -       | -    |              |          |
| 5        | 203.9| -   | -    | -       | -    |              |          |
| 6        | 73.6 | -   | -    | -       | -    |              |          |
| 7        | 48.4 | 3.43| d    | 3.0     | CH   |              |          |
| 8        | 70.9 | -   | -    | -       | -    |              |          |
| 9        | 202.8| -   | -    | -       | -    |              |          |
| 10       | 53.9 | 2.91| q    | 7.1     | CH   |              | 9, 11, 15|
| 11       | 209.4| -   | -    | -       | -    |              |          |
| 12       | 77.4 | -   | -    | -       | -    |              |          |
| 13       | 9.4  | 1.18| s    | -       | CH₃  | 3, 4, 5, 8, 10|
| 14       | 24.8 | 1.27| s    | -       | CH₃  | 1, 5, 6, -   |
| 15       | 13.6 | 1.38| d    | 7.1     | CH₃  | 9, 10, 11, 10|
| 16       | 26.6 | 1.36| s    | -       | CH₃  | 7, 11, 12, - |
| 1'       | 173.1| -   | -    | -       | -    |              |          |
| 2'       | 117.9| 6.16| d    | 7.1     | CH   | 1', 4', 5', 3'|
| 3'       | 145.6| 7.42| m    | -       | CH   | 1', 4', 2'   |
| 4' = 4'' | 142.4| 7.27| dd   | 10.9    | CH   | 2', 5', 2''  |
| 5' = 5'' | 130.6| 6.25| m    | -       | CH   | 2', 6', 3', 4', 6' |
| 6' = 6'' | 19.3  | 1.91| d    | 6.7     | CH₃  | 4', 5', 5'   |
| 1''      | 196.9| -   | -    | -       | -    |              |          |
| 2''      | 123.1| 6.33| m    | -       | CH   | 1'', 3'', 4'', 5'', 5'' |
| 3''      | 147.4| 7.37| m    | -       | CH   | 1'', 4'', 2'' |
**Figure S36**: $^1$H-NMR spectrum for 3a recorded at 500 MHz in CDCl$_3$. 

**Diagram Description**: The spectrum shows the chemical shifts and multiplicity of various nuclei in compound 3a. The peaks correspond to different hydrogen atoms with their respective chemical environments, which are critical for assigning the structure of the compound.
Figure S37: $^{13}$C-NMR spectrum for 3a recorded at 125 MHz in CDCl$_3$. 
Figure S38: HSQC spectrum for 3a recorded at 500 MHz in CDCl₃.
Figure S39: HMBC spectrum for 3a recorded at 500 MHz in CDCl₃.
Figure S40: $^1$H-$^1$H COSY spectrum for 3a recorded at 500 MHz in CDCl$_3$.

UV $\lambda_{max}$ (MeOH): 266 nm, 295 nm, 382 nm

HRMS (ESI) $m/z$ (M-H) calcd for C$_{28}$H$_{33}$O$_8$: 497.2175, found: 497.2155
Figure S41: UV-absorption (top) and fragmentation pattern of 3b in ES+ TIC (middle) and ES- TIC (bottom).

Figure S42: HRMS data for 3b.
Table S8: Summarized NMR data for 3b recorded in CDCl₃.

| Position | δC  | δH  | M   | J_H_H/Hz | HSQC  | HMBC | H-H COSY |
|----------|-----|-----|-----|----------|-------|------|----------|
| 1        | 41.2| 3.72| d   | 3.4      | CH    | 2, 5, 6, 14, 1' | 7 |
| 2        | 108.9| -   | -   | -        | -     | -    | -        |
| 3        | 194.3| -   | -   | -        | -     | -    | -        |
| 4        | 64.4 | -   | -   | -        | -     | -    | -        |
| 5        | 203.2| -   | -   | -        | -     | -    | -        |
| 6        | 73.2 | -   | -   | -        | -     | -    | -        |
| 7        | 47.0 | 3.49| d   | 3.4      | CH    | 1, 2, 8 11, 12, 16, 1'' |
| 8        | 71.7 | -   | -   | -        | -     | -    | -        |
| 9        | 202.8| -   | -   | -        | -     | -    | -        |
| 10       | 53.7 | 2.95| q   | 3.4      | CH    | 9, 11, 15 12, 15 |
| 11       | 209.2| -   | -   | -        | -     | -    | -        |
| 12       | 77.4 | -   | -   | -        | -     | -    | -        |
| 13       | 9.4  | 1.21| s   | -        | CH₃   | 3, 4, 5, 8 |
| 14       | 24.6 | 1.30| s   | -        | CH₃   | 1, 5, 6 |
| 15       | 13.4 | 1.36| d   | 7.1      | CH₃   | 9, 10, 11 10 |
| 16       | 26.9 | 1.41| s   | -        | CH₃   | 7, 11, 12 |
| 1'       | 173.6| -   | -   | -        | -     | -    | -        |
| 2'       | 117.9| 6.21| d   | 6.8      | CH    | 1', 4', 5' 3' |
| 3'       | 145.7| 7.44| dd  | 10.0     | CH    | 1', 4', 5', 2' |
| 3'       | 15.0 | -   | -   | -        | -     | 6'    | -        |
| 4'       | 142.6| 7.30| dd  | 10.9, 14.9| CH    | 3', 5' 5' |
| 5'       | 131.1| 6.31| m   | -        | CH    | 2', 4', 6' 3', 4', 6' |
| 6'       | 19.1 | 1.91| d   | 6.6      | CH₃   | 4', 5' 5' |
| 1''      | 205.7| -   | -   | -        | -     | -    | -        |
| 2''      | 40.3 | 2.65| m   | -        | CH₂   | 1'', 3'', 4'' |
| 3''      | 27.5 | 2.32| m   | -        | CH₂   | 1'', 4'', 2'', 4'' |
| 4''      | 129.4| 5.37| m   | -        | CH    | 2'', 3'', 5'', 6'', 2'', 4'' |
| 5''      | 126.6| 5.47| m   | -        | CH    | 3'', 5'', 6'' |
| 6''      | 18.0 | 1.64| d   | 6.8      | CH₃   | 4'', 5'' 5'' |
Figure S43: $^1$H-NMR spectrum for 3b recorded at 500 MHz in CDCl$_3$. 
Figure S44: $^{13}$C-NMR spectrum for 3b recorded at 125 MHz in CDCl$_3$. 
Figure S45: HSQC spectrum for 3b recorded at 500 MHz in CDCl₃.
Figure S4: HMBC spectrum for 3b recorded at 500 MHz in CDCl₃.
**Figure S47**: $^1$H-$^1$H COSY spectrum for $3b$ recorded at 500 MHz in CDCl$_3$.

**tetradihydrobisorbicillinol 3c**

Chemical Formula: C$_{28}$H$_{36}$O$_8$

Exact Mass: 500.2410

UV$_{\text{max}}$ (MeOH): 222 nm, 271 nm, 383 nm

HRMS (ESI) $m/z$ (M-H): calcd for C$_{28}$H$_{35}$O$_8$: 499.2332, found: 499.2337
Figure S48: UV-absorption (top) and fragmentation pattern of 3c in ES+ TIC (middle) and ES- TIC (bottom).

Figure S49: HRMS data for 3c.
Table S9: Summarized NMR data for 3c recorded in CDCl₃.

| Position | δC  | δH  | M   | JHH/Hz | HSQC | HMBC H to C | H-H COSY |
|----------|-----|-----|-----|--------|------|-------------|----------|
| 1        | 41.5 | 3.66| d   | 3.2    | CH   | 2, 3, 5, 6, 8 (14) | 7        |
| 2        | 109.8 | -   | -   | -      | -    | -           | -        |
| 3        | 190.3 | -   | -   | -      | -    | -           | -        |
| 4        | 63.2  | -   | -   | -      | -    | -           | -        |
| 5        | 202.7 | -   | -   | -      | -    | -           | -        |
| 6        | 72.4  | -   | -   | -      | -    | -           | -        |
| 7        | 47.3  | 3.44| d   | 3.1    | CH   | 2, 5, 9, 11, 12, 16, 1'' | 1        |
| 8        | 71.5  | -   | -   | -      | -    | -           | -        |
| 9        | 202.7 | -   | -   | -      | -    | -           | -        |
| 10       | 53.6  | 2.87| q (buried) | 12.4 | CH   | 9, 11, 15 | 15       |
| 11       | 209.9 | -   | -   | -      | -    | -           | -        |
| 12       | 77.0  | -   | -   | -      | -    | -           | -        |
| 13       | 9.2   | 1.18| s   | -      | CH₃  | 3, 4, 5, 8, 14 | -        |
| 14       | 25.1  | 1.29| s   | -      | CH₃  | 1, 5, 6 | -        |
| 15       | 13.4  | 1.36| d   | 1.4    | CH₃  | 8, 9, 11, 12 | 10       |
| 16       | 27.1  | 1.38| s   | -      | CH₃  | 4, 7, 11, 12 | -        |
| 1''      | 181.9 | -   | -   | -      | -    | -           | -        |
| 2''      | 42.7  | 2.67| m   | -      | CH₂  | 3', 4' | -        |
| 3''      | 26.8  | 2.28| m   | -      | CH₂  | 4', 5' | 4'       |
| 4'' = 4''| 129.7 | 5.42| m   | -      | CH   | 6'    | 3', 4', 6' |
| 5'' = 5''| 126.3 | 5.51| m   | -      | CH   | 6'    | 3', 4', 6' |
| 6'' = 6''| 18.1  | 1.64| m   | -      | CH₃  | 4', 5' | 4', 5'   |
| 1'''     | 205.7 | -   | -   | -      | -    | -           | -        |
| 2'''     | 38.1  | 2.92| m   | -      | CH₂  | 1'', 3'', 4'' | 3''      |
| 3'''     | 27.1  | 2.37| m   | -      | CH₂  | 1'', 4'', 5'', 2'', 4'', 5'' | -        |
Figure S50: $^1$H-NMR spectrum for 3c recorded at 600 MHz in CDCl$_3$. 

SUPPORTING INFORMATION
**Figure S5**: $^{13}$C-NMR spectrum for 3c recorded at 125 MHz in CDCl$_3$. 
Figure S52: HSQC spectrum for 3c recorded at 600 MHz in CDCl₃.
Figure S53: HMBC spectrum for 3c recorded at 600 MHz in CDCl₃.
Figure S54: $^1$H-$^1$H COSY spectrum for 3c recorded at 600 MHz in CDCl$_3$.

bisvertinol 4a

Chemical Formula: $C_{28}H_{34}O_8$
Exact Mass: 498.2254

UV$_{\text{max}}$ (MeOH): 202 nm, 223 nm, 272 nm, 304 nm, 404 nm
HRMS (ESI) $m/z$ (M-H) calcd for $C_{28}H_{34}O_8$: 497.2175, found: 497.2174
Figure S55: UV-absorption (top) and fragmentation pattern of 4a in ES+ TIC (middle) and ES- TIC (bottom).

Figure S56: HRMS data for 4a.
Table S10: Summarized NMR data for 4a recorded in CDCl₃.

| Position | δC  | δH  | M   | J_H-H/Hz | HSQC | HMBC  | H-H COSY |
|----------|-----|-----|-----|----------|------|-------|----------|
|          |     |     |     |          |      |       |          |
| 1        | 110.5 | -   | -   | -        | -    | -     | -        |
| 2        | 162.7 | -   | -   | -        | -    | -     | -        |
| 3        | 79.7  | -   | -   | -        | -    | -     | -        |
| 4        | 53.4  | 3.62| s   | CH       | 2, 3, 6, 7, 13, 14, 1' |
| 5        | 100.5 | -   | -   | -        | -    | -     | -        |
| 6        | 191.6 | -   | -   | -        | -    | -     | -        |
| 7        | 53.4  | -   | -   | -        | -    | -     | -        |
| 8        | 106.2 | -   | -   | -        | -    | -     | -        |
| 9        | 73.8  | -   | -   | -        | -    | -     | -        |
| 10       | 36.7  | 2.60| s   | CH₂      | 8, 9, 11, 12, 16, 1' |
| 11       | 103.7 | -   | -   | -        | -    | -     | -        |
| 12       | 191.6 | -   | -   | -        | -    | -     | -        |
| 13       | 6.9   | 1.52| s   | CH₃      | 1, 2, 6 |
| 14       | 25.7  | 1.47| s   | CH₃      | 2, 3, 4, 7 |
| 15       | 19.2  | 1.33| s   | CH₃      | 4, 7, 8, 12 |
| 16       | 22.3  | 1.27| s   | CH₃      | 8, 9, 10 |
| 1'       | 169.2 | -   | -   | -        | -    | -     | -        |
| 2'       | 120.4 | 6.38| d   | 15.6     | CH   | 1', 4', 3' |
| 3'       | 139.2 | 7.29| m   | CH       | 1', 5', 2', 4' |
| 4'       | 131.0 | 6.28| m   | CH       | 2', 6', 3', 5' |
| 5'       | 137.1 | 6.12| dq  | 6.8, 13.9| CH   | 3', 6', 6', 4' |
| 6' = 6'' | 19.1  | 1.88| m   | CH₃      | 4', 5', 5', 5', 4', 4'', 5'' |
| 1''      | 180.3 | -   | -   | -        | -    | -     | -        |
| 2''      | 120.4 | 6.16| d   | 14.9     | CH   | 1'', 4'', 3'' |
| 3''      | 143.1 | 7.30| m   | CH       | 1'', 4'', 5'', 2'', 4'' |
| 4''      | 130.7 | 6.34| m   | CH       | 2'', 3'', 3'', 5'', 6'' |
| 5''      | 141.3 | 6.36| m   | CH       | 3'', 6'', 6'' |

SUPPORTING INFORMATION
Figure S57: $^1$H-NMR spectrum for 4a recorded at 500 MHz in CDCl$_3$. 

**Figure S57:** $^1$H-NMR spectrum for 4a recorded at 500 MHz in CDCl$_3$. 

**Figure S57:** $^1$H-NMR spectrum for 4a recorded at 500 MHz in CDCl$_3$. 

**Figure S57:** $^1$H-NMR spectrum for 4a recorded at 500 MHz in CDCl$_3$. 

**Figure S57:** $^1$H-NMR spectrum for 4a recorded at 500 MHz in CDCl$_3$. 

**Figure S57:** $^1$H-NMR spectrum for 4a recorded at 500 MHz in CDCl$_3$.
Figure S58: $^{13}$C-NMR spectrum for 4a recorded at 125 MHz in CDCl$_3$. 
Figure S59: HSQC spectrum for 4a recorded at 500 MHz in CDCl₃.
Figure S60: HMBC spectrum for 4a recorded at 500 MHz in CDCl₃.
Figure S61: $^1$H–$^1$H COSY spectrum for 4a recorded at 500 MHz in CDCl$_3$.

Isomer B was identified by comparing NMR data with those reported by Hirota et al.\cite{9}

UV$_{\text{max}}$ (MeOH): 361 nm
HRMS (ESI) $m/z$ (M+H)+ calcd for C$_{25}$H$_{29}$O$_{10}$: 489.1761, found: 489.1764

Isomer B was identified by comparing NMR data with those reported by Hirota et al.\cite{9}
Figure S62: UV-absorption (top) and fragmentation pattern of 6b in ES- TIC (middle) and ES+ TIC (bottom).

Figure S63: HRMS data for 6b.
Table S11: Summarized NMR data for 6b recorded in CDCl₃.

| Position | δC  | δH  | M  | J_H/Hz | HSQC | HMBC H to C | H-H COSY |
|----------|-----|-----|----|--------|------|-------------|----------|
| 1        | 39.9| 3.27| t  | 2.8    | CH   | 2, 3, 4, 5, 6, 7, 8, 17, 1' | -        |
| 2        | 109.8| -   | -  | -      | -    | -           | -        |
| 3        | 193.3| -   | -  | -      | -    | -           | -        |
| 4        | 69.5 | -   | -  | -      | C    | -           | -        |
| 5        | 205.7| -   | -  | -      | C    | -           | -        |
| 6        | 74.4 | -   | -  | -      | C    | -           | -        |
| 7a       | 40.0 | 2.42| dd | 2.5, 14.1| CH₂ | 1, 2, 6, 8, 9 | 7b       |
| 7b       | 40.0 | 2.87| dd | 3.3, 14.0| CH₂ | 1, 2, 6, 8, 9 | 7a       |
| 8        | 81.6 | -   | -  | -      | C    | -           | -        |
| 9        | 165.6 or 167.8 | -   | -  | -      | C    | -           | -        |
| 10       | 70.1 | 3.98| td | 6.4, 9.9| CH   | 11, 12, 15 | 11, 15   |
| 11       | 83.2 | 4.35| dd | 7.9, 10.2| CH   | 12, 15 | 10, 12    |
| 12       | 70.3 | 4.55| m  | -      | CH   | 11, 13, 14 | 11, 13   |
| 13       | 136.7| 6.73| m  | -      | CH   | 11, 14, 15 | 12, 15   |
| 14       | 128.9| -   | -  | -      | C    | -           | -        |
| 15a      | 30.8 | 2.31| m  | -      | CH₂  | 10, 11, 18 | 10       |
| 15b      | 30.8 | 3.01| dd | 6.4, 17.7| CH₂ | 10, 11, 18 | 10       |
| 16       | 8.6  | 1.35| s  | -      | CH₃  | 3, 4, 5, 8 | -        |
| 17       | 24.4 | 1.29| s  | -      | CH₃  | 1, 5, 6   | -        |
| 18       | 165.6| -   | -  | -      | C    | -           | -        |
| 19       | 52.6 | 3.78| s  | -      | CH₃  | 18         | -        |
| 1'       | 165.6 or 167.8 | -   | -  | -      | C    | -           | -        |
| 2'       | 117.8| 6.12| d  | 14.9   | CH   | 2, 1', 3', 4' | 3'     |
| 3'       | 143.0| 7.33| dd | 10.6, 14.9 | CH | 1', 2', 4', 5' | 2', 4' |
| 4'       | 130.9| 6.27| m  | -      | CH   | 6', 2'   | 5', 3'   |
| 5'       | 140.3| 6.21| m  | -      | CH   | 6', 3'   | 4', 6'   |
| 6'       | 19.1 | 1.90| d  | 6.3    | CH₃  | 4', 5'   | 5'      |
Figure S64: $^1$H-NMR spectrum for 6b recorded at 500 MHz in CDCl$_3$. 

SUPPORTING INFORMATION
Figure S65: $^{13}$C-NMR spectrum for 6b recorded at 125 MHz in CDCl$_3$. 

[Diagram of the 13C-NMR spectrum for 6b]
Figure S66: HSQC spectrum for 6b recorded at 500 MHz in CDCl₃.
Figure S67: HMBC spectrum for 6b recorded at 500 MHz in CDCl₃.
Figure S68: $^1$H–$^1$H COSY spectrum for 6b recorded at 500 MHz in CDCl$_3$.

epoxysorbicillinol 10a

Chemical Formula: C$_{14}$H$_{17}$O$_5$
Exact Mass: 264.0998

UV$_{\text{max}}$ (MeOH): 289 nm
HRMS (ESI) $m/z$ ($M$+H)$^+$ calcd for C$_{14}$H$_{17}$O$_5$: 265.1076, found: 265.1099
Figure S69: UV-absorption (top) and fragmentation pattern of 10a in ES+ TIC (middle) and ES- TIC (bottom).

Figure S70: HRMS data for 10a.
Table S12: Summarized NMR data for 10a recorded in DMSO-d6.

| Position | δC    | δH    | M  | J_{H-H}/Hz | HSQC | HMBC H to C | H-H COSY |
|----------|-------|-------|----|------------|------|--------------|----------|
| 1        | 187.5 | -     | -  | -          | -    | -            | -        |
| 2        | 104.1 | -     | -  | -          | -    | -            | -        |
| 3        | 172.7 | -     | -  | -          | -    | -            | -        |
| 4        | 62.7  | -     | -  | -          | -    | -            | -        |
| 5        | 60.9  | 3.62  | s  | -          | CH   | 3, 4, 6,     | -        |
| 6        | 68.7  | -     | -  | -          | -    | -            | -        |
| 7        | 8.0   | 1.55  | s  | -          | CH₃  | 1, 2, 3      | -        |
| 8        | 26.1  | 1.39  | s  | -          | CH₃  | 3, 4, 5      | -        |
| 1'       | 192.3 | -     | -  | -          | -    | -            | -        |
| 2'       | 124.1 | 6.32  | d  | 5.6        | CH   | 1', 3', 4', 3' | -        |
| 3'       | 144.1 | 7.15  | dd | 10.0, 15.3 | CH   | 1', 2', 4', 2', 4' | -        |
| 4'       | 130.2 | 6.33  | m  | -          | CH   | 2', 3', 6', 3', 5' | -        |
| 5'       | 142.4 | 6.33  | m  | -          | CH   | 2', 3', 4', 4', 6' | -        |
| 6'       | 18.7  | 1.84  | d  | 5.6        | CH₃  | 2', 3', 4', 5, 4', 5' | -        |

Figure S71: $^1$H-NMR spectrum for 10a recorded at 500 MHz in DMSO-d6.
Figure S72: $^{13}$C-NMR spectrum for 10a recorded at 125 MHz in DMSO-d$_6$. 
Figure S73: HSQC spectrum for 10a recorded at 500 MHz in DMSO-d6.
Figure S74: HMBC spectrum for 10a recorded at 500 MHz in DMSO-d6.
Figure S75: $^1$H–$^1$H COSY spectrum for 10a recorded at 500 MHz in DMSO-d$_6$.

2', 3'-dihydroepoxyisorbicillinol 10b

Chemical Formula: C$_{14}$H$_{16}$O$_5$

Exact Mass: 266.1154

$\lambda_{\text{max}}$ (MeOH): 277 nm

HRMS (ESI) $m/z$ (M+H)$^+$ calcd for C$_{14}$H$_{16}$O$_5$: 265.1076, found: 265.1099
Figure S76: UV-absorption (top) and fragmentation pattern of 10b in ES+ TIC (middle) and ES- TIC (bottom).

Figure S77: HRMS data for 10b.
### Table S1: Summarized NMR data for 10b recorded in DMSO-d6.

| Position | δ_C | δ_H | M  | J_H-H/Hz | HSQC | HMBC | H-H COSY |
|----------|-----|-----|----|----------|------|------|----------|
| 1        | -   | -   | -  | -        | -    | -    | -        |
| 2        | 104.3 | -   | -  | -        | -    | -    | -        |
| 3        | 173.7 | -   | -  | -        | -    | -    | -        |
| 4        | 62.8  | -   | -  | -        | -    | -    | -        |
| 5        | 60.8  | 3.67 | s  | -        | CH   | 4, 6, 8, - |
| 6        | 68.6  | -   | -  | -        | -    | -    | -        |
| 7        | 7.9   | 1.55 | s  | -        | CH_3 | 2, 3 | -        |
| 8        | 26.0  | 1.38 | s  | -        | CH_3 | 3, 5, 6 | -       |
| 1'       | 202.9 | -   | -  | -        | -    | -    | -        |
| 2'a      | 40.1  | 2.57 | dd | 8.0, 6.7 | CH_2 | 1', 4'; 5' 2'b, 3' |
| 2'b      | 40.1  | 2.62 | m  | -        | CH_2 | 1', 4'; 5' 2'a, 3' |
| 3'       | 25.5  | 2.17 | m  | -        | CH_2 | 1', 2', 4'; 2', 4'; 5' |
| 4'       | 125.2 | 5.42 | m  | -        | CH   | 3', 6' 3', 5', 6' |
| 5'       | 129.7 | 5.40 | m  | -        | CH   | 3', 6' 3', 4', 6' |
| 6'       | 17.7  | 1.61 | d  | 4.9      | CH_3 | 4', 5' 4', 5' |

**Figure S78:** $^1$H-NMR spectrum for 10b recorded at 500 MHz in DMSO-d6.
Figure S79: $^{13}$C-NMR spectrum for 10b recorded at 125 MHz in DMSO-d$_6$. 
Figure S8: HSQC spectrum for 10b recorded at 500 MHz in DMSO-d6.
Figure S81: HMBC spectrum for 10b recorded at 500 MHz in CDCl₃.
**Supporting Information**

Figure S8: $^1$H-$^1$H COSY spectrum for 10b recorded at 500 MHz in DMSO-d6.

**Figure**: 3D structure of scytolide 12.

- Chemical Formula: C$_{11}$H$_{12}$O$_6$
- Exact Mass: 240.0634

UV$_{	ext{max}}$ (MeOH): 214 nm, 245 nm
Figure S83: UV-absorption (top) and fragmentation pattern of 12 in ES+ TIC (middle) and ES- TIC (bottom).

Table S14: Summarized NMR data for 12 recorded in CDCl₃.

| Position | δ_H  | M   | J_H,H/Hz | H-H COSY |
|----------|------|------|-----------|----------|
| 1a       | 5.12 | s    |           | 1b       |
| 1b       | 5.69 | s    |           | 1a       |
| 2        | 4.41 | dd   | 10.3, 7.9 | 3, 4     |
| 3        | 4.11 | td   | 10.1, 6.5 | 2, 6a, 6b|
| 4        | 4.60 | m    |           | 3, 5     |
| 5        | 6.78 | s    |           | 4, 6     |
| 6a       | 3.08 | dd   | 17.6, 6.3 | 3, 6     |
| 6b       | 2.42-| m    |           | 3, 6     |
| 7        | 3.79 | s    | -         | -        |
Figure S8: $^1$H-NMR spectrum for 12 recorded at 600 MHz in CDCl$_3$. 
Figure S8: $^1$H-$^1$H COSY spectrum for 12 recorded at 600 MHz in CDCl$_3$.

trichopyrone 13

Chemical Formula: C$_{11}$H$_{12}$O$_3$

Exact Mass: 192.0786

$\lambda_{\text{max}}$ (MeOH): 250 nm, 350 nm

HRMS (ESI) $m/z$ (M-H)· calcd for C$_{11}$H$_{12}$O$_3$: 191.0708, found: 191.0714
Figure S86: UV-absorption (top) and fragmentation pattern of 13 in ES+ TIC (middle) and ES− TIC (bottom).

Figure S87: HRMS data for 13.
### Table S15: Summarized NMR data for 13 recorded in DMSO-d6.

| Position | δ_C   | δ_H   | M  | J_H-HHz | HSQC | HMBC H to C | H-H COSY |
|----------|-------|-------|----|---------|------|-------------|----------|
| 1        | 164.9 |       |    |         |      |             |          |
| 2        | 98.3  |       |    |         |      |             |          |
| 3        | 164.1 |       |    |         |      |             |          |
| 4        | 100.8 | 6.11  | s  |         | CH   | 2, 5, 6     |          |
| 5        | 155.8 |       |    |         |      |             |          |
| 6        | 120.8 | 6.20  | d  | 15.4    | CH   | 5, 8        | 7        |
| 7        | 133.8 | 6.87  | dd | 10.7, 15.4 | CH   | 5, 6, 8, 9, 6, 8 |
| 8        | 130.8 | 6.23  | m  |         | CH   | 10          | 9        |
| 9        | 135.5 | 6.10  | m  |         | CH   | 7, 10       | 10       |
| 10       | 18.4  | 1.82  | d  | 6.9     | CH<sub>3</sub> | 8, 9     | 8, 9     |
| 11       | 8.7   | 1.79  | s  |         | CH<sub>3</sub> | 1, 2, 3  |          |

**Figure S88:** ¹H-NMR spectrum for 13 recorded at 400 MHz in DMSO-d6.
Figure S89: $^{13}$C-NMR spectrum for 13 recorded at 125 MHz in DMSO-d6.
Figure S90: HSQC spectrum for 13 recorded at 400 MHz in DMSO-d6.
Figure S9: HMBC spectrum for 13 recorded at 400 MHz in DMSO-d6.
Figure S9. $^1$H-$^1$H COSY spectrum for 13 recorded at 400 MHz in DMSO-d6.

Chemical Formula: C_{13}H_{15}O_{4}
Exact Mass: 236,1049

UV$_{\text{max}}$ (MeOH): 243 nm, 343 nm
HRMS (ESI) $m/z$ (M-H): calcd for C_{13}H_{15}O_{4}: 235.0970, found: 235.0991
Figure S93: UV-absorption (top) and fragmentation pattern of 14 in ES+ TIC (middle) and ES− TIC (bottom).

Figure S94: HRMS data for 14.
Table S16: Summarized NMR data for 14 recorded in DMSO-d6.

| Position | Position (δ_C) | Position (δ_H) | Multiplicity (M) | J_H,H/Hz | HSQC | HMBC | H to C | COSY |
|----------|----------------|----------------|------------------|-----------|------|------|--------|------|
| 1        | 165.7          | -              | -                | -         | -    | -    | -      | -    |
| 2        | 92.2           | -              | -                | -         | -    | -    | -      | -    |
| 3        | 175.0          | -              | -                | -         | -    | -    | -      | -    |
| 4        | 107.9          | 5.61           | s                | -         | CH   | 2, 5 | -      | -    |
| 5        | 153.5          | -              | -                | -         | -    | -    | -      | -    |
| 6        | 47.1           | 3.43           | s                | -         | CH₂  | 4, 5, 7 | -    | -    |
| 7        | 205.2          | -              | -                | -         | -    | -    | -      | -    |
| 8        | 41.2           | 2.52           | d                | 7.3       | CH₂  | 7, 9, 10 | 9    | -    |
| 9        | 26.0           | 2.13           | m                | -         | CH₂  | 7, 8, 10, 8, 10 | 11 | -    |
| 10       | 129.9          | 5.39           | m                | -         | CH   | 12   | 9, 11, 12 | -    |
| 11       | 125.1          | 5.39           | m                | -         | CH   | 12   | 9, 10, 12 | -    |
| 12       | 17.7           | 1.59           | d                | 5.1       | CH₃  | 10, 11 | 10, 11 | -    |
| 13       | 9.2            | 1.59           | s                | -         | CH₃  | 1, 2, 3 | -    | -    |

Figure S95: ¹H-NMR spectrum for 14 recorded at 600 MHz in DMSO-d6.
Figure S96: $^{13}$C-NMR spectrum for 14 recorded at 125 MHz in DMSO-d$_6$. 

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[SUPPORTING INFORMATION page with Figure S96]
Figure S97: HSQC spectrum for 14 recorded at 600 MHz in DMSO-d6.
Figure S98: HMBC spectrum for 14 recorded at 600 MHz in DMSO-d6.
Figure S99: $^1$H-$^1$H COSY spectrum for 14 recorded at 600 MHz in DMSO-d6.

**Reduced sorbicillinol 15a**

Chemical Formula: C$_{14}$H$_{18}$O$_{4}$

Exact Mass: 250.1205

$\lambda_{\text{max}}$(MeOH): 222 nm, 265 nm, 374 nm

HRMS (ESI) $m/z$ (M-H)$^-$ calcd for C$_{14}$H$_{17}$O$_4$: 249.1127, found: 249.1135
Figure S100: UV-absorption (top) and fragmentation pattern of 15a in ES+ TIC (middle) and ES− TIC (bottom).

Figure S101: HRMS data for 15a.
Table S17: Summarized NMR data for 15a recorded in DMSO-d6.

| Position | δ_C  | δ_H  | M  | J_H-H/Hz | HSQC | HMBC  | H-H  |
|----------|------|------|----|----------|------|-------|------|
|          |      |      |    |          |      |       |      |
| 1        | 190.9| -    | -  | -        | -    | -     | -    |
| 2        | 106.1| -    | -  | -        | -    | -     | -    |
| 3        | 175.9| -    | -  | -        | -    | -     | -    |
| 4        | 69.5 | -    | -  | -        | -    | -     | -    |
| 5a       | 37.2 | 2.56 | d  | 14.3     | CH_2 | 4, 6, 8, 1' | -   |
| 5b       | 37.2 | 2.72 | d  | 14.3     | CH_2 | 1, 3, 4, 5a | 6, 8, 1' |
| 6        | 103.2| -    | -  | -        | -    | -     | -    |
| 7        | 7.7  | 1.61 | s  | -        | -    | -     | -    |
| 8        | 25.8 | 1.19 | s  | -        | -    | -     | -    |
| 1'       | 164.6| -    | -  | -        | -    | -     | -    |
| 2'       | 121.0| 6.42 | d  | 14.9     | CH   | 6, 1', 4' | 3'  |
| 3'       | 136.7| 6.97 | dd | 11.0, 14.9| CH   | 1', 2', 4', 2', 4' | 3'  |
| 4'       | 131.1| 6.33 | m  | -        | CH   | 2, 3', 6' | 3', 5', 6' |
| 5'       | 135.4| 6.06 | dq | 6.8, 14.0| CH   | 3', 6'  | 4', 6' |
| 6'       | 18.4 | 1.82 | d  | 6.8      | CH_3 | 4', 5'  | 4', 5' |
Figure S102: $^1$H-NMR spectrum for 15a recorded at 600 MHz in DMSO-d$_6$. 
Figure S103: $^{13}$C-NMR spectrum for 15a recorded at 125 MHz in DMSO-d6.
Figure S104: HSQC spectrum for 15a recorded at 600 MHz in DMSO-d6.
Figure S10: HMBC spectrum for 15a recorded at 600 MHz in DMSO-d6.
Figure S10: \(^1\text{H}-^1\text{H}\) COSY spectrum for 15a recorded at 600 MHz in DMSO-d6.

monomethylated reduced sorbicillinol 15a*

Chemical Formula: C_{13}H_{20}O_4
Exact Mass: 264.1362
Table S18: Summarized NMR data for 15a* recorded in DMSO-d6.

| Position | δC  | δH  | M   | J_H_H/Hz | HSQC | HMBC H to C | H-H COSY |
|----------|-----|-----|-----|-----------|------|-------------|----------|
| 1        | 190.8 | -  | -   | -         | -    | -           | -        |
| 2        | 115.9 | -  | -   | -         | -    | -           | -        |
| 3        | 173.8 | -  | -   | -         | -    | -           | -        |
| 4        | 69.9  | -  | -   | -         | -    | -           | -        |
| 5        | 38.5  | 2.66 | d  | 5.1     | CH₂  | 1, 3, 4, 5 | 6, 8, 1' |
| 6        | 103.3 | -  | -   | -         | -    | -           | -        |
| 7        | 8.8   | 1.70 | s  | -        | CH₃  | 1, 2, 3    | -        |
| 8        | 25.9  | 1.29 | s  | -        | CH₃  | 3, 4, 5    | -        |
| 9        | 60.4  | 3.97 | s  | -        | CH₃  | 3          | -        |
| 1'       | 166.8 | -  | -   | -         | -    | -           | -        |
| 2'       | 120.6 | 6.44 | d  | 14.8    | CH   | 8, 1', 4', 3' | -        |
| 3'       | 138.3 | 7.06 | dd | 11.0, 14.9 | CH   | 1', 4', 5', 2', 4' | -        |
| 4'       | 131.1 | 6.35 | m  | -        | CH   | 2', 6', 3', 5' | -        |
| 5'       | 136.8 | 6.13 | dq | 6.8, 14.0 | CH   | 3', 6', 4', 6' | -        |
| 6'       | 18.5  | 1.82 | d  | 6.9     | CH₃  | 2', 4', 5', 5' | -        |

Figure S107: ¹H-NMR spectrum for 15a* recorded at 500 MHz in DMSO-d6.
Figure S108: $^{13}$C-NMR spectrum for 15a* recorded at 125 MHz in DMSO-d$_6$. 

**Diagram Description:** 
- The spectrum shows various peaks at different chemical shifts, indicating the presence of different functional groups and chemical environments.
- The molecular structure of 15a* is depicted, highlighting the positions of the peaks corresponding to specific atoms.
- The 13C-NMR spectrum provides insights into the chemical shift and coupling constants, aiding in the assignment of signals to specific carbon atoms within the molecule.
Figure S109: HSQC spectrum for 15a* recorded at 500 MHz in DMSO-d6.
Figure S11: HMBC spectrum for 15a* recorded at 500 MHz in DMSO-d6.
Figure S11: $^1$H–$^1$H COSY spectrum for 15a* recorded at 500 MHz in DMSO-d6.

vertinolide 16a

Chemical Formula: $C_{16}H_{18}O_4$
Exact Mass: 250.1205

UV$_{\text{max}}$ (MeOH): 232 nm, 274 nm, 358 nm
HRMS (ESI) $m/z$ (M-H) calcd for $C_{16}H_{18}O_4$: 273.1127, found: 273.1132
Figure S112: UV-absorption (top) and fragmentation pattern of 16a in ES+ TIC (middle) and ES- TIC (bottom).

Figure S113: HRMS data for 16a.
Table S19: Summarized NMR data for 16a recorded at 400 MHz in DMSO-d6.

| Position | δ_C  | δ_H  | M   | J_H-H/Hz | HSQC | HMBC  | H-H COSY |
|----------|------|------|-----|----------|------|-------|-----------|
| 1        | 174.9| -    | -   | -        | -    | -     | -         |
| 2        | 97.5 | -    | -   | -        | -    | -     | -         |
| 3        | 176.4| -    | -   | -        | -    | -     | -         |
| 4        | 82.8 | -    | -   | -        | -    | -     | -         |
| 5        | 30.9 | 2.14 | m   | -        | CH_2 | 3, 4, 6, 6 |
|          |      |      |     |          |      | 7, 14 |
| 6        | 34.0 | 2.48, 2.66 | m, m | -        | CH_2 | 4, 5, 7, 5 |
| 7        | 201.8| -    | -   | -        | -    | -     | -         |
| 8        | 127.0| 6.04 | d   | 15.5     | CH   | 6, 7, 9, 9, 10 |
| 9        | 145.4| 7.17 | dd  | 10.5, 15.5 | CH | 7, 10, 11, 8, 10 |
| 10       | 142.7| 6.26 | m   | -        | CH   | 9, 11, 12, 9, 11, 12 |
| 11       | 130.2| 6.20 | m   | -        | CH   | 9, 10, 12, 9, 10, 12 |
| 12       | 19.1 | 1.88 | d   | 6.7      | CH_3 | 9, 10, 11, 10, 11 |
| 13       | 6.1  | 1.71 | s   | -        | CH_3 | 1, 2, 3 | -         |
| 14       | 23.4 | 1.48 | s   | -        | CH_3 | 3, 4, 5 | -         |

Figure S114: ^1H-NMR spectrum for 16a recorded at 400 MHz in DMSO-d6.
Figure S115. $^{13}$C-NMR spectrum for 16a recorded at 125 MHz in DMSO-d6.
Figure S116: HSQC spectrum for 16a recorded at 400 MHz in DMSO-d6.
Figure S11: HMBC spectrum for 16a recorded at 400 MHz in DMSO-d6.
Figure S118: $^1$H-$^1$H COSY spectrum for 16a recorded at 400 MHz in DMSO-d6.

2', 3'-dihydrovertinolide 16b

Chemical Formula: C$_{14}$H$_{21}$O$_4$
Exact Mass: 252.1362

$\text{UV}_{\lambda\text{max}}$ (MeOH): 226 nm, 274 nm, 367 nm
HRMS (ESI) $m/z$ (M-H)$^-$ calcd for C$_{14}$H$_{21}$O$_4$: 253.1440, found: 253.1453
Figure S119: UV-absorption (top) and fragmentation pattern of 16b in ES+ TIC (middle) and ES- TIC (bottom).

Figure S120: HRMS data for 16b.
Table S20: Summarized NMR data for 16b recorded in DMSO-d6.

| Position | δ_C  | δ_H  | M     | J_H-H/Hz | HSQC | HMBC H to C | H-H COSY |
|----------|------|------|-------|----------|------|-------------|----------|
| 1        | 174.5| -    | -     | -        | -    | -           | -        |
| 2        | 97.8 | -    | -     | -        | -    | -           | -        |
| 3        | 176.0| -    | -     | -        | -    | -           | -        |
| 4        | 82.6 | -    | -     | -        | -    | -           | -        |
| 5        | 30.0 | 2.08 | m (broad) | -  | CH₂ | 3, 4, 6, 7, 14 | 6        |
| 6        | 36.8 | 2.35, 2.48 | m, m  | -  | CH₂ | 3, 4, 7 | 5        |
| 7        | 211.4| -    | -     | -        | -    | -           | -        |
| 8        | 42.9 | 2.47 | m (broad) | -  | CH₂ | 7, 9, 10 | 9        |
| 9        | 26.9 | 2.22 | m     | -        | CH₂ | 7, 8, 10, 8, 10 | 11       |
| 10       | 129.2| 5.36 | m     | -        | CH  | 9, 9, 10, 12, 12 | 9, 11     |
| 11       | 126.5| 5.43 | m     | -        | CH  | 8, 9, 10, 10, 12 | 10, 12    |
| 12       | 18.1 | 1.64 | d (broad) | 9.3 | CH₃ | 8, 9, 10, 10, 11, 9 | 11        |
| 13       | 6.1  | 1.71 | s     | -        | CH₃ | 1, 2, 3 | -        |
| 14       | 23.5 | 1.47 | s     | -        | CH₃ | 3, 4, 5 | -        |

Figure S121: 1H-NMR spectrum for 16b recorded at 500 MHz in DMSO-d6.
Figure S122: $^{13}$C-NMR spectrum for 16b recorded at 125 MHz in DMSO-d6.
Figure S12: HSQC spectrum for 16b recorded at 500 MHz in DMSO-d6.
Figure S12: HMBC spectrum for 16b recorded at 500 MHz in DMSO-d6.
Figure S125: $^1$H–$^1$H COSY spectrum for 16b recorded at 500 MHz in DMSO-d6.

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Author Contributions

Conception of the work, data collection, data analysis and interpretation (LK, EB, EJS, RJC); Drafting the article (LK, EB, EJS, RJC); Critical revision of the article (LK, EB, EJS, RJC); Funding acquisition (EJS, RJC).