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Chemoenzymatic Asymmetric Total Synthesis of (*R*)-Lasiodiplodin Methyl Ether through a Sulfatase-Based Deracemization Process

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(*R*)-Lasiodiplodin methyl ether, a precursor of the antileukemic agent lasiodiplodin, was synthesized through a seven-step linear sequence. Chirality was introduced through a sulfatase-based deracemization process, in which a functionalized (*rac*)-*sec*-sulfate ester was enzymatically hydrolyzed with inversion of the stereocenter using an alkyl sulfatase. The remaining sulfate ester enantiomer was hydrolyzed with retention of configuration under acidic conditions, yielding the chiral key building block as the sole product in 93 % *ee* The total synthesis was completed through Negishi cross-coupling and ring-closing metathesis.

Introduction

Salicylic acid derivatives are important bioactive compounds and thus are popular synthetic targets. In addition to the most prominent representative – Aspirin® (acetyl salicylic acid) – closely related resorcylic acid lactones have gained attention since several natural products of this type were indentified as pharmacophores (Figure 1). For example, (*S*)-zearalenone exhibits anabolic, estrogenic, uterotropic, and antibacterial activity,[1–4] and the nonsteroidal animal growth-promoting antagonist zeranol is in clinical trials for the treatment of (post)menopausal syndrome.[5] *cis*-Resorcylide inhibits plant growth[6,7] and radicicol constitutes an important antitumor agent.[8,9] Another member of this family is lasiodiplodin, which displays antileukemia activity,[10] and its demethylated congener, which efficiently inhibits prostaglandin biosynthesis.[11]

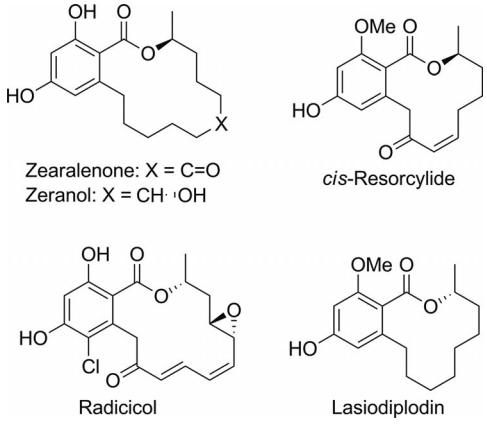

Figure 1. Bioactive resorcylic acid lactones.

Many of the synthetic routes to lasiodiplodin either lead to the racemate[12–15] or are based on chiral starting materials (e.g., epoxides or alcohols)[16–19] or on chiral sulfoxide auxiliaries.[20] The first catalytic asymmetric synthesis, reported by Jones and Huber, was based on Cr-catalyzed enantioselective addition of Me_2Zn onto an aldehyde (86% *ee*).[21] Recently, the group of Feringa reported a highly stereoselective Cu-catalyzed allylic alkylation.[22,23]

To expand the catalytic toolbox for the preparation of lasiodiplodin, we envisaged applying a chemoenzymatic deracemization process, which is based on inverting alkyl sulfatases. Sulfatases are a heterogenic group of enzymes that catalyze the cleavage of sulfate esters, yielding an alcohol and hydrogen sulfate.[24–27] Depending on their mode of action, sulfatases either cleave the S–O bond (leading to retention of configuration at the *sec*-alcohol), or the C–O bond (causing inversion at the stereogenic carbon atom).[25] The sulfatase "Pisa1" from *Pseudomonas* sp. DSM 6611 was recently identified as the first inverting *sec*-alkyl sulfatase possessing a broad substrate spectrum. Enantioconvergence was achieved in combination with retaining acid-catalyzed hydrolysis of the nonconverted sulfate ester to yield the corresponding *sec*-alcohol in high yield and enantiomeric purity as the sole product (Scheme 1).[28,29]

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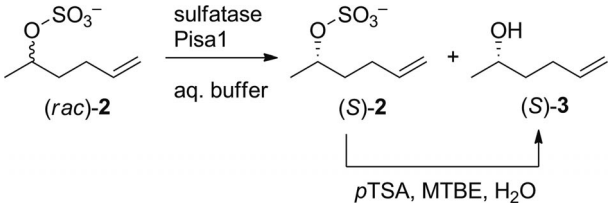
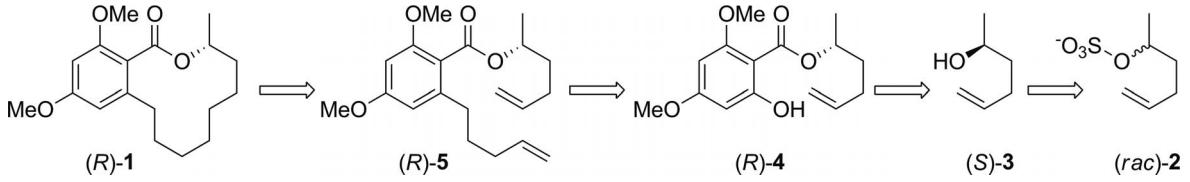


Scheme 1. Enantioconvergent sulfate ester hydrolysis for the preparation of chiral alcohol **3**.

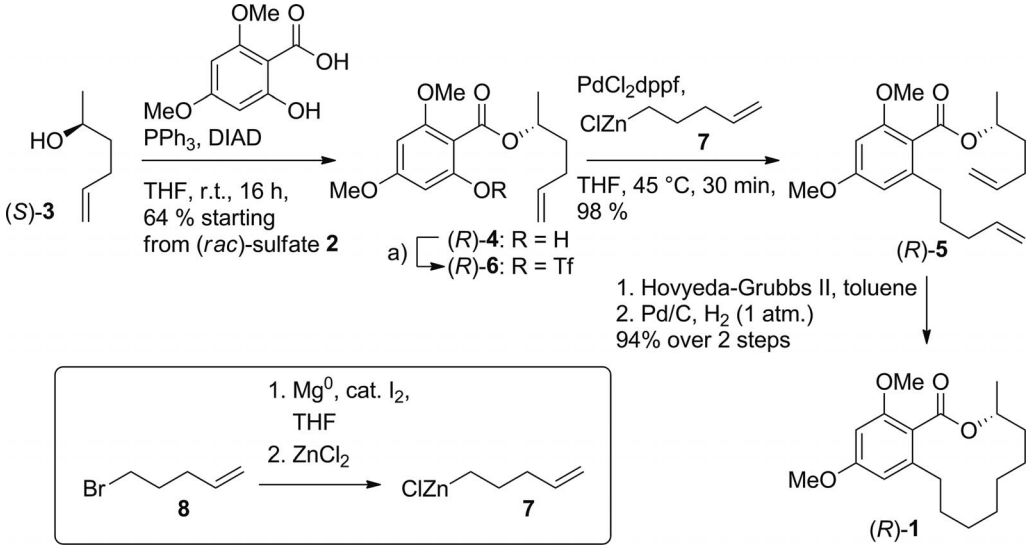
Results and Discussion

Our retrosynthetic analysis is outlined in Scheme 2. The macrolactone is formed through ring-closing metathesis, and the open-chain precursor **5** is prepared through Negishi cross-coupling and Mitsunobu esterification from chiral alcohol (*S*)-**3**, which may be obtained by the sulfatase-based deracemization process mentioned above.

To assess the feasibility of this strategy, the sulfatase-process was upscaled as follows: Alkyl sulfatase Pisa1 perfectly inverted the (*R*)-enantiomer of (*rac*)-**2** [> 99% *ee* of (*S*)-**3** at 50% conversion[30]], leaving (*S*)-**2** untouched. For the acid-catalyzed hydrolysis of the latter, two protocols were established: (i) A one-pot, two-step protocol, in which acidic hydrolysis of (*S*)-**2** was performed in the presence of (*S*)-**3** formed during enzymatic hydrolysis, and (ii) a two-step process involving extractive separation of enzymatically formed (*S*)-**3** prior to acidic hydrolysis of (*S*)-**2**.[28] Initial attempts using the one-pot procedure gave low yields due to the volatility of (*S*)-**3** causing losses during reflux at 60 °C overnight. Consequently, the two-step process requiring only 40 °C for two hours was amended by cautiously concentrating the solution of (*S*)-**3**, which was directly employed in the subsequent Mitsunobu esterification step to furnish (*R*)-**4** in 64% isolated yield and 93% *ee* over three steps (Scheme 3). Because the enantiomeric excess of alcohol (*S*)-**3** obtained by deracemization was > 99%, the slight drop of the enantiomeric excess must have been incurred during the Mitsunobu inversion. Activation of the phenol moiety in (*R*)-**4** as the triflate gave (*R*)-**6** in 95% yield. Employing $[Pd(PPh_3)_4]$ for Negishi cross-coupling with 4-pentenyl Zn^{II} chloride (**7**) gave only 20% of the desired alkylation product (*R*)-**5** but mainly furnished "dehalogenated" and homocoupling products. The latter was most likely due to transmetallation from palladium to zinc, which is facilitated by the presence of oxygen-containing substituents in the *ortho*-position relative to the (pseudo)halide.[31] Thus, the newly formed Zn species couples with remaining triflate, yielding the undesired homocoupling product. Additionally, literature analysis revealed that this type of coupling was successfully applied to salicylic triflates only in one case, when an amide group was present in the molecule, which provided an alternative coordination site for the zinc metal core.[32] To overcome these problems, optimization studies were performed, which revealed the following crucial points: (i) Replacement of the triphenylphosphane ligand of the Pd catalyst by 1,1′-bis(diphenylphosphino)ferrocene (dppf), and



Scheme 2. Retrosynthetic analysis for the chemoenzymatic synthesis of (*R*)-lasiodiplodin methyl ether (**1**).



Scheme 3. Synthesis of (*R*)-lasidiplodin methyl ether (**1**). *Reagents and conditions:* (a) Tf_2O, 2,6-lutidine, CH_2Cl_2, 30 min, 95%.

(ii) higher dilution of the reaction mixture (0.1 M triflate **6**) strongly favored cross-coupling over undesired side reactions. Additionally, the preparation of the Zn reagent had a significant impact on the product distribution, because the use of Zn dust, according to Knochel et al.,[33] either favored the second transmetallation step[31] or (more likely) remaining Zn dust inserted into the Ar–OTf bond, yielding undesired Ar-Zn species, which promote side-product formation. As a consequence, when the organometallic reagent **7** was prepared through transmetallation of the corresponding Grignard compound, an almost quantitative isolated yield of diene (*R*)-**5** was obtained. In agreement with our observations, it has been shown that different cations (i.e., Li^+ vs. Mg^{2+}) can alter the outcome of similar cross-coupling reactions significantly.[34]

To form the macrolactone ring, diene (*R*)-**5** was subjected to ring-closing metathesis, which gave a mixture of *E*/*Z* isomers (65:35). However, it was difficult to isolate more than 80% of the unsaturated macrolactone intermediate despite the fact that full conversion was observed by GC analysis. Because we assumed that the latter might be caused by Ru-catalyzed dimerization or oligomerization of the initially formed macrocyclic alkene upon concentration of the reaction mixture, we envisaged two possible solutions: (i) Removal of the Ru catalyst by filtration through silica gel prior to concentration of the solution, which led to an isolated yield of 91%, indicating that we were focusing on the right parameters. (ii) In addition, we combined ring-closing metathesis and hydrogenation of the macrocyclic C=C bond in a one-pot fashion, which gave lasiodiplodin methyl ether (*R*)-**1** in 93% *ee* in 94% isolated yield in two steps.

Conclusions

We have successfully employed a sulfatase-based deracemization process in the chemoenzymatic asymmetric total synthesis of (*R*)-lasiodiplodin methyl ether. The latter can be easily transformed into lasiodiplodin and its demethylated congener,[17,20] which are natural products possessing anti-leukemic and prostaglandin-inhibitor activities, respectively. The title compound was obtained in 44% overall yield and 93% *ee* over a seven-step linear synthesis. Yields could be significantly improved by performing several steps in a one-pot fashion without intermediate product isolation.

Experimental Section

General: All chemicals were purchased from Sigma Aldrich, Acros Organics, or Alfa Aesar and used as received; solvents were obtained from Roth. All moisture- or air-sensitive operations were conducted under dry argon in heat-dried glassware. Anhydrous THF was distilled from potassium/benzophenone prior to use, toluene was dried with molecular sieves (3 Å) after evaporation of the toluene/water azeotrope. Biocatalytic reactions were performed in a HT Infors Unitron AJ 260 incubator at 120 rpm shaking (horizontal position) and 30 °C.

NMR spectra were recorded with a Bruker NMR instrument operating at 300 (^{1}H) and 75 (^{13}C) MHz; chemical shifts (δ values) are given in ppm and coupling constants (*J*) are given in Hz. GC–MS measurements were performed with an Agilent 7890A GC system, equipped with an Agilent 5975C mass-selective detector (EI 70 eV) and a HP-5-MS column (30 m × 0.25 mm × 0.25 μm film) using He at a flow rate of 0.55 mL/min. Temperature program: 200 °C, hold 0.5 min, 5 °C/min 300 °C, hold 2 min, inlet temperature: 300 °C. High-resolution mass spectra were recorded with a Waters Synapt HDMS Q-TOF mass spectrometer (ESI ion source, positive mode, capillary voltage 2.6 kV) using a syringe pump to directly infuse the sample, which was dissolved in MeCN. Chiral GC-FID analysis was carried out with an Agilent Technologies 7890A GC-FID equipped with an Agilent Technologies 7693B autosampler fitted with a Varian Chirasil Dex CB column (25 m × 0.32 mm × 0.25 μm film) after derivatization using the following parameters: injector temperature: 200 °C, H_2 flow rate 2.0 mL/min, injection pressure 0.60 bar, temperature program: 80 °C, hold for 1.0 min, 3 °C/min to 95 °C, 15 °C/min, to 150 °C. Chiral HPLC analysis was performed with a Shimadzu HPLC system using the columns and methods as specified below. Flash chromatography was performed using Merck silica gel 60 (mesh size 40–63 μm). Petroleum ether had a boiling range of 60–95 °C. His-tagged alkyl sulfatase Pisa1 was prepared as recently reported.[29]

(*rac*)-5-Hexen-2-yl Sulfate [(*rac*)-2]: Sodium hydride (0.400 g, 60% dispersion in mineral oil, 9.99 mmol) was suspended in 1,4-dioxane (20 mL) under an atmosphere of argon. To the suspension, (*rac*)-**3** (1.21 mL, 1000 mg, 9.99 mmol) was added dropwise through a septum by using a syringe. The reaction was stirred for 1 h and a solution of sulfurtrioxide triethylamine complex (1.63 g, 9.0 mmol) in 1,4-dioxane (15 mL) was added dropwise over a period of 15 min. The mixture was stirred for 16 h, followed by quenching with distilled H_2O (ca. 5 mL). The obtained solution was concentrated until all dioxane was removed. The aqueous solution was diluted with additional distilled H_2O (15 mL) and lyophilized to give (*rac*)-**3** as a white powder (1.56 g, 7.7 mmol, 77%) with physical properties as reported recently.[28] ^{1}H NMR (300 MHz, D_2O): δ = 5.85–5.72 (m, 1 H), 5.03–4.84 (m, 2 H), 4.44–4.34 (m, 1 H), 2.10–1.98 (m, 2 H), 1.69–1.50 (m, 2 H), 1.22 (d, *J* = 7.3 Hz, 3 H) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 138.6, 114.7, 78.0, 35.2, 28.8, 19.9 ppm.

Enantioconvergent Preparation of (*S*)-5-Hexen-2-ol [(*S*)-3]: (*rac*)-5-Hexen-2-yl sulfate [(*rac*)-**2**; 200 mg, 494 mmol] was dissolved in Tris/HCl buffer (40 mL, 100 mM, pH 8.0) in a round-bottomed flask. Pisa1 enzyme (5.2 mg in 640 μL of the same buffer) was added and the flask was sealed with a glass stopper fitted with a teflon ring and shaken for 24 h at 120 rpm and 30 °C (vertical position). The aqueous layer was extracted with *t*BuOMe (3 × 5 mL) and the combined organic phase was kept on ice (organic phase **A**). The remaining aqueous phase was evaporated at 60 °C and 5 mbar, and the residue was dissolved in water (2.8 mL), *t*BuOMe (76 mL), and 1,4-dioxane (10 μL). *p*-Toluenesulfonic acid monohydrate (1.4 g, 7.4 mmol) was added, the flask was fitted with a reflux condenser, and the mixture was stirred at 40 °C for 2 h. After cooling to room temperature, saturated aqueous K_2CO_3 (15 mL) was added, the phases were separated, the aqueous phase was extracted with *t*BuOMe (2 × 5 mL), and the combined organic phases (including organic phase **A**) were collected. 1,4-Dioxane (6 mL) was added and the reaction mixture was concentrated to 6–8 mL end volume. The latter solution was directly used for the subsequent Mitsunobu esterification. For compound characterization, no 1,4-dioxane was added and concentration under reduced pressure gave

(*S*)-**3** (41 mg, 41% yield, > 99% *ee*) as a colorless oil. $[\alpha]_D^{20}$ = +15.6 (*c* = 1.0, Et_2O); ref.[35] +17.3 (*c* = 1.026, Et_2O). ^1H NMR ($CDCl_3$, 300 MHz): δ = 5.91–5.78 (m, 1 H), 5.68–4.96 (m, 2 H), 3.83 (sext, *J* = 6.2 Hz, 1 H), 2.16 (nonet, *J* = 7.1 Hz, 2 H), 1.68 (br. s, 1 H), 1.64–1.47 (m, 2 H), 1.20 (d, *J* = 6.3 Hz, 3 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 138.5, 114.8, 67.7, 38.3, 30.2, 23.5 ppm.

Derivatization of 3 for Chiral GC-FID Analysis: A solution of alcohol **3** in *t*BuOMe was treated with 4-(dimethylamino)pyridine (2 mg, 0.016 mmol) and acetic anhydride (100 μL, 108 mg, 1.06 mmol) and the mixture was shaken overnight at 120 rpm and 30 °C. The reaction was quenched by addition of H_2O (300 μL), the phases were separated, and the organic phase was dried with Na_2SO_4 prior to injection. t_R = 5.07 (*R*), 4.07 (*S*) min.

(*R*)-5-Hexen-2-yl 2-Hydroxy-4,6-dimethoxybenzoate [(*R*)-4]: 2-Hydroxy-4,6-dimethoxy benzoic acid (198 mg, 1.0 mmol) was dissolved in THF (3.5 mL) in a 100 mL round-bottomed flask. Diisopropyl azodicarboxylate (203 μL, 209 mg, 1.03 mmol) was added and the flask was capped with a rubber septum. PPh_3 (338 mg, 1.26 mmol) was dissolved in the solution of (*S*)-5-hexen-2-ol obtained from the enantioconvergent process and the solution was added by using a syringe and cannula over a period of 1.5 h. After complete addition, the reaction mixture was stirred overnight at room temperature. The mixture was concentrated under reduced pressure and the residue was taken up in EtOAc (10 mL) and washed with an aqueous H_2O_2 solution (10%, 10 mL) to oxidize remaining PPh_3. The aqueous phase was extracted with EtOAc (3× 10 mL) and the combined organic phase was dried with Na_2SO_4, filtered, and concentrated under reduced pressure. The remaining brown oil was subjected to dry flash column chromatography through a silica gel pad (1 cm diameter, 5 cm height; petroleum ether/EtOAc, 10:1, 100 mL) to give pure (*R*)-**4** (179 mg, 0.64 mmol, 64%, 93% *ee*). $[\alpha]_D^{20}$ = –38.3 (*c* = 1.0, acetone); HPLC analysis (Diacel Chiralcel OJ; 18 °C; 0.3 mL/min; *n*-heptane/2-PrOH, 99:1): t_R = 25.64 (*S*), 28.12 (*R*) min. ^1H NMR ($CDCl_3$, 300 MHz): δ = 12.07 (s, 1 H), 6.12 (d, *J* = 2.4 Hz, 1 H), 5.97 (d, *J* = 2.4 Hz, 1 H), 5.92–5.79 (m, 1 H), 5.16 (sext, *J* = 6.3 Hz, 1 H), 5.08–4.97 (m, 2 H), 3.82 (s, 3 H), 3.81 (s, 3 H), 2.32–2.13 (m, 2 H), 1.91–1.65 (m, 2 H), 1.37 (d, *J* = 6.3 Hz, 3 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 170.8, 165.8, 165.1, 162.3, 138.0, 114.9, 97.2, 93.3, 91.6, 71.6, 55.9, 55.4, 35.1, 29.5, 20.0 ppm. GC-MS (EI; t_R = 5.89 min): *m*/*z* (%) = 280 (11), 198 (6), 180 (100), 166 (1), 152 (13), 137 (10), 123 (2), 95 (3). HRMS (ESI): *m*/*z* calcd for $C_{15}H_{21}O_5^+$ 281.1389 $[M + H]^+$; found 281.1377.

(*R*)-5-Hexen-2-yl 4,6-Dimethoxy-2-(trifluoromethylsulfonyloxy)-benzoate [(*R*)-6]: Compound (*R*)-**4** (233 mg, 0.83 mmol) was placed into an argon-flushed 50 mL round-bottomed flask and dissolved in CH_2Cl_2 (6 mL). The reaction mixture was cooled to 0 °C (ice bath), 2,6-lutidine (250 μL, 230 mg, 2.1 mmol) and triflic anhydride (250 μL, 419 mg, 1.5 mmol) were added and the mixture was stirred for 20 min at 0 °C, quenched with saturated ammonium chloride (5 mL), and the organic phase was separated. The aqueous layer was extracted with CH_2Cl_2 (2× 10 mL) and the combined organic phase was washed with 5 M HCl (2× 20 mL), dried with Na_2SO_4, and concentrated under reduced pressure. The crude product was purified by flash chromatography (petroleum ether/EtOAc, 10:1) to give triflate (*R*)-**6** as a pale-yellow oil (324 mg, 0.79 mmol, 95%). $[\alpha]_D^{20}$ = –12.4 (*c* = 1.0, acetone). ^1H NMR ($CDCl_3$, 300 MHz): δ = 6.48 (d, *J* = 2.1 Hz, 1 H), 6.45 (d, *J* = 2.1 Hz, 1 H), 5.91–5.78 (m, 1 H), 5.19 (sext, *J* = 6.3 Hz, 1 H), 5.09–4.98 (m, 2 H), 3.86 (s, 3 H), 3.85 (s, 3 H), 2.26–2.01 (m, 2 H), 1.91–1.81 (m, 1 H), 1.74–1.62 (m, 1 H), 1.37 (d, *J* = 6.3 Hz, 3 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 162.9, 162.2, 159.3, 147.8, 137.8, 118.5 (q, *J* = 319 Hz, C–F coupling), 115.0, 111.0, 98.7, 98.4, 72.7, 56.3, 55.9, 35.0, 29.5, 19.7 ppm. GC-MS (EI; t_R = 6.38 min): *m*/*z* (%) = 412 (1), 331 (51), 313 (100), 286 (3), 180 (48), 152 (11), 137 (20), 82 (24). HRMS (ESI): *m*/*z* calcd for $C_{16}H_{19}SO_7F_3Na^+$ 435.0701 $[M + Na]^+$; found 435.0712.

4-Pentenylzinc(II) Chloride (7): A 25 mL round-bottomed flask, equipped with a reflux condenser and a glass stopper was dried in high vacuum using a heat gun. The apparatus was vented with dry argon, the stopper was removed, and magnesium turnings (111 mg, 4.57 mmol) were added under a positive stream of argon. The whole apparatus was again dried using a heat gun under high vacuum and vented with argon, followed by the addition of one small crystal of iodine (ca. 2 mg, sublimed using a heat gun). The flask was allowed to cool until all the iodine vapor had settled down and anhydrous THF (2.2 mL) was added. The mixture was stirred at room temperature during slow addition of 5-bromopent-1-ene (504 μL, 643 mg, 4.25 mmol) in THF (2 mL). After addition of ca. 200 μL, the mixture was heated using a heat gun to initiate the metal insertion and the reaction was kept at moderate reflux by adding further 5-bromopent-1-ene over the time of addition. After completion of the addition, the mixture was stirred at room temperature for an additional 10 min, then cooled to 0 °C (ice bath) and a solution of $ZnCl_2$ (698 mg, 5.12 mmol) in anhydrous THF (5 mL) was added over a period of 1 min. The obtained white slurry was taken into a syringe by using a cannula and septum and used as such for the next reaction step.

(*R*)-5-Hexen-2-yl 2,4-Dimethoxy-6-(4-pentenyl)benzoate [(*R*)-5]: Triflate **6** (412 mg, 1.0 mmol) was added to a 20 mL biotage vial equipped with a stir bar. The vial was capped and crimped with a teflon septum and primed with argon for 45 min. $[PdCl_2dppf]$ (36 mg, 0.05 mmol) was dissolved in anhydrous THF (2 mL) and added to the starting material, followed by addition of 4-pentenylzinc(II) chloride (**7**; 0.42 M in anhydrous THF, 8 mL, 3.36 mmol) prepared as described above. The vial was placed into a preheated oil bath (45 °C bath temperature) and stirred at this temperature. After completion of the reaction (0.5–1 h as checked by GC analysis) the vial was opened and the reaction was quenched by addition of saturated ammonium chloride (10 mL). Extraction with EtOAc (3× 100 mL), drying of the combined organic phase over Na_2SO_4, and concentration under reduced pressure, gave the crude product, which was purified by flash chromatography (petroleum ether/EtOAc, 10:1) to give diene (*R*)-**5** as a pale-yellow oil (326 mg, 0.98 mmol, 98% yield). $[\alpha]_D^{20}$ = –19.4 (*c* = 1.0, acetone). ^1H NMR ($CDCl_3$, 300 MHz): δ = 6.34 (m, 2 H), 5.92–5.76 (m, 2 H), 5.20 (sext, *J* = 6.3 Hz, 1 H), 5.10–4.96 (m, 4 H), 3.82 (s, 3 H), 3.80 (s, 3 H), 2.63–2.57 (m, 2 H), 2.28–2.06 (m, 4 H), 1.88–1.60 (m, 4 H), 1.36 (d, *J* = 6.3 Hz, 3 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 168.0, 161.2, 157.8, 142.2, 138.3, 137.9, 117.1, 115.0, 114.8, 105.7, 96.2, 71.2, 55.7, 55.4, 35.2, 33.6, 33.3, 30.4, 29.6, 20.1 ppm. GC-MS (EI; t_R = 8.59 min): *m*/*z* (%) = 332 (35), 278 (7), 263 (1), 250 (27), 233 (67), 211 (37), 196 (100), 191 (77), 178 (18), 165 (13), 152 (67), 137 (12), 120 (12), 91 (14), 77 (12). HRMS (ESI): *m*/*z* calcd for $C_{20}H_{28}O_4Na^+$ 355.1885 $[M + Na]^+$; found 355.1887.

(*R*)-12,14-Dimethoxy-3-methyl-3,4,5,8,9,10-hexahydro-1*H*-benzo[*c*][1]oxacyclododecin-1-one: A two-necked 500 mL round-bottomed flask equipped with a reflux condenser and a stopper was dried under high vacuum using a heat gun and the apparatus was vented with dry argon. The stopper was replaced by a rubber septum, anhydrous toluene (120 mL) was introduced by using a syringe and cannula, and heated to 85 °C (oil bath temperature). Diene (*R*)-**5** (82 mg, 0.25 mmol) was dissolved in anhydrous toluene (20 mL), Hoveyda–Grubbs catalyst (2nd generation, 8 mg, 0.013 mmol) was

dissolved in anhydrous toluene (8 mL) and both solutions were added in portions over a period of 1 h. After completion of the addition, the mixture was stirred for an additional 10 min at 85 °C and then allowed to cool to room temperature. The mixture was filtered through a pad of silica gel (ca. 1 g), the pad was washed with EtOAc (20 mL), and the filtrate was concentrated under reduced pressure. The remaining crude product was subjected to column chromatography (petroleum ether/EtOAc, 15:1) to give an *E*/*Z* mixture of the title compound (69 mg, 0.23 mmol, 91% yield), which was separated by preparative TLC (Silica gel GF plates; 20 × 20 cm; 1500 microns; petroleum ether/EtOAc, 20:1, eluted sevenfold) to give the single isomers.

(*E*)-Isomer: Yield 17 mg (0.06 mmol, 23%). $[\alpha]_D^{20}$ = +16.8 (*c* = 1.0, acetone).^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.28 (d, *J* = 2.1 Hz, 1 H), 6.23 (d, *J* = 2.1 Hz, 1 H), 5.26 (dt, J_1 = 31.4, J_2 = 10.6 Hz, 2 H), 4.84 (septet, *J* = 5.7 Hz, 1 H), 3.73 (s, 3 H), 3.71 (s, 3 H), 2.53–2.36 (m, 3 H), 2.11 (sext, *J* = 9.0 Hz, 1 H), 1.78–1.57 (m, 6 H), 1.31 (d, *J* = 6.0 Hz, 3 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 168.6, 161.0, 157.2, 142.0, 131.7, 129.4, 119.1, 105.5, 96.3, 70.1, 55.9, 55.3, 33.6, 31.0, 29.8, 23.8, 23.1, 20.4 ppm. GC-MS (EI; t_R = 9.69 min): *m*/*z* (%) = 304 (66), 287 (13), 275 (26), 259 (21), 247 (39), 233 (11), 220 (29), 203 (35), 191 (100), 178 (59), 164 (20), 152 (66), 135 (15), 120 (10), 105 (6), 91 (17), 77 (20). HRMS (ESI): *m*/*z* calcd for $C_{18}H_{25}O_4^+$ 305.1753 $[M + H]^+$; found 305.1771.

(*Z*)-Isomer: Yield 27 mg (0.09 mmol, 35%). $[\alpha]_D^{20}$ = –30.4 (*c* = 1.4, acetone). ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.38 (d, *J* = 2.0 Hz, 1 H), 6.31 (d, *J* = 2.0 Hz, 1 H), 5.42–5.33 (m, 1 H), 5.26–5.18 (m, 1 H), 5.16–5.09 (m, 1 H), 3.81 (s, 3 H), 3.79 (s, 3 H), 2.67–2.64 (m, 2 H), 2.36–2.31 (m, 1 H), 2.20–2.06 (m, 2 H), 1.91–1.65 (m, 5 H), 1.35 (d, *J* = 6.3 Hz, 3 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 168.3, 161.1, 157.7, 141.5, 133.5, 127.1, 118.7, 104.7, 96.2, 73.1, 55.9, 55.3, 34.5, 31.2, 29.2, 28.9, 28.4, 20.9 ppm. GC-MS (EI; t_R = 9.49 min): *m*/*z* (%) = 304 (75), 287 (14), 275 (28), 259 (22), 247 (40), 233 (13), 220 (30), 203 (42), 191 (100), 178 (57), 164 (21), 152 (75), 135 (14), 120 (11), 105 (8), 91 (19), 77 (23). HRMS (ESI): *m*/*z* calcd for $C_{18}H_{25}O_4^+$ 305.1753 $[M + H]^+$; found 305.1760.

(*R*)-Lasiodiplodin Methyl Ether [(*R*)-12,14-Dimethoxy-3-methyl-3,4,5,6,7,8,9,10-octahydro-1*H*-benzo[*c*][1]oxacyclododecin-1-one; (*R*)-1]: A two-necked 500 mL round-bottomed flask equipped with a reflux condenser and a stopper was dried under high vacuum using a heat gun and the apparatus was vented with dry argon. The stopper was replaced by a rubber septum, anhydrous toluene (120 mL) was introduced by using a syringe and cannula and heated to 85 °C (oil bath temperature). Diene (*R*)-**4** (82 mg, 0.25 mmol) was dissolved in anhydrous toluene (20 mL), Hoveyda–Grubbs catalyst (2nd generation, 8 mg, 0.013 mmol) was dissolved in anhydrous toluene (8 mL) and both solutions were added portionwise over a period of 1 h. After completion of the addition, the mixture was stirred for an additional 10 min at 85 °C and then allowed to cool to room temperature, Pd/C (10%, 40 mg) was added and the apparatus was placed under vacuum and vented with H_2 five times and the mixture was stirred at room temperature under a hydrogen atmosphere (1 atm) for 1.5 days. The mixture was filtered through a pad of silica gel (ca. 1 g), the pad was washed with EtOAc (20 mL) and the filtrate was concentrated under reduced pressure. The remaining crude product was subjected to column chromatography (petroleum ether/EtOAc, 15:1) to give lasiodiplodin methyl ether [(*R*)-**1**; 72 mg, 23 mmol, 94%, 93% *ee*]. $[\alpha]_D^{20}$ = +7.17 (*c* = 1.0, $CHCl_3$), ref.[19] +8.7 (*c* = 1.63, CH_3Cl); HPLC analysis (Diacel Chiralcel OD-H; 20 °C; 0.7 mL/min; *n*-heptane/2-PrOH, 85:15): t_R = 6.44 (*R*), 7.59 (*S*) min. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 6.34 (d, *J* = 2.1 Hz, 1 H), 6.32 (d, *J* = 2.1 Hz, 1 H), 5.34–5.25 (m, 1 H), 3.81 (s, 3 H), 3.80 (s, 3 H), 2.74 (dt, J_1 = 13.5, J_2 = 7.8 Hz, 1 H), 2.55 (dt, J_1 = 13.5, J_2 = 6.6 Hz, 1 H), 2.01–1.90 (m, 1 H), 1.73–1.60 (m, 4 H), 1.54–1.37 (m, 5 H), 1.34 (d, *J* = 6.3 Hz, 3 H), 1.31–1.22 (m, 2 H) ppm. ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 168.5, 161.1, 157.7, 142.8, 118.1, 105.8, 96.3, 72.0, 55.9, 55.3, 32.3, 30.6, 26.5, 25.5, 24.2, 21.2, 19.5 ppm. GC-MS (EI; t_R = 9.78 min): *m*/*z* (%) = 306 (73), 291 (11), 277 (2), 261 (4), 219 (8), 196 (100), 191 (61), 178 (22), 165 (19), 152 (90), 135 (12), 120 (12), 91 (13), 77 (12).

Supporting Information (see footnote on the first page of this article): ^{1}H and ^{13}C NMR spectra, chiral GC- and HPLC-chromatograms.

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[1] S. A. Hitchcock, G. Pattenden, *J. Chem. Soc. Perkin Trans. 1* **1992**, 1323–1328.
[2] E. Keinan, S. Sinha, A. Sinhabachi, *J. Chem. Soc. Perkin Trans. 1* **1991**, 3333–3339.
[3] K. C. Nicolaou, N. Winssinger, J. Pastor, F. Murphy, *Angew. Chem.* **1998**, *110*, 2677; *Angew. Chem. Int. Ed.* **1998**, *37*, 2534–2537.
[4] D. Taub, N. N. Girotra, R. D. Hoffsommer, C. H. Kuo, H. L. Slates, S. Weber, N. L. Wendler, *Tetrahedron* **1968**, *24*, 2443–2461.
[5] W. H. Utian, *Br. Med. J.* **1973**, *1*, 579–581.
[6] C. J. Barrow, *J. Nat. Prod.* **1997**, *60*, 1023–1025.
[7] H. Oyama, T. Sassa, M. Ikeda, *Agric. Biol. Chem.* **1978**, *42*, 2407–2409.
[8] H. G. Cutler, R. F. Arrendale, J. P. Springer, P. D. Cole, R. G. Roberts, T. R. Hanlin, *Agric. Biol. Chem.* **1987**, *51*, 3331–3338.
[9] P. Delmotte, J. Delmotte-Plaquée, *Nature* **1953**, *171*, 344.
[10] K.-H. Lee, N. Hayashi, M. Okano, I. H. Hall, R.-Y. Wu, A. T. McPhail, *Phytochemistry* **1982**, *21*, 1119–1121.
[11] Y. Xin-Sheng, Y. Ebizuka, H. Noguchi, F. Kuichi, Y. Iitaka, U. Sanakawa, *Tetrahedron Lett.* **1983**, *24*, 2407–2410.
[12] S. J. Danishefsky, S. J. Etheredge, *J. Org. Chem.* **1979**, *44*, 4716–4717.
[13] A. Fürstner, O. R. Thiel, N. Kindler, B. Bartkowska, *J. Org. Chem.* **2000**, *65*, 7990–7995.
[14] H. Gerlach, A. Thalmann, *Helv. Chim. Acta* **1977**, *60*, 2866–2866.
[15] T. Takahashi, K. Kasuga, J. Tsuji, *Tetrahedron Lett.* **1978**, *19*, 4917–4920.
[16] F. Bracher, B. J. Schulte, *J. Chem. Soc. Perkin Trans. 1* **1996**, 2619–2622.
[17] M. Braun, U. Mahler, S. Houben, *Liebigs Ann. Chem.* **1990**, 513–517.
[18] A. Fürstner, N. Kindler, *Tetrahedron Lett.* **1996**, *37*, 7005–7008.
[19] A. Fürstner, G. Seidel, N. Kindler, *Tetrahedron* **1999**, *55*, 8215–8230.
[20] A. R. Solladié, M. C. Carreno, J. L. G. Ruano, *Tetrahedron: Asymmetry* **1990**, *1*, 187–198.
[21] G. B. Jones, R. S. Huber, *Synlett* **1993**, 367–368.
[22] K. Geurts, S. P. Fletcher, B. L. Feringa, *J. Am. Chem. Soc.* **2006**, *128*, 15572–15573.

[23] Y. Huang, A. J. Minnaard, B. L. Feringa, *Synthesis* **2011**, 1055–1058.
[24] P. Bojarova, S. J. Williams, *Curr. Opin. Chem. Biol.* **2008**, *12*, 573–581.
[25] P. Gadler, K. Faber, *Trends Biotechnol.* **2007**, *25*, 83–88.
[26] S. R. Hanson, M. D. Best, C.-H. Wong, *Angew. Chem.* **2004**, *116*, 5858; *Angew. Chem. Int. Ed.* **2004**, *43*, 5736–5763.
[27] M. A. Kertesz, *FEMS Microbiol. Rev.* **2000**, *24*, 135–175.
[28] M. Schober, T. Knaus, M. Toesch, P. Macheroux, U. Wagner, K. Faber, *Adv. Synth. Catal.* **2012**, *354*, 1737–1742.
[29] M. Schober, P. Gadler, T. Knaus, H. Kayer, R. Birner-Grünberger, C. Gülly, P. Macheroux, U. Wagner, K. Faber, *Org. Lett.* **2011**, *13*, 4296–4299.
[30] The conversion was calculated from the *ee* value of the product and the *ee* of the remaining sulfate ester, see: A. J. J. Straathof, J. A. Jongejan, *Enzyme Microb. Technol.* **1997**, *21*, 559–571.
[31] Q. Liu, Y. Lan, J. Liu, G. Li, Y.-D. Wu, A. Lei, *J. Am. Chem. Soc.* **2009**, *131*, 10201–10210.
[32] H. Wehlan, E. Jezek, N. Lebrasseur, G. Pavé, E. Roulland, A. J. P. White, J. N. Bourrows, A. G. M. Barrett, *J. Org. Chem.* **2006**, *71*, 8151–8158.
[33] S. Sase, M. Jaric, A. Metzger, V. Malakhov, P. Knochel, *J. Org. Chem.* **2008**, *73*, 7380–7382.
[34] L. Jin, C. Liu, J. Liu, F. Hu, Y. Lan, A. S. Batsanov, J. A. K. Howard, T. B. Marder, A. Lei, *J. Am. Chem. Soc.* **2009**, *131*, 16656–16657.
[35] P. Conti, C. Dallanoce, M. De Amici, C. De Micheli, G. Carrea, F. Zambianchi, *Tetrahedron: Asymmetry* **1998**, *9*, 657–665.

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