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ORIGINAL PAPER

Intramolecular Diels–Alder cyclization of biodihydroxylated benzoic acid derivatives towards novel heterocyclic scaffolds

Thomas C. M. Fischer · Hannes G. Leisch · Marko D. Mihovilovic

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Abstract The potential of biodihydroxylated benzoic acid derivatives as versatile precursors for the construction of polycyclic scaffolds was investigated. *Ralstonia eutropha* B9 was used to biodihydroxylate sodium benzoate to sodium (1*S*,2*R*)-1,2-dihydroxycyclohexa-3,5-diene-1-carboxylate in high optical purity (>95% *ee*). The required cyclization precursors could be obtained in moderate to excellent yields after protection of the intermediate and subsequent functional group transformation. Intramolecular Diels–Alder cylizations were carried out under thermal or microwave conditions leading to enantiomerically pure products with five chiral centers.

Keywords Bioorganic chemistry · Cycloadditions · Enzymes · Biosynthesis · Dioxygenases

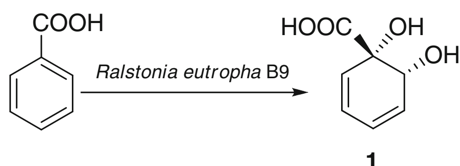
Introduction

Whereas microbial 2,3-dihydroxylation of aromatic compounds has been shown to be a highly valuable method for organic synthesis of highly versatile chiral intermediates [1, 2], the enzymatic 1,2-dioxygenation of aryl carboxylates has been neglected in comparison. The asymmetric building blocks obtained by the microbial 2,3-dihydroxylation reaction have been used in organic synthesis to achieve a wide variety of complex target molecules with highly diverse biological activity [3–9]. The 1,2-dihydroxylation of aromatic systems was first reported as early as 1971 by Reiner and Hegemann using a mutant strain of *Ralstonia eutropha* (strain B9, formerly known as *Alcaligenes eutrophus*). This benzoate 1,2-dioxygenase is a typical Rieske dioxygenase system composed of a benzoate-1,2-dioxygenase (BZDO) and benzoate-1,2-direductase (BZDR). BZDO is a 200-kDa multimeric protein with an $(\alpha\beta)_3$ quaternary structure in which each α subunit contains a Rieske-type [2Fe–2S] cluster and a mononuclear non-heme iron active site [10]. BZDR is a 38-kDa protein containing an FAD and a [2Fe–2S] cluster with all-cysteine ligation [11]. Substrate binding and oxygen activation occur at the mononuclear iron site [12]. Although transformation of planar aromatic carboxylates into chiral diols has been known for almost four decades, only two applications in the synthesis of bioactive compounds have been reported utilizing such chiral metabolites [13–15].

In order to avoid over-metabolism of benzoic acid to the corresponding catechol, in accordance with the routine pathway, the 1,2-dihydroxylation reaction is performed with mutant microorganisms deficient in a dihydroxydiol dehydrogenase, hence avoiding re-aromatization and enabling high-yielding accumulation of the enantiomerically pure valuable intermediate (1*S*,2*R*)-1,2-dihydroxycyclohexa-3,5-diene-1-carboxylic acid **1** (DCDC) (Scheme 1). Several mutant strains have been investigated in subsequent studies by different groups, particularly the groups of Reiner and Knackmuss, which were able to transform several substituted aryl carboxylates [2, 13–20].

Apart from these applications in bioactive compound synthesis, few papers have been published investigating systematically the chemistry of DCDC. Widdowson et al. [21] first determined the absolute stereochemistry as (1*S*,2*R*)-DCDC in 1995 by X-ray diffraction and used derivatives of the metabolite for intermolecular Diels–Alder cyclizations. In 2001 Meyers et al. [22] reported the

T. C. M. Fischer · H. G. Leisch · M. D. Mihovilovic (✉)
Institute of Applied Synthetic Chemistry,
Vienna University of Technology, Getreidemarkt 9/163-OC,
1060 Vienna, Austria
e-mail: mmihovil@pop.tuwien.ac.at



Scheme 1

use of DCDC as precursor for the synthesis of a large number of highly functionalized synthons by sequential oxygenation reactions; this approach was later elaborated into a novel synthetic strategy to tetracyclines [11]. A multi-gram-scale fermentation was also outlined in this paper. The synthesis of carba-β-l-fructopyranose was reported in 2004 by Parker et al. [10]; this was subsequently converted to a synthetic scaffold of carbacyclic topiramate.

Finally, the potential of the biodioxygenation product was briefly investigated by our group in intramolecular cycloaddition reactions towards heterocyclic structures [23]. In this contribution, we provide a more detailed summary of these cyclization studies towards novel heterocyclic scaffolds.

Results and discussion

Up-scaling of benchtop fermentation with Ralstonia eutropha B9

Further improvements in up-scaling and applicability of the whole-cell biotransformation to multi-gram quantities of 1,2-dihydroxycyclohexadiene carboxylic acid (**1**) were required for further synthetic investigations. The described biotransformation was previously conducted in Hutner's mineral base as culture medium and scaled up to a substrate concentration of 4 g/dm^3 and a yield of 73% by Leisch [23] in shake-flask experiments and to a titer of 6.7 g/dm^3 and a yield of 74% in a 7 dm^3 fermentation experiment. Under similar conditions a yield of only 39% in a 80 dm^3 fermentation experiment was reported by Myers et al. [22]. Alternative growth and substrate feed conditions and a simplified work-up procedure were reported by Payer [24].

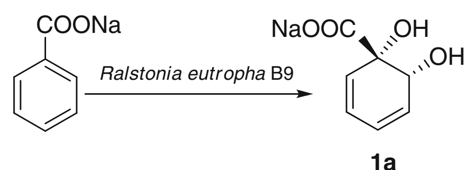
Careful assessment and combination of several previously disclosed fermentation conditions enabled further improvement of the biotransformation efficiency, with regard to isolated DCDC yield, and operational stability, in particular during the work-up process.

With a fed-batch biotransformation procedure utilizing sodium benzoate as substrate and D-fructose as carbon source it was possible to increase the final substrate concentration to 76.8 mmol/dm^3, which equals a yield/culture volume ratio of 9.4 g/dm^3 compared with the previously published ratios of 4 g/dm^3 and 6.7 g/dm^3, respectively. A significant advantage of the described biotransformation by Payer [20] is the simple work-up procedure in comparison with previous literature [22, 23]. A major obstacle in product isolation is the limited stability of DCDC at low pH. Standard fermentation work-up is initiated by separation of cells by centrifugation followed by extraction of DCDC with EtOAc at pH < 3. Under these conditions, partial re-aromatization of DCDC to salicylic acid as side product was difficult to prevent and large quantities of EtOAc are required for the extraction. Also, re-aromatization is observed during evaporation of the organic solvent, even at low temperatures and reduced pressure, because of the acidity of the biotransformation product; this property of the product also leads to limited storage capability of the isolated material. This is also a limitation of some previous biotransformation studies using various host strains, as isolated product yields are usually significantly lower (or product mixtures are obtained with re-aromatized material as major contaminant) than substrate titer and conversion data may indicate [25, 26].

By precipitating the sodium salt **1a**, and not the free acid **1**, on addition of isopropanol to the fermentation supernatant, the product is obtained in a preferable storage form, based on the higher stability, and re-aromatization could be circumvented (Scheme 2).

Mineral minimal medium (1 mmol/dm^3 phosphate buffer) utilized by Payer [20] was also used to increase the final substrate concentration from 76.8 mmol/dm^3 to the described 300 mmol/dm^3 product formation, but this failed because the higher concentration of sodium benzoate seemed to inhibit the transformation, which was attributed to substrate toxicity.

Therefore, the culture medium was changed back to Hutner's mineral base as described by Meyers et al. [22] and us, previously, and feeding of substrate and D-fructose was repeated multiple times (47×) to keep substrate concentration under possible toxicity limits. The reaction could be easily monitored via control of oxygen saturation, because after each addition of sodium benzoate solution the oxygen concentration decreased rapidly as oxygen is consumed for insertion of the two hydroxyl groups, as shown in Fig. 1.



Scheme 2

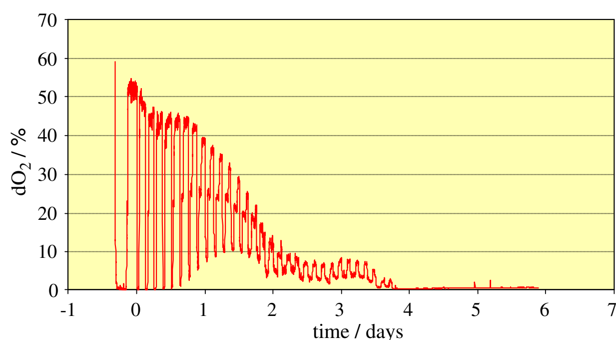


Fig. 1 Monitoring of the oxygen concentration during fed batch fermentation, every spike of the oxygen concentration curve indicates addition of substrate

The decrease in amplitude of the graph in Fig. 1 may be explained by partial jamming of the oxygen sensor membrane by biomass.

After complete conversion of the substrate the oxygen concentration increases again and drops sharply after the next substrate addition. With Hutner's mineral base as medium it was possible to increase the final substrate concentration to 88.6 mmol/dm^3, which equals a yield/culture volume ratio of 12.8 g/dm^3. These values are calculated with the maximum amounts of medium and solutions (sodium benzoate and D-fructose solution) added. The expected volume of the fermentation broth after complete fermentation time should have been 1.75 dm^3; however, only about 1.55 dm^3 was left in the fermenter because of evaporation over the long fermentation time (four days) despite the presence of an exhaust cooler. Considering this amount of liquid the final substrate concentration was 100 mmol/dm^3, which equals a yield/culture volume ratio of 14.4 g/dm^3 under operational conditions.

The isolated yield cannot be reported exactly because of co-precipitation of salts originating from the medium; moreover HMB medium is a sodium–potassium buffer and product **1a** was precipitated as a mixture of sodium and/or potassium salts; therefore only 100% conversion determined by HPLC analysis can be reported.

The weight of the precipitation product was 41.5 g; the maximum weight would be 42.8 g which consists of a maximum of 30.25 g product **1a** as the potassium salt and 12.6 g medium salts. It could be concluded that 73% of the precipitate was product **1a,** which could also be confirmed by NMR which displayed only the expected product.

We were able to double the yield per liter culture medium with this feed batch procedure from below 7–14.4 g/dm^3 while ensuring 100% conversion.

Preparation of precursors for intramolecular Diels–Alder cyclization

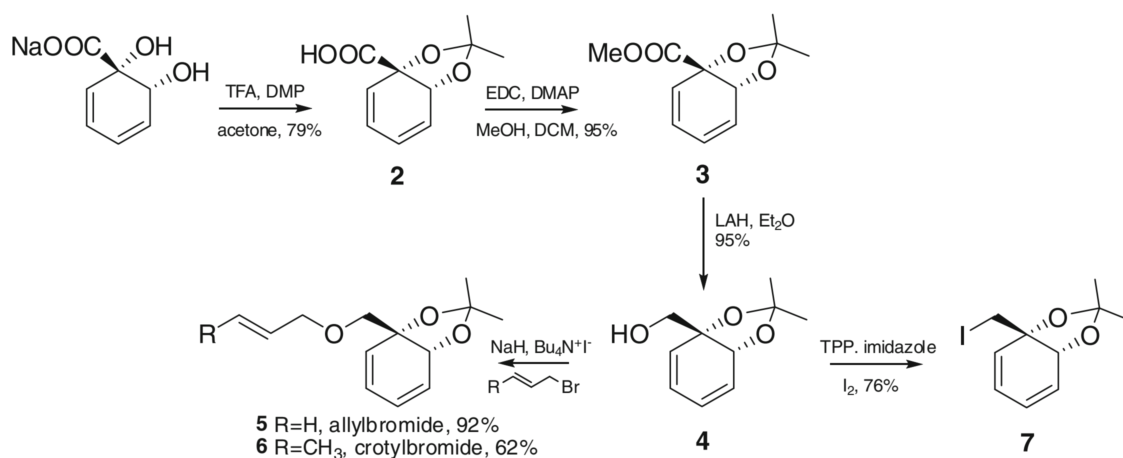
Protection of biodihydroxylated compound **1a** is necessary for further synthetic elaboration, because of the inherent instability of the free dihydroxylated acid (predominantly leading to re-aromatization). The outlined work-up procedure requires a different protection procedure compared with previous literature reports, because the sodium salt **1a** is insoluble under standard ketalization conditions (mixture of dimethoxypropane and acetone) and therefore does not undergo facile protection with the literature procedure [22, 23]. It seems that the free acid moiety is needed for the protection reaction in order to ensure efficient Lewis-type catalysis. Several acids, in varying concentrations and total amounts, were tested but only the use of concentrated trifluoroacetic acid (1.5 equivalents) in a mixture of 2,2-dimethoxypropane and acetone gave efficient conversion to acetonide **2** in 79% yield starting form the biooxidation product as carboxylate salt (Scheme 3). Subsequent esterification of compound **2** with methanol using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (EDC) as activating reagent [27] gave (3a*S*,7a*R*)-2,2-dimethyl-7a*H*-benzo[1,3]dioxole-3a-carboxylic acid methyl ester **3** in 92% yield as colorless solid (Scheme 3).

Reduction to alcohol **4** as key intermediate for the subsequent synthesis of the precursors for the intramolecular Diels–Alder cyclization was conducted according to a modified procedure by Widdowson [21]; increasing the reaction time and reducing the reaction temperature led to a significantly improved yield of 95%.

Allylation of compound **4** with allyl bromide under standard ether-formation conditions was initially hampered by the fact that predominantly re-aromatization with subsequent O-allylation was observed and only minor amounts of ether **5** could be obtained. To circumvent this problem a modified procedure by Martin [28] was applied using tetrabutylammonium iodide as catalyst to give compound **5** in excellent yields up to 92%. The corresponding crotyl ether **6** could also be obtained in 62% yield by use of this procedure with crotyl bromide. Substitution of the hydroxyl group by iodine to obtain iodo-acetonide **7** was conducted according to the procedure of Marco-Contelles et al. [29] using triphenylphospine, imidazole, and iodine under reflux conditions. This procedure gave the desired compound **7** in 76% yield.

Intramolecular Diels–Alder cyclization reactions

Utilization of the above prepared precursors in intramolecular Diels–Alder reaction offers the prospect of concomitant formation of three to four asymmetric centers



Scheme 3

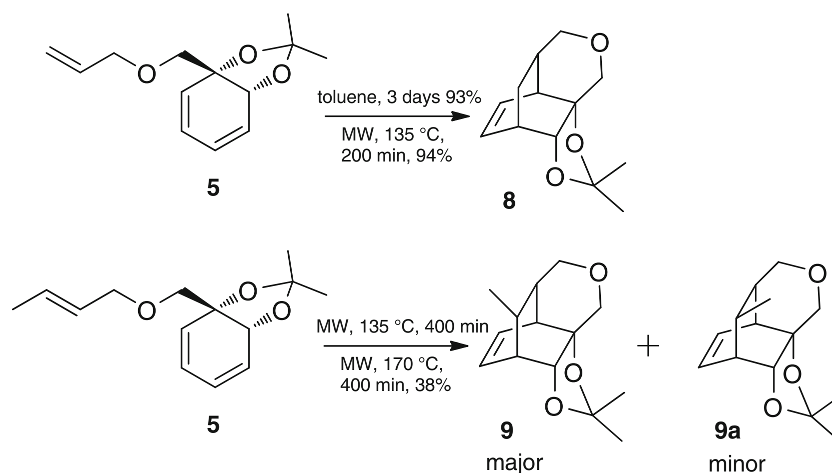
(in addition to the two chiral centers generated in the biooxidation step) because the regioselectivity of the cyclization is fully controlled; this high control of regiochemistry is in marked contrast with previous intermolecular Diels–Alder reactions, which are largely limited by the moderate difference of electron density in the diene system.

Compounds **5** and **6** were either reacted under microwave irradiation or under thermal conditions by heating under reflux in toluene.

Allyl ether compound **5** could be cyclized in a straightforward manner to the polycyclic Diels–Alder product [3a*R*-(3aα,4*β*,6aα,7*β*,10aα)]-3a,4,7,8-tetrahydro-2,2-dimethyl-4,7-methano-6a*H*,10*H*-1,3-dioxolo[4,5-*i*][2]benzopyran (**8**) in excellent yields under both microwave (94%) and under thermal (93%) conditions. Reaction times could be significantly reduced from 3 days to 200 min when using a microwave reactor (Scheme 4).

The cyclization reaction with substituted allyl ether **6** was also conducted under microwave irradiation but led to the expected polycyclic compound **9** with the methyl group in the *anti* position relative to the oxygen-containing bridge, and to the unexpected product **9a** with the methyl adopting a *syn* configuration. The inseparable product was a 4:1 mixture of **9** and **9a**, respectively, and the presence of the unexpected isomer may indicate the possibility of a cyclization process, which does not exclusively follow the classical concerted Diels–Alder pathway but rather proceeds via a step-wise mechanism. The total yield of 38% reflects an increase in steric repulsion because of the presence of the additional methyl group. The major *anti* product, **9**, which is expected for a concerted mechanism, was identified by advanced NMR techniques.

The two isomers could be easily structurally differentiated by NOE experiments as shown in Fig. 2. The correlation of H-3a (4.20 ppm) with H-11 (1.62–1.72 ppm)



Scheme 4

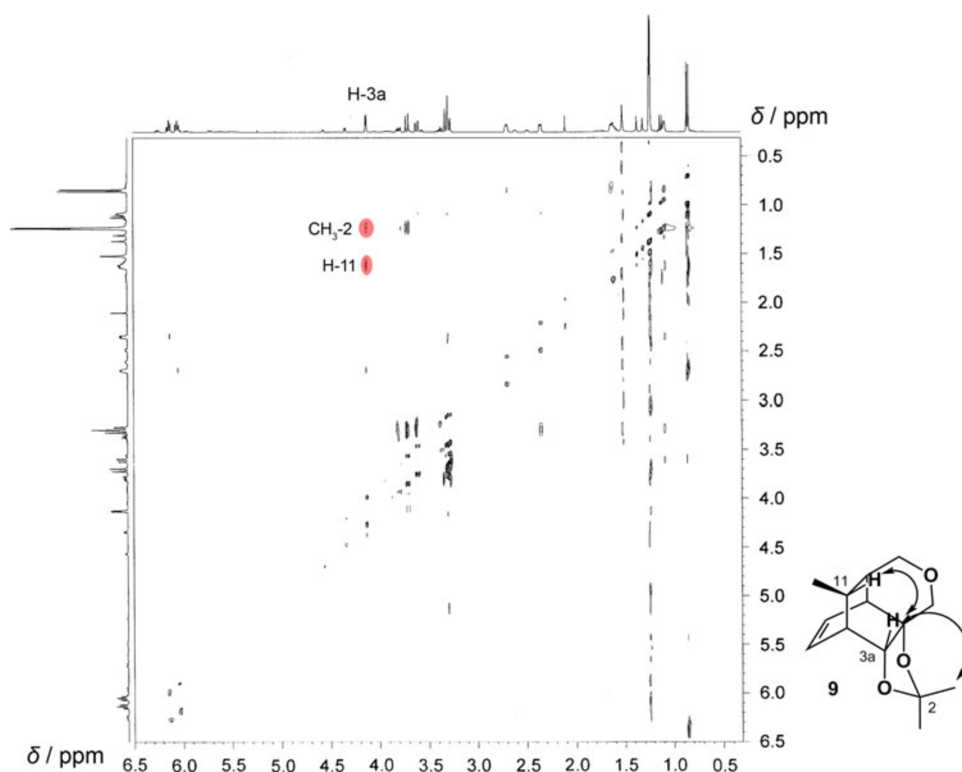


Fig. 2 NOESY spectral data for compounds **9** and **9a**

is only possible (gray), when the configuration is *anti* relative to the acetonide group, which is the case for compound **9**. If the configuration is *syn*, as in compound **9a**, no correlation should be observed.

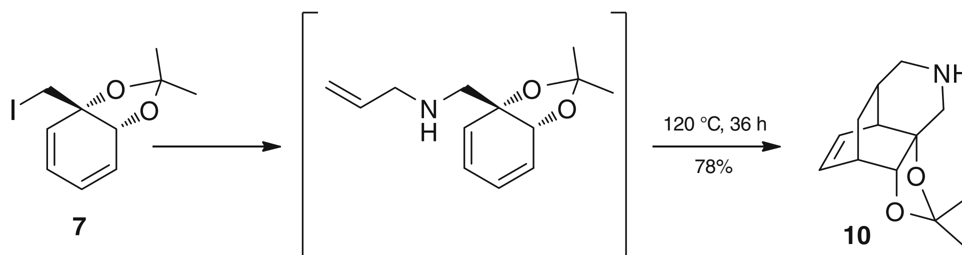
For further investigation to introduce an amino side chain, compound **7** was chosen as starting material for formation of the corresponding cyclization precursor. The neopentyl structural motif in **7** was expected to affect the efficiency of the nucleophilic substitution reaction. Hence, the reaction partner was provided in large excess using allylamine as reactant and solvent [30]. According to GC–MS, reaction at 120 °C for 36 h resulted in full conversion. Surprisingly, immediate cyclization was observed, because the polycyclic amine **10** was obtained as sole product in 78% yield and excellent optical purity (Scheme 5).

The structure of this compound was also determined by 2D NMR techniques and comparison with a previously published lactam analog, for which the configuration has been confirmed by X-ray diffraction [23].

Conclusion

The efficiency of a whole-cell-based biotransformation of benzoic acid to a chiral diol metabolite was significantly improved by increasing both substrate titer and isolated yield of asymmetric product, and by simplifying the work-up procedure in order to provide a more stable form of the biooxygenation product.

We were able to develop a facile synthetic pathway to construct functionalized polycyclic bridged heterocycles of a novel structural type containing up to six chiral centers which were generated de novo from a planar starting material in a minimum number of steps.



Scheme 5

Excellent control of five stereogenic centers could be achieved in a microwave and thermally assisted intramolecular Diels–Alder cyclization, and initial chirality was introduced within the biodihydroxylation step in high optical purity (>95% *ee*).

Experimental

Materials and methods

Unless otherwise noted, chemicals and microbial growth media were purchased from commercial suppliers and used without further purification. All solvents were distilled before use. Flash column chromatography was performed on silica gel 60 from Merck (40–63 µm). Basic silica gel was obtained by mixing Et_3N (5% of solvent volume), silica gel, and the desired solvent mixture. Melting points were determined using a Kofler-type Leica Galen III micro hot-stage microscope.

NMR spectra were recorded from $CDCl_3$, DMSO-d_6, MeOH-d_4, or D_2O solutions on a Bruker AC 200 (200 MHz) or Bruker Avance UltraShield 400 (400 MHz) spectrometer and chemical shifts are reported in ppm using TMS as internal standard. Peak assignment is based on correlation experiments. Ambiguous assignment is marked with an asterisk.

Combustion analysis was carried out in the Microanalytical Laboratory, University of Vienna. Microwave reactions were carried out in a CEM Explorer PLS™ microwave oven.

General conversion control and examination of purified products were performed with GC Top 8000/MS Voyager (quadrupole, EI+) or GC Focus/MS DSQ II (quadrupole, EI+) using a standard capillary column BGB5 (30m × 0.32 mm ID) and a standardized temperature profile (short run: 100 °C (2 min) → 18 °/min → 280 °C (2 min)).

HPLC analyses were recorded on a Thermo Finnigan Surveyor Plus using a Phenomenex Luna C18(2) column (10 µm, 250 mm × 4.6 mm diameter). Detection was performed on a Thermo Finnigan PDA Plus photodiode-array detector (standard method: 1 cm^3/min, H_2O (0.1% TFA)–ACN = 85:15 1 min → H_2O (0.1% TFA)–ACN = 20:80 9 min → H_2O (0.1% TFA)–ACN = 20:80 3 min → H_2O (0.1% TFA)–ACN = 85:15 3 min → H_2O (0.1% TFA)–ACN = 85:15 5 min).

HRMS were recorded using a Shimadzu LC prominence (Phenomenex Luna C 18(2) 5 µm, 300 mm × 4.6 mm diameter)/MS-IT-TOF. The MS was calibrated in ESI mode and measurements where recorded in APCI mode; therefore a regular deviation of about 10 ppm between calculated and found values was encountered.

Large-scale fermentations were performed in a New Brunswick Bioflow 110 fermenter equipped with pH probe, oxygen probe, flow controller, and temperature control. Monitoring of all fermentation conditions was performed by use of Biocommand Plus 3.30 software by New Brunswick.

Centrifugations for removing of cell debris were realized on Sigma 6K15 (rotor 12500). Specific rotation $[\alpha]_D^{20}$ was determined using a Perkin Elmer Polarimeter 241.

Sodium (1S,2R)-1,2-dihydroxycyclohexa-3,5-diene-1-carboxylate (**1a,** $C_7H_7NaO_4$)

LB(II) medium [24] (100 cm^3) inoculated with a single colony of *Ralstonia eutopha* B9 grown on LB(II) agar plates, grown for 3 days at 30 °C, was shaken at 30 °C at 120 rpm on an orbital shaker for 3 days. An aliquot (40 cm^3) of the cellular suspension was used to inoculate 1.400 cm^3 HMB medium [23] (pH 7.4) and D-fructose solution (2.52 g, 10 mmol/dm^3) was added. The culture medium was grown at 30 °C, pH 7.4, agitation rate 300 rpm, and sterile air was introduced at a rate of 3 dm^3/min until an $OD_{256} = 2.8$ was reached (about 16 h). The fermentation broth was then induced with sodium benzoate (7 cm^3, 1 M) and D-fructose solution (2.52 g, 10 mmol/dm^3). After about 6.5 h sodium benzoate was completely transformed to the dihydroxy product and repetitive feeding was started with sodium benzoate (3.2 cm^3, 0.49 M, 0.75 mmol/dm^3 h) and D-fructose (3.2 cm^3, 0.28 M, 0.8 mmol/dm^3 h). Sodium benzoate and D-fructose were repeatedly added every 180 min—47 times over a period of 141 h.

The fermentation broth was centrifuged in portions at 9,600 rpm (=17,000*g*) for 20 min and the supernatant was concentrated at reduced pressure to a volume of 150 cm^3 at 40 °C. Isopropanol (850 cm^3) was added to the concentrate and the product precipitated within 1 h. After isolation of the precipitate by filtration and drying at 70 °C under reduced pressure, the product was obtained as a pale beige solid (41.5 g); 73% of the precipitate is product **1a** which corresponds to 30.25 g (100%) of mixed salts. ^{1}H NMR (D_2O, 200 MHz): $\delta = 5.72$–6.19 (m, 4H, H-3, H-4, H-5, H-6), 4.87 (s, 1H, H-2) ppm; ^{13}C NMR (D_2O, 50 MHz): $\delta = 176.3$ (s, COO), 121.6, 124.9, 127, 133.3 (4×d, C-3, C-4, C-5, C-6), 70.5 (d, C-2), 73.6 (s, C-1) ppm.

(3aS,7aR)-2,2-Dimethyl-1,3-benzodioxole-3a(7aH)-carboxylic acid (**2**)

Trifluoroacetic acid (1.08 cm^3, 14.55 mmol) was added dropwise to a solution of 1.74 g **1a** (9.7 mmol) in 10 cm^3 2,2-dimethoxypropane and 2 cm^3 acetone at 0 °C under inert (N_2) gas atmosphere. After stirring the mixture overnight at room temperature the solvent was removed at reduced pressure. The crude residue was dissolved in the minimum amount of water and extracted with

dichloromethane. The organic layer was extracted twice with saturated sodium bicarbonate solution. The aqueous phase was cooled on an ice bath, then acidified with cold 2 M HCl and extracted rapidly with dichloromethane. Drying over sodium sulfate and evaporation of the solvent at reduced pressure afforded **2** (1.50 g, 79%) as pale yellow crystals. M.p.: 44–46 °C; $[\alpha]_D^{20} = -345.5\ 10^{-1}\ ^\circ\ cm^2/g$ ($c = 0.96$, $CHCl_3$); spectral properties of the compound matched previous reports [21, 22].

(3aS,7aR)-2,2-Dimethyl-1,3-benzodioxole-3a(7aH)-carboxylic acid methyl ester (**3**)

EDC (806 mg, 4.2 mmol, 1.1 equiv.), 47 mg DMAP (0.38 mmol, 0.1 equiv.), and 184 mg methanol (5.73 mmol, 1.5 equiv.) were added sequentially to a solution of 750 mg **2** (3.82 mmol) dissolved in 10 cm^3 dry dichloromethane at 0 °C under nitrogen atmosphere. The reaction mixture was cooled for one additional hour and the solution was stirred at room temperature overnight. The reaction mixture was diluted with dichloromethane and washed with satd. NH_4Cl solution, satd. $NaHCO_3$ solution, and water. The organic layer was dried over sodium sulfate and the solvent was evaporated at reduced pressure. Purification by flash column chromatography (SiO_2, LP–EtOAc 25:1) gave **3** (739 mg, 92%) as a colorless solid. M.p.: 49–51 °C ([21] 51.5–52.5 °C); $[\alpha]_D^{20} = -417.2\ 10^{-1}\ ^\circ\ cm^2/g$ ($c = 1$, $CHCl_3$); spectral properties of the compound matched previous reports [21, 22].

(3aR,7aR)-2,2-Dimethyl-1,3-benzodioxole-3a(7aH)-methanol (**4**)

A solution of 300 mg **3** (1.43 mmol) in 4 cm^3 dry diethyl ether was added dropwise to a suspension of 65 mg LAH (1.71 mmol, 1.2 equiv.) in 4 cm^3 dry Et_2O at 0 °C. After 20 min TLC analysis indicated full conversion of the starting material. EtOAc was added and the mixture stirred for a further 10 min, then 2 M HCl was added until a precipitate was formed. The reaction mixture was filtered through a bed of Celite® and the Celite pad was thoroughly washed with EtOAc. The combined organic layers were dried over sodium sulfate and the solvent was evaporated at reduced pressure. Purification by flash column chromatography on NEt_3 impregnated silica gel (25:1; LP–EtOAc) gave **4** (247 mg, 95%, [21] 84%) as a colorless oil, which crystallized in the fridge. M.p.: 42–43 °C ([21] 41–44 °C); $[\alpha]_D^{20} = -215.9\ 10^{-1}\ ^\circ\ cm^2/g$ ($c = 1.03$, $CHCl_3$); spectral properties of the compound matched previous reports [21].

(3aR,7aR)-3a,7a-Dihydro-2,2-dimethyl-3a-[(2-propenyloxy)methyl]-1,3-benzodioxole (**5**, $C_{13}H_{18}O_3$)

Compound **4** (50 mg, 0.27 mmol), tetrabutylammonium iodide (catalytic amount), and 170 mg 3-bromopropene (1.40 mmol, 5 equiv.) were dissolved in 5 cm^3 dry THF. Sodium hydride (13 mg, 0.54 mmol, 2 equiv.) was added in one portion at 0 °C. After 2 h of stirring at room temperature the reaction was quenched by addition of 1 cm^3 water at 0 °C. The reaction mixture was extracted repeatedly with EtOAc. The organic layer was washed with brine and dried over sodium sulfate. Evaporation of the solvent and purification by flash column chromatography (SiO_2, 35:1; LP–EtOAc) gave **5** (56 mg, 92%) as a colorless oil. ^1H NMR ($CDCl_3$, 200 MHz): $\delta = 5.70$–6.16 (m, 5H, H-4, H-5, H-6, H-7, $C\underline{H}{=}CH_2$), 5.08–5.22 (m, 2H, $C\underline{H_2}{=}CH$), 4.49 (d, $J = 4.3$ Hz, 1H, H-7a), 4.05 (dd, $J = 1.7$ Hz, $J = 5.5$ Hz, 2H, $C\underline{H_2}$–CH=), 3.04 (dd, $J = 10.2$ Hz, $J = 18.8$ Hz, 2H, H-α), 1.45 (s, 3H, CH_3), 1.37 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 122.4$, 124.6, 125.1, 129.4, 134.5 (5×d, C-4, C-5, C-6, C-7, $\underline{C}H{=}CH_2$), 116.9 (t, $\underline{C}H_2{=}CH$), 106.2 (s, C-2), 79.5 (s, C-3a), 72.7, 73.2 (2×t, C-α, $\underline{C}H_2$-O), 71.9 (d, C-7a), 27.1 (q, CH_3), 26.5 (q, CH_3) ppm; $[\alpha]_D^{20} = -265.6\ 10^{-1}\ ^\circ\ cm^2/g$ ($c = 1$, $CHCl_3$).

(3aR,7aR)-3a-[(2-Buten-1-yloxy)methyl]-3a,7a-dihydro-2,2-dimethyl-1,3-benzodioxole (**6**, $C_{14}H_{20}O_3$)

Compound **4** (100 mg, 0.55 mmol, 1 equiv.), 371 mg crotyl bromide (2.75 mmol, 5 equiv.), and tetrabutylammonium iodide (catalytic amount) were dissolved in 10 cm^3 dry THF. Sodium hydride (27 mg, 1.1 mmol, 2 equiv.) was added in one portion at 0 °C. After 2 h of stirring at 0 °C the reaction temperature was allowed to rise to room temperature and stirred overnight. The reaction was quenched by addition of 2 cm^3 water at 0 °C. After extraction with EtOAc, the organic layer was washed with brine and dried over sodium sulfate. Evaporation of the solvent and purification by flash column chromatography (SiO_2, 40:1; LP–EtOAc) gave **6** (80 mg, 62%) as a light yellow oil. ^1H NMR ($CDCl_3$, 200 MHz): $\delta = 5.85$–6.07 (m, 3H, H-4, H-5, H-6), 5.37–5.75 (m, 3H, H-4′, H-5′, H-7), 4.42 (d, $J = 4.5$ Hz, 1H, H-3a), 3.81–4.09 (m, 2H, H-3′), 3.22–3.41 (m, 2H, H-1′), 1.54–1.69 (m, 3H, H-6′), 1.37 (s, 3H, CH_3), 1.29 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 127.4$, 129.4 (2×d, $-\underline{C}H{=}\underline{C}H-$), 122.4, 124.7, 125.1, 129.5 (4×d, C-4, C-5, C-6, C-7), 106.2 (s, C-2), 79.6 (s, C-3a), 72.6, 73.1 (2×t, C-α, $\underline{C}H_2$–CH=), 72.1 (d, C-7a), 27.2 (q, CH_3), 26.6 (q, CH_3), 17.7 (q, CH_3) ppm; $[\alpha]_D^{20} = -67.6\ 10^{-1}\ ^\circ\ cm^2/g$ ($c = 1.43$, $CHCl_3$).

(3aS,7aR)-3a,7a-Dihydro-3a-(iodomethyl)-2,2-dimethyl-1,3-benzodioxole (**7**, $C_{10}H_{13}IO_2$)

Compound **4** (800 mg, 4.4 mmol, 1 equiv.) was dissolved in 70 cm^3 dry toluene and treated with 3.46 g triphenylphosphine (13.2 mmol, 3 equiv.), 899 mg imidazole (13.2 mmol, 4 equiv.), and 2.23 g iodine (8.8 mmol, 2 equiv.) at reflux for 10 min. After cooling to room temperature the reaction mixture was washed with satd. $NaHCO_3$ and a 5% aqueous solution of $Na_2S_2O_3$. The organic layer was dried over sodium sulfate and evaporated

at reduced pressure. The residue was submitted to flash column chromatography (SiO_2, LP–EtOAc; 100:1) to give **7** (976 mg, 76%) as a colorless oil. ^{1}H NMR ($CDCl_3$, 200 MHz): $\delta = 5.95$–6.17 (m, 3H, H-4, H-5, H-6), 5.75 (d, $J = 9.8$ Hz, 1H, H-7), 4.44 (d, $J = 4.1$ Hz, 1H, H-7a), 3.32 (d, $J = 10.6$ Hz, 1H, H-α), 3.15 (d, $J = 10.6$ Hz, 1H, H-α), 1.51 (s, 3H, CH_3), 1.33 (s, 3H, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 50 MHz): $\delta = 123.3$, 125.0, 125.2, 129.1 (4×d, C-4, C-5, C-6, C-7), 107.2 (s, C-2), 77.5 (s, C-3a), 74.9 (d, C-7a), 27.1 (q, 2× CH_3), 12.9 (t, C-α) ppm; $[\alpha]_D^{20} = -127.8$ 10^{-1} ° cm^2/g ($c = 1.15$, $CHCl_3$).

[3aR-(3aα,4β,6aα,7β,10aα)]-3a,4,7,8-Tetrahydro-2,2-dimethyl-4,7-methano-6aH,10H-1,3-dioxolo[4,5-i][2]-benzopyran (**8**, $C_{13}H_{18}O_3$)

Method A Compound **5** (100 mg, 0.45 mmol) dissolved in 4 cm^3 toluene was treated in the microwave oven at 135 °C for 200 min. The solvent was evaporated and the residue was purified by flash column chromatography (SiO_2, 40:1; LP–EtOAc) to give **8** (94 mg, 94%) as a colorless oil.

Method B Compound **5** (80 mg, 0.36 mmol) dissolved in 20 cm^3 toluene was heated under reflux for 72 h. The solvent was evaporated and the residue was purified by flash column chromatography (SiO_2, 40:1; LP–EtOAc) to give **8** (74 mg, 93%) as a colorless oil.

^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 6.17$–6.26 (m, 2H, H-5, H-6), 4.22 (d, $J = 10.5$ Hz, 1H, H-3a), 3.79 (d, $J = 10.5$ Hz, 1H, H-10), 3.67 (d, $J = 11.1$ Hz, 1H, H-8), 3.36–3.42 (m, 2H, H-8, H-10), 2.90–2.97 (m, 1H, H-4), 2.45–2.51 (m, 1H, H-6a), 1.68–1.75 (m, 1H, H-7), 1.49–1.57 (dt, $J = 3.5$ Hz, $J = 12$ Hz, 1H, H-11), 1.34–1.40 (m, 1H, H-11), 1.32 (bs, 6H, 2× CH_3) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 131.4$, 132.9 (d, C-5, C-6), 109.0 (s, C-2), 83.4 (d, C-3a), 81.2 (s, C-10a), 73.8 (t, C-10), 71.1 (t, C-8), 42.5 (d, C-6a), 35.8 (d, C-4), 33.4 (d, C-7), 28.4 (q, CH_3), 26.8 (q, CH_3), 25.5 (t, C-11) ppm; $[\alpha]_D^{20} = -11.95$ 10^{-1} ° cm^2/g ($c = 0.95$, $CHCl_3$).

[3aR-(3aR,4S*,6aS*,7R*,10aR*,11R*)]-4,6a,7,8-Tetrahydro-2,2,11-trimethyl-4,7-methano-3aH,10H-1,3-dioxolo[4,5-i][2]benzopyran* (**9**, $C_{14}H_{20}O_3$) *and [3aR-(3aR*,4S*,6aS*,7R*,10aR*,11S*)]-4,6a,7,8-Tetrahydro-2,2,11-trimethyl-4,7-methano-3aH,10H-1,3-dioxolo[4,5-i][2]benzopyran* (**9a**, $C_{14}H_{20}O_3$)

Compound **6** (24 mg, 0.1 mmol) was dissolved in 2 cm^3 dry toluene and heated by microwave irradiation at 135 °C under stirring for 400 min and then 400 min at 170 °C. After evaporation of the solvent the residue was submitted to column chromatography for purification and 9 mg (38%) **9** and **9a** was obtained as an inseparable mixture in the ratio of 4:1 as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz): major *anti* product **9**: $\delta = 6.20$ (t, 1H, $J = 7.9$ Hz, H-6), 6.11 (t, 1H, $J = 7.2$ Hz, H-5), 4.20 (d, 1H, $J = 3.5$ Hz, H-3a), 3.76 (d, 1H, $J = 10.8$ Hz, H-10), 3.65–3.70 (m, 1H, H-8), 3.33–3.40 (m, 2H, H-8, H-10), 2.73–2.79 (m, 1H, H-4), 2.39–2.44 (m, 1H, H-6a), 1.62–1.72 (m, 1H, H-11), 1.31 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.13–1.17 (m, 1H, H-7), 0.91 (d, 3H, $J = 7.0$ Hz, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): major *anti* product **9**: $\delta = 131.1$ (d, C–5), 130.5 (d, C-6), 109.2 (s, C-2), 83.4 (d, C-3a), 80.7 (s, C-10a), 73.6 (t, C-10), 69.8 (t, C-8), 42.9 (d, C-6a), 42.7 (d, C-4), 42.3 (d, C-7), 31.9 (d, C-11), 28.1 (q, CH_3), 26.5 (q, CH_3), 21.1 (q, CH_3) ppm; ^{1}H NMR ($CDCl_3$, 400 MHz): minor *syn* product **9a**: $\delta = 6.28$–6.34 (m, 1H, H-5), 5.99–6.05 (m, 1H, H-6), 4.41 (d, 1H, $J = 3.5$ Hz, H-3a), 3.82–3.89 (m, 2H, H-8, H-10), 3.35–3.49 (m, 2H, H-8, H-10), 2.64–2.69 (m, 1H, H-4), 2.52–2.57 (m, 1H, H-6a), 1.75–1.87 (m, 1H, H-11), 1.62–1.72 (m, 1H, H-7), 1.44 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 1.20 (d, 3H, $J = 7.6$ Hz, CH_3) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): minor *syn* product **9a**: $\delta = 135.3$ (d, C-5), 129.4 (d, C-6), 108.8 (s, C-2), 81.2 (s, C-10a), 80.0 (d, C-3a), 73.9 (t, C-10), 68.1 (t, C-8), 43.4 (d, C-6a), 42.9 (d, C-4), 34.4 (d, C-7), 31.5 (d, C-11), 28.3 (q, CH_3), 26.6 (q, CH_3), 13.7 (q, CH_3) ppm.

[3aR-(3aR,4S*,6aS*,7R*,10aR*)]-4,6a,7,8,9,10-Hexahydro-2,2-dimethyl-4,7-methano-3aH-1,3-dioxolo[4,5-i]isoquinoline* (**10**, $C_{13}H_{19}O_2N$)

Compound **7** (63 mg, 0.22 mmol) was dissolved in 2 cm^3 allylamine and stirred at 120 °C for 36 h. After completion of the reaction allylamine was removed under reduced pressure and purification was performed by flash column chromatography under basic conditions (SiO_2, LP–EtOAc 2:1 → EtOAc) yielding **10** (37 mg, 78%) as an orange oil. ^{1}H NMR ($CDCl_3$, 400 MHz): $\delta = 6.15$–6.26 (m, 2H, H-5, H-6), 4.21 (d, $J = 3.8$ Hz, 1H, H-3a), 2.89–2.95 (m, 1H, H-4), 3.04 (d, 1H, $J = 11.7$ Hz, H-10), 2.82 (d, 1H, $J = 11.7$ Hz, H-10), 2.65–2.81 (m, 2H, H-8), 2.38–2.42 (m, 1H, H-6a), 1.72–1.79 (m, 1H, H-7), 1.46–1.55 (m, 1H, H-11), 1.33 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.24–1.29 (m, 1H, H-11) ppm; ^{13}C NMR ($CDCl_3$, 100 MHz): $\delta = 131.1$, 131.2 (2×d, C-5, C-6), 112.3 (t, $=\underline{C}H_2$), 108.5 (s, C-2), 82.8 (d, C-3a), 81.9 (s, C-10a), 54.2 (t, C-10), 49.0 (t, C-8), 43.3 (d, C-6a), 35.7 (d, C-4), 32.2 (d, C-7), 28.2 (q, CH_3), 26.4 (q, CH_3), 25.1 (t, C-11) ppm; $[\alpha]_D^{20} = +3.0$ 10^{-1} ° cm^2/g ($c = 0.73$, $CHCl_3$); HRMS: [M + H$^+$] calc. 222.1494, found 222.1217.

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