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Efficient Generation of a Trisporoid Library by Combination of Synthesis and Biotransformation

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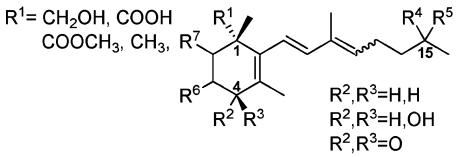

substitutent / series	R^4	R^5	R^6	R^7
A	H	H	H	H
B	O		H	H
C	H	OH	H	H
D	O		OH	H
E	H	OH	H	OH

Trisporic acids and their biosynthetic precursors represent a family of powerful fungal pheromones and morphogenetic factors. A highly flexible synthetic protocol is described that (i) provides rapid access to nonfunctionalized early trisporoids from β-ionone, (ii) includes a regiospecific oxidative functionalization of β-ionone leading to 1-acetoxy-β-ionone giving access to functionalized trisporoids, and (iii) utilizes a biotransformation of early synthetic trisporoids by growing cells of *Blakeslea trispora* to prepare late trisporoids including trisporic acids. The same protocol also provides deuterium-labeled trisporoids such as trisporin B [$^2H_3$]-**19**. Administration of [$^2H_3$]-**19** to growing cells of the (−)-mating type of *B. trispora* resulted in the formation of the labeled trisporols [$^2H_3$]-**20** and [$^2H_3$]-**21**. Growing cultures containing both mating types can be used to prepare trisporic acids from early precursors.

Introduction

Carotenoid-derived compounds (Scheme 1) regulate the first stages of sexual development and stimulate the production of zygophores in the fungal phylum Zygomycota, for example, in *Blakeslea trispora*,[1] *Phygomyces blakesleeanus*,[2] *Zygorhynchus moelleri*,[3] and *Mucor mucedo*.[1,4] Sexual reactions of these fungi are controlled by a family of pheromones, the trisporic acids, resulting from oxidative degradation of β-carotene. The compounds are involved in recognition of mating partners, induce the first steps of sexual differentiation, maintain the development of sexual structures, and even mediate the recognition between Zygomycetes and their parasites (e.g., *Parasitella parasitica*).[5]

Pioneering analytical work with *Blakeslea trispora* and *Mucor mucedo* revealed a chemical dialogue between the sexual partners that required an exchange of early trisporoids as precursors for the bioactive trisporic acids. As outlined in Scheme 1, both mating types are at least competent to produce 4-dihydrotrisporin (**2**) but lack the ability to generate the downstream compounds.[7]

Instead, the biosynthesis of the late trisporoids was assumed to require a diffusive exchange of "prohormones" between the mating partners and results in a "cooperative biosynthesis"[8,9] that offers additional regulatory possibilities and molecular diversification at the level of metabolites.

In fact, the currently known group of trisporoids is structurally and functionally highly diverse but the specific role of the individual compounds in the sexual development and function of the different Zygomycetes is largely unknown. Trisporoids were grouped within A, B, C, D, and E series according to their substitution pattern at R^4, R^5, R^6, and R^7 and according to further oxidative functionalization of the ring system (R^1, R^2, R^3) in trisporols, trisporic acids, (4-dihydro)-methyltrisporates, and (4-dihydro)-trisporins (Scheme 2).[2,7,8,10−12]

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(1) Austin, D. J.; Bulock, J. D.; Gooday, G. W. *Nature* **1969**, *223*, 1178−1179.
(2) Miller, M. L.; Sutter, R. P. *J. Biol. Chem.* **1984**, *259*, 6420−6422.
(3) Schipper, M. A. *Trans. Br. Mycol. Soc.* **1971**, *56*, 157−159.
(4) Gooday, G. W. *Phytochemistry* **1968**, *7*, 2103−2105.
(5) Schultze, K.; Schimek, C.; Wostemeyer, J.; Burmester, A. *Gene* **2005**, *348*, 33−44.
(6) van den Ende, H. *Sexual Interactions in Plants (Experimental Botany)*; Academic Press: New York, 1976; Vol. 9, p 64−71.
(7) Bulock, J. D.; Jones, B. E.; Winskill, N. *Pure Appl. Chem.* **1976**, *47*, 191−202.
(8) Nieuwenhuis, M.; van den Ende, H. *Arch. Microbiol.* **1975**, *102*, 167−169.
(9) Werkman, B. A. *Arch. Microbiol.* **1976**, *109*, 209−213.
(10) Bulock, J. D.; Austin, D. J.; Snatzke, G.; Hruban, L. *J. Chem. Soc. D, Chem. Commun.* **1970**, 255−256.

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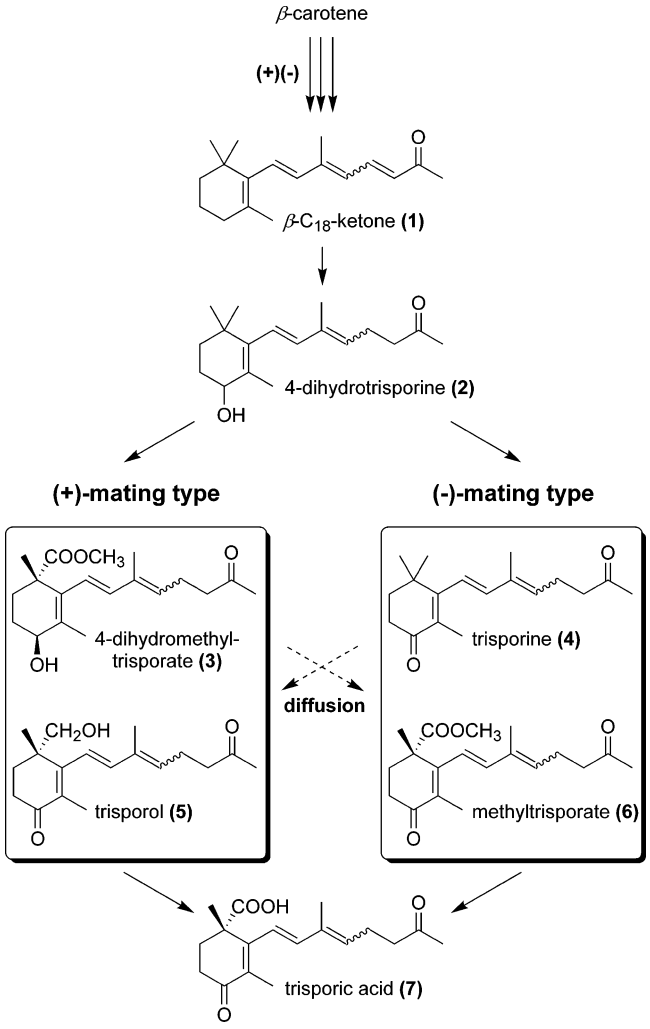
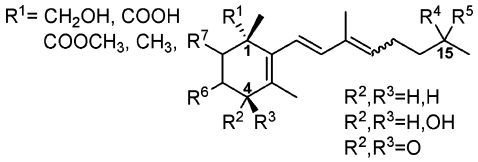
SCHEME 1. Postulated Biosynthetic Pathway for Trisporic Acid Series B[6]



SCHEME 2. Functionalization Patterns of Trisporoids[a]



substitutent / series	R^4	R^5	R^6	R^7
A	H	H	H	H
B	O		H	H
C	H	OH	H	H
D	O		OH	H
E	H	OH	H	OH

[a] Characters A−E represent the different series.

Until now, systematic studies correlating structural features with biological activities have been missing and only certain aspects have been investigated.[13] Besides the configurational isomers, natural trisporoids possess chiral centers at C1, C4, and C15 of the most important A, B, and C series.[7,10,14] However, the very low concentrations of the hormones hampered their direct structural characterization and made the chemical synthesis imperative for physiological studies. Several routes to the late trisporoids such as methyl trisporates, trisporols, and free trisporic acids have been developed and reported.[7,15−23] The disadvantage of previous routes is the large number of steps required for the synthesis of the final targets and the lack of flexibility to produce early and late trisporoids along the same protocol using common intermediates. Especially, the lack of efficient routes to trisporoids with position-specific isotopic labeling hampered systematic in vivo studies and kinetic analyses from the early trisporoids to the late bioactive signals in the competent mating types.

Here, we present a novel and highly flexible concept that is based on three key elements: (i) rapid access to the nonfunctionalized C_{18}-apocarotenoid skeleton from commercial β-ionone, (ii) regiospecific oxidative functionalization of the cyclohexene moiety of the apocarotenoids at C4, and (iii) biotransformation of early synthetic trisporoids by growing cultures of the zygomycete *Blakeslea trispora*. The approach has no need for complex functionalized or chiral precursors because the growing cells of the fungus are expected to oxidize the correct methyl group at C1 of the cyclohexene. The method also provides a rapid access to deuterium-labeled compounds as valuable tools for the analysis of trisporic acid biosynthesis in mucoraceous fungi.

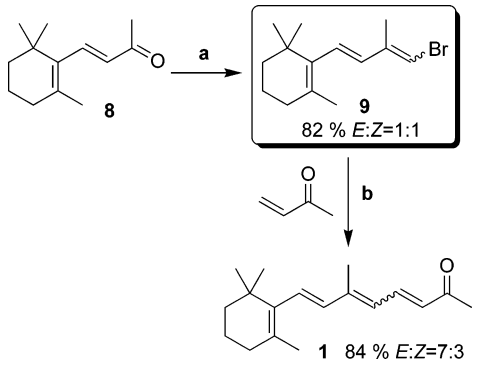
Results

Synthesis of Early Trisporoids. The concept for the synthesis of trisporoids was designed to provide the different groups of trisporoids shown in Scheme 1, namely, the nonfunctionalized early trisporoids (e.g., the β-C_{18}-ketone), the group of C4- and/or C15-modified intermediates, and finally the group of late trisporoids that are functionalized at the methyl group at C1. A particular simple and highly flexible access to the apocarotenoid C_{18}-skeleton is possible from the vinyl bromide **9** that allows the completion of differently functionalized side chains in a single operation with limited or no protecting group strategies (Scheme 3 and Scheme 4). Moreover, β-ionone (**8**) is an ideal target for an isotope exchange reaction yielding [$^2H_3$]-β-ionone (**18**)[24] which can be used for the synthesis of labeled trisporoids[25,26] along the same protocol.

The central building block **9** (*E*/*Z* = 1:1) is obtained by Wittig olefination of β-ionone (**8**) with bromomethylidenetriphenyl-

(11) Sutter, R. P. *Science* **1970**, *168*, 1590−1592.
(12) Sutter, R. P.; Dadok, J.; Bothnerby, A. A.; Smith, R. R.; Mishra, P. K. *Biochemistry* **1989**, *28*, 4060−4066.
(13) Schachtschabel, D.; Schimek, C.; Wostemeyer, J.; Boland, W. *Phytochemistry* **2005**, *66*, 1358−1365.
(14) Reschke, T. *Tetrahedron Lett.* **1969**, 3435−3439.
(15) Edwards, J. A.; Schwarz, V.; Fajkos, J.; Maddox, M. L.; Fried, J. H. *J. Chem. Soc. D, Chem. Commun.* **1971**, 292−293.
(16) Isoe, S.; Hayase, Y.; Sakan, T. *Tetrahedron Lett.* **1971**, 3691−3694.
(17) Secrist, J. A.; Hickey, C. J.; Norris, R. E. *J. Org. Chem.* **1977**, *42*, 525−527.
(18) Prisbylla, M. P.; Takabe, K.; White, J. D. *J. Am. Chem. Soc.* **1979**, *101*, 762−763.
(19) Trost, B. M.; Ornstein, P. L. *Tetrahedron Lett.* **1983**, *24*, 2833−2836.
(20) Takabe, K.; White, J. D. *Tetrahedron Lett.* **1983**, *24*, 3709−3712.
(21) White, J. D.; Takabe, K.; Prisbylla, M. P. *J. Org. Chem.* **1985**, *50*, 5233−5244.
(22) Takahashi, S.; Oritani, T.; Yamashita, K. *Agric. Biol. Chem.* **1987**, *51*, 2291−2293.
(23) Reeder, M. R.; Meyers, A. I. *Tetrahedron Lett.* **1999**, *40*, 3115−3118.
(24) Bergen, H. R.; Furr, H. C.; Olson, J. A. *J. Labelled Compd. Radiopharm.* **1988**, *25*, 11−21.
(25) Ramamurthy, V.; Liu, R. S. H. *Tetrahedron* **1975**, *31*, 201−206.
(26) Camps, J.; Font, J.; Demarch, P. *Tetrahedron* **1981**, *37*, 2493−2499.

SCHEME 3. Synthesis of the β-C_{18}-Ketone (1)[a]



[a] Reagents and conditions: (a) $(BrCH_2PPh_3)^+Br^-$, LiHMDS, THF, −78 °C for 1 h, room temperature for 12 h; (b) $Pd(Ac)_2$, PPh_3, Et_3N, THF, 60 °C, 120 h.

phosphorane using LiHMDS as a soft base for deprotonation of the phosphonium salt. The vinyl bromide **9** is an excellent component for transition-metal-catalyzed alkylations, and in the case of the β-C_{18}-ketone (**1**), this is easily achieved by a Heck-type alkylation of **9** with but-3-en-2-one.[27] The corresponding iodide proved to be too unstable and decomposed during workup.

The alkylation sequence could be successfully extended to the acetoxy-β-ionone **10** which is readily available from β-ionone (**8**) by allylic oxidation with benzoquinone and Pd-$(O_2CCF_3)_2$/*o*-methoxyacetophenone.[28] Alternative methods proved to be less satisfactory.[29−33] The key intermediate **11** is obtained by Wittig olefination in 82% yield as described for the synthesis of **9** followed by deacetylation with methanolic KOH.[34]

The third building block, namely, the ketone **12**, was obtained best by oxidation of **11** with MnO_2.[35] Direct oxidation of the vinyl bromide **9** with CrO_3·pyridine was possible, but the yield was low (35%).[36] Using either **11** or **12** for the Pd(0)-catalyzed cross-coupling with organozincates, the two series of C4-hydroxylated (**2**, **15**, **16**) or C4-keto (**4**, **13**, **14**) trisporoids were obtained in a single operation and high yields (ca. 95%, GC-MS).[4,37] The required organozincates were obtained from the corresponding iodides without the need for protecting groups.[38] This minimized the number of transformations on the generally acid- and base-sensitive trisporoid backbone. For example, the trisporoids **2** and **4** were available from **11** or **12** by alkylation with an organozincate derived from 4-iodo-butan-2-one.[39] The same strategy was followed to generate the secondary alcohols **14** and **16** or to produce trisporoids with aliphatic side chains of different length for structure−function studies.[13] For trisporin A (**13**) and **15**, an almost quantitative conversion (99% GC-MS) was observed. The trisporoids **14** and **16** resulted from alkylation of **11** or **12** with an organozincate derived from (4-iodobutan-2-yloxy)(*tert*-butyl)dimethylsilane. The latter is obtained from methyl-3-hydroxymethylbutanoate by protection with *tert*-butyldimethylsilylchloride,[40] reduction of the methyl ester moiety with DIBALH,[41] and iodination of the resulting alcohol with I_2/PPh_3 to (4-iodobutan-2-yloxy)(*tert*-butyl)dimethylsilane.[42] The sequence has the advantage that the racemate and both enantiomers of methyl-3-hydroxybutanoate are available, thus allowing also the synthesis of both enantiomers of **14** and **16**. In all alkylation reactions, the original ratio of the *E*/*Z*-isomers (ca. 1:1) of **11** and **12** was nearly maintained in the products.

Deuterium-labeled trisporoids were obtained from β-ionone (**8**) or 1-acetoxy-β-ionone **10** as outlined in Scheme 5. Exchange of the acidic protons in β-ionone (**8**) by deuterium atoms was achieved in CH_3OD in the presence of *N*-ethyl-diisopropylamine and resulted in $[^2H_3]$-**17** or $[^2H_3]$-**18** (>97% ^2H per position according to NMR).

The subsequent Wittig reaction with bromomethylidenetriphenylphosphorane and the final alkylation with but-3-en-2-one under Heck conditions generated $[^2H_3]$-**19** and proceeded without exchange of deuterium atoms.[43] Owing to the flexibility of the synthetic protocol, almost all early and late (vide infra) trisporoids can be prepared in a few steps and high overall yields.

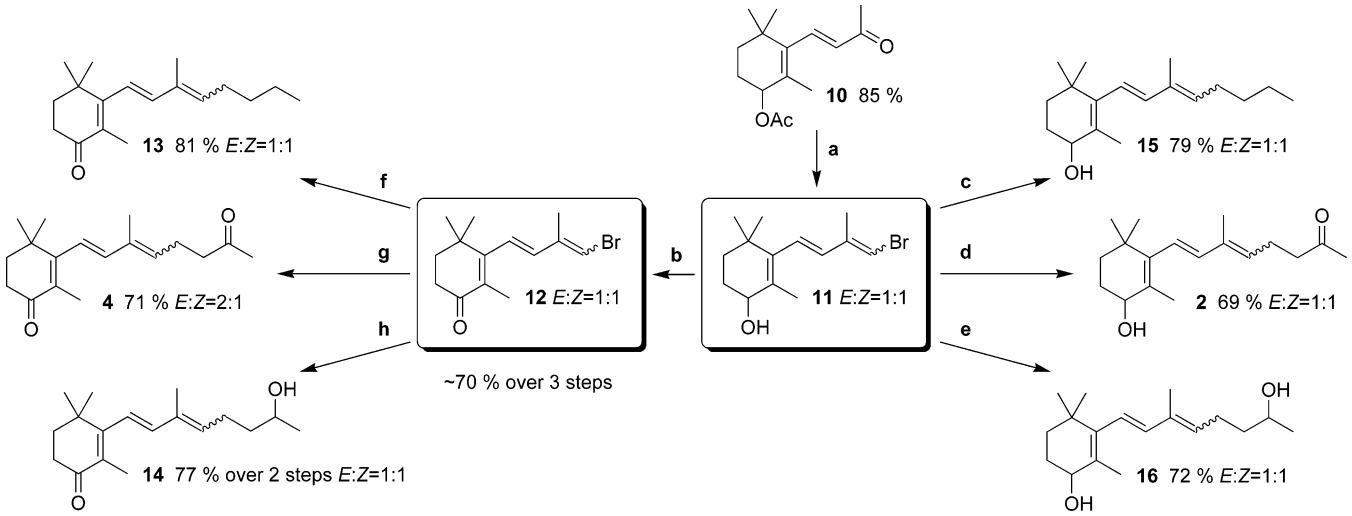
Biotransformation of Early Trisporoids by Growing Cultures of *Blakeslea trispora.* The last important aspect of the envisaged uniform concept from early to late trisporoids was the successful administration and transformation of the synthetic precursors by growing cells of *Blakeslea trispora*. In a representative feeding experiment, $[^2H_3]$-trisporin B (**20**) was added to growing cells of the (−)-mating type of *B. trispora* precultivated on a SUP medium for 3 days (Scheme 6).[44] After 48 h, the mycelia was removed and the culture broth was adjusted to pH 8. The produced trisporols $[^2H_3]$-**21** and $[^2H_3]$-**22** were extracted with chloroform/isopropanol (10% yield, equal amounts of $[^2H_3]$-**21** and $[^2H_3]$-**22**). The presence of the deuterium atoms corroborated the origin of the $[^2H_3]$-trisporols from **20** and confirmed the feasibility of the synthetic concept.

The formation of **21** and **22** by cells representing the (−)-mating type of *B. trispora* is not consistent with the originally proposed cooperative biosynthetic pathway (Scheme 1) but experimentally confirms the revised proposed interaction scheme.[7,45]

Under the same conditions, growing cultures of the (+)-mating type produced the corresponding trisporic acid type C in acceptable yield (ca. 10%). With respect to the CD spectra of **6** and methyltrisporate C, the *pro-S* methyl group at C1 of the cyclohexene was converted into the −COOH group, consistent with the established absolute stereochemistry of the

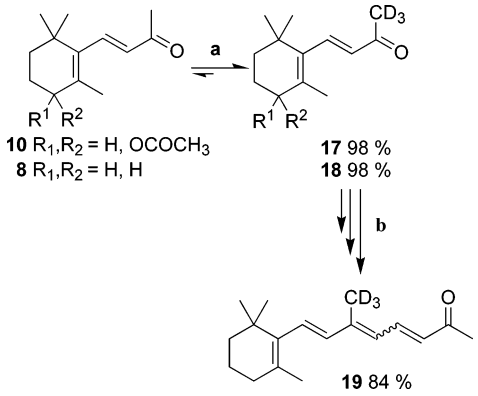
(27) Naora, H.; Ohnuki, T.; Nakamura, A. *Bull. Chem. Soc. Jpn.* **1988**, *61*, 2859−2863.
(28) McMurry, J. E.; Kocovsky, P. *Tetrahedron Lett.* **1984**, *25*, 4187−4190.
(29) Kim, T. H.; Asaka, Y. *Chem. Express* **1991**, *6*, 125−128.
(30) Guirado, A.; Moss, G. P.; Utley, J. H. P. *J. Chem. Soc., Chem. Commun.* **1987**, 41−42.
(31) Oritani, T.; Yamashita, K. *Phytochemistry* **1983**, *22*, 1909−1912.
(32) Haag, A.; Eschenmoser, W.; Eugster, C. H. *Helv. Chim. Acta* **1980**, *63*, 10−15.
(33) Jurado-Gonzalez, M.; Sullivan, A. C.; Wilson, J. R. H. *Tetrahedron Lett.* **2003**, *44*, 4283−4286.
(34) Barrero, A. F.; Alvarez-Manzaneda, E. J.; Alvarez-Manzaneda, R.; Chahboun, R.; Meneses, R.; Aparicio, M. *Synlett* **1999**, 713−716.
(35) Takahashi, S.; Oritani, T.; Yamashita, K. *Tetrahedron* **1988**, *44*, 7081−7088.
(36) Sarett, L. H.; Arth, G. E.; Lukes, R. M.; Beyler, R. E.; Poos, G. I.; Johns, W. F.; Constantin, J. M. *J. Am. Chem. Soc.* **1952**, *74*, 4974−4976.
(37) Knochel, P.; Perea, J. J. A.; Jones, P. *Tetrahedron* **1998**, *54*, 8275−8319.
(38) Huo, S. Q. *Org. Lett.* **2003**, *5*, 423−425.
(39) Irifune, S.; Kibayashi, T.; Ishii, Y.; Ogawa, M. *Synthesis-Stuttgart* **1988**, 366−369.
(40) Kobayashi, Y.; Kumar, G. B.; Kurachi, T.; Acharya, H. P.; Yamazaki, T.; Kitazume, T. *J. Org. Chem.* **2001**, *66*, 2011−2018.
(41) Ernst, B.; Wagner, B. *Helv. Chim. Acta* **1989**, *72*, 165−171.
(42) Mori, K.; Maemoto, S. *Liebigs Ann. Chem.* **1987**, 863−869.
(43) Akita, H. *Chem. Pharm. Bull.* **1983**, *31*, 1796−1799.
(44) Wöstemeyer, J. *Eur. J. Biochem.* **1985**, *146*, 443−448.
(45) Sutter, R. P.; Whitaker, J. P. *Naturwissenschaften* **1981**, *68*, 147−148.

SCHEME 4. Building Block Strategy for the Synthesis of the Early Trisporoids of the Series A, B, and C[a]



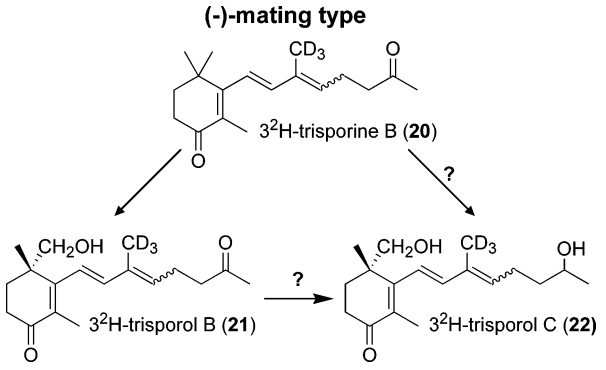
[a] Reagents and conditions: (a) (i) $(BrCH_2PPh_3)^+Br^-$, LiHMDS, THF, −78 °C for 1 h, room temperature for 12 h, (ii) KOH, MeOH, room temperature, 6 h; (b) MnO_2, CH_2Cl_2, room temperature, 12 h; (c) and (f) Bu_2Zn, $Pd[PPh_3]_4$, THF, −78 °C to room temperature, 3−12 h; (d) and (g) 4-iodobutan-2-one-zincate, $Pd[PPh_3]_4$, THF, −78 °C to room temperature, 3−12 h; (e) and (h) (i) ((4-iodobutan-2-yloxy)(*tert*-butyl)dimethylsilane)-zincate, $Pd[PPh_3]_4$, THF, −78 °C to room temperature, 3−12 h, (*ii*) aq HF in MeCN (0.1%), room temperature, 12 h.

SCHEME 5. Synthesis of [$^2H_3$]-β-C_{18}-Ketone (19)[a]



[a] Reagents and conditions: (a) CH_3OD, *N*-ethyl-diisopropylamine, 3 days; (b) **18**, $Pd(Ac)_2$, but-3-en-2-one, PPh_3, Et_3N, THF, 60 °C, 120 h.

SCHEME 6. Feeding Experiments with Growing Cultures of the (−)-Mating Type of *B. trispora*



family of trisporoids. Because the trisporic acids represent the last products of the biosynthetic sequence, the earlier trisporols **21** and **22** have the same absolute configuration.

In summary, we have shown that growing cultures of zygomycete fungi can be successfully used to transform early trisporoids into trisporic acids. By using cultures of either the (+)- or (−)-mating types, the transformation can be terminated at the level of the trisporols or trisporic acids, respectively. Moreover, the conversion of the β-C_{18}-ketone (**1**) or downstream intermediates such as **4**, **5**, and **6** establishes these compounds as intermediates en route to the terminal trisporic acids.

Experimental Section

Formation of Zincates, Representative Procedure.[38] **(4-Iodobutan-2-one)-zincate.** In a dry 25 mL Schlenk tube were placed under argon dry DMAP (4 mL), I_2 (63.5 mg, 0.25 mmol), and Zn dust (477.5 mg, 7.58 mmol). The mixture was stirred at room temperature until the red color of the I_2 disappeared. 4-Iodo-butan-2-one (1 g, 5.05 mmol) was added to the 0 °C cold solution, and stirring was continued for 15 min. For the (4-iodobutan-2-yloxy)-(*tert*-butyl)dimethylsilane, the zincate formation was performed at 40 °C for 3 h. Completion of the metal insertion was indicated by GC-MS analysis of the hydrolyzed and iodinated mixture.

Wittig Olefination, General Procedure. 2-(4-Brom-3-methylbuta-1,3-dienyl)-1,3,3-trimethylcyclohex-2-ene (9). A suspension of bromomethyltriphenylphosphonium bromide (13.2 g, 30.3 mmol) in dry THF (60 mL) was stirred at −78 °C, and a freshly prepared solution of $LiN(SiMe_3)_2$ (28.5 mL, 1.03 M in THF) was slowly added. To achieve complete deprotonation, the suspension was stirred for 30 min at −78 °C and allowed to warm to 0 °C for 30 min. After recooling to −78 °C, β-ionone (**8**) (3.89 g, 18.2 mmol) in dry THF (5 mL) was slowly added via syringe. After 1 h, the mixture was allowed to reach room temperature and stirring was continued overnight. The solution was hydrolyzed with aq NH_4Cl, extracted with 3 × 50 mL of Et_2O, and dried with Na_2SO_4, and the solvent was removed under reduced pressure. The triphenylphosphanoxide was precipitated with pentane and removed by filtration over Alox N using petroleum ether for elution. Because the product readily decomposed upon contact with chromatographic materials, the crude bromide was used for further transformations. Yield: ~4.47 g (16.6 mmol; 82%).

3-((1*E*,3*E*/*Z*)-4-Bromo-3-methylbuta-1,3-dienyl)-2,4,4-trimethylcyclohex-2-one (12). The same procedure as described for **9** was followed with bromomethyltriphenylphosphonium bromide (13.2 g, 30.4 mmol), dry THF (60 mL), $LiN(SiMe_3)_2$ (28.5 mL, 1.03 M

in THF), and 1-acetoxy-β-ionone **10** (5.1 g, 18.2 mmol in 5 mL of dry THF). The triphenylphosphanoxide was precipitated with pentane and removed by filtration on Alox N using petroleum ether/ether (6:1, v/v) for elution. A solution of 3-((1*E*,3*E*/*Z*)-4-bromo-3-methylbuta-1,3-dienyl)-2,4,4-trimethylcyclohex-2-enylacetate (5.0 g, 15.3 mmol) in MeOH (20 mL) was alkalinized with methanolic KOH (10 M). After stirring for 6 h, aq NH_4Cl was added and the water phase was extracted with Et_2O. The extract was dried (Na_2SO_4), and the solvent was evaporated under reduced pressure. Flash filtration on Alox N with petroleum ether/ether (1:1, v:v) afforded **11** as a yellow oil. **11** (4.0 g, 14 mmol) was dissolved in CH_2Cl_2 (100 mL), and MnO_2 (28 g, 0.45 mol) was added. The suspension was stirred for 12 h. The MnO_2 was filtered over celite, and after removal of solvent, the ketone **12** was used without further purification for alkylation. Yield from **10**: ~70%.

(3*E*,5*E*,7*E*/*Z*)-6-Methyl-8-(2,6,6-trimethylcyclohex-1-enyl)-octa-3,5,7-trien-2-one (β-C_{18}-Ketone) (1). In absolute THF (25 mL) were placed palladium(II) acetate (12 mg, 56 μmol), PPh_3 (60 mg, 0.23 mmol), the vinyl halide (**9**) (300 mg), triethylamine (180 μL, 1.34 mmol), and but-3-en-2-one (237 mg, 1.35 mmol). The mixture was heated for 120 h at 60 °C. The reaction was quenched with saturated NH_4Cl solution (30 mL) and extracted with Et_2O (3 × 30 mL) and dried (Na_2SO_4), and the solvent was removed under reduced pressure. Purification was achieved on silica using petroleum ether/ether (9:1,v:v) for elution. Yield: 241 mg (84%) as a mixture of *E*/*Z*-isomers (7:3). IR [cm^{-1}]: 2927, 2863, 1659, 1588, 1449, 1360, 1253, 975. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.03 (s, 6H), 1.45−1.49 (m, 2H), 1.58−1.67 (m, 2H), 1.71 and 1.73 (s, 3H), 2.01−2.05 (t, ^3J = 6.33 Hz, 2H), 2.06 (s, 3H), 2.29 (s, 3H), 6.05−6.18 (m, 2.8H), 6.37−6.43 (2d, ^3J = 15.8 Hz, 1H), 6.6−7.72 (d, ^3J = 15.8 Hz, 0.2H), 7.53 and 7.60 (dd, ^3J = 11.92 Hz and ^3J = 12.28 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.1, 19.1, 21.7, 27.6, 28.9, 33.1, 34.3, 39.6, 126.2, 127.7, 128.6, 129.3, 130.9, 131.2, 132.4, 136.7, 137.5, 138.0, 139.2, 144.5, 145.5, 198.4. EI-MS $[M]^+$ 258 (100), 243 (61), 215 (34), 199 (14), 189 (26), 187 (24), 185 (24), 175 (28), 173 (39), 159 (54), 157 (26), 145 (47), 143 (26), 133 (24), 131 (26), 123 (22), 119 (28), 115 (18), 109 (28), 107 (18), 105 (27), 93 (12), 91 (26), 81 (10), 77 (11). HRMS (EI) *m*/*z* calcd for $C_{18}H_{26}O$ 258.198366, found 258.197555.

[1,1-$^2H_3$]-(3*E*)-4-(2,6,6-Trimethylcyclohex-1-enyl)but-3-en-2-one (18). β-Ionone (**8**) (500 mg, 2.6 mmol) and ethyl-diisopropylamine (100 μL) were dissolved in deuterated methanol (5 mL) and stirred for 3 days. D_2O (10 mL) was added, and the aqueous phase was extracted with Et_2O (5 × 10 mL). The unified organic extracts were dried (Na_2SO_4), and the solvent was removed under reduced pressure. Yield: 496 mg (2.5 mmol; 98%, >97% ^{2}H). IR [cm^{-1}]: 2931, 2866, 2252, 1687, 1662, 1607, 1457, 1297, 1262, 1176, 1027, 980. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.06 (s, 6H), 1.48 (m, 2H), 1.62 (m, 2H), 1.76 (s, 3H), 2.06 (t, ^3J = 6.3 Hz, 2H), 6.11 (d, ^3J = 16.4 Hz, 1H), 7.26 (d, ^3J = 16.4 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.9, 21.7, 26.2−26.8, 28.8, 33.5, 34.0, 39.7, 131.6, 135.9, 136.0, 143.2, 198.8. EI-MS $[M]^+$: 195 (8), 181 (16), 180 (100), 179 (16), 162 (5), 149 (6), 138 (10), 133 (9), 125 (7), 121 (5), 119 (6), 107 (6), 105 (6), 95 (6), 93 (11), 91 (10), 79 (6), 77 (7), 69 (4), 65 (4), 55 (5). HRMS (EI) *m*/*z* calcd for $C_{13}H_{17}D_3O$ 195.170246, found 195.170017.

2,4,4-Trimethyl-3-(oxobut-1-enyl)cyclohex-2-enylacetate (10). To a solution of 1,4-benzoquinone (2 g, 18.5 mmol) and β-ionone (**8**) (3.55 g, 18.5 mmol) in acetic acid (60 mL) were added palladiumbis(trifluoroacetate) (300 mg, 0.93 mmol) and *o*-methoxyacetophenone (560 mg, 3.7 mmol). The mixture was heated to 70 °C for 12 h. NaOH solution (200 mL, 6 N) was added, and the water phase was extracted with 5 × 50 mL of Et_2O. The unified organic extracts were washed with 30 mL of saturated $NaHCO_3$ solution. The crude product was purified on silica (petroleum ether/ether (2:1; v:v)). Yield: 3.93 g (15.7 mmol; 85%). IR [cm^{-1}]: 2966, 2934, 2862, 1722, 1677, 1451, 1366, 1245, 1019, 962, 860. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.08 (s, 3H), 1.04 (s, 3H), 1.42−1.48 (m, 1H), 1.60−1.67 (m, 1H), 1.69 (s, 3H), 1.70−1.77 (m, 1H), 1.87−1.96 (m, 1H), 2.08 (s, 3H), 2.30 (s, 3H), 5.21−5.24 (t, ^3J = 4.77 Hz, 1H), 6.10−6.14 (d, ^3J = 16.44 Hz, 1H), 7.15−7.19 (d, ^3J = 16.44 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 18.2, 21.2, 25.1, 27.3, 27.4, 28.7, 34.5, 34.7, 71.9, 129.9, 133.3, 141.7, 142.0, 170.8, 198.0. EI-MS $[M]^+$: 235 (29), 208 (19), 190 (100), 175 (80), 161 (12), 151 (16), 147 (46), 133 (42), 123 (15), 119 (36), 109 (42), 105 (24), 91 (25), 77 (12), 65 (7), 55 (10). HRMS (EI) *m*/*z* calcd for $C_{15}H_{22}O_3$ 235.133420, found 235.133965.

Synthesis of Trisporins, General Procedure. **3-((1*E*,3*E*/*Z*)-(*R*,*S*)-7-(*tert*-Butyldimethyl-silanoyl)-3-methylocta-1,3-dienyl)-2,4,4-trimethylcyclohex-2-enone.** To a −78 °C cold solution of the vinyl halide **12** (200 mg, 0.71 mmol) and $Pd[PPh_3]_4$ (46.2 mg, 40 μmol) in absolute THF (20 mL) was added the zincate solution (2.2 mL, 2.2 mmol, 1 M). The mixture was allowed to come to room temperature within 3 h, and stirring was continued for 12 h. The reaction mixture was quenched with saturated NH_4Cl solution (25 mL) and extracted with Et_2O (3 × 20 mL). After removal of the solvent, the oil was purified on silica with petroleum ether/ether (6:1,v:v). Yield: 227 mg (0.58 mmol, 82%). IR [cm^{-1}]: 2958, 2929, 2857, 1663, 1465, 1254, 1135, 1091, 1032, 835, 775. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.01 and 0.02 (2s, 6H), 0.85−0.87 (m, 9H), 1.10−1.11 (m, 2H), 1.16−1.17 (m, 6H), 1.43−1.49 (m, 2H), 1.80−1.87 (m, 9H), 2.10−2.13 (m, 1H), 2.25−2.27 (m, 1H), 2.47−2.51 (m, 2H), 3.75−3.79 (m, 1H), 5.49−5.52 (t, ^3J = 7.44 Hz, 0.8H), 5.55−5.59 (t, ^3J = 7.20 Hz, 0.2H), 6.03−6.06 (d, ^3J = 16.10 Hz, 0.2H), 6.13−6.16 (d, ^3J = 16.22 Hz, 0.8H), 6.18−6.21 (d, ^3J = 16.10 Hz, 0.2H), 6.60−6.63 (d, ^3J = 16.22 Hz, 0.8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ −4.8, −4.4, 13.8, 18.1, 20.1, 23.9, 25.81, 25.84, 27.50, 27.53, 34.2, 35.6, 37.2, 39.9, 68.1, 121.9, 124.7, 129.6, 131.5, 133.3, 135.5, 141.3, 161.9, 199.5. EI-MS $[M]^+$: 390 (48), 375 (7), 333 (22), 258 (30), 243 (100), 231 (19), 215 (14), 201 (15), 187 (26), 185 (21), 173 (21), 159 (29), 145 (15), 133 (9), 119 (14), 103 (12), 91 (7), 75 (47). HRMS (EI) *m*/*z* calcd for $C_{24}H_{42}O_2Si$ 390.295409, found 390.295766.

2,4,4-Trimethyl-3-((1*E*/*Z*)-3-methylocta-1,3-dienyl)cyclohex-2-enone (Trisporin A) (13). Following the general procedure for trisporins, **12** (200 mg, 0.71 mmol) and $Pd[PPh_3]_4$ (46.2 mg, 40 μmol) were dissolved in absolute THF (20 mL) and the Bu_2Zn solution (2.2 mL, 2.2 mmol, 1 M) was added. After removal of the solvent, the oil was purified on silica with petroleum ether/ether (9:1, v/v). Yield: 159 mg (0.61 mmol, 86%) as a mixture of *E*/*Z* isomers (1:1). IR [cm^{-1}]: 2958, 2860, 1666, 1586, 1461, 1353, 1333, 1308, 1198, 1093, 1031, 968. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89−0.93 (m, 3H), 1.17 and 1.18 (2s, 6H), 1.29−1.43 (m, 4H), 1.81−1.86 (m, 8H), 2.13−2.21 (m, 2H), 2.47−2.52 (m, 2H), 5.50 (t, ^3J = 7.19 Hz, 0.5H), 5.56 (t, ^3J = 7.37 Hz, 0.5H), 6.04 (d, ^3J = 16.19 Hz, 0.5H), 6.13−6.22 (d, ^3J = 16.07 Hz, 1H), 6.62 (d, ^3J = 16.19 Hz, 0.5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.0, 13.7, 13.9, 20.1, 22.2, 22.4, 27.2, 27.5, 28.2, 31.6, 32.0, 34.2, 35.6, 37.3, 121.9, 124.5, 129.6, 131.5, 133.5, 133.7, 135.9, 141.4, 161.7, 199.4. EI-MS $[M]^+$: 260 (70), 245 (38), 227 (13), 217 (23), 204 (36), 203 (73), 190 (18), 189 (100), 175 (27), 171 (12), 163 (24), 161 (45), 148 (15), 147 (45), 145 (13), 134 (14), 133 (88), 131 (12), 119 (68), 117 (11), 107 (13), 105 (27), 93 (11), 91 (29), 81 (13), 79 (11), 77 (13), 69 (14), 55 (13). HRMS (EI) *m*/*z* calcd for $C_{18}H_{28}O$ 260.214016, found 260.213158.

2,4,4-Trimethyl-3-((1*E*/*Z*)-3-methyl-7-oxo-octa-1,3-dienyl)cyclohex-2-enone (Trisporin B) (4). The reaction was performed according to the general procedure for trisporins with **12** (100 mg, 0.35 mmol) and $Pd[PPh_3]_4$ (23.1 mg, 20 μmol) in absolute THF (20 mL), and 4-iodobutan-2-onezincate (2.2 mL, 2.2 mmol, 1 M) was added. The crude product was purified over silica using petroleum ether/ether (2:1,v/v) for elution. Yield: 78 mg (0.28 mmol, 81%) as a mixture of *E*/*Z* isomers (2:1). IR [cm^{-1}]: 2925, 2867, 1716, 1662, 1353, 1334, 1199, 1160, 1095, 1032, 969. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15 and 1.16 (s, 6H), 1.80−1.86 (m, 8H), 2.12 and 2.14 (2s, 3H), 2.39−2.56 (m, 6H), 5.47−5.50 (t, ^3J = 6.52 Hz, 0.65H), 5.42−5.45 (t, ^3J = 7.56 Hz, 0.35H), 6.06−

6.09 (d, ^3J = 16.03 Hz, 0.35H), 6.15−6.20 (2d, ^3J = 16.25 Hz, ^3J = 16.03 Hz, 1H), 6.58−6.62 (d, ^3J = 16.25 Hz, 0.65H). ^{13}C NMR (100 MHz, CDCl3) δ 12.1, 13.7, 20.1, 22.7, 27.5, 29.9, 34.3, 35.7, 37.3, 43.0, 43.6, 122.8, 125.5, 129.6, 130.9, 132.7, 133.1, 134.4, 140.7, 161.5, 199.4, 207.9. EI-MS [M]$^+$: 274 (100), 259 (27), 241 (16), 231 (62), 217 (21), 203 (51), 201 (55), 200 (29), 199 (44), 189 (29), 185 (23), 175 (31), 173 (32), 161 (33), 157 (30), 147 (40), 145 (29), 133 (53), 119 (52), 105 (37), 91 (48), 79 (25), 77 (29), 69 (26), 55 (36). HRMS (EI) *m*/*z* calcd for $C_{18}H_{26}O_2$ 274.193280, found 274.193399.

3-((1*E*/*Z*)-7-Hydroxy-3-methylocta-1,3-dienyl)-2,4,4-trimethylcyclohex-2-enone (Trisporin C) (14). To a solution of 3-((1*E*,3*E*/*Z*)-(*R*,*S*)-7-(*tert*-butyldimethyl-silanoyl)-3-methylocta-1,3-dienyl)-2,4,4-triethylcyclohex-2-enone (20 mg, 0.051 mmol) in MeCN (10 mL) was added HF (100 μL, aq 40%). The solution was stirred for 6 h and quenched with water (20 mL). The aqueous phase was extracted with Et_2O (3 × 20 mL), dried (Na_2SO_4), and purified on Florisil using petroleum ether/ether (1:1, v/v) for elution. Yield: 13.3 mg (0.047 mmol, 95%) as a mixture of *E*/*Z* isomers (3:2). IR [cm^{-1}]: 3428, 2963, 2925, 2865, 1657, 1452, 1355, 1334, 1310, 1093, 969. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.15 and 1.16 (2s, 6H), 1.17 and 1.21 (2d, 3H), 1.45−1.60 (m, 2H), 1.81−1.88 (m, 8H), 2.16 (s, 1H), 2.21−2.34 (m, 2H), 2.47−2.51 (m, 2H), 2.47−2.51 (m, 2H), 3.77−3.85 (m, 1H), 5.49−5.52 (t, ^3J = 7.32 Hz, 0.6H), 5.55−5.58 (t, ^3J = 7.33 Hz, 0.4H), 6.04−6.07 (d, ^3J = 16.13 Hz, 0.4H), 6.15 and 6.21 (2d, ^3J = 16.26 Hz, ^3J = 16.13 Hz, 0.4H and 0.6H), 6.61−6.64 (d, ^3J = 16.26 Hz, 0.6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.1, 13.8, 20.1, 23.5, 23.9, 24.8, 27.5, 34.2, 35.6, 37.1, 38.6, 39.1, 67.5, 122.3, 124.9, 131.9, 132.7, 133.1, 133.7, 134.8, 141.1, 161.9, 199.6. EI-MS [M]$^+$: 276 (100), 243 (37), 232 (32), 220 (39), 205 (33), 203 (29), 201 (26), 187 (29), 173 (32), 163 (39), 162 (25), 161 (32), 159 (30), 147 (38), 145 (31), 133 (50), 121 (25), 119 (48), 107 (21), 105 (35), 93 (28), 91 (45), 81 (25), 79 (27), 77 (25), 69 (31), 55 (38). HRMS (EI) *m*/*z* calcd for $C_{18}H_{28}O_2$ 276.208930, found 276.209442.

Representative Procedure for 4-Dihydrotrisporins. 2,4,4-Trimethyl-3-((1*E*,3*E*/*Z*)-3-methylocta-1,3-dienyl)cyclohex-2-enol (4-Dihydrotrisporin A) (15). To a −78 °C cold solution of **11** (200 mg, 0.7 mmol) and Pd[PPh_3]$_4$ (46.2 mg, 40 μmol) in absolute THF (20 mL) was added zincate solution (2.2 mL, 2.2 mmol, 1 M). The mixture was allowed to come to room temperature within 60 min, and stirring was continued for 48 h. The reaction mixture was quenched with saturated NH_4Cl solution (25 mL) and extracted with Et_2O. After removal of the solvent, the oil was purified on Florisil with petroleum ether/ether (2:1, v/v) for elution. Yield: 144 mg (0.55 mmol, 79%) as mixture of *E*/*Z*-isomers (19:1) after chromatography. The compound is very sensitive to light and oxygen and should be handled under argon. IR [cm^{-1}]: 3331, 2930, 2860, 1454, 1022, 965, 740. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.87−0.92 (m, 3H), 1.00 and 1.03 (2s, 6H), 1.30−1.36 (m, 4H), 1.39−1.44 (m, 1H), 1.57 (s, 1H), 1.61−1.94 (m, 9H), 2.12−2.16 (m, 2H), 3.99−4.01 (t, ^3J = 4.23 Hz, 1H), 5.35−5.38 (t, ^3J=7.67 Hz, 1H), 6.01−6.04 (d, ^3J = 16.02 Hz, 1H), 6.42−6.45 (d, ^3J = 16.02 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ13.9, 18.6, 20.3, 22.3, 27.1, 27.4, 28.5, 29.0, 32.2, 34.5, 34.7, 70.3, 125.8, 129.2, 130.7, 131.4, 131.9, 142.3. EI-MS [M − 18]$^+$: 244 (100), 229 (50), 201 (22), 187 (45), 173 (56), 159 (68), 145 (71), 131 (29), 119 (44), 105 (17), 91 (14). HRMS (EI) *m*/*z* calcd for $C_{18}H_{28}$ 244.219101, found 244.218601.

(7*E*/*Z*)-8-(3-Hydroxy-2,6,6-trimethylcyclohex-1-enyl)-6-methylocta-5,7-dien-2-one (4-Dihydrotrisporin B) (2). Following the procedure for **15**, **2** was prepared from **11** (200 mg, 0.7 mmol), Pd[PPh_3]$_4$ (46.2 mg, 40 μmol) in absolute THF (20 mL), and 4-iodobutan-2-one-zincate (2.2 mL, 2.2 mmol, 1 M). The crude product was purified over silica using petroleum ether/ether (6:1, v/v) for elution. Yield: 133 mg (0.48 mmol, 69%) as a mixture of *E*/*Z* isomers (1:1). The compound is very sensitive to light and oxygen and should be handled under argon. IR [cm^{-1}]: 3411, 2958, 2926, 2853, 1713, 1449, 1360, 1266, 1159, 1023, 964. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 and 1.01 (2s, 6H), 1.38−1.44 (m, 1H), 1.59−1.73 (m, 3H), 1.73−1.94 (m, 7H), 2.13 and 2.14 (2s, 3H), 2.38−2.43 (m, 2H), 2.47−2.54 (m, 2H), 3.96−4.00 (m, 1H), 5.28 and 5.37 (2t, ^3J = 7.03 Hz, ^3J = 7.09 Hz, 1H), 5.92−6.07 (3d, ^3J = 16.01 Hz, ^3J = 16.01 Hz, ^3J = 16.26 Hz, 1.5H), 6.38−6.42 (d, ^3J = 16.26 Hz, 0.5H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.2, 18.6, 20.3, 21.7, 22.5, 27.3, 28.4, 28.9, 29.7, 29.9, 34.4, 43.3, 43.8, 70.1, 123.9, 126.8, 127.9, 129.3, 129.9, 130.7, 133.1, 138.4, 142.2, 208.5. EI-MS [M]$^+$: 276 (15), 258 (64), 243 (7), 225 (6), 218 (10), 215 (15), 200 (16), 187 (21), 185 (100), 173 (26), 171 (13), 170 (14), 163 (11), 159 (53), 157 (57), 147 (23), 145 (43), 144 (24), 143 (32), 142 (14), 141 (12), 135 (17), 133 (24), 131 (26), 129 (23), 128 (20), 122 (15), 121 (31), 119 (50), 117 (16), 115 (17), 109 (16), 107 (28), 105 (40), 95 (26), 93 (22), 91 (36), 81 (17), 79 (21), 77 (20), 55 (13). HRMS (EI) *m*/*z* calcd for $C_{18}H_{28}O_2$ 276.208930, found 276.207939.

3-((1*E*,3*E*)-7-Hydroxy-3-methylocta-1,3-dienyl)-2,4,4-trimethylcyclohex-2-enol (4-Dihydrotrisporin C) (16). Prepared according to the synthetic procedure for **15**, from **11** (200 mg, 0.7 mmol) with Pd[PPh_3]$_4$ (46.2 mg, 40 μmol) in absolute THF (20 mL) and (4-iodobutan-2-yloxy)(*tert*-butyl)dimethylsilane-zincate (2.2 mL, 2.2 mmol, 1 M). After the workup procedure, the crude product was diluted with MeCN (10 mL), containing HF (100 μL, aq 40%). The solution was stirred for 6 h and quenched with water (20 mL). The aqueous phase was extracted with Et_2O (3 × 20 mL), dried (Na_2SO_4), and purified on Florisil using petroleum ether/ether (1:1, v/v) for elution. Yield: 140 mg (0.5 mmol, 72%). The compound is very sensitive to light and oxygen and should be handled under argon. Due to the high sensitivity, **16** was derivatized with MSTFA to the corresponding bistrimethylsilylether. IR [cm^{-1}]: 2957, 2917, 2849, 1266, 838, 742. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.11 (s, 9H), 0.15 (s, 9H), 0.97 (s, 3H), 1.02 (s, 3H), 1.15−1.16 (d, ^3J = 5.95 Hz, 3H), 1.43 (s, 3H), 1.53−1.57 (m, 2H), 1.61−1.69 (m, 3H), 1.71 and 1.74 (2s, 3H), 1.96−2.06 (m, 1H), 2.09−2.23 (m, 2H), 3.78−3.81 (m, 1H), 4.01−4.05 (m, 1H), 5.33 (t, ^3J = 6.52 Hz, 0.3H), 5.40 (t, ^3J = 7.56 Hz, 0.7H), 5.96 (d, ^3J = 16.03 Hz, 0.7H), 6.02 and 6.04 (2d, ^3J = 16.03 Hz, ^3J = 16.26 Hz, 1H), 6.43 (d, ^3J = 16.26 Hz, 0.3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 12.3, 14.1, 23.8, 23.9, 24.8, 29.7, 30.3, 31.9, 35.2, 39.9, 39.9, 68.1, 71.1, 123.7, 125.5, 129.8, 131.0, 131.6, 133.9, 135.0, 138.6, 141.2. EI-MS [M]$^+$: 422 (82), 407 (8), 366 (2), 351 (2), 332 (6), 317 (26), 311 (11), 289 (5), 277 (55), 261 (40), 247 (16), 237 (60), 224 (85), 211 (100), 197 (30), 187 (30), 171 (49), 157 (29), 143 (40), 129 (14), 117 (24), 107 (10), 95 (9), 73 (84), 55 (6). HRMS (EI) *m*/*z* calcd for $C_{24}H_{46}O_2Si_2$ 422.303638, found 422.303223.

Biotransformation of 20 to Trisporol B (21) and Trisporol C (22). Cells of the (−)-mating type of *Blakeslea trispora* were precultivated on a SUP medium for 3 days. The grown mycelia were sterile filtered off from the medium and placed on a maltose medium. [$^2H_3$]-Trisporin B (**20**) (10 mg) was added and incubated for 48 h. The mycelia were filtered off, and the broth was adjusted to pH 8 with NaOH and extracted with chloroform/*i*-propanol (20:1, v/v). After drying and removal of the solvent, the products were purified and separated by chromatography on silica with petroleum ether/ether (1:1, v/v). Yield: 1.0 mg (ca. 10%, 1:1 mixture of [$^2H_3$]-**21** and [$^2H_3$]-**22**). The substance is very sensitive to light and oxygen and should be handled under argon. The spectra were identical with the literature data[18,21] except for a singlet at 1.81 ppm owing to substitution by deuterium atoms.

Isolation of Trisporic Acids. Cells of the (+)- and (−)-mating type of *Blakeslea trispora* were separately precultivated on a SUP medium for 3 days. The grown mycelium was sterile filtered off from the media and both were placed together on maltose media and shaken for the following 6 days. The mycelium was removed, and the medium was adjusted to pH 8 with NaOH and extracted with chloroform/*i*-propanol (20:1, v/v) for removal of the precursors. Next, the medium was adjusted to pH 2 and extracted with chloroform/isopropanol (20:1, v/v). After drying and removal of

the solvent, the products were purified and separated on silica with petroleum ether/ether (1:1, v/v).

2-((1*E*,3*E*)-7-Hydroxy-3-methylocta-1,3-dienyl)-1,3-dimethyl-4-oxocyclohex-2-enecarboxylic Acid (Trisporic Acid C, As Methyl Ester). IR [cm^{-1}]: 3445, 2922, 2852, 1730, 1660, 1454, 1351, 1308, 1250, 1188, 1134, 1097, 1024, 969. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.21−1.22 (d, ^3J = 6.3 Hz, 3H), 1.42−1.55 (m, 2H), 1.56 (s, 3H), 1.70 (s, 1H), 1.86 (s, 3H), 1.92−1.98 (m, 4H), 2.15−2.25 (m, 1H), 2.28−2.35 (m, 1H), 2.37−2.45 (m, 1H), 2.50−2.56 (m, 2H), 3.68 (s, 3H), 3.75−3.86 (m, 1H), 5.60−5.64 (t, ^3J = 6.3 Hz, 1H), 6.42−6.38 (d, ^3J = 16.34 Hz, 1H), 6.82−6.86 (d, ^3J = 16.34 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) 12.1, 14.1, 19.8, 22.6, 23.7, 33.2, 34.5, 39.4, 46.7, 52.5, 67.5, 124.7, 132.2, 132.5, 132.6, 134.7, 152.4, 177.0, 197.7. EI-MS $[M]^+$: 320 (100), 305 (10), 287 (12), 276 (12), 261 (11), 247 (8), 243 (13), 233 (5), 227 (14), 220 (46), 215 (14), 202 (15), 201 (18), 199 (14), 187 (34), 175 (22), 173 (35), 162 (25), 161 (25), 159 (45), 147 (33), 145 (31), 135 (25), 133 (20), 131 (22), 119 (25), 107 (21), 105 (28), 93 (23), 91 (37), 85 (13), 81 (26), 79 (23), 77 (21), 55 (36). HRMS (EI) *m*/*z* calcd for $C_{19}H_{28}O_4$ 320.198760, found 320.199165. CD spectra were identical with the reported data.[14]

Methyl-4-hydroxy-2-((1*E*,3*E*)-7-hydroxy-3-methylocta-1,3-dienyl)-1,3-dimethylcyclohex-2-enecarboxylate (4-Dihydromethyltrisporate C). Isolated trisporic acid C (50 mg, 0.16 mmol) was derivatized with diazomethane and diluted in dry THF (5 mL), and $NaBH_4$ (1.5 mg, 0.04 mmol) was added and stirred for 30 min. HCl (0.1 M) was slowly added until the precipitate was dissolved. The aqueous phase was extracted with ether and dried, and the solvent was evaporated under reduced pressure. The product was purified by chromatography on Florisil with petroleum ether/ether (1:1, v/v) for elution. Yield: 46.8 mg (0.14 mmol, 91%). IR [cm^{-1}]: 3382, 2932, 2869, 1714, 1455, 1377, 1260, 1197, 1122, 1018, 911. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18−1.19 (d, ^3J = 5.95 Hz, 3H), 1.40 (s, 3H), 1.46−1.51 (m, 2H), 1.65−1.79 (m, 3H), 1.82 (s, 3H), 1.93 (s, 3H), 1.95−2.03 (m, 1H), 2.13−2.28 (m, 2H), 3.65 (s, 3H), 3.76−3.80 (m, 1H), 4.14−4.16 (t, ^3J = 5.49 Hz, 1H), 5.39−5.42 (t, ^3J = 7.56 Hz, 1H), 6.17−6.20 (d, ^3J = 16.26 Hz, 1H), 6.45−6.49 (d, ^3J = 16.26 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.5, 20.1, 23.6, 23.8, 24.3, 28.3, 32.5, 39.4, 46.1, 52.1, 67.5, 70.2, 125.3, 128.5, 130.6, 132.7, 134.4, 135.1, 177.9. EI-MS $[M - 18]^+$: 322 (1), 304 (85), 289 (18), 245 (7), 229 (9), 204 (100), 189 (18), 173 (11), 171 (12), 161 (27), 145 (26), 133 (24), 125 (20), 119 (57), 105 (34), 91 (32), 85 (36), 55 (67). HRMS (EI) *m*/*z* calcd for $C_{19}H_{28}O_3$ 322.214410, found 322.214348.

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Supporting Information Available: Infrared spectra, mass spectra, and ^{1}H NMR and ^{13}C NMR spectra of compounds **1**, **2**, **4**, **10**, **13**−**15**, **17**−**19**, and 3-((1*E*,3*E*/*Z*)-(*R*,*S*)-7-(*tert*-butyldimethylsilanoyl)-3-methylocta-1,3-dienyl)-2,4,4-trimethylcyclohex-2-enone, 2,4,4-trimethyl-3-((1*E*,3*E*)-3-[$^2H_3$]-ethylocta-1,3-dienyl)-cyclohex-2-enone, 2,4,4-trimethyl-3-((1*E*,3*E*/*Z*)-3-[$^2H_3$]-methylocta-1,3-dienyl)cyclohex-2-enol, methyl-2-((1*E*,3*E*)-7-hydroxy-3-methylocta-1,3-dienyl)-1,3-dimethyl-4-oxocyclohex-2-enecarboxylate, and methyl-4-hydroxy-2-((1*E*,3*E*)-7-hydroxy-3-methylocta-1,3-dienyl)-1,3-dimethylcyclohex-2-enecarboxylate. This material is available free of charge via the Internet at http://pubs.acs.org.

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