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Comprehensive Study of Okaspirodiol: Characterization, Total Synthesis, and Biosynthesis of a New Metabolite from *Streptomyces*

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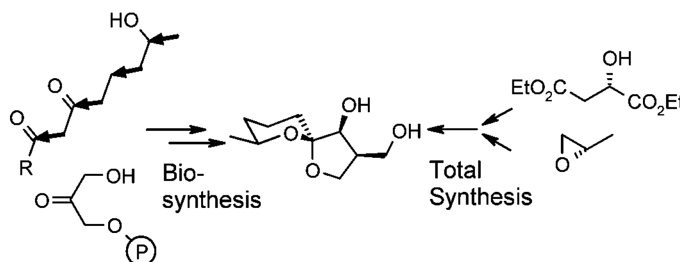

The new spiro[4.5]acetal okaspirodiol (**4**) was isolated from *Streptomyces* sp. Gö TS 19 as a secondary metabolite in yields up to 380 mg/L. The structure of this cryptic ketotetrol was elucidated by different methods including X-ray analysis, and its equilibration under mildly acidic conditions furnishing three additional isomers was thoroughly studied. Although metabolite **4** is not the thermodynamically favored isomer, a high-yielding total synthesis was accomplished comprising a stereoselective spiro-acetalization under equilibrium conditions. This approach benefits from the important influence of an intramolecular hydrogen bond on the stabilization of the spiro[4.5]acetal moiety. The biosynthesis of **4** was investigated by feeding experiments with ^{13}C-labeled precursors proving its origin from a new type of the rare mixed acetate−glycerol biosynthetic pathway. All results are discussed on the basis of the structural diversity of spiroacetals in nature and their chemical properties.

Introduction

Spiroacetals occur ubiquitously in nature as subunits of miscellaneous natural products from different sources such as microbes, fungi, plants, insects, and marine organisms, and some of these compounds and/or their derivatives exhibit strong biological activities.[1] This comprises the reveromycins,[2] inhibitors of the mitogenic activity of epidermal growth factor, and the cephalostatins,[3] which are highly potent cell growth inhibitors. Moreover, the telomerase-inhibiting activity of griseorhodin and rubromycin is attributed to the presence of a spiroacetal moiety in these natural products.[4] Whereas the latter are highly functionalized, polycyclic compounds, various spiroacetals from insects are volatile, simple molecules and act as pheromones.[5] In contrast, the biological function of several other naturally occurring spiroacetals has not been disclosed so far. Therefore,

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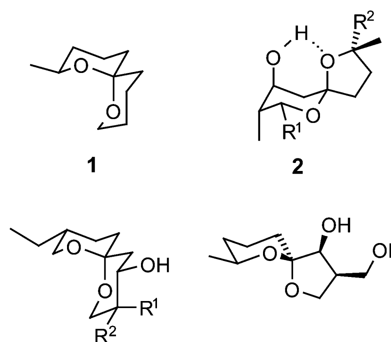
the interest in the chemistry and pharmacology of this important class of compounds is steadily growing.

Regarding their synthesis, the major challenge frequently is the stereoselective assembly of the spirocyclic structure with a linking carbon atom which usually is a stereogenic center but can easily isomerize under mildly acidic conditions. Luckily though, in most cases, the natural products possess the thermodynamically favored configuration and conformation of the spiro center,[6] thus paving the way for a ring closure under equilibrium conditions. The structural preferences are influenced by steric and stereoelectronic (anomeric) effects[7] which have been most thoroughly studied in the case of simple spiro[5.5] compounds[8] such as (*E*)-2-methyl-1,7-dioxaspiro[5.5]undecane (**1**). Yet, in the case of compounds bearing hydroxyl groups at appropriate positions, intramolecular hydrogen bonds and chelating effects can lead to an unexpected conformation as in the monensin−water complex **2** with its axial arrangement of the hydroxyl group.[9] Therefore, the determination of the stabilizing factors turns out to be essential for synthetic work with spiroacetal natural products as has been shown for the reveromycins[2b−e] and the talaromycins (**3a**,**b**), a group of spiro[5.5]acetals from the fungus *Talaromyces stipitatus* which recently attracted extensive interest in synthesis.[10] Herein, we describe the isolation, chemical characterization, and enantioselective total synthesis of the new natural product okaspirodiol (**4**), a spiro[4.5]acetal bearing two hydroxyl groups which are responsible for intramolecular hydrogen bonds and isomerization processes.

Focusing on the question of how nature synthesizes okaspirodiol (**4**), some alternative biosynthetic pathways are likely to be responsible for the formation of this new natural product. The biosynthetic origin of such an unusually substituted spiroacetal has not yet been investigated. At present, many microbial spiroacetal metabolites are derived from acetate;[5,11] however, structure **4** also implies carbohydrate precursors such as glycerol or even a heptose. We addressed these alternatives efficiently by feeding experiments with ^{13}C-labeled glycerol and acetate. The putative biosynthetic mechanism is discussed in the light of the enzymatic basis of microbial secondary metabolite formation.



3a: R^1 = CH_2OH, R^2 = H **4:** R = H
3b: R^1 = H, R^2 = CH_2OH **5:** R = *p*-Br-Bz

Results and Discussion

Isolation and Chemical Characterization. Okaspirodiol (**4**) was detected by detailed analysis of the metabolite pattern of the *Streptomyces* sp. strain Gö TS 19 by methods of chemical screening.[12] Fermentation of this strain, which was isolated from a Bavarian soil sample and found to be most closely related to *S. kanamyceticus* (DSM 40500^{T}),[13] was carried out in a malt/glucose/yeast medium (medium A). Okaspirodiol (**4**) was isolated from the culture broth and purified by column chromatography on Sephadex LH-20 and silica gel yielding 60 mg/L of a colorless solid. The production of **4** was significantly enhanced by extraordinary high aeration (6.0 vvm) to afford an improved yield of up to 275 mg/L. Feeding of glycerol to the growing cultures raised the yield of **4** even further to 380 mg/L, a particularly high yield efficiently achieved with the OSMAC method (one-strain-many-compounds).[14] Staining with orcin reagent led to an intense brown color, but okaspirodiol (**4**) was not detectable under UV light on silica gel TLC plates. The ESI mass spectrum of **4** showed an ion peak at $m/z = 225$ ($[M + Na]^+$), and the molecular formula was determined by ESI−HRMS to be $C_{10}H_{18}O_4$. The constitution of **4** was derived from ^{1}H NMR and ^{13}C NMR experiments disclosing the scaffold of a spiro[4.5]acetal with a signal of a quaternary carbon at 102.9 ppm. From 2D-COSY and HMBC experiments, the structure of **4** was finally deduced to be a 3-hydroxymethyl-7-methyl-1,6-dioxaspiro[4.5]decan-4-ol. Thus, okaspirodiol is a new natural product with an unprecedented combination of rings and substituents not found in nature or in synthetic products before. It is distinguished from the spiro[4.5]acetals from insects by its hydroxyl groups[5] and only shows some similarities with the talaromycins (**3a**,**b**) mentioned above in that they bear comparable substituents but comprise just pyran rings.[10]

The absolute configuration of **4** was assigned on the basis of the analysis of selected derivatives. Esterification with (*R*)- and (*S*)-2-phenylbutyric acid and ^{1}H NMR analysis of the thus obtained diastereomeric products according to the method of Helmchen[15] indicated the (*S*)-configuration of C-4. Various

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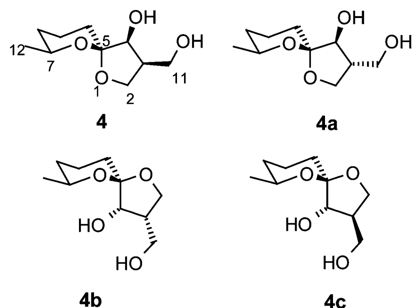


	4	4a	4b	4c
δ[a] (7-H)	3.94	3.91	3.71	3.70
δ[a] (4-H)	3.84	3.42	4.32	4.14
δ (C-5)	102.9	102.9	109.8	109.1
A[b]	29	47	5	19
B[b]	7.7	85	2.6	4.7
C[b]	23	77	-	-

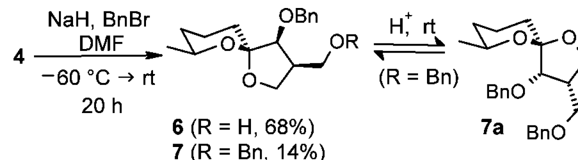
[a]NMR spectra recorded in CD_3OD. [b]Equilibration cond. **A**: $CDCl_3$, rt (ratio after chromatography). – **B**: HCl, CD_3OD, rt. – **C**: CSA, CD_2Cl_2, rt.

FIGURE 1. Isomerization studies of okaspirodiol (**4**).

attempts to obtain suitable crystals of **4** failed, but finally, X-ray analysis of the *p*-bromobenzoate **5** of okaspirodiol was accomplished (see Supporting Information).[16] These results altogether showed the microbial metabolite **4** to possess the all-(*S*)-configuration.

As expected for a spiroacetal, okaspirodiol (**4**) readily isomerizes under mildly acidic conditions. When stored in $CDCl_3$ at room temperature for 48 h (condition **A**), three additional isomers were formed which could readily be separated by column chromatography on silica gel to yield a 29:47:5:19 ratio of **4**−**4c** (Figure 1). To achieve a complete equilibration under thermodynamic control, okaspirodiol was treated with both methanolic HCl (condition **B**) and 10-camphorsulfonic acid (CSA)/CD_2Cl_2 (condition **C**) at room temperature. Under the former condition, the same four isomers were obtained, yet **4a** was by far the main component. In contrast, only **4** and **4a** were detected after isomerization under the aprotic condition **C** as determined by ^{13}C NMR analysis of the reaction mixtures. The isomers were characterized by ^1H NMR, ^{13}C NMR, and NOESY experiments. Most enlightening are the chemical shifts of 7-H which show a downfield shift when 7-H is placed in a 1,3-diaxial orientation with O-1 as in **4** and **4a**.[17] Additionally, the *cis* relationship of O-6 with the oxygen substituent at C-4 exerts a shielding effect on C-5 and 4-H.[18] Finally, the respective relationship of the C-4 hydroxyl and the C-3 hydroxymethyl group was assigned on the basis of the experimental work and NOE studies (see Supporting Information). Thus, an epimerization of the spiro carbon C-5 and an unusual transacetalization leading to the epimerization of C-3 create the four isomers **4**−**4c**. Quite remarkably, the frequently observed isomerization of substituents at the α-carbons of spiroacetals via the open-chain ketodiol[19] was not found for the hydroxyl group at C-4.

SCHEME 1. Benzylation of Okaspirodiol (4) and Isomerization of the Dibenzylated 7



Conditions	7	7a
HCl, CD_3OD	60	40
CSA, CD_2Cl_2	71	29

All isomers possess the chairlike conformation of the six-membered ring together with the sterically favored equatorially oriented methyl group. Okaspirodiol (**4**) and its isomer **4a** with their (*S*)-configuration at C-5 benefit from two anomeric effects because of the axial−quasi−axial arrangement of the spiro C−O bonds and therefore always outbalance the (*R*)-configured minor isomers **4b** and **4c**. Interestingly, the natural product **4** is not the thermodynamically most stable isomer which is **4a** presumably because of the steric preference of a *trans* relationship of the substituents at C-3 and C-4. For evaluating the influence of hydrogen bonding on the structural stabilization, okaspirodiol (**4**) was benzylated to give a mixture of mono- and dibenzylated products **6** and **7** (Scheme 1).[20] Exposure of **7** to the equilibrating conditions **B** and **C**, respectively, yielded only one additional isomer **7a** as expected because of suppression of the epimerization of C-3. Yet, the ratio of isomer(s) with the (*S*)-configuration at C-5 to the one(s) with the (*R*)-configuration significantly dropped from 93:7 (condition **B**) or >95:5 (condition **C**) in the case of okaspirodiol (**4**) to just 60:40 (condition **B**) or 71:29 (condition **C**) in the case of the dibenzylated **7**. Therefore, the (*S*)-configuration in **4** and **4a** is not only stereoelectronically favored but also must be extensively stabilized by an intramolecular hydrogen bond between the C-4 hydroxyl group and O-6, especially in an aprotic medium. DFT calculations on the B3LYP/6-31G* level of theory confirmed this result revealing distances of 1.88 Å (isomer **4**) and 2.10 Å (isomer **4a**) between O-6 and the relevant hydrogen atom, although the calculated absolute energies of isomers **4**−**4c** did not reflect the observed equilibrium ratios (see Supporting Information). It has been known for a long time that Lewis acids can chelate and thus favor the *cis* orientation of the hydroxyl group and O-6 in 4-hydroxy-spiro[4,5]acetals,[18a] yet this seems to be the first evidence for a stabilization just by hydrogen bonding.

Enantioselective Total Synthesis. From the isomerization studies, it was concluded that a selective total synthesis of **4** would be possible involving the spirocyclization of an acyclic or monocyclic precursor under equilibrium conditions. Even though the natural product is not the thermodynamically favored isomer, it should indeed selectively be formed if the hydroxymethyl group ending up at C-3 is protected and the C-4 hydroxyl group can exert its directing influence. A variety of methods

(16) CCDC 288701 contains the supplementary crystallographic data for **5**. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, U.K.; fax: (+44) 1223−336−033 or e-mail: deposit@ccdc.cam.ac.uk).

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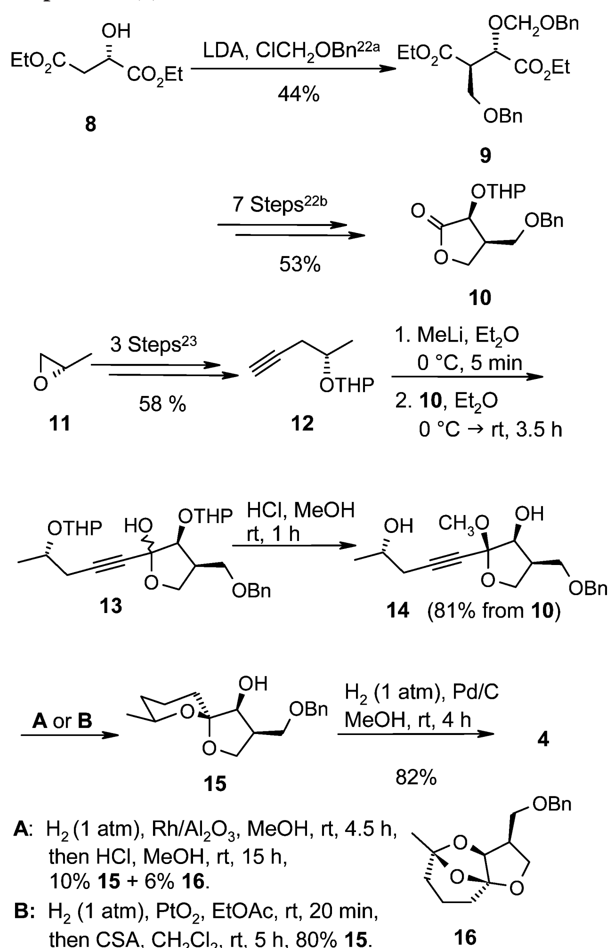
SCHEME 2. Enantioselective Total Synthesis of Okaspirodiol (4)



for the synthesis of spiroacetals have been elaborated over the past decades,[1] yet the concept of adding a lithiated terminal alkyne bearing a protected hydroxyl group to a suitable lactone, followed by hydrogenation of the triple bond and ring closure, appeared to be the most attractive one.[21] Thus, both fragments of okaspirodiol can separately be prepared according to known procedures and then combined in a very late stage of the synthesis. A diastereoselective alkylation of (*S*)-diethyl malate (**8**) according to Seebach et al.[22a] furnishes diester **9** which was transformed to the desired lactone **10** in seven high-yielding steps (53% overall yield from **9**)[22b] (Scheme 2). Furthermore, starting from (*S*)-propylene oxide (**11**), three straightforward transformations yielded the desired THP-protected, (*S*)-configured alkynol **12** (58% overall yield).[23] This substrate was then lithiated with ethereal MeLi and added to the lactone **10**.

Because the crude product **13** could not be hydrogenated, it was treated with methanolic HCl to give the acetal **14** which was obtained as a single diastereomer, most likely having an (*R*)-configured anomeric carbon. The hydrogenation of this substrate was still challenging,[24] and thus different catalysts were investigated. The commonly employed Rh/Al_2O_3[21a] led to incomplete conversions as can be seen from the isolation of the tricyclic diacetal **16** as a side product after cyclization. Better results were achieved using the Adams catalyst PtO_2 in ethyl acetate, but the reaction had to be carefully monitored by TLC to prevent a hydrogenation of the phenyl ring. After subsequent cyclization, the desired compound **15** was obtained as a single isomer in an excellent yield of 80%. Finally, hydrogenolysis of the benzyl ether moiety was accomplished using Pd/C in MeOH to furnish okaspirodiol (**4**). The analytical data of the synthetic compound entirely matched those of the natural product, e.g., the optical rotation $[\alpha]_D^{20}$ −53 (*c* 1.0, MeOH). Thus, a completely stereoselective total synthesis was achieved furnishing the novel spiro[4.5]acetal **4** in 52% yield over the five steps from the known precursors **10** and **12**. Resubjecting the monobenzylated okaspirodiol **15** to CSA in CH_2Cl_2 yielded only traces of a second isomer (20:1 ratio according to HPLC) which must be the product of an epimerization of C-5.

Biosynthesis. Okaspirodiol (**4**) is the first small spiroacetal isolated from actinomycetes. Only very few biosynthetic studies on similar structures from nature have been carried out to date. The biosynthetic pathway to low-boiling spiro compounds isolated from fruit flies was investigated by De Voss et al. and most likely involves several oxidation steps starting from fatty acids.[25] Another example is blazeispirol A isolated from the fungus *Agaricus blazei*. In this case, the spiroacetal was found to be of mevalonate origin.[26]

The carbon skeleton of okaspirodiol (**4**) indicates that the biosynthesis possibly proceeds by the condensation of a glycerol unit with an intermediate from the sugar pool like sedoheptulose or from the carboxylic acid pool. Feeding experiments were carried out by continuously adding sterile aqueous solutions of [1-^{13}C]acetate, [2-^{13}C]acetate, or [U-$^{13}C_3$]glycerol, respectively, to growing cultures of *Streptomyces* sp. Gö TS 19 during the period of highest production of **4**. NMR analysis of **4** isolated from the [U-$^{13}C_3$]glycerol feeding experiment showed ^{13}C−^{13}C couplings for all carbon atoms derived from intact incorporation of C_2- or C_3-units of the precursor, but C-4 showed no coupling (Table 1). The resulting labeling pattern does not match a sedoheptulose-based biosynthetic pathway. In the case of a sedoheptulose precursor, two C_3- (C-12/C-7/C-8, C-2/C-3/C-11) and two C_2-units would form the carbon skeleton (see Supporting Information). In addition, feeding ^{13}C-labeled acetate

TABLE 1. ^{13}C NMR Analyses (150.8 MHz, CD_2Cl_2) of Enriched 4 from Feeding Experiments

carbon atom	δ_C (ppm)	[1-^{13}C]acetate[a]	[2-^{13}C]acetate[a]	[U-$^{13}C_3$]glycerol, $J_{(C,C)}$ (Hz)
2	61.1			37.5
3	42.1			37.5; 32.5
4	77.9		1.66	
5	102.9	2.96		45.5
7	67.6	2.34		40.5
8	32.6		1.17	32.5
9	20.0	1.83		32.5
10	30.9		1.16	45.5
11	66.9			32.5
12	21.7		1.42	40.5

[a] Specific incorporation.[27]

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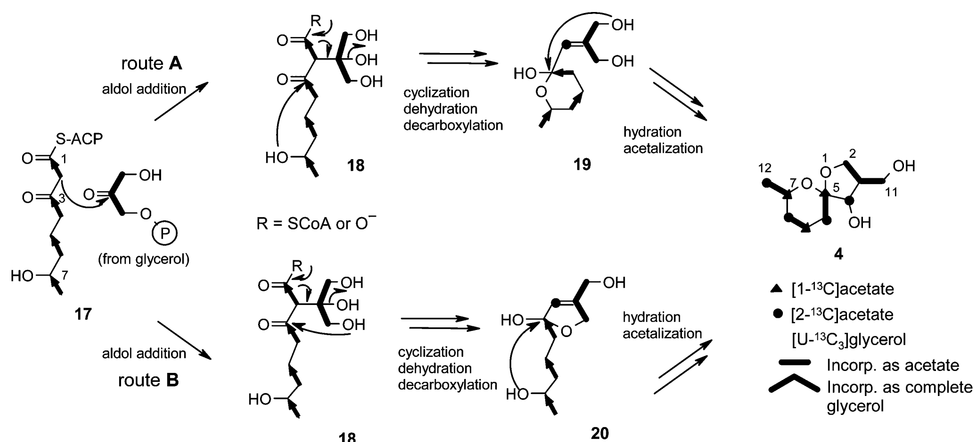
SCHEME 3. Labeling Pattern and Proposed Series of Biosynthetic Steps of Okaspirodiol (4)



gave okaspirodiol with a specific incorporation pattern[27] and thus confirmed that a polyketide chain and a glycerol unit form the spiroacetal skeleton of okaspirodiol (**4**) (Scheme 3).

The incorporation pattern of **4** gives evidence that surprisingly it is originated from the mixed acetate−glycerol biosynthetic pathway which has recently gained new interest.[28] From this, two plausible biosynthetic routes (**A**, **B**) suggest a partially reduced tetraketide **17** derived from a polyketide synthase (or a fatty acid like pathway)[28a] which undergoes an aldol addition of a dihydroxyacetone-derived 3-carbon unit (Scheme 3). The proposed C_{11}-product **18** is cyclized, dehydrated, and decarboxylated to form the hemiacetal **19**. Rehydration with inverse regioselectivity and intramolecular acetalization then lead to okaspirodiol (**4**) concordantly with the observed incorporation pattern (pathway **A**). Along the plausible alternative route (pathway **B**), the five-membered ring is formed at first, so that subsequent dehydration and decarboxylation give **20** which finally forms **4** via rehydration and acetalization. Admittedly, an alternative order of reactions and thus alternative structures of the intermediates are also possible, e.g., thioester hydrolysis or formation.

Some similarities for this biosynthetic pathway can be found in the literature,[29,30] such as for the well-known biosynthesis of the virginiae butanolides. In addition, the mixed acetate−glycerol biosynthetic pathway was recently found to be responsible for the formation of a new diversity of furan and lactone metabolites from actinomycetes (**21**−**23**)[31] in addition to the known autoregulators such as the A-factor (**24**) or virginiae butanolide A[29] (**25**) (Figure 2). Okaspirodiol (**4**) expands the class of compounds from the mixed acetate−glycerol biosynthetic pathway for the first time by the scaffold of a spiroacetal. Prior to the present studies, no natural product has been known whose biosynthesis comprises a similar set of reactions: an unbranched tetraketide as a precursor for the aldol condensation with a glycerol-derived C_3-unit, a decarboxylation step, and an acetalization, which occur within the mixed acetate−glycerol pathway to yield the new hydroxylated spiro[4.5]acetal **4**.

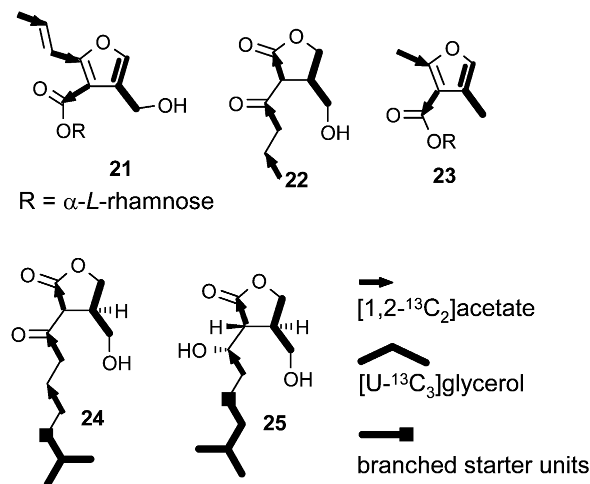


FIGURE 2. Microbial metabolites from other types of the mixed acetate−glycerol biosynthetic pathway.

The biosynthetic and structural similarity of okaspirodiol (**4**) with the "microbial hormones" **24** and **25** encouraged us to screen for regulatory activity on growing actinomycetes strains. No unambiguous results were obtained so far. No antibacterial activity of **4** was found in plate diffusion assays with *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Candida albicans*; however, growth inhibition was observed for some

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fungi. Further work to specify this antifungal activity is now in progress with an expanded screening approach. The particular ecological advantage of the high production (up to 380 mg/L) of **4** and some other members of the mixed acetate−glycerol biosynthetic pathway (e.g., **21**−**23**) for the producing organism needs further investigation.

Conclusion

The cryptic ketotetrol okaspirodiol (**4**) is a highly interesting spiroacetal because of its two free hydroxyl groups which are responsible for the unique isomerization pattern: the C-4 hydroxyl group permits a very effective stabilization of the configuration and conformation of the spiro center through hydrogen bonds. The C-3 hydroxymethyl group allows for a transacetalization, a particular *ansa isomerization*, leading to an epimerization at this carbon atom and the formation of the thermodynamically more stable isomer **4a**. The isomerization studies also revealed the remarkable fact that the strain *Streptomyces* sp. Gö TS 19 selectively forms the less stable isomer **4**, and no isomerization products were isolated from the fermentation broth. Additionally, the understanding of the principles of stabilization strongly eased the total synthesis, furnishing the new natural product in 52% yield over only five highly stereoselective steps from known precursors.

Because okaspirodiol (**4**) is the first spiroacetal built from an as yet unknown type of the rare mixed acetate−glycerol biosynthetic pathway, it is an important example to emphasize the variability of the secondary metabolite biosynthesis. Starting from simple building blocks, a new structure is formed which has not been found in nature before and has not yet been invented by synthetic chemists' ideas.

Experimental Section

Fermentation. Strain Gö TS 19 (*Streptomyces* sp.) was incubated at 28 °C for 7 days and maintained on medium A agar plates. A 1 cm^2 agar piece was used to inoculate 50 mL of preculture (medium A, rotary shaker, 180 rpm, 48 h, 28 °C) in 300 mL Erlenmeyer flasks (with three flow spoilers). A portion of 100 mL of the preculture was used to inoculate a 2 L fermenter, containing 900 mL of medium A. Standard fermentation conditions: 72−86 h, 500 rpm, aeration 6.0 vvm, 28 °C. Medium A: 10 g/L of malt extract; 4 g/L of glucose; 4 g/L of yeast extract; culture plates: additional 10 g/L of agar.

Feeding Experiments. Feeding experiments with [1-^{13}C]acetate and [U-$^{13}C_3$]glycerol were carried out under the conditions described above. In general, precursors were administered to the fermentation as sterile aqueous solutions. Continuous feeding with a low rate pump was carried out with the ^{13}C-labeled precursors between the 26th and 50th hour of fermentation. [1-^{13}C]acetate: 560 mg/L in 100 mL of water (6.8 mmol/L culture broth), yielding 22 mg of labeled **4**. [U-$^{13}C_3$]glycerol: 400 mg of unlabeled glycerol was added to 100 mg of ^{13}C-labeled glycerol and dissolved in 100 mL of water (1.05 mmol/L culture broth), yielding 380 mg of labeled **4**. [2-^{13}C]acetate: 250 mg of the ^{13}C-labeled compound was dissolved in 60 mL of water and added to a fermentation of 300 mL of medium A (1 L Erlenmeyer flasks, 250 rpm, 28 °C) which was inoculated with 15 mL of preculture, yielding 42 mg of labeled **4**.

Isolation and Purification. The culture broth was separated from the mycelium by filtration, and the mycelium was discarded. The culture filtrate was passed through an Amberlite XAD-2 column, and impurities were washed out with deionized water. The metabolites were eluted with MeOH, and evaporation yielded crude extracts (ca. 1.5 g/L) which were applied to gel chromatography on Sephadex LH-20 (column: 100 × 2.5 cm, MeOH). The fractions containing the spiroacetal **4** as detected by TLC were further purified by column chromatography on silica gel (*c*-Hex/EtOAc 1:1) yielding pure okaspirodiol (**4**).

(3*S*,4*S*,5*S*,7*S*)-3-Hydroxymethyl-7-methyl-1,6-dioxaspiro[4.5]-decan-4-ol (4). The title compound was isolated as described above as a colorless, crystalline solid: R_f = 0.42 (CH_2Cl_2/MeOH 9:1); mp 98 °C; $[\alpha]_D^{20}$ −53 (*c* 1.0, MeOH); ^{1}H NMR (600 MHz, CD_3OD) δ 1.14 (d, ^3J = 6.1 Hz, 3 H, CH_3), 1.21 (m_c, 1 H, 8-H), 1.48 (m_c, 1 H, 10-H), 1.58 (m_c, 1 H, 8-H), 1.64−1.75 (m, 2 H, 9(10)-H), 1.80 (m_c, 1 H, 9-H), 2.49 (m_c, 1 H, 3-H), 3.69 (dd, ^3J = 7.8, ^2J = 11.0 Hz, 1 H, 2-H), 3.73−3.79 (m, 2 H, 2-H, CH_2OH), 3.84 (d, ^3J = 9.0 Hz, 1 H, 4-H), 3.94 (ddq, ^3J = 2.0, ^3J = 6.1, ^3J = 12.5 Hz, 1 H, 7-H), 3.98 (t, J = 8.5 Hz, 1 H, CH_2OH); ^{1}H NMR (600 MHz, CD_2Cl_2) δ 1.13 (d, ^3J = 6.1 Hz, 3 H, CH_3), 1.16−1.27 (m, 1 H, 8-H), 1.52−1.62 (m, 5 H, 8(9,10)-H), 2.51 (m_c, 1 H, 3-H), 2.83 (d, ^3J = 9.0 Hz, 1 H, 4-OH), 2.89 (dd, ^3J = 5.0, ^3J = 7.0 Hz, 1 H, CH_2O*H*), 3.59−3.65 (m, 2 H, 2-H, CH_2OH), 3.69−3.72 (m, 1 H, 2-H), 3.88−3.95 (m, 2 H, 4-H, CH_2OH), 3.95 (ddq, ^3J = 2.5, ^3J = 6.1, ^3J = 12.5 Hz, 1 H, 7-H); ^{13}C NMR (150.8 MHz, CD_2Cl_2) δ 20.0 (C-9), 21.7 (CH_3), 30.9 (C-10), 32.6 (C-8), 42.1 (C-3), 61.1 (C-2), 66.9 (CH_2OH), 67.6 (C-7), 77.9 (C-4), 102.9 (C-5); IR (cm^{-1}, KBr) 3445, 2934, 1441, 1386, 1128, 1078; MS (ESI) *m*/*z* (%) 225.1 (100) $[M + Na]^+$. Anal. Calcd for $C_{10}H_{18}O_4$: 225.109746 $[M + Na]^+$ (correct mass according to ESI−HRMS).

(3*R*,4*S*,5*S*,7*S*)-4-Hydroxy-7-methyl-1,6-dioxaspiro[4.5]dec-3-yl-methyl 4-Bromobenzoate (5) and (3*R*,4*S*,5*S*,7*S*)-4-(4-Bromophenylcarbonyloxy)-7-methyl-1,6-dioxaspiro[4.5]dec-3-yl-methyl 4-Bromobenzoate (5a). To a solution of spiroacetal **4** (59.6 mg, 0.295 mmol) in dichloromethane (4 mL) were subsequently added *p*-bromobenzoic acid (119 mg, 0.592 mmol), ethyl-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDCI, 116 mg, 0.605 mmol), and a catalytic amount of 4-(dimethylamino)pyridine (3 mg). Because of an incomplete conversion, additional amounts of *p*-bromobenzoic acid (60.0 mg, 0.301 mmol) and EDCI (60.0 mg, 0.313 mmol) were added after 4 h. The reaction mixture was stirred overnight at room temperature and then diluted with dichloromethane (20 mL) and water (10 mL). The phases were separated, and the organic phase was washed with water (10 mL), saturated NH_4Cl solution (10 mL), and brine (10 mL), dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by flash column chromatography on silica gel (20 g, *c*-Hex/EtOAc 3:1). Two fractions were collected containing 26.8 mg (16%) of the diester **5a** (R_f = 0.51) and 71.2 mg (63%) of the monoester **5** (R_f = 0.26) as colorless solids. **5**: mp 91 °C; $[\alpha]_D^{20}$ −12 (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, CD_2Cl_2) δ 1.14 (d, ^3J = 6.1 Hz, 3 H, CH_3), 1.16−1.28 (m, 1 H, 8-H), 1.50−1.90 (m, 5 H, 8(9,10)-H), 2.66−2.80 (m, 2 H, 3-H, OH), 3.77 (dd, ^3J = 7.5, ^2J = 9.0 Hz, 1 H, 2-H), 3.91 (t, J = 8.5 Hz, 1 H, 2-H), 3.93 (ddq, ^3J = 2.1, ^3J = 6.1, ^3J = 12.5 Hz, 1 H, 7-H), 4.02 (dd, J = 8.0, J = 9.0 Hz, 1 H, CH_2OBz), 4.28 (dd, J = 8.0, J = 11.0 Hz, 1 H, CH_2OBz), 4.56 (dd, ^3J = 5.5, ^3J = 7.0 Hz, 1 H, 4-H), 7.63−7.66 (m, 2 H, Ar−H), 7.82−7.86 (m, 2 H, Ar−H); ^{13}C NMR (75.5 MHz, CD_2Cl_2) δ 20.0 (C-9), 21.9 (CH_3), 31.2 (C-10), 32.7 (C-8), 40.4 (C-3), 64.7 (C-2), 67.7 (C-7), 68.7 (CH_2OBz), 76.9 (C-4), 103.2 (C-5), 128.1 (CH, C−Ar), 129.6 (CH, C−Ar), 131.3 (C_{quat}, C−Ar), 132.0 (C_{quat}, C−Ar), 165.9 (CO_2); IR (cm^{-1}, KBr) 3417, 2964, 2930, 2867, 1716, 1591, 1387, 1331, 1272, 1082; MS (ESI) *m*/*z* (%) 407.1 (100) $[M + Na]^+$, 792.7 (40) $[2M + Na]^+$, 1340.0 (34) $[4M−C_7H_4BrO_2]^+$. **5a**: mp 55 °C; $[\alpha]_D^{20}$ −134 (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, CD_2Cl_2) δ 1.16 (d, ^3J = 6.1 Hz, 3 H, CH_3), 1.20−1.32 (m, 1 H, 8-H), 1.50−1.90 (m, 5 H, 8(9,10)-H), 3.08 (m_c, 1 H, 3-H), 3.82 (dd, ^3J = 6.1, ^2J = 9.0 Hz, 1 H, 2-H), 3.93 (ddq, ^3J = 2.1, ^3J = 6.1, ^3J = 12.5 Hz, 1 H, 7-H), 4.20 (t, J = 9.0 Hz, 1 H, 2-H), 4.37 (dd, ^3J = 7.0, ^2J = 11.0 Hz, 1 H, CH_2OBz), 4.58 (dd, ^3J = 7.5, ^2J = 11.0 Hz, 1 H, CH_2OBz), 5.12 (d, ^3J = 9.0 Hz, 1 H, 4-H), 7.46−7.56 (m, 4 H, Ar−H), 7.63−7.66 (m, 2 H, Ar−H), 7.82−7.86 (m, 2 H, Ar−H); ^{13}C NMR (75.5 MHz, CD_2Cl_2) δ 20.2 (C-9), 22.0 (CH_3), 30.7 (C-10), 32.5 (C-8), 37.8 (C-3), 64.9 (C-2),

66.9 (C-7), 67.8 (CH_2OBz), 77.2 (C-4), 103.3 (C-5), 128.0 (CH, C−Ar), 128.4 (CH, C−Ar), 129.1 (CH, C−Ar), 129.2 (CH, C−Ar), 131.1 (C_{quat}, C−Ar), 131.5 (C_{quat}, C−Ar), 131.7 (C_{quat}, C−Ar), 131.9 (C_{quat}, C−Ar), 165.6 (2 C, CO_2); IR (cm^{-1}, KBr) 3424, 2931, 2875, 1723, 1592, 1396, 1291, 1127; MS (ESI) *m/z* (%) 591 (65) [M + Na]$^+$, 1158.5 (100) [2M + Na]$^+$; CD (MeOH) λ_{extr} ([Θ]) = 254 (−126 044), 238 (45 741), 224 (16 486), 211 (39 208) nm.

(3*S*,4*S*,5*S*,7*S*)-4-Benzyloxy-3-hydroxymethyl-7-methyl-1,6-dioxaspiro[4.5]decane (6) and (3*S*,4*S*,5*S*,7*S*)-4-Benzyloxy-3-benzyloxymethyl-7-methyl-1,6-dioxaspiro[4.5]decane (7). The spiroacetal **4** (33.6 mg, 0.166 mmol) was dissolved in DMF (4 mL) and cooled to −60 °C, and sodium hydride (60% in mineral oil, 16.7 mg, 0.42 mmol) was added. After being stirred for 1 h at −60 °C, the mixture was allowed to warm to −10 °C and kept at this temperature for another 30 min. The reaction mixture was again cooled to −60 °C, and a solution of benzyl bromide (49 μL, 71 mg, 0.41 mmol) in DMF (1 mL) was added. The resulting mixture was stirred for 20 h at room temperature, cooled to 0 °C, quenched with water (2 mL), and stirred for 5 min at room temperature. The reaction mixture was diluted with water (20 mL) and dichloromethane (20 mL). The aqueous phase was extracted with dichloromethane (2 × 25 mL), and the combined organic layers were washed with water (2 × 20 mL), dried over Na_2SO_4, and concentrated in vacuo. The crude product was purified by flash column chromatography on silica gel (10 g, *c*-Hex/EtOAc 5:1). Two fractions were collected containing 8.6 mg (14%) of the dibenzylated derivative **7** (R_f = 0.20) and 33 mg (68%) of the monobenzylated compound **6** (R_f = 0.11) as colorless oils. **6**: $[\alpha]_D^{20}$ −13 (*c* 1.0, MeOH); ^{1}H NMR (600 MHz, CD_2Cl_2) δ 1.09 (d, ^3J = 6.1 Hz, 3 H, CH_3), 1.11−1.21 (m, 1 H, 8-H), 1.36−1.39 (m, 1 H, 10-H), 1.48−1.52 (m, 1 H, 8-H), 1.54−1.60 (m, 1 H, 9-H), 1.64−1.75 (m, 2 H, 9(10)-H), 2.52 (m_c, 1 H, 3-H), 3.29 (d, br, ^3J = 7.5 Hz, 1 H, OH), 3.40 (dt, ^3J = 4.5, ^2J = 11.0 Hz, 1 H, CH_2OH), 3.64 (d, ^3J = 9.5 Hz, 1 H, 4-H), 3.83 (dd, ^3J = 2.5, ^2J = 11.0 Hz, 1 H, CH_2OH), 3.83−3.92 (m, 2 H, 2-H), 3.87 (ddq, ^3J = 2.1, ^3J = 6.1, ^3J = 12.5 Hz, 1 H, 7-H), 4.54 (d, ^2J = 11.8 Hz, 1 H, CH_2Ph), 4.57 (d, ^2J = 11.8 Hz, 1 H, CH_2Ph), 7.21−7.32 (m, 5 H, Ar−H); ^{13}C NMR (75.5 MHz, CD_2Cl_2) δ 20.3 (C-9), 21.5 (CH_3), 31.3 (C-10), 32.4 (C-8), 40.0 (C-3), 59.6 (C-2), 66.0 (C-7), 66.9 (CH_2OBn), 73.5 (C-4), 84.0 (CH_2Ph), 102.0 (C-5), 128.1 (CH, C−Ar), 128.2 (CH, C−Ar), 128.7 (CH, C−Ar), 138.3 (C_{quat}, C−Ar); MS (ESI) *m/z* (%) 293.1 (100) [M + H]$^+$, 606.9 (40) [2M + Na]$^+$. **7**: $[\alpha]_D^{20}$ −147 (*c* 1.0, MeOH); ^{1}H NMR (300 MHz, CD_3COCD_3) δ 1.02 (d, ^3J = 6.1 Hz, 3 H, CH_3), 1.05−1.18 (m, 1 H, 8-H), 1.35−1.42 (m, 1 H, 10-H), 1.48−1.82 (m, 4 H, 8(9,10)-H), 2.71 (m_c, 1 H, 3-H), 3.63−3.78 (m, 4 H, 2(4)-H, CH_2OBn), 3.83 (ddq, ^3J = 2.1, ^3J = 6.1, ^3J = 12.5 Hz, 1 H, 7-H), 3.95 (t, J = 8.5 Hz, 1 H, 2-H), 4.42 (d, ^2J = 11.8 Hz, 1 H, CH_2Ph), 4.50 (d, ^2J = 11.8 Hz, 1 H, CH_2Ph), 4.60 (s, 2 H, CH_2Ph), 7.22−7.40 (m, 10 H, Ar−H); ^{13}C NMR (75.5 MHz, CD_3COCD_3) δ 20.7 (C-9), 22.2 (CH_3), 31.8 (C-10), 33.1 (C-8), 39.5 (C-3), 66.7 (C-7), 69.6 (C-2), 71.2 (CH_2OBn), 73.4 (CH_2Ph), 73.5 (CH_2Ph), 84.3 (C-4), 103.3 (C-5), 128.0 (CH, C−Ar), 128.3 (CH, C−Ar), 128.3 (CH, C−Ar), 128.7 (CH, C−Ar), 129.0 (CH, C−Ar), 139.6 (C_{quat}, C−Ar), 139.9 (C_{quat}, C−Ar); MS (ESI) *m/z* (%) 405.3 (100) [M + Na]$^+$, 383.3 (5) [M + H]$^+$. Anal. Calcd (%) for $C_{24}H_{30}O_4$ (382.50): C, 75.36; H, 7.91. Found: C, 75.05; H, 7.61.

(2*R*,3*S*,4*S*)-4-Benzyloxymethyl-2-[(*S*)-4-hydroxypent-1-ynyl]-2-methoxytetrahydrofuran-3-ol (14). A solution of (*S*)-2-[(tetrahydro-2*H*-pyran-2-yl)oxy]-4-pentyne (**12**, 61 mg, 0.36 mmol) in diethyl ether (20 mL) was cooled to 0 °C, and methyllithium (0.35 mL, 0.34 mmol, 0.97 M in pentane) was added. After being stirred for 5 min at 0 °C, the solution was transferred by cannula to second flask containing a precooled solution (0 °C) of (2*S*,3*S*)-3-benzyloxymethyl-2-[(tetrahydro-2*H*-pyran-2-yl)oxy]-4-butanolide (**10**, 111 mg, 0.362 mmol) in diethyl ether (20 mL). The resulting mixture was stirred for 20 min at 0 °C and then for 3 h at room temperature. An aqueous solution of NH_4Cl (20%, 10 mL) was added, and the mixture was stirred for 10 min. The layers were separated, and the organic phase was washed with saturated $NaHCO_3$ solution (10 mL) and brine (10 mL) and dried over Na_2SO_4. Concentration in vacuo yielded 150 mg of a yellow oil, which was dissolved in MeOH (15 mL). Concentrated hydrochloric acid (20 μL) was added to this solution, and the mixture was stirred for 60 min at room temperature. When the reaction had ceased, saturated $NaHCO_3$ solution (10 mL) was added and the mixture was diluted with diethyl ether (100 mL). The phases were separated, and the aqueous phase was extracted with diethyl ether (2 × 50 mL). The combined organic layers were washed with saturated $NaHCO_3$ solution (10 mL) and brine (10 mL) and dried over Na_2SO_4. Concentration in vacuo yielded 88 mg (81%) of the title compound **14** as a colorless oil, whose purity was >95%: R_f = 0.20 (*c*-Hex/EtOAc 1:2); $[\alpha]_D^{20}$ +21 (*c* 1.0, MeOH); ^{1}H NMR (250 MHz, CD_2Cl_2) δ 1.21 (d, ^3J = 8.3 Hz, 3 H, 5′-H), 2.38 (dd, ^3J = 6.5, ^2J = 16.5 Hz, 1 H, 3′-H), 2.48 (dd, ^3J = 4.6, ^2J = 16.5 Hz, 1 H, 3′-H), 2.85 (m_c, 1 H, 4-H), 3.38 (s, 3 H, OCH_3), 3.58 (dd, ^3J = 6.5, ^2J = 9.3 Hz, 1 H, CH_2-OBn), 3.78 (dd, ^3J = 7.0, ^2J = 9.3 Hz, 1 H, CH_2OBn), 3.80−4.02 (m, 3 H, 5(4′)-H), 4.25 (d, ^3J = 4.6 Hz, 1 H, 3-H), 4.51 (s, 2 H, CH_2Ph), 7.21−7.40 (m, 5 H, Ar−H); ^{13}C NMR (62.9 MHz, CD_2Cl_2) δ 22.3 (C-5′), 28.8 (C-3′), 41.6 (C-4), 50.4 (OCH_3), 65.9 (C-4′), 67.8 (CH_2OBn), 69.7 (C-5), 73.1 (CH_2Ph), 76.2 (C-2′), 77.5 (C-3), 85.5 (C-1′), 106.2 (C-2), 127.6 (CH, C−Ar), 128.3 (CH, C−Ar), 138.3 (C_{quat}, C−Ar); IR (cm^{-1}, film) 3444, 2937, 2268, 1634, 1453, 1370, 1282, 1222, 1110. Anal. Calcd for $C_{18}H_{24}O_5$: 343.15147 [M + Na]$^+$ (correct mass according to ESI−HRMS).

(3*S*,4*S*,5*S*,7*S*)-3-Benzyloxymethyl-7-methyl-1,6-dioxaspiro[4.5]-decan-4-ol (15), Method A. The alkyne **14** (107 mg, 334 μmol) was dissolved in MeOH (10 mL), and Rh on activated Al_2O_3 (5%, 40.2 mg) was added. The mixture was vigorously stirred for 4.5 h at room temperature under a hydrogen atmosphere (1 atm) and then filtered through 2 cm Celite. The solids were washed with MeOH (20 mL), and the filtrate was concentrated in vacuo to a volume of approximately 15 mL. Then, concentrated hydrochloric acid was added (10 μL), and the mixture was stirred for 15 h at room temperature. The solution was diluted with diethyl ether (200 mL) and water (20 mL). The layers were separated, and the organic phase was washed with saturated $NaHCO_3$ solution (10 mL) and brine (10 mL) and dried over Na_2SO_4. The solvent was removed in vacuo, and the residue was separated by flash column chromatography on silica gel (10 g, *c*-Hex/EtOAc 3:1) to yield 6.0 mg (6%) of (1*S*,4*S*,5*S*,7*R*)-4-benzyloxymethyl-7-methyl-2,6,11-trioxatricyclo[5.3.1.0^{1,5}]undecane (**16**) (R_f = 0.58, *c*-Hex/EtOAc 1:1) and 9.8 mg (10%) of the title compound **15** (R_f = 0.40, *c*-Hex/EtOAc 1:1) as colorless oils. **15**: $[\alpha]_D^{20}$ −17 (*c* 1.0, MeOH); ^{1}H NMR (250 MHz, CD_2Cl_2) δ 1.15 (d, ^3J = 6.1 Hz, 3 H, CH_3), 1.14−1.25 (m, 1 H, 8-H), 1.48−1.82 (m, 5 H, 8(9,10)-H), 2.58 (m_c, 1 H, 3-H), 2.68 (d, ^3J = 8.5 Hz, 1 H, 4-OH), 3.46 (t, J = 9.1 Hz, 1 H, CH_2-OBn), 3.70 (t, J = 9.1 Hz, 1 H, CH_2OBn), 3.75 (dd, ^3J = 7.5, ^2J = 8.8 Hz, 1 H, 2-H), 3.81 (t, J = 8.8 Hz, 1 H, 2-H), 3.93 (ddq, ^3J = 2.0, ^3J = 6.1, ^3J = 12.5 Hz, 1 H, 7-H), 3.98 (dd, ^3J = 7.9, ^3J = 8.5 Hz, 1 H, 4-H), 4.45 (d, ^2J = 14.0 Hz, 1 H, CH_2Ph), 4.51 (d, ^2J = 14.0 Hz, 1 H, CH_2Ph), 7.20−7.40 (m, 5 H, Ar−H); ^{13}C NMR (62.9 MHz, CD_2Cl_2) δ 19.8 (C-9), 21.6 (CH_3), 31.1 (C-10), 32.5 (C-8), 40.9 (C-3), 67.2 (C-7), 69.1 (C-2), 69.6 (CH_2OBn), 73.1 (CH_2Ph), 76.8 (C-4), 102.9 (C-5), 127.5 (CH, C−Ar), 127.6 (CH, C−Ar), 128.3 (CH, C−Ar), 138.7 (C_{quat}, C−Ar); IR (cm^{-1}, KBr) 2934, 1735, 1617, 1457, 1385, 1229, 1087; MS (ESI) *m/z* (%) 293.1 (100) [M + H]$^+$, 315.2 (40) [M + Na]$^+$. Anal. Calcd (%) for $C_{17}H_{24}O_4$ (292.37): C, 69.84; H, 8.27. Found: C, 69.68; H, 8.22. **16**: ^{1}H NMR (250 MHz, CD_2Cl_2) δ 1.38 (s, 3 H, CH_3), 1.52−2.00 (m, 6 H, 8(9,10)-H), 2.45 (m_c, 1 H, 4-H), 3.39 (t, J = 9.1 Hz, 1 H, CH_2OBn), 3.67 (dd, ^3J = 6.2, ^2J = 9.1 Hz, 1 H, CH_2-OBn), 3.98 (t, J = 8.5 Hz, 1 H, 3-H), 4.18 (t, J = 8.5 Hz, 1 H, 3-H), 4.47−4.53 (m, 3 H, 5-H, CH_2Ph), 7.20−7.40 (m, 5 H, Ar−H); ^{13}C NMR (62.9 MHz, CD_2Cl_2) δ 19.4 (C-9), 23.5 (CH_3), 28.7 (C-10), 34.2 (C-8), 40.5 (C-4), 69.3 (CH_2OBn), 73.3 (CH_2Ph), 73.4 (C-3), 80.2 (C-5), 110.0 (C-7), 113.7 (C-1), 127.5 (CH, C−Ar), 127.7 (CH, C−Ar), 128.3 (CH, C−Ar), 138.6 (C_{quat}, C−Ar); MS

(DCI, 70 eV) m/z (%) 291.2 (10) $[M + H]^+$, 308.2 (100) $[M + NH_4]^+$, 598.5 (65) $[2M + NH_4]^+$. Anal. Calcd for $C_{17}H_{22}O_4$: 291.15894 $[M + H]^+$ (correct mass according to ESI−HRMS).

Method B. The alkyne **14** (10.0 mg, 31.2 μmol) was dissolved in ethyl acetate (2 mL). PtO_2 (3.0 mg, 13 μmol) was added, and the resulting suspension was stirred for 20 min at room temperature under a hydrogen atmosphere (1 atm). The reaction mixture was filtered through 1.5 cm Celite and washed with 20 mL of MeOH, and the filtrate was concentrated in vacuo. Dichloromethane (1.5 mL) and (+)-10-camphorsulfonic acid (3.5 mg, 15 μmol) were added, and the mixture was stirred for 5 h at room temperature under TLC control. When the reaction had ceased, the solution was diluted with diethyl ether (50 mL), washed with saturated $NaHCO_3$ solution (5 mL), water (2 × 10 mL), and brine (10 mL), and dried over Na_2SO_4. The solvent was removed in vacuo, and the residue was purified by flash column chromatography on silica gel (10 g, *c*-Hex/EtOAc 4:1) to yield 7.3 mg (80%) of the title compound **15**.

Synthesis of Okaspirodiol (4). The benzylated precursor **15** (15 mg, 51 μmol) was dissolved in MeOH (3 mL), and Pd/C (10%, 3 mg) was added. After being stirred for 4 h at room temperature under a hydrogen atmosphere (1 atm), the reaction mixture was filtered through 1.5 cm of Celite and washed with 20 mL of MeOH. The solvent was removed in vacuo, and the residue was purified by flash column chromatography on silica gel (5 g, *c*-Hex/EtOAc 4:1) to furnish 8.5 mg (82%) of the title compound **4**. The optical rotation $\{[\alpha]_D^{20}\ -53\ (c\ 1.0,\ \text{MeOH})\}$ and all spectroscopic data were consistent with the data of okaspirodiol (**4**) isolated from *Streptomyces* sp. Gö TS 19. Anal. Calcd (%) for $C_{10}H_{18}O_4$ (202.25): C, 59.39; H, 8.97. Found: C, 59.68; H, 8.92.

Computations. Ab initio DFT calculations were performed using Spartan 04 for Windows (Wavefunction Inc., Irvine, CA). All isomers of **4** were geometry minimized using the B3LYP method with a 6-31G* basis set.

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Supporting Information Available: General experimental methods; procedures for the isomerization of okaspirodiol (**4**) and analytical data of isomers **4a**−**4c**; ^{1}H NMR and ^{13}C NMR spectra of all new compounds including the ^{13}C NMR spectrum of the natural product from the [U-$^{13}C_3$]glycerol feeding experiment; a short graphical explanation for the exclusion of a sedoheptulose-based biosynthesis; tabularly summary of HMBC and COSY correlations for okaspirodiol **4**; NOESY spectra and a graphical summary of observed NOE cross-peaks for compounds **4**−**4c**; structure, total energies, and Cartesian coordinates for calculated structures **4**−**4c**; and ORTEP drawing, X-ray data, and details for the X-ray data acquisition for **5** (CIF format). This material is available free of charge via the Internet at http://pubs.acs.org.

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