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Synthesis and Structural Revision of the Fungal Tetramic Acid Metabolite Spiroscytalin

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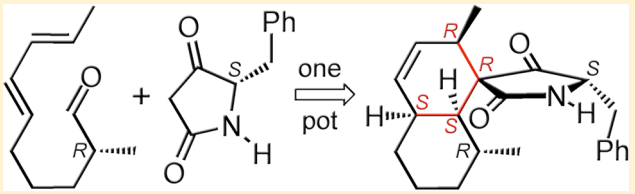
Supporting Information

ABSTRACT: Spiroscytalin, a natural 3-spirotetramic acid of hitherto uncertain absolute configuration, was synthesized for the first time by a one-pot Knoevenagel–IMDA reaction of an L-phenylalanine-derived tetramic acid and (*R*)-2-methyl-deca-6*E*,8*E*-dienal. Its absolute configuration was assigned by the known configurations of the starting compounds and by NOESY correlations. Its identity with the natural isolate was proved by the comparison of the NMR and circular dichroism spectra and of the specific optical rotations. Its absolute configuration (3*R*,5*S*,6*S*,7*R*,11*S*,14*R*) is enantiomeric to that originally proposed by the isolating group. This natural isomer of spiroscytalin showed moderate activity against *Candida albicans* and good activity against an export-deficient mutant of *Escherichia coli*.



INTRODUCTION

Spiroscytalin was isolated, among other bioactive metabolites, from solid-phase cultures of the filamentous fungus *Scytalidium cuboideum* (MSX 68345).[1] It features a phenylalanine-derived tetramic acid, which is 3-spiro-annulated to a dimethyl substituted *cis*-octalin. The natural isolate was assigned the structure **1** with the configuration 3*S*,5*R*,6*R*,7*S*,11*R*,14*S* on the grounds of NOESY experiments, electronic circular dichroism studies, and computational calculations (Figure 1).[1] Its spiro structure sets it apart from the related, yet more common natural tetramic acids[2] with 3-octalinoyl residues such as methiosetin (**2**), recently synthesized by our group,[3] or vinylogous variants such as zopfiellamide (**3**).[4] Natural spiroscytalin was found to exhibit moderate antiproliferative activity against three human tumor cell lines with double-digit micromolar IC_{50} concentrations. The antimicrobial activities were not assessed yet. We now report the first synthesis of the spiroscytalin featuring analytical data consistent with those of the natural isolate, which clarifies the absolute configuration of the latter.

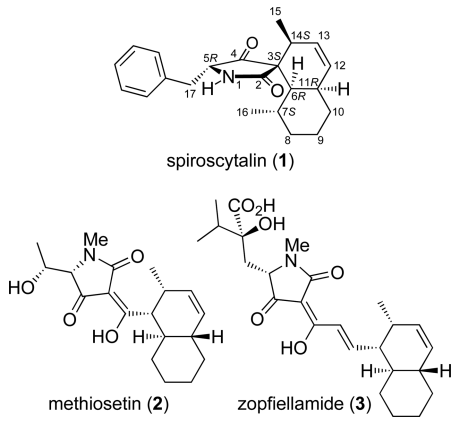


Figure 1. Proposed structures of spiroscytalin (**1**), methiosetin (**2**), and zopfiellamide (**3**).

RESULTS AND DISCUSSION

Scheme 1 delineates a retrosynthetic approach to spiroscytalin with stereocenters 5 and 7 configured as proposed by Oberlies et al. for their natural isolate.[1] This route was to establish the residual four stereocenters in a late stage intramolecular Diels–Alder (IMDA) cycloaddition of unknown stereospecificity. The IMDA precursor, 3-decatrienyltetramic acid **4**, can be obtained by a Knoevenagel condensation of tetramic acids (*R*)-**5**, readily available from Boc-protected D-phenylalanine **6** and Meldrum's acid according to Jouin,[5] with the known[6] enantiopure aldehyde (*S*)-**7**. The latter was to be prepared by a stereoselective α-alkylation of the RAMP hydrazone **8** of deca-6*E*,8*E*-dienal **10**.

Deca-6*E*,8*E*-dienal (**10**)[3] reacted with neat (*R*)-1-amino-2-methoxymethylpyrrolidin (RAMP) **9** to give hydrazone (*R*)-**11** in over 80% yield (Scheme 2). The treatment of the latter with LDA at −18 °C for 15 h and subsequent methylation at −100 °C according to a protocol by Enders[7] afforded hydrazone (*R*,*S*)-**8** in over 90% yield after column chromatography. It was treated with excess methyl iodide to give the corresponding quaternary hydrazonium salt, which was subjected to acidic hydrolysis in a two-phase system.[8] Column chromatography of the organic phase left the enantiopure aldehyde (*S*)-**7** in over 50% yield.

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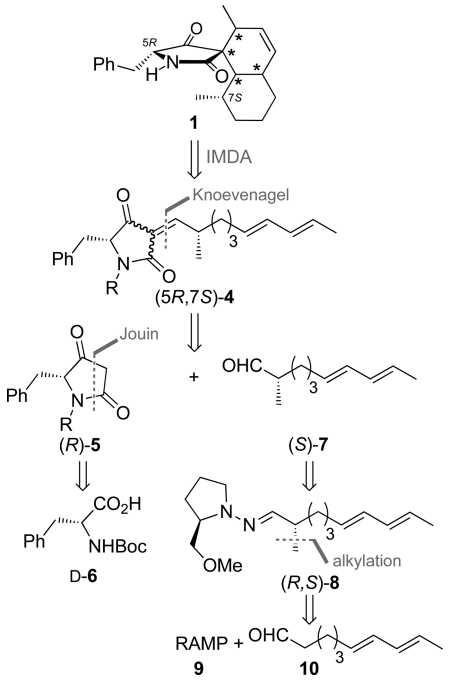
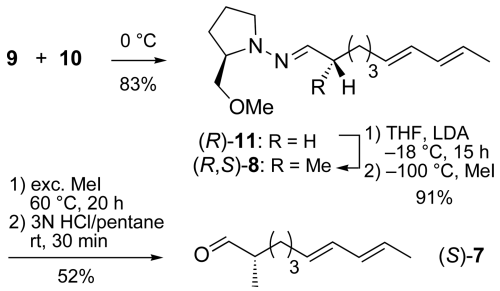
Scheme 1. Retrosynthesis of Spiroscytalin



Scheme 2. Synthesis of Aldehyde (*S*)-7



Tetramic acids (*R*)-**5a** (R = Boc) and (*R*)-**5b** (R = H) were prepared by the reaction of protected D-phenylalanine **6** with Meldrum's acid followed by deprotection with TFA in the case of **5b** as described by Tønder et al.[9] It turned out to be impossible to prepare 3-decatrienyltetramic acids (5*R*,7*S*)-**4** by the Knoevenagel condensation between aldehyde (*S*)-**7** and tetramic acids (*R*)-**5** without a spontaneously ensuing IMDA reaction. This is in keeping with a report by Ramachary et al.[10] on a similar domino sequence of Knoevenagel and intermolecular Diels–Alder reactions, yet contrasts the poor reactivity of 5-*exo*-methylenetetramic acids in intermolecular Diels–Alder reactions to give 5-spirotetramic acids reported by Moody et al.[11] We then optimized the conditions for a purposeful, stereoselective one-pot consecutive Knoevenagel–IMDA reaction between compounds (*R*)-**5** and (*S*)-7 by varying the basic catalyst for the Knoevenagel reaction (pyridine or L-proline), the Lewis acid catalyst for the IMDA reaction (Me_2AlCl, $ZnCl_2$, or none), and the tetramic acids (N-protected **5a** and N-unprotected **5b**). Scheme 3 and Table 1 summarize the results.

In each case, two major product diastereomers were formed. The crude Boc-protected spirotetramic acids obtained in runs A–C were not purified but were deprotected with TFA right away. Column chromatography followed by preparative HPLC afforded the pure stereoisomers **1**, **12**, and **13**. Their

Scheme 3. One-Pot Knoevenagel–IMDA Synthesis of Spiroscytalin Stereoisomers Derived from D-Phenylalanine and Aldehyde (*S*)-7 and Probable Transition States

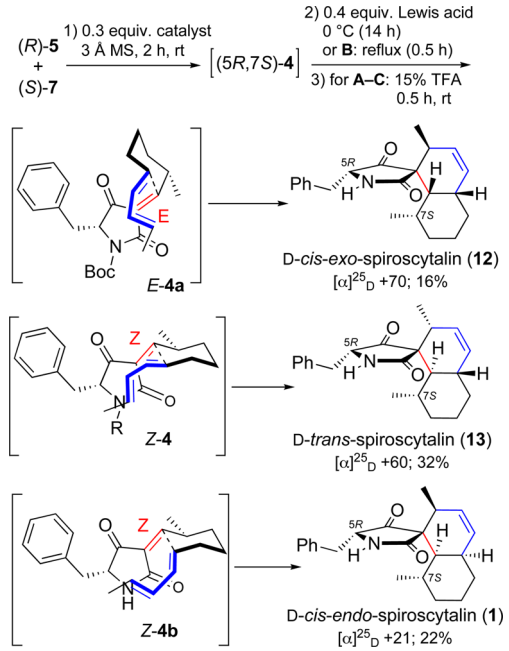


Table 1. Reagents and Products

entry	tetramic acid	solvent	catalyst	Lewis acid	product (% yield)
A	(*R*)-**5a**	$CHCl_3$	pyridine	Me_2AlCl	**12** (16) + **13** (32)
B	(*R*)-**5a**	MeOH	L-proline		**12** (16) + **13** (32)
C	(*R*)-**5a**	$CHCl_3$	pyridine	$ZnCl_2$	**12** (10) + **13** (20)
D	(*R*)-**5b**	$CHCl_3$	pyridine	Me_2AlCl	**1** (22) + **13** (14)

configurations were assigned on the grounds of the knowledge of the configurations of C-5 and C-7 and of a careful analysis of their ^1H, ^{13}C, and NOESY NMR spectra (cf. the Supporting Information). Scheme 3 depicts these stereoisomers annotated as D with respect to the starting D-phenylalanine, as *cis* or *trans* with respect to the octalin moiety and as *endo* or *exo* with respect to the cyclohexane ring pointing toward or away from the amide segment of the heterocycle. The runs A–C afforded D-*cis*-*exo*-spiroscytalin (**12**) and D-*trans*-spiroscytalin (**13**) in a 1:2 ratio. Interestingly, run D starting from N-unprotected tetramic acid (*R*)-**5b** furnished a 1:1.5 mixture of D-*trans*-spiroscytalin (**13**) and D-*cis*-*endo* isomer **1** that was originally assumed by Oberlies et al.[1] to be the natural product. The observed selectivities can be rationalized by assuming transition states as shown in Scheme 3. The most important directing group is the benzyl residue leaving space only for the diene to approach from the opposite side. Another decisive group is the α-methyl residue (C-16) of aldehyde **7**, which should adopt an equatorial position on the newly formed 6-ring. The formation of the product isomers **1** and **13** from the reaction of unprotected tetramic acid **5b** is explicable by assuming transition states with a *Z*-configured alkene C3–C6, which is favored in the Knoevenagel step due to an accelerated intramolecular protonation of the leaving hydroxy group by the enol of the tetramic acid. The transition states *Z*-**4** and *Z*-**4b** differ only in a 180° diene flip. The bulky Boc group of tetramic acid **5a** should lead to the formation of transition states *E*-**4a**, which cyclizes to **12**, and *Z*-**4a**, which yields **13**.

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While the NMR data of isomer **1** closely resembled that of the natural isolate, their optical rotations differed considerably: $[\alpha]_D^{25}$ +21 for **1** and $[\alpha]_D^{25}$ −7.7 for the isolate. Figure 2 depicts

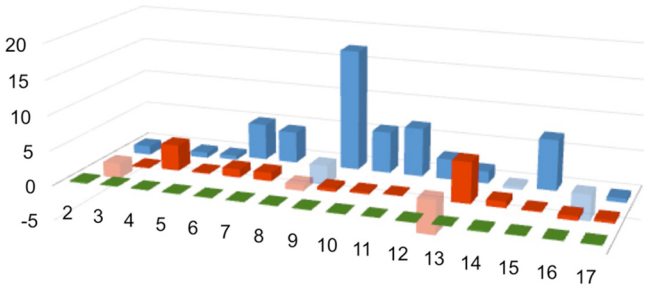


Figure 2. Difference in ppm (*y*-axis) of the ^{13}C NMR shifts of carbon atoms 2−17 (*x*-axis) of stereoisomers **1** (green), **12** (red), and **13** (blue) relative to those reported for the natural isolate.[1]

the deviations of the chemical shifts of carbon atoms 2−17 of the stereoisomers **1**, **12**, and **13** from those reported for the natural isolate. It shows that the *trans*-octalin derivative **13** deviates more from the natural isolate than the *cis-exo* derivative **12** and that the two *cis*-octalin derivatives **1** and **12** differ mainly in the shifts of the olefinic carbons C-12 and C-13 and the carbonyl carbon atoms C-2 and C-4. This finding is supported by a markedly different sterical encumbrance of these atoms in MM2-optimized molecular models of isomers **1** and **12** (Figure 3). Hence, we concluded that the natural isolate might actually

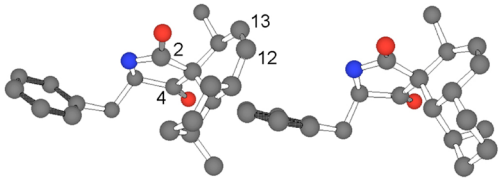


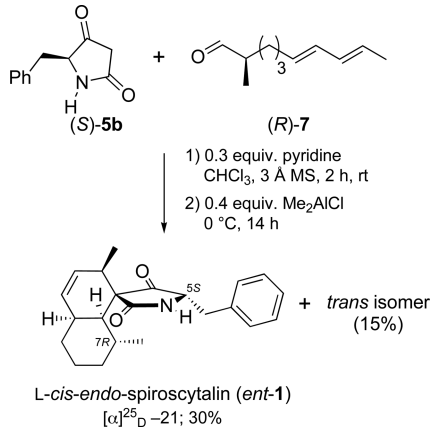
Figure 3. MM2-optimized molecular models of isomers **1** (left) and **12** (right).

be the enantiomer of isomer **1**. This would make sense also in terms of the biosynthesis being more likely to start from natural L-phenylalanine. The relatively minor deviation of the optical rotation of −8, measured by the isolating group, from −21 for the hypothetical *ent*-**1** may be due to chiral impurites that would also explain the reported UV absorbance of the natural sample at 376 nm despite the absence of a corresponding chromophore.

Finally, we actually synthesized *ent*-**1** starting from aldehyde (*R*)-**7**, prepared analogously to its enantiomer from aldehyde **10** and SAMP and the known N-unprotected tetramic acid (*S*)-**5b**.[5] Their one-pot Knoevenagel−IMDA reaction catalyzed by pyridine and Me_2AlCl left a 2:1 mixture of *ent*-**1** and an unspecified *trans* isomer. The separation via HPLC afforded 30% of pure *ent*-**1**, showing a specific optical rotation of $[\alpha]_D^{25}$ −21 and an NMR spectra identical to those of the natural isolate (Scheme 4).

Since Oberlies et al.[1] had based their stereochemical assignment for spiroscytalin upon the congruence of experimental and calculated electronic circular dichroism (ECD) spectra, we also measured the ECD spectra of the four stereoisomers **1**, *ent*-**1**, **12**, and **13** and found a perfect match of the spectral curves of *ent*-**1** and of the natural isolate.[1] These curves rise monotonically from negative values at 210 nm with

Scheme 4. One-Pot Knoevenagel−IMDA Synthesis of the Spiroscytalin Isomer *ent*-1



a zero-crossing at 214 nm to a positive local maximum at ca. 225 nm, whence they fall again through a zero-crossing at 254 nm to finally reach a flat negative local minimum at 311 nm. The spectra of **12** and **13** are distinctly different (Figure 4). This is the proof that natural spiroscytalin is identical to our synthetic isomer *ent*-**1** with the absolute configuration (3R,5S,6S,7R,11S,14R).

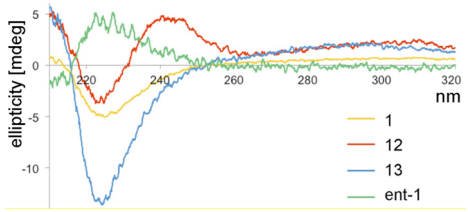


Figure 4. ECD spectra of stereoisomers **1**, *ent*-**1**, **12**, and **13**.

The four synthetic stereoisomers **1**, *ent*-**1**, **12**, and **13** of spiroscytalin were tested for antifungal activity against *Candida albicans*. Only the natural product *ent*-**1** showed a moderate antifungal effect with an IC_{50} concentration of 64 μg/mL, whereas the other isomers were inactive. The isomers *ent*-**1** and **12** were also tested against the export-defective, drug-susceptible ΔTolC mutant of *Escherichia coli*. Both compounds were of similar activity with IC_{50} values of 4.9 ± 1.5 μg/mL (*ent*-**1**) and 3.9 ± 0.05 μg/mL (**12**) (cf. Supporting Information for the experimental details).

■ CONCLUSIONS

We devised a one-pot Knoevenagel−IMDA synthesis of the 3-spiro-octalinyltetramic acid spiroscytalin, which establishes four out of its six stereocenters. Couples of stereoisomers of spiroscytalin were obtained starting from either D- or L-phenylalanine-derived tetramic acids and (*R*)- or (*S*)-2-methyldeca-6*E*,8*E*-dienal. Their absolute configurations were assigned by NOESY correlations. The substituent on the nitrogen atom of the tetramic acid appears to be decisive for the stereoisomers to be formed. The natural spiroscytalin stereoisomer was identified by the comparison of its optical rotation and NMR and CD spectra with those of the natural isolate. Its absolute configuration is enantiomeric to that proposed in the literature.

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EXPERIMENTAL SECTION

IR spectra were recorded with an FT-IR spectrophotometer equipped with an ATR unit. Chemical shifts of NMR signals were given in parts per million (δ) downfield from tetramethylsilane for ^{1}H and ^{13}C NMR spectra. Mass spectra were obtained under EI (70 eV) conditions. High resolution mass spectra were obtained with a UPLC/Orbitrap MS system in ESI mode. Optical rotations were measured at 589 nm (Na-D line) using solutions in methanol. Electronic circular dichroism spectra were recorded on a Jasco Spectropolarimeter J-710 using solutions in MeOH at concentrations of 0.2 mg/mL. Energy minimization for structures **1** and **12** was done using the MM2 force field module in Chem3D (PerkinElmer). For chromatography, silica gel 60 (230−400 mesh) was used. All reagents were purchased from commercial sources and were used without further purification. All anhydrous solvents were dried over molecular sieves. The known tetramic acids **5a**[9] and **5b**[5] were prepared according to literature.

Analytical HPLC: Beckman System Gold Programmable Solvent Module 126 with Phenomenex Kinetex C-18-HPLC column, length 250 × 4.6 mm, pore size 100 Å, particle size 5 μm. Chiral HPLC: Beckman System Gold Programmable Solvent Module 125 with Phenomenex Lux Amylose-1-HPLC column, length 100 × 4.6 mm, pore size 100 Å, particle size 5 μm. Detection in either case: Beckman Instruments Diode Array Detection Module 168. Programs used for the separation of spiroscytalin diastereomers: flow rate 0.7 mL/min. Entries A−C: 77% MeOH, 23% H_2O (10 min) then 80% MeOH, 20% H_2O, R_t = 16.6 min (F1)/17.9 min (F2). Entry D: 55% MeCN, 45% H_2O (25 min) then 70% MeCN, 30% H_2O, R_t = 26.1 min (F1)/29.3 min (F2).

Preparative HPLC: Knauer WellChrom K-1800 with Phenomenex Kinetex C-18-HPLC-column, length 200 × 21.1 mm, pore size 100 Å, particle size 5 μm. Detection: Knauer WellChrom UV-detector K-2600. Programs used for purification of spiroscytalin diastereomers: flow rate 14.95 mL/min. Entries A−C: 77% MeOH, 23% H_2O (10 min) then 80% MeOH, 20% H_2O, R_t = 17.3 min (F1)/18.3 min (F2). Entry D: 45% MeCN, 55% H_2O (2 min) then 55% MeCN, 45% H_2O (22 min) then 70% MeCN, 30% H_2O, R_t = 27.0 min (F1)/29.5 min (F2).

(*S*,6*E*,8*E*)-2-Methyl-deca-6,8-dienal (*S*)-7. Hydrazone (*R*,*S*)-**8** (1.55 g, 5.6 mmol) was treated with an excess of methyl iodide and stirred at 60 °C for 14 h. The salt obtained upon evaporation was dissolved and hydrolyzed in a two-phase system (3 N HCl, *n*-pentane) by vigorous stirring for 30 min.[8] After separation of the phases and extraction of the aqueous one with *n*-pentane, the combined organic layers were dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography to give aldehyde (*S*)-**7** (0.48 g, 2.9 mmol, 52%) as a colorless oil: R_f = 0.76 (cyclohexane/ethyl acetate 3:1). $[\alpha]_D^{25}$ +15 (*c* 1.0, CH_2Cl_2); IR (ATR) ν_{max} 3018, 2960, 2926, 2856, 1727, 1493, 1457, 1377, 1363, 1261, 1188, 1081, 1020, 987, 967, 803, 716 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.61 (d, *J* = 1.9 Hz, 1 H), 5.97−6.05 (m, 2 H), 5.51−5.61 (m, 2 H), 2.30−2.38 (m, 1 H), 2.08 (q, *J* = 6.9 Hz, 2 H), 1.73 (d, *J* = 6.4 Hz, 3 H), 1.64−1.69 (m, 2 H), 1.37−1.45 (m, 3 H), 1.09 (d, *J* = 7.0 Hz, 3 H); HRMS (ESI) *m*/*z* [M + H]$^+$ calcd for $C_{11}H_{19}O^+$ 167.14304, found 167.14295.

(*R*,6*E*,8*E*)-2-Methyl-deca-6,8-dienal (*R*)-7. (*R*)-**7** (0.50 g, 3.0 mmol, 54%) was obtained analogously to its enantiomer from hydrazone (*S*,*R*)-**8** (1.55 g) as a colorless oil: $[\alpha]_D^{25}$ −15 (*c* 1.0, CH_2Cl_2); IR (ATR) ν_{max} 3018, 2960, 2926, 2856, 1727, 1493, 1457, 1377, 1363, 1261, 1188, 1081, 1020, 987, 967, 803, 716 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 9.60 (d, *J* = 1.9 Hz, 1 H), 5.92−6.07 (m, 2 H), 5.47−5.62 (m, 2 H), 2.32 (sxd, *J* = 6.8, 1.9 Hz, 1 H), 2.07 (q, *J* = 7.0 Hz, 2 H), 1.72 (d, *J* = 6.4 Hz, 3 H), 1.63−1.71 (m, 2 H), 1.31−1.46 (m, 3 H), 1.08 (d, *J* = 7.0 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 205.1, 131.4, 131.0, 130.8, 127.2, 46.2, 32.4, 29.9, 26.7, 18.0, 13.3; HRMS (ESI) *m*/*z* [M + H]$^+$ calcd for $C_{11}H_{19}O^+$ 167.1430, found 167.1429.

(1*E*,2*S*,6*E*,8*E*)-*N*-((*R*)-2-(Methoxymethyl)pyrrolidin-1-yl)-2-methyldeca-6,8-dien-1-imine (*R*,*S*)-8. RAMP **9** (1.0 g, 7.7 mmol) was treated dropwise with deca-6,8-dienal[3] **10** (1.29 g, 7.7 mmol) while stirring at 0 °C. After 2 h, the crude product was dissolved in CH_2Cl_2, and the resulting solution was dried over Na_2SO_4, concentrated *in vacuo*, and finally purified by column chromatography (silica gel, cyclohexane/ethyl acetate 3:1; R_f = 0.49) to give hydrazone (6*E*,8*E*)-*N*-((*R*)-2-(methoxymethyl)pyrrolidin-1-yl)deca-6,8-dien-1-imine (*R*)-**11** (1.68 g, 6.35 mmol, 83%) as a colorless oil. It was dissolved in dry THF (15 mL), and the resulting solution was added to 20 mL of a cooled (0 °C) solution of LDA[7] in THF, prepared from diisopropylamine (1.07 mL, 7.62 mmol) and 2.91 mL of BuLi (2.4 M in hexane). After stirring for 1 h at 0 °C and 14 h at −18 °C, the mixture was cooled to −100 °C and treated with a solution of MeI (0.51 mL, 8.3 mmol) in THF (10 mL). The mixture was stirred at this temperature for 3 h, allowed to warm to rt, and partitioned between ether and water (20 mL each). The organic layer was separated, and the aqueous one was extracted with ether. The combined organic fractions were collected, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford the α-alkylated hydrazine (*R*,*S*)-**8** after column chromatography (silica gel, cyclohexane/ethyl acetate 3:1) as a colorless oil (1.61 g, 5.8 mmol, 91%): R_f = 0.62; $[\alpha]_D^{25}$ +100 (*c* 1.0, MeOH); IR (ATR) ν_{max} 2925, 1604, 1458, 1377, 1340, 1302, 1196, 1118, 986, 926, 904, 737, 668, 607; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.45 (d, *J* = 6.6 Hz, 1 H), 5.90−6.06 (m, 2 H), 5.53 (dqui, *J* = 13.1, 6.5 Hz, 2 H), 3.55 (dd, *J* = 8.8, 3.3 Hz, 1 H), 3.38 (dt, *J* = 8.8, 7.0 Hz, 1 H), 3.35 (s, 3 H), 3.26−3.38 (m, 2 H), 2.69 (q, *J* = 8.3 Hz, 1 H), 2.28 (qd, *J* = 6.9, 6.6 Hz, 1 H), 2.03 (q, *J* = 6.6 Hz, 2 H), 1.74−1.95 (m, 4 H), 1.70 (d, *J* = 6.6 Hz, 3 H), 1.31−1.45 (m, 4 H), 1.01 (d, *J* = 6.9 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 144.1, 131.8, 131.7, 130.3, 126.6, 74.7, 63.4, 59.1, 50.3, 36.9, 35.1, 32.5, 26.9, 26.5, 22.0, 19.0, 17.9; HRMS (ESI) *m*/*z* [M + H]$^+$ calcd for $C_{17}H_{31}N_2O^+$ 279.2431, found 279.2421.

(1*E*,2*R*,6*E*,8*E*)-*N*-((*S*)-2-(Methoxymethyl)pyrrolidin-1-yl)-2-methyldeca-6,8-dien-1-imine (*S*,*R*)-8. (*S*,*R*)-**8** (1.60 g, 5.8 mmol, 91%) was prepared analogously to its enantiomer from SAMP (1.0 g, 7.7 mmol), aldehyde **10** (1.29 g, 7.7 mmol), and methyl iodide (0.51 mL, 8.3 mmol): R_f = 0.62; $[\alpha]_D^{25}$ −100 (*c* 1.0, MeOH); IR (ATR) ν_{max} 2925, 1604, 1458, 1377, 1340, 1302, 1196, 1118, 986, 926, 904, 737, 668, 607; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.45 (d, *J* = 6.6 Hz, 1 H), 5.90−6.05 (m, 2 H), 5.53 (dqui, *J* = 13.1, 6.5 Hz, 2 H), 3.54 (dd, *J* = 8.8, 3.3 Hz, 1 H), 3.38 (dt, *J* = 8.8, 7.0 Hz, 1 H), 3.34 (s, 3 H), 3.26−3.38 (m, 2 H), 2.69 (q, *J* = 8.5 Hz, 1 H), 2.28 (qd, *J* = 6.9, 6.6 Hz, 1 H), 2.02 (q, *J* = 6.3 Hz, 2 H), 1.84−1.96 (m, 4 H), 1.70 (d, *J* = 6.6 Hz, 3 H), 1.34−1.44 (m, 4 H), 1.00 (d, *J* = 6.9 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 144.0, 131.8, 131.7, 130.3, 126.5, 74.7, 63.4, 59.1, 50.3, 36.9, 35.0, 32.5, 26.9, 26.5, 22.0, 19.0, 17.9; HRMS (ESI) *m*/*z* [M + H]$^+$ calcd for $C_{17}H_{31}N_2O^+$ 279.2431, found 279.2424.

D-*cis*-*exo*-Spiroscytalin 12. *Method A.* A solution of (*R*)-5-benzyl-1-*t*-butoxycarbonylpyrrolidine-2,4-dione (*R*)-**5a** (145 mg, 0.5 mmol) in $CHCl_3$ (2 mL) was treated with a 3 Å molecular sieve, pyridine (12 μL, 0.15 mmol), and aldehyde (*S*)-**7** (76 mg, 0.5 mmol). The mixture was stirred at rt for 2 h and then cooled to 0 °C. Me_2AlCl (200 μL, 0.2 mmol, 1 M in hexane) was added, and the resulting mixture was left to stir for 14 h at the same temperature.

Method B. A solution of (*R*)-**5a** (145 mg, 0.5 mmol) in MeOH (2 mL) was treated with a 3 Å molecular sieve, L-proline[10] (12 mg, 0.10 mmol), and aldehyde (*S*)-**7** (76 mg, 0.5 mmol). The mixture was stirred at rt for 2 h and then heated to reflux for 30 min before it was left to stand for 14 h at rt.

Method C. A solution of (*R*)-**5a** (145 mg, 0.5 mmol) in $CHCl_3$ (2 mL) was treated with a 3 Å molecular sieve, pyridine (12 μL, 0.15 mmol), and aldehyde (*S*)-**7** (76 mg, 0.5 mmol). The mixture was stirred at rt for 2 h and then cooled to 0 °C. $ZnCl_2$ (27 mg, 0.2 mmol) was added, and the resulting mixture was left to stir for 14 h at the same temperature.

Uniform Workup. The mixture was diluted with CH_2Cl_2 and washed with water. The aqueous phase was re-extracted with CH_2Cl_2, and the combined organic layers were dried over Na_2SO_4 and evaporated. The crude remainder was dissolved in 15% TFA in CH_2Cl_2 (4 mL) and stirred for 30 min at rt. The volatiles were removed under reduced pressure, and the residue obtained was purified by column chromatography on silica gel to give a 2:1 diastereomeric mixture of **13**:**12** (R_f = 0.30; cyclohexane/ethyl acetate

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3:1) as a colorless oil (methods A and B: 0.08 g, 0.24 mmol, 48%. Method C: 0.05 g, 0.15 mmol, 30%). This mixture was quantitatively separated by preparative HPLC to give isomer **13** (methods A and B: 53 mg, 32%. Method C: 33 mg, 20%) and isomer **12** (methods A and B: 27 mg, 16%. Method C: 17 mg, 10%).

Data of Isomer 12: $[\alpha]_D^{25}$ +70 (*c* 1.0, MeOH); IR (ATR) ν_{max} 3204 (br), 3089, 3029, 2923, 2850, 1764, 1690, 1605, 1498, 1451, 1377, 1339, 1288, 1260, 1207, 1148, 1090, 1030, 903, 876, 799, 749, 731, 698, 617, 587, 562; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.27−7.38 (m, 3 H), 7.18−7.22 (m, 2 H), 6.09 (br s, 1 H), 5.76 (ddd, *J* = 10.1, 3.8, 3.0 Hz, 1 H), 5.37 (dt, *J* = 10.1, 2.0 Hz, 1 H), 3.96 (ddd, *J* = 10.9, 3.3, 1.1 Hz, 1 H), 3.32 (dd, *J* = 13.7, 3.3 Hz, 1 H), 2.64 (dd, *J* = 13.7, 10.9 Hz, 1 H), 2.54−2.62 (m, 1 H), 2.41−2.52 (m, 1 H), 2.23 (d, *J* = 6.3 Hz, 1 H), 1.97−2.12 (m, 1 H), 1.45−1.62 (m, 6 H), 1.35 (d, *J* = 11.3 Hz, 1 H), 1.05 (d, *J* = 7.1 Hz, 3 H), 0.91 (d, *J* = 7.4 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 215.9, 175.0, 136.2, 132.0, 129.2 (×2), 129.1 (×2), 127.4, 126.9, 64.4, 58.8, 45.0, 38.9, 38.1, 31.7, 31.4, 27.6, 27.3, 20.8, 19.1, 16.4; HRMS (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{22}H_{28}NO_2^+$ 338.2115, found 338.2109.

D-*trans*-Spiroscytalin 13. **13** was prepared alongside isomer **12** by methods A−C (cf. protocols for compound **12**): $[\alpha]_D^{25}$ +60 (*c* 1.0, MeOH); IR (ATR) ν_{max} 3204 (br), 3089, 3029, 2923, 2850, 1764, 1690, 1605, 1498, 1451, 1377, 1339, 1288, 1260, 1207, 1148, 1090, 1030, 903, 876, 799, 749, 731, 698, 617, 587, 562; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.27−7.36 (m, 3 H), 7.18−7.23 (m, 2 H), 6.10 (br, s, 1 H), 5.73 (dt, *J* = 9.5, 2.3 Hz, 1 H), 5.50 (ddd, *J* = 9.5, 4.1, 3.0 Hz, 1 H), 4.01 (dd, *J* = 10.9, 3.4 Hz, 1 H), 3.44 (dd, *J* = 14.0, 3.4 Hz, 1 H), 2.63 (dd, *J* = 14.0, 10.9 Hz, 1 H), 2.52 (qdd, *J* = 7.4, 4.1, 2.3 Hz, 1 H), 2.32 (ddddd, *J* = 12.7, 10.4, 3.3, 3.0, 2.3 Hz, 1 H), 1.96 (dtt, *J* = 12.7, 3.3, 2.3 Hz, 1 H), 1.81 (ddqd, *J* = 11.5, 10.4, 6.6, 3.8 Hz, 1 H), 1.67−1.74 (m, 2 H), 1.51 (t, *J* = 10.4 Hz, 1 H), 1.44 (dt, *J* = 12.7, 3.8 Hz, 1 H), 1.22 (td, *J* = 12.7, 3.6 Hz, 1 H), 1.09−1.15 (m, 1 H), 1.04 (d, *J* = 7.4 Hz, 3 H), 0.80 (d, *J* = 6.6 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 211.4, 175.9, 136.8, 135.4, 129.2 (×2), 128.7 (×2), 127.9, 127.3, 63.6, 58.0, 49.0, 38.5, 37.9, 37.6, 36.9, 34.7, 33.4, 25.8, 23.4, 17.6; HRMS (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{22}H_{28}NO_2^+$ 338.2115, found 338.2113.

D-*cis-endo*-Spiroscytalin 1. *Method D.* A solution of (*R*)-5-benzyl-pyrrolidine-2,4-dione (*R*)-**5b** (95 mg, 0.5 mmol) in $CHCl_3$ (2 mL) was treated with a 3 Å molecular sieve, pyridine (12 μL, 0.15 mmol), and aldehyde (S)-7 (76 mg, 0.5 mmol). The mixture was stirred at rt for 2 h and then cooled to 0 °C. Me_2AlCl (200 μL, 0.2 mmol, 1 M in hexane) was added, and the resulting mixture was left to stir for 14 h at the same temperature. Then it was diluted with CH_2Cl_2 and washed with water. The aqueous phase was re-extracted with CH_2Cl_2, and the combined organic layers were dried over Na_2SO_4 and evaporated. The crude remainder was purified by column chromatography on silica gel to give a 1.5:1 diastereomeric mixture of **1**:**13** (R_f = 0.30; cyclohexane/ethyl acetate 3:1) as a colorless oil (0.06 g, 0.18 mmol, 36%). This mixture was quantitatively separated by preparative HPLC to afford isomer **1** (37 mg, 22%) and isomer **13** (23 mg, 14%). Data of isomer **1**: $[\alpha]_D^{25}$ +21 (*c* 1.0, MeOH); IR (ATR) ν_{max} 3203 (br), 3018, 2961, 2924, 2852, 1761, 1694, 1605, 1497, 1453, 1375, 1287, 1260, 1229, 1091, 1029, 942, 867, 798, 753, 721, 700, 661, 619, 584, 563; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.16−7.42 (m, 5 H), 6.15 (br, s, 1 H), 5.77 (dt, *J* = 10.0, 3.3 Hz, 1 H), 5.35 (dt, *J* = 10.0, 2.0 Hz, 1 H), 3.78 (dd, *J* = 11.4, 3.2 Hz, 1 H), 3.36 (dd, *J* = 13.6, 3.2 Hz, 1 H), 2.74 (qddd, *J* = 7.4, 3.3, 2.7, 2.0 Hz, 1 H), 2.51 (dd, *J* = 13.6, 11.4 Hz, 1 H), 2.42−2.50 (m, 1 H), 2.29 (d, *J* = 6.9 Hz, 1 H), 1.91 (qdd, *J* = 7.1, 3.8, 2.7 Hz, 1 H), 1.69−1.83 (m, 1 H), 1.57−1.65 (m, 1 H), 1.45−1.56 (m, 2 H), 1.27−1.42 (m, 2 H), 1.13 (d, *J* = 7.1 Hz, 3 H), 0.91 (d, *J* = 7.4 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 212.1, 177.0, 136.5, 132.4, 129.2 (×2), 128.9 (×2), 127.4, 126.1, 64.1, 58.9, 43.7, 38.4, 37.8, 31.6, 30.1, 28.4, 27.5, 21.3, 19.5, 16.1; HRMS (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{22}H_{28}NO_2^+$ 338.2115, found 338.2108.

Natural (3R,5S,6S,7R,11S,14R)-Spiroscytalin ent-1. *ent*-**1** was prepared analogously to its entantiomer **1** from (*S*)-**5b** (95 mg, 0.5 mmol) and aldehyde (*R*)-7 (76 mg, 0.5 mmol): yield 49 mg (30%) alongside a separable *trans*-isomer (25 mg, 15%); $[\alpha]_D^{25}$ −21 (*c* 1.0, MeOH); IR (ATR) ν_{max} 3208 (br), 3018, 2961, 2924, 2871, 1760, 1694, 1605, 1497, 1455, 1376, 1288, 1261, 1229, 1091, 1029, 942, 909, 796, 728, 700, 647, 620, 582, 560; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28−7.40 (m, 3 H), 7.20−7.25 (m, 2 H), 6.11 (br, s, 1 H), 5.77 (dt, *J* = 10.0, 3.3 Hz, 1 H), 5.35 (dt, *J* = 10.0, 2.0 Hz, 1 H), 3.78 (ddd, *J* = 11.5, 3.0, 0.6 Hz, 1 H), 3.36 (dd, *J* = 13.4, 3.0 Hz, 1 H), 2.75 (qddd, *J* = 7.4, 3.3, 2.7, 2.0 Hz, 1 H), 2.51 (dd, *J* = 13.4, 11.5 Hz, 1 H), 2.42−2.50 (m, 1 H), 2.29 (d, *J* = 6.6 Hz, 1 H), 1.91 (qdd, *J* = 7.1, 3.8, 2.7 Hz, 1 H), 1.69−1.83 (m, 1 H), 1.54−1.58 (m, 1 H), 1.46−1.53 (m, 2 H), 1.29−1.39 (m, 2 H), 1.13 (d, *J* = 7.1 Hz, 3 H), 0.91 (d, *J* = 7.4 Hz, 3 H); ^{13}C NMR ($CDCl_3$, 75.5 MHz) δ 212.1, 177.0, 136.5, 132.4, 129.2 (×2), 128.9, 128.9, 127.4, 126.1, 64.1, 58.9, 43.7, 38.4, 37.8, 31.6, 30.1, 28.4, 27.5, 21.3, 19.5, 16.1; HRMS (ESI) *m*/*z* $[M + H]^+$ calcd for $C_{22}H_{28}NO_2^+$ 338.2115, found 338.2112.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.7b00727.

^{1}H and ^{13}C NMR spectra and HPLC chromatograms of **1**, *ent*-**1**, (*S*)-**7**, (*R*,*S*)-**8**, (*S*,*R*)-**8**, **12**, and **13**; ^{1}H shifts of isolated natural spiroscytalin and synthetic **1** and *ent*-**1**; tests of *ent*-**1** and **12** for antimicrobial activity (PDF)

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Notes

The authors declare no competing financial interest.

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