A Modular Approach to the Antifungal Sphingofungin Family: Concise Total Synthesis of Sphingofungin A and C

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Abbreviations

C18: octadecyl carbon chain bonded silica
CITU: 1,1,3,3-tetramethyl-2-(4,5,6,7-tetrachloro-1,3-dioxoisooindolin-2-yl)isouronium-hexafluorophosphate(V)
DCM: dichloromethane
DIPEA: N,N-diisopropylethylamine
DMF: dimethylformamide
EtOAc: ethyl acetate
IPA: isopropanol
MeCN: acetonitrile
MeOH: methanol
MW: microwave
NMP: N-methyl-2-pyrrolidone
PPTS: pyridinium p-toluenesulfonate
TBME: tert-butyl methyl ether
TFA: trifluoroacetic acid
THF: tetrahydrofuran
TIPS: triisopropylsilane
TMSOTf: trimethylsilyl trifluoromethanesulfonate
1. General Information

NMR measurements were performed on a Bruker AVANCE II 300 MHz, a Bruker AVANCE III 500 MHz and a Bruker AVANCE III 600 MHz spectrometer, equipped with a Bruker Cryoplateform. The chemical shifts are reported in parts per million (ppm) relative to the solvent residual peak of $^{13}$C$_6$D$_6$ ($^{1}$H: 7.16 ppm, $^{13}$C: 128.06 ppm), DMSO-$d_6$ ($^{1}$H: 2.50 ppm, quintet; $^{13}$C: 39.52 ppm, septet), CDCl$_3$ ($^{1}$H: 7.26 ppm, singlet; $^{13}$C: 77.16 ppm, triplet) and MeOD-$d_4$ ($^{1}$H: 3.31 ppm, quintet; $^{13}$C: 49.00 ppm, septet). LC-ESI-HRMS measurements were carried out on an Accela UPLC system (Thermo Scientific) coupled with a Kinetex Phenyl-Hexyl column (50 x 2.1 mm, particle size 1.7 µm) combined with a Q-Exactive mass spectrometer (Thermo Scientific) equipped with an electrospray ion (ESI) source. UHPLC-MS measurements were performed on a Shimadzu LCMS-2020 system equipped with single quadrupole mass spectrometer using a Phenomenex Kinetex C18 column (50 x 2.1 mm, particle size 1.7 µm, pore diameter 100 Å). Column oven was set to 40 °C; scan range of MS was set to m/z 150 to 2,000 with a scan speed of 10,000 u/s and event time of 0.25 s under positive and negative mode. DL temperature was set to 250 °C with an interface temperature of 350 °C and a heat block of 400 °C. The nebulizing gas flow was set to 1.5 L/min and dry gas flow to 15 L/min. Semi-preparative HPLC was performed on a Shimadzu HPLC system using a Phenomenex Luna C18(2) (250 x 30 mm, particle size 5 µm, pore diameter 100 Å) column and Phenomenex Luna Phenyl-Hexyl (250 x 21.2 mm, particle size 5 µm, pore diameter 100 Å) column. Flash chromatography was performed on a Biotage Isolera Prime. IR spectra were recorded on an FT/IR-4100 ATR spectrometer (JASCO). Optical rotations were recorded on a P-1020 polarimeter (JASCO).

Chemicals

All reagents and solvents for synthesis were purchased from Acros Organics, Alfa Aesar, Carl Roth, Sigma Aldrich, TCI, Th. Geyer, Strem Chemicals and VWR and used without further purification. Unless otherwise stated standard Schlenk techniques were used. (R)-pentadec-14-en-7-ol (8a)$^{[1]}$, tert-butyl glyoxylate (9)$^{[2]}$, both enantiomers of 2,4,6-trimethylbenzenesulfonamide ((R)-10 and (S)-10)$^{[3]}$, Weinreb amide 11$^{[4]}$ and tetrachloro-$N$-hydroxyphthalimide tetramethyluronium hexafluorophosphate (CITU)$^{[5]}$ were prepared according to the literature. Nickel catalysts were purchased from Sigma Aldrich or Strem Chemicals. Unless otherwise stated, all anhydrous solvents were purchased from Acros Organics.
2. Synthesis

General Procedure 1 (GP1) for the preparation of sulfinyl imines \((R)-7\) and \((S)-7\)

Sulfinyl imines 7 were prepared according to a modified literature procedure.\(^6\) A heat gun-dried flask charged with sulfinamide 10 (1.0 eq) and molecular sieves (1.0 g/mmol, 4 Å) was evacuated and backfilled with argon. This process was repeated (3x). Anhydrous DCM (0.3 M) was added followed by freshly prepared tert-butyl glyoxylate 9 (1.4 eq). The reaction was quickly heated to 50 °C to depolymerize tert-butyl glyoxylate. Next, pyrrolidine (0.1 eq) was added and the mixture was stirred at r.t. overnight. The reaction was filtered over a pad of Celite and the filtrate was evaporated. The crude product was purified by flash chromatography over a silica gel column (eluant: cyclohexane/EtOAc 100:0 to 80:20 to 0:100). The appropriate fractions were collected and evaporated to afford the corresponding sulfinyl imine 7.

General Procedure 2 (GP2) for the decarboxylative coupling

The decarboxylative coupling was performed according to a modified literature procedure.\(^6\) A heat gun-dried flask was charged with carboxylic acid 13 (1.0 eq) and anhydrous NMP (0.4 M) under argon atmosphere. To the resulting solution were subsequently added CITU (1.1 eq), a solution of the corresponding sulfinyl imine 7 (1.1 eq) in anhydrous NMP (0.4 M), Ni(OAc)\(_2\)·4H\(_2\)O (0.25 eq) and Zinc dust (3.0 eq) followed immediately by N-methylmorpholine (2.2 eq). The resulting mixture was stirred at r.t. for 1.5 h. The reaction mixture was diluted with EtOAc and poured on 10% aq. citric acid solution. The aq. layer was removed and extracted with EtOAc (2x). The org. layers were combined, washed with brine, dried over Na\(_2\)SO\(_4\), filtered and evaporated. The crude product was adsorbed on silica and purified by flash chromatography over a silica column (eluant: cyclohexane/(TBME/IPA 10:1) 100:0 to 50:50). The appropriate fractions were combined and evaporated to afford a yellowish solid. To remove the remaining tetrachlorophthalimide impurity, the solid was suspended in DCM and filtered over a cotton pad. The pad was rinsed with DCM and the combined filtrate was evaporated to afford a yellowish solid. This solid was adsorbed on silica and purified a second time by flash chromatography under the same conditions. The appropriate fractions were collected evaporated to afford the pure, corresponding protected amino ester.
General Procedure 3 (GP3) for the cross metathesis

To a solution of (2S)-protected amino ester (2S)-14 (1.0 eq) and corresponding alkene (8a-8d) (1.1 eq or 5.0 eq) in anhydrous DCM (0.1 M) under argon atmosphere was added copper iodide (0.4 eq). The resulting suspension was heated to 40 °C and then Grubbs 2nd generation catalyst (0.2 eq) was added in 5 portions every 2 h. Upon completed addition, the resulting mixture was stirred at 40 °C overnight. Volumes above 2.0 mL were concentrated under vacuum. Otherwise, the reaction was directly purified by flash chromatography over a silica gel column (eluant: cyclohexane/EtOAc). The appropriate fractions were collected and evaporated to afford the corresponding protected sphingofungin derivative. Frequently, (2S)-protected amino ester (starting material) (2S)-14 was recovered as well.

General procedure 4 (GP4) for the global deprotection

A culture tube or vial charged with the corresponding protected sphingofungin derivative (1.0 eq) was evacuated and backfilled with argon. Next, anhydrous DCM (0.03 M) was added and the resulting solution was cooled to -78 °C. At this temperature, BCl₃ (4.0 eq, 1.0 M solution in DCM) was added dropwise and the reaction was stirred at -78 °C for 30 min. After this time, the reaction was added to a vigorously stirred ice cold mixture of MeOH (50x volume of added BCl₃ solution) and H₂O (5x volume of added BCl₃ solution). The resulting solution was concentrated to in vacuo to 1/10 of the volume and then diluted with H₂O. The resulting mixture was pre-purified by flash chromatography over a C18 column (eluant: H₂O/MeCN + 0.1% formic acid 95:5 to 0:100). The appropriate fractions were collected evaporated to afford a colorless solid. The product was further purified by preparative HPLC over a Phenyl-Hexyl-column (Phenomenex Luna, 5 µm, 250 x 21.2 mm, 100 Å) (eluant: H₂O/MeCN + 0.1% formic acid). The appropriate fractions were collected evaporated and lyophilized to afford the corresponding sphingofungin C derivative as a colorless solid.
(R)-sulfanyl imine [(R)-7]

(R)-sulfanyl imine (R)-7 was synthesized according to GP1 using (R)-2,4,6-trimethylbenzenesulfinamide (R)-10 (1.0 g, 5.46 mmol) to afford (1.3 g, 4.56 mmol, 84% yield) a colorless solid.

\[ \text{1H-NMR (600 MHz, CDCl}_3\text{): } \delta 8.15 (s, 1H, 2-H), 6.86 (s, 2H, 2x 5-H), 2.45 (s, 6H, 2x 4-Me), 2.28 (s, 3H, 6-Me), 1.55 (s, 9H, -O}t\text{Bu) ppm.} \]

\[ \text{13C-NMR (150 MHz, CDCl}_3\text{): } \delta 160.6 (s, C-1), 155.5 (d, C-2), 142.5, 138.9, 133.4 (3s, C-6, 2x C-4, C-3), 131.1 (d, 2x C-5), 84.2 (s, -OtBu), 28.1, 21.3, 19.0 (3q, -OtBu, 6-Me, 2x 4-Me) ppm.} \]

\[ \text{HRMS (ESI, m/z): calc'd for C}_{15}\text{H}_{22}\text{NO}_3\text{S [M+H]}^+ 296.1315; found 296.1305.} \]

\[ \text{IR (ATR) } \nu_{\text{max}}: 2979, 2930, 1718, 1596 \text{ cm}^{-1}.} \]

\[ \text{OR } [\alpha]_D^{21} = -236 (c 1.6, CHCl}_3\text{).} \]

(S)-sulfanyl imine [(S)-7]

(S)-sulfanyl imine (S)-7 was synthesized according to GP1 using (S)-2,4,6-trimethylbenzenesulfinamide (S)-10 (900 mg, 4.91 mmol) to afford (1.2 g, 4.21 mmol, 86% yield) a colorless solid.

\[ \text{1H-NMR (500 MHz, CDCl}_3\text{): } \delta 8.15 (s, 1H, 2-H), 6.86 (s, 2H, 2x 5-H), 2.46 (s, 6H, 2x 4-Me), 2.28 (s, 3H, 6-Me), 1.55 (s, 9H, -O}t\text{Bu) ppm.} \]

\[ \text{13C-NMR (125 MHz, CDCl}_3\text{): } \delta 160.6 (s, C-1), 155.5 (d, C-2), 142.5, 138.9, 133.4 (3s, C-6, 2x C-4, C-3), 131.1 (d, 2x C-5), 84.2 (s, -OtBu), 28.1, 21.2, 19.0 (3q, -OtBu, 6-Me, 2x 4-Me) ppm.} \]

\[ \text{HRMS (ESI, m/z): calc'd for C}_{15}\text{H}_{22}\text{NO}_3\text{S [M+H]}^+ 296.1315; found 296.1304.} \]

\[ \text{IR (ATR) } \nu_{\text{max}}: 2979, 2926, 1718, 1596 \text{ cm}^{-1}.} \]

\[ \text{OR } [\alpha]_D^{21} = +234 (c 1.8, CHCl}_3\text{).} \]
Allyl alcohol (12)

Allyl alcohol 12 was prepared according to a modified literature procedure. A heat gun dried flask was charged with literature known Weinreb amide 11 (1.0 g, 3.62 mmol) and anhydrous THF (20.0 mL) under argon atmosphere. The resulting solution was cooled to -78 °C. Next, a solution of vinylmagnesium bromide (12.0 mL, 5.43 mmol, 0.5 M in THF) and anhydrous THF (3.4 mL) was added dropwise at -78 °C to the solution over 5 min. Upon completed addition, the reaction was stirred at -78 °C for 45 min. The reaction was poured on a vigorously stirred, ice-cold mixture of 1.0 M HCl and TBME (1:1). The aq. layer was extracted with EtOAc (2x). The org. layers were washed with brine, combined, dried over Na₂SO₄, filtered and evaporated to afford the ketone intermediate as yellowish oil.

In a separate flask, to an ice-cold solution of CeCl₃·7H₂O (4.6 g, 12.3 mmol) in anhydrous MeOH (28.0 mL) was added NaBH₄ (521 mg, 13.8 mmol) in portions. The resulting suspension was stirred at 0 °C for 10 min. After this time, the suspension was added dropwise over 10 min via serological pipette to a flask charged with a solution of the ketone intermediate in anhydrous MeOH (28.0 mL) at -78 °C. The resulting mixture was stirred at -78 °C for 1 h. The reaction was quenched by dropwise adding H₂O (10.0 mL) at -78 °C. The resulting mixture was allowed to reach r.t. and extracted with EtOAc (3x). The org. layers were washed with brine, combined, dried over Na₂SO₄, filtered and evaporated. The crude product was purified by flash chromatography over a silica gel column (eluant: cyclohexane/EtOAc 70:30 to 40:60). The appropriate fractions were collected and evaporated to afford allyl alcohol 12 (538 mg, 2.19 mmol, 61% yield over two steps, dr >20:1) as a colorless oil.

**¹H-NMR (600 MHz, CDCl₃):** δ 5.93 (ddd, J = 17.2, 10.5, 5.5 Hz, 1H, 5-H), 5.39 (br. d, J = 17.2 Hz, 1H, 6-Hₐ), 5.25 (br. d, J = 10.5 Hz, 1H, 6-Hₐ), 4.76 (m, 1H, 2-H), 4.49 (m, 1H, 3-H), 4.25-4.22 (m, 1H, 4-H), 3.74 (s, 3H, -OMe), 3.23 (s, 3H, -N(Me)-), 2.31 (br. s, 1H, -OH), 1.49 (s, 3H, 7-Me), 1.46 (s, 3H, 7-Me) ppm.

**¹³C-NMR (150 MHz, CDCl₃):** δ 170.5 (s, C-1), 137.1 (d, C-5), 117.0 (t, C-6), 111.6 (s, C-7), 80.7, 73.9, 72.0 (3d, C-3, C-2, C-4), 61.9, 32.6, 27.3, 26.3 (4q, -OMe, -N(Me)-, 2x 7-Me) ppm.

**HRMS (ESI, m/z):** calc’d for C₁₁H₂₀NO₅ [M+H]⁺ 246.1336; found 246.1326.

**IR (ATR) νmax:** 3442, 3084, 2986, 2938, 1659 cm⁻¹.

**OR [α]D²⁰:** -2.2 (c 1.2, CHCl₃).
Carboxylic acid (13)

To a solution of allyl alcohol 12 (430 mg, 1.75 mmol) in THF (15.0 mL) and H₂O (2.9 mL) was added LiOH (63.0 mg, 2.63 mmol). The reaction was stirred at r.t. for 30 min. After this time, the reaction was poured on 0.5 M aq. HCl and the resulting mixture was extracted with EtOAc (3x). The org. layers were washed with brine, combined, dried over MgSO₄, filtered and evaporated.

The residue was dissolved in anhydrous pyridine (35.0 mL) and cooled to 0 °C. At this temperature, acetic anhydride (2.5 mL, 2.7 g, 29.3 mmol) was added dropwise and the reaction was stirred at r.t. for 30 min. Next, the solution was diluted with cyclohexane (150 mL) and the mixture was evaporated to azeotropically remove pyridine. This step was repeated twice to remove most of the pyridine. The resulting residue was dissolved in EtOAc and 0.5 M aq. HCl and extracted with EtOAc (2x). The combined org. layers were washed with H₂O and brine, dried over Na₂SO₄, filtered and evaporated. The crude product was purified by flash chromatography over a silica gel column (eluant: cyclohexane/EtOAc + 0.1% formic acid 80:20 to 0:100). The appropriate fractions were collected and evaporated to afford carboxylic acid 13 (424 mg, 1.74 mmol, 99% yield, dr >99:1) as a colorless oil.

¹H-NMR (600 MHz, CDCl₃): δ 5.88 (ddd, J = 17.3, 10.6, 6.3 Hz, 1H, 5-H), 5.50 (br. dd, J = 6.3, 4.8 Hz, 1H, 4-H), 5.44 (br. d, J = 17.3 Hz, 1H, 6-Hₐ), 5.37 (br. d, J = 10.6 Hz, 1H, 6-Hₐ), 4.46 (dd, J = 6.3, 4.8 Hz, 1H, 3-H), 4.41 (d, J = 6.3 Hz, 1H, 2-H), 2.13 (s, 3H, -OAc), 1.50 (s, 3H, 7-Me), 1.43 (s, 3H, 7-Me) ppm.

¹³C-NMR (150 MHz, CDCl₃): δ 174.9, 170.2 (2s, C-1, -OAc), 131.7 (d, C-5), 120.1 (t, C-6), 112.8 (s, C-7), 79.3, 75.0, 73.4 (3d, C-3, C-2, C-4), 26.8, 25.9, 21.2 (3q, 2x 7-Me, -OAc) ppm.

HRMS (ESI, m/z): calc’d for C₁₁H₁₅O₆ [M-H] - 243.0874; found 243.0869.

IR (ATR) νmax: 3174, 3027, 2992, 2937, 1740 cm⁻¹.

OR [α]D²²: + 5.1 (c 2.4, CHCl₃).

SI-10
(2R)-Protected amino ester [(2R)-14]

(2R)-Protected amino ester (2R)-14 was synthesized according to GP2 using carboxylic acid 13 (416 mg, 1.70 mmol) and (R)-sulfinyl imine (R)-7 (554 mg, 1.87 mmol) to afford (439 mg, 0.89 mmol, 52% yield) a yellow solid.

$^1$H-NMR (500 MHz, C$_6$D$_6$): $\delta$ 6.50 (s, 2H, 2x 10-H), 5.97 (ddd, $J$ = 17.2, 10.5, 6.5 Hz, 1H, 6-H), 5.88 (m, 1H, 5-H), 5.57 (d, $J$ = 8.7 Hz, 1H, -NH-), 5.39 (dt, $J$ = 17.2, 1.2 Hz, 1H, 7-H$_A$), 5.15 (dt, $J$ = 10.5, 1.2 Hz, 1H, 7-H$_B$), 4.56 (dd, $J$ = 8.2, 5.7 Hz, 1H, 4-H), 4.49 (dd, $J$ = 8.2, 2.1 Hz, 1H, 3-H), 4.30 (dd, $J$ = 8.7, 2.1 Hz, 1H, 2-H), 2.65 (s, 6H, 2x 9-Me), 1.93 (s, 3H, 11-Me), 1.76 (s, 3H, -OAc), 1.34 (s, 9H, -OtBu), 1.29 (s, 3H, 12-Me), 1.16 (s, 3H, 12-Me) ppm.

$^{13}$C-NMR (125 MHz, C$_6$D$_6$): $\delta$ 169.9, 169.2, 140.6, 138.7, 137.1 (5s, C-1, -OAc, C-11, C-8, 2x C-9), 132.7, 131.2 (2d, C-6, 2x C-10) 119.4 (t, C-7), 110.1, 82.6 (2s, C-12, -OtBu), 78.6, 77.7, 73.9, 57.4 (4d, C-3, C-4, C-5, C-2), 27.9, 27.1, 27.0, 20.8, 20.5, 19.5 (6q, -OtBu, 12-Me, 12-Me, 11-Me, -OAc, 2x 9-Me) ppm.

HRMS (ESI, m/z): calc’d for C$_{25}$H$_{38}$NO$_7$S [M+H]$^+$ 496.2364; found 496.2349.

IR (ATR) $\nu_{\text{max}}$: 3368, 2988, 2966, 2930, 1736, 1712 cm$^{-1}$.

OR [α]$_D^{21}$: -74.9 (c 1.0, CHCl$_3$).
(2S)-Protected amino ester [(2S)-14]

(2S)-Protected amino ester (2S)-14 was synthesized according to GP2 using carboxylic acid 13 (708 mg, 2.90 mmol) and (S)-sulfinyl imine (S)-7 (942 mg, 3.19 mmol) to afford (774 mg, 1.56 mmol, 54% yield) a yellow oil.

$^1$H-NMR (500 MHz, C$_6$D$_6$): δ 6.50 (s, 2H, 2x 10-H), 5.85 (ddd, J = 17.3, 10.5, 6.9 Hz, 1H, 6-H), 5.64-5.62 (m, 1H, 5-H), 5.38 (d, J = 9.9 Hz, 1H, -NH-), 5.24 (dt, J = 17.3, 1.2 Hz, 1H, 7-H$_a$), 5.01 (br. d, J = 10.5 Hz, 1H, 7-H$_b$), 4.48 (dd, J = 6.2, 5.1 Hz, 1H, 4-H), 4.27-4.24 (m, 1H, 3-H), 4.21 (dd, J = 9.9, 6.3 Hz, 1H, 2-H), 2.56 (s, 6H, 2x 9-Me), 1.93 (s, 3H, 11-Me), 1.58 (s, 3H, -OAc), 1.44 (s, 9H, -OtBu), 1.41 (s, 3H, 12-Me), 1.38 (s, 3H, 12-Me) ppm.

$^{13}$C-NMR (125 MHz, C$_6$D$_6$): δ 170.5, 169.5, 140.7, 138.6, 137.1 (5s, C-1, -OAc, C-11, C-8, 2x C-9), 133.3, 131.2 (2d, C-6, 2x C-10), 119.3 (t, C-7), 111.0, 82.6 (2s, C-12, -OtBu), 80.3, 79.2, 74.4, 61.1 (4d, C-4, C-3, C-5, C-2), 28.0, 27.9, 27.4, 20.8, 20.4, 19.4 (6q, -OtBu, 12-Me, 12-Me, 11-Me, -OAc, 2x 9-Me) ppm.

HRMS (ESI, m/z): calc'd for C$_{25}$H$_{38}$NOS [M+H]$^+$ 496.2364; found 496.2350.

IR (ATR) $\nu_{\text{max}}$: 2981, 2932, 1734 cm$^{-1}$.

OR [$\alpha$]$_D^{22}$: +112 (c 1.4, CHCl$_3$).
Protected sphingofungin C (15) was synthesized according to GP3 using (2S)-protected amino ester (2S)-14 (59.0 mg, 0.119 mmol) and literature known (R)-pentadec-14-en-7-ol (8a)\(^{[1]}\) (29.6 mg, 0.131 mmol) to afford protected sphingofungin C 15 (48.0 mg, 0.069 mmol, 58% yield, brsm: 80% yield) as a brown oil and starting material (2S)-14 (16.0 mg, 0.032 mmol, 27% yield) as a brown oil.

\(^1\)H-NMR (500 MHz, C\(_6\)D\(_6\)): \(\delta\) 6.52 (s, 2H, 2x 23-H), 5.81 (dt, \(J = 15.2, 6.7\) Hz, 1H, 7-H), 5.68 (dd, \(J = 8.2, 6.1\) Hz, 1H, 5-H), 5.60 (br. dd, \(J = 15.2, 8.2\) Hz, 1H, 6-H), 5.39 (d, \(J = 10.1\) Hz, 1H, -NH-), 4.58 (t, \(J = 6.1\) Hz, 1H, 4-H), 4.29 (t, \(J = 6.3\) Hz, 1H, 3-H), 4.23 (dd, \(J = 10.1, 6.3\) Hz, 1H, 2-H), 3.47 (m, 1H, 14-H), 2.58 (s, 6H, 2x 22-Me), 1.96 (s, 3H, 24-Me), 1.86-1.82 (m, 2H, 8-H), 1.66 (s, 3H, -OAc), 1.46 (s, 9H, -OtBu), 1.44 (s, 6H, 2x 25-Me), 1.40-1.19 (m, 20H, 9-H - 13-H, 15-H - 19-H), 0.91 (t, \(J = 6.9\) Hz, 3H, 20-H) ppm.

\(^{13}\)C-NMR (125 MHz, C\(_6\)D\(_6\)): \(\delta\) 170.5, 169.6, 140.7, 138.7 (4s, C-1, -OAc, C-24, C-21), 137.9 (d, C-7), 137.1 (s, 2x C-22), 131.2, 125.3 (2d, 2x C-23, C-6), 111.1, 82.6 (2s, C-25, -OtBu), 80.7, 79.8, 75.3, 71.5, 61.3 (5d, C-4, C-3, C-5, C-14, C-2), 38.2, 38.1 (2t, C-13, C-15)*, 32.5, 32.3 (2t, C-8, C-18), 29.9, 29.8, 29.5 (3t, C-10, C-11, C-17)*, 28.9 (t, C-9), 28.0, 27.5 (2q, -OtBu, 2x 25-Me), 26.2, 26.0 (2t, C-12, C-16)*, 23.1 (t, C-19), 20.9, 20.7, 19.4, 14.4 (4q, 24-Me, -OAc, 2x 22-Me, C-20) ppm. *could not be unambiguously assigned

HRMS (ESI, \(m/z\)): calc’d for C\(_{38}\)H\(_{64}\)NO\(_8\)S [M+H]\(^+\) 694.4347; found 694.4329.

IR (ATR) \(\nu_{\text{max}}\): 3429, 2980, 2927, 2855, 1732 cm\(^{-1}\).

OR \([\alpha]_{D}^{22}\): +93.1 (c 1.7, CH\(_2\)Cl\(_2\)).
Sphingofungin C (3)

Sphingofungin C 3 was synthesized according to GP4 using protected sphingofungin C 15 (67.0 mg, 0.097 mmol) to afford (14.0 mg, 0.032 mmol, 34% yield) a colorless solid.

HRMS (ESI, m/z): calc’d for C_{22}H_{42}NO_7 [M+H]^+ 432.2956; found 432.2940.

IR (ATR) \( \nu_{\text{max}} \): 3239, 2923, 2852, 1716, 1615, 1507 cm\(^{-1}\).

OR [\( \alpha \)]\(_{D}^{22} \): +8.5 (c 0.1, MeOH).

Table S1. NMR signal comparison of isolated and synthetic sphingofungin C 3 (MeOD-\( d_4 \)).

| Position | isolated sphingofungin C\(^a\) \(^{1}\) H-NMR\(^a\) \(\delta, m, H (J \text{ in Hz})\) | synthesized sphingofungin C \(^{1}\) H-NMR\(^a\) \(\delta, m, H (J \text{ in Hz})\) | isolated sphingofungin C \(^{13}\) C-NMR\(^b\) \(\delta, m\) | synthesized sphingofungin C \(^{13}\) C-NMR\(^b\) \(\delta, m\) |
|----------|----------------------------------|----------------------------------|----------------|------------------|
| 1        |                                  |                                  | 171.6, s       | 171.6, s         |
| 2        | 3.78, d, 1H (4.8)                | 3.78, d, 1H (4.8)                | 61.1, d        | 61.1, d          |
| 3        | 4.17, dd, 1H (4.8, 1.6)           | 4.17, m, 1H                      | 67.9, d        | 68.0, d          |
| 4        | 3.82, dd, 1H (8.3, 1.6)           | 3.83, m, 1H                      | 74.5, d        | 74.5, d          |
| 5        | 5.40-5.36, m, 2H                  | 5.41-5.37, m, 2H                 | 77.8, d        | 77.8, d          |
| 6        |                                  |                                  | 125.7, d       | 125.8, d         |
| 7        | 5.88, m, 1H                      | 5.88, m, 1H                      | 138.8, d       | 138.8, d         |
| 8        | 2.08-2.04, m, 2H                  | 2.07-2.02, m, 2H                 | 38.44, t       | 38.46            |
| 14       | 3.50, m, 1H                      | 3.50, m, 1H                      | 72.4, d        | 72.4, d          |
| 20       | 0.91, t, 3H (7.0)                | 0.91, t, 3H (7.0)                | 14.4, q        | 14.4, q          |
| -OAc     | 2.05, s, 3H                      | 2.05, s, 3H                      | 172.4, s       | 172.4, s         |
|          |                                  |                                  | 21.3, q        | 21.3, q          |
| 9-13     |                                  |                                  | 38.41, 33.4, 33.0, | 38.43, 33.4, 33.1, |
| & 14     | 1.45-1.29, m, 20H                | 1.45-1.31, m, 20H                | 30.62, 30.55, 30.3, | 30.64, 30.56, 30.3, |
| 15-19     |                                  |                                  | 29.9, 26.79, 26.74, | 29.9, 26.80, 26.75, |
|          |                                  |                                  | 23.7, 10t       | 23.7, 10t        |

\(^a\) 600 MHz; \(^b\) 150 MHz; \(^c\) isolated from Aspergillus sp.
Tested global deprotection conditions

Table S2. Comprehensive list of the tested deprotection conditions to afford sphingofungin C (3). First Brønsted acids were tested to remove all protecting groups, but either only amine deprotection was observed or the product decomposition. A stepwise approach, where the free amine was resubjected to further deprotection, was not successful. Addition of most Lewis acids caused only partial deprotection, except treatment with a BCl₃-solution at -78 °C yielded sphingofungin C with matching spectroscopic data.

| entry | condition | yield | entry | condition | yield |
|-------|-----------|-------|-------|-----------|-------|
| 1     | DCM/TFA/H₂O (10:3:1) | side products | 14 | 1.0 M aq. HCl, THF | side products |
|       | 0 °C to r.t., overnight | |       | r.t., 4 days | |
| 2     | 2 M aq. HCl | side products | 15 | AcOH (80%), THF | side products |
|       | MW 120 °C, 10 min | |       | MW 110 °C, 30 min then r.t., 3 days | |
| 3     | TFA/H₂O (3:1) | partially deprotected | 16 | Dowex 50W, H₂O, MeOH | decomposition |
|       | r.t., overnight | |       | r.t. to MW 110 °C, 15 min | |
| 4     | TFA/H₂O/TIPS (8:1:1) | partially deprotected | 17 | AcOH (60%), THF | partially deprotected |
|       | 0 °C to r.t., on | |       | 60 °C, 900 mbar, 2.5 h | |
| 5     | ZnBr₂, DCM | traces | 18 | TFA/H₂O (9:1) | side products |
|       | then H₂O | |       | 0 °C to r.t., 2.5 h | |
|       | r.t., overnight | |       | then r.t., 4 h | |
| 6     | TIPS, DCM/TFA (3:2) | traces | 19 | 4.0 M HCl in dioxane, THF | free amine (crude) |
|       | then 1.0 M aq HCl | |       | r.t., 20 min then TiCl₄, DCM | no product |
|       | 0 °C to r.t., 90 min then r.t. overnight | |       | 0 °C, 2.5 h | |
| 7     | InCl₃, H₂O, MeCN | partially deprotected | 20 | FeCl₃, DCM | decomposition |
|       | r.t., 24 h | |       | r.t., 1 h | |
| 8     | TiCl₄, DCM, 0 °C, 3 h | side products | 21 | AcCl, MeOH, then conc. HCl (37%) | side products |
|       | | |       | r.t., 2 days | |
| 9     | Et₃SiH, DCM/TFA (3:2) | side products | 22 | TMSOTf, 2,6-lutidine, DCM |-78 to 0 °C to r.t., overnight | side products |
|       | 0 °C to r.t., overnight | |       | then | |
| 10    | 4.0 M HCl in dioxane | free amine (40% yield) | 23 | BF₃·OEt₂, HS(CH₂)₂SH, DCM | traces |
|       | r.t., 1 h then Et₃SiH, DCM/TFA (3:2) | |       | 0 °C to r.t., overnight | |
|       | r.t., overnight | |       | side products | |
| 11    | conc. aq. HCl (37%), THF, 0 °C to r.t., 2 h | traces | 24 | BF₃·OEt₂, DCM | side product |
|       | | |       | 0 °C to r.t., 6 h | |
| 12    | 4.0 M HCl in dioxane, THF | free amine (62% yield) | 25 | B-Bromocatecholborane, DCM | traces |
|       | r.t., 20 min then ZnBr₂, DCM then H₂O | |       | 0 °C, 3.5 h | |
|       | r.t., 18 h then 2 h then InCl₃, H₂O, MeCN r.t., overnight | |       | side product | |
| 13    | 4.0 M HCl in dioxane, MeOH r.t., 2 h | side products (loss of acetyl group) | 26 | PPTS, MeOH | 18% |
|       | | |       | 0 °C to r.t. to reflux, overnight, then BCls, DCM | |
|       | | |       | -78 to -40 °C, 2 h | |

*all reactions were monitored by LC/MS and run until the desired product mass appeared or stated otherwise in the table; MW: microwave*
Figure S1. Summary of total synthesis and step counts.
Sphingofungin D (4)

To a solution of sphingofungin C 3 (16.0 mg, 0.037 mmol) in MeOH (742 µL) was added Et₃N (185 µL) and the resulting mixture was stirred at r.t. for 1 h. After this time, the reaction mixture was evaporated in vacuo and the resulting mixture was purified by preparative HPLC over a C18 column (eluant: H₂O/MeCN + 0.1% formic acid 95:5 to 0:100). The appropriate fractions were collected evaporated and lyophilized to afford a colorless solid. Since the NMR spectra showed a mixture of products, the colorless solid was dissolved in MeOH (3.0 mL) and Et₃N (750 µL) was added. The resulting solution was stirred at r.t. for 3 h and then evaporated under vacuum. The residue was dissolved in MeOH (1.0 mL) and H₂O (1.0 mL) and the resulting mixture was purified by flash chromatography over a C18 column (eluant: H₂O/MeOH 50:50, isocratic). The appropriate fractions were combined, evaporated and lyophilized to afford sphingofungin D 4 (6.0 mg, 0.014 mmol, 38% yield) as a colorless solid.

¹H-NMR: See table Table S3.

¹³C-NMR: See table Table S3.

HRMS (ESI, m/z): calc'd for C₂₂H₄₂NO₇ [M+H]⁺ 432.2956; found 432.2944.

IR (ATR) νmax: 3287, 2922, 2851, 1653, 1541 cm⁻¹.

OR [α]D²²: +11.6 (c 0.1, MeOH); Lit[8]: +16 (c 0.3, MeOH).
Table S3. NMR signal comparison of reported[8] and synthetic sphingofungin D (4) (solvent: MeOD-d4).

| Position | reported[8] sphingofungin D | synthesized sphingofungin D | reported[8] sphingofungin D | synthesized sphingofungin D |
|----------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|          | ¹H-NMR³⁺ | ¹H-NMR⁵⁺ | ¹³C-NMR⁷⁺ | ¹³C-NMR⁹⁺ |
|          | δ, m, H  | δ, m, H  | δ, m  | δ, m  |
|          | (J in Hz) | (J in Hz) |       |       |
| 1        | 176.7    | 176.4, s |       |       |
| 2        | 4.39, d, 1H (6.8) | 4.43, d, 1H (6.7) | 58.0 | 58.0, d |
| 3        | 3.81, dd, 1H (6.8, 1.5) | 3.84, br. d, 1H (6.7) | 72.6 | 72.53, d |
| 4        | 3.43, dd, 1H (7.3, 1.5) | 3.44, br. d, 1H (7.0) | 75.5 | 75.5, d |
| 5        | 4.14, dd, 1H (7.6, 7.3) | 4.15, dd, 1H (7.5, 7.5) | 75.2 | 75.2, d |
| 6        | 5.46, dd, 1H (15.6, 7.6) | 5.47, dd, 1H (15.4, 7.5) | 130.3 | 130.3, d |
| 7        | 5.77, dt, 1H (15.6, 6.8) | 5.78, dt, 1H (15.4, 6.7) | 135.4 | 135.4, d |
| 8        | 2.04, m, 2H | 2.06, m, 2H | 33.5 | 33.5, t |
| 14       | 3.49, m, 1H | 3.50, m, 1H | 72.4 | 72.46, d |
| 20       | 0.90, t, 3H (6.6) | 0.91, t, 3H (6.8) | 14.4 | 14.4, q |
| -NHAc    | 2.01, s, 3H | 2.01, s, 3H | 173.4 | 173.4, s |
|          |            |            | 22.9 | 22.8, q |
| 9-13     |            |            | 38.5, 33.1, 30.7, | 38.5, 33.1, 30.7, |
| &        | 1.48-1.24, m, 20H | 1.45-1.30, m, 20H* | 30.5, 30.3, 30.2, | 30.6, 30.3, 30.2, |
| 15-19    |            |            | 26.8, 23.7 | 26.8, 23.7, 8t |

¹unknown MHz; ²500 MHz; ³unknown MHz; ⁴125 MHz; ⁵Integrates for ca. 32H instead of 20H due to residual Et3N;
Sphingofungin B (2)

A vial charged with sphingofungin C 3 (9.0 mg, 0.021 mmol) and MeCN (695 µL) was cooled to 0 °C. At this temperature, 3 M aq. HCl (348 µL) was added dropwise and the reaction was stirred at 4 °C overnight and then at r.t. for 8 h. Consumption of the starting material proceeded slowly. Hence, the reaction was heated at 40 °C for 20 h. After this time, the reaction was evaporated under vacuum and the residue redissolved in MeOH (695 µL). To the resulting solution was slowly added 10% aq. NaOH (463 µL) and the reaction was stirred at r.t. for 2 h. The reaction was neutralized by slowly adding formic acid (46 µL) and diluted with MeOH and H₂O. The mixture was purified by preparative HPLC over a phenyl-hexyl column (Luna, 5 µm, 250 x 21.2 mm, 100 Å) (eluant: H₂O/MeCN + 0.1% formic acid 80:20 to 50:50). The appropriate fractions were collected evaporated to afford sphingofungin B 2 (2.0 mg, 0.005 mmol, 25% yield) as a colorless solid.

¹H-NMR: See table Table S4.

¹³C-NMR: See table Table S4.

HRMS (ESI, m/z): calc'd for C₂₀H₄₀NO₆ [M+H]⁺ 390.2850; found 390.2845.

IR (ATR) νₘₐₓ: 3214, 2922, 2851, 1616, 1507 cm⁻¹.

OR [α]D²₁: -14.6 (c 0.1, MeOH); Lit[1]: [α]D²₅: -13.6 (c 1.0, MeOH).
Table S4. NMR signal comparison of isolated and synthetic sphingofungin B (2) (solvent: MeOD-d$_4$).

![Sphingofungin Structure]

| Position | isolated sphingofungin B$^e$ | synthesized sphingofungin B | isolated sphingofungin B | synthesized sphingofungin B |
|----------|------------------------------|-----------------------------|--------------------------|----------------------------|
|          | $^1$H-NMR$^a$ | $^1$H-NMR$^b$ | $^{13}$C-NMR$^c$ | $^{13}$C-NMR$^d$ |
|          | $\delta$, m, H | $\delta$, m, H | $\delta$, m | $\delta$, m |
|          | ($J$ in Hz) | ($J$ in Hz) | ($J$ in Hz) | ($J$ in Hz) |
| 1        | 3.78, d, 1H (4.8) | 3.77, d, 1H (4.8) | 171.9, s | 171.8, s |
| 2        | 4.17-4.15, m, 2H | 4.17-4.14, m, 2H | 60.8, d | 60.8, d |
| 3        | 3.62, dd, 1H (6.8, 1.1) | 3.62, br. d, 1H (6.8) | 76.0, d | 76.0, d |
| 5        | 4.17-4.15, m, 2H | 4.17-4.14, m, 2H | 75.2, d | 75.2, d |
| 6        | 5.48, dd, 1H (15.4, 7.6) | 5.48, dd, 1H (15.4, 7.6) | 130.2, d | 130.2, d |
| 7        | 5.81-5.76, m, 1H (15.4, 6.8) | 5.79, dt, 1H (15.4, 6.8) | 135.5, d | 135.5, d |
| 8        | 2.06, m, 2H | 2.06, m, 2H | 33.5, t | 33.5, t |
| 14       | 3.50, m, 1H | 3.50, m, 1H | 72.4, d | 72.5, d |
| 20       | 0.90, m, 3H (6.8) | 0.91, t, 3H (6.8) | 14.4, q | 14.4, q |
| 9-13     | 1.44-1.29, m, 20H | 1.45-1.31, m, 20H | 26.78, 26.76, 23.7, 9t | 26.80, 26.77, 23.7, 9t |
| 15-19    | 38.4, 33.0, 30.7, 30.5, 30.4, 30.2, | 38.5, 33.1, 30.7, 30.6, 30.4, 30.2, |
|          | & 26.78, 26.76, 23.7, 9t | & 26.80, 26.77, 23.7, 9t |

$^a$600 MHz; $^b$500 MHz; $^c$150 MHz; $^d$125 MHz; $^e$isolated from Aspergillus sp.
To a solution of sphingofungin B (9.0 mg, 0.023 mmol) in anhydrous DMF (205 µL) under argon atmosphere were subsequently added DIPEA (11.0 µL, 8.4 mg, 0.065 mmol) and 1H-pyrazole-1-carboxamidine hydrochloride (4.8 mg, 0.032 mmol). The resulting solution was stirred at r.t. for 24 h. Next, the reaction mixture was diluted with H2O (2.0 mL) and the resulting mixture was purified by flash chromatography over a C18 column (eluant: H2O/MeOH 95:5 to 0:100). The appropriate fractions were collected evaporated to afford sphingofungin A (9.0 mg, 0.021 mmol, 90% yield) as a colorless solid.

1H-NMR (500 MHz, MeOD-d4): δ 5.78 (dt, J = 15.5, 6.8 Hz, 1H, 7-H), 5.48 (dd, J = 15.5, 7.5 Hz, 1H, 6-H), 4.17 (m, 1H, 5-H), 4.04 (d, J = 6.2 Hz, 1H, 2-H), 3.93 (dd, J = 6.2, 1.4 Hz, 1H, 3-H), 3.50 (m, 1H, 14-H), 3.45 (dd, J = 6.9, 1.4 Hz, 1H, 4-H), 2.06 (m, 2H, 8-H), 1.44-1.31 (m, 20H, 9-H - 13-H, 15-H - 19-H), 0.91 (t, J = 6.7 Hz, 3H, 20-H) ppm.

13C-NMR (125 MHz, MeOD-d4): δ 174.5, 159.1 (2s, C-1, C-21), 135.4, 130.3, 75.2, 75.1, 72.6, 72.5, 60.4 (7d, C-7, C-6, C-4, C-5, C-3, C-14, C-2), 38.5, 38.4 (2t, C-13, C-15)*, 33.4, 33.1 (2t, C-8, C-18), 30.7, 30.6, 30.3, 30.2 (4t, C-9 - C11, C-17)*, 26.8 (t, C-12, C-16)*, 23.7 (t, C-19), 14.4 (q, C-20) ppm. *could not be unambiguously assigned.

HRMS (ESI, m/z): calc’d for C21H42N3O6 [M+H]+ 432.3068; found 432.3054.

IR (ATR) νmax: 3324, 3170, 2924, 2852, 1654, 1616 cm⁻¹.

OR [α]D²²: +15.3 (c 0.1, MeOH).
A culture tube charged with (2R)-protected amino ester (2R)-14 (74.0 mg, 0.149 mmol) and (R)-pentadec-14-en-7-ol (8a)[1] (37.0 mg, 0.164 mmol) was evacuated and backfilled with argon. Anhydrous DCM (1.5 mL) was added followed by Grubbs 2nd generation catalyst (13.0 mg, 0.015 mmol) and the resulting mixture was stirred at r.t. overnight. Next, additional Grubbs 2nd generation catalyst (6.3 mg, 0.007 mmol) was added and the resulting reaction was stirred at r.t. for 5 h. Again, (R)-pentadec-14-en-7-ol (8a) (37.0 mg, 0.164 mmol) was added and the reaction was stirred at r.t. for additional 3 h. Finally, (R)-pentadec-14-en-7-ol (8a) (37.0 mg, 0.164 mmol) and Grubbs 2nd generation catalyst (5.0 mg, 0.006 mmol) were added and the resulting reaction was stirred at reflux overnight. The reaction mixture was adsorbed on silica and the mixture on silica was purified by flash chromatography over a silica gel column (eluant: cyclohexane/EtOAc 100:0 to 60:40). The appropriate fractions were combined and evaporated to afford starting material (2R)-protected amino ester (2R)-14 (22.0 mg) and crude protected 2-epi sphingofungin C 17a as a brown oil. 17a was repurified by flash chromatography over a C18 column (eluant: H2O/MeCN 20:80 to 0:100). The appropriate fractions were combined and evaporated to afford pure protected 2-epi sphingofungin C 17a (44.0 mg, 0.063 mmol, 42% yield, brsm: 60%) as a colorless oil.

1H-NMR (500 MHz, C6D6): δ 6.49 (s, 2H, 2x 23-H), 5.97 (dt, J = 15.4, 6.8 Hz, 1H, 7-H), 5.86 (m, 1H, 5-H), 5.71 (br. dd, J = 15.4, 8.0 Hz, 1H, 6-H), 5.61 (d, J = 8.7 Hz, 1H, -NH-), 4.58 (dd, J = 7.9, 7.2 Hz, 1H, 4-H), 4.49 (dd, J = 7.9, 1.9 Hz, 1H, 3-H), 4.31 (dd, J = 8.7, 1.9 Hz, 1H, 2-H), 3.56-3.51 (m, 1H, 14-H), 2.65 (s, 6H, 2x 22-Me), 2.05-2.01 (m, 2H, 8-H), 1.92 (s, 3H, 24-Me), 1.78 (s, 3H, -OAc), 1.45-1.28 (m, 20H, 9-H - 13-H, 15-H - 19-H), 1.39 (s, 9H, -O-tBu), 1.33 (s, 3H, 25-Me), 1.17 (s, 3H, 25-Me), 0.90 (t, J = 6.9 Hz, 3H, 20-H) ppm.

13C-NMR (125 MHz, C6D6): δ 169.9, 169.4, 140.7, 138.6 (4s, C-1, -OAc, C-24, C-21), 138.4 (d, C-7), 137.0 (s, 2x C-22), 131.2, 124.6 (2d, 2x C-23, C-6), 110.2, 82.7 (2s, C-25, -O-tBu), 79.3, 78.3, 75.4, 71.6, 57.5 (5d, C-3, C-4, C-5, C-14, C-2), 38.4, 38.3 (2t, C13, C15)*, 32.6, 32.4 (2t, C-8, C-18), 30.0, 29.8, 29.4 (3t, C-10, C-11, C17)*, 28.9 (t, C-9), 28.0, 27.1, 27.0 (3q, -O-tBu, 25-Me, 25-Me), 26.3, 26.0 (2t, C-12, C16)*, 23.1 (t, C-19), 20.8** (t, 24-Me, -OAc)*, 19.6, 14.4 (2q, 22-Me, C-20) ppm. *could not be unambiguously assigned; **DEPT135 shows two rotamers (24-Me, -OAc)
HRMS (ESI, m/z): calc’d for C_{38}H_{64}O_{8}S [M+H]^+ 694.4347; found 694.4329.

IR (ATR) \( \nu_{\text{max}} \): 3447, 3310, 2980, 2927, 2855, 1739 cm\(^{-1}\).

OR [\alpha]_D^{22}: -37.7 (c 0.7, CH\(_2\)Cl\(_2\)).

**Pentadec-14-en-7-one (8b)**

A flask charged with literature known (R)-pentadec-14-en-7-ol (8a)\(^{[1]} \) (94.0 mg, 0.415 mmol) was evacuated and backfilled with argon. Next, DCM (4.2 mL) was added and to the resulting solution was added Dess-Martin periodinane (211 mg, 0.498 mmol) in one portion. The reaction was stirred at r.t. for 30 min. The reaction was poured on a diluted aq. NaHCO\(_3\) solution and the aq. layer was extracted with DCM (3x). The org. layers were combined, dried over NaSO\(_4\), filtered and evaporated. The crude product was purified by flash chromatography over a silica gel column (eluant: cyclohexane/EtOAc 100:0 to 80:20). The appropriate fractions were combined and evaporated to afford pentadec-14-en-7-one 8b (86.0 mg, 0.383 mmol, 92% yield) as a yellowish liquid.

\(^1\)H-NMR (500 MHz, CDCl\(_3\)): \( \delta \) 5.80 (ddt, \( J \) = 17.1, 10.2, 6.7 Hz, 1H, 2-H), 4.99 (ddd, \( J \) = 17.1, 3.4, 1.7 Hz, 1H, 1-H\(_A\)), 4.93 (m, 1H, 1-H\(_B\)), 2.38 (t, \( J \) = 7.5 Hz, 4H, 8-H, 10-H), 2.04 (m, 1H, 3-H), 1.59-1.53 (m, 4H, 7-H, 11-H), 1.39-1.35 (m, 2H, 4-H), 1.33-1.26 (m, 10H\(^*\), 5-H, 6-H, 12-H - 14-H), 0.88 (t, \( J \) = 6.9 Hz, 3H, 15-H) ppm. *Integrates for 12H instead of 10H.

\(^{13}\)C-NMR (125 MHz, CDCl\(_3\)): \( \delta \) 211.8 (s, C-9), 139.2 (d, C-2), 114.4 (t, C-1), 43.0, 42.9 (2t, C-8, C-10\(^*\)), 33.9, 31.8 (2t, C-3, C-13), 29.3, 29.1, 29.0, 28.9 (4t, C-4 - C-6, C-12\(^*\)), 24.02, 23.98 (2t, C-7, C-11\(^*\)), 22.6 (t, C-14), 14.2 (q, C-15) ppm. *could not be unambiguously assigned.

HRMS (ESI, m/z): calc’d for C\(_{15}\)H\(_{29}\)O [M+H]\(^+\) 225.2213; found 225.2206.

IR (ATR) \( \nu_{\text{max}} \): 3077, 2925, 2855, 1714 cm\(^{-1}\).
Protected 14-oxo sphingofungin C (17b) was synthesized according to GP3 using (2S)-protected amino ester (2S)-14 (51.0 mg, 0.103 mmol) and pentadec-14-en-7-one 8b (35.0 mg, 0.154 mmol) to afford protected 14-oxo sphingofungin C 17b (54.0 mg, 0.078 mmol, 76% yield) as a brownish oil.

^1^H-NMR (600 MHz, C_6D_6): δ 6.53 (s, 2H, 2x 23-H), 5.77 (dt, J = 15.4, 6.8 Hz, 1H, 7-H), 5.67 (dd, J = 8.1, 5.8 Hz, 1H, 5-H), 5.59 (br. dd, J = 15.4, 8.1 Hz, 1H, 6-H), 5.36 (d, J = 10.2 Hz, 1H, -NH-), 4.56 (m, 1H, 13-H, 15-H), 4.29 (m, 1H, 2-H), 2.59 (s, 6H, 2x 22-Me), 2.04 (q, J = 7.5 Hz, 4H, 12-H, 16-H), 1.96 (s, 3H, 24-Me), 1.79 (m, 2H, 8-H), 1.64 (s, 3H, -OAc), 1.56-1.50 (m, 4H, 4-H, 12-H, 16-H), 1.46 (s, 9H, -OtBu), 1.44 (s, 6H, 2x 25-Me), 1.27-1.24 (m, 2H, 19-H), 1.20-1.18 (m, 6H, 9-H, 10-H, 18-H), 1.12-1.10 (m, 4H, 11-H, 17-H)*, 0.88 (t, J = 7.2 Hz, 3H, 20-H) ppm. *could not be unambiguously assigned.

^13^C-NMR (150 MHz, C_6D_6): δ 208.7, 170.6, 169.6, 140.6, 138.8 (5s, C-14, C-1, -OAc, C-24, C-21), 137.6 (d, C-7), 137.1 (s, 2x C-22), 131.2, 125.4, (2d, 2x C-23, C-6), 111.2, 82.5 (2s, C-25, -OAc), 80.9, 79.6, 75.2, 61.3 (4d, C-4, C-3, C-5, C-2), 42.7, 42.6 (2t, C-13, C-15)*, 32.5, 32.1 (2t, C-8, C-18), 29.39, 29.36, 29.35 (3t, C-10, C-11, C-17)*, 28.9 (t, C-9), 28.03, 28.00, 27.6 (3q, 25-Me, -OAc, 25-Me), 24.1, 24.0 (2t, C-12, C-16)*, 22.9 (t, C-19), 20.9, 20.7, 19.4, 14.3 (4q, 24-Me, -OAc, 2x 22-Me, C-20) ppm. *could not be unambiguously assigned.

HRMS (ESI, m/z): calc’d for C_{38}H_{52}NO_{8}S [M+H]^+ 692.4191; found 692.4173.

IR (ATR) ν_{max}: 3052, 2929, 2855, 1733, 1713 cm⁻¹.

OR [α]_D^{22}: +90.5 (c 1.3, CH_2Cl_2).
Protected 14-desoxy sphingofungin C (17c) was synthesized according to GP3 using (2S)-protected amino ester (2S)-14 (64.0 mg, 0.129 mmol) and 1-pentadecene (8c) (174 µL, 136 mg, 0.646 mmol) to afford protected 14-desoxy sphingofungin C 17c (56.0 mg, 0.083 mmol, 64% yield) as a brown oil.

\[ ^1H-NMR \ (500 \text{ MHz, } \text{C}_6\text{D}_6): \delta \ 6.52 \ (s, \ 6H, \ 2x \ 23-H), \ 5.79 \ (dt, \ J = 15.3, 6.8 \text{ Hz}, \ 1H, \ 7-H), \ 5.68 \ (dd, \ J = 8.2, 5.6 \text{ Hz}, \ 1H, \ 5-H), \ 5.60 \ (br. dd, \ J = 15.3, 8.2 \text{ Hz}, \ 1H, \ 6-H), \ 5.36 \ (d, \ J = 10.2 \text{ Hz}, \ 1H, \ -NH-), \ 4.55 \ (m, \ 1H, \ 4-H), \ 4.30 \ (m, \ 1H, \ 3-H), \ 4.23 \ (dd, \ J = 10.2, 6.6 \text{ Hz}, \ 1H, \ 2-H), \ 2.59 \ (s, \ 6H, \ 2x \ 22-Me), \ 1.95 \ (s, \ 3H, \ 24-Me), \ 1.82 \ (m, \ 2H, \ 8-H), \ 1.64 \ (s, \ 3H, \ -OAc), \ 1.46 \ (s, \ 9H, \ -O\text{tBu}), \ 1.45 \ (s, \ 6H, \ 2x \ 25-Me), \ 1.32-1.17 \ (m, \ 22H, \ 9-H \ - 19-H), \ 0.92 \ (t, \ J = 6.9 \text{ Hz}, \ 3H, \ 20-H) \text{ ppm.} \]

\[ ^{13}C-NMR \ (125 \text{ MHz, } \text{C}_6\text{D}_6): \delta \ 170.6, \ 169.6, \ 140.6, \ 138.8 \ (4s, \ C-1, \ -OAc, \ C-24, \ C-21), \ 137.8 \ (d, \ C-7), \ 137.1 \ (s, \ 2x \ C-22), \ 131.2, \ 125.3 \ (2d, \ 2x \ C-23, \ C-6), \ 111.2, \ 82.5 \ (2s, \ C-25, \ -O\text{tBu}), \ 80.9, \ 79.6, \ 75.1, \ 61.4 \ (4d, \ C-4, \ C-3, \ -OAc, \ C-2), \ 32.6, \ 32.4 \ (2t, \ C-8, \ C-18), \ 30.20, \ 30.16, \ 30.09, \ 29.9, \ 29.8, \ 29.6 \ (6t, \ C-10 - C-17), \ 29.2 \ (t, \ C-9), \ 28.0, \ 27.6 \ (2q, \ -O\text{tBu}, \ 2x \ 25-Me), \ 23.1 \ (t, \ C-19), \ 20.8, \ 20.7, \ 19.4, \ 14.4 \ (4q, \ 24-Me, \ -OAc, \ 2x \ 22-Me, \ C-20) \text{ ppm.} \]

HRMS (ESI, \text{m/z}): calc’d for C_{38}H_{64}NO_7S [M+H]^+ 678.4398; found 678.4379.

IR (ATR) \text{v}_{\text{max}}: \ 2983, \ 2924, \ 2853, \ 1739 \text{ cm}^{-1}.

OR [\alpha]^D_{21}: +99.3 (c 0.7, CH_2Cl_2).
Protected short sphingofungin C (17d)

Protected short sphingofungin C \textbf{17d} was synthesized according to \textbf{GP3} using \textit{(2S)-protected amino ester \textit{(2S)-14}} (53.0 mg, 0.107 mmol) and 1-octene \textbf{(8d)} (83.9 µL, 60.0 mg, 0.535 mmol) to afford protected short sphingofungin C \textbf{17d} (53.0 mg, 0.091 mmol, 85% yield) as a brown oil.

\textbf{1H-NMR (600 MHz, \textit{C}_6\textit{D}_6)}: \(\delta\) 6.51 (s, 2H, 2x 16-H), 5.77 (dt, \(J = 15.4, 6.8\) Hz, 1H, 7-H), 5.67 (dd, \(J = 8.2, 5.7\) Hz, 1H, 5-H), 5.58 (br. dd, \(J = 15.4, 8.2\) Hz, 1H, 6-H), 5.35 (d, \(J = 10.2\) Hz, 1H, -NH-), 4.54 (m, 1H, 4-H), 4.29 (m, 1H, 3-H), 4.23 (dd, \(J = 10.2, 6.7\) Hz, 1H, 2-H), 2.58 (s, 6H, 2x 15-Me), 1.94 (s, 3H, 17-Me), 1.79 (m, 2H, 8-H), 1.63 (s, 3H, -OAc), 1.46 (s, 9H, -OtBu), 1.44 (s, 6H, 2x 18-Me), 1.25-1.12 (m, 8H, 9-H-12-H), 0.88 (t, \(J = 7.3\) Hz, 3H, 13-H) ppm.

\textbf{13C-NMR (150 MHz, \textit{C}_6\textit{D}_6)}: \(\delta\) 170.6, 169.6, 140.6, 138.8 (4s, C-1, -OAc, C-17, C-14), 137.8 (d, C-7), 137.1 (s, 2x C-15), 131.1, 125.2 (2d, 2x C-16, C-6), 111.2, 82.5 (2s, C-18, -OtBu), 80.9, 79.5, 75.1, 61.4 (4d, C-4, C-3, C-5, C-2), 32.6, 32.0 (2t, C-8, C-11), 29.2, 29.1 (2t, C-9, C-10)*, 28.04, 28.00, 27.6 (3q, 18-Me, -OtBu, 18-Me), 23.0 (t, C-12), 20.8, 20.7, 19.4, 14.3 (4q, 17-Me, -OAc, 2x 15-Me, C-13) ppm. *could not be unambiguously assigned.

\textbf{HRMS (ESI, \textit{m/z})}: calc'd for \textit{C}_{31}\textit{H}_{50}\textit{NO}_{7}\textit{S} [M+H]^+ 580.3303; found 580.3283.

\textbf{IR (ATR) \nu_{\text{max}}}: 2977, 2955, 2925, 2855, 1733 cm\(^{-1}\).

\textbf{OR [\alpha]}\textbf{D}^{22}: +97.6 (c 1.5, CH\textsubscript{2}Cl\textsubscript{2}).
A vial charged with protected 2-epi sphingofungin C 17a (14.0 mg, 0.020 mmol) was evacuated and backfilled with argon. Next, anhydrous DCM (2.0 mL) was added and the resulting solution was cooled to -78 °C. At this temperature, BCl₃ (202 µL, 0.202 mmol, 1.0 M solution in DCM) was added dropwise and the reaction was stirred at -78 °C for 1 h. Reaction control by LC/MS showed product as well as intermediates. Hence, additional BCl₃ (40.0 µL, 0.040 mmol, 1.0 M solution in DCM) was added dropwise and the reaction was stirred at -78 °C for additional 1.5 h. The reaction was quenched at -78 °C by adding dropwise a mixture of MeCN/H₂O. The resulting mixture was allowed to reach r.t. and then carefully concentrated under vacuum. The residue was diluted with H₂O. The resulting mixture was purified by flash chromatography over a C18 column (eluant: H₂O/MeCN + 0.1% formic acid 90:10 to 0:100). The appropriate fractions were collected evaporated to afford 2-epi sphingofungin C 18a (5.0 mg, 0.012 mmol, 57% yield) as a colorless solid.

**1H-NMR (500 MHz, MeOD-d₄):** δ 5.89 (dt, J = 15.2, 6.8 Hz, 1H, 7-H), 5.44 (br. dd, J = 15.2, 7.9 Hz, 1H, 6-H), 5.37 (m, 1H, 5-H), 4.22 (dd, J = 3.2, 1.5 Hz, 1H, 3-H), 3.84 (dd, J = 8.6, 1.5 Hz, 1H, 4-H), 3.66 (d, J = 3.2 Hz, 1H, 2-H), 3.50 (m, 1H, 14-H), 2.09-2.05 (m, 2H, 8-H), 2.06 (s, 3H, -OAc), 1.42-1.31 (m, 20H, 9-H - 13-H, 15-H - 19-H), 0.91 (t, J = 7.0 Hz, 3H, 20-H) ppm.

**13C-NMR (125 MHz, MeOD-d₄):** δ 172.5, 172.3 (2s, C-1, -OAc), 138.8, 125.7, 77.6, 76.5, 72.5, 68.9, 59.7 (7d, C-7, C-6, C-5, C-4, C-14, C-3, C-2), 38.5, 38.4 (2t, C-13, C-15)*, 33.4, 33.1 (2t, C-8, C-18), 30.64, 30.56, 30.3 (3t, C-10, C-11, C-17)*, 29.9 (t, C-9), 26.80, 26.75 (2t, C-12, C-16)*, 23.7 (t, C-19), 21.3, 14.4 (2q, -OAc, C-20) ppm. *could not be unambiguously assigned.

**HRMS (ESI, m/z):** calc’d for C₂₂H₄₂NO₇ [M+H]+ 432.2956; found 432.2941.

**IR (ATR) v max:** 3172, 2923, 2851, 1716, 1646, 1517 cm⁻¹.

**OR [α]D²²:** +11.1 (c 0.1, MeOH).
14-oxo sphingofungin C (18b)

14-oxo sphingofungin C 18b was synthesized according to GP4 using protected 14-oxo sphingofungin C 17b (38.0 mg, 0.055 mmol) to afford (9.0 mg, 0.021 mmol, 38% yield) a colorless solid.

$^1$H-NMR (500 MHz, MeOD-$d_4$): $\delta$ 5.87 (m, 1H, 7-H), 5.42-5.37 (m, 2H, 5-H, 6-H), 4.17 (m, 1H, 3-H), 3.83 (m, 1H, 4-H), 3.77 (d, $J = 4.8$ Hz, 1H, 2-H), 2.44 (t, $J = 7.4$ Hz, 4H, 13-H, 15-H), 2.07-2.03 (m, 2H, 8-H), 2.05 (s, 3H, -OAc), 1.57-1.51 (m, 4H, 14-H, 16-H), 1.43-1.37 (m, 2H, 9-H), 1.34-1.29 (m, 10H, 10-H, 11-H, 17-H - 19-H), 0.90 (t, $J = 6.9$ Hz, 3H, 20-H) ppm.

$^{13}$C-NMR (125 MHz, MeOD-$d_4$): $\delta$ 212.9, 171.0, 170.1 (3s, C-14, -OAc, C-1), 137.3, 124.4, 76.4, 73.1, 66.6, 59.7 (6d, C-7, C-6, C-5, C-4, C-3, C-2), 42.1, 42.0 (2t, C-13, C-15)*, 31.9, 31.4 (2t, C-8, C-18), 28.7, 28.6, 28.3 (3t, C-9 - C11, C-17)*, 23.5, 23.4 (2t, C-12, C-16)*, 22.2 (t, C-19), 19.9, 13.0 (2q, -OAc, C-20) ppm. *could not be unambiguously assigned.

HRMS (ESI, $m/z$): calc’d for C$_{22}$H$_{40}$NO$_7$ [M+H]$^+$ 430.2799; found 430.2786.

IR (ATR) $\nu_{\text{max}}$: 3165, 2923, 2851, 1703, 1615, 1507 cm$^{-1}$.

OR $[\alpha]_D^{22}$: +8.9 (c 0.1, MeOH).

14-desoxy sphingofungin C (18c)

14-desoxy sphingofungin C 18c was synthesized according to GP4 using protected 14-desoxy sphingofungin C 17c (24.0 mg, 0.035 mmol) to afford (5.0 mg, 0.012 mmol, 34% yield) a colorless solid.

$^1$H-NMR (500 MHz, MeOD-$d_4$): $\delta$ 5.88 (m, 1H, 7-H), 5.41-5.36 (m, 2H, 5-H, 6-H), 4.17 (dd, $J = 4.8$, 1.2 Hz, 1H, 3-H), 3.83 (m, 1H, 4-H), 3.77 (d, $J = 4.8$ Hz, 1H, 2-H), 2.07-2.03 (m, 2H, 8-H), 2.04 (s, 3H, -OAc), 1.41-1.29 (m, 22H, 9-H - 19-H), 0.90 (t, $J = 7.0$ Hz, 3H, 20-H) ppm.
$^{13}$C-NMR (125 MHz, MeOD-$d_4$): δ 172.4, 171.5 (2s, -OAc, C-1), 138.9, 125.7, 77.9, 74.6, 67.9, 61.2 (6d, C-7, C-6, C-5, C-4, C-3, C-2), 33.5, 33.1 (2t, C-8, C-18), 30.8, 30.7, 30.6, 30.5, 30.3, 30.0 (6t, C-9 - C-17), 23.7 (t, C-19), 21.3, 14.4 (2q, -OAc, C-20) ppm.

HRMS (ESI, $m/z$): calc’d for C$_{22}$H$_{42}$NO$_6$ [M+H]$^+$ 416.3007; found 416.2994.

IR (ATR) $\nu_{\text{max}}$: 3532, 3306, 3168, 2918, 2849, 1717, 1623, 1595, 1504 cm$^{-1}$.

OR $[\alpha]_D^{21}$: +16.0 (c 0.1, MeOH).

**Short sphingofungin C (18d)**

Short sphingofungin C 18d was synthesized according to GP4 using protected short sphingofungin C 17d (34.0 mg, 0.059 mmol) to afford (8.0 mg, 0.025 mmol, 43% yield) a colorless solid.

$^1$H-NMR (500 MHz, MeOD-$d_4$): δ 5.88 (m, 1H, 7-H), 5.41-5.36 (m, 2H, 5-H, 6-H), 4.17 (m, 1H, 3-H), 3.83 (m, 1H, 4-H), 3.77 (m, 1H, 2-H), 2.07-2.03 (m, 2H, 8-H), 2.04 (s, 3H, -OAc), 1.41-1.36 (m, 2H, 9-H), 1.34-1.28 (m, 6H, 10-H - 12-H), 0.90 (t, $J = 6.9$ Hz, 3H, 13-H) ppm.

$^{13}$C-NMR (125 MHz, MeOD-$d_4$): δ 172.4, 171.6 (s, -OAc, C-1), 138.9, 125.7, 77.8, 74.5, 68.0, 61.1 (6d, C-7, C-6, C-5, C-4, C-3, C-2), 33.4, 32.8 (2t, C-8, C-11), 30.0, 29.9 (2t, C-9, C-10)*, 23.6 (t, C-12), 21.2, 14.4 (2q, -OAc, C-13) ppm. *could not be unambiguously assigned.

HRMS (ESI, $m/z$): calc’d for C$_{15}$H$_{28}$NO$_6$ [M+H]$^+$ 318.1911; found 318.1900.

IR (ATR) $\nu_{\text{max}}$: 3527, 3304, 3157, 2957, 2925, 2855, 1717, 1648, 1624, 1590 cm$^{-1}$.

OR $[\alpha]_D^{22}$: +5.8 (c 0.1, MeOH).

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3. Structure Determinations by X-Ray

The intensity data for the compound (2R)-14 were collected on a Nonius KappaCCD diffractometer using graphite-monochromated Mo-Kα radiation. Data were corrected for Lorentz and polarization effects; absorption was taken into account on a semi-empirical basis using multiple-scans\[^{9-11}\]. The structure was solved by intrinsic phases (SHELXT\[^{12}\]) and refined by full-matrix least squares techniques against Fo\(^2\) (SHELXL-2018\[^{13}\]). All hydrogen atoms were included at calculated positions with fixed thermal parameters. All non-hydrogen atoms were refined anisotropically\[^{13}\]. The absolute configuration could not be determined directly. The determination was made by the known configuration of the educt. XP\[^{14}\] was used for structure representations.

![Figure S2. ORTEP representation of (2R)-14 showing hydrogens at the stereogenic centers.](image)

**Crystal Data for (2R)-14**: C\(^{25}\)H\(^{37}\)NO\(^{7}\)S, Mr = 495.61 gmol\(^{-1}\), colourless prism, size 0.104 x 0.072 x 0.068 mm\(^3\), monoclinic, space group P 2\(_1\), a = 11.0523(4), b = 5.9384(2), c = 20.2447(6) Å, β = 96.079(1)°, V = 1321.25(8) Å\(^3\), T= -140 °C, Z = 2, ρ\(_{\text{calcd.}}\) = 1.246 g/cm\(^3\), μ (Mo-Kα) = 1.65 cm\(^{-1}\), multi-scan, transmin: 0.5477, transmax: 0.7456, F(000) = 532, 13161 reflections in h(-14/14), k(-7/7), l(-26/26), measured in the range 2.023° ≤ Θ ≤ 27.483°, completeness Θ\(_{\text{max}}\) = 99.8%, 5764 independent reflections, R\(_{\text{int}}\) = 0.0814, 5047 reflections with F\(_{0}\) > 4σ(F\(_{0}\)), 326 parameters, 1 restraints, R\(_{1}\)\(_{\text{obs}}\) = 0.0734, wR\(^2\)\(_{\text{obs}}\) = 0.1851, R\(_{1}\)\(_{\text{all}}\) = 0.0883, wR\(^2\)\(_{\text{all}}\) = 0.1980, GOOF = 1.089, Flack-parameter -0.16(10), largest difference peak and hole: 0.560 / -0.514 e Å\(^{-3}\).

Crystallographic data (excluding structure factors) has been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC-2106584 for (2R)-14. Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [E-mail: deposit@ccdc.cam.ac.uk].
4. Biological Methods

Antibacterial and antifungal assays

All sphingofungins were tested in a disk diffusion assay against bacterial strains *Staphylococcus aureus* 134/94 R9 (MRSA), *Enterococcus faecalis* 1528 R10 (VRE) and *Mycobacterium vaccae* 10670 M4 and fungal strains *Candida albicans* H8 and *Aspergillus fumigatus* ATCC46645.[15]

Table S5. Antimicrobial growth inhibition activities of sphingofungin A - D (1 - 4) and compounds 18a - 18d.[ab]

| compound | MRSA [mm] | VRE [mm] | *M. vaccae* [mm] | *C. albicans* [mm] | *A. fumigatus* [mm] |
|----------|-----------|----------|------------------|--------------------|---------------------|
| 1        | 0         | 0        | 0                | 15p-P              | 0/A                 |
| 2        | 0         | 0        | 0                | 25p                | 0/A                 |
| 3        | 0         | 0        | 0                | 25p                | 0/A                 |
| 3        | 0         | 0        | 0                | 25p                | 0/A                 |
| 4        | 12P       | 0        | 0                | 0                  | 0/A                 |
| 18a      | 12P       | 0        | 0                | 0                  | 0/A                 |
| 18b      | 0         | 0        | 0                | 15p                | 0/A                 |
| 18c      | 0         | 0        | 0                | 11P                | 0/A                 |
| 18d      | 12P       | 0        | 0                | 0                  | 0/A                 |

positive control 0c | 16Fc | 23p | 22d | 20d
negative controla | 11P | 12p | 11p | 0 | 12p

* All compounds were dissolved in DMSO and tested at a concentration of 1 mg/mL; b p=presence of colonies in the inhibition zone, P=presence of many colonies in the inhibition zone, A=indication of inhibition, F=facilitation; c ciprofloxacin (5 µg/mL in H2O); d amphotericin B (10 µg/mL in DMSO/MeOH); a DMSO.

Minimal inhibitory concentrations of the most active compounds from the disk diffusion assay were determined against the corresponding strains (*Candida albicans* H8 and *Aspergillus fumigatus* ATCC46645) using a standard broth dilution method from the CLSI (Clinical and Laboratory Standards Institute) guideline.[16]

Table S6. MIC’s of sphingofungin A-C (1-3) and derivative 18b.[ab]

| compounds | *C. albicans* [µg/mL] | *A. fumigatus* [µg/mL] |
|-----------|-----------------------|------------------------|
| 1         | 50                    | 50                     |
| 2         | 25                    | 25                     |
| 3         | 50                    | 25                     |
| 18b       | 50                    | 25                     |
| positive control | <0,05 | 0,78 |
| negative control | 50 | 50 |

* a all compounds have been dissolved in DMSO/MeOH; b starting concentration: 100 µg/mL; c amphotericin B; d DMSO/MeOH.
Antiprotozoal assays

All compounds (except 18c) were tested against *Trypanosoma brucei rhodesiense* STIB 900 (trypomastigotes), *Trypanosoma cruzi* Tulahuen C4 (amastigotes), *Leishmania donovani* MHOM-ET-67/L82 (amastigotes) and *Plasmodium falciparum* NF54 (intraerythrocytic forms).[17]

**Activity against Trypanosoma brucei rhodesiense STIB900**[18]

This stock was isolated in 1982 from a human patient in Tanzania and after several mouse passages cloned and adapted to axenic culture conditions. Minimum Essential Medium (50 μL) supplemented with 25 mM HEPES, 1 g/L additional glucose, 1% MEM non-essential amino acids (100x), 0.2 mM 2-mercaptoethanol, 1 mM Na-pyruvate and 15% heat inactivated horse serum was added to each well of a 96-well microtiter plate. Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. Then 4x10^3 bloodstream forms of *T. b. rhodesiense* STIB 900 in 50 μL was added to each well and the plate incubated at 37 °C under a 5 % CO₂ atmosphere for 70 h. 10 μL resazurin solution (resazurin, 12.5 mg in 100 mL double-distilled water) was then added to each well and incubation continued for a further 2–4 h. Then, the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. Data were analyzed with the graphic programme Softmax Pro (Molecular Devices Cooperation, Sunnyvale, CA, USA), which calculated IC₅₀ values by linear regression (Huber 1993) and 4-parameter logistic regression from the sigmoidal dose inhibition curves. Melarsoprol (Arsobal Sanofi-Aventis, received from WHO) is used as control.

**Activity against T. cruzi**[19]

Rat skeletal myoblasts (L-6 cells) were seeded in 96-well microtiter plates at 2000 cells/well in 100 μL RPMI 1640 medium with 10% FBS and 2 mM l-glutamine. After 24 h the medium was removed and replaced by 100 μL per well containing 5000 trypomastigote forms of *T. cruzi* Tulahuen strain C2C4 containing the β-galactosidase (Lac Z) gene. After 48 h the medium was removed from the wells and replaced by 100 μL fresh medium with or without a serial drug dilution of eleven 3-fold dilution steps covering a range from 100 to 0.002 μg/mL. After 96 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterility. Then the substrate CPRG/Nonidet (50 μL) was added to all wells. A color reaction developed within 2–6 h and could be read photometrically at 540 nm. Data were analyzed with the graphic programme Softmax Pro (Molecular Devices), which calculated IC₅₀ values by linear regression and 4-parameter logistic regression from the sigmoidal dose inhibition curves. Benznidazole is used as control (IC₅₀ 0.5±0.2 μg/mL).

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Activity against *L. donovani* axenic amastigotes

Amastigotes of *L. donovani* strain MHOM/ET/67/L82 are grown as axenic culture at 37 °C in SM medium at pH 5.4 supplemented with 10% heat-inactivated fetal bovine serum under an atmosphere of 5% CO₂ in air. One hundred microlitres of culture medium with 10⁵ amastigotes from axenic culture with or without a serial drug dilution are seeded in 96-well microtitre plates. Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 μg/mL are prepared. After 70 h of incubation the plates are inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10 μL of resazurin (12.5 mg resazurin dissolved in 100 mL distilled water) are then added to each well and the plates incubated for another 2 h. Then the plates are read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wave length of 536 nm and an emission wave length of 588 nm. From the sigmoidal inhibition curves the IC₅₀ values are calculated by linear regression (Huber 1993) and 4-parameter logistic regression using SoftmaxPro software (Molecular Devices Cooperation, Sunnyvale, CA, USA).

Activity against *P. falciparum*

In vitro activity against erythrocytic stages of *P. falciparum* was determined using a ³H-hypoxanthine incorporation assay, using the drug sensitive NF54 strain and the standard drugs chloroquine (Sigma C6628) and artesunate (Sigma A3731). Compounds were dissolved in DMSO at 10 mg/ml and further diluted in medium before added to parasite cultures incubated in RPMI 1640 medium without hypoxanthine, supplemented with HEPES (5.94 g/L), NaHCO₃ (2.1 g/L), neomycin (100 U/mL), Albumax® (5 g/L) and washed human red cells A⁺ at 2.5% haematocrit (0.3% parasitaemia). Serial drug dilutions of eleven 3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. The 96-well plates were incubated in a humidified atmosphere at 37 °C; 4% CO₂, 3% O₂, 93% N₂. After 48 h 50 μL of ³H-hypoxanthine (=0.5 μCi) was added to each well of the plate. The plates were incubated for a further 24 h under the same conditions. The plates were then harvested with a Betaplate™ cell harvester (Wallac, Zurich, Switzerland), and the red blood cells transferred onto a glass fibre filter then washed with distilled water. The dried filters were inserted into a plastic foil with 10 ml of scintillation fluid, and counted in a Betaplate™ liquid scintillation counter (Wallac, Zurich, Switzerland). IC₅₀ values were calculated from sigmoidal inhibition curves by linear regression using Microsoft Excel. Chloroquine and artemisinin are used as control.

Antiproliferative and cytotoxic assays

In *vitro cytotoxicity with L-6 cells*

Assays were performed in 96-well microtiter plates, each well containing 100 μL of RPMI 1640 medium supplemented with 1% L-glutamine (200mM) and 10% fetal bovine serum, and 4000 L-6 cells (a primary cell line derived from rat skeletal myoblasts. Serial drug dilutions of eleven
3-fold dilution steps covering a range from 100 to 0.002 μg/mL were prepared. After 70 h of incubation the plates were inspected under an inverted microscope to assure growth of the controls and sterile conditions. 10 μL of resazurin was then added to each well and the plates incubated for another 2 h. Then the plates were read with a Spectramax Gemini XS microplate fluorometer (Molecular Devices Cooperation, Sunnyvale, CA, USA) using an excitation wavelength of 536 nm and an emission wave length of 588 nm. The IC$_{50}$ values were calculated by linear regression (Huber 1993) and 4-parameter logistic regression from the sigmoidal dose inhibition curves using SoftmaxPro software (Molecular Devices Cooperation, Sunnyvale, CA, USA). Podophyllotoxin (Sigma P4405) is used as control.

In vitro cytotoxicity with human umbilical vein endothelial cells

The antiproliferative and cytotoxic was performed according to the method reported by Krieg et al.\cite{15} All compounds were assayed using human umbilical vein endothelial cells HUVEC (ATCC CRL-1730), human chronic myeloid leukemia cells K-562 (DSM ACC 10) for their antiproliferative effects (GI50) as well as using human cervix carcinoma cells HeLa (DSM ACC 57) for their cytotoxic effects (CC50).

Cells and culture conditions

Cell proliferation and cytotoxicity assays are applied to assess the efficacy and potency of natural products. We used following cell lines:

| Cell line            | cell culture medium          |
|----------------------|------------------------------|
| Huvec (ATCC CRL-1730) | DMEM (CAMBREX 12-614F)      |
| K-562 (DSM ACC 10)    | RPMI 1640 (CAMBREX 12-167F) |
| HeLa (DSM ACC 57)     | RPMI 1640 (CAMBREX 12-167F).|

Cells were grown in the appropriate cell culture medium supplemented with 10 mL/L ultraglutamine 1 (Cambrex 17-605E/U1), 550 µL/L (50 mg/mL gentamicin sulfate (CAMBREX 17-518Z), and 10% heat inactivated fetal bovine serum (GIBCO Life Technologies 10270-106) at 37 °C in 5% CO$_2$ in high density polyethylene flasks (NUNC 156340).

To decide if the compounds have an antiproliferative and/or a cytotoxic effect on human cells, we measured the cytotoxicity (cell death) and the antiproliferative activity (retardation of cell proliferation). For this, we used different colorimetric assays for determining the number of viable cells. Under our experimental conditions, the optical density measured from the CellTiter-Blue® reagent and methylene blue assay is proportional to the number of viable cells.

Proliferation assay

The test substances were dissolved in DMSO before being diluted in cell culture medium. The adherent cells were harvested at the logarithmic growth phase after soft trypsinization using 0.25 % trypsin in PBS containing 0.02 % EDTA (Biochrom KG L2163). For each experiment,
approximately 10,000 cells were seeded with 0.1 mL culture medium per well of the 96-well microplates (HUVEC: flat bottomed NUNC 167008, K-562: round bottomed NUNC 163320). To test the antiproliferative effect of natural products on HUVEC and K-562, the cells were incubated for 72 hours in plates prepared with control and different dilutions of test substances. The GI_{50} values were defined as being where the inhibition of proliferation is 50 % compared to untreated control.

**Cytotoxicity assay**

For the cytotoxicity assay, HeLa cells were preincubated for 48 hours without the test substances. To test the cytotoxic effect of natural products on HeLa, the dilutions of the compounds were carried out carefully on the subconfluent monolayers of HeLa cells after the preincubation time. After incubation time, the cytolytic effect of compounds were analysed in compare to negative control. The 50 % cytotoxicity concentration (CC_{50}) was defined as the test compound concentration required for destruction in 50 % of the cell monolayer compared to untreated control.

**Condition of incubation**

The cells were incubated with dilutions of the natural products in microplates for 72 h at 37 °C in a humidified atmosphere and 5% CO_{2}. This incubation was found to be an optimum time for the evaluation of the cytotoxicity and the inhibition of cell proliferation by finding out the number of viable cells stained with CellTiter-Blue® reagent or methylene blue.

**Methods of evaluation**

For estimating the influence of natural products on cell proliferation of K-562, we determinate the numbers of viable cells present in multiwell plates via CellTiter-Blue® assay (PROMEGA). It uses the indicator dye resazurin to measure the metabolic capacity of cells as indicator of cell viability. Viable cells of untreated control retain the ability to reduce resazurin into resorufin, whereas nonviable cells rapidly lose metabolic capacity and do not reduce the indicator dye. The absorption maximum for resazurin is 605 nm and the absorption maximum for resorufin is 573 nm. Thus, the absorbance measurements at 570 nm and using 600 nm as a reference wavelength can be used to monitor results. Values are compared to blank well containing CellTiter-Blue® reagent without cells.

The adherent HUVEC and HeLa cells were fixed by glutaraldehyde (MERCK 1.04239.0250) and stained with a 0.05% solution of methylene blue (SERVA 29198) for 15 min. After gently washing, the stain was eluted by 0.2 mL of 0.33 N HCl in the wells. The optical densities were measured at 660 nm (methylene blue) in SUNRISE microplate reader (TECAN).

Under our experimental conditions, the signals from the methylene blue and CellTiter-Blue® reagent are proportional to the number of viable cells.
A repeat determination has been conducted in all experiments, four replicates were assayed. The calculations of the different values of GI$_{50}$ and CC$_{50}$ were performed with the software Magellan (TECAN).

**Table S7.** Antiproliferative effect and cytotoxicity of sphingofungin A-D (1 - 4) and compounds 18a - 18d.$^a$

| compound | antiproliferative effect | cytotoxicity |
|----------|-------------------------|--------------|
|          | HUVEC       | K-562       | HeLa       |
|          | GI$_{50}$   | GI$_{50}$   | CC$_{50}$  |
| [µg/mL]  | [µg/mL]     | [µg/mL]     |
| 1        | > 50        | > 50        | 41.4 (± 1.3) |
| 2        | > 50        | > 50        | > 50       |
| 3        | > 50        | > 50        | > 50       |
| 4        | > 50        | > 50        | > 50       |
| 18a      | > 50        | > 50        | > 50       |
| 18b      | > 50        | > 50        | > 50       |
| 18c      | 34.0 (± 5.2) | > 50        | 46.6 (± 2.4) |
| 18d      | > 50        | > 50        | > 50       |

$^a$ all compounds were dissolved in DMSO.
5. NMR Spectra

Figure S3. $^1$H-NMR of compound (R)-7 (600 MHz) in CDCl$_3$.

Figure S4. $^{13}$C-NMR of compound (R)-7 (150 MHz) in CDCl$_3$. 
Figure S5. $^1$H-NMR of compound (S)-7 (500 MHz) in CDCl$_3$.

Figure S6. $^{13}$C-NMR of compound (S)-7 (125 MHz) in CDCl$_3$. 
Figure S7. $^1$H-NMR of compound 12 (600 MHz) in CDCl$_3$. 
**Figure S8.** $^{13}$C-NMR of compound 12 (150 MHz) in CDCl$_3$.

**Figure S9.** $^1$H-NMR of compound 13 (600 MHz) in CDCl$_3$.

**Figure S10.** $^{13}$C-NMR of compound 13 (150 MHz) in CDCl$_3$. 
Figure S11. $^1$H-NMR of compound (2R)-14 (500 MHz) in C$_6$D$_6$.

Figure S12. $^{13}$C-NMR of compound (2R)-14 (125 MHz) in C$_6$D$_6$. 
Figure S13. $^1$H-NMR of compound (2S)-14 (500 MHz) in C$_6$D$_6$.

Figure S14. $^{13}$C-NMR of compound (2S)-14 (125 MHz) in C$_6$D$_6$. 

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Figure S15. $^1$H-NMR of compound 15 (500 MHz) in C$_6$D$_6$.

Figure S16. $^{13}$C-NMR of compound 15 (125 MHz) in C$_6$D$_6$. 
**Figure S17.** $^1$H-NMR of sphingofungin C (3) (600 MHz) in MeOD-$d_4$.

**Figure S18.** $^{13}$C-NMR of sphingofungin C (3) (150 MHz) in MeOD-$d_4$. 
**Figure S19.** $^1$H-NMR of sphingofungin D (4) (500 MHz) in MeOD-$d_4$.

**Figure S20.** $^{13}$C-NMR of sphingofungin D (4) (125 MHz) in MeOD-$d_4$. 
Figure S21. $^1$H-NMR of sphingofungin B (2) (500 MHz) in MeOD-$d_4$.

Figure S22. $^{13}$C-NMR of sphingofungin B (2) (125 MHz) in MeOD-$d_4$. 
Figure S23. $^1$H-NMR of sphingofungin A (1) (500 MHz) in MeOD-$d_4$.

Figure S24. $^{13}$C-NMR of sphingofungin A (1) (125 MHz) in MeOD-$d_4$. 
Figure S25. $^1$H-NMR of compound 17a (500 MHz) in C$_6$D$_6$.

Figure S26. $^{13}$C-NMR of compound 17a (125 MHz) in C$_6$D$_6$. 
Figure S27. $^1$H-NMR of compound 8b (500 MHz) in CDCl$_3$.

Figure S28. $^{13}$C-NMR of compound 8b (125 MHz) in CDCl$_3$. 
Figure S29. $^1$H-NMR of compound 17b (600 MHz) in C$_6$D$_6$.

Figure S30. $^{13}$C-NMR of compound 17b (150 MHz) in C$_6$D$_6$. 
Figure S31. $^{1}$H-NMR of compound 17c (500 MHz) in Cd$_6$.

Figure S32. $^{13}$C-NMR of compound 17c (125 MHz) in Cd$_6$. 
Figure S33. $^1$H-NMR of compound 17d (600 MHz) in C$_6$D$_6$.

Figure S34. $^{13}$C-NMR of compound 17d (150 MHz) in C$_6$D$_6$. 

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Figure S35. $^1$H-NMR of compound 18a (500 MHz) in MeOD-$d_4$.

Figure S36. $^{13}$C-NMR of compound 18a (125 MHz) in MeOD-$d_4$. 
Figure S37. $^1$H-NMR of compound 18b (500 MHz) in MeOD-$d_4$.

Figure S38. $^{13}$C-NMR of compound 18b (125 MHz) in MeOD-$d_4$. 

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Figure S39. \(^1\)H-NMR of compound 18c (500 MHz) in MeOD-\(d_4\).

Figure S40. \(^{13}\)C-NMR of compound 18c (125 MHz) in MeOD-\(d_4\).
Figure S41. $^1$H-NMR of compound 18d (500 MHz) in MeOD-$d_4$.

Figure S42. $^{13}$C-NMR of compound 18d (125 MHz) in MeOD-$d_4$.
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