Supplemental Material for

Exploiting Enzymatic Promiscuity to Engineer a Focused Library of Highly Selective Antifungal and Antiproliferative Aureothin Analogues

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Experimental Section

General material and methods. All reagents were purchased from commercial suppliers and used without further purification. NMR spectra (in $CDCl_3$) were measured on Bruker Avance DRX 300 and DPX 500 instruments. Chemical shifts are reported in ppm. When peak multiplicities are reported, the following abbreviations are used: s, singlet; d, doublet; t, triplet; m, multiplet; br, broadened. The NMR numbering system refers to the general structure shown in Figure 4. HR-MS (ESI) was recorded on a triple quadrupole mass spectrometer TSQ Quantum Ultra AM (Thermo Electron). LC-MS experiments were performed on a LCQ mass spectrometer equipped with an ESI source and an ion trap (Thermo Electron). Further analytical and preparative HPLC experiments were carried out on a Gilson machine. IR spectra were measured using a JASCO FT/IR-4100 spectrometer supplemented with the ATR system. Therefore, substances were charged as solids or thin films (highly concentrated solutions in volatile solvents). Optical rotation was determined with a JASCO P-1020 polarimeter supplemented with a thermostat (50 mm cuvette; c [g 100 mL^{-1}]). Analytical thin layer chromatography (TLC) was carried out on Merck silica gel 60 F254 TLC plates. TLC visualization was accomplished using 254/366 nm UV light or charring solutions of molybdatophosphoric acid. Preparative column chromatography was performed on (0.04-0.063 mm, 230-400 mesh) silica gel normal phase.

Plasmids and General DNA Procedures. DNA isolation, plasmid preparation, restriction digests, gel electrophoresis and ligation reactions were conducted according to standard methods. pBluescript II SK(-) (Stratagene, Amsterdam, NL), pGEM®-T Easy (Promega, Mannheim) and pMOSBlue (Amersham Biosciences, Freiburg) were the routine vectors for subcloning and preparation of DNA templates for sequencing. Restriction enzyme-digested DNA fragments were recovered from agarose gel by the GFX PCR DNA and Gel Band Purification Kit (Amersham).

Bacterial Strains and Culture Conditions. *S. lividans* ZX1 and *S. albus* served as host strains for all heterologous expression experiments. *S. lividans* ZX1 was cultured on R5 agar and YEME liquid medium for protoplast transformation and on MS (mannitol soya flour) for all other experiments. Transformants were selected with apramycin and/or thiostreptone according to standard protocols. *E. coli* strains DH5α and XL1 blue served as hosts for routine subcloning. *E. coli* strains were grown in LB medium supplemented with ampicillin (100 µg mL^{-1}), or apramycin (50 µg mL^{-1}) for selection of plasmids. For metabolite production, wild-type and mutant strains were cultivated in appropriate media for 5 days at 28 °C with shaking. Media composition: J: 100 g sucrose, 10 g yeast extract, 30 g tryptone soya broth, 10 g $MgCl_2(6H_2O)$, 1000 mL purified water; M10: 4 g glucose, 4 g yeast extract, 10 g malt extract, 1000 ml purified water, pH = 7.3; R2YE: 103 g sucrose, 0.25 g K_2SO_4, 10.12 g $MgCl_2(6H_2O)$, 10 g glucose, 0.1 g casaminosacids, 5 g yeast extract, 5.73 g TES-buffer, 2 mL trace element solution, 1000 mL purified water (trace element solution: 40 mg $ZnCl_2$, 200 mg $FeCl_3(6H_2O)$, 10 mg $CuCl_2(2H_2O)$, 10 mg $MnCl_2(4H_2O)$, 10 mg $Na_2B_4O_7(10H_2O)$, 10 mg $(NH_4)_6Mo_7O_{24}(4H_2O)$, 1000 mL purified water), after autoclaving addition of: 15 mL 20% L-proline, 10 mL 0.5% KH_2PO_4, 7 mL 1 N NaOH, 4 mL 5 M $CaCl_2(2H_2O)$; E1: 20 g glucose, 20 g soluble starch, 5 g Pharmamedia®, 2.5 g yeast extract, 3 g $CaCO_3$, 1 g NaCl, 1 g $MgSO_4(7H_2O)$, 1 g $K_2HPO_4(3H_2O)$, 1000 mL purified water, pH 7.5.

Construction of *aurF/aurH* null mutant. ORF *aurH* was inactivated by partial digestion of a vector, pHJ65 containing a truncated *aur* gene cassette (*aurAGHB*), with *Nsp*I. Then, the inactivated *aurH* was cut with *Sgf*I and the yielded 2.845 kb fragment was ligated into the *Sgf*I site of the pHJ79 vector (pSET152 derivative; *aurF* null mutant). The resulting plasmid, pMZ01, was introduced into *S. lividans* ZX1 and *S. albus* by PEG-induced protoplast transformation.

Complementation of *aurF/aurH* null mutant. The *aurF* gene including the native ribosome binding site (RBS) was amplified by PCR using primer FW (5' aat cta gaA TG CCA CGA CAC CGC GGG 3'; *Xba*I restriction site) and RV (5' aat cta gaA CGC GGC GTC GGG GTC AAC G 3'; *Xba*I restriction site). The PCR product was cloned into pGEM-T Easy Vector (Promega) for sequencing. The 1.16 kb *Xba*I fragment was then

ligated into the *Xba*I site of pHJ110 (*aurH*; pWHM4* derivative). The resulting construct, pMZ04, was cotransformed into *S. albus*::pMZ01 by PEG-induced protoplast transformation.

Shake flask culture of *S. albus*::pMZ01. 50 mL of medium (R2YE or E1) supplemented with apramycin sulphate (c = 30 µg mL^{-1}) for plasmid selection was inoculated with 50 µL spore suspension (*S. albus*::pMZ01) and incubated at 30 °C for 20-24 hours with shaking. An inoculum (2.5 mL) of the preculture was transferred into 50 mL fresh medium and continuously incubated at 30 °C. After 2 days of growing the culture was fed with a precursor dissolved in 25% aq DMSO under pulse feeding on 2 following days (6 µmol / 3 µmol, c = 1 mg 100 μL^{-1}, saponification with 1 M NaOH) and incubated for another 5 days on a rotary shaker. Mycelia and culture filtrate were extracted three times with an equal volume of EtOAc (80 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was dissolved in 1 mL MeOH and characterized via LC (Phenomenex Luna C18(2) 10 µm, 250 x 4.6 mm; eluent ACN/H_2O gradient, flow rate 1 mL min^{-1}).

Fermentation of *S. albus*::pMZ01. Fermentations were performed using 2 x 20 L (per 30 L fermentor) or 60 L media (75 L fermentor) depending on the secondary metabolite. For production *S. albus*::pMZ01 was grown with stirring at 28 °C in R2YE or E1 media supplemented with 30 mg L^{-1} apramycin sulphate for plasmid selection. A 3 day old seed culture of *S. albus*::pMZ01 (1/100) was used to inoculate the fermentation media and the approach was incubated for 2 days. Then, the culture was fed with the precursor dissolved in 25% aq DMSO under pulse feeding on 2 following days (0.12 mmol L^{-1} and 0.06 mmol L^{-1}, c = 1 mg 100 μL^{-1}, saponification with 1 M NaOH). After incubation for another 5 days the culture was neutralized with 1 N HCl and separated in mycelia und culture filtrate. After extraction with EtOAc the organic layers were dried with Na_2SO_4 and concentrated to dryness under reduced pressure. The crude extracts were subjected to chromatography on silica gel using a $CHCl_3$/MeOH gradient as eluent. All fractions containing the preferred product were combined and further purified by preparative RP-HPLC (Phenomenex Luna C18(2) 10 µm, 250 x 21.2 mm; eluent: ACN/H_2O gradient, flow rate 20 mL min^{-1}).

Biotransformation for α- and γ-pyrone methylation. ORF *aurI*, including the ribosome binding site, was PCR-amplified from genomic DNA of *S. thioluteus*.. Primers: *aurI*, forward: CCC GAC ACC GAC AGG AAC AAA ATG; *aurI*, reverse: CGC TCC CCC GGT ATT TGT CAG. The amplicons were subcloned as *Eco*RI fragments and introduced into the *Eco*RI site downstream of the ermE promoter of pWHM4*, yielding plasmid pHJ95 (*aurI*). A 1.8 kb *Eco*RI fragment was recovered from cosmid 9A6 and introduced into *Eco*RI digested pWHM4*, yielding plasmid pHJ72. pHJ72, pHJ95, and pWHM4* were introduced into *S. albus* (wt) via PEG mediated protoplast transformation. For feeding experiments, *S. albus*/pHJ72, *S. albus*/pHJ95 and *S. albus*/pWHM4* were grown in 50 mL R5 media (supplemented with 10 µL 25 mg mL^{-1} thiostreptone stock solution). After 1 day the substrate (nordeoxyaureothin, 1 mg in DMSO) was added and further incubated for 5 days. HPLC-MS analysis of the EtOAc extract of the culture broth was performed on an Agilent Technologies 1100 series LC/MSD equipped with a DAD, an electrospray ion source operating in positive mode and a quadrupole mass analyzer by using the column Knauer Eurospher 100 C-18 5µm (125 x 3 mm) and gradient elution (MeCN/0.1% HCO_2H-H_2O 0.05/99.5, in 16 min to MeCN/0.1% HCO_2H-H_2O 99.5/0.5, for 1 min at MeCN/0.1% HCO_2H-H_2O 99.5/0.5, in 5 min to MeCN/0.1% HCO_2H-H_2O 0.05/99.5) at a flow rate of 1 mL min^{-1}. Mass peaks corresponding to deoxyaureothin/isodeoxyaureothin could be detected when supplementing *S. albus*/pHJ72 and *S. albus*/pHJ95 cultures with nordeoxyaureothin, respectively. In culture of *S. albus*/pWHM4* only the administered substrates were found.

Cross-complementation of Δ*aurI* mutant with *encK*. Plasmid pHJ72 was introduced into *S. lividans* ZX1::pHJ47 by PEG mediated protoplast transformation. The resulting strain, *S. lividans* ZX1::pHJ47/pHJ72, was cultivated in 20 L M10 medium at 28 °C for 7 days. Mycelium and culture filtrate were separated and extracted with EtOAc. The extracts were dried with Na_2SO_4, filtrated and the solvent was removed under reduced pressure. Two subsequent open column chromatographic separations were performed (1st column: silica gel, $CHCl_3$:MeOH = 9:1; 2nd column: silica gel, $CHCl_3$:MeOH = 98:2). Selected fractions were pooled and subjected to preparative RP-HPLC (Macherey Nagel Nucleosil C18 100 5 µm, 250 x 21 mm; eluent: ACN/H_2O gradient; flow rate 10 mL min^{-1}).

Small-scale biotransformation using AurH (whole cell approach). 50 mL of J medium supplemented with thiostreptone (c = 5 µg mL^{-1}) for plasmid selection was inoculated with 50 µL spore suspension (*S. albus*/pHJ110) and incubated at 30 °C for 2 days with shaking. An inoculum (2.5 mL) of the preculture was transferred into 50 mL fresh M10 medium and continuously incubated at 30 °C. After 2 days of growing the culture was fed with a precursor dissolved in DMSO (1.3 µmol, c = 1 mg 25 μL^{-1}) and incubated for another 5 days on a rotary shaker. Mycelia and culture filtrate were extracted three times with an equal volume of EtOAc (80 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to dryness under reduced pressure. The residue was dissolved in 1 mL MeOH and characterized via LC (Phenomenex Luna C18(2) 10 µm, 250 x 4.6 mm; eluent: ACN/H_2O gradient, flow rate 1 mL min^{-1}).

Preparative-scale biotransformation using AurH (whole cell approach). Cultivations were performed using 8 x 250 mL batches. For production, *S. albus*/pHJ110 was grown with shaking at 30 °C in J media supplemented with 5 µg mL^{-1} thiostreptone for plasmid selection. After 2 days of growing an inoculum (12.5 mL) of the preculture was transferred into 250 mL fresh M10 media and the approach was incubated for 2 days. Then, the culture was fed with the precursor dissolved in DMSO (6.5 µmol, c = 1 mg 25 μL^{-1}). After incubation for another 5 days the culture was separated in mycelia und culture filtrate. After extraction with EtOAc the organic layers were dried with Na_2SO_4 and concentrated to dryness under reduced pressure. The crude extracts were subjected to chromatography on silica gel using a $CHCl_3$/MeOH gradient as eluent. All fractions containing the preferred product were combined and further purified by semipreparative RP-HPLC (Macherey Nagel Nucleosil C18 100 5 µm, 250 x 10 mm; eluent: ACN/H_2O gradient, flow rate 5 mL min^{-1}).

Physicochemical characterization of isolated aureothin derivatives.

7 (Deoxyaureonitrile) ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.82 (s, 3H, 3a-CH_3), 1.88 (d, *J*(H,H) = 1.22 Hz, 3H, 9a-CH_3), 1.91 (d, *J*(H,H) = 1.18 Hz, 3H, 11a-CH_3), 1.94 (s, 3H, 5a-CH_3), 2.4 (br t, *J*(H,H) = 7.53 Hz, 2H, 8-CH_2), 2.75 (br t, *J*(H,H) = 7.57 Hz, 2H, 7-CH_2), 3.93 (s, 3H, OMe), 5.75 (br s, 1H, 10-CH), 6.24 (br s, 1H, 12-CH), 7.31 (d, *J*(H,H) = 8.29 Hz, 2H, 14,18-CH), 7.57 (d, *J*(H,H) = 8.36 Hz, no determination of long range coupling constants, 2H, 15,17-CH) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 6.82 (1C, 3a-CH_3), 9.92 (1C, 5a-CH_3), 18.03 (1C, 9a-CH_3), 19.03 (1C, 11a-CH_3), 29.57 (1C, 7-CH_2), 37.72 (1C, 8-CH_2), 55.28 (1C, OMe), 99.49 (1C, 3-C), 109.56 (1C, 16-C-CN), 118.58 (1C, 5-C), 119.03 (1C, 13-C), 127.56 (1C, 12-CH), 129.4 (2C, 14,18-CH), 130.36 (1C, 10-CH), 131.86 (2C, 15,17-CH), 136.37 (1C, 9-C), 138.47 (1C, 11-C), 142.59 (1C, CN), 157.42 (1C, 6-C), 162.05 (1C, 2-C), 180.83 (1C, 4-C=O) ppm; IR: ν = 3045 (vw, =C-H), 3028 (w, =C-H), 2957/2929/2914/2882/2854/2834 (m-w, CH_3, CH_2), 2221 (s-m, C≡N), 1668 (s, C=O), 1619 (w, C=C), 1584 (vs, C=C), 1502 (w, C=C), 1461/1453/1415/1385/1373/1369 (m, CH_3, CH_2), 1335/1320/1250/1206/1164/1140/1043/1030/981 (m-w, C-O-C), 906 (m, =C-H), 855/833/799 (w, =C-H), 768 (m-w, =C-H), 704/665 (w, =C-H) cm^{-1}; HR-MS (ESI): calcd. for $C_{23}H_{26}N_1O_3$: 364.1907, observed: 364.1907.

8 (Fluorodeoxyaureothin) ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.83 (s, 3H, 3a-CH_3), 1.88 (d, *J*(H,H) = 1.14 Hz, 6H, 9a-CH_3, 11a-CH_3), 1.95 (s, 3H, 5a-CH_3), 2.38 (br t, *J*(H,H) = 7.54 Hz, 2H, 8-CH_2), 2.74 (br t, *J*(H,H) = 7.59 Hz, 2H, 7-CH_2), 3.94 (s, 3H, OMe), 5.74 (br s, 1H, 10-CH), 6.21 (br s, 1H, 12-CH), 6.99 (m, no determination of long range or H-F coupling constants, 2H, 15,17-CH), 7.2 (m, no determination of long range or H-F coupling constants, 2H, 14,18-CH) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 6.84 (1C, 3a-CH_3), 9.92 (1C, 5a-CH_3), 17.92 (1C, 9a-CH_3), 18.71 (1C, 11a-CH_3), 29.69 (1C, 7-CH_2), 37.76 (1C, 8-CH_2), 55.28 (1C, OMe), 99.49 (1C, 3-C), 114.96 (d, 2C, *J*(C,F) = 21.28 Hz, 15,17-CH), 118.54 (1C, 5-C), 128.1 (1C, 12-CH), 130.44 (d, 2C, *J*(C,F) = 7.85 Hz, 14,18-CH), 130.73 (1C, 10-CH), 133.94 (d, 1C, *J*(C,F) = 3.39 Hz, 13-C), 134.88 (1C, 9-C), 134.94 (d, 1C, *J*(C,F) = 1.36 Hz, 11-C), 157.65 (1C, 6-C), 161.32 (d, 1C, *J*(C,F) = 259.01 Hz, 16-C-F), 162.09 (1C, 2-C), 180.92 (1C, 4-C=O) ppm; IR: ν = 3033 (vw, =C-H), 2953/2925/2856 (m-w, CH_3, CH_2), 1740 (br w, =C-H), 1666 (vs, C=O), 1594 (vs, C=C). 1539 (vw, C=C). 1505 (s, C=C), 1460/1409/1376 (m, CH_3, CH_2), 1227 (m, C-F), 1317/1246/1158/1094/1031(br)/984 (m-w, C-O-C), 889/836/768/670 (m-w, =C-H) cm^{-1}; HR-MS (ESI): calcd. for $C_{22}H_{26}FO_3$: 357.1860, observed: 357.1861.

9 (Chlorodeoxyaureothin) ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.83 (s, 3H, 3a-CH_3), 1.88 (t, *J*(H,H) = 1.63 Hz, 6H, 9a-CH_3, 11a-CH_3), 1.95 (s, 3H, 5a-CH_3), 2.39 (br t, *J*(H,H) = 7.54 Hz, 2H, 8-CH_2), 2.74 (br t, *J*(H,H) = 7.6 Hz, 2H, 7-CH_2), 3.94 (s, 3H, OMe), 5.74 (br s, 1H, 10-CH), 6.2 (br s, 1H, 12-CH), 7.16 (d, *J*(H,H) = 8.46 Hz, no determination of long range coupling constants, 2H, 14,18-CH), 7.26 (d, *J*(H,H) = 8.54 Hz, no determination of long range coupling constants, 2H, 15,17-CH) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 6.8 (1C, 3a-CH_3), 9.88 (1C, 5a-CH_3), 17.91 (1C, 9a-CH_3), 18.77 (1C, 11a-CH_3), 29.63 (1C, 7-CH_2), 37.72 (1C, 8-CH_2), 55.24 (1C, OMe), 99.47 (1C, 3-C), 118.51 (1C, 5-C), 127.96 (1C, 12-CH), 128.2 (2C, 15,17-CH), 130.44 (2C, 14,18-CH), 130.63 (1C, 10-CH), 131.94 (1C, 16-C-Cl), 135.14 (1C, 9-C), 135.72 (1C, 11-C), 136.3 (1C, 13-C), 157.56 (1C, 6-C), 162.04 (1C, 2-C), 180.87 (1C, 4-C=O) ppm; IR: ν = 3021 (vw, =C-H), 2952/2924/2854 (m-w, CH_3, CH_2), 1732 (br w, =C-H), 1666 (vs, C=O), 1596 (vs, C=C), 1539 (vw, C=C), 1489 (m, C=C), 1459/1408/1376 (m, CH_3, CH_2), 1342/1317/1246/1162/1136/1011/984 (m-w, C-O-C), 1091 (m, C-Cl), 889/852/800 (w, =C-H), 768 (m, =C-H), 723 (w, =C-H), 670 (br w, =C-H) cm^{-1}; HR-MS (ESI): calcd. for $C_{22}H_{26}ClO_3$: 373.1565, observed: 373.1566.

10 (Bromodeoxyaureothin) ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.83 (s, 3H, 3a-CH_3), 1.87 (m, 6H, 9a-CH_3, 11a-CH_3), 1.94 (s, 3H, 5a-CH_3), 2.38 (br t, *J*(H,H) = 7.54 Hz, 2H, 8-CH_2), 2.74 (br t, *J*(H,H) = 7.59 Hz, 2H, 7-CH_2), 3.93 (s, 3H, OMe), 5.74 (br s, 1H, 10-CH), 6.18 (br s, 1H, 12-CH), 7.1 (d, *J*(H,H) = 8.4 Hz, no determination of long range coupling constants, 2H, 14,18-CH), 7.42 (d, *J*(H,H) = 8.45 Hz, no determination of long range coupling constants, 2H, 15,17-CH) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 6.84 (1C, 3a-CH_3), 9.92 (1C, 5a-CH_3), 17.96 (1C, 9a-CH_3), 18.82 (1C, 11a-CH_3), 29.66 (1C, 7-CH_2), 37.77 (1C, 8-CH_2), 55.28 (1C, OMe), 99.49 (1C, 3-C), 118.54 (1C, 5-C), 120.1 (1C, 16-C-Br), 128.03 (1C, 12-CH), 130.52 (2C, 14,18-CH), 130.67 (1C, 10-CH), 131.19 (2C, 15,17-CH), 135.24 (1C, 9-C), 135.88 (1C, 11-C), 136.8 (1C, 13-C), 157.59 (1C, 6-C), 162.07 (1C, 2-C), 180.89 (1C, 4-C=O) ppm; IR: ν = 3020 (vw, =C-H), 2950/2924/2856 (m-w, CH_3, CH_2), 1739 (br w, =C-H), 1666 (vs, C=O), 1595 (vs, C=C), 1538 (vw, C=C), 1485 (m-w, C=C), 1458/1408/1376 (m, CH_3, CH_2), 1341/1316/1246/1162/1136/1030(br)/1008/984 (m-w, C-O-C), 1072 (m-w, C-Br), 889/851/830/820/789 (w, =C-H), 768 (m, =C-H), 679 (br w, =C-H) cm^{-1}; HR-MS (ESI): calcd. for $C_{22}H_{26}BrO_3$: 417.1060, observed: 417.1064.

11 (Iododeoxyaureothin) ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.83 (s, 3H, 3a-CH_3), 1.88 (m, 6H, 9a-CH_3, 11a-CH_3), 1.94 (s, 3H, 5a-CH_3), 2.38 (br t, 2H, *J*(H,H) = 7.55 Hz, 8-CH_2), 2.74 (br t, 2H, *J*(H,H) = 7.59 Hz, 7-CH_2), 3.93 (s, 3H, OMe), 5.74 (br s, 1H, 10-CH), 6.16 (br s, 1H, 12-CH), 6.97 (d, *J*(H,H) = 8.28 Hz, no determination of long range coupling constants, 2H, 14,18-CH), 7.62 (d, *J*(H,H) = 8.38 Hz, no determination of long range coupling constants, 2H, 15,17-CH) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 6.85 (1C, 3a-CH_3), 9.93 (1C, 5a-CH_3), 17.97 (1C, 9a-CH_3), 18.84 (1C, 11a-CH_3), 29.67 (1C, 7-CH_2), 37.79 (1C, 8-CH_2), 55.29 (1C, OMe), 91.49 (1C, 16-C-I), 99.5 (1C, 3-C), 118.55 (1C, 5-C), 128.13 (1C, 12-CH), 130.69 (1C, 10-CH), 130.79 (2C, 14,18-CH), 135.29 (1C, 9-C), 136.03 (1C, 11-C), 137.18 (2C, 15,17-CH), 137.39 (1C, 13-C), 157.58 (1C, 6-C), 162.07 (1C, 2-C), 180.89 (1C, 4-C=O) ppm; IR: ν = 3067/3055 (vw, =C-H), 3041/3020 (vw, =C-H), 2995/2953/2924/2853 (m-w, CH_3, CH_2), 1747 (br vw, =C-H), 1665 (vs, C=O), 1610 (vw, C=C), 1582 (vs, C=C), 1534 (vw, C=C), 1467/1415/1373 (s-m, CH_3, CH_2), 1320/1248/1164/1006/978 (m-w, C-O-C), 1065 (w, C-I), 909 (w, =C-H), 891 (m, =C-H), 851 (w, =C-H), 797/764 (m, =C-H), 719 (w, =C-H), 670 (br w, =C-H) cm^{-1}; HR-MS (ESI): calcd. for $C_{22}H_{26}IO_3$: 465.0921, observed: 465.0924.

14 (Phenyldeoxyaureothin) ^{1}H-NMR (500 MHz, $CDCl_3$): δ 1.84 (s; 3H; 3a-CH_3); 1.9 (s; 3H; 9a-CH_3); 1.902 (s; 3H; 11a-CH_3); 1.96 (s; 3H; 5a-CH_3); 2.39 (br t; *J*(H,H) = 7.54 Hz; 2H; 8-CH_2); 2.75 (br t; *J*(H,H) = 7.59 Hz; 2H; 7-CH_2); 3.94 (s; 3H; OMe); 5.77 (br s; 1H; 10-CH); 6.28 (br s; 1H; 12-CH); 7.25 (m; 14,15,17,18-CH) ppm. ^{13}C-NMR (125 MHz, $CDCl_3$): δ 6.87 (1C; 3a-CH_3); 9.94 (1C; 5a-CH_3); 17.95 (1C; 9a-CH_3); 18.85 (1C; 11a-CH_3); 29.74 (1C; 7-CH_2); 37.82 (1C; 8-CH_2); 55.3 (1C; OMe); 99.53 (1C; 3-C); 118.56 (1C; 5-C); 128.11 (2C; 15,17-CH); 128.93 (1C; 13-C); 128.97 (2C; 14,18-CH); 129.27 (1C; 12-CH); 130.94 (1C; 10-CH); 134.81 (1C; 9-C); 135.07 (1C; 11-C); 137.95 (1C; 16-CH); 157.72 (1C; 6-C); 162.12 (1C; 2-C); 180.98 (1C; 4-C=O) ppm. IR: ν = 2955/2925/2853 (w, CH_3, CH_2); 1666 (s, C=O); 1597 (s, C=C); 1541 (vw, C=C);

1458/1410/1377 (m, CH_3, CH_2); 1318/1247/1220/1163/1137/1030/983 (m-w, C-O-C); 918/879 (vw, =C-H); 770 (vs, =C-H); 745 (w, =C-H); 698 (s-m, =C-H); 669 (m, =C-H) cm^{-1}. HRMS (ESI) calcd. for $C_{22}H_{27}O_3$: 339.1955; observed: 339.1966.

15 (2-Naphthodeoxyaureothin) ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.85 (s, 3H, 3a-CH_3), 1.94 (d, *J*(H,H) = 1.28 Hz, 3H, 9a-CH_3), 1.97 (s, 3H, 5a-CH_3), 2.01 (d, *J*(H,H) = 1.2 Hz, 3H, 11a-CH_3), 2.41 (br t, *J*(H,H) = 7.56 Hz, 2H, 8-CH_2), 2.76 (br t, *J*(H,H) = 7.59 Hz, 2H, 7-CH_2), 3.95 (s, 3H, OMe), 5.82 (br s, 1H, 10-CH), 6.43 (br s, 1H, 12-CH), 7.42 (m, 3H, Ar-H`s), 7.69 (br s, 1H, Ar-H), 7.79 (m, 3H, Ar-H`s) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 6.83 (1C, 3a-CH_3), 9.9 (1C, 5a-CH_3), 17.98 (1C, 9a-CH_3), 18.97 (1C, 11a-CH_3), 29.68 (1C, 7-CH_2), 37.8 (1C, 8-CH_2), 55.26 (1C, OMe), 99.43 (1C, 3-C), 118.48 (1C, 5-C), 125.6/126.01/127.48/127.8 (4C, Ar–CH`s), 129.27 (1C, 12-CH), 130.93 (1C, 10-CH), 131.99/133.31/135.42 (3C, Ar- C`s), 134.94 (1C, 9-C), 135.52 (1C, 11-C), 157.66 (1C, 6-C), 162.07 (1C, 2-C), 180.89 (1C, 4-C=O) ppm; IR: ν = 3052 (w, =C-H), 2951/2925/2855 (m-w, CH_3, CH_2), 1666 (s, C=O), 1593 (vs, C=C), 1538 (vw, C=C), 1504 (vw, C=C), 1459/1409/1376 (m, CH_3, CH_2), 1341/1317/1246/1182/1163/1137/1031(br)/984 (m-w, C-O-C), 902 (w, =C-H), 860 (vw, =C-H), 818 (w, =C-H), 768/750 (m, =C-H), 667 (br vw, =C-H) cm^{-1}; HR-MS (ESI): calcd. for $C_{26}H_{29}O_3$: 389.2117, observed: 389.2123.

17 (Isodeoxyaureothin). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.9 (s, 3H, C(C**H**$_3$)-CH_2), 1.93 (s, 3H, CH2-CO-C(C**H**$_3$)), 1.97 (s, 3H, Ar-CH=C(C**H**$_3$)), 2.05 (s, 3H, C(C**H**$_3$)-C=O), 2.41 (t, J = 7.75 Hz, 2H, C**H**$_2$-CH_2-CO), 2.67 (m, Hz, 2H, CO-C**H**$_2$), 3.8 (s, 3H, O-C**H**$_3$), 5.79 (s, 1H, C**H**=C(CH3)), 6.32 (s, 1H, Ar-C**H**=), 7.37 (d, J = 10.97 Hz, 2H, Ar-C**H**), 8.17 (d, J = 11.37 Hz, 2H) Ar-C**H** (C**H**-C(NO_2)) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 10.22 (1C, CH2-CO-C(**C**H$_3$)), 10.22 (1C, C(**C**H$_3$)-C=O), 18.54 (1C, C(**C**H$_3$)-CH_2), 19.18 (1C, Ar-CH=C(**C**H$_3$)), 29.92 (1C, CO-**C**H$_2$) 38.15 (1C, **C**H$_2$-CH_2-CO), 60.20 (1C, O-**C**H$_3$), 109.5 (1C, **C**-CO(CH_3)), 110 (1C, **C**-C=O), 123.4 (2C, Ar-**C**H), 127.17 (1C Ar-**C**H=C(CH_3)), 129.46 (2C, Ar-**C**H-C(NO_2)), 130.22 (1C C(CH_3)-**C**=C(CH_3)), 137.29 (1C, **C**-CH_2-CH_2), 139.07 (1C, Ar-CH=**C**(CH_3)), 144.62 (1C, Ar-**C**), 146 (1C, **C**-NO_2), 158.03 (1C, CH_2-**C**O), 165 (1C, **C**=O) ppm. HR-MS (ESI): calcd. for $C_{22}H_{26}N_1O_5$: 384.1805, observed: 384.1814

20 (7-Hydroxydeoxyisoaureothin). $[\alpha]_D^{20}$ = +17.77 (c = 0.132 in MeOH). ^{1}H-NMR (300 MHz, $CDCl_3$): δ 1.92 (s, 3H, C(C**H**$_3$)-CH_2), 1.97 (s, 3H, Ar-CH=C(C**H**$_3$)), 1.98 (s, 3H, CH(OH)-CO-C(C**H**$_3$)), 2.05 (s, 3H, C(C**H**$_3$)-C=O), 2.53 (d, J = 5.5 Hz, 1 H) C**H**$_2$-CH_2-CO), 2.61 (d, J = 8.26 Hz, 1H, C**H**$_2$-CH_2-CO), 3.82 (s, 3H, O-C**H**$_3$), 4.82 (dd, J = 6.18, J = 7.92 Hz, 1H, CO-C**H**$_2$), 5.87 (s, 1H, C**H**=C(CH3)), 6.34 (s, 1H, Ar-C**H**=), 7.39 (d, J = 8.65 Hz, 2H, Ar-C**H**), 8.06 (d, J = 8.86 Hz, 2H) Ar-C**H** (C**H**-C(NO_2)) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ 9,54 (1C, CH2-CO-C(**C**H$_3$)), 10.42 (1C, C(**C**H$_3$)-C=O), 18.54 (1C, C(**C**H$_3$)-CH_2), 19.18 (1C, Ar-CH=C(**C**H$_3$)), 46.56 (1C, **C**H$_2$-C(OH)), 60.44 (1C, O-**C**H$_3$), 67.02 (1C, **C**(OH)), 110.2 (1C, **C**-CO(CH_3)), 110.9 (1C, **C**-C=O), 123.53 (2C, Ar-**C**H), 127.67 (1C Ar-**C**H=C(CH_3)), 129.5 (2C, Ar-**C**H-C(NO_2)), 133.09 (1C, C(CH3)-**C**=C(CH_3)), 133.67 (1C, **C**-CH_2-C(OH)), 139.07 (1C, Ar-CH=**C**(CH_3)), 144.62 (1C, Ar-**C**), 146 (1C, **C**-NO_2), 156.2 (1C, CH_2-**C**O), 165 (1C, **C**=O) ppm. HR-MS(ESI): calcd. for $C_{22}H_{26}N_1O_6$: 400.1755, observed: 400.1771.

22 (Fluoroaureothin) $[\alpha]_D^{20}$ = - 23.0 (c 0.224 in MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.83 (s, 3H, 3a-CH_3), 1.96 (s, 3H, 11a-CH_3), 2.01 (s, 3H, 5a-CH_3), 2.89 (br dd, *J*(H,H) = 6.12/15.86 Hz, 1H, 8-C**H$_A$**H$_B$), 3.01 (br dd, *J*(H,H) = 7.14/15.9 Hz, 1H, 8-CH$_A$**H$_B$**), 3.92 (s, 3H, OMe), 4.71 (br d, *J*(H,H) = 13.93 Hz, 1H, 9a-C**H$_A$**H$_B$), 4.83 (br d, *J*(H,H) = 13.93 Hz, 1H, 9a-CH$_A$**H$_B$**), 5.11 (t, *J*(H,H) = 6.86 Hz, 1H, 7-CH), 6.13 (br s, 1H, 10-CH), 6.27 (br s, 1H, 12-CH), 7.01 (t, *J*(H,H) = 8.62, no determination of long range or H-F coupling constants, 2H, 15,17-CH), 7.19 (m, 2H, 14,18-CH) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 6.88 (1C, 3a-CH_3), 9.4 (1C, 5a-CH_3), 17.36 (1C, 11a-CH_3), 38.21 (1C, 8-CH_2), 55.24 (1C, OMe), 70.1 (1C, 9a-CH_2), 73.25 (1C, 7-CH), 99.97 (1C, 3-C), 115.16 (d, 2C, *J*(C,F) = 21.5 Hz, 15,17-CH), 119.98 (1C, 5-C), 126.38 (1C, 10-CH), 129.46 (1C, 12-CH), 130.58 (d, 2C, *J*(C,F) = 7.92 Hz, 14,18-CH), 133.48 (d, 1C, *J*(C,F) = 3.4 Hz, 13-C), 134.73 (1C, 11-C), 138.18 (1C, 9-C), 155.02 (1C, 6-C), 161.55 (d, 1C, *J*(C,F) = 246.89 Hz, 16-C-F), 162.09 (1C, 2-C), 180.6 (1C, 4-C=O) ppm; IR: ν = 2957/2920/2865 (w, CH_3, CH_2), 1748 (br w, =C-H), 1670 (s, C=O), 1608 (vs, C=C), 1539 (vw, C=C), 1505 (s-m, C=C), 1456/1413/1377/1369 (m-w, CH_3, CH_2), 1221 (m-w, C-F), 1321/1250/1181/1156/1047/1031/986/970 (m-w, C-O-C), 926 (vw, =C-H), 891/816/766/733 (w, =C-H), 665 (vw, =C-H) cm^{-1}; HR-MS (ESI): calcd. for $C_{22}H_{24}FO_4$: 371.1659, observed: 371.1643.

23 (Chloroaureothin) $[\alpha]_D^{20}$ = + 25.7 (c 0.638 in MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.83 (s, 3H, 3a-CH_3), 1.96 (s, 3H, 11a-CH_3), 2.0 (s, 3H, 5a-CH_3), 2.89 (br dd, *J*(H,H) = 5.69/15.74 Hz, 1H, 8-C**H$_A$**H$_B$), 3.01 (br dd, *J*(H,H) = 7.02/15.78 Hz, 2H, 8-CH$_A$**H$_B$**), 3.91 (s, 3H, OMe), 4.7 (br d, *J*(H,H) = 13.93 Hz, 2H, 9a-C**H$_A$**H$_B$), 4.82 (br d, *J*(H,H) = 13.95 Hz, 1H, 9a-CH$_A$**H$_B$**), 5.1 (t, *J*(H,H) = 6.82 Hz, 1H, 7-CH), 6.13 (br s, 1H, 10-CH), 6.25 (br s, 1H, 12-CH), 7.15 (d, *J*(H,H) = 8.23 Hz, 2H, 14,18-CH), 7.28 (d, *J*(H,H) = 8.27 Hz, 2H, 15,17-CH) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 6.87 (1C, 3a-CH_3), 9.38 (1C, 5a-CH_3), 17.43 (1C, 11a-CH_3), 38.2 (1C, 8-CH_2), 55.23 (1C, OMe), 70.08 (1C, 9a-CH_2), 73.24 (1C, 7-CH), 99.95 (1C, 3-C), 119.98 (1C, 5-C), 126.31 (1C, 10-CH), 128.38 (2C, 15,17-CH), 129.3 (1C, 12-CH), 130.25 (2C, 14,18-CH), 132.46 (1C, 16-C-Cl), 135.45 (1C, 11-C), 135.87 (1C, 13-C), 138.58 (1C, 9-C), 154.95 (1C, 6-C), 162.07 (1C, 2-C), 180.57 (1C, 4-C=O) ppm; IR: ν = 2956/2920/2855 (m-w, CH_3, CH_2), 1736 (br vw, =C-H), 1668 (s, C=O), 1606 (vs, C=C), 1536 (vw, C=C), 1489 (m-w, C=C), 1457/1413/1368 (m, CH_3, CH_2), 1321/1252/1182/1157/1045/1030/1007/986/969 (s-m, C-O-C), 1090 (m, C-Cl), 925 (vw, =C-H), 897/805/766/733 (w, =C-H), 706 (vw, =C-H), 665 (w-vw, =C-H) cm^{-1}; HR-MS (ESI): calcd. for $C_{22}H_{24}ClO_4$: 387.1358, observed: 387.1352.

24 (Bromoaureothin) $[\alpha]_D^{20}$ = + 27.4 (c 0.48 in MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.82 (s, 3H, 3a-CH_3), 1.95 (br s, 3H, 11a-CH_3), 2.0 (s, 3H, 5a-CH_3), 2.89 (br dd, *J*(H,H) = 6.15/15.93 Hz, 1H, 8-C**H$_A$**H$_B$), 3.01 (br dd, *J*(H,H) = 6.64/15.94 Hz, 1H, 8-CH$_A$**H$_B$**), 3.91 (s, 3H, OMe), 4.7 (br d, *J*(H,H) = 13.93 Hz, 1H, 9a-C**H$_A$**H$_B$), 4.82 (br d, *J*(H,H) = 13.99 Hz, 1H, 9a-CH$_A$**H$_B$**), 5.1 (t, *J*(H,H) = 6.88 Hz, 1H, 7-CH), 6.13 (br s, 1H, 10-CH), 6.23 (br s, 1H, 12-CH), 7.09 (d, *J*(H,H) = 8.4 Hz, no determination of long range coupling constants, 2H, 14,18-CH), 7.44 (d, *J*(H,H) = 8.44 Hz, no determination of long range coupling constants, 2H, 15,17-CH) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 6.87 (1C, 3a-CH_3), 9.39 (1C, 5a-CH_3), 17.44 (1C, 11a-CH_3), 38.21 (1C, 8-CH_2), 55.23 (1C, OMe), 70.09 (1C, 9a-CH_2), 73.24 (1C, 7-CH), 99.96 (1C, 3-C), 119.99 (1C, 5-C), 120.59 (1C, 16-C-Br), 126.32 (1C, 10-CH), 129.33 (1C, 12-CH), 130.57 (2C, 14.18-CH), 131.33 (2C, 15,17-CH), 135.55 (1C, 11-C), 136.32 (1C, 13-C), 138.64 (1C, 9-C), 154.93 (1C, 6-C), 162.07 (1C, 2-C), 180.58 (1C, 4-C=O) ppm; IR: ν = 2954/2921/2856 (m-w, CH_3, CH_2), 1747 (br vw, =C-H), 1668 (s, C=O), 1605 (vs, C=C), 1536 (vw, C=C), 1486 (w, C=C), 1457/1412/1377/1368 (m, CH_3, CH_2), 1320/1251/1182/1158/1044/1032/11004/986/970 (s-m, C-O-C), 1073 (w, C-Br), 925 (vw, =C-H), 896/802/766/732 (w, =C-H), 665 (vw, =C-H) cm^{-1}; HR-MS (ESI): calcd. for $C_{22}H_{24}BrO_4$: 431.0852, observed: 431.0850.

25 (Iodoaureothin) $[\alpha]_D^{20}$ = + 28.5 (c 0.517 in MeOH); ^{1}H-NMR (300 MHz, $CDCl_3$): δ = 1.83 (s, 3H, 3a-CH_3), 1.96 (d, *J*(H,H) = 1.13 Hz, 3H, 11a-CH_3), 2.0 (s, 3H, 5a-CH_3), 2.89 (br dd, *J*(H,H) = 6.17/6.48/16.12 Hz, 1H, 8-C**H$_A$**H$_B$), 3.01 (br dd, *J*(H,H) = 7.31/15.95 Hz, 1H, 8-CH$_A$**H$_B$**), 3.91 (s, 3H, OMe), 4.69 (br d, *J*(H,H) = 14.0 Hz, 1H, 9a-C**H$_A$**H$_B$), 4.82 (br d, *J*(H,H) = 14.08 Hz, 1H, 9a-CH$_A$**H$_B$**), 5.1 (t, *J*(H,H) = 6.72 Hz, 1H, 7-CH), 6.12 (br s, 1H, 10-CH), 6.22 (br s, 1H, 12-CH), 6.96 (d, *J*(H,H) = 8.28 Hz, no determination of long range coupling constants, 2H, 14,18-CH), 7.64 (d, *J*(H,H) = 8.41 Hz, no determination of long range coupling constants, 2H, 15,17-CH) ppm; ^{13}C-NMR (75 MHz, $CDCl_3$): δ = 6.88 (1C, 3a-CH_3), 9.4 (1C, 5a-CH_3), 17.46 (1C, 11a-CH_3), 38.23 (1C, 8-CH_2), 55.25 (1C, OMe), 70.1 (1C, 9a-CH_2), 73.26 (1C, 7-CH), 92.03 (1C, 16-C-I), 100.0 (1C, 3-C), 120.01 (1C, 5-C), 126.34 (1C, 10-CH), 129.44 (1C, 12-CH), 130.81 (2C, 14,18-CH), 135.68 (1C, 11-C), 136.92 (1C, 13-C), 137.33 (2C, 15,17-CH), 138.7 (1C, 9-C), 154.95 (1C, 6-C), 162.09 (1C, 2-C), 180.59 (1C, 4-C=O) ppm; IR: ν = 2950/2920/2859 (m-w, CH_3, CH_2), 1747 (br vw, =C-H), 1668 (s, C=O), 1605 (vs, C=C), 1536 (vw, C=C), 1482 (w, C=C), 1456/1411/1377/1367 (m, CH_3, CH_2), 1320/1251/1181/1158/1043/1033/1000/986 (s-m, C-O-C), 1063 (vw, C-I), 925 (vw, =C-H), 896/800/766/732 (w, =C-H), 665 (vw, =C-H) cm^{-1}; HR-MS (ESI): calcd. for $C_{22}H_{24}IO_4$: 479.0714, observed: 479.0694.

26 (2-Naphthoaureothin) $[\alpha]_D^{20}$ = + 37.3 (c 0.306 in MeOH); ^{1}H-NMR (500 MHz, $CDCl_3$): δ = 1.84 (s, 3H, 3a-CH_3), 2.02 (s, 3H, 5a-CH_3), 2.08 (br s, 11a-CH_3), 2.92 (br dd, *J*(H,H) = 5.59/5.98/15.84 Hz, 1H, 8-C**H$_A$**H$_B$), 3.05 (br dd, *J*(H,H) = 6.54/15.87 Hz, 1H, 8-CH$_A$**H$_B$**), 3.94 (s, 3H, OMe), 4.76 (br d, *J*(H,H) = 13.98 Hz, 1H, 9a-C**H$_A$**H$_B$), 4.88 (br d, *J*(H,H) = 13.96 Hz, 1H, 9a-CH$_A$**H$_B$**), 5.13 (t, *J*(H,H) = 6.8 Hz, 1H, 7-CH), 6.21 (br s, 1H, 10-CH), 6.48 (br s, 1H, 12-CH), 7.36 (m, 1H, Ar-CH), 7.44 (m, 2H, Ar-CH`s), 7.68 (br s, 1H, Ar-CH), 7.79 (m, 3H, Ar-CH`s) ppm; ^{13}C-NMR (125 MHz, $CDCl_3$): δ = 6.89 (1C, 3a-CH_3), 9.41 (1C, 5a-CH_3), 17.63 (1C, 11a-CH_3), 38.27 (1C, 8-CH_2), 55.28 (1C, OMe), 70.18 (1C, 9a-CH_2), 73.24 (1C, 7-CH), 99.96 (1C, 3-C), 119.95 (1C, 5-C), 126.65 (1C, 10-CH), 125.93/126.23/127.33/127.6/127.7/127.88 (7C, Ar-CH`s), 130.69 (1C, 12-CH), 132.2/133.28 (2C, Ar-C`s), 134.98 (1C, 13-C), 135.25 (1C, 11-C), 138.21 (1C, 9-C), 155.1 (1C, 6-C), 162.12 (1C, 2-C), 180.63 (1C, 4-C=O) ppm; IR: ν = 3052 (w, =C-H), 2957/2924/2855 (m-w, CH_3, CH_2), 1747 (w, =C-H), 1662 (s, C=O), 1584 (vs, C=C), 1537 (vw, C=C), 1505 (vw, C=C), 1463/1412/1374 (m, CH_3, CH_2), 1324/1257/1161/1049/1033/976 (s-m, C-O-C), 898 (w, =C-H), 862 (vw, =C-H), 815/767/749 (m-w, =C-H), 668 (vw, =C-H) cm^{-1}; HR-MS (ESI): calcd. for $C_{26}H_{26}O_4$: 403.1904, observed: 403.1891.

Table S1. Comparison of NMR data for α- and γ-pyrones.

	Isodeoxyaureothin (**17**)		Deoxyaureothin (**6**)	
C-Atom numbering	^{13}C-NMR 75 MHz	^{1}H-NMR 300 MHz	^{13}C-NMR 75 MHz	^{1}H-NMR 300 MHz
2	**165.0**	-	**162.1**	-
2a	-	-	55.3	3.95 (s, 3H)
3	110.0	-	99.6	-
3a	10.2	2.05 (s, 3H)	6.9	1.84 (s, 3H)
4	**168.0**	-	**180.9**	-
4a	60.2	3.8 (s, 3H)	-	-
5	109.5	-	118.7	-
5a	10.1	1.93 (s, 3H)	10.0	1.96 (s, 3H)
6	158.0	-	157.4	-
7*	29.9	2.67 (m, Hz, 2H)	29.6	2.77 (t, *J* = 7.5Hz, 2H)
8*	38.2	2.41 (t, *J* = 7.75 Hz, 2H)	37.8	2.42 (t, *J* = 7.5Hz, 2H)
9*	137.3	-	136.7	-
9a	18.5	1.9 (s, 3H)	18.1	1.96 (s, 3H)
10*	130.2	5.79 (s, 1H)	130.4	5.78 (s, 1H)
11	139.1	-	139.3	-
11a	19.2	1.97 (s, 3H)	19.2	1.91 (s, 3H, 11-CH3)
12	127.2	6.32 (s, 1H)	127.3	6.30 (s, 1H)
13	144.6	-	144.7	-
14*	129.5	7.37 (d, *J* = 8.7 Hz, 1H)	129.5	7.38 (d, *J* = 8.7 Hz, 1H)
15*	123.4	8.17 (d, *J* = 8.9 Hz, 1H)	123.5	8.17 (d, *J* = 8.8 Hz, 1H)
16	146.0	-	145.9	-
17*	123.4	8.17 (d, *J* = 8.9 Hz, 1H)	123.5	8.17 (d, *J* = 8.8 Hz, 1H)
18*	129.5	7.37 (d, *J* = 8.7 Hz, 1H)	129.5	7.38 (d, *J* = 8.7 Hz, 1H)

* minor stereoisomers (*E*/*Z*) could be detected

NMR-Spectra

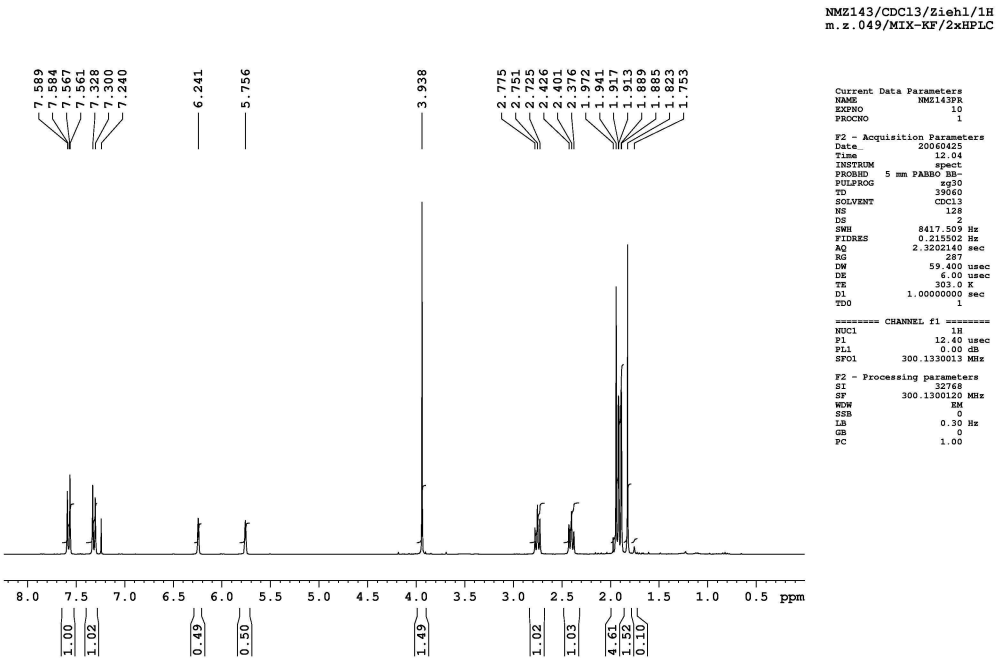

Figure S1. [1]H NMR spectrum of **7** (Deoxyaureonitrile).

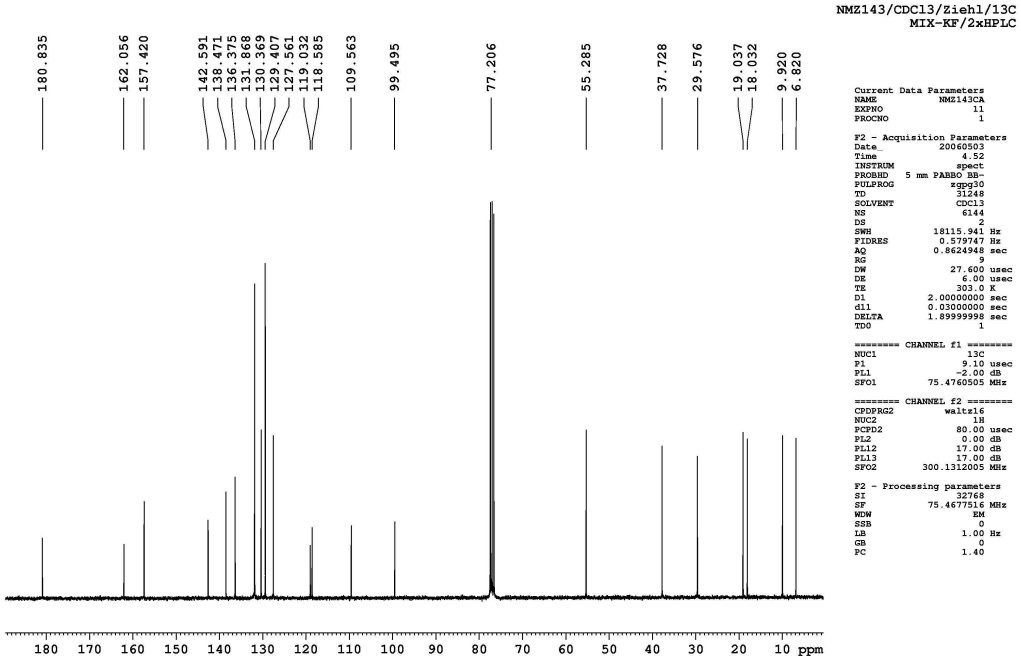

Figure S2. [13]C NMR spectrum of **7** (Deoxyaureonitrile).

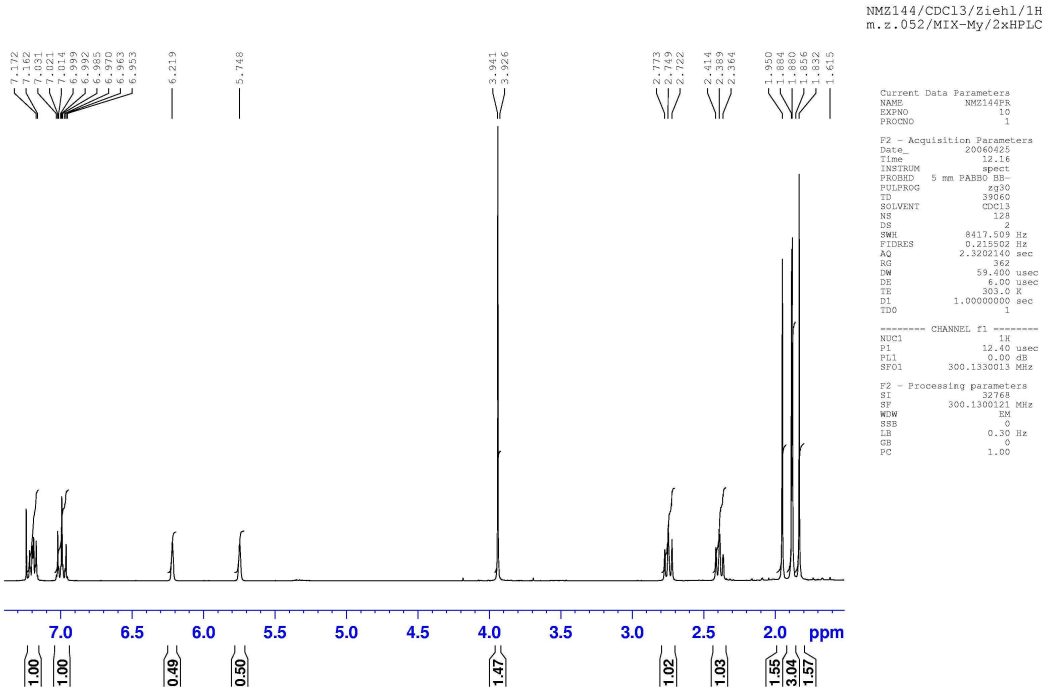

Figure S3. [1]H NMR spectrum of **8** (Fluorodeoxyaureothin).

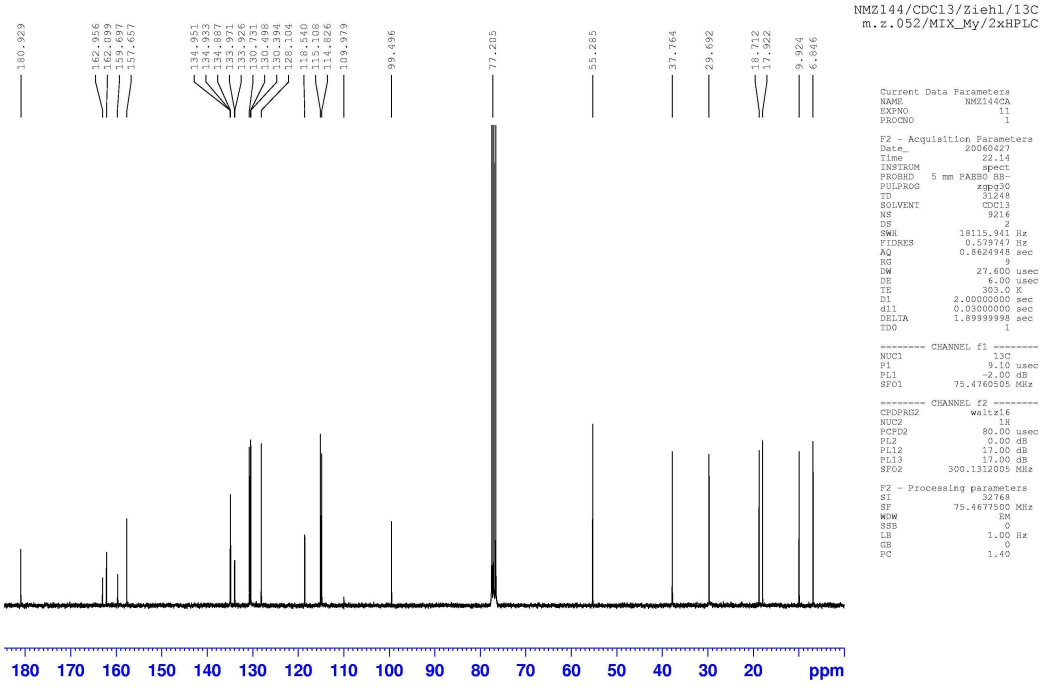

Figure S4. [13]C NMR spectrum of **8** (Fluorodeoxyaureothin).

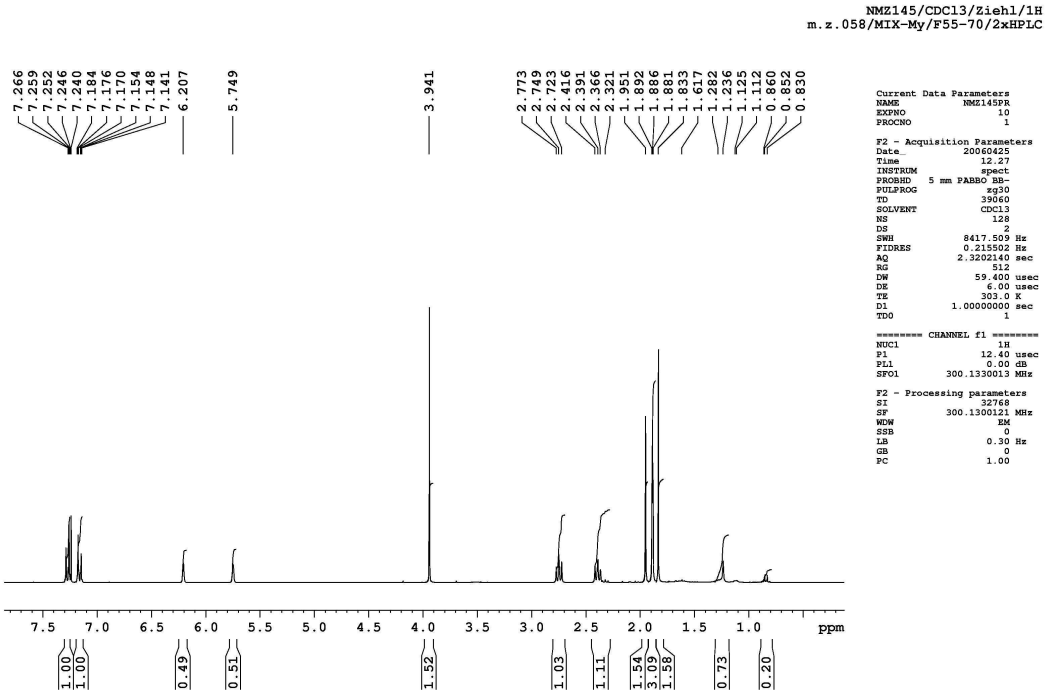

Figure S5. ^{1}H NMR spectrum of **9** (Chlorodeoxyaureothin).

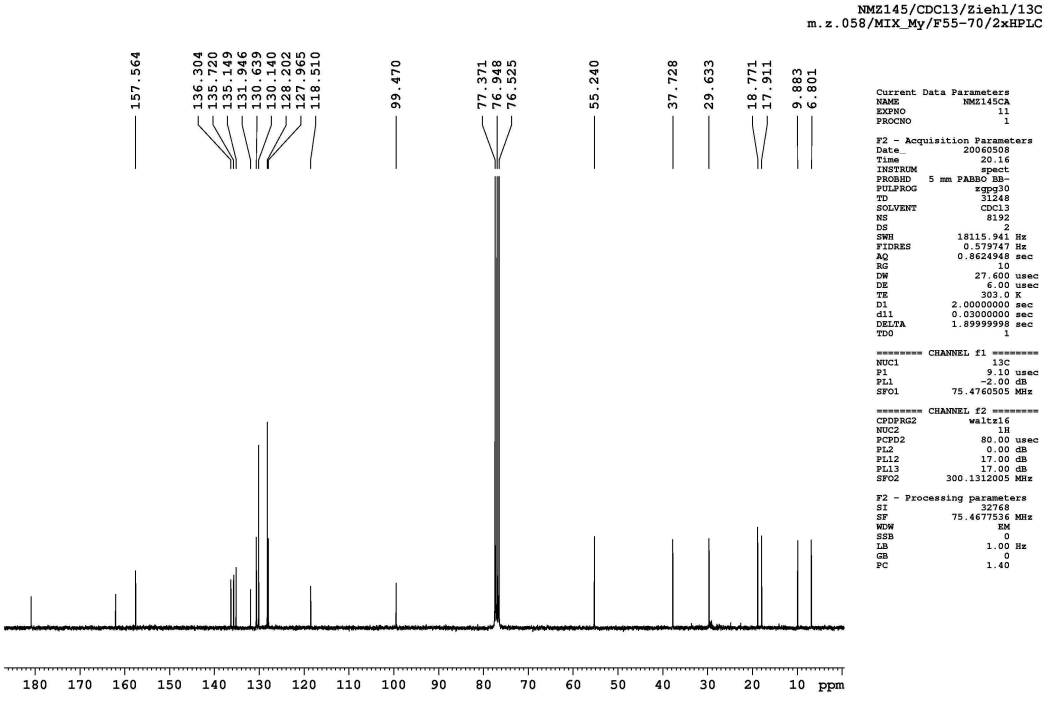

Figure S6. ^{13}C NMR spectrum of **9** (Chlorodeoxyaureothin).

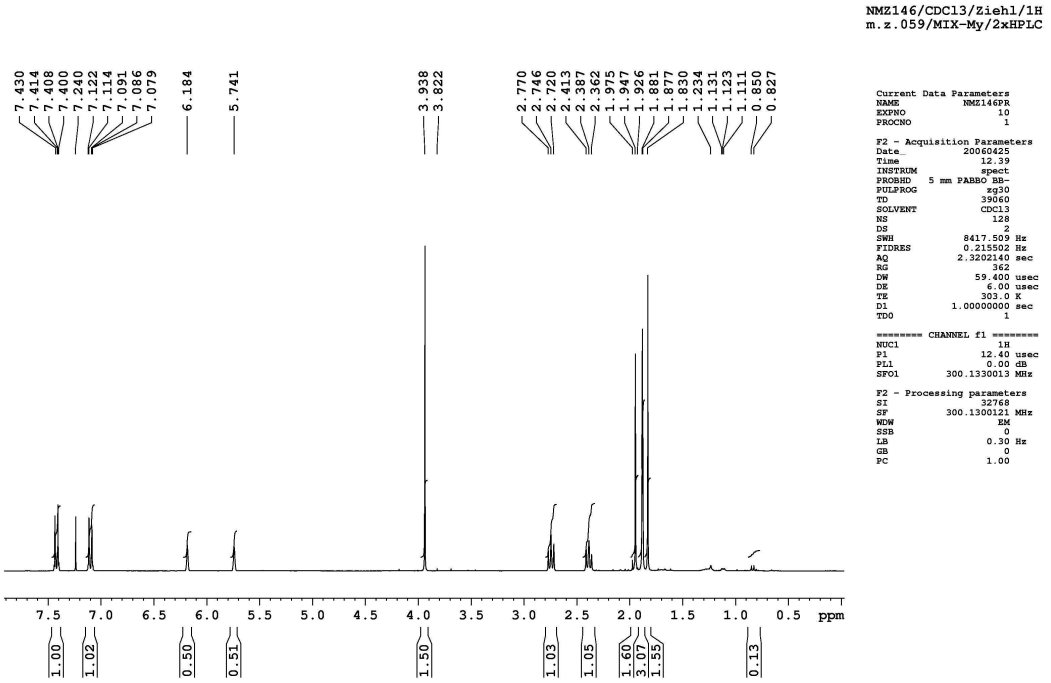

Figure S7. ^{1}H NMR spectrum of **10** (Bromodeoxyaureothin).

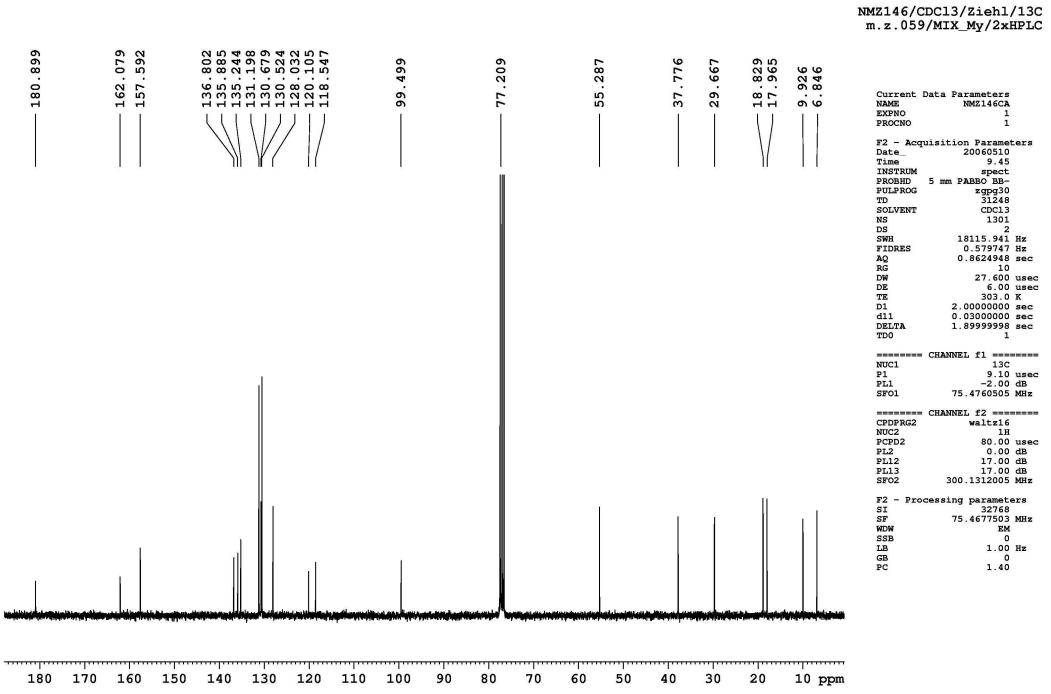

Figure S8. ^{13}C NMR spectrum of **10** (Bromodeoxyaureothin).

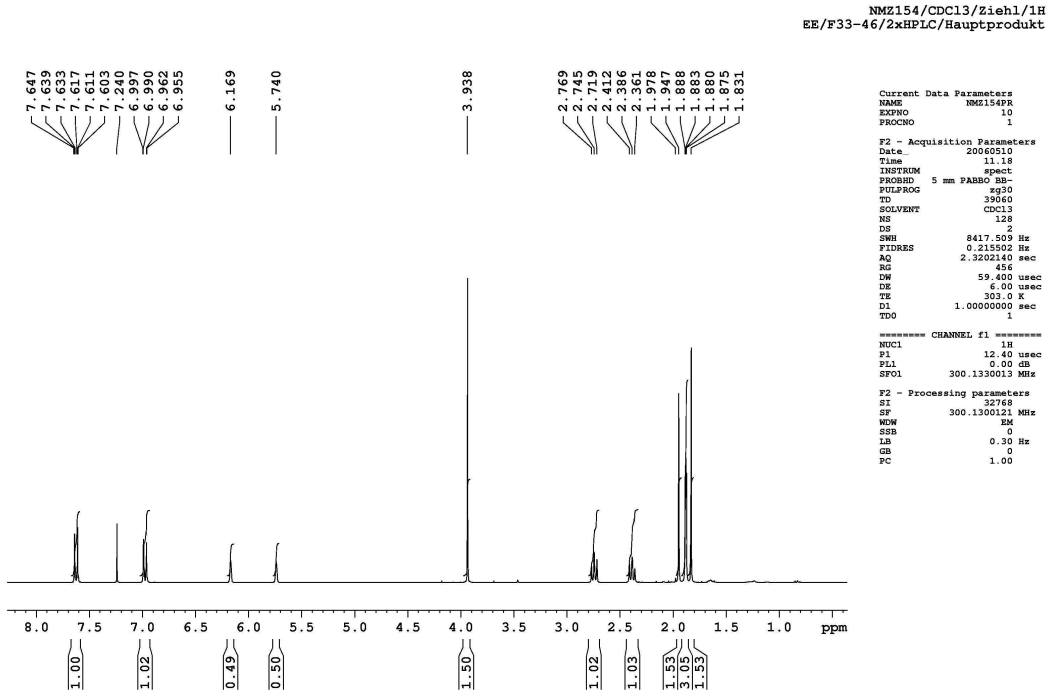

Figure S9. [1]H NMR spectrum of **11** (Iododeoxyaureothin).

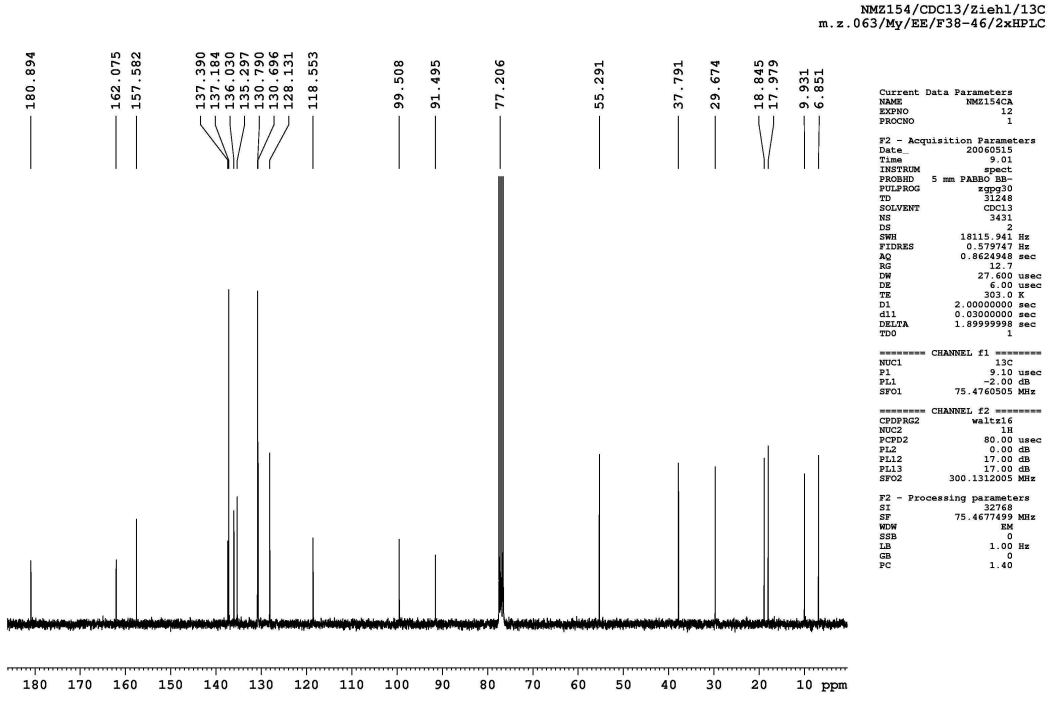

Figure S10. [13]C NMR spectrum of **11** (Iododeoxyaureothin).

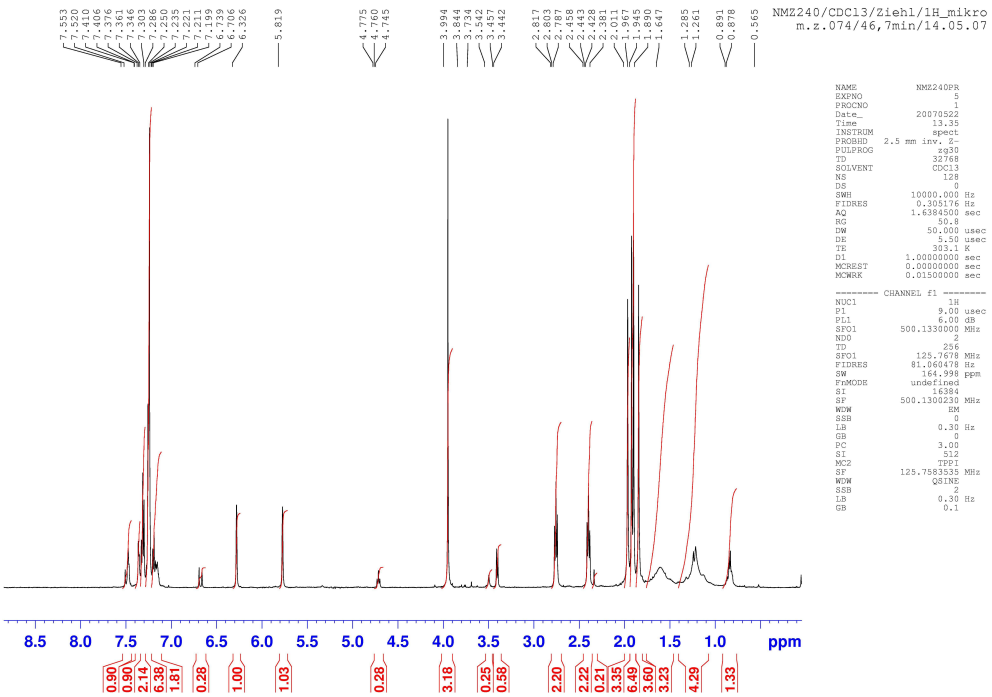

Figure S11. [1]H NMR spectrum of **14** (Phenyldeoxyaureothin).

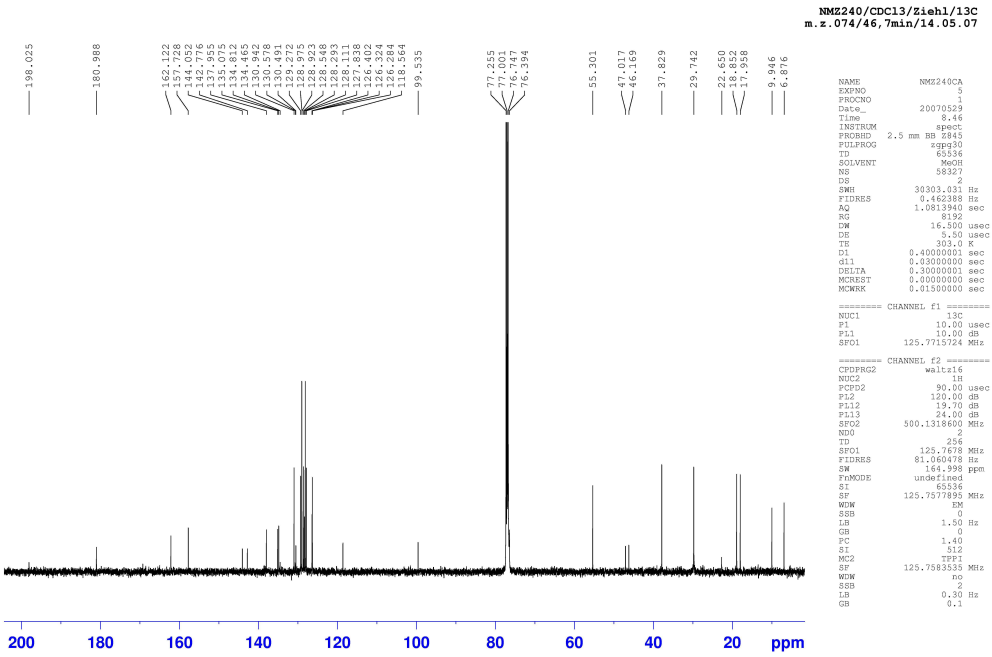

Figure S12. [13]C NMR spectrum of **14** (Phenyldeoxyaureothin).

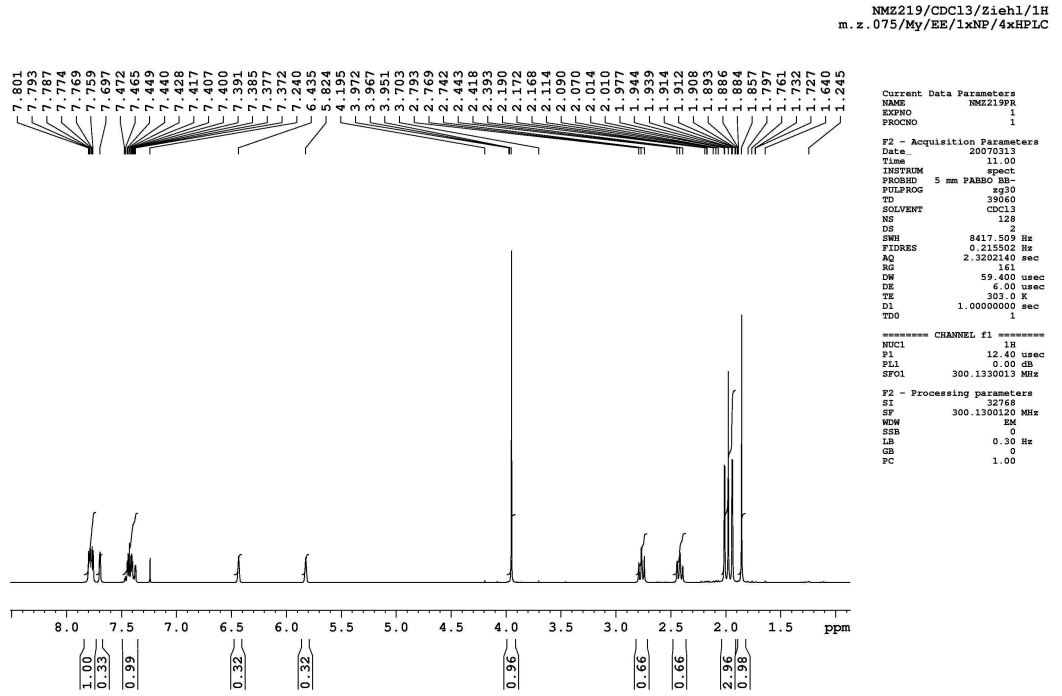

Figure S13. [1]H NMR spectrum of **15** (2-Naphthodeoxyaureothin).

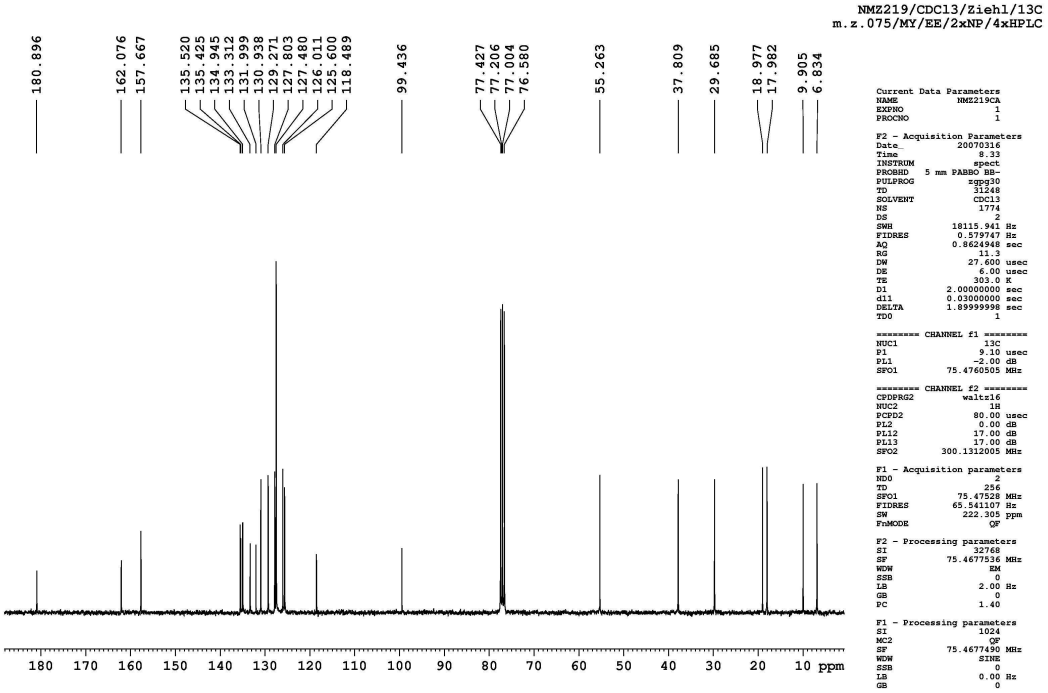

Figure S14. [13]C NMR spectrum of **15** (2-Naphthodeoxyaureothin).

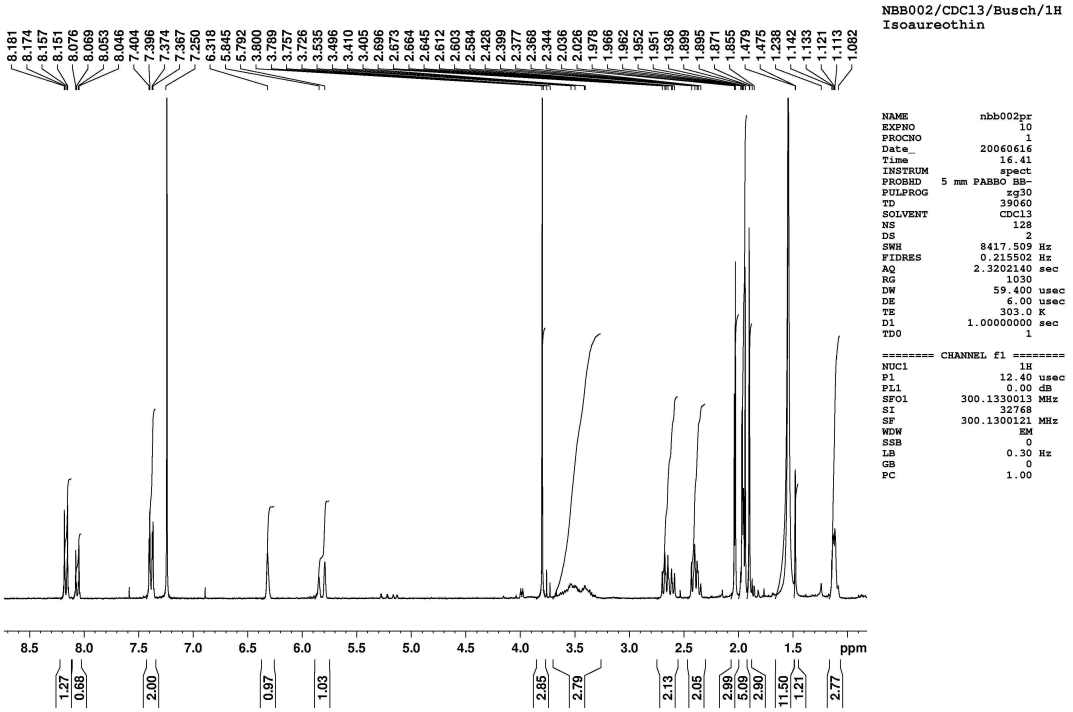

Figure S15. ^{1}H NMR spectrum of **17** (Isodeoxyaureothin).

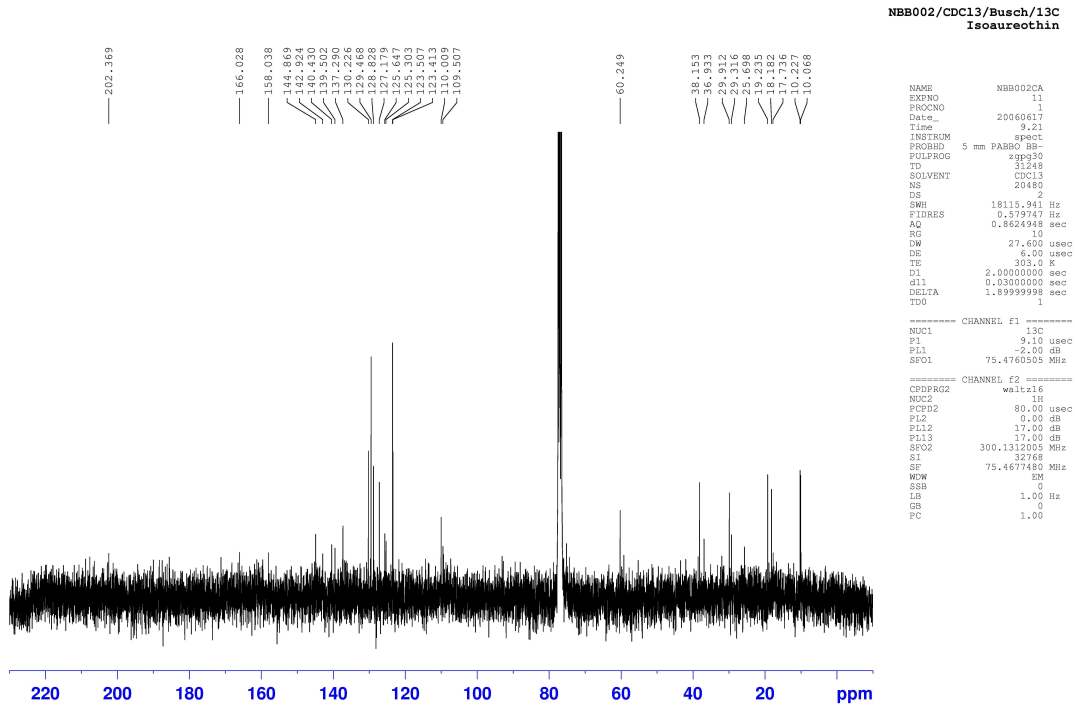

Figure S16. ^{13}C NMR spectrum of **17** (Isodeoxyaureothin).

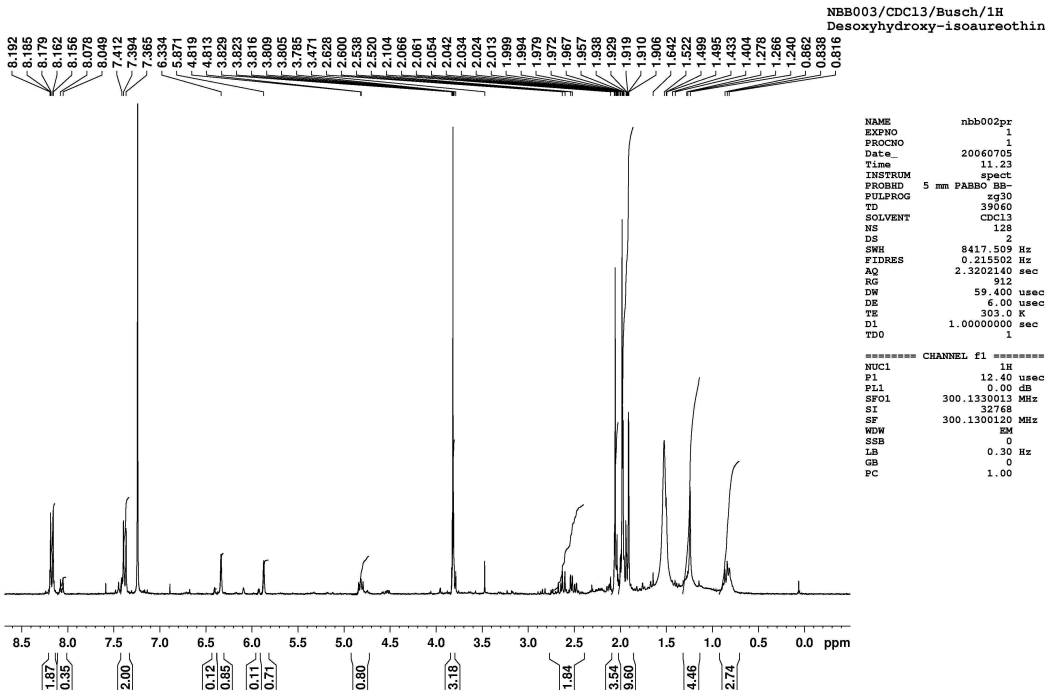

Figure S17. ^{1}H NMR spectrum of **20** (7-Hydroxydeoxyisoaureothin).

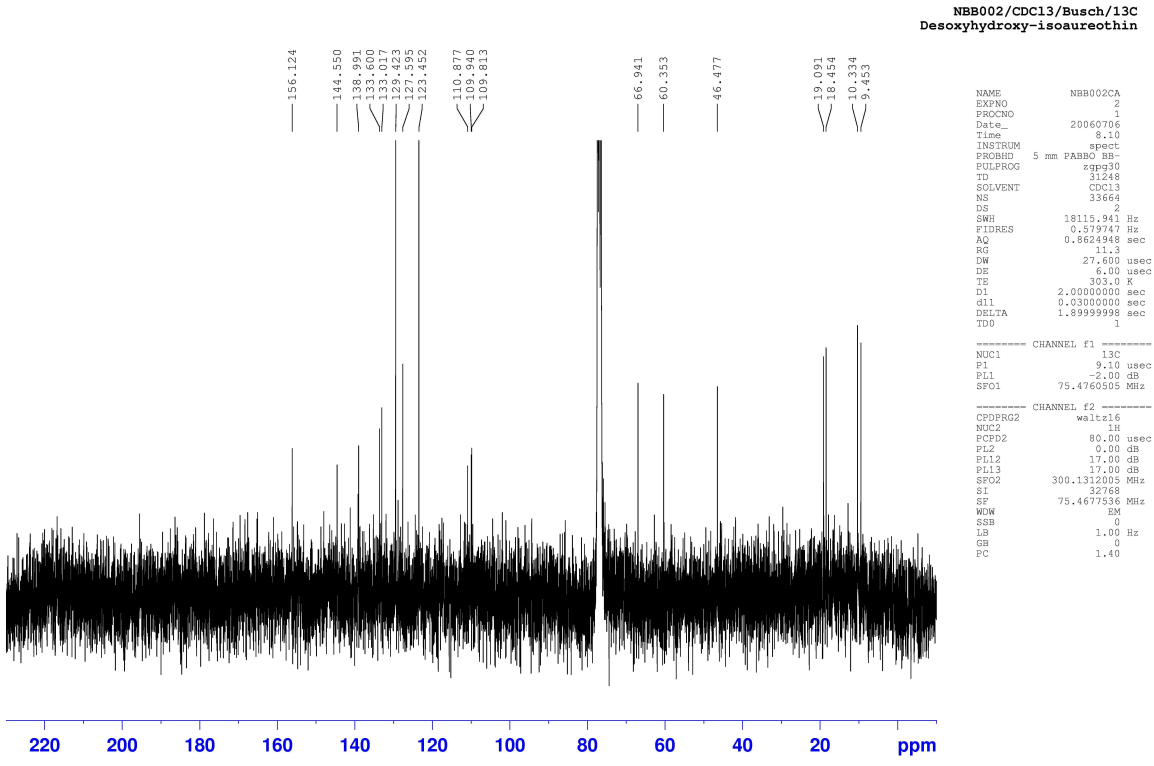

Figure S18. ^{13}C NMR spectrum of **20** (7-Hydroxydeoxyisoaureothin).

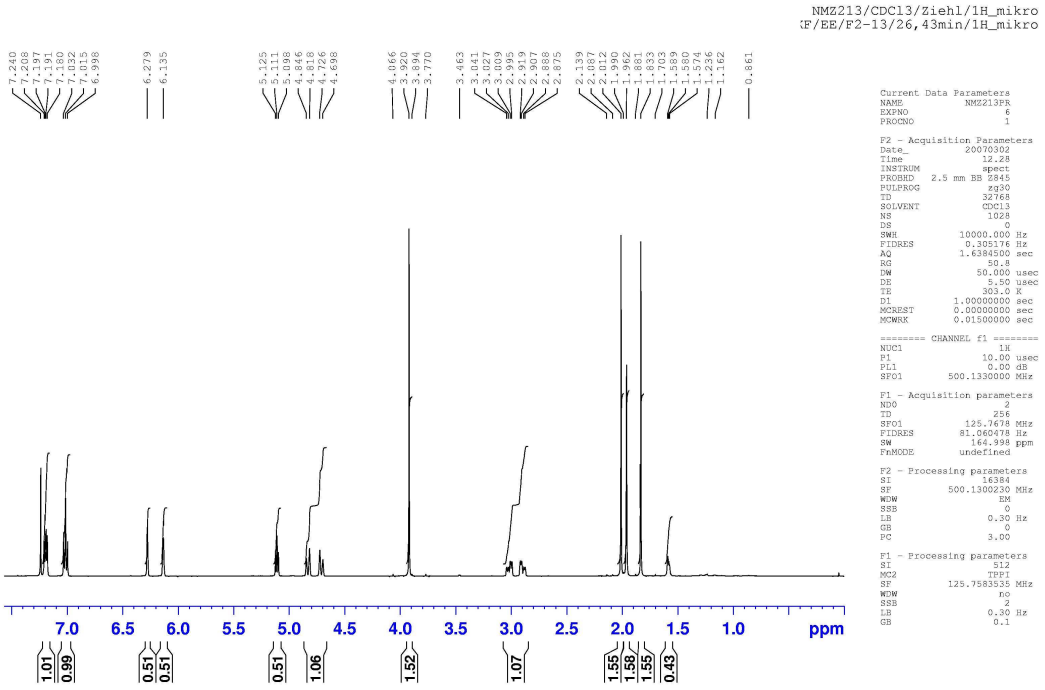

Figure S19. ^{1}H NMR spectrum of **22** (Fluoroaureothin).

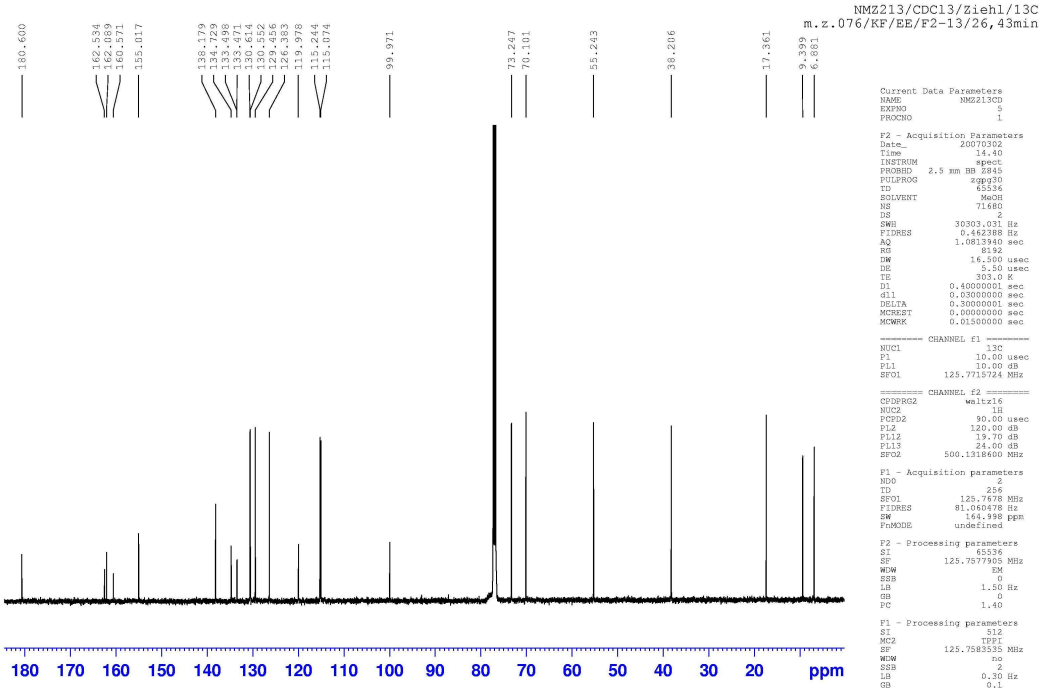

Figure S20. ^{13}C NMR spectrum of **22** (Fluoroaureothin).

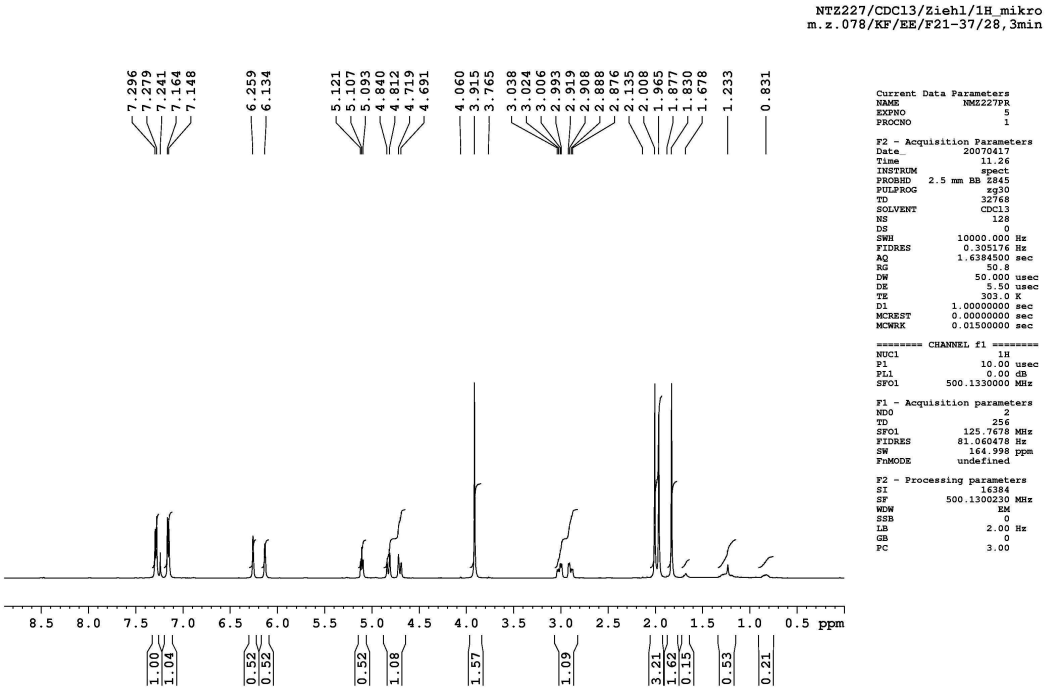

Figure S21. [1]H NMR spectrum of **23** (Chloroaureothin).

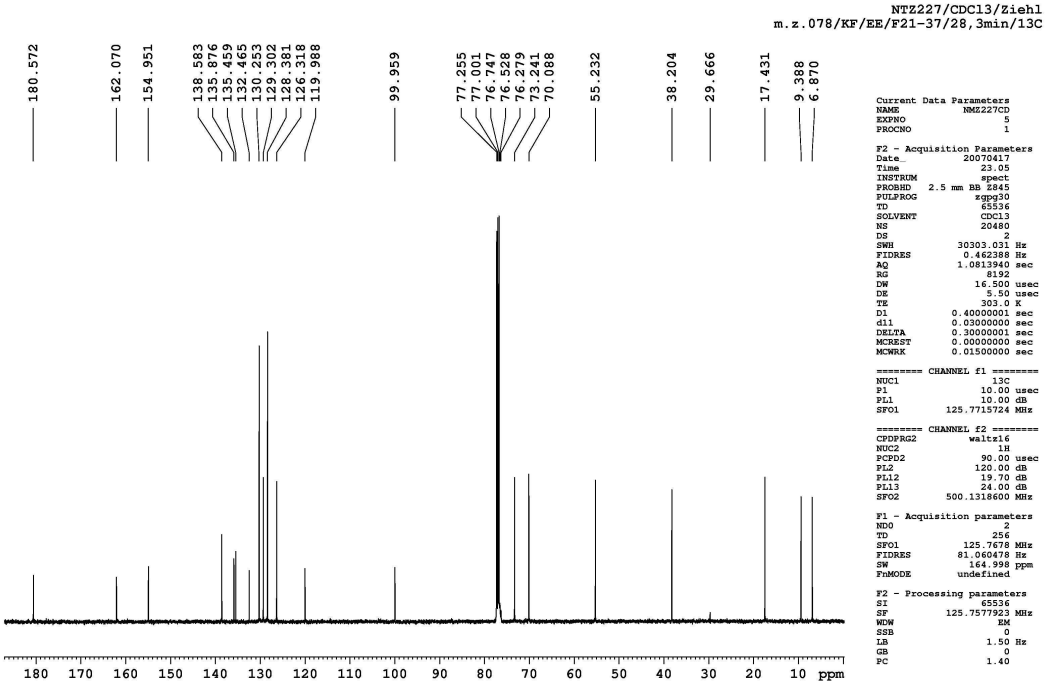

Figure S22. [13]C NMR spectrum of **23** (Chloroaureothin).

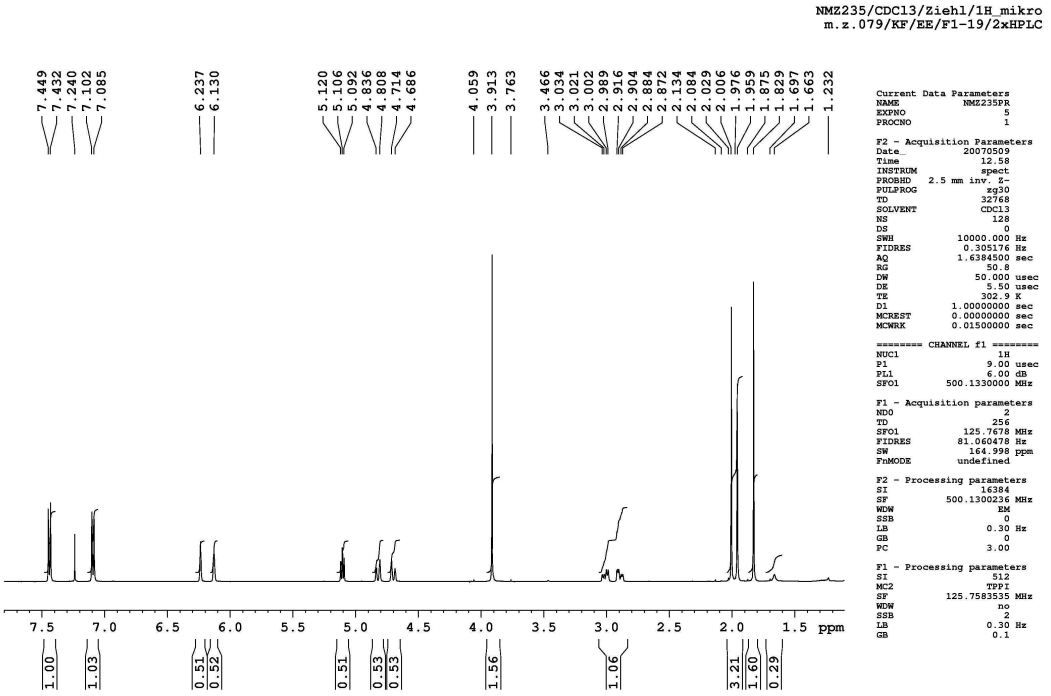

Figure S23. ^{1}H NMR spectrum of **24** (Bromoaureothin).

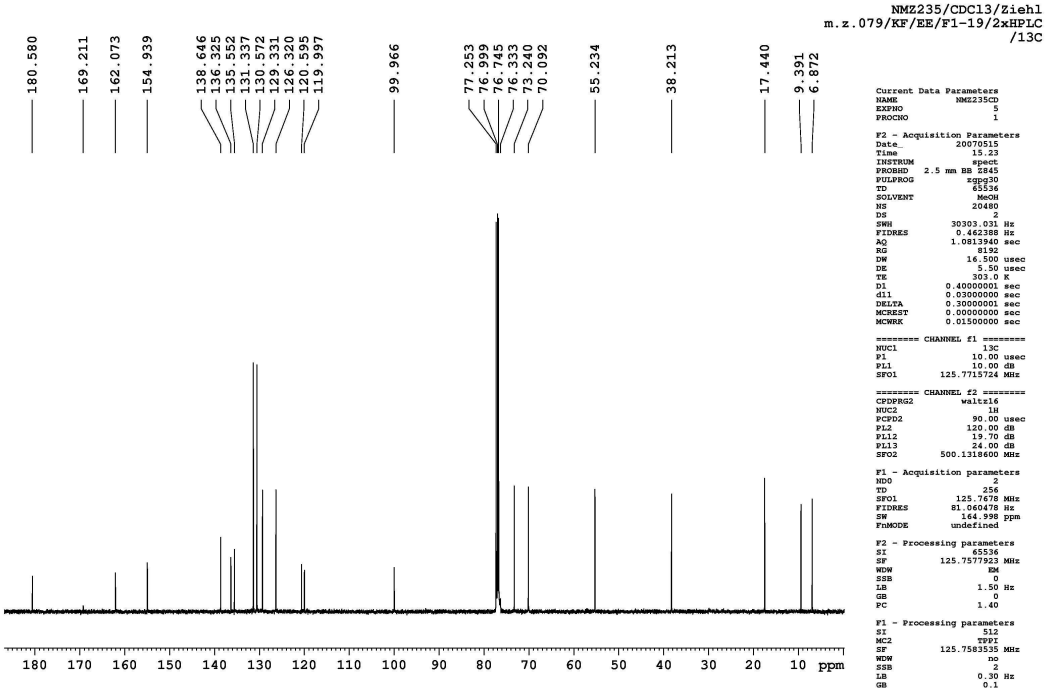

Figure S24. ^{13}C NMR spectrum of **24** (Bromoaureothin).

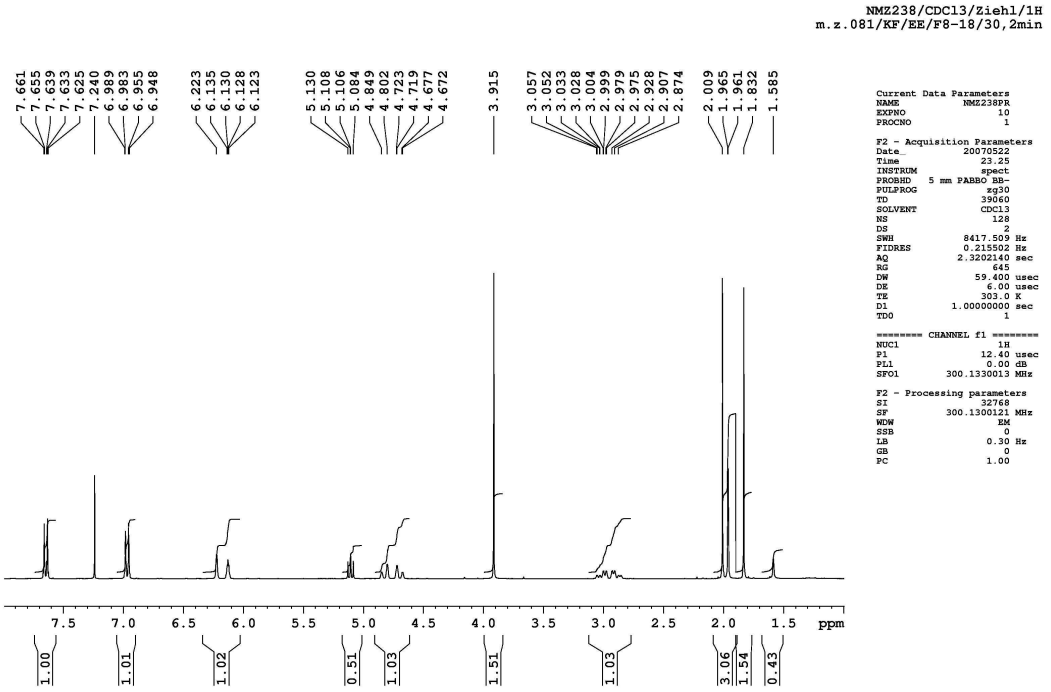

Figure S25. ^{1}H NMR spectrum of **25** (Iodoaureothin).

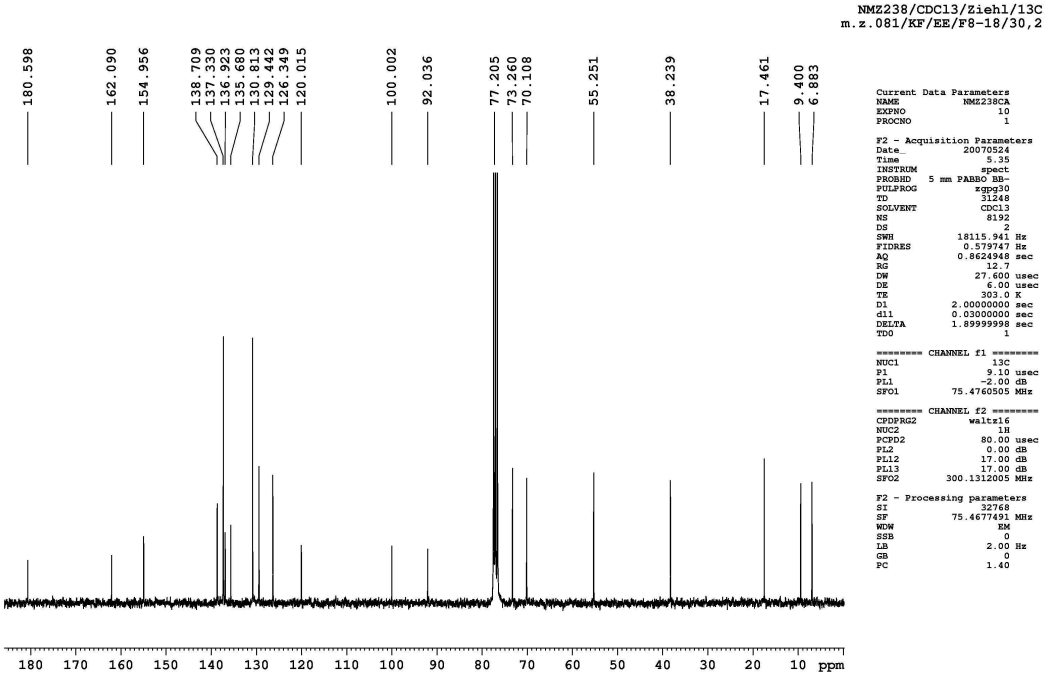

Figure S26. ^{13}C NMR spectrum of **25** (Iodoaureothin).

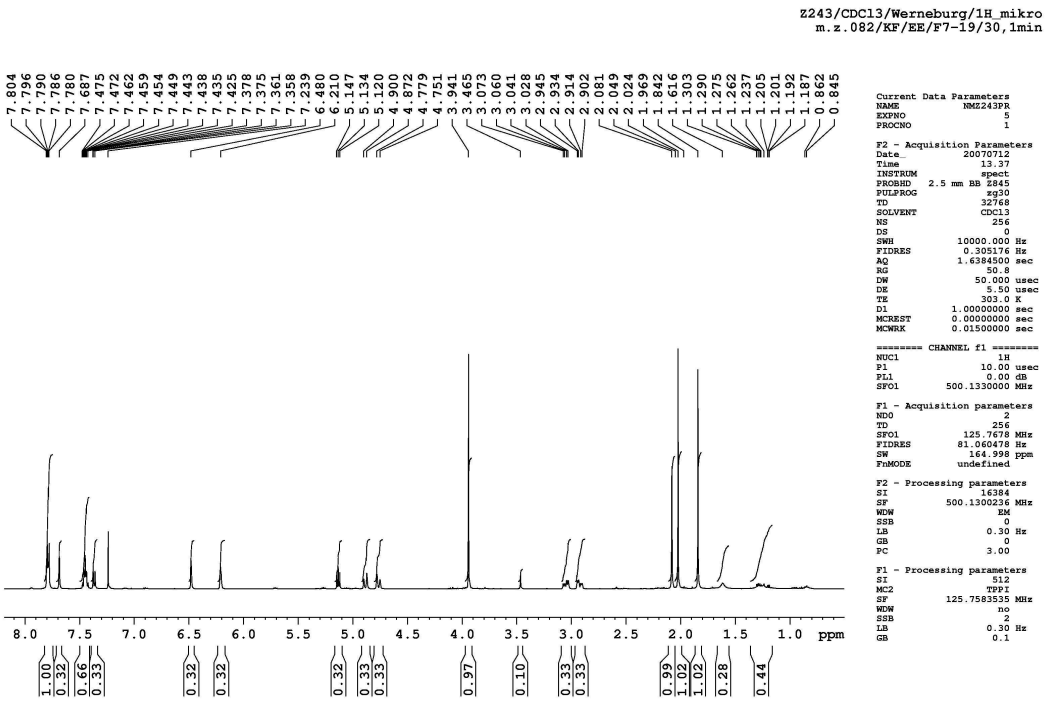

Figure S27. ^{1}H NMR spectrum of **26** (2-Naphthoaureothin).

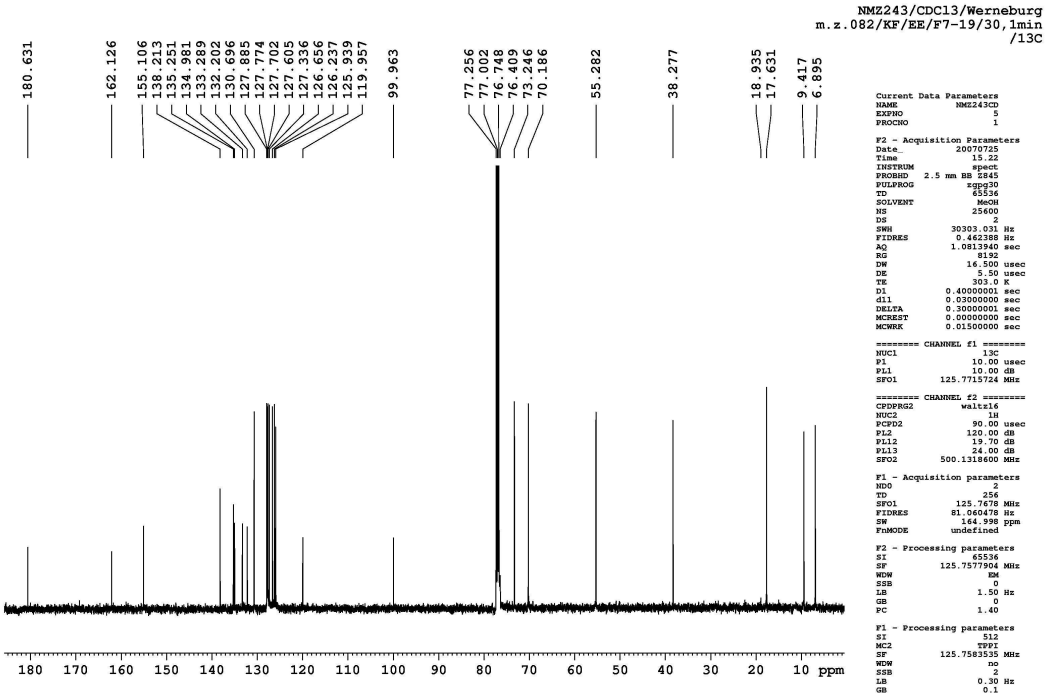

Figure S28. ^{13}C NMR spectrum of **26** (2-Naphthoaureothin).

Biological activity assays

Antimicrobial assays. Antimicobial activities were studied qualitatively by agar diffusion tests and quantitatively by determination of minimal inhibitory concentration (MIC) according to the NCCLS guidelines and DIN 58940 using the broth microdilution method.

Antiproliferative assays. The test substances are dissolved in DMSO before being diluted in DMEM. 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 (NUNC 167008). Cells / culture medium: Huvec (ATCC CRL-1730) / DMEM (CAMBREX 12-614F); K-562 (DSM ACC 10) / RPMI 1640 (CAMBREX 12-167F).

Cytotoxic assay. For the cytotoxic assay, HeLa cells were 48 hours preincubated without the test substances. The dilutions of the compounds were carried out carefully on the subconfluent monolayers of HeLa cells after the preincubation time. Cells / culture medium: HeLa (DSM ACC 57) / RPMI 1640 (CAMBREX 12-167F).

Cells and culture conditions. Cells were grown in the appropiate cell culture medium supplemented with 10 mL L^{-1} ultraglutamine 1 (Cambrex 17-605E/U1), 500 µL L^{-1} gentamicin sulphate (CAMBREX 17-518Z), and 10% heat inactivated fetal bovine serum (PAA A15-144) at 37 °C in high density polyethylene flasks (NUNC 156340).

Condition of incubation and method of evaluation. The cells were incubated with dilutions of the test substances for 72 hours at 37 °C in a humidified atmosphere and 5% CO_2. For estimating the influence of chemical compounds on cell proliferation of K-562, we determined the numbers of viable cells present in multi-well plates via CellTiter-Blue® assay. 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, which is highly fluorescent. Nonviable cells rapidly lose metabolic capacity, do not reduce the indicator dye, and thus do not generate a fluorescent signal. Under our experimental conditions, the signal from the CellTiter-Blue® reagent is proportional to the number of viable cells. The adherent Huvec and HeLa cells were fixed by glutaraldehyde and stained with a 0.05% solution of methylene blue 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 in SUNRISE microplate reader (TECAN). The GI_{50} and CC_{50} values were defined as being where the concentration-response curve intersected the 50% line, compared to untreated control. Furthermore, the 50% cytotoxicity concentration (CC_{50}) indicates the test compound concentration required for destruction in 50% of the cell monolayer compared to untreated control. These comparisons of the different values were performed with software Magellan (TECAN). A repeat determination has been conducted in all cases.

Table S2. Survey of antimicrobial activity in an extended bacterial, fungal and yeast test assay in comparison to Nystatin and Amphotericin B. The minimal growth inhibition zone (mm) was measured. (p = partial inhibition zone; P = intermingled inhibition zone; A = indication of an inhibition zone; diff = diffuse growth boundary, nt = not tested)

Compoundsg	c [µg/mL] (MeOH / DMSO)	*Bacillus subtilis* 6633	*Staphylococcus aureus* 511	*Escherichia coli* 458	*Pseudomonas aeruginosa* SG 127	*Pseudomonas aeruginosa* K 799/61	MRSA *Staphylococcus aureus* 134/93	VRSA *Enterococcus faecalis* 1528	*Mycobacterium vaccae* 10670	*Sporobolomyces salmonicolor* 549	*Candida albicans* BMSY 212	*Penicillium notatum* JP36	c [µg/mL] (MeOH / DMSO)	*Aspergillus fumigatus* ATCC 46645	*Aspergillus terreus* DSM 826	*Aspergillus niger* DSM 737	*Penicillium avellaneum* UC 4376	*Fusarium culmorum* JP 15	*Glomerella cingulata*	*Phoma destructiva* 10670	*Saccharomyces cerevisiae* GI 300	*Klyveromyces marxianus* IMET 25148	*Rhodotorula rubra* IMET 25030
6	100	0	12p	0	0	0	12p	0	12A	20P	0	21	100	15P	0	15P	20P	15P	12P	0/0	0	0	15P
7	100	0	12P	0	0	0	11.5 /14p	0	14	27p	14P	26p	100	20P	0	25P	20 /27p	17P	15P	0/20	0	0	21P
8	100	11	10 /12P	0	0	0	10.5	0	16	10	0	12P	100	15P	0	Ap	17p	14P	Ap	0/12	0	0	0
9	100	0	10 /12P	0	0	0	10 /12p	0	16	15A	0	13P	100	14P	0	Ap	12P	14P	Ap	0/12	0	0	0
10	100	11	9.5 /12P	0	0	0	12p	0	16	14p	0	14P	100	12P	0	Ap	AP	14P	Ap	0/19	0	0	0
11	100	0	10.5 /11P	0	0	0	13p	0	14.5	13A	0	14P	100	12P	0	15P	AP	14P	Ap	0/18	0	0	0
15	100	0	0	0	0	0	13A	0	14	0	0	10A	nt	-	-	-	-	-	-	-	-	-	-
14	100	0	10	0	0	0	13P	0	16p	0	0	10.5 /13A	nt	-	-	-	-	-	-	-	-	-	-
1	100	0	0	0	0	0	0	0	0	38P	32p	36 /40p	100	27P	26 /35p	40P	28 /48p	15p /30P	20 /30p	0/42	0	0	35P
21	100	0	0	0	27p	0	0	0	0	39P	33p	34 /44p	100	nt	nt	nt	nt	25p	28P	0	nt	0	34P
22	100	0	0	0	0	0	0	0	0	29p diff	23p	26p	100	19P	20P	20P	nt	nt	18A	nt	nt	nt	nt
23	100	0	10	0	0	0	10,5 /12p	0	16	13 /43P	19P	29 /38p	100	30P	35 /40p	35P	nt	nt	32 /43p	nt	nt	nt	nt
24	100	0	0	0	0	0	10 /15P	0	11 /13P	12 /40P	23P	25 /36p	100	22P	30 /38p	30P	nt	nt	26 /37p	nt	nt	nt	nt
25	100	0	0	0	0	0	0	0	0	13 /34P	18P	21 /28p	100	22P	25 /30p	23P	nt	nt	20 /28p	nt	nt	nt	nt
26	100	9.5/ 12A	0	0	0	9.5	0	0	13p	17 /28p	17p	16 /25p	nt	-	-	-	-	-	-	-	-	-	-
17	0	0	0	0	0	0	0	0	0	0	0	0	nt	-	-	-	-	-	-	-	-	-	-
20	0	0	0	0	0	0	0	0	0	0	0	0	nt	-	-	-	-	-	-	-	-	-	-
Ampho-tericin B	10	nt	nt	nt	nt	nt	nt	nt	nt	14p	20	14p	10	nt	nt	nt	nt	nt	0	nt	nt	nt	nt
Cipro-floxacin	5	26	17	23/ 30p	27	27/ 34p	0	16	14 /23p	nt	nt	nt											
Ny													100	16 (50 µg/ mL)	18	19 (25 µg/ mL)	29 (200 µg/ mL)	17p /25P	23 (400 µg/ mL)	31	15/ 22p	22	20

Table S3. Survey of antiproliferative and cytotoxic activity. (First value is an individual value. The second value was determined as duplicate; nt = not tested.)

Compound	Antiproliferative activity			Cytotoxicity
	Huvec GI_{50} [µg mL^{-1}]	L-929 GI_{50} [µg mL^{-1}]	K-562 GI_{50} [µg mL^{-1}]	HeLa CC_{50} [µg mL^{-1}]
Deoxyaureothin derivates				
6	6.2 / 3.0	9 .3	1 .8 / 3.1	16.5 / 8.0
7	1.8	5.0	1.0	7.8
8	4.3 / 3.5	10.0	0.5 / 3.8	11.8 / 13.3
9	8.1	12.1	0.8	15.3
10	11.3	7.0	0.5	10.5
11	40.6	7.9	0.5	12.8
15	10.2 / 5.2	nt	8.0 / 4.8	9.3 / 8.0
14	4.1	nt	8.7	18.0
Aureothin derivates				
1	>50 / 14.9	10.0	10.0 / 25.0	10.0 / >25
21	nt	10.2	3.5	35.4
22	16.2 / 4.3	nt	6.8 / 7.3	25.2 / 17.4
23	8.1	nt	6.0	38.0
24	18.3	nt	>50	41.4
25	8.0	nt	5.7	36.2
26	9.2 / 5.8	nt	12.6 / 4.7	14.7 / 15.1