

SUPPLEMENTARY MATERIAL

Ordering the Reductive and Cytochrome P450 Oxidative Steps in Demethylsterigmatocystin Formation Yields General Insights into the Biosynthesis of Aflatoxin and Related Fungal Metabolites

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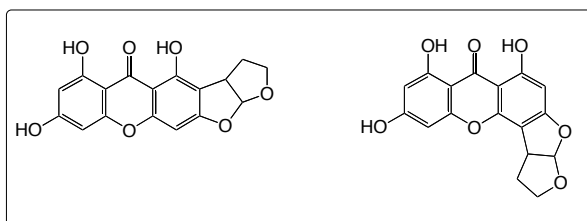
Charles and 34th Streets, Baltimore, MD 21218

Experimental Section

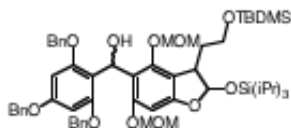
General Materials and Methods: Melting points were determined with a Thomas-Hoover oil bath apparatus in open capillaries and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Varian Unity^{Plus} 400 MHz spectrometer and are referenced to CDCl_3 (7.27 and 77.0 ppm) or d_6 -acetone (2.04 and 29.9 ppm) as indicated. High and low resolution mass spectra were recorded by Dr. Joseph Kachinski of the Department of Chemistry, The Johns Hopkins University using a VG Instruments 70-S 250 GC/MS at 70 eV in EI^+ , CI^+ , or FAB operating modes. Additional exact mass determinations were performed by Dr. Christopher Hadad of the Ohio State University and by Dr. Ronald Cerny of the University of Nebraska. Infrared spectra were recorded on a Perkin-Elmer 1600 Series FTIR spectrophotometer. Combustion analyses were performed by Atlantic Microlab, Inc. (Norcross, GA). Flash chromatography was

performed using EM silica gel 60 (230-400 mesh). Thin-layer chromatography (TLC) was performed on Analtech Uniplate glass plates containing fluorescent indicator. TLC plates were photographed by James Van Rensselaer, The Johns Hopkins University. Reagents and aflatoxin standards were purchased from Aldrich, Inc. Media components were purchased from Difco (Detroit, MI) or Fisher (Pittsburg, PA). Nonaqueous reactions were performed in flame-dried glassware under an atmosphere of N₂ or Ar. Solvents were distilled immediately prior to use (THF and Et₂O from sodium/benzophenone ketyl, and CH₂Cl₂ and CH₃CN from CaH₂). During workup organic solutions were dried over anhydrous Na₂SO₄. HPLC was performed with a Perkin-Elmer 235C diode array detector and Series 410 LC pump using a Phenomenex (Torrance, CA) reverse-phase column (Prodigy 5 μ ODS(3) 100Å, 250 x 4.60 mm, 5 micron). Mini-prep columns were purchased from Qiagen (Valencia, CA).

SYNTHETIC PROCEDURES

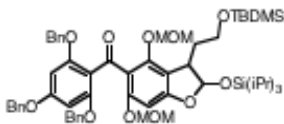


Synthesis of (*Linear*) 4,6,8-Trihydroxy-2,3,3a,12a-tetrahydrofuro[2,3-b]furo-xanth-5-one (**24**) and (*Angular*) 4,6,8-Trihydroxy-1,2,3a,12c-tetrahydrofuro[2,3-c]furo-xanth-7-one (**25**).



5-[1-(*R,S*)-{2',4',6'-Tris-(*O*-benzyl)}-1-hydroxy-methyl]-3-(*t*-butyldimethylsilyloxy-2-ethyl)-4,6-bis-(*O*-methoxymethyl)-2,3-dihydro-benzofuran-2-yloxy]-triisopropyl-silane (13**). (*Procedure A*) A pentane (50 mL) solution of bromide **9** (1.86 g, 2.88 mmol) was cooled to $-40\text{ }^{\circ}\text{C}$ under argon. *n*-BuLi (1.26 M, 2.29 mL, 2.88 mmol) was added over 30 s. After 1 min a THF (15 mL) solution of 2,4,6-*tris*(*O*-benzyl)-benzaldehyde (**11**, 1.22 g, 2.88 mmol, $-40\text{ }^{\circ}\text{C}$) was cannulated into the pentane solution. The resulting mixture was stirred 15 min at $-40\text{ }^{\circ}\text{C}$ and then 15 min at $0\text{ }^{\circ}\text{C}$ before it was quenched with aq NaHCO_3 . The product mixture was diluted with Et_2O and washed with water and brine, then concentrated and passed through a column of silica gel with 10% EtOAc in hexanes. The benzophenolic pair **13** was isolated together as a faintly yellow oil (1.7 g, 59%). TLC R_f 0.55, 0.59 (20% EtOAc in hexanes). ^1H NMR (400 MHz, CD_3COCD_3) δ 7.4 (m, 15H), 6.62 (t, 1H, $J = 10.5\text{ Hz}$), 6.44 (m, 2H), 5.93, 5.91 (s, 1H), 5.40, 5.32 (d, 1H, $J = 10.4\text{ Hz}$), 5.0 (m, 10H), 3.7 (m, 2H) 3.5 (m, 1H), 3.47, 3.40 (s, 3H), 3.02, 3.00 (s, 3H), 2.87 (s, 1H), 1.95 (m, 1H), 1.51 (m, 1H), 1.12 (m, 21H), 0.92 (m, 9H), 0.07 (m, 6H). ^{13}C NMR (100 MHz, CD_3COCD_3) δ 160.7, 160.6, 160.2, 160.0, 159.8, 159.1, 158.4, 157.8, 155.8, 154.7, 154.6, 138.9, 138.7, 130.0, 129.8, 129.2, 129.0, 128.8, 121.2, 120.0, 116.2, 115.6, 115.4, 114.8, 112.1, 107.8, 107.2, 106.9, 100.5, 100.2, 97.5, 96.0, 95.6, 95.4, 95.0, 94.9, 94.5, 93.8, 71.9, 71.7, 71.5, 71.2, 71.0, 68.4, 66.0, 62.3, 62.2, 62.1, 58.1, 57.0, 56.7, 56.4, 56.3, 49.9, 49.8, 48.5, 35.9, 27.0, 19.5, 19.0, 18.9, 13.7, 13.5, -4.5 . IR (neat) 3544 (br), 2947, 2894, 1603, 1498, 1151,**

1112, 1054 cm^{-1} . HRMS-EI (m/z): M^+Na calcd for $\text{C}_{57}\text{H}_{78}\text{O}_{11}\text{Si}_2$, 1017.4975; found, 1017.4975. Anal. Calcd for $\text{C}_{57}\text{H}_{78}\text{O}_{11}\text{Si}_2$: C, 68.78; H, 7.90. Found: C, 68.72; H, 7.99.



5-Keto-[2',4',6'-tris-(*O*-benzyl)-phenyl]-3-(*t*-butyldimethyl-silyloxy-2-ethyl)-4,6-bis-(*O*-methoxymethyl)-2,3-dihydro-benzofuran-2-yloxy]-triisopropyl-silane (15**).**

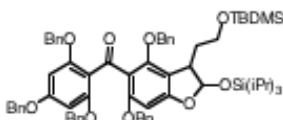
(*Procedure B*) Benzophenols **13** (1.65 g, 1.66 mmol) were dissolved in CH_2Cl_2 (50 mL) and cooled to 0 °C. Dess-Martin periodinane (0.77 g, 1.83 mmol) was added and the mixture was stirred 1 h and warmed to ambient temperature. The mixture was washed with aq NaHCO_3 , water, and brine, then concentrated and passed through a column of silica gel with 10% EtOAc in hexanes. Benzophenone **15** was isolated as a yellow oil (1.0 g, 60%). TLC R_f 0.55 (20% EtOAc in hexanes). ^1H NMR (400 MHz, CD_3COCD_3) δ 7.35 (m, 15H), 6.42 (s, 2H), 6.38 (s, 1H), 6.01 (s, 1H), 5.11 (s, 2H), 5.04 (s, 4H), 4.80 (m, 4H), 3.74 (m, 2H), 3.51 (dd, 1H, $J = 3.5, 9.6$ Hz.) 3.27 (s, 3H), 3.04 (s, 3H), 1.98 (m, 1H), 1.53 (m, 1H), 1.14 (m, 21H), 0.90, 0.89 (s, 9H), 0.03, 0.02 (s, 6H). ^{13}C NMR (100 MHz, CD_3COCD_3) δ 190.77, 163.0, 162.1, 161.7, 161.3, 159.7, 159.4, 158.6, 154.4, 138.1, 138.0, 137.8, 129.5, 129.4, 129.3, 129.2, 129.1, 128.9, 128.8, 128.7, 128.6, 128.4, 128.2, 128.2, 121.3, 117.5, 115.6, 107.2, 107.1, 100.1, 95.5, 94.3, 94.2, 71.5, 71.0, 70.7, 61.6, 57.2, 56.0, 48.8, 35.3, 26.4, 18.9, 18.4, 18.3, 12.9, -5.2. IR (neat) 2947, 2866, 1660, 1600, 1153, 1115, 1047 cm^{-1} . HRMS-EI (m/z): M^+Na calcd for $\text{C}_{57}\text{H}_{76}\text{O}_{11}\text{Si}_2$, 1015.4818; found, 1015.4857. Anal. Calcd for $\text{C}_{57}\text{H}_{76}\text{O}_{11}\text{Si}_2$: C, 68.92; H, 7.71. Found: C, 68.89; H, 7.96.



5-Keto-[2',4',6'-tris-(*O*-benzyl)-phenyl]-3-(*t*-butyldimethyl-silyloxy-2-ethyl)-4-(*O*-benzyl)-6-(*O*-methoxymethyl)-2,3-dihydro-benzofuran-2-yloxy]-triisopropyl-silane and 5-Keto-[2',4',6'-tris-(*O*-benzyl)-phenyl]-3-(*t*-butyldimethyl-silyloxy-2-ethyl)-6-(*O*-benzyl) 4-(*O*-methoxymethyl)-2,3-dihydro-benzofuran-2-yloxy]-triisopropyl-silane.

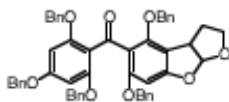
(*Procedure C*) Benzophenone **15** (1.0 g, 1.0 mmol) in CH₂Cl₂ (50 mL) was cooled to 0 °C under argon. BCl₃ (2M in CH₂Cl₂, 0.55 mL, 1.1 mmol) was added over several minutes and the ice bath was removed. After 20 min the reaction was quenched with aq NaHCO₃. The organic layer was separated and washed with water and brine, then dried, concentrated, and passed through a column of silica gel with 20% EtOAc in hexanes. The resulting mixture of monophenols was isolated as a clear oil. TLC R_f 0.37 (10% EtOAc in hexanes). The mixture was treated with benzyl bromide (2 mL, 16.7 mmol) and DMF (5 mL) and then cooled to 0 °C. NaH (60% suspension, 1.0 g) was added and the ice bath was removed. The mixture was quenched 30 min later with aq NaHCO₃ then diluted with ethyl acetate and water. The organic layer was washed with water and brine, then dried, concentrated and passed through a column of silica gel with 10-30% EtOAc in hexanes. The regioisomeric mixture of tetrabenzyl ethers (0.77 g, 80% over two steps) was isolated as a clear oil. TLC R_f 0.24 (10% EtOAc in hexanes). ¹H NMR (400 MHz, CD₃COCD₃) δ 7.26 (m, 20H), 6.42 (s, 1H), 6.35 (s, 2H), 6.04 (d, 1H, *J* = 1.2 Hz), 5.11 (s, 2H), 4.98, 4.88 (d, 4H, *J* = 6.1 Hz), 4.81 (m, 4H), 3.68 (m, 2H), 3.52 (dd, 1H, *J* = 3.6, 9.6 Hz), 3.13 (s, 3H), 1.98 (m, 1H), 1.64 (m, 1H), 1.18 (m, 21H), 0.83, 0.82 (s, 9H), 0.00, -0.02 (s, 6H). ¹³C NMR (100 MHz, CD₃COCD₃) δ 190.5, 162.1, 161.9, 159.5, 158.6, 155.9, 138.7, 138.1, 129.4, 129.2, 128.8, 128.8, 128.6, 128.3,

121.7, 117.4, 114.8, 107.5, 107.4, 95.5, 94.3, 94.2, 74.9, 70.8, 70.7, 61.3, 56.0, 48.7, 35.2, 26.4, 18.4, 12.9, -5.2. IR (neat) 2952, 1660, 1602, 1153, 1113 cm^{-1} . HRMS-EI (m/z): M^+Na calcd for $\text{C}_{62}\text{H}_{78}\text{O}_{10}\text{Si}_2$, 1061.5026; found, 1061.4941. Anal. Calcd for $\text{C}_{62}\text{H}_{78}\text{O}_{10}\text{Si}_2$: C, 71.64; H, 7.56. Found: C, 71.66; H, 7.38.

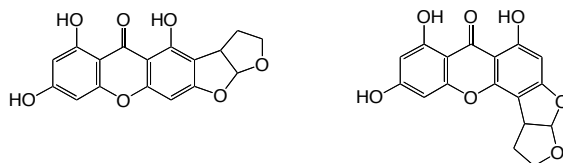


5-Keto-[2',4',6'-tris-(*O*-benzyl)-phenyl]-3-(*t*-butyldimethyl-silyloxy-2-ethyl)-4,6-bis-(*O*-benzyl)-2,3-dihydro-benzofuran-2-yloxy]-triisopropyl-silane (17**).**

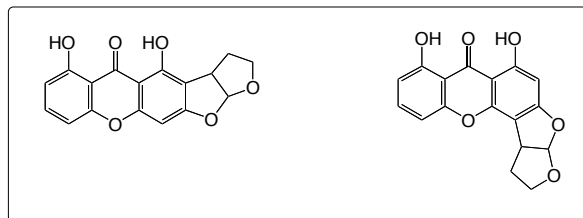
Pentabenzylether **17** (clear oil, 0.55 g, 0.51 mmol, 75%, TLC R_f 0.28 - 10% EtOAc in hexanes) was prepared from mixture of tetrabenzylethers prepared above (0.7 g, 0.67 mmol) according to *Procedure C*. ^1H NMR (400M Hz, CD_3COCD_3) δ 7.32 (m, 25H), 6.41 (s, 1H), 6.29 (s, 2H), 6.05 (d, 1H, $J = 1.2$ Hz), 5.11 (s, 2H), 4.83 (s, 6H), 4.80, 4.60 (d, 2H, $J = 12.0$ Hz), 3.70 (m, 2H), 3.51 (dd, 1H, $J = 2.4, 9.3$ Hz), 2.01 (m, 1H), 1.64 (m, 1H), 1.20 (m, 21H), 0.85 (s, 9H), 0.00, -0.02 (s, 6H). ^{13}C NMR (100 MHz, CD_3COCD_3) δ 190.5, 185.1, 162.1, 162.0, 160.2, 159.5, 156.2, 138.8, 138.1, 137.8, 129.4, 129.1, 129.0, 128.8, 128.6, 128.4, 128.3, 128.3, 128.2, 121.1, 117.5, 113.9, 107.4, 94.1, 92.2, 74.9, 71.2, 70.8, 70.7, 61.3, 48.7, 35.2, 26.4, 18.8, 18.4, 18.3, 12.9, -5.2. IR (neat) 2955, 1660, 1602, 1153, 1113 cm^{-1} . HRMS-EI (m/z): M^+Na calcd for $\text{C}_{67}\text{H}_{80}\text{O}_9\text{Si}_2$, 1107.5233; found, 1107.5215. Anal. Calcd for $\text{C}_{67}\text{H}_{80}\text{O}_9\text{Si}_2$: C, 74.13; H, 7.43. Found: C, 74.39; H, 7.51.



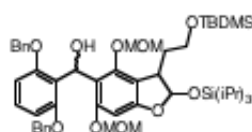
5-Keto-[2',4',6'-tris-(*O*-benzyl)-phenyl]-4,6-bis-(*O*-benzyl)-[2,3-b]-benzo-2,3,3a,8a-tetrahydro-bisfuran (19**). (*Procedure D*) To pentabenzylether **17** (0.5 g, 0.46 mmol) and THF (10 mL) was added TBAF (1 M in THF, 1.4 mL, 1.4 mmol). The mixture was heated to reflux (*ca.* 1 h) until conversion to the diol was complete (TLC R_f 0.05 - 20% EtOAc in hexanes). The reaction mixture was then cooled to 0 °C and aq HCl (3.0 M, 2 mL) was added. The ice bath was removed and the mixture was stirred 30 min, then diluted with Et₂O and quenched with aq NaHCO₃. The etherial layer was washed with water and brine, then dried, concentrated, and passed through a column of silica gel with 20% EtOAc in hexanes. Tetrahydrobisfuran **19** was isolated (0.31 g, 85% yield, mp = 58-60 °C) as a white solid. TLC R_f = 0.30 (20% EtOAc in hexanes). ¹H NMR (400 MHz, CD₃COCD₃) δ 7.31 (m, 25H), 6.34 (s, 2H), 6.33 (s, 1H), 6.31 (s, 1H), 5.14 (s, 2H), 4.84 (s, 6H), 4.78, 4.60 (d, 2H, J = 11.4 Hz), 3.96 (m, 2H), 3.42 (q, 1H, J = 8.4 Hz), 2.05 (m, 1H), 2.00 (m, 1H). ¹³C NMR (100 MHz, CD₃COCD₃) δ 190.3, 163.2, 162.3, 160.2, 159.8, 156.0, 138.9, 138.1, 138.0, 137.8, 129.4, 129.1, 129.0, 128.9, 128.8, 128.7, 128.5, 128.4, 121.1, 117.3, 113.0, 112.3, 94.2, 91.1, 74.9, 71.2, 71.0, 70.8, 67.8, 45.9, 32.8. IR (neat) 2955, 1660, 1602, 1153, 1113 cm⁻¹. HRMS-EI (m/z): M⁺Na calcd for C₅₂H₄₄O₈, 819.2928; found, 819.2934. Anal. Calcd for C₅₂H₄₄O₈: C, 78.37; H, 5.57. Found: C, 78.34; H, 5.64.**



(Linear) 4, 6, 8-Trihydroxy-2,3,3a,12a-tetrahydrofuro[2,3-b]furo-xanth-5-one (24) and (Angular) 4, 6, 8-Trihydroxy-1,2,3a,12c-tetrahydrofuro[2,3-c]furo-xanth-7-one (25). (*Procedure E*) Tetrahydrobisfuran **19** (100 mg, 0.12 mmol), Pd black (100 mg, 0.94 mmol), and EtOAc (3 mL) were shaken 12 h under H₂ (30 psi). The product mixture was gravity filtered, washed with EtOAc and concentrated. This crude mixture was used a source of the angular xanthone **25** [TLC R_f = 0.04 ; 20% EtOAc in hexanes, ¹H NMR (400 MHz, CD₃COCD₃) δ 12.2 (s, 1H), 11.8 (s, 1H), 11.1 (s, 1H), 6.51 (d, 1H, J = 8.4 Hz), 6.46 (d, 1H, J = 2.1 Hz), 6.28 (d, 1H, J = 2.1 Hz), 6.21 (s, 1H), 4.1 (m, 2H), 3.5 (m, 1H), 2.1 (m, 2H)]. Pure **24** was isolated as a white solid (10.3 mg, 0.03 mmol, 24%, 250 °C dec; TLC R_f = 0.10; 20% EtOAc in hexanes) following elution of the crude product mixture through a plug of silica gel with 60% EtOAc in hexanes. ¹H NMR (400 MHz, CD₃COCD₃) δ 12.2 (s, 1H), 11.7 (s, 1H), 11.0 (s, 1H), 6.50 (d, 1H, J = 8.4 Hz), 6.44 (d, 1H, J = 2.1 Hz), 6.39 (s, 1H), 6.28 (d, 1H, J = 2.1 Hz), 4.1 (m, 2H), 3.5 (m, 1H), 2.1 (m, 2H). ¹³C NMR (100 MHz, CD₃SOCD₃) δ 182.4, 166.3, 165.9, 162.0, 157.4, 157.2, 156.6, 113.5, 109.0, 101.8, 100.4, 98.6, 94.2, 89.0, 67.2, 42.7, 30.5. HRMS-EI (m/z): M⁺ Na calcd for C₁₇H₁₂O₇, 351.0475; found, 351.0493.



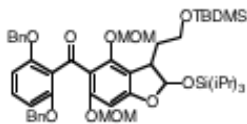
Synthesis of (*Linear*) 4,6-Dihydroxy-2,3,3a,12a-tetrahydrofuro[2,3-b]furo-xanth-5-one (21) and (*Angular*) 6,8-Dihydroxy-1,2,3a,12c-tetrahydrofuro[2,3-c]furo-xanth-7-one (22).



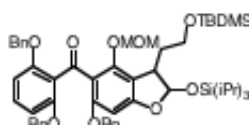
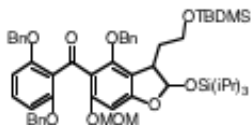
5-[1-(*R,S*)-{2',6'-*Bis*-(*O*-benzyl)}-1-hydroxy-methyl]-3-(*t*-butyldimethyl-silyloxy-2-ethyl)-4,6-bis-(*O*-methoxymethyl)-2,3-dihydro-benzofuran-2-yloxy]-triisopropyl-silane (12).

Benzophenols **12** (clear oil, 3.35 g, 3.77 mmol, 92%, TLC R_f 0.53, 0.54 - 20% EtOAc in hexanes) were prepared from bromide **9** (2.64 g, 4.08 mmol) and 2,6-bis-(*O*-benzyl)-benzaldehyde (**10**, 1.3 g, 4.08 mmol) according to *Procedure A*. ^1H NMR (400 MHz, CD_3COCD_3) δ 7.35 (m, 10H), 6.77, 6.75 (s, 2H), 6.67 (m, 1H), 6.40, 6.39 (s, 1H), 5.94, 5.92 (d, $J = 2.0$ Hz, 1H), 5.52, 5.43 (d, $J = 10.4$ Hz, 1H), 5.2 (m, 6H), 5.04 (m, 2H), 3.78 (m, 2H), 3.62, 3.60 (s, 1H), 3.5 (m, 1H), 2.99, 2.95, 2.85 (s, 6H), 1.87 (m, 1H), 1.79 (m, 1H) 1.2 (m, 21H), 0.85 (m, 9H), 0.05 (s, 6H). ^{13}C NMR (100 MHz, CD_3COCD_3) δ 160.1, 159.9, 158.7, 158.6, 158.4, 158.2, 157.7, 154.7, 154.7, 139.0, 138.8, 138.8, 129.9, 129.8, 129.5, 129.3, 129.1, 128.9, 128.8, 128.7, 123.0, 121.9, 119.7, 115.5, 115.2, 107.5, 107.3, 107.1, 106.9, 100.4, 100.1, 95.4, 95.2, 94.8, 94.7, 71.6, 71.4, 68.6, 66.2, 62.3, 62.2, 58.0, 56.2, 49.8, 49.7, 38.6, 35.8, 26.9, 28.8, 18.9, 18.8, 15.0, 13.5, 13.4, -4.6. IR (neat) 3558, 2953, 1593, 1463, 1380, 1092 cm^{-1} . HRMS-EI (m/z):

M^+Na calcd for $C_{50}H_{72}O_{10}Si_2$, 911.4556; found, 911.4543. Anal. Calcd for $C_{50}H_{72}O_{10}Si_2$: C, 67.53; H, 8.16. Found: C, 68.24; H, 8.04.

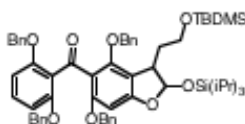


5-Keto-[2',6'-bis-(*O*-benzyl)-phenyl]-3-(*t*-butyldimethyl-silyloxy-2-ethyl)-4,6-bis-(*O*-methoxymethyl)-2,3-dihydro-benzofuran-2-yloxy]-triisopropyl-silane (14**). Benzophenone **14** (faintly yellow oil, 0.57 g, 0.65 mmol, 50%, TLC R_f 0.53 - 20% EtOAc in hexanes) was prepared from benzophenols **12** (1.15 g, 1.3 mmol) according to *Procedure B*. 1H NMR (400 MHz, CD_3COCD_3) δ 7.25 (m, 11H), 6.73 (d, 2H, J = 8.5 Hz), 6.41 (s, 1H), 6.04 (d, 1H, J = 1.3 Hz), 5.05 (s, 4H), 4.8 (m, 4H), 3.73 (m, 2H), 3.53 (dd, 1H, J = 3.0, 9.6 Hz), 3.31 (s, 3H), 3.01 (s, 3H), 1.99 (m, 1H), 1.65 (s, 1H), 1.1 (m, 21H), 0.90, 0.88 (s, 9H), 0.03, 0.02 (s, 6H). ^{13}C NMR (100 MHz, CD_3COCD_3) δ 191.4, 162.2, 159.1, 157.9, 155.0, 138.2, 131.1, 129.3, 129.2, 129.1, 128.3, 128.1, 124.3, 120.3, 115.8, 107.2, 106.7, 100.2, 95.3, 94.1, 71.0, 61.5, 57.2, 55.9, 48.7, 35.1, 26.4, 18.9, 18.4, 18.3, 12.9, -5.2. IR (neat) 2947, 2866, 1668, 1593, 1465, 1255, 1099 cm^{-1} . HRMS-EI (m/z): M^+Na calcd for $C_{50}H_{70}O_{10}Si_2$, 909.4399; found, 909.4360. Anal. Calcd for $C_{50}H_{70}O_{10}Si_2$: C, 67.68; H, 7.95. Found: C, 67.96; H, 7.98.**



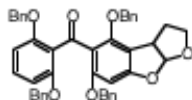
5-Keto-[2',6'-bis-(*O*-benzyl)-phenyl]-3-(*t*-butyldimethyl-silyloxy-2-ethyl)-4-(*O*-benzyl)-6-(*O*-methoxymethyl)-2,3-dihydro-benzofuran-2-yloxy]-triisopropyl-silane and **5-**

Keto-[2',6'-bis-(*O*-benzyl)-phenyl]-3-(*t*-butyldimethyl-silyloxy-2-ethyl)-6-(*O*-benzyl)-4-(*O*-methoxymethyl)-2,3-dihydro-benzofuran-2-yloxy]-triisopropyl-silane. The isomeric tribenzylethers shown above (clear oil, 0.64 g, 0.69 mmol, 74%, TLC R_f 0.21 - 20% EtOAc in hexanes) were prepared from benzophenone **14** (0.82 g, 0.92 mmol) according to *Procedure C*. ^1H NMR (400 MHz, CD_3COCD_3) δ 7.3 (m, 16H), 6.68 (s, 1H), 6.44 (d, $J = 8.4$ Hz, 2H), 6.06 (d, $J = 1.4$ Hz, 1H), 4.97 (dd, $J = 4.6, 16.6$ Hz, 4H), 4.82 (m, 4H), 3.66 (m, 2H), 3.51 (dd, $J = 3.5, 9.6$ Hz, 1H), 3.08 (s, 3H), 1.98 (m, 1H), 1.63 (m, 1H), 1.2 (m, 21H), 0.85, 0.84 (s, 9H), 0.00, -0.02 (s, 6H). ^{13}C NMR (100 MHz, CD_3COCD_3) 191.2, 185.2, 162.4, 160.7, 159.0, 158.0, 156.4, 138.7, 138.2, 131.1, 129.2, 128.8, 128.4, 128.3, 128.2, 128.1, 124.2, 120.8, 115.0, 107.4, 106.8, 106.6, 95.3, 94.1, 74.9, 70.8, 61.3, 56.0, 48.7, 35.1, 26.4, 18.4, 18.3, 12.9, -5.2. IR (neat) 2947, 2866, 1667, 1593, 1469, 1377, 1256, 1100 cm^{-1} . HRMS-EI (m/z): M^+Na calcd for $\text{C}_{55}\text{H}_{72}\text{O}_9\text{Si}_2$, 955.4607; found, 955.4556. Anal. Calcd for $\text{C}_{55}\text{H}_{72}\text{O}_9\text{Si}_2$: C, 70.78; H, 7.78. Found: C, 70.96; H, 7.76.

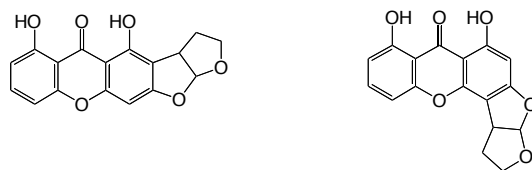


5-Keto-[2',6'-bis-(*O*-benzyl)-phenyl]-3-(*t*-butyldimethyl-silyloxy-2-ethyl)-4,6-bis-(*O*-benzyl)-2,3-dihydro-benzofuran-2-yloxy]-triisopropyl-silane (16**).** Tetrabenzylether **16** (clear oil, 0.68 g, 0.70 mmol, 78%, TLC R_f 0.25 - 20% EtOAc in hexanes) was prepared from the mixture of tribenzylethers prepared above (0.84 g, 0.90 mmol) according to *Procedure C*. ^1H NMR (400 MHz, CD_3COCD_3) δ 7.2 (m, 21H), 6.60 (d, $J = 8.4$ Hz, 2H), 6.40 (s, 1H), 6.03 (s, 1H), 4.80 (s, 6H), 4.63 (m, 2H), 3.65 (m, 2H), 3.47 (dd, $J = 8.6$ Hz, 1H), 1.95 (m, 1H), 1.61 (m, 1H), 1.15 (m, 21H), 0.81 (s, 9H), 0.00, -0.02 (s, 6H). ^{13}C NMR (100 MHz, CD_3COCD_3) δ 191.2,

185.1, 162.5, 160.6, 158.1, 156.6, 138.8, 138.2, 137.7, 131.0, 129.1, 129.0, 128.8, 128.4, 128.2, 124.1, 120.3, 114.1, 107.4, 106.7, 92.2, 74.9, 72.7, 71.2, 70.7, 61.3, 48.7, 35.1, 26.4, 18.8, 18.4, 18.3, 12.9, -5.2. IR (neat) 2946, 2866, 1663, 1592, 1453, 1376, 1255, 1100 cm^{-1} . HRMS-EI (m/z): $\text{M}^+ \text{Na}$ calcd for $\text{C}_{60}\text{H}_{74}\text{O}_8\text{Si}_2$, 1001.4814; found, 1001.4829. Anal. Calcd for $\text{C}_{60}\text{H}_{74}\text{O}_8\text{Si}_2$: C, 73.58; H, 7.62. Found: C, 73.67; H, 7.56.



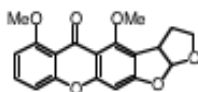
5-Keto-[2',6'-bis-(O-benzyl)-phenyl]-4,6-bis-(O-benzyl)-[2,3-b]-benzo-2,3,3a,8a-tetrahydro-bisfuran (18). Tetrahydrobisfuran **18** (white solid, mp = 58-60 °C, 0.30 g, 0.43 mmol, 86%, TLC R_f 0.30 - 20% EtOAc in hexanes) was prepared from disilylether **16** (0.48 g, 0.50 mmol) according to *Procedure D*. ^1H NMR (400 MHz, CD_3COCD_3) δ 7.2 (m, 20H), 7.06 (m, 1H), 6.65 (d, J = 8.4 Hz, 2H), 6.34 (d, J = 5.6 Hz, 1H), 6.32 (s, 1H), 4.74 (m, 8H), 3.94 (m, 2H), 3.42 (m, 1H), 1.99 (m, 2H). ^{13}C NMR (100MHz, CD_3COCD_3) δ 191.1, 163.6, 160.6, 158.3, 156.4, 138.9, 138.1, 137.6, 131.3, 129.1, 129.0, 128.9, 128.5, 128.4, 128.4, 128.3, 123.9, 120.4, 113.0, 112.5, 106.8, 91.1, 74.9, 71.2, 70.9, 67.8, 45.8, 32.5. IR (neat) 2975, 2873, 1662, 1592, 1452, 1376, 1249, 1097 cm^{-1} . HRMS-EI (m/z): $\text{M}^+ \text{Na}$ calcd for $\text{C}_{45}\text{H}_{38}\text{O}_7$, 713.2510; found, 713.2543. Anal. Calcd for $\text{C}_{45}\text{H}_{38}\text{O}_7$: C, 78.24; H, 5.54. Found: C, 78.19, H, 5.56.



(Linear) 4,6-Dihydroxy-2,3,3a,12a-tetrahydrofuro[2,3-b]furo-xanth-5-one (21) and (Angular) 6,8-Dihydroxy-1,2,3a,12c-tetrahydrofuro[2,3-c]furo-xanth-7-one (22).

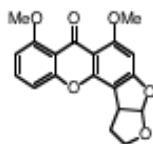
Tetrahydrobisfuran (**18**, 100 mg 0.14 mmol), Pd black (100 mg, 0.94 mmol), and EtOAc (4 mL) were shaken 12 h under H₂ (30 psi). The product mixture was gravity filtered, washed with EtOAc and concentrated. This crude mixture was used as a source of the angular xanthone **22** [TLC *R_f* 0.33; 20% EtOAc in hexanes, ¹H NMR (400 MHz, CDCl₃) δ 12.16 (s, 1H), 11.89 (s, 1H), 7.72 (t, *J* = 8.4 Hz, 1H), 7.05 (d, *J* = 8.4 Hz, 1H), 6.81 (d, *J* = 8.4 Hz, 1H), 6.54 (d, *J* = 5.6 Hz, 1H), 6.26 (s, 1H), 4.15 (m, 2H), 3.63 (m, 1H), 2.15 (m, 2H)]. Pure **21** was isolated as a white solid (9 mg, 0.03 mmol, 20% yield, 245 °C dec, TLC *R_f* 0.43; 20% EtOAc in hexanes) following elution of the crude product mixture through a plug of silica gel with 60% EtOAc in hexanes. ¹H NMR (400 MHz, CDCl₃) δ 12.07 (s, 1H), 11.75 (s, 1H), 7.70 (t, *J* = 8.4 Hz, 1H), 7.00 (d, *J* = 8.4 Hz, 1H), 6.80 (d, *J* = 8.4 Hz, 1H), 6.57 (s, 1H), 6.53 (d, *J* = 5.6 Hz, 1H), 4.10 (m, 2H), 3.51 (m, 1H), 2.14 (m, 2H). ¹³C NMR (100 MHz, CD₃SOCD₃) δ 184.0, 167.0, 160.2, 157.7, 156.7, 155.6, 137.4, 113.7, 110.7, 109.3, 107.1, 106.9, 102.5, 89.2, 67.3, 42.7, 30.5. HRMS-EI (*m/z*): M⁺H calcd for C₁₇H₁₂O₆, 313.0707; found, 313.0737.

Preparation of bis-*O*-methylxanthone derivatives (Figure 2).



4,6-Dimethoxy-2,3,3a,12a-tetrahydrofuro[2,3-b]furo-xanth-5-one.

Dihydroxyxanthone **21** (approximately 0.5 mg), dimethylsulfate (0.25 mL), and DMF (3 mL) were stirred at 0 °C and then NaH (0.5 g) was added. The ice bath was removed and stirring was continued 90 min. The mixture was then diluted with EtOAc and quenched with aq NaHCO₃. The organic layer was washed with water and brine, then dried over Na₂SO₄, and concentrated. White needles (approximately 0.3 mg) were recrystallized from EtOAc and hexanes. TLC *R_f* 0.31 – 50% EtOAc in hexanes (the dimethylether emits blue fluorescence under short wave UV light). ¹H NMR (400 MHz, CD₃COCD₃) δ 7.62 (t, *J* = 8.4 Hz, 1H), 6.99 (d, *J* = 8.4 Hz, 1H), 6.93 (d, *J* = 8.4 Hz, 1H), 6.54 (s, 1H), 6.44 (d, *J* = 5.6 Hz, 1H), 4.06 (m, 2H), 3.60 (m, 1H), 2.28 (m, 2H).



6,8-Dimethoxy-1,2,3a,12c-tetrahydrofuro[2,3-c]furo-xanth-7-one.

Sterigmatocystin (**30**) (approximately 2.0 mg), 10% Pd on carbon (20 mg) and EtOAc (2 mL) were shaken 5 h under H₂ (30 psi). The mixture was gravity filtered, and washed with EtOAc. The resulting filtrate was concentrated and eluted through a silica plug with 30% EtOAc and hexanes. The sterigmatocystin TLC *R_f* was 0.32 and the reduced tetrahydrosterigmatocystin TLC *R_f* was 0.24 (30% EtOAc in hexanes). The resulting tetrahydrobisfuran was then combined with dimethylsulfate (0.25 mL) and DMF (3 mL). The mixture was stirred at 0 °C and NaH (0.5 g) was added. The ice bath was then removed and stirring was continued 90 min. The mixture was diluted with EtOAc and quenched with aq NaHCO₃. The organic layer was washed with

water and brine, dried over Na_2SO_4 and concentrated. *O*-Methyldihydrohydrosterigmatocystin was eluted through a plug of silica gel with 50% EtOAc and hexanes. (TLC R_f 0.24; 50% EtOAc in hexanes; the dimethylether emits blue fluorescence under short wave UV light). ^1H NMR (400 MHz, CD_3COCD_3) δ 7.59 (t, $J = 8.4$ Hz, 1H), 7.02 (d, $J = 8.4$ Hz, 1H), 6.92 (d, $J = 8.4$ Hz, 1H), 6.47 (d, $J = 5.6$ Hz, 1H), 6.41 (s, 1H), 4.1 (m, 2H), 3.6 (m, 1H), 2.3 (m, 2H).

Microbiological Procedures

Organisms, media, and buffers were detailed in the accompanying report. Likewise, the whole-cell and ground-cell feeding experiments were conducted as described in that paper.