

Supplementary Methods

Dauer recovery assay. Nematodes were cultured for about 14 d in liquid media at 20 °C on a rotary shaker. When most of the worms became dauers, the culture media was centrifuged, the pellet was resuspended in small amount of S medium, and then layered on top of the 15% (w/w) ficoll solution in 0.1% sodium chloride, allowing the dauers to settle to the lower layer. The upper layer and interface were removed, and dauers in the ficoll layer were washed with S medium three times. About 100-200 dauer larvae were placed on NGM-agar plates (with 0.05% yeast extract, but without peptone) containing test samples and incubated at 25 °C for about 48 h. Dauer and non-dauer larvae were then counted.

Dauer formation assay. Synthetic ascarosides or vehicle (ethanol) were added to 100 μ L of water at 30 times the assay concentration. This 100 μ L was then mixed into 3 mL of NGM-agar (without peptone) at 60 °C in a 35 x 10 mm plate, and the agar was allowed to set. A stock of heat-killed OP50 was made by diluting OP50 to 8 mg / mL with S basal and heating to 95 °C for 30 min, with vortexing every 5 min. The plates and OP50 stock were stored overnight at 4 °C. 20 μ L of the OP50 stock was added to each plate, and plates were allowed to dry in a hood for 15 min. 5 adult worms were placed on each plate, allowed to lay eggs for 3-4 h, and removed. These adult worms were semi-synchronized for the egg lay usually by performing another egg lay 3d prior to the assay. The assay plates were incubated at 20 °C for 92-96 h or at 25 °C for 68-72 h, and dauers were identified based on size, shape, and lack of pharyngeal pumping. In the titration

experiments, in at least one experiment per concentration, the number of dauers was verified by treatment with 1% sodium dodecyl sulfate for 45 min. Data was included only from assay plates that had between 40 and 100 worms. Performing the assay using agar plates made with agar that was less refined than Noble agar (BD Biosciences) resulted in fewer dauers in response to ascaroside treatment, but did not change the relative activity of the different ascarosides.

Preparation of (-)-5*R*-(tetrahydro-6'*S*-methyl-3'*R*,5'*R*-di-(4-nitrobenzoyloxy)-2*H*-pyran-2'*R*-yloxy)-2-hexanone (4). Compound **1** (1.0 mg) and a catalytic amount of 4-dimethylaminopyridine were added to 300 μ l of a stirred solution of 4-nitrobenzoyl chloride in pyridine (10 mg/mL). The reaction mixture was kept at room temperature for 6 h. Subsequently, 100 μ L of water was added and the mixture was dried *in vacuo*. The resulting residue was chromatographed on silica gel with hexane and ethylacetate (1:2) to afford derivative **4**.

Quantitative analysis of ascarosides 1-3 in conditioned media extract. Conditioned media extract derived from 2 L of media was suspended in 0.35 ml of methanol- d_4 and stirred for 2 h at 20 °C. The resulting yellow suspension was centrifuged, and the supernatant was separated. The pellet was re-suspended by addition of another 0.35 mL of methanol- d_4 and after additional stirring the suspension was again centrifuged, and the supernatant was removed. Further extraction of the pellet did not yield significant additional quantities of ascarosides. The combined supernatants were evaporated to dryness, and the resulting oily residue was re-dissolved in 0.6 ml of methanol- d_4 (99.99%

d) for NMR-spectroscopic analysis using a high-resolution dqfCOSY (0.6 s acquisition time, 8 ppm sweep width, 512 increments). Using identical parameters, reference dqfCOSY spectra were acquired using 0.29 mg and 0.021 mg samples of synthetic **1** and 0.10 mg and 0.020 mg samples of synthetic **2** or **3**. The amount of **1** in the media extract samples was determined via signal-to-noise measurements of cross sections of the H-3/H-4 cross peak of **1**, and the amounts of **2** and **3** were determined from signal-to-noise measurements of cross sections of the H-2/H-3 cross peaks of **2** and **3**. These cross peaks were chosen for analysis because they did not overlap significantly with other signals in the spectra and showed characteristic and clearly recognizable splitting patterns. In two different 2 L cultures, 104 µg and 187 µg of **1**, 48 µg and 76 µg of **2**, and less than 10 µg and 20 µg of **3** were detected.

Synthesis of (-)-5R-(tetrahydro-3'R,5'R-dihydroxy-6'S-methyl-2H-pyran-2'R-yloxy)-2-hexanone (1) and (-)-8R-(tetrahydro-3'R,5'R-dihydroxy-6'S-methyl-2H-pyran-2'R-yloxy)-2E-nonenoic acid (2).

Synthesis of (-)-5R-(tetrahydro-3'R,5'R-dihydroxy-6'S-methyl-2H-pyran-2'R-yloxy)-2-hexanone (1)¹. Trichloroacetonitrile (2.1 mmol, 210 µl,) and DBU (0.1 mmol, 15 µl) were added *via* syringe to a stirred solution of 2,4-di-O-benzoylascarylose² (tetrahydro-3'R,5'R-dibenzoyloxy-6'S-methyl-2H-pyrane (**5**), 0.98 mmol, 345 mg) in dry dichloromethane (8 ml) at room temperature. After 30 min of stirring, the dichloromethane was evaporated at room temperature *in vacuo*, and the residue was quickly chromatographed over a short (10 cm) silica gel column (solvent ethyl acetate:

hexane = 1:4). Yield: 404 mg (0.81 mmol, 81%) of N-(tetrahydro-3'R,5'R-dibenzoyloxy-6'S-methyl-2H-pyran-2'S-yloxy)-2,2,2-trichloroacetimide (**6**) as a viscous colorless oil. ¹H NMR (500 MHz, acetone-d₆): δ 9.48 (s, 1H, N-H), 8.14 (m, 2H), 8.04 (m, 2H), 7.71 (m, 1H), 7.66 (m, 1H), 7.59 (m, 2H), 7.53 (m, 2H), 6.39 (m, 1H, 2'-H), 5.42 (m, 1H, 3'-H), 5.24 (ddd, J = 11.5, 9.8, 4.5 Hz, 1H, 5'-H), 4.36 (dq, J = 9.8, 6.3 Hz, 1H, 6'-H), 2.66 (m, 1H, 4'-H), 2.34 (ddd, J = 14.1, 11.5, 3.1 Hz, 1H, 4'-H), 1.32 (d, J = 6.35 Hz, 3H, 6'-CH₃); ¹³C NMR (126 MHz, acetone-d₆): δ 166.12, 165.95, 160.15, 134.68, 134.43, 130.92, 130.86, 130.68 (2C), 130.57 (2C), 129.87 (2C), 129.84 (2C), 94.66, 91.96, 70.93, 70.51, 70.22, 30.55, 18.39.

A stirred solution of N-(tetrahydro-3'R,5'R-dibenzoyloxy-6'S-methyl-2H-pyran-2'S-yloxy)-2,2,2-trichloroacetimide (**6**) (0.54 mmol, 265 mg) and 2R,5R-hexanediol (1.3 mmol, 154 mg) in 4 ml of dry dichloromethane was treated with trimethylsilyltriflate (0.06 mmol, 10.9 μl) at 0 °C¹. After 30 min at this temperature, saturated aqueous NaHCO₃ (0.2 ml) was added. The resulting two-phase system was stirred vigorously for 5 min, and subsequently filtered over a pad (1 g) of dry NaHCO₃. The filtrate was evaporated *in vacuo* at room temperature and chromatographed over silica gel (solvent ethyl acetate:hexane = 1:2) yielding 160 mg (0.35 mmol, 64%) of 5R-(tetrahydro-3'R,5'R-dibenzoyloxy-6'S-methyl-2H-pyran-2'R-yloxy)-2R-hexanol (**7**) as a colorless oil. ¹H NMR (600 MHz, acetone-d₆): δ 8.11 (m, 2H), 8.03 (m, 2H), 7.69 (m, 1H), 7.65 (m, 1H), 7.57 (m, 2H), 7.53 (m, 2H), 5.14 (ddd, J = 11.5, 9.8, 4.5 Hz, 1H, 5'-H), 5.11 (m, 1H, 3'-H), 5.00 (m, 1H, 2'-H), 4.19 (dq, J = 9.8, 6.3 Hz, 1H, 6'-H), 3.92 (m, 1H, 5-H), 3.78 (m, 1H, 2-H), 2.45 (m, 1H, 4'-H), 2.22 (ddd, J = 13.6, 11.5, 3.0 Hz, 1H, 4'-H), 1.78 (m, 1H, 4-H), 1.64-1.52 (m, 3H, 3-H and 4-H), 1.26 (d, J = 6.35 Hz, 3H, 6'-CH₃), 1.21 (d, J = 6.2

Hz, 3H, 6-H), 1.17 (d, J = 6.2 Hz, 3H, 1-H); ^{13}C NMR (151 MHz, acetone- d_6): δ 166.20 (2C), 134.46, 133.33, 131.28, 131.22, 130.58 (2C), 130.46 (2C), 129.76 (2C), 129.70 (2C), 94.73, 73.46, 71.70, 70.83, 67.95, 67.42, 36.38, 34.33, 30.64, 24.37, 19.80, 18.45.

Crushed molecular sieves (4 Å, 0.2 g) were added to a stirred solution of 5*R*-(tetrahydro-3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-2*H*-pyran-2'*R*-yloxy)-2*R*-hexanol (**7**) (0.35 mmol, 160 mg) in dry dichloromethane (5 ml) at room temperature. After 5 min of stirring, pyridinium dichromate (300 mg) was added in one portion³. The resulting mixture was stirred for 2 h, then diluted with hexane (5 ml) and filtered over a short silica gel column (ethyl acetate:hexane = 1:3). The filtrate was evaporated *in vacuo*, yielding 142 mg (0.31 mmol, 89%) of (-)-5*R*-(tetrahydro-3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-2*H*-pyran-2'*R*-yloxy)-2-hexanone (**8**) as a colorless oil; $[\alpha]_D^{25}$ -3.6, *c* 4.9 (dichloromethane); ^1H NMR (600 MHz, acetone- d_6): δ 8.09 (m, 2H), 8.03 (m, 2H), 7.63 (m, 1H), 7.61 (m, 1H), 7.52 (m, 2H), 7.48 (m, 2H), 5.13 (ddd, J = 11.5, 9.8, 4.5 Hz, 1H, 5'-H), 5.10 (m, 1H, 3'-H), 4.96 (m, 1H, 2'-H), 4.12 (dq, J = 9.8, 6.3 Hz, 1H, 6'-H), 3.90 (sext, J = 6.2 Hz, 1H, 5-H), 3.78 (m, 1H, 2-H), 2.63 (m, 2H, 3-H), 2.42 (m, 1H, 4'-H), 2.20 (ddd, J = 13.6, 11.5, 3.0 Hz, 1H, 4'-H), 2.15 (s, 3H, 1-H), 1.86-1.80 (m, 2H, 4-H), 1.26 (d, J = 6.35 Hz, 3H, 6'-CH₃), 1.19 (d, J = 6.2 Hz, 3H, 6-H); ^{13}C NMR (151 MHz, acetone- d_6): δ 207.86, 165.91, 165.87, 133.93, 133.81, 130.88, 130.76, 130.23 (2C), 130.12 (2C), 129.24 (2C), 129.12 (2C), 94.21, 72.10, 71.89, 71.30, 67.67, 39.83, 31.56, 30.36, 29.90, 19.11, 18.03.

A solution of (-)-5*R*-(tetrahydro-3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-2*H*-pyran-2'*R*-yloxy)-2-hexanone (**8**) (0.31 mmol, 142 mg) in 1 M methanolic KOH (3 ml) was stirred at room temperature for 3 h. Subsequently, NaHCO₃ (100 mg) was added and the mixture was evaporated to dryness *in vacuo*. The residue was chromatographed over a

silica gel column (solvent dichloromethane:methanol = 12:1), yielding 59 mg (0.24 mmol, 78%) of (-)-5*R*-(tetrahydro-3'*R*,5'*R*-dihydroxy-6'*S*-methyl-2*H*-pyran-2'*R*-yloxy)-2-hexanone (**1**) as a colorless oil; $[\alpha]_D^{23} = -78.4$, c 1.2 (dichloromethane); HR-ESIMS (m/z): $[M+Na]^+$ calcd. for $C_{12}H_{22}O_5Na$, 269.1365; found, 269.1381. 1H and ^{13}C NMR spectroscopic data were identical with those of the natural product.

(-)-8*R*-(tetrahydro-3'*R*,5'*R*-dihydroxy-6'*S*-methyl-2*H*-pyran-2'*R*-yloxy)-2*E*-nonenoic acid (2**).** A stirred solution of N-(tetrahydro-3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-2*H*-pyran-2'*S*-yloxy)-2,2,2-trichloroacetimide (**6**) (0.16 mmol, 80.12 mg) and 8*R*-hydroxy-2*E*-nonenoic acid ethyl ester (**9**) (0.15 mmol, 29.5 mg) in 4 ml of dry dichloromethane was treated with trimethylsilyltriflate (0.016 mmol, 2.85 μ l) at 0 °C. After 60 min at this temperature, saturated aqueous $NaHCO_3$ (0.2 ml) was added. The resulting two-phase system was stirred vigorously for 5 min, and subsequently filtered over a pad (1 g) of dry $NaHCO_3$. The filtrate was evaporated *in vacuo* at room temperature and chromatographed over silica gel (solvent ethyl acetate:hexane = 1:4) yielding 64 mg (0.12 mmol, 74%) of (-)-8*R*-(tetrahydro-3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-2*H*-pyran-2'*R*-yloxy)-2*E*-nonenoic acid ethyl ester (**10**) as a colorless oil; $[\alpha]_D^{25} = -7.5$, c 3.2 (dichloromethane); 1H NMR (600 MHz, acetone- d_6): δ 8.10 (m, 2H), 8.04 (m, 2H), 7.68 (m, 1H), 7.64 (m, 1H), 7.56 (m, 2H), 7.52 (m, 2H), 6.96 (dt, $J = 15.7, 7.0$ Hz, 1H, 3-H), 5.86 (dt, $J = 15.7, 1.6$ Hz, 1H, 2-H), 5.14 (ddd, $J = 11.5, 9.8, 4.5$ Hz, 1H, 5'-H), 5.11 (m, 1H, 3'-H), 5.00 (m, 1H, 2'-H), 4.17 (dq, $J = 9.8, 6.3$ Hz, 1H, 6'-H), 4.09 (q, $J = 7.1$ Hz, 2H, CH_3CH_2), 3.90 (m, 1H, 8-H), 2.45 (m, 1H, 4'-H), 2.28 (m, 2H, 4-H), 2.21 (ddd, $J = 13.6, 11.5, 3.0$ Hz, 1H, 4'-H), 1.67 (m, 1H), 1.60-1.45 (m, 5H), 1.26 (d, $J = 6.35$ Hz, 3H,

6'-CH₃), 1.20 (d, J = 6.2 Hz, 3H, 6-H), 1.19 (t, J = 7.1 Hz, 3H, CH₃CH₂); ¹³C NMR (151 MHz, acetone-d₆): δ 166.78, 166.19 (2C), 149.84, 134.43, 134.32, 131.21 (2C), 130.57 (2C), 130.51 (2C), 129.75 (2C), 129.63 (2C), 122.48, 94.71, 73.26, 72.50, 71.68, 67.99, 60.61, 37.87, 32.89, 30.61, 28.97, 26.27, 19.77, 18.48, 14.85.

A solution of (-)-8*R*-(tetrahydro-3'*R*,5'*R*-dibenzoyloxy-6'*S*-methyl-2*H*-pyran-2'*R*-yloxy)-2*E*-nonenoic acid ethyl ester (**10**) (50.0 mg, 0.093 mmol) in a mixture of THF (3 ml) and 2 M aqueous LiOH (5 ml) was stirred at 63 °C for 6 h. The mixture was cooled to room temperature and the pH was adjusted to 3.5 *via* drop wise addition of 0.5 N aqueous HCl. NaCl (1 g) was added and the resulting mixture was extracted with three 10-ml portions of ethyl acetate. The combined extracts were evaporated *in vacuo* and the residue was chromatographed over silica gel (solvent dichloromethane:methanol = 10:1), yielding 19 mg (0.063 mmol, 68%) of (-)-8*R*-(tetrahydro-3'*R*,5'*R*-dihydroxy-6'*S*-methyl-2*H*-pyran-2'*R*-yloxy)-2*E*-nonenoic acid (**2**) as a colorless oil; [α]_D²⁵ = -84.2, *c* 1.1 (acetone); HR-ESIMS (*m/z*): [M+Na]⁺ calcd. for C₁₅H₂₆O₆Na, 325.1627; found 325.1614.

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2. Sznajdman, M., Cirelli, A.F., De Lederkremer, R.M. Carbon-13 NMR spectral studies of di- and trideoxyaldohexoses. *Journal of Carbohydrate Chemistry* **5**, 249-55 (1986).
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