Supporting Information

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SI Text

***Panagrellus redivivus* Cultures and Worm Water Collection.** Ten nematode growth medium plates seeded with *Escherichia coli* (OP50 strain) were chunked and incubated at room temperature (RT) for 3 d. Worms were washed off the plates with 25 mL S-complete medium and transferred to a 250-mL Erlenmeyer flask containing 3% (0.75 g) *E. coli* (HB101 strain); 10,000 worms/mL were incubated at 22 °C for 3 d and then transferred to a 2-L Erlenmeyer flask containing 250 mL S-complete medium and 3% (7.5 g) HB101. Mixed populations of *P. redivivus* were cultured for 12 d, and 3% HB101 was added every third day. After 12 d of incubation, the worms were separated from bacteria by sucrose flotation. Clean *P. redivivus* were placed in M9 buffer for 30 min to clear their gut. Worms were washed three times with double-distilled water (ddH_20) and then incubated for 1 h in ddH_20 with a density of ~30,000 worms/mL to collect worm exometabolites. Worm water was filtered with a 0.2-μm nylon syringe filter and stored at −80 °C for additional analysis. A working unit called worm equivalents (WEs) was used to keep track of relative concentrations of unknown compounds. One WE is the volume of worm water that contains the compounds secreted by one worm in 1 h.

Activity-Guided C18 Fractionation. The purification involved a series of fractionation steps guided by the attraction bioassay. The first step used a Varian Mega BE C18 column, which has a 5-mL void volume. A constant pressure was applied to the column by applying pressure to a 50-mL syringe, which was attached to the column with a modified rubber stopper covered with Teflon tape. The C18 column was first washed with 50 mL 90% methanol followed by 50 mL ddH_20 for equilibration. Conditioned water from 4,000,000 WE *P. redivivus* was lyophilized and resuspended in H_2O, and then, it was added to the equilibrated column; the flow-through was collected. The column was washed with 25 mL ddH_20 and then eluted with 25 mL 50% methanol followed by 25 mL 90% methanol. The 50% and 90% methanol fractions were collected and tested for activity. Bioassays showed the pheromone activity in the 50% methanol fraction. All of the samples were collected in glass vials with Teflon caps and stored at −80 °C.

Ion exchange fractionation. The active 50% methanol fraction was further fractionated using anion and cation columns from Alltech. Both columns were conditioned with 5–10 mL ddH_2O and then coupled together (cation column on top) with an adapter. The 50% methanol fraction was diluted to 20% methanol and then added to the cation column. The syringe was placed on top of the cation column, and constant pressure was applied to push the sample through both columns. The flow-through was collected, and the columns were decoupled. Each column was eluted with 2 mL 250 mM KCl, 500 mM KCl, and 1 M KCl. Six fractions were then desalted using C18 column. A total of seven fractions, including the flow-through, were tested for bioactivity. Bioassays showed the pheromone activity in the 1 M KCl anion fraction.

HPLC purification. The active 1 M KCl anion fraction was purified by C18 reversed-phase column (PLRP-S polymeric reversed-phase column, 250 × 4.6 mm i.d.; Varian) using the LC-MS system with the conditions described below. A lyophilized sample was resuspended in 100 μL 50% methanol, and 10 μL were injected through the column to determine the retention time of the active component, which was tested for activity using the male/female attraction assay. LC-MS analysis of the active 50% fraction showed the presence of two major peaks that had retention times of 8.5 and 9.7 min. The peak with RT:8.5 min showed the female attraction, and the peak with RT:9.7 min showed the male attraction. The remaining samples were injected in succession (30 μL × 3) to do peak-based fractionation of these two active components. The samples were stored at −80°C.

NMR Spectroscopy. NMR spectra were acquired at 27 °C using a Bruker 600 MHz Avance II spectrometer equipped with a 5-mm TXI CryoProbe in the Advanced Magnetic Resonance Imaging and Spectroscopy (AMRIS) Facility at the University of Florida and the NHMFL or Varian INOVA 600 (600 MHz), Varian INOVA 500 (500 MHz), or Varian INOVA 400 (400 MHz) spectrometers in Cornell University's NMR facility. We collected standard 1D and 2D NMR experiments [double quantum filtered-correlated spectroscopy (dqf-COSY), total correlation spectroscopy (TOCSY), heteronuclear single quantum correlation (HSQC), heteronuclear multiple bond correlation (HMBC), nuclear Overhauser effect spectroscopy (NOESY), and rotating Overhauser effect spectroscopy (ROESY)] on the active components from the activity-guided purification. ^1H NMR chemical shifts are reported in parts per million (δ) relative to residual solvent peaks (δ = 7.26 ppm for chloroform-*d*, δ = 3.31 ppm for methanol-d_4). NMR spectroscopic data are reported as follows: chemical shift, multiplicity (d, doublet; m, multiplet; q, quartet; s, singlet; t = triplet), coupling constants (Hertz), and integration. ^{13}C NMR chemical shifts are reported in parts per million (δ) relative to $CDCl_3$ (δ = 77.2) in chloroform-*d* and CD_3OD (δ = 49.0) in methanol-d_4.

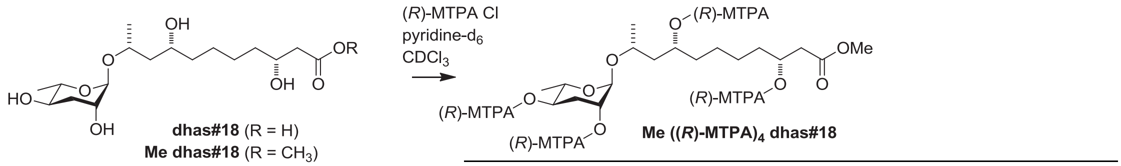
LC-MS. A Thermo Finnigan LCQ Deca XP Max was used with electrospray ionization in positive ion mode in the 50–1,000 AMU range. The Thermo Separation spectra HPLC system consisted of a P4000 quaternary pump, an AS 3000 autosampler, and a UV 6000 diode array detector. The solvents were (*i*) water with 0.1% formic acid and (*ii*) 90% acetonitrile–10% water with 10 mM ammonium formate. The column temperature was maintained at 60 °C and a solvent flow of 1.0 mL/min. The reversed-phase column (PLRP-S polymeric reversed-phase column, 250 × 4.6 mm i.d.; Varian) was eluted with a solvent composition starting with 90:10 (*i*:*ii*) for 2 min followed by a gradient to 5:95 in 20 min and an additional 5 min at 5:95. UV absorption was monitored at 190 and 400 nm, and solvent flow between the UV detector and MS electrospray interface split 9:1 with a low-volume microneedle P450 splitter valve (Upchurch Scientific), making it possible to obtain spectra of eluted compounds and simultaneously collect 90% of the injected material for bioassay.

Sex-Specific Identification. For preparation of *P. redivivus* sex-specific worm media, 300–500 young adult males and females were picked and incubated separately in M9 at a concentration of 1 worm per 1 μL for 6 h. Worms were filtered out, and worm media were lyophilized separately. The resulting residues were taken up in 100 μL methanol, filtered, and analyzed by LC-MS using single-ion recording mode.

HPLC-ESI-MS/MS. High-performance liquid chromatography-electrospray ionization-mass spectrometry (HPLC-ESI-MS)/MS was performed using an Agilent 1100 Series HPLC system equipped with an Agilent Eclipse XDB-C18 column (9.4 × 250 mm, 5-μm particle diameter) connected to a Quattro II mass spectrometer (Micromass/Waters) using a 10:1 split. A 0.1% acetic acid–acetonitrile solvent gradient was used at a flow rate of 3.6 mL/min starting with an acetonitrile content of 5% for 5 min, which was increased to 100% over a period of 40 min. Exudates were analyzed by HPLC-ESI-MS in negative and positive ion modes using a capillary voltage of 3.5 kV and a cone voltage of −40 and +20 V, respectively. LC-MS/MS screening for precursor ions of m/z = 73.0 (negative mode) was performed using argon as the collision gas at 2.1 mtorr and 30 eV.

Isolation of *P. redivivus* Ascarosides. Ascarosides (ascr#1, ascr#10, dhas#18, bhas#18, and bhas#10) were isolated from media supernatant extracts of *P. redivivus* by HPLC using an Agilent 1100 Series HPLC system equipped with an Agilent Eclipse XDB-C18 column (9.4 × 250 mm, 5-μm particle diameter) coupled to a Teledyne ISCO Foxy 200 fraction collector. A 0.1% acetic acid–acetonitrile solvent gradient was used at a flow rate of 3.6 mL/min starting with an acetonitrile content of 5% for 5 min, which was increased to 100% over a period of 40 min (identical to the conditions used for HPLC-ESI-MS/MS analysis).

Synthesis of Mosher Esters.

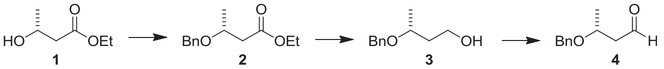


dhas#18 (R = H)
Me dhas#18 (R = CH_3)
Me ((*R*)-MTPA)$_4$ dhas#18

A solution of synthetic dhas#18 (182 μg, 0.5 μmol) in a mixture of methanol (250 μL) and toluene (250 μL) was treated with 2.0 M (trimethylsilyl)diazomethane in diethyl ether (10 μL, 20 μmol). After stirring for 30 min, excess reagent was quenched by the addition of acetic acid (10 μL), and the solution was concentrated in vacuo. The residue was taken up in dry $CDCl_3$ (1.6 mL), treated with pyridine-d_5 (2 μL, 25 μmol), and split in one-half, and each portion was treated with either (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride or (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (3.7 μL, 20 μmol).

Simultaneously, a solution of natural dhas#18 (*ca.* 35 μg, 0.1 μmol), isolated from *P. redivivus*, in a mixture of methanol (50 μL) and toluene (50 μL) was treated with 2.0 M (trimethylsilyl) diazomethane in diethyl ether (2 μL, 4 μmol). After stirring for 30 min, excess reagent was quenched by the addition of acetic acid (2 μL), and the solution was concentrated in vacuo. The residue was taken up in dry $CDCl_3$ (0.8 mL) and treated with pyridine-d_5 (0.4 μL, 5 μmol) and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (0.8 μL, 4 mmol). After stirring at room temperature for 3 h, samples were transferred to NMR tubes and analyzed by ^1H NMR spectroscopy.

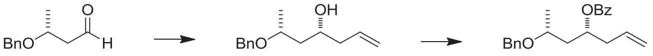
Synthesis of 3*R*-benzyloxybutanal (4).



1 **2** **3** **4**

A solution of ethyl (*R*)(−)-3-hydroxybutyrate (**1**; 1.01 g, 7.57 mmol) and benzyl 2,2,2-trichloroacetimidate (3.82 g, 15.13 mmol) in a 2:1 mixture (vol/vol) of cyclohexane and dichloromethane (75 mL) was treated with triflic acid (100 μL) and stirred for 40 h (1, 2). The reaction was quenched with saturated aqueous $NaHCO_3$ solution, extracted with dichloromethane (3 × 100 mL), dried over Na_2SO_4, and concentrated in vacuo. Flash column chromatography on silica using a gradient of 0–20% ethyl acetate in hexanes afforded **2** (1.57 g, 7.06 mmol, 93%) as a colorless oil. A solution of **2** (1.18 g, 5.31 mmol) in freshly distilled tetrahydrofuran (THF) (5 mL) was brought to 0 °C, treated slowly with a cooled solution of $LiAlH_4$ (398 mg, 10.5 mmol) in THF (15 mL), and stirred for 30 min at 0 °C. The mixture was diluted with diethyl ether (20 mL) and quenched by the addition of ice. The mixture was acidified with concentrated acetic acid, extracted with diethyl ether, dried over Na_2SO_4, and concentrated in vacuo. Flash column chromatography on silica using a gradient of 10–40% ethyl acetate in hexanes afforded **3** (860 mg, 4.82 mmol, 91%) as a colorless oil. To a Schlenk flask charged with argon, alcohol **3** (835 mg, 4.69 mmol) and dry dichloromethane (25 mL) were added and stirred. The mixture was brought to 0 °C, treated with *N*,*N*-diisopropylethylamine (1.9 mL, 14.0 mmol), and allowed to stir. After 15 min, the mixture was treated at 0 °C with sulfur trioxide pyridine complex (2.24 g, 14.1 mmol) in dry dimethyl sulfoxide (5.0 mL) and allowed to stir for 30 min, at which time the reaction was quenched with brine (20 mL) and extracted with dichloromethane (20 mL) and then ethyl acetate (3 × 25 mL). The combined organic layer was dried over Na_2SO_4, concentrated in vacuo, and purified by flash column chromatography on silica using a gradient of 0–20% ethyl acetate in hexanes yielding **4** (750 mg, 4.26 mmol, 91%) as a colorless oil. ^1H NMR (500 MHz, chloroform-*d*): δ (ppm) 9.81–9.78 (m, 1H), 7.38–7.23 (m, 5H), 4.61 (d, J = 11.6 Hz, 1H), 4.48 (d, J = 11.6 Hz, 1H), 4.13–4.05 (m, 1H), 2.71 (ddd, J = 16.4, 7.4, 2.5 Hz, 1H), 2.53 (ddd, J = 16.4, 5.0, 1.8 Hz, 1H), 1.31 (d, J = 6.2 Hz, 3H). ^{13}C NMR (125 MHz, chloroform-*d*): δ (ppm) 201.2, 138.1, 128.2, 127.43, 127.42, 70.3, 70.0, 50.2, 19.5.

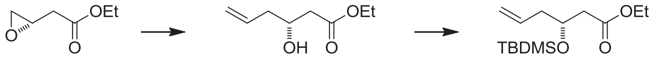
Synthesis of (4*R*,6*R*)-6-(benzyloxy)hept-1-en-4-yl benzoate (6).



4 **5** **6**

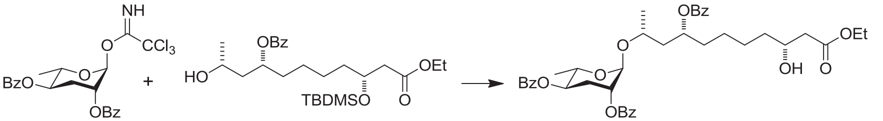
A solution of **4** (190 mg, 1.07 mmol) in dichloromethane (15 mL) was brought to −78 °C, treated with $TiCl_4$ (120 μL, 1.12 mmol), and stirred for 10 min at −78 °C (1). The mixture was treated with allyltriphenylstannane (880 mg, 2.25 mmol) in dichloromethane (2 mL) over 5 min and stirred at −78 °C. After 4 h, the mixture was treated with saturated aqueous $NaHCO_3$ solution (10 mL). After the reaction mixture reached RT, acetonitrile (15 mL) and KF (400 mg, 6.9 mmol) were added to the mixture and were allowed to stir. After 10 h, the mixture was diluted with diethyl ether and filtered over celite. The filtrate was extracted with diethyl ether (3 × 20 mL), and the organic layer was dried over Na_2SO_4 and concentrated in vacuo. Flash column chromatography on silica using a gradient of 0–30% ethyl acetate in hexanes afforded **5** (194 mg, 0.88 mmol, 82%) as a colorless oil. A solution of **5** (194 mg, 0.88 mmol) in pyridine (5 mL) was brought to 0 °C, treated with benzoyl chloride (200 μL, 1.72 mmol), allowed to warm to RT, and stirred. After 6.5 h, the mixture was quenched with brine (20 mL) and diluted with diethyl ether (20 mL). The organic layer was successively washed with 5% aqueous HCl (30 mL), brine (20 mL), saturated $NaHCO_3$ (20 mL), and brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. Flash column chromatography on silica using a gradient of 0–10% ethyl acetate in hexanes afforded **6** (280 mg, 0.86 mmol, 97%) as a colorless oil. ^1H NMR (500 MHz, chloroform-*d*): δ (ppm) 8.04–8.00 (m, 2H), 7.58–7.52 (m, 1H), 7.46–7.40 (m, 2H), 7.35–7.31 (m, 2H), 7.29–7.24 (m, 2H), 7.22–7.16 (m, 1H), 5.83 (ddt, J = 17.1, 10.0, 7.2 Hz, 1H), 5.51–5.45 (m, 1H), 5.12–5.03 (m, 2H), 4.52 (d, J = 11.2 Hz, 1H), 4.36 (d, J = 11.2 Hz, 1H), 3.68–3.60 (m, 1H), 2.53–2.41 (m, 2H), 1.91–1.79 (m, 2H), 1.23 (d, J = 6.1 Hz, 3H). ^{13}C NMR (125 MHz, chloroform-d): δ (ppm) 166.1, 138.6, 133.6, 132.8, 130.7, 129.6, 128.40, 128.39, 128.1, 127.6, 118.1, 71.4, 71.2, 71.0, 41.7, 39.5, 20.0.

Synthesis of ethyl-(*R*)-3-*tert*-butyldimethylsilyloxyhex-5-enoate (9).



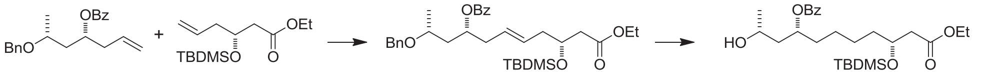
7 8 9

A solution of CuBr·SMe$_2$ (868 mg, 4.22 mmol) in a 3:5 mixture (vol/vol) of THF:diethyl ether (40 mL) was brought to −78 °C under Ar atmosphere, treated with vinyl magnesium bromide (1.0 M in THF, 8.45 mL), and allowed to stir. After stirring at −78 °C for 5 min, the reaction was brought to −40 °C and allowed to stir for 2 h at −40 °C. Subsequently, ethyl (*S*)-2-oxiranylacetate (**7**; 500 mg, 3.84 mmol) was added at −40 °C, and the reaction was stirred at −40 °C for an additional 6 h. The reaction mixture was gradually warmed up to RT over 4 h, and then, it was brought to 0 °C and quenched with cold saturated NH$_4$Cl (50 mL). The resulting mixture was extracted with diethyl ether (3 × 50 mL), dried over Na$_2$SO$_4$, and concentrated in vacuo. Flash column chromatography on silica using a gradient of 5–40% ethyl acetate in hexanes afforded **8** (440 mg, 2.78 mmol, 72%) as a colorless oil. A solution of **8** (440 mg, 2.78 mmol) in dry DMF (20 mL) was cooled to 0 °C, treated with imidazole (270 mg, 4.00 mmol), and stirred for 5 min. This mixture was treated with *tert*-butyldimethylsilyl chloride (543 mg, 3.60 mmol) and stirred for 6.5 h. The reaction was quenched with brine (30 mL), extracted with diethyl ether, dried over Na$_2$SO$_4$, and concentrated in vacuo. Flash column chromatography on silica using a gradient of 0–25% ethyl acetate in hexanes afforded **9** (707 mg, 2.59 mmol, 93%) as a colorless oil. ^{1}H NMR (500 MHz, chloroform-d): δ (ppm) 5.85–5.75 (m, 1H), 5.09–5.04 (m, 2H), 4.23–4.17 (m, 1H), 4.15–4.07 (m, 2H), 2.47–2.38 (m, 2H), 2.30–2.25 (m, 2H), 1.26 (t, J = 7.2 Hz, 3H), 0.86 (s, 9H), 0.07 (s, 3H), 0.04 (s, 3H). ^{13}C NMR (125 MHz, chloroform-d): δ (ppm) 171.8, 134.2, 117.8, 69.1, 60.4, 42.30, 42.25, 25.8, 18.1, 14.3, −4.4, −4.8.



12 11 13

Synthesis of ethyl (3*R*,8*R*,10*R*)-8-benzoyloxy-3-*tert*-butyldimethylsilyloxy-10-hydroxyundecanoate (11).



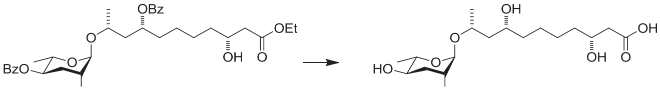
6 9 10 11

A solution of **6** (160 mg, 0.49 mmol) and **9** (368 mg, 1.35 mmol) in dry dichloromethane (15 mL) was treated with 1,4-benzoquinone (5 mg, 46 μmol) in dichloromethane (1 mL) and stirred for 10 min. To the mixture, Grubbs second-generation catalyst (20 mg, 24 μmol) was added and allowed to stir at 40 °C. After 40 h, the mixture was filtered over a small pad of silica and concentrated in vacuo. Flash column chromatography on silica using a gradient of 0–25% ethyl acetate in hexanes afforded **10** (70 mg, 123 μmol, 27%). A solution of **10** (70 mg, 123 μmol) was dissolved in ethanol (6 mL), treated with Pd/C [130 mg, 10% (wt/wt)], and hydrogenated for 11 h. The mixture was filtered over cotton and concentrated in vacuo. Flash column chromatography on silica using a gradient of 5–40% ethyl acetate in hexanes afforded **11** (41 mg, 86 μmol, 70%) as a colorless oil. ^{1}H NMR (500 MHz, chloroform-d): δ (ppm) 8.07–8.02 (m, 2H), 7.60–7.54 (m, 1H), 7.47–7.42 (m, 2H), 5.35–5.27 (m, 1H), 4.15–4.04 (m, 3H), 3.76–3.67 (m, 1H), 2.42 (dd, J = 14.6, 7.0 Hz, 1H), 2.37 (dd, J = 14.6, 5.6 Hz, 1H), 1.81–1.60 (m, 4H), 1.52–1.31 (m, 6H), 1.23 (t, J = 7.1 Hz, 3H), 1.17 (d, J = 6.3 Hz, 3H), 0.84 (s, 9H), 0.02 (s, 3H), 0.01 (s, 3H). ^{13}C NMR (125 MHz, chloroform-d): δ (ppm) 171.9, 167.8, 133.3, 130.0, 129.9, 128.5, 72.5, 69.3, 63.4, 60.4, 44.9, 42.8, 37.5, 35.2, 25.9, 25.8, 24.9, 23.0, 18.1, 14.3, −4.4, −4.7.

Synthesis of 13.

A solution of 2,4-di-*O*-benzoyl-ascarylose-1-(2,2,2-trichloroacetimide) (**12**; 50 mg, 100 μmol) (2) in dry dichloromethane (2 mL) at −10 °C was treated with **11** (41 mg, 86 μmol) and trimethylsilyloxy triflate (7 μL). After 3 h, the solution was washed with saturated NaHCO$_3$ solution (400 μL), dried over Na$_2$SO$_4$, and concentrated in vacuo. Flash column chromatography on silica using a gradient of 10–40% ethyl acetate in hexane afforded **13** (17 mg, 24 μmol, 28%) as a colorless oil. ^{1}H NMR (400 MHz, chloroform-d): δ (ppm) 8.23–7.96 (m, 6H), 7.64–7.34 (m, 9H), 5.47–5.38 (m, 1H), 5.15–5.05 (m, 2H), 4.96 (br s, 1H), 4.15 (q, J = 7.2 Hz, 2H), 4.07–3.95 (m, 2H), 3.95–3.86 (m, 1H), 2.47 (dd, J = 16.4 3.2 Hz, 1H), 2.43–2.31 (m, 1H), 2.38 (dd, J = 16.4 8.9 Hz, 1H), 2.26–2.16 (m, 1H), 1.99–1.86 (m, 2H), 1.85–1.67 (m, 2H), 1.58–1.38 (m, 6H), 1.25 (t, J = 7.2 Hz, 3H), 1.25 (d, J = 6.1 Hz, 3H), 0.95 (d, J = 6.2 Hz, 3H). ^{13}C NMR (100 MHz, chloroform-d): δ (ppm) 173.2, 166.3, 165.90, 165.89, 133.4, 133.3, 133.0, 130.6, 130.2, 129.98, 129.94, 129.92, 129.6, 128.6 (×2), 128.5, 92.7, 71.7, 71.3, 70.5, 67.9, 67.5, 67.2, 60.8, 41.9, 41.4, 36.5, 35.0, 29.8, 25.6, 25.0, 19.3, 17.6, 14.3.

Synthesis of dhas#18.



13 dhas#18

A solution of **13** (9 mg, 13 μmol) in tetrahydrofuran (70 μL) was treated with LiOH (11 mg, 260 μmol) in water (110 μL) and 1,4-dioxane (700 μL) at 67 °C for 4.5 h. The reaction mixture was acidified with acetic acid (200 μL), concentrated in vacuo, treated with methanol (2 mL), and concentrated in vacuo. Flash column chromatography on silica using a gradient of 10–50% methanol in dichloromethane with 0.5% glacial acetic acid followed by HPLC purification afforded **dhas#18** (2 mg, 5.5 μmol, 42%) as a colorless oil. ^{1}H NMR (600 MHz, chloroform-d): δ (ppm) 4.68 (br s, 1H), 4.09–4.03 (m, 1H), 4.01–3.95 (m, 1H), 3.84–3.79 (m, 1H), 3.73–3.71 (m, 1H), 3.68 (dq, $J = 9.4$, 6.2 Hz, 1H), 3.52 (ddd, $J = 11.3$, 9.4, 4.5 Hz, 1H), 2.44 (dd, $J = 15.2$, 4.7 Hz, 1H), 2.37 (dd, $J = 15.2$, 8.2 Hz, 1H), 1.98–1.93 (m, 1H), 1.79–1.73 (m, 1H), 1.64 (dd, $J = 15.0$, 7.2 Hz, 1H), 1.47 (dd, $J =$ 15.0, 13.7 Hz, 1H), 1.55–1.36 (m, 8H), 1.23 (d, $J = 6.2$ Hz, 3H), 1.15 (d, $J = 6.1$ Hz, 3H). ^{13}C NMR (151 MHz, chloroform-d): δ (ppm) 175.7, 96.6, 71.0, 69.7, 69.1, 68.7 (×2), 68.1, 45.8, 43.2, 39.1, 37.9, 35.8, 26.5, 26.4, 19.4, 17.9. $\alpha_D^{20} = -61.6$ (c = 0.002, methanol).

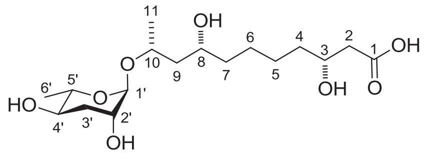
1. Keck GE, Murry JA (1991) Total synthesis of (-)-colletol. *J Org Chem* 56(23):6606–6611.

2. Butcher RA, Fujita M, Schroeder FC, Clardy J (2007) Small-molecule pheromones that control dauer development in Caenorhabditis elegans. *Nat Chem Biol* 3(7):420–422.

Table S1. NMR spectroscopic data for dhas#18

Position	δ ^{13}C (ppm)	δ ^{1}H (ppm)	^{1}H-^{1}H coupling constants (Hz)	Key HMBC correlations
1	175.7	—		
2	43.2	2a = 2.44	$J_{2a,2b} = 15.5$, $J_{2a,3} = 4.8$	C-1, C-3, C-4
		2b = 2.37	$J_{2b,3} = 8.2$	
3	69.1	3.98		
4	37.9	1.50		
5	26.5	1.53–1.28		
6	26.5	1.53–1.28		
7	39.1	1.53–1.28		
8	68.7	3.81	$J_{8,9a} = 9.3$, $J_{8,9b} = 3.0$	
9	45.8	9a = 1.47	$J_{9a,9b} = 14.3$, $J_{9a,10} = 3.4$,	C-7, C-8, C-11
		9b = 1.64	$J_{9b,10} = 9.2$	
10	68.7	4.05	$J_{10,11} = 6.0$	C-9, C-1′
11	19.4	1.15		C-9, C-10
1′	96.6	4.68	$J_{1',2'} = 2.4$	C-10, C-3′, C-5′
2′	69.7	3.72	$J_{2',3'(ax)} = 6.2$, $J_{2',3'(eq)} = 6.2$	C-10
3′	35.8	1.76 (ax)	$J_{3'(ax),3'(eq)} = 12.7$, $J_{3'(ax),4'} = 11.1$	C-1′, C-2′, C-4′, C-5′
		1.96 (eq)	$J_{3'(eq),4'} = 3.8$	
4′	68.1	3.52	$J_{4',5'} = 9.3$	C-3′, C-5′, C-6′
5′	71.0	3.68	$J_{5',6'(eq)} = 6.2$	C-1′, C-3′, C-4′, C-6′
6′	17.9	1.23		C-4′, C-5′

^{1}H (600 MHz), ^{13}C (151 MHz), and HMBC NMR spectroscopic data for **dhas#18** in methanol-d_4. Chemical shifts were



referenced to (CD$_2$H̲OD) = 3.31 ppm and (C̲D$_3$OD) = 49.00 ppm.

Fig. S1. *P. redivivus* bioassays. *P. redivivus* males and females are attracted to supernatant isolated from the opposite sex but do not show attraction to their own sex; 25 virgin female and 25 male young adults were separately incubated in 25 mL M9 buffer for 6 h and then applied to a two-spot bioassay as previously described in the work by Choe et al. (1).

Fig. S1

1. Choe A, et al. (2012) Ascaroside signaling is widely conserved among nematodes. *Curr Biol* 22(9):772–780.

Fig. S2. Activity-guided fractionation of male- and female-specific attractants in *P. redivivus*. Overall schematic of the protocol with active fractions in red. Bioassay results are shown in the text (Fig. 2*A*).

Fig. S2

Fig. S3. (*A*) LC-MS data of natural, synthetic, and spike ascr#1. SIM, selective ion monitor; TIM, total ion monitor. (*B*) MS spectrum of natural, synthetic, and spike ascr#1. (*C*) 1D NMR data of natural, synthetic, and spike ascr#1.

Fig. S3

Fig. S4. (*A*) LC-MS analysis of natural, synthetic, and spike dhas#18. SIM, selective ion monitor; TIM, total ion monitor. (*B*) MS spectrum of natural, synthetic, and spike dhas#18. (*C*) 1D NMR of natural dhas#18. (*D*) COSY-dqf NMR of natural, synthetic, and spike dhas#18. (*E*) COSY -dfq NMR of natural dhas#18. (*F*) COSY 2D NMR data of partial structure 1 of natural dhas#18. (*G*) COSY 2D NMR data of partial structure 1 of natural dhas#18. (*H*) COSY 2D NMR data of ascaroside ring 1 of natural dhas#18. (*I*) COSY 2D NMR data of ascaroside ring 2 of dhas#18.

Fig. S4

Fig. S5. (*A*) ^{1}H NMR spectrum (500 MHz; chloroform-*d*) of **4**. (*B*) ^{13}C NMR spectrum (125 MHz; chloroform-*d*) of **4**. (*C*) ^{1}H NMR spectrum (500 MHz; chloroform-*d*) of **6**. (*D*) ^{13}C NMR spectrum (125 MHz; chloroform-*d*) of **6**. (*E*) ^{1}H NMR spectrum (500 MHz; chloroform-*d*) of **9**. (*F*) ^{13}C NMR spectrum (125 MHz; chloroform-*d*) of **9**. (*G*) ^{1}H NMR spectrum (500 MHz; chloroform-*d*) of **11**. (*H*) ^{13}C NMR spectrum (125 MHz; chloroform-*d*) of **11**. (*I*) ^{1}H NMR spectrum (500 MHz; chloroform-*d*) of **13**. (*J*) ^{13}C NMR spectrum (125 MHz; chloroform-*d*) of **13**. (*K*) ^{1}H NMR spectrum (600 MHz; methanol-d_4) of **dhas#18**. (*L*) dqf-COSY spectrum (600 MHz; methanol-d_4) of **dhas#18**. (*M*) HMQC spectrum (600 MHz for ^{1}H and 151 MHz for ^{13}C; methanol-d_4) of **dhas#18**. (*N*) HMBC spectrum (600 MHz for ^{1}H and 151 MHz for ^{13}C; methanol-d_4) of **dhas#18**.

Fig. S5