Supporting Information

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SI Text

General Methods. All reactions were performed under a nitrogen atmosphere. Chemicals and solvents were purchased from commercial suppliers and used as received. Methylene chloride and tetrahydrofuran were dried by passing through an activated alumina column before use. All flash chromatography was performed using 230–400 mesh silica gel (EMD Chemicals).

Synthesis of Intermediate 5. A mixture of 228.4 mg (0.641 mmol) of dibenzoyl ascarylose (1), 83 μl (0.96 mmol) of 3-buten-1-ol, and 50 mg of powdered 3-Å molecular sieves in 3 ml of methylene chloride was cooled to 0°C in an ice bath. 0.3 mL $BF_3{\cdot}OEt_2$ (2 mmol) was then added at once, and the resulting mixture was stirred at 0°C for 1 h. Triethylamine (0.8 ml) was added, and the resulting mixture was diluted with 15 ml of methylene chloride and filtered. Evaporation of volatiles afforded 683 mg of an oil. Silica gel chromatography (30–60% diethyl ether in methylene chloride) afforded 150.2 mg of intermediate **5** as an oil (57% yield). $[\alpha]_D^{20}$ = +4.1, *c* 0.97 (methanol); HR-ESIMS (*m*/*z*): $[M+Na]^+$ calculated for $C_{24}H_{26}O_6Na$ 433.1627, found 433.1632; ^1H NMR (300 MHz, $CDCl_3$): 8.12 (d, 2H, J = 6.9 Hz); 8.04 (d, 2H, J = 6.9 Hz); 7.59 (m, 2H); 7.47 (m, 4H); 5.89 (m, 1H); 5.08–5.24 (m, 4H); 4.86 (s, 1H); 4.09 (dq, 1H, J = 9.9Hz, J = 6.3 Hz); 3.82 (dt, 1H, J = 9.6 Hz, J = 6.9 Hz); 3.60 (dt, 1H, J = 9.6 Hz, J = 6.6 Hz); 2.42 (m, 3H); 2.21 (t, 1H, J = 12.3 Hz); 1.31 (t, 3H, J = 6.3 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): 165.7; 165.6; 134.8; 133.2; 133.1; 130.0; 129.9; 129.8; 129.6; 128.4; 116.7; 96.4; 70.58; 70.56; 67.2; 66.8; 33.9; 29.7; 17.9.

Synthesis of Intermediate 6. To a suspension of 70.5 mg (0.172 mmol) of **5** and 43 mg (0.51 mmol) of sodium bicarbonate in 7 ml of acetone at 23°C, 136 mg (0.86 mmol) of potassium permanganate was added at once. The resulting mixture was stirred for 42 min at 23°C. Hydrochloric acid (1 M, 5 ml) was then added. The resulting mixture was extracted with 5 × 10 ml of ethyl acetate. The organic extracts were then dried over anhydrous magnesium sulfate and filtered. Evaporation of volatiles afforded 72 mg of an oil. Silica gel chromatography of the oil (1–2% isopropanol in methylene chloride) afforded 52.0 mg of intermediate **6** as an oil (71% yield). $[\alpha]_D^{20}$ = +13.8, *c* 0.37 (methanol); HR-ESIMS (*m*/*z*): $[M+Na]^+$ calculated for $C_{23}H_{24}O_8Na$ 451.1369, found 451.1367; ^1H NMR (300 MHz, $CDCl_3$): 8.12 (d, 2H, J = 7.7 Hz); 8.05 (d, 2H, J = 7.8 Hz); 7.58 (m, 2H); 7.47 (m, 4H); 5.14–5.22 (m, 2H); 4.87 (s, 1H); 4.10 (m, 2H); 3.80 (dt, 1H, J = 10.2 Hz, J = 6.0 Hz); 2.74 (t, 2H, J = 6.0 Hz); 2.41 (dt, 1H, J = 13.5 Hz, J = 3.6 Hz); 2.18 (t, 1H, J = 12.5 Hz); 1.30 (d, 3H, J = 6.3 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): 176.6; 165.7; 165.6; 133.3; 133.2; 130.0; 129.9; 129.8; 129.6; 128.5; 128.4; 96.6; 70.5; 70.4; 67.1; 62.9; 34.6; 29.7; 17.8.

Synthesis of Ascaroside C3 (4). 24.9 mg (58.1 μmol) of **6** was dissolved in 2 ml of tetrahydrofuran, to which 3 ml of 2 M lithium hydroxide was added. The resulting mixture was warmed to reflux. After refluxing for 2 h, the reaction mixture was allowed to cool to room temperature. Concentrated hydrochloric acid was then carefully added until pH 2. Solid sodium chloride was added until the solution was saturated. The solution was then extracted with 6 × 5 ml of ethyl acetate. The organic extracts were dried over anhydrous magnesium sulfate and filtered. Evaporation of volatiles afforded 18.2 mg of a solid. Silica gel chromatography (10–20% methanol in methylene chloride) afforded 10.5 mg of ascaroside C3 (**4**) as an oil (82% yield). $[\alpha]_D^{20}$ = −73.6, *c* 0.11 (methanol); HR-ESIMS (*m*/*z*): $[M+Na]^+$ calculated for $C_9H_{16}O_6Na$ 243.0845, found 243.0840; for NMR data see Table S1 and Fig. S2. The shifts of H-2, H-3a, and H-3b for synthetic ascaroside C3 are slightly different in Table S1 and Fig. S2 because the shifts depend on the concentration of the sample. The synthetic sample that was used to generate the proton data in Table S1 was less concentrated than the synthetic sample that was used to generate the spectra in Fig. S2 (1 mg/ml versus 10 mg/ml).

1. Jeong PY, *et al.* (2005) Chemical structure and biological activity of the *Caenorhabditis elegans* dauer-inducing pheromone. *Nature* 433:541–545.

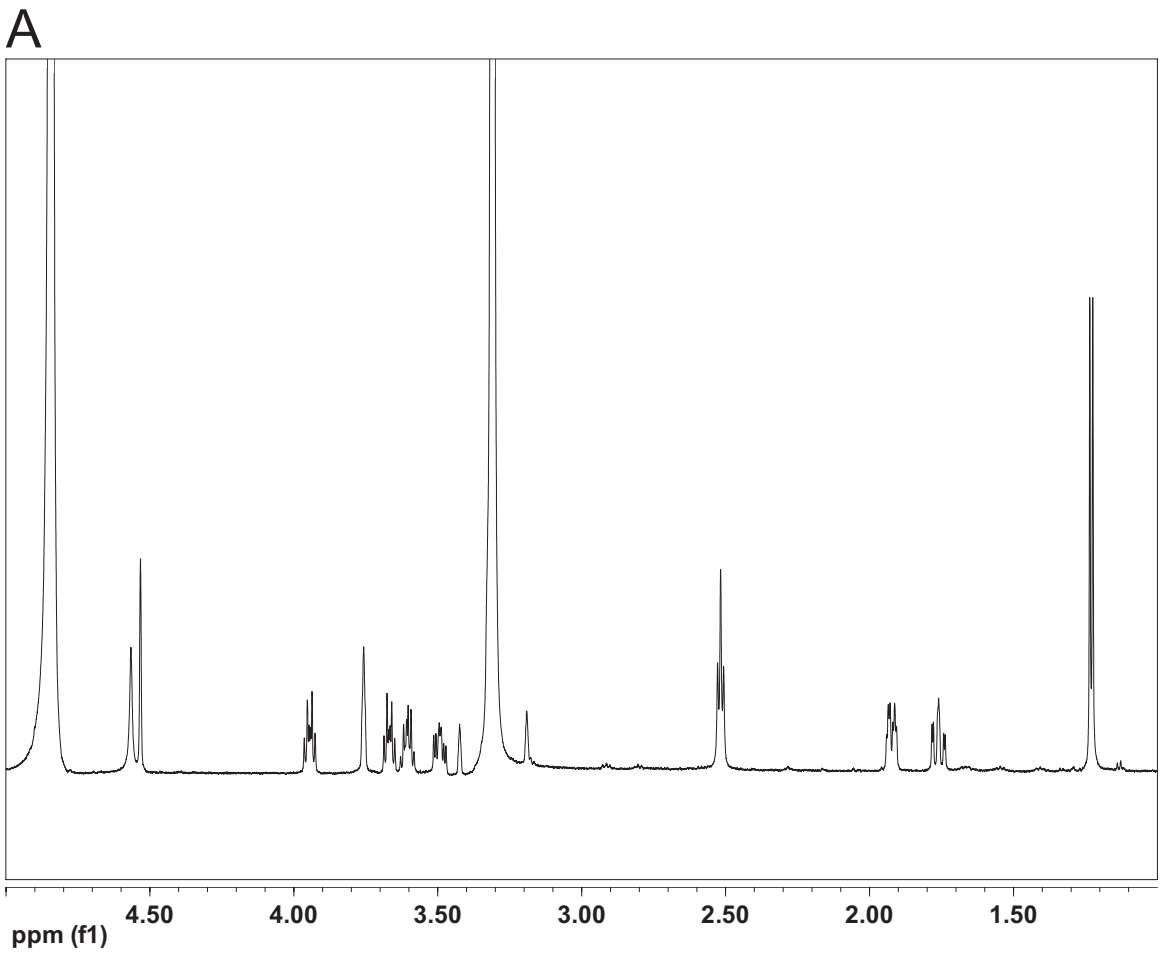
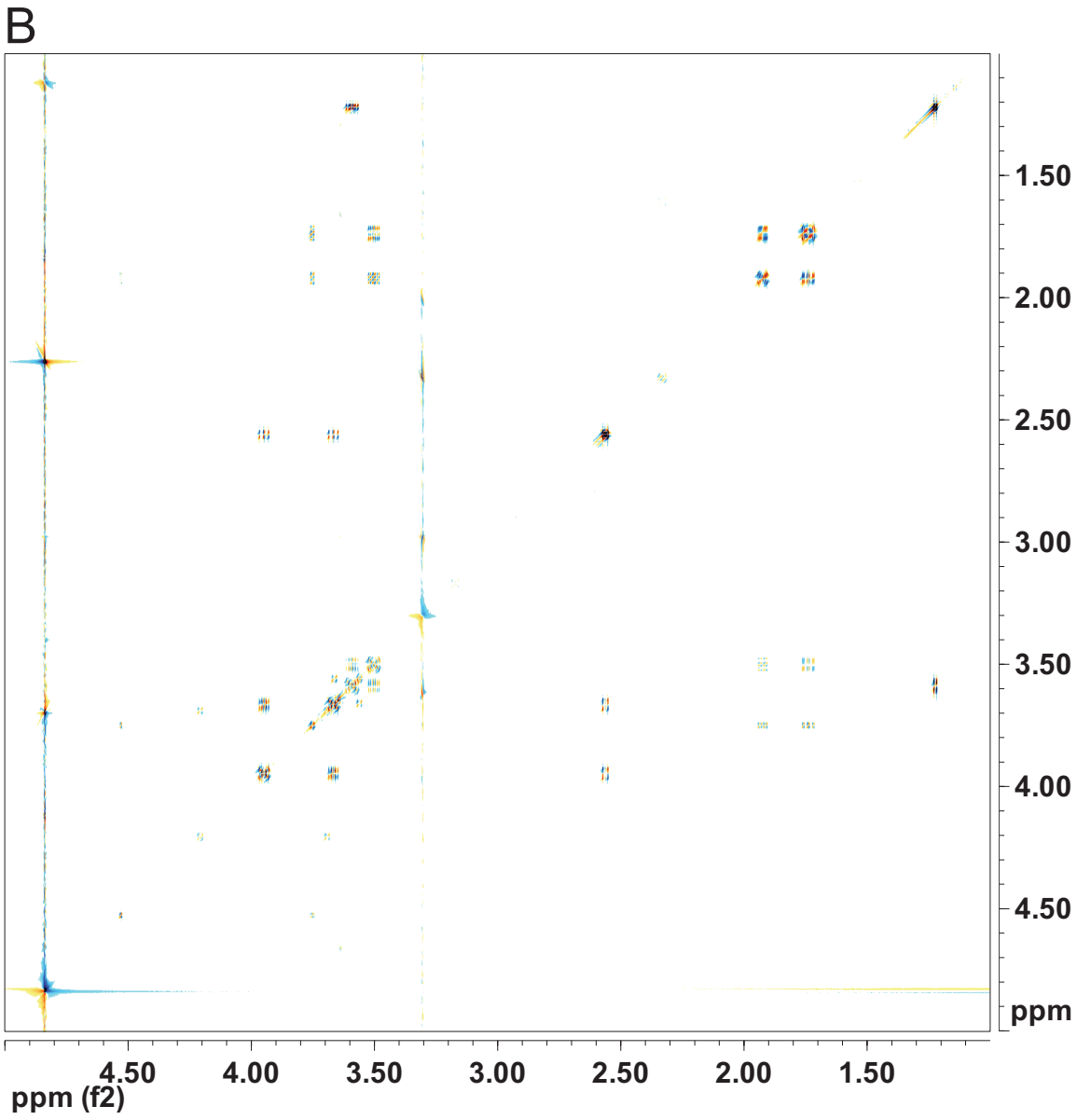


Fig. S1A. NMR spectra of natural ascaroside C3 (**4**) at 1 mg/ml in methanol-d_4. (*A*) The ^{1}H NMR spectrum of natural **4** in methanol-d_4. The peak at 4.57 ppm is a contaminant. (*B*) dqf-COSY spectrum of natural **4** in methanol-d_4. (*C*) gHMQC spectrum of natural **4** in methanol-d_4. (*D*) gHMBC spectrum of natural **4** in methanol-d_4. (*E*) NOESY spectrum of natural **4** in methanol-d_4.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ppm (f2)

Fig. S1B.

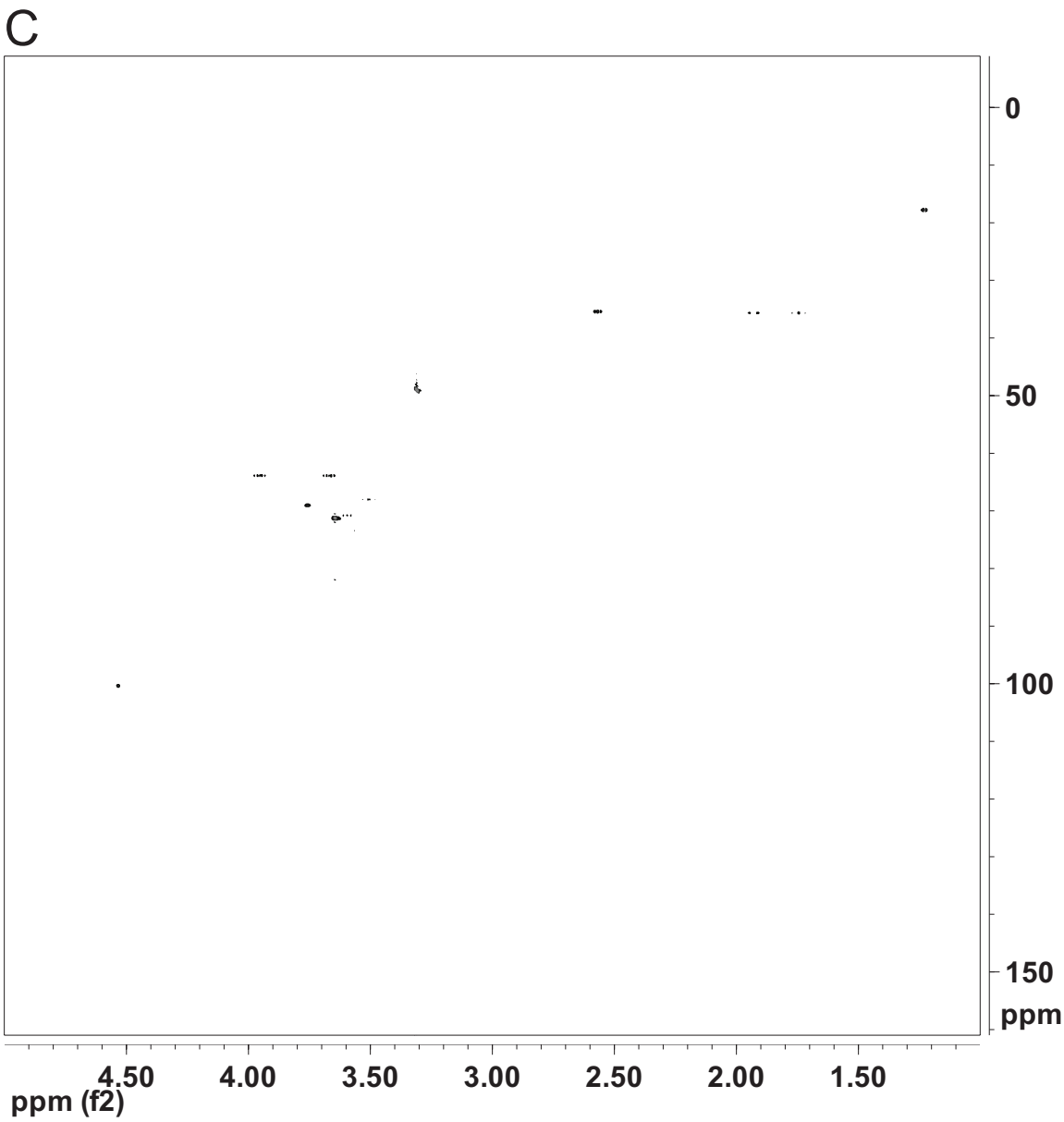

Fig. S1C.

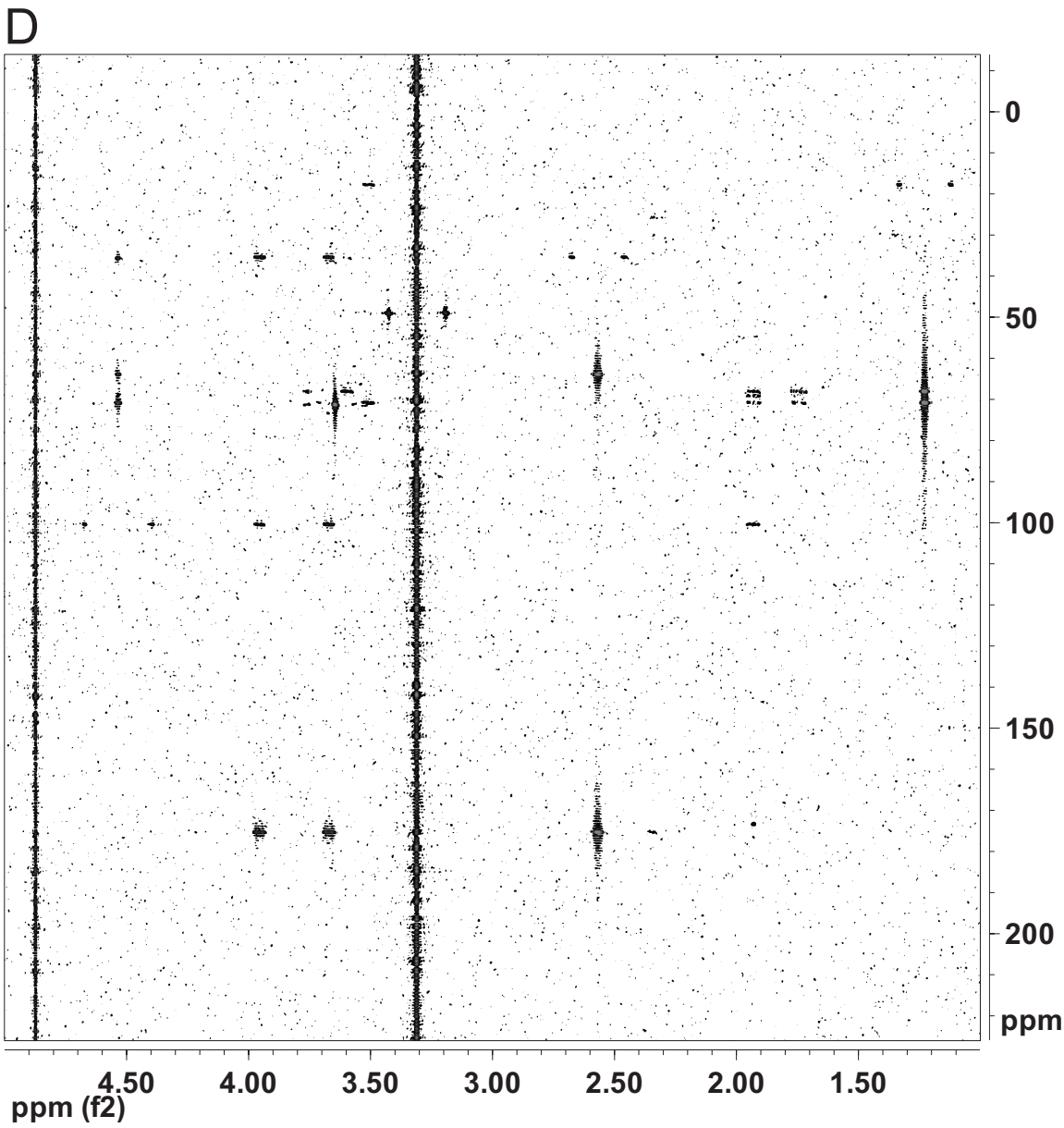

Fig. S1D.

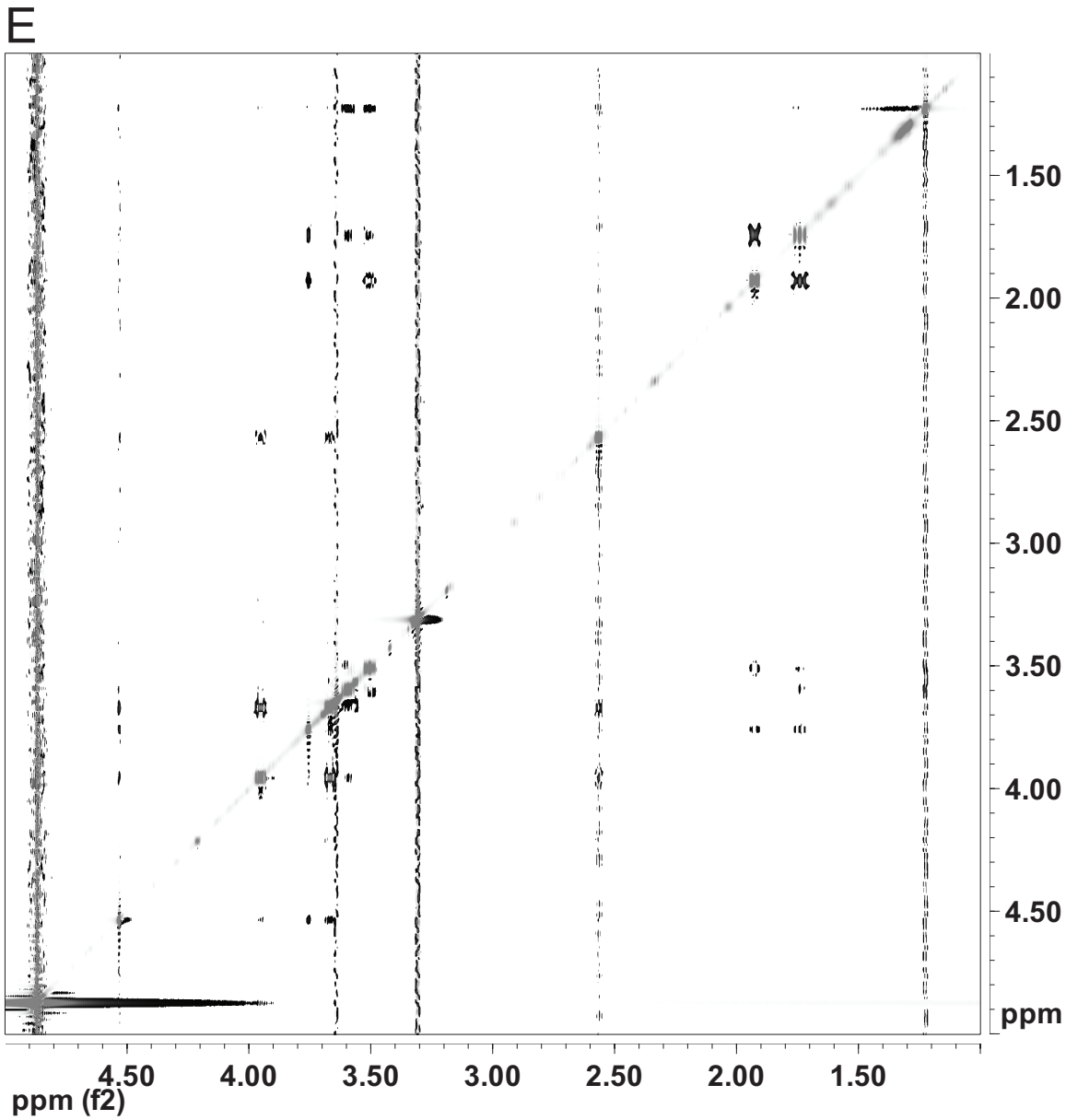
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Fig. S1E.

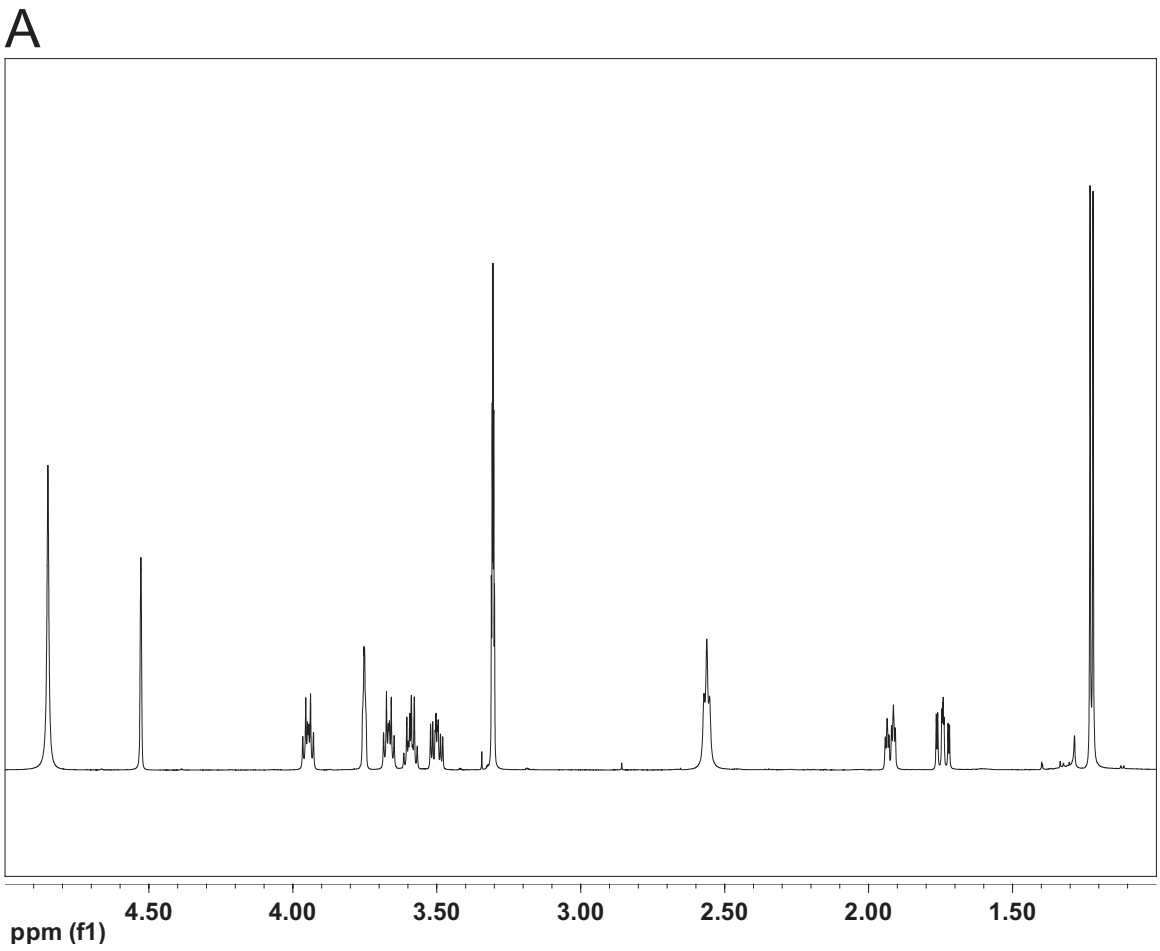
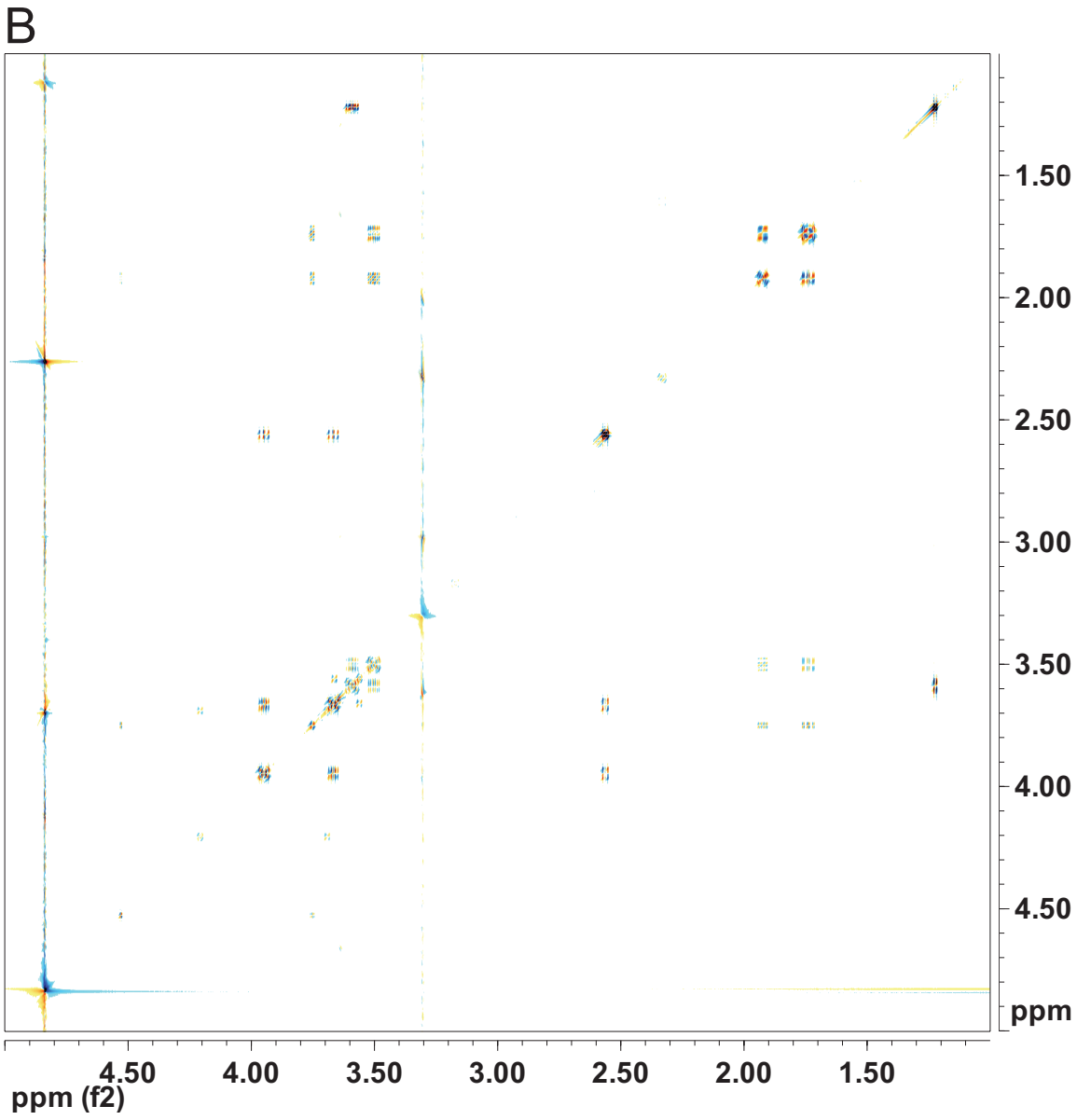


Fig. S2A. NMR spectra of synthetic ascaroside C3 (**4**) at 10 mg/mL in methanol-d_4. (*A*) The ^{1}H NMR spectrum of synthetic **4** in methanol-d_4. (*B*) dqf-COSY spectrum of synthetic **4** in methanol-d_4. (*C*) gHMQC spectrum of synthetic **4** in methanol-d_4. (*D*) gHMBC spectrum of synthetic **4** in methanol-d_4.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ppm (f2)

Fig. S2B.

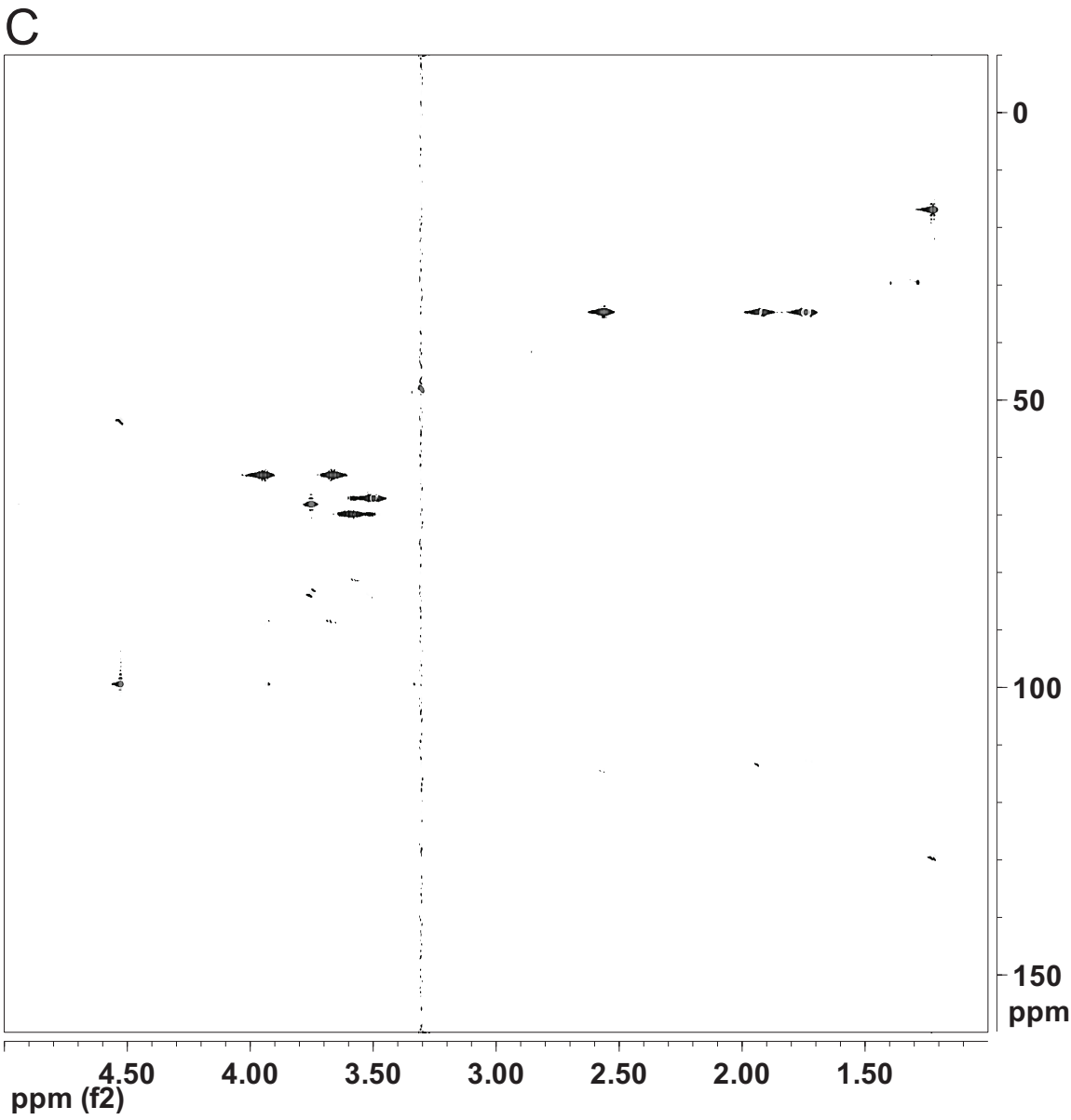

Fig. S2C.

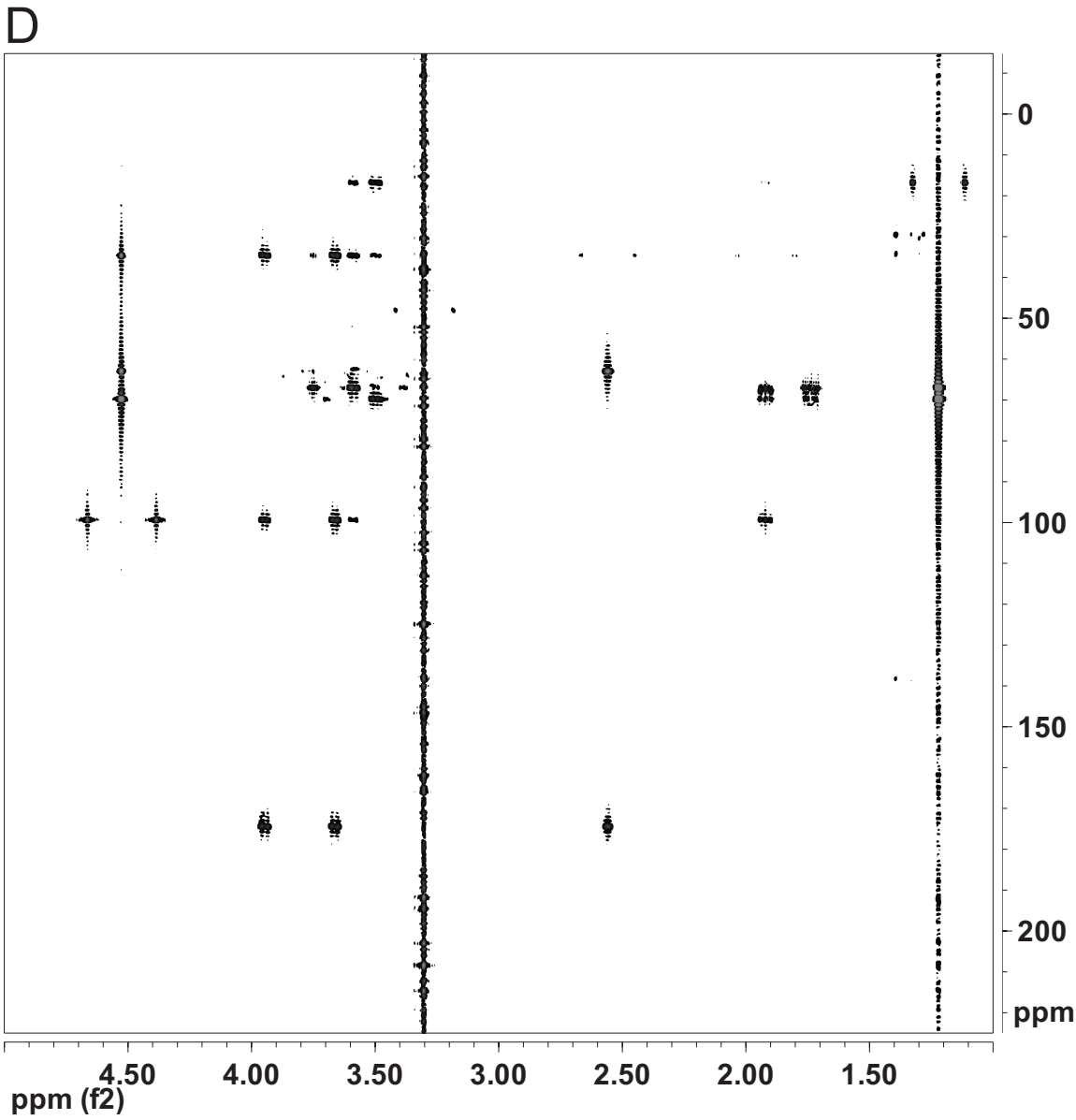

Fig. S2D.

Table S1. The ^{1}H and ^{13}C shifts derived from ^{1}H, dqf-COSY, gHMQC, and gHMBC spectra of natural and synthetic ascaroside C3 (4) in methanol-d_4

	Natural ascaroside C3 (**4**)			Synthetic ascaroside C3 (**4**)		
No.	δ_H mult. [*J* (Hz)]	δ_C	HMBC	δ_H mult. [*J* (Hz)]	δ_C	HMBC
1		175.1			175.4	
2	2.52, t ($J_{2,3a} = 6.3$, $J_{2,3b} = 6.3$)	35.5	C-1,3	2.53, t ($J_{2,3a} = 6.3$, $J_{2,3b} = 6.3$)	35.7	C-1,3
3a	3.67, dt ($J_{3a,3b} = 9.7$)	64.0	C-1,2,1′	3.67, dt ($J_{3a,3b} = 9.8$)	64.1	C-1,2,1′
3b	3.95, dt		C-1,1′	3.95, dt		C-1,2,1′
1′	4.53, br s	100.4	C-3,2′,3′,5′	4.53, br s	100.4	C-3,2′,3′,5′
2′	3.76, dt ($J_{1',2'} = 1.3$)	69.2	C-4′	3.76, dt ($J_{1',2'} = 1.3$)	69.1	
3′ax	1.76, ddd ($J_{2',3'ax} = 2.9$, $J_{3'ax,3'eq} = 13.1$)	35.7	C-4′,5′	1.76, ddd ($J_{2',3'ax} = 2.9$, $J_{3'ax,3'eq} = 13.1$)	35.8	C-2′,4′,5′
3′eq	1.92, ddd ($J_{2',3'eq} = 2.9$)		C-1′,2′,4′,5′	1.93,ddd ($J_{2',3'eq} = 2.9$)		C-1′,4′,5′,6′
4′	3.49, ddd ($J_{3'ax,4'} = 9.5$, $J_{3'eq,4'} = 4.4$)	68.2	C-5′,6′	3.50, ddd ($J_{3'ax,4'} = 9.5$, $J_{3'eq,4'} = 4.4$)	68.1	C-3′,5′,6′
5′	3.61, dq ($J_{4',5'} = 9.4$)	70.9	C-3′,4′	3.60, dq ($J_{4',5'} = 9.4$)	70.8	C-1′,3′,4′,6′
6′	1.23, d ($J_{5',6'} = 6.2$)	18.0	C-4′,5′	1.23, d ($J_{5',6'} = 6.2$)	17.9	C-4′,5′

Table S2. Activity of the ascarosides in Daf-c and Daf-d strain backgrounds in the dauer formation assay

Genotype	Conc, μM	Temp.	Control (vehicle only)	C3 (**4**)	C6 (**1**)	C9 (**2**)
N2 (wild type)	0.67	25°C	0 (± 0)	50 (± 10)	42 (± 16)	29 (± 18)
daf-3 (e1376)	0.67	25°C	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
daf-5 (e1386)	0.67	25°C	0 (± 0)	1 (± 1)	2 (± 2)	1 (± 1)
daf-12 (m20)	0.67	25°C	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
daf-16 (mu86)	0.67	25°C	0 (± 0)	0 (± 0)	0 (± 0)	0 (± 0)
N2 (wild type)	6	16°C	0 (± 0)	91 (± 5)	15 (± 5)	30 (± 10)
daf-7 (e1372)	6	16°C	72 (± 14)	81 (± 1)	82 (± 11)	83 (± 4)
daf-2 (e1370)	6	16°C	1 (± 1)	84 (± 12)	57 (± 0)	68 (± 5)
N2 (wild type)	0.22	20°C	0 (± 0)	10 (± 5)	n.d.	n.d.
daf-2 (e1370)	0.22	20°C	40 (± 9)	86 (± 4)	n.d.	n.d.
N2 (wild type)	0.67	20°C	0 (± 0)	n.d.	10 (± 0)	2 (± 3)
daf-2 (e1370)	0.67	20°C	40 (± 9)	n.d.	93 (± 10)	94 (± 1)
N2 (wild type)	6	20°C	0 (± 0)	95 (± 5)	43 (± 10)	55 (± 16)
tax-4 (p678)	6	20°C	16 (± 2)	11 (± 12)	18 (± 9)	28 (± 6)
tax-2 (p691) tax-4 (p678)	6	20°C	15 (± 6)	11 (± 1)	17 (± 0)	17 (± 3)
N2 (wild type)	0.67	20°C	0 (± 0)	37 (± 10)	n.d.	n.d.
tax-4 (p678)	0.67	20°C	16 (± 2)	18 (± 0)	n.d.	n.d.
tax-2 (p691) tax-4 (p678)	0.67	20°C	15 (± 6)	10 (± 7)	n.d.	n.d.

The data represent the average of two experiments (± one standard deviation). n.d., not determined.