Supplemental Data

A Conserved Endocrine Mechanism Controls the Formation of Dauer and Infective Larvae in Nematodes

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Supplemental Experimental Procedures

Nematode strains and cultures

Worms were grown on NGM plates with a lawn of *E. coli* strain OP50[1]. The strains used in this study are as follows: natural isolates of *P. pacificus*, PS312 (the wild type *P. pacificus* strain), PS1843 (for SNP mapping); *C. elegans* N2; dauer formation constitutive strains *Ppa-dfc-1(tu391), Ppa-dfc-2(tu392), Ppa-dfc-3(u393), Ppa-dfc-4(tu394)*; *Ppa-daf-12* strains *Ppa-daf-12(tu381, tu389, tu390). S. papillosus* animals were maintained as previously described using rabbits as hosts [2].

Preparation of concentrated dauer pheromone extracts

We prepared concentrated dauer pheromone extracts following the method previously reported [3] with some modifications. Worms collected from 20-40 fully-grown NGM plates were inoculated into S-medium containing 1% (w/v) OP50, 50μg/ml streptomycin, and 50μg/ml nystatin and grown at room temperature for 3 days with an additional feeding of OP50 after two days. The supernatant was collected with centrifugation and passed through a 0.22 μm filter. Hydrated active charcoal (1/20 volume; Fluka) was added to the supernatant and stirred for an hour. The supernatant was discarded and the charcoal was washed extensively with water.

Pheromone bound to the charcoal was sequentially eluted with ethanol, and 1:1:1 mixture of ethanol, acetone and toluene. Eluted samples were combined, dried under vacuum and re-suspended in water (1/250-1/500 of original volume).

Dauer formation assays

For dauer formation assays on pheromone plates, either NGM agar without peptone, or NGM agar without peptone and cholesterol was combined with streptomycin or kanamycin (50 μg/ml final concentration) and pheromone extract, and solidified. OP50 was treated with 2 mg/ml kanamycin in S-medium without cholesterol for 1.5 hours at 25° C with gentle agitation. The killed OP50 was washed once and re-suspended in 50μg/ml kanamycin in S-medium without cholesterol to 2% (w/v). The suspension was spotted on the pheromone plates and used for the assay. Five to 30 animals were picked on the plate and allowed to lay eggs for 2.5-5 hours. The parents were removed and the plates were incubated at 15-25° C. Numbers of dauer and non-dauer larvae were counted after 2-6 days. The response to pheromones varied depending on the pheromone preparations and the amount of the pheromone extract added. Therefore all the experiments in a single case were performed in parallel using plates prepared from the same pheromone-agar mixture. Note that the experiments described in Figures 2 A, B and C were performed with different batches and different amounts of pheromone. For assays with Daf-c mutants, OP50 grown in LB medium was centrifuged and re-suspended in 1/5 volume of 0.9% NaCl. 90 μl of re-suspended bacteria and either 10 μl of 75 μM Δ-4 dafachronic acid, 75 μM Δ-7 dafachronic acid (dissolved in ethanol; synthesized as described previously [4, 5]) , or ethanol were combined, and spotted on an NGM plate (3ml NGM-agar in a 35 mm Petri dish). The eggs were transferred to assay plates and incubated at 20 °C for 3-4 days. In the assay with fully

penetrant Daf-c mutants, F1 and F2 eggs from heterozygous animals were collected and mixed well on an OP50 lawn. From a common pool, eggs were transferred to each assay plate. For dauer formation assays with conditioned medium, supernatants of PS312 or N2 liquid culture were prepared and filtered as described above. Hypochlorite-treated eggs or J2 larvae (allowed to hatch overnight in medium without food bacteria) of *P. pacificus* were cultured in the conditioned medium or M9 medium supplemented with 20% (w/v) OP50 in S-medium to the final concentration of 0.1-0.5%. Statistical analyses were done with the program R. Fisher's exact test was used to test independence. All the 95% confidence intervals are exact binomial confidence intervals.

Infective larvae formation assay with S. papillosus

Young larvae and adults of *S. papillosus* were prepared as previously described [2]. For assays with the progeny of parasitic females, feces of parasitized rabbits were moisturized, mixed with sawdust and incubated at 25° C for 4 hours. Larvae were collected with Baermann funnels overnight, washed with water, and transferred to NGM plates containing Δ-4 DA, Δ-7 DA, or ethanol (prepared as described above), and incubated at 25° C. The numbers of infective and non-infective larvae on each plate were counted on the next day. For assays with the progeny of free-living adults, feces were moisturized, mixed with sawdust and incubated at 25° C for 24 hours. Adults were collected with Baermann funnels, transferred to assay plates, and allowed to lay eggs overnight. Adults were removed from plates and two days later, infective and non-infective larvae on each plate were collected with Baermann funnels.

Mutant isolation and positional mapping

PS312 animals were mutagenized with either 50 mM ethylmethanesulfonate or 1 mM ethylnitrosourea in M9 buffer for 4 hours at 20° C. For screening for Daf-c mutants one to two F1 animals were picked on NGM plates, incubated at 25° C for four days and screened for the presence of dauer larvae. From plates containing dauer larvae, multiple adult worms were picked to recover 100% penetrant Daf-c mutants. Four strains isolated in the screening turned out to be incompletely penetrant and kept as homozygous mutant strains. The rest of the strains was kept by picking heterozygous animals carrying the mutation. For screening for Daf-d mutants, the F2 animals were picked individually into the wells of 96-well plates containing 0.5% (w/v) OP50, 50μg/ml streptomycin, and 50μg/ml nystatin in S-medium with or without cholesterol. After incubation for several days with additional rounds of feeding with OP50, the wells were screened for the absence of dauer larvae. The mutant strains were backcrossed at least twice before further analyses. Positional mapping was carried out using single strand conformational polymorphisms.

RT-PCR

Total RNA was isolated from mixed stage PS312 animals and reverse transcribed with random hexaoligonucleotide for 5' rapid amplification of cDNA ends (RACE) or primers (5'-CCAGTGAGCAGAGTGACGAGGACTCGAGCTCAAGCTTTTTTTTTTTTTTT(A/G/C)-3') for 3' RACE. For 5' RACE, the first round PCR was conducted with a primer containing a 5' part of SL1 splice leader sequence, 5'-GTCGACGGTTTAATTACCCAAG-3' and an outer 3' gene specific primer for *Ppa-daf-12*, 5'-ACCGTCTTCATCAGAGGAGTG-3' downstream the predicted stop codon. For the second PCR, a partially overlapping SL1 primer, 5' GGTTTAATTACCCAAGTTTGAG-3' and an inner 3' gene specific primer 5'-CGAGTATGAGTGGTTCGGATT-3' were used. Amplified fragments were cloned into

pCR2.1-TOPO (Invitrogen) and sequenced. The 3' UTR sequence was obtained by directly sequencing a fragment amplified by 3' RACE with the primers 5'-CCAGTGAGCAGAGTGACG-3' and 5'-TCTGTACGGTGAGGATGCAC-3'for the first PCR, and 5'-GAGGACTCGAGCTCAAGC-3' and 5'-TCGTGCCCTTGAACTTCTTC-3'for the second PCR respectively. For the PCR cloning of *S. papillosus daf-12*, young progeny of parasitic adults were collected from rabbit feces and incubated at 25° C on a NGM plate seeded with OP50. On the next day worms were collected before infective larvae started to appear, and total RNA was prepared. Initially two pairs of nested primers (5'-CAAAAAACTTGTCGGGTTTG-3', 5'-CAATTGTTCTTCATTTAAAATCCA-3', 5'-TGTCGGGTTTGTGGCGACCA-3' and 5'-CATTTAAAATCCATTCTTTTTTCAT-3') were designed on the coding sequence for the DBD of *S. stercoralis* DAF-12 and successfully amplified a partial cDNA corresponding to the DBD of *Spa-DAF-12*. Also the same 5' primers could be successfully used for 3' RACE of *Spa-DAF-12* cDNA. Two new nested primers (5'-TGGAAGCATTTCTTCAAACG-3' and 5'-TCTTCAAACGACATTTTTGACA-3') were designed on the *Spa-DAF-12* DBD. These primers and a primer containing the start codon and 37 bp 5' UTR sequence of Sst-*DAF-12* cDNA (5'-TTATGACTCTACTTTTGGTTAAAAATCAAAATTTAATATG-3') could successfully amplify the 5' coding sequence for *Spa-DAF-12*. These three primers were also used for testing the expression of *Spa-daf-12* in the progeny of free-living adults. For this, male and female progeny of parasitic females was placed on an NGM plate and incubated at 25° C overnight. Parental animals were removed from the plate, and eggs laid on the plate were incubated at 25° C. On the next day, the young larvae destined to become infective larvae were collected and

total RNA was prepared. The sequence data were deposited in GeneBank under the accession numbers EU805523 to EU805537 and FJ224097.

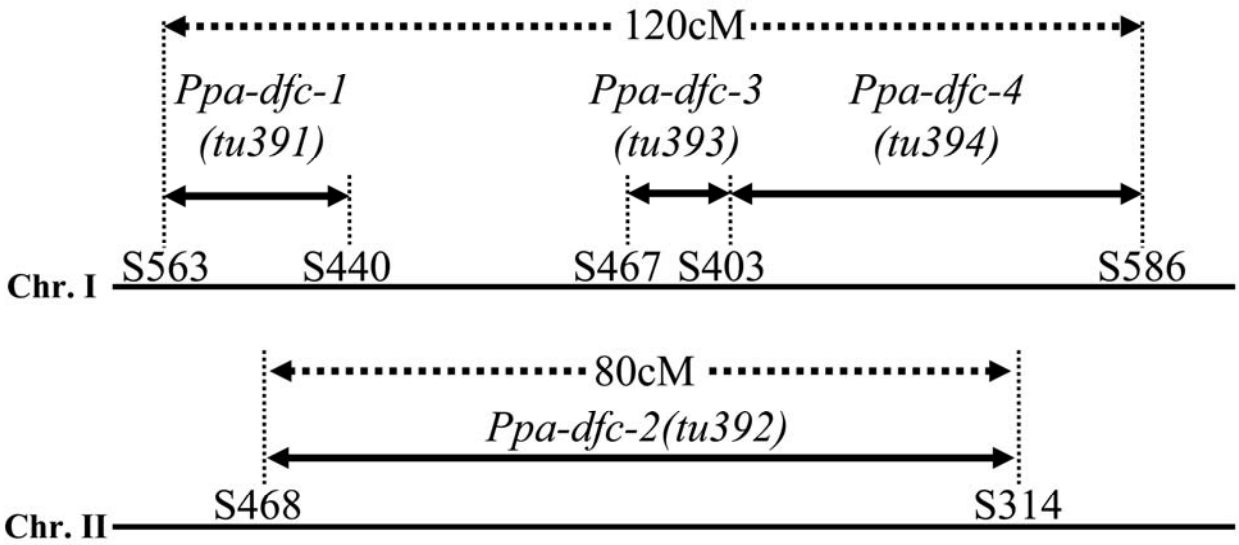


Figure S1. Genetic map positions of *P. pacificus* Daf-c mutants. Positions of four Daf-c mutations used in dauer formation assays are indicated with the positions of genetic markers (13).

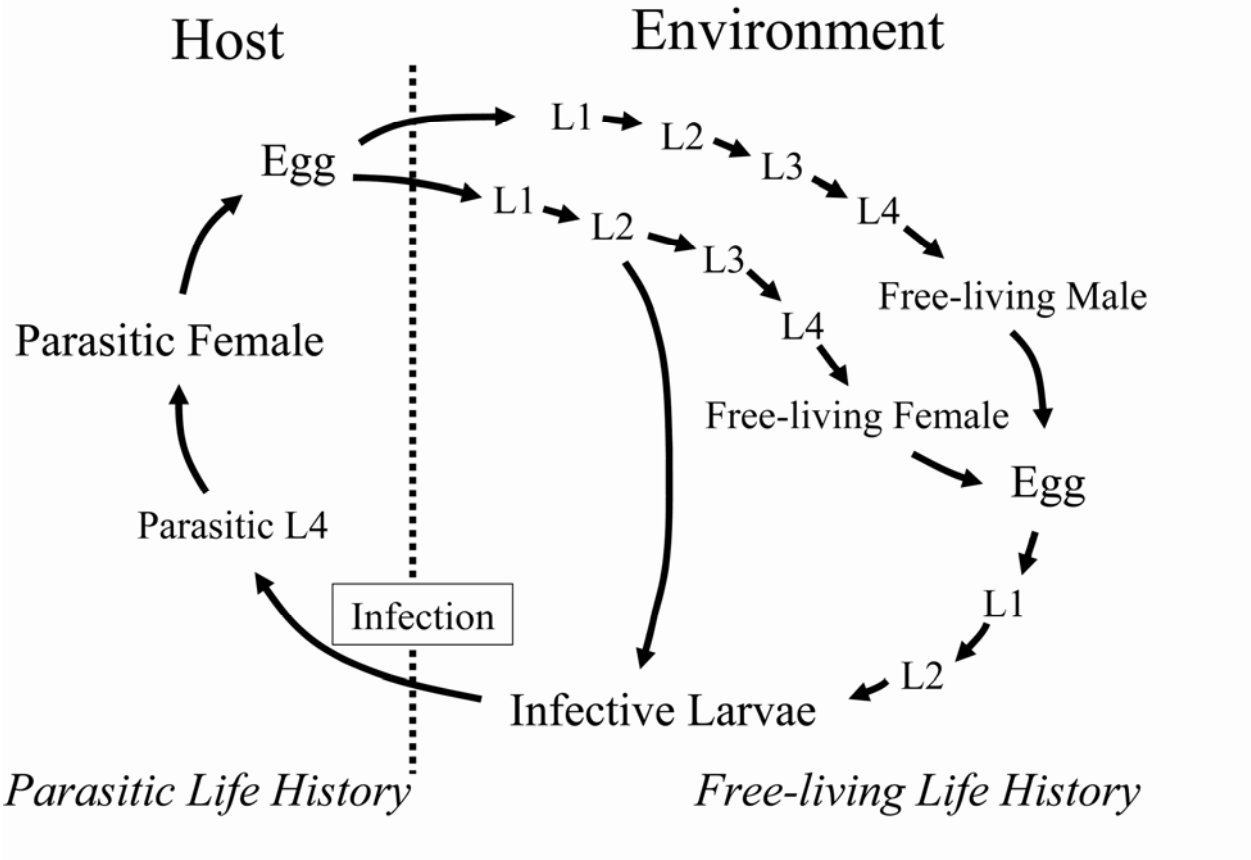


Figure S2. Life cycle of *S. papillosus.* Progeny of parasitic females can either develop into infective larvae (direct development) or into free-living adults (indirect development). All the progeny from free-living adults forms infective larvae. Infective larvae in the host develop to parasitic female and parthenogenetically reproduce.

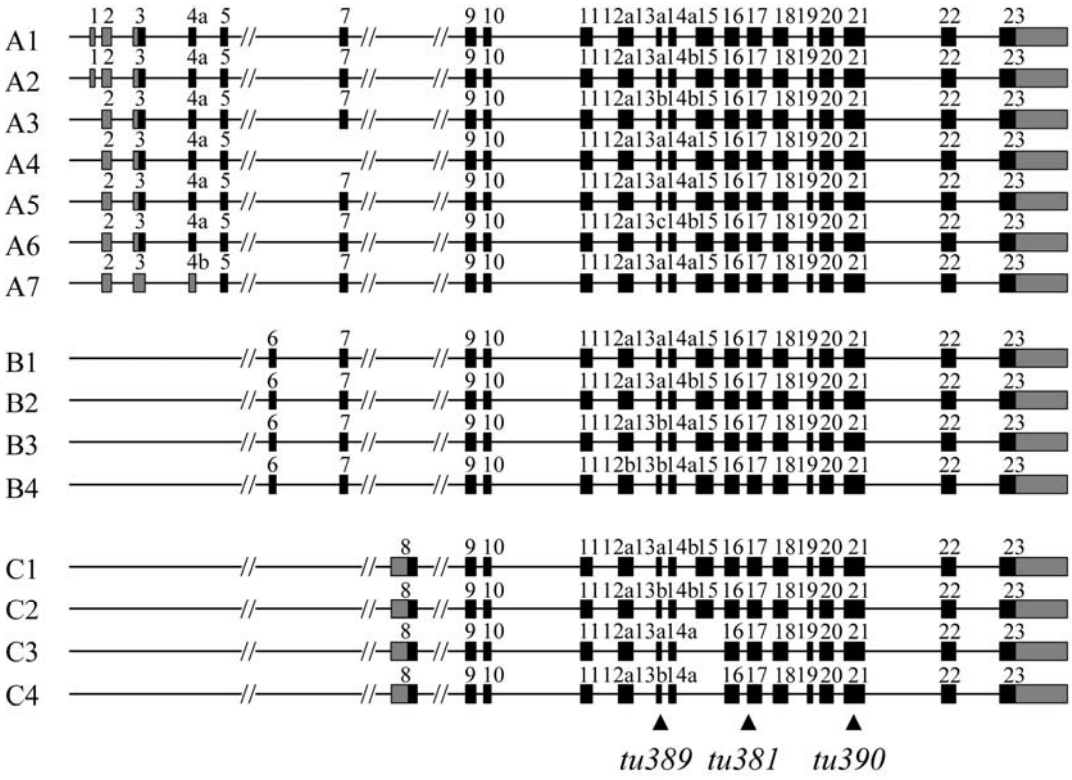
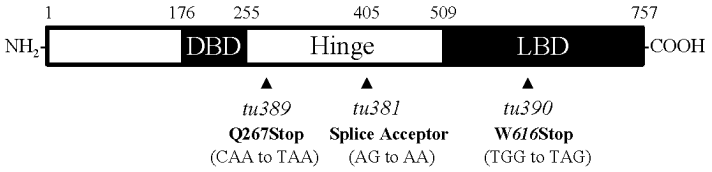


Figure S3. Gene and cDNA structures of *Ppa-daf-12* and observed splice variants. Exons with polymorphic splice sites are indicated by letters (e.g. 12a), un-translated regions are shown as grey boxes. Triangles indicate the positions of the three molecular lesions. Note that *tu389* affects all isoforms of exon 13.

A



B

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P. pacificus  176 CRVCGDHATGYNFNVITCESCKAFFRRNALRPKEFKCPYSED 217
C. elegans    118 CRVCGDHATGYNFNVITCESCKAFFRRNALRPKEFKCPYSED 159
S. papillosus 113 CRVCGDHATGYNFNVITCESCKAFFRRNALRPKEFKCPYSDD 154

P. pacificus  218 CDINSVSRRFCQKCRLRKCFSVGMKKEWILNEEQLRRR 255
C. elegans    160 CEINSVSRRFCQKCRLRKCFTVGMKKEWILNEEQLRRR 197
S. papillosus 155 CEINSVSRRFCQKCRLKKCFQVGMKKEWILNEEQLRRR 192
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C

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P. pacificus  509 IHPGDK-LSFQLNTAELRALDNVREAFSGMNETLDSGKQRESF 550
C. elegans    502 VPPAERNINYQLNAAELKALDAVREAFYGMDDPMEQGRQMQSF 544
S. papillosus 496 TFSLKTERDYTLSDKDLKELDSIRDSFQCMNEPLDNDQQASTL 538

P. pacificus  551 MKNNKTPTDIMNIMDITMRRLVKMSKKLPAFNELSNEGKFALL 593
C. elegans    545 LKANKTPADIMNIMDVTMRRFVKVAKGVPAFREVSQEGKFSLL 587
S. papillosus 539 AKKEHNPTDILNVMDITMRRLVKMAKRLGAFNEISEAGKFSLL 581

P. pacificus  594 KGGMVEMLTVRGVTRYDASSNSWKTPVVPDQYNVPV---SMFD 633
C. elegans    588 KGGMIEMLTVRGVTRYDASTNSFKTPTIKGQ-NVSVNVDDMFA 629
S. papillosus 582 KGGMIEMLTIRGVTVFNADKGVWQTPVDGHS-QISFN---MFD 620

P. pacificus  634 QLNMDCRDEQKHRFMQFVSALHEDLRKNELAISLIMLIVLFSH 676
C. elegans    630 KLNANAQ-AQKAKCLEFFGFFDEEIKKNELAVYLVMLAVLFSV 671
S. papillosus 621 KLRPDIKDKQKRGFLDFFNLLHSDVRKNDLAIDIIVLMVLFDS 663

P. pacificus  677 R-DNVNSPNDRILIDKHHRDYSALLFRYLESLYGEDARKFQEI 718
C. elegans    672 RSDPPMNENDVRIVTERHNHFMSLLNRYLESLFGEQARRIFER 714
S. papillosus 664 KREGLVSQQDKETVEKLHRNYESLLHRYLYSIHKEEAEQRFAS 706

P. pacificus  719 LPRALELLHVISDHSITLFMGTVKTEEAEPLPREFFK 755
C. elegans    715 IPKALGLLNEIARNAGMLFMGTVRSGEAEELPGEFFK 751
S. papillosus 707 IPKALVALRKVAENAVTLFLGAGNTTEAASLPKEFFA 743
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Figure S4. (A) Domain structure of *Ppa*-DAF-12. The DNA binding domain (DBD) and ligand binding domain (LBD) are separated by a hinge region. Filled triangles indicate the positions of the three molecular lesions. The nature of the molecular lesions are as follows: G to A transition in the splice acceptor site of exon 17 in *tu381*, CAA (Q) to TAA (stop) transition in *tu389*, and TGG (W) to TAG (Stop) transition in *tu390*. **B-C** Alignment of amino acid sequences of two functional domains of *Ppa*-DAF-12, *Cel*-DAF-12 and *Spa*-DAF-12. Amino acids identical

between the three species are indicated in bold. **(B)** Alignment of the DBD. Three motifs important for DNA recognition, the P box (ESCKA), the D box (PYS(E/D)D), and the DR box (GDHATGYNFNVIT), are indicated with lines. One splice variant (B4; Fig. S3) has a four amino acids insertion (TTSQ) in the position indicated by an open triangle. **(C)** Alignment of the LBD.

Figure	Condition/Strain	# of dauer/IL3	# of non-dauer/IL3	n	% dauer/IL3
Fig. 2A	**312 ph./312**	56	28	84	66.7
	312 ph./N2	0	82	82	0.0
	N2 ph./312	0	63	63	0.0
	N2 ph./N2	61	20	81	75.3
Fig. 2B	**Chol. +**	80	116	196	40.8
	Chol. -	164	36	200	82.0
Fig. 2C	**PS312**	20	56	76	26.3
	tu381	0	81	81	0
	tu389	0	98	98	0
	tu390	0	51	51	0
Fig. 4A	**E (*dfc-1*)**	146	52	198	73.7
	Δ4 (*dfc-1*)	0	137	137	0.0
	Δ7 (*dfc-1*)	0	170	170	0.0
	E (*dfc-2*)	144	17	161	89.4
	Δ4 (*dfc-2*)	0	287	287	0.0
	Δ7 (*dfc-2*)	0	300	300	0.0
	E (*dfc-3*)	34	231	265	12.8
	Δ4 (*dfc-3*)	75	354	429	17.5
	Δ7 (*dfc-3*)	0	270	270	0.0

	E (*dfc-4*)	35	185	220	15.9
	Δ4 (*dfc-4*)	56	255	311	18.0
	Δ7 (*dfc-4*)	0	191	191	0.0
Fig. 4B	**E**	42	136	178	23.6
	Δ4	35	107	142	24.6
	Δ7	0	156	156	0.0
Fig. 4C	**E**	237	21	258	91.9
	Δ4	200	69	269	74.3
	Δ7	0	52	52	0.0

Table S1. Summary of dauer/infective larvae formation assays. This Table provides the details of the data shown in Figures 2 and 4. For description of the experiments see the legend to Fig.

Supplemental References

1. Brenner, S. (1974). The genetics of Caenorhabditis elegans. Genetics *77*, 71-94.
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3. Kawano, T., Kataoka, N., Abe, S., Ohtani, M., Honda, Y., Honda, S., and Kimura, Y. (2005). Lifespan extending activity of substances secreted by the nematode Caenorhabditis elegans that include the dauer-inducing pheromone. Biosci Biotechnol Biochem *69*, 2479-2481.
4. Giroux, S., and Corey, E.J. (2007). Stereocontrolled synthesis of dafachronic acid A, the ligand for the DAF-12 nuclear receptor of Caenorhabditis elegans. J Am Chem Soc *129*, 9866-9867.
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