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De novo asymmetric synthesis and biological analysis of the daumone pheromones in *Caenorhabditis elegans* and in the soybean cyst nematode *Heterodera glycines*

Haibing Guo [a], James J. La Clair [b], Edward P. Masler [c], George A. O'Doherty [d,*], Yalan Xing [e,*]

[a] School of Nuclear Technology and Chemistry & Life Science, Hubei University of Science and Technology, Xianning 437100, China
[b] Xenobe Research Institute, PO Box 3052, San Diego, CA 92163-1052, USA
[c] Nematology Laboratory, USDA-ARS-NEA, Beltsville, MD 20705, USA
[d] Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115, USA
[e] Department of Chemistry, William Paterson University, 300 Pompton Rd, Wayne, NJ 07470, USA

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ABSTRACT

The de novo asymmetric total syntheses of daumone **1**, daumone **3** along with 5 new analogs are described. The key steps of our approach are: the diastereoselective palladium catalyzed glycosylation reaction; the Noyori reduction of 2-acetylfuran and an ynone, which introduce the absolute stereochemistry of the sugar and aglycon portion of daumone; and an Achmatowicz rearrangement, an epoxidation and a ring opening installing the remaining asymmetry of daumone. The synthetic daumones **1** and **3** as well as related analogs were evaluated for dauer activity in *Caenorhabditis elegans* and for effects on hatching of the related nematode *Heterodera glycines*. This data provides additional structure activity relationships (SAR) that further inform the study of nematode signaling.

1. Introduction

Daumones comprise a family of small molecule pheromones that can control multiple behaviors in the free-living nematode *Caenorhabditis elegans*.[1] Under unfavorable growth conditions, a mixture of pheromones including **1**–**3** signal *C. elegans* to enter the dauer stage, an enduring and non-ageing stage of the nematode life cycle with distinctive adaptive features and extended life.[2] In 2005, Jeong isolated, characterized and completed a total synthesis of daumone **1**.[3,4] Later that year we reported a de novo asymmetric[5] approach to **1**.[6] In 2007, daumone pheromones **2** and **3** were isolated and characterized by the Clardy group and were shown to have superior dauer inducing properties.[2a] In 2009, Riddle reported that synthetic daumone **1** did not fully represent the same biological effects from the isolated extracts.[7] Specifically, synthetic daumone **1** required near lethal doses to induce dauer effects, suggesting that other pheromone impurities (e.g., **2** and **3**) were present in the isolated samples of daumone **1**. More recently, other daumone-like compounds have been isolated and reported to have pheromone properties in *C. elegans* and related nematodes.[8]

Dauer formation in *C. elegans* is analogous to a protective behavior of the soybean cyst nematode *Heterodera glycines*, which is an obligate plant parasite. Embryos within *H. glycines* eggs can undergo a developmental arrest that prevents hatching of the infective stage until environmental conditions are optimal and host plants are available. This nematode is the most important pathogen of soybeans worldwide, and causes annual losses of over $1 billion in the United States alone. Worldwide, *H. glycines* and other plant-parasitic nematodes account for more than $100 billion in crop losses annually (Fig. 1).

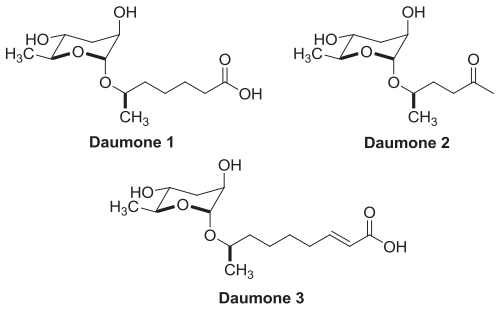

Fig. 1. Daumone natural products.

* Corresponding authors. E-mail addresses: g.odoherty@neu.edu (G.A. O'Doherty), XINGY@wpunj.edu (Y. Xing).

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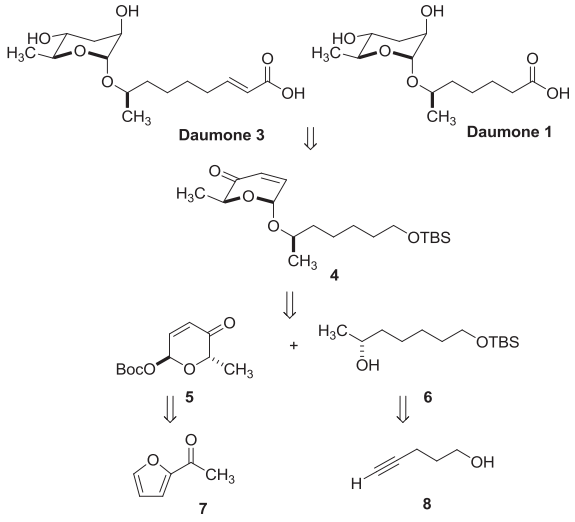
0040-4020/

With great pressure to discover novel agents that effectively suppress plant-parasitic nematodes and are environmentally responsible, biological targets such as egg hatching offer promise.[9] Although the genetics underlying the *C. elegans* and *H. glycines* developmental arrest behaviors appear not to be identical,[2] it is intriguing to consider what molecular mechanisms the *C. elegans* dauer formation and *H. glycines* embryonic developmental arrest might share, and how daumones **1**–**3** can be used to discover such mechanisms. This biological hypothesis involving daumones, along with their interesting structures, inspired us to pursue their de novo synthesis. Herein we fully describe the de novo asymmetric synthesis of daumones **1** and **3** and related analogs[10] and their effects on *C. elegans* and *H. glycines* biology.

Our strategy for the synthesis of daumones **1** and **3** is shown in Scheme 1. Retrosynthetically, both daumones **1** and **3** could be established from pyranone **4**, which, in turn, could be stereoselectively derived by a palladium-catalyzed coupling of the pyranone **5** and aglycon alcohol **6**.[11] The synthesis of the building blocks **5** and **6** was envisioned from ketone **7**,[12] and 4-pentynol **8**, respectively, using a Noyori reduction to install the stereochemical features within both components.

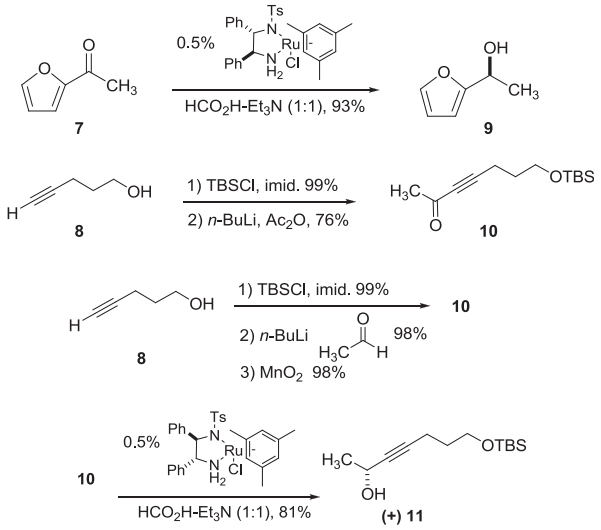


Scheme 1. Retrosynthetic analysis of daumone **1** and **3**.

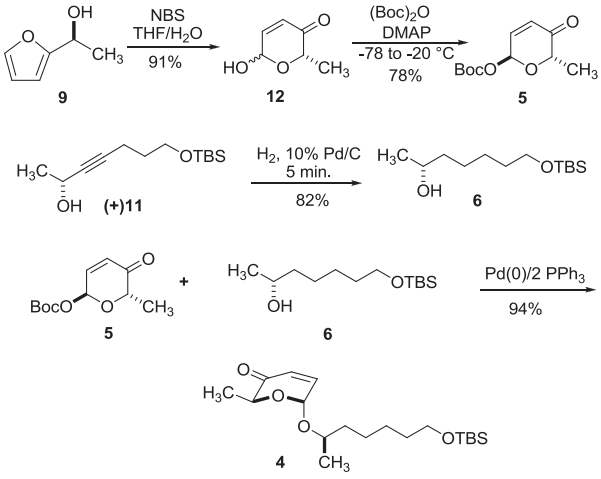
2. Results and discussion

We began by exploring the Noyori reduction to install the absolute stereochemistry in furan alcohol **9** and propargyl alcohol **11**, respectively (Scheme 2).[13] Using this approach, alcohol **9** was obtained in 93% yield (>96% ee) from acylfuran **7** using the Noyori (*S,S*) catalyst. Similar methods also allowed us to prepare the fatty alcohol side chain. We began by preparing ynone **10** from 4-pentynol **8** by two approaches, either in one-step by quenching with acetic anhydride in 75% yield, or metalation of the TBS ether with *n*-BuLi and then quenching with acetaldehyde followed by oxidation with MnO_2 in 95% yield. Noyori reduction of **10** provided alcohol **11** in 81% yield (>96% ee).

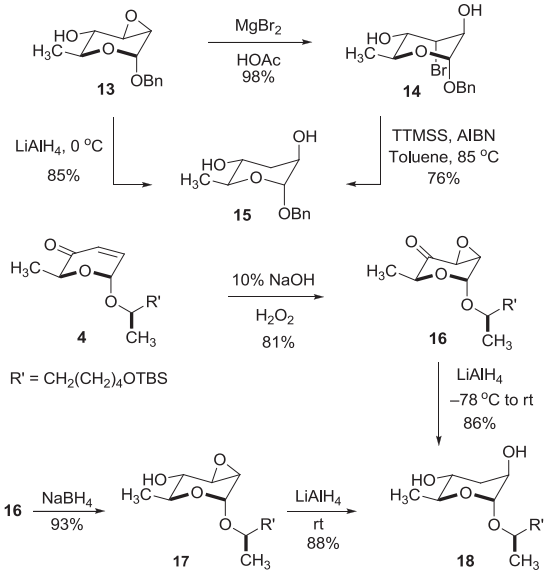
Next, we turned our attention towards elaborating **9** into the desired glycosylation precursor **5** (Scheme 3). Application of the Achmatowicz reaction (NBS in buffered aq. THF) to **9** afforded the ring-expanded pyranone product **12** in excellent yield (91%).[14] Hemiacetal **12** was then converted to its corresponding Boc-protected pyranone **5**. The second precursor **6** was then completed by reduction (H_2 10% Pd/C) of propargyl alcohol **11** in 82% yield. The palladium-catalyzed glycosylation of **5** with **6** occurred under Pd catalysis providing enone **4** as a single diastereomer in 94% yield.[15]



Scheme 2. Noyori reduction to install stereochemistry.



Scheme 3. Palladium catalyzed glycosylation.

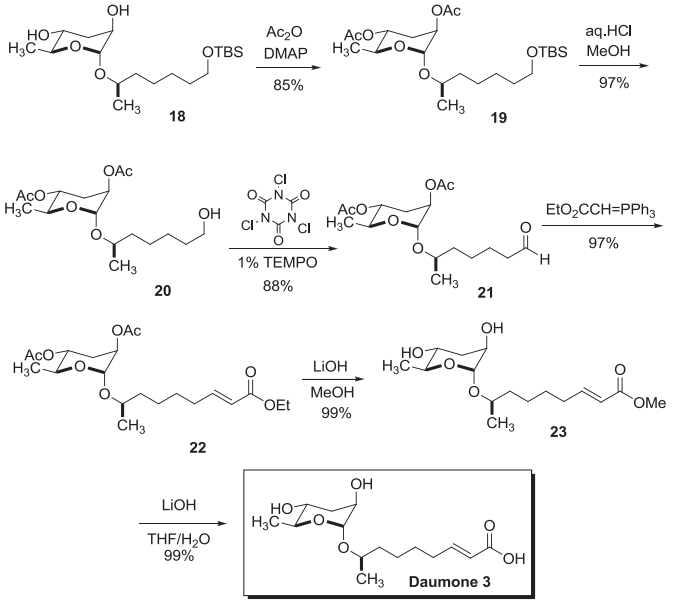


Scheme 4. Redox hydration strategy to install the ascaroside.

Our next operation focused on the installation of the required C-2 hydroxyl of the ascaroside ring.[3] As shown in Scheme 4, we began by using **13** as a model. Our initial studies indicated that we were able to open epoxide **13** to its corresponding bromohydrin **14** and subsequently reduce this material to the desired ascaroside **15** under radical conditions using tris(trimethylsilyl)silane (TTMSS).[16]

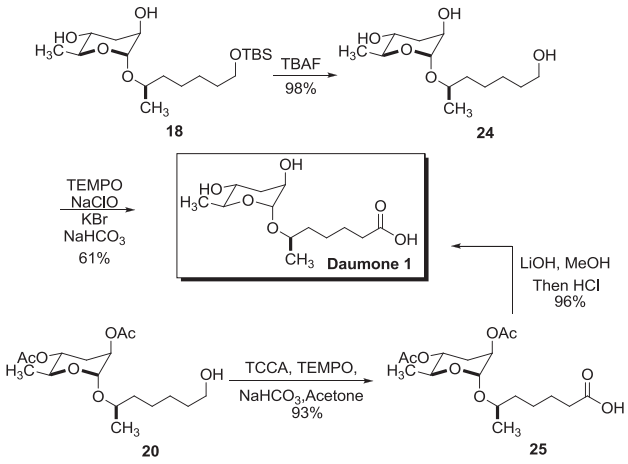
We also found a second solution through the regioselective and diastereoselective reduction of **14** to **15** by $LiAlH_4$.[17] We were then able to apply the latter procedure to the preparation of **18**. First, enone **4** was diastereoselectively epoxidized to afford **16** in 81% yield by treatment with H_2O_2 in the presence of a catalytic amount of NaOH.[18] Fortunately, treatment of **16** with $LiAlH_4$ returned the correct isomer **18** in a manner that directly mimicked the product obtained by reduction of the carbonyl first with $NaBH_4$ followed by opening of the epoxide **17** with $LiAlH_4$.

With diol **18** in hand, we were able to complete the synthesis of **3** using a six-step procedure. As shown in Scheme 5, this began by protection using acetic anhydride to provide the bis-acetate **19**. At this stage, removal of the TBS protection with aq HCl set the stage for installation of the terminal unsaturation. Alcohol **20** was oxidized to aldehyde **21** followed by treatment with Horner–Wadsworth–Emmons (HWE) reaction that afforded ethyl ester **22**. Completion of the synthesis of **3** arose through tandem acetate deprotection and *trans*-esterification to methyl ester **23** followed by hydrolysis with LiOH in THF/H_2O. Overall this six step process delivered **3** in 69% overall yield from the central intermediate **18**.



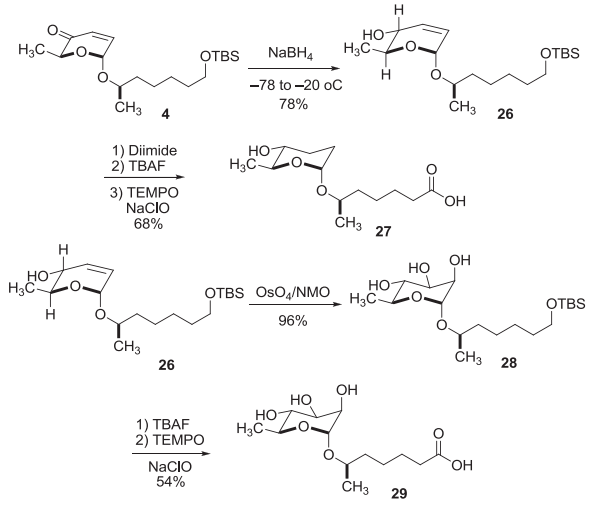
Scheme 5. Synthesis of daumone **3**.

We previously reported the preparation of daumone **1** from triol **24**, via a two-step TBS-group deprotection (**18**, 98% yield) and chemoselective oxidation (61% yield).6 Herein, we report an improved synthesis of daumone **1** from bis-acetate **20** (89% yield over two steps). Thus a trichloroisocyanuric acid (TCCA)/TEMPO oxidation of the primary alcohol in the suitably protected **20** to carboxylic acid **25** occurred in 93% yield.[19] Deprotection of the two acetate groups in **25** with LiOH gave the desired product daumone **1** in 96% yield (Scheme 6).



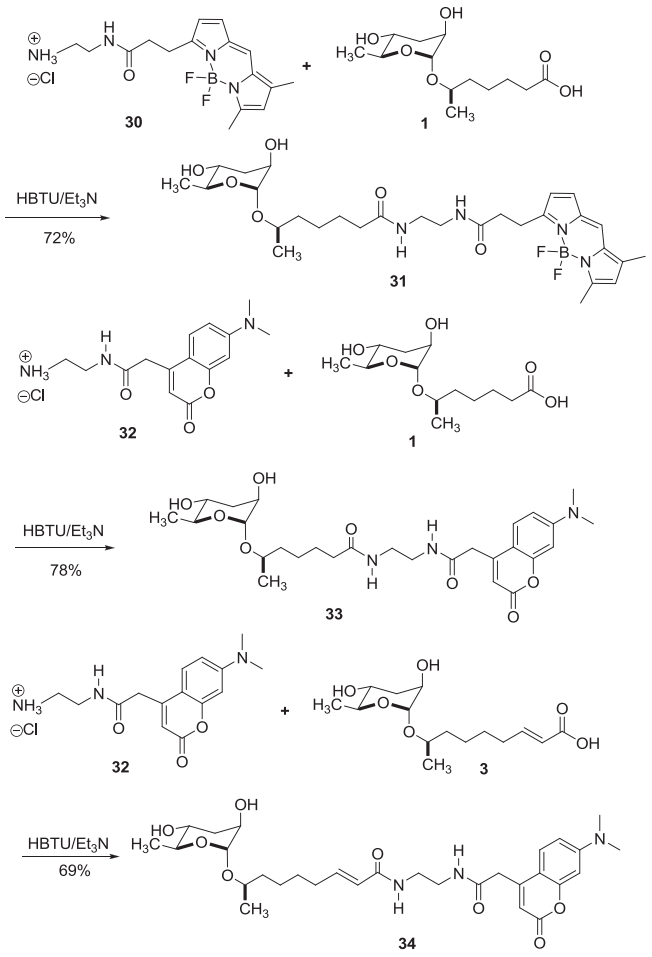
Scheme 6. Synthesis of daumone **1**.

This de novo approach enabled access to a series of daumone analogues (Scheme 7) and fluorescent probes (Scheme 8), which have been used in dauer inducing experiments to probe the structure activity relationships as well as explore the physiological mode of action.[20] Both C-2 deoxy- and C-3 hydroxy-daumone (**27** and **29**) were obtained from the palladium glycosylation product **4**. Reduction of **4** with $NaBH_4$ gave allylic alcohol **26** as a single diastereomer (78%). The deoxy-analogue **27** was established from **26** by diimide reduction of the pyran double bond followed by TBS-ether deprotection and oxidation to carboxylic acid **27** (53% overall yield from enone **4**). Alternatively, OsO_4 was used to diastereoselectively oxidize the double bond of **26** to the rhamnose isomer **28** in excellent yield (96%). The deprotection of the TBS-group with TBAF followed by oxidation with TEMPO provided the C-3 hydroxy daumone analogue **29** (42% overall yield from enone **4**).



Scheme 7. Synthesis of daumone analogues.

Three fluorescent daumone analogs **31**, **33** and **34** (Scheme 8) were synthesized in parallel by a peptide coupling of green and blue fluorescent amines **30** and **32** with daumone **1**, and amine **32** with daumone **3**, in the presence of HBTU and Et_3N, respectively, in good yield (**31**: 72%, **33**: 78%, **34**: 69%). Previously, we showed that fluorescent compound **34** can serve as a probe to track the uptake and localization of dauer-inducing pheromones in *C. elegans*.[20]



Scheme 8. Synthesis of fluorescent daumones.

Next, we applied an in vivo assay to gain an understanding of the structure-activity relationships (SAR) associated with dauer induction. In order to visualize the spacial and temporal effect using the fluorescent analogues, we developed a specific dosing assay that allowed the washing away of excess drug.[20] While many of the published assays use treatment during multiple stages or long-term growth,[1,2,4] our studies have found that the uptake of these materials is rapid and does not require constant exposure. We have found that the *C. elegans* treated with daumones **1** or **3** remain in the dauer state long after the worms have been washed with fresh media. In fact, time course imagining studies have shown the fluorescent analogues **33** and **34** are retained in *C. elegans* for greater than 24 h.

Following this liquid based assay,[20] *C. elegans* worms were exposed to a 1.0 μM solution of **1**, **3**, **23**, **29**, **31**, **33** and **34** and the percent of dauer formation was ascertained after transfer to agar media (Fig. 2). This assay allowed us to test the effects of the compounds at different stages of worm development. For comparative purposes,[20] the activity is reported as compared to compound **3** (positive control) and solvent alone (negative control).

From this screen, we found that three compounds, **3**, **23**, and **34**, were identified as having significant effects on dauer induction. Data from these studies revealed two key SAR features. First, the inclusion of a terminal-unsaturation within the olefinic side chain was key to improving the dauer activity. Second, these studies provided further confirmation that the addition of functionality at the carboxylic acid terminus either via an ester in **23** or amide in **34** were tolerated.[8] Combined these observations provide an important next step in establishing the development of fluorescent probes for the further study of nematode biology. In addition, the design of this assay allowed us to specifically identify that **3**, **23**, and **34** are active when presented not only during L1 but also at the egg stage or at entry to L1. While the current convention,[2a,4,7] screen over multiple stages, this assay provides the additional confirmation as to the stage and timing of the dauer induction.

Daumones **1** and **3** were also evaluated in a *H. glycines* hatch assay.[21] Eggs were continuously exposed to aqueous preparations (1, 2, 6, 10 or 1000 μM) of either daumone in vitro. Each treatment was replicated 16–24 times over 4 experiments. Percent hatch means were compared by 1-way ANOVA. After 6 days, daumone **3** stimulated hatching from treated eggs by a mean of 21±1% above control ($P<0.05$) at all doses between 1 μM and 10 μM. Notably, higher doses (e.g., 1 mM) had no effect. Daumone **1** had no effect on the hatching at any dose. Given the mechanical barriers to chemical penetration of nematode eggs, hatch stimulation by daumone **3** is significant. Whether hatch stimulation is the result of the daumone penetrating the egg and directly affecting the embryo, or the effect of a change in eggshell permeability related to the daumone molecule, are open and important questions[22] relating to mode of action. Daumone **3** is likely effective at less than 1 μM in *H. glycines*, with an effective concentration range of ~10-fold. Daumone **3** was effective at similar doses in inducing dauer formation in *C. elegans*.[20] The transition from hatch stimulation occurs at >10 μM. This loss of effect may not involve general toxicity, since hatched worms in the presence of 1 mM daumone **3** exhibited the same behavior as control groups.

3. Conclusion

In conclusion, de novo asymmetric total syntheses of daumones **1**, **3**, and five analogs were accomplished. This includes the development of new synthetic routes to **3**, **29**, **31**, **33**, and **34**. These

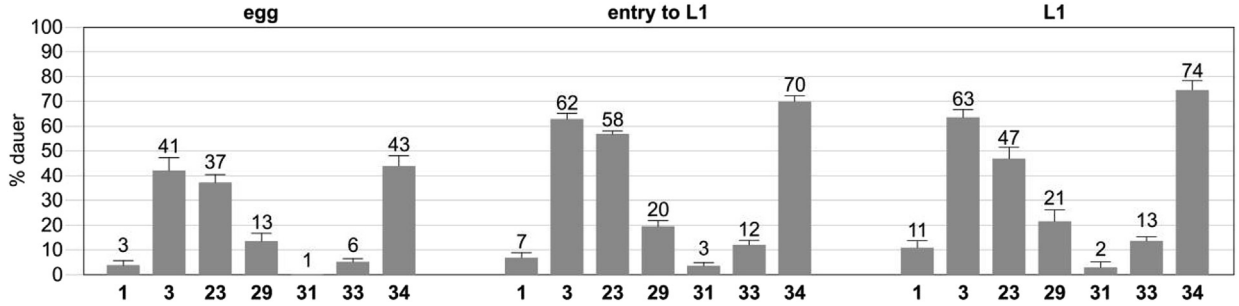


Fig. 2. Dauer induction assays. Compounds were screened by treating 200–300 worms as egg, during hatching, or in L1 stage with 1 μM of the ascribed compound in a volume of 200 μL of M9 media for 20 min, as previously reported.[20] The worms were then washed with fresh media, transferred to blank NG agar plates, and incubated for 72 h at 23 °C. The percentage of dauer worms was determined from the average of five repetitions of this assay. Each screen was conducted with a solvent control at 0–2% dauer formation (negative control) and established daumone **3** (positive control). Dauer states were counted in agar based upon their characteristic morphology and verified by counting the worms after treatment with 1% (w/v) aqueous SDS solution. The average and deviation of both plate and SDS-treated larvae are presented.

syntheses take advantage of our highly enantio- and diastereo-controlled palladium catalyzed glycosylation reaction and Noyori reduction of two ketones. Daumones **1** and **3** and the fluorescent analogs were evaluated in dauer inducing and egg hatching assays. Daumone **3** is an effective inducer of dauer formation in *C. elegans* and of hatch stimulation in *H. glycines*. Conversely, daumone **1** was less effective than daumone **3** in each of the two nematode species.

4. Experimental section

4.1. General information

^{1}H and ^{13}C NMR spectra were recorded on 270 MHz and 600 MHz spectrometers. Chemical shifts are reported relative to $CDCl_3$ (δ 7.26 ppm) for ^{1}H NMR and $CDCl_3$ (δ 77.0 ppm) for ^{13}C NMR. Infrared (IR) spectra were obtained on FT-IR spectrometer. Optical rotations were measured with a digital polarimeter in the solvent specified. Only new procedures and compounds are reported in this experimental section. For compounds not included in this section, please see the Supplementary data.

4.2. (2*R*,3*R*,5*R*,6*S*)-2-((*R*)-7-(*tert*-Butyldimethylsilyloxy)heptan-2-yloxy)-6-methyltetrahydro-2*H*-pyran-3,5-diyl diacetate (19)

To a solution of diol **18** (752 mg, 2 mmol) in pyridine (2 mL), was added Ac_2O (1 mL) and DMAP (35 mg). The reaction mixture was stirred for 12 h. Water was added to destroy the excess acetic anhydride, extracted (3×50 mL) with Et_2O washed with 40 mL of saturated $CuSO_4$ solution for three times, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified using silica gel flash chromatography eluting with 5% ether/hexane to give acetate **19** (912 mg, 1.98 mmol, 99%); a colorless oil; R_f=0.49 (10% EtOAc/hexane); $[\alpha]_D^{25}$=−66 (*c*=1.1, CH_2Cl_2); IR (thin film, cm^{-1}), 2958, 1743, 1371, 1309, 1037, 835; 1H NMR (600 MHz, $CDCl_3$) 4.74 (m, 1H), 4.70 (ddd, *J*=11.4, 10.2, 4.2 Hz, 1H), 4.65 (*s*, 1H), 3.85 (dq, *J*=9.6, 6.6 Hz, 1H), 3.76 (m, 1H), 3.60 (t, *J*=6.6 Hz, 2H), 2.11 (ddd, *J*=13.2, 3.6, 3.6 Hz, 1H), 2.10 (s, 3H), 2.05 (s, 3H), 1.93 (ddd, *J*=13.2, 11.4, 3 Hz, 1H), 1.58 (m, 2H), 1.54 (m, 2H), 1.43 (m, 2H), 1.34 (m, 2H), 1.17 (d, *J*=6.6 Hz, 3H), 1.12 (d, *J*=6.0 Hz, 3H), 0.89 (s, 9H), 0.04 (s, 6H); 13C NMR (150 MHz, $CDCl_3$) 170.4, 170.1, 93.7, 72.4, 70.7, 70.0, 68.8, 63.3, 37.2, 32.9, 29.5, 26.1, 26.0, 25.9, 25.6, 21.3, 21.2, 19.1, 18.4, 17.7, −5.2; HRMS: calcd for [$C_{23}H_{44}O_7SiNa^+$]: 483.2749, found: 483.2755.

4.3. 2-((*R*)-7-Hydroxyheptan-2-yloxy)-tetrahydro-6-methyl-2*H*-pyran-3,5-diacetate (20)

To a solution of TBS-ether **19** (460.6 mg, 1 mmol) in dry MeOH (2 mL), 6 N HCl (2 mL) was added at room temperature under the argon atmosphere. After 4 h, the reaction mixture was diluted with ether (20 mL) and quenched with saturated $NaHCO_3$, extracted (3×5 mL) with Et_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by passing a silica gel pad eluting with pure ether to give alcohol **20** (350 mg, 0.97 mmol, 97%); a colorless oil, $[\alpha]_D^{25}$=−86 (*c*=1.12, CH_2Cl_2); IR (thin film, cm^{-1}) 2975, 2935, 2861, 1739, 1230, 1105, 1032, 983; ^{1}H NMR (600 MHz, $CDCl_3$) 4.81 (m, 1H), 4.77 (ddd, *J*=11.4, 10.2, 4.2 Hz, 1H), 4.72 (s, 1H), 3.84 (dq, *J*=9.6, 6.6 Hz, 1H), 3.74 (m, 1H), 3.62 (t, *J*=6.6 Hz, 2H), 2.10 (ddd, *J*=13.2, 3.6, 3.6 Hz, 1H), 2.09 (s, 3H), 2.03 (s, 3H), 1.92 (ddd, *J*=13.2, 11.4, 3 Hz, 1H), 1.57 (m, 2H), 1.54 (m, 2H), 1.43 (m, 2H), 1.36 (m, 2H), 1.15 (d, *J*=6.6 Hz, 3H), 1.11 (d, *J*=6.0 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) 170.4, 170.1, 93.7, 72.3, 70.7, 70.0, 66.8, 62.7, 37.1, 32.7, 29.4, 25.8, 25.5, 21.2, 21.1, 19.1, 17.7; HRMS: calcd for [$C_{17}H_{30}O_7Na^+$]: 369.1884, found: 369.1891.

4.4. 2-Methyl-6-((*R*)-7-oxoheptan-2-yloxy)-tetrahydro-2*H*-pyran-3,5-yl diacetate (21)

A solution of alcohol **20** (240 mg, 0.693 mmol) and trichloroisocyanuric acid (171.2 mg, 1.386 mmol) in ethyl ether (30 mL) was stirred at −30 °C, and then 2,2,6,6-tetramethylpeperidinooxy (5 mg, 0.034 mmol) was added in one portion. The mixture was stirred for 20 min and then quenched with 30 mL saturated sodium carbonate. The organic layer was separated and the aqueous layer was extracted with ether. Combined the organic layer and washed with brine, dried (Na_2SO_4), filtered and concentrated. Chromatography on silica gel (20% EtOAc/Hexane) gave aldehyde **21** (210 mg, 0.61 mmol, 88%) as colorless oil; R_f=0.66 (40% EtOAc/Hexane); $[\alpha]_D^{25}$=−41 (*c*=1.0, MeOH); IR (thin film, cm^{-1}), 2929, 1741, 1234, 1040; ^{1}H NMR (600 MHz, $CDCl_3$) δ 9.76 (t, *J*=1.8 Hz, 1H), 4.82 (m, 1H), 4.78 (ddd, *J*=11.4, 10.2, 4.8 Hz, 1H), 4.72 (s, 1H), 3.82 (dq, *J*=12.6, 6 Hz, 1H), 3.76 (ddq, *J*=6.0, 6.0, 6.0 Hz, 1H), 2.44 (dt, *J*=7.2, 1.8 Hz, 1H), 2.09 (m, 3H), 2.04 (m, 3H), 1.91 (ddd, *J*=13.8, 11.4, 3 Hz, 1H), 1.68−1.46 (m, 8H), 1.33 (d, *J*=6.6 Hz, 6H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 202.6, 170.6, 170.3, 93.8, 72.3, 70.8, 70.0, 67.0, 44.0, 37.0, 29.6, 25.4, 22.3, 21.4, 21.3, 19.2, 17.9. HRMS: calcd for [$C_{17}H_{28}O_7Na^+$]: 367.1227, Found: 367.1733.

4.5. (8*R*,*E*)-Ethyl-8-(3,5-diacetoxy-6-methyl-tetrahydro-2*H*-pyran-2-yloxy)non-2-enoate (22)

Ethyl (triphenylphosphoranylidene)acetate (396.7 mg, 1.14 mmol) was dissolved in 0.6 mL of CH_2Cl_2, into which aldehyde **21** (190 mg, 0.55 mmol) was added. The reaction was stirred vigorously at room temperature for 12 h before solvent was removed in vacuum. Chromatography on silica gel (30% EtOAc/Hexane) gave ethyl ester **22** as colorless oil (220 mg, 0.53 mmol, 96.5%). R_f=0.57 (40% EtOAc/Hexane); $[\alpha]_D^{25}$=−22 (*c*=1.0, MeOH); IR (thin film, cm^{-1}), 2936, 1743, 1233, 1038; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.96 (dt, *J*=13.8, 7.2 Hz, 1H), 5.82 (dt, *J*=15.6, 1.8 Hz, 1H), 4.83 (m, 1H), 4.79 (ddd, *J*=10.8, 9.6, 4.2 Hz, 1H), 4.73 (s, 1H), 4.18 (q, *J*=7.2 Hz, 2H), 3.84 (dq, *J*=12, 6 Hz, 1H), 3.76 (ddq, *J*=6.0, 6.0, 6.0 Hz, 1H), 2.22 (dt, *J*=7.2, 1.2 Hz, 1H), 2.11 (m, 3H), 2.06 (m, 3H), 1.93 (ddd, *J*=13.8, 11.4, 3 Hz, 1H), 1.60−1.35 (m, 8H), 1.28 (t, *J*=7.2 Hz, 3H); 1.17 (d, *J*=6.6 Hz, 3H), 1.13 (d, *J*=6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 170.3, 170.1, 166.7, 148.9, 121.5, 93.6, 72.2, 70.6, 69.8, 66.8, 60.4, 60.2, 36.8, 32.2, 29.3, 27.9, 25.3, 21.2, 19.0, 17.6, 14.3; HRMS: calcd for [$C_{21}H_{34}O_8Na^+$]: 437.2146, Found: 437.2151.

4.6. (8*R*,*E*)-Methyl-8-(3,5-dihydroxy-6-methyl-tetrahydro-2*H*-pyran-2-yloxy)non-2-enoate (23)

To a 20 mL flask was added ethyl ester **22** (136 mg, 0.328 mmol), MeOH (3 mL), Lithium hydroxide (30 mg, 1.25 mmol). The resulting solution was stirred at room temperature for 24 h. Aqueous HCl (0.5 M, 3 mL) was added before solvent was removed in vacuum. Chromatography on silica gel (80% EtOAc/Hexane) gave methyl ester **23** as colorless oil (103 mg, 0.309 mmol, 99%). R_f=0.58 (EtOAc); $[\alpha]_D^{25}$=−81 (*c*=1.0, MeOH); IR (thin film, cm^{-1}), 3404, 2934, 2180, 1991, 1723, 1655, 1438, 1273, 1203, 1101, 1128, 1029, 984, 854; ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.96 (dt, *J*=15.6, 6.6 Hz, 1H), 5.83 (dt, *J*=15.6, 1.2 Hz, 1H), 4.69 (s, 1H), 3.80−3.77 (m, 1H), 3.72 (s, 3H), 3.65 (dt, *J*=6.6, 3.0 Hz, 1H), 3.59−3.57 (m, 1H), 2.22 (ddd, *J*=9.0, 7.2, 1.8 Hz, 1H), 1.88−1.72 (m, 2H), 1.61 (m, 2H), 1.55−1.35 (m, 6H), 1.27 (d, *J*=6. Hz, 3H), 1.22 (d, *J*=6 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$) δ 167.3, 149.5, 121.7, 96.1, 71.3, 70.0, 69.3, 69.1, 51.5, 36.9, 35.2, 32.0, 27.8, 25.0, 20.0, 17.7; HRMS: calcd for [$C_{16}H_{28}O_6Na^+$]: 339.1778, Found: 339.1784.

4.7. (8*R*,*E*)-8-(3,5-Dihydroxy-6-methyl-tetrahydro-2*H*-pyran-2-yloxy)non-2-enoic acid (daumone 3)

To a 20 mL flask was added methyl ester **23** (13 mg, 0.04 mmol), THF/H_2O (4:1, 0.4 mL), Lithium hydroxide (1.44 mg, 0.06 mmol). The resulting solution was stirred at room temperature for 24 h. Aqueous HCl (0.5 M, 0.4 mL) was added to adjust the pH to 7, and then the solvent was removed in vacuum. Chromatography on silica gel (5% MeOH/EtOAc) gave acid **1** as colorless oil (12 mg, 0.0397 mmol, 99%). R_f=0.28(EtOAc); $[\alpha]_D^{25}$=−62 (*c*=1.0, MeOH); IR (thin film, cm^{-1}), 3703, 3364, 2930, 2357, 2164, 2084, 2010, 1954, 1696, 1272, 1127, 981, 667; ^{1}H NMR (600 MHz, CD_3OD) 6.93 (dt, *J*=15.6, 7.2 Hz, 1H), 5.79 (dt, *J*=15.6, 1.2 Hz, 1H), 4.62 (s, 1H), 3.78−3.75 (m, 2H), 3.69 (s, 1H), 3.63−3.58 (m, 1H), 3.49 (ddd, *J*=10.8, 9.6, 4.8 Hz, 1H), 2.23−2.21 (m, 2H), 2.05−1.91 (m, 2H), 1.74 (ddd, *J*=12.6, 11.4, 2.4 Hz, 1H), 1.58−1.23 (m, 4H), 1.19 (d, *J*=6.6 Hz, 3H), 1.11 (d, *J*=6 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) 169.9, 148.8, 122.4, 96.4, 71.3, 70.0, 68.8, 67.2, 36.9, 34.8, 31.9, 28.0, 25.2, 18.2, 16.9; HRMS: calcd for [$C_{15}H_{26}O_6Na^+$]: 325.1622, Found: 325.1627.

4.8. (*R*)-*N*-(2-(3-(4,4-Difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-s-indacene-3-propionyl) propanamido)ethyl)-6-(tetrahydro-3,5-dihydroxy-6-methyl-2*H*-pyran-2-yloxy) heptanamide (31)

To a solution of amine **30** (5 mg, 0.013 mmol) in THF (0.1 mL), was added Et_3N (3.9 μl), HBTU (10.2 mg, 0.027 mmol) and daumone **2** (7.4 mg, 0.027 mmol). The reaction mixture was stirred overnight, concentrated under reduced pressure, purified using silica gel flash chromatography eluting with 12% MeOH/Et_2O to give amide **31** (5.5 mg, 0.009 mmol, 72%); R_f=0.54 (20% MeOH/EtOAc); IR (thin film, cm^{-1}) 3397, 2967, 2930, 2861, 1607, 1469, 1140, 1043, 850; ^{1}H NMR (600 MHz, CD_3OD) δ 7.50 (s, 1H), 7.08 (d, *J*=3.6 Hz, 1H), 6.40 (d, *J*=3.6 Hz, 1H), 6.28 (s, 1H), 4.70 (s, 1H), 3.84 (ddq, *J*=6.0, 6.0, 6.0 Hz, 1H), 3.77 (m, 1H), 3.68 (dq, *J*=9.6, 6.0 Hz 1H), 3.58 (ddd, *J*=10.2, 10.2, 4.2 Hz, 1H), 3.35 (t, *J*=6.0 Hz, 2H), 3.34 (t, *J*=6.0 Hz, 2H), 3.29 (t, *J*=7.8 Hz, 2H), 2.68 (t, *J*=7.8 Hz, 2H), 2.58 (s, 3H), 2.35 (s, 3H), 2.24 (t, *J*=7.8 Hz, 2H), 2.02 (ddd, *J*=13.2, 3.6, 3.6 Hz, 1H), 1.83 (ddd, *J*=13.2, 11.4, 3.0 Hz, 1H), 1.67−1.58 (m, 2H), 1.57−1.50 (m, 2H), 1.46−1.33 (m, 2H), 1.28 (d, *J*=6.0 Hz, 3H), 1.18 (d, *J*=6.0 Hz, 3H); HRMS: calcd for [$C_{29}H_{43}BF_2N_4O_6Na^+$]: 615.3136, found: 615.3140.

4.9. (*R*)-*N*-(2-(2-(7-(Dimethylamino)-2-oxo-2*H*-chromen-4-yl) acetamido)ethyl)-6-(tetrahydro-3,5-dihydroxy-6-methyl-2*H*-pyran-2-yloxy) heptanamide (33)

To a solution of amine **32** (20 mg, 0.05 mmol) in THF (0.5 mL), was added Et_3N (8.4 μl), HBTU (22.7 mg, 0.06 mmol) and daumone **2** (16.4 mg, 0.06 mmol). The reaction mixture was stirred overnight, concentrated under reduced pressure, purified using silica gel flash chromatography eluting with 20% MeOH/Et_2O to give amide **33** (21 mg, 0.039 mmol, 78%); R_f=0.47 (10% MeOH/EtOAc); $[\alpha]_D^{25}$=−40 (*c*=1.6, MeOH); IR (thin film, cm^{-1}) 3352, 2931, 2865, 1611, 1404, 1131, 1026, 980; ^{1}H NMR (600 MHz, D_2O) δ 7.36 (d, *J*=9.0 Hz, 1H), 6.66 (dd, *J*=9.0, 2.4 Hz, 1H), 6.33 (d, *J*=2.4 Hz, 1H), 6.01 (s, 1H), 4.77 (s, 1H), 3.89 (m, 1H), 3.80 (ddq, *J*=6.0, 6.0, 6.0 Hz, 1H), 3.68 (dq, *J*=9.6, 6.0 Hz 1H), 3.67 (s, 2H), 3.65 (ddd, *J*=10.2, 10.2, 4.2 Hz, 1H), 3.41 (t, *J*=6.0 Hz, 2H), 3.35 (t, *J*=6.0 Hz, 2H), 3.01 (s, 6H), 2.09 (ddd, *J*=13.2, 3.6, 3.6 Hz, 1H), 2.07 (t, *J*=7.8 Hz, 2H), 1.79 (ddd, *J*=13.2, 11.4, 3.0 Hz, 1H), 1.51−1.44 (m, 1H), 1.43−1.36 (m, 2H), 1.35−1.29 (m, 1H), 1.29−1.17 (m, 2H), 1.28 (d, *J*=6.0 Hz, 3H), 1.16 (d, *J*=6.0 Hz, 3H); ^{13}C NMR (67.5 MHz, CD_3OD) δ 176.6, 171.6, 164.4, 157.3, 154.9, 152.8, 127.0, 110.7, 110.6, 109.9, 98.9, 97.5, 72.4, 71.3, 70.0, 68.5, 40.5, 40.4, 40.3, 40.0, 38.1, 37.2, 36.2, 27.0, 26.7, 19.5, 19.4, 18.3; HRMS: calcd for [$C_{28}H_{41}N_3O_8Na^+$]: 570.2789, found: 570.2788.

4.10. (8*R*,*E*)-8-(3,5-Dihydroxy-6-methyl-tetrahydro-2*H*-pyran-2-yloxy)-*N*-(2-(2-(7-(dimethylamino)-2-oxo-2*H*-chromen-4-yl)acetamido)ethyl)non-2-enamide (34)

To a solution of amine **32** (6 mg, 0.015 mmol) in THF (0.125 mL), was added Et_3N (3 ul), HBTU (4.7 mg, 0.0125 mmol) and acid **3** (5 mg, 0.0125 mmol). The reaction mixture was stirred overnight, concentrated under reduced pressure, purified using silica gel flash chromatography eluting with 10% MeOH/EtOAc to give amide **34** (5 mg, 0.00872 mmol, 69%); R_f=0.55 (10% MeOH/EtOAc); $[\alpha]_D^{25}$=−30 (*c*=0.5, MeOH); IR (thin film, cm^{-1}), 3781, 3358, 2933, 2252, 2149, 1997, 1699, 1615, 1531, 1453, 1403, 1252, 1128, 1030, 983, 834; ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.32 (d, *J*=9 Hz, 1H), 6.92 (dt, *J*=15.6, 7.2 Hz, 1H), 6.77−6.71 (m, 1H), 6.54 (d, *J*=3 Hz, 1H), 6.03 (s, 1H), 5.81 (dt, *J*=15.6, 1.2 Hz, 1H), 4.63 (s, 1H), 3.79−3.78 (m, 1H), 3.70 (s, 1H), 3.67−3.60 (m, 2H), 3.41 (t, *J*=6.0 Hz, 2H), 3.34 (t, *J*=6.0 Hz, 2H), 3.06 (s, 6H), 2.26−2.22 (m, 1H), 1.97−1.88 (m, 2H), 1.78−1.73 (m, 1H), 1.58−1.54 (m, 2H), 1.50−1.45 (m, 2H), 1.43−1.38 (m, 2H), 1.28 (m, 2H), 1.20 (d, *J*=6.6 Hz, 3H), 1.11 (d, *J*=6 Hz, 3H); ^{13}C NMR (150 MHz, CD_3OD) δ 170.3, 167.9, 163.1, 156.0, 153.6, 151.4, 149.2, 144.6, 125.7, 123.5, 121.9, 109.5, 108.7, 97.6, 96.4, 71.2, 70.0, 68.8, 67.2, 39.3, 39.0, 38.7, 36.9, 34.8, 31.8, 28.1, 28.0, 25.2, 18.1, 16.9; HRMS: calcd for [$C_{30}H_{43}O_8N_3Na^+$]: 596.2942, Found: 596.2948.

4.11. *C. elegans* protocols

C. elegans Bristol variety, strain N2, were grown on nematode growth media (NGM) agar plates with *E. coli* (OP50) as a food source under standard uncrowded and well-fed conditions at 20 °C unless otherwise stated.

4.12. Dauer formation assay

Adult worms were placed on a plate containing 3 mL of NGM agar and incubated at 20 °C for 48−96 h. After incubation, adult worms were removed, and the eggs or entry to-L1, L1-staged progeny were collected by centrifugation after suspension of the agar in 5 mL of M9 media. Eggs, entry to-L1 stage or L1 stage worms were then resuspended in 190 μL of M9 media in a 96 well plate and treated with a 10 μL solution of the desired compound suspended in 50% (v/v) aqueous DMSO or 50% (v/v) aqueous ethanol. After incubation for 4 h at 23 °C, the solution was transferred to a 1 mL NGM agar plate, and the entry to-L1 stage or L1 stage larvae were then incubated for 52−72 h at 23 °C. The number of eggs or larvae was counted before and after incubation. A 1% (w/v) aqueous SDS solution (1.0 mL) was added to the plate, and the surviving worms were counted as dauer larvae.[2a,4,7]

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Supplementary data

Supplementary data (experimental procedures and characterization for the synthesis of all compounds, and copies of ^{1}H NMR, ^{13}C NMR for all new compounds can be found in the supplementary data) associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.tet.2016.03.033.

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3. In this manuscript we refer to the naturally occurring ascarosides with dauer inducing properties as daumones. There is an effort to change the natural product class name from 'daumones' to 'ascarosides'. This new naming convention derives it name from ascarylose, the unique sugar associated with this class of natural products. Unfortunately, the name ascarosides is already assigned by carbohydrate nomenclature conventions to any natural and unnatural ascarylose with a group attached to the anomeric oxygen (i.e., ascaroside is akin to glucoside).
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