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Comparative Ascaroside Profiling of *Caenorhabditis* Exometabolomes Reveals Species-Specific (ω) and (ω – 2)-Hydroxylation Downstream of Peroxisomal β-Oxidation.

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ABSTRACT

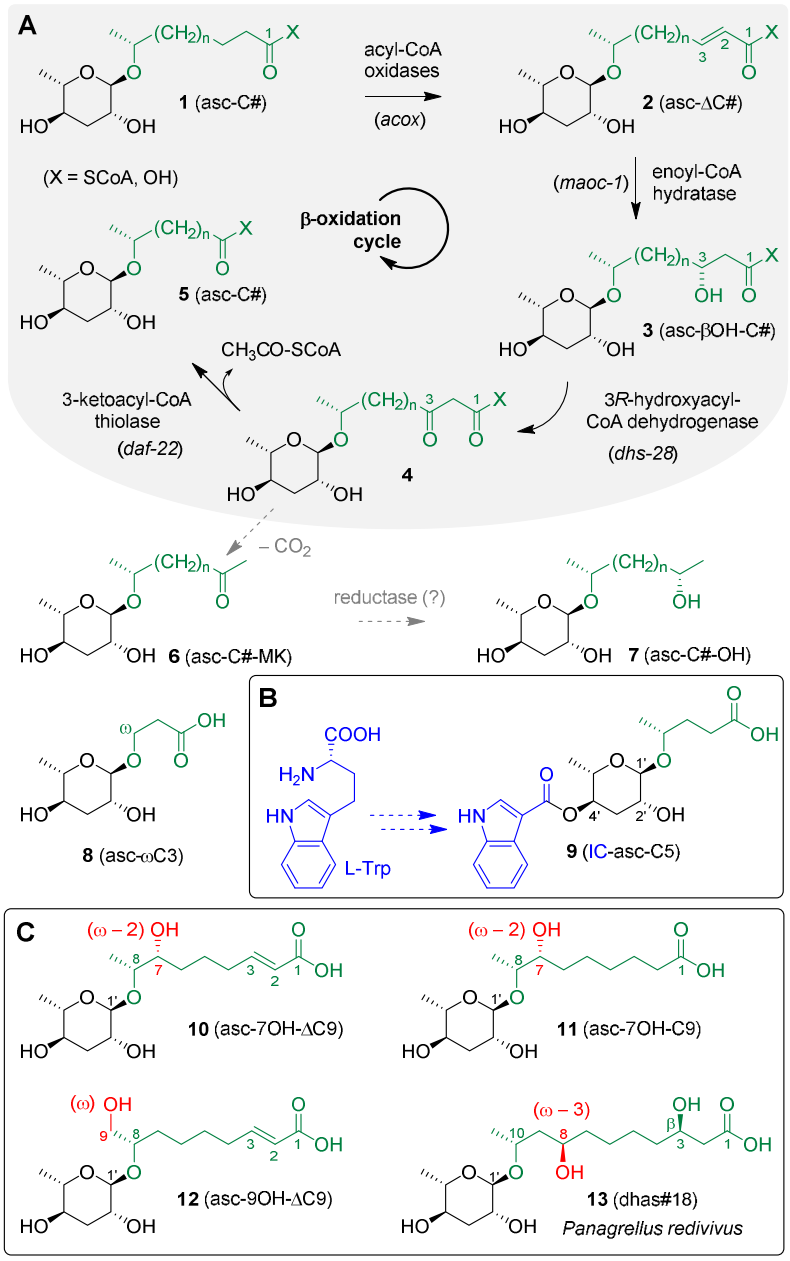
Chemical communication in nematodes, such as the model organism *Caenorhabditis elegans*, is modulated by a variety of glycosides based on the dideoxysugar L-ascarylose. Comparative ascaroside profiling of nematode exometabolome extracts using a GC-EIMS screen reveals that several basic components including ascr#1 (asc-C7), ascr#2 (asc-C6-MK), ascr#3 (asc-ΔC9), ascr#5 (asc-ωC3), and ascr#10 (asc-C9) are highly conserved among the *Caenorhabditis*. Three novel, side chain hydroxylated ascaroside derivatives were exclusively detected in the distantly related *C. nigoni* and *C. afra*. Molecular structures of these species-specific, putative signaling molecules were elucidated by NMR spectroscopy and confirmed by total synthesis and chemical correlations. Biological activities were evaluated using attraction assays. The identification of (ω)- and (ω – 2)-hydroxyacyl ascarosides demonstrates how GC-EIMS-based ascaroside profiling facilitates the detection of novel ascaroside components and exemplifies how species-specific hydroxylation of ascaroside aglycones downstream of peroxisomal β-oxidation increases the structural diversity of this highly conserved class of nematode signaling molecules.

INTRODUCTION

Research with the model organism *Caenorhabditis elegans* and the development of novel analytical techniques promoted the characterization of ascarosides, a modular glycolipid library based on the 3,6-dideoxysugar L-ascarylose linked to fatty acid derived aglycones (Scheme 1).[1] In *C. elegans*, ascarosides modulate a large diversity of biological responses including dauer formation,[2-6] reproduction,[6,7] stress resistance,[7,8] lifespan,[8,9] and behavior.[10-14] The diversity of biological responses modulated by ascarosides is paralleled by their large structural diversity. Even small changes in molecular structures,[7,14,15] synergistic effects,[10,11,16]

and variations in ascaroside compositions[7,17,18] can dramatically alter their biological activity. Homologous series originate from chain shortening of very long chain precursors upon peroxisomal β-oxidation (Scheme 1A) to furnish ascarosides carrying acyl (**1**, asc-C#), enoyl (**2**, asc-ΔC#), and (3*R*)-hydroxyacyl (**3**, asc-βOH-C#) aglycones.[19-23] 2-Ketoalkyl (**6**, asc-C#-MK) and 2-hydroxyalkyl (**7**, asc-C#-OH) aglycones are presumably produced via decarboxylation and subsequent reduction of labile 3-ketoacyl intermediates (**4**). Furthermore, (ω)-ascarosides such as asc-ωC3 (**8**, ascr#5) demonstrate that (ω – 1) and (ω)-linked components enter the peroxisomal β-oxidation cycle. Downstream of the peroxisomal β-oxidation cycle, the resulting basic ascaroside skeletons serve as scaffolds for the attachment of additional structural units derived from primary metabolic pathways to furnish a modular library of species-specific signaling components such as the indole ascaroside IC-asc-C5 (**9**, icas#9) (Scheme 1B).[5,12,16,22,24]

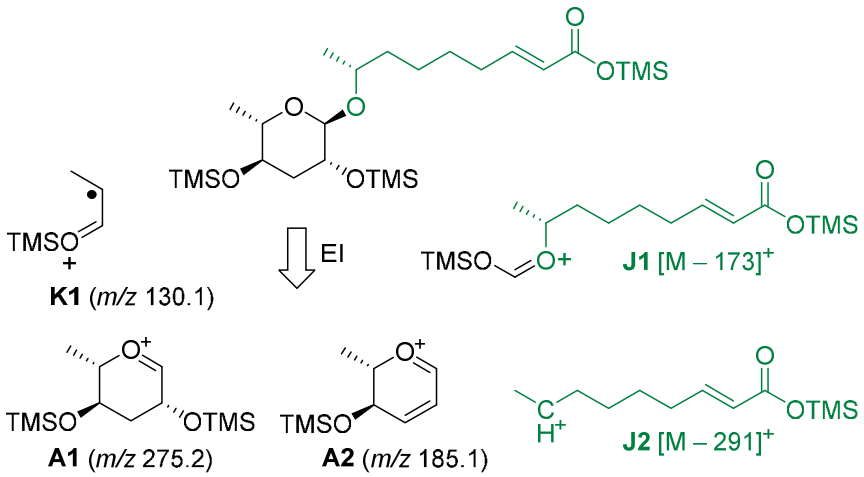
Scheme 1. Ascaroside diversity originating from: (A) side chain modification and shortening via peroxisomal β-oxidation, (B) subsequent attachment of additional metabolic units from primary metabolism, or (C) specific (ω), (ω – 2), or (ω – 3)-hydroxylation downstream of peroxisomal β-oxidation.



Ascaroside signaling is highly conserved in intraspecies nematode communication,[16,25-29] and also involved in inter-genotypic competition[30,31] and cross kingdom interactions,[32-34] demonstrating that ascarosides represent key regulators of nematode chemical ecology. Considering the small amounts of ascarosides present in nematode exometabolomes, the large diversity of homologous structures, and the complexity of the background matrix, mass spectrometric techniques are indispensable for comprehensive ascaroside analysis. Mass

spectrometric screens that employ specific fragment ions as markers are capable to highlight putative nematode-derived ascarosides and their biosynthetic precursors and mask the background matrix. We have previously developed a HPLC-ESI-(-)-MS/MS precursor ion screen that employs an ascarylose-derived fragment ion at *m/z* 73.1 $[C_3H_5O_2]^-$ for the selective detection of known as well as yet unidentified components.[22] ESI-(–)-MS/MS precursor ion screening has been employed in various studies[22,25-27,29,33,34] but ultimately requires a triple quadrupole instrument. Considering the importance of ascaroside signaling in nematode chemical ecology, we recently developed a complementary GC-EIMS technique[35] that employs an ascarylose-derived K1-fragment ion at *m/z* 130.1 $[C_6H_{14}OSi]^{+\bullet}$ along with A1 and A2 fragment ions at *m/z* 275.1 $[C_{12}H_{27}O_3Si_2]^+$ and *m/z* 185.1 $[C_9H_{17}O_2Si]^+$ as characteristic markers to facilitate selective ascaroside profiling in trimethylsilyl (TMS) derivatized crude nematode exometabolome extracts (Scheme 2). Furthermore, aglycone-specific fragment ions for a rearranged oxonium ion (J1) at $[M - 173]^+$ and a carbocation (J2) at $[M - 291]^+$ facilitate the identification of compound specific sidechains. Here we employ the GC-EIMS screen for comparative ascaroside profiling in a variety of *Caenorhabditis* species to demonstrate its potential for the discovery of novel ascaroside components in crude unfractionated nematode exometabolome extracts.

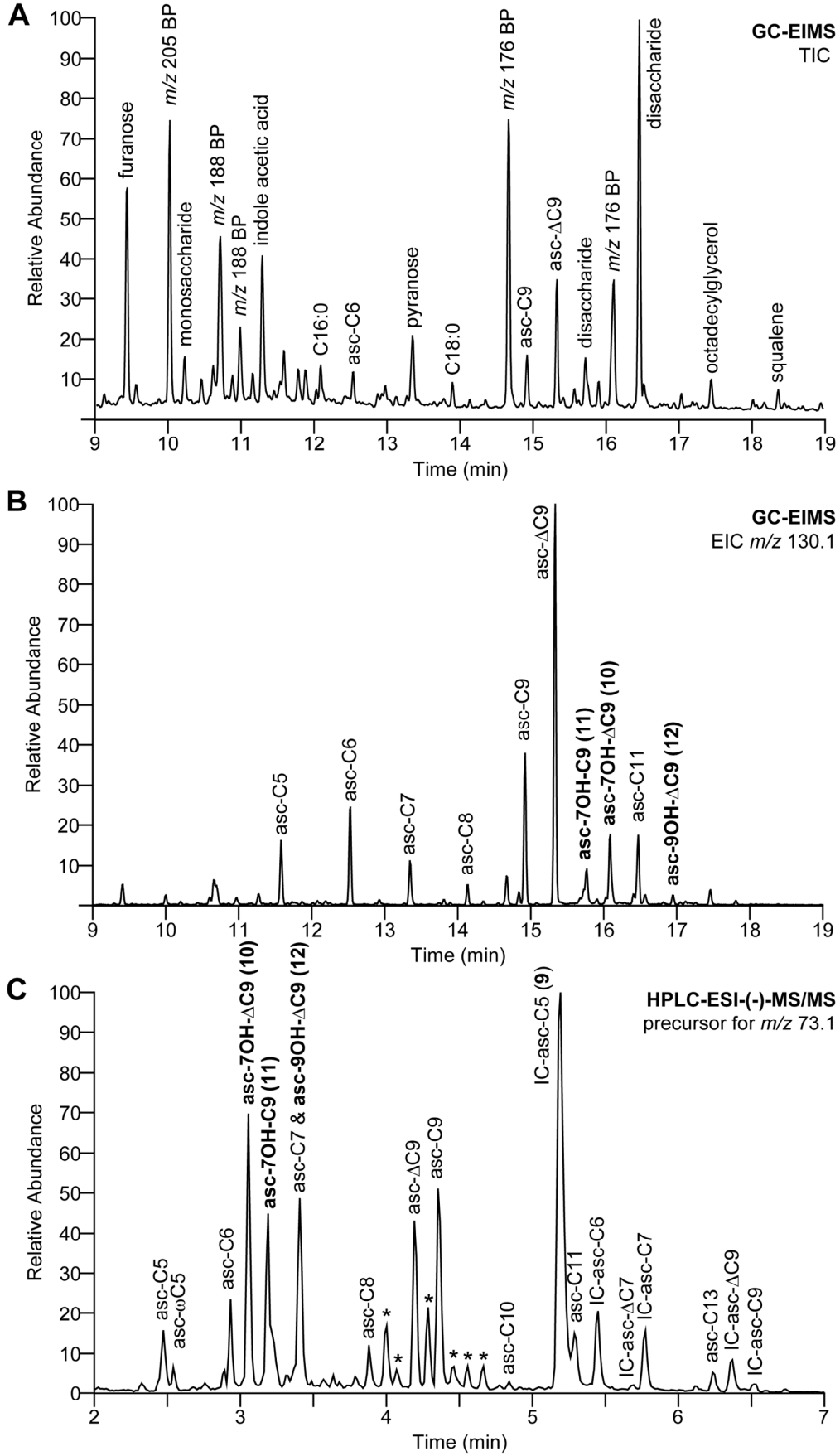
Scheme 2. EI-induced fragmentation of TMS-derivatized ascarosides.



RESULTS AND DISCUSSION

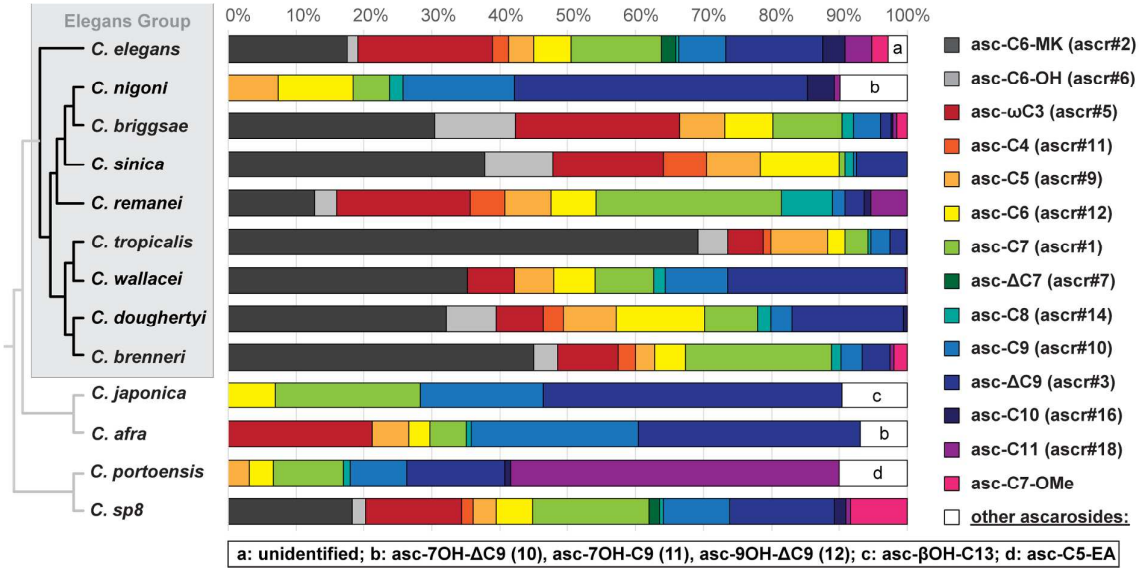
Following a phylometabolomic approach we performed a GC-EIMS-based comparative analysis of exometabolome extracts from 13 *Caenorhabditis* species with a strong focus on the Elegans group that harbors the model organism *C. elegans*.[36,37] Liquid cultures were established in S-medium and propagating nematodes were fed with concentrated *E. coli* OP50 for 7 days, after which cultures were starved for another 7 days.[38] The media supernatant representing the exometabolome was collected, lyophilized, and extracted with methanol. Crude exometabolome extracts were converted into their TMS derivatives using *N*-Methyl-*N*-(trimethylsilyl) trifluoroacetamide (MSTFA) and subsequently analyzed by GC-EIMS to show a large diversity of primary and secondary metabolites, some of which could be tentatively identified using the NIST 14 mass spectral library (Figure 1A). Putative ascarosides were detected by inspection of the extracted ion chromatograms for the highly characteristic ascarylose-derived K1 fragment ion at *m/z* 130.1 $[C_6H_{14}OSi]^{+\bullet}$ (Figures 1B and S1). Individual ascaroside structures were identified based on their aglycone-specific J1 $[M - 173]^+$ and J2 $[M - 291]^+$ fragment ion signals and Kovats retention indices by comparison with a collection of more than 200 components that carry (ω – 1)- or (ω)-linked acyl (**1**), enoyl (**2**), (3*R*)-hydroxyacyl (**3**), 2-ketoalkyl (**6**), or 2-hydroxyalkyl (**7**) sidechains previously characterized in *C. elegans* wild-type and peroxisomal β-oxidation mutants.[35]

Figure 1: Ascaroside profiling of the *C. nigoni* exometabolome. (A) Total ion chromatogram (TIC) of the TMS-derivatized *C. nigoni* crude exometabolome; (B) Extracted ion chromatogram (EIC) for the K1 fragment at *m/z* 130.1 $[C_6H_{14}OSi]^{+\bullet}$; (C) HPLC-ESI-MS/MS precursor ion screen for *m/z* 73.1 $[C_3H_5O_2]^-$.


A
GC-EIMS
TIC
Relative Abundance
Time (min)
furanose
monosaccharide
m/z 205 BP
m/z 188 BP
m/z 188 BP
indole acetic acid
C16:0
asc-C6
pyranose
C18:0
m/z 176 BP
asc-C9
asc-ΔC9
disaccharide
m/z 176 BP
disaccharide
octadecylglycerol
squalene
B
GC-EIMS
EIC m/z 130.1
asc-C5
asc-C6
asc-C7
asc-C8
asc-C9
asc-ΔC9
asc-7OH-C9 (11)
asc-7OH-ΔC9 (10)
asc-C11
asc-9OH-ΔC9 (12)
C
HPLC-ESI-(-)-MS/MS
precursor for m/z 73.1
asc-C5
asc-ωC5
asc-C6
asc-7OH-ΔC9 (10)
asc-7OH-C9 (11)
asc-C7 & asc-9OH-ΔC9 (12)
asc-C8
asc-ΔC9
asc-C9
asc-C10
IC-asc-C5 (9)
asc-C11
IC-asc-C6
IC-asc-ΔC7
IC-asc-C7
asc-C13
IC-asc-ΔC9
IC-asc-C9

These analyses demonstrate that basic ascarosides with sidechains ranging from 3 to 11 carbons are highly conserved in all *Caenorhabditis* wild-type isolates tested, although their relative compositions vary significantly between the different species (Figure 2). A set of five compounds, asc-C7 (**1**, n = 2, X = OH; ascr#1, daumone#1),[2] asc-C9 (**1**, n = 4, X = OH; ascr#10),[12] asc-ΔC9 (**2**, n = 4, X = OH; ascr#3, daumone#3),[3] asc-C6-MK (**6**, n = 2; ascr#2, daumone#2),[3] and asc-ωC3 (**8**, asc#5)[4] consistently represent the dominating components, and, taken together, account for more than 60% of the total ascarosides identified. An exception is the *C. portoensis* metabolome that predominantly contains asc-C11 (**1**, n = 6, X = OH; ascr#18). Furthermore, asc-C6-MK (**6**, n = 2; ascr#2) is absent in *C. nigoni*, *C. japonica*, *C. afra*, and *C. portoensis*, whereas asc-ωC3 (**8**, asc#5) is absent in *C. nigoni*, *C. japonica*, and *C. portoensis*. Asc-C6-OH (**7**, n = 2; ascr#6) was exclusively detected alongside asc-C6-MK (**6**, n = 2; ascr#2) supporting the assumption of a common biosynthetic origin (Scheme 1). While these basic ascarosides are common in most *Caenorhabditis* species tested, GC-EIMS based ascaroside profiling also revealed some highly species-specific components including a yet unidentified component with a J2 fragment at *m/z* 186.1 from *C. elegans*, asc-βOH-C13 (**3**, n = 8, X = OH; bhas#22) from *C. japonica*, and asc-C5-EA (**1**, n = 0, X = $NHCH_2CH_2OH$; easc#9) from *C. portoensis*.[35]

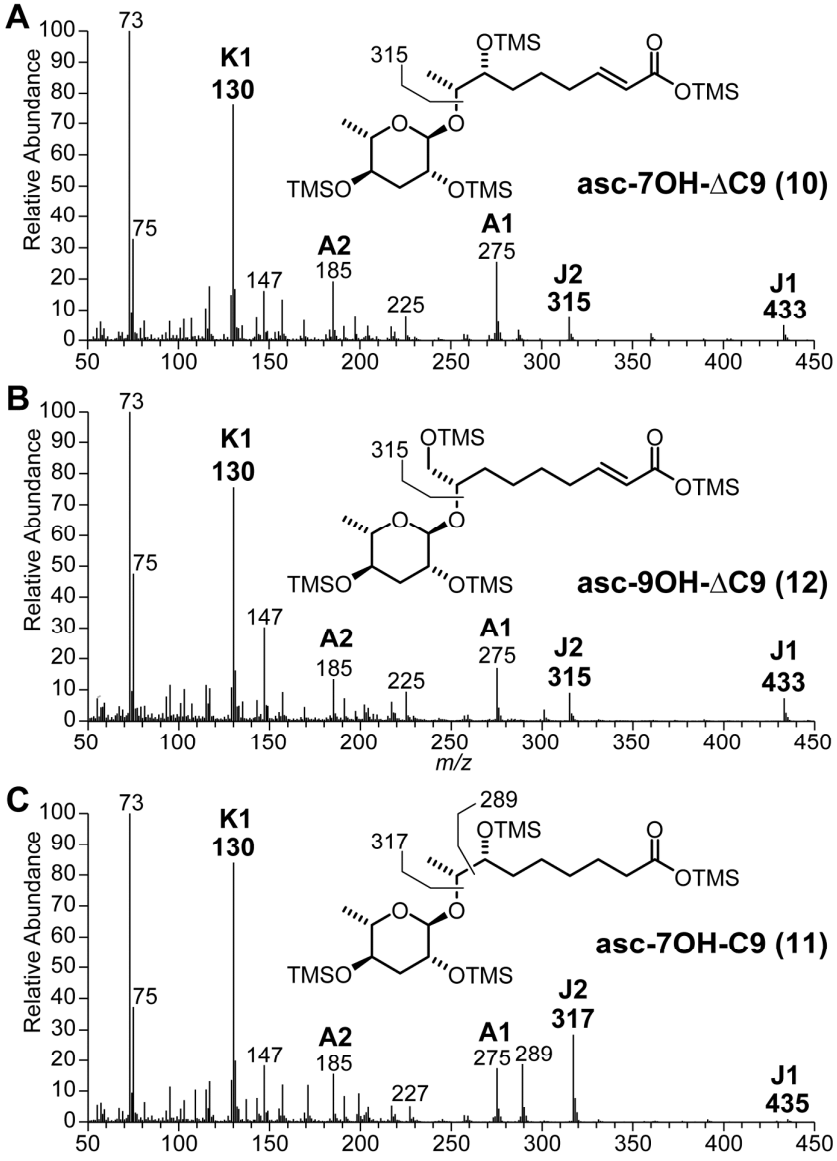
Figure 2: Comparative GC-EIMS ascaroside profiling of 13 *Caenorhabditis* exometabolomes.



In addition, we observed three putative ascarosides that accounted for around 10% of the total ascarosides detected in the exometabolome of *C. nigoni* (Figures 2 and 1B) but did not match any of the more than 200 basic ascaroside structures previously identified in *C. elegans* wild-type and mutant metabolomes.[35] Inspection of their 70 eV EIMS spectra (Figure 3) revealed dominating K1-fragment ion signals at *m/z* 130.1 $[C_6H_{14}OSi]^{+\bullet}$, along with A1-fragments at *m/z* 275.2 $[C_{12}H_{27}O_3Si_2]^+$, and A2-fragments at *m/z* 185.1 $[C_9H_{17}O_2Si]^+$ that are characteristic for the ascarylose unit (Scheme 2). Several aglycone-specific signals for J1 fragment ions at *m/z* 433.2 $[C_{19}H_{41}O_5Si_3]^+$, J2 fragment ions at *m/z* 315.2 $[C_{15}H_{31}O_3Si_2]^+$, and (J2 – TMSOH) fragment ions at *m/z* 225.1 $[C_{12}H_{21}O_2Si]^+$ indicated nine carbon side chains with one additional unit of unsaturation and one trimethylsilyloxy moiety for compounds **10** and **12**. The third compound **11** displayed diagnostic signals for a J1 fragment at *m/z* 435.3 $[C_{19}H_{43}O_5Si_3]^+$, a J2 fragment at *m/z* 317.2 $[C_{15}H_{33}O_3Si_2]^+$, and a (J2 – TMSOH) fragment at *m/z* 227.1 $[C_{12}H_{23}O_2Si]^+$ indicative for a trimethylsilyloxy-substituted nine carbon side chain. Comparison with the known (3*R*)-hydroxylated asc-βOH-C9 (**3**, n = 4, X = OH; bhas#10),

previously characterized in the exometabolome of *Panagrellus redivivus*,[26,35] demonstrated that both compounds are different (Figure S2) and excluded a β-oxidation-derived 3-hydroxyacyl aglycone due to the lack of the characteristic fragment ion at *m/z* 233.1 $[C_9H_{21}O_3Si_2]^+$ derived from α-cleavage. However, the identification of a homologous fragment ion signal at *m/z* 289.2 $[C_{13}H_{29}O_3Si_2]^+$ suggested a 7-hydroxyacyl structure for asc-7OH-C9 (**11**) from *C. nigoni*, demonstrating how EIMS fragmentation can aid in structure assignment.

Figure 3: GC-EIMS spectra of (ω) and (ω – 2) hydroxyacyl ascarosides from *C. nigoni.*



In conclusion, comparative GC-EIMS-based ascaroside screening revealed three species-specific, side chain hydroxylated compounds in the *C. nigoni* exometabolome. The same components with molecular ion signals at *m/z* 317.2 $[M - H]^-$ for **10** and **12** and *m/z* 319.2 $[M - H]^-$ for **11** were also detected using the HPLC-ESI-(–)-MS/MS precursor ion screen for *m/z* 73.1 $[C_3H_5O_2]^-$ (Figure 1C), demonstrating that the GC-EIMS and HPLC-MS/MS methods complement each other. However, in contrast to GC-EIMS, the MS/MS precursor ion screen also revealed additional derivatives including large amounts of indole ascarosides such as IC-

asc-C5 (**9**, icas#9), a male attractant in *C. nigoni*,[16] demonstrating that GC-EIMS is restricted to the most basic ascaroside compounds.

To identify the molecular structures of the species-specific ascarosides (**10-12**), the exometabolome extract of 1.6 L *C. nigoni* liquid culture supernatant was fractionated by solid phase extraction (RP-C_{18}-SPE) using a 10%-stepwise gradient of aqueous methanol as eluent. Fractions were screened for ascarosides by GC-EIMS (Figure S3) and ^{1}H NMR spectroscopy (Figure S4) using the K1 fragment ion at *m/z* 130.1 $[C_6H_{14}OSi]^{+\bullet}$ and the anomeric proton at approximately δ_H 4.65 ppm (s, 1H) as characteristic markers, respectively. These analyses confirmed the assignment of several known ascarosides and traced the target compounds to a fraction eluted with 40% methanol (Figure S3) that contained predominantly asc-C7 (**1**, n = 2 , X = OH; ascr#1) along with a diversity of additional metabolites such as indole acetic acid (IAA, auxine) and anthranilic acid (Figure S5). Subsequent separation by semipreparative HPLC using a C18 column furnished fractions of sufficient purity to facilitate structure assignment.

Table 1. NMR data for (ω) and (ω – 2)-hydroxyacyl ascarosides (**10-12**) isolated from *C. nigoni* (400 MHz, CD_3OD).

	***threo*-asc-7OH-ΔC9 (10)**			***threo*-asc-7OH-C9 (11)**			**asc-9OH-ΔC9 (12)**		
Position	δ_C[a]		δ_H[b], mult (*J*, Hz)	δ_C[a]		δ_H[b], mult (*J*, Hz)	δ_C[a]		δ_H[b], mult (*J*, Hz)
1	*nd*	C	-	*nd*	C	-	*nd*	C	-
2	124.8	CH	5.83 d (15.5)	37.5	CH_2	2.21 t (7.6)	122.9	CH	5.81 d (15.6)
3	148.1	CH	6.86 dt (15.5, 7.0)	26.9	CH_2	1.63 m	150.7	CH	6.95 dt (15.6, 6.9)

4	32.7	CH_2	2.25 m	30.4	CH_2	1.40 m	33.1	CH_2	2.25 m
5	25.6	CH_2	1.54-1.68 m	27.0	CH_2	1.40 m	29.3	CH_2	1.52 m
6	32.8	CH_2	1.48-1.62 m	32.9	CH_2	1.48-1.53 m	26.3	CH_2	1.50 m
7	74.8	CH	3.53 m	75.2	CH	3.52 m	32.8	CH_2	1.59 m
8	75.5	CH	3.74 dq (3.9, 6.3)	75.6	CH	3.73 dq (3.8, 6.2)	78.6	CH	3.69 m
9	14.5	CH_3	1.14 d (6.3)	14.6	CH_3	1.14 d (6.2)	64.6	CH_2	3.50 dd (11.7, 5.6) 3.60 dd (11.7, 4.2)
1’	97.9	CH	4.65 s	97.8	CH	4.65 s	99.8	CH	4.75 s
2’	69.7	CH	3.76 s.br	69.8	CH	3.76 s.br	69.6	CH	3.84 s.br
$3'_{ax}$ $3'_{eq}$	35.8	CH_2	1.95 dt (13.0, 3.8) 1.80 ddd (13.0, 11.4, 3.0)	35.8	CH_2	1.95 dt (13.0, 3.8) 1.81 ddd (13.0, 11.4, 3.0)	35.9	CH_2	1.95 dt (13.0, 3.8) 1.78 ddd (13.0, 11.2, 3.1)
4’	68.2	CH	3.52 ddd (11.3, 9.3, 4.3)	68.4	CH	3.51 ddd (11.4, 9.5, 4.4)	68.4	CH	3.53 m
5’	71.3	CH	3.64 dq (9.3, 6.3)	71.4	CH	3.66 dq (9.5, 6.3)	71.4	CH	3.67 m
6’	17.8	CH_3	1.22 d (6.2)	17.8	CH_3	1.22 d (6.2)	18.1	CH_3	1.22 d (6.1)

a from HSQC spectrum. b from ^{1}H NMR and *dqf*-COSY spectra.

The molecular formula of $C_{15}H_{26}O_7$ for compound **10** (~275 µg) was established by HR-EIMS. Inspection of one- and two-dimensional NMR spectra (^{1}H NMR, *dqf*-COSY, HSQC) confirmed an α-configured ascarylose moiety along with an (ω – 1)-linked α,β-unsaturated C9 side chain (Table 1). Furthermore, the (ω – 2)-position of the additional hydroxy group was deduced based on *dqf*-COSY correlations from the terminal (ω)-methyl group at δ_H 1.14 ppm (d, J = 6.3 Hz, 3H), δ_C 14.5 ppm to the (ω – 1)-oxymethine group at δ_H 3.74 ppm (dq, J = 3.9 Hz, J = 6.3 Hz, 1H), δ_C 75.5 ppm and further on to the (ω – 2)-position at δ_H 3.53 ppm (m, 1H), δ_C 74.8 ppm. While all (ω – 1)-linked homologous ascarosides that have been identified

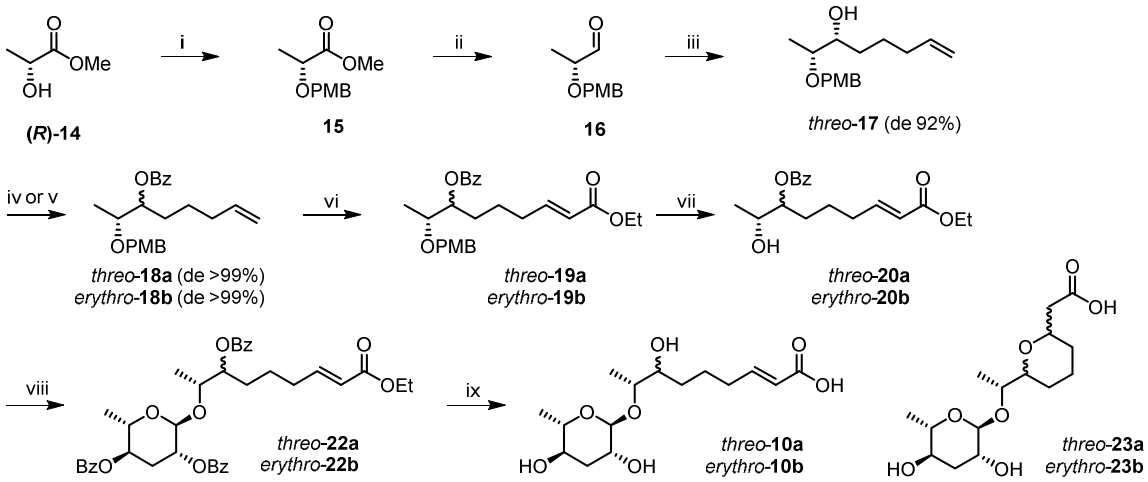
so far share the same (*R*)-configuration at the penultimate carbon,[1] the stereochemistry of the (ω – 2)-hydroxymethine group of asc-7OH-ΔC9 (**10**) could not be unambiguously assigned based solely on the vicinal H,H-coupling constant of 3.9 Hz.[39]

Compound **11** (~110 μg) with a molecular formula of $C_{15}H_{28}O_7$ according to ESI-HRMS exhibits almost identical NMR data for the ascarylose unit and the (ω)-part of the sidechain, but displays a triplet signal at 2.21 (t, J = 7.6 Hz, 2H) instead of the signals for an α,β-unsaturated enoyl moiety (Table 1), thus, suggesting the corresponding dihydro-derivative structure asc-7OH-C9 (**11**). Compound **12** (~130 μg) with a molecular formula of $C_{15}H_{26}O_7$ according to ESI-HRMS was obtained as a 1:1 mixture with asc-7OH-C9 (**11**). Comparative analysis of their *dqf*-COSY spectra indicated an ascarylose unit with considerably different chemical shifts (Table 1). Furthermore, an α,β-unsaturated side chain was identified due to δ_H 5.81 (d, J_E = 15.6 Hz, 1H) and 6.95 (dt, J_E = 15.6 Hz, J = 6.9 Hz, 1H), along with a hydroxymethylene group at δ_H 3.50 (dd, ^2J = 11.7 Hz, ^3J = 5.6 Hz, 1H), 3.60 (dd, ^2J = 11.7 Hz, ^3J = 4.2 Hz, 1H), and δ_C 64.6 ppm, that displayed vicinal H,H-coupling correlation to the (ω – 1)-oxymethine proton at δ_H 3.69 (m, 1H) and δ_C 78.6 ppm, thus indicating the terminal (ω)-position for the hydroxylation of the aglycone in asc-9OH-ΔC9 (**12**). In conclusion, analysis of one and two-dimensional NMR spectra revealed three novel side chain modified ascarosides that carry additional hydroxy functions at the 7-position (ω – 2) and the 9-position (ω), the structures of which were finally established by total synthesis and chemical correlations.

Both diastereomeric (ω – 2)-hydroxy ascarosides, (7*R*,8*R*)-*threo*-**10a** and (7*S*,8*R*)-*erythro*-**10b** were synthesized as shown in Scheme 3. (*R*)-Methyl lactate (**14**) was converted to the *para*-methoxybenzyl (PMB) ether **15** and reduced to the aldehyde **16** using DIBAL-H. Addition of 4-pentenylmagnesium bromide afforded (6*R*,7*R*)-*threo*-6-hydroxy-7-PMB-*O*-1-octene (**17**) with a diastereomeric excess of *de* 92% due to asymmetric induction via a Cram chelate

complex.[40] Esterification of **17** with benzoyl chloride and pyridine or benzoic acid under Mitsunobu conditions (PPh_3, DIAD) afforded the diastereomeric benzoates (6*R,*7*R*)-*threo*-**18a** or (6*S,*7*R*)-*erythro*-**18b**, respectively, in excellent diastereomeric purities of *de* >99% after column chromatography (Figure S6). Cross metathesis with ethyl acrylate using Grubbs 2nd generation catalyst[41] furnished the corresponding ethyl 7-Bz-*O*-8-PMB-*O*-(2*E*)-nonenoates (7*R,*8*R*)-*threo*-**19a** or (7*S,*8*R*)-*erythro*-**19b** that were subsequently deprotected using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)[42] to afford (7*R,*8*R*)-*threo*-**20a** or (7*S,*8*R*)-*erythro*-**20b**. Coupling with 2,4-di-*O*-benzoyl protected ascarylose via the trichloroacetimidate route[43] furnished the diastereomeric ascarosides (**22a** or **22b**) that were finally deprotected using alkaline hydrolysis to give the desired (ω-2)-hydroxy ascarosides (7*R,*8*R*)-*threo*-**10a** or (7*S,*8*R*)-*erythro*-**10b**. In addition, an undesired intramolecular cyclization product (**23a** or **23b**) was obtained. Comparison of the NMR (Figure S7) and GC-EIMS (Figure S8) data of diastereomeric (7*R,*8*R*)-*threo*-**10a** and (7*S,*8*R*)-*erythro*-**10b** with those of the natural product (**10**) isolated from the *C. nigoni* exometabolome confirmed its (7*R,*8*R*)-*threo* configuration. Furthermore, the (7*R,*8*R*)-*threo*-configuration of the dihydro-derivative asc-7OH-C9 (**11**) from *C. nigoni* was unambiguously established by comparison of the mass spectra and GC retention times with those of the hydrogenation products of (7*R,*8*R*)-*threo*-**10a** and (7*S,*8*R*)-*erythro*-**10b** (Figure S9).

Scheme 3: Synthesis of (ω – 2)-hydroxyacyl ascarosides *threo*-asc-7OH-ΔC9 (**10a**) and *erythro*-asc-7OH-ΔC9 (**10b**).

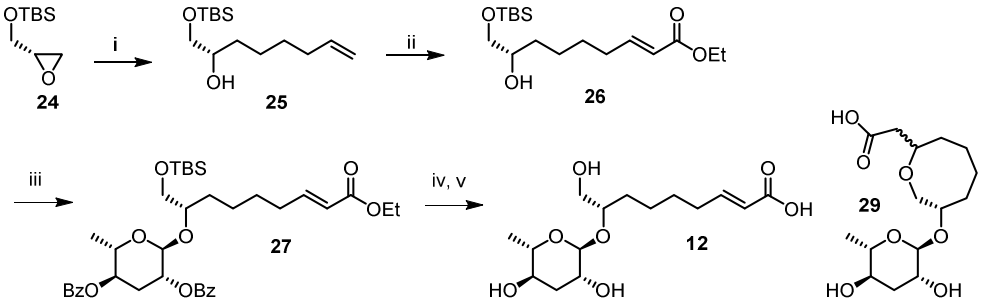


Reagents and conditions: (i) 4-methoxybenzyl trichloroacetimidate, trimethylsilyl triflate, CH_2Cl_2, 0 °C, 3 h, 62%; (ii) DIBAL-H, CH_2Cl_2, -78 °C, 0.5 h, 100%; (iii): 4-pentenylmagnesium bromide, Et_2O, 0 °C, 1 h, 58%; (iv) benzoyl chloride, pyridine, CH_2Cl_2, 0 °C, 12 h, 87% (**18a**); (v) benzoic acid, PPh_3, DIAD, THF, 4 h, 55% (**18b**); (vi): ethyl acrylate, Grubbs 2nd generation catalyst, CH_2Cl_2, 40 °C, 9 h, 76% (**19a**) and 81% (**19b**); (vii): DDQ, CH_2Cl_2/H_2O, 1 h, 70% (**20a**) and 72% (**20b**); (viii): 2,4-di-*O*-benzoyl-ascarosyl-1-(2,2,2-trichloroacetimidate) (**21**), trimethylsilyl triflate, CH_2Cl_2, 0 °C, 3 h, 68% (**22a**) and 64% (**22b**); (ix): lithium hydroxide, water, MeOH, 12 h, 36% (**10a**), 46% (**10b**), 39% (**23a**), and 42% (**23b**).

The (ω)-hydroxylated ascaroside asc-9OH-ΔC9 (**12**) was synthesized as shown in Scheme 4. Copper(I)-catalyzed addition of pentenyl magnesium bromide to *tert*-butyldimethylsilyl (*S*)-glycidyl ether (**24**) afforded (*S*)-8-*tert*-butyldimethylsilyloxy-7-hydroxy-1-octene (**25**). Cross metathesis with ethyl acrylate using Grubbs 2nd generation catalyst[41] gave the corresponding (8*S*,2*E*)-ethyl 8-hydroxy-9-*tert*-butyldimethylsilyloxy-2-nonenoate (**26**). Coupling of **26** to 2,4-di-*O*-benzoyl-ascarosyl trichloroacetimidate (**21**) furnished **27**, which was subsequently

deprotected using tetrabutylammonium fluoride (TBAF) to give the alcohol **28**, followed by alkaline hydrolysis to give asc-9OH-ΔC9 (**12**) identical to the natural product from *C. nigoni* as shown by comparison of the GC-EIMS and NMR data (Figures S8 and S10). In addition, an undesired intramolecular cyclization product **29** with an oxacyclooctan ring was obtained.

Scheme 4. Synthesis of the (ω)-hydroxyacyl ascaroside asc-9OH-ΔC9 (**12**).



Reagents and conditions: (i) 4-pentenylmagnesium bromide, copper(I)iodide, THF, 0 °C, 3 h, 94%; (ii) ethyl acrylate, Grubb's 2nd generation catalyst, CH_2Cl_2, 40 °C, 9 h, 81%; (iii) 2,4-di-*O*-benzoylascarosyl-1-(2,2,2-trichloroacetimidate) (**21**), trimethylsilyl triflate, CH_2Cl_2, 0 °C, 3 h, 53%; (iv) tetrabutylammonium fluoride, THF, 3 h, 78%; (v) lithium hydroxide, water, MeOH, 12 h, 25% (**12**) and 69% (**29**).

Using the synthetic compounds as reference standards, *threo*-asc-7OH-ΔC9 (**10a**), *threo*-asc-7OH-C9 (**11a**), and asc-9OH-ΔC9 (**12**) could also be identified in the exometabolome extract of *Caenorhabditis afra* (sp.7) strain JU1286 from Ghana (Figures S11-12), a member of the distantly related Japonica group (Figure 2)[36,37] suggesting that (ω) and (ω – 2)-hydroxylation in *C. nigoni* and *C. afra* has a polyphyletic origin. Targeted screening for homologous compounds using GC-EIMS and HPLC-MS demonstrated that (ω) and (ω – 2)-hydroxylation in *C. nigoni* and *C. afra* is tightly controlled and exclusively affects C9 and ΔC9 aglycones,

indicating that this species-specific modification of ascaroside aglycones occurs downstream of peroxisomal β-oxidation.

Aiming to characterize the biological functions of the novel ascarosides, behavioral response of *C. nigoni* males or females to 1 μM asc-(7*R*)-OH-ΔC9 (**10a**), asc-(7*S*)-OH-ΔC9 (**10b**), asc-9OH-ΔC9 (**12**), as well as asc-ΔC9 (**2**, n = 4, X = OH; ascr#3) was evaluated using a spot attraction assay. While these analyses demonstrate that the common asc-ΔC9 (**2**, n = 4, X = OH; ascr#3) acts as a potent male attractant in *C. nigoni*, reminiscent of the attraction of *C. elegans* males,[10] the species-specific (ω – 2) and (ω)-hydroxylated derivatives (**10a**, **10b**, and **12**) are not active (Figure S13). Additional experiments will be required to clarify the biological functions of these compounds and unravel the ecological significance of species-specific (ω) and (ω – 2)-hydroxylation in *C. nigoni* and *C. afra.*

CONCLUSION

Our results demonstrate that comparative GC-EIMS-based ascaroside profiling represents a powerful technique to characterize ascaroside diversity and detect novel species-specific components in TMS-derivatized crude nematode exometabolomes. We have isolated three new (ω) and (ω – 2)-hydroxylated ascarosides from *C. nigoni* and determined their structures using NMR spectroscopy. Structure assignments were unambiguously established by total synthesis and chemical correlations. Comparative analysis of 13 *Caenorhabditis* species demonstrates that within the Elegans group both (ω)- and (ω – 2)-hydroxylated ascarosides (**10-12**) are highly specific for *C. nigoni* (sp.9), but the same compounds were also detected in the rather distantly related *C. afra* (sp.7), a member of the Japonica group, thus, suggesting a polyphyletic origin for the hydroxylation steps. In addition, we found that (ω)- and (ω – 2)-hydroxylation in *C. nigoni* and *C. afra* is highly specific for ascarosides carrying C9 and ΔC9

sidechains, strongly suggesting that the hydroxylation step occurs downstream of peroxisomal β-oxidation. While traces of (3*R*)-hydroxyacyl ascarosides, intermediates of the peroxisomal β-oxidation cycle, are widespread in nematode exometabolomes, sidechain hydroxylation downstream of β-oxidation such as (ω)- and (ω – 2)-hydroxylation in *C. nigoni* and *C. afra* has so far only been described as male-specific (ω – 3)-hydroxylation in *Panagrellus redivivus* (Scheme 1C).[26] However, while the dihydroxylated dhas#18 (**13**) represents a male-produced female-attractant in *Panagrellus redivivus*,[26] the hydroxy ascarosides (**10**-**12**) did not attract *C. nigoni* males or females. Additional research will be required to elucidate the biological functions of these species-specific components and decipher the ecological significance of (ω)- and (ω – 2)-hydroxylation of ascaroside aglycones as a means to further increase structural diversity of this highly conserved class of nematode signaling molecules.

EXPERIMENTAL SECTION

Preparation of exometabolome extracts. Wild-type isolates of thirteen *Caenorhabditis* species were cultivated at 23 °C on NGM agar seeded with *E. coli* OP50: *C. elegans* N2 (Bristol), *C. nigoni* (sp.9) JU1422, *C. briggsae* AF16, *C. sinica* (sp.5) JU727, *C. remanei* PB4641, *C. tropicalis* (sp.11) JU1373, *C. wallacei* (sp.16) JU1904, *C. doughertyi* (sp.10) JU1771, *C. brenneri* (sp.4) PB2801, *C. japonica* DF5081, *C. afra* (sp.7) JU1286, *C. portoensis* (sp.6) EG4788, and *C. n. sp. 8* (sp.8) QX1182. Mixed stage nematodes from five 10 cm plates collected in M9 buffer served as inoculums for liquid cultures grown in 100 ml S-medium at 23 °C and 150 rpm. Concentrated *E. coli* OP50 bacteria pellet from an overnight culture in LB medium at 37 °C and 170 rpm was provided as food from day 1 to day 7, after which the cultures were starved for 7 days. After 14 days, nematodes were separated by centrifugation (5 min at 5000 g). The filtered supernatant representing the exometabolome

was frozen at -80 °C, lyophilized, and extracted with 3 x 100 ml methanol for 12 h each. The combined extract was filtered, concentrated to dryness at 40 °C under reduced pressure, reconstituted in 1 ml methanol, and aliquots were analyzed by HPLC-HRMS, HPLC-MS/MS precursor ion screening for *m/z* 73.1, as well as GC-EIMS. All experiments were performed in triplicate.

Preparation of Trimethylsilyl (TMS) Derivatives for GC-EIMS Analysis. Aliquots of crude nematode exometabolome extracts, *C. nigoni* exometabolome fractions, and synthetic ascaroside standards were concentrated to dryness. The residues were treated with 10 µl N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) at 60 °C for 40 minutes, diluted with 10 µl DCM, and 1 µl of the solution analyzed by GC-EIMS.

Gas Chromatography-Electron Impact-Mass Spectrometry (GC-EIMS). Separation of volatile TMS derivatives and acquisition of their 70 eV electron impact mass spectra was performed using a Trace GC 2000 series (Thermo Scientific) equipped with a Zebron ZB-5 Guardian column (15 m, 0.25 mm ID, 0.25 µm film thickness; with 10 m guardian end) coupled to a single quadrupole ThermoQuest Trace MS (Finnigan). Helium was used as the carrier gas at a flow rate of 1 ml/min. A temperature program starting at 130 °C for 5 min, followed by a linear gradient of +10 °C/min to 350 °C was applied. A total volume of 1 µl was injected using a 10:1 split ratio and an injector temperature of 250 °C. Electron ionization (EI, 70 eV) mass spectra were acquired from *m/z* 35–650 amu. Data were analyzed with the Xcalibur 3.1 software (Thermo Fisher Scientific).

Liquid Chromatography-Electrospray Ionization-High Resolution-Mass Spectrometry (HPLC-ESI-HRMS). HPLC-ESI-HRMS analysis of crude nematode exometabolome extracts and *C. nigoni* exometabolome fractions was performed using a Dionex UltiMate 3000 HPLC instrument coupled to a Bruker Maxis ultrahigh resolution (UHR) qTOF mass

spectrometer equipped with an electrospray ionization (ESI) unit operated in positive or negative mode. Chromatographic separations were achieved using an Agilent ZORBAX Eclipse XDB-C18 column (250 x 3 mm, 5 µm particle diameter) with a flow rate of 400 µl/min and gradient elution starting at 3% acetonitrile in 0.5% aqueous acetic acid for 5 minutes followed by a linear increase to 100% acetonitrile with 0.5% acetic acid within 35 minutes. Data were analyzed with the Compass DataAnalysis 4.3 software (Bruker).

Liquid Chromatography Electrospray Ionization Precursor Ion Screening. HPLC-MS/MS precursor ion screening for *m/z* 73.1 was performed using an Agilent 1260 HPLC instrument (Agilent Technologies) coupled to an API5000 Triple Quadrupole LC/MS/MS mass spectrometer (AB Sciex, Darmstadt) equipped with an electrospray ionization (ESI) unit operated in negative mode. A CID energy of -34 was applied. Chromatographic separations were achieved using an Agilent ZORBAX Eclipse XDB-C18 column (50 x 4.6 mm, 1.8 µm particle diameter) (Agilent Technologies) with a flow rate of 1.1 ml/min and gradient elution starting at 5% acetonitrile in 0.05% aqueous formic acid followed by a linear in-crease to 95% acetonitrile with 0.05% formic acid within 10 minutes. Data were analyzed with the Analyst 1.6 software (AB Sciex).

NMR spectroscopy. NMR spectra were recorded in CD_3OD or $CDCl_3$ at 400 MHz for ^1H and 100 MHz for ^{13}C using a Bruker AMX400 instrument. Residual solvent signals were used as internal standard with ^1H at 3.31 ppm and ^{13}C at 49.05 ppm for CD_3OD or ^1H at 7.26 ppm and ^{13}C at 77.16 ppm for $CDCl_3$. Two-dimensional homonuclear double quantum filtered (*dqf*)-COSY spectra were recorded using phase cycling for coherence selection. For the isolated compounds a total of 32 scans were acquired using a time domain of 8k in F2 (acquisition time of 1.2 s) and 512 increments in F1. For two dimensional heteronuclear HSQC spectra 96 scans were acquired using a time domain of 1k in F2 and 256 increments in F1. Spectra were zero-filled to 8k x 4k (COSY) or 4k x 2k (HSQC) prior to Fourier

transformation, phased manually, and baseline corrected using the Topspin 3.2 (Bruker) and MNova 9.0 (Mestrelab Research) software.

Spot Retention Assay. Assays were performed as described previously.[10,44] 50-60 larval-stage 4 (L4) worms were segregated by sex and stored at 20 °C for 5 hours to overnight to be assayed as young adults. 0.6 µL of vehicle control or ascaroside solution was placed in each scoring region. As the working stock of ascaroside was made in MilliQ-purified ultrapure H_2O, this was used as the vehicle control. Five animals were placed on each "X" of the assay plate, which was then transferred to a microscope containing a camera and recorded for 20 minutes. Each sex and compound was assayed over three plates per day on at least three different days.

Isolation of hydroxy ascarosides from the *C. nigoni* exometabolome. Hydroxyacyl ascarosides of *C. nigoni* were isolated from 1.6 L of the liquid culture supernatant. The filtered supernatant was frozen at -80 °C, lyophilized, and the residue extracted with 3 x 100 ml methanol for 12 h each. The filtered extract was concentrated to dryness under reduced pressure and the resulting *C. nigoni* exometabolome extract was adsorbed onto 2 g of celite and fractionated by reverse phase chromatography on 5 g RP-C_{18}-SPE cartridges (Chromabond, Macherey-Nagel) using increasing concentrations of methanol in water as eluent to afford 10 fractions (20 ml each). Aliquots of 10 µl were concentrated to dryness under reduced pressure, treated with 10 µl MSTFA at 60 °C for 30 min, diluted with 10 µl DCM, and analyzed by GC-EIMS (Figure S3). Fractions were concentrated to dryness under reduced pressure and analyzed by ^{1}H NMR spectroscopy (Figure S4). The 40% methanol fraction containing the target components according to GC-EIMS was subsequently submitted to semi-preparative HPLC using an Agilent HP-1100 HPLC instrument equipped with a Grom-Sil 120 ODS-4 HE column (250 x 8 mm, 5 µm) coupled to a Gilson 206 Abimed fraction collector. A flow rate of 2 ml/min with gradient elution was used starting at 3%

acetonitrile in 0.5% aqueous acetic acid for 3 minutes, followed by a linear increase to 100% acetonitrile with 0.5% acetic acid within 30 minutes. Aliquots of 10 µl were analyzed by GC-EIMS and HPLC-ESI-(-)-HR-MS as described before. Fractions containing the target compounds were concentrated to dryness, dissolved in 650 µl CD_3OD, and analyzed by one- and two-dimensional NMR spectroscopy.

(7*R*,8*R*,2*E*)-*threo*-8-[(3',6'-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-7-hydroxy-2-nonenoic acid (*threo*-asc-7OH-ΔC9, **10**) isolated from the *C. nigoni* exometabolome (275 µg, c = ~720 nmol/L), for ^{1}H and ^{13}C NMR data see table 1; HRMS (ESI-TOF) *m/z* $(M - H)^-$ Calcd for $C_{15}H_{25}O_7$ 317.1606, found 317.1619.

(7*R*,8*R*)-*threo*-8-[(3',6'-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-7-hydroxynonanoic acid (*threo*-asc-7OH-C9, **11**) isolated from the *C. nigoni* exometabolome (110 µg, ~285 nmol/L), for ^{1}H and ^{13}C NMR data see table 1; HRMS (ESI-TOF) *m/z* $(M - H)^-$ Calcd for $C_{15}H_{27}O_7$ 319.1762, found 319.1771.

(2*E*,8*S*)-8-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-9-hydroxy-2-nonenoic acid (asc-9OH-ΔC9, **12**) isolated from the *C. nigoni* exometabolome (130 µg, ~340 nmol/L), for ^{1}H and ^{13}C NMR data see table 1; HRMS (ESI-TOF) *m/z* $(M - H)^-$ Calcd for $C_{15}H_{25}O_7$ 317.1606, found 317.1617.

(*R*)-Methyl 2-(4-methoxybenzyloxy)propanoate (15).[45] Under argon atmosphere a solution of (*R*)-(+)-methyl 2-hydroxypropanoate (**14**) (1.04 g, 10 mmol) and 4-methoxybenzyl 2,2,2-trichloroacetimidate (2.8 g, 10 mmol) in dry DCM (15 ml) at 0 °C was treated with trimethylsilyl triflate (10 µl). After stirring at 0 °C for 3 h the reaction was quenched by addition of saturated $NaHCO_3$ solution (1 ml), the mixture was diluted with DCM (15 ml), and washed with saturated $NaHCO_3$ solution (2 x 10 ml), dried over Na_2SO_4, and concentrated under reduced pressure. The product was isolated by column chromatography

(silica gel, 9:1 v/v hexane/ethyl acetate elution, R_f = 0.22) to afford **15** (1.38 g, 6.2 mmol, 62%) as a yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.28 (d, J = 8.7 Hz, 2H), 6.87 (d, J = 8.7 Hz, 2H), 4.61 (d, J = 11.3 Hz, 1H), 4.38 (d, J = 11.3 Hz, 1H), 4.05 (q, J = 6.8 Hz, 1H), 3.79 (s, 3H), 3.75 (s, 3H), δ 1.41 (d, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 159.5, 129.72, 129.70, 113.9, 73.7, 71.8, 55.4, 52.0, 18.8.[45]

(*R*)-2-(4-Methoxybenzyloxy)propanal (16).[45] Under argon atmosphere a solution of **15** (672 mg, 3 mmol) in dry DCM (10 ml) at -78 °C was treated dropwise with a 1 M DIBAL-H solution (3.3 ml, 3.3 mmol) in toluene. After stirring at -78 °C for 30 minutes the reaction was quenched with methanol (0.5 ml) and saturated sodium potassium tartrate solution (10 ml) and stirred for 1 h and extracted with DCM (2 x 20 ml). The combined organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography (silica gel, DCM elution) afforded **16** (580 mg, 3 mmol, 100% yield) as a colorless oil that was directly used for the next step. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.63 (d, J = 1.8 Hz, 1H), 7.29 (d, J = 8.7 Hz, 2H), 6.89 (d, J = 8.7 Hz, 2H), 4.57 (d, J =11.4 Hz, 1H), 4.54 (d, J = 11.4 Hz, 1H), 3.87 (dq, J = 1.8 Hz, J = 7.0 Hz, 1H), 3.80 (s, 3H), 1.31 (d, J = 7.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 203.7, 159.7, 129.8, 129.5, 114.1, 79.3, 71.9, 55.4, 15.4.[45]

(2*R*,3*R*)-*threo*-3-Hydroxy-2-(4-methoxybenzyloxy)-7-octene (17). Under argon atmosphere a solution of 4-pentenylmagnesium bromide (6 mmol) in diethyl ether (10 ml), prepared from 5-bromo-1-pentene (900 mg, 6 mmol) and magnesium (150 mg, 6.2 mmol), was cooled to 0 °C and treated dropwise with **16** (580 mg, 3 mmol) in Et_2O (2 ml) over the course of 5 min. The resulting mixture was stirred at 0 °C for 1 h, quenched with saturated aqueous NH_4Cl solution (10 ml) and the aqueous layer extracted with ethyl acetate (2 x 10 ml). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The product was isolated by column chromatography (silica gel, 4:1 v/v hexane/ethyl acetate elution, R_f= 0.42) to afford **17** (460 mg, 1.74 mmol, 58% yield) with a diastereoisomeric excess of *de* =

92% as determined by ^{1}H NMR spectroscopy. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 (d, J = 8.6 Hz, 2H), 6.88 (d, J = 8.6 Hz, 2H), 5.80 (ddt, J = 17.2 Hz, J = 10.2 Hz, J = 6.7 Hz, 1H), 5.00 (dbr, J = 17.2 Hz, 1H), 4.94 (dbr, J = 10.3 Hz, 1H), 4.60 (d, ^2J = 11.1 Hz, 1H), 4.36 (d, ^2J = 11.1 Hz, 1H), 3.80 (s, 3H), 3.40 (m, 1H), 3.34 (dq, J = 6.0, J = 6.3 Hz, 1H), 2.09 (m, 2H), 1.62 (m, 1H), 1.47 (m, 1H), 1.44 (m, 2H), 1.17 (d, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 159.4, 138.9, 130.6, 129.5, 114.6, 114.0, 78.2, 74.9, 70.8, 55.3, 33.8, 32.4, 24.9, 15.7.

(2*R*,3*R*)-*threo*-3-Benzoyloxy-2-(4-methoxybenzyloxy)-7-octene (18a). A solution of **17** (316.5 mg, 1.2 mmol) and dry pyridine (290 µl, 3.6 mmol) in dry DCM (2 ml) at 0 °C was treated with benzoylchloride (280 µl, 2.4 mmol) in dry DCM (1 ml). After stirring at RT for 12 h the mixture was diluted with DCM (10 ml), washed with 1 M HCl (10 ml), saturated aqueous $NaHCO_3$ solution (10 ml), dried over Na_2SO_4, and concentrated under reduced pressure. Column chromatography of the residue (silica gel, 9:1 v/v hexane/ethyl acetate elution, R_f = 0.37) afforded (2*R*,3*R*)-*threo*-**18a** (386 mg, 1.05 mmol, 87% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 7.6 Hz, 2H), 7.59 (m, 1H), 7.47 (m, 2H), 7.26 (d, J = 8.5 Hz, 2H), 6.86 (d, J = 8.5 Hz, 2H), 5.79 (ddt, J = 17.0 Hz, J = 10.3 Hz, J = 6.7 Hz, 1H), 5.25 (m, 1H), 5.01 (d, J = 17.1 Hz, 1H), 4.96 (d, J = 10.3 Hz, 1H), 4.62 (d, J = 11.5 Hz, 1H), 4.49 (d, J =11.5 Hz, 1H), 3.81 (s, 3H), 3.74 (dq, J = 4.9 Hz, J = 6.3 Hz, 1H), 2.10 (m, 2H), 1.77 (m, 2H), 1.46 (m, 2H), 1.23 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.4, 159.3, 138.5, 133.0, 130.7, 129.8, 129.4, 129.0, 128.5, 114.9, 113.9, 76.1, 74.6, 70.9, 55.4, 33.7, 29.0, 25.0, 15.5.

(2*R*,3*S*)-*erythro*-3-Benzoyloxy-2-(4-methoxybenzyloxy)-7-octene (18b). Under argon atmosphere a solution of **17** (294.5 mg, 1.11 mmol), triphenylphosphine (668.8 mg, 2.55 mmol), and benzoic acid (300.4 mg, 2.46 µmol) in dry THF (6 ml) at 0 °C was treated with 440 µl diisopropyl azodicarboxylate (DIAD, 440 µl, 2.23 mmol). After stirring at RT for 4 h

the solvent was removed under reduced pressure. Column chromatography of the residue (silica gel, 9:1 v/v hexane/ethyl acetate elution, R_f = 0.37) afforded (2*R*,3*S*)-*erythro*-**18b** (223.5 mg, 606.6 μmol, 55% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, J = 7.6 Hz, 2H), 7.59 (m, 1H), 7.47 (m, 2H), 7.27 (d, J = 8.5 Hz, 2H), 6.85 (d, J = 8.5 Hz, 2H), 5.80 (ddt, J = 17.0 Hz, J = 10.2 Hz, J = 6.7 Hz, 1H), 5.28 (m, 1H), 5.03 (d, J = 17.1 Hz, 1H), 4.98 (d, J = 10.3 Hz, 1H), 4.59 (d, J = 11.5 Hz, 1H), 4.54 (d, J =11.5 Hz, 1H), 3.79 (s, 3H), 3.74 (dq, J = 3.8 Hz, J = 6.3 Hz, 1H,), 2.11 (m, 2H), 1.80 (m, 2H), 1.52 (m, 2H), 1.28 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.3, 159.2, 138.5, 132.9, 130.68, 130.74, 129.8, 129.4, 128.5, 114.9, 113.9, 76.2, 75.4, 70.8, 55.3, 33.7, 29.3, 25.0, 16.0.

(7*R*,8*R*,2*E*)-*threo*-Ethyl 7-benzoyloxy-8-(4-methoxybenzyloxy)-2-nonenoate (19a). Under argon atmosphere a solution of **18a** (368.5 mg, 1 mmol) and ethyl acrylate (545 μl, 5 mmol) in DCM (30 ml) was treated with Grubbs-II catalyst (50 mg, 59 μmol) and stirred at 40 °C for 9 h. The solution was concentrated under reduced pressure and the residue purified by chromatography (silica gel, 4:1 v/v hexane/ethyl acetate elution, R_f = 0.40) to afford **19a** (335.7 mg, 762 μmol, 76% yield) as a green oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 7.2 Hz, 2H), 7.57 (m, 1H), 7.47 (m, 2H), 7.25 (d, J = 8.6 Hz, 2H), 6.93 (dt, J = 15.6, J = 7.0 Hz, 1H), 6.86 (d, J = 8.6 Hz, 2H), 5.82 (d, J = 15.6 Hz, 1H), 5.24 (m, 1H), 4.62 (d, J = 11.5 Hz, 1H), 4.48 (d, J = 11.5 Hz, 1H), 4.19 (q, J = 7.0 Hz, 2H), 3.81 (s, 3H), 3.73 (dq, J = 4.6, J = 6.4 Hz, 1H), 2.23 (m, 2H), 1.78 (m, 2H), 1.52 (m, 2H), 1.30 (t, J = 6.9 Hz, 3H), 1.23 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7, 166.4, 159.3, 148.6, 133.1, 130.7, 130.4, 129.8, 129.5, 128.5, 121.8, 113.9, 75.8, 74.4, 70.9, 60.3, 55.4, 32.1, 29.1, 24.2, 15.4, 14.4.

(7*S*,8*R*,2*E*)-*erythro*-Ethyl 7-benzoyloxy-8-(4-methoxybenzyloxy)-2-nonenoate (19b). Under argon atmosphere a solution of **18b** (223.5 mg, 607 μmol) and ethyl acrylate (330 μl, 3 mmol) in DCM (20 ml) was treated with Grubbs-II catalyst (30 mg, 35 μmol) and stirred at 40 °C for 9 h. The solution was concentrated under reduced pressure and the residue purified by

chromatography (silica gel, 4:1 v/v hexane/ethyl acetate elution, R_f = 0.40) to afford **19b** (218.2 mg, 495 μmol, 81% yield) as a brownish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, J = 7.2 Hz, 2H), 7.59 (m, 1H), 7.47 (m, 2H), 7.26 (d, J = 8.5 Hz, 2H), 6.94 (dt, J = 15.6, J = 6.9 Hz, 1H), 6.84 (d, J = 8.5 Hz, 2H), 5.82 (d, J = 15.6 Hz, 1H), 5.24 (dt, J = 9.1 Hz, J = 3.9 Hz, 1H), 4.55 (s, 2H), 4.19 (q, J = 7.1 Hz, 2H), 3.79 (s, 3H), 3.72 (dq, J = 3.9 Hz, J = 6.4 Hz, 1H), 2.25 (m, 2H), 1.79 (m, 2H), 1.56 (m, 2H), 1.29 (t, J = 7.0 Hz, 3H), 1.26 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7, 166.2, 159.2, 148.5, 133.0, 130.6, 130.5, 129.7, 129.4, 128.5, 121.8, 113.8, 76.0, 75.3, 70.8, 60.2, 55.3, 32.0, 29.3, 24.2, 16.0, 14.4.

(7*R*,8*R*,2*E*)-*threo*-Ethyl 7-benzoyloxy-8-hydroxy-2-nonenoate (20a). A solution of **19a** (330 mg, 750 μmol) in DCM (5 ml) was treated with water (260 μl) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (187 mg, 825 μmol). After stirring at RT for 1 h the mixture was quenched with water (1 ml) and the aqueous phase extracted with DCM (5 x 1 ml). The combined organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography (silica gel, 2:1 v/v hexane/ethyl acetate elution, R_f = 0.42) afforded **20a** (168.7 mg, 527 μmol, 70% yield) as a yellowish oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (m, 2H); 7.57 (m, 1H), 7.45 (m, 2H), 6.91 (dt, J = 15.7, J = 7.0 Hz, 1H), 5.80 (d, J = 15.7 Hz, 1H), 5.05 (dt, J = 7.8 Hz, J = 5.0 Hz, 1H), 4.16 (q, J = 7.2 Hz, 2H), 3.94 (dq, J = 4.8, J = 6.4 Hz, 1H), 2.23 (m, 2H), 1.77 (m, 2H), 1.55 (m, 2H), 1.26 (t, J = 7.0 Hz, 3H), 1.23 (d, J = 6.5 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7, 166.6, 148.4, 133.3, 130.1, 129.8, 128.6, 121.9, 77.9, 69.0, 60.3, 32.0, 30.2, 24.1, 19.6, 14.4; HRMS (ESI-TOF) m/z $(M + NH_4)^+$ Calcd for $C_{18}H_{28}NO_5$ 338.1962, found 338.1979.

(7*S*,8*R*,2*E*)-*erythro*-Ethyl 7-benzoyloxy-8-hydroxy-2-nonenoate (20b). A solution of **19b** (218.2 mg, 495 μmol) in DCM (5 ml) was treated with water (260 μl) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (124 mg, 545 μmol). After stirring at RT for 1 h the mixture was

quenched with 1 ml water and the aqueous phase extracted with DCM (5 x 1 ml). The combined organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. Column chromatography (silica gel, 2:1 v/v hexane/ethyl acetate elution, R_f = 0.42) afforded **20b** (113.5 mg, 354 µmol, 72% yield) as a yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.05 (m, 2H), 7.57 (m, 1H), 7.45 (m, 2H), 6.91 (dt, J = 15.7, J = 6.9 Hz, 1H), 5.80 (d, J = 15.7 Hz, 1H), 5.11 (dt, J = 9.3 Hz, J = 3.7 Hz, 1H), 4.15 (q, J = 7.2 Hz, 2H), 4.00 (dq, J = 3.8, J = 6.4 Hz, 1H), 2.24 (m, 2H), 1.76 (m, 2H), 1.57 (m, 2H), 1.26 (t, J = 7.1 Hz, 3H), 1.23 (d, J = 6.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7, 166.7, 148.4, 133.2, 130.1, 129.7, 128.5, 121.9, 78.2, 69.3, 60.2, 31.9, 29.2, 24.2, 18.3, 14.3; HRMS (ESI-TOF) *m/z* $(M + NH_4)^+$ Calcd for $C_{18}H_{28}NO_5$ 338.1962, found 338.1977.

***O*-(2,4-di-*O*-Benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl) trichloroacetimidate (21).** A solution of 2,4-di-*O*-benzoyl-ascarylose (53.5 mg, 150 µmol) in DCM (1 ml) was treated with trichloroacetonitrile (32 µl, 320 µmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (5 µl, 33.4 µmol). After stirring for 30 min the yellowish solution was concentrated under reduced pressure. Column chromatography of the residue (silica gel, 4:1 v/v hexane/ethyl acetate elution) afforded **20** (61.2 mg, 122.4 µmol, 81% yield) as a colorless oil that was directly used for the next steps.

(7*R*,8*R*,2*E*)-*threo*-Ethyl 7-benzoyloxy-8-[(2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-nonenoate (22a). A solution of **20a** (14.7 mg, 45.9 µmol) and **21** (15.3 mg, 30.6 µmol) in dry DCM (1 ml) at 0 °C was treated with trimethylsilyl triflate (5 µl) and stirred for 3 h. The reaction was quenched with sat. $NaHCO_3$ solution (100 µl), dried over Na_2SO_4, and concentrated under reduced pressure. Column chromatography (silica gel, 4:1 v/v hexane/ethyl acetate elution, R_f = 0.24) afforded **22a** (13.8 mg, 20.9 µmol, 68% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (m, 2H), 8.10 (m, 2H), 7.80 (d, J = 7.6 Hz, 2H), 7.58 (m, 3H), 7.47 (m, 2H), 7.46 (m, 2H), 7.42 (m, 2H), 6.94 (dt, J = 15.7, J = 7.0 Hz,

1H), 5.83 (d, J = 15.6 Hz, 1H), 5.31 (m, 1H), 5.15 (s.br, 1H), 5.10 (ddd, J = 11.0, J = 9.8 Hz, J = 4.7 Hz, 1H), 4.98 (s, 1H), 4.16 (q, J = 7.1 Hz, 2H), 4.08 (m, 1H), 4.01 (dq, J = 9.7 Hz, J = 6.3 Hz, 1H), 2.41 (dt, J = 13.7 Hz, J = 3.8 Hz, 1H), 2.28 (m, 2H), 2.19 (m, 1H), 1.80 (m, 2H), 1.61 (m, 2H), 1.29 (d, J = 6.5 Hz, 3H), 1.26 (t, J = 7.0 Hz, 3H), 1.03 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.6, 166.1, 165.9, 165.7, 148.3, 133.4, 133.2, 133.2, 130.4, 130.3, 130.0, 129.91, 129.88, 129.7, 128.6, 128.5, 122.0, 93.3, 75.9, 72.5, 71.1, 70.4, 67.3, 60.3, 32.0, 29.9, 29.8, 24.0, 17.8, 15.0, 14.4; HRMS (ESI-TOF) m/z $(M + NH_4)^+$ Calcd for $C_{38}H_{46}NO_{10}$ 676.3116, found 676.3133.

(7*S*,8*R*,2*E*)-*erythro*-Ethyl 7-benzoyloxy-8-[(2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-nonenoate (22b). A solution of **20b** (14.7 mg, 45.9 μmol) and **21** (15.3 mg, 30.6 μmol) in dry DCM (1 ml) at 0 °C was treated with trimethylsilyl triflate (5 μl) and stirred for 3 h. The reaction was quenched with sat. $NaHCO_3$ solution (100 μl), dried over Na_2SO_4 and concentrated under reduced pressure. The residue was chromatographed (silica gel, 4:1 v/v hexane/ethyl acetate elution, R_f = 0.26) to afford **22b** (12.9 mg, 19.5 μmol, 64% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (m, 2H), 8.09 (m, 2H), 7.99 (d, J = 7.8 Hz, 2H), 7.58 (m, 3H), 7.46 (m, 6H), 6.96 (dt, J = 15.8, J = 7.2 Hz, 1H), 5.85 (d, J = 15.5 Hz, 1H), 5.22 (dt, J = 15.2 Hz, J = 4.0 Hz, 1H), 5.15 (s.br, 1H), 5.12 (m, 1H), 4.94 (s, 1H), 4.16 (q, J = 7.1 Hz, 2H), 4.16 (dq, J = 3.8 Hz, J = 6.4 Hz, 1H), 4.13 (m, 1H), 2.41 (dt, J = 13.2 Hz, J = 3.8 Hz, 1H), 2.30 (m, 2H), 2.19 (ddd, J =13.0 Hz, J = 11.7 Hz, J = 2.8 Hz, 1H), 1.93 (m, 1H), 1.82 (m, 1H), 1.64 (m, 2H), 1.29 (d, J = 6.5 Hz, 3H), 1.26 (t, J = 7.1 Hz, 3H), 1.03 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.7, 166.2, 165.9, 165.7, 148.4, 133.4, 133.3, 133.2, 130.4, 130.1, 130.0, 129.8, 128.6, 128.5, 122.0, 93.6, 76.6, 72.5, 71.0, 70.6, 67.2, 60.3, 32.1, 29.8, 28.6, 24.4, 17.7, 14.8, 14.4; HRMS (ESI-TOF) m/z $(M + NH_4)^+$ Calcd for $C_{38}H_{46}NO_{10}$ 676.3116, found 676.3129.

(7*R*,8*R*,2*E*)-*threo*-8-[(3',6'-Dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-7-hydroxy-2-nonenoic acid ((7*R*,8*R*)-*threo*-10a). A solution of **22a** (13.8 mg, 20.9 μmol) in methanol (2 ml) was treated with LiOH monohydrate (7.0 mg, 167 μmol) in H_2O (100 μl). After stirring for 12 h the reaction mixture was acidified with acetic acid and concentrated under reduced pressure. The product was isolated by a combination of column chromatography on silica gel using a mixture of 15% methanol in dichloromethane with 0.1% acetic acid and solid phase extraction on reverse phase C18 using increasing concentrations of methanol in water as eluent to afford *threo*-**10a** (2.4 mg, 7.5 μmol, 36% yield) along with the intramolecular cyclization product **23a** (2.5 mg, 7.9 μmol, 39% yield).

(7*R*,8*R*)-*threo*-**10a**: ^{1}H NMR (400 MHz, CD_3OD) δ 6.95 (dt, J = 15.6 Hz, J = 7.0 Hz, 1H), 5.82 (d, J = 15.6 Hz, 1H), 4.65 (s, 1H), 3.75 (s.br, 1H), 3.74 (dq, J = 3.9 Hz, J = 6.1 Hz, 1H), 3.64 (dq, J = 9.3 Hz, J = 6.2 Hz, 1H), 3.53 (m, 1H), 3.52 (m, 1H), 2.27 (m, 2H), 1.95 (dt, J = 13.1 Hz, J = 3.8 Hz, 1H), 1.80 (ddd, J = 13.1 Hz, J = 11.0 Hz, J = 3.0 Hz, 1H), 1.70 (m, 1H), 1.54 (m, 3H), 1.22 (d, J = 6.2 Hz, 3H), 1.14 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 170.6, 150.5, 123.2, 97.8, 75.3, 74.9, 71.4, 69.9, 68.3, 35.9, 33.1, 33.0, 25.9, 18.1, 14.7; HRMS (ESI-TOF) *m/z* (M - H)$^-$ Calcd for $C_{15}H_{25}O_7$ 317.1606, found 317.1618.

2-((6*R*)-6-((*R*)-1-[(3,6-Dideoxy-α-L-*arabino*-hexopyranosyl)oxy]ethyl)tetrahydro-2H-pyran-2-yl)acetic acid (23a): ^{1}H NMR (400 MHz, CD_3OD) δ 4.66 (s, 1H), 3.76 (s.br, 1H), 3.65(m, 1H), 3.74 (m, 1H), 3.65 (m, 1H), 3.52 (m, 2H), 2.50 (ddd, J = 15.0 Hz, J = 7.2 Hz, J = 2.6 Hz, 1H), 2.41 (ddd, J = 15.0 Hz, J = 5.6 Hz, J = 2.7 Hz, 1H), 1.95 (dt, J = 13.1 Hz, J = 3.8 Hz, 1H), 1.81 (ddd, J = 13.1 Hz, J = 11.3 Hz, J = 3.0 Hz, 1H), 1.58 (m, 3H), 1.51 (m, 2H), 1.46 (m, 1H), 1.22 (d, J = 6.2 Hz, 3H), 1.14 (d, J = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.6, 97.8, 79.32/79.37, 75.33/75.38, 75.1, 71.4, 69.9, 68.4, 40.4, 35.9, 35.0, 33.4, 22.83/22.87, 18.1, 14.7; HRMS (ESI-TOF) *m/z* (M - H)$^-$ Calcd for $C_{15}H_{25}O_7$ 317.1606, found 317.1614.

(7*S*,8*R*,2*E*)-*erythro*-8-[(3',6'-Dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-7-hydroxy-2-nonenoic acid ((7*S*,8*R*)-*erythro*-10b). A solution of **22b** (12.9 mg, 19.5 μmol) in methanol (2 ml) was treated with LiOH monohydrate (6.6 mg, 156 μmol) in H_2O (100 μl). After stirring for 12 h the reaction mixture was acidified with acetic acid and concentrated under reduced pressure. The product was isolated by a combination of column chromatography on silica gel using a mixture of 15% methanol in dichloromethane with 0.1% acetic acid and solid phase extraction on reverse phase C18 using increasing concentrations of methanol in water as eluent to afforded *erythro*-**10b** (2.9 mg, 9.1 μmol, 46% yield) along with the intramolecular cyclization product **23b** (2.6 mg, 8.2 μmol, 42% yield).

(7*S*,8*R*)-*erythro*-**10b**: ^{1}H NMR (400 MHz, CD_3OD) δ 6.96 (dt, J = 15.5 Hz, J = 7.1 Hz, 1H), 5.82 (d, J = 15.5 Hz, 1H), 4.66 (s, 1H), 3.75 (s.br, 1H), 3.66 (m, 1H), 3.63 (dq, J = 9.5 Hz, J = 6.2 Hz, 1H), 3.53 (m, 1H), 3.52 (m, 1H), 2.27 (m, 2H), 1.95 (dt, J = 13.3 Hz, J = 3.7 Hz, 1H), 1.80 (ddd, J = 13.1 Hz, J = 11.1 Hz, J = 3.0 Hz, 1H), 1.65 (m, 2H), 1.54 (m, 1H), 1.43 (m, 1H), 1.22 (d, J = 6.2 Hz, 3H), 1.15 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 170.4, 150.6, 123.0, 97.6, 75.6, 75.2, 71.3, 69.8, 68.4, 35.9, 33.6, 33.0, 25.6, 18.1, 14.2; HRMS (ESI-TOF) *m/z* $(M - H)^-$ Calcd for $C_{15}H_{25}O_7$ 317.1606, found 317.1615.

2-((6*S*)-6-((*R*)-1-[(3,6-Dideoxy-α-L-*arabino*-hexopyranosyl)oxy]ethyl)tetrahydro-2H-pyran-2-yl)acetic acid (23b): ^{1}H NMR (400 MHz, CD_3OD) δ 4.66 (s, 1H), 3.75 (s.br, 1H), 3.66 (m, 2H), 3.64 (m, 1H), 3.52 (m, 2H), 2.50 (ddd, J = 15.0 Hz, J = 7.1 Hz, J = 2.1 Hz, 1H), 2.41 (ddd, J = 15.1 Hz, J = 5.4 Hz, J = 2.2 Hz, 1H), 1.95 (1H, dt, J = 13.1 Hz, J = 3.9 Hz, 1H), 1.80 (ddd, J = 13.1 Hz, J = 11.3 Hz, J = 3.1 Hz, 1H), 1.59 (m, 1H), 1.57 (m, 2H), 1.44 (m, 1H), 1.41 (m, 2H), 1.22 (d, J = 6.2 Hz, 3H), 1.15 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 175.7, 97.6, 79.3, 75.57/75.61, 75.4, 71.3, 69.8, 68.4, 40.4, 35.9, 34.9, 34.1, 22.6, 18.1, 14.1; HRMS (ESI-TOF) *m/z* $(M - H)^-$ Calcd for $C_{15}H_{25}O_7$ 317.1606, found 317.1622.

(7*R*,8*R*)-*threo*- or (7*S*,8*R*)-*erythro*-8-[(3',6'-Dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-7-hydroxynonanoic acid (*threo*-11a or *erythro*-11b). Aliquots (10 μg) of synthetic (7*R*,8*R*)-*threo*-asc-7OH-ΔC9 (**10a**) or (7*S*,8*R*)-*erythro*- asc-7OH-ΔC9 (**10b**) in methanol (500 μl) were treated with 10% palladium on carbon (10 mg) and hydrogenated under atmospheric pressure for 1 h. The mixture was filtered over a small patch of silica, concentrated to dryness, and the resulting (7*R*,8*R*)-*threo*-asc-7OH-C9 (**11a**) or (7*S*,8*R*)-*erythro*- asc-7OH-C9 (**11b**) submitted to TMS derivatization for chemical correlation with the natural product isolated from *C. nigoni*.

(7*S*)-7-*tert*-Butyldimethylsilyloxy-6-hydroxy-1-octene (25). Under argon atmosphere a solution of 4-pentenylmagnesium bromide (1.68 mmol) in THF (2 ml), prepared from 5-bromo-1-pentene (250 mg, 1.68 mmol) and magnesium (45 mg, 1.88 mmol), was added slowly to a mixture of copper(I)iodide (32 mg, 168 μmol) and (*S*)-3-*tert*-butyldimethylsilyloxy-1,2-epoxypropane (**24**) (210 mg, 1.12 mmol) in THF (2 ml) at 0 °C. After stirring at 0 °C for 3 h, the solution was quenched with saturated ammonium chloride solution, stirred for 1 h, and the aqueous phase extracted with diethyl ether. The organic phase was washed with brine, concentrated under reduced pressure and the residue chromatographed (silica gel, DCM elution, R_f = 0.43) to afford **25** (273 mg, 1.05 mmol, 94% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.76 (ddt, J = 17.0 Hz, J = 10.3 Hz, J = 6.7 Hz, 1H), 4.95 (ddt, J = 17.1 Hz, J = 2.0 Hz, J = 1.5 Hz, 1H), 4.89 (ddt, J = 10.2 Hz, J = 2.0 Hz, J = 1.5 Hz, 1H), 3.58 (m, 2H), 3.36 (m, 1H), 2.02 (dt, J = 6.7 Hz, J = 7.1 Hz, 2H), 1.26-1.48 (m, 6H), 0.87 (s, 9H), 0.03 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.9, 114.4, 71.8, 67.4, 33.8, 32.7, 29.1, 26.0, 25.1, 18.3, -5.3, -5.4.

(2*E*,8*S*)-Ethyl 9-*tert*-butyldimethylsilyloxy-8-hydroxy-2-nonenoate (26). Under argon atmosphere a solution of **25** (258.5 mg, 1 mmol) and ethyl acrylate (545 μl, 5 mmol) in DCM (30 ml) was treated with Grubbs 2nd generation catalyst (50 mg, 58.9 μmol) and stirred at 40

°C for 9 h. The solution was concentrated under reduced pressure and the residue chromatographed (silica gel, 4:1 v/v hexane/ethyl acetate elution, R_f = 0.51) to afford **26** (268 mg, 810 mmol, 81% yield) as a yellowish oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (dt, J = 15.7 Hz, J = 6.9 Hz, 1H), 5.80 (d, J = 15.7 Hz, 1H), 4.17 (q, J = 7.1 Hz, 2H), 3.62 (m, 1H), 3.60 (dd, J = 10.6 Hz, J = 3.3 Hz, 1H), 3.37 (dd, J = 10.6 Hz, J = 8.3 Hz, 1H), 2.20 (m, 2H), 1.33-1.54 (m, 6H), 1.27 (t, J = 7.1 Hz, 3H), 0.89 (s, 9H), 0.06 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 149.2, 121.6, 71.8, 67.4, 60.3, 32.7, 32.2, 28.3, 26.0, 25.3, 18.4, 14.4, -5.2, -5.3; HRMS (ESI-TOF) m/z $(M + NH_4)^+$ Calcd for $C_{17}H_{38}NO_4Si$ 348.2565, found 348.2572.

(2*E*,8*S*)-Ethyl 8-[(2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-9-*tert*-butyldimethylsilyloxy-2-nonenoate (27). Under argon atmosphere a solution of **26** (30.3 mg, 91.8 μmol) and **21** (30.6 mg, 61.2 μmol) in dry DCM (1 ml) at 0 °C was treated with trimethylsilyl triflate (5 μl) and stirred for 3 h. The reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution (100 μl), dried over Na_2SO_4, and concentrated under reduced pressure. The product was isolated by column chromatography (silica gel, 4:1 v/v hexane/ethyl acetate elution, R_f = 0.50) to afford **27** (21.7 mg, 32.4 μmol, 53% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, J = 8.0 Hz, 2H), 8.03 (d, J = 7.9 Hz, 2H), 7.58 (m, 2H), 7.46 (m, 4H), 6.99 (dt, J = 15.6 Hz, J = 7.0 Hz, 1H), 5.85 (d, J = 15.6 Hz, 1H), 5.22 (s.br, 1H), 5.15 (ddd, J = 11.4 Hz, J = 10.3 Hz, J = 4.4 Hz, 1H), 5.10 (s, 1H), 4.16 (q, J = 7.1 Hz, 2H), 4.15 (m, 1H) 3.78 (m, 1H), 3.65 (dd, J = 10.5 Hz, J = 5.9 Hz, 1H), 3.61 (dd, J = 10.6 Hz, J = 5.0 Hz, 1H), 2.43 (dt, J = 13.3 Hz, J = 3.9 Hz, 1H), 2.26 (m, 2H), 2.19 (ddd, J = 13.5 Hz, J = 11.6 Hz, J = 3.0 Hz, 1H), 1.40-1.70 (m, 6H), 1.28 (d, J = 6.9 Hz, 3H), 1.26 (t, J = 7.1 Hz, 3H), 0.85 (s, 9H), 0.06 (s, 3H), 0.05 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 165.8, 165.7, 149.1, 133.3, 130.2, 130.1, 130.0, 129.8, 128.6, 128.5, 121.7, 96.4, 78.4, 71.0, 70.8, 67.3, 65.5, 60.3, 32.3, 31.8, 29.8, 28.2, 25.9, 25.8, 25.3, 18.3, 18.0, 14.4, -5.3, -5.4; HRMS (ESI-TOF) m/z $(M + NH_4)^+$ Calcd for $C_{37}H_{56}NO_9Si$ 686.3719, found 686.3735.

(2*E*,8*S*)-Ethyl 8-[(2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-9-hydroxy-2-nonenoate (28). Under argon atmosphere a solution of **27** (21.7 mg, 32.4 μmol) in dry THF (1 ml) was treated with 1 M tetrabutylammonium fluoride (50 μl, 50 μmol) in dry THF (1 ml) and stirred at RT for 3 h. The solution was concentrated under reduced pressure and the residue separated by column chromatography (silica gel, 2:1 v/v hexane/ethyl acetate elution) to afford **28** (14.0 mg 25.2 μmol, 78% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* = 7.8 Hz, 2H), 8.05 (d, *J* = 7.7 Hz, 2H), 7.59 (m, 2H), 7.46 (m, 4H), 6.97 (dt, *J* = 15.6 Hz, *J* = 6.9 Hz, 1H), 5.83 (d, *J* = 15.7 Hz, 1H), 5.20 (s.br, 1H), 5.19 (ddd, *J* = 11.3 Hz, *J* = 10.5 Hz, *J* = 4.5 Hz, 1H), 5.08 (s, 1H), 4.16 (q, *J* = 7.1 Hz, 2H), 4.13 (m, 1H), 3.82 (m, 1H), 3.76 (dd, *J* = 12.0 Hz, *J* = 2.9 Hz, 1H), 3.61 (dd, *J* = 12.0 Hz, *J* = 5.6 Hz, 1H), 2.44 (dt, *J* = 13.5 Hz, *J* = 3.9 Hz, 1H), 2.25 (m, 2H), 2.21 (ddd, *J* = 13.5 Hz, *J* = 11.6 Hz, *J* = 3.0 Hz, 1H), 1.40-1.75 (m, 6H), 1.29 (d, *J* = 6.8 Hz, 3H), 1.26 (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 166.8, 166.1, 165.8, 148.9, 133.5, 133.4, 130.1, 130.0, 129.82, 129.79, 128.6, 121.7, 95.9, 79.3, 71.2, 70.6, 67.5, 64.5, 60.3, 32.2, 31.4, 29.8 28.2, 25.3, 18.0, 14.4; HRMS (ESI-TOF) *m/z* $(M + NH_4)^+$ Calcd for $C_{31}H_{42}NO_9$ 572.2854, found 572.2871.

(2*E*,8*S*)-8-[(3,6-Dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-9-hydroxy-2-nonenoic acid (asc-9OH-ΔC9) (12). A solution of **28** (14.0 mg, 25.3 μmol) in methanol (2 ml) was treated with LiOH monohydrate (8.5 mg, 202.4 μmol) in water (100 μl) and stirred for 12 h. The mixture was acidified with acetic acid, and concentrated to dryness under reduced pressure. The residue was separated by column chromatography (silica gel, 15% v/v methanol in dichloromethane with 0.1% acetic acid as eluent) and solid phase extraction (reverse phase C18, 10% stepwise increase of methanol in water as eluent) to afford **12** (2.0 mg, 6.3 μmol, 25 % yield) identical to the natural product from *C. nigoni*, along with its intramolecular cyclization product **28** (5.6 mg, 17.6 μmol, 69% yield).

asc-9OH-ΔC9 (**12**): colorless oil, ^{1}H NMR (400 MHz, CD_3OD) δ 6.95 (dt, *J* = 15.6 Hz, *J* = 6.9 Hz, 1H), 5.81 (d, *J* = 15.6 Hz, 1H), 4.75 (s, 1H), 3.84 (s.br, 1H), 3.69 (m, 1H), 3.67 (m, 1H), 3.60 (dd, *J* = 11.7 Hz, *J* = 4.2 Hz, 1H), 3.53 (m, 1H), 3.50 (dd, *J* = 11.7 Hz, *J* = 5.6 Hz, 1H), 2.25 (m, 2H), 1.95 (dt, *J* = 13.0 Hz, *J* = 3.8 Hz, 1H), 1.78 (ddd, *J* = 13.0 Hz, *J* = 11.2 Hz, *J* = 3.1 Hz, 1H), 1.59 (m, 2H), 1.52 (m, 2H), 1.50 (m, 2H), 1.22 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 170.3, 150.7, 122.9, 99.8, 78.6, 71.4, 69.6, 68.4, 64.6, 35.9, 33.1, 32.8, 29.3, 26.3 18.1; HRMS (ESI-TOF) *m/z* (M - H)$^-$ Calcd for $C_{15}H_{25}O_7$ 317.1606, found 317.1614.

(7*S*)-7-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]oxocan-2-yl)-acetic acid (**29**): colorless oil. ^{1}H NMR (400 MHz, CD_3OD) *δ* 4.75 (s, 1H), 3.84 (s.br, 1H), 3.69 (m, 1H), 3.68 (m , 1H), 3.65 (m, 1H), 3.53 (m, 1H), 3.52 (m, 1H), 2.49 (dd, *J* = 15.2 Hz, *J* = 7.2 Hz, 1H), 2.40 (dd, *J* = 15.2 Hz, 5.6 Hz, 1H), 1.95 (dt, *J* = 13.0 Hz, *J* = 4.0 Hz, 1H), 1.78 (m, 1H), 1.59 (m, 1H), 1.57 (m, 2H), 1.40-1.60 (m, 4H), 1.22 (d, *J* = 6.2 Hz, 3H), ^{13}C NMR (100 MHz, CD_3OD) *δ* 175.6, 99.8, 79.29/79.32, 78.73/78.77, 71.37/71.40, 69.6, 68.4, 64.6, 40.4, 36.0, 34.9, 32.8, 26.8, 26.3, 18.2; HRMS (ESI-TOF) *m/z* (M - H)$^-$ Calcd for $C_{15}H_{25}O_7$ 317.1606, found 317.1619.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.XXXXXXX.

Supporting figures as indicted in the text,

NMR spectra of isolated ascarosides (**10 – 12**) and synthetic compounds (**10a, 10b, 12, 15 – 29**).

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Notes

The authors declare no competing financial interest.

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