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Epimerization of an Ascaroside-Type Glycolipid Downstream of the Canonical β-Oxidation Cycle in the Nematode *Caenorhabditis nigoni*

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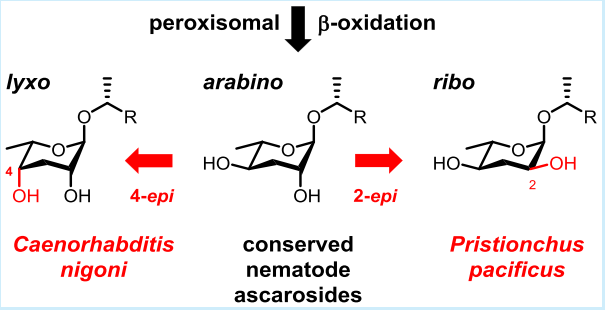
Supporting Information

ABSTRACT: A species-specific ascaroside-type glycolipid was identified in the nematode *Caenorhabditis nigoni* using HPLC-ESI-(−)-MS/MS precursor ion scanning, HR-MS/MS, and NMR techniques. Its structure containing an L-3,6-dideoxy-*lyxo*-hexose unit was established by total synthesis. The identification of this novel 4-*epi*-ascaroside (caenorhabdoside) in *C. nigoni* along with the previous identification of 2-*epi*-ascarosides (paratosides) in *Pristionchus pacificus* indicate that nematodes can generate highly specific signaling molecules by epimerization of the ascarylose building block downstream of the canonical β-oxidation cycle.



3,6-Dideoxyaldohexoses constitute essential building blocks of bacterial lipopolysaccharides[1] and nematode glycolipids.[2] Of the eight theoretically possible isomers (Figure 1), six have

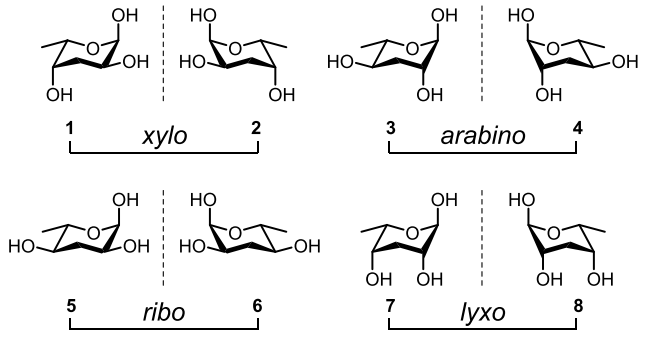


Figure 1. Eight possible stereoisomers of 3,6-dideoxyaldohexose, of which five were identified from microorganisms (**1**−**4**, **6**) and four from nematodes (**3**−**5**, and **7**, this work).

previously been described, which represent three pairs of enantiomers (**1**−**6**). In many Gram-negative bacteria, 3,6-dideoxyhexoses located at the terminal ends of lipopolysaccharide chains constitute major epitopes that determinate antigen activity[3] such as L-colitose (**1**, 3,6-dideoxy-L-*xylo*-hexose) from *E. coli* O111,[4] D-abequose (**2**, 3,6-dideoxy-D-*xylo*-hexose) from *Salmonella abortus equi*,[5] L-ascarylose (**3**, 3,6-dideoxy-L-*arabino*-hexose) from *Yersinia pseudotuberculosis*,[6] D-tyvelose (**4**, 3,6-dideoxy-D-*arabino*-hexose) from *Salmonella typhi*,[5,7] and D-paratose (**6**, 3,6-dideoxy-D-*ribo*-hexose) from *Salmonella paratyphi*.[8] Within the animal kingdom, D-tyvelose (**4**) is known as a terminal glycan unit of glycoproteins of the parasitic nematode *Trichinella spiralis*.[9] The enantiomeric L-ascarylose (**3**) was first described from lipophilic glycolipids located in the eggs of parasitic nematodes of the *Ascaris* genus.[10] Over the past decade, L-ascarylose (**3**) has been recognized as the core building block of a large diversity of nematode-derived glycolipids, commonly known as ascarosides, which represent key signaling molecules in nematode chemical ecology.[2] Nematode ascarosides include a large diversity of homologous fatty acid like aglycones derived from the peroxisomal β-oxidation cycle and, in addition, incorporate a variety of building blocks from primary metabolic pathways to form a modular library of signaling molecules.[11] Recently, a sixth 3,6-dideoxysugar, the enantiomeric L-paratose (**5**, 3,6-dideoxy-L-*ribo*-hexose), was identified as a rare building block of 2-*epi*-ascaroside-type glycolipids such as **12** from the nematode *Pristionchus pacificus*.[12] Here we describe the identification of a novel 4-*epi*-ascaroside-type glycolipid **11** from the nematode *Caenorhabditis nigoni* that represents the first natural product carrying the L-3,6-dideoxy-*lyxo*-hexose unit (**7**) that we like to call L-caenorhabdose. The exclusive specificity of these rare glycolipids for certain aglycones suggests that nematodes can generate species-specific 3,6-dideoxyaldohexoses via 2- and 4-epimerization of the L-ascarylose building block (**3**) downstream of the highly conserved peroxisomal β-oxidation cycle.

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Comprehensive ascaroside profiling of the *Caenorhabditis nigoni* exometabolome extract using two complementary techniques, a HPLC-ESI-(−)-MS/MS precursor ion scan[13] for the ascaroside specific fragment ion at m/z 73.0 $[C_3H_5O_2]^-$ (Figure S1) and GC-EIMS analysis of TMS-derivatized crude metabolome extracts[14] using extracted ion chromatograms for the K1 fragment ion at m/z 130.1 $[C_6H_{14}OSi]^{\bullet+}$, resulted in the identification of several common simple ascarosides (**9**, n = 0−7), along with some species-specific derivatives such as the hydroxyacyl ascaroside asc-7OH-ΔC9 (**10**) (Figure 2).[15]

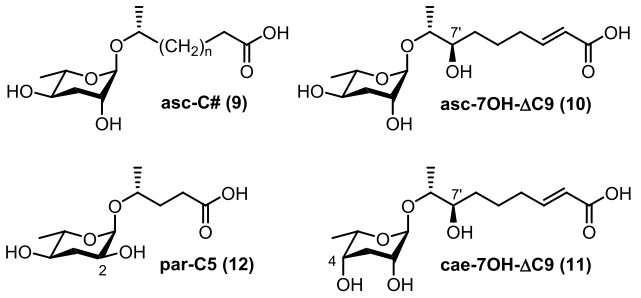

Figure 2. Core structure of nematode ascarosides (**9**) along with the species-specific hydroxyacyl ascaroside asc-7OH-ΔC9 (**10**) and 4-epimeric hydroxyacyl caenorhabdoside cae-7OH-ΔC9 (**11**) from *Caenorhabditis nigoni*, as well as the 2-epimeric L-paratoside par-C5 (**12**) from *Pristionchus pacificus*.

One additional putative ascaroside-type component (**11**) was enriched from the *C. nigoni* exometabolome extract by reverse-phase C18 solid-phase extraction (SPE) and finally isolated as a 1:1 mixture along with coeluting asc-C7 (SMID:[16] ascr#1, **9**, n = 3) using semipreparative RP-C18-HPLC (Figure S2). The molecular formula of $C_{15}H_{26}O_7$ was established by HRMS (found m/z 317.1603 $[M - H]^-$, Δ 0.9 ppm), whereas HR-MS/MS analysis suggested a hydroxynonenoic acid aglycone (Figure S3). Differential analysis of the ^{1}H NMR spectrum with those of a preceding fraction rich in asc-C7 (**9**, n = 3) highlighted signals corresponding to the target compound **11** (Figure S4). Comparative analysis of the high-resolution *dqf*-COSY spectrum of **11** with those of asc-7OH-ΔC9 (**10**), previously identified from *C. nigoni*,[15] suggested the presence of a similar (ω − 1)-linked 7OH-ΔC9 aglycone, whereas the 7,8-*threo*-configuration was derived from the vicinal coupling constant of ^3J = 3.9 Hz by comparison with the *threo*- and *erythro*-configured synthetic standards prepared as previously described (Figure S5).[15] Because the target compound **11** was different from *threo*-asc-7OH-ΔC9 (**10**), the stereochemistry of its 3,6-dideoxyhexose moiety was reevaluated based on the chemical shifts and ^{1}H,^{1}H-coupling constants (Table 1, Figure S6). Structure assignment was complicated by the fact that the chemical shifts for H-2 and H-4 were almost indistinguishable, but both methines were found to exhibit small coupling constants with both anisochoric H-3 methylene protons at δ_H 1.93 ppm (*dt*, ^2J = 14.2 Hz, ^3J = 3.5 Hz) and 2.06 ppm (*dt*, ^2J = 14.2 Hz, ^3J = 3.1 Hz). Furthermore, the methine proton H-5 at δ_H 3.99 ppm appeared as a broad quartet with $^3J_{5,6}$ = 6.6 Hz, instead of the *dq*-signal with $^3J_{5,4}$ = 9.3 Hz and $^3J_{5,6}$ = 6.2 Hz commonly observed in ascarosides, indicating that the vicinal coupling constant $^3J_{5,4}$ is small. Along with the very small (unresolved) coupling constant for the anomeric proton H-1, these results indicated the α-configured 4-epimer of ascarylose for the sugar moiety that we like to call caenorhabdose (**7**)

Table 1. 400 MHz NMR Data of the Hydroxyacyl Ascaroside-Type Glycolipids asc-7OH-ΔC9 (10)[15] and cae-7OH-ΔC9 (11) from *C. nigoni* (in CD_3OD)

	asc-7OH-ΔC9 (**10**)		cae-7OH-ΔC9 (**11**)	
	^{1}H	^{13}C	^{1}H	^{13}C
1	4.65 s	97.8	4.78 s	98.8
2	3.75 s.br	69.9	3.57 s.br	68.2
3ax	1.80 ddd, 13.1, 11.0, 3.0	35.9	2.06 dt, 14.3, 3.1	32.3
3eq	1.95 dt, 13.1, 3.8		1.93 ddt, 14.3, 1.0, 3.4	
4	3.52 ddd, 11.3, 9.3, 4.3	68.3	3.57 s.br	69.1
5	3.64 dq, 9.3, 6.2	71.4	3.99 dq, 1.0, 6.6	68.5
6	1.22 d, 6.2	18.1	1.18 d, 6.6	17.2
1′	-	170.6	-	170.8
2′	5.82 d, 15.6	123.2	5.82 d, 15.6	123.7
3′	6.95 dt, 15.6, 7.0	150.5	6.91 dt, 15.6, 7.0	150.0
4′	2.27 m	33.1	2.26 m	33.0
5′	1.54−1.68 m	25.9	1.53−1.70 m	25.8
6′	1.48−1.62 m	33.0	1.52−1.59 m	33.0
7′	3.53 m	74.9	3.52 m	75.0
8′	3.74 dq, 3.9, 6.2	75.3	3.75 dq, 3.9, 6.3	75.7
9′	1.14 d, 6.2	14.7	1.15 d, 6.3	14.8

and, thus, suggested the cae-7OH-ΔC9 structure for caenorhabdoside **11** (SMID:[16] caen#1).

The structure assignment of cae-7OH-ΔC9 (**11**) was unambiguously established by total synthesis as shown in Scheme 1. The *threo*-configured aglycone (**19**) was prepared in 9.5% yield over six steps as previously described (Scheme 1a).[15] (+)-Methyl D-lactate (**13**) was converted to the *para*-methoxybenzyl (PMB) ether (**14**), reduced to the aldehyde **15**, and reacted with 4-pentenyl magnesium bromide to furnish the *threo*-configured 2-*O*-PMB-3-hydroxy-oct-7-ene (**16**) with a diastereoisomeric excess of de > 92% as determined by ^{1}H NMR. Benzoylation of **16** gave **17** with de > 99% after column chromatography. Cross metathesis of **17** with ethyl acrylate using Grubbs second-generation catalyst furnished the non-enoate ester **18**, which was converted to the aglycone unit **19** upon selective cleavage of the PMB group. The 2,4-di-*O*-benzoyl protected L-caenorhabdose building block (**28**) was prepared in 1% yield over eight steps as shown in Scheme 1b. Commercially available L-rhamnose (**20**) was converted to the 1-*O*-benzyl rhamnoside (**21**) and the 2,3-positions protected as isoproylidene ketal (**22**). After formation of the 4-trifluoromethylsulfonate (triflate) ester (**23**) and hydrolysis of the ketal, the resulting **24** was cyclized to the oxirane (**25**) under slightly alkaline conditions.[17] Regioselective oxirane ring opening using lithium triethylborohydride furnished the L-1-*O*-benzyl caenorhabdoside (**26**).[18]

After benzoylation to the ester **27**, palladium-catalyzed hydrogenation furnished the 2,4-di-*O*-benzoyl-caenorhabdose building block (**28**) as a 7:3 mixture of the α- and β-anomers. Glycoside formation between the aglycone (**19**) and the caenorhabdose (**28**) unit was accomplished using the trichloroacetimidate route[19] to furnish **29** as a 3.4:1 mixture of the α- and β-isomers. Subsequent deprotection of isolated **29** under alkaline conditions afforded cae-7OH-ΔC9 (**11**), which displayed identical NMR (Figure S7) and LC-MS (Figure S8) data as the natural product isolated from *C. nigoni*.

Potential behavioral activity was evaluated using a holding assay that quantifies nematode retention in cae-7OH-ΔC9 (**11**) conditioned scoring regions as well as a two-spot chemotaxis assay that quantifies nematode preference for

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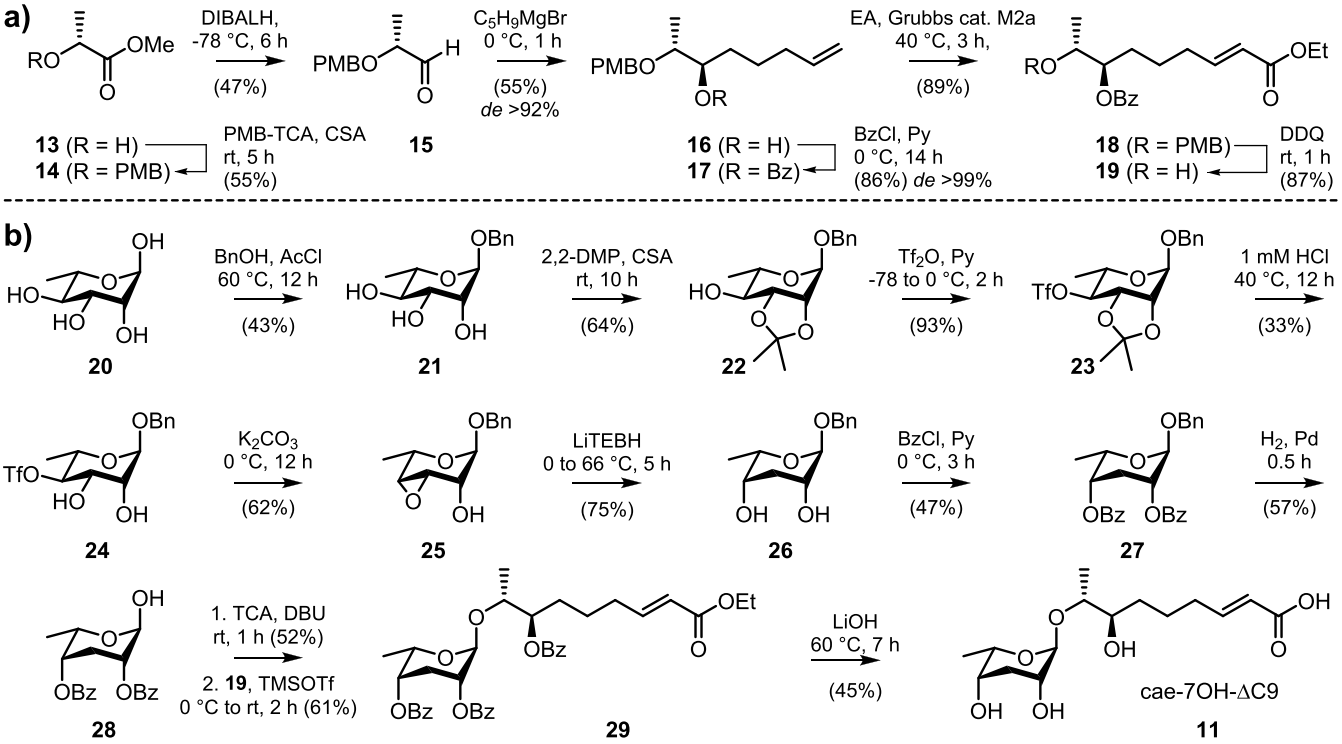
Scheme 1. Total Synthesis of Caenorhabdoside cae-7OH-ΔC9 (11) from *C. nigoni*[a]



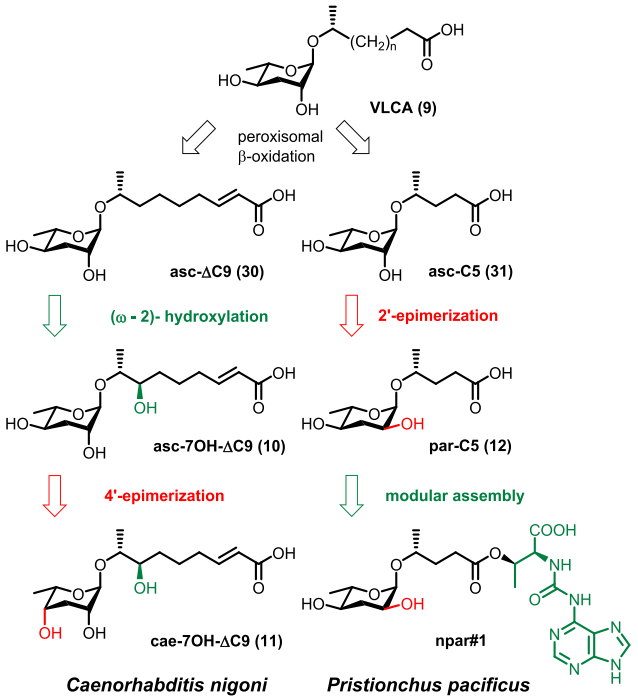
[a]PMB-TCA: *para*-methoxybenzyl trichloroacetimidate, CSA: camphor-10-sulfonic acid, EA: ethyl acrylate. Grubbs cat. M2a: Grubbs second-generation catalyst, 2,2-DMP: 2,2-dimethoxypropane, LiTEBH: lithium triethylborohydride, TCA: trichloroacetonitrile, DBU: 1,8-diazabicyclo-undec-7-ene.

feeding in cae-7OH-ΔC9 (**11**) conditioned *E. coli* spots in comparison with solvent-treated control. We found that cae-7OH-ΔC9 (**11**), individually as well as in combination with asc-7OH-ΔC9 (**10**), does not elicit any significant response in adult *C. nigoni* males or females. Additional experiments will be required to unravel the biological activity and ecological function of these highly species-specific potential signaling compounds.

In conclusion, chemical analysis of the *C. nigoni* metabolome resulted in the identification of a novel 4-*epi*-ascaroside-type glycolipid (**11**), the first natural product carrying the 3,6-dideoxy-*lyxo*-hexose unit (**7**). While the biogenesis of various 3,6-dideoxyhexoses in bacteria (**1**−**4**, **6**) is well understood,[20] the *de novo* pathway to L-ascarylose (**3**) as the core building block of nematode ascarosides remains enigmatic. Functional characterization of *C. elegans* genes with homology to those implicated in the biogenesis of bacterial 3,6-dideoxyhexoses demonstrated that they are involved in L-rhamnose (6-deoxymannose) biosynthesis.[21]

Comprehensive ascaroside profiling of the *C. nigoni* metabolome demonstrated that formation of the 4-epimeric caenorhabdoside **11** is highly specific for the 7-OH-ΔC9 aglycone (Scheme 2). While its biosynthetic precursor asc-ΔC9 (SMID: ascr#3, **30**) is highly abundant, the corresponding 4-epimeric caenorhabdoside with a ΔC9 aglycone could not be detected (Figure S9), indicating that 4-epimerization occurs downstream from the *β*-oxidation cycle and side-chain hydroxylation. Similarly, analysis of *P. pacificus daf-22*, a mutant defective in peroxisomal *β*-oxidation, did not reveal any long-chain paratoside precursors,[22] which suggests that par-C5 (SMID: part#9, **12**) is derived from the corresponding asc-C5 (SMID: ascr#9, **31**) via 2-epimerization downstream of the *β*-oxidation cycle. Enzymes capable of catalyzing epimerization of nonactivated hydroxymethine groups are well-known from

Scheme 2. Postulated Biosynthesis of Highly Species-Specific Signaling Molecules in the Nematodes *C. nigoni* and *P. pacificus* Based on Epimerization of the L-Ascarylose Building Block Downstream of the *β*-Oxidation Cycle



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carbohydrate metabolism.[23] Taken together, these results demonstrate how epimerization of the L-ascarylose building block acts along with peroxisomal β-oxidation, side chain hydroxylation, and modular assembly to generate highly species-specific glycolipids that form the chemical language of nematodes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.9b03808.

Detailed experimental procedures, supporting tables and figures as described in the mail text, and NMR spectra of isolated and synthetic compounds (PDF)

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Notes

The authors declare no competing financial interest.

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