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Dimerization of conserved ascaroside building blocks generates species-specific male attractants in *Caenorhabditis* nematodes†

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Comparative ascaroside profiling of *Caenorhabditis* nematodes using HPLC-ESI-(–)-MS/MS precursor ion scanning revealed a class of highly species-specific ascaroside dimers. Their 2- and 4-isomeric, homo- and heterodimeric structures were identified using a combination of HPLC-ESI-(+)-HR-MS/MS spectrometry and high-resolution *dqf*-COSY NMR spectroscopy. Structure assignments were confirmed by total synthesis of representative examples. Functional characterization using holding assays indicated that males of *Caenorhabditis remanei* and *Caenorhabditis nigoni* are exclusively retained by their conspecific ascaroside dimers, demonstrating that dimerization of conserved monomeric building blocks represents a yet undescribed mechanism that generates species-specific signaling molecules in the *Caenorhabditis* genus.

Introduction

Chemical communication in nematodes is mediated by ascarosides, glycolipids of the 3,6-dideoxy-*arabino*-aldohexose L-ascarylose linked to a wide range of homologous aglycones derived from the peroxisomal β-oxidation cycle (Fig. 1).[1–8]

Ascaroside signalling is widely conserved in nematodes[9] and modulates a large diversity of biological functions.[7,8] Over the past decade the development of sensitive ascaroside-selective screens based on characteristic fragment ions observed during electrospray ionization-tandem mass spectrometry (ESI-MS/MS)[10,11] or electron ionization-mass spectrometry (EI-MS)[12] along with comparative analysis of wild-type and peroxisomal β-oxidation mutant metabolomes[13–15] has facilitated the identification of several hundreds of ascaroside structures, most of which have not yet been characterized with respect to their potential biological functions. Systematic bioassays with ascarosides, while limited in number and scope, established that even minor changes in molecular structure dramatically impact their biological activities,[16] which demonstrates that ascaroside signalling represents a complex "chemical language". Comparative analysis of several closely related *Caenorhabditis* species revealed that most simple ascarosides are highly conserved. Short chain ascarosides carrying (ω–1)-linked odd numbered C5, C7, C9, and C11 acyl chains as aglycones (1) are particularly abundant and widely distributed.[17] These common ascarosides serve as scaffolds for species-specific modifications including the hydroxylation of the aglycone[17,18] and epimerization of the L-ascarylose unit (*e.g.* 2),[19] as well as the attachment of additional building

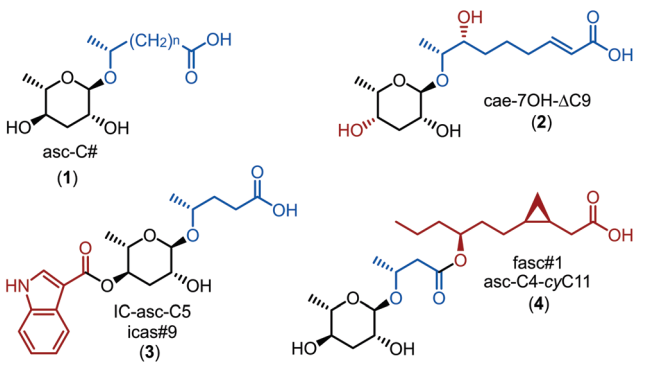

Fig. 1 General structure of common ascarosides (**1**) along with species-specific derivatives from *C. nigoni* (**2** and **3**) and *C. remanei* (**4**); L-ascarylose unit in black, aglycone moiety in blue, and species-specific modifications in red.

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blocks from diverse primary metabolic pathways to generate complex modular libraries (*e.g.* **3**).[13,15,20–22] Here we show that homo- and heterodimerization of conserved ascaroside building blocks represents another mechanism that creates species-specific signalling molecules in the *Caenorhabditis*.

Results and discussion

Comparative ascaroside profiling reveals species-specific dimers

Comparative ascaroside profiling of the nematode culture supernatants (the exometabolomes) of *Caenorhabditis nigoni* and *Caenorhabditis remanei* (Fig. 2) using HPLC-ESI-(−)-MS/MS precursor ion scanning for the highly characteristic fragment ion at *m/z* 73.0 $[C_3H_5O_2]^-$[10] revealed a diversity of common simple ascarosides with (ω–1)-linked side chains ranging from C4 to C11 (**1**, *n* = 1–8). In addition, some species-specific derivatives could be identified, including hydroxyacyl ascarosides[17] and the corresponding 4-epimeric caenorhabdoside cae-7OH-ΔC9 (**2**),[19] as well as indole ascarosides dominated by IC-asc-C5 (**3**)[23] from *C. nigoni* or the fatty acid ascarosides dominated by asc-C4-cyC11 (**4**) from *C. remanei*.[24]

A yet uncharacterized class of putative ascarosides was observed in small quantities in both *C. nigoni* and *C. remanei* (Fig. 2) as well as other *Caenorhabditis* species. Their universal molecular formula $C_{(20+n)}H_{(34+2n)}O_{11}$ (*n* = 0–10) was determined by high resolution mass spectrometry (HR-MS), which suggested a homologous series of ascaroside dimers (Table S1†). Systematic analysis of the ESI-(−)-HR-MS/MS spectra (Fig. 3) revealed neutral loss of an ascarylose unit [M − $C_6H_{13}O_4$] to form ion **I** along with a monomeric ascaroside building block **II** from cleavage of the ester linkage, thus, facilitating the differentiation of isomeric homo and heterodimers. ESI-(+)-HR-MS/MS results in an oxonium ion **III** corresponding to the esterified ascaroside building block, as well as the loss of the terminal aglycone to yield fragment ion **IV** composed of both ascarylose units connected by their aglycone linker, which ultimately facilitates the assignment of both monomeric building blocks along with their order of attachment.

Comparative profiling of fourteen *Caenorhabditis* species demonstrated a high degree of species-specificity. Eight out of

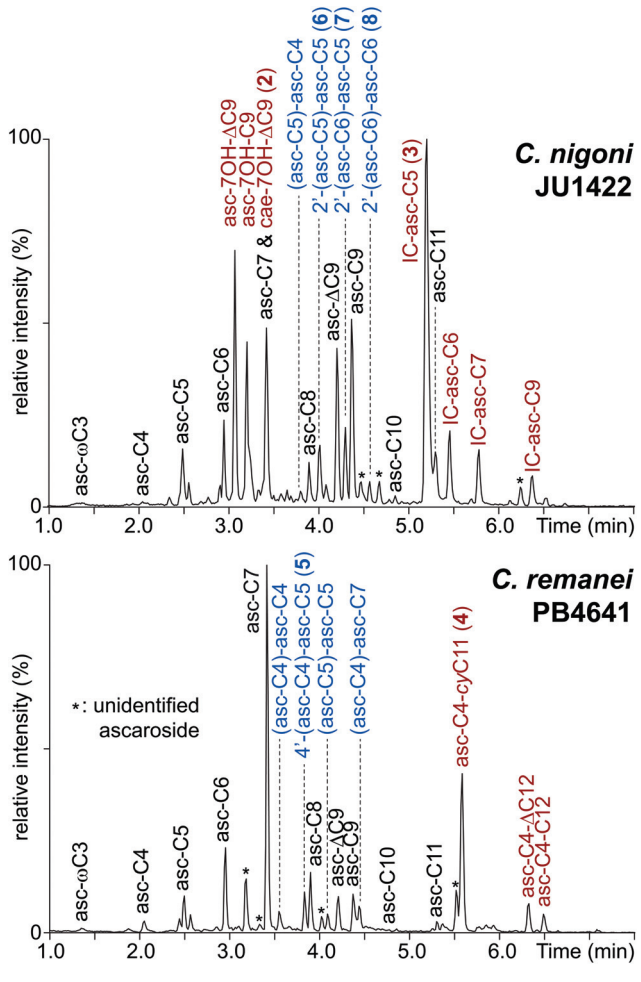

Fig. 2 Ascaroside profiling in *C. nigoni* and *C. remanei* by MS/MS precursor ion scanning for *m/z* 73.0 $[C_3H_5O_2]$ reveals common ascarosides (black) along with species-specific components (red) including homo- and heterodimers (blue).

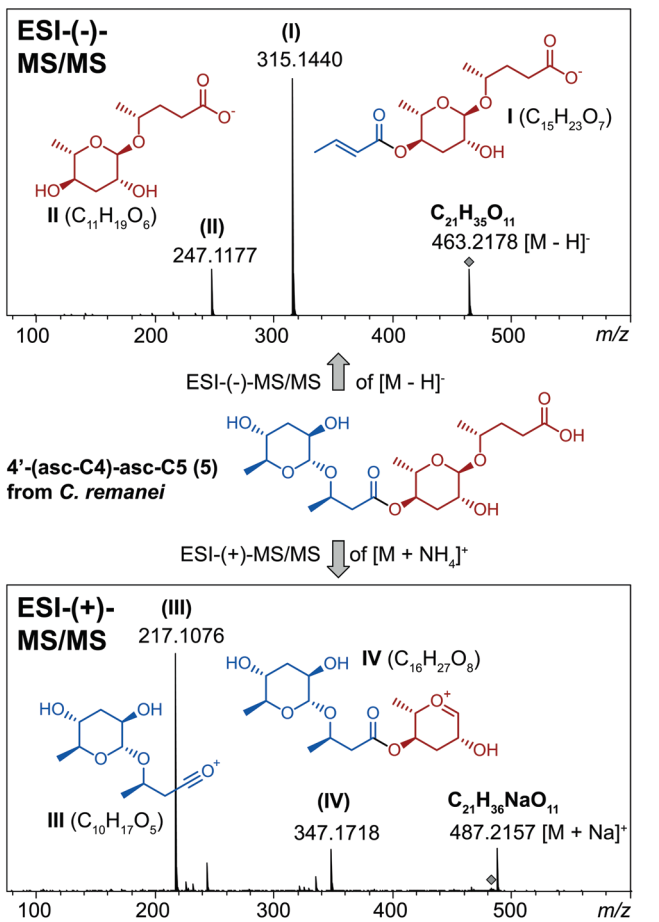

Fig. 3 ESI-MS/MS analysis facilitates structure assignment of dimeric ascarosides as shown for 4'-(asc-C4)-asc-C5 (**5**) from *C. remanei* (monomeric units in red and blue).

fourteen species were found to be devoid of dimeric ascarosides, which are abundant in members of the Elegans group but could not be detected in the model organism *C. elegans* (Fig. S1†). Monomeric building blocks and their order of assembly were identified by HPLC-ESI-(+)-MS/MS (Fig. S2†), which resulted in the characterization of 32 dimeric ascarosides (Table S1†). The dominating ascaroside dimers were identified as 4′-(asc-C4)-asc-C5 (**5**) in *C. remanei*, 2′-(asc-C6)-asc-C5 (**7**) in *C. nigoni*, (asc-C4)-asc-C4 in *C. brenneri*, (asc-C5)-asc-C5 in *C. tropicalis*, (asc-C6)-asc-C6 in *C. sinica*, and (asc-C7)-asc-C9 in *C. briggsae*, (Fig. S3†). Considering that the monomeric building blocks with sidechains ranging from C4 to C11 are conserved in *Caenorhabditis* spp.[17] this specific-specificity demonstrates that the biogenesis of ascaroside dimers is tightly controlled. Dimeric ascarosides carrying unsaturated side chains (such as (asc-ΔC9)-asc-C7) were also detected (Table S1†). Furthermore, traces of three ascaroside trimers in *C. nigoni* were identified as ((asc-C6)-asc-C6)-asc-C*x* (*x* = 4, 5, and 6) (Fig. S4, Table S4†).

Structure assignments were confirmed by isolation of representative components (Fig. 4) from crude *Caenorhabditis* exometabolome extracts by solid phase extraction (SPE) and semi-preparative HPLC on RP-C18. Fractionation of 1.5 L of the *C. remanei* liquid culture supernatant afforded approximately 130 μg of the 4-linked 4′-(asc-C4)-asc-C5 (**5**) (Fig. S5†). Inspection of high resolution *dqf*-COSY spectra indicated that the characteristic 4-linkage of **5** (that could not be derived from the MS/MS data) could already be deduced in the partially purified metabolome mixture obtained after the 1st SPE fractionation step (Fig. S6†), which was utilized for the structure assignment of additional ascaroside dimers. Combined SPE and HPLC fractionation of 1.6 L of the *C. nigoni* liquid culture supernatant gave mixtures enriched in 2-linked 2′-(asc-C5)-asc-C5 (**6**), 2′-(asc-C6)-asc-C5 (**7**), and 2′-(asc-C6)-asc-C6 (**8**) (Fig. S7†) that were identified using a combination of ESI-(+)-HR-MS/MS (Fig. S8†) and *dqf*-COSY techniques (Fig. S9, Table S2†), demonstrating that their species-specific biosynthesis in the *Caenorhabditis* spp. includes the formation of homo- and heterodimeric 2- and 4-isomers (Fig. 4).

Total synthesis of ascaroside dimers

In order to unambiguously establish their structure assignments and to obtain pure materials for the characterization of biological activities, 2- and 4-isomeric pairs of homodimeric 2′/4′-(asc-C6)-asc-C6 and heterodimeric 2′/4′-(asc-C6)-asc-C5 were synthesized as shown in Fig. 5. Glycosylation of homologous (*R*)-2-hydroxyalkenes with 2,4-di-*O*-benzoyl-ascarylose (**9**, prepared in 8 steps from commercially available L-(+)-rhamnose)[25,26] was accomplished using the trichloroacetimidate route[27] to afford the (ω–1)-linked terminal alkenyl ascarosides (**10a/b**). After alkaline hydrolysis, cross metathesis with benzyl acrylate catalysed by Grubbs 2nd generation catalyst[28] furnished the α,β-unsaturated benzyl esters **11a/b** that served as monomeric building blocks for the synthesis of ascaroside dimers. Furthermore, one part of the hexenoate homolog **11b** was derivatized with *tert*-butyldimethylsilyl chloride and *O*-debenzylated by palladium catalysed hydrogenation to yield the second monomeric building block **12**. Coupling of the homologous ascaroside benzyl esters (**11a** or **11b**) with the 2,4-di-*O*-TBS protected ascaroside (**12**) by Steglich esterification[29] afforded mixtures of the trimeric 2,4-diester and both dimeric monoesters. Using an optimized 2 : 1 ratio of the monomeric building blocks **11** and **12** afforded predominantly the 2- and 4-isomeric dimers that were separated by column chromatography on silica gel. Next, palladium catalysed hydrogenation furnished the free acids that were finally deprotected using hydrogen fluoride in pyridine to give the 2-isomeric (**7** and **8**) and 4-isomeric (**13a/b**) ascaroside dimers that were iso-

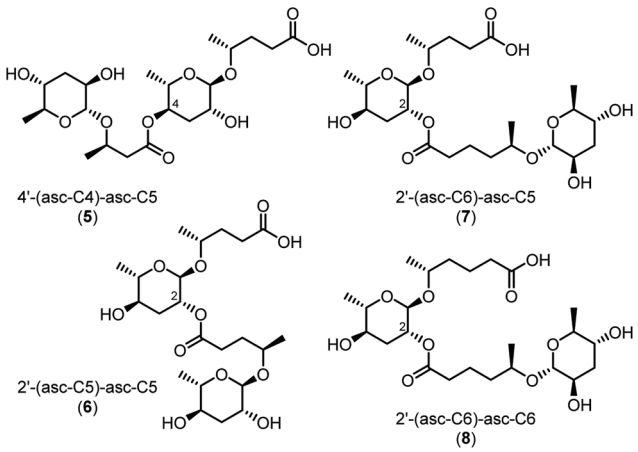

Fig. 4 Ascaroside dimers isolated from *Caenorhabditis remanei* PB4641 (**5**) and *Caenorhabditis nigoni* JU1422 (**6–8**).

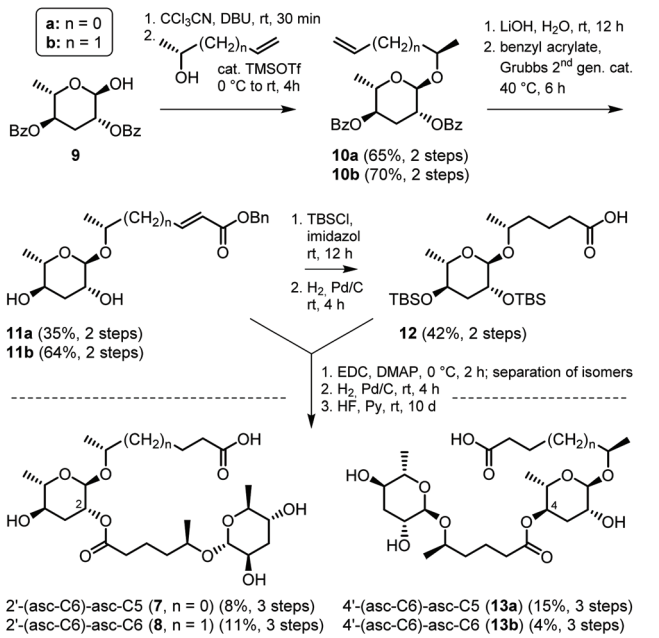

Fig. 5 Total synthesis of homo- and hetero-dimeric ascarosides from *C. nigoni*.

lated by RP-C18-SPE. Comparative NMR and HPLC-MS analysis confirmed that synthetic 2′-(asc-C6)-asc-C5 (**7**) and 2′-(asc-C6)-asc-C6 (**8**) are identical to the natural products isolated from *C. nigoni* (Fig. S10 and S11†).

Functional characterization of ascaroside dimers

Potential behavioural activities were characterized using a holding assay that quantifies nematode retention in ascaroside conditioned scoring regions in comparison to solvent control (Fig. 6). Females of *C. remanei* and *C. nigoni* did not respond to any of the dimeric ascarosides tested. *C. remanei* males were retained by 100 fmol of the conspecific 4′-(asc-C4)-asc-C5 (**5**), but not by the hetero-specific 2′-(asc-C6)-asc-C5 (**7**) and 2′-(asc-C6)-asc-C6 (**8**). In contrast *C. nigoni* males were retained by 100 fmol of the conspecific 2′-(asc-C6)-asc-C5 (**7**) and 2′-(asc-C6)-asc-C6 (**8**) but not by any of the hetero-specific 4-linked components such as 4′-(asc-C4)-asc-C5 (**5**), 4′-(asc-C6)-asc-C5 (**13a**), and 4′-(asc-C6)-asc-C6 (**13b**), demonstrating that males of both species are exclusively retained by their own conspecific ascaroside dimers.

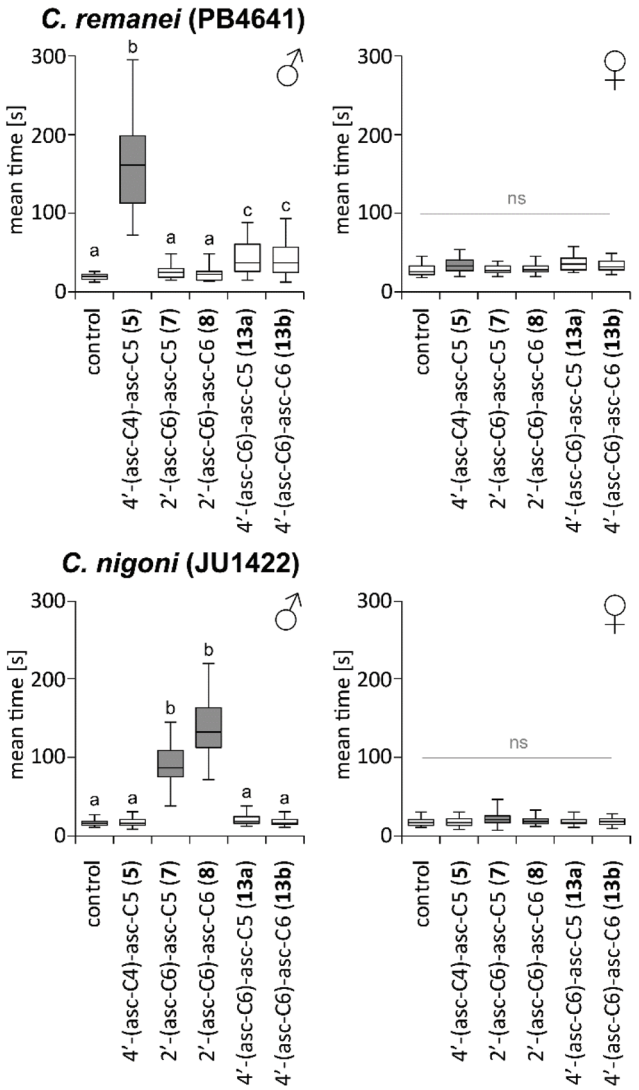

Fig. 6 Species-specific retention of *C. remanei* and *C. nigoni* in response to 100 fmol of the ascaroside dimers shows male-specific retention to conspecific components (different letters indicate statistically significant differences between groups, $P < 0.01$, ANOVA with Dunett's *post hoc* test, $n = 20$, values represent means ± 1 SD).

Conclusions

In conclusion, combination of HR-MS/MS and NMR techniques along with total synthesis facilitated the identification of 2- and 4-isomeric homologous series of ascaroside dimers in *Caenorhabditis* spp. The homodimeric 4′-(asc-C7)-asc-C7 (SMID: dasc#1)[30] has previously been shown to act as a modulator of mouth form dimorphism in the androdioecious nematode *Pristionchus pacificus*, diplogastridae, which also produces a variety of 2-linked ascaroside dimers that carry an additional 4-linked ureido-isobutyrate moiety.[31,32] Among the gonochoristic *C. remanei* and *C. nigoni* the males are exclusively retained by ecologically significant amounts of their conspecific ascaroside dimers, demonstrating that dimerization represents an effective mechanism to generate species-specific signalling molecules. Considering just the most prevalent monomeric ascaroside building blocks with (ω–1)-linked acyl aglycones ranging from 4 to 11 carbons, their dimerization *via* the 2- or 4-position results in 128 theoretically possible structures of which 27 were now characterized in *Caenorhabditis* species using HR-MS/MS techniques. Incorporation of unsaturated aglycones as well as the assembly of ascaroside trimers further expands the structural diversity of these components. Remarkably, dimers carrying even numbered aglycones were particularly prevalent, although the corresponding monomeric building blocks such as asc-C4 (**1**, $n = 1$) and asc-C6 (**1**, $n = 3$) are rare and cannot be produced by canonical β-oxidation of the predominating homologous series of odd numbered ascarosides. Along with the hydroxylation of the ascaroside aglycones, epimerization of the ascarylose unit, and attachment of additional building blocks from primary metabolic pathways, the dimerization of monomeric units represents a highly effective mechanism to generate species-specific ascarosides, which form a complex chemical language in nematode communication.

Experimental

Organisms

Wild-type *Caenorhabditis* isolates of *C. afra* JU1286 (Ghana), *C. brenneri* PB2801 (Costa Rica), *C. briggsae* AF16 (India), *C. doughertyi* JU1771 (India), *C. elegans* N2 (UK), *C. japonica* DF5081 (Japan), *C. monodelphis* JU1667 (Germany), *C. nigoni* JU1422 (India), *C. portoensis* EG4788 (Portugal), *C. remanei* PB4641 (United States), *C. sinica* JU727 (China), *C. tropicalis* JU1373 (La Réunion), *C. virilis* JU1968 (France), and *C. wallacei* JU1904 (Indonesia), as well as *Escherichia coli* OP50 (uracil aux-

otroph) were obtained from the Caenorhabditis elegans Genetics Center (CGC).

Preparation of exometabolome extracts

Fourteen wild-type *Caenorhabditis* species were cultivated at 23 °C on Nematode Growth Medium (NGM) agar[33] seeded with *E. coli* OP50. Mixed stage nematodes from five 10 cm plates were collected in M9 phosphate buffer[33] to serve as inoculums for liquid cultures grown in 100 ml S-medium[33] at 23 °C and 150 rpm. Concentrated *E. coli* OP50 bacteria pellet (from an overnight culture in lysogeny broth (LB) medium at 37 °C and 170 rpm and collected by centrifugation at 5000*g* for 10 min) 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 × 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 analysed by HPLC-HR-MS/MS in positive and negative mode, as well as HPLC-ESI-(−)-MS/MS precursor ion scanning for *m*/*z* 73.0 [$C_3H_5O_2$].

Liquid chromatography-electrospray ionization-high resolution-tandem mass spectrometry (HPLC-ESI-HR-MS/MS)

HPLC-ESI-HRMS analysis of crude nematode exometabolome extracts and 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 × 3 mm, 5 μm particle diameter) with a flow rate of 400 μl min^{-1} and gradient elution starting at 3% acetonitrile in 0.5% aqueous acetic acid (v/v) for 5 minutes followed by a linear increase to 100% acetonitrile with 0.5% acetic acid (v/v) within 35 minutes. Data were analysed with the Compass DataAnalysis 4.3 software (Bruker).

Liquid chromatography electrospray ionization precursor ion scanning (HPLC-ESI-MS/MS)

MS/MS precursor ion scanning for *m*/*z* 73.0 [$C_3H_5O_2$] was performed using an Agilent 1260 HPLC instrument (Agilent Technologies) coupled to an API5000 Triple Quadrupole mass spectrometer (AB Sciex, Darmstadt) equipped with an electrospray ionization (ESI) unit operated in negative mode. A collision energy of −34 was applied. Chromatographic separations were achieved using an Agilent ZORBAX Eclipse XDB-C18 column (50 × 4.6 mm, 1.8 μm particle diameter) (Agilent Technologies) with a flow rate of 1.1 ml min^{-1} and gradient elution starting at 5% acetonitrile in 0.05% aqueous formic acid (v/v) followed by a linear in-crease to 95% acetonitrile with 0.05% formic acid (v/v) within 10 minutes. Data were analysed with the Analyst 1.6 software (AB Sciex).

NMR spectroscopy

NMR spectra were recorded in CD_3OD at 400 MHz for ^1H and 100 MHz for ^{13}C using a Bruker Avance III HD 400 instrument equipped with a 5 mm BBFO probe (MPICE), Bruker Avance II 400 instrument equipped with a 5 mm BBFO probe (NPAC, UniNE), or at 700 MHz for ^1H and 175 MHz for ^{13}C using a Bruker Avance III HD 700 instrument equipped with a 1.7 mm TCI microcryoprobe (MPICE). Residual solvent signals with ^1H at 3.31 ppm and ^{13}C at 49.05 ppm were used as internal standard. 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. Spectra were zero-filled prior to Fourier transformation, phased, and baseline corrected using the Topspin 3.2 (Bruker) and MNova 9.0 (Mestrelab Research) software.

Enrichment of dimeric ascarosides from *Caenorhabditis* exometabolome extracts

Dimeric ascarosides were enriched from exometabolome extracts obtained from 1.6 L of a *Caenorhabditis nigoni* JU1422 liquid culture supernatant[17] and 1.5 L of a *Caenorhabditis remanei* PB4641 liquid culture supernatant[24] obtained as previously described. The filtered supernatant was frozen at −80 °C, lyophilized, and the residue extracted with 3 × 100 ml methanol for 12 h each. The filtered extracts were concentrated to dryness under reduced pressure and the resulting exometabolome extract were adsorbed onto 2 g of Celite and fractionated by solid phase extraction (SPE) on 5 g reverse phase C18 cartridges (Chromabond, Macherey-Nagel) using a stepwise gradient of methanol in water as eluent (0 to 100% in 10% steps, v/v) to afford 10 fractions (20 ml each). Fractions were concentrated to dryness under reduced pressure and analyzed by HPLC-ESI-HR-MS/MS. Fraction eluting with 40–70% methanol that were found to be rich in dimeric ascarosides were either fractionated again by solid phase extraction (SPE) on 1 g reverse phase C18-endcapped cartridges (Chromabond, Macherey-Nagel) using a stepwise gradient of methanol in water as eluent (0 to 100% in 10% steps, v/v) to afford 10 fractions (5 ml each) and/or fractions containing the target components according to HPLC-ESI-(−)-HR-MS were subsequently submitted to semi-preparative HPLC using an Agilent HP-1100 HPLC instrument equipped with a Grom-Sil 120 ODS-4 HE column (250 × 8 mm, 5 μm) coupled to a Gilson 206 Abimed fraction collector. A flow rate of 2 ml min^{-1} with gradient elution was used starting at 3% acetonitrile in 0.5% aqueous acetic acid (v/v) for 3 minutes, followed by a linear increase to 100% acetonitrile with 0.5% acetic acid (v/v) within 30 minutes. Aliquots of 10 μl were analysed by HPLC-ESI-(−)-HR-MS as described before. Fractions containing the target compounds were concentrated to dryness under reduced pressure, dried in high vacuum, and the residues were dissolved in 550 μl CD_3OD and analysed by one- and two-dimensional NMR spectroscopy.

(4*R*)-4-[[3,6-Dideoxy-4-*O*-[(3*R*)-3-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxo-butyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (4′-(asc-C4)-asc-C5, 5) isolated from the *C. remanei* PB4641 exometabolome (approximately 130 μg); ^{1}H NMR (700 MHz, CD_3OD) δ 1.15 (3H, d, J = 6.1 Hz), 1.16 (3H, d, J = 6.1 Hz), 1.21 (3H, d, J = 6.2 Hz), 1.22 (3H, d, J = 6.2 Hz), 1.72 (ddd, J = 13.0 Hz, J = 11.7 Hz, J = 3.7 Hz), 1.83 (2H, m), 1.92 (1H, dt, J = 13.3 Hz, J = 3.9 Hz), 1.92 (1H, ddd, J = 13.2 Hz, J = 11.4 Hz, J = 3.8 Hz), 2.07 (1H, dt, J = 12.6 Hz, J = 3.8 Hz), 2.22 (1H, ddd, J = 14.9 Hz, J = 9.8 Hz, J = 6.1 Hz), 2.35 (1H, ddd, J = 15.1 Hz, J = 10.0 Hz, J = 6.4 Hz), 2.50 (1H, dd, J = 15.1 Hz, J = 5.4 Hz), 2.56 (1H, dd, J = 15.0 Hz, J = 7.5 Hz), 3.51 (1H, ddd, J = 11.6 Hz, J = 9.5 Hz, J = 4.8 Hz), 3.62 (1H, dq, J = 9.6 Hz, J = 6.2 Hz), 3.70 (1H, s.br), 3.72 (1H, s.br), 3.82 (1H, m), 3.90 (1H, dq, J = 9.6 Hz, J = 6.1 Hz), 4.23 (1H, m), 4.67 (1H, s), 4.69 (1H, s), 4.87 (1H, ddd, J = 11.3 Hz, J = 9.6 Hz, J = 6.1 Hz); ^{13}C NMR (175 MHz, CD_3OD) δ 18.0, 18.0, 18.7, 18.7, 32.7, 35.0, 35.2, 35.6, 43.1, 67.8, 67.9, 69.0, 69.2, 69.3, 71.0, 71.4, 72.2, 96.8, 97.1, 171.8, 182.1 (Table S2†); ESI-(−)-HR-MS: obs. m/z 463.2176 $[M - H]^-$, calc. m/z 463.2185 for $C_{21}H_{35}O_{11}$ (Table S1†).

(4*R*)-4-[[3,6-Dideoxy-2-*O*-[(4*R*)-4-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxo-pentyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (2′-(asc-C5)-asc-C5, 6) enriched from the *C. nigoni* JU1422 exometabolome (approximately 50 μg) along with dominating asc-C8; ^{1}H NMR (400 MHz, CD_3OD) δ 1.14 (3H, d, J = 6.1 Hz), 1.14 (3H, d, 6.3 Hz), 1.22 (3H, d, J = 6.2 Hz), 1.23 (3H, d, J = 6.2 Hz), 1.77 (1H, m), 1.80 (4H, m), 1.87 (1H, ddd, J = 13.2 Hz, J = 11.4 Hz, J = 3.2 Hz), 1.96 (1H, m), 2.01 (1H, dt, J = 13.1 Hz, J = 4.1 Hz), 2.32 (2H, dt, J = 5.0 Hz, J = 7.2 Hz), 2.39 (2H, m), 3.41 (ddd, J = 11.4 Hz, J = 9.6 Hz, J = 4.6 Hz), 3.52 (1H, m), 3.62 (1H, m), 3.68 (1H, dq, J = 9.7 Hz, J = 6.3 Hz), 3.72 (1H, s.br), 3.80 (1H, m), 3.83 (1H, m), 4.65 (1H, s), 4.71 (1H, s), 4.79 (1H, s.br) (Table S2†); ESI-(−)-HR-MS: obs. m/z 477.2348 $[M - H]^-$, calc. m/z 477.2341 for $C_{22}H_{37}O_{11}$ (Table S1†).

(4*R*)-4-[[3,6-Dideoxy-2-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxo-hexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (2′-(asc-C6)-asc-C5, 7) enriched from the *C. nigoni* JU1422 exometabolome (approximately 100 μg) along with dominating asc-ΔC9; ^{1}H NMR (400 MHz, CD_3OD) δ 1.14 (3H, d, J = 6.2 Hz), 1.14 (3H, d, J = 6.3 Hz), 1.22 (3H, d, J = 6.2 Hz), 1.22 (3H, d, J = 6.2 Hz), 1.56 (2H, m), 1.70 (1H, m), 1.77 (1H, ddd, J = 13.3 Hz, J = 11.4 Hz, J = 3.0 Hz), 1.80 (3H, m), 1.90 (1H, ddd, J = 13.3 Hz, J = 11.2 Hz, J = 3.4 Hz), 1.96 (1H, dt, J = 13.1 Hz, J = 4.1 Hz), 2.01 (1H, dt, J = 13.2 Hz, J = 3.9 Hz), 2.32 (2H, m), 2.39 (2H, dt, J = 3.1 Hz, J = 7.4 Hz), 3.40 (ddd, J = 11.5 Hz, J = 9.7 Hz, J = 4.9 Hz), 3.52 (1H, ddd, J = 11.3 Hz, J = 9.6 Hz, J = 4.7 Hz), 3.62 (1H, dq, J = 9.5 Hz, J = 6.2 Hz), 3.71 (1H, dq, J = 9.7 Hz, J = 6.3 Hz), 3.73 (1H, s.br), 3.81 (1H, m), 3.83 (1H, m), 4.65 (1H, s), 4.71 (1H, s), 4.79 (1H, s.br) (Table S2†); ESI-(−)-HR-MS: obs. m/z 491.2495 $[M - H]^-$, calc. m/z 491.2498 for $C_{23}H_{39}O_{11}$ (Table S1†).

(5*R*)-5-[[3,6-Dideoxy-2-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxo-hexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid (2′-(asc-C6)-asc-C6, 8) isolated from the *C. nigoni* JU1422 exometabolome (approximately 100 μg) along with minor amounts of asc-C9; ^{1}H NMR (400 MHz, CD_3OD) δ 1.14 (3H, d, J = 6.3 Hz), 1.14 (3H, d, J = 6.3 Hz), 1.22 (3H, d, J = 6.2 Hz), 1.22 (3H, d, J = 6.2 Hz), 1.57 (4H, m), 1.65 (1H, m), 1.69 (1H, m), 1.76 (1H, m), 1.77 (1H, ddd, J = 13.0 Hz, J = 11.5 Hz, J = 3.1 Hz), 1.79 (1H, m), 1.89 (1H, ddd, J = 13.4 Hz, J = 11.5 Hz, J = 3.3 Hz), 1.95 (1H, dt, J = 13.2 Hz, J = 3.9 Hz), 2.00 (1H, dt, J = 13.3 Hz, J = 3.9 Hz), 2.32 (2H, t, J = 7.2 Hz), 2.39 (2H, dt, J = 3.0 Hz, J = 7.5 Hz), 3.40 (ddd, J = 11.5 Hz, J = 9.7 Hz, J = 4.9 Hz), 3.51 (1H, ddd, J = 11.5 Hz, J = 9.7 Hz, J = 4.9 Hz), 3.62 (1H, dq, J = 9.7 Hz, J = 6.3 Hz), 3.70 (1H, dq, J = 9.7 Hz, J = 6.3 Hz), 3.72 (1H, s.br), 3.79 (1H, m), 3.80 (1H, m), 4.65 (1H, s), 4.71 (1H, s), 4.78 (1H, s.br) (Table S2†); ESI-(−)-HR-MS: obs. m/z 505.2664 $[M - H]^-$, calc. m/z 505.2654 for $C_{24}H_{41}O_{11}$ (Table S1†).

Holding assay to evaluate nematode behavioural response

Nematode preference for environments conditioned with 100 fmol of ascaroside dimers was measured using a modified holding assay. On a 6 cm Petri dish filled with 6 ml peptone-free nematode growth medium (NGM) agar, circular scoring regions of 9 mm diameter were marked. Next, 1 μl of 10% aqueous methanol (v/v, as solvent control) or 100 nM solutions of ascaroside dimers such as natural 4′-(asc-C4)-asc-C5 (**5**) isolated from *C. remanei* PB4641, synthetic 2′-(asc-C6)-asc-C5 (**7**) and 2′-(asc-C6)-asc-C6 (**8**) identical to the natural products isolated from *C. nigoni* JU1422, or their synthetic 4-isomers 4′-(asc-C6)-asc-C5 (**13a**) and 4′-(asc-C6)-asc-C6 (**13b**) in 10% aqueous methanol (v/v) were placed in the centre of the scoring areas onto the agar and left to dry for 5 minutes. Young adult nematodes from non-starved and non-crowded 6 cm NGM agar plates seeded with *E. coli* OP50 were sorted by sex and transferred to peptone-free unseeded NGM agar plates for approximately 30 min before being used for the assay to minimize the amount of concomitant bacteria. Individual worms (up to 3) were placed into the centre of the conditioned scoring region and the time required for the nematodes to leave the scoring region was recorded. Nematodes were defined to have left the scoring area when no part of the nematode was still within the circular boundary. A total number of 20 worms per condition were analysed and experiments were repeated on two separate days with comparable results. A one-way ANOVA with Dunett's post-test was performed using the SPSS statistics software version 26 (IBM) to evaluate the effect of ascaroside dimers *versus* solvent control on mean times nematode spent in scoring regions.

(3*R*)-3-[(2,4-Di-*O*-benzoyL-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-butene (10a). A solution of 2,4-di-*O*-benzoyL-3,6-dideoxy-α-L-*arabino*-hexopyranose (**9**, 395 mg, 1.11 mmol) (synthesized in 6 steps from L-rhamnose[25,26]) in dry dichloromethane (15 ml) was treated with trichloroacetonitrile (222 μL, 2.22 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 16 μL 0.11 mmol). After stirring for 30 min, the solution was concentrated under reduced pressure and the residue was quickly chromatographed over a short column of silica gel using 10% (v/v) ethyl acetate in hexane as eluent to afford 2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl-1-(2,2,2-trichloroethanimidate) (462 mg, 0.92 mmol, 83% yield) as a colourless oil that was immediately used for the next step.

A solution of 2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl-1-(2,2,2-trichloroethanimidate) (462 mg, 0.92 mmol) and (*R*)-(−)-3-butene-2-ol (104 mg, 1.44 mmol) in dry dichloromethane (15 mL) at 0 °C was treated with catalytic amounts of trimethylsilyl triflate (5 μL). After stirring at 0 °C for 1 h and another 3 h at room temperature the mixture was concentrated under reduced pressure and fractionated by column chromatography on silica gel using 2.5% (v/v) ethyl acetate in toluene as eluent to afford (3*R*)-3-[(2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-butene (**10a**, 297 mg, 0.72 mmol, 78% yield) as a colourless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 1.26 (3H, d, J = 6.2 Hz), 1.31 (3H, d, J = 6.4 Hz), 2.23 (1H, m), 2.42 (1H, td, J = 13.5 Hz, J = 3.8 Hz), 4.13 (1H, dq, J = 9.7 Hz, J = 6.1 Hz), 4.31 (1H, m), 4.98 (1H, s), 5.14 (1H, d, J = 10.3 Hz), 5.19 (2H, m), 5.28 (1H, d, J = 17.2 Hz), 5.96 (1H, ddd, J = 6.3 Hz, J = 10.6 Hz, J = 17.1 Hz), 7.47 (4H, m), 7.59 (2H, m), 8.04 (2H, d, J = 7.2 Hz), 8.11 (2H, d, J = 7.1 Hz); ^{13}C {^{1}H} NMR (100 MHz, CD_3OD) δ 17.7, 19.9, 29.7, 66.9, 70.6, 70.9, 74.3, 94.6, 114.8, 128.4 (4C), 129.6 (2C), 129.9 (2C), 130.0, 133.15, 133.24, 140.1, 165.7, 165.8; ESI-(+)-HR-MS: obs. m/z 433.1631 [M + Na]$^+$, calc. m/z 433.1622 for $C_{24}H_{26}NaO_6$.

(4*R*)-4-[(2,4-Di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-pentene (10b). A solution of 2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl-1-(2,2,2-trichloroethanimidate) (462 mg, 0.92 mmol; see **10a** for its preparation) and (*R*)-(−)-4-pentene-2-ol (126 mg, 1.46 mmol) in dry dichloromethane (15 mL) at 0 °C was treated with catalytic amounts of trimethylsilyl triflate (5 μL). After stirring at 0 °C for 1 h and another 3 h at room temperature the mixture was concentrated under reduced pressure. The residue was fractionated by column chromatography on silica gel using 2.5% (v/v) ethyl acetate in toluene as eluent to afford (4*R*)-4-[(2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-pentene (**10b**, 332 mg, 0.78 mmol, 85% yield) as a colourless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 1.20 (3H, d, J = 6.1 Hz), 1.24 (3H, d, J = 6.2 Hz), 2.19 (1H, ddd, J = 13.5 Hz, J = 11.3 Hz, J = 2.8 Hz), 2.31 (1H, m), 2.40 (2H, m), 3.92 (1H, tq, J = 9.2 Hz, J = 6.1 Hz), 4.20 (1H, dq, J = 9.7 Hz, J = 6.2 Hz), 4.96 (1H, s), 5.12 (4H, m), 5.92 (1H, ddt, J = 17.2 Hz, J = 9.9 Hz, J = 7.2 Hz), 7.50 (4H, m), 7.62 (2H, m), 8.01 (2H, d, J = 7.2 Hz), 8.09 (2H, d, J = 7.2 Hz); ^{13}C {^{1}H} NMR (100 MHz, CD_3OD) δ 18.1, 19.3, 30.6, 42.7, 68.2, 72.0, 72.7, 73.8, 95.2, 117.6, 129.7 (4C), 130.5 (2C), 130.7 (2C), 131.2, 134.48, 134.54, 136.3, 167.0, 167.1; ESI-(+)-HR-MS: obs. m/z 447.1782 [M + Na]$^+$, calc. m/z 447.1778 for $C_{25}H_{28}NaO_6$.

Benzyl (2*E*,4*R*)-4-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-pentenoate (11a). A solution of (3*R*)-3-[(2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-butene (**10a**, 180 mg, 0.439 mmol) in methanol (1.8 ml) was treated with 820 μL of saturated aqueous lithium hydroxide (105 mg, 4.39 mmol) solution. The mixture was stirred for 12 h at room temperature, the pH neutralized with acetic acid, and the solution concentrated under reduced pressure. The product was purified by column chromatography on silica gel using 10% methanol (v/v) in dichloromethane as eluent to afford (3*R*)-3-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-butene (64.7 mg, 0.320 mmol, 73% yield) as a colourless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 1.19 (3H, d, J = 6.2 Hz), 1.22 (3H, d, J = 6.4 Hz), 1.79 (1H, ddd, J = 13.2 Hz, J = 11.2 Hz, J = 3.1 Hz), 1.95 (1H, ddd, J = 13.1 Hz, J = 3.9 Hz, J = 3.9 Hz), 3.51 (1H, ddd, J = 11.1 Hz, J = 9.5 Hz, J = 4.6 Hz), 3.61 (1H, qd, J = 9.3 Hz, J = 6.0 Hz), 3.75 (1H, br s), 4.23 (1H, qd, J = 6.3 Hz, J = 6.4 Hz), 4.66 (1H, s), 5.06 (1H, ddd, J = 10.5 Hz, J = 1.6 Hz, J = 1.6 Hz), 5.22 (1H, ddd, J = 17.3 Hz, J = J = 1.5 Hz), 5.90 (1H, ddd, J = 17.2 Hz, J = 10.5 Hz, J = 6.1 Hz); ESI-(+)-HR-MS: obs. m/z 225.1103 [M + Na]$^+$, calc. m/z 225.1097 for $C_{10}H_{18}NaO_4$.

A solution of (3*R*)-3-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-butene (64.7 mg 0.320 mmol) in dry dichloromethane (6.5 ml) was treated with benzyl acrylate (240 μL, 259.6 mg, 1.6 mmol) and Grubbs 2nd generation catalyst (27.2 mg, 32.0 μmol, 10 mol%). After stirring at reflux for 6 h the solution was concentrated under reduced pressure and the residue fractionated on silica gel using 5% (v/v) methanol in dichloromethane as eluent to afford benzyl (2*E*,4*R*)-4-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-pentenoate (**11a**, 51.2 mg, 0.152 mmol, 48% yield) as a colourless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 1.15 (3H, d, J = 5.80 Hz), 1.28 (3H, d, J = 6.6 Hz), 1.79 (1H, ddd, J = 13.3 Hz, J = 10.9 Hz, J = 3.1 Hz), 1.96 (1H, m), 3.52 (2H, m), 3.78 (1H, br.s), 4.44 (1H, m), 4.68 (1H, s), 5.18 (2H, s), 6.07 (1H, dd, J = 15.8 Hz, J = 1.7 Hz), 6.99 (1H, dd, J = 15.7 Hz, J = 5.4 Hz), 7.34 (5H, m); ^{13}C {^{1}H} NMR (100 MHz, CD_3OD) δ 17.9, 19.6, 36.0, 67.3, 68.2, 69.6, 71.4, 72.4, 98.6, 120.6, 129.26, 129.31 (2C), 129.6 (2C), 151.6, 167.8; ESI-(+)-HR-MS: obs. m/z 359.1474 [M + Na]$^+$, calc. m/z 359.1465 for $C_{18}H_{24}NaO_6$.

Benzyl (2*E*,5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-hexenoate (11b). A solution of (4*R*)-4-[(2,4-di-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-pentene (**10b**, 200 mg, 0.471 mmol) in methanol (2 mL) was treated with 881 μL of saturated aqueous lithium hydroxide (112 mg, 4.71 mmol) solution. The mixture was stirred for 12 h at room temperature, the pH neutralized with acetic acid, and the solution concentrated under reduced pressure. The product was purified by column chromatography on silica gel using 10% (v/v) methanol in dichloromethane as eluent to afford (4*R*)-4-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-pentene (88.8 mg, 0.411 mmol, 87% yield) as a colourless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 1.13 (3H, d, J = 6.1 Hz), 1.20 (3H, d, J = 6.2 Hz), 1.76 (1H, ddd, J = 13.1 Hz, J = 11.3 Hz, J = 3.1 Hz), 1.95 (1H, ddd, J = 12.8 Hz, J = 3.3 Hz, J = 0.9 Hz), 2.28 (2H, m), 3.51 (1H, ddd, J = 11.3 Hz, J = 9.5 Hz, J = 4.6 Hz), 3.65 (1H, dq, J = 9.5 Hz, J = 6.4 Hz), 3.72 (1H, br s), 3.82 (1H, m), 4.64 (1H, s), 5.06 (2H, m), 5.88 (1H, ddt, J = 17.2 Hz, J = 10.3 Hz, J = 7.2 Hz); ESI-(+)-HR-MS: obs. m/z 239.1266 [M + Na]$^+$, calc. m/z 239.1254 for $C_{11}H_{20}NaO_4$.

A solution of (4*R*)-4-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-pentene (98.8 mg 0.457 mmol) in dry dichloromethane (10 mL) was treated with benzyl acrylate (343 μL, 370 mg, 2.28 mmol) and Grubbs 2nd generation catalyst (38.8 mg, 45.7 μmol, 10 mol%). After stirring at reflux for 6 h the solution was concentrated under reduced pressure and the residue fractionated on silica gel using 5% methanol in dichloromethane (v/v) as eluent to afford benzyl (2*E*,5*R*)-5-[(3,6-dideoxy-

α-L-*arabino*-hexopyranosyl)oxy]-2-hexenoate (**11b**, 118.9 mg 0.339 mmol, 74% yield) as a colourless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 1.16 (3H, d, J = 5.9 Hz), 1.17 (3H, d, J = 6.1 Hz), 1.74 (1H, ddd, J = 13.1 Hz, J = 11.0 Hz, J = 2.9 Hz), 1.94 (1H, dt, J = 12.9 Hz, J = 3.9 Hz), 2.45 (2H, m), 3.50 (1H, ddd, J = 11.1 Hz, J = 9.4 Hz, J = 4.5 Hz), 3.59 (1H, dq, J = 9.6 Hz, J = 6.0 Hz), 3.72 (1H, br.s), 3.94 (1H, m), 4.63 (1H, s), 5.17 (2H, s), 5.97 (1H, d, J = 15.7 Hz), 7.05 (1H, dt, J = 15.6 Hz, J = 7.3 Hz), 7.35 (5H, m); ^{13}C {^{1}H} NMR (100 MHz, CD_3OD) δ 18.1, 19.5, 35.9, 40.8, 67.2, 68.3, 69.9, 71.4, 72.4, 98.2, 124.0, 129.18, 129.21 (2C), 129.5 (2C), 147.9, 167.7; ESI-(+)-HR-MS: obs. m/z 373.1629 $[M + Na]^+$, calc. m/z 373.1622 for $C_{19}H_{26}NaO_6$.

(5*R*)-5-[(2,4-Di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-hexanoic acid (12). A solution of benzyl (2*E*,5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-hexenoate (**11b**, 55 mg, 157 μmol) in dry dichloromethane (5 mL) was treated with imidazole (53 mg, 785 μmol) and *tert*-butyldimethylsilyl chloride (118 mg, 785 μmol). After stirring overnight, the reaction was quenched with water (1 mL) and the organic phase dried over sodium sulphate and concentrated under reduced pressure. The residue was fractionated by column chromatography on silica gel using 15% (v/v) ethyl acetate in hexane as eluent to afford benzyl (2*E*,5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-hexenoate (40 mg, 69.1 μmol, 44% yield) as a colourless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 0.07 (12H, m), 0.89 (9H, s), 0.92 (9H, s), 1.12 (3H, d, J = 6.0 Hz), 1.14 (3H, d, J = 6.2 Hz), 1.74 (1H, ddd, J = 12.9 Hz, J = 10.2 Hz, J = 2.7 Hz), 1.82 (1H, ddd, J = 12.9 Hz, J = J = 3.7 Hz), 2.43 (2H, ddd, J = 7.3 H z, J = 6.2 Hz, J = 1.2 Hz), 3.59 (1H, dq, J = 8.9 Hz, J = 5.9 Hz), 3.65 (1H, ddd, J = 9.9 Hz, J = 9.2 Hz, J = 4.3 Hz), 3.80 (1H, br.s), 3.92 (1H, tq, J = 6.2 Hz, J = 6.1 Hz), 4.53 (1H, s), 5.13 (1H, d, J = 12.5 Hz), 5.17 (1H, d, J = 12.5 Hz), 5.96 (1H, dt, J = 15.7 Hz, J = 1.7 Hz), 7.03 (1H, dt, J = 15.5 Hz, J = 7.4 Hz), 7.32 (5H, m); ^{13}C {^{1}H} NMR (100 MHz, CD_3OD) δ −4.71 (2C), −4.4, −3.8, 18.6, 18.8, 18.9, 19.6, 26.3, 26.4 (3C), 37.9, 40.8, 67.1, 70.1, 71.2, 71.6, 72.7, 98.3, 124.1, 129.14 (2C), 129.15, 129.5 (2C), 137.6, 147.9, 167.5; ESI-(+)-HR-MS: obs. m/z 601.3334 $[M + Na]^+$, calc. m/z 601.3351 for $C_{31}H_{54}NaO_6Si_2$.

A solution of benzyl (2*E*,5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-hexenoate (40 mg, 69.1 μmol) in MeOH (0.5 mL) was treated with 10 mol% palladium on carbon (20 mg) and hydrogenated under H_2 atmosphere (1 atm). After stirring for 4 h the catalyst was removed by filtration over Celite and the product was obtained after concentration under reduced pressure to afford (5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-hexanoic acid (**12**, 32.7 mg, 66.7 μmol, 96% yield) as a colourless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 0.09 (12H, m), 0.91 (9H, s), 0.92 (9H, s), 1.13 (3H, d, J = 6.1 Hz), 1.17 (3H, d, J = 6.0 Hz), 1.45–1.78 (3H, m), 1.85 (1H, ddd, J = 12.7 Hz, J = J = 3.8 Hz), 2.31 (2H, td, J = 7.7 Hz, J = 2.4 Hz), 3.63 (1H, m), 3.67 (1H, m), 3.79 (1H, m), 3.80 (1H, s.br), 4.54 (1H, s); ^{13}C {^{1}H} NMR (100 MHz, CD_3OD) δ −4.80, −4.78, −4.4, −3.9, 18.5, 18.8, 18.9, 19.2, 26.25 (3C), 26.30 (3C), 31.3, 33.4, 38.0, 70.2, 71.3, 71.6, 71.7, 97.8, 177.4; ESI-(+)-HR-MS: obs. m/z 513.3047 $[M + Na]^+$, calc. m/z 513.3038 for $C_{24}H_{50}NaO_6Si_2$.

Benzyl (2*E*,4*R*)-4-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-pentenoate & benzyl (2*E*,4*R*)-4-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldi-methylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-pentenoate. A solution of (5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-hexanoic acid (12, 5.0 mg, 10.2 μmol) in dry dichloromethane (0.5 mL) at 0 °C was treated with 4-dimethylaminopyridine (DMAP, 2.5 mg, 20.4 μmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC HCl, 3.9 mg, 20.4 μmol). After stirring for 5 minutes a solution of benzyl (2*E*,4*R*)-4-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-pentenoate (**11a**, 6.9 mg, 20.4 μmol) in dry dichloromethane (0.5 mL) was added. After 2 h at 0 °C, the solution was concentrated under reduced pressure and the residue was fractionated by column chromatography on silica gel using a stepwise gradient from 10% to 30% (v/v) ethyl acetate in hexane as eluent to afford the 2-linked benzyl (2*E*,4*R*)-4-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-pentenoate (2.1 mg, 2.57 μmol, 25% yield) and the 4-linked benzyl (2*E*,4*R*)-4-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-pentenoate (2.6 mg, 3.18 μmol, 31% yield) as colourless oils.

Benzyl (2*E*,4*R*)-4-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-pentenoate. ^{1}H NMR (400 MHz, CD_3OD) δ 0.08 (12H, s), 0.90 (9H, s), 0.92 (9H, s), 1.12 (3H, d, J = 6.0 Hz), 1.16 (3H, d, J = 6.4 Hz), 1.17 (3H, d, J = 6.1 Hz), 1.29 (3H, d, J = 6.5 Hz), 1.55 (2H, m), 1.78 (5H, m), 2.02 (1H, m), 2.40 (2H, t, J = 7.0 Hz), 3.42 (1H, m), 3.63 (3H, m), 3.79 (2H, m), 4.44 (1H, m), 4.54 (1H, s), 4.76 (1H, s), 4.86 (1H, s.br), 5.19 (2H, s), 6.08 (1H, d, J = 15.3 Hz), 6.98 (1H, dd, J = 15.7 Hz, J = 5.3 Hz), 7.36 (5H, m); ESI-(+)-HR-MS: obs. m/z 831.4512 $[M + Na]^+$, calc. m/z 831.4504 for $C_{42}H_{72}ONaO_{11}Si_2$.

Benzyl (2*E*,4*R*)-4-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-pentenoate. ^{1}H NMR (400 MHz, CD_3OD) δ 0.07 (12H, s), 0.90 (9H, s), 0.93 (9H, s), 1.07 (3H, d, J = 6.4 Hz), 1.11 (3H, d, J = 5.9 Hz), 1.17 (3H, d, J = 6.0 Hz), 1.29 (3H, d, J = 6.4 Hz), 1.53 (2H, m), 1.67 (1H, m), 1.80 (4H, m), 2.06 (1H, dt, J = 12.9 Hz, J = 3.3 Hz), 2.36 (2H, m), 3.64 (2H, m), 3.78 (4H, m), 4.44 (1H, m), 4.54 (1H, s), 4.72 (1H, s), 4.85 (1H, m), 5.19 (2H, s), 6.06 (2H, d, J = 15.2 Hz), 6.99 (1H, dd, J = 15.4 Hz, J = 5.7 Hz), 7.36 (5H, m); ESI-(+)-HR-MS: obs. m/z 831.4515 $[M + Na]^+$, calc. m/z 831.4504 for $C_{42}H_{72}ONaO_{11}Si_2$.

Benzyl (2*E*,5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-hexenoate & Benzyl (2*E*,5*R*)-5-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldi-methylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexo-pyranosyl]oxy]-2-hexenoate. A solution of (5*R*)-5-

[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-hexanoic acid (**12**, 4.5 mg, 9.2 μmol) in dry dichloromethane (0.5 mL) at 0 °C was treated with 4-dimethylaminopyridine (DMAP, 2.3 mg, 18.4 μmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC HCl, 3.5 mg, 18.4 μmol). After stirring for 5 minutes a solution of benzyl (2*E*,5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-hexenoate (**11b**, 6.5 mg, 18.4 μmol) in dry dichloromethane (0.5 mL) was added. After 2 h at 0 °C the solution was concentrated under reduced pressure and the residue fractionated by column chromatography on silica gel using a stepwise gradient from 10% to 30% (v/v) ethyl acetate in hexane as eluent to afford the 2-linked benzyl (2*E*,5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethyl-silyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-hexenoate (2.4 mg, 2.89 μmol, 31% yield) and the 4-linked benzyl (2*E*,5*R*)-5-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl) oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-hexenoate (1.1 mg, 1.32 μmol, 14% yield) as colourless oils.

Benzyl (2*E*,5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-hexenoate. ^{1}H NMR (400 MHz, CD_3OD) δ 0.08 (12H, s), 0.90 (9H, s), 0.92 (9H, s), 1.12 (3H, d, J = 6.0 Hz), 1.17 (6H, m), 1.46–1.89 (9H, m), 1.99 (1H, dt, J = 13.5 Hz, J = 3.5 Hz), 2.40 (1H, t, J = 6.6 Hz), 2.46 (1H, t, J = 6.6 Hz), 3.42 (1H, m), 3.65 (3H, m), 3.78 (2H, m), 3.80 (1H, s), 3.94 (1H, q, J = 6.1 Hz), 4.54 (1H, s), 4.71 (1H, s), 4.78 (1H, s.br), 5.17 (2H, s), 5.98 (1H, d, J = 15.7 Hz), 7.05 (1H, dt, J = 15.5 Hz, J = 7.8 Hz), 7.34 (5H, m); ESI-(+)-HR-MS: obs. m/z 845.4670 $[M + Na]^+$, calc. m/z 845.4662 for $C_{43}H_{74}ONaO_{11}Si_2$.

Benzyl (2*E*,5*R*)-5-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-hexenoate. ^{1}H NMR (400 MHz, CD_3OD) δ 0.08 (12H, s), 0.90 (9H, s), 0.92 (9H, s), 1.07 (3H, d, J = 6.2 Hz), 1.10 (3H, d, J = 6.1 Hz), 1.17 (3H, d, J = 5.3 Hz), 1.19 (3H, d, J = 5.1 Hz), 1.46 (2H, m), 1.54 (2H, m), 1.63 (1H, m), 1.79 (2H, m), 2.02 (1H, dt, J = 11.9 Hz, J = 4.1 Hz), 2.27 (2H, m), 2.45 (2H, t, J = 7.1 Hz), 3.63 (2H, m), 3.72 (1H, s. br), 3.79 (3H, m), 3.95 (1H, m), 4.53 (1H, s), 4.68 (1H, s), 4.84 (1H, m), 5.19 (2H, s), 5.98 (1H, d, J = 15.8 Hz), 7.07 (1H, dt, J = 14.4 Hz, J = 7.7 Hz), 7.31 (5H, m); ESI-(+)-HR-MS: obs. m/z 845.4675 $[M + Na]^+$, calc. m/z 845.4662 for $C_{43}H_{74}ONaO_{11}Si_2$.

(4*R*)-4-[[3,6-Dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid. A solution of benzyl (2*E*,4*R*)-4-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-pentenoate (2.6 mg, 3.21 μmol) in methanol (0.5 mL) was treated with 10 mol% palladium on carbon (10 mg) and hydrogenated under H_2 atmosphere (1 atm). After stirring for 4 h the catalyst was removed by filtration over Celite and the product was obtained without further purification by concentration under reduced pressure to afford (4*R*)-4-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (2.1 mg, 2.91 μmol, 90% yield) as a colourless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 0.08 (12H, s), 0.90 (9H, s), 0.92 (9H, s), 1.12 (3H, d, J = 6.1 Hz), 1.14 (3H, d, J = 6.0 Hz), 1.16 (3H, d, J = 6.0 Hz), 1.18 (3H, d, J = 6.1 Hz), 1.49 (2H, m), 1.62 (1H, m), 1.81 (6H, m), 2.04 (1H, td, J = 12.2 Hz, J = 4.0 Hz), 2.36 (4H, m), 3.63 (2H, m), 3.72 (1H, s.br), 3.82 (3H, m), 4.54 (1H, s), 4.69 (1H, s), 4.88 (1H, m); ^{13}C NMR (100 MHz, CD_3OD) δ −4.8 (4C), 17.9, 18.4, 18.5, 19.1, 22.2, 26.0 (6C), 32.5, 32.9, 34.0, 34.6, 37.4, 37.7, 68.1, 69.3, 70.0, 71.0, 71.2, 71.4, 71.9, 72.1, 97.1, 97.7; ESI-(−)-HR-MS: obs. m/z 719.4237 $[M - H]^-$, calc. m/z 719.4227 for $C_{35}H_{67}O_{11}Si_2$.

(4*R*)-4-[[3,6-Dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid. Benzyl (2*E*,4*R*)-4-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-pentenoate (2.1 mg, 2.60 μmol) was hydrogenated as described for the 2′-(asc-C6)-asc-C5 derivative to afford (4*R*)-4-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (1.8 mg, 2.50 μmol, 96% yield) as a colourless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 0.08 (12H, s), 0.90 (9H, s), 0.93 (9H, s), 1.12 (3H, d, J = 6.4 Hz), 1.14 (3H, d, J = 6.8 Hz), 1.18 (3H, d, J = 5.8 Hz), 1.23 (3H, d, J = 6.1 Hz), 1.55 (2H, m), 1.68 (1H, m), 1.79 (5H, m), 1.90 (1H, m), 2.00 (1H, m), 2.25 (1H, m), 2.34 (1H, m), 2.40 (2H, t, J = 7.0 Hz), 3.39 (1H, m), 3.66 (3H, m), 3.81 (3H, m), 4.54 (1H, s), 4.72 (1H, s), 4.78 (1H, s br); ^{13}C NMR (100 MHz, CD_3OD) δ −4.8 (4), 17.8, 18.3, 18.8, 19.0, 22.0, 26.0 (6), 33.0, 34.6, 34.6, 34.7, 37.1, 37.7, 68.5, 70.0, 70.4, 71.2, 71.3, 71.9, 72.2, 72.5, 94.1, 97.8; ESI-(−)-HR-MS: obs. m/z 719.4241 $[M - H]^-$, calc. m/z 719.4227 for $C_{35}H_{67}O_{11}Si_2$.

(5*R*)-5-[[3,6-Dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic-acid. Benzyl (2*E*,5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-hexenoate (2.4 mg, 2.89 μmol) was hydrogenated as described for the 2′-(asc-C6)-asc-C5 derivative to afford (5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid (1.5 mg, 2.04 μmol, 70% yield) as a colourless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 0.08 (12H, s), 0.90 (9H, s), 0.92 (9H, s), 1.12 (3H, d, J = 5.8 Hz), 1.13 (3H, d, J = 5.8 Hz), 1.17 (3H, d, J = 6.0 Hz), 1.22 (3H, d, J = 6.1 Hz), 1.59 (6H, m), 1.83 (5H, m), 2.00 (1H, m), 2.21 (2H, m), 2.40 (2H, m), 3.39 (1H, m), 3.66 (3H, m), 3.80 (3H, m), 4.54 (1H, s), 4.71 (1H, s), 4.78 (1H, s.br); ^{13}C NMR (100 MHz, CD_3OD) δ −4.9 (4C), 17.9, 18.3 (2C), 18.9, 22.1, 23.6, 26.0 (6C), 32.7, 34.8, 37.7, 37.7, 38.1, 38.5, 67.8, 69.3, 69.9, 70.9, 71.1, 71.3, 72.1, 72.4, 97.3, 97.7; ESI-(−)-HR-MS: obs. m/z 733.4399 $[M - H]^-$, calc. m/z 733.4384 for $C_{36}H_{69}O_{11}Si_2$.

(5*R*)-5-[[3,6-Dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid. Benzyl (2*E*,5*R*)-

5-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-2-hexenoate (1.1 mg, 1.32 μmol) was hydrogenated as described for the 2′-(asc-C6)-asc-C5 derivative to afford (5*R*)-5-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid (0.9 mg, 1.22 μmol, 92% yield) as a colourless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 0.08 (12H, s), 0.90 (9H, s), 0.92 (9H, s), 1.12 (9H, m), 1.14 (9H, d, J = 6.1 Hz), 1.17 (3H, d, J = 6.1 Hz), 1.49 (2H, m), 1.62 (4H, m), 1.76 (3H, m), 1.85 (2H, m), 2.03 (1H, m), 2.20 (2H, t, J = 7.3 Hz), 2.35 (2H, m), 3.62 (2H, m), 3.71 (1H, s br), 3.80 (3H, m), 3.87 (1H, m), 4.54 (1H, s), 4.68 (1H, s), 4.86 (1H, m); ^{13}C NMR (100 MHz, CD_3OD) δ −4.9 (4C), 17.9, 18.3, 18.6, 18.9, 22.1, 23.6, 26.0 (6C), 32.7, 34.8, 37.7, 37.7, 38.1, 38.5, 67.8, 69.3, 69.9, 70.9, 71.1, 71.3, 72.1, 72.4, 97.3, 97.7; ESI-(−)-HR-MS: obs. m/z 733.4392 $[M - H]^-$, calc. m/z 733.4384 for $C_{36}H_{69}O_{11}Si_2$.

(4*R*)-4-[[3,6-Dideoxy-2-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (2′-(asc-C6)-asc-C5, 7). In a 2 ml polypropylene cryovial a solution of (4*R*)-4-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexo-pyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (2.1 mg, 2.91 μmol) in dry tetrahydrofuran (500 μL) was treated with dry pyridine (40 μL, 500 μmol) and Olah's reagent (70% (w/w) hydrogen fluoride in pyridine, 13 μl, 500 μmol). After stirring for 10 days the reaction was quenched by transfer into a 4 ml glass vial and addition of saturated aqueous sodium hydrogencarbonate solution (0.1 mL), dried over sodium sulphate, filtered, and concentrated under reduced pressure. The residue was fractionated by solid phase extraction (SPE) on a 100 mg reverse phase C18ec cartridge (Chromabond, Macherey Nagel) using a stepwise gradient of methanol in water (0–100% in 10% steps, v/v) as eluent to afford (4*R*)-4-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (7, 0.5 mg, 1.02 μmol, 35% yield) as a colourless oil identical to the natural product from *C. nigoni*. ^{1}H NMR (400 MHz, CD_3OD) δ 1.14 (3H, d, J = 6.1 Hz), 1.14 (3H, d, 6.1 Hz), 1.23 (3H, d, J = 6.1 Hz), 1.23 (3H, d, J = 6.1 Hz), 1.56 (2H, m), 1.70 (1H, m), 1.77 (1H, ddd, J = 13.2 Hz, J = 11.1 Hz, J = 3.0 Hz), 1.80 (3H, m), 1.90 (1H, ddd, J = 13.4 Hz, J = 11.8 Hz, J = 3.4 Hz), 1.96 (1H, dt, J = 13.2 Hz, J = 3.7 Hz), 2.01 (1H, dt, J = 13.0 Hz, J = 3.8 Hz), 2.34 (1H, m), 2.37 (1H, m), 2.39 (2H, dt, J = 3.2 Hz, J = 7.3 Hz), 3.40 (ddd, J = 11.4 Hz, J = 9.6 Hz, J = 4.4 Hz), 3.52 (1H, ddd, J = 11.2 Hz, J = 9.5 Hz, J = 4.4 Hz), 3.62 (1H, dq, J = 9.3 Hz, J = 6.2 Hz), 3.71 (1H, dq, J = 9.6 Hz, J = 6.2 Hz), 3.73 (1H, s.br), 3.81 (1H, m), 3.83 (1H, m), 4.65 (1H, s), 4.71 (1H, s), 4.79 (1H, s.br); ^{13}C NMR (100 MHz, CD_3OD) δ 17.9, 17.9, 18.7, 18.7, 21.8, 33.4, 34.0, 34.6, 34.9, 35.1, 37.2, 68.2, 68.2, 70.1, 70.1, 70.9, 72.2, 72.2, 72.3, 94.1, 97.6 (Table S3†); ESI-(−)-HR-MS: obs. m/z 491.2502 $[M - H]^-$, calc. m/z 491.2498 for $C_{23}H_{39}O_{11}$.

(5*R*)-5-[[3,6-Dideoxy-2-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid (2′-(asc-C6)-asc-C6, 8). (5*R*)-5-[[3,6-Dideoxy-2-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid (1.5 mg, 2.03 μmol) was treated as described for 7 to afford (5*R*)-5-[[3,6-dideoxy-2-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid (**8**, 0.5 mg, 0.99 μmol, 49% yield) as a colourless oil identical to the natural product from *C. nigoni*. ^{1}H NMR (400 MHz, CD_3OD) δ 1.14 (3H, d, J = 6.1 Hz), 1.14 (3H, d, 6.2 Hz), 1.22 (3H, d, J = 6.3 Hz), 1.22 (3H, d, J = 6.3 Hz), 1.57 (4H, m), 1.65(1H, m), 1.70 (1H, m), 1.76 (1H, m), 1.77 (1H, ddd, J = 13.3 Hz, J = 11.2 Hz, J = 3.1 Hz), 1.79 (1H, m), 1.88 (1H, ddd, J = 13.4 Hz, J = 11.5 Hz, J = 3.0 Hz), 1.95 (1H, dt, J = 13.4 Hz, J = 3.8 Hz), 2.00 (1H, dt, J = 13.2 Hz, J = 3.6 Hz), 2.24 (2H, t, J = 7.3 Hz), 2.39 (2H, dt, J = 3.1 Hz, J = 7.3 Hz), 3.41 (ddd, J = 11.3 Hz, J = 9.7 Hz, J = 4.7 Hz), 3.51 (1H, ddd, J = 11.1 Hz, J = 9.6 Hz, J = 4.6 Hz), 3.62 (1H, dq, J = 9.5 Hz, J = 6.3 Hz), 3.70 (1H, dq, J = 9.5 Hz, J = 6.3 Hz), 3.72 (1H, s.br), 3.79 (1H, m), 3.80 (1H, m), 4.65 (1H, s), 4.71 (1H, s), 4.78 (1H, s.br); ^{13}C NMR (100 MHz, CD_3OD) δ 17.8, 17.8, 18.7, 18.7, 22.6, 22.6, 33.1, 34.7, 35.7, 36.8, 37.4, 37.4, 68.3, 68.5, 70.1, 70.1, 70.9, 72.2, 72.2, 72.3, 94.3, 97.4 (Table S3†); ESI-(−)-HR-MS: obs. m/z 505.2657 $[M - H]^-$, calc. m/z 505.2654 for $C_{24}H_{41}O_{11}$.

(4*R*)-4-[[3,6-Dideoxy-4-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (4′-(asc-C6)-asc-C5, 13a)

(4*R*)-4-[[3,6-Dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexo-pyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (1.8 mg, 2.50 μmol) was treated as described for 7 to afford (4*R*)-4-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-pentanoic acid (**13a**, 0.6 mg, 1.22 μmol, 49% yield) as a colourless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 1.13 (3H, d, J = 6.2 Hz), 1.14 (3H, d, J = 6.3 Hz), 1.16 (3H, d, J = 6.2 Hz), 1.22 (3H, d, J = 6.2 Hz), 1.56 (2H, m), 1.67 (1H, m), 1.76 (1H, ddd, J = 13.1 Hz, J = 11.1 Hz, J = 3.0 Hz), 1.79 (1H, m), 1.80 (2H, m), 1.85 (1H, ddd, J = 13.3 Hz, J = 11.3 Hz, J = 3.0 Hz), 1.95 (1H, dt, J = 13.0 Hz, J = 3.8 Hz), 2.04 (1H, dt, J = 12.9 Hz, J = 4.2 Hz), 2.35 (2H, m), 2.40 (2H, t, J = 7.4 Hz), 3.51 (1H, ddd, J = 11.0 Hz, J = 9.5 Hz, J = 4.6 Hz), 3.61 (1H, dq, J = 9.4 Hz, J = 6.2 Hz), 3.72 (1H, s.br), 3.73 (1H, s.br), 3.80 (1H, m), 3.85 (1H, m), 3.85 (1H, dq, J = 9.7 Hz, J = 6.2 Hz), 4.64 (1H, s), 4.69 (1H, s), 4.87 (1H, ddd, J = 11.3 Hz, J = 9.7 Hz, J = 4.5 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 17.8, 18.5, 18.5, 18.5, 22.3, 31.4, 33.2, 33.2, 34.8, 35.6, 37.3, 68.2, 68.2, 69.6, 69.6, 71.1, 71.1, 71.7, 71.9, 97.2, 97.4 (Table S3†); ESI-(−)-HR-MS: obs. m/z 491.2503 $[M - H]^-$, calc. m/z 491.2498 for $C_{23}H_{39}O_{11}$.

(5*R*)-5-[[3,6-Dideoxy-4-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid (4′-(asc-C6)-asc-C6, 13b). (5*R*)-5-[[3,6-Dideoxy-4-*O*-[(5*R*)-5-[(2,4-di-*O*-*tert*-butyldimethylsilyl-3,6-dideoxy-α-L-*arabino*-hexo-pyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid (0.9 mg, 1.28 μmol) was treated as described for 7 to afford (5*R*)-5-[[3,6-dideoxy-4-*O*-[(5*R*)-5-[(3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-oxohexyl]-α-L-*arabino*-hexopyranosyl]oxy]-hexanoic acid (**13b**, 0.2 mg, 0.39 μmol, 30% yield) as a colourless oil. ^{1}H NMR (400 MHz, CD_3OD) δ 1.13 (3H, d, J = 6.2 Hz), 1.14 (3H, d, J = 6.2 Hz), 1.14 (3H, d, J = 6.2 Hz), 1.22

(3H, d, J = 6.2 Hz), 1.57 (4H, m), 1.67 (2H, m), 1.76 (1H, ddd, J = 13.2 Hz, J = 11.2 Hz, J = 3.0 Hz), 1.77 (1H, m) 1.78 (1H, m), 1.85 (1H, ddd, J = 12.9 Hz, J = 1.4 Hz, J = 2.9 Hz), 1.95 (1H, dt, J = 13.2 Hz, J = 3.9 Hz), 2.03 (1H, dt, J = 12.8 Hz, J = 4.0 Hz), 2.25 (2H, t, J = 7.2 Hz), 2.35 (2H, dt, J = 5.2 Hz, J = 7.2 Hz), 3.51 (1H, ddd, J = 11.2 Hz, J = 9.4 Hz, J = 4.5 Hz), 3.61 (1H, dq, J = 9.5 Hz, J = 6.1 Hz), 3.72 (2H, s.br), 3.80 (2H, m), 3.86 (1H, dq, J = 9.7 Hz, J = 6.2 Hz), 4.64 (1H, s), 4.69 (1H, s), 4.86 (1H, ddd, J = 11.4 Hz, J = 9.7 Hz, J = 4.6 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 17.9, 18.7, 18.7, 18.7, 22.6, 22.6, 32.9, 34.8, 35.8, 36.8, 37.6, 37.6, 67.9, 68.0, 69.5, 69.5, 71.0, 71.3, 72.1, 72.1, 97.3, 97.3 (Table S3†); ESI-(−)-HR-MS: obs. m/z 505.2656 $[M - H]^-$, calc. m/z 505.2654 for $C_{24}H_{41}O_{11}$.

Conflicts of interest

There are no conflicts to declare.

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