Organic & Biomolecular Chemistry

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/d5ob00271k

Identification and synthesis of 4′-*ortho*-aminobenzoyl ascarosides as sex pheromones of gonochoristic *Caenorhabditis nigoni*†

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Received 14th February 2025,
Accepted 12th March 2025

DOI: 10.1039/d5ob00271k

rsc.li/obc

Using a combination of RP-C18 chromatography, MS and NMR techniques, a new class of homologous modular ascarosides carrying a 4′-*ortho*-aminobenzoyl moiety was identified from *Caenorhabditis nigoni* and *Caenorhabditis tropicalis*. These compounds could not be detected using targeted ascaroside screens based on precursor ion screening for *m/z* 73.0294 $[C_3H_5O_2]^-$, which highlighted a limitation of the current protocols. Their structure assignment was established by total synthesis of AB-asc-C5 (SMID: abas#9) as a representative example in about 1% yield over 14 steps. To achieve this aim, a new method for the synthesis of orthogonally protected ascarosides has been developed which provides methyl 2-benzoyl-ascaroside as a highly versatile building block for regioselective ascaroside synthesis. Furthermore, a new synthesis for short chain C5 ascarosides was developed that employs selective reduction and Grubbs cross metathesis. The identity of synthetic AB-asc-C5 and the natural product isolated from *C. nigoni* was established by an NMR mixing experiment. Retention of *C. nigoni* males by the exclusively female produced AB-asc-C5 suggests a function as a sex pheromone component. Along with the indole ascarosides (icas), the new class of 4′-*ortho*-aminobenzoyl ascarosides (abas) represents a mechanism to translate bacterial food dependent L-tryptophan availability into species-specific signaling molecules.

Introduction

Chemical signaling in nematodes (roundworms) involves complex mixtures of glycolipids based on the L-3,6-dideoxy-*arabino*-aldohexose called L-ascarylose. Simple ascarosides (**1**) carrying (ω-1)-linked acyl aglycones ranging from 5 to 11 carbons are highly conserved in nematodes[1–3] and represent key regulators in nematode chemical ecology, modulating nematode behavior[4,5] and developmental plasticity,[6] as well as interactions with microorganisms,[7–10] plants,[10–13] insects,[14–16] and mammals.[17] In addition, a constantly increasing number of complex ascaroside derivatives have recently been described, particularly in the most extensively studied androdioecious model organisms *Caenorhabditis elegans* (Maupas 1900[18])[19–21] and *Pristionchus pacificus* (Sommer, Carta, Kim & Sternberg 1996[22]).[3,23–25] These modular ascarosides are derived from the combinatorial assembly of additional building blocks attached to the ascarylose core structure or the acyl moiety of the aglycone upon reaction with carboxylesterase (*cest*) enzymes located in lysosome related organelles (LROs).[26–29] Comparative analysis of closely related *Caenorhabditis* spp.[2,30–32] and *Pristionchus* spp.[3] demonstrated that these components can be highly species-specific, suggesting important ecological functions in species-specific interactions. Only a small number of ascaroside pheromones have been identified using classical bioactivity guided fractionation methodologies[33–37] and most components are currently detected by mass spectrometric analysis. Discovery of yet unidentified ascarosides depends strongly on comparative metabolomics utilizing mutants defective in short chain ascaroside pheromone biosynthesis like *daf-22*,[19,20,38–41] molecular networks[20,21,27] or mass spectrometric screens that utilize specific fragment ions derived from the ascarylose core structure as diagnostic marker ions.[19,42] ESI-(−)-MS/MS precursor ion screening for *m/z* 73.0294 $[C_3H_5O_2]^-$ has been shown to

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†Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d5ob00271k

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represent a most useful ascaroside screen[19,30,43] but its limitations have never been systematically explored.

Our previous analysis of the exometabolome of gonochoristic (male/female) *Caenorhabditis nigoni* (Félix, Braendle & Cutter, 2014) using ESI-(−)-MS/MS precursor ion screening for *m*/*z* 73.0294 $[C_3H_5O_2]^-$ revealed highly species specific (ω-2)-hydroxy asc-ΔC9 (**2**),[2] a unique 4′-*epi*-ascaroside (**3**),[44] and male attracting 2′-*O*-linked homo- and heterodimeric ascarosides 2′-(asc-C6)-asc-C5 (**4a**) and 2′-(asc-C6)-asc-C6 (**4b**)[32] as well as large amounts of indole ascarosides, predominantly the male attracting IC-asc-C5 (**5**, SMID: icas#9)[30] (Fig. 1).

Herein, we describe the identification of a homologous series of 4′-*ortho*-aminobenzoyl ascarosides (AB-asc-Cx, SMID: abas, **6**) as species-specific components of *C. nigoni* (and *Caenorhabditis tropicalis*) that could not be readily detected using ESI-(−)-MS/MS precursor ion screening. Instead, the homologous series was observed using an ESI-(+)-MS screen for the highly characteristic marker ion signal at *m*/*z* 250.1074 $[C_{13}H_{16}NO_4]^+$ from in-source fragmentation. Molecular structures were assigned based on the interpretation of HR-MS/MS and one- and two-dimensional ^{1}H NMR spectra and subsequently confirmed by total synthesis of AB-asc-C5 (**6a**, *n* = 2) as a representative example using newly developed procedures for the preparation of orthogonally protected ascarylose building blocks (**16a–c**) and short chain asc-C5 derivatives.

Results and discussion

Identification of *ortho*-aminobenzoyl ascarosides (abas) from *C. nigoni*

The bacterivorous *C. nigoni* (Félix, Braendle & Cutter, 2014[45]) (formerly *C.* sp. 9) strain JU1422, an inbred line of the type specimen JU1325 collected in 2007 in Kerala, India,[45,46] was propagated at 23 °C in a monoxenic liquid culture supplemented with *Escherichia coli* OP50 as the bacterial diet. The lyophilized exometabolome from 1.6 L of culture supernatant representing the entirety of excreted metabolites was extracted with methanol and fractionated by reverse phase chromatography on RP-C18, and the resulting fractions were analyzed by HPLC-ESI-(−)-MS/MS precursor ion screening for *m*/*z* 73.0294 $[C_3H_5O_2]^-$, which resulted in the identification of a diversity of common basic ascarosides (Fig. 1), *e.g.* asc-ΔC9 (**1a**, SMID: ascr#3),[2] along with some highly species-specific (ω)- and (ω-2)-hydroxyascarosides, *e.g.* asc-7-HO-ΔC9 (**2**),[2] and the 4′-epimeric 4′-*epi*-asc-7-HO-ΔC9 (**3**, SMID: caen#1) carrying the unique L-caenorhabdose unit.[44] In addition, several 2′-*O*-linked homo- and heterodimeric ascarosides (**4**, SMID: dasc) were identified[32] along with modular indole ascarosides dominated by IC-asc-C5 (**5**, SMID: icas#9).[30]

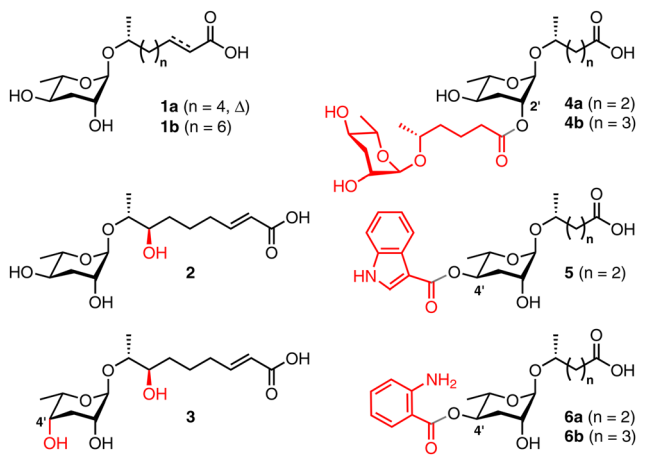

Fig. 1 Selected ascarosides from *Caenorhabditis nigoni*.

During the isolation of these components, complementary ^{1}H NMR spectroscopy revealed that the RP-C18 fraction eluted with 70% methanol contained a class of yet unidentified 4′-*O*-substituted modular ascarosides (Fig. S1†), which, however, could not be detected using the standard ESI-(−) precursor ion screen. Instead of the characteristic marker ion signal for a C3-fragment at *m*/*z* 73.0294 $[C_3H_5O_2]^-$ commonly observed for basic ascarosides (*e.g.* **1–3**) and the large majority of complex modular ascarosides identified so far (*e.g.* **4** and **5**), the ESI-(−)-HR-MS/MS spectra of these new modular ascarosides (**6**) revealed characteristic signals for an aminobenzoyl substituted C6-fragment at *m*/*z* 248.0928 $[C_{13}H_{14}NO_4]^-$ (**I**) and aminobenzoyl substituted C3-fragments at *m*/*z* 192.0666 $[C_{10}H_{10}NO_3]^-$ (**II**) and *m*/*z* 174.0561 $[C_{10}H_8NO_2]^-$ (**III**), along with an aminobenzoate anion at *m*/*z* 136.0404 $[C_7H_6NO_2]^-$ (**IV**) (Fig. 2a and b and S2†). Complementary ESI-(+)-HR-MS and MS/MS analysis revealed a highly characteristic fragment ion at *m*/*z* 250.1074 $[C_{13}H_{16}NO_4]^+$ for the tetrahydropyrylium cation (**V**) from the loss of the homologous aglycones upon in-source fragmentation, which furnished an amino-benzoylium cation (**VI**) at *m*/*z* 120.0444 $[C_7H_6NO]^+$ upon collision induced dissociation tandem mass spectrometry (Fig. 2a and b and S3†). Screening the ESI-(+)-HR-MS chromatogram of the *C. nigoni* exometabolome for this high intensity marker ion (**V**) at *m*/*z* 250.1074 $[C_{13}H_{16}NO_4]^+$ revealed a homologous series of aminobenzoyl substituted ascarosides (SMID: abas) with aglycone side chains ranging from C3 to C11 (Fig. 2c and Table S1†). Comparative analysis of exometabolome extracts from nine different *Caenorhabditis* spp. of the Elegans group using the ESI-(+)-MS screen for *m*/*z* 250.1074 $[C_{13}H_{16}NO_4]^+$ (Fig. S4†) demonstrated that the homologous series of aminobenzoyl substituted ascarosides is abundant in gonochoristic *C. nigoni* and hermaphroditic *C. tropicalis* (Fig. 2c), which are not directly related (Fig. S5†).[45,46] Traces of the aminobenzoyl ascarosides AB-asc-ωC3 (abas#5), AB-asc-C9 (abas#10), and AB-asc-ΔC9 (abas#3) were also detected in the model organism *C. elegans* (N2) (Fig. S4†), demonstrating that the analysis of related *Caenorhabditis* species represents an effective method to identify trace components in *C. elegans*.

To determine the molecular structures of the species-specific aminobenzoyl ascarosides, the RP-C18 fraction eluted with 70% methanol was further separated by semipreparative HPLC to yield approximately 100 µg AB-asc-C5 (**6a**, *n* = 2, abas#9) as a mixture with asc-C11 (**1b**, ascr#18), as well as approximately 100 µg AB-asc-C6 (**6b**, *n* = 3, abas#12). Analysis of their ^{1}H NMR (Fig. S6†) and high resolution *dqf*-COSY

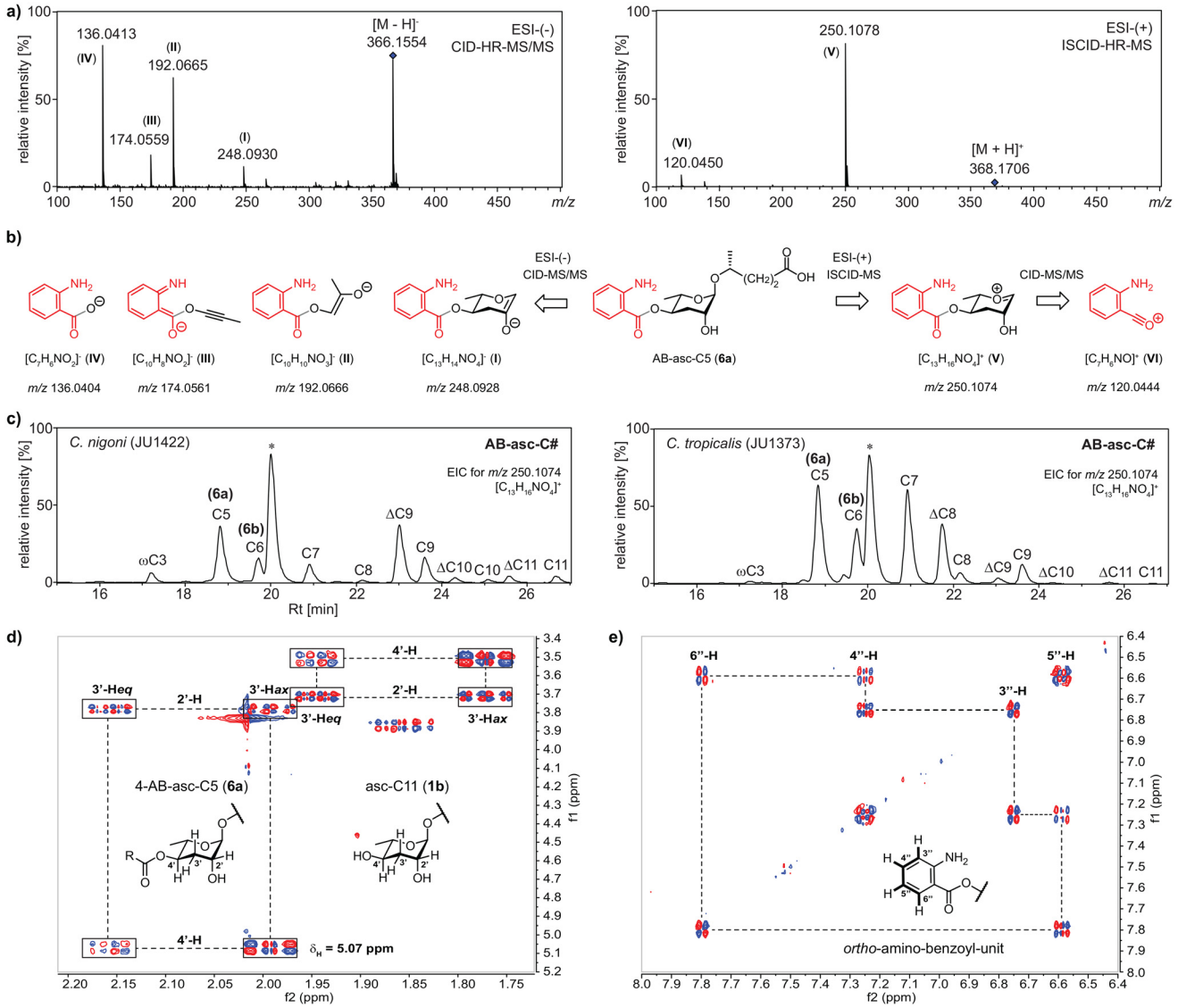

Fig. 2 Structure assignment of 4′-*ortho*-aminobenzoyl ascarosides (abas) based on ESI-HR-MS/MS spectra of AB-asc-C5 (**6a**, abas#9) as a representative (a), which indicate characteristic fragment ion structures (b), of which the tetrahydropyrylium ion (**V**) at *m*/*z* 250.1074 $[C_{13}H_{16}NO_4]^+$ was employed as a marker ion in ESI-(+)-MS screens for homologous 4′-*ortho*-aminobenzoyl ascarosides in *C. nigoni* and *C. tropicalis* (c), *: *E. coli* derived N^6-(Δ^2-isopentenyl)-2-methylthioadenosine;[67,68] sections of the *dqf*-COSY spectrum of AB-asc-C5 (**6a**, abas#9) enriched along with asc-C11 (**1b**) shows the presence of a 4′-*O*-acyl substituted ascaroside (d) along with an *ortho*-aminobenzoate moiety (e).

spectra (Fig. S7†) confirmed a 4′-substituted ascarylose moiety with δ_H = 5.07 ppm (ddd) for 4′-H (Fig. 2d) and revealed an *ortho*-aminobenzoyl (anthranilate) moiety with δ_H = 6.73 ppm (d, 3″-H), 7.23 ppm (dd, 4″-H), 6.58 ppm (dd, 5″-H), and 7.76 ppm (d, 6″-H) (Fig. 2e), indicating a homologous series of 4′-*ortho*-aminobenzoyl ascarosides (**6**, AB-asc-Cx, SMID: abas#).

Total synthesis of the 4′-*ortho*-aminobenzoyl ascaroside AB-asc-C5 (6a, abas#9)

Structure assignment of the homologous series of 4′-*ortho*-aminobenzoyl ascarosides was subsequently confirmed by total synthesis of AB-asc-C5 (**6a**, abas#9) as a representative example in about 1% yield over 14 steps (Fig. 3). The orthogonally protected 2-*O*-benzoyl-4-*O*-*tert*-butyldiphenysilyl-ascarylose building block (**16a**) was prepared from commercially available L-rhamnose (**7**) in 12% yield over 9 steps *via* regioselective borohydride reduction of cyclic sulfate ester **13** following a literature procedure (Fig. 3a).[47] Alternatively, **16a** was preferably obtained in 30% yield over 6 steps from L-rhamnose (**7**) using the Hanessian–Hullar benzylidene ring opening following a modified literature procedure (Fig. 3b).[48] Regioselective fragmentation of methyl 2,3-*O*-benzylidene-rhamnoside (**17**) upon reaction with *N*-bromosuccinimide and $CaCO_3$ furnished methyl 2-*O*-benzoyl-3-bromo-ascaroside (**18**) in 89% yield. 3-Debromination of **18** using $LiAlH_4$ has previously been reported to furnish methyl ascaroside in 75% yield *via* the 2,3-

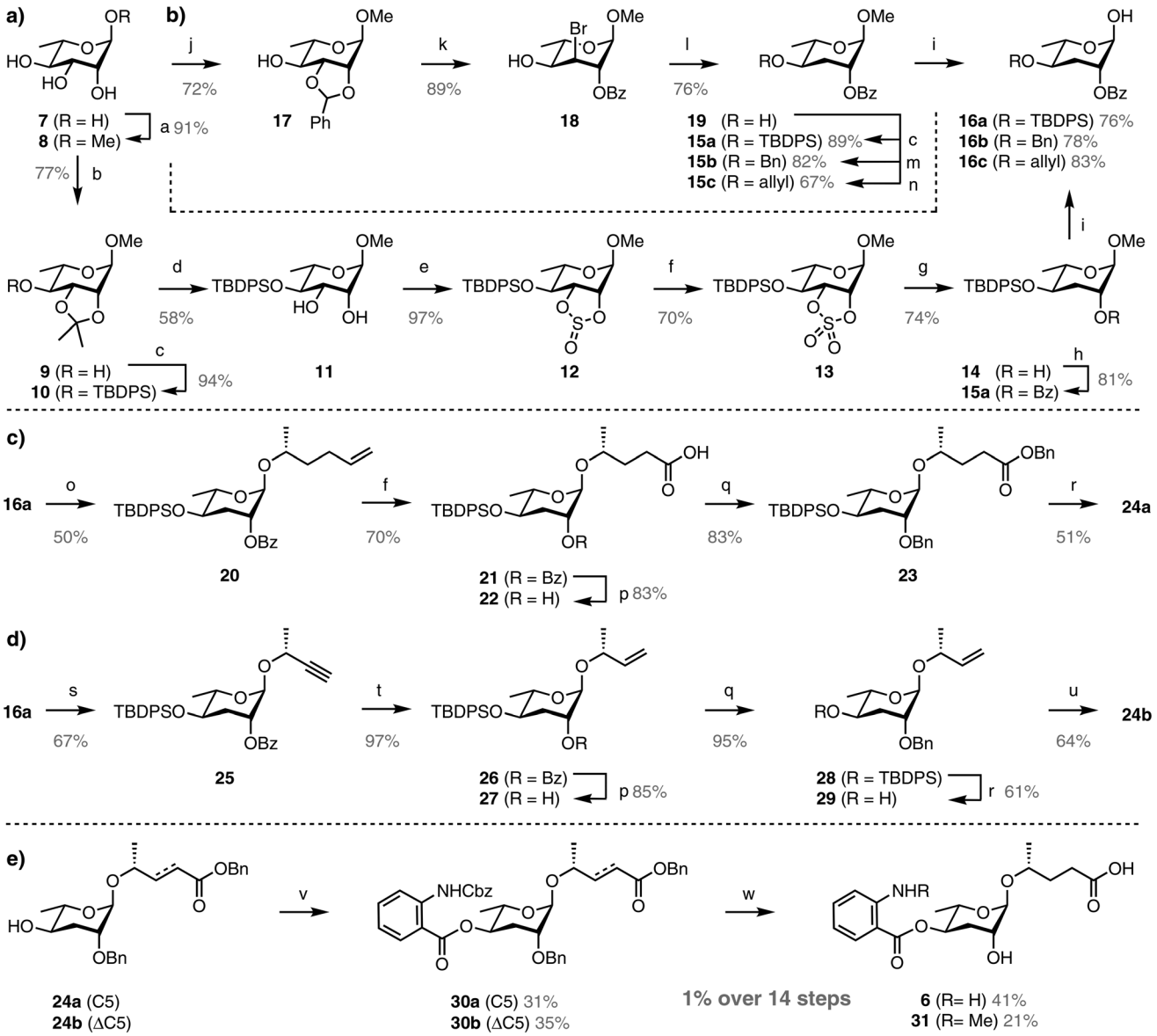

Fig. 3 Total synthesis of the 4′-*ortho*-aminobenzoyl ascaroside AB-asc-C5 (**6a**, abas#9) from *C. nigoni* using the orthogonally protected ascarylose building block **16a** obtained from L-rhamnose (**7**) either *via* borohydride reduction of **13** (a) or *via* radical dehalogenation of **18** to furnish highly versatile methyl 2-*O*-benzoyl ascaroside (**19**) as a precursor for **16a** and derivatives (b), which was employed for the synthesis of 2′-*O*-benzyl-ascaroside C5-*O*-benzyl ester (**24a**) and 2′-*O*-benzyl-ascaroside ΔC5-*O*-benzyl ester (**24b**) *via* ruthenium catalyzed oxidation of alkenyl ascarosides (c) or cross metathesis (d), respectively, both of which were 4′-*O*-acylated with *N*-Cbz-protected *ortho*-amino-benzoic acid and deprotected to furnish AB-asc-C5 (**6**, abas#9) (e); reagents: a: methanol, AcCl; b: 2,2-dimethoxypropane, CSA; c: TBDPSCl, imidazole; d: TFA; e: $SOCl_2$, pyridine; f: $RuCl_3$, $NaIO_4$; g: $(Bu_4N)BH_4$; h: BzCl, pyridine; i: BBr_3 or 3.0 M HCl; j: benzaldehyde dimethylacetal, PTSA; k: NBS, $CaCO_3$; l: Bu_3SnH, AIBN; m: Bn-OPT, MgO; n: *O*-allyl 2,2,2-trichloroacetimidate, TfOH; o: 1. TCA, DBU, 2. (*R*)-5-hexen-2-ol, TMSOTf; p: LiOH; q: NaH, BnBr; r: TBAF; s: 1. TCA, DBU, 2. (*R*)-3-butyn-2-ol, TMSOTf; t: H_2, Lindlar cat.; u: benzyl acrylate, Grubbs 2nd Gen. catalyst; v: *N*-Cbz-*ortho*-amino-benzoic acid, EDC, DMAP; w: H_2, Pd/C.

anhydro intermediate.[48,49] However, aiming for orthogonally protected ascarylose building blocks, we found that selective 3-debromination of **18** was readily accomplished at the multigram scale with good yield (78%) using radical dehalogenation with tributyltin hydride,[49] and could also be performed using nickel boride reduction (77% yield),[49] or palladium catalyzed hydrogenation under alkaline conditions (79% yield),[50] furnishing methyl 2-benzoyl ascaroside (**19**). The versatility of **19** as an orthogonally protected ascarylose building block with an α-glycoside directing 2-*O*-benzoyl moiety was subsequently demonstrated by synthesis of derivatives carrying *tert*-butyldiphenylsilyl (**15a**, 89% yield),[51] benzyl (**15b**, 82% yield),[52] or allyl (**15c**, 67% yield)[53] protecting groups at the 4-*O*-position. Subsequent 1-*O*-demethylation was effected with boron tribromide for 2-*O*-benzoyl-4-*O*-*tert*-butyldiphenysilyl-ascarylose (**16a**, 76% yield),[47] whereas 3.0 M hydrochloric acid was preferable

for 2-*O*-benzoyl-4-*O*-benzyl-ascarylose (**16b**, 78% yield) and 2-*O*-benzoyl-4-*O*-allyl-ascarylose (**16c**, 83% yield).

Starting from orthogonally protected 2-*O*-benzoyl-4-*O*-*tert*-butyldiphenysilyl-ascarylose (**16a**), the corresponding (5*R*)-1-hexen-5-yl ascaroside (**20**) was prepared (Fig. 3c) using the trichloroacetimidate method,[54] followed by ruthenium-catalyzed periodate oxidation[55] to the acyl derivative, the 2′-*O*-benzoyl-4′-*O*-*tert*-butyldiphenysilyl-ascaryloside C5 (**21**). Alkaline hydrolysis furnished the 4′-*O*-*tert*-butyldiphenysilyl-ascaryloside C5 (**22**) that was exhaustively *O*-benzylated to give the 2′-*O*-benzyl-4′-*O*-*tert*-butyldiphenysilyl-ascaryloside C5 *O*-benzyl ester (**23**). 4′-*O*-Desilylation with tetrabutylammonium fluoride (TBAF) furnished the 2′-*O*-benzyl-ascaryloside C5 *O*-benzyl ester (**24a**) as a known building block for selective 4′-*O*-acylation.[47] Because modular and dimeric ascarosides carrying the (ω-1)-linked C5 sidechain are prominent in *Caenorhabditis* spp.[30,32] and *Pristionchus* spp.[3] a complementary synthesis was developed that replaces the oxidation and esterification steps with a reduction and Grubbs' cross metathesis to install the short chain aglycone directly as the desired *O*-benzyl ester (Fig. 3d). Commercially available (*R*)-3-butyn-2-ol was coupled with 2-*O*-benzoyl-4-*O*-*tert*-butyldiphenysilyl-ascarylose (**16a**) using the trichloroacetimidate method[54] and the resulting (2*R*)-3-butyn-2-yl glycoside **25** was subsequently reduced to the (2*R*)-3-buten-2-yl glycoside **26** by selective hydrogenation with Lindlar's catalyst.[56] Alkaline hydrolysis furnished the (2*R*)-3-buten-2-yl 4′-*O*-*tert*-butyldiphenysilyl-ascaryloside (**27**) that was 2′-*O*-benzylated to **28** and subsequently 4′-*O*-desilylated with TBAF to furnish the (2*R*)-3-buten-2-yl 2′-*O*-benzyl-ascaryloside (**29**). Cross metathesis with benzyl acrylate using Grubbs 2nd generation catalyst[57] furnished the 2′-*O*-benzyl-ascaryloside ΔC5 *O*-benzyl ester (**24b**) as an alternative building block for selective 4′-*O*-acylation. Both, the 2′-*O*-benzyl-ascaryloside C5 *O*-benzyl ester (**24a**) and 2′-*O*-benzyl-ascaryloside ΔC5 *O*-benzyl ester (**24b**) were subsequently 4′-*O*-acylated with *N*-Cbz-protected *ortho*-aminobenzoic acid using Steglich esterification[58] (Fig. 3e). Finally, *O*-debenzylation (in parallel to the reduction of the double bond in the case of **30b**) upon palladium catalyzed hydrogenation furnished AB-asc-C5 (**6a**, abas#9) along with its *N*-methyl derivative (**31**).

The identity of the synthetic AB-asc-C5 (**6a**, abas#9) with the natural product isolated from *C. nigoni* JU1422 was unambiguously established by comparative analysis of their HPLC-MS (Fig. S8†) and ^{1}H NMR data (Fig. S9†). Although ^{1}H NMR spectra of isolated and synthetic AB-asc-C5 (**6a**) in CD_3OD displayed considerable differences in chemical shifts, a mixing experiment demonstrated that these are due to a pronounced salt and pH dependency and that the synthetic compound exhibits identical NMR data to the natural material once measured under identical experimental conditions (Fig. 4 and S9), which demonstrates the identity of the natural and synthetic material, and thereby confirms the structure assignment of the homologous series of 4′-*ortho*-aminobenzoyl ascarosides in *C. nigoni* and *C. tropicalis*.

Female produced *ortho*-aminobenzoyl ascaroside AB-asc-C5 (6, abas#9) attracts *C. nigoni* males

Sex-specific analysis of the gonochoristic *C. nigoni* JU1422 indicated that the dominating AB-asc-C5 (**6a**, abas#9) and IC-asc-C5 (**5**, icas#9) are (almost) exclusively produced by females (Fig. 5a). Potential behavioral activities of AB-asc-C5 (**6a**) were evaluated using a holding assay that characterizes nematode retention in ascaroside conditioned scoring regions in comparison with the solvent control. Statistically significant ($p < 0.023$) retention of males by 10 pmol of the female-produced AB-asc-C5 (**6a**) (Fig. 5b) suggests a potential function as part of the female sex pheromone blend. Because required amounts are relatively high (Fig. S10†), it is conceivable that AB-asc-C5 (**6a**) functions along (or even in synergism) with additional female-produced pheromone components, such as the other

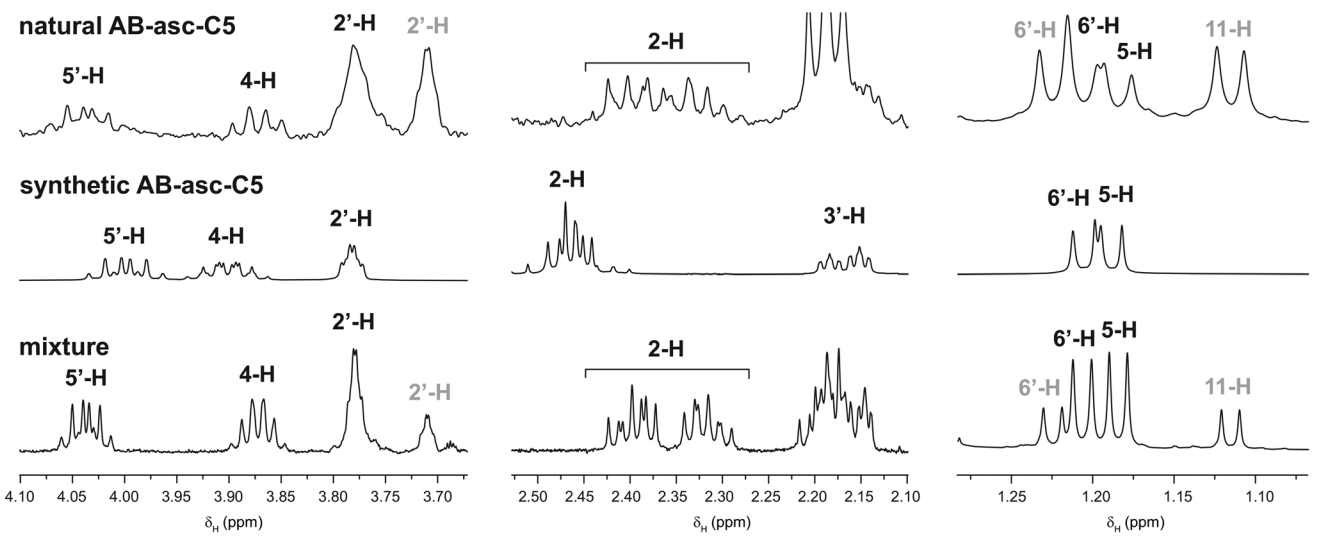

Fig. 4 Comparison of the ^{1}H NMR spectra of natural and synthetic AB-asc-C5 (**6a**) revealed differences in chemical shifts, which were shown to result from strong pH dependency by a mixing experiment (signals of asc-C11 (**1b**) marked in grey).

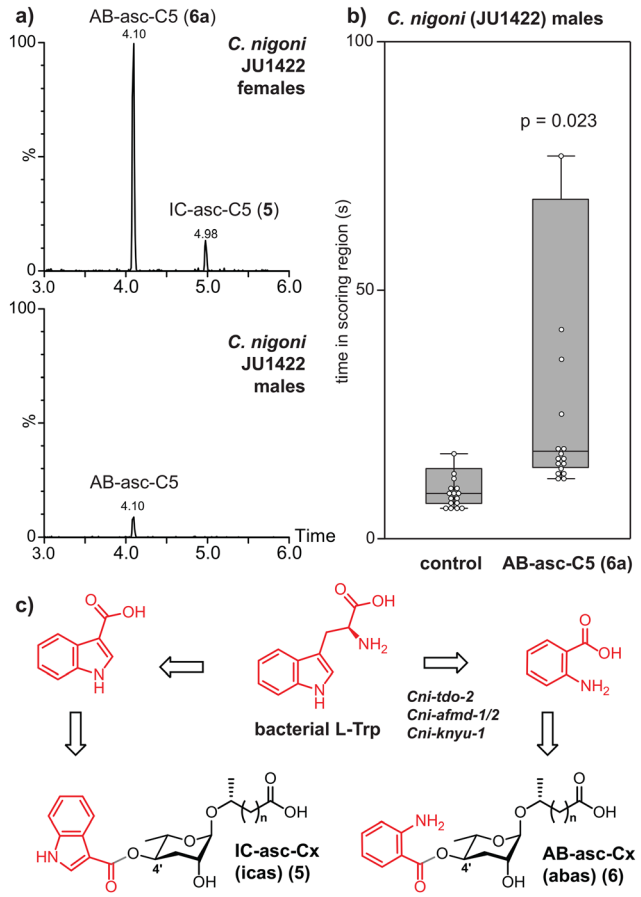

Fig. 5 Sex-specific biosynthesis of AB-asc-C5 (**6a**) and IC-asc-C5 (**5**) by female *C. nigoni* (JU1422) as shown by EICs for 366.120 and 390.188 ± 0.02 Da, respectively, suggests potential signaling functions (a), such as male-specific retention of *C. nigoni* by 10 pmol of female-produced AB-asc-C5 (**6a**) (b). Hypothetical biosynthesis of indole ascarosides (**5**, icas) and *ortho*-amino-benzoyl ascarosides (**6**, abas) from bacteria-derived L-tryptophan (c).

homologous *ortho*-aminobenzoyl ascarosides (abas) (Fig. 2c), or the indole ascarosides (icas), such as the dominating IC-asc-C5 (**5**, icas#9) previously shown to retain *C. nigoni* JU1422 males at 10 pmol.[30] In contrast, *C. nigoni* JU1422 females display only insignificant responses to ecologically relevant amounts of AB-asc-C5 (**6a**) (Fig. S11†) or IC-asc-C5.[30] The hermaphroditic *C. tropicalis* JU1373 did not exhibit any response to AB-asc-C5 (**6a**) at any of the concentrations tested (Fig. S12†).

Conclusions

In conclusion, using a combination of reverse phase C18 chromatography and careful analysis of ^{1}H NMR and HR-MS/MS spectra, a homologous series of species-specific 4′-*ortho*-aminobenzoyl ascarosides (AB-asc-Cx, abas) was identified (Fig. 2). Interestingly, these new modular ascarosides could not be detected using the standard ESI-(−)-precursor ion screen for *m*/*z* 73.0 $[C_3H_5O_2]^-$,[19] demonstrating a considerable limitation that extends beyond the fact that certain ascarosides simply do not ionize in ESI-(−).[42] Depending on the fragmentation behavior of the ester bond, certain 4′-*O*-substituted ascarosides, such as AB-asc-C5 (**6a**, abas#9), fail to produce a fragment ion signal at *m*/*z* 73.0 $[C_3H_5O_2]^-$ upon collision induced dissociation (CID) of ESI-(−)-produced $[M - H]^-$ ions and instead display only an *O*-acyl substituted C3-fragment, thus, preventing their detection with conventional screening methods. While limiting the versatility of the ESI-(−)-MS/MS precursor ion screening, this observation confirms our previous assumption that the $[C_3H_5O_2]^-$ fragment is derived from C4–C5–C6 of the ascarylose moiety. Consequently, observation of *O*-acyl substituted C3-fragment ions also provides a potential methodology for HR-MS/MS-based structure assignment of yet unidentified 4′-*O*-substituted ascarosides available in trace quantities only, including species-specific ascarosides from nematodes that could not be cultivated in sufficient quantities to facilitate their NMR-based structure assignment.

Molecular structures of the new 4′-*ortho*-aminobenzoyl ascarosides (abas) were unambiguously established by total synthesis of AB-asc-C5 (**6a**) as a representative using a new methodology for the preparation of an orthogonally protected ascarylose building block that facilitates the installation of diverse protecting groups at the 4-*O*-position and thereby complements currently available procedures.[43,59] Furthermore, a new protocol for the synthesis of (ω-1)-linked short chain C5 ascarosides using Grubbs metathesis was developed and employed for the synthesis of AB-asc-C5 (**6a**), which was finally shown to be identical to the natural product isolated from *C. nigoni* using ^{1}H NMR mixing experiments.

Behavioral bioassays with the synthetic material of the female-produced AB-asc-C5 (**6a**) revealed significant retention of *C. nigoni* males by 10 pmol, suggesting a function as part of the sex pheromone blend, potentially along with the male attracting female-produced IC-asc-C5 (**5**).[30] Both the indole-3-carboxylic acid (IC) and *ortho*-aminobenzoic acid (AB) moieties of indole ascarosides (**5**, icas) and *ortho*-aminobenzoyl ascarosides (**6**, abas), respectively, are known to depend on the metabolism of bacteria derived L-tryptophan (Fig. 5c). Incorporation of L-tryptophan into indole ascarosides (icas) by *C. elegans* has been demonstrated.[60,61] As L-tryptophan is an essential amino acid for *Caenorhabditis* spp. that is exclusively derived from the bacterial diet, its incorporation into indole ascarosides (**5**, icas) and *ortho*-aminobenzoyl ascarosides (**6**, abas) reveals two complementary mechanisms of bacterivorous *Caenorhabditis* nematodes to translate microbial L-tryptophan biosynthesis into species-specific signaling molecules. While indole ascarosides (icas) are rather common within the Elegans group,[30] the *ortho*-aminobenzoyl ascarosides (abas) are highly species-specific for *C. nigoni* and *C. tropicalis*. Free *ortho*-aminobenzoic acid (anthranilic acid) has previously been detected in the *C. nigoni* exometabolome.[2] Furthermore, the *ortho*-aminobenzoyl moiety has been recognized as a building block of the pentamodular ascaroside pasa#9 from *P. pacificus*,[25] as well as anthranilate glucoside (angl#1)[62] and structurally related modular glucosides (*mogl*) retained in the *C. elegans* endometabolome.[27,63,64]

Experimental

Organisms

Wild-type *Caenorhabditis* isolates including *C. nigoni* JU1422 (India), *C. elegans* N2 (UK), *C. remanei* PB4641 (USA), *C. briggsae* AF16 (India), *C. sinica* JU727 (China), *C. tropicalis* JU1373 (La Réunion), *C. wallacei* JU1904 (Indonesia), *C. doughertyi* JU1771 (India), *C. brenneri* PB2801 (Costa Rica), as well as the uracil auxotroph *Escherichia coli* OP50 strain were obtained from the *Caenorhabditis elegans* Genetics Center (CGC).

Preparation of exometabolome extracts

Wild-type *Caenorhabditis* isolates were cultivated at 23 °C on Nematode Growth Medium (NGM) agar[65] seeded with *E. coli* OP50. Mixed stage nematodes from five 10 cm plates collected in M9 buffer served as inoculums for liquid cultures grown in 100 mL S-medium[65] at 23 °C and 150 rpm. Concentrated *E. coli* OP50 bacteria pellets (from an overnight culture in lysogeny broth (LB) medium at 37 °C and 170 rpm) were 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 analyzed by HPLC-HR-MS, MS/MS, and HPLC-MS/MS precursor ion scanning. All experiments were performed in triplicate.

High-performance liquid chromatography-electrospray ionization-high resolution (tandem) mass spectrometry

HPLC-ESI-HR-MS and MS/MS 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 operating in the 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 for 5 minutes followed by a linear increase to 100% acetonitrile with 0.5% acetic acid within 35 minutes. Data were analyzed using the Compass Data analysis 4.3 software (Bruker).

High-performance liquid chromatography electrospray ionization tandem mass spectrometry precursor ion scanning

HPLC-ESI-MS/MS precursor ion scanning 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 operating in the negative mode. 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 followed by a linear increase to 95% acetonitrile with 0.05% formic acid within 10 minutes. Data were analyzed using the Analyst 1.6 software (AB Sciex).

Ultra high-performance liquid chromatography-electrospray ionization-high resolution (tandem) mass spectrometry

UHPLC-ESI-HR-MS and MS/MS analyses were performed using an Acquity ultra-high pressure liquid chromatography system coupled to a Synapt G2 QTOF mass spectrometer (Waters, Milford, MA, USA) controlled by MassLynx 4.1. Chromatographic separations were achieved using a Waters Acquity HSS T3 column (100 × 2.1 mm, 1.8 μm particle diameter) maintained at 30 °C with gradient elution starting at 0% acetonitrile in 0.05% aqueous formic acid followed by a linear increase to 100% acetonitrile with 0.05% formic acid within 10 minutes. The flow rate was set to 0.5 mL min^{-1} and the injection volume was 2.5 μL. Detection was performed using electrospray ionization (ESI) in the positive or negative ionization mode using a source temperature of 120 °C, desolvation gas and temperature at 15 L min^{-1} and 400 °C, and capillary voltage at 2.5 kV for ESI-(+) or −2.0 kV for ESI-(−). Mass spectra were recorded from *m*/*z* 50 to *m*/*z* 1200. Data-independent (broad band collision induced dissociation) fragmentation data were collected using a collision energy of 8–55 V in the positive mode and 10–60 V in the negative mode. Data were analyzed using the MassLynx software (Waters, Milford, MA, USA).

NMR spectroscopy

NMR spectra were recorded at 400 MHz for ^{1}H and 100 MHz for ^{13}C using a Bruker AVANCE III HD 400 instrument equipped with a 5 mm BBFO probe (MPICE), a Bruker AVANCE II 400 instrument equipped with a 5 mm BBFO+ probe (NPAC, UniNE), or at 600 MHz for ^{1}H and 150 MHz for ^{13}C using a Bruker Avance Neo Ascend 600 instrument equipped with a 5 mm BBI probe (NPAC, UniNE). Residual solvent signals were used as an internal reference with ^{1}H at 3.31 ppm and ^{13}C at 49.05 ppm for CD_3OD or ^{1}H at 7.26 ppm and ^{13}C at 77.16 ppm for $CDCl_3$. Two-dimensional high-resolution double quantum filtered *dqf*-COSY spectra were recorded using phase cycling for coherence selection. Spectra were zero-filled prior to Fourier transformation, phased manually, and baseline corrected using the Topspin 3.2 (Bruker) and MNova 9.0 and 12.0 (Mestrelab Research) software.

Isolation of *ortho*-aminobenzoyl ascarosides (abas) from *C. nigoni*

4′-*ortho*-Aminobenzoyl ascarosides (**6**, *n* = 2–3) were isolated from 1.6 L of a *C. nigoni* JU1422 liquid culture grown for 2 weeks. The filtered media supernatant was frozen at −80 °C and lyophilized, and the powdered residue was extracted with 3 × 100 mL methanol for 12 h each. The filtered extract was concentrated to dryness under reduced pressure and the

resulting exometabolome extract was adsorbed onto 2 g of Celite and fractionated on 5 g RP-C18 cartridges (Chromabond, Macherey-Nagel) using a stepwise increase of methanol concentrations in water as the eluent (0–100% in 10% steps) to afford 10 fractions of 20 mL each. Fractions were concentrated to dryness using a centrifugal vacuum concentrator and analyzed by ^{1}H NMR and *dqf*-COSY spectroscopy. The 70% methanol fraction containing the target component according to NMR was subsequently subjected 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 for 3 minutes, followed by a linear increase to 100% acetonitrile with 0.5% acetic acid within 30 minutes. Aliquots of 100 μl were analyzed 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 analyzed by one- and two-dimensional NMR spectroscopy.

(4*R*)-4-[(4-*O*-(2-Amino-benzoyl)-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-pentanoic acid (AB-asc-C5 (SMID: abas#9) (6a, *n* = 2)). ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 7.78 (d, J = 8.2 Hz, 1H, 6″-H), 7.23 (dd, J = 8.4 Hz, J = 7.1 Hz, 1H, 4″-H), 6.73 (d, J = 8.4 Hz, 1H, 3″-H), 6.57 (dd, J = 8.2 Hz, J = 7.1 Hz, 1H, 5″-H), 5.07 (ddd, J = 11.1 Hz, J = 9.7 Hz, J = 4.8 Hz, 1H, 4′-H), 4.74 (br.s, 1H, 1′-H), 4.04 (dq, J = 9.7 Hz, J = 6.4 Hz, 1H, 5′-H), 3.87 (m, 1H, 4-H), 3.77 (br.s, 1H, 2′-H), 2.42 (m, 1H, 2-Ha), 2.33 (m, 1H, 2-Hb), 2.17 (ddd, J = 13.8 Hz, J = 4.8 Hz, J = 2.8 Hz, 1H, 3′-Heq), 1.98 (ddd, J = 13.8 Hz, J = 11.0 Hz, J = 3.0 Hz, 1H, 3′-Hax), 1.90–1.84 (m, 2H, 3-H), 1.20 (d, 6.7 Hz, 3H, 6′-H), 1.18 (d, J = 6.2 Hz, 3H, 5-H); HRMS (ESI-TOF) m/z $(M + H)^+$ calcd for $C_{18}H_{26}NO_7$ 368.1704, found 368.1706, Δ 0.5 ppm, $(M - H)^-$ calcd for $C_{18}H_{24}NO_7$ 366.1558, found 366.1564, Δ 1.6 ppm.

(5*R*)-5-[(4-*O*-(2-Amino-benzoyl)-3,6-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-hexanoic acid (AB-asc-C6 (SMID: abas#12) (6b, *n* = 3)). ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 7.76 (d, J = 8.1 Hz, 1H, 6″-H), 7.23 (dd, J = 8.4 Hz, J = 7.1 Hz, 1H, 4″-H), 6.73 (d, J = 8.3 Hz, 1H, 3″-H), 6.58 (dd, J = 8.0 Hz, J = 7.1 Hz, 1H, 5″-H), 5.06 (ddd, J = 11.1 Hz, J = 9.6 Hz, J = 4.8 Hz, 1H, 4′-H), 4.76 (br.s, 1H, 1′-H), 4.03 (dq, J = 9.6 Hz, J = 6.5 Hz, 1H, 5′-H), 3.86 (m, 1H, 5-H), 3.78 (br.s, 1H, 2′-H), 2.27 (*t*, 2H, 2-H), 2.16 (ddd, J = 13.0 Hz, J = 5.4 Hz, J = 2.8 Hz, 1H, 3′-Heq), 1.97 (ddd, J = 13.0 Hz, J = 11.1 Hz, J = 3.0 Hz, 1H, 3′-Hax), 1.80 (m, 2H, 3-H), 1.63 (m, 1H, 4-Ha), 1.55 (m, 1H, 4-Hb), 1.20 (d, J = 6.5, 3H, 6′-H), 1.17 (d, J = 6.5 Hz, 3H, 6-H); HRMS (ESI-TOF) m/z $(M + H)^+$ calcd for $C_{19}H_{28}NO_7$ 382.1860, found 382.1865, Δ 1.3 ppm, $(M - H)^-$ calcd for $C_{19}H_{26}NO_7$ 380.1715, found 367.1728, Δ 3.4 ppm.

Sex specific compounds from *C. nigoni*

For sex specific exometabolome analysis of *C. nigoni* JU1422, a total of 100 male and 100 female adult worms were collected from non-starved cultures on NGM agar seeded with *E. coli* OP50 using a 0.25 mm platinum iridium (90 : 10 wt%) wire pick. Nematodes were incubated for 15 h at 20 °C and 180 rpm in 500 μL S-medium supplemented with 100 μL of an *E. coli* OP50 overnight culture in LB medium. Cultures were filtered over cotton to remove the nematodes and the medium was frozen at −20 °C, lyophilized, and extracted with 500 μL methanol. Filtered extracts were concentrated under reduced pressure and the residues were taken up in 100 μL of methanol for UPLC-ESI-(−)-HR-MS analysis.

Retention assay to evaluate nematode behavioral response

Nematode preference for environments conditioned with known amounts of AB-asc-C5 (**6a**, n = 2, abas#9) was measured using a retention assay. On a 6 cm Petri dish filled with 6 mL peptone free NGM agar, circular scoring regions of 9 mm diameter were marked. Next, 1 μl of methanol (as negative control) or AB-asc-C5 (**6a**, n = 2) in methanol was placed in the center 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 plates seeded with *E. coli* OP50 were transferred to peptone-free NGM agar without any food for *ca.* 15 min before being used for the assay to minimize the amount of concomitant bacteria. Individual worms were placed in the center of the conditioned scoring region and the time required for the nematode to leave the scoring region was measured. Nematodes were defined to have left the scoring area when no part of the nematode was still within the circular boundary. A total of 20 worms per condition were analyzed. A one-way between subjects ANOVA with Dunett's posttest was performed using the SPSS software to evaluate the effect of indole ascarosides on mean times nematode spent in scoring regions.

Total synthesis of AB-asc-C5 (6a, abas#9) from *C. nigoni*

1-*O*-Methyl-6-deoxy-α-L-*arabino*-hexopyranoside (8, methyl rhamnoside). Under an N_2 atmosphere, a solution of L-rhamnose (7, 15.0 g, 91 mmol) in methanol (230 mL) was treated with acetyl chloride (25.5 mL, 364.5 mmol) and the mixture was refluxed overnight. The reaction was quenched with $NaHCO_3$ (30.6 g) and the mixture was filtered and concentrated to dryness under reduced pressure. The resulting residue was purified through a silica plug using 10% methanol in dichloromethane (v/v) as the eluent to afford **8** (14.8 g, 83.1 mmol, 91% yield, α/β: 7/1) as a yellow oil. ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 4.55 (d, J = 1.6 Hz, 1H), 3.78 (dd, J = 3.5 Hz, J = 1.7 Hz, 1H), 3.60 (dd, J = 9.5, J = 3.5 Hz, 1H), 3.53 (dq, J = 9.5 Hz, J = 6.2 Hz, 1H), 3.35 (dd, J = 9.5 Hz, J = 9.5 Hz, 1H) 3.34 (s, 1H), 1.27 (d, J = 6.2 Hz, 3H); identical to reported data.[66]

1-*O*-Methyl-6-deoxy-2,3-(*O*-isopropylidene)-α-L-*arabino*-hexopyranoside (9). Under an N_2 atmosphere, a solution of **8** (14.8 g, 83.1 mmol) in dry acetone (300 mL) was treated with camphorsulfonic acid (202 mg, 0.87 mmol) followed by 2,2-dimethoxypropane (57.8 mL, 465 mmol). After stirring overnight at room temperature, the mixture was neutralized with 1 M sodium hydroxide solution and concentrated under reduced pressure. The residue was dissolved in dichloromethane and

the organic phase was washed with water and brine and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using 35% ethyl acetate in hexane (v/v) as the eluent to afford **9** (14.0 g, 64.1 mmol, 77% yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 4.83 (br.s, 1 H), 4.14–4.08 (m, 1H), 4.05 (dd, J = 7.1 Hz, J = 5.8 Hz, 1H), 3.62 (dq, J = 9.2 Hz, J = 6.3 Hz, 1H), 3.37 (s, 3H), 3.37 (dd, J = 9.5 Hz, J = 7.2 Hz, 1H), 1.51 (s, 3H), 1.34 (s, 3H), 1.29 (d, J = 6.4 Hz, 3H); identical to reported data.[47]

1-*O*-Methyl-6-deoxy-2,3-*O*-(isopropylidene)-4-*O*-*tert*-butyldiphenylsilyl-α-L-*arabino*-hexopyranoside (10). Under an N_2 atmosphere, a solution of **9** (14.0 g, 64.1 mmol) in dichloromethane (150 mL) was treated with *tert*-butyldiphenylchlorosilane (25 mL, 96 mmol) and imidazole (8.8 g, 128 mmol). After stirring overnight at room temperature, the mixture was filtered and the filtrate was concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using 20% ethyl acetate in hexane (v/v) as the eluent to afford **10** (27.6 g, 60.4 mmol, 94% yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.75–7.70 (m, 2H), 7.69–7.65 (m, 2H), 7.44–7.32 (m, 6H), 4.75 (br.s, 1H), 4.19 (dd, J = 6.9 Hz, J = 5.8 Hz, 1H), 4.06 (dd, J = 5.8 Hz, J = 0.8 Hz, 1H), 3.67 (dqd, J = 9.6 Hz, J = 6.3 Hz, J = 0.6 Hz, 1H), 3.38 (dd, J = 9.6 Hz, J = 6.9 Hz, 1H), 3.36 (s, 3H), 1.23 (s, 3H), 1.09 (s, 3H), 1.07 (s, 9H), 1.05 (d, J = 6.3 Hz, 3H); identical to reported data.[47]

1-*O*-Methyl-6-deoxy-4-*O*-*tert*-butyldiphenylsilyl-α-L-*arabino*-hexopyranoside (11). A solution of **10** (27.6 g, 60.4 mmol) in dichloromethane (600 mL) was treated with a solution of trifluoroacetic acid (3.1 mL, 40 mmol) containing 1% water. After stirring overnight, the mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography on silica gel using a gradient of 10 to 50% of ethyl acetate in hexane (v/v) as the eluent to afford **11** (14.5 g, 34.8 mmol, 58% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.77–7.73 (m, 2H), 7.72–7.66 (m, 2H), 7.48–7.38 (m, 6H), 4.59 (br.s, 1H), 3.83 (dd, J = 8.5 Hz, J = 3.6 Hz, 1H), 3.78 (dd, J = 3.5 Hz, J = 1.6 Hz, 1H), 3.74 (dq, J = 9.2 Hz, J = 6.3 Hz, 1H), 3.54 (dd, J = 9.2 Hz, J = 8.5 Hz, 1H), 3.38 (s, 3H), 1.26 (d, J = 6.3 Hz, 3H), 1.07 (s, 9H); identical to reported data.[47]

1-*O*-Methyl-6-deoxy-2,3-(*O*-sulfite)-4-*O*-*tert*-butyldiphenylsilyl-α-L-*arabino*-hexopyranoside (12). Under an N_2 atmosphere, a solution of **11** (14.5 g, 34.8 mmol) in ethyl acetate (185 mL) and pyridine (5.6 mL, 69.6 mmol) was cooled to 0 °C and treated with thionyl chloride (3.8 mL, 52.2 mmol). After stirring for 30 min, ethyl acetate was added, and the organic phase was washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using 35% ethyl acetate in hexane (v/v) as the eluent to give **12** (15.64 g, 33.8 mmol, 97% yield) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.72–7.65 (m, 4H), 7.47–7.34 (m, 6H), 4.91 (dd, J = 7.6 Hz, J = 5.5 Hz, 1H), 4.92 (br.s, 1H), 4.73 (dd, J = 5.5 Hz, J = 0.9 Hz, 1H), 3.75 (dq, J = 9.7 Hz, J = 6.3 Hz, 1H), 3.40 (s, 3H), 3.14 (dd, J = 9.6 Hz, J = 7.6 Hz, 1H), 1.12 (d, J = 6.3 Hz, 3H), 1.06 (s, 9H); identical to reported data.[47]

1-*O*-Methyl-6-deoxy-2,3-(*O*-sulfate)-4-*O*-*tert*-butyldiphenylsilyl-α-L-*arabino*-hexopyranoside (13). A vigorously stirred solution of **12** (15.6 g, 33.8 mmol) in dichloromethane (110 mL), acetonitrile (110 mL) and water (96 mL) was treated with ruthenium(III) chloride hydrate (348 mg, 1.6 mmol) in water (14 mL) followed by sodium periodate (37.4 g). After 20 min of stirring, water was added and the mixture was extracted with dichloromethane. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using a gradient of 10 to 35% ethyl acetate (v/v) in hexane as the eluent to afford **13** (11.3 g, 23.6 mmol, 70% yield) as an oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.70–7.65 (m, 4H), 7.47–7.36 (m, 6H), 4.96 (dd, J = 7.3 Hz, J = 5.4 Hz, 1H), 4.90 (dd, J = 5.4 Hz, J = 0.9 Hz, 1H), 4.85 (br.s, 1H), 3.81 (dd, J = 9.6 Hz, J = 7.3 Hz, 1H), 3.73 (dq, J = 9.7, J = 6.1 Hz, 1H), 3.38 (s, 3H), 1.05 (s, 9H), 1.02 (d, J = 6.1 Hz, 3H); identical to reported data.[47]

1-*O*-Methyl-4-*O*-*tert*-butyldiphenylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranoside (14). Under an N_2 atmosphere, a solution of **13** (11.3 g, 23.6 mmol) in tetrahydrofuran (110 mL) at 55 °C was treated with tetrabutylammonium borohydride (12.1 g, 47.2 mmol). After stirring for 5 h, the mixture was cooled to 0 °C and quenched with acetone. Concentrated sulfuric acid (4.4 mL) was added and the resulting mixture was stirred for 1 h. The mixture was treated with methanol (4.4 mL) and stirred for 3 h. The solution was neutralized with saturated aqueous $NaHCO_3$ solution and the product was extracted with ethyl acetate, washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using 20% ethyl acetate in hexane (v/v) as the eluent to afford **14** (6.99 g, 17.4 mmol 74% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.72–7.64 (m, 4H), 7.46–7.34 (m, 6H), 4.37 (br. s, 1H), 3.74 (dq, J = 8.9 Hz, J = 6.3 Hz, 1H), 3.70–3.66 (m, 1H), 3.64 (ddd, J = 10.7 Hz, J = 9.1 Hz, J = 4.7 Hz, 1H), 3.38 (s, 3H), 1.86 (ddd, J = 13.5, J = 10.7 Hz, J = 3.1 Hz, 1H), 1.78 (ddd, J = 13.5 Hz, J = 4.7 Hz, J = 3.5 Hz, 1H), 1.19 (d, J = 6.2 Hz, 3H), 1.06 (s, 9H); identical to reported data.[47]

1-*O*-Methyl-2-*O*-benzoyl-4-*O*-*tert*-butyldiphenylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranoside (15a). Under an N_2 atmosphere, a solution of **14** (6.99 g, 17.4 mmol) in pyridine (310 mL) at 0 °C was treated dropwise with benzoyl chloride (5.7 mL, 52.2 mmol). After stirring for 1 h, the reaction was quenched with water and the aqueous phase was extracted with hexane. The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using 20% ethyl acetate in hexane (v/v) as the eluent to afford **15a** (7.2 g, 14.3 mmol, 81% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.77–7.71 (m, 2H), 7.70–7.63 (m, 4H), 7.58–7.53 (m, 1H), 7.42–7.35 (m, 3H), 7.35–7.28 (m, 5H), 4.96–4.92 (m, 1H), 4.54 (br.s, 1H), 3.84 (dq, J = 9.2, J = 6.3 Hz, 1H), 3.66 (ddd, J = 11.1 Hz, J = 9.2 Hz, J = 4.4 Hz, 1H), 3.41 (s, 3H), 2.03 (ddd, J = 13.6, J = 11.2, J = 3.0 Hz, 1H), 1.87 (ddd, J

= 13.6 Hz, J = 4.4 Hz, J = 3.2 Hz, 1H), 1.29 (d, J = 6.2 Hz, 1H), 1.05 (s, 9H); identical to reported data.[47]

1-*O*-Methyl-6-deoxy-2,3-(*O*-benzylidene)-α-L-*arabino*-hexopyranoside (17). A solution of **8** (3.0 g, 16.8 mmol) in dimethylformamide (11 mL) was treated with benzaldehydedimethylacetal (3 mL, 20.2 mmol) and *para*-toluenesulfonic acid (81 mg, 0.42 mmol), and the mixture was stirred overnight at 60 °C under reduced pressure (20 mbar) using a rotary evaporator. After the completion of the reaction, the mixture was neutralized with saturated aqueous $NaHCO_3$ solution, extracted with dichloromethane, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using 35% ethyl acetate in hexane (v/v) as the eluent to afford a diastereoisomeric mixture of **17** (3.2 g, 12.0 mmol, 72% yield) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 7.53–7.50 (m, 2H), 7.45–7.43 (m, 2H), 7.41–7.34 (m, 6H), 6.16 (s, 1H), 5.91 (s, 1H), 4.98 (br.s, 1H), 4.92 (br.s, 1H), 4.38 (dd, J = 7.2 Hz, J = 2.8 Hz, 1H), 4.24 (dd, J = 6.5 Hz, J = 2.9 Hz, 1H), 4.21(dd, J = 2.9 Hz, J = 1.1 Hz, 1H), 4.11 (dd, J = 2.8 Hz, J = 1.2 Hz, 1H), 3.74 (qd, J = 9.3 Hz, J = 6.4 Hz, 1H), 3.71 (qd, J = 8.8 Hz, J = 6.3 Hz, 1H), 3.56 (ddd, J = 9.3 Hz, J = 7.2 Hz, J = 4.2 Hz, 1H), 3.46 (ddd, J = 8.8 Hz, J = 6.5 Hz, J = 4.9 Hz, 1H), 3.42 (s, 3H), 3.39 (s, 3H), 2.40 (d, J = 4.2 Hz, 1H), 2.34 (d, J = 4.9 Hz, 1H), 1.36 (d, J = 6.3 Hz, 3H), 1.31 (d, J = 6.4 Hz, 3H), ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 138.7, 137.2, 129.6, 129.4, 128.62, 128.59, 126.8, 126.2, 104.3, 103.2, 98.3, 98.2, 79.7, 78.2, 77.9, 75.5, 74.5, 72.1, 66.3, 65.5, 55.19, 55.18, 17.8, 17.6, HRMS (ESI-TOF) m/z (M + Na)$^+$ calcd for $C_{14}H_{18}O_5Na$ 289.1046, found 289.1049, Δ −1.0 ppm.

1-*O*-Methyl-2-*O*-benzoyl-3-bromo-3,6-dideoxy-*altro*-hexopyranoside (18). Under an N_2 atmosphere, a solution of **17** (2.0 g, 7.5 mmol) in dry acetonitrile (100 mL) was treated with freshly dried calcium carbonate (900 mg, 9 mmol) and *N*-bromosuccinimide (1.55 g, 8.7 mmol). After refluxing for 1 h, the mixture was cooled to room temperature and filtered and the filtrate was diluted with dichloromethane. The organic phase was washed with water, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using 35% of ethyl acetate in hexane (v/v) as the eluent to afford **18** (2.3 g, 6.7 mmol, 89% yield) as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 8.04 (dd, J = 8.3 Hz, J = 1.8 Hz, 2H), 7.60 (tt, J = 7.6 Hz, J = 1.8 Hz, 1H), 7.47 (dd, J = 8.3 Hz, J = 7.6 Hz, 2H), 5.52 (dd, J = 3.6 Hz, J = 1.6 Hz, 1H), 4.72 (br.s, 1H), 4.54 (dd, J = 4.4 Hz, J = 3.6 Hz, 1H), 4.05 (dq, J = 8.9 Hz, J = 6.4 Hz, 1H), 3.59 (dd, J = 8.9 Hz, J = 4.4 Hz, 1H), 3.44 (s, 3H), 1.39 (d, J = 6.4 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 165.0, 133.8, 130.0, 128.7, 99.2, 73.2, 69.8, 65.9, 55.7, 52.7, 17.3; HRMS (ESI-TOF) m/z (M + Na)$^+$ calcd for $C_{14}H_{17}BrNaO_5$ 367.0152, found 367.0146, Δ −1.6 ppm.

1-*O*-Methyl-2-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranoside (19) using Bu_3SnH. Under an N_2 atmosphere, a solution of **18** (4.0 g, 11.6 mmol) in dry toluene (170 mL) was treated with tri-*n*-butyltin hydride (6.0 mL, 23 mmol) and 2,2′-azobis (2-methylpropionitrile) (AIBN) (300 mg, 1.8 mmol). After stirring for 2 h at 80 °C, the mixture was cooled to room temperature and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using 35% ethyl acetate in hexane (v/v) as the eluent to afford **19** (2.4 g, 9.0 mmol, 76% yield) as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 8.04 (dd, J = 8.3 Hz, 1.6 Hz, 2H), 7.57 (tt, J = 7.5 Hz, J = 1.6 Hz, 1H), 7.45 (dd, J = 8.3 Hz, J = 7.5 Hz, 2H), 5.17 (ddd, J = 3.2 Hz, J = 3.2 Hz, J = 1.6 Hz, 1H), 4.66 (br.s, 1H), 3.72–3.66 (m, 2H), 3.42 (s, 3H), 2.23 (ddd, J = 13.3 Hz, J = 4.0 Hz, J = 3.2 Hz, 1H), 1.99 (ddd, J = 13.3 Hz, J = 10.8 Hz, J = 3.2 Hz, 1H), 1.35 (s, 3H), ^{13}C NMR(150 MHz, $CDCl_3$): δ (ppm) 165.8, 133.4, 129.9, 128.6, 97.5, 71.1, 69.4, 68.5, 55.0, 32.9, 17.9, HRMS (ESI-TOF) m/z (M + Na)$^+$ calcd for $C_{14}H_{18}O_5Na$ 289.1046 found 289.1052, Δ 2.1 ppm.

1-*O*-Methyl-2-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranoside (19) using nickel boride. Under an N_2 atmosphere, a solution of **18** (40 mg, 116 μmol) in ethanol (10 mL) was slowly treated with sodium borohydride (26 mg, 650 μmol) and nickel chloride hexahydrate (59 mg, 260 μmol). After stirring the solution at room temperature for 25 min, the mixture was concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using 20% ethyl acetate in hexane (v/v) as the eluent to afford **19** (24 mg, 90 μmol, 77% yield).

1-*O*-Methyl-2-*O*-benzoyl-3,6-dideoxy-α-L-*arabino*-hexopyranoside (19) using Pd-catalyzed hydrogenation. Under an N_2 atmosphere, a solution of **18** (10 mg, 29 μmol) in ethanol (1 mL) was treated with palladium on charcoal (5 mg) and triethylamine (7 μl, 49 μmol). After stirring under a hydrogen atmosphere (1 atm) for 13 h, the mixture was filtered over a cotton plug and the catalyst was rinsed with ethanol. The filtrate was washed with water, dried over Na_2SO_4 and then concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using 35% ethyl acetate in hexane (v/v) as the eluent to afford **19** (6 mg, 23 μmol, 79% yield) as a yellow oil.

1-*O*-Methyl-2-*O*-benzoyl-4-*O*-*tert*-butyldiphenylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranoside (15a). Under an N_2 atmosphere, a solution of **19** (5 mg, 18.8 μmol) in dichloromethane (500 μl) was treated with *tert*-butyldiphenylchlorosilane (10 μl, 37.6 μmol) and imidazole (2.6 mg, 37.6 μmol). After stirring for 19 h, the mixture was filtered and then concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using 20% ethyl acetate in hexane (v/v) as the eluent to afford **15a** (8.5 mg, 16.8 μmol, 89% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.77–7.71 (m, 2H), 7.70–7.63 (m, 4H), 7.58–7.53 (m, 1H), 7.42–7.35 (m, 3H), 7.35–7.28 (m, 5H), 4.96–4.92 (m, 1H), 4.54 (br.s, 1H), 3.84 (dq, J = 9.2, J = 6.3 Hz, 1H), 3.66 (ddd, J = 11.1 Hz, J = 9.2 Hz, J = 4.4 Hz, 1H), 3.41 (s, 3H), 2.03 (ddd, J = 13.6, J = 11.2, J = 3.0 Hz, 1H), 1.87 (ddd, J = 13.6 Hz, J = 4.4 Hz, J = 3.2 Hz, 1H), 1.29 (d, J = 6.2 Hz, 1H), 1.05 (s, 9H); identical to reported data.[47]

1-*O*-Methyl-2-*O*-benzoyl-4-*O*-benzyl-3,6-dideoxy-α-L-*arabino*-hexopyranoside (15b). Under an N_2 atmosphere, a solution of **19** (300 mg, 1.1 mmol) in α,α,α-benzotrifluoride (2.3 mL) was treated with 2-benzyloxy-1-methylpyridiniumtriflate (780 mg,

2.3 mmol) and MgO (91 mg, 2.3 mmol). After stirring the solution for 14 h at 83 °C, the mixture was cooled to room temperature and filtered. The solid was washed with dichloromethane and the filtrate was concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using 20% ethyl acetate in hexane as the eluent to afford **15b** (330 mg, 0.93 mmol, 82% yield) as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 8.04 (dd, J = 8.0 Hz, J = 2.7 Hz, 2H), 7.60 (tt, J = 7.6 Hz, J = 2.7 Hz, 1H), 7.47 (dd, J = 8.0 Hz, J = 7.6 Hz, 2H), 7.35–7.27 (m, 5H), 5.19 (ddd, J = 3.4 Hz, J = 3.1 Hz, J = 1.4 Hz, 1H), 4.68 (br.s, 1H), 4.65 (d, J = 11.2 Hz, 1H), 4.51 (d, J = 11.2 Hz, 1H), 3.85 (dq, J = 9.0 Hz, J = 6.2 Hz, 1H), 3.49 (ddd, J = 11.4 Hz, J = 9.0 Hz, J = 6.4 Hz, 1H), 3.43 (s, 1H), 2.37 (ddd, J = 13.7 Hz, J = 6.4 Hz, J = 3.1 Hz, 1H), 2.02 (ddd, J = 13.7 Hz, J = 11.4 Hz, J = 3.4 Hz, 1H), 1.37 (d, J = 6.2 Hz, 3H); ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 165.7, 138.4, 138.2, 133.3, 129.9, 128.51, 128.49, 128.0, 97.4, 75.0, 71.2, 71.0, 68.0, 54.8, 29.6, 18.3; HRMS (ESI-TOF) m/z (M + Na)$^+$ calcd for $C_{21}H_{24}O_5$ 379.1516, found 379.1504, Δ −3.2 ppm.

1-*O*-Methyl-2-*O*-benzoyl-4-*O*-allyl-3,6-dideoxy-α-L-*arabino*-hexopyranoside (15c). Under an N_2 atmosphere, a solution of **19** (20 mg, 75 μmol) in dichloromethane (0.3 mL) and cyclohexane (0.6 mL) was treated with *O*-allyl 2,2,2-trichloroacetimidate (38.3 μl, 188 μmol) followed by trifluoromethanesulfonic acid (5 μl). After stirring the solution for 20 h, the mixture was filtered, and the filtrate was neutralized with saturated aqueous $NaHCO_3$ solution, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using 20% ethyl acetate in hexane as the eluent to afford **15c** (15 mg, 50 μmol, 67%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ (ppm) 8.05 (dd, J = 8.3 Hz, J = 1.5 Hz, 2H), 7.58 (tt, J = 7.4 Hz, J = 1.5 Hz, 1H), 7.46 (dd, J = 8.3 Hz, J = 7.4 Hz, 2H), 5.89 (dddd, J = 17.2 Hz, J = 10.4 Hz, J = 5.8 Hz, J = 5.6 Hz, 1H), 5.26 (dddd, J = 17.2 Hz, J = 1.7 Hz, J = 1.4 Hz, J = 1.2 Hz, 1H), 5.16 (ddd, J = 3.1 Hz, J = 2.7 Hz, J = 1.5 Hz, 1H), 5.16 (dddd, J = 10.4 Hz, J = 1.7 Hz, J = 1.1 Hz, J = 1.0 Hz, 1H), 4.64 (br.s, 1H), 4.10 (dddd, J = 12.4 Hz, J = 5.8 Hz, J = 1.2 Hz, J = 1.1 Hz, 1H), 3.96 (dddd, J = 12.4 Hz, J = 5.8 Hz, J = 1.4 Hz, J = 1.0 Hz, 1H), 3.78 (dq, J = 9.3 Hz, J = 6.2 Hz, 1H), 3.41 (s, 3H), 3.39 (ddd, J = 11.2 Hz, J = 9.3 Hz, J = 4.7 Hz, 1H), 2.30 (ddd, J = 14.1 Hz, J = 4.7 Hz, J = 2.7 Hz, 1H), 1.95 (ddd, J = 14.1 Hz, J = 11.2 Hz, J = 3.1 Hz, 1H), 1.33 (d, J = 6.2 Hz, 3H), ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 165.8, 135.0, 133.3, 130.2, 129.9, 128.6, 117.1, 97.5, 75.2, 71.0, 70.3, 68.0, 54.9, 29.8, 18.3, HRMS (ESI-TOF) m/z (M + Na)$^+$ calcd for $C_{17}H_{22}O_5Na$ 329.1359, found 329.1357, Δ −0.6 ppm.

2-*O*-Benzoyl-4-*O*-*tert*-butyldiphenylsilyl-3,6-dideoxy-α-L-*arabino*-hexopyranose (16a). Under an N_2 atmosphere, a solution of **15a** (7.2 g, 14.3 mmol) in dichloromethane (170 mL) at −78 °C was treated dropwise with 1 M boron tribromide solution in dichloromethane (11.7 mL, 28.1 mmol). After stirring for 3 h, the reaction was quenched with saturated aqueous $NaHCO_3$ solution and the aqueous phase was extracted with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using 20% ethyl acetate in hexane (v/v) as the eluent to afford **16a** (5.2 g, 10.7 mmol, 76% yield, α/β mixture 4/1) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) for the dominating α-anomer: δ (ppm) 7.77–7.64 (m, 7H), 7.45–7.23 (m, 8H), 5.08 (br.s, 1H), 5.00–4.97 (m, 1H), 4.09 (dq, J = 8.9 Hz, J = 6.2 Hz, 1H), 3.68 (ddd, J = 11.4 Hz, J = 8.9 Hz, J = 4.4 Hz, 1H), 2.13 (ddd, J = 13.8 Hz, J = 11.4 Hz, J = 3.0 Hz, 1H), 1.89 (dt, J = 13.8 Hz, J = 3.9 Hz, 1H), 1.27 (d, J = 6.2 Hz, 3H), 1.06 (s, 9H); identical to reported data.[47]

2-*O*-Benzoyl-4-*O*-benzyl-3,6-dideoxy-α-L-*arabino*-hexopyranose (16b). A solution of **15b** (170 mg, 0.48 mmol) in acetic acid (6.8 mL) was treated with a 3 M solution of hydrochloric acid (630 μl, 1.89 mmol). After stirring the solution for 2 h at 80 °C, the mixture was cooled to room temperature, quenched with $NaHCO_3$ (180 mg) and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using 20% ethyl acetate in hexane (v/v) as the eluent to afford **16b** (130 mg, 0.38 mmol, 78%, α/β mixture 3/1) as a yellow oil. ^{1}H NMR (600 MHz, $CDCl_3$) for the dominating α-anomer: δ (ppm) 8.01 (dd, J = 8.6 Hz, J = 2.8 Hz, 2H), 7.59 (tt, J = 7.7 Hz, J = 2.8 Hz, 1H), 7.46 (dd, J = 8.6 Hz, J = 7.7 Hz, 2H), 5.20 (dd, J = 3.2 Hz, J = 3.1 Hz, 1H), 5.19 (br.s, 1H), 4.65 (d, J = 11.4 Hz, 1H), 4.50 (d, J = 11.4 Hz, 1H), 4.09 (dq, J = 8.7 Hz, J = 6.5 Hz, 1H), 3.48 (ddd, J = 11.2 Hz, J = 8.7 Hz, J = 4.4 Hz, 1H), 2.34 (ddd, J = 13.8 Hz, J = 4.4 Hz, J = 3.2 Hz, 1H), 2.09 (ddd, J = 13.8 Hz, J = 11.2 Hz, J = 3.1 Hz, 1H), 1.33 (d, J = 6.5 Hz, 3H), ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 165.8, 138.2, 133.4, 129.9, 128.6, 128.1, 91.2, 75.1, 71.3, 71.2, 68.3, 29.0, 18.3, HRMS (ESI-TOF) m/z (M + Na)$^+$ calcd for $C_{20}H_{22}O_5Na$ 365.1359, found 365.1356, Δ −0.8 ppm, $(M_2 + Na)^+$ calcd for $C_{40}H_{44}O_{10}Na$ 707.2827, found 707.2830, Δ 0.4 ppm.

2-*O*-Benzoyl-4-*O*-allyl-3,6-dideoxy-α-L-*arabino*-hexopyranose (16c). A solution of **15c** (5 mg, 16.3 μmol) in acetic acid (180 μl) was treated with a 3 M solution of hydrochloric acid (18 μl, 54 μmol). After stirring the solution for 2 h at 80 °C, the mixture was cooled to room temperature, quenched with $NaHCO_3$ (5 mg) and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using 20% ethyl acetate in hexane (v/v) as the eluent to afford **16c** (4 mg, 13.6 μmol, 83%, α/β mixture 3/1) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) for the dominating α-anomer: δ (ppm) 8.05 (dd, J = 8.2 Hz, J = 1.4 Hz, 2H), 7.59 (tt, J = 7.2 Hz, J = 1.4 Hz, 1H), 7.46 (dd, J = 8.2 Hz, J = 7.2 Hz, 2H), 5.90 (dddd, J = 17.4 Hz, J = 10.2 Hz, J = 5.8 Hz, J = 5.6 Hz, 1H), 5.27 (dddd, J = 17.4 Hz, J = 1.7 Hz, J = 1.3 Hz, J = 1.3 Hz, 1H), 5.21 (dd, J = 3.2 Hz, J = 2.9 Hz, 1H), 5.19 (br.s, 1H), 5.17 (dddd, J = 10.2 Hz, J = 1.7 Hz, J = 1.1 Hz, J = 1.0 Hz, 1H), 4.11 (dddd, J = 12.5 Hz, J = 5.6 Hz, J = 1.3 Hz, J = 1.0 Hz, 1H), 4.04 (dq, J = 8.8 Hz, J = 6.3 Hz, 1H), 3.98 (dddd, J = 12.5 Hz, J = 5.8 Hz, J = 1.3 Hz, J = 1.1 Hz, 1H), 3.41 (ddd, J = 11.2 Hz, J = 8.8 Hz, J = 5.0 Hz, 1H), 2.31 (ddd, J = 13.8 Hz, J = 5.0 Hz, J = 2.9 Hz, 1H), 2.05 (ddd, J = 13.8 Hz, J = 11.2 Hz, J = 3.2 Hz, 1H), 1.32 (d, J = 6.3 Hz, 3H), ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 166.5, 159.8, 149.4, 138.4, 130.4, 128.6, 128.0, 127.9, 120.1, 114.1, 96.8, 75.1, 71.3, 70.7, 68.9, 68.7, 66.3, 55.4, 32.0, 19.1, 18.1, HRMS (ESI-TOF) m/z (M + Na)$^+$ calcd for $C_{16}H_{20}O_5Na$ 315.1203, found

315.1198, Δ −1.6 ppm, HRMS (ESI-TOF) *m*/*z* $(M_2 + Na)^+$ calcd for $C_{32}H_{40}O_{10}Na$ 607.2514, found 607.2515, Δ 0.2 ppm.

(5*R*)-5-[(2′-*O*-Benzoyl-4′-*O*-*tert*-butyldiphenylsilyl-3′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-hexene (20). Under an N_2 atmosphere, a solution of **16a** (100 mg, 0.20 mmol) in dry dichloromethane (4 mL) was treated with trichloroacetonitrile (42 μL, 0.40 mmol) and 1,8-diazabicyclo (5.4.0) undec-7-ene (1.2 μL, 7 μmol). After stirring for 1 h, the mixture was concentrated under reduced pressure and the residue was purified *via* flash column chromatography on silica gel using 10% ethyl acetate in hexane (v/v) as the eluent to furnish trichloracetimidate (100 mg) that was directly used for the next step. The purified trichloroacetimidate in dry dichloromethane (3 mL) at 0 °C was treated with (*R*)-5-hexene-2-ol (51 μl, 0.41 mmol) and 5 μL trimethylsilyltriflate. The mixture was stirred from 0 °C to room temperature until completion of the reaction and then the mixture was quenched with saturated aqueous $NaHCO_3$ solution and diluted with dichloromethane. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using 20% ethyl acetate in hexane (v/v) as the eluent to afford **20** (58.4 mg, 0.10 mmol, 50% yield) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): *δ* (ppm) 7.74–7.71 (m, 2H), 7.70–7.67 (m, 2H), 7.67–7.64 (m, 2H), 7.55 (tt, *J* = 8.8 Hz, *J* = 1.3 Hz, 1H), 7.40–7.36 (m, 3H), 7.34–7.31 (m, 3H), 7.31–7.27 (m, 2H), 5.86 (ddt, *J* = 17.1 Hz, *J* = 10.2 Hz, *J* = 6.7 Hz, 1H), 5.04 (ddt, *J* = 17.1 Hz, *J* = 1.9 Hz, *J* = 1.6 Hz, 1H), 4.98 (ddt, *J* = 10.2 Hz, *J* = 1.9 Hz, *J* = 1.2 Hz, 1H), 4.87 (dd, *J* = 3.7 Hz, *J* = 2.9 Hz, 1H), 4.74 (br.s, 1H), 3.89 (dq, *J* = 9.0 Hz, *J* = 6.2 Hz, 1H), 3.79 (m, 1H), 3.65 (ddd, *J* = 11.2 Hz, *J* = 9.0 Hz, *J* = 4.6 Hz, 1H), 2.15–2.08 (m, 2H), 2.04 (ddd, *J* = 13.7 Hz, *J* = 11.2 Hz, *J* = 2.9 Hz, 1H), 1.87 (ddd, *J* = 13.7 Hz, *J* = 4.6 Hz, *J* = 3.7 Hz, 1H), 1.50–1.40 (m, 2H), 1.26 (d, *J* = 6.2 Hz, 3H), 1.12 (d, *J* = 6.1 Hz, 3H), 1.06 (s, 9H); ^{13}C NMR (150 MHz, $CDCl_3$): *δ* (ppm) 165.4, 139.0, 135.9, 135.9, 134.1, 133.2, 129.9, 129.74, 129.72, 129.6, 128.2, 127.6, 127.5, 114.5, 93.4, 72.1, 71.9, 70.3, 69.8, 31.0, 33.9, 33.3, 28.8, 27.0, 25.3, 19.3, 19.2, 18.4; identical to reported data.[47]

(4*R*)-4-[(2′-*O*-Benzoyl-4′-*O*-*tert*-butyldiphenylsilyl-3′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-pentanoic acid (21). A solution of **20** (149 mg, 0.26 mmol) in dichloromethane (2 mL), acetonitrile (2 mL), and water (1.5 mL) was treated with ruthenium (III) chloride hydrate (5.4 mg, 0.03 mmol) in water (0.5 mL) while stirring vigorously. After 5 min, sodium periodate (278 mg, 1.3 mmol) was added to the mixture. After stirring for 16 h, water (6 mL) was added and the aqueous phase was extracted with dichloromethane (2 × 6 mL). The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using 40% ethyl acetate in hexane (v/v) as the eluent to afford **21** (107 mg, 0.18 mmol, 70% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* (ppm) 7.77–7.71 (m, 2H), 7.71–7.60 (m, 4H), 7.55 (*t*, *J* = 7.8 Hz, 1H), 7.42–7.27 (m, 8H), 4.88 (br.s, 1H), 4.76 (br.s, 1H), 3.92–3.81 (m, 2H), 3.67 (ddd, *J* = 11.1 Hz, *J* = 9.8 Hz, *J* = 4.3 Hz, 1H), 2.64–2.47 (m, 2H), 2.01 (m, 1H), 1.95–1.84 (m, 3H), 1.28 (d, *J* = 6.5 Hz, 3H), 1.17 (d, *J* = 6.0 Hz, 3H), 1.07 (s, 9H), ^{13}C NMR(100 MHz, $CDCl_3$): *δ* (ppm) 179.1, 165.5, 136.1, 136.0, 134.2, 133.4, 133.1, 130.0, 129.9, 129.84, 129.77, 128.3, 127.8, 127.6, 93.5, 71.9, 71.2, 70.31, 70.28, 33.4, 32.0, 27.2, 27.1, 19.5, 19.1, 18.4, 14.3; identical to reported data.[47]

(4*R*)-4-[(4′-*O*-*tert*-Butyldiphenylsilyl-3′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-pentanoic acid (22). A solution of **21** (107 mg, 0.18 mmol) in 1,4-dioxane (5 mL) was treated with a solution of lithium hydroxide monohydrate (30.2 mg, 0.72 mmol) in water (780 μl). After stirring for 20 h at 60 °C, the mixture was cooled to room temperature, glacial acetic acid (540 μl) was added and the mixture was concentrated under reduced pressure. The resulting mixture was purified by flash column chromatography on silica gel using 10% ethyl acetate in hexane (v/v) as the eluent to afford **22** (74 mg, 0.15 mmol, 83% yield). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* (ppm) 7.72–7.64 (m, 4H), 7.47 (*t*, *J* = 7.6 Hz, 1H), 7.44–7.34 (m, 5H), 4.59 (br.s, 1H), 3.85 (m, 1H), 3.75 (dq, *J* = 6.3 Hz, *J* = 8.6 Hz, 1H), 3.69–3.59 (m, 2H), 2.61–2.45 (m, 2H), 1.93–1.83 (m, 3H), 1.78 (m, 1H), 1.16 (d, *J* = 6.3 Hz, 3H), 1.14 (d, *J* = 6.1 Hz, 3H), 1.05 (s, 9H); identical to reported data.[47]

Benzyl (4*R*)-4-[(2′-*O*-benzyl-4′-*O*-*tert*-butyldiphenylsilyl-3′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-pentanoate (23). Under an N_2 atmosphere, a solution of **22** (52 mg, 90 μmol) in dimethylformamide (3 mL) was treated with benzyl bromide (5 mL, 660 μmol) and sodium hydride (52 mg, 1.3 mmol). After stirring for 13 h, the solution was cooled to 0 °C, quenched with 1 M hydrochloric acid solution (1 mL) and extracted with ethyl acetate. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using 5% ethyl acetate in hexane (v/v) as the eluent to afford **23** (52.5 mg, 78 μmol, 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$): *δ* (ppm) 7.74–7.67 (m, 4H), 7.42–7.31 (m, 10H), 7.26–7.18 (m, 3H), 7.08–7.04 (m, 2H), 5.18 (d, *J* = 12.2 Hz, 1H), 5.13 (d, *J* = 12.2 Hz, 1H), 4.64 (br.s, 1H), 4.14 (d, *J* = 12.2 Hz, 1H), 4.05 (d, *J* = 12.2 Hz, 1H), 3.86–3.68 (m, 3H), 3.28 (br.s, 1H), 2.60–2.42 (m, 2H), 1.92–1.76 (m, 3H), 1.65 (ddd, *J* = 13.5 Hz, *J* = 10.6 Hz, *J* = 2.7 Hz, 1H), 1.25 (d, *J* = 5.8 Hz, 3H), 1.07 (d, *J* = 6.2 Hz, 3H), 1.05 (s, 9H), ^{13}C NMR(100 MHz, $CDCl_3$): *δ* (ppm) 173.6, 138.4, 138.3, 136.2, 136.08, 136.06, 136.04, 136.03, 134.8, 133.9, 129.9, 129.7, 128.7, 128.6, 127.9, 127.8, 127.7, 127.6, 94.5, 75.6, 72.3, 70.5, 70.3, 70.13, 70.10, 69.4, 32.4, 32.3, 30.7, 27.1, 19.5, 18.8, 18.5; identical to reported data.[47]

Benzyl (4*R*)-4-[(2′-*O*-benzyl-3′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-pentanoate (24a). Under an N_2 atmosphere, a solution of **23** (52.5 mg, 79 μmol) in tetrahydrofuran (2 mL) was treated with a 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran (280 μl, 280 μmol). After stirring overnight at room temperature, water was added and the mixture was extracted with dichloromethane. The organic phase was washed with Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using 20% ethyl acetate in hexane (v/v) as the eluent to afford **24a** (17 mg, 40 μmol, 51% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): *δ* (ppm)

7.42–7.25 (m, 10H), 5.12 (s, 2H), 4.78 (br.s, 1H), 4.60 (d, J = 12.4 Hz, 1H), 4.56 (d, J = 12.4 Hz, 1H), 3.83 (m, 1H), 3.68–3.52 (m, 2H), 3.50 (br.s, 1H), 2.54–2.39 (m, 2H), 2.12 (dt, J = 13.1 Hz, J = 3.6 Hz, 1H), 1.92–1.77 (m, 2H), 1.69 (ddd, J = 13.4 Hz, J = 10.8 Hz, J = 3.0 Hz, 1H), 1.25 (d, J = 6.0 Hz, 3H), 1.10 (d, J = 6.0 Hz, 3H); identical to reported data.[47]

(3*R*)-3-[(2′-*O*-Benzoyl-4′-*O*-*tert*-butyldiphenylsilyl-3′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-butyne (25). Under an N_2 atmosphere, a solution of **16a** (1.0 g, 2.0 mmol) in dry dichloromethane (10 mL) was treated with trichloroacetonitrile (0.42 mL, 4 mmol) and 1,8-diazabicyclo (5.4.0) undec-7-ene (25 μL, 0.1 mmol). After stirring for 30 min, the mixture was concentrated under reduced pressure and the residue was quickly purified by short silica gel flash column chromatography with 10% ethyl acetate in hexane and directly used for the next step. The purified trichloroacetimidate (970 mg, 1.53 mmol, 77% yield) in dry dichloromethane (20 mL) at 0 °C was treated with (*R*)-3-butyn-2-ol (156 mg, 2.25 mmol) in dry dichloromethane (2.3 mL) and 67 μL trimethylsilyltriflate. The mixture was stirred from 0 °C to room temperature until completion of the reaction; then the mixture was quenched with saturated aqueous $NaHCO_3$ solution and diluted with dichloromethane. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using 20% ethyl acetate in hexane (v/v) as the eluent to afford **25** (720 mg, 1.33 mmol, 67% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.75–7.71 (m, 2H), 7.71–7.65 (m, 4H), 7.56 (m, 1 H), 7.42–7.26 (m, 8H), 4.93 (dd, J = 3.6 Hz, J = 3.1 Hz, 1H), 4.80 (br.s, 1H), 4.43 (dq, J = 6.7 Hz, J = 2.2 Hz, 1H), 4.07 (dq, J = 9.2 Hz, J = 6.2 Hz, 1H), 3.68 (ddd, J = 11.2 Hz, J = 9.2 Hz, J = 4.4 Hz, 1H), 2.49 (d, J = 2.2 Hz, 1H), 2.09 (ddd, J = 13.9 Hz, J = 11.2 Hz, J = 3.1 Hz, 1H), 1.89 (ddd, J = 13.9 Hz, J = 4.4 Hz, J = 3.1 Hz, 1H), 1.49 (d, J = 6.7 Hz, 3H), 1.28 (d, J = 6.2 Hz, 3H), 1.06 (s, 9H), ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 165.4, 136.1, 136.0, 134.2, 133.4, 133.1, 129.9, 128.3, 127.8, 127.6, 95.8, 84.2, 72.5, 71.4, 70.4, 70.3, 63.8, 33.4, 27.2, 22.2, 19.5, 18.2, HRMS (ESI-TOF) m/z (M + NH_4)$^+$ calcd for $C_{33}H_{42}NO_5Si$ 560.2827, found 560.2831, Δ 0.7 ppm.

(3*R*)-3-[(2′-*O*-Benzoyl-4′-*O*-*tert*-butyldiphenylsilyl-3′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-butene (26). Under an N_2 atmosphere, a solution of **25** (304 mg, 560 μmol) in ethyl acetate (4 mL) was added to a mixture of Lindlar's catalyst (300 mg) and quinoline (300 μl) in ethyl acetate (4 mL). After stirring vigorously under a hydrogen atmosphere (1 atm) for 24 h, the mixture was filtered over a silica plug and the catalyst was washed with ethyl acetate. The filtrate was concentrated under reduced pressure and the residue was poured into a 10% hydrochloric acid solution. The mixture was extracted with dichloromethane. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by column flash chromatography on silica gel using 10% ethyl acetate in hexane (v/v) as the eluent to afford **26** (301 mg, 543 μmol, 97% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.73 (dd, J = 8.3 Hz, J = 1.3 Hz, 2H), 7.66 (m, 4H), 7.55 (tt, J = 7.2 Hz, J = 1.4 Hz, 1H), 7.38 (dd, J = 7.7 Hz, J = 7.2 Hz, 2H), 7.31 (m, 6H), 5.94 (ddd, J = 17.1 Hz, J = 10.5 Hz, J = 6.2 Hz, 1H), 5.25 (dd, J = 17.3 Hz, J = 1.4 Hz, 1H), 5.13 (dd, J = 10.5 Hz, J = 1.4 Hz, 1H), 4.92 (dd, J = 3.9 Hz, J = 3.0 Hz, 1H), 4.78 (s, 1H), 4.24 (dq, J = 6.4 Hz, J = 6.2 Hz, 1H), 3.90 (dq, J = 9.2 Hz, J = 6.2 Hz, 1H), 3.66 (ddd, J = 11.2 Hz, J = 9.2 Hz, J = 4.3 Hz, 1H), 2.07 (ddd, J = 14.0 Hz, J = 11.2 Hz, J = 3.0 Hz, 1H), 1.88 (ddd, J = 14.0 Hz, J = 3.9 Hz, J = 3.0 Hz, 1H), 1.26 (d, J = 6.4 Hz, 3H), 1.24 (d, J = 6.3 Hz, 3H), 1.05 (s, 9H), ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 165.5, 140.4, 136.1, 136.0, 133.1, 129.9, 128.3, 127.5, 127.6, 114.7, 94.5, 73.9, 71.8, 70.5, 69.9, 33.4, 27.2, 20.0, 19.5, 18.3, HRMS (ESI-TOF) m/z (M + NH_4)$^+$ calcd for $C_{33}H_{44}NO_5Si$ 562.2983, found 562.2991, Δ 1.4 ppm.

(3*R*)-3-[(4′-*O*-*tert*-Butyldiphenylsilyl-3′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-butene (27). A solution of **26** (147 mg, 270 μmol) in 1,4-dioxane (7 mL) was treated with a solution of lithium hydroxide monohydrate (23 mg, 540 μmol) in water (1.5 mL). The mixture was heated to 60 °C. After stirring for 24 h, the mixture was cooled to room temperature, glacial acetic acid was added and the mixture was concentrated under reduced pressure. The resulting mixture was purified by flash column chromatography on silica gel using 10% ethyl acetate in hexane (v/v) as the eluent to afford **27** (101 mg, 229 μmol, 85% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.67 (m, 4H), 7.39 (m, 6 H), 5.92 (ddd, J = 17.2 Hz, J = 10.5 Hz, J = 6.0 Hz, 1H), 5.24 (dd, J = 17.2 Hz, J = 1.5 Hz, 1H), 5.12 (dd, J = 10.5 Hz, J = 1.5 Hz, 1H), 4.62 (s, 1H), 4.23 (dq, J = 6.3 Hz, J = 6.0 Hz, 1H), 3.81 (dq, J = 9.0 Hz, J = 6.3 Hz, 1H), 3.66 (dd, J = 3.8 Hz, J = 3.0 Hz, 1H), 3.64 (ddd, J = 10.7 Hz, J = 9.1 Hz, J = 4.2 Hz, 1H), 1.88 (ddd, J = 13.7 Hz, J = 10.8 Hz, J = 3.1 Hz, 1H), 1.78 (ddd, J = 13.4 Hz, J = 4.2 Hz, J = 3.8 Hz, 1H), 1.22 (d, J = 6.3 Hz, 3H), 1.15 (d, J = 6.2 Hz, 3H), 1.05 (s, 9H), ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm) 140.4, 136.1, 133.9, 130.3, 129.9, 129.8, 128.6, 127.8, 127.6, 114.5, 97.1, 73.3, 70.1, 70.1, 69.3, 35.8, 27.1, 20.6, 19.8, 19.5, 18.2, HRMS (ESI-TOF) m/z (M + NH_4)$^+$ calcd for $C_{26}H_{40}NO_4Si$ 458.2721, found 458.2731, Δ 2.2 ppm.

(3*R*)-3-[(2′-*O*-benzyl-4′-*O*-*tert*-Butyldiphenylsilyl-3′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-1-butene (28). Under an N_2 atmosphere, a solution of **27** (85.7 mg, 194 μmol) in dry dimethylformamide (3.7 mL) was treated with benzyl bromide (68.4 μl, 570 μmol) followed by sodium hydride (29.6 mg, 1.24 mmol). After stirring the solution for 14 h at room temperature, 1 M hydrochloric solution (1.5 mL) was added to the mixture and the mixture was extracted with ethyl acetate. The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using 20% ethyl acetate in hexane (v/v) as the eluent to afford **28** (97.8 mg, 184 μmol, 95% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.76–7.66 (m, 4H), 7.40–7.34 (m, 11H), 5.92 (ddd, J = 17.3, J = 10.5, J = 5.8 Hz, 1H), 5.22 (ddd, J = 17.3 Hz, J = 1.5 Hz, J = 1.5 Hz, 1H), 5.10 (ddd, J = 10.5, J = 1.5 Hz, J = 1.5 Hz, 1H), 4.69 (br.s, 1H), 4.24 (qd, J = 6.4 Hz, J = 5.8 Hz, 1H), 4.17 (d, J = 12.2 Hz, 1H), 4.09 (d, J = 12.2 Hz, 1H), 3.77 (ddd, J = 10.5 Hz, J = 9.2 Hz, J = 4.8 Hz, 1H), 3.80 (dq, J = 9.2 Hz, J = 6.1 Hz, 1H), 3.36 (dd, J = 3.8 Hz, J = 3.1 Hz, 1H), 1.84 (ddd, J = 13.5

Hz, J = 4.8 Hz, J = 3.8 Hz, 1H), 1.74 (ddd, J = 13.5, J = 10.5, J = 3.1 Hz, 1H), 1.24 (d, J = 6.1 Hz, 3H), 1.19 (d, J = 6.4 Hz, 3H), 1.06 (s, 9H), ^{13}C NMR (150 MHz, $CDCl_3$): δ (ppm) 140.5, 136.1, 136.0, 128.6, 127.9, 114.3, 95.4, 75.4, 72.5, 70.3, 70.2, 70.1, 32.3, 27.1, 19.5, 18.3, HRMS (ESI-TOF) m/z $(M + NH_4)^+$ calcd for $C_{33}H_{46}NO_4Si$ 548.3191, found 548.3202, Δ 2.0 ppm.

(3*R*)-3-[(2′-*O*-benzyl-3′,6′-Dideoxy-α-L-*arabino*-hexopyranosyl) oxy]-1-butene (29). Under an N_2 atmosphere, a solution of **28** (97.8 mg, 184 μmol) in tetrahydrofuran (5 mL) was treated with a 1 M solution of tetrabutylammonium fluoride in tetrahydrofuran (0.54 mL, 540 μmol). After stirring overnight at room temperature, water was added and the mixture was extracted with dichloromethane. The combined organic phases were dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography on silica gel using 20% ethyl acetate in hexane (v/v) as the eluent to afford **29** (32.7 mg, 112 μmol, 61% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.37–726 (m, 5 H), 5.87 (ddd, J = 17.2 Hz, J = 10.5 Hz, J = 6.0 Hz, 1H), 5.20 (ddd, J = 17.3 Hz, J = 1.5 Hz, J = 1.5 Hz, 1H), 5.07 (ddd, J = 10.5 Hz, J = 1.5 Hz, J = 1.5 Hz, 1H), 4.82 (br.s, 1H), 4.59 (s, 2H), 4.25 (qd, J = 6.4 Hz, J = 6.0 Hz, 1H), 3.65 (ddd, J = 10.5 Hz, J = 9.5 Hz, J = 4.3 Hz, 1H), 3.64 (dq, J = 9.5 Hz, J = 5.9 Hz, 1H), 3.58 (ddd, J = 3.8 Hz, J = 3.2 Hz, J = 1.7 Hz, 1H), 2.14 (ddd, J = 13.2 Hz, J = 4.3 Hz, J = 3.7 Hz, 1H), 1.77 (ddd, J = 13.2 Hz, J = 10.5 Hz, J = 3.1 Hz, 1H), 1.26 (d, J = 5.9 Hz, 3H), 1.20 (d, J = 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 140.4, 138.4, 128.5, 127.8, 127.8, 114.4, 94.9, 75.9, 72.7, 71.3, 69.9, 68.6, 32.9, 19.7, 17.7; HRMS (ESI-TOF) m/z $(M + Na)^+$ calcd for $C_{17}H_{24}NaO_4$ 315.1567, found 315.1567, Δ 0.0 ppm.

Benzyl (4*R*)-4-[(2′-*O*-benzyl-3′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-pentenoate (24b). Under an N_2 atmosphere, a solution of **29** (32.7 mg, 112 μmol) and benzyl acrylate (89 mg, 0.55 mmol) in dry dichloromethane (5 mL) was treated with Grubb's 2nd generation catalyst (8.8 mg, 10 μmol). The solution was refluxed overnight, cooled to room temperature, and concentrated under reduced pressure. The resulting residue was fractionated by flash column chromatography on silica gel using 35% ethyl acetate in hexane (v/v) as the eluent to afford **24b** (30.5 mg, 71.5 μmol, 64% yield) as a brown oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 7.41–7.27 (m, 10H), 6.97 (dd, J = 15.7 Hz, J = 5.3 Hz, 1H), 6.04 (dd, J = 15.7 Hz, J = 1.7 Hz, 1H), 5.20 (d, J = 11.8 Hz, 1H), 5.19 (d, J = 11.8 Hz, 1H) 4.82 (s, 1H), 4.61 (d, J = 12.1 Hz, 1H), 4.57 (d, J = 12.0 Hz, 1H), 4.43 (dq, J = 6.5 Hz, J = 5.3 Hz, 1H), 3.64 (ddd, J = 11.0 Hz, J = 9.5 Hz, J = 4.5 Hz, 1H), 3.59 (ddt, J = 3.9 Hz, J = 3.0 Hz, J = 1.7 Hz, 1H), 3.55 (dq, J = 9.5 Hz, J = 6.1 Hz, 1H), 2.17 (ddd, J = 13.1 Hz, J = 4.5 Hz, J = 3.9 Hz, 1H), 1.76 (ddd, J = 13.1 Hz, J = 11.0 Hz, J = 3.0 Hz, 1H), 1.24 (d, J = 6.5 Hz, 3H), 1.23 (d, J = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 166.4, 149.9, 128.7, 128.6, 128.5, 128.4, 127.9, 127.8, 119.8, 94.9, 75.7, 71.3, 70.5, 70.2, 68.4, 66.5, 32.9, 19.1, 17.8; HRMS (ESI-TOF) m/z $(M + NH_4)^+$ calcd for $C_{25}H_{34}NO_6$ 444.2381, found 444.2386, Δ 1.1 ppm.

Benzyl (4*R*)-4-[(2′-*O*-benzyl-4′-(2″-*N*-(benzyloxycarbonylamino)benzoyl)-3′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-pentanoate (30a). Under an N_2 atmosphere, a solution of *N*-benzyloxycarbonylanthranilic acid (21 mg, 80 μmol) in dichloromethane (7 mL) was treated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (15 mg, 80 μmol) and 4-dimethylaminopyridine (9.3 mg, 80 μmol). The mixture was stirred for 30 min and **24a** (17 mg, 40 μmol) in dichloromethane (3 mL) was added. After stirring for 22 h, the mixture was quenched with water, extracted with dichloromethane, dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using 50% ethyl acetate in hexane (v/v) as the eluent to afford **30a** (8.5 mg, 12.5 μmol, 31% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.47 (dd, J = 8.6 Hz, J = 0.9 Hz, 1H), 7.97 (dd, J = 8.0 Hz, J = 1.7 Hz, 1H), 7.54 (ddd, J = 8.8 Hz, J = 7.3 Hz, J = 1.7 Hz, 1H), 7.47–7.42 (m, 2H), 7.41–7.27, (m, 13H), 7.00 (ddd, J = 8.2 Hz, J = 7.3 Hz, J = 1.1 Hz, 1H), 5.23 (s, 2H), 5.15 (d, J = 12.3 Hz, 1H), 5.10 (d, J = 12.3 Hz, 1H), 5.05 (ddd, J = 10.7 Hz, J = 9.6 Hz, J = 4.6 Hz, 1H), 4.82 (br.s, 1H), 4.70 (d, J = 12.3 Hz, 1H), 4.58 (d, J = 12.3 Hz, 1H), 3.95 (dq, J = 10.7 Hz, J = 6.2 Hz, 1H), 3.86 (m, 1H), 3.54 (ddd, J = 3.7 Hz, J = 2.8 Hz, J = 1.8 Hz, 1H), 2.58–2.41 (m, 2H), 2.35 (ddd, J = 13.0 Hz, J = 4.6 Hz, J = 2.8 Hz, 1H), 1.91–1.84 (m, 2H), 1.85 (ddd, J = 13.0 Hz, J = 10.7 Hz, J = 3.7 Hz, 1H), 1.21 (d, J = 6.2 Hz, 3H), 1.11 (d, J = 6.1 Hz, 3H), ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 173.4, 167.2, 153.6, 142.1, 138.2, 136.3, 136.1, 134.8, 130.4, 128.74, 128.71, 128.50, 128.46, 128.43, 128.40, 128.37, 121.8, 119.1, 94.5, 75.1, 71.7, 71.2, 70.6, 67.3, 67.1, 66.5, 32.3, 30.7, 29.3, 18.8, 18.0, HRMS (ESI-TOF) m/z $(M + H)^+$ calcd for $C_{40}H_{44}NO_9$ 682.3011, found 682.3008, Δ −0.4 ppm, $(M + NH_4)^+$ calcd for $C_{40}H_{47}N_2O_9$ 699.3276, found 699.3278, Δ 0.3 ppm.

Benzyl (4*R*)-4-[(2′-*O*-benzyl-4′-(2″-*N*-(benzyloxycarbonylamino) benzoyl)-3′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-2-pentenoate (30b). Under an N_2 atmosphere, a solution of **24b** (30.5 mg, 72 μmol) in dichloromethane (800 μl) at 0 °C was treated with *N*-benzyloxycarbonylanthranilic acid (39 mg, 288 μmol) and 4-dimethylaminopyridine (24 mg, 36 μmol). The mixture was stirred for 5 min, when dicyclohexylcarbodiimide (59 mg, 288 μmol) in dichloromethane (500 μl) was added. After stirring for 21 h, the mixture was diluted with dichloromethane, filtered and water was added. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using 10% of ethyl acetate in hexane (v/v) as the eluent to afford **30b** (17 mg, 25 μmol, 35%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 8.47 (d, J = 8.5 Hz, 1H), 7.98 (dd, J = 8.0 Hz, J = 1.6 Hz, 1H), 7.54 (ddd, J = 8.0 Hz, J = 7.5 Hz, J = 1.5 Hz, 1H), 7.48–7.27 (m, 15H), 7.02 (ddd, J = 8.5 Hz, J = 7.5 Hz, J = 1.6 Hz, 1H), 7.00 (dd, J = 15.9 Hz, J = 5.4 Hz, 1H), 6.06 (dd, J = 15.9, J = 1.6 Hz, 1H), 5.23 (s, 2H), 5.21 (s, 2H), 5.07 (ddd, J = 10.4 Hz, J = 9.5 Hz, J = 4.4 Hz, 1H), 4.85 (br.s, 1H), 4.71 (d, J = 12.1 Hz, 1H), 4.59 (d, J = 12.1 Hz, 1H), 4.44 (qd, J = 6.4 Hz, J = 5.4 Hz, 1H), 3.92 (dq, J = 9.5 Hz, J = 6.2 Hz, 1H), 3.62 (dd, J = 3.7 Hz, J = 3.0 Hz, 1H), 2.38 (ddd, J = 13.2, J = 10.4 Hz, J = 3.0 Hz, 1H), 1.90 (ddd, J = 13.0, J = 4.4 Hz, J = 3.7 Hz, 1H), 1.26 (d, J = 6.4 Hz, 3H), 1.18 (d, J = 6.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ (ppm) 167.1, 166.3, 153.5, 149.6, 142.0,

137.9, 136.2, 134.7, 130.9, 128.61, 128.57, 128.5, 128.32, 128.26, 127.84, 127.79, 121.6, 119.9, 118.9, 114.6, 95.4, 74.8, 71.4, 71.2, 71.0, 67.2, 67.0, 66.4, 29.3, 19.1, 17.7; HRMS (ESI-TOF) m/z $(M + Na)^+$ calcd for $C_{40}H_{41}NO_9Na$ 702.2674, found 702.2679, Δ 0.7 ppm.

(4*R*)-4-[(4′-*O*-(2″-Amino-benzoyl)-3′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-pentanoic acid (6a). Under an N_2 atmosphere, a solution of **30a** (17.5 mg, 25 μmol) or **30b** (17.0 mg, 25 μmol) in methanol (1.5 mL) and ethyl acetate (250 μl) was treated with a suspension of 10% palladium on activated carbon (25.5 mg) in methanol (2 mL). After stirring under a hydrogen atmosphere (1 atm) for 3 h, the mixture was filtered and the catalyst was washed with methanol. The filtrate was concentrated under reduced pressure and the residue was purified using semi-preparative chromatography on a Waters Breeze HPLC system (1525 binary HPLC pump with a 2487 dual wavelength absorbance detector) equipped with a reverse-phase Xterra MS C18 OBD column (19 × 150 mm, 5 μm) coupled with a Gilson FC 203B fraction collector. Separation was achieved using gradient elution with 15 to 60% (v/v) acetonitrile in water with 0.05% trifluoroacetic acid (v/v) over 50 min and then 60 to 100% over 20 min at a flow rate of 8 mL min^{-1} to afford **6a** (3.8 mg, 10.3 μmol, 41%) along with its *N*-methyl derivative **31** (2 mg, 5 μmol, 21%).

(4*R*)-4-[(4′-*O*-(2″-Amino-benzoyl)-3′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-pentanoic acid (6a). ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 7.77 (dd, J = 8.2 Hz, J = 1.8 Hz, 1H), 7.23 (ddd, J = 8.4 Hz, J = 7.1 Hz, J = 1.8 Hz, 1H), 6.74 (dd, J = 8.4 Hz, J = 1.2 Hz, 1H), 6.57 (ddd, J = 8.2 Hz, J = 7.1 Hz, J = 1.2 Hz, 1H), 5.07 (ddd, J = 11.1 Hz, J = 9.7 Hz, J = 4.8 Hz, 1H), 4.74 (br.s, 1 H), 4.00 (dq, J = 9.7 Hz, J = 6.4 Hz, 1H), 3.90 (m, 1H), 3.78 (dd, J = 3.9 Hz J = 3.1 Hz, 1H), 2.55–2.39 (m, 1H), 2.17 (ddd, J = 13.5 Hz, J = 4.8 Hz, J = 3.9 Hz, 1H), 1.97 (ddd, J = 13.5 Hz, J = 11.1 Hz, J = 3.2 Hz, 1H), 1.93–1.77 (m, 1H), 1.19 (d, J = 6.4 Hz, 3H), 1.18 (d, J = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 177.5, 168.6, 132.0, 111.2, 97.5, 71.7, 71.0, 69.5, 68.7, 33.4, 33.2, 19.0, 18.1; HRMS (ESI-TOF) m/z $(M + H)^+$ calcd for $C_{18}H_{26}NO_7$ 368.1704, found 368.1711, Δ 1.9 ppm, $(M - H)^-$ calcd for $C_{18}H_{24}NO_7$ 366.1558, found 366.1563, Δ 1.4 ppm.

(4*R*)-4-[(4′-*O*-(2″-*N*-Methylamino-benzoyl)-3′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)oxy]-pentanoic acid (31). ^{1}H NMR (400 MHz, CD_3OD): δ (ppm) 7.85 (dd, J = 8.1 Hz, J = 1.7 Hz, 1H), 7.38 (ddd, J = 8.6 Hz, J = 7.1 Hz, J = 1.7 Hz, 1H), 6.71 (d, J = 8.6 Hz, 1H), 6.57 (dd, J = 8.1 Hz, J = 7.1 Hz, 1H), 5.06 (ddd, J = 11.1 Hz, J = 9.6 Hz, J = 4.7 Hz, 1H), 4.74 (br.s, 1H), 4.00 (dq, J = 9.6 Hz, J = 6.2 Hz, 1H), 3.90 (m, 1H), 3.78 (dd, J = 3.8 Hz J = 3.0 Hz, 1H), 2.90 (s, 3H), 2.55–2.39 (m, 1H), 2.15 (ddd, J = 12.9 Hz, J = 4.7 Hz, J = 3.8 Hz, 1H), 1.97 (ddd, J = 12.9 Hz, J = 11.1 Hz, J = 3.0 Hz, 1H), 1.93–1.78 (m, 1H), 1.184 (d, J = 6.2 Hz, 3H), 1.18 (d, J = 6.1 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD): δ (ppm) 135.7, 132.6, 115.4, 111.5, 97.4, 71.3, 70.9, 69.3, 68.5, 33.0, 31.0, 29.5, 18.6, 18.1; HRMS (ESI-TOF) m/z $(M + H)^+$ calcd for $C_{19}H_{28}NO_7$ 382.1860, found 382.1972, Δ 3.1 ppm, $(M - H)^-$ calcd for $C_{19}H_{26}NO_7$ 380.1715, found 380.1720, Δ 1.3 ppm.

2-*N*-Benzyloxycarbonylamino-benzoic acid (*N*-Cbz *ortho*-amino-benzoic acid). A suspension of anthranilic acid (1.37 g, 10 mmol) and sodium hydrogen carbonate (1.68 g, 20 mmol) in 25 mL THF at 0 °C was treated with benzyl chloroformate (2.05 g, 12 mmol). After stirring at 0 °C for 2 h, the mixture was poured into 50 mL water and extracted with 2 × 50 mL ethyl acetate. The organic phase was washed with 2 × 10 mL diluted HCL, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography using 50% of ethyl acetate in hexane (v/v) as the eluent to afford *N*-Cbz *ortho*-amino-benzoic acid (2.41 g, 8.9 mmol, 89% yield) as a white solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ (ppm) 10.34 (s, 1H), 8.49 (d, J = 8.6 Hz, 1H), 8.09 (d, J = 8.6 Hz, 1H), 7.59 (t, J = 7.7 Hz, 1H), 7.31–7.46 (m, 5H), 7.07 (t, J = 7.7 Hz, 1H), 5.23 (s, 2H).

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Funding by the University of Neuchâtel and the Max Planck Society is gratefully acknowledged. C. Dong acknowledges support from the National Natural Science Foundation of China (Grants No. 22107011, 22407016 & 22477010) and the Department of Education of Guangdong Province (Grant No. 2021KQNCX269). S. Bandi acknowledges support from the Swiss National Science Foundation (SNSF-169700). All nematode strains were provided by the Caenorhabditis Genetics Center, which is funded by the NIH Office of Research Infrastructure Programs (P40 OD010440).

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