

# Improved Synthesis for Modular Ascarosides Uncovers Biological Activity

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**(5)** Supporting Information

**ABSTRACT:** A versatile synthesis of modular ascarosides, a family of signaling molecules from *Caenorhabditis elegans* and other nematodes, via hydrogenolysis of a cyclic sulfate derived from methyl- $\alpha$ -L-rhamnopyranoside is reported. The route enables selective introduction of different side chains at the 1, 2, and 4 positions of the sugar, as demonstrated for ascarosides from *C. elegans* and *Pristionchus pacificus*. Bioassays with synthetic samples of 4'-tigloyl ascaroside mbas#3 revealed its role as an avoidance or dispersal signal.

ver the past decade, a group of specialized metabolites, the nematode-derived modular metabolites (NDMMs), have been identified from the model organism C. elegans and other nematodes.<sup>1,2</sup> These compounds are derived from glycosides of the dideoxy sugars L-ascarylose and L-paratose, which are decorated with diverse building blocks from amino acid, nucleoside, carbohydrate, and fatty acid metabolism (Figure 1).<sup>1,3</sup> NDMMs function as signaling molecules that regulate many aspects of nematode life history, including development,<sup>4–7</sup> lifespan,<sup>8,9</sup> morphology,<sup>10</sup> social communication,<sup>6,7,11-15</sup> as well as interactions with other species.<sup>16-18</sup> Since even small changes in the chemical structures of NDMMs can greatly affect biological activity and NDMMs can be active at extremely low (femtomolar) concentrations,<sup>12,19</sup> requirements for the purity of synthetic samples for bioassays are particularly stringent. Previous syntheses of ascaroside- and paratoside-based NDMMs employed 2,4-di-O-benzoylascarylose 2 as a key intermediate, which was obtained in six steps from 1 (Figure 2).<sup>4,20–22</sup> An improved version of this route was reported by Knölker et al.<sup>23</sup> However, 2 does not permit regioselective modification or introduction of other substituents at the 2'- and 4'-positions and, thus, is ill-suited for the selective synthesis of 2'- or 4'-substituted NDMMs.

We aimed to develop a synthesis for a key intermediate that would enable straightforward differentiation of the 2' and 4' positions. Recognizing that the crucial 3' deoxygenation would be facilitated by using an activating group that could double as a protecting group for the 2' hydroxy group, we turned our attention to Klemer–Rodemeyer-like transformations. Based on literature precedent,<sup>24,25</sup> inverse Klemer–Rodemeyer fragmentation of 2,3-O-benzylidinerhamnose derivative **3** was



expected to furnish ascarylose derivative 5 but instead produced the dihydropyran 4 as the major product, likely as a result of abstraction of the benzylic proton (Scheme 1). Inspired by the work of van Boom and co-workers,<sup>26</sup> we then considered a strategy based on regioselective opening of cyclic sulfates, whose utility is being increasingly recognized in carbohydrate chemistry.<sup>27</sup> Commercially available methyl L-rhamnopyranoside 6 was treated with 2,2-dimethoxypropane to afford 7, followed by TBDPS protection. In the presence of excess TBDPSCl, acetonide deprotection occurred during silica gel chromatography to directly afford diol 8 in nearly quantitative yield. Treatment of 8 with thionyl chloride led to cyclic sulfite 9, which was subsequently oxidized with sodium periodate in the presence of a catalytic amount of ruthenium trichloride to give cyclic sulfate 10. In addition, the corresponding 4methoxybenzyl-protected derivative 14 was prepared as described by van Boom and co-workers.<sup>26</sup> Reduction of 14 with tetrabutylammonium borohydride produced the two regioisomers 15 and 16 in a ratio of about 6:1, as reported.<sup>26</sup> Better regioselectivity (15:1, easily separable) was achieved when the corresponding 4'-TBDPS-protected derivative 10 was used.

Next, we explored the effect of different protective groups in position 2' on the outcome of subsequent glycosylation reactions. Although the use of the 2'-benzyl derivative would offer advantages for the synthesis of some modular ascarosides, we expected 2'-benzoyl protection to be preferable, as

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Figure 1. Modular ascarosides incorporating building blocks from neurotransmitter (osas#9), folate (ascr#8), tryptophan (icas#3), and short-chain fatty acid (mbas#3) metabolism from *C. elegans* and, from the satellite model organism *P. pacificus*, dimeric ascarosides ubas#1 and dasc#1.



Figure 2. Previous approach to ascaroside synthesis and the desired key intermediate.

anchimeric assistance of the benzoyl carbonyl would provide better stereoselectivity of the glycosylation step. In test reactions with (2*R*)-5-hexen-2-ol, we found that the 2'-benzyl derivative produced acceptable yields of the desired  $\alpha$ -anomer (ratio  $\alpha:\beta = 7:1$ ); however, we ultimately proceeded using the 2'-benzoyl derivative **13** because of better yields in subsequent steps, in addition to better stereoselectivity of the glycosylation (ratio  $\alpha:\beta > 50:1$ ).

To demonstrate the synthetic utility of **13**, we selected three modular ascarosides: icas#9 and mbas#3 from *C. elegans* and dasc#1 from *P. pacificus*. The dimeric ascaroside dasc#1 has recently been shown to regulate mouth form development in *P. pacificus*,<sup>10</sup> and synthetic dasc#1 is urgently needed for the study of the underlying signaling pathways, which are of great interest for understanding development of morphology in animals.<sup>28,29</sup> The indole ascaroside icas#9 is one of the most abundant indole ascarosides, acts as a strong hermaphrodite attractant in *C. elegans*, and contributes to induction of larval diapause (dauer).<sup>12,30</sup> In contrast, nothing is known about the

Scheme 1. Inverse Klemer–Rodemeyer Fragmentation of 2,3-O-Benzylidinerhamnose Derivative 8 (a) and Preparation of Ascaroside Derivatives via Methoxyrhamnose-Derived Cyclic Sulfates (b, c)



biological activity of mbas#3,<sup>19</sup> in part because previous syntheses yielded insufficient material for bioassays.

Starting from benzoyl derivative 13, icas#9 was synthesized in nine steps with an overall yield of 15% (Scheme 2). After removal of the anomeric methyl using BBr<sub>3</sub> and glycosylation of (2R)-5-hexen-2-ol, the terminal double bond was oxidized to the corresponding carboxylic acid 24. Following debenzoylation and dibenzylation, the TBDPS group was removed to yield intermediate 27. Finally, attachment of the indole carboxy moiety and subsequent debenzylation afforded icas#9. Using an analogous sequence, dasc#1 was synthesized from key intermediate 13 in eight steps with an overall yield of 7% (Scheme 2). For the synthesis of mbas#3, we had to take into account that the presence of the tigloyl and unsaturated side chain preclude a hydrogenation step. Therefore, we here employed 4-methoxybenzyl for 2'-protection, which could be easily removed using trifluoroacetic acid. In this way, mbas#3 was synthesized from key intermediate 13 in eight steps with an overall yield of 22% (Scheme 2).

Next, we explored the biological properties of mbas#3 in bioassays with *C. elegans*. Since it is structurally related to icas#3 (Figure 1), differing only in the nature of the 4'-substituent, we tested mbas#3 in an assay that had previously been used to characterize icas#3 as an attractive pheromone (Figure 3).<sup>12</sup> Strikingly, we found that, in contrast to icas#3, which was tested in parallel, mbas#3 is not attractive and instead triggers avoidance behavior. Avoidance behavior was observed in assays with the common laboratory strain N2 Bristol as well as in CB4856, a *C. elegans* strain originally collected in Hawaii that differs from the laboratory strain N2 in that it has a higher



Scheme 2. Synthesis of mbas#3, dasc#1, and icas#9 from 13

propensity to aggregate on food.<sup>31</sup> In order to exclude the possibility that the observed behavioral effects of mbas#3 are due to synergy with other ascarosides produced by the worms during the assays, we also tested the effect of mbas#3 on *daf-22* mutant worms that do not produce any ascaroside-based signaling molecules because of a defect in peroxisomal  $\beta$ -oxidation.<sup>19</sup> Similar to the results we obtained for N2 Bristol and CB4856, mbas#3 triggered avoidance behavior in *daf-22* mutant worms.

These results suggest that mbas#3 serves as a dispersal signal in *C. elegans* that may antagonize the attractive properties of indole ascarosides such as icas#3 and icas#9 in a manner similar to the antagonistic effects of high concentrations of ascr#3 on icas#3-mediated attraction.<sup>12</sup> Avoidance behavior induced by mbas#3 also differs from previously described avoidance behavior triggered by another ascaroside, osas#9. osas#9, an ascaroside specifically produced in large quantities by starved L1 larvae, triggers avoidance behavior only in starved worms, but not in worms that have access to food,<sup>11</sup> whereas mbas#3, which is produced by *C. elegans* larvae and adults, triggers avoidance even in the presence of food.

In summary, we report a synthetic strategy that enables selective preparation of 2'- or 4'-substituted ascarosides, based



**Figure 3.** Quadrant assay used to measure attractive and repulsive properties of icas#3 and mbas#3. (a) Agar plates were divided into quadrants, with opposite quadrants containing icas#3 at 1 pM or mbas#3 at 10 nM. Worms were placed into the center of the plate and their location tracked over time. (b) Chemotaxis indices measured for icas#3 and mbas#3. See Srinivasan et al.<sup>12</sup> for methods.

on reductive opening of a rhamnose-derived cyclic sulfate. The new approach enabled facile access to synthetic samples of very high purity of three modular ascarosides, icas#3, dasc#1, and mbas#3. Testing of synthetic mbas#3 revealed that this ascaroside mediates a novel, unexpected behavioral phenotype. Availability of synthetic mbas#3 and other ascarosides of high purity will enable further, more detailed studies of their biological roles.

## ASSOCIATED CONTENT

## **Supporting Information**

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.or-glett.7b01009.

Detailed experimental procedures and full spectroscopic data for all major compounds (PDF)

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#### **Organic Letters**

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