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Stereospecific Synthesis of the Saccharosamine-Rhamnose-Fucose Fragment Present in Saccharomicin B

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Abstract

A synthetic route has been developed for constructing the D-saccharosamine-L-rhamnose-D-fucose (Sac-Rha-Fuc) trisaccharide fragment present in the antibacterial natural product saccharomicin B. The Sac monosaccharide was synthesized through a modified nine step procedure starting from D-rhamnal in 23% overall yield. 1-*O*-TBS Sac donors were used to construct the β -linked Sac-Rha disaccharide. This disaccharide was coupled to a Fuc acceptor under BSP/Tf₂O conditions to afford a trisaccharide properly functionalized for elaboration to saccharomicin B.

Bacteria strains resistant to currently approved antimicrobial therapies continue to present a worldwide health crisis.^{1a} This has prompted calls for both the development of new antimicrobials and the reinvestigation of previously discarded classes of natural products as potential leads for antimicrobial development.^{1b} One class of molecules that holds potential to serve as next-generation antibacterial agents is the saccharomicins. Isolated by Kong et al. in 1998 from actinomycete *Saccharothrix espanaensis* from a rare soil in Puerto Llano, Spain,² these compounds exhibited good to moderate activity against a variety of Gram-positive (MICs 0.12–8.0 μ g/mL) and Gram-negative bacteria (MICs 0.25–32 μ g/mL), as well as resistant strains such as MRSA (MICs 0.12–0.5 μ g/mL).^{2,3} Unfortunately, the saccharomicins have an extremely narrow therapeutic window with a median lethal dose (LD₅₀) of 16 mg/kg in mice.^{2,3} Although the saccharomicins currently cannot be used as antibiotics, analogs of these natural products still hold potential promise given the pressing need for rapid development of next-generation antibacterial agents. The biosynthesis of the saccharomicins has not been fully elucidated, however, and only the enzymes responsible for aglycone formation have been studied in depth.^{4,5} Due to these reasons, chemical synthesis

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ASSOCIATED CONTENT

Supporting Information

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Experimental procedures, synthetic details, characterization data, and copies of ¹H and ¹³C NMR spectra for all new compounds (PDF)

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represents the only avenue for producing material to answer questions about the mechanism of toxicity and structure–activity relationships of the saccharomicins.

Saccharomicin B is a heptadecasaccharide containing five different deoxy sugar monosaccharides. D-Saccharosamine (Sac) and 4-*epi*-L-vancosamine (Eva) are unique 2,6-dideoxy sugars bearing a tertiary C-3 carbon possessing methyl and amino substitution. The other three monosaccharides present in the molecule are D-fucose (Fuc), L-rhamnose (Rha), and L-digitoxose (Dig). To date, only three fragments of the saccharomicins have been chemically synthesized. In 2005, McDonald et al. constructed the Fuc-aglycone fragment through a Schmidt glycosylation.⁶ Subsequent studies from the McDonald group resulted in the synthesis of the Sac-Fuc-Dig trisaccharide fragment of the molecule in high yield and excellent selectivity.⁷ While both of these approaches utilized enantiomers of the monosaccharides found in saccharomicin B, they did help establish the absolute stereochemistry of the natural product.^{6,7} More recently, our own lab has developed a route to the Fuc-8 to Dig-10 branching point of saccharomicin B.⁸ As part of our ongoing studies directed at the total synthesis of saccharomicin B, here we report the development of a route to the Sac-Rha-Fuc fragment of the molecule corresponding to residues 7–5.

Retrosynthetically, we envisioned the Sac-Rha-Fuc trisaccharide fragment **2** could be synthesized starting from disaccharide donor **3** and the acceptor **4** through a benzenesulfinyl piperidine (BSP)/trifluoromethanesulfonic anhydride (Tf₂O)-mediated glycosylation (Scheme 1).⁹ Disaccharide **3** would in turn arise from coupling of an appropriately modified Sac donor **5** with Rha **6**. We initially envisioned that this could be achieved using our *p*-toluenesulfonyl chloride (TsCl) mediated reagent-controlled dehydrative glycosylation methodology using hemiacetal donor **5a** for synthesizing the Sac-Rha disaccharide.^{10a}

While routes have been previously established for synthesizing the Rha monosaccharide,¹¹ we needed to develop scalable approaches to the appropriately functionalized Sac and Fuc monosaccharides. The synthesis of the Sac monosaccharide is the more challenging of the two due to the tertiary center present in the molecule. To date, only five approaches for the construction of this molecule have been reported.^{12–14} McDonald et al. and Parker et al. developed routes involving tungsten mediated cyclizations to form Sac glycols.¹² McDonald's approach^{12a} relied on installation of the amine before cyclization, whereas Parker^{12b} carried out cyclization right before amine installation. Nicolaou et al. and Takahashi et al. used IBX-mediated C–H insertion reactions to afford Sac.¹³ Most recently, Wan et al. developed a more direct approach starting from D-rhamnal **7**.¹⁴ The key steps in the synthetic route involve formation of a cyclic sulfamidate imine, followed by Grignard addition to construct the tertiary carbon center.

We envisioned Sac could arise from known D-rhamnal **7** as well, through a shorter sequence (Scheme 2). To this end, selective oxidation of the allylic alcohol on D-rhamnal **7**¹⁵ followed by acetylation afforded ketone **8**.¹⁶ Initial attempts at isolating the ketone product without protecting the C4 hydroxyl led to extremely low-yielding reactions and issues with purification due to the instability and volatility of this species. Subjecting **8** to a two-step one-pot conjugate addition and oxime forming reaction afforded **9**.¹⁷ The acetate protecting group was removed under Zemplén conditions and afforded the oxime **10**. Subsequent

installation of the 3-methyl substituent on D-saccharosamine to provide **11** was achieved using a combination of MeLi and CeCl₃ as described by Scharf et al. for the synthesis of D-decilonitrose.^{18a} After forming the tertiary center, **11** was subjected to a three-step sequence where the N–O bond was reduced, the resulting amine was subjected to diazo transfer, and the C-4 alcohol was protected as a naphthylmethyl ether to afford compound **12** in three steps and 62% overall yield.^{19,20} Finally, thioglycoside hydrolysis using NBS and wet acetone afforded hemiacetal donor **5a**.²¹

With **5a** in hand, we sought to apply our reagent-controlled methodology to synthesize the β -linked Sac-Rha disaccharide.¹⁰ Given the differences between **5a** and the D-olivose and D-oleandrose donors used in our previous studies,^{10a} we initially chose to examine a model glycosylation reaction. To this end, we examined the reaction between **5a** and *p*MeO-PhOH as a model acceptor. Surprisingly, all attempts to subject **5a** to the TsCl-mediated glycosylation reaction led the formation of the product as a mixture of anomers (see Supporting Information). Despite our lack of success with the model reaction, we chose to examine the TsCl-promoted reaction between **5a** and Rha acceptor **6**. Interestingly, under these conditions the disaccharide product was formed as a single isomer. However, it possessed the undesired α -stereochemistry (Scheme 3). The α -selectivity was confirmed by the coupling constant of the anomeric proton of the D-Sac residue ($J_{\text{H1}} = 4.6$ Hz).^{14,22}

Our inability to synthesize the β -linked disaccharide using our sulfonate-mediated glycosylation reactions led us to examine other glycosylation chemistries. After several unfruitful approaches, we turned our attention to the use of a 1-*O*-TBS 2-deoxy glycoside donor. Although to our knowledge the use of this donor had not been previously reported for β -linked disaccharide construction, Monneret et al. demonstrated that 1-*O*-TBS D-ribo glycosides underwent β -selective glycosylations with aglycone acceptors.^{23a} To this end, the hemiacetal oxygen of Sac **5a** was protected with a TBS group using TBSCl and imidazole to afford TBS glycoside **14** as a single β -anomer (Scheme 4). In addition, we also chose to examine the alternative Sac donor **5b** possessing an acetate protecting group on the C4 position for ease of removal after glycosylation.^{23a}

Sac donors **14** and **5b** were both initially tested using the conditions described by Monneret et al.^{23a} To this end the BF₃·OEt₂-promoted reaction between donor **14** or donor **5b** and acceptor **6** led to selective formation of the desired β -linked disaccharides **17** and **3** exclusively, albeit in low yields (Table 1, entries 1 and 2). The coupling constants of the anomeric proton for the Sac residue were $J_{\text{H1}} = 2.0$ and 9.5 Hz, indicative of a β -linked product. At this point, we decided to continue the synthesis with donor **5b** due to the higher yield of the glycosylation reaction with this substrate and the ease of removal of the C4 acetate group. Having established this chemistry would afford the product with the desired diastereoselectivity, we turned our attention to optimizing the yield of the reaction. Using excess donor we were able to obtain the desired product **3** in excellent yield, again as a single β -anomer (Table 1, entries 3 and 4). We also examined the use of excess acceptor **6** to determine if similar yields could be achieved using the more readily accessible coupling partner in excess (Table 1, entries 5 and 6). Again, we found good yields and excellent selectivity could be obtained under these conditions.

With disaccharide **3** in hand, we turned our attention to the construction of trisaccharide **2**. We initially examined a thioglycoside Fuc acceptor, despite concerns that this compound might undergo intermolecular aglycone transfer to the acceptor.²⁴ This concern proved to be well-founded, and our attempt at BSP/Tf₂O-mediated glycosylation using a Rha thioglycoside donor and C3 Fuc thioglycoside acceptor resulted in decomposition of the substrates. Due to our inability to use a thioglycoside acceptor in this reaction, we chose to convert **18** to the corresponding 1-*O*-TBS ether **4** (Scheme 5). Initial attempts at transforming the thioglycoside directly into the hemiacetal using *N*-bromosuccinimide (NBS)²¹ resulted in decomposition. We therefore opted to use a two-step procedure to convert **18** to the corresponding hemiacetal. To this end, thioglycoside **18** was converted into an anomeric acetate **19** using *N*-iodosuccinimide (NIS)/AcOH. Deacetylation under Zemplén conditions then afforded hemiacetal **20**.²⁵ Anomeric TBS protection of the hemiacetal formed Fuc **21** exclusively as the β -anomer. Finally, the naphthylmethyl ether group was removed using DDQ to form the C3 Fuc acceptor **4**.^{11,26}

With both coupling partners in hand, we turned our attention to the synthesis of trisaccharide **2**. To this end, **3** was preactivated using BSP/Tf₂O at -40 °C followed by addition of acceptor **4** and subsequent warming to 0 °C (Scheme 6).²⁷ Pleasingly, these conditions led to formation of the desired trisaccharide **2** in 60% yield as a single α -anomer. The stereochemistry of the glycosidic linkages in trisaccharide fragment **2** was deduced from the anomeric ¹H-¹³C coupling constants in the proton-coupled ¹³C NMR spectrum. The *J*_{C1-H1} coupling constant of 170 Hz for the anomeric carbon of the Rha confirmed the α -stereochemistry of this linkage. In addition, the *J*_{C1-H1} coupling constant for the anomeric carbon of Sac was 162 Hz, indicating the β -linkage of this residue was not affected by the reaction conditions.

In summary, we have synthesized the Sac-Rha-Fuc trisaccharide **2** fragment of saccharomicin B. A modified approach for synthesizing Sac donor **5b** has been developed starting from D-rhamnal **7** that proceeds in nine steps and 23% overall yield. The Sac-Rha disaccharide **3** was obtained in good yield as a single β -anomer by BF₃·OEt₂ mediated glycosylation coupling of 1-*O*-TBS Sac donor **5b** and Rha acceptor **6**. Subsequent coupling of **3** with Fuc acceptor **4** under BSP/Tf₂O conditions afforded trisaccharide **2** in good yield and excellent α -selectivity. In combination with our previously reported synthesis of the C-8 to C-10 fragment of saccharomicin B,⁸ these studies are allowing us to probe the utility of chemistries developed for the direct construction of 2-deoxy-sugar linkages in the context of real systems. In addition, the presence of the Fuc 1-*O*-TBS and Sac C-4 acetate in **2** render it appropriately functionalized to be readily converted into either a glycosyl donor or a glycosyl acceptor for further elaboration into the saccharomicin backbone. The elaboration of this molecule into larger fragments of saccharomicin B is currently under investigation in our laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Scheme 1.
Retrosynthetic Outline of Sac-Rha-Fuc Fragment in Saccharomicin B (1)

Scheme 2.
 Synthesis of Sac Monosaccharide 5a

Scheme 3.

Sac-Rha Disaccharide Glycosylation Attempt Using TsCl/KHMDS

Scheme 5.
Synthesis of Fuc 1-*O*-TBS Protected C3 Acceptor 4

Scheme 6.
Synthesis of Sac-Rha-Fuc Trisaccharide Fragment 2

Table 1.

Sac-Rha Disaccharide 3 Optimizations

entry	donor (equiv)	acceptor 6	product	time	yield (%)
1	14 (1.1)	1 equiv	17	5 h	23
2	5b (1.1)	1 equiv	3	5 h	28
3	5b (2)	1 equiv	3	5 h	62
4	5b (3)	1 equiv	3	6 h	70
5	5b (1)	2 equiv	3	5 h	41
6	5b (1)	3 equiv	3	6 h	66