OL Organic Letters

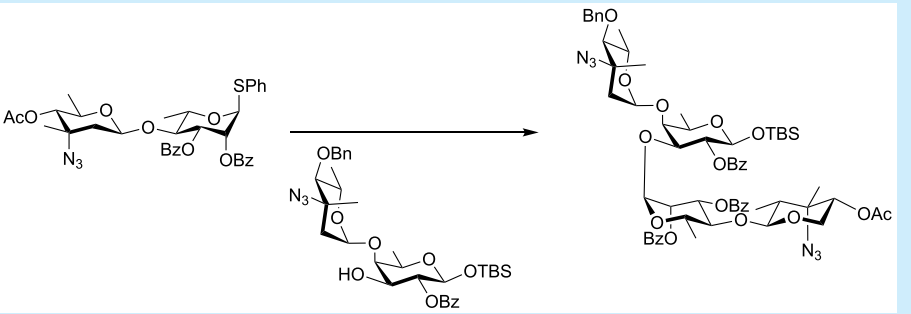
pubs.acs.org/OrgLett Letter

Synthesis of the Branched Tetrasaccharide Fragment of Saccharomicin A

Brian P. Garreffi, Akash P. Maney, and Clay S. Bennett*

Cite This: *Org. Lett.* 2023, 25, 369–372 Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information



ABSTRACT: A synthesis of the branched tetrasaccharide fragment of saccharomicin A using 1-OTBS donors to stereoselectively synthesize both α- and β-linked disaccharides is reported. The disaccharides were united using BSP/Tf_2O to afford the tetrasaccharide fragment as a single α-anomer in 72% yield. This branched tetrasaccharide fragment can be used as donor and acceptor species to synthesize larger fragments of saccharomicin A.

Saccharomicin A is a heptadecasaccharide isolated by Kong and co-workers[1] that possesses broad spectrum antimicrobial activity against both Gram-positive and Gram-negative pathogens (Figure 1).[2] The continued global threat of

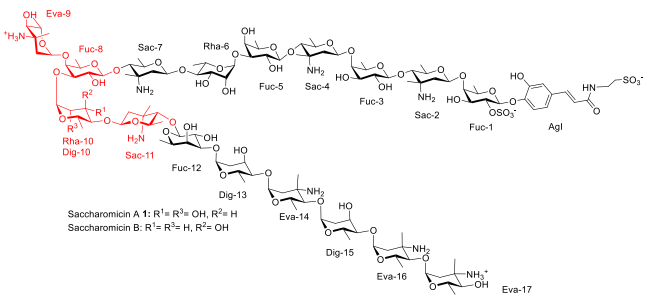


Figure 1. Structure of saccharomicin A 1. The target branched tetrasaccharide fragment is colored red.

antimicrobial resistance has created a pressing need for new antimicrobials,[3−5] and saccharomicin A or its analogue holds promise to help fill this role. Before saccharomicin can serve as a lead compound, however, it will be necessary to develop a route that is flexible enough for analogue production. While the glycosyltransferases responsible for the installation of the sugars have been identified, the complete biosynthesis has yet to be elucidated.[3,4] As a consequence, the saccharomicins continue to attract attention from the synthetic community.[5−8]

Our group has had an ongoing interest in the total synthesis of saccharomicins.[9−11] To date, we have synthesized three fragments corresponding to Fuc-5-Sac-7, Eva-9-Dig-10, and Fuc-12-Eva-17. While the Fuc-5-Sac-7 and Fuc-12-Eva-17 fragments are set for elaboration into either saccharomicin, the branched tetrasaccharide connecting these residues has yet to be synthesized. Here we report the first synthesis of the Eva-9-Sac-11 fragment of saccharomicin A (Figure 1). Notably, this fragment is ready for conjugation to the other saccharomicin fragments our group has synthesized.

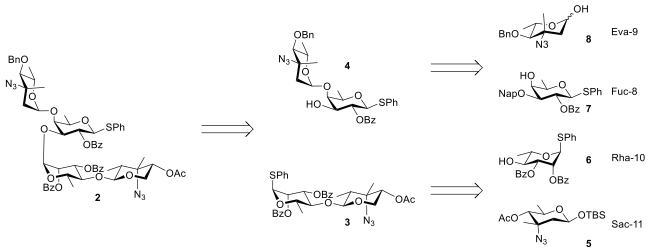
Retrosynthetically, we envisioned that **2** would arise form a [2+2] coupling between disaccharides **3** and **4** (Scheme 1). Disaccharide donor **3** would arise through reaction between donor **5** and acceptor **6** using Lewis acid-mediated activation as described by Monneret and later utilized by us in our synthesis of the Fuc5-Sac7 fragment of the molecule.[12] In a similar vein, union of donor **8** with acceptor **7** would afford **4**.

In the forward direction, we chose to use compound **6** as an acceptor as we reasoned that the C2 and C3 benzoates would be easier to remove in our end game than the Piv ester and benzyl ether used in our previous synthesis.[10] Compound **6**

Received: December 1, 2022
Published: January 10, 2023

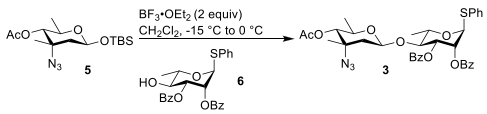
https://doi.org/10.1021/acs.orglett.2c04081
Org. Lett. 2023, 25, 369–372

Scheme 1. Retrosynthesis of the Branched Tetrasaccharide Fragment



was synthesized through a modification of a literature procedure (see the Supporting Information).[13] Initially, we sought to synthesize **3** using our method for the synthesis of the Sac-7-Rha-6 disaccharide, but the change in the protecting group led to the synthesis of **3** in only 46% yield with the major byproducts being species resulting from the degradation of **5** (Table 1, entry 1).[10] Increasing the amount of donor from

Table 1. Optimization of the β-Stereoselective Glycosylation of 1-*O*-TBS D-Saccharosamine Donor 5 with L-Rhamnose Acceptor 6



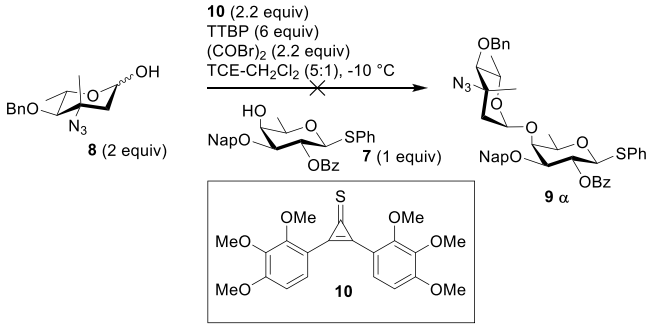
entry	5:6 ratio	yield of 3 (%)
1	3:1	46 (β only)
2	4:1	31 (β only)
3	1:3	75 (β only)

3 to 4 equiv resulted in a further decrease in the yield of **3** and a subsequent increase in the level of degradation of **5** (Table 2, entry 2). In an effort to improve the yield and prevent degradation, we next examined using acceptor **6** in excess (Table 1, entry 3). Pleasingly, this strategy worked, and we were able to obtain the target disaccharide in 75% yield as a single β-anomer.

We next turned our attention to the construction of **4**. To this end, we chose to use acceptor **7** to minimize protecting group manipulations later in the synthesis. Compound **7** was synthesized through a modification of a literature procedure (see the Supporting Information).[14,15] Initial attempts to unify **7** with hemiacetal **8** using our previously reported oxalyl bromide/cyclopropenethione **10** chemistry proved to be problematic upon scale-up (Scheme 2).[9] In an attempt to find a more robust chemistry, we were again attracted to the work of Monneret, who demonstrated that 1-OTBS donors possessing equatorial azide at position 3 reacted with nucleophiles to afford α-linked products.[12]

We therefore turned our attention to using 1-OTBS glycoside **11** as a donor. To this end, TBS protection of the anomeric hydroxyl in **8** afforded **11** in 92% yield (Scheme 3). We initially attempted the glycosylation of **11** with **7** at −78 °C in CH_2Cl_2 for 16 h, which afforded disaccharide **9** in 70% yield and moderate selectivity (4:1 α:β) (Table 2, entry 1). To improve the α selectivity of the reaction, we attempted to take advantage of the "ether effect" by using THF as a co-solvent.[16] Unfortunately, using a 1:1 CH_2Cl_2/THF solvent ratio resulted in no product formation (Table 2, entry 2). Reasoning that the low selectivity may be the result of in situ anomerization, we

Scheme 2. Synthesis of α(1,4)-Linked 4-*epi*-L-Vancosamine D-Fucose Disaccharide 9α



Scheme 3. Synthesis of α(1,4)-Linked 4-*epi*-L-Vancosamine D-Fucose Disaccharide 4

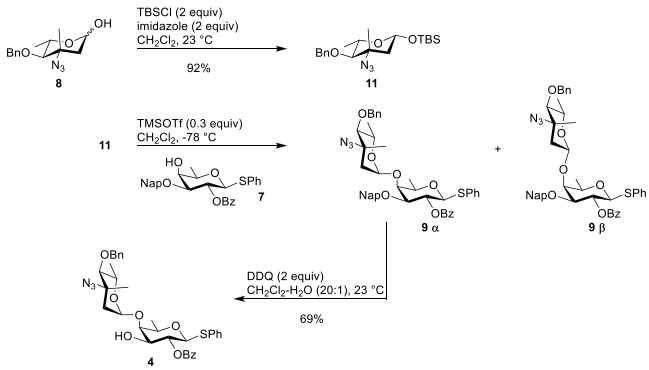


Table 2. Optimization of the α-Stereoselective Glycosylation of 1-*O*-TBS 4-*epi*-L-Vancosamine Donor 11 with D-Fucose Acceptor 7

entry	time (h)	donor:acceptor	yield of 9 (%) (α:β)
1	16	1:2	70 (4:1)
2[c]	16	1:2	NR[b]
3[a]	16	1:2	NR[b]
4	8	1:2	64 (16:1)
5	5	1:2	93 (8:1)
6	5	1:1.5	92 (8:1)

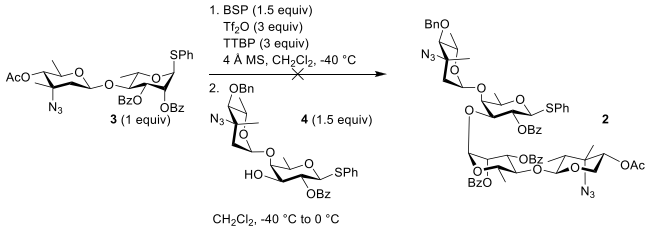
[a]The reaction is run with the addition of TTBP. [b]No reaction. [c]With a 1:1 CH_2Cl_2/THF solvent.

added the proton scavenger 2,4,6-tri-*tert*-butylpyrimidine (TTBP) to the reaction mixture; however, this led to no product formation (Table 2, entry 3). Shortening the reaction time to 8 h improved the selectivity of the reaction, albeit with a small decrease in the yield (Table 2, entry 4). A further decrease in the reaction time to 5 h led to the production of **9** in 93% yield as an 8:1 (α:β) mixture of anomers (Table 2, entries 5 and 6). Removal of the Nap ether using DDQ in a 20:1 CH_2Cl_2/H_2O solvent afforded disaccharide acceptor **4** in 69% yield (Scheme 3).[17,18]

With both coupling partners in hand, we turned our attention to the synthesis of the branched tetrasaccharide fragment of saccharomicin A **2**. On the basis of our previous experience with the saccharomicins, we sought to unite these fragments using benzenesulfinyl piperidine (BSP) and triflic anhydride (Tf_2O).[10,19,20] To this end, we reacted disaccharide donor **3** with BSP and Tf_2O in the presence of TTBP before adding disaccharide acceptor **4** (Scheme 4). Unfortunately, all

https://doi.org/10.1021/acs.orglett.2c04081
Org. Lett. 2023, 25, 369−372

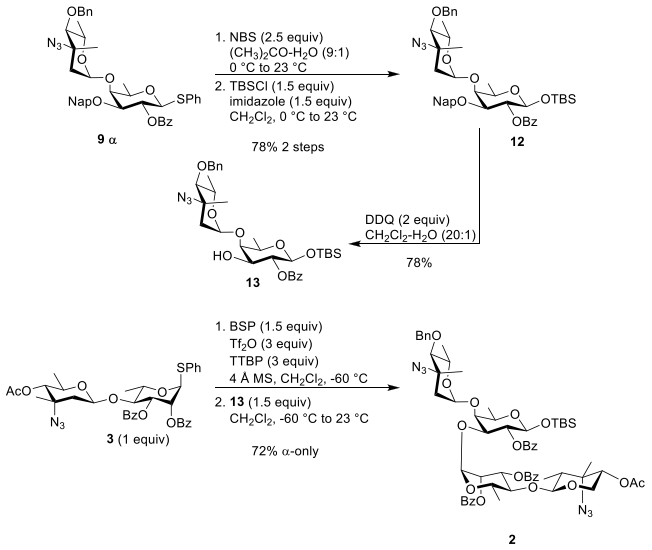
Scheme 4. Initial Attempts to Synthesize the Branched Tetrasaccharide Fragment 2



attempts to use this chemistry led to degradation of the coupling partners through intramolecular aglycone transfer.[21] Further attempts to unite **3** and **4** using *N*-iodosuccinimide/triflic acid (NIS/TfOH) failed to deliver any product.

Reasoning that the aglycone transfer process could be suppressed by using a different, less reactive aglycone, we converted **9α** into 1-OTBS glycoside **13** (Scheme 5). To this

Scheme 5. Synthesis of Disaccharide Acceptor 13 and Synthesis of Branched Tetrasaccharide Fragment 2



end, a two-step sequence involving *N*-bromosuccinimide-mediated (NBS) thioglycoside hydrolysis followed by TBS protection afforded **12** in 78% yield over two steps (Scheme 5). As with our previous studies, the 1-OTBS glycoside was formed as a single β anomer. Removal of the Nap protecting group using DDQ in a 20:1 CH_2Cl_2/H_2O solvent afforded acceptor **13** in 78% yield (Scheme 5). With the new disaccharide acceptor in hand, we again turned our attention to the synthesis of the tetrasaccharide.[19,20] To our delight, with the new 1-OTBS acceptor, we were able to unite **3** and **13** to afford **2** in 72% yield as a single α-anomer (Scheme 5).

In conclusion, we have synthesized the branched tetrasaccharide fragment of saccharomicin A. Key to this synthesis was the use of 1-OTBS glycoside activation for the construction of both α-linked Eva-9-Fuc-8 **9α** and β-linked Sac-11-Rha-10 **3**. Both reactions were highly selective, although the underlying cause of the different selectivities is unclear at this time.[12] Notably, this advanced fragment is properly functionalized to permit the construction of the Fuc5-Eva17 fragment of the saccharosamine backbone, bringing us one step closer to the completion of the total synthesis.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.orglett.2c04081.

Experimental details and characterization data (PDF)

FAIR data, including the primary NMR FID files, for compounds **S14**–**S16**, **7**, **S17**, **6**, **8**, **11**, **5**, **3**, **9α**, **9β**, **4**, **12**, **13**, and **2** (ZIP)

■ AUTHOR INFORMATION

Corresponding Author

Clay S. Bennett − *Department of Chemistry, Tufts University, Medford, Massachusetts 02155, United States;* orcid.org/0000-0001-8070-4988; Email: clay.bennett@tufts.edu

Authors

Brian P. Garreffi − *Department of Chemistry, Tufts University, Medford, Massachusetts 02155, United States*

Akash P. Maney − *Department of Chemistry, Tufts University, Medford, Massachusetts 02155, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.orglett.2c04081

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors thank the National Science Foundation (NSF CHE-1954841) for its generous financial support.

■ REFERENCES

(1) Kong, F.; Zhao, N.; Siegel, M. M.; Janota, K.; Ashcroft, J. S.; Koehn, F. E.; Borders, D. B.; Carter, G. T. Saccharomicins, Novel Heptadecaglycoside Antibiotics Effective against Multidrug-Resistant Bacteria. *J. Am. Chem. Soc.* **1998**, *120* (51), 13301−13311.

(2) Singh, M. P.; Petersen, P. J.; Weiss, W. J.; Kong, F.; Greenstein, M. Saccharomicins, Novel Heptadecaglycoside Antibiotics Produced by Saccharothrix Espanaensis: Antibacterial and Mechanistic Activities. *Antimicrob. Agents Chemother.* **2000**, *44* (8), 2154−2159.

(3) Zhao, J.; Mo, T.; Li, X.; Ding, W.; Zhang, Q. Dissection of the Glycosylation in the Biosynthesis of the Heptadecaglycoside Antibiotic Saccharomicin A. *J. Org. Chem.* **2021**, *86* (16), 11117−11124.

(4) Strobel, T.; Al-Dilaimi, A.; Blom, J.; Gessner, A.; Kalinowski, J.; Luzhetska, M.; Pühler, A.; Szczepanowski, R.; Bechthold, A.; Rückert, C. Complete Genome Sequence of Saccharothrix Espanaensis DSM 44229T and Comparison to the Other Completely Sequenced Pseudonocardiaceae. *BMC Genomics* **2012**, *13* (1), 465.

(5) Zeng, J.; Wang, R.; Zhang, S.; Fang, J.; Liu, S.; Sun, G.; Xu, B.; Xiao, Y.; Fu, D.; Zhang, W.; Hu, Y.; Wan, Q. Hydrogen-Bonding-Assisted Exogenous Nucleophilic Reagent Effect for β-Selective Glycosylation of Rare 3-Amino Sugars. *J. Am. Chem. Soc.* **2019**, *141* (21), 8509−8515.

(6) Balthaser, B. R.; McDonald, F. E. Brønsted Acid-Promoted Glycosylations of Disaccharide Glycal Substructures of the Saccharomicins. *Org. Lett.* **2009**, *11* (21), 4850−4853.

(7) Pletcher, J. M.; McDonald, F. E. Synthesis of the Saccharomicin Fucose−Aglycon Conjugate and Determination of Absolute Configuration. *Org. Lett.* **2005**, *7* (21), 4749−4752.

(8) Barpuzary, B.; Kim, M.; Rhee, Y. H. Synthetic Study toward Saccharomicin Based upon Asymmetric Metal Catalysis. *Org. Lett.* **2021**, *23* (15), 5969−5972.
(9) Soliman, S. E.; Bennett, C. S. Reagent-Controlled Synthesis of the Branched Trisaccharide Fragment of the Antibiotic Saccharomicin B. *Org. Lett.* **2018**, *20* (11), 3413−3417.
(10) Bylsma, M.; Bennett, C. S. Stereospecific Synthesis of the Saccharosamine-Rhamnose-Fucose Fragment Present in Saccharomicin B. *Org. Lett.* **2018**, *20* (15), 4695−4698.
(11) Jana, M.; Bennett, C. S. Synthesis of the Non-Reducing Hexasaccharide Fragment of Saccharomicin B. *Org. Lett.* **2018**, *20* (23), 7598−7602.
(12) Daley, L.; Guminski, Y.; Demerseman, P.; Kruczynski, A.; Etiévant, C.; Imbert, T.; Hill, B. T.; Monneret, C. Synthesis and Antitumor Activity of New Glycosides of Epipodophyllotoxin, Analogues of Etoposide, and NK 611. *J. Med. Chem.* **1998**, *41* (23), 4475−4485.
(13) Frihed, T. G.; Pedersen, C. M.; Bols, M. Synthesis of All Eight Stereoisomeric 6-Deoxy- L -Hexopyranosyl Donors - Trends in Using Stereoselective Reductions or Mitsunobu Epimerizations: Synthesis of 6-Deoxy- L -Hexopyranosyl Donors. *Eur. J. Org. Chem.* **2014**, *2014* (35), 7924−7939.
(14) Tilbrook, M. G.; Stick, R. V.; Williams, S. J. β-Acarbose. VIII.The Synthesis of Some N-Linked Carba-Oligosaccharides. *Aust. J. Chem.* **1999**, *52* (9), 895.
(15) Wang, J.; Li, J.; Chen, H.-N.; Chang, H.; Tanifum, C. T.; Liu, H.-H.; Czyryca, P. G.; Chang, C.-W. T. Glycodiversification for the Optimization of the Kanamycin Class Aminoglycosides. *J. Med. Chem.* **2005**, *48* (20), 6271−6285.
(16) Elferink, H.; Pedersen, C. M. l -Rhamnosylation: The Solvent Is the Solution: l -Rhamnosylation: The Solvent Is the Solution. *Eur. J. Org. Chem.* **2017**, *2017* (1), 53−59.
(17) Kim, H. M.; Kim, I. J.; Danishefsky, S. J. Total Syntheses of Tumor-Related Antigens N3: Probing the Feasibility Limits of the Glycal Assembly Method. *J. Am. Chem. Soc.* **2001**, *123* (1), 35−48.
(18) Xia, J.; Abbas, S. A.; Locke, R. D.; Piskorz, C. F.; Alderfer, J. L.; Matta, K. L. Use of 1,2-Dichloro 4,5-Dicyanoquinone (DDQ) for Cleavage of the 2-Naphthylmethyl (NAP) Group. *Tetrahedron Lett.* **2000**, *41* (2), 169−173.
(19) Crich, D.; Li, L. 4,6- *O* -Benzylidene-Directed β-Mannopyranosylation and α-Glucopyranosylation: The 2-Deoxy-2-Fluoro and 3-Deoxy-3-Fluoro Series of Donors and the Importance of the O2−C2−C3−O3 Interaction. *J. Org. Chem.* **2007**, *72* (5), 1681−1690.
(20) Crich, D.; Smith, M. 1-Benzenesulfinyl Piperidine/Trifluoromethanesulfonic Anhydride: A Potent Combination of Shelf-Stable Reagents for the Low-Temperature Conversion of Thioglycosides to Glycosyl Triflates and for the Formation of Diverse Glycosidic Linkages. *J. Am. Chem. Soc.* **2001**, *123* (37), 9015−9020.
(21) Li, Z.; Gildersleeve, J. C. Mechanistic Studies and Methods To Prevent Aglycon Transfer of Thioglycosides. *J. Am. Chem. Soc.* **2006**, *128* (35), 11612−11619.

https://doi.org/10.1021/acs.orglett.2c04081
Org. Lett. 2023, 25, 369−372