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Reagent-Controlled Synthesis of the Branched Trisaccharide Fragment of the Antibiotic Saccharomicin B

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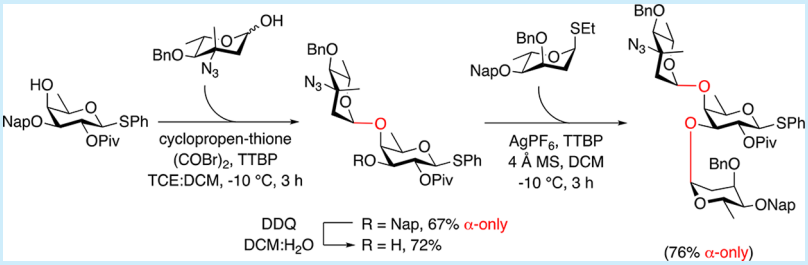
Supporting Information



ABSTRACT: A concise synthesis of a branched trisaccharide, α-L-Dig-(1 → 3)-[α-L-Eva-(1 → 4)]-β-D-Fuc, corresponding to saccharomicin B, has been developed via reagent-controlled α-selective glycosylations. Starting from the D-fucose acceptor, L-*epi*-vancosamine was selectively installed using 2,3-bis(2,3,4-trimethoxyphenyl)cyclopropene-1-thione/oxalyl bromide mediated dehydrative glycosylation. Following deprotection, L-digitoxose was installed using the $AgPF_6$/TTBP thioether-activation method to produce the trisaccharide as a single α-anomer. This highly functionalized trisaccharide can potentially serve as both a donor and an acceptor for the total synthesis of the antibiotic saccharomicin B.

Saccharomicin B, a heptadecasaccharide antibiotic, was first isolated and characterized in 1998 by Kong and co-workers from the cultured broths of the rare actinomycete *Saccharothrix espanaensis* (Figure 1).[1] This class of antibiotics was found to possess broad-spectrum activity, both *in vitro* and *in vivo*, against a wide range of Gram-positive and Gram-negative bacteria, including multidrug resistant strains of staphylococci and enterococci.[2] Given the pressing need for the development of new classes of antimicrobials, saccharomicin B holds the potential to serve as a next-generation antibiotic, especially if a route could be developed that was flexible enough to provide both the natural material and analogues. The biosynthesis of saccharomicin B has yet to be fully elucidated,[3] and the promiscuity of the enzymes involved in the synthesis is unknown. This leaves chemical synthesis as the only avenue for the production of analogues. Before such analogue synthesis will be possible, however, an efficient route to the parent compound needs to be devised.

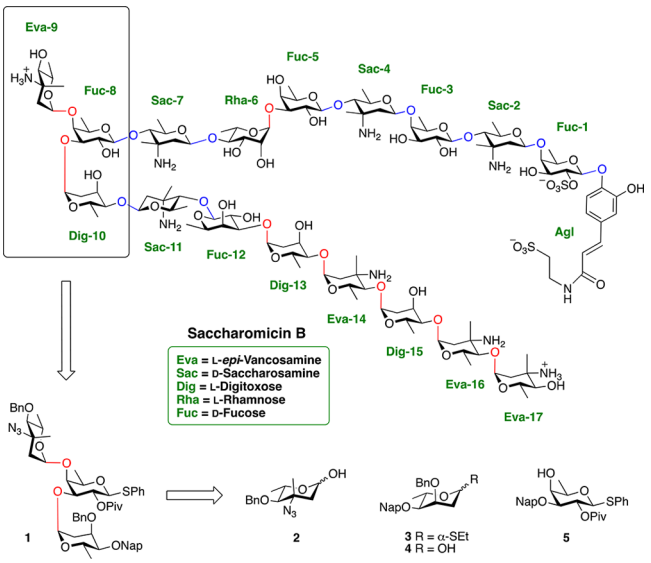


Figure 1. Structures of saccharomicin B, the target trisaccharide **1**, and the key deoxy-sugar monosaccharide building blocks (**2**−**5**).

This daunting molecule possesses several structural elements which would pose a major challenge to any attempted synthesis. Saccharomicin B is composed of five different deoxy-sugars including the rare L-4-*epi*-vancosamine (Eva), D-saccharosamine (Sac), and L-digitoxose (Dig), which are difficult to synthesize and install in a stereocontrolled fashion. Historically, it has been extremely difficult to control the stereochemical outcome of glycosylation reactions using deoxy-sugar donors without recourse to lengthy sequences involving temporary prosthetic groups or *de novo* synthesis,[4] which introduces several steps between each glycosylation reaction. As a consequence, only two reports of studies directed at fragments of saccharomicins have been reported to date, both from McDonald's lab.[5,6] The first of these studies was directed at the synthesis of the fucose−aglycone terminus of the molecule. A later study was directed at the synthesis of both the

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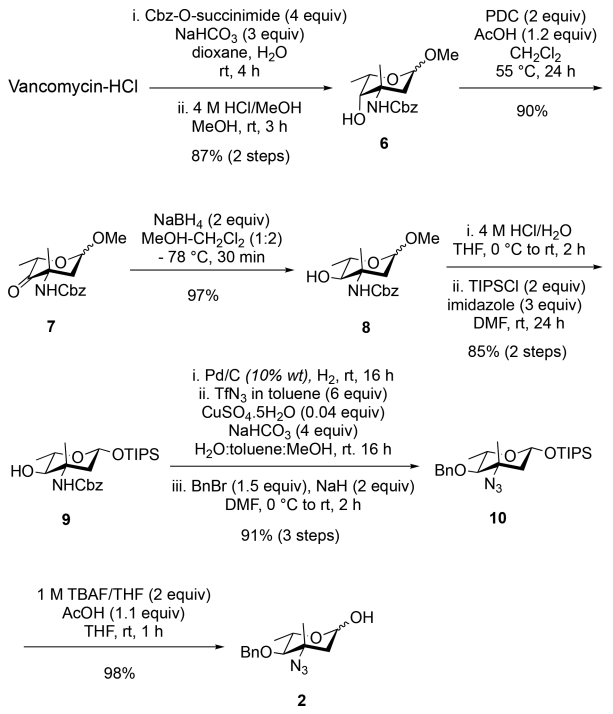
antipodal fucose−saccharosamine and fucose−saccharosamine−digitoxose structures.

As part of a synthetic campaign directed at the total synthesis of saccharomicin B, we sought to target the highly congested trisaccharide **1** using our reagent-controlled α-selective dehydrative glycosylation strategy.[7] The challenges involved in assembling the branched trisaccharide fragment **1** are, first, the synthesis of a set of rare, suitably functionalized deoxy-sugar building blocks **2**−**5**, followed by the stereospecific construction of the two α-glycosidic linkages.

Synthesis of the trisaccharide began with the construction of L-4-*epi*-vancosamine. This is a rare, branched-chain 2,3,6-trideoxy-aminosugar with an amine-bearing C-3 tertiary center.[8−10] Synthesis of the L-4-*epi*-vancosamine hemiacetal building block **2** was effectively accomplished starting from vancomycin-hydrochloride (Scheme 1). To this end, the amino

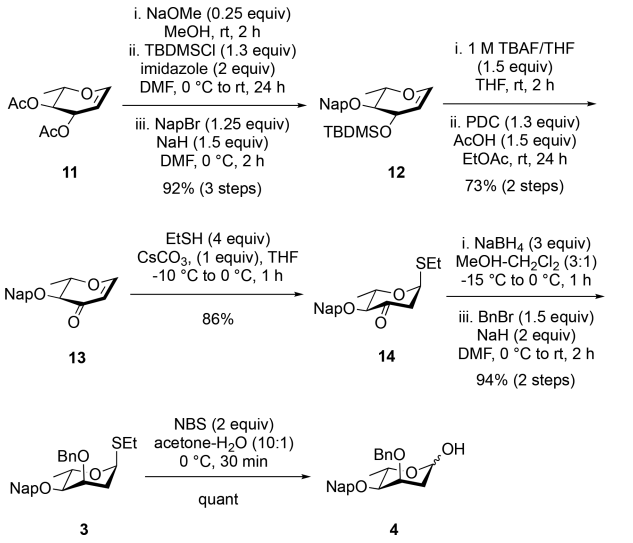
Scheme 1. Synthetic Pathway for the L-4-*epi*-Vancosamine Building Block



group of the vancosamine moiety on vancomycin-HCl was subjected to Cbz carbamate protection, followed by acidic methanolysis of the vancosaminyl sugar under anhydrous conditions[11] to give the corresponding methyl glycoside **6** in 87% yield.[8a,12] Inversion of configuration at the C-4 atom of **6** was achieved via a stereospecific oxidation−reduction sequence, as previously described by Kahne and Walsh.[8b] Thus, pyridinium dichromate (PDC) mediated oxidation, in the presence of acetic acid, afforded the 4-ketosugar **7** in 90% yield, which upon subsequent reduction with sodium borohydride ($NaBH_4$) in a mixture of MeOH−CH_2Cl_2 (1:2) at −78 °C furnished selectively the 4-*epi*-vancosamine derivative **8** in 97% yield. Acid hydrolysis (4 M aq HCl, THF, 0 °C) of the methyl glycoside **8**, followed by regioselective O-silylation of the anomeric hydroxyl group with triisopropylsilyl chloride (TIPSCl) and imidazole in dry DMF, gave the silyl derivative **9** as a single β-anomer (85% over two steps). Catalytic hydrogenolysis (Pd/C, H_2) of the N-Cbz carbamate produced a primary amine, which was readily transformed into the corresponding azide by the copper(II)-catalyzed diazotransfer from freshly prepared trifluoromethanesulfonyl azide (TfN_3) solution in toluene.[13,14] Subsequent benzylation under standard conditions (BnBr, NaH, DMF, 0 °C) afforded the azide derivative **10** (91% yield, over three steps). Finally, selective removal of the anomeric TIPS group from **10** with tetra-*n*-butylammonium fluoride (TBAF, 1 M in THF) in the presence of acetic acid at room temperature cleanly provided the desired L-4-*epi*-vancosamine hemiacetal **2** in 98% yield.

Synthesis of the 2,6-dideoxy-sugar, L-digitoxose,[15] building blocks **3** and **4** is outlined in Scheme 2. Zemplén de-O-

Scheme 2. Synthetic Pathway for L-Digitoxose Building Blocks



acetylation of the commercially available 3,4-di-*O*-acetyl-L-rhamnal (**11**) using catalytic sodium methoxide, followed by regioselective 3-*O*-silylation of the resulting diol with *tert*-butyldimethylsilyl chloride (TBDMSCl) in the presence of imidazole at 0 °C[16] and subsequent etherification with 2-naphthylmethyl bromide (NapBr) and sodium hydride in anhydrous DMF at 0 °C, gave the 4-*O*-Nap ether protected derivative **12** in excellent yield (92%, over three steps). Desilylation (TBAF/THF) of the 3-*O*-TBDMS group, followed by PDC/AcOH oxidation of the resulting allylic alcohol in ethyl acetate at room temperature, gave the hex-1-en-3-ulose **13** in 73% yield over two steps. Low-temperature cesium carbonate-promoted Michael-type addition of ethanthiol to the enone derivative **13** in THF[17] produced the thioethyl 2-deoxy-3-uloside **14** in 86% yield as a single α-isomer. Sodium borohydride ($NaBH_4$) reduction of ketone **14** in a mixture of MeOH−CH_2Cl_2 (3:1) at −15 °C was achieved with excellent stereoselectivity. ^1H and ^{13}C NMR spectra of the crude product showed a single product, containing an axial C3-OH, corresponding to the desired L-digitoxose stereoisomer. Subsequent benzylation of the crude alcohol furnished the thioglycoside **3** in 94% yield over two steps. Chemoselective hydrolysis of the anomeric phenyl thioether group with *N*-bromosuccinimide (NBS) in wet acetone (10:1 acetone−water)[18,19] at 0 °C smoothly afforded the L-digitoxose hemiacetal **4** in quantitative yield.

Synthesis of the D-fucose acceptors (Scheme 3) commenced with the regioselective alkylation of the equatorial 3-hydroxyl

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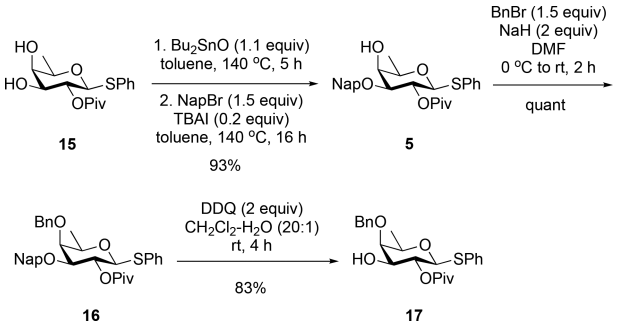
Scheme 3. Synthetic Pathway for the D-Fucose Building Blocks



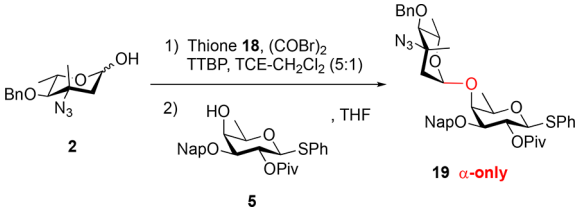
group of diol[20] **15**, via dibutylstannylene acetal formation under Dean–Stark conditions,[21,22] using 2-naphthylmethyl bromide (NapBr) to afford the 3-*O*-Nap ether building block **5** in 93% yield. Benzylation (BnBr, NaH, DMF, 0 °C) of **5** at the 4-position gave quantitatively the fully protected derivative **16**, and subsequent oxidative cleavage of the Nap ether with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) in a mixture of CH_2Cl_2–H_2O (20:1) afforded **17** in 83% yield.

With the appropriate deoxy-sugar glycosyl donors and acceptors in hand, we turned our attention to disaccharide formation. To this end, 2,3-bis(2,3,4-trimethoxyphenyl)-cyclopropene-1-thione **18** was initially activated with oxalyl bromide and then treated with the *epi*-vancosaminyl hemiacetal donor **2** and 2,4,6-tri-*tert*-butylpyrimidine (TTBP) in a mixture of 1,1,2-trichloroethylene (TCE) and CH_2Cl_2, followed by the addition of the Fuc-C4-acceptor **5** in THF at room temperature. This dehydrative glycosylation condition afforded the desired disaccharide **19** with complete α-stereoselectivity, albeit in a modest 16% yield along with a substantial amount of trehalose (Table 1, entry 1). After rapid screening of different reaction temperatures and times (Table 1, entries 2–6), we were able to achieve a satisfactory isolated yield (65%), with no impact on selectivity, by performing all the additions at −10 °C and then maintaining the reaction temperature at −10 °C for 1 h before warming to room temperature (Table 1, entry 6). Increasing the donor-to-acceptor ratio from 2:1 to 3:1 did not offer any improvement to the yield (Table 1, entry 7). Interestingly, our best result for the α-1,4-selective glycosylation of **2** with **5** was obtained when the donor **2** was coactivated in the presence of the acceptor **5** (Table 1, entry 8), affording disaccharide **19** in 67% yield. The α-(1 → 4) glycosidic linkage in **19** was confirmed by the ^{1}H NMR spectrum, which showed a doublet (δ 5.14 ppm) with a vicinal coupling constant $J_{1,2}$ for H-1′ of 4.3 Hz.

Having established the conditions for the construction of disaccharide **19**, we turned our attention to the assembly of trisaccharide **1**. To this end, unmasking of the Nap protecting group on **19** using DDQ afforded disaccharide **20** in 72% yield (Scheme 4). With both the disaccharide acceptor **20** and the L-digitoxose hemiacetal donor **4** in hand, we initially examined the dehydrative glycosylation to directly forge the target trisaccharide fragment. However, condensation of **20** with **4** under our cyclopropenium activation conditions proceeded in poor yield, favoring the formation of the β-isomer. In hindsight, this was not unexpected as digitoxose is known to preferentially undergo β-selective glycosylation reactions.[23]

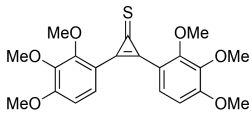
Attempts to optimize our cyclopropenium cation-mediated glycosylation on a model system failed to produce the desired

Table 1. Optimization of α-Stereoselective Dehydrative Glycosylation for the Synthesis of Disaccharide 19[a]

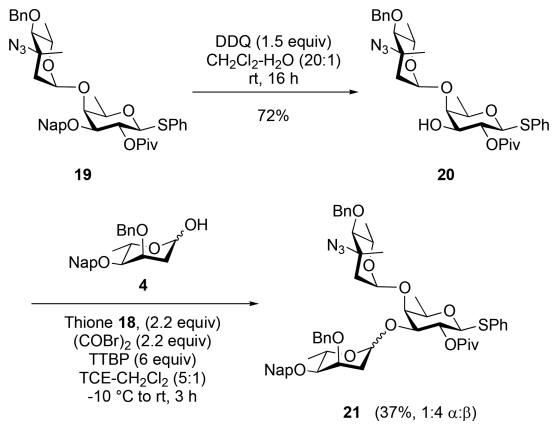


entry	temp (°C)	time (h)	yield (%)[b]
1	rt	2	16
2	0	2	39
3	−10	2	52
4	−20	18	26
5	−10 → rt	2	58
6[c]	−10 → rt	3	65
7[c,d]	−10 → rt	3	63
8[c,e]	−10 → rt	3	67

[a]Acceptor (1 equiv), donor (2 equiv), thione (2.2 equiv), oxalyl bromide (2.2 equiv), and TTBP (6 equiv) unless otherwise noted. [b]Yield of isolated product after purification. [c]Reaction mixture was stirred at −10 °C for 1 h before being allowed to warm to rt. [d]Acceptor (1 equiv), donor (3 equiv), thione (3.3 equiv), oxalyl bromide (3.3 equiv), and TTBP (9 equiv). [e]Donor was coactivated in the presence of acceptor; THF was not used.



Scheme 4. Dehydrative Glycosylation of Disaccharide Acceptor 20 with L-Digitoxose Hemiacetal Donor 4



anomer (see Supporting Information). Our inability to utilize our cyclopropenium method for the construction of **1** led us to consider alternative approaches. To this end, we were attracted to the mild activation of deoxy thioglycosides using silver hexafluorophosphate ($AgPF_6$) reported by Hirama et al.[24a] This approach takes advantage of the different reactivity between 2-oxy- and 2-deoxy-thioglycosides toward $AgPF_6$. After evaluating the scope of the reaction with the two reported[24] non-nucleophilic bases (DTBMP and TTBP) using D-fucose thioglycoside **17** as a model C-3 acceptor (Table 2), we found that the $AgPF_6$/TTBP combination is the optimal glycosylation promoter system for the stereoselective formation of the L-Dig-α-(1 → 3)-D-Fuc linkage.

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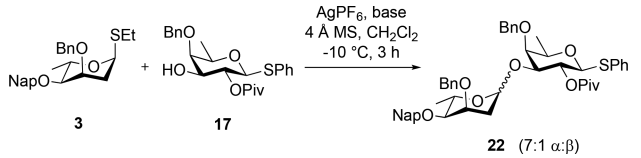
Table 2. Optimization of $AgPF_6$-Mediated Glycosylation Using D-Fucose Thioglycoside 17 as a Model C-3 Acceptor[a]



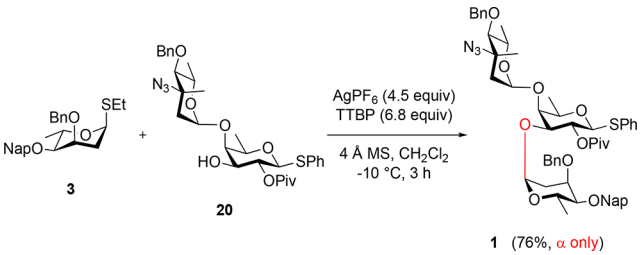
3 17 22 (7:1 α:β)

entry	base	yield (%)[b]
1	DTBMP	62
2[c]	DTBMP	44
3	TTBP	72

[a]Acceptor (1 equiv), donor (1.5 equiv), $AgPF_6$ (3 equiv based on donor), and base (4 equiv based on donor) unless otherwise noted. [b]Yield of isolated product after purification. [c]$AgPF_6$ (3 equiv based on acceptor) and base (4 equiv based on acceptor).

Next, we focused on applying the optimized $AgPF_6$ thioether-activation method to effect the final glycosylation to assemble the target trisaccharide **1**. To this end, a mixture of disaccharide acceptor **20** and glycosyl donor **3** in the presence of TTBP and 4 Å molecular sieves was treated with $AgPF_6$ at −10 °C for 3 h to give exclusively the desired trisaccharide **1** as a single α-anomer in 76% yield (Scheme 5). Again, selectivity

Scheme 5. Synthesis of the Target Trisaccharide 1



3 20 1 (76%, α only)

was confirmed by ^1H NMR, which showed a doublet for the anomeric proton of the digitoxose residue (δ 4.78 ppm) with a vicinal coupling constant $J_{1,2}$ of 4.7 Hz. In addition, ^{13}C NMR showed the anomeric carbon signals for the digitoxose and the *epi*-vancosamine residues at δ 98.6 ppm ($J_{C\text{-}1,H\text{-}1}$ 168.7 Hz) and δ 97.2 ppm ($J_{C\text{-}1,H\text{-}1}$ 173.0 Hz), respectively.

In conclusion, we have developed an efficient strategy for the stereoselective synthesis of a branched trisaccharide **1**, of potential value for a future synthesis of the antibiotic saccharomicin B, via reagent-controlled α-selective glycosylations of 2,6-dideoxy- and 2,3,6-trideoxy-sugars. The rare deoxy sugar building blocks were readily constructed from commercially available materials. Our cyclopropenium cation-mediated reagent-controlled dehydrative glycosylation between L-4-*epi*-vancosamine hemiacetal **2** and D-fucose C4-acceptor **5** gave **19** exclusively as the α-anomer. Following DDQ-mediated oxidative cleavage of the 3-*O*-Nap ether, glycosylation of the formed disaccharide acceptor **20** with the L-digitoxose thioglycoside **3**, promoted by $AgPF_6$/TTBP, afforded the desired α-linked trisaccharide **1** as a single diastereomer. This highly functionalized trisaccharide can potentially serve as both a donor and an acceptor for the total synthesis of saccharomicin B, which is under investigation in our laboratory.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b01355.

Experimental procedures, characterization data, and ^1H and ^{13}C NMR spectra for all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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