Published in final edited form as:

Org Lett. 2018 December 07; 20(23): 7598–7602. doi:10.1021/acs.orglett.8b03333.

Synthesis of the Non-Reducing Hexasaccharide Fragment of Saccharomicin B

Manas Jana and Clay S. Bennett*

Department of Chemistry, Tufts University, 62 Talbot Avenue, Medford, Massachusetts 02155, United States

Abstract

A synthesis of the nonreducing end hexasaccharide of saccharomicin B, α -L-Eva-(1 $\rightarrow$ 4)- α -L-Eva-(1 $\rightarrow$ 4)- α -L-Dig-(1 $\rightarrow$ 4)- α -L-Eva-(1 $\rightarrow$ 4)- α -L-Dig-(1 $\rightarrow$ 4)- β -D-Fuc, has been developed. Selective glycosylations of L-digitoxose (L-Dig) using AgPF₆/TTBP-mediated thioether activation and L-4-*epi*-vancosamine (L-Eva) using Tf₂O/DTBMP-mediated sulfoxide activation produced the hexasaccharide as a single diastereomer in very good yield. This hexasaccharide is properly functionalized to serve as a glycosyl donor for the total synthesis of saccharomicin B.

Graphical Abstract

The heptadecasaccharide antibiotics saccharomicins A and B were first isolated from the rare actinomycete *Saccharothrix espanaensis* bacteria by Kong and co-workers.¹ These molecules possess significant antibacterial activity against Gram-positive and Gram-negative bacteria.² While an unknown mode of toxicity precluded their further development as antibiotics, it is possible that saccharomicin analogues could have potential antimicrobial activity without undesirable side effects. In order to understand the antibacterial activity, mechanism of toxicity, and structure–activity relationships of the saccharomicins, it is essential to access a significant quantity of the material, which is technically challenging to isolate from natural sources with adequate purity, as fermentation produces 5.6 mg/L of saccharomicin B.¹ Since the biosynthesis of saccharomicin B is unknown,³ chemical synthesis remains the best option for obtaining this material for answering questions about the saccharomicins.

*Corresponding Author: clay.bennett@tufts.edu.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.8b03333. Experimental procedures, characterization data, and ¹H and ¹³C NMR spectra for all new compounds (PDF)

Notes

The authors declare no competing financial interest.

Saccharomicin B consists five different deoxy sugars that include *L*-rhamnose (Rha) and the rare monosaccharides *D*- fucose (Fuc), *L*-4-*epi*-vancosamine (Eva), *L*-digitoxose (Dig), and *D*-saccharosamine (Sac). To date, the synthesis of four fragments of the saccharomicins have been reported. McDonald et al. have reported the construction of the Fuc- aglycone moiety and a Dig-10 to Fuc-12 fragment of the molecule in high yield and excellent selectivity.⁴ Very recently, our laboratory has reported efficient and stereoselective routes to the Fuc-8 to Dig-10 branching point and the Fuc-5 to Sac-7 fragment of saccharomicin B (Figure 1).⁵ Herein, we report the first synthetic route to the nonreducing hexasaccharide fragment of saccharomicin B which corresponds to the Fuc- 12 to Eva-17 sector, which would be the largest fragment of the molecule so far synthesized.

Retrosynthetically, we envisioned that the hexasaccharide fragment could be obtained from a 4 + 2 coupling between the tetrasaccharide 3 and disaccharide 4.⁶ Tetrasaccharide 3 could ultimately be traced back to three suitably functionalized monosaccharides: *L*-digitoxose 6, *L*-4-*epi*-vancosamine 8, and *D*- fucose 10. The *L*-4-*epi*-vancosamine⁷ can be synthesized from vancomycin-HCl using our group's modification^{5a} to the procedure described by Kahne and Walsh.⁸ Similarly, *L*- digitoxose can be obtained from *L*-rhamnol as most recently described by our group^{5a} (for the preparation of the monosaccharides, see Supporting Information). Meanwhile, disaccharide 4 could be obtained through glycosylation between *L*-4-*epi*-vancosamines 8 and 9.^{6,8}

Our initial studies focused on construction of the disaccharide 7. Initial attempts to couple donor 6 and acceptor 10 using AgPF₆ in the presence of 2,6-di-*tert*-butyl-4- methylpyridine (DTBMP)⁹ afforded disaccharide 11 in moderate yield as a mixture of anomers (Table 1, entries 1 and 2). After several unfruitful approaches, both the yield and α -selectivity were recovered by replacing DTBMP with less basic 2,4,6-tri-*tert*-butylpyrimidine (TTBP) at 0 °C.^{10,11} Under these conditions, 11 was produced in 72–85% yield as a single α -anomer (Table 1, entries 3 and 4). Interestingly, we found that running the reaction for too long (5.5 vs 4 h) resulted in lower yield, presumably due to product decomposition. Treating 11 with DDQ to unmask the Nap protecting group afforded the disaccharide acceptor 7 without anomerization in 93% yield (Scheme 1).¹²

We initially envisioned that the *L*-4-*epi*-vancosamine could be installed on this disaccharide 7 using our previously described cyclopropenium cation mediated dehydrative glycosylation.¹³ All attempts to carry out this coupling failed, however, which led us to reconsider our strategy. After examining several approaches, we ultimately drew inspiration from the Kahne group, who reported the construction of the vancomycin disaccharide through activating a *L*-vancosamine sulfoxide donor with Tf₂O.⁸

To this end, in a two step activation, first the thioglycoside donor 8 was converted to sulfoxide using *m*-CPBA, which was activated using Tf₂O and glycosylated with acceptor 7 to produce the trisaccharide 12 in moderate yield (38%) as a single α -isomer.⁸ After optimization, we found that using a 2:1 donor/acceptor ratio, the addition of molecular sieves, and a shorter reaction time provided an optimum yield (61%) of the desired trisaccharide (Table 2, entry 5). In addition, we also found that addition of a proton scavenger, such as β -pinene (A)¹⁴ or 4-allyl-1,2-dimethoxybenzene (B),¹⁵ minimized

unwanted side reactions (Table 2, entries 4–6).¹⁶ Finally, changing the order of addition of donor did not have any additional impact on the yield (Table 2, entry 6).¹⁷ The coupling constant of the anomeric proton of the *L*-Eva residue at δ 4.90 ppm ($J_{H1} = 4.5$ Hz) and *L*-Dig residue at δ 4.84 ppm ($J_{H1} = 3.5$ Hz) confirmed the α -stereochemistry of the newly formed linkage. Finally, removal of the Nap protecting group on 12 using DDQ afforded trisaccharide acceptor 5 in 90% yield (Scheme 2).¹²

With the trisaccharide acceptor 5 in hand, we examined its coupling to *L*-digitoxose 6 using AgPF₆ and DTBMP⁹ in DCM at -5 °C. Under these conditions, we were able to obtain tetrasaccharide 13 in 66% yield as a single α -isomer. Shortening the reaction time and increasing the amount of donor in the reaction helped to improve the yield to 76% without compromising selectivity (Table 3, entry 3). The stereochemistry of the glycosidic linkages in the tetrasaccharide fragment 13 was deduced from the anomeric ¹H–C coupling constants in the proton-coupled ¹³C NMR spectrum.¹⁸ The anomeric carbons of the two *L*-Dig residues show J_{C1-H1} coupling constants of 167.5 and 170.0 Hz, while J_{C1-H1} for *L*-Eva was 166.2 Hz, which confirmed the α -stereochemistry of those linkages. The coupling constant of the anomeric proton of the two *L*-Dig residues at δ 5.16 ppm ($J_{H1} = 4.0$ Hz), 4.85 ppm ($J_{H1} = 4.5$ Hz) and *L*-Eva residue at δ 4.90 ppm ($J_{H1} = 4.0$ Hz) further confirmed the α -stereochemistry. Finally, Nap removal under standard conditions (DDQ) gave tetrasaccharide acceptor 3 in 88% yield (Scheme 3).¹²

With 3 in hand, we initially chose to examine a linear synthesis of the molecule. To this end, we turned our attention to the synthesis of pentasaccharide 14. Coupling 8 and 3 using a two-step activation method led to an inseparable mixture of the pentasaccharide 14 and what appeared to be a trehalose derivative 15 in a modest 33% yield (Scheme 3). Attempts to improve upon this result failed. We therefore chose to examine a more convergent 4 + 2 route to the molecule.

This approach began with construction of the disaccharide 16. To this end, sequential oxidation and Tf₂O-mediated coupling of donor 8 with acceptor 9 produced disaccharide 16 in a good yield of 58% and moderate selectivity (Table 4 entry 1). With further optimization, we found that using a 2:1 donor/acceptor ratio slightly improved the yield (Table 4, entry 2). Finally, a shorter reaction time and addition of the scavenger 4-allyl-1,2-dimethoxybenzene (Table 4, entries 3 and 4) improved the disaccharide and afforded the product in 77% yield as an inseparable 3:1 (α/β) mixture of isomers.^{15,17} Following Nap removal, it was possible to isolate the desired α - anomer 18, which was converted to hemiacetal donor 19 under standard conditions. Next, we converted the hemiacetal donor 19 into the corresponding acetate 20 in anticipation of converting this species to the requisite thioglycoside.¹⁹ Attempts to synthesize thioglycoside 4 using BF₃·OEt₂ failed, instead affording a mixture of Eva thioglycosides 21 and 22 (Scheme 4).¹⁹ Given the apparent sensitivity of this compound to Lewis acids, we chose to directly convert the hemiacetal 19 into the corresponding thioglycoside 4 using our *p*-toluenesulfonyl chloride (TsCl) mediated dehydrative glycosylation methodology.²⁰ Under these conditions, we were able to obtain thioglycoside 4 in a moderate 37% yield as 2:1 (α/β) mixture of anomers.²¹

With both coupling partners in hand, we set about examining the 4 + 2 glycosylation for the construction of the hexasaccharide target. Pleasingly, subsequent oxidation of the thiol 4 by *m*-CPBA^{8a,b} and activation with Tf₂O in the presence of the proton scavenger 4-allyl-1,2-dimethoxybenzene in DCM at -78 °C furnished the target hexasaccharide 2 in excellent yield 50% and α -selectivity (Scheme 5).^{8,15,17} All of the anomeric carbon linkages were confirmed by the proton- coupled HSQC NMR spectrum. The J_{C1-H1} coupling constant for the anomeric carbons in the proton-coupled HSQC NMR spectrum are 172.5 Hz (C-1_{Eva}), 171.1 Hz (C-1_{Eva}), 172.3 Hz (C-1_{Dig}), 172.2 Hz (C-1_{Eva}), 170.7 Hz (C-1_{Dig}), and 155.7 Hz (C-1_{Fuc}), respectively. The coupling constants of the anomeric proton of the hexasaccharide are δ 5.14 ppm (two protons overlapped, H-1, H-1), 4.90 ppm (J = 4.5 Hz, H-1), 4.86 ppm (J_{H1} = 5.0 Hz, H-1_{Dig}), 4.79 ppm (J_{H1} = 4.5 Hz, H-1_{Eva}), and 4.66 (J_{H1} = 10.0 Hz, H-1_{Fuc}), further confirming the stereochemistry of the linkages.

In summary, we have developed an expedient chemical synthesis of the hexasaccharide 2, which corresponds to the nonreducing end of saccharomicin B. This compound, which is the largest fragment of saccharomicin synthesized to date, bears a thioglycoside aglycone, which will allow it to directly serve as a donor for elaboration to the natural product. The chemistry reported above also probes the efficiency of different glycosylations using deoxy-sugar donors in a challenging setting. In particular, it demonstrates the utility of both the Kahne sulfoxide glycosylation reaction and AgPF₆-mediated thioglycoside activation in the construction of highly complex deoxy-sugar oligosaccharides. Studies to elaborate 2 into saccharomicin B are currently underway in our laboratory.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We thank the National Institutes of Health (R01-GM115779) for generous financial support.

REFERENCES

- (1). (a)Kong F; Zhao N; Siegel MM; Janota K; Ashcroft JS; Koehn FE; Borders DB; Carter GT J. Am. Chem. Soc 1998, 120, 13301.(b)Shi SD-H; Hendrickson CL; Marshall AG; Siegel MM; Kong F; Carter GT J. Am. Soc. Mass Spectrom 1999, 10, 1285.
- (2). Singh MP; Petersen PJ; Weiss WJ; Kong F; Greenstein M Antimicrob. Agents Chemother 2000, 44, 2154. [PubMed: 10898690]
- (3). (a)Strobel T; Al-Dilaimi A; Blom J; Gessner A; Kalinowski J; Luzhetska M; Pühler A; Szczepanowski R; Bechthold A; Rückert C. BMC Genomics 2012, 13, 465. [PubMed: 22958348] (b)Berner M; Krug D; Bihlmaier C; Vente A; Müller R; Bechthold A J. Bacteriol 2006, 188, 2666. [PubMed: 16547054]
- (4). (a)Pletcher JM; McDonald FE Org. Lett 2005, 7, 4749. [PubMed: 16209526] (b)Balthaser BR; McDonald FE Org. Lett 2009, 11, 4850. [PubMed: 19780574]
- (5). (a)Soliman S; Bennett CS Org. Lett 2018, 20, 3413. [PubMed: 29790762] (b)Bylsma M; Bennett CS Org. Lett 2018, 20, 4695. [PubMed: 30015496]
- (6). (a)Kahne D; Walker S; Cheng Y; Van Engen D J. Am. Chem. Soc 1989, 111, 6881.(b)Yang D; Kim SH; Kahne D J. Am. Chem. Soc 1991, 113, 4715.(c)Raghavan S; Kahne D J. Am. Chem. Soc 1993, 115, 1580.(d)Kim SH; Augeri D; Yang D; Kahne D J. Am. Chem. Soc 1994, 116, 1766.

- (7). (a)Nishimura Y; Ishii K; Kondo S J. Antibiot 1990, 43, 54. [PubMed: 2307630] (b)Avenzoza A; Busto JH; Corzana F; Peregrina JM; Sucunza D; Zurbano MM Tetrahedron: Asymmetry 2003, 14, 1037.
- (8). (a)Ge M; Thompson C; Kahne D J. Am. Chem. Soc 1998, 120, 11014.(b)Thompson C; Ge M; Kahne D J. Am. Chem. Soc 1999, 121, 1237.(c)Losey HC; Peczu MW; Chen Z; Eggert US; Dong SD; Pelczar I; Kahne D; Walsh CT Biochemistry 2001, 40, 4745. [PubMed: 11294642]
- (9). (a)Lear MJ; Yoshimura F; Hiram M Angew. Chem., Int. Ed 2001, 40, 946.(b)Fan E; Shi W; Lowary TL J. Org. Chem 2007, 72, 2917. [PubMed: 17373847] (c) Ren F; Hogan PC; Anderson AJ; Myers AG J. Am. Chem. Soc 2007, 129, 5381. [PubMed: 17417855]
- (10). (a)Zhang G; Fang L; Zhu L; Aimiwu JE; Shen J; Cheng H; Muller MT; Lee GE; Sun D; Wang PG J. Med. Chem 2005, 48, 5269. [PubMed: 16078845] (b)Zhu L; Cao X; Chen W; Zhang G; Sun D; Wang PG Bioorg. Med. Chem 2005, 13, 6381. [PubMed: 16055335] (c)Zhang G; Fang L; Zhu L; Zhong Y; Wang PG; Sun D J. Med. Chem 2006, 49, 1792. [PubMed: 16509594]
- (11). Crich D; Smith M; Yao Q; Picione J Synthesis 2001, 2001, 323.
- (12). (a)Xia J; Abbas SA; Locke RD; Piskorz CF; Alderfer JL; Matta KL Tetrahedron Lett 2000, 41, 169.(b)Kim HM; Kim IJ; Danishefsky SJ J. Am. Chem. Soc 2001, 123, 35. [PubMed: 11273599] (c)Csá vá M; Borba A; Szilágyi L; Lipták A Synlett 2002, 2002, 887.(d)Fang T; Mo K-F; Boons GJ J. Am. Chem. Soc 2012, 134, 7545. [PubMed: 22475263] (e)Cattaneo V; Oldrini D; Corrado A; Berti F; Adamo R Org. Chem. Front 2016, 3, 753.(f)Lloyd D; Bylsma M; Bright DK; Chen X; Bennett CS J. Org. Chem 2017, 82, 3926. [PubMed: 28281755]
- (13). (a)Nogueira JM; Nguyen SH; Bennett CS Org. Lett 2011, 13, 2814. [PubMed: 21548642] (b)Nogueira JM; Issa JP; Chu A-HA; Sisel JA; Schum RS; Bennett CS Eur. J. Org. Chem 2012, 2012, 4927.(c)Nogueira JM; Bylsma M; Bright DK; Bennett CS Angew. Chem., Int. Ed 2016, 55, 10088.
- (14). (a)Gu X; Chen L; Wang X; Liu X; You Q; Xi W; Gao L; Chen G; Chen Y-L; Xiong B; Shen JJ Org. Chem 2014, 79, 1100.(b)Chen G; Yin Q; Yin J; Gu X; Liu X; You Q; Chen Y-L; Xiong B; Shen J Org. Biomol. Chem 2014, 12, 9781. [PubMed: 25370689]
- (15). Gildersleeve J; Smith A; Sakurai K; Raghavan S; Kahne D J. Am. Chem. Soc 1999, 121, 6176.
- (16). While yields were not improved in presence of the scavenger, it minimized side reactions, which greatly facilitated purification of the product.
- (17). Gildersleeve J; Pascal RA Jr.; Kahne DJ Am. Chem. Soc 1998, 120, 5961.
- (18). Bock K; Pedersen CJ J. Chem. Soc., Perkin Trans. 2 1974, 2 (0), 293.
- (19). Nicolaou KC; Mitchell HJ; Jain NF; Bando T; Hughes R; Winssinger N; Koumbis AE Chem. - Eur. J 1999, 5, 2648.
- (20). Lloyd D; Bennett CS Chem. - Eur. J 2018, 24, 7610. [PubMed: 29572995]
- (21). While the p-toluenesulfonyl chloride promoted reaction proceeded in low yield, it may be possible to improve this with the use of a different sulfonyl chloride. These studies are currently under examination in our laboratory.

Figure 1. Retrosynthetic outline of hexasaccharide fragment in saccharomicin B. Fragments circled in green have been previously synthesized by our group. The fragment in red is this work.

Scheme 1.
Synthesis of Disaccharide Acceptor **7**

Scheme 2.
Successful Synthesis of Trisaccharide Acceptor **5**

Scheme 3.
Glycosylation Attempt To Synthesize Pentasaccharide 14

Scheme 4.
Optimization of Eva-Eva Disaccharide Thioglycoside 4

Scheme 5.

Successful Attempt for the Synthesis of Hexasaccharide Fragment 2

Table 1.

Optimization of Disaccharide 11 Synthesis^a

entry	D/A	base	temp (°C)	time (h)	yield % ($\alpha:\beta$) ^b
1 ^c	1.1/1	DTBMP	-10	10	41 (4:1)
2 ^c	1/1.5	DTBMP	-10 to 0	22	43 (3:1)
3 ^d	1/1.5	TTBP	0	5.5	72 (1:0)
4 ^d	1/1.5	TTBP	0	4	85 (1:0)

^a AgPF_6 (3.0 equiv), 4 Å molecular sieves unless otherwise noted.^b Yield of isolated product after purification.^c DTBMP (5.0 equiv).^d TTBP (4.0 equiv).

Table 2.

Optimization of Trisaccharide Fragment 12 Synthesis^a

entry	donor (equiv)	scavenger	time (h)	yield ^b (%)
1 ^c	1.5		3	38
2 ^{c,d}	2		2	51
3	2		1	59
4	2	A	1	56
5	2	B	1	61
6 ^e	2	B	1	59

^a *m*-CPBA (1.0 equiv), acceptor (1.0 equiv), DTBMP (4.0 equiv), Tf₂O (2.0 equiv), reaction temp -78 °C, 4 Å molecular sieves unless otherwise noted.

^b Yield of isolated product after purification.

^c Without molecular sieves.

^d Donor was preactivated over 5 min.

^e Donor was added dropwise to the solution of acceptor, DTBMP, scavenger, and Tf₂O in DCM at -78 °C. A: β-pinene, B: 4-allyl-1,2-dimethoxybenzene.

Table 3.Optimization of Tetrasaccharide Fragment 13 Synthesis^a

entry	donor (equiv)	time (h)	yield ^b (%)
1	2	2.5	66
2	2	1	70
3	2.5	1	76

^aAcceptor (1.0 equiv), DTBPM (5.0 equiv), AgPF₆ (3.0 equiv), reaction temp -5 °C, 4 Å molecular sieves unless otherwise noted.^bYield of isolated product after purification.

Table 4.

Optimization of Eva-Eva Disaccharide 16^a

entry	donor (equiv)	scavenger	time (h)	yield ^b (%)
1 ^{c,d}	1.1		2	58
2 ^{e,f}	2		2	61
3 ^{d,e}	2	B	1	72
4 ^{d,e}	2	B	1	77

^a *m*-CPBA (1.0 equiv), acceptor (1.0 equiv), DTBMP (4.0 equiv), reaction temp -78 °C, 4 Å molecular sieves unless otherwise noted.

^b Yield of isolated product after purification.

^c Tf₂O (1.2 equiv)

^d Donor was added dropwise to the solution of acceptor, DTBMP, scavenger, and Tf₂O in DCM at -78 °C.

^e Tf₂O (2.2 equiv).

^f Donor was coactivated with acceptor, DTBMP, and scavenger (B). B: 4-allyl- 1,2-dimethoxybenzene.