Saccharomicins, Novel Heptadecaglycoside Antibiotics Effective against Multidrug-Resistant Bacteria

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Abstract: Two novel heptadecaglycoside antibiotics, saccharomicins A (**1**) and B (**2**), were isolated from a new species of *Saccharothrix*. Their structures were determined by a combination of chemical degradation and spectroscopic methods. Saccharomicin B (**2**) was identified as *N*-(*m*,*p*-dihydroxycinnamoyl) taurine *p*-*O*-[α-4-*epi*-vancosaminopyranosyl(1→4)-α-4-*epi*-vancosaminopyranosyl(1→4)-α-digitoxopyranosyl(1→4)-α-4-*epi*-vancosaminopyranosyl(1→4)-α-digitoxopyranosyl(1→3)-*β*-fucopyranosyl(1→4)-*β*-saccharosaminopyranosyl(1→4)-α-digitoxopyranosyl(1→3)]-[α-4-*epi*-vancosaminopyranosyl(1→4)]-*β*-fucopyranosyl(1→4)-*β*-saccharosaminopyranosyl(1→4)-α-rhamnopyranosyl(1→3)-*β*-fucopyranosyl(1→4)-*β*-saccharosaminopyranosyl(1→4)-*β*-fucopyranosyl(1→4)-*β*-saccharosaminopyranosyl(1→4)-*β*-2-sulfate-fucopyranoside. Saccharomicin A (**1**) differs from saccharomicin B (**2**) only in the 10th sugar residue, where α-digitoxopyranosyl(1→3) is replaced with α-rhamnopyranosyl(1→3). The novel amino sugar residue, designated saccharosamine, was identified by NMR spectroscopy and X-ray crystallography. Both saccharomicins A (**1**) and B (**2**) showed antimicrobial activity against Gram-positive bacteria.

Introduction

Rapid emergence of bacterial resistance to antimicrobial agents has been recognized as an epidemic of global proportions.[1] Particularly in hospital-acquired infections, many pathogenic bacteria are multiply resistant to several classes of antibiotics, effectively narrowing therapeutic options. For multiply resistant *Staphylococcus aureus*, vancomycin currently represents the last line of defense, whereas for certain resistant strains of *Mycobacterium tuberculosis* and vancomycin-resistant enterococci, no viable alternatives exist.[2] One response to this dilemma has been the issuance of new guidelines for antimicrobial usage to curtail the rate of development of resistance.[3] In addition to efforts to control the emergence of resistance to established classes of antimicrobial agents, there is renewed emphasis on the discovery of novel antibiotics. As part of a systematic reevaluation of antibacterial leads generated over five decades of infectious disease research, an antibiotic complex designated LL-C19004 was investigated. From the complex, produced by the rare actinomycete *Saccharothrix espanaensis*, two novel heptadecaglycoside antibiotics, saccharomicins A (**1**) and B (**2**), were isolated (Figure 1). Saccharomicins represent a novel class of antibiotics, unrelated to all known families of antibiotics, and show activity both in vitro and in vivo against multiply resistant strains of *S. aureus* as well as vancomycin-resistant enterococci. This paper describes the isolation, purification, and structure elucidation of saccharomicins A (**1**) and B (**2**). It will be shown that the oligosaccharide portion of saccharomicins has unique chemical reactivity. Spectroscopic analysis of the degradation products has provided a means to a gradual stepwise construction of the final structure of the intact molecules. Taxonomy and fermentation of the producing organism as well as the biological activity of the saccharomicins will be reported fully elsewhere.

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Results and Discussion

Isolation of LL-C19004 Complex, Initial Chemical Characterization, and Separation of Saccharomicins. Saccharomicins A (**1**) and B (**2**) were originally isolated as an antibiotic complex, designated LL-C19004, from the fermentation filtrate by adsorption on a cation exchange column of Amberlite IRC-(NH_4^+) and elution with dilute ammonium hydroxide. The active fractions were further purified on a column of CM Sephadex (NH_4^+) by gradient elution with water−1.5 M ammonium hydroxide. The purified complex was obtained in the free base form as an amorphous white powder following lyophilization.

Initially, chemical characterization of the antibiotics was carried out on the purified LL-C19004 complex.[4] Elemental analysis demonstrated that the complex contained carbon, hydrogen, nitrogen, oxygen, and sulfur. FABMS data suggested that the components of the complex were in the molecular weight range of 2200−2800 amu and contained polysaccharide units. The UV profiles seen in the intact antibiotic complex were similar to those for a substituted cinnamoyl chromophore. Comparison of the UV absorption spectra with those of isoferulic acid (*p*-methoxy-*m*-hydroxycinnamic acid) confirmed that the bathochromic shift patterns of the two chromophores were comparable, suggesting that the *p*-OH group in the complex was masked. To confirm the postulated structure for the chromophore, this unit was isolated.[5] The antibiotic complex was refluxed in the presence of zinc in strong alkali for 16 h under nitrogen followed by acidification and ether extraction.

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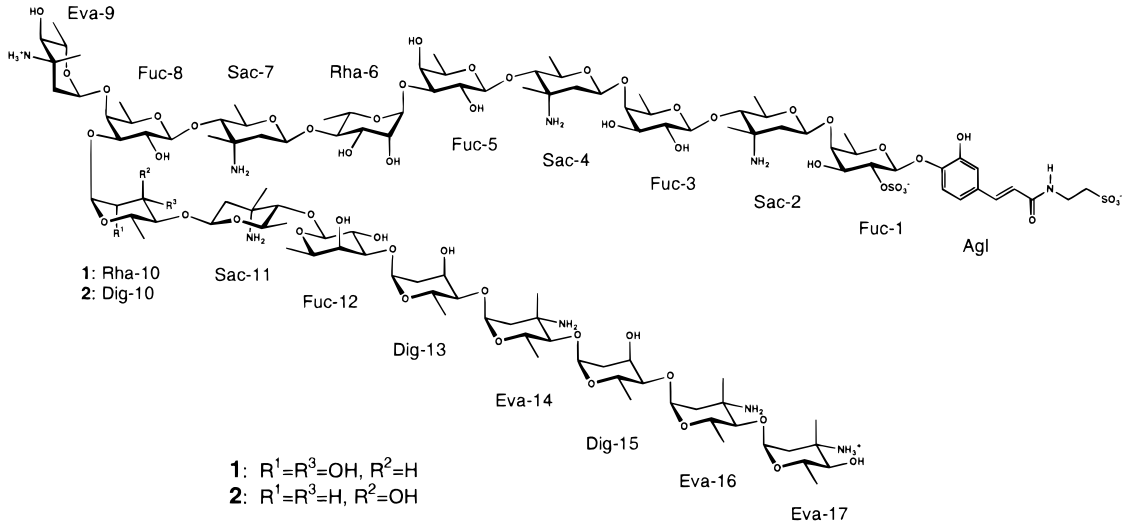
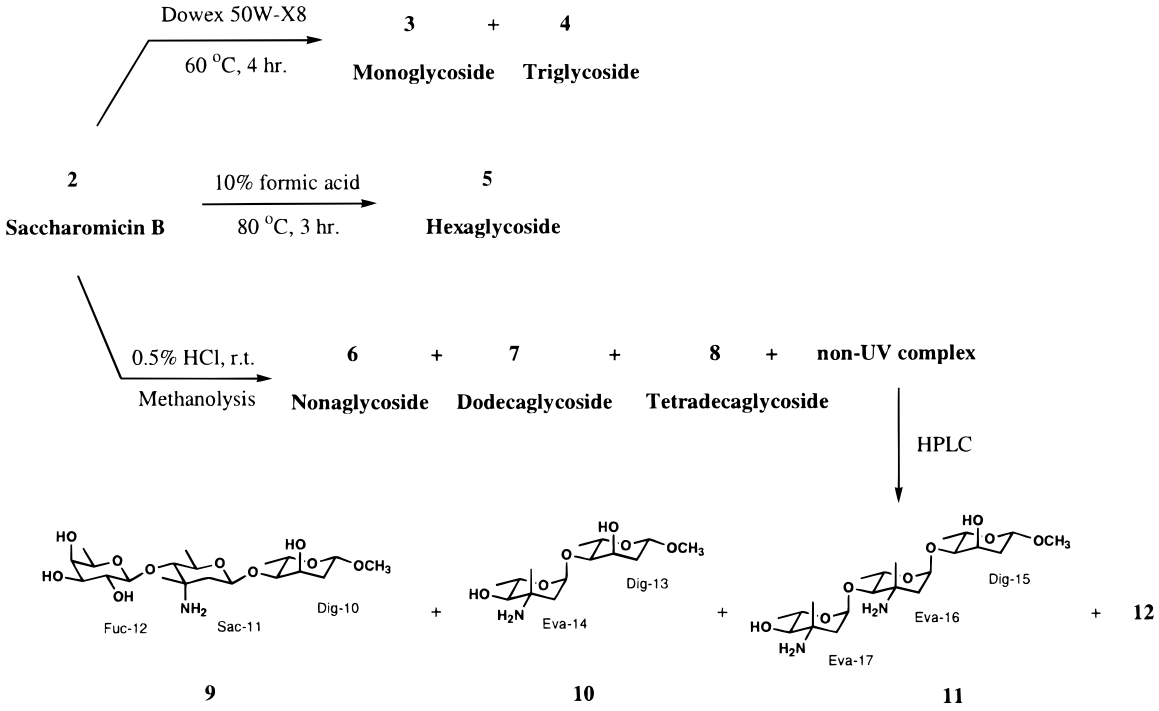


Figure 1. Structure of saccharomicins A (**1**) and B (**2**): Agl = aglycon, Fuc = fucose, Sac = saccharosamine, Rha = rhamnose, Eva = 4-*epi*-vancosamine, Dig = digitoxose.

Scheme 1



UV spectra and TLC analysis confirmed that the isolated LL-C19004 chromophore was caffeic acid (*m*,*p*-dihydroxycinnamic acid) by comparison with the reference compound. Characterization of the polysaccharide portion of the LL-C19004 antibiotic complex was carried out by *N*-acetylation and methanolysis.[6] Products obtained were subjected to column chromatography, and the purified components were studied by TLC, NMR, MS, and X-ray crystallography. Compounds recovered included peracetyl methyl glycosides of fucose, rhamnose, and the derivatized disaccharide of fucose and a novel amino sugar 3-amino-2,3,6-trideoxy-3-*C*-methyl-*ribo*-hexopyranose, designated saccharosamine.[7]

Extremely careful calibration of the reversed-phase HPLC conditions eventually yielded complete resolution of the LL-C19004 complex into individual pure components, saccharomicins A (**1**) and B (**2**). The ratio of **1** to **2**, produced in fermentations, varied from 3:1 to 1:1. Both compounds are highly polar and dissolve readily in water. Saccharomicin A (**1**) is more polar than saccharomicin B (**2**) as judged by HPLC mobility. Both compounds showed identical UV spectra. Low-resolution electrospray mass spectrometry (LRESIMS) indicated that saccharomicin A (**1**) had a molecular weight of 2795 amu and saccharomicin B (**2**) 2779 amu.

Chemical Degradation of Saccharomicin B (2) and Spectral Characterization of the Degradation Products. To fully determine the antibiotic structures by NMR techniques, a series of chemical degradation reactions was performed predominantly on saccharomicin B (**2**). Mild acid hydrolysis of saccharomicin B (**2**) in water using Dowex 50W-X8 resin as a catalyst at 60

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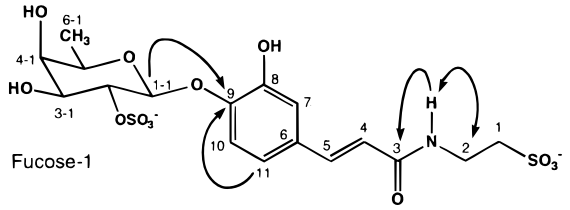

Figure 2. Structure of compound **3** and key HMBC ($^1H \rightarrow ^{13}C$) and COSY ($^1H \leftrightarrow ^1H$) correlations.

°C for 4 h yielded a mixture of neutral and basic degradation products.[8] All neutral products remained in the reaction solution, while the basic components adsorbed onto the Dowex resin which was readily separated by filtration. A preparative HPLC separation of the components present in the neutral aqueous phase gave two UV-absorbing products, monoglycoside **3** and triglycoside **4**, that had UV chromophores identical to that of the starting material **2** (Scheme 1).

The negative low-resolution ESIMS of compound **3** showed a low abundance of singly charged molecular ion at *m*/*z* 512 $[M - H]^-$ and a very intense doubly charged molecular ion at *m*/*z* 255.5 $[M - 2H]^{2-}$ appropriate for a molecular formula $C_{17}H_{23}NO_{13}S_2$. This result suggested the presence of two strongly acidic functionalities that were predominantly deprotonated under the MS conditions. This MS evidence together with analysis of NMR data acquired for **3** led to identification of two sulfur-containing groups, a sulfate and a sulfonic acid moiety, consistent with the elemental analysis performed on the antibiotic complex LL-C19004.

The proton NMR spectrum of **3** showed five downfield resonances identical to those of the intact antibiotics **1** and **2**. Two olefinic proton resonances were observed at δ 6.42 (H4) and δ 7.25 (H5) with a coupling constant of 15.7 Hz, indicating an *E* configuration. Three aromatic proton resonances were observed: a broad singlet at δ 6.99 (H7), a sharp doublet at δ 7.05 (H10), and a broad doublet at δ 6.94 (H11). The COSY spectrum showed that H11 coupled strongly to H10 and weakly to the meta proton H7. In the ^{13}C NMR spectrum, all nine sp^2 carbon resonances accounting for the *m*,*p*-dihydroxycinnamoyl moiety were observed. Detailed analysis of the 2D NMR data confirmed the presence of the UV chromophore, a substituted cinnamic acid (Figure 2).

A deshielded triplet at δ 8.01 with a coupling constant of 5.4 Hz was assigned to an amide NH proton because it did not show a correlation to any carbons in the HMQC spectrum. An IR band at 1657 cm^{-1} supported the presence of an amide group. A COSY correlation was observed between this NH proton and a methylene proton resonance at δ 3.43 (H2) that was further coupled to another methylene proton resonance at δ 2.71 (H1), both integrating for two protons. In the HMQC experiment, the methylene triplet H1 was correlated to C1 at δ 50.8 and H2 correlated to C2 at δ 35.6, typical chemical shifts for a taurine unit according to the assignments published previously.[9] A two-bond HMBC correlation observed from the NH proton to the carbonyl carbon C3 at δ 165.6 demonstrated that the taurine unit was linked to *m*,*p*-dihydroxycinnamic acid unit via an amide bond.

The remaining proton NMR signals were due to the presence of one sugar moiety. A deshielded doublet at δ 4.76 was assigned to the anomeric proton H1-1 that was correlated to a resonance of δ 101.4 (C1-1), typical for an anomeric carbon. A coupling constant of 7.5 Hz indicated the sugar was in the β configuration. In the COSY spectrum, the anomeric proton H1-1 was correlated to a triplet at δ 4.30 (H2-1), which was attached to a carbon at 76.2 ppm. The downfield chemical shifts for both H2-1 and C2-1 suggested that the sulfate group, indicated by the MS data, was attached at the 2-position.[10] The remainder of the spin system for the sugar residue was readily identified by analysis of the COSY spectrum. The proton coupling constants and NOESY data demonstrated that H3-1 at δ 3.56 and H5-1 at δ 3.79 were axial, while H4-1 (broad singlet) at δ 3.52 was equatorial. On the basis of this NMR evidence, the first sugar unit was identified as β-2-sulfate-fucopyranosyl (fucose-1). A three-bond HMBC correlation observed between the anomeric proton H1-1 and aromatic carbon C9 established the glycosidic linkage. Therefore, compound **3** was identified as *p*-*O*-β-2-sulfate-fucopyranoside with an unprecedented *N*-(*m*,*p*-dihydroxycinnamoyl)taurine as an aglycon (Figure 2).

Compound **4** had the same UV chromophore as that of **3**, indicating the same aglycon. Unlike the monoglycoside **3**, compound **4** exhibited an abundant singly charged ion at *m*/*z* 801 $[M - H]^-$ in the negative low-resolution ESIMS, corresponding to the molecular formula $C_{30}H_{46}N_2O_{19}S_2$. This was due to the presence of an amino group in compound **4** that was protonated under the MS conditions, offsetting one of two negative charges derived from the sulfate and sulfonic acid groups. Examination of the NMR data revealed that **4** had all the resonances nearly identical to those identified for **3** except for the chemical shift assigned to C4-1 (**4**, δ 76.6; **3**, 70.3), indicating C4-1 in **4** was glycosylated. In addition, the spectrum of compound **4** contained two extra sets of monosaccharide resonances.

Detailed analysis of the 2D NMR data led to complete assignment of the resonances for **4**. A broad doublet at δ 5.08 was assigned to the anomeric proton H1-2, which correlated to a carbon resonance at δ 96.9 (C1-2). The coupling constant of 9.3 Hz indicated that the anomeric proton H1-2 was axial. This proton H1-2 showed COSY correlations strongly to one of the two methylene protons $H2_{ax}$-2 at δ 1.68 and weakly to another methylene proton $H2_{eq}$-2 at δ 2.25. The lack of further COSY correlations for these methylene protons suggested that the spin system was interrupted by a quaternary carbon. In the HMBC spectrum, a methyl singlet at δ 1.36 (H7-2) showed three cross-peaks at carbon resonances δ 40.8, 55.4, and 81.2, assigned to the methylene carbon C2-2, the quaternary carbon C3-2, and the methine carbon C4-2, respectively. Further HMBC correlations were observed between C4-2 and a methyl doublet (H6-2) at δ 1.31 and between H6-2 and another methine carbon resonance at δ 68.4, assigned to C5-2. These correlations in conjunction with the HMQC experiment completed the ^1H and ^{13}C NMR assignments for the second sugar unit designated saccharosamine-2. Although the signal for H5-2 at δ 3.73 was obscured by other proton resonances, H4-2 at δ 3.24 was represented by a well-resolved doublet with a coupling constant of 9.7 Hz, suggesting an axial relationship between H4-2 and H5-2. The methyl group C7-2 at δ 25.0 was assigned to be in equatorial orientation based on its diagnostic lowfield chemical shift.[11] A three-bond HMBC correlation between H4-1 at δ 3.75 and C1-2 at δ 96.9 established the glycosidic linkage (Figure 3).

The third sugar moiety in **4** was identified as fucose-3. Relative to H1-1, the anomeric proton H1-3 resonated upfield

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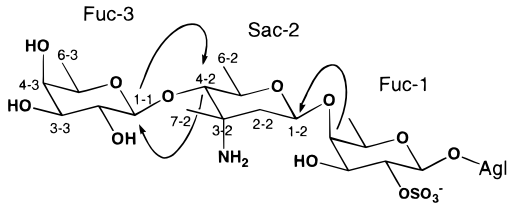

Figure 3. Structure of triglycoside **4** and key HMBC ($^1H \rightarrow ^{13}C$) correlations.

at δ 4.22, reflecting the lack of the influence from the benzene ring's anisotropic effect. The magnitude of its coupling constant (7.4 Hz) indicated its axial orientation. The signals for H2-3 and C2-3 also appeared upfield at δ 3.38 and 70.4, respectively, compared with those of the 2-sulfated-fucose-1 at 4.24 δ (H2-1) and 76.2 (C2-1). The rest of the NMR assignments were comparable with those of fucose-1 discussed for **3**. Mutual HMBC correlations between the anomeric proton H1-3 at δ 4.22 and C4-2 at δ 81.2 and between H4-2 at δ 3.24 and C1−3 at δ 103.9 identified the position of the glycosidic bond. Therefore, the terminal disaccharide moiety of **4** is equivalent to the disaccharide unit previously established by X-ray crystallographic analysis of the derivative methyl β-*N*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-β-fucopyranosyl)saccharosaminopyranoside.[7]

Hydrolysis of saccharomicin B (**2**) with 10% formic acid in water at 80 °C for 3 h generated a small amount of hexaglycoside **5** (Scheme 1). Hexaglycoside **5** was purified by HPLC using MeOH−tetrafluoroacetic acid (TFA) buffer to protonate all amino groups present in the molecule. Compound **5** showed a molecular ion at *m*/*z* 1236.5 $[M - H]^-$ in the low-resolution ESIMS, corresponding to a molecular formula of $C_{49}H_{79}N_3O_{29}S_2$. Subtraction of the atoms present in triglycoside **4** ($C_{30}H_{46}N_2O_{19}S_2$) from the molecular formula of hexaglycoside **5** suggested that **5** contained three additional monosaccharide units accounting for a composition of $C_{19}H_{33}NO_{10}$. Detailed analysis of the ^1H, ^{13}C, DEPT-135, COSY, HMQC, HMBC, and NOESY data led to the proposed structure for **5**. The three extra sugar units were identified as one more extension of the disaccharide β-fucopyranosyl(1→4)-β-saccharosaminopyranosyl(1→4) and a rhamnose residue.

Assignments for the second set of disaccharide β-fucopyranosyl(1→4)-β-saccharosaminopyranosyl(1→4) NMR signals in **5** were similar to the discussion for **4**. Identification of the rhamnose residue was based on interpretation of the ^1H and COSY data. A deshielded broad singlet at δ 4.85 was assigned to the anomeric proton H1-6. The COSY spectrum showed the whole spin system. The signal for methine proton H4-6 was a well-resolved triplet with a coupling constant of 9.5 Hz, requiring H3-6, H4-6, and H5-6 all to be axial. From the coupling constant of 3.3 Hz between H3-6 and H2-6, an equatorial position for H2-6 could be deduced. Finally, the anomeric proton H1-6 was also determined as equatorial on the basis of its chemical shift and one-bond $^1H-^{13}C$ coupling constant of 171 Hz.[12] The ^{13}C NMR data provided further support for this assignment by comparison with literature values.[13]

HMBC and NOESY correlations were used to determine the sequence within this hexaglycoside. All of the expected HMBC correlations between the anomeric protons and the corresponding glycosidic carbons were observed. The glycosidic carbons showed a diagnostic ~6 ppm downfield shift over normal hydroxylated carbon resonances. In addition, strong NOESY correlations between each pair of the anomeric protons and glycosidic methine protons corroborated the sugar−sugar connectivities.

Rhamnose-6 was shown to be linked to fucose-5 at the 3-position (C3-5). The lack of an HMBC correlation between a resonance at δ 80.3 (C3-5) and any methyl proton doublets ruled out the possibility that this carbon was located at the 4-position. In conjunction with the COSY and HMQC data, a strong HMQC-TOCSY correlation between the anomeric proton H1-5 at δ 4.28 and carbon resonance at δ 80.3 secured this carbon (C3-5) within fucose-5.

Saccharomicins A (**1**) and B (**2**) are highly susceptible to hydrolysis under acidic conditions. The effect of exposure of saccharomicin B (**2**) to 10% TFA was immediately apparent with the appearance of three new compounds by HPLC. LC-MS analysis of these peaks indicated the molecular weights of 1670.5, 2090.0, and 2363.4 amu, corresponding to nonaglycoside **6**, dodecaglycoside **7**, and tetradecaglycoside **8**, respectively. Methanolysis of saccharomicin B (**2**) in 0.5% HCl at room temperature for 1 h yielded the three targeted products, **6**−**8**, plus the non-UV absorbing components (Scheme 1). Each product was separated and purified for structure characterization.

Nonaglycoside **6** showed an abundant ion of *m*/*z* of 835.7 $[M + 2H]^{2+}$ in the low-resolution ESIMS, corresponding to a molecular formula of $C_{69}H_{115}N_5O_{37}S_2$. Subtraction of the composition of hexaglycoside **5** ($C_{49}H_{79}N_3O_{29}S_2$) from the molecular formula of **6** ($C_{69}H_{115}N_5O_{37}S_2$) revealed that the remaining three-sugar unit had an elemental composition of $C_{20}H_{36}N_2O_8$ equivalent to two amino sugar units ($C_{14}H_{26}N_2O_4$) and one 6-deoxy sugar unit ($C_6H_{10}O_4$). Detailed analysis of ^1H, ^{13}C, DEPT-135, COSY, HMQC, HMBC, and NOESY data led to determination of the structure for **6**. The seventh and eighth sugar units were identified as the disaccharide unit β-fucopyranosyl(1→4)-β-saccharosaminopyranosyl(1→4), and the ninth sugar residue was 4-*epi*-vancosamine-9.

The disaccharide unit of β-fucopyranosyl(1→4)-β-saccharosaminopyranosyl(1→4) was readily identified as discussed for compound **4**. Identification of the 4-*epi*-vancosamine-9 residue was based on NMR analysis and comparison with literature data. Its anomeric proton (H1-9) signal at δ 5.18 had a proton−proton coupling constant of 4.5 Hz and a one-bond $^1H-^{13}C$ coupling constant of 172 Hz, indicating its equatorial orientation. The methyl group C7-9 at δ 18.1 was assigned as axial, diagnosed from its upfield chemical shift.[11] Again, HMBC and NOESY correlations were used to determine the sugar linkages. For example, correlations were observed between H1-9 and C4-8 (HMBC) and between H1-9 and H4-8 (NOESY). Finally, a comparison with the literature data for 4-*epi*-vancosamine gave support for the above assignment.[14]

Once the structures of the first nine sugar residues and aglycon were determined, it was possible to elucidate the entire structure of saccharomicin B (**2**) by analysis of spectroscopic data collected for the intact natural product and comparison with the described fragments (vide infra). Therefore, it is not necessary to discuss the details here of structure characterization for the degradation products dodecaglycoside **7** and tetradecaglycoside **8**.

Rechromatography of the UV-transparent fraction (Scheme 1) obtained from the methanolysis reaction mixture of saccharomicin B (**2**) yielded three major products: methyl β-fucopy-

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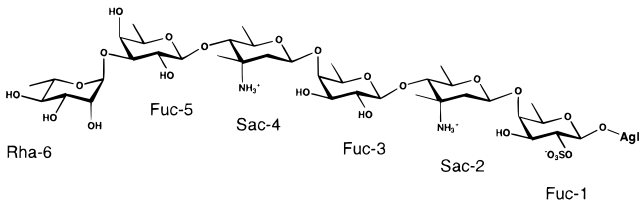

Figure 4. Structure of hexaglycoside **5**.

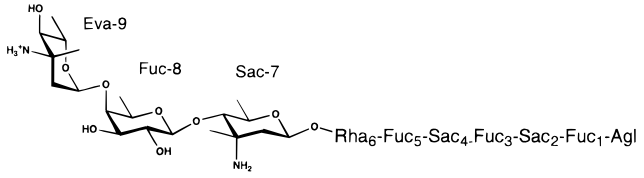

Figure 5. Structure of nonaglycoside **6**.

ranosyl(1→4)-β-saccharosaminopyranosyl(1→4)-β-digitoxopyranoside (**9**), methyl α-4-*epi*-vancosaminopyranosyl(1→4)-β-digitoxopyranoside (**10**), and methyl α-4-*epi*-vancosaminopyranosyl(1→4)-α-4-*epi*-vancosaminopyranosyl(1→4)-β-digitoxopyranoside (**11**), corresponding to cleavage at three digitoxose residues of the molecule. Their structures were determined by analyses of the MS and NMR data. The β configuration for each of the methyl glycosides, deduced from the anomeric proton chemical shifts and coupling constants, was considered the result of S_N2-type inversion at the anomeric carbons during the mild methanolysis. Inversion at an anomeric carbon was also observed in the case of **12**, the terminal octasaccharide, isolated in minute quantity from the methanolysis. The ^{1}H NMR spectrum of **12** showed that only the methyl glycoside anomeric proton H1-10 was axial, resonating at δ 4.61 with coupling constants of 9.1 Hz (axial−axial) and 1.9 Hz (axial−equatorial), while the intact anomeric protons of digitoxose-13 and digitoxose-15 were both equatorial based on the lower field chemical shifts at δ 5.04 for H1-13 and δ 5.13 for H1-15 and smaller coupling constants. The anomeric ^{1}H−^{13}C coupling constants of 161, 168, and 169 Hz for digitoxose-10, digitoxose-13, and digitoxose-15, respectively, were consistent with the structure shown in Figure 6.

Structure Elucidation of Saccharomicin B (2). Saccharomicin B (**2**) was isolated as an optically active white powder. Its molecular formula was first deduced to be $C_{121}H_{207}N_9O_{58}S_2$ by detailed analysis of NMR data and later confirmed by high-resolution FT-ICR (Fourier transform ion cyclotron resonance) mass spectrometry that gave a molecular weight of 2778.29643 after electron mass correction, appropriate for the molecular formula of **2** (calcd 2778.29641). All 1D and 2D NMR data for **2** were acquired at 50 °C, which proved to be crucial for achieving the best resolution for both proton and carbon spectra. Examination of the ^{1}H and ^{13}C NMR spectra of **2** quickly identified the lowfield signals attributable to the aglycon *N*-(*m*,*p*-dihydroxycinnamoyl)taurine as discussed for compound **3**. An intensive analysis of the ^{1}H NMR, ^{13}C NMR, DEPT-135, COSY, HMQC, HMBC, TOCSY, HMQC-TOCSY, NOESY, HMQC-NOESY, and coupled-HMQC data in conjunction with comparison of the NMR assignments of the degradation products led to identification of 17 sugar residues: five fucoses, four saccharosamines, four 4-*epi*-vancosamines, three digitoxoses, and a rhamnose residue. Since the substructures and sequence of the first nine sugar residues were already determined in compound **6**, what remained to be defined in the intact molecule are the terminal eight sugar residues and their linkages.

The 4-*epi*-vancosamine residues were readily identified owing to their well-resolved anomeric proton and carbon resonances. Thus, the anomeric proton H1-16 showed COSY correlations to both methylene protons $H2_{ax}$-16 and $H2_{eq}$-16. In the HMBC spectrum, it (H1-16) was correlated to C3-16 and C5-16. Both of these carbons were further correlated to a proton doublet at δ 3.08 (H4-16). This proton doublet was attached to a deshielded carbon resonance at δ 86.6 (C4-16), indicative of a glycosylated carbon. For the other three 4-*epi*-vancosamine residues, the 4-position protons all resonated at δ 3.13 in the ^{1}H NMR spectrum but correlated to three different carbon resonances at δ 75.1 (C4-9), 75.2 (C4-17), and 83.2 (C4-14). Unambiguous assignment was achieved by HMBC correlations between the anomeric proton resonances and well-resolved quaternary carbons, between the quaternary carbons and methyl proton singlets, and between the methyl proton singlets and the 4-position carbons. The chemical shifts of 75.1 and 75.2 ppm for C4-9 and C4-17, respectively, indicated that these two were the terminal sugars. The higher field chemical shifts observed for the carbons β to the amino groups in these terminal sugars, as compared to those for the corresponding carbons in Eva-14 and Eva-16, suggested that the two terminal amino sugar residues were protonated.[15]

The anomeric proton resonances for the three digitoxose residues were obscured by a broad peak at δ 5.05 ppm. Fortunately, the 4-position proton signals were all resolved, and the proton coupling information indicated that they were axial. The ^{1}H−^{1}H COSY correlations were routinely employed to establish the proton spin system and relative configurations for each sugar unit. For example, H4-15 at δ 3.22 was strongly correlated to H5-15 at δ 4.09, the latter further coupled to a methyl proton resonance at δ 1.14 (H6-15), and weakly to H3-15 at δ 4.02. The proton H3-15 showed additional weak COSY but strong NOESY correlations to both methylene protons at δ 1.79 ($H2_{ax}$-15) and 2.05 ($H2_{eq}$-15), indicating that H3-15 was equatorial. Owing to the superposition of signals at δ 5.05, the measurement of proton coupling constants for H1-15 was impossible. However, a coupling constant of 169 Hz for this anomeric proton−carbon bond revealed its equatorial orientation. The fact that the glycosidic bonds of the digitoxose residues were highly susceptible to acid was consistent with their assigned α-glycosidic linkages. The HMQC experiment was employed to determine the chemical shifts of C2-15, C3-15, C4-15, C5-15, and C6-15 at δ 34.3, 61.4, 76.0, 64.3, and 17.5, respectively, which indicated that C4-15 was the most likely site for the glycosidic linkage. Finally, an HMBC correlation observed between H5-15 and C1-15 completed the ^{1}H and ^{13}C NMR assignments for the digitoxose-15 residue. Similarly, the other two digitoxose residues were identified (see Table 1).

The 11th and 12th sugar residues in **2** were found to be the disaccharide unit β-fucopyranosyl(1→4)-β-saccharosaminopyranosyl(1→4). The anomeric proton and carbon resonances of saccharosamine-11 appeared at relatively higher field at δ 4.92 (H1-11) and 94.9 (C1-11) than those of the other saccharosamines. A proton coupling constant of 9.5 Hz for H1-11 defined its axial stereochemistry. The NMR resonances for the fucose residues were all very similar. The slight but important differences between the anomeric proton resonances played a key role in the identification of intraglycosidic sites (within the fucose) via HMQC-TOCSY correlations and interglycosidic sites (fucosylation sites) via HMQC-NOESY correlations. Thus, an HMQC-NOESY correlation between H1-12 and C4-11 revealed this glycosidic linkage (Figure 7, superimposed with the correlation between C4-4 and H1-5).

Identification of the positions in the sequence for the terminal eight sugar residues was straightforward. A strong HMBC

(15) Wehrli, F. W.; Wirthlin, T. *Interpretation of Carbon-13 NMR Spectra*; Heyden & Son Ltd.: London, 1976; p 30.

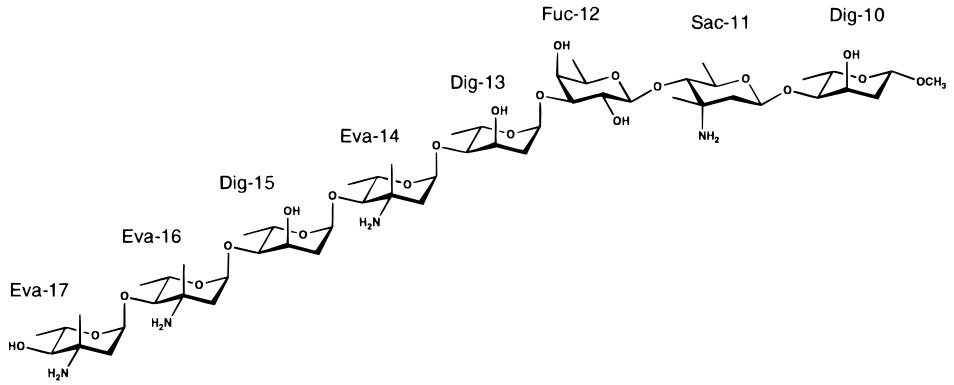

Figure 6. Structure of octasaccharide **12**.

correlation between the anomeric proton H1-17 at δ 5.20 and the most downfield oxygenated carbon C4-16 at δ 86.6 revealed the last sugar linkage. Although an HMBC correlation between the anomeric proton H1-16 and a carbon resonance at δ 76.0 (C4-15) was inconclusive due to superposition with other carbon signals (C2-1 at δ 76.0 and C4-1 at δ 76.2), the observation of an HMBC correlation between the anomeric carbon C1-16 and H4-15 firmly established the connectivity. The NOESY experiment was also very important in identification of the interglycosidic connectivities, which gave all the expected correlations. The HMQC-NOESY experiment further clarified the assignments, which virtually converted the severely overlapping proton−proton signals to relatively dispersed proton−carbon correlations. NOESY correlations were observed between H1-15 and H4-14 (overlapped), which was clarified by an HMQC-NOESY observation between C1-15 and H4-14, and between H1-14 and H4-13. An additional HMQC-NOESY correlation observed between C1-13 and H3-12 and a NOESY correlation observed between H1-11 and H4-10 established the glycosidic linkages for the terminal eight sugar residues as shown in Figure 7.

The anomeric proton H1-8 at δ 4.26 showed HMQC-TOCSY correlation to C3-8 at δ 77.7, which is in the expected chemical shift range for a glycosidic carbon. Since the attachment of 4-*epi*-vancosamine-9 to fucose-8 at the 4-position (C4-8) was already determined in nonaglycoside **6**, it was apparent that the terminal octasaccharide (Eva-17 to Dig-10) was linked to fucose-8 at the 3-position (C3-8) in **2**. An HMQC-NOESY correlation observed between H1-10 and C3-8 affirmed the glycosidic linkage. Further evidence for this linkage was observed from NMR correlations between Eva-9 and Dig-10. The signal for H1-9 at δ 5.35 showed NOESY correlations to H4-8 at δ 3.54 and to a proton resonance at 3.97 (H3−10 or H5-10, superimposed) as well as a correlation to C5-10 at δ 63.2 (resolved) in the HMQC-NOESY spectrum as shown in Figure 8.

Additional evidence for the 4-*epi*-vancosamine-9 branch was obtained from the high-resolution ESI-MS/MS experiment, which confirmed the sequence of the terminal nine sugar residues as shown in Figure 9.[16] The fragmentation pattern reflected preferential cleavage of the weak α-sugar linkages. Three diagnostic fragment ions present in the IRMPD (infrared multiphoton dissociation) spectrum were at *m*/*z* 2635.1, 2492.0, and 2348.9, corresponding to cleavages of the α-linkages of 4-*epi*-vancosamine-17, 4-*epi*-vancosamine-16, and 4-*epi*-vancosamine-9, respectively, consistent with the proposed oligosaccharide sequence.

Structure Elucidation of Saccharomicin A (1). Saccharomicin A (**1**) was isolated as an optically active amorphous white powder. It gave a molecular weight of 2794.2916 by high-resolution FT-ICR mass spectrometry appropriate for a molecular formula of $C_{121}H_{207}N_9O_{59}S_2$ (calcd 2794.2916, −0.07 ppm), differing from that of saccharomicin B (**2**) by the addition of one oxygen atom. Detailed analysis of the NMR data as well as the chemical degradation and high-resolution ESI-MS/MS of saccharomicin A (**1**) revealed that it contained a rhamnose residue in place of the digitoxose-10 residue in compound **2**. The 1D and 2D ^{1}H and ^{13}C NMR data obtained for saccharomicin A (**1**) showed a close correspondence to the data obtained for saccharomicin B (**2**). The only significant differences in the data for the two compounds were the chemical shifts of the resonances assigned to the 10th sugar residue. A well-resolved carbon resonance at δ 100.8 was assigned to the anomeric carbon of rhamnose-10 that correlated to a proton resonance at δ 5.01 (H1-10) in the HMQC spectrum. This anomeric proton H1-10 showed COSY correlation to H2-10 at δ 3.72 and HMQC-TOCSY correlation to C2-10 at δ 70.2. In the HMBC spectrum, it (H1-10) showed strong correlations to C3-10 at δ 70.2, C5-10 at δ 67.7, and C3-8 at δ 77.9 of fucose-8. This evidence indicated that the only glycosidic site on rhamnose-10 was at the 4-position (C4-10, δ 78.0). Other notable differences were that both anomeric proton and carbon resonances of saccharosamine-11 (H1-11/ C1-11) showed downfield shifts to δ 5.03/98.2 in **1** from δ 4.92/94.9 in **2** and that the anomeric proton resonance of 4-*epi*-vancosamine-9 (H1-9) was shifted upfield to δ 5.02 in **1** from δ 5.35 in **2**. Methanolysis of saccharomicin A (**1**) in 0.5% hydrochloric acid at room temperature yielded only small amount of nonaglycoside **6** and mainly dodecaglycoside **13** and tetradecaglycoside **14**, as well as methyl glycosides **10** and **11**. High-resolution ESI-MS/MS of saccharomicin A (**1**) obtained by IRMPD gave fragments corresponding to cleavage of the terminal six sugar residues and the side chain 4-*epi*-vancosamine-9 residue. These chemical degradation and ESI-MS/MS data demonstrated that the digitoxosyl bond is more vulnerable than that of the rhamnose residue.

Biological Activities. Saccharomicins exhibited potent antibacterial activity in vitro against a panel of pathogenic Gram-positive organisms, including *S. aureus* (MIC: 0.2−2 μg/mL) and *Streptococcus* spp. (MIC: 1−2 μg/mL), and were somewhat less potent versus Gram-negative bacteria, e.g. *Escherichia coli* (MIC: 16−32 μg/mL). Saccharomicins also demonstrated protection against lethal Gram-positive infections in mice: *S. aureus* Smith (ED_{50}: 0.06 mg/kg), *S. aureus* Rose (ED_{50}: 2.6

(16) Shi, S. D.-H.; Hendrickson, C. L.; Marshall, A. G.; Siegel, M. M.; Kong, F.; Carter, G. T. *Proceedings of the American Society for Mass Spectrometry and Allied Topics, 46th ASMS Conference*, Orlando, FL, May 31−June 4, 1998; ThPF 200.

Table 1. ^{1}H and ^{13}C NMR Spectral Data for Saccharomicin B (**2**)

C	^{1}H	^{13}C	C	^{1}H	^{13}C	C	^{1}H	^{13}C
aglycon			Rha-6			Fuc-12		
1	2.65, t, $J = 7.2$ Hz	50.7	1-6	4.86, bs	101.8	1-12	4.22	103.4
2	3.41	35.6	2-6	3.74	70.8	2-12	3.43	70.0
NH	7.81, t, $J = 5.4$ Hz		3-6	3.72	70.4	3-12	3.40	80.1
3		165.1	4-6	3.43	78.4	4-12	3.50	70.5
4	6.4, d, $J = 15.6$ Hz	120.7	5-6	3.64	66.7	5-12	3.53	69.9
5	7.26, d, $J = 15.6$ Hz	138.7	6-6	1.13	18.0	6-12	1.12	16.0
6		130.3	Sac-7			Dig-13		
7	6.98, bs	114.0	1-7	5.07, bd	98.2	1-13	5.05	98.1
8		147.2	2_{ax}-7	1.44, dd, $J = 9.7, 13.5$ Hz	43.5	2_{ax}-13	1.79	34.7
9		146.8	2_{eq}-7	1.97, bd, $J = 13.5$ Hz		2_{eq}-13	2.05	
10	7.04, d, $J = 8.2$ Hz	116.3	3-7		51.7	3-13	4.03	61.4
11	6.93, dd, $J = 1.4, 8.2$ Hz	120.0	4-7	3.04	83.4	4-13	3.18, dd, $J = 2.1, 9.2$ Hz	75.5
Fuc-1			5-7	3.76	68.8	5-13	4.11, dq, $J = 9.4, 6.0$ Hz	62.0
1-1	4.76, d, $J = 7.6$ Hz	101.1	6-7	1.27	18.0	6-13	1.16	18.0
2-1	4.27, t, $J = 7.8$ Hz	76.0	7-7	1.19, s	29.4	Eva-14		
3-1	3.70	72.1	Fuc-8			1-14	4.98, bd, $J = 4.2$ Hz	91.5
4-1	3.74	76.2	1-8	4.26, d, $J = 7.7$ Hz	102.5	2_{ax}-14	1.79	44.0
5-1	3.87, q, $J = 6.6$ Hz	69.3	2-8	3.40	70.5	2_{eq}-14	1.92	
6-1	1.22	16.6	3-8	3.52	77.7	3-14		52.6
Sac-2			4-8	3.54	77.7	4-14	3.13, d, $J = 9.4$ Hz	83.2
1-2	5.05	98.1	5-8	3.61	68.8	5-14	3.60	65.1
2_{ax}-2	1.29	44.0	6-8	1.12	16.3	6-14	1.19	18.0
2_{eq}-2	1.81		Eva-9			7-14	1.22, s	21.4
3-2		51.4	1-9	5.35, bs	96.8	Dig-15		
4-2	3.03	84.3	2_{ax}-9	1.79	40.0	1-15	5.05	97.7
5-2	3.75	68.8	2_{eq}-9	2.07		2_{ax}-15	1.78	34.3
6-2	1.27	18.0	3-9		54.5	2_{eq}-15	2.05	
7-2	1.09	30.4	4-9	3.13, d, 9.4 Hz	75.1	3-15	4.02	61.4
Fuc-3			5-9	3.75	65.6	4-15	3.22, dd, $J = 2.7, 8.0$ Hz	76.0
1-3	4.18, d, $J = 7.5$ Hz	103.6	6-9	1.14	17.5	5-15	4.09, dq, $J = 8.0, 6.5$ Hz	64.3
2-3	3.26, t, $J = 8.4$ Hz	71.0	7-9	1.35, s	18.6	6-15	1.14	17.5
3-3	3.40	73.3	Dig-10			Eva-16		
4-3	3.61	77.2	1-10	5.05	96.4	1-16	4.94, bd, $J = 5.0$ Hz	93.2
5-3	3.54	69.2	2_{ax}-10	1.79	34.8	2_{ax}-16	1.67, dd, $J = 4.4, 13.6$ Hz	44.0
6-3	1.12	16.4	2_{eq}-10	1.94		2_{eq}-16	1.81	
Sac-4			3-10	3.97	62.4	3-16		52.1
1-4	5.03, bd	98.2	4-10	3.32, bd, $J = 8.0$ Hz	77.0	4-16	3.08, d, $J = 9.2$ Hz	86.6
2_{ax}-4	1.38, dd, $J = 9.7, 13$ Hz	43.6	5-10	3.97	63.2	5-16	3.63	65.2
2_{eq}-4	1.89		6-10	1.12	17.5	6-16	1.18	18.0
3-4		51.5	Sac-11			7-16	1.15, s	22.1
4-4	3.05	84.5	1-11	4.92, bd, $J = 9.5$ Hz	94.9	Eva-17		
5-4	3.75	68.8	2_{ax}-11	1.38	44.0	1-17	5.20, bd, $J = 4.5$ Hz	98.4
6-4	1.27	18.0	2_{eq}-11	1.80		2_{ax}-17	1.80	40.0
7-4	1.16, s	30.2	3-11		52.1	2_{eq}-17	2.11, bd, $J = 13.2$ Hz	
Fuc-5			4-11	3.04	84.5	3-17		54.0
1-5	4.22	103.4	5-11	3.75	68.8	4-17	3.13, d, $J = 9.4$ Hz	75.2
2-5	3.43	70.0	6-11	1.27	18.0	5-17	3.63	65.9
3-5	3.38	80.1	7-11	1.15, s	30.2	6-17	1.15	18.2
4-5	3.46	69.8				7-17	1.29, s	18.9
5-5	3.53	69.9						
6-5	1.12	16.0						

[a] Spectra recorded at 500 MHz at 50 °C (*J* values in Hz).

mg/kg), *Streptococcus pyogenes* C203 (ED_{50}: 0.9 mg/kg), and *Streptococcus pneumoniae* SV1 (ED_{50}: 0.85 mg/kg) (ED_{50} = median effective dose). The compounds do exhibit toxic effects in mice at elevated doses. The median lethal dose (LD_{50}) value in mice for a single subcutaneous dose is approximately 16 mg/kg, and therefore, for some infections, the safety margins are inadequate.

Conclusion

The saccharomicins represent a new family of antibiotics. The aglycon *N*-(*m*,*p*-dihydroxycinnamoyl)taurine is the first reported example of an amide bond linkage between cinnamic acid and taurine. In addition, the polysaccharide portion of the molecules also exhibits unique features. All 17 monosaccharide residues are 6-deoxy sugars. Except for the sulfated fucose residue that is directly linked to the aglycon, the other 16 sugar residues are evenly divided into two categories: eight amino sugars and eight non-amino sugars, which are further separated into four groups: four fucoses, four saccharosamines, four 4-*epi*-vancosamines, and four of a combination of rhamnose(s) and digitoxoses. It is interesting to note that the fucoses are always connected to saccharosamines via a strong $\beta-\beta$ glycosidic linkage, as this portion of the molecule can survive harsh acidic conditions, e.g. refluxing in 5% methanolic HCl. In contrast, the glycosidic linkages of digitoxoses are the most labile as they are in the α-configuration and sterically less hindered. Two of the four 4-*epi*-vancosamine residues, the branching residue Eva-9 and the terminal one Eva-17, are both protonated as indicated by their ^{13}C NMR data. This suggests that the molecules exist as a quadruply charged zwitterion. The antimicrobial activity of the saccharomicins may be attributed to their zwitterionic property that presumably folds the molecules

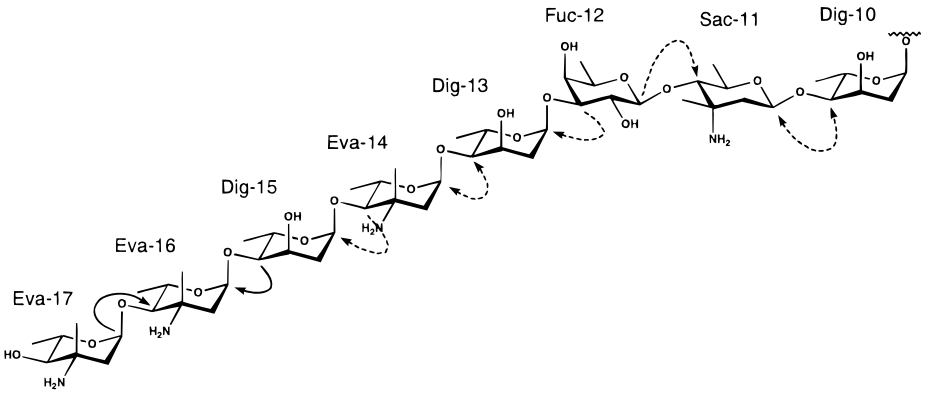

Figure 7. Key HMBC ($^1H \rightarrow ^{13}C$), NOESY ($^1H \leftrightarrow ^1H$), and HMQC-NOESY ($^1H \rightarrow ^{13}C$) correlations for establishing the sugar sequence of the terminal portion of saccharomicin **2**.

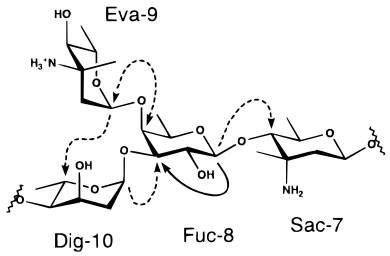

Figure 8. Key NOESY ($^1H \leftrightarrow ^1H$), HMQC-NOESY ($^1H \rightarrow ^{13}C$), and HMQC-TOCSY ($^1H \rightarrow ^{13}C$) correlations for establishing the branched portion of the oligosaccharide for **2**.

into two loops. The macrocyclic backbone conformation may interact with bacterial cell walls and thus cause damage. This hypothesis is supported by our observation of antibiotic activity for the tetradecaglycoside fragment which has activity comparable to that of the intact antibiotics, whereas the dodecaglycoside fragment is less active, and smaller degradation products show no activity.

The sugar residues are depicted arbitrarily in their most abundant natural forms, that is D-fucose, L-rhamnose, L-4-*epi*-vancosamine, and L-digitoxose. The configuration of saccharosamine was shown to be the same as that of the fucose on the basis of the crystal structure of disaccharide derivative methyl *β*-*N*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-*β*-fucopyranosyl)saccharosaminopyranoside. Determination of the absolute stereochemistry of the sugar residues is under investigation.

Experimental Section

Physical Measurements. Infrared spectra were recorded as KBr disks using a Nicolet 710 FT-IR spectrometer. UV spectra were obtained with a Hewlett-Packard Model 8453 spectrometer. Optical rotations were taken on a Jasco DIP-370 digital polarimeter. FAB mass spectra were measured on a VG-ZAB SE instrument and a VG 11-250 data system. Electrospray mass spectra were measured on a VG Quattro-I triple-quadrupole mass spectrometer. High-resolution mass spectra were recorded on a 9.4-T electrospray FT-ICR mass spectrometer. High-resolution MS/MS of the oligosaccharide was obtained by IRMPD with a 40-W continuous-wave CO_2 laser. NMR spectra were recorded on a Bruker DRX 500 or AMX 300 spectrometer in deuterated solvents. ^1H and ^{13}C chemical shifts were measured in parts per million relative to partially deuterated solvent peaks of DMSO-d_6 at δ 2.49 ppm (^1H) and δ 39.5 ppm (^{13}C) or methanol-d_4 at δ 3.30 ppm (^1H) and δ 49.0 ppm (^{13}C) for ^1H and ^{13}C NMR signals, respectively. $^1H-^1H$ coupling constants were measured from 1D proton spectra, and one bond $^1H-^{13}C$ coupling constants were extracted from 2D coupled HMQC spectra given in hertz. A typical NMR data set measured for a compound included carbon, DEPT, proton, COSY, TOCSY (40 ms mixing time), HMQC, HMBC, NOESY or ROESY, HMQC-TOCSY, HMQC-NOESY, and coupled HMQC spectra. All 2D experiments were run nonspinning.

HPLC Systems. A Hewlett-Packard 1090M LC system with diode array detection employing a Rainin Microsorb-MV (C18) reversed phase column (5 μm, 4.6 × 150 mm) or MetaChem Inertsil ODS-3 (C18) reversed phase column (5 μm, 4.6 × 250 mm) was used for analysis of fractions and degradation products. Preparative HPLC separations were accomplished on a Rainin-Dynamax or Waters-4000 system using Microsorb (C18, 41.4 × 250 mm), Dynamax-60A (C18, 20 × 250 mm), or Inertsil ODS-3 (C18, 10 × 250 mm) column. Fractions from all columns were generally collected by hand according to observed peaks. All solvents used were obtained from J. T. Baker, Inc., and were of the highest commercially available purity.

Antibacterial Evaluation. The in vitro antibacterial effects were determined by standard agar dilution methods against clinical isolates. The inoculum of each culture was approximately (1−5) × 10^4 colony forming units applied with the Steers multiple inocula replicator to plates containing the antibiotic in Mueller-Hinton agar. The in vivo activity was assessed in female mice, strain CD-1, weighing 20 g each, infected intraperitoneally with sufficient bacterial cells suspended in broth to kill 95−100% of untreated mice within 48 h. The antibiotic was administered in a single subcutaneous dose 30 min post-infection. Seven-day survival ratios from three or four separate tests were pooled for the determination of the median effective dose (ED_{50}).

Isolation of Antibiotic LL-C19004 Complex. To the whole mash (about 820 L) of LL-C19004 fermentation was added a 41-kg portion of diatomaceous earth, and the mixture was filtered and the cake washed with 123 L of water. The filtrate and wash were combined (900 L), then passed at a flow rate of 575−625 mL/min through a column containing a 14-L bed volume of Amberlite IRC 50(NH_4^+) ion-exchange resin. The column was next washed with 40 L of water at a flow rate of 515−625 mL/min and was then eluted with 60 L of 2 N ammonium hydroxide, at a flow rate of 575−625 mL/min. The active fractions were concentrated and then freeze-dried to yield 114 g of solid. A 5.0-g portion of the solid was mixed with 25 mL of water, adjusted to pH 7.0 with hydrochloric acid, and placed on a 3 × 45 cm column of CM Sephadex (NH_4^+). Pump pressure was applied to force the solution down the column. The column was eluted first with 250 mL of water and then with a linear gradient of water−1.5 M ammonium hydroxide for 2 h. Pooling of fractions was based on an autoturb assay with *S. aureus* 209P, giving 1.25 g of LL-C19004 complex as a white powder after lyophilization.

Isolation of the Chromophore. A solution of LL-C19004 complex (6 g) in 250 mL of 10% sodium hydroxide in the presence of 4 g of zinc was heated under reflux in a nitrogen atmosphere for 16 h. The hydrolysate was acidified to pH 2.0 with concentrated sulfuric acid and extracted with ether (200 mL × 2). The ether solution was extracted with 5% sodium bicarbonate solution (100 mL × 2). The alkaline solution was acidified to pH 2.0 with 5 N sulfuric acid and the ether extraction repeated. The ether solution was dried over sodium sulfate, benzene added, and the mixture concentrated. A small volume of hexane was added and the organic layer concentrated and dried to give 122 mg of an orange solid. The orange solid (90 mg) was dissolved in

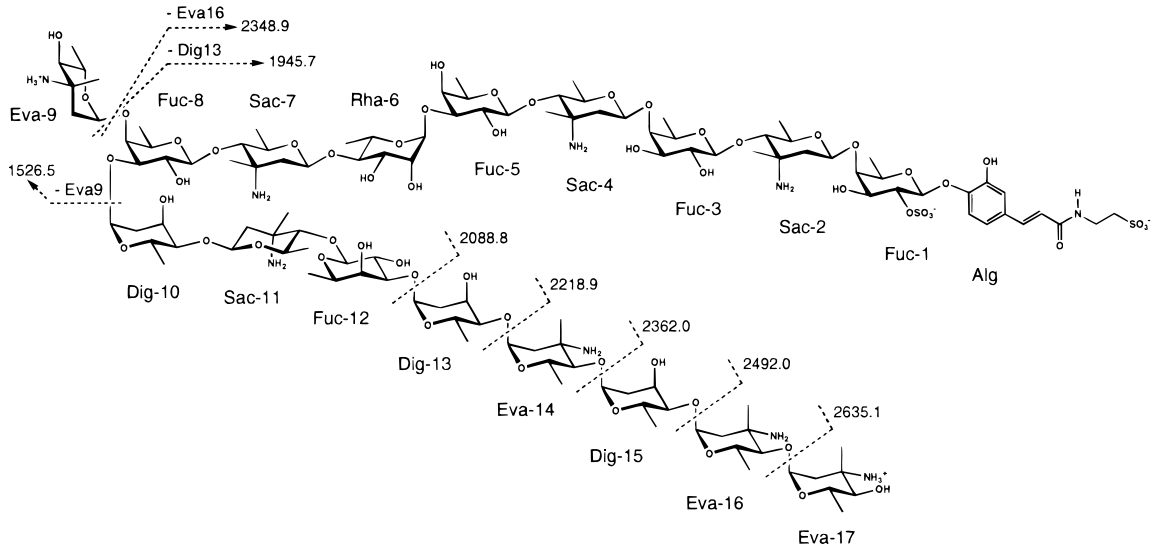

Figure 9. IRMPD-MS/MS fragmentation pattern of saccharomicin B (**2**).

methanol and spotted on silica gel F254 glass-backed plate followed by development in the solvent system benzene/methanol/glacial acetic acid (45:8:4). By direct comparison with an authentic sample of *m,p*-dihydroxycinnamic acid, the LL-C19004 chromophore was located at R_f 0.10. The amount recovered was 12.9 mg.

Methanolysis of N-Acetylated Complex. N-acetylated LL-C19004 complex (4.0 g) was refluxed in dry methanol containing 5 wt % HCl gas for 4 h. After the solution had cooled to room temperature, it was neutralized by batchwise addition of solid $(Ag)_2CO_3$ (15 g) with stirring. The resulting suspension was filtered, and the filtrate was evaporated in vacuo to a black syrup. This syrup was partitioned between water (120 mL) and ethyl acetate (100 mL). Upon evaporation, the ethyl acetate layer yielded 300 mg of residue which was chromatographed on a silica gel column (Woelm TSC 100 g, 2.5 × 35 cm) developed with methanol:methylene chloride (1:40) at 1.5 mL/min to afford methyl α-*N*-acetyl saccharosaminopyranoside (20 mg). The aqueous layer was also evaporated in vacuo to a syrup which was dissolved in pyridine (10 mL) and acetic anhydride (6 mL) and heated at 50 °C for 17 h. After removal of the reagents, the reaction residue was chromatographed repeatedly on silica gel columns using either ethyl acetateexane (1:4) or toluene/ethyl acetate/ethanol (18:6:1) to yield methyl 2,3,4-tri-*O*-acetyl-α-rhamnopyranoside (60 mg), methyl 2,3,4-tri-*O*-acetyl-α-fucopyranoside (200 mg), and methyl α-*N*-acetyl-4-*O*-(2,3,4-tri-*O*-acetyl-*β*-fucopyranosyl)saccharosaminopyranoside (400 mg).

Separation of LL-C19004 Complex into Saccharomicins A (1) and B (2). LL-C19004 complex (31 g) was dissolved in 300 mL of a mixture of water and acetonitrile (2:1). The solution was chromatographed on a reversed phase column (Microsorb, 41.4 × 250 mm) using a step gradient of acetonitrile and 0.1 M ammonium acetate buffer, adjusted to pH 5.3 with TFA, at a flow rate of 80 mL/min. The fractions were analyzed by an HP-1090 HPLC using a Microsorb-MV column (C18, 5m, 4.6 × 150 mm). A linear gradient of (A) aqueous ammonium acetate (0.05 M, pH 5.3) and (B) 7:3 acetonitrile/water (0.05 M, pH 5.3) with 20% B at the start to 40% B over 20 min at 1.0 mL/min was used, and the peaks were detected by UV absorbance at 280 nm. Fractions were pooled according to the analytical HPLC, concentrated under reduced pressure, and then desalted on an LH-20 column (45 × 550 mm, methanol/water = 9:1) and lyophilized to yield saccharomicin A (**1**, 5.0 g), saccharomicin B (**2**, 5.5 g), and the unresolved starting material.

Saccharomicin A (1): white amorphous powder; $[\alpha]_D = -105°$ (*c* 1.0, MeOH); HRMS (FT-ICR) 2794.2914 ($C_{121}H_{207}N_9O_{59}S_2$, calcd 2794.2916, −0.07 ppm); LRESIMS *m/z* 1396.8 ($[M - 2H]^{2-}$, relative intensity 100%), 930.8 ($[M - 3H]^{3-}$, 55%); 1398.5 ($[M + 2H]^{2+}$, 5%), 932.6 ($[M + 3H]^{3+}$, 60%), 699.8 ($[M + 4H]^{4+}$, 100%), 560.2 ($[M + 5H]^{5+}$, 48%); UV (λ_{max}, nm) 218, 237, 287, 316; IR (KBr, cm^{-1}) 3404, 2979, 2937, 1562, 1452, 1406, 1342, 1306, 1273, 1208, 1169, 1132, 1065, 988; ^{1}H NMR (500 MHz, DMSO-d_6, 50 °C) fucose-8: δ 4.26 (d, 7.6 Hz, H1-8), 3.41 (H2-8), 3.56 (H3-8), 3.55 (H4-8), 3.62 (H5-8), 1.12 (H6-8); 4-*epi*-vancosamine-9: 5.02 (H1-9), 1.79 ($H2_{ax}$-9), 2.04 ($H2_{eq}$-9), 3.16 (d, 9.3 Hz, H4-9), 3.74 (H5-9), 1.14 (H6-9), 1.32 (s, H7-9); rhamnose-10: 5.01 (bs, H1-10), 3.72 (H2-10), 3.43 (H3-10), 3.44 (H4-10), 3.44 (H5-10), 1.18 (H6-10); saccharosamine-11: 5.03 (H1-11), 1.29 ($H2_{ax}$-11), 1.80 ($H2_{eq}$-11), 3.02 (d, 10.8 Hz, H4-11), 3.73 (H5-11), 1.27 (H6-11), 1.17 (s, H7-11); the remaining proton resonances, same as for **2** (see Table 1); ^{13}C NMR (125 MHz, DMSO-d_6, 50 °C) fucose-8: δ 102.4 (C1-8), 70.5 (C2-8), 77.9 (C3-8), 78.3 (C4-8), 68.8 (C5-8), 16.0 (C6-8); 4-*epi*-vancosamine-9: 97.1 (C1-9), 40.0 (C2-9), 54.0 (C3-9), 75.3 (C4-9), 65.8 (C5-9),17.6 (C6-9), 18.8 (C7-9); rhamnose-10: 100.8 (C1-10), 70.2 (C2-10), 70.2 (C3-10), 78.0 (C4-10), 67.7 (C5-10), 17.6 (C6-10); saccharosamine-11: 98.2 (C1-11), 43.9 (C2-11), 52.0 (C3-11), 84.5 (C4-11), 68.8 (C5-11), 18.0 (C6-11), 30.1 (C7-11); the remaining carbon resonances, same as for **2** (see Table 1).

Saccharomicin B (2): white amorphous powder; $[\alpha]_D = -101°$ (*c* 0.9, MeOH); HRMS (FT-ICR) 2778.29643 ($C_{121}H_{207}N_9O_{58}S_2$, calcd 2778.29641, +0.007 ppm); LRESIMS *m/z* 1388.8 ($[M - 2H]^{2-}$, 100%), 925.5 ($[M - 3H]^{3-}$, 61%); 1390.6 ($[M + 2H]^{2+}$, 4%), 927.3 ($[M + 3H]^{3+}$, 57%), 695.8 ($[M + 4H]^{4+}$, 100%), 557.0 ($[M + 5H]^{5+}$, 49%); UV (λ_{max}, nm) 218, 237, 287, 316; IR (KBr, cm^{-1}) 3404, 3190, 2980, 2937, 1657, 1563, 1452, 1404, 1341, 1273, 1267, 1206, 1169, 1133, 1065, 1012, 987; ^{1}H NMR and ^{13}C NMR data are listed in Table 1.

Preparation of 3 and 4. Saccharomicin B (**2**, 1 g) was dissolved in 100 mL of water containing 20 mL of Dowex 50W-X8 resin (100−200 mesh, H^+ form) and heated at 60 °C for 4 h with stirring under nitrogen. The reaction mixture was filtered, and the Dowex resin was washed with water. The filtrates and wash were combined, concentrated under reduced pressure, and lyophilized to yield a neutral fraction (0.39 g). The neutral fraction was then chromatographed by a preparative HPLC (Microsorb 41.1 × 250 mm, C18 column) using a gradient of methanol/water with 10% at the start to 60% methanol over 60 min at 30.0 mL/min and detection by UV at 285 nm to afford compounds **3** (retention time 14 min, 80 mg) and **4** (36 min., 60 mg) after routine workup.

Monoglycoside 3: colorless glass; LRESIMS *m/z* 512 ($[M - H]^-$, relative intensity 8%, $C_{17}H_{23}NO_{13}S_2$, calcd 513.0611), 432 ($[M - SO_3 - H]^-$, 60%), 286 ([aglycon − H]$^-$, 58%), 255.6 ($[M - 2H]^{2-}$, 100%); ^{1}H NMR (300 MHz, DMSO-d_6, 27 °C) aglycon: δ 2.71 (t, 7.2 Hz, H1), 3.43 (H2), 8.01 (d, 5.4 Hz, amide-H), 6.42 (d, 15.7 Hz, H4), 7.25 (d, 15.7 Hz, H5), 6.99 (bs, H7), 7.05 (d, 8.1 Hz, H10), 6.94 (bd, 8.1 Hz, H11); fucose-1: 4.76 (d, 7.5 Hz, H1-1), 4.30 (t, 8.4 Hz, H2-1), 3.56 (bd, 9.5 Hz, H3-1), 3.52 (bs, H4-1), 3.79 (q, 6.3 Hz, H5-1), 1.20 (d, 6.3 Hz, H6-1); ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C) aglycon: δ 50.8 (C1), 35.6 (C2), 165.6 (C3), 120.6 (C4), 138.7 (C5), 130.1 (C6),

113.9 (C7), 147.4 (C8), 147.0 (C9), 116.3 (C10), 119.9 (C11); fucose-1: 101.4 (C1-1), 76.2 (C2-1), 72.2 (C3-1), 70.3 (C4-1), 70.3 (C5-1), 16.6 (C6-1).

Triglycoside 4: colorless glass; LRESIMS *m/z* 801 ([M − H]$^-$, relative intensity 100%, $C_{30}H_{46}N_2O_{19}S_2$, calcd 802.2136), 721 ([M − SO_3 − H]$^-$, 11%), 286 ([aglycon − H]$^-$, 9%), 400 ([M − 2H]$^{2-}$, 61%); ^{1}H NMR (300 MHz, DMSO-d_6, 27 °C) aglycon: same as for **3**; fucose-1: 4.79 (d, 7.4 Hz, H1-1), 4.24 (t, 8.3 Hz, H2-1), 3.72 (H3-1), 3.75 (H4-1), 3.85 (q, 6.1 Hz, H5-1), 1.19 (d, 6.1 Hz, H6-1); saccharosamine-2: 5.08 (bd, 9.3 Hz, H1-2), 1.68 (bt, 11.8 Hz, $H2_{ax}$-2), 2.25 (bd, 14.1 Hz, $H2_{eq}$-2), 3.24 (d, 9.7 Hz, H4-2), 3.73 (H5-2), 1.31 (d, 5.8 Hz, H6-2), 1.36 (s, H7-2); fucose-3: 4.22 (d, 8 Hz, H1-3), 3.38 (t, 8.3 Hz, H2-3), 3.36 (H3-3), 3.44 (H4-3), 3.54 (q, 6.5 Hz, H5-3), 1.12 (d, 6.3 Hz, H6-3); ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C) aglycon: same as for **3**; fucose-1: 100.9 (C1-1), 76.2 (C2-1), 71.9 (C3-1), 76.6 (C4-1), 69.3 (C5-1), 16.7 (C6-1); saccharosamine-2: 96.9 (C1-2), 40.8 (C2-2), 55.4 (C3-3), 81.2 (C4-2), 68.4 (C5-2), 17.8 (C6-2), 25.0 (C7-2); fucose-3: 103.9 (C1-3), 70.4 (C2-3), 73.9 (C3-3), 70.8 (C4-3), 70.0 (C5-3), 16.3 (C6-3).

Preparation of 5. Saccharomicin B (**2**, 0.1 g) was dissolved in 10 mL of water and 1.1 mL of formic acid with stirring at room temperature. The reaction mixture was monitored by analytical HPLC for 3 days, and no meaningful amount of hexaglycoside **5** was detected. The reaction was accelerated by heating at 80 °C with stirring for 3 h, concentrated to dryness by evaporation under reduced pressure, and chromatographed by reversed phase HPLC (Dynamax-60A, C18, 20 × 250 mm) detected by UV absorbance at 285 nm. Isocratic elution for 40 min with mobile phase consisting of 30% methanol (A) and 70% water with 0.05% TFA (B), followed by gradient elution to 80% methanol (A) in 30 min at 5.5 mL/min yielded hexaglycoside **5** (68 min, 8 mg).

Hexaglcoside 5: colorless glass; LRESIMS *m/z* 1236.5 ([M − H]$^-$, 39%, $C_{49}H_{79}N_3O_{29}S_2$, calcd 1237.4241), 617.9 ([M − 2H]$^{2-}$, 100%); ^{1}H NMR (500 MHz, DMSO-d_6, 27 °C) aglycon: same as for **3**; fucose-1: 4.84 (d, 7.2 Hz, H1-1), 4.24 (H2-1), 3.75 (H3-1), 3.76 (H4-1), 3.87 (q, 6.3 Hz, H5-1), 1.18 (d, 6.2 Hz, H6-1); saccharosamine-2: 5.09 (bd, 9.6 Hz, H1-2), 1.70 (dd, 14.0, 9.6 Hz, $H2_{ax}$-2), 2.29 (bd, 14.0 Hz, $H2_{eq}$-2), 3.27 (d, 9.9 Hz, H4-2), 3.75 (H5-2), 1.33 (d, 6.2 Hz, H6-2), 1.37 (s, H7-2); fucose-3: 4.23 (d, 7.8 Hz, H1-3), 3.32 (t, 8.1 Hz, H2-3), 3.46 (H3-3), 3.64 (H4-3), 3.63 (H5-3), 1.12 (H6-3); saccharosamine-4: 5.01 (bd, 9.7 Hz, H1-4), 1.60 (dd, 15.0, 9.7 Hz, $H2_{ax}$-4), 2.20 (bd, 15.0 Hz, $H2_{eq}$-4), 3.24 (d, 9.8 Hz, H4-4), 3.73 (H5-4), 1.30 (d, 6.0 Hz, H6-4), 1.37 (s, H7-4); fucose-5: 4.28 (d, 7.8 Hz, H1-5), 3.50 (H2-5), 3.40 (H3-5), 3.49 (H4-5), 3.59 (H5-5), 1.13 (H6-5); rhamnose-6: 4.85 (bs, H1-6), 3.75 (H2-6), 3.56 (H3-6), 3.20 (t, 9.5 Hz, H4-6), 3.63 (H5-6), 1.12 (H6-6); ^{13}C NMR (125 MHz, DMSO-d_6, 27 °C) aglycon: same as for **3**; fucose-1: 100.9 (C1-1), 76.4 (C2-1), 72.0 (C3-1), 76.8 (C4-1), 69.3 (C5-1), 16.8 (C6-1); saccharosamine-2: 97.0 (C1-2), 40.8 (C2-2), 55.4 (C3-2), 81.3 (C4-2), 68.8 (C5-2), 18.0 (C6-2), 25.0 (C7-2); fucose-3: 104.0 (C1-3), 70.4 (C2-3), 73.4 (C3-3), 77.9 (C4-3), 69.7 (C5-3), 16.6 (C6-3); saccharosamine-4: 97.0 (C1-4), 40.8 (C2-4), 55.4 (C3-4), 81.5 (C4-4), 68.6 (C5-4), 18.0 (C6-4), 25.0 (C7-4); fucose-5: 103.8 (C1-5), 70.3 (C2-5), 80.3 (C3-5), 70.7 (C4-5), 69.7 (C5-5), 16.3 (C6-5); rhamnose-6: 102.4 (C1-6), 70.6 (C2-6), 70.6 (C3-6), 72.1 (C4-6), 68.8 (C5-6), 18.0 (C6-6).

Preparation of 6−12. To a solution of MeOH (25 mL) and saccharomicin B (**2**, 0.1 g) was added 25 mL of MeOH containing 1% concentrated HCl. The reaction mixture was stirred at room temperature for 1 h and then quenched with NH_4OH/MeOH solution to neutralize. After concentration under reduced pressure, the reaction mixture was loaded onto a reversed phase HPLC column (Inertsil ODS-3, C18, 10 × 250 mm). The column was first eluted isocratically at 3 mL/min with a solvent system of 40% MeOH and 0.1 M NH_4OAc buffer and detected by both RI and UV detectors for 25 min. Compounds eluted in sequence were methyl α-4-*epi*-vancosaminopyranosyl(1→4)-*β*- digitoxopyranoside **10** (3.0 mg), methyl α-4-*epi*-vancosaminopyranosyl-(1→4)-α-digitoxopyranoside (trace), methyl α-4-*epi*-vancosaminopyranosyl(1→4)-α-4-*epi*-vancosaminopyranosyl(1→4)-*β*-digitoxopyranoside **11** (9.0 mg), methyl α-4-*epi*-vancosaminopyranosyl(1→4)-α-4-*epi*-vancosaminopyranosyl(1→4)-α-digitoxopyranoside (1.0 mg), and methyl *β*-fucopyranosyl(1→4)-*β*-saccharosaminopyranosyl(1→4)-*β*-digitoxopyranoside **9** (2.0 mg). The column was then eluted with a step gradient of MeOH and 0.1 M NH_4OAc buffer from 58% MeOH to 73% MeOH over 25 min to yield nonaglycoside **6** (14 mg), a mixture (16 mg) of dodecaglycoside **7** and methyl octasaccharide **12**, tetradecaglycoside **8** (12 mg), and the starting material **2** (∼20 mg). The mixture was rechromatographed on the same column eluting isocratically with a solvent system of 64% MeOH and 36% 0.1 M NH_4OAc buffer at 3 mL/min to give dodecaglycoside **7** (9 mg) and methyl octasaccharide **12** (3 mg).

Nonaglycoside 6: colorless glass; LRESIMS *m/z* 835.7 ([M + 2H]$^{2+}$, 100%, $C_{69}H_{115}N_5O_{37}S_2$, calcd 1669.6712); ^{1}H NMR (500 MHz, DMSO-d_6, 50 °C) aglycon: same as for **2**; fucose-1: 4.80 (H1-1), 4.27 (H2-1), 3.74 (H3-1), 3.75 (H4-1), 3.86 (H5-1), 1.22 (Hz, H6-1); saccharosamine-2: 5.10 (H1-2), 1.62 ($H2_{ax}$-2), 2.20 ($H2_{eq}$-2), 3.21 (H4-2), 3.76 (H5-2), 1.31 (H6-2), 1.32 (s, H7-2); fucose-3: 4.22 (H1-3), 3.31 (H2-3), 3.44 (H3-3), 3.61 (H4-3), 3.60 (H5-3), 1.13 (H6-3); saccharosamine-4: 5.03 (H1-4), 1.47 ($H2_{ax}$-4), 2.01 ($H2_{eq}$-4), 3.12 (H4-4), 3.73 (H5-4), 1.28 (H6-4), 1.26 (H7-4); fucose-5: 4.24 (H1-5), 3.46 (H2-5), 3.38 (H3-5), 3.46 (H4-5), 3.55 (H5-5), 1.12 (H6-5); rhamnose-6: 4.86 (bs, H1-6), 3.77 (H2-6), 3.71 (H3-6), 3.43 (H4-6), 3.66 (H5-6), 1.13 (H6-6); saccharosamine-7: 5.06 (H1-7), 1.37 ($H2_{ax}$-7), 1.93 ($H2_{eq}$-7), 3.11 (H4-7), 3.75 (H5-7), 1.28 (H6-7), 1.15 (H7-7); fucose-8: 4.24 (H1-8), 3.29 (H2-8), 3.46 (H3-8), 3.53 (H4-8), 3.61 (H5-8), 1.12 (H6-8); 4-*epi*-vancosamine-9: 5.18 (bd, 4.5 Hz, H1-9), 1.82 ($H2_{ax}$-9), 2.13 ($H2_{eq}$-9), 3.17 (H4-9), 3.75 (H5-9), 1.13 (H6-9), 1.38 (H7-9); ^{13}C NMR (125 MHz, DMSO-d_6, 50 °C) aglycon: same as for **2**; fucose-1: 100.9 (C1-1), 76.0 (C2-1), 72.0 (C3-1), 76.3 (C4-1), 69.3 (C5-1), 16.5 (C6-1); saccharosamine-2: 96.9 (C1-2), 41.7 (C2-2), 54.3 (C3-2), 82.1 (C4-2), 68.4 (C5-2), 17.5 (C6-2), 26.0 (C7-2); fucose-3: 103.6 (C1-3), 70.5 (C2-3), 73.8 (C3-3), 77.3 (C4-3), 68.9 (C5-3), 16.6 (C6-3); saccharosamine-4: 97.8 (C1-4), 42.4 (C2-4), 53.3 (C3-4), 82.8 (C4-4), 68.5 (C5-4), 18.0 (C6-4), 27.7 (C7-4); fucose-5: 103.8 (C1-5), 70.2 (C2-5), 80.1 (C3-5), 69.8 (C4-5), 69.7 (C5-5), 16.3 (C6-5); rhamnose-6: 101.9 (C1-6), 70.5 (C2-6), 70.6 (C3-6), 78.6 (C4-6), 66.8 (C5-6), 17.5 (C6-6); saccharosamine-7: 97.8 (C1-7), 43.2 (C2-7), 52.5 (C3-7), 82.3 (C4-7), 68.6 (C5-7), 17.7 (C6-7), 30.4 (C7-7); fucose-8: 102.8 (C1-8), 70.9 (C2-8), 73.5 (C3-8), 78.4 (C4-8), 69.4 (C5-8), 16.0 (C6-8); vancosamine-9: 96.8 (C1-9), 40.0 (C2-9), 54.8 (C3-9), 74.3 (C4-9), 65.4 (C5-9), 17.8 (C6-9), 18.1 (C7-9).

Dodecaglycoside 7: colorless glass; LRESIMS *m/z* 1043.5 ([M − 2H]$^{2-}$, 100%, $C_{88}H_{148}N_6O_{46}S_2$, calcd 2088.8868); 1045.2 ([M + 2H]$^{2+}$, 55%), 697.3 ([M + 3H]$^{3+}$, 100%); ^{1}H NMR (500 MHz, DMSO-d_6, 50 °C) aglycon and the rest sugar residues: same as for **6**; fucose-8: 4.27 (H1-8), 3.39 (H2-8), 3.52 (H3-8), 3.53 (H4-8), 3.62 (H5-8), 1.12 (H6-8); 4-*epi*-vancosamine-9: 5.38 (bs, H1-9), 1.78 ($H2_{ax}$-9), 2.08 ($H2_{eq}$-9), 3.14 (H4-9), 3.76 (H5-9), 1.14 (H6-9), 1.36 (H7-9); digitoxose-10: 5.04 (H1-10), 1.78 ($H2_{ax}$-10), 1.92 ($H2_{eq}$-10), 3.97 (H3-10), 3.31 (H4-10), 3.96 (H5-10), 1.11 (H6-10); saccharosamine-11: 4.91 (H1-11), 1.40 ($H2_{ax}$-11), 1.85 ($H2_{eq}$-11), 3.03 (H4-11), 3.74 (H5-11), 1.27 (H6-11), 1.17 (H7-11); fucose-12: 4.17 (H1-12), 3.27 (H2-12), 3.30 (H3-12), 3.52 (H4-12), 3.50 (H5-12), 1.12 (H6-12); ^{13}C NMR (125 MHz, DMSO-d_6, 50 °C), aglycon and the rest sugar residues, same as for **6**; fucose-8: 102.3 (C1-8), 70.9 (C2-8), 77.5 (C3-8), 77.6 (C4-8), 69.4 (C5-8), 16.0 (C6-8); vancosamine-9: 96.4 (C1-9), 39.4 (C2-9), 54.8 (C3-9), 74.6 (C4-9), 65.2 (C5-9), 17.6 (C6-9), 18.2 (C7-9); digitoxose-10: 96.3 (C1-10), 34.2 (C2-10), 62.1 (C3-10), 77.1 (C4-10), 62.8 (C5-10), 17.3 (C6-10); saccharosamine-11: 94.5 (C1-11), 43.6 (C2-11), 53.2 (C3-11), 84.0 (C4-11), 68.7 (C5-11), 17.9 (C6-11), 29.7 (C7-11); fucose-12: 103.1 (C1-12), 70.5 (C2-12), 73.5 (C3-11), 69.6 (C4-12), 69.4 (C5-12), 16.1 (C6-12).

Tetradecaglycoside 8: colorless glass; LRESIMS *m/z* 1182.0 ([M + 2H]$^{2+}$, 23%, $C_{101}H_{171}N_7O_{51}S_2$, calcd 2362.0444), 788.2 ([M + 3H]$^{3+}$, 100%), 591.7 ([M + 4H]$^{4+}$, 87%); ^{1}H NMR (500 MHz, DMSO-d_6, 27 °C) and ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C), recorded but no 2D NMR characterization.

Compound 9: colorless glass; LRESIMS *m/z* 452.3 ([M + H]$^+$, 100%, $C_{20}H_{37}NO_{10}$, calcd 451.2418), 903.1 ([2M + H]$^+$, 23%); ^{1}H NMR (300 MHz, DMSO-d_6, 27 °C) digitoxose-10: 4.62 (dd, 9.3, 2.2 Hz, H1-10), 1.48 ($H2_{ax}$-10), 1.85 ($H2_{eq}$-10), 4.05 (H3-10), 4.48 (OH), 3.25 (H4-10), 3.72 (H5-10), 1.12 (H6-10), 3.30 (s, OCH_3); saccharosamine-11: 4.91 (d, 9.1 Hz, H1-11), 1.43 ($H2_{ax}$-11), 1.88 ($H2_{eq}$-11),

3.07 (d, 9.5 Hz, H4-11), 3.74 (H5-11), 1.28 (d, 6.3 Hz, H6-11), 1.20 (s, H7-11); fucose-12: 4.18 (H1-12), 3.29 (H2-12), 3.30 (H3-12), 3.41 (bs, H4-12), 4.29 (OH), 3.48 (bq, 6.3 Hz, H5-12), 1.11 (d, 6.3 Hz, H6-12); ^{13}C NMR (100 MHz, DMSO-d_6, 27 °C) digitoxose-10: 97.5 (C1-10), 37.0 (C2-10), 62.4 (C3-10), 76.7 (C4-10), 66.9 (C5-10), 17.8 (C6-10), 54.8 (OCH_3); saccharosamine-11: 93.5 (C1-11), 43.0 (C2-11), 53.2 (C3-11), 82.9 (C4-11), 68.1 (C5-11), 17.8 (C6-11), 28.5 (C7-11); fucose-12: 103.0 (C1-12), 70.0 (C2-12), 73.1 (C3-12), 70.2 (C4-12), 69.1 (C5-12), 16.0 (C6-12).

Compound 10: colorless glass; LRESIMS *m*/*z* 306.2 ($[M + H]^+$, 100%, $C_{14}H_{27}NO_6$, calcd 305.1838); ^{1}H NMR (300 MHz, DMSO-d_6, 27 °C) digitoxose-13: 4.61 (bd, 9.1 Hz, H1-13), 1.44 (dt, 2.3, 11.3 Hz, $H2_{ax}$-13), 1.85 (bd, 13.7 Hz, $H2_{eq}$-13), 4.12 (bs, H3-13), 3.11 (dd, 2.0, 9.1 Hz, H4-13), 3.76 (dq, 9.1, 6.3 Hz, H5-13), 1.14 (d, 6.3 Hz, H6-13), 3.30 (s, OCH_3); 4-*epi*-vancosamine-14: 4.92 (bs, H1-14), 1.64 ($H2_{ax}$-14), 1.85 ($H2_{eq}$-14), 2.98 (bd, 8.7 Hz, H4-14), 3.49 (m, H5-14), 1.11 (H6-14), 1.13 (H7-14); ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C) digitoxose-13: 97.9 (C1-13), 37.4 (C2-13), 60.7 (C3-13), 75.0 (C4-13), 67.2 (C5-13), 18.7 (C6-13), 55.1 (OCH_3); 4-*epi*-vancosamine-14: 91.4 (C1-14), 40.0 (C2-14), 52.1 (C3-14), 77.4 (C4-14), 65.8 (C5-14), 18.2 (C6-14), 21.0 (C7-14).

Compound 11: colorless glass; LRESIMS *m*/*z* 449.2 ($[M + H]^+$, 100%, $C_{21}H_{40}N_2O_8$, calcd 448.2785), 897.3 ($[2M + H]^+$, 25%); ^{1}H NMR (300 MHz, DMSO-d_6, 27 °C) digitoxose-15: 4.61 (dd, 1.9, 9.3 Hz, H1-15), 1.44 (dt, 2.4, 10.8 Hz, $H2_{ax}$-15), 1.84 ($H2_{eq}$-15), 4.11 (d, 2.7 Hz, H3-15), 3.10 (dd, 2.7, 9.5 Hz, H4-15), 3.75 (dq, 9.0, 6.1 Hz, H5-15), 1.15 (d, 6.2 Hz, H6-15), 3.30 (s, OCH_3); 4-*epi*-vancosamine-16: 4.87 (d, 4.1 Hz, H1-16), 1.62 (dd, 4.1, 14.0 Hz, $H2_{ax}$-16), 1.76 (d, 14.0 Hz, $H2_{eq}$-16), 3.01 (d, 9.3 Hz, H4-16), 3.52 (m, H5-16), 1.15 (d, 6.2 Hz, H6-16), 1.07 (s, H7-16); 4-*epi*-vancosamine-17: 5.17 (d, 4.0 Hz, H1-17), 1.67 (dd, 4.0, 13.4 Hz, $H2_{ax}$-17), 1.99 (d, 13.4 Hz, $H2_{eq}$-17), 3.03 (d, 9.5 Hz, H4-17), 3.57 (m, H5-17), 1.11 (d, 6.1 Hz, H6-17), 1.18 (s, H7-17); ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C), digitoxose-15: 98.6 (C1-15), 37.7 (C2-15), 61.1 (C3-15), 75.5 (C4-15), 67.6 (C5-15), 18.7 (C6-15), 55.5 (OCH_3); 4-*epi*-vancosamine-16: 92.0 (C1-16), 44.1 (C2-16), 51.8 (C3-16), 86.9 (C4-16), 65.4 (C5-16), 18.5 (C6-16), 22.5 (C7-16); 4-*epi*-vancosamine-17: 99.0 (C1-17), 39.5 (C2-17), 52.5 (C3-17), 77.3 (C4-17), 66.4 (C5-17), 18.2 (C6-17), 20.6 (C7-17).

Compound 12: colorless glass; LRESIMS *m*/*z* 141.5 ($[M + H]^+$, 53%, $C_{53}H_{96}N_4O_{22}$, calcd 1140.6516), 571.5 ($[M + 2H]^{2+}$, 100%); ^{1}H NMR (500 MHz, DMSO-d_6, 47 °C) digitoxose-10: 4.61 (dd, 1.9, 9.1 Hz, H1-10), 1.48 (ddd, 2.7, 9.6, 12.8 Hz, $H2_{ax}$-10), 1.84 ($H2_{eq}$-10), 4.04 (H3-10), 3.25 (dd, 2.9, 9.1 Hz, H4-10), 3.71 (m, H5-10), 1.13 (H6-10), 3.30 (s, OCH_3); saccharosamine-11: 4.89 (dd, 1.1, 9.4 Hz, H1-11), 1.32 (dd, 9.5, 13.1 Hz, $H2_{ax}$-11), 1.74 ($H2_{eq}$-11), 2.97 (d, 9.6 Hz, H4-11), 3.74 (m, H5-11), 1.26 (d, 6.3 Hz, H6-11), 1.12 (s, H7-11); fucose-12: 4.20 (d, 7.0 Hz, H1-12), 3.41 (H2-12), 3.38 (H3-12), 3.49 (bs, H4-12), 4.48 (OH), 3.53 (bq, 6.5 Hz, H5-12), 1.12 (H6-12); digitoxose-13: 5.04 (d, 3.8 Hz, H1-13), 1.76 ($H2_{ax}$-13), 2.04 ($H2_{eq}$-13), 4.03 (H3-13), 3.17 (d, 9.4 Hz, H4-13), 4.10 (m, H5-13), 1.15 (H6-13); 4-*epi*-vancosamine-14: 4.94 (d, 3.3 Hz, H1-14), 1.69 ($H2_{ax}$-14), 1.73 ($H2_{eq}$-14), 3.03 (d, 9.4 Hz, H4-14), 3.57 (m, H5-14), 1.17 (H6-14), 1.13 (H7-14); digitoxose-15: 5.13 (t, 3.7 Hz, H1-15), 1.75 ($H2_{ax}$-15), 2.03 ($H2_{eq}$-15), 3.99 (m, H3-15), 3.19 (dd, 2.8, 9.0 Hz, H4-15), 4.06 (m, H5-15), 1.13 (H6-15); 4-*epi*-vancosamine-16: 4.91 (d, 4.4 Hz, H1-16), 1.61 (dd, 4.5, 14.0 Hz, $H2_{ax}$-16), 1.74 (m, $H2_{eq}$-16), 2.99 (d, 9.6 Hz, H4-16), 3.59 (m, H5-16), 1.16 (H6-16), 1.12 (s, H7-16); 4-*epi*-vancosamine-17: 5.15 (d, 4.0 Hz, H1-17), 1.57 ($H2_{ax}$-17), 1.86 ($H2_{eq}$-17), 2.89 (d, 9.5 Hz, H4-17), 3.57 (m, H5-17), 1.11 (H6-17), 1.12 (s, H7-17); ^{13}C NMR (from 2D HMQC and HMBC, 500 MHz, DMSO-d_6, 47 °C), digitoxose-10: 98.5 (C1-10), 37.6 (C2-10), 63.0 (C3-10), 77.1 (C4-10), 67.9 (C5-10), 17.8 (C6-10), 55.5 (OCH_3); saccharosamine-11: 94.7 (C1-11), 44.9 (C2-11), 51.4 (C3-11), 85.3 (C4-11), 69.1 (C5-11), 18.2 (C6-11), 31.3 (C7-11); fucose-12: 103.8 (C1-12), 70.2 (C2-12), 80.5 (C3-12), 70.5 (C4-12), 69.7 (C5-12), 16.5 (C6-12); digitoxose-13: 98.4 (C1-13), 34.8 (C2-13), 61.7 (C3−13), 75.7 (C4-13), 62.3 (C5-13), 18.1 (C6-13); 4-*epi*-vancosamine-14: 91.9 (C1-14), 44.9 (C2-14), 52.1 (C3-14), 85.1 (C4-14), 65.5 (C5-14), 18.6 (C6-14), 22.7 (C7-14); digitoxose-15: 98.0 (C1-15), 34.8 (C2-15), 61.7 (C3-15), 76.4 (C4-15), 64.2 (C5-15), 18.1 (C6-15); 4-*epi*-vancosamine-16: 92.6 (C1-16), 44.2 (C2-16), 52.4 (C3-16), 87.1 (C4-16), 65.5 (C5-16), 18.2 (C6-16), 22.7 (C7-16); 4-*epi*-vancosamine-17: 99.6 (C1-17), 42.8 (C2-17), 53.0 (C3-17), 79.3 (C4-17), 66.7 (C5-17), 18.2 (C6-17), 22.2 (C7-17).

Dodecaglycoside 13: colorless glass; LRESIMS *m*/*z* 1053.7 ($[M + 2H]^{2+}$, 100%, $C_{88}H_{148}N_6O_{47}S_2$, calcd 2104.8817); ^{1}H NMR (500 MHz, DMSO-d_6, 50 °C) partially characterized, 4-*epi*-vancosamine-9: 5.03 (H1-9); rhamnose-10: 5.02 (H1-10), 3.42 (H4-10), 3.43 (H5-10); saccharosamine-11: 5.02 (H1-11); fucose-12: 4.17 (H1-12), 3.27 (H2-12), 3.30 (H3-12), 3.52 (H4-12), 3.50 (H5-12), 1.12 (H6-12); ^{13}C NMR (125 MHz, DMSO-d_6, 50 °C) partially characterized, vancosamine-9: 96.9 (C1-9); rhamnose-10: 100.8 (C1-10), 78.3 (C4-10), 67.5 (C5-10); saccharosamine-11: 98.2 (C1-11); fucose-12: 103.4 (C1-12), 70.5 (C2-12), 73.5 (C3-12), 69.6 (C4-12), 69.4 (C5-12), 16.1 (C6-12).

Tetradecaglycoside 14: colorless glass; LRESIMS *m*/*z* 1190.4 ($[M + 2H]^{2+}$, 100%, $C_{101}H_{171}N_7O_{52}S_2$, calcd 2378.0393), 794.0 ($[M + 3H]^{3+}$, 62%); 1188.0 ($[M - 2H]^{2-}$, 100%), 792.2 ($[M - 3H]^{3-}$, 66%); ^{1}H NMR (500 MHz, DMSO-d_6, 50 °C) aglycon and the rest sugar residues: same as for **1**; fucose-8: 4.26 (H1-8); 4-*epi*-vancosamine-9: 5.02 (H1-9); rhamnose-10: 5.01 (H1-10), 3.42 (H4-10), 3.42 (H5-10), 1.11 (H6-10); saccharosamine-11: 5.03 (H1-11); fucose-12: 4.20 (H1-12); digitoxose-13: 5.04 (H1-13); 4-*epi*-vancosamine-14: 5.03 (H1-9), 1.8 ($H2_{ax}$-14), 2.0 ($H2_{eq}$-14), 3.12 (H4-14), 3.74 (H5-14), 1.14 (H6-14); ^{13}C NMR (125 MHz, DMSO-d_6, 50 °C) aglycon and the rest sugar residues: same as for **1**; fucose-8: 102.5 (C1-8); vancosamine-9: 97.2 (C1-9); rhamnose-10: 100.8 (C1-10), 70.2 (C2-10), 70.4 (C3-10), 78.3 (C4-10), 67.5 (C5-10), 17.6 (C6-10); saccharosamine-11: 98.2 (C1-11); fucose-12: 103.4 (C1-12); digitoxose-13: 98.0 (C1-13); 4-*epi*-vancosamine-14: 91.2 (C1-14), 39.4 (C2-14), 53.8 (C3-14), 75.6 (C4-14), 65.6 (C5-14), 17.8 (C6-14), 19.4 (C7-14).

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Supporting Information Available: Relevant spectra for 1, 2, 6, and 12 (28 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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