Emmyguyacins A and B: Unusual Glycolipids from a Sterile Fungus Species That Inhibit the Low-pH Conformational Change of Hemagglutinin A during Replication of Influenza Virus

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Two novel glycolipids, emmyguyacin A (**1a**) and emmyguyacin B (**1b**), were isolated at concentrations of 1.51 g/L from a potato dextrose agar fermentation of a sterile fungus species. The compounds inhibit replication of influenza A virus (A/X31) in MDCK cells by inhibiting the pH-dependent conformational change of hemagglutinin A (IC_{50} 9 μM). The structures were deduced using one- and two-dimensional NMR techniques and mass spectrometric analyses on both the parent compounds and a host of degradation products and derivatives. A novel and unusual oxalic acid ester of a monohydroxylated fatty acid (**5**, 17-oxalyloxydocosanoic acid) is reported. The first isolation and characterization of the fatty acid 17-hydroxydocosanoic acid (**3**) itself is also reported as a saponification product of **1**.

Influenza continues to significantly impact public health. Annually, 20,000 deaths and 100,000 hospitalizations are attributed to flu in the United States alone.[1a] Airborne transmission,[1a] facile viral mutation,[1a] vaccine shortages,[1b] and actual and perceived side effects and limitations of both vaccines[1c] and prophylactic drugs[1d] contribute to the drive for new therapies and preventative medicines for influenza.

Research during the last fifteen years has elucidated many of the mechanisms by which the influenza virus invades, captures, and mobilizes the replication capabilities of a host cell, providing new targets in the search for antiviral treatments.[2a−c] Influenza virus belongs to the Orthomyxoviradae family and consists of a core of ribonucleoprotein segments surrounded by a matrix protein layer all neatly packaged in a lipid bilayer. This lipid "envelope" incorporates three viral proteins: hemagglutinin (HA), neuraminidase (NA), and either the M2 protein (type A) or the analogous NB (type B).[2d]

HA, which along with NA protrudes as spikes from the virion surface, plays a central role in viral infection of a host cell. HA undergoes a proteolytic cleavage which generates two subunit chains (HA1 and HA2) linked by disulfide bonds and conformationally constrained by ionic and hydrogen bonding interactions such that HA2 is hidden within the folded protein.[2d] Viral adsorption to a cell surface is accomplished via HA1 binding to the sialic acid terminus common to many cell surface glycoproteins. Endocytosis follows, the virion gains entry to the cell, and it must effect its escape before being destroyed by the host cell lysosomes. Obligingly, the pH of the suicidal endosome falls, triggering not only M2-mediated release of the viral RNP[2b,d] from the matrix protein but also the fusion of the viral and endosomal membranes.[2d] As the endosomal pH decreases, noncovalent bonds destabilize, allowing an energetically favorable, irreversible conformational change which results in the hydrophobic HA2 subunit being thrust into the endosomal membranes. This fusion allows the release of viral genetic material into host cell cytosol, whence it enters the nucleus and replicates.[2d] Several antiviral strategies targeting HA have been proposed.[2a−d] We were interested in identifying compounds that would inhibit the conformational change of HA at low pH,[2e,f] thus preventing the fusion of viral and endosomal membranes and the resulting release of viral RNP.

Results and Discussion

Mechanism-based in vitro high-throughput assay[3] of 80,000 fungal extracts identified 23 extracts that inhibited the pH-induced conformational change of HA. These were subsequently subjected to a cell-based assay utilizing MDCK cells infected with influenza virus (A/X31).[4] The crude MeOH extract (CR) of fungus OSI 55538 significantly inhibited viral replication (IC_{50} <4.5 μg/mL). This extract was partitioned between hexanes (K1), $CHCl_3$ (K2), and aqueous MeOH (K3). While some antiviral activity was detected in both K1 and K2 (IC_{50} 120 and 90 μg/mL, respectively), the aqueous MeOH fraction contained the bulk of the activity (IC_{50} <4.5 μg/mL). This suggested a polar compound, in contrast to its strong lipophilic retention on octadecylsilylated silica gel (ODS). This latter characteristic allowed the use of ODS in the solid-phase extraction mode, retaining the active component while eluting the polar inactive constituents with water. Subsequent elution of the ODS with MeOH provided material (fraction C3) which was chromatographed by ODS HPLC (H_2O−MeOH) to give a clean mixture of two closely related glycolipids (apparent 3:1 ratio), only partially separable by HPLC. Sequential HPLC separations allowed the isolation of the major component emmyguyacin A (**1a**) in ∼95% purity (1.51 g/L).

Gross Structure Determination. ^{1}H NMR spectroscopy of the major component **1a** (Table 1) indicated the presence of at least two sugars attached to a fatty chain via an ester linkage. ^{13}C NMR spectroscopy (Table 1) showed two anomeric methines (δ 94.60 and 94.98), nine oxygen-bearing methines, two oxygen-bearing methylenes, three carbonyl resonances (δ 175.5, 166.1, and 165.2), one alkyl methyl, and numerous overlapping alkyl methylenes. Thus, at least two sugars and three esters or acids were

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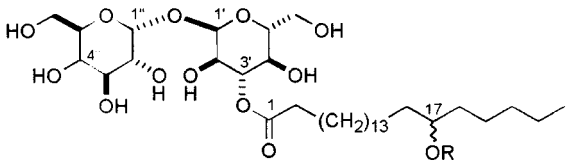
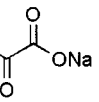
Table 1. Proton (400 MHz) and Carbon (100 MHz) NMR Data for Emmyguyacin A (**1a**) (CD_3OD, 25 °C)

atom	^{13}C (δ)[a]	mult	^{1}H (δ)[a]	int	mult	J_{H-H} (Hz)	$^{1}H-^{13}C$ HMBC correlations (δ)
1	175.50	s					
2	35.33	t	2.40	2H	t	7.5	175.5, 30.75, 26.15
3	26.15	t	1.66	2H	m		175.5, 35.33, 30.75
4	30.75	t	1.36	2H	m		
5−15	*b*	t	1.25−1.31		br		
16	35.26[c]	t	1.63[c]	2H	m		
17	76.39	d	4.90	1H	m		166.1, 35.26
18	35.19[c]	t	1.58[c]	2H	m		
19	*b*	t	1.25−1.31		br		
20	*b*	t	1.25−1.31		br		
21	32.95	t	1.31	2H	m		
22	14.5	q	0.89	3H	br t	6.9	23.67, 32.95
glu							
1′	94.60	d	5.17	1H	d	3.7	94.98, 76.68, 73.56
2′	71.32	d	3.62	1H	dd	10.1, 3.7	76.68
3′	76.68	d	5.28	1H	dd	9.8, 9.6	175.5, 71.32, 69.72
4′	69.72	d	3.48	1H	t	9.6	76.68, 73.56, 62.15
5′	73.56	d	3.86	1H	m		69.72
6′	62.15	t	3.72	1H	dd	11.4, 7.0	69.72
			3.66	1H	dd	obsc, 4.9	73.56
gal							
1″	94.98	d	5.16	1H	d	3.9	94.60, 70.72, 72.43
2″	69.76	d	3.85	1H	dd	9.8, 3.8	70.72
3″	70.72	d	3.98	1H	dd	10.0, 3.2	69.76
4″	71.16	d	3.90	1H	d	2.6	70.72
5″	72.43	d	4.04	1H	br t	6	94.98, 71.16, 62.80
6″	62.80	t	3.79	1H	dd	12.0, 2.3	72.43
			3.69	1H	dd	obsc, 5.1	71.16
oxal							
1‴	166.1	s					
2‴	165.2	br s					

[a] Referenced to residual solvent peaks: proton, 3.30 ppm; carbon, 49.0 ppm. [b] Unassigned carbon resonances (δ) for the aliphatic chain: 23.61, 23.67, 26.08, 26.46, 30.19, 30.48, 30.59, 30.62 (×2), 30.68, 30.73 (multiple overlapping). Broad proton resonance at 1.25−1.31 ppm integrated for ~30H. [c] Assignments may be interchanged.



1a β-OH (C-4"), R= 

1b α-OH (C-4"), R=(CO)(CO)ONa

2 β-OH (C-4"), R=H

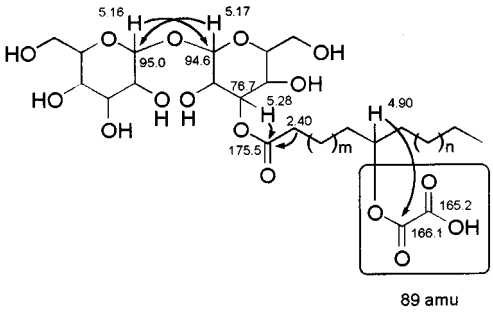


Figure 1. Key HMBC correlations for **1**.

present in the molecule. Two-dimensional $^{1}H-^{1}H$ COSY, $^{1}H-^{1}H$ TOCSY, $^{1}H-^{13}C$ HMQC, and $^{1}H-^{13}C$ HMBC NMR experiments allowed the assignment of the proton and carbon shifts (although not all the multiplicities or coupling constants) for the disaccharide. Multibond correlations between the anomeric protons and carbons revealed a C1−O1−C1′ ether-linked disaccharide (Figure 1).

Similarly, the fatty acid was found to be attached via an ester linkage to the C3 oxygen of one of the sugars. Although the fatty acid itself was also determined to bear an esterified hydroxyl functionality, neither its exact position nor the exact length of the aliphatic chain could be determined by NMR. Furthermore, only one set of protons *alpha* to a carbonyl (δ 175.5) could be identified (δ 2.40); a second carbonyl (δ 166.1) showed proton correlations only through its ester oxygen, and the third carbonyl (δ 165.2) manifested no correlations at all (Figure 1). To complete the structure, it was necessary not only to locate the position of the isolated carbonyl (δ 165.2) but also to determine the identities of the disaccharide (including absolute stereochemistries), the fatty acid (including the total length of the aliphatic chain, the relative lengths of the linker (m) and tail (n) regions, and the stereochemistry at the ester-bearing carbon), and the substituent on the carbonyl side of the fatty acid ester (δ 166.1).

Mass Spectrometry of the Parent Compound. The isolated parent material was subjected to APCI, ES, and FABMS, and the results are summarized in Table 2. The discrepancy between the apparent molecular weights in the negative vs positive ionization modes was initially puzzling. The negative modes suggested a molecular weight (MW) of 752 amu, while the positive modes suggested either a MW of 774 with $[M + Na]^+$ of 797 or a MW of 752 with $[M + Na]^+$ of 775 and $[M - H + 2Na]^+$ of 797. The problem was solved by the introduction of lithium into the probe. Observation of ions with *m/z* 781 $[774 + Li]^+$ and 797 $[774 + Na]^+$ suggested that the isolated parent compound actually contained a sodium ion—*m/z* 775 was not attributable solely to formation of a mass spectrometry adduct. In the negative mode, this ionically bound sodium was stripped away, and the de-sodiated parent anion was detected at *m/z* 751.

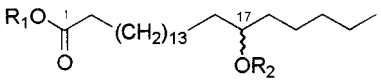
Table 2. Mass Spectrometric Analysis of Emmyguyacin A (**1a**)

mode (matrix)	obsvd *m/z*	ion[a]
APCI (−)	751	$[M - H]^-/[M^{Na} - Na]^-$
ES (−)	751	$[M - H]^-/[M^{Na} - Na]^-$
FAB (+)	775[b]	$[M + Na]^+/[M^{Na} + H]^+$
(glycerol)	797	$[M^{Na} + Na]^+$
FAB (+)		
(*m*NBA)	797	$[M^{Na} + Na]^+$
FAB (+)	781	$[M^{Na} + Li]^+$
(*m*NBA + Li)	797	$[M^{Na} + Na]^+$

[a] M and M^{Na} represent the parent compound as the free acid and the sodium salt, respectively. [b] Denotes high-resolution peak matching.

This was strong evidence for a carboxylate ion in the molecule and suggested an assignment for the uncorrelated carbonyl (δ 165.2) in the ^{13}C NMR spectrum. Further evidence for this functionality was found in the IR spectrum of the parent in which two carbonyl absorbances were observed at 1718 (ester) and 1651 cm^{-1}, the latter characteristic of a carboxylate stretch. Thus, the free acid would have a molecular weight of 752, and the isolated sodium salt a molecular weight of 774. High-resolution mass spectrometry of the *m/z* 775 $[M^{Na} + H]^+$ ion gave a molecular formula of $C_{36}H_{64}O_{16}Na$. With the molecular weight of the parent compound in hand, subtraction of the disaccharide and fatty acid weights would allow molecular weight determination for the "unaffiliated" ester on the fatty acid chain.

Identification of the Fatty Acid. The parent glycolipid mixture (fraction C3) was saponified with 1 M NaOH in MeOH, the latter to facilitate dissolution. Upon complete conversion of starting material, the reaction was acidified to pH ~3.5 and extracted exhaustively with $CHCl_3$. Both the aqueous and organic portions were concentrated in vacuo to yield a beige foam and a white filmy powder, respectively.



3 R_1=H, R_2=H
4 R_1=CH_3, R_2=H
5 R_1=H, R_2=(CO)(CO)OH

The $CHCl_3$ extract yielded a mixture of the fatty acid and its methyl ester (**3** and **4**). Treatment of this mixture with diazomethane produced pure methyl ester (**4**). Of note was the significant shielding of the hydroxyl-bearing methinic proton, which shifted from δ 4.90 in the parent compound (**1**) to δ 3.60 in **4**. This suggested that hydrolysis of the unknown side-chain ester had occurred under the saponification conditions, thereby allowing for the identification of the core fatty acid (**3**).

Mass spectrometry analysis of the fatty acid/methyl ester mixture prior to treatment with diazomethane is shown in Table S1 (Supporting Information). The parent molecular weights of 356 amu (free acid) and 370 amu (methyl ester) suggested a 22-carbon chain. This was confirmed by high-resolution peak matching (FAB) of the $[M^{ME} + H]^+$ and $[M^{ME} + Na]^+$ ions, which gave formulas of $C_{23}H_{47}O_3$ and $C_{23}H_{46}O_3Na$, respectively.

Analysis of EIMS fragmentation patterns of **4** in conjunction with high-resolution daughter-ion MS allowed the placement of the hydroxyl group on the sixth carbon from the tail of the fatty acid chain (Figure 2). Ions at *m/z* 299 and 270 resulted from cleavage of the C-17/C-18 and C-16/C-17 bonds, respectively. Further supporting evidence was

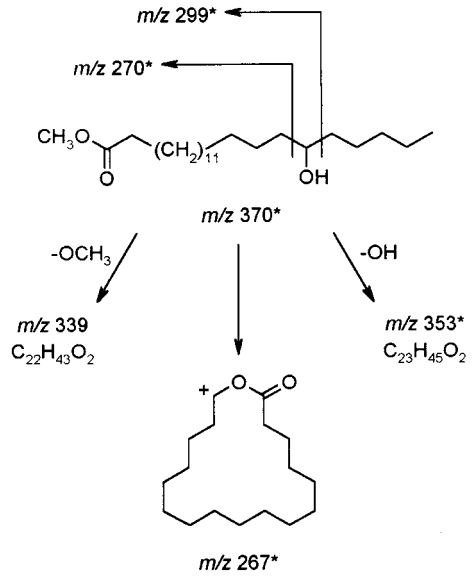


Figure 2. Electron impact mass spectrometry fragmentation of methyl 17-hydroxydocosanoate (**4**). Ions marked by an asterisk were confirmed by high-resolution peak matching.

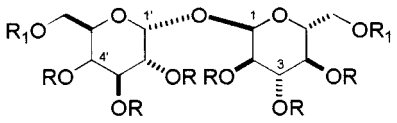
discerned from a prominent peak at *m/z* 267, resulting from a β-scission-like expulsion of pentane $[C_5H_{11}]^+$ and subsequent ring closure of the hydroxyl onto the methyl ester carbonyl to form the 18-membered lactone shown in Figure 2.

Thus, the fatty acid moiety was identified as 17-hydroxydocosanoic acid (**3**). This compound has never before been isolated, being reported only as a trace component of platelet lipids (2 picomoles/10^9 platelets) and detected by LC-EIMS.[5a,b] The stereochemistry of the hydroxyl group could not be definitively determined. Optical rotation of the methyl ester **4** in $CHCl_3$ was zero. This did not necessarily indicate a racemic mixture, however, as the stereocenter was quite distal from the absorbing chromophore. Preparation of the *R* and *S* Mosher esters also did not provide unequivocal evidence for the stereochemistry. Treatment of **3** separately with the (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) chlorides provided two products, which were indistinguishable by proton (^1H), carbon (^{13}C), and fluorine (^{19}F) NMR spectroscopy. The ^1H and ^{19}F NMR spectra of the two Mosher products appeared to represent single, pure compounds. The ^{13}C NMR spectra, however, exhibited many additional resonances, several of which were attributed to doubling. For example, in the parent alcohol **4**, the shifts of C-16 and C-18 occurred at δ 37.60 and 37.56. In both Mosher products, these were shielded and appeared doubled at δ 33.87, 33.82, 33.60, and 33.56. This suggested the presence of diastereomers and thus pointed toward a mixture of alcohol epimers. Circular dichroism curves of the two Mosher esters were virtually identical—but with opposite signs—deviating slightly only in the region below 220 nm^{-1}. This accumulation of data was consistent with the assignment of **1** as a mixture of C-17 epimers in undefined proportions.

Identification of the Disaccharides. One- and two-dimensional NMR analysis of the aqueous fraction from the alkaline hydrolysis of fraction C3 revealed a mixture of two sugars,[6] the major component of which was a 1−1′ ether-linked unsymmetrical hexose disaccharide (APCI (−), *m/z* 341 $[M - H]^-$). A fully coupled ^{13}C NMR spectrum of the unacylated disaccharide provided proton−carbon coupling constants for the anomeric carbons (J_{CH} = 171 Hz) and thus fixed the C1−O1−C1′ linkage as axial−axial ($J_{CHaxial} \approx 165-170$ Hz; $J_{CHequatorial} \approx 158-162$ Hz).[7]

Overlapping resonances, complicated by signals from the minor component, necessitated derivatization.

Treatment of a portion of the disaccharide mixture with *o*-toluic acid chloride (2.2 equiv) followed by HPLC purification yielded a clean sample of the major disaccharide acylated on the primary C-6 hydroxyls only (**7**), clarifying the sugar region by deshielding the four methylene resonances by 0.5−0.8 ppm. Comparison of the coupling constants of **7** with those of **1a** fixed the attachment of the fatty acid on the C-3 hydroxyl of an α-glucopyranose.



6 R=R$_1$=H, β-OH (C-4')
7 R=H, β-OH (C-4'), R$_1$=*o*-toluyl
8 R=R$_1$=Ac, β-OH (C-4')
9 R=R$_1$=H, α-OH (C-4')
10 R=R$_1$=Ac, α-OH (C-4')

The remaining sugar unit was tentatively identified as α-galactose on the basis of analysis of its coupling constants ($J_{H1'eq-H2'ax}$ = 3.9 Hz, $J_{H2'ax-H3'ax}$ = 9.4 Hz, $J_{H3'ax-H4'eq}$ = 3.4 Hz). Insignificant couplings among the remaining protons suggested a toluyl-induced ring distortion, and heating the sample to 40 °C gave a clean set of resonances, allowing the final assignments. Exhaustive acetylation of the sugar mixture from the alkaline hydrolysis followed by HPLC purification yielded as its major component 2,3,4,6-*O*-tetraacetyl,1-*O*-(2,3,4,6-*O*-tetraacetyl-α-D-glucopyranosyl)-α-D-galactopyranoside (**8**), which compared favorably with literature by proton NMR[8] and optical rotation.[8]

A minor peracetate (**10**), corresponding to the sugar portion of the minor component in the parent glycolipid mixture, was also isolated. Though apparently a monohexose,[6] strong HMBC correlations between the anomeric proton and its carbon revealed a symmetrical disaccharide, and FABMS (positive mode) in the presence of lithium gave a molecular ion of 685 amu $[C_{28}H_{38}O_{19} + Li]^+$. Coupling constant analysis in the proton NMR spectrum allowed the assignment of 2,3,4,6-*O*-tetraacetyl,1-*O*-(2,3,4,6-*O*-tetraacetyl-α-D-glucopyranosyl)-α-D-glucopyranoside (**10**, peracetylated α,α-trehalose). Standard α,α-trehalose obtained from SIGMA was identical by ^{13}C NMR to the minor component in the free sugar mixture.[6] Thus, the two major components of the parent glycolipid mixture appeared to differ only in the composition of disaccharides and were produced in ca. 6:1 ratio of the glucosylgalactose (**1a**) to the glucosylglucose (**1b**) compounds.

Identifying the Remainder of the Molecule. When the molecular weights of the disaccharide ($C_{12}H_{21}O_{11}$, 341 amu) and the fatty acid backbone ($C_{22}H_{42}O$, 322 amu) fragments were subtracted from that of the parent compound (752 amu), a fragment of 89 amu remained to be assigned. The known ester on the fatty acid chain (−OC═O) accounted for 44 amu, and the unassigned carbonyl (C═O) consumed another 28 amu, leaving a total of 17 amu to be assigned as a hydroxyl. Thus, an oxalate fragment was proposed (Figure 1), explaining both the ester HMBC correlation and the "isolated" carbonyl (δ 165.2) observed in the ^{13}C NMR spectrum and further providing a site for the ionically bound sodium in the parent compound.

Once the presence of the oxalic acid moiety was suspected, attempts were made to "trap" it in order to directly confirm its presence. The failure of a variety of methods to prepare the methyl oxalate of both **1a** and its peracetate may be attributed to the absence of an acidic proton, further supporting the inference from mass spectrometry data that the compound occurs naturally as the sodium salt.

Treatment of the parent mixture with K_2CO_3 and MeOH gave a complex mixture of hydrolysis and transesterification products. Acid hydrolysis, on the other hand, yielded, in addition to several transesterification products, two compounds, the structures of which were determined by ^{1}H NMR spectroscopy to be the free fatty acid bearing only a hydroxyl (**3**) and the free fatty acid bearing an oxalyl ester (**5**). The ^{1}H NMR spectra (CD_3OD) of the two compounds were quite similar, but **3** manifested a one-proton resonance typical for a hydroxylated methine (δ 3.50), while in the spectrum of **5**, the corresponding methine was deshielded to δ 4.90, suggesting esterification of the hydroxyl. Additionally, the four protons vicinal to H-17 in **5** were deshielded by ca. 0.2 ppm relative to those in **3**. No other proton resonances attributable to the alkyl component of an ester (−OCO*R*) were observed. Furthermore, while both compounds **3** and **5** exhibited in the infrared spectrum a typical carboxylic acid carbonyl stretch (1739 cm^{-1}), the esterified compound (**5**) further displayed a carbonyl absorbance at 1662 cm^{-1}, consistent with a carboxylate functionality.

On the basis of the above evidence, the structures of the two active components from culture OSI 55538 PDa were proposed as **1a** (emmyguyacin A) and **1b** (emmyguyacin B). Several 1−1-linked disaccharides esterified with fatty acids are known, but the vast majority are at least diacylated. None contain **3** or **5** as an ester. Furthermore, to the best of our knowledge, a fatty acid esterified with oxalic acid has never been reported. The high quantity in which this material is produced is noteworthy—a clean ca. 6:1 mixture of **1a** and **1b** has been isolated at concentrations of 1.51 g/L.

Biological Activity of the Emmyguyacins. A 95:5 mixture of emmyguyacins A (**1a**) and B (**1b**) inhibited the replication of influenza virus (A/X31) in confluent MDCK cells at concentrations of ca. 9 μM. The same mixture showed no toxicity in confluent cells (determined by XTT cell viability assay) at more than 4 times that concentration (CC_{50} >40 μM). A similar sample manifested toxicity in replicating MDCK cells (CC_{50} 15 μM). Both influenza replication and dividing cells cultured in the presence of FCS were unaffected by emmyguyacins A and B. This indicated that the biological activity of the mixture was abrogated by serum proteins. Studies to probe the serum-protein binding properties of the emmyguyacins and to confirm the inhibition of the hemagglutinin A conformational change in more detail will be published separately.

To selectively remove and thus assess the role of the oxalyl group in the toxicity observed in replicating MDCK cells, an aqueous ethanol solution of **1a** was treated with sodium cyanoborohydride in ammonium acetate buffer (0 °C). Subsequent purification by HPLC (ACN−H_2O gradient) yielded clean desoxylate **2**. There appeared to be no significant difference in the activities of **1** and **2** with respect to either viral inhibition or toxicity.

Experimental Section

General Experimental Procedures. Optical rotations were obtained in the appropriate solvent on a Jasco DIP-1000 polarimeter. IR spectra were obtained on a Jasco 420 FTIR spectrophotometer. Low- and high-resolution FAB and EI mass spectrometric measurements were made on a modified Finnegan MAT-212 high-resolution double-focusing spectrometer (70

eV, 3 keV acceleration voltage, direct introduction probe using best sample temperature). APCI and ES mass spectra were obtained on a Hewlett-Packard 1050 LC system (ODS columns from various suppliers, typically 3 μm apd, 4 mm diameter × 5 cm length, H_2O−ACN elution, 0.5 mL/min flow rate) coupled to a Fissons VG Platform quadrapole operating under MassLynx (ver. 2.2). Proton, carbon, and fluorine NMR spectra were obtained on a Varian Mercury 400 MHz system using 5 mm Varian inverse-detection pulse field gradient or broad band probes tuned to the nucleus of interest. Spectra were referenced to residual solvent resonances: $CDCl_3$, δ_H 7.27, δ_C 77.0 ppm; D_2O, δ_H 4.71 ppm; CD_3OD, δ_H 3.31, δ_C 49.0 ppm. ^{19}F NMR shifts are reported in ppm relative to an external TFA standard.

Organism. OSI 55538 was isolated on May 26, 1991, from leaf litter debris lodged between rocks in an intermittent stream channel. The site was on a nature trail at Chimney Picnic Ground, Route 441, Great Smoky Mountains National Park, TN, in an old growth mesic forest dominated by *Aesculus octandra, Acer saccharum, Betula lenta,* and hemlock. A voucher specimen of OSI 55538 is preserved at Mycosynthetix, Inc. (Durham, NC). OSI 55538 was a sterile culture grown on five media: malt extract agar (10 g malt extract, 18 g agar, 1 L distilled H_2O), potato dextrose agar (39 g potato dextrose agar, 1 L distilled H_2O), yeast extract peptone dextrose agar (10 g yeast extract, 20 g glucose, 18 g agar, 20 g meat peptone, 1 L distilled H_2O), Czapek's agar plus dextrose (3 g $NaNO_3$, 1 g K_2HPO_4, 500 mg $MgSO_4 \cdot 7H_2O$, 500 mg KCl, 10 mg $FeSO_4 \cdot 7H_2O$, 30 g sucrose, 20 g glucose, 28 g agar, 1 L distilled H_2O), and cornmeal/tomato paste agar (20 g corn meal, 20 g tomato paste, 10 g yeast extract, 18 g agar, 1 L distilled H_2O). Growth diameters of colonies after 14 days averaged 6, 6, 7, 5, and 7 mm, respectively. All colonies were white, minutely tomentose, but those on potato dextrose agar and yeast extract peptone dextrose agar also had regions of warm sepia[9] hyphae near colony centers. All cultures were composed of septate branching hyphae. Hyphae were linear, occasionally septate, and ranged from 0.8 to 2.5 μm in diameter, except for the pigmented hyphae, which were considerably more septate and were up to 3.0 μm in diameter.

Fermentation. A detailed description of the fermentation conditions is provided in the Supporting Information. Basically, the fungal strain OSI 55538 was grown on a malt agar slant and stored at room temperature until transfer to YESD seed broth. The culture was grown and scaled up once over two weeks before being homogenized and used to inoculate 3 L of potato dextrose agar (Becton Dickinson 211550) in 500 mL Nunc plates. The plates were incubated at 22 °C for 11 days, at which time they were frozen at −80 °C, lyophilized, and extracted overnight with MeOH. The MeOH extract was filtered to remove solids and then evaporated in vacuo to yield a dark brown sticky residue.

Isolation of Emmyguyacins. The concentrated MeOH extract from 3 L of fermentation of fungal strain OSI 55538 was dissolved in distilled H_2O (150 mL) and MeOH (150 mL). The resulting solution was extracted with hexanes (2 × 250 mL) and subsequently with $CHCl_3$ (2 × 250 mL). The concentrated aqueous MeOH fraction was dissolved in H_2O (50 mL) and chromatographed over ODS silica gel (90 g), eluting sequentially with H_2O (600 mL), 20% MeOH−80% H_2O (500 mL), and MeOH (500 mL). The MeOH fraction was concentrated to dryness to yield 4.53 g of a beige amorphous powder (fraction C3). Five percent of this material (~225 mg) was chromatographed by HPLC (Rainin Dynamax Microsorb ODS column 25.4 mm diameter × 25.0 cm length with 25.4 mm diameter × 5.0 cm length guard cartridge, H_2O−MeOH gradient, UV detection at $\lambda = 225$ nm). An apparent (^1H NMR spectroscopy) 3:1 mixture of two closely related compounds (~200 mg) was obtained. This was iteratively chromatographed, combining leading and tailing fractions until ~100 mg of material enriched in **1a** (>95%) was obtained. A fraction containing ~20 mg of material slightly enriched (~70%) in **1b** was also obtained.

Emmyguyacin A (1-*O*-(3-*O*-(1-oxo-17-oxalyloxydocosyl)-α-D-glucopyranosyl)-α-D-galactopyranoside) (1a): white amorphous powder isolated as sodium salt; IR (MeOH) ν_{max} 3366, 2919, 2849, 1718, 1651, 1235, 1080, 1044, 1002 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; MS, see Table 2; HRFABMS *m/z* 775.40918 (calcd for $C_{36}H_{64}O_{16}Na$, 775.40918).

Emmyguyacin B (1-*O*-(3-*O*-(1-oxo-17-oxalyloxydocosyl)-α-D-glucopyranosyl)-α-D-glucopyranoside) (1b): beige amorphous powder inseparable from **1a**; identified through degradation studies.

Saponification of Emmyguyacin (1a,b) Mixture. Fraction C3 (250 mg) was treated with MeOH (1 mL) and 1 M NaOH (1 mL) for 15 min at 55 °C. TLC analysis (silica gel, 5:2.5:0.1 $CHCl_3$−MeOH−AcOH) indicated the absence of starting material, the reaction solution was divided into halves, and the MeOH was removed in vacuo. One-half was partitioned between aqueous acid (HCl, 10 mL, pH 3.5) and $CHCl_3$ (3 × 10 mL), the latter yielding a mixture of the free fatty acid (**3**) and the fatty acid methyl ester (**4**) as a white solid (fraction A; MS data, see Table S1). The aqueous portion was filtered and concentrated to a beige residue (fraction D), which appeared by ^1H NMR to be a mixture of sugars, at least one of which was a disaccharide. Alternatively, treatment of fraction C3 (520 mg) with 1 M NaOH in the absence of MeOH provided both a hygroscopic beige foam (identical by ^1H NMR to fraction D) and a clean sample of **3** (165.8 mg) without concomitant formation of **4**. However, due to solubility problems, the reaction formed a sludge, requiring multiple acid/base/organic solvent manipulations and the use of ion exchange columns for isolation of **3**.

Methylation of Fatty Acid. Treatment of **3** (90 mg) with diazomethane in Et_2O gave **4** as a waxy white film (66 mg).

Methyl 17-hydroxydocosanoate (4): waxy white film; $[\alpha]^{25}_D$ 0° (*c* 0.96, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.68 (3H, s, COO*CH*$_3$), 3.60 (1H, br m, H-17), 2.41 (2H, t, $J = 7.6$ Hz, H-2), 1.63 (2H, m, H-3), 1.50−1.37 (6H, br), 1.37−1.24 (24H, br), 0.90 (3H, t, $J = 6.8$ Hz, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.1 (s, C-1), 72.0 (d, C-17), 51.5 (q, COO*CH*$_3$), 37.60 (t, C-16*), 37.56 (t, C-18*), 34.2 (t, C-2), 32.0 (t, C-19), 29.8 (t, C-4), 29.76 (3C, t), 29.75 (2C, t), 29.73 (t), 29.70 (t), 29.6 (t), 29.4 (t), 29.3 (t), 25.8 (t), 25.5 (t), 25.1 (t, C-3), 22.8 (t, C-21), 14.23/14.20 (q, C-22); MS, see Table S1 (Supporting Information); HRFABMS (*m*NBA) *m/z* 393.33456 (calcd for $C_{23}H_{46}O_3Na$, 393.33457), *m/z* 371.35247 (calcd for $C_{23}H_{47}O_3$, 371.35247).

Acidic Hydrolysis of Emmyguyacin (1a,b) Mixture. A 190 mg portion of fraction C3 was dissolved in MeOH (1 mL) and 1 M HCl (1 mL) and heated to 55 °C. TLC analysis (silica gel, 5:2.5:0.1 $CHCl_3$−MeOH−AcOH, phosphomolybdic acid/Δ) indicated total conversion of starting material after 4.5 h, and the MeOH was removed in vacuo. The reaction mixture was diluted with H_2O, extracted with $CHCl_3$, and eluted through a Bakerbond NH/NH_2 solid-phase extraction column (500 mg) to yield a solution of pH 6.5, which was concentrated to a sticky residue (49 mg). Trituration with CD_3OD provided a complex mixture (fraction E-M, 13 mg) of what appeared by ^1H NMR to be unreacted starting material and transesterification products. Fraction E-M was chromatographed by HPLC (Zorbax ODS column 10.0 mm diameter × 25.0 cm length, H_2O−MeOH gradient, UV detection at $\lambda = 215$) to provide several fractions comprising diacylated emmyguyacins, probably resulting from acid-catalyzed transesterification. Additionally, two compounds eluting at 11 and 21 min were identified as **5** and **3**, respectively.

17-Oxalyloxydocosanoic acid (5): white film; IR ($CHCl_3$) ν_{max} 2924, 2853, 1739, 1662, 1591, 1464, 1376, 1232 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz, $T = 40$ °C) δ 4.90 (1H, m, H-17), 2.31 (2H, t, $J = 8$ Hz, H-2), 1.61 (8H, br m), 1.5−1.25 (28H), 0.9 (3H, t, $J = 7$ Hz, H-22).

17-Hydroxydocosanoic acid (3): white film; IR ($CHCl_3$) ν_{max} 2916, 2848, 1739, 1463 cm^{-1}; ^1H NMR (CD_3OD, 400 MHz, $T = 40$ °C) δ 3.50 (1H, br, H-17), 2.30 (2H, t, $J = 8$ Hz, H-2), 1.60 (2H, br m), 1.49−1.39 (8H, br), 1.39−1.21 (26H, br), 0.92 (3H, t, $J = 7$ Hz, H-22); APCI-MS (−) *m/z* 355 $[M]^+$, HRFABMS (+) *m/z* 356.36312 (calcd for $C_{22}H_{44}O_3$, 356.36314).

Preparation of Mosher Derivatives of 4. Typically, 31.3 mg (0.086 mmol) of alcohol was dissolved in dry pyridine (500 μL) and $CHCl_3$ (220 μL), to which was added either (*R*)- or (*S*)-MTPA-Cl (2 equiv). After stirring at RT for 20 h, the reactions were concentrated in vacuo to oils, diluted with $CHCl_3$ (4 mL), quenched with H_2O (0.5 mL), and extracted with 10% $NaHCO_3$ (3 × 1 mL). The organic was washed with 0.5 M HCl until the wash was acidic (3 × 1 mL), washed with H_2O (2 × 1 mL), and dried over Na_2SO_4 before being concentrated to dryness in vacuo.

Methyl 17-((*R*)-methoxytrifluoromethylphenylacetoxy)-docosanoate: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.55 (2H, m, H-5′), 7.40 (3H, m, H-4′, H-6′), 5.10 (1H, m, H-17), 3.66 (3H, s), 3.57 (3H, s), 2.30 (2H, t, J = 8 Hz, H-2), 1.7−1.45 (6H, m), 1.38−1.0 (30H), 0.9/0.85 (3H, overlapping t, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 174.0 (s, C-1), 166.1 (s, C-1′), 132.4 (s, C-3′), 129.3 (d, C-6′), 128.1 (d, 2 × C-5′), 127.2 (d, 2 × C-4′), 123.3 (q, CF_3, J_{C-F} = 286 Hz), 77.6 (d, C-17), 55.4 (q, OC′H_3), 51.4 (q, OCH_3), 34.2, 33.9, 33.8, 33.60, 33.56, 31.77, 31.69, 31.62, 29.75 (multiple C), 29.72, 29.70, 29.62, 29.59, 29.58, 29.55, 29.53, 29.46, 29.36, 29.3, 25.4, 25.3, 25.1, 25.00, 24.97, 24.6, 22.64, 22.60, 22.55, 14.15, 14.06, 14.03; ^{19}F NMR ($CDCl_3$, 373 Hz) δ 4.7 (CF_3).

Methyl 17-((*S*)-methoxytrifluoromethylphenylacetoxy)-docosanoate: ^{1}H, ^{13}C, and ^{19}F NMR spectra indistinguishable from those of the *R*-Mosher ester.

Toluylation of Disaccharide from Saponification of Emmyguyacin Mixture. The disaccharide mixture (fraction D, 36.8 mg) isolated from saponification of fraction C3 was dissolved in pyridine (1 mL). *o*-Toluyl chloride (30 μL) was added by 5 μL drops at RT to the stirred pyridine solution. After 8.5 h, the pyridine was removed under vacuum, and the resulting residue was suspended in H_2O (3 mL) and extracted with $CHCl_3$ (2 × 3 mL). The $CHCl_3$ solution was washed with H_2O (1 × 3 mL). Both the aqueous and organic portions were concentrated in vacuo and chromatographed by HPLC (ODS, H_2O−MeOH gradient, UV detection at λ = 254 nm). Four peaks from the aqueous portion were cursorily identified as mono- and diacylated disaccharides; none of the latter were acylated solely on the C-6 primary hydroxyls. Two peaks from the organic portion were identified as a triacylated disaccharide, and the disaccharide was acylated on both C-6 primary hydroxyls (**7**), the latter of which was characterized by 1D and 2D NMR spectroscopy.

1-*O*-(6-*O*-*o*-Toluyl-α-D-glucopyranosyl)-6-*O*-*o*-toluyl-α-D-galactopyranoside (7): ^{1}H NMR (CD_3OD, 400 MHz, T = 25 °C) δ 7.93 (2H, dd, J = 7.8, 1.2 Hz, tol-H6), 7.42 (1H, br dd, J = 7.5, 1.4 Hz, tol-H3), 7.39 (1H, br dd, J = 7.5, 1.4 Hz, tol-H3), 7.28 (2H, br m, tol-H4), 7.24 (2H, br m, tol-H5), 5.17 (1H, d, J = 3.8 Hz, gal-H1), 5.13 (1H, d, J = 3.9 Hz, glu-H1), 4.57 (1H, dd, J = 12.0, 5.1 Hz, glu-H6), 4.42 (1H, obsc, gal-H5), 4.42 (2H, obsc ABX, gal-H6), 4.40 (1H, dd, J = 12.0, 2.1 Hz, glu-H6), 4.16 (1H, ddd, J = 9.8, 5.1, 2.1 Hz, glu-H5), 3.98 (1H, br s, gal-H4), 3.97 (1H, dd, J = 8.7, 3.4 Hz, gal-H3), 3.89 (1H, m, gal-H2), 3.81 (1H, dd, J = 9.8, 9.0 Hz, glu-H3), 3.46 (1H, dd, J = 9.8, 3.9 Hz, glu-H2), 3.42 (1H, dd, J = 9.8, 9.0 Hz, glu-H4), 2.56 (6H, s, tol-CH_3); ^{1}H NMR (CD_3OD, 400 MHz, T = 40 °C) δ 7.90 (2H, d, J = 7.9 Hz, tol-H6), 7.42 (1H, d, J = 7.3 Hz, tol-H3), 7.38 (1H, d, J = 6.8 Hz, tol-H3), 7.30−7.20 (2H, br m, tol-H4), 7.30−7.20 (2H, br m, tol-H5), 5.17 (1H, d, J = 3.8 Hz, gal-H1), 5.13 (1H, d, J = 3.8 Hz, glu-H1), 4.57 (1H, dd, J = 11.9, 2.1 Hz, glu-H6), 4.4 (1H, obsc, gal-H5), 4.43 (2H, br s, gal-H6), 4.41 (1H, dd, J = 12.3, 5.7 Hz, glu-H6), 4.16 (1H, ddd, J = 9.9, 5.1, 1.9 Hz, glu-H5), 3.98 (1H, br s, gal-H4), 3.97 (1H, obsc dd, gal-H3), 3.88 (1H, dd, J = 9.4, 3.8 Hz, gal-H2), 3.80 (1H, t, J = 9.4 Hz, glu-H3), 3.46 (1H, dd, J = 9.7, 3.8 Hz, glu-H2), 3.41 (1H, t, J = 9.0 Hz, glu-H4), 2.58 (6H, s, tol-CH_3); ^{13}C NMR (CD_3OD, 100 MHz, T = 25 °C) δ 169.2 (s, tol-C7), 141.2 (s, tol-C2), 133.1 (d, tol-C3), 132.5 (d, tol-C4), 131.5 (d, tol-C6), 131.0 (s, tol-C1), 126.7 (d, tol-C5), 95.4 (d, gal-C1), 95.1 (d, glu-C1), 74.7 (d, glu-C3), 73.1 (d, glu-C2), 72.1 (d, glu-C4), 71.4 (d, glu-C5), 71.0 (d, gal-C4), 70.9 (d, gal-C3), 70.2 (d, gal-C5), 69.7 (d, gal-C2), 65.1 (t, gal-C6), 64.9 (t, glu-C6), 21.5 (q, tol-CH_3).

Acetylation of Disaccharide Mixture and Separation of Disaccharide Peracetates. The disaccharide mixture (fraction D, 56.2 mg) isolated from saponification of fraction C3 was dissolved in pyridine (1 mL, dried over KOH), and acetic anhydride (1.0 mL) was added. The solution was stirred at RT for 3 days, at which time the reaction was concentrated in vacuo to dryness. The resulting residue was partitioned between H_2O (2 mL) and $CHCl_3$ (2 × 2 mL). The organic portion was concentrated to a beige foam (32.2 mg) and chromatographed by HPLC (Zorbax ODS column 10.0 mm diameter × 25.0 cm length, H_2O−MeOH gradient, UV detection at λ = 215 nm). Compounds **10** (f21) and **8** (f23, 6.9 mg) were isolated in ca. 1:4 ratio.

2,3,4,6-*O*-Tetraacetyl, 1-*O*-(2,3,4,6-*O*-tetraacetyl-α-D-glucopyranosyl)-α-D-glucopyranoside (10): white film; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.51 (1H, t, J = 10.3 Hz, H-3), 5.30 (1H, d, J = 3.9 Hz, H-1), 5.06 (1H, dd, J = 10.3, 3.9 Hz, H-2), 5.04 (1H, dd, J = 10.3, 9.8 Hz, H-4), 4.25 (1H, dd, J = 11.7, 5.5 Hz, H-6), 4.06 (1H, ddd, J = 10.3, 5.5, 2.1 Hz, H-5), 4.02 (1H, dd, J = 11.7, 2.1 Hz, H-6), 2.18 (3H, s, OAc), 2.09 (3H, s, OAc), 2.06 (3H, s, OAc), 2.05 (3H, s, OAc); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.5 (s, C═O), 169.9 (s, C═O), 169.50 (s, C═O), 169.49 (s, C═O), 92.2 (d, C-1), 69.94 (d, C-3), 69.85 (d, C-4), 68.5 (d, C-2), 68.2 (d, C-5), 61.7 (t, C-6), 20.7 (q, OAc), 20.64 (q, OAc), 20.63 (q, OAc), 20.60 (q, OAc); FABMS (+, glycerol) m/z 637 $[M - COCH_2]^+$, 617 $[M - 2COCH_2 + Na]^+$, 595 $[M - 2COCH_2]^+$, 575 $[M - 3COCH_2 + Na]^+$, 553 $[M - 3COCH_2]^+$, 511 $[M - 4COCH_2]^+$; FABMS (+, *m*NBA, lithium) m/z 685 $[M + Li]^+$, 643 $[M - COCH_2 + Li]^+$, 601 $[M - 2COCH_2 + Li]^+$, 559 $[M - 3COCH_2 + Li]^+$, 517 $[M - 4COCH_2 + Li]^+$.

2,3,4,6-*O*-Tetraacetyl, 1-*O*-(2,3,4,6-*O*-tetraacetyl-α-D-glucopyranosyl)-α-D-galactopyranoside (8): white film; $[\alpha]^D_{26}$ +141° (*c* 0.69, $CHCl_3$), lit.[8] $[\alpha]^D_{26}$ +196° (*c* 0.25, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 5.53 (1H, br d, J = 3.3 Hz), 5.49 (1H, t, J = 9.7 Hz), 5.37 (1H, dd, J = 11.2, 3.2 Hz), 5.35 (1H, br d, J = 3.6 Hz), 5.31 (1H, t, J = 3.6 Hz), 5.30 (1H, dd, J = 7.4, 3.6 Hz), 5.05 (1H, dd, J = 10.6, 3.8 Hz), 5.04 (1H, t, J = 9.3 Hz), 4.28 (1H, t, J = 6.7 Hz), 4.23 (1H, dd, J = 12.1, 5.9 Hz), 4.11 (1H, dd, J = 11.3, 7.2 Hz), 4.08−4.01 (3H, overlapping), 2.16 (3H, s, OAc), 2.12 (3H, s, OAc), 2.10 (3H, s, OAc), 2.09 (3H, s, OAc), 2.05 (3H, s, OAc), 2.04 (3H, s, OAc), 2.03 (3H, s, OAc), 2.02 (3H, s, OAc), comparable to literature values.[8]

Desoxalylation of 1a. A saturated, buffered solution (1 N NH_4OAc, pH 7, 2 mL) of sodium cyanoborohydride was added dropwise to a solution of **1a** (100 mg in 80% H_2O−EtOH, 5 mL) at 0 °C. After 2 h, the solution was passed through an ODS sep pac column and washed with a H_2O−MeOH step gradient. The material eluting in the 85% MeOH fraction was further purified by HPLC (H_2O−ACN gradient) to yield pure **2** (9.5 mg, 10% yield).

1-*O*-(3-*O*-(1-Oxo-17-hydroxydocosyl)-α-D-glucopyranosyl)-α-D-galactopyranoside (2): UV (MeOH) λ_{max} (log ϵ) 224 (3.48); ^{1}H NMR (CD_3OD, 400 MHz) δ 5.28 (1H, dd, J = 9.5, 9.5 Hz, H-3′) 5.17 (1H, d, J = 4 Hz, H-2′), 5.16 (1H, d, J = 4 Hz, H-1″), 4.04 (1H, m, H-5″), 3.98 (1H, dd, J = 10, 3 Hz, H-3″), 3.90 (1H, m, H-4″), 3.86 (1H, m, H-5′), 3.85 (1H, m, H-2″), 3.79 (1H, dd, H-6″a), 3.72 (1H, dd, H-6′a), 3.69 (1H, m, H-6″b), 3.66 (1H, m, H-6′b), 3.62 (1H, m, H-2′), 3.50 (1H, m, H-14), 3.48 (1H, dd, J = 9, 9 Hz, H-4′), 2.41 (2H, t, J = 7.5 Hz, H-2), 1.62 (2H, m, H-3), 1.50 (2H, m, H-15), 1.40 (2H, m, H-18), 1.32 (2H, m, H-21), 1.31−1.28, (20H, br), 1.30 (2H, m, H-16), 1.28 (2H, m, H-19), 1.28 (2H, m, H-20), 1.20 (2H, m, H-4), 0.90 (3H, t, J = 7 Hz, H-22); ^{13}C NMR (CD_3OD, 100 MHz) δ 175.85 (s, C-1), 95.43 (d, C-1′), 95.39 (d, C-1″), 76.69 (d, C-3′), 73.82 (d, C-5′), 73.43 (d, C-5″), 72.72 (d, C-4″), 72.61 (d, C-17), 71.68 (d, C-3″), 71.43 (d, C-10), 70.07, (d, C-2″), 70.01 (d, C-4′), 63.07 (t, C-6″), 62.39 (t, C-6′), 38.56 (t, C-18), 35.47 (t, C-16), 33.29 (C-16), 30.98 (t, C-20), 30.78 (t, C-19), 30.9−30.7 (10C), 26.64 (t, C-21), 26.23 (t, C-3), 23.87 (t, C-23), 14.60 (q, C-24); ESIMS m/z 681 $[M + H]^+$.

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Supporting Information Available: Detailed fermentation conditions for OSI 55538 and table of MS data for **3** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(3) The general procedure for the high-throughput screening assay for inhibition of HA follows: Virus (X31) was coated on the bottom of 96-well plates and exposed to the crude MeOH extracts of fungal fermentations. Subsequently, the pH was lowered to 5 by addition of acetic acid to induce the irreversible conformational change of the HA. Following incubation and washing, the wells were treated with a primary monoclonal antibody (LC89) which specifically bound the conformationally altered HA protein. Treatment with a secondary anti-mouse antibody conjugated with horseradish peroxidase (HRP) followed by addition of HRP substrate and colorimetric detection at 405 and 490 nm was used to determine the degree of antibody binding. Decreased transmission indicated inhibition of the pH-induced conformational change of viral HA.

(4) The general procedure for the cell-based assay for inhibition of influenza replication follows: MDCK cells were plated in monolayers in 96-well plates and when confluent were infected with influenza virus (A/X31) in serum-free culture medium supplemented with 1 μg/mL trypsin. Dilutions of the test extracts were added in serum-free medium, and the plates incubated at 37 °C for 16 h. After removal of the medium, the cells were fixed with glutaraldehyde and incubated with IgG monoclonal antibody specific for X31 HA. The wells were washed and incubated with protein A conjugated with horseradish peroxidase (HRP). Addition of HRP substrate followed by colorimetric detection allowed the determination of the degree of viral suppression.

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(6) NMR data[a] for the disaccharide mixture from saponification of fraction C3: ^{1}H NMR (D_2O, 400 MHz) δ 5.14 (1H, d, J = 4.0 Hz), 5.13 (1H, d, J = 4.1 Hz), 5.11[b] (d, J = 4 Hz), 3.99 (1H, t, J = 6.1 Hz), 3.96−3.91 (2H, overlapping), 3.82 (1H, dd, J = 10.5, 4.1 Hz), 3.79 (1H, br), 3.77 (2H, br s), 3.76−3.71 (2H, br), 3.68 (1H, dd, J = 11.4, 5.3), 3.67 (1H, br d, J = 5.3), 3.57 (1H, dd, J = 10.1, 3.8), 3.37 (1H, t, J = 9.2 Hz); ^{13}C NMR (D_2O, 100 MHz) δ 173.5[c] (s), 94.2 (d), 94.14[b] (d), 94.06 (d), 73.49 (d), 73.48[b] (d), 73.12[b] (d), 73.08 (d), 72.3 (d), 72.04 (d), 72.01[b] (d), 70.66 (d), 70.66[b] (d), 70.2 (d), 69.9 (d), 68.9 (d), 62.2 (d), 61.5 (t), 61.5[b] (t). [a]Resonances of the major component compare favorably with literature values[10] for 1-*O*-(α-D-glucopyranosyl)-α-D-galactopyranoside. [b]Resonances attributable to minor component; identical to those observed in the ^{13}C NMR spectrum of commercially available α,α-trehalose. [c]Single resonance unaffiliated with any other resonance; possibly oxalate from the saponification.

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