Amino Acid Conjugated Sophorolipids: A New Family of Biologically Active Functionalized Glycolipids

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Sophorolipids (SLs) are extra cellular glycolipids produced by *Candida bombicola* ATCC 22214 when grown in the presence of glucose and fatty acids. These compounds have a disaccharide head group connected to a long-chain hydroxyl−fatty acid by a glycosidic bond. To explore structure−activity of modified SLs, a new family of amino acid−SL derivatives was prepared. Synthesized analogs consist of amino acids linked by amide bonds formed between their α-amino moiety and the carboxyl group of ring-opened SL fatty acids. Their preparation involved the following: (i) hydrolysis of a natural SL mixture with aqueous alkali to give SL free acids, (ii) coupling of free acids to protected amino acids using dicarbodiimide, and (iii) removing amino acid carboxyl protecting groups. These conjugates were evaluated for their antibacterial, anti-HIV, and spermicidal activity. All tested analogs showed antibacterial activity against both gram +ve and gram −ve organisms. Leucine-conjugated SL was most efficient. For example, the minimum inhibitory concentrations (MIC) for *Moraxella* sp. and *E. coli* were 0.83 and 1.67 mg/mL, respectively. Among the alkyl esters of amino acid conjugated SLs, the ethyl ester of leucine−SLs was most active. Against *Moraxella* sp., *S. sanguinis*, and *M. imperiale*, MIC values are 7.62×10^{-4}, 2.28×10^{-3} and 1.67 mg/mL, respectively. All compounds displayed virus-inactivating activity with 50% effective concentrations (EC_{50}) below 200 μg/mL. The EC_{50} of leucine−SL ethyl ester was 24.1 μg/mL, showing that it is more potent than commercial spermicide nonoxynol-9 ($EC_{50} \approx 65$ μg/mL).

INTRODUCTION

Sophorolipids (SLs) are glycolipids produced by the yeast *Candida bombicola* when cultured on carbohydrate and lipidic substrates. Structurally, SLs are composed of dimeric sophorose (2′-*O*-β-D-glucopyranosyl-β-D-glucopyranose) linked to the hydroxyl group at the penultimate position of, most often, a monounsaturated C18 fatty acid (*1*). First described by Gorin et al. (*2*), SLs exist as mixtures of eight major components (lactone and acid forms) with varying degrees of acetylation at the 6′- and 6″-positions of the sophorose moiety (Chart 1).

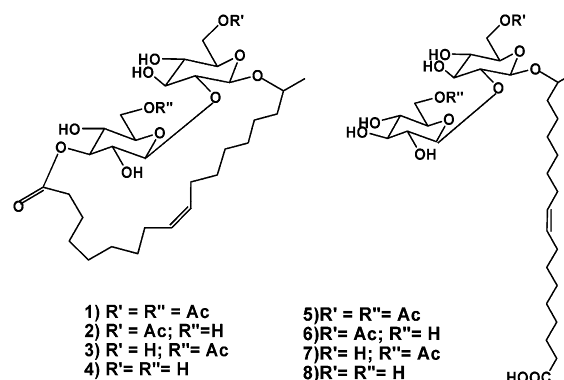
Our ongoing research with SLs and their derivatives have shown that they have immense potential as therapeutic agents that can function as septic shock antagonists (*3*), anticancer agents (*4*), and antibacterial (*5*), antiviral (*6*, *7*), spermicidal (*6*, *7*), and antifungal agents (*8*). However, more structural derivatives of SLs are needed to better define structural−biological activity relationships and thereby enhance SL efficacy and its spectrum of action.

Attempts have been made to perform in vivo modifications of SL structure during biosynthesis. Selective feeding during fermentations of lipophilic substrates such as alkanes and fatty acids did bring about changes in the sophorolipid structure (*9*−*11*). Although this approach is promising, thus far it has affected a change in only a small fraction of the SLs produced.

Thus far, in vitro modification of SLs has been most successful. Earlier reports from our laboratories described SL modifications by chemoenzymatic strategies (*12*, *13*). Bisht et al. synthesized several alkyl esters of SLs using sodium alkoxide/alkanol mixtures under reflux conditions. The resulting SL alkyl esters were subjected to Novozyme 435 catalyzed acylation in anhydrous THF to furnish C-6′ and C-6″ acylated compounds in high yields with excellent chemoselectivity (*13*). Singh et al. synthesized SL−amide derivatives by lipase-catalyzed amidation of SL ethyl ester in dry THF. Amines used included phenethylamine, tyramine, and 2-(*p*-tolyl)ethylamine (*12*). Recently, Nunez et al. reported the synthesis of galactose conjugated SL ester. They successfully transesterified SL methyl ester with 1,2-3,4-di-*O*-isopropylidene-D-galactopyranose in THF using an immobilized lipase catalyst (*14*).

In this paper, a new family of SL derivatives consisting of SL−amino acid and SL−amino acid ethyl ester conjugates were prepared by linking the α-NH_2 group of amino acids to the carboxyl terminus of SL fatty acids. These compounds were assayed to ascertain their antibacterial, anti-HIV, and spermicidal activities. The results of this work provide important insights that expand our knowledge of SL structure−bioactivity relationships.

Chart 1. Structures of Sophorolipids Produced by *Candida bombicola*



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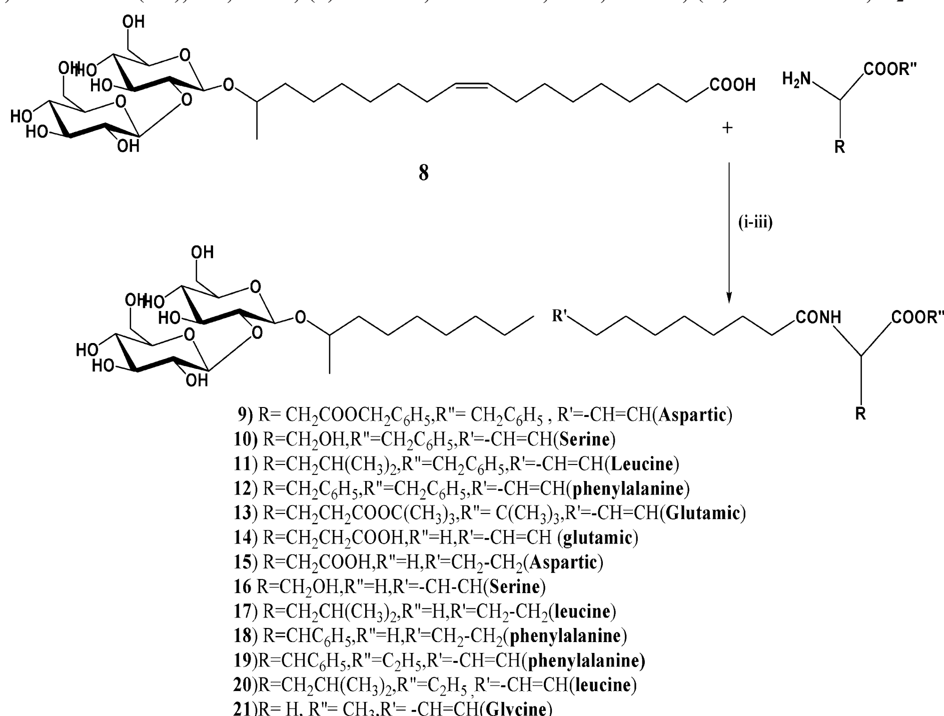
Scheme 1. (i) DCC, DCM/DMF (4:1), RT, 24 hr; (ii) 13 → 14, TFA/DCM, 0 °C, 30 min; (iii) Pd/C ethanol, H_2



EXPERIMENTAL SECTION

General Chemicals and Procedures. Anhydrous solvents, methylene chloride, *N,N*-dimethylformamide, hydrochloride form of carboxyl group protected amino acids, dicyclohexylcarbodiimide (DCC), 1-hydroxybenzotriazole (HOBt), and 4-(dimethylamino)pyridine (DMAP) were purchased from Aldrich Chemical Company in the highest available purity and were used as received unless otherwise noted. Amino acid alkyl ester hydrochlorides were converted to the free-amine form by treatment with an equivalent amount of *N*-methylmorpholine. All liquid reagents were transferred by syringe under N_2 atmosphere. Proton (^{1}H) NMR spectra were recorded on Bruker-300 and Varian 400 M spectrometers at 300 and 400 MHz, respectively. Carbon (^{13}C) NMR spectra were recorded on a Bruker-300 spectrometer at 75 MHz. Chemical shifts are reported in parts per million downfield from 0.00 ppm using trimethylsilane (TMS) as the internal standard. Coupling constants are expressed in hertz (Hz). Column chromatographic separations were conducted with silica gel 60 (Aldrich Chemical Co.). Analytical thin layer chromatography (TLC) were performed on Merck silica gel $^{60}F_{254}$ plates. The spots were developed by spraying with α-naphthol/sulfuric acid (for 100 mL solution, 500 mg of α-naphthol in 5% sulfuric acid solution in ethanol) detecting system. IR spectra were recorded using a Perkin-Elmer FT-IR 1600 series. The samples were dissolved in THF and deposited as thin films on KBr discs. Optical rotations were determined at 598 nm on a Perkin-Elmer 241 digital polarimeter with a 2 dm cell. Values of $[\alpha]_D$ are given in units of 10^{-1} deg cm^2 g^{-1}.

Sophorolipids Synthesis. SLs were synthesized by fermentation of *Candida bombicola* (*12*, *13*). The fermentation media contained glucose (100 g), yeast extract (10 g), urea (1 g), and oleic acid (40 g) per 1000 mL water. After 10 day fermentations, SLs were extracted three times with ethyl acetate. The extracts were pooled and the solvent removed. The resulting product was washed with hexane to remove residual fatty acids.

Isolation of SL-COOH (structure 8). Acidic SLs were synthesized from the natural mixture by alkaline hydrolysis following a literature procedure (*12*, *13*). The hydrolyzed SLs were acidified, the solution was cooled in an ice bath, and the precipitate was further purified by silica gel column chromatography using a chloroform/methanol gradient (3:22 to 4:22, v/v). The product was identified by NMR and LC-MS, which were identical to previously published spectra.

General Procedure for DCC Coupling of SL-COOH (structure 8) with C-Terminal Protected Amino Acids (structures 9−13, 19−21). Amino acid alkyl ester hydrochloride (1 mmol) was suspended in a 4:1 mixture of DCM/DMF (15 mL). The hydrochloride salt was neutralized with *N*-methylmorpholine (1 mmol, 108 μL) and stirred for 15 min giving a clear solution. SL-COOH (**8**, 622 mg, 1 mmol), HOBt (135 mg, 1 mmol), DCC (1.2 mmol, 244 mg), and DMAP (0.1 mmol, 12 mg) were then added to the clear solution. Stirring was continued at room temperature for 24 h. Water (2 mL) was added, and the reaction was stirred for an additional 10 min. Filtration of DCU followed by solvent evaporation gave a transparent syrup which was diluted with ethyl acetate and subsequently washed with cold 0.1 N HCl (20 mL). The ethyl acetate layer was dried over $MgSO_4$, filtered, and concentrated. Purification was performed by flash chromatography to give the desired compounds in 72−78% yields.

Removal of *tert*-Butyl Protecting Group. A solution of **13** (500 mg, 0.58 mmol) in 10 mL CH_2Cl_2/TFA (7:3) was stirred at 0 °C for 30 min. After completion of the reaction, the solution was concentrated under vacuum and twice evaporated with anhydrous toluene to give **14** (380 mg, 87% yield).

Removal of Benzyl Protection and Hydrogenation of cis Double Bond. A solution of **9−12** in 5 mL dry ethanol was stirred at room temperature under H_2 atmosphere in the presence of 10% pd/C (50 mg) for 72 h. Reaction progress was monitored by TLC, and on completion, the solids were removed from the reaction mixture by passing it through a celite pad. The filtrate was concentrated by rotoevaporation under vacuum to give compounds **15−18** in quantitative yields.

Liquid Chromatography−Mass Spectrophotometry Analysis of SLs. A Waters ZQ LC/MS system was used for analysis and identification of individual sophorolipids and amino acid conjugated sophorolipids. The system includes 2795 Alliance HT separation module, 2996 photodiode array detector, and

Waters (Micromass) ZQ 2000 online mass detector. Separation was carried out on a 250 × 4.6 mm polymeric reversed-phase PLRP-S column (particle size 8 μm, pore size 1000 Å, Polymer Laboratories). The water/acetonitrile mobile phase was programmed as follows: 60% water to 50% water in 20 min, then 60% water for 5.0 min (re-equilibration). The HPLC eluent (1 mL/min) was split at the outlet of the column by means of a zero-dead-volume tee splitter to introduce eluent to PDA detector and mass spectrometer. LC-MS was operated with an atmospheric pressure chemical ionization (APCI) probe in positive ion mode. Probe and source temperature were set to 450 and 50 °C, respectively. Cone voltage, corona current, and nebulizer gas flow were set at 60 V, 0.66 μA, and 300 L/h, respectively. Sophorolipids were detected in scan mode with a scan duration of 2.0 s, interscan delay of 0.1 s, and scan range 170−850 *m/z*. Data acquisition and mass spectrometric evaluation were performed with *MassLynx Software 4.0*. The samples for LC/MS were dissolved in 50:50 acetonitrile/water at a concentration of 40−200 μg/mL and were passed through a 0.45 μm PTFE filter before injection.

17-L-[(2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-*cis*-9-octadecenoic Acid (structure 8). $[\alpha]_D^{25}$ = −12.8 (*c* = 0.0104 g/mL in MeOH); IR [KBr plate, cm^{-1} (% *T*)] 3378, 2924, 2853, 1702, 1550, 1412, 1074, 722, 626. ^1H NMR (CH_3OH-d_4): 5.35 (2H, m, H-9 and H-10), 4.63 (1H, d, *J* = 8.1 Hz, H-1″), 4.44 (1H, d, *J* = 7.5 Hz, H-1′), 3.82−3.87 (3H, m, H-6′b, H-6″b, and H-17), 3.63−3.68 (3H, m, H-6′a, H-6″a, and H-5″), 3.53−3.58 (2H, m, H-2′ and H-3′), 3.21−3.41 (5H, m, H-3″, H-2″, H-4″, H-4′, and H-5′), 2.27 (2H, t, *J* = 7.3 Hz, H-2), 2.03−2.05 (4H, m, H-8 and H-11), 1.57−1.62 (4H, m, H-3 and H-16), 1.30−1.46 (16H, m, H-4−7 and H-12−15), 1.24 (3H, d, *J* = 6.0 Hz, H-18). ^{13}C NMR (CH_3OH-d_4): 178.42 (C═O), 130.84, 130.93 (CH═CH), 104.70 (C-1″), 102.76 (C-1′), 81.96, 78.89, 78.36, 78.27, 77.82, 77.78, 75.90, 71.82, 70.52, 63.14, 62.81, 37.85, 35.17, 30.94, 30.85, 30.80, 30.43, 30.33, 30.27, 30.20, 28.18, 28.14, 26.28, 26.19, 21.94 (C-18). LC/APCI-MS: R_t 4.2 min, *m/z* 645.39 $[M + Na]^+$; 4.6 min, *m/z* 645.41-$[M + Na]^+$.

Aspartic Acid-*N*-{17-L-[(2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide}dibenzyl Ester (Structure 9). $[\alpha]_D^{25}$ = −14.7 (*c* = 0.0112 g/mL in MeOH); IR [KBr plate, cm^{-1} (% T)] 3357, 2925, 2853, 2360, 1741, 1650, 1543, 1377, 1227, 1169, 1078, 894. ^1H NMR (CH_3OH-d_4): 7.30−7.39 (10H, m, $-CH_2C_6H_5$ × 2), 5.34(2H, m, H-9 and H-10), 5.09 and 5.12 (4H, 2s, $-CH_2C_6H_5$ × 2), 4.84 (1H, overlapping triplet with HDO peak, CONHC*H*), 4.63 (1H, d, *J* = 8.1 Hz, H-1″), 4.44 (1H, d, *J* = 7.5 Hz, H-1′), 3.81−3.87 (3H, m, H-6′b, H-6″b, and H-17), 3.62−3.68 (3H, m, H-6′a, H-6″a, and H-5″), 3.35−3.58 (4H, m, H-2′, H-3′, H-3″, and H-2″), 3.21−3.33 (3H, m, H-4″, H-4′, and H-5′), 2.90 (2H, dd, *J* = 7.2 and 11.7 Hz CH_2 protons from aspartic acid moiety), 2.15 (2H, t, *J* = 7.2 Hz, H-2), 2.03−2.05 (4H, m, H-8 and H-11), 1.59−1.65 (4H, m, H-3 and H-16), 1.30−1.46 (16H, m, H-4−7 and H-12−15), 1.24 (3H, d, *J* = 6.3 Hz, H-18). ^{13}C NMR (CH_3OH-d_4): 176.05, 171.83 and 171.59 (CO × 3), 137.04, 136.84, 130.76, 129.22, 129.45, 104.57 (C-1″ or C-1′), 102.57 (C-1″ or C-1′), 81.84 (C-2′), 79.55, 79.11, 78.70, 78.12, 75.70, 71.66, 71.34, 68.21, 67.25, 63.00, 62.66, 37.68, 36.98, 36.66, 30.73, 30.12, 26.75, 26.16, 21.89 (C-18). LC/APCI-MS: R_t 15.4 min, *m/z* 940.56 $[M + Na]^+$ and 16.9 min, 940.56 $[M + Na]^+$.

Serine-*N*-{17-L-[(2′-O-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-*cis*-9-octadecenamide} Benzyl Ester (structure 10). $[\alpha]_D^{25}$ = −6.8 (*c* = 0.0128 g/mL in MeOH); IR [KBr plate, cm^{-1} (% T)] 3561, 2920, 2849, 2390, 1713, 1624, 1537, 1414, 1242, 1148, 1077, 786. ^1H NMR (CH_3OH-d_4): 7.32−7.34 (5H, m, $CH_2C_6H_5$), 5.36 (2H, m, H-9 and H-10), 5.18 (2H, s, $CH_2C_6H_5$), 4.63 (1H, d, *J* = 8.1 Hz, H-1″), 4.56 (1H, t, *J* = 4.5 Hz, $NHCHCOOCH_2C_6H_5$), 4.45 (1H, d, *J* = 7.5 Hz, H-1′), 3.79−3.93 (5H, m, CH_2OH from serine, H-6′b, H-6″b, and H-17), 3.58−3.69 (3H, m, H-6′a, H-6″a, and H-5″), 3.35−3.58 (4H, m, H-2′, H-3′, H-3″, and H-2″), 3.21−3.33 (3H, m, H-4″, H-4′, and H-5′), 2.26 (2H, t, *J* = 7.2 Hz, H-2), 2.03−2.04 (4H, m, H-8 and H-11), 1.57−1.62 (4H, m, H-3 and H-16), 1.31−1.46 (16H, m, H-4−7 and H-12−15), 1.24 (3H, d, *J* = 6.3 Hz, H-18). ^{13}C NMR (CH_3OH-d_4): 176.55, 171.83, 137.25, 129.57, 129.93, 129.15, 104.76 (C-1″ or C-1′), 102.73 (C-1″ or C-1′), 82.09 (C-2′), 78.82, 78.27, 77.83, 75.92, 71.86, 71.58, 68.05, 63.14, 62.85, 56.38, 37.82, 36.85, 30.84, 30.38, 30.25, 28.19, 26.89, 26.27, 21.92 (C-18). LC/APCI-MS: R_t 5.5 min, *m/z* $[M + Na]^+$ 822.51 and 6.1 min, *m/z* 822.49 $[M + Na]^+$.

Leucine-*N*-{17-L-[(2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-*cis*-9-octadecenamide} Benzyl Ester (structure 11). $[\alpha]_D^{25}$ = −7.5 (*c* = 0.0160 in MeOH). ^1H NMR (CH_3OH-d_4): 7.30−7.36 (5H, m, $CH_2C_6H_5$), 5.36 (2H, m, H-9 and H-10), 5.14 (2H, s, $CH_2C_6H_5$), 4.63 (1H, d, *J* = 8.1 Hz, H-1″), 4.38−4.50 (1H, m, $NHCHCOOCH_2C_6H_5$), 4.45 (1H, d, *J* = 7.5 Hz, H-1′), 3.82−3.87 (3H, m, H-6′b, H-6″b, and H-17), 3.58−3.69 (3H, m, H-6′a, H-6″a, and H-5″), 3.35−3.58 (4H, m, H-2′, H-3′, H-3″, and H-2″), 3.21−3.33 (3H, m, H-4″, H-4′, and H-5′), 2.21 (2H, t, *J* = 7.2 Hz, H-2), 2.03−2.04 (4H, m, H-8 and H-11), 1.58−1.68 (7H, m, $CH_2CH(CH_3)_2$, H-3 and H-16), 1.31−1.46 (16H, m, H-4−7 and H-12−15), 1.24 (3H, d, *J* = 6.3 Hz, H-18), 0.93 and 0.89 (6H, 2d, *J* = 6 Hz each, $CH_2CH(CH_3)_2$). ^{13}C NMR (CH_3OH-d_4): 176.86, 174.47, 137.70, 129.51, 130.15, 130.76, 104.76, 102.73, 82.34, 79.28, 78.67, 78.63, 78.14, 76.28, 72.20, 71.89, 63.48, 63.17, 52.46, 42.21, 38.23, 37.82, 31.21, 30.86, 30.64, 27.38, 27.01, 26.71, 26.50, 23.90, 22.34, 22.12. LC/APCI-MS: R_t 14.4 min, *m/z* 848.94 $[M + Na]^+$ and 13.02 min, *m/z* 848.91 $[M + Na]^+$.

Phenylalanine-*N*-{17-L-[(2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-*cis*-9-octadecenamide} Benzyl Ester (structure 12). $[\alpha]_D^{25}$ = −34.8 (*c* = 0.0117 in MeOH). ^1H NMR (CH_3OH-d_4): 7.28−7.36 (5H, m, $CH_2C_6H_5$), 7.15−7.25 (5H, m, C_6H_5), 5.36 (2H, m, H-9 and H-10), 5.12 (2H, s, $CH_2C_6H_5$), 4.69−4.74 (1H, t, merging with HDO signal, $NHCHCOOCH_2$-C_6H_5), 4.63 (1H, d, *J* = 8.1 Hz, H-1″), 4.45 (1H, d, *J* = 7.5 Hz, H-1′), 3.82−3.86 (3H, m, H-6′b, H-6″b, and H-17), 3.58−3.69 (3H, m, H-6′a, H-6″a, and H-5″), 3.35−3.47 (4H, m, H-2′, H-3′, H-3″, and H-2″), 3.21−3.33 (3H, m, H-4″, H-4′, and H-5′), 2.98−2.93 (2H, m, $C_6H_5CH_2$ phenylalanine moiety), 2.15 (2H, t, *J* = 7.2 Hz, H-2), 2.03−2.03 (4H, m, H-8 and H-11), 1.44−1.65 (7H, m, $CH_2CH(CH_3)_2$, H-3 and H-16), 1.33−1.40 (16H, m, H-4−7 and H-12−15), 1.24 (3H, d, *J* = 6.3 Hz, H-18). ^{13}C NMR (CH_3OH-d_4): 176.67, 173.34, 138.58, 137.48, 131.29, 130.68, 128.29, 129.99, 129.75, 105.11 (C-1″ or C-1′), 103.05 (C-1″ or C-1′), 82.55, 79.12, 78.62, 78.09, 76.26, 72.16, 71.98, 68.38, 65.87, 63.43, 63.15, 55.71, 44.85, 38.75, 38.19, 37.18, 31.23, 30.76, 30.59, 28.60, 27.33, 26.67, 22.27. LC/APCI-MS: R_t 14.3 min, *m/z* 882.59 $[M + Na]^+$ and 15.7 min, *m/z* 882.56 $[M + Na]^+$.

Glutamic Acid-*N*-{17-L-[(2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-*cis*-9-octadecenamide} *tert*-Butyl Ester (structure 13). $[\alpha]_D^{25}$ = −13.3 (*c* = 0.0125 g/mL in MeOH). IR [KBr plate, cm^{-1} (% T)] 3347, 2927, 2854, 2360, 1731, 1650, 1593, 1540, 1368, 1255, 1154, 1074, 894. ^1H NMR (CH_3OH-d_4): 5.34 (2H, m, H-9 and H-10), 4.63 (1H, d, *J* = 8.1 Hz, H-1″), 4.44 (1H, d, *J* = 7.5 Hz, H-1′), 4.30 (1H, dd, *J* = 5.4 and 9.6 Hz, CONHC*H*), 3.81−3.87 (3H, m, H-6′b, H-6″b, and H-17), 3.62−3.68 (3H, m, H-6′a, H-6″a, and H-5″), 3.35−3.58 (4H, m, H-2′, H-3′, H-3″, and H-2″), 3.21−3.33 (3H, m, H-4″, H-4′, and H-5′), 2.31 (2H, t, *J* = 7.5 Hz, CH_2 protons from glutamic acid moiety), 2.15 (2H, t, *J* = 7.2 Hz, H-2), 2.03−2.11 (5H, m, CH_a from γ methylene of glutamic acid moiety, H-8 and H-11), 1.80−1.90 (1H, m, CH_b from β methylene of

glutamic acid moiety), 1.59−1.65 (4H, m, H-3 and H-16), 1.45 and 1.46 (18H, 2s, $C(CH_3)_3 \times 2$), 1.30−1.48 (16H, m, H-4−7 and H-12−15), 1.24 (3H, d, J = 6.3 Hz, H-18). ^{13}C NMR (CH_3OH-d_4): 176.38, 173.66 and 172.65 (C═O × 3), 130.87, 104.71 (C-1′′ or C-1′), 102.74 (C-1′′ or C-1′), 82.85, 81.90 (C-2′), 78.84, 78.30, 77.82, 75.91, 71.83, 71.34, 63.13, 62.79, 53.71, 37.83, 36.81, 30.84, 30.29, 28.35, 28.31, 28.19, 27.86, 27.00, 26.61, 26.27, 26.16, 21.93 (C-18). LC/APCI-MS: R_t 10.9, m/z 886.62 [M + Na]$^+$ and 12.2 min, m/z 886.60 [M + Na]$^+$.

Glutamic-*N*-{17-L-[(2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)oxy]-*cis*-9-octadecenamide} Acid (structure 14). $[\alpha]_D^{25}$ = −12.3 (c = 0.0121 g/mL in MeOH). ^{1}H NMR (CH_3OH-d_4): 5.35 (2H, m, H-9 and H-10), 4.63 (1H, d, J = 8.1 Hz, H-1′′), 4.43−4.60 (2H, m, CONHC*H*COOH, H-1′), 3.82−3.87 (3H, m, H-6′b, H-6′′b, and H-17), 3.62−3.68 (3H, m, H-6′a, H-6′′a, and H-5′′), 3.35−3.58 (4H, m, H-2′, H-3′, H-3′′, and H-2′′), 3.21−3.34 (3H, m, H-4′′, H-4′, and H-5′), 2.40 (2H, t, J = 7.5 Hz, CH_2 protons from glutamic acid moiety), 2.25 (2H, t, J = 7.2 Hz, H-2), 2.18−2.22 (1H, m, CH_a from β methylene of glutamic acid moiety), 1.89−2.04 (5H, m, CH_b from β methylene of glutamic acid moiety H-8 and H-11), 1.59−1.70 (4H, m, H-3 and H-16), 1.30−1.48 (16H, m, H-4−7 and H-12−15), 1.25 (3H, d, J = 6.3 Hz, H-18). ^{13}C NMR (CH_3OH-d_4): 176.38, 175.0 and 174.32 (C═O × 3), 130.89, 104.74 (C-1′′ or C-1′), 102.74 (C-1′′ or C-1′), 82.05, 78.86, 78.29, 77.80, 75.92, 71.86, 71.57, 65.19, 63.15, 62.82, 44.00, 37.84, 36.86, 31.29, 30.85, 30.38, 30.27, 28.19, 27.89, 26.92, 26.28, 21.93 (C-18). LC/APCI-MS: R_t 2.9 min, m/z 774.54 [M + Na]$^+$ and 4.1 min, m/z 774.57 [M + Na]$^+$.

Aspartic-*N*-{17-L-[(2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)oxy]octadecanamide} Acid (structure 15). $[\alpha]_D^{25}$ = −5.7 (c = 0.1050 in MeOH). ^{1}H NMR (CH_3OH-d_4): 4.80−4.87 (1H, overlapping multiplet with HDO signal, CONHC*H*COOH), 4.63 (1H, d, J = 8.1 Hz, H-1′′), 4.45 (1H, d, J = 7.5 Hz, H-1′), 3.81−3.89 (3H, m, H-6′b, H-6′′b, and H-17), 3.62−3.68 (3H, m, H-6′a, H-6′′a, and H-5′′), 3.35−3.55 (4H, m, H-2′, H-3′, H-3′′, and H-2′′), 3.21−3.35 (3H, m, H-4′′, H-4′, and H-5′), 2.80 (2H, dd, J = 6.6 and 9.3 Hz CH_2 protons from aspartic acid), 2.23 (2H, t, J = 7.2 Hz, H-2), 1.59−1.74 (4H, m, H-3 and H-16), 1.30−1.46 (24H, m, H-4−15), 1.24 (3H, d, J = 6.3 Hz, H-18). ^{13}C NMR (CH_3OH-d_4): 176.12 (CONH), 174.39 and 174.52 (CO × 2), 104.57 (C-1′′ or C-1′), 102.57 (C-1′′ or C-1′), 82.02 (C-2′), 78.87, 78.34, 77.80, 75.92, 71.86, 71.57, 63.13, 62.82, 37.84, 37.29, 36.90, 34.78, 30.79, 30.23, 26.88, 26.78, 26.31, 26.08, 21.91 (C-18). LC/APCI-MS: R_t 2.8 min, m/z 762.39 [M + Na]$^+$ and 3.1 min, 762.55 [M + Na]$^+$.

Serine-*N*-{17-L-[(2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)oxy]octadecanamide} (structure 16). $[\alpha]_D^{25}$ = −10.4 (c = 0.0156 g/mL in MeOH). ^{1}H NMR (CH_3OH-d_4): 4.60 (1H, d, J = 8.1 Hz, H-1′′), 4.45 (1H, t, J = 4.28 Hz, NHC*H*COOH), 4.40 (1H, d, J = 7.5 Hz, H-1′), 3.74−3.88 (5H, m, C*H*$_2$OH from serine, H-6′b, H-6′′b, and H-17), 3.58−3.69 (3H, m, H-6′a, H-6′′a, and H-5′′), 3.35−3.58 (4H, m, H-2′, H-3′, H-3′′, and H-2′′), 3.16−3.33 (3H, m, H-4′′, H-4′, and H-5′), 2.26 (2H, t, J = 7.2 Hz, H-2), 1.57−1.62 (4H, m, H-3 and H-16), 1.31−1.46 (24H, m, H-4−15), 1.21 (3H, d, J = 6.3 Hz, H-18). ^{13}C NMR (CH_3OH-d_4): 176.82, 173.89, 105.04 (C-1′′ or C-1′), 103.11 (C-1′′ or C-1′), 82.30 (C-2′), 79.29, 78.65, 78.14, 76.28, 72.20, 71.89, 63.47, 63.36, 56.43, 8.23, 37.27, 31.17, 30.32, 30.67, 27.24, 26.68, 22.29 (C-18). LC/APCI-MS: R_t 2.4 min, m/z [M + Na]$^+$ 734.76 and 3.2 min, m/z 734.78 [M + Na]$^+$.

Leucine-*N*-{17-L-[(2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)oxy]octadecanamide} (structure 17). $[\alpha]_D^{25}$ = −8.2 (c = 0.0151 g/mL in MeOH). IR [KBr plate, cm^{-1} (% T)] 3352, 2924, 2853, 2390, 1713, 1644, 1538, 1466, 1235, 1163, 1077, 720. ^{1}H NMR (CH_3OH-d_4): 4.58 (1H, d, J = 8.1 Hz, H-1′′), 4.40 (1H, d, J = 7.5 Hz, H-1′), 4.34−4.40 (1H, m, NHC*H*COOH), 3.78−3.86 (3H, m, H-6′b, H-6′′b, and H-17), 3.57−3.64 (3H, m, H-6′a, H-6′′a, and H-5′′), 3.36−3.53 (4H, m, H-2′, H-3′, H-3′′, and H-2′′), 3.19−3.33 (3H, m, H-4′′, H-4′, and H-5′), 2.18 (2H, t, J = 7.2 Hz, H-2), 1.54−1.67 (7H, m, C*H*$_2$C*H*(CH_3)$_2$, H-3 and H-16), 1.31−1.46 (24H, m, H-4−15), 1.19 (3H, d, J = 6.3 Hz, H-18), 0.90 and 0.87 (6H, 2d, J = 6.0 Hz each, $CH_2CH(CH_3)_2$). ^{13}C NMR (CH_3OH-d_4): 176.86, 176.76, 105.07, 103.11, 82.35, 79.28, 78.67, 78.63, 78.14, 76.28, 72.20, 71.89, 63.49, 63.17, 52.46, 42.05, 38.24, 37.24, 31.22, 30.86, 30.64, 27.39, 27.01, 26.71, 26.50, 23.90, 22.34, 22.25. LC/APCI-MS: R_t 3.2 min, and 3.9 min, m/z 760.64 [M + Na]$^+$.

Phenylalanine-*N*-{17-L-[(2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)oxy]octadecanamide} (structure 18). $[\alpha]_D^{25}$ = −11.3 (c = 0.0142 g/mL in MeOH). IR [KBr plate, cm^{-1} (% T)] 3416, 2917, 2849, 2390, 1730, 1643, 1542, 1468, 1238, 1158, 1080, 864. ^{1}H NMR (CH_3OH-d_4): 7.13−7.17 (5H, m, C_6H_5 from phenylalanine), 4.54−4.56 (2H, m, H-1′′ and NHC*H*COOH), 4.35 (1H, d, J = 7.5 Hz, H-1′), 3.82−3.86 (3H, m, H-6′b, H-6′′b, and H-17), 3.58−3.69 (3H, m, H-6′a, H-6′′a, and H-5′′), 3.35−3.47 (4H, m, H-2′, H-3′, H-3′′, and H-2′′), 3.21−3.33 (3H, m, H-4′′, H-4′, and H-5′), 3.02 (2H, m, CH_2 phenylalanine moiety), 2.15 (2H, t, J = 7.2 Hz, H-2), 1.50−1.52 (4H, m, H-3 and H-16), 1.33−1.40 (24H, m, H-4−15), 1.21(3H, d, J = 6.3 Hz, H-18). ^{13}C NMR (CH_3OH-d_4): 176.56, 175.71, 139.05, 130.63, 129.78, 128.11, 105.06, 103.12, 82.33, 79.25, 78.63, 78.17, 72.20, 71.92, 65.52, 63.47, 63.15, 58.71, 44.35, 38.83, 38.22, 37.27, 31.17, 30.82, 30.48, 27.28, 26.68, 22.28. LC/APCI-MS: R_t 2.8 min, m/z 794.62 [M + Na]$^+$ and 3.2 min, m/z 794.60 [M + Na]$^+$.

Phenylalanine-*N*-{17-L-[(2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)oxy]-*cis*-9-octadecanamide} Ethyl Ester (structure 19). $[\alpha]_D^{25}$ = −8.6 (c = 0.0152 g/mL in MeOH). IR [KBr plate, cm^{-1} (% T)] 3331, 2925, 2854, 2360, 1738, 1650, 1603, 1542, 1454, 1376, 1172, 1074, 1034. ^{1}H NMR (CH_3OH-d_4): 7.29−7.19 (5H, m, C_6H_5 from phenylalanine), 5.35 (2H, m, H-9 and H-10), 4.68−4.62 (2H, m, H-1′′ and NHC*H*COOH), 4.44 (1H, d, J = 7.5 Hz, H-1′), 4.13 (2H, q, J = 7.2 Hz, OC*H*$_2$-CH_3), 3.82−3.87 (3H, m, H-6′b, H-6′′b, and H-17), 3.58−3.69 (3H, m, H-6′a, H-6′′a, and H-5′′), 3.35−3.47 (4H, m, H-2′, H-3′, H-3′′, and H-2′′), 3.21−3.33 (3H, m, H-4′′, H-4′, and H-5′), 3.02 (2H, t, J = 5.1 Hz, CH_2 phenylalanine moiety), 2.15 (2H, t, J = 7.2 Hz, H-2), 2.03−2.05 (4H, m, H-8 and H-11), 1.44−1.65 (4H, m, H-3 and H-16), 1.33−1.40 (16H, m, H-4−7 and H-12−15), 1.24−1.26 (6H, m, OCH$_2$C*H*$_3$ and H-18). ^{13}C NMR (CH_3OH-d_4): 176.57, 173.63, 138.68, 131.23, 130.59, 129.84, 128.22, 105.08, 103.11, 82.37, 79.25, 78.71, 78.65, 78.21, 76.27, 72.21, 71.90, 63.50, 63.15, 62.71, 55.58, 38.82, 37.09, 31.29, 31.20, 30.77, 30.71, 30.56, 30.46, 28.55, 27.26, 26.63, 22.28. LC/APCI-MS: R_t 8.9 min, m/z 820.87 [M + Na]$^+$ and 10.0 min, m/z 820.87 [M + Na]$^+$.

Leucine-*N*-{17-L-[(2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)oxy]-*cis*-9-octadecanamide} Ethyl Ester (structure 20). $[\alpha]_D^{25}$ = −6.4 (c = 0.0181 g/mL in MeOH). IR [KBr plate, cm^{-1} (% T)] 3316, 2927, 2855, 2360, 1737, 1649, 1560, 1446, 1375, 1266, 1196, 1087, 940, 857. ^{1}H NMR (CH_3OH-d_4): 5.34−5.38 (2H, m, H-9 and H-10), 4.65 (1H, d, J = 7.8 Hz, H-1′′), 4.41−4.48 (2H, m, NHC*H*COOCH$_2$CH$_3$ and H-1′), 4.18 (2H, q, OC*H*$_2$CH$_3$), 3.88−3.84 (3H, m, H-6′b, H-6′′b, and H-17), 3.71−3.64 (3H, m, H-6′a, H-6′′a, and H-5′′), 3.36−3.55 (4H, m, H-2′, H-3′, H-3′′, and H-2′′), 3.19−3.33 (3H, m, H-4′′, H-4′, and H-5′), 2.04−2.06 (4H, m, H-8 and H-11), 2.18 (2H, t, J = 7.2 Hz, H-2), 1.54−1.67 (7H, m, C*H*$_2$C*H*(CH_3)$_2$, H-3 and H-16), 1.31−1.46 (16H, m, H-4−7 and H-12−15), 1.24−1.30 (6H, m, OCH$_2$C*H*$_3$ and H-18), 0.93 and 0.97 (6H, 2d, J = 6.0 Hz each, $CH_2CH(CH_3)_2$). ^{13}C NMR (CH_3OH-d_4): 176.82, 174.78, 131.32, 131.24, 105.10, 103.11, 82.42, 79.23, 78.69, 78.63, 78.20, 78.14, 76.28, 72.23, 71.90, 63.53, 63.19,

62.63, 52.48, 41.80, 38.22, 37.14, 31.32, 31.22, 30.80, 30.74, 30.66, 30.59, 27.38, 26.66, 26.45, 23.78, 22.36, 22.28. LC/APCI-MS: R_t 7.7 min, *m/z* 786.79 $[M + Na]^+$ and 8.7 min, *m/z* 786.89 $[M + Na]^+$.

Glycine-*N*-{17-L-[(2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)oxy]-*cis*-9-octadecenamide} Methyl Ester (structure 21). $[\alpha]_D^{25} = -8.6$ ($c = 0.0136$ g/mL in MeOH). IR [KBr plate, cm^{-1} (% T)] 3357, 2925, 2853, 1741, 1650, 1543, 1377, 1227, 1169, 1078, 894. ^1H NMR (CH_3OH-d_4): 5.36 (2H, m, H-9 and H-10), 4.63 (1H, d, $J = 8.1$ Hz, H-1″), 4.45 (1H, d, $J = 7.5$ Hz, H-1″), 3.92 (2H, s, $NHCH_2COOCH_3$), 3.81−3.87 (3H, m, H-6′b, H-6″b, and H-17), 3.72 (3H, s, $NHCH_2COOCH_3$), 3.63−3.74 (3H, m, H-6′a, H-6″a, and H-5″), 3.35−3.58 (4H, m, H-2′, H-3′, H-3″, and H-2″), 3.21−3.33 (3H, m, H-4″, H-4′, and H-5′), 2.25 (2H, t, $J = 7.2$ Hz, H-2), 2.03−2.05 (4H, m, H-8 and H-11), 1.59−1.65 (4H, m, H-3 and H-16), 1.30−1.46 (16H, m, H-4−7 and H-12−15), 1.24 (3H, d, $J = 6.3$ Hz, H-18). ^{13}C NMR (CH_3OH-d_4): 176.83, 171.99, 130.95, 130.90 (*C*H=*C*H), 104.85, 102.73, 82.27 (C-2′), 79.08 (C-17), 78.68, 78.64 (C-3″ and C-5′), 78.26, 78.16 (C-3′ and C-5″), 76.29 (C-2″), 72.28, 72.01 (C-4″ and C-4′), 63.54, 63.24 (C-6′ and C-6″), 52.93 (OCH_3), 42.26 ($NHCH_2COOCH_3$), 38.18, 37.16, 31.26, 31.17, 30.96, 30.75, 30.67, 30.57, 28.54, 27.19, 26.62, 22.25 (C-18). LC/APCI-MS: R_t 4.1 min, *m/z* 716.38 $[M + Na]^+$ and 4.3 min, *m/z* 716.38 $[M + Na]^+$.

Antimicrobial Assay. To determine antimicrobial activities, *Escherichia coli, Bacillus subtilis, Salmonela cholerasesuis*, *Microbacterium imperiale* (obtained from ATCC, U.S.A.), *Moraxella* sp., *Streptococcus agalactiae*, and *Streptococcus sanguinis* (obtained as gift cultures from Dr. Maja Nowakowski, SUNY downstate medical center, NY) were used.

The minimum inhibitory concentrations (MIC) of the amino acid conjugated SLs were determined by the broth serial dilution method performed in 96-well microtiter plates. A stock solution of SL (15 mg/mL) was prepared in 200 mg/mL sucrose solution (pH 9.0). Cultures were grown o/n and diluted to 0.1 OD before experiments. Serial dilutions (50 μL) of each sample were made in the broth, and plates incubated at 30 °C. After 18−24 h, the plates were examined for growth of the organisms. The concentration that inhibited more then 90% growth of the organism was record as the MIC. Positive control (cells plus medium) and negative control (medium only) were performed with each experiment. Percent inhibition was calculated by comparing the experimental readings with the positive control. Three replicates were performed for each SL derivative against each test organism.

Spermicidal Assay. The spermicidal activity of SLs was evaluated using a modification of the method by Sander and Cramer (*15*). Semen samples were collected from normal donors by masturbation, allowed to liquefy at room temperature, and assessed for motility. The protocol was approved by the Eastern Virginia Medical School Institutional Review Board. Stock solutions, typically 10 mg/mL, were prepared in DMSO. Serial 2-fold dilutions in 0.9% NaCl of test compounds were incubated with semen aliquots for 30 s. Motility was evaluated under the microscope during the first incubation and after dilution in buffer and further incubation for 1 h. Only those dilutions that immobilized 100% of the spermatozoa pass the test. Results were expressed as minimum effective concentrations (MEC or ED100).

HIV Inactivation Assay. Anti-HIV activity was evaluated by incubating serial dilutions of the SL compounds with HIV-1 (IIIB) and CD4/β-galactosidase-expressing HeLa cells for 2 h in 12-well plates (*16*). After this incubation, virus and compounds were washed away, and the cells were resuspended and cultured in fresh RPMI 1640 medium supplemented with 10% fetal bovine serum for 48 h at 37 °C and 5% CO_2. These cells express β-galactosidase under the control of the HIV long terminal repeat (LTR). Thus, expression of this enzyme is linked to HIV infection. After the 48 h incubation, cells were washed in PBS, and β-galactosidase activity was measured using the Galacto-Star β-galactosidase reporter gene assay (Applied Biosystems, Foster City, CA). Briefly, the cells were lysed and their contents incubated with Galacto-Star substrate. Chemiluminescence was quantified in a luminometer. Cells incubated with virus and no compounds provided values representing 100% infection. Fifty percent effective concentrations (EC_{50}) were calculated using a linear regression model.

RESULTS AND DISCUSSION

Synthesis. SL mixture produced by the fermentation of *C. bombicola* on glucose/oleic acid contains at least fourteen different compounds that exist mainly in the lactonic and acidic forms. These compounds also have a variable degree of acetylation at the primary hydroxyl groups at 6′ and 6″ positions. The aliphatic component of SLs is composed of 17-hydroxyoleic acid that exists in hydrogenated and non-hydrogenated forms. To free carboxylic groups of lactonic sophorolipids as well as to reduce natural sophorolipid mixture heterogeneity, alkaline hydrolysis was performed according to a published procedure (*17*). During alkaline hydrolysis, acetate groups of sophorose units were removed with concomitant opening of the lactonic ring to give 17-L-[(2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-*cis*-9-octadecenoic acid (SL-COOH, **8**). The downfield shift (δ 3.66) for the C-1 carbon of **8** (δ 178.42), when compared to the cyclic form (δ 174.76), confirmed that opening of the lactonic ring occurred (*18*). All peaks due to carbohydrate protons match well with other ring-open derivatives synthesized in the past and for which rigorous assignments had been made using $^1H-^1H$ COSY NMR and DEPT 135 experiments (*12*, *13*). Finally, before conjugation, purity of the SL-COOH was determined by LC/APCI (*m/z* 645.39 and 645.41). Two peaks of the same mass at different retention times were observed. This is attributed to attachment of dimeric sophorose to the hydroxyl group at either the penultimate or terminal fatty acid carbon where the later is the minor component (*19*).

The free carboxylic group was used as a handle to anchor various amino acids using DCC coupling. The coupling was performed without protecting free hydroxyl groups of sophorose moieties. SL-COOH is sparingly soluble in DCM; however, addition of anhydrous DMF renders the compound completely soluble, yielding a homogeneous solution. Conjugation of amino acids to sophorolipids was confirmed by identifying relevant NMR signals of the amino acid moiety. For example, coupling of dibenzylaspartic ester to SL-COOH gave a double doublet at 2.90 ppm (2H) that corresponds to the aspartate methylene of the side chain. Furthermore, ^{13}C NMR showed three peaks in the carbonyl region, two at 171.83 and 171.59 ppm due to esters and one at 176.05 ppm due to newly formed amide. Deprotection of sophorolipid−amino acid conjugates with benzyl ester protecting groups was performed in dry ethanol by hydrogenation over Pd/C. This method resulted in both the removal of benzyl ester groups and the reduction of cis double bonds of the SL fatty acid moiety. Removal of *tert*-butyl protecting groups was carried out in DCM/TFA at 0 °C. Performing this reaction under mild acidic conditions avoided the hydrolysis of anomeric linkages of SLs.

To further broaden the range of SL−amino acid conjugates for bioactivity studies, amino acid ethyl esters were coupled to SL-COOH. SL−amino acid conjugates where the amino acid carboxylate groups are ethyl esters increases compound hydrophobicity relative to its corresponding carboxylate salt form. Furthermore, introduction of ethyl esters was not considered problematic, since liberation of ethanol by metabolism gives readily metabolized byproducts.

Table 1. Antimicrobial Activity of Different Amino Acid Conjugated SLs

SL sample	14	15	16	17	18	19	20	21	MAEE-SL[a]
Culture	Minimal Inhibitory Concentration (mg/mL)								
E. coli	0.56	5	5	1.67	5	>5	>5	>5	5
B. subtilis	>7.5	>7.5	>7.5	5	>7.5	5	1.67	1.67	0.06
Moraxella sp.	1.67	5	7.5	0.83	5	5	0.000762	NA	0.02
S. cholerasesuis	>7.5	>7.5	>7.5	5	>7.5	>5	>5	>5	5
S. agalactiae	5	5	5	5	>7.5	1.67	ND[b]	5	0.007
S. sanguinis	>7.5	>7.5	5	2.5	7.5	5	0.00228	0.56	ND[b]
M. imperiale	>7.5	>7.5	5	5	>7.5	>5	1.67	5	ND[b]

[a] MAEE-SL = ethyl 17-L-([2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl]oxy)-*cis*-9-octadecenoate-6′,6″-diacetate. The compound was synthesized using protocol described elsewhere.[13] [b] ND = not determined.

This family of new SL−amino acid conjugates was assessed to understand structure−bioactivity relationships by performing studies of antibacterial, anti-HIV, and spermicidal activity.

Biological Activity. Biosurfactants represent an emerging class of important compounds in biomedical science that offer antifungal, antibacterial, antimycoplasmal, and antiviral properties (*20*). The proposed primary mechanism of action of these surfactants is membrane lipid order perturbation, which compromises the viability of microorganisms (*21*). Some amino acid conjugated SLs display bactericidal and virucidal activity. However, none of these compounds are significantly spermicidal, showing a certain degree of membrane specificity in their lytic action.

Antibacterial Activity. All tested analogues showed antibacterial activity against both gram +ve and gram −ve organisms (Table 1). Comparing the efficiencies of amino acid conjugated SLs shows that leucine-conjugated SL is most efficient with MIC of 5 mg/mL or less for all seven of the organisms tested. MIC for *Moraxella* sp. was 0.83 mg/mL, and for *E. coli*, it was 1.67 mg/mL. This derivative was also the most effective of those tested against *B. subtilis* and *S. sanguinis* (5 mg/mL and 2.5 mg/mL, respectively). Among the alkyl esters of amino acid conjugated SLs, the ethyl ester of leucine−SLs was most active. It is also the most effective compound of those evaluated against *Moraxella* sp., *S. sanguinis*, and *M. imperiale* with MIC values of 7.62×10^{-4}, 2.28×10^{-3}, and 1.67 mg/mL, respectively. In earlier studies by our laboratory, monoacetylated ethyl ester SL (MAEE-SL) was identified as an efficient antibacterial compound.[5] After comparing the activity of ethyl ester leucine−SL to that of MAEE-SL, it is evident that ethyl ester−leucine SL is more potent against *Moraxella* sp. However, the antibacterial activity of MAEE relative to ethyl ester−leucine SL was higher against the other microorganisms tested. Therefore, future experiments will be directed toward preparing amino acid−SLs whose sophorose head group contains various extents of acetylation.

Anti-HIV Activity. As shown in Table 2, all compounds displayed virus inactivating activity with 50% effective concentrations (EC_{50}) below 200 μg/mL. Comparing potencies of amino acid conjugated SLs and their alkyl esters showed that esterified derivatives were more effective than corresponding non-esterified compounds. The ethyl ester of leucine-conjugated SL was most potent with an EC_{50} of 24.1 μg/mL. This analogue was more potent than the commercial spermicide nonoxynol-9, which displays virucidal anti-HIV activity with an $EC_{50} \approx$ 65 μg/mL. Dimethyl sulfoxide (DMSO) did not display antiviral activity at the concentrations present in the tested compound dilutions.

Spermicidal Activity. Spermicidal activity was measured using a modified Sander−Cramer test. None of the compounds displayed significant spermicidal activity beyond that shown by the solvent (DMSO) control. In contrast, nonoxynol-9 (N-9) and MAEE, included as positive controls, revealed the minimum effective concentration (MEC) values of around 300 and 500 μg/mL, respectively.

Table 2. Anti-HIV and Spermicidal Activity of Different Amino Acid Conjugated SLs

SL sample	anti-HIV activity EC_{50} (μg/mL)	spermicidal activity MEC*a* (mg/mL)
14	194.9	ND[e]
15	182.3	7.5 ± 1.0
16	177.4	7.5 ± 1.0
17	116.9	7.0 ± 1.1
18	148.7	6.7 ± 1.0
19	65.9	5.8 ± 0.8
20	24.1	7.5 ± 1.0
21	92	9.2 ± 0.8
MAEE	ND[e]	0.5 ± 0.04
DMSO[c]	>1000[b]	7.5 ± 1.0[b]
N-9[d]	ND[e]	0.339 ± 0.057

[a] Minimum effective concentration. [b] Solute equivalent concentration. [c] Dimethyl sulfoxide. [d] Nonoxynol-9. [e] Not determined.

SUMMARY OF RESULTS

By changing the amino acid from hydrophilic (L-glutamic, L-aspartic, L-serine) to hydrophobic (L-phenylalanine, L-leucine), improved antimicrobial properties were achieved. The leucine-conjugated SL ethyl ester derivative was the most potent bactericidal and HIV-1 virucidal compound studied herein. Nevertheless, the potency of this analog must be further enhanced before it can be considered as an important candidate for advanced therapy. The results of structure−activity relationships from this work provide important guidance in how to engineer SL derivatives to achieve effective new therapeutics. Work is in progress to determine how the length of leucine alkyl esters conjugated to SL, and acetylating sophorose head groups of amino acid−SL conjugates, modulates SL biological properties.

Supporting Information Available: NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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