Enzyme-Mediated Regioselective Acylations of Sophorolipids

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Enzymatic synthesis of well-defined sophorolipid analogues for evaluation of their bioactivities and as new building blocks for the preparation of glycolipid-based amphiphilic polymers is described. Lipase Novozym 435 from *Candida antarctica* has been shown to be an efficient catalyst for acylation of sophorolipids esters. A mixture of sophorolipids produced by *Torulopsis bombicola* was esterified by reaction with sodium alcoxide. The alkyl esters of sophorose lipids were subjected to Novozym 435 catalyzed acylation in dry tetrahydrofuran (THF) with vinyl acrylate and vinyl acetate to diacyl derivatives. The reactions were highly regioselective, and exclusive acylation of the hydroxyl groups on C-6′ and C-6″ took place. Methyl ester in the absence of the acylating agent, or with the agent at a concentration less than equimolar, gave sophorolactone (**9**). Careful analysis of the spectral data revealed it to be a synthetic analogue of microbially produced macrolactone. Sophorolactone (**9**) differs in the site at which the sophorose ring is attached to the fatty acid. Specifically, in **9**, unlike the natural sophorolipids, the fatty acid carboxyl carbon is linked to the C-6″ hydroxyl, not to the C-4″ hydroxyl. Subsequent acrylation of **9** catalyzed by Novozym 435 led to the formation of the C-6′ monoacryl derivative linked only to the primary site.

Introduction

Sophorolipids are microbial extracellular surface-active glycolipids. Cells of *Torulopsis bombicola* produce sophorolipids when they are grown on sugars, hydrocarbons, vegetable oils, or mixtures thereof. First described by Gorin et al.[1] in 1961, sophorolipids occur as a mixture of macrolactone and free acid structures that are acetylated to various extents at the sophorose ring primary hydroxyl positions (Figure 1). Careful examinations have revealed that at least eight structurally different sophorolipids are produced.[2] The main component was 17-hydroxyoctadecanoic acid and its corresponding lactone.[3] It has been suggested that these lipids are not only essential for the growth of *T. bombicola* on water-insoluble alkanes but that they also inhibit the growth of other alkane-utilizing yeasts.[4] Recent advances in microbial fermentation methods have made it possible to generate up to 700 g/L of sophorolipids.[5]

Work has been carried out to "tailor" sophorolipid (SL) structure during in vivo formation. These studies have mainly involved the selective feeding of different lipophilic substrates. For example, changing the cosubstrate from sunflower to canola oil resulted in a large increase (50% to 73%) of the lactonic portion of SLs.[6] Also, unsaturated C-18 fatty acids of oleic acid may be transferred unchanged into sophorolipids.[7] Thus, to date, physiological variables during fermentations have provided routes to the variation of sophorolipid composition but have not led to well-defined pure compounds. Our interest in sophorolipids arises due to their intriguing structures and potential application in a wide variety of fields. Sophorolipids are routinely prepared in our laboratory by fermentation of *T. bombicola* on glucose/oleic acid mixtures following a literature procedure.[8]

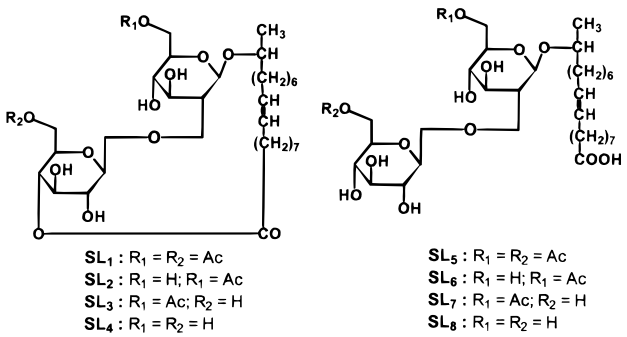

Figure 1. Structures of sophorolipids produced by *Torulopsis* sp.

One of our goals was the development of suitable synthetic methods so that well-defined sophorolipid analogues would be in-hand for subsequent evaluation of their bioactivities. Review of the existing data on the use of glycolipids to treat very severe immune disorders is certainly promising. In summary, the glycolipids have

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been reported to be of interest for the following: (i) in vivo cancer treatment/antitumor cell activity by cytokine upregulation;[9] (ii) treatment of autoimmune disorders (putatively via regulation of cytokine profiles);[10] (iii) in vivo and in vitro antiendotoxic (septic) shock activity (via cytokine downregulation);[11] (iv) regulation of angiogenesis (via modification of cytokine profile);[12] and (v) apoptosis induction by modification of cytokine profiles.[13]

An alternative strategy to the tailoring of sophorolipid structure during the in vivo processing step is to develop methods for the regioselective modification of sophorolipids after microbial synthesis. Surprisingly, only one report exists that describes the enzyme-catalyzed synthesis of a monoacetylated sophorolipid derivative. This involved deacetylation in a biphasic environment using acetylesterase as the catalyst. Low yields (25−30%) were reported after long reaction times.[8]

In this paper, we describe highly regioselective syntheses by the acylation of sophorolipids. These reactions were catalyzed by the lipase from *Candida antarctica* (Novozym 435) in anhydrous tetrahydrofuran at room temperature. The aim was to develop new sophorolipid analogues that would be available for subsequent studies of bioactivity. In addition, analogues were prepared that should be homo- or copolymerizable using conventional polymerization chemistries. The biological properties of these sophorolipid analogues, as well as their use for polymer-forming reactions, will be described elsewhere.

Rationale and Significance

The development of a family of new glycolipid-based building blocks for the preparation of amphiphilic polymers has tremendous potential for use in a wide range of industrial and pharmaceutical applications.[14] Polysaccharide modifications for the synthesis of amphiphilic polymers have been practiced for over a century. However, in view of the complex nature of polysaccharides, their regioselective modification to form well-defined products is tedious and currently only practiced as an academic curiosity. The creation of an arsenal of new glycolipid-based monomers is expected to be useful for the formation of well-defined amphiphilic polymers. These polymers will be of interest as an alternative route to regioselective polysaccharide modifications. Also, polymers bearing sugar residues have been reported to be of great importance for pharmacological applications where the sugar groups play an important role.[14d,e]

Recent studies with microbe- and human-derived glycolipids have demonstrated that these structures are of great interest for both humoral and cellular immune response activation/modulation.[15] Generally, there is a need to better understand how glycolipids participate in the immune response. The methods developed in this paper for the selective modification of sophorolipids, as well as the glycolipid analogues generated herein, will be useful in subsequent studies directed toward elucidating relationships between glycolipid structure and biological properties.

Experimental Section

General Chemicals and Procedures. All chemicals and solvents were analytical grade and were used as received unless otherwise noted. Zeolite, a gift from Rohm and Haas Co., was dried over P_2O_5 in an oven desiccator (0.1 mmHg; 38 h; 50 °C) prior to use. Sophorolipids were synthesized by fermentation of *Torulopsis bombicola* on glucose/oleic acid mixtures following a literature procedure.[8] The biomass was harvested by centrifugation and freeze-drying and then it was extracted with three 100 mL portions of ethyl acetate. The ethyl acetate extracts were combined and the solvent was removed to give a white powder. The residual fermentation fatty acids were removed from this powder by Soxhlet extraction using hexane to give the 'crude' product. Prior to their use, the sophorolipids were dried over P_2O_5 in a vacuum desiccator (0.1 mmHg, 38 h, room temperature).

Column chromatographic separations were performed over silica gel 60 (Aldrich Chemical Co.). In a typical separation, 50 g of silica gel was used to pack a glass column (5 cm × 50 cm) in the eluent ($CHCl_3$/MeOH mixture). Then, 200 mL of the eluent was run through the column before the crude compound (500 mg) dissolved in a minimal volume of eluent was loaded onto the top of the silica bed in the column. Different fractions were subsequently eluted (1 mL/ min) and monitored by thin-layer chromatography (TLC). Fractions containing the purified compound were pooled together, and the solvent was evaporated to give the pure compound.

Porcine pancreatic lipase (PPL) Type II Crude (activity = 61 units/mg protein) and *Candida rugosa* lipase (CCL) Type VII (activity = 4570 u/mg protein) were obtained from the Sigma Chemical Co. The lipases PS-30, AK, and MAP-10 from *Pseudomonas cepacia*, *Pseudomonas fluorescens*, and *Mucor javanicus*, respectively, were obtained from Amano Enzymes Co., Ltd. Specified activities at pH 7.0 were 30 000 units/g, 20 000 units/g and 10 000 units/g, respectively. Immobilized lipases from *C. antarctica* (Novozym 435) and *Mucor miehei*

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(Lipozyme IM) were gifts from Novo Nordisk Inc. All enzymes, prior to their use, were dried over P_2O_5 (0.1 mmHg, 25 °C, 16 h).

Nuclear Magnetic Resonance. ^{1}H and ^{13}C NMR spectra were recorded using Bruker ARX-250 and DRX-500 spectrometers. Chemical shifts in parts per millions are reported downfield from 0.00 ppm using trimethylsilane (TMS) as the internal reference. Unambiguous assignments were derived from COSY and HETCOR spectra. The following abbreviations are used to present the spectral data: s = singlet, brs = broad singlet, d = doublet, dd = doublet of doublet, t = triplet, dt = doublet of triplet, q = quartet, and p = quintet.

Mass Spectrometry Instrumentation. Mass analyses were performed using a Bruker Biflex MALDI-TOF spectrometer. Spectra were acquired using the machine in linear (1.8 m), delayed extraction (150 ns), positive ion mode with a 337 nm laser (3 ns pulse width, Laser Science Inc.). The source voltage was 20 kV for the source plate, 18.5 kV for the extraction plate, and 7.1 kV for the post-source ion lens. The detector (linear) was a dual-microchannel plate type operated at 1.6 kV. The samples were prepared by the following procedure. A solution of the product in THF (10 mg/mL) was mixed 1:5 (v/v) with chloroform/acetonitrile (1:6 v/v) that contained 2,5-dihydroxybenzoic acid (10 mg/mL). This mixture (1 μL) was applied to a polished stainless steel target. Removal of solvents with a stream of dry warm air produced uniform films of small crystals.

Other Instrumental Methods. Optical rotations were measured using a Perkin-Elmer 241 digital polarimeter. Infrared spectra were recorded using a Perkin-Elmer FT-IR spectrometer model 1760 X. The samples for infrared spectroscopy were dissolved in THF and deposited as thin films on NaCl optical disks.

Synthesis of Methyl 17-L-([2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (SL-Me, 1).[3b] In a typical reaction, to a 100 mL round-bottomed flask equipped with a reflux condensor were added 10 g of dry crude sophorolipid and 10 mL 0.022 N sodium methoxide in methanol solution. The reaction assembly was protected from atmospheric moisture by a $CaCl_2$ guard tube. The reaction mixture was refluxed for 3 h, cooled to room temperature (30 °C), and acidified using glacial acetic acid. The reaction mixture was concentrated by rotoevaporation and poured with stirring into 100 mL of ice-cold water that resulted in the precipitation of the sophorolipid methylester as a white solid. The precipitate was filtered, washed with ice−water, and lyophilized (8.77 g, yield 95.0%): $[\alpha]^{25}_D$ −9.77 (c = 0.0145 g/mL, THF); IR [cm^{-1} (%T)] 3354 (1.7), 2929 (2.0), 2860 (3.4), 1741 (4.4), 1165 (4.8), 1074 (2.0), 1030 (3.2); ^{1}H NMR (500 MHz, CD_3OD) δ 1.28 (3H, d, J = 7.0 Hz, H-18), 1.38 (14H, brs, H-4−7 and -12−14), 1.48 (2H, m, H-15), 1.62 (4H, p, J = 7.0 Hz, H-3 and -16), 2.04 (4H, dt, J = 7.0 Hz, H-8 and -11), 2.34 (2H, t, J = 7.5 Hz, H-2), 3.23−3.38 (4H, m, H-2″,-4″,-4′, and -5′), 3.40 (1H, t, J = 8.4 Hz, H-3″), 3.48 (1H, t, J = 8.4 Hz, H-2′), 3.58 (1H, t, J = 8.4 Hz, H-3′), 3.65−3.73 (3H, m, H-6′a, -6″a, and -5″), 3.68 (3H, s, OCH_3), 3.80−3.96 (3H, m, H-6′b, -6″b, and -17), 4.47 (1H, d, J = 7.0 Hz, H-1′), 4.66 (1H, J = 6.7 Hz, H1″) and 5.38 (2H, m, H-9 and -10); ^{13}C NMR (125.8 MHz) δ 20.89, 25.02, 25.26, 27.12, 27.17, 29.13, 29.16, 29.22, 29.39, 29.86, 29.90, 29.95, 33.85, 36.80, 50.99, 61.82, 62.13, 70.56, 70.85, 74.89, 76.76, 76.83, 77.24, 77.31, 77.76, 81.12, 101.69, 103.75, 130.45, 130.60, 175.04; MALDI-TOF m/z 659.84 (M + Na)$^+$.

Synthesis of Ethyl 17-L-([2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (SL-Et, 2). A procedure identical to the one described above for the methyl ester was used to prepare the sophorolipid ethyl ester. Crude sophorolipid (2 g) in 2.5 mL of 0.021 N sodium ethoxide in ethanol was refluxed for 3 h. The identical workup as for analogue **1** gave 1.85 g of the ethyl ester (yield 85%): $[\alpha]^{25}_D$ −10.51 (c = 0.0138 g/mL, THF); IR [cm^{-1} (%T)] 3359 (68.2), 29.20 (67.9), 2850 (70.2), 1738 (70.4), 1076 (65.9), 1040 (67.2); ^{1}H NMR (250 MHz, CD_3OD) δ 1.15 (3H, t, J = 6 Hz, OCH_2CH_3), 1.20 (3H, d, J = 7.2 Hz, H-18), 1.28 (14H, brs, H-4−7 and -12−14), 1.32 (2H, m, H-15), 1.51 (4H, p, J = 7.0 Hz, H-3 and -16), 2.00 (4H, dt, J = 7.0 Hz, H-8 and -11), 2.28 (2H, t, J = 7.5 Hz, H-2), 2.95−3.71 (13H, m, H-Sug and -17), 4.05 (2H, q, J = 7.0 Hz, OCH_2CH$_3$), 4.32 (1H, d, J = 7.0 Hz, H-1′), 4.39 (1H, d, J = 6.9 Hz, H1″) and 5.32 (2H, m, H-9 and -10); ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 14.04, 21.16, 24.36, 24.46, 26.49, 26.56, 28.33 (double), 28.40, 28.58, 28.95, 29.05, 29.09, 33.47, 36.05, 59.49, 61.00, 61.12, 69.98, 70.04, 74.91, 75.66, 76.06, 76.20, 76.41, 76.85, 82.02, 100.92, 103.91, 129.49, 129.61, 172.74; MALDI-TOF m/z 673.30 (M + Na)$^+$.

Synthesis of Butyl 17-L-([2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (SL-Bu, 3). Similarly to the synthesis of the methyl/ethyl esters, 2.5 mL of 0.038 N sodium butoxide in butanol and 2 g of the dry crude sophorolipid were added to a round-bottom flask fitted with a reflux condensor. The reaction mixture was stirred for 3 h at 65 °C. The usual workup procedures gave 1.5 g of the butyl ester (yield 51%): $[\alpha]^{25}_D$ −9.22 (c = 0.0141 g/mL, THF); R_f = 0.38 (chloroform/methanol/water, 32.5:7.5:1); IR [cm^{-1} (%T)] 3369 (49.9), 2929 (46.7), 2856 (56.1), 1737 (56.2), 1462 (67.6), 1379 (66.5), 1173 (60.4), 1077 (43.5); ^{1}H NMR (250 MHz, DMSO-d_6) δ 0.90 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.14 (3H, d, J = 7.2 Hz, H-18), 1.28 (16H, brs, CH_2CH_3, H- 4−7, and -12−14), 1.34 (4H, m, H-15 and $CH_2CH_2CH_3$), 1.55 (4H, p, J = 7.0 Hz, H-3 and -16), 2.00 (4H, dt, J = 7.0 Hz, H-8 and -11), 2.28 (2H, t, J = 7.5 Hz, H-2), 3.00−3.74 (12H, m, H-Sug and -17), 4.05 (2H, t, J = 6.5 Hz, OCH_2CH_2), 4.31 (1H, d, J = 7.2 Hz, H-1′), 4.40 (1H, J = 7.0 Hz, H1″) and 5.32 (2H, m, H-9 and -10); ^{13}C NMR (62.9 MHz, DMSO-d_6) δ 13.44, 18.58, 21.20, 24.44, 24.51, 26.53, 26.61, 28.36 (double), 28.43, 28.63, 28.99, 29.14, 29.25, 30.21, 33.53, 36.10, 61.95, 62.07, 64.17, 70.93, 70.99, 75.86, 76.60, 76.99, 77.14, 77.34, 77.78, 82.98, 101.87, 104.88, 130.41, 130.54, 173.72; MALDI-TOF m/z 702.25 (M + Na)$^+$.

Methyl 17-L-([2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl]-oxy)-*cis*-9-octadeceno-ate 6′,6″-diacrylate (4). To a 50 mL round-bottom flask under dry argon were added 1 g of Novozym 435 and a solution of **1** (500 mg) in 20 mL of dry THF. Excess of vinyl acrylate (2 mL) was then added to the reaction mixture, and the contents were stirred magnetically at 35 °C for 96 h. The reaction setup was secluded from the light with black paper. The usual workup procedure led to the isolation of 0.51 g of the crude product. Product purification by column chromatography yielded 0.48 g (yield 87%) of **4**: $[\alpha]^{25}_D$ −1.38 (c = 0.0125 g/mL, THF); IR [cm^{-1} (%T)] 3394 (26.9), 2928 (16.6), 2856 (26.8), 1729 (9.8), 1636 (54.9), 1618 (61.2), 1438 (42.1), 1408 (28.4), 1375 (41.3), 1297 (25.5), 1276 (31.2), 1198 (13.9), 1082 (9.8); ^{1}H NMR (250 MHz, CD_3OD) δ 1.20 (3H, d, J = 6.5 Hz, H-18), 1.38 (16H, brs, H-4−7 and -12−15), 1.62 (4H, p, J = 7.0 Hz, H-3 and -16), 2.04 (4H, dt, J = 7.0 Hz, H-8 and -11), 2.34 (2H, t, J = 7.5 Hz, H-2), 3.23−3.65 (8H, m, H-2″−5″ and -2′−5′), 3.68 (3H, s, OCH_3), 3.76 (1H, m, H-17), 4.25−4.94 (4H, m, H-6′ and 6″), 4.49 (1H, d, J = 7.0 Hz, H-1′), 4.58 (1H, J = 6.7 Hz, H1″), 5.38 (2H, m, H-9 and -10), 5.92 (2H, 2dd, J = 10.1 and 2.0 Hz, COCH=CH_{2cis}), 5.92 (2H, 2dd, J = 17.0 and 10.0 Hz, COCH=CH$_2$) and 6.44 (1H, 2dd, 17.0 and 2.0 Hz, COCH=C$H_{2\ trans}$); ^{13}C NMR (62.9 MHz, CD_3OD) δ 20.81, 25.01, 25.27, 27.11, 27.15, 29.10, 29.13, 29.18, 29.36, 29.73 (double), 29.85, 33.85, 36.81, 50.98, 63.91, 64.02, 70.46, 70.67, 73.96, 74.69, 75.11, 76.64, 76.89, 77.41, 82.97, 101.49, 104.74, 128.44 (double), 129.79, 129.95, 130.63, 130.75, 166.51, 166.57, 175.04; MALDI-TOF m/z 767.55 (M + Na)$^+$.

Methyl 17-L-([2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl]-oxy)-*cis*-9-octadeceno-ate 6′,6″-diacetate (5). Compound **1** (500 mg) was dissolved in 20 mL of dry THF. To this solution were added vinyl acetate (2 mL) and Novozym 435 (1 g), and the suspension was stirred magnetically at 35 °C for 96 h. The enzyme was filtered off, the solvent was evaporated, and the product was purified by column chromatography (eluent $CHCl_3$/MeOH, 9:1) to give 515 mg (yield 91%) of **5**: $[\alpha]^{25}_D$ −7.22 (c = 0.0115 g/mL, THF); IR [cm^{-1} (%T)] 3392 (43.8), 2927 (35.0), 2855 (44.0), 1742 (26.4), 1437 (54.8), 1370 (46.9), 1244 (34.7), 1173 (49.0), 1081 (31.7), 1037 (34.7); ^{1}H NMR (250 MHz, CD_3OD) δ 1.20 (3H, d, J = 6.5 Hz, H-18), 1.30 (18H, brs, H- 4−7 and -12−15), 1.62 (4H, p, J = 7.0 Hz, H-3 and -16), 2.00 (4H, m, H-8 and -11), 2.08 (6H, s, COCH_3),

2.30 (2H, t, J = 7.5 Hz, H-2), 3.23−3.60 (8H, m, H-2″−5″ and -2′−5′), 3.66 (3H, s, OCH_3), 3.76 (1H, m, H-17), 3.90−4.40 (4H, m, H-6′ and 6″), 4.46 (1H, d, J = 7.1 Hz, H-1′), 4.54 (1H, 7.2 Hz, H1″), 5.38 (2H, m, H-9 and -10); ^{13}C NMR (62.9 MHz, CD_3-OD) δ 19.69 (double), 20.08, 24.04, 24.38, 26.30 (double), 28.19 (triple), 28.41, 28.77 (double), 28.85, 33.23, 35.53, 50.52, 62.85, 62.90, 69.06, 69.50, 72.67, 72.96, 73.74, 75.13, 75.26, 76.34, 80.82, 99.94, 103.06, 128.95 (double), 171.75, 171.95, 174.33; MALDI-TOF m/z 744.22 $(M + Na)^+$.

Ethyl 17-L-([2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl]-oxy)-*cis*-9-octadeceno-ate 6′,6″-diacetate (6). Compound **2** (500 mg) was dissolved in 20 mL of dry THF. To this solution were added vinyl acetate (2 mL) and Novozym 435 (1 g), and the suspension was stirred magnetically at 35 °C for 96 h. The enzyme was filtered off, the solvent was evaporated, and the product was purified by column chromatography (eluent $CHCl_3$/MeOH, 9:1) to give 490 mg (yield 87%) of **6**: $[\alpha]^{25}_D$ −2.11 (c = 0.0121 g/mL, THF); IR [cm^{-1} (%T)] 3392 (29.8), 2928 (20.4), 2855 (32.5), 1740 (10.5), 1446 (49.7), 1370 (31.4), 1244 (17.3), 1177 (34.7), 1082 (9.8), 1037 (16.0); ^{1}H NMR (250 MHz, $CDCl_3/C_6D_6$) δ 1.16 (3H, t, J = 7.0 Hz, OCH_2CH_3), 1.32 (19H, brs, H- 4−7 and -12−15, 18), 1.62 (4H, p, J = 7.0 Hz, H-3 and -16), 2.00 (3H, s, $COCH_3$, 2.09 (4H, m, H-8 and -11), 2.23 (2H, t, J = 7.2 Hz, H-2), 3.33−3.65 (8H, m, H-2″−5″ and -2′−5′), 3.81 (1H, m, H-17), 4.07 (2H, q, J = 7.0 Hz, OCH_2CH_3), 4.40−4.60 (5H, m, H-6′, 6″, and 1′), 4.70 (1H, d, J = 7.0 Hz, H1″), 5.45 (2H, m, H-9 and -10); ^{13}C NMR (62.9 MHz, C_6D_6) δ 14.4, 20.79, 20.99, 21.61, 25.39, 25.98, 27.71, 27.82, 29.51, 29.54, 29.61, 29.96, 30.16, 30.29, 30.37, 34.52, 37.09, 60.08, 64.31(double), 70.96, 71.15, 74.17, 75.04, 75.40, 76.77, 77.19, 77.56, 83.77, 101.54, 105.34, 130.28, 130.32, 171.75, 171.95, 174.33; MALDI-TOF m/z 757.36 $(M + Na)^+$.

Butyl 17-L-([2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl]-oxy)-*cis*-9-octadeceno-ate 6′,6″-diacetate (7). Compound **3** (500 mg) was dissolved in 20 mL of dry THF. To this solution were added vinyl acetate (2 mL) and Novozym 435 (1 g), and the suspension was stirred magnetically at 35 °C for 96 h. The enzyme was filtered off, the solvent was evaporated, and the product was purified by column chromatography (eluent $CHCl_3$/MeOH, 9:1) to give 483 mg (yield 86%) of **7**: $[\alpha]^{25}_D$ −1.63 (c = 0.0183 g/mL, THF); IR [cm^{-1} (%T)] 3373 (39.3), 2929 (29.4), 2856 (41.5), 1740 (20.0), 1454 (56.0), 1370 (43.2), 1244 (28.0), 1175 (43.3), 1082 (24.0), 1037 (27.8); ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.95 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.24 (3H, d, J = 7.2 Hz, H-18), 1.30 (16H, brs, H-4−7, CH_2CH_3, and -12−14), 1.45 (4H, m, H-15 and -$CH_2CH_2CH_3$), 1.60 (4H, p, J = 7.0 Hz, H-3 and -16), 2.00 (4H, dt, J = 7.0 Hz, H-8 and -11), 2.10 (6H, s, $COCH_3$), 2.28 (2H, t, J = 7.5 Hz, H-2), 3.23−3.65 (8H, m, H-2″−5″ and -2′−5′), 4.10 (2H, t, J = 6.5 Hz, H-OCH_2CH_2), 3.76 (1H, m, H-17), 4.25−4.44 (4H, m, H-6′ and 6″), 4.45 (1H, d, J = 7.2 Hz, H-1′), 4.52 (1H, d, J = 7.0 Hz, H1″) and 5.32 (2H, m, H-9″ and -10″); ^{13}C NMR (62.9 MHz, $CDCl_3$) δ 13.69, 19.18, 20.90 (double), 21.13, 25.05, 25.36, 27.26 (double), 29.15 (double), 29.18, 29.36, 29.71 (double), 29.78, 30.79, 34.44, 36.36, 63.46, 63.61, 64.14, 70.00, 70.56, 73.52 (double), 74.71, 75.89, 76.12, 77.28, 81.44, 100.69, 103.79, 129.82, 129.93, 171.75, 171.95, 174.33; MALDI-TOF m/z 786.46 $(M + Na)^+$.

Methyl 17-L-([2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl]-oxy)-*cis*-9-octadeceno-ate 6′,6″-disuccinate (8). Compound **1** (500 mg) was dissolved in 20 mL of dry THF. To this solution were added succinic anhydride (2 mL) and Novozym 435 (1 g), and the suspension was stirred magnetically at 35 °C for 96 h. The enzyme was filtered off, the solvent was evaporated, and the product was purified by column chromatography (eluent $CHCl_3$/MeOH, 8:2) to give 492 mg (yield 75%) of **8**: $[\alpha]^{25}_D$ −3.57 (c = 0.0110 g/mL, THF); IR [cm^{-1} (%T)] 3373 (56.9), 2927 (50.9), 2855 (58.4), 1735 (40.7), 1374 (63.9), 1168 (51.1), 1078 (49.7); ^{1}H NMR (250 MHz, CD_3OD) δ 1.12 (3H, d, J = 7.0 Hz, H-18), 1.25 (16H, brs, H- 4−7 and -12−15), 1.52 (4H, p, J = 7.0 Hz, H-3 and -16), 1.98 (4H, dt, 7.0 Hz, H-8 and -11), 2.20 (2H, t, J = 7.5 Hz, H-2), 2.50 (8H, m, $COCH_2CH_2$-COOH), 3.10−3.60 (8H, m, H-2″−5″ and -2′−5′), 3.56 (3H, s, OCH_3), 3.68 (1H, m, H-17), 4.15−4.30 (4H, m, H-6′ and -6″), 4.35 (1H, d, J = 7.0 Hz, H-1′), 4.50 (1H, d, J = 6.7 Hz, H1″), 5.28 (2H, m, H-9 and -10); ^{13}C NMR (62.9 MHz, CD_3OD) δ 20.86, 25.01, 25.27, 27.11, 27.17, 28.85, 28.90, 28.98, 29.12, 29.18 (double), 29.37 (double), 29.76, 29.86, 33.85, 36.84, 51.00, 63.97, 63.99, 70.48, 70.59, 73.98, 74.68, 74.90, 76.59, 76.85, 77.47, 82.36, 101.48, 104.35, 129.81, 129.97, 171.75, 171.95, 174.33; MALDI-TOF m/z 861.29 $(M + Na)^+$.

Novozym 435 catalyzed synthesis of 17-L-([2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoic acid 1′, 6″-lactone (sophorolactone, 9). For the following, an inert atmosphere was maintained using a glovebag and dry argon. To an oven dried 100 mL round-bottomed flask were transferred 1.5 g of methyl **17-L-**([2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (**1**), 2.2 g of zeolite, 2 g of Novozym 435 (dried in a vacuum desiccator, 0.1 mmHg, 25 °C, 16 h), and dry THF (50 mL), and the round-bottomed flask was immediately stoppered. The flask was then placed in a constant temperature oil bath maintained at 35 °C for 96 h, and the contents were stirred magnetically. A control reaction was set up as described above except Novozym 435 was not added. TLC ($CHCl_3$/MeOH, 7:3) was used to follow the progress of the reaction. The reaction was quenched by removing the enzyme and zeolite by vacuum filtration (glass fritted filter, medium porosity), the enzyme was washed 3−4 times with 5 mL portions of THF, the filtrates were combined, and solvent was removed in vacuo to give 1.45 g of the product. The crude product (1.45 g) was purified by column chromatography over silica gel (100 g, 130−270 mesh, 60 Å, Aldrich) using a gradient solvent system of chloroform/methanol (2 mL/minute) with increasing order of polarity to give 1.2 g (yield 84%) of purified product: $[\alpha]^{25}_D$ −4.25 (c = 0.0141 g/mL, THF); IR [cm^{-1} (%T)] 3360 (50.1), 2927 (44.0), 2855 (52.0), 1735 (53.1), 1458 (65.5), 1375 (64.4), 1174 (58.9), 1076 (39.3); ^{1}H NMR (500 MHz, CD_3OD) δ 1.24 (3H, d, J = 7.0 Hz, H-18), 1.38 (14H, brs, H-4−7 and -12−14), 1.42 (2H, m, H-15), 1.62 (4H, p, J = 7.0 Hz, H-3 and -16), 2.08 (4H, dt, J = 7.0 Hz, H-8 and -11), 2.36 (2H, t, J = 7.5 Hz, H-2), 3.23−3.48 (4H, m, H-2″,-4″,-2″, and -5′), 3.48 (2H, m, H-3′ and -5″), 3.58 (2H, m, H-3″ and -4′), 3.7 (1H, m, H-6′a), 3.85 (2H, m, H-6′b and -17), 4.20 (1H, m, H-6″a), 4.42 (1H, m, H-6″b), 4.49 (1H, d, J = 7.0 Hz, H-1′), 4.52 (1H, d, J = 6.7 Hz, H1″) and 5.38 (2H, m, H-9 and -10); ^{13}C NMR (125.8 MHz) δ 20.71, 24.70, 25.47, 26.15, 27.21, 27.88, 28.21, 28.66, 28.97, 29.57, 29.72, 29.99, 33.99, 36.49, 61.76, 63.38, 70.12, 70.40, 74.69, 75.24, 76.58, 76.67 (double), 77.05, 83.87, 101.11, 105.41, 129.78, 130.29, 174.30; MALDI-TOF m/z 627.95 $(M + Na)^+$.

17-L-([2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoic acid 1′,6″-lactone 6′-acrylate (10). To an oven-dried 100 mL round-bottomed flask were added **9** (1.0 g), 2.0 g of zeolite, and 3 g of Novozym 435 that was dried (0.1 mmHg, 25 °C, 16 h). Then, dry THF (30 mL) was added, and the round-bottomed flask was immediately stoppered. Vinyl acrylate (2.5 mL) was added, and the round-bottomed flask was wrapped with black paper to seclude it from the light. This flask was then placed in a constant temperature oil bath (35 °C) with magnetic stirring for 96 h. A control reaction was set up as was described above except that Novozym 435 was not added. Progress of the reaction was followed by TLC ($CHCl_3$/MeOH, 9:1). The reaction was quenched by removing the enzyme and zeolite by vacuum filtration (glass fritted filter, medium porosity), the enzyme was washed 3−4 times with 5 mL portions of THF, the filtrates were combined, and solvent was removed in vacuo to give 1.5 g of the product. The crude product (1.05 g) was purified by column chromatography over silica gel (50 g, 130−270 mesh, 60 Å, Aldrich) using a gradient solvent system of chloroform/methanol (2 mL/minute) with increasing order of polarity to give 0.9 g (yield 83%) of purified product: $[\alpha]^{25}_D$ −2.81 (c = 0.0116 g/mL, THF); IR [cm^{-1} (%T)] 3378 (49.6), 2928 (42.8), 2854 (50.1), 1735 (39.8), 1710 (52.0), 1457 (63.4), 1410 (61.9), 1354 (60.3), 1295 (55.3), 1185 (49.6), 1077 (29.7); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.22 (3H, d, J = 7.0 Hz, H-18), 1.38 (14H, brs, H-4−7 and -12−14), 1.42 (2H, m, H-15), 1.66 (4H, p, J = 7.0 Hz, H-3 and -16), 2.08 (4H, dt, J = 7.0 Hz, H-8 and -11), 2.36 (2H, t, 7.5 Hz, H-2), 3.23−3.70 (8H, m, H-2″−5″ and -2′−5′), 3.78 (1H, q, H-17), 4.10−4.64 (6H, m, H-6′, -6″, -1′, and -1″), 5.38 (2H, m, H-9 and -10), 5.92 (1H,

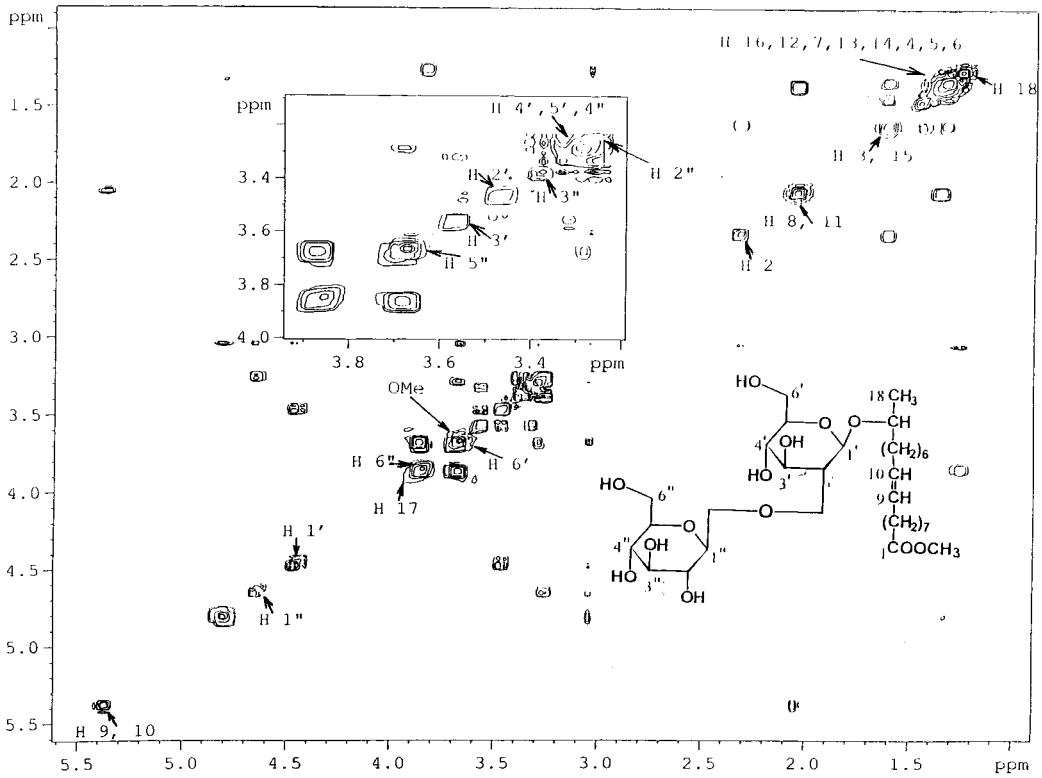


Figure 2. $^1H-^1H$ COSY NMR spectrum of **1** (500 MHz, CD_3OD).

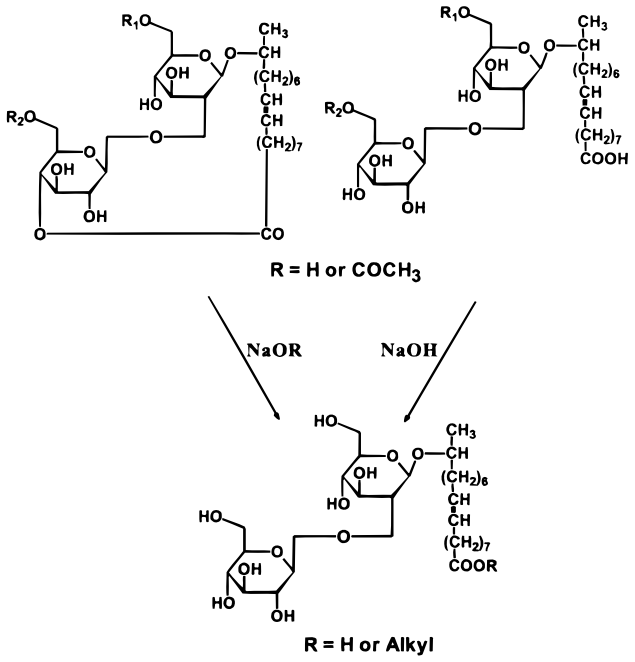
dd, J = 10.1 and 2.0 Hz, COCH=CH_{2cis}), 5.92 (1H, dd, J = 17.0 and 10.0 Hz, COC*H*=CH_2) and 6.44 (1H, dd, J = 17.0 and 2.0 Hz, COCH=CH_{2trans}); ^{13}C NMR (125.8 MHz) δ 20.66, 24.67, 25.48, 26.12, 27.15, 27.88, 28.18, 28.67, 28.92, 29.47, 29.61, 29.92, 33.97, 36.64, 63.41, 63.88, 70.18, 70.67, 73.99, 74.69, 75.34, 76.69, 76.92, 77.00, 83.57, 101.24, 105.28, 128.42, 129.77, 130.25, 130.58, 166.58, 174.80; MALDI-TOF *m/z* 681.90 $(M + Na)^+$.

Results and Discussion

Sophorolipids produced by the yeast *T. bombicola* grown on oleic acid and glucose give a mixture of at least eight different compounds. The products exist mainly in the lactonic and acidic forms with variable degrees of acetylation at position(s) 6′ and 6″ (Figure 1).[2b] Scheme 1 shows the strategy that was conceived for the preparation of well-defined ring-opened sophorolipids that are esterified at the fatty acid carboxyl terminus. Hence, Scheme 1 shows how pure compounds can be prepared from a complex mixture of sophorolipids. This route was considered as an alternative to the isolation and purification of individual pure components from the sophorolipid mixture. Because pure natural sophorolipid structures are of considerable interest, one goal of this work was to use the ester intermediates to generate a range of pure sophorolipids that would either be identical or closely related to various natural structures.

Initially, hydrolysis of the mixture with NaOH was performed following a literature procedure[3b] to obtain the deacetylated acidic sophorolipid (SL_8). Unfortunately, this product was soluble only in highly polar solvents such as water, DMF, DMSO, and pyridine. Because lipase activity is known to be drastically reduced in polar aprotic solvents and water is not a good medium for esterification reactions, an alternative strategy for generating a single pure compound from the sophorolipid mixture was required. Hence, the synthesis of methyl and ethyl sophorolipid esters was conducted by reaction of the mixture with sodium methoxide and ethoxide (Scheme 1).

Scheme 1



Synthesis of Ester Sophorolipid Derivatives. The sophorolipid mixture was refluxed with an alcoholic solution of sodium methoxide to yield the methyl ester (SL-Me, **1**).[3b] The optically active compound, $[\alpha]^{25}_D$ −9.77, was isolated in 95% yield. The product was soluble in anhydrous THF and was identified on the basis of the results of a MALDI-TOF mass spectrum (*m/z* 659.85 $(M + Na)^+$) and detailed spectral data. This sophorolipid methyl ester was described earlier, but its complete

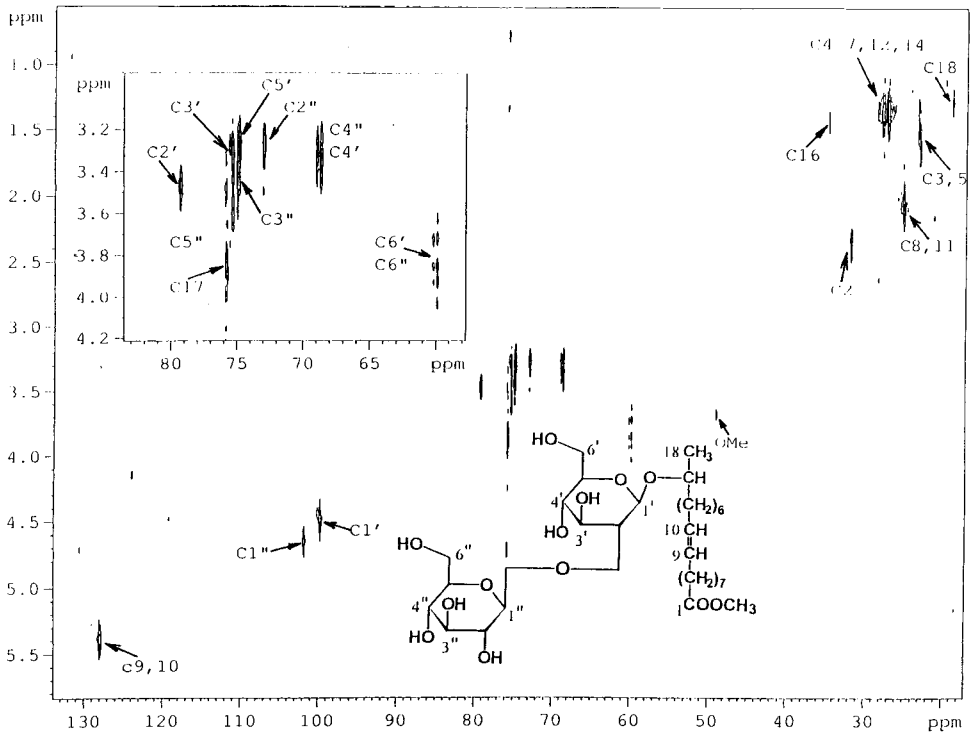
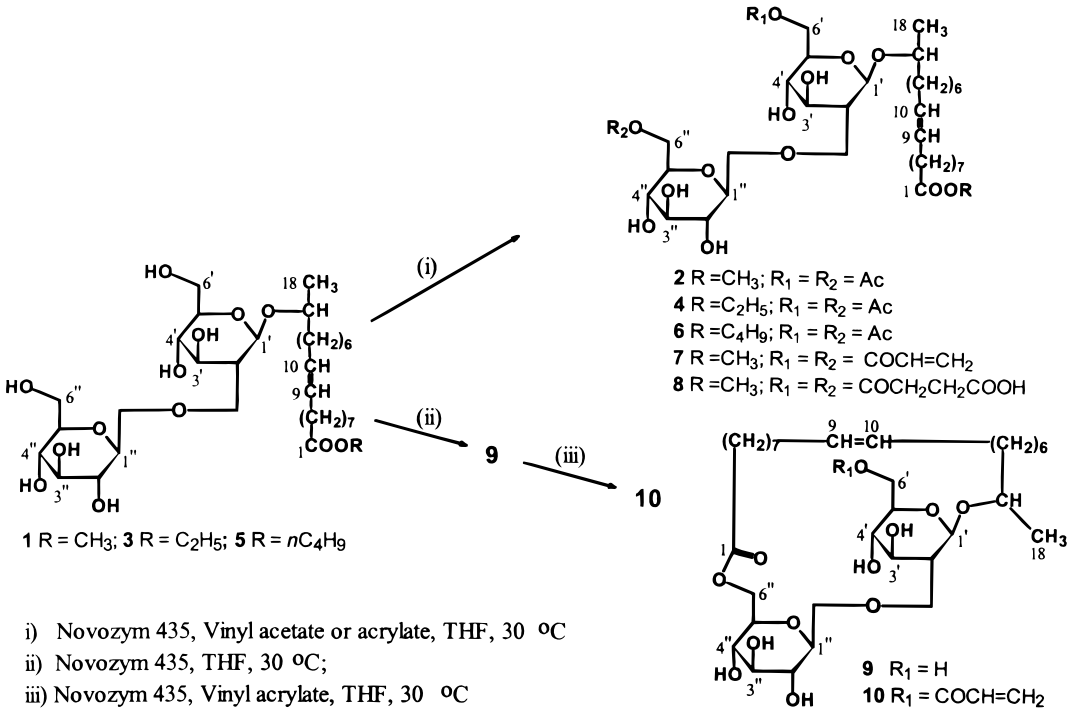


Figure 3. $^{1}H-^{13}C$ HETCOR NMR spectrum of **1** (500 MHz, CD_3OD). Insert shows resonances for the methine carbons of the sophorose rings.

Scheme 2



spectral data was not given.[3b] The ^{1}H NMR spectrum of **1** was complex, and chemical shifts were spread over the 1.2−5.5 ppm range. The acetyl group resonances observed around 2.2 ppm for the natural sophorolipid mixture were no longer seen, and the methyl ester group was a singlet at 3.68 ppm. The assignments shown were derived from a $^{1}H-^{1}H$ COSY 45 NMR spectrum (Figure 2). The ^{13}C NMR spectrum of **1** showed a signal corresponding to the methyl group at 50.99 ppm. The carbonyl group (C-1, Scheme 2) resonance was observed at 175.04 ppm. Assignments of the resonances in the ^{13}C NMR spectrum were made on the basis of a $^{1}H-^{13}C$ HETCOR experiment (Figure 3). By similarly reacting the natural sohorolipid mixture with sodium ethoxide ($NaOC_2H_5$) and sodium butoxide ($NaOC_4H_9$), the ester derivatives ethyl **17**-L-([2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (SL-Et, **2**) and butyl **17**-L-([2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (SL-Bu, **3**) were formed.

Enzyme Screening. The SL-Me was next subjected to lipase-catalyzed esterification with vinyl acrylate in dry THF (Scheme 2). A study to determine the efficiency

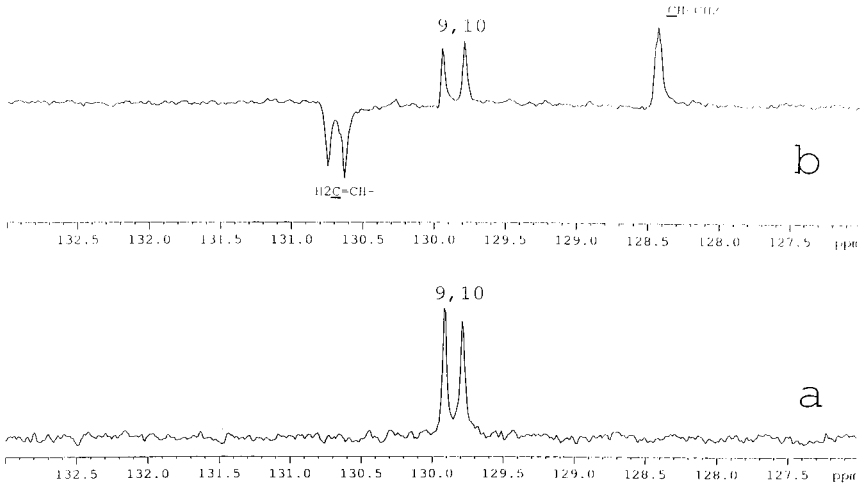


Figure 4. Regions at 126−134 ppm of the DEPT 135 spectra of (a) the SL-Me, **1** and (b) the 6′,6″-diacrylate derivative, **4**.

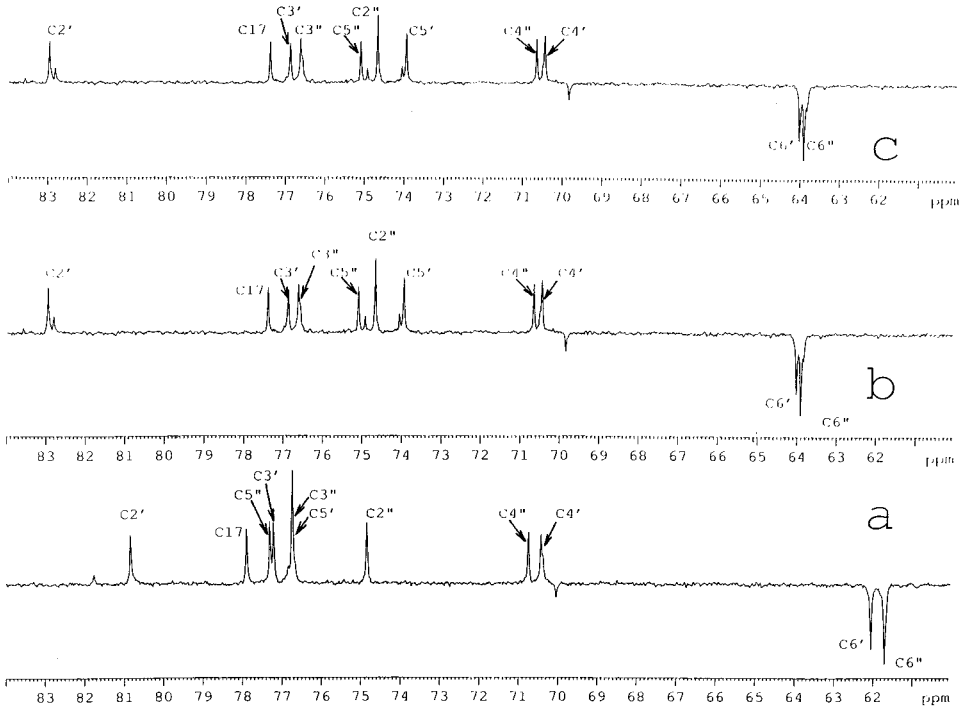


Figure 5. Regions at 61−84 ppm of the DEPT 135 spectra of (a) the SL-Me, **1**; (b) the 6′,6″-diacrylate derivative, **4**, and (c) the 6′,6″-diacetate derivative, **5**.

of different lipases (PPL, CCL, PS-30, AK, MAP-10, Novozym 435, and Lipozyme IM) for the acylation of **1** in dry organic solvent at room temperature was performed. In 48 h, all lipases showed conversion, and the extent of conversion varied considerably with the source (PPL ≈ 40%, CCL < 30%, PS-30 ≈ 50%, AK ≈ 50%, MAP-10 < 10%, and Novozym 435 > 70%). Hence, Novozym 435 was found to be the lipase of choice for the acylation with vinyl acrylate.

Lipase-Catalyzed Regioselective Acylations. Initial attempts at the synthesis of the monoacryl sophorolipid derivative were conducted using excess vinyl acrylate and Novozym 435 as the catalyst (Scheme 2). The resulting product was purified by column chromatography over silica gel and was identified from its proton and carbon NMR spectra. The compound had an $(M + Na)^+$ ion peak at *m*/*z* 767.55 from MALDI-TOF, which is 43 mass units higher than that calculated for the monoacryl derivative. The proton NMR of the compound showed resonances at 5.92−6.44 ppm due to two acryl groups in the molecule. The proton NMR spectrum of the compound showed a downfield shift in the resonance positions of both the C-6′ and C-6″ protons. However, conclusive determination of the position of the acryl groups in the molecule was not possible from the proton NMR spectrum due to its complexity. The ^{13}C NMR spectrum of this product was edited using a DEPT 135 pulse sequence to separate out the resonances due to the methine and methyl carbons from those due to the methylene carbons. Expanded regions of the DEPT 135 ^{13}C NMR spectra of compounds **1** and **4** are shown in Figures 4 and 5. The DEPT 135 spectrum of the product clearly showed peaks due to two acryl groups ($COCH{=}CH_2$) at 129.79, 129.95, 130.63, and 130.75 (Figure 4b). The peaks due the methine carbons appear inverted. Additionally, carbons C-9 and C-10 in the lipid chain of the molecule appeared at 129.79 and 129.95 ppm. Importantly, the resonances at 63.91 and 64.02 ppm for C-6′ and C-6″, respectively,

were 2.0 ppm downfield compared to the corresponding methyl ester (Figure 5b). Hence, these results suggest the formation of a diacryl product that is acylated at positions C-6′ and C-6″. Interestingly, it was observed that the resonance position of C-2′ (carbon bearing no free hydroxyl group) was shifted downfield by ~2 ppm upon formation of the acyl ester at C-6′ and C-6″. This downfield shift in the resonance position of C-2′ might very well be due to the conformational changes in the lipid structure upon acylation. Furthermore, an upfield shift of 2.5 ppm for the resonances corresponding to C-5′ and C-5″ and no significant changes in the resonance positions of the other carbons in the molecule were found (Figure 5b). The upfield shift in the resonance positions of C-5′ and C-5″ is consistent with the γ-effect that is caused by the attachment of the acryl groups at the C-6′ and C-6″ hydroxyls. Therefore, these NMR results showed that the Novozym 435 catalyzed acylation proceeded in a highly regioselective fashion where only the C-6′ and C-6″ hydroxyls in the molecule were acrylated. This product (**4**) was therefore conclusively identified as methyl **17**-L-([2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate 6′,6″-diacrylate.

The above reaction was further extended to prepare other related 6′,6″-diacylated derivatives. Methyl **17**-L-([2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate 6′,6″-diacetate (**5**) was prepared by the Novozym 435 catalyzed acylation of methyl **17**-L-([2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate (SL-Me, **1**), using vinyl acetate in dry THF. Compound **5** was isolated by column chromatography over silica gel (*m*/*z* 744.22 $(M + Na)^+$, $[\alpha]^{25}_D$ −7.22). The ^{1}H NMR spectrum of **5** showed resonances similar to those of **1**. The two acetyl groups appeared as a singlet at 2.08 (6H) ppm. In the ^{13}C NMR spectrum, the resonances for the C-6′ and C-6″ protons were observed ~0.5 ppm downfield relative to **1**. The DEPT 135 ^{13}C NMR spectrum of **5** showed the presence of the two acetyl groups in the molecule (19.69 (double) ppm) and also confirmed the positions of acylation. The DEPT 135 spectrum of **5**, when compared to that of **1**, showed a downfield shift of about 2.0 ppm in the resonance position of C-6′ and C-6″ and an upfield shift of about 2.5 ppm in the resonance position of C-5′ and C-5″ (Figure 5c). Furthermore, except for the downfield shift of ~2 ppm in the resonance position of C-2′, there were no observable changes in the resonance position of the other carbons in the molecule. Hence, it was concluded that the acetylation of **1** with vinyl acetate catalyzed by the lipase Novozym 435 was highly regioselective.

Acetylation of SL-Et (**2**) and SL-Bu (**3**) with vinyl acetate in dry THF catalyzed by Novozym 435 also led to the formation of ethyl 17-L-([2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate 6′,6″-diacetate (**6**, *m*/*z* 757.36 $(M + Na)^+$, $[\alpha]^{25}_D$ −2.11 (c = 0.0121 g/mL)) and butyl 17-L-([2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate 6′,6″-diacetate (**7**, *m*/*z* 786.46 $(M + Na)^+$, $[\alpha]^{25}_D$ −1.63 (c = 0.0183 g/mL)) in a regioselective fashion. Spectral data for **6** and **7** were similar to that observed for **5** with additional resonances that correspond to the ester alkyl group. The DEPT 135 spectra of **6** and **7**, when compared to that of **1**, also showed a downfield shift of about 2.0 ppm in the resonance position of C-6′ and C-6″ and an upfield shift of about 2.5 ppm in the resonance position of C-5′ and C-5″.

The preparation of regioselectively modified sophorolipids that contain carboxyl functionalities is of interest. Hence, the acylation of **1** as above was conducted using succinic anhydride as the acylating agent to prepare methyl 17-L-([2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl]-oxy)-*cis*-9-octadecenoate 6′,6″-disuccinate (**8**). Compound **8** was purified by column chromatography which gave a viscous colorless liquid (*m*/*z* 861.29 $(M + Na)^+$, $[\alpha]^{25}_D$ −3.57). In the ^{1}H NMR spectrum of **8**, the methylene protons of the succinate groups appeared at 2.50 (8H) ppm. Also, the resonances for the C-6′ and C-6″ protons were shifted downfield (~0.5 ppm) from those observed in **1**. The assignment of the resonances in the ^{1}H NMR spectrum was made from a ^{1}H−^{1}H COSY 45 experiment performed on **8** (Figure 6). The position of the succinate groups at C-6′ and C-6″ was established from the DEPT 135 ^{13}C NMR spectrum. When compared to the DEPT 135 ^{13}C NMR spectrum of **1**, the resonances for the C-6′ and C-6″ in **8** were shifted downfield by about 2.0 ppm and resonances for the C-5′ and C-5″ were shifted upfield by 2.5 ppm. The observed NMR studies established that succinylation of the C-6′ and C-6″ hydroxyl groups did indeed occurred regioselectively.

Synthesis of Sophorolactone. One of the objectives of this work was the site-selective synthesis of a monoacryl derivative of **1**. Such a product could be used as a glycolipid monomer that is polymerizable to linear chains through well-established free radical methods. For this purpose, reactions of **1** were conducted where the ratio of **1** to vinyl acrylate was varied. When the ratio of **1** to vinyl acrylate was 1:1 or less, **9** ($[\alpha]^{25}_D$ −4.25, *m*/*z* 627.95 $(M + Na)^+$) was formed (see Scheme 2). Compound **9** was separated from the unreacted SL-Me by column chromatography. Comparison of the ^{1}H NMR spectra for **9** and **1** showed substantial differences in the appearance of the proton signals in the 3.25−4.5 ppm region that are due to carbohydrate protons. Assignments to resonances were made from a ^{1}H−^{1}H COSY NMR spectrum (Figure 7). The two doublets assigned to H-1′ and -1″ at 4.49 and 4.52, respectively, were shifted so that they are in closer proximity for **1** than for **9**. Furthermore, the ^{1}H NMR spectrum of **9** did not indicate that acrylation occurred and had no resonances corresponding to the methyl ester. These anomalous features of the ^{1}H NMR spectrum of **9** suggested the formation of a lactone where the ester linkage was between the carboxylic acid end group of the fatty acid chain and one of the hydroxyl groups of the sophorose ring. The ^{1}H NMR spectrum of **9** also showed a 0.5 ppm downfield shift in the resonance position of the C-6″ protons, suggesting participation of the C-6″ hydroxyl group in the formation of the lactone ring. However, conclusive determination of the hydroxyl group(s) on the sophorose moiety that took part in lactone formation required additional studies by ^{13}C NMR. Specifically, the ^{13}C NMR of **9** was recorded after editing by the DEPT 135 pulse sequence. This permitted resolution of the resonances due to the methyl, methylene, and methine carbons. The DEPT 135 spectra of **1** and **9** were compared (Figures 5a and 8a). A downfield shift of 1.5 ppm in the resonance position of the C-6″ was accompanied by an upfield shift of 1.3 ppm in the resonance position of the C-5″ (Figure 8a). This observation confirmed that a bond between the hydroxyl of C-6″ and the lipid carbonyl carbon (C-1, Scheme 2) formed the lactone ester.

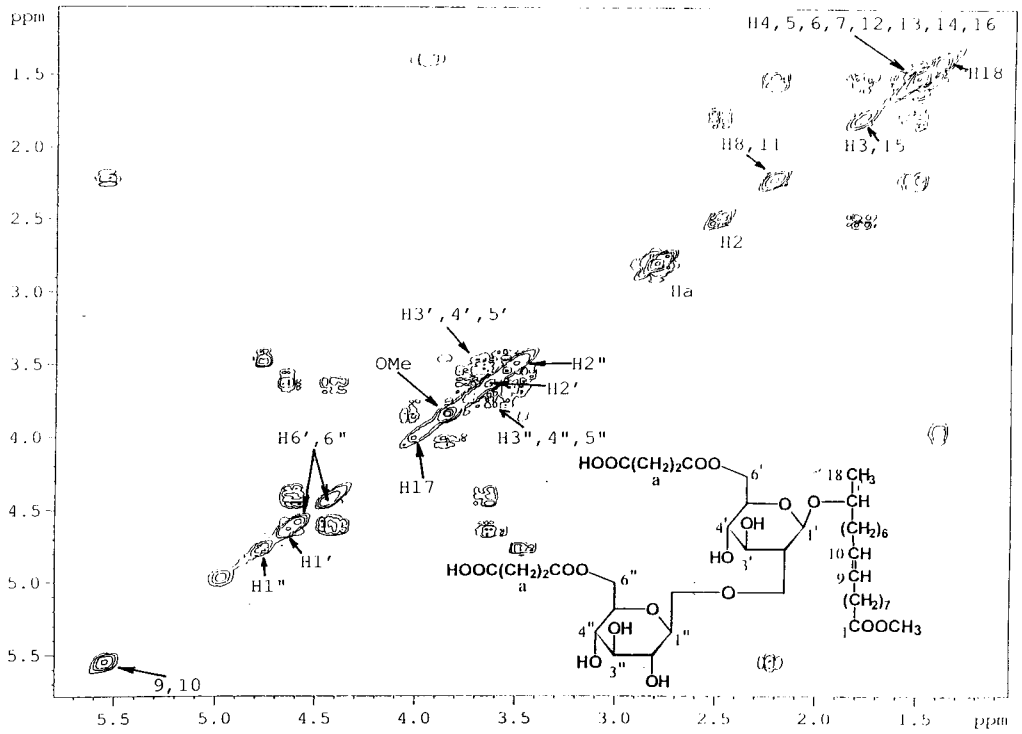


Figure 6. $^1H-^1H$ COSY NMR spectrum of **8** (250 MHz, CD_3OD).

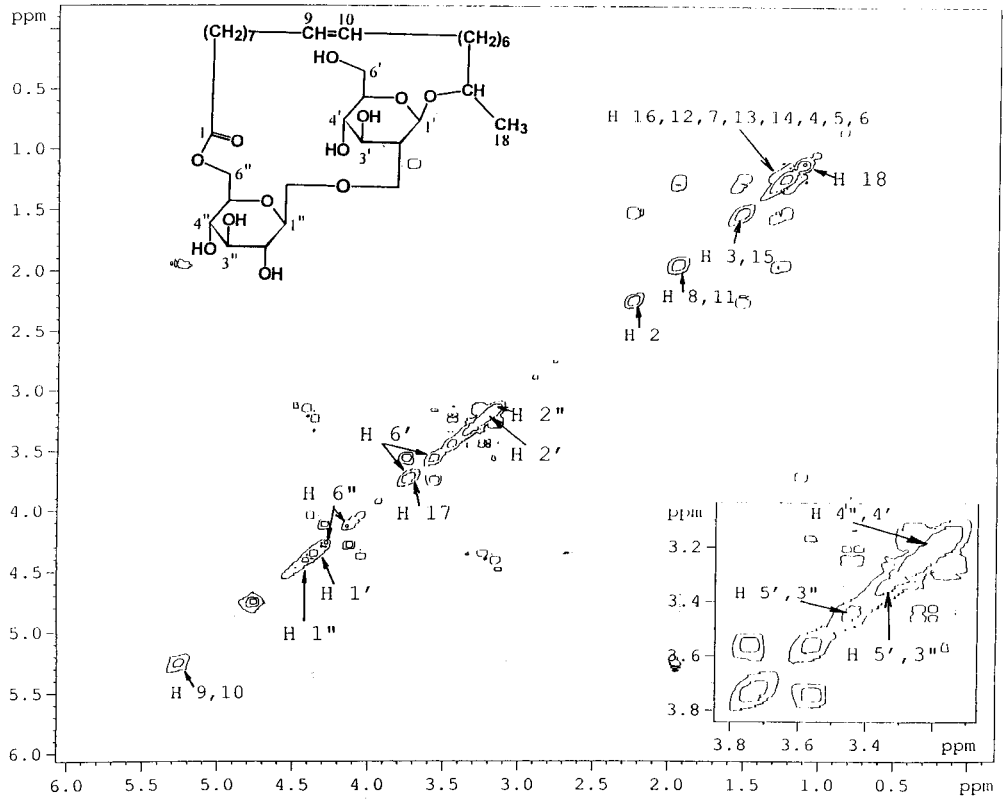


Figure 7. $^1H-^1H$ COSY NMR spectrum of sophorolactone **9** (250 MHz, CD_3OD).

The structure of the lactone **9** is very interesting, as it is an unnatural analogue of the microbially produced macrolactone. The lactone **9** differs in the site at which the sophorose ring is attached to the fatty acid. Specifically, in **9**, unlike the natural sophorolipids, the fatty acid carboxyl carbon (C-1) is linked to the C-6″ hydroxyl, not to the C-4″ hydroxyl. Interestingly, Jones[16] proposed a structure based on the lactonic structure **9** for a diacetyl sophorolipid isolated from a strain of *Torulopsis gropengiesseri*. The structure was proposed to be a 1,6″-lactone having acetate groups at the hydroxyls of C-6′ and C-3″. However, Tulloch et al.[3b] later isolated the same com-

(16) Jones, D. F. *J. Chem. Soc. C* **1967**, 479.

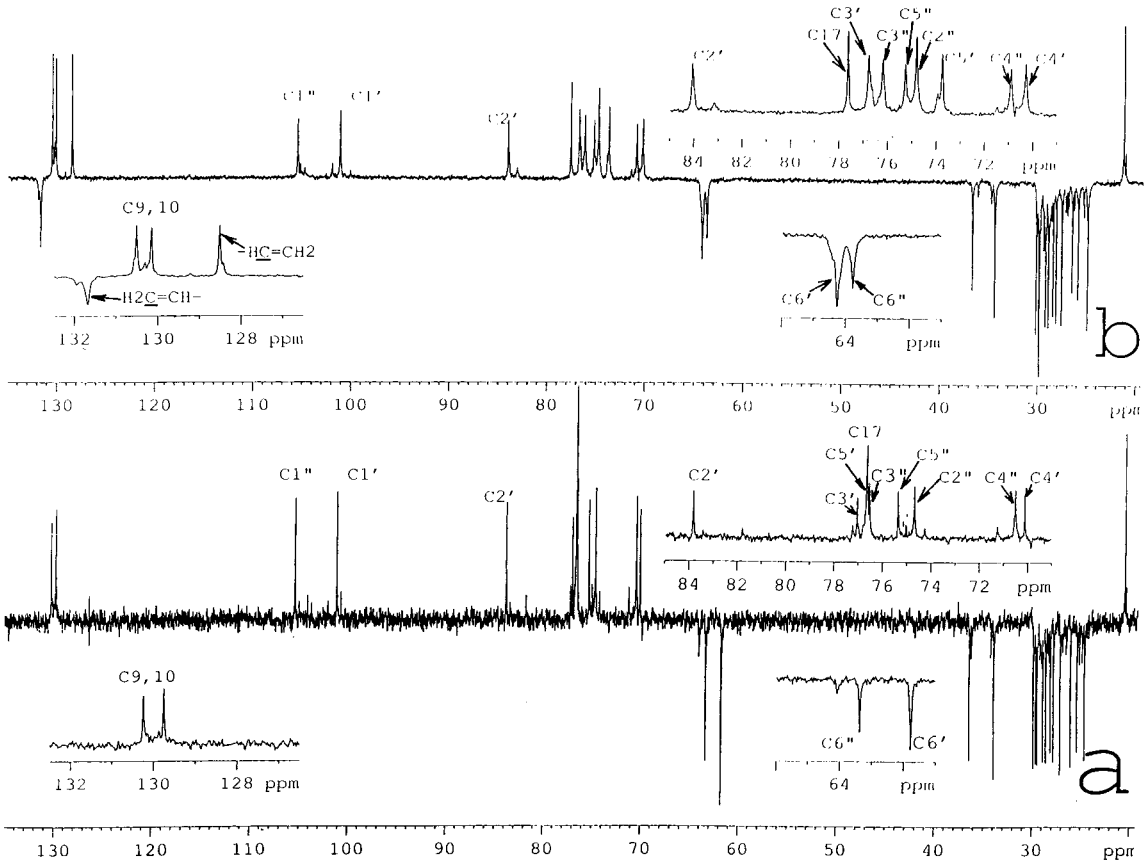


Figure 8. DEPT 135 spectra of (a) the sophorolactone, **9** and (b) the 6′-acrylate derivative, **10**.

pound; after detailed analysis, they showed that the structure of the compound differed from that described by Jones and was instead SL_1 (Figure 1).

The successful synthesis of **9** provided a sophorolipid analogue that had only one primary hydroxyl group. Hence, this compound was an excellent candidate for the regioselective conversion of **9** to the corresponding monoacryl derivative linked only to the one remaining primary site. Indeed, the reaction of **9** with vinyl acrylate catalyzed by Novozym 435 in dry THF gave **10** ($[\alpha]^{25}_D$ −2.81, *m/z* 681.90 $(M + Na)^+$). The ^{1}H NMR spectrum of **10** confirmed that the monoacrylation did indeed take place: 5.92 (1H, dd), 6.25 (1H, dd), and 6.44 (1H, dd). In addition, when compared to the ^{1}H NMR spectrum of **9**, the resonance position of the methylene on carbon 6′ for **10** showed a 0.7 ppm downfield shift. Further proof of the acrylation position for **10** was obtained by comparison of the ^{13}C DEPT 135 NMR spectra for **9** and **10** (Figure 8). The spectrum of **10** showed a downfield shift of 2.0 ppm in the resonance position of the C-6′ carbon and an upfield shift of 2.5 ppm in the resonance position of the C-5′ (Figure 8b).

Conclusions

In the present study, we have successfully demonstrated the regioselectivity of Novozym 435 catalyzed reactions that provided a wide range of new sophorolipid analogues. Surprisingly, this is the first report of lipase-catalyzed sophorolipid modifications. Important concepts that motivated this work were the following: (i) It is relatively simple to convert the mixture of sophorolipids to a pure intermediate. (ii) The choice of a sophorolipid intermediate that is readily soluble in moderately polar solvents facilitates further lipase-catalyzed reactions that do not proceed well in polar aprotic media. (iii) Sophorolipids are produced in large amounts by fermentation methods. It was also envisioned that the proposed sophorolipid analogues would lead to a new family of glycolipids that may be important immunomodulators. Furthermore, it was thought that certain analogues, such as the mono- and difunctional acryl sophorolipid derivatives, would be useful as monomers for the preparation of unusual amphiphilic vinyl homo- and copolymers. With these ideas in mind, a convergent synthesis that provided organosoluble sophorolipid analogues that were esterified at the lipid terminal carboxyl position was carried out. Then, these sophorolipid esters were used for Novozym 435 catalyzed regioselective acylations at the C-6′and C-6″ hydroxyl groups. An interesting outcome of this work was the fact that attempts to prepare a monoacryl derivative led to the formation of an unnatural sophorolactone. This sophorolactone was found to have the lactone ester link between C-1 of the 17-hydroxy-*cis*-9-octadecenoic acid chain and the C-6″ hydroxyl group of the sophorose moiety. To the best of our knowledge, seven compounds (**4**−**10**) are reported for the first time.

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Supporting Information Available: ^{1}H and ^{13}C/DEPT 135 NMR spectra of compounds (16 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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