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Regioselective Enzyme-Catalyzed Synthesis of Sophorolipid Esters, Amides, and Multifunctional Monomers

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Novel enzyme-mediated synthetic routes were developed to provide a new family of sophorolipid derivatives and glycolopid-based amphiphilic monomers. These compounds are of great interest for their potential use in immunoregulation, as well as for other biological properties. In the present work, an efficient lipase-catalyzed conversion of sophorolipid ethyl ester to (a) the 6′-monoacylated derivatives using Novozym 435, (b) 6″-monoacylated derivatives using Lipase PS-C, (c) secondary amide derivatives using Novozym 435, and (d) 6′,6″-diacylated amide derivatives using Novozym 435 in an one-pot reaction and (e) the regioselective monoacylation of an amide derivative at the 6′- and 6″-positions using Novozym 435 and Lipase PS-C, respectively, are described. The ethyl ester produced by esterification of the sophorolipid mixture with sodium ethoxide was subjected to acylation catalyzed by Novozym 435 in dry tetrahedrofuran (THF) with vinyl acetate and vinyl methacrylate to produce 6′-monoacylated derivatives. In contrast, Lipase PS-C catalyzed acylations of sophorolipid ethyl ester in dry THF with vinyl acetate and vinyl methacrylate to give the corresponding 6′-monoacylated derivatives. Novozym 435 mediated amidation of sophorolipid ethyl ester in dry THF with phenethylamine, tyramine, *p*-methoxyphenethylamine, 2-(*p*-tolyl)ethylamine, and *p*-fluorophenethylamine generated the corresponding secondary amides but not tertiary amides. The formation of diacyl derivatives of amides was achieved by their treatment with vinyl acetate and vinyl methacrylate in dry THF using Novozym 435 as catalyst. The conversion of sophorolipid ethyl ester to the same diacyl derivatives of amide (i.e., both amidation and acylation) in high yield was also demonstrated in dry THF by a one-pot reaction using Novozym 435. Furthermore, regioselective monoacylation of a sophorolipid amide at 6′ and 6″ in dry THF with vinyl acetate and vinyl methacrylate using Novozym 435 and Lipase PS-C was also demonstrated.

Introduction

Sophorolipids are a group of extracellular nonionic and anionic glycolipids. They are produced by resting cells of *Candida bombicola* when cultured on carbohydrates, fatty acids, hydrocarbons, or mixtures thereof.[1] Sophorolipids consist of a sophorose linked to the hydroxyl group at the penultimate position of, most often, a C18 fatty acid.[2] Sophorolipids, first described by Gorin et al.,[1] exist as a mixture of eight major components (lactone and acid forms) with varying degrees of acetylation at the 6′- and 6″-positions of the sophorose moiety (Figure 1). Analytical resolution of sophorolipids from *Candida bombicola* by reverse phase HPLC and GC-MS revealed 8 major and 15 minor components.[3] Sophorolipids are reported to be useful in improving microbe growth on water-insoluble substrates[4] and are likely involved in the regulation of energy metabolism.[5] Manipulation of physiological parameters and fermentation conditions resulted in im-

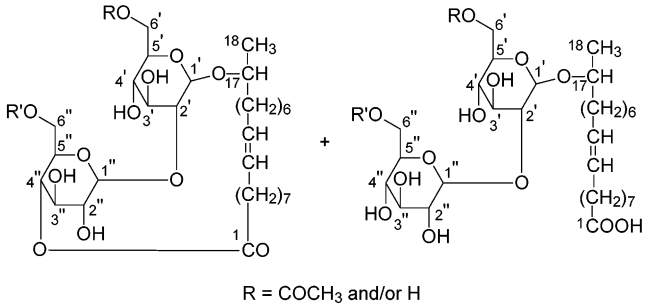

FIGURE 1. Structure of lactonic and acidic forms of Sophorolipid mixture produced by *Candida bombicola*.

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provement of sophorolipid production,[6] altering the structure and affecting the distribution of various fractions in sophorolipids.[7] Many fermentation strategies have been developed to improve the volumetric yield and efficiency of microbial sophorolipid synthesis.[8] The best result thus far reported in our laboratory was a sophorolipid yield of 350 g/L by a fed-batch fermentation.[9]

Synthesis of glycolipids and their derivatives are of great interest due to their varied biological activities and potential use in other applications. Biological applications of glycolipids and their analogues are in (i) cancer treatment by cytokine upregulation/macrophage activation,[10] (ii) treatment of autoimmune disorders,[11] (iii) treatment of antiendotoxic (septic) shock by cytokine down regulation,[12] (iv) regulation of angiogenesis,[13] and (v) apoptosis induction.[14] In addition to the above, sophorolipids are of interest for use in enhanced oil recovery,[15] cosmetics,[16] germicidal preparations,[17] and detergents.[18]

Attempts have been made to perform an in vivo modification of sophorolipid structure during biosynthesis. Selective feeding during fermentations of lipophilic substrates such as alkanes and fatty acids did bring about changes in the sophorolipid structure.[19a−l] Although this approach is promising, thus far it has affected a change in a small fraction of the sophorolipids produced. Also, any modification accomplished during in vivo biosynthesis has been limited to changes in the lipid structure. An alternate approach, which is the subject of this paper, is to use in vitro enzymatic methods for structural modifications of sophorolipids after their microbial synthesis. Previously, our group reported chemical and enzyme-catalyzed modifications of a natural sophorolipid mixture.[20,21] The synthesis of alkyl esters, their regioselective diacylation at 6′- and 6″-positions,[20] and polymerization of a 6-*O*-acryloyl derivative were reported.[21] Also, the enzyme-catalyzed deacylation of a sophorolactone (Figure 1, R = Ac) in a biphasic medium using an acetylesterase was shown to give low yields (25−30%) even after 10 days.[22] Rau et al.[23] has reported the conversion of sophorolipids into a glucose lipid in two steps (i.e., alkaline hydrolysis of sophorolipids to acidic sophorolipid (Figure 1, R = H, acidic form) and its enzyme-catalyzed conversion to a glucose lipid using several glycosidases. The most effective of the glycosidases studied was herperinidase (*Aspergillus niger*), which gave the glucose lipid in 80% yield. More recently, Furstner et al.[24] reported the multistep synthesis of the natural sophorolipid lactone by ring-closing alkyne metathesis.

In this paper, we report chemoenzymatic methods that significantly expand the current knowledge of how to prepare well-defined sophorolipid analogues. The importance of this work lies in the potential use of sophorolipid analogues in a wide array of medical applications. By using in vitro lipase-catalysis, highly regioselective acetylations were accomplished exclusively at the 6′-, 6″-, or both 6′- and 6″-positions. This gave previously unknown monoacetate derivatives of the sophorolipid ethyl ester. These biotransformations were generally conducted in dry THF using Novozym 435 or Lipase PS-C at 40−50 °C. Also, a new series of sophorolipid amides were prepared by the mild lipase-catalyzed amidation reaction between primary amines and the sophorolipid ethyl ester

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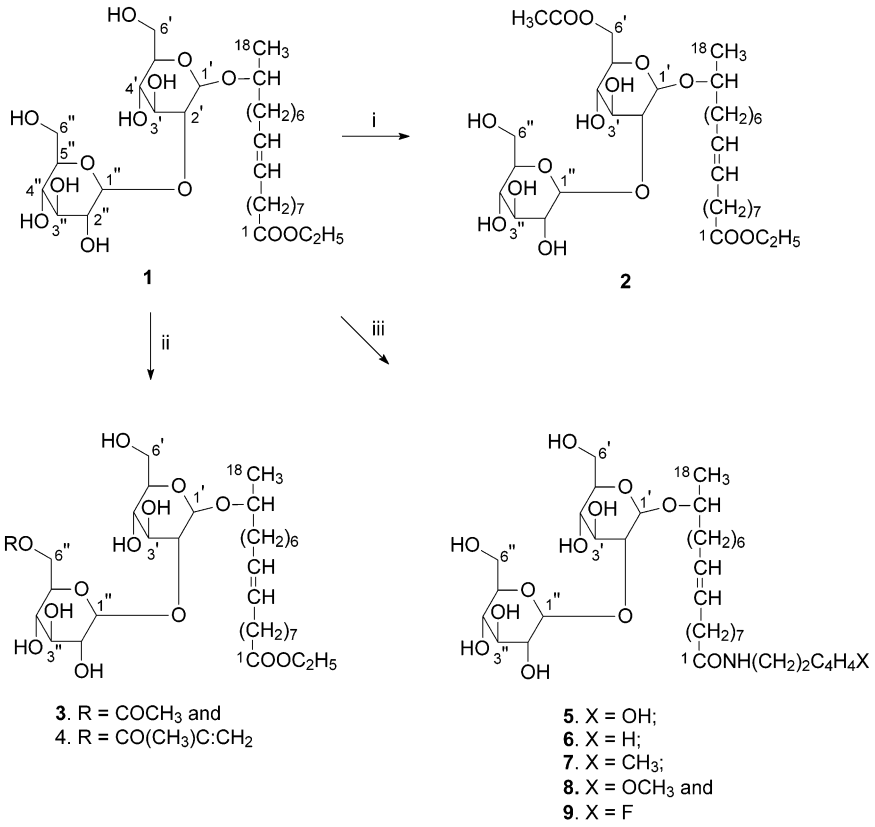
SCHEME 1[a]



[a] (i) vinyl acetate, Novozym 435, dry THF, 40 °C, 2.5 h; (ii) vinyl ester (vinyl acetate for **3** and vinyl methacrylate for **4**), Lipase PS-C, dry THF, 40 °C, 72 h; (iii) primary amine (tyramine for **5**, phenethylamine for **6**, (*p*-tolyl)amine for **7**, *p*-methoxyphenethylamine for **8**, *p*-fluorophenethylamine for **9**), Novozym 435, dry THF, 50 °C, 24 h.

1. Further regioselective acetylations of sophorolipid amides were performed to provide well-defined monoacetylated (at 6′ or 6″) and diacetylated (at 6′ and 6″) derivatives. The biological activities of these sophorolipid analogues as well as their use as monomers and surfmers will be described elsewhere.

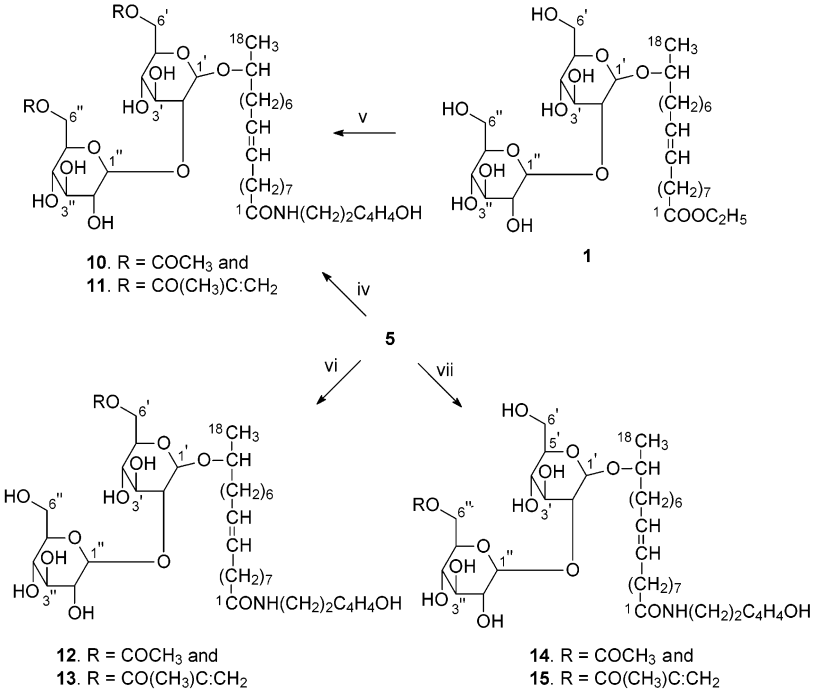
Result and Discussion

The sophorolipid mixture produced by the fermentation of *C. bombicola* on glucose/oleic acid mixtures[19a] contains at least eight components that exist mainly in the lactonic and acidic forms. These compounds also have variable degrees of acetylation at the primary hydroxyl groups at 6′- and 6″-positions. In an earlier report,[20] our group described the preparation of pure alkyl esters from a natural sophorolipid mixture. Also, the regioselective lipase-catalyzed diacylation of the sophorolipid ethyl ester (**1**, Scheme 1) at the 6′- and 6″-positions was reported.[20] Furthermore, the lipase-catalyzed cyclization of **1** to form an unnatural sophorolipid macrolactone linked between the fatty acid carboxyl group at C-1 and the 6″-hydroxyl was described.[20] To gain improved precision of group placement during sophorolipid acetylation reactions, we attempted to monoacylate **1** without concurrent formation of the unnatural sophorolipid macrolactone esterified at the 6″ hydroxyl position. In addition, we investigated the feasibility of using mild lipase-catalysis to convert **1** to a family of secondary amide sophorolipid analogues. Furthermore, the monoacylation and diacylation of sophorolipid amides were also studied.

The sophorolipid ethyl ester, **1**, obtained by treating the microbial sophorolipid mixture with sodium ethoxide following the procedure reported earlier by our group,[20] was used as a synthon for enzyme-mediated transformations to form corresponding sophorolipid monoacetate, amide, and other monoacyl and diacyl derivatives (Schemes 1 and 2). Monoacylation of **1** at the 6′-position to form analogue **2**, amidation of **1** with primary amines (tyramine, phenethylamine, 2-(*p*-tolyl)ethylamine, *p*-methoxyphenethylamine, *p*-fluorophenethylamine) to form analogues **5**−**9**, diacylation of amide **5** at the 6′- and 6″-positions to form **10** (from vinyl acetate) and **11** (from vinyl methacrylate), and monoacylation of amide **5** at the 6′-position to form **12** (from vinyl acetate) and **13** (from vinyl methacrylate) were all effectively catalyzed by a physically immobilized form of *Candida antartica* lipase B (Novozym 435) (Schemes 1 and 2). To perform monoacylation reactions at position 6″ of ethyl ester **1** and amide **5**, Lipase PS-C (Lipase PS on ceramic support) was used in place of Novozym 435 to give **3** and **4**, and **14** and **15**, respectively (Schemes 1 and 2). Attempts to prepare amides by chemical methods (e.g., by treatment of substrate with amines in dry THF at 40−60 °C, refluxing the substrate and amine mixture in ethanol at 80 °C for 10 days) failed.

Synthesis of Sophorolipid Ethyl Ester 1. The preparation and thorough structural elucidation (using

SCHEME 2[a]



[a] (iv) vinyl acetate for **10** and vinyl methacrylate for **11**, Novozym 435, dry THF, 50 °C, 80 h; (v) tyramine, Novozym 435, dry THF, 50 °C, 24 h; vinyl ester (vinyl acetate for **10** and vinyl methacrylate for **11**), 50 °C, 80 h (one-pot reaction); (vi) vinyl ester (vinyl acetate for **12** and vinyl methacrylate for **13**), Novozym 435, dry THF, 40 °C, 20 h; (vii) vinyl ester (vinyl acetate for **14** and vinyl methacrylate for **15**), Lipase PS-C, dry THF, 40 °C, 72 h.

^{1}H and ^{13}C NMR, DEPT, ^{1}H−^{1}H COSY, HETCOR, IR and MS) of ethyl ester **1** was previously reported by our group.[20] Compound **1** was prepared for the present study using the method identical to that in ref 20, and spectral data confirmed its structure and purity.

Novozym 435 Catalyzed Monoacylation of Sophorolipid Ethyl Ester 1 at the 6′-Position. Our group already reported the diacylation of ethyl ester **1** at the 6′- and 6″-positions.[20] To further improve our ability to precisely control the degree and site of modification of sophorolipid analogues, studies were carried out to prepare monoacylated derivatives from **1**. Ethyl ester **1** was treated with excess vinyl acetate in dry THF at 40 °C for 2.5 h using Novozym 435 as the catalyst. By this method, monoacetate **2** modified at the 6′-position was formed in 77% yield (Scheme 1). Also, a small amount (9%) of the corresponding diacetate modified at both the 6′- and 6″-positions was found.[20] The optically active compound **2** ($[\alpha]^{25}_D$ −10.80) was identified by an LC-APCI mass spectrum (*m*/*z* 715.51 [M + Na]$^+$ and 693.52 [M + H]$^+$) and detailed NMR spectral analyses. The appearance in ^{1}H NMR of additional resonances at 1.90−2.18 ppm (m) for three protons and the downfield shift of two protons of compound **1** at 3.20−3.95 ppm (m) to 4.21 (1H, m) and 4.39 (1H, m) for compound **2** showed the presence of one acetyl group in the molecule. ^{13}C NMR of compound **2** (see Experimental Section for **2** and Figure S2 in Supporting Information) showed a downfield shift of ∼2 ppm in C-6′ (65.06 ppm) as compared to that for **1** (63.24 ppm). Also, there was no significant change in C-6″. The occurrence of monoacetylation at C-6′ was further supported by ^{13}C NMR resonances at 21.08 and 172.72 ppm corresponding to the acetyl *C*H$_3$ and *C*═O, respectively. Monoacetylation at C-6′ also resulted in an upfield shift of ∼2 ppm of C-5′, which is explained by the γ-effect between C-5′ and the neighboring acetyl *C*═O atom. A downfield shift of ∼2 ppm in C-2′ (carbon bearing no free hydroxyl group) from 81.85 ppm in **1** to 84.00 ppm in **2** was also observed and might be associated with a conformational change upon acetylation at C-6′.[20] The assignment of protons and carbons were made by comparison of ^{1}H and ^{13}C signals of precursor **1** with **2** and by careful inspection of the ^{1}H−^{1}H COSY, ^{1}H−^{13}C HETCOR spectra of **2**. Metheine, methylene, and methyl groups were distinguished by a ^{13}C NMR DEPT spectrum of the molecule (Figure 2a). The resonances in the ^{1}H NMR of **2** at 5.35 ppm (m) for two protons were due to H-9 and H-10. These assignments were further supported by ^{13}C peaks at 130.91 and 131.04 corresponding to C-9 and C-10. Thus, these results showed that Novozym 435 was an effective catalyst for the monoacylation of **1** at C-6′**,** and compound **2** was identified as ethyl 17-L-[(2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]-*cis*-9-octadecenoate-6′-acetate.

Lipase PS-C Catalyzed Regioselective Monoacylation of the Sophorolipid Ethyl Ester 1 at C-6″. Compound **1** was treated at 40 °C for 72 h with an excess of vinyl acetate in dry THF using Lipase PS on a ceramic support (Lipase PS-C) (Scheme 1). This gave monoacetate **3** [($[\alpha]^{25}_D$ −9.67); *m*/*z* 715.51 [M + Na]$^+$], selectively modified at C-6″, in 89% yield. The appearance in the ^{1}H NMR for **3** of additional resonances at 1.90−2.18 ppm for three protons and the downfield shift of two protons of compound **1** at 3.20−3.95 ppm to 4.20 (1H) and 4.37 (1H) for **3** showed the presence of one acetyl group in the molecule. The ^{13}C NMR of compound **3** (see Experimental

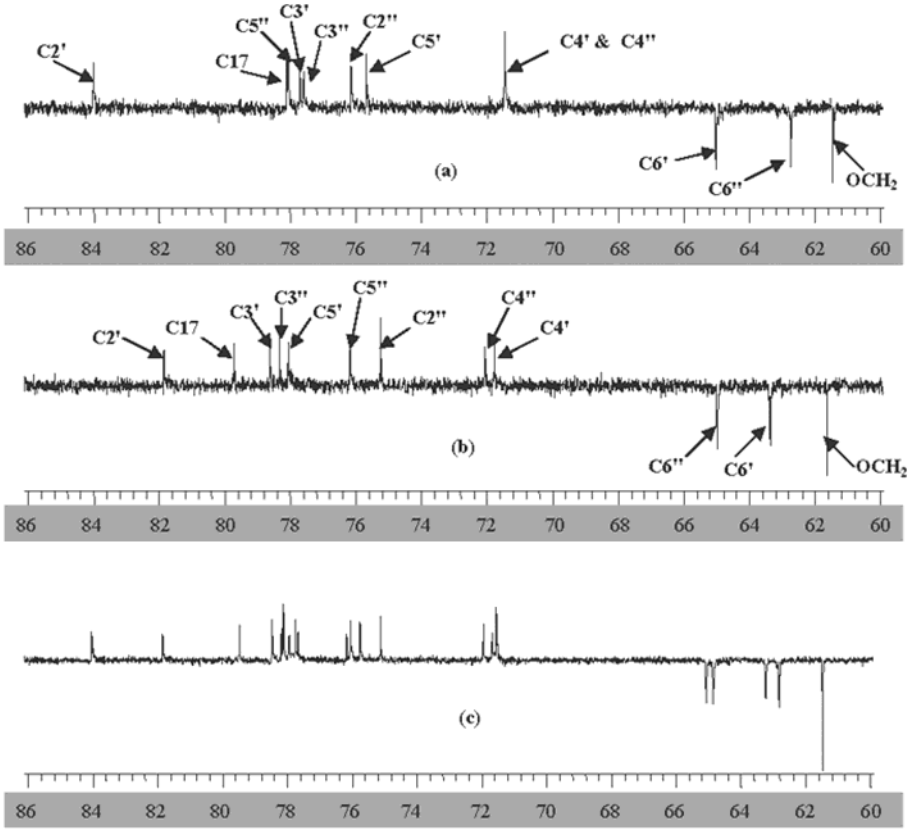


FIGURE 2. DEPT 135 spectra of the region 60.00−86.00 ppm for (a) **2**, (b) **3,** and (c) a mixture of **2** and **3** in MeOH-d_4.

Section for **3**, its ^{13}C DEPT NMR in Figure 2b and Figure S4 in Supporting Information) when compared to **1** showed a downfield shift of ∼2 ppm in C-6″ (62.77 to 64.87 ppm). Also, there was no significant change in the peak position of C-6′. The occurrence of monoacetylation at C-6″ was further supported by ^{13}C NMR resonances at 20.88 and 172.73 ppm corresponding to the acetyl CH_3 and C═O, respectively. Monoacetylation at C-6″ also resulted in an upfield shift of ∼2 ppm for C-5″ that is due to the γ-effect (see above). The assignment of protons and carbons were made by comparison of ^{1}H and ^{13}C signals of precursor **1** with **3** and by careful inspection of the ^{1}H−^{1}H COSY, ^{1}H−^{13}C HETCOR spectra of **3**. Metheine, methylene, and methyl groups were distinguished by a ^{13}C NMR DEPT spectrum of the molecule (Figure 2b). No significant changes were observed for compounds **1**, **2**, and **3** in the positions of protons and carbons associated with the sophorolipid fatty acid CH═CH moiety. Also, to further substantiate the difference in the NMR spectra of **2** and **3**, they were mixed in equimolar amounts. Study of the ^{13}C DEPT 135 of this mixture showed distinctly resolved signals for **2** at 65.06 (C-6′), 62.78 (C-6″), and C-2′ (84.00) and for **3** at 63.23 (C-6′), 64.87 (C-6″), and C-2′ (81.84) (see Figure 2c and Experimental Section for **2** and **3** and Figures S3 and S5 in Supporting Information). On the basis of these observations compound **3** was identified as ethyl 17-L-[(2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]-*cis*-9-octadecenoate-6″-acetate. Thus, these results showed that Lipase PS-C was an effective catalyst for the acetylation of **1** at C-6.

The above reaction was further extended to the preparation of ethyl 17-L-[(2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]-*cis*-9-octadecenoate-6″-methacrylate **4** ([α]$^{25}_D$ −5.67; *m*/*z* 741.52 [M + Na]$^+$) in 86% yield (Scheme 1) by Lipase PS-C mediated acryloylation of **1** using an excess of vinyl methacrylate with the same set of reaction condition. This compound had ^{1}H NMR resonances similar to those of **3** except those that appeared at 1.93 (s), 5.63(s), and 6.11(s) ppm in **4** due to replacement of an acetyl group in **3** by a methacryl group at C-6″ (see Experimental Section for **4** and Figure S6 in Supporting Information). The resonances in the ^{13}C NMR spectrum of **4** at 18.56, 126.43, 137.85, and 168.67 ppm further support the presence of a methacryl moiety in the molecule. Moreover, the ^{13}C NMR showed a significant downfield shift for C-6″ (from 62.77 ppm in **1** to 65.15 ppm in **4**), an upfield shift for the C-5″-signal, and no change for C-2′. Therefore, it was concluded that Lipase PS-C is an excellent catalyst for highly regioselective acylation of **1** at C-6″ using vinyl acetate or vinyl methacrylate.

Enzyme Screening for Amidation Reaction. For amidation of ethyl ester **1** with primary amines (tyramine, phenethylamine, *p*-methoxyphenethylamine, 2-(*p*-tolyl)-ethylamine, *p*-fluorophenethylamine), different lipases (PPL, CCL, PS-30, Lipase AK, Lipase PS, MAP-10, Novozym 435, and Lipozyme IM) in dry organic solvent at different temperatures (room temperature, 30, 40, and 50 °C) were evaluated. Only Novozym 435 was able to catalyze the formation of amide in high yield. A preferable condition for the reaction was in dry THF at 50 °C for 24 h. Amidation did not occur when the lipases other than Novozym 435 were used.

Novozym 435 Catalyzed Amidation of Sophorolipid Ethyl Ester 1. Novozym 435 was used to catalyze

mild amidation reactions between primary amines (tyramine, phenethylamine, *p*-methoxyphenethylamine, 2-(*p*-tolyl)ethylamine, *p*-fluorophenethylamine) and the sophorolipid ethyl ester **1 (**Scheme 1). When tyramine was used, the secondary amide **5** ($[\alpha]^{25}_D$ −12.33) was formed in 92% yield (Scheme 1). Compound **5** was purified by column chromatography over silica gel with methanol/chloroform (1:9, v/v) as eluent. Study of the LC-APCI mass spectrum of **5** showed an ion peak at 742.27 ($[M + H]^+$). The ^{1}H NMR spectrum of **5** showed the absence of resonances for O−CH_2CH_3 of **1**.[20] Instead, protons corresponding to the tyramine amide moiety at 2.68 ppm ([C═O]HNCH_2CH_2, t), 3.20−3.72 ppm ([C═O]-HNCH_2CH_2, m), and the aromatic region at 6.71 ppm (2H, d, J = 6.71 Hz) and 7.01 ppm (2H, d, J = 6.71 Hz) were found. Similarly, the ^{13}C NMR spectrum of **5** showed the absence of resonances for O−CH$_2$$CH_3$ of **1**.[20] Instead, carbons corresponding to the tyramine amide moiety were found at positions that correspond with the expected product structure (see Experimental Section for compound **5** and Figure S7 in Supporting Information). IR analysis of **5** showed an absorption peak at 1643 cm^{-1} that corresponds to the amide-I band of the NH−C═O group. The retention of the disaccharide structure for **5** was evident by study of the sugar ring protons and carbons (see Experimental Section for compound **5** and Figures S7 and S8 in Supporting Information). For example, the acetal carbons C-1′ and C-1″ showed signals at 102.82 and 104.75 ppm that are consistent with that expected on the basis of comparisons to **1**. Representative ^{13}C and ^{1}H NMR signals for the fatty acid moiety of **5** were also observed. For example, resonances in the ^{1}H NMR of **5** corresponding to the CH═CH moiety at 5.35 ppm (2H, m) are due to H-9 and H-10. Also, in the ^{13}C NMR spectrum, resonances at 130.94 and 131.00 due to C-9 and C-10, respectively, were observed. Furthermore, the ^{13}C resonance of the (*C*═O)-NH group was observed at 176.35 ppm. On the basis of these results and other spectral observations (see Experimental Section for compound **5**, and Figures S7 and S8 in Supporting Information), compound **5** was identified as *p*-hydroxy phenethyl 17-L-[(2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide). Thus, it was concluded that Novozym 435 catalyzed the amidation of **1** with tyramine to give **5**.

With a view to establish a general method for amide preparation, the above reaction was extended to prepare phenethyl 17-L-[(2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide (**6**), *p*-(tolyl)ethyl 17-L-[(2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide (**7**), *p*-methoxyphenethyl 17-L-[(2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide (**8**), and *p*-fluorophenethyl 17-L-[(2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide (**9**) by Novozym 435 catalyzed amidation of **1** with phenethylamine, *p*-(tolyl)ethylamine, *p*-methoxyphenethylamine, and *p*-fluorophenethylamine, respectively, using the same set of reaction condition. Spectral data for **6**−**9** were identical with that observed for **1** with additional resonances that appeared as a result of replacement of the $-OCH_2CH_3$ group by $-CONH(CH_2)_2C_6H_5$, $-NH(CH_2)_2C_6H_4CH_3$, $-NH(CH_2)_2C_6H_4OCH_3$, and $-NH(CH_2)_2C_6H_4F$ groups, respectively (see Experimental Section). Hence, Novozym 435 was found to be an excellent catalyst for the synthesis of secondary amides from reactions between **1** and primary amines.

Novozym 435 Catalyzed Diacylation (at 6′ and 6″-Positions) of 5. As part of a previous study, our laboratory reported the selective diacetylation of **1** along with detailed spectral data.[20] To further extend the level of structural control attainable with the new sophorolipid amides described herein, the diacylation of sophorolipid amide **5** was studied. Compound **5** was treated with excess vinyl acetate in dry THF at 50 °C for 80 h using Novozym 435 as catalyst. By this method, the corresponding diacetyl derivative **10** ($[\alpha]^{25}_D$ −6.27) was formed in high yield (91%) (Scheme 2). The LC-APCI mass spectrum of **10** gave *m*/*z* 826.56 $[M + H]^+$, which is 82 mass units higher than **5**, which corresponds to the addition of two acetyl groups. The ^{1}H NMR of **10** showed additional resonances at 1.92−2.12 ppm that correspond to the six acetyl hydrogens. As was the case for the diacyl derivative of **1** reported earlier,[20] the ^{13}C NMR of compound **10** when compared to that of **5** showed downfield shifts of ∼2 ppm for C-6′ and C-6″ (from 62.83 and 63.18 ppm in **5** to 64.89 and 65.07 ppm in **10**, respectively). The acetylation at both C-6′ and C-6″ was further supported by ^{13}C NMR resonances at 20.90, 21.06, and 172.84 (two nonresolved signals) that correspond to two acetyl CH$_3$ and two C═O groups, respectively. The assignment of protons and carbons were made by comparison of ^{1}H and ^{13}C signals of precursor **5** with **10** and by careful inspection of the ^{1}H−^{1}H COSY, ^{1}H−^{13}C HETCOR spectra of **10**. Metheine, methylene, and methyl groups were distinguished by a ^{13}C NMR DEPT spectrum of **10**. Thus, Novozym 435 was an effective catalyst for the diacetylation of **5** at C-6′ and C-6″, and this compound was identified as *p*-hydroxy phenethyl 17-L-[(2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)-oxy-*cis*-9-octadecenamide-6′,6″-diacetate. This study also showed that the replacement of the ethyl ester group in **1** by an amide group in **5** did not have a significant effect on the Novozym 435 catalyzed regioselective acetylation of these substrates (see Experimental Section for compound **5** and **10** and Figures S7, S8, S17, and S18 in Supporting Information).

The above reaction was further extended for the preparation of the corresponding dimethacrylate derivative, *p*-hydroxy phenethyl 17-L-[(2′-*O*-*β*-D-glucopyranosyl-*β*-D-glucopyranosyl)-oxy-*cis*-9-octadecenamide-6′,6″-dimethacrylate (**11**, $[\alpha]^{25}_D$ −3.00; *m*/*z* 900.51 $[M + Na]^+$, 878.53 $[M + H]^+$) in 88% yield by Novozym 435 mediated acryloylation of **5** using an excess of vinyl with the same set of reaction conditions (Scheme 2). The presence of two methacryl groups at C-6′ and C-6″ in **11** were identified by ^{1}H NMR resonances at 1.93, 1.94, 5.52, 6.12, and 6.14 ppm and the ^{13}C NMR spectrum at 18.57, 18.69, 126.47, 126.68, 137.78, 137.83, 168.76, and 168.83 ppm. Relative to precursor **5**, the ^{13}C NMR of compound **11** showed significant downfield shifts of >2 ppm for C-6′ and C-6″ (see Experimental Section for compound **5** and **11** and Figures S7, S8, S19, and 20 in Supporting Information).

Since Novozym 435 worked well for both amidation as well as acylation, we attempted the synthesis of **11** from **1** by a one-pot two-step process. First, the amidation of **1** to form **5** was performed exactly as above. Subsequently, vinyl methacrylate was added in excess to the reaction without isolation of **5** or further change in the

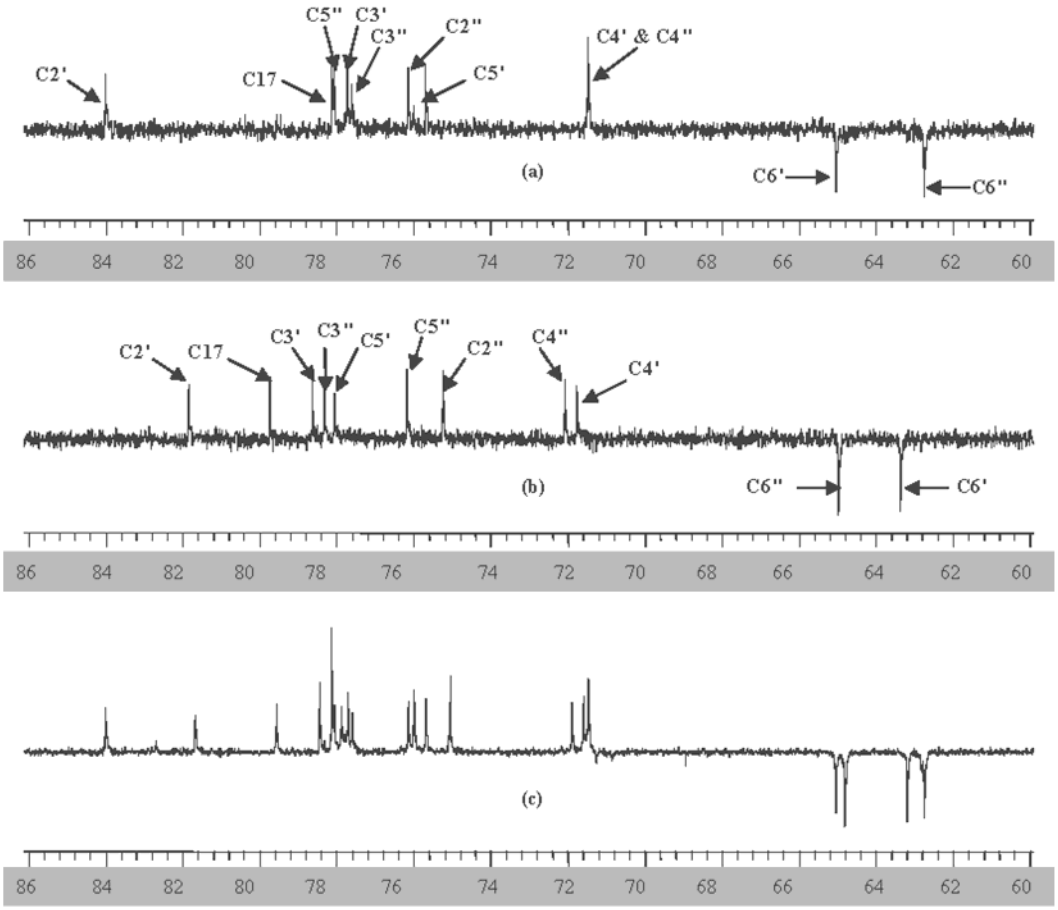


FIGURE 3. DEPT 135 spectra of the region 60.00−86.00 ppm for (a) **12**, (b) **14**, and (c) a mixture of **12** and **14** in MeOH-d_4.

reaction conditions. By this method, compound **11** was obtained in high yield.

Novozym 435 Catalyzed Regioselective Monoacylation of 5 at 6′-Position. After success in monoacylation of **1** the method was extended for the synthesis of monoacyl derivative of amide **5**. The sophorolipid amide **5** was treated with excess vinyl acetate in dry THF at 40 °C for 20 h using Novozym 435 as the catalyst. By this method, monoacetate **12** modified at the 6′-position was formed in 83% yield (Scheme 2). Also, a small amount (7%) of the corresponding diacetate **10** was found. The optically active compound **12** ($[\alpha]^{25}_D$ −8.60) was identified by an LC-APCI mass spectrum (*m*/*z* 784.56 (M + H)$^+$, which is 41 mass unit higher than that for **5**, indicating the presence of one acetyl groups in the molecule) and detailed NMR spectral analyses. The proton NMR of the compound showed additional resonences at 1.92−2.10 for three protons due to one acetyl groups in the molecule, which was further supported by resonances at 21.07, 172.77 in ^{13}NMR spectrum of the molecule. Furthermore ^{13}C NMR spectrum of **12** showed downfield shift of ∼2 ppm in 6′ -position (from resonance at 63.18 to 65.09 ppm) indicating acylation at this position. Monoacetylation at C-6′ also resulted in an upfield shift of ∼2 ppm of C-5′ due to the γ-effect between C-5′ and the neighboring acetyl *C*═O atom. A downfield shift of ∼2 ppm in C-2′ (carbon bearing no free hydroxyl group) was also observed, which might be associated with a conformational change upon acetylation at C-6′.[20] The assignment of protons and carbons were made by comparison of ^{1}H and ^{13}C signals of precursor **5** with **12** and by careful inspection of the ^{1}H−^{1}H COSY, ^{1}H−^{13}C HETCOR spectra of **12**. Metheine, methylene, and methyl groups were distinguished by a ^{13}C NMR DEPT spectrum of the molecule (Figure 3a). Thus, these results showed that Novozym 435 was an effective catalyst for the monacylation of **5** at C-6′ and this compound was identified as *p*-hydroxy phenethyl 17-L-[(2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide-6′-acetate. Therefore, Novozym 435 is not only an excellent catalyst for highly regioselective diacylation but also monoacylation at the 6′-position of **5**.

The above reaction was further extended for the preparation of *p*-hydroxy phenethyl 17-L-[(2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)oxy]-*cis*-9-octadecenamide-6′-monomethacrylate (**13**, $[\alpha]^{25}_D$ −5.00; *m*/*z* 810.25 (M + H)$^+$) in 80% yield by Novozym 435 mediated acryloylation of **5** using an excess vinyl methacrylate and the same set of reaction condition (Scheme 2). Also a small amount of dimathacrylate **11** (7%) was formed. This compound had ^{1}H and ^{13}C NMR resonances similar to those of **12** except those that appearing as a result of replacement of an acetyl group in **12** by one methacryl group (^{1}H NMR 1.94 (3H, s), 5.63 (1H, s), 6.14 (1H, s) ppm and ^{13}C NMR 18.70, 126.71, 137.71, 137.76, 168.79 ppm) in **13** at C-6′ (see Experimental Section for compound **12** and **13**, and Figures S21−S24 in Supporting Information). Furthermore ^{13}C NMR showed significant downfield shift in C-6″ and upfield shift of >2 ppm in C-5″-position but no change in C-2′ was noticed. Meth-

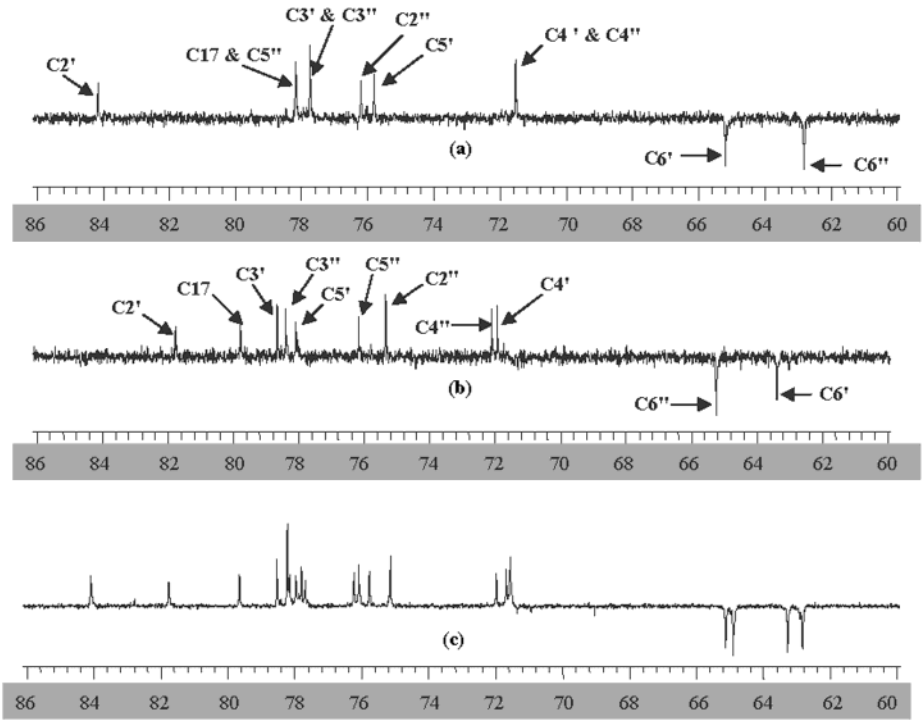


FIGURE 4. DEPT 135 spectra of the region 60.00−86.00 ppm for (a) **13**, (b) **15,** and (c) a mixture of **13** and **15** in MeOH-d_4.

eine, methylene, and methyl groups were distinguished by a ^{13}C NMR DEPT spectrum of the molecule (Figure 4a). Therefore, it was concluded that Novozym 435 is an excellent catalyst for regioselective monoacylation of **5** using different acylating agents.

Novozym 435 Catalyzed Regioselective Monoacylation of 5 at 6′′-C. After success in monoacylation of **1** at the 6′′-position, the method was extended to the synthesis of similar monoacyl derivative **14** (*m*/*z* 784.52 $(M + Na)^+$, $([\alpha]^{25}_D$ −8.07), selectively modified at C-6′′, in 87% yield by treating compound **5** with an excess of vinyl acetate in dry THF using Lipase PS on a ceramic support (Lipase PS-C) at 40 °C for 72 h. The appearance of additional resonance at 2.00−2.10 ppm of three protons in the ^{1}H NMR for **14** and the downfield shift of two protons of compound **5** at 3.20−3.89 (m) to 4.18 (1H, m) and 4.37 (1H, m) ppm for **14**, showed the presence of one acetyl group in the molecule. Furthermore ^{13}C NMR of compound **14** (see Experimental Section for compounds **5** and **14**, Figures S7, S8, S25, and S26 in Supporting Information and its ^{13}C DEPT NMR in Figure 3b) showed a downfield shift of ∼2 ppm in C-6′′ (64.88 ppm) compared to that for **5** (62.83 ppm). Also, there was no significant change in the peak position of C-6′. The occurrence of monoacetylation at C-6′′ was further supported by ^{13}C NMR resonances at 20.87 and 172.76 ppm corresponding to the acetyl *C*H$_3$ and *C*═O, respectively. Monoacetylation at C-6′′ also resulted in an upfield shift of ∼2 ppm of C-5′′, which is due to the γ-effect. The assignment of protons and carbons were made by comparison of ^{1}H and ^{13}C signals of precursor **5** with **14**. Metheine, methylene, and methyl groups were distinguished by a ^{13}C NMR DEPT spectrum of the molecule (Figure 3b). Also, to further substantiate the difference in the NMR spectra of **12** and **14**, they were mixed in equimolar amounts. Study of the ^{13}C DEPT 135 of this mixture showed distinctly resolved signals for **12** at 65.09 (C-6′), 62.82 (C-6′′), and C-2′ (84.01) and for **14** at 64.88 (C-6′), 63.26 (C-6′′), and C-2′ (81.84) (see Figure 3c and Experimental Section for **5**, **12**, and **14**). No significant changes were observed for compounds **5** and **14** in the positions of protons and carbons associated with the sophorolipid fatty acid CH═CH moiety (C-9 and C-10). Thus, these results showed that Lipase PS-C was an effective catalyst for the acetylation of **5** at C-6′′. Compound **14** was identified as *p*-hydroxy phenethyl 17-L-[(2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)]-oxy]-*cis*-9-octadecenamide-6′′-acetate.

The above reaction was further extended to the preparation of related monomethacrylated derivative *p*-hydroxy phenethyl 17-L-[(2′-*O*-β-D-glucopyranosyl-β-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide-6′′-methacrylate (**15**, $[\alpha]^{25}_D$ −4.34; *m*/*z* 832.53 $(M + Na)^+$, 810.55 $(M + H)^+$) in 88% yield by Lipase PS-C mediated acryloylation of **5** using an excess of vinyl methacrylate and the same set of reaction condition (Scheme 2). This compound had ^{1}H NMR resonances similar to **14** except those that appeared as a result of replacement of an acetyl group in **14** by one methacryl group at 1.93 (3H, s), 5.62 (1H, s), 6.11 (1H, s) ppm in **15** at C-6′′ (see Experimental Section). The resonances in ^{13}C NMR spectrum of **15** at 18.57, 126.46, 137.84, 168.70 further supported the presence of a methacryl moiety in the molecule. Furthermore ^{13}C NMR showed significant downfield shift in C-6′′, upfield shift in C-5′′, but no change in C-2′ was noticed. Metheine, methylene, and methyl groups were distinguished by a ^{13}C NMR DEPT spectrum of the molecule (Figure 4b). Also, to further substantiate the difference in the NMR spectra of **13** and **15**, they were mixed in equimolar amounts. Study of the ^{13}C DEPT 135 of this mixture showed distinctly resolved signals for **13** at 65.19 (C-6′), 62.80 (C-6′′), and C-2′ (84.15) and for **15** at 63.28

(C-6′), 65.15 (C-6″), and C-2′ (81.77) (see Figure 4c and Experimental Section for **13**−**15**). Therefore, it was concluded that Lipase PS-C is an excellent catalyst for regioselective monoacylation of **5** using different acylating agents.

All compounds synthesized in this study were analyzed by elemental analysis and were found to show good agreement between experimental and calculated values (see Experimental Section for numerical values).

Conclusions

In the present study, lipase-catalysis was used to perform highly selective transformations on complex glycolipid substrates produced by yeasts. These glycolipids, known as sophorolipids, pose significant synthetic challenges because they consist of readily hydrolyzable acetal moieties that link the glucose residues of sophorose as well as the 17-hydroxy oleic acid to sophorose. Of the five secondary and two primary hydroxyls of the sophorolipid ethyl ester, lipase-catalysis allowed the selective placement of an acetyl or a methacryl group at either of the two primary hydroxyl positions. This was accomplished by the identification of lipases that have different regioselectivities. The preparation of 6′-monoacylated derivatives was accomplished by using Novozym 435. To prepare 6″-monoacylated derivatives, Lipase PS-C was found to be highly selective. Novozym 435 also catalyzed the reaction between primary amines and the sophorolipid ethyl ester to form a new family of sophorolipid amide derivatives. These amides were also found to be suitable substrates for highly precise lipase-catalyzed acetylation and methacryl reactions at either the 6′- or 6″-hydroxyl positions. In addition, a one-pot two-step process was demonstrated where a sophorolipid amide derivative was first formed, followed by selective acetylation and methacrylation at both the 6′- and 6″-hydroxyl positions. To the best of our knowledge this is the first report of a one-pot reaction involving amidation as well as acylation using the same enzyme. The sophorolipid derivatives prepared in this work have immense potential to provide therapeutic agents that can function as tunable immunoregulators. What is striking is the relative ease by which these glycolipid analogues can be generated with a diverse range of structural attributes. The placement of methacryl groups on sophorolipids provides options to incorporate these molecules into polymers by conventional or controlled free-radical polymerizations. Furthermore, the methacryl and tyrosine groups that were introduced into sophorolipid analogues allow these molecules to function as reactant surfactants also known as surfmers. Biological activities of the new compounds prepared herein as well as polymerizations of the new mono- and divinyl sophorolipids are under investigation and will be reported separately. To the best of our knowledge, all 14 compounds synthesized during this investigation are new.

Experimental Section

General Chemicals. Sophorolipid mixture was synthesized by fermentation of *C. bombicola* on glucose/oleic acid mixtures following a literature procedure.[20,22] All chemicals and solvents used for this investigation were analytical grade and were used as received unless otherwise noted. All enzymes, prior to their use, were dried at high vacuum.

Experimental Procedure. Ethyl 17-L-[(2′-*O*-*β*-D-Glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenoate-6′-acetate (2). To a solution of ethyl ester, **1** (325.4 mg, 0.5 mmol), prepared from sophorolipid mixture by our group following the procedure reported earlier,[22] and vinyl acetate (230.9 μL, 2.5 mmol) in dry THF (5 mL) was added Novozym 435 (100 mg), and the mixture was stirred at 40 °C for 2.5 h under nitrogen atmosphere. The enzyme was filtered out followed by washing with THF (3 × 3 mL). After the removal of solvent, the residue was charged to a silica gel column chromatography with MeOH/$CHCl_3$ (1:24 to 2:23, v/v) to afford diacetate (33.0 mg, 9%)[20] and monoacetate **2** as a white solid (267.0 mg, 77%): $[\alpha]^{25}_D$ −10.80 (c 0.015, MeOH); IR (KBr plate) [cm^{-1} (%T)] 3414, 2930, 2860, 2795, 2523, 1741, 1464, 1372, 1242, 1160, 1079, 1030; ^{1}H NMR (300 MHz, MeOH-d_4) δ 1.22 (3H, d, J = 6.1 Hz, H-18), 1.23 (3H, t, J = 7.2 Hz, $COCH_2CH_3$), 1.24−1.50 (16H, m, H-4−7 and-12−15), 1.50−1.70 (4H, m, H-3 and H-16), 1.90−2.18 (7H, m, H-8, H-11 and $COCH_3$), 2.29 (2H, t, J = 7.4 Hz, H-2), 3.20−3.74 (9H, m, H-2′-5′, -2″-5″ and H-6″$_a$), 3.75−3.95 (2H, m, H-6″$_b$ and H-17), 4.11 (2H, q, J = 7.0 Hz, OCH_2CH_3), 4.21 (1H, m, H-6′$_a$), 4.39 (1H, m, H-6′$_b$), 4.45 (1H, d, J = 7.6 Hz, H-1′), 4.55 (1H, d, J = 7.7 Hz, H-1″), 5.35 (2H, m, H-9 and -10); ^{13}C NMR (MeOH-d_4) δ 14.73, 21.08, 21.95, 26.15, 26.33, 28.22, 28.28, 30.24, 30.33, 30.51, 30.86, 30.93, 30.99, 35.24, 37.76, 61.49, 62.78, 65.06, 71.49, 75.69, 76.12, 77.60, 77.67, 78.05, 78.11, 84.00, 102.43, 105.73, 130.91, 131.04, 172.72, 175.66; LC-APCI-MS *m*/*z* (relative intensity) 715.51 [(M + Na)$^+$, 11], 693.52 [(M + H)$^+$, 4], 327.45 (60), 309.43 (100), 263.36 (44), 245.33 (14), 213.27 (5), 205.15 (9). Anal. Calcd for $C_{34}H_{60}NO_{14}$: C, 58.94; H, 8.73. Found: C, 58.63; H, 8.47.

Ethyl 17-L-[(2′-*O*-*β*-D-Glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenoate-6″-acetate (3). To a solution of ethyl ester, **1** (325.4 mg, 0.5 mmol), and vinyl acetate (230.9 μL, 2.5 mmol) in dry THF (5 mL) was added Lipase PS-C (100 mg), and the mixture was stirred at 40 °C for 72 h under nitrogen atmosphere. The enzyme was filtered out, followed by washing with THF (3 × 3 mL). After removal of solvent the residue was subjected to silica gel column chromatography with MeOH/$CHCl_3$ (1:9, v/v) to afford amide **3** as a white solid (308 mg, 89%): $[\alpha]^{25}_D$ −9.67 (*c* 0.015, MeOH); IR (KBr plate) [cm^{-1} (%T)] 3376, 2930, 2854, 2512, 1741, 1470, 1410, 1367, 1232, 1160, 1079, 1030, 894, 726, 671; ^{1}H NMR (MeOH-d_4) δ 1.23 (6H, m, J = 7.2 Hz, H-18 and $COCH_2CH_3$), 1.24−1.50 (16H, m, H-4-7 and -12-15), 1.50−1.70 (4H, m, H-3 and H-16), 1.90−2.18 (7H, m, H-8 and -11, $COCH_3$), 2.29 (2H, t, J = 7.5 Hz, H-2), 3.20−3.70 (9H, m, H-2′-5′, -2″-5″, H-6′$_a$), 3.76 (1H, m, H-17), 3.83 (H, m, H-6′$_b$), 4.11 (2H, q, J = 7.2 Hz, OCH_2CH_3), 4.20 (1H, m, H-6″$_a$), 4.37 (1H, m, H-6″$_b$), 4.45 (1H, d, J = 7.5 Hz, H-1′), 4.63 (1H, d, J = 7.5 Hz, H-1″), 5.35 (2H, m, H-9 and -10); ^{13}C NMR (MeOH-d_4) δ 14.72, 20.88, 22.06, 26.16, 26.45, 28.24, 28.28, 30.26, 30.35, 30.51, 30.88, 31.03, 35.25, 38.11, 61.49, 63.23, 64.87, 71.67, 71.93, 75.10, 76.03, 77.92, 78.16, 78.44, 79.45, 81.84, 102.98, 104.76, 130.93, 131.04, 172.73, 175.68; LC-APCI-MS *m*/*z* (relative intensity) 715.52 [(M + Na)$^+$, 6], 531.50 (17), 513.50 (5), 495.50 (4), 327.46 (100), 309.44 (97), 263.36 (37), 245.33 (13), 213.3 (4), 205.15 (5). Anal. Calcd for $C_{34}H_{60}NO_{14}$·1.0 H_2O: C, 58.94; H, 8.73. Found: C, 59.03; H, 8.51.

Ethyl 17-L-[(2′-*O*-*β*-D-Glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenoate-6″-methacrylate (4). To a solution of ethyl ester, **1** (325.4 mg, 0.5 mmol), and vinyl methacrylate (230.9 μL, 2.5 mmol) in dry THF (8 mL) was added Lipase PS-C (100 mg), and the mixture was stirred at 40 °C for 72 h under nitrogen atmosphere. The enzyme was filtered out, followed by washing with THF (3 × 3 mL). After the removal of solvent the residue was subjected to silica gel column chromatography with MeOH/$CHCl_3$ (2:23, v/v) to afford **4** as a white solid (318 mg, 86%): $[\alpha]^{25}_D$ −5.67 (*c* 0.015, MeOH); IR (KBr plate) [cm^{-1} (%T)] 3435, 3359, 2919, 2860, 1736, 1638,

1459, 1377, 1345, 1301, 1160, 1149, 1079, 1077, 1081, 1016, 1021, 932, 894, 813, 731, 661; ^{1}H NMR (MeOH-d_4) δ 1.21 (3H, d, J = 6.1 Hz, H-18), 1.23 (3H, t, d, J = 7.1 Hz, $COCH_2CH_3$), 1.24−1.50 (16H, m, H-4−7 and-12−15), 1.50−1.70 (4H, m, H-3 and H-16), 1.93 (3H, s, $COCH_3$), 1.95−2.12 (4H, m, H-8 and -11), 2.29 (2H, t, J = 7.5 Hz, H-2), 3.20−3.68 (9H, m, H-2′-5′, -2″-5″, H-6′$_a$), 3.75 (1H, m, H-17), 3.83 (1H, m, H-6′$_b$), 4.11 (2H, q, J = 7.1 Hz, OCH_2CH_3), 4.25 (1H, m, H-6″$_a$), 4.44−4.55 (2H, m, H-1′ and -6″$_b$), 4.64 (1H, d, J = 7.8 Hz, H-1″), 5.35 (2H, m, H-9 and -10), 5.63 (1H, s, =CH$_a$), 6.11 (1H, s, =CH$_b$); ^{13}C NMR (MeOH-d_4) δ 14.72, 18.56, 22.12, 26.18, 26.48, 28.25, 28.28, 30.28, 30.37, 30.52, 30.89, 31.04, 35.26, 38.13, 61.51, 63.27, 65.15, 71.84, 71.98, 75.23, 76.04, 77.97, 78.25, 78.49, 79.51, 81.81, 103.01, 104.75, 126.43, 130.95, 131.06, 137.85, 168.67, 175.71; LC-APCI-MS m/z (relative intensity) 741.52 [(M + Na)$^+$, 23], 557.49 (24), 327.45 (100), 309.43 (73), 263.35 (25), 245.33 (10), 231.38 (8), 213.27 (6). Anal. Calcd for $C_{36}H_{62}O_{14}$: C, 60.15; H, 8.69. Found: C, 60.17; H, 8.64.

Preparation of Amides (5−9). To a solution of ethyl ester, **1** (325.4 mg, 0.5 mmol), and respective primary amine (0.51 mmol; tyramine for **5**, phenethylamine for **6**, *p*-(tolyl)ethylamine for **7**, *p*-methoxyphenethylamine for **8**, *p*-fluorophenethylamine for **9**) in dry THF (5 mL) was added Novozym 435 (100 mg), and the mixture was stirred at 50 °C for 24 h under nitrogen atmosphere. The enzyme was filtered out, followed by washing with THF (3 × 3 mL). After the removal of solvent the residue was subjected to silica gel column chromatography with MeOH/$CHCl_3$ (1:9, v/v) to afford amides **5**−**9**.

***p*-Hydroxy Phenethyl 17-L-[(2′-*O*-*β*-D-Glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide (5).** White solid (341.3 mg, 92%); $[\alpha]^{25}_D$ −12.33 (*c* 0.015, MeOH); IR (KBr plate) [cm^{-1} (%T)] 3424, 3316, 2930, 2853, 1643, 1622, 1546, 1519, 1464, 1247, 1155, 1073, 1030, 981, 927, 900, 829, 720, 671; ^{1}H NMR (MeOH-d_4) δ 1.24 (3H, d, J = 6.2 Hz, H-18), 1.26−1.50 (16H, m, H-4-7 and H-12-15), 1.51−1.62 (4H, m, H-3 and -16), 2.04 (4H, m, H-8 and -11), 2.13 (2H, t, J = 7.5 Hz, H-2), 2.68 (2H, t, J = 7.4 Hz, NCH_2CH_2), 3.20−3.72 (12H, m, H-2′-5′, H-2″-5″, NCH_2, H-6′$_a$ and -6″$_a$), 3.78−3.89 (3H, m, H-17, H-6′$_b$ and -6″$_b$), 4.44 (1H, d, J = 7.6 Hz, H-1′), 4.63 (1H, d, J = 7.5 Hz, H-1″), 5.35 (2H, m, H-9 and -10), 6.71 (2H, d, J = 8.5 Hz 2xAr−H), 7.01(2H, d, J = 8.5 Hz, 2 × Ar-H); ^{13}C NMR (MeOH-d_4) δ 22.05, 26.35, 27.18, 28.27, 30.34, 30.44, 30.51, 30.88, 30.92, 31.01, 35.82, 37.27, 37.91, 42.32, 62.83, 63.18, 71.55, 71.88, 75.96, 77.82, 77.87, 78.32, 78.40, 79.00, 82.00, 102.82, 104.75, 116.34, 130.84, 130.94, 131.00, 131.32, 156.97, 176.35; LC-APCI-MS m/z (relative intensity) 742.27 [(M + H)$^+$, 100], 580.26 (35), 418.25 (42), 400.25 (5). Anal. Calcd for $C_{38}H_{63}NO_{13}$: C, 61.52; H, 8.56; N, 1.89. Found: C, 61.22; H, 8.74; N, 1.69.

Phenethyl 17-L-[(2′-*O*-*β*-D-Glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide (6). White solid (327 mg, 90%); $[\alpha]^{25}_D$ −13.93 (*c* 0.015, MeOH); IR (KBr plate) [cm^{-1} (%T)] 3386, 3327, 2925, 2854, 1638, 1546, 1456, 1459, 1377, 1263, 1160, 1079, 1030, 927, 900, 786, 748, 699, 690; ^{1}H NMR (MeOH-d_4) δ 1.24 (3H, d, J = 6.4 Hz, H-18), 1.26−1.50 (16H, m, H-4-7 and H-12-15), 1.51−1.62 (4H, m, H-3 and -16), 2.04 (4H, m, H-8 and -11), 2.13 (2H, t, J = 7.5 Hz, H-2), 2.78 (2H, t, J = 7.4 Hz, NCH_2CH_2), 3.20−3.72 (12H, m, H-2′-5′, H-2″-5″, NCH_2, H-6′$_a$ and -6″$_a$), 3.78−3.90 (3H, m, H-17, H-6′$_b$ and -6″$_b$), 4.44 (1H, d, J = 7.6 Hz, H-1′), 4.63 (1H, d, J = 7.6 Hz, H-1″), 5.35 (2H, m, H-9 and -10), 7.15−7.30 (5H, m, 5 × Ar-H); ^{13}C NMR (MeOH-d_4) δ 22.04, 26.35, 27.15, 28.26, 30.32, 30.43, 30.50, 30.71, 30.81, 30.86, 30.92, 31.01, 36.65, 37.25, 37.89, 41.98, 62.86, 63.18, 71.57, 71.89, 75.97, 77.82, 77.88, 76.31, 78.39, 78.91, 82.09, 102.79, 104.78, 127.42, 129.56, 129.86, 129.93, 130.93, 131.00, 140.61, 176.32. LC-APCI-MS m/z (relative intensity) 748.72 [(M + Na)$^+$, 8], 726.73 [(M + H)$^+$, 34], 564.63 (35), 402.57 (100), 384.56 (30). Anal. Calcd for $C_{38}H_{63}NO_{12}$: C, 62.87; H, 8.75; N, 1.93. Found: C, 62.53; H, 8.70; N, 1.86.

***p*-(Tolyl)ethyl 17-L-[(2′-*O*-*β*-D-Glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide (7).** White solid (340.4 mg, 92%); $[\alpha]^{25}_D$ −11.20 (*c* 0.015, MeOH); IR (KBr plate) [cm^{-1} (%T)] 3376, 3310, 2925, 2854, 1643, 1551, 1459, 1452, 1410, 1377, 1399, 1079, 1030, 920, 894, 807; ^{1}H NMR (MeOH-d_4) δ 1.24 (3H, d, J = 6.1 Hz, H-18), 1.26−1.50 (16H, m, H-4-7 and H-12-15), 1.51−1.60 (4H, m, H-3 and -16), 2.04 (4H, m, H-8 and -11), 2.13 (2H, t, J = 7.5 Hz, H-2), 2.28 (3H, s, Ar-CH_3), 2.73 (2H, t, J = 7.4 Hz, NCH_2CH_2), 3.20−3.77 (12H, m, H-2′-5′, H-2″-5″, NCH_2, H-6′$_a$ and -6″$_a$), 3.82−3.98 (3H, m, H-17, H-6′$_b$ and -6″$_b$), 4.44 (1H, d, J = 7.7 Hz, H-1′), 4.63 (1H, d, J = 8.0 Hz, H-1″), 5.35 (2H, m, H-9 and -10), 7.08 (4H, m, 4 × Ar-H); ^{13}C NMR (DMSO-d_6) δ 20.57, 21.23, 24.52, 25.26, 26.57, 26.59, 28.51, 28.58, 28.65, 29.11, 29.17, 34.76, 35.38, 36.11, 60.96, 61.03, 69.93, 74.98, 75.82, 76.04, 76.21, 76.45, 76.94, 82.02, 101.01, 103.97, 128.45, 128.79, 129.58, 129.65, 134.85, 136.40, 171.99; LC-APCI-MS m/z (relative intensity) 762.77 [(M + Na)$^+$, 10], 740.78 [(M + H)$^+$, 37), 578.67 (35), 416.61 (100), 398.59 (28). Anal. Calcd for $C_{39}H_{65}NO_{12}$: C, 63.31; H, 8.85; N, 1.89. Found: C, 63.00; H, 8.86; N, 1.80.

***p*-Methoxyphenethyl 17-L-[(2′-*O*-*β*-D-Glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide (8).** White solid (351.5 mg, 93%); $[\alpha]^{25}_D$ −13.00 (*c* 0.015, MeOH); IR (KBr plate) [cm^{-1} (%T)] 3365, 3305, 3001, 2925, 2854, 1643, 1372, 1546, 1525, 1464, 1410, 1301, 1247, 1160, 1079, 1035, 1003, 959, 927, 894, 816; ^{1}H NMR (MeOH-d_4) δ 1.24 (3H, d, J = 6.1 Hz, H-18), 1.26−1.57 (16H, m, H-4-7 and H-12-15), 1.54−1.72 (4H, m, H-3 and -16), 2.03 (4H, m, H-8 and -11), 2.13 (2H, t, J = 7.4 Hz, H-2), 2.71 (2H, t, J = 7.4 Hz, NCH_2CH_2), 3.28−3.73 (12H, m, H-2′-5′, H-2″-5″, NCH_2, H-6′$_a$ and -6″$_a$), 3.75 (3H, s, OCH_3), 3.80−3.90 (3H, m, H-17, H-6′$_b$ and -6″$_b$), 4.44 (1H, d, J = 7.6 Hz, H-1′), 4.63 (1H, d, J = 7.6 Hz, H-1″), 5.35 (2H, m, H-9 and -10), 6.83 (2H, d, J = 8.5 Hz, 2 × Ar-H), 7.11 (2H, d, J = 8.5 Hz, 2 × Ar-H); ^{13}C NMR (MeOH-d_4) δ 22.04, 26.40, 27.20, 28.30, 30.36, 30.48, 30.54, 30.82, 30.97, 31.05, 35.81, 37.31, 37.95, 42.20, 55.85, 62.94, 63.26, 71.69, 71.99, 76.05, 77.92, 77.98, 78.40, 78.48, 78.96, 82.19, 102.87, 104.88, 115.07, 130.90, 130.98, 131.06, 132.65, 159.89, 176.41; LC-APCI-MS m/z (relative intensity) 756.47 [(M + H)$^+$, 74), 594.43 (44), 432.40 (100), 414.39 (13). Anal. Calcd for $C_{39}H_{65}NO_{13}\cdot 1.0H_2O$: C, 60.52; H, 8.73; N, 1.81. Found: C, 60.44; H, 8.57; N, 1.79.

***p*-Fluorophenethyl 17-L-[(2′-*O*-*β*-D-Glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide (9).** White solid (331 mg, 89%); $[\alpha]^{25}_D$ −12.27 (*c* 0.015, MeOH); IR (KBr plate) [cm^{-1} (%T)] 3398, 3314, 3001, 2930, 2854, 1643, 1551, 1513, 1470, 1377, 1231, 1157, 1084, 1040, 1030, 894, 822, 729; ^{1}H NMR (MeOH-d_4) δ 1.24 (3H, d, J = 6.2 Hz, H-18), 1.26−1.50 (16H, m, H-4-7 and H-12-15), 1.50−1.60 (4H, m, H-3 and -16), 2.04 (4H, m, H-8 and -11), 2.13 (2H, t, J = 7.5 Hz, H-2), 2.78 (2H, t, J = 7.4 Hz, NCH_2CH_2), 3.22−3.72 (12H, m, H-2′-5′, H-2″-5″, NCH_2, H-6′$_a$ and -6″$_a$), 3.73−3.98 (3H, m, H-17, H-6′$_b$ and -6″$_b$), 4.44 (1H, d, J = 7.6 Hz, H-1′), 4.63 (1H, d, J = 7.6 Hz, H-1″), 5.35 (2H, m, H-9 and -10), 6.93−7.02 (2H, m, 2 × Ar-H), 7.17−7.25 (2H, m, 2 × Ar-H); ^{13}C NMR (MeOH-d_4) δ 21.91, 26.25, 27.03, 28.16, 30.21, 30.82, 35.68, 37.41, 37.81, 41.81, 77.77, 78.24, 78.81, 82.03, 102.71, 104.72, 115.87, 116.15, 130.83, 130.91, 131.42, 131.52, 136.45, 161.39, 164.60, 176.25; LC-APCI-MS m/z (relative intensity) 766.75 [(M + H)$^+$, 13], 744.76 [(M + H)$^+$, 25], 582.65 (33), 420.59 (100), 402.57 (35). Anal. Calcd for $C_{38}H_{62}FNO_{12}$: C, 61.35; H, 8.40; N, 1.88. Found: C, 61.04; H, 8.49; N, 1.74.

***p*-Hydroxy Phenethyl 17-L-[(2′-*O*-*β*-D-Glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide-6′,6″-diacetate (10).** To a solution of **5** (325.4 mg, 0.5 mmol) and vinyl acetate (369.5 μL, 4 mmol) in dry THF (5 mL) was added Novozym 435 (115 mg), and the reaction mixture was stirred at 50 °C for 80 h under nitrogen atmosphere. The enzyme was filtered out, followed by washing of enzyme with THF (3 × 3 mL). After the removal of solvent the residue was subjected to silica gel chromatography with MeOH/$CHCl_3$ (1:24, v/v) as eluent to afford **10** as white solid (375.8 mg, 91%): $[\alpha]^{25}_D$ −6.27 (*c* 0.015, MeOH); IR (KBr plate) [cm^{-1} (%T)] 3370, 2925, 2849, 1747, 1631, 1516, 1456, 1372, 1247, 1171, 1079, 1030, 916, 834, 726, 655; ^{1}H NMR (MeOH-d_4) δ 1.19 (3H, d, J = 6.2 Hz, H-18),

1.22−1.50 (16H, m, H-4-7 and H-12-15), 1.50−1.70 (4H, m, H-3 and -16), 1.92−2.12 (10H, m, H-8 and -11, 2 × $COCH_3$), 2.14 (2H, t, J = 7.5 Hz, H-2), 2.68 (2H, t, J = 7.4 Hz, NCH_2CH_2), 3.22−3.70 (10H, m, H-2′-5′, H-2″-5″, NCH_2), 3.75 (1H, m, H-17), 4.18 (1H, m, H-6′$_a$ and -6″$_a$), 4.36 (2H, m, H-6′$_b$ and -6″$_b$), 4.44 (1H, d, J = 7.6 Hz, H-1′), 4.56 (d, J = 7.6 Hz, H-1″), 5.35 (2H, m, H-9 and -10), 6.70 (2H, d, J = 8.5 Hz, 2 × Ar-H), 7.01 (2H, d, J = 8.5 Hz, 2 × Ar-H); ^{13}C NMR (MeOH-d_4) δ 20.90, 21.06, 21.96, 26.39, 27.18, 28.29, 30.32, 30.44, 30.52, 30.94, 31.01, 35.84, 37.29, 37.98, 42.32, 64.89, 65.07, 71.54, 71.64, 75.01, 75.71, 76.19, 77.66, 77.93, 78.65, 79.58, 83.88, 102.67, 105.75, 116.37, 130.85, 130.91, 130.96, 130.97, 131.02, 131.39, 156.99, 172.84, 176.35; LC-APCI-MS *m*/*z* (relative intensity) 826.56 [(M + H)$^+$, 100], 784.55 (16), 742.55 (3), 664.52 (6), 622.51 (24), 580.49 (7), 418.47 (62), 400.46 (8), 121.09 (3). Anal. Calcd for $C_{42}H_{67}NO_{15}$: C, 61.07; H, 8.18; N, 1.70. Found: C, 60.99; H, 7.94; N, 1.55.

***p*-Hydroxy Phenethyl 17-L-[(2′-*O*-*β*-D-Glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide-6′,6″-dimethacrylate (11).** To a solution of **5** (325.4 mg, 0.5 mmol) and vinyl methacrylate (480.7 μL, 4 mmol) in dry THF (5 mL) was added Novozym 435 (130 mg), and the reaction mixture was stirred at 50 °C for 80 h under nitrogen atmosphere. The enzyme was filtered out, followed by washing of enzyme with THF (3 × 3 mL). After the removal of solvent residue was subjected to silica gel chromatography with MeOH/$CHCl_3$ (1:24, v/v) as eluent to afford **11** as white solid (395.1 mg, 90%).

One-Pot Synthesis of 11 from 1. To a solution of ethyl ester **1** (325.4 mg, 0.5 mmol) and tyramine (69.9 mg, 0.51 mmol) in dry THF (5 mL) was added Novozym 435 (230 mg), and the mixture was stirred at 50 °C for 24 h. After addition of vinyl methacrylate (480.7 μL, 4 mmol) in dry THF (2 mL), the whole reaction mixture was stirred at 50 °C for 80 h. The enzyme was filtered out, followed by washing of enzyme with THF (4 × 3 mL). After removal of solvent, the residue was subjected to silica gel chromatography with MeOH/$CHCl_3$ (1:24, v/v) as eluent to afford pure **11** as a white solid (386 mg, 88%): $[\alpha]^{25}_D$ −3.00 (*c* 0.015, MeOH); IR (KBr plate) [cm^{-1} (%T)] 3386, 2925, 2860, 2501, 2360, 2333, 1719, 1638, 1557, 1519, 1377, 1329, 1305, 1170, 1084, 1018, 814; ^{1}H NMR (MeOH-d_4) δ 1.16 (3H, d, J = 6.1 Hz, H-18), 1.18−1.40 (16H, m, H-4-7, -12-15), 1.42−1.67 (4H, m, H-3 and H-16), 1.93 (3H, s, =C(CH_3)), 1.94 (3H, s, =C(CH_3)), 2.00−2.10 (4H, m, H-8 and H-11), 2.13 (1H, t, J = 7.4 Hz, H-2), 2.57 (2H, t, J = 7.3 Hz, NCH_2CH_2), 3.22−3.64 (10H, m, H-2′-5′, H-2″-5″, NCH_2), 3.68 (1H, m, H-17), 4.25 (2H, m, H-6′$_a$ and -6″$_a$), 4.40−4.57 (3H, m, H-6′$_b$ and -6″$_b$ and H-1′), 4.47 (1H, d, J = 7.6 Hz, H-1″), 5.35 (2H, m, H-9 and H-10), 5.52 (2H, s, 2 × =CH_a), 6.12 and 6.14 (2H, 2s, 2 × =CH_b), 6.68 (2H, d, J = 8.5 Hz, 2 × Ar-H), 7.01 (2H, d, J = 8.5 Hz, 2 × Ar-H); ^{13}C NMR (MeOH-d_4) δ 18.57, 18.69, 22.01, 26.44, 27.20, 28.31, 30.38, 30.47, 30.53, 30.82, 30.86, 30.97, 31.04, 35.87, 37.31, 38.02, 42.34, 65.22, 65.24, 71.60, 71.86, 75.13, 75.81, 76.26, 77.79, 78.07, 78.68, 84.01, 102.72, 105.86, 116.38, 126.47, 126.68, 130.86, 130.97, 131.06, 131.41, 137.78, 137.83, 157.03, 168.76, 168.83, 176.37; LC-APCI-MS *m*/*z* (relative intensity) 900.51 [(M + Na)$^+$, 28], 878.53 [(M + H)$^+$, 100], 810.51 (10), 716.51 (4), 648.48 (23), 580.45 (5), 418.42 (64), 400.41 (13), 231.07 (4). Anal. Calcd for $C_{46}H_{71}NO_{15}$: C, 62.92; H, 8.15; N, 1.60. Found: C, 62.83; H, 8.11; N, 1.67.

***p*-Hydroxy Phenethyl 17-L-[(2′-*O*-*β*-D-Glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide-6′-acetate (12).** To a solution of **5** (100.2 mg, 0.135 mmol) and vinyl acetate (61.9 μL, 0.67 mmol) in dry THF (5 mL) was added Novozym 435 (35 mg), and the reaction mixture was stirred at 40 °C for 20 h under nitrogen atmosphere. The enzyme was filtered out, followed by washing of enzyme with THF (3 × 3 mL). After the removal of solvent, residue was subjected to silica gel chromatography with MeOH/$CHCl_3$ (1:24 to 2:23, v/v) as eluent to afford **11** (8.3 mg, 7%) and **12** as a white solid (87.8 mg, 83%): $[\alpha]^{25}_D$ −8.60 (*c* 0.015, MeOH); IR (KBr plate) [cm^{-1} (%T)] 3392, 3006, 2854, 2490, 1741, 1643, 1637, 1519, 1470, 1367, 1247, 1176, 1084, 1030, 894, 838, 726; ^{1}H NMR (MeOH-d_4) δ 1.21 (3H, d, J = 6.2 Hz, H-18), 1.22−1.50 (16H, m, H-4-7 and H-12-15), 1.50−1.70 (4H, m, H-3 and -16), 1.92−2.10 (7H, m, H-8 and -11 and $COCH_3$), 2.14 (2H, t, J = 7.5 Hz, H-2), 2.68 (2H, t, J = 7.4 Hz, NCH_2CH_2), 3.22−3.74 (11H, m, H-2′-5′, H-2″-5″, NCH_2, H-6″$_a$), 3.75 (2H, m, H-6″$_b$, H-17), 4.18 (1H, m, H-6′$_a$), 4.38 (1H, m, H-6′$_b$), 4.44 (1H, d, J = 7.6 Hz, H-1′), 4.56 (1H, d, J = 7.7 Hz, H-1″), 5.35 (2H, m, H-9 and -10), 6.70 (2H, d, J = 8.5 Hz, 2 × Ar-H). 7.01 (2H, d, J = 8.5 Hz, 2 × Ar-H); ^{13}C NMR (MeOH-d_4) δ 21.07, 21.97, 26.34, 27.17, 28.28, 30.32, 30.36, 30.44, 30.52, 30.94, 31.01, 35.84, 37.29, 37.78, 42.32, 62.82, 65.09, 71.54, 75.73, 76.15, 77.64, 77.72, 78.13, 84.01, 102.46, 105.75, 116.37, 130.84, 130.97, 131.02, 131.36, 156.99, 172.77, 176.33; LC-APCI-MS *m*/*z* (relative intensity) 784.56 [(M + H)$^+$, 85], 742.56 (10), 622.51 (8), 580.49 (19), 418.47 (100), 400.46 (12), 121.08 (3). Anal. Calcd for $C_{40}H_{65}NO_{14}$: C, 61.28; H, 8.36; N, 1.79. Found: C, 61.06; H, 8.49; N, 1.62.

***p*-Hydroxy Phenethyl 17-L-[(2′-*O*-*β*-D-Glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide-6′-methacrylate (13).** To a solution of **5** (100.2 mg, 0.135 mmol) and vinyl methacrylate (80.5μL, 0.67 mmol) in dry THF (5 mL) was added Novozym 435 (35 mg), and reaction mixture was stirred for 20 h under nitrogen atmosphere. Then enzyme was filtered out and washed with THF (3 × 3 mL). The solvent was removed under vacuum, and the residue was subjected to column chromatography on silica gel with MeOH/$CHCl_3$ (1:24 to 2:23, v/v) to afford **11** (7.1 mg, 6%) and **13** as a white solid (87.5 mg, 80%): $[\alpha]^{25}_D$ −5.00 (*c* 0.015, MeOH); IR (KBr plate) [cm^{-1} (%T)] 3384, 2933, 2848, 2778, 1729, 1641, 1551, 1512, 1452, 1367, 1301, 1170, 1080, 1010, 945, 830, 815, 720; ^{1}H NMR (MeOH-d_4) δ 1.17 (3H, d, J = 6.8 Hz, H-18), 1.20−1.45 (16H, m, H-4-7 and H-12-15), 1.50−1.65 (4H, m, H-3 and -16), 1.94 (3H, s, =C(CH_3)), 2.04 (4H, m, H-8 and -11), 2.13 (2H, t, J = 7.3 Hz, H-2), 2.68 (2H, t, J = 7.2 Hz, NCH_2CH_2), 3.22−3.70 (11H, m, H-2′-5′, H-2″-5″, NCH_2, H-6″$_a$), 3.77 (1H, m, H-17), 3.84 (1H, m, H-6″$_b$), 4.37 (1H, m, H-6′$_a$), 4.45−4.52 (2H, m, H-1′ and H-6′$_b$), 4.56 (1H, d, J = 7.6 Hz, H-1″), 5.35 (2H, m, H-9 and -10), 5.63 (1H, s, =CH_a), 6.14 (1H, s, =CH_b), 6.70 (2H, d, J = 8.5 Hz, 2 × Ar-H), 7.01 (2H, d, J = 8.5 Hz, 2 × Ar-H); ^{13}C NMR (MeOH-d_4) δ 18.70, 21.97, 26.33, 27.17, 28.28, 30.35, 30.45, 30.52, 30.73, 30.86, 30.94, 31.01, 35.84, 37.29, 37.81, 42.32, 62.80, 65.19, 71.49, 75.78, 77.70, 78.14, 78.19, 84.15, 102.51, 105.85, 116.37, 126.71, 130.84, 130.94, 131.03, 131.36, 137.71, 137.76, 156.97, 168.79, 176.33; LC-APCI-MS *m*/*z* (relative intensity) 810.25 [(M + H)$^+$, 100], 742.27 (9), 648.27 (5), 580.26 (23), 418.27 (100), 400.27 (7), 231.00 (3), 121.0 (4). Anal. Calcd for $C_{42}H_{67}NO_{14}\cdot 0.25H_2O$: C, 61.94; H, 8.35; N, 1.72. Found: C, 61.86; H, 8.45; N, 1.62.

***p*-Hydroxy Phenethyl 17-L-[(2′-*O*-*β*-D-Glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide-6″-acetate (14).** To a solution of **5** (100.2 mg, 0.135 mmol) and vinyl acetate (41.6 μL, 0.45 mmol) in dry THF (5 mL) was added Lipase PS-C (35 mg), and the reaction mixture was stirred at 40 °C for 72 h under nitrogen atmosphere. The enzyme was filtered out, followed by washing of enzyme with THF (3 × 3 mL). After the removal of solvent, the residue was subjected to silica gel chromatography with MeOH/$CHCl_3$ (2:23, v/v) as eluent to afford **14** as white solid (92.1 mg, 87%): $[\alpha]^{25}_D$ −8.07 (*c* 0.015, MeOH); IR (KBr plate) [cm^{-1} (%T)] 3345, 2925, 2850, 2479, 2426, 1741, 1644, 1547, 1516, 1454, 1458, 1361, 1246, 1162, 1074, 1034, 924, 897, 826, 725; ^{1}H NMR (MeOH-d_4) δ 1.22 (3H, d, J = 6.2 Hz, H-18), 1.24−1.50 (16H, m, H-4-7 and H-12-15), 1.50−1.70 (4H, m, H-3 and -16), 2.00−2.10 (7H, m, H-8, H-11 and $COCH_3$), 2.13 (2H, t, J = 7.5 Hz, H-2), 2.68 (2H, t, J = 7.3 Hz, NCH_2CH_2), 3.22−3.70 (13H, m, H-2′-5′, H-2″-5″, NCH_2, H-6′$_a$), 3.74 (1H, m, H-17), 3.83 (1H, m, H-6′$_b$ and 4.18 (1H, m, H-6″$_a$), 4.37 (1H, m, H-6″$_b$), 4.44 (1H, d, J = 7.6 Hz, H-1′), 4.64 (1H, d, J = 7.6 Hz, H-1″), 5.35 (2H, m, H-9 and -10), 6.70 (2H, d, J = 8.5 Hz, 2 × Ar-H). 7.01 (2H, d, J = 8.5 Hz, 2 × Ar-H); ^{13}C NMR (MeOH-d_4) δ 20.87, 26.46, 27.19, 28.28, 30.33, 30.37, 30.45, 30.53, 30.91, 30.95,

30.99, 31.05, 35.86, 37.30, 38.14, 42.34, 63.26, 64.88, 71.69, 71.97, 75.13, 76.06, 77.96, 78.19, 78.48, 79.52, 81.84, 103.02, 104.79, 116.37, 130.86, 130.98, 131.03, 131.39, 157.03, 172.76, 176.36; LC-APCI-MS *m*/*z* (relative intensity) 784.52 [$(M + H)^+$, 100], 742.08 (23), 622.12 (72), 580.13 (22), 460.17(4), 418.15 (91), 400.16 (30). Anal. Calcd for $C_{40}H_{65}NO_{14}$: C, 61.28; H, 8.36; N, 1.79. Found: C, 60.88; H, 8.17; N, 1.69.

***p*-Hydroxy Phenethyl 17-L-[(2′-*O*-*β*-D-Glucopyranosyl-*β*-D-glucopyranosyl)-oxy]-*cis*-9-octadecenamide-6″-methacrylate (15).** To a solution of **5** (100.2 mg, 0.135 mmol) and vinyl methacrylate (54.1 μL, 0.45 mmol) in dry THF (5 mL) was added Lipase PS-C (35 mg), and reaction mixture was stirred for 72 h under nitrogen atmosphere. Then enzyme was filtered out and washed with THF (3 × 3 mL). The solvent was removed under vacuum, and the residue was subjected to column chromatography on silica gel with MeOH/$CHCl_3$ (2:23, v/v) to afford **15** as white solid (96.2 mg, 88%): $[\alpha]^{25}_D$ −4.34 (*c* 0.0152, MeOH); IR (KBr plate) [cm^{-1} (%T)] 3457, 3348, 3006, 2930, 2854, 2558, 2469, 2425, 1725, 1551, 1519, 1457, 1459, 1388, 1245, 1296, 1236, 1162, 1079, 1069, 1030, 938, 900, 834, 815; ^{1}H NMR (MeOH-d_4) δ 1.21 (3H, d, J = 6.1 Hz, H-18), 1.22−1.50 (16H, m, H-4-7, H-12-15), 1.51−1.60 (4H, m, H-3 and -16), 1.93 (3H, s, CH_3), 2.00 (4H, m, H-8 and -11), 2.14 (2H, t, J = 7.3 Hz, H-2), 2.68 (2H, t, J = 7.3 Hz, NCH_2CH_2), 3.22−3.70 (11H, m, H-2′-5′, H-2″-5″ and NCH_2, H-6′$_a$), 3.77 (1H, m, H-17), 3.80 (1H, m, H-6′$_b$), 4.25 (1H, m, H-6″$_a$), 4.44−4.55 (2H, m, H-1′ and H-6″$_b$), 4.64 (1H, d, J = 7.5 Hz, H-1″), 5.35 (2H, m, H-9 and -10), 5.62 (1H, s, $=CH_a$), 6.11 (1H, s, $=CH_b$), 6.71 (2H, d, J = 8.5 Hz, 2 × Ar-H), 7.01 (2H, d, J = 8.5 Hz, 2 × Ar-H); ^{13}C NMR (MeOH-d_4) δ 18.57, 22.13, 26.49, 27.20, 28.29, 30.34, 30.38, 30.47, 30.53, 30.90, 30.96, 31.06, 35.87, 37.30, 38.14, 42.34, 63.28, 65.15, 71.84, 71.99, 75.23, 76.05, 77.98, 78.26, 78.51, 79.58, 81.77, 103.03, 104.75, 116.38, 126.46, 130.86, 130.96, 130.98, 131.03, 131.40, 137.84, 157.04, 168.70, 176.37; LC-APCI-MS *m*/*z* (relative intensity) 832.53 $(M + Na)^+$, 810.55 [$(M + H)^+$, 72], 742.54 (4), 648.52 (63), 580.50 (8), 418.49 (100), 400.48 (26), 231.18 (4). Anal. Calcd for $C_{42}H_{67}NO_{14}\cdot 1.5\ H_2O$: C, 60.27; H, 8.43; N, 1.67. Found: C, 60.51; H, 8.18; N, 1.62.

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Supporting Information Available: ^{1}H and ^{13}C NMR spectra of all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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