Glycolipids from *Candida bombicola*: Polymerization of a 6-*O*-Acryloyl Sophorolipid Derivative

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Polymers with amphiphilic properties are of great interest since they are important components of a wide range of industrial and pharmaceutical products.[1] The modification of naturally occurring polysaccharides, which has been practiced for over a century, is a viable route to amphiphilic glycolipid containing polymers. However, in view of the complex nature of polysaccharides, their regioselective modification to form well-defined products is tedious and is generally practiced only as an academic curiosity. Incorporation of sugars in synthetic polymers is a viable alternative for the generation of amphiphilic polymers.[2] However, selectivity in such reactions often requires complex multistep synthetic pathways.[3] Enzyme-catalyzed transformations can provide high selectivity. Furthermore, chemoenzymatic strategies have been developed for preparation of sugars that are linked to vinyl polymers.[4] Vinyl monomers with sugar groups, such as glucosylethyl methacrylate, alkyl, or aryl 6-*O*-acryloyl-α-D-glucopyranosides have been prepared.[5] A problem often encountered in such work is that polar aprotic solvents such as DMF and pyridine are required to dissolve carbohydrates. Unfortunately, these solvents do not support the catalytic activity of lipases, and therefore, slow reactions and low yields were reported.[4,6] Moreover, the use of polar aprotic solvents restricts the practical adoption of such technology by industry.

Sophorolipids are microbial extracellular glycolipids produced by resting cells of *Candida bombicola*.[7] First described by Gorin et al.[7] in 1961, sophorolipids occur as a mixture of macrolactones and free acid structures that are acetylated to various extents at the primary hydroxyl sophorose ring positions (Figure 1).[7] Our laboratories are currently engaged in both the biosynthesis and selective modification of sophorolipids.[8] Our hypothesis is that the unique structures of sophorolipids will find applications in many areas such as cleaning technology, bioremediation, and bioactive therapeutic agents. Considering that sophorolipids have complex structures, their modification to monovinyl compounds that will be polymerizable to linear polymers is challenging. In this paper we report an efficient chemoenzymatic route that led to an intriguing 6-*O*-acryloyl sophorolipid macrolactone analogue. The homopolymerization of this monomer as well as its copolymerization with acrylic acid and acrylamide is also reported.

The strategy that was developed for the site-selective incorporation of an acryl group in the sophorolipid molecule is shown in Scheme 1. The methyl ester of the sophorolipid ($[\alpha]^{25}_D$ −9.77; *m/z* 659.84 ($M + Na^+$); 95%)

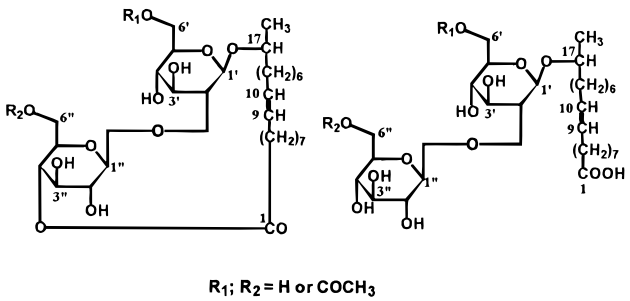

Figure 1. Sophorolipids produced by *Turolopsis bombicola* when grown on a mixture of glucose and oleic acid.

was synthesized by refluxing the sophorolipid mixture with an alcoholic solution of sodium methoxide.[8] The sophorolipid methyl ester was then subjected to acryloylation with vinyl acrylate (≥2 equiv) in dry THF. The ability of the lipases PPL, CCL, PS-30, AK, MAP-10, Novozym-435, and Lipozyme IM to catalyze this transformation was studied.[9] Of the lipases evaluated, Novozym-435 was found to be the preferred catalyst. Acryloylation with an excess of vinyl acrylate (vinyl acrylate:sophorolipid methyl ester ≥2:1) using Novozym-435 as the catalyst gave 6′,6″-diacryloylate as the primary product.[8] Surprisingly, when monoacryloylation was attempted with 1 mol equiv of vinyl acrylate, compound **1** was isolated by column chromatography ($[\alpha]^{25}_D$ −4.25; *m/z* 627.95 ($M + Na)^+$; 84%).[10] The ^{1}H NMR spectrum of compound **1** lacked signals corresponding to an acryloyl group, and there were significant differences relative to the sophorolipid methyl ester in the region 3.25−4.5 ppm. Also, the ^{1}H NMR spectrum of compound **1** lacked resonances corresponding to the methyl ester group. These anomalous features in the ^{1}H NMR of **1** suggested the formation of a lactone ring between the carboxylic acid end group of the fatty acid chain with one of the hydroxyl groups of the sophorose ring. The ^{1}H NMR spectrum of **1** also showed a 0.5 ppm downfield shift relative to the sophorolipid methyl ester in the resonance position of C-6″ protons, suggesting participation of a C-6″ hydroxyl in the formation of the lactone ring. Its ^{13}C NMR spectrum (edited by a DEPT-135 pulse sequence) provided conclusive evidence toward exclusive participation of the C-6″ hydroxyl in the lactone ring formation.

The structure of the lactone **1** is very interesting, as it is an unnatural analogue of the microbially produced macrolactone. Specifically, in **1**, unlike the natural sophorolipids, the fatty acid carboxyl carbon (C-1) is linked to the C-6″ hydroxyl, not to the C-4″ hydroxyl. The successful synthesis of **1** provided a sophorolipid analogue that had only one primary hydroxyl group. Hence, this compound was an excellent candidate for the regioselective conversion of **1** to the corresponding monoacryloyl derivative linked only to the one remaining primary site. The Novozym-435-catalyzed acryloylation of lactone **1** using vinyl acrylate in dry THF was conducted to give **2** ($[\alpha]^{25}_D$ −2.81, *m/z* 681.90 ($M + Na)^+$). The ^{1}H NMR shifts at 5.92 (1H, dd, 10.1 and 2.0 Hz, COCH=CH_{2cis}), 6.25 (1H, dd, 17.0 and 10.0 Hz, COC*H*=CH_2), and 6.44 ppm (1H, dd, 17.0 and 2.0 Hz, COCH=CH_{2trans}) confirmed monoacryloylation. In addition, the ^{1}H NMR spectrum of **2** showed a 0.7 ppm downfield shift in the resonance position of the methylene on carbon 6′

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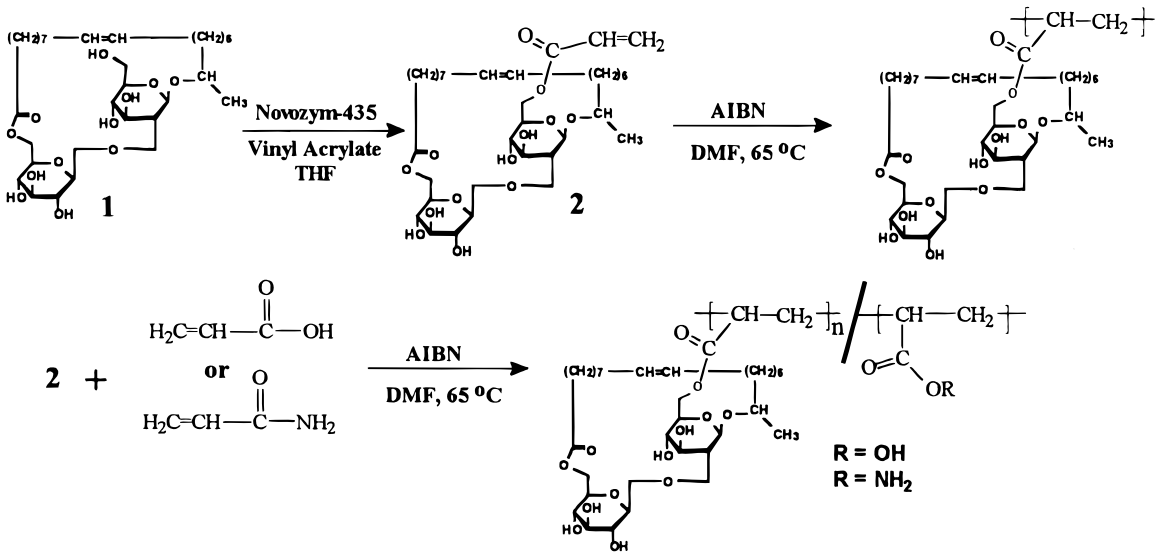
Scheme 1. Lipase-Catalyzed Regioselective Acryloylation and Subsequent Copolymerization of 6-*O*-Acryloyl Sophorolipid Derivative



when compared to the ^{1}H NMR spectrum of **1**. The position of acryloylation in **2** was conclusively identified from comparisons with the corresponding ^{13}C NMR spectrum of **1**. A downfield shift of 1.5 ppm in the resonance position of the 6′ carbon together with an upfield shift of 1.5 ppm in the resonance position of carbon 5′ confirmed that the acryloylation occurred at the 6′ hydroxyl position.

The homopolymerization of **2** was conducted by using AIBN as the initiator in dry DMF. In a representative example, to a small flame-dried flask, a solution of 250 mg of **2** in 3 mL of DMF was added. The solution was degassed (freeze/pump/thaw cycles), and 0.1% (w/v) AIBN was added. The flask was then placed in an oil bath maintained at 65 °C. The polymerization was continued for 16 h. Precipitating the polymer in ethyl acetate terminated the reaction, and the white precipitate was washed with acetone to yield 200 mg (80% yield) of the polymer. The ^{1}H NMR spectrum of the homopolymer did not show resonances due to the acryloyl protons (see Figure 2b).[11] In addition, inspection of the corresponding resonances showed that the integrity of the sophorolipid moiety was maintained under the reaction conditions used. The ^{13}C NMR spectrum of the polymer further supported that the sugar moiety was not disturbed and that the [−(−CH−CH$_2$−)−] carbon backbone was formed. The polymer was soluble in DMF and DMSO but was insoluble in water. The lack of solubility of this polymer in water is explained by the presence of the large 1′,6″-lactone ring that introduces substantial hydrophobic character. It is noteworthy to mention that the unusual amphiphilic character of the polymer side groups may provide unique inter- and intramolecular interactions.

Copolymers of **2** with acrylic acid and acrylamide were prepared to generate products having diverse solution properties and increased hydrophilic character. In other words, for some copolymer compositions, it was anticipated that the products would be water-soluble. The copolymerizations were conducted by procedures similar to the one described above for homopolymerization using AIBN as the initiator in dry DMF. In addition, adjusting the monomer feed ratio controlled the copolymer composition. Because of signal overlap and poor resolution, it was not possible to determine the composition of the

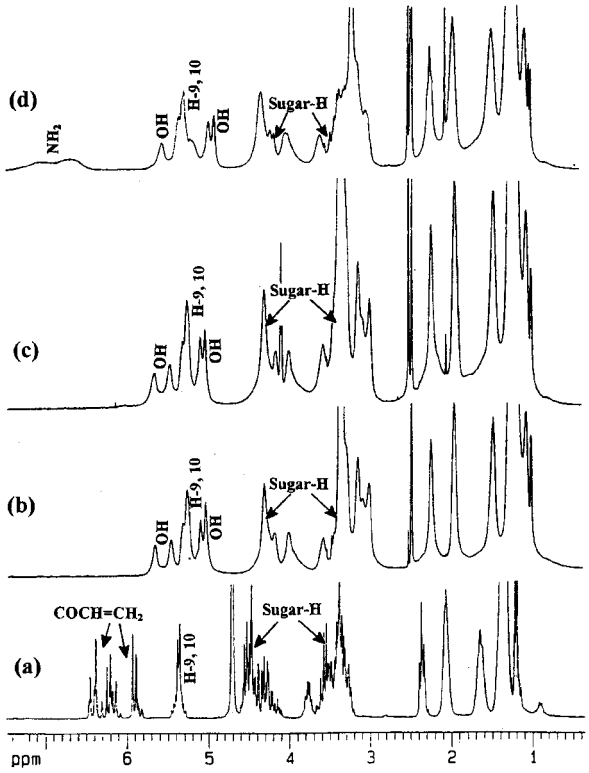


Figure 2. ^{1}H NMR spectra of **2** ($CDCl_3$, a), poly **2** (DMSO-d_6, b), 1:1 copolymer **2**/acrylic acid (DMSO-d_6, c), and 1:1 copolymer **2**/acrylamide (DMSO-d_6, d).

copolymers from the ^{1}H NMR spectra (Figure 2d). The compositions of acrylamide/**2** copolymers therefore were determined from the percent nitrogen of the copolymers.[12] Table 1 shows the copolymer composition in the acrylamide/**2** copolymers along with the percent nitrogen. Interestingly, only copolymers with <3 mol % of **2** showed solubility in water.

Following the above-described procedure, copolymers of **2** and acrylic acid were prepared. Copolymers were generated with 3, 5, 10, and 50 mol % of **2** in the feed.[14] Efforts to determine the composition of the copolymers from their ^{1}H NMR were unsuccessful due to overlapped signals (Figure 2c). The copolymers were isolated by precipitation in ethyl acetate in almost quantitative

Table 1. Composition for Acrylamide/2 Copolymers

entry	**2** in the feed (mol %)	**2** in the copolymer (mol %)[a]	nitrogen (%)[a]	yield (%)	M_n[b]	DP[b]	M_w/M_n[b]
1	50	46.5	2.17	78	7.5×10^3	105	1.3
2	5	4.2	14.01	92			
3	3	2.1	16.40	87	4.2×10^4	59	3.0
4	2	3.2	15.12	97			
5	1	0.7	18.55	90	3.2×10^4	45	3.8
6	0.5	0.9	18.09	94			

[a] From CHN analysis. [b] GPC analysis of the hydrolyzed polymer.[13,14]

yield. The solution properties of these novel polymers are currently under evaluation in our laboratories. Analysis by ^{13}C NMR of the diad and triad sequences for acrylic/**2** and acrylamide/**2** copolymers was attempted. Spectra were recorded in DMSO-d_6 (300 K, 125.77 MHz) on a Bruker DRX 500 spectrometer. Observation of the carbonyl regions for the acrylic/**3** copolymer (1/1 feed ratio) showed signals at 175.7 and 173.8 ppm. These peak positions are identical to that of the respective homopolymers. In other words, the signals at 175.7 and 173.8 ppm correspond to poly(acrylic acid) and poly(**2**), respectively. Similarly, the carbonyl region for the copolymer of acrylamide/**2** (46.5 mol % of **2**, entry 1 in Table 1) showed signals at 176.0 and 173.9 ppm. Again, these peaks occur at identical positions as the corresponding homopolymers. These results suggest that the copolymers formed are blocky in nature. However, we cannot exclude the possibility that diads of acrylamide/**2** and acrylic acid/**2** are present but are not resolved.

Solubility and conformational changes associated with different molar compositions made it difficult to analyze and compare different copolymer samples by gel permeation chromatography (GPC). It is speculated that the conformation changes associated with the large polar SL groups might be responsible for the solubility differences among the compositional copolymers. It was therefore decided to treat the polymer with 2 N NaOH to hydrolyze the SL groups.[13] The hydrolysis resulted in formation of acryl polymers with similar structures but without the pendent SL groups which were subsequently analyzed by GPC.[14] Table 1 and ref 14 list results of the GPC analyses for the representative samples of acrylamide/**2** and acrylic acid/**2** copolymers, respectively. High molecular weight copolymers were formed. Increasing acrylamide or acrylic acid ratio in the monomer feed led to increase in degree of polymerization and the polydispersity index of the copolymers (Table 1).[14]

In conclusion, we have successfully carried out enzymatic transformations on sophorolipids with high regioselectivity. To the best of our knowledge, this is the first report of any attempt toward the incorporation of naturally derived glycolipids into polymers. The copolymer structures generated have controlled quantities of a unique amphiphilic structure that will be of interest in solution studies that will follow. It is also noteworthy to point out that this work made use of a chemoenzymatic approach, exploiting the best of enzyme and chemical methods.

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Supporting Information Available: Expanded regions of the ^{13}C NMR spectra of copolymers of **2** with acrylamide and acrylic acid. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Porcine pancreatic (PPL) type II crude (activity = 61 units/mg of protein) and *Candida rugosa* lipases (CCL) type VII (activity = 4570 units/mg of protein) were obtained from 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 (USA) Co., Ltd. (specified activities at pH 7.0 were 30 000, 20 000, and 10 000 units/g, respectively). Immobilized lipases from *C. antarctica* (Novozym-435) and *Mucor miehei* (Lipozyme IM) were gifts from Novo Nordisk Bioindustrials, Inc. All enzymes, prior to their use, were dried over P_2O_5 (0.1 mmHg, 25 °C, 16 h).

(10) Synthesized from **1** by Novozym 435 catalyzed acryloylation with vinyl acrylate. In a representative example: A solution of 1.3 g of **1** and 2.5 mL of vinyl acrylate in 30 mL of THF was stirred magnetically at 35 °C for 96 h in the presence of 3 g of Novozym 435. The reaction setup secluded from the light and followed by TLC. The reaction was quenched by removing the enzyme by vacuum filtration. The solvent was removed in vacuo to give 1.5 g of the crude biotransformation product, which was purified by column chromatography over silica gel to give 1.2 g of purified **2**.

(11) ^1H NMR spectra were recorded using Bruker ARX-250 and DRX-500 spectrometer at 250 and 500 MHz, respectively. ^{13}C NMR spectra were recorded at 62.9 or 125.7 MHz with chemical shifts in ppm referenced relative to TMS at 0.00 ppm.

(12) CHN analyses were performed in duplicate at Atlantic Microlabs, Inc., Georgia.

(13) A 2 N NaOH solution (3 mL) was added to 4 mg of the polymer sample. The reaction vial was sealed and kept at 55 °C for 24 h. Dilute HCl solution was used to neutralize the reaction mixture to pH = 7. The polymer samples were recovered after removing water in vacuo at 55 °C.

(14) GPC samples were made in 0.05 M sodium acetate buffer solution (pH = 7.0). A waters GPC system, 510 HPLC pump, U6K injector, 410 differential refractometer, equipped with shodex OHpak KB-802.5 and two KB-80M GPC columns in series, was used for the molecular weight analyses. Sodium acetate solution (0.05 M, pH = 7.0) was used as eluent at a flow rate of 1.0 mL/min. Sample concentrations were about 0.5%, and the injection volume was 10 μL. Ten narrow dispersity poly(ethylene oxide) (PEO) samples in molecular weights ranging from 8.85×10^5 to 961 were used as standards. Waters Millennium version 2.15 was used for calculations. Acrylic acid/**2**: (97/3) $M_n = 2.4 \times 10^4$, $M_w = 4.8 \times 10^4$, $M_w/M_n = 1.9$; (1/1) $M_n = 6.2 \times 10^3$, $M_w = 7.2 \times 10^3$, $M_w/M_n = 1.2$.

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