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Communications to the Editor

Glycolipid Polymer Synthesized from Natural Lactonic Sophorolipids by Ring-Opening Metathesis Polymerization

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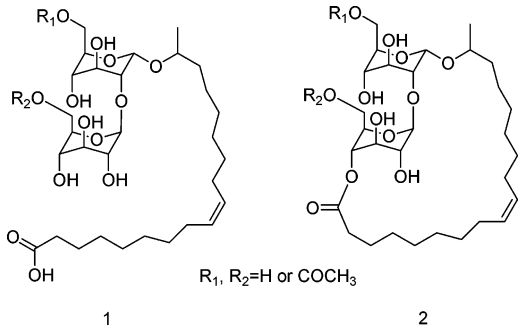
In the past two decades, ring-opening metathesis polymerization (ROMP) has proven to be an efficient method for control of polymer structure and bulk properties.[1] Ruthenium-based catalysts introduced by Grubbs permit metathesis reactions in polar and nonpolar reaction media in addition to being tolerant toward many polar functional groups under ambient reaction conditions. To polymerize monomers that bear biologically active moieties such as carbohydrates or peptides, the bioactive molecule is chemically conjugated to a polymerizable entity. For ROMP, biologically active molecules have been appended to polymerizable substances such as norbenene.[2] However, this approach leads to polymers whose main chain is nondegradable in biological systems.

Here, we describe the ROMP of a 26-membered ring glycolipid monomer by using a ruthenium catalyst. The glycolipid monomer is the natural lactonic form of sophorolipids, a microbial biosurfactant. Sophorolipids are most often constructed from the disaccharide sophorose that is glycosidically linked to the hydroxyl group at the penultimate carbon of a mono-unsaturated C_{18} chain-length fatty acid[3] (Scheme 1). They are fermentatively produced by yeasts such as *Candida bombicola*.[3] Sophorolipids have shown great promise in a wide range of therapeutic functions that include (i) septic shock antagonists, (ii) antibacterial, (iii) antifungal, (iv) antiviral (HIV-1), (v) antispermicidal, and (vi) anticancer agents.[4] Chemoenzymatic methods have been developed to synthesize a range of pure sophorolipid analogues from the microbially produced natural mixture.[5]

Scheme 1. Sophorolipids from *Candida bombicola* Are Synthesized as a Mixture of Ring-Opened (1) and Lactonic (2) Molecules That Are Acetylated to Variable Degrees at Sophorose 6′- and 6″-Positions



Previously, our laboratory reported the synthesis of polyvinyls with pendent sophorolipid groups.[6] The monomer, 6-*O*-acryloyl sophorolipid, was prepared in three steps from a natural sophorolipid mixture. Pendant bioactive glycolipid moieties were appended to carbon−carbon main chains that are nondegradable. This paper describes an efficient alternative route to sophorolipid polymers that have very different structures from those synthesized by free-radical polymerization. Natural lactonic sophorolipids, highly abundant within the mixture of products formed by *Candida bombicola*, were directly polymerized. The resulting polymers were prepared in high molecular weights and are soluble in common organic media. Furthermore, the polymer is designed to bioresorb in biologically active milieus due to the regular occurrence of disaccharide and ester links along chains.

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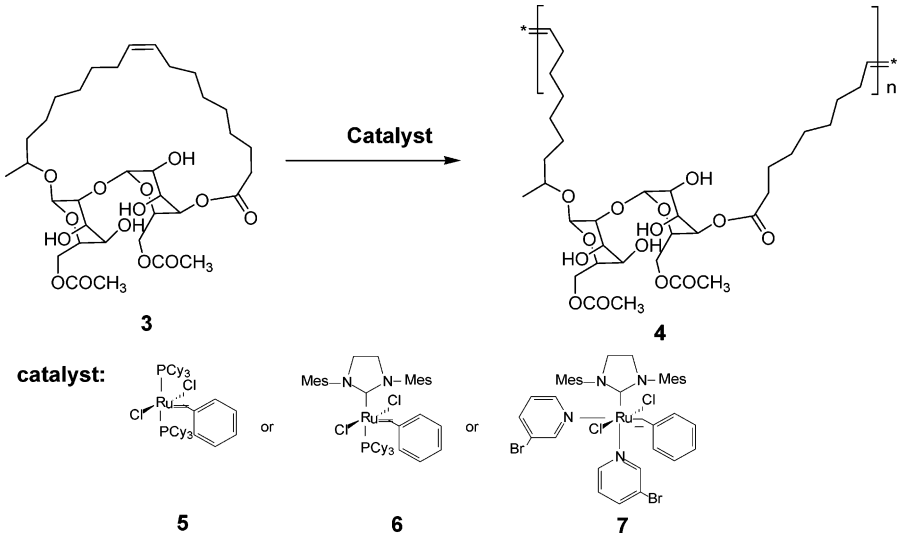
Scheme 2. ROMP of Lactonic Sophorolipids To Form Poly(sophorolipid)



Sophorolipids were synthesized by fermentation of *Candida bombicola* by a method described in detail elsewhere.[5b] The lactonic fraction of the product mixture was purified by silica gel column chromatography using a methanol/chloroform mixture as eluent.

LC/MS analysis of column-purified materials was performed using an atmospheric pressure chemical ionization (APCI) probe in the positive ion mode with detection in the scan mode (range 170−850 *m*/*z*). Three major peaks were observed in the total ion chromatograph (TIC). They were assigned to (i) two isomers of lactonic diacetate sophorolipid with monounsaturated 9-octadecenoic acid (peaks *a* and *b* in the Supporting information Figure S-1a, *m*/*z* at 711) and (ii) lactonic diacetate sophorolipid with a saturated lipid moiety (peak *c*, *m*/*z* = 713). According to LC/MS/MS analysis of sophorolipids by Hu and Ju,[7] peaks *a* and *b* are distinguished by lipidic moieties with 17- and 18-hydroxy-9-octadecenoic acid, respectively (see Supporting Information). The ^{1}H NMR spectrum of column-purified sophorolipids confirms they are lactonic with a high degree of acetylation at sophorose 6′- and 6″-positions.[5a] Column-purified lactonic sophorolipids, hereinafter called lactonic sophorolipids, were used as monomers for ROMP polymerization studies.

Polymerizations of lactonic sophorolipids were conducted in dichloromethane using three different ruthenium catalysts (Scheme 2). Briefly, lactonic sophorolipids and catalyst were dissolved in dichloromethane (CH_2Cl_2), and reaction vessels were purged with nitrogen. After mixing solutions of monomer and catalyst, depending on reaction conditions, gels were formed in 2−60 min. All reactions were run for 5 min at 25 °C. Polymers were isolated by dissolving reaction mixtures in THF, precipitation by slow addition of THF/CH_2Cl_2/polymer solutions into ethanol, filtration, and stripping of solvent. M_n and M_w/M_n (PD) of isolated products were determined by size exclusion chromatography (SEC) using polystyrene standards. Table 1 summarizes reaction conditions investigated, product yield, M_n, and PDI of precipitated polymers.

Lactonic sophorolipids were polymerized by all three ruthenium catalysts forming polymers with $M_n \geq 42\,200$ g/mol and yield ≥ 67%. Catalyst **6** (see Scheme 2) gave poly(sophorolipids) in highest yield (89%) and molecular weight (M_n 103 000). The yield of precipitated polymer and molecular weight averages increased with increased monomer concentration at constant monomer-to-catalyst ratio (88, see entries 2 and 3). Monomer molar concentrations used herein are below that typical used for ROMP polymerizations of low ring-strain monomers (e.g., 4−5 M). Low monomer solubility in the reaction medium partly due to their large molar mass restricted exploration of higher lactonic sophorolipid solution concentrations. Attempts to polymerize lactonic sophorolipids at concentrations below 0.2 M resulted in either low yields or no product (data not shown).

Table 1. Synthesis of Poly(sophorolipids)[a]

entry	[M][b] (mol/L)	cat.[c]	[M]/[cat.] (mol/mol)	yield[d] (%)	M_n[e] (g/mol)	PDI[e]
1	0.54	5	88	86	67 500	2.2
2	0.54	6	88	89	103 000	2.0
3	0.31	6	88	86	55 200	1.8
4	0.79	6	130	86	83 000	1.9
5	0.52	7	81	87	42 200	1.8
6	0.67	7	105	67	54 900	1.8

[a] Reactions were performed for 5 min at 25 °C in methylene chloride. [b] Monomer concentration in reaction mixture. [c] Ruthenium catalyst for ROMP (see Scheme 2). [d] Ethanol-insoluble product. [e] Determined by SEC relative to polystyrene standards.

The small fraction of lactonic sophorolipids with saturated fatty acids formed by *Candida bombicola* should not be polymerized by ROMP. To test this, nonpolymerized monomer was recovered from the ethanol-soluble fraction and was analyzed by LC-MS. Indeed, nonpolymerized lactonic sophorolipids were highly enriched in the saturated analogue (see Figure S-1b, Supporting Information).

Proton NMR spectra (400 MHz, d_6-DMSO with 0.5% D_2O) of the lactonic monomer and poly(sophorolipid) are similar (see Figure S-2, Supporting Information). However, differences were observed in the spectral region corresponding to vinyl proton resonances H-9 and H-10 (−C*H*═C*H*−, see Supporting Information Figure S-2a,b). For the monomer, the double bond is purely *cis* as expected based on fatty acid metabolism. The *cis* vinyl proton resonances appear as a complex multiplet of overlapped signals between 5.25 and 5.35 ppm, centered at 5.31 ppm (Figure S-2a). For poly(sophorolipid), a weak signal at 5.31 ppm was observed as well as a new signal at 5.35 ppm assigned to *trans* vinyl protons.[8] Formation of both *cis* and *trans* −C*H*═C*H*− isomers in poly(sophorolipid) is consistent with that these ROMP catalysts are not specific with respect to double bond isomer structure.[9]

Thermodynamics, that is, the relative stabilities of the cyclic monomer and linear polymer, determines whether a ring-opening polymerization will proceed.[10] For example, formation of polymer when liquid cycloalkenes are converted to corresponding linear semicrystalline polymers depends on changes in enthalpy, entropy, and free energy (ΔH_{lc}, ΔS_{lc}, and ΔG_{lc}, respectively). A question that arose during this study is, what drives the formation of poly(sophorolipid) given the low strain energy of the 26-membered lactonic monomer? One explanation is that the product formed is much more stable than the monomer (large ΔH_{lc}). Indeed, previous work by our laboratory showed that ring-opened "acidic" sophorolipids acted as bolaamphiliphiles ("bolas") and formed giant twisted and helical ribbons of 5−11 μm width and several hundred micrometers in length.[11] Furthermore, differential scanning calorimetry (see thermogram in Supporting Information, Figure S-3) shows that poly(sophorolipids) are semicrystalline materials with a relatively high glass transition (T_g, 48 °C), melting point (T_m, 135 °C), and heat of fusion (ΔH_f, 28.9 J/g). Therefore, it appears that strong enthalpic interactions between poly(sophorolipid) molecules arise that drives ROMP of 26-membered lactonic sophorolipids. Besides the thermodynamic aspect, the low activation energy associated with metathesis conversions is also important to allow the facile polymerization of lactonic sophorolipid.

In summary, lactonic sophorolipid, derived from fermentation of *Candida bombicola*, was polymerized by ROMP catalysis. Poly(sophorolipid) was prepared in yield and M_n up to 89% and 103 000, respectively. By this chemo-biocatalytic route, the complexity of this natural bioactive glycolipid was transferred to polymer chains that have disaccharide, ester, and monounsaturated hydrocarbon moieties. Poly(sophorolipids) are semicrystalline materials that melt at 135 °C. The facile polymerization of low ring-strain 26-membered lactonic sophorolipids can be explained by strong enthalpic interactions that develop between poly(sophorolipid) molecules. Work is in progress to characterize the crystalline structure, solid state, and biological properties of poly(sophorolipids).

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Supporting Information Available: LC/MS method and spectra of purified lactonic sopholipids and unreacted lactonic sophorolipid monomer recovered form ROMP; proton NMR spectra of purified lactonic sophorolipid and poly(sophorolipid). This material is available free of charge via the Internet at http://pubs.acs.org.

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