

“Sweet Silicones”: Biocatalytic Reactions to Form Organosilicon Carbohydrate Macromers

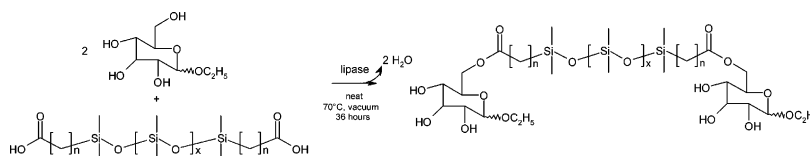
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ABSTRACT



Immobilized lipase B from *Candida antarctica* (Novozyme 435) catalyzed the regioselective formation of ester bonds between organosilicon carboxylic diacids and a C1–O-alkylated sugar under mild reaction conditions (i.e., low temperature, neutral pH, solventless). Specifically, the acid-functionalized organosilicones reacted with the primary hydroxyl group at the C6 position of α,β -ethyl glucoside during the regioselective esterification. The pure organosilicon–sugar conjugates were prepared in a one-step reaction without protection–deprotection steps and without activation of the acid groups with the integrity of the siloxane bonds.

Historically, reaction conditions have inhibited the synthesis of structurally defined organosilicon carbohydrates. Typically, esters and polyesters are synthesized with an acid or base catalyst in a one-step process at high temperatures for long periods.¹ Although these reaction conditions favor the equilibrium of condensation, they also promote uncontrolled side reactions. While reasonable reaction rates and conversions may be achieved through acid or base catalysis, these catalysts may induce the decomposition of potentially useful functional groups and bonds (e.g., siloxane). In addition, the usual acid and base catalysts are not regioselective and may catalyze esterification at all reactive groups on a multifunctional monomer. Consequently, the ability to control the structure of the material is essentially lost.

Given the ability to self-assemble, amphiphilic organosilicon carbohydrates were documented to have unusual

properties in solution or as neat materials.^{2,3} The physical properties of these “sweet silicones”² are dependent on the structure of the attached carbohydrate. They may be used as surfactants, adhesion promoters, or chiral templates. Braunmuhl et al.^{2,3} synthesized poly(dimethylsiloxane)s with pendent maltoheptaoside or maltoheptaonamide groups by hydrosilylation and/or amidation. Subsequently, potato phosphorylase was used to enzymatically catalyze the formation of poly(dimethylsiloxane)-graft-($\alpha,1\rightarrow4$)-D-glucopyranose molecules with α -D-glucose-1-phosphate in a citrate buffer at 37 °C. The amylose side chains were determined to have helical structure. While this represents an elegant strategy for the synthesis of amphiphilic organosilicon carbohydrates, the need for multiple steps and activation chemistry would be problematic at larger reaction scales.

Enzyme catalysis can circumvent conventional protection–deprotection steps during conjugation of carbohydrates to

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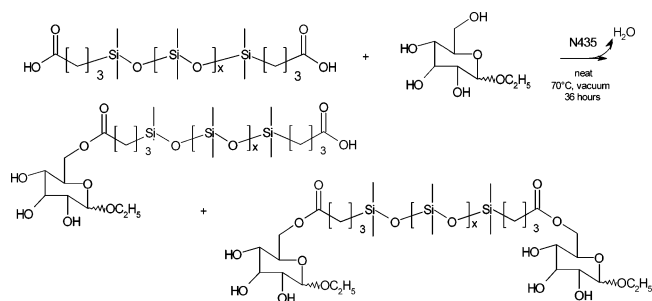
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organosilicones. Methods of engineering of an enzyme's environment within reactions by its incorporation within micelles, immobilization on or within solid supports, and by shifting the polarity of the solvent have all been effectively used to optimize the handling and activity of a broad range of biocatalysts.^{4–7}

In this paper, the lipase-catalyzed esterification of organosilicon carboxylic diacids and the C1–O-alkylated α,β -ethyl glucoside was studied. The length of the organosilicon segment was varied, and its effect on the conversion to esterified products and/or the regioselectivity of the transformation was studied. Carbon (¹³C) nuclear magnetic resonance (NMR) was used to determine the regioselectivity of the esterification reactions. Electrospray MS and SEC methods were also used to determine the regioselective esterification of ethyl glucoside with PDMS end-blocked acid. The catalyst was physically immobilized lipase B from *Candida antarctica* (Novozyme 435).

All reagents were purchased in the highest available purity and used as received. α,β -Ethylglucoside was prepared and purified according to a literature method.^{8,9} 1,3-Bis(3-carboxypropyl)tetramethyldisiloxane (PDMS diacid with $x = 0$, product #SIB1027.0, CAS #3353-68-2) and PDMS diacids with $x_{av} = 7$ and 65 (product #XG-0886 bulk and product #XG-0889 bulk, respectively) were purchased from Gelest, Inc. (Tullytown, PA). Novozyme 435 (N435) was a gift from Novozymes (Bagsvaerd, Denmark). N435 is a commercial source of *C. antarctica* lipase B immobilized on acrylic resin beads.

Scheme 1. Lipase-Catalyzed Esterification between α,β -Ethylglucoside and Diacid-Terminated Siloxanes (PDMS Diacids) where $x = 0, 7,$ and 65



The reactions were conducted in vacuo with constant stirring in two-neck round-bottom flasks heated by an external oil bath. The neat (solventless) reactions were performed with a 1:2 molar ratio of the PDMS diacid to α,β -ethylglucoside. Furthermore, the ratio of N435 to the

reactants was 1:10 w/w. Prior to reactions, the enzyme was transferred to an oven-dried vial and dried in vacuo (0.1 mmHg) at 25 °C for 48 h. α,β -Ethyl glucoside was added to the organosilicon reactant at 70 °C. After these components formed a monophasic mixture, dried N435 was added, and the reaction mixture was maintained at 70 °C for 2 h and then maintained in vacuo (70 °C, 65 mmHg) with magnetic stirring for an additional 34 h. The temperature 70 °C was selected since this resulted in a low-viscosity reaction mixture. At temperatures below 50 °C, ethylglucoside behaves as an oily adhesive, making stirring difficult. Furthermore, previous work by us and others has shown that 70 °C is a preferred temperature for condensation reactions.^{10,11} The reactions were monitored by thin-layer chromatography (TLC). After reactions, the mixtures were filtered to remove N435, and products were purified by flash silica gel column chromatography (silica gel 60, Aldrich). In a typical separation, 50 g of silica gel was used to pack a glass column (5 × 50 cm) in the eluent (CHCl₃/MeOH mixture). Then, 200 mL of eluent was run through the column before the crude compound (500 mg), dissolved in a minimal amount of eluent, was loaded onto the top of the column silica bed. Different fractions were subsequently eluted (1 mL/min) and monitored by thin-layer chromatography (TLC). Fractions containing the purified compounds were pooled together, and the solvent was evaporated to give the pure product. The chromatographic separations of products from esterifications of 1,3-bis(3-carboxypropyl)tetra-methyldisiloxane ($x = 0$) and the corresponding higher molar mass PDMS diacids ($x_{av} = 7$ and 65) were performed using chloroform/methanol mixtures of 9/2 and 9.5/0.5 v/v, respectively.

For the PDMS diacid with $x = 0$, the mono- ($R_f = 0.6$, CHCl₃/MeOH 9:2, isolated yield 19%) and diester ($R_f = 0.4$, CHCl₃/MeOH 9:2, isolated yield 44%) were formed during the esterification. The products from N435-catalyzed esterifications of the other PDMS diacids ($x = 7$ and 65) were also isolated by flash column chromatography, and the overall yields were 79 and 83%, respectively. For control reactions performed under similar conditions at 70 °C but without enzyme, no acylation of the product was observed. The organosilicon carbohydrate conjugates were tan fluids. Structural analyses of these substances formed by the biotransformations were performed by nuclear magnetic resonance (NMR), mass spectroscopy (MS), and size exclusion chromatography (SEC) experiments relative to polystyrene as a standard, and THF was the solvent. Furthermore, the products were characterized by thermal analytical methods (see below).

Regioselectivity of esterification reactions was determined by recording ¹³C DEPT NMR spectra (Figure 1). In the ¹³C DEPT NMR spectrum of α,β -ethylglucoside (Figure 1B), the signals of the 6- α,β -carbons both occurred at 61.4 ppm. After esterification, the 6- α,β -carbons shift downfield by 2.1 ppm. Concurrently, due to the γ -effect caused by substitution

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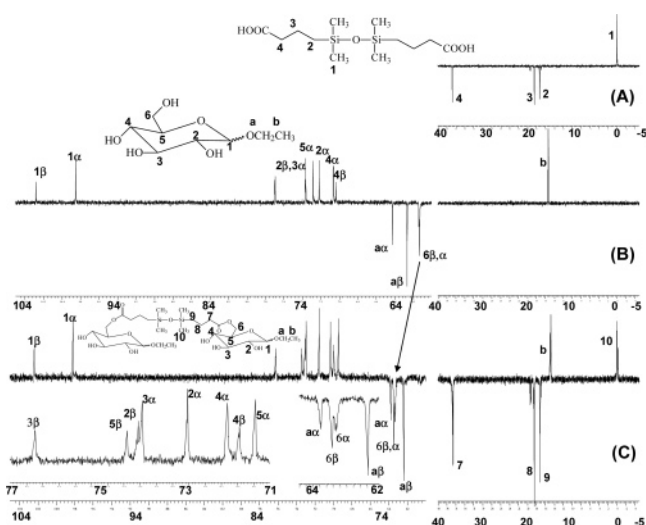


Figure 1. ¹³C DEPT NMR (DMSO-*d*₆, 75.5 MHz) spectra of 1,3-bis(carboxypropyl)-tetramethyldisiloxane (A), α,β-ethylglucoside (B), and 1,3-bis(1'-ethylglycosyl-6'-propionate) tetramethyldisiloxane (C).

at the 6-hydroxyl position, the 5α- and 5β-carbons shift upfield by approximately 3.4 ppm. Since the chemical shifts of the other α,β-ethylglucoside carbons remained nearly unchanged, we conclude that the esterification occurred with >98% regioselectivity for the primary hydroxyl position (C6). Similarly, ¹³C DEPT NMR spectra were recorded for the mono- and diesterified PDMS diacids with *x* = 7 and 65. Comparison of these spectra to the spectrum in Figure 1C showed identical chemical shifts and relative signal areas for carbons corresponding to esterified α,β-ethylglucoside carbons (see Figure S-1 in Supporting Information for the enlarged view of the ¹³C DEPT of 1,3-bis(1'-ethylglycosyl-6'-propionate) tetramethyldisiloxane). Hence, for the range of PDMS chain lengths studied, N435-catalyzed esterifications with α,β-ethylglucoside occurred with high regioselectivity. Comparatively similar chemical shifts and relative signal areas were previously reported by our laboratory for α,β-ethyl glucoside conjugated by esterification at the primary C6 hydroxyl group to the carboxyl chain end of poly(ε-caprolactone).^{10,11} In the later example, α,β-ethyl glucoside replaced water as the initiator for ε-caprolactone polymerizations.

The products of N435-catalyzed esterification reactions between α,β-ethylglucoside and PDMS diacids were characterized by electrospray ionization mass spectrometry (ESI MS). Figure 2 displays the ESI MS spectrum of products from N435-catalyzed reaction of 1,3-bis(3-carboxypropyl)-tetramethyldisiloxane with α,β-ethyl glucoside. An *m/z* signal at 495.2 corresponds to a monoester with a nonreactive cyano functionality present in the starting material.

The presence of mono- and diesters in the product mixture was confirmed by signals at *m/z* 514 and 704, respectively. The absence of a *m/z* signal for the product corresponding to two PDMS diacids linked at two sugar positions supports that the esterification is very selective.

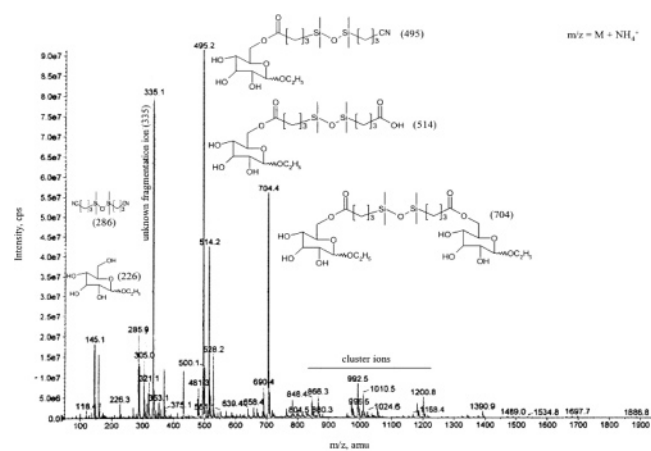


Figure 2. ESI MS analysis of the lipase-catalyzed regioselective esterification of 1,3-bis(3-carboxypropyl)tetramethyldisiloxane and α,β-ethyl glucoside.

Figure 3 shows the ESI-MS spectrum of products from the N435-catalyzed esterification of the *x* = 7 PDMS diacid and α,β-ethylglucoside. Furthermore, the spectrum shows the distribution of mono- (Δ) and diester (O) products along with the number and distribution of differing chain length species in the product. The observation that there was no

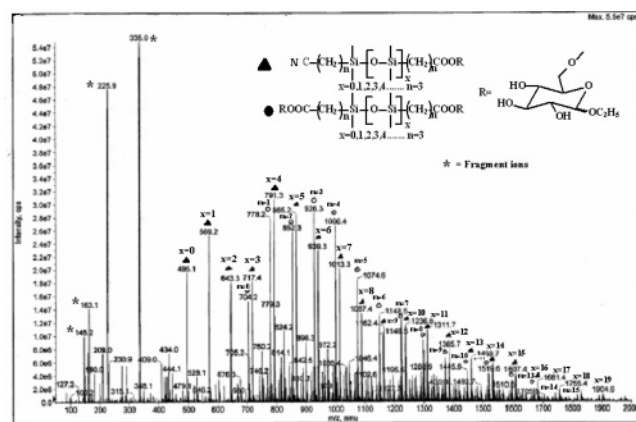


Figure 3. ESI-MS analysis of the lipase-catalyzed esterification of diacid end-blocked poly(dimethylsiloxane) and α,β-ethylglucoside. The spectrum shows the distribution of (D) monoester cyano and ethylglucoside functional PDMS and (O) diester ethylglucoside functional PDMS products along with the number and distribution of differing chain length species.

change in the distribution from the starting material and the product proves that the mild reaction conditions caused little or no exchange reactions. The esterification is almost 99%, as the monoester functional has the cyano and ethylglucoside functionalities, whereas the diester has ethylglucoside groups at both chain ends. Thus, as we increase the chain length of the dimethylsiloxane, the extent of esterification resulting from N435 catalysis increased to almost 99%.

The distribution of different molecular weight species was also studied by size exclusion chromatography (SEC). SEC was used to determine the number and weight average molecular weights and the polydispersity (M_n , M_w , and M_w/M_n , respectively) of the starting materials and products. Following the esterification of the $x = 7$ PDMS diacid, the M_n and M_w/M_n of the organosilicon carbohydrate conjugates were 1320 and 1.4, respectively. The M_n and M_w/M_n of the $x = 7$ PDMS starting material were 902 and 1.5, respectively. Comparatively, a theoretical esterification that occurs for the diester product at one of the four hydroxyl groups of α,β -ethyl glucoside would result in a product with $M_n = 1310$. Similarly, SEC chromatographs of the $x = 65$ PDMS diacid prior to and after the esterification reaction showed a small increase in M_w (from 12 400 to 12 700) and little change in M_w/M_n (from 6.0 to 6.8). The similarity of M_w/M_n values as well as theoretical and measured M_n values for the starting oligomers and products supports that the esterification reactions are regioselective. If the reactions were nonregioselective and, instead, occurred at multiple sites of α,β -ethyl glucoside, large increases in M_n and shifts in M_w/M_n would have been observed.

On the basis of thermal gravimetric analyses (TGAs) and differential scanning calorimetry (DSC) results, the conjugate of 1,3-bis(1'-ethylglycosyl-6'-propionate) tetramethyldisiloxane and α,β -ethylglucoside experienced a critical mass loss at 184 °C versus 164 °C for the PDMS diacid reactant (see Tables 1 and 2 and Figure S-3 in Supporting Informa-

Table 1. TGA Analysis of Organosilicon Carbohydrates

| product (av DP ^a) | °C | weight remaining in N ₂ | | | | | | |
|----------------------------------|----|------------------------------------|-----|-----|-----|-----|-----|-----|
| | | 100 | 200 | 300 | 400 | 500 | 600 | 700 |
| $x = 0^a$ | % | 98 | 92 | 49 | 5 | 1 | 1 | 2 |
| $x = 7^a$ | % | 98 | 94 | 65 | 32 | 4 | 2 | 1 |
| $x = 65^a$ | % | 100 | 99 | 95 | 87 | 71 | 4 | 1 |

^a Av DP = the diacid siloxane average degree of polymerization = $x + 2$.

tion). On the basis of the derivative curve, weight loss occurred at two temperatures (281 and 395 °C) for the product versus one temperature (226 °C) for the reactant (see Figure S-2 in Supporting Information). The T_g values of the product and the corresponding PDMS diacid were -26 and -76 °C, respectively. Hence, more energy was required to achieve molecular motion for the ester derivative. During the DSC heating cycles, the esterified product had a crystalline phase with a peak melting temperature (T_m) and enthalpy of fusion (ΔH_f) of 13 °C and 1.7 J/g, respectively (see Figure S-5 in Supporting Information). In contrast, a cold crystallization ($T_{cc} = -15$ °C, 50.4 J/g) and two crystalline phases ($T_m = 30$ °C, 45 J/g; $T_m = 46$ °C, 8.6 J/g) were detected for the corresponding PDMS diacid reactant (see Figure S-4 in Supporting Information).

A similar analysis for the $x = 7$ PDMS diacid showed a critical mass loss at 201 °C versus 197 °C for the PDMS diacid reactant. Comparatively, the weight loss occurred at

Table 2. DSC Analysis of Organosilicon Carbohydrates

| product (av DP ^a) | DSC results |
|----------------------------------|--|
| $x = 0^a$ | $T_g = -26^\circ\text{C}$; $T_m = 13$ to 17°C T_m (peak maximum) at 13°C , $\Delta H_f = 1.7$ J/g. |
| $x = 7^a$ | $T_g = -34^\circ\text{C}$; $T_m = 18$ to 27°C T_m peak maximum at 20° and 26°C |
| $x = 65^a$ | $T_g = -123^\circ\text{C}$; $T_{cc} = -107$ to -55°C , $T_m = -54$ to -32°C , T_{cc} (peak maximum) at -79°C , $\Delta H_f = 29$ J/g, T_m (peak maximum) at -47 and -38°C , $\Delta H_f = 30$ J/g. |

^a Average DP of diacid siloxane = $x + 2$.

three temperatures (218, 268, and 417 °C) for the product versus two temperatures (249 and 563 °C) for the starting PDMS diacid. The T_g values of the product and reactant were -111 and -34 °C, respectively. During DSC heating cycles, two melting endotherms with peaks at 20 and 26 °C were observed in the product. Given the small ΔH_f values of these melting transitions, the region of order within the product is either small or the molecular interactions are weak.

When the PDMS chain length was extended to $x = 65$ ($n = 3$), the ethylglycosyl conjugate products showed a critical mass loss at 264 °C versus 172 °C for the diacid reactant. Comparatively, the weight loss occurred at three temperatures (324, 397, and 529 °C) in the product versus two temperatures (216 and 591 °C) in the starting material. The T_g of both the product and the diacid disiloxane were -123 °C.

During the DSC heating cycles, a cold crystallization ($T_{cc} = -79$ °C, 29 J/g) of the product showed two melting endotherms with peaks at -47 and -38 °C (30 J/g). Thus, the thermal characteristics of PDMS sugar conjugates with $x = 65$ are dominated by properties of the long PDMS chain.

Conclusion

Pure organosilicon-sugar conjugates were prepared in a one-step reaction, without protection-deprotection steps. This simplification of an otherwise tedious reaction was a result of the inherent regioselectivity of the lipase catalyst. The lipase-catalyzed reactions did not require activation of the acid groups. In comparison to organic materials, the hydrophobic organosilicones were acceptable substrates. Given the ability to perform a selective reaction and maintain the integrity of the siloxane bonds with lipase, the ability to synthesize structurally defined organosilicon carbohydrates with a diversified set of functional groups may be used to create new materials such as fibers, films, coatings, gels, and surfactants with novel properties.

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Supporting Information Available: Experimental details and Figures S-1–5. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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