Enzymatic *Grafting* of Amylose *from* Poly(dimethylsiloxanes)

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ABSTRACT: The synthesis of poly(dimethylsiloxane-*graft*-(α,1→4)-glucopyranose) by enzymatic polymerization with potato phosphorylase (E.C. 2.4.1.1) has been successfully performed using a polyinitiator based on polysiloxane with statistically distributed maltoheptaonamide and maltoheptaoside. Three different pathways are described to obtain suitable polyinitiators. Polysiloxanes with pendant gluconamide or maltoheptaonamide groups are prepared from acetyl-protected *N*-allylaldonamides by hydrosilation using a rhodium catalyst. Maltoheptaonamide-modified siloxanes of high molecular weight remain water insoluble. However, in an aqueous suspension the carbohydrate side chains are solvated and are accessible for phosphorylase to polymerize the amylose grafts.

1. Introduction

Amphiphilic polymers in which supramolecular self-assembly is achieved by molecular recognition show a variety of unusual properties in bulk and in solution.[1] A new class of such amphiphilic polymers is based on poly(dimethylsiloxanes) (PDMS) as a hydrophobic backbone and carbohydrate derivatives as hydrophilic side chains.[2] Similar hybrid systems of synthetic polymers and carbohydrates have attracted some attention. Kobayashi[3] synthesized polystyrene with pendant carbohydrate groups. These polymers enable hepatocytes to grow *in vitro* for several days. Thus, pathobiochemical studies become possible without the disadvantages of *in vivo* investigations. The use of PDMS as a backbone may be of advantage in similar applications. As a biocompatible material PDMS is characterized by thermal stability and high oxygen affinity. The high segmental mobility results in a low glass transition temperature. This makes the use of PDMS as a low-temperature elastomer feasible. Gruber[4] reported the synthesis of PDMS networks with statistically functionalized saccharose as a cross-linking agent. These materials show mechanical stability, optical homogeneity, and excellent biocompatibility.

On the other side, the unique properties of saccharides in biological systems are based on the specific hydrogen bond interaction defined by the stereochemistry of carbohydrates. A suitable reaction pathway to attach glycosides of various carbohydrates onto PMDS in a well-defined manner was reported recently.[2]

The hydrophilic−lipophilic balance of covalent "sugar siloxanes" can be adjusted by four parameters, the molecular weight of the PDMS backbone, the degree of substitution, the kind of carbohydrate attached, and the length of the saccharide side chains. The physicochemical properties of the "sweet silicone" in bulk and in solution[5] strongly depend on the structure of the carbohydrate attached. In addition, these polymers have interesting potential as functional systems, such as surface modifiers or chiral templates.

In the present paper we report on the first results of the enzymatic *grafting* of amylose *from* the PDMS backbone.

The *in vitro* synthesis of starch (poly-(α,1→4)-D-glucopyranose) from α-D-glucose-1-phosphate (Glc-1-P) by primer-dependent enzymatic catalysis is well-known and was investigated first by Cori[6] in 1940. The enzyme catalyzes the reversible reaction

$$((\alpha,1\rightarrow4)\text{-Glc})_n + \text{Glc-1-P} \rightleftharpoons ((\alpha,1\rightarrow4)\text{-Glc})_{n+1} + \text{P}$$

where a glucose unit is transferred from inorganic phosphate (P) to the nonreducing 4-OH terminus of an (α,1→4)-glucan chain. The phosphate is released as HPO_4^{2-}. Applications of this reaction to various chemically synthesized primers and detailed studies on the mechanism and kinetics were carried out by Pfannemüller.[7,8] Under standard reaction conditions the enzymatic glucan synthesis provides a linear correlation between the reaction time and chain length. The number of glucose units of the side chains can be determined by the amount of liberated phosphate. The polydispersity of glucans synthesized enzymatically is described by a Poisson distribution, if the reaction is not allowed to exceed 30% monomer consumption. $\bar{M}_w/\bar{M}_n$ is expected to be lower than 1.1.

To use this reaction as a tool for the synthesis of amphiphilic graft copolymers onto a PDMS backbone, the primer—which serves as the initiator for the enzymatic polymerization—has to be attached to the polymer. While a primer of DP = 4 is sufficient for the enzymatic polymerization,[7] we will use a slightly longer oligoglucan which, in addition, serves as a spacer between the hydrophobic backbone and the reactive site.

2. Results and Discussion

The first synthetic challenge is to attach oligoglucans of DP > 4 with a structurally defined linkage to the hydrophobic PDMS backbone. In these oligomeric primers the 4-OH group of the terminal glucose moiety must be accessible to the enzymatic condensation. Thus, the linkage to the PDMS is preferably made via C-1 of the reducing terminus. Linear maltoheptaose (Glc_7; **3**) which can be used as a starting material is obtained from cyclomaltoheptaose (Schardinger β-cyclodextrin) by hydrolysis under acidic conditions[9] in high yield. Several different attempts to link the maltoheptaose to PDMS either via an acetal (**1**) or amide (**2**) linkage have been realized and will be discussed in the following (Figure 1).

2.1. Synthesis without Protective Groups. Reducing carbohydrates can be easily converted to the corresponding aldonic acid lactones by electrolytic oxidation according to a technique elaborated first by

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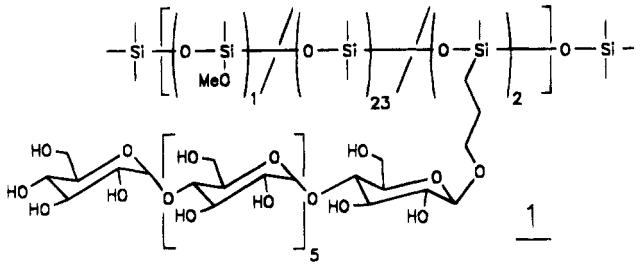


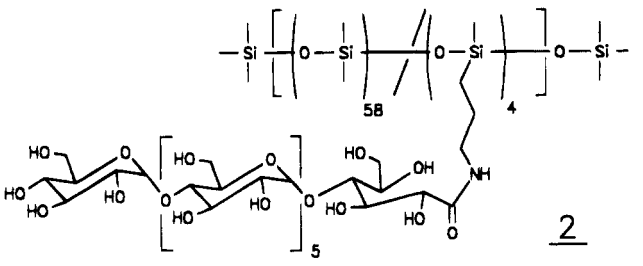
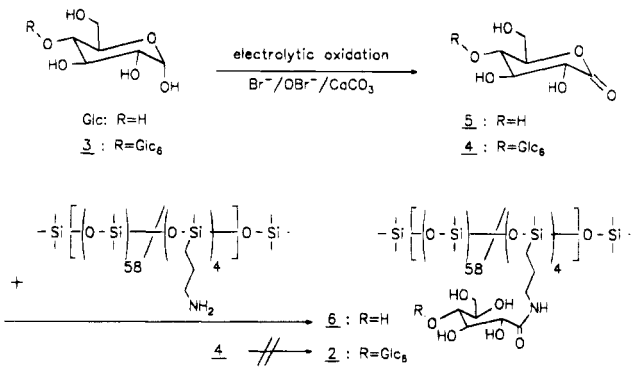


Figure 1. Oligosaccharide side chains attached to polysiloxane. **1**: 7% maltoheptaoside comonomeric units, $\bar{P}_n = 27$. **2**: 6% maltoheptaonamide comonomeric units, $\bar{P}_n = 62$.

Scheme 1. Reaction Sequence for the Addition of Unprotected Aldonolactones to Amino-Functionalized PDMS



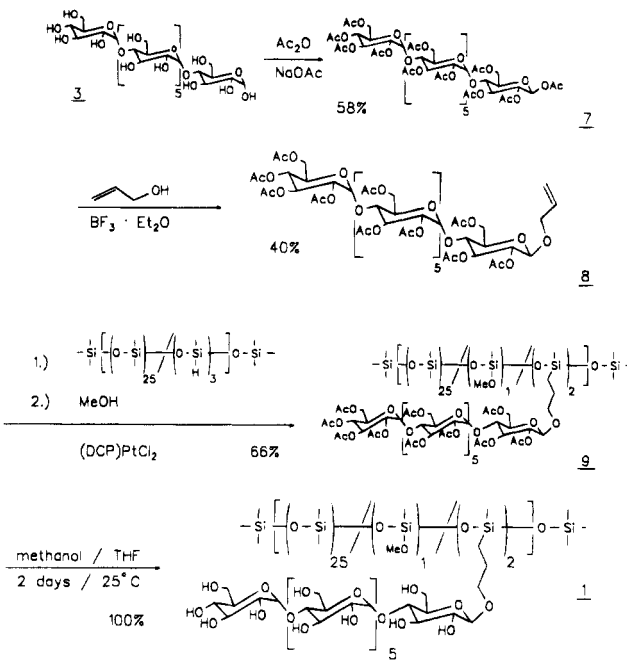
Frush[10] and optimized by Pfannemüller[11] (Scheme 1). This reaction also works for maltoheptaose (**3**) giving the corresponding maltoheptaonolactone (**4**; Glc_6GlcA-lactone), but the reaction is rather slow and oxidation of C-6 to the uronic acid will occur. Thus the oxidation must be stopped before quantitative conversion is achieved.

These unprotected aldonolactones selectively react with amino nucleophiles to provide the corresponding aldonic acid amides. Upon addition of amino-functionalized PDMS to a solution of δ-gluconolactone (GlcA-lactone; **5**) in THF, gluconamide modified PDMS (**6**; GlcA-*N*-PDMS) could be obtained. However, differences in solubility between aldonolactones and PDMS are the limiting factors for this very simple technique. We failed to synthesize Glc_6GlcA-*N*-PDMS (**2**) using OH-unprotected maltoheptaonolactone (**4**) because **4** is completely insoluble in organic solvents (except NMP or DMF at 60 °C, which are nonsolvents for PDMS). In solvent mixtures with hydroxyl-containing cosolvents (e.g., methylcellosolve, which is a poor solvent for PDMS) and at prolonged reaction times (3 weeks) trace amounts of ester formation with the cosolvent were observed. Obviously protective group chemistry has to be applied to overcome the differences in polarity.

2.2. Hydrosilation of 1-*O*-Allylglycosides. Applying the reaction pathway reported previously,[2] it is possible to synthesize a poly(dimethylsiloxane) with pendant maltoheptaoside moieties (Glc_7-1-*O*-PDMS; **1**) in moderate yields, according to Scheme 2. Maltoheptaose (**3**) was peracetylated to obtain **7**, which was converted to the β-1-*O*-allylmaltoheptaoside (**8**). The allylic double bond is added to PDMS containing Si–H groups under platinum catalysis. The resulting O-acetylated saccharide–siloxane (**9**) can be deprotected to obtain the carbohydrate–PDMS hybrid (**1**; Glc_7-1-*O*-PDMS).

In this reaction sequence the glycosilation reaction with allyl alcohol (**7** → **8**) under acidic catalysis with

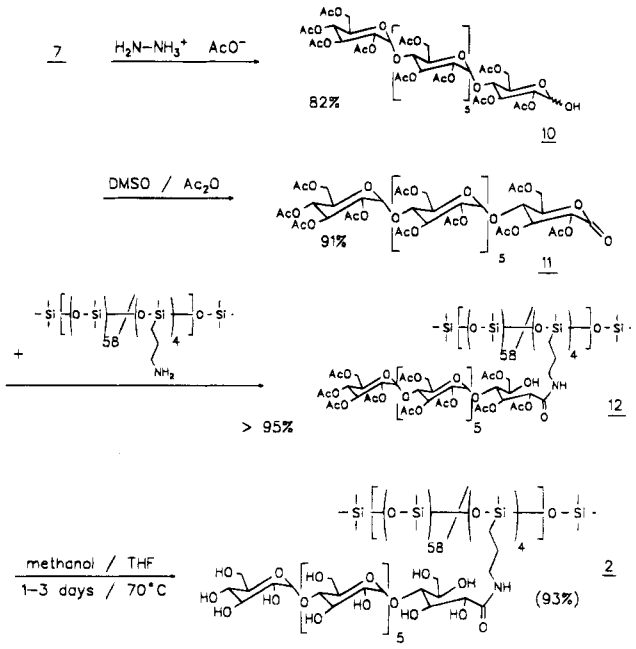
Scheme 2. Reaction Sequence for the Formation of Glc_7-1-*O*-PDMS (1).



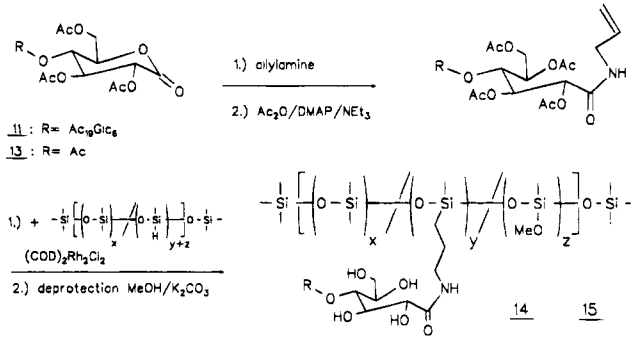
boron trifluoride is a crucial step. This reagent cleaves glycosidic linkages as well. In addition hydrosilation of 1-*O*-allylmaltoheptaoside proceeds up to a conversion degree of 66% at a Si–H content of 11 mol % in the starting polymer. This is probably due to sterical hindrance of the bulky carbohydrate. Thus the yield over these two steps (**7** → **8** → **9**) is only 27%.

2.3. Coupling by Amide Linkage. An alternative pathway to obtain PDMS hybrids with glucan primer side groups is based on the work of Frush et al.,[10] Onodera et al.,[12] and Kuzuhara et al.[13] Poly(dimethylsiloxane-*co*-methyl((3-maltoheptaonamide)propyl)siloxane) (**2**; Glc_6GlcA-*N*-PDMS) is obtained in a five-step synthesis in good yields over all steps using acetyl protective groups (see Scheme 3). First, the anomeric lactol group of the peracetylated maltoheptaose (**7**) was deblocked by hydrazinium acetate[14] to get 1-OH deprotected **10** in 82% yield after purification by MPLC. Oxidation with acetic anhydride and DMSO leads to the peracetylated maltoheptaonolactone (**11**). The electron-withdrawing effect of the acetates increases the reactivity of the aldonolactones. This lactone reacts with numerous nucleophiles like R–NH_2 and R–OH. Thus, purification is complicated and solvent mixtures containing alcohols cannot be used. Reaction of the lactone **11** with amino-functionalized PDMS (Genesee Polymers GP-4) leads to the acetylated sugar–siloxane (**12**). Cleavage of the protective groups provides the siloxane–amylose primer (**2**; Glc_6GlcA-*N*-PDMS); see Scheme 3.

Scheme 3. Reaction Sequence for the Formation of Glc_6GlcA-*N*-PDMS (2).



Scheme 4. Reaction Scheme for Hydrosilation of *N*-allylaldonamides[a]



[a] **14**: R = Glc_6; $x = 880$; $y = 9$; $z = 10$. **15a**: R = H; $x = 190$; $y = 19$; $z = 0$. **15b**: R = H; $x = 880$; $y = 19$; $z = 0$.

2.4. Hydrosilation of *N*-Allylaldonamides. In a third approach allylamine was added to the protected lactones of gluconic and maltoheptaonic acid **11** (**13** and **11**; Scheme 4). The 5-OH group of the resulting amide was acetylated with Ac_2O/DMAP in nearly quantitative yield. These derivatives were coupled in a hydrosilation reaction onto siloxanes containing Si–H groups.

The use of poly(hydromethylsiloxane-*co*-dimethylsiloxane) (P(DMS-*co*-HMS)) instead of amino-functionalized PDMS is of considerable advantage because the former is accessible in a wide range of chain length and Si–H content. However, the hydrosilation via addition of Si–H to C=C double bonds using a platinum catalyst is complicated by the presence of heteroatoms other than oxygen. Olefins containing amido groups will not react with Si–H under Pt catalysis. Using a rhodium complex, even *N*-allylamides can be hydrosilated.[15] Under catalysis of bis(1,5-cyclooctadiene)dirhodium(I) dichloride ($(COD)_2Rh_2Cl_2$) *N*-allylgluconamide was added to various siloxanes in nearly quantitative yields (93–100%). After deprotection the structure identical to **6** (Scheme 1) was obtained in the polymers **15a** and **15b** (GlcA-*N*-PDMS; Scheme 4).

The hydrosilation with peracetylated *N*-allylmaltoheptaonamide does not lead to quantitative conversion of Si–H. At prolonged reaction time at low temperature (66 °C) increasing amounts of decomposition products are observed. In refluxing toluene (110 °C) conversion to the desired Si–C bond up to 48% was possible in 2 days at a Si–H content of 2 mol % in the starting polymer.

Some characteristics of the sweet silicones synthesized by the methods described are summarized in Table 1. **1** was obtained starting with a rather short siloxane, because it is known that even a modification by maltose units provides a water-soluble material.[2] Dicyclopentadieneplatinum(II) dichloride ((DCP)$PtCl_2$) (0.1% molar ratio) was used for Si–H addition. In the case of **2** and **6** we started from the commercially available amino-functionalized "GP-4" from Genesee Polymers (Flint, MI) to yield either a glucan starter **2** or a gluconamide-modified siloxane **6** for reference to investigate the structures obtained in **15a,b** using $(COD)_2Rh_2Cl_2$. **14**, **15a**, and **15b** were synthesized using equilibration products from octamethylcyclotetrasiloxane (D_4), hexamethyldisiloxane as end capper, and a poly(hydromethylsiloxane) (PHMS; $\bar{P}_n \approx 40$) in the desired ratio. Hydrosilation was carried out using 1 mol % of the rhodium catalyst $(COD)_2Rh_2Cl_2$.

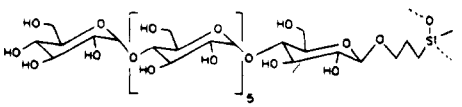
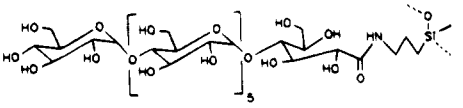
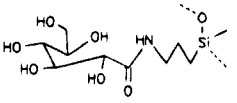
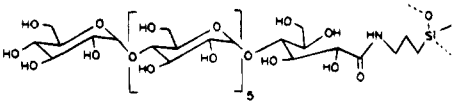
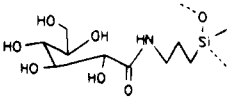
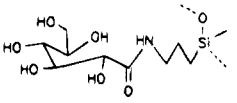
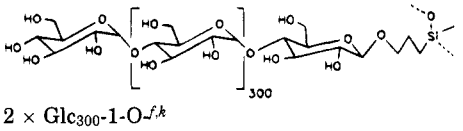
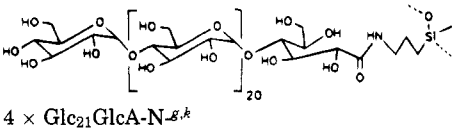
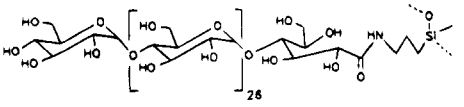
Enzymatic Grafting of Glucose-1-P. So far enzymatic amylose graft copolymers have only been obtained if a hydrophilic polymer backbone has been used,[8,16] as the enzymatic reaction proceeds in a homogeneous solution. In case of **1** where the PDMS backbone is rather short ($\bar{P}_n = 27$) the "plurifunctional initiator" is water soluble. Thus, the standard reaction conditions as described by Pfannemüller[17] could be applied. The saccharide side chains of **1** are recognized by potato phosphorylase (E.C. 2.4.1.1) as initiators for the enzymatic polymerization to obtain "farinaceous silicone", poly(dimethylsiloxane-*graft*-(α,1→4)-D-glucopyranose) (Glc_{300}-1-*O*-PDMS, **16**).

The polymerization from the maltoheptaoside side chains of the siloxane **1** by E.C. 2.4.1.1 was carried out in aqueous citrate buffer (pH = 6.20) at 37 °C. In a parallel run unmodified maltoheptaose as a natural substrate was used as amylose primer to get information about the enzymatic activity as a reference. Using the carbohydrate–siloxane hybrid material **1**, the phosphate liberated is 70% of the reference. This shows that the enzymatic activity is only slightly reduced by the presence of the silicone backbone (Figure 2).

In preparative runs the reaction was stopped before reacting equilibrium (80% monomer consumption) to obtain a narrow molecular weight distributed graft copolymer. Up to 300 glucose units could be polymerized onto each side chain starting with **1**. The average degree of polymerization $\bar{P}_n = 300$ is derived from the ratio of 10 μmol of polymer-bound saccharide primer to 3.0 mmol of phosphate, which was liberated during the reaction period. The concentration of inorganic phosphate in a sample taken from the reaction mixture was photometrically determined, according to Pfannemüller.[17] The amount of phosphate liberated by phosphorylase without primer from Glc-1-P is negligible.

To obtain the resulting material, the homogeneous solution was heated to reflux to denaturate the enzyme. Coagulated protein was removed by filtration. Pfannemüller found that amylose in the range of DP ≈ 80 up to 200 precipitates rapidly,[18] in most cases during the enzymatic reaction. In preparations of high molecular weight glucans the reaction mixtures remain homogeneous, but precipitation of the product can be achieved by treatment with *n*-butanol. Further, aging of these

Table 1. Carbohydrate-Modified Poly(dimethylsiloxanes)

starting polymer (ratio of comonomeric units)	MW × 10^{-3}	saccharide structure (saccharides per siloxane chain)	wt % (Sac)	MW (hybrid) × 10^{-3}	possible solvent	sample
P(DMS-*co*-HMS)[a] 24:3 (11%)	2.0[d]	 2 × Glc_7-1-O-[f]	53	4.3	H_2O, THF	**1**
P(DMS-*co*-H_2N-pr-MS)[b] 58:4 (6%)	4.6[d]	 4 × Glc_6GlcA-N-[g]	50	9.2	(none)	**2**
P(DMS-*co*-H_2N-pr-MS)[b] 58:4 (6%)	4.6[d]	 4 × GlcA-N-[h]	13	5.3	toluene, THF	**6**
P(DMS-*co*-HMS)[c] 860:19 (2%)	65[e]	 9 × Glc_6GlcA-N-[i]	14	75	(none)	**14**
P(DMS-*co*-HMS)[c] 170:19 (10%)	14[e]	 19 × GlcA-N-[i]	20	17	toluene, THF	**15a**
P(DMS-*co*-HMS)[c] 860:19 (2%)	65[e]	 19 × GlcA-N-[i]	5	68	toluene, THF	**15b**
P(DMS-*co*-HMS)[a] 24:3 (11%)	2.0[d]	 2 × Glc_{300}-1-O-[f,k]	98	98	hot glycerol	**16**
P(DMS-*co*-H_2N-pr-MS)[b] 58:4 (6%)	4.6[d]	 4 × $Glc_{21}GlcA$-N-[g,k]	75	19	(none)	**17a**
P(DMS-*co*-H_2N-pr-MS)[b] 58:4 (6%)	4.6[d]	 4 × $Glc_{27}GlcA$-N-[g,k]	80	23	(none)	**17b**

[a] PS 123.5, purchased from ABCR. [b] GP-4, purchased from Genesee Polymers. [c] Equilibration product of D4 and PHMS with F_3C-SO_3H. [d] Stated by manufacturer. [e] Determined by GPC, PDMS calibration. [f] Si−H addition to 1-*O*-allyl-glycoside, 0.1% (DCP)$PtCl_2$. [g] $-NH_2$ addition to peracetylated aldonolactone. [h] $-NH_2$ addition to unprotected aldonolactone. [i] Si−H addition to *N*-allyl-aldonamide, 1% $(COD)_2Rh_2Cl_2$. [k] Enzymatic grafting.

precipitates decreases solubility, presumably because intra- and intermolecular hydrogen bonds are formed. Thus, the conformation of the macromolecule does not allow water molecules to solubilize the glucan chains.

In a first attempt we tried to apply this precipitation procedure to the aqueous solution of **16** after removal of the protein. In analogy to amylose, **16** precipitates on treatment with butanol, but it was not possible to dissolve the dry product again. We tested several solvents, e.g., water, DMF, dioxane, THF, and NMP which are known as good solvents for amylose, but because of the hydrophobic siloxane backbone these attempts were unsuccessful. One possibility to "renaturate" insoluble amylose is treatment with hot glycerol, where at 130−190 °C a homogeneous solution is formed. This behavior can also be observed in the case of **16**. But these conditions would affect the glycosidic linkages and were not applied during workup.

However, the water-insoluble modification of amylose as well as **16** can be swollen with aqueous J_2/KJ solution to form a deep blue complex. The UV/vis spectra of the blue amylose iodine complex (helical (α,1→4)-glucan) and of the **16** iodine complex are very similar. The broad charge-transfer absorption ($\lambda_{max} \approx 620$ nm) is only observed in cases where iodine is enclosed in amylose helices. The spectrum of the cellulose iodine complex is dominated by strong UV absorption ($\lambda_{max} < 380$ nm). It is similar to one taken from J_2/KJ solution. In the case of cellulose iodine is only attached to the surface of nonhelical (β,1→4)-glucan chains. From this investigation a helical structure of the carbohydrate side chains can be derived. A possible structure of product **16** is suggested in Figure 3.

The higher molecular weight siloxanes with the maltoheptaonamide side chains (**2**) are not soluble in water. Two different attempts were made for the enzymatic polymerization onto high molecular weight **2**. To retain a homogeneous reaction mixture, organic cosolvents were added to the buffered solution of the enzyme to test which solvents are tolerated in the

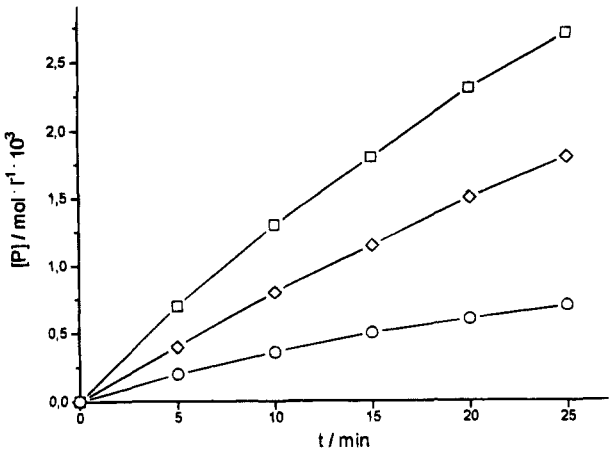


Figure 2. Monomer consumption with various primers. Conditions are as follows: 0.1 mM oligosaccharide chains; 10 mM Glc-1-P; T = 37 °C; 500 units/L of phosphorylase in 0.05 M sodium citrate buffer (pH = 6.20). □: Glc_7 **3**. ◇: Glc_7-1-*O*-PDMS **1**. ○: Glc_6GlcA-*N*-PDMS **2**.

Table 2. Relative Activity of Phosphorylase with Several Solvent Mixtures[a]

	content of organic solvents in sodium citrate buffer (vol %)		
solvent	10%	20%	30%
isopropyl alcohol	15	–	–
acetonitrile	28	–	–
dioxane	34	20	–
NMP	75	35	–
THF	60	40	–
dimethoxyethane	91	68	56

[a] – indicates inactivation of enzyme. The amount of phosphate liberated during the first 5 min of the polyreaction was determined and correlated to enzyme activity. The average value obtained using pure sodium citrate (pH = 6.20) is set to 100% for reference. Conditions: 0.05 M sodium citrate; 100 μM Glc_7(**3**); 10 mM Glc-1-*P*; 500 units/L of phosphorylase; 37 °C.

enzymatic polyreaction. Polar protic solvents (e.g., isopropyl alcohol) suppress the enzymatic activity even at low concentrations. Aprotic solvents with a high dipole moment (e.g., NMP) are tolerated at low concentrations, while aprotic solvents with low polarity (e.g., 1,2-dimethoxyethane, DME) can be applied up to more than 20% (v/v). The results are summarized in Table 2.

However, these mixtures are not suitable to dissolve the plurifunctional siloxane initiator. Alternatively, a suspension of the glucan starter **2** was used. In the biological system (potato) the amylose synthesis is also a heterogeneous (suspension) polymerization[19] and the natural substrate of phosphorylase is a glycoprotein in the amyloplasts. Formally the siloxane takes the "function" of the protein.

Indeed, amylose can be polymerized enzymatically onto high molecular weight glucan starter **2** suspended in aqueous citrate buffer. The rate of polymerization is about 25% compared with **1** (Figure 2). The carbohydrate side chains of **2** seem to be solvated in this solvent and are able to coordinate the glucan storage site of phosphorylase. This is in accordance with the NMR results from a suspension of **2** in D_2O (Figure 4a). The signals of the only partially averaged siloxane methyl protons are very broad (δ = 0.5 ppm, width at half-height = 16 Hz), while the resonances of the carbohydrate protons (δ = 3.7–4.5 and 5.7 ppm, width = 12 Hz) are well resolved, as in a solution of higher concentration.

Characterization of the enzymatically synthesized sugar siloxanes is very difficult because of the insolubility of these materials. Typical siloxane signals as well as the amide linkage and the OH sugar absorptions were detected by infrared spectroscopy (Figure 5). In the range from 3700 up to 2400 cm^{-1} the very broad absorption of strongly associated OH groups can be observed. (α,1→4)-Glucans stabilize their chain conformation by 3′-OH/2-OH hydrogen bond interaction. Considering these interactions as a chemical bond (α,1→4)-glucan chains consist of annelated six- and eight-membered rings. Thus, the O–H stretching vibration is shifted to lower frequencies. The sharp signal of ν_{C-H} (2960 cm^{-1}) is superposed on the broad O–H resonance. In the carbonyl range the typical absorptions of an amide linkage appear (1658 and 1548 cm^{-1}, amides I and II). The siloxane absorptions dominate the fingerprint range by Si–O (1050 cm^{-1}) and Si–C (800 cm^{-1}) stretching vibrations. The Si–C deformation vibration results in a very sharp signal at 1260 cm^{-1}.

^{1}H-NMR analysis of **17b** (Figure 4b) suspended in D_2O provides the same structural information as the spectrum of **2**, with Si–CH_3 signals appearing with less intensity. The line width of the siloxane methyl protons is increased in the larger polymer to 42 Hz. The initial length of six glucose units and one gluconamide was elongated to 21 and 27 carbohydrate moieties in the polymers **17a** and **17b**, determined by the amount of liberated phosphate during the reaction.

In six analytical runs the kinetic data of the phosphorylase reaction upon varying the concentration of the siloxane primer **2** were studied. From each run six samples were taken in a time interval of 5 min. K_M and v_{max} were extrapolated as first demonstrated by Lineweaver and Burk as

$$K_M = 6.1 \times 10^{-4} \text{ mol/L}$$

and

$$v_{max} = 9.8 \times 10^{-5} \text{ mol/L·min}$$

The apparent kinetic value of K_M is similar to that determined using maltoheptaose as glucan primer relative to Glc-1-P, $K_M = 5.7 \times 10^{-4}$ mol/L. Therefore, it seems that polymer-bound maltoheptaonamide is accepted by the enzyme as well as freely dissolved maltoheptaose.

v_{max} in the suspension polymerization is less than half of the value obtained in homogeneous solution ($v_{max} = 2.0 \times 10^{-4}$ mol/L·min).

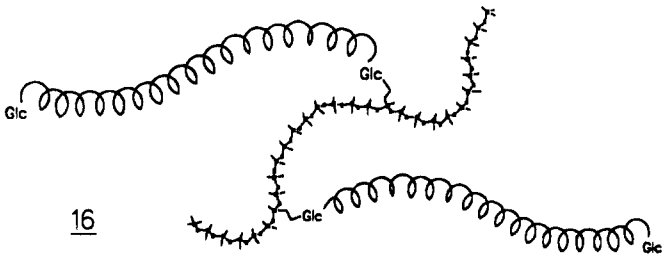
Investigations of the mechanical and other bulk properties of these materials are in progress and will be published elsewhere.

Experimental Section

All solvents and liquid reagents were purified by distillation under nitrogen before use. DMSO was kept for 2 weeks over 3-Å molecular sieves before distillation to remove traces of water. DMF was stirred 2 weeks over calcium hydride before distillation under argon and exclusion of light.

Enzymes were prepared according to the simplified procedure of Pfannemüller.[7] All solutions were purged with nitrogen for several hours before use. Only sterilized flasks were used. During the whole preparation the enzyme solution was kept at 0–4 °C in an ice bath.

Determination of enzymatic activity was carried out by detection of liberated phosphate, which was quantitatively measured by a photometrical method elaborated by Fiske and Subbarow.[20] In this work "1 unit" is defined as the amount of



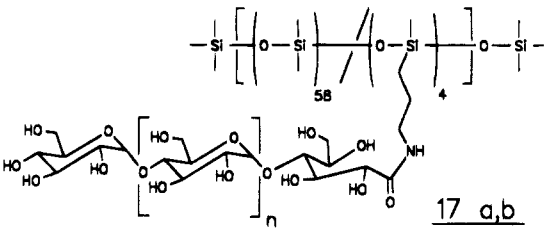


Figure 3. Proposed structure of **16**: Helical amylose conformation. *N*-PDMS–oligoglucan (enzymatically synthesized): **17a**, $n = 20$; **17b**, $n = 26$.

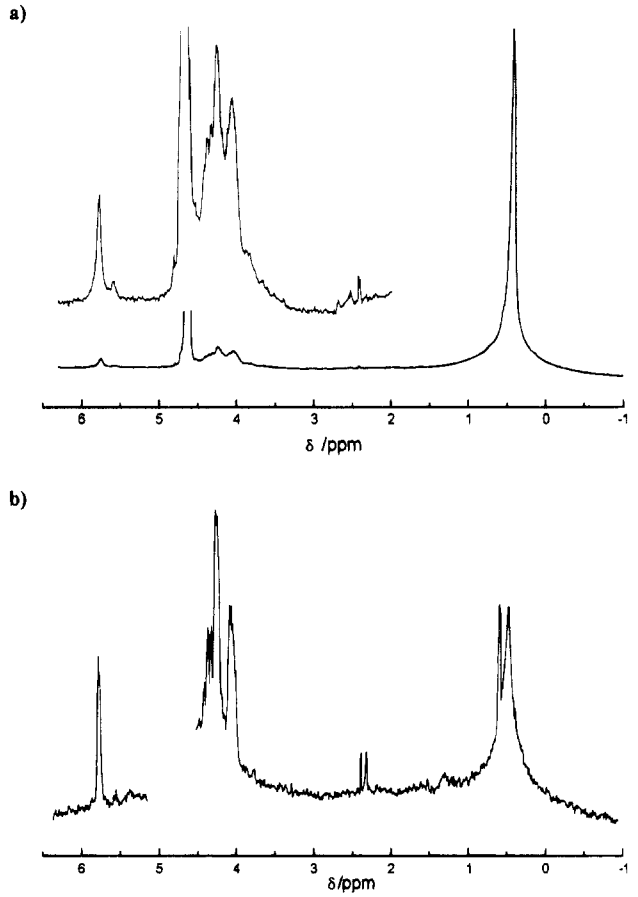


Figure 4. (a) 200-MHz ^{1}H-NMR spectrum of **2** (suspension in D_2O at 80 °C, ppm): δ 5.7 (Glc H-1 (line width = 12 Hz)), 5.55 (GlcA H-2 (?), 4.7 (HDO), 4.5–3.8 (Glc H-2...H-6), 3.7 (NCH_2-), 2.4 ($-CH_2CH_2CH_2-$), 0.8 ($-CH_2Si$), 0.5 ($SiCH_3$ (line width = 16 Hz)). (b) 200-MHz ^{1}H-NMR spectrum of **17b** (suspension in D_2O at 80 °C, HDO peak skipped, ppm): δ 5.8 (Glc H-1), 3.9–4.5 (Glc H-2...H-6), 3.8 (NCH_2-), 2.3 ($-CH_2CH_2-CH_2-$), 0.6 ($-CH_2Si$), 0.4 ($SiCH_3$ (line width = 42 Hz)).

enzyme suspension, which liberates 0.1 mg of phosphate from Glc-1-P in 3 min at 37 °C, according to Hidy and Day.[21]

From 1 kg of potato tubers (Solanum tuberosum L. var. Christa and Attika, organically grown by a local farmer) about 1700 units of phosphorylase activity can be obtained in 1 day of work. A suspension of the enzyme (30 mL from 1 kg of potatoes) in 0.05 M sodium citrate buffer can be kept for several weeks without significant loss of activity at 4 °C stabilized with 10 mg of L-glutathione (reduced).

Glucose-1-P was prepared as described by Husemann[17] with minor changes of the procedure: The reaction solution was kept under nitrogen, so covering with a layer of toluene was not necessary. Instead of using crude potato juice and precipitated Zulkowsky starch, we incubated a pure phosphorylase solution with partially hydrolized native potato starch (10 mM HCl at 100 °C for 30 min). Thus, no impurities of the potato juice must be removed and purification becomes easier.

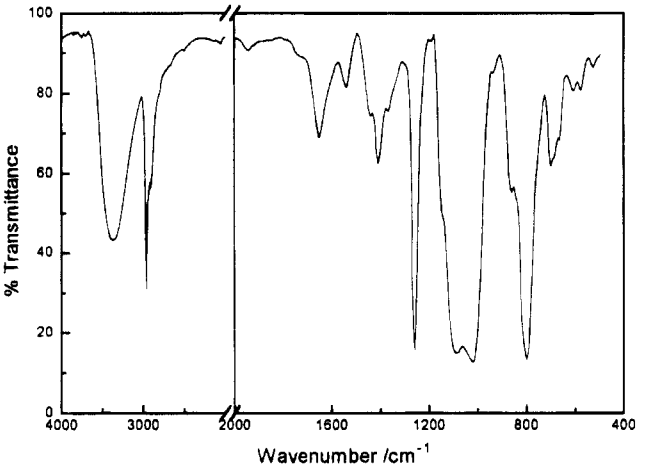


Figure 5. IR spectrum of an aldonamide-modified siloxane (**2**). Wavenumber (cm^{-1}): 3700–2400 (vb, $\nu_{O-H...O}$), 2960 (s, ν_{C-H}), 1658 (w, $\nu_{C=O}$, amide I), 1548 (w, δ_{N-H}, amide II), 1260 (s, δ_{Si-C}), 1100/1020 (b, $\nu_{Si-O} + \nu_{C-O}$), 860 (vw, δ_{1-C-H}), 802 (s, ν_{Si-C}), 662 (vw, $\delta_{O-H...O}$).

IR spectra were recorded on a Bruker IFS 48 Fourier transform spectrometer and NMR spectra on a Bruker AC 200.

Maltoheptaose (3) from β-Cyclodextrin.[9] The preparation was carried out in a simplified way. A total of 200 g (180 mmol) of cyclomaltoheptaose is dissolved in 800 mL of 0.01 M hydrochloric acid and kept under reflux for exactly 2 h. The solution is neutralized with the calculated amount of 1 M NaOH (8 mL) and buffered to pH = 7.0 with Na_2HPO_4/NaH_2PO_4. The color changes to a pale yellow. The solution is stored at 4 °C for 12 h. A total of 85% of the educt crystallizes from the solution. The regenerated cyclodextrin is filtered off and collected for the next run. The remaining solution is warmed up to 60 °C, saturated with *p*-xylene (approximately 1 mL), and stirred for several hours. Crystallization of the cyclodextrin is brought to completion in the refrigerator overnight. The *p*-xylene/cyclodextrin complex is removed from the solution by filtration over Celite 535 (Fluka). The water is removed under reduced pressure to $^1/_{10}$ of the initial volume (80 mL). After further saturation with *p*-xylene, final traces of the cyclical educt crystallize overnight at 4 °C and are removed by filtration. Evaporation of the water under reduced pressure and drying *in vacuo* provide crude maltoheptaose which is contaminated only by traces of oligoglucans (Glc_2 up to Glc_6) and sodium chloride/phosphate. The crop is dissolved in 40 mL of water and added dropwise to 1500 mL of ethanol (p.a.). Pure precipitated maltoheptaose (**3**) is removed by filtration and dried *in vacuo* for several days.

Yield: 10% of maltoheptaose; 85% crystalline cyclodextrin; 5% cyclodextrin/*p*-xylene complex on Celite. Mp: 220 °C. ^{13}C-NMR (in D_2O, ppm): δ 99.5 (C-1 chain), 95.6/91.7 (α/β C^I-1), 76.0–69.7 (C-2–C-5), 69.1 (C^{VII}-4), 60.3 (C-6).

To recover cyclodextrin from the *p*-xylene complex adsorbed on Celite, the material is dissolved in hot water and filtered to remove Celite. *p*-Xylene is removed by codistillation with water from the remaining solution under reduced pressure. Recrystallization from water provides colorless crystals, suitable for the next run.

Maltoheptaonolactone 4.[11] To a solution of 23 g (20 mmol) of **3** in 150 mL of water are added 0.8 g (3.4 mmol) of

$CaBr_2$ and 1.4 g (14 mmol) of $CaCO_3$. The well-stirred suspension is equipped with two carbon electrodes and electrolyzed for 5 h and 20 min at a current of 200 mA (≅3840 C ≅40 mmol of electrons). The temperature is kept at 15–20 °C. The solution is degassed at 60 mbar for half an hour, and undissolved $CaCO_3$ is filtered off. The turbid solution is chromatographed over a short column of Amberlite IR-120/H^+ ion exchange resin to remove calcium. The eluate (300 mL) is concentrated to one-third of its volume under reduced pressure and stirred with 3.9 g (14 mmol) of Ag_2CO_3 for 30 min in the dark. Precipitated AgBr is filtered off, and the turbid solution is chromatographed over Amberlite IR-120/H^+ ion exchange resin to remove silver ions. The turbid brown eluate is now filtered through an ultramembrane (Filtron 3kDa) to remove colloidal silver. The clear and colorless solution is evaporated to dryness and dried *in vacuo* to obtain **4**.

Yield: 20.2 g (87% of saccharides with 83% conversion to the lactone, determined by titration). ^{13}C-NMR (D_2O, ppm): δ 175.4 (C^{I}-1), 100.2 (C^{II}-1), 99.4 (C-1), 81.4–70.7 (C-2–C-5), 69.1 (C^{VII}-4), 61.8 (C^{I}-6), 60.3 (C-6). FT-IR (cm^{-1}): 3500–2500 (vb) $\nu_{O-H...O}$), 2926 (s), ν_{C-H}, 1735 (s), $\nu_{C=O}$ lactone), 1360 (w, ring vib), 1024 (vb, ν_{C-O}).

Siloxane Sugar 6. To a solution of 4.6 g (with 4 mmol of $-NH_2$) of GP-4 (Genesee Polymers) in 80 mL of dry THF is added under nitrogen 0.8 g (4.5 mmol) of δ-gluconolactone (**5**; Merck), and the suspension is heated to reflux for 3 h while the lactone dissolves. Now THF is removed under reduced pressure, and crude **6** is dissolved in 100 mL of toluene. The turbid solution is extracted with water to remove unreacted gluconic acid. The organic phase is dried with $MgSO_4$, and the solvent is removed under reduced pressure. Drying *in vacuo* provides pure **6**.

Yield: 5.2 g (98% theoretical). FT-IR (cm^{-1}): 3500–2500 (vb, ν_{O-H}), 2960 (s, ν_{C-H}), 1650 (w, $\nu_{C=O}$, amide I); 1540 (w, δ_{N-H}, amide II), 1410 (w, δ_{C-H}), 1260 (s, δ_{Si-C}), 1025/1090 (b, ν_{C-O} + ν_{Si-O}), 802 (s, ν_{Si-C}).

Peracetylated Maltoheptaose (7).[22] A total of 11.5 g (140 mmol) of sodium acetate is added to 140 mL (1.4 mol) of acetic anhydride and heated to reflux. After removal of the heating device, 26.5 g (23 mmol; with 530 mmol of OH groups) of maltoheptaose (**3**) is added at a rate which keeps the reaction mixture boiling. If the reaction does not proceed, which can be recognized by the presence of unreacted undissolved **3**, heating the flask at a single spot will reactivate the acetylation. After all of the carbohydrate is added, the mixture is kept for 30 min under reflux and than allowed to cool down at room temperature.

The reaction mixture is poured into 500 mL of well-stirred ice and water and stirred for 3–4 h. A first crude crop can be filtered off. The aqueous solution is extracted two times with 50 mL of ethyl acetate, which is combined with the first solid crop. The organic solution is extracted twice with 50 mL of water, 50 mL of a saturated $NaHCO_3$ solution, and again two times with water. Drying of the organic layer with $MgSO_4$ and evaporation of the ethyl acetate under reduced pressure provides a pale yellow powder of crude peracetylated Glc_7 (**7**).

Triple recrystallization from isopropyl alcohol and purification by MPLC (toluene/acetone, 3:1 (v/v)) provides 28 g (13 mmol, 58% theoretical) of pure **7** (ratio of anomers = 15% α:85% β).

Mp: 126 °C. 200-MHz ^{1}H-NMR ($CDCl_3$, ppm): δ 6.2 (d, αH-1, J = 3.7 Hz (15%)), 5.7 (d, βH-1, J = 8.0 Hz (85%), 1H), 5.3 (m, H-1 chain, 6H), 5.1–4.0 (m, H-2...H-5, 28H), 3.9 (d, H-6, 14H), 2.3–2.0 (m, acetyl-CH_3, 69H).

1-OH Deprotected Maltoheptaose (10).[14] A total of 15 g (7 mmol) of peracetylated maltoheptaose (**7**) is dissolved in 70 mL of DMF at room temperature under nitrogen. A total of 0.77 g (8.4 mmol) of hydrazinium acetate is added to the stirred solution, which is heated to 60 °C for 40 min. Upon cooling the homogeneous reaction mixture to room temperature, 150 mL of ethyl acetate is added. The organic solution is extracted with 150 mL of water, which is extracted twice with ethyl acetate. The combined organic solutions are extracted with a 0.1 M KH_2PO_4 solution and three times with water. Drying with $MgSO_4$ and evaporation of the solvent under reduced pressure provide crude **10**, which may contain small amounts of the solvent. DMF is removed as an azeotrope with toluene. The product is only contaminated with **7** (DC). Purification via MPLC (toluene/acetone, 5:2 (v/v)) provides 12 g (5.8 mmol, 82% theoretical) of the lactol free carbohydrate.

Mp: 118 °C. 200-MHz ^{1}H-NMR ($CDCl_3$, ppm): δ 5.6 (t, H^{I}-1, 1H), 5.3 (m, H-1, 6H), 4.9–4.0 (m, H-2...H-5, 28H), 3.9 (m, H-6, 14H), 2.1 (m, acetyl-CH_3, 66H). FT-IR (cm^{-1}): 3470 (s, ν_{O-H}), 2968 (s, ν_{C-H}), 1750 (s, $\nu_{C=O}$ acetyl), 1374 (w, ring vib), 1050 (vb, ν_{C-O}).

Peracetylated Maltoheptaonolactone (11).[13] A total of 6.6 g (3.2 mmol) of **10** is dissolved in 50 mL (580 mmol) of DMSO stirred gently at room temperature in a flask equipped with a drying tube. A total of 30 mL (300 mmol) of acetic anhydride is added dropwise, and the reaction mixture is kept at room temperature for 12 h.

A total of 300 mL of ethyl acetate and 150 mL of water are added, and the emulsion is stirred for 30 min. The aqueous layer is removed and extracted with 50 mL of ethyl acetate. The combined organic solutions are extracted 20 times with 50 mL of water to remove dimethyl sulfide. The ethyl acetate solution is dried over $MgSO_4$ and evaporated under reduced pressure to dryness. Codistillation with 100 mL of dry toluene for 10 times removes DMSO and dimethyl sulfide. For the last purification, the crude lactone is dissolved in 20 mL of toluene and added dropwise at 0 °C to 500 mL of petroleum benzin (boiling range 40–60 °C) under vigorous stirring. Filtration provides 6 g (2.9 mmol, 91% theoretical) of **11**.

Mp: 111 °C. 200-MHz ^{1}H-NMR ($CDCl_3$, ppm): δ 6.4 (d, H^{I}-2, 1H), 5.3 (m, H-1, 6H), 5.1–4.0 (m, H-2...H-5, 28H), 3.9 (m, H-6, 14H), 2.1 (m, acetyl-CH_3, 66H).

Acetylated Siloxane Sugar (12). A total of 4.2 g (2.0 mmol) of **11** and 2.5 g (with 1.9 mmol amino groups) of GP-4 silicone (Genesee Polymers) are dried *in vacuo* overnight. The lactone is dissolved in 10 mL of dry toluene in a reaction flask under an inert gas atmosphere. A solution of GP-4 in 5 mL of the same solvent is added dropwise with gentle stirring. The mixture is kept for 12 h at room temperature. Evaporation of the solvent provides a pale yellow glassy material, containing unreacted **11**. Using GP-4 (molecular weight = 4600, according to product information provided by Genesee Polymers) purification was not possible by precipitation. Separation of the low molecular weight impurities was carried out by preparative gel permeation chromatography on SDV-Gel (25 μm, 1000 Å).

Yield: 5.7 g (85% of siloxane with >95% conversion of amino groups). ^{13}C-NMR ($CDCl_3$, ppm): δ 170.6–169.4 (acetyl C=O), 166.5 (C^{I}-1), 95.7 (C-1), 79.9–68.1 (C-2...C-5), 61.5–62.5 (C-6), 42.2 (NCH_2–), 23.1 ($-CH_2CH_2CH_2-$), 20.7 (acetyl-CH_3), 14.5 ($-CH_2Si$), 1.6 to −0.7 ($SiCH_3$). FT-IR (cm^{-1}): 3450 (w, ν_{N-H}), 2950 (s, ν_{C-H}), 1750 (s, $\nu_{C=O}$, acetyl), 1660 (w, $\nu_{C=O}$, amide I), 1534 (w, δ_{N-H}, amide II), 1430 (w, δ_{C-H}), 1240 (s, δ_{Si-C}), 1050 (b, ν_{C-O} + ν_{Si-O}), 800 (s, ν_{Si-C}).

Siloxane Sugar (2). A total of 5.6 g (with 37 mmol acetyl groups) of **12** is dissolved in 50 mL of dry THF under argon. A total of 100 mL of dry methanol and 30 mg of dry potassium carbonate are added with gentle stirring, and the reaction mixture is heated to reflux. The degree of conversion can be detected by IR spectroscopy of a sample by decrease of $\nu_{C=O}$ at 1735 cm^{-1} with a corresponding increase of ν_{O-H} at 3500–3000 cm^{-1}. As a side reaction, methanolysis of the amide linkage (observation of the ester formation by IR) may occur after prolonged reaction time.

After about 20 h 50 mL of water is added and THF and methanol are removed in a rotavapor. Crude **2** is obtained by lyophilization. Using impure **12** as well as **12** purified by GPC will provide **2** containing maltoheptaonic acid derivatives.

They are removed by suspending crude **2** in 100 mL of a 0.1 M NaCl solution and dialysis against distilled water until the disappearance of ν_{COO^-} at 1600 cm^{-1}. Freeze drying provides pure **2**.

Yield: 2.6 g (65% theoretical). 200-MHz ^{1}H-NMR (suspension in D_2O at 80 °C, ppm): δ 5.7 (H-1), 5.55 (H^{I}-2(?), 4.5–3.8 (H-2...H-6), 3.7 (NCH_2–), 2.4 (CH_2CH_2), 0.8 ($-CH_2Si$), 0.5 ($SiCH_3$). FT-IR (cm^{-1}): 3700–2400 (vb, ($\nu_{O-H...O}$), 2960 (s, ν_{C-H}), 1658 (w, $\nu_{C=O}$, amide I), 1548 (w, δ_{N-H}, amide II), 1260 (s,

δ_{Si-C}), 1100/1020 (b, $\nu_{Si-O} + \nu_{C-O}$), 860 (vw, δ_{1-C-H}), 802 (s, ν_{Si-C}), 662 (vw), $\delta_{O-H...O}$).

Sugar Siloxanes 14 and 15. A total of 1 g of P(DMS-*co*-HMS) is dissolved in 10 mL of dry THF or toluene under an inert atmosphere (nitrogen or argon) and 1 mol % (according to SiH in the copolymer) bis(1,5-cyclooctadiene)dirhodium(I) dichloride is added under gentle stirring. After 5 min a solution of the protected *N*-allylaldonamide (10% excess, according to SiH) in the same solvent is added and the reaction mixture is heated to 70 °C. The degree of conversion is followed by IR spectroscopy by the decrease of ν_{Si-H} at 2157 cm^{-1}. After approximately 20 h the brown solution is filtered to remove precipitated rhodium, and in the case of THF the solvent is evaporated and substituted by toluene. The organic solution is first stirred with 0.1 M Na–EDTA (Titriplex III; Merck) for 12 h. The aqueous layer is removed by centrifugation. This procedure is repeated three times with water for 30 min. The remaining organic phase is dried over $MgSO_4$ and evaporated under reduced pressure to dryness. In the case of high molecular weight siloxanes with a lower degree of modification purification can be achieved by precipitation of the toluene solution with isopropyl alcohol or methanol. Using low molecular weight siloxanes or a high degree of modification unreacted saccharides are removed by preparative GPC (SDV-Gel (25 μm, 1000 Å), eluent toluene). Yield: 80–95% (theoretical).

Deprotection of the saccharide side chains is carried out analogously to siloxane sugar (**2**).

^{13}C-NMR (THF-d_8, ppm): 173.2 (C^I-1), 75.0–71.8 (C-2...C-5), 65.9 (C-6), 42.5 (NCH_2–), 24.1 ($-CH_2CH_2CH_2$); 15.4 ($-CH_2$-Si), 1.9 to −1.5 ($SiCH_3$). FT-IR: Identical to **6**.

Farinaceous Siloxane 16. A total of 22 mg (with 10 μmol of Glc_7 primer) of **1**[2] is dissolved in 100 mL of sodium citrate buffer (0.05 M, pH = 6.20) and heated to 37 °C. After addition of 3.7 g (10 mmol) Glc-1-P and 400 units of phosphorylase, the reaction flask is incubated for 4 h at 37 °C. During this period 3.0 mmol of phosphate is liberated. To stop polymerization, the mixture is heated to reflux. After five min the coagulated protein is filtered off and the warm solution is saturated with 10 mL of *n*-butanol. **16** precipitates during the night in the refrigerator. It is washed with methanol and diethyl ether and dried *in vacuo*, yield 0.6 g. Insoluble **16** forms a deep blue complex with iodine.

FT-IR (cm^{-1}): 3700–2300 (vb, $\nu_{O-H...O}$), 2940 (s, ν_{C-H}), 1596 (w, ring vib), 1412 (b, $\delta_{C-H} + \delta_{O-H}$), 1150–1028 (b, $\nu_{C-O} + \nu_{Si-O}$), 792 (vw, ν_{Si-C}).

Farinaceous Siloxanes 17a,b. A total of 0.7 g (with 290 μmol of Glc_7 primer) of **2** is suspended in 100 mL of sodium citrate buffer (0.05 M, pH = 6.20) and heated to reflux for 10 h. After cooling in a water bath to 37 °C 2.2 g (5.9 mmol, **17a**) [4.5 g, 12 mmol (**17b**)] of Glc-1-P and after dissolving 390 units (5-mL suspension) phosphorylase are added. After vigorous stirring for 100 min at 37 °C 4.3 mmol (**17a**) [6.0 mmol (**17b**)] of phosphate is liberated. The reaction is stopped by heating the mixture with a hot oil bath (130 °C) to reflux for 5 min to denaturate the enzyme. Dialysis of the suspension against water for 2 weeks removes citrate buffer and Glc-1-P. The product is isolated by freeze drying. Yield: 1.6 g (**17a**) [1.9 g (**17b**)]. From the ratio of 4.3 mmol (**17a**) [6.0 mmol (**17b**)] of new carbohydrate units polymerized onto siloxane with 0.29 mmol of starting Glc_6GlcA, an average degree of polymerization $\bar{P}_n = 15$ (**17a**) [$\bar{P}_n = 21$ (**17b**)] is derived. Thus, the side chains can be described as $Glc_{21}GlcA$ (**17a**) [$Glc_{27}GlcA$ (**17b**)].

200-MHz ^{1}H-NMR (D_2O, ppm): δ 5.8 (H-1), 3.9–4.5 (H-2...H-6), 3.8 (NCH_2–), 2.3 ($-CH_2CH_2CH_2-$), 0.6 ($-CH_2Si$), 0.5 ($SiCH_3$). FT-IR (cm^{-1}): 3700–2300 (vb, $\nu_{O-H...O}$), 2960 (s, ν_{C-H}), 1654 (s, $\nu_{C=O}$, amide I), 1540 (w, δ_{N-H}, amide II), 1420 (b, $\delta_{C-H} + \delta_{O-H}$), 1258 (s, δ_{Si-C}), 1050 (vb, $\nu_{Si-O} + \nu_{C-O}$); 802 (s, ν_{Si-C}).

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