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An Efficient Method for the Syntheses of Novel Amphiphilic Polysaccharides by Regio- and Thermoselective Modifications of Chitosan

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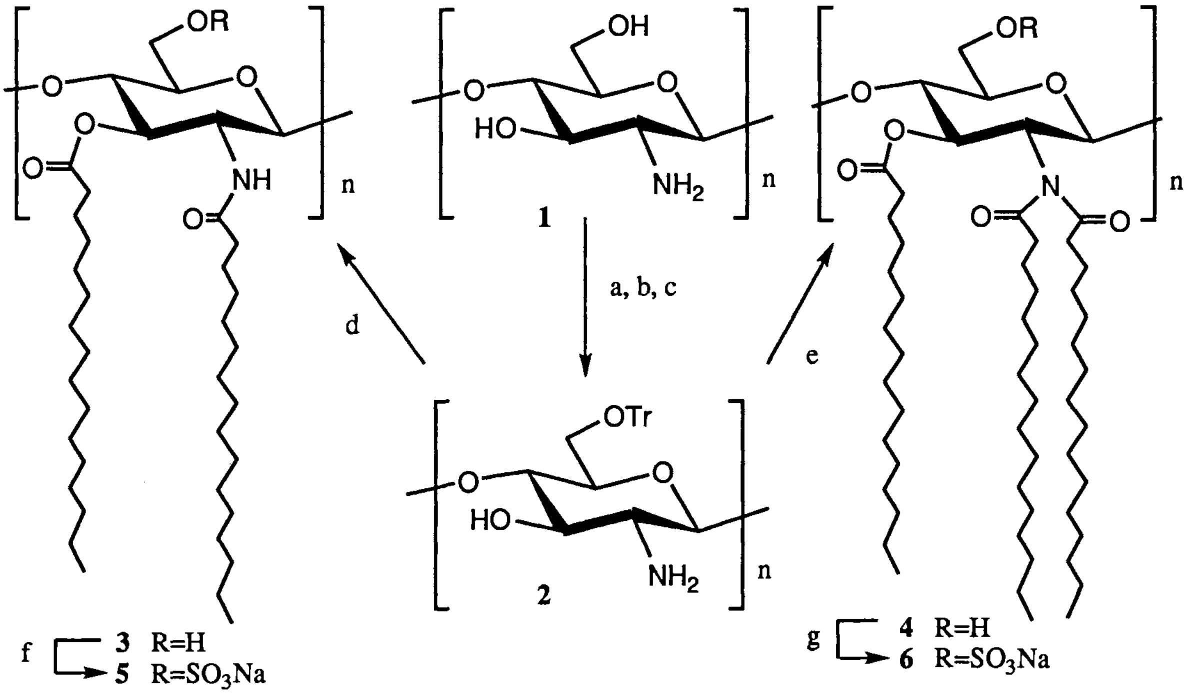
The first example of an unique and efficient method for the preparation of amphiphilic polysaccharides having self-assembling nature is described. Regio- and thermospecific chemical manipulations of a standardized intermediate derived from chitosan markedly facilitated the synthetic procedures of novel types of polysaccharide architectures. Artificial glycolipid-type polymers showed excellent properties to form stable monolayer membranes at air/water interface.

Specific structures and functions based on self-organization of various synthetic amphiphiles result in the formation of biomembrane mimetics.1) Langmuir-Blodgett films, liquid crystals, and polymer vesicles have been the subject of extensive investigation. Research efforts have increased drastically in the past decade because of possible applications in nonlinear optical devices, microlithography, biosensors, and drug delivery systems.2) Polymeric oriented systems containing sugar moieties are of great interest as chiral scaffoldings for biomimetic molecules with much higher specificity for molecular recognition. Carbohydrates and related carriers of biological information are expected to be one of the most effective and specific reagents in antibacterial, antitumor, and anti-Human Immunodeficiency Virus (HIV) therapies.3) In fact, certain naturally occurring polysaccharides bearing hydrophobic anchors have been found to remarkably enhance not only the stability but the cell specificity of liposomes.4) According to current dogma, our attention is now directed toward the molecular design of self-organizing systems from polysaccharide amphiphiles and their functions. We describe herein the first synthetic approach to artificial glycolipid-type polymers having a capacity to form monolayer membranes using regioselective chemical manipulations of a natural aminopolysaccharide resource, chitosan.

While remarkable progress has been made in chitin technology,5) a fully satisfying, straightforward solution to synthesize finely designed and high performance materials from this polysaccharide still remains elusive. Since the intractability of this rigid and insoluble polymer had long made regioselective chemical modifications impossible, the recent use of N-phthaloyl chitosan6) exhibiting excellent solubility in common organic solvents was seen to have considerable advantages as a convenient starting material. A major advantage of compound **1** in chitin chemistry lies in the ease of further regioselective and quantitative manipulations to



Scheme 1. Reagents and conditions: (a) 3 equiv of $C_6H_4(CO)_2O$, DMF, 130 °C, (b) 10 equiv of $(C_6H_5)_3CCl$, pyridine, 80 °C, (c) excess of N_2H_4 H_2O, 100 °C, (d) i) 4 equiv of $CH_3(CH_2)_{14}COCl$, pyridine, 45 °C, 17 h, ii) $Cl_2CHCOOH$, r.t., 2 h, (e) i) 9 equiv of $CH_3(CH_2)_{14}COCl$, pyridine, 70 °C, 17 h, ii) $Cl_2CHCOOH$, r.t., 2 h, (f) i) 3 equiv of SO_3Me_3N, pyridine, 90 °C, 24 h, ii) 1 N NaOH aq., (g) i) 3 equiv of SO_3Me_3N, pyridine, 60 °C, 24 h, ii) 1 N NaOH aq.

provide a ready series of intermediates.[7)]

Scheme 1 indicates the synthetic route for the new polysaccharide architectures in this work. When a key intermediate, **2**, (1→4)-2-amino-2-deoxy-6-O-trityl-β-D-glucopyranan, was treated with excess palmitoyl chloride in pyridine to regioselectively introduce a lipophilic moiety at C-2 and C-3 positions, degrees of substitution were found to be markedly affected by the reaction temperature as shown in the scheme.[8)] A particularly attractive feature of the thermo-controlled acylation is that the palmitoylation reaction proceeds smoothly in homogeneous solution. Subsequent removal of trityl protecting groups at C-6 position gave the unique tri-substituted derivative **4** in addition to a desired di-substituted derivative **3**.[9)] Typical IR spectra of a series of novel chitosan derivatives having N, O-

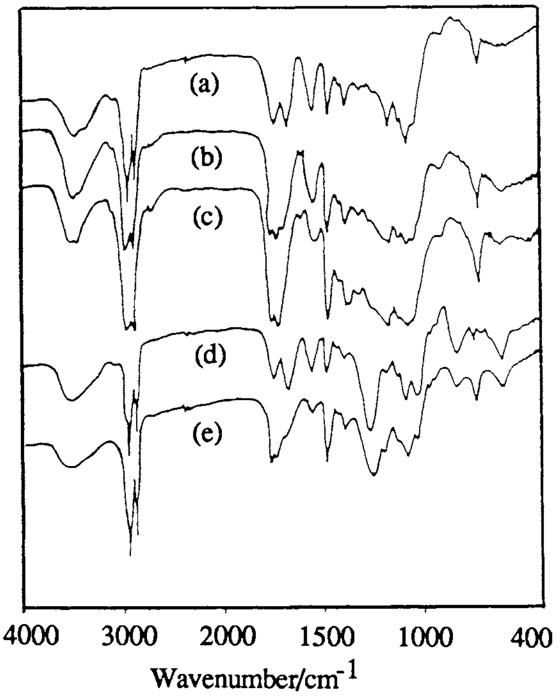


Fig. 1. IR spectra of new chitosan derivatives: (a) **3**, (b) a product obtained at 55 °C, (c) **4**, (d) **5**, and (e) **6**.

palmitoyl chains clearly support the reasonable chemical structures of diacyl **3** (45 °C), triacyl derivative **4** (70 °C), and the structural mixture of amido and imido type products obtained at 55 °C (Fig. 1).[10] Introduction of O-sulfate groups at C-6 positions of derivative **3** and **4** was efficiently achieved by employing SO_3-trimethylamine complex as a sulfating reagent in dimethylformamide (DMF) as shown in the IR spectra. Rigorous purification of the products by gel permiation chromatography over Sephadex LH-20 afforded the anionic polysaccharide amphiphiles.[11] As anticipated, a polysaccharide amphiphile **6** having triple acyl-chains exhibited better solubility in common organic solvents such as chloroform, dichloromethane, and benzene than that of double-chain type compound **5**.[12]

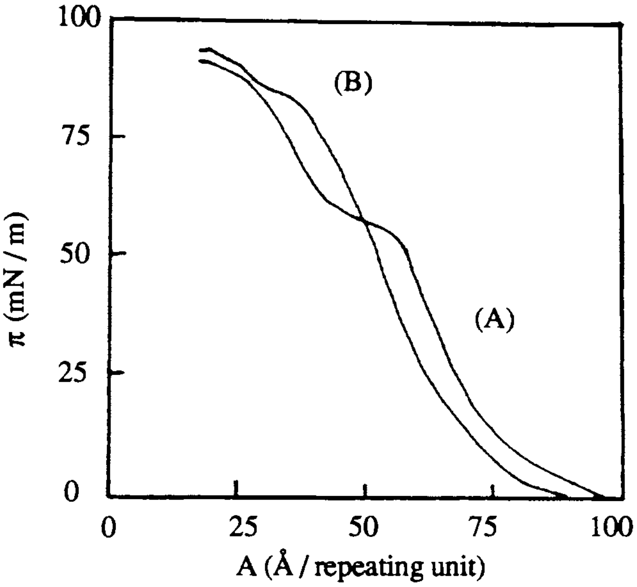


Fig. 2. Compression isotherms of compounds (A) **4** and (B) **6** at air/water interface.

Excellent solubility of compound **4** and **6** bearing tripalmitoyl groups in organic solvents prompted us to preliminarily examine their capacities to form monomolecular films.[13] Deposition of each polymer on a Langmuir film balance afforded stable monolayers with high collapse pressures. Surface pressure-area isotherms of compound **4**, shown in Fig. 2, extrapolate to molecular areas of approximately 60 Å / monomer repeating unit at the collapse pressure (ca. 65 mN/m). This suggests that the long acyl chains may align along the molecular axis with a slight increase in diameter. Moreover, two polysaccharide amphiphiles show distinctly different monolayer behavior dependent on the nature of the hydrophilic groups at C-6 positions. The compression curve of **4** is typical for passage from a liquid expanded state, through a liquid compressed state, to a solid compact state. Stable monolayers of **6** in the liquid compressed state could also be transferred to silicone-coated glass plates by conventional vertical dipping methods at a rate of 3.3 mm/min. The interlayer distance for Langmuir-Blodgett films (20 layers) calculated from the 2θ value of X-ray diffraction patterns was estimated to be ca. 41 Å, which corresponds well with theoretical value (approximately 40 Å) for the interlayer distance of multilamellar bilayer membranes.

Since these finely designed polysaccharide amphiphiles are expected to form a variety of polymeric assemblies such as polymer vesicles or liquid crystals,[14] novel chitosan derivatives described herein are of growing interest from the viewpoints of chiral scaffoldings not only for membrane mimetics in biomedical field but for polymer supports in chemical purposes including chiral separations and asymmetric reactions. As an initial step, we are currently investigating sugar-dependent molecular recognition of proteins (lectins) by these organized polysaccharide systems containing N-acetyl-D-glucosamine.

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8) With respect to direct acylations of chitosan with long acylchlorides, see, S. Fujii, H. Kumagai, and M. Noda, *Carbohydr. Res.*, **83**, 389 (1980).

9) Much lower reactivity of the amide-protons at C-2 positions of compound **3** seems to be affected by intramolecular hydrogen bonds in addition to steric hinderance. Chemical shifts and signal-shape of the amide-protons drastically changed above 70 ℃ by ^{1}H-NMR experiments in pyridine-d_5 at various temperatures.

10) Although the synthetic details including all physical data will appear in a future publication as a full paper, all new compounds prepared here gave satisfactory spectral and elemental analyses. For example; Compound **3**: Anal. Found: C, 67.62; H, 10.83; N, 2.55%. Calcd for $C_{38}H_{71}O_6N\ H_2O$: C, 67.77; H, 11.14; N, 2.08%. Compound **4**: Anal. Found: C, 74.43; H, 11.61; N, 1.75%. Calcd for $C_{54}H_{101}O_7N$: C, 74.01; H, 11.62; N, 1.60%.

11) The degrees of sulfation (*ds*) of compound **5** and **6** were estimated to be *ds* 0.77 (S, 3.47%) and *ds* 0.85 (S, 2.82%) based on the data of sulfur analyses, respectively.

12) Compounds **3** and **5** are soluble in pyridine and few cosolvent systems.

13) Langmuir-type film balance (Joyce-Loebl monomolecular layer, UK), deposition of 6.78 x 10^{14} molecules (calculated from the repeating residue) in chloroform at the surface (20 ℃); deposition followed by 20 min of equilibration, compression rate 0.33 cm/min. The subphase was water, purified by distillation and subsequent filtration through a Milli-Q purification system.

14) We have preliminarily found that some of the amphiphiles from chitosan showed a capacity to form lyotropic liquid crystals and liposome-like vesicles, and their properties and functions will be published in somewhere.

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