Enzymatic Synthesis of Surface Active and Biodegradable Glucosaminide Fatty Acid Esters

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Abstract: Methyl, ethyl and butyl β-D-glucosaminide 6-O-fatty acid esters were prepared by the novel direct transglycosylation reaction of chitosan with methanol, ethanol and butanol using resting cells of the chitosan-assimilating strain, Penicillium sp. KS018 as the enzyme source of exo-β-D-GlcNase followed by the transesterification reaction with the fatty acid methyl ester using lipase. Transesterification by the lipase depended on enzyme origin, and reaction conditions, such as temperature and reaction pressure. Methyl, ethyl and butyl β-D-glucosaminide 6-O-fatty acid monoesters demonstrated excellent surface activity in aqueous solution. The antimicrobial properties of the 6-O-dodecanoyl-glucosaminides were stronger compared to dodecyl β-D-glucoside. The methyl, ethyl and butyl glucosaminide 6-O-fatty acid esters were all readily biodegradable by activated sludge.

Key words: enzymatic synthesis, transglycosylation, chitosan, sugar based surfactant, surface activity, glucosaminide fatty acid ester, biodegradation

1 Introduction

The greening of chemistry requires the discovery and development of new synthetic pathways using alternative feedstocks, reaction conditions and catalysts for improved selectivity and energy minimization and the designing bio/environmentally compatible chemicals. Some challenges for this include application of biocatalysts and use of naturally abundant and renewable resources, such as polysaccharides and plant oils, as alternative feedstocks.

Chitosan is produced by the deacetylation of chitin which is one of the most abundant biomass forms next to cellulose. D-Glucosamine is a monomeric component of chitosan and has wide application as a hydrophilic moiety of amphiphilic molecules having biological properties and molecular recognition abilities. Such amphiphilic molecules that form supramolecular assemblies involve long chain alkyl glycosides and short or medium chain alkyl glycoside fatty acid esters (1,2). Long chain fatty acid esters with various sugars, such as glucose, sucrose and raffinose, show excellent surface activities and biodegradability (3). Some are used as edible surfactants, such as an emulsifier for food processing. The biodegradation mechanism of sugar esters, such as sucrose esters and the α-sulfofatty acid sucrose ester, has also been extensively studied (4).

Though short chain alkyl D-glucosaminides are soluble in water and in organic solvents, the short chain alkyl D-glucosaminides may become versatile interme-
diates for the production of novel biologically active and environmentally benign amphipiles. The direct preparation of such glycosides from chitosan and an alcohol using an enzyme may contribute to the establishment of a new field of green chemistry. Glycosides should be produced instead of free sugar units when the hydrolase enzyme has transglycosylation activity and an alcohol is present instead of water in the reaction mixture. The enzymatic preparation of the alkyl glycoside by transglycosylation of polysaccharides, such as xylan (5,6), chitosan (7-9) and cellulose (10), and an alcohol has been reported by us. The short chain alkyl β-D-glucosaminide 6-O-fatty acid ester (R’-RGlcN) was prepared by direct transglycosylation of chitosan and an alcohol using exo-β-D-glucosaminidase (exo-β-D-GlcNase) from the chitosan-assimilating strain Pseudomonas fluorescens KS018 with subsequent esterification with fatty acid. Physico-chemical properties as surfactant, and biodegradability and antimicrobial activity were evaluated. The concept of this study and preparation of the surfactant, R’-RGlcN, are shown in Scheme 1.

2 Experimental

2.1 Materials and Measurements

Methyl octanoate and methyl dodecanoate were purchased from Tokyo Kasei Kogyo Co., Ltd. and distilled under reduced pressure before use. Chitosan was purchased from Katokichi Co., Ltd. (Japan). Porcine pancreatic lipase (41 U/mg protein, according to the supplier) was from Sigma Chemical Co. (St. Louis, MO, USA). Candida antarctica lipase immobilized on acrylic resin [Novozym 435 (triaclylglycerol hydrolase + carboxylesterase) having 10,000 PLU/g (propyl laurate units: lipase activity based on ester synthesis)] and Rhizomucor miehei lipase immobilized on a macroporous anion exchange resin (Lipozyme RM IM) were kindly supplied by Novozymes Japan Ltd. (Chiba, Japan). Lipase AK (Pseudomonas fluorescens) was kindly supplied by Amano Pharmaceutical Co., Ltd. (Nagoya, Japan). The enzyme was dried in vacuum over P2O5 at 25°C for 1 d. 1H and 13C NMR spectra were recorded with a JEOL spectrometer (Tokyo, Japan) at 300 and 75 MHz, respectively.

Short chain alkyl β-D-glucosamides (RGlcN) and their 6-O-fatty acid esters (R’-RGlcN) were analyzed by a high-performance liquid chromatography (HPLC) with a refractive index detector and a commercial HPLC column (TSK-GEL NH2-60, TOSOH, Co., Ltd., Tokyo, Japan, acetonitrile-water as the eluent) calibrated with the chemically synthesized authentic standards.

2.2 Isolation of Chitosan-Assimilating Microbe and Preparation of Resting Cells as Enzyme Source of Exo-β-D-GlcNase

Chitosan-assimilating microbes were isolated from soil using enrichment culture techniques with chitosan as the sole carbon source. The most active fungal strain, KS018, was selected as the chitosan-assimilating strain having transglycosylation activity. The KS018 strain, identified as Penicillium sp. by NCIMB Japan Co., Ltd. (Shimizu-shi, Japan), was grown in an inorganic medium (0.2% NH4Cl, 0.02% K2HPO4, 0.02% MgSO4·7HO, 0.0002% CaCl2, 0.0001% FeSO4·7H2O, 0.0002% MnSO4·4H2O, 0.0007% ZnSO4·7H2O, and trace of CuSO4 and Vitamin B1·HCl) containing 0.5% chitosan as the growing substrate (200 mL) in a shake flask with reciprocal shaking at 30°C. Initial pH of the medium was adjusted to 6.0 with NaOH. At 4-days incubation, the cells were harvested by centrifugation (9,000 g, 15 min, 4°C), washed with saline to obtain 10 g of wet cells as the enzyme source of exo-β-D-glucosaminidase (exo-β-D-GlcNase) for the preparation of alkyl β-D-glucosaminide (RGlcN). The resting cells showed exo-β-D-GlcNase activity as measured using chitotritol (11).

2.3 Preparation of RGlcN by Transglycosylation of Chitosan and Alcohol Using Resting Cells of Penicillium sp. KS018

RGlcN was prepared by direct transglycosylation of chitosan and an alcohol using resting cells as biocatalyst as shown in Scheme 1. A typical preparation of methyl β-D-glucosaminide (MeGlcN) is as follows. A mixture of 50 mL 0.5% aqueous chitosan solution (pH 4.0), 30 mL methanol, 10 g wet cells and 10 mL acetate buffer (100 mM, pH 6.0) was incubated in a shake flask with stirring at 30°C for 48 h. Yield of MeGlcN in the incubation mixture was directly determined by HPLC using an authentic standard. The product was purified by column chromatography and its structure analyzed. After the reaction, the unreacted chitosan was filtered and the filtrate evaporated and purified by column chromatography [(SiO2, CHCl3/CH3OH/28% NH3OH = 10/4/1 (v/v), Rf=0.2] to obtain MeGlcN in a yield of
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132 mg (527 mg/g chitosan). The isolated product was analyzed by HPLC, elemental analysis, IR, $^1$H NMR and $^{13}$C NMR spectroscopies. Results for MeGlcN: $^1$H NMR (270 MHz: D$_2$O): $\delta$=2.50 (1H, m), 3.20-3.35 (3H, m), 3.40 (3H, s), 3.59-3.79 (1H, dd, $J$=5.6), 3.78-4.02 (1H, dd, $J$=2.2, 12.2), 4.20 (1H, d, $J$=8.28).

Ethyl $\beta$-D-glucosaminide (EtGlcN) and butyl $\beta$-D-glucosaminide (BuGlcN) were prepared by the reaction of chitosan with ethanol and butanol, respectively, using resting cells. EtGlcN: $^1$H NMR (270 MHz: D$_2$O): $\delta$=1.15 (3H, t, $J$=7.28), 2.52 (1H, m), 3.20-3.35 (3H, m), 3.40 (3H, s), 3.50-3.60 (1H, dd, $J$=2.2, 5.6), 3.78 (1H, dd, $J$=2.2, 12.2), 4.20 (1H, d, $J$=8.28). BuGlcN: $^1$H NMR (270 MHz: D$_2$O): $\delta$=0.93 (3H, t, $J$=8.0), 1.41 (2H, m), 1.63 (2H, m), 2.64 (1H, dd, $J$=9.6), 3.34-3.50 (3H, m), 3.63-3.79 (2H, m), 3.90-4.02 (2H, m), 4.39 (1H, d, $J$=8.2).

2.4 Preparation of Methyl, Ethyl and Butyl 6-O-Acyl-$\beta$-D-Glucosaminides (R’-RGlcN) Using Lipase

RGlcN and the fatty acid methyl ester were stirred with lipase at constant temperature under a reduced pressure as shown in Scheme 1. Transesterification products were periodically analyzed by HPLC. After the reaction, an aliquot of ethanol was added to the reaction mixture and the insoluble enzyme was removed by filtration through a Celite pad. The ethanol was evaporated to obtain the crude product which was purified by column chromatography (silica gel C-300, CHCl$_3$-CH$_2$OH-28% NH$_3$OH=20:5:1, v/v).

The chemical structure of the isolated product was found by HPLC, elemental analysis, and IR, $^1$H NMR and $^{13}$C NMR spectroscopy. MeGlcN (100 mg), three equivalents methyl dodecanoate (333 mg) and 100% Lipozyme RM IM (100 mg, relative to MeGlcN) were stirred at 70°C and 100 Torr for 7 h. After the reaction, 20 mL ethanol were added and the insoluble enzyme was removed by filtration through a Celite pad. The ethanol was evaporated to obtain the crude product (400 mg). This was further purified using silica gel column chromatography to obtain methyl 6-O-dodecanoyl-$\beta$-D-glucosaminide (C12-MeGlcN) in a yield of 63.4% (122.3 mg) as a white powder. A series of R’-RGlcNs were prepared similarly. Results for R’-RGlcN are shown in Table 1.

2.5 Interfacial Properties

2.5.1 Static surface tension and occupation area of a molecule at surface ($A_{\text{min}}$)

Static surface tension was measured with an automatic digital Kyowa Precise Surface Tensiometer by the CBVP Method (Kyowa Kagaku Co., Ltd., Tokyo, Japan) at 25°C. Measurement was carried out using the Wilhelmy vertical plate technique and sandblasted glass plate. The test solutions were aged at 25°C for at least 1 h before measurement. Each measurement was made three times and the mean value was taken.

The occupation area of a molecule at a surface ($A_{\text{min}}$) was calculated according to the literature (12). The surface excess concentration ($I_{\text{max}}$) in mole/cm$^2$, and the corresponding $A_{\text{min}}$ in nm$^2$, at the liquid/air interface were calculated using equations 1 and 2:

$$I_{\text{max}} = \left(1/2.303 RT\right) \left(\partial g/\partial \log C\right)_T$$  (1)

$$A_{\text{min}} = 10^{14}/N I$$  (2)

where ($\partial g/\partial \log C$)$_T$ is the slope of the surface tension vs. concentration curves below the cmc at a constant temperature and $g$ is the surface tension.

2.5.2 Foaming power and emulsification power

Foam properties were measured by the semi-micro TK method at 25°C according to Yano and Kimura (13). Initial foam volume in mL expressed the foam production and foam volume after 5 min expressed foam stability.

Emulsification power was determined by the height of the emulsion layer after shaking a 0.1% aqueous

Scheme 1 Preparation of R’-RGlcN and Eco-Cycle System.
solution (5 mL) with hexane (5 mL) for 1 min at 25°C. Volumes of emulsion layer, toluene layer and aqueous layer were each measured after a 5-minute stand.

2.6 Antimicrobial Activity
The antimicrobial activity of the R’-RGlcN was evaluated by the agar dilution method (14). Gram-positive bacterial strains, Staphylococcus aureus KB210, Bacillus subtilis KB211 and Micrococcus luteus KB212, gram-negative bacterial strains, Escherichia coli KB213, Salmonella typhimurium KB220 and Pseudomonas aeruginosa KB115 and five fungal strains, Candida albicans KF1, Saccharomyces cerevisiae KF25, Trichophyton mentagrophytes KF213, Penicillium chrysogenum KF270 and Aspergillus niger KF103 were used. Nutrient agar and Sabouraud dextrose agar were used for bacterial and fungi, respectively. Antimicrobial activity was expressed as minimum inhibitory concentration (MIC).

2.8 Biodegradation
R’-RGlcN biodegradability was evaluated by biochemical oxygen demand (BOD). BOD was determined with a BOD Tester (Model 200F; TAITEC Corp., Koshigaya-shi) using the oxygen consumption method, according to the Modified MITI Test (15). Activated sludge was obtained from a municipal sewage plant in Yokohama City.
3 Results and Discussion

3.1 Preparation of RGlCN by Transglycosylation of Chitosan and Alcohol Using Resting Cells

Resting cells of *Penicillium* sp. KS018 were capable of transglycosylation and MeGlcN was produced by the direct reaction of chitosan and methanol with a yield of 530 mg/g chitosan. No MeGlcN was produced using monomeric D-glucosamine or N-acetyl-D-glucosamine in place of chitosan. Transglycosylation between chitosan and methanol using resting cells of *Penicillium* sp. KS018 was analyzed with respect to the reaction conditions. Yield of RGlCN gradually increased with incubation time, and after a 2-day incubation, reached constant value. The yield increased with temperature up to 30°C and then decreased probably due to deactivation of the enzyme. Reaction temperature, giving the highest yield of RGlCN, was 30°C. The yield quickly increased to maximum value with alcohol concentration from 0.2 to 10% and then slightly decreased due to deactivation of the enzyme by high alcohol concentration. Figure 1 shows yields of RGlCN using methanol, ethanol and butanol during a 2-day incubation at 30°C. Yield decreased with chain length of the alcohol.

3.2 Enzymatic Synthesis of Methyl, Ethyl and Butyl 6-O-Acyl-β-D-Glucosaminides

Methyl, ethyl and butyl β-D-glucosaminides (RGlCN) were reacted with methyl dodecanoate and octanoate by lipase to produce the corresponding 6-O-acylated β-D-glucosaminides (R’-RGlCN). Esterification was significantly influenced by enzyme origin, reaction temperature, molar ratio of the two reactants, reaction time, enzyme concentration, reaction pressure and organic solvent.

3.2.1 Effects of enzyme origin

Lipases from various origins were screened for 6-O-monoesterification of RGlCN and methyl dodecanoate at 70°C. Table 2 shows 6-O-esterification results for MeGlcN with methyl dodecanoate. Lipozyme RM IM showed the best results for the 6-O-monoester synthesis. Without lipase, no significant 6-O-esterification occurred suggesting the lipase (Lipozyme RM IM) to actually catalyzed esterification.

3.2.2 Effects of reaction temperature

The reaction temperature significantly influenced

![Graph showing yields of RGlCN using methanol, ethanol and butanol during a 2-day incubation at 30°C.](image)

**Figure 1** Preparation of RGlCN by Transglycosylation of Chitosan with Methanol, Ethanol and Butanol Using Resting Cells of *Penicillium* sp. KS 018 at 30°C for 2 Days.

### Table 2 Effects of Enzyme Origin on Yield of Methyl 6-O-dodecanoyl-β-D-glucosaminide (C12-MeGlcN)

<table>
<thead>
<tr>
<th>Entry</th>
<th>Lipase</th>
<th>Molar ratio of MeDo/MeGlcN</th>
<th>Solvent</th>
<th>Pressure (Torr)</th>
<th>Time (h)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lipozyme RM IM</td>
<td>5/1</td>
<td>—</td>
<td>80</td>
<td>5</td>
<td>73</td>
</tr>
<tr>
<td>2</td>
<td>Novozym 435</td>
<td>5/1</td>
<td>—</td>
<td>80</td>
<td>5</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>lipase AK</td>
<td>5/1</td>
<td>—</td>
<td>80</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>PPL</td>
<td>5/1</td>
<td>—</td>
<td>80</td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>5</td>
<td>Lipozyme RM IM</td>
<td>1/1</td>
<td>—</td>
<td>80</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>6</td>
<td>Lipozyme RM IM</td>
<td>1/1</td>
<td>THF</td>
<td>760</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Novozym 435</td>
<td>1/1</td>
<td>THF</td>
<td>760</td>
<td>24</td>
<td>52</td>
</tr>
<tr>
<td>8</td>
<td>lipase AK</td>
<td>1/1</td>
<td>THF</td>
<td>760</td>
<td>24</td>
<td>0</td>
</tr>
</tbody>
</table>

a) Esterification carried out in bulk using methyl dodecanoate (MeDo) and MeGlcN with 100% lipase (relative to MeGlcN) at 70°C
yield of the 6-O-monoester (R’-RGlcN). Figure 2 shows the relationship between yield and temperature for Me, Et and BuGlcN with methyl dodecanoate and octanoate. The highest yield was obtained by the esterification around 70°C. 6-O-Monoester yield decreased with temperature higher than 70°C probably due to deactivation of the enzyme.

3.2.3 Effects of substrate molar ratio

The molar ratio of fatty acid methyl ester and RGlcN was responsible for 6-O-monoester yield due to equilibrium shift of the reaction system. Figure 3 shows the relationship between yield and molar ratio of the two reactants. The same was observed such that 6-O-monoester yield increased with methyl dodecanoate ratio. At least 3 equivalents methyl dodecanoate versus RGlcN were necessary to obtain a 60% 6-O-monoester yield.

3.2.4 Time course of esterification

Figure 4 shows the time course of the transesterification of RGlcN and methyl dodecanoate by Lipozyme RM IM at a molar ratio of 5. A mixture of methyl dodecanoate (214 mg, 10 mmol), RGlcN (2 mmol) and Lipozyme RM IM (100% relative to RGlcN) was stirred at 70°C and 80 Torr. Aliquots of the reaction mixture were periodically withdrawn and directly analyzed by HPLC. Yield of the 6-O-monoester gradually increased to an equilibrium value after 6 h. The yield of monoester was then almost constant.

3.2.5 Effects of enzyme concentration

Enzyme concentration was responsible for the rate of the reaction as shown in Fig. 5. At least 100% immobilized lipase was necessary for efficient esterification. More enzyme caused no increase in ester yield. The present experiment requires a relatively large amount of enzyme. However, the enzyme could be repeatedly used without significant decrease in activity. In order to establish a practical method for esterification, a continuous bioreactor using the immobilized enzyme is now under study.

3.2.6 Effects of the solvent

Organic solvent may facilitate transesterification by
diffusion of the two substrates. Therefore, an equimolar mixture of the two substrates was used for the reaction instead of excess fatty acid esters when using an organic solvent. Lipases from various origins were compared with respect to monoester synthesis in tetrahydrofuran (THF) at 70°C. Only Novozym 435 exhibited activity for monoester synthesis in THF. However, no esterification occurred using Lipozyme RM IM which showed excellent activity under bulk conditions, possibly due to deactivation of the enzyme by organic solvent. The effects of the organic solvent on monoester yields were compared for various solvents, such as THF, acetonitrile, dimethyl formamide, toluene and tert-butanol, at 70°C for a 24-h reaction. The results are summarized in Table 2. The best results were obtained when THF was used as the solvent. It was confirmed that 6-O-dodecanoyl MeGlcN (C12-MeGlcN) was obtained in 54% yield by reaction of an equal molar methyl dodecanoate and MeGlcN in THF at 70°C for 24 h. Without solvent, excess methyl ester as solvent was essential for esterification.

3.2.7 Effects of reaction pressure

Reaction pressure influences esterification by equilibrium shift of the reaction system. Figure 6 shows the effects of the reaction pressure on reaction of RGlcN and methyl dodecanoate at 70°C between 760 and 40 Torr. The highest yield was obtained when RGlcN and methyl dodecanoate reacted at 100 Torr.

### 3.3 Interfacial Properties of R’-RGlcN in Aqueous Solution

RGlcN octanoic and dodecanoic acid 6-O-monoesters (R’-RGlcN) exhibited surface activity, such as surface tension depression, micelle formation and foaming properties. From surface tension versus concentration plots for fatty acid 6-O-monoesters in distilled water, critical micelle concentration (cmc) determined from inflection of surface tension versus concentration curve, surface tension at cmc (γ_{cmc}), efficiency of adsorption at the surface (pC_{20}) [pC_{20}, negative log of C_{20} the surfactant molar concentration required to reduce surface tension by 20 mN/m] (16, 17), and A_{min} of fatty acid 6-O-monoesters are summarized in Table 3 along with reference data for n-dodecyl poly(oxyethylene) ether (n=6) (C12EO6) measured under the same conditions. Cmc was dependent on length of the acyl chain length and the aglycones of RGlcN. The shorter the acyl chain, the higher was cmc. γ_{cmc} of RGlcN fatty acid 6-O-monoesters were lower compared to C12EO23.

Foaming properties were examined by the semimicro TK method at cmc at 25°C and are summarized in Fig. 7. Excellent foam production and stability were observed for C12-EtGlcN. C12-BuGlcN showed high foam production but low stability similar to the polyethylene glycol monoalkyl ether, such as C12EO6 and n-dodecyl poly(oxyethylene) ether (n=25) (C12EO25).
Emulsification power was determined by shaking 0.1% aqueous sample solution (5 mL) and hexane (5 mL) for 1 min at 25°C. Volumes of the emulsion, toluene and aqueous layers after a 5-minute stand are shown in Fig. 8. The dodecyl ester showed excellent emulsification power compared to conventional polyoxyethylene dodecyl ether type nonionics. C12-BuGlcN showed the strongest emulsification power for the hexane-water system.

3.4 Hydrolytic Degradation

The characteristic feature of the ester-type surfactant may be the hydrolytic degradability, possibly responsible for quick biodegradation. A hydrolytic degradation test was carried out by dissolving the esters in buffer solutions at pH 4, 7, 8 and 10. The results are shown in Fig. 9. R’-RGlcNs were quite stable at pHs 4 and 7 but gradually hydrolyzed at pH 8 and quickly at pH 10.

3.5 Biodegradation

A quick and complete biodegradation after use is one of the indispensable factors for the next generation surfactants, because water-soluble surfactants are generally difficult to recover or recycle. The biodegradation of R’-RGlcN occurs by hydrolytic cleavage of ester bonds to liberate fatty acid and the corresponding RGlcN. A convenient way to predict aerobic biodegradability is to measure BOD. The biodegradability of R’-RGlcN was evaluated based on BOD. BOD was measured with a BOD tester, using activated sludge at substrate concentration of 15 mg/L. Figure 10 shows the time course of the biodegradation (BOD/ThOD × 100) of R’-RGlcN based on BOD and theoretical oxygen demand (ThOD). All samples were equally biodegraded at more than 60% after 10 d, which can be considered as readily biodegradable. On the other hand, the biodegradation rate of a conventional ethoxylated alcohol, such as C12EO6, was less than that of R’-RGlcN under the same conditions.

3.6 Antimicrobial Activity

R’-RGlcNs were screened for antimicrobial activity toward gram-positive and gram-negative bacterial and

<table>
<thead>
<tr>
<th>Compound</th>
<th>cmc (mmol/L)</th>
<th>γcmc (mN/m)</th>
<th>pC20</th>
<th>Amin × 10² (nm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C8-MeGlcN</td>
<td>8.58</td>
<td>28.2</td>
<td>3.2</td>
<td>43.8</td>
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<tr>
<td>C8-EtGlcN</td>
<td>5.39</td>
<td>29.5</td>
<td>3.4</td>
<td>49.8</td>
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<tr>
<td>C8-BuGlcN</td>
<td>2.76</td>
<td>26.0</td>
<td>3.9</td>
<td>48.7</td>
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<tr>
<td>C12-MeGlcN</td>
<td>1.68</td>
<td>26.8</td>
<td>3.7</td>
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<tr>
<td>C12-EtGlcN</td>
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<td>28.0</td>
<td>4.7</td>
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<tr>
<td>C12-BuGlcN</td>
<td>0.0719</td>
<td>27.6</td>
<td>5.7</td>
<td>55.9</td>
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<tr>
<td>C12EO6</td>
<td>0.110</td>
<td>31.3</td>
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<td>66.7</td>
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</table>

Table 3: Surface Activity of 6-O-acylated Glucosaminides at 25°C.
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fungal strains. Minimum inhibitory concentrations (MIC) are given in Table 4. C12-RGlCN with a dodecanoyl group showed a broad spectrum of antimicrobial activity. Antimicrobial activity of C12-RGlCNs was greater than that of 1-dodecyl β-D-glucopyranoside (C12βGlc) (18) or conventional n-dodecyl tetra(oxyethylene) ether (C12EO4) (18) toward Escherichia coli and Salmonella typhimurium.

4 Conclusions

Methyl, ethyl and butyl β-D-glucosaminide 6-O-fatty acid esters were prepared by the novel direct transglycosylation reaction of chitosan with methanol, ethanol and butanol using resting cells of the chitosan-assimilating strain, Penicillium sp. KS018 followed by the transesterification reaction with the fatty acid methyl ester using Lipozyme RM IM under reduced pressure. The methyl, ethyl and butyl 6-O-acyl-β-D-glucosaminides exhibited excellent surface activity and

Table 4 Antimicrobial Activity of 6-O-acylated β-D-glucosaminides.

<table>
<thead>
<tr>
<th>Compounds/organism</th>
<th>MIC (mg/mL)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>C8-MeGlcN</td>
</tr>
<tr>
<td>S. aureus</td>
<td>&gt;400</td>
</tr>
<tr>
<td>B. subtilis</td>
<td>&gt;400</td>
</tr>
<tr>
<td>M. luteus</td>
<td>&gt;400</td>
</tr>
<tr>
<td>E. coli</td>
<td>&gt;400</td>
</tr>
<tr>
<td>S. typhimurium</td>
<td>&gt;400</td>
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<tr>
<td>P. aeruginosa</td>
<td>&gt;400</td>
</tr>
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<td>C. albicans</td>
<td>&gt;400</td>
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<td>S. cerevisiae</td>
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<td>T. mentagrophytes</td>
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<tr>
<td>P. chrysogenum</td>
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<tr>
<td>A. niger</td>
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</table>

<sup>a</sup>C12βGlc: Dodecyl β-D-glucopyranoside (18)
biodegradability.

Acknowledgments

Immobilized lipases from Candida antarctica (Novozym 435) and from Rhizomucor miehei (Lipozyme RM IM) were supplied by Novozymes Japan Ltd. (Chiba, Japan). This work was partially supported by a Grant-in-Aid for the 21st Century COE Program “KEIO LCC” from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

References