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Synthesis of Alkyl-β-D-thioglucopyranosides, a Series of New Nonionic Detergents

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As a part of our search for new types of detergent useful for biological applications, a series of alkyl-β-D-thioglucopyranosides was synthesized in several steps from glucose. The overall yield was about 80%. Critical micelle concentrations of *n*-hexyl-, *n*-heptyl-, *n*-octyl-, and *n*-nonyl-β-D-thioglucopyranoside were determined. Because of their electroneutrality, high solubility in water and high critical micelle concentration, *n*-heptyl- and *n*-octyl-β-D-thioglucopyranoside seem to be potentially useful detergents for applications in biological systems.

Keywords——alkylthioglucoside; synthesis; nonionic detergent; critical micelle concentration; solubilization

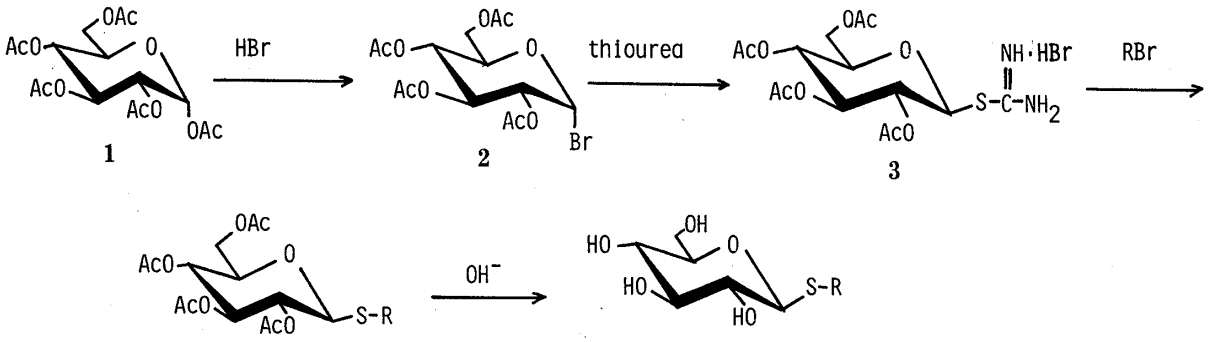
Detergents are important in the solubilization of hydrophobic materials. Although numerous detergents are commercially available, only a few detergents are suitable for use in studies of membrane proteins.[1] Ideally such a detergent should possess strong solubilizing power and nondenaturing character. Furthermore, desirable properties of a detergent for use in the solubilization and reconstitution of membrane proteins are as follows: high solubility of the detergent itself; a high critical micelle concentration to permit rapid removal of the detergent by dialysis; electrical neutrality to permit the use of ion exchange chromatography and isoelectric focusing; optical transparency to allow spectrophotometric analysis of materials of interest; a well-defined chemical composition and high purity to ensure experimental reproducibility;[2] and absence of interference with protein determination. Among the detergents currently used, *n*-octyl-β-D-glucopyranoside possesses all of the desirable properties described above, and has been proved to be an excellent detergent for use in membrane biochemistry.[3-5] However, because the synthesis of *n*-octyl-β-D-glucopyranoside involves a step which requires a silver catalyst,[6] this detergent is very expensive. Furthermore, *n*-octyl-β-D-glucopyranoside is hydrolyzed by β-glucosidase. Therefore care is necessary when this detergent is used in biological systems.

We tried to synthesize analogs of *n*-octyl-β-D-glucopyranoside without using expensive materials or a catalyst to obtain a suitable detergent at low cost. Here we report the synthesis of a series of alkyl-β-D-thioglucopyranosides, which are S-substituted analogs of alkyl-β-D-glucopyranosides. The properties of the compounds suggest that they may have considerable potential for use in membrane research and other fields.

Results and Discussion

Synthesis

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**2**) was synthesized by the modification of a published procedure.[7] The treatment of **2** with thiourea gave crystalline



1 2 3

4: R = *n*-C_6H_{13}
5: R = *n*-C_7H_{15}
6: R = *n*-C_8H_{17}
7: R = *n*-C_9H_{19}
8: R = *n*-$C_{10}H_{21}$

9: R = *n*-C_6H_{13}
10: R = *n*-C_7H_{15}
11: R = *n*-C_8H_{17}
12: R = *n*-C_9H_{19}
13: R = *n*-$C_{10}H_{21}$

Chart 1

TABLE I. MS Spectral Data for the Acetates

4	388 (t[a], $M^+ - CH_3COOH$), 331 (7.3, $M^+ - SC_6H_{13}$), 170 (5.6), 169 (60.4), 127 (13.7), 115 (5.3), 109 (46.0), 81 (5.1), 43 (100)
5	402 (t, $M^+ - CH_3COOH$), 342 (14.2, $M^+ - CH_3COOH \times 2$), 331 (11.7, $M^+ - SCH_7H_{15}$), 269 (12.9), 227 (9.5), 211 (6.4), 169 (95.1), 145 (7.2), 97 (7.9), 85 (6.1), 83 (5.5), 81 (7.9), 57 (7.7), 43 (100)
6	416 (t, $M^+ - CH_3COOH$), 356 (4.7, $M^+ - CH_3COOH \times 2$), 331 (14.4, $M^+ - SC_8H_{17}$), 283 (14.2), 241 (11.1), 169 (100), 139 (8.1), 127 (21.6), 109 (77.4), 103 (6.1), 97 (8.1), 69 (7.5), 57 (5.5), 55 (5.7), 43 (100)
7	430 (t, $M^+ - CH_3COOH$), 370 (3.4, $M^+ - CH_3COOH \times 2$), 331 (11.1, $M^+ - SC_9H_{19}$), 297 (10.3), 211 (5.4), 169 (80.9), 145 (5.5), 139 (5.6), 127 (14.9), 109 (54.5), 97 (5.6), 81 (5.8), 43 (100)
8	444 (t, $M^+ - CH_3COOH$), 384 (3.9, $M^+ - CH_3COOH \times 2$), 331 (14.1, $M^+ - SC_{10}H_{21}$), 331 (12.0), 269 (9.4), 211 (6.4), 169 (92.7), 145 (6.0), 139 (6.2), 127 (15.8), 109 (58.7), 97 (6.4), 81 (6.1), 55 (5.7), 43 (100)

a) t = trace.

thiopseudourea hydrobromide (**3**),[8,9] which was reacted with *n*-octylbromide to obtain *n*-octylthioglucoside tetraacetate (**6**) (Chart 1). The infrared (IR) spectrum ($CHCl_3$) of **6** showed an absorption at 1760 cm^{-1} (C=O), and the mass (MS) spectrum showed fragment ions at *m/e* 416 ($M^+ - CH_3COOH$), 356 ($M^+ - CH_3COOH \times 2$), and 331 ($M^+ - SC_8H_{17}$) (Table I). The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **6** showed the signals of four acetyl groups (δ 2.08, 2.06, 2.03 and 2.01) and various alkyl protons (δ 2.65, 1.58, 1.27 and 0.88) (Table II). The anomeric proton showed a coupling constant of 9.5 Hz, which suggested that the alkyl-thio group has β-configuration. Other thioglucoside acetates **4**, **5**, **7** and **8** were prepared by the same method as **6**.

Hydrolysis of **6** with 5 mM NaOH in MeOH containing a small amount of water gave the deacetyl compound **11**. Compound **11** showed no C=O band but gave an OH band at 3600—3100 cm^{-1} in the IR spectrum. The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum showed the signals of sugar skeleton carbons at δ 86.4 (C-1), 80.7 (C-2 or C-5), 78.3 (C-3), 70.2 (C-4), 73.4 (C-5 or C-2), and 61.9 (C-6), and those of eight alkyl carbons at δ 32.6, 30.7, 30.6, 30.0, 30.0, 29.7, 23.4, and 14.6 (Table III). Gas liquid chromatography (GLC) of **11**

TABLE II. ^{1}H-NMR Data (270 MHz)[a]

	H-1	H-2	H-3	H-4	H-5	H-6	H-6′	Acetyl	Alkyl
4	4.48 d 9.5[b]	5.03 t 9.5	5.22 t 9	5.08 t 9	3.70 m	4.25 dd 12.5, 5	4.13 dd 12.5, 2	2.09, 2.06 2.03, 2.01	2.67 (SCH_2) 1.60 ($CH_2\times1$) 1.35 ($CH_2\times1$) 1.29 ($CH_2\times2$) 0.88 (t, 6.6, CH_3)
5	4.48 d 9.5	5.03 t 9.5	5.22 t 9	5.08 t 9	3.71 m	4.25 dd 12.5, 5	4.13 dd 12.5, 2	2.08, 2.06 2.03, 2.01	2.67 (SCH_2) 1.60 ($CH_2\times1$) 1.28 ($CH_2\times4$) 0.88 (t, 6.6, CH_3)
6	4.48 d 10	5.03 t 10	5.22 t 9.5	5.08 t 9.5	3.70 m	4.24 dd 12.5, 5	4.13 dd 12.5, 2.5	2.08, 2.06 2.03, 2.01	2.65 (SCH_2) 1.58 ($CH_2\times1$) 1.27 ($CH_2\times5$) 0.88 (t, 6.6, CH_3)
7	4.48 d 10	5.03 t 10	5.22 t 9.5	5.09 t 9.5	3.71 m	4.25 dd 12.5, 5	4.13 dd 12.5, 3	2.08, 2.06 2.03, 2.01	2.67 (SCH_2) 1.59 ($CH_2\times1$) 1.26 ($CH_2\times6$) 0.88 (t, 6.6, CH_3)
8	4.48 d 10	5.03 t 10	5.22 t 10	5.09 t 10	3.71 m	4.25 dd 12.5, 5	4.13 dd 12.5, 3	2.08, 2.06 2.03, 2.01	2.68 (SCH_2) 1.59 ($CH_2\times1$) 1.26 ($CH_2\times7$) 0.88 (t, 6.6, CH_3)

a) All spectra were in $CDCl_3$. *b*) Coupling constants are given in Hz.

TABLE III. ^{13}C-NMR (67.8 MHz) Data (in D_2O Containing Internal Dioxane (67.4 ppm))

	C-1	C-2	C-3	C-4	C-5	C-6	Alkyl carbons
9	86.3[a]	80.7 or 73.3	78.3	70.3	73.3 or 80.7	61.9	32.0, 30.6, 30.4, 29.1, 23.1, 14.5
10	86.3	80.8 or 73.4	78.4	70.3	73.4 or 80.8	61.9	32,4 30.5, 30.5, 29.5, 29.4, 23.2, 14.5
11	86.4	80.7 or 73.4	78.3	70.2	73.4 or 80.7	61.9	32.6, 30.7, 30.6, 30.0, 30.0, 29.7, 23.4, 14.6
12	86.4	80.7 or 73.4	78.3	70.2	73.4 or 80.8	61.9	32.7, 30.8, 30.7, 30.5, 30.2, 30.2, 29.9, 23.4, 14.6
13	86.4	80.8 or 73.4	78.3	70.2	73.4 or 80.8	61.9	32.6, 30.6, 30.6, 30.4, 30.4, 30.1, 30.1, 29.8, 23.3, 14.6

a) Chemical shifts (ppm) were obtained from dioxane as the standard.

showed only one peak at a relative retention time of 6.9 with respect to 1,2,3,4,6-pentakis-*O*-trimethylsilyl-α-D-glucopyranoside (Table IV). Other alkyl thioglucosides **9**, **10**, **12** and **13** were prepared from the corresponding acetates **4**, **5**, **7** and **8**, respectively, by the same method as **11**.

Elemental analyses of all these compounds (**4**—**13**) showed good agreement with

Table IV. GLC[a] Data for the Thioglucosides[b]

9	10	11	12	13
4.3[c]	5.9	6.9	10.9	15.1

a) OV-1 (2%, 2 m, O.D. 1/8″), 200 °C, H_2 40 ml/min, N_2 50 ml/min, air 250 ml/min.
b) All samples were injected after trimethylsilylation.
c) Relative retention time with respect to 1,2,3,4,6-pentakis-*O*-trimethylsilyl-α-D-glucopyranose.

Table V. Elemental Analyses

Compound	Formula	Calcd	Found
4	$C_{20}H_{32}O_9S$	C, 53.56; H, 7.19	C, 53.24; H, 6.89
5	$C_{21}H_{34}O_9S$	C, 54.53; H, 7.41	C, 54.35; H, 7.39
6	$C_{22}H_{36}O_9S$	C, 55.44; H, 7.61	C, 55.27; H, 7.59
7	$C_{23}H_{38}O_9S$	C, 56.31; H, 7.81	C, 56.28; H, 7.82
8	$C_{24}H_{40}O_9S$	C, 57.12; H, 8.03	C, 56.99; H, 8.03
9	$C_{12}H_{24}O_5S$	C, 51.40; H, 8.63	C, 50.98; H, 8.42
10	$C_{13}H_{26}O_5S$	C, 53.03; H, 8.90	C, 52.88; H, 8.90
11	$C_{14}H_{28}O_5S$	C, 54.52; H, 9.15	C, 54.41; H, 8.98
12	$C_{15}H_{30}O_5S$	C, 55.87; H, 9.38	C, 55.66; H, 9.33
13	$C_{16}H_{32}O_5S$	C, 57.11; H, 9.59	C, 56.89; H, 9.51

Table VI. CMC Values of Alkyl-β-D-thioglucopyranosides

Alkyl group	CMC (M)
$-C_6H_{13}$	1.0×10^{-1}
$-C_7H_{15}$	3.0×10^{-2}
$-C_8H_{17}$	9.0×10^{-3}
$-C_9H_{19}$	2.5×10^{-3}

calculated values (Table V). Thus, we synthesized alkyl-β-D-thioglucopyranosides without using an expensive catalyst or other materials in high yield (overall yield, about 80%).

Properties

For the use of detergents in the solubilization of membrane proteins or other hydrophobic materials, the detergent itself should be soluble in water. Among the alkyl-β-D-thioglucopyranosides we synthesized, *n*-hexyl-, *n*-heptyl- and *n*-octyl-β-D-thioglucopyranoside were highly soluble in water. *n*-Nonyl-β-D-thioglucopyranoside was rather insoluble, and decyl-β-D-thioglucopyranoside was insoluble. Thus, *n*-hexyl-, *n*-heptyl- and *n*-octyl-β-D-thioglucopyranoside seem to be suitable for use in membrane research.

Critical micelle concentration (CMC) is a convenient parameter for assessing detergents. A detergent with high CMC is desirable for solubilization, purification and reconstitution of membrane proteins. Thus, we measured the CMCs of alkyl-β-D-thioglucopyranosides (Table VI). The CMC decreased to 1/3.4 (average) per one additional methylene group, similarly to the case of alkyl-β-D-glucosides.[10] The CMCs of *n*-hexyl-, *n*-heptyl- and *n*-octyl-β-D-thioglucopyranoside seem to be high enough for practical use. The CMC of *n*-octyl-β-D-glucopyranoside has been reported to be 25 mM.[10] The high CMCs suggest that these detergents could be removed fairly easily by dialysis. The low CMC of *n*-nonyl-β-D-

thioglucopyranoside suggests difficulty in its use in membrane biochemistry. We tested the solubilizing power and whether or not these detergents are easily removable, and obtained very satisfactory results with *n*-heptyl- and *n*-octyl-β-D-thioglucopyranoside.[11]

These detergents possessed strong solubilizing power for membrane proteins of *Escherichia coli*, and did not denature the solubilized proteins.[11] Since these detergents are electrically neutral, it is possible to use them in ion exchange chromatography and isoelectric focusing. Furthermore, the detergents are optically transparent, and thus do not interfere with analysis by spectrophotometry.

Although the properties of *n*-octyl-β-D-thioglucopyranoside were very similar to those of *n*-octyl-β-D-glucopyranoside, the former was superior to the latter in several respects (for example, stability, applicability and cost) for practical use.[11]

Experimental

Chemicals——All chemicals and solvents were of reagent grade and were obtained from commercial sources. TMS-HT kit (silylating reagent) was from Tokyo Kasei Co., Ltd.

Analyses——Analytical thin-layer chromatography (TLC) was performed on Merck Kieselgel 60 F_{254}. Kieselgel 60 (Merck, 70—230 mesh ASTM) was used for column chromatography. Melting points were determined with a Yanaco micro melting point apparatus.

^{1}H-NMR (270 MHz) spectra of samples in $CDCl_3$ solution containing internal tetramethylsilane and ^{13}C-NMR (67.8 MHz) spectra of samples in D_2O solution containing internal dioxane were recorded with a JEOL JNM-GX 270 FT NMR spectrometer. MS spectra were obtained with an LKB type 9000 spectrometer at an ionizing energy of 70 eV by the direct inlet method. GLC was done on a Hewlett Packard 5710A gas chromatograph. IR spectra were recorded with Hitachi 215 grating infrared spectrophotometer.

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl Bromide (2)——α-D-Glucopyranose pentaacetate (**1**) was prepared by Zémplen's method.[12] Compound **2** was synthesized by a published procedure[7] with some modification as described below. Compound **1** (150 g, 0.38 mol) was added at room temperature to a stirred suspension of red phophorus (10 g, 0.32 mol) and bromine (18 ml, 0.34 mol) in acetic anhydride (300 ml), then aqueous hydrogen bromide (47%, 15 ml) was added dropwise. The resulting suspension was stirred for 1 h at 40—50 °C. Dichloromethane (500 ml) was added to the reaction mixture, and the whole was filtered. The filtrate was washed successively with water, saturated aqueous $NaHCO_3$, and water, then dried over Na_2SO_4, and filtered. The filtrate was evaporated under reduced pressure at 40 °C to give a hard crystalline mass. Recrystallization from ether afforded pure tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**2**, 145 g, yield 91.7%), mp 88—90 °C.

2-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)thiopseudourea Hydrobromide (3)——A mixture of **2** (140 g, 0.34 mol), thiourea (30 g, 0.40 mol), and acetone (500 ml) was boiled under reflux for 30 min, then cooled in an ice bath. The solid product was separated by filtration. Recrystallization from acetone gave **3** in 95% yield; mp 216—217 °C (dec.); lit.,[8] mp 205 °C.

***n*-Octyl 2,3,4,6-Tetra-*O*-acetyl-1-thio-β-D-glucopyranoside (6)**——K_2CO_3 (3 g, 21.7 mmol) and $NaHSO_3$ (2 g, 19.2 mmol) were added in that order to a solution of **3** (10 g, 21 mmol) and *n*-octylbromide (4.5 g, 23.3 mmol) in 1 : 1 (v/v) acetone–water (500 ml), and the mixture was kept overnight at room temperature. Analysis by TLC (benzene–acetone 8 : 2) showed that 1-thio-D-glucose (generated from the thioisourea derivative) was no longer present after this reaction. The mixture was extracted with dichloromethane (200 ml × 2). The combined organic extracts were washed with water, dried over Na_2SO_4 and evaporated to give a residue. The residue was dissolved in benzene and applied to a silica gel column. Column chromatography (benzene–acetone, gradient up to 5% acetone) followed by recrystallization from *n*-hexane gave colorless needles (**6**); mp 71—72 °C, yield 96%.

n-Hexyl- (**4**, mp 64—65 °C), *n*-heptyl- (**5**, mp 72—74 °C; lit.,[13] mp 69—70 °C), *n*-nonyl- (**7**, mp 71—73 °C), and *n*-decyl-β-D-thioglucopyranoside tetraacetate (**8**, mp 76—77 °C) were obtained by the same method as described for **6** in yields of more than 94%.

***n*-Octyl 1-Thio-β-D-glucopyranoside (11)**——Compound **6** (8 g, 16.8 mmol) was dissolved in 5 mM NaOH in MeOH (100 ml), and allowed to stand overnight at room temperature. The mixture was neutralized by addition of acetic acid and evaporated to give a residue. The residue was dissolved in dichloromethane (20 ml) and subjected to silica gel column chromatography. Compound **11** was eluted with 5% MeOH in CH_2Cl_2. The eluates were evaporated under a vacuum to give quantitatively a colorless residue (**11**).

Compounds **9**, **10**, **12** and **13** were obtained by the same method as **11** from **4**, **5**, **7** and **8**, respectively.

Determination of CMC——The CMCs were determined by testing the effect of the detergents on the fluorescence intensity of 8-anilino-1-naphthalenesulfonic acid. The changes in the fluorescence intensity were measured at excitation and emission wavelengths of 375 and 480 nm, respectively.[14]

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