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ARTICLE

Significantly Improved Equilibrium Yield of Long-Chain Alkyl Glucosides via Reverse Hydrolysis in a Water-Poor System Using Cross-Linked Almond Meal as a Cheap and Robust Biocatalyst

WANG Qinqqin[1], YU Huilei[1,a], ZHAO Na[2], LI Chunxiu[1], SHANG Yazhuo[2], LIU Honglai[2], XU Jianhe[1,b]

[1]State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China
[2]Department of Chemistry and Molecular Engineering, East China University of Science and Technology, Shanghai 200237, China

Abstract: An array of ten β-D-glucopyranosides with varied alkyl chain lengths were enzymatically synthesized. It was found that for longer alkyl chains a lower initial rate and final yield of glucoside was obtained except for methyl glucoside because of the severe toxicity of methanol to the enzyme. From a thermodynamics point of view, the equilibrium constant and Gibbs free energy variation of the glucoside syntheses were systematically investigated. To improve the final yields of the glucosides containing long alkyl chains the equilibrium of the enzymatic glucoside synthesis was altered. The equilibrium yield of decyl β-D-glucoside increased from 1.9% to 6.1% when the water content was reduced from 10% to 5% (v/v) using *tert*-butanol as a cosolvent and 0.10 mol/L of glucose as a substrate. As for the other longer alkyl chain glucosides, heptyl β-D-glucoside was found to have significant surface activity as well.

Key words: alkyl β-D-glucosides; decyl β-D-glucoside; enzymatic synthesis; reverse hydrolysis; water-poor system; equilibrium yield

As a type of non-ionic surfactant, alkyl β-D-glucosides are non-toxic and biodegradable [1] and have antimicrobial properties [2,3]. They are widely used as biocompatible detergents in foods, detergents, and in the pharmaceutical industry [4,5].

Traditionally, alkyl β-D-glucosides have been produced by a chemical process but this leads to a mixture of products [6]. To obtain more defined compounds several protection and deprotection steps for the reactive hydroxyl groups and the activation of anomeric carbon are needed [7]. Recently, much work on the environmentally benign enzymatic synthesis of alky1 glucosides has appeared in the literature. The synthesis can be achieved through two pathways, the first of which is reverse hydrolysis (a thermodynamic approach) and the other is transglycosylation (a kinetic approach) [8]. Vulfson et al. [9] used almond β-glucosidase to catalyze the alkyl β-glucoside synthesis in a two-phase system of water/organic solvent. Vic et al. [8] synthesized alkyl β-D-glucosides from D-glucose and alcohols by reverse hydrolysis using the commercially available almond β-D-glucosidase in a homogeneous medium of acetonitrile-water (9:1, v/v). Thereafter, more reports on the synthesis of alkyl β-glucosides have been published. Among them, hexyl [10–20] and octyl [12–24] β-D-glucosides were more extensively studied by comparison with the other glucosides containing long alkyl chains. However, the equilibrium constants and Gibbs free energies of glucosides with various alkyl chain lengths have never been reported or compared systematically in terms of thermodynamics.

Not all alkyl β-D-glucosides have obvious surfactant properties. Only octyl, nonyl, and decyl β-D-glucosides show significant surface activity among all the reported β-D-glucosides. The enzymatic synthesis of glucosides containing long alkyl chains has not been reported much perhaps because of their extremely low yields [10,14,17–19,25]. It is especially difficult to synthesize decyl β-D-glucoside containing a highly hydro-

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[a]Corresponding author. Tel/Fax: +86-21-6425-0840; Email: huileiyu@ecust.edu.cn
[b]Corresponding author. Tel/Fax: +86-21-6425-0840; Email: jianhexu@ecust.edu.cn
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phobic chain enzymatically using a general glucosidase. The reported yield of decyl glucoside was 0.8% even after 14 d of reaction by the reverse-hydrolysis pathway [16].

In our work, various alkyl *β*-D-glucosides were synthesized by the direct condensation of glucose with corresponding alcohols using almond meal as a cheap biocatalyst. Meanwhile, a method to tune the enzymatic synthesis of alkyl *β*-D-glucosides was found by a systematic investigation into the reaction kinetics of ten *n*-alkyl *β*-D-glucosides. Furthermore, important factors that affect the equilibrium yield were also identified and optimized to improve the final yield of surface active *β*-D-glucosides containing long alkyl chains.

1 Experimental

Fresh almonds were peeled and powdered, washed three times with ethyl acetate and twice with acetone. The powder was dried in a vacuum desiccator and stored at 4 °C. A powder of the dry almond meal between 40 and 200 mesh was used in this work. The hydrolytic activity of the almond meal was assayed as reported previously [18]. The almond meal was further cross-linked with 30 mmol/L glutaraldehyde for 1 h [26] to improve the biocatalyst's stability.

Ten home-made *n*-alkyl *β*-D-glucopyranosides (from methyl to decyl) were used as standards for high performance liquid chromatography (HPLC) analysis. All other chemicals were obtained commercially and were reagent grade or better.

1.1 Enzymatic synthesis of alkyl *β*-D-glucosides

In a typical experiment, an alkyl alcohol was used as the substrate and the medium. The reaction mixture was composed of 18 ml of primary alcohol and 2 ml of water containing 0.25 mol/L glucose (Scheme 1). After 60 mg almond meal was added, the mixture was incubated in a 100-ml flask and constantly shaken at 180 r/min at 50 °C. At certain intervals, 50 μl of the mixture was withdrawn and analyzed by HPLC.

For the synthesis of methyl *β*-D-glucoside, 2 ml of methanol, 16 ml of acetonitrile, and 2 ml of water containing 0.25 mol/L glucose were mixed.

1.2 Equilibrium between glucoside hydrolysis and reverse-hydrolysis

For the hydrolytic reaction of glucosides, 0.25 mol/L of propyl, hexyl, or octyl glucoside was dissolved in a mixture of 18 ml of the corresponding alcohol and 2 ml of water containing 60 mg almond meal. The reaction was performed in a 100-ml flask that was constantly shaken at 180 r/min at 50 °C. After the mixture was incubated for 72 and 144 h respectively, 30 mg almond meal was supplemented. The reverse hydrolysis of the propyl, hexyl, and octyl glucosides was performed as in the *n*-alkyl *β*-D-glucosides synthesis.

1.3 HPLC analysis

Glucose as well as methyl, ethyl, propyl, butyl, pentyl, and hexyl glucosides were determined by HPLC (Agilent Technologies 1200 Series) using an Aminex HPX-87H Column (Bio-Rad, *φ*300 mm × 7.8 mm, 9 μm). They were eluted with 5 mmol/L H_2SO_4 at a flow rate of 0.6 ml/min at 65 °C and detected using a refractive index detector.

Heptyl, octyl, nonyl, and decyl glucosides were determined by HPLC (Agilent Technologies 1200 Series) using an Agilent XDB-C18 Column (*φ*4.6 mm × 150 mm, 5 μm). They were eluted with MeOH-H_2O (2:1, v/v) at a flow rate of 0.8 ml/min at 30 °C and detected using a refractive index detector.

1.4 Optimization of the decyl *β*-D-glucoside synthesis

The effects of water content, solvent content, glucose concentration, pH, and temperature on the enzymatic synthesis of decyl glucoside were systematically investigated.

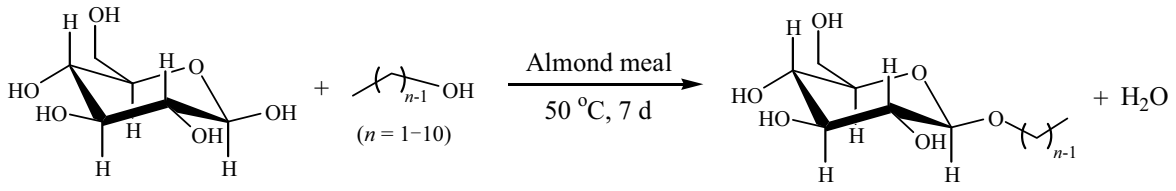
To choose the most suitable water content for the reaction system, the effect of water content on the initial rate and final yield were determined. Various amounts of water including 5%, 8%, 10%, 15%, 20%, 30%, and 50% were added to the reaction system (5 ml), in which deionized water was used instead of a potassium phosphate buffer.

To determine the best solvent, the synthesized decyl glucoside was measured using a standard assay after being incubated in a decanol solution with different solvents (1:1, v/v) at 50 °C for 48 h.

To determine a suitable glucose concentration, various concentrations of glucose (0.05, 0.10, 0.25, and 0.75 mol/L) were used for the synthesis of decyl glucoside.

1.5 Characterization of the alkyl *β*-D-glucosides

Optical rotations were measured using an Autopol I Po-



Scheme 1. Enzymatic synthesis of alkyl *β*-glucosides by reverse hydrolysis.

larimeter (Rudolph Research Analytical, Inc. USA). For surface tension measurements [27], the surfactant was dissolved in double-distilled water. Surface tension was measured at 25 °C with a DCA315 system (Thermo-Cahn Instruments, Inc. USA). Before the measurement, the equipment was calibrated using double-distilled water at 25 °C.

A plot of surface tension versus concentration was used to determine the critical micelle concentration (cmc). The free energy associated with the micelle formation per mole of monomer unit (G) was evaluated according to the equation [28]: $\Delta G_m^0 = RT\ln(\text{cmc})$.

2 Results and discussion

2.1 Synthesis of alkyl β-D-glucosides

Synthetic reaction profiles for the ten *n*-alkyl β-D-glucosides (C_1–C_{10}) are shown in Fig. 1. Their final yields (Table 1) were significantly affected by the alkyl chain lengths of the alcohols that functioned as glycosidyl acceptors [29]. Longer alkyl chains led to lower reaction rates and final yields with the exception of the synthesis of methyl β-D-glucoside. Both the rate and the yield for the synthesis of methyl β-D-glucoside were lower than that of the ethyl, propyl, and butyl glucosides. This may be due to the severe toxicity of methanol to the almond glucosidase.

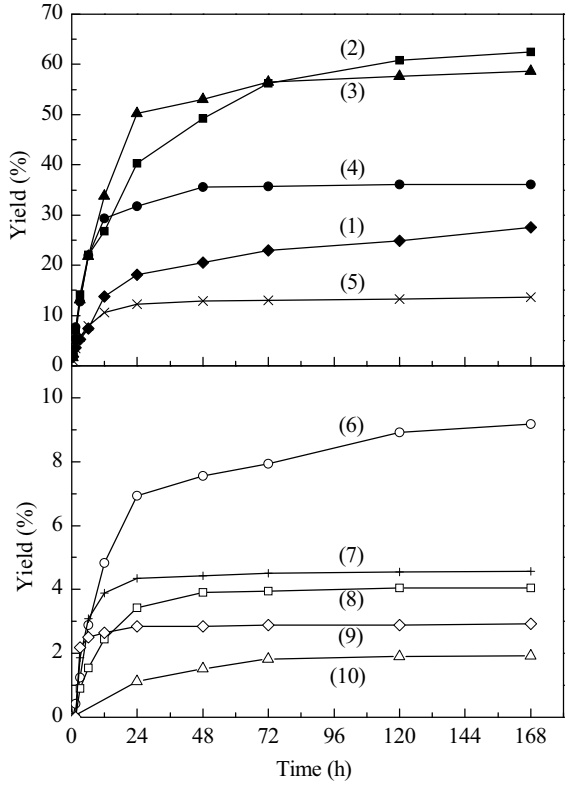


Fig. 1. Progress curves for *n*-alkyl β-D-glucosides synthesis (C_1–C_{10}). (1) Methyl glucoside; (2) Ethyl glucoside; (3) Propyl glucoside; (4) Butyl glucoside; (5) Pentyl glucoside; (6) Hexyl glucoside; (7) Heptyl glucoside; (8) Octyl glucoside; (9) Nonyl glucoside; (10) Decyl glucoside.

The purity of all these alkyl glucosides was detected by HPLC in Table 1 while propyl β-D-glucoside, butyl β-D-glucoside, and pentyl β-D-glucoside were also characterized by ^{1}H NMR as follows.

Propyl β-D-glucoside. ^{1}H NMR (500 MHz, D_2O): δ 0.85–0.87 (m, 3H), 1.54–1.62 (m, 2H), 3.19–3.22 (m, 1H), 3.30–3.34 (m, 1H), 3.38–3.45 (m, 2H), 3.56–3.61 (m, 1H), 3.64–3.68 (m, 1H), 3.80–3.88 (m, 2H), 4.41 (d, J = 8.0 Hz, 1H).

Butyl β-D-glucoside. ^{1}H NMR (500 MHz, D_2O): δ 0.84–0.87 (m, 3H), 1.28–1.36 (m, 2H), 1.53–1.58 (m, 2H), 3.18–3.22 (m, 1H), 3.28–3.34 (m, 1H), 3.38–3.45 (m, 2H), 3.60–3.68 (m, 2H), 3.85–3.90 (m, 2H), 4.40 (d, J = 8.0 Hz, 1H).

Pentyl β-D-glucoside. ^{1}H NMR (500 MHz, D_2O): δ 0.83–0.85 (m, 3H), 1.27–1.29 (m, 4H), 1.57–1.58 (m, 2H), 3.19–3.22 (m, 1H), 3.31–3.35 (m, 1H), 3.38–3.45 (m, 2H), 3.60–3.68 (m, 2H), 3.85–3.89 (m, 2H), 4.40 (d, J = 8.0 Hz, 1H).

2.2 Equilibrium between glucoside hydrolysis and reverse hydrolysis

The yield of alkyl β-D-glucoside is controlled by the thermodynamic equilibrium and the biocatalyst activity [16]. To verify the equilibrium yield of alkyl β-D-glucoside, both the hydrolysis and reverse-hydrolysis of propyl, hexyl, and octyl glucosides were performed (Fig. 2). On this basis, 30 mg almond meal was added twice at 72 and 144 h to avoid the effect of biocatalyst activity loss. With an increase in the alkyl chain length the yield of *n*-alkyl β-D-glucosides synthesis decreased sharply while the yield and rate of glucoside hydrolysis increased greatly. The equilibrium yield did not change after the addition of almond meal. Therefore, the final yield of alkyl β-D-glucosides was affected not by the residual activity of the biocatalyst but by the thermodynamic equilibrium.

Table 1 Thermodynamic data for the synthesis of alkyl-β-D-glucosides

Product	Yield (%)	K	ΔG_m^θ/(kJ/mol)
Methyl β-D-glucoside	27.6	1.03	–0.073
Ethyl β-D-glucoside	62.5	0.797	0.609
Propyl β-D-glucoside	58.7	0.685	1.02
Butyl β-D-glucoside	36.1	0.326	3.01
Pentyl β-D-glucoside	13.7	0.107	6.01
Hexyl β-D-glucoside	9.17	0.079	6.82
Heptyl β-D-glucoside	4.57	0.042	8.51
Octyl β-D-glucoside	4.05	0.041	8.58
Nonyl β-D-glucoside	2.92	0.033	9.20
Decyl β-D-glucoside	1.93	0.023	10.1

Yield = C_P/C_{G0}, where C_P is the final concentration of glucoside and C_{G0} is the initial concentration of glucose.

K is the equilibrium constants of ten *n*-alkyl β-D-glucosides; $K = C_P C_W/(C_G C_A)$, where C_P is the final concentration of glucoside, C_W is the final concentration of water, C_G is the final concentration of glucose, and C_A is the final concentration of *n*-alcohol.

$\Delta G_m^\theta = -RT\ln K$, where the temperature was 50 °C.

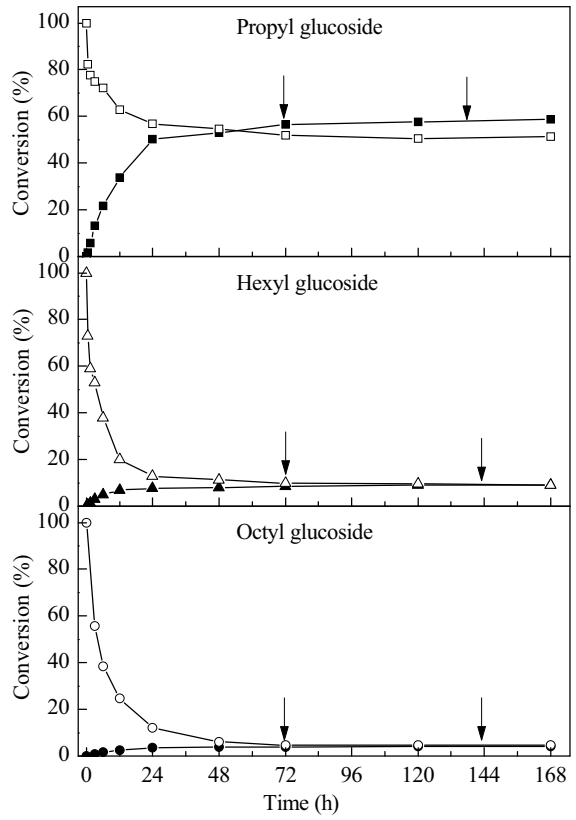


Fig. 2. Reaction profiles of glucoside hydrolysis and reverse hydrolysis glucoside synthesis. Open symbols represent the glucoside hydrolysis and the filled symbols represent the reverse hydrolysis while the arrowhead represents the extra addition of enzyme (30 mg each).

Moreover, the equilibrium constants and Gibbs free energy of ten *n*-alkyl *β*-D-glucosides were calculated and are shown in Table 1. The equilibrium constant decreased and the Gibbs free energy increased greatly when the alkyl chain became longer. The obtained values differed from the constants reported by Panintrarux et al. [29] as the enzymatic synthesis of alkyl glycosides in this study was carried out in monophasic systems instead of biphasic systems.

Longer alkyl chains resulted in lower final equilibrium yields and higher Gibbs free energies were required. To improve the final yield of the glucosides with long alkyl chains, the reaction system was optimized for the reduction of the Gibbs free energy.

2.3 Optimized synthesis of decyl glucoside with cross-linked almond meal

Water content is a critical factor that influences the activity of the catalyst and the equilibrium of the reaction [17–20]. At a higher water content (5%–15%, v/v), a faster initial rate was observed (Fig. 3). With further increase in water content (above 5%, v/v) the conversion of glucose decreased gradually. By optimizing the water content the equilibrium yield of decyl

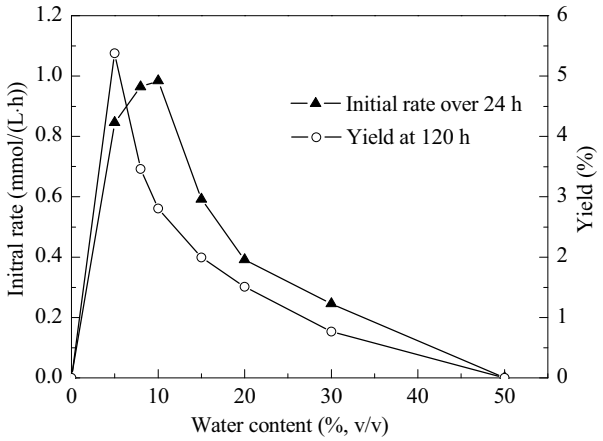


Fig. 3. Synthetic yield of decyl *β*-D-glucoside as a function of water content. Reaction conditions: immobilized biocatalyst 60 mg, reaction system 5 ml, glucose 0.25 mol/L, 50 °C.

β-D-glucoside increased significantly from 1.9% to 5.4% when the water content was reduced from 10% to 5% (v/v).

A cosolvent was added to the reaction system to decrease the amount of required alcohol and to increase the solubility of the hydrophobic substrate. Meanwhile, a suitable organic cosolvent should not affect the activity of the enzyme and should be easily removed and reused [8,22–24]. The influence of dioxane, *tert*-butanol, acetone, and acetonitrile on the reaction was investigated. The highest yield at 48 h was obtained in a *tert*-butanol system, as shown in Fig. 4.

The glucose concentration is also an important factor that affects the equilibrium of the reaction. Generally, in a reversible-hydrolysis synthesis reaction the product concentration increases with an increase in the glucose concentration [21]. However, the yield of decyl glucoside formation was the lowest at the highest glucose concentration (0.75 mol/L) used in this study (Fig. 5). The separate phase became more increas-

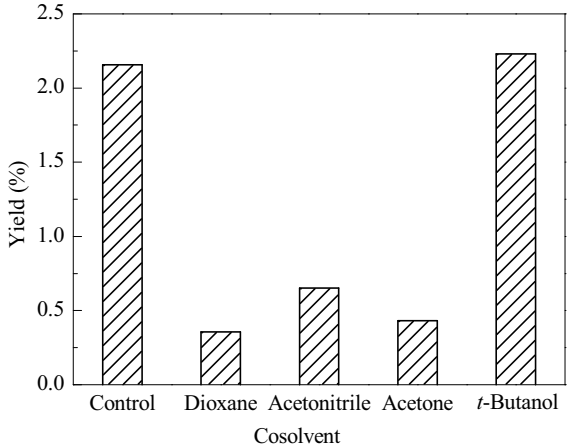


Fig. 4. Effect of various cosolvents on the enzymatic synthesis of decyl *β*-D-glucoside. Reaction conditions: cosolvent to decanol volume ratio 1:1, 0.25 mol/L glucose, 5% water, reaction system 5 ml, 50 °C, 48 h. The control represents the reaction system without any cosolvent.

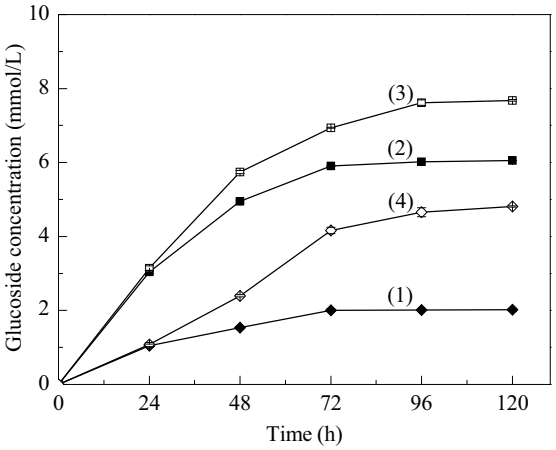


Fig. 5. Effect of glucose concentration on the enzymatic synthesis of decyl glucoside. Reactions were performed in a 20-ml water-decanol system with 5% water and 60 mg/ml cross-linked almond meal at 50 °C. Initial concentration of glucose (mol/L): (1) 0.05; (2) 0.1; (3) 0.25; (4) 0.75.

ingly viscous at higher glucose concentrations resulting in a poor distribution of the separate phase in the bulk organic phase. Therefore, a reduction in the reaction rate might be attributable to mass transfer resistance [21].

2.4 Surfactant properties of alkyl *β*-D-glucosides

The physical and chemical properties of glucosides with different chain lengths are shown in Table 2. The optical rotations of the glucosides were measured and we found that the ten *β*-D-glucosides were all levo-compounds. Alkyl glucosides with alkyl chains ranging from methyl to pentyl were water-soluble while the solubility of the hexyl to decyl glucosides was poor, and they were dissolved in dichloromethane to measure their optical rotations. The cmc of the commonly used surfactants ranged between 1 and 100 mmol/L. Only glucosides with long alkyl chains (> C_6) showed significant surface activity. The surface tension and cmc decreased greatly when the alkyl chain length increased. This report is the first to describe heptyl *β*-D-glucoside having significant surface activity in addition to octyl and decyl *β*-D-glucoside with longer alkyl chains. The cmc of heptyl *β*-D-glucoside is lower than that of many commonly used surfactants.

3 Conclusions

From a thermodynamic point of view, we investigated the equilibrium constant and Gibbs free energy of glucosides synthesis. The final yield of alkyl glucosides was not determined by the residual activity of the biocatalyst but by the thermodynamic equilibrium and the equilibrium yield was significantly affected by the alkyl chain length. Moreover, to enhance the synthesis yield of the glucoside containing a long alkyl chain we optimized the important factors that affect the equilibrium yield systematically. The surface activity of the alkyl glucosides was measured and we found that heptyl *β*-D-glucoside has significant surface activity.

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Table 2 Physical and chemical properties of glucosides with different chain lengths

Product	$[\alpha]_D^{28}$	Lit. $[\alpha]_D^{20}$	cmc[c] (mmol/L)	Lit. cmc (mmol/L)	ΔG_m^0/(kJ/mol)
Methyl *β*-D-glucoside	–30.0[a]	–33.5 [10]	—	—	—
Ethyl *β*-D-glucoside	–32.7[a]	–35.7 [30]	—	—	—
Propyl *β*-D-glucoside	–37.7[a]	–39.5 [31]	—	—	—
Butyl *β*-D-glucoside	–32.8[a]	–36.5 [18]	—	—	—
Pentyl *β*-D-glucoside	–48.4[a]	–35.5 [10]	—	—	—
Hexyl *β*-D-glucoside	–44.0[b]	–33.9 [18]	—	—	—
Heptyl *β*-D-glucoside	–40.0[b]	—	40	—	–7.89
Octyl *β*-D-glucoside	–30.2[b]	–31.7 [18]	20	23.0 [32]	–9.70
Nonyl *β*-D-glucoside	–34.8[b]	—	10	—	–11.4
Decyl *β*-D-glucoside	–38.7[b]	—	2	2.20 [32]	–15.4

[a]The glucosides were dissolved in water.
[b]The glucosides were dissolved in dichloromethane.
[c]The measurement temperature was 25 °C

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