JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Food and Beverage Chemistry/Biochemistry

Enzymatic Preparation of an Homologous Series of Long-chain 6-O-Acylglucose Esters and their Evaluation as Emulsifiers

Min-Yi Liang, Yongsheng Chen, Martin G Banwell, Yong Wang, and Ping Lan

J. Agric. Food Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jafc.8b00913 • Publication Date (Web): 30 Mar 2018

Just Accepted

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Enzymatic Preparation of an Homologous Series of Long-chain 6-*O*-Acylglucose Esters and their Evaluation as Emulsifiers

Min-Yi Liang[†,‡], Yongsheng Chen[†], Martin G. Banwell[§,#], Yong Wang*[†,‡], and Ping Lan*[†]

[†]Guangdong Saskatchewan Oil Seed Joint Laboratory, Department of Food Science and Engineering, Jinan University, Guangzhou, 510632, China

[‡]Guangdong Engineering Technology Research Center for Oils and Fats Biorefinery, Guangzhou 510632, China

[§]College of Pharmacy, Jinan University, Guangzhou, 510632, China

[#]Research School of Chemistry, Institute of Advanced Studies, The Australian National University, Canberra, ACT 2601, Australia

*Corresponding author (Telephone: +86-20-85221367. Fax: +86-20-85224766. E-mail: ping.lan@jnu.edu.cn)

ABSTRACT

Sugar fatty acid esters are non-ionic surfactants that are widely exploited in the food and cosmetics industries, as well as in the oral care and medical supply fields. Accordingly, new methods for their selective synthesis and the "tuning" of their emulsifying properties are of considerable interest. Herein we report simple and irreversible enzymatic esterifications of D-glucose with seven fatty acid vinyl esters. The foaming and emulsifying effects of the resulting 6-*O*-acylglucose esters were then evaluated. In accord with expectations, when the length of alkyl side chain associated with the 6-*O*-acylglucose esters increases then their HLB values decrease while the stabilities of the derived emulsions improve. In order to maintain good foaming properties alkyl side chains of at least 9 to 11 carbons in length are required. In the first such assays on 6-*O*-acylglucose esters, most of those described herein are shown to be non-toxic to the HepG2, MCF-7, LNacp, SW549, and LO-2 cell lines.

KEYWORDS: 6-*O*-acylglucose esters, foaming properties, emulsion stability, structure-function profiles

INTRODUCTION

Those sugar fatty acid esters used in industry are normally obtained by combining sustainably produced and inexpensive carbohydrates such as sucrose or glucose with lauric, palmitic or stearic acids or their corresponding low molecular weight esters via esterification or trans-esterification reactions, respectively. Long-chain fatty acid esters of sucrose, in particular, are high volume commodity chemicals and act as non-ionic surfactants with the sucrose moiety serving as a hydrophilic head group and the long-chain fatty acid residue as the lipophilic tail. The ensuing amphipathic properties mean such esters are especially important in the food and cosmetics industries as well as in the oral care and medical supply fields, not least because they exhibit good emulsifying properties while also being readily biodegradable and non-toxic.[1-3] Moreover, their functional characteristics can be "dialed up" over a wide range through the attachment of varying numbers and types of fatty acyl moieties to the sucrose core.[4]

Most of the sucrose esters on the market are currently manufactured by very traditional chemical techniques.[5] Given that there are eight non-equivalent hydroxyl groups associated with the sucrose molecule, the fundamental challenge associated with the required derivatization process arises from the often concurrent formation of mono-, di-, tri- and even higher-order esters.[6,7] None of the current industrial processes is particularly selective for the formation of any given sucrose ester, thus dramatically reducing the yield of the target compound(s) as well requiring the

application of purification regimes. Of course, the formation of by-products also creates waste steams and so further increasing the cost-of-goods.[8]

The deficiencies associated with the current methods for manufacturing sucrose esters have attracted considerable attention, particularly from academic laboratories. Manifold efforts have been made to improve the current methods of manufacture by focusing on, for example, alternate acyl donors[7] and reaction solvents,[9,10] new modes of promoting reaction (including through ultrasonic irradiation),[11] and the use of biological catalysts (enzymes).[12-16] The main difficulties encountered in attempts to improve the yields of enzymatic couplings arises from the low activity of the biological catalysts in polar solvents such as DMSO and DMF that are required to dissolve the carbohydrates.[13] To prevent enzyme deactivation it has been suggested that these highly polar solvents are doped with alcoholic ones such as 2-butanol[12] or *t*-amyl alcohol[1,14] that serve as adjuvants. Increasing the concentration of sucrose in the reaction medium so as to improve throughput whilst retaining the activity of the enzyme represents another challenge. The highest sucrose loading successfully deployed to date is 0.7 M (in DMSO) although a "pre-treated" lipase was required to establish an effective process but thereby delivering sucrose mono-caprate at useful scale and rate.[13] The reaction times required for the enzymatic generation of sucrose esters has reportedly ranged from 6 h to several days, with the longer periods representing a major drawback for industrial development.[15,16]

While there have been extensive studies on the enzyme-mediated synthesis of sucrose monoesters, the corresponding glucose-derived systems are much less studied.

Compared to sucrose, glucose displays higher regioselectivity in the trans-esterification process since there is only one primary hydroxyl group present, whereas there are three in sucrose.[17] Most efforts to prepare glucose fatty acid esters have focused on enzymatic approaches, since these offer the possibility of employing mild reaction conditions and could, in principle at least, proceed at high volume and with excellent regioselectivities. A range of commercially available and immobilized lipases, most notably Novozym435, Lipozyme RMIM and Lipozyme LIM, has been used to catalyze the regioselective acylation of glucose with various series of long-chain fatty acids.[15-20] Most of these delivered the 6-*O*-acylglucose ester as the major product. For instance, Ren *et al* reported[17] enzymatic syntheses of the 6-*O*-palmitoyl-, 6-*O*-lauroyl- and 6-*O*-hexanoyl-glucose esters in DMSO and *t*-amyl alcohol media using the corresponding fatty acids as the acyl donor. Significantly, a stand-alone but highly efficient Novozym435-catalyzed synthesis of 6-*O*-myristoylglucopyranose was achieved by Davis *et al*[20] using the relevant enol ester as the acyl donor and a mixture of THF/pyridine as the solvent system. Surprisingly, this attractive process does not appear to have been deployed more extensively even though it should be capable of delivering a series of such glucose mono-esters and so allowing for the systematic study of the impacts of varying the fatty acid chain on the emulsification properties of this monosaccharide. Accordingly, the aims of the current study were to undertake an in-depth investigation of the structure-function profiles of an homologous series 6-*O*-acylglucose esters and

thereby gain insights into their potential utility in the food industry as, for example, emulsifiers.

MATERIALS AND METHODS

Materials. D-glucose (anhydrous, 99.0%), pyridine, tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF), benzene, *t*-amyl alcohol, *t*-BuOH, Span 20, Span 40, Span 60, Span 80, Tween 20 and Tween 80 were purchased from the Energy Chemical Co., Ltd (Shanghai, China). Vinyl hexanoate (>99.0%), vinyl *n*-octanoate (>99.0%), vinyl decanoate (>99.0%), vinyl laurate (>99.0%), vinyl myristate (>99.0%), vinyl palmitate (>96.0%) and vinyl stearate (>95.0%) were purchased from the Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan) while Novozym435 was supplied by Sigma-Aldrich (St. Louis, MO). Pure canola oil was purchased from a local grocery store while commercial sucrose stearic esters S-570 and S-770 were obtained from the Mitsubishi-Chemical Foods Corporation (Tokyo, Japan). Cell culture media were purchased from the Gibco U.S. Biotechnology Co. (Waltham, MA) while foetal bovine serum (FBS) was obtained from the Tianhang Biotech Co. Inc. (Zhejiang, China).

General Protocols. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded at 25 °C on a Bruker spectrometer (Billerica, MA) operating at 500 MHz for proton and 125 MHz for carbon nuclei. For ^{1}H NMR spectra, the residual protio-solvent signals were used as internal standards. Low-resolution ESI mass spectra were recorded on an Agilent single-quadrupole liquid chromatograph-mass spectrometer (Santa Clara, CA).

Synthesis of the 6-*O*-Acylglucose Esters. Enzymatic syntheses of the seven 6-*O*-acylglucose esters were conducted (Figure 1) using modifications of a previously reported method.[20] In brief, commercially available Novozym435 was used as the catalyst while seven long alkyl chain (5 to 17 carbons) fatty acid vinyl esters, namely vinyl hexanoate, vinyl octanoate, vinyl decanoate, vinyl laurate, vinyl myristate, vinyl palmitate and vinyl stearate, were selected as the acyl donors. The use of vinyl esters has proven advantageous since, in each instance, the trans-esterification reactions are irreversible and the by-product is acetaldehyde that acts as an internal water scavenger, and so thus promoting the desired transformation.[20] THF/pyridine (4:1 v/v) served as the reaction solvent and allowed for effective solubilization of the free glucose without adversely impacting on the activity of the enzyme. Under such conditions, complete reaction was achieved within 48 h on multi-gram scales and delivered each 6-*O*-acylglucose ester in a completely regioselective manner. The products were isolated by trituration with diethyl ether, affording the target compounds in high purity, as determined by NMR spectroscopic analysis. A typical protocol was as follows:

A mixture of anhydrous D-glucose (1.00 g, 5.6 mmol) and THF/pyridine (40 mL, 4:1 v/v) contained in a 100 mL round-bottom flask was subject to sonication and once dissolution had taken place (*ca*. 5 min), Novozym435 (50 to 200 mg) was added along with the relevant fatty acid vinyl ester (3.0 equiv., 16.8 mmol). The ensuing mixture was stirred magnetically at 40 °C until all the glucose had been consumed (normally 24 to 48 h) as determined by TLC analysis. The ensuing solution was decanted from the bulk of the solid residue and the residual enzyme removed by filtration and the

solid thus retained washed with methanol (2 x 10 mL) then dichloromethane (1 x 10 mL). The combined filtrates were dried (Na_2SO_4), filtered then concentrated under reduced pressure to afford a yellow solid, trituration of which (with 100 mL of diethyl ether) followed by suction filtration gave the corresponding 6-*O*-acylglucose ester **2-8** as an amorphous white solid in high yield. The optimization of the reaction through varying the enzyme loading, the solvent(s) and/or reaction times is detailed in Table 1.

6-*O*-Hexanoylglucopyranose, **2.** Amorphous white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.34 (d, J = 4.7 Hz, 1H, 1-OH), 5.03 (d, J = 5.7 Hz, 1H, 4-OH), 4.90 (app. t, J = 4.5 Hz, 1H, H1), 4.75 (d, J = 4.8 Hz, 1H, 3-OH), 4.53 (d, J = 6.7 Hz, 1H, 2-OH), 4.27 (dd, J = 11.6 and 1.6 Hz, 1H, H6′), 3.99 (dd, J = 11.6 and 6.3 Hz, 1H, H6), 3.76 (ddd, J = 9.9, 6.1 and 2.0 Hz, 1H, H5), 3.43 (td, J = 9.1 and 4.8 Hz, 1H, H3), 3.12 (m, 1H, H2), 3.03 (m, 1H, H4), 2.27 (td, J = 7.3 and 2.2 Hz, 2H), 1.52 (m, 2H), 1.26 (m, 4H), 0.86 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.4, 92.8 (C1), 73.3 (C3), 72.6 (C2), 71.0 (C4), 69.6 (C5), 64.3 (C6), 33.9, 31.1, 24.6, 22.3, 14.3. MS (ESI, +ve): *m/z* 279 ($[M+H]^+$, 20%), 296 ($[M+H_2O]^+$, 70), 301 ($[M+Na]^+$, 100).

6-*O*-Octanoylglucopyranose, **3.** Amorphous white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.33 (d, J = 4.7 Hz, 1H, 1-OH), 5.03 (d, J = 5.7 Hz, 1H, 4-OH), 4.90 (app. t, J = 4.5 Hz, 1H, H1), 4.74 (d, J = 4.8 Hz, 1H, 3-OH), 4.52 (d, J = 6.7 Hz, 1H, 2-OH), 4.26 (dd, J = 11.7 and 2.0 Hz, 1H, H6′), 3.99 (dd, J = 11.7 and 6.2 Hz, 1H, H6), 3.76 (ddd, J = 10.1, 6.2 and 2.0 Hz, 1H, H5), 3.42 (td, J = 9.1 and 4.9 Hz, 1H, H3), 3.12 (m, 1H, H2), 3.03 (m, 1H, H4), 2.27 (td, J = 7.3 and 2.3 Hz, 2H), 1.52 (m,

2H), 1.26 (m, 8H), 0.86 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.4, 92.8 (C1), 73.3 (C3), 72.7 (C2), 71.0 (C4), 69.6 (C5), 64.3 (C6), 33.9, 31.6, 28.9, 28.8, 24.9, 22.5, 14.4. MS (ESI, +ve): *m/z* 307 ($[M+H]^+$, 10%), 324 ($[M+H_2O]^+$, 100), 329 ($[M+Na]^+$, 50).

6-*O*-Decanoylglucopyranose, **4.** Amorphous white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.34 (d, J = 5.2 Hz, 1H, 1-OH), 5.03 (d, J = 5.7 Hz, 1H, 4-OH), 4.90 (m, 1H, H1), 4.75 (d, J = 4.8 Hz, 1H, 3-OH), 4.52 (d, J = 6.7 Hz, 1H, 2-OH), 4.26 (dd, J = 11.7 and 2.0 Hz, 1H, H6′), 3.99 (dd, J = 11.7 and 6.3 Hz, 1H, H6), 3.76 (ddd, J = 10.1, 6.3 and 2.0 Hz, 1H, H5), 3.43 (td, J = 9.2 and 4.8 Hz, 1H, H3), 3.12 (m, 1H, H2), 3.04 (m, 1H, H4), 2.27 (m, 2H), 1.52 (m, 2H), 1.26 (m, 12H), 0.86 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.4, 92.8 (C1), 73.3 (C3), 72.7 (C2), 71.0 (C4), 69.6 (C5), 64.3 (C6), 33.9, 31.7, 29.3, 29.2, 29.1, 28.9, 24.9, 22.6, 14.4. MS (ESI, +ve): *m/z* 335 ($[M+H]^+$, 10%), 352 ($[M+H_2O]^+$, 100), 357 ($[M+Na]^+$, 50).

6-*O*-Lauroylglucopyranose, **5.** Amorphous white solid. ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.35 (d, J = 4.3 Hz, 1H, 1-OH), 5.05 (d, J = 5.7 Hz, 1H, 4-OH), 4.95 (m, J = 4.7 Hz, 1H, H1), 4.76 (d, J = 4.8 Hz, 1H, 3-OH), 4.54 (d, J = 6.7 Hz, 1H, 2-OH), 4.26 (m, 1H, H6′), 3.99 (m, 1H, H6), 3.76 (m, 1H, H5), 3.43 (m, 1H, H3), 3.16 (m, 1H, H2), 3.04 (m, 1H, H4), 2.27 (m, 2H), 1.52 (m, 2H), 1.26 (m, 16H), 0.86 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.4, 92.8 (C1), 73.9 (C3), 72.7 (C2), 71.0 (C4), 69.6 (C5), 64.4 (C6), 33.9, 31.8, 29.5, 29.4, 29.2, 29.1, 28.9, 24.9(4), 24.9(2), 22.6, 14.4. MS (ESI, +ve): *m/z* 363 ($[M+H]^+$, 40%), 380 ($[M+H_2O]^+$, 50), 385 ($[M+Na]^+$, 100).

185 **6-*O*-Myristoylglucopyranose**, **6.** Amorphous white solid. ^{1}H NMR (500 MHz,
186 DMSO-d_6) δ. 6.34 (broad s, 1H, 1-OH), 5.03 (broad s, 1H, 4-OH), 4.89 (m, 1H, H1),
187 4.30 (d, J = 8.8 Hz, 1H, 2-OH), 4.27 (d, J = 11.6 Hz, 1H, H6′), 3.99 (dd, J = 11.6 and
188 6.3 Hz, 1H, H6), 3.76 (m, 1H, H5), 3.43 (t, J = 9.1, 1H, H3), 3.12 (m, 1H, H2), 3.04
189 (m, 1H, H4), 2.27 (m, 2H), 1.52 (m, 2H), 1.26 (m, 2-OH), 0.86 (t, J = 7.0 Hz, 3H). ^{13}C
190 NMR (125 MHz, DMSO-d_6) δ 173.4, 92.8 (C1), 75.2 (C3), 73.3 (C2), 71.0 (C4), 69.6
191 (C5), 64.3 (C6), 33.9, 31.8, 29.5(3), 29.5(0), 29.4(8), 29.4(7), 29.3(7), 29.2, 29.1, 28.9,
192 24.9, 22.6, 14.4. MS (ESI, +ve): *m/z* 391 ($[M+H]^+$, 30%), 408 ($[M+H_2O]^+$, 50), 413
193 ($[M+Na]^+$, 70), 647 (100).

194 **6-*O*-Palmitoylglucopyranose**, **7.** Amorphous white solid. ^{1}H NMR (500 MHz,
195 DMSO-d_6) δ 6.34 (d, J = 4.3 Hz, 1H, 1-OH), 5.03 (d, J = 5.7 Hz, 1H, 4-OH), 4.90 (t, J
196 = 4.3 Hz, 1H, H1), 4.75 (d, J = 4.8 Hz, 1H, 3-OH), 4.53 (d, J = 6.7 Hz, 1H, 2-OH),
197 4.27 (dd, J = 11.7 and 2.0 Hz, 1H, H6′), 3.99 (dd, J = 11.7 and 6.2 Hz, 1H, H6), 3.76
198 (ddd, J = 10.0, 6.2 and 2.0 Hz, 1H, H5), 3.43 (td, J = 9.2 and 4.6 Hz, 1H, H3), 3.13
199 (ddd, J = 9.9, 6.5 and 3.5 Hz, 1H, H2), 3.04 (m, 1H, H4), 2.27 (m, 2H), 1.52 (m, 2H),
200 1.26 (m, 24H), 0.86 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.4, 92.8
201 (C1), 73.3 (C3), 72.7 (C2), 71.0 (C4), 69.6 (C5), 64.3 (C6), 33.9, 31.8, 29.5 (x5), 29.4,
202 29.3, 29.2, 29.1, 28.9, 24.9, 22.6, 14.4. MS (ESI, +ve): *m/z* 419 ($[M+H]^+$, 70%), 436
203 ($[M+H_2O]^+$, 30), 441 ($[M+Na]^+$, 100).

204 **6-*O*-Stearoylglucopyranose**, **8.** Amorphous white solid. ^{1}H NMR (500 MHz,
205 DMSO-d_6) δ 6.34 (d, J = 5.3 Hz, 1H, 1-OH), 5.03 (d, J = 5.7 Hz, 1H, 4-OH), 4.90 (dd,
206 J = 5.3 and 3.4 Hz, 1H, H1), 4.76 (d, J = 4.8 Hz, 1H, 3-OH), 4.53 (d, J = 6.7 Hz, 1H,

2-OH), 4.27 (dd, J = 11.7 and 2.0 Hz, 1H, H6′), 3.99 (dd, J = 11.7 and 6.2 Hz, 1H, H6), 3.76 (ddd, J = 10.0, 6.2 and 2.0 Hz, 1H, H5), 3.43 (td, J = 9.2 and 4.7 Hz, 1H, H3), 3.13 (ddd, J = 9.9, 7.0 and 3.5 Hz, 1H, H2), 3.04 (ddd, J = 10.1, 8.7 and 5.5 Hz, 1H, H4), 2.27 (m, 2H), 1.52 (m, 2H), 1.26 (m, 28H), 0.86 (t, J = 7.0 Hz, 3H). ^{13}C NMR (125 MHz, DMSO-d_6) δ 173.3, 92.7 (C1), 73.3 (C3), 72.7 (C2), 71.0 (C4), 69.6 (C5), 64.4 (C6), 33.9, 31.8, 29.5(3) (x7), 29.5(0), 29.4, 29.2, 29.1, 28.9, 24.9, 22.6, 14.4. MS (ESI, +ve): *m/z* 447 ($[M+H]^+$, 10%), 469 ($[M+Na]^+$, 70), 758 (100).

Determination of Hydrophilic-Lipophilic Balance (HLB) Values. The HLB values of the seven products obtained as detailed above, namely esters **2**-**8**, were determined experimentally using the water number method.[21] Thus, 100 mg of each compound was dissolved in DMF/benzene (40 mL, 100:5 v/v). The resulting solution was titrated with distilled water while being maintained at 20 ±1 °C until a permanent turbidity was obtained. All results were reproducible to within 0.5 units of HLB. A plot of consumed water number vs HLB was then established using emulsifiers with known HLB values, including Span 80 (HLB = 4.3), Span 60 (HLB = 4.8), Span 40 (HLB = 6.7), Span 20 (HLB = 8.6), Tween 80 (HLB = 15.6) and Tween 20 (HLB = 16.7), for calibration purposes. The HLB values of 6-*O*-acylglucose esters **2**-**8**, as well as the commercial sucrose stearic esters S-570 and S-770, were then read from this plot. All the experiments were performed in triplicate.

Evaluation of Foamability and Foam Stability. Evaluation of the foamability and foam stability of esters **2**-**8** together with controls S-570, S-770, Span20 and Span80 were undertaken using a previously described method.[22,23] Specifically, 10 mL

of 0.1%, 0.2% and 0.5% w/w aqueous dispersions of each sample were placed in a 50 mL, flat-bottomed measuring cylinder and the height of solution (H_1) was measured before being homogenized, using a blender, at 3000 rpm for 2 min. The initial foam height (H_2) was measured immediately. Following resting periods of 10, 20, and 30 min, the foam heights (H_3) were then recorded. All the experiments were performed in triplicate. The foamability and foaming stabilities of these compounds were calculated using the following equations:

Foamability (%) = $H_2/ H_1 \times 100\%$

Foam stability (%) = $H_3/ H_2 \times 100\%$

Determination of Emulsion Stability Index. The emulsifying stability indices of esters **2-8** as well as the four controls were determined using minor modifications of literature protocols.[17,24] Specifically, 10.0 g of 0.1%, 0.2% and 0.5% w/w aqueous solutions of the test compounds were each mixed with 1.0 g of canola oil while a solution lacking a test material was used as the control. The ensuing mixtures were homogenized for 2 min at 20,000 rpm using a blender and a 20 μL sample of the resulting emulsion was diluted with 2 mL deionized water before being drawn into a clean spectrometric cuvette. The absorbance of the emulsion at 500 nm was measured at 0 min and 20 min. All the experiments were performed in triplicate. The emulsion stability indices (ESI) was calculated using the following equation:

ESI (%) = $A_0 \times 20/[A_0 - A_{20}] \times 100\%$

where A_0 and A_{20} are the absorbances obtained at 0 min and 20 min.

Evaluation of Cytotoxicities. In order to determine the biosafety of the 6-*O*-acylglucose esters **2**-**8** at the cellular level, their cytotoxicities were measured. The methylene blue method of analysis was employed with some modifications. Specifically, cells were seeded at 4×10^4/well on a 96-well plate in 100 μL of complete medium (DMEM+ 5% FBS, 50 units/mL penicillin, 50 μg /mL streptomycin, 100 μg /mL gentamicin). After being incubated for 24 h at 37 °C, the medium was removed and the cells were washed once with 100 μL of sterile and cold phosphate buffer solution (PBS, 0.01M, pH7.4). Free FBS medium containing the test material was then added to the well while the same medium lacking any test compound served as the control. After an additional 24 h of incubation, the medium was removed and the wells were washed with PBS. 50 μL of methylene blue (Hank's balanced salt solution + 1.25% glutaraldehyde + 0.6% methylene blue) was added to each well, then the cells were incubated for 1 h at 37 °C in 5% CO_2. After incubation, the staining agent was removed and the cells were washed six times with deionized water then elution buffer (100 μL) was added to each well. The plates were placed on a table oscillator for 20 min, and absorbance was measured at 570 nm using a microplate reader. Concentrations of samples that decrease the absorbance by >10% when compared to the blank control were considered to be cytotoxic. All the experiments were performed in triplicate.

Statistical Analysis. Each evaluation experiment was performed three times. The results are presented as the mean values ± standard deviation (SD). Statistical analyses were performed using SPSS software (SPSS, Inc., Chicago, IL) to identify any

significant differences. All values were assessed by one-way analysis of variance (ANOVA) in conjunction with Duncan's new multiple-range test (MRT).

RESULTS AND DISCUSSION

Syntheses and Characterization of Products. The optimization of the enzymatic acylation of glucose at C-6 was explored by varying the enzyme loading, the solvent(s) and/or reaction times. In each instance, three equivalents of the relevant fatty acid vinyl ester were used as the acyl donor. As shown in Table 1, when 5% w/w of the enzyme was used in anhydrous THF/pyridine then good to excellent yields of the target compound were obtained within 24 h. When DMSO/*t*-BuOH, was used as the solvent system then yields ranging from 47-57% were observed and 48 h was required for reactions to go to completion. Similar trends were observed in the DMSO/*t*-amyl alcohol solvent system (entry 4). Higher enzyme loadings (entry 3) only resulted in marginally higher yields. The reaction seemed to be particularly water sensitive, as lower yields (entry 5) were obtained when undried/reagent-grade THF/pyridine was used. Among the seven vinyl esters, the laurate and myristate-based systems exhibited the best reactivity and productivity.

The structures of all products **2**-**8** were confirmed by undertaking MS spectrometric and NMR spectroscopic analyses, including those arising from the outcomes of ^{1}H-^{1}H COSY and HMBC experiments. In all instances, two sets of chemical shifts were observed in the NMR spectra, reflecting the presence of both the α- and β-anomeric forms of the product monosaccharide.[17] With the exception of 6-*O*-lauroylglucose, the former anomer predominated. Key elements of the ^{1}H-^{1}H

294 COSY and HMBC correlation spectra derived from 6-*O*-palmitoylglucopyranose are
295 shown in Figure 2. These are representative of the series as a whole. As revealed
296 through HMBC experiments, the protons at C-6 of the pyranose ring interact with the
297 carbonyl carbon of the ester moiety and thus establishing the site of esterification. The
298 ^{1}H-^{1}H COSY data allowed for the assignment of the resonances due to the
299 oxymethine protons as well as those arising from the side-chain protons.

300 The protocols defined here are robust and offer the capacity to generate a
301 significant number of variants of the title 6-*O*-acylglucose esters in high yields.
302 Furthermore, they are capable of producing such esters in a completely regioselective
303 fashion and at multi-gram scale. Additionally, product purification is simple and
304 efficient.

305 **Hydrophile-Lipophile Balance (HLB) Values.** The hydrophilic-lipophilic
306 balance (HLB) represents one of the standard means for emulsifier classification and
307 selection.[25] This value is a measure of the degree to which a potential emulsifier is
308 hydrophilic (high HLB values are above 11) or lipophilic (low HLB values are below
309 9). Those in the range 9–11 are considered intermediate.[26] The HLB values of each
310 product derived by the water number method are shown in Figure 3 and reveal that as
311 the length of the hydrophobic side-chain increases from esters **2**-**8**, the HLB values
312 decrease from 9.0 to 5.7. Since the potential utility of emulsifiers is often defined
313 using the HLB system, these values suggest that esters **2**-**8** could have practical
314 applications. Based on the Griffin method[26,27] of predicting surfactant properties,
315 esters **2** and **3** have the potential to act as oil-in-water emulsifiers (HLB >8), while

esters **4**-**8** might be used as water-in-oil emulsifiers (HLB <8). Compounds **2**-**5** could also be expected to serve as wetting and spreading agents.

Foaming Properties. Surfactants stabilize the foams by preventing bubble coalescence.[28-30] Given their significance, foams are generally described in terms of their "foamability", which is defined as the capacity of the surfactants to form foams. The stability of a foam, which tracks variations in foam height or volume over time, is first monitored immediately after foam generation.[22] The issues of foamability and foam stability are vital for food emulsifiers since certain foods such as ice cream, Chantilly cream, mousses and marshmallow need to possess good shelf-lives and maintain their appearance even when subject to variations in storage conditions.[31,32] The foaming properties of each product, as well as the four controls, were evaluated in terms of their "foamability" at different concentrations and the outcomes of such studies are shown in Figure 4. Unsurprisingly, foamability is positively correlated with sample concentration. At all three concentrations, ester **2** displayed the weakest foamability even though it had the highest HLB value. Compound **3** showed superior properties but these were no better than those of the four controls. When the length of alkyl side chain increased to 9 carbons (as seen in **4**), the highest foamability was observed (39.1% at 0.5% w/w). Indeed, ester **4** had remarkably better foamability than the controls at the same concentrations. Nevertheless, it is clear that foamability begins to decrease when products with longer (>9 carbons) alkyl side chains are involved. Although ester **5** exhibited a slightly higher foamability than those of controls under all concentrations tested, ester **6** was only comparable to such controls

at high concentrations (29.4% at 0.5%). When the alkyl side chains were increased to 15 carbons (as in **7**) or 17 carbons (as in **8**), their foamabilities were lower than most of the other samples. These results are rather counterintuitive since there appears to be no correlation between the HLB values and foamability. The inverted V-type profile revealed in Figure 4 is also intriguing in that while esters **2**, **3**, S-570, S-770 and Span20 are regarded as more hydrophilic than compounds **4** and **5**, the last pair exhibited the best foamability. Presumably these outcomes are due to the differing surface tensions of the compounds. Those with higher surface tensions, such as compounds **2**, **3**, **7**, **8**, are less able to increase surface area to facilitate foam generation and, thus, display poorer foamability.[33]

Foaming Stability. The foaming stability (FS) of esters **2**-**8** as well as the four controls were measured over 10 min at 0.1%, 0.2%, and 0.5% w/w concentrations and the outcomes of the relevant experiments are shown in Figure 5A. While ester **2** failed to give any meaningful data, the FS of all the remaining esters increased with concentration although the differences were very modest, if detectable, at the 0.2% and 0.5% concentration levels. As was the case with the studies described above, an inverted V-shaped profile was observed in moving through the full series of glucose esters. For esters **3**-**5**, their FSs increased steadily from 30% through to 97.5% and thus indicating the benefits of longer side chains in promoting foaming stability. In contrast, with esters **6**-**8** the increasing length of the respective side-chains significantly reduced the capacity to maintain foams. Since foaming stability is influenced by standing time, these were determined for all samples at 0.2% w/w

concentration over 10, 20, 30 min. The outcomes of the relevant set of experiments are shown in Figure 5B and reveal that the FSs for all esters decreased significantly between the standing times of 10 and 30 min. At the 30 min mark the foams generated using both esters **3** and **8** disappeared completely, indicating the poor stability of the derived foaming systems. In contrast, the four commercial controls exhibited excellent stability over time.

Emulsion Stability. Emulsion stability refers to the ability of an emulsion to resist change in its properties over time.[34] Such stability is highly dependent upon liquid droplet size and distribution, emulsion processing conditions, the emulsifiers' physical characteristics, solvents, the phase volume ratio and continuous phase viscosity.[35] The ability to form stable emulsions is a classic characteristic of good surfactants.[33] The emulsifying stability index of an emulsifier provides a measure of stability, over a defined period of time, for the emulsion. Accordingly, the emulsion stability indices (ESIs) of each product, as well as the four controls, were determined at 0.1%, 0.2%, and 0.5% w/w concentrations in an oil-in-water system. The data so obtained are shown in Figure 6 and indicate that in each instance the test samples displayed higher ESIs than the blank control and reveal that there is a good correlation between the length of side chain and the ESI. In all instances, the ESIs were proportional to concentration. Among the seven glucose esters, congener **2** showed the lowest ESIs. A relatively high ESI (421.1 ± 12.9) was seen when ester **8** was tested at low concentration (0.1% w/w) and this rose to 919.3 ± 34.7 at 0.5% w/w, thus representing the highest ESI value observed. This profile could be attributed to the

stronger hydrophobic interactions between the longer alkyl side chains in independent molecules of this form and with the oil droplets thus assuming a more compact structure and so resulting in higher emulsion stability.[17] The ESIs of esters **2**-**8** are significantly lower than those of the four controls at every concentration. This is consistent with a previous study[17] that revealed, after measuring the diameters of the oil droplets in emulsions derived from two glucose esters, that unlike the sucrose esters, the former delayed but did not ultimately prevent the coalescence of oil-in-water emulsions.

Cytotoxicity. Food emulsifiers must not only possess suitable functional properties but, above all else, must be non-toxic.[36] Sugar fatty acid esters are generally accepted as safe emulsifiers in the food and cosmetics industries because they are converted into harmless carbohydrates and fatty acids in the human stomach. However, the cellular toxicities (cytotoxicities) of the glucose fatty acid esters have not yet been established. In the present study, the cytotoxicities of the title esters were evaluated against four cancer cell lines, specifically HepG2 (a liver cancer cell line), MCF-7 (a breast cancer cell line), LNacp (a prostate cancer cell line) and SW549 (a thyroid cancer cell line) as well as LO-2 (a normal liver cell line). As shown in Table 2, all seven 6-*O*-acylglucose esters were devoid of significant cytotoxic effects. Specifically, ester **4** showed weak cytotoxic activity towards all the cell lines employed, the concentration required to inhibit cell proliferation at the 10% level ranging from 210 to 600 μmol/L. Ester **8** displayed weak inhibitory activity toward SW549 but was inactive against the other four cell lines. The remaining esters had no

notable cytotoxicity towards any of the five cell lines. Therefore, esters **2**, **3**, **5**, **6**, **7** can be regarded as non-toxic toward all of the cell lines screened in this study.

The structure-function profile of the 6-*O*-acylglucose esters, as established through this study, can be summarized as follows: when the length of alkyl side chain of the 6-*O*-acylglucose esters increases, their HLB values decrease, while, contrarily, the stabilities of the derived emulsions increase. To have good foaming properties, a 9 or 11 carbon alkyl side chain is required. The presence of a side chain with less or more carbons than these decreases the foaming properties. The protocols and data presented here should provide an important reference point for further studies on the preparation and evaluation of fatty acid esters derived from di- and higher order-saccharides.

ASSOCIATED CONTENT

Supporting Information

^{1}H and ^{13}C NMR Spectra of compounds **2**-**8**, HMBC, ^{1}H-^{1}H COSY spectra of compound **7**. This material is available free of charge via the internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Telephone: +86-20-85221367. Fax: +86-20-85224766.

E-mail: twyong@jnu.edu.cn (Y. W.)

E-mail: ping.lan@jnu.edu.cn (P. L.)

Funding

We gratefully acknowledge financial support from the National Key Research and Development Program of China under grant 2017YFD0400200, and the Department of Science and Technology of Guangdong Province under grants 2017B090907018 and 2014A010107014 as well as the Fundamental Research Funds for the Central Universities, China.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Ferrer, M.; Cruces, M. A.; Bernabe, M.; Ballesteros, A.; Plou, F. J. Lipase-catalyzed regioselective acylation of sucrose in two-solvent mixture. *Biotechnol. Bioeng.* **1999**, *65*, 10–16.

(2) Yang, Z.; Huang, Z. L. Enzymatic synthesis of sugar fatty acid esters in ionic liquids. *Catal. Sci. Technol.* **2012**, *2*, 1767–1775.

(3) Polat, T.; Linhardt, R. J. Syntheses and applications of sucrose-based esters. *J. Surfactants Deterg.* **2001**, *4*, 415−421.

(4) Kobayashi, T. Lipase-catalyzed syntheses of sugar esters in non-aqueous media. *Biotechnol. Lett.* **2011**, *33*, 1911–1919.

(5) Zhao, R.; Chang, Z.; Jin, Q.; Li, W.; Dong, B.; Miao, X. Heterogeneous base catalytic transesterification synthesis of sucrose ester and parallel reaction control. *Int. J. Food Sci. Technol.* **2014**, *49*, 854–860.

(6) Yan, Y.; Bornscheuer, U. T.; Stadler, G.; Lutz-Wahl, S.; Reuss, M.; Schmid, R. D. Production of sugar fatty acid esters by enzymatic esterification in a stirred-tank

membrane reactor: optimization of parameters by response surface methology. *J. Am. Oil. Chem. Soc.* **2001**, *78*, 147–152.

(7) Cruces, M. A.; Plou, F. J.; Ferrer, M.; Bernabé, M.; Ballesteros, A. Improved synthesis of sucrose fatty acid monoesters. *J. Am. Oil Chem. Soc.* **2001**, *78*, 541–546.

(8) Khan, R. Chemistry and new uses of sucrose: how important? *Pure Appl. Chem.* **1984**, *56*, 833–844.

(9) Park, S.; Kazlauskas, R. J. Improved preparation and use of room-temperature ionic liquids in lipase-catalyzed enantio- and regio-selective acylations. *J. Org. Chem.* **2001**, *66*, 8395–8401.

(10) Liu, Q.; Janssen, M. H. A.; van Rantwijk, F.; Sheldon, R. A. Room-temperature ionic liquids that dissolve carbohydrates in high concentrations. *Green Chem*. **2005**, *7*, 39–42.

(11) Huang, D.; Jiang, X.; Zhu, H.; Fu, X.; Zhong, K.; Gao, W. Improved synthesis of sucrose fatty acid monoesters under ultrasonic irradiation. *Ultrason. Sonochem.* **2010**, *17*, 352–355.

(12) Zhong, X.; Qian, J.; Liu, M.; Ma, L. *Candida rugosa* lipase catalyzed synthesis of sucrose-6-acetate in a 2-butanol/buffer two phase system. *Eng. Life Sci*. **2013**, *13*, 563–571.

(13) Inprakhon, P.; Wongthongdee, N.; Amornsakchai, T.; Pongtharankul, T.; Sunintaboon, P.; Wiemann, L. O.; Durand, A.; Sieber, V. Lipase-catalyzed synthesis of sucrose monoester: increased productivity by combining enzyme

pretreatment and non-aqueous biphasic medium. *J. Biotechnol.* **2017**, *259*, 182–190.

(14) Plou, F. J.; Cruces, M. A.; Ferrer, M.; Fuentes, G.; Pastor, E.; Bernabé, M.; Christensen, M.; Comelles, F.; Parra, J. L.; Ballesteros, A. Enzymatic acylation of di- and tri-saccharides with fatty acids: choosing the appropriate enzyme, support and solvent. *J. Biotechnol*. **2002**, *96*,55-66.

(15) Habulin, M.; Šabeder, S.; Knez, Ž. Enzymatic synthesis of sugar fatty acid esters in organic solvents and in supercritical carbon dioxide and their antimicrobial activity. *J. Supercrit. Fluids* **2008**, *45*, 338–345.

(16) Shi, Y. G.; Li, J. R.; Chu, Y. H. Enzyme-catalyzed regioselective synthesis of sucrose-based esters. *J. Chem. Technol. Biotechnol.* **2011**, *86*, 1457–1468.

(17) Ren, K.; Lamsal, B. P. Synthesis of some glucose-fatty acid esters by lipase from *Candida antarctica* and their emulsion functions. *Food Chem.* **2017**, *214*, 556–563.

(18) Ganske, F.; Bornscheuer, U. T. Optimization of lipase-catalyzed glucose fatty acid ester synthesis in a two-phase system containing ionic liquids and *t*-BuOH. *J. Mol. Catal. B: Enzym.* **2005**, *36*, 40–42.

(19) Poehnlein, M.; Ulrich, J.; Kirschhoefer, F.; Nusser, M.; Muhle-Goll, C.; Kannengiesser, B.; Brenner-Weiss, G.; Luy, B.; Liese, A.; Syldatk, C.; Hausmann, R. Lipase-catalyzed synthesis of glucose-6-*O*-hexanoate in deep eutectic solvents. *Eur. J. Lipid Sci. Technol.* **2015**, *117*, 161–166.

(20) Davis, R. A.; Lin, C. H.; Gervay-Hague, J. Chemoenzymatic synthesis of

492 cholesteryl-6-*O*-tetradecanoyl-α-D-glucopyranoside: a product of host cholesterol
493 efflux promoted by *Helicobacter pylori*. *Chem. Commun.* **2012**, *48*, 9083–9085.
494 (21) Gupta, R. K.; James, K.; Smith, F. J. Sucrose esters and sucrose ester/glyceride
495 blends as emulsifiers. *J. Am. Oil Chem. Soc.* **1983**, *60*, 862–869.
496 (22) Zhang, X.; Song, F.; Taxipalati, M.; Wei, W.; Feng, F. Comparative study of
497 surface-active properties and antimicrobial activities of disaccharide monoesters.
498 *PLoS One* **2014**, *9*, 1–19.
499 (23) Lajnaf, R.; Picart-Palmade, L.; Cases, E.; Attia, H.; Marchesseau, S.; Ayadi, M. A.
500 The foaming properties of camel and bovine whey: the impact of pH and heat
501 treatment. *Food Chem.* **2018**, *240*, 295–303.
502 (24) Pearce, K. N.; Kinsella, J. E. Emulsifying properties of proteins: evaluation of a
503 turbidimetric technique. *J. Agric. Food Chem.* **1978**, *26*, 716–723.
504 (25) Chun, A. H.; Martin, A. N. Measurement of hydrophile-lipophile balance of
505 surface-active agents. *J. Pharm. Sci.* **1961**, *50*, 732–736.
506 (26) Griffin, W. C. Classification of surface active agents by HLB. *J. Soc. Cosmet.*
507 *Chem.* **1949**, *1*, 311–326.
508 (27) Griffin, W. C. Calculation of HLB values of nonionic surfactants. *Am. Perfum.*
509 *and Essent. Oil Rev.* **1995***, 65*, 26–29.
510 (28) Wege, H. A.; Kim, S.; Paunov, V. N.; Zhong, Q.; Velev, O. D. Long-term
511 stabilization of foams and emulsions with in-situ formed microparticles from
512 hydrophobic cellulose. *Langmuir* **2008**, *24*, 9245–9253.
513 (29) Belhaij, A.; Al-Mahdy, O. Foamability and foam stability of several surfactants

514 solutions: the role of screening and flooding. *J. Pet. Environ. Biotechnol.* **2015**, *6*,
515 1–6.
516 (30) Malysa, K.; Lunkenheimer, K. Foams under dynamic conditions. *Curr. Opin.*
517 *Colloid Interface Sci.* **2008**, *13*, 150–162.
518 (31) Hutzler, S.; Lösch, D.; Carey, E.; Weaire, D.; Hloucha, M.; Stubenrauch, C.
519 Evaluation of a steady-state test of foam stability. *Philos. Mag.* **2011**, *91*, 537–
520 552.
521 (32) Foegeding, E. A.; Luck, P. J.; Davis, J. P. Factors determining the physical
522 properties of protein foams. *Food Hydrocolloids* **2006**, 20, 284–292.
523 (33) Kothekar, S. C.; Ware, A. M.; Waghmare, J. T.; Momin, S. A. Comparative
524 analysis of the properties of tween-20, tween-60, tween-80, arlacel-60, and
525 arlacel-80. *J. Dispersion Sci. Technol.* **2007**, *28*, 477–484.
526 (34) Silvestre, M. P. C.; Decker, E. A.; McClements, D. J. Influence of copper on the
527 stability of whey protein stabilized emulsions. *Food Hydrocolloids* **1999**, *13*,
528 419–424.
529 (35) Avramenko, N. A.; Low, N. H.; Nickerson, M. T. The effects of limited
530 enzymatic hydrolysis on the physicochemical and emulsifying properties of a
531 lentil protein isolate. *Food Res. Int.* **2013**, *51*, 162–169.
532 (36) Kaur, K.; Kaur, J.; Kumar, R.; Mehta, S. K. Formulation and physiochemical
533 study of α-tocopherol based oil in water nanoemulsion stabilized with non toxic,
534 biodegradable surfactant: sodium stearoyl lactate. *Ultrason. Sonochem.* **2017**, *38*,
535 570–578.

536 **FIGURE CAPTIONS**

537 **Figure 1.** Enzymatic syntheses of the 6-*O*-acylglucose esters **2**-**8**.

538 **Figure 2.** Key HMBC and ^{1}H-^{1}H COSY correlations for 6-*O*-palmitoylglucopyranose

539 **7**.

540 **Figure 3.** The HLB values of compounds **2**-**8** and the commercial sucrose stearic

541 esters S-570 and S-770 (mean ± SD, n = 3).

542 **Figure 4.** The foamability of compounds **2**-**8** and the commercial sucrose stearic

543 esters S-570 and S-770 as well as span20 and span80 at 0.1%, 0.2%, and 0.5% w/w

544 concentrations (mean ± SD, n = 3).

545 **Figure 5.** (A) The foaming stability of compounds **2**-**8** and the commercial sucrose

546 stearic esters S-570 and S-770 as well as span20 and span80 over 10 min at 0.1%,

547 0.2%, and 0.5% w/w concentrations; and (B) over 10, 20, 30 min at 0.2% w/w

548 concentration (mean ± SD, n = 3).

549 **Figure 6.** The emulsion stability index of compounds **2**-**8** and the commercial sucrose

550 stearic esters S-570 and S-770 as well as span20 and span80 over 20 min at 0.1%,

551 0.2%, and 0.5% w/w concentrations (mean ± SD, n = 3).

Table 1. Investigations of Acylation Conditions[a]

Entry	Enzyme Loading (w/w %)	Solvents (v/v ratio)	Time (h)	Yields (%)						
				2	**3**	**4**	**5**	**6**	**7**	**8**
1	5	THF/pyridine[b] (4:1)	24	78	84	82	88	88	80	72
2	5	DMSO/*t*-BuOH[b] (1:1)	48	47	54	56	57	57	51	52
3	10	THF/pyridine[b] (4:1)	24	82	89	87	90	92	90	75
4	10	DMSO/*t*-amyl alcohol[b] (4:1)	48	41	48	56	55	52	47	43
5	20	THF/pyridine[c] (4:1)	48	44	51	57	60	58	52	29
6	20	THF/pyridine[b] (4:1)	48	89	94	95	95	95	87	82

[a]The reactions were carried out using glucose (1 mmol) and the relevant fatty acid vinyl ester (3 mmol) under the conditions shown above. The products were normally purified via trituration but in the cases involving incomplete reaction, column chromatographic separations were performed. [b]Strictly anhydrous solvents used. [c]Reagent-grade solvents used.

Table 2. The Cytotoxic (> 10% Inhibition) Concentrations of Glucose Esters **2-8**

	Cell Line				
Sample	HepG2 (μM)	LNacp (μM)	MCF-7 (μM)	SW549 (μM)	LO2 (μM)
2	**>** 2300±3	**>** 2300±70	**>** 2300±20	2260±9	**>** 2300±10
3	1800±30	**>** 2120±20	**>** 2120±2	**>** 2120±6	1240±50
4	360±2	600±30	510±2	480±9	210±30
5	1240±10	1740±20	830±10	880±3	**>** 1760±60
6	**>** 1650±20	**>** 1650±10	**>** 1650±8	1640±10	**>** 1650±50
7	**>** 1220±10	**>** 1220±6	**>** 1220±10	**>** 1220±20	**>** 1220±90
8	1120±20	**>** 1130±20	**>** 1130±6	560±3	**>** 1130±30

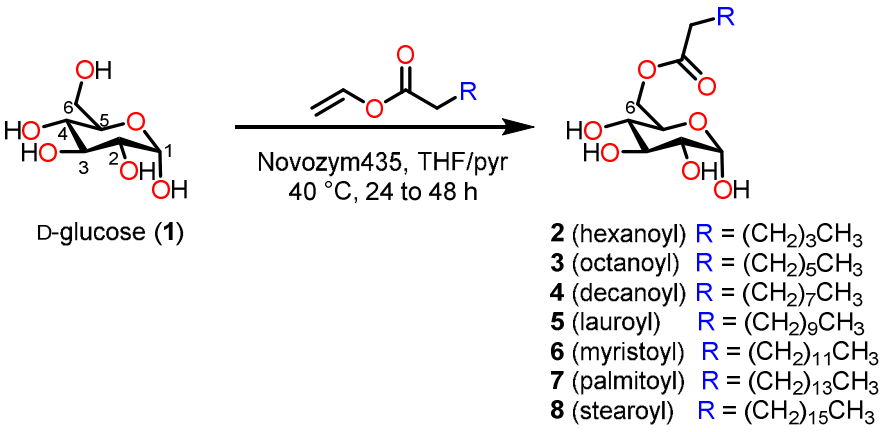
Novozym435, THF/pyr
40 °C, 24 to 48 h
D-glucose (1)
2 (hexanoyl) R = (CH2)3CH3
3 (octanoyl) R = (CH2)5CH3
4 (decanoyl) R = (CH2)7CH3
5 (lauroyl) R = (CH2)9CH3
6 (myristoyl) R = (CH2)11CH3
7 (palmitoyl) R = (CH2)13CH3
8 (stearoyl) R = (CH2)15CH3

Figure 1.

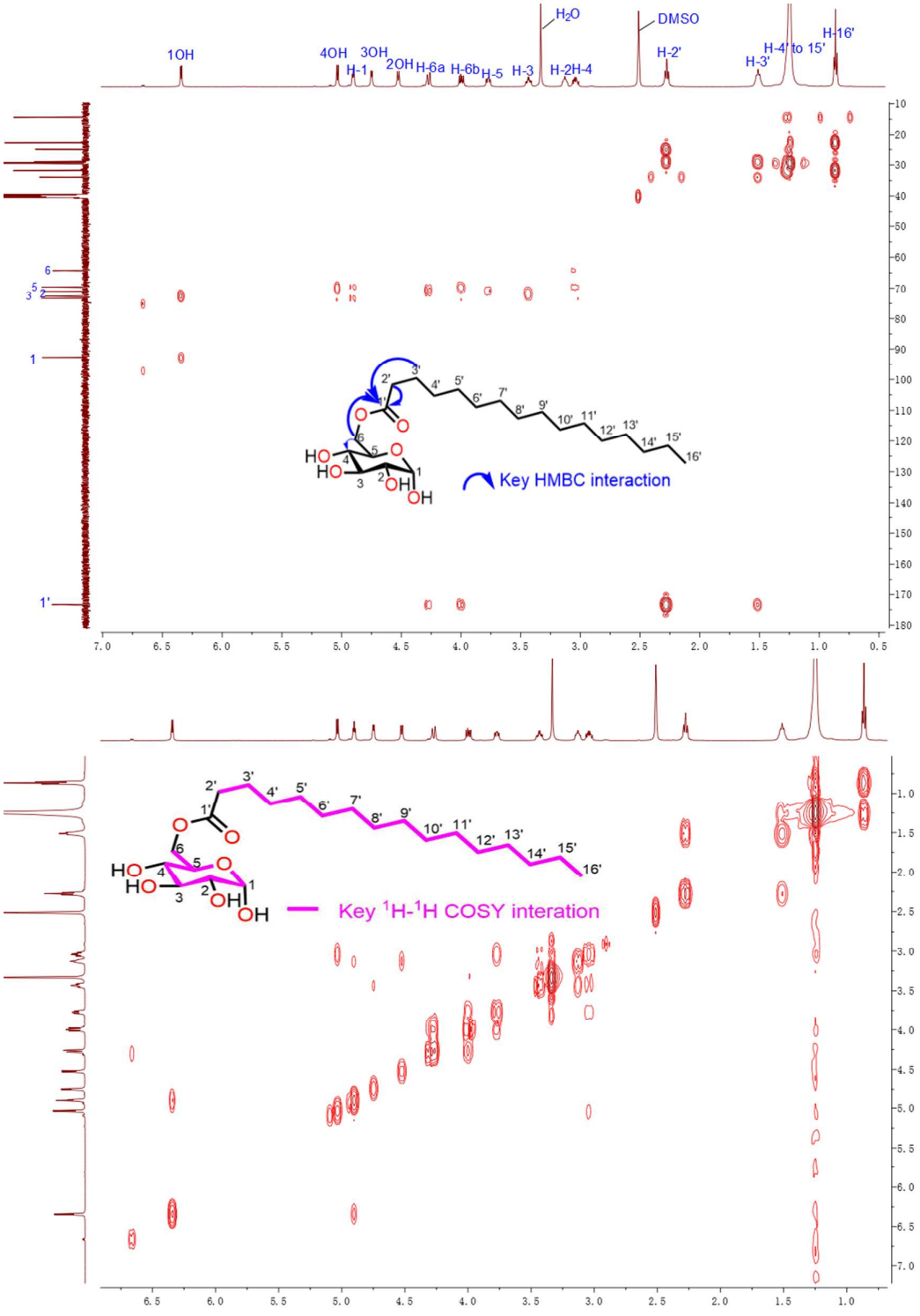
1OH
4OH
3OH
H-1
2OH
H-6a
H-6b
H-5
H-3
H-2
H2O
DMSO
H-2'
H-3'
H-4' to 15'
H-16'
Key HMBC interaction
Key 1H-1H COSY interation

Figure 2.

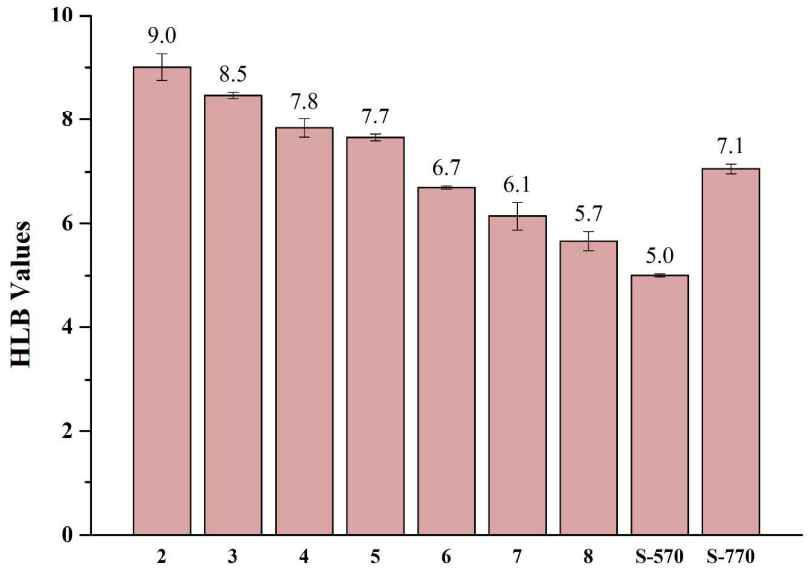

Figure 3.

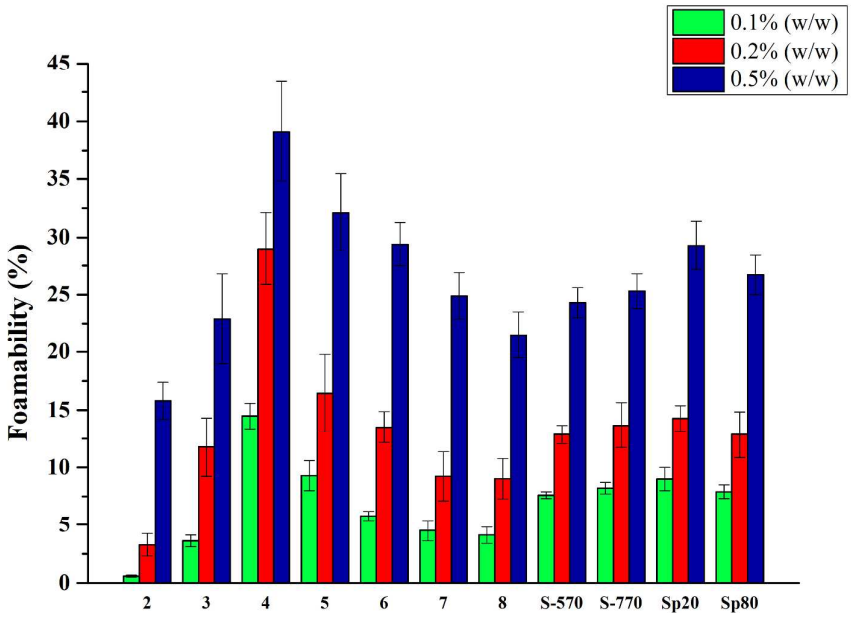
0.1% (w/w)
0.2% (w/w)
0.5% (w/w)
Foamability (%)
0
5
10
15
20
25
30
35
40
45
2
3
4
5
6
7
8
S-570
S-770
Sp20
Sp80

Figure 4.

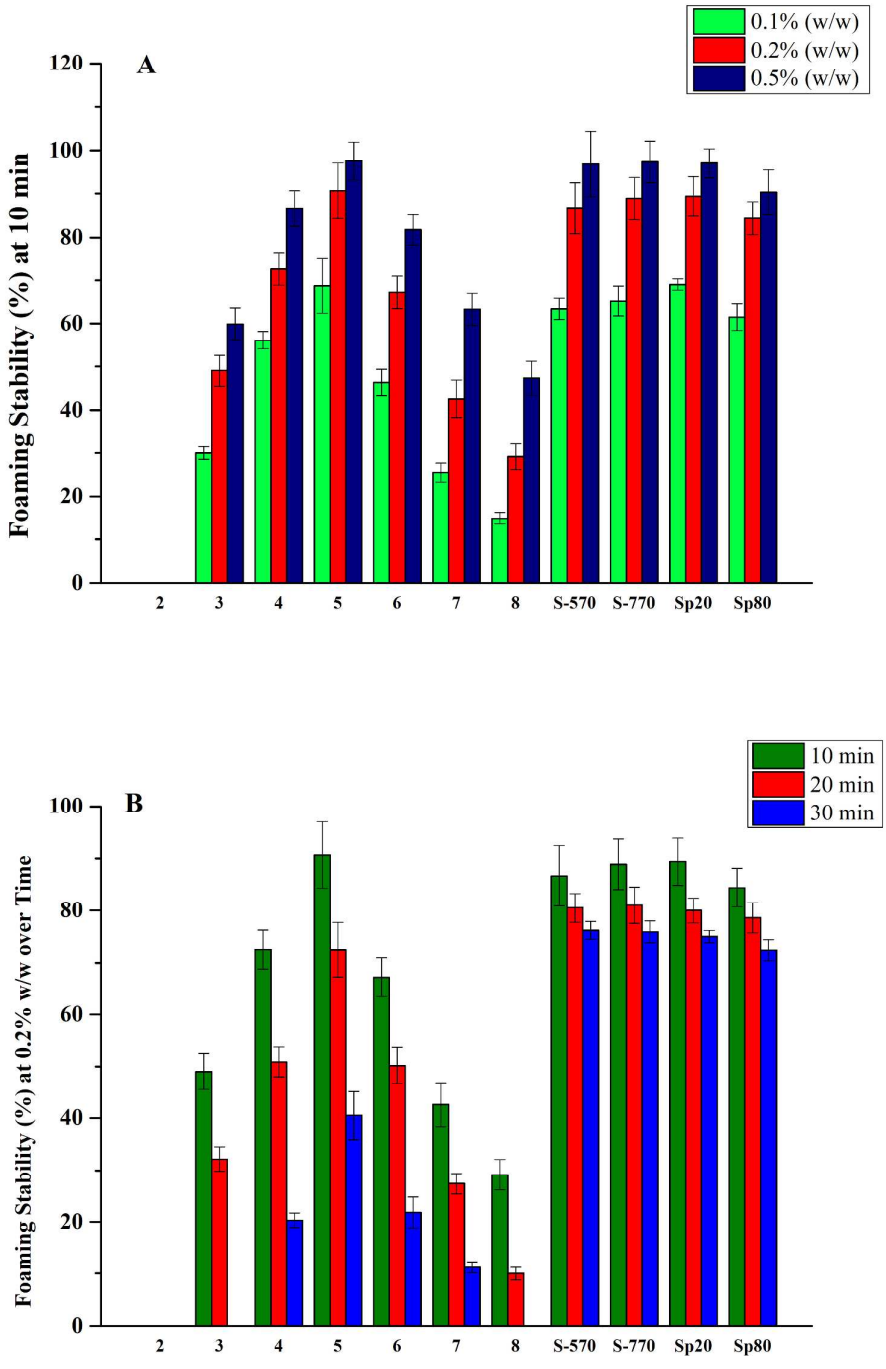
A
0.1% (w/w)
0.2% (w/w)
0.5% (w/w)
Foaming Stability (%) at 10 min
120
100
80
60
40
20
0
2
3
4
5
6
7
8
S-570
S-770
Sp20
Sp80
B
10 min
20 min
30 min
Foaming Stability (%) at 0.2% w/w over Time
100
80
60
40
20
0
2
3
4
5
6
7
8
S-570
S-770
Sp20
Sp80

Figure 5.

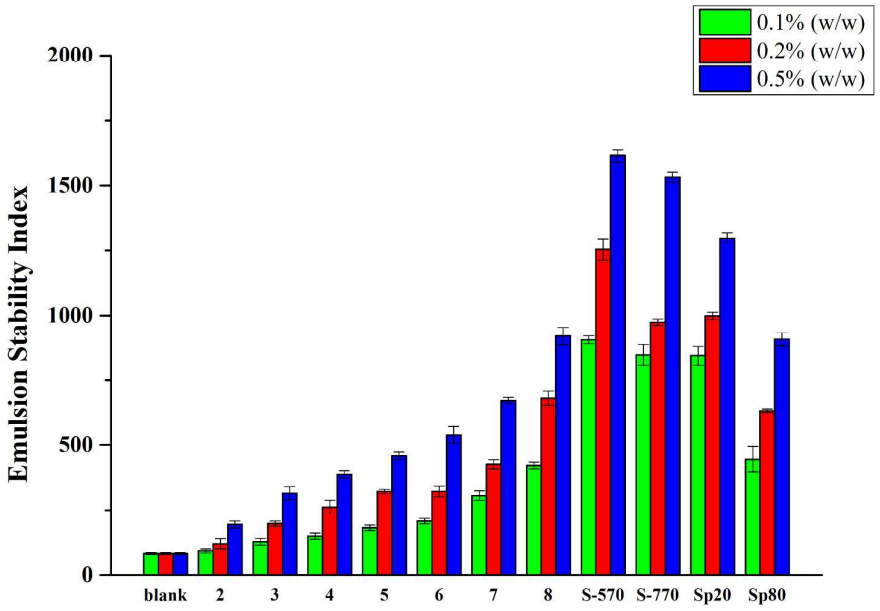
0.1% (w/w)
0.2% (w/w)
0.5% (w/w)
Emulsion Stability Index
2000
1500
1000
500
0
blank
2
3
4
5
6
7
8
S-570
S-770
Sp20
Sp80

Figure 6.

TABLE OF CONTENTS GRAPHIC