Enzymatic Synthesis of Puerarin Glucosides Using Cyclodextrin Glucanotransferase with Enhanced Antiosteoporosis Activity

Wei Huang, Qi He, Zhen-Ru Zhou, Hai-Bin He, and Ren-Wang Jiang*

ABSTRACT: Puerarin (PU) is the most abundant isoflavone from the root of Pueraria lobata and exhibits a broad range of pharmacological activities. However, poor water solubility and low bioavailability limit its use. Enzymatic transglycosylation is emerging as a new strategy to improve the pharmacodynamic and pharmacokinetic properties of natural products for drug development. In this study, three PU glucosides (PU-G, PU-2G, and PU-3G) were synthesized by using a cyclodextrin glucanotransferase from Bacillus licheniformis with PU as the acceptor and α-cyclodextrin as the sugar donor. The transglycosylation products were isolated and structurally identified by mass spectrometry and nuclear magnetic resonance. The water solubilities of PU-G, PU-2G, and PU-3G were 15.6, 100.9, and 179.1 times higher than that of PU, respectively. Moreover, the antiosteoporosis activities of these glucosides were tested, and PU-G was found to show much more potent antiosteoporosis activity as compared to the original PU.

INTRODUCTION

Isoflavones are a group of natural phenolic compounds that are widely distributed in various plants and foods, for example, legumes, soybean, and soy foods. Isoflavones have been found to show significant health benefits, including prevention of heart disease, daily antioxidants, prevention of osteoporosis, and reduction of postmenopausal syndrome in women.1-7

Pueraria lobata is a famous traditional medical plant, whose extracts are used as in various dietary supplements.8,9 Puerarin (daidzein 8-C-glucoside, PU) is the most abundant isoflavone in the root of P. lobata, which has been used to treat coronary heart disease, angina pectoris, cardiac infarction, sudden deafness, ocular blood flow problem, and alcoholism.10 Contemporary research demonstrated that PU has important functions including antioxidative, antiallergic, antitumor, antiosteoporosis, and anti-inflammatory activities.11-13 PU injection and other preparations have been approved by the China Food and Drug Administration (CFDA) and used in the clinics.14 Although PU has wide beneficial effects for human health, the low bioavailability due to poor water solubility restricts its applications.15

Recently, a lot of studies have been carried out to improve the physicochemical properties and bioactivities of PU via structural modifications.16,17 Glycosylation is emerging as an important approach to prepare water-soluble and stable glycosides from water-insoluble and unstable compounds.18,19 The sugar moiety plays a significant role in their physicochemical properties and biological activities.20-22 Compared to chemical glycosylation, enzymatic transglycosylation has some unique advantages, for example, high specificity and efficiency, less byproducts, and environmental friendliness.23,24 Cyclodextrin glucanosyltransferase (CGTase: EC 2.4.1.19) belongs to family 13 of the glycoside hyrolases, which also includes α-amylase, isoamylase, pullulanases, and branching enzymes.25 CGTase could catalyze four types of reactions, namely, cyclization, coupling, disproportionation, and hydrolysis. Cyclodextrins (α-, β-, and γ-CD) were synthesized via cyclization reactions. The coupling and disproportionation activities of CGTase could be used as an applicable tool for the transglycosylation of the suitable acceptor molecules.26-29 Many studies have reported the use of CGTase-catalyzed transglycosylation of phenolic compounds to improve their physicochemical properties and biological activities. Astragalin glucoside has been synthesized using CGTase and showed enhanced anti-inflammatory effects and aldose reductase inhibitory activity.30 The glycosylated genistein has been synthesized using CGTase catalysis, and its glucosides were found to show improved water solubility and stability at 4 °C.31 Various α-glucosylated derivatives of epigallocatechin gallate were synthesized by a CGTase from Thermoanaerobacter sp.31 To the best of our knowledge, enzymatic transglycosylation of PU catalyzed by CGTase has not yet been reported.

In this study, we report the enzymatic synthesis of PU glucosides using a CGTase from Bacillus licheniformis. Three new PU glucosides (PU-G, PU-2G, and PU-3G) were biosynthesized and characterized by high-resolution electro-

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Article

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INTRODUCTION

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Recently, a lot of studies have been carried out to improve the physicochemical properties and bioactivities of PU via structural modifications.16,17 Glycosylation is emerging as an important approach to prepare water-soluble and stable glycosides from water-insoluble and unstable compounds.18,19 The sugar moiety plays a significant role in their physicochemical properties and biological activities.20-22 Compared to chemical glycosylation, enzymatic transglycosylation has some unique advantages, for example, high specificity and efficiency, less byproducts, and environmental friendliness.23,24 Cyclodextrin glucanosyltrans-
spray ionization mass spectrometry (HR-ESI-MS) and nuclear magnetic resonance (NMR) for the first time. Moreover, we compared the water solubility PU-G, PU-2G, and PU-3G with PU. In addition, the inhibitory effects of PU and its glucosides on osteoporosis were evaluated.

### MATERIALS AND METHODS

**Materials.** PU was purchased from Baoji Chenguang Biotech Co., Ltd. (Baoji, China). α-CD, β-CD, γ-CD, CD, starch, source, maltose, β-glucose, and l-glucose were purchased from J & K Scientific Ltd. (Beijing, China). The recombinant enzyme CGTase (GenBank accession no. X15752.1) was overexpressed and purified as previously described by our group (Figure S1).

Cell counting kit-8 was obtained from Dojindo Laboratories (Dousindo, Japan). Alkaline phosphatase (ALP) detection kit was bought from Jiancheng Bioengineering Institute (Nanjing, China). High-performance liquid chromatography (HPLC)-grade acetonitrile was purchased from Merck KGaA (Darmstadt, Germany). Water was prepared with a Milli-Q, plus Ultrapure Water System (Millipore, Billerica, MA). Other reagents and chemicals were purchased from Sigma-Aldrich and J & K.

**Enzymatic Transglycosylation of PU.** Enzymatic transglycosylation of PU was carried out in a reaction volume of 200 μL consisted of 50 mM Tris·HCl (pH 7.5), 2 mg/mL of α-CD, 1 mM of PU, and 100 μL of crude protein of CGTase. Two control reactions that withheld either CGTase or α-CD were conducted in parallel. After incubation at 37 °C for 12 h, all the reactions were terminated with 200 μL of ice-cold MeOH and analyzed by HPLC.

**Optimization of Transglycosylation Reaction.** The effects of reaction conditions for the transglycosylation of PU were optimized, including temperature (ranged from 25 to 65 °C), pH (ranged from 3.0 to 11.0), divalent metal ions (Mn²⁺, Mg²⁺, Zn²⁺, Ca²⁺, Cu²⁺, Pb²⁺, and Co²⁺), sugar donor (α-CD, β-CD, γ-CD, starch, maltose, sucrose, β-glucose, and l-glucose), reaction times (ranged from 5 min to 12 h), and sugar donor concentrations (from 500 μg/mL to 10 mg/mL with a fixed PU concentration of 1 mM). All the enzymatic reactions were terminated with 200 μL of ice-cold MeOH and analyzed by HPLC. The yields were calculated according to the area of HPLC peak and the following equation

\[
Yield(\%) = \frac{[product]}{[PU]} \times 100\%
\]

In this equation, [product] and [PU] indicate the peak areas of transglycosylation products and original PU in the HPLC chromatogram, respectively.

**HPLC Analysis.** HPLC analyses were performed on an Agilent 1200 series HPLC system (Agilent Technologies, USA) coupled with a reverse-phase C18 column (Ultimate Polar RP C18 column 250 mm × 4.6 mm I.D., 5 μm, Welch Materials, Inc. China). The injection volume was 10 μL. The mobile phase was a mixture of water (0.1% formic acid; A) and acetonitrile (B). The separation was achieved through the gradient 10–75% B at 0–20 min and 75–100% B at 20–25 min with a flow rate of 1 mL/min and detection at 254 nm.

**Synthesis and Isolation of PU Glucosides.** To identify the PU glucosides and assay their water solubility and biological activity, the transglycosylation reaction was scaled-up to 175 mL under optimal condition: 1 mM PU, 4 mg/mL of α-CD, 35 mg of purified CGTase, and 50 mM Tris·HCl (pH 7.0) at 40 °C for 2 h. The reaction was quenched by the addition of an equal volume of ice-cold MeOH and centrifuged at 12,000 rpm for 20 min. The supernatant was concentrated to 5 mL under reduced pressure and filtered by a 0.45 μm membrane. Then, the filtered solution was purified by a reverse-phase semipreparative HPLC system (Wufeng Scientific Instruments Co., Ltd, China) equipped with a COSMOSIL Packed SC18-MS-II column (250 mm × 10.0 mm I.D., 5 μm, Nacalai Tesque, Inc., Japan). The mobile phase was a mixture of water (0.1% formic acid)/acetonitrile (8:1). The flow rate was 3 mL/min, and the detection wavelength was 254 nm. The purified products were further identified by HR-ESI-MS and NMR.

**High-Resolution Electrospray Ionization Mass Spectrometry.** The molecular mass of PU glucosides was determined using a mass spectrometer coupled to a hybrid QTOF analyzer (AB SCIEX). The samples were dissolved in MeOH (containing 0.1% formic acid) and ionized by ESI in positive mode.

**Nuclear Magnetic Resonance.** Approximately 30.0 mg of PU-G, 26 mg of PU-2G, or 12.0 mg of PU-3G were dissolved in 450 μL of DMSO-d₆ and transferred to 3 mm NMR tube. NMR measurements were performed on a 400 MHz spectrometer (Bruker, Inc. Switzerland), and the glycosidic linkages of PU-G, PU-2G, and PU-3G were characterized using the two-dimensional (2D) NMR (HSQC and HMBC).

**Cell Culture.** The mouse preosteoblast cell line MC3T3-E1 was purchased from the Cell Bank of the Chinese Academy of Science (Shanghai Institute for Biological Science, China). MC3T3-E1 was cultured in Dulbecco’s modified Eagle’s medium (DMEM, Hyclone, Miami, Fl, USA) supplemented with 10% fetal bovine serum (HyClone, USA), 100 U/mL of penicillin, and 100 μg/mL of streptomycin at 37 °C under 5% CO₂:humidified air. The culture medium was changed every 3 days.

**Cell Viability.** MC3T3-E1 cells were seeded at a density of 5000 cells/well in 96-well culture plates (each well 100 μL) and incubated for 24 h. Then, the culture medium was replaced with or without the samples diluted in the culture medium. After 48 h of incubation, the medium was changed to DMEM. Then, 10 μL of CCK-8 was added to each well and incubated for 2 h after which the optical density (OD) was measured at 450 nm using a microplate reader. The percent of proliferation or viability was determined by the formula: ratio (%) = [(ODs − ODb)/(ODc − ODb)] × 100%. ODs, ODc, and ODb indicated the OD of the cell lines incubated with samples, vehicle control, and blank, respectively.

**Water Solubility Analysis.** Excess PU and its glucosides were mixed with 100 μL of distilled water in a 1.5 mL Eppendorf tube at room temperature under sonication for 1 h at room temperature, and then, the tubes were kept at room temperature overnight. In the next day, the solution was centrifuged at 12,000 rpm for 10 min. The supernatants were diluted with MeOH and filtered through the 0.45 μm syringe filter. The concentration of the compound in the supernatant was calculated according to the HPLC analysis.

**ALP Detection Assay.** MC3T3-E1 cells (5.0 × 10⁴/well in six-well plates) were treated with samples (PU-G, PU-2G, and PU-3G) for 48 h. Then, the medium supernatant was collected and analyzed according to the protocol of the manufacturer.

**Runt-Related Transcription Factor 2 mRNA Expression.** Extraction of total RNA from MC3T3-E1 cells, reverse transcription, and quantitative polymerase chain reaction analysis of mRNA expression levels were performed as previously described. The emitted fluorescence signal was collected by the Roche LightCycler 480 Detection System.
(Germany). The mRNA level of runt-related transcription factor 2 (Runx2) was normalized by GADPH as an internal control. The following primers were used:

Runx2-Forward: 5′-CGGACGAGGCAAGAGTTTCA-3′
Runx2-Reverse: 5′-GGATGAGGAATGCGCCCTAA-3′
GADPH-Forward: 5′-CATGGCCTTCCGTGTTCCTA-3′
GADPH-Reverse: 5′-CCTGCTTCACCACCTTCTTGAT-3′.

Statistical Analysis. SPSS 20.0 version was used for statistical analysis. The data were presented as the mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance, followed by a post hoc Fisher’s least significant difference test. Differences were considered to be statistically significant at P < 0.05.

RESULTS AND DISCUSSION

Enzymatic Transglycosylation of PU. The transglycosylation reaction was performed using PU as a substrate, α-CD as the sugar donor, and the recombinant CGTase as the catalysis. The reaction mixture was terminated with ice-cold MeOH and analyzed by HPLC−diode-array detector (DAD) and LC−MS, which showed that PU was successfully converted into four new peaks in the experimental group. The new peaks were named PU-G, PU-2G, PU-3G, and PU-4G (Figure 1A). These new peaks (PU-G, PU-2G, PU-3G, and PU-4G) exhibited pseudomolecular ions with 162, 324, 486, and 648 mass units more than that of PU (Figure 1B), indicating that they were the mono-, di-, tri-, and tetra-O-glucosides of PU, respectively. These results showed that PU could be glycosylated under the catalysis of CGTase, which is reported here for the first time. Compared to the previously reported methods (chemical synthesis,34,35 cell culture,36 and bacterial lysate37 to modify PU), our glycosylation catalyzed by CGTase in this paper reduced the generation of byproducts and simplified the purification processes.

Optimization of the Transglycosylation Reaction. To improve the yield of the PU glucosides, the transglycosylation reaction conditions were optimized. The effect of reaction temperature was studied, and the total yield of PU glucosides reached the maximum when the temperature was set at 45 °C (Figure 2A). The optimum pH was found to be 6.5, though the recombinant CGTase has a broad pH activity ranging from 6 to 10 (Figure 2B). The effect of reaction time was investigated, and the highest yield of PU glucosides was achieved with a reaction time of 2 h. When the reaction time was extended to 10 h, there is no significant difference in the yield (Figure 2C). Sugar donor is a very important factor in glycosylation reactions. In our experiments, α-CD was determined to be the most suitable sugar donor (Figure 2D). The effect of divalent metal ions was investigated. Mn2+, Mg2+, and Ca2+ could increase the yields of transglycosylated products remarkably, and the Mn2+ was the best for this reaction (Figure 2E). We also investigated the effect of the concentration of sugar donor. The optimal concentration of α-CD was determined to be 4 mg/mL when the concentration of PU was fixed at 1 mM (Figure 2F). Accordingly, the optimal transglycosylation conditions were as follows: 50 mM phosphate buffer, pH 6.5; 5 mM MnCl2; 4 mg/mL α-CD; 1 mM PU; and 100 μg purified CGTase in 200 μL final volume at 45 °C for 2 h. Under the above-optimized conditions, the yields of PU-G, PU-2G, PU-3G, and PU-4G were 19.6, 12.5, 6.1, and 1.1%, respectively. It is noteworthy that CGTase was used in this study because CGTase could maintain strong activity in a wide temperature range (30−45 °C), and its expression level in Escherichia coli was very high (30 mg/L). Furthermore, PU could be catalyzed by this CGTase to a variety

![Figure 1.](https://example.com/figure1.png)
of new products, enriching the structural diversity of PU derivatives.

**Structural Analysis of PU Glucosides.** To further confirm the structure of PU glucosides, we scaled-up the transglycosylation reaction. The products were isolated from the reaction mixture by preparative HPLC and then freeze-dried as white powders. The yield of PU-4G is very low (1.1%), and thus, we could not obtain enough quantities for both HMBC testing and activity testing. The structures of PU-G, PU-2G, and PU-3G were further determined by 1H and 13C NMR spectra and 2D NMR spectra (Figures S2−S13). It is noteworthy that although the PU glucosides with α-1,6-glycosidic linkages have been synthesized by Leuconostoc dextranucrase, the α-1,4-glycosidic linkage of PU glucosides was synthesized here for the first time (Figure 3). The newly formed α(1→4) glycosidic linkage of our transfer product can be hydrolyzed in humans by α-amylase and α-glucosidases, implying that the PU derivatives are metabolized in the same way as PU itself in the human body.30,41

The HR-ESI-MS spectrum of PU-G showed a pseudomolecular ion [M + H]⁺ peak at m/z 741.2241, indicating a molecular formula of C33H40O19 (calcd 741.2245 for C27H30O14), which suggested that PU-2G was a PU diglucoside. Compared to PU-G, the 1H and 13C NMR spectra of PU-2G showed an additional anomic proton signal at δ 5.12 (1H, d, J = 3.4 Hz, H-1‴) and an anomic carbon signal at δ 100.87 (C-1‴, overlap). This additional glucosyl residue of PU-2G was linked to 4‴-OH according to the HMBC correlation of H-1‴ with C-4‴ (δC = 79.98). The resonance for C-4‴ was shifted to downfield (9.00 ppm) as compared to that of PU-G due to glycosyl substitution. These data confirm that the third α-D-glucopyranosyl residue and the inner β-D-glucopyranosyl residue were 1,4-linked. Thus, compound PU-2G was identified as α-D-glucosyl-(1→4)-puerarin.

The HR-ESI-MS spectrum of PU-2G showed a pseudomolecular ion [M + H]⁺ peak at m/z 741.2241, indicating a molecular formula of C33H40O19 (calcd 741.2245 for C27H30O14), which suggested that PU-2G was a PU diglucoside. Compared to PU-G, the 1H and 13C NMR spectra of PU-2G showed an additional anomic proton signal at δ 5.12 (1H, d, J = 3.4 Hz, H-1‴) and an anomic carbon signal at δ 100.87 (C-1‴, overlap). This additional glucosyl residue of PU-2G was linked to 4‴-OH according to the HMBC correlation of H-1‴ with C-4‴ (δC = 79.98). The resonance for C-4‴ was shifted to downfield (9.00 ppm) as compared to that of PU-G due to glycosyl substitution. These data confirm that the third α-D-glucopyranosyl residue and the inner β-D-glucopyranosyl residue were 1,4-linked. Thus, compound PU-2G was identified as α-D-glucosyl-(1→4)-puerarin.
The HR-ESI-MS spectrum of PU-3G showed a pseudomolecular ion [M + H]+ peak at m/z 903.3503, indicating a molecular formula of C_{39}H_{50}O_{24} (calcld. 903.3507 for C_{39}H_{50}O_{24}), which suggested that PU-3G was a PU triglucoside. The 1H and 13C NMR data of PU and PU-3G were compared in Table 1. The 1H NMR spectrum of PU-3G showed four anomeric proton signals at δ 5.09 (1H, d, J = 3.4 Hz, H-1″‴), 5.02 (1H, d, J = 3.4 Hz, H-1″″), 5.01 (1H, d, J = 3.4 Hz, H-1‴), and 4.83 (1H, d, J = 9.7 Hz, H-1″), suggesting the presence of three α-anomers and one β-anomer in the sugar moiety. The 13C NMR chemical shifts of C-4″ (δ 79.48), C-4‴ (δ 79.70), and C-4″″ (δ 79.97) were shifted to downfield. The HMBC experiment showed correlations among H-1″ (δ 4.83), C-7 (δ 161.83), and C-9 (δ 157.20), between H-1‴ (δ 5.01) and C-4″ (δ 79.48), between H-1″″ (δ 5.02) and C-4‴ (δ 79.70), and between H-1‴‴ (δ 5.09) and C-4″″ (δ 79.97). Thus, compound PU-3G was identified as α-D-maltotriosyl-(1 → 4)-puerarin.

Water Solubility of PU and Its Glucosides. The water solubilities of PU-G, PU-2G, and PU-3G were determined and compared with that of PU. The result showed that the solubility of PU was 5.24 mg/mL, whereas those of PU-G, PU-2G, and PU-3G were 81.76, 528.54, and 938.26 mg/mL, respectively. The solubility of PU-G, PU-2G, and PU-3G was approximately 16.5, 100.9, and 179.1 times higher than that of PU, respectively (Table 2). These results indicated that the addition of glucosyl group could remarkably increase the water solubility of PU. These PU glucosides with higher water solubility might have broad applications in industries.

Antiosteoporosis Activity of PU and Its Glucosides. PU is one of the major isoflavonoid phytoestrogen extracted from the P. lobata (Willd.) Ohwi, which has attracted increasing attention because of its beneficial effects on antiosteoporosis,42,43 and PU were shown to have higher estrogenic activity than daidzein and genistein in MCF-7 cells.44 Like other isoflavones, PU has low water solubility and permeability, which limits its bioavailability and industrial applications.15 In our study, three new transglycosylation products PU-G, PU-2G, and PU-3G with α (1 → 4) glycosidic linkages were obtained. Their water solubilities were significantly enhanced as compared to PU. To investigate whether glycosylation altered the antiosteoporosis activity of PU, we studied the antiosteoporosis activity of PU and its glucoside using MC3T3-E1 cells. The cells treated with 0.1 μM of PU and its glucosides had the highest viability among the three concentrations (0.1, 1, and 10 μM), suggesting that a lower concentration of PU and its glucosides might have a better promoting effect (Figure S14). Therefore, we chose 0.1 μM for further study. ALP is a representative enzyme for the indication of osteoblast differentiation, which plays a vital role in the stimulation of late-stage osteoblast genesis elements.45 The ALP activity was selected as a biomarker.
to investigate the antiosteoporosis effects of PU and its glucosides. After 48 h of treatment with PU, PU-G, PU-2G, and PU-3G, the enzyme activity of ALP increased to 120.75, 154.79, 127.98, and 121.10%, respectively, compared to the control group (Figure 4A). Moreover, PU-G exhibited much more potent antiosteoporosis activity than PU. Runx2 is a key bone growth regulator involved in the proliferation and differentiation of osteoblasts.45 We further investigated the effect of PU and its glucosides on the mRNA expression level of runx2. The results showed that the expression of runx2 increased in MC3T3-E1 cells after treatment with 0.1 μM PU and its glucosides for 48 h. We can also observe that PU-G can more significantly increase the mRNA expression of runx2 as

| Table 1. $^1$H and $^{13}$C NMR Data of PU and PU-3G |
|-----------------------------------------------|
| carbon no. | (δH) | (δC) | (δH) | (δC) |
| PU | 2 | 152.67 | 152.57 |
| | 3 | 122.53 | 122.59 |
| | 4 | 174.90 | 174.91 |
| | 5 | 7.94 (1H, d, J = 8.8 Hz) | 126.23 | 7.93 (1H, d, J = 8.6 Hz) |
| | 6 | 115.02 | 115.02 |
| | 7 | 161.12 | 161.83 |
| | 8 | 112.66 | 112.30 |
| | 9 | 157.16 | 157.20 |
| | 10 | 116.56 | 116.56 |
| PU-3G | 1′ | 7.39 (1H, d, J = 8.6 Hz) | 123.07 | 7.39 (1H, d, J = 8.6 Hz) |
| | 2′ | 6.80 (1H, d, J = 8.6 Hz) | 115.02 | 6.78 (1H, d, J = 8.6 Hz) |
| | 3′ | 161.12 | 161.83 |
| | 4′ | 112.66 | 112.30 |
| | 5′ | 157.16 | 157.20 |
| | 6′ | 116.56 | 116.56 |
| | 1″ | 4.81 (1H, d, J = 9.8 Hz) | 73.43 | 4.84 (1H, d, J = 9.8 Hz) |
| | 2″ | 70.76 | 71.77 |
| | 3″ | 78.77 | 78.52 |
| | 4″ | 70.50 | 79.48 |
| | 5″ | 81.87 | 80.49 |
| | 6″ | 61.45 | 60.92 |
| α-β-glucose (1) | 1‴ | 5.01 (1H, d, J = 3.4 Hz) | 100.65 |
| | 2‴ | 70.33 |
| | 3‴ | 73.48 |
| | 4‴ | 79.70 |
| | 5‴ | 73.12 |
| | 6‴ | 60.83 |
| α-β-glucose (2) | 1″‴ | 5.02 (1H, d, J = 3.4 Hz) | 100.81 |
| | 2″‴ | 70.30 |
| | 3″‴ | 73.33 |
| | 4″‴ | 79.97 |
| | 5″‴ | 72.56 |
| | 6″‴ | 60.83 |
| α-β-glucose (3) | 1″‴ | 5.09 (1H, d, J = 3.4 Hz) | 100.81 |
| | 2″‴ | 69.95 |
| | 3″‴ | 73.20 |
| | 4″‴ | 60.92 |
| | 5″‴ | 72.11 |
| | 6″‴ | 60.28 |

Table 2. Water Solubility of PU and Its Glucosides

| compound | solubility in water (mg/mL) | relative solubility |
|-----------|------------------------------|-------------------|
| PU        | 5.24 ± 1.32                 | 1.0               |
| PU-G      | 81.76 ± 5.36                | 15.6              |
| PU-2G     | 528.54 ± 18.24              | 100.9             |
| PU-3G     | 938.26 ± 25.53              | 179.1             |

"Mean ± SD.

Figure 4. Antiosteoporosis activity of PU and its glucosides. (A) Effect of PU and its glucosides on the ALP activity. (B) Effect of PU and its glucosides on the expression of runx2. The data presented are mean ± standard error of the mean (n = 3). * P < 0.05, ** P < 0.01, and *** P < 0.001 are relative to the control group.
Bacillus licheniformis CGTase from PU-2G glucosides were tested, and optimized. The reaction conditions including temperature, reaction time, pH, sugar donor, and ratio of sugar donor and substrate were optimized. PU-G, PU-2G, and PU-3G showed enhanced water solubility. Moreover, the antiosteoporosis activities of these glucosides were tested, and PU-G was found to show much more potent antiosteoporosis activity as compared to the original PU. This study provides a new CGTase-catalyzed method for the preparation of PU transglycosylation derivatives and extends the application of PU in the industry and daily life.

## ASSOCIATED CONTENT

#### Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00950.

Recombinant CGTase analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis; ¹H NMR, ¹³C NMR, and 2D NMR data of PU glucosides, and effects of MC3T3-E1 cell viability (PDF)

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### Notes

The authors declare no competing financial interest.

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