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# Using ionic liquid cosolvents to improve enzymatic synthesis of arylalkyl β-D-glucopyranosides

Rong-Ling Yang, Ning Li*, Min-Hua Zong*

*State Key Laboratory of Pulp and Paper Engineering, College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China*

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ABSTRACT

Enzymatic synthesis of various arylalkyl β-D-glucopyranosides catalyzed by prune (*Prunus domestica*) seed meal via reverse hydrolysis in the mixture of organic solvent, ionic liquid (IL) and phosphate buffer was described. Among four hydrophilic organic solvents tested, ethylene glycol diacetate (EGDA) was found to be the most suitable for enzymatic synthesis of salidroside, a bioactive compound of commercial interest, from D-glucose and tyrosol. The effects of the nature of ionic liquids and their contents on the enzymatic glucosylation were studied. The addition of a suitable amount of ILs including denaturing ones was favorable to shift the reaction equilibrium toward the synthesis, thus improving the yields. Among the examined ILs, the novel IL [BMIm]I proved to be the best. And this IL was applied as the solvent in biocatalysis for the first time. The yields were found to be enhanced between 0.2-fold and 0.5-fold after the addition of 10% (v/v) [BMIm]I. In 10% (v/v) [BMIm]I-containing system, the desired arylalkyl β-D-glucopyranosides were synthesized with 15–28% yields, among which salidroside was obtained with a yield of 22%.



## 1. Introduction

Glycosides are of commercial interest in the food, cosmetic and pharmaceutical industries. For example, alkyl glycosides are an important group of nonionic surfactants due to the excellent properties such as good surfactant performance, low toxicity and high biodegradability [1]. Salidroside (4-hydroxyphenethyl β-D-glucopyranoside) is known as the main active component of the plant *Rhodiola*, which has been used as the traditional medicinal herb in China for more than 1000 years. Recently, this compound was found to have neuron- and cardioprotection [2,3], anticancer and antiviral activities [4,5], etc. As compared to chemical methods [6], enzymatic synthesis of these compounds attracted more attention owing to the simplicity, high stereo- and regioselectivity, mild reaction conditions [7].

Compared to glycosyl transferase-catalyzed synthesis of glycosides, the processes mediated by glycosidases display greater application potential since these enzymes are inexpensive, available in large quantities, generally stable and have low specificity toward aglycones [8]. In addition, non-activated sugars such as monosaccharides or disaccharides can be used directly as glycosyl donors to synthesize glycosides. Reverse hydrolysis is the condensation reaction of monosaccharides with alcohols to furnish alkyl glycosides as the desired products and water as the byproduct that would not interfere with the downstream processing, whereas the detrimental byproducts (monosaccharides) would form in the transglycosylation of alcohols and disaccharides [7]. However, thermodynamically controlled reverse hydrolysis generally affords lower yields than the transglycosylation controlled kinetically. Considerable efforts have been made to improve the equilibrium yields [9–11], among which reducing water activities by adding organic cosolvents is one of the most efficient strategies [12,13].

Ionic liquids (ILs) represent a class of promising and "green" nonmolecular solvents [14,15]. Like conventional water-miscible organic solvents, these ionic solvents enabled to work as the cosolvents to control the water activities, thus inhibiting the second hydrolysis of the glycosylated products and improving the yields [16,17]. In addition, this type of solvents has proven to be the media with positive effects on the enzymes, such as improving the activity, selectivity and/or the stability [18,19]. Recently, we found that a variety of fruit seed meals, especially prune seed meal, were excellent biocatalysts for the synthesis of various alkyl and arylalkyl β-D-glucopyranosides from D-glucose and alcohols (data unpublished). In the present work, we focused our interest on the enhancement of the enzymatic synthesis of arylalkyl β-D-glucopyranosides by using ILs as the cosolvents, which were able to control water activities of the systems, thus improving the yields (Scheme 1).

* Corresponding authors. Tel.: +86 20 8711 1452; fax: +86 20 2223 6669.
*E-mail addresses:* lining@scut.edu.cn (N. Li), btmhzong@scut.edu.cn (M.-H. Zong).

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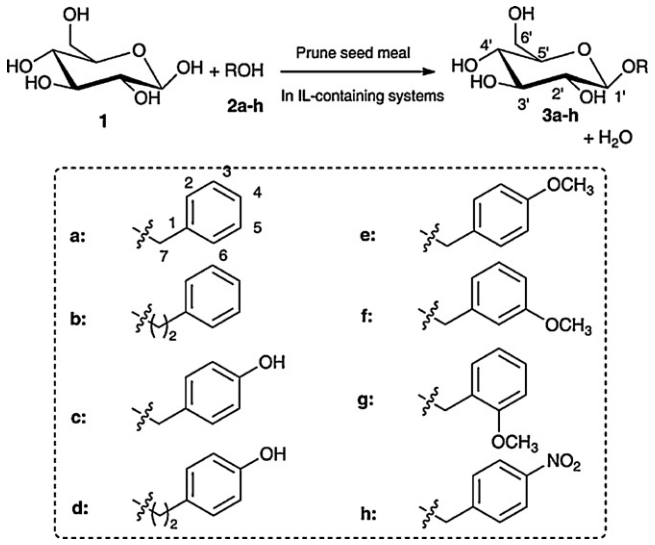


**Scheme 1.** Enzymatic synthesis of various arylalkyl β-D-glucopyranosides in IL-containing systems.

## 2. Materials and methods

### 2.1. Materials

1-Butyl-3-methylimidazole tetrafluoroborate ([BMIm]$BF_4$, ≥97%), 1-butyl-2, 3-dimethylimidazole tetrafluoroborate ([BMMIm]$BF_4$, ≥97%), 1-butyl-3-methylimidazole iodide ([BMIm]I, ≥98%), 1,3-dimethylimidazole methylsulfate ([MMIm]$MeSO_4$, ≥98%) and salidroside were purchased from Sigma–Aldrich (USA). 1-Butyl-3-methylimidazole octylsulfate ([BMIm]$C_8SO_4$, ≥98%) was from Merck (Germany). 1-Butyl-3-methylimidazole hexafluorophosphate ([BMIm]$PF_6$, ≥96%), 1-hexyl-3-methylimidazole tetrafluoroborate ([HMIm]$BF_4$, ≥96%), 1-butyl-3-methylimidazole chloride ([BMIm]Cl, ≥96%), 1-ethoxyethyl-3-methylimidazole chloride ([EOEMIm]Cl, ≥96%), and 1-acetoxyethyl-3-methylimidazole chloride ([AcOEMIm]Cl, ≥96%) were from Lanzhou Institute of Chemical Physics (China). 4-Nitrophenol, 4-nitrobenzyl alcohol and tyrosol were from Linfeng Chemical Co., (Shanghai, China). 4-Hydroxybenzyl alcohol, 4-methoxybenzyl alcohol, 3-methoxybenzyl alcohol, 2-methoxybenzyl alcohol and phenethyl alcohol were obtained from Haiqu Chemical Co., (Shanghai, China). Ethylene glycol diacetate (EGDA) was from TCI (Japan). Prune seeds were from local food processing company. The defatted prune seed meal (23.7 U/g) was prepared and the enzyme activity was assayed as described by Yu et al. [11]. One unit of hydrolytic activity was defined as the amount of enzyme that released 1 μmol 4-nitrophenol per minute at pH 7.0 and 50 °C. All other chemicals are of high purity commercially available.

### 2.2. General procedure for enzymatic glucosylation

D-Glucose (0.5 mmol) and alcohol (5 mmol) were added to 2 ml mixture of 10% (v/v) phosphate buffer (pH 6.0, 50 mM) and organic solvent. The reaction was initiated by adding 5.5 U crude enzyme (defatted prune seed meal) at 50 °C and 200 rpm. Aliquots were withdrawn from the reaction mixture at specified time intervals, and then diluted by 25-fold with the corresponding mobile phase prior to HPLC analysis.

### 2.3. HPLC analysis

HPLC analysis was carried out on an Agilent 1100 chromatograph with a variable wavelength detector and a Zorbax Eclipse Plus C18 column (4.6 mm × 250 mm, 5 μm, Agilent). The flow rate and column temperature were 1.0 ml/min and 30 °C, respectively. The absorption wavelength for HPLC analysis was 257 nm, with the exception of the analysis of salidroside **3d** at 275 nm. A gradient elution with methanol/water being 60/40 (v/v) from 0 to 4 min, and then methanol/water being 80/20 (v/v) at 6.0 min was used. The retention times of the compounds **3a–h** were 3.02, 3.47, 2.52, 2.58, 3.06, 3.20, 3.28 and 3.09 min, respectively. The peak of salidroside **3d** was identified by comparison with the retention time of authentic standard. Yields were calculated by the ratio of the actual product concentration to the theoretic product concentration based on D-glucose. The analytical errors were less than 1%.

### 2.4. Structure characterization

The structures of glycosides were determined by $^1H$ and $^{13}C$ NMR (Bruker AVANCE Digital 400 MHz NMR spectrometer, Germany) at 400 and 100 MHz, respectively. NMR data of the compound **3a** is the same as reported previously [20].

*Phenethyl β-D-glucopyranoside* **3b**. $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.80–2.90 (m, 2H, $H_7$), 2.94–2.99 (m, 1H, $H_{4'}$), 3.01–3.17 (m, 3H, $H_{2'} + H_{3'} + H_{5'}$), 3.41–3.46 (m, 1H, 10.45, $H_{6'}$), 3.62–3.68 (m, 2H, $H_8$), 3.91–3.98 (m, 1H, $H_{6'}$), 4.19 (d, 1H, $J$ = 7.6 Hz, $H_{1'}$), 4.47 (t, 1H, $J$ = 6.0 Hz, $OH_{6'}$), 4.88 (d, 1H, $J$ = 4.8 Hz, $OH_{3'}$), 4.91 (d, 1H, $J$ = 4.4 Hz, $OH_{4'}$), 4.96 (d, 1H, $J$ = 4.8 Hz, $OH_{2'}$), 7.19–7.21 (m, 1H, $H_4$), 7.27–7.30 (m, 4H, $H_2 + H_3 + H_5 + H_6$). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 35.62 ($C_2$), 61.10 ($C_{6'}$), 69.39 ($C_{4'}$), 70.12 ($C_{2'}$), 73.14 ($C_{3'}$), 76.77 ($C_1$), 76.84 ($C_{5'}$), 102.80 ($C_{1'}$), 125.97 ($C_4$), 128.14 ($C_3 + C_5$), 128.83 ($C_2 + C_6$), 138.69 ($C_1$).

*4-Hydroxybenzyl β-D-glucopyranoside* **3c**. $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.97–3.15 (m, 4H, $H_{2'} + H_{3'} + H_{4'} + H_{5'}$), 3.43–3.49 (m, 1H, $H_{6'}$), 3.68–3.72 (m, 1H, $H_{6'}$), 4.19 (d, 1H, $J$ = 7.8 Hz, $H_{1'}$), 4.45 (d, 1H, $J$ = 11.4 Hz, $H_7$), 4.53 (t, 1H, $J$ = 5.8 Hz, $OH_{6'}$), 4.70 (d, 1H, $J$ = 11.4 Hz, $H_7$), 4.91 (apparent dd, 2H, $OH_{3'} + OH_{4'}$), 5.01 (d, 1H, $J$ = 4.8 Hz, $OH_{2'}$), 6.73 (apparent d, 2H, $H_3 + H_5$), 7.18 (apparent d, 2H, $H_2 + H_6$), 9.35 (s, 1H, $OH_4$). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 61.08 ($C_{6'}$), 69.31 ($C_{4'}$), 70.11 ($C_{2'}$), 73.38 ($C_{3'}$), 76.71 ($C_7$), 76.78 ($C_{5'}$), 101.57 ($C_{1'}$), 114.74 ($C_3 + C_5$), 127.97 ($C_1$), 129.37 ($C_2 + C_6$), 156.64 ($C_4$).

*4-Methoxylbenzyl β-D-glucopyranoside* **3e**. $^1H$ NMR (DMSO-$d_6$): $\delta$ 2.99–3.14 (m, 4H, $H_{2'} + H_{3'} + H_{4'} + H_{5'}$), 3.44–3.50 (m, 1H, $H_{6'}$), 3.68–3.73 (m, 1H, $H_{6'}$), 3.74 (s, 3H, $OCH_3$), 4.20 (d, 1H, $J$ = 7.7 Hz, $H_{1'}$), 4.49–4.53 (m, 2H, $H_7 + OH_{6'}$), 4.76 (d, 1H, $J$ = 11.7 Hz, $H_7$), 4.89 (d, 1H, $J$ = 4.7 Hz, $OH_{4'}$), 4.92 (d, 1H, $J$ = 4.6 Hz, $OH_{3'}$), 5.04 (d, 1H, $J$ = 4.9 Hz, $OH_{2'}$), 6.91 (apparent d, 2H, $H_3 + H_5$), 7.32 (apparent d, 2H, $H_2 + H_6$). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 54.94 ($OCH_3$), 61.07 ($C_{6'}$), 69.04 ($C_{4'}$), 70.08 ($C_7$), 73.37 ($C_{2'}$), 76.68 ($C_{3'}$), 76.81 ($C_{5'}$), 101.65 ($C_{1'}$), 113.41 ($C_3 + C_5$), 129.22 ($C_2 + C_6$), 129.76 ($C_1$), 158.56 ($C_4$).

*3-Methoxylbenzyl β-D-glucopyranoside* **3f**. $^1H$ NMR (DMSO-$d_6$): $\delta$ 3.01–3.17 (m, 4H, $H_{2'} + H_{3'} + H_{4'} + H_{5'}$), 3.44–3.50 (m, 1H, $H_{6'}$), 3.67–3.72 (m, 1H, $H_{6'}$), 3.75 (s, 3H, $OCH_3$), 4.22 (d, 1H, $J$ = 7.7 Hz, $H_{1'}$), 4.53 (t, 1H, $J$ = 5.9 Hz, $OH_{6'}$), 4.59 (d, 1H, $J$ = 12.6 Hz, $H_7$), 4.81 (d, 1H, $J$ = 12.6 Hz, $H_7$), 4.89 (d, 1H, $J$ = 4.6 Hz, $OH_{4'}$), 4.93 (d, 1H, $J$ = 4.6 Hz, $OH_{3'}$), 5.13 (d, 1H, $J$ = 4.9 Hz, $OH_{2'}$), 6.84 (dd, 1H, $J$ = 2.4, 8.2 Hz, $H_4$), 6.95 (d, 1H, $J$ = 7.6 Hz, $H_2$), 7.01 (s, 1H, $H_6$), 7.26 (t, 1H, $J$ = 7.9 Hz, $H_5$). $^{13}C$ NMR (DMSO-$d_6$): $\delta$ 55.63 ($OCH_3$), 61.96 ($C_{6'}$), 69.91 ($C_{4'}$), 71.03 ($C_7$), 74.19 ($C_{2'}$), 77.50 ($C_{3'} + C_{5'}$), 102.74 ($C_{1'}$), 113.57 ($C_4$), 113.67 ($C_2$), 120.20 ($C_6$), 129.59 ($C_5$), 140.37 ($C_1$), 159.94 ($C_3$).

*2-Methoxylbenzyl β-D-glucopyranoside* **3g**. $^1H$ NMR (DMSO-$d_6$): $\delta$ 3.03–3.18 (m, 4H, $H_{2'} + H_{3'} + H_{4'} + H_{5'}$), 3.43–3.49 (m, 1H, $H_{6'}$), 3.67–3.72 (m, 1H, $H_{6'}$), 3.78 (s, 3H, $OCH_3$), 4.25 (d, 1H, $J$ = 7.7 Hz, $H_{1'}$), 4.52 (t, 1H, $J$ = 5.9 Hz, $OH_{6'}$), 4.60 (d, 1H, $J$ = 13.5 Hz, $H_7$), 4.83 (d, 1H, $J$ = 13.5 Hz, $H_7$), 4.89 (d, 1H, $J$ = 4.8 Hz, $OH_{4'}$), 4.92 (d, 1H,

$J$ = 4.7 Hz, $OH_{3'}$), 5.08 (d, 1H, $J$ = 4.9 Hz, $OH_{2'}$), 6.91–6.98 (m, 2H, $H_3 + H_4$), 7.23–7.28 (m, 1H, $H_5$), 7.48–7.50 (m, 1H, $H_6$). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 55.05 ($OCH_3$), 60.96 ($C_{6'}$), 64.25 ($C_{4'}$), 70.11 ($C_{2'}$), 70.06 ($C_{3'}$), 73.30 ($C_7$), 76.54 ($C_{5'}$), 102.14 ($C_{1'}$), 110.28 ($C_3$), 119.74 ($C_5$), 126.05 ($C_4$), 127.89 ($C_1 + C_6$), 156.12 ($C_2$).

*4-Nitrobenzyl β-D-glucopyranoside* **3h**. H NMR (DMSO-$d_6$): $\delta$ 3.05–3.20 (m, 4H, $H_{2'} + H_{3'} + H_{4'} + H_{5'}$), 3.44–3.49 (m, 1H, $H_{6'}$), 3.68–3.72 (m, 1H, $H_{6'}$), 4.29 (d, 1H, $J$ = 7.7 Hz, $H_{1'}$), 4.55 (t, 1H, $J$ = 5.8 Hz, $OH_{6'}$), 4.77 (d, 1H, $J$ = 14.0 Hz, $H_7$), 4.92–4.98 (m, 3H, $OH_{3'} + OH_{4'} + H_7$), 5.21 (d, 1H, $J$ = 4.7 Hz, $OH_{2'}$), 7.69 (apparent d, 2H, $H_2 + H_6$), 8.22 (apparent d, 2H, $H_3 + H_5$). $^{13}$C NMR (DMSO-$d_6$): $\delta$ 61.04 ($C_{6'}$), 68.39 ($C_{4'}$), 70.02 ($C_{2'}$), 73.42 ($C_{3'}$), 76.59 ($C_7$), 76.94 ($C_{5'}$), 102.36 ($C_{1'}$), 123.17 ($C_3 + C_5$), 127.96 ($C_2 + C_6$), 146.29 ($C_1$), 146.65 ($C_4$).

## 3. Results

### 3.1. Effects of organic solvents and their contents on enzymatic synthesis of salidroside

Four water-miscible organic solvents including EGDA, *t*-butanol, dioxane and acetone were tested as the cosolvents for the enzymatic synthesis of salidroside (Fig. 1). As shown in Fig. 1a, the initial reaction rates were comparable in the media containing organic solvents of the same content. In 90% (v/v) organic solvent-containing systems, for example, the initial reaction rates ranged from 3.1 to 3.5 mM/h. Besides, the initial reaction rates increased with the decrease of the contents of organic solvents and to the highest when the content of organic solvents decreased to 80% (v/v), with the exception of dioxane-containing systems. The highest initial reaction rate (3.9 mM/h) was afforded in 80% (v/v) EGDA-containing system.

On the contrary, the yields decreased significantly with the decrement of the contents of organic solvents (Fig. 1b). For example, a yield of 17% was achieved in 90% (v/v) *t*-butanol-containing system, while the yield decreased to 12% in the presence of 70% (v/v) *t*-butanol. And the best yield (18%) was furnished in 90% (v/v) EGDA-containing system. The results showed that among four organic solvents, EGDA was the best for the enzymatic synthesis of salidroside.

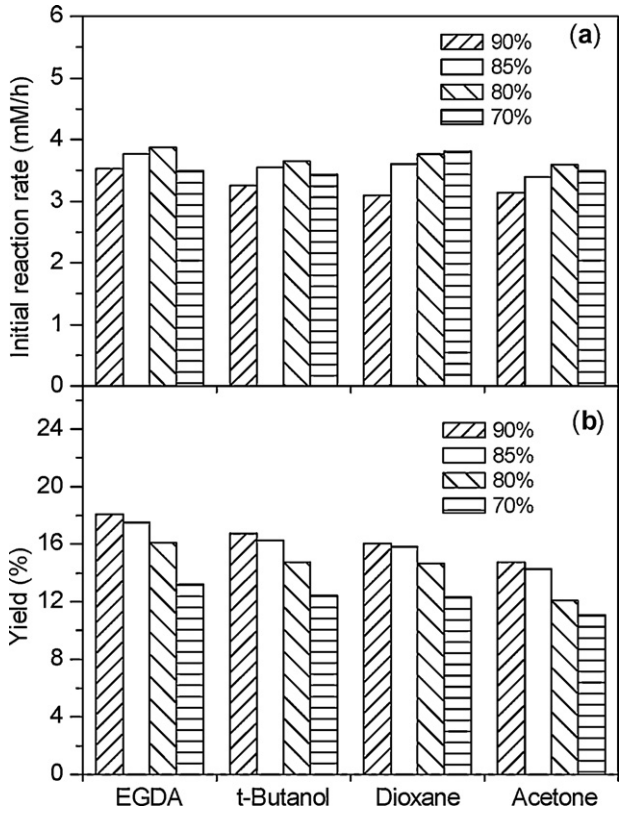


**Fig. 1.** Effects of organic solvents and their contents on the initial reaction rate (a) and yield (b) in the enzymatic synthesis of salidroside **3d**. Conditions: 0.5 mmol glucose, 5 mmol tyrosol, 5.5 U prune seed meal, organic solvent-phosphate buffer (pH 6.0, 50 mM), total volume 2 ml, 50 °C, 200 rpm. 90%, 85%, 80% and 70% represent volume percent of corresponding organic solvents in the reaction systems.

### 3.2. Effects of ILs and their contents on enzymatic synthesis of glucosides

Table 1 showed the effect of a variety of ILs on the enzymatic synthesis of salidroside. It was found that the initial reaction rates (3.0–3.3 mM/h) decreased slightly upon addition of several widely used ILs such as those containing $BF_4^-$ and $PF_6^-$ anions, but the improved yields (20–21%) were obtained except for that (17%) in [BMIm]$PF_6$-containing system (Table 1, entries 2–5). As shown in Table 1 (entries 6–8), similarly, the addition of [BMIm]$C_8SO_4$, [MMIm]$MeSO_4$ and [BMIm]I resulted in slower reaction rates and higher yields (20–22%). However, the enzyme displayed extremely low activities and even no activity in the presence of 10% (v/v) denaturing ILs such as [BMIm]Cl, [EOEMIm]Cl and [AcOEMIm]Cl, and the enzymatic reactions afforded low yields (Table 1, entries 9–11).

With the enzymatic synthesis of salidroside **3d** and 4-nitrobenzyl β-D-glucopyranoside **3h** as model reactions, the contents of ILs were optimized (Table 2). In the synthesis of **3d**, the optimum contents of [BMIm]$BF_4$, [BMMIm]$BF_4$, and [BMIm]$C_8SO_4$ were 10–15% (v/v) and the yields were increased to 21%. In the cases of [BMIm]$C_8SO_4$- and [BMIm]I-containing systems, the optimum contents of the two ILs were 10% (v/v). And among all the examined IL-containing systems, the best result (a yield of 22%) was obtained in 10% (v/v) [BMIm]I-containing system. However, the optimum content of the hydrophobic IL [BMIm]$PF_6$ was 5% (v/v), in which the improvement of the yield was negligible. Interestingly, such "denaturing" ILs as [BMIm]Cl, [EOEMIm]Cl and [AcOEMIm]Cl were found to enhance the enzymatic glucosylation at the optimum contents, affording higher yields (20%).

It was found that in the enzymatic synthesis of **3h**, the optimum contents of all the ILs were the same as those in the synthesis of **3d**. For example, the optimum content of [BMIm]$BF_4$ and [EOEMIm]Cl were 15% and 5% (v/v), respectively. It seemed not to be occasional since similar phenomena occurred in the synthesis of **3e** (Table 1S, available as supplementary material). Also, the highest yield (15%) was obtained in 10% (v/v) [BMIm]I-containing system.

**Table 1**
Effect of ILs on enzymatic synthesis of salidroside **3d**.

| Entry | IL | $V_0$ (mM/h) | Yield (%) |
|---|---|---|---|
| 1 | – | 3.5 | 18 |
| 2 | [BMIm]$BF_4$ | 3.0 | 21 |
| 3 | [BMMIm]$BF_4$ | 3.0 | 21 |
| 4 | [HMIm]$BF_4$ | 3.3 | 20 |
| 5 | [BMIm]$PF_6$ | 3.2 | 17 |
| 6 | [BMIm]$C_8SO_4$ | 2.9 | 20 |
| 7 | [MMIm]$MeSO_4$ | 3.0 | 21 |
| 8 | [BMIm]I | 3.1 | 22 |
| 9 | [BMIm]Cl | 0 | 0 |
| 10 | [EOEMIm]Cl | 0.5 | 9 |
| 11 | [AcOEMIm]Cl | 0.4 | 2 |

Conditions: 0.5 mmol glucose, 5 mmol tyrosol, 5.5 U prune seed meal, EGDA-10% (v/v) IL-10% (v/v) phosphate buffer (pH 6.0, 50 mM), total volume 2 ml, 50 °C, 200 rpm. Reaction time: 72 h.

**Table 2**
Effects of ILs and their contents on the enzymatic synthesis of the compounds **3d** and **3 h**.

| IL | **3d** | | **3h** | |
|---|---|---|---|---|
| | Optimum content (%, v/v) | Yield (%)/relative yield (%) | Optimum content (%, v/v) | Yield (%)/relative yield (%) |
| The control | – | 18/100 | – | 10/100 |
| [BMIm]$BF_4$ | 10–15 | 21/117 | 15 | 14/140 |
| [BMMIm]$BF_4$ | 10–15 | 21/117 | 15 | 13/130 |
| [BMIm]$C_8SO_4$ | 15 | 21/117 | 15 | 13/130 |
| [BMIm]I | 10 | 22/122 | 10 | 15/150 |
| [HMIm]$BF_4$ | 10 | 20/111 | 10 | 12/120 |
| [BMIm]$PF_6$ | 5 | 18/100 | 5 | 11/110 |
| [BMIm]Cl | 2.5 | 20/111 | 2.5 | 12/120 |
| [EOEMIm]Cl | 5 | 20/111 | 5 | 12/120 |
| [AcOEMIm]Cl | 5 | 20/111 | 5 | 12/120 |

Conditions: 0.5 mmol glucose, 5 mmol alcohol, 5.5 U prune seed meal, EGDA-various content of IL-10% (v/v) phosphate buffer (pH 6.0, 50 mM), total volume 2 ml, 50 °C, 200 rpm.

### 3.3. Enzymatic synthesis of arylalkyl glycosides in [BMIm]I-containing system

The synthesis of a group of arylalkyl β-D-glucopyranosides catalyzed by prune seed meal was performed in 10% (v/v) [BMIm]I-containing system (Table 3). The enzymatic glucosylation of benzyl alcohol afforded the product **3a** with a 21% yield (Table 3, entry 1). And the yield decreased to 17% with the elongation of alkyl chain (Table 3, entry 2). The highest yield (28%) was obtained after 68 h in the synthesis of 4-hydroxybenzyl β-D-glucopyranoside **3c** (Table 3, entry 3). As shown in Table 3 (entries 5–7), in the enzymatic glycosylation of a series of methoxy-substituted benzyl alcohol, the highest yield (26%) was furnished for 4-methoxybenzyl β-D-glucopyranoside **3e**, and lower (22%) for 3-methoxybenzyl β-D-glucopyranoside **3f**, and the lowest (15%) for 2-methoxybenzyl β-D-glucopyranoside **3g**. Besides, 4-nitrobenzyl β-D-glucopyranoside **3h** was synthesized with a yield of 15% after 50 h (Table 3, entry 8).

## 4. Discussion

*t*-Butanol and dioxane were widely used as organic cosolvents in glycosides synthesis [11,20]. However, little attention was paid to the enzymatic glycosylation in EGDA-containing systems [21]. The results showed that EGDA as the cosolvent was slightly better than other three organic solvents at the same content. Besides, compared to dioxane that is classified by the international agency for research on cancer (IARC) as a Group 2B carcinogen, EGDA is a green and safe solvent. And it has many good features such as slow evaporating rate, good flow properties and biodegradability. More importantly, unlike *t*-butanol, EGDA is partially miscible with water. So upon completion of the reaction, two phases would be formed by adding water, thus remarkably simplifying the downstream processing. In addition, the initial reaction rates increased, with decreasing contents of the organic solvents, while the yields deceased significantly. The possible reason might be that reverse hydrolysis is thermodynamically controlled, and a low water activity is desirable to shift the reaction equilibrium toward synthesis, thus leading to a higher yield [7,8]. On the other hand, the glycosidases are amenable to denaturation at a low water activity [13,22].

**Table 3**
Enzymatic synthesis of various glucosides in [BMIm]I-containing system.

| Entry | Glucoside | Time (h) | Yield (%) |
|---|---|---|---|
| 1 | **3a** | 42 | 21 |
| 2 | **3b** | 42 | 17 |
| 3 | **3c** | 68 | 28 |
| 4 | **3d** | 72 | 22 |
| 5 | **3e** | 50 | 26 |
| 6 | **3f** | 50 | 22 |
| 7 | **3g** | 50 | 15 |
| 8 | **3h** | 50 | 15 |

Conditions: 0.5 mmol glucose, 5 mmol alcohol, 5.5 U prune seed meal, EGDA-10% (v/v) [BMIm]I-10% (v/v) phosphate buffer (pH 6.0, 50 mM), total volume 2 ml, 50 °C, 200 rpm.

Zhao proposed that like the ions, the effects of ILs on the enzyme activity and stability followed the Hofmeister series in aqueous solutions of hydrophilic ILs: kosmotropic anions and chaotropic cations stabilize enzymes while chaotropic anions and kosmotropic cations destabilize them [23]. The kosmotropicity of the anions follow a decreasing order: $Cl^- > I^- > BF_4^- > PF_6^-$; according to the abovementioned principle, the enzyme activity should be the highest in $Cl^-$-containing system, and lower in $I^-$-containing system, the lowest in $PF_6^-$-containing system. Nevertheless, indeed, no activity was observed in [BMIm]Cl-containing system (Table 1, entry 9), and the enzyme activity in [BMIm]$PF_6$-containing system was comparable to those in [BMIm]$BF_4$- and [BMIm]I-containing systems (Table 1, entries 2, 5 and 8). Obviously, the effects of ILs on the enzyme activity did not follow the Hofmeister series in the present work, which might be ascribed to the presence of low content of water (10%, v/v) in the systems, thus preventing ILs from dissociating into individual ions.

As compared to that (3.5 mM/h) in the control, the initial reaction rates (2.9–3.3 mM/h) were slightly lower in IL-containing systems (Table 1, entries 2–8), which might stem from the high viscosities of ILs. The most extensively used ILs such as those with $BF_4^-$ were able to promote the enzymatic reactions, thus leading to higher yields (Table 1, entries 2–4), while a slightly lower yield (17%) was obtained in [BMIm]$PF_6$-containing system (Table 1, entry 5). The possible reason is that [BMIm]$PF_6$ is a hydrophobic IL, which has more poor ability of lowering water activity than those hydrophilic ILs. Previously, [MMIm]$MeSO_4$ was demonstrated to enable to improve the yields and/or the enzyme stability in the enzymatic glycosylation reactions [16,17]. Also, a higher yield (21%) was achieved with this IL as the cosolvent (Table 1, entry 7). Interestingly, unlike its counterparts containing $Cl^-$ and $Br^-$ anions, the novel IL [BMIm]I did not denature the enzyme. In addition, the enzymatic glycosylation of tyrosol was enhanced by adding this IL, affording a higher yield (Table 1, entry 8). To the best of our knowledge, the biocatalytic application of this IL was reported for the first time. The significant denaturation of the enzyme in the systems containing $Cl^-$ anion (Table 1, entries 9–11) might be attributed to high hydrogen bond basicity of this anion [14,24–26].

Table 2 showed that the optimization led to significant increment of the yields in the systems containing the denaturing ILs [BMIm]Cl, [EOEIm]Cl and [AcOEMIm]Cl. In the enzymatic synthesis of salidroside **3d**, the denaturation of the enzyme was remarkably

relieved by reducing the contents of these ILs, thus giving satisfactory yields (20%). Likewise, slightly higher yields (12%) were achieved in the synthesis of **3h** under the optimum contents of these ILs. To date, few enzymes were reported to be active in the denaturing ILs [27]. The striking results revealed that this biocatalyst (the prune seed meal) was strongly resistant to nonaqueous media including the denaturing ILs, displaying great synthetic application potential. Although the yield was only increased by 4–5% in the absolute value, which was not as significant as we expected, the addition of IL cosolvents led to significant improvements of the relative yields within the range from 0.2- to 0.5-fold. It was expected that the yields would be improved further by coupling the "solvent engineering" with *in situ* product removal [10,11,28].

As shown in Table 3, the effect of the structures of the glycosyl acceptors, especially the chain length of the alkyl, on the enzymatic reaction is remarkable. For example, the enzymatic glucosylation of benzyl alcohol **2a** afforded the product **3a** with a yield of 21%, whereas the yield (17%) was lower with phenethyl alcohol **2b** as the glycosyl acceptor. Similarly, the glucosylation yield (28%) of 4-hydroxybenzyl alcohol **2c** was higher than that (22%) of 4-hydroxyphenethyl alcohol **2d**. Sheldon and coworkers proposed that the equilibrium yield was reduced by about 40% for each extra carbon atom [7]. In addition, the enzyme preferred catalyzing the glucosylation of 4-hydroxy substituted aryl alcohols to that of no substituted analogs (Table 3, entries 1 and 3, 28% *vs.* 21%; entries 2 and 4, 22% *vs.* 17%). Besides, 4-methoxy substitution resulted in a higher yield (26%, Table 3, entry 5). The substitution pattern of the methoxyl on phenyl ring exerted a great effect on the yields (Table 3, entries 5–7). The yield was the highest for the *para*-substituted benzyl alcohol, and lower for the *meta*-substituted counterpart, and the lowest for the *ortho*-substituted counterpart. It might derive from the steric hindrance. The lowest yield (15%) was obtained in the glucosylation of 4-nitrobenzyl alcohol, which might be attributed to the electronic effect and/or steric strain of the strong electron-withdrawing $NO_2$ group [29]. Similarly, Xu and coworkers reported that the yield was the lowest for the glucosylation of 4-nitrobenzyl alcohol catalyzed by apple seed meal among a series of 4-substituted benzyl alcohol [20].

## 5. Conclusions

In conclusion, the ILs proved to be excellent cosolvents to lowering the water activity of the reaction system, thus improving the reaction equilibrium yields. Interestingly, the IL with halo anion [BMIm]I not only did not inactivate the enzyme, but also promoted the enzymatic glucosylation. Besides, the crude enzyme from prune seeds was highly active in the systems containing lower contents (2.5–5%) of denaturing ILs such as [BMIm]Cl, [EOEIm]Cl and [AcOEMIm]Cl, displaying great application potential in synthetic organic chemistry.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molcatb.2011.08.009.

## References

[1] V.M. Dembitsky, Lipids 39 (2004) 933–953.
[2] S. Yu, M. Liu, X. Gu, F. Ding, Cell. Mol. Neurobiol. 28 (2008) 1067–1078.
[3] J.P. Zhang, A.H. Liu, R.R. Hou, J. Zhang, X. Jia, W.F. Jiang, J.Z. Chen, Eur. J. Pharmacol. 607 (2009) 6–14.
[4] H.B. Wang, Y.Y. Ding, J. Zhou, X.L. Sun, S.W. Wang, Phytomedicine 16 (2009) 146–155.
[5] X. Hu, S. Lin, D. Yu, S. Qiu, X. Zhang, R. Mei, Cell Biol. Toxicol. 26 (2010) 499–507.
[6] X. Zhu, R.R. Schmidt, Angew. Chem. Int. Ed. 48 (2009) 1900–1934.
[7] F. van Rantwijk, M.W.V. Oosterom, R.A. Sheldon, J. Mol. Catal. B: Enzym. 6 (1999) 511–532.
[8] B.M. de Roode, M.C.R. Franssen, A. van der Padt, R.M. Boom, Biotechnol. Prog. 19 (2003) 1391–1402.
[9] A. Millqvist-Fureby, I.S. Gill, E.N. Vulfson, Biotechnol. Bioeng. 60 (1998) 190–196.
[10] B.M. de Roode, J. van Beek, A. van der Padt, M.C.R. Franssen, R.M. Boom, Enzyme Microb. Technol. 29 (2001) 513–520.
[11] H.L. Yu, J.H. Xu, W.Y. Lu, G.Q. Lin, J. Biotechnol. 133 (2008) 469–477.
[12] G. Vic, D. Thomas, D.H.G. Crout, Enzyme Microb. Technol. 20 (1997) 597–603.
[13] A. Ismail, S. Soultani, M. Ghoul, J. Biotechnol. 69 (1999) 145–149.
[14] S. Park, R.J. Kazlauskas, Curr. Opin. Biotechnol. 14 (2003) 432–437.
[15] Z. Yang, W.B. Pan, Enzyme Microb. Technol. 37 (2005) 19–28.
[16] M. Lang, T. Kamrat, B. Nidetzky, Biotechnol. Bioeng. 95 (2006) 1093–1100.
[17] N. Kaftzik, P. Wasserscheid, U. Kragl, Org. Process Res. Dev. 6 (2002) 553–557.
[18] F. van Rantwijk, R.A. Sheldon, Chem. Rev. 107 (2007) 2757–2785.
[19] U. Kragl, M. Eckstein, N. Kaftzik, Curr. Opin. Biotechnol. 13 (2002) 565–571.
[20] A.M. Tong, W.Y. Lu, J.H. Xu, G.Q. Lin, Bioorg. Med. Chem. Lett. 14 (2004) 2095–2097.
[21] T. Balogh, L. Boross, J. Kosary, Tetrahedron 60 (2004) 679–682.
[22] G. Ljunger, P. Adlercreutz, B. Mattiasson, Enzyme Microb. Technol. 16 (1994) 751–755.
[23] H. Zhao, J. Mol. Catal. B: Enzym. 37 (2005) 16–25.
[24] N. Li, D. Ma, M.H. Zong, J. Biotechnol. 133 (2008) 103–109.
[25] S. Lee, S. Ha, S. Lee, Y.-M. Koo, Biotechnol. Lett. 28 (2006) 1335–1339.
[26] M.B. Turner, S.K. Spear, J.G. Huddleston, J.D. Holbrey, R.D. Rogers, Green Chem. 5 (2003) 443–447.
[27] A.R. Toral, A.P. de los Rios, F.J. Hernandez, M.H.A. Janssen, R. Schoevaart, F. van Rantwijk, R.A. Sheldon, Enzyme Microb. Technol. 40 (2007) 1095–1099.
[28] F. Ahmed, A. Stein, G.J. Lye, J. Chem. Technol. Biotechnol. 76 (2001) 971–977.
[29] M.B. Smith, J. March, March's Advanced Organic Chemistry: Reactions, Mechanisms and Structure, 6th ed., John Wiley & Sons, Inc, Hoboken, New Jersey, 2007.