Highly regioselective glucosylation of 2′-deoxynucleosides by using the crude β-glycosidase from bovine liver

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ABSTRACT

An enzymatic regioselective approach for the glucosylation of a series of 2′-deoxynucleosides was described by using the crude β-glycosidase from bovine liver that is less expensive and can be simply prepared in a standard organic laboratory. With the glucosylation of 2′-deoxyuridine as a model reaction, the effects of several key factors on the enzymatic reaction were examined. The optimum enzyme dosage, buffer pH and temperature were 0.05 U/ml, 9.5 and 42 °C, respectively. The presence of alkali β-glycosidase as the main active component in the crude enzyme extract might account for the high glucosylation activity at pH 9.5. In addition, the desired 5′-*O*-glucosylated derivatives of 2′-deoxynucleosides were synthesized with the yields of 22–72% and exclusive 5′-regioselectivities (>99%).

1. Introduction

Nucleoside analogs constitute a vital class of anticancer and antiviral agents (Sanghvi and Cook, 1993; Yoo and Jones, 2006). For example, floxuridine (5-fluoro-2′-deoxyuridine, FUdR) has been used extensively in the clinical treatment of colon carcinoma and hepatic metastases for many years (Kemeny et al., 1999); and idoxuridine has proven to be a useful antiviral agent in the treatment of herpes simplex virus and varicella-zoster virus infections (De Clercq, 2004). However, these nucleoside agents generally suffer from poor bioavailability, low selectivity and various side effects, etc. in the clinical practice; thus, the chemical modified nucleosides without such drawbacks represent a promising direction for their pharmaceutical applications (De Clercq and Field, 2006; Landowski et al., 2005). It was reported that glycosylated derivatives of many active compounds could mask their toxicity and/or improve their pharmacokinetic properties. For instance, the toxicity of 5′-*O*-β-D-galactosyl-5-fluorouridine was more than 100-fold less than the parent compound to bone marrow cells in Balb/c mice (Abraham et al., 1994). In addition, it has been demonstrated that the cellular uptake of many active components could be significantly enhanced after glycosylation modification, due to the active absorption mediated by glucose transport system (Mizuma et al., 1992). D-Glucose conjugates of 7-chlorokynurenic acid, a potential neuroprotective agent, were able to facilitate the transport of this active compound across the blood–brain barrier (Battaglia et al., 2000).

Additionally, disaccharide nucleosides are an important group of natural compounds, which consist of a disaccharide that is linked to a base moiety through an *N*-glycosidic bond. Such compounds were often found as minor structural elements in a variety of biopolymers such as tRNA and poly(ADP-ribose) (Efimtseva et al., 2009), although their biological roles were still obscure. Moreover, a number of biologically active disaccharide nucleosides have been isolated and characterized (Efimtseva and Mikhailov, 2004). For example, the amicetin group antibiotics possess a broad spectrum of biological activities such as antibacterial, fungicidal, herbicidal, antitumor and antiviral activities; and adenophostins A and B, two disaccharide nucleosides, are the most powerful agonists of inositol 1,4,5-triphosphate receptors, which play an important role in Ca^{2+} release (Efimtseva and Mikhailov, 2002). Hence, the synthesis of such compounds is attracting increasing interest in the field of structural biology of biopolymer as well as in the pharmaceutical industry.

Enzymatic modification of the parent drugs, by virtue of simplicity, mild reaction conditions, exquisite selectivity and being environmentally friendly, is a promising alternative to chemical methods (Diaz-Rodriguez et al., 2005; Li et al., 2008, 2009a). Biocatalytic modification of nucleosides was reviewed comprehensively by our group (Li et al., 2010) and Ferrero et al. (Ferrero and Gotor, 2000). It was found that there were only few reports on enzymatic glycosylation of nucleosides in the two decades. Binder et al.,

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for example, described the synthesis of four *O*-β-D-galactosyl-nucleosides using β-galactosidase from *Aspergillus oryzae* through transgalactosylation, but the yields (3–7%) were pretty low (Binder et al., 1995). Andreotti et al. (2007) successfully synthesized a group of 5′-*O*-β-D-galactosylated nucleoside derivatives using the marine β-galactosidase from *Aplysia fasciata*. The yields generally ranged from low to good (12–60%), with the exception of 5′-*O*-β-D-galactosyl-uridine with a yield of 80%.

Recently, our group reported the regioselective galactosylation of FUdR catalyzed by a commercial β-galactosidase from bovine liver with a high yield (75%) and an excellent 5′-regioselectivity (>99%) (Zeng et al., 2010). Herein, we continued to extend our interest to the glucosylation modification of a group of 2′-deoxynucleosides by using the crude β-glycosidase from bovine liver, a less expensive biocatalyst (Scheme 1). To the best of our knowledge, the enzymatic synthesis of β-D-glucosyl-containing disaccharide nucleosides was reported for the first time.

2. Materials and methods

2.1. Materials

p-Nitrophenyl β-D-glucopyranoside (pNPGlc) was supplied by Alfa Aesar (USA). *o*-Nitrophenyl β-D-galactoside (oNPGal) was from Genebase Bioscience (Guangzhou, China). FUdR was purchased from Shanghai Hanhong (China). 2′-Deoxyuridine, thymidine and idoxuridine were bought from Tuoxin Biotechnology & Science (China). 5-Bromo-2′-deoxyuridine and the commercial β-galactosidase were obtained from Sigma–Aldrich (USA). All other chemicals were obtained from commercial sources and with the highest purity available.

2.2. Preparation of the crude β-glycosidase

Fresh bovine liver was obtained from a local market, diced into approximately 1 cm cubes and stored at −20 °C prior to use. All procedures were performed at 4 °C or on ice unless otherwise stated. To 100 ml phosphate buffer (10 mM, pH 6.0) containing protease inhibitors [1 mM EDTA, 1 mM β-mercaptoethanol, 0.02% NaN_3, 17 mM PMSF, 1% (v/v) isopropanol], 100 g bovine liver was added, and homogenized. The homogenate was centrifuged at 10 000 × *g* for 30 min at 4 °C, and the pellet was discarded. The supernatant was re-centrifuged at 30 000 × *g* for 60 min at 4 °C. The lipid layer on the surface of the supernatant was carefully removed with a pipette, and then the extract was incubated at 50 °C with gentle stir for 20 min, followed by treatment on ice for another 20 min. Then the extract was centrifuged at 15 000 × *g* for 20 min at 4 °C, and ammonium sulfate was added to the supernatant to 75% saturation. After incubation for 20 h, the precipitate was collected by centrifugation for 20 min at 8 000 × *g* and dissolved in Na_2HPO_4–citric acid buffer (50 mM, pH 6.0). The solution was dialyzed against this buffer overnight and the crude extract was collected by centrifugation at 15 000 × *g* for 10 min at 4 °C.

2.3. Enzyme activity assay of the crude β-glycosidase

To 0.5 ml phosphate buffer (100 mM, pH 7.0), 20 μl the crude enzyme solution and 0.5 ml pNPGlc solution (50 mM) were added. The reaction was conducted for 10 min at 37 °C and 200 rpm, and then stopped by adding 8.98 ml Na_2CO_3 (1 M). The released *p*-nitrophenol was assayed spectrophotometrically at 400 nm. One unit of glycosidase activity was defined as the amount of enzyme required to catalyze the release of 1 μmol *p*-nitrophenol per min under the conditions given. The specific activity of the crude β-glycosidase was 1.50 U/ml.

2.4. General procedure for enzymatic glucosylation of 2′-deoxynucleosides

In a typical experiment, 2 ml glycine–NaOH buffer (100 mM, pH 9.5) containing 0.04 mmol 2′-deoxynucleoside, 0.02 mmol pNPGlc and 0.1 U enzyme was incubated in a 15 ml Erlenmeyer shaking flask capped with a septum at 200 rpm and 42 °C. Aliquots were withdrawn at specified time intervals from the reaction mixture. The samples were treated at 100 °C for 5 min to denature the enzyme, and then diluted by 25 times with the corresponding mobile phase prior to HPLC analysis. Yields were calculated by the ratio of the actual product concentration to the theoretic product concentration based on the pNPGlc. The regioselectivity was defined as the percentage of the HPLC peak area of the desirable product in all the glucosylated products. All the enzymatic glucosylation experiments were carried out duplicate.

2.5. Synthesis, purification and structure determination of disaccharide nucleosides

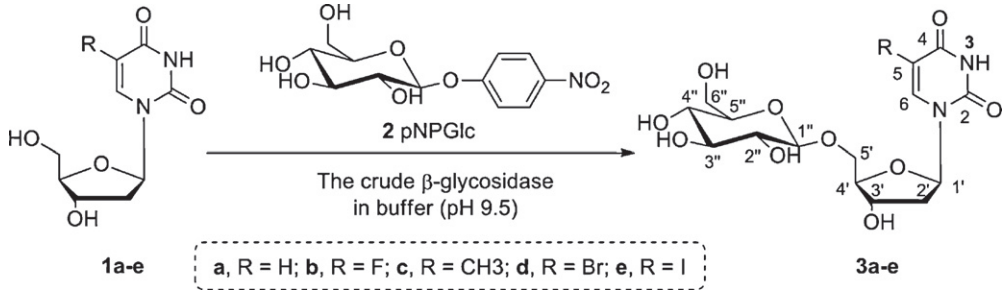
The product structure was determined by ^1H and ^{13}C NMR (Bruker AVANCE Digital 400 MHz NMR spectrometer, Germany) at 400 and 100 MHz, respectively.

5′-*O*-β-D-glucosyl-2′-deoxyuridine **3a** (13% isolated yield). To 20 ml phosphate buffer (100 mM, pH 9.5) containing pNPGlc (0.2 mmol, 60 mg) and 2′-deoxyuridine (0.4 mmol, 91.2 mg), 1.0 U the crude β-glycosidase was added. The reaction was conducted at 42 °C and 200 rpm for 11 h (monitored by HPLC). The mixture was treated at 100 °C for 10 min to denature the enzyme, filtered and then evaporated under reduced pressure leading to a residue, which was purified by column chromatography on silica gel. ^1H NMR (D_2O): δ 2.45–2.48 (m, 2H, H2′), 3.32–3.55 (m, 4H, H2″ + H3″ + H4″ + H5″), 3.74–3.79 (m, 1H, H4′), 3.87–3.99 (m, 2H, H6″), 4.21–4.26 (m, 2H, H5′), 4.55 (d, *J* = 7.6 Hz, 1H, H1″), 4.58–4.62 (m, 1H, H3′), 5.93 (d, *J* = 8.0 Hz, 1H, H6), 6.33 (t, *J* = 6.4 Hz, H1′), 7.96 (d, *J* = 8.0 Hz, 1H, H5). ^{13}C NMR (D_2O): δ 38.96 (C2′), 61.07 (C6″), 69.30 (C5′), 69.95 (C4″), 71.09 (C3′), 73.44 (C2″), 75.98 (C5″), 76.13 (C3″), 85.67 (C4′), 86.13 (C1′), 102.18 (C5), 102.51 (C1″), 142.36 (C6), 151.71 (C2), 166.41 (C4).

5′-*O*-β-D-glucosyl-FUdR **3b** (34% isolated yield). The reaction conditions were same as those for the synthesis of the compound **3a** except for FUdR (0.4 mmol, 98.4 mg). The reaction time was 11 h. ^1H NMR (D_2O): δ 2.37–2.49 (m, 2H, H2′), 3.33–3.57 (m, 4H, H2″ + H3″ + H4″ + H5″), 3.72–3.76 (m, 1H, H4′), 3.90–3.99 (m, 2H, H6″), 4.26 (apparent d, 2H, H5′), 4.56 (d, *J* = 8.0 Hz, 1H, H1″), 4.62 (br s, 1H, H3′), 6.31 (t, *J* = 6.4 Hz, 1H, H1′), 8.12 (d, *J* = 6.4 Hz, 1H, H6). ^{13}C NMR (D_2O): δ 38.84 (C2′), 60.82 (C6″), 68.46 (C5′), 69.66 (C4″), 70.55 (C3′), 73.11 (C2″), 75.59 (C5″), 75.79 (C3″), 85.46 (C4′), 85.84 (C1′), 101.89 (C1″), 125.59, 125.93 (C6), 139.22, 141.53 (C5), 149.73 (C2), 158.97, 159.23 (C4).

5′-*O*-β-D-glucosyl-thymidine **3c** (15% isolated yield). The reaction conditions were same as those for the synthesis of the compound **3a** except for thymidine (0.4 mmol, 96.8 mg). The reaction time was 22 h. ^1H NMR (D_2O): δ 1.95 (s, 3H, H7), 2.39–2.50 (m, 2H, H2′), 3.34–3.45 (m, 2H, H2″ + H4″), 3.50–3.58 (m, 2H, H3″ + H5″), 3.74–3.78 (m, 1H, H4′), 3.88–4.00 (m, 2H, H6″), 4.24 (t, 2H, H5′), 4.56 (d, *J* = 8.0 Hz, 1H, H1″), 4.61–4.64 (m, 1H, H3′), 6.35 (t, *J* = 6.8 Hz, 1H, H1′), 7.73 (s, 1H, H6); ^{13}C NMR (D_2O): 11.84 (C7), 38.76 (C2′), 61.09 (C6″), 69.44 (5′), 70.03 (C4″), 71.19 (C3′), 73.51 (C2″), 76.01 (C5″), 76.18 (C3″), 85.51 (C4′), 85.59 (C1′), 102.57 (C5), 111.41 (C1″), 137.68 (C6), 151.76 (C2), 166.53 (C4).

5′-*O*-β-D-glucosyl-5-bromo-2′-deoxyuridine **3d** (10% isolated yield). The reaction conditions were same as those for the synthesis of the compound **3a** except for 5-bromo-2′-deoxyuridine (0.4 mmol, 112.8 mg). The reaction time was 22 h. ^1H NMR (D_2O): δ 2.45–2.48 (m, 2H, H2′), 3.44–3.59 (m, 4H, H2″ + H3″ + H4″ + H5″),



Scheme 1. Enzymatic regioselective glucosylation of 2′-deoxynucleosides catalyzed by the crude β-glycosidase from bovine liver.

3.76–3.80 (m, 1H, H4′), 3.90–4.00 (m, 2H, H6″), 4.25–4.31 (m, 2H, H5′), 4.56 (d, J = 8.0 Hz, 1H, H1″), 4.65–4.68 (m, 1H, H3′), 6.33 (t, J = 6.4 Hz, 1H, H1′), 8.36 (s, 1H, H6); ^{13}C NMR (D_2O): δ 36.65 (C2′), 58.48 (C6″), 66.04 (C5′), 67.31 (C4″), 67.98 (C3′), 70.68 (C2″), 73.29 (C5″), 73.47 (C3″), 83.09 (C4′), 83.46 (C1′), 93.76 (C5), 99.64 (C1″), 138.60 (C6), 148.24 (C2), 159.17 (C4).

5′-*O*-β-D-glucosyl-idoxuridine **3e** (8% isolated yield). The reaction conditions were same as those for the synthesis of the compound **3a** except for idoxuridine (0.4 mmol, 141.6 mg). The reaction time was 30 h. ^{1}H NMR (D_2O): δ 2.48 (apparent t, 2H, H2′), 3.53–3.62 (m, 4H, H2″ + H3″ + H4″ + H5″), 3.81–3.85 (m, 1H, H4′), 3.91–4.03 (m, 2H, H6″), 4.27–4.33 (m, 2H, H5′), 4.58 (d, J = 6.8 Hz, 1H, H1″), 4.67–4.70 (m, 1H, H3′), 6.35 (t, J = 6.4 Hz, 1H, H1′), 8.39 (s, 1H, H6); ^{13}C NMR (D_2O): δ 39.59 (C2′), 61.43 (C6″), 68.40 (C5), 69.09 (C5′), 70.24 (C4″), 70.97 (C3′), 73.58 (C2″), 76.27 (C5″), 76.39 (C3″), 85.97 (C1′), 86.21 (C4′), 102.67 (C1″), 146.33 (C6), 151.70 (C2), 163.39 (C4).

2.6. HPLC analysis

The mixture of enzymatic glucosylation was analyzed by RP-HPLC on an XBridge™ C18 column (4.6 mm × 250 mm, 5 μm, Waters) using a Waters 1525 pump and a Waters 2489 UV detector with a flow rate of 1 ml/min.

A gradient elution [25 mM ammonium acetate solution/methanol: 80/20 (v/v) from 0 to 3.5 min; 80/20 (v/v) to 45/55 (v/v) from 3.5 to 4.5 min; 45/55 (v/v) from 4.5 to 9.5 min] was used for the analysis of the mixture of enzymatic glucosylation of 2′-deoxyuridine and FUdR. The retention times of 5′-*O*-β-D-glucosylated derivative, 2′-deoxynucleoside, pNPGlc and *p*-nitrophenol were 2.82, 3.04, 7.18 and 8.75 min (2′-deoxyuridine); 2.98, 3.35, 7.28 and 8.81 min (FUdR), respectively. Another gradient elution [25 mM ammonium acetate solution/methanol: 70/30 (v/v) from 0 to 4.2 min; 70/30 (v/v) to 45/55 (v/v) from 4.2 to 5.0 min; 45/55 (v/v) from 5.0 to 9.5 min] was used for the analysis of the mixture of enzymatic glucosylation of thymidine, 5-bromo-2′-deoxyuridine and idoxuridine. The retention times of 5′-*O*-β-D-gucosylated derivative, 2′-deoxynucleoside, pNPGlc and *p*-nitrophenol were 2.81, 3.16, 5.09 and 8.71 min (thymidine); 2.86, 3.48, 5.12 and 8.73 min (5-bromo-2′-deoxyuridine); 3.02, 3.74, 5.10 and 8.78 min (idoxuridine), respectively. The absorption wavelength for the analysis is 261 nm (2′-deoxyuridine), 267 nm (thymidine), 269 nm (FUdR), 279 nm (5-bromo-2′-deoxyuridine), 280 nm (idoxuridine), 300 nm (pNPGlc), 400 nm (*p*-nitrophenol), respectively.

3. Results and discussion

3.1. Effect of enzyme dosage

The structures of these disaccharide nucleosides obtained could be confirmed by ^{1}H and ^{13}C NMR spectra. The $J_{1''-2''}$ values (7.6 or 8.0 Hz) of anomeric proton of the glycosyl moiety in the ^{1}H NMR signal showed that the anomeric links of all the derivatives were of β-orientation (Hirata et al., 1999). Lichtenthaler et al. (1981) described the assignment rule for the *O*-glycosylation sites in the regioselective glycosylation of nucleosides by using ^{13}C NMR, where the glycosylation of the hydroxyl group in the ribose moiety resulted in a significant downfield shift for the appended ^{13}C nucleus, and smaller upfield displacements for the adjacent carbons. For example, chemical shift of C5′ of 2′-deoxyuridine changed from 61.81 ppm to 69.30 ppm after glucosylation modification, while chemical shift of C4′ decreased from 87.26 ppm to 85.67 ppm. According to Lichtenthaler's assignment rule, the product was 5′-*O*-β-D-glycosylated derivative. Similarly, other desired products could be assigned as 5′-*O*-β-D-glycosylated derivatives.

With the glucosylation of 2′-deoxyuridine as a model reaction, effects of several key factors on the enzymatic reaction were explored.

Firstly, the influence of enzyme dosage on this enzymatic reaction was investigated (Fig. 1). It was shown that the initial reaction rate increased rapidly with the increase of enzyme dosage in the range of 0.01–0.08 U/ml. The highest yield was achieved when the enzyme dosage increased to 0.05 U/ml, and further increase of the enzyme dosage led to comparable yields. However, the enzyme dosage had no effect on the 5′-regioselectivity. The optimum enzyme dosage was 0.05 U/ml.

3.2. Effect of buffer pH

It was well known that pH was one of the crucial effectors on the enzyme performance (Tipton and Dixon, 1979). It influenced not only the catalytic activity and the stability of the enzyme, but also the selectivity by altering the ionization state of the enzyme and the

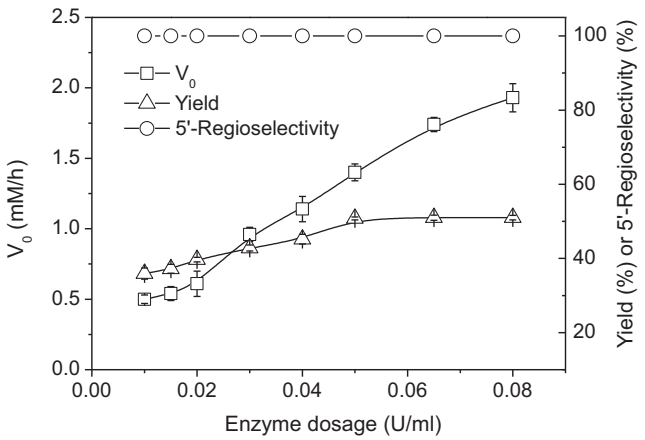


Fig. 1. Effect of enzyme dosage on the enzymatic glucosylation of 2′-deoxyuridine. Reaction conditions: 2 ml phosphate buffer (100 mM, pH 6.5) containing 0.02 mmol pNPGlc, 0.04 mmol 2′-deoxyuridine and the crude enzyme at 42 °C and 200 rpm.

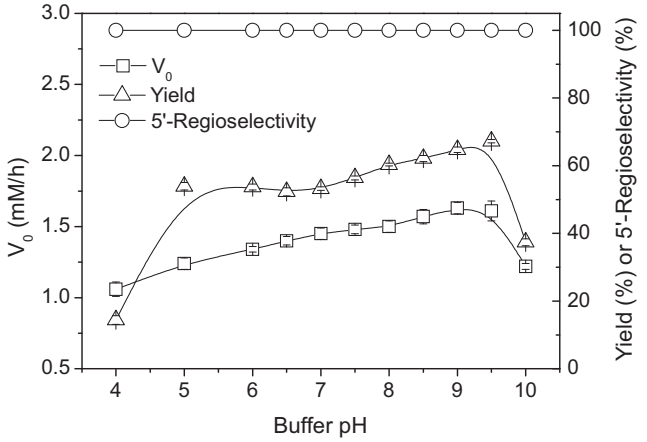


Fig. 2. Effect of buffer pH on the enzymatic glucosylation of 2′-deoxyuridine. Reaction conditions: 2 ml buffer (100 mM, Na_2HPO_4–citric acid, pH 4.0 and 5.0; phosphate buffer pH 6.0–8.0; glycine–NaOH, pH 8.5–10.0) containing 0.02 mmol pNPGlc, 0.04 mmol 2′-deoxyuridine and 0.1 U the crude enzyme at 42 °C and 200 rpm.

substrate(s). The effect of buffer pH on the enzymatic glucosylation of 2′-deoxyuridine was shown in Fig. 2. The results revealed that the enzymatic reaction was accelerated significantly when the buffer pH increased from 4.0 to 9.0. Further increase (pH 9.5) resulted in a slight decrement of the initial reaction rate. And the initial reaction rate dramatically decreased to 1.2 mM/h at pH 10.0. Likewise, the influence of the buffer pH on the yield was remarkable. For example, a yield of 14% was obtained at pH 4.0, and then the yield went up drastically with the increment of buffer pH. Both the high reaction rate and best yield (67%) were achieved at pH 9.5, which was the optimum. Previously, we found that the optimum pH of the commercial β-galactosidase from bovine liver was 6.5 in the enzymatic galactosylation of FUdR (Zeng et al., 2010). Apparently, the optimum pH of the enzyme responsible for the reaction in the crude enzyme of bovine liver is quite different from that of the commercial β-galactosidase. Is there a novel enzyme in the crude enzyme? Hence, the following experiments were designed to answer the question.

The optimum pH of the two biocatalysts was assayed spectrophotometrically with two glycosides (pNPGlc and oNPGal) as the hydrolytic substrate, respectively (Fig. 3). As shown in Fig. 3a, with pNPGlc as the substrate, the optimum pH of the commercial β-galactosidase was 7.0–7.5 while the crude β-glycosidase extract displayed the highest activity at 9.0–9.5. However, with oNPGal as the substrate, the aforementioned two biocatalysts had an optimum pH of 6.0 and 8.5, respectively (Fig. 3b). It was reported that the optimum pH of the enzyme might be altered by using different model reactions (Dwevedi and Kayastha, 2009). Indeed, the optimum pH of the crude enzyme made a slightly acidic shift with oNPGal as the substrate, as compared to that with pNPGlc. And a similar phenomenon appeared in the commercial β-galactosidase. But obviously, such changes due to the usage of different model reactions could not fully account for the great difference of the optimum pH of the two biocatalysts.

It was worth noting that the effect of pH on the commercial β-galactosidase-catalyzed hydrolysis of both pNPGlc and oNPGal did not give classical bell-shaped curves, but gave double bell-shaped ones. For instance, in Fig. 3a, two peaks of the activity appeared at pH 7.5 and pH 9.0, respectively; in Fig. 3b, there were also two activity peaks at pH 6.0 and pH 8.5, respectively. It could be explained by the fact that the commercial β-galactosidase from bovine liver was not purified to homogeneity according to the Sigma technical service. The results suggested the presence of at least two

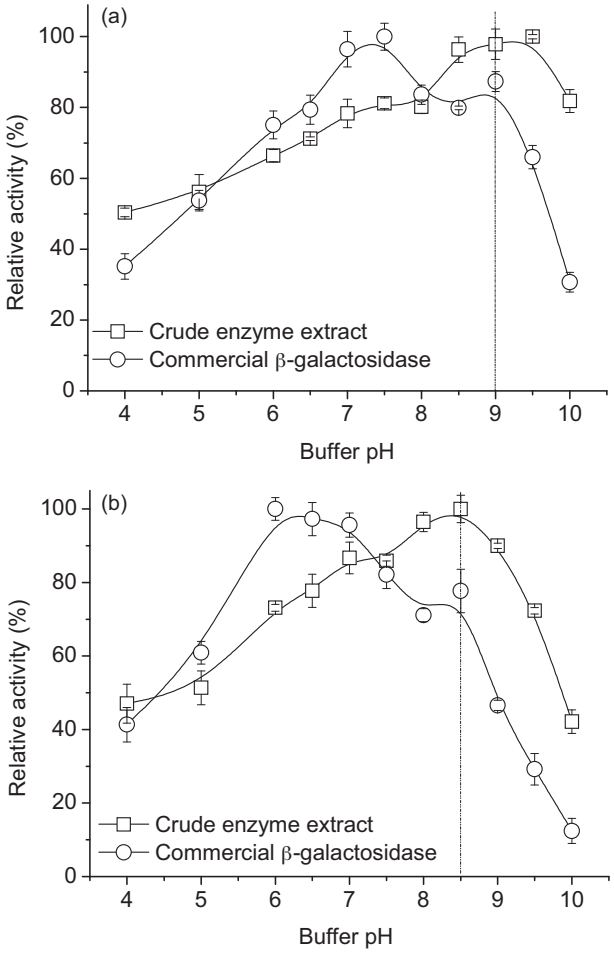


Fig. 3. Effect of buffer pH on the enzymatic hydrolysis of glycosides: (a) pNPGlc; (b) oNPGal. Reaction conditions: 1 ml buffer (100 mM, Na_2HPO_4–citric acid, pH 4.0 and 5.0; phosphate buffer pH 6.0–8.0; glycine–NaOH, pH 8.5–10.0) containing 0.01 mmol glycosides and 0.05 U enzyme at 42 °C and 200 rpm.

β-glycosidases in this commercial enzyme preparation, in which the major fraction was active at 6.0 or 7.5 while the minor have the high activity at basic pH. Interestingly, as shown in Fig. 3, the minor fraction in this commercial enzyme preparation was just corresponding to the major one in the crude enzyme extract of bovine liver. In addition, unlike commercial enzyme, the crude enzyme displayed the bell-shaped pH curves. It might stem from the different bovine specie and preparation method. In the crude extract, for example, most of the enzyme of acid pH optima might be removed during preparation. The β-glycosidase at alkaline pH seemed to be unique, because the pH optima of most β-glycosidases were located within the acidic or neutral range (Gekas and Lopez-Leiva, 1985; Joo et al., 2009; O'Connell and Walsh, 2010; Yu et al., 2007). The purification and characterization of this active fraction are under process in our laboratory, which might be a highly potential biocatalyst for the enzymatic glycosylation of nucleosides.

3.3. Effect of temperature

As shown in Fig. 4, the effect of reaction temperature on the initial reaction rate and yield was remarkable. The initial reaction rate increased rapidly with increasing temperature within the range from 35 to 55 °C. Nevertheless, the product yield altered marginally when the temperature increased from 35 to 45 °C. Further increase of temperature resulted in sharp decrement of the yield, possibly due to the denaturation of the biocatalyst. The best result was

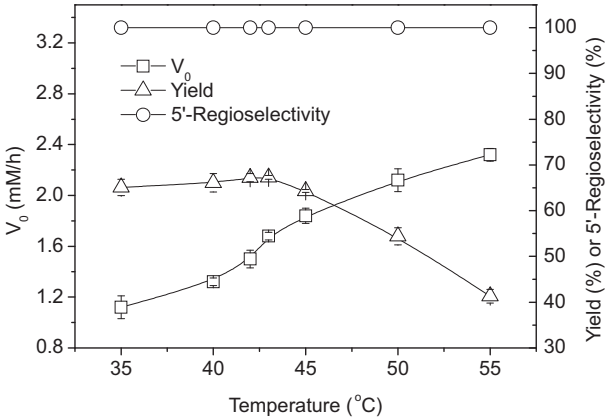


Fig. 4. Effect of temperature on the enzymatic glucosylation of 2′-deoxyuridine. Reaction conditions: 2 ml glycine–NaOH buffer (100 mM, pH 9.5) containing 0.02 mmol pNPGlc, 0.04 mmol 2′-deoxyuridine and 0.1 U the crude enzyme at 200 rpm and corresponding temperature.

obtained at around 42 °C, which was the optimum reaction temperature. Upon optimization of the key conditions, the yield was significantly improved from less than 50% to 67% with 100% 5′-regioselectivity retaining.

3.4. Effect of nucleoside structure

A group of glucosyl-containing disaccharide nucleosides was synthesized by using crude enzyme extract of bovine liver (Table 1). Interestingly, it was found that the initial reaction rate decreased with increasing bulk of R-group of 2′-deoxynucleosides **1a**–**e**. For example, the highest initial reaction rate was observed in the glucosylation of 2′-deoxyuridine **1a** with H as R-group (1.5 mM/h, entry 1), and the rate decreased in the glucosylation of FUdR **1b** with F as R-group (0.9 mM/h, entry 2), and decreased further in the glucosylation of thymidine **1c** with methyl as R-group (0.6 mM/h, entry 3), and finally to the lowest in the glucosylation of idoxuridine **1e** with I as R-group (0.2 mM/h, entry 5). In lipase-catalyzed regioselective acylation of 2′-deoxynucleosides, a similar effect of the R-group on the reaction rate was observed previously (Li et al., 2009a,b). The lower reaction rate might be ascribed to unfavorable steric hindrance of the larger R-group. The enzymatic glucosylation of 2′-deoxyuridine **1a** afforded a yield of 67% (entry 1); and FUdR **1b** was glucosylated enzymatically with a yield of 72% (entry 2); the lower yields of 33%, 49% and 22% were achieved in the enzymatic glucosylation of thymidine **1c**, 5-bromo-2′-deoxyuridine **1d** and idoxuridine **1e**, respectively (entries 3–5)). It was particularly worth noting that this biocatalyst displayed excellent regioselectivities (>99%) in the enzymatic glucosylation of all 2′-deoxynucleosides.

Table 1
Enzymatic glucosylation of 2′-deoxynucleosides catalyzed by the crude extract of bovine liver.

Entry	Nucleosides	R-	V_0 (mM/h)	Yield[a] (%)	5′-Regioselectivity (%)
1	**1a**	H	1.53 ± 0.05	67 ± 1	>99
2	**1b**	F	0.91 ± 0.06	72 ± 2	>99
3	**1c**	CH_3	0.58 ± 0.03	33 ± 2	>99
4	**1d**	Br	0.34 ± 0.03	49 ± 3	>99
5	**1e**	I	0.23 ± 0.02	22 ± 2	>99

Reaction conditions: 2 ml glycine–NaOH buffer (100 mM, pH 9.5) containing 0.02 mmol pNPGlc, 0.04 mmol nucleoside and 0.1 U the crude enzyme at 42 °C and 200 rpm.

[a] Yield determined by HPLC.

4. Conclusions

A group of disaccharide nucleosides have been successfully synthesized through an enzymatic approach. The crude β-glycosidase from bovine liver proved to be a versatile biocatalyst for the regioselective glucosylation of 2′-deoxynucleoside. In addition, this is the first report of the enzymatic synthesis of *O*-β-D-glucosylated derivatives of nucleosides. Particularly, the enzymatic glucosylation reactions occurred exclusively at the 5′-hydroxyl group of 2′-deoxynucleosides, furnishing excellent 5′-regioselectivities (>99%). As compared to chemical methods, our enzymatic approach exhibits great application potential in organic synthesis due to its simplicity, exquisite selectivity and being environmentally friendly.

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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.jbiotec.2011.06.031.

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