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A facile enzymatic process for the preparation of ibuprofen ester prodrug in organic media

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Abstract

A novel glucopyranoside derivative of ibuprofen, ibuprofen methyl α-D-glucopyranoside ester was synthesized via immobilized lipase-catalyzed esterification between racemic ibuprofen and methyl α-D-glucopyranoside in non-aqueous medium. An appropriate product concentration (4.6 mg ml^{-1}) was achieved by optimization of reaction conditions, such as solvent type, reaction temperature, enzyme concentration and initial concentration of substrates. Comparing with the parent drug ibuprofen in physicochemical properties, the glucopyranoside derivative of ibuprofen has better hydrophilicity. The chemical structure of the ibuprofen ester was confirmed to be methyl 6-*O*-(2′-(4′-isobutylphenyl) propionyl) α-D-glucopyranoside.

Keywords: Glucopyranoside derivative; Ibuprofen; Lipase; Esterification; Prodrug

1. Introduction

Ibuprofen is one of widely used non-steroidal anti-inflammatory drugs (NSAIDs) for the treatment of rheumatoid arthritis (RA). Like most other NSAIDs, it causes gastrointestinal (GI) ulceration and hemorrhage on long-term oral administration [1,2]. It is generally believed that these side effects resulted from the direct contact effect, which can be attributed to a combination of local irritation produced by the free carboxylic group of the NSAIDs and by local inhibition of the cytoprotective action of prostaglandin in the GI tract [3–8]. Consequently, it is necessary to develop their bioreversible derivatives such as prodrugs in order to decrease the toxicity induced by NSAIDs.

Prodrugs are defined as pharmacologically inactive derivatives of parent drugs that require chemical or enzymatic transformation within the body to release the active drugs [6,9–11]. The prodrug for temporarily masking the carboxylic group of NSAIDs is promising to reduce or abolish the GI toxicity due to the localized effect [3,4]. Ester and amide prodrugs are synthesized for this aim because they neither possess a free carboxylic group nor inhibit the prostaglandin biosynthesis. Moreover, toxicological and pharmacological profiles of some esters and amides of ibuprofen have been reported [2,3,11,12]. Some *N*,*N*-substituted glycoamide esters represented potentially useful derivatives of ibuprofen in reducing the GI toxicity, maintaining their therapeutic activity and improving their delivery characteristics [8].

In the development of NSAIDs prodrugs, it is necessary to carefully select alcohol or amine as the acyl acceptor, because an appropriate aqueous solubility and lipophilicity of the prodrugs is essential to give an acceptable bioavailability [4,8]. However, most of the prodrugs of NSAIDs reported in literatures have poor or limited water solubility [10,13]. The amphiphilic structure of methyl α-D-glucopyranoside makes it particularly efficient for such a purpose. This glucose derivative contains several reactive hydroxyl groups and can therefore be acylated by means of lipase-catalyzed reactions [14]. It has shown that the glucopyranoside derivatives of ibuprofen exhibited lower toxicity than ibuprofen on GI mucosa [15,16]. However, at least two synthesis steps are needed to obtain the glucopyranoside derivatives of ibuprofen by using chemical methods. Furthermore, regioselective synthesis of carbohydrate derivatives by chemical methods is complicated by the requirement of protection and deprotection steps due to the presence of several hydroxyl groups of

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similar reactivity [17,18]. Therefore, it is of considerably practical interest to develop alternative regioselective, enzymatic approaches [17,19,20]. In the present report, we developed a facile enzymatic process to directly synthesis the methyl α-D-glucopyranoside ester of ibuprofen from the racemic ibuprofen using immobilized lipase in the organic media with the benefit of only one synthesis step instead of the above two steps.

2. Materials and methods

2.1. Lipases and chemicals

Novozym 435 (lipase B from *Candida antarctica*, a non-specific lipase immobilized on a macroporous acylic resin with a specific activity 10,000 propyl laurate unit (PLU) g^{-1} and water content 1–2% (w/w), PLU is based on a reaction between propyl alcohol and lauric acid), Lipozyme IM (lipase from *Mucor miehei*, immobilized on a macroporous anion resin, specific activity 5–6 BAUN g^{-1}; water content 2–3% (w/w), Batch Acidolsis Unit Novo (BAUN) is based on a reaction between high oleic sunflower oil and decanoic acid at 70 °C for 60 min) were supplied by Novo Nordisk Bioindustrials Inc. Lipase from *Candida* sp. was immobilized by adsorption on siliceous earth [21]. *Porcine pancreas* lipase Type II was obtained from Sigma and *Candida lipolytica* lipase was produced and purified in our lab.

Methyl α-D-glucopyranoside was obtained from Sigma, and racemic ibuprofen was obtained from Shandong Pharmaceutical Co. Ltd. (China). (*S*)-Ibuprofen (purity, >99.9%) was obtained from Hubei Biocause Pharmaceutial Co., Ltd. All organic solvents were obtained from commercial suppliers (Shanghai Chemical Company, China) and were analytical grade or purer.

2.2. Analytical procedures

2.2.1. TLC analysis

Qualitative analysis of reaction mixtures was made by thin-layer chromatography (TLC) on silica gel plates eluted with $CHCl_3/CH_3OH/H_2O$ (65/15/2, v/v/v). TLC plates were visualized under UV lamp and/or visualized by heating (110 °C, 5 min) after spraying H_2SO_4/methanol (1:1). Under these conditions, ibuprofen and its ester had an R_f of 0.9 and 0.65, respectively.

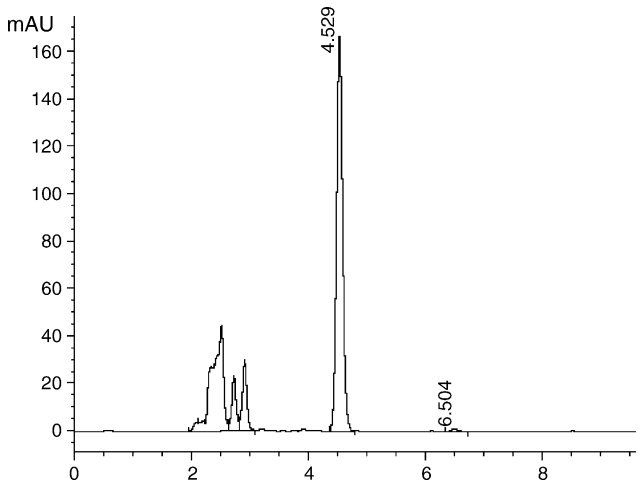


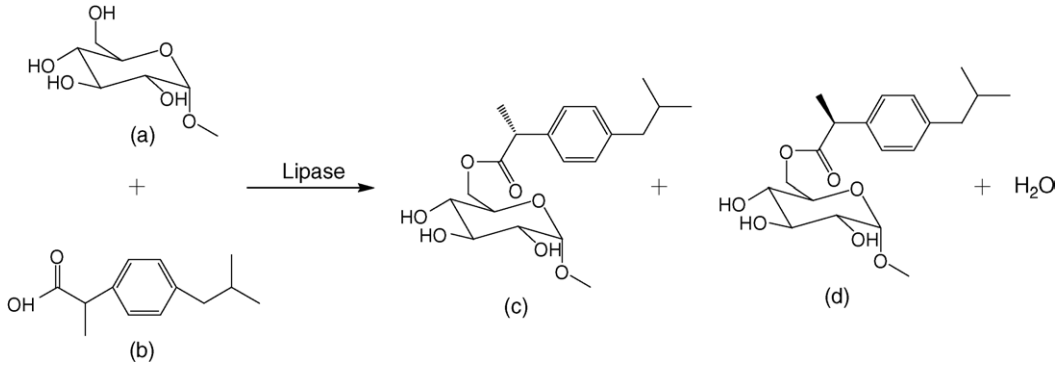
Fig. 1. HPLC of the purified product. The retention times of total (*R*, *S*)-ibuprofen and total (*R*, *S*)-esters were 6.504 and 4.528 min, respectively.

2.2.2. HPLC analysis

Quantitative analysis of samples was made by HPLC on a reverse phase column (Zorbax SB-C18, 5 μm, 4.6 mm × 250 mm, Agilent, USA) using Agilent 1100 series (USA) equipped with a DAD detector at 254 nm and ambient temperature. Elution was conducted with mixture of 0.02 M sodium acetate solution (pH 2.5, 40%) and acetonitrile (60%) at a flow rate of 1.0 ml min^{-1}. The volume of the injected sample was 10 μl. The concentration of ibuprofen was determined from the peak area based on calibration curve prepared using standard ibuprofen, as well as ibuprofen methyl α-D-glucopyranoside ester solution in organic medium. The result of HPLC is shown in Fig. 1. The retention times of total (*R*, *S*)-ibuprofen and total (*R*, *S*)-esters were 6.504 and 4.528 min, respectively.

2.3. Synthesis of (R, S)-ibuprofen ester

Unless otherwise indicated, in a typical synthesis of methyl α-D-glucopyranoside ester of ibuprofen, reactions were carried



Scheme 1. Route of synthesis of ibuprofen glucopyranside derivative. The percents of *R*-enantiomer (c) and *S*-enantiomer (d) in the product were 69.9 and 30.1%, respectively. (a) Methyl α-D-glucopyranoside, (b) ibuprofen, (c) 6-*O*-(2′*R*-(4′-isobutylphenyl) propionyl) α-D-glucopyranoside and (d) 6-*O*-(2′*S*-(4′-isobutylphenyl) propionyl) α-D-glucopyranoside.

out by mixing the indicated amount of corresponding (*R*, *S*)-ibuprofen with methyl α-D-glucopyranoside in a stopped glass bottle as shown in Scheme 1. To this reaction mixture was added 20.0 ml of organic media of interest. The mixture was incubated in a thermoconstant orbital shaker at the given temperature and shaking speed. When most of substrates had dissolved, the reaction was started by the addition of indicated amount of lipase. All experiments were carried out in duplicate. A blank control without enzyme was done with the same amount of substrates and reacted as described above. At the indicated time intervals, samples were withdrawn for HPLC analysis. At the end of the reaction, the mixture was extracted with CH_2Cl_2 by stirring at ambient temperature for 20 min. Hereby, the immobilized lipase was separated by flotation from the reaction mixture allowing an easy recovering of the biocatalyst. Organic solvent from the supernatant was removed in vacuo and the crude product was purified by silica gel chromatography (ethyl acetate/methanol, 10:1, v/v).

2.4. Purification and characterization of the product

Methyl α-D-glucopyranoside ester of ibuprofen is not commercially available. In order to obtain pure molecule (standard), purification of this compound was achieved by using a silica gel liquid chromatography column (300 mm × 20 mm). Eluent solution consisted of an ethyl acetate/methanol mixture (10:1, v/v) with 1 ml min^{-1} flow rate. Fractions containing product were pooled and the solvent was evaporated, a unique product was obtained at purity above 98% (w/w) (Fig. 1). The structure of purified product was also confirmed by ^1H and ^{13}C NMR.

2.5. Determination of aqueous solubility and partition coefficient

The solubility of ibuprofen and synthesized ester was determined in water at 25 °C by adding an excess of compound to water and vigorously shaking for 4 h at 27 °C on a mechanical shaker to initially exceed solubility at 25 °C. The mixture was then stored at 25 °C for 2 h to attain equilibrium at this temperature. After filtration/centrifugation, the aqueous layer was analyzed using the HPLC.

Partition coefficient was determined in octanol–water system using the shake flask method. Accurately weighted compound was dissolved in 1-octanol, and it was shaken with a water layer at 25 ± 2 °C for 1 h in a 50-ml conical flask with an ungreased glass stopper. Two layers were separated by centrifugation and the aqueous layer withdrawn using Eppendoff pipettes. The amount of compound in aqueous layer was quantified using HPLC and partition coefficient determined from the formula, $P = C_O \times V_W / C_W \times V_O$. C_O and C_W are solute concentrations in octanol and water phase after equilibration, respectively. V_W and V_O are the volume of water and octanol, respectively.

2.6. Chemical structure analysis

The product of interest was analyzed by mass spectrometry (MS) using Perkin-Elmer SCIEX API 100 for ESI measurements.

Structure of the synthesized and purified product was established by ^1H and ^{13}C NMR using Brücker AM 500 spectrometer (Karlsruhe, Germany) in $CDCl_3$ with TMS as internal standard.

Melting point (mp) was determined with electrothermal apparatus (Beijing Keyi Electro-optic Instrument Plant) and is uncorrected.

Infrared (IR) spectrum of purified compound was obtained on Nicolet Magna-IR550 (solvent, $CHCl_3$).

The enantiomeric purity of product was analyzed by automatic polarimeter WZZ-1S (Shanghai Precision Scientific Instrument Co., Ltd.).

3. Results and discussion

3.1. Selection of biocatalysts

Lipases have been successfully applied as catalysts to esterify substrates that contain at least one hydroxy or carboxyl group [17]. Four kinds of lipases from different origin were investigated in the esterification of ibuprofen and methyl α-D-glucopyranoside.

The product concentration catalyzed by the four lipases ranged from 0.2 to 3.1 mg ml^{-1} (Table 1). The best result was obtained with Novozym 435 lipase from *C. antarctica*, followed by lipase from *C.* sp. These two immobilized *Candida* lipase gave higher product concentration than the immobilized *M. miehei* (Lipozyme IM) and the other non-immobilized lipases, which supports the observation of Akoh and Mutua [22]. In addition, lipase from *C. antarctica* has the high activity and regioselectivity towards glucopyranoside [23]. Therefore, immobilized lipase Novozym 435 was used in all subsequent experiments.

3.2. Selection of suitable organic solvents

A suitable organic solvent should dissolve enough substrates for the lipase-catalyzed esterification, and while the solvent should not affect lipase activity and stability.

In order to find an appropriate solvent, we determined the solubility of methyl α-D-glucopyranoside and ibuprofen in various organic solvents at 25 °C (Table 2). As expected, the solubility of methyl α-D-glucopyranoside is very low and never exceeded more than 10.0 mg ml^{-1} (in *tert*-amyl alcohol). However, the solubility of ibuprofen was generally much higher than

Table 1
Effect of lipases from different resources on the esterification of methyl α-D-glucopyranosideand with ibuprofen

Enzyme	Concentration of product (mg ml^{-1})	Reaction time (h)
C. lipolytic	0.2	>200
M. miehei (Lipozyme IM)	0.3	>200
P. pancreas	0.3	>200
Candida sp.	0.4	>200
Novozym 435 (*C. antarctica*)	3.1	144

Reaction conditions: reaction was carried out in 20.0 ml acetonitrile containing 1.0 mmol methyl α-D-glucopyranoside and ibuprofen in the presence of 10 g l^{-1} enzyme at 50 °C and 200 rpm.

Table 2
Comparison of solubility and conversion with different organic solvents as reaction medium

Solvent	log *P*	Solubility (mg ml^{-1})		Concentration of product (mg ml^{-1})
		Ibuprofen	Methyl α-D-glucopyranoside	
Acetonitrile	−0.36	45	1.6	3.1
2-Butanone	0.29	240	1.2	0.7
t-Amyl alcohol	1.4	330	8.1	2.3
Cyclohexane	3.2	30[a]	<1.0	0
Hexane	3.5	35[a]	<1.0	0
Heptane	4.0	24[a]	<1.0	0
Isooctane	4.5	10[a]	<1.0	0

Reaction conditions: ibuprofen methyl α-D-glucopyranoside ester synthesis was performed in 20.0 ml organic solvent containing 1.0 mmol methyl α-D-glucopyranoside and ibuprofen in the presence of Novozym 435 (10.0 g l^{-1}). The mixture was incubated at 50 °C and 200 rpm for 144 h. (log *P* was taken as an indicator of solvent polarity.)
[a] Data from Higgins et al. [24].

that of methyl α-D-glucopyranoside in various organic solvents. Despite this, we found high conversions in these organic media.

Table 2 shows that there is no apparent correlation between the polarity of the solvent, represented as log *P* (octanol–water partition coefficient), and the activity of lipase. In apolar solvents, such as hexane and isooctane, no product can be detected. The absence of conversion in most hydrophobic solvents in the present study can therefore be ascribed to the very low solubility of methyl α-D-glucopyranoside rather than to the lack of activity of lipase (Table 2). Among all the solvents tested, acetonitrile (log $P = -0.36$) gives the highest product concentration (3.1 mg ml^{-1}). Synthesis was thus further studied in acetonitrile. The single product appears as a dark spot on TLC plates.

3.3. Effect of reaction temperature

Esterification of methyl α-D-glucopyranoside and ibuprofen catalyzed by Novozym 435 was investigated at different temperatures (30–70 °C) (Fig. 2). More than 3.1 mg ml^{-1} of ibuprofen methyl α-D-glucopyranoside ester could be obtained within 6 days when reaction temperature exceeded 50 °C. However, the concentration of product at 70 °C was half of the concentration at 60 °C, which may be due to enzyme inactivation. Thus, 50 °C was chosen for further studies.

3.4. Effect of lipase concentration

High enzyme concentrations involve shorter reaction times, but increase process cost. Therefore, it is necessary to reach a compromise between productivity and enzyme input. For this reason, increasing Novozym concentrations (in the range of 5–50 g l^{-1}) were used to acylate methyl α-D-glucopyranoside with ibuprofen in acetonitrile. As shown in Fig. 3, yield increased as increasing enzyme concentration. In fact, the enzyme concentration does not influence the equilibrium of reaction. It indicated that increase of enzyme amount could shorten the time of reaching the reaction equilibrium. At high enzyme concentration, the enzyme consumption number (e.c.n.) [25], however, increased

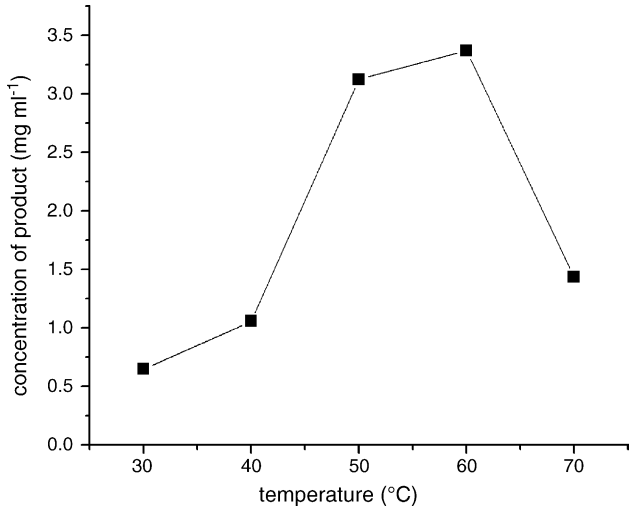


Fig. 2. Effect of temperature on the product concentration of the reaction. Reaction conditions: ibuprofen methyl α-D-glucopyranoside ester synthesis was performed in 20.0 ml acetonitrile containing 1.0 mmol of α-D-glucopyranoside and ibuprofen in the presence of Novozym 435 (10.0 g l^{-1}) at 200 rpm for 144 h.

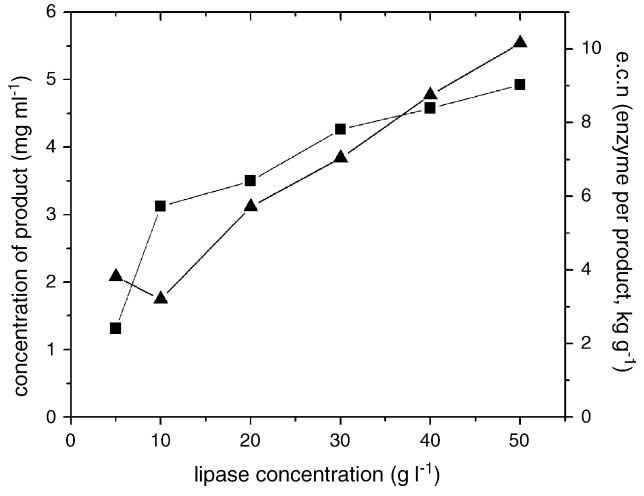


Fig. 3. Effect of lipase concentration on the esterification of ibuprofen with methyl α-D-glucopyranoside: (■) concentration of product; (▲) e.c.n. Reaction conditions: ibuprofen methyl α-D-glucopyranoside ester synthesis was performed in 20.0 ml acetonitrile containing 1.0 mmol of α-D-glucopyranoside and ibuprofen. The mixture was incubated at 50 °C and 200 rpm for 144 h.

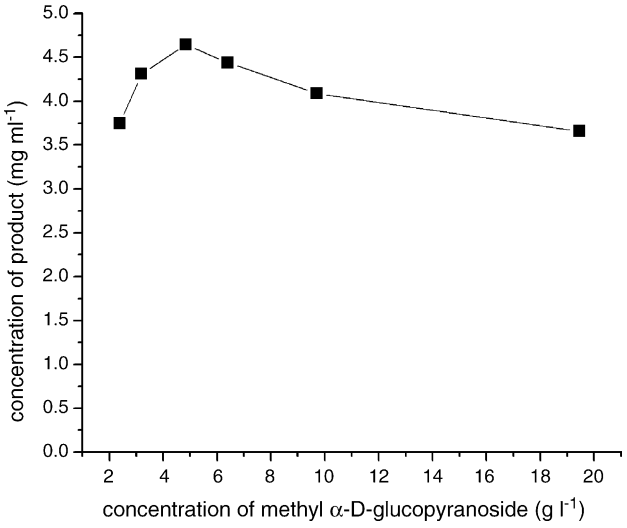


Fig. 4. Effect of initial glucopyranoside concentration on product yield. Reaction conditions: ibuprofen methyl α-D-glucopyranoside ester synthesis was performed in 20.0 ml acetonitrile containing 100 mM of ibuprofen in the presence of Novozym 435 (10.0 g l^{-1}). The mixture was incubated at 50 °C and 200 rpm for 144 h.

significantly. It may be due to the difficulty to maintain uniform suspension of the biocatalysts.

3.5. Influence of initial methyl α-D-glucopyranoside concentration on ibuprofen ester synthesis

The final quantity of ibuprofen methyl α-D-glucopyranoside ester obviously depends on the amount that can be converted by enzyme. So, it is important to study the effect of initial methyl α-D-glucopyranoside concentration on the yield for process optimization. The concentration of ibuprofen was held constant in all cases. The concentration of product increased as increasing methyl α-D-glucopyranoside concentration from 2.4 to 4.8 g l^{-1} (Fig. 4). A maximal concentration of product (4.6 mg ml^{-1}) is obtained with an initial concentration of 4.8 g l^{-1}.

The solubility of methyl α-D-glucopyranoside in the reaction system was approximately 2.0 mg ml^{-1}. Therefore, 2.4 or 4.8 g l^{-1} of methyl α-D-glucopyranoside added gave the same initial concentration of soluble glucopyranoside. The higher product concentration observed at 4.8 g l^{-1} methyl α-D-glucopyranoside could be explained by the excess of unsolubilized glucopyranoside which was continuously solubilized as the reaction proceeded, thereby providing a high substrate concentration during the entire reaction time. Higher concentration did not increase the yield, which may be due to the inhibition of the enzyme by the glucopyranoside [26,27] and/or the low solubility of methyl α-D-glucopyranoside in acetonitrile.

3.6. Study of lipase stability

For the determination of the operation stability, the model synthesis of ibuprofen ester prodrug was studied for four subsequent reaction cycles (each 144 h) in acetonitrile containing 0.5 mmol of methyl α-D-glucopyranoside and 1 mmol ibuprofen with Novozym 435 (0.2 g) at 50 °C. After completion of each reaction, the enzyme separated from the reaction mixtures (as outlined in the experimental section) was dried in vacuo immediately and used for the next cycle. Novozym 435 activity decreased from 3.38 g ml^{-1} (87.3%, first cycle) to 1.23 mg ml^{-1} (31.7%, fourth cycle), product yields at the second cycle and the third cycle are 1.96 and 1.25 mg ml^{-1}, respectively.

Table 3
Physicochemical properties of ibuprofen (I) and its glucopyranoside derivative (II)

Compound	Aqueous solubility (mg ml^{-1})	log *P*	Melting point (°C)
I[a]	0.028	3.71	75–77
II	0.934	−0.943	52–55

[a] Data from Higgins et al. [24].

3.7. Physicochemical properties of the glucopyranoside derivative of ibuprofen

The enzymatic hydrolysis of esters is highly sensitive to the hydrophilicity of substrate itself. The glucopyranoside derivative of ibuprofen is more soluble in water than is ibuprofen and has lower octanol–water partition coefficient (log *P*) value (Table 3). The solubility of racemic ibuprofen is about 32 times lower than that of its glucopyranoside derivative in the water at 25 °C, which may facilitate biomembrane transport and make the parent drug more bioavailable from the site of administration, such as the GI tract, the rectum, the blood–brain barrier, the skin, or the eyes.

3.8. Product analysis

After acylation of methyl α-D-glucopyranoside (1 mmol) with ibuprofen (1 mmol) using Novozym 435 (0.2 g) as biocatalyst, optical rotation of residual ibuprofen was analyzed. The specific rotations ($[\alpha]_D^{25}$ ($c = 1.0$ in ethanol)) of residual ibuprofen and (*S*)-Ibuprofen were $+21.2^0$ and $+53.3^0$, respectively. Then, the optical purity of product was 39.7%, and the percent of *R*-enantiomer in the product was 69.9%.

The product purified by silica gel liquid chromatography was analyzed by MS, NMR and IR.

3.8.1. Mass spectrometry and IR

Mass spectrometry data gave a molecular ion at $m/z = 382$, corresponding exactly to ibuprofen methyl α-D-glucopyranoside ester's molecular mass.

Fig. 5 presents the IR spectra of methyl α-D-glucopyranoside ibuprofen ester. Absorption bands were at 3000–2850 cm^{-1} (C–H stretch in CH_3 and/or CH_2), 1734 cm^{-1} (ester C=O), 1460 cm^{-1} (C–H stretch in CH_3 and/or CH_2) and 920 cm^{-1} (pyranose ring).

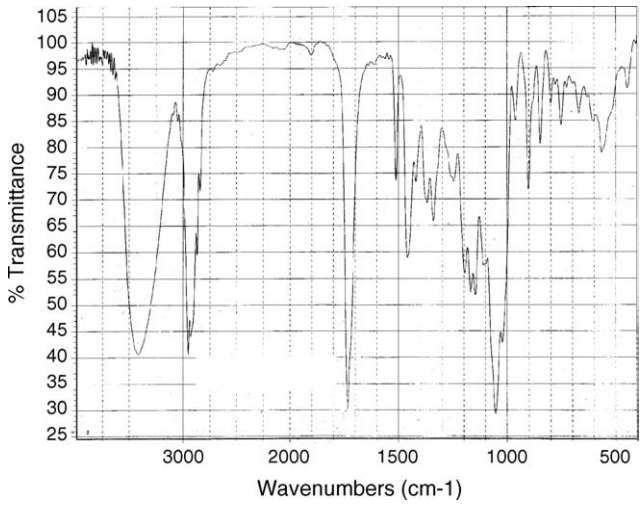


Fig. 5. IR spectrum of ibuprofen methyl α-D-glucopyranoside ester.

3.8.2. Nuclear magnetic resonance (NMR)

Thanks to NMR data, it was possible to show that ibuprofen was exclusively grafted onto the C^6 position of the methyl α-D-glucopyranoside. ^{1}H NMR data are as follows (500 MHz, $CDCl_3$): δ 7.19 (d, 2H, J = 7.97 Hz, H-2′, H-6′), 7.10 (d, 2H, J = 7.96 Hz, H-3′, H-5′), 4.64 (d, 1H, J = 3.60 Hz, H-1), 4.26–4.38 (3H, H-6a, H-6b, H-5), 3.74 (q, 1H, J = 7.17, ROOC-C$\underline{H}$CH$_3$-R′), 3.63 (3H, OH-2,3,4), 3.43 (2H, H-2, H-4), 3.24 (t, 1H, J = 9.47 Hz, H-3) 3.20 (s, 3H, ROC$\underline{H}_3$), 2.42 (d, 2H, J = 7.13 Hz, C$\underline{H}_2$CH(CH$_3$)$_2$), 1.82 (m, 1H, J = 6.74 Hz, CH$_2$C$\underline{H}$(CH$_3$)$_2$), 1.48 (d, 3H, J = 7.15 Hz, ROOC-CHC$\underline{H}_3$-R′), 0.88 (d, 6H, J = 6.60 Hz, CH$_2$CH(C$\underline{H}_3$)$_2$).

The product was purified and analyzed by ^{13}C NMR spectrometry to identify the position esterified with ibuprofen (Fig. 6). The ^{13}C NMR chemical shift data (ppm) of the product were as follows (500 MHz, $CDCl_3$): δ 175.7 (C=O), 141.2 (C-4′), 138.1 (C-1′), 130.0 (C-2′, C-6′), 127.9 (C-3′, C-5′), 99.9 (C-1), 74.6 (C-2, C-5), 72.5 (C-3), 71.1 (C-4), 70.3 (C-6), 64.6 (RO$\underline{C}$H$_3$), 55.6 ($\underline{C}$H$_2$CH(CH$_3$)$_2$), 45.7 (ROOC-$\underline{C}$HCH$_3$-R′), 30.8 (CH$_2\underline{C}$H(CH$_3$)$_2$), 23.0 (CH$_2$CH($\underline{C}$H$_3$)$_2$), 19.2 (ROOC-CH$\underline{C}$H$_3$-R′).

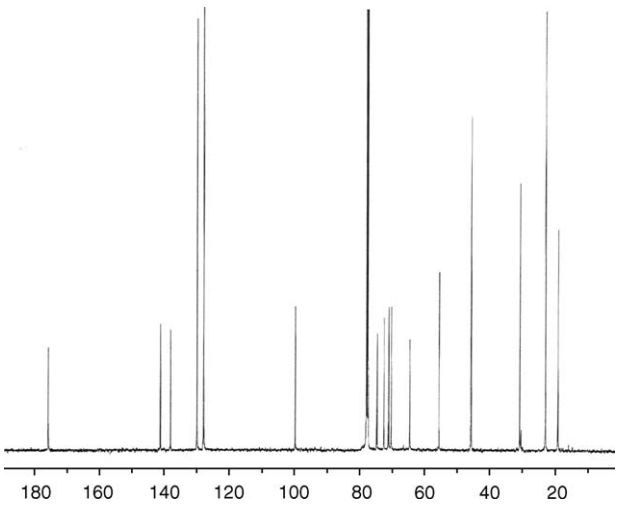


Fig. 6. ^{13}C NMR spectrum of ibuprofen methyl α-D-glucopyranoside ester in $CDCl_3$–TMS.

These data confirmed the structure of the product to be methyl 6-*O*-(2′-(4′-isobutylphenyl) propionyl) α-D-glucopyranoside.

4. Conclusions

In this work, we established a new and potential program of enzymatic esterification of glucopyranoside and ibuprofen.

The approach concerning synthesis of glucopyranoside derivative of ibuprofen provided a promising way for grafting alkyl glucopyranoside onto ibuprofen directly, which will be superior to chemical method in many aspects. In addition, the related process is environmentally benign compared with the traditional chemical method: the biocatalyst and the formed esters are non-toxic and biodegradable, and the used solvent has low toxicity and is easily recovered.

Investigation is now in progress to test the therapeutic and anti-inflammatory properties of the above product.

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