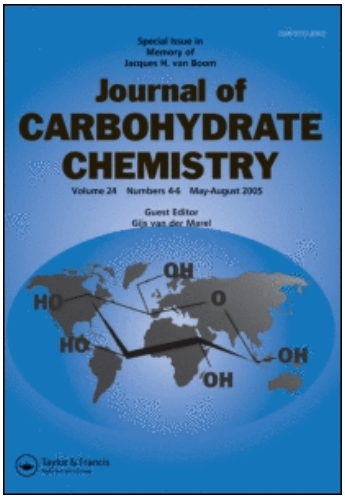

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Guoling Zhao[a]; Weidong Yan[b]
[a] China Tobacco Hunan Ind. Corp., Changsha, China [b] Department of Chemistry, Zhejiang University, Hangzhou, China

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Synthesis of Betulin-3-yl-ß-D-Glucopyranoside

Guoling Zhao[1] and Weidong Yan[2]

[1]China Tobacco Hunan Ind. Corp., Changsha 410001, China
[2]Department of Chemistry, Zhejiang University, Hangzhou 310027, China

Two concise routes toward betulin-3-yl-ß-D-glucopyranoside, being different in the protection of primary alcohol of betulin, were developed. The synthesis adopted a stepwise glycosidation method employing glycosyl trichloroacetimidate as donor.

Keywords Betulin; Glycosylation; Schmidt method

INTRODUCTION

The high content of betulin (Fig. 1) in birch bark (up to 20%) and its exceedingly simple extraction have stimulated research on its use as starting material for synthesizing biologically active compounds.[1] Triterpenoid of the lupeol group (betulin, lupeol, betulinic acid, and their derivatives) have recently been reported to possess high biological activity (anti-inflammatory, cholagogic, antiviral, antitumor, etc.).[2] Unfortunately, betulin suffers a low water solubility, resulting in inefficient biological efficacy.[3] Since it is generally accepted that glycosides are more water soluble than the respective aglycones, the introduction of a glycoside chain has become a popular tool for increasing water solubility and bioavailability.[4] Recently, it was reported that the glycoside of many triterpenoids and steroids are biologically more available to living organisms than their starting aglycons.[5] An enormous amount of precise biological studies testify that several important biological and pharmacological activities lie in the sugar moieties: sugars seem to play a key role in the interaction of the drugs with their receptors.[6]

Several approaches have been taken with success for the chemical synthesis of betulin glycosides, the majority of synthetic studies focused on glycosylation using the Koenigs-Knorr method.[7–9] Uvarova et al. reported on the modifications of the Koenigs-Knorr reaction used for glycosylation

Address correspondence to Guoling Zhao, China Tobacco Hunan Ind. Corp., Changsha 410001, China. E-mail: zhaogaigai@hotmail.com

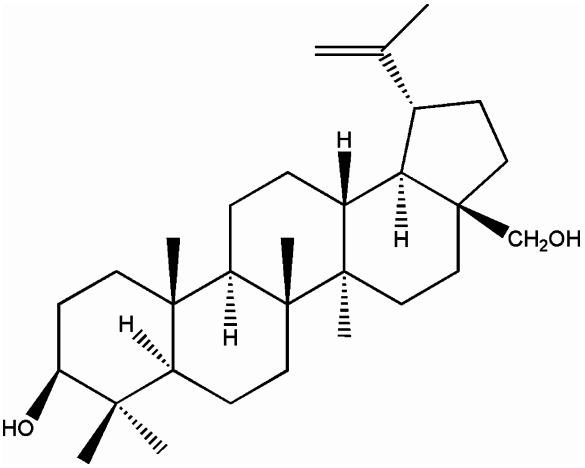


Figure 1: Structure of betulin in white birch bark.

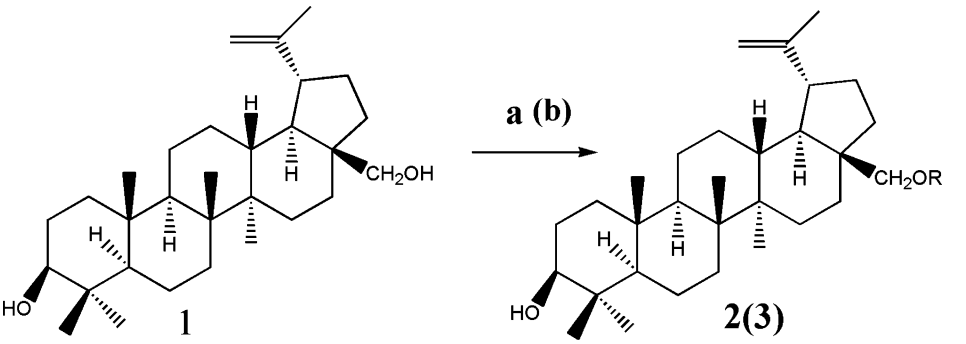
of steroid and triterpenoid alcohols. Six betulin glycosides have been prepared.[7]

Little work has been published concerning the preparation of betulin glycosides using the Schmidt method. Trichloroacetimidate-mediated glycosylation was reported by Schmidt and his coworkers in 1980 as an alternative useful method to the classical Koenigs-Knorr procedure and now appears to be one of the most ideal glycosylation protocols.[10] Our research group is interested in the synthesis of triterpenoid saponins of the lupane type. In this article employing a procedure similar to that used for the previous synthesis of α-hederin, 3-*O*-ß-D-glucopyranoside of betulin was readily obtained from protected betulin and 2, 3, 4, 6-tetra-*O*-benzoyl-β-D-glucopyranosyl trichloroacetimidate under the promotion of TMSOTf at low temperature.[11]

RESULTS AND DISCUSSION

From the structure of betulin, we can see that betulin has two hydroxyl groups (C-3, C-28). To perform the glycosidation at the C-3 position of betulin, we need to protect the hydroxyl at C-3 and prepare the corresponding mono-acetate and trityl ether at first. As the reactivity of the C-28 hydroxyl group of betulin is much higher than the one at C-3, 28-acetoxybetulin was obtained in moderate yield (73%) by using an excess of acetic anhydride in CH_2Cl_2 at rt.[12] Accordingly, tritylation of betulin at C-28 was also obtained using trityl chloride in anhydrous pyridine overnight at 80°C (Sch. 1).[13]

Previous experience has shown that construction of the glycosidic linkage with the aglycone is critical in the synthesis of saponins. The conditions for glycosylation of the aglycone are best developed at the monosaccharide level.[14–16]

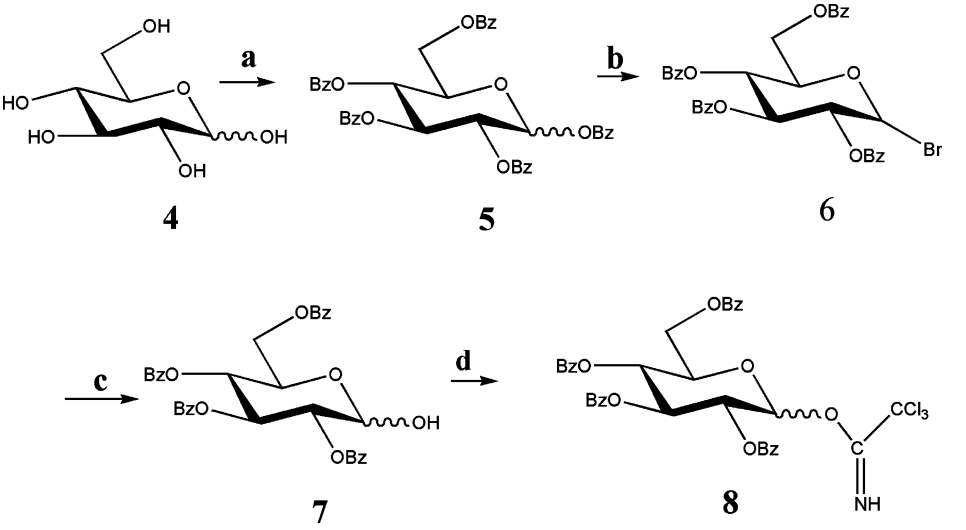


Scheme 1. Reagent and reaction conditions: (a) acetic anhydride, dichloromethane, rt; (b) trityl chloride, pyridine, 80°C, overnight.

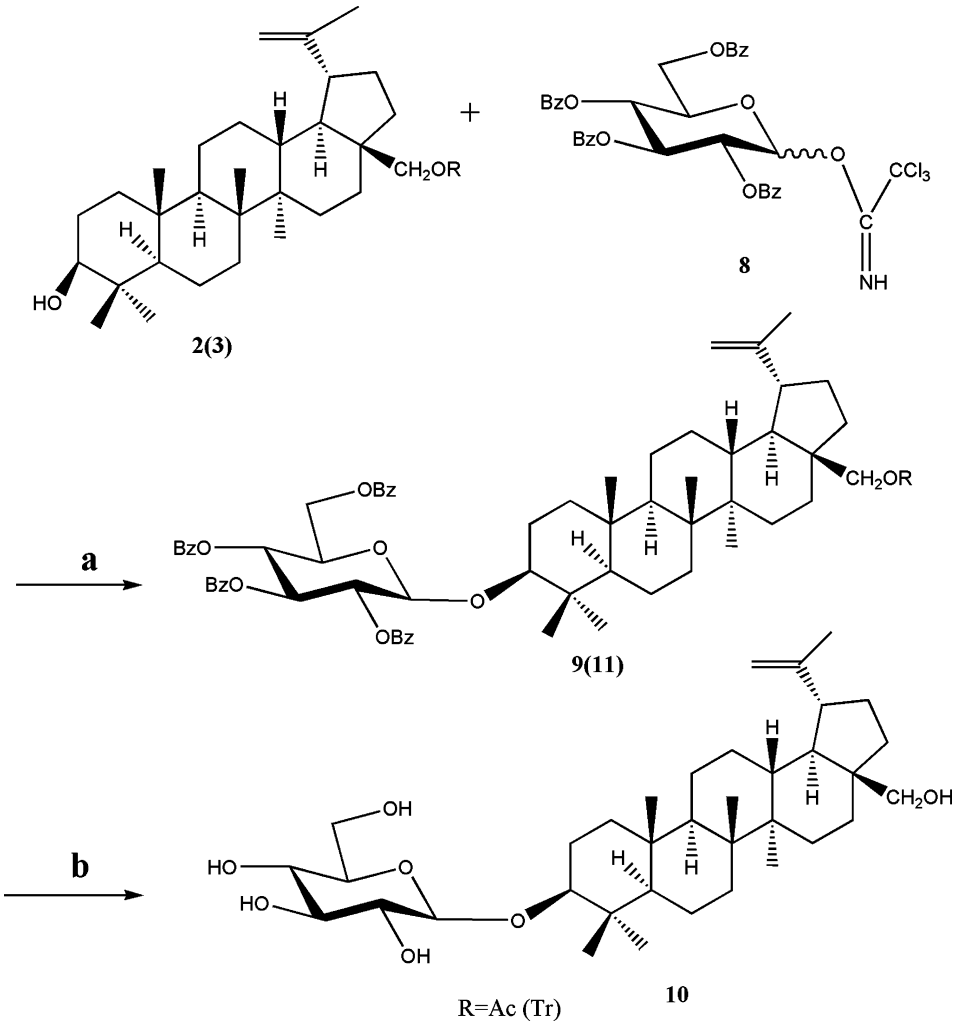
Secondly protected glucopyranose derivative was prepared for glycosidic linkage. Perbenzoylated trichloroacetimidate was chosen as sugar donor, which can be easily prepared from glucopyranose (Sch. 2).[17]

With the acceptor and donor in hand, synthesis of betulin-3-yl-ß-D-glucopyranoside proceeded smoothly in the presence of a catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a promoter.

The first synthetic route toward betulin-3-yl-ß-D-glucopyranoside is depicted in Scheme 3; esterification of betulin followed by glycosylation with 2,



Scheme 2. Reagent and reaction conditions: (a) benzoyl chloride, pyridine, 0°C; (b) hydrogen bromide/acetic acid, dichloromethane, rt; (c) silver carbonate, acetone, water; (d) trichloroacetonitrile, DBU.



Scheme 3. Reagent and reaction conditions: (a) TMSOTf, powdered 4 Å molecular sieves, dichloromethane, −5°C; (b) 0.1 M sodium methoxide in methanol, neutralized with ion resin (H^+ form).

3, 4, 6-tetra-*O*-benzoyl-*β*-D-glucopyranosyl trichloroacetimidate under the promotion of TMSOTf gave **9** in an excellent yield. Deprotection of the benzoyl and acetyl group of **9** was performed by NaOMe in methanol to furnish betulin-3-yl-ß -D-glucopyranoside.

Alternatively, primary alcohol of betulin was protected by a bulky trityl group, using trityl chloride in anhydrous pyridine to afford 28-*O*-trityl-betulin selectively in 85% yield. For construction of the glycosidic bond at the 3-OH of betulin, the glycosylation condition was similar to the above. Deprotection of trityl group was performed by PTS in CH_2Cl_2/CH_3OH, and benzoyl group was

removed by NaOMe in methanol affording betulin-3-yl-ß-D-glucopyranoside (Sch. 3).

In conclusion, two concise routes toward betulin-3-yl-ß -D-glucopyranoside, being different in the protection of primary alcohol of betulin, were developed.

EXPERIMENTAL

General Methods

Air- and water-sensitive reactions were performed in flame-dried glassware under argon atmosphere. Moisture-sensitive reagents were introduced via a dry syringe. Solvents were dried prior to use in the usual way. Boiling range of petroleum ether was 60–90°C. Column chromatography was carried out using 100–200 mesh silica gel. Analytical TLC was performed with silica gel (300–400 mesh, Yantai, China) and visualized by dipping in a solution of sulfuric acid in ethanol and then heating at 100°C. ESI-MS were recorded using a thermofinnigan quadripolar mass spectrometer. NMR spectra were recorded on a Bruker Avance DMX 500.

Isolation of Betulin (1)

A sample of 100 g finely crushed birch out bark was extracted in 1 L of 95% ethanol (v/v) under reflux. Crude betulin obtained from extraction was recrystallized from chloroform and methanol to provide purified betulin.

Preparation of 28-*O*-acetyl-betulin (2)

To a CH_2Cl_2 solution (120 mL) containing betulin (2.0 g, 4.51 mmol) was added acetic anhydride (50 mL, 520 mmol). After stirring at rt overnight, the mixture was washed exhaustively with saturated $NaHCO_3$ solution and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The crude product was purified by column chromatography (10:1.25 petroleum ether-EtOAc) to give 28-*O*-acetyl-betulin as a white powder (1.60 g, 73%). m.p. 210–212°C; $[\alpha]_D^{20} + 8.5$(*c* 1.58, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 4.69, 4.59 (br s, each 1H, H-29), 4.26 (d, $J = 11.1$ Hz, 1H, H-28), 3.87 (d, $J = 10.8$ Hz, 1H, H-28), 3.20 (dd, 1H, $J = 4.7$, 4.8 Hz, H-3), 2.44 (m, 1H, H-19), 2.07 (s, 3H, Ac), 1.68 (s, 3H, H-30), 1.05–1.97 (complex, CH_2, CH), 0.76, 0.83, 0.97, 0.98, 1.03, 1.68 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-30); ^{13}C NMR ($CDCl_3$): δ 171.7 (Ac), 150.2 (C-20), 109.9 (C-29), 79.1 (C-3), 14.8, 15.4, 16.1, 16.2, 18.3, 19.2, 20.9, 21.1, 25.3, 27.1, 27.5, 28.1, 29.6, 29.8, 34.2, 34.6, 37.2, 37.6, 38.7, 38.9, 40.9, 42.8, 46.4, 47.7, 48.8, 50.4, 55.4, 62.8. EI-MS (m/z): 484.8 [M^+] (calcd for $C_{32}H_{52}O_3$, 484.4).

Preparation of 28-*O*-trityl-betulin (3)

To a dry pyridine solution (50 mL) containing betulin (5 g, 11.3 mmol) was added trityl chloride (10 g, 35.9 mmol) and DMAP (0.15 g, 1.22 mmol). After stirring at 80°C overnight, the mixture was extracted with EtOAc; washed with cold 1 N HCl, saturated $NaHCO_3$, and brine; subsequently dried ($MgSO_4$); filtered; and concentrated in vacuo. The crude product was purified by column chromatography (10:1.25 petroleum ether-EtOAc) to afford 28-*O*-trityl-betulin as colorless needles (6.57 g, 85%). m.p. 132–133°C; $[\alpha]_D^{20}$ − 5.4(*c* 0.3 $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 7.49 (d, 6H, $J = 8.3$ Hz, trityl H′-2, H′-6), 7.31 (dd, 6H, $J = 7.3$, 7.8 Hz, trityl H′-3, H′-5), 7.24 (dd, 3H, $J = 6.8$, 7.1 Hz, trityl H′-4), 4.59, 4.53 (br s, each 1H, H-29), 3.16, 3.14 (d, each 1H, $J = 8.8$ Hz, H-28), 2.92 (dd, 1H, $J =$ 10.0, 4.2 Hz, H-3), 2.18–2.21 (m, 3H, H-2 and H-19), 1.09–1.64 (complex, CH_2, CH), 0.76, 0.83, 0.97, 0.98, 1.03, 1.68 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-30);^{13}C NMR ($CDCl_3$): δ 151.1 (C-20), 144.7 (trityl C′-1), 129.0 (trityl C′-3), 128.2 (trityl C′-5), 127.9 (trityl C′-2), 127.5 (trityl C′-6), 127.0 (trityl C′-4), 109.6 (C-29), 86.9 (trityl C-α), 79.1 (C-3), 14.9, 15.6, 16.1, 16.3, 18.5, 19.3, 20.9, 22.9, 25.4, 27.1, 27.6, 28.2, 30.1, 30.4, 34.3, 35.4, 35.5, 37.3, 37.5, 38.8, 39.1, 40.8, 42.6, 47.8, 47.9, 49.1, 50.5, 55.4, 59.7. ESI-MS (m/z): 707.3 $[M+Na]^+$ (calcd for $C_{49}H_{64}O_2Na$, 707.3).

Preparation of 2, 3, 4, 6-tetra-*O*-benzoyl-β-D-glucopyranosyl trichloroacetimidate (8)

Glucopyranose (10 g, 55.5 mmol) was treated with benzoyl chloride (36 mL, 310.4 mmol) and pyridine (200 mL) for 12 h at rt. The perbenzoylated glucopyranose was dissolved in a solution of 33% HBr in AcOH (30 mL). After stirring 1.5 h, the mixture was washed with saturated $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was dissolved in acetone (250 mL) and H_2O (10 mL); Ag_2CO_3 (20 g, 60.0 mmol) was added portionwise. The hydrolysis was performed for 2 h at rt, then the mixture was filtered and the combined filtrate was concentrated, and the residue was dissolved in a solution of dried CH_2Cl_2 (70 mL) containing CCl_3CN (9.1 mL, 90.8 mmol) and DBU (680 μL, 4.62 mmol). After stirring for 4 h at rt, the mixture was concentrated and purified by flash chromatography (CH_2Cl_2) to give **8** as a white crystalline powder (25.2 g, 75%). m.p. 65.0–68.2°C $[\alpha]_D^{20}$ + 76.5(*c* 1.67,$CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.66 (s, 1H, NH), 8.18–7.26 (20H, C_6H_5), 6.84 (d, 1H,$J = 3.0$ Hz, H-1), 6.33 (t, 1H, $J = 10.0$ Hz, H-3), 5.86 (t, 1H, $J = 9.8$ Hz, H-4), 5.69 (dd, 1H, $J = 10.2$, 3.5 Hz, H-2), 4.63 (d, 2H, $J = 10.3$ Hz, H-6), 4.48 (dd, 1H, 12.8, $J = 5.4$ Hz, H-5); ^{13}C NMR ($CDCl_3$): δ 166.2, 165.7, 165.4, 165.2 (4 × CO), 160.80 (C=N), 133.4, 130.3, 129.6, 128.9 (C_6H_5), 103.4 (C-1), 79.0 (C-3), 77.2 (C-5), 73.6 (C-2), 70.7 (C-4), 63.3 (C-6). ESI-MS (m/z) 764.3 $[M + Na]^+$ (calcd for $C_{36}H_{28}NO_{10}Cl_3Na$, 764.3).

Preparation of 28-*O*–acetoxy-betulin-3-yl-2,3,4,6-benzoyl-ß-D-glucopyranoside (9)

A mixture of acceptor **2** (500 mg, 1.03 mmol) and 2, 3, 4, 6-tetra-*O*-benzoyl-β-D-glucopyranosyl trichloroacetimidate **8** (1.15 g, 1.55 mmol) and powdered 4 Å molecular sieves (3 g) in dry CH_2Cl_2 (50 mL) was stirred for 40 min at rt, then the mixture was cooled to –5°C for 30 min, followed by the dropwise addition of dry CH_2Cl_2 solution (1 mL) containing TMSOTf (10 μL, 0.052 mmol) under Ar while keeping rigorous anhydrous conditions. The reaction mixture was stirred at this temperature until TLC indicated the disappearance of the acceptor. Triethylamine (0.3 mL) was added and the mixture was filtered through Celite and the solvents were evaporated. The crude residue was purified by column chromatography (10:1.25 petroleum ether–EtOAc) to give **9** as a white amorphous solid (350 mg, 70%).$[\alpha]_D^{20} + 5.4$(*c* 1.2, $CHCl_3$)^{1}H NMR ($CDCl_3$): δ 8.04–7.83, 7.55–7.28 (2m, 20H, C_6H_5), 5.94 (t, 1H, $J = 8.0$ Hz, H′-1), 5.57 (m, 2H, H′-3, H′-4), 4.86 (dd, 1H, $J = 8.0$, 8.2 Hz, H′-2), 4.72 (br s, 1H, betulin-H-29), 4.64 (br s, 1H, betulin-H-29), 4.59 (dd, 2H, $J = 12.4$, 12.2 Hz, H′-6), 4.25 (d, 1H,$J = 10.9$ Hz, betulin-H-28), 4.16 (m, 1H, H′-5), 3.87 (d, 1H, $J = 9.2$ Hz, betulin-H-28), 3.08 (dd, 1H, $J = 9.5$ Hz, betulin-H-3), 2.46 (m, 1H, betulin-H-19), 2.06 (s, 3H, Ac), 0.94–1.84 (complex, CH_2, CH), 0.62, 0.68, 0.76, 0.94, 0.99, 1.65 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-30); ^{13}C NMR ($CDCl_3$): δ 171.7 (Ac), 166.2, 166.1, 165.5, 165.2 (4 × CO), 150.4 (C-20), 134.1, 133.4, 130.1, 128.6 (C_6H_5), 110.1 (C-29), 103.4 (C′-1), 90.9 (C-3), 77.5 (C′-5), 72.8 (C′-2), 71.1 (C′-4), 63.6 (C′-6), 14.9, 15.4, 16.2, 18.2, 19.4, 20.9, 21.2, 25.4, 26.2, 27.2, 27.7, 29.8, 30.1, 34.3, 34.8, 36.9, 37.7, 38.8, 39.1, 41.0, 42.8, 46.5, 47.8, 48.9, 50.6, 55.7, 62.9. ESI-MS (m/z):1085.3 $[M + Na]^+$ (calcd for $C_{66}H_{78}O_{12}Na$, 1085.3).

Preparation of betulin-3-yl-ß -D-glucopyranoside (10)

To a solution of **9** (500 mg, 0.47 mmol) in 1:1 $CHCl_3$-CH_3OH (20 mL) was added 0.1 M NaOMe in methanol (5 mL), and the reaction mixture was stirred for 48 h at rt. TLC (4:1 petroleum ether–EtOAc) then revealed the absence of **9** and the presence of a slower-running compound. The solution was neutralized with ion-exchange resin (H^+), filtered, and concentrated in vacuo. The crude product was purified by column chromatography (10:1, $CHCl_3$–MeOH) to afford **10** as a white amorphous solid (300 mg, 60%). m.p. 174–175°C; $[\alpha]_D^{20} + 2.7$(*c* 0.58, CH_3OH). ^{1}H NMR (CD_3OD) δ 4.66 (br s, 1H, H-29), 4.53 (br s, 1H, H-29), 4.34 (d, 1H, $J = 7.8$ Hz, H′-1), 4.12 (d, 1H,$J = 10.9$ Hz, betulin-H-28), 3.64 (t, 1H, $J = 9.8$ Hz, H′-6), 3.52 (d, 1H,$J = 10.9$ Hz, betulin-H-28), 3.11 (t, 1H, $J = 9.8$ Hz, H′-3), 3.09 (dd, 1H, $J = 11.9$, 5.1 Hz, H?-6), 3.07 (t, 1H, $J = 11.7$ Hz, H′-4), 3.03 (t, 1H, $J = 11.7$ Hz, H′-5), 3.00 (t, 1H, $J = 11.7$ Hz, H′-2), 2.94 (dd, 1H, $J = 11.2$ Hz, betulin-H-3), 2.50 (m, 1H, H-19),

0.74–1.98 (complex, CH_2, CH), 0.84, 0.88, 1.02,1.05, 1.08, 1.69 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-30); ^{13}C NMR (CD_3OD) δ: 151.1 (C-20), 110.2 (C-29), 103.4 (C′-1), 90.9 (C-3), 79.0 (C′-3), 77.2 (C′-5), 73.6 (C′-2), 70.7 (C′-4), 63.3 (C′-6), 60.9 (C-28), 14.9, 15.6, 16.1, 16.3, 18.5, 19.3, 20.9, 22.9, 25.4, 27.1, 27.6, 28.2, 30.1, 30.4, 34.3, 35.4, 36.8, 37.3, 38.9, 40.8, 42.6, 47.9, 49.1, 50.5, 55.6, 59.7. ESI-MS (m/z): 627.1 $[M + Na]^+$ (calcd for $C_{36}H_{60}O_7Na$, 627.1).

Preparation of 28-*O*-trityl-betulin–3-yl-2,3,4,6-benzoyl-ß-D-glucopyranoside (11)

A mixture of acceptor **3** (500 mg, 0.73 mmol) and 2, 3, 4, 6-tetra-*O*-benzoyl-β-D-glucopyranosyl trichloroacetimidate **8** (814.8 mg, 1.09 mmol) and powdered 4 Å molecular sieves (3 g) in dry CH_2Cl_2 (40 mL) was stirred for 40 min at rt, then the mixture was cooled to –5°C for 30 min, followed by the dropwise addition of dry CH_2Cl_2 solution (1.0 mL) of TMSOTf (10 μL, 0.052 mmol) under Ar while keeping rigorous anhydrous conditions. The reaction mixture was stirred at this temperature until TLC indicated the disappearance of the acceptor. Triethylamine (0.3 mL) was added and the mixture was filtered through Celite and the solvents were evaporated. The crude residue was purified by column chromatography (10:1.25 petroleum ether–EtOAc) to give **11** as a white amorphous solid (65%, 325 mg). m.p. 132–133°C; $[\alpha]_D^{20} - 4.2$(*c* 0.2 $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.14–7.92, 7.29–7.56 (2m, 35H, C_6H_5), 6.29 (t, 1H,J = 9.9 Hz, H′-1), 5.92 (t, 1H, J = 10.4 Hz, H′-4), 5.31 (dd, 1H, J = 9.5, 10.4 Hz, H′-3), 4.71 (m, 2H,J = 2.1 Hz, H′-2, betulin-H-29), 4.65 (br s, 1H, betulin-H-29), 4.60 (m, 2H, H′-6), 4.55 (m, 1H, H′-5), 3.13 (d, 1H,J = 9.5 Hz, betulin-H-28), 3.08 (dd, 1H, J = 9.5 Hz, betulin-H-3), 2.92 (d, 1H, J = 9.2 Hz, betulin-H-28), 2.22 (m, 1H, betulin-H-19), 0.94–1.84 (complex, CH_2, CH), 0.62, 0.68, 0.76, 0.94, 0.99, 1.69 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-30); ^{13}C NMR ($CDCl_3$): δ 166.2, 165.7, 165.4, 165.2 (4 × CO), 150.9 (C-20), 144.67, 144.65, 139.9, 134.0, 133.4, 127.0–130.3 (C_6H_5), 109.6 (C-29), 103.4 (C′-1), 86.9 (trityl C-α), 90.9 (C-3), 79.0 (C′-3), 77.2 (C′-5), 73.6 (C′-2), 70.7 (C′-4), 63.3 (C′-6), 61.9 (C-28), 14.9, 15.6, 16.1, 16.3, 18.5, 19.3, 20.9, 22.9, 25.4, 27.1, 27.6, 28.2, 30.1, 30.4, 34.3, 35.4, 36.8, 37.3, 38.9, 40.8, 42.6, 47.9, 49.1, 50.5, 55.6, 59.7. ESI-MS (m/z): 1285.4 $[M + Na]^+$ (calcd for $C_{83}H_{90}O_{11}Na$, 1285.4).

Preparation of betulin-3-yl-2,3,4,6-benzoyl-ß-D- glucopyranoside (12)

Compound **11** (500 mg, 0.039 mmol) was dissolved in 1:3 CH_2Cl_2-CH_3OH (20 mL), the mixture was vigorously stirred, and the pH was maintained at 2–3 by addition of PTS. After 1 day the mixture was concentrated and the residue was purified by column chromatography (10:1 petroleum ether–EtOAc) to give **12** a white amorphous solid (350 mg ,70%).$[\alpha]_D^{20} + 3.2$(*c* 0.5 $CHCl_3$); ^{1}H NMR

($CDCl_3$): δ 8.14–7.92, 7.29–7.56 (2m, 20H, C_6H_5), 6.29 (t, 1H,J = 9.9 Hz, H′-1), 5.92 (t, 1H, J = 10.4 Hz, H′-4), 5.31 (dd, 1H, J = 9.5, 10.4 Hz, H′-3), 4.71 (m, 2H,J = 2.1 Hz, H′-2, betulin-H-29), 4.65 (br s, 1H, betulin-H-29), 4.60 (m, 2H, H′-6), 4.55 (m, 1H, H′-5), 4.12 (d, 1H,J = 10.9 Hz, betulin-H-28), 3.42 (d, 1H, J = 9.2 Hz, betulin-H-28), 3.08 (dd, 1H, J = 9.5 Hz, betulin-H-3), 2.22 (m, 1H, betulin-H-19), 0.94–1.84 (complex, CH_2, CH), 0.62, 0.68, 0.76, 0.94, 0.99, 1.69 (all s, each 3H, H-23, H-24, H-25, H-26, H-27, H-30); ^{13}C NMR ($CDCl_3$): δ 166.2, 165.7, 165.4, 165.2 (4 × CO), 150.9 (C-20), 133.4, 130.3, 129.6, 128.9 (C_6H_5), 109.6 (C-29), 103.4 (C′-1), 90.9 (C-3), 79.0 (C′-3), 77.2 (C′-5), 73.6 (C′-2), 70.7 (C′-4), 63.3 (C′-6), 60.9 (C-28), 14.9, 15.6, 16.1, 16.3, 18.5, 19.3, 20.9, 22.9, 25.4, 27.1, 27.6, 28.2, 30.1, 30.4, 34.3, 35.4, 36.8, 37.3, 38.9, 40.8, 42.6, 47.9, 49.1, 50.5, 55.6, 59.7. ESI-MS (m/z):1043.4 $[M + Na]^+$ (calcd for $C_{64}H_{76}O_{11}Na$, 1043.4).

Preparation of betulin-3-yl-ß -D-glucopyranoside (10)

Compound **10** was prepared from **12** in the same manner as that described for compound **9**.

REFERENCES

1. Zhao, G.L.; Yan, W.D.; Cao, D. Simultaneous determination of betulin and betulinic acid in white birch bark using RP-HPLC. *J. Pharmaceut. Biomed.* **2007**, *43*, 959–962.

2. Tolstikova, T.G.; Sorokina, L.V.; Tolstikov, G.A.; Tolstikov, A.G,; Flekhter, O.B. Biological activity and pharmacological prospects of lupane terpenoids: I. Natural lupane derivatives.*Russ. J. Bioorg. Chem.* **2006**, *32*, 42–55.

3. Jeong, H.J.; Chai, H.B.; Park, S.Y.; Kim, D.H.L. Preparation of amino acid conjugates of betulinic acid with activity against human melanoma. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1201–1204.

4. Grun, J.; Revell, J.D.; Conza, M.; Wennemers, H. Peptide–polyethylene glycol conjugates: synthesis and properties of peptides bearing a C-terminal polyethylene glycol chain. *Bioorg. Med. Chem.* **2006**, *14*, 6197–6201.

5. Cheng, M.-S.; Yan, M.-C.; Liu, Y.; Zheng, L.-G.; Liu, J. Synthesis of β-hederin and hederacolchiside A_1: triterpenoid saponins bearing a unique cytotoxicity-inducing disaccharide moiety. *Carbohydr. Res.* **2006**, *341*, 60–67.

6. Pellissier, H. The glycosylation of steroids. *Tetrahedron* **2004**, *60*, 5123–5162.

7. Uvarova, N.I.; Oshitok, G.I.; Elyakov, G.B. Synthesis of steroid and triterpenoid glycosides by the orthoester method. *Carbohydr. Res.* **1973**, *27*, 79–87.

8. Uvarova, N.I.; Atopkina, L.N.; Elyakov, G.B. Synthesis of triterpene and steroid glycosides. *Carbohydr. Res.* **1980**, *83*, 33–42.

9. Samoshina, N.F.; Denisenko, M.V.; Denisenko, V.A.; Uvarova, N. I Synthesis of glycosides of lupane-type triterpene acids. *Chem. Nat. Compd.* **2003**, *39*, 575–582.

10. Schmidt, R.R.; Michel, J. Facile synthesis of α-and β-O-glycosyl imidates; preparation of glycosides and disaccharides. *Angew. Chem. Int. Ed. Engl.* **1980**, *19*, 731–732.

11. Ple, K.; Chwalek, M.; Voutquenne-Nazabadioko, L. Synthesis and hemolytic activity of some hederagenin diglycosides. *Eur. J. Org. Chem.* **2004**, 1588–1603.

12. Hiroya, K.; Takahashi, T.; Miura, N.; Naganuma, A.; Sakamoto, T. Synthesis of betulin derivatives and their protective effects against the cytotoxicity of cadmium. *Bioorg. Med. Chem.* **2002**, *10*, 3229–3236.

13. Hata, K.; Hori, K.; Takahashi, S. Differentiation- and apoptosis-inducing activities by pentacyclic triterpenes on a mouse melanoma cell line. *J. Nat. Prod.* **2002**, *65*, 645–648.

14. Saito, S.; Ebashi, J.; Sumita, S.; Furumoto, T.; Nagamura, Y.; Nishida, K.; Isiguro, I. Comparison of cytoprotective effects of saponins isolated from leaves of Aralia elata Seem. (Araliaceae) with synthesized bisdesmosides of oleanolic acid and hederagenin on carbon tetrachloride in duced hepatic injury. *Chem. Pharm. Bull.* **1993**, *41*, 1395–1401.

15. Yu, B.; Xie, J.; Deng, S.; Hui, Y. First synthesis of a bidesmosidic triterpene saponin by a highly efficient procedure. *J. Am. Chem. Soc.* **1999**, *121*, 12196–12197.

16. Atopkina, L.N.; Uvarova, N.I.; Elyakov, G.B. Simplified preparation of the ginsenoside-Rh_2 minor saponin from ginseng. *Carbohydr. Res.* **1997**, *303*, 449–451.

17. Ziegler, T.; Jurisch, C. Chemoenzymatic synthesis of enantiomerically enriched aminoalkenols and glycosides thereof. *Tetrahedron Asymm.* **2000**, *11*, 3403–3418.