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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF DIHYDROBETULIN *N*-ACETYLGLUCOSAMINIDES

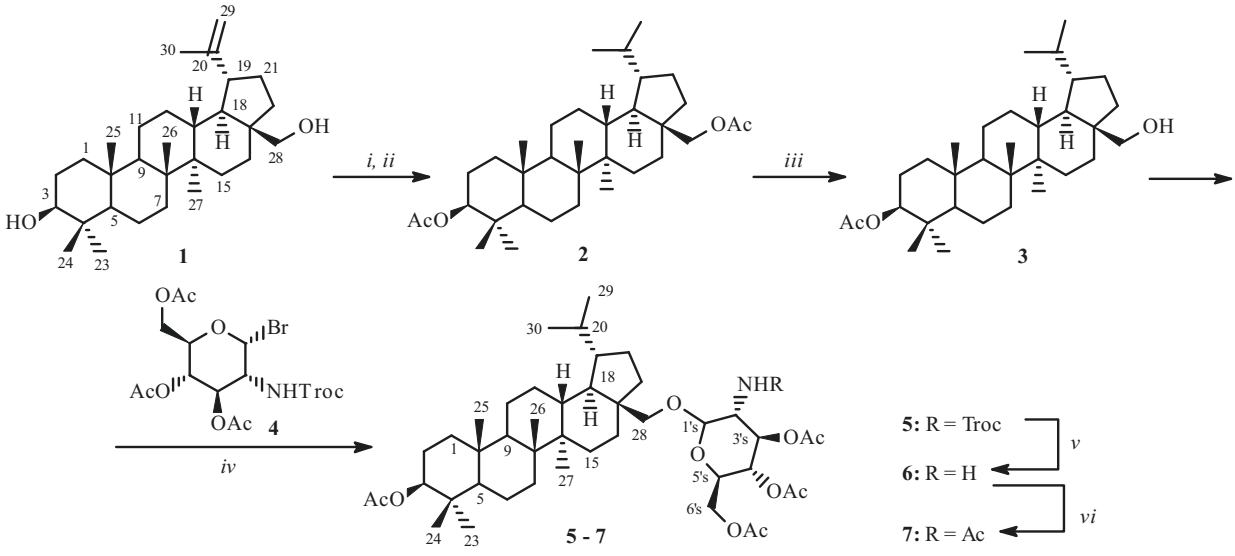
I. Yu. Strobykina, B. F. Garifullin, R. R. Sharipova, A. D. Voloshina, A. S. Strobykina, A. B. Dobrynin, and V. E. Kataev*

Dihydrobetulin N-*acetylglucosaminides were synthesized for the first time. A study of their antimicrobial activity against a standard set of Gram-positive and Gram-negative bacteria and fungi showed highly selective bacteriostatic activity for glycosides* ***9*** *and* ***13*** *against* Staphylococcus aureus *ATCC 209p.*

Keywords: betulin, dihydrobetulin, D-glucosamine, antimicrobial activity.

Many betulin glycosides have now been synthesized and studied [1–9]. The used glycones include D-glucopyranose [1–4, 7–9], D-galactopyranose [4, 8, 9], D-mannopyranose [4, 8, 9], D-rhamnopyranose [4, 8], and D-arabinopyranose [4, 5, 8]. Many glycosides exhibited cytostatic activity against various human cancer cell lines [4, 6–8]. Several glycosides were investigated for hypocholesterolemic [2] and growth-regulating activity [3]. The betulin glycosides were not studied for other types of biological activity.

In continuation of a series of publications on the synthesis of glucosamine conjugates with natural terpenoids [10, 11], we turned our attention to the triterpenoid betulin (**1**). The double bond of **1** was hydrogenated over Pd by the literature method [13] to prevent a Wagner–Meerwein rearrangement of **1** to form allobetulin [12] during a $ZnCl_2$-activated Koenigs–Knorr reaction of α-D-glucopyranosyl bromide with glycosyl acceptors [10, 11]. Then, the hydroxyl of dihydrobetulin, which was obtained in 67% yield, was acylated as before [13]. Next, the C-28 acyl protecting group was selectively removed from dihydrobetulin diacetate (**2**) by magnesium methoxide in THF according to the literature [14] to produce in 61% yield 3β-*O*-acetyldihydrobetulin (**3**).



i. H_2, Pd–C, THF, CH_3OH; *ii*. Ac_2O – Py; *iii*. $Mg(OCH_3)_2$, CH_3OH, THF; *iv*. $ZnCl_2$, CH_2Cl_2; *v*. Zn, AcOH; *vi*. Ac_2O, Et_3N, CH_2Cl_2

A. E. Arbuzov Institute of Organic and Physical Chemistry, Kazan Scientific Center, Russian Academy of Sciences, 8 Arbuzova St., Kazan, 420088, Russian Federation, fax: (843) 273 22 53, e-mail: kataev@iopc.ru. Translated from *Khimiya Prirodnykh Soedinenii*, No. 6, November–December, 2017, pp. 936–940. Original article submitted February 7, 2017.

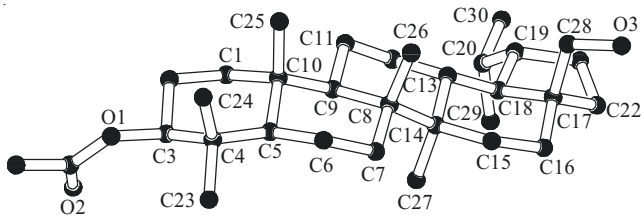
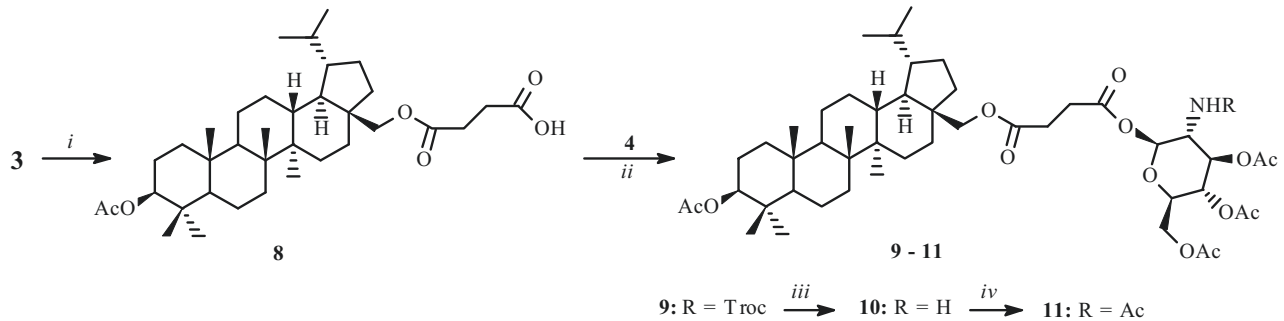


Fig. 1. X-ray molecular structure of 3β-*O*-acetyldihydrobetulin **3** (H atoms and molecule DMSO not shown).

An X-ray crystal structure analysis (XSA) of this triterpenoid was performed because its molecular structure had not been reported (Fig. 1). In the next step, **3** was glycosylated by 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-α-D-glucopyranosyl bromide (**4**), which was prepared from commercially available glucosamine hydrochloride as before [15].

The $ZnCl_2$-activated Koenigs–Knorr reaction of glycosyl donor **4** and glycosyl acceptor **3** was carried out as before [11]. Glycoside **5** was obtained in 35% yield. A peak in the electrospray-ionization (ESI) mass spectrum (MS) at *m*/*z* 970.5 for $[M + Na]^+$ ($C_{47}H_{72}Cl_3NO_{12}$, MM 947.41) indicated that it had formed. The anomeric proton of glycoside **7** resonated in the PMR spectrum as a doublet at 4.84 ppm with vicinal SSCC 3.6 Hz. This indicated that the glycoside bond had the α-orientation. Next, the 2,2,2-trichloroethoxycarbonyl (Troc) protecting group was removed using Zn dust in glacial AcOH as before [10]. The resulting amine **6** was acylated without further purification as before [11]. Glycoside **7** was obtained in 79% yield. Peaks in the MALDI MS at *m*/*z* 838.5 for $[M + Na]^+$ and 854.4 for $[M + K]^+$ ($C_{46}H_{73}NO_{11}$, MM 815.52) indicated that it had formed. The conversion from **5** to **7** was accompanied by the disappearance in the PMR spectrum of a multiplet at 4.66–4.76 ppm for the Troc methylene protons, the appearance of a singlet at 1.93 ppm for the acetamide acyl group, and a low-field shift of the resonance for the amide proton that appeared as a doublet at 5.60 ppm (^3J = 9.3 Hz). The anomeric proton of **7** resonated as a doublet at 4.80 ppm with vicinal SSCC 3.7 Hz. This indicated that the α-orientation of the glycoside bond was retained.

In the next step, the reaction of 3β-*O*-acetyldihydrobetulin (**3**) with an excess of succinic anhydride in Py produced in 38% yield 3β-*O*-acetyl-28-*O*-succinyldihydrobetulin (**8**), the formation of which was indicated by a peak in the ESI-MS at *m*/*z* 609.6 for $[M + Na]^+$ ($C_{36}H_{58}O_6$, MM 586.42). Then, glycosyl donor **4** underwent a reaction catalyzed by tetrabutylammonium bromide (TBAB) with the carboxylic acid of **8** to give in 38% yield glycoside **9**, the formation of which was indicated by peaks in the MALDI MS at *m*/*z* 1070.8 for $[M + Na]^+$ and 1086.8 for $[M + K]^+$ ($C_{51}H_{76}Cl_3NO_{15}$, MM 1047.4). The anomeric proton of glycoside **9** resonated in the PMR spectrum as a doublet at 5.76 ppm with vicinal SSCC 8.7 Hz, indicating that the resulting glycoside bond had the β-orientation. This agreed with the literature on the course of the Koenigs–Knorr reaction between glucopyranoside bromide and a carboxyl-containing glycosyl acceptor under phase-transfer conditions with TBAB catalysis and K_2CO_3 [16, 17]. Then, the Troc protection on **9** was removed using Zn dust in glacial AcOH as before [10]. The resulting amine **10** was acylated without further purification according to the literature [11] for afford in 43% yield glycoside **11**. Peaks in the MALDI MS at *m*/*z* 938.6 for $[M + Na]^+$ and 954.6 for $[M + K]^+$ ($C_{50}H_{77}NO_{14}$, MM 915.53) indicated that it had formed. The conversion from **9** to **11** was accompanied by the disappearance in the PMR spectrum of doublets at 4.67 ppm (^3J = 12.1 Hz) and 4.80 ppm (^3J = 12.1 Hz) corresponding to Troc methylene proton resonances and the appearance of a singlet at 1.94 ppm that corresponded to the resonance of the acetamide acyl protons. Also, a singlet belonging to the amide proton of **11** underwent a low-field shift and was observed as a doublet of doublets at 5.51 ppm (^3J = 9.0 Hz). The anomeric proton of **11** resonated as a doublet at 5.72 ppm with vicinal SSCC 9.0 Hz, indicating that the glycoside bond had the β-orientation.



i. Succinic anhydride, DMAP, Py; *ii*. TBAB, K_2CO_3, H_2O, CH_2Cl_2; *iii*. Zn, AcOH; *iv*. Ac_2O, Et_3N, CH_2Cl_2

Antimicrobial activity of synthesized glycosides **3**, **7**, **8**, **11**, and **12** [18] was studied against Gram-positive bacteria *Staphylococcus aureus* ATCC 209p and *Bacillus cereus* ATCC 8035; Gram-negative bacteria *Escherichia coli* CDCF-50 and *Pseudomonas aeruginosa* ATCC 9027; and fungi *Aspergillus niger* BKMF-1119, *Trichophyton mentagrophytes* var. *Gypseum* 1773, and *Candida albicans* 855–653.

Glycosides **7** and **11** inhibited the growth of only *S. aureus* with the activity of **11** (MIC = 15.6 μg/mL) exceeding by four times and that of glycoside **7** (MIC = 7.8 μg/mL) by eight times that of the antibiotic chloramphenicol (MIC = 62.5 μg/mL). Betulin derivatives **3** and **8** exhibited antibacterial activity only if they were glycosylated. They themselves and 1,3,4,6-tetra-*O*-acetyl-2-deoxy-2-acetamido-D-glucopyranose (**12**), which was the aglycone of **7** and **11** and was synthesized by analogy with the literature [18], were inactive.

EXPERIMENTAL

The XSA of **3** was performed at the Spectro-Analytical Joint Use Center of the RFFI (JUC SAC) on a Bruker Smart APEX II CCD diffractometer (λ Mo Kα = 0.71073 Å, ω-scanning, 2θ < 52°, R_{int} = 0.048). A total of 12,707 reflections were measured, of which 6,356 were independent. The number of observed reflections with $I > 2\sigma(I)$ was 4,352. Absorption corrections were made using the SADABS program [19]. The structure was solved by direct methods using the SIR program [20] and refined by full-matrix least-squares methods using the SHELXL97 program [21]. Hydroxyl H atoms were found in an electron-density difference synthesis and refined isotropically. The coordinates of other H atoms were calculated geometrically and refined using a rider model. All calculations used the WinGX [22] and APEX2 programs [23]. The final agreement parameters R = 0.1344, wR_2 = 0.3975, GOF = 1.41. The number of refined parameters was 364 with Flack parameter 0.4(4). Crystals of **3** ($C_{32}H_{54}O_3 \cdot C_2H_6OS$, MM 564.89) were monoclinic at 296K with a = 13.291(8), b = 7.257(5), c = 17.757(11) Å, β = 104.484(9)°, V = 1658.3(18) Å^3, Z = 2, space group $P2_1$, d_{calcd} = 1.131 g/cm^3, μ = 0.132 mm^{-1}, and $F(000)$ = 624. A complete dataset for the structure of **3** was deposited in the Cambridge Structural Database (registration No. CCDC 1530699).

PMR and ^{13}C NMR spectra were recorded on Avance-400 and Avance-600 spectrometers (Bruker, Germany) at operating frequencies 400 and 600 MHz for ^{1}H and 100 MHz for ^{13}C using the solvent [CD(H)Cl$_3$] for calibration. Resonances in the spectra were attributed according to the literature [13, 15, 24]. MALDI mass spectra were obtained on an UltraFlex III TOF/TOF time-of-flight mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) in linear mode. The Nd YAG laser had λ = 355 nm. Data were processed using the FlexAnalysis 3.0 program (Bruker Daltonik GmbH, Bremen, Germany). Measurements were made in positive-ion mode in the range m/z 200–6000. The matrix was 2,5-dihydroxybenzoic acid (DHB) and *p*-nitroaniline (*p*-NA). Samples were dissolved in CH_2Cl_2 at a concentration of 10^{-3} mg/mL. A solution of the matrix in MeCN at a concentration of 10 mg/mL was prepared. Samples were deposited by the dried-drop method. The matrix solution was applied using a 0.5-μL pipette onto an Anchor Chip target (Bruker Daltonik GmbH, Bremen, Germany). Analyte solution (0.5 μL) was deposited on the target after the solvent evaporated. Electrospray ionization (ESI) mass spectra were obtained on an AmazonX mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany). ESI-MS were recorded in positive-ion mode in the range m/z 100–2800 at capillary potential 4500 V. The drying gas (N_2) was at 250°C with flow rate 8 L/min. The eluent was MeOH–H_2O (70:30) at flow rate 0.2 mL/min. Samples were diluted with MeOH to a concentration of 10^{-6} g/L. The injected sample volume was 20 μL. Data were processed using the DataAnalysis 4.0 program (Bruker Daltonik GmbH, Bremen, Germany). The course of reactions and purity of compounds were monitored by TLC on Sorbfil plates (OOO Imid, Krasnodar, Russia). Compounds were detected by treating plates with H_2SO_4 solution (5%) followed by heating to 120°C. Specific rotation was measured on a Model 341 polarimeter (PerkinElmer, USA) in a thermostatted cell at 20°C using λ = 589 nm. Melting points were determined on a Boetius apparatus.

Betulin (**1**) was supplied by Cand. N. I. Medvedeva (UIC, RAS). 3β-*O*-Acetyl-28-*O*-acetyldihydrobetulin (**2**), 3β-*O*-acetyldihydrobetulin (**3**), and 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2′,2′,2′-trichloroethoxycarbonylamino)-α-D-glucopyranosyl bromide (**4**) were prepared in analogy with the literature [13–15]. Constants and spectral characteristics of **2**, **3**, and **4** agreed with the literature [13, 15]. Commercial glucosamine hydrochloride and $ZnCl_2$ (Acros, Belgium) were used.

3-*O*-Acetyl-28-*O*-[3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-(2″,2″,2″-trichloroethoxycarbonylamino)-α-D-glucopyranosyl]-dihydrobetulin (5). A solution of **3** (0.15 g, 0.31 mmol) and glycoside donor **4** (0.15 g, 0.37 mmol) in anhydrous CH_2Cl_2 (5 mL) under Ar was treated with $ZnCl_2$ (0.05 g, 0.37 mmol). The reaction mixture was stirred in the dark for 28 h at 20°C; diluted with CH_2Cl_2; washed successively with $NaHCO_3$ solution (5%), H_2O, saturated NaCl solution, and water; dried over $CaCl_2$; and concentrated at reduced pressure. Flash chromatography of the residue over a dry column (silica gel,

hexane–EtOAc, from 20:1 to 5:1) produced **5** (0.10 g, 35%) as a white amorphous powder, $[\alpha]_D^{20}$ +73.9° (*c* 1.2, CH_2Cl_2). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.80–1.95 (26H, m, CH, CH_2), 0.77 (3H, d, J = 6.7, H_3-29 or H_3-30), 0.83–0.86 (12H, m, 4 CH_3), 0.96, 1.01 (3H each, s, CH_3), 2.01, 2.03, 2.04, 2.10 [3H each, s, 4 $CH_3C(O)$], 3.05 (1H, d, J = 9.5, H_A-28), 3.89–3.96 (2H, d, H-5′s, H_B-28), 4.01–4.09 (2H, m, H-6′s, 2′s), 4.32 (1H, dd, J = 12.4, 3.9, H-6′s), 4.47 (1H, dd J = 10.5, 5.6, H-3), 4.66–4.76 (2H, m, CH_2CCl_3), 4.84 (1H, d, J = 3.6, H-1′s), 5.07–5.15 (2H, m, NH, H-4′s), 5.20 (1H, t, J = 10.1, H-3′s). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 14.7, 14.9, 16.1, 16.5, 18.1, 20.5, 20.6, 20.7, 20.8, 21.2, 21.3, 21.9, 22.9, 23.6, 26.7, 27.0, 27.9, 29.4, 30.2, 34.2, 37.0, 37.1, 37.8, 38.3, 40.9, 42.8, 44.7, 47.1, 48.1, 49.9, 54.3, 55.3, 61.8, 67.6, 67.9, 68.1, 71.0, 74.6, 80.8, 80.9, 95.4, 97.9, 154.1, 169.3, 170.6, 170.9 (2 C). Mass spectrum (ESI) *m/z*: 970.5 $[M + Na]^+$ (calcd $[M + Na]^+$ 970.4). $C_{47}H_{72}Cl_3NO_{12}$.

3β-*O*-Acetyl-28-(3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-amino-α-D-glucopyranosyl)-dihydrobetulin (6). A solution of **5** (0.1 g, 0.1 mmol) in glacial AcOH (4 mL) under Ar was treated with activated Zn dust (0.5 g, 7.6 mmol), stirred for 3 h at 20°C, and concentrated at reduced pressure. The residue was diluted with CH_2Cl_2 (10 mL); washed with $NaHCO_3$ solution (5%) and H_2O; dried over $MgSO_4$; and concentrated at reduced pressure to afford **6** (0.08 g, 94%), which was used without further purification.

3β-*O*-Acetyl-28-(3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-acetamido-α-D-glucopyranosyl)-dihydrobetulin (7). A solution of **6** (1 mmol) in CH_2Cl_2 (5 mL) under Ar was treated with Et_3N (3 mmol) and Ac_2O (10 mmol); stirred for 2 h at 20°C; diluted with CH_2Cl_2 (10 mL); washed successively with saturated $NaHCO_3$ solution, HCl solution (1 M), and H_2O; dried over Na_2SO_4; and concentrated at reduced pressure. Glycoside **7** was isolated by chromatography (silica gel, hexane–EtOAc, from 5:1 to 1:1) as a white amorphous powder. Yield 0.064 g (79%), $[\alpha]_D^{20}$ +44.9° (*c* 1.07, CH_2Cl_2). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.88–2.08 (25H, m, CH, CH_2), 0.76 (3H, d, J = 6.8, H_3-29 or H_3-30), 0.82–0.87 (12H, m, 4 CH_3), 0.97, 1.01 (3H each, s, CH_3), 1.93, 2.02, 2.03, 2.04, 2.10 [3H each, s, 5 $CH_3C(O)$], 3.02 (1H, d, J = 9.4, H_A-28), 3.86–3.93 (2H, m, H-5′s, H_B-28), 4.04 (1H, dd, J = 12.4, 2.0, H-6′s), 4.26–4.35 (2H, m, H-2′s, 6′s), 4.47 (1H, dd, J = 10.5, 5.5, H-3), 4.80 (1H, d, J = 3.7, H-1′s), 5.09–5.20 (2H, m, H-3′s, 4′s), 5.60 (1H, d, J = 9.3, NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 14.6, 14.9, 16.0, 16.1, 16.5, 18.1, 20.6, 20.7 (2 C), 20.8, 21.3, 21.7, 22.9, 23.1, 23.7, 26.8, 27.0, 27.9, 29.4, 30.2, 34.3, 34.8, 37.0, 37.1, 37.8, 38.4, 40.9, 42.8, 44.7, 47.2, 48.1, 49.9, 52.3, 55.3, 61.9, 67.3, 67.9, 68.0, 71.4, 80.9, 97.9, 169.2, 169.8, 170.7, 171.0, 171.4. Mass spectrum (MALDI): *m/z* 838.5 $[M + Na]^+$ (calcd $[M + Na]^+$ 838.5), *m/z* 854.4 $[M + K]^+$ (calcd $[M + K]^+$ 854.5). $C_{46}H_{73}NO_{11}$.

3β-*O*-Acetyl-28-*O*-succinyldihydrobetulin (8). A solution of **3** (0.64 g, 1.3 mmol), succinic anhydride (0.39 g, 3.9 mmol), and DMAP (0.48 g, 3.9 mmol) in anhydrous Py (10 mL) was refluxed for 16 h, cooled, acidified with HCl solution (10%), poured into ice water, and extracted with $CHCl_3$. The organic layer was washed successively with H_2O, HCl solution (5%), saturated NaCl solution, and H_2O; dried over $MgSO_4$; and concentrated at reduced pressure. Recrystallization from MeOH produced **8** as a white amorphous powder. Yield 0.33 g (43%), $[\alpha]_D^{20}$ –10.5° (*c* 1.33, CH_2Cl_2). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.80–1.90 (27H, m, CH, CH_2), 0.77 (3H, d, J = 6.7, H_3-29 or H_3-30), 0.83 (3H, d, J = 6.7, H_3-29 or H_3-30), 0.84, 0.85, 0.86, 0.96, 1.04 (3H each, s, 5 CH_3), 2.04 [3H, s, $CH_3C(O)$], 2.60–2.72 (4H, m, $OCCH_2CH_2CO$), 3.86 (1H, d, J = 10.9, H_A-28), 4.30 (1H, d, J = 10.9, H_B-28), 4.44–4.51 (1H, dd, J = 10.4, 6.1, H-3). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 14.6, 14.9, 16.0, 16.1, 16.5, 18.2, 20.8, 21.3, 21.6, 22.9, 23.7, 26.8, 26.9, 27.9, 28.9, 29.1, 29.4, 29.8, 31.6, 34.2, 37.0, 37.2, 37.8, 38.4, 40.9, 42.9, 44.5, 46.6, 48.2, 50.0, 55.3, 63.3, 81.0, 171.1, 172.4, 177.4. Mass spectrum (ESI), *m/z* 609.6 $[M + Na]^+$ (calcd $[M + Na]^+$ 609.4). $C_{36}H_{58}O_6$.

3β-*O*-Acetyl-28-*O*-{succinyl-[3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-(2″,2″,2″-trichloroethylcarbonylamino)-β-D-glucopyranosyl]}-dihydrobetulin (9). A solution of **4** (0.2 g, 0.37 mmol) in anhydrous CH_2Cl_2 (4 mL) under Ar was treated with K_2CO_3 (0.063 g, 0.46 mmol), H_2O (5 mL), and TBAB (0.025 g, 0.08 mmol); stirred vigorously, treated dropwise with a solution of **8** (0.18 g, 0.31 mmol) in CH_2Cl_2 (6 mL), stirred for 10 h at 20°C, and diluted with CH_2Cl_2 (10 mL). The aqueous layer was separated and extracted with CH_2Cl_2. The combined organic layers were washed with H_2O and dried over $CaCl_2$. The solvent was distilled at reduced pressure. Flash chromatography of the residue over a dry column (silica gel, hexane–EtOAc, from 10:1 to 2:1) produced **9** as a white amorphous powder. Yield 0.10 g (38%), $[\alpha]_D^{20}$ +0.8° (*c* 1.33, $CHCl_3$). ^{1}H NMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.83–2.0 (26H, m, CH, CH_2), 0.76 (3H, d, J = 6.8, H_3-29 or H_3-30), 0.83 (3H, d, J = 6.8, H_3-29 or H_3-30), 0.84, 0.85, 0.86, 0.95, 1.04 (3H each, s, 5 CH_3), 2.04 [9H, br.s, 3 $CH_3C(O)$], 2.09 [3H, s, $CH_3C(O)$], 2.58–2.73 (4H, m, $OCCH_2CH_2CO$), 3.79–3.86 (2H, m, H-5′s, H_A-28), 3.88–3.98 (1H, m, H-2′s), 4.08–4.14 (1H, dd, J = 12.5, 1.4, H-6′s), 4.25–4.33 (2H, m, H-6′s, H_B-28), 4.47 (1H, dd, J = 10.4, 6.0, H-3), 4.67 (1H, d, J = 12.1, CH_ACCl_3), 4.80 (1H, d, J = 12.1, CH_BCCl_3), 5.09–5.17 (2H, m, H-4′s, NH), 5.24 (1H, t, J = 9.9, H-3′s), 5.76 (1H, d, J = 8.7, H-1′s). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 14.6, 14.8, 16.1 (2C), 16.5, 18.2, 20.5, 20.6, 20.7, 20.8, 21.2, 21.6, 22.9, 23.7,

26.8, 26.9, 27.9, 28.8, 29.1, 29.4, 29.8, 34.2, 34.6, 37.0, 37.2, 37.8, 38.4, 40.9, 42.9, 44.5, 46.5, 48.1, 49.9, 55.3, 61.6, 63.2, 67.9, 72.0, 72.9, 74.6, 80.9, 81.0, 92.5, 95.4, 154.1, 169.3, 170.5, 170.7 (2 C), 171.0, 172.1. Mass spectrum (MALDI): *m/z* 1070.8 $[M + Na]^+$ (calcd $[M + Na]^+$ 1070.4), *m/z* 1086.8 $[M + K]^+$ (calcd $[M + K]^+$ 1086.4). $C_{51}H_{76}Cl_3NO_{15}$.

3*β*-*O*-Acetyl-28-*O*-[succinyl-(3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-amino-*β*-D-glucopyranosyl)]-dihydrobetulin (10). A solution of **9** (0.1 g, 0.1 mmol) in glacial AcOH (4 mL) under Ar was treated with activated Zn dust (0.5 g, 7.6 mmol), stirred for 3 h at 20°C, and concentrated at reduced pressure. The residue was diluted with CH_2Cl_2 (10 mL), washed with $NaHCO_3$ solution (5%) and H_2O, dried over $MgSO_4$, and concentrated at reduced pressure to afford **10** (0.04 g, 42%) that was used without further purification.

3*β*-*O*-Acetyl-28-*O*-[succinyl-(3′,4′,6′-tri-*O*-acetyl-2′-deoxy-2′-acetamido-*β*-D-glucopyranosyl)]-dihydrobetulin (11). A solution of **10** (1 mmol) in CH_2Cl_2 (5 mL) under Ar was treated with Et_3N (3 mmol) and Ac_2O (10 mmol); stirred for 2 h at 20°C; diluted with CH_2Cl_2 (10 mL); washed successively with saturated $NaHCO_3$ solution, HCl solution (1 M), and H_2O; dried over Na_2SO_4, and concentrated at reduced pressure. Glycoside **11** was isolated by chromatography (silica gel, hexane–EtOAc, from 5:1 to 1:1) as a white amorphous powder. Yield 0.016 g (43%), $[\alpha]_D^{20}$ +2.8° (*c* 0.65, CH_2Cl_2). ^{1}H NMR spectrum (600 MHz, $CDCl_3$, δ, ppm, J/Hz): 0.83–1.90 (26 H, m, dihydrobetulin core), 0.76 (3H, d, J = 7.0, H_3-29 or H_3-30), 0.83 (3H, d, J = 7.0, H_3-29 or H_3-30), 0.84, 0.85, 0.86, 0.94, 1.02 (3H each, s, 5 CH_3), 1.94, 2.03, 2.037, 2.041, 2.09 [3H each, s, 5 $CH_3C(O)$], 2.58–2.72 (4H, m, $COCH_2CH_2CO$), 3.77–3.81 (1H, m, H-5′s), 3.83 (1H, d, J = 11.0, H_A-28), 4.11 (1H, dd, J = 12.5, 2.0, H-6′s), 4.25–4.34 (3H, m, H-2′s, 6′s, H_B-28), 4.47 (1H, dd, J = 11.0, 5.5, H-3), 5.11–5.19 (2H, m, H-3′s, 4′s), 5.51 (1H, d, J = 9.0, NH), 5.72 (1H, d, J = 9.0, H-1′s). ^{13}C NMR spectrum (100 MHz, $CDCl_3$, δ, ppm): 14.6, 14.8, 16.0, 16.1, 16.5, 18.2, 20.5, 20.6, 20.7, 20.8, 21.3, 21.6, 22.9, 23.2, 23.7, 26.8, 26.9, 27.9, 28.7, 29.1, 29.4, 29.7, 34.2, 34.6, 37.0, 37.2, 37.8, 38.4, 40.9, 42.9, 44.6, 46.6, 48.2, 50.0, 53.2, 55.3, 61.6, 63.2, 67.7, 72.5, 73.0, 80.9, 92.8, 169.2, 170.2, 170.6, 171.0, 171.08, 171.10, 172.3. Mass spectrum (MALDI): *m/z* 938.6 $[M + Na]^+$ (calcd $[M + Na]^+$ 938.5), *m/z* 954.6 $[M + K]^+$ (calcd $[M + K]^+$ 954.5). $C_{50}H_{77}NO_{14}$.

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