EFFECTIVE SYNTHESIS OF METHYL 3β-AMINO-3-DEOXYBETULINATE

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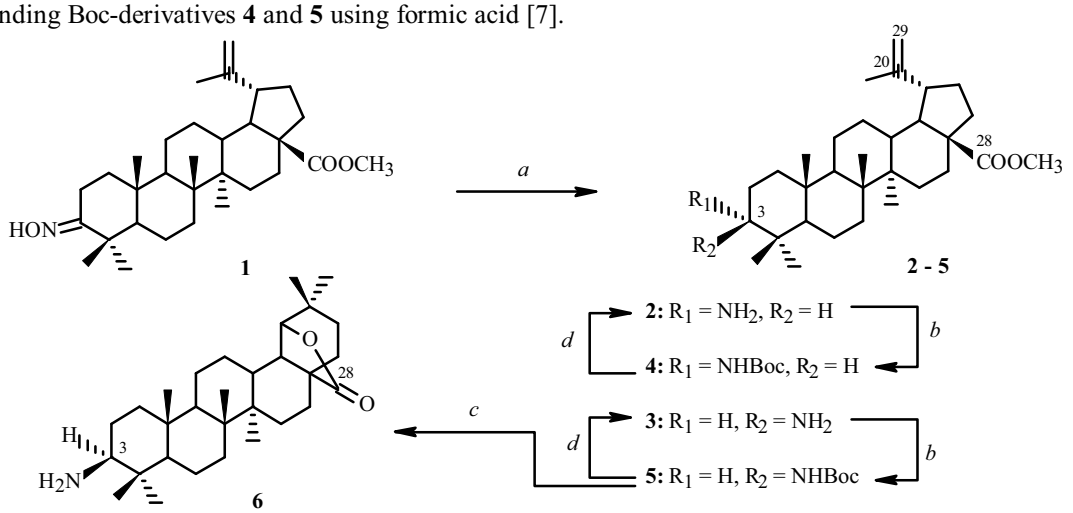
UDC 547.597:542.952.3

A practical method for preparing methyl 3β-amino-3-dexoybetulinate that is based on column chromatographic separation of 3α- and 3β-t-butoxycarbonates and subsequent removal of the protecting group using formic acid is proposed. The structure of methyl 3β-N-(t-butoxycarbonyl)-3-deoxybetulinate was established by an x-ray structure analysis.

Key words: synthesis, aminotriterpenoids, betulin, methyl 3β-*N*-(*t*-butoxycarbonyl)-3-deoxybetulinate, x-ray structure analysis.

The synthesis of pure 3α- and 3β-aminotriterpenoids is practically always complicated by their poor solubility and similar chromatographic mobility. Considering that 3-aminotriterpenoids are promising compounds for the synthesis of practically valuable derivatives [1, 2], the development of effective methods for preparing them is a timely task. The most suitable method for preparing 3β-aminotriterpenoids is reductive amination of C3-ketones to give a mixture of isomers in 80-90% yield [3-5].

We propose the following approach for preparing pure methyl 3α- and 3β-amino-3-deoxybetulinate. Reduction of methylbetulonate oxim (**1**) by sodium cyanoborohydride, analogously as before [3], produced a mixture of amines **2** and **3** in a 1:9 ratio (3α-NH_2:3β-NH_2) in 85% yield. Then, the mixture of **2** and **3** was esterified with di-*t*-butylcarbonate to form 3α- and 3β-*N*-(*t*-butoxycarbonyl) derivatives **4** and **5**, which were separated well by column chromatography. Slow evaporation of the benzene solution of **5** produced crystals, the structure of which was established by an x-ray structure analysis (Fig. 1). Treatment of **5** with trifluoroacetic acid removed the Boc protecting group and isomerized ring E to form 3β-amino-3-deoxy-28-oxoallobetulin (**6**). Trifluoroacetic acid was proposed by us previously as a catalyst for isomerizing betulin into allobetulin [6]. Methyl esters of 3α- and 3β-amino-3-deoxybetulinic acid (**2** and **3**) were prepared pure previously in quantitative yield from the corresponding Boc-derivatives **4** and **5** using formic acid [7].



a. $NaBH_3CN$, NH_4OAc, 15% $TiCl_3$, MeOH, 24 h, 0°C; *b.* Boc_2O, CH_2Cl_2, 12 h, 20°C; *c.* CF_3COOH, CH_2Cl_2, 20°C; *d.* HCOOH, MeOH, 2 h, 20°C

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0009-3130/08/4405-0603

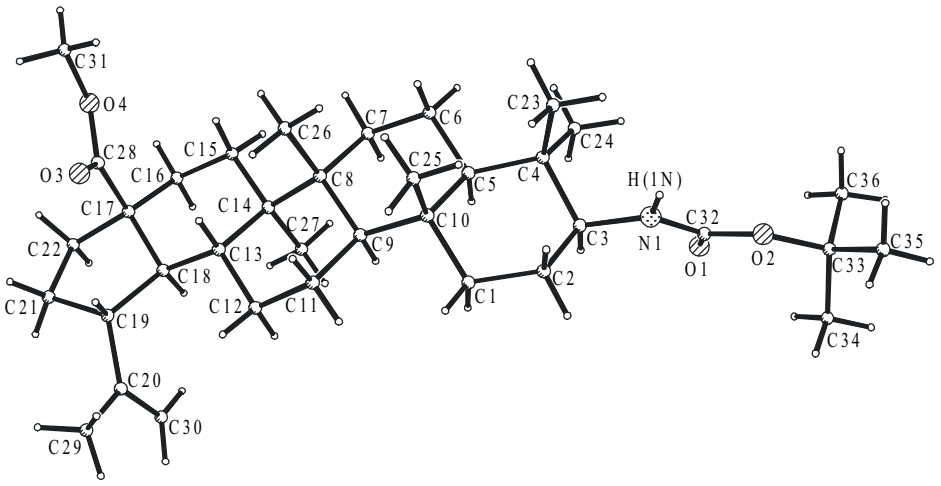

Fig. 1. Structure of methyl 3β-*N*-(*t*-butoxycarbonyl)-3-deoxybetulinate (**5**).

EXPERIMENTAL

PMR and ^{13}C NMR spectra in $CDCl_3$ and CD_3OD were recorded on a Bruker AM-300 spectrometer (300 and 75.5 MHz, respectively) with TMS internal standard. Melting points were determined on a Boetius microstage. Optical density was measured on a Perkin—Elmer 241 MC polarimeter using a 1-dm tube. TLC was performed on Silufol plates (Chemapol, Czech. Rep.) using $CHCl_3$:CH_3OH (25:1, system A; 25:3, system B). Compounds were developed using phosphotungstic acid in ethanol (5%) with subsequent heating at 100-120°C for 2-3 min. Methyl 3-hydroxyiminobetulonate (**1**) was prepared as before [8].

Methyl esters of 3-amino-3-deoxybetulinic acid (2 and 3) were prepared by reduction of **1** by $NaBH_3CN$—NH_4OAc—$TiCl_3$ by the literature method [3]. PMR spectrum (CD_3OD, δ, ppm): 0.58, 0.61, 0.65, 0.69, 0.78 (15H, 5s, $5CH_3$), 0.82-1.68 (21H, m, CH_2, CH), 1.49 (3H, s, CH_3), 1.73-2.01 (3H, m, H-13, H-16), 2.48-2.54 (0.9H, m, H-3α), 2.55-2.68 (0.1H, m, H-3β), 2.92-3.01 (1H, m, H-19), 3.34 (3H, s, OCH_3), 4.32-4.47 (2H, m, $N\underline{H}_2$), 4.60 and 4.73 (1H each, both s, H-29).

Methyl 3α- and 3β-*N*-(*t*-butoxycarbonyl)-3-deoxybetulinate (4 and 5). A solution of **2** and **3** (0.57 g, 1 mmol) that were produced by the above method in CH_2Cl_2 (20 mL) was treated with Boc_2O (350 mg, 1.6 mmol) and stirred at room temperature for 12 h. Solvent was evaporated in vacuo using a water aspirator. The solid was chromatographed over a column of Al_2O_3 with elution by benzene, $CHCl_3$, and $CHCl_3$:CH_3OH (100:1).

Compound 4. Yield 0.05 g (9%), R_f 0.59 (system A), mp 147-150°C. $C_{36}H_{59}NO_4$. PMR spectrum ($CDCl_3$, δ, ppm): 0.72, 0.78, 0.88, 0.92, 0.98 (15H, 5s, $5CH_3$), 1.20-2.00 (21H, m, CH_2, CH), 1.45 and 1.48 (9H, both s, $3CH_3$), 1.67 (3H, s, CH_3), 2.12-2.33 (3H, m, H-13, H-16), 2.92-3.07 (1H, m, H-19), 3.52 (1H, s, H-3β), 3.64 (3H, s, OCH_3), 4.32-4.42 (1H, m, NH), 4.60 and 4.73 (1H each, both br, H-29).

Compound 5. Yield 0.5 g (87%), R_f 0.84 (system A), mp 155-157°C, $[\alpha]_D^{20}$ +10.6° (*c* 0.80, $CHCl_3$). $C_{36}H_{59}NO_4$. PMR spectrum ($CDCl_3$, δ, ppm): 0.72, 0.78, 0.88, 0.92, 0.98 (15H, 5s, $5CH_3$), 1.20-2.00 (21H, m, CH_2, CH), 1.45 and 1.48 (9H, both s, $3CH_3$), 1.67 (3H, s, CH_3), 2.12-2.33 (3H, m, H-13, H-16), 2.92-3.07 (1H, m, H-19), 3.14-3.31 (1H, m, H-3β), 3.64 (3H, s, OCH_3), 4.32-4.42 (1H, m, NH), 4.60 and 4.73 (1H each, both br, H-29). ^{13}C NMR spectrum (δ, ppm): 14.6, 15.8, 16.1, 18.5, 19.2, 20.7, 25.4, 25.9, 27.7, 28.2 (2 × CH_3), 28.3 (CH_3), 29.5, 30.4, 32.0, 34.1, 36.9, 37.2, 37.8, 38.1, 39.1, 40.5, 40.7, 42.2, 46.8, 49.3, 50.3, 51.1, 56.1, 56.4, 58.0 (C-3), 78.7 [$(CH_3)_3\underline{C}O$], 109.5 (C-29), 150.4 (C-20), 155.7 (–NH$\underline{C}$OO), 176.5 (C-28).

X-ray Structure Analysis of Methyl 3β-*N*-(*t*-butoxycarbonyl)-3-deoxybetulinate (5). Colorless needle-like crystals, $C_{36}H_{59}NO_4$ (MW = 569.84), orthorhombic at 293 K, a = 8.547(11) Å, b = 18.44(2), c = 21.97(3), V = 3464(8) Å^3, space group $P2_12_12_1$, Z = 4, d_{calc} = 1.093 g/cm^3. A data set of reflections was collected on a CAD4 Enraf—Nonius diffractometer at 120 K (λ Mo Kα-radiation, $2\theta_{max}$ = 49°) using a single crystal (0.55 × 0.40 × 0.30 mm). Equivalent reflections were averaged to produce 6488 independent reflections (R_{int} = 0.0732) that were used to solve and refine the structure. The structure was solved by direct methods and refined anisotropically over F^2_{hkl} (H atoms were placed in geometrically calculated positions and

refined isotropically using the rider model). The final agreement factors were $R_1 = 0.0544$ [calculated over F_{hkl} for 3865 reflections with $I > 2\sigma(I)$], $wR_2 = 0.1225$ [calculated over F^2_{hkl} for all 6488 reflections], GOOF = 1.014 for 370 refined parameters. All calculations were carried out using the SHELXTL Plus 5 programs. Atomic coordinates and temperature factors were deposited in the Cambridge Crystallographic Data Center (CCDC 630827).

3β-Amino-3-deoxy-28-oxoallobetulin (6). A solution of **5** (0.57 g, 1 mmol) in CH_2Cl_2 (10 mL) was stirred, treated with CF_3COOH (1 mL), neutralized after 10 min with saturated Na_2CO_3 solution (TLC monitoring), washed with water, dried over $CaCl_2$, and evaporated in vacuo. Yield 0.43 g (95%), R_f 0.34 (system B), mp >330°C, $[\alpha]_D^{20}$ +18.2° (*c* 0.62, CH_3OH). $C_{30}H_{49}NO_2$. PMR spectrum (CD_3OD, δ, ppm): 0.75, 0.78, 0.81, 0.86, 0.90, 0.98, 1.03 (21H, 7s, $7CH_3$), 1.20-1.90 (22H, m, CH_2, CH), 2.45-2.57 (1H, m, H-3β), 3.55 (1H, s, H-19), 4.34-4.45 (2H, m, NH_2).

Methyl 3α-amino-3-deoxybetulinate (2). A solution of **4** (0.57 g, 1 mmol) in HCOOH (95%, 2 mL) was stirred for 2 h at room temperature and diluted with water (100 mL). The precipitate was filtered off, washed with water until neutral, and dried. Yield 0.43 g (92%), R_f 0.46 (system B), mp 83-85°C, $[\alpha]_D^{20}$ +19.2° (*c* 0.76, CH_3OH). $C_{31}H_{51}NO_2$. PMR spectrum (CD_3OD, δ, ppm): 0.78, 0.81, 0.85, 0.89, 0.98 (15H, 5s, $5CH_3$), 1.20-2.00 (21H, m, CH_2, CH), 1.69 (3H, s, CH_3), 2.13-2.33 (3H, m, H-13, H-16), 2.74 (1H, s, H-3β), 2.92-3.06 (1H, m, H-19), 3.64 (3H, s, OCH_3), 4.32-4.47 (2H, m, NH_2), 4.60 and 4.73 (1H each, both s, H-29).

Methyl 3β-amino-3-deoxybetulinate (3) was prepared from **5** by the above method. Yield 0.42 g (89%), R_f 0.28 (system B), mp 215-218°C (lit. [9] mp 210.5-213.5°C), $[\alpha]_D^{20}$ +10.2° (*c* 0.41, CH_3OH). $C_{31}H_{51}NO_2$. PMR spectrum (CD_3OD, δ, ppm): 0.79, 0.83, 0.85, 0.91, 0.98 (15H, 5s, $5CH_3$), 1.20-2.00 (21H, m, CH_2, CH), 1.70 (3H, s, CH_3), 2.15-2.30 (3H, m, H-13, H-16), 2.48-2.54 (1H, m, H-3β), 2.92-3.06 (1H, m, H-19), 3.66 (3H, s, OCH_3), 4.30-4.43 (2H, m, NH_2), 4.62 and 4.75 (1H each, both s, H-29).

ACKNOWLEDGMENT

The work was supported financially by the RFBR (Project No. 08-03-00868).

REFERENCES

1. C.-M. Ma, N. Nakamura, and M. Hattori, *Chem. Pharm. Bull.*, **48**, 1681 (2000).
2. C.-M. Ma, S.-Q. Cai, R.-Q. Wang, P.-F. Tu, M. Hattori, and M. Daneshtalab, *Eur. J. Med. Chem.*, **40**, 582 (2005).
3. M. Tsukahara, T. Nishino, I. Furuhashi, H. Inoue, T. Sato, and H. Matsumoto, *Chem. Pharm. Bull.*, **53**, 1103 (2005).
4. D. S. H. L. Kim, J. M. Pezzuto, and E. Pisha, *Bioorg. Med. Chem. Lett.*, **8**, 1707 (1998).
5. Y. Kashiwada, J. Chiyo, Y. Ikeshiro, T. Nagao, H. Okabe, L. M. Cosentino, K. Fowke, S. L. Morris-Natschke, and K.-H. Lee, *Chem. Pharm. Bull.*, **48**, 1387 (2000).
6. N. I. Medvedeva, O. B. Flekhter, O. S. Kukovinets, F. Z. Galin, G. A. Tolstikov, I. Baglin, and K. Kave, *Izv. Akad. Nauk, Ser. Khim.*, **4**, 804 (2007).
7. T. Honda, T. Janosik, Y. Honda, J. Han, K. T. Liby, C. R. Williams, R. D. Couch, A. C. Anderson, M. B. Sporn, and G. W. Gribble, *J. Med. Chem.*, **47**, 4923 (2004).
8. I. Valterova, J. Klinot, and A. Vystruil, *Collect. Czech. Chem. Commun.*, **48**, 649 (1983).
9. U. Wrzeciono and A. Mikolajewska, *Rocz. Chem.*, **46**, 1285 (1972).