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Synthesis and Antitubercular and Antibacterial Activities of Triethylammonium 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyl Decyl Phosphate

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Abstract—Phosphorylation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucose at the anomeric hydroxy group gave previously unknown triethylammonium 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyl phosphonate, and successive treatment of the latter with decan-1-ol and aqueous iodine afforded triethylammonium 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl phosphate.

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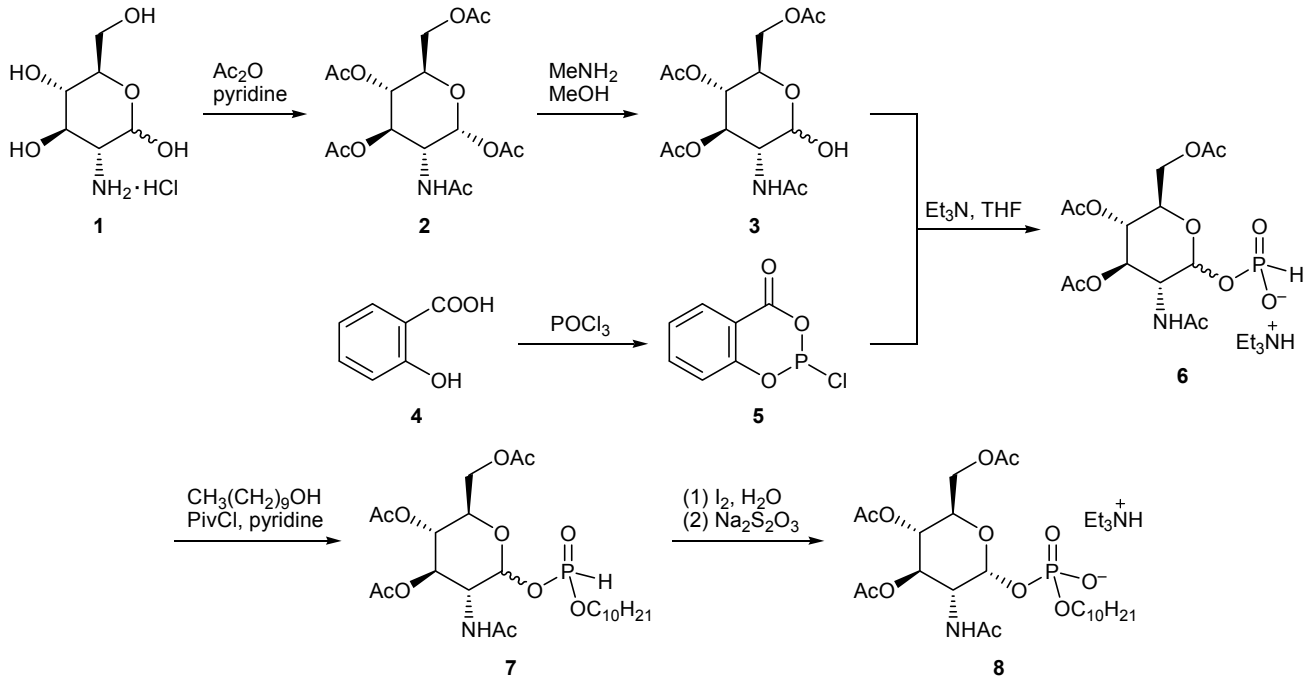
We have recently reported the synthesis of phosphorylated glycolipids based on glucuronic acid, which showed antitubercular activity against *Mycobacterium tuberculosis* H37Rv (MBT) comparable with the activity of the known antitubercular drug pyrazinamide (MIC 12.5 μg/mL) [1]. In continuation of our studies in the field of synthesis and antitubercular activity of glucosamine derivatives [2, 3], herein we describe a novel approach to phosphorylated glucosamine-based glycolipids. This approach utilizes commercially available glucosamine hydrochloride (**1**) as starting compound which was acylated with acetic anhydride in pyridine to pentaacetyl derivative **2**. Regioselective removal of the acetyl protection from the anomeric hydroxyl of **2** by the action of methylamine in methanol gave tetraacetyl glucosamine **3**. The latter reacted with 2-chloro-2*H*,4*H*-1,3,2-benzodioxaphosphinin-4-one (**5**) [4] acording to the procedure described in [1] to give triethylammonium 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α/β-D-glucopyranosyl phosphonate (**6**) in 34% yield (after isolation by chromatography). The ^{1}H NMR spectrum of **6** contained signals typical of tetraacetyl glucosamine **3**, as well as signals of protons of the triethylammonium cation and PH proton (δ 6.90 ppm, d, $^1J_{HP}$ = 668 Hz). The ^{31}P NMR spectrum of **6** showed a singlet at δ_P 1.93 ppm. The anomeric proton in the carbohydrate moiety of **6** resonated as a multiplet in the region δ 5.61–5.69 ppm, indicating formation of a mixture of anomers. In the next stage, phosphonate **6** was activated with pivaloyl chloride and treated with decan-1-ol. Glycolipid **7** thus formed was oxidized without isolation with aqueous iodine to produce 23% (after isolation by chromatography) of target phosphorylated glycolipid **8**. The formation of **8** was confirmed primarily by the disappearance of PH singlet at δ_P 1.93 ppm from the ^{31}P NMR spectrum and appearance of a singlet at δ_P –2.27 ppm, which is typical of phosphate monoanion (PO_4^-). In the MALDI mass spectrum of **8** we observed the molecular ion peak $[M]^-$ with *m*/*z* 566.4 (calcd.: *m*/*z* 566.2, $C_{24}H_{41}NO_{12}P^-$). The anomeric proton signal of **8** was located in the ^{1}H NMR spectrum at δ 5.51 ppm ($^3J_{HP}$ = 7.34, $J_{1,2}$ = 3.22 Hz), in keeping with α-orientation of the glycoside bond.

The described method of synthesis of glycolipids **8** essentially differs from the known procedures for the preparation of phosphorylated glucosamine-based glycolipids containing a phosphate or pyrophosphate (diphosphate) fragment [5–11]. First, the key glucosamine derivative with protected hydroxyl groups in positions *3*, *4*, and *6* and free anomeric hydroxyl group (monosaccharide **3**), which was subjected to phosphorylation, was readily prepared by regioselective deprotection of the anomeric hydroxyl group of penta-

Scheme 1.



acetylglucosamine **2** by the action of methylamine in methanol. Second, by analogy with [1], we utilized a different procedure for the synthesis of glycolipids with a phosphate group from tetraacetylated glucosamine **3**. Monosaccharide **3** is usually phosphorylated by reaction with dibenzyl *N*,*N*-diisopropylphosphoramidite and 1*H*-tetrazole [9], followed by oxidation with hydrogen peroxide, debenzylation, and treatment with tetrabutylammonium hydroxide. The salt thus obtained is then alkylated with alkyl bromide in the presence of molecular sieves. In our case, the alkylation involved not glucosamine phosphate but phosphonate which was readily synthesized from tetraacetylglucosamine **3** and phosphorochloridite **5**. The synthesis of target compound **8** from salt **6** was carried out in a one-pot fashion with the oxidation of phosphonate **7** to phosphate with iodine in the final stage.

The antimicrobial activity of glycolipid **8** against gram positive and gram negative bacteria and fungi, as well as its antitubercular activity, was studied. Compound **8** inhibited the growth of *S. aureus* at a minimum inhibitory concentration (MIC) of 62.5 μg/mL, which is comparable to the activity of Chloramphenicol used as control. However, compound **8** turned out to be inactive against other microorganisms. It should be noted that no data on antibacterial activity of glucosamine derivatives were reported previously. Glycolipid **8** inhibited the growth of MBT at a MIC of 3.1 μg/mL; i.e., its antitubercular activity was much lower than the activity of the first line antitubercular drug isoniazid (MIC 0.1 μg/mL) but 4 times higher than that of the second line antitubercular drug pyrazinamide [12] and macrocyclic glucosamine derivatives [3] (MIC 13 μg/mL). We are continuing studies on the synthesis and biological activity of phosphorylated glycolipids based on glucosamine.

EXPERIMENTAL

The ^{1}H, ^{13}C, and ^{31}P NMR spectra were recorded on a Bruker Avance-400 spectrometer (Germany) at 400 (^{1}H) and 100.6 MHz (^{13}C, ^{31}P) using the solvent signals as reference ($CDCl_3$). Signals were assigned on the basis of published data [1, 3, 13]. The mass spectra (MALDI) were obtained on a Bruker Daltonik UltraFlex III TOF/TOF instrument operating in the linear mode (Nd:YAG laser, λ 355 nm); the data were processed by Bruker Daltonik FlexAnalysis 3.0; a.m.u. range 200–6000, negative ion detection, metal target, *p*-nitroaniline matrix; samples were dissolved in methanol to a concentration of 10 μg/mL. The optical rotations were measured on a Perkin Elmer-341 polarimeter at λ 589 nm (temperature 20°C). The progress of reactions was monitored, and the purity of the isolated compounds was checked, by thin-layer chromatography on Sorbfil plates; spots were detected by treatment with 5% sulfuric acid, followed by heating to 120°C.

Glucosamine hydrochloride was commercial product (Acros Organics). Compounds **2**, **3**, and **5** were synthesized as described in [14–16].

Triethylammonium 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α/β-D-glucopyranosyl phosphonate (6). Compound **5**, 0.42 g (2 mmol), was added with stirring to a solution of 0.67 g (2 mmol) of tetraacetylglucosamine **3** and 1.87 mL (18.5 mmol) of triethylamine in 10 mL of THF. The mixture was stirred for 3 h, 1 mL of water was added, and the mixture was stirred for 1 h more. The mixture was then evaporated to dryness, and the product was isolated by chromatography on silica gel using methylene chloride–methanol (40:1 to 5:1 with addition of 1% of triethylamine) as eluent. Yield 0.33 g (34%), colorless oily material, $[\alpha]_D^{20}$ = 56.7 (c = 0.8, $CHCl_3$). ^{1}H NMR spectrum, δ, ppm: 1.36 t (9H, C**H**$_3$CH$_2$N, J = 7.30 Hz); 1.97 s (3H), 2.01 s (6H), and 2.07 s (3H) (CH_3CO); 3.08 q (6H, CH_3C**H**$_2$N, J = 7.30 Hz), 4.09–4.27 m (3H, 2-H, 6-H), 4.36–4.47 m (1H, 5-H), 5.17 t (1H, 4-H, J = 9.62 Hz), 5.30 t (1H, 3-H, J = 9.92 Hz), 5.61–5.69 m (1H, 1-H), 6.90 d (1H, PH, J = 668.04 Hz), 6.90–7.05 m [1H, N**H**C(O)CH$_3$], 11.73 br.s (1H, **H**NEt$_3$). ^{13}C NMR spectrum, δ$_C$, ppm: 8.67 s (3C, **C**H$_3$CH$_2$N), 20.72 br.s (2C, **C**H$_3$CO), 20.83 s (**C**H$_3$CO), 23.05 s (**C**H$_3$CONH), 45.88 s (3C, CH$_3$**C**H$_2$N), 52.09 s (C^2), 61.93 s (C^6), 68.38 s (C^4), 68.99 s (C^3), 71.21 s (C^5), 93.05 d (C^1, J_{CP} = 3.91 Hz); 169.55 s, 170.15 s, 171.19 s, 171.27 s (CH$_3$**C**O). ^{31}P NMR spectrum: δ$_P$ 1.93 ppm (PH). Found, %: C 47.05; H 7.49; N 5.53; O 33.95; P 5.98. $C_{20}H_{37}N_2O_{11}P$. Calculated, %: C 46.87; H 7.28; N 5.47; O 34.34; P 6.04.

Triethylammonium 2-acetamido-3,4,6-triacetyl-2-deoxy-α-D-glucopyranosyl decyl phosphate (8). A solution of 0.37 g (0.72 mmol) of phosphonate **6** in 10 mL of pyridine was cooled to –20°C, 0.2 g (1.26 mmol) of decan-1-ol was added with stirring, and a solution of 0.3 mL (2.5 mmol) of pivaloyl chloride in 5 mL of pyridine was then added. The mixture was stirred for 1 h, 1 mL of water and 0.18 g (0.71 mmol) of iodine were added, the mixture was stirred for 2 h, and a 1 M solution of $Na_2S_2O_3$ was added dropwise until the iodine color disappeared. The light yellow mixture was evaporated to dryness, and the product was isolated from the residue by chromatography on silica gel using methylene chloride–methanol (40:1 to 5:1 with addition of 1 vol % of triethylamine) as eluent. Yield 0.11 g (23%), colorless oily material, $[\alpha]_D^{20}$ = 26.6 (c = 0.642, MeOH). ^{1}H NMR spectrum, δ, ppm: 0.86 t [3H, C**H**$_3$(CH$_2$)$_9$, J = 7.0 Hz], 1.18 s (4H, CH$_3$C**H**$_2$C**H**$_2$), 1.21–1.29 m [12H, [(C**H**$_2$)$_6$CH$_2$O], 1.32 t (9H, C**H**$_3$CH$_2$N, J = 7.3 Hz); 1.94 s, 1.98 s, 1.99 s, and 2.05 s (3H each, CH_3CO); 3.06 q (6H, CH$_3$C**H**$_2$N, J = 7.31 Hz), 3.83–3.94 m (2H, CH$_2$O), 4.05–4.10 m (1H, 2-H), 4.18-4.25 m (2H, 6-H), 4.30–4.40 m (1H, 5-H), 5.16 t (1H, 4-H, J = 9.80 Hz), 5.30 t (1H, 3-H, J = 10.04 Hz), 5.51 d.d (1H, 1-H, J = 7.34, 3.22 Hz), 7.06 d [1H, N**H**C(O)CH$_3$, J = 9.35 Hz], 11.98 br.s (1H, **H**NEt$_3$). ^{13}C NMR spectrum, δ$_C$, ppm: 8.71 s (3C, **C**H$_3$CH$_2$N), 14.14 s [**C**H$_3$(CH$_2$)$_9$], 20.68 s (**C**H$_3$CO), 20.78 s (**C**H$_3$CO), 22.71 s (**C**H$_3$CO), 22.99 s (**C**H$_3$CONH), 25.88 s [CH$_3$**C**H$_2$(CH$_2$)$_8$], 27.41 s (CH$_3$CH$_2$**C**H$_2$), 29.36 s (CH$_3$CH$_2$CH$_2$**C**H$_2$)), 29.47 s [CH$_3$(CH$_2$)$_3$**C**H$_2$], 29.62 s [CH$_3$(CH$_2$)$_4$**C**H$_2$], 29.68 s [**C**H$_2$(CH$_2$)$_3$O], 30.88 s (**C**H$_2$CH$_2$CH$_2$O), 31.93 s (**C**H$_2$CH$_2$O), 45.71 s (3C, CH$_2$N), 52.15 s (C^2), 61.96 s (C^6), 66.16 s (CH$_2$O), 68.36 s (C^4), 68.65 s (C^5), 71.53 s (C^3), 94.16 d (C^1, J_{CP} = 5.42 Hz), 169.48 s (CH$_3$**C**O), 170.84 s (CH$_3$**C**O), 171.02 s (CH$_3$**C**O), 182.24 s (CH$_3$**C**O). ^{31}P NMR spectrum (100.6 MHz, $CDCl_3$): δ$_P$ –2.27 ppm (PO_4^-). Mass spectrum: m/z 566.4 (I_{rel} 100%) [M – Et$_3$N]$^-$. $C_{24}H_{41}NO_{12}P$. Calculated: M – Et$_3$N 566.2. Found, %: C 54.09; H 8.78; N 4.21; O 28.37; P 4.55. $C_{30}H_{57}N_2O_{12}P$. Calculated, %: C 53.88; H 8.59; N 4.19; O 28.71; P 4.63.

The antimicrobial activity of glycolipid **8** was evaluated by the serial dilution method on liquid nutrient media according to the procedures described in [17, 18]; the minimum inhibitory concentrations were determined. The test cultures were gram positive bacteria *S. aureus* ATCC 209p and *B. cereus* ATCC 8035, gram negative bacteria *E. coli* CDC F-50 and *P. aeruginosa* ATCC 9027, and fungi *A. niger* BKMF-1119, *T. mentagrophytes var. gypseum* 1773, and *C. albicans* 855–653.

The antitubercular activity of glycolipid **8** against *Mycobacterium tuberculosis* H37Rv (MBT) laboratory strain was evaluated by the vertical diffusion method on *Novaya* solid nutrient medium. The medium was placed in 5-mL test tubes which were inoculated with 0.1 mL of a suspension of mycobacteria diluted to a turbidity of 10 GKI units; the test tubes were incubated for 24 h to grow MBT. The test tubes were set vertically, and 0.3 mL of a solution of **8** in DMSO with a concentration of 12.5, 6.2, 3.1, 1.5, 0.7, 0.35, or 0.1 μg/mL was added dropwise to each test tube. The test tubes were then placed in a thermostat and incubated for 10 days at 37°C under sterile conditions. The growth of MBT was evaluated according to a standard procedure, according to which an inhibition zone of larger than 10 mm indicates tuberculostatic

activity. The MBT growth inhibition zone (mm) is proportional to the tuberculostatic activity. An inhibition zone of 100 mm and larger is regarded as the complete inhibition. The antitubercular drug isoniazid (MIC 0.1 μg/mL) was used as control.

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REFERENCES

1. Izmest'ev, E.S., Andreeva, O.V., Sharipova, R.R., Kravchenko, M.A., Garifullin, B.F., Strobykina, I.Yu., Kataev, V.E., and Mironov, V.F., *Russ. J. Org. Chem*., 2017, vol. 53, p. 51.
2. Garifullin, B.F., Strobykina, I.Yu., Sharipova, R.R., Kravchenko, M.A., and Kataev, V.E., *Macroheterocycles*, 2016, vol. 9, p. 320.
3. Garifullin, B.F., Strobykina, I.Yu., Sharipova, R.R., Kravchenko, M.A., Andreeva, O.V., Bazanova, O.B., and Kataev, V.E., *Carbohydr. Res*., 2016, vol. 431, p. 15.
4. Young, R.W., *J. Am. Chem. Soc*., 1952, vol. 74, p. 1672.
5. Beletskaya, I.P., Karlstedt, N.B., Nifant'ev, E.E., Khodarev, D.V., Kukhareva, T.S., Nikolaev, A.V., and Ross, A.J., *Russ. J. Org. Chem*., 2006, vol. 42, p. 1780.
6. Montoya-Peleaz, P.J., Riley, J.G., Szarek, W.A., Valvano, M.A., Schutzbach, J.S., and Brockhausen, I., *Bioorg. Med. Chem. Lett*., 2005, vol. 15, p. 1205.
7. Brockhausen, I., Larsson, E.A., and Hindsgaul, O., *Bioorg. Med. Chem. Lett*., 2008, vol. 18, p. 807.
8. Riley, J.G., Xu, C., and Brockhausen, I., *Carbohydr. Res*., 2010, vol. 345, p. 586.
9. Dumbre, S., Derouaux, A., Lescrinier, E., Piette, A., Joris, B., Terrak, M., and Herdewijn, P., *J. Am. Chem. Soc*., 2012, vol. 134, p. 9343.
10. Vinnikova, A.N., Torgov, V.I., Utkina, N.S., Veselovsky, V.V., Druzhinina, T.N., Wang, S., Brockhausen, I., and Danilov, L.L., *Russ. J. Bioorg. Chem*., 2015, vol. 41, p. 105.
11. Chen, C., Liu, B., Xu, Y., Utkina, N., Zhou, D., Danilov, L., Torgov, V., Veselovsky, V., and Feng, L., *Carbohydr. Res*., 2016, vol. 430, p. 36.
12. Donald, P.R., *Tuberculosis*, 2010, vol. 90, p. 279.
13. Coxon, B., *Carbohydr. Res*., 2005, vol. 340, p. 1714.
14. Cao, Z., Qu, Y., Zhou, J., Liu, W., and Yao, G., *J. Carbohydr. Chem*., 2015, vol. 34, p. 28.
15. Gorityala, B.K., Lu, Z., Leow, M.L., Ma, J., and Liu, X.-W., *J. Am. Chem. Soc*., 2012, vol. 134, p. 15229.
16. Donahue, M.G. and Johnston, J.N., *Bioorg. Med. Chem. Lett*., 2006, vol. 16, p. 5602.
17. National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility. Tests for bacteria that grow aerobically – Sixth edition: Approved standard. M7-A5, NCCLS, Wayne, PA, USA. 2000.
18. National Committee for Clinical Laboratory Standards. Reference method for broth dilution antifungal susceptibility testing of conidium-forming filamentous fungi: Proposed standard. M38-P, NCCLS, Wayne, PA, USA. 1998.