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Synthesis and Biological Activity of 3,4,-Tri-*O*-Acetyl-*N*-Acetylglucosamine and Tetraacetylglucopyranose Conjugated with Alkyl Phosphates

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Abstract—Conjugates of 3,4,6-tri-*O*-acetyl-*N*-acetylglucosamine and tetraacetyl glucopyranose with alkyl phosphates were synthesized. The dependence of their antibacterial and antituberculosis activities on the length of the alkyl substituent at the phosphate group was found. The conjugates with a decyl substituent exhibited in vitro the highest antituberculosis activity against *Mycobacterium tuberculosis* H37Rv (MIC 3 μg/mL) but the weakest effect towards *Streptococcus aureus* and *Bacillus cereus* (≤MIC 125 μg/mL). Vice versa, the conjugates with a cetyl substituent demonstrated the highest antibacterial activity in vitro towards *S. aureus* and *B. cereus* (MIC 16 μg/mL) but showed the lowest antituberculosis activity (MIC 12 μg/mL) among the compounds under study.

Keywords: antibacterial activity, antituberculosis activity, glucosamine, glucopyranose, glycoconjugates, *Mycobacterium tuberculosis*

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INTRODUCTION

Peptidoglycan is an important macromolecule, which surrounds bacteria and mycobacteria, shapes them and protects from the damaging effect of their own high osmotic pressure. Inhibition of the PG synthesis results in the lysis of bacterial cells. Therefore, all the enzymes involved in its synthesis are attractive targets for designing new antibacterial and antimycobacterial (antituberculosis) agents. Of the wide enzyme spectrum, the attention of researchers is focused on GlmU-T and PG-Ts, which catalyze the latest stages of PG biosynthesis [1–3].

In the recent decade, a series of Glm-U and PG-Ts modified at carbohydrate residues, phosphate groups or alkyl substituents has been synthesized (Fig. 1) [3–9]. The most known inhibitors bear a disaccharide residue composed of GlcNAc and *N*-acetylmuramic acid fragments (MurNAc) (compounds (**I**) [4]) or contain a single GlcNAc fragment (compounds (**II**) and (**III**) [5–8]). Also, the known inhibitors are divided into diphosphates (compounds (**II**) [5–8]) and monophosphates (compounds (**I**) and (**III**) [4, 7]). It was shown that inhibitors (**II**) and (**III**) are alternative substrates for bacterial glucosyl and galactosyl transferases and could inhibit to some extent the activity of various bacterial enzymes [4–8]. It is interesting that the most structurally simple compound (**IV**) (Fig. 1) can inhibit *M. tuberculosis* GlmU-T at millimolar concentrations [9]. It should seem that if a compound could inhibit any enzyme on the way to bacterial PG, it would inhibit bacterial growth as well. Surprisingly, we have not found any publications describing how the synthesized inhibitors of bacterial glucosyl or galactosyl transferases inhibited the growth of these bacteria. We only found one comparative report of antienzymatic and antibacterial activities of glycosyl transferase inhibitors [4], the results of which were amazing. In particular, compounds (**III**), the best inhibitors of *E. coli* PBP1b PG-T, did not demonstrate antibacterial activity [4].

Taking into consideration these data, we decided to check whether the acetylation of carbohydrate hydroxyl groups results in the appearance of antibacterial activity of the compounds of type (**III**).

Abbreviations: GlcNAc, *N*-acetylglucosamine; GlmU-T, GlcNAc-1*P*-uridyl transferase; PG-T, PG glycosyl transferase; GlcNAc-1-*P*-alkyl, *N*-acetylglucosamine 1-alkylphosphate; MIC, minimal inhibitory concentration; PG, peptidoglycan; TMS-OTF, trimethylsilyl trifluoromethanesulfonate.

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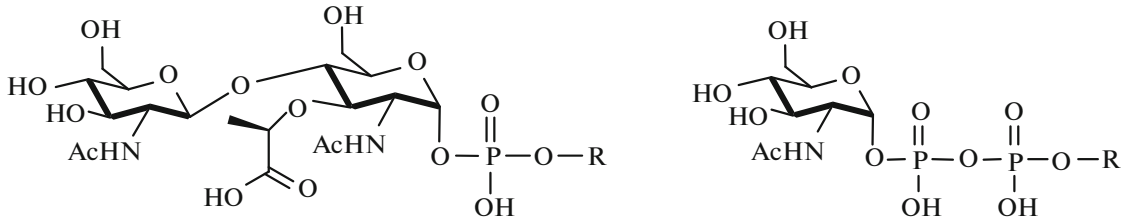
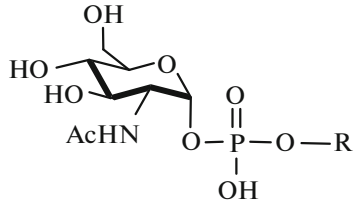
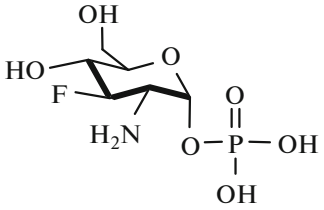


GlcNAc-1-4-MurNAc-1-*P*-R **(I)**

GlcNAc-1-*PP*-R **(II)**



GlcNAc-1-*P*-R **(III)**



(IV)

R = prenyl ($-C_5H_9$); farnesyl ($-C_{15}H_{25}$); phenoxyhexyl ($-C_6H_{12}OPh$); decyl ($-C_{10}H_{21}$); phenoxyundecyl ($C_{11}H_{22}OPh$); cetyl ($-C_{16}H_{33}$); phenoxycetyl ($C_{16}H_{32}OPh$)

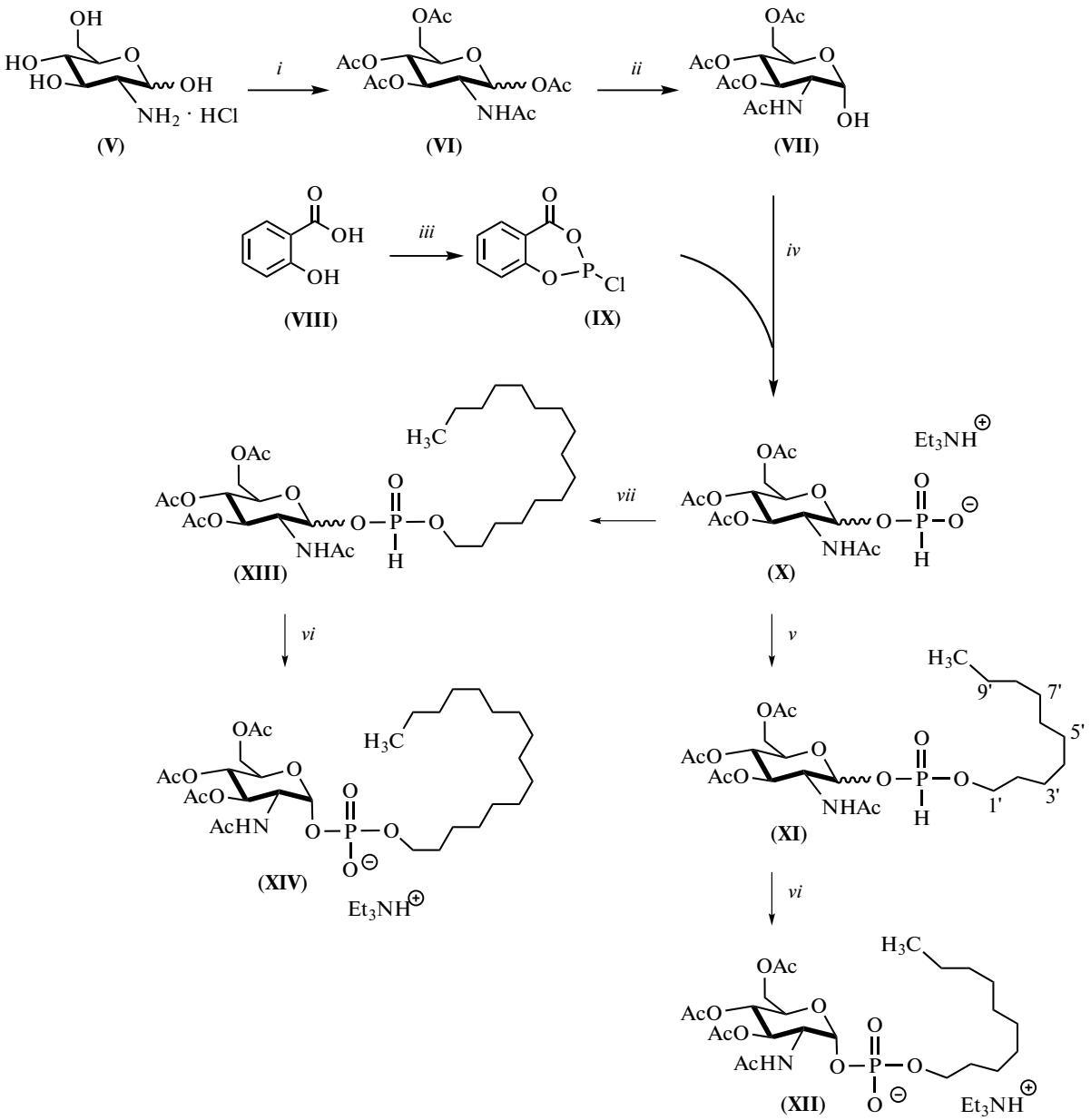
Fig. 1. Some examples of enzyme inhibitors involved in the biosynthesis of bacterial and mycobacterial peptidoglycans.

RESULTS AND DISCUSSION

In this work, we described the synthesis, antibacterial and antituberculosis activities of conjugates of 3,4,6-tri-*O*-acetyl-*N*-acetyl-D-glucosamine and tetraacetylated D-glucopyranose with alkyl phosphates.

As a starting compound for the synthesis of the target conjugates (hereafter glycophosphates) on the basis of 3,4,6-tri-*O*-acetyl-*N*-acetyl-D-glucosamines (**XII**) and (**XIV**) we used commercial glucosamine hydrochloride (**V**) (Scheme 1). It was acetylated as described in [10] and the resulting 1,3,4,6-tetra-*O*-acetyl-*N*-acetyl-D-glucosamine (**VI**) was transformed to 3,4,6-tri-*O*-acetyl-*N*-acetyl-D-glucosamine (**VII**) by the regioselective removal of the protective group from the anomeric hydroxyl group using methylamine in methanol similarly to [11]. Compound (**VII**) was phosphonylated at its anomeric hydroxyl group with 2-chloro-2*H*,4*H*-1,3,2-benzodioxaphosphorin-4-one (**IX**) [12], which was prepared from salicylic acid (**VIII**) as described in [13]. *H*-Phosphonate (**X**) was isolated after chromatography in 34% yield. In the ^{1}H NMR spectrum of phosphonate (**X**), a triplet at 1.36 ppm ($J_{H\text{-}H}$ 7.3 Hz) and a quartet at 3.08 ppm ($J_{H\text{-}H}$ 7.3 Hz), which corresponded to the resonances of triethylammonium protons, and a doublet at 6.9 ppm ($J_{P\text{-}H}$ 668 Hz) corresponding to the proton resonance of the P–H group were seen along with characteristic resonances of 3,4,6-tri-*O*-acetyl-*N*-acetylglucosamine (**VII**). The anomeric proton was observed as a multiplet at 5.61–5.69 ppm, which implied the formation of the anomeric mixture. The ^{31}P NMR of compound (**X**) contained a singlet at 1.93 ppm inherent for a phosphorus atom of *H*-phosphonates.

At the next step, *H*-phosphonate (**X**) was activated with pivaloyl chloride and treated with decanol. The resulting glycophosphonate (**XI**) was oxidized with aqueous iodine solution and the target glycophosphate (**XII**) was obtained after chromatography in 23% yield. Its structure was confirmed in the first place by the disappearance in the ^{1}H NMR spectrum of a proton resonance of the P–H group at 6.9 ppm and the appearance in the ^{31}P NMR spectrum of a singlet at –2.27 ppm corresponding to the phosphate group in a monoanionic form (PO_4^-). Mass spectral data also indicated the formation of glycophosphate (**XII**). The MALDI spectrum of glycophosphate (**XII**) contained a peak at *m/z* 566.4 $[M]^-$ ($C_{24}H_{41}NO_{12}P$). The resonance of the anomeric proton of glycophosphate (**XII**) was observed as a doublet of doublets at 5.51 ppm ($^3J_{H1,P}$ 7.34 Hz, $^3J_{H1,H2}$ 3.22 Hz), which implied the formation of the individual α-anomer.

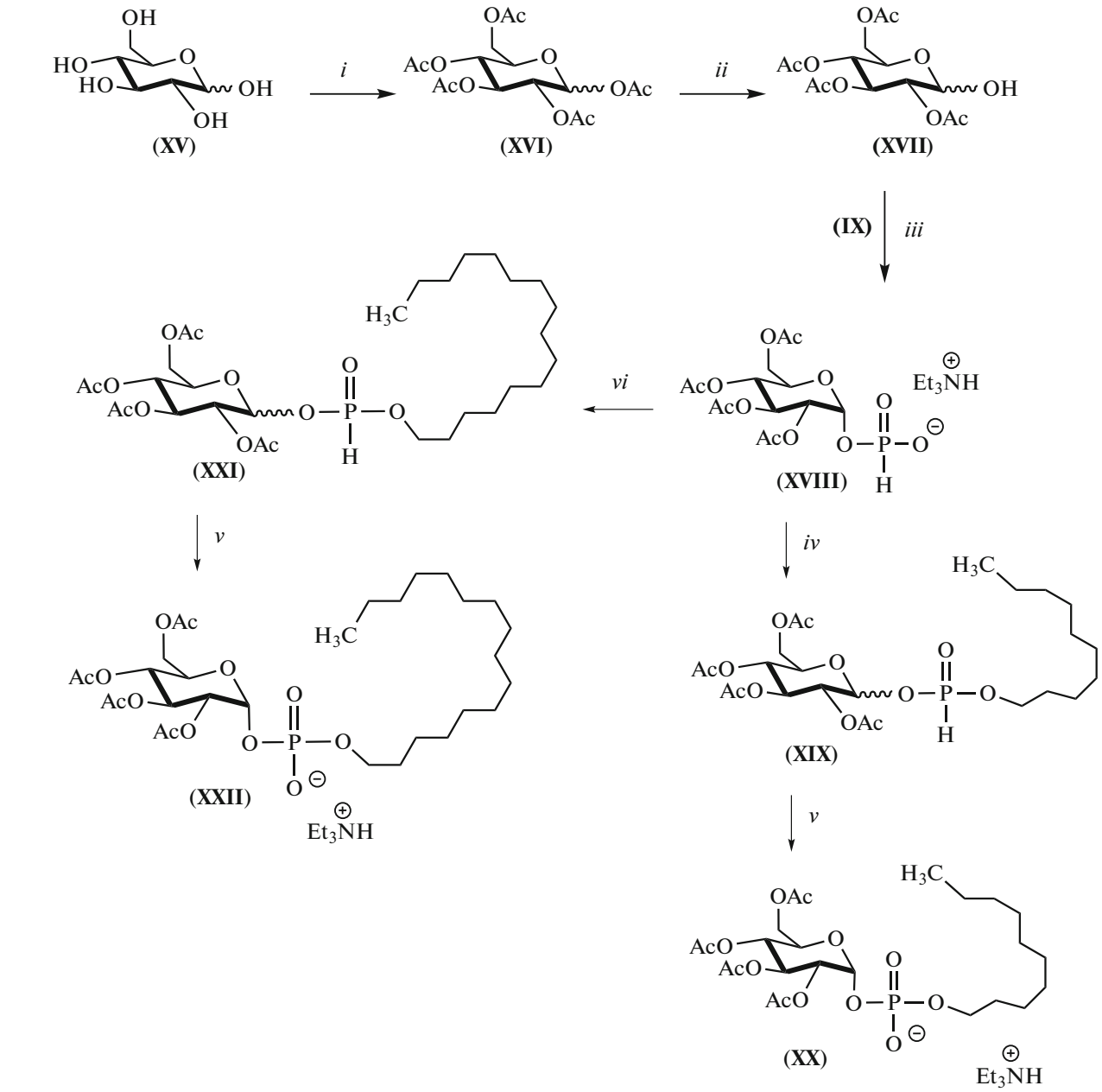


Scheme 1. The synthesis of glycophosphates (**XII**) and (**XIV**). Conditions and reagents: (*i*) Ac_2O, Py, 0 → 20°C, 24 h; (*ii*) CH_3NH_2, CH_3OH, THF, *rt*, 0.5 h; (*iii*) PCl_3, toluene, 110°C, 12 h; (*iv*) Et_3N, H_2O, THF, *rt*, 12 h; (*v*) $CH_3(CH_2)_9OH$, PivCl, Py, −15°C, 2 h; (*vi*) 1. I_2, H_2O, 20°C, 1 h; 2. $Na_2S_2O_3$; (*vii*) $CH_3(CH_2)_{15}OH$, PivCl, Py, −15°C, 2 h, *rt*.

For the evaluation of the impact of the length of alkyl substituents on the biological activity we synthesized, in addition to glycophosphate (**XII**), glycophosphate (**XIV**) by the interaction of *H*-phosphonate (**X**) with cetyl alcohol. After chromatography, it was isolated in 10% yield. Its formation was confirmed by the lack of the P-H resonance in the ^{1}H NMR spectrum, and the presence of a singlet at −2.43 ppm in the ^{31}P NMR spectrum corresponding to the phosphate group in a monoanionic form (PO_4^-). The MALDI spectrum of glycophosphate (**XIV**) contained a peak at *m/z* 650.6 $[M]^-$ ($C_{30}H_{53}NO_{12}P$). The resonance of the anomeric proton of glycophosphate (**XIV**) was observed as a doublet of doublets at 5.51 ppm ($^3J_{H1,P}$ 7.18 Hz, $^3J_{H1,H2}$ 3.53 Hz), which implied the α-configuration of the glycoside bond.

For the evaluation of the influence of a glucosamine residue on the biological activity of glycophosphates (**XII**) and (**XIV**) we synthesized glycophosphates (**XX**) and (**XXII**), which contained tetracetylated *D*-glucopyranose instead of 3,4,6-tri-*O*-acetylglucosamine (Scheme 2). The starting D-glucopyranose (**XV**) was acetylated as described in [14], and the anomeric protective group was removed with

hydrazine acetate as described in [15]. The reaction of the resulting 2,3,4,6-tetra-*O*-acetyl-α/β-D-glucopyranose (**XVII**) with salicyl chlorophosphite (**IX**) led to *H*-phosphonate (**XVIII**) isolated by column chromatography in 44% yield. In its ^{1}H NMR spectrum, a triplet at 1.28 ppm ($J_{H\text{-}H}$ 7.3 Hz) and a quartet at 3.0 ppm ($J_{H\text{-}H}$ 7.3 Hz) corresponding to a triethylammonium group as well as a doublet at 6.91 ppm ($J_{P\text{-}H}$ 638.9 Hz) corresponding to the proton of the P–H group were observed along with characteristic resonances of tetracetylated glucopyranose (**XVII**). The ^{31}P NMR spectrum of compound (**XVIII**) contained a singlet at 0.77 ppm corresponding to the phosphorus resonance in *H*-phosphonates. In the MALDI spectrum of glycophosphate (**XVIII**) a peak at *m/z* 513.2 $[M]^-$ ($C_{20}H_{36}NO_{12}P$) was present. The anomeric proton observed as a doublet of doublets at 5.75 ppm ($J_{H1,P}$ 8.9 Hz, $J_{H1,H2}$ 3.4 Hz) evidenced the formation of the α-anomer.

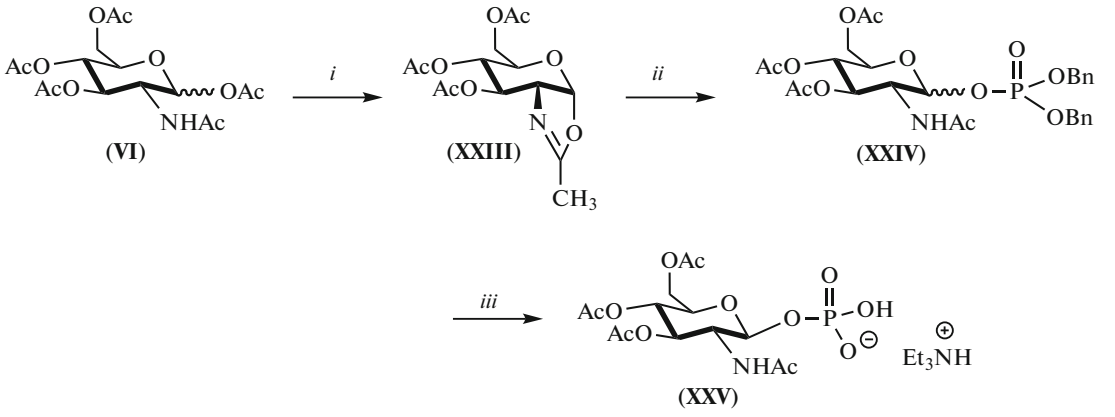


Scheme 2. The synthesis of glycophosphates (**XX**) and (**XXII**). Conditions and reagents: (*i*) Ac_2O, Py; 0 → 20°C, 24 h; (*ii*) $H_3N^+NH_3^+2AcO^-$, DMF, 0 → 20°C, 3 h; (*iii*) THF, Et_3N, 20°C, 12 h; (*iv*) $CH_3(CH_2)_9OH$, PivCl, Py, −15°C, 2 h; (*v*) 1. H_2O, I_2, 20°C, 1 h, 2. $Na_2S_2O_3$; (*vi*) $CH_3(CH_2)_{15}OH$, PivCl, Py, −15°C, 2 h.

Glycophosphates (**XX**) and (**XXII**) were obtained from *H*-phosphonate (**XVIII**) similarly to the synthesis of glycophosphates (**XII**) and (**XIV**) (Scheme 1) described above. Glycophosphate (**XX**) was isolated after chromatography in 53% yield. Its MALDI spectrum contained a peak at *m/z* 567.1 $[M]^-$ ($C_{24}H_{40}NO_{13}P$). The resonance of the phosphorus atom in the ^{31}P NMR spectrum was seen as a singlet at −2.69 ppm, which indicated the presence of the phosphate group in a monoanionic form (PO_4^-). The resonance of the anomeric proton was observed as a doublet of doublets at 5.72 ppm ($^3J_{H1,P}$ 7.8 Hz, $^3J_{H1,H2}$ 3.3 Hz), which implied the formation of the individual α-anomer. Glycophosphate (**XXII**) was obtained in 35% yield after chromatography. Its MALDI spectrum contained a peak at *m/z* 675.2 $[M + Na]^+$ ($C_{30}H_{53}NaO_{13}P$). In the ^{31}P NMR spectrum of glycophosphate (**XXII**) only one singlet at −2.31 ppm was present. The resonance of the anomeric proton in the ^1H NMR spectrum was observed as a doublet of doublets at 5.57 ppm ($^3J_{H1,P}$ 7.9 Hz, $^3J_{H1,H2}$ 3.5 Hz), which implied the formation of glycophosphate (**XXII**) in the form of the individual α-anomer.

We would like to emphasize the difference between our method of synthesis of glycophosphates (**XII**) and (**XIV**) and the known syntheses of glucosamine conjugates with phosphate or diphosphate fragments [4–8, 16]. First, it was the preparation of the key glucosamine derivative (**VII**) with protected hydroxyl groups at positions C3, C4, and C6 and the free anomeric hydroxyl group, which was phosphonylated at the next stage. 3,4,6-Tri-*O*-acetyl-*N*-acetylglucosamine (**VII**) was easily obtained by the regioselective removal of the acetyl protective group from the monosaccharide (**VI**) anomeric hydroxyl group using methylamine in methanol. Second, we used another approach [12] for the synthesis of conjugates of glucosamine derivative (**VII**) and a phosphate group. Normally (for example, [4]), monosaccharide (**VII**) was phosphorylated by the reaction with dibenzyl *N,N*-diisopropylphosphoramidate and 1*H*-tetrazole followed by oxidation with hydrogen peroxide, debenzylation, and treatment with *tert*-butylammonium hydroxide. The resulting salt was subjected to the reaction with aliphatic bromides in the presence of molecular sieves. We did not alkylate glucosamine phosphate but *H*-phosphonate (**X**) which was easily prepared by the interaction of 3,4,6-tri-*O*-acetyl-*N*-acetylglucosamine (**VII**) with salicyl chlorophosphite (**IX**) [13]. The target glycophosphates (**XII**) and (**XIV**) were obtained from *H*-phosphonate (**X**) using oxidation of *H*-phosphonates (**XI**) and (**XIII**) with iodine at the final stage. Glycophosphates (**XX**) and (**XXII**) were prepared from 2,3,4,6-tetraacetyl-α-D-glucopyranose in a similar manner. The yields of glycophosphates (**XII**) and (**XIV**) were small, but the reaction conditions will be updated.

For the evaluation of the effect of alkyl substituents in glycophosphates (**XII**) and (**XIV**) on the biological activity we synthesized glycophosphate (**XXV**) (Scheme 3). Oxazoline (**XXIII**) was obtained by the reaction of 1,3,4,6-tetra-*O*-acetyl-*N*-acetyl-D-glucosamine (**VI**) with TMS-OTF as described in [17]. Glycophosphate (**XXV**) was synthesized from oxazoline (**XXIII**) as described in [18] but without isolating and purifying dibenzyl phosphate (**XXIV**). Glycophosphate (**XXV**) as a trimethylammonium salt was obtained in 27% yield. In its ^{31}P NMR spectrum a singlet at 1.94 ppm was present, which corresponded to the monophosphate group in a monoanionic form (PO_4^-). In its MALDI spectrum a peak at *m/z* 426.3 $[M]^-$ ($C_{14}H_{21}NO_{12}P$) was observed. Unlike glycophosphates (**XII**), (**XIV**), (**XX**), and (**XXII**), the anomeric proton resonance of glycophosphate (**XXV**) was seen as a triplet at 5.18 ppm ($^3J_{H1,H2} = {}^3J_{H1,P} = 10$ Hz), which indicated the formation of the β-anomer.



Scheme 3. The synthesis of phosphate (**XXV**). Conditions and reagents: (*i*) TMS-OTF, $C_2H_4Cl_2$, 50°C, 30 h; (*ii*) $(PhCH_2O)_2P(O)OH$, Ag_2CO_3, $CH_2Cl_2/CH_3CN/Et_2O$, 0–20°C, 18 h; (*iii*) H_2, Pd/C, MeOH, Et_3N, 20°C, 4–8 h.

Compounds (**VI**), (**X**), (**XII**), (**XIV**), (**XX**), (**XXII**), (**XXV**), and (**XXVI**) were examined for antituberculosis activity against *Mycobacterium tuberculosis* H37Rv (MBT), as well as for antimicrobial activity, 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). All the compounds under study inhibited in vitro the MBT growth (Table 1). Although their antituberculosis activity was 30–125 times lower than the activity of isoniazid, the antituberculosis drug, the activities of compounds (**X**), (**XIV**), (**XXII**), and (**XXV**) were equal to that of pyrazinamide, another antituberculosis drug. Moreover, the antituberculosis activities of compounds (**VI**) and (**XXVI**) were twice as high as the pyrazinamide activity, whereas the activities of compounds (**XII**) and (**XX**) fourfold exceeded that of pyrazinamide (Table 1). Compounds (**VI**), (**XX**), (**XXV**), and (**XXVI**) were inactive against all the bacteria and fungi used in the antimicrobial testing. Only glycophosphates (**XII**), (**XIV**), and (**XXII**) demonstrated antibacterial activity against *S. aureus* and *B. cereus* (Table 1). In regard to the other bacteria and fungi used in the screening these compounds were inactive. The analysis of MIC values (Table 1) allowed the following conclusions. First, it is the alkyl substituent that leads to the appearance of antibacterial activity of tetraacetylated glucopyranose and 3,4,6-tri-*O*-acetyl-*N*-acetylglucosamine. Glucosamine derivatives (**VI**) and (**XXVI**) and glycophosphates (**XII**) and (**XX**) demonstrated approximately the same antituberculosis activity. Second, glycophosphates (**XIV**) and (**XXII**) bearing a hexadecyl substituent showed higher antibacterial activity (MIC 16 μg/mL) than glycophosphates (**XII**) and (**XX**) with a decyl substituent. In particular, glycophosphates (**XIV**) and (**XXII**) with a hexadecyl substituent inhibited in vitro the growth of *S. aureus* four times more effectively than antibiotic chloramphenicol, whereas the activity of glycolipid (**XII**) bearing a decyl spacer was nearly equal to that of chloramphenicol, and glycophosphate (**XX**) was inactive against the bacteria and fungi tested. Third, among the compounds under study, the nature of the glycophosphate carbohydrate residue did not affect its antibacterial activity, the length of the alkyl substituent being more important. For example, one can see that the in vitro antibacterial activities towards *S. aureus* and *B. cereus* of glycophosphate (**XIV**) with a glucosamine residue and glycophosphate (**XXII**) containing a glucopyranosyl fragment were the same. Fourth, the length of the alkyl substituent significantly impacted antituberculosis activity of the compounds under study. Glycophosphates (**XII**) and (**XX**) with a decyl substituent inhibited in vitro the MBT growth four times more effectively than glycophosphates (**XIV**) and (**XXII**) with a hexadecyl group (MIC 3 μg/mL vs 12 μg/mL respectively). Fifth, the length of the alkyl substituent in the glycophosphates under study affected the antituberculosis and antibacterial activities in different ways. In particular, glycophosphates (**XII**) and (**XX**) with a decyl chain exibited in vitro the highest antituberculosis activity against MBT (MIC 3 μg/mL) and at the same time the weakest antibacterial activity against *S. aureus* (MIC 62 and ≤500 μg/mL respectively) and *B. cereus* (MIC 125 and ≤500 μg/mL respectively). Conversely, glycophosphates (**XIV**) and (**XXII**) with a cetyl substituent demonstrated the highest antibacterial activity against *S. aureus* (MIC 16 μg/mL) and *B. cereus* (MIC 62.5 μg/mL) and the lowest antituberculosis activity against MBT (MIC 12 μg/mL).

To summarize, we found that acetylation of carbohydrate hydroxyl groups in glycophosphates of type (**III**) [4] supported the appearance of antibacterial and antituberculosis activities.

EXPERIMENTAL

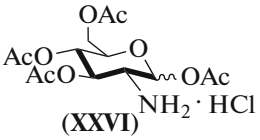
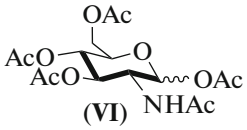
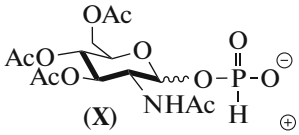
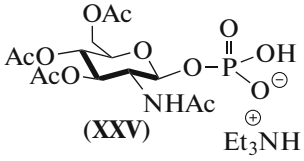
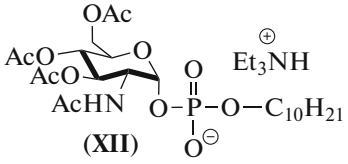
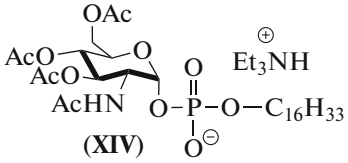
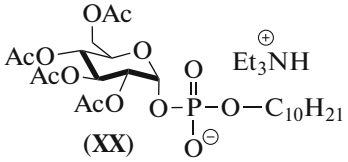
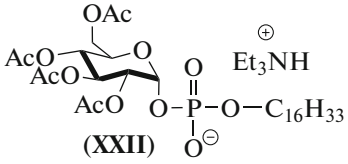
^{1}H, ^{13}C, and ^{31}P NMR spectra (δ, ppm; *J*, Hz) were registered on an Avance-400 spectrometer (Bruker, Germany) with a frequency of 400 MHz (^{1}H) and 100.6 MHz (^{13}C and ^{31}P) in $CDCl_3$. Mass spectra MALDI TOF were registered on an UltraFlex III TOF/TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) in a linear mode. The laser was Nd:YAG, λ 355 nm. The data were interpreted using the FlexAnalysis 3.0 program (Bruker Daltonik GmbH, Bremen, Germany). The measurements were conducted in the *m/z* range of 200 to 6000 with the registration of negatively charged ions. A metal target was used with *p*-nitroaniline as a matrix. The samples were dissolved in methanol (10^{-3} mg/mL). Optical rotation was measured on a PerkinElmer-341 polarimeter (PerkinElmer, United States) at λ 589 nm at 20°C. The reaction completeness and product purity were monitored by TLC on Sorbfil plates (Imid LLC, Krasnodar, Russia). The compounds were developed with 5% sulfuric acid followed by heating to 120°C.

The reactions sensitive to air and/or moisture were performed in an argon atmosphere in anhydrous solvents, which were preliminarily purified and dried (if necessary) according to the standard procedures.

Compounds (VI) [10], (VII) [11], (IX) [13], (XVI) [14], (XVII) [15], (XXIII) [17], and (XXVI) [19, 20] were synthesized using published procedures. Spectral characteristics of compounds (**VI**), (**VII**), and (**IX**) agreed with the published data [10, 11, 13]. Spectral parameters of compounds (**XVI**), (**XVII**), and (**XXIII**) correlated with the data in [21–23]. Characteristics of compound (**XXVI**) corresponded to [19, 20]. Commercial *D*-glucosamine hydrochloride (**V**) and *D*-glucopyranose (**XV**) were from Acros (Belgium).

3,4,6-Tri-*O*-acetyl-2-deoxy-2-acetamido-α/β-D-glucopyranosyl *H*-phosphonate, triethylammonium salt (X).

Table 1. Antituberculosis and antimicrobial activities of the compounds synthesized

	MIC, μg/mL		
	M. tuberculosis H37Rv	*S. aureus* ATCC 209p	*B. cereus* ATCC 8035
 **(XXVI)**	6	>500	>500
 **(VI)**	6	>500	>500
 **(X)**	12	–	–
 **(XXV)**	12	>500	>500
 **(XII)**	3	62	125
 **(XIV)**	12	16	62
 **(XX)**	3	>500	>500
 **(XXII)**	12	16	62
Isoniazid	0.1	–	–
Pyrazinamide	12	–	–
Chloramphenicol	–	62	62

Salicyl chlorophosphite (**IX**) (0.42 g, 2 mmol) [13] was added under stirring to a solution of 3,4,6-tri-*O*-acetyl-*N*-acetylglucosamine (**VII**) (0.67 g, 2 mmol) [11] and triethylamine (1.87 mL, 18.5 mmol) in dry THF (10 mL) and the mixture was stirred for 3 h. Water (1 mL) was added and the mixture was stirred for 1 h and evaporated to dryness. The residue was chromatographed on silica gel eluting with CH_2Cl_2–CH_3OH (40 : 1 → 5 : 1 and 1 vol % Et_3N) to give 0.33 g (34%) of compound (**X**) as colorless oil, $[\alpha]_D^{20}$ + 56.7 (*c* 0.8, $CHCl_3$). Found, %: C 47.05; H 7.49; N 5.53; P 5.98. $C_{20}H_{37}N_2O_{11}P$. Calc., %: C 46.87; H 7.28; N 5.47; P 6.04. ^{1}H NMR: 1.36 [t, 9H, *J* 7.30, $N^+(CH_2CH_3)_3$], 1.97, 2.01, 2.07 (all s, 12H, $4CH_3CO$), 3.08 [q, 6H, *J* 7.3, $N^+(CH_2CH_3)_3$], 4.09–4.27 (m, 3H, H_2, H_{6a}, H_{6b}), 4.36–4.46 (m, 1H, H5), 5.17 (t, 1H, *J* 9.62, H4), 5.27–5.35 (m, 1H, H3), 5.61–5.69 (m, 1H, H1), 6.90 (d, 1H, $J_{1,P}$ 668.04, P–H), 6.90–7.05 [m, 1H, $NHC(O)CH_3$], 11.73 (br s, 1H, HN^+). ^{13}C NMR: 8.7 ($CH_3CH_2N^+$), 20.7, 20.8 [3 $C(O)CH_3$], 23.0 ($CH_3C(O)NH$), 45.9 (CH_2N^+), 52.1 (C2), 61.9 (C6), 68.4 (C4), 69.0 (C3), 71.2 (C5), 93.1 (C1, *J* 3.9), 169.6, 170.9, 171.1, 171.2 (4 C=O). ^{31}P NMR: +1.93.

General procedure for the synthesis of (XII) and (XIV). Decan-1-ol or hexadecane-1-ol (2 eq) and a solution of pivaloyl chloride (4.3 eq) in pyridine (5 mL) were successively added to a solution of *H*-phosphonate (**X**) (1 eq) in pyridine (10 mL) cooled to –20°C. The mixture was stirred for 1 h and water (1 mL) and iodine (1 eq) were added. The mixture was stirred for 2 h and 1M $Na_2S_2O_3$ was added dropwise until the iodine color disappeared. The pale yellow reaction mixture was evaporated to dryness and the residue was chromatographed on silica gel eluting with CH_2Cl_2–CH_3OH (40 : 1 → 5 : 1 and 1 vol % Et_3N) to give decyl glycophosphate (**XII**) (23%) as colorless oil and hexadecyl glycophosphate (**XIV**) (10%) as a colorless oil.

2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-*D*-glucopyranosyl decyl phosphate, triethylammonium salt (XII). $[\alpha]_D^{20}$ + 26.6 (*c* 0.642, CH_3OH). Found, %: C 54.09; H 8.78; N 4.21; P 4.55. $C_{30}H_{57}N_2O_{12}P$. Calc., %: C 53.88; H 8.59; N 4.19; P, 4.63. MALDI-TOF MS: *m/z* 566.4 $[M]^-$. Calc. M^- 566.2 ($C_{24}H_{41}NO_{12}P^-$). ^{1}H NMR: 0.86 (t, 3H, *J* 7.0, H10'), 1.18 (br s, 4H, H-9', H-8'), 1.21–1.28 (m, 10H, H3'–H-7'), 1.32 [t, 9H, *J* 7.30, $N^+(CH_2CH_3)_3$], 1.56–1.64 (m, 2H, H2'), 1.94, 1.98, 1.99, 2.06 (all s, 12H, 4 CH_3CO), 3.06 [q, 6H, *J* 7.31, $N^+(CH_2CH_3)_3$], 3.83–3.94 (m, 2H, H1'), 4.05–4.10 (m, 1H, H2), 4.18–4.25 (m, 2H, H6a, H6b), 4.30–4.40 (m, 1H, H5), 5.16 (t, 1H, *J* 9.80, H4), 5.26–5.32 (m, 1H, H3), 5.51 (dd, 1H, $J_{1,P}$ 7.34, $J_{1,2}$ 3.22, H1), 7.06 [d, 1H, $J_{2,NH}$ 9.35, $NHC(O)CH_3$], 11.98 (br s, 1H, HN^+). ^{13}C NMR: 8.7 ($CH_3CH_2N^+$), 14.1 (C10'), 20.8, 22.7, 23.0 [3 $C(O)CH_3$, $CH_3C(O)NH$], 25.9, 27.4, 29.4, 29.5, 29.6, 29.7, 31.9 (C2'–C9'), 45.7 (CH_2N^+), 52.2 (C2), 61.9 (C6), 66.2 (C1'), 68.4 (C4), 68.7 (C3), 71.5 (C5), 94.1 (d, C1, $J_{C,P}$ 5.4), 169.5, 170.8, 171.0, 182.2 (4 C=O). ^{31}P NMR: –2.27.

2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl cetyl phosphate, triethylammonium salt (XIV). $[\alpha]_D^{20}$ + 10.5 (*c* 0.846, CH_2Cl_2). Found, %: C 57.35; H 9.20; N 3.63; P 4.08. $C_{36}H_{69}N_2O_{12}P$. Calc., %: C 57.43; H 9.24; N 3.72; P 4.11. MALDI-TOF MS: *m/z* 650.6 $[M]^-$. Calc. M^- 650.3 ($C_{30}H_{53}NO_{12}P^-$). ^{1}H NMR: 0.86 (t, 3H, *J* 6.8, H16'), 1.19 (br s, 4H, H15', H14'), 1.22–1.27 (m, 22H, H3'–H13'), 1.36 [t, 9H, *J* 7.33, $N^+(CH_2CH_3)_3$], 1.55–1.65 (m, 2H, H2'), 1.94, 1.98, 1.99, 2.06 (all s, 12H, 4 CH_3CO), 3.07 [q, 6H, *J* 7.33, $N^+(CH_2CH_3)_3$], 3.85–3.94 (m, 2H, H1'), 4.05–4.11 (m, 1H, H2), 4.17–4.27 (m, 2H, H6a, H6b), 4.32–4.40 (m, 1H, H5), 5.16 (t, 1H, *J* 9.68, H4), 5.29 (t, 1H, *J* 9.96, H3), 5.51 (dd, 1H, $J_{1,P}$ 7.18, $J_{1,2}$ 3.53, H1), 7.17 (d, 1H, $J_{2,NH}$ 9.64, $NHC(O)CH_3$). ^{13}C NMR: 8.8 ($CH_3CH_2N^+$), 14.2 (C16'), 20.8, 20.9 [3 $C(O)CH_3$, $NHC(O)CH_3$], 22.8, 25.9, 27.4, 29.5, 29.8, 32.0 (C2'–C15'), 45.8 (CH_2N^+), 52.3 (C2), 62.0 (C6), 63.6 (C1'), 68.7 (C3, C4, C5), 94.2 (C1), 169.5, 170.9, 182.6 (4 C=O). ^{31}P NMR: –2.43.

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl *H*-phosphonate, triethylammonium salt (XVIII). Salicyl chlorophosphite (**IX**) (3.23 g, 16 mmol) [13] was added under stirring to a solution of glucopyranose tetraacetate (**XVII**) (6.2 g, 17.8 mmol) [15] and triethylamine (17.3 mL, 124.6 mmol) in dry THF (35 mL) and the mixture was stirred for 9 h. Water (15.4 mL) was added and the reaction mixture was stirred for 1 h. The mixture was evaporated to dryness, and the residue was chromatographed on silica gel eluting with CH_2Cl_2–CH_3OH (50 : 1 and 1 vol % Et_3N) to give 4 g (44%) of compound (**XVIII**) as a hygroscopic white powder, $[\alpha]_D^{20}$ + 48.4 (*c* 0.9, $CHCl_3$). Found, %: C 46.82; H 7.11; N 2.81; P 6.00. $C_{20}H_{36}NO_{12}P$. Calc., %: C 46.78; H 7.07; N 2.73; P 6.03. MALDI-TOF MS: *m/z* 513.2 [*M*]. Calc. *M* 513.2 ($C_{20}H_{36}NO_{12}P$). ^{1}H NMR: 1.28 [t, 9H, *J* 7.30, $N^+(CH_2CH_3)_3$], 1.96, 1.98, 2.01, 2.04 (all s, 12H, $4CH_3CO$), 3.0 [q, 6H, *J* 7.30, $N^+(CH_2CH_3)_3$], 4.07 (dd, 1H, $J_{6a,6b}$ 12.4, $J_{6a,5}$ 2.3, H6a), 4.19 (dd, 1H, $J_{6a,6b}$ 12.4, $J_{6b,5}$ 3.39, H6b), 4.26–4.30 (m, 1H, H5), 4.90–4.94 (m, 1H, H2), 5.07 (t, 1H, *J* 9.8, H4), 5.50 (t, 1H, *J* 9.8, H3), 5.75 (dd, 1H, $J_{1,P}$ 8.9, $J_{1,2}$ 3.4, H1), 6.91 (d, 1H, $J_{1,P}$ 638.9, P–H). ^{13}C NMR: 8.5 ($CH_3CH_2N^+$), 20.49, 20.57, 20.59, 20.61 [4 $C(O)CH_3$], 45.7 (CH_2N^+), 61.6 (C6), 68.2 (C3), 68.3 (C4), 70.1 (C5), 70.5 (C2), 91.1 (C1), 169.5, 169.8, 169.9, 170.6 (4 C=O). ^{31}P NMR: +0.77.

General procedure for the synthesis of glycophosphates (XX), (XXII). Decan-1-ol or hexadecane-1-ol (2 eq) and a solution of pivaloyl chloride (4.3 eq) in pyridine (5 mL) were successively added under stirring

to a solution of *H*-phosphonate (**XVIII**) (1 eq) in pyridine (10 mL) cooled to –20°C. The mixture was stirred for 1 h and water (1 mL) and iodine (1 eq) were added. The mixture was stirred for 2 h and 1M $Na_2S_2O_3$ was added dropwise until the iodine color disappeared. The pale yellow reaction mixture was evaporated to dryness and the residue was chromatographed on silica gel to give glycophosphates (**XX**) (53%, colorless oil) and (**XXII**) (35%, colorless oil).

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl decyl phosphate, triethylammonium salt (XX). Phosphate (**XX**) was isolated as colorless oil in a yield of 53% by flash chromatography on silica gel eluting with CH_2Cl_2/CH_3OH (100 : 1 → 100 : 2, and 1 vol % Et_3N); $[\alpha]_D^{20}$ + 18.2° (*c* 1.00, CH_2Cl_2). Found, %: C 53.79; H 8.49; N 2.05; P 4.68. $C_{30}H_{56}NO_{13}P$. Calc., %: C 53.80; H 8.43; N 2.09; P 4.62. MALDI-TOF MS: *m/z* 567.1 $[M]^-$. Calc. M^- 567.2 ($C_{24}H_{40}O_{13}P^-$). ^{1}H NMR: 0.86 (t, 3H, *J* 6.9, H10'), 1.18–1.23 (m, 14H, H3'–H-7'), 1.31 [t, 9H, *J* 7.3, $N^+(CH_2C\boldsymbol{H}_3)_3$], 1.39–1.44 (m, 2H, H-8'), 1.56–1.65 (m, 2H, H2'), 1.98, 1.99, 2.02, 2.05 (all s, 12H, 4C$\boldsymbol{H}_3$CO), 3.07 [q, 6H, *J* 7.3, $N^+(C\boldsymbol{H}_2CH_3)_3$], 3.84–3.95 (m, 2H, H1'), 4.05–4.34 (m, 3H, H5, H6a, H6b), 4.89–4.96 (m, 1H, H2), 5.11 (t, 1H, *J* 9.8, H4), 5.52 (t, 1H, *J* 9.8, H3), 5.72 (dd, 1H, $J_{1,P}$ 7.8, $J_{1,2}$ 3.3, H1). ^{13}C NMR: 8.5 ($\boldsymbol{C}H_3CH_2N^+$), 14.1 (C10'), 20.6, 20.7 [4 C(O)$\boldsymbol{C}H_3$], 22.6, 25.7, 27.1, 29.3, 29.4, 29.6, 30.8, 31.9 (C2'–C9'), 45.6 ($\boldsymbol{C}H_2N^+$), 61.6 (C6), 67.9 (C1'), 68.3 (C4), 68.3 (C3), 70.2 (C5), 70.3 (C2), 91.9 (C1), 169.6, 170.1, 170.7 (4 C=O). ^{31}P NMR: –2.69.

2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl cetyl phosphate, triethylammonium salt (XXII). Phosphate (**XXII**) (35%, colorless oil) was isolated by flash chromatography on silica gel eluting with CH_2Cl_2/CH_3OH (100 : 1 → 100 : 1.5, 1 vol % Et_3N); $[\alpha]_D^{20}$ + 11.2° (*c* 1.00, CH_2Cl_2). Found, %: C 57.41; H 9.01; N 1.78; P 4.15. $C_{36}H_{68}NO_{13}P$. Calc., %: C 57.35; H 9.09; N 1.86; P 4.11. MALDI-TOF MS: *m/z* 675.2 $[M + Na]^+$. Calc. $[M + Na]^+$ 675.3 ($C_{30}H_{53}NaO_{13}P^+$). ^{1}H NMR: 0.75 (t, 3H, *J* 7.0, H16'), 1.03–1.23 (m, 26H, H3'–H15'), 1.28 [t, 9H, *J* 7.3, $N^+(CH_2C\boldsymbol{H}_3)_3$], 1.45–1.53 (m, 2H, H2'), 1.88, 1.90, 1.92, 1.95 (all s, 12H, 4C$\boldsymbol{H}_3$CO), 3.07 [q, 6H, *J* 7.3, $N^+(C\boldsymbol{H}_2CH_3)_3$], 3.52–3.76 (m, 2H, H1'), 3.96–4.16 (m, 3H, H5, H6a, H6b), 4.76–4.80 (m, 1H, H2), 4.98 (t, 1H, *J* 9.7, H4), 5.38 (t, 1H, *J* 9.6, H3), 5.57 (dd, 1H, $J_{1,P}$ 7.9, $J_{1,2}$ 3.5, H1). ^{13}C NMR: 8.7 ($\boldsymbol{C}H_3CH_2N^+$), 14.2 (C10'), 20.8 [C(O)$\boldsymbol{C}H_3$], 22.8, 25.9, 27.4, 29.5, 29.6, 29.8, 30.1, 30.8, 32.0 (C2'–C15'), 45.9 ($\boldsymbol{C}H_2N^+$), 61.8 (C6), 66.2 (C1'), 68.5 (C4), 68.9 (C3), 70.3 (C5), 70.8 (C2), 92.0 (C1), 169.8, 170.3, 170.9 (4 C=O). ^{31}P NMR: –2.31.

2-Acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-*O*-phosphate, triethylammonium salt (XXV). Dibenzyl phosphate (1.71 g, 6.16 mmol) was added to a solution of oxazoline (**XXIII**) [17] (1.69 g, 5.13 mmol) in 1,2-dichloroethane (37 mL) and the reaction mixture was stirred at room temperature for 19 h. The mixture was concentrated and dried in vacuum. The resulting (**XXIV**) was used without further purification.

Phosphate (**XXIV**) (1.7 g, 2.8 mmol) was dissolved in methanol (50 mL) and 10% Pd/C (1.6 g) was added in an argon atmosphere. The solution was hydrogenated at room temperature under vigorous stirring for 3 h and filtered through celite. Celite was washed with hot methanol (10 mL) and trimethylamine (0.4 mL) and the solvents were removed in vacuum. The residue was washed with a CH_2Cl_2–ether mixture, filtered, and the filtrate was evaporated in vacuum to give 0.4 g (27%) of compound (**XXV**) as colorless syrup, $[\alpha]_D^{20}$ + 34.4 (*c* 0.9, CH_3OH). Found, %: C 45.51; H 7.01; N 5.23; P 5.89. $C_{20}H_{37}N_2O_{12}P$. Calc., %: C 45.45; H 7.06; N 5.30; P 5.86. MALDI-TOF MS: *m/z* 426.3 $[M]^-$. Calc. M^- 426.1 ($C_{14}H_{21}NO_{12}P^-$). ^{1}H NMR: 1.17 [t, 9H, *J* 7.3, $N^+(CH_2C\boldsymbol{H}_3)_3$], 1.86, 1.87, 1.89, 1.95 (all s, 12H, C$\boldsymbol{H}_3$CO), 2.95 (q, 6H, *J* 7.3, $N^+(C\boldsymbol{H}_2CH_3)_3$), 3.91–3.99 (m, 1H, H6a), 4.05–4.17 (m, 3H, H2, H5, H6b), 4.98 (t, 1H, *J* 9.5, H4), 5.08 (d, 1H, *J* 3.2, H3), 5.18 (t, 1H, *J* 10, H1), 6.44 (d, 1H, $J_{2,NH}$ 9.5, NH), 9.37 (br s, 1H, N^+H). ^{13}C NMR: 8.7 ($\boldsymbol{C}H_3CH_2N^+$), 20.7, 20.8, 22.8 [3 C(O)$\boldsymbol{C}H_3$], 23.2 ($\boldsymbol{C}H_3C(O)NH$), 45.5 ($\boldsymbol{C}H_2N^+$), 52.6 (C2), 62.4 (C6), 67.3 (C4), 68.7 (C3), 71.6 (C5), 91.6 (d, *J* 6.7, C1), 169.6 (NH$\boldsymbol{C}$=O), 170.7, 170.9, 171.4 (3 C=O). ^{31}P NMR: +1.94.

Biological activity. Antimicrobial activity of compounds (**VI**), (**XII**), (**XIV**), (**XX**), (**XXII**), (**XXV**), and (**XXVI**) was studied using serial dilutions in liquid nutrient media as described in [24, 25] followed by calculations of MIC values impeding the growth and development of the test microorganisms. Particularly, *Staphylococcus aureus* ATCC 209p and *Bacillus cereus* ATCC 8035 were used as Gram-positive cultures; *Escherichia coli* CDC F-50 and *Pseudomonas aeruginosa* ATCC 9027 as Gram-negative cultures; and *Aspergillus niger* BKMF-1119, *Trichophyton mentagrophytes var. gypseum* 1773, *and Candida albicans* 855-653 as fungi. Antibiotic chloramphenicol was used as a control.

The study of antituberculosis activity of compounds (**VI**), (**X**), (**XII**), (**XIV**), (**XX**), (**XXII**), (**XXV**), and (**XXVI**) was conducted by the method of vertical diffusion [26] on a thick nutrient medium "New" using the laboratory MBT strain H37Rv. The nutrient medium was poured into tubes, 5 mL in each, seeded with suspensions of mycobacteria (0.1 mL per each) diluted in accordance with the opacity standard of 10 GKI units. The tubes were placed in a thermostat for 24 h to grow MBT. In a day, the tubes were vertically positioned and 12.5, 6.2, 3.1, 1.5, 0.7, 0.35, 0.1 μg/mL suspensions of compounds (**VI**), (**X**), (**XII**), (**XIV**), (**XX**), (**XXII**), (**XXV**), and (**XXVI**) in

aqueous DMSO were dropped in the tubes following their free edges. The tubes were placed in a thermostat and stored at sterile conditions at 37°C for 10 days. The MBT growth was evaluated using a standard approach, according to which the appearance of areas of the MBT delayed growth exceeding 10 mm evidenced the tuberculostatic properties. Antituberculosis drugs isoniazid and pyrazinamide inhibiting the MBT growth at MIC 0.1 and 12 μg/mL, respectively, were used as controls.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflict of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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