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Dedicated to Full Member of the Russian Academy of Sciences N.S. Zefirov on his 80th anniversary

Development of Synthetic Approaches to Macrocyclic Glycoterpenoids on the Basis of Glucuronic Acid and Diterpenoid Isosteviol

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Abstract—An approach has been developed to the synthesis of macrocyclic glycoterpenoids containing diterpenoid isosteviol and glucuronic acid fragments. Selective screening revealed compounds exhibiting antitubercular activity against H37RV, *M. Avium*, and *M. Terrae* strains at a level comparable to the known antitubercular drugs isoniazid, ofloxacin, and pyrazinamide. The compound possessing the highest antitubercular activity is non-cytotoxic toward human erythrocytes.

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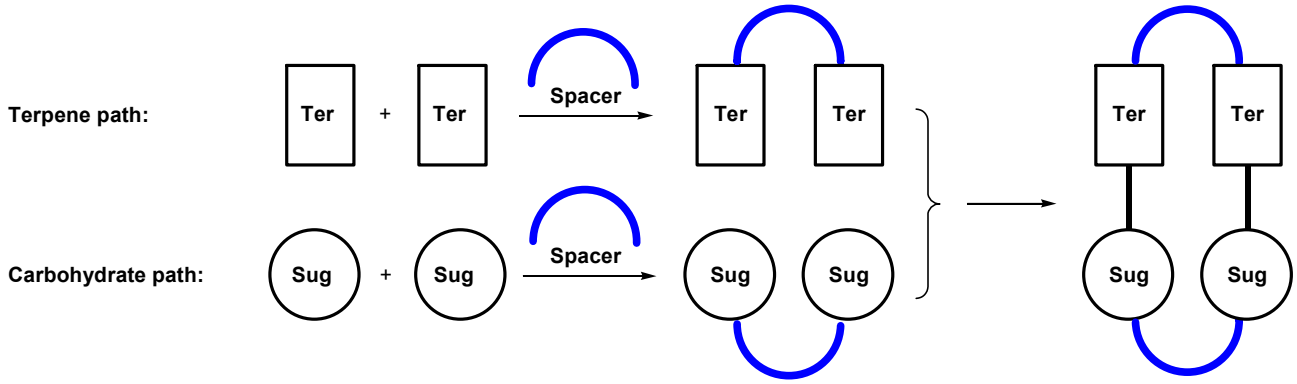
Glucuronic acid plays a very important role in human biochemistry; it metabolizes xenobiotics by converting them into nontoxic water-soluble glycosides (glucuronides). The ability of glucuronic acid to transform hydrophobic substances into water-soluble ones is successfully used by chemists as a method for ensuring bioavailability of new therapeutic agents [1]. A large number of structurally diverse glucuronides that can be regarded as prodrugs have been synthesized and studied [1, 2]. Glucuronic acid derivatives at the carboxy group, such as glucuronamides, have been the subjects of considerably less studies. In particular, some glycolipids [3, 4] and glycopeptides [5] have been reported. Taking into account that the synthesis of macrocyclic analogs and macrocyclic derivatives of natural compounds [6, 7] has become a rapidly developing field of new drug design, we focused on synthetic macrocyclic derivatives of glucuronic acid where two glucopyranuronic fragments are linked by a polymethylene, alkenylene, or alkynylene spacer at the anomeric centers and by 1,4-phenylene- or 1,4-xylylenediamide spacer at the carboxy groups [8, 9]. These macrocycles were called glycophanes due to the presence in their molecules of aromatic rings and carbohydrate moieties and were initially studied as receptors for aromatic molecules [8]. Addition of lactose residues to the 3-OH groups of the glucopyranuronic acid fragments has made the macrocycles capable of inhibiting lectin binding to tumor cells and lectin-dependent erythrocyte aggregation [8, 9]. In the present article we report the synthesis of macrocyclic glucuronic acid derivatives containing several isosteviol fragments. Isosteviol (**1**, 16-oxo-*ent*-beyeran-19-oic acid) [10] is a diterpenoid exhibiting a broad spectrum of biological activity, including hypotensive, antihypertensive, insulinotropic, cardio- and neuroprotective, and tuberculostatic activities [11]. Antitubercular activity was also found for various isosteviol derivatives [12, 13], including macrocyclic ones. We presumed that combination of two natural metabolites of the carbohydrate and terpene series into a single macrocyclic system should give rise to a new class of biologically active compounds which, by analogy with [14], were called macrocyclic glycoterpenoids.

On the basis of our experience accumulated while synthesizing macrocyclic derivatives of isosteviol [15, 16], we initially tried to obtain macrocyclic glyco-

Scheme 1.

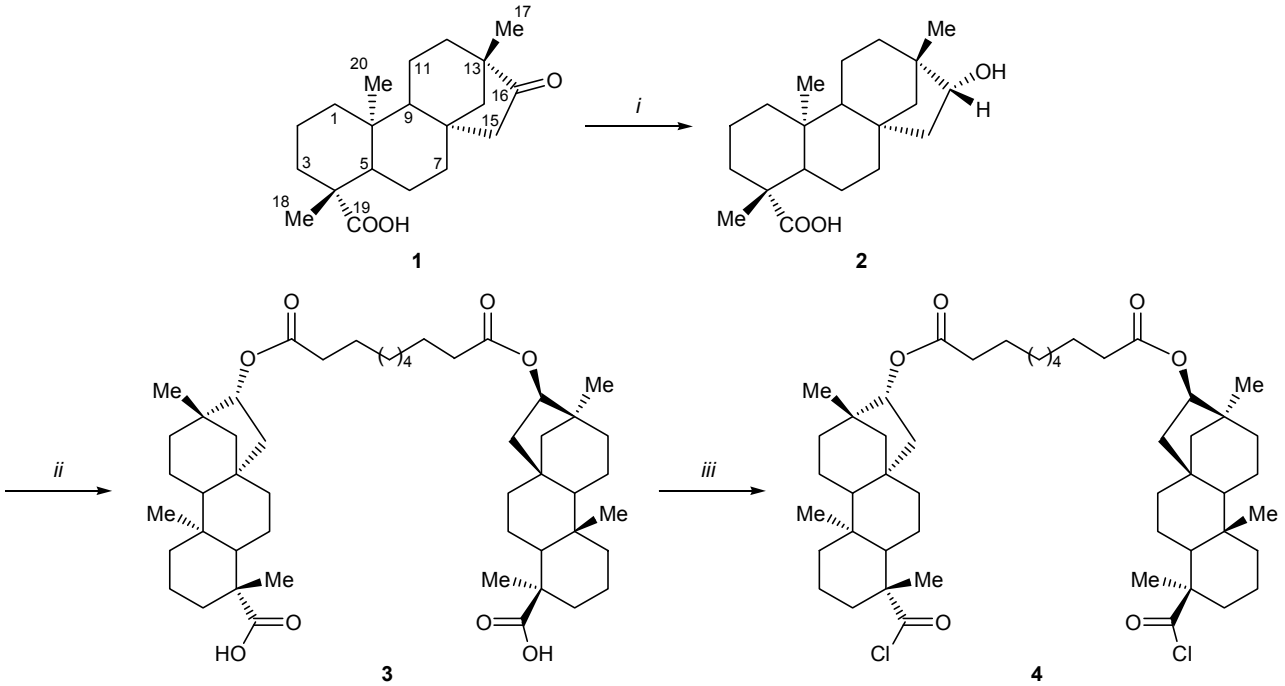


terpenoids according to the convergent approach which consisted of two paths, terpene and carbohydrate (Scheme 1). Both paths should lead to the formation of binuclear structures (precursors) in which two terpene or carbohydrate molecules are linked through a spacer. It is important that both terpene and hydrocarbon precursors would have free reactive groups capable of being involved in macrocyclization in the final step of the convergent synthesis.

As starting material in the terpene path we selected isosteviol (**1**). Chemoselective reduction of **1** with sodium tetrahydridoborate in methanol [17] gave dihydroisosteviol **2** which reacted with sebacoyl chloride to afford bis-acid **3**, and the latter was converted into diacyl dichloride **4** which was defined as terpene precursor (Scheme 2).

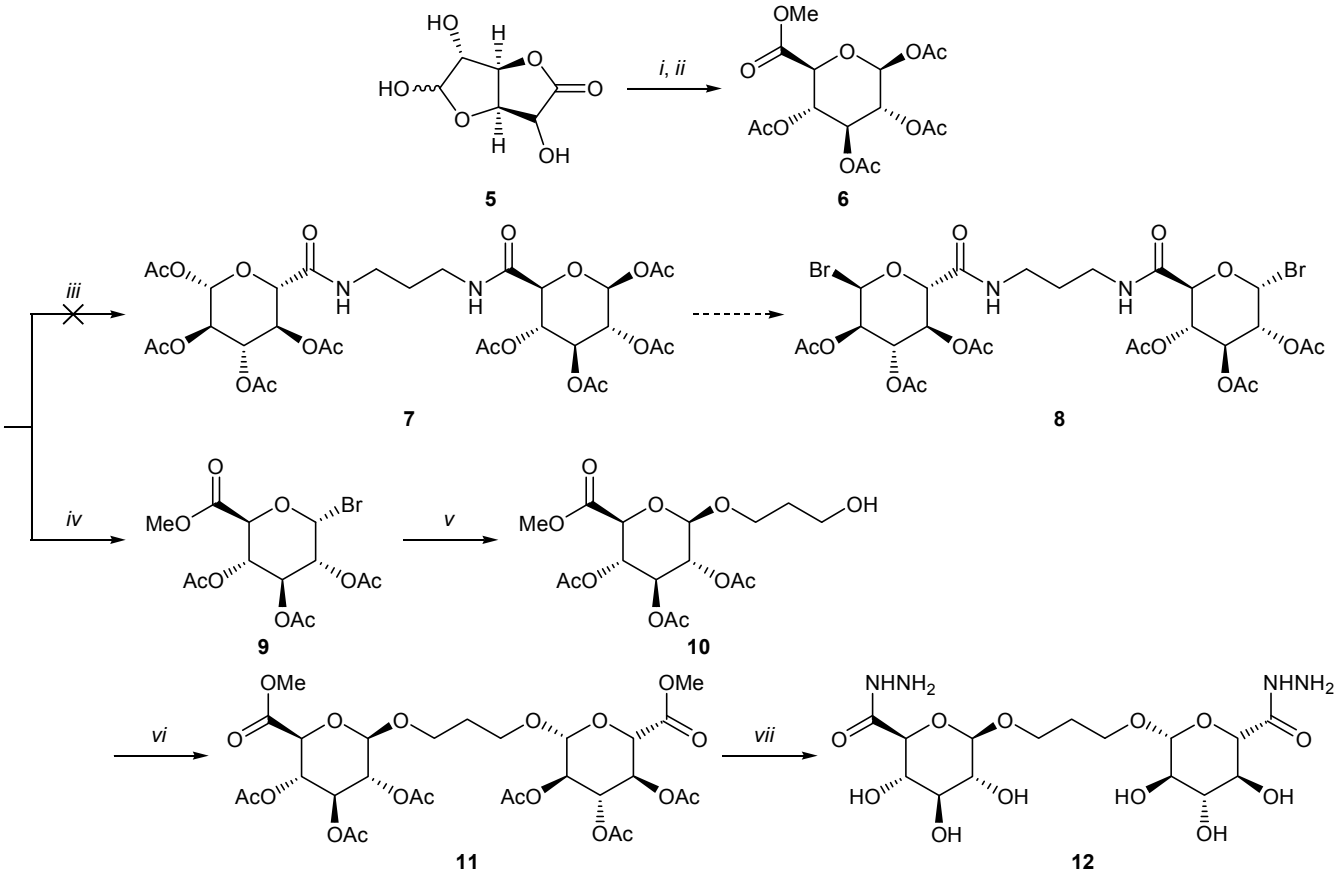
The carbohydrate path was started from D-glucurono-6,3-lactone (**5**) (Scheme 3). By treatment of **5** with sodium methoxide according to [18] we obtained glucuronic acid methyl ester which was subjected to acetylation. The ^{1}H NMR spectrum of tetra-*O*-acetyl derivative **6** contained only one set of signals, and the anomeric proton resonated as a doublet at δ 5.77 ppm with a vicinal coupling constant of 7.7 Hz; these findings unambiguously indicated stereospecific opening of lactone **5** and β-configuration of methyl 1,2,3,4-tetra-*O*-acetyl-D-glucopyranuronate (**6**). We planned to react **6** with a diamine to obtain binuclear structure **7**

Scheme 2.



i: $NaBH_4$, MeOH; *ii*: $ClC(O)(CH_2)_8C(O)Cl$, DMAP, pyridine, CH_2Cl_2; *iii*: $SOCl_2$, CH_2Cl_2, Δ.

Scheme 3.



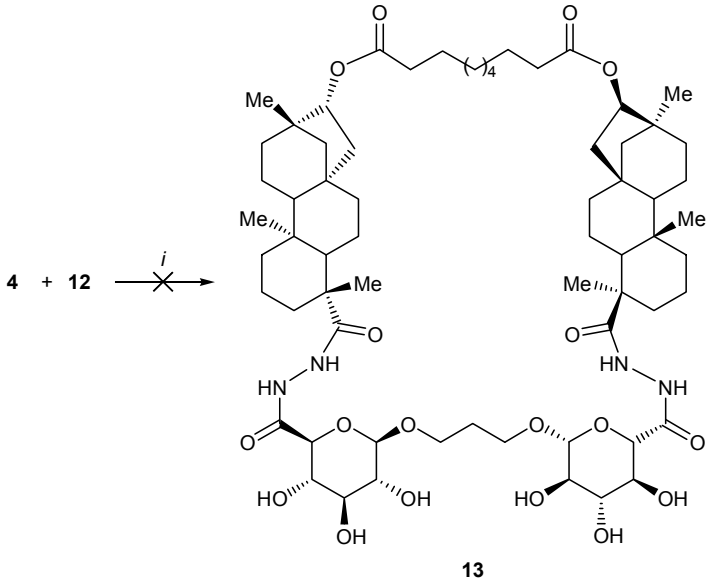
i: MeONa, MeOH, 20°C; *ii*: Ac_2O, $HClO_4$; *iii*: $H_2N(CH_2)_3NH_2$, MeOH, Δ; *iv*: 33% HBr–AcOH, 0°C; *v*: $HO(CH_2)_3OH$, $AgCO_3$, 4-Å molecular sieves, $CHCl_3$, Δ; *vi:* **9**, Ag_2CO_3, 3-Å molecular sieves, $CHCl_3$, Δ; *vii*: $NH_2NH_2 \cdot H_2O$, CH_2Cl_2, Δ.

and replace acetoxy groups at the anomeric carbon atoms by bromine; dibromide **8** was assumed to be the carbohydrate precursor. However, the reaction of **6** with propane-1,3-diamine under standard conditions for the amination of esters afforded no diamide **7**. We then tried a different way to synthesize carbohydrate precursor. By reaction of **6** with HBr in acetic acid we obtained methyl 2,3,4-tri-*O*-acetyl-1-bromo-α-D-glucopyranuronate (**9**) which was involved in the Königs–Knorr reaction with propane-1,3-diol (Scheme 3).

Insofar as the Königs–Knorr synthesis was carried out in excess diol, the product was glucuronoside **10** (yield 75%). It showed in the ^{1}H NMR spectrum multiplets at δ 1.78–1.84, 3.68–3.77, and 4.00–4.06 ppm due to protons in the $OCH_2CH_2CH_2O$ fragment, and the electron impact mass spectrum of **10** contained a ion peak with *m*/*z* 391 $[M - H]^+$ which results from elimination of proton from the hydroxy group. Further fragmentation includes cleavage of bonds in the hydroxypropyl substituent and finally cleavage of C–C and C–O bonds in the tetrahydropyran ring. The anomeric proton in **10** resonated in the ^{1}H NMR spectrum as a doublet at δ 4.58 ppm with a vicinal coupling constant of 7.6 Hz, which unambiguously indicated β-orientation of the glycoside bond. The subsequent Königs–Knorr reaction of **10** with bromide **9** at a ratio of 2:1 in boiling chloroform produced 25% of bis-glucuronoside **11**. The latter reacted with hydrazine hydrate in boiling methylene chloride, and the resulting bis-hydrazide **12** (yield 80%) was the carbohydrate precursor.

As the final step of the convergent synthesis we planned to react terpene precursor **4** with carbohydrate precursor **12** (Scheme 4). Unfortunately, bis-hydrazide **12** is insoluble in organic solvents (it is soluble only in water), so that the reaction was carried out under heterogeneous conditions, and the desired macrocyclic glycoterpenoid **13** was formed only in trace amount. It was identified by the ion peak with *m*/*z* 1253 $[M + Na]^+$ (calculated for $C_{65}H_{102}N_4O_{18}$: *m*/*z* 1250 $[M + Na]^+$) in the MALDI mass spectrum of the reaction mixture.

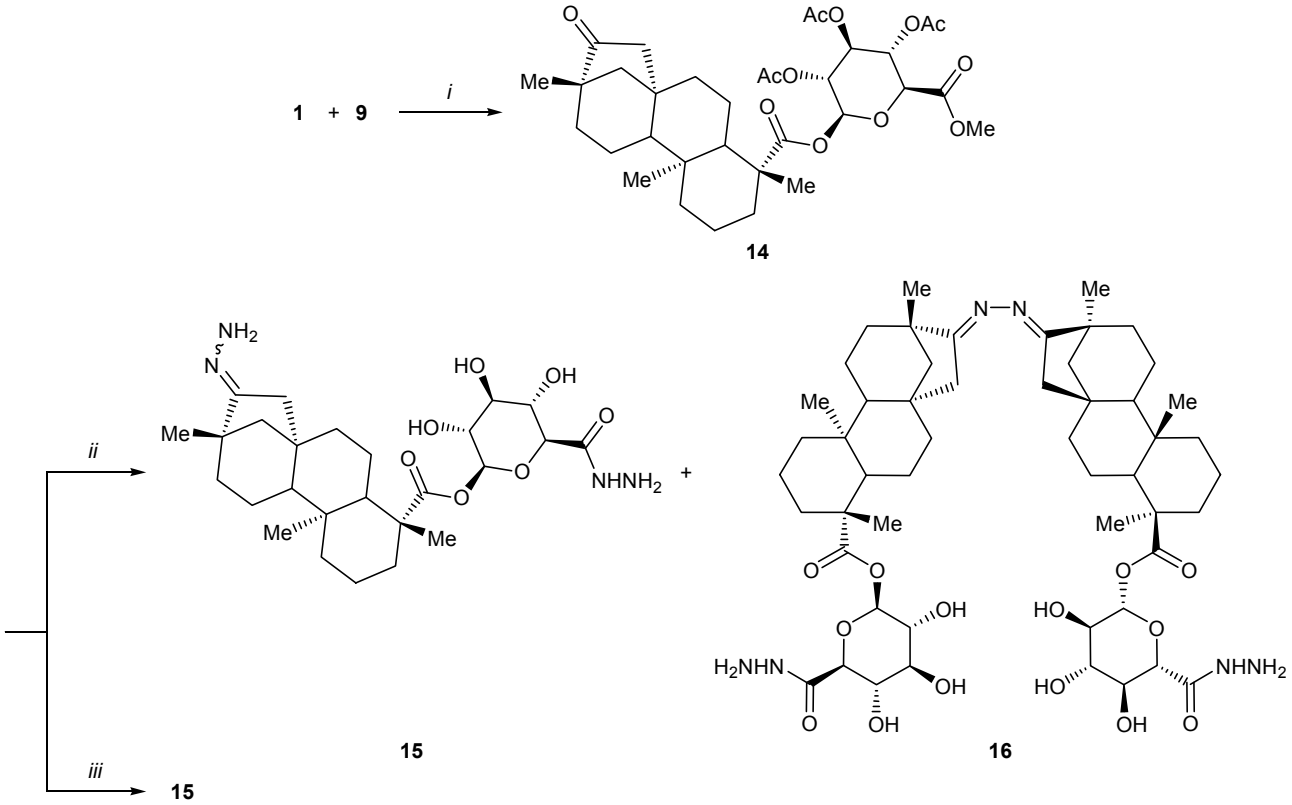
Scheme 4.



i: CH_2Cl_2, Et_3N, Δ.

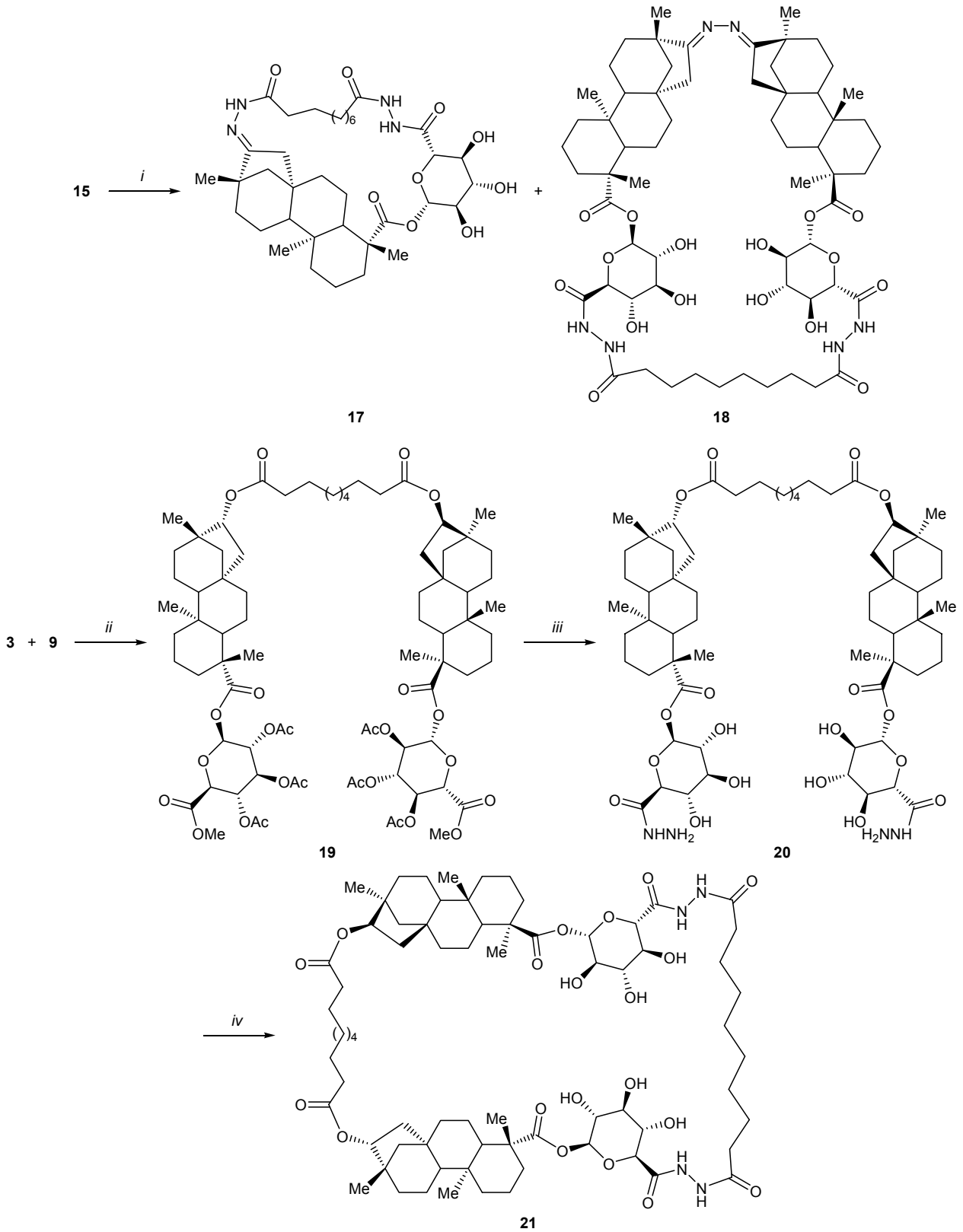
Having failed to implement convergent synthesis of macrocyclic glycoterpenoid, we made an attempt to connect the terpene and carbohydrate components in the initial step. For this purpose, isosteviol (**1**) was subjected to glycosylation at the carboxy group by reaction with bromide **9** in the presence of potassium carbonate and tetrabutylammonium bromide (TBAB) [19] (Scheme 5). Glucuronoside **14** was thus syn-

Scheme 5.



i: TBAB, K_2CO_3, CH_2Cl_2–H_2O, Δ; *ii*: CH_2Cl_2, $NH_2NH_2 \cdot H_2O$, Δ, 2 h; *iii*: $NH_2NH_2 \cdot H_2O$, MeOH, 20°C, 1 h.

Scheme 6.



i: $Cl(O)C(CH_2)_8C(O)Cl$, CH_2Cl_2, DMAP, 20°C, then reflux; *ii*: TBAB, K_2CO_3, CH_2Cl_2–H_2O, Δ; *iii*: MeOH–$CHCl_3$, $NH_2NH_2 \cdot H_2O$, 20°C; *iv*: $Cl(O)C(CH_2)_8C(O)Cl$, $CHCl_3$–pyridine, 4-Å molecular sieves, 20°C.

thesized in 85% yield. The isosteviol and acetylated glucuronic acid methyl ester fragments each showed in the ^{1}H NMR spectrum one set of signals, and the anomeric proton resonated as a doublet at δ 5.77 ppm with a vicinal coupling constant of 8.0 Hz. These data unambiguously indicated stereospecific glucuronidation of **1** with exclusive formation of β-glucuronoside **14**. Reactive groups necessary for the macrocyclization were introduced into molecule **14** via reaction with hydrazine hydrate in boiling methylene chloride. This reaction led to the formation of expected hydrazonohydrazide **15** in a mixture with azine **16**. Compounds **15** and **16** were identified in the reaction mixture by MALDI mass spectrometry (**15**: *m*/*z* 523 $[M]^+$; calcd.: *M* 522, $C_{26}H_{42}N_4O_7$; **16**: *m*/*z* 1035 $[M + Na]^+$; calcd.: *M* + Na 1036, $C_{52}H_{80}N_6O_{14}$). We failed to isolate glucuronosides **15** and **16** in the pure state, for they decomposed during chromatographic separation on silica gel. Presumably, azine **16** was formed by reaction of hydrazone **15** with initial glucuronoside **14**. When the reaction of **14** with hydrazine hydrate was performed at room temperature, compound **15** was obtained in 90% yield as a single product (Scheme 5).

Macrocyclization of hydrazonohydrazide **15** was attempted using sebacoyl chloride in methylene chloride in the presence of 4-dimethylaminopyridine (DMAP) at room temperature. After 6 h, no products were detected in the reaction mixture, so that the temperature was raised to 40°C. After 16 h at 40°C, we detected macrocyclic glycoterpenoids **17** and **18** (Scheme 6) by MALDI mass spectrometry (**17**: *m*/*z* 711 $[M + Na]^+$; calcd.: *M* + Na 711, $C_{36}H_{56}N_4O_9$; **18**: *m*/*z* 1180 $[M + Na]^+$; calcd.: *M* + Na 1180, $C_{62}H_{94}N_6O_{16}$). We failed to separate compounds **17** and **18** by column chromatography due to instability of the hydrazonohydrazide and azine fragments on silica gel.

Next we tried to synthesize a macrocycle containing neither hydrazonohydrazide nor azine fragment which hamper isolation of the product by column chromatography. For this purpose, the glucuronic acid fragment was attached directly to the terpene precursor. The reaction of methyl 2,3,4-tri-*O*-acetyl-1-bromo-α-D-glucopyranuronate **9** with bis-acid **3** gave 56% (after column chromatography) of bis-glucuronide **19** (Scheme 6). The anomeric protons in **19** resonated in the ^{1}H NMR spectrum as a single doublet at δ 5.73 ppm with a vicinal coupling constant of 8.0 Hz, indicating β-orientation of the glycoside bonds. Compound **19** was treated with hydrazine hydrate in methanol–chloroform at room temperature to obtain 90% of bis-hydrazide **20**. The reaction involved only the ester groups of **19**; therefore, β-orientation of the glycoside bonds was retained in **20**. This followed from the anomeric proton signal in the ^{1}H NMR spectrum, which appeared as a single doublet at δ 3.78 ppm (^3J = 9.6 Hz), in keeping with published data for various β-glycosides derived from nonacylated glucuronic acid methyl ester (δ 4.26 ppm, ^3J = 9.4 Hz [20]) and 1-*O*-acetyl-α-D-glucuronic acid hydrazide. The anomeric proton in the latter (β-orientation) resonated at δ 5.02 ppm as a doublet with a considerably lower vicinal coupling constant (^3J = 4.2 Hz) [21]. As expected, the hydrazidation was accompanied by deacetylation of the carbohydrate moieties, and the ^{1}H NMR spectrum of **20** lacked signals assignable to acetoxy groups.

In the final step, bis-hydrazide **20** was subjected to macrocyclization with sebacoyl chloride in chloroform–pyridine at room temperature. The progress of the reaction was monitored by IR spectroscopy, following the disappearance of the ν(C=O) band (1800 cm^{-1}) typical of carbonyl stretching vibrations of sebacoyl chloride from the IR spectrum of the reaction mixture. Macrocyclic glycoterpenoid **21** was isolated in 30% yield by column chromatography on silica gel. The anomeric protons therein resonated in the ^{1}H NMR spectrum as a single doublet at δ 3.88 ppm (^3J = 9.5 Hz), indicating β-orientation of the glycoside bonds. The NH signals appeared as multiplets at δ 7.31–7.38 and 7.71–7.77 ppm, as in the spectra of macrocyclic isosteviol derivatives containing analogous fragments [15]. This suggests that macrocycle **21** exists as several conformers due to restricted rotation about the C(O)–NH and C(O)HN–HNC(O) bonds.

Isosteviol derivatives containing hydrazide and hydrazonohydrazide fragments, as well as macrocyclic isosteviol derivatives having no nitrogen-containing groups, are known to exhibit antitubercular activity [12, 13]. Therefore, some of the synthesized compounds were tested for antitubercular activity. The results showed (see table) tuberculostatic activity of compound **15** against H37RV, *M. Avium*, and *M. Terrae* laboratory strains and MLU clinical strain at a level comparable to the known antitubercular drugs isoniazid and ofloxacin. The data on hemolytic effect (cytotoxicity) of compound **15** toward human erythrocytes in a saline blood solution are given below. It is seen that compound **15** in the concentration range 0.9–

Concentration of **15**, μg/mL	250	125	62.5	31.3	7.8	3.9	1.9	0.9
Hemolysis, %	2.7	0.9	0.6	0.5	0.2	0	0	0

Tuberculostatic activity of compounds **15**, **20**, and **21**

Compound no.	Minimum inhibitory concentration (μg/mL) against *M. tuberculosis* strains			
	H37RV	*M. Avium*	*M. Terrae*	MLU[a]
15	0.7	0.7	0.35	0.7
20	12.5	12.5	12.5	12.5
21	12.5	12.5	12.5	12.5
Isoniazid	0.1	0.1	0.1	0.1
Ofloxacin	0.1	0.1	0.1	–

[a] MLU is a *M. tuberculosis* strain isolated from patients under treatment at the clinics of the Ural Research Institute for Phthisiopulmonology.

125 μg/mL is not cytotoxic to human erythrocytes. The antitubercular activity of **20** and **21** was comparable to that of pyrazinamide [22].

In summary, we have synthesized the first representative of macrocyclic glycoterpenoids containing glucuronic acid and *ent*-beyerane diterpenoid (isosteviol) fragments. The developed synthetic approach makes it possible to obtain macrocycles with different sizes by variation of the dicarboxylic acid spacer. Selective screening has revealed compounds whose antitubercular activity was comparable to the activity of known antitubercular drugs (isoniazide, ofloxacin, and pyrazinamide).

EXPERIMENTAL

The ^{1}H NMR spectra were recorded on Bruker Avance-400 and Avance-500 spectrometers (Germany). The MALDI mass spectra (a.m.u. range 400–3000) were obtained on a Bruker Daltonik UltraFlex III TOF/TOF mass spectrometer (Germany); samples were dissolved in chloroform or chloroform–methanol (1 : 1) to a concentration of 10^{-3} mg/mL and were applied by the dried drop method; *p*-nitroaniline was used as matrix; the data were processed by FlexAnalysis 3.0 (Bruker Daltonik, Germany). The electron impact mass spectra (70 eV) were recorded on a DFS Thermo Electron Corporation instrument (Germany) with direct sample admission into the ion source (ion source temperature 280°C, direct inlet probe temperature 250°C). The optical rotations were measured on a Perkin Elmer-341 polarimeter (USA). The IR spectra (400–4000 cm^{-1}) were obtained on a Bruker Vector 22 spectrometer (Germany) from samples prepared as films. The melting points were determined on a Boetius micro hot stage. The progress of reactions and the purity of products were monitored by TLC on Sorbfil PTSKh-AF-A plates (Krasnodar, Russia); spots were developed by treatment with 5% H_2SO_4 followed by heating. The products were isolated by flash chromatography on KSK silica gel (0.063–0.125 mm; manufactured by *KhromLab* Ltd., Russia).

Bis-acid **3** was synthesized according to the procedure described in [13]. mp 108–109°C, $[\alpha]_D^{20}$ = –97.7° (*c* = 1.1, $CHCl_3$); published data [23]: mp 108–110°C, $[\alpha]_D^{20}$ –99° (*c* = 0.17, C_6H_6). Compounde **6** was synthesized as described in [18]. mp 177°C (from MeOH), $[\alpha]_D^{20}$ = +8.1° (*c* = 1.1, $CHCl_3$); published data: mp 176°C [18]; mp 177–178°C (from EtOH), $[\alpha]_D^{25}$ = +7.4° (*c* = 2.0, $CHCl_3$) [24]. Compound **9** was prepared as reported in [25]; mp 105–106°C (from EtOAc); published data [24]: mp 106–107°C (from EtOH). (+)-D-Glucurono-6,3-lactone (**5**) was commercial product (Acros, Belgium).

Methyl 1-*O*-3-hydroxypropyl-β-D-glucopyranuronate (10). Silver carbonate, 0.5 g (1.81 mmol), was added to a solution of 0.5 g (1.26 mmol) of bromide **9** and 1 g (13.15 mmol) of propane-1,3-diol in 50 mL of freshly distilled chloroform, 4-Å molecular sieves were added, and the mixture was stirred for 1 h at 20°C and was then heated for 10 h under reflux. The mixture was cooled, washed with water, and dried over anhydrous $MgSO_4$, and the solvent was removed to leave a crystalline product. Yield 0.36 g (75%), mp 95–97°C, $[\alpha]_D^{20}$ = –20.2° (*c* = 1.7, $CHCl_3$). ^{1}H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm: 1.78–1.84 m (2H, 1′-$OCH_2C\mathbf{H}_2$), 2.01 s (3H, CH_3CO), 2.02 s (3H, CH_3CO), 2.05 s (3H, CH_3CO), 3.68–3.77 m (3H, 1′-OCH_2, $C\mathbf{H}_2OH$), 3.75 s (3H, OCH_3), 4.00–4.06 m (1H, 1′-OCH_2), 4.05 d (1H, 5′-H, *J* = 9.5 Hz), 4.58 d (1H, 1′-H, *J* = 7.6 Hz), 4.98–5.02 m (1H, 2′-H), 5.20–5.28 m (2H, 3′-H, 4′-H). Mass spectrum, *m*/*z* (I_{rel}, %): 391 (0.03) $[M - H]^+$, 361 (0.01) $[M - CH_2OH]^+$, 333 (0.14) $[M - (CH_2)_3OH]^+$, 317 (1.32) $[M - O(CH_2)_3OH]^+$, 291 (0.16) $[M - (CH_2)_3OHC(O)CH_2]^+$, 257 (1.9) $[M - O(CH_2)_3OHOC(O)CH_4]^+$, 229 (3.9) $[C_{10}H_{13}O_6]^+$, 213 (9.9) $[C_{10}H_{13}O_5]^+$, 127 (77.3) $[C_7H_{11}O_2]^+$, 101 (52.6) $[C_5H_9O_2]^+$, 87 (100) $[C_4H_7O_2]^+$, 73 (13.3) $[C_3H_5O_2]^+$, 59 (10.8) $[C_3H_7O]^+$. Found, %: C 48.90; H 6.21. $C_{16}H_{24}O_{11}$. Calculated, %: C 48.98; H 6.17. *M* 392.35.

Dimethyl 6,6′-[propane-1,3-diylbis(oxy)]bis-(3,4,5-tri-acetoxytetrahydro-2*H*-pyran-2-carboxylate) (11). Silver carbonate, 0.25 g (0.91 mmol), was added to a solution of 0.26 g (0.66 mmol) of compound **10** and 0.13 g (0.33 mmol) of bromide **9** in anhydrous chloroform, 4-Å molecular sieves were added, and the mixture was heated for 4 h under reflux.

The mixture was filtered, the filtrate was evaporated under reduced pressure, and the residue was subjected to silica gel chromatography using petroleum ether–ethyl acetate (1:1) as eluent. Yield 0.11 g (25%), mp 219–221°C, $[\alpha]_D^{20}$ = –19.4° (*c* = 1, CH_2Cl_2). ^{1}H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm: 1.81–1.87 m (2H, 1′-$OCH_2C\mathbf{H}_2$), 2.013 s (6H, CH_3CO), 2.015 s (6H, CH_3CO), 2.049 s (6H, CH_3CO), 3.58–3.64 m (2H, 1′-OCH_2), 3.75 s (6H, OCH_3), 3.86–3.92 m (2H,1′-OCH_2), 4.04 d (2H, 5′-H, *J* = 9.5 Hz), 4.52 d (2H, 1′-H, *J* = 7.6 Hz), 4.96–5.01 m (2H, 2′-H), 5.19–5.27 m (4H, 3′-H, 4′-H). Mass spectrum: *m*/*z* 731.02 $[M + Na]^+$. Found, %: C 49.68; H 6.11. $C_{29}H_{40}O_{20}$. Calculated, %: C 49.15; H 5.69. *M* 708.21.

6,6′-[Propane-1,3-diylbis(oxy)]bis(3,4,5-triacetoxytetrahydro-2*H*-pyran-2-carbohydrazide) (12). Hydrazine hydrate, 0.5 mL (10 mmol), was added to a solution of 0.22 g (0.31 mmol) of compound **11** in 20 mL of methylene chloride. The mixture was heated for 2 h under reflux with stirring and kept for 10 h at 20°C, and the precipitate was filtered off and washed with methanol. Yield 0.11 g (80%), mp 234–237°C, $[\alpha]_D^{20}$ = –11.4° (*c* = 0.7, DMSO). IR spectrum, ν, cm^{-1}: 3375, 3273, 3086 (NH_2), 1662, 1619, 1553 [C(O)NH]. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm: 1.70–1.78 m (2H, 1′-$OCH_2C\mathbf{H}_2$), 2.98 t (2H, 2′-H, *J* = 8.3 Hz), 3.17 t (2H, 3′-H, *J* = 8.8 Hz), 3.36 m (2H, 4′-H, *J* = 9.2 Hz), 3.48–3.56 m (4H, 1′-OCH_2, 5′-H), 3.71–3.79 m (2H, 1′-OCH_2), 4.16 d (2H, 1′-H, *J* = 7.9 Hz), 9.03 s and 9.24 s (2H, NH). Mass spectrum: *m*/*z* 478.73 $[M + Na]^+$. Found, %: C 38.98; H 6.34. $C_{15}H_{28}N_4O_{12}$. Calculated, %: C 39.47; H 6.18. *M* 456.40.

3,4,5-Triacetoxy-6-methoxycarbonyltetrahydro-2*H*-pyran-2-yl 16-oxo-*ent*-beyeran-19-oate (14). Potassium carbonate, 0.21 mg (1.5 mmol), and tetrabutylammonium bromide (TBAB), 0.06 g (0.2 mmol), were added to a solution of 0.21 g (0.52 mmol) of bromide **9** and 0.13 g (0.4 mmol) of isosteviol (**1**) in 20 mL of methylene chloride, and 2 mL of water was added under stirring. The mixture was heated for 4 h under reflux, cooled, diluted with chloroform, washed with water, and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure, and the residue was recrystallized from ethanol. Yield 0.21 g (85%), mp 271–272°C, $[\alpha]_D^{20}$ = –47.4° (*c* = 1.2, $CHCl_3$). ^{1}H NMR spectrum (500 MHz, $CDCl_3$), δ, ppm: 0.86–1.90 m (18H, *ent*-beyerane), 0.69 s (3H, $C^{20}H_3$), 0.97 s (3H, $C^{17}H_3$), 1.21 s (3H, $C^{18}H_3$), 2.02 s (6H, CH_3CO), 2.03 s (3H, CH_3CO), 2.16 d (1H, 3-H_{eq}, *J* = 13.3 Hz), 2.56 d.d (1H, 16-H, *J* = 18.6, 3.9 Hz), 3.72 s (3H, OCH_3), 4.13 d (1H, 2′-H, *J* = 9.7 Hz), 5.18–5.33 m (3H, 3′-H, 4′-H, 5′-H), 5.77 d (1H, 1′-H, *J* = 8.0 Hz). Mass spectrum: *m*/*z* 657.20 $[M + Na]^+$. Found, %: C 61.89; H 7.90. $C_{33}H_{46}O_{12}$. Calculated, %: C 62.45; H 7.30. *M* 634.71.

3,4,5-Trihydroxy-6-(hydrazinecarbonyl)tetrahydro-2*H*-pyran-2-yl 16-hydrazinylidene-*ent*-beyeran-19-oate (15). Hydrazine hydrate, 1 mL (20 mmol), was added to a solution of 0.3 g (0.47 mmol) of compound **14** in 30 mL of methanol. The mixture was kept for 48 h at 20°C, and the precipitate was filtered off and washed with methanol. Yield 0.22 g (90%), mp 149–151°C, $[\alpha]_D^{20}$ = –29.2° (*c* = 1.1, MeOH). ^{1}H NMR spectrum (400 MHz, CD_3OD), δ, ppm: 0.83–1.96 m (18H, *ent*-beyerane), 0.84 s (3H, $C^{20}H_3$), 1.01 s (3H, $C^{17}H_3$), 1.21 s (3H, $C^{18}H_3$), 2.18 d (1H, 3-H_{eq}, *J* = 13.8 Hz), 2.77 d.d (1H, 16-H, *J* = 17.9, 2.7 Hz), 3.31–3.33 m (1H, 2′-H), 3.41–3.44 m (2H, 3-H, 4′-H), 3.57–3.63 m (1H, 5′-H), 3.76 d (1H, 1′-H, *J* = 9.7 Hz), 5.42–5.45 m (1H, OH). Mass spectrum: *m*/*z* 545.20 $[M + Na]^+$. Found, %: C 60.21; H 8.82; N 10.31. $C_{26}H_{42}N_4O_7$. Calculated, %: C 59.75; H 8.10; N 10.72. *M* 522.63.

Bis{19-[3,4,5-triacetoxy-6-(methoxycarbonyl)-tetrahydro-2*H*-pyran-2-yloxy]-19-oxo-*ent*-beyeran-16-yl} decanedioate (19). Potassium carbonate, 0.55 g (4 mmol), and TBAB, 0.4 g (1.26 mmol), were added to a solution of 0.5 g (1.26 mmol) of bromide **9** and 0.51 g (0.63 mmol) of bis-acid **3** in 50 mL of freshly distilled methylene chloride, 5 mL of water was then added, and the mixture was stirred for 1 h at 20°C and heated for 16 h under reflux. The mixture was cooled, diluted with chloroform, washed with water, and dried over anhydrous $MgSO_4$, the solvent was removed under reduced pressure, and the residue was subjected to silica gel chromatography using petroleum ether–ethyl acetate (2:1) as eluent. After drying under reduced pressure, the product was a white powder. Yield 0.5 g (56%), mp 114–115°C, $[\alpha]_D^{20}$ = –39.9° (*c* = 1.1, $CHCl_3$). IR spectrum: ν 1759 cm^{-1} (C=O). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm: 0.70–1.90 m [50H, *ent*-beyerane, $(CH_2)_6$], 0.66 s (6H, $C^{20}H_3$), 0.87 s (6H, $C^{17}H_3$), 1.16 s (6H, $C^{18}H_3$), 1.96 s (6H, CH_3CO), 1.98 s (6H, CH_3CO), 1.99 s (6H, CH_3CO), 2.11 d (2H, 3-H_{eq}, *J* = 13.1 Hz), 2.25 t [4H, 16-$OC(O)CH_2$, *J* = 7.4 Hz], 3.68 s (6H, C^7H_3), 4.06–4.14 m (2H, 5′-H), 4.66 d.d (2H, 16-H, *J* = 10.7, 4.1 Hz), 5.14–5.29 m (6H, 2′-H, 3′-H, 4′-H), 5.73 d (2H, 1′-H, *J* = 8.0 Hz). Mass spectrum: *m*/*z* 1461.8 $[M + Na]^+$. Found, %: C 62.11; H 7.71. $C_{76}H_{110}O_{26}$. Calculated, %: C 63.40; H 7.70. *M* 1438.72.

Bis{19-[3,4,5-trihydroxy-6-(hydrazinecarbonyl)-tetrahydro-2*H*-pyran-2-yloxy]-19-oxo-*ent*-beyeran-16-yl} decanedioate (20). Hydrazine hydrate, 0.5 mL, was added to a solution of 0.5 g (0.35 mmol) of bis-glucuronide **19** in a mixture of 30 mL of methanol and 10 mL of chloroform. The mixture was kept at 20°C, and the crystalline solid was filtered off and washed with methanol. Yield 0.37 g (90%), white powder, mp 131–132°C, $[\alpha]_D^{20}$ = –41.2° (*c* = 0.7, $CHCl_3$–MeOH, 1:1). IR spectrum, ν, cm^{-1}: 3323 (O–H, N–H), 1759 (C=O) 1674, 1610, 1373 [C(O)NH]. ^1H NMR spectrum (400 MHz, $CDCl_3$–CD_3OD, 1:1), δ, ppm: 0.80–1.90 m [50H, *ent*-beyerane, $(CH_2)_6$], 0.77 s (6H, $C^{20}H_3$), 0.89 s (6H, $C^{17}H_3$), 1.19 s (6H, $C^{18}H_3$), 2.16 d (2H, 3-H_{eq}, *J* = 13.3 Hz), 2.30 t [4H, 16-OC(O)CH_2, *J* = 7.4 Hz], 3.31–3.35 m (2H, 2′-H), 3.39–3.46 m (4H, 3′-H, 4′-H), 3.55–3.61 m (2H, 5′-H), 3.78 d (2H, 1′-H, *J* = 9.6 Hz), 4.67 d.d (2H, 16-H, *J* = 10.6, 4.2 Hz), 5.39–5.42 m (2H, OH), 7.69–7.73 m (2H, NH). Mass spectrum: *m*/*z* 1209.5 $[M + Na]^+$. Found, %: C 62.03; H 8.72; N 4.81. $C_{62}H_{98}N_4O_{18}$. Calculated, %: C 62.71; H 8.32; N 4.72. *M* 1186.68.

17^3,17^4,17^5,34^3,34^4,34^5-Hexahydroxy-2,13,16,35-tetraoxa-19,20,31,32-tetraaza-1,14(16,4α)di(19-nor-*ent*-beyerana)-17,34(2,6)di(tetrahydropyrana)-cyclohexatriacontaphane-3,12,15,18,21,30,33,36-octaone (21). Dihydrazide **20**, 0.3 g (0.25 mmol), was kept for 3 h under reduced pressure and dissolved in a mixture of 50 mL of anhydrous chloroform and 5 mL of freshly distilled pyridine, 4-Å molecular sieves were added, the mixture was kept for 1 h, and 0.06 g (0.25 mmol) of sebacoyl chloride was added. The mixture was heated for 72 h at 20°C, the solvent was removed under reduced pressure, and the residue was subjected to silica gel chromatography using ethyl acetate–methanol (4:1) as eluent to isolate compound **21** as a white amorphous powder. Yield 0.1 g (30%), mp 176–178°C, $[\alpha]_D^{20}$ = –34.9° (*c* = 0.55, MeOH). IR spectrum, ν, cm^{-1}: 3290 (O–H, N–H), 1730 (C=O), 1670, 1630, 1372 [C(O)NH]. ^1H NMR spectrum (400 MHz, $CDCl_3$–CD_3OD, 1:1), δ, ppm: 0.60–1.80 m [62H, *ent*-beyerane, $(CH_2)_6$], 0.67 s (6H, $C^{20}H_3$), 0.79 s (6H, $C^{17}H_3$), 1.11 s (6H, $C^{18}H_3$), 2.08 d (2H, 3-H_{eq}, *J* = 12.8 Hz), 2.11–2.25 m [8H, 16-OC(O)CH_2, NHC(O)CH_2], 3.38–3.48 m (4H, 2′-H, 3′-H), 3.51–3.62 m (4H, 4′-H, 5′-H), 3.88 d (2H, 1′-H, *J* = 9.5 Hz), 4.58 d.d (2H, 16-H, *J* = 10.2, 4.7 Hz), 5.38 d (2H, OH, *J* = 7.5 Hz), 7.31–7.38 m and 7.71–7.77 m [2H each, C(O)NH]. Mass spectrum: *m*/*z* 1375.7 $[M + Na]^+$. Found, %: C 64.35; H 8.61; N 4.29. $C_{72}H_{112}N_4O_{20}$. Calculated, %: C 63.88; H 8.34; N 4.14. *M* 1352.78.

Compounds **15**, **20**, and **21** were tested for antitubercular activity by the vertical diffusion method on a *Novaya* solid nutrient medium using H37RV, *M. Avium*, and *M. Terrae* laboratory strains and MLU clinical strain. The nutrient medium was placed in 5-mL test tubes and inoculated with 0.1 mL of test cultures diluted to a turbidity of 10 units (according to the standard developed by the Tarasevich State Scientific Research Institute for Standardization and Quality Control of Biologicals), and the test tubes were incubated for 24 h to grow tuberculosis bacteria. The test tubes were then set vertically, and 0.3 mL of a solution of compound **15**, **20**, or **21** in aqueous alcohol with a concentration of 12.5, 6.2, 3.1, 1.5, 0.7, 0.35, or 0.1 μg/mL was added dropwise. The test tubes were incubated for 10–12 days at 37°C under sterile conditions, and the zone of bacterial growth inhibition was measured. An inhibition zone of longer than 10 mm indicated tuberculostatic activity, and an inhibition zone of 100 mm and longer was regarded as complete inhibition. Antituberculosis drugs isoniazid and ofloxacin were used as controls.

The cytotoxicity (hemolytic effect) of compound **15** toward human erythrocytes was evaluated by comparing the optical density of a solution of **15** and human erythrocyte suspension in saline with the optical density of blood for 100% hemolysis according to the procedure described in [26]. According to GOST R ISO 10993 4-99, a compound is considered to be cytotoxic toward human erythrocytes if the hemolysis exceeds 2%.

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