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Isoniazid Conjugates with D-Arabinofuranose

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Abstract—Glycoconjugates with isoniazid attached to the hydroxy group at the C^5 atom of D-arabinofuranose via the spacers differing in length have been synthesized.

Keywords: isoniazid, arabinofuranose, glycoconjugates

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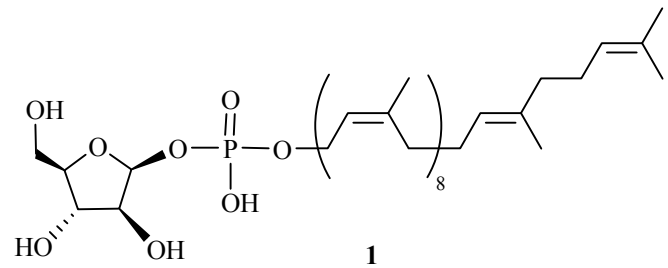
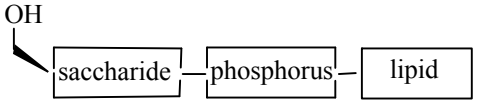
β-D-Arabinofuranosyl-1-*O*-monophosphodecaprenol **1** has been recognized as an exclusive source of D-arabinose involved in the synthesis of arabinogalactan and lipoarabinomannan, major components of cell wall of mycobacteria [1, 2]. Since the biosynthesis of these glycans is decisive in the survival of mycobacteria, it has been suggested that the inhibitors of this process should be efficient antibacterial and antimycobacterial agents [3]. Such inhibitors are searched among mimetics of decaprenylphosphoryl-arabinose **1**, glycolipids of general formula **2** with arabinofuranose [2, 4], glucopyranose [5], glucosamine [6–8], galactopyranose [9, 10], mannopyranose [11, 12], or glucuronic acid [13] as the carbohydrate block. Phosphate [5, 11–13] and diphosphate [6–9] groups have been used as the phosphorus-containing fragment. The lipid moiety included prenyl, farnesyl, decyl, or cetyl fragment as well as other polymethylene chains. Since the hydroxy group at the C^5 atom of the arabinofuranose moiety of decaprenylphosphorylara-binose **1** is involved in the growth of the oligo-saccharide chain of arabinogalactans, it has been

Scheme 1.



1



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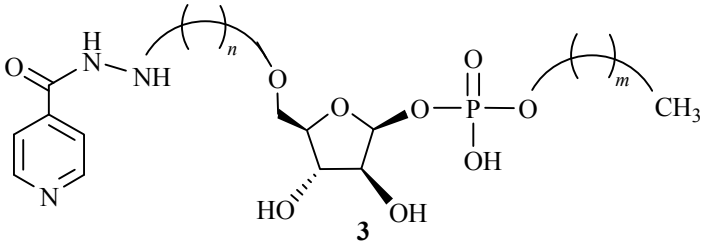

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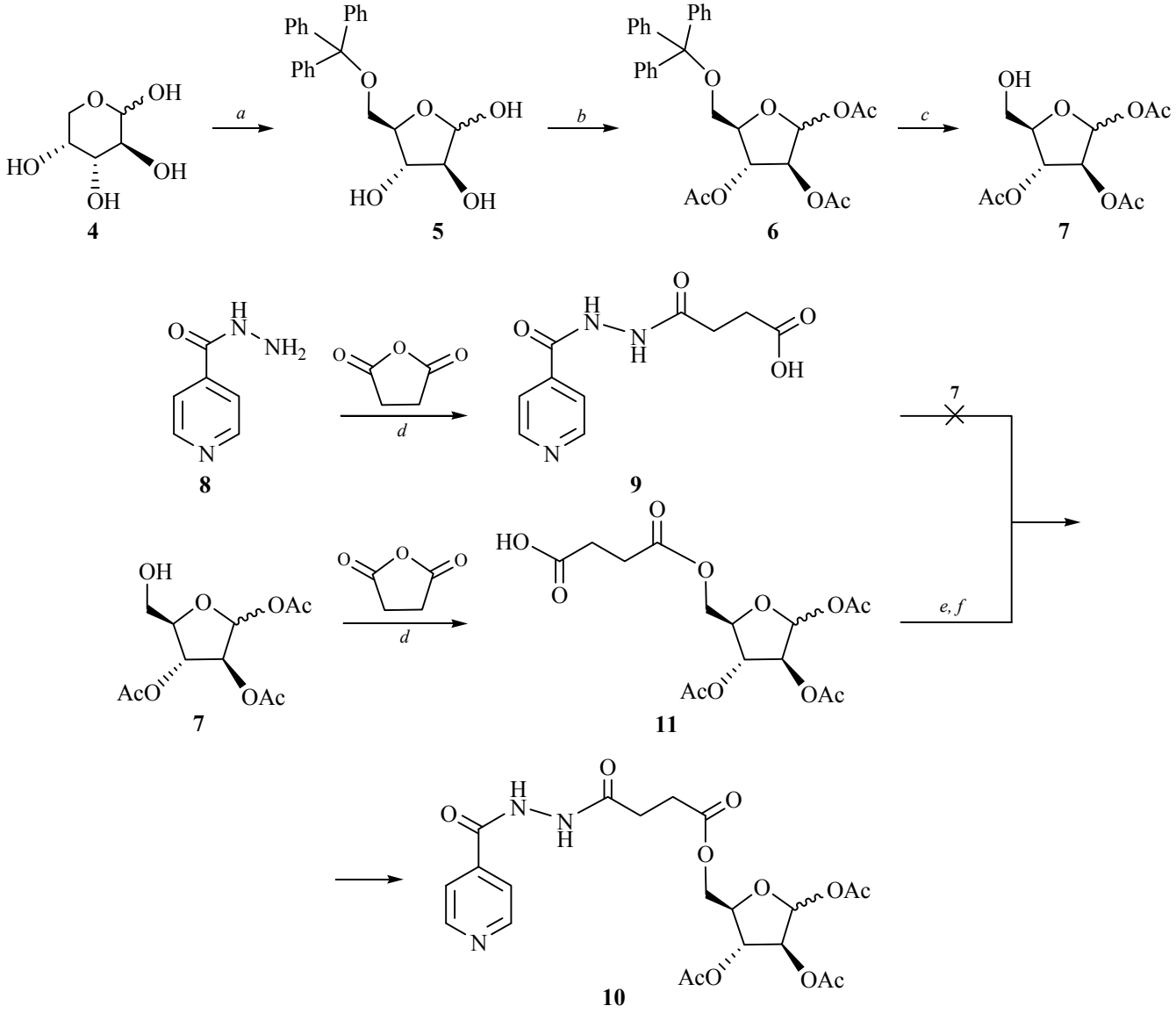
Scheme 2.



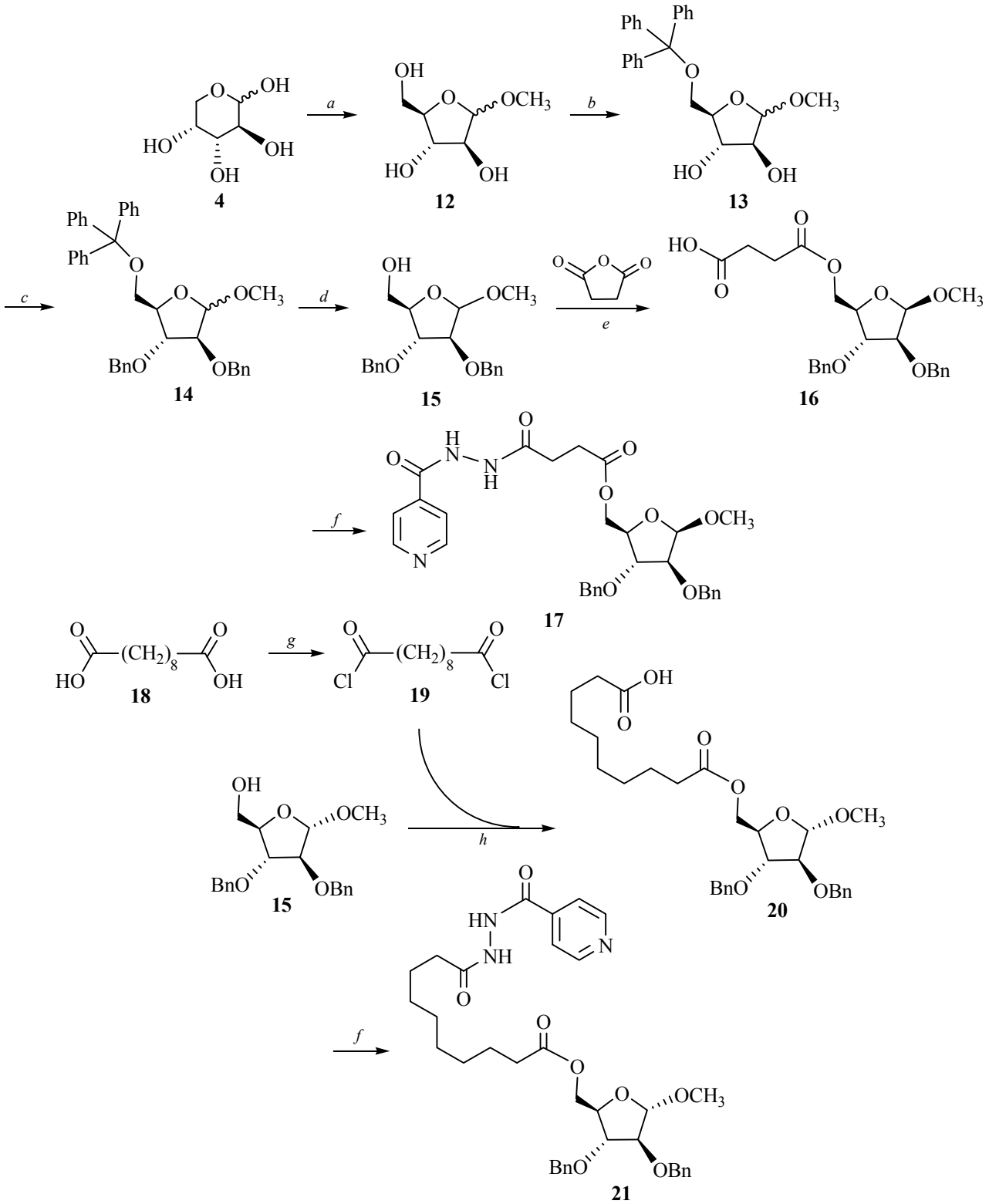
a, TrCl, Py, 25°C; *b*, Ac_2O, Py, 0°C; *c*, AcOH, H_2O, 80°C; *d*, CH_2Cl_2, Py, DMAP, 25°C; *e*, CO_2Cl_2, CH_2Cl_2, 25°C; *f*, isoniazid **8**, Py, CH_2Cl_2, 25°C.

substituted with a halogen atom, methoxy, amino, azido, acetamido, or other groups in certain mimetics to inhibit the said process [4, 14].

In continuation of the search of antituberculous agents among phosphorylated glycolipids [13], we started the synthesis of mimetics of decaprenylphosphorylarabinose **1** of general formula **3**, with isoniazid molecule attached to the hydroxyl at the C^5 atom of arabinofuranose via the spacers differing in length. Here we report on the synthesis of semi-products of the target glycolipids **3**, conjugates of isoniazid and D-arabinofuranose **10**, **17**, and **21**.

By analogy with [4], the first stage of the conjugates synthesis involved the conversion of commercial D-arabinose **4** into D-arabinofuranose **5** with simultaneous selective trityl protection of the C^5 hydroxyl. The other hydroxy groups were then converted into acetyl ones, and the trityl protection of the arabinofuranoside **6** was removed with 80% solution of acetic acid, with the formation of 1,2,3-tri-

Scheme 3.



a, CH_3OH, HCl, 25°C; *b*, TrCl, Py, 25°C; *c*, DMF, NaH, $C_6H_5CH_2Br$, 0°C; *d*, AcOH, H_2O, 80°C; *e*, CH_2Cl_2, Py, DMAP, 25°C; *f*, DCC, DMAP, Py, isoniazid **8**, 25°C; *g*, $SOCl_2$, CH_2Cl_2, 25°C; *h*, CH_2Cl_2, Et_3N, 25°C.

O-acetyl-5-hydroxy-D-arabinofuranose **7** (Scheme 2). The second stage involved the reaction of isoniazid **8** with succinic anhydride. Dioxane, ethyl acetate, or mixture of ethyl acetate with water are usually used as solvents for the reactions of hydrazides with cyclic anhydrides [15, 16]. However, following [17], we performed the reaction in ethanol under reflux, which allowed isolation of pure product **9** from the reaction mixture without additional purification. Unfortunately, acid **9** was insoluble in organic solvents, and it was impossible to perform its reaction with arabinofuranoside **7** to prepare conjugate **10**.

In view of that, we performed the interaction of arabinofuranoside **7** with succinic anhydride, leading to 1,2,3-tri-*O*-acetyl-5-succinyl-D-arabinofuranoside **11** in 66% yield (Scheme 2). Its formation was confirmed by the presence of a single peak in the ESI mass spectrum, at *m*/*z* 399.2 $[M + Na]^+$ ($C_{15}H_{20}NaO_{11}$, *M* 399.09). The presence of two types of signals in the ^{1}H NMR spectrum of arabinofuranoside **11** assigned to the resonance of anomeric proton evidenced the formation of approximately equal amounts of α- and β-anomer forms. According to the reference data of [18–20] the singlet at 6.09 ppm was assigned to the α-anomer, and the doublet at 6.28 ppm with the vicinal spin-spin interaction constant 4.4 Hz was assigned to the β-anomer.

The interaction of arabinofuranoside **11** with isoniazid **8** performed as described in [21] gave conjugate **10** (Scheme 2) according to the mass spectrometry data. The MALDI mass spectrum of the reaction mixture contained the peaks at *m*/*z* 518.1 $[M + Na]^+$ ($C_{21}H_{25}N_3NaO_{11}$, *M* 518.14) and *m*/*z* 534.1 $[M + K]^+$ ($C_{21}H_{25}KN_3O_{11}$, *M* 534.11). Unfortunately, we failed to isolate pure conjugate **10**. To avoid the formation of the open-chain form, methoxy group was attached at the C^1 anomeric atom of arabinofuranose via the interaction of D-arabinose **4** with methanol [22].

Further selective protection-deprotection (as in [23]) afforded methyl 2,3-di-*O*-benzyl-D-arabinofuranoside **15** (Scheme 3), and its α- and β-anomers were isolated by chromatography on silica gel. ^{1}H NMR spectra of isomers of arabinofuranoside **15** showed the resonance of the anomeric proton of the β-anomer at 4.60 ppm as a doublet with a vicinal coupling constant of 4.5 Hz, and the anomeric proton of the α-isomer appeared as a singlet at 4.96 coinciding with the reference data ([18] and [19, 20], respectively).

The β-anomer of arabinofuranoside **15** was then involved in the reaction with succinic anhydride, having afforded methyl 2,3-di-*O*-benzyl-5-succinyl-β-D-arabinofuranoside **16** in 86% yield. Its formation was evidenced by the presence of the signals at *m*/*z* 467.2 $[M + Na]^+$ ($C_{24}H_{28}NaO_8$, *M* 467.17) and *m*/*z* 483.2 $[M + K]^+$ ($C_{24}H_{28}KO_8$, *M* 483.14) in the mass spectrum. The interaction of arabinofuranoside **16** with isoniazid **8** performed similarly to [21] gave (according to the mass spectral data) conjugate **17** (Scheme 3) isolated by chromatography in 27% yield. Its formation was confirmed by the presence of a single signal at *m*/*z* 586.3 $[M + Na]^+$ ($C_{30}H_{33}N_3NaO_8$, *M* 586.22) in the mass spectrum. ^{1}H NMR spectrum of conjugate **17** contained the multiplets at 7.7 and 8.6 ppm corresponding to the resonance of the protons of the pyridine ring of the isonicotinoyl fragment, on top of the signals assigned to the protons of the arabinofuranose and the succinic acid moieties.

To prepare the conjugate of isoniazid with D-arabinofuranose containing a longer spacer, methyl 2,3-di-*O*-benzyl-α-D-arabinofuranoside **15** was involved in the reaction with sebacyl chloride **19** (Scheme 3) prepared via the interaction of sebacic acid **18** with thionyl chloride. The reaction led to the formation of methyl 2,3-di-*O*-benzyl-5-sebacyl-α-D-arabinofuranoside **20** isolated by chromatography with 33% yield. Its formation was confirmed by the presence of the signals at *m*/*z* 551.17 $[M + Na]^+$ ($C_{30}H_{40}NaO_8$, *M* 551.26) and *m*/*z* 567.18 $[M + K]^+$ ($C_{30}H_{40}KO_8$, *M* 567.24) in the mass spectrum. In the ^{1}H NMR spectrum, the anomeric proton appeared as a singlet at 4.94 ppm evidencing the existence of arabinofuranoside **20** as the α-anomer.

Finally, the acid **20** was involved in the reaction with isoniazid **8** under conditions similar to the synthesis of conjugate **17**. The chromatography on silica gel afforded conjugate **21** in 25% yield. Its formation was confirmed by the presence of a single signal at *m*/*z* 670.2 $[M + Na]^+$ ($C_{36}H_{45}N_3NaO_8$, *M* 670.31) in the mass spectrum. The ^{1}H NMR spectrum of conjugate **21** contained the doublets at 7.62 ppm (vicinal spin-spin coupling constant 5.5 Hz) and 8.68 ppm (vicinal spin-spin coupling constant 4.9 Hz) corresponding to the resonance of the protons of the pyridine ring of the isonicotinoyl fragment, on top of the signals assigned to the protons of the arabinofuranose and sebacic acid moieties. The anomeric proton of conjugate **21** appeared as a singlet at 4.92 ppm in the ^{1}H NMR spectrum confirming the preservation of the α-orientation of the glycoside bond.

In summary, we performed the first step of the synthesis of target mimetics of decaprenylphosphoryl-

arabinose **1** of general formula **3**. The first conjugates of isoniazid with D-arabinofuranose **17** and **21** were prepared. The second step which will be described in the upcoming report will include the substitution of the anomeric methoxy group of the conjugates **17** and **21** with bromine atoms, phosphorylation of the obtained conjugates with esters of phosphoric acid containing lipid groups, and removal of the benzyl protection.

EXPERIMENTAL

^{1}H and ^{13}C NMR spectra were recorded using Avance-400 and Avance-600 spectrometers (Bruker, Germany). MALDI mass spectra were registered in the linear mode using a UltraFlex III TOF/TOF mass spectrometer (Bruker Daltonik GmbH, Germany) equipped with a Nd:YAG laser, λ 355 nm. The data were processed using FlexAnalysis 3.0 software (Bruker Daltonik GmbH, Germany). The measurements were performed over the *m*/*z* 200–6000 Da range in the positive ions mode using a metallic target, with 2,5-dihydroxybenzoic acid and *para*-nitroaniline as the matrix. Electrospray ionization (ESI) mass spectra were registered using an AmazonX mass spectrometer (Bruker Daltonik GmbH, Germany). The measurements were performed over the *m*/*z* 100–1500 Da range in the negative ions mode, the capillary voltage being 4500 V. Optical rotation was measured using a Perkin Elmer-341 polarimeter (USA). The reactions completeness and the products purity were monitored by thin layer chromatography on Sorbfil plates (Imid, Russia); the substances were visualized by treatment with 5% solution of sulfuric acid followed by heating to 120°C or by UV irradiation.

Compounds **5**, **6**, and **7** were prepared as described elsewhere [4]. Compound **9** was prepared by a method described in [17]; its melting point and spectral properties coincided with the reference data [15, 16]. Arabinofuranoside **12** was prepared as described in [22], and arabinofuranosides **13** and **14** were synthesized following [23]; their spectral parameters coincided with the reference data. Arabinofuranoside **15** was obtained as described in [23]; its protons signals coincided with the reference data [18–20]. Commercial D-arabinose **4** (Acros, Belgium) and isoniazid **8** (Merck, Germany) were used.

1,2,3-Tri-*O*-acetyl-5-succinyl-α/β-D-arabinofuranose (11). A solution of 0.6 g (6 mmol) of succinic anhydride and 0.6 g (5.3 mmol) of DMAP in 10 mL of pyridine was added to a solution of 0.4 g (1.4 mmol) of arabinofuranoside **7** in 5 mL of methylene chloride. The reaction mixture was stirred at room temperature during 18 h, 3 mL of water was then added, and the stirring was continued for 3 h. The mixture was concentrated at a reduced pressure, dissolved in 100 mL of methylene chloride, washed with water, and dried over $MgSO_4$. Methylene chloride was removed at a reduced pressure, and the residue was recrystallized from a 1 : 1 mixture of petroleum ether and ethyl acetate. DMAP crystals were filtered off, the solvent was removed from the filtrate, and the residue was dried at a reduced pressure. Yield 0.67 g (66%), colorless substance, $[\alpha]_D^{20}$ 6.5° (*c* 1.1, CH_3OH). ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm: 1.98–2.04 m (18H, CH_3CO), 2.58 q (8H, CH_2, *J* = 3.0 Hz), 4.10–4.36 m (8H, $H^{4,5}$), 4.98 d.d (1H, $H^{3\alpha}$, *J* = 4.5, 0.9 Hz), 5.12 d (1H, $H^{2\alpha}$, *J* = 1.3 Hz), 5.22–5.30 m (2H, $H^{2\beta,3\beta}$), 6.09 s (1H, $H^{1\alpha}$), 6.28 d (1H, $H^{1\beta}$, *J* = 4.4 Hz), 13.1 br.s (2H, OH). ^{13}C NMR spectrum (100 MHz, CD_3Cl), δ_C, ppm: 20.23, 20.48, 20.49, 20.52, 20.82, 20.85 (OCO<u>C</u>H$_3$), 28.89, 28.92 (CH_2CH_2), 53.34 (OCH_3), 62.99, 64.56 (C^2), 74.67, 75.21 (C^5), 76.72, 79.44 (C^3), 80.45, 82.15 (C^4), 93.56, 99.18 (C^1), 169.13, 169.37, 169.61, 169.82, 170.09, 171.88 (C=O). Mass spectrum (ESI), *m*/*z* (I_{rel}, %): 399.2 (100) $[M + Na]^+$. Found, %: C 47.96; H 5.93. $C_{15}H_{20}O_{11}$. Calculated, %: C 47.88; H 5.36.

Methyl 2,3-di-*O*-benzyl-5-succinyl-β-D-arabinofuranoside (16). A solution of 0.4 g (4 mmol) of succinic anhydride and 0.1 g (0.8 mmol) of DMAP in 10 mL of pyridine was added to a solution of 0.3 g (0.9 mmol) of compound **15** in 5 mL of methylene chloride. The reaction mixture was stirred at room temperature during 18 h, 3 mL of water was then added, and the stirring was continued for 3 h. The mixture was concentrated at a reduced pressure, dissolved in 100 mL of methylene chloride, washed with water, and dried over $MgSO_4$. Methylene chloride was removed at a reduced pressure, and the residue was recrystallized from a 1 : 1 mixture of petroleum ether and ethyl acetate. DMAP crystals were filtered off, the solvent was removed from the filtrate, and the residue was dried at a reduced pressure. Yield 0.3 g (86%), pale yellow substance. ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm: 2.61 br.s (4H, CH_2CH_2), 3.33 s (3H, OCH_3), 4.02–4.21 m (5H, H^{2-5}), 4.58–4.72 m (5H, C<u>H</u>$_2C_6H_5$, H^1), 7.23–7.38 m (10H, C_6H_5), 11.3 br.s (1H, OH). ^{13}C NMR spectrum (100 MHz, CD_3Cl), δ_C, ppm: 28.79, 28.90 (CH_2CH_2), 54.87 (O<u>C</u>H$_3$), 63.85 (C^2), 72.00, 72.21 (<u>C</u>H$_2C_6H_5$), 79.22 (C^5), 83.28 (C^3), 87.84 (C^4), 107.24 (C^1), 127.89, 127.91, 127.94,

127.97, 128.45, 128.49, 137.38, 137.56 ($2C_6H_5$), 171.93, 177.00 (C=O). Mass spectrum (MALDI), *m*/*z* (I_{rel}, %): 467.21 (50) $[M + Na]^+$, 483.15 (100) $[M + K]^+$. Found, %: C 64.91; H 6.43. $C_{24}H_{28}O_8$. Calculated, %: C 64.85; H 6.35.

Methyl 2,3-di-*O*-benzyl-5-(isonicotinoylhydrazinocarbonylethylenecarboxyl)-β-D-arabinofuranoside (17). 0.65 g (3.1 mmol) of *N*,*N*'-dicyclohexylcarbodiimide and 0.05 g (0.4 mmol) of DMAP was added to a solution of 0.35 g (0.79 mmol) of acid **16** and 0.45 g (3.2 mmol) of isoniazid **8** in 30 mL of pyridine. The solution was stirred during 24 h on a bath at 30°C. Pyridine was then removed at a reduced pressure, and the residue was purified by chromatography on silica gel (eluent: petroleum ether—ethyl acetate, 5 : 1 to 1 : 3 and ethyl acetate). Yield 0.12 g (27%), yellow substance. ^{1}H NMR spectrum (400 MHz, CD_3OD), δ, ppm: 2.60–2.72 m (4H, CH_2CH_2), 3.33 s (3H, OCH_3), 4.03–4.21 m (5H, H^{2-5}), 4.53–4.69 m (5H, $C\underline{H}_2C_6H_5$, H^1), 7.22–7.38 m (10H, C_6H_5), 7.75–7.79 m (2H, C_5H_4N), 8.65–8.69 m (2H, C_5H_4N). ^{13}C NMR spectrum (100 MHz, CD_3OD), δ_C, ppm: 29.39, 30.03 (CH_2CH_2), 55.46 (OCH_3), 66.82 (C^2), 73.46, 73.61 ($\underline{C}H_2C_6H_5$), 80.23 (C^5), 83.27 (C^3), 85.55 (C^4), 103.06 (C^1), 123.12 (C_5H_4N), 128.84, 129.05, 129.43, 129.47 ($2C_6H_5$), 139.13, 139.49, 151.01 (C_5H_4N), 166.77 [C(O)NH], 173.41, 173.75 (C=O). Mass spectrum (MALDI), *m*/*z* (I_{rel}, %): 586.28 (100) $[M + Na]^+$. Found, %: C 63.78; H 5.98; N 7.39. $C_{30}H_{33}N_3O_8$. Calculated, %: C 63.93; H 5.90; N 7.46.

Methyl 2,3-di-*O*-benzyl-5-sebacyl-α-D-arabinofuranoside (20). A solution of arabinofuranoside **15** in 20 mL of methylene chloride and three drops of triethylamine were slowly added to a solution of 0.3 g sebacyl dichloride **19** in 60 mL of anhydrous methylene chloride. The mixture was stirred at room temperature. When arabinofuranoside **15** was completely consumed, methylene chloride was removed at a reduced pressure, and the residue was purified by chromatography on silica gel (eluent: petroleum ether–ethyl acetate, 10 : 1 to 6 : 1). Yield 0.13 g (33%), pale yellow substance. ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm: 1.29 br.s (8H, CH_2), 1.54–1.67 m (4H, CH_2), 2.26–2.36 m (4H CH_2C=O), 3.39 s (3H, OCH_3), 3.85 d.d (1H, H^2, J = 2.8, 5.1 Hz), 4.01 d.d (1H, H^4, J = 1.1, 2.9 Hz), 4.15–4.32 m (3H, $H^{3,5}$), 4.46–4.51 m (4H, $C\underline{H}_2C_6H_5$), 4.94 s (1H, H^1), 7.26–7.39 m (10H, C_6H_5). ^{13}C NMR spectrum (100 MHz, $CDCl_3$), δ_C, ppm: 24.86, 28.99, 34.02 (CH_2), 54.93 (OCH_3), 63.54 (C^2), 72.04, 72.25 ($\underline{C}H_2C_6H_5$), 79.33 (C^5), 82.30 (C^3), 88.02 (C^4), 101.70 (C^1), 127.22, 127.81, 127.83, 127.89, 128.15, 128.39, 128.41 ($2C_6H_5$), 173.48, 174.21 (C=O). Mass spectrum (MALDI), *m*/*z* (I_{rel}, %): 551.17 (100) $[M + Na]^+$, 567.18 (30) $[M + K]^+$. Found, %: C 68.31; H 7.49. $C_{30}H_{40}O_8$. Calculated, %: C 68.16; H 7.63.

Methyl 2,3-di-*O*-benzyl-5-(isonicotinoylhydrazinocarbonyloctamethylenecarcoxyl)-α-D-arabinofuranoside (21). 0.1 g (0.48 mmol) of *N*,*N*'-dicyclohexylcarbodiimide and 0.06 g (0.54 mmol) of DMAP were added to a solution of 0.13 g (0.25 mmol) of arabinofuranoside **20** and 0.07 g (0.51 mmol) of isoniazid **8** in 50 mL of pyridine. The solution was stirred during 24 h on a bath at 30°C. Pyridine was removed at a reduced pressure, and the residue was purified by chromatography on silica gel (eluent: petroleum ether—ethyl acetate, 10 : 1 to 1 : 2, ethyl acetate). Yield 0.04 g (25%), yellow substance. ^{1}H NMR spectrum (600 MHz, $CDCl_3$), δ, ppm: 1.27 br.s (8H, CH_2), 1.54–1.69 m (4H, CH_2), 2.25–2.33 m (4H CH_2C=O), 3.37 s (3H, OCH_3), 3.82 d.d (1H, H^2, J = 2.7, 6.6 Hz), 3.99 d (1H, H^4, J = 2.2 Hz), 4.13–4.22 m (3H, H^5), 4.27 d.d (1H, H^3, J = 2.7, 11.5 Hz), 4.45–4.59 m (4H, $C\underline{H}_2C_6H_5$), 4.92 s (1H, H^1), 7.24–7.38 m (10H, C_6H_5), 7.62 d (2H, C_5H_4N, J = 5.5 Hz), 8.68 d (2H, C_5H_4N, J = 4.9 Hz), 8.95 br.s (1H, NH), 10.03 br.s (NH). ^{13}C NMR spectrum (100 MHz, $CDCl_3$), δ_C, ppm: 24.67, 28.93, 33.96 (CH_2), 54.91 (OCH_3), 63.64 (C^2), 72.04, 72.24 ($\underline{C}H_2C_6H_5$), 79.32 (C^5), 83.44 (C^3), 87.88 (C^4), 107.29 (C^1), 120.96 (C_5H_4N), 127.81, 127.83, 127.9, 128.39, 128.41 ($2C_6H_5$), 137.55, 150.46, 150.51 (C_5H_4N), 162.83 [C(O)NH], 171.51, 173.52 (C=O). Mass spectrum (MALDI), *m*/*z* (I_{rel}, %): 670.19 (100) [*M* + Na]. Found, %: C 66.67; H 7.12, N 6.43. $C_{36}H_{45}N_3O_8$. Calculated, %: C 66.75; H 7.00, N 6.49.

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CONFLICT OF INTERESTS

No conflict of interest was declared by the authors.

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