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Synthesis of P^1-(11-phenoxyundecyl)-P^2-(2-acetamido-2-deoxy-3-*O*-α-D-rhamnopyranosyl-α-D-glucopyranosyl) diphosphate and P^1-(11-phenoxyundecyl)-P^2-(2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-galactopyranosyl) diphosphate for the investigation of biosynthesis of O-antigenic polysaccharides in *Pseudomonas aeruginosa* and *Escherichia coli* O104

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ABSTRACT

Two new phenoxyundecyl diphosphate sugars were synthesized for the first time: P^1-(11-phenoxyundecyl)-P^2- (2-acetamido-2-deoxy-3-*O*-α-D-rhamnopyranosyl-α-D-glucopyranosyl) diphosphate and P^1-(11-phenoxyundecyl)-P^2-(2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-galactopyranosyl) diphosphate to study the third step of biosynthesis of the repeating units of O-antigenic polysaccharides in *Pseudomonas aeruginosa* and *E.coli* O104 respectively.

1. Introduction

The biosynthesis of enterobacterial O-antigenic polysaccharides occurs *via* intermediate formation of polyprenyl diphosphate sugars anchored into the cell membrane. The assembly of the repeating units of the polysaccharides is catalyzed by specific glycosyltransferases. Elucidating the mechanisms of this process opens a way to development of a new antibacterial drug generation. The use of native polyprenyl diphosphate sugars for this purpose is practically impossible due to their extremely low concentrations and short lifetime in bacterial cells. Data concerning the possibility of replacing polyprenyl group with phenoxyundecyl group in the abovementioned acceptor substrates have been published recently [1].

We have previously synthesized a number of phenoxyundecyl diphosphate monosaccharides Sug-PP-$(CH_2)_{11}$OPh (Sug = GlcNAc, GalNAc, Glc or Gal). It was demonstrated that these synthetic analogues can function as acceptor substrates for the corresponding bacterial glycosyltransferases catalysing the second step of O-antigenic polysaccharide repeating unit biosynthesis [1–7].

This work is a continuation of our research on the chemical synthesis and investigation of the biological properties of non-

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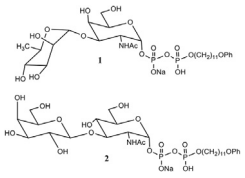
isoprenoid analogues of undecaprenyl diphosphate sugars. Two new compounds of this series contain a phenoxyundecyl residue instead of undecaprenyl, and the disaccharide residue as the sugar moiety: P^1-(11-phenoxyundecyl)-P^2-(2-acetamido-2-deoxy-3-*O*-α-D-rhamnopyranosyl-α-D-glucopyranosyl) diphosphate [D-Rha(α1→3)-D-GlcNAcα-PP-UnOPh, **1**] and P^1-(11-phenoxyundecyl)-P^2-(2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-galactopyranosyl) diphosphate [D-Gal(β1→3)-D-GalNAcα-PP-UnOPh, **2**]. These compounds were synthesized to identify and to study glycosyltransferases that catalyze the third step of biosynthesis of O-antigenic polysaccharide repeating units in *Pseudomonas aeruginosa* and *E.coli* O104 respectively.



D-Rhamnose polymers can be found in virtually all *Pseudomonas aeruginosa* species but also in variety of other bacteria, e.g. *Stenotrophomonas maltophilia, Azospirillum brasilense, Azorhizobium caulinodans, Pantoea agglomerans and E.coli O99.* Thus, the synthetic compound D-Rha(α1→3)-D-GlcNAcα-PP-UnOPh **1** can potentially serve as an adaptor structure for the extension of D-Rhamnose oligosaccharides or polymers in many different bacteria. The Galβ1-3GalNAc-structure at reducing end is found in *E.coli* serotypes O5, O22, O36, O69, O71, O107, O117, O155, O160, O171, O174, and O178. In addition, it is found in other serotypes as internal structure. The synthetic compound D-Gal(β1→3)-D-GalNAcα-PP-UnOPh **2** can be a substrate for many enzymes that extend the O-antigen repeating unit chain. Toxicity is unknown but since the enzymatic studies are done in vitro, the toxicity is not relevant.

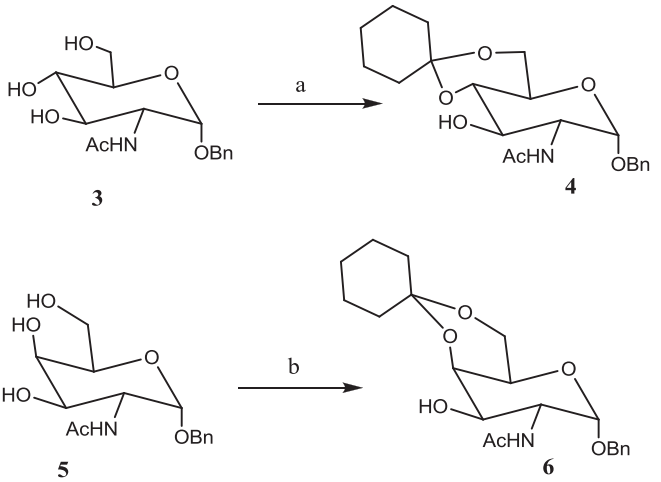
2. Results and discussion

To synthesize the target disaccharide derivatives, the Helferich method [8] (glycosylation of an alcohol using a glycosyl halide as the glycosyl donor and a mercury cyanide as the promoter) was used.

Benzyl 2-acetamido-4,6-*O*-cyclohexylidene-2-deoxy-α-D-glucopyranoside **4** and benzyl 2-acetamido-4,6-*O*-cyclohexylidene-2-deoxy-α-D-galactopyranoside **6** were chosen as glycosyl acceptors for the synthesis of the corresponding protected disaccharides since they can easily be obtained by the interaction of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside [12] **3** or benzyl 2-acetamido-2-deoxy-α-D-galactopyranoside [12] **5** with cyclohexanone diethyl ketal in acetonitrile in the presence of catalytic amounts of TsOH (Scheme 1) according to the procedure described in Ref. [13]. A lower yield of **6** was apparently a result of the competitive formation of the 3,4-*O*-cyclohexylidene derivative as a by-product which was separated by column chromatography on SiO_2.

2,3,4-Tri-*O*-acetyl-α-D-rhamnopyranosyl bromide **7** was obtained by reaction of 1,2,3,4-tetra-*O*-acetyl-α-D-rhamnopyranose [10] with 40% solution of HBr in glacial AcOH and without purification was glycosylated with benzyl 2-acetamido-4,6-*O*-cyclohexylidene-2-deoxy-α-D-glucopyranoside **4** in dry acetonitrile in the presence of $Hg(CN)_2$ affording benzyl 2-acetamido-4,6-*O*-cyclohexylidene-2-deoxy-3-*O*-(2,3,4-tri-*O*-acetyl-α-D-rhamnopyranosyl)-α-D-glucopyranoside **8** in a yield of 40%.

2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide **9** was obtained analogously from 1,2,3,4,6-penta-*O*-acetyl-β-D-galactopyranose and was condensed with benzyl 2-acetamido-4,6-*O*-cyclohexylidene-2-deoxy-α-D-galactopyranoside **6** under similar conditions affording benzyl 2-acetamido-2-deoxy-4,6-*O*-cyclohexylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)- α-D-galactopyranoside **10** in a yield of 42% (Scheme 2).



Scheme 1. Reagents and conditions: a) cyclohexanone diethyl ketal, TsOH, MeCN, 20° C, **4**: 98%; b) cyclohexanone diethyl ketal, TsOH, MeCN, 20° C, **6**: 45%.

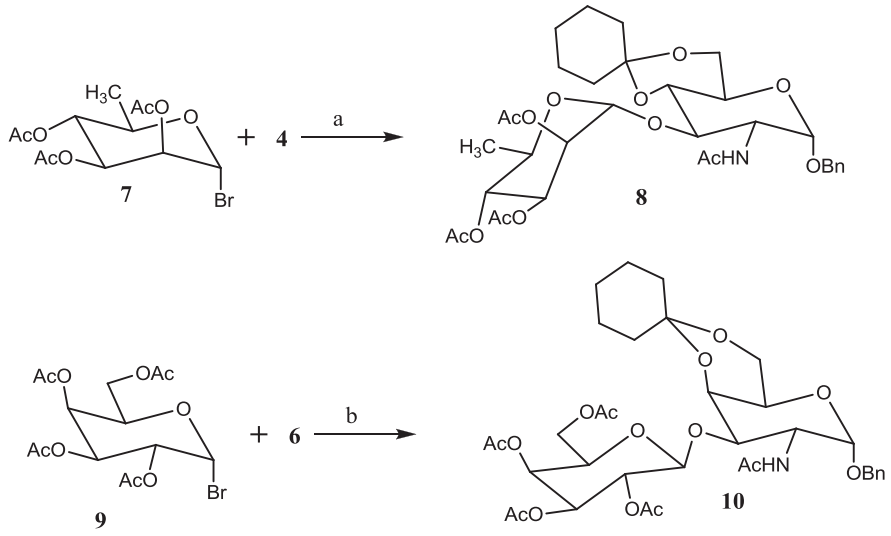
After removal of the cyclohexylidene protecting group from **8** and **10** with 90% CF_3COOH, diols **11**(95%) and **12** (93.6%) were obtained, and were further converted to derivatives **13** and **14** in a yield of 98%.

Removing the benzyl group from **13** or **14** by hydrogenolysis afforded **15** or **16** in a yield of 90%. The derivatives were treated with bis(benzyloxy)(diisopropylamino)phosphine [10] in the presence of tetrazole followed by oxidation with *m*-chloroperbenzoic acid (mCPBA), and dibenzyl α-glycosyl phosphates **17** or **18** were obtained in a yield of 85%. Hydrogenolysis of the latter compounds in the presence of 10% $Pd(OH)_2/C$ in glacial acetic acid resulted in acetylated α-glycosyl dihydrogen phosphates **19** or **20** in a yield of 90% (Scheme 3).

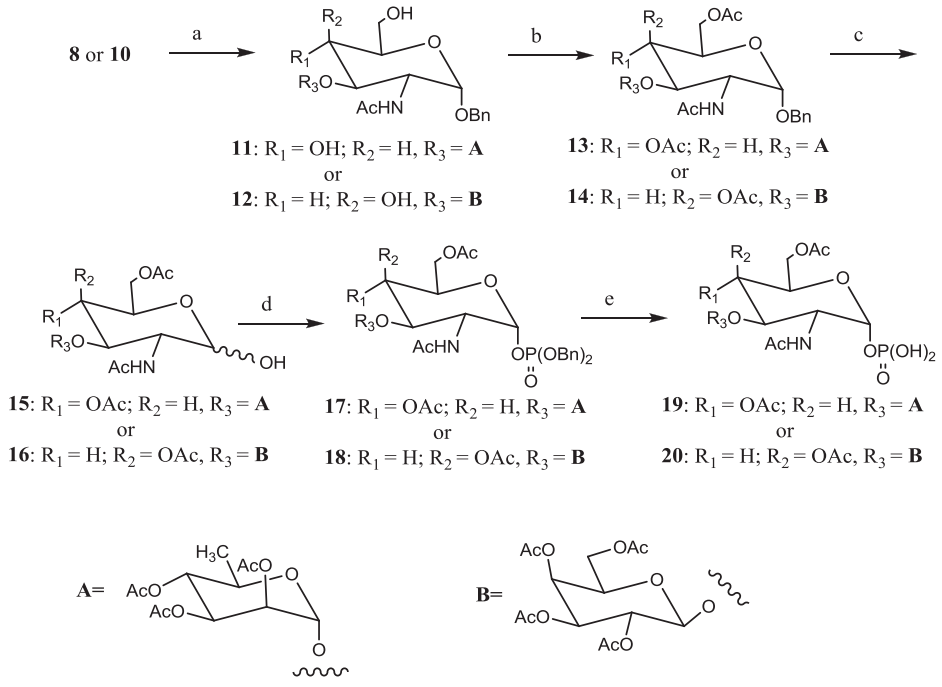
Activating 11-phenoxyundecyl phosphate **21** [2] with 1,1-carbonyldiimidazole (CDI) for 2 h led to phosphoroimidazolidate intermediate **22** (R_f 0.9, $CHCl_3$/MeOH/H_2O 6:4:0.5). Following the condensation of crude **22** with acetylated glycosyl phosphates **19** or **20** at 37 °C for 28 h resulted in protected diphosphates **23** or **24** (Rf 0.6). Small quantities of the symmetrical bis-(phenoxyundecyl) diphosphate intermediate **22** (R_f 0.7), and acetylated sugar phosphates **19** or **20** (R_f 0.3) were also present in the reaction mixture. Reaction mixtures containing **23** and **24** were deacetylated with sodium methoxide in methanol followed by neutralization with AcOH. The desired sodium salts of phenoxyundecyl diphosphate disaccharides **1** or **2** were isolated by reversed-phase chromatography on 5 mL C18 Sep-Pak cartridges in total yields of 60% for **1** and of 59% for **2** (Scheme 4).

The structure of **1** was confirmed by NMR and mass spectrometry data. The ^1H NMR spectrum in CD_3OD represented a superposition of the (2-acetamido-2-deoxy-3-*O*-α-D-rhamnopyranosyl-α-D-glucopyranosyl) phosphate and 11-phenoxyundecyl phosphate (**21**) spectra with the ratio of the components 1: 1. The ^{31}P NMR spectrum contained two doublets at δ −9.9 ($J_{P1,P2}$ 20.2 Hz) and δ −12.4 ($J_{P2,P1}$ 20.2 Hz) ppm, which are typical for non-symmetrical P^1,P^2-diesters of pyrophosphoric acid. The HRESIMS spectrum (negative ion mode) of **1** contained a signal m/z 772.2691, which corresponds to $[M - H]^-$ for compound (**1**).

The structure of the compound **2** was also confirmed by NMR and MS data. The ^1H NMR spectrum in CD_3OD represented a



Scheme 2. Reagents and conditions: a) CH_3CN, $Hg(CN)_2$, **8**: 40%; b) CH_3CN, $Hg(CN)_2$, **10**: 42%.



Scheme 3. Reagents and conditions: a) 90% CF_3COOH, **11** (95%), **12** (42%); b) Ac_2O/Py, **13** or **14**: 98%; c) H_2- $Pd(OH)_2$/C, EtOH, **15** or **16**:90%; d) *i*) $(BnO)_2PN(iPr)_2$, tetrazole, CH_2Cl_2; *ii*) mCPBA, **17** or **18**: 85%; e) H_2-$Pd(OH)_2$/C, AcOH, **19** or **20**: 90%.

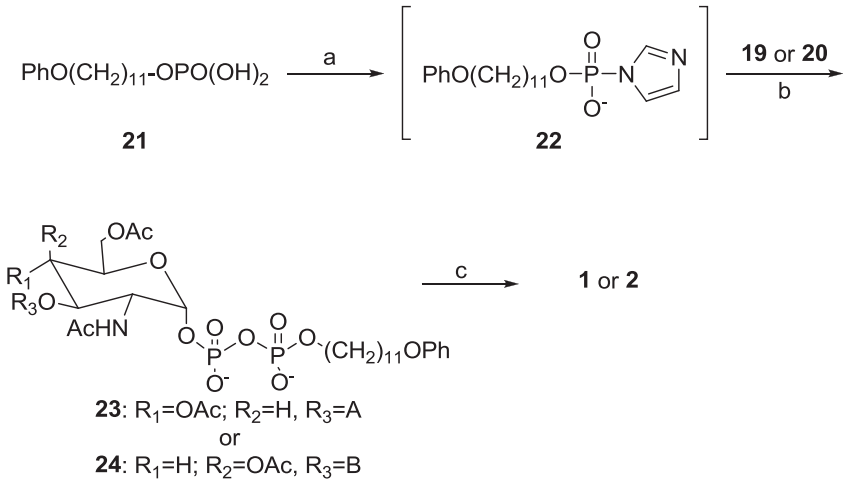
superposition of the (2-acetamido-2-deoxy-3-*O*-β-D-galactopyranosyl-α-D-galacopyranosyl) phosphate and 11-phenoxyundecyl phosphate (**21**) spectra with the ratio of the components 1: 1. The ^{31}P NMR spectrum contained two doublets at δ −10.05 ($J_{P1,P2}$ 20.2 Hz) and δ −12.4 ($J_{P2,P1}$ 20.2 Hz) ppm, which are typical for non-symmetrical P^1,P^2-diesters of pyrophosphoric acid. The HRESIMS spectrum (positive ion mode) of **2** contained a signal m/z 812.2618, which corresponds to $[M + Na]^+$ for compound (**2**).

The target compounds **1** and **2** are currently examined as acceptor substrates in biochemical assays. Compound **1** is the adaptor compound for the extension of the common polysaccharide antigen by D-rhamnosyltransferases from *Pseudomonas aeruginosa* [4]. Compound **2** is the acceptor substrate for α2,3-sialyltransferase WbwA from *Escherichia coli* serotype O104 [9].

3. Experimental

3.1. General

The reactions were performed with the use of commercial reagents (Aldrich, Fluka, Acros Organics). Concentration of organic solutions was performed under reduced pressure below 40 °C. Column chromatography was carried out on a silica gel (40–60 μm, Acros Organics). Thin-layer chromatography was performed using glass-based Silica Gel 60 F_{254} plates (Merck, Germany). The ^1H, ^{13}C, and ^{31}P NMR spectra were recorded for solutions in $CDCl_3$ (in



Scheme 4. Reagents and conditions: a) *i*) *i*Pr_2NH, C_6H_6; *ii*) CDI/THF; *iii*) MeOH; b) *i*Pr_2NH, THF; c) *i*) MeONa/MeOH; *ii*) AcOH; *iii*) C18 Sep-Pak/water-MeOH, sodium salt **23:** total yield 60%; sodium salt **24**: total yield 59%; A and B see Scheme 3.

CD_3OD for compounds **1** and **2**) on a Bruker AM-300 instrument (300.13 MHz for ^1H) or on a Bruker AVANCE 600 spectrometer (for ^1H, 600.13, for ^{13}C, 150.90, and for ^{31}P, 242.9 MHz). Chemical shifts (δ) are reported in parts per million (ppm), and signals are described as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), and m (multiplet). ^1H NMR chemical shifts were referenced to residual signal of $CHCl_3$ (δ_H 7.27) or CD_2HOD (δ_H 3.33). ^{13}C chemical shifts were referenced to the central resonance of $CDCl_3$ (δ_C 77.0) and CD_3OD (δ_C 49.0). Phosphorus chemical shifts are given relative to those of 85% phosphoric acid: δ = 0 ppm. Assignments of the signals in the NMR spectra were confirmed using 2D-spectroscopy (COSY, HSQC, HMBC). High-resolution mass spectra (electrospray ionization, HRESIMS) were recorded in positive mode (negative mode for **1**) on a Bruker micrOTOF II mass spectrometer for 2×10^{-5} M solutions in MeCN or MeOH. Optical rotations were measured using a JASCO P-2000 automatic digital polarimeter (Japan) for solutions in $CDCl_3$ with concentration c = 1 unless stated otherwise. $[\alpha]_D^{20}$ values are given in units of 10^{-1}deg cm^3g^{-1}. Reverse-phase chromatography was conducted with C18 cartridges (Sep-Pak, Waters, United States, 5 mL).

3.2. Benzyl 2-acetamido-4,6-O-cyclohexylidene-2-deoxy-α-D-glucopyranoside (*4*)

Compound **4** was obtained from benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside [12] (**3**) according to the procedure described [13] in a yield of 98%. R_f 0.3 (toluene/acetone 1: 1); $[\alpha]_D^{20}$ +100.3; HRESIMS: m/z = 392.2055 $[M + H]^+$, calcd for $C_{21}H_{29}NO_6$: 391.1995; m/z = 414.1870 $[M + Na]^+$, calcd for $C_{21}H_{29}NO_6Na$:414.1887; ^1H NMR: δ 7.20–7.50 (m, 5H, CH_2Ph); 5.90 (d, 1H, *J* 9 Hz, NH); 4.90 (d, 1H, *J* 4 Hz, H-1); 4.75–4.40 (m, 2H, PhCH_2O); 4.20 (m, 1H, H-5); 3.90–3.60 (m, 5H, H-6, H-6′, H-4, H-3, H-2); 3.00 (s, 1H, OH); 2.00 (s, 3H, NHCOCH_3); 2.00–1.30 (m, 10H, C_6H_{10}).

3.3. Benzyl 2-acetamido-4,6-O-cyclohexylidene-2-deoxy-α-D-galactopyranoside (*6*)

Compound **6** was synthesized from benzyl 2-deoxy-2-acetamido-α-D-galactopyranoside [12] (**5**) according to the procedure described [13] in a yield of 45%. R_f 0.3 (toluene/acetone 1: 1); $[\alpha]_D^{20}$ +120.6; HRESIMS: m/z = 392.2056 $[M + H]^+$, calcd for $C_{21}H_{29}NO_6$: 391.1995; m/z = 414.1873 $[M + Na]^+$, calcd for $C_{21}H_{29}NO_6Na$: 414.1887; m/z = 430.1615 $[M + K]^+$, calcd for $C_{21}H_{29}NO_6K$: 430.1626; ^1H NMR: δ 7.50–7.20 (m, 5H, CH_2Ph); 5.80 (d, 1H, *J* 9 Hz, NH); 5.00 (d, 1H, *J* 4 Hz, H-1); 4.72–4.50 (m, 2H, PhCH_2O); 4.50–4.40 (m, 1H, H-5); 4.20 (d,1H, *J* 2 Hz, H-4); 4.10–3.70 (m, 4H, H-6, H-6′, H-3, H-2); 2.70 (s, 1H, OH); 2.00 (s, 3H, NHCOCH_3); 2.00–1.30 (m, 10H, C_6H_{10}).

3.4. Benzyl 2-acetamido-4,6-O-cyclohexylidene-2-deoxy-3-O-(2,3,4-tri-O-acetyl-α-D-rhamnopyranosyl)-α-D-glucopyranoside (*8*)

1,2,3,4-Tetra-*O*-acetyl-α-D-rhamnopyranose [10] (500 mg, 1.5 mmol) was dissolved in glacial AcOH (1 mL), cooled to 0 °C, and 5 mL of a 40% solution of HBr in glacial AcOH was added with stirring. After 2 h at rt TLC (toluene/acetone 4:1) showed the presence of one chromatographic zone (R_f 0.5) corresponding to bromide **7.** The reaction mixture was freeze-dried. The suspension of **4** (390 mg, 1 mmol), $Hg(CN)_2$ (500 mg, 2 mmol), molecular sieves 3 Å (500 mg, powder) in dry acetonitrile (2 mL) was stirred for 30 min at rt, a solution of **7** in dry acetonitrile (2 mL) was added dropwise and stirring was continued overnight. The reaction mixture was diluted with $CHCl_3$ (50 mL), filtered and washed consecutively with sat. aq. KI (50 mL) and H_2O (2 × 25 mL). The organic phase was dried with $MgSO_4$, and the solvent was evaporated. The residue was subjected to column chromatography (toluene/acetone 1: 0 → 1: 1) affording compound **8** as a foam (265 mg, 40%): R_f 0.5 (toluene/acetone 2:1); $[\alpha]_D^{20}$ +80.6°; HRESIMS: m/z = 664.2950 $[M+H]^+$, calcd for $C_{33}H_{45}NO_{13}$: 663.2891; m/z = 686.2770 $[M+Na]^+$, calcd for $C_{33}H_{45}NO_{13}Na$ 686.2783; ^1H NMR (D-Rha = II; GlcNAc = I): δ 7.40–7.30 (m, 5H, CH_2Ph); 5.64 (d, 1H, *J* 9.6 Hz, NH); 5.39 (br s, 1H, H-2^{II}); 5.19 (dd, 1H, $J_{3,2}$ 3.6 Hz, $J_{3,4}$ 9.6 Hz, H-3^{II}); 5.02 (br s, 1H, H-1^{II}), 5.02 (t, 1H, $J_{4,3}$ 9.6 Hz, $J_{4,5}$ 9.6 Hz, H-4^{II}); 4.87 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1^I); 4.71–4.44 (m, 2H, OCH_2Ph); 4.33–4.30 (m, 1H, H-2^I); 3.92–3.90 (m, 1H, H-5^{II}); 3.81–3.80 (m, 5H, H-3^I,4^I,5^I,6^I,$6^{\prime I}$); 2.12 (s, 3H, OAc); 2.04 (s, 3H, OAc); 1.95 (s, 3H, OAc); 1.94 (s, 3H, NHAc); 2.00–1.30 (m, 10H, C_6H_{10}): 1.19 (d, 3H, $J_{6,5}$ 6.6 Hz, H-6^{II}); ^{13}C NMR: δ 100.1 (quaternary carbon atom), 99.3 (C-1^{II}); 97.4 (C-1^I); 76.8 (C-3^I); 73.9 (C-5^I); 70.8 (C-4^{II}); 69.8 (CH_2Ph); 69.6 (C-2^{II}); 69.1 (C-3^{II}); 66.7 (C-5^{II}); 63.8 (C-4^I); 61.5 (C-6^I); 51.7 (C-2^I); 23.1 (NHCOCH_3); 20.7 (OCOCH_3); 17.5 (C-6^{II}).

3.5. Benzyl 2-acetamido-2-deoxy - 3-O-(2,3,4-tri-O-acetyl-α-D-rhamnopyranosy)-α-D-glucopyranoside (**11**)

Protected disaccharide **8** (250 mg, 0.38 mmol) was dissolved in 90% aqueous CF_3COOH (1 mL) and the reaction mixture was stirred at rt. After 15 min TLC (toluene/acetone 2: 1) showed complete reaction. Ethanol (10 mL) and toluene (10 mL) were added, and the reaction mixture was evaporated to dryness. The syrupy residue was subjected to column chromatography with linear gradient (toluene→acetone) affording **11** as a foam (208 mg, 95%): R_f 0.5 (toluene/acetone 1: 1).

3.6. Benzyl 2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4-tri-O-acetyl-α-D-rhamnopyranosyl)-α-D-glucopyranoside (**13**)

A solution of **11** (208 mg, 0.316 mmol) in a mixture of dry pyridine (1 mL) and Ac_2O (1 mL) was stirred overnight at room temperature. Ethanol (10 mL) and toluene (10 mL) were added and the reaction mixture was evaporated to dryness. The syrupy residue was subjected to column chromatography ($CHCl_3$ → acetone), affording **13** as a foam (235 mg, 98%): R_f 0.7 ($CHCl_3$/acetone 4: 1); $[\alpha]_D^{20}$ +160.1; HRESIMS: m/z = 668.2550 $[M+H]^+$, calcd for $C_{31}H_{41}NO_{15}$: 667.2476; m/z = 690.2349 $[M+Na]^+$, calcd for $C_{31}H_{41}NO_{15}Na$:690.2368; ^1H NMR (Rha = II, GlcNAc = I): δ 7.40–7.30 (m, 5H, CH_2Ph); 5.63 (d, 1H, *J* 9 Hz, NH); 5.16 (dd, 1H, $J_{3,2}$ 3 Hz, $J_{3,4}$ 9.6 Hz, H-3^{II}); 5.12 (dd, 1H, $J_{4,3}$ 9.6 Hz, $J_{4,5}$ 9.6 Hz, H-4^{I}); 5.03 (br s, 1H, H-2^{II}); 5.02 (t, 1H, $J_{4,3}$ 10.2 Hz, $J_{4,5}$ 10.2 Hz, H-4^{II}); 4.97 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1^{I}); 4.78 (br s, 1H, H-1^{II}); 4.69–4.47 (m, 2H, OCH_2Ph); 4.34–4.30 (m, 1H, H-2^{I}); 4.20–4.17 (m, 2H, H-6^{I}), 3.93–3.88 (m, 2H, H-5^{II}, H-5^{I}); 3.88 (t, 1H, $J_{3,4}$ 9.6 Hz, $J_{3,2}$ 9.6 Hz, H-3^{I}); 2.14–1.90 (m, 18H, OCOCH_3, NHCOCH_3); 1.17 (d, 3H, $J_{6,5}$ 6.6 Hz, H-6^{II}); ^{13}C NMR: δ 99.9 (C-1^{II}); 96.7 (C-1^{I}); 80.4 (C-3^{I}); 70.8 (C-4^{II}); 70.1 (C-2^{II}); 70.1 (CH_2Ph); 70.0 (C-4^{I}); 68.6 (C-3^{II}); 68.3 (C-5^{I}); 67.2 (C-5^{II}); 62.1 (C-6^{I}); 52.2 (C-2^{I}); 23.4 (NHCOCH_3); 20.6 (OCOCH_3); 17.7 (C-6^{II}).

3.7. 2-Acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4-tri-O-acetyl-α-D-rhamnopyranosyl)-α-D-glucopyranose (**15**)

A solution of **13** (235 mg, 0.35 mmol) in ethanol (10 mL) was hydrogenated in the presence of 10% $Pd(OH)_2/C$ (50 mg) at 36 °C with stirring until the benzyl group was completely removed (TLC: $CHCl_3$/acetone 8: 2). The catalyst was separated by centrifugation at 10,000 rpm, and the supernatant was evaporated to dryness affording compound **15** (180 mg, 90%): R_f 0.1 ($CHCl_3$/acetone 4: 1); $[\alpha]_D^{20}$ +40.5; HRESIMS: m/z = 578.2079 $[M+H]^+$, calcd for $C_{24}H_{35}NO_{15}$: 577.2007; m/z = 600.1898 $[M+Na]^+$, calcd for $C_{24}H_{35}NO_{15}Na$: 600.1899. The product was used in the next step of the synthesis without purification.

3.8. 2-Acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4-tri-O-acetyl-α-D-rhamnopyranosyl)-α-D-glucopyranosyl dibenzyl phosphate (**17**)

To a stirred solution of **15** (180 mg, 0.31 mmol) and tetrazole (230 mg, 3.0 mmol) in dry CH_2Cl_2 (5 mL) under an argon atmosphere and −60 °C bis (benzyloxy)(diisopropylamino)phosphine [11] (640 mg, 2 mmol) was added dropwise with stirring. The solution was allowed to warm to rt and stirred for 5 h under argon atmosphere. The reaction mixture was cooled to −60 °C, mCPBA (400 mg, 2 mmol) was added with stirring followed by warming to rt. After 18 h of stirring the solution was diluted with $CHCl_3$ (100 mL) and washed with sat. aq. $Na_2S_2O_3$ (50 mL), sat. aq. $NaHCO_3$, and water. The organic phase was concentrated. The residue was subjected to column chromatography (toluene/EtOAc 1: 1 → 0: 1) affording **17** (220 mg, 85%): R_f 0.5 (EtOAc); $[\alpha]_D^{20}$ +44.4; HRESIMS: m/z = 838.2658 $[M+H]^+$, calcd for $C_{38}H_{48}NO_{18}P$: 837.2609; m/z = 860.2488 $[M+Na]^+$, calcd for $C_{38}H_{48}NO_{18}PNa$: 860.2501; ^1H NMR (Rha = II, GlcNAc = I): δ 7.40–7.30 (m, 5H, CH_2Ph); 5.69 (dd, 1H, $J_{1,2}$ 3.0 Hz, $J_{1,P}$ 5.4 Hz, H-1^{I}); 5.64 (d, 1H, *J* 9 Hz, NH); 5.15–5.07 (m, 8H, H-2^{II}, 3^{II}, 4^{II}, 4^{I}, OCH_2Ph); 4.74 (br s, 1H, H-1^{II}); 4.33–4.30 (m, 1H, H-2^{I}); 4.10–4.07 (m, 1H, H-6^{I}); 3.93–3.85 (m, 3H, H-5^{II}, 5^{I}, $6'^{I}$); 3.67 (t, 1H, $J_{3,4}$ 10.2 Hz, $J_{2,3}$ 10.2 Hz, H-3^{I}); 2.15–1.75 (m, 18H, COCH_3, NHCOCH_3); 1.16 (d, 3H, $J_{6,5}$ 6.6 Hz, H-6^{II}); ^{13}C NMR: δ 100.5 (C-1^{II}); 96.6 (d, $J_{1,P}$ 6.8 Hz, C-1^{I}); 79.0 (C-3^{I}); 61.5 (C-6^{I}); 52.2 (d, $J_{2,P}$ 7.1 Hz, C-2^{I}); 23.0 (NHCOCH_3); 20.5 (OCOCH_3); 17.6 (C-6^{II}); ^{31}P NMR: −2.4 (s).

3.9. 2-Acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-α-D-rhamnopyranosyl)-α-D-glucopyranosyl dihydrogen phosphate (**19**)

A solution of **17** (130 mg, 0.155 mmol) in glacial AcOH (5 mL) was hydrogenated in the presence of 10% $Pd(OH)_2/C$ (50 mg) at 36 °C with shaking until the benzyl groups were completely removed (TLC: EtOAc). The catalyst was separated by centrifugation at 10,000 rpm and the supernatant was freeze-dried. Removal of benzyl group decreases R_f (EtOAc) value of compound to 0, the starting material disappears affording compound **15** as a white powder (180 mg, 90%): R_f 0; HRESIMS: m/z = 658.1755 $[M+H]^+$, calcd for $C_{24}H_{36}NO_{18}P$: 657.1670; m/z = 680.1567 $[M+Na]^+$, calcd for $C_{24}H_{36}NO_{18}PNa^+$: 680.1562; ^{31}P NMR: −1.4 (br s). The product was used at the next step of the synthesis without additional purification.

3.10. Sodium P^1-(11-phenoxyundecyl)-P^2-(2-acetamido-2-deoxy-3-O-α-D-rhamnopyranosyl-α-D-glucopyranosyl) diphosphate (**1**)

Dry diisopropylamine (0.3 mL) was added to a solution of 11-phenoxyundecyl dihydrogen phosphate [2] (**21**, 76 mg, 0.22 mmol) in dry benzene (4 mL). The mixture was stirred under argon atmosphere for 5 min and then freeze-dried. The resulting white powder was dissolved in dry THF (6 mL), and CDI (270 mg, 1.66 mmol) was added. The reaction mixture was stirred at rt under argon atmosphere. After 2 h TLC ($CHCl_3$/MeOH/H_2O 6:4:0.5) showed total conversion of **21** (R_f 0.5) into intermediate phosphoroimidazolidate **22** (R_f 0.85). Dry MeOH (1 mL) was added and the mixture was stirred for 30 min at rt. The solvent was removed in vacuum, the residue was dissolved in dry benzene (6 mL) and freeze-dried affording crude 11-phenoxyundecyl phosphoroimidazolidate **22**.

A mixture of glycosyl dihydrogen phosphate **19** (92 mg, 0.14 mmol), dry benzene (6 mL) and dry diisopropylamine (0.3 mL) was stirred under argon atmosphere and freeze-dried. Solution of crude phosphoroimidazolidate **22** in dry THF (3 mL) was added to the lyophilizate with stirring under argon atmosphere. The reaction mixture was left at 37 °C for 48 h, the solvent was evaporated under reduced pressure, the oily residue was dissolved in MeOH (3 mL) and 1 M solution of MeONa in dry methanol (200 μL) was added. After stirring for 1 h at rt glacial AcOH (0.6 mL) was added, the solution was stirred for 15 min and evaporated to dryness in vacuum. The solid residue was dissolved in water (40 mL), applied on C18 Sep-Pak cartridge preactivated with EtOH and elution was carried out from water (40 mL) to 5, 10, 15, 20 and 30% v/v solution of EtOH in water (20 mL of each); the desired product was eluted with 10 and 15% solution of EtOH in water. The combined fractions were evaporated under reduced pressure, the residue was dissolved in 5 mL of water, and the solution was freeze-dried affording **1** as a white powder (68 mg, 60%): R_f 0.65 (CH_3Cl/MeOH/H_2O 10:10:3); $[\alpha]_D^{20}$ +62.0; HRESIMS: m/z = 772.2691 $[M-H]^-$, calcd for

$C_{31}H_{52}NO_{17}P_2$: 772.2716; ^{1}H NMR (Rha = II, GlcNAc = I): δ 8.85 (s, 1H, H-2 imidazole salt); 7.52 (s, 2H, H4, H-5 imidazole salt) 7.25–7.22 (m, 2H, PhO); 6.90–6.85 (m, 3H, PhO); 5.51 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{1,P}$ 6.6 Hz, H-1^I); 5.13 (bs, 1H, H-1^{II}); 4.10–4.05 (m, 1H, H-2^I); 4.02–3.98 (m, 2H, CH_2OP); 4.00–3.90 (m, 4H, H-2^{II}, 5^I, PhOCH_2); 3.83–3.76 (m, 2H, H-6^I, 3^I); 3.70–3.60 (m, 3H, H-6′^{I}, 3^{II}, 5^{II}); 3.46 (t, 1H, $J_{4,3}$ 10.2 Hz, $J_{4,5}$ 10.2 Hz, H-4^I); 3.34 (t, 1H, $J_{4,3}$ 10.2 Hz, $J_{4,5}$ 10.2 Hz, H-4^{II}), 2.05 (s, 3H, NHCOCH_3), 1.80–1.30 (m, 18H, CH_2); 1.27 (d, 3H, $J_{5,6}$ 6 Hz, H-6^{II}); ^{13}C NMR: δ 174.1 (CO); 135.7 (C-2, imidazolium salt); 130.5 (C-4, C-5, imidazolium salt); 121.6, 120.6, 115.7 (PhO); 103.2 (C-1^{II}); 96.60 (d, $J_{1,P}$ 6.3 Hz, C-1^I); 79.8 (C-3^I); 75.0 (C-5^I); 74.2 (C-4^{II}); 72.9 (C-4^I); 72.6 (C-3^{II}); 72.4 (C-2^{II}); 70.3 (C-5^{II}); 69.1 (PhOCH_2); 67.4 (d, $J_{P,C}$ 6 Hz, CH_2OP); 62.6 (C-6^I); 53.9 (d, $J_{2,P}$ 8.5 Hz, C-2^I); 32.0 (d, $J_{C,P2}$ 5.8 Hz, CH_2CH_2OP); 30.85, 30.83, 30.39,30.64, 30.58, 27.31, 27.04 (CH_2); 23.4 (NHCOCH_3), 18.3 (C-6^{II}); ^{31}P NMR: δ −9.9 (d, $J_{P2,P1}$ 20.2 Hz, P-2); −12.4 (d, $J_{P1,P2}$ 20.2 Hz, P-1).

3.11. Benzyl 2-acetamido-4,6-O-cyclohexylidene-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside (**10**)

A mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**9**) [14] (620 mg, 1.5 mmol), **4** (490 mg, 1.25 mmol), $Hg(CN)_2$ (311 mg, 1.25 mmol), molecular sieves 3 Å (100 mg) and acetonitrile (15 mL) was stirred overnight at room temperature. The reaction mixture was diluted with $CHCl_3$ (50 mL), the precipitate was filtered off and the filtrate was washed with sat. aq. KI (50 mL) and H_2O (2 × 25 ml). The organic phase was dried ($MgSO_4$) and the solvent was evaporated. The residue was subjected to column chromatography (toluene → EtOAc) affording **10** as a foam (380 mg, 42%): R_f 0.7 (toluene/EtOAc 2:1); $[\alpha]_D^{20}$ +88.2; HRESIMS: m/z = 722.3018 $[M+H]^+$, calcd for $C_{35}H_{47}NO_{15}$: 721.2946; m/z = 744.2836 $[M+Na]^+$, calcd for $C_{35}H_{47}NO_{15}Na^+$:744.2843, m/z = 760.2577 $[M+K]^+$, calcd for $C_{35}H_{47}NO_{15}K^+$:760.2583; ^{1}H NMR (Gal = II, GalNAc = I): δ 7.50–7.20 (m, 5H, CH_2Ph); 5.55 (d, 1H, *J* 9 Hz, NH); 5.36 (d, 1H, $J_{3,4}$ 3.6 Hz, H-4^{II}); 5.15 (dd, 1H, $J_{1,2}$ 8.4 Hz, $J_{2,3}$ 10.2 Hz, H-2^{II}); 5.10 (d, 1H, $J_{1,2}$ 4 Hz, H-1^I); 4.98 (dd, 1H, $J_{2,3}$ 10.2, $J_{3,4}$ 3.6 Hz, H-3^{II}); 4.71 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1^{II}); 4.71–4.50 (m, 2H, OCH_2Ph); 4.27 (bd, 1H, $J_{3,4}$ 3 Hz, H-4^I); 3.90–3.80 (m, 2H, H-3^{II}, 5^{II}); 2.20–1.90 (m, 15H, OCOCH_3, NHCOCH_3), 2.00–1.30 (m, 10H, C_6H_{10}).

3.12. Benzyl 2-acetamido-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside (**12**)

Compound **10** (300 mg, 0.42 mmol) was treated with 90% CF_3COOH as described for synthesis of **11** affording diol **12** as a foam (250 mg, 93.6%): R_f 0.7 (toluene/ethyl acetate 2:1); $[\alpha]_D^{20}$ +65.2; HRESIMS: m/z = 642.2398 $[M+H]^+$, calcd for $C_{29}H_{39}NO_{15}$: 641.2320; m/z = 664.2225 $[M+Na]^+$, calcd for $C_{29}H_{39}NO_{15}Na$: 664.2217.

3.13. Benzyl 2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranoside (**14**)

A solution of **12** (200 mg, 0.31 mmol) in a mixture of dry pyridine (1 mL) and Ac_2O (1 mL) was stirred overnight at rt, ethanol (10 mL) and toluene (10 mL) was added and the reaction mixture was evaporated to dryness. The syrupy residue was subjected to column chromatography (toluene → acetone) affording **14** as a foam (220 mg, 98%): R_f 0.5 (toluene/EtOAc 2:1); $[\alpha]_D^{20}$ +85.3; HRESIMS: m/z = 726.2613 $[M+H]^+$, calcd for $C_{33}H_{43}NO_{17}$: 725.2531; m/z = 748.2425 $[M+Na]^+$, calcd for $C_{33}H_{43}NO_{17}Na$: 748.2429; ^{1}H NMR (Gal = II, GalNAc = I): δ 7.40–7.30 (m, 5H, CH_2Ph); 5.64 (d, 1H, *J* 9.0 Hz, NH); 5.38 (bd, 1H, $J_{3,4}$ 3.0 Hz, H-4^I); 5.34 (bd, 1H, $J_{3,4}$ 3,6 Hz, H-4^{II}); 5.12 (dd, 1H, $J_{2,1}$ 8.40 Hz, $J_{2,3}$ 10.2 Hz, H-2^{II}); 5.04 (d, 1H, $J_{1,2}$ 4.2 Hz, H-1^I); 4.95 (dd, 1H, $J_{3,4}$ 3.6 Hz, $J_{2,3}$ 10.2 Hz, H-3^{II}); 4.58 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1^{II}); 4.55–4.51 (m, 1H, H-2^I); 4.20–4.10 (m, 3H, H-5^I, H-6′^{I}, H-6^{II}); 4.04–4.01 (m, 1H, H-6^I); 4.00 (dd, 1H, $J_{3,4}$ 3.0 Hz, $J_{3,2}$ 11.4 Hz, H-3^I); 3.86–3.85 (m, 1H, H-5^{II}); 2.14–1.96 (m, 21H, COCH_3, NHCOCH_3); ^{13}C NMR: δ 100.5 (C-1^{II}), 97.2 (C-1^I), 72.8 (C-3^I), 71.0 (C-5^I), 70.9 (C-3^{II}), 70.8 (CH_2Ph), 68.8 (C-4^I), 68.5 (C-2^{II}), 67.6 (C-5^I), 66.8 (C-4^{II}), 62.9 (C-6^I), 61.1 (C-6^{II}), 48.5 (C-2^I), 23.3 (NHCOCH_3), 20.5 (OCOCH_3).

3.14. 2-Acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranose (**16**)

Compound **14** (200 mg, 0.28 mmol) was hydrogenated as described for synthesis of **15** affording compound **16** (160 mg, 90%): R_f 0.05 (toluene/EtOAc 2:1); $[\alpha]_D^{20}$ +45.3; HRESIMS: m/z = 636.2143 $[M+H]^+$, calcd for $C_{26}H_{37}NO_{17}$: 635.2061; m/z = 658.1963 $[M+Na]^+$, calcd for $C_{26}H_{37}NO_{17}Na$: 658.1959. The compound was used without further purification.

3.15. 2-Acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl dibenzyl phosphate(**18**)

Treatment of **16** (95 mg, 0.15 mmol) with bis(benzyloxy)(diisopropylamino)phosphine [11] (320 mg, 1 mmol) was conducted analogously to the synthesis of **17** affording compound **18** as a foam (115 mg, 85%): R_f 0.5 (EtOAc), $[\alpha]_D^{20}$ +53.3; HRESIMS: m/z = 896.2739 $[M+H]^+$, calcd for $C_{40}H_{50}NO_{20}P$: 895.2664; m/z = 918.2565 $[M+Na]^+$, calcd for $C_{40}H_{50}NO_{20}PNa$: 918.2561; ^{1}H NMR (Gal = A II, GalNAc = B I): δ 7.40–7.30 (m, 5H, CH_2Ph); 5.79 (d, 1H, *J* 9.0 Hz, NH); 5.75 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{1,P}$ 6.0 Hz, H-1^I); 5.40 (br d, 1H, $J_{3,4}$ 3.0 Hz, H-4^I), 5.36 (br d, 1H, $J_{3,4}$ 3,6 Hz, H-4^{II}); 5.10 (dd, 1H, $J_{1,2}$ 8.4 Hz, $J_{2,3}$ 10.2 Hz, H-2^{II}); 5.09–5.01 (m, 4H, OCH_2Ph); 4.96 (dd, 1H, $J_{3,4}$ 3.6 Hz, $J_{2,3}$ 10.2 Hz, H-3^{II}), 4.58 (d, 1H, $J_{1,2}$ 8.4 Hz, H-1^{II}); 4.57–4.52 (m, 1H, H-2^I), 4.25 (t, 1H, H-5^I); 4.16–4.09 (m, 4H, H-6^{II}, 6^I); 3.93–3.88 (m, 2H, H-5^{II},3^I); 2.15–1.80 (m, 21H, OCOCH_3, NHCOCH_3); ^{13}C NMR: δ 170.5–169.6; 128.7–128,2 (aromatic); 100.5 (C-1^{II}); 97.5 (d, $J_{1,P}$ 6.8 Hz, C-1^I); 71.6 (C-3^I); 71.0 (C-5^{II}); 70.8 (C-3^{II}); 66.8 (C-4^{II}); 62.1 (C-6^I); 61.2 (C-6^{II}); 48.7 (d, $J_{P,2}$7Hz, C-2^I); 22.8 (NHCOCH$_3$); 20.5 (OCOCH$_3$); ^{31}P NMR: −2.4 (s).

3.16. 2-Acetamido-4,6-di-O-acetyl-2-deoxy-3-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-galactopyranosyl phosphate (**20**)

A solution of **18** (50 mg, 0.056 mmol) in glacial AcOH (5 mL) was hydrogenated with 10% $Pd(OH)_2$/C (10 mg) as described for **17** affording dihydrogen phosphate **20** as a white powder (36 mg, 90%): R_f 0 (EtOAc); ^{31}P NMR: −1.5 (br s). The compound was used without further purification.

3.17. Sodium P^1-(11-phenoxyundecyl)-P^2-(2-acetamido-2-deoxy-3-O-β-D-galactopyranosyl-α-D-galactopyranosyl) diphosphate (**2**)

Diphosphate **2** was prepared similarly to the procedure described for the synthesis of diphosphate **1** from 11-phenoxyundecyl dihydrogen phosphate [2] (28 mg, 0.08 mmol), CDI (100 mg, 0.61 mmol) and freshly made glycosyl dihydrogen phosphate **20** (36 mg, 0.05 mmol). The target compound was eluted from C18 Sep-Pak cartridge with 10, 15 and 20% of EtOH. Fractions containing the target compound were concentrated and freeze-dried affording **2** as a white powder (25 mg, 59%): R_f 0.6 (CH_3Cl/MeOH/H_2O 10:10:3), $[\alpha]_D^{20}$ +68.5; HRESIMS: m/z = 812.2618 $[M+Na]^+$, calcd for $C_{31}H_{52}NNaO_{18}P_2$: 811.2557; m/z = 834.2448 $[M+2Na]^+$, calcd for $C_{31}H_{52}NNa_2O_{18}P_2$: 834.2450; ^{1}H NMR (Gal = II, GalNAc = I): δ 8.83 (s, 1H, H-2 imidazole salt); 7.51 (s, 2H, H4, H-5

imidazole salt) 7.25–7.22 (m, 2H, PhO); 6.89–6.87 (m, 3H, PhO); 5.60 (dd, 1H, $J_{1,2}$ 3.6 Hz, $J_{1,P}$ 6.6 Hz, H-1^{I}); 4.55–4.52 (m, 1H, H-2^{I}); 4.42 (d, 1H, $J_{1,2}$ 7.2 Hz, H-1^{II}); 4.20–4.16 (m, 1H, H-5^{I}); 4.17 (br d, 1H, $J_{3,4}$ 2.4 Hz, H-4^{I}); 3.99–3.94 (m, 4H, CH_2OP, PhOCH_2), 3.85 (br d, 1H, $J_{3,4}$ 3,0 Hz, H-4^{II}); 3.55–3.54 (m, 1H, H-5^{II}); 2.03 (s, 3H, NHCOCH_3); 1.78–1.28 (m, 18H, CH_2); ^{13}C NMR: δ 135.8 (C-2, imidazolium salt); 130.5 (C-4, C-5, imidazolium salt); 121.6, 120.7, 115.7 (PhO); 106.7 (C-1^{II}), 96.9 (d, $J_{1,P}$ 5.9 Hz, C-1^{I}); 79.3 (C-3^{I}); 76.8 (C-5^{II}); 74.9 (C-5^{I}); 73.5 (C-2^{II}); 70.5, 70.4 (C-4^{II}, 4^{I}); 69.0 (PhOCH_2); 67.4 (d, *J* 6.2 Hz, CH_2OP); 63.0 (C-6^{I}); 62.6 (C-6^{II}); 50.2 (d, $J_{2,P}$ 7.8 Hz, C-2^{I}); 32.0; 30.9; 30.8; 30.7; 30.5; 27.3; 27.1; 19.6 (CH_2); 22.8 (NHCOCH_3); ^{31}P NMR: δ −10.1 (d, $J_{P1,P2}$ 20.2 Hz, P-2), −12.4 (d, $J_{P1,P2}$ 20.2 Hz, P-1).

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References

[1] P. Montoya-Peleaz, J.G. Riley, W.A. Szarek, M. Valvano, J.S. Schutzbach, I. Brockhausen, Identification of a UDP-Gal: GlcNAc-R galactosyltransferase activity in *Escherichia coli* VW187, Bioorg. Med. Chem. Lett. 15 (2005) 1205–1211.

[2] T.N. Druzhinina, L.L. Danilov, V.I. Torgov, N.S. Utkina, N.M. Balagurova, V.V. Veselovsky, A.O. Chizhov, 11-Phenoxyundecyl phosphate as a 2-acetamido-2-deoxy-α-D-glucopyranosyl phosphate acceptor in O-antigen repeating unit assembly of *Salmonella arizonae* O:59, Carbohydr. Res. 345 (2010) 2636–2640.

[3] N.S. Utkina, L.L. Danilov, T.N. Druzhinina, V.V. Veselovsky, Simple synthesis of P^1-(11-phenoxyundecyl)-P^2-(2-acetamido-2-deoxy- α-*D*-galactopyranosyl) diphosphate, Russ. J. Bioorg. Chem. 36 (2010) 783–785.

[4] S. Wang, Y. Hao, J.S. Lam, J.Z. Vlahakis, W.A. Szarek, A. Vinnikova, V.V. Veselovsky, I. Brockhausen, Biosynthesis of the common polysaccharide antigen of *Pseudomonas aeruginosa* PAO1: characterization and role of WbpZ – a GDP-d-rhamnose: GlcNAc/GalNAc-diphosphate-lipid α1,3-D-rhamnosyltransferase WbpZ, J. Bacteriol. 197 (2015) 2012–2019.

[5] S. Wang, D. Czuchry, B. Liu, A. Vinnikova, Y. Gao, J.Z. Vlahakis, W.A. Szarek, L. Feng, L. Wang, I. Brockhausen, Characterization of two UDP-Gal: GalNAc-diphosphate-lipid β1,3-galactosyltransferases WbwC from *Escherichia coli* serotypes O104 and O5, J. Bacteriol. 196 (2014) 3122–3133.

[6] N.S. Utkina, L.L. Danilov, V.V. Veselovsky, V.I. Torgov, T.N. Druzhinina, Synthesis of P^1-(11-phenoxyundecyl)-P^2-(α-D-galactopyranosyl)diphosphate and P^1-(11- phenoxyundecyl)-P^2-(α-D-glucopyranosyl)diphosphate and investigation of their acceptor properties in the reaction of mannosyl residue transfer catalyzed by mannosyltransferase from *Salmonella nawport*, Russ. J. Bioorg. Chem. 4 (2012) 412–416.

[7] Y. Gao, B. Liu, S. Strum, J.S. Schutzbach, T.N. Druzhinina, N.S. Utkina, V.I. Torgov, L.L. Danilov, V.V. Veselovsky, J.Z. Vlahakis, W.A. Szarek, L. Wang, I. Brockhausen, Biochemical characterization of WbdN, a β 1,3-glucosyltransferase involved in O-antigen synthesis in enterohemorrhagic *Escherichia coli* O157, Glycobiology 22 (2012) 1092–1102.

[8] B. Helferich, J. Zirner, Zur Synthese von Tetraacetyl-hexosen mit freiem 2-Hydroxyl. Synthese einiger Disaccharide, Chem. Ber. 95 (1962) 2604–2611.

[9] D. Czuchry, P. Desormeaux, M. Stuart, D. Jarvis, K.L. Matta, W.A. Szarek, I. Brockhausen, Identification and biochemical characterization of the novel α2,3-sialyltransferase WbwA from pathogenic *Escherichia coli* serotype O104, J. Bacteriol. 197 (2015) 3760–3768.

[10] T. Nishio, Y. Miyake, K. Kubota, M. Yamai, S. Miki, T. Ito, T. Oku, Synthesis of the 4-, 6-deoxy, and 4,6-dideoxy derivatives of d-mannose, Carbohydr. Res. 280 (1996) 357–363.

[11] W. Bannwarth, A. Trzeciak, Simple and effective chemical phosphorylation procedure for biomolecules, Helvetica Chim. Acta 70 (1987) 175–186.

[12] S.K. Mamidyala, S. Dutta, B.A. Chrunik, C. Preville, H. Wang, Glycomimetic ligands for the human asialoglycoprotein receptor, J. A. C. S. 134 (2012) 1978–1981.

[13] P.I. Abronina, A.I. Zinin, D.A. Romashin, N.N. Malysheva, A.O. Chizhov, L.O. Kononov, Novel benzyl-free glycosyl donors for highly stereoselective 1,2-*cis*-fucosylation, Synlett 26 (2015) 2267–2271.

[14] H. Ohle, W. Maracek, W. Bourjau, Über Schwefelsäure-Verbindungen der Zucker, II. Mittel.: eine Reaktion zur Unterscheidung ring-isomerer acylierter Halogen-zucker, Chem. Ber. 62 (1929) 833–854.