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Artificial *N*-functionalized UDP-glucosamine analogues as modified substrates for *N*-acetylglucosaminyl transferases

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Dedicated to Professor András Lipták on the occasion of his 70th birthday

Abstract—Analogues of UDP-GlcNAc modified at the 2-acetamido group of the GlcNAc moiety were prepared in order to study their role in the mechanism of *N*-acetylglucosaminyl transferase mediated glycosylation reactions. The structural analogues with *N*-formyl-, *N*-propionyl-, *N*-butyryl- and *N*-isobutyryl-groups were synthesized, utilizing the morpholidate coupling method starting from D-glucosaminyl-1-phosphate after selective N-acylation of its amino group with the appropriate *N*-acyloxysuccinimide esters as well as a chlorinated formylformiate.

Keywords: GlcNAc-1-phosphate; Acylamidoglucose; UDP-glucosamine analogues; Morpholidate coupling; Carbohydrate-binding proteins

1. Introduction

Approaches for efficient syntheses of carbohydrate building blocks up to complex oligosaccharides still receive increasing attention. In addition to naturally occurring carbohydrate structures displayed on cell surfaces and involved in molecular recognition processes mediated by binding events[1–5] unnatural oligosaccharide target compounds became the goal of both chemical and enzymatic approaches.[6–13] Some of these compounds show potential as drug candidates since they interfere in cellular recognition either by modulation or inhibition and give rise to more effective artificial analogues than their natural substrates. A promising and still developing approach besides classical linear and convergent glycosylation paths involves the use of transferases that employ nucleoside diphosphohexoses (NDP hexoses) as activated carbohydrate donors.[14–22] Such transferases of the Leloir pathway display high stereospecificity and regioselectivity in directing an activated carbohydrate donor to a specific position of a recognized acceptor, eliminating the need for numerous protection and deprotection steps. However, this methodology suffers in the sense that these enzymes preclude the use of alternative carbohydrate donors that differ significantly in structure from their naturally occurring counterparts, making enzymatic synthesis of unnatural oligosaccharides a still challenging and difficult endeavour. By use of their naturally occurring glycosylation donor substrate *N*-acetylhexosaminyl transferases take part in the stepwise glycosylation towards glycoconjugates, among them sphingolipids and gangliosides, regulating the essential lipid metabolism in human nervous system cells. A number of disease phenotypes, based on deficient degradation of specific gangliosides are known, including the many well characterized hydrolases taking part in this process.[23] Their counter parts, the ganglioside generating and membrane bound transferases are so far less well investigated since they are not applicable to common methods such as X-ray crystallography and require alternative investigative techniques based on affinity labelling and enzyme–substrate interaction studies. Modified structural analogues of UDP-GlcNAc applicable for this purpose are described in this contribution.

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Natural and unnatural NDP-sugars have been synthesized by the classical method[24] and many more recent variations such as by Wittmann and Wong.[25] In our previous work we reported the syntheses of several structural analogues of UDP-GalNAc, displaying 2-amino-2-deoxy, 2-azido-2-deoxy, *N*-bromoacetyl, *N*-propionyl and *N*-butyryl functionalities by N-acylation of D-galactosaminyl-1-phosphate, in the latter three cases with corresponding *N*-hydroxysuccinimide esters with high *N*-selectivity and good yields.[26] This conception was transferred to D-glucosaminyl-1-phosphate giving rise to *N*-acylamidoglucosyl phosphates all of which were converted to uridine glucosaminyl nucleotides under morpholidate coupling conditions.

Recently in the UDP-GlcNAc series, UDP-*N*-trifluoracetylglucosamine[27] and 2-azido analogue of UDP-GlcNAc were prepared.[28] Attempts for their further processing by UDP-GlcNAc transferases showed success for the N-trifluoracetylated UDP derivative. However, neither the corresponding non-acylated UDP-2-aminoglucose nor the UDP-2-azidoglucose sugar nucleotides proved to be transferable by transferases. This indicates the presence of an acylamido group to be critical since it serves as a necessary recognition element in order to be processed by GlcNAc transferases. UDP-*N*-Formamido-, UDP-*N*-propionylamido-, UDP-*N*-butyramido- and UDP-*N*-isobutyramidoglucose, described in this paper are therefore expected to be potentially transferable in transferase reactions to give oligosaccharide analogues with potential bioactive properties.

2. Results and discussion

2.1. Syntheses of phosphates

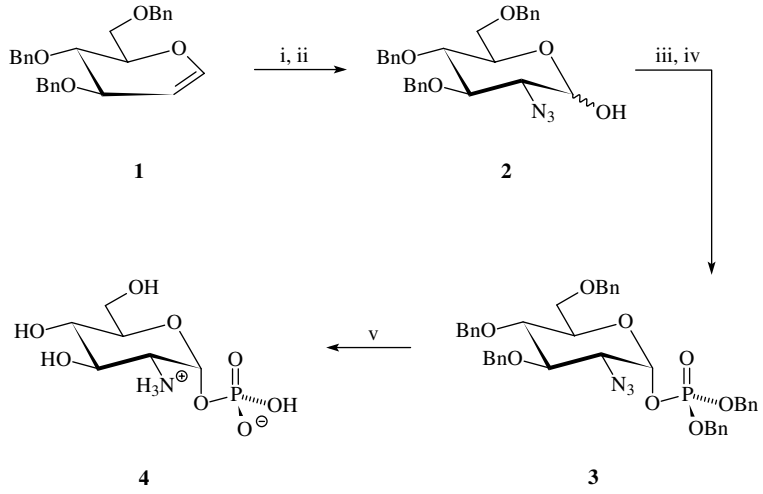
The selective N-acylation of unprotected aminosugars with esters derived from *N*-hydroxysuccinimide as activated acylating agents has proven to be a versatile and general approach to acylamidosugars, since such esters display moderate reactivity making them ideal reactants for N-nucleophiles. A wide range of acyl groups can be introduced and many other functionalities such as labile phosphate groups at the anomeric position within the aminosugar are compatible to this reaction as we have shown in our recent work on the synthesis of selectively N-acylated galactosamines.[26]

For the syntheses of *N*-acylamidoglucopyranosyl phosphates and further towards their corresponding uridine diphospho derivatives D-glucosaminyl-1-phosphate (**4**) was chosen as the building block and proved to be a suitable common starting material for the structural analogues of UDP-GlcNAc reported in this paper. The synthesis of D-glucosaminyl-1-phosphate (**4**) started from perbenzylated D-glucal (**1**) and followed a pathway via azidonitration.[29,30] After regioselective introduction of the azide in equatorial manner into the 2-position the 2-azidoglucopyranosyl nitrate obtained was subjected to reductive hydrolysis yielding the benzylated 2-azidoglucose **2** after treatment with sodium nitrite in an aqueous dioxane solution. The following stereoselective phosphorylation was carried out employing dibenzyl-*N*,*N*′-di-*iso*-propylphosphoramidite in the presence of 1*H*-tetrazole as catalyst via an initially formed phosphate. This was oxidized with *meta*-chloroperbenzoic acid to the protected α-phosphate **3** in a one-pot reaction, in which no formation of the corresponding β-phosphate was observed. Complete debenzylation and reduction of the azide functionality was carried out by hydrogenolysis using 10% palladium on activated charcoal affording glucosaminyl-1-phosphate (**4**) in one step, which turned out to be the most critical of the whole synthetic protocol regarding yield and completion of deprotection. The same conditions [50 bar H_2 atmosphere, solvent system ethylacetate, methanol, water (1:2:1) and excessive amount of palladium catalyst] previously reported by us for the synthesis of galactosaminyl-1-phosphate by a similar synthetic route were applied leading to the unprotected amine **4** in 67% yield (Scheme 1).

The subsequent selective N-acylation of the aminophosphate **4** utilizing *N*-propionyl-oxysuccinimide, *N*-butyroxysuccinimide and *N*-isobutyrosuccinimide, respectively, gave the 2-acylamidophosphates **6**–**8** in yields ranging from 80% to 85% at pH 7.0 in a solvent system of THF and water (1:10) dissolving both the esters and the highly polar aminophosphate **4**.

No hydrolysis of the phosphate group and no side products arising from O-acylation at the unprotected 3-, 4- and 6-positions could be monitored as expected. The appropriate *N*-acyloxysuccinimide esters were easily prepared by reacting *N*-hydroxysuccinimide with either propionyl chloride, butyryl chloride and pivaloyl chloride in the presence of triethylamine followed by column chromatographic purification or recrystallization. N-Formylation of the aminophosphate **4** was carried out by use of 2,4,5-trichlorophenylformiate[31] as formylating reagent under improved conditions as described for the preparation of the phosphates **6**–**8**, thus leading to the *N*-formamido modified aminoglucosyl-1-phosphate **5** in 78% yield after Biogel P2 purification procedures and lyophilization in a DIPEA mediated transesterification step. Foregoing attempts to prepare the N-formylated glucosaminyl-1-phosphate by use of the more common formylating reagents such as formyl acetate or *p*-nitrophenyl formiate were unsuccessful or led to unsatisfactory yields and side products.

The acylamido phosphates **5**–**8** were isolated as ammonium salts due to size exclusion chromatography with 250 mM ammonium hydrogencarbonate solution and were converted to triethylammonium salts by ion



Scheme 1. Reagents and conditions: (i) $Ce^{IV}(NH_4)_2(NO_3)_6$, NaN_3, MeCN, −25 °C, 1 day; (ii) $NaNO_2$, dioxane, water, 80 °C, 1 day; (iii) $(^iPr)_2NP(OBn)_2$, 1*H*-tetrazole, CH_2Cl_2, 5 h; (iv) *m*CPBA, CH_2Cl_2, 0 °C, 1 h, **3**: 58%; (v) H_2, Pd/C (10%), EtOAc/MeOH/water (1:2:1), 65 bar, rt, 2 d, **4**: 67%.

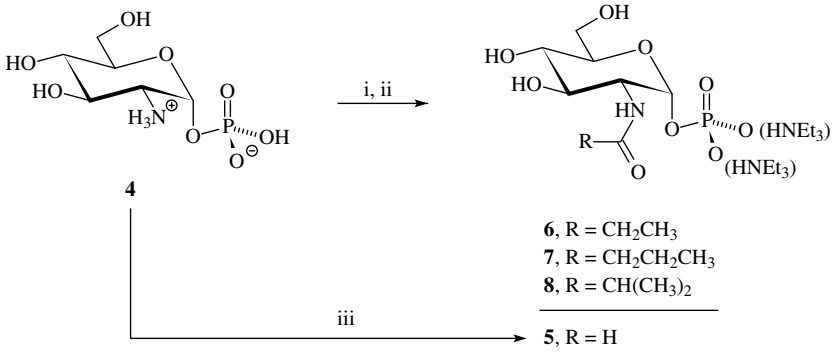
exchange chromatography for solubility reasons in the subsequent nucleotide coupling with UMP morpholidate (Scheme 2).

^1H, ^{13}C and ^{31}P NMR data for the unequivocal structural assignments of the phosphates **5**–**8** in the form of their ammonium salts are summarized in the upper half of Tables 1 and 2.

By mass spectrometric investigation of the azidophosphate **3** an unusual fragmentation pattern by Maldi-TOF analysis was observed. In addition to the expected mass peaks 774 $[M+K]^+$ and 758 $[M+Na]^+$ further masses at 746 $[M-N_2+K]^+$, 730 $[M-N_2+Na]^+$, 459 $[M-O_2P(OBn)_2]^+$ and 430 $[M-O_2P(OBn)_2-N_2]^+$ appeared. Very likely the latter four correspond to cationic species arising from **3** upon loss of either nitrogen or a dibenzylphosphate group as well as both functionalities. Thus a fragmentation path either by an initial rearrangement of a nitrene via an imine and subsequent dephosphorylation to an imino-carboxonium ion or vice versa by initial phosphate loss via an aziridinium ion following rearrangement towards an aziridino-carboxonium ion may be concluded (Scheme 3).

2.2. Syntheses of UDP glucosamines

The syntheses of the UDP-*N*-acylamidoglucoses **9**–**12** from their triethylammonium phosphate precursors **5**–**8** were performed under modified Khorana conditions[24–28] with the 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt of uridine monophospho-morpholidate as the activated UMP source under argon atmosphere in a solution of anhydrous DMF/anhydrous pyridine (∼2:1) at room temperature. The coupling reactions were worked up after 5 days by evaporation of the organic solvents without heating and subsequent freeze drying, followed by size exclusion chromatography and subsequent desalting on Biogel P2. The modified unnatural UDP glucosamines were thus obtained in a yield range around 30% as colourless ammonium salt solids (Scheme 4).



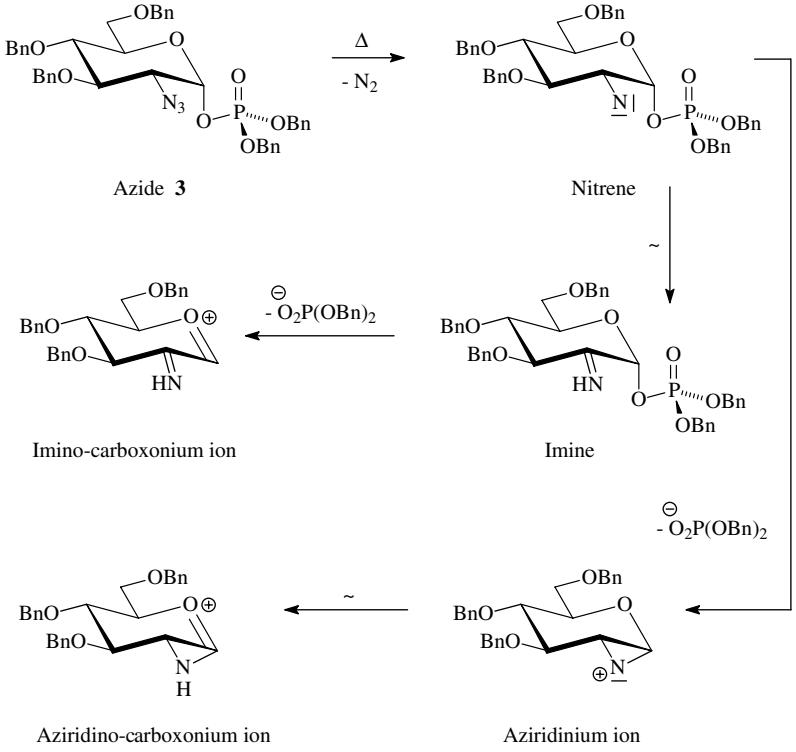
Scheme 2. Reagents and conditions: (i) 1.5–2.5 equiv R–CO_2–succinimide, THF/water, pH 7.0–7.5, rt, 1–2 days; (ii) Dowex 50W-X8 (triethylammonium form), **6**: 79%, **7**: 86%, **8**: 84%; (iii) 1 equiv 2,4,5-trichlorophenylformiate, 1.2 equiv di-*iso*-propylethylamine, DMF, rt, 1–2 days, **5**: 78%.

Table 1. ^{1}H and ^{31}P NMR chemical shifts (δ in ppm) and coupling constants (J in Hz) for compounds **5**–**12** in D_2O as solvents

Compound	Acylamido	Glucopyranose ring							Ribofuranose ring				Uracil	
		H-1	H-2	H-3	H-4	H-5	H-6a, H-6b	^{31}P	H-1′	H-2′, H-3′	H-4′	H-5′a, H-5′b	H-5″	H-6″
5		5.52, dd $J_{1,2} = 3.3$ $J_{1,P} = 7.1$	4.01, m $J_{2,3} = 10.3$	3.83, dd $J_{3,4} = 9.7$	3.57, dd $J_{4,5} = 9.6$	3.97, m $J_{5,6a} = 4.3$ $J_{5,6b} = 2.6$	3.89, m $J_{6a,6b} = 12.7$	1.58						
6	2.51, q 1.28, t	5.55, dd $J_{1,2} = 3.3$ $J_{1,P} = 7.1$	4.02, m $J_{2,3} = 10.3$	3.83, dd $J_{3,4} = 9.7$	3.57, dd $J_{4,5} = 9.6$	3.97, m $J_{5,6a} = 5.3$ $J_{5,6b} = 2.7$	3.90, m $J_{6a,6b} = 12.9$	0.27						
7	3.40, t 1.70, m 1.01, t	5.52, dd $J_{1,2} = 3.3$ $J_{1,P} = 7.1$	4.01, m $J_{2,3} = 10.3$	3.83, dd $J_{3,4} = 9.7$	3.59, dd $J_{4,5} = 9.6$	3.96, dt $J_{5,6a} = 4.1$ $J_{5,6b} = 2.7$	3.88, m $J_{6a,6b} = 12.9$	1.86						
8	2.02, m 1.11, d	5.53, dd $J_{1,2} = 3.1$ $J_{1,P} = 7.1$	4.02, m $J_{2,3} = 10.3$	3.86, dd $J_{3,4} = 9.7$	3.60, dd $J_{4,5} = 9.6$	3.97, ddd $J_{5,6a} = 4.3$ $J_{5,6b} = 2.7$	3.92, m $J_{6a,6b} = 12.7$	2.15						
9		5.53, dd $J_{1,2} = 3.3$ $J_{1,P} = 7.3$	4.02, m $J_{2,3} = 10.5$	3.83, dd $J_{3,4} = 9.7$	3.59, $J_{4,5} = 9.6$	3.96, m $J_{5,6a} = 4.3$ $J_{5,6b} = 2.5$	3.90, m $J_{6a,6b} = 12.9$	−10.4 −12.1	5.98, d $J_{1',2'} = 4.1$	4.37, m	4.30, m	4.24, m	5.98, d $J_{5'',6''} = 8.1$	7.96, d
10	2.50, q 1.27, t	5.53, dd $J_{1,2} = 3.3$ $J_{1,P} = 7.1$	4.00, m $J_{2,3} = 10.4$	3.82, dd $J_{3,4} = 9.7$	3.57, $J_{4,5} = 9.6$	3.95, m $J_{5,6a} = 4.3$ $J_{5,6b} = 2.7$	3.89, m $J_{6a,6b} = 12.7$	−10.6 −12.3	5.98, d $J_{1',2'} = 4.1$	4.37, m	4.30, m	4.24, m	5.97, d $J_{5'',6''} = 8.1$	7.95, d
11	3.41, t 1.72, m 1.03, t	5.54, dd $J_{1,2} = 3.3$ $J_{1,P} = 7.1$	4.02, m $J_{2,3} = 10.4$	3.85, dd $J_{3,4} = 9.7$	3.59, m $J_{4,5} = 9.6$	3.97, m $J_{5,6a} = 4.3$ $J_{5,6b} = 2.7$	3.90, m $J_{6a,6b} = 12.7$	−10.2 −12.1	5.99, d $J_{1',2'} = 4.1$	4.37, m	4.32, m	4.25, m	5.98, d $J_{5'',6''} = 8.1$	7.98, d
12	2.01, m 1.10, d	5.55, dd $J_{1,2} = 3.3$ $J_{1,P} = 7.3$	4.04, m $J_{2,3} = 10.1$	3.87, dd $J_{3,4} = 9.6$	3.61, dd $J_{4,5} = 9.6$	3.98, m $J_{5,6a} = 4.3$ $J_{5,6b} = 2.7$	3.92, m $J_{6a,6b} = 12.5$	−10.3 −12.3	6.00, d $J_{1',2'} = 4.0$	4.36, m	4.33, m	4.25, m	5.98, d $J_{5'',6''} = 8.1$	7.99, d

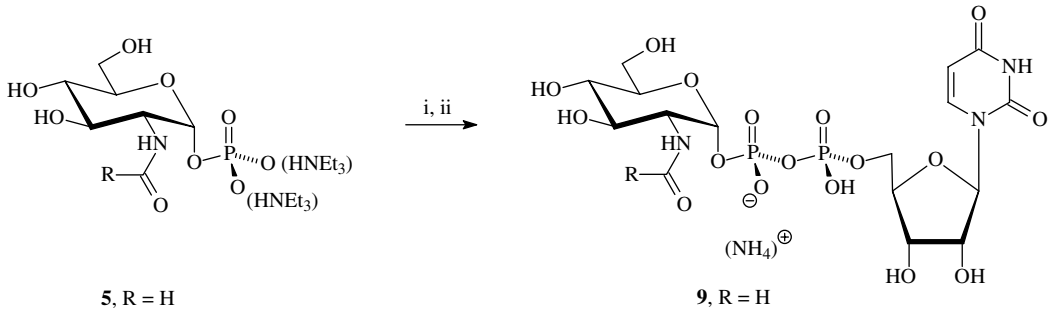
Table 2. ^{13}C NMR chemical shifts (δ in ppm) and coupling constants between carbon and phosphorus (J in Hz) for compounds **5**–**12** in D_2O as solvents

Compound	Acylamido	Glucopyranose ring						Ribofuranose ring					Uracil			
		C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-2″	C-4″	C-5″	C-6″
5	179.1, CO	94.7, d $J_{1,P} = 6.1$	53.6, d $J_{2,P} = 8.3$	71.2	69.6	73.2	60.2									
6	178.9, CO 29.6, CH_2 9.9, CH_3	94.7, d $J_{1,P} = 5.7$	53.7 $J_{2,P} = 8.1$	71.1	69.8	73.2	60.7									
7	178.1, CO 37.8, CH_2 19.3, CH_2 13.2, CH_3	94.6, d $J_{1,P} = 6.1$	53.7, d $J_{2,P} = 8.1$	71.0	69.7	73.1	60.5									
8	178.6, CO 28.7, CH 12.6, CH_3	94.7, d $J_{1,P} = 6.1$	53.9, d $J_{2,P} = 8.3$	71.2	69.9	73.1	60.6									
9	179.2, CO	94.5, d $J_{1,P} = 6.1$	53.7, d $J_{2,P} = 8.1$	71.0	69.5	73.1	60.3	88.5	74.0	69.6	83.3, d $J_{4',P} = 9.1$	65.1, d $J_{5',P} = 5.3$	152.4	166.7	102.8	141.6
10	179.3, CO 29.7, CH_2 9.9, CH_3	94.8, d $J_{1,P} = 6.1$	53.9, d $J_{2,P} = 8.3$	71.1	69.7	73.3	60.6	88.7	74.2	69.8	83.5, d $J_{4',P} = 9.1$	65.2, d $J_{5',P} = 5.3$	152.5	166.7	102.9	141.7
11	177.8, CO 37.7, CH_2 19.1, CH_2 13.1, CH_3	94.6, d $J_{1,P} = 6.3$	53.7, d $J_{2,P} = 8.3$	71.0	69.7	73.1	60.4	88.5	74.1	69.8	83.4, d $J_{4',P} = 9.1$	65.1, d $J_{5',P} = 5.1$	152.3	166.9	103.0	141.6
12	178.8, CO 28.8, CH 12.7, CH_3	94.6, d $J_{1,P} = 6.3$	53.8, d $J_{2,P} = 8.5$	71.1	69.7	73.1	60.5	88.4	74.2	69.9	83.4, d $J_{4',P} = 9.1$	65.1, d $J_{5',P} = 5.1$	152.2	166.4	103.2	141.5



Scheme 3. Fragmentation pattern of the azidophosphate **3** observed by Maldi-TOF spectrometry in positive mode and DHB as matrix.

^1H, ^{13}C, ^{31}P NMR and coupling constants data of the UDP glucosamines **9**–**12** are summarized in the lower half of Tables 1 and 2. Additional syntheses of novel and unnatural UDP-hexosamine analogues are in progress and will be reported in due course.



5, R = H
6, R = CH_2CH_3
7, R = $CH_2CH_2CH_3$
8, R = $CH(CH_3)_2$

9, R = H
10, R = CH_2CH_3
11, R = $CH_2CH_2CH_3$
12, R = $CH(CH_3)_2$

Scheme 4. Reagents and conditions: (i) 1.6 equiv 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium UMP-morpholidate, DMF/pyridine (2:1), rt, 5 days; (ii) 1. Biogel P2, 250 mM NH_4HCO_3, 2. Biogel P2, deionized water, **9**: 28%, **10**: 30%, **11**: 33%, **12**: 30%.

3. Experimental

3.1. General methods

TLC was performed on silica gel 60-coated aluminium sheets (E. Merck) using the given eluent mixtures. Spots were visualized under UV light at 366 nm and by spraying with 10% H_2SO_4 in EtOH and subsequent heating. Column chromatography was performed on silica gel 60 (230–240 mesh, grain size 0.040–0.063 nm, E. Merck). Size exclusion chromatography and desalting procedures were performed on Biogel P2 (Bio-Rad) either with aqueous 250 mM NH_4HCO_3 solution or deionized water as eluent. Optical rotations were measured on a Perkin–Elmer Polarimeter 243, with $[\alpha]_D$ values given in units of 10^{-1} deg cm^2 g^{-1}. NMR spectra were recorded on a Bruker AMX-400 NMR spectrometer. Chemical shifts are referred to the solvents used. Maldi-TOF spectra were measured on a Bruker Biflex-II spectrometer equipped with a N_2 laser unit (337 nm) with DHB as matrix both in positive and negative modes.

3.2. Procedure A: selective N-acylation

D-Glucosamine-1-phosphate (0.1 mmol) was dissolved in water (0.5 mL) and treated with a solution of the *N*-acyloxysuccinimide (0.15 mmol) of choice in THF/water (0.5 mL, 5:1). The pH was adjusted to 7.0 using 400 mM solution of potassium hydroxide in water. After stirring overnight at rt the resulting solution was if, necessary, treated with some more *N*-acyloxysuccinimide (0.1 mmol) in THF/water (0.5 mL, 5:1) with subsequent pH adjustment to 7.0 and stirring overnight. The resulting solution was purified on Biogel P2 with water as eluent, and after freeze drying the product containing fractions yielded 80–85% of the desired *N*-acylamido glucosyl phosphate.

3.3. Procedure B: UMP morpholidate coupling of glucosaminyl phosphates

The phosphate (0.18 mmol) obtained by procedure A was dissolved in water (1 mL) and passed through a column (1 cm × 5.5 cm) of Dowex 50W-X8 (triethylammonium form) to give the phosphate in form of the corresponding triethylammonium salt in quantitative yield. The phosphate was dissolved together with uridine-5′-monophosphomorpholidate (4-morpholine-*N*,*N*′-dicyclohexyl carboxamidinium salt, 1.6 equiv, 0.22–0.29 mmol) in anhydrous pyridine (10 mL) and concentrated to dryness without heating under reduced pressure. Ventilation to normal pressure was carried out with dry argon. After repeating this procedure three times, the resulting syrup-like residue was dissolved in anhydrous pyridine/anhydrous DMF (~3 mL, 1:2) and stirred for 5 days at rt sealed under an argon atmosphere. Removal of the solvents without heating under reduced pressure followed by dissolving the residue in water, filtration and subsequent separation on Biogel P2 with first 250 mM NH_4HCO_3 solution and afterwards for desalting purpose with deionized water as eluent, yielded the uridine diphosphoglucosamine in the form of its ammonium salt as a colourless and highly hygroscopic powder.

3.4. Synthesis of glycosyl phosphates

3.4.1. (2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl) dibenzyl phosphate (3). 1*H*-Tetrazole (307 mg, 4.38 mmol) was suspended in dry dichloromethane (3 mL) under argon and stirring at rt, followed by the addition of dibenzyl-*N*,*N*′-diisopropylphosphoramidite (0.73 mL, 745 mg, 2.59 mmol). Within 15 min the suspension became a clear solution and 2-azido-3,4,5-tri-*O*-benzyl-2-deoxy-α/β-D-glucopyranose[29,30] (427 mg, 0.9 mmol, $M = 475.54$ g/mol) in dry dichloromethane

(3 mL) was added rapidly. After stirring for 4 h at rt the solution was cooled to 0 °C and 3-chloroperbenzoic acid (617 mg, 2.5 mmol) was added in small doses, followed by stirring for 1 h at rt. Removal of the solvent under reduced pressure at 30 °C bath temperature and purification by column chromatography yielded compound **3** (382 mg, 58%) as a colourless syrup. $[\alpha]_D^{20}$ −42.5 (*c* 1.0 $CHCl_3$); Maldi-TOF (DHB, positive mode): $m/z =$ 774 $[M+K]^+$, 758 $[M+Na]^+$, 746 $[M-N_2+K]^+$, 730 $[M-N_2+Na]^+$, 459 $[M-O_2P(OBn)_2]^+$, 430 $[M-O_2P(OBn)_2-N_2]^+$, IR (KBr): $\nu = 2114\ cm^{-1}$ (N_3); ^1H NMR (acetone-d_6): δ 7.19 (m, 25H, 5 × Ph); 5.73 (dd, 1H, H-1, $J_{1,2} = 3.3$ Hz, $J_{1,P} = 6.1$ Hz); 4.98, 4.95 (2 × d, 4H, 2 × $POCH_2Ph$); 4.78, 4.76, 4.62, 4.46, 4.35, 4.28 (6 × d, 6H, 3 × OCH_2Ph); 3.86 (m, 1H, H-5, $J_{4,5} = 9.8$ Hz, $J_{5,6a} = 4.3$ Hz, $J_{5,6b} = 2.7$ Hz); 3.84 (dd, 1H, H-3, $J_{2,3} = 10.7$ Hz, $J_{3,4} = 10.1$ Hz); 3.74 (dd, 1H, H-2); 3.71 (dd, 1H, H-4); 3.67 (m, 1H, H-6a, $J_{6a,6b} = 13.1$ Hz); 3.54 (m, 1H, H-6b); ^{13}C NMR (acetone-d_6): δ 97.8 (d, C-1, $J_{C\text{-}1,P} = 6.1$ Hz), 81.7 (C-3); 75.8 (C-4); 74.0 (C-5); 69.2 (C-6); 65.1 (d, C-2, $J_{C\text{-}2,P} = 8.3$ Hz); ^{31}P NMR (acetone-d_6): δ −0.87.

3.4.2. 2-Amino-2-deoxy-α-D-glucopyranosyl phosphate (4). (2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-glucopyranosyl) dibenzylphosphate (**3**, 379 mg, 0.52 mmol) was dissolved in a mixture of water/MeOH/EtOAc (25 mL, 1:2:1). This solution was treated with palladium on activated charcoal (650 mg, 10%) and hydrogenated under 65 bar pressure for 3 days. After filtration and evaporation of the organic solvents under reduced pressure followed by lyophilization the crude product was purified on Biogel P2 with water as eluent resulting in compound **4** (89 mg, 67%) as a colourless foamy solid. $[\alpha]_D^{20}$ −36.5 (*c* 1.0 water); ^1H NMR (D_2O): δ 5.77 (dd, 1H, H-1, $J_{1,2} = 3.5$ Hz, $J_{1,P} = 6.6$ Hz); 3.97 (dd, 1H, H-3, $J_{2,3} = 10.4$ Hz, $J_{3,4} = 9.4$ Hz); 3.94 (m, 1H, H-5, $J_{4,5} = 9.9$ Hz, $J_{5,6a} = 4.0$ Hz, $J_{5,6b} = 2.3$ Hz); 3.90 (dd, 2H, H-6a, $J_{6a,6b} = 12.5$ Hz); 3.86 (dd, 2H, H-6b); 3.60 (dd, 1H, H-4); 3.43 (m, 1H, H-2, $J_{2,P} = 2{,}3$ Hz). ^{13}C NMR (D_2O): δ 92.2 (d, C-1, $J_{1,P} = 6.1$ Hz); 73.2 (C-5); 69.9 (C-3); 69.4 (C-4); 60.3 (C-6); 54.5 (dd, C-2, $J_{2,P} = 9.1$ Hz); ^{31}P NMR (D_2O): δ 2.70.

3.4.3. 2-Deoxy-2-formylamido-α-D-glucopyranosyl phosphate (5). The triethylammonium salt of 2-amino-2-deoxy-α-D-glucopyranosyl phosphate **4** (27 mg, 0.1 mmol) together with 2,4,5-trichlorophenylformiate (27 mg, 0.1 mmol) and di-*iso*-propylethylamine (15.5 mg, 20.5 μL, 0.12 mmol) in 3 mL anhydrous DMF were stirred for 2 days at room temperature. Upon dilution with 50 mL deionized water the resulting solution was neutralized with 1 M HCl solution, following freeze drying and desalting on Biogel P2 with 250 mM aqueous ammonium hydrogencarbonate solution to give the title ammonium salt **5** (30 mg, 78%) as a colourless foamy solid; $[\alpha]_D^{20}$ +66.5 (*c* 1.0 water).

3.4.4. 2-Deoxy-2-propionylamido-α-D-glucopyranosyl phosphate (6). According to procedure A 2-amino-2-deoxy-α-D-glucopyranosyl phosphate **4** (75 mg, 0.29 mmol) and *N*-propionyloxysuccinimide ester were reacted to give the title compound **6** (80 mg, 79%) as a colourless hygroscopic powder; $[\alpha]_D^{20}$ +35 (*c* 1.0 water).

3.4.5. 2-Butyramido-2-deoxy-α-D-glucopyranosyl phosphate (7). According to procedure A 2-amino-2-deoxy-α-D-glucopyranosyl phosphate **4** (73 mg, 0.28 mmol) and *N*-butyroxy-succinimide were converted to the title compound **7** in form of a colourless hygroscopic solid (88 mg, 86%); $[\alpha]_D^{20}$ +44 (*c* 1.0 water).

3.4.6. 2-Deoxy-2-isobutyramido-α-D-glucopyranosyl phosphate (8). 2-Amino-2-deoxy-α-D-glucopyranosyl phosphate **4** (72 mg, 0.28 mmol) was reacted with *N*-*iso*-butyroxysuccinimide as described by procedure A thus to give title compound **7** (85 mg, 84%) as a colourless hygroscopic powder; $[\alpha]_D^{20}$ +19.5 (*c* 1.0 water).

3.4.7. Uridine 5′-(2-deoxy-2-formylamido-α-D-glucopyranosyl diphosphate) (9). According to procedure B the triethylammonium salt of *2-deoxy-2-formylamido-α-D-glucopyranosyl phosphate* (**5**, 38 mg, 78 μmol) was reacted with the UMP morpholidate reagent (85 mg, 125 μmol) resulting in the title compound **9** (14 mg, 28%) as a colourless solid.

3.4.8. Uridine 5′-(2-deoxy-2-propionylamido-α-D-glucopyranosyl diphosphate) (10). 2-Propionylamido-2-deoxy-α-D-glucopyranosyl phosphate (**6**) in form of its triethylammonium salt (83 mg, 166 μmol) was reacted with the UMP morpholidate coupling reagent (183 mg, 266 μmol) as described in procedure B to give compound **10** (36 mg, 30%) as a colourless powder.

3.4.9. Uridine 5′-(2-butyramido-2-deoxy-α-D-glucopyranosyl diphosphate) (11). The triethylammonium salt of 2-butyramido-2-deoxy-α-D-glucopyranosyl phosphate (**7**, 71 mg, 133 μmol) was reacted with the UMP morpholidate reagent (147 mg, 215 μmol) according to procedure B to give the title compound **11** (30 mg, 33%) as a colourless powder.

3.4.10. Uridine 5′-(2-isobutyramido-2-deoxy-α-D-glucopyranosyl diphosphate) (12). The triethylammonium salt of 2-isobutyramido-2-deoxy-α-D-glucopyranosyl phosphate (**8**, 60 mg, 113 μmol) was reacted with the UMP morpholidate reagent (125 mg, 183 μmol) according to procedure B to give the title compound **12** (23 mg, 30%) as a colourless powder.

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