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Syntheses of unnatural N-substituted UDP-galactosamines as alternative substrates for *N*-acetylgalactosaminyl transferases

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Dedicated to Professor Derek Horton on the occasion of his 70th birthday

Abstract

UDP-GalNAc analogues with slight modifications in the 2-acetamido group of the GalNAc moiety are prepared in order to study their role in the mechanism of the *N*-acetylgalactosaminyl transferase mediated glycosylation step. The analogues with *N*-propionyl-, *N*-butyryl- and *N*-bromoacetyl-groups were synthesized, utilizing Khorana's morpholidate coupling method starting from D-galactosaminyl-1-phosphate after selective N-acylation of its amino group with the appropriate *N*-acyloxysuccinimides. Furthermore, in addition to UDP-galactosamine its 2-azido analogue has been efficiently prepared involving a metal catalyzed diazo transfer reaction.

Keywords: GalN-1-P; Acylamidosugars; UDP-sugars; Morpholidate coupling; Diazo transfer

1. Introduction

Molecular recognition in organisms is known to be mediated via binding events involving complex carbohydrates on cell surfaces.[1–5] Compounds that interfere with these recognition processes by means of modulation or inhibition may be candidates for drugs in a variety of diseases. Therefore strategies for the synthesis of such oligosaccharide target compounds have received increasing attention in recent years, including syntheses of unnatural carbohydrates as potentially more effective agents, which mimic their natural occurring analogues.[6–11]

Next to purely synthetic approaches by means of linear and convergent glycosylation steps, an alternative and promising methodology involves the use of transferases that employ nucleotide hexoses as activated carbohydrate donors.[12–14] The main advantage for this approach is that transferase enzymes display high stereospecifity and regioselectivity in directing the activated carbohydrate donor to the suitable position of the recognized acceptor. This approach eliminates the need for numerous protection and deprotection steps required in non-enzymatic syntheses. However, it suffers in the sense, that these enzymes preclude the use of alternative donor carbohydrates that differ significantly from their natural occurring counterparts, making enzymatic synthesis of some unnatural oligosaccharides a challenging and difficult task. *N*-Acetylgalactosamine (GalNAc) is a wide-spread component of natural oligosaccharides and glycoconjugates that are generated by *N*-acetylgalactosaminyl transferases utilizing uridine diphospho-*N*-acetylgalactosamine (UDP-GalNAc) as donor by transfer of GalNAc to a recognized precursor of the glycosylated product formed.

It has been shown in recent chemo-enzymatic work, that in the UDP-HexNAc series, UDP-*N*-trifluoracetylgalactosamine and UDP-*N*-trifluoracetylglucosamine were successfully transferred, whereas in the latter case, the corresponding non-acylated UDP-amine proved to be non-transferable, most likely due to its charged ammonium-ion form.[13]

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 HexNAc trans-

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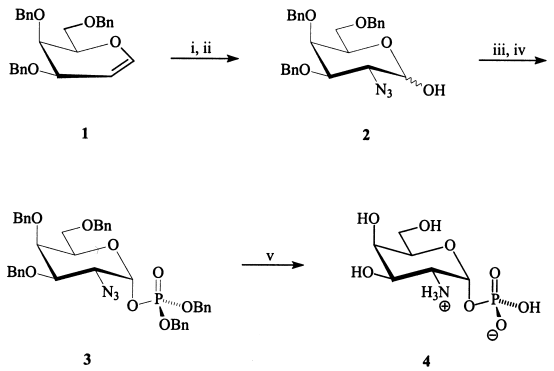
ferases. UDP-*N*-propionyl-, UDP-*N*-butyryl- and UDP-*N*-bromoacetylgalactose, described in this paper are therefore expected to be potentially transferable in transferase reactions to give oligosaccharides with potential bioactive properties.

2. Results and discussion

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.[14] A wide range of acyl groups can be introduced and many other functionalities such as labile phosphate groups within the aminosugar are applicable to this reaction. For the syntheses of *N*-acylamidogalactopyranosyl phosphates and further towards their corresponding uridine diphospho derivatives, D-galactos-aminyl-1-phosphate (**4**) was chosen as a key intermediate and proved to be a suitable common starting material for the range of UDP-GalNAc analogues reported in this paper.

The synthesis of D-galactosaminyl-1-phosphate (**4**) started from perbenzylated D-galactal (**1**) and followed the pathway via azidonitration initially established by Lemieux et al.[15] and extended to perbenzylated glycals by Schmidt et al.[16] The azide was regioselectively and preferentially introduced in an equatorial manner into the 2-position of the pyranose. The 2-azidogalactosyl nitrate obtained as the main product in this reaction was subjected to reductive hydrolysis and led to the formation of the benzylated 2-azidogalactose **2** after treatment with sodium nitrite in an aqueous dioxane solution (Scheme 1).

The benzylated 2-azidogalactose **2** was phosphorylated employing dibenzyl-*N*,*N*′-diisopropylphosphoramidite and 1*H*-tetrazole as a catalyst. Initially the phosphite was formed, which was oxidized with 3-chloroperbenzoic acid to the protected phosphate **3** in a one-pot reaction. Exclusively the α-phosphate could be isolated, whereas the formation of the corresponding β-phosphate was not observed. A preceding attempt to obtain the α-phosphate **3** via trichloroacetimidate formation from **2** followed by phosphorylation with dibenzyl phosphate failed, since under a variety of conditions only the α-trichloroacetimidate arose, which in turn gave the pure β-phosphate in the subsequent phosphorylation step.

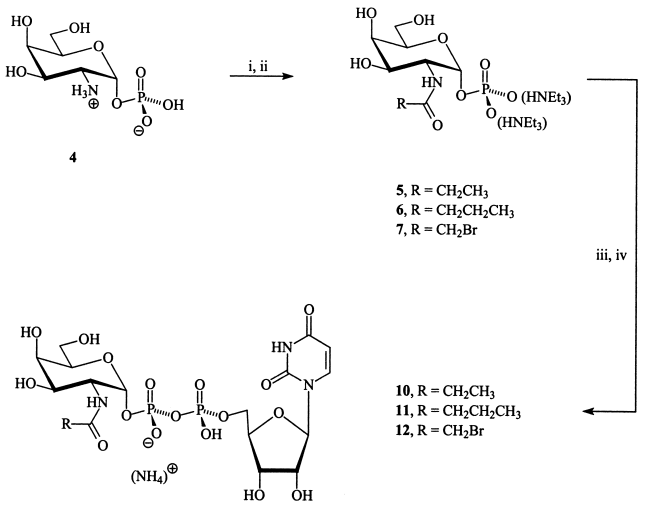


Scheme 1. (i) $Ce^{IV}(NH_4)_2(NO_3)_6$, NaN_3, MeCN, −25 °C, 1 day; (ii) $NaNO_2$, dioxane, water, 80 °C, 1 day; (iii) $(^iPr)_2NP(OBn)_2$, 1*H*-tetrazole, CH_2Cl_2, 5 h; (iv) *m*CPBA, CH_2Cl_2, 0 °C, 1 h, **3**: 62%; (v) H_2, Pd/C, (1:2:1) EtOAc–MeOH–water, 50 bar, 1.5 days, **4**: 71%.

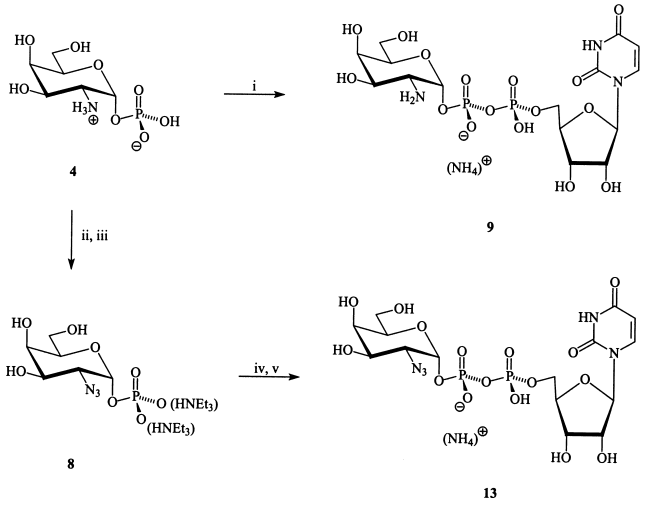
Complete debenzylation and reduction of the azide functionality was carried out in a single reaction step by hydrogenolysis using palladium on activated charcoal affording galactosamine-1-phosphate (**4**). This reaction step turned out to be critical regarding yield and completion of deprotection, since the azide is initially turned into an amine, which inhibits further debenzylation. Impurities in the starting material and varying catalyst qualities showed to have a significant inhibition effect on the hydrogenation step leading to longer reaction times and resulting in lower yields. Another difficulty is due to the stepwise debenzylation which first occurs to the benzyl ester groups of the phosphate moiety, thus leading to an β-amino-phosphoric acid inner salt resulting in the need for a solvent system to dissolve both compounds **3** and **4** which differ considerably in polarity. In compliance with the difficulties stated, hydrogenation was performed under a hydrogen atmosphere of 50 bar pressure with an excessive amount of palladium catalyst in a homogenous phase solvent mixture ethyl acetate, (1:2:1) methanol and water leading to the unprotected amine **4** in 2 g scale and in 71% yield without formation of side products or partially debenzylated material.

The subsequent selective N-acylation of the aminophosphate **4** utilizing *N*-propionyloxysuccinimide, *N*-butyroxysuccinimide and *N*-(2-bromoacetoxy)succinimide, respectively, gave the 2-acylamidophosphates **5**–**7** in an average yield around 85%. Under such mild reaction conditions, no cleavage of the phosphate and no side products arising from O-acylation at the unprotected 3-, 4- and 5-positions could be monitored. The appropriate *N*-acyloxysuccinimide esters were easily prepared by reacting *N*-hydroxysuccinimide with either propionylchloride or butyrylchloride in the presence of triethylamine as an auxiliary base in case of the *N*-propionyloxysuccinimide and *N*-butyroxysuccinimide, whereas *N*-(2-bromo-acetoxy)succinimide was obtained by standard DCC coupling conditions from 2-bromoacetic acid and *N*-hydroxysuccinimide, all of which were used after column chromatographic purification or recrystallization (Scheme 2).

In order to synthesize the 2-azido analogue of UDP-galactosamine **13**, the amino group in galactosamine-1-



Scheme 2. (i) R-CO_2-succinimide, THF–water, pH 7.0, 1 day; (ii) Dowex 50W-X8 (triethylammonium form), **5**: 90%, **6**: 84%, **7**: 82%; (iii) 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium UMP-morpholidate, (1:1) DMF–pyridine, several days; (iv) (1) Biogel P2, 0.25 M NH_4HCO_3, (2) Biogel P2, water, **10**: 31%, **11**: 32%, **12**: 30%.



Scheme 3. (i) UDP-Glc, galactose-1-phosphate uridyl transferase (E.C. 2.7.7.12), 5 mM $HSCH_2CH_2OH$, pH 8.7, 30 °C, 3 days, **9**: 12%; (ii) TfN_3, $CuSO_4$, K_2CO_3, CH_2Cl_2–MeOH–water, 2 days; (iii) Dowex 50W-X8 (triethyl-ammonium form), **8**: 59%; (iv) 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium UMP-morpholidate, (1:1) DMF–pyridine, 5 days; (v) (1) Biogel P2, 0.25 M NH_4HCO_3, (2) Biogel P2, water, **13**: 25%.

phosphate (**4**) was reversibly converted to an azido group to give 2-azidogalactose-1-phosphate (**8**) employing a copper(II) salt-catalyzed diazo transfer reaction with triflic azide, copper(II) sulfate and potassium carbonate, which ensures the amine not to be present in the form of its ammonium ion. Due to the insolubility of triflic azide in aqueous solution, a one-phase mixture from dichloromethane, methanol and water served as the solvent medium. Under these conditions, azide formation occurred maintaining the galacto configuration in the azide **8** as reported by Vasella et al. and Wong et al.[17,18]

The presence of the azido group in **8** was established by IR analysis ($\nu = 2087\ cm^{-1}$). ^1H and ^{13}C NMR data for the unequivocal structural assignments of the phosphates **5**–**8** are summarized in Tables 1 and 2 (upper parts).

Syntheses of UDP hexoses.—The syntheses of the *N*-acylamido-UDP-galactoses **10**–**12** and 2-azido-UDP-galactose **13** from their phosphate precursor **5**–**8** were performed under standard Khorana conditions[19] with the 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium salt of uridine monophospho morpholidate as the activated UMP source under an argon atmosphere in a solution of (1: 1) anhydrous DMF–pyridine at room temperature. Prior to the reaction, the phosphates were converted to their triethylammonium salts, thus increasing the nucleophilicity and enabling solubility in the organic solvent mixture. The reactions were usually worked up after several days by evaporation of the organic solvents, followed by size-exclusion chromatography and subsequent desalting on Biogel P2. In addition to mostly unreacted starting material, the UDP hexoses were obtained in a yield range of 25–32%. The products were always accompanied by minor amounts of symmetrical uridine–diphospho–uridine (UPPU) formed upon self-condensation of the UMP morpholidate reagent. Thus it was increasingly difficult to isolate the product by chromatography, mainly due to its similarity to UPPU concerning polarity and molecular size. Galactosamine-1-phosphate (**4**) itself failed to react under the described morpholidate coupling conditions. Therefore it was converted to UDP-galactosamine (**9**) upon incubation with galactose-1-phosphate uridyl transferase (E.C. 2.7.7.12) under improved and simpler reaction conditions than those reported by Whitesides et al.[20] Galactose-1-phosphate uridyl transferase transfers UMP from UDP-Glc to galactose-1-phosphate its natural substrate, and it is known to accept GalN-1-P, but not GalNAc-1-P with a relative reaction rate in the order of 5% for GalN-1-P compared to Gal-1-P and the K_m value being 30 times higher in the case of GalN-1-P.[21,22] This is reflected in an observed yield of 12% UDP-galactosamine (**12**) after an incubation period of 3 days at 30 °C and pH 8.7 in a 5 mM 2-mercaptoethanol solution, since formation of **12** is not favored by ways of equilibrium. At 37 °C the yield dropped even further accompanied by the release of UMP as a side product (Scheme 3).

In contrast to the previously reported results, this enzyme-based approach displayed a severe sensitivity towards upscaling. Whereas a gram scale attempt failed and led only to traces of the desired product, the before mentioned yield was only achieved in scales of 10–70

Table 1
^{1}H and ^{31}P NMR chemical shifts (δ in ppm) and coupling constants (J in Hz) for compounds **4**–**13** in D_2O as solvent

Compound	Acylamido protons	Galactopyranose 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″
4		5.38, dd $J_{1,2}$ 3.2 $J_{1,P}$ 6.9	3.20, m $J_{2,3}$ 10.7	3.80, dd $J_{3,4}$ 3.2	3.72, dd $J_{4,5}<2$	3.88, dt $J_{5,6a}$ 6.9 $J_{5,6b}$ 5.1	3.45, m $J_{6a,6b}$ 12.0	2.66							
5	1.99, q 5.12, dd 0.79, t	3.88, m $J_{1,2}$ 3.3 $J_{1,P}$ 7.4	3.63, dd $J_{2,3}$ 10.9	3.71, dd $J_{3,4}$ 3.1	3.83, dt $J_{4,5}<2$	3.43, m $J_{5,6a}$ 6.4 $J_{5,6b}$ 6.1	0.35 $J_{6a,6b}$ 11.7								
6	2.23, t 1.55, m 0.86, t	5.35, dd $J_{1,2}$ 3.6 $J_{1,P}$ 7.4	4.12, m $J_{2,3}$ 10.6	3.86, dd $J_{3,4}$ 3.3	3.94, dd $J_{4,5}<2$	4.12, m $J_{5,6a}$ 5.3 $J_{5,6b}$ 4.7	3.67, m $J_{6a,6b}$ 12.7	2.63							
7	2.60, m	5.47, dd $J_{1,2}$ 3.6 $J_{1,P}$ 7.6	4.25, m $J_{2,3}$ 10.9	3.97, dd $J_{3,4}$ 3.3	4.01, dd $J_{4,5}<2$	4.12, dt $J_{5,6a}$ 6.4 $J_{5,6b}=6.1$	3.73, m $J_{6a,6b}$ 11.7	2.88							
8		5.49, dd $J_{1,2}$ 3.6 $J_{1,P}$ 7.9	3.44, m $J_{2,3}$ 10.9	4.02, dd $J_{3,4}$ 3.3	3.95, dd $J_{4,5}<2$	4.08, ddd $J_{5,6a}$ 7.4 $J_{5,6b}$ 5.1	3.67, m $J_{6a,6b}$ 12.0	−0.26							
9		5.55, dd $J_{1,2}$ 3.6 $J_{1,P}$ 6.9	3.22, m $J_{2,3}$ 10.7	3.78, dd $J_{3,4}$ 3.3	3.75, dd $J_{4,5}<2$	3.90, m $J_{5,6a}$ 5.7 $J_{5,6b}=5.1$	3.44, m $J_{6a,6b}$ 12.0	−9.90 −12.2	5.68, d $J_{1',2'}$ 4.6	4.07, m	3.98, m	3.94, m		5.66, d $J_{5'',6''}$ 8.1	7.65, d
10	2.49, q 1.25, t	5.67, dd $J_{1,2}$ 3.3 $J_{1,P}$ 7.1	4.37, m $J_{2,3}$ 10.9	4.10, dd $J_{3,4}$ 3.1	4.17, dd $J_{4,5}<2$	4.33, m $J_{5,6a}$ 6.4 $J_{5,6b}$ 5.6	3.89, m $J_{6a,6b}$ 11.7	−10.0 −12.2	6.11, d $J_{1',2'}$ 4.3	4.55, m	4.41, m	4.37, m		6.09, d $J_{5'',6''}$ 8.1	8.09, d
11	3.45, t 1.75, m 1.05, t	5.66, dd $J_{1,2}$ 3.4 $J_{1,P}$ 7.0	4.36, m $J_{2,3}$ 11.0	4.09, dd $J_{3,4}$ 3.3	4.18, m $J_{4,5}<2$	4.32, m $J_{5,6a}$ 6.4 $J_{5,6b}$ 5.3	3.90, m $J_{6a,6b}$ 11.7	−10.7 −12.2	6.10, d $J_{1',2'}$ 5.3	4.50, m	4.40, m	4.36, m		6.09, d $J_{5'',6''}$ 8.1	8.09, d
12	2.70, m	5.64, dd $J_{1,2}$ 3.3 $J_{1,P}$ 7.1	4.38, m $J_{2,3}$ 10.7	4.06, dd $J_{3,4}$ 3.3	4.09, dd $J_{4,5}<2$	4.25, m $J_{5,6a}$ 6.4 $J_{5,6b}$ 5.9	3.81, m $J_{6a,6b}$ 12.0	−10.1 −11.6	6.01, d $J_{1',2'}<5.5$	4.54, m	4.40, m	4.29, m		6.00, d $J_{5'',6''}$ 8.1	8.09, d
13		5.66, dd $J_{1,2}$ 3.5 $J_{1,P}$ 7.3	3.54, m $J_{2,3}$ 10.7	4.05, m $J_{3,4}$ 3.2	4.00, m $J_{4,5}<2$	4.14, m $J_{5,6a}$ 6.1 $J_{5,6b}$ 6.0	3.68, m $J_{6a,6b}$ 12.0	−10.3 −12.1	5.93, d $J_{1',2'}<5.5$	4.32, m	4.22, m	4.18, m		5.93, d $J_{5'',6''}$ 8.2	7.92, d

Table 2
^{13}C NMR chemical shifts (δ in ppm) and coupling constants between carbon and phosphorus (J in Hz) for compounds **4–13** in D_2O as solvent

Compound	Acylamido	Galactopyranose 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″
4		91.5, d $J_{1,P}$ 4.6	51.7, d $J_{2,P}$ 7.4	66.8	68.6	71.8	61.4									
5	178.2, CO 29.5, CH_2 9.8, CH_3	94.0, d $J_{1,P}$ 5.9	50,3, d $J_{2,P}$ 7.7	67.8	68.9	72.0	61.8									
6	178.3, CO 38.1, CH_2 19.3, CH_2 13.2, CH_3	93.8, d $J_{1,P}$ 5.9	50.3 $J_{2,P}$ 7.8	68.2	69.1	71.8	61.7									
7	171.5, CO 74.9, CH_2	94.0, d $J_{1,P}$ 5.7	50.4, d $J_{2,P}$ 8.1	67.6	68.8	72.2	61.6									
8		94.0, d $J_{1,P}$ 5.6	60.4, d $J_{2,P}$ 7.8	67.7	69.3	71.8	61.6									
9		93.6, d $J_{1,P}$ 5.6	51.3, d $J_{2,P}$ 9.2	68.4	67.1	72.6	61.2	88.9	70.0	74.1	83.5, d $J_{4',P}$ 8.7	65.5, d $J_{5',P}$ 5.6	150.3	165.2	103.0	142.0
10	179.3, CO 29.5, CH_2 9.89, CH_3	95.2, d $J_{1,P}$ 6.3	50.0, d $J_{2,P}$ 8.4	68.0	68.8	72.4	61.4	88.9	74.2	70.1	83.6, d $J_{4',P}$ 9.1	65.4, d $J_{5',P}$ 5.4	152.5	166.6	103.0	142.1
11	178.4, CO 38.0, CH_2 19.3, CH_2 13.2, CH_3	95.2, d $J_{1,P}$ 6.5	50.0, d $J_{2,P}$ 8.6	67.9	68.8	72.4	61.4	88.9	74.1	70.0	83.6, d $J_{4',P}$ 9.2	65.3, d $J_{5',P}$ 5.6	160.7	160.7	103.0	142.0
12	171.6, CO 74.8, CH_2	94.8, d $J_{1,P}$ 6.2	50.2, d $J_{2,P}$ 8.4	67.8	68.8	72.5	61.4	88.9	74.2	70.0	83.6, d $J_{4',P}$ 9.0	65.4, d $J_{5',P}$ 5.3	152.2	166.6	103.0	142.1
13		95.0, d $J_{1,P}$ 6.1	60.1, d $J_{2,P}$ 8.7	67.7	69.1	72.3	61.3	88.8	70.1	74.2	83.7, d $J_{4',P}$ 8.7	65.3, d $J_{5',P}$ 5.6	152.2	166.6	103.1	142.1

mg of the starting material. The reaction was therefore carried out with 450 mg GalN-1-P, and was done in 40 parallel reactions with an absolute volume of 0.5 mL each. Thus UDP-GalN-1-P (**9**) was obtained in modest yields. The ^{13}C NMR chemical shifts for both the phosphates **4** and **8**, as well as their corresponding UDP derivatives **9** and **13**, were almost identical apart from C-2 being 10 ppm shifted downfield when attached to an azido group compared to an amino group in both cases. ^{1}H and ^{13}C NMR data of the UDP hexoses **9**–**13** are summarized in Tables 1 and 2 (lower parts). Attempts to convert the UDP-azide **13** to the UDP-amine 9 by reduction methods were not yet successful. Further work in this direction and additional syntheses of novel *N*-acylamido-UDP-hexoses are in progress.

3. Experimental

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). Petroleum ether refers to the fraction with distillation range 5–-70 °C. Biogel separation was performed on Biogel P2 (Bio-Rad) either with 0.25 M NH_4HCO_3 solution or 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}. Elemental analyses were performed by the microanalytical laboratory of the University of Hamburg. IR absorptions were recorded on a ATI Matteson FT-IR (Genesis Series). 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 with DHB as matrix.

Procedure A: preparation of triflic azide in dichloromethane.—NaN_3 (960 mg, 14.8 mmol) was dissolved in water (2.7 mL) and after addition of CH_2Cl_2 (2 mL) cooled to ca. 2 °C. Under vigorous stirring, trifluoromethanesulfonic anhydride (0.49 mL) was added dropwise within 5 min. The mixture was stirred another 2 h, followed by separation of the organic layer and twice extracting the aqueous layer with CH_2Cl_2 (2 mL), thus obtaining triflic azide in CH_2Cl_2 (8 mL). This solution was washed once with a solution of satd Na_2CO_3 (2 mL), and after separation, used without further purification.

Procedure B: selective N-acylation.—D-Galactosamine-1-phosphate (0.1 mmol) was dissolved in water (0.5 mL) and treated with a solution of the *N*-acyloxysuccinimide (0.15 mmol) in 1:4 THF–water (0.5 mL). The pH was adjusted to 7.0 using a 0.4-M solution of potassium hydroxide in water. After stirring over night at rt, the resulting solution was, if necessary, treated with some more *N*-acyloxysuccinimide (0.1 mmol) in 1:4 THF–water (0.5 mL) with subsequent pH adjustment to 7.0 and stirring over night. The resulting solution was purified on Biogel P2 with water as eluent, and after freeze drying the product containing fractions, yielded 80–90% of the desired acylamido hexosyl phosphate.

Procedure C: morpholidate coupling of hexosyl phosphates.—The phosphate (0.18 mmol) obtained by procedure B was dissolved in water (1 mL) and passed through a column (1 × 5.5 cm) of Dowex 50W-X8 (triethylammonium form) to give the phosphate in the 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 anhyd 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 1:1 anhyd pyridine–anhyd DMF (3 mL) and stirred for 5–7 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 0.25 M NH_4HCO_3 solution, and afterwards for desalting purpose with water as eluent, yielded the uridine diphosphohexose in form of its ammonium salt.

*(2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-*D*-galactopyranosyl) dibenzyl phosphate* (**3**).—1*H*-Tetrazole (368 mg, 5.25 mmol) was suspended in dry CH_2Cl_2 (3 mL) under argon and stirring at rt, followed by the addition of dibenzyl-*N*,*N*′-diisopropylphosphoramidite (0.87 mL, 894 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-galactopyranose[16] in dry CH_2Cl_2 (3 mL) was added rapidly. After stirring for 5 h at rt, the solution was cooled to 0 °C and 3-chloro-perbenzoic acid (740 mg, 3 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** (493 mg, 62%) as a colorless syrup. $[\alpha]_D^{20}$ + 76° (*c* 1.0, $CHCl_3$); MALDI-TOF (DHB, positive mode): *m*/*z* 774 $[M+K]^+$, 758 $[M+Na]^+$, 746 $[M-N_2+K]^+$, 730 $[M-N_2+Na]^+$, 684 $[M-C_7H_7+K]^+$, 668 $[M-C_7H_7+Na]^+$, IR (KBr): *ν* 2114.1 cm^{-1} (N_3); ^{1}H NMR (acetone-d_6): δ 7.20 (m, 25 H, 5 × Ph), 5.75 (dd, 1 H, H-1, $J_{1,2}$ 3.1, $J_{1,P}$ 6.1 Hz), 4.99, 4.95 (2 × d, 4 H, 2 × $POCH_2Ph$), 4.80, 4.77, 4.62, 4.47, 4.36, 4.29 (6 × d, 6 H, 3 × OCH_2Ph), 4.18 (d, 1 H,

H-4, $J_{4,5} < 2$ Hz); 4.11 (m, 1 H, H-5, $J_{5,6a}$ 6.6, $J_{5,6b}$ 6.1 Hz), 3.99 (m, 2 H, H-2 and H-3, $J_{2,3}$ 10.7, $J_{3,4}$ 3.1 Hz), 3.54 (m, 1 H, H-6a, $J_{6a,6b}$ 9.4 Hz), 3.39 (m, 1 H, H-6b); ^{13}C NMR (acetone-d_6): δ 91.5 (C-1), 71.8 (C-5), 68.6 (C-4), 66.8 (C-3), 61.4 (C-6), 51.7 (C-2); ^{31}P NMR (acetone-d_6): δ −1.05.

2-Amino-2-deoxy-α-D-galactopyranosyl phosphate (**4**).

—(2-Azido-3,4,6-tri-*O*-benzyl-2-deoxy-α-D-galactopyranosyl) dibenzylphosphate (**3**, 470 mg, 0.64 mmol) was dissolved in a solvent mixture 1:2:1 water–MeOH–EtOAc (20 mL). This solution was treated with palladium on activated charcoal (740 mg, 10%) and hydrogenated under 50 bar pressure for 2.5 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** (117 mg, 71%) as a colorless foamy solid. $[\alpha]_D^{20}$ +108° (*c* 1.0, water).

2-Deoxy-2-propionylamido-α-D-galactopyranosyl phosphate (**5**).—According to procedure B, 2-amino-2-deoxy-α-D-galactopyranosyl phosphate (**4**, 88 mg, 0.34 mmol) and *N*-propionyloxysuccinimide were reacted as above to give the title compound **5** (96 mg, 90%). $[\alpha]_D^{20}$ +98° (*c* 1.0, water).

2-Butyramido-2-deoxy-α-D-galactopyranosyl phosphate (**6**).—According to procedure B, 2-amino-2-deoxy-α-D-galactopyranosyl phosphate (**4**, 81 mg, 0.31 mmol) and *N*-butyroxy-succinimide were converted to the title compound **6** (86 mg, 84%). $[\alpha]_D^{20}$ +79° (*c* 1.0, water).

2-(2-Bromoacetamido)-2-deoxy-α-D-galactopyranosyl phosphate (7).—2-Amino-2-deoxy-α-D-galactopyranosyl phosphate (**4**, 103 mg, 0.40 mmol) was reacted with *N*-(2-bromo-acetoxy) succinimide as in procedure B, thus obtaining the title compound **7** (124 mg, 82%). $[\alpha]_D^{20}$ +62° (*c* 1.0, water).

2-Azido-2-deoxy-α-D-galactopyranosyl phosphate (**8**).—Prior to the reaction, a fresh solution of triflic azide was prepared by procedure A within the given quantity. This solution was added dropwise to a solution of 2-amino-2-deoxy-α-D-galactopyranosyl phosphate (**4**, 158 mg, 0.61 mmol), K_2CO_3 (127 mg, 0.92 mmol) and a catalytic amount of $CuSO_4 \cdot 5\ H_2O$ in 3:2 water–MeOH (10 mL). In addition, MeOH (14.5 mL) and water (7 mL) were added resulting in a cloudy solution which was stirred for 2 days at rt. Purification was performed utilizing Biogel P2 chromatography with 0.25 M NH_4HCO_3 as eluent to give the azide **8** (115 mg, 59%). IR (KBr): ν 2087 cm^{-1} (N_3).

Uridine 5′-(2-amino-2-deoxy-α-D-galactopyranosyl diphosphate) (**9**).—Both 2-amino-2-deoxy-α-D-galactopyranosyl phosphate (442 mg, 1.6 mmol) as well as UDP glucose (732 mg, 1.2 mmol) were each separately dissolved in a solution of 2-mercaptoethanol in water (5 mM, 20 mL). 0.5 mL of both solutions were combined (20 × 1 mL in whole) and each treated with galactose-1-phosphate uridyl transferase (0.5 U, E.C. 2.7.7.12) followed by adjusting the pH to 8.7 and shaking at 30 °C for 3 days. Within this time, the pH was readjusted to 8.7 every 24 h. The combined solutions were purified twice over Biogel P2 with 0.25 M NH_4HCO_3 as eluent followed by desalting on Biogel P2 with water yielding UDP-GalN (112 mg, 12%) as a colorless hygroscopic solid.

Uridine 5′-(2-deoxy-2-propionylamido-α-D-galactopyranosyl diphosphate) (**10**).—2-Propionylamido-2-deoxy-α-D-galactopyranosyl phosphate (**5**) in form of its triethylammonium salt (92 mg, 177 μmol) was reacted with the morpholidate coupling reagent (195 mg, 284 μmol) as in procedure C to give compound **10** (35 mg, 31%).

Uridine 5′-(2-butyramido-2-deoxy-α-D-galactopyranosyl diphosphate) (**11**).—The triethylammonium salt of 2-butyramido-2-deoxy-α-D-galactopyranosyl phosphate (**6**, 89 mg, 167 μmol) was reacted with the morpholidate reagent (181 mg, 264 μmol) according to procedure C to give the title compound **11** (35 mg, 32%) as a white solid.

Uridine 5′-(2-(2-bromoacetamido)-2-deoxy-α-D-galactopyranosyl diphosphate) (**12**).—According to procedure C, the triethylammonium salt of 2 - (2 - bromoacetamido) - 2 - deoxy-α-D-galactopyranosyl phosphate (**7**, 39 mg, 66.9 μmol) was reacted with the morpholidate reagent (77 mg, 112 μmol) resulting in the title compound **12** (14 mg, 30%) as a white solid.

Uridine 5′-(2-azido-2-deoxy-α-D-galactopyranosyl diphosphate) (**13**).—According to procedure C, the triethylammonium salt of 2-azido-2-deoxy-α-D-galactopyranosyl phosphate (**8**, 90 mg, 185 μmol) was reacted with the morpholidate reagent (203 mg, 295 μmol) resulting in the title compound **13** (28 mg, 25%) as a white solid.

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