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Carbohydrate Research 342 (2007) 1831–1840
Carbohydrate RESEARCH

Synthesis of fluorescently labelled and internally quenched UDP-Gal probes

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Received 15 February 2007; received in revised form 27 March 2007; accepted 31 March 2007
Available online 11 April 2007

Abstract—The preparation of fluorescently labelled and internally quenched UDP-Gal probes bearing a fluorescence emitter and a quencher is described. The rate of transfer using several galactosyltransferases was examined. Our results demonstrate that galactose-modified, sugar-nucleotide-modified and double modified UDP-Gal analogues are recognized as weak substrates by blood group B α-(1→3) galactosyltransferase, α-(1→3) galactosyltransferase and milk bovine β-(1→4) galactosyltransferase.

Keywords: Carbohydrates; Nucleotides; Glycosylations; Galactosyltransferases; Oligosaccharides; UDP-Gal mimetics

1. Introduction

Carbohydrates in the form of glycoconjugates are involved in many intracellular and intercellular processes such as the trafficking of proteins, bacterial and viral infection, normal cell differentiation, tumour progression and metastasis.[1] The biosynthesis of mammalian oligosaccharides in vivo is mediated by glycosyltransferases that sequentially transfer a single pyranosyl residue from a sugar nucleotide donor to a growing carbohydrate chain acceptor. Although, many synthetic approaches using modified acceptor structures have been reported, very little is known about the acceptance of unnatural sugar nucleotide structures.[2] In the past a variety of assays such as radiochemical assays,[3] ELISA assays,[4] coupled enzyme assays,[5] and recently capillary electrophoresis (CE) with laser induced fluorescence detection[6] have been developed to measure the activity of glycosyltransferases. However, there is a demand for sensitive assays amenable to high throughput screening. To search for sensitive methods to determine glycosyltransferase activity, we became interested in developing a glycosyltransferase assay based on internal fluorescence quenching. Internal fluorescence quenching[7] assays have previously been used to determine the enzymatic activity of hydrolytic enzymes such as peptidases and proteases[8] DNA-joining reactions[9] and sialyltransferases.[10] The assay requires a substrate bearing a fluorescent emitter and a fluorescent quencher on each side of the cleavable bond. Enzymatic cleavage of the molecule is followed by release of the fluorescence as the proximity of the two chromophores decreases. A variety of donor/quencher pairs such as 5-(2-aminoethyl)aminonapthalene-1-sulfonic acid (Edans)/4-(4-dimethylaminophenylazo)benzoic acid (Dabsyl)[8a] and *o*-aminobenzamide (ABz)/3-nitrotryrosine [Tyr(NO_2)][8b] have been previously described to have ideal spectroscopic properties for internal fluorescence quenching.

This paper describes the synthesis of the UDP-Gal sugar nucleotide analogue **1** bearing an emitter (ABz) on the galactose moiety and a quencher [Tyr(NO_2)] on the uracil portion (Fig. 1). The sugar nucleotide analogues **2** and **3** were also prepared. A preliminary account without experimental details on the synthesis of fluorescence labelled UDP-Gal analogues was recently communicated.[11]

The modified sugar nucleotide analogue **1** was designed based on the following rationale: (a) both fluorophores are linked to the sugar nucleotide via short

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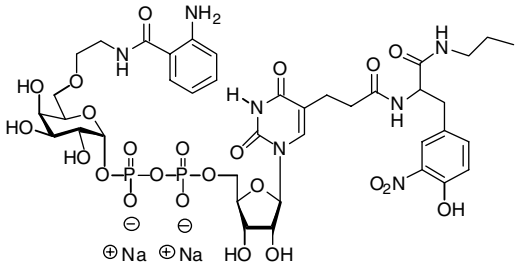
doi:10.1016/j.carres.2007.03.029



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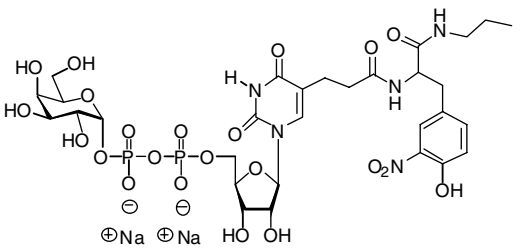

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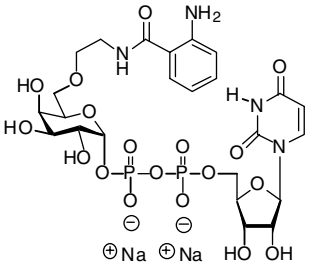

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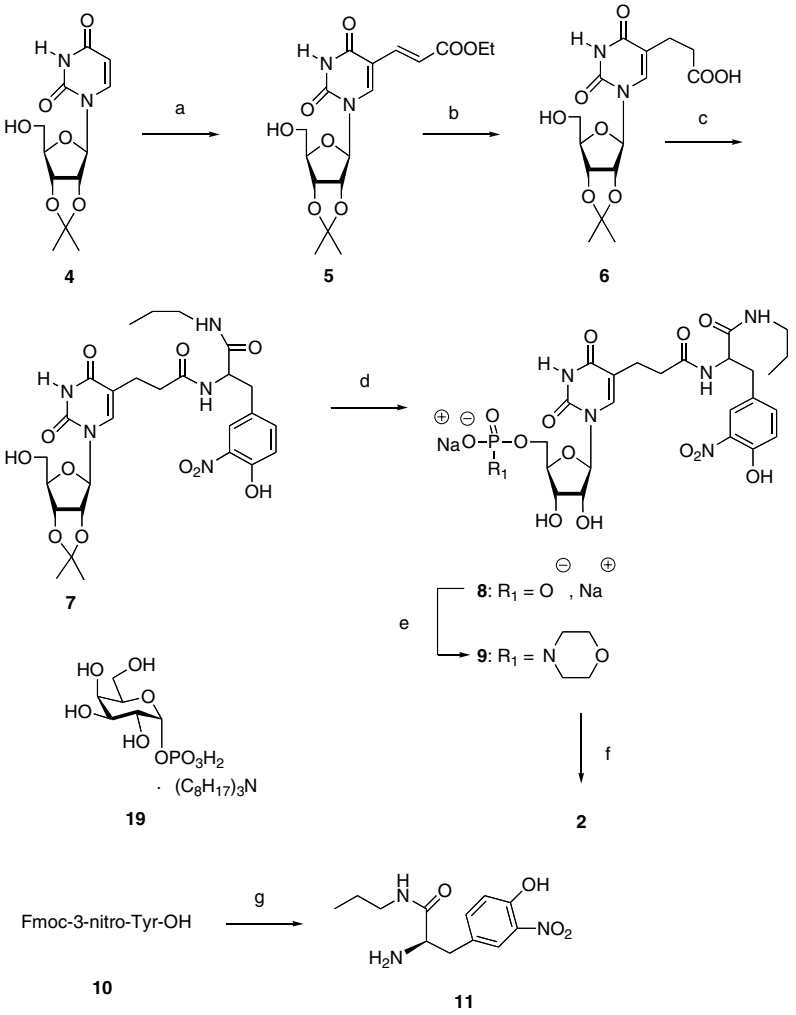
Figure 1. Structure of internally fluorescence-quenched UDP-Gal analog **1** and fluorescence labelled UDP-Gal analogs **2** and **3**.

linkers to ensure maximal fluorescence quenching; (b) the ABz-fluorophore is linked to the 6-position of galactose because it is expected that modifications at the 6-position would have the best chance of retaining enzymatic activity; (c) the quencher is linked to the 5-position of uridine as 5-modified uridine analogues are well documented in the literature.[12]

2. Results and discussion

The synthesis of the uridine-modified portion of the sugar nucleotide containing the quencher is outlined in Scheme 1. Palladium-catalyzed oxidative coupling of 2′,3′-*O*-isopropylidene uridine with methyl acrylate in acetonitrile afforded the 5-substituted uridine **5** in 51% yield.[13] Hydrogenation of the double bond using Pearlman's catalyst and ester hydrolysis (0.5 M KOH) provided the acid **6**. Compound **6** was then used to install the quencher moiety. This was achieved through coupling of the amine **11** (prepared in a two step procedure from commercially available *N*-Fmoc-*p*-nitro-phenylalanine) with the acid **6** to yield the amide **7** in 81% yield. Phosphorylation of the 5′-positon of ribose was achieved through reaction with phosphorus oxychloride in triethylphosphate,[14] followed by deblocking of the isopropylidene protecting group (2 N HCl/H_2O) and ion exchange chromatography to afford phosphate **8**. Compound **8** was activated as the nucleoside 5′monophosphomorpholidate **9** by treatment with 1,3-dicyclohexylcarbodiimide and morpholine in *tert*-butyl alcohol at elevated temperature. Subsequently, the activated nucleoside monophosphate was coupled to the galactose 1-phosphate **19** (Sigma) under 1*H*-tetrazole catalyzed conditions[15] to furnish the uracil-modified sugar nucleotide **2** in 33% yield.

The synthesis of the galactose-modified portion of the sugar nucleotide containing the emitter moiety is outlined in Scheme 2. O-Cyanomethylation[16] of 1,2:3,4-di-*O*-isopropylidene-D-galactopyranose (NaH, CH_3CN, $BrCH_2CN$) afforded the cyano compound **13** in 81% yield. Compound **13** was effectively reduced to the amine using the borane–methyl sulfide complex in tetrahydrofuran before being converted into the hydrochloride salt **14**. Compound **14** was then used to install the emitter moiety by coupling to Fmoc-protected *o*-amino benzoic acid. This was achieved by using 2-(1*H*-benzotriazole-1yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU) as the coupling reagent to afford the amide **15** in 83% yield. Deblocking of the galactose portion (80% HOAc, 90 °C) followed by acetylation and selective deacetylation ($BnNH_2$, THF) of the anomeric hydroxyl group provided the hemiacetal **16** in 52% overall yield after three steps. The phosphate group was installed by treatment of **16** with lithium bis(trimethylsilyl)amide (1 M solution in hexane) in THF at −78 °C followed by trapping of the intermediate anion with dibenzylphosphoryl chloride to produce the phosphate ester **17** in 50% yield. Deblocking of the compound was achieved by a three-step procedure. First, the benzyl protecting groups on the phosphate were removed by hydrogenation using Pearlman's catalyst followed by treatment with 20% piperidine in DMF to ensure complete cleavage of the Fmoc-protecting group and purification by chromatography. The remaining acetate protecting groups were cleaved by exposure to a ternary solvent mixture ($Et_3NH/MeOH/H_2O$, 1:3:1) to produce the galactose-modified emitter moiety, which was subsequently converted into the sodium form **18** for stability reasons. The pyridinium salt of monophosphate **18** was then coupled to UMP (uridine 5′-monophosphomorpholidate 4-morpholino-*N*,*N*′dicyclohexylcarboxamidine salt, Sigma) in pyridine to afford the emitter-modified UDP-Gal analogue **3** in 40% yield. The same coupling conditions were applied to couple the uridine-modified quencher moiety **9** with the pyridinium salt of **18** to produce the target compound **1** in an overall yield of 21% yield.

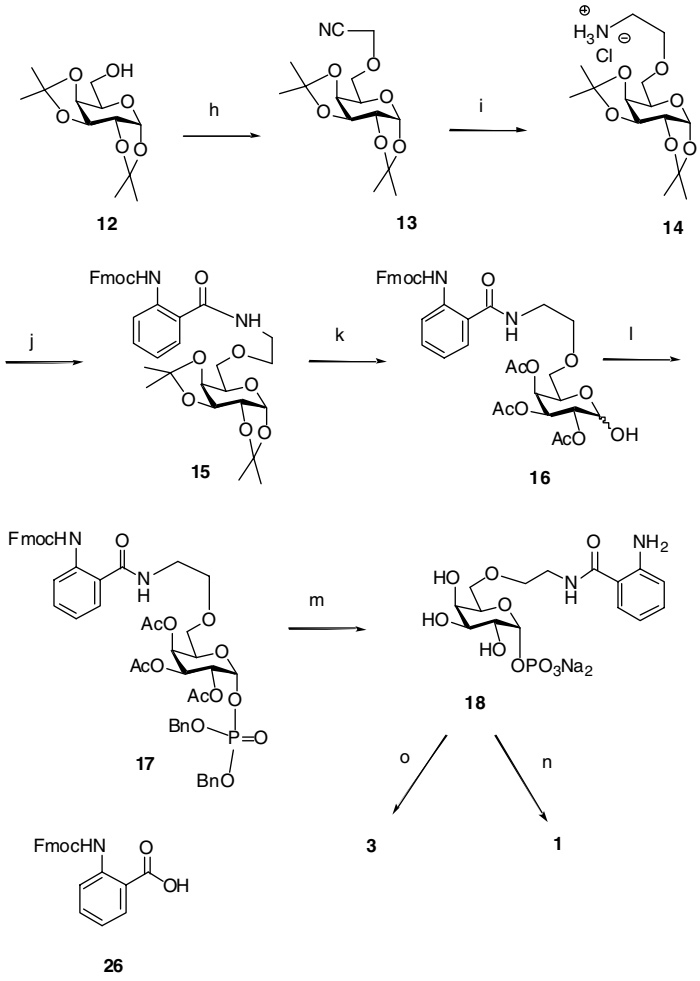


Scheme 1. Synthesis of UDP-Gal analogue **2**. Reagents and conditions: (a) $Pd(OAc)_2$, $CH_2CHCOOEt$, CH_3CN (51%); (b) (1) $H_2/Pd(OH)_2$; (2) KOH (94%); (c) **11**, TBTU, DMF, DIEA (81%); (d) (1) $POCl_3$, $PO(OEt)_3$; (2) HCl (51%); (e) DCC, morpholine, *tert*-butanol/H_2O; 1:1, 95 °C (89%); (f) **19**, 1*H*-tetrazole (33%); (g) (1) TBTU, DMF, $CH_3(CH_2)_2NH_2$; (2) 20% piperidine/DMF (80%).

With UDP-Gal analogues **1**–**3** in hand, they were then studied as potential substrates for galactosyltransferase reactions. The enzymes selected were as follows: (1) blood group B α-(1→3) galactosyltransferase (blood group B α-(1→3) GalT E.C. 2.4.1.37),[17] which catalyzes the transfer of D-galactopyranose to from UDP-Gal to the 3-hydroxyl group of α-L-Fuc*p*-(1→2)-β-D-Gal*p*-OR; (2) α-(1→3) galactosyltransferase,[18] which catalyzes the transfer of D-galactopyranose from UDP-Gal to the 3′-hydroxyl group of β-D-Gal*p*-(1→4)-β-D-Glc*p*-OR; (3) milk bovine β-(1→4) GalT,[19] which catalyzes the transfer of D-galactopyranose from UDP-Gal to the 4-hydroxyl group of Glc*p*(NAc)-OR. Initially, the transfer reactions of UDP-Gal analogues **1**–**3** with substrates **20**–**22** were studied as shown in Scheme 3. In all cases except for the milk β-(1→4) GalT catalyzed reactions of donors **3** and **1** with acceptor **22**, transfer of the galactose unit from the UDP-Gal donor analogues **1**–**3** to the acceptors **20**–**22** was observed as evidenced by MALDI TOF analysis of the reaction products. In the case of UDP-Gal analogue **2**, it was possible to monitor and measure (see Table 1) the relative rate of transfer when compared to unmodified UDP-Gal by TLC. For all other transfer reactions TLC quantification was not possible due to the small amount of product formed during the transfer reaction. Due to the low transfer rates of UDP-Gal modified donors **1**–**3**, no other quantification methods such as CE and fluorescence quenching were attempted.

3. Conclusion

In conclusion, a route for the synthesis of galactose-, uracil-modified UDP-Gal analogues as well as an internally quenched UDP-Gal analogue have been developed. Most of the UDP-Gal analogues act as weak



Scheme 2. Synthesis of UDP-Gal analogues **1** and **3**. Reagents and conditions: (h) CH_3CN, NaH, CH_2BrCN (81%); (i) (1) $BH_3{\cdot}Me_2S$, THF; (2) HCl (75%); (j) **26**, TBTU, DIEA, DMF (83%); (k) (1) 80% HOAc, 80 °C; (2) Ac_2O, pyridine; (3) $BnNH_2$, THF (52%); (l) LiHMDS, THF, $POCl(OBn)_2$, −49 °C (50%); (m) (1) $Pd(OH)_2$, H_2; (2) 20% piperdine/DMF; (3) $Et_3N/MeOH/H_2O$; 1:3:3; (4) ion exchange (78%); (n) **9**, 1*H*-tetrazole, pyridine (21%); (o) uridine 5′monophosphomorpholidate-4-morpholino-*N*,*N*′cyclohexylcarbox-aminidine salt, pyridine, 1*H*-tetrazole (40%).

substrates for three selected galactosyltransferases and permit transfer of their galactose unit to the acceptor.

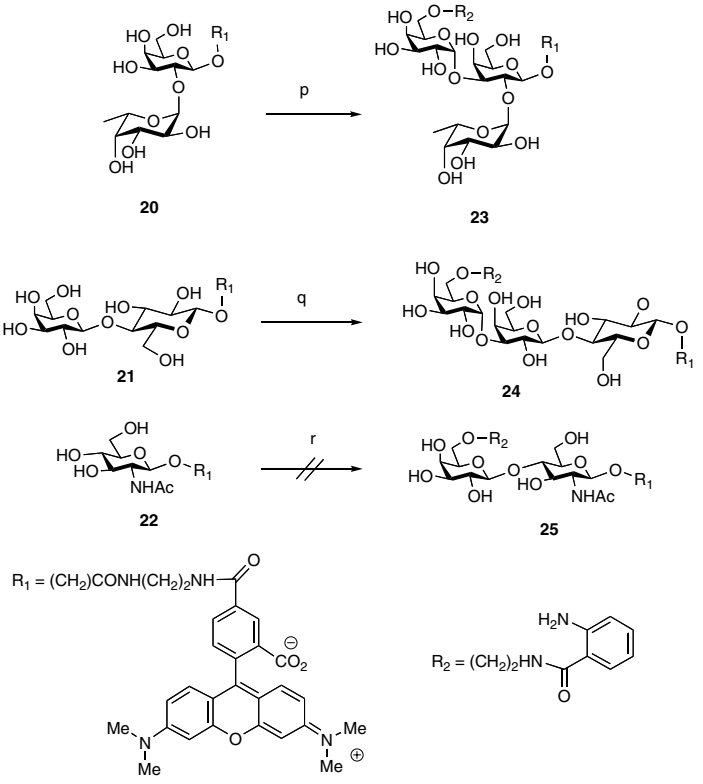
4. Experimental

4.1. General methods

CH_2Cl_2 was distilled from calcium hydride. Organic solutions were concentrated under diminished pressure at <40 °C (bath temperature). NMR spectra were recorded at 360 MHz or 500 MHz for ^1H and at 75 MHz for ^{13}C. Chemical shifts are reported relative to $CHCl_3$ [δ_H 7.26, δ_C (centre of triplet) 77.0] or to CH_3OH [δ_H 73.35, δ_C (centre of septet) 49.0] to acetone as internal standard (D_2O) or phosphoric acid. TLC was performed on E. Merck Silica Gel 60 F_{254} with detection by charring with 8% H_2SO_4 acid. Silica gel (0.040–0.063 mm) was used for column chromatography.

4.2. 5-(2-Propenoic acid)-2′,3′-*O*-isopropylidene uridine ethyl ester (5)

Commercially available **4** (1420 mg, 5 mmol) and $Pd(OAc)_2$ (1380 mg, 6.14 mmol) were suspended in acetonitrile (5 mL) and heated under reflux for 5 h. The solution was cooled and filtered over Celite. The Celite was washed with 50% EtOAc in MeOH (250 mL) and the organic washings were combined. The solvent was removed under reduced pressure and the crude product was crystallized from 10% MeOH in EtOAc to afford compound **5** (970 mg, 2.53 mmol, 51%) as transparent crystals. Compound **5**: ^1H NMR (DMSO): δ 1.22 (t, 3H, Me), 1.48 (s, 3H, Me), 1.65 (s, 3H, Me), 3.54–3.59 (m, 1H, H-5′a), 3.62–3.66 (m, 1H, H-5′b), 4.10–4.16 (m, 3H, H-4′, –OC*H*$_2$CH$_3$), 4.77 (dd, 1H, $J = 3.5$ Hz, $J = 6.6$ Hz, H-3′), 4.95 (dd, 1H, $J = 2.5$ Hz, H-2′), 5.20 (br t, OH, exchanges with D_2O), 5.85 (d, 1H, H-1′), 6.82 (d, 1H, $J = 15.9$ Hz,



Scheme 3. Glycosyltransferase-catalyzed reactions of UDP-Gal analogue **1** with acceptors **20**–**22** to products **23**–**24**. (p) **1**, blood group B α-(1→3) GalT; (q) **1**, α-(1→3) GalT; (r) **1**, milk β-(1→4) GalT.

Table 1. Relative rates of transfer of donor **2** with acceptors **20**–**22** compared to unmodified UDP-Gal

Enzyme	Donor	v_{rel} (%)
Calf thymus α-(1→3) GalT	**2**	1
Blood group B α-(1→3) GalT	**2**	1
Milk bovine β-(1→4) GalT	**2**	3

CHC*H*CO_2Et), 7.31 (d, 1H, C*H*CHCO_2Et), 8.33 (s, 1H, H-6), 11.45 (br, 1H, NH, exchanges with D_2O); ESIMS $[M+H]^+$: 383.1. Anal. Calcd for $C_{17}H_{22}N_2O_8$: C, 53.40; H, 5.80; N, 7.33. Found: 53.21, H, 5.96; N, 7.49.

4.3. 5-(Propanoic acid)-2′,3′-*O*-isopropylidene uridine (6)

Compound **5** (950 mg, 2.54 mmol) was dissolved in 40% MeOH in EtOAc (180 mL). Pearlman's catalyst (200 mg) was added and the alkene was hydrogenated at atmospheric pressure for 5 h. The reaction mixture was filtered and the solvent was removed under reduced pressure. The solid residue (950 mg) was dissolved in 0.5 M KOH (50 mL). After 3 h the solution was acidified with concentrated hydrochloric acid and the aqueous layer was extracted with EtOAc (6 × 50 mL). The combined organic layers were washed with brine and concentrated under reduced pressure to afford compound **6** (850 mg, 2.38 mmol, 94%) as a white solid. ^{1}H NMR (DMSO): δ 1.22 (t, 3H, Me), 1.48 (s, 3H, Me), 1.65 (s, 3H, Me), 2.35–2.45 (m, 4H, –CH_2CH_2COOH), 3.58 (m, 1H, $J = 4.7$ Hz, $J = 11.8$ Hz, H-5′a), 4.05 (dd, 1H, $J = 4.5$ Hz, $J = 11.89$ Hz, H-5′b), 4.77 (dd, 1H, $J = 3.7$ Hz, $J = 6.5$ Hz, H-3′), 4.88 (dd, 1H, $J = 2.8$ Hz, $J = 6.5$ Hz, H-2′), 5.20 (br s, OH, exchanges with D_2O), 5.84 (d, 1H, $J = 2.8$ Hz, H-1′), 7.67 (s, 1H, H-6), 10.5 (br s, COOH, exchanges with D_2O), 11.45 (br, 1H, NH, exchanges with D_2O); ESIMS *m*/*z* calcd for $[C_{15}H_{21}N_2O_8+Na]^+$: 379.1117; found, 379.1118.

4.4. HTyr(NO_2)-NHpropyl (11)

Commercially available Fmoc-3-nitro-Tyr-OH (200 mg, 0.44 mmol), TBTU (186 mg, 0.58 mmol) and propylamine (130 μL, 1.6 mmol) were dissolved in DMF (5 mL). The reaction was stirred for 2 h. The solvent was removed under reduced pressure and the solid residue was codistilled with toluene (3×) before being concentrated under reduced pressure. The solid residue was taken into CH_2Cl_2 (25 mL) and 1 M hydrochloric acid (10 mL) was added. The organic layer separated and the solvent was removed under reduced pressure. The crude orange residue was dissolved in a mixture containing CH_2Cl_2 (10 mL) and piperidine (3 mL). After 40 min the solvent was removed under reduced pressure and the crude material was purified by column chroma-

tography using 3% MeOH in CH_2Cl_2 as eluent to afford **11** (45 mg, 80%) which was directly used for the coupling to **6**.

4.5. 2′,3′-*O*-Isopropylidene uridine-5-(ethylcarboxamide *N*-(Tyr-(3-nitro)) *N*′-propylamide) (7)

Compound **6** (1.00 g, 2.81 mmol) and compound **11** (0.98 g, 3.65 mmol) and TBTU (1.17 g, 3.64 mmol) were dissolved in DMF (20 mL) and diisopropylethylamine (733 μL) was added. After 2 h the solvent was removed under reduced pressure and the crude residue was codistilled with toluene (3 × 30 mL). The product was purified by column chromatography using 3% MeOH in CH_2Cl_2 as eluent to afford compound **7** (1.38 g, 2.28 mmol, 81%). ^{1}H NMR (DMSO): δ 0.78 (t, 3H, $CH_2CH_2CH_3$), 1.35 (s, 3H, Me), 1.35–1.45 (m, 2H, $CH_2CH_2CH_3$), 1.55 (s, 3H, Me), 2.52–2.67 (m, 4H, –$CH_2CH_2CO_2NH$–), 2.88 (dd, 1H, $J = 6.1$ Hz, $J = 13.6$ Hz, ArCH_2–), 2.94–3.03 (m, 2H, ArCH_2–, –CONHCH_2), 3.26–3.29 (m, 1H, CONHCH_2), 3.82 (dd, 1H, $J = 2.9$ Hz, $J = 12.5$ Hz, H-5′a), 3.92 (dd, 1H, 2.0 Hz, $J = 12.5$ Hz, H-5′b), 4.20 (s, 1H, OH, exchanges with D_2O), 4.30 (m, 1H, H-4′), 4.46 (m, 1H, Hα(Tyr(NO_2))), 4.85 (dd, 1H, $J = 3.1$ Hz, $J = 6.3$ Hz, H-2′), 4.93 (1H, dd, $J = 6.3$ Hz, 2.6 Hz, H-3′), 5.86 (d, 1H, $J = 3.1$ Hz, H-1′), 6.30 (br t, 1H, NH, exchanges with D_2O), 7.02 (d, 1H, $J = 8.6$ Hz, Ar-H), 7.39 (dd, 1H, $J = 2.1$ Hz, $J = 8.6$ Hz), 7.50 (s, 1H, Ar-H), 7.95 (dd, 1H, $J = 2.1$ Hz, Ar-H), 8.09 (br d, 1H, NH, exchanges with D_2O), 10.1 (br s, 1H, exchanges with D_2O), 10.5 (br s, 1H exchanges with D_2O); ESIMS $[M+H]^+$: 606.2. Anal. Calcd for $C_{27}H_{35}N_5O_{11}$: C, 53.55; H, 5.83; N, 11.56. Found: C, 53.21, H, 5.74; N, 11.39.

4.6. Disodium 2′,3′-*O*-isopropylidene-5′-*O*-phosphate uridine-5-(ethylcarboxamide *N*-(Tyr-(3-nitro)) *N*′-propylamide) (8)

Compound **7** (1.38 g, 2.27 mmol) was dissolved in triethylphosphate (10 mL) and the reaction mixture was cooled to 0 °C. Phosphoryl chloride ($POCl_3$, 850 mL, 9 mmol) was slowly added and the ice bath was removed immediately after addition. After 16 h water (100 mL) and ether (50 mL) were added. The aqueous layer was concentrated to dryness and the crude solid residue was dissolved in 2 M hydrochloric acid (60 mL) and left for 18 h. Solid sodium bicarbonate was added to neutralize the pH and the solution was evaporated to dryness. The crude material was desalted on a C-18 column using $H_2O \rightarrow 50\%$ MeOH/H_2O as eluent. The combined fractions containing a yellow material were concentrated. Subsequently, the material was converted into the disodium salt using the AG 50W-X2 resin (Bio Rad). The collected yellow fractions were filtered through a paper filter concentrated and purified by a P2 column (Bio Rad) to afford compound **8** (800 mg, 1.16 mmol, 51%). ^{1}H NMR (D_2O): δ 0.72 (t, 3H, $CH_2CH_2CH_3$), 1.30–1.40 (m, 2H, $CH_2CH_2CH_3$), 2.50–2.68 (m, 4H, –$CH_2CH_2CO_2NH$–), 2.92–3.01 (m, 3H, 2 × ArCH_2, –CONHCH_2), 3.10–3.16 (m, 1H, –CONHCH_2), 3.98–4.04 (m, 2H, H′5a, H5′b), 4.21–4.25 (m, 1H, H-4′), 4.31–4.35 (m, 1H, H-3′), 4.36–4.41 (m, 2H, H-2′, Hα(Tyr(NO_2))), 5.93 (d, 1H, $J = 5.5$ Hz, H-1′), 6.94 (d, 1H, $J = 8.6$ Hz, Ar-H), 7.36 (d, 1H, $J = 8.6$ Hz, Ar-H), 7.74 (s, 1H, Ar-H), 7.82 (s, 1H, Ar-H); ^{13}C NMR (D_2O): δ 10.5, 15.3, 15.4, 34.0, 36.0, 41.1, 55.4, 65.4 (d, $J_{C,P} = 6.5$ Hz), 70.2, 73.6, 84.0 (d, $J_{C,P} = 7.5$ Hz), 88.3, 113.6, 122.8, 125.0, 125.9, 135.2, 137.7, 138.2, 151.7, 158.8, 165.5, 172.8, 175.1; ESIMS *m*/*z* calcd for $[C_{24}H_{32}N_5O_{14}P+Na]^+$: 668.1581; found, 668.1587.

4.7. Sodium 2′,3′-*O*-isopropylidene-5′-*O*-monophosphomorpholidate uridine-5-(ethylcarboxamide *N*-Tyr-(3-nitro) *N*′-propylamide) (9)

Compound **8** (disodium form, 85 mg, 0.131 mmol) was passed through a BIO Rad AG 50W-X2 cation exchange column (H^+, 1.5 × 10 cm) and the solution was concentrated. The residue was dissolved in a 1:1 mixture of water (2 mL) and *tert*-butanol (2 mL), morpholine (50 μL, 0.58 mmol) and *N*,*N*′-dicyclohexylcarbodiimide (105 mg, 0.51 mmol) was added and the reaction mixture was refluxed for 2 h. TLC (isopropanol/1 M ammonium acetate, 2:1) showed complete conversion into a higher moving spot. The solution was filtered and partitioned with ether. The aqueous layer was dried under reduced pressure, dissolved in water and passed through a BIO Rad AG 50W-X2 cation exchange column (Na^+, 1.5 × 10 cm). Evaporation of the solvent under reduced pressure provided **9** (86 mg, 89%) that was directly used for the next reaction. ^{1}H NMR (D_2O): δ 0.66 (t, 3H, –$CH_2CH_2CH_3$), 1.22–1.35 (m, 2H, –$CH_2CH_2CH_3$), 2.46–2.62 (m, 4H, –$CH_2CH_2CO_2NH$–), 2.82 (dd, 1H, $J = 8.7$ Hz, $J = 13.6$ Hz, ArCH_2–), 2.86–2.95 (m, 2H, ArCH_2–, –CONHCH_2–), 3.01–3.06 (m, 4H, 2 × –NCH_2CH_2O–), 3.07–3.13 (m, 1H, –CONHCH_2–), 3.60–3.70 (m, 4H, 2 × –NCH_2CH_2O–), 3.96–4.08 (m, 2H, H5′a, H5′b), 4.17–4.22 (m, 1H, H-4′), 4.28 (dd, 1H, $J = 5.3$ Hz, $J = 5.5$ Hz, H-3′), 4.31–4.35 (m, 2H, H-2′, Hα(Tyr(NO_2))), 5.95 (d, 1H, $J = 5.3$ Hz, H-1′), 6.72 (d, 1H, $J = 8.9$ Hz, Ar-*H*), 7.17 (dd, 1H, $J = 2.4$ Hz, 8.9 Hz, Ar-*H*), 7.49 (s, 1H, Ar-H), 7.70 (d, 1H, $J = 2.4$ Hz, Ar-H); ^{31}P NMR (D_2O, H_3PO_4): δ 3.40 (s); ESIMS *m*/*z* calcd for $[C_{28}H_{39}N_6O_{14}P+Na]^+$: 737.2159; found, 737.2162.

4.8. Synthesis of UDP-Gal analogue (2)

Dipotassium-α-D-galactosyl phosphate analogue **18** (42 mg, 125 μmol) was passed through a BIO Rad AG

50W-X2 cation exchange column (pyridinium form, 1.5×5 cm) and the solution was concentrated and codistilled (2×8 mL) with dry pyridine. Trioctylamine (55 μL, 125 μmol) and dry pyridine (5 mL) was added, concentrated and the mixture was co-evaporated with dry pyridine (3×6 mL) to form **19**. Uridine 5′-monophosphomorpholidate-4-morpholino-*N*,*N*′-dicyclohexylcarboxamidinium salt analogue **9** (191 mg, 189 μmol) was added and the mixture was co-evaporated with dry pyridine (3×6 mL). 1*H*-Tetrazole (30 mg, 426 μmol) and dry pyridine (2 mL) were added and the solution was stirred at rt. The reaction was monitored by TLC (2:1, *i*-PrOH/1 M NH_4OAc). After 20 h, the mixture was diluted with water (2 mL) and evaporated. The residue was suspended in 4 mL of 100 mM NH_4HCO_3 and extracted with ether (3 mL). After evaporation, the residue was purified on a Bio-Gel P-2 column (2×65 cm), eluted with 100 mM NH_4HCO_3. The product-containing fractions were concentrated, dissolved in H_2O and further purified using gradient reverse phase column chromatography on C-18 silica ($H_2O \rightarrow 50\%$ H_2O/MeOH) before ion exchanged with a BIO Rad AG 50W-X2 cation exchange column (sodium form, 1.5×5 cm) and lyophilized to provide the UDP-Gal analogue **2** (39.4 mg, 33% yield). ^{1}H NMR (D_2O): δ 0.66 (t, 3H, $-CH_2CH_2CH_3$), 1.30–1.40 (m, 2H, $-CH_2CH_2CH_3$), 2.50–2.68 (m, 4H, $-CH_2CH_2CO_2NH-$), 2.94–3.04 (m, 3H, $2 \times ArCH_2-$, $CONHCH_2-$), 3.10–3.16 (m, 1H, $-CONHCH_2-$), 3.72 (dd, 1H, $J = 4.9$ Hz, $J = 12.3$ Hz, H-6a galactose), 3.76 (dd, 1H, $J = 6.6$ Hz, $J = 12.3$ Hz, H-6b galactose), 3.79–3.83 (m, 1H, H-2 galactose), 3.91 (dd, 1H, $J = 9.9$ Hz, $J = 2.4$ Hz, H-3 galactose), 4.0 (dd, $J = 2.4$ Hz, $J \sim 1$ Hz, H-4 galactose), 4.14–4.18 (m, 1H, H-5 galactose), 4.20–4.30 (m, 3H, $2 \times$ H-5 ribose, H-3 ribose), 4.34–4.39 (m, 2H, H-2 ribose, H-4 ribose), 4.40–4.44 (m, 1H, Hα(Tyr(NO_2))), 5.66 (dd, 1H, $J_{H1,H2} = 3.8$ Hz, $J_{H1,P} = 6.1$ Hz, H-1 galactose), 5.93 (d, 1H, $J = 5.3$ Hz, H-1 ribose), 7.0 (d, 1H, $J = 7.7$ Hz, Ar-H), 7.43 (dd, 1H, $J = 7.7$ Hz, $J \sim 1$ Hz, Ar-H), 7.66 (s, 1H, Ar-H), 7.88 (d, 1H, $J \sim 1$ Hz); ^{31}P NMR (D_2O, H_3PO_4): δ −12.6 (d, $J_{P,P} = 20.8$ Hz, P attached to galactose), −11.2 (d, $J_{P,P} = 20.8$ Hz, P attached to uridine); ESIMS *m*/*z* calcd for $[C_{30}H_{41}N_5Na_3O_{22}P_2]^+$: 954.1412; found, 954.1411.

4.9. 6-*O*-(Cyanomethyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside (13)

Alcohol **12** (500 mg, 1.92 mmol) was dissolved in acetonitrile (10 mL) and sodium hydride (400 mg, 60% suspension in mineral oil) was added and the reaction was stirred at 0 °C. After 30 min bromoacetonitrile (600 μL, 9.6 mmol) was added dropwise at 0 °C. After 2 h CH_2Cl_2 (40 mL) was added and the solution was filtered over Celite and concentrated under reduced pressure. Column chromatography using 25% EtOAc in hexane provided compound **13** (453 mg, 81%). ^{1}H NMR ($CDCl_3$): δ 1.29 (s, 6H, $2 \times$ Me), 1.40 (s, 3H, Me), 1.48 (s, 3H, Me), 3.67 (dd, 1H, $J = 7.5$ Hz, $J = 10.4$ Hz, H-6a), 3.76 (dd, 1H, $J = 4.4$ Hz, $J = 10.1$ Hz, H-6b), 3.94 (m, 1H, H-5), 4.17 (dd, 1H, $J = 2.0$ Hz, $J = 8.0$ Hz, H-4), 4.25 (d, 1H, $J = 16.2$ Hz, OCH_2CN), 4.28 (dd, 1H, $J = 2.6$ Hz, 5.0 Hz, H-2), 4.32 (d, 1H, $J = 16.2$ Hz, OCH_2CN), 4.56 (dd, 1H, $J = 2.6$ Hz, $J = 8.0$ Hz, H-3), 5.48 (d, 1H, $J = 5.0$ Hz, H-1); ^{13}C NMR ($CDCl_3$): δ 24.46, 24.92, 25.98, 26.05, 56.69, 67.03, 70.35, 70.51, 70.68, 71.09, 96.31, 108.80, 109.61, 115.96 (CN); ESIMS *m*/*z* calcd for $[C_{14}H_{21}NO_6+Na]^+$: 322.1266; found, 322.1261. Anal. Calcd for $C_{14}H_{21}NO_6$: C, 56.18; H, 7.07; N, 4.68. Found: 56.35, H, 7.15; N, 4.51.

4.10. 6-*O*-(Aminoethyl)-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside (14)

Nitrile **13** (108 mg, 0.36 mmol) was dissolved in tetrahydrofuran (4 mL) and borane–dimethyl sulfide complex (140 μL, 1.33 mmol) was added and the solution was refluxed in an inert atmosphere (Ar). After 3 h additional borane–dimethyl sulfide complex (140 μL, 1.33 mmol) was added and the reflux continued for 3 h. The solution was cooled and MeOH (1 mL) was slowly added. The condenser was removed and the solution was stirred overnight. On the next day the solvent was removed under reduced pressure and the compound was purified by column chromatography using a ternary solvent mixture (chloroform/MeOH/ammonia; 1000:100:1). The amine was converted into the hydrochloride salt by addition of hydrochloric acid to afford **14** (90 mg, 75%) as a white salt. ^{1}H NMR ($CDCl_3$, as hydrochloride salt): δ 1.30 (s, 3H, Me), 1.33 (s, 3H, Me), 1.43 (s, 3H, Me), 1.55 (s, 3H, Me), 3.20–3.28 (m, 2H), 3.62 (dd, 1H, $J = 7.5$ Hz, $J = 10.3$ Hz, H-6a), 3.68 (dd, 1H, $J = 4.6$ Hz, $J = 10.3$ Hz, H-6b), 3.75–3.80 (m, 1H), 3.81–3.86 (m, 1H), 3.94–3.99 (m, 1H, H-5), 4.23 (dd, 1H, $J = 2.0$ Hz, $J = 8.0$ Hz, H-4), 4.30 (dd, 1H, $J = 2.5$ Hz, $J = 5.0$ Hz, H-2), 4.58 (dd, 1H, $J = 2.5$ Hz, 8.0 Hz, H-3), 5.52 (d, 1H, $J = 5.0$ Hz, H-1), 8.30 (br s, 3H); ^{13}C NMR ($CDCl_3$ as free amine): δ 24.48, 24.94, 26.00, 26.10, 41.76, 66.74, 69.56, 70.56, 70.67, 71.23, 73.51, 96.39, 108.58, 109.28; ESIMS $[M+H]^+$: 304.2.

4.11. 6-*O*-[*N*(Fmoc)-(ABz)aminoethyl]-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside (15)

Compound **14** (110 mg, 0.32 mmol), TBTU (110 mg, 0.34 mmol), *N*(Fmoc)-ABz (97.7 mg, 0.272 mmol) and DIEA (130 μL, 0.64 mmol) were dissolved in DMF (2 mL) and stirred for 2 h. The solvent was removed under reduced pressure and the residue was taken into

water (5 mL) and extracted with EtOAc (5 × 3 mL). The combined organic layer was dried over sodium sulfate, filtered and purified by column chromatography using 40% EtOAc in hexane as eluent to afford **15** (145 mg, 0.23 mmol, 83%). ^{1}H NMR ($CDCl_3$): δ 1.30 (s, 3H, Me), 1.34 (s, 3H, Me), 1.43 (s, 3H, Me), 1.50 (s, 3H, Me), 3.62–3.76 (m, 6H, H-6a, H-6b, 2 × –OC*H*$_2$CH$_2$NH–, 2 × –OCH$_2$C*H*$_2$NH–), 3.96–4.0 (m, 1H, H-5), 4.21 (dd, 1H, $J = 2.0$ Hz, 8.0 Hz, H-4), 4.30 (t, 1H, Fmoc), 4.32 (dd, 1H, $J = 2.5$ Hz, 5.0 Hz, H-2), 4.41 (d, 2H, Fmoc), 4.60 (dd, 1H, $J = 2.5$ Hz, $J = 8.0$ Hz, H-3), 5.51 (d, 1H, $J = 5.0$ Hz, H-1), 6.96–7.02 (m, 2H, NH exchanges with D_2O, Ar-H), 7.31 (t, 2H, $J = 7.5$ Hz, Fmoc), 7.38 (t, 2H, $J = 7.5$ Hz, Fmoc), 7.44 (t, 1H, $J = 7.4$ Hz, Ar-H), 7.57 (dd, 1H, $J = 1.5$ Hz, $J = 8.0$ Hz, Ar-H), 7.65 (d, 2H, $J = 8.2$ Hz, Fmoc), 7.75 (d, 2H, $J = 7.5$ Hz, Fmoc), 8.33 (d, 1H, Ar-H), 10.9 (s, 1H, NH, exchanges with D_2O); ESIMS $[M+H]^+$: 645.3; Anal. Calcd for $C_{36}H_{40}N_2O_9$: C, 67.07; H, 6.25; N, 4.35. Found: C, 66.96, H, 6.47; N, 4.57.

4.12. Dibenzyl (2,3,4-tri-*O*-acetyl 6-*O*-[*N*(Fmoc)-(ABz)aminoethyl]-α-D-galactopyranosyl)-phosphate (17)

Compound **15** (560 mg, 0.88 mmol) was dissolved in 80% acetic acid (10 mL) and heated for 12 h at 95 °C. The reaction was cooled and water (30 mL) was added. The aqueous layer was extracted (3×) with EtOAc and the combined organic layers were dried (sodium sulfate) and concentrated under reduced pressure. The residue was dissolved in a mixture containing pyridine (2 mL) and Ac_2O (1 mL) and left for 2 h before the solvent was removed under reduced pressure and codistilled with toluene to afford 680 mg of a crude material. Crude material (530 mg) was dissolved in tetrahydrofuran (10 mL) and benzylamine (103 μL, 0.94 mmol) was added and left for 12 h. The solvent was removed under reduced pressure and the anomeric mixture was purified by gradient column chromatography using 50–30% hexane in EtOAc as eluent to afford compound **16** (340 mg, 52%) as an anomeric mixture. Anomeric mixture **16** was directly used for the conversion into the dibenzylphosphate derivative **17**. Anomeric mixture **16** (59 mg, 0.085 mmol) was dissolved in tetrahydrofuran (2 mL) and cooled to −78 °C before lithium bis(trimethylsilyl) amide (1.0 M in tetrahydrofuran, 90 μL) was added. After 5 min dibenzylphosphoryl chloride (850 μL) of a 10% weight solution in tetrahydrofuran was added and the temperature was raised to −49 °C. After 20 min TLC (33% hexane in EtOAc) showed the absence of starting material and acetic acid (500 μL) and MeOH (1 mL) was added. The solvent was removed under reduced pressure and the product was purified by column chromatography using 50% mixture of hexane and EtOAc to afford **17** (41 mg, 50%) as a white powder. ^{1}H NMR ($CDCl_3$): δ 1.90 (s, 3H, OAc), 2.00 (s, 3H, OAc), 2.05 (s, 3H, OAc), 3.35 (dd, 1H, $J = 6.9$ Hz, 9.9 Hz, H-6a), 3.38 (dd, 1H, $J = 5.2$ Hz, $J = 9.9$ Hz, H-6b), 3.42–3.61 (m, 4H, 2 × –OC*H*$_2$CH$_2$NH–, 2 × –OCH$_2$C*H*$_2$NH–), 4.22–4.26 (m, 1H, H-5), 4.29 (t, 1H, Fmoc), 4.40 (d, 2H, Fmoc), 4.98–5.08 (m, 4H, 4 × –OC*H*$_2$Ph), 5.20 (ddd, 1H, $J = 10.8$ Hz, $J = 3.3$ Hz, $J_{H2,P} = 6.3$ Hz, H-2), 5.32 (dd, 1H, 3.2 Hz, 10.8 Hz, H-3), 5.53 (dd, 1H, $J = 3.2$ Hz, 1.2 Hz, H-4), 5.93 (dd, 1H, $J = 3.3$ Hz, $J_{H1,P} = 6.3$ Hz, H-1), 6.93 (br t, 1H, NH, exchanges with D_2O), 7.03 (ddd, 1H, $J = 1.2$ Hz, $J = 8.0$ Hz, $J = 8.0$ Hz, Ar-H), 7.27–7.36 (m, 12H, Bn and Fmoc), 7.37 (t, 2H, Fmoc), 7.43 (ddd, 1H, $J = 8.0$ Hz, $J = 8.0$ Hz, 1.4 Hz, Ar-H), 7.58 (dd, 1H, $J = 8.0$ Hz, $J = 1.4$ Hz, Ar-H), 7.65 (d, 2H, $J = 7.5$ Hz, Fmoc), 7.75 (d, 2H, $J = 7.6$ Hz, Fmoc), 8.32 (d, 1H, Ar-H), 10.9 (s, 1H, NH, exchanges with D_2O); ^{31}P NMR ($CDCl_3$): δ −0.3 (s); ESIMS $[M+H]^+$: 951.3. Anal. Calcd for $C_{50}H_{51}N_2O_{15}P$: C, 63.15; H, 5.41; N, 2.95. Found: C, 63.39, H, 5.66; N, 3.07.

4.13. Disodium (6-*O*-[*N*(Fmoc)-(ABz)aminoethyl]-α-D-galactopyranosyl)-phosphate (18)

Compound **17** (118 mg, 0.12 mmol) and Pd/C (5%) were suspended in 25% EtOAc in MeOH (volume: 8 mL) and hydrogenated at atmospheric pressure for 4 h. The solution was filtered over Celite and the solvent was removed under reduced pressure. The residue was dissolved in DMF (3 mL) and piperidine (1 mL) and the solution was stirred for 35 min. Subsequently, the solvent was removed under reduced pressure and the solid residue was purified by gradient column chromatography on iatrobeads using EtOAc/MeOH/water (16:4:1→4:2:1). The remaining acetate groups were removed by exposure to a ternary mixture containing Et_3N/MeOH/water (1:3:3) for 12 h. The solvent was removed under reduced pressure and the residue was dissolved in water and passed through a BIO Rad AG 50W-X2 cation exchange column (sodium form, 1.5 × 5 cm) and lyophilized to provide **18** (39 mg, 78%) as a white powder. ^{1}H NMR (D_2O): δ 3.52–3.62 (m, 2H, H-6a, H-6b), 3.70–3.78 (m, 5H), 3.88 (dd, 1H, $J = 3.1$ Hz, $J = 10.2$ Hz, H-3), 3.98 (d, 1H, $J = 3.1$ Hz, H-4), 4.27 (t, 1H), 5.48 (dd, 1H, $J = 3.4$ Hz, $J_{H1,P} = 6.8$ Hz), 6.84 (t, 1H, $J = 7.6$ Hz, Ar-H), 6.88 (d, 1H, $J = 8.2$ Hz, Ar-H), 7.33 (t, 1H, $J = 8.3$ Hz, Ar-H), 7.44 (d, 1H, $J = 8.2$ Hz); ESIMS m/z calcd for $[C_{16}H_{27}N_2NaO_{10}P]^+$: 461.1301; found, 461.1303.

4.14. General procedure for the synthesis of UDP-Gal analogues 1 and 3

Disodium-α-D-galactosyl phosphate analogue **18** (65 mg, 139 μmol) was passed through a BIO Rad AG 50W-X2 cation exchange column (pyridinium form, 1.5 × 5 cm) and the solution was concentrated and codis-

tilled (2 × 8 mL) with dry pyridine. Uridine 5′-monophosphomorpholidate-4-morpholino-*N*,*N*′-dicyclohexylcarboxamidinium salt (190 μmol) or analogue **9** (191 mg, 189 μmol) was added and the mixture was coevaporated with dry pyridine (3 × 6 mL). 1*H*-Tetrazole (35 mg, 447 μmol) and dry pyridine (2 mL) were added and the solution was stirred at rt. The reaction was monitored by TLC (2:1, *i*-PrOH/1 M NH_4OAc) and NMR by removing small aliquots (50 μL) from the reaction mixture after 12 h, 48 h, 72 h and 96 h. After 20 h (compound **3**) and 120 h (compound **1**) the mixture was diluted with water (3 mL) and evaporated. The residue was suspended in 4 mL of 100 mM NH_4HCO_3 and extracted with ether (3 mL). After evaporation, the residue was purified on a Bio-Gel P-2 column (2 × 65 cm), eluted with 100 mM NH_4HCO_3. The product-containing fractions were concentrated, redissolved in H_2O and further purified using gradient reverse phase column chromatography on C-18 silica (H_2O → 50% H_2O/MeOH). The product-containing fractions were concentrated and the material was further purified by repeating the previously described Bio-Gel P-2 column separation followed by a final reverse phase column chromatography on C-18 silica as described earlier. Lyophilization of the sample provided the desired target molecules **1** and **3**.

Compound **3**: ^{1}H NMR (D_2O): δ 3.52–3.62 (m, 2H), 3.70–3.82 (m, 5H), 3.90 (dd, 1H, $J = 10.2$ Hz, $J = 3.2$ Hz, H-3 galactose), 4.01 (d, 1H, $J = 3.2$ Hz, H-4 galactose), 4.15–4.27 (m, 3H), 4.29 (t, 1H, $J = 6.3$ Hz, H-5 galactose), 4.31–4.35 (m, 2H), 5.62 (dd, 1H, $J = 3.5$ Hz, $J_{H1,P} = 7.3$ Hz, H-1 galactose), 5.92 (d, 1H, $J = 5.6$ Hz, H-1 ribose), 5.93 (d, 1H, $J = 8.1$ Hz, uridine), 6.82–6.90 (m, 2H, 2 × Ar-H), 7.32 (t, 1H, $J = 7.7$ Hz, Ar-H), 7.45 (d, 1H, Ar-H), 7.91 (d, 1H, $J = 8.1$ Hz, uridine); ^{31}P NMR (D_2O, H_3PO_4): δ −12.6 (d, $J_{P,P} = 18.9$ Hz, P attached to galactose), −11.0 (d, $J_{P,P} = 18.9$ Hz, P attached to uridine); ESIMS *m*/*z* calcd for $[C_{24}H_{33}N_4Na_2O_{18}P_2]^+$: 773.1055; found, 773.1054.

Compound **1**: ^{1}H NMR (D_2O): δ 0.66 (t, 3H, –$CH_2CH_2CH_3$), 1.30–1.40 (m, 2H, –$CH_2CH_2CH_3$), 2.50–2.68 (m, 4H, –$CH_2CH_2CO_2NH$–), 2.93–3.14 (m, 4H, 2 × ArCH_2–, 2 × CONHCH_2–), 3.50–3.60 (m, 2H), 3.72–3.77 (m, 4H), 3.78–3.82 (m, 1H, H-2 galactose), 3.91 (dd, 1H, $J = 10.2$ Hz, $J = 3.2$ Hz, H-3 galactose), 4.0 (dd, 1H, $J = 3.2$ Hz, $J < 1$ Hz, H-4 galactose), 4.18–4.26 (m, 3H), 4.28–4.33 (m, 2H, H-5), 4.33–4.36 (t, 1H, $J = 5.2$ Hz, H-4 ribose), 4.40–4.44 (m, 1H, Hα-(Tyr(NO_2))), 5.66 (dd, 1H, $J_{H1,H2} = 3.7$ Hz, $J_{H1,P} = 7.3$ Hz, H-1 galactose), 5.93 (d, 1H, $J = 5.2$ Hz, H-1 ribose), 6.80 (m, 1H, $J = 7.9$ Hz, $J < 1$ Hz, Ar-H), 6.85 (d, 1H, $J = 7.9$ Hz, Ar-H), 7.10 (d, 1H, $J = 8.6$ Hz, Ar-H), 7.29 (m, 1H, $J = 1.4$ Hz, $J = 8.3$ Hz, Ar-H), 7.43 (m, 1H, $J = 1.2$ Hz, $J = 7.8$ Hz, Ar-H), 7.51 (m, 1H, $J = 2.1$ Hz, $J = 8.6$ Hz, Ar-H), 7.62, (s, 1H, Ar-H), 7.92 (m, 1H, $J = 2.1$ Hz); ^{31}P NMR (D_2O, H_3PO_4): δ −12.4 (d, $J_{P,P} = 20.5$ Hz, P attached to galactose), −11.1 (d, $J_{P,P} = 20.5$ Hz, P attached to uridine); ESIMS *m*/*z* calcd for $[C_{39}H_{52}N_7O_{23}Na_2P_2]^+$: 1094.2385; found, 1094.2386.

4.15. General procedure for the galactosyltransferase catalyzed reactions

4.15.1. α-(1→3) GalT assay. α-(1→3) GalT was isolated according to the literature procedure.[1] UDP-Gal analogues (**1**–**3**, 20 nmol) and lactose-TMR (**21**, 20 nmol) were incubated with calf thymus α(1→3) GalT[6] (80 mU) in buffer A (10 μL) at room temperature (Buffer A: 35 mM sodium cacodylate, 20 mM $MnCl_2$, 0.1% Triton X-100, 0.8 mg/mL BSA, pH 6.5). The progress of the reactions was monitored by TLC (65:35:7 $CHCl_3$/MeOH/H_2O) and compared to the reaction of unmodified UDP-Gal (Sigma, 20 nmol) and lactose-TMR[6] (**21**, 20 nmol) incubated with calf thymus α-(1→3) GalT (800 μU). Aliquots (1 μL) were taken after 2, 5, 8,15, 29, 25, 55, 100, 150 min and starting materials and products were separated by TLC (65:35:7 $CHCl_3$/MeOH/H_2O). Relative transfer rates were measured by integration of the new product spots using SNAPSCAN and compared with unmodified UDP-Gal. Observed MS Data (MALDI TOF) for substrate **21** reacting with donor **1**–**3**. $[M+H]^+ = 1277.6$; $[M+H]^+ = 1115.5$; $[M+H]^+ = 1277.6$.

4.15.2. Blood group B α-(1→3) GalT assay. Recombinant blood group B α-(1→3) GalT were prepared as previously described.[2] UDP-Gal analogues (**1**–**3**, 20 nmol) and Fuc-Gal-TMR (**20**, 20 nmol) were incubated with blood Group B α-(1→3) GalT (22 mU) in buffer B (10 μL) at room temperature (Buffer B: 50 mM sodium cacodylate, 20 mM $MnCl_2$, 1 mg/mL BSA, pH 6.8). The progress of the reactions was monitored by TLC (65:35:5, $CHCl_3$/MeOH/H_2O) and compared to the reaction of unmodified UDP-Gal (Sigma, 20 nmol) and Fuc-Gal-TMR[6] (**20**, 20 nmol) incubated with blood group B α(1→3) GalT (220 μU). Aliquots (1 μL) were taken after 2, 5, 8, 15, 29, 25, 55, 100, 150 min and starting materials and products were separated by TLC (65:35:5, $CHCl_3$/MeOH/H_2O). Relative transfer rates were measured by integration of the new product spots using SNAPSCAN and compared with unmodified UDP-Gal. Observed MS Data (MALDI TOF) for substrate **20** reacting with donor **1**–**3**. MS $[M+H]^+$: 1261.6; $[M+H]^+$: 1099.5; $[M+H]^+$: 1261.6.

4.15.3. Milk bovine β-(1→4) GalT assay. Milk bovine β-(1→4) GalT was prepared according to the previously published procedure.[3] UDP-Gal analogues (**1**–**3**, 20 nmol) and GlcNAc-TMR (**22**, 20 nmol) were incubated with blood Group B α-(1→3) GalT (44 mU) in

buffer B (10 μL) at room temperature (Buffer B: 50 mM sodium cacodylate, 20 mM $MnCl_2$, 1 mg/mL BSA, pH 6.8). The progress of the reactions was monitored by TLC (65:35:7, $CHCl_3$/MeOH/H_2O) and compared to the reaction of unmodified UDP-Gal (Sigma, 20 nmol) and GlcNAc-TMR (**22**, 20 nmol) incubated with milk bovine β-(1→4) GalT (440 μU). Aliquots (1 μL) were taken after 2, 5, 8,15, 29, 25, 55, 100 and 150 min and starting materials and products were separated by TLC (65:35:7, $CHCl_3$/MeOH/H_2O). Relative transfer rates were measured by integration of the new product spots using SNAPSCAN and compared with unmodified UDP-Gal. Observed MS Data (MALDI TOF) for substrates **22** and donor **2** reacting with milk bovine β-(1→4) GalT: MS $[M+H]^+$: 994.5.

Acknowledgements

Financial support of this project was provided by NSERC. The author thanks Dr. Ole Hindsgaul and Dr. Monica Palcic for their technical assistance and expertise and the University of Alberta for providing the facilities to perform the work.

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