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Non-isosteric *C*-glycosyl analogues of natural nucleotide diphosphate sugars as glycosyltransferase inhibitors

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Abstract—A series of *C*-glycosyl ethylphosphonophosphate analogues of UDP-Glc, UDP-Gal, UDP-GlcNAc and GDP-Fuc were synthesized from the corresponding *C*-glycosyl ethylphosphonic acids. Analogues were obtained as α-anomers through either diastereoselective photo-induced radical addition of glycosyl bromides (D-Glc, D-Gal and L-Fuc) to diethyl vinylphosphonate, or a multi-step sequence (D-GlcNAc), with subsequent coupling with morpholidate-activated nucleotide monophosphates. The in vitro inhibitory activity of UDP-Gal, GDP-Fuc and UDP-GlcNAc analogues towards glycosyltransferases (β-1,4-GalT, FUT3 and *LgtA*) was evaluated through a competition fluorescence assay and IC_{50} values of 40 μM, 2 mM and 3.5 mM were obtained, respectively.

1. Introduction

Formation of glycosidic linkages in the biosynthesis of oligosaccharides, glycolipids and glycoproteins usually occurs through the Leloir pathway[1] under the control of glycosyltransferases,[2–5] by transfer of a carbohydrate from a nucleotide diphosphate sugar (NDP sugar) as donor to the acceptor substrate, releasing the corresponding nucleotide diphosphate. Glycosyltransferases catalyze the transfer of sugar units to acceptors with either inversion or retention of the anomeric configuration with respect to the NDP sugar donor. The proposed mechanism[6–8] of glycosyl transfer with inversion of configuration is occurring through weakening of the donor glycosidic bond implicating an acidic residue in the enzyme active site, followed by a S_N2-type transition state involving an oxonium ion. Final attack of the acceptor's hydroxyl group with assistance of a basic group in the enzyme catalytic site provides the glycosylated acceptor (Fig. 1).

Since glycoconjugates are involved in numerous cell–cell recognition and communication processes, the rational design of glycosyltransferase inhibitors has been intensively investigated[9–33] for a better understanding of biochemical pathways implicating these enzymes and also for potential therapeutical applications. Glycosyltransferase inhibitors are generally designed based on analogies between the three different moieties composing the NDP sugar natural substrates, mimicking either the carbohydrate part,[9–12,31] the diphosphate linkage,[13–20] the nucleoside moiety or combinations of these.[21–30] The diphosphate linkage, essential for most glycosyltransferases' activity, interacts via its dianionic charge with metallic cations, for example, Mn^{2+}, coordinated with two aspartate residues within the active site (Fig. 1). Several mimics of the dianionic charge of NDP sugars have been designed ranging from phosphonates,[32] methylpyrophosphates,[19,33] methylenediphosphonates,[14] to malonates, tartrates or even monosaccharidic structures.[13–20] Even though several syntheses of glycoconjugates mimicking the diphosphate linkage have been described in the literature, the biological evaluation of these analogues towards glycosyltransferases is rather limited.[13–15,32,33]

C-Glycosyl analogues are useful mimetics due to their higher stability towards acid- or enzyme-catalyzed

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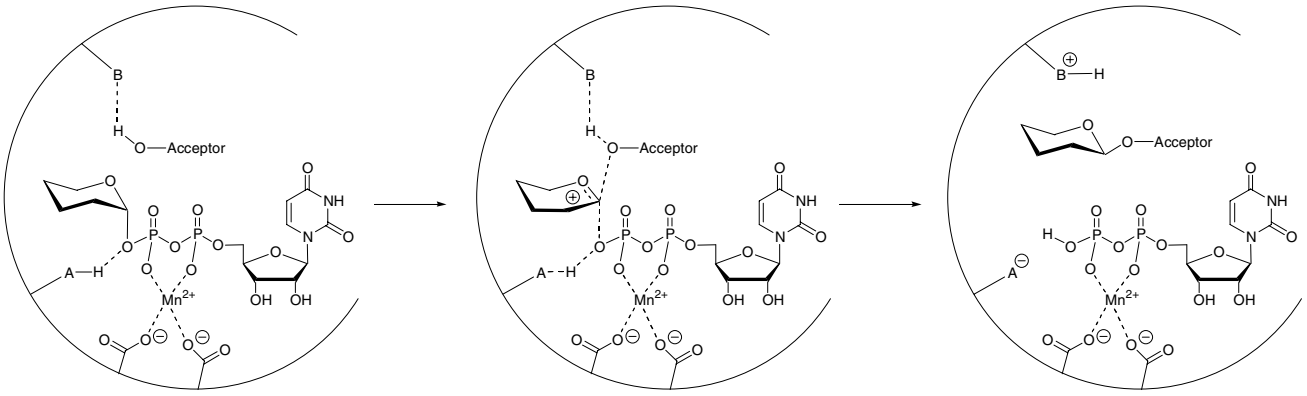


Figure 1. Proposed mechanism for inverting glycosyltransferases.[26]

hydrolysis compared to *O*-glycosides and several approaches have been developed for their stereocontrolled synthesis.[34–39] Being involved in a continuing programme focusing on a series of glycosyltransferases with mostly α-configured natural substrates, we wanted to prepare analogues with the same α-configuration. The use of radical chemistry,[40] for example, addition of glycosyl radicals to suitable alkenes, is probably the most efficient method[41–48] due to mild conditions, atom economy and high stereocontrol. Although this approach is leading predominantly to α-configured *C*-glycosyl derivatives, we could achieve the synthesis of the β-configured analogues[41] under very mild conditions using photo-induced activation.

Based on our previously developed radical chemistry,[41] we are now reporting on the design of donor substrate analogues, with a modification at the diphosphate linkage (Fig. 2). These compounds are not isosteric to natural substrates due to the replacement of the oxygen atom by an ethylene moiety, resulting in an increased distance between the sugar anomeric centre and the phosphorus atom. Therefore, it was assumed that these structures would mimic the lengthening of the anomeric C–O bond which occurs in the S_N2-type transition state (Fig. 1) involved for the natural substrate according to the proposed mechanism.[26]

2. Results and discussion

The preparation of three *C*-glycosyl analogues (D-Glc, D-Gal and L-Fuc) of NDP sugar was achieved through photo-induced radical addition of glycosyl bromides to diethyl vinylphosphonate,[41] the GlcNAc derivative being obtained by a multi-step route, to afford *C*-glycosyl ethylphosphonates. The corresponding phosphonic acids were then coupled to activated nucleotide monophosphates in order to obtain the desired analogues for enzymatic studies.

2.1. Synthesis of UDP-Glc and UDP-Gal analogues (4a,b)

Reaction of commercially available acetobromoglycoses **1a**,**b** with diethyl vinylphosphonate under photo-induced conditions (Scheme 1) yielded the desired α-configured *C*-glycosyl ethylphosphonates **2a**,**b**.[41] Deprotection[49] of the diethylphosphonates upon treatment with Me_3SiBr in MeCN followed by deacetylation (H_2O/MeOH/Et_3N) and C_{18}-reverse phase column chromatography afforded the fully deprotected *C*-glycosyl ethylphosphonic acids **3a**,**b** in high yields. Final coupling of **3a**,**b** and UMP-morpholidate[50,51] provided the desired analogues **4a**,**b** after purification[52] by ion exchange resin chromatography using DEAE-Sephadex A-25 (HCO_3^- form).

2.2. Synthesis of GDP-L-Fuc analogue (8)

Preparation of the GDP-L-fucose analogue **8** was also achieved through a similar procedure (Scheme 2). Acetylation of L-fucose followed by conversion of **5** into L-fucosyl bromide[53] and subsequent treatment under photochemical conditions[41] in the presence of diethyl vinylphosphonate afforded the L-fucosyl ethylphosphonates in good yield, as a mixture of anomers predominantly α (α/β, 9:1).[47] The α-anomer **6** could be easily purified

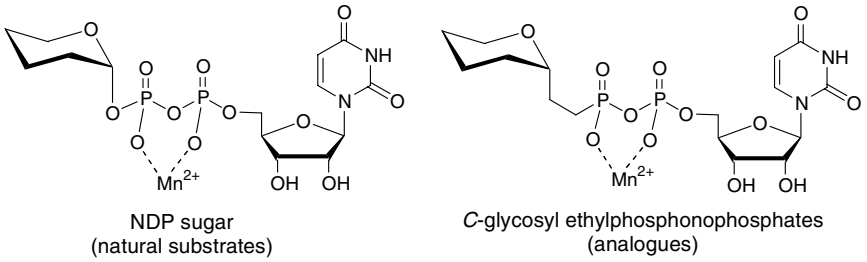
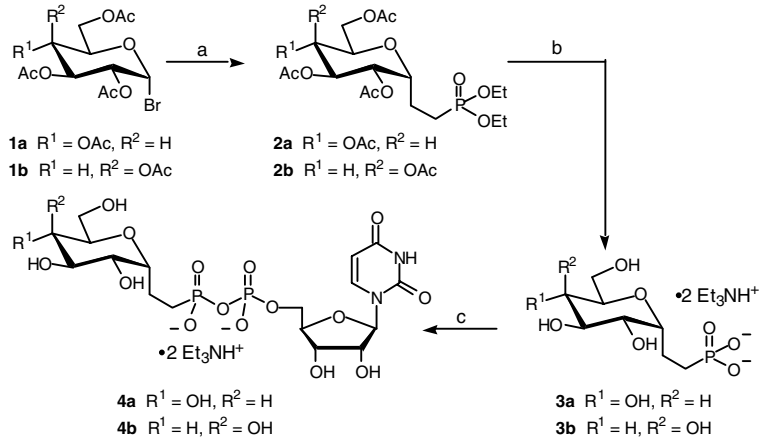
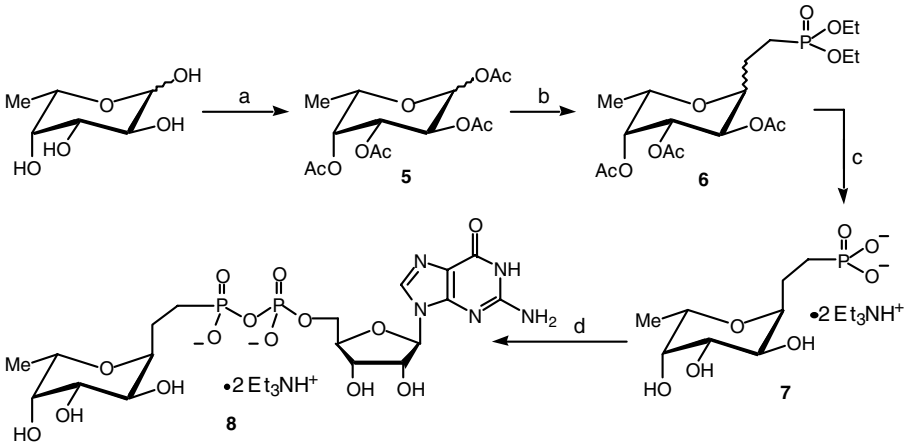


Figure 2. Glycosyltransferase natural substrates and *C*-glycosyl ethylphosphonophosphate analogues.



Scheme 1. Synthesis of *C*-glycosyl ethylphosphonophosphate analogues of UDP-Glc **4a** and UDP-Gal **4b**. Reagents: (a) diethyl vinylphosphonate, $NaBH_3CN$, *n*-Bu_3SnCl, AIBN, *t*-BuOH, hν, 72–75%; (b) Me_3SiBr, C_5H_5N, MeCN then MeOH, H_2O, Et_3N, 92–94%; (c) 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphomorpholidate, tetrazole, C_5H_5N, 10–24%.



Scheme 2. Synthesis of GDP-L-fucose analogue **8**. Reagents and conditions: (a) Ac_2O, C_5H_5N, quantitative; (b) HBr/AcOH, CH_2Cl_2 then diethyl vinylphosphonate, $NaBH_3CN$, *n*-Bu_3SnCl, AIBN, *t*-BuOH, hν, 59%, α/β 9:1; (c) Me_3SiBr, C_5H_5N, MeCN then MeOH, H_2O, Et_3N, 89%; (d) 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium guanosine 5′-monophosphomorpholidate, tetrazole, C_5H_5N, 24%.

by silica gel chromatography and its stereochemistry was assigned by a detailed analysis of the ^1H NMR data. The observed coupling constant between H-1 and H-2 ($^3J_{1,2}$ = 5.6 Hz) is in agreement with the α-configuration at the anomeric centre as noted for similar *C*-glycosyl derivatives.[54] Deprotection[49] of the diethylphosphonate followed by deacetylation provided the fully deprotected phosphonic acid **7**. Coupling with GMP-morpholidate[50,51] afforded the desired analogue **8**. As compared to uracil derivatives **4a**,**b** and **10**, we noticed a relatively poor stability of analogue **8** resulting in partial decomposition into GMP and **7** when stored for 2 weeks at ~+4 °C. Slow decomposition is probably due to the sensitivity of the P–O–P–O linkage and the increased basicity and nucleophilicity of the guanine moiety, in comparison to uracil.

2.3. Synthesis of UDP-GlcNAc analogue (10)

Synthesis of the GlcNAc-based analogue **10** using the radical approach was ineffective. When either the chloro- or bromo-activated *N*-acetylglucosamine derivative **A** (Fig. 3) were subjected to coupling under our standard conditions[41] in the presence of a large excess of diethyl vinylphosphonate (up to 15 equiv), the major compound isolated after irradiation was the oxazoline **B** in >80% yield. Despite several attempts using acetamide or trifluoroacetamide derivatives, we were not able to obtain the expected *C*-glycosyl ethylphosphonate **C**, even though Junker and Fessner[47] previously reported the radical coupling of the glucosamine trifluoroacetamide derivative with diethyl vinylphosphonate. Radical reactions in the GlcNAc series being highly sensitive to different parameters,[42,46] one can therefore assume that using different conditions (Et_2O/5 × 50 W halogen lamps under Fessner's conditions[47] and *t*-BuOH/450 W UV lamp in our case) would greatly alter the outcome of the reaction.

Nevertheless, the protected GlcNAc analogue could be prepared through a multi-step approach[55,56] and coupling of the corresponding phosphonic acid **9** with

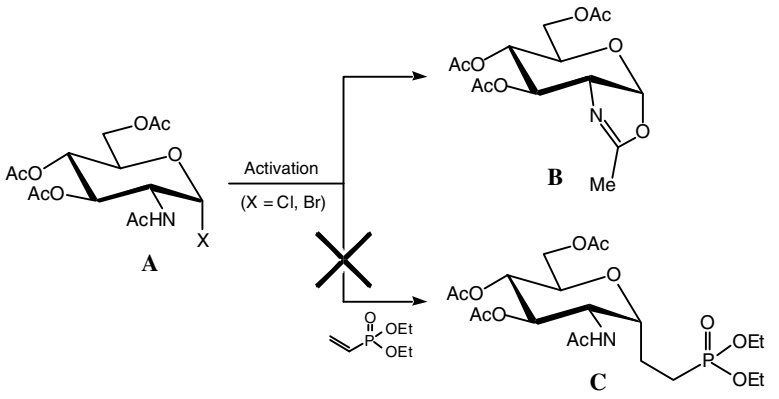


Figure 3. Attempted syntheses of GlcNAc-based *C*-glycosyl ethylphosphonates using photo-induced radical conditions.

UMP-morpholidate afforded the desired GlcNAc *C*-glycosyl ethylphosphonophosphate analogue **10** (Scheme 3).

The formation of the P–O–P bond in *C*-glycosyl ethylphosphonophosphate analogues **4a**,**b**, **8** and **10** could be easily evidenced by ^{31}P NMR spectroscopy displaying two doublets resonating at $\delta \sim -10$ (phosphate) and $\delta \sim 19$ ppm (phosphonate) with a $^{2}J_{P,P} = 26$ Hz. The presence of the desired phosphonophosphates in the crude reaction mixtures was checked by $^{31}P \times {}^{31}P$ 2D COSY NMR analysis with observation of the expected correlation between these two doublets.

3. Biological evaluations

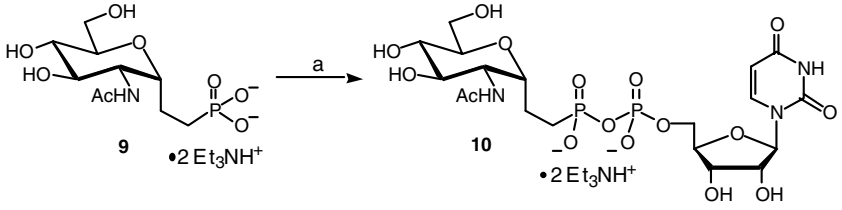
3.1. Inhibitory activity of UDP-Gal analogue (*4b*)

Compound **4b** is a non-isosteric α-configured analogue of the sugar nucleotide donor UDP-Gal, the natural substrate of bovine milk β-1,4-galactosyltransferase (β-1,4-GalT, EC 2.4.1.22). This inverting enzyme catalyzes the transfer, with the β-configuration, of a galactose unit from UDP-Gal to the 4-position of a GlcNAc residue. Human β-1,4-GalT plays a role in the biosynthesis of blood group antigens and sialyl Lewis x (sLe^x) but is also involved in some pathologies such as arthritis and cancer.[31] The biological evaluation of **4b** towards bovine milk β-1,4-GalT was carried out through a competition assay using a fluorescent acceptor substrate, GlcNAc-β-*O*-$(CH_2)_6$-NH-dansyl,[57] where the reaction product was quantified by RP-HPLC. K_m constants were measured for bovine milk β-1,4-galactosyltransferase and the two other enzymes studied (vide infra), and compared to the corresponding K_m values reported in the literature (Table 1). In each case, a fairly good agreement was observed, thus proving the validity of the applied method. The IC_{50} value of 40 μM observed for **4b** is similar to the K_m value[58] for the natural donor substrate, UDP-Gal (Table 1). This compound is therefore a good inhibitor of β-1,4-GalT. It is noteworthy that non-isosteric α- and β-configured analogues of UDP-Gal displaying an extra methylene between the sugar and UDP have no inhibitory activity against recombinant pig α(1–3)-galactosyltransferase (pα(1–3)GalT).[59] On the other hand, the *C*-glycosyl ethylphosphonic acid **3b**, evaluated in the same conditions up to 16 mM concentration, did not display any inhibitory activity towards β-1,4-GalT, thus

Table 1. Inhibitory activities (IC_{50}) of NDP-sugar analogues **4b**, **8** and **10**

Glycosyltransferases	K_m[a] (μM)	K_m[b] (μM)	Analogues	IC_{50}
β-1,4-GalT	44[58]	51	**4b**	40 μM[c]
FUT3	33[61]	43	**8**	2 mM[d]
LgtA	220[62]	540	**10**	3.5 mM[e]

[a] K_m values previously reported in the literature.
[b] K_m values measured with our enzymatic assay using fluorescent acceptor substrates, under similar experimental conditions as described for the measurement of the IC_{50} values of **4b**, **8** and **10**.
[c] Measured at 0.2 mM dansylated acceptor substrate and 0.04 mM UDP-Gal.
[d] Measured at 0.2 mM dansylated acceptor substrate and 0.05 mM GDP-Fuc.
[e] Measured at 0.8 mM dansylated acceptor substrate and 0.25 mM UDP-GlcNAc.



Scheme 3. Synthesis of UDP-GlcNAc analogue **10**. Reagents: (a) 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphomorpholidate, tetrazole, C_5H_5N, 13%.

indicating that the nucleotide moiety of UDP-Gal analogues cannot be omitted without altering the inhibitory activity.

3.2. Inhibitory activity of GDP-Fuc analogue (8)

The inhibitory activity of GDP-Fuc analogue **8** towards recombinant human Galβ(1 → 3/4)GlcNAc-α-1,4/3-fucosyltransferase[60] (FUT3, EC 2.4.1.65) was determined. This enzyme catalyzes the transfer of a fucose unit from GDP-Fuc, with the α-configuration, to the 3- or 4-position of a GlcNAc residue belonging either to disaccharide acceptors Galβ(1 → 3)GlcNAc or Galβ(1 → 4)GlcNAc. Inhibition tests were similarly carried out through a competition assay using a fluorescent acceptor substrate, Galβ(1 → 3)GlcNAc-β-*O*-$(CH_2)_6$-NH-dansyl,[57] in which the reaction product was quantified by RP-HPLC. An IC_{50} value of 2 mM, about 2 orders of magnitude higher than the K_m for GDP-Fuc,[61] was found for GDP-Fuc analogue **8** (Table 1). The weak inhibitory activity of **8** against FUT3 could be due to the α-anomeric configuration of this *C*-glycosyl analogue, opposite to the β-configuration of the natural donor substrate GDP-Fuc, although an α-anomer analogue of GDP-Fuc has been reported to inhibit significantly human fucosyltransferases,[31] even though their natural substrates possess the opposite β-configuration. The elongation of the distance between the guanosine and the sugar moiety in **8** can also account for the lower inhibition observed.[12]

3.3. Inhibitory activity of UDP-GlcNAc analogue (10)

The inhibitory activity of UDP-GlcNAc analogue **10** against recombinant *Neisseria meningitidis* Galβ(1 → 4)Glc-β-1,3-*N*-acetyl-glucosaminyltransferase[57] (*LgtA*, EC 2.4.1.56) was measured according to the same procedure with Galβ(1 → 4)Glcβ-*O*-$(CH_2)_6$-NH-dansyl[57] as the fluorescent acceptor substrate. This enzyme catalyzes the transfer of a GlcNAc residue from the sugar nucleotide donor UDP-GlcNAc to the 3-position of the terminal galactose of a lactose unit. With a IC_{50} value of 3.5 mM that is about 1 order of magnitude higher than the K_m value for UDP-GlcNAc,[62] the analogue **10** turned out to be only a poor inhibitor towards the bacterial enzyme *LgtA* (Table 1), unlike the analogue **4b** that exhibited a significant inhibitory activity against the bovine β-1,4-GalT. Considering that both glycosyltransferases are inverting enzymes that require manganese ions for proper activity, a different spatial arrangement of the amino acid residues involved in the enzyme active sites may account for this discrepancy. Other elongated non-isosteric compounds have been shown to be weaker inhibitors of UDP-GlcNAc 2-epimerase, as compared to analogues which closely resemble UDP-GlcNAc.[29,33]

4. Conclusion

A photo-induced addition of glycosyl radicals to diethyl vinylphosphonate followed by deprotection and coupling with activated nucleotide monophosphates afforded three *C*-glycosyl ethylphosphonophosphate analogues of glycosyltransferase donor substrates (**4a** for UDP-Glc, **4b** for UDP-Gal and **8** for GDP-Fuc), the UDP-GlcNAc analogue **10** being obtained through a multi-step sequence. The radical-based approach allowed for the syntheses of *C*-glycosyl ethylphosphonates with high diastereoselectivity in favour of the α-anomer. Biological evaluations of UDP-Gal, GDP-Fuc and UDP-GlcNAc analogues (**4b**, **8** and **10**, respectively) were achieved using a fluorescence assay and IC_{50} values of 40 μM, 2 mM and 3.5 mM were determined for β-1,4-GalT, FUT3 and *LgtA*, respectively. Based on these results, the replacement of the O–P oxygen–phosphorus bond in the natural donor substrates by C–C–P carbon–carbon–phosphorus bonds led to non-isosteric analogues displaying weak inhibitory activities, except for UDP-Gal analogue **4b** which turned out to be an interesting candidate for β-1,4-galactosyltransferase inhibition, at least in vitro. Further investigations of the biological properties of these glycoconjugates are being pursued in our efforts to better understand the mechanism of action of glycosyltransferases and their biological implications.

5. Materials and methods

5.1. General methods

tert-Butanol was distilled over CaH_2 and under argon atmosphere. Diethyl vinylphosphonate (97% grade) and $NaBH_3CN$ (95% grade) were purchased from Aldrich and used as received. Air was removed from the reaction media by bubbling argon into organic solutions. Thin-layer chromatography (TLC) was carried out on aluminium sheets coated with silica gel 60 F_{254} (Merck). TLC plates were inspected under UV light and developed by charring after spraying with 5% H_2SO_4 in EtOH. Preparative C_{18}-reversed phase chromatography (RP-18) was performed using a 15 × 15 mm column of fully endcapped silica gel 100 C_{18} (>400 mesh, Fluka). Ion exchange chromatography was performed using a 25 × 200 mm column of DEAE-Sephadex A25 resin (HCO_3^- form, Pharmacia). ^1H and ^{13}C NMR spectra were recorded using Bruker AC200, DRX300 or DRX500 spectrometers with the residual solvent as the internal standard.[63] The chemical shifts are expressed on the δ scale in parts per million (ppm). ^{31}P NMR spectra were recorded with a Bruker spectrometer at 80 MHz (AC200) or 120 MHz (DRX300) from D_2O or $CDCl_3$ solutions with H_3PO_4 as the external reference. The following abbreviations are used to explain the observed multiplicities: s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublet of doublets; t, triplet; q, quadruplet; m, multiplet; br, broad. NMR solvents were purchased from Eurisotop (Saint Aubin, F-91194, France). Mass spectra were measured with a Finnigan Mat95XL spectrometer. Optical rotations were measured using a Perkin-Elmer polarimeter. Reversed phase HPLC (RP-HPLC) analyses were performed using a Waters 600 pump equipped with a reversed phase column (C_{18}, Nucleosil, 5 μm, 3.9 mm id × 300 mm; mobile phase, H_2O–MeCN:

73:27; flow rate, 1 mL/min), with a Luminescence Spectrometer LS50B (Perkin-Elmer) as the detection device. Fluorescence of substrates and products was read at 385 nm excitation/540 nm emission. β-1,4-GalT was purchased from Sigma. *LgtA*[64] and FUT3[60] were prepared according to previously described procedures.[52] Structure elucidation was deduced from 1D and 2D NMR spectroscopy which allowed, in most cases, complete signal assignments based on COSY, HSQC and HMBC correlations. The glycopyranosyl rings existed in all cases under a chair conformation ($^4C_1$ or $^1C_4$), as deduced from the observed values of the vicinal H–H coupling constants. Abbreviations Glc, Gal, Fuc, Rib and Ura were used for the NMR assignments of D-glucose, D-galactose, L-fucose, D-ribose and uracil protons and carbons, respectively.

5.2. Bis(triethylammonium) 2-(α-D-glucopyranosyl)-ethylphosphonate (3a)

To a solution of **2a**[41] (1.854 g, 3.73 mmol) in MeCN (40 mL) was added C_5H_5N (3.12 mL, 37.88 mmol) at 0 °C followed by dropwise addition of Me_3SiBr (5 mL, 37.88 mmol). The solution was stirred at 0 °C for 2 h then quenched with H_2O/C_5H_5N (9:1/30 mL). The mixture was evaporated to dryness and the white powder purified (RP-18/H_2O then H_2O/MeOH, 1:1) to obtain the acetylated phosphonic acid intermediate. The product was stirred in H_2O/MeOH/Et_3N (7:3:1/30 mL) for 16 h then evaporated to dryness and the white powder purified (RP-18/H_2O) to afford **3a** (933 mg, 92%) as a colourless foam. $[\alpha]_D$ +37 (*c* 1, H_2O). ^1H NMR (D_2O, 300 MHz): δ 1.23 (t, 18H, J = 7.3 Hz, NCH_2CH_3), 1.38–1.92 (m, 4H, CH_2CH_2P), 3.15 (q, 12H, J = 7.3 Hz, NCH_2CH_3), 3.28 (t, 1H, $J_{4,3} = J_{4,5}$ = 9.8 Hz, H-4), 3.46 (ddd, 1H, $J_{5,6'}$ = 2.2 Hz, $J_{5,6}$ = 5.9 Hz, $J_{5,4}$ = 9.8 Hz, H-5), 3.58–3.72 (m, 3H, H-2, H-3, H-6), 3.82 (dd, 1H, $J_{6',6}$ = 12.3 Hz, H-6′), 3.94 (m, 1H, H-1). ^{13}C NMR (D_2O, 75 MHz): δ 9.1 (NCH_2CH_3), 19.4 (d, $J_{C,P}$ = 3.4 Hz, CH_2CH_2P), 24.4 (d, $J_{C,P}$ = 174.2 Hz, CH_2CH_2P), 47.5 (NCH_2CH_3), 62.0 (C-6), 71.3 (C-4), 72.1, 74.0 (2s, C-2, C-3), 73.2 (C-5), 77.0 (d, $J_{C,P}$ = 16.5 Hz, C-1). ^{31}P NMR (D_2O, 80 MHz): δ 26.2. LSIMS (negative mode, glycerol) *m/z*: 271 $[M-2HNEt_3+H]^-$. HRLSIMS (negative mode, glycerol) *m/z*: calcd for $C_8H_{16}O_8P_1$ $[M-2HNEt_3+H]^-$, 271.0583; found, 271.0584.

5.3. Bis(triethylammonium) 2-(α-D-galactopyranosyl)-ethylphosphonate (3b)

To a solution of **2b**[41] (668 mg, 1.34 mmol) in MeCN (20 mL) was added C_5H_5N (1.11 mL, 13.4 mmol) at 0 °C followed by Me_3SiBr (1.8 mL, 13.4 mmol). The solution was stirred at 0 °C for 1 h then quenched with H_2O/C_5H_5N (9:1/10 mL). The mixture was evaporated to dryness and the white powder purified (RP-18/H_2O then H_2O/MeOH, 1:1) to obtain the acetylated phosphonic acid intermediate. The product was stirred in H_2O/MeOH/Et_3N (7:3:1/30 mL) for 16 h then evaporated to dryness and the white powder purified (RP-18/H_2O) to afford **3b** (342 mg, 94%) as a colourless foam and as its mono-triethylammonium salt. $[\alpha]_D$ +53 (*c* 1, H_2O). ^1H NMR (D_2O, 300 MHz): δ 1.17 (t, 9H, J = 7.3 Hz, NCH_2CH_3), 1.30–1.85 (m, 4H, CH_2CH_2P), 3.08 (q, 6H, J = 7.3 Hz, NCH_2CH_3), 3.57–3.65 (m, 3H, H-5, H-6, H-6′), 3.70 (dd, 1H, $J_{2,1}$ = 3.4 Hz, $J_{2,3}$ = 9.3 Hz, H-2), 3.83–3.94 (m, 3H, H-1, H-3, H-4). ^{13}C NMR (D_2O, 75 MHz): δ 8.6 (NCH_2CH_3), 18.8 (d, $J_{C,P}$ = 2.8 Hz, CH_2CH_2P), 24.3 (d, $J_{C,P}$ = 135.0 Hz, CH_2CH_2P), 47.0 (NCH_2CH_3), 61.6 (C-6), 68.7 (C-2), 69.5, 70.0 (2s, C-3, C-4), 72.0 (C-5), 76.2 (d, $J_{C,P}$ = 16.6 Hz, C-1). ^{31}P NMR (D_2O, 80 MHz): δ 26.2. LSIMS (negative mode, glycerol) *m/z*: 271 $[M-2HNEt_3+H]^-$. HRLSIMS (negative mode, glycerol) *m/z*: calcd for $C_8H_{16}O_8P_1$ $[M-2HNEt_3+H]^-$, 271.0583; found, 271.0584.

5.4. General procedure for the synthesis of 4a,b, 8 and 10

A water solution of **3a**,**b**, **7** or **9** was evaporated to dryness then dried under high vacuum over P_2O_5 for 24 h. The morpholidate-activated nucleotide monophosphate and tetrazole were added. The solvent was evaporated off and the mixture dried under high vacuum over P_2O_5 for 24 h. The solid residue was dissolved in anhydrous C_5H_5N (10 mL) and stirred under argon at room temperature for 10 days. The reaction mixture was diluted with H_2O (100 mL) then evaporated to dryness and the yellow powder purified (Sephadex DEAE-A25 HCO_3^- form/0 to 1 M TEAB) to obtain **4a**,**b**, **8** or **10** as white foams.

5.4.1. Bis(triethylammonium) [2-(α-D-glucopyranosyl)-ethylphosphono]uridin-5′-yl phosphate (4a). A mixture of **3a** (509 mg, 1.87 mmol), 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphomorpholidate (470 mg, 0.68 mmol) and tetrazole (16.2 mL, 7.27 mmol, 0.45 M in MeCN) was treated according to the general procedure described above to obtain **4a** (95 mg, 24%). $[\alpha]_D$ +18 (*c* 1, H_2O). ^1H NMR (D_2O, 300 MHz): δ 1.24 (t, 18H, J = 7.3 Hz, NCH_2CH_3), 1.58–2.00 (m, 4H, CH_2CH_2P), 3.13 (q, 12H, J = 7.3 Hz, NCH_2CH_3), 3.28–3.36 (m, 1H, H-4_{Glc}), 3.52 (ddd, 1H, $J_{5,4}$ = 9.7 Hz, $J_{5,6}$ = 5.7 Hz, $J_{5,6'}$ = 2.2 Hz, H-5_{Glc}), 3.62–3.75 (m, 3H, H-2_{Glc}, H-3_{Glc}, H-6_{Glc}), 3.84 (dd, 1H, $J_{6',6}$ = 12.3 Hz, H-$6'_{Glc}$), 3.92–4.03 (m, 1H, H-1_{Glc}), 4.15–4.21 (m, 2H, H-5_{Rib}, H-$5'_{Rib}$), 4.21–4.28 (m, 1H, H-4_{Rib}), 4.30–4.39 (m, 2H, H-2_{Rib}, H-3_{Rib}), 5.88 (d, 1H, J = 7.8 Hz, H-5_{Ura}), 6.00 (d, 1H, $J_{1,2}$ = 4.6 Hz, H-1_{Rib}), 7.83 (d, 1H, J = 7.7 Hz, H-6_{Ura}). ^{13}C NMR (D_2O, 125 MHz): δ 8.7 (NCH_2CH_3), 18.9 (d, $J_{C,P}$ = 3.0 Hz, CH_2CH_2P), 24.1 (d, $J_{C,P}$ = 139.5 Hz, CH_2CH_2P), 47.0 (NCH_2CH_3), 61.6 (C-6_{Glc}), 65.1 (d, $J_{C,P}$ = 5.3 Hz, C-5_{Rib}), 70.0, 74.1 (C-2_{Rib}, C-3_{Rib}), 70.9 (C-4_{Glc}), 71.7 (C-2_{Glc}), 72.7 (C-5_{Glc}), 73.6 (C-3_{Glc}), 76.5 (d, $J_{C,P}$ = 17.5 Hz, C-1_{Glc}), 83.6 (d, $J_{C,P}$ = 9.1 Hz, C-4_{Rib}), 88.8 (C-1_{Rib}), 103.0 (C-5_{Ura}), 142.1 (C-6_{Ura}), 152.1 (C-2_{Ura}), 166.5 (C-4_{Ura}). ^{31}P NMR (D_2O, 80 MHz): δ −10.8 (d, $J_{P,P}$ = 26.3 Hz, CPO*P*O), 19.6 (d, $J_{P,P}$ = 26.3 Hz, C*P*O-PO). LSIMS (negative mode, glycerol) *m/z*: 577 $[M-2HNEt_3+H]^-$. HRLSIMS (negative mode, glycerol) *m/z*: calcd for $C_{17}H_{27}N_2O_{16}P_2$ $[M-2HNEt_3+H]^-$, 577.0836; found, 577.0834.

5.4.2. Bis(triethylammonium) [2-(α-D-galactopyranosyl)-ethylphosphono]uridin-5′-yl phosphate (4b). A mixture of **3b** (177 mg, 0.65 mmol), 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphomor-

pholidate (250 mg, 0.36 mmol) and tetrazole (5.6 mL, 2.53 mmol, 0.45 M in MeCN) was treated according to the general procedure described above to obtain **4b** (21 mg, 10%). $[\alpha]_D$ +39 (*c* 1.05, H_2O). ^1H NMR (D_2O, 300 MHz): δ 1.23 (t, 18H, J = 7.3 Hz, NCH_2CH_3), 1.30–2.00 (m, 4H, CH_2CH_2P), 3.10 (q, 12H, J = 7.3 Hz, NCH_2CH_3), 3.66–3.75 (m, 3H, H-5_{Gal}, H-6_{Gal}, H-$6'_{Gal}$), 3.80 (dd, 1H, $J_{3,4}$ = 3.4 Hz, $J_{3,2}$ = 9.4 Hz, H-3_{Gal}), 3.90–3.93 (m, 1H, H-4_{Gal}), 3.94–4.04 (m, 2H, H-1_{Gal} H-2_{Gal}), 4.10–4.20 (m, 2H, H-5_{Rib}, H-$5'_{Rib}$), 4.20–4.26 (m, 1H, H-4_{Rib}), 4.29–4.38 (m, 2H, H-2_{Rib}, H-3_{Rib}), 5.87 (d, 1H, J = 7.7 Hz, H-5_{Ura}), 6.00 (d, 1H, $J_{1,2}$ = 4.6 Hz, H-1_{Rib}), 7.82 (d, 1H, J = 7.7 Hz, H-6_{Ura}). ^{13}C NMR (D_2O, 125 MHz): δ 8.6 (NCH_2CH_3), 18.7 (d, $J_{C,P}$ = 2.4 Hz, CH_2CH_2P), 24.3 (d, $J_{C,P}$ = 139.4 Hz, CH_2CH_2P), 47.1 (NCH_2CH_3), 61.6 (C-6_{Gal}), 65.1 (d, $J_{C,P}$ = 5.2 Hz, C-5_{Rib}), 68.7 (C-2_{Gal}), 69.5 (C-4_{Gal}), 69.99 (C-3_{Gal}), 70.02, 74.2 (C-2_{Rib}, C-3_{Rib}), 72.0 (C-5_{Gal}), 76.1 (d, $J_{C,P}$ = 18.1 Hz, C-1_{Gal}), 83.7 (d, $J_{C,P}$ = 9.0 Hz, C-4_{Rib}), 88.8 (C-1_{Rib}), 103.0 (C-5_{Ura}), 142.1 (C-6_{Ura}), 152.2 (C-2_{Ura}), 166.6 (C-4_{Ura}). ^{31}P NMR (D_2O, 120 MHz): δ −10.3 (d, $J_{P,P}$ = 26.8 Hz, CPO*P*O), 20.3 (d, $J_{P,P}$ = 26.8 Hz, C*P*OPO). LSIMS (negative mode, glycerol) *m/z*: 577 $[M-2HNEt_3+H]^-$. HRLSIMS (negative mode, glycerol) *m/z*: calcd for $C_{17}H_{27}N_2O_{16}P_2$ $[M-2HNEt_3+H]^-$, 577.0836; found, 577.0832.

5.4.3. Diethyl 2-(2,3,4-tri-*O*-acetyl-α-L-fucopyranosyl)-ethylphosphonate (6). A solution of **5**[53] (1.5 g, 4.52 mmol) and HBr (5 mL, 33% wt in AcOH) in CH_2Cl_2 (5 mL) was stirred at 0 °C for 10 min, then warmed to rt and stirred for an additional 45 min. The mixture was poured into saturated $NaHCO_3$ (100 mL) and extracted with CH_2Cl_2 (2 × 100 mL). The organic layers were combined, dried (Na_2SO_4), filtered and evaporated to dryness. The crude orange gum was used for the next step without further purification. A solution of the crude fucosyl bromide (1.6 g, 4.52 mmol), $NaBH_3CN$ (426 mg, 6.77 mmol), AIBN (445 mg, 2.71 mmol), *n*-Bu_3SnCl (370 μL, 1.35 mmol) and diethyl vinylphosphonate (3.5 mL, 22.6 mmol) in *t*-BuOH (80 mL) was introduced into a quartz tube, then degassed (argon) for 15 min and finally irradiated for 5 h with a medium pressure mercury lamp (Hanovia/450 W) equipped with a Vycor filter (λ > 254 nm) and inserted in a two-wall jacket made of quartz with water flow for cooling. The mixture was evaporated to dryness and purified (SiO_2/petroleum ether then EtOAc, then EtOAc/MeOH, 95:5) to afford the pure α-anomer (718 mg) and a second crop (450 mg) of a 77:23 mixture of α/β-anomers, respectively, as determined by ^1H NMR. Compound **6** (1.17 g) was obtained in 59% total yield with an α/β global ratio of 9:1. The α-anomer was used for characterization and the next synthetic steps. $[\alpha]_D$ −70 (*c* 1, $CHCl_3$). ^1H NMR ($CDCl_3$, 300 MHz): δ 0.98 (d, 3H, $J_{6,5}$ = 6.4 Hz, H-6), 1.17 (dt, 6H, J_{CH_2,CH_3} = 7.3 Hz, $J_{CH_3,P}$ = 1.5 Hz, $POCH_2CH_3$), 1.40–1.91 (m, 4H, CH_2CH_2P), 1.82, 1.89, 1.98 (3s, 3× 3H, CH_3CO), 3.74 (dq, 1H, $J_{5,4}$ = 1.5 Hz, $J_{5,6}$ = 6.4 Hz, H-5), 3.94 (qd, 4H, J_{CH_2,CH_3} = 7.3 Hz, $J_{CH_3,P}$ = 2.7 Hz, $POCH_2CH_3$), 3.90–4.00 (m, 1H, H-1), 5.01 (dd, 1H, $J_{2,3}$ = 10.1 Hz, $J_{3,4}$ = 3.3 Hz, H-3), 5.08 (dd, 1H, $J_{4,5}$ = 1.5 Hz, H-4), 5.14 (dd, 1H, $J_{1,2}$ = 5.6 Hz, H-2). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 15.7 (C-6), 16.1 (d, $J_{C,P}$ = 5.8 Hz, $POCH_2CH_3$), 18.3 (d, $J_{C,P}$ = 3.8 Hz, CH_2CH_2P), 20.3, 20.4 (2s, CH_3CO), 21.3 (d, $J_{C,P}$ = 136.6 H z, CH_2CH_2P), 61.3, 61.4 (2d, $J_{C,P}$ = 6.3 Hz, $POCH_2CH_3$), 65.1 (C-5), 67.6 (C-2), 68.0 (C-3), 70.2 (C-4), 72.1 (d, $J_{C,P}$ = 16.9 Hz, C-1), 169.4, 169.6, 170.0 (3s, CH_3CO). ^{31}P NMR ($CDCl_3$, 80 MHz): δ 31.8. LSIMS (positive mode, glycerol) *m/z*: 439 $[M+H]^+$, 461 $[M+Na]^+$. HRLSIMS (positive mode, glycerol) *m/z*: calcd for $C_{18}H_{32}O_{10}P_1$ $[M+H]^+$, 439.1733; found, 439.1732.

5.4.4. Bis(triethylammonium) 2-(α-L-fucopyranosyl)-ethylphosphonate (7). To a solution of **6** (630 mg, 1.44 mmol) in MeCN (20 mL) was added C_5H_5N (1.18 mL, 14.42 mmol) at 0 °C followed by Me_3SiBr (1.90 mL, 14.42 mmol). The solution was stirred at 0 °C for 1 h then quenched with H_2O/C_5H_5N (9:1/15 mL). The mixture was evaporated to dryness and the white powder purified (RP-18/H_2O then H_2O/MeOH, 1:1) to obtain the acetylated phosphonic acid intermediate. The product was stirred in H_2O/MeOH/Et_3N (7:3:1/25 mL) for 16 h then evaporated to dryness and the white powder purified (RP-18/H_2O) to afford **7** (459 mg, 89%) as a colourless foam and as its mono-triethylammonium salt. $[\alpha]_D$ −62 (*c* 1, H_2O). ^1H NMR (D_2O, 300 MHz): δ 1.03 (d, 3H, $J_{6,5}$ = 6.4 Hz, H-6), 1.12 (t, 9H, J = 7.3 Hz, NCH_2CH_3), 1.20–1.82 (m, 4H, CH_2CH_2P), 3.03 (q, 6H, J = 7.3 Hz, NCH_2CH_3), 3.59–3.84 (m, 5H, H-1 to H-5). ^{13}C NMR (D_2O, 75 MHz): δ 8.6 (NCH_2CH_3), 16.2 (C-6), 18.8 (d, $J_{C,P}$ = 2.1 Hz, CH_2CH_2P), 24.6 (d, $J_{C,P}$ = 134.8 Hz, CH_2CH_2P), 46.9 (NCH_2CH_3), 67.2, 68.3, 70.1, 72.1 (4s, C-2 to C-5), 76.6 (d, $J_{C,P}$ = 17.1 Hz, C-1). ^{31}P NMR (D_2O, 80 MHz): δ 25.9. LSIMS (negative mode, glycerol) *m/z*: 255 $[M-2HNEt_3+H]^-$. HRLSIMS (negative mode, glycerol) *m/z*: calcd for $C_8H_{16}O_7P_1$ $[M-2HNEt_3+H]^-$, 255.0634; found, 255.0631.

5.4.5. Bis(triethylammonium) [2-(α-L-fucopyranosyl)-ethylphosphono]guanosin-5′-yl phosphate (8). A mixture of **7** (114 mg, 0.44 mmol), 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium guanosine 5′-monophosphomorpholidate (90 mg, 0.12 mmol) and tetrazole (2.8 mL, 1.24 mmol, 0.45 M in MeCN) was treated according to the general procedure described above to obtain **8** (17.6 mg, 24%) as 1:3 mixture with excess TEAB salts. ^1H NMR (D_2O, 300 MHz): δ 1.14 (d, 3H, $J_{6,5}$ = 6.4 Hz, H-6_{Fuc}), 1.31 (t, 18H, J = 7.3 Hz, NCH_2CH_3), 1.50–2.00 (m, 4H, CH_2CH_2P), 3.50 (q, 12H, J = 7.3 Hz, NCH_2CH_3), 3.68–3.83 (m, 4H, H-2_{Fuc} to H-5_{Fuc}), 3.90–3.99 (m, 2H, H-1_{Fuc}, H-5_{Rib}), 4.16–4.23 (m, 2H, H-4_{Rib}, H-$5'_{Rib}$), 4.30–4.38 (m, 1H, H-3_{Rib}), 4.52 (dd, 1H, $J_{2,1}$ = 6.0 Hz, $J_{2,3}$ = 3.3 Hz, H-2_{Rib}), (d, 1H, $J_{1,2}$ = 6.0 Hz, H-1_{Rib}), 8.13 (s, 1H, H-8_{Gua}). ^{13}C NMR (D_2O, 125 MHz): δ 15.8 (C-6), 18.4 (d, $J_{C,P}$ = 3.4 Hz, CH_2CH_2P), 24.1 (d, $J_{C,P}$ = 140.0 Hz, CH_2CH_2P), 65.3 (d, $J_{C,P}$ = 5.2 Hz, C-5_{Rib}), 66.9 (C-5_{Fuc}), 70.4 (C-3_{Rib}), 68.2, 69.9, 71.9 (C-2_{Fuc} to C-4_{Fuc}), 73.9 (C-2_{Rib}), 76.3 (d, $J_{C,P}$ = 17.2 Hz, C-1_{Fuc}), 83.7 (d, $J_{C,P}$ = 8.6 Hz, C-4_{Rib}), 87.3 (C-1_{Rib}), 137.4 (C-8_{Gua}), 151.4, 153.8, 157.8, 158.5 (C-2_{Gua}, C-4_{Gua}, C-5_{Gua}, C-6_{Gua}). ^{31}P NMR (D_2O,

80 MHz): δ −10.7 (d, $J_{P,P}$ = 26.2 Hz, CPO*P*O), 19.7 (d, $J_{P,P}$ = 26.2 C*P*OPO). LSIMS (negative mode, glycerol) *m/z*: 600 [M−2HNEt$_3$+H]$^-$. HRLSIMS (negative mode, glycerol) *m/z*: calcd for $C_{18}H_{28}N_5O_{14}P_2$ [M−2HNEt$_3$+H]$^-$, 600.1108; found, 600.1104.

5.4.6. Bis(triethylammonium) [2-(2-acetamido-2-deoxy-α-D-glucopyranosyl)-ethylphosphono]uridin-5′-yl phosphate (10). A mixture of **9**[56] (214 mg, 0.68 mmol), 4-morpholine-*N*,*N*′-dicyclohexylcarboxamidinium uridine 5′-monophosphomorpholidate (440 mg, 0.64 mmol) and tetrazole (14.2 mL, 6.41 mmol, 0.45 M in MeCN) was treated according to the general procedure described above to obtain **10** (50 mg, 13%). [α]$_D$ +35 (*c* 1, H$_2$O). ^{1}H NMR (D$_2$O, 300 MHz): δ 1.22 (t, 18H, *J* = 7.3 Hz, NCH$_2$C*H*$_3$), 1.47–1.94 (m, 4H, C*H*$_2$C*H*$_2$P), 1.99 (s, 3H, C*H*$_3$CONH), 3.11 (q, 12H, *J* = 7.3 Hz, NC*H*$_2$CH$_3$), 3.35 (t, 1H, $J_{4,3} = J_{4,5}$ = 9.5 Hz, H-4$_{Glc}$), 3.42–3.50 (m, 1H, H-5$_{Glc}$), 3.67 (dd, 1H, $J_{6,5}$ = 5.5 Hz, $J_{6,6'}$ = 12.4 Hz, H-6$_{Glc}$), 3.73 (t, 1H, $J_{3,4} = J_{3,2}$ = 9.5 Hz, H-3$_{Glc}$), 3.83 (dd, 1H, $J_{6',5}$ = 1.5 Hz, H-6′$_{Glc}$), 3.75–3.80 (m, 2H, H-1$_{Glc}$, H-2$_{Glc}$), 4.05–4.15 (m, 2H, H-5$_{Rib}$, H-5′$_{Rib}$), 4.15–4.20 (m, 1H, H-4$_{Rib}$), 4.25–4.30 (m, 2H, H-2$_{Rib}$, H-3$_{Rib}$), 5.86 (d, 1H, *J* = 7.7 Hz, H-5$_{Ura}$), 5.98 (d, 1H, $J_{1,2}$ = 4.4 Hz, H-1$_{Rib}$), 7.81 (d, 1H, *J* = 7.7 Hz, H-6$_{Ura}$). ^{13}C NMR (D$_2$O, 125 MHz): δ 8.7 (NCH$_2$*C*H$_3$), 19.6 (d, $J_{C,P}$ = 3.0 Hz, *C*H$_2$CH$_2$P), 22.3 (*C*H$_3$CONH), 24.3 (d, $J_{C,P}$ = 139.6 Hz, CH$_2$*C*H$_2$P), 47.0 (N*C*H$_2$CH$_3$), 53.8 (C-2$_{Glc}$), 61.5 (C-6$_{Glc}$), 65.1 (d, $J_{C,P}$ = 5.3 Hz, C-5$_{Rib}$), 70.0, 74.1 (C-2$_{Rib}$, C-3$_{Rib}$), 71.0 (C-3$_{Glc}$), 71.4 (C-4$_{Glc}$), 72.7 (C-5$_{Glc}$), 74.5 (d, $J_{C,P}$ = 18.3 Hz, C-1$_{Glc}$), 83.6 (d, $J_{C,P}$ = 9.1 Hz, C-4$_{Rib}$), 88.8 (C-1$_{Rib}$), 103.0 (C-5$_{Ura}$), 142.1 (C-6$_{Ura}$), 152.1 (C-2$_{Ura}$), 166.5 (C-4$_{Ura}$), 174.8 (CH$_3$*C*ONH). ^{31}P NMR (D$_2$O, 80 MHz): δ −10.7 (d, $J_{P,P}$ = 26.4 Hz, CPO*P*O), 19.2 (d, $J_{P,P}$ = 26.4 Hz, C*P*OPO). LSIMS (positive mode, glycerol) *m/z*: 620 [M−2HNEt$_3$+3H]$^+$, 721 [M−HNEt$_3$+2H]$^+$, 823 [M+H]$^+$. LSIMS (negative mode, glycerol) *m/z*: 618 [M−2HNEt$_3$+H]$^-$. HRLSIMS (negative mode, glycerol) *m/z*: calcd for $C_{19}H_{30}N_3O_{16}P_2$ [M−2HNEt$_3$+H]$^-$, 618.1101; found, 618.1108.

5.5. General procedure for the determination of IC_{50} values

Solutions were prepared and incubated as indicated below. Incubation was halted by immersion for 2 min in a boiling water bath. The solution was diluted with water (100 μL) and filtered. The samples were analyzed by RP-HPLC using fluorescence detection. The percentage of conversion was quantified from the fluorescence intensity of the peaks corresponding, respectively, to acceptor substrate and product. A control reaction without inhibitor was run simultaneously and measurements for each concentration were done in triplicate. In each assay, the amount of the disaccharide formed was less than 20% of the total amount of donor substrate (NDP-sugar).

5.6. Inhibition of β-1,4-galactosyltransferase (EC 2.4.1.22, β-1,4-GalT)

Enzyme assay conditions: 10 mM $MnCl_2$, 50 mM Hepes buffer (pH 7.4), 2 g/mL BSA, 200 μM β-GlcNAc-O-$(CH_2)_6$-dansyl,[57] 40 μM UDP-Gal, 0.1 mU β-1,4-GalT and inhibitor **4b** (1, 5, 10, 25, 50 and 100 μM) in 50 μL total volume. Assays were incubated at 30 °C for 14 min.

5.7. Inhibition of Galβ(1 → 3/4)GlcNAc-α-1,4/3-fucosyltransferase (EC 2.4.1.65, FUT3)

Enzyme assay conditions: 10 mM $MnCl_2$, 25 mM cacodylate buffer (pH 6), 200 μM Galβ(1 → 3)GlcNAcβ-*O*-$(CH_2)_6$-NH-dansyl,[57] 50 μM GDP-Fuc, 0.2 mU FUT3 and inhibitor **8** (0.5, 1, 2, 4 and 8 mM) in 50 μL total volume. Assays were incubated at 37 °C for 9 min.

5.8. Inhibition of Galβ(1 → 4)Glc-β-1,3-*N*-acetyl-glucosaminyltransferase (EC 2.4.1.56, *LgtA*)

Enzyme assay conditions: 15 mM $MnCl_2$, 50 mM cacodylate buffer (pH 7.2), 800 μM Galβ(1 → 4)Glcβ-*O*-$(CH_2)_6$-NH-dansyl,[57] 250 μM UDP-GlcNAc, 0.4 mU *LgtA* and inhibitor **10** (1, 2, 4, 8 and 16 mM) in 50 μL total volume. Assays were incubated at 37 °C for 6 min.

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