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# Synthesis of 2-fluoro and 4-fluoro galactopyranosyl phosphonate analogues of UDP-Gal

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ABSTRACT

Two novel nonisosteric UDP-Gal analogues, (2-deoxy-2-fluoro- and 4-deoxy-4-fluoro-α-D-galactopyranosyl) phosphonoyl phosphates, were synthesized by optimized multistep procedures starting from 3,4,6-tri-O-benzyl-D-galactal and allyl 2,3,6-tri-O-benzyl-α-D-glucopyranoside, respectively. The key steps were a Michaelis-Arbuzov reaction of respective deoxy-fluoro-D-galactopyranosyl acetate with triethyl phosphite followed by a Moffatt-Khorana coupling reaction with UMP-morpholidate. The structure of all new compounds was confirmed by NMR and mass spectroscopies..



## 1. Introduction

Glycosyltransferases are essential enzymes for the biosynthesis of cell–surface complex carbohydrates.[1] The galactosyltransferase (GalT) family transfers, in the presence of a metal ion, D-galactopyranosyl residue from uridine-diphosphate-α-D-galactose (UDP-Gal) to specific hydroxyl groups of a particular acceptor via β1–4, β1–3, α1–3 and α1–4 linkages.[2] Derivatives and mimics of UDP-Gal have been therefore synthesized very frequently as potential GalT inhibitors for drug discovery.[3,4] The replacement of β-phosphate in UDP-Gal with a phosphonate group seems to be a general strategy for the generation of hydrolytically stable analogues.[5–8] Furthermore, the substitution of a D-galactopyranose hydroxyl group with fluorine especially at position 2 can imply dramatic changes in binding ability.[9–12] UDP-Gal2F was found to be a reversible inhibitor of α-1,3-GalT, giving $K_i$ 245 μM.[13]

As a further study on the synthesis of new UDP-Gal analogues combining the structural features of a hydrolytically stable C1–P bond and an activation effect of fluorine attached at the position 2 or 4 of the galactopyranosyl unit, we report herein the synthesis of uridine 5′-(4-deoxy-4-fluoro-α-D-galactopyranosyl)phosphonoyl phosphate (**1**) and uridine 5′-(2-deoxy-2-fluoro-α-D-galactopyranosyl)phosphonoyl phosphate (**2**).

## 2. Results and discussion

The synthesizing strategy was dictated by a modification that was accomplished first. Fluorine can be introduced by numerous nucleophilic substitutions and the Michaelis–Arbuzov reaction is generally a suitable tool for the creation of hexopyranosyl phosphonate.[14] According to preliminary experiments, the fluorination was chosen as the first modification and benzyl protecting groups were preferred over acetyls (Scheme 1).

The key intermediates, 1-*O*-acetyl-2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-D-galactopyranose (**7**) and 1-*O*-acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-2-fluoro-D-galactopyranose (**8**) (Scheme 1) could yield in the Michaelis–Arbuzov reaction with triethyl phosphite and Lewis acid the corresponding anomeric diethyl phosphonates **5** and **6**, respectively. After separation and deesterification, the phosphonic acids **3** and **4** will be coupled with uridine 5′-phosphate (UMP) to the target compounds **1** and **2**.

### 2.1. Synthesis of acetates 7 and 8

Methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside[15] seemed to be initially a good starting compound for the synthesis of acetate **7** (Scheme 2). However, the final hydrolysis or acetolysis of methyl galactopyranoside **9** yielded the respective pyranose **12** or 1-acetate **7** only with very low yields. The loss of valuable intermediate **9** urged us to search for a more suitable starting compound. Allyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside[16] was esterified providing allyl 2,3,6-tri-*O*-benzyl-4-*O*-triflyl-α-D-glucopyranoside (not isolated and characterized), which was transformed into allyl 4-deoxy-4-fluoro-α-D-galactopyranoside **10** by treatment with TASF.[17] Allyl glycoside **10** was then treated with a catalytic amount of $(Ph_3P)_3RhCl$ in the presence of DABO[18] to give prop-1-en-1-yl 2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-α-D-galactopyranoside (**11**) as a mixture of (*E*)- and (*Z*)-isomers that was cleaved without seperation to produce the corresponding galactopyranose **12** with

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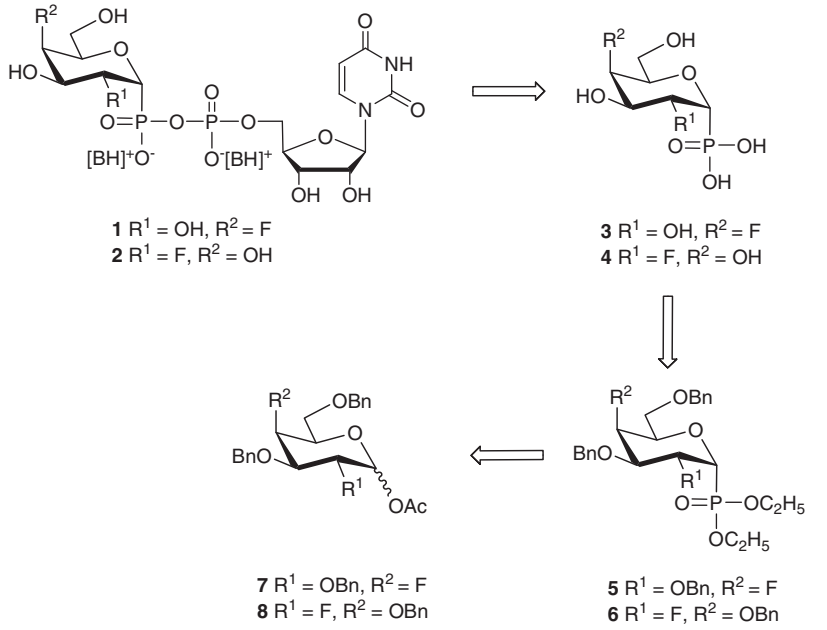
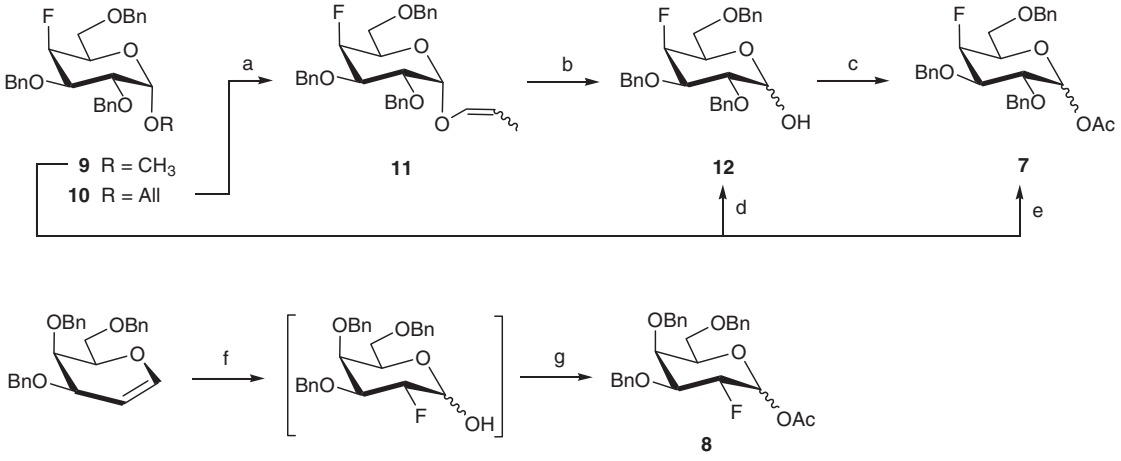



**Scheme 1.** Retrosynthesis.




**Scheme 2.** Reagents and conditions: (a) EtOH–Tol–$H_2O$ (7:3:1), $(Ph_3P)_3RhCl$, DABO, 92%; (b) acetone–1 M HCl (10:1), 86%; (c) Py, $Ac_2O$, 91%; (d) 80% AcOH, 1 M HCl, 36%; (e) AcOH–$Ac_2O$ (3:1), $CH_2Cl_2$, $H_2SO_4$, 15%; (f) $CH_3NO_2$, Selectfluor™, $H_2O$; (g) Py, $Ac_2O$, 54%.

an 86% two-step overall yield. In contrast, direct deprotection of allyl glycoside **10** with palladium chloride in diluted acetic acid[19] gave only 44% aldose **12**. Sequential acetylation of **12** provided acetate **7** with a 91% yield.

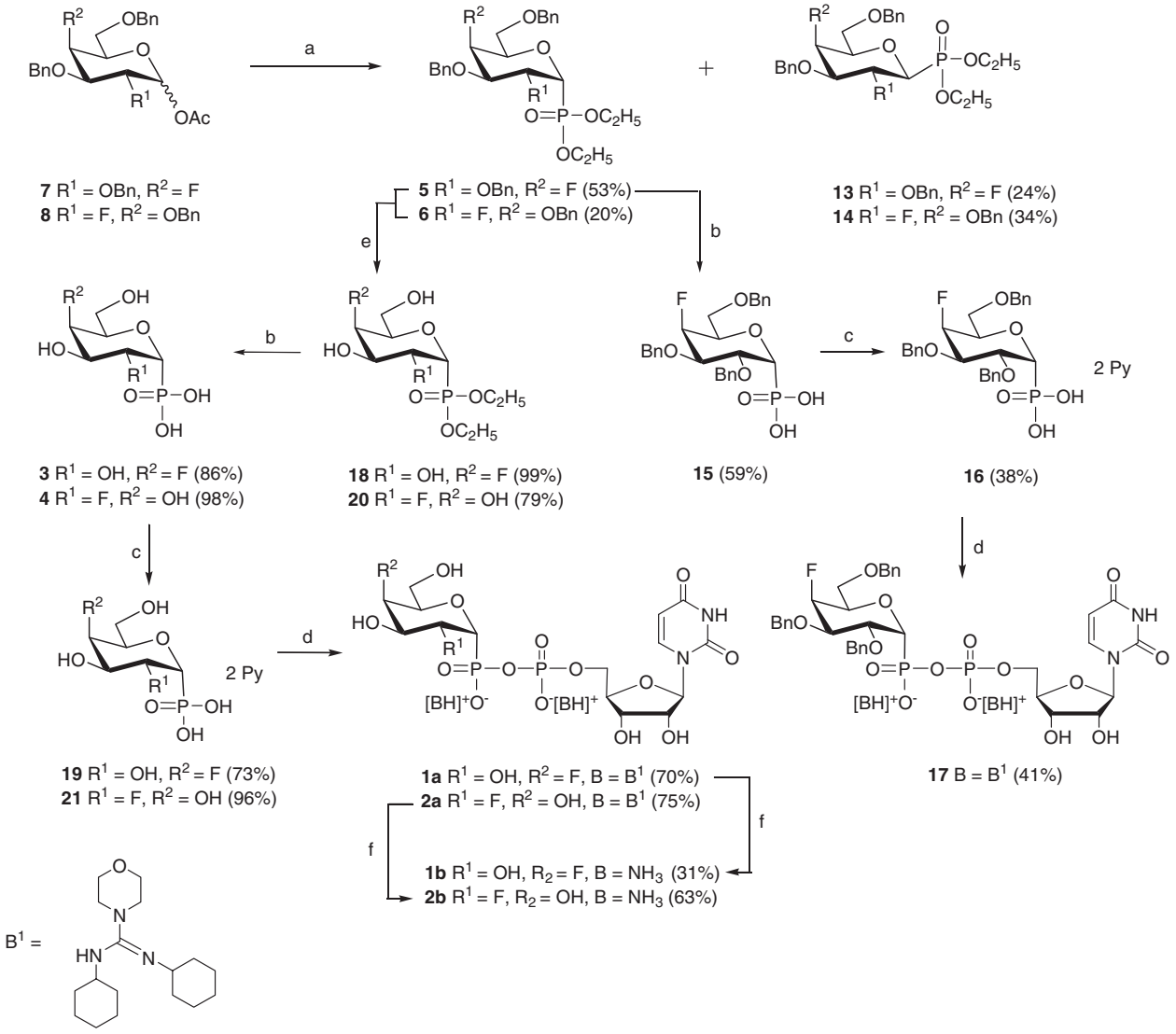
Contrary to the 4-deoxy-4-fluorohexose **7**, the synthesis of its 2-deoxy-2-fluoro isomer **8** has been a very active area for many years. Commercially available 3,4,6-tri-*O*-benzyl-D-*lyxo*-hex-1-enitol (3,4,6-tri-*O*-benzyl-D-galactal) was subjected to the treatment of Selectfluor™ in a slightly modified protocol.[20] Instead of *N*,*N*-dimethylformamide, nitromethane was used as a solvent due to its greater evaporation rate. 3,4,6-Tri-*O*-benzyl-2-deoxy-2-fluoro-D-galactose was not isolated in a pure state but the crude reaction mixture was immediately acetylated and the target acetate **8** was obtained with a 54% overall yield, as a mixture of the α/β anomers in the ratio of ca 1.2 (Scheme 2).

## 2.2. Preparation of C1-phosphonates by the Michaelis–Arbuzov reaction

A direct nucleophilic substitution of the C-1 acetoxy group of acetates **7** and **8** with a dialkyl phosphonate group could be accomplished by the Michaelis–Arbuzov reaction; the mechanism of which has been proposed.[21–23]

Treatment of the anomeric acetates **7** ($\alpha/\beta = 1.4$) with triethyl phosphite and TMSOTf in dichloromethane primarily at 0 °C, later at rt gave diethyl phosphonates **5** and **13** with a very good yield of 77%, that is comparable with the 80% described for diethyl (2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)phosphonates[14] (Scheme 3).

Diethyl 4-deoxy-4-fluoro-α-D-galactopyranosyl phosphonate **5** and its β-D-isomer **13** were easily separated by column chromatography. Both phosphonates **5** and **13** showed in NMR spectra signals characteristic for the presence of fluorine on C-4 and phosphorus on C-1. The comparison of the chemical shifts and coupling constants $J_{C,P}$ of the C-1 signals is highly valuable for the determination of the anomeric configuration in **5** and **13**. The coupling constant $J_{C1,P}$ for α-phosphonate **5** was lower than that for β-phosphonate **13** in accord with the data published for other phosphonates.[14,24] This assignment corresponds also with the generally accepted rule that the signal of carbon C-1 of pyranose with α-D-configuration appears at a higher field than the C-1 signal of the corresponding β-D-anomer.[25]




**Scheme 3.** Reagents and conditions: (a) $CH_2Cl_2$, $(C_2H_5O)_3P$, TMSOTf; (b) $CH_2Cl_2$, $BrSiMe_3$, acetone–$H_2O$ (9:1) (2 steps); (c) Py; (d) Py, UMP-morpholidate, 1*H*-tetrazole, Hünigs base; (e) $H_2$, MeOH, 20% $Pd(OH)_2/C$; (f) Dowex 1-X8, $HCOO^-$, elution with 0–1 M $NH_4HCO_3$.

Preparation of the isomeric 2-deoxy-2-fluoro-α-D-galactopyranosyl phosphonate **6** and 2-deoxy-2-fluoro-β-D-galactopyranosyl phosphonate **14** proceeded starting from acetate **8**, analogous to the procedure for phosphonates **5** and **13** described above (Scheme 3). Unfortunately, we observed a complete loss of stereoselectivity resulting in a ratio of **6**/**14** = 3:5. In addition, the preparative yield was low, reaching only 54% due to the presence of several by-products. Our results confirm the previous finding that a ratio of products arising from the Michaelis–Arbuzov reaction does not depend substantially on the anomeric configuration of incoming glycosyl acetate.[26] For comparison, an anomeric mixture of 1-*O*-acetyl-2,3,4,6-tetra-*O*-benzyl-D-galactopyranose ($\alpha/\beta = 2$) gave the corresponding diethyl phosphonates in the ratio $\alpha/\beta = 1.5$.[14] A ratio varying from 20: 1 to 10: 1 in favour of the 1,2-*cis*-configurated dimethyl glycosyl phosphonates (i.e., α-D-*gluco*, α-D-*galacto*, or β-D-*manno*), was established for the respective protected 1-*O*-acetyl hexopyranoses.[24]

### 2.3. Preparation of target phosphonoyl phosphates 1 and 2

The phosphono-phosphate bond in **1** and **2** could be created by the reaction of ammonium or pyridinium salt of the respective phosphonic acid with an activated form of UMP following the Moffatt–Wong protocol.[27] Deesterification of the diethyl phosphonate **5** by treatment with bromotrimethylsilane produced phosphonic acid **15** (Scheme 3). Phosphonic acid **15** was then transformed into pyridinium salt **16** and its coupling with UMP-morpholidate was finished after 8 days. The phosphonophosphate **17** was isolated as a bis(4-morpholine-*N*,*N*′-dicyclohexyl carboximidamide) salt. Subsequently, the salt **17** was debenzylated by catalytic hydrogenation but the deprotection was accompanied with the partial reduction of the uracil double bond. Because of that, the deprotected phosphonic acids **3** and **4** had to be used in the coupling reaction.

Thus, diethyl phosphonate **5** was hydrogenated on $Pd(OH)_2/C$ in MeOH and produced 4-deoxy-4-fluoro-α-D-galactopyranosyl phosphonate **18** (Scheme 3). In the next step ethyl ester groups in **18** were cleaved with trimethylsilyl bromide giving phosphonic acid **3**. Finally, bis(pyridinium) (4-deoxy-4-fluoro-α-D-galactopyranosyl)phosphonate (**19**) was obtained after repeated co-evaporation of **3** with pyridine. Coupling reaction of **19** with UMP-morpholidate under activation by 1*H*-tetrazole was finished after 8 days. After repeated LC purification on RP-18 phase, only impure 4-morpholine-*N*,*N*′-dicyclohexyl carboximidamidium salt **1a**

contaminated with UMP-morpholidate was obtained. In $^1$H NMR spectra there were characteristic signals of protons on the double bond of uracil, doublet of the D-ribofuranose H-1 proton, signal of H-4 of 4-deoxy-4-fluoro-D-galactose and dominant signals belonging to the protons of the base moiety. The further purification was ineffective therefore the dicyclohexyl carboximidamidium salt **1a** was transformed to ammonium salt **1b** using ion-exchange resin Dowex 1-X8 in the formiate form and ammonium bicarbonate solution as eluent in a concentration gradient.[28] In the first fractions eluted with water only, 4-morpholine-*N*,*N*′-dicyclohexyl carboximidamidium formiate was identified. Diammonium uridin-5′-[(4-deoxy-4-fluoro-α-D-galactopyranosyl)phosphonyl]phosphate (**1b**) was then obtained with a 31% yield, followed by the spare UMP-morpholidate.

The synthesisizing route for the preparation of the analogue **2** followed the same strategy already developed for **1** (Scheme 3). Benzyl groups in diethyl phosphonate **6** were removed by catalytic hydrogenation followed by deesterification of **20** and transformation of phosphonic acid **4** into bis(pyridinium) salt **21**. The coupling reaction of the bis(pyridinium) phosphonate **21** with UMP-morpholidate produced 4-morpholine-*N*,*N*′-dicyclohexyl carboximidamide salt **2a**. The diammonium salt **2b** was then prepared with a 63% yield by passing salt **2a** through an ion-exchange resin.

In summary, we have synthesized two novel hydrolytically stable UDP-Gal mimics having fluorine atom at the sensitive positions of the galactopyranosyl unit. Biological evaluation is currently under investigation and the results will be reported in due course.

## 3. Experimental

### 3.1. General methods

Melting points were determined with a Kofler apparatus, and are not corrected. Optical rotations were measured with a Autopol VI (Rudolph) digital polarimeter in appropriate solvents, at a temperature of 20 °C and the 589 nm sodium line, in 1 dm cuvettes and are given at $10^{-1}$ deg cm$^2$ g$^{-1}$. Silica gel (100–160 μm, Merck) was used for column chromatography, C-18 silica gel (200–400 mesh, Sigma Aldrich) for chromatography on reversed phase.

NMR spectra were recorded with a Bruker DRX 500 Avance spectrometer operating at 500.1 MHz for $^1$H, 125.8 MHz for $^{13}$C, 470.4 MHz for $^{19}$F and 202.4 MHz for $^{31}$P, or with a Varian Gemini 300HC spectrometer operating at 299.9 MHz for $^1$H, 282.2 MHz for $^{19}$F and 121.4 MHz for $^{31}$P, in the solvents $CDCl_3$, $CD_3OD$, or $D_2O$. Chemical shifts were stated in ppm and referenced to tetramethylsilane, trichlorofluoromethane or phosphoric acid, respectively ($\delta$ = 0 ppm). Coupling constants ($J$) are reported in Hertz (Hz). Assignment of the signals was accomplished by means of COSY, HETCOR, APT and HMQC experiments. In the description of spectra of the potential inhibitors, H or C nuclei of the D-ribose residue are apostrophized, nuclei on the uracil moiety are marked in brackets as (U); the nuclei of the deoxyfluorogalactose are presented without special marking.

Mass spectra were measured with a Q-Tof Micro (Waters, USA) or LTQ Velos Orbitrap (Thermo Fisher Scientific, UK) instruments equipped with LockSpray in ES+ and ES− modes with the mobile phase of methanol and a flow rate of 100 μL min$^{-1}$.

HPLC-MS system consisted of a Hewlett-Packard HP/Agilent Technologies (USA) 1100 HPLC instrument which was coupled on line to HP mass selective single quadrupole detector (model G1946 A) and controlled by ChemStation software (revision B.02.01). Column RP C18 (30 × 2.1 mm) Zorbax SB-C18 (3.5 μm particle size) was used with mobile phases of 50% methanol (A) and 100% methanol (B), each containing 5 mmol ammonium formiate and a flow rate 0.3 mL min$^{-1}$. Capillary voltage for electrospray ionization was set at 3.5 kV.

Unless otherwise stated, solvents were evaporated at 40 °C and 2 kPa and compounds were dried at 60 °C and 2 kPa. Reactions were monitored by thin-layer chromatography (TLC) on the silica gel plates with Merck Stahl 10–40 μm. Compound traces on the TLC plates were visualized by exposure or with an aqueous solution of $Ce(SO_4)_2$ in 10% $H_2SO_4$ followed by charring, or with UV radiation at a wavelength of 254 nm.

### 3.2. Synthesis of acetates 7 and 8

#### 3.2.1. Methyl 2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-α-D-galactopyranoside (9)

Methyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside[15] (2.22 g, 4.78 mmol) in a solution of pyridine (2.4 mL) and $CH_2Cl_2$ (30 mL) was converted into the 4-*O*-triflyl derivative by treatment with triflic anhydride (1 mL, 5.9 mmol + 2 mL $CH_2Cl_2$) at −10 °C. After 1 h the reaction was judged by TLC (3:1, petroleum ether–EtOAc) to be complete. The reaction mixture was then diluted with $CH_2Cl_2$ (60 mL), washed with aqueous HCl (2 M, 30 mL), satd $NaHCO_3$ (30 mL), $H_2O$ (30 mL), dried and concentrated. To a solution of raw 4-*O*-triflyl derivative (2.72 g, 96%) in cold $CH_2Cl_2$ (60 mL), TASF (4 g, 14.52 mmol) was added. The solution was heated under reflux (60 °C) and the reaction was judged to be complete by TLC after 20 min. Product was obtained in the usual manner and purified by flash chromatography (3:1, petroleum ether–EtOAc,) to give syrup **9** (1.68 g, 75%), $[\alpha]_D^{20}$ +63 (*c* 1.1, MeOH). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.43–7.29 (m, 15H, aromatic protons), 4.86 (br d, 1H, $^2J_{4,F}$ = 49.8, H-4), 4.84 (d, 1H, $J$ = 12.1, PhC*H*), 4.83 (d, 1H, $J$ = 12, PhC*H*), 4.77 (d, 1H, $J$ = 12, PhC*H*), 4.70 (d, 1H, $J$ = 12.1, PhC*H*), 4.67 (d, 1H, $J_{1,2}$ = 2.9, H-1), 4.60 (d, 1H, $J$ = 11.8, PhC*H*), 4.56 (d, 1H, $J$ = 11.8, PhC*H*), 3.95–3.86 (m, 3H, H-2, H-3, H-5), 3.69 (dd, 1H, $J_{6a,5}$ = 7.2, $J_{6a,6b}$ = 9.5, H-6a), 3.62 (dd, 1H, $J_{6b,5}$ = 6.5, $J_{6b,6a}$ = 9.5, H-6′), 3.40 (s, 3H, $OCH_3$); NMR (125 MHz, $CDCl_3$) $\delta$: 138.26, 138.14, 137.79 (C-quart.), 128.4–127.57 (C-arom.), 98.71 (C-1), 87.55 (d, $^1J_{C,F}$ = 182.5, C-4), 75.94 (d, $^2J_{C\text{-}3,F}$ = 17.6, C-3), 75.7 (C-2), 73.84, 73.58, 72.74 (3 × O*C*H$_2$Ph), 68.01 (d, $J_{C\text{-}6,F}$ = 6.8, C-6), 67.92 (d, $^2J_{C\text{-}5,F}$ = 19.5, C-5), 55.52 (–$OCH_3$); $^{19}$F NMR (470 MHz) $\delta$: −219.1 (ddd, $J_{F,H\text{-}4}$ = 50.3, $J_{F,H\text{-}3}$ = $J_{F,H\text{-}5}$ = 30.4, F-4); ESI-MS, *m*/*z*: calcd for $C_{28}H_{31}FNaO_5$ [**9**+Na]$^+$: 489.20, found: 489.24. Anal. Calcd for $C_{28}H_{31}FO_5$: C, 72.08; H, 6.70. Found: C, 72.11; H, 6.56.

#### 3.2.2. Allyl 2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-α-D-galactopyranoside (10)

A solution of allyl 2,3,6-tri-*O*-benzyl-α-D-glucopyranoside[16] (475 mg, 0.97 mmol) in pyridine (1.2 mL) and $CH_2Cl_2$ (10.7 mL) was converted into the 4-*O*-triflyl derivative by treatment with $Tf_2O$ (0.53 mL, 3.1 mmol + 1.06 ml $CH_2Cl_2$) at −10 °C. After 1 h, the reaction was completed as checked by TLC (3:1, *n*-hexane–EtOAc). The reaction mixture was then diluted with $CH_2Cl_2$ (13 mL), the solution stepwise washed with 2 m aq HCl (7 mL), satd $NaHCO_3$ (7 mL), $H_2O$ (7 mL) and dried ($MgSO_4$). After filtration and evaporation of the solvent, the residual syrup triflate (584 mg, yield 97%) was diluted with cold $CH_2Cl_2$ (15 mL) and TASF (878 mg, 3.18 mmol) was added. The solution was heated at 60 °C and the reaction was judged to be complete by TLC (3:1, *n*-hexane–EtOAc) after 20 min and then concentrated. Purification by column chromatography (3:1, *n*-hexane–EtOAc) gave **10** as a syrup (436 mg, 91%), $[\alpha]_D^{20}$ +37 (*c* 1.4, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.40–7.26 (m, 15H, aromatic protons), 5.91 (m, 1H, –C*H*═), 5.30 (m, 1H, ═C*H*$_2$), 5.20 (m, 1H, ═C*H*$_2$), 4.86 (dd, 1H, $J_{F,4}$ = 49.6, H-4), 4.85 (d, 1H, overlap., H-1), 4.83 (d, 1H, $J$ = 11.5, PhC*H*), 4.82 (d, 1H, $J$ = 10.8, PhC*H*), 4.80 (d, 1H, $J$ = 10.2, PhC*H*), 4.75 (d, 1H, $J$ = 10.8, PhC*H*), 4.66 (d, 1H, $J$ = 12, PhC*H*), 4.55 (d, 1H, $J$ = 11.6, PhC*H*), 4.15 (m, 1H, OC*H*$_2$), 4.02 (m, 1H, OC*H*$_2$), 4.00–3.88 (m, 3H, H-2, H-3, H-5), 3.67 (dd, 1H, $J$ = 9.4, 7.2, H-6a), 3.59 (dd, 1H, $J$ = 9.5, 6.5, H-6b); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 138.35, 138.23, 137.83

(C-quart.), 133.66 (–*C*H=), 128.41–127.64 (C-arom.), 118.17 (*C*$H_2$=), 96.28 (C-1), 87.54 (d, $^1J_{C,F}$ = 182.5, C-4), 76.14 (d, $^2J$ = 17.6, C-3), 75.76 (C-2), 73.64, 73.57 and 72.77 (3 × Ph*C*$H_2$), 68.50 (–O*C*$H_2$–), 68.11 (d, $^2J$ = 17.8, C-5), 67.96 (d, $J_{C\text{-}6,F}$ = 5.5, C-6); $^{19}$F NMR (470 MHz, $CDCl_3$) $\delta$: −218.04 (ddd, $J_{F,3} = J_{F,5}$ = 29.9, $J_{F,4}$ = 49.6); ESI-MS, *m/z*: calcd for $C_{30}H_{33}FNaO_5$ [**10**+Na]$^+$: 515.22, found: 515.30. Anal. Calcd for $C_{30}H_{33}FO_5$: C, 73.15; H, 6.75. Found: C, 72.71; H, 6.57.

### 3.2.3. Prop-1-*en*-1-yl 2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-α-D-galactopyranoside (11)

To a solution of allyl 2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-α-D-galactopyranoside (**10**) (1.55 g, 3.14 mmol) in EtOH–toluene–$H_2O$ (7:3:1) (185 mL), tris(triphenylphosphine)-rhodium(I)chloride (239 mg) and 1,4-diazabicyclo[2.2.2]octane (77 mg) were added. The mixture was stirred under reflux at 100 °C until the starting compound was consumed (ca 5 h). After concentration the residue (1.94 g) was extracted between water and diethyl ether. The organic phase was dried ($MgSO_4$) and the solvent evaporated. The residue (1.71 g) was purified by chromatography (3:1, *n*-hexane–EtOAc) to give prop-1-e*n*-1-yl 2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-α-D-galactopyranoside (**11**) (1.57 g, 91.8%, mixture of (*E*) and (*Z*) isomers in a ratio about 1.6:1) which was directly used in the next reaction for the preparation of free aldose **12**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.41–7.25 (m, aromatic protons), 6.14 (dq, *J* = 12.3, 1.7, –O–C*H*= (*E*)), 6.03 (dq, *J* = 6.2, 1.7, –O–C*H*= (*Z*)), 5.16 (m, Σ*J* = 33.1, =*H*C$CH_3$ (*E*)), 5.0–4.58 (m, H-4, H-1, =*H*C–$CH_3$ (*Z*) and 3 × OC$H_2$Ph), 4.19–3.86 (m, H-2, H-3, H-5), 3.69–3.42 (m, H-6a, H-6b), 1.61 (dd, *J* = 7, 1.7, C$H_3$–C= (Z)), 1.54 (dd, *J* = 7.9, 1.5, C$H_3$–C= (E)). ESI-MS, *m/z*: calcd for $C_{30}H_{34}FO_5$ [**11**+H]$^+$: 493.23, found: 493.24. Anal. Calcd for $C_{30}H_{33}FO_5$: C, 73.15; H, 6.75. Found: C, 72.95; H, 6.78.

### 3.2.4. 2,3,6-Tri-*O*-benzyl-4-deoxy-4-fluoro-D-galactopyranose (12)

**3.2.4.1. From methyl glycoside 9 by treatment with AcOH–HCl–$H_2O$.** Methyl 2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-α-D-galactopyranoside (**10**) (107 mg, 0.23 mmol) was heated in a steam bath at 100 °C with a mixture of 80% acetic acid (2 mL) and hydrochloric acid (1 m, 0.65 mL). The reaction mixture was monitored by TLC (2:1, *n*-hexane–EtOAc; **9**: $R_f$ = 0.86; **12**: $R_f$ = 0.65). The mixture was cooled after 20 h, diluted with $CHCl_3$ (12 mL) and washed with aq $NaHCO_3$ (3 × 20 mL) and brine (10 mL). After drying and concentration, a dark yellow syrup (118 mg) resulted, which after purification by column chromatography on silica gel (3:1, *n*-hexane–EtOAc) gave 21.7 mg (36%) of the hexose **12**. $^1$H NMR (300 MHz, $CDCl_3$) $\delta$: 7.42–7.25 (m, aromatic protons), 5.24 (d, 1H, $J_{1,2}$ = 2.7, H-1α), 4.95–4.68 (m, 7H, 6 × PhC*H*, H-4, $^2J_{4,F}$ =50,6), 4.63 (d, 1H, $J_{1,2}$ = 7.4, H-1β), 4.25–4.16 (ddd, 1H, H-5, $J_{5,F}$ = 30 ).

**3.2.4.2. From allyl glycoside 10 by treatment with $PdCl_2$.** Palladium chloride (496 mg, 3.12 mmol) was added to a solution of glycoside **10** (492 mg, 1.00 mmol) in 95% aq AcOH (9.95 mL) containing AcONa (312 mg).[19] The dark suspension was stirred until TLC evidenced complete conversion of the reactant into a slower moving spot (ca 4 h). The reaction mixture was filtered through a bed of Celite, diluted with $CH_2Cl_2$ (15 mL) and washed with water (8 mL), satd $NaHCO_3$ soln (2 × 8 mL) and water (5 mL), dried ($MgSO_4$) and concentrated. The resulting dark yellow syrup was purified by flash chromatography *n*-hexane–EtOAc, 3:1 to provide aldose **12** (100 mg, 44%, α/β 2.5). $[\alpha]_D^{20}$ +56 (*c* 0.84, acetone); $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 7.25–7.40 (m, aromatic protons), 5.22 (d, *J* = 2.6, H-1α), 4.88–4.66 (m, PhC$H_2$), 4.84 (d, $^2J$ = 50.4, H-4α), 4.63 (dd, *J* = 7.4, H-1β), 4.56 (d, *J* = 11.9, PhC$H_2$), 4.52 (d, *J* = 11.9, PhC$H_2$), 4.16 (ddd, $J_{5,F}$ = 29.73, $J_{5,6} = J_{5,6'}$ = 6.5, H-5α), 3.90–3.86 (m, $J_{3,4}$ = 3.2, $J_{3,2}$ = 10.5, H-2, H-3), 3.67–3.62 (m, H-6a, H-6b), 3.16 (s, OH); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$: 128.59–127.55 (C-arom.), 97.36 (C-1β), 91.84 (C-1α), 87.17 (d, $^1J_{C,F}$ = 183.2, C-4α), 86.74 (d, $^1J_{C,F}$ = 183.0, C-4β), 75.68 (C-3α), 73.83 (Ph$CH_2$α), 75.32 (Ph$CH_2$β), 73.72 (Ph$CH_2$β), 73.59 (Ph$CH_2$α), 72.37 (Ph$CH_2$α/Ph$CH_2$β), 68.19 (C-5α), 68.01 (C-6α), 67.9 (C-6 β); $^{19}$F NMR (470 MHz, $CDCl_3$) δ:-219.4 (ddd, $J_{F,4}$ = 50.5, $J_{F,3} = J_{F,5}$ = 30.5, F-4α); −216.7 (ddd, $J_{F,4}$ = 50.1, $J_{F,3} = J_{F,5}$ = 27.9, F-4β); ESI-MS, *m/z*: calcd for $C_{27}H_{30}FO_5$ [**12**+H]$^+$ 453.20, found: 453.21. Anal. Calcd for $C_{27}H_{29}FO_5$: C, 71.66; H, 6.46. Found: C, 71.95; H, 6.78.

**3.2.4.3. From prop-1-en-1-yl 2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-α-D-galactopyranoside (11).** Galactopyranoside **11** (4.035 g, 8.19 mmol) was dissolved in acetone-aq 1 M HCl (10:1, 238 mL) and stirred under reflux at 100 °C for 40 min. The course of the reaction was monitored by TLC (3:1, *n*-hexane–EtOAc). After cooling the solution was neutralized with $NaHCO_3$ and then evaporated. The residue was diluted with water and then extracted with diethylether, the organic phase was dried and concentrated. Column chromatography (3:1, *n*-hexane–EtOAc) gave **12** (2.96 g, 86%). $^1$H NMR data were consistent with those obtained in Section 3.2.4.2.

### 3.2.5. 1-*O*-Acetyl-2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-D-galactopyranose (7)

**3.2.5.1. From methyl 2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-α-D-galactopyranoside (9).** To a solution of methyl glycoside **9** (200 mg, 0.43 mmol) in AcOH–$Ac_2O$, 3:1 (3 mL) and $CH_2Cl_2$ (30 mL), concentrated $H_2SO_4$ (0.1 mL) was added dropwise. After vigorous stirring for 1 h at room temperature the reaction mixture was monitored by TLC (2:1, *n*-hexane–EtOAc; **9**: $R_f$ = 0.54, **7**: $R_f$ = 0.71). The reaction was quenched by the addition of ice (15 g) and the mixture was extracted with $CH_2Cl_2$ (3 × 10 mL), the extract was washed with satd $NaHCO_3$ soln (3 × 10 mL) and $H_2O$ (3 × 10 mL), concentrated and dried. The raw product (151 mg) was purified by flash chromatography (3:1, petroleum ether–EtOAc) to give acetate **7** (30 mg, 15%) as a syrup, the TLC and NMR data were consistent with the product obtained in Section 3.2.5.2.

**3.2.5.2. From 2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-D-galactopyranose (12).** A solution of aldose **12** (396 mg, 0.876 mmol) in pyridine (2 mL) with $Ac_2O$ (2 mL) was stirred at room temperature for 1 h, then was concentrated and dried (407 mg). The residue was purified by flash chromatography on silica gel (3:1, *n*-hexane–EtOAc) to give acetate **7** as a colourless syrup, (390 mg, 91%, α/β = 1.4). $[\alpha]_D^{20}$ +63 (*c* 1.06, MeOH); $^1$H NMR (500 MHz, $CDCl_3$) $\delta$: 7.39–7.25 (m, aromatic protons), 6.33 (d, $J_{1,2}$ = 3.5, H-1α), 5.58 (d, $J_{1,2}$ = 8.0, H-1β), 4.94 (bdd, $J_{4,3}$ = 2.2, $J_{H,F}$ = 50, H-4α), 4.89 (bdd, $J_{4,3}$ = 2.1, $J_{H,F}$ = 50, H-4β), 4.84 (d, *J* = 11.2, PhC*H*–), 4.79–4.68 (m, PhC*H*–), 4.56–4.50 (m, PhC*H*–), 4.08–3.95 (m, H-5α), 4.01 (dd, $J_{2,1}$ = 3.5, $J_{2,3}$ = 10.6, H-2α), 3.84 (ddd, $J_{3,4}$ = 2.2, $J_{3,2}$ = 9.9, $J_{H,F}$ = 28.3, H-3α), 3.84 (dd, $J_{2,3}$ = 9.7, $J_{2,1}$ = 8, H-2β), 3.78–3.54 (m, H-6aβ, H-6bβ, H-6aα, H-6bα, H-5β, H-3β), 2.11 (s, $COCH_3$α), 2.04 (s, $COCH_3$β); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$: 169.30 (COMe), 169.17 (COMe), 138.1–137.53 (C-arom. quart.), 128.45–127.69 (C-arom.); α anomer: 90.37 (C-1), 86.68 (d, $^1J_{C,F}$ = 183.75, C-4), 75.53 (d, $^2J_{C,F}$ = 17.6, C-3), 74.62 (C-2), 73.69, 73.63, 72.52 (3 × Ph*C*$H_2$–), 70.34 (d, $^2J_{C,F}$ = 18, C-5), 67.4 (d, $J_{C,F}$ = 5.5, C-6), 21.06 (*C*O*C*$H_3$); β anomer: 93.73 (C-1), 85.32 (d, $^1J_{C,F}$ = 183.75, C-4), 79.37 (d, $^2J_{C,F}$ = 18.1, C-3), 77.53 (C-2), 75.44, 73.63, 72.28 (3 × Ph*C*$H_2$–), 72.74 (d, $^2J_{C,F}$ = 18.4, C-5), 66.95 (d, $J_{C,F}$ = 5.5, C-6), 20.9 (*C*O*C*$H_3$); $^{19}$F NMR data (470 MHz, $CDCl_3$) $\delta$: α anomer −214.76 (ddd, $J_{F,H\text{-}3} = J_{F,H\text{-}5}$ = 28.6, $J_{F,H\text{-}4}$ = 50.2); β anomer: −213.32 (ddd, $J_{F,H\text{-}3} = J_{F,H\text{-}5}$ = 27.6, $J_{F,H\text{-}4}$ = 49.6); ESI-MS, *m/z*: calcd for $C_{29}H_{31}FNaO_6$ [**7**+Na]$^+$: 517.20, found: 517.32. Anal. Calcd for $C_{29}H_{31}FO_6$: C, 70.43; H, 6.32. Found: C, 70.11; H, 6.56.

#### 3.2.6. 1-*O*-Acetyl-3,4,6-tri-*O*-benzyl-2-deoxy-2-fluoro-D-galactopyranose (8)

A suspension of water (5 mL) and 1-chloromethyl-4-fluoro-1,4-diazoniabicyclo[2,2,2]octane bis(tetrafluoroborate) (Selectfluor™, 1.7 g, 4.76 mmol) were added to a solution of 3,4,6-tri-*O*-benzylgalactal (1.084 g, 2.66 mmol) in $CH_3NO_2$ (25 mL) and the reaction was allowed to proceed for 15 min at 100 °C. The reaction was monitored by TLC (4:1, *n*-hexane–EtOAc) and quenched with water (20 mL). Then the mixture was extracted with EtOAc (3 × 30 mL). The combined organic extracts were dried with $MgSO_4$ and concentrated (1.256 g, 2.78 mmol). The residue was dissolved in pyridine (4 mL) and $Ac_2O$ (4 mL) was added. After 16 h, the reaction was finished by the addition of MeOH (8 mL) and $CH_2Cl_2$ (40 mL). After washing with 5% $H_2SO_4$ (2 × 10 mL) and satd $NaHCO_3$ soln (2 × 10 mL) the organic phase was dried ($MgSO_4$). Purification on silica gel column (7:1, *n*-hexane–EtOAc) gave **8** (610 mg, 54%, $\alpha/\beta$ 1) as a colourless oil, $[\alpha]_D^{20}$ +48 (*c* 3.0, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$: 7.30–7.19 (m, aromatic protons), 6.32 (d, $J_{1,2}$ = 3.6, H-1α), 5.59 (dd, $J_{1,2}$ = 7.8, $J_{1,F}$ = 4.8, H-1β), 5.01 (ddd, $J_{2,1}$ = 3.8, $J_{2,3}$ = 9.8, $J_{2,F}$ = 49.5, H-2α), 4.86 (d, *J* = 8.7, PhC*H*), 4.84 (d, *J* = 8.7, PhC*H*), 4.75 (ddd, $J_{2,1}$ = 8.7, $J_{2,3}$ = 9.1, $J_{2,F}$ = 51.9, H-2β), 4.73 (d, *J* = 11.9, PhC*H*–), 4.68 (d, *J* = 11.5, PhC*H*), 4.65–4.59 (m, 2 × PhC*H*), 4.53 (d, *J* = 11.5, PhC*H*), 4.49 (d, *J* = 11.3, PhC*H*), 4.41–4.30 (m, 4 × PhC*H*), 4.0 (br s, H-4α), 3.96–3.87 (m, H-5α, H-4β, H-3α), 3.64 (dd, $J_{5,6a} = J_{5,6b}$ = 6.7, H-5β), 3.60 (ddd, $J_{3,4}$ = 2.6, $J_{3,2} = J_{3,F}$ = 9.6, H-3β), 3.55–3.43 (m, H-6aβ, H-6bβ, H-6aα, H-6bα,), 2.06, 2.04 (2 × s, 2 × COC*H*$_3$); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$: 169.27, 169.12 (2 × *C*OMe), 138.2–137.58 (6 × C-quart. arom.), 128.44–127.45 (C-arom.); 91.99 (d, $^2J_{C,F}$ = 25.4, C-1β), 90.55 (d, $^1J_{C,F}$ = 183.6, C-2β), 89.73 (d, $^2J_{C,F}$ = 22.5, C-1α), 88.07 (d, $^1J_{C,F}$ = 188.0, C-2α), 79.97 (d, $^2J_{C,F}$ = 15.6, C-3β), 76.87 (d, $^2J_{C,F}$ = 15.8, C-3α), 75.06, 74.9 (2 × PhC$H_2$), 74.93 (d, $J_{C,F}$ = 8.3, C-4α), 74.27 (C-5β), 73.87 (d, $J_{C,F}$ = 8.4, C-4β), 73.58, 73.50, 72.86, 72.80 (4 × PhC$H_2$), 71.78 (C-5α), 67.93 (C-6α), 67.55 (C-6β), 20.93, 20.86 (2 × $CH_3$CO–); $^{19}$F NMR (282 MHz, $CDCl_3$) $\delta$: α anomer: −208.9 (ddd, *J* = 3.8, 10.0, 48.8); β anomer: −207.3 (ddd, *J* = 3, 10.5, 52.5); ESI-MS, *m*/*z*: calcd for $C_{29}H_{35}FNO_6$ [**8**+$NH_4$]$^+$: 512.24, found: 512.24. Anal. Calcd for $C_{29}H_{31}FO_6$: C, 70.43; H, 6.32. Found: C, 69.99; H, 6.35.

### 3.3. Diethyl galactosyl phosphonates 5, 13, 6 and 14. Debenzylated diethyl galactosyl phosphonates 18 and 20

#### 3.3.1. General procedure for phosphonylation of galactosyl acetates 7 and 8

To a solution of anomeric acetates **7** or **8** (0.2–1.2 mmol) in $CH_2Cl_2$ (4 mL/mmol of substrate), $(EtO)_3P$ (2.6 mol equiv) was added under Ar. After cooling to −1 °C, TMSOTf (2 mol equiv) was added dropwise for 1 h and the mixture stirred at room temperature for 1 day and monitored by TLC until all the starting material was consumed and two products had been formed. The reaction was finished by the addition of a mixture of water and EtOAc (1:10, 25 mL/mmol of substrate). After washing two times with a satd soln of $NaHCO_3$ and two times with a satd soln of NaCl, the organic phase was dried ($MgSO_4$), evaporated and the residue was separated on a silica gel column.

#### 3.3.1.1. Diethyl (2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-α-D-galactopyranosyl)phosphonate (5) and diethyl (2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-β-D-D-galactopyranosyl)phosphonate (13).

From acetate **7** (128 mg, 0.25 mmol) a crude mixture of the phosphonates **5** and **13** (198 mg) was obtained, ($R_f$ = 0.15, 0.45; 3:1, *n*-hexane–EtOAc). Separation on silica gel column (elution with *n*-hexane: EtOAc 3:1) yielded:

α-Anomer **5** (75.6 mg, 53%), mp 103–105 °C; $[\alpha]_D^{20}$ +53 (*c* 1.0, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$: 7.4–7.28 (m, 15H, aromatic protons), 4.92 (br d, 1H, H-4, $^2J_{4,F}$ = 49.8), 4.79 (m, 4H, 2 × PhC*H*), 4.61–4.54 (m, 2H, PhC*H*), 4.47–4.30 (m, 3H, H-1, H-3, H-5), 4.28–4.09 (m, 4H, H-2, 3 × $CH_3$C*H*), 4.03 (m, 1H, $CH_3$C*H*) 3.70 (dd, 1H, $J_{6a,6b}$ = 10, $J_{6a,5}$ = 6.6, H-6a), 3.64 (dd, 1H, $J_{6b,5}$ = 10, $J_{6b,5}$ = 5.8, H-6b), 1.26 (t, 3H, *J* = 7.1, $CH_3$), 1.24 (t, 3H, *J* = 7.1, $CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$: 138.08, 137.90, 137.86 (C-arom. quart.), 128.34–127.61 (C-arom.), 87.21 (d, $^1J_{C,F}$ = 182.7, C-4), 76.31 (d, $^2J_{C,F}$ = 17.3, C-3), 74.62 (C-2), 73.94, 73.36, 72.63 (3 × PhC$H_2$–), 73.57 (d, $^2J_{C,F}$ = 18.4, C-5), 71.52 (d, $^1J_{C,P}$ = 153.7, C-1), 68.15 (d, $J_{C,F}$ = 4.8, C-6), 62.72 (d, $J_{C,P}$ = 6.4, $CH_3CH_2$), 62.14 (d, $J_{C,P}$ = 6.9, $CH_3CH_2$), 16.40 (d, $J_{C,P}$ = 6.3, $CH_3$), 16.33 (d, $J_{C,P}$ = 6.0, $CH_3$); $^{19}$F NMR (470 MHz, $CDCl_3$, dec) $\delta$: −212.7 (F-4); $^{31}$P NMR (202.5 MHz, $CDCl_3$, dec) $\delta$: 21.6 (P-1); ESI-MS, *m*/*z*: calcd for $C_{31}H_{38}FNaO_7P$ [**5**+$Na$]$^+$: 595.22, found: 595.35. Anal. Calcd for $C_{31}H_{38}FO_7P$: C, 65.02; H, 6.69. Found: C, 64.96; H, 6.52.

β-Anomer **13** (34.4 mg, 24%), syrup, $[\alpha]_D^{20}$ +40 (*c* 1.0, acetone). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$: 7.39–7.27 (m, 15H, H-aromatic protons), 4.93 (d, 1H, *J* = 10.3, PhC*H*), 4.91 (br d, 1H, $^2J_{4,F}$ = 46.5, H-4, overlap.), 4.86 (d, 1H, *J* = 10.3, PhC*H*), 4.78 (d, 1H, *J* = 11.7, PhC*H*), 4.69 (d, 1H, *J* = 11.7, PhC*H*), 4.57–4.51 (m, 2H, 2 × PhC*H*), 4.25–4.2 (m, 5H, H-2, 4 × $CH_3$C*H*), 3.75–3.5 (m, 5H, H-1, H-3, H-5, H-6a, H-6b), 1.27 (t, 3H, *J* = 7, $CH_3$), 1.23 (t, 3H, *J* = 7, $CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$): $\delta$ 138.21, 137.63, 137.57 (C-arom. quart.), 128.49–127.58 (C-arom.), 85.63 (d, $^1J_{C,F}$ = 183.6, C-4), 81.38 (dd, $^2J_{C,F}$ = 17.6, $J_{C,P}$ = 17.7, C-3), 77.46 (d, $J_{C,P}$ = 16.9, C-5), 75.42 (PhC$H_2$), 74.96 (d, $^1J_{C,P}$ = 171.4, C-1), 74.91 (C-2), 73.67, (PhC$H_2$), 71.84 (PhC$H_2$), 67.64 (d, $J_{C,F}$ = 5.4, C-6), 63.27 (d, $J_{C,P}$ = 5.8, $CH_3CH_2$), 62.45 (d, $J_{C,P}$ = 6.5, $CH_3CH_2$), 16.38 (d, $J_{C,P}$ = 5.8, $CH_3$), 16.27 (d, $J_{C,P}$ = 6.4, $CH_3$); $^{19}$F NMR (470 MHz, $CDCl_3$) $\delta$: −218.40 (ddd, *J* = 47, 27.6, F-4); $^{31}$P NMR (202.5 MHz, $CDCl_3$) $\delta$: 19.60 (m, P-1); TOF-ESI: *m*/*z* calcd for $C_{31}H_{38}FNaO_7P$ [**13**+$Na$]$^+$: 595.22, found: 594.93. Anal. Calcd for $C_{31}H_{38}FO_7P$: C, 65.02; H, 6.69. Found: C, 65.36; H, 6.74.

#### 3.3.1.2. Diethyl (3,4,6-tri-*O*-benzyl-2-deoxy-2-fluoro-α-D-galactopyranosyl)phosphonate (6) and diethyl (3,4,6-tri-*O*-benzyl-2-deoxy-2-fluoro-β-D-galactopyranosyl)phosphonate (14).

From acetate **8** (550 mg, 1.11 mmol) a raw mixture of the phosphonates **5** and **14** (743 mg) were obtained, ($R_f$ = 0.09, 0.19; *n*-hexane:EtOAc 4:1). Separation on silica gel column (elution with *n*-hexane:EtOAc 2:1) yielded:

α-Anomer **6** (128 mg, 20%), syrup, $[\alpha]_D^{20}$ +26 (*c* 1.1, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$: 7.26–7.17 (m, 15H, aromatic protons), 4.99 (dddd, 1H, $^2J_{2,F}$ = 48.0, $J_{2,P}$ = 12.8, $J_{2,3}$ = 6.0, $J_{2,1}$ = 3.8, H-2), 4.64–4.62 (m, 3H, 3 × PhC*H*), 4.49–4.39 (m, 3H, 3 × PhC*H*), 4.34–4.31 (m, 1H, H-5), 4.29 (ddd, $J_{1,2}$ = 3.7, $^2J_{1,P}$ = 14.3, $J_{1,F}$ = 23.3, H-1), 4.15–4.03 (m, 5H, H-3, 4 × $CH_3$C*H*), 3.93 (ddd, 1H, *J* = 3.5, H-4), 3.79 (dd, 1H, $J_{6a,6b}$ = 11.1, $J_{6a,5}$ = 8.1, H-6a), 3.55 (dd, 1H, $J_{6b,6a}$ = 11.1, $J_{6b,5}$ = 4.0, H-6b), 1.2 (m, 6H, 2 × $CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$: 138.19, 137.96, 137.87 (C-arom. quart.), 128.41–127.59 (C-arom.), 88.67 (dd, $^1J_{C,F}$ = 182.4, $^2J_{C,P}$ = 2.5, C-2), 75.56 (d, $J_{C,P}$ = 8.6, C-5), 75.35 (br d, $^2J_{C,F}$ = 24.4, C-3), 73.92 (br d, $J_{C,F}$ = 3.4, C-4), 73.42, 73.21 (3 × PhC$H_2$), 67.52 (dd, $^1J_{C,P}$ = 166.6, $^2J_{C,F}$ = 22.9, C-1), 66.51 (C-6), 63.24 (d, $J_{C,P}$ = 6.2, $CH_3CH_2$), 62.63 (d, $J_{C,P}$ = 6.7, $CH_3CH_2$), 16.41 (d, $J_{C,P}$ = 8.19, $CH_3$), 16.34 (d, $J_{C,P}$ = 8.6, $CH_3$); $^{19}$F NMR (282 MHz, $CDCl_3$) $\delta$: −200.2 (m, F-2); $^{31}$P NMR (202.5 MHz) $\delta$: 19.01 (m, P-1); ESI-MS, *m*/*z*: calcd for $C_{31}H_{42}FNO_7P$ [**6**+$NH_4$]$^+$: 590.27, found: 590.23. Anal. Calcd for $C_{31}H_{38}FO_7P$: C, 65.02; H, 6.69. Found: C, 65.01; H, 6.61.

β-Anomer **14** (218 mg, 34%), syrup, $[\alpha]_D^{20}$ +9 (*c* 1.2, $CHCl_3$). $^1$H NMR (500 MHz, $CDCl_3$) $\delta$: 7.28–7.17 (m, 15H, aromatic protons), 5.05 (dddd, 1H, $^2J_{2,F}$ = 50.7, $J_{2,P} = J_{2,1} = J_{2,3}$ = 9.8, H-2), 4.85, 4.74, 4.61, 4.48 (4 × d, 4H, 4 × PhC*H*), 4.37–4.31 (m, 2H, 2 × PhC*H*), 4.14–4.06 (m, 4H, 4 × $CH_3$C*H*), 3.90 (dd, 1H, $J_{4,3} = J_{4,5}$ = 2.7, H-4), 3.60 (ddd, 1H, $J_{1,2} = J_{1,F}$ = 9.8, $^2J_{1,P}$ = 4.5, H-1), 3.57–3.45 (m, 4H, H-3, H-5, H-6a, H-6b), 1.22 (t, 3H, *J* = 7.07, $CH_3$), 1.18 (t, 3H, *J* = 7.08, $CH_3$); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$: 138.5, 138.06, 137.76 (C-arom.

quart.), 128.44–127.51 (C-arom.), 88.91 (dd, $^1J_{C,F}$ = 182.5, $^2J_{C,P}$ = 3.0, C-2), 81.36 (dd, $^2J_{C,F}$ = $J_{C,P}$ = 15.9, C-3), 79.05 (d, $J_{C,P}$ = 16.0, C-5), 74.76 (PhCH$_2$), 74.67 (d, $J_{C,F}$ = 9.6, C-4), 73.84 (dd, $^1J_{C,P}$ = 171.54, $^2J_{C,F}$ = 25.3, C-1), 73.53 (PhCH$_2$), 72.91 (PhCH$_2$), 68.22 (C-6), 63.24, 63.19 (2 × × d, $J_{C,P}$ = 6.5, 2 × CH$_3$CH$_2$), 16.37 (d, $J_{C,P}$ = 9.32, CH$_3$), 16.29 (d, $J_{C,P}$ = 9.07, CH$_3$); $^{19}$F NMR (282 MHz, CDCl$_3$) $\delta$: −200.70 (m, F-2); $^{31}$P NMR (202.5 MHz, CDCl$_3$) $\delta$: 17.71 (m, P-1); ESI-MS, *m*/*z*: calcd for $C_{31}H_{42}FNO_7P$ [**14**+NH$_4$]$^+$: 590.27, found: 590.16. Anal. Calcd for $C_{31}H_{38}FO_7P$: C, 65.02; H, 6.69. Found: C, 64.86; H, 6.64.

### 3.3.2. General procedure for debenzylation of galactosyl phosphonates 5 and 6

A vigorously stirred mixture of diethyl glycosyl phosphonate **5** or **6** (0.5–6 mmol), 20% palladium hydroxide on carbon (100 mg/1 mmol of substrate) and MeOH (10 mL/1 mmol of substrate) was degassed under vacuum and saturated with hydrogen. The suspension was stirred at rt for 1 day under a slight overpressure of hydrogen. On TLC (10:1, CHCl$_3$–MeOH) one spot was observed with $R_f$ ~0.14 under the spot of the starting compound ($R_f$ ~0.9). Reaction mixture was filtered through Super-Cel. The filter cake was washed with toluene (3 × 20 mL) and EtOH (3 × 25 mL). The combined filtrate was evaporated to dryness to give corresponding debenzylated diethyl glycosyl phosphonate **18** or **20** of sufficient purity.

**3.3.2.1. Diethyl (4-deoxy-4-fluoro-α-D-galactopyranosyl)phosphonate (18).** From diethyl (2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-α-D-galactopyranosyl)phosphonate (**5**) (1.83 g, 6.04 mmol), diethyl (4-deoxy-4-fluoro-α-D-galactopyranosyl)phosphonate (**18**) was obtained (950 mg, 99%) as white crystals, mp 77–79 °C; $[\alpha]_D^{20}$ +82 (*c* 1.0, MeOH). $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 4.80 (br d, 1H, H-4, $^2J_{4,F}$ = 49.8), 4.38 (dd, 1H, $J_{1,2}$ = 6.7, $^2J_{1,P}$ = 11.4, H-1), 4.23 (ddd, 1H, $J_{3,2}$ = 9.7, $^4J_{3,P}$ =2.7, $J_{3,F}$ = 25, H-3), 4.20–4.08 (m, 5H, 4 × CH$_3$C*H*, H-2), 4.04 (ddd, 1H, $J_{5,6a}$ = $J_{5,6b}$ = 6.7, $J_{F,5}$ = 27, H-5), 3.68 (dd, 1H, $J_{6a,6b}$ = 11.4, $J_{6a,5}$ = 6.7, H-6a), 3.63 (dd, 1H, $J_{6b,6a}$ = 11.4, $J_{6b,5}$ = 6.5, H-6b), 1.34 (t, 3H, *J* = 7, CH$_3$), 1.31 (t, 3H, *J* = 7, CH$_3$); $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta$: 90.41 (d, $^1J_{C,F}$ = 180.2, C-4), 76.90 (d, $^2J_{C,F}$ = 18.2, C-5), 74.63 (d, $^1J_{C,P}$ = 151.5, C-1), 70.59 (d, $^2J_{C,F}$ = 17.7, C-3), 68.77 (C-2), 63.91 (d, $J_{C,P}$ = 7, CH$_3$CH$_2$), 63.77 (d, $J_{C,P}$ = 7, CH$_3$CH$_2$), 61.16 (d, *J* = 5.9, C-6), 16.77 (d, $J_{C,P}$ = 5.4, CH$_3$), 16.73 (d, $J_{C,P}$ = 5.3, CH$_3$); $^{19}$F NMR (282.2 MHz, CD$_3$OD): $\delta$ −221.3 (ddd, *J* = 27.9, 49.7, F-4); $^{31}$P NMR (202.5 MHz, CD$_3$OD): $\delta$ 24.0 (m, P-1); ESI-MS, *m*/*z*: calcd for $C_{10}H_{20}FNaO_7P$ [**18**+Na]$^+$: 325.08, found: 325.06. Anal. Calcd for $C_{10}H_{20}FO_7P$: C, 39.74; H, 6.67. Found: C, 39.63; H, 6.56.

**3.3.2.2. Diethyl (2-deoxy-2-fluoro-α-D-galactopyranosyl)phosphonate (20).** From diethyl (3,4,6-tri-*O*-benzyl-2-deoxy-2-fluoro-α-D-galactopyranosyl)phosphonate (**6**) (310 mg, 0.54 mmol), diethyl (2-deoxy-2-fluoro-α-D-galactopyranosyl)phosphonate (**20**) was obtained (129 mg, 79%) as a colourless syrup, $[\alpha]_D^{20}$ +46 (*c* 1.0, CHCl$_3$). $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 4.90 (dddd, 1H, $J_{2,1}$ = 5.4, $J_{2,3}$ = 7.3, $J_{2,P}$ = 20.5, $^2J_{2,F}$ = 49.0, H-2), 4.53 (ddd, 1H, $J_{1,2}$ = 5.3, $^2J_{1,P}$ = $J_{1,F}$ = 13.7, H-1), 4.25–4.14 (m, 5H, 2 × CH$_3$C*H*$_2$, H-3), 4.05–3.98 (m, 2H, H-5, H-4), 3.83 (dd, 1H, $J_{6a,5}$ = 7.2, $J_{6a,6b}$ = 12.1, H-6a), 3.69 (dd, 1H, $J_{6b,5}$ = 4.4, $J_{6b,6a}$ = 12.1, H-6b), 1.33 (m, 6H, 2 × CH$_3$); $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta$: 90.68 (dd, $^1J_{C,F}$ = 180.6, $^2J_{C,P}$ = 1.6, C-2), 78.87 (d, $J_{C,P}$ = 5.7, C-5), 69.95 (dd, $^2J_{C,F}$ = 20.8, $J_{C,P}$ = 3.8, C-3), 69.86 (dd, $^1J_{C,P}$ = 161.1, $^2J_{C,F}$ = 23.5, C-1), 69.32 (d, $J_{C,F}$ = 5.7, C-4), 64.63 (d, $J_{C,P}$ = 6.8, CH$_3$CH$_2$), 64.15 (d, $J_{C,P}$ = 7.2, CH$_3$CH$_2$), 61.02 (C-6), 16.72 (dd, $J_{C,P}$ = 6.3, CH$_3$); $^{19}$F NMR (282.2 MHz, CD$_3$OD) $\delta$: −204.75 (ddd, $J_{F,H\text{-}2}$ = 49.1, F-2); $^{31}$P NMR (CD$_3$OD, 202.5 MHz, dec) $\delta$: 21.25 (d, $J_{P,F}$ = 4.8, P-1); ESI-MS, *m*/*z*: calcd for $C_{10}H_{21}FO_7P$ [**20**+H]$^+$: 303.10, found: 303.14. Anal. Calcd for $C_{10}H_{20}FO_7P$: C, 39.74; H, 6.67. Found: C, 39.74; H, 7.14.

## 3.4. Glycosylphosphonic acids and bis(pyridinium) salts

### 3.4.1. General procedure for deesterification of diethyl esters 5, 18 and 20 and for preparation of bis(pyridinium) salts 16, 19 and 21

To a solution of diethyl phosphonate **5**, **18** or **20** in CH$_2$Cl$_2$ (10 mL/mmol substrate), stirred under Ar at 0 °C, BrSiMe$_3$ (15 mmol/mmol substrate) was added within 30 min. The reaction mixture was then stirred at rt under Ar for ca 4 h. The course of the reaction was monitored by TLC (1:1, *n*-hexane–EtOAc). After the disappearance of the starting ester, the mixture was concentrated to give bis(trimethylsilyl) phosphonate which was directly hydrolysed in an acetone–water mixture (9:1, 20 mL/mmol of substrate) under stirring at rt for 2 h (monitoring by TLC, 1:1, *n*-hexane–EtOAc), then the mixture was concentrated. By preparative LC on RP-18 (1:1 → 3:1, MeOH–H$_2$O), the target phosphonic acid **15**, **3** or **4** was obtained.

For preparation of the corresponding bis(pyridinium) salts, the phosphonic acid **15, 3** or **4** was repeatedly evaporated with dry pyridine.

### 3.4.2. (2,3,6-Tri-*O*-benzyl-4-deoxy-4-fluoro-α-D-galactopyranosyl)phosphonic acid (15) and its bis(pyridinium) salt 16

From the diethyl phosphonate **5** (296 mg, 0.52 mmol) the phosphonic acid **15** was obtained (156 mg, 59%), $[\alpha]_D^{20}$ +35 (*c* 0.3, MeOH). $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 7.42–7.16 (m, 15H, aromatic protons), 4.92 (br d, 1H, $^2J_{4,F}$ = 50, H-4), 4.86–4.55 (m, 6H, 6 × PhC*H*), 4.38–4.29 (m, 3H, H-1, H-3, H-5), 4.13 (ddd, 1H, $J_{2,P}$ = 27, $J_{2,3}$ = 9, $J_{2,1}$ = 6, H-2), 3.71 (dd, 1H, $J_{6a,6b}$ = 9.7, $J_{6a,5}$ = 6.7, H-6a), 3.62 (dd, 1H, $J_{6b,6a}$ = 10, $J_{6b,5}$ = 6, H-6b); $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta$: 139.73, 139.66, 139.25 (arom. quart. C), 129.54–128.71 (C-arom.), 88.65 (d, $^1J_{C,F}$ = 181.4, C-4), 77.57 (d, $^2J_{C,F}$ = 16.9, C-5), 76.23 (C-2), 74.42 (PhCH$_2$), 74.39 (PhCH$_2$), 74.25 (d, $^2J_{C,F}$ = 16.6, C-3), 73.73 (PhCH$_2$), 72.75 (d, $^1J_{C,P}$ = 155.9, C-1), 69.12 (d, *J* = 5.8, C-6); $^{19}$F NMR (470 MHz) $\delta$:-216.93 (dec, F-2); $^{31}$P NMR (202.5 MHz) $\delta$: 18.32 (m, P-1); ESI-MS, *m*/*z*: calcd for $C_{27}H_{29}FO_7P$ [**15**–H]$^-$: 515.16, found: 515.14. Anal. Calcd for $C_{27}H_{30}FO_7P$: C, 62.79; H, 5.85. Found: C, 62.35; H, 5.31.

**3.4.2.1. Bis(pyridinium) salt 16.** Syrup, $[\alpha]_D^{20}$ +51 (*c* 1.0, MeOH). $^1$H NMR (500 MHz, CD$_3$OD) $\delta$: 8.61 (br s, 2H, Py), 8.12 (br t, 2H, *J* = 7.5, Py), 7.63 (m, 2H, Py) 7.49–7.23 (m, 15H, H-aromatic protons), 4.93 (br d, 1H, $^2J_{4,F}$ = 50.0, H-4), 4.83 (d, 1H, *J* = 11.6, PhCH), 4.70 (m, 2H, 2 × PhCH), 4.63 (d, 1H, *J* = 11.6, PhCH), 4.54 (m, 2H, 2 × PhCH), 4.43–4.31 (m, 3H, H-1, H-3, H-5), 4.14 (ddd, 1H, $J_{2,3}$ = 9.1 $J_{2.1}$ = 6.7, $J_{2,P}$ = 26.9, H-2), 3.71 (dd, 1H, $J_{6a,6b}$ = 9.7, $J_{6a,5}$ = 6.8, H-6a), 3.62 (dd, 1H, $J_{6b,6a}$ = 9.8, $J_{6b,5}$ = 5.9, H-6b); $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta$: 147.25 (Py), 142.03 (Py), 139.75, 139.69, 139.31 (C-arom. quart.), 126.69 (Py) 129.54–128.71 (C-arom.), 88.66 (d, $^1J_{C,F}$ = 181.6, C-4), 77.54 (d, $^2J_{C,F}$ = 16.7, C-5), 76.44 (C-3), 74.46, 74.38 (PhCH$_2$), 74.25 (d, $^2J_{C,P}$ = 18.3, C-2), 73.65 (PhCH$_2$), 72.78 (d, $^1J_{C,F}$ = 152.4, C-1), 69.11 (d, *J* = 5, C-6); $^{19}$F NMR (470 MHz, CD$_3$OD, dec) $\delta$: −216.74 (F-4); $^{31}$P NMR (202.5 MHz, CD$_3$OD) $\delta$: 17.96 (m, P-1). ESI-MS, *m*/*z*: calcd for $C_{27}H_{29}FO_7P$ [**15**–H]$^-$: 515.16, found 515.17. Anal. Calcd for $C_{37}H_{40}FN_2PO_7$: C, 65.87; H, 5.98; N, 4.15. Found: C, 65.82; H, 6.19; N, 4.03.

### 3.4.3. (4-Deoxy-4-fluoro-α-D-D-galactopyranosyl)phosphonic acid (3) and its bis(pyridinium) salt 19

From the diethyl phosphonate **18** (813 mg, 2.69 mmol), phosphonic acid **3** was obtained (572 mg, 86%) as a foamy syrup, $[\alpha]_D^{20}$ +50 (*c* 1, MeOH). $^1$H NMR (500 MHz, D$_2$O) $\delta$: 4.88 (br d, 1H, $^2J_{4,F}$ = 49.6, H-4), 4.30 (dd, 1H, $J_{1,2}$ = 6.5, $^2J_{1,P}$ = 11.7, H-1), 4.22 (ddd, 1H, $^4J_{3,P}$ = 2.6, $J_{3,2}$ = 9.6, $J_{3,F}$ = 28.8, H-3), 4.21–4.10 (m, 2H,

H-2, H-5), 3.75 (dd, 1H, $J_{6a,5}$ = 7.3, $J_{6a,6b}$ = 11.4, H-6a), 3.69 (dd, 1H, $J_{6b,5}$ = 5.3, $J_{6b,6a}$ = 11.5, H-6b); $^{13}$C NMR (125 MHz, $D_2O$) $\delta$: 91.38 (d, $^1J_{C,F}$ = 177.4, C-4), 75.99 (d, $^2J$ = 18.0, C-5), 74.56 (d, $^1J_{C,P}$ = 148.1, C-1), 70.34 (d, $^2J_{C,F}$ = 17.5, C-3), 68.79 (C-2), 61.41 (d, $J$ = 5.3, C-6); $^{19}$F NMR (470 MHz, $D_2O$) $\delta$: −219.9 (dec, F-4), $^{31}$P NMR (202.5 MHz, $D_2O$) $\delta$: 17.97 (dd, $J$ = 26.8, 10.7, P-1). ESI-MS, $m/z$: calcd for $C_6H_{11}FO_7P$ [**3**–H]$^-$: 245.02, found: 245.04. Anal. Calcd for $C_6H_{12}FO_7P$: C, 29.28; H, 4.91. Found: C, 29.12; H, 5.16.

**3.4.3.1. Bis(pyridinium) salt 19.** Syrup, $[\alpha]_D^{20}$ +68 (*c* 1.0, MeOH); $^1$H NMR (500 MHz, $D_2O$) $\delta$: 8.59 (br s, 4H, Py), 8.19 (2H, Py), 7.71 (4H, Py), 4.87 (d, 1H, $^2J_{4,F}$ = 49.9, H-4), 4.33–4.04 (m, 4H, H-1, H-2, H-3, H-5), 3.76 (dd, 1H, $J_{6a,6b}$ = 11.9, $J_{6a,5}$ = 7.3, H-6a), 3.65 (dd, 1H, $J_{6b,6a}$ = 11.9, $J_{6b,5}$ = 5, H-6b); $^{13}$C NMR (125 MHz, $D_2O$) $\delta$: 146.21, 143.88, 127.37 (Py), 91.48 (d, $^1J_{C,F}$ = 177.4, C-4), 75.51 (d, $^2J_{C,F}$ = 17.9, C-5), 74.30 (d, $^1J_{C,P}$ = 145.1, C-1), 70.49 (d, $^2J_{C,F}$ = 17.2, C-3), 69.41 (C-2), 61.2 (d, $J_{C,F}$ = 4.6, C-6); $^{19}$F NMR (470 MHz, $D_2O$) $\delta$: −219.75 (m, F-4); $^{31}$P NMR (202 MHz, $D_2O$) $\delta$: 14.81 (dec, P-1); ESI-MS, $m/z$: calcd for $C_6H_{11}FO_7P$ [**3**–H]$^-$: 245.02, found: 245.06. Anal. Calcd for $C_{16}H_{22}FN_2O_7P$: C, 47.53; H, 5.48; N, 6.93. Found C, 47.20; H, 5.61; N, 7.07.

### 3.4.4. (2-Deoxy-2-fluoro-α-D-galactopyranosyl)phosphonic acid (4) and its bis(pyridinium) salt 21

From the diethyl phosphonate **20** (102 mg, 0.34 mmol), phosphonic acid **4** (81 mg, 98%) was obtained as a syrup, $[\alpha]_D^{20}$ +45 (*c* 1.3, MeOH). $^1$H NMR (500 MHz, $D_2O$) $\delta$: 4.89 (dddd, 1H, $J_{2,1}$ = 5.1, $J_{2,3}$ = 8.4, $J_{2,P}$ = 18.3, $^2J_{2,F}$ = 48.9, H-2), 4.34 (ddd, 1H, $^2J_{1,P} = J_{1,F}$ = 13.0, $J_{1,2}$ = 5.1, H-1), 4.22 (bdd, $J_{3,2}$ = 8.4, $J_{3,F}$ = 10.1, H-3), 4.02 (m, 2H, H-4, H-5), 3.78 (dd, 1H, $J_{6a,5}$ = 7.7, $J_{6a,6b}$ = 12, H-6a), 3.61 (dd, 1H, $J_{6b,5}$ = 2.8, H-6b); $^{13}$C NMR (125 MHz, $D_2O$) $\delta$: 89.54 (d, $^1J_{C,F}$ = 177.8, C-2), 76.50 (C-5), 69.02 (dd, $^1J_{C,P}$ = 163.5, $^2J_{C,F}$ = 24.1, C-1), 68.36 (d, $^2J_{C,F}$ = 20.5, C-3), 67.94 (d, $J_{C,F}$ = 5.9, C-4), 59.66 (C-6); $^{19}$F NMR (282 MHz, $D_2O$) $\delta$: −203.65 (br d, $J_{F,H\text{-}2}$ = 49.1, F-2); $^{31}$P NMR (202.5 MHz, $D_2O$, dec) $\delta$: 16.33 (P-1). ESI-MS, $m/z$: calcd for $C_6H_{16}FNO_7P$ [**4**+$NH_4$]$^+$: 264.06, found: 264.08; calcd for $C_6H_{11}FO_7P$ [**4**−H]$^-$: 245.02, found: 244.8. Anal. Calcd for $C_6H_{12}FO_7P$: C, 29.28; H, 4.91. Found: C, 28.99; H, 5.11.

**3.4.4.1. Bis(pyridinium) salt 21.** Syrup, $[\alpha]_D^{20}$ +38 (*c* 1.4, MeOH); $^1$H NMR (500 MHz, $D_2O$) $\delta$: 8.66 (br s, 4H, Py), 8.38 (2H, Py), 7.86 (4H, Py), 4.88 (dddd, 1H, $J_{2,1}$ = 5.8, $J_{2,3}$ = 6.3, $J_{2,P}$ = 15.5, $J_{2,F}$ = 49.1, H-2), 4.28–4.18 (m, 2H, H-1, H-3), 4.08–4.03 (m, 2H, H-4, H-5), 3.84 (dd, 1H, $J_{6a,6b}$ = 12.2, $J_{6a,5}$ = 8.3, H-6a), 3.62 (dd, 1H, $J_{6b,6a}$ = 12.2, $J_{6b,5}$ = 2.8, H-6b); $^{13}$C NMR (125 MHz, $D_2O$) $\delta$: 144.73, 143.06, 126.68 (Py), 89.92 (d, $^1J_{C,F}$ = 177.8, C-2), 76.25 (C-5), 68.95 (dd, $^2J_{C,F}$ = 21.7, $^1J_{C,P}$ = 161.1, C-1), 68.32 (d, $J_{C,F}$ = 21.7, C-3), 67.82 (d, $J_{C,F}$ = 5.5, C-4), 59.34 (C-6); $^{19}$F NMR (282 MHz, $D_2O$) $\delta$: −202.9 (br d, $J_{F,H\text{-}2}$ = 49.2, F-2); $^{31}$P NMR (202 MHz, $D_2O$) $\delta$: 13.8 (dec, P-1); ESI-MS, $m/z$: calcd for $C_6H_{11}FO_7P$ [**4**−H]$^-$: 245.02, found: 245.04. Anal. Calcd for $C_{16}H_{22}FN_2O_7P$: C, 47.53; H, 5.48; N, 6.93. Found C, 47.61; H, 5.55; N, 6.97.

## 3.5. Coupling reaction of the bis(pyridinium) salts 16, 19 and 21 with UMP-morpholidate

### 3.5.1. General procedure

The crude salt **16**, **19** or **21** was coevaporated 3 times with dry pyridine (10 mL/mmol of substrate). After addition of UMP-morpholidate (1 mol equiv), the mixture was again coevaporated 3 times with dry pyridine (10 mL/mmol of substrate) and dried under high vacuum for 5 h. Subsequently, the solid was taken up in dry pyridine (25 mL/mol of substrate) under Ar, and to this solution dried 1*H*-tetrazole (6 mL/mmol of substrate) was added. Thereafter, the solution was stirred at room temperature for 7 days under Ar. For the termination of the reaction a mixture of Hünigs base and water (0.8 and 25 mL resp.,/mmol of substrate) was added, and then the solution was concentrated in vacuo. The residue was coevaporated again with the same amount of Hünigs base–water, dried under vacuum and then purified by preparative LC on RP-18 (1:1 → 3:1, MeOH–$H_2O$). The products were obtained as salts with the base 4-morpholine-*N,N*′-dicyclohexyl carboximidamide **17**, **1a** or **2a**.

### 3.5.2. Bis(4-morpholine-*N,N*′-dicyclohexyl carboximidamidium) uridine-5′-[(2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-α-D-galactopyranosyl)phosphonoyl]phosphate (17)

Starting from pyridinium phosphonate **16** (412 mg. 0.61 mmol), the salt **17** (351 mg, 41%) was obtained as white yellow solid, $[\alpha]_D^{20}$ +19 (*c* 1.1, MeOH); $^1$H NMR (500 MHz, $CD_3OD$) $\delta$: signals of the nucleotide moiety: 8.11 (d, 1H, $J$ = 8.1, H-6(U)), 7.48–7.21 (m, 15H, aromatic protons), 5.94 (d, 1H, $J$ = 4.9, H-1′), 5.84 (d, 1H, $J$ = 8.1, H-5(U)), 5.04 (d, 1H, $J$ = 12.2, PhC*H*), 4.93 (br d, 1H, H-4), 4.77 (d, 1H, $J$ = 11.6, PhC*H*), 4.72 (d, 1H, $J$ = 10.2, PhC*H*), 4.67 (d, 1H, $J$ = 10.5, PhC*H*), 4.65 (d, 1H, $J$ = 11.6, PhC*H*), 4.60 (d, 1H, $J$ = 12, PhC*H*), 4.42 (dd, 1H, $J'_{2',3} = J'_{3',4}$ = 4.6, H-3′), 4.36 (dd, $J$ = 10.5, 6.0, H-1), 4.25 (dd, 1H, $J'_{2',1} = J'_{2',3}$ = 4.9, H-2′), 4.22 (dd, 1H, $J$ = 11.7, H-5′), 4.08 (br s, 1H, H-4′), 3.78 (bdd, 1H, $J$ = 9.0, 5.0, H-6a), 3.69 (bdd, overlapped, H-5), 3.57 (bdd, 1H, $J$ = 9.0, 8.0, H-6b); other signals (nuclei of the base moieties): 3.73 (dd, 8H, $J$ = 9.1, 4 × $CH_2O$), 3.41 (dd, 8H, $J$ = 9.1, 4 × $CH_2N$), 3.30 (overlap., CHN), 1.93 (m, 8H, 4 × $CH_2$), 1.82 (m, 8H, 4 × $CH_2$), 1.67 (m, 4H, 2 × $CH_2$), 1.44–1.30 (m, 20H, 10 × $CH_2$); $^{13}$C NMR (125 MHz, $CD_3OD$) $\delta$: signals of the nucleotide moiety: 159.37 (C=O(U)), 143.01 (C-6(U)), 129.38–128.21 (C-arom.), 103.24 (C-5(U)), 89.74 (C-1′)), 88.3 (d, $^1J_{4,F}$ = 181.1 Hz, C-4), 78.96 (C-5), 77.15 (C-3), 75.79 (C-2′), 74.14, 74.0 (3 × $CH_2$Ph), 71.12 (C-3′); other signals: 67.29 ($CH_2O$ (morph.)), 56.03 (N=CH), 49.52 ($CH_2N$ (morph.)), 34.46, 26.3, 26.14 ($CH_2$ (cyclohexyl)); $^{19}$F NMR (470 MHz, $CD_3OD$) $\delta$: −217.28 (dec, F-4); $^{31}$P NMR (202.5 MHz, $CD_3OD$) $\delta$: −10.73 (d, $J'_{P,P}$ = 29.5, phosphate), 5.95 (d, $J'_{P,P}$ = 29.5, phosphonate); ESI-MS, $m/z$: calcd for $C_{36}H_{40}FN_2O_{15}P_2$ [**17**−2B−H]$^-$: 821.2, found: 821.2; calcd for $C_{17}H_{32}N_3O$ [B+H]$^+$: 294.3, found: 294.3. Anal. Calcd for $C_{70}H_{103}FN_8O_{17}P_2$: C, 59.65; H, 7.37; N, 7.95. Found: C, 59.27; H, 7.30; N, 7.65.

### 3.5.3. Bis(4-morpholine-*N,N*′-dicyclohexyl carboxamidimidium) uridine-5′-[(4-deoxy-4-fluoro-α-D-galactopyranosyl)phosphonoyl] phosphate (1a)

Starting from the pyridinium salt **19** (512 mg, 1.27 mmol) the salt **1a** was obtained as colourless syrup (1.01 g, 70%), $[\alpha]_D^{20}$ +11 (*c* 1.1, water). $^1$H NMR (500 MHz, $D_2O$) $\delta$: signals of the nucleotide moiety: 7.92 (d, 1H, $J$ = 8.1, H-6(U)), 5.94 (d, 1H, $J$ = 4.9, H-1′), 5.90 (d, 1H, $J$ = 8.1, H-5(U)), 4.89 (br d, 1H, $^2J_{4,F}$ = 49.9, H-4), 4.34–4.19 (m, 8H, H-2′, H-3′, H-4′, H-1, H-2, H-3, H-4, H-5), 4.10 (ddd, 1H, $J$ = 2.4, 4.2, 11.5, H-5a′), 4.02 (ddd, 1H, $J$ = 2.9, 5.3, 11.6, H-5b′), 3.78 (dd, 1H, $J$ = 7.5, 12.1, H-6a), 3.68 (dd, 1H, $J$ = 4.3, 12.1 Hz, H-6b); other signals: 7.87 (d), 5.89 (d), 3.90 (dd), 3.74 (dd), 3.66 (dddd), 3.40 (dd), 3.25 (dd), 3.15 (q), 1.88 (bd), 1.56 (bd), 1.29(m); $^{13}$C NMR (125 MHz, $D_2O$) $\delta$: signals of the nucleotide moiety: 167.65 (C-4 (U)), 159.24 (C-2 (U)), 143.06 (C-6 (U)), 103.98 (C-5 (U)), 91.49 (d, $^1J_{C,F}$ = 177.3, C-4), 89.9 (C-1′), 84.83 (d, $J$ = 8.9, C-4′), 75.54 (d, $^2J_{C,F}$ = 17.6, C-5), 75.26 (C-2′), 74.39 (d, $^1J_{C,P}$ = 145.5, C-1), 71.11 (d, $^2J_{C,P}$ = 14, C-2), 70.47 (d, $^2J_{C,F}$ = 16.9, C-3), 69.36 (C-3′), 65.52 (C-5′), 61.23 (C-6); other signals: 153.24, 67.37, 65.02, 55.87, 55.80, 49.42, 44.58, 43.97, 34.30, 26.05, 19.15, 17.68, 13.54; $^{19}$F NMR (470 MHz, $D_2O$, $^1$H dec) $\delta$: −218.2 (F-4); $^{31}$P NMR (202.5 MHz, $D_2O$, $^1$H dec) $\delta$: 14.8, 0.84, −10.8; ESI-MS, $m/z$: calcd for $C_{15}H_{22}FN_2O_{15}P$ [**1a**−2B−H]$^-$: 551.05, found: 551.11; calcd for $C_9H_{12}N_2O_9P$ [**UMP**−H]$^-$: 323.03, found: 323.05; calcd for $C_6H_{11}FO_7P$ [**3**−H]$^-$: 245.02, found: 245.04; calcd for $C_{17}H_{32}N_3O$ [**B**+H]$^+$: 294.25, found: 294.70.

### 3.5.4. Bis(4-morpholine-*N,N′*-dicyclohexylcarboxamidimidium) uridine-5′-[(2-deoxy-2-fluoro-α-D-galactopyranosyl)phosphonoyl] phosphate (2a)

Starting from the pyridinium salt **21** (102 mg, 0.25 mmol), salt **2a** (217 mg, 75%) was obtained as a colourless syrup, $[\alpha]_D^{20}$ +5 (*c* 1.4, MeOH). $^1$H NMR (500 MHz, $D_2O$) $\delta$: signals of the nucleotide moiety: 7.85 (d, 1H, $J$ = 8.1, H-6(U)), 5.91 (m, 2H, H-1′, H-5(U)), 4.92 (dddd, 1H, $J_{2,1}$ = 5, $J_{2,3}$ = 7 Hz, $J_{2,P}$ = 17.5, $^2J_{2,F}$ = 47.5, H-2), 4.45 (ddd, $J_{1,2}$ = 5, $J_{1,P}$ = $J_{1,F}$ = 15, H-1), 4.34– 4.05 (m, 8H, H-2′, H-3′, H-4′, H-5a′, H-5b′, H-3, H-4, H-5), 3.85 (H-6a, overlap.), 3.59 (H-6b, overlap.); other signals: 7.90 (d), 5.88 (m), 3.88 (dd), 3.64 (dddd), 3.24 (dd), 3.12 (q), 1.26 (m); $^{13}$C NMR (125 MHz, $D_2O$) $\delta$: signals of the nucleotide moiety: 166.05 (C-4 (U)), 151.78 (C-2(U)), 141.66 (C-6 (U)), 102.68 (C-5 (U)), 89.56 (dd, $J_{C,P}$ = 13, $^1J_{C,F}$ = 179.1, C-2), 88.4 (C-1′), 83.67 (d, $J$ = 8.7, C-4′), 76.31 (C-5), 73.80 (C-2′), 69.01 (dd, $^1J_{C,P}$ = 162.9, $^2J_{C,F}$ = 19.2, C-1), 68.37 (d, $J$ = 21.1, C-3), 67.9 (d, $J$ = 12.8, C-4), 64.89 (br s, C-5′), 59.44 (d, $J$ = 8.2, C-6); other signals: 166.18, 151.73, 141.62, 102.62, 88.3, 83.18, 73.75, 54.40, 54.32, 43.19, 43.12, 42.57, 42.51, 17.77, 17.69, 16.31, 16.23; $^{19}$F NMR (470 MHz, $D_2O$) $\delta$: −217.28 (dec, 1F); $^{31}$P NMR (202.5 MHz, $D_2O$, $^1$H dec) $\delta$: −10.75, 5.57; ESI-MS, *m/z*: calcd for $C_{15}H_{22}FN_2O_{15}P$ [**1a**−2B−H]$^-$: 551.05, found: 551.11; calcd for $C_{17}H_{32}N_3O$ [**B**+H]$^+$: 294.25, found: 294.29.

### 3.5.5. General procedure for transformation of the bis(4-morpholine-*N,N′*-dicyclohexyl carboximidamide-ium) salts 1a and 2a to the diammonium salts 1b and 2b

The salt **1a** or **2a** (0.145–0.180 mmol) were applied in water solution (10 mL) on a column of ion-exchange resin Dowex 1-X8 (200–400 mesh, $HCO_2^-$ form, 1.2 × 10 cm). The column was eluted with aq ammonium bicarbonate in the gradient of 0–1 M.[28] In the first fraction eluted with water only 4-morpholine-*N,N′*-dicyclohexyl carboxamidimidium formiate was separated. The fractions eluted with ca 0.4 M $NH_4HCO_3$ were combined and evaporated to yield the desired diammonium salts **1b** or **2b**. Elution with 0.8–1 M $NH_4HCO_3$ produced unreacted UMP salt.

### 3.5.6. Diammonium uridine-5′-[(4-deoxy-4-fluoro-α-D-galactopyranosyl)phosphonoyl] phosphate (1b)

From the salt **1a** (165 mg, 0.145 mmol) the diammonium salt **1b** (51 mg, 31%) was obtained as a colourless syrup, $[\alpha]_D$ +20 (*c* 1.2, water). $^1$H NMR (500 MHz, $D_2O$) $\delta$: 7.93 (d, 1H, $J$ = 8.1, H-6 (U)), 5.94 (d, 1H, $J$ = 4.9, H-1′), 5.90 (d, 1H, $J$ = 8.1, H-5 (U)), 4.89 (br d, 1H, $^2J_{4,F}$ = 50, H-4), 4.32 (dd, 1H, $J$ = 5.1, H-2′), 4.29 (m, 2H, H-3′, H-5), 4.2– 4.06 (m, 4H, H-4′, H-1, H-2, H-3), 4.09 (ddd, 1H, $J$ = 2.3, 4.2, 11.5, H-5a′), 4.01 (ddd, 1H, $J$ = 3.0, 5.0, 11.5, H-5b′), 3.78 (dd, 1H, $J$ = 7.5, 11.9, H-6a), 3.69 (dd, 1H, $J$ = 5.0, 11.9, H-6b); $^{13}$C NMR (125 MHz, $D_2O$) $\delta$: 166.26 (C-4 (U)), 151.86 (C-2 (U)), 141.72 (C-6 (U)), 102.61 (C-5 (U)), 90.14 (d, $^1J_{C,F}$ = 177.5, C-4), 88.54 (C-1′), 83.51 (d, $J$ = 8.8, C-4′), 74.11 (d, $^2J_{C,F}$ = 15.7, C-5), 73.88 (C-2′), 72.91 (d, $^1J$ = 144.5, C-1), 69.82 (C-3′), 69.14 (d, $^2J_{C,F}$ = 16.5, C-3), 68.04 (d, $J$ = 3.2, C-2), 64.05 (d, $J$ = 4.7, C-5′), 59.82 (d, $J$ = 6.0, C-6); $^{19}$F NMR (282 MHz, $D_2O$) $\delta$: −218.35 (ddd, F-4); $^{31}$P NMR (202.5 MHz, $^1$H dec) $\delta$: 14.75, 1.18; ESI-MS, *m/z*: calcd for $C_{15}H_{22}FN_2O_{15}P_2$ [**1b**−2B−H]$^-$: 551.05, found: 551.17; calcd for $C_9H_{12}N_2O_9P$ [**UMP**−H]$^-$: 323.03, found: 323.59; calcd for $C_6H_{11}FO_7P$ [**3**−H]$^-$: 245.02, found: 245.33; calcd for $C_{15}H_{24}FN_2O_{15}P_2$ [**1b**-2B+H]$^+$: 553.06, found: 553.06. Anal. Calcd for $C_{15}H_{29}FN_4O_{15}P_2.4H_2O$: C, 27.36; H, 5.66; N, 8.51. Found: C, 27.35; H, 5.76; N, 8.25.

### 3.5.7. Diammonium uridine-5′-[(2-deoxy-2-fluoro-α-D-galactopyranosyl)phosphonoyl] phosphate (2b)

From the salt **2a** (203 mg, 0.178 mmol) the diammonium salt **2b** (129 mg, 63%) was obtained as a colourless syrup, $[\alpha]_D$ +6 (*c* 0.9, water). $^1$H NMR (500 MHz, $D_2O$) $\delta$: 7.92 (d, 1H, $J$ = 8.1, H-6(U)), 5.96–5.94 (m, 2H, H-1′, H-5(U)), 4.94 (dddd, 1H, $J_{2,1}$ = 5.3, $J_{2,3}$ = 7.2, $J_{2,P}$ = 18.0, $^2J_{2,F}$ = 48.8, H-2), 4.48 (ddd, $J_{1,2}$ = 5.3, $^2J_{1,P}$ = $J_{1,F}$ = 14.6, H-1), 4.37–4.33 (m, 3H, H-2′, H-3′, H-3), 4.28 (br s, 1H, H-4′), 4.23 (ddd, H, $J$ = 2.5, 4.3, 11.8, H-5a′), 4.19 (ddd, 1H, $J$ = 3.0, 5.4, 11.8, H-5b′), 4.16 (dd, $J_{5,6a}$ = 8.3, $J_{5,6b}$ = 3.5, H-5), 4.12 (ddd, 1H, $J_{4,5}$ = $J_{4,3}$ = $J_{4,F}$ = 3.2, H-4), 3.92 (dd, 1H, $J_{6a,5}$ = 8.3, $J_{6a,6b}$ = 12.4, H-6a), 3.66 (dd, 1H, $J_{6b,5}$ = 3.6, $J_{6b,6a}$ = 12.4, H-6b); $^{13}$C NMR (125 MHz, $D_2O$) $\delta$: 166.28 (C-4(U)), 151.91 (C-2(U)), 141.76 (C-6(U)), 102.75 (C-5(U), 89.77 (d, $^1J_{C,F}$ = 178.5, C-2), 88.58 (C-1′), 83.36 (d, $J_{C,P}$ = 8.8, C-4′), 76.42 (d, $J_{C,P}$ = 5.3, C-5), 73.83 (C-2′), 69.77 (C-3′), 68.90 (dd, $^1J_{C,P}$ = 164.1, $^2J_{C,F}$ = 23.0, C-1), 68.19 (d, $J$ = 19.8, C-3), 67.97 (d, $J_{C,F}$ = 5.7, C-4), 64.96 (d, $J_{C,P}$ = 4.9, C-5′), 59.52 (C-6); $^{19}$F NMR (470 MHz, $D_2O$, dec) $\delta$: −202.8 (F-2); $^{31}$P NMR (202.5 MHz, $D_2O$, $^1$H dec) $\delta$: −10.61 (d, $J$ = 28.8,), 5.75 (d, $J$ = 28.8 ); ESI-MS, *m/z*: calcd for $C_{15}H_{22}FN_2O_{15}P_2$ [**2b**−2B−H]$^-$: 551.05, found: 551.29; calcd for $C_9H_{12}N_2O_9P$ [**UMP**−H]$^-$: 323.03, found: 323.56;.

Anal. Calcd for $C_{15}H_{29}FN_4O_{15}P_2.H_2O$: C, 29.81; H, 5.17; N, 9.27. Found: C, 29.53; H, 5.42; N, 8.96.

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