Preparation of fluorinated galactosyl nucleoside diphosphates to study the mechanism of the enzyme galactopyranose mutase†

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A novel latent→active phosphorylation strategy has been employed for the preparation of two fluorinated nucleoside diphosphates (compounds I and II). The strategy is based on the isomerisation of substituted allyl to vinyl glycosides which were subsequently phosphorylated by treatment with dibenzyl hydrogen phosphate, *N*-iodosuccinimide and a catalytic amount of trimethylsilyl triflate. This methodology is very suitable for the preparation of nucleoside diphosphates that have a modification in the saccharide moiety since the allyl moiety serves first as an anomeric protecting group, allowing for protecting-group manipulation and functionalisation of the sugar ring, but after isomerisation to the corresponding vinyl glycoside it acts as an anomeric leaving group. The 2-F and 4-F Gal-UDP derivatives I and II do not inhibit the enzyme galactopyranose mutase in the direction pyranose ⟶ furanose but both compounds have been found to inhibit the reverse reaction.

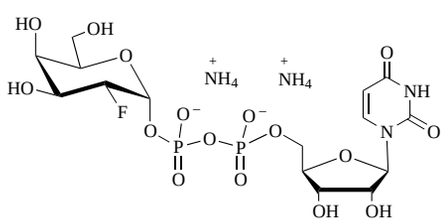
Introduction

D-Galactose in the furanose form is found in oligo- and polysaccharides and glycoconjugates of certain bacteria, protozoa and fungi,[1] however it is not a constituent of mammalian saccharides. The first polysaccharide found to contain galactofuranose was galactocarolose which is an extracellular β-D-(1→5)-linked polygalactofuranose produced by *Penicillium charlesii*.[2] Galactofuranoside is also present in the oligosaccharide core of glycoinositol phospholipids of *Trypanosoma cruzi* which is the infective agent of Chagas' disease.[3] *Mycobacterium tuberculosis* is another pathogen that contains this rather unusual sugar, and an arabinogalactan consisting of ~30 α-D-galactofuranose and ~60 α-D-arabinofuranose residues which is linked to a peptidoglycan *via* a unique diglycosyl phosphoryl bridge has been isolated from this organism.[4]

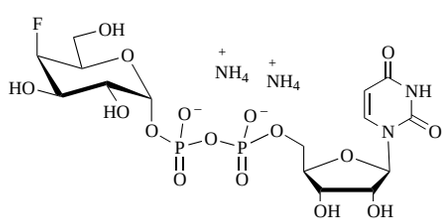
The restriction of the five-membered-ring configuration of galactose to parasites makes the galactofuranose biosynthetic pathway an important target for the design of novel antibacterial, antiprotozoal and antifungal drugs. However, a detailed knowledge of the biosynthesis of galactofuranose-containing oligosaccharides is needed.

It has been proposed that the activated sugar for incorporation of α-D-galactofuranosides into oligosaccharides is uridine 5′-diphosphate (UDP)-galactofuranose which is probably derived *via* the ring contraction of a galactopyranose derivative. Recently, a galactopyranose mutase enzyme that catalyses the interconversion of UDP-galactopyranose and UDP-galactofuranose has been cloned and overexpressed.[5] The equilibrium facilitated by this enzyme is biased in favour of the pyranose isomer, and at equilibrium 10% of the galactopyranose is converted into the furanose form. The reaction mechanism of ring contraction is not yet known but it has been proposed that the 2-hydroxy group of galactose is involved.[6]

In this paper we report the synthesis of fluorinated UDP-galactopyranose derivatives **I** and **II** which will be valuable compounds in an investigation, at a molecular level, of the enzymic interconversion of UDP-galactopyranose and UDP-galactofuranose. The galactopyranose nucleoside diphosphate **I**, having a fluorine atom at the 2-position of galactose, may facilitate mechanistic investigation of the action of galactopyranose mutase and help establish the involvement of the 2-hydroxy group in the ring contraction. Furthermore, the fluorine label will enable any interconversion to be monitored by the use of ^{19}F NMR spectroscopy. The derivative **II** has been substituted with a fluorine atom at the 4-position of galactose and this modification should have a very minor effect on the structural and electronic properties of the molecule. Therefore, it is to be expected that compound **II** will be recognised by the galactopyranose mutase and act as an inhibitor of the enzyme, since the 4-hydroxy group is required for the furanose ring to be formed.



I



II

Synthetic targets: UDP-2-deoxy-2-fluorogalactose **I** and UDP-4-deoxy-4-fluorogalactose **II**

Results and discussion

It has been found that vinyl glycosides are efficient glycosyl donors for the synthesis of O-glycosidic linkages.[7] Recently we described a new, latent→active anomeric phosphorylation strategy based on the isomerisation of substituted allyl to

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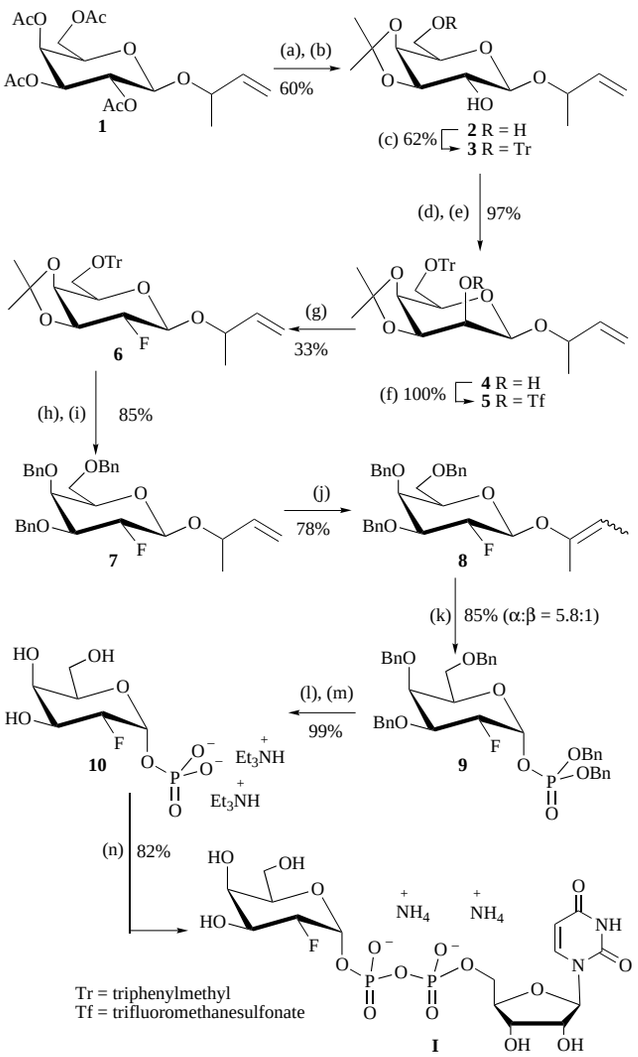
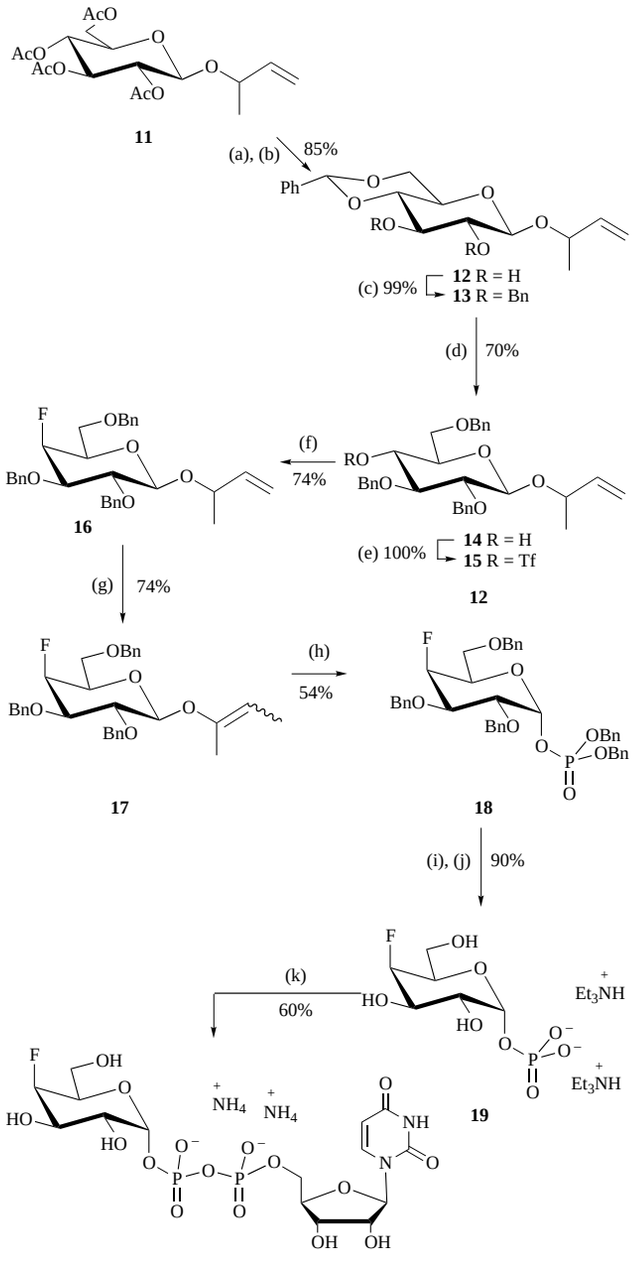
vinyl glycosides which were subsequently phosphorylated by treatment with dibenzyl hydrogen phosphate, *N*-iodosuccinimide (NIS) and a catalytic amount of trimethylsilyl trifluoromethanesulfonate (TMSOTf).[8,9] In this strategy, the allyl moiety serves two purposes: (i) it acts as an anomeric protecting group, allowing for protecting group manipulation and functionalisation of the sugar ring and (ii) it functions as a latent leaving group, since isomerisation gives a vinyl glycoside which can be used as a glycosyl donor.

It was envisaged that the features of allyl and vinyl glycosides would make them versatile synthons for the preparation of glycosyl phosphates **9** and **18** (Schemes 1 and 2) and, after deprotection, it should be easy to convert these anomeric phosphates into the corresponding nucleotide diphosphates **I** and **II** *via* a standard method.[10]



Scheme 1 Synthesis of diammonium 2-deoxy-2-fluoro-α-D-galactopyranosyl uridin-5′-yl diphosphate **I**. *Reagents and conditions:* (a) KO^{t}Bu, MeOH; (b) 2,2-dimethoxypropane, acetonitrile, CSA; (c) trityl chloride, pyridine; (d) (i) oxalyl dichloride, DMSO, DCM (−78 °C), (ii) Et_3N; (e) $LiAlH_4$, THF; (f) Tf_2O, pyridine, DCM (−78 °C→room temp.); (g) TEAF, acetonitrile (50 °C); (h) AcOH, aq. TFA; (i) benzyl bromide, NaH, DMF; (j) $Rh(PPh_3)_3Cl$, BuLi, THF; (k) $(BnO)_2PO_2H$, NIS, TMSOTf, DCM, molecular sieves (4 Å); (l) 10% Pd/C, H_2, ethyl acetate, propan-2-ol, water (4:6:2 v/v); (m) DOWEX 1-X8[Et_3NH^+]; (n) uridine 5′-monomorpholidophosphate, pyridine.



Scheme 2 Synthesis of diammonium 4-deoxy-4-fluoro-α-D-galactopyranosyl uridin-5′-yl diphosphate **II**. *Reagents and conditions:* (a) KO^{t}Bu, MeOH; (b) benzaldehyde dimethyl acetal, acetonitrile, CSA; (c) benzyl bromide, NaH, DMF; (d) TFA, triethylsilane, DCM (−20 °C→room temp.); (e) Tf_2O, pyridine, DCM (−78 °C→room temp.); (f) TBAF, THF; (g) $Rh(PPh_3)_3Cl$, BuLi, THF; (h) $(BnO)_2PO_2H$, NIS, TMSOTf, DCM, molecular sieves (4 Å); (i) 10% Pd/C, H_2, ethyl acetate–propan-2-ol–water (4:6:2 v/v); (j) DOWEX 1-X8[Et_3NH^+]; (k) uridine 5′-monomorpholidophosphate, pyridine.

Glycosyl donor **8**, which is the precursor of the anomeric phosphate **9**, was prepared from the readily available allyl galactoside **1**.[8] Racemic but-3-en-2-ol was used for the preparation of compound **1**, resulting in the formation of a mixture of diastereoisomers. While the diastereoisomeric nature of the glycosides does not affect the chemistry, it complicates the interpretation of the NMR spectra. Multi-gram quantities of optically pure but-3-en-2-ol, however, can easily be obtained by a literature procedure.[11] Deacetylation of compound **1** with potassion *tert*-butoxide in methanol, followed by treatment with acetone, 2,2-dimethoxypropane and a catalytic amount of acid yielded the partially protected allyl glycoside **2** in good yield. Subsequent regioselective tritylation with trityl chloride in pyridine gave compound **3**, the hydroxy group of which was substituted by a fluorine atom (⟶ **6**) in a double-inversion procedure. Thus, Swern oxidation of secondary alcohol **3** gave the corresponding ketone which, after purification using silica gel column chromatography, was reduced with $LiAlH_4$ in tetrahydrofuran (THF) to provide, in almost quantitative yield,

the talose derivative **4**. The ^{1}H NMR spectrum of compound **4** showed that none of the isomeric galactoside was present, and the coupling constant $J_{1,2}$ 1.8 Hz, typical of a 1,2-*cis* linkage, confirmed the stereochemistry of the product. Next, fluorine was introduced, first by treatment of compound **4** with trifluoromethanesulfonic anhydride and pyridine in dichloromethane (DCM) to give the corresponding triflate **5** which was immediately substituted by reaction with tetraethylammonium fluoride (TEAF) in acetonitrile at 50 °C to furnish the galactoside derivative **6**. It is important to note that the isopropylidene moiety of the intermediate triflate prevents a competing elimination reaction.[12] Compound **6** was converted into the benzylated allyl glycoside **7** by treatment with a mixture of acetic acid and trifluoroacetic acid (TFA) in water followed by benzylation with benzyl bromide and sodium hydride in dimethylformamide (DMF). The allyl moiety of compound **7** remained intact throughout the synthetic manipulations described above, but was now readily converted into an efficient anomeric leaving group by isomerisation to give the vinyl glycoside **8**. The isomerisation of compound **7** was conveniently performed with a catalytic amount of Wilkinson's catalyst, treated with BuLi in refluxing THF[13] and, after a reaction time of 30 min, compound **8** was isolated in 78% yield. Under these isomerisation conditions, we believe that Wilkinson's catalyst is converted into a rhodium hydride species which is superior for isomerisation. It is also noteworthy that these isomerisation conditions are compatible with the presence of a fluorine atom in the glycoside.

The anomeric phosphate was introduced by reaction of a solution of compound **8** in DCM with dibenzyl hydrogen phosphate, NIS and a catalytic amount of TMSOTf. When the reaction was performed at −20 °C, the anomeric phosphate **9** was isolated in a good yield but the undesired β-anomer was the main product (65%; α : β = 1 : 2). Fortunately, an acceptable α-selectivity could be achieved by performing the reaction at room temperature (85%; α : β = 5.8 : 1) and the anomers could be separated by flash chromatography. It is conceivable that when the reaction is performed at room temperature, the initially produced β-anomer may anomerise to give the thermodynamically more stable α-anomer. However, attempted isomerisation of the β-phosphate by treatment with a catalytic amount of hydrochloric acid failed and starting material was recovered together with some trehalose. It should be noted that others have successfully isomerised related compounds under similar conditions.[9a,b]

Next, the anomeric phosphate **9** was debenzylated by catalytic hydrogenation over Pd/C in the presence of sodium acetate, and the phosphate **10** was obtained as a triethylammonium salt in a good yield after treatment of the product with Dowex 1-X8[Et_3NH^+] and purification by size-exclusion column chromatography (LH-20). Finally, condensation of compound **10** with uridine 5′-monomorpholidophosphate in pyridine for 5 days gave, after ion-exchange column chromatography, the requisite UDP-derivative **I** in a very pleasing yield of 82%. The high yield in this coupling may be attributed to the stabilising effect of the fluorine atom on the anomeric linkage. The spectral analysis obtained for this compound confirmed it to be the target compound **I**. The fluorine coupling constants in the ^{1}H and ^{13}C NMR spectra were consistent with a 2-deoxy-2-fluoro glycoside and the α-glycosidic linkage was confirmed by the anomeric coupling constants; $J_{1,P}$ 7.4 Hz, $J_{1,2}$ 3.7 Hz and $J_{1,F}$ < 0.5 Hz. The presence of two multiplets of similar intensity in the ^{31}P NMR spectrum confirmed the presence of a diphosphate linkage and matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) spectrometry gave a negative ion that corresponded in *m/z* to the singly protonated diphosphate. The presence of a small amount of side-product was also observed in the sample of compound **I** and it is thought that this corresponded to the symmetric by-product P^1,P^2-bis-5′-uridine diphosphate (UPPU).

Having successfully prepared the UDP-2-deoxy-2-fluorogalactose **I**, we focused our attention on the synthesis of the UDP-galactose derivative having a fluorine at the 4-position of the galactose. In this case, the allyl glucoside **11** was selected as the starting material and it was envisaged that the galactose configuration could be obtained by conversion of the 4-hydroxy group of a glucoside into a good leaving group followed by substitution with a fluoride nucleophile. The glucoside **14** is the appropriate precursor for this transformation and was obtained by a four-step procedure. Thus, deacetylation of compound **11** with KO^{t}Bu in methanol followed by treatment with benzaldehyde dimethyl acetal and a catalytic amount of camphor-10-sulfonic acid (CSA) gave regioselectively the partially protected saccharide **12**. Benzylation of compound **12** under standard conditions afforded the fully protected species **13**, the benzylidene acetal of which was reductively opened with triethylsilane and TFA[14] to yield the required compound **14**. The fluorine of intermediate target **16** was conveniently introduced by treatment of compound **14** with trifluoromethanesulfonic anhydride and pyridine in DCM, to give triflate **15** and immediate nucleophilic displacement with tetrabutylammonium fluoride (TBAF) in THF. The anomeric phosphate was obtained by isomerisation of the allyl moiety of compound **16** to give the vinyl glycoside **17**, which was phosphorylated by reaction with dibenzyl hydrogen phosphate, NIS and a catalytic amount of TMSOTf. The success of this phosphorylation also depended strongly on the reaction temperature. When the phosphorylation was performed at −20 °C, compound **18** was isolated as a single anomer but in a disappointing yield of 35%. The yield could be improved to 54% by performing the reaction at room temperature. A substantial amount of the undesired β-anomer is probably formed at low reaction temperatures and which, in this case, hydrolyses during the work-up and purification procedure. As for vinyl glycoside **8**, the phosphorylation of compound **17** at a higher temperature gave better α-selectivity which here is reflected in a higher yield. Having compound **18** in hand, the synthesis of target **II** was completed as performed for compound **I**. Thus, hydrogenolysis of the benzyl ethers of compound **18** yielded compound **19**, which was condensed with uridine 5′-monomorpholidophosphate under standard conditions to give, after purification by ion-exchange chromatography, the requisite UDP-4-fluoro-4-deoxygalactose **II**. Compound **II** was also found to be contaminated with a small amount of side-product (UPPU) and the spectral data obtained were in accord with those published previously.[15]

Preliminary inhibition studies[5] show that derivatives **I** and **II** do not inhibit the enzyme galactopyranose mutase in the direction pyranose ⟶ furanose but both compounds were found to inhibit the reverse reaction. At concentrations of 166 μM of inhibitor and 35 μM UDP-galactofuranose, 55 and 48% inhibition of the transformation furanose ⟶ pyranose was observed for compounds **I** and **II** respectively. The K_m-value for the enzyme is 1 mM. Currently, we are examining whether compound **I** can be converted by the enzyme into the analogous furanose derivative and this experiment will firmly establish the importance of the 2-hydroxy group for the enzymic transformation. The full experimental detail of the inhibition studies will be reported elsewhere.

Conclusions

We have described the preparation of two modified UDP-galactosyluridine diphosphates (**I** and **II**) using a novel latent→active phosphorylation strategy. A latent anomeric allyl moiety functioned as an efficient anomeric protecting group but could be converted into a leaving group by a Rh-catalysed isomerisation to give a vinyl glycoside. The vinyl glycoside underwent clean phosphorylation and the anomeric outcome of the reaction could be controlled by the reaction temperature. The ano-

meric phosphates **9** and **18** were converted by a known procedure into target nucleoside diphosphates **I** and **II**.

Initial biochemical investigations have revealed that derivatives **I** and **II** do not inhibit the enzyme galactopyranose mutase in the direction pyranose ⟶ furanose but both compounds were found to inhibit the reverse reaction.

Experimental

Reagents were purchased from Aldrich, Fluka and Lancaster. All reaction solvents were distilled prior to use: DCM, acetonitrile, pyridine, diethyl ether and DMF from calcium hydride, THF from lithium aluminium hydride, acetone from potassium carbonate and methanol from its magnesium Grignard. Flash silica gel column chromatography was carried out on silica gel (Merck 9385). Thin layer chromatography (TLC) analysis was carried out on silica gel-coated aluminium plates (Merck 12.05554 Kieselgel 60 F254). Compounds were visualised by UV light (254 nm) and/or by dipping in H_2SO_4–methanol (1:10 v/v) and subsequent charring. NMR spectra were recorded on Bruker AC 300 and 250 spectrometers equipped with Aspect 3000 computers. For ^{1}H and ^{13}C spectra, chemical shifts are given in parts per million (δ) relative to tetramethylsilane. For ^{19}F spectra, chemical shifts are given in parts per million (δ) relative to $CFCl_3$, and for ^{31}P spectra, chemical shifts are given in parts per million (δ) relative to H_3PO_4. Presaturation of water signals was used on all D_2O spectra. *J*-values are given in Hz. Fast-atom bombardment (FAB) mass spectra were recorded using a VG Zabspec spectrometer with *m*-nitrobenzyl alcohol as matrix. Chemical ionisation (CI) mass spectra were recorded on a VG Prospec spectrometer using ammonia as reagent gas. Negative-ion MALDI-TOF mass spectra were recorded using a Kratos Kompact instrument using an indole–acrylic acid matrix. Light petroleum refers to the 40–60 °C fraction.

(*R/S*)-But-3-en-2-yl 3,4-*O*-isopropylidene-β-D-galactopyranoside 2

Potassium *tert*-butoxide (0.56 g, 5.00 mmol) was added to a stirred solution of (*R/S*)-but-3-en-2-yl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside **1** (6.00 g, 14.93 mmol) in dry methanol (20 ml), and the resulting mixture was stirred at room temperature under argon. After 3.5 h, TLC analysis (acetone–DCM, 1:19 v/v) showed the complete conversion of the starting material (R_f 0.47) into a product (R_f 0.00). The reaction mixture was neutralised with Dowex 1-X8[H^+] and, after filtration, concentrated to dryness *in vacuo* to yield a solid, which was dissolved in acetone (10 ml) and to the resulting solution was added 2,2-dimethoxypropane (3.0 ml, 24.4 mmol), followed by a catalytic amount of CSA (~5 mg). After stirring of the mixture for 2 h under argon, TLC analysis (methanol–DCM, 1:9 v/v) showed the conversion of the starting material (R_f 0.06) into a major product (R_f 0.48). The reaction was quenched by the addition of pyridine (0.30 ml) and the reaction mixture was concentrated to dryness *in vacuo* to give a yellow oil, which was redissolved in ethyl acetate (30 ml) and the solution was washed successively with saturated aq. sodium hydrogen carbonate (2 × 10 ml) and brine (2 × 10 ml). The organic phase was dried ($MgSO_4$), and concentrated to dryness *in vacuo* to yield a yellow oil. Purification using flash chromatography (100 g SiO_2; eluent ethyl acetate–light petroleum, 3:7 v/v) and concentration of the appropriate fractions yielded *title compound* **2** as a solid (2.45 g, 60%), δ_H(300 MHz; $CDCl_3$) 6.01–5.62 (m, 1 H, C*H*=CH_2), 5.23–5.03 (m, 2 H, CH=CH_2), 4.38–4.17 (m, 1 H, C*H*CH_3), 4.23 (2 d, $J_{1,2}$ 8.1, 1 H, H-1), 4.13–3.02 (m, 2 H, H-3 and -4), 3.97–3.86 (m, 1 H, H-2), 3.84–3.69 (m, 2 H, H_2-6), 3.56–3.48 (m, 1 H, H-5), 1.50–1.46 (2 s, 3 H, CH_3CO_2), 1.39–1.25 (2 s, 3 H, CH_3CO_2) and 1.24–1.12 (m, *J* 6, 3 H, CHCH_3); δ_C(75 MHz; $CDCl_3$) 140.6 and 138.7 (*C*H=CH_2), 117.7 and 115.0 (*C*H_2=CH), 110.4 (CH_3CO_2), 101.0 and 98.9 (C-1), 79.1–75.0 (C-2, -3, -4 and *C*HCH$_3$), 73.7–73.5 (C-5 and -6), 28.1 and 26.3 (CH_3CO_2) and 21.6 and 20.4 (CH*C*H$_3$); FAB *m/z* 297 ([M + Na]$^+$) (Found: C, 57.00; H, 8.13. $C_{13}H_{22}O_6$ requires C, 56.91; H, 8.08%).

(*R/S*)-But-3-en-2-yl 3,4-*O*-isopropylidene-6-*O*-trityl-β-D-galactopyranoside 3

Compound **2** (2.45 g, 9.27 mmol) was dissolved in pyridine (10 ml) and trityl chloride (2.58 g, 9.27 mmol) was added. The mixture was stirred under argon and TLC analysis (acetone–DCM, 1:49 v/v) after 16 h showed the conversion of the starting material (R_f 0.07) into a major product (R_f 0.34). The solution was concentrated to dryness *in vacuo*, the residue was redissolved in ethyl acetate (20 ml), and the resulting solution was washed successively with saturated aq. sodium hydrogen carbonate (2 × 10 ml) and brine (2 × 10 ml). The organic phase was dried ($MgSO_4$), and concentrated to dryness *in vacuo* to yield a sticky yellow solid. Purification using flash chromatography (100 g SiO_2; eluent ethyl acetate–light petroleum, 1:3 v/v) and concentration of the appropriate fractions yielded *compound* **3** (2.96 g, 62%) as a solid, δ_H(300 MHz; $CDCl_3$) 7.52–7.20 (m, 15 H, ArH), 6.07–5.52 (m, 1 H, C*H*=CH_2), 5.28–5.04 (m, 2 H, CH=CH_2), 4.44–4.23 (m, 1 H, C*H*CH_3), 4.20 (2 d, $J_{1,2}$ 8.5, 1 H, H-1), 4.18–3.98 (m, 2 H, H-3 and -4), 3.77–3.68 (m, 1 H, H-5), 3.60–3.34 (m, 3 H, H-2 and H_2-6), 1.55–1.52 (2 s, 3 H, CH_3CO_2), 1.39–1.25 (2 s, 3 H, CH_3CO_2) and 1.24–1.12 (m, *J* 6, 3 H, CHCH_3); δ_C(75 MHz; $CDCl_3$) 144.0 (ArC quaternary), 140.1 and 138.9 (*C*H=CH_2), 128.8–127.0 (ArCH), 117.6 and 115.2 (*C*H_2=CH), 110.1 (CH_3CO_2), 100.6 and 98.9 (C-1), 86.7 (Ph$_3$*C*O), 78.8–72.6 (C-2, -3, -4, -5 and *C*HCH$_3$), 62.9 (C-6), 25.6 and 25.5 (*C*H_3CO_2) and 21.1 and 20.2 (CH*C*H$_3$); FAB *m/z* 539 ([M + Na]$^+$) (Found: C, 74.26; H, 6.91. $C_{32}H_{36}O_6$ requires C, 74.39; H, 7.02%).

(*R/S*)-But-3-en-2-yl 3,4-*O*-isopropylidene-6-*O*-trityl-β-D-talopyranoside 4

To a cooled (−60 °C) solution of oxalyl dichloride (0.55 ml, 6.33 mmol) in DCM (5 ml) was added dimethyl sulfoxide (DMSO) (0.88 ml, 10.23 mmol). After 10 min, a solution of compound **3** (2.85 g, 5.52 mmol) in DCM (10 ml) was added dropwise and the solution was stirred under argon at reduced temperature (−60 °C). After 2 h, triethylamine (5 ml) was added and TLC analysis (acetone–DCM, 1:49 v/v) showed the conversion of starting material (R_f 0.34) into an intermediate product (R_f 0.48). The solution was concentrated to dryness *in vacuo* and ethyl acetate (20 ml) was added. The resulting solution was washed successively with saturated aq. sodium hydrogen carbonate (2 × 10 ml) and brine (2 × 10 ml). The organic phase was dried ($MgSO_4$), and concentrated to dryness *in vacuo* to yield a sticky yellow solid. Purification using flash chromatography (100 g SiO_2; eluent ethyl acetate–light petroleum, 1:3 v/v) and concentration of the appropriate fractions gave a solid, which was dissolved in THF (5 ml) and to the resulting, stirred solution was added lithium aluminium hydride (725 mg, 19.0 mmol). TLC analysis (acetone–DCM, 1:24 v/v) immediately after the addition showed the conversion of the starting material (R_f 0.76) into a major product (R_f 0.67). Ethyl acetate (20 ml) was added to the reaction mixture and the resulting solution was washed successively with 1 M aq. NaOH (2 × 10 ml) and brine (2 × 10 ml). The organic phase was dried ($MgSO_4$), and concentrated to dryness *in vacuo* to yield a waxy yellow solid. Purification using flash chromatography (100 g SiO_2; eluent ethyl acetate–light petroleum, 1:3 v/v) and concentration of the appropriate fractions yielded *compound* **4** (2.76 g, 97%) as a solid, δ_H(300 MHz; $CDCl_3$) 7.53–7.20 (m, 15 H, ArH), 6.07–5.60 (m, 1 H, C*H*=CH_2), 5.28–5.02 (m, 2 H, CH=CH_2), 4.57 (2 d, $J_{1,2}$ 1.8, 1 H, H-1), 4.42–4.08 (m, 3 H, H-3, -4 and C*H*CH_3), 3.78–3.68 (m, 2 H, H_2-6), 3.62–3.30 (m, 2 H, H-2 and -5), 1.60–1.52 (2 s, 3 H, CH_3CO_2), 1.40–1.30 (2 s, 3 H, CH_3CO_2) and 1.27–1.12 (m, *J* 6, 3 H, CHCH_3); δ_C(75 MHz;

$CDCl_3$) 144.0 (ArC quaternary), 140.5 and 139.1 (*C*H=CH_2), 128.8–127.1 (ArCH), 117.4 and 114.8 (*C*H_2=CH), 110.0 (CH_3CO_2), 98.0 and 96.6 (C-1), 86.8 (Ph_3CO), 79.7–71.8 (C-2, -3, -4, -5 and *C*HCH_3), 63.1 (C-6), 25.6 and 25.5 (CH_3CO_2) and 21.1 and 20.2 (CH*C*H_3); FAB *m/z* 539 ([M + Na]$^+$) (Found: C, 74.14; H, 7.15. $C_{32}H_{36}O_6$ requires C, 74.39; H, 7.02%).

(*R/S*)-But-3-en-2-yl 2-deoxy-2-fluoro-3,4-*O*-isopropylidene-6-*O*-trityl-β-D-galactopyranoside 6

Compound **4** (2.68 g, 5.18 mmol) was dissolved in DCM (20 ml), and pyridine (3 ml) was added. The stirred solution was cooled (−78 °C) under argon and trifluoromethanesulfonic anhydride (Tf_2O) (0.78 ml, 1.30 g, 5.20 mmol) was added. The reaction mixture was allowed to warm to room temperature and TLC analysis (acetone–DCM, 1:49 v/v) after 40 min showed the complete conversion of starting material (R_f 0.32) into an intermediate product (R_f 0.76). The reaction mixture was diluted with DCM (50 ml) and washed successively with saturated aq. sodium hydrogen carbonate (2 × 10 ml) and brine (2 × 10 ml). The organic phase was dried ($MgSO_4$), and concentrated to dryness *in vacuo* to yield a sticky yellow solid. Purification using flash chromatography (SiO_2 100 g; eluent ethyl acetate–light petroleum, 1:3 v/v) and concentration of the appropriate fractions gave compound **5** as a solid. The solid was immediately dissolved in acetonitrile (30 ml), and the solution was stirred under argon and heated (50 °C). TEAF hydrate (3.00 g, 20.10 mmol) was added to the solution and TLC analysis (acetone–DCM, 1:49 v/v) after 2 h showed the conversion of compound **5** (R_f 0.76) into a major product (R_f 0.71). The reaction mixture was concentrated to dryness *in vacuo*, the residue was re-dissolved in ethyl acetate (30 ml) and the solution was washed successively with saturated aq. sodium hydrogen carbonate (2 × 10 ml) and brine (2 × 10 ml). The organic phase was dried ($MgSO_4$), and concentrated to dryness *in vacuo* to yield a sticky yellow solid. Purification using flash chromatography (50 g SiO_2; eluent ethyl acetate–light petroleum, 1:3 v/v) and concentration of the appropriate fractions yielded *compound* **6** (887 mg, 33%) as a solid, δ_H(300 MHz; $CDCl_3$) 7.53–7.20 (m, 15 H, ArH), 6.07–5.65 (m, 1 H, C*H*=CH_2), 5.32–5.05 (m, 2 H, CH=CH_2), 4.46–4.18 (m, 5 H, H-1, -2, -3, -4 and C*H*CH_3), 3.76–3.69 (m, 1 H, H-5), 3.56–3.32 (m, 2 H, H_2-6), 1.60–1.52 (2 s, 3 H, CH_3CO_2), 1.40–1.30 (2 s, 3 H, CH_3CO_2) and 1.27–1.12 (m, *J* 6, 3 H, CH*C*H_3); δ_C(75 MHz; $CDCl_3$) 143.9 (ArC quaternary), 140.0 and 138.8 (*C*H=CH_2), 128.8–125.2 (ArCH), 117.4 and 115.4 (*C*H_2=CH), 110.6 (CH_3CO_2), 96.8 (d, $J_{1,F}$ 23.7, C-1), 93.5 (d, $J_{2,F}$ 184.8, C-2), 86.8 (Ph_3CO), 77.8 and 74.9 (C-4 and -5), 72.4 (d, $J_{3,F}$ 12.4, C-3), 62.7 (C-6), 25.6 and 25.5 (CH_3CO_2) and 21.1 and 20.2 (CH*C*H_3); δ_F(282 MHz; $CDCl_3$) −218.6 (ddd, $J_{2,F}$ 48.3, $J_{3,F}$ 22.9, $J_{1,F}$ 3.8); FAB *m/z* 541 ([M + Na]$^+$) (Found: C, 74.02; H, 6.87. $C_{32}H_{35}FO_5$ requires C, 74.11; H, 6.80%).

(*R/S*)-But-3-en-2-yl 3,4,6-tri-*O*-benzyl-2-deoxy-2-fluoro-β-D-galactopyranoside 7

To a solution of compound **6** (460 mg, 0.86 mmol) in acetic acid–water (10 ml, 4:1 v/v) was added TFA dropwise until a yellow colour persisted. TLC analysis (methanol–DCM, 1:9 v/v) showed the complete conversion of starting material (R_f 0.98) into an intermediate product (R_f 0.21). Water (50 ml) was added and the resulting solution was concentrated *in vacuo*, with continual addition of water, until no acid persisted; the solution was then concentrated to dryness. Purification using flash chromatography (50 g SiO_2; eluent ethyl acetate) and concentration of the appropriate fractions gave a solid, which was dissolved in DMF (2 ml), and sodium hydride (120 mg; 60% dispersion in oil; 2.98 mmol) was added to the cooled (0 °C) solution, followed by benzyl bromide (0.29 ml, 2.45 mmol). After stirring of the mixture under argon for 3 h, TLC analysis (acetone–DCM, 1:49 v/v) showed the complete conversion of starting material (R_f 0.00) into a product (R_f 0.68). The reaction mixture was poured onto crushed ice (5 g), extracted with diethyl ether (20 ml), and the extract was washed successively with saturated aq. sodium hydrogen carbonate (2 × 10 ml) and brine (2 × 10 ml). The organic phase was dried ($MgSO_4$), and concentrated to dryness *in vacuo* to yield a sticky yellow solid. Purification using flash chromatography (30 g SiO_2; eluent ethyl acetate–light petroleum, 1:5 v/v) and concentration of the appropriate fractions yielded *compound* **7** (382 mg, 85%) as a solid, δ_H(300 MHz; $CDCl_3$) 7.37–7.20 (m, 15 H, ArH), 6.02–5.62 (m, 1 H, C*H*=CH_2), 5.27–5.03 (m, 2 H, CH=CH_2), 4.96–4.23 (m, 7 H, H-1, -2, C*H*CH_3 and ArCH_2), 3.95–3.90 (m, H-5), 3.66–3.50 (m, 4 H, H-3, -4 and H_2-6) and 1.36–1.21 (2 d, *J* 6, 3 H, CHCH_3); δ_C(75 MHz; $CDCl_3$) 140.2 and 139.1 (*C*H=CH_2), 138.4–137.9 (ArC quaternary), 128.4–127.6 (ArCH), 117.0 and 115.1 (*C*H_2=CH), 99.3 and 98.4 (d, $J_{1,F}$ 23.2, C-1), 92.0 (d, $J_{2,F}$ 183.7, C-2), 80.3 (d, $J_{3,F}$ 7.9, C-3), 77.5 and 73.6 (C-4 and -5), 74.7–72.7 (ArCH_2), 68.7 (C-6) and 21.7 and 20.4 (CH*C*H_3); δ_F(282 MHz; $CDCl_3$) −205.5 (ddd, $J_{2,F}$ 52.14, $J_{3,F}$ 12.71, $J_{1,F}$ < 1); FAB *m/z* 529 ([M + Na]$^+$) (Found: C, 73.41; H, 7.11. $C_{31}H_{35}FO_5$ requires C, 73.49; H, 6.96%).

(*E/Z*)-But-2-en-2-yl 3,4,6-tri-*O*-benzyl-2-deoxy-2-fluoro-β-D-galactopyranoside 8

Butyllithium (1.5 M in THF; 1000 μl, 1.5 mmol) was added to a stirred, orange solution of Wilkinson's catalyst (500 mg, 0.05 mmol) in THF (1 ml) under argon. The resulting brown solution was stirred at 20 °C for 5 min and was then transferred *via* syringe into a refluxing solution of compound **7** (175 mg, 0.35 mmol) in THF (10 ml) under argon. TLC analysis (acetone–DCM, 1:49 v/v) after 30 min showed complete conversion of starting material (R_f 0.73) into a product (R_f 0.75). The reaction mixture was diluted with DCM (20 ml) and concentrated to afford an orange-red residue, which was purified by flash chromatography (20 g SiO_2; eluent DCM) to afford the *isomeric compound* **8** (136 mg, 78%) as a solid, δ_H(300 MHz; $CDCl_3$) 7.37–7.20 (m, 15 H, ArH), 4.97–4.37 (m, 9 H, H-1, -2, ArCH_2 and C=C*H*), 3.99–3.92 (m, 1 H, H-5), 3.68–3.57 (m, 4 H, H-3, -4 and H_2-6), 1.92–1.82 (m, 3 H, CH_3CO) and 1.67–1.49 (m, 3 H, CH_3CH); δ_C(75 MHz; $CDCl_3$) 151.6 and 149.5 [O*C*(CH_3)=C], 138.4–137.8, (ArC quaternary), 138.8–127.6 (ArCH), 106.7, 98.9 (=*C*HCH_3), 98.8 and 98.1 (d, $J_{1,F}$ 24.3, C-1), 92.4, 98.1 (d, $J_{2,F}$ 183.7, C-2), 80.3 (2 d, $J_{3,F}$ 11.3, C-3), 74.2 and 73.8 (C-4 and -5), 74.8–72.8 (Ar*C*H_2), 68.5 (C-6), 18.6 and 15.6 (*C*H_3CO) and 11.9 and 10.3 (CH*C*H_3); δ_F(282 MHz; $CDCl_3$) −204.3 and −204.7 (2 ddd, $J_{4,F}$ 52.1, $J_{3,F}$ 14.0, $J_{5,F}$ 12.7); FAB *m/z* 529 ([M + Na]$^+$) (Found: C, 73.59; H, 6.89. $C_{31}H_{35}FO_5$ requires C, 73.49; H, 6.96%).

Dibenzyl 3,4,6-tri-*O*-benzyl-2-deoxy-2-fluoro-α-D-galacto-pyranosyl phosphate 9

A mixture of vinyl glycoside **8** (135 mg, 0.24 mmol) in DCM (1 ml) and molecular sieves (100 mg, 4 Å powdered) were stirred for 1 h under argon. NIS (200 mg, 0.88 mmol) was suspended in DCM (2 ml) by sonication in a separate reaction vessel. Dibenzyl hydrogen phosphate (225 mg, 0.81 mmol) was added to the suspension and the mixture was allowed to equilibrate under argon for 5 min. TMSOTf (18.7 μl, 0.094 mmol) was then added and the suspension was stirred for a further 30 s. The resulting solution (1.1 ml) was then added by syringe to the ready prepared solution of the vinyl glycoside **8**. Immediate TLC analysis (acetone–DCM, 1:99 v/v) of the reaction mixture showed the conversion of the starting material (R_f 0.70) into a major product (R_f 0.47). The reaction mixture was neutralised by the addition of triethylamine, and ethyl acetate (20 ml) was added. The resulting solution was washed successively with aq. sodium metabisulfate (2 × 5 ml) and brine (2 × 5 ml). The combined organic phases were dried ($MgSO_4$), concentrated to dryness *in vacuo*, and the resulting oil was purified using flash chromatography (25 g SiO_2; eluent THF–toluene, 1:9 v/v). Concentration of the appropriate fractions yielded *compound* **9** (121 mg,

71%) as a clear oil [a significant amount of the β-phosphate (21 mg, 14%) was also isolated], δ_H(300 MHz; $CDCl_3$) 7.41–7.17 (m, 25 H, ArH), 5.95 (dd, $J_{1,P}$ 6.3, $J_{1,2}$ 3.7, 1 H, H-1), 5.18–4.83 (m, 1 H, H-2), 5.10–5.05 (m, $J_{H,P}$ 7.4, 4 H, ArCH_2OP), 4.80–4.73 (m, 6 H, ArCH_2), 4.12 (ddd, $J_{2,3} = J_{3,F} = 6.6$, $J_{3,4} < 1$, 1 H, H-3), 4.02 (dd, $J_{4,5}$ 2.9, 1 H, H-4), 3.95 (dt, 1 H, H-5, $J_{5,6}$ 10.3) and 3.60–3.40 (m, 2 H, H_2-6); δ_C(75 MHz; $CDCl_3$) 139.1–138.6 (ArC quaternary), 128.6–127.8 (ArCH), 96.3 (C-1, $J_{C-1,P}$ 5.7, $J_{C-1,F}$ 23.18), 88.5 (C-2, $J_{C-4,F}$ 188.8, $J_{C-2,P}$ 9.0), 76.4 (C-3, $J_{C-3,F}$ 17.5), 74.9 (C-4, $J_{C-3,F}$ 7.9), 72.0 (C-5), 75.2, 73.5 and 73.0 (ArCH_2), 69.4–68.1 (ArCH_2OP, $J_{C,P}$ 5.1) and 68.1 (C-6); δ_F(282 MHz; $CDCl_3$) −206.7 (ddd, $J_{2,F}$ 49.6, $J_{3,F}$ 6.6, $J_{5,F} < 1$); δ_P(121 MHz; $CDCl_3$) −2.0 (^{1}H decoupled); FAB *m/z* 735 ([M + Na]$^+$) (Found: M$^+$, 735.2482. $C_{41}H_{42}FNaO_8P$ requires *m/z* 735.2499).

Bis(triethylammonium) 2-deoxy-2-fluoro-α-D-galactopyranosyl phosphate 10

Compound **9** (120 mg, 0.17 mmol) was dissolved in a solvent mixture of ethyl acetate–propan-2-ol–water (10 ml, 4:6:2 v/v/v), and sodium acetate (78 mg, 0.95 mmol) was added. 10% Pd/C (100 mg) was added and hydrogen was bubbled through the suspension for 24 h. The solution was filtered, then concentrated to dryness *in vacuo*, the residue was re-dissolved in methanol (10 ml), and the solution was treated with Dowex 1-X8[Et_3NH^+] and concentrated to dryness *in vacuo*. Purification of the residue on a Sephadex LH-20 column (100 g; eluent MeOH–water, 4:1 v/v) yielded compound **10** (77 mg, 99%) as a solid, δ_H(300 MHz; D_2O) 5.61 (dd, $J_{1,P}$ 7.72, $J_{1,2}$ 3.7, 1 H, H-1), 4.76–4.74 (m, 1 H, H-4), 4.58 (ddd, $J_{2,F}$ 49.6, $J_{2,3}$ 9.6, 1 H, H-2), 4.15–3.95 (m, 2 H, H-3 and -5), 3.65–3.60 (m, 2 H, H_2-6), 3.13 (q, *J* 5.9, 12 H, CH_2CH$_3$) and 1.22 (t, 18 H, CH$_3$CH$_2$); δ_C(75 MHz; D_2O) 95.7 (C-1, $J_{C-1,P}$ 5.7, $J_{C-1,F}$ 22.6), 91.5 (C-2, $J_{C-2,F}$ 178.0, $J_{C-2,P}$ 9.0), 70.8 (C-3, $J_{C-3,F}$ 17.5), 72.9 (C-4, $J_{C-4,F}$ 8.5), 69.8 (C-5) and 56.7 (C-6); δ_P(121 MHz; $CDCl_3$) −0.75 (^{1}H decoupled).

Diammonium 2-deoxy-2-fluoro-α-D-galactopyranosyl uridin-5′-yl diphosphate I

Compound **10** (77 mg, 0.16 mmol) and uridine 5′-monomorpholidophosphate (100 mg, 0.15 mmol) were separately dried by co-evaporation from pyridine twice (2 × 5 ml). They were then dissolved in pyridine (5 ml each) and the solutions combined. The resulting reaction mixture was stirred under argon for 5 days before being concentrated to dryness *in vacuo* and the resulting syrup was diluted with water (10 ml), applied to a column of Dowex 1-X8[HCO_3^-] (1 × 12 cm) and eluted with a linear gradient of aq. NH_4HCO_3 (0–1 M). The appropriate fractions were combined and lyophilised to yield title compound **I** (82 mg, 82%) as a solid, δ_H(300 MHz; D_2O) 7.89 (d, $J_{5,6}$ 8.1, 1 H, U:H-6), 5.91–5.85 (m, 2 H, rib:H-1′, U:H-5), 5.75 (dd, $J_{1,P}$ 7.4, $J_{1,2}$ 3.7, $J_{1,F} < 0.5$, 1 H, gal:H-1), 4.65 (ddd, $J_{2,F}$ 45, $J_{2,3}$ 9.2, 1 H, gal:H-2), 4.38–3.77 (m, 8 H, gal, H-3, -4, -5 and rib-H-2′, -H-3′, -H-4′ and -H_2-5′) and 3.72–3.65 (m, 2 H, H_2-6); δ_C(75 MHz; D_2O) 169.9 (U:C-2), 155.4 (U:C-4), 145.2 (U:C-6), 105.9 (U:C-5), 96.0 (gal:C-1, $J_{C-1,P}$ 5.7, $J_{C-1,F}$ 22.0), 91.7 (rib:C-1′), 91.3 (gal:C-2, $J_{C-2,F}$ 185.9, $J_{C-2,P}$ 7.4), 86.4 (rib:C-4′, $J_{C-4',P}$ 10.2), 76.9 and 72.8 (rib:C-2′, -3′), 75.0 (gal:C-5), 70.7 (gal:C-3, $J_{C-3,F}$ 17.0), 68.0 (gal:C-6) and 63.8 (rib:C-5′, $J_{C-5',P}$ 5.9); δ_P(121 MHz; $CDCl_3$) −10.6 and −12.2 (2 m) (^{1}H decoupled); MALDI-TOF *m/z* 567 [M]$^-$.

(*R/S*)-But-3-en-2-yl 4,6-*O*-benzylidene-β-D-glucopyranoside 12

Potassium *tert*-butoxide (0.56 g, 5.00 mmol) was added to a stirred solution of (*R/S*)-but-3-en-2-yl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **11** (6.00 g, 14.93 mmol) in dry methanol (20 ml), and the resulting reaction mixture was stirred at room temperature, under argon. After 3.5 h, TLC analysis (acetone–DCM, 1:19 v/v) showed the complete conversion of the starting material (R_f 0.47) into an intermediate (R_f 0.00). The reaction mixture was neutralised with Dowex 1-X8[H^+] and, after filtration, concentrated to dryness *in vacuo* to yield a solid. The intermediate product was dissolved in acetonitrile (10 ml), and benzaldehyde dimethyl acetal (2.40 ml, 15.78 mmol) was added followed by a catalytic amount of CSA (~5 mg). After stirring of the mixture for 2 h under argon, TLC analysis (methanol–DCM, 1:9 v/v) showed complete conversion of the starting material (R_f 0.05) into a product (R_f 0.32). The reaction was quenched by the addition of pyridine (0.30 ml) and the resulting solution was concentrated to dryness *in vacuo*. The resulting yellow oil was re-dissolved in ethyl acetate (30 ml) and the solution was washed successively with saturated aq. sodium hydrogen carbonate (2 × 10 ml) and brine (2 × 10 ml). The organic phase was dried ($MgSO_4$), and concentrated to dryness *in vacuo* to yield a yellow oil. Purification using flash column chromatography (100 g SiO_2; eluent ethyl acetate–light petroleum, 3:7 v/v) and concentration of the appropriate fractions yielded *compound* **12** (4.08 g, 85%) as a solid, δ_H(300 MHz; $CDCl_3$) 7.53–7.26 (m, 5 H, ArH), 5.98–5.15 (m, 1 H, C*H*=CH$_2$), 5.52 (s, 1 H, ArC*H*O$_2$), 5.32–5.11 (m, 2 H, CH=CH_2), 4.47 (d, $J_{1,2}$ 8, 1 H, H-1), 4.42–4.27 (m, 2 H, H-4, C*H*CH$_3$), 3.87–3.53 (m, 2 H, H-2 and -3), 3.61–3.29 (m, 3 H, H-5, H_2-6) and 1.38–1.30 (2 d, *J* 6, 3 H, CHCH_3); δ_C(75 MHz; $CDCl_3$) 139.7 and 138.6 (*C*H=CH$_2$), 137.0 (ArC quaternary), 129.3–126.3 (ArCH), 117.7, 115.5 (*C*H$_2$=CH), 101.9 (Ar*C*HO$_2$), 101.4 and 100.0 (C-1), 80.6–66.3 (*C*HCH$_3$, C-2, -3, -4 and -5), 68.7 (C-6) and 21.6 and 20.2 (CH*C*H$_3$); FAB *m/z* 345 ([M + Na]$^+$) (Found: C, 63.33, H, 6.79. $C_{17}H_{22}O_6$ requires C, 63.34; H, 6.88%).

(*R/S*)-But-3-en-2-yl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside 13

Sodium hydride (1.00 g; 60% dispersion in oil; 24.8 mmol) was added to a cooled solution (0 °C) of compound **12** (3.06 g, 9.50 mmol) in DMF (20 ml). Benzyl bromide (2.43 ml, 20.45 mmol) was added and after stirring of the mixture under argon for 3 h, TLC analysis (acetone–DCM, 1:49 v/v) showed the complete conversion of starting material (R_f 0.00) into product (R_f 0.48). Methanol (30 ml) was added to the reaction mixture and stirring was continued for a further 30 min. The mixture was concentrated to dryness *in vacuo*, the resulting solid was dissolved in diethyl ether (50 ml), and the solution was washed with brine (2 × 50 ml). The organic phase was dried ($MgSO_4$), and concentrated to dryness *in vacuo* to yield a yellow oil. Purification using flash chromatography (100 g SiO_2; eluent ethyl acetate–light petroleum, 1:4 v/v) and concentration of the appropriate fractions yielded *compound* **13** (4.76 g, 99%) as a solid, δ_H(300 MHz; $CDCl_3$) 7.52–7.20 (m, 15 H, ArH), 6.00–5.67 (m, 1 H, C*H*=CH$_2$), 5.56 (s, 1 H, ArC*H*O$_2$), 5.29–5.10 (m, 2 H, CH=CH_2), 4.95–4.74 (m, 4 H, ArCH_2), 4.61–4.54 (2 d, $J_{1,2}$ 8, 1 H, H-1), 4.39–4.26 (m, 2 H, H-4 and C*H*CH$_3$), 3.83–3.64 (m, 3 H, H-3 and H_2-6), 3.49 (dd, $J_{2,3}$ 8, 1 H, H-2), 3.41–3.32 (m, 1 H, H-5) and 1.37–1.32 (2 d, *J* 6, 3 H, CHCH_3); δ_C(75 MHz; $CDCl_3$) 142.8 and 140.0 (*C*H=CH$_2$), 138.3–137.3 (ArC quaternary), 128.0–126.0 (ArCH), 117.3 and 115.3 (*C*H$_2$=CH), 100.8 (Ar*C*HO$_2$), 101.1 and 102.2 (C-1), 82.2–81.1 (*C*HCH$_3$, C-2, -3, -4 and -5), 77.4–68.8 (ArCH_2), 66.0 (C-6) and 21.6 and 20.4 (CH*C*H$_3$); FAB *m/z* 525 ([M + Na]$^+$) (Found: C, 73.93; H, 6.69. $C_{31}H_{34}O_6$ requires C, 74.07; H, 6.82%).

(*R/S*)-But-3-en-2-yl 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside 14

Compound **13** (4.75 g, 9.46 mmol) was dissolved in DCM (30 ml) and the solution was cooled (−20 °C) and stirred under argon. Subsequently, triethylsilane (7.80 ml, 47.3 mmol) and TFA (3.80 ml, 47.3 mmol) were added dropwise. The reaction mixture was allowed to warm to room temperature and stirring was continued for 2 h. TLC analysis (acetone–DCM, 1:49 v/v) showed the complete conversion of starting material (R_f 0.81) into product (R_f 0.45). Ethyl acetate (50 ml) was added to the reaction mixture and the resulting solution was washed succes-

sively with saturated aq. sodium hydrogen carbonate (2 × 10 ml) and brine (2 × 10 ml). The organic phase was dried ($MgSO_4$), and concentrated to dryness *in vacuo* to yield a sticky yellow solid. Purification using flash chromatography (100 g SiO_2; eluent ethyl acetate–light petroleum, 1:3 v/v) and concentration of the appropriate fractions yielded *compound* **14** (3.37 g, 70%) as a solid, δ_H(300 MHz; $CDCl_3$) 7.38–7.21 (m, 15 H, ArH), 6.00–5.67 (m, 1 H, C*H*=CH_2), 5.27–5.04 (m, 2 H, CH=C*H*$_2$), 4.98–4.47 (m, 7 H, H-1 and ArC*H*$_2$), 4.42–4.26 (m, 1 H, C*H*CH_3), 3.78–3.33 (m, 6 H, H-2, -3, -4, -5 and H_2-6) and 1.37–1.31 (2 d, *J* 6, 3 H, CHC*H*$_3$); δ_H(75 MHz; $CDCl_3$) 140.3 and 139.1 (*C*H=CH_2), 138.7–138.0 (ArC quaternary), 128.6–127.7 (ArCH), 117.2 and 115.0 (*C*H$_2$=CH), 101.9 and 100.5 (C-1), 84.3–71.8 (*C*HCH_3, C-2, -3, -4 and -5), 75.3–73.6 (Ar*C*H$_2$), 70.4 (C-6) and 21.6 and 20.4 (CH*C*H$_3$); FAB *m/z* 527 ($[M + Na]^+$) (Found: C, 73.72, H, 7.15. $C_{31}H_{36}O_6$ requires C, 73.78; H, 7.19%).

(*R/S*)-But-3-en-2-yl 2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-β-D-galactopyranoside 16

To a solution of compound **14** (2.50 g, 4.96 mmol) in DCM (20 ml) was added pyridine (3 ml). The resulting solution was cooled (−78 °C) and stirred under argon, and Tf_2O (0.74 ml, 4.96 mmol) was added. The reaction mixture was allowed to warm to room temperature and, after 40 min, TLC analysis (ethyl acetate–cyclohexane, 3:7 v/v) showed complete conversion of starting material (R_f 0.66) into compound **15** (R_f 0.88). The reaction mixture was diluted with DCM (50 ml) and was washed successively with saturated aq. sodium hydrogen carbonate (2 × 10 ml) and brine (2 × 10 ml). The organic phase was dried ($MgSO_4$), and concentrated to dryness *in vacuo* to yield compound **15** as a sticky yellow solid. Compound **15** was immediately dissolved in THF (20 ml) stirred under argon, and TBAF solution (1.1 M in THF, dried over molecular sieves 4 Å; 4.5 ml, 4.95 mmol) was added. A colour change from yellow to deep red was observed and immediate TLC analysis (ethyl acetate–cyclohexane, 3:7 v/v) showed complete conversion of the intermediate (R_f 0.88) into a major product (R_f 0.63). The reaction mixture was concentrated to dryness *in vacuo*, the resulting brown solid was dissolved in DCM (50 ml), and the solution was washed with brine (2 × 10 ml). The organic phase was dried ($MgSO_4$), and concentrated to dryness *in vacuo* to yield a sticky yellow solid. Purification using flash chromatography (100 g SiO_2; eluent ethyl acetate–cyclohexane, 1:9 v/v) and concentration of the appropriate fractions yielded *compound* **16** (1.75 g, 70%) as a solid, δ_H(250 MHz; $CDCl_3$) 7.42–7.22 (m, 15 H, ArH), 6.02–5.63 (m, 1 H, C*H*=CH_2), 5.24–5.02 (m, 2 H, CH=C*H*$_2$), 4.98–4.47 (m, 7 H, H-1 and ArC*H*$_2$), 4.86 (ddd, $J_{3,4}$ 4, $J_{4,5}$ < 0.5, $J_{4,F}$ 48, 1 H, H-4), 4.48–4.42 (2 d, $J_{1,2}$ 8, H-1), 4.42–4.23 (m, 1 H, C*H*CH_3), 3.79–3.37 (m, 5 H, H-2, -3, -5 and H_2-6) and 1.36–1.21 (2 d, *J* 6, 3 H, CHC*H*$_3$); δ_C(63 MHz; $CDCl_3$) 140.2 and 139.0 (*C*H=CH_2), 138.4–137.7 (ArC quaternary), 128.4–127.7 (ArCH), 117.0 and 114.4 (*C*H$_2$=CH), 101.7 and 100.2 (C-1), 86.0 (2 d, $J_{C\text{-}4,F}$ 183, C-4), 79.0 (d, $J_{C\text{-}3,F}$ 19, C-3), 78.9 (*C*HCH_3), 77.2 (C-2), 72.1 (d, $J_{C\text{-}5,F}$ 18, C-5), 67.7 (C-6), 75.3–72.2 (Ar*C*H$_2$) and 21.8 and 20.4 (CH*C*H$_3$); CI *m/z* 524 ($[M + NH_4]^+$) (Found: C, 73.80; H, 6.99. $C_{31}H_{35}FO_5$ requires C, 73.50; H, 6.96%).

(*E/Z*)-But-2-en-2-yl 2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-β-D-galactopyranoside 17

Butyllithium (1.5 M in THF; 1 ml, 1.5 mmol) was added to a stirred, orange solution of Wilkinson's catalyst (500 mg, 0.50 mmol) in THF (1 ml) which was placed under argon. The resulting brown solution was stirred at 20 °C for 5 min and was then transferred *via* syringe into a refluxing solution of compound **16** (1.66 g, 3.28 mmol) in THF (10 ml) under argon. TLC analysis (acetone–DCM, 1:49 v/v) after 30 min showed complete conversion of starting material (R_f 0.71) into product (R_f 0.79). The reaction mixture was diluted with DCM (20 ml) and concentrated to afford an orange-red residue. The residue was purified by flash chromatography (20 g SiO_2; eluent DCM) to afford the required compound **17** (1.23 g, 74%) as a solid, δ_H(250 MHz; $CDCl_3$) 7.42–7.10 (m, 15 H, ArH), 4.98–4.48 (m, 9 H, H-1, -4, ArC*H*$_2$, C=CH), 3.89–3.42 (m, 5 H, H-2, -3, -5 and H_2-6), 1.92–1.82 (m, 3 H, C*H*$_3$C=CH) and 1.67–1.49 (m, 3 H, C*H*$_3$CH); δ_C(63 MHz; $CDCl_3$) 151.5 and 149.1 [O*C*(CH_3)=C], 138.4–137.8 (ArC quaternary), 128.4–127.8 (ArCH), 105.4 and 100.1 (=*C*HCH_3), 100.3 and 98.1 (C-1), 85.8 (d, $J_{C\text{-}4,F}$ 183, C-4), 78.9 (d, $J_{C\text{-}3,F}$ 18, C-3), 78.9 (*C*HCH_3), 77.2 (C-2), 72.2 (d, $J_{C\text{-}5,F}$ 22, C-5), 72.0 (C-6), 75.5–73.2 (Ar*C*H$_2$), 18.6 and 15.6 (*C*H$_3$C=CH) and 11.9 and 10.3 (CH*C*H$_3$); δ_F(282 MHz; $CDCl_3$) −194.9 (2 ddd, $J_{4,F}$ 48, $J_{3,F}$ 28, $J_{5,F}$ 20); CI *m/z* 524 ($[M + NH_4]^+$) (Found: C, 73.52; H, 7.01 $C_{31}H_{35}FO_5$ requires C, 73.49; H, 6.96%).

Dibenzyl 2,3,6-tri-*O*-benzyl-4-deoxy-4-fluoro-α-D-galactopyranosyl phosphate 18

A mixture of vinyl glycoside **17** (125 mg, 0.22 mmol) in DCM (1 ml) and molecular sieves (100 mg; 4 Å powdered) was stirred for 1 h. In a separate reaction vessel, NIS (200 mg, 0.88 mmol) was suspended in DCM (2 ml) by sonication. Dibenzyl hydrogen phosphate (225 mg, 0.81 mmol) was added to the suspension and the mixture was allowed to equilibrate under argon for 5 min. TMSOTf (18.7 μl, 0.094 mmol) was then added and the suspension was stirred for a further 30 s. The resulting suspension (1.1 ml) was then added by syringe to the ready prepared solution of the vinyl glycoside **17**. Immediate TLC analysis (acetone–DCM, 1:99 v/v) of the reaction mixture showed the conversion of the starting material (R_f 0.79) into a major product (R_f 0.50). The reaction mixture was neutralised by the addition of triethylamine and diluted with ethyl acetate (20 ml). The resulting mixture was filtered, and washed successively with aq. sodium metabisulfite (2 × 5 ml) and brine (2 × 5 ml). The organic phase was dried ($MgSO_4$), concentrated to dryness *in vacuo*, and the resulting oil was purified using flash chromatography (25 g SiO_2; eluent acetone–DCM, 1:49 v/v). Concentration of the appropriate fractions yielded *compound* **18** (89 mg, 54%) as a clear oil, δ_H(300 MHz; $CDCl_3$) 7.60–7.03 (m, 25 H, ArH), 5.95 (dd, $J_{1,P}$ 6.6, $J_{1,2}$ 3.3, 1 H, H-1), 5.11–4.99 (m, $J_{H,P}$ 7.4, 4 H, ArC*H*$_2$OP), 4.89 (ddd, $J_{4,F}$ 50, $J_{3,4}$ 2.2, $J_{4,5}$ < 1, 1 H, H-4), 4.80–4.73 (m, 4 H, ArC*H*$_2$), 4.48 (s, 2 H, ArC*H*$_2$), 4.09 (dt, $J_{5,F}$ 29.4, $J_{5,6}$ 6.6, 1 H, H-5), 3.99 (ddd, $J_{2,3}$ 9.6, $J_{2,P}$ 2.6, 1 H, H-2), 3.83 (ddd, $J_{3,F}$ 27.6, 1 H, H-3) and 3.69–3.47 (m, 2 H, H_2-6); δ_C(75 MHz; $CDCl_3$) 137.8–135.8 (ArC quaternary), 128.5–127.8 (ArCH), 96.2 (C-1, $J_{C\text{-}1,P}$ 6.2), 86.8 (C-4, $J_{C\text{-}4,F}$ 183.4), 75.1 (C-3, $J_{C\text{-}3,F}$ 18.0), 75.1 (C-2, $J_{C\text{-}2,P}$ 8.5), 70.1 (C-5, $J_{C\text{-}5,F}$ 18.1), 73.7, 73.6 and 72.5 (Ar*C*H$_2$), 69.4–69.2 (Ar*C*H$_2$OP, J_{C-P} 5.8 and 5.1) and 67.5 (C-6, $J_{C\text{-}6,F}$ 5.7); δ_F(282 MHz; $CDCl_3$) −218.6 (ddd, $J_{4,F}$ 48, $J_{3,F}$ 29.3, $J_{5,F}$ 27.6); δ_P(121 MHz; $CDCl_3$) −1.8 (^{1}H decoupled); FAB *m/z* 735 ($[M + Na]^+$) (Found: $[M + Na]^+$, 735.2482. $C_{41}H_{42}FNaO_8P$ requires *m/z*, 735.2499).

Bis(triethylammonium) 4-deoxy-4-fluoro-α-D-galactopyranosyl phosphate 19

Compound **18** (170 mg, 0.24 mmol) was dissolved in a mixture of ethyl acetate–propan-2-ol–water (10 ml; 4:6:2 v/v/v), and sodium acetate (78 mg, 0.95 mmol) was added. 10% Pd/C (100 mg) was added and hydrogen was bubbled through the suspension for 24 h. The solution was filtered, then concentrated to dryness *in vacuo*, the residue was re-dissolved in methanol (10 ml) and treated with Dowex 1-X8[Et_3NH^+], and the mixture was concentrated to dryness *in vacuo*. Purification on a Sephadex LH-20 column (100 g; eluent MeOH–water, 4:1 v/v) yielded compound **19** (98 mg, 90%) as a solid, δ_H(300 MHz; D_2O) 5.51 (dd, $J_{1,P}$ 6,8, $J_{1,2}$ 3.7, 1 H, H-1), 4.89 (ddd, $J_{4,F}$ 50, $J_{3,4}$ 2.2, $J_{4.5}$ < 1, 1 H, H-4), 4.15 (dt, $J_{5,F}$ 31.6, $J_{5,6}$ 5.9, 1 H, H-5), 3.97 (ddd, $J_{2,3}$ 9.6, $J_{2,P}$ 2.6, 1 H, H-2), 3.82 (ddd, $J_{3,F}$ 27.6, 1 H, H-3), 3.74–3.72 (m, 2 H, H_2-6), 3.13 (q, *J* 5.9, 12 H, C*H*$_2$CH$_3$)

and 1.22 (t, 18 H, CH_3CH_2); δ_C(75 MHz; D_2O) 97.4 (C-1, $J_{C\text{-}1,P}$ 6.2), 92.9 (C-4, $J_{C\text{-}4,F}$ 183.4), 72.8 (C-3, $J_{C\text{-}3,F}$ 18.1), 71.2 (C-2, $J_{C\text{-}2,P}$ 8.5), 70.5 (C-5, $J_{C\text{-}5,F}$ 18.1) and 62.5 (C-6); δ_P(121 MHz; $CDCl_3$) −0.22 (^{1}H decoupled).

Diammonium 4-deoxy-4-fluoro-α-D-galactopyranosyl uridin-5′-yl diphosphate II

Compound **19** (89 mg, 0.19 mmol) and uridine 5′-monomorpholidophosphate (130 mg, 0.19 mmol) were separately dried by co-evaporation from pyridine (2 × 5 ml). They were each dissolved in pyridine (10 ml) and the solutions were combined. The reaction mixture was stirred under argon for 5 days and was then concentrated to dryness *in vacuo*. The resulting syrup was diluted with water (10 ml) and applied to a column of Dowex 1-X8[HCO_2^-] (1 × 12 cm) and eluted with a linear gradient of aq. NH_4HCO_3 (0–1 M). The appropriate fractions were combined and lyophilised to yield compound **II** (69 mg, 60%) as a solid, δ_H(300 MHz; D_2O) 7.91 (d, 1 H, U : H-6, $J_{5,6}$ 8.1), 5.95–5.91 (m, 2 H, rib : H-1′, U : H-5), 5.63 (dd, $J_{1,P}$ 7.0, $J_{1,2}$ 3.3, 1 H, gal : H-1), 4.88 (ddd, $J_{4,F}$ 50, $J_{3,4}$ 2.2, $J_{4.5}$ < 1, 1 H, gal : H-4), 4.05 (dt, $J_{5,F}$ 31.6, $J_{5,6}$ 5.9, 1 H, gal : H-5), 3.82 (ddd, $J_{3,F}$ 27.6, 1 H, gal : H-3), 4.38–3.77 (m, 6 H, gal : H-2, rib : H-2′, -3′, -4′ and H_2-5′) and 3.80–3.70 (m, 2 H, H_2-6); δ_C(75 MHz; D_2O) 169.1 (U, C-2), 154.7 (U, C-4), 144.3 (U, C-6), 105.4 (U, C-5), 98.3 (gal : C-1, $J_{C\text{-}1,P}$ 7.3), 92.9 (gal : C-4, $J_{C\text{-}4,F}$ 183.4), 91.2 (rib : C-1′), 85.8 (rib : C-4′, $J_{C\text{-}4',P}$ 8.5), 76.5 and 72.3 (rib : C-2′ and -3′), 73.3 (gal : C-3, $J_{C\text{-}3,F}$ 18.1), 71.0 (gal: C-2, $J_{C\text{-}2,P}$ 8.5), 70.7 (gal : C-5, $J_{C\text{-}5,F}$ 18.1), 67.6 (gal : C-6) and 62.5 (rib : C-5′, $J_{C\text{-}5',P}$ 5.9); δ_P(121 MHz; $CDCl_3$) −10.3 and 11.9 (2 d, $J_{P,P}$ 19.4) (^{1}H decoupled); MALDI-TOF *m/z* 566 $[M]^-$.

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