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A New Methodology for the Synthesis of Fluorinated *exo*-Glycals and Their Time-Dependent Inhibition of UDP-Galactopyranose Mutase

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Abstract: Fluorinated carbohydrates constitute a very important class of mechanistic probes for glycosyl-processing enzymes. In this study, we describe the first synthesis of fluorinated and phosphonylated *exo*-glycals and their corresponding nucleotide sugars in the galactofuranose series. The synthetic protocol that we have developed is a Selectfluor-mediated fluorination/elimination sequence on phosphonylated *exo*-glycals, and it offers a new entry into fluorinated carbohydrate chemistry. The challenging *E*/*Z* stereochemical assignment of the resulting tetrasubstituted alkenes, which bear an alkoxy, an alkyl, a fluoro, and a phosphonyl group, has been achieved through NMR experiments. The corresponding (*E*)- and (*Z*)-nucleotide fluorosugars have been prepared and tested as inhibitors of UDP-galactopyranose mutase (UGM). UGM is a flavoenzyme that catalyzes the isomerization of uridine diphosphate(UDP)-galactopyranose into UDP-galactofuranose, a key step of the biosynthesis of important mycobacterial cell-wall glycoconjugates. The two diastereomeric molecules were found to display time-dependent inactivation of UGM, as expected from preliminary results using non-fluorinated *exo*-glycal nucleotides. The inhibitory properties of the two fluorinated molecules led us to suggest that the inactivation mechanism proceeds through two-electron processes, despite the presence of the flavin cofactor within the UGM catalytic site.

Keywords: glycals • inhibitors • mechanistic probes • nucleotides • Selectfluor

Introduction

The biosynthesis of poly-galactofuranose (Gal*f*) glycoconjugates has recently received particular and increasing attention due to their essential role in maintaining the integrity of mycobacteria.[1–7] From the biocatalytic viewpoint, the quest to unravel the biogenesis of galactofuranose (Gal*f*) led to the discovery of a unique enzymatic ring-contraction/expansion, namely the interconversion between uridine diphosphate(UDP)-galacto*pyranose* and the corresponding *furanose*, which is catalyzed by UDP-galactopyranose mutase (UGM or Glf). UGM is a flavoprotein but, unexpectedly, no redox process has been evidenced to date.[8–10] Several mechanisms have been proposed to describe this intriguing reaction: the involvement of a 1,4-anhydro-galactose as a low-energy intermediate,[11] the formation of an anomeric radical after a single-electron transfer from the reduced flavin to an intermediate oxycarbenium species,[8] and the formation of a covalent adduct between the enzyme[12] (or its cofactor)[9,13] and the substrate. In a previous study, we described the synthesis and binding properties of a constrained analogue of UDP-galactose locked in a $^{1,4}B$ boat conformation, to probe the conformational itinerary of the galactose residue during its enzymatic interconversion.[14] Recently, a mechanistic study using radiolabeled UDP-galactose and aimed at trapping a covalent intermediate led to the characterization of an adduct between the flavin cofactor and the galactose residue.[13] Whereas a stepwise mechanism involving both an SET[8] and the formation a covalent adduct[9,12,13] may be considered, it was concluded from the recently resolved three-dimensional structure of reduced UGM that, given the mode of bending of the flavin cofactor upon reduction, the formation of a covalent intermediate and the galactose anomeric position seemed unlikely.[15] Nevertheless, time-dependent inactivation of UGM has been observed by Liu et al. with the fluorinated UDP-Gal*f* analogues **1** and **2**[16] and by ourselves using UDP-*exo*-glycal

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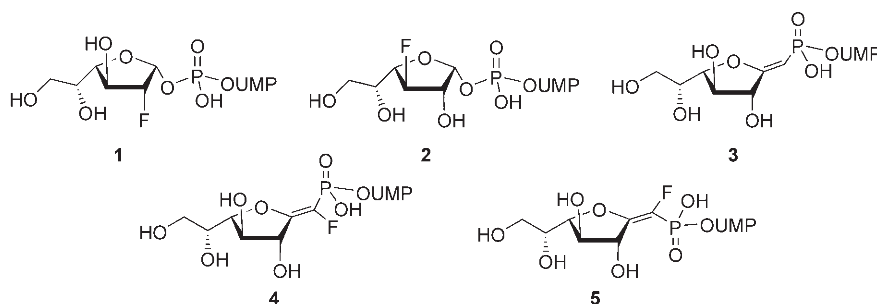
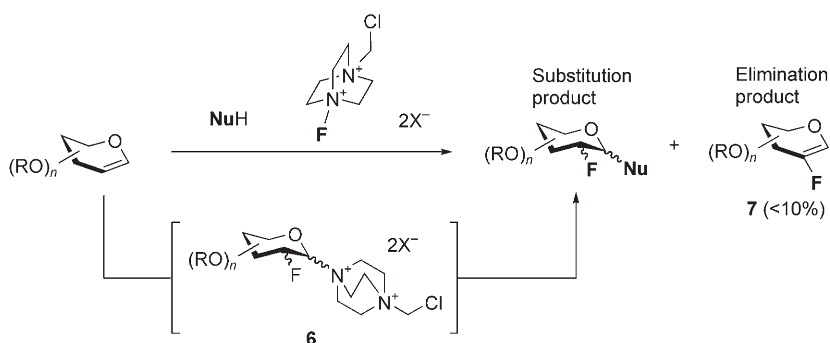
3.[17] Although these results were only observed when UGM was used in its native (oxidized) state, they strongly suggested that a covalent bond was transiently formed between the substrate and a catalytic nucleophile such as the flavin itself.



In view of the interesting properties of *exo*-glycal **3**, we pursued our investigation by designing a new generation of UDP-*exo*-glycals that would behave as inactivators of UGM and would clarify the inactivation mechanism of UGM by *exo*-glycals. Thus, the fluorinated *exo*-glycals **4** and **5** were naturally envisaged, considering the unique stabilizing properties of the fluorine atom. In mechanistic studies of retaining glycosidases, 2-fluoro-glycosides now constitute key tools to evidence a transient covalent process as well as to identify the catalytic nucleophiles.[18] Unfortunately, this approach (molecules **1** and **2**) did not allow the characterization of a covalent adduct with UGM by means of mass spectrometry.[16] From a more general perspective, since fluorinated substrate analogues constitute a very important class of probes for the elucidation of enzyme mechanisms,[19] and *exo*-glycal nucleotides derived from neuraminic acid display excellent inhibition properties against sialyl transferases,[20] we decided to explore the chemistry of *exo*-glycal fluorination. In addition, developing new techniques for the fluorination of *exo*-glycals might lead to applications in inhibition/inactivation studies of numerous glycosyl-processing enzymes. Herein, we describe a new methodology leading to the synthesis of fluorinated nucleotide sugars **4** and **5**, their stereochemical assignment, and their inhibition properties against UGM.

Results and Discussion

A survey of the literature describing the synthesis of fluorinated enol ethers did not lead us to find a general method for preparing tetrasubstituted alkenes bearing a fluoro, a phosphonyl, an alkoxy, and an alkyl group. Moreover, to the best of our knowledge, this kind of functionality has not yet been explored in enzyme inhibition or inactivation studies. On the other hand, the past few years have seen a great development in the field of carbohydrate fluorinations, thanks to the development of a new generation of fluorinating reagents. For instance, the fluorination of *endo*-glycals by Selectfluor is noteworthy.[21,22] This reagent appeared to be the best fluorinating molecule for the preparation of 2-fluoroglycosides functionalized at the anomeric position with a broad range of substituents (see Scheme 1).[21] The mechanism of this electrophilic addition/nucleophilic substitution is now well understood and involves a 2-fluoro-1-trialkylammonium intermediate adduct **6**. Given that this chemistry was developed with *endo*-glycals, we reasoned that Selectfluor should also be a very good fluorinating agent for *exo*-glycals, even when phosphonylated. In fact, the only reported case in which an *endo*-glycal was transformed into a fluoro-*endo*-glycal was a side reaction giving poor yields of fluoroalkene **7** (Scheme 1).[22] As a first approach, we reasoned that if the fluorination were to be performed on *exo*-glycal **8** with a base instead of a nucleophile, an addition–elimination should occur, directly yielding the desired fluorinated *exo*-glycal **9** (Scheme 2). The starting tetrasilylated *exo*-glycal **8** could be easily prepared in two steps from tetrasilylated γ-galactonolactone.[17] We thus screened the reaction conditions (solvent, temperature, several non-nucleophilic bases, etc.) to evaluate this new reaction, but the expected fluorinated *exo*-glycals **9** were never isolated when the reaction was run under basic conditions. This result might be attributed to the incompatibility of Selectfluor with the different bases that we used. Adding the base (1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), trialkylamines, *t*BuOK, Py, 2,6-di-*tert*-butylphenol (2,6-DTBP), etc.) after enol ether **8** had completely reacted with



Scheme 1. **Nu**H = ROH, RNH_2, RSH, $(RO)_2P(O){-}OH$; X = BF_4^-, TfO^-.

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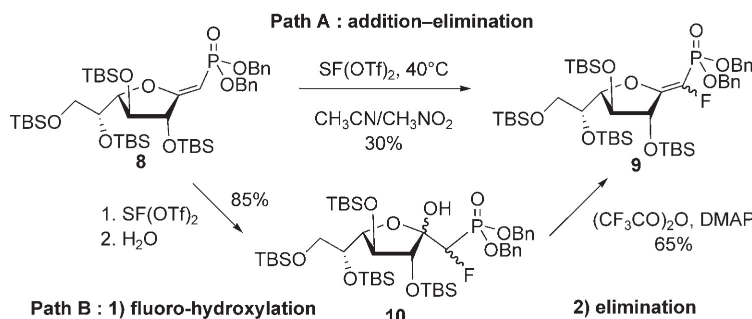

Scheme 2. TBS = *tert*-butylsilyl, DMAP = 4-dimethylaminopyridine.

Selectfluor did not give the expected molecules, but rather a complex mixture of inseparable products. Somewhat surprisingly, we finally observed the formation of the addition/elimination products **9**, in 30% overall yield, when no base was added to promote the desired elimination (reaction performed in anhydrous CH_3CN/CH_3NO_2, 1:1, at 40 °C). This result can be explained by the fact that the leaving group in intermediate **6** is a 1,4-diazabicyclo[2.2.2]octane (DABCO) derivative that can itself play the role of a base. However, the yield was only 30% and the fluorinated enol ethers were very difficult to separate from side products: under these conditions, some 40% of ketols **10** could be isolated, demonstrating that the limiting step was indeed the elimination (Scheme 2, path A).

Fluoro-hydroxylation reaction: In view of the above result, we had to envisage the preparation of the target molecules **9** through a two-step sequence. In our hands, the best procedure to produce phosphonylated *exo*-glycals proved to be the method developed by Lin et al., which involves activating an anomeric ketol by trifluoroacetylation under basic conditions, giving *exo*-glycals directly in good yields with a *Z* stereospecificity.[23–25] For this reaction, the nature of the activating reagent, generating an anomeric leaving group, is of extreme importance since other common leaving groups, such as sulfonates, do not cleanly produce *exo*-glycals. The synthesis of **9** therefore implied the two-step sequence depicted in Scheme 2 (path B): i) synthesis of a fluorinated and phosphonylated intermediate ketol **10**; ii) application of the Lin elimination procedure to generate product **9**.[23,24] Thus, we chose to perform a fluoro-hydroxylation of tetrasilylated *exo*-glycal **8**. Such a transformation has been described by Wong et al. for several *endo*-glycals but, surprisingly, applying the published procedures did not allow the generation of the desired fluoro-ketol **10** (Table 1).[26,27] The typical reaction conditions (DMF/H_2O, CH_3CN/H_2O or CH_3NO_2/H_2O; excess Selectfluor) led only to a complex mixture of side products (Table 1, entries 1–5). Knowing that Selectfluor reactivity can be strongly modulated by all of the reaction parameters, especially the solvent system and the temperature,[22] we carried out an extensive screening of the reaction conditions (see Table 1). We eventually found the appropriate conditions, which gave a satisfactory 85% yield (Table 1, entry 11) after purification. Unexpectedly, the key parameter that ensured a successful and reproducible fluoro-hydroxylation was that this reaction had to be performed under strictly anhydrous conditions. Acetonitrile was found to be the solvent of choice, either pure or mixed with anhydrous nitromethane (Table 1, entries 6–12). This is in marked contrast to literature reports, which describe the same reaction with water as cosolvent. Moreover, nitromethane had been described as the ideal solvent for electrophilic fluorination/nucleophilic substitution of *endo*-glycals.[22] For the fluoro-hydroxylation of phosphonylated *exo*-glycal **8**, running the reaction in pure nitromethane led only to moderate yields of fluoro-ketol **10**, which was conta-

Table 1. Fluoro-hydroxylation of tetrasilylated *exo*-glycal **8**.

8 → **10** + (*Z*,*E*)-**9**

Entry	MS[a]	Solvent	SF[b] [equiv]	T [°C]	Time [h]	Conv.[c] [%]	**10**[d] [%]	**9**[d] [%]	Side product[f] [%]
1	–	DMF/H_2O	4	RT	36	100[e]	0	0	100
2	–	DMF/H_2O	4	50	12	100[e]	0	0	100
3	–	CH_3NO_2/H_2O	4	RT	48	100[e]	0	0	100
4	–	CH_3CN/H_2O	1.3	RT	48	100[e]	0	0	100
5	–	CH_3CN/H_2O	1.3	50	12	100[e]	0	0	100
6	4 Å	CH_3NO_2	3	RT	72	50	20[f]	0	30
7	4 Å	CH_3NO_2	3	40	12	80	48[f]	10	12
8	4 Å	CH_3NO_2	6	40	12	85	50[f]	10	25
9	4 Å	CH_3NO_2	10	40	12	88	43[f]	7	38
10	4 Å	CH_3CN	3	30	48	93	82	4	7
11	4 Å	CH_3CN/CH_3NO_2[g]	3	30	12	90	85	5	0
12	4 Å	CH_3CN/CH_3NO_2[g]	3	40	24	93	62	16	7
13	–	CH_3CN/CH_3NO_2[g]	3	40	24	–[d]	0	0	100

[a] Molecular sieves. [b] Selectfluor (TfO^- form). [c] Determined by ^{31}P NMR of the crude reaction mixture. [d] Yield of isolated product. [e] The starting material was totally decomposed. [f] Ketols **10** were inseparable. [g] 1:1 (v/v). [h] Detected by ^{31}P and ^{19}F NMR spectroscopy.

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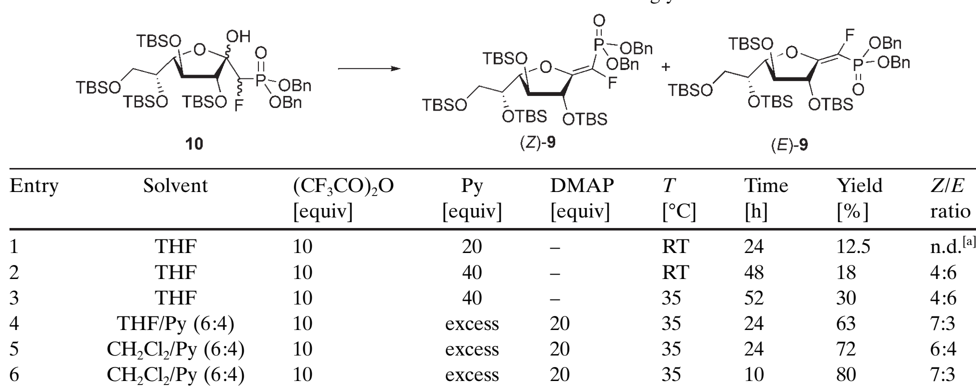
minated with inseparable side products (Table 1, entries 6–9). When the reaction was monitored by ^{19}F NMR spectrometry, we did not observe the characteristic signals of the intermediate adduct **6**, even though such an adduct has been purified and characterized with other carbohydrates.[22] This probably signifies that this intermediate is rather unstable in this case. Surprisingly, after filtering off the required molecular sieves, the best yields were not obtained by addition of aqueous solutions, but rather by diluting the crude reaction mixture with dichloromethane and treating it with silica gel. Fluoro-ketol **10** was isolated as a mixture of two diastereomers in a ratio of 1:1.3.

Standard conditions[14,28] used for the preparation of *exo*-glycal **6** were applied to generate the fluorinated molecules **9**, but the reaction proved very sluggish (Table 2, entries 1–

Table 2. Conversion of the fluoro-ketol **10** to the desired fluorinated *exo*-glycal **9**.

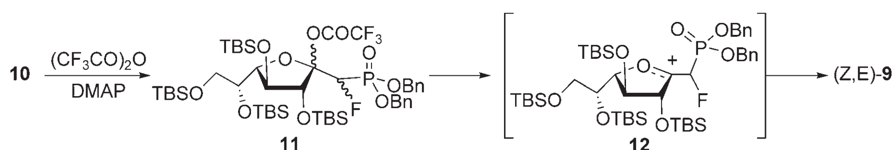


Entry	Solvent	$(CF_3CO)_2O$ [equiv]	Py [equiv]	DMAP [equiv]	*T* [°C]	Time [h]	Yield [%]	*Z*/*E* ratio
1	THF	10	20	–	RT	24	12.5	n.d.[a]
2	THF	10	40	–	RT	48	18	4:6
3	THF	10	40	–	35	52	30	4:6
4	THF/Py (6:4)	10	excess	20	35	24	63	7:3
5	CH_2Cl_2/Py (6:4)	10	excess	20	35	24	72	6:4
6	CH_2Cl_2/Py (6:4)	10	excess	20	35	10	80	7:3

[a] Not determined.

3). Although pyridine and triethylamine are the usual bases for this elimination, an excess of DMAP gave by far the best results (Table 2, entries 4–6). The fact that the reaction is much slower when applied to fluoro-ketol **10** as compared to its non-fluorinated homologue[17] is interesting from a mechanistic point of view. Indeed, it strongly suggests that this elimination involves an oxycarbenium species **12** as an intermediate (Scheme 3): the electron-withdrawing character of the fluorine atom disfavors the formation of a cationic intermediate and thus reduces the reaction rate. A competition between an E1-type and a concerted E2-type mechanism has been previously discussed by Lin et al., but was not fully demonstrated.[28] A second feature reinforces the suggestion of an E1-type stepwise mechanism: we were able to isolate intermediate **11**, whereas, to date, such quaternary anomeric trifluoroacetates have never been observed or characterized, probably because they are too labile. This shows that when the elimination is performed on fluoro-ketol **10**, the rate-determining step is the elimination of the trifluoroacetate, whereas when applied to non-fluorinated ketols,[17,23,24,28] the acylation determines the elimination kinetics. All of these observations are consistent with an E1-type mechanism, but do not rule out an E1cb mechanism. The desired *exo*-glycals **9** were obtained in 65 % yield in a *Z*/*E* ratio of 64:36.

***E*/*Z* structural assignment**: Because of the lack of ^{1}H–^{1}H coupling constants, the stereochemistry of tetrasubstituted alkenes is generally difficult to establish. Determining the configuration of *exo*-glycals **9** proved to be even more difficult since the exocyclic double bond bears three heteroelements. The stereochemistry of the starting (*Z*)-*exo*-glycal **8** was conclusively assigned on the basis of ^{1}H NMR and NOE experiments. Furthermore, given the novelty of this kind of functionality, we did not know if the coupling constants between ^{19}F, ^{31}P, and the two olefinic ^{13}C were characteristic of the stereochemistry. Moreover, we had to face another difficulty: the non-fluorinated (*E*)-*exo*-glycal (*E*)-**8** had never been synthesized since the methods for producing it are *Z* stereospecific. We reasoned that with (*E*)-*exo*-glycal (*E*)-**8** in hand, we would strengthen our assignments based on NMR methods such as NOE experiments. In a related project aimed at producing *C*-glycosidic Gal*f*-1-phosphate analogues bearing a leaving group at the anomeric position to generate potential suicide inhibitors of UGM, we fortuitously prepared and fully characterized the (*E*)-*exo*-glycal (*E*)-**8** (Scheme 4). The reaction of ketol **13** with (diethylamino)sulfurtrifluoride (DAST) under classical conditions gave the 1-fluoro-phosphonate **14** in 41 % yield. The stereochemistry at the anomeric position could be easily assigned by virtue of the characteristic ^3J coupling constant between H-2 and the anomeric fluorine atom.[29] Under these conditions, an elimination also occurred, leading to a mixture of diastereomeric *exo*-glycals **8** in a *Z*/*E* ratio of 2:1. The yield of the *E* diastereomer was poor, but sufficient to produce enough material for a full NMR characterization. Interestingly, the *E*/*Z* diastereomers were very easily separated since they displayed very different R_f values (ΔR_f >0.3) upon silica gel chromatography. The (*Z*)-*exo*-glycals **8** (with different protective groups) were always more polar than their *E* isomers. We



Scheme 3.

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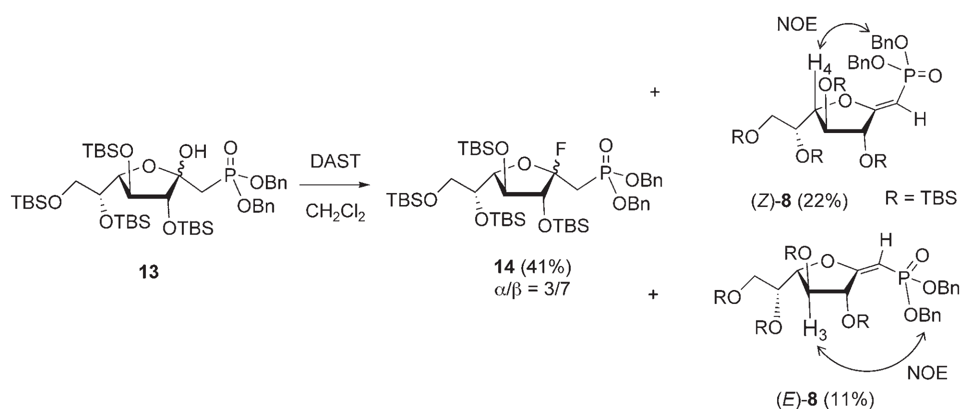


Scheme 4.

showed poor inhibition percentages (< 10% at [I] = [UDP-Galf] = 1 mM). Under non-reducing conditions, a time-dependent inactivation was observed (Figure 1), as in the case of *exo*-glycal **3**[17] or Liu's fluorinated UDP-Gal*f* analogues **1** and **2**.[16] Unfortunately, we were unable to find evidence of a covalent adduct between the inactivators and the protein, or the cofactor, by mass spectrometry. This result indicates that the still putative adduct is not sufficiently stable under mass spectrometry analysis conditions, despite the presence of a fluorine atom in the molecule. Both the (*Z*)- and (*E*)-fluorinated *exo*-glycals **4** and **5** displayed much slower inactivation kinetics compared to the non-fluorinated analogue **3**. Indeed, a complete loss of UGM residual activity was only observed after 50–70 h of incubation with the fluorinated molecules **4** and **5** (Figure 1), whereas under the same conditions UGM was inactivated within 15 h by non-fluorinated *exo*-glycal **3**.[17] Since molecules **3** and **4** are structurally very similar, they should display similar binding properties towards UGM. Therefore, the significant difference in their inhibition kinetics must originate from the inactivation mechanism itself. It is reasonable to conclude that the electron-withdrawing character of the fluorine atom present in molecule **4** is the cause of the dramatic decrease in the inactivation rate. Since captodative (or push–pull) effects stabilize radical intermediates, the presence of the fluorine atom should not decrease the rate of a radical process. On the contrary, if the inactivation mechanism is a two-electron process, such as a pro-

tentatively assigned the *Z* configuration to the slower-migrating fluorinated species **9**. This assignment was eventually confirmed by NOE experiments. Indeed, distinct NOEs were observed between the benzylic protons and H-4 of (*Z*)-**8** and (*Z*)-**9** (Scheme 4). These effects were not seen with (*E*)-**8** and (*E*)-**9**, but instead NOEs between the benzylic protons and H-3 were observed for both *E* diastereomers. The clear similarities in migratory aptitudes and NOEs constitute convincing evidence for the assignment of the *E*/*Z* stereochemistry of molecules **9**.

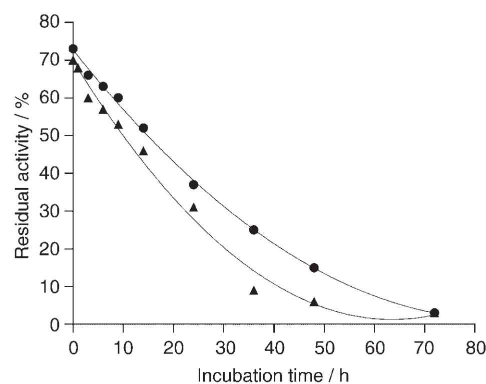
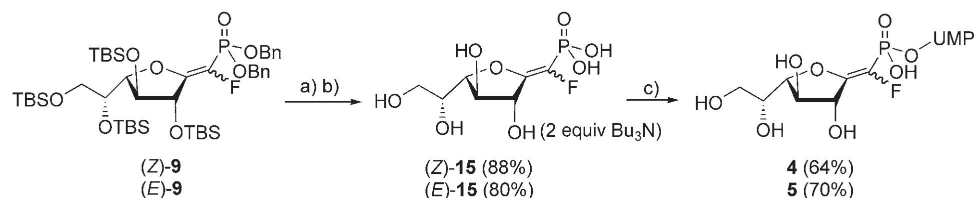


Figure 1. Time-dependent inactivation of **4** (●)and **5** (▲).

Synthesis of target nucleotide sugars: From the UGM inhibition perspective, we were satisfied by the fact that the elimination leading to *exo*-glycals **9** was not stereoselective. As a matter of fact, no efficient method has been described for the preparation of (*E*)-phosphonylated *exo*-glycals. Therefore, when we described the synthesis and the inactivation properties of (*Z*)-*exo*-glycal **3**, we were unable to study the inhibition of its *E* isomer. We now have the two fluorinated diastereomers at our disposal. As in the synthesis of UDP-(*Z*)-*exo*-glycal-Gal*f* **3**,[17] (*E*)-**9** and (*Z*)-**9** could be efficiently deprotected if the hydrogenolysis step was performed first, followed by desilylation (compounds (*E*)-**15** and (*Z*)-**15**, Scheme 5). The corresponding nucleotide sugars were then obtained in yields of 64% and 70% for molecules **4** and **5**, respectively, using the Bogachev procedure.[14,30]

UGM inactivation study: Fluorinated *exo*-glycals **4** and **5** were found to display the same type of inhibition properties as the non-fluorinated analogue **3**:[17] under reducing conditions (obtained by dithionite addition), the two molecules



Scheme 5. Reagents and conditions: a) H_2, Pd/C, Et_3N CH_2Cl_2, RT; b) nNu$_4$NF·3H_2O, THF, 0°C; c) UMP-*N*-methylimidazolium, CH_3CN, RT.

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tonation (Scheme 6), the presence of the fluorine atom should strongly alter the inactivation kinetics. It is well-established that enol ethers are readily activated by acids: their intrinsic basicity can indeed be strongly decreased if an electron-withdrawing group, such as a fluorine atom, is directly linked to the double bond (their stabilities towards acids are then improved). The decrease in basicity of the enol ether functionality in molecules **4** and **5**, compared to **3**, is likely to be the origin of the observed difference in inhibition kinetics. Therefore, the inactivation mechanism of UGM with fluorinated molecules **4** and **5** is unlikely to involve a single-electron transfer or radical chemistry (Scheme 6, pathways B and C). Instead, a protonation (Scheme 6, pathway A) can reasonably be invoked as the first step of the inactivation mechanism of UGM by *exo*-glycals.

In conclusion, we have described an efficient methodology for the synthesis of phosphonylated and fluorinated *exo*-glycals, using Selectfluor as fluorinating agent. This new reaction sequence has been exploited for the synthesis of both diastereomers of UDP-fluoro-*exo*-galactal, **4** and **5**. As expected, these two molecules display time-dependent inactivation of UDP-galactopyranose mutase. Comparison with the inhibition properties of the non-fluorinated analogue **3** has led to a clarification of the inactivation mechanism of UGM by UDP-*exo*-glycals: despite the presence of the FAD cofactor close to the galactose binding site, the mechanism of inactivation most probably proceeds through a sequence of two-electron processes. We are now investigating application of this new methodology to other carbohydrates, with a view to generating inhibitors or mechanistic probes of other glycosyl-processing enzymes.

Experimental Section

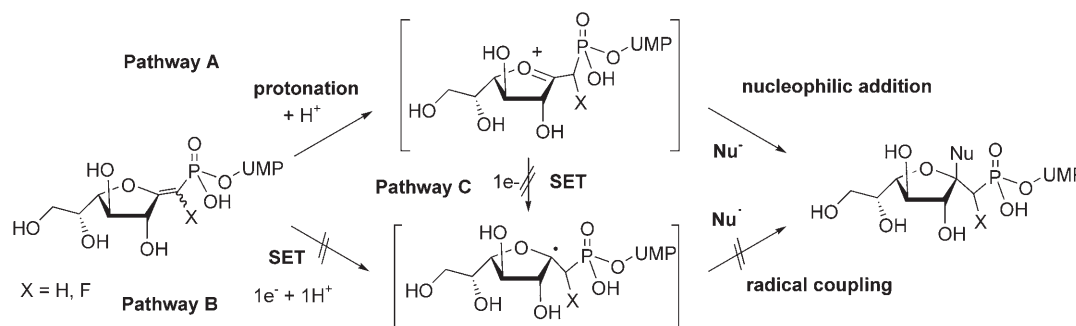
Materials and procedures: All chemicals were purchased from Sigma, Aldrich or Fluka and were used without further purification. Tetrahydrofuran, diethyl ether, and toluene were freshly distilled from sodium benzophenone, dichloromethane from P_2O_5, and acetonitrile from CaH_2. ^1H, ^{13}C, ^{19}F, and ^{31}P NMR spectra were recorded with Bruker AC-250 and AMX-400 spectrometers. All compounds were characterized by ^1H, ^{13}C, ^{19}F, and ^{31}P NMR as well as by ^1H–^1H and ^1H–^{13}C correlation experiments. Specific optical rotations were measured on a Perkin-Elmer 241 polarimeter with solutions in a 1 dm cell. Melting points were determined with a Büchi 535 apparatus. Column chromatography was performed on silica gel (Kieselgel Si 60; 40–63 μm). Size-exclusion chromatography was performed on a Sephadex G15 column (2.5×60 cm) using a Pharmacia Biotech Äkta FPLC apparatus; fractions containing uridine derivatives were detected with UV light. When required, nucleotide sugars were purified by semipreparative HPLC using a Waters Delta prep 4000 chromatography system equipped with a NovaPak C18 (1×10 cm) column (eluent: 50 mM triethylammonium acetate, pH 6.8). For enzymatic assays, a Waters 600E analytical apparatus equipped with a Zorbax C18-SB column (25×0.46 cm, 5 μm) was used (eluent: 50 mM triethylammonium acetate, pH 6.8). UDP-Gal*f* was prepared according to known procedures.[31,32]

Enzyme kinetics and inhibition assays: The conditions described by Liu et al. were followed.[16] All assays were performed using a potassium phosphate buffer (100 mM, pH 6.8) at $T = 21\,°C$. To obtain reductive conditions, freshly prepared sodium dithionite solutions were used to allow a final concentration of 20 mM. When native mutase was used (without sodium dithionite), reactions were conducted in the dark under aerobic conditions. All inhibition studies involved measuring the conversion of pure starting UDP-Gal*f* into UDP-Gal*p* (compared with a commercially available sample) by analytical HPLC (C18 column, elution with 50 mM triethylammonium acetate, pH 6.8; detection at 262 nm). Substrate and inhibitor concentrations were adjusted to 1 mM from titrated mother solutions. As a control, the relative concentrations of any species in the reaction mixtures could be determined titrimetrically using UMP as an internal standard. Enzyme concentrations were adjusted to produce a conversion of UDP-Gal*p* between 5 and 15%. Reactions were stopped by freezing the solution in liquid nitrogen. Residual enzyme activities were then measured in the presence of inhibitors at three different times, compared with the same experiment conducted without inhibitors, and the values were averaged.

Inactivation assays: Several reactors containing the enzyme (5.4 μM) and the inactivator **4** or **5** (3.56 mM) were incubated for different times (0, 3, 6, 9, 14, 24, 36, 48, and 72 h). UDP-α-D-Gal*f* (1 mM) was then added to initiate the reaction and the sample was freeze-dried in liquid nitrogen to stop the enzymatic reaction. The residual activity of the enzyme was determined as described previously for the inhibition assays.[17]

Test for reversibility of the inactivation by UDP-1′-fluoro-*exo*-glycal-D-galactofuranose: Two samples containing UDP-1(1′*Z*)-[1′F]-*exo*-glycalGal*f* **4** (3.2 mM) and UDP-1(1′*E*)-[1′F]-*exo*-glycalGal*f* **5** (3.2 mM) were individually incubated with the mutase (5.4 μM) in phosphate buffer (total volume 60 μL, 100 mM, pH 7.4) for 72 h, cooled in ice and in the dark under aerobic conditions. Similar experiments were performed without the inactivator. The resulting solutions were applied to filters of a Vivaspin 0.5 mL concentrator equipped with a 10000 MWCO PES membrane; they were diluted with 300 mL of phosphate buffer and centrifuged (2000 *g* for 2 min) to obtain a final volume of 60 μL. This procedure was performed four times to remove all small molecules. The residual enzyme activity was measured before and after filtration (with and without dithionite).[16,17]

Atom and position numberings: We have systematically numbered the phosphonate methylene group as 1′ and adopted the usual numbering for carbohydrates from 1 to 6, with 1 for the anomeric position. For nucleotide sugars, we used the conventional ribose and pyrimidine numberings:



Scheme 6.

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1′ for the anomeric position, 1″ for the nitrogen atom linked to the ribose.

(1(1′)*Z*)-2,3,5,6-Tetra-*O*-*tert*-butyldimethylsilyl-1-(dibenzyloxyphosphoryl)methylidene-D-galactofuranose (8): A cooled (−70 °C) solution of dibenzyl methylphosphonate (4.96 g, 18.0 mmol) in THF (36 mL) under argon atmosphere was treated first with *n*BuLi (6.9 mL, 2.5 M solution in hexane, 17.3 mmol) and, after 20 min, with a solution of 2,3,5,6-tetra-*O*-*tert*-butyldimethylsilyl-D-galactono-1,4-lactone (4.56 g, 7.19 mmol) in THF (7 mL). The temperature was maintained at −70 °C for 10 min, and then the mixture was allowed to warm to −40 °C over a period of 1 h. The solution was then diluted with 1 M phosphate buffer (pH 7; 170 mL) and extracted with CH_2Cl_2 (2×350 mL). The combined organic phases were dried over $MgSO_4$, filtered, concentrated, and dried overnight in vacuo. The crude material was then dissolved in THF (50 mL) at 0 °C, and pyridine (5.81 mL, 71.9 mmol) and trifluoroacetic anhydride (5.0 mL, 36.0 mmol) were added. After 4 h at 0 °C, the reaction was stopped by adding saturated aqueous $NaHCO_3$, and the mixture was extracted with EtOAc (350 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Purification by chromatography on silica gel (cyclohexane/EtOAc, 5:1) afforded **8** (4.55 g, 71 % yield) as a colorless syrup.

$[\alpha]_D^{21}$ = +6.4 (*c* = 1.1 in $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.38–7.30 (m, 10 H; H arom.), 5.09 (ABX, *J*(H,P) = 7.4 Hz, *J*(H,H) = 12.0 Hz, 2 H; CH_2Ph), 5.02 (ABX, *J*(H,P) = 7.9 Hz, *J*(H,H) = 12.0 Hz, 2 H; CH_2Ph), 4.61 (dd, *J*(1′,2) = 1.1 Hz, *J*(1′,P) = 10.5 Hz, 1 H; H-1′), 4.47 (dt, *J*(1′,2) = 1.1 Hz, *J*(2,P) = *J*(2,3) = 3.9 Hz, 1 H; H-2), 4.41 (t, *J*(3,4) = *J*(4,5) = 3.9 Hz, 1 H; H-4), 4.33 (dt, *J*(2,3) = *J*(3,4) = 3.9 Hz, *J*(3,P) = 0.6 Hz, 1 H; H-3), 3.92 (dt, *J*(5,6a) = 7.0 Hz, *J*(5,6b) = 4.6 Hz, 1 H; H-5), 3.77 (ABX, *J*(5,6a) = 7.0 Hz, *J*(6a,6b) = 10.2 Hz, 1 H; H-6a), 3.65 (ABX, *J*(5,6b) = 4.6 Hz, *J*(6a,6b) = 10.2 Hz, 1 H; H-6b), 0.94 (s, 9 H; Si-*t*Bu), 0.92 (s, 9 H; Si-*t*Bu), 0.90 (s, 9 H; Si-*t*Bu), 0.88 (s, 9 H; Si-*t*Bu), 0.16 (s, 6 H; 2 Si-Me), 0.13 (s, 3 H; Si-Me), 0.12 (s, 3 H; Si-Me), 0.11 (s, 3 H; Si-Me), 0.09 (s, 3 H; Si-Me), 0.08 (s, 3 H; Si-Me), 0.02 ppm (s, 3 H; Si-Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.03 (d, *J*(1,P) = 2.6 Hz; C-1), 136.93 (d, *J*(C,P) = 7.3 Hz; C^q arom.), 136.91 (d, *J*(C,P) = 7.5 H; C^q arom.), 128.23–127.57 (10 CH arom.), 90.27 (C-4), 81.78 (d, *J*(1′,P) = 195.7 Hz; C-1′), 81.05 (d, *J*(2,P) = 13.9 Hz; C-2), 76.39 (C-3), 72.57 (C-5), 66.63 (d, *J*(C,P) = 4.5 Hz; CH_2Ph), 66.60 (d, *J*(C,P) = 5.0 Hz; CH_2Ph), 64.39 (C-6), 25.93 (Si-C(CH_3)$_3$), 25.82 (Si-C(CH_3)$_3$), 25.66 (2 Si-C(CH_3)$_3$), 18.28 (Si-*C*(CH_3)$_3$), 18.05 (Si-*C*(CH_3)$_3$), 17.83 (Si-*C*(CH_3)$_3$), 17.72 (Si-*C*(CH_3)$_3$), −3.82 (Si-Me), −4.02 (Si-Me), −4.34 (Si-Me), −4.38 (Si-Me), −4.41 (Si-Me), −4.81 (Si-Me), −5.40 (Si-Me), −5.42 ppm (Si-Me); MS (DCI-NH_3): *m*/*z*: 893 [*M*+H]$^+$; elemental analysis calcd (%) for $C_{45}H_{81}O_8PSi_4$: C 60.49, H 9.14; found: C 60.36, H 9.27.

1-(Dibenzyloxyphosphoryl)fluoromethyl-2,3,5,6-tetra-*O*-*tert*-butyldimethylsilyl-D-galactofuranose (10): *exo*-Glycal **8** (2.5 g, 2.80 mmol) was dissolved in a mixture of anhydrous MeCN (100 mL) and nitromethane (100 mL) under argon atmosphere in the presence of 4 Å molecular sieves (6.6 g). The suspension was stirred for 3 h at room temperature, and then Selectfluor (bis(triflate), 4.0 g, 8.41 mmol) was added at 0 °C. The resulting solution was stirred for 12 h at 30 °C, then diluted with EtOAc and filtered through a pad of Celite. The filtrate was concentrated under reduced pressure, then diluted with CH_2Cl_2 (2 mL), and silica gel (ca. 2 g) was added. The residue was purified by chromatography on silica gel (cyclohexane/EtOAc, 15:1 → 12.5:1) to give colorless **10** (2.08 g, 80 % yield) as a mixture of two diastereomers A and B in a ratio of 1:1.25.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.40–7.30 (m, 20 H; H arom.), 5.28–5.11 (m, 8 H; 4 CH_2Ph), 5.11 and 5.01 (2 d, *J*(1′,P) = 3.8 Hz, *J*(1′,F) = 44.2 Hz, 1 H; H-1′$_A$), 4.98 and 4.87 (2 d, *J*(1′,P) = 6.4 Hz, *J*(1′,F) = 44.6 Hz, 0.8 H; H-1′$_B$), 4.98 (br s, 1 H; OH-1$_A$), 4.57 (d, *J*(OH,2) = 2.0 Hz, 0.8 H; OH-1$_B$), 4.48 (dd, *J*(2,3) = 4.3 Hz, *J*(2,OH) = 2.0 Hz, 0.8 H; H-2$_B$), 4.33 (dd, *J*(2,3) = 4.3 Hz, *J*(3,4) = 3.1 Hz, 0.8 H; H-3$_B$), 4.31 (dd, *J*(3,4) = 1.0 Hz, *J*(4,5) = 5.4 Hz, 1 H; H-4$_A$), 4.20 (m, 1 H; H-3$_A$), 4.17 (s, 1 H; H-2$_A$), 4.14 (t, *J*(3,4) = *J*(4,5) = 3.1 Hz, 0.8 H; H-4$_B$), 3.91 (q, *J*(4,5) = *J*(5,6a) = *J*(5,6b) = 5.4 Hz, 1 H; H-5$_A$), 3.84 (ddd, *J*(4,5) = 3.1 Hz, *J*(5,6a) = 6.8 Hz, *J*(5,6b) = 5.4 Hz, 0.8 H, H-5$_B$), 3.77 (ABX, *J*(5,6a) = 6.8 Hz, *J*(6a,6b) = 10.2 Hz, 0.8 H; H-6a$_B$), 3.73 (ABX, *J*(5,6a) = 5.4 Hz, *J*(6a,6b) = 10.2 Hz, 1 H; H-6a$_A$), 3.62 (ABX, *J*(5,6b) = 5.4 Hz, *J*(6a,6b) = 10.2 Hz, 1.8 H; H-6b$_{A,B}$), 0.97 (s, 7.2 H; Si-*t*Bu), 0.96 (s, 7.2 H; Si-*t*Bu), 0.95 (s, 9 H; Si-*t*Bu), 0.94 (s, 9 H; Si-*t*Bu), 0.93 (s, 9 H; Si-*t*Bu), 0.92 (s, 9 H; Si-*t*Bu), 0.92 (s, 7.2 H; Si-*t*Bu), 0.87 (s, 7.2 H; Si-*t*Bu), 0.25 (s; Si-Me), 0.21 (s; Si-Me), 0.19 (s; 2 Si-Me), 0.18 (2 s; 2 Si-Me), 0.17 (2 s; 2 Si-Me), 0.16 (s; Si-Me), 0.14 (s; Si-Me), 0.13 (s; Si-Me), 0.09 (s; 2 Si-Me), 0.08 (s; Si-Me), 0.04 (s; Si-Me), 0.02 ppm (s; Si-Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 136.4 (d, *J*(C,P) = 6.4 Hz; C^q arom.), 136.15 (d, *J*(C,P) = 6.2 Hz; 2 C^q arom.), 136.12 (d, *J*(C,P) = 6.5 Hz; C^q arom.), 128.42–127.70 (20 CH arom.), 105.59 (dd, *J*(1,P) = 2.2 Hz, *J*(1,F) = 24.3 Hz; C-1$_A$), 102.13 (d, *J*(1,P) = 1.0 Hz, *J*(1,F) = 20.5 Hz; C-1$_B$), 88.49 (C-4$_A$), 88.19, 86.28 (2 d, *J*(1′,P) = 162.8 Hz, *J*(1′,F) = 191.9 Hz; C-1′$_B$), 87.80, 85.97 (2 d, *J*(1′,P) = 162.7 Hz, *J*(1′,F) = 184.0 Hz; C-1′$_A$), 84.62 (C-4$_B$), 82.82 (d, *J*(2,P) = 7.5 Hz; C-2$_A$), 79.61 (d, *J*(2,P) = 3.5 Hz, *J*(2,F) = 5.3 Hz, C-2$_B$), 78.90 (C-3$_A$), 78.05 (C-3$_B$), 73.61 (C-5$_B$), 73.23 (C-5$_A$), 68.61 (d, *J*(C,P) = 5.9 Hz; CH_2Ph), 68.50 (d, *J*(C,P) = 5.8 Hz, CH_2Ph), 68.38 (d, *J*(C,P) = 6.3 Hz; CH_2Ph), 67.67 (d, *J*(C,P) = 6.3 Hz; CH_2Ph), 65.92 (C-6$_A$), 64.40 (C-6$_B$), 26.07 (Si-C(CH_3)$_3$), 25.98 (Si-C-(CH_3)$_3$), 25.90 (2 Si-C(CH_3)$_3$), 25.86 (Si-C(CH_3)$_3$), 25.77 (2 Si-C(CH_3)$_3$), 25.68 (2 Si-C(CH_3)$_3$), 18.48 (Si-*C*(CH_3)$_3$), 18.27 (Si-*C*(CH_3)$_3$), 18.25 (Si-*C*-(CH_3)$_3$), 18.14 (Si-*C*(CH_3)$_3$), 18.03 (Si-*C*(CH_3)$_3$), 17.94 (Si-*C*(CH_3)$_3$), 17.81 (Si-*C*(CH_3)$_3$), 17.79 (Si-*C*(CH_3)$_3$), −3.97 (Si-Me), −4.18 (2 Si-Me), −4.22 (2 Si-Me), −4.26 (Si-Me), −4.29 (3 Si-Me), −4.49 (Si-Me), −4.54 (Si-Me), −4.65 (Si-Me), −4.66 (Si-Me), −5.19 (Si-Me), −5.22 (Si-Me), −5.35 (Si-Me), −5.39 ppm (2 Si-Me); ^{31}P NMR (101 MHz, $CDCl_3$): δ = 17.76 (d, *J*(P,F) = 75.9 Hz; major diastereomer), 16.00 ppm (d, *J*(P,F) = 72.9 Hz; minor diastereomer); ^{19}F NMR (235 MHz, $CDCl_3$): δ = −210.33 (dd, *J*(F,P) = 75.3 Hz, *J*(F,H1) = 44.7 Hz; major diastereomer), −211.12 ppm (dd, $J_{F\text{-}P}$ = 72.9 Hz, $J_{F\text{-}H1'}$ = 44.7 Hz; minor diastereomer); MS (DCI-NH_3): *m*/*z* (%): 929 (100) [*M*+H]$^+$, 946 (95) [*M*+NH_4]$^+$; elemental analysis calcd (%) for $C_{45}H_{82}FO_9PSi_4$: C 58.15, H 8.89; found: C 57.75, H 9.22.

1-(Dibenzyloxyphosphoryl)fluoromethyl-1-*O*-trifluoroacetyl-2,3,5,6-tetra-*O*-*tert*-butyldimethylsilyl-D-galactofuranose (11): The mixture of ketols **10** (100 mg, 0.11 mmol) was dissolved in anhydrous THF (2.5 mL), and anhydrous pyridine (349 μL, 4.40 mmol) and trifluoroacetic anhydride (150 μL, 1.10 mmol) were successively added at 0 °C. The solution was stirred for 3 h at 0 °C and then allowed to warm to room temperature. After concentration under vacuum, the crude reaction mixture was purified by flash chromatography on silica gel (cyclohexane/EtOAc, 11:1) to give compound **11** (68 mg, 62 % yield) as a colorless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.37–7.30 (m, 10 H; H arom.), 6.02 (dd, *J*(1′,P) = 14.6 Hz, *J*(1′,F) = 45.7 Hz, 1 H; H-1′), 5.76 (dd, *J*(2,3) = 3.0 Hz, *J* = 9.8 Hz, 1 H; H-2), 5.17–4.96 (m, 4 H; 2 CH_2Ph), 5.07 (d, *J*(3,4) = 5.7 Hz, 1 H; H-4), 4.29–4.26 (m, 2 H; H-3 and H-5), 4.00 (ABX, *J*(5,6a) = 1.7 Hz, *J*(6a,6b) = 11.4 Hz, 1 H; H-6a), 3.73 (ABX, *J*(5,6b) = 1.8 Hz, *J*(6a,6b) = 11.4 Hz, 1 H; H-6b), 0.94 (s, 18 H; 2 Si-*t*Bu), 0.92 (s, 9 H; Si-*t*Bu), 0.86 (s, 9 H; Si-*t*Bu), 0.23 (s, 3 H; Si-Me), 0.19 (s, 3 H; Si-Me), 0.13 (s, 3 H; Si-Me), 0.12 (s, 3 H; Si-Me), 0.11 (s, 3 H; Si-Me), 0.09 (s, 3 H; Si-Me), 0.08 (s, 3 H; Si-Me), 0.07 (s, 3 H; Si-Me); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 197.97 (d, *J*(C,F) = 12.5 Hz; C-1), 157.04 (q, *J*(C,F) = 41.9 Hz; *C*O-CF_3), 135.16 (d, *J*(C,P) = 6.0 Hz; C^q arom.), 135.08 (d, *J*(C,P) = 6.1 Hz; C^q arom.), 128.71–127.72 (10 CH arom.), 114.54 (q, *J*(C,F) = 284.1 Hz; CO-*C*F_3), 90.88 (dd, *J*(1′,P) = 145.5 Hz, *J*(1′,F) = 199.2 Hz; C-1′), 79.37 (C-2), 78.58 (C-4), 73.25 (C-3), 69.49 (d, *J*(C,P) = 7.5 Hz; CH_2Ph), 69.10 (d, *J*(C,P) = 6.8 Hz; CH_2Ph), 68.74 (C-5), 65.86 (C-6), 26.01 (Si-C(CH_3)$_3$), 25.79 (Si-C(CH_3)$_3$), 25.71 (Si-C-(CH_3)$_3$), 25.67 (Si-C(CH_3)$_3$), 18.47 (Si-*C*(CH_3)$_3$), 18.20 (Si-*C*(CH_3)$_3$), 17.82 (Si-*C*(CH_3)$_3$), 17.61 (Si-*C*(CH_3)$_3$), −5.02 (Si-Me), −5.04 (Si-Me), −5.12 (2 Si-Me), −5.20 (Si-Me), −5.34 (Si-Me), −5.47 (Si-Me), −5.75 (Si-Me); ^{31}P NMR (101 MHz, $CDCl_3$): δ = 9.77 ppm (d, *J*(P,F1′) = 64.8 Hz); ^{19}F NMR (235 MHz, $CDCl_3$): δ = −74.55 (d, *J*(F,F1′) = 14.8 Hz; OCOCF_3), −210.17 ppm (ddd, *J*(F,F1′) = 14.8 Hz, *J*(F1′,H1′) = 45.8 Hz, *J*(F1′,P) = 64.6 Hz; F-1′); MS (DCI-NH_3): *m*/*z* (%): 1025 (55) [*M*+H]$^+$, 1042 (100) [*M*+NH_4]$^+$; HRMS: *m*/*z*: calcd for $C_{47}H_{82}O_{10}F_4Si_4P$: 1025.4659; found 1025.4646.

(1(1′)*Z*)-1-Deoxy-1-(dibenzyloxyphosphoryl)fluoromethylidene-2,3,5,6-tetra-*O*-*tert*-butyldimethylsilyl-D-galactofuranose ((*Z*)-9) and (1(1′)*E*)-1-

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deoxy-1-(dibenzyloxyphosphoryl)fluoromethylidene-2,3,5,6-tetra-*O*-*tert*-butyldimethylsilyl-D-galactofuranose ((*E*)-9): Compound **10** (826 mg, 0.89 mmol) was dissolved in a mixture of anhydrous CH_2Cl_2 and pyridine (6:4, 25 mL) under argon atmosphere. The solution was cooled to 0°C, and then DMAP (2.18 g, 17.8 mmol) and trifluoroacetic anhydride (1.2 mL, 8.9 mmol) were successively added. The reaction mixture was stirred for 24 h at 35°C, and then the solvents were evaporated under reduced pressure. The residue was diluted with CH_2Cl_2 (60 mL) and the organic layer was washed with water, dried over $MgSO_4$, filtered, and concentrated. The residue was then purified by chromatography on silica gel (cyclohexane/EtOAc, 9:1) to give (*Z*)-**9** (381 mg, 46% yield) and (*E*)-**9** (146 mg, 26% yield) in a ratio of 64:36 (72% overall yield).

(*Z*)-9: $[\alpha]_D^{18} = -7.3$ ($c = 1.0$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.38$–7.35 (m, 10H; H arom.), 5.17 (ABX, J(H,P) = 6.5 Hz, J(H,H) = 11.7 Hz, 2H; 2C*H*Ph), 5.12 (ABX, J(H,P) = 7.7 Hz, J(H,H) = 11.7 Hz, 1H; C*H*Ph), 5.05 (ABX, J(H,P) = 7.0 Hz, J(H,H) = 11.7 Hz, 1H; C*H*Ph), 4.78 (d, 1H; H-3), 4.41 (dd, J(3,4) = 1.6 Hz, J(4,5) = 9.1 Hz, 1H; H-4), 4.27 (d, J(2,3) = 0.7 Hz, 1H; H-2), 4.01 (td, J(4,5) = 9.1 Hz, J(5,6a,b) = 4.0 Hz, 1H; H-5), 3.74 (AX, J(5,6a,b) = 4.0 Hz, 2H; H-6a and H-6b), 0.94 (s, 9H; Si-*t*Bu), 0.92 (s, 9H; Si-*t*Bu), 0.89 (s, 9H; Si-*t*Bu), 0.87 (s, 9H; Si-*t*Bu), 0.20 (s, 3H; Si-Me), 0.15 (2s, 6H; 2Si-Me), 0.14 (s, 3H; Si-Me), 0.11 (s, 3H; Si-Me), 0.10 (s, 6H; 2Si-Me), 0.04 ppm (s, 3H; Si-Me); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 160.85$ (dd, $J_{1\text{-P}} = 21.6$ Hz, $J_{1\text{-F}} = 37.3$ Hz; C-1), 136.03 (d, $J_{\text{C-P}} = 8.3$ Hz; 2C^q arom.), 133.57 (dd, $J_{1'\text{-P}} = 232.4$ Hz, $J_{1'\text{-F}} = 241.4$ Hz; C-1′), 128.53–127.61 (10C arom.), 95.91 (C-4), 76.75 (C-3), 76.10 (C-2), 72.35 (C-5), 67.80 (d, $J_{\text{C-P}} = 4.5$ Hz; CH_2Ph), 67.71 (d, $J_{\text{C-P}} = 5.1$ Hz; CH_2Ph), 66.50 (C-6), 26.11 (Si-C(CH_3)$_3$), 25.89 (Si-C(CH_3)$_3$), 25.61 (Si-C(CH_3)$_3$), 25.55 (Si-C(CH_3)$_3$), 18.56 (Si-*C*-(CH_3)$_3$), 18.07 (Si-*C*(CH_3)$_3$), 17.93 (Si-*C*(CH_3)$_3$), 17.75 (Si-*C*(CH_3)$_3$), −4.55 (Si-Me), −4.66 (Si-Me), −4.70 (Si-Me), −4.88 (Si-Me), −5.14 (Si-Me), −5.17 (Si-Me), −5.22 (Si-Me), −5.27 ppm (Si-Me); ^{31}P NMR (101 MHz, $CDCl_3$): $\delta = 6.81$ ppm (d, J(P,F) = 94.2 Hz); ^{19}F NMR (235 MHz, $CDCl_3$): $\delta = -174.10$ ppm (d, J(P,F) = 94.1 Hz); MS (DCI-NH_3): m/z (%): 911 (20) $[M+H]^+$, 928 (100) $[M+NH_4]^+$; HRMS: m/z: calcd for $C_{45}H_{81}O_8FSi_4P$: 911.4730; found 911.4722.

(*E*)-9: $[\alpha]_D^{18} = -40.4$ ($c = 1.0$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.41$–7.38 (m, 10H; H arom.), 5.25 (s, 1H; H-3), 5.19 (ABX, J(H,P) = 6.8 Hz, J(H,H) = 11.9 Hz, 1H; C*H*Ph), 5.15 (ABX, J(H,P) = 6.2 Hz, J(H,H) = 11.7 Hz, 2H; 2C*H*Ph), 5.04 (ABX, J(H,P) = 6.8 Hz, J(H,H) = 11.7 Hz, 1H; C*H*Ph), 4.47 (d, J(4,5) = 9.9 Hz, 1H; H-4), 4.23 (d, J(2,3) = 3.3 Hz, 1H; H-2), 4.17 (td, J(4,5) = 9.9 Hz, J(5,6a,b) = 3.5 Hz, 1H; H-5), 3.81 (AX, J(5,6a,b) = 3.5 Hz, 2H; H-6a and H-6b), 0.98 (s, 9H; Si-*t*Bu), 0.97 (s, 9H; Si-*t*Bu), 0.94 (s, 9H; Si-*t*Bu), 0.91 (s, 9H; Si-*t*Bu), 0.30 (s, 3H; Si-Me), 0.29 (s, 3H; Si-Me), 0.20 (s, 3H; Si-Me), 0.17 (s, 3H; Si-Me), 0.16 (2s, 6H; 2Si-Me), 0.15 (s, 3H; Si-Me), 0.10 ppm (s, 3H; Si-Me); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 159.15$ (dd, J(1,P) = 4.4 Hz, J(1,F) = 48.4 Hz; C-1), 135.82 (d, J(C,P) = 7.4 Hz; C^q arom.), 135.79 (d, J(C,P) = 8.8 Hz; C^q arom.), 131.30 (dd, J(1′,P) = 247.6 Hz, J(1′,F) = 254.9 Hz; C-1′), 128.45–127.67 (10C arom.), 94.52 (C-4), 77.27 (d, J(3,P) = 2.6 Hz; C-3), 76.73 (C-2), 72.30 (C-5), 67.90 (d, J(C,P) = 5.2 Hz; CH_2Ph), 67.64 (d, J(C,P) = 4.2 Hz; CH_2Ph), 64.00 (C-6), 26.10 (Si-C(CH_3)$_3$), 25.87 (Si-C(CH_3)$_3$), 25.80 (Si-C(CH_3)$_3$), 25.55 (Si-C(CH_3)$_3$), 18.56 (Si-*C*(CH_3)$_3$), 18.24 (Si-*C*(CH_3)$_3$), 17.95 (Si-*C*(CH_3)$_3$), 17.79 (Si-*C*-(CH_3)$_3$), −4.65 (Si-Me), −4.70 (Si-Me), −4.73 (3Si-Me), −4.75 (Si-Me), −5.27 ppm (2Si-Me); ^{31}P NMR (101 MHz, $CDCl_3$): $\delta = 7.85$ ppm (d, J(P,F) = 78.0 Hz); ^{19}F NMR (235 MHz, $CDCl_3$): $\delta = -163.69$ ppm (d, J(P,F) = 77.7 Hz); MS (DCI-NH_3): m/z (%): 911 (100) $[M+H]^+$, 928 (20) $[M+NH_4]^+$; HRMS: m/z: calcd for $C_{45}H_{81}O_8FSi_4P$: 911.4730; found 911.4732.

Dibenzyl (1-deoxy-1-fluoro-2,3,5,6-tetra-*O*-TBDMS-D-galactofuranosyl)-methyl phosphonate (14): DAST (41 µL, 0.312 mmol) was gradually added to a cooled (0°C) solution of lactol **10** (238 mg, 0.260 mmol) in CH_2Cl_2 (3 mL), and the mixture was stirred for 1 h at 0°C. After washing with saturated aqueous $NaHCO_3$ solution, the aqueous phase was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel (cyclohexane/EtOAc, 5:1) to furnish monofluoride **14** (98 mg, 41%), (*Z*)-*exo*-glycal (*Z*)-**8** (49 mg, 22%), and (*E*)-*exo*-glycal (*E*)-**8** (24 mg, 11%), respectively.

^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.36$ (m; 10H, ArH), 4.97–5.15 (m; $ArCH_2$), 4.56 (d, J(2,3) = 5.5 Hz, J(2,F) = 13.7 Hz; H-2$_\alpha$), 4.39 (dt, J(2,3) = 5.5 Hz, J(3,F) = 1.0 Hz; H-3$_\alpha$), 4.35 (d, J(2,F) = 5.4 Hz; H-2$_\beta$), 4.09–4.14 (m; H-4$_\alpha$ and H-4$_\beta$), 4.07 (brs; H-3$_\beta$), 3.86–3.90 (m; H-5$_\alpha$), 3.81–3.90 (m; H-4$_\beta$ and H-5$_\beta$), 3.59–3.74 (m; H-6$_\alpha$ and H-6$_\beta$), 2.51–2.74 (m; H-1′$_\alpha$ and H-1′$_\beta$), 0.88–0.94 (m; MeSi), 0.03–0.20 ppm (m; *t*BuSi); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 136.6\times3$, 136.5, 136.4×2, 136.3×2, 128.5, 128.4, 128.3, 128.2×3, 128.1, 128.0×3, 127.8, 120.4×2, 118.2×2, 115.4, 113.1, 82.3×2, 81.9×2, 81.0×2, 80.8×2, 78.5, 76.4, 74.2, 67.7, 67.6×3, 66.9, 66.8×3, 65.8, 64.5, 34.7, 34.3, 33.3, 32.9, 31.8, 31.6, 30.4, 30.1, 26.0×2, 25.9×3, 25.8×2, 25.6, 18.4, 18.3, 18.0, 17.9×2, 17.8, −3.7×2, −4.0×2, −4.1×2, −4.2, −4.3×2, −4.6, −4.8, −5.2, −5.3, −5.4×2 ppm; ^{19}F NMR (235 MHz, $CDCl_3$): $\delta = -95.1$ (dd, J(2,F) = 9.4 Hz, J(1′,F) = 23.5 Hz; F$_\alpha$), −99.0 ppm (td, J(2,F) = 4.7 Hz, J(P,F) = 14.1 Hz, J(1′,F) = 23.5 Hz; F$_\beta$); ^{31}P NMR (101 MHz, $CDCl_3$): $\delta = 24.95$ (s, P$_\beta$), 23.96 ppm (d, J(P,F) = 4.7 Hz; P$_\alpha$).

(1(1′)*E*)-2,3,5,6-Tetra-*O*-TBDMS-1-(dibenzyloxyphosphoryl)methylidene-D-galactofuranose ((*E*)-8): $[\alpha]_{23}^{D} = -21.7$ ($c = 0.5$ in $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): $\delta = 7.36$ (m, 10H; ArH), 4.94–5.07 (m; $ArCH_2$), 4.90 (d, J(1′,P) = 7.7 Hz, 1H; H-1′), 4.31 (d, J(4,5) = 9.8 Hz, 1H; H-4), 4.13 (brs, 1H; H-2), 4.10 (td, J(4,5) = 9.8 Hz, J(5,6) = 3.6 Hz, 1H; H-5), 3.75 (d, J(5,6) = 3.6 Hz, 2H; H-6), 0.89–0.94 (m; MeSi), 0.06–0.29 ppm (m; *t*BuSi); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 176.1$, 175.8, 128.8, 128.4×2, 128.1, 128.0, 93.4, 84.7, 82.7, 76.4, 75.8, 72.5, 66.6×2, 66.5, 66.3, 26.1, 25.9×2, 25.6, −4.5, −4.6×2, −4.7×2, −5.3 ppm; ^{31}P NMR (101 MHz, $CDCl_3$): $\delta = 21.51$ ppm (s); MS (DCI-NH_3): m/z: 893 $[M+H]$; HRMS (DCI): m/z: calcd for $C_{49}H_{50}O_8P$ (MH^+): 893.4834; found: 893.4824.

((1(1′)*Z*)-1-Deoxy-1-methylidene-1′-fluoro-D-galactofuranosyl)phosphonic acid ((*Z*)-15): A solution of compound (*Z*)-**9** (297 mg, 0.33 mmol) in anhydrous CH_2Cl_2 (22 mL) was stirred in the presence of Et_3N (91 µL, 0.66 mmol, 2 equiv) and Pd/C (10%, 90 mg) for 1 h at room temperature under H_2 atmosphere (1 bar). The suspension was then filtered through a pad of Celite and the filtrate was concentrated to quantitatively afford a viscous oil (304 mg). Tetrabutylammonium fluoride (433 mg, 1.39 mmol, 4.2 equiv) was added to a solution of this compound in THF (15 mL) at 0°C. After stirring for 1 h at room temperature, the reaction mixture was concentrated under reduced pressure, and the residue was redissolved in the minimum amount of water. This solution was filtered through paper, and the aqueous filtrate was freeze-dried. The residue, redissolved in the minimum amount of water, was applied to a short column of Dowex 50WX8-200 (Na^+ form). The appropriate fractions (detected by TLC, eluting with $EtOH/NH_4OH/H_2O$, 5:3:1) were pooled and freeze-dried. The compound was then purified on Sephadex G15 (eluent: H_2O) and subsequently applied to a column of Dowex 50WX8-200 (Bu_3NH^+ form). The desired fractions were combined and freeze-dried to give (*Z*)-**15** (170 mg, 88% yield, 1.7 equivalents tributylammonium salt) as a white hygroscopic solid.

$[\alpha]_D^{22} = -14.1$ ($c = 0.8$ in H_2O, 1.7 equiv Bu_3N); ^1H NMR (400 MHz, D_2O): $\delta = 4.92$ (m, 1H; H-2), 4.22 (t, J(2,3) = J(3,4) = 4.6 Hz, 1H; H-3), 4.17 (t, J(3,4) = J(4,5) = 4.6 Hz, 1H; H-4), 3.86 (td, J(4,5) = J(5,6a) = 4.6 Hz, J(5,6b) = 6.5 Hz, 1H; H-5), 3.73 (ABX, J(5,6a) = 4.6 Hz, J(6a,6b) = 11.8 Hz, 1H; H-6a), 3.68 (ABX, J(5,6b) = 6.6 Hz, J(6a,6b) = 11.8 Hz, 1H; H-6a), 3.11 (m; CH_2(d), Bu_3N), 1.65 (m; CH_2(c), Bu_3N), 1.35 (sext., J(H,H) = 7.5 Hz; CH_2(b), Bu_3N), 0.91 ppm (t, J(H,H) = 7.5 Hz; CH_3(a), Bu_3N); ^{13}C NMR (100 MHz, D_2O): $\delta = 153.10$ (dd, J(1,P) = 18.9 Hz, J(1,F) = 37.7 Hz; C-1), 142.93 (dd, J(1′,P) = 236.8 Hz, J(1′,F) = 247.9 Hz; C-1′), 86.67 (C-4), 76.08 (C-3), 74.74 (d, J(2,P) = 5.5 Hz; C-2), 70.92 (C-5), 62.46 (C-6), 53.02 (CH_2(d), Bu_3N), 25.54 (CH_2(c), Bu_3N), 19.63 (CH_2(b), Bu_3N), 13.15 ppm (CH_3(a), Bu_3N); ^{31}P NMR (101 MHz, D_2O): $\delta = 0.16$ ppm (d, J(P,F) = 85.1 Hz); ^{19}F NMR (235 MHz, D_2O): $\delta = -169.80$ ppm (dd, J(P,F) = 84.7 Hz, J(F,H2) = 2.4 Hz); MS (FAB$^-$): m/z: 273 $[M-H]^-$; HRMS: m/z: calcd for $C_7H_{11}O_8FP$: 273.0176; found 273.0172.

UDP-(1(1′)*Z*)-1′-fluoro-*exo*-glycal-D-galactofuranose (4): A suspension of 5′-UMP (triethylammonium salt, 65.1 mg, 0.153 mmol) in a mixture of freshly distilled MeCN (800 µL), *N*,*N*-dimethylaniline (77 µL, 0.61 mmol),

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and Et_3N (43 μL, 0.31 mmol) was stirred under argon atmosphere at 0 °C. Trifluoroacetic anhydride (128 μL, 0.92 mmol) was then slowly added dropwise. The reaction mixture was stirred for a few minutes at room temperature, after which time a red-brown coloration was observed. Excess trifluoroacetic anhydride and trifluoroacetic acid were removed from the reaction mixture under vacuum (using an oil pump and a liquid nitrogen trap). In a separate flask, a mixture of *N*-methylimidazole (61 μL, 0.76 mmol) and Et_3N (128 μL, 0.92 mmol) in anhydrous MeCN (150 μL) was prepared, cooled to 0 °C, and then added to the flask containing the mixed phosphoryl anhydride. The reaction mixture was stirred for 10 min at 0 °C, after which time a bright-yellow solution was obtained. In the meantime, in another flask a mixture of (*Z*)-**15** (tributylammonium salt, 71.8 mg, 0.118 mmol) and preactivated 4 Å molecular sieves in MeCN (800 μL) was stirred for 30 min at 0 °C. The solution of the UMP-*N*-methylimidazolium species was then added dropwise to the solution containing (*Z*)-**15**. The resulting mixture was stirred at 0 °C under argon for 2 h, and then allowed to warm to room temperature. The reaction was complete after 20 h, whereupon it was quenched with cold aqueous ammonium formate (3 mL, 250 mM, pH 7). After filtration through a Celite pad, the amines were extracted from the aqueous phase with CH_2Cl_2 (3 mL). The organic phase was washed with cold aqueous ammonium formate (2 mL, 250 mM, pH 7) and the combined aqueous phases were pooled and freeze-dried. The residue was purified by size-exclusion chromatography (Sephadex G15) eluting with a 50 mM triethylammonium acetate buffer (pH 6.8). The appropriate fractions were pooled and freeze-dried. Compound **4** was further purified by HPLC on a C_{18} column, using 50 mM triethylammonium acetate buffer (pH 6.8) containing 1 % MeCN as eluent at a flow rate of 1 mL min^{-1}. This protocol afforded compound **4** with the (*Z*)-configuration as a white solid (triethylammonium salt) in 64 % yield.

^{1}H NMR (400 MHz, D_2O): δ = 7.97 (d, *J*(5'',6'') = 8.2 Hz, 1H; H-6''), 6.00 (d, *J*(1',2') = 4.6 Hz, 1H; H-1'), 5.98 (d, *J*(5'',6'') = 8.2 Hz, 1H; H-5''), 4.95 (dd, *J*(2,3) = 3.4 Hz, *J*(2,P) = 7.1 Hz, 1H; H-2), 4.39–4.36 (m, 2H; H-2' and H-3'), 4.29–4.25 (m, 2H; H-3 and H-4'), 4.22 (t, *J*(3,4) = *J*(4,5) = 4.8 Hz, 1H; H-4), 4.18 (ABXX', *J*(4',5') = 5.6 Hz, *J*(5'a,5'b) = 9.0 Hz, *J*(5',P) = 2.6 Hz, 2H; H-5'a,b), 3.88 (td, *J*(4,5) = *J*(5,6a) = 4.8 Hz, *J*(5,6b) = 6.7 Hz, 1H; H-5), 3.77 (ABX, *J*(5,6a) = 4.8 Hz, *J*(6a,6b) = 11.8 Hz, 1H; H-6a), 3.71 (ABX, *J*(5,6b) = 6.7 Hz, *J*(6a,6b) = 11.8 Hz, 1H; H-6b), 3.21 (q, *J* = 7.3 Hz; CH_2, Et_3N), 1.29 ppm (t, *J* = 7.3 Hz, CH_3, Et_3N); ^{13}C NMR (100 MHz, D_2O): δ = 166.61 (C-4''), 154.96 (dd, *J*(1,P) = 20.6 Hz, *J*(1,F) = 36.9 Hz; C-1), 152.22 (C-2''), 142.01 (C-6''), 140.60 (dd, *J*(*C*F,P) = 232.9 Hz, *J*(*C*F,F) = 239.7 Hz; *C*-F), 103.05 (C-5''), 88.61 (C-1'), 86.88 (C-4), 83.73 (d, *J*(4',P) = 9.1 Hz; C-4'), 76.11 (d, *J*(2,P) = 7.1 Hz, C-2), 75.99 (C-3), 74.17 (C-3'), 70.97 (C-5), 70.10 (C-2'), 65.18 (d, *J*(5',P) = 5.5 Hz, C-5'), 62.46 (C-6), 47.04 (CH_2, Et_3N), 8.60 ppm (CH_3, Et_3N); ^{31}P NMR (101 MHz, D_2O): δ = −8.87 (dd, *J*(P_α,P_β) = 24.3 Hz, *J*(P_α,F) = 90.1 Hz; P_α), −11.69 ppm (d, *J*(P_α,P_β) = 25.3 Hz; P_β); ^{19}F NMR (235 MHz, D_2O): δ = −172.06 ppm (dd, *J*(F,P_α) = 91.8 Hz, *J*(F,H2) = 2.35 Hz); MS (ESI$^+$): *m*/*z*: 603 [*M*+Na]$^+$; HRMS: *m*/*z*: calcd for $C_{16}H_{23}O_{16}FN_2P_2Na$: 603.0405; found 603.0432.

((1(1')*E*)-1-Deoxy-1-methylidene-1'-fluoro-*exo*-glycal-D-galactofuranosyl)phosphonic acid ((*E*)-15): Compound (*E*)-**9** (294 mg, 0.32 mmol) was hydrogenated (reaction time: 3 h) and desilylated (reaction time: 1.5 h) following the same procedures as used for the preparation of compound (*Z*)-**15**. After purification of the crude reaction mixture, first by size-exclusion chromatography (Sephadex G15, eluent H_2O) and then by ion-exchange chromatography (Dowex Na^+ and Bu_3NH^+ forms), compound (*Z*)-**15** (166 mg, 80 % yield, bis(tributylammonium) salt) was obtained as a hygroscopic white solid.

$[\alpha]_D^{22}$ = −19.5 (*c* = 0.77 in H_2O, 2 equiv Bu_3N); ^{1}H NMR (400 MHz, D_2O): δ = 4.81 (m, 1H; H-2), 4.25 (t, *J*(2,3) = *J*(3,4) = 6.6 Hz, 1H; H-3), 4.08 (dd, *J*(3,4) = 6.6 Hz, *J*(4,5) = 4.0 Hz, 1H; H-4), 3.85 (ddd, *J*(5,6a) = 4.9 Hz, *J*(5,6b) = 7.1 Hz, 1H; H-5), 3.72 (ABX, *J*(5,6a) = 4.9 Hz, *J*(6a,6b) = 11.8 Hz, 1H; H-6a), 3.67 (ABX, *J*(5,6b) = 7.1 Hz, *J*(6a,6b) = 11.8 Hz, 1H; H-6a), 3.09 (m; CH_2(d), Bu_3N), 1.64 (m; CH_2(c), Bu_3N), 1.34 (sext, *J*(H,H) = 7.3 Hz; CH_2(b), Bu_3N), 0.90 ppm (t, *J*(H,H) = 7.3 Hz; CH_3(a), Bu_3N); ^{13}C NMR (100 MHz, D_2O): δ = 149.26 (dd, *J*(1,P) = 7.2 Hz, *J*(1,F) = 37.1 Hz; C-1), 141.38 (dd, *J*(1',P) = 216.7 Hz, *J*(1',F) = 254.9 Hz; C-1'), 84.00 (C-4), 75.65 (C-3), 74.75 (C-2), 70.43 (C-5), 62.76 (C-6), 52.96 (CH_2(d), Bu_3N), 25.52 (CH_2(c), Bu_3N), 19.65 (CH_2(b), Bu_3N), 13.00 ppm (CH_3(a), Bu_3N); ^{31}P NMR (101 MHz, D_2O): δ = 0.95 ppm (d, *J*(P,F) = 82.0 Hz); ^{19}F NMR (235 MHz, D_2O): δ = −155.43 ppm (d, *J*(P,F) = 82.4 Hz); MS (FAB$^-$): *m*/*z*: 273 [*M*−H]$^-$; HRMS: *m*/*z*: calcd for $C_7H_{11}O_8FP$: 273.0176; found 273.0186.

UDP-(1(1')*E*)-1'-fluoro-*exo*-glycal-D-galactofuranose (5): The coupling procedure was essentially identical to that described for compound **4**. The reaction time for the coupling of *exo*-glycal (*E*)-**15** (62.7 mg, 0.12 mmol) with activated UMP (0.15 mmol) was 24 h at room temperature. The crude mixture was purified by size-exclusion chromatography (Sephadex G15, eluent H_2O). Compound **5** was further purified by HPLC on a C_{18} column, eluting with 50 mM triethylammonium acetate buffer (pH 6.8) containing 1 % MeCN at a flow rate of 1 mL min^{-1}. This protocol afforded compound **5** as a white solid (triethylammonium salt) in 70 % yield.

^{1}H NMR (400 MHz, D_2O): δ = 7.97 (d, *J*(5'',6'') = 8.1 Hz, 1H; H-6''), 6.01 (d, *J*(1',2') = 4.7 Hz, 1H; H-1'), 5.97 (d, *J*(5'',6'') = 8.1 Hz, 1H; H-5''), 5.01 (dd, *J*(2,3) = 1.7 Hz, *J*(2,P) = 2.9 Hz, 1H; H-2), 4.38–4.34 (m, 2H; H-2' and H-3'), 4.32 (dd, *J*(2,3) = 1.7 Hz, *J*(3,4) = 1.7 Hz, 1H; H-3), 4.30–4.27 (m, 2H; H-4 and H-4'), 4.23 (ABXX', *J*(4',5'a) = 4.3 Hz, *J*(5'a,5'b) = 11.9 Hz, *J*(5'a,P) = 2.5 Hz, 1H; H-5'a), 4.17 (ABXX', *J*(4',5'b) = 5.2 Hz, *J*(5'a,5'b) = 11.9 Hz, *J*(5'b,P) = 2.5 Hz, 1H; H-5'b), 3.92 (td, *J*(4,5) = *J*(5,6a) = 4.4 Hz, *J*(5,6b) = 6.5 Hz, 1H; H-5), 3.76 (ABX, *J*(5,6a) = 4.4 Hz, *J*(6a,6b) = 11.9 Hz, 1H; H-6a), 3.70 (ABX, *J*(5,6b) = 6.5 Hz, *J*(6a,6b) = 11.9 Hz, 1H; H-6b), 3.16 (q, *J* = 7.3 Hz; CH_2, Et_3N), 1.23 ppm (t, *J* = 7.3 Hz, CH_3, Et_3N); ^{13}C NMR (100 MHz, D_2O): δ = 166.57 (C-4''), 153.04 (dd, *J*(1,P) = 6.3 Hz, *J*(1,F) = 43.4 Hz; C-1), 152.22 (C-2''), 141.98 (C-6''), 137.88 (dd, *J*(*C*F,P) = 239.5 Hz, *J*(*C*F,F) = 255.1 Hz; C-F), 103.07 (C-5''), 88.57 (C-1'), 87.84 (C-4), 83.79 (d, *J*(4',P) = 9.2 Hz; C-4'), 75.79 (C-3), 75.38 (C-2), 74.27 (C-3'), 70.93 (C-5), 70.27 (C-2'), 65.30 (d, *J*(5',P) = 5.4 Hz; C-5'), 62.72 (C-6), 47.04 (CH_2, Et_3N), 8.61 ppm (CH_3, Et_3N); ^{31}P NMR (101 MHz, D_2O): δ = −7.84 (dd, *J*(P_α,P_β) = 23.3 Hz, *J*(P_α,F) = 84.0 Hz; P_α), −11.63 ppm (d, *J*(P_α,P_β) = 22.3 Hz; P_β); ^{19}F NMR (235 MHz, D_2O): δ = −156.70 ppm (d, *J*(F,P_α) = 84.7 Hz); MS (ESI$^+$): *m*/*z*: 603 [*M*+Na]$^+$; HRMS: *m*/*z*: calcd for $C_{16}H_{23}O_{16}FN_2P_2Na$: 603.0405; found 603.0385.

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