Synthesis of a novel fluorinated phosphonyl *C*-glycoside, (3-deoxy-3-fluoro-β-D-glucopyranosyl)methylphosphonate, a potential inhibitor of β-phosphoglucomutase

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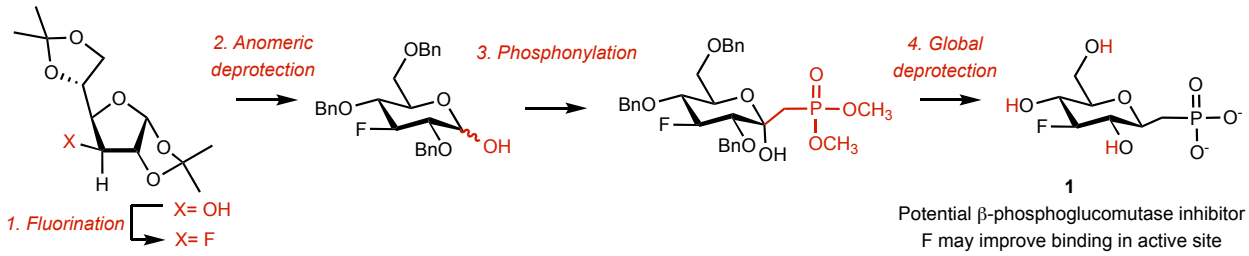

Abstract: β-phosphoglucomutase (βPGM) catalyzes the conversion of β-glucose 1-phosphate (βG1P) to glucose-6-phosphate (G6P), a universal source of cellular energy, in a two-step process. Transition state analogue (TSA) complexes formed from substrate analogues and a metal fluoride (MgF_3^- and AlF_4^-) enable analysis of each of these enzymatic steps independently. Novel substrate analogues incorporating fluorine offer opportunities to interrogate the enzyme mechanism using 19F NMR spectroscopy. Herein, the synthesis of a novel fluorinated phosphonyl *C*-glycoside (3-deoxy-3-fluoro-β-D-glucopyranosyl)methylphosphonate (**1**), in 12 steps (0.85 % overall yield) is disclosed. A four-stage synthetic strategy was employed, involving: 1) fluorine addition to the monosaccharide, 2) selective anomeric deprotection, 3) phosphonylation of the anomeric centre, and 4) global deprotection. Analysis of βPGM and **1** will be reported in due course.

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1. Introduction

β-phosphoglucomutase (βPGM) catalyzes the conversion of β-glucose 1-phosphate (βG1P) to glucose-6-phosphate (G6P), a universal source of cellular energy [1,2]. Upon binding βG1P, the structure of βPGM changes from its "cap-open" to "cap-closed" conformation [3, Figure 1A]. A phosphorylated aspartic acid residue then transfers its phosphate onto the C6 alcohol of βG1P, forming the β-glucose-1,6-bisphosphate (βG16BP) intermediate. βG16BP reorients itself in the enzyme active site to allow for dephosphorylation of C1 by the aspartic acid residue. To study the two-step mechanism of this transformation, transition state analogue (TSA) complexes have been prepared using a substrate analogue and a metal fluoride [4,5, Figure 1B]. MgF_3^- and AlF_4^- are used to mimic the charge and geometry of the transferred phosphate [6,7, Figure 1C]. Previously in the Jakeman lab, a mutant 5-fluorotryptophan (5FW) labeled β-PGM (5FW-W216F 5FWβPGM) was prepared that incorporates a ^{19}F probe directly into the enzyme to enable monitoring of the transition state analogue complexes by ^{19}F NMR spectroscopy, and to compare the molar quantities of protein and ligand in a single spectrum [5]. To study the first transition state, it was not possible to use βG1P as a substrate as it is readily turned over by the enzyme [8]. Instead, synthetic sugars β-D-glucopyranosylmethylphosphonate (βG1CP) and 1-β-phosphonofluromethylene-1-deoxy-D-glucopyranose (βG1CF$_s$P) were utilized as substrates to probe the transition state of the first step (TSA1). TSA1 complexes of these substrates with 5FW- W216F 5FWβPGM and MgF_3^- or AlF_4^- were observed by ^{19}F NMR spectroscopy. In the spectra, several fluorine resonances were observed for the enzyme, indicating that it exists in a mixture of its cap-open and cap-closed conformations [5].

In the current work, a novel fluorinated phosphonyl *C*-glycoside (3-deoxy-3-fluoro-β-D-glucopyranosyl)methylphosphonate, ammonium salt (**1**), was synthesized and fully characterized. This compound is structurally similar to the previously studied 5FW- W216F 5FWβPGM substrate G1CP [5,9], with the only difference being a fluorine atom in place of the hydroxyl group on C3 of G1CP. C1 phosphonates (eg. G1CP) are stable against phosphorylation by βPGM unlike the analogous phosphates (eg. βG1P) [4]. Fluorine is an effective bioisostere for a hydroxyl group, with fluorinated sugar derivatives often been used to inhibit or probe the mechanism of enzymes [10,11]. Incorporation of fluorine into the substrate provides an additional signal which can be monitored via ^{19}F NMR spectroscopy during complexation studies. This substitution of a hydroxyl group for a fluorine will potentially lead to better binding in the active site, due to the increased electronegativity of fluorine leading to improved hydrogen bonding with residues in this area of the active site and a different ratio of cap-open and cap-closed conformations[4]. Future work will include formation of the **1**-W216F 5FWβPGM complex. If the binding of **1** in the active site is stronger, a complex more stable than those studied previously would be expected to form [5] and therefore greater conversion of cap-open to cap-closed enzyme would be observed in the ^{19}F NMR spectrum of the **1**-W216F 5FWβPGM TSA complex than observed for the TSA complexes with G1CP and G1CF$_s$P [5].

The synthesis of a small library of α-deoxyfluoro hexopyranosyl 1-phosphates (including deoxyfluorination at C2, C3, C4, or C5) has been reported [12]. However, few examples of fluorinated methylenephosphonate glucosides have been prepared, consisting only of substrates fluorinated at the C1' position [4,9]. Thus, the synthesis of a C3-deoxyfluorinated methylenephosphonate glucoside (**1**) will fill a gap in existing libraries of fluorinated glucosides.

2. Results and Discussion

The synthetic scheme for **1** was devised based on methods which were successful in previous syntheses of fluorinated phosphates and phosphonates [4,9,12-17]. Several reviews have been published on the syntheses and biological properties of fluorosugars [18-22] as well as phosphonylated *C*-glycosides [23, 24]. Eight fluorinated α-D-glucopyranosyl 1-phosphate analogues were previously synthesized using a four-stage synthetic strategy: 1) fluorine addition to the monosaccharide, 2) selective anomeric deprotection, 3) phosphorylation of the anomeric centre, and 4) global deprotection [12]. A similar synthetic strategy was employed in the synthesis of **1**, apart from the third step being phosphonylation rather that phosphorylation. Diacetone-D-glucose was selected as the starting material because of the accessibility of the 3-OH for fluorination. After fluorination, the monosaccharide was converted into a protected fluorinated gluconolactone, in preparation for phosphonate anion addition. Subsequently, a Barton-McCombie radical deoxygenation successfully reduced the anomeric centre to enable global deprotection. These key transformations were used to design a twelve-step synthesis for (3-deoxy-3-fluoro-β-D-glucopyranosyl)methylphosphonate, ammonium salt (**1**, Scheme 1).

Compounds **2-5** were synthesized as previously described in the literature [25-29], whereas **6-14** and **1** were novel compounds. The first stage of the synthesis involved installation of the fluorine at C3. To introduce this functionality in the equatorial position, the stereochemistry at C3 of diacetone-D-glucose had to be inverted. This was accomplished by first oxidizing the C3 hydroxyl using Dess-Martin Periodinane (DMP) in dichloromethane with sodium bicarbonate, producing **2,** followed by reduction using sodium borohydride in ethanol-water, thus inverting the stereochemistry and yielding **3** (94 % over two steps, Scheme 1). The fluorine was then incorporated into **3** either by fluoro-de-hydroxylation using diethylaminosulfur trifluoride (DAST) in dichloromethane-pyridine (45 % yield) or by consecutive nucleophilic substitution

reactions using triflic anhydride followed by tetrabutylammonium fluoride (TBAF), giving **4** (46 % yield). In the second set of transformations, the furanose **4** would be converted to a pyranose ring and the anomeric hydroxyl group would be protected. We planned to protect the C2, C4, and C6 hydroxyl groups as benzyl ethers and required a protecting group for the C1 hydroxyl group which would be deprotected under different conditons, thus we initially selected a methoxy group for C1 protection. After all of the molecule's hydroxyl groups were protected, the methoxy group could be deprotected selectively under acidic conditions, leaving a single hydroxyl group available for phosphonylation. The transformation of **4** to the C1-protected pyranose was accomplished by refluxing in methanol with acetyl chloride, yielding **5** (66 %, Scheme 2). The three benzyl protecting groups on C2, C4, and C6 were then introduced to **5** using sodium hydride and benzyl bromide in dry dimethyl formamide (DMF) with tetrabutylammonium iodide (TBAI) as a catalyst, providing **6** (72 %). The methyl glucoside **6** proved recalcitrant to selective deprotection of the methyl ether using literature conditions of sulfuric acid in acetic acid at 85°C [30,31] or variations thereof; therefore an alternate protecting group was introducted offering orthogonal deprotection opportunities (Scheme 1). For this route, we forewent the methoxy group and selected an allyl ether for C1 protection instead. Allyl ethers require different deprotection conditions from benzyl ethers and are relatively easy to remove [32]. Using hydrochloric acid in allyl alcohol, the acetal **4** was converted to a pyranose ring and the allyl group was introduced at the anomeric position, giving **7** (38 %). The three benzyl protecting groups were introduced using the same method described for the production of **6**, giving **8** (61 %). In the third stage of the synthesis, the anomeric allyl group was selectively deprotected without affecting the benzyl ethers. This was accomplished by isomerizing the allyl group to a prop-1-en-1-yl group followed by hydrolysis (in one pot), using

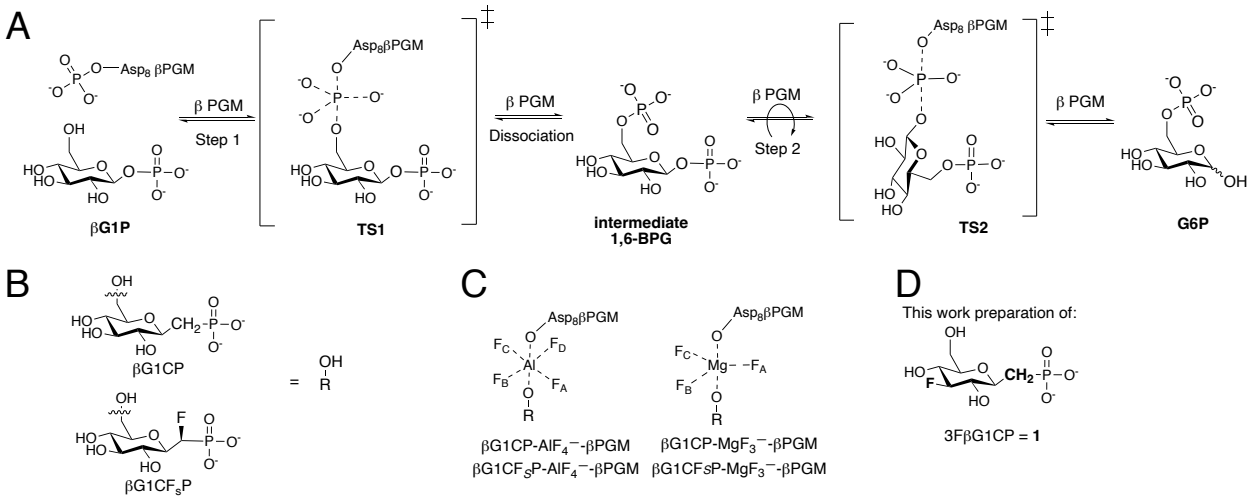
tetrakis(triphenylphosphine)palladium(0) as a catalyst along with acetic acid as both co-catalyst and solvent, to give **9** (54 %). The fourth stage was introduction of the phosphono group to the anomeric position. This transformation was performed by oxidizing **9** to the gluconolactone **10** (97 %) using DMP in dichloromethane. This was followed by treating dimethyl methylphosphonate with *n*-butyllithium, generating a phosphonate nucleophile *in situ* which then underwent addition to the lactone of **10**, furnishing **11** (58 %). The axial hydroxyl group which formed during the phosphonylation was unwanted in the final product, therefore the next step was to reduce it. Triethyl silane reduction of the hemi-acetal was unsuccessful and consequently a two-step method was implemented. First, an oxalate ester was formed at the anomeric position of **11** by addition of methyl chlorooxoacetate in CH_2Cl_2-pyridine. Second, azobisisobutyronitrile (AIBN) and tributyltin hydride in refluxing toluene were used to convert the crude oxalate ester **12** to the C1 radical, which terminated as a mixture of unsaturated and saturated phosphonates **13** and **14** (28 %). **13** was formed diastereoselectively as the *Z* isomer, which was deteremined by analysis of the alkenyl coupling constants, consistent with previously reported *Z*-configured phosphonylated *exo*-glycals [33]. The **13**/**14** mixture was hydrogenated over a period of four hours using H_2 gas (1 atm) and catalytic palladium on carbon in order to saturate the double bond of **13**, converting the entire mixture to **14** without deprotecting the benzyl ethers. **14** was obtained regioselectively as the "β"-C-glycoside, congruent with reported hydrogenations of phosphonylated *exo*-glycals [9,13,17,33]. **13**/**14** were recovered several times after failed hydrogenation attempts, leading to residual loss which negatively impacted the yield of the final step. Now the fifth and final stage of our synthesis had been reached, requiring only global deprotection and ion-exchange to reach the final product. The benzyl groups of **14** were deprotected by treatment with trimethylsilyl iodide (TMSI) followed by deprotection of the

methyl ethers by refluxing with hydrochloric acid. The phosphonate functionality was converted to the ammonium salt form by titrating with ammonium hydroxide (pH= 8) to give the final product **1** (28 %). The yield for the final step has the potential to be improved in future syntheses as a higher yield (66 %) is reported for similar transformations in the literature [9].

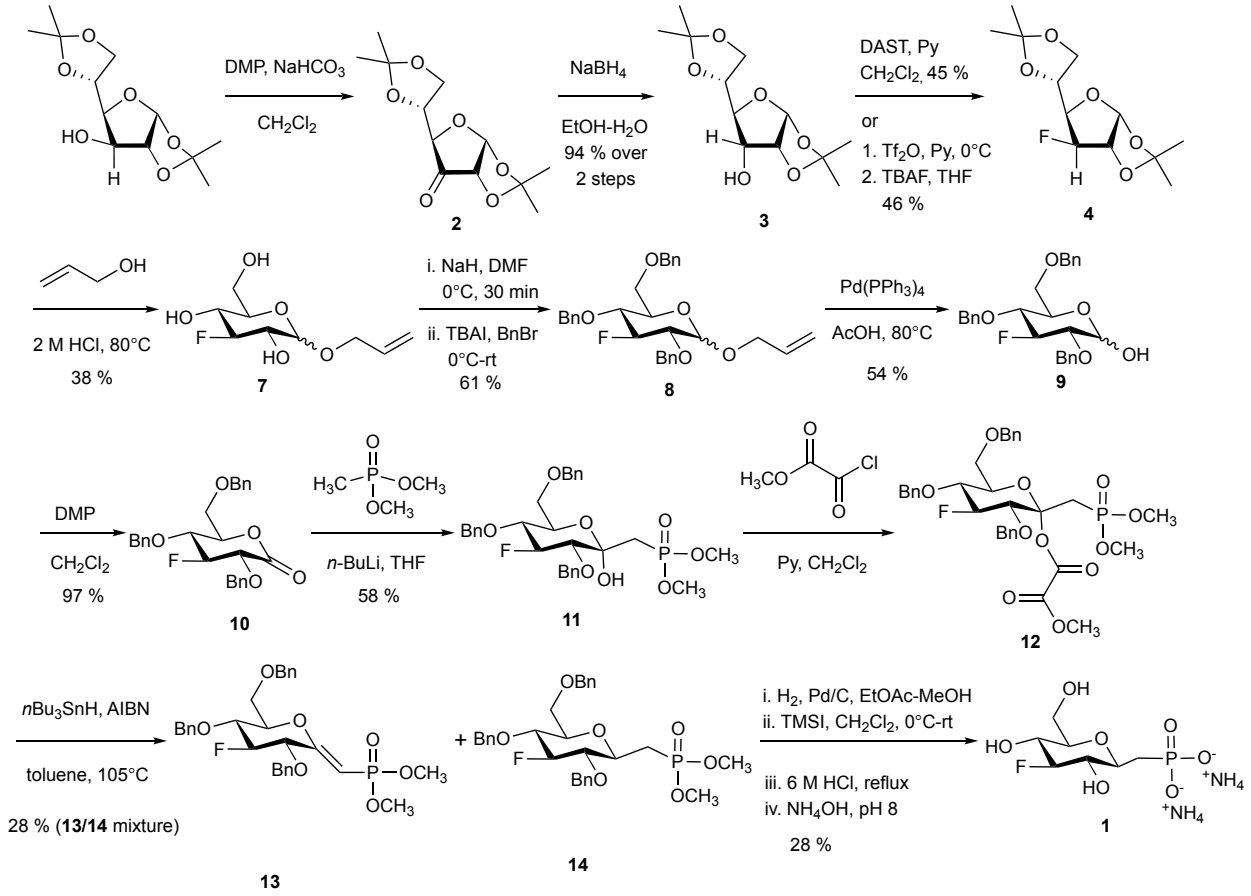
3. Conclusions

The first synthesis of **1**, a potential inhibitor of β-phosphoglucomutase, is reported in twelve steps with an overall yield of 0.85 %. In future research, **1** will be evaluated kinetically to determine whether it will inhibit the W216F mutant β-phosphoglucomutase. The formation of the transition state analogue complex between **1** and W216F 5FWβPGM will be monitored using ^{19}F NMR spectroscopy to provide valuable insight into the structure of the first transition state in the βPGM-catalyzed conversion of βG1P to G6P.

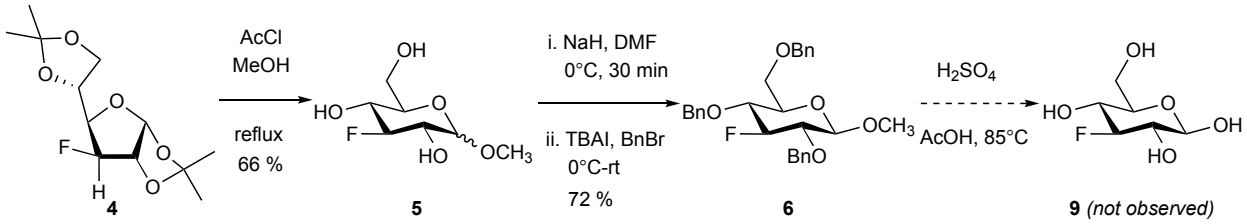
Figure 1. beta-Phosphoglucomutase mechanism, isosteric phosphonate analogues of βG1P, metal fluoride transition state analogues, and the phosphonate prepared herein. (A) Conversion of βG1P to G6P via two transition states with dissociation and rebinding of βG16BP, (B) previously synthesized phosphonates, (C) metal fluoride complexes as transition-state analogues for step 1 (TSA1) formed with (fluoro)phosphonates in B[4], (D) 3-deoxy-3-fluoro-β-D-glucopyranosyl)methylphosphonate (**1**) presented in this work.



Scheme 1. Twelve step synthesis of (3-deoxy-3-fluoro-β-D-glucopyranosyl)methylphosphonate (**1**)



Scheme 2. Preparation of the anomeric methyl ether **6** and selective deprotection attempt.



4. Experimental

4.1. General experimental methods

Reactions were performed in oven-dried glassware and monitored for completion using TLC. Reactions under an inert atmosphere were conducted using nitrogen gas. Glass-backed normal phase silica TLC plates were visualized using UV light, *p*-anisaldehyde stain, or potassium permanganate stain. NMR spectra were obtained using Bruker AV-300 or AV-500 instruments. Two-dimensional NMR spectra (COSY and HSQC for all compounds as well as HMBC for **10**) were used to aid in the assignment of the ^{1}H and $^{13}C\{^{1}H\}$ NMR spectra of new compounds. High resolution mass spectra were acquired by Xiao Feng on a Bruker microTOF Focus Mass Spectrometer, using an ESI^{+} or ESI^{-} source.

4.2. Synthetic procedures and spectroscopic data

1,2:5,6-di-*O*-isopropylidene-α-D-allofuranose (3)

1,2:5,6-di-*O*-isopropylidene-α-D-*xylo*-hexofuranos-3-ulose (**2**) was synthesized using the procedure from the literature [25]. The crude **2** was subjected to the next reaction without further purification. Compound **3** was synthesized following the literature procedure [25]. **3** (4.73 g) was isolated in a 94 % yield over the two steps. ^{1}H NMR (500 MHz, $CDCl_3$) spectral data matched that reported [25].

3-deoxy-3-fluoro-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (4)

Compound **4** was synthesized using a modified literature procedure [34]. Compound **3** (8.35 g, 32.1 mmol) was dissolved in pyridine (64 mL, anhydrous) and placed under an inert atmosphere. The mixture was cooled to 0°C and triflic anhydride (1.15 eq, 36.9 mmol, 6.18 mL) was added slowly. The reaction mixture was stirred until complete conversion was observed via TLC (1 hour), then it was diluted with ethyl acetate (145 mL) and washed with $NaHCO_3$ (sat. aq, 145 mL) and brine (80 mL). The organic phase was dried using $MgSO_4$ and then concentrated *in*

vacuo. The residue was subjected directly to the next reaction without further purification. The crude triflate was placed under an inert atmosphere and TBAF (1 M in THF, 3 eq, 96.3 mmol, 96.3 mL) was added. It was stirred for 42.5 hours (at rt for the first 19 hours, then the temperature was increased to 60°C for the remaining reaction time). The reaction mixture was cooled to 0°C and diluted with Et_2O (300 mL), washed with NH_4Cl (sat. aq, 3 x 200 mL) and brine (200 mL). The organic phase was dried using $MgSO_4$ and then concentrated *in vacuo*. The residue was lyophilised, taken up in Et_2O, and filtered to remove tetrabutylammonium salts. The filtrate was concentrated *in vacuo*, yielding **4** (3.83 g, 46%). ^{19}F NMR (470 MHz, $CDCl_3$) δ = -207.7 (ddd, $^3J_{H2\text{-}F}$= 10.6 Hz, $^3J_{H4F}$= 29.2 Hz, $^2J_{H3\text{-}F}$= 49.9 Hz); ^1H NMR (500 MHz, $CDCl_3$) spectral data matched that reported [27].

Methyl 3-deoxy-3-fluoro-α/β-D-glucopyranoside (5)

Compound **5** was prepared following a modified literature procedure [29]. Acetyl chloride (4.3 mL) and methanol (20 mL, anhydrous) were placed under an inert atmosphere, cooled to 0°C, and stirred for 30 minutes. Then, a solution of **4** (692 mg, 2.64 mmol) in methanol (20 mL, anhydrous) was added. The reaction mixture was stirred at reflux (70°C, oil bath) under an inert atmosphere for 4 hours. Then, the reaction mixture was cooled to room temperature and concentrated *in vacuo*. Silica gel column chromatography in 9 CH_2Cl_2: 1 MeOH was used to isolate **5** (341 mg, 66 %, 2α:1β as determined by integration of the ^1H NMR spectrum). ^{19}F NMR (470 MHz, $CDCl_3$) δ= -196.6 (dt, $^3J_{H\text{-}H}$= 13.3 Hz, $^2J_{H\text{-}F}$= 53.0 Hz, β-anomer), -200.6 (dt, $^3J_{H\text{-}H}$= 13.3 Hz, $^2J_{H\text{-}F}$= 54.2 Hz, α anomer); ^1H NMR (500 MHz, $CDCl_3$) of the α anomer matched the reported spectral data [35]; for the β-anomer: ^1H NMR (500 MHz, $CDCl_3$) δ= 4.41 (dt, $^2J_{H\text{-}F}$= 53.1 Hz, $^3J_{H\text{-}H}$= 8.8 Hz, 1Hβ, H3β), 4.26 (d, $^3J_{H1\text{-}H2}$= 7.8 Hz, 1Hβ, H1β), 3.97-3.62 (m, includes 4Hβ, H2β, H4β, H6aβ, H6bβ, overlaps with α anomer signals), 3.58 (s, 3Hβ, OCH_3β). In the ^1H

NMR signals from 0.00 to 2.50 ppm were impurities that were not successfully removed upon column chromatography.

Methyl 2,4,6-tri-*O*-benzyl-3-deoxy-3-fluoro-β-D-glucopyranoside (6)

Compound **6** was synthesized following a benzylation procedure for a similar compound [36]. Compound **5** (64 mg, 0.33 mmol) was dissolved in DMF (7 mL, anh.). The mixture was cooled to 0°C, and NaH was added (60 % in mineral oil, 4 eq, 1.32 mmol, 52.8 mg), followed by stirring for 30 minutes at this temperature. Tetrabutylammonium iodide (TBAI, 0.01 eq, 3.3 µmol, 1.2 mg) was added and the reaction mixture was placed under an inert atmosphere. Benzyl bromide (4 eq, 1.32 mmol, 157 µL) was added dropwise to the mixture at 0°C. The reaction mixture was stirred at room temperature overnight and then cooled to 0°C. MeOH (1.6 mL) was added dropwise to quench. Extracted the reaction mixture with water (25 mL) and washed with ethyl acetate (3 x 25 mL). The organic layers were combined and washed with brine (25 mL). The organic fraction was dried over $MgSO_4$ and the solvent was removed *in vacuo*. Compound **6** was purified using silica gel column chromatography in 7 hexanes: 3 ethyl acetate (110 mg, 72 %). ^{19}F NMR (470 MHz, $CDCl_3$) δ= -188.7 (dt, $^3J_{H-H}$= 14.4 Hz, $^2J_{H-F}$ =52.1 Hz); ^1H NMR (500 MHz, $CDCl_3$) δ= 7.44 (m, 15H, aromatic), 5.00-4.87 (m, CH_2), 4.78 (dt, $^3J_{H-H}$= 8.8 Hz , $^2J_{H-F}$= 51.9 Hz, H3), 4.75-4.64 (m, CH_2), 4.40 (d, J= 7.8 Hz, 1H, H1), 3.85 (m, 3H, H4, H6a, H6b), 3.68 (s, 3H, OCH_3), 3.62 (dt, J= 8.4 Hz, 16.7 Hz, 1H, H2), 3.54 (m, 1H, H5); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ= 138.3-138.0 (3 x OCH_2-Ph), 128.5-127.8 (15 x Ph) 103.8 (d, $^3J_{CF}$=12.3 Hz, C1), 98.1 (d, $^1J_{CF}$= 185.3 Hz, C3), 79.8 (d, $^2J_{CF}$= 17.7 Hz, C2), 76.2 (d, $^2J_{CF}$= 17.4 Hz, C4), 74.44 (2 x CH_2), 73.6-73.5 (1 x CH_2 + C5), 68.6 (C6), 57.3 (OCH_3); HRESIMS$^+$ (m/z): calcd for $C_{28}H_{31}FNaO_5$ [**12**+Na]$^+$: 489.2053, found: 489.2055.

Allyl 3-deoxy-3-fluoro-α/β-D-glucopyranoside (7)

Compound **7** was synthesized using a literature allylation procedure along with isopropylidene deprotection conditions [37,38]. Compound **4** (456 mg, 1.74 mmol) was dissolved in allyl alcohol (7.0 mL) and placed under an inert atmosphere. Concentrated HCl (1.39 mL, 2 M) was added and the reaction mixture was stirred at 80ºC (oil bath) for 3 hours. The mixture was then cooled to room temperature and neutralized using $NaHCO_3$ (s), stirred for 20 minutes, and filtered. The filtrate was concentrated *in vacuo* and the residue was purified via silica gel column chromatography in CH_2Cl_2-MeOH (93:7 followed by 4:1) to give **7** (147 mg, 38 %, 1α:1β as determined by integration of the ^{1}H NMR spectrum). ^{19}F (470 MHz, $CDCl_3$) δ= -196.1 (dt, $^3J_{H\text{-}F}$=13.7 Hz, $^2J_{H3\text{-}F}$= 52.6 Hz), -200.3 (dt, $^3J_{H\text{-}F}$=13.0 Hz, $^2J_{H3\text{-}F}$= 54.3 Hz); ^{19}F{^{1}H} NMR (470 MHz, CD_3OD) δ= -195.7, -200.0; for the α-anomer: ^{1}H NMR (500 MHz, CD_3OD) δ= 5.99 (m, 1H, C<u>H</u>=CH_2), 5.37 (m, 1H, CH=C<u>H</u>$_2$), 5.21 (m, 1H, CH=C<u>H</u>$_2$), 4.91 (t, $^3J_{H1\text{-}H2}$= 3.5 Hz, 1H, H1α), 4.54 (dt, $^3J_{H\text{-}F}$= 8.7 Hz, $^2J_{H3\text{-}F}$= 54.5 Hz, 1H, H3α), 4.43-4.05 (m, 2H, O-C<u>H</u>$_2$-CH), 3.94-3.56 (m, 4H, H2, H4, H6a, H6b), 3.47 or 3.30 (m, 1H, H5α); for the β-anomer: ^{1}H NMR (500 MHz, CD_3OD) δ= 5.99 (m, 1H, C<u>H</u>=CH_2), 5.37 (m, 1H, CH=C<u>H</u>$_2$), 5.21 (m, 1H, CH=C<u>H</u>$_2$), 4.43-4.05 (m, 3H, H3β, O-C<u>H</u>$_2$-CH), 4.37 (d, J= 7.9 Hz, 1H, H1β), 3.94-3.56 (m, 4H, H2, H4, H6a, H6b), 3.47 or 3.30 (m, 1H, H5β); ^{13}C{^{1}H} NMR (125 MHz, CD_3OD) δ= 135.5 and 135.2 (2 x s, 2 x <u>C</u>H=CH_2), 117.7 and 117.6 (2 x s, 2 x CH=<u>C</u>H$_2$), 102.5 (d, J= 12.2 Hz, C1), 99.2 (d, J= 10.5 Hz, C1), 98.4 (d, J= 183.1 Hz, C3), 96.6 (d, J= 180.6 Hz, C3), 76.5 (d, J= 8.1 Hz, C5), 73.5 (d, J= 17.9 Hz, C2 or C4), 73.2 (d, J= 7.2 Hz, C5), 71.7 (d, J= 17.2 Hz, C2 or C4), 71.1 (s, O-C<u>H</u>$_2$), 69.8 (d, J= 14.1 Hz, C4 or C2), 69.6 (d, J= 14.3 Hz, C4 or C2), 69.4 (s, O-C<u>H</u>$_2$), 62.2 and 62.1 (2 x s, 2 x C6); HRESIMS$^+$ (m/z): calcd for $C_9H_{15}FNaO_5$ [**13**+Na]$^+$: 245.0801, found: 245.0801.

Allyl 2,4,6-tri-*O*-benzyl-3-deoxy-3-fluoro-α/β-D-glucopyranoside (8)

Compound **8** was synthesized from **7** following the same literature procedure used for the synthesis of **6**. Compound **7** (1.49 g, 6.69 mmol) was dissolved in DMF (30 mL, anh.), cooled to 0°C, and NaH was added (60 % in mineral oil, 5 eq, 33.5 mmol, 1.34 g), followed by stirring for 30 minutes. Tetrabutylammonium iodide (TBAI, 0.01 eq, 67 µmol, 25 mg) was added and the reaction mixture was placed under an inert atmosphere. Benzyl bromide (5 eq, 33.5 mmol, 3.98 mL) was added dropwise at 0°C. The reaction mixture was stirred at room temperature overnight and then cooled to 0°C. MeOH (24 mL) was added dropwise to quench. H_2O (240 mL) was added to the reaction mixture, followed by washing with ethyl acetate (3 x 180 mL). The combined the organic layers were washed with brine (180 mL). The organic phase was dried over $MgSO_4$ and concentrated *in vacuo*. Compound **8** was purified using silica gel column chromatography in 10 hexanes: 1 ethyl acetate (1.51 g, 61 %, 1α:1β was maintained upon benzylation). $^{19}F\{^1H\}$ NMR (470 MHz, $CDCl_3$) δ= -188.7, -192.8; ^1H NMR (500 MHz, $CDCl_3$) δ= 7.34 (m, 16H, 15 Ph, $CHCl_3$), 6.00 (m, 1H, C<u>H</u>=CH_2), 5.41 (m, 1H, CH=C<u>H</u>$_2$), 5.28 (m, 1H, CH=C<u>H</u>$_2$), 5.15-4.48 (m, 9H, 6 Ph-C<u>H</u>, H3, H1, solvent/impurity), 4.21 (m, 1H, O-C<u>H</u>$_2$-CH), 4.06 (m, 1H, O-C<u>H</u>$_2$-CH), 3.87-3.47 (m, 5H, H2, H4, H5, H6a, H6b); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ= 138.2 (3 x quaternary Ph), 134.1 and 133.7 (<u>C</u>H=CH_2), 128.6-127.9 (15 x tertiary Ph), 118.2 and 117.5 (CH=<u>C</u>H$_2$), 101.9 and 96.4 (2 x d, J= 12.0 and 11 Hz, C1), 98.2 and 96.5 (2 x d, J= 184 and 183 Hz, C3), 79.9 (d, J= 17.5 Hz, C2), 77.4 and 77.1 (2 x d, J= 31 Hz, C4), 76.2, 74.6, 70.5, and 69.6 (C<u>H</u>$_2$-Ph), 73.7 and 73.2 (C5), 68.6 and 68.4 (d, J= 27 and 36 Hz, C6); HRESIMS$^+$ (m/z): calcd for $C_{30}H_{33}FNaO_5$ [**14**+Na]$^+$: 515.2210, found: 515.2191.

2,4,6-tri-*O*-benzyl-3-deoxy-3-fluoro-α/β-D-glucopyranoside (9)

Compound **9** was synthesized following a literature procedure for allyl deprotection [39]. Compound **8** (96 mg, 0.19 mmol) was dissolved in glacial acetic acid (7.6 mL). $Pd(PPh_3)_4$ (0.3

eq, 59 µmol, 68 mg) was added to the reaction mixture and the flask contents were placed under an inert atmosphere with stirring at 80°C (oil bath) for 23 hours. The reaction mixture was concentrated *in vacuo* and the residue was taken up in H_2O (45 mL), extracted with ether (3 x 25 mL), and the organic fractions combined. The organic phase was washed with $NaHCO_3$ (sat. aq, 22 mL) and brine (22 mL), dried with $MgSO_4$, and concentrated *in vacuo*. Compound **9** was purified using silica gel column chromatography in 7 hexanes: 1 ethyl acetate (48 mg, 54 %, 4α:1β as determined by integration of the ^{19}F NMR spectrum). ^{19}F NMR (470 MHz, $CDCl_3$) δ= -189.0 (dt, $^3J_{H-F}$= 13.7 Hz, $^2J_{H-F}$= 51.8 Hz, β anomer), -193.8 (dt, $^3J_{H-F}$= 13.1 Hz, $^2J_{H-F}$= 53.5 Hz, α anomer). ^1H NMR (500 MHz, $CDCl_3$) (assignments are for the major α anomer) δ= 7.34 (15H, m, Ph), 5.27 (1H, t, J= 3.7 Hz, H1), 4.99 (1H, dt, $^3J_{H-H}$ = 8.7 Hz, $^2J_{H-F}$= 53.6 Hz, H3), 4.88, 4.86, 4.73, 4.64, 4.55, 4.52 (6H, 6 x d, $^2J_{H-H}$= 10-12 Hz, 6 x O-C$\underline{H}_2$-Ph), 3.59-3.82 (3H, m, H5, H6a, H6b), 3.77 (1H, m, H4), 3.67 (1H, m, H2); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) (assignments are for α anomer) δ= 138.1 (3 x s, 3 x quaternary Ph), 128.9-127.9 (m, 15 x Ph), 96.8 (d, J= 183 Hz, H3), 92.0 (d, J= 11 Hz, C1), ~77 (obscured by $CDCl_3$ peak, H2), 76.0 (d, J= 17.0 Hz, H4), 74.7, 73.5, 73.8 (3 x s, 3 x CH_2), 69.7 (d, J= 8.6 Hz, C5), 68.4 (s, C6); HRESIMS$^+$ (m/z): calcd for $C_{27}H_{29}FNaO_5$ [**15**+Na]$^+$: 475.1897, found: 475.1884.

2,4,6-tri-*O*-benzyl-3-deoxy-3-fluoro-D-gluconolactone (10)

Compound **10** was initially synthesized by following a literature procedure for Swern oxidation [40,41]. Purified via silica column chromatography in 3 hexanes: 1 ethyl acetate to give **10** (9 mg, 9 %). An improved synthesis of **10** was later performed using Dess-Martin Periodinane (DMP) in CH_2Cl_2 [42]. Compound **9** (243 mg, 0.539 mmol) was combined with DMP (0.3 M in CH_2Cl_2, 1.5 eq, 0.809 mmol, 2.70 mL) under an inert atmosphere. The reaction mixture was stirred for 24 hours at room temperature, then diluted with Et_2O (11 mL) and washed with

$Na_2S_2O_3$ (sat. aq, 7.5 mL). The aqueous layer was extracted with Et_2O (3 x 6 mL). The combined the organic extracts were washed with water (10 mL) and brine (6 mL), dried with $MgSO_4$, and concentrated *in vacuo.* The product was purified by taking up in Et_2O (16 mL), filtering off the precipitate, and concentrating the organics *in vacuo*. Performed the purification procedure a second time, using minimal Et_2O (12 mL). Compound **10** was then isolated (235 mg, 97 %, contains a small amount of DMP and/or iodinane byproduct). $^{19}F\{^1H\}$ NMR (470 MHz, $CDCl_3$) δ= -183.5; ^1H NMR (500 MHz, $CDCl_3$) δ= 7.35 (15H, m, Ph), 5.02, 4.85, 4.77, 4.60, 4.59, 4.50 (6H, 6 x d, J= 11-12 Hz, 6 x O-C$\underline{H}_2$-Ph), 4.92 (1H, dt, $^3J_{H-H}$= 7.2 Hz, $^2J_{H-F}$= 51.0 Hz, H3), 4.39 (1H, dt, $^3J_{H5-H4}$= 8.6 Hz, $^3J_{H5-H6}$= 2.5 Hz, H5), 4.21 (1H, dd, $^3J_{H2-H3}$= 7.3 Hz, $^3J_{HF}$= 15.6 Hz, H2), 4.12 (1H, ddd, $^3J_{H4-H3}$= 7.2 Hz, $^3J_{H4-H5}$= 8.5 Hz, $^3J_{H-F}$= 16.3 Hz, H4), 3.75 (2H, m, $H6_{a\&b}$); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ= 168.0 (s, C1), 136.5, 137.0, 137.3 (3 x s, 3 x quaternary Ph), 128.2 (m, 15 x Ph), 93.8 (d, $^1J_{C-F}$=181 Hz, H3), 77.3 (overlapping with $CDCl_3$ peak, C5), 75.7 (d, $^2J_{C-F}$= 23.9 Hz, C2), 74.5 (d, $^2J_{C-F}$= 20.8 Hz, C4), 74.0, 73.7, 73.6 (3 x s, 3 x OCH_2), 67.7 (s, $H6_{a\&b}$); HRESIMS$^+$ (m/z): calcd for $C_{27}H_{27}FNaO_5$ [**16**+Na]$^+$: 473.1740, found: 473.1719.

3,5,7-tri-*O*-benzyl-1,4-dideoxy-1-dimethoxyphosphoryl-4-fluoro-α-D-gluco-2-heptulopyranose (11)

Compound **11** was synthesized following a literature procedure for phosphonylation [9]. Dimethyl methylphosphonate (distilled, 3 eq, 4.87 mmol, 0.53 mL) was combined with THF (anh., 15.5 mL) under an inert atmosphere. The solution was cooled to -78°C and then *n*-BuLi (2.5 M in hexanes, 3 eq, 4.87 mmol, 1.95 mL) was added gradually, over one minute. The reaction mixture was stirred for 30 minutes at -78°C. A solution of **10** (731 mg, 1.62 mmol) in THF (anh., 7.8 mL) was added. The reaction mixture was stirred for 2 h at -78°C. Then, quenched with NH_4Cl (sat. aq, 10 mL). The contents of the flask were transferred to a separatory

funnel and the two layers separated. The organic phase was washed with water (37 mL). The combined aqueous phases were washed with CH_2Cl_2 (3 x 19 mL). The combined organic phases were then dried with $MgSO_4$ and concentrated *in vacuo*. Product **11** was purified via silica gel column chromatography in 1 hexanes: 1 ethyl acetate (540 mg, 58 %). $^{31}P\{^1H\}$ NMR (202 MHz, $CDCl_3$) δ= 30.8 (s); $^{19}F\{^1H\}$ NMR (470 MHz, $CDCl_3$) δ= -192.2 (d, $^5J_{F-P}$= 4.1 Hz); ^{19}F NMR (470 MHz, $CDCl_3$) δ= -192.2 (dt, $^3J_{H-F}$= 13.0 Hz, $^2J_{H3-F}$= 54.0 Hz); ^1H NMR (500 MHz, $CDCl_3$) δ= 7.40-7.28 (15H, m, 15 x Ph), 5.81 (1H, s, OH), 5.11 (1H, dt, $^3J_{H-H}$= 8.6 Hz, $^2J_{H-F}$= 53.9 Hz, H3), 4.98, 4.87, 4.66, 4.58 (4 x 1H, 4 x d, J= 10.9-11.7 Hz, 4 x OC<u>H</u>-Ph), 4.49 (2H, s, OC<u>H</u>$_2$-Ph), 4.07 (1H, m, H5), 3.80 (1H, m, H4), 3.73 (1H, m, H6a), 3.68, 3.65 (2 x 3H, 2 x d, J= 4.8 Hz, P(O)(OC<u>H</u>$_3$)$_2$), 3.61 (1H, m, H6b), 3.36 (1H, dd, $^3J_{H2-H3}$= 9.1 Hz, $^3J_{H-F}$= 11.7 Hz, H2), 2.46 (1H, dd, J= 15.3Hz, J= 17.5 Hz, H1'a), 1.18 (1H, dd, J= 15.2 Hz, J= 18.6 Hz, H1'b); $^{13}C\{^1H\}$ NMR (125 MHz, $CDCl_3$) δ= 138.2, 138.0, 137.7 (3 x s, 3 x quaternary Ph), 129.0-127.9 (m, 15 x tertiary Ph), 98.2 (dd, $^2J_{C-F}$= 180 Hz, $^4J_{C-P}$= 4.3 Hz, H3), 97.1 (dd, J= 7.9 Hz, 12.2 Hz, C1), 80.3 (t, J= 15.0 Hz, C2), 76.3 (d, J= 16.9 Hz, C4), 74.6, 74.4, 73.6 (3 x O<u>C</u>H$_2$Ph), 70.2 (d, J= 8.7 Hz, C5), 68.4 (s, C6), 53.6, 52.1 (2 x d, J= 5.6 Hz and J= 6.4 Hz respectively, 2 x P-O<u>C</u>H$_3$), 32.7 (d, J= 136.0 Hz, C1'); HRESIMS$^+$ (m/z): calcd for $C_{30}H_{36}FNaO_8P$ [**17**+Na]$^+$: 597.2030, found: 597.2017.

3,5,7-tri-*O*-benzyl-1,4-dideoxy-1-dimethoxyphosphoryl-2-methyloxalate-4-fluoro-α-D-gluco-2-heptulopyranose (12)

Compound **12** was synthesized following a literature procedure for the synthesis of a similar compound [3]. Compound **11** (71.4 mg, 0.124 mmol) was dissolved in CH_2Cl_2 (anh., 180 µL) and pyridine (anh., 50 µL). Methyl chlorooxoacetate (10 eq, 1.24 mmol, 114 µL) was added dropwise while stirring vigorously. The reaction mixture was stirred overnight at room

temperature, under an inert atmosphere. EtOH (40 μL) was added to the reaction mixture and the solution was stirred for 10 minutes. The mixture was diluted with ethyl acetate (4 mL), washed with $NaHCO_3$ (sat. aq, 5 mL), water (4 mL), and brine (3 mL). The organic phase was dried with anhydrous $MgSO_4$ and then concentrated *in vacuo* to give **12** (crude: 70 mg, 85 %). The residue was directly subjected to the next reaction without further purification. $^{31}P\{^1H\}$ NMR (202 MHz, $CDCl_3$) δ= 24.4 (s); $^{19}F\{^1H\}$ NMR (470 MHz, $CDCl_3$) δ= -193.9 (s); HRESIMS$^+$ (m/z): calcd for $C_{33}H_{38}FNaO_{11}P$ [**18**+Na]$^+$: 683.2, found: 683.2.

[1(1')*Z*]-2,4,6-tri-*O*-benzyl-1,3-deoxy-3-fluoro-1-(dimethoxyphosphoryl)methylidene-D-glucopyranose (13) and dimethyl *C*-(2,4,6-tri-*O*-benzyl-3-deoxy-3-fluoro-β-D-glycopyranosyl)methylphosphonate (14)

Compounds **13/14** were synthesized using conditions from the literature for a radical based substitution reaction with an oxalate ester [4]. Compound **12** (0.145 mmol) was combined with AIBN (0.19 eq, 2.75 x 10^{-2} mmol, 4.5 mg) and dissolved in toluene (anh., 5.4 mL) under an inert atmosphere. Tributyl tin hydride (2 eq, 0.290 mmol, 78 μL) was added, and the reaction mixture was refluxed for 1 hour at 105°C (oil bath) under an inert atmosphere. The reaction mixture was cooled to room temperature and quenched with water (12 mL). It was then extracted with ethyl acetate (3 x 6 mL). The combined organic phases were dried with anhydrous $MgSO_4$ and then concentrated *in vacuo*. The product was purified via silica gel-KF (10 % KF w/w) column chromatography [43] (49 CH_2Cl_2: 1 MeOH) to give a mixture of **13** and **14** (23 mg, 28 %). This chromatographic procedure was selected as a column using a silica gel-KF stationary phase has been reported to reduce organotin impurities to a level which is undetectable by ^1H NMR [43]. Upon running the column, the excess tributyl tin hydride reacts in a nucleophilic substitution reaction with KF, producing tributyl tin fluoride, which is insoluble in organic solvents, thus

allowing for the chromatographic separation of these impurities from the organic-soluble products [44]. A fraction of pure **13** was isolated: $^{31}P\{^{1}H\}$ NMR (202 MHz, $CDCl_3$) δ= 21.9 (s); $^{19}F\{^{1}H\}$ NMR (470 MHz, $CDCl_3$) δ= -177.8 (s); ^{1}H NMR (500 MHz, $CDCl_3$) δ= 7.26-7.39 (17H, m, $CHCl_3$ and 15 x Ph), 5.57 (1H, dd, $^{5}J_{H1\text{-}H3}$= 1.4 Hz, $^{2}J_{H\text{-}P}$= 13.4 Hz, H1), 5.17 (1H, d, $^{3}J_{H2\text{-}H3}$= 6.8 Hz, H2), 4.99 (1H, dt, $^{3}J_{HH}$= 2.6 Hz, $^{2}J_{H\text{-}F}$= 46.9 Hz, H3), 4.79 (2H, s, OC<u>H</u>$_2$-Ph), 4.77, 4.64, 4.57, 4.56 (4 x 1H, 4 x d, $^{2}J_{HH}$= 11.3-12.2 Hz, 4 x OC<u>H</u>-Ph), 4.60 (1H, m, H5), 3.90 (1H, ddd, $^{3}J_{HH}$= 3.4 Hz, $^{3}J_{HH}$= 10.4 Hz, $^{3}J_{HF}$= 25.6 Hz, H4), 3.80 (1H, d, $^{2}J_{HH}$= 11.2 Hz, H6a), 3.75, 3.72 (6H, 2 x d, J= 11.4 Hz, P(OC<u>H</u>$_3$)$_2$), 3.72 (d, $^{2}J_{HH}$= 11.2 Hz, H6b); $^{13}C\{^{1}H\}$ NMR (125 MHz, $CDCl_3$) δ= 165.3 (d, $^{2}J_{CP}$= 27.2 Hz, C1), 138.0, 137.8, 137.3 (3 x s, 3 x quaternary Ph), 128.6-128.0 (m, 15 x tertiary Ph), 93.0 (d, $^{1}J_{CF}$= 182.5 Hz, C3), 92.2 (d, $^{2}J_{CF}$= 20.5 Hz, C2), 76.5 (d, $^{2}J_{CF}$= 27.2 Hz, C4), 74.3 (d, $^{3}J_{CF}$= 7.5 Hz, C5), 73.7, 72.5, 71.7 (3 x s, 3 x <u>C</u>H$_2$Ph), 72.6 (d, J= 32.7 Hz, C1'), 52.3, 52.4 (2 x d, $^{2}J_{CP}$= 6.0 Hz, 2 x P(O<u>C</u>H$_3$)); HRESIMS$^+$ (m/z): calcd for $C_{30}H_{34}FNaO_7P$ [**19**+Na]$^+$: 579.1924, found: 579.1906. A second fraction with a mixture of **14** and **13** was isolated; characterization is for **14**: $^{31}P\{^{1}H\}$ NMR (202 MHz, $CDCl_3$) δ= 31.0 (s); $^{19}F\{^{1}H\}$ NMR (470 MHz, $CDCl_3$) δ= -185.3 (d, $^{5}J_{F\text{-}P}$= 3.4 Hz); Selected ^{1}H NMR (500 MHz, $CDCl_3$) δ= 2.33 (1H, ddd, J= 2.5 Hz, 15.7 Hz, 18.3 Hz, H1'a), 1.92 (1H, ddd, J= 9.2 Hz, 16.2 Hz, 25.4 Hz, H1'b); HRESIMS$^+$ (m/z): calcd for $C_{30}H_{36}FNaO_7P$ [**20**+Na]$^+$: 581.2080, found: 581.2063.

(3-deoxy-3-fluoro-β-D-glucopyranosyl)methylphosphonate, ammonium salt (15)

Compound **21** was synthesized by subsequent hydrogenation, benzyl deprotection, methyl deprotection, and ion exchange conditions from the literature [9]. First, the **13/14** mixture (18.3 mg, 3.29 x 10^{-2} mmol) was dissolved in ethyl acetate (0.5 mL) and MeOH (0.5 mL). Pd/C (10 wt. %) was added (20 mol %, 7.0 mg). The reaction mixture was degassed under vacuum (using

a Schlenk line connected to a vacuum pump) and saturated with hydrogen at atmospheric pressure. The mixture was reacted for 4 hours at room temperature, after which it was filtered through celite and washed with ethyl acetate/ MeOH (1:1). The filtrate was then concentrated *in vacuo*. ^{1}H NMR ($CDCl_3$, 500 MHz) of the residue was used to confirm complete conversion to the saturated form by the disappearance of the alkenyl H1 signal. Second, the residue was subjected to benzyl deprotection without further purification. The concentrated filtrate was combined with TMSI (1 M in CH_2Cl_2, 29 eq, 0.924 mmol, 0.95 mL) at 0^{o}C under an inert atmosphere. The reaction mixture was allowed to warm to room temperature and stirred for 4 h, after which it was quenched with MeOH (0.4 mL) and concentrated *in vacuo*. The residue was taken up in H_2O (4 mL) and washed with Et_2O (8 x 4 mL). The aqueous layer was concentrated *in vacuo* and lyophilized. ^{1}H NMR (D_2O, 500 MHz) of the residue demonstrated that benzyl deprotection had been successful by the absence of benzyl signals (both aromatic and methylene). Third, methyl deprotection was performed: HCl (6M, 1.5 mL) was added to the residue and the reaction mixture was heated at 60^{o}C (oil bath) for 6 hours. The reaction mixture was cooled to room temperature and concentrated *in vacuo*. Fourth, the residue was taken up in water (1 mL) and the pH adjusted to 8 using NH_4OH (2 M, aq.). The aqueous solution was concentrated *in vacuo* and lyophilized to obtain **15** (2.7 mg, 28 %). ^{31}P{^{1}H} NMR (202 MHz, D_2O) δ= 21.3 (s); ^{19}F{^{1}H} NMR (470 MHz, D_2O) δ= -192.8 (s); ^{19}F NMR (470 MHz, D_2O) δ= -192.8 (dt, $^{3}J_{H-F}$= 13.3 Hz, $^{2}J_{H-F}$= 53.0 Hz); ^{1}H NMR (500 MHz, D_2O) δ= 4.47 (1H, dt, $^{2}J_{HF}$= 53.3 Hz, $^{3}J_{HH}$= 8.5 Hz, H3), 3.96 (1H, d, J= 12.0 Hz, H6a), 3.75 (2H, m, H4, H6a), 3.67 (1H, m, H1), 3.57 (1H, m, H2), 3.49 (1H, m, H5), 2.23 (t, J= 17.4 Hz, H1'a), 1.90 (1H, m, H1'b); ^{13}C{^{1}H} NMR (125 MHz, D_2O) δ= 97.9 (d, $^{1}J_{C-F}$= 182.2 Hz, C3), 78.7 (d, $^{3}J_{C-F}$= 7.3 Hz, C5), 75.2 (d,

C1), 72.8 (d, $^{2}J_{C\text{-}F}$= 17 Hz, C4), 68.3 (d, $^{2}J_{C\text{-}F}$= 16.9 Hz, C2), 60.6 (s, C6), 31.1 (C1'); HRESIMS^{-} (m/z): calcd for $C_7H_{13}FO_7P^-$ [**21**-H]$^-$: 259.0388, found: 259.0377.

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Supporting information: ^{1}H NMR spectra are available for **1**, **3**-**11**, **13**-**14**. $^{13}C\{^{1}H\}$ spectra are available for **1**, **6**-**11**, **13**-**14**. ^{19}F spectra are available for **4**-**7**, **9**. $^{19}F\{^{1}H\}$ spectra are available for **1**, **8**, **10**-**14**. $^{31}P\{^{1}H\}$ spectra are available for **1**, **11**-**14**. HSQC NMR spectrum is available for **1**. High resolution mass spectra are available for **1**, **6**-**14**.

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