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α-Bromophosphonate analogs of glucose-6-phosphate are inhibitors of glucose-6-phosphatase

A. Michael Downey, Christopher W. Cairo *

Alberta Glycomics Centre, Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2G2, Canada

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ABSTRACT

Glucose-6-phosphatase (G6Pase) is an essential metabolic enzyme that has upregulated activity in Type II diabetes. Synthetic analogs of the G6Pase substrate, glucose-6-phosphate (G6P), may provide new tools to probe enzyme activity, or lead to specific inhibitors of glycosylphosphatase enzymes. Here we have developed synthetic routes to a panel of non-hydrolyzable G6P analogs containing α-bromo, α,α-dibromo, and α-bromo-α,β-unsaturated phosphonates compatible with a carbohydrate nucleus. We confirm that these functionalities have potency as inhibitors of G6Pase in vitro, providing a series of new phosphate isosteres that can be exploited for inhibitor design.

1. Introduction

Glucose-6-phosphatase (G6Pase) is a pivotal regulator of glucose metabolism, catalyzing the last step of gluconeogenesis and glycogenolysis by dephosphorylation of glucose-6-phosphate (G6P), generating glucose and inorganic phosphate (P_i).[1,2] It has been shown that G6Pase activity is upregulated in the livers of Type II diabetic patients; however, few therapeutic strategies have been developed to exploit this finding.[3,4] Furthermore, due to its tight association with the endoplasmic reticulum (ER), the structure of G6Pase remains to be fully elucidated.[5,6] As a result, inhibitors of G6Pase are of interest as potential therapeutics, as research tools for exploring glucose metabolism, and for use in structural studies of the enzyme. We set out to rationally design and test new, non-hydrolyzable isosteres of the glycosylphosphate group that could be used in the design of inhibitors of G6Pase.

A general strategy for producing competitive inhibitors of protein phosphatase enzymes has been to create substrate mimics that replace the hydrolyzable C–O–P bond of the phosphate group with a non-hydrolyzable C–C–P bond to generate a phosphonate moiety.[7] Improvements on this strategy have explored installation of fluorine atoms at the phosphonate α-carbon to more closely mimic the electronics of the phosphoester linkage.[8–10] Many G6P analogs that incorporate these functional groups have been studied (CH_2–P,[11–16] CH_2–CH_2–P,[13–21] CH_2–CHF–P,[21,22] and CH_2–CF_2–P).[20,23,24] These compounds were found to be substrates for glucose-6-phosphate dehydrogenase[21] and 2-deoxy-*scyllo*-inosose synthase;[15,16] however, none have been tested as inhibitors of G6Pase.

Although glycosyl α-fluorophosphonate derivatives have been synthesized, the analogous α-bromophosphonate moiety has only been examined in non-carbohydrate substrates.[25–31] Importantly, the α-bromophosphonate functional group has been found to be a potent inhibitor of protein tyrosine phosphatases (PTPs). We[27] and others[25,26] have reported benzylic α-bromophosphonate derivatives which act as irreversible inhibitors of PTPs. As a result, they have been exploited to generate selective activity-based probes.[26] Previous studies have found α-fluorophosphonates show improved potency against PTPs[8–10] relative to their phosphonate counterparts. However, there are no reports of the activity of α-bromophosphonates as inhibitors of glycosylphosphatases. We expected these analogs would have some of the features of their α-fluorophosphonate counterparts—including a more electron deficient phosphonate relative to the alkylphosphonate. Additionally, the weaker C–Br bond might allow these compounds to act as covalent inhibitors. In PTPs a nucleophilic cysteine residue has been postulated to form a covalent adduct with α-bromophosphonate inhibitors, thus deactivating the enzyme.[25,26] G6Pase is known to contain a nucleophilic histidine residue within the active site.[5] The postulated mechanism for enzymatic hydrolysis of G6P into glucose and P_i involves key amino acid residues Arg83, Arg170, His119, and His176 (Fig. 1). The Arg83 and Arg170 residues are proposed to stabilize the anionic oxygens of the G6P phosphate moiety preparing His176 for nucleophilic attack on the phosphorus, displacing the glycoside. Subsequent hydrolysis of the phosphoenzyme intermediate liberates the phosphate.[5]

* Corresponding author. Tel.: +1 780 492 0377; fax: +1 780 492 8231.
E-mail address: ccairo@ualberta.ca (C.W. Cairo).

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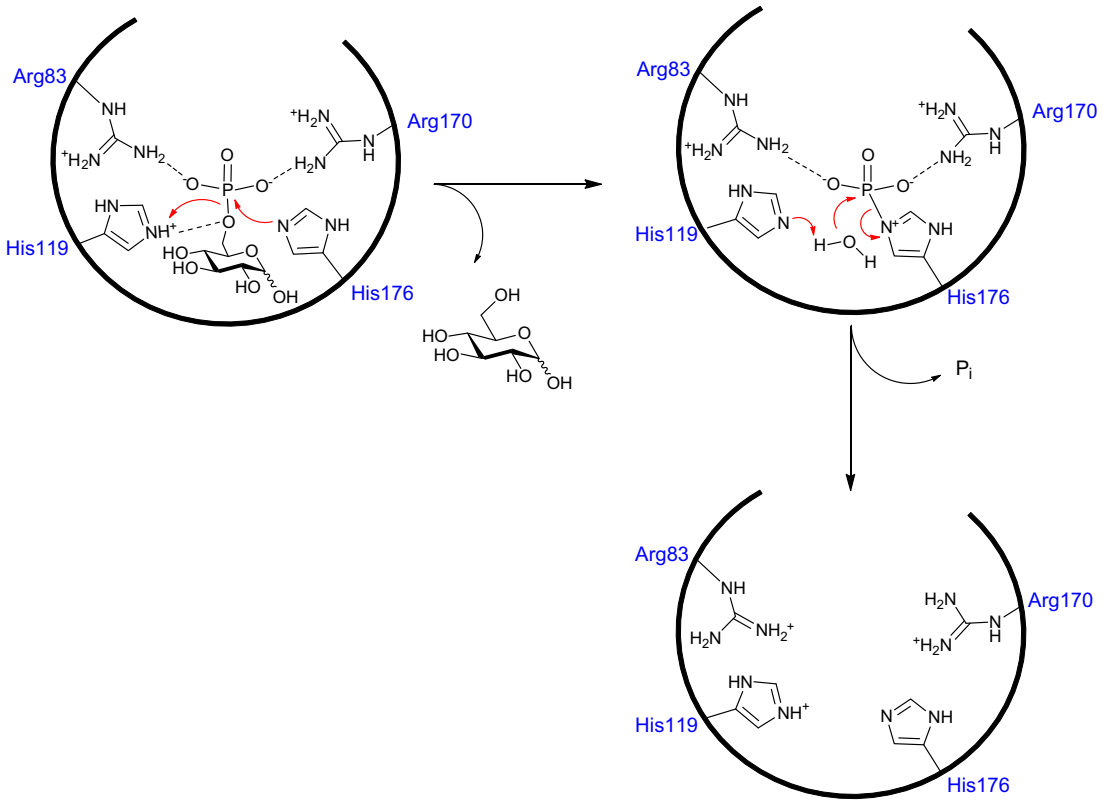


Figure 1. Proposed catalytic mechanism of G6Pase (modified from Ghosh et al., 2002).[5]

Covalent modification of histidine is known for alkylchlorides and alkylbromides.[32,33] Therefore, we postulated that an α-bromophosphonate moiety on a G6P substrate analog would be worthy of exploration as a competitive or covalent inhibitor of the enzyme (**1**, Fig. 2). Furthermore, we were unable to identify any reports of the incorporation of an α-bromophosphonate into a carbohydrate nucleus. Thus, we set out to obtain synthetic access to this novel glycoside functionality.

We envisioned that access to this functional group could be obtained via one of three alternate strategies (Fig. 2): (i) bromination of an α-hydroxy phosphonate, (ii) a Horner–Wadsworth–Emmons (HWE) reaction and subsequent reduction, or (iii) through α-halogenation of a phosphorus-stabilized carbanion. Furthermore, in the design of this target, we considered that G6Pase catalyzes the last step in glycogenolysis where the glycogen substrate contains α(1–4)- and α(1–6)-linkages.[34] Thus, we planned to synthesize the analog as a methyl-α-glucopyranoside, which has the benefit of preventing mutarotation of the anomeric position during synthetic manipulations.

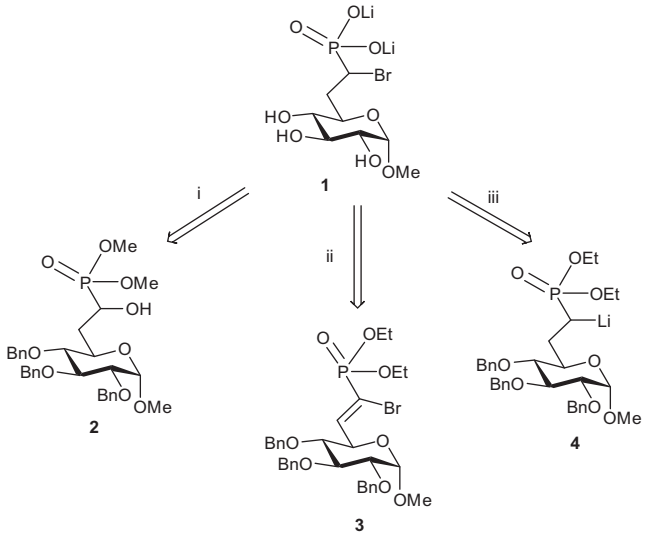
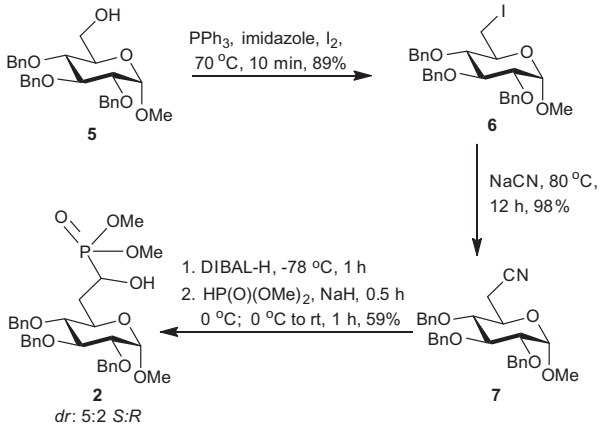


Figure 2. Potential synthetic routes to glycosyl α-bromophosphonates.

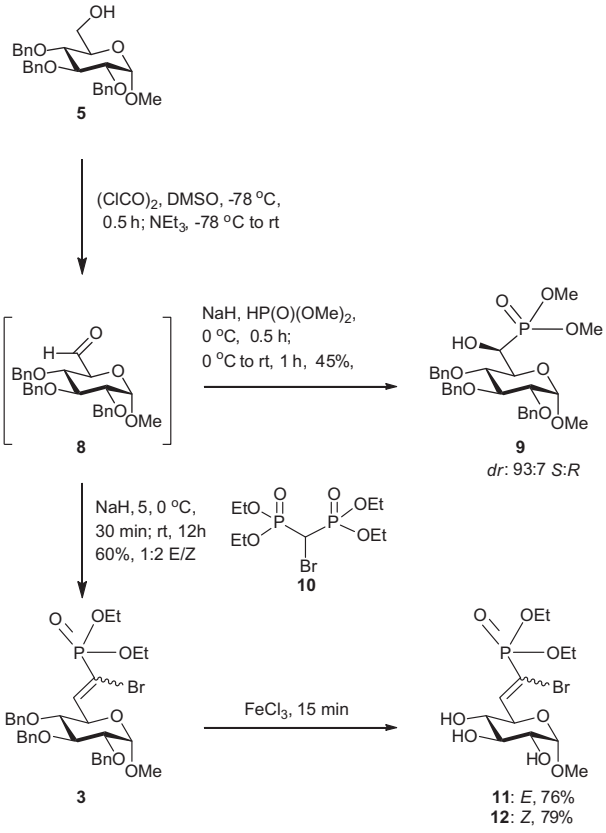
2. Results and discussion

Following previous routes to glycosyl α-fluorophosphonates, we first attempted to brominate via an α-hydroxyphosphonate intermediate. The benzyl-β-glucopyranoside analog of α-hydroxyphosphonate, compound **2**, has previously been used to access the corresponding α-fluorophosphonate.[21] This work provided us with synthetic inspiration and allowed us to assign the composition of our diastereomeric mixture.[21] We arrived at **2** in 4 steps from methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside (**5**)[35,36] and 51% overall yield as inseparable *C*7 diastereomers (5:2 *S*:*R*; Scheme 1). We postulated that the slight diastereomeric excess observed was due to the preferred approach of the phosphite anion in the Pudovick reaction[37] following the Cram model. We were able to gain additional support for this hypothesis by synthesizing the *C*6 α-hydroxyphosphonate derivative (**9**) which gave the corresponding product in a 93:7 (*S*:*R*) ratio when subjected to the same Pudovik reaction conditions (Scheme 2). Although the α-hydroxy functionalization in substrate **2** has previously been used to access the α-fluorophosphonate, all attempts to convert compounds **2** and **9** to the corresponding α-bromophosphonate were unsuccessful (conditions are summarized in Supplementary data).

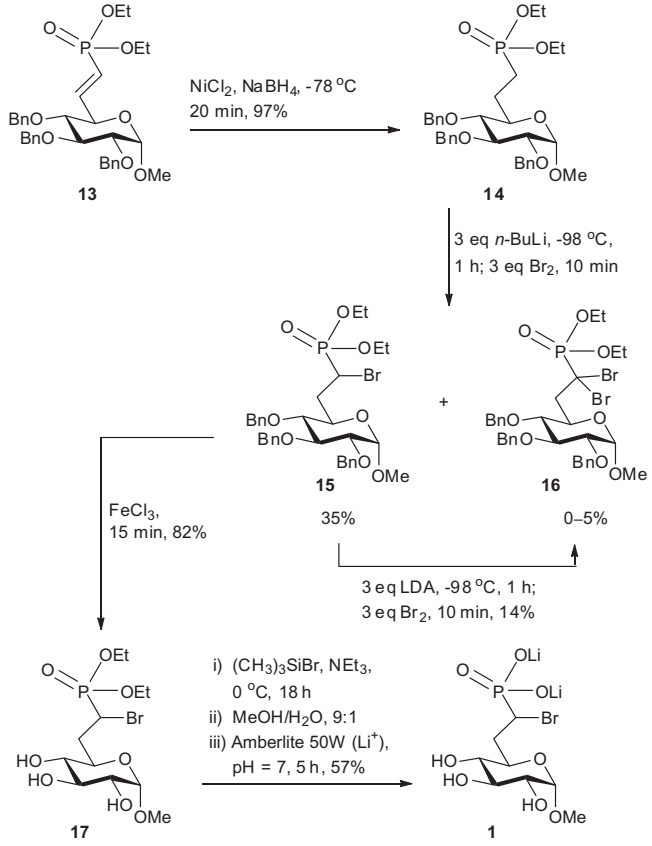
We next turned our attention to pathway **ii** (Scheme 2). Starting again from the 2,3,4-benzyl protected compound, **5**,[35,38] we were able to access aldehyde **8** via Swern oxidation. Crude aldehyde **8**



Scheme 1. Synthesis of bromination precursor **2**.



Scheme 2. Synthesis of analog **9**, and access to α-bromo-α,β-unsaturated phosphonates **11** and **12**.



Scheme 3. Synthesis of target monobromo adduct **1**.

was then subjected to HWE addition using known monobrominated precursor, **10**,[39] to obtain the α,β-unsaturated phosphonate, compound **3**, in good yield and a 1:2 (*E*:*Z*) diastereomeric ratio determined using hydrogen–phosphorus coupling constants derived previously.[40] Fortuitously, the isomers were easily separable by column chromatography. Unfortunately, all attempts to reduce the olefin and debenzylate the carbohydrate nucleus without cleavage of the C–Br bond failed. We attempted to circumvent this issue using a *para*-methoxybenzyl protected glucopyranoside; however, competing side products in the HWE reaction, and our inability to reduce the olefin made this route to compound **1** non-viable. Undaunted, we recognized that the α,β-unsaturated phosphonate was unprecedented on a carbohydrate nucleus, and could itself act as a potential analog of the phosphate group. Additionally, a recent report demonstrates that electron deficient olefins can be used to covalently and reversibly inhibit cysteine thiols.[41] Since standard Pd- or Pt-catalyzed hydrogenation conditions resulted in debromination of compound **3**, we turned to Lewis acid conditions for debenzylation.[42,43] Treatment of **3** with stoichiometric anhydrous $FeCl_3$ provided debenzylated bromo compounds **11** (*E;* 76%) and **12** (*Z;* 79%) in good yield.

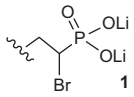
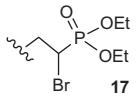
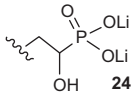
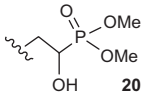
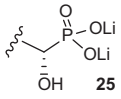
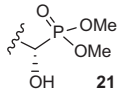
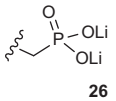
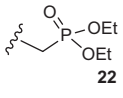
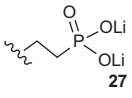
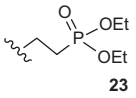
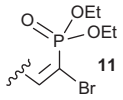
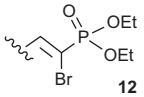
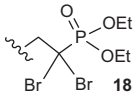
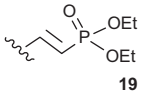
We next focused on developing a monobromination strategy that not require subsequent olefin reduction (pathway **iii**). We first synthesized a fully protected glycoside containing an α,β-unsaturated phosphonate moiety, **13**, according to known methods.[18] Subsequent reduction to compound **14** was performed using $NiCl{\cdot}6H_2O$ and $NaBH_4$ in nearly quantitative yield (Scheme 3).[44,45] We were pleased to find that monobromination at *C*7 could be achieved with careful dropwise addition of a solution of **14** in THF at −98 °C to generate a phosphorus-stabilized carbanion in situ.[46] Subsequent quench of the phosphorus-stabilized carbanion in the presence of Br_2 furnished the target monobromo adduct, **15**, in moderate yield as an inseparable 1:1 mixture of diastereomers. Precise control of temperature and rate of addition of **14** was pivotal to reproducibility. Attempts to increase the yield using alternative bases (lithium diisopropylamide (LDA), *s*-BuLi, *t*-BuLi), or bromine sources (dibromoethane, *N*-bromosuccinimide) were unsuccessful. Close examination of the crude spectra for product **15** suggested the formation of a small amount of dibromo adduct, **16**, under these conditions. As this functionality had only been reported twice previously,[47,48] and never in complex molecules or glycosides, we set out to improve the yield of compound **16** for later testing. By re-subjecting crude phosphonate **15** to LDA and Br_2 at −98 °C, we could increase the yield of **16** to a moderate 14%, and recycle monobromo compound **15** in 30% yield (Scheme 3).

To obtain compounds for testing, we deprotected the phosphonate derivatives described above. We again employed Lewis acid debenzylation conditions to obtain the phosphonate esters of the monobromo (**17**; 82%), dibromo (**18**; 64%), and the α,β-unsaturated phosphonate (**19**; 82%). We also synthesized the *C*7 and *C*6 α-hydroxyphosphonate esters (**20**, **21**; see Schemes S1 & S2 in the Supplementary data) and saturated *C*6 and *C*7 phosphonate esters (**22**, **23**; see Schemes S3 & S4) for comparison using precedented methods.[18,49,50] Phosphonate ester cleavage of **20**–**23** to the corresponding phosphonic acids (**24**–**27**; Schemes S1–S4), was achieved following a modification of known $(CH_3)_3SiBr$-mediated methods.[27,49] Phosphonic acid derivative **1** was obtained in this way, providing our initial target compound in 6 steps and 13% overall yield.

With a panel of novel phosphonic acids and phosphonate ester analogs of G6P in hand, we set out to test the biological activity of these compounds against a glycosyl phosphatase enzyme, G6Pase.[51] We first tested the phosphonic acid derivatives (**1**, **24**–**27**; Table 1) against G6Pase from Wister rat microsomes. Inhibitors were tested at a concentration of 2.5 mM in the presence of G6P substrate at 4 mM. Thus, inhibiton of the enzyme under these conditions would indicate that the compounds have significant activity over the native enzyme substrate in the range of its K_m (3.2 mM).[52,53] In our first experiments, we found that the five phosphonic acid compounds displayed weak inhibition of G6Pase after a short incubation (15 min; data not shown). However, longer incubations revealed that compound **1** substantially reduced G6Pase activity to 25 ± 5%. Compounds **24** (81 ± 4%, entry 5) and **27** (85 ± 5%, entry 2) were the only other phosphonic acid analogs that measurably reduced G6Pase activity. The activity of the alkylphosphonic acids (**26**, **27**) suggested that the phosphonate group alone was not sufficient for inhibition of G6Pase. We also tested compound **1** for evidence of irreversible inhibition[54–56] using dilution experiments;[55] however, we found no evidence of irreversible inhibition at incubations as long as 72 h. These findings could be consistent with **1** acting as a slow-binding, non-covalent inhibitor, or with a slow partitioning of the inhibitor to the enzyme active site.[56,57] Although we were encouraged that compound **1** showed increased activity for the enzyme over the native substrate, the long incubation required to observe activity was a potential drawback. We considered that the polar nature of the phosphonic acid group could reduce the potency of these compounds in vivo, thus we set out to test the activity of the neutral phosphonate ester derivatives.

Assay of the phosphonate ester analogs of G6P at 2.5 mM (**11**, **12**, and **17**–**23**) with a G6P concentration of 3.3 mM revealed that these compounds displayed increased potency over native G6P with a far shorter timeframe of inhibition than the phosphonic acids. We were pleased to find that after only a 1 h incubation, G6Pase activity was reduced by α-bromophosphonate analog **17** to 68 ± 4% and α,α-dibromophosphonate analog **18** to 64 ± 4%. These results are consistent with our hypothesis that the α- and α,α-bromophosphonates can act as analogs of the phosphate group. Interestingly, α-hydroxyphosphonate analog **21** (47 ± 3%) had significantly improved activity over both **17** and **18**. We note that compounds **20** and **21**, which showed significant differences in activity, differ only in the homologation of a methylene group. We postulate that this observation is consistent with the hydroxyl group gaining a specific contact in the active site which would normally stabilize the phosphate leaving group. Likely candidates are R83 or R170 (Fig. 1) which have been found to be necessary for catalysis.[5] The novel α-bromo-α,β-unsaturated analogs **11** (66 ± 11) and **12** (51 ± 8%) also displayed inhibition of G6Pase. Testing of the four α-bromophosphonate esters (**11**–**12**, **17**, and **18**) as well as the most active inhibitor, **21**, for irreversible inhibition using the same dilution experiment as for compound **1** with a 1 h incubation, was negative.

Table 1
G6Pase activity after incubation with inhibitors

R[a,b]	% Activity[c] (60 h)	R[a,b]	% Activity[c] (1 h)
 **1**	25 ± 5	 **17**	68 ± 4
 **24**	81 ± 4	 **20**	74 ± 12
 **25**	>95	 **21**	47 ± 3
 **26**	>95	 **22**	72 ± 10
 **27**	85 ± 5	 **23**	67 ± 7
		 **11**	66 ± 11
		 **12**	51 ± 8
		 **18**	64 ± 4
		 **19**	69 ± 12

[a] Substituent attached at *C*6 of methyl-α-glucoside.
[b] See Supplementary data for synthetic details of compounds not discussed in the text.
[c] Inhibition data were obtained using the Taussky–Shorr method for phosphate release (See Supplementary data). Compounds (2.5 mM) were incubated with disrupted Wister rat microsomes in MOPS buffer at pH 6.5 at 4 °C for the specified time. Inhibition is expressed as % activity ± SE relative to G6P (n = 3–4).

Activity assays for all compounds were performed in disrupted microsomes, allowing us to conclude that the improved activity of the phosphonate esters was not likely to be the result of changes in membrane permeability. By using disrupted microsome in all experiments we are able to conclude that the reduction in activity results from direct interaction with G6Pase, not from inhibition of the membrane G6P transporter.[2,58]

To further probe the origin of the phosphonate ester activity, we incubated a sample of **17** with a microsome sample for 12 h. We were able to observe substantial cleavage of the ester to the phosphonic acid in this time course as determined by ^{31}P NMR. Thus, the phosphonate esters may be active as the phosphonic acid after processing by a native esterase enzyme. Future studies will address the specific mechanism of inhibition involved; however, our results clearly demonstrate that glycosylphosphonate analogs, particularly α-bromophosphonate-based analogs, can act as mimics of the G6P substrate.

3. Conclusions

We have developed synthetic methodology to access α-bromo, α,α-dibromo, and α-bromo-α,β-unsaturated glycosylphospho-

nates. This is the first report of the incorporation of these functional groups into glycosides. We provide evidence that these novel functional groups can act as mimics of the phosphate group that displayed improved activity for the G6Pase active site over the native substrate, G6P. Our results expand the range of glycosylphosphate analogs available for medicinal chemistry studies of carbohydrate metabolism. The mode of inhibition by these new derivatives will be explored in future studies.

4. Experimental

4.1. General methods

All reagents were purchased from commercial sources and used without purification unless otherwise stated. Anhydrous solvents used were purified by successive passage through columns of alumina and copper under argon. If reactions were run under an inert environment using anhydrous solvents, it is noted. Reactions were monitored by analytical thin layer chromatography (TLC) using silica gel (60-F_{254}, 0.25 mm, Silicycle, Quebec, Canada) as a medium, and spots were visualized under ultraviolet light (254 nm) or stained by charring with a ceric ammonium molybdate (CAM) solution. Products were purified by column chromatography as indicated using silica gel (230–400 mesh, Silicycle, Quebec, Canada). All NMR spectra were obtained using Varian instruments. ^{1}H NMR spectra were performed at 400, 500, or 700 MHz as indicated. ^{13}C NMR spectra were performed at 125 MHz. ^{31}P NMR spectra were recorded at 160 or 202 MHz as indicated. ^{1}H NMR data are reported as first order, and the peak assignments were made on the basis of 2D-NMR (^{1}H–^{1}H COSY and HSQC) experiments. Electrospray mass spectra were recorded on Agilent Technologies 6220 TOF using CH_2Cl_2, MeOH, or H_2O as solvent with added NaCl. Optical rotations were measured at 22 ± 2 °C at the sodium D line (589 nm) and are in units of deg·mL(dm·g)$^{-1}$.

4.2. Experimental procedures

4.2.1. Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-iodo-α-D-glucopyranoside (6)

This procedure was adapted from that of Ko et al.[50] To a solution of **5** (187 mg, 0.403 mmol, 1.0 equiv) in toluene (4 mL) was added triphenylphosphine (158 mg, 0.604 mmol, 1.5 equiv), imidazole (69 mg, 1.01 mmol, 2.5 equiv) and iodine (153 mg, 0.604 mmol, 1.5 equiv) with stirring and the reaction was heated to 80 °C for 15 min. After 15 min an oily brown precipitate persisted that indicated the reaction had gone to completion. The toluene was then removed in vacuo and the residue was dissolved in EtOAc (20 mL). The organic layer was washed with 1 M $Na_2S_2O_3$ solution (10 mL), a concentrated $NaHCO_3$ solution (10 mL) and brine (10 mL), dried over Na_2SO_4, and concentrated in vacuo to furnish a crude yellow oil. The residue was purified using column chromatography (1:9 EtOAc–hexane) to provide **6** (205 mg, 0.357 mmol, 89%) as a colorless oil. R_f = 0.32 (1:9 EtOAc–hexane); $[\alpha]_D$ +32.58 (*c* 0.34, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–7.29 (m, 15H, *Ph*CH_2), 5.02 (d, 1H, *J* = 10.8 Hz, C*H*$_2$Ph) 4.97 (d, 1H, *J* = 10.9 Hz, C*H*$_2$-Ph), 4.83(1) (d, 1H, *J* = 10.8 Hz, C*H*$_2$Ph), 4.82(6) (d, 1H, *J* = 12.1 Hz, C*H*$_2$Ph), 4.70 (app t, 2H, *J* = 11.5 Hz, C*H*$_2$Ph), 4.64 (d, 1H, *J* = 3.6 Hz, H-1), 4.04 (app t, 1H, *J* = 9.4 Hz, H-3), 3.57 (dd, 1H, *J* = 9.4, 3.6 Hz, H-2), 3.50 (dd, 1H, *J* = 10.4, 2.3 Hz, H-6a), 3.48 (app dt, 1H, *J* = 9.4, 3.2 Hz, H-5), 3.45 (s, 3H, OC*H*$_3$), 3.37 (app t, *J* = 9.4, 1H, H-4), 3.32 (dd, *J* = 10.4, 6.4 Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.6, 138.0(5), 138.0(2), 128.5(4), 128.5(3), 128.4(7), 128.1(1), 128.0(2), 128.0(0), 127.9(6), 127.7(3), 98.1 (C-1), 81.6 (C-3), 81.5 (C-4), 80.1, (C-2), 75.8 (*C*H$_2$Ph), 75.4 (*C*H$_2$Ph), 73.5 (*C*H$_2$-Ph), 69.3 (C-5), 55.5 (OCH$_3$), 7.7 (C-6). HR ESIMS: *m*/*z* [M+Na$^+$] calcd for $C_{28}H_{31}O_5INa$: 597.11084. Found: 597.11016.

4.2.2. Methyl 2,3,4-tri-*O*-benzyl-6-cyano-6-deoxy-α-D-glucopyranoside (7)

To a solution of **6** (583 mg, 1.01 mmol, 1.0 equiv) in DMF (7.5 mL) was added NaCN (100 mg, 2.03 mmol, 2.0 equiv) with stirring and the reaction was heated at 70 °C for 12 h. After 12 h the DMF was removed in vacuo using a high vacuum rotary evaporator in a water bath at 60 °C. The residue was dissolved in 30 mL of CH_2Cl_2 and the organic layer was washed with H_2O (2 × 15 mL) and brine (15 mL), dried over Na_2SO_4, and concentrated to yield **7** (469 mg, 0.990 mmol, 98%) as a colorless oil that required no further purification. R_f = 0.31 (1:4 EtOAc–hexane); $[\alpha]_D$ +58.23 (*c* 2.52, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37–7.30 (m, 15H, *Ph*CH_2), 5.01 (d, 1H, *J* = 11.0, C*H*$_2$Ph), 4.94 (d, 1H, *J* = 11.2 Hz, C*H*$_2$-Ph), 4.81 (d, 1H, 11.0 Hz, C*H*$_2$Ph), 4.79 (d, 1H, *J* = 12.1 Hz, C*H*$_2$Ph), 4.65 (d, 1H, 12.1 Hz, C*H*$_2$Ph), 4.61 (d, 1H, *J* = 11.2 Hz, C*H*$_2$Ph), 4.58 (d, 1H, 3.6 Hz, H-1), 3.98 (app t, 1H, *J* = 9.2 Hz, H-3), 3.80 (ddd, 1H, *J* = 9.9, 7.1, 3.4 Hz, H-5), 3.56 (dd, 1H, *J* = 9.2, 3.6 Hz, H-2), 3.40 (s, 3H, OC*H*$_3$), 3.33 (dd, 1H, *J* = 9.9, 9.2 Hz, H-4), 2.63 (dd, 1H, *J* = 17.0, 3.4 Hz, H-6a), 2.43 (dd, 1H, *J* = 17.0, 7.1 Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.4, 137.9, 137.6(3), 128.6(1), 128.5(4), 128.4(5), 128.1(2), 128.1(0), 128.0(7), 127.9, 127.7, 116.7 (*C*≡N), 98.1 (C-1), 81.6 (C-3), 79.9(5) (C-4), 79.9(2) (C-2), 75.7 (*C*H$_2$Ph), 75.2(*C*H$_2$Ph), 73.5(*C*H$_2$Ph), 66.3 (C-5) 55.5 (OCH$_3$), 20.8 (C-6). HR ESIMS: *m*/*z* [M+Na$^+$] calcd for $C_{29}H_{31}NO_5Na$: 496.2094. Found: 496.2098.

4.2.3. Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-7-dimethoxyphosphoryl-D/L-*glycero*-α-D-*gluco*-heptopyranoside (2)

A solution of **7** (390 mg, 0.824 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (15 mL) under N_2 was cooled to −78 °C and diisobutylaluminum hydride (1.0 M in hexanes, 2.47 mL, 2.47 mmol, 3.0 equiv) was added dropwise over 15 min. Stirring was continued at −78 °C for 1 h at which point the reaction was quenched using 1 M HCl (7.5 mL, 9.0 equiv) and the reaction was allowed to warm to rt. After stirring at rt for 15 min the reaction was diluted further with CH_2Cl_2 (15 mL) and the organic layer was washed with 1 M HCl (2 × 15 mL). The HCl solution was rinsed with CH_2Cl_2 (2 × 10 mL) and the combined organic layers were washed with brine (15 mL), dried over Na_2SO_4, and concentrated in vacuo to yield the crude aldehyde as a colorless oil that was used immediately without further purification. Dimethyl phosphite (151 μL, 1.65 mmol, 2.0 equiv) was dissolved in anhydrous THF (5 mL) under an N_2 atmosphere and the flask was cooled to 0 °C before NaH (60% in mineral oil, 49 mg, 1.24 mmol, 1.5 equiv) was added in one portion with vigorous stirring. After 30 min a pre-dissolved solution of the crude aldehyde (0.824 mmol, 1.0 equiv) in anhydrous THF (5 mL) was added dropwise via syringe over 15 min. The reaction was warmed to rt and stirring was continued for 1 h. After 1 h the THF was removed in vacuo. The residue was redissolved in CH_2Cl_2 (30 mL) and the organic layer was washed with 1 M HCl (15 mL) and brine (15 mL), dried over Na_2SO_4, and concentrated. The residue was purified using column chromatography (1:1 EtOAc–hexane then 50:50:2 EtOAc–hexane–AcOH) to afford **2** as a faint brown oil (285 mg, 0.486 mmol, 59% from **7**) which was an inseparable 5:2 *S*–*R* mixture of diastereomers that co-eluted. R_f = 0.17 (4:1 EtOAc–hexane); $[\alpha]_D$ +43.96 (*c* 1.00, CH_2Cl_2). HR ESIMS: *m*/*z* [M+Na$^+$] calcd for $C_{31}H_{43}O_9PNa$: 609.2224. Found: 609.2216. Spectroscopic data for the major *S* isomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37–7.25 (m, 15H, *Ph*CH_2), 4.98 (d, 1H, *J* = 10.8 Hz, C*H*$_2$Ph), 4.90 (d, 1H, *J* = 11.0 Hz, C*H*$_2$Ph), 4.80 (d, 1H, *J* = 10.8 Hz, C*H*$_2$Ph), 4.79 (d, 1H, *J* = 12.2 Hz, C*H*$_2$Ph), 4.66 (d, 1H, *J* = 12.1 Hz, C*H*$_2$Ph), 4.60 (d, 1H, *J* = 11.0 Hz, C*H*$_2$Ph), 4.53 (d, 1H, *J* = 3.6 Hz, H-1), 4.17 (ddd, 1H, *J* = 11.1, 6.6, 2.2 Hz, H-7), 4.02–3.97 (m, 2H, H3 + H5), 3.80–3.75 (m, 6H, 2 × C*H*$_3$OP), 3.50 (dd, 1H, *J* = 9.5, 3.6 Hz, H-2), 3.39 (s, 3H, C*H*$_3$O), 3.22 (app t, 1H, *J* = 9.5 Hz, H-4), 2.17 (dddd, 1H, *J* = 15.3, 9.4, 6.6, 2.6 Hz, H-6a),

1.78 (dddd, 1H, J = 15.3, 8.4, 7.4, 2.2 Hz, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$): δ 136.2, 135.6(2), 135.6(1), 126.0, 125.9(9), 125.9(7), 125.9(6), 125.9(3), 125.9(1), 125.6, 125.5(4), 125.5(2), 125.4(6), 125.4(3), 125.3(7), 125.2(5), 125.2(2), 125.1(7), 95.4 (C-1), 79.4 (C-3), 79.0 (C-4), 77.6 (C-2), 73.3 (CH_2Ph), 72.6 (CH_2Ph), 70.9 (CH_2-Ph), 63.4 (d, J_{C-P} = 14.2 Hz, C-5), 61.8 (d, J_{C-P} = 164.2 Hz, C-7), 52.8 (CH_3O), 50.9 (d, J_{C-P} = 7.0 Hz, CH_3OP), 50.7 (d, J_{C-P} = 7.0 Hz, CH_3OP), 30.7 (d, J_{C-P} = 2.1 Hz, C-6); ^{31}P NMR (202 MHz, $CDCl_3$) δ 24.37. Spectroscopic data for the minor *R* isomer: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37–7.25 (m, 15H, $PhCH_2$), 4.98 (d, 1H, J = 10.8 Hz, CH_2-Ph), 4.90 (d, 1H, J = 11.0 Hz, CH_2Ph), 4.80 (d, 1H, J = 10.8 Hz, CH_2Ph), 4.79 (d, 1H, J = 12.2 Hz, CH_2Ph), 4.64 (d, 1H, J = 11.8 Hz, CH_2Ph), 4.60 (d, 1H, J = 11.0 Hz, CH_2Ph), 4.53 (d, 1H, J = 3.6 Hz, H-1), 4.14 (ddd, 1H, J = 10.9, 7.1, 2.6 Hz, H-7), 3.95 (d, 1H, J = 9.2 Hz, H-3), 3.90–3.82 (m, 1H, H-5), 3.80-3.75 (m, 6H, 2 × CH_3OP), 3.50 (dd, 1H, J = 9.5, 3.6 Hz, H-2), 3.38 (s, 3H, CH_3O), 3.27 (app t, 1H, J = 9.2 Hz, H-4), 2.34 (dddd, 1H, J = 14.8, 7.1, 2.8, 2.8 Hz, H-6a), 1.84 (m, 1H, H-6b); ^{13}C NMR (125 MHz, $CDCl_3$): δ 136.2, 135.6(2), 135.6(1), 126.0, 125.9(9), 125.9(7), 125.9(6), 125.9(3), 125.9(1), 125.6, 125.5(4), 125.5(2), 125.4(6), 125.4(3), 125.3(7), 125.2(5), 125.2(2), 125.1(7), 95.7 (C-1), 79.2 (C-3), 79.1 (C-4), 77.3 (C-2), 73.3 (CH_2Ph), 72.8 (CH_2Ph), 71.0 (CH_2Ph), 68.9 (d, J_{C-P} = 17.3 Hz, C-5), 65.4 (d, J_{C-P} = 170.0 Hz, C-7), 53.0 (CH_3O), 51.0 (d, J_{C-P} = 7.0 Hz, CH_3OP), 50.7 (d, J_{C-P} = 7.5 Hz, CH_3OP), 30.7 (C-6); ^{31}P NMR (202 MHz, $CDCl_3$) δ 22.69.

4.2.4. Methyl 2,3,4-tri-*O*-benzyl-6-dimethoxyphosphoryl-L-*glycero*-α-D-*gluco*-pyranoside (9)

A solution of oxalyl chloride (0.14 mL, 1.592 mmol, 4.0 equiv) in anhydrous CH_2Cl_2 (3 mL) under an N_2 atmosphere was cooled to –78 °C and anhydrous DMSO (0.23 mL, 31.8 mmol, 8.0 equiv) was added dropwise with rigorous stirring. After stirring at –78 °C for 20 min, a pre-dissolved solution of **5** (0.185 g, 0.398 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (3 mL) was added dropwise via syringe. Stirring was continued for 1 h before the reaction was quenched with NEt_3 (0.89 mL, 6.37 mmol, 16.0 equiv). Stirring was continued at –78 °C for 15 min before the reaction was warmed to room temperature. The reaction was diluted with 20 mL of CH_2Cl_2 and the organic layer was washed with concentrated NH_4Cl (10 mL). The aqueous layer was rinsed with CH_2Cl_2 (5 mL) and the combined organic layers were washed with brine (2 × 10 mL), dried over Na_2SO_4, and concentrated. The crude aldehyde, **8**, was isolated as a dark orange oil that was freshly prepared each time, dried on a high vacuum, and subsequently used without purification.

To a solution of dimethyl phosphite (73 μL, 0.796 mmol, 2.0 equiv) in anhydrous THF (2 mL) under an N_2 atmosphere was added NaH (60% in mineral oil, 24 mg, 0.597 mmol, 1.5 equiv) with vigorous stirring for 30 min. After 30 min a pre-dissolved solution of crude aldehyde **7** was added to the reaction by cannula. Stirring was continued for 12 h, after which time the THF was removed in vacuo. The crude residue was diluted with EtOAc (25 mL) and the organic layer was rinsed with 1 M HCl (20 mL) and brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo to afford a pale brown oil. The residue was purified using flash chromatography (4:1 EtOAc–hexane to EtOAc) to furnish **9** (102 mg, 0.178 mmol, *dr*: 93:7 *S*:*R*, 45%) as a colorless oil. R_f = 0.18 (4:1 EtOAc–hexane); HR ESIMS: *m*/*z* [M+Na$^+$] calcd for $C_{30}H_{37}O_9PNa$: 595.2067. Found: 595.2060. Spectroscopic data provided are for the L-*glycero* isomer: $[\alpha]_D$ +10.60 (*c* 1.20, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.40–7.29 (m, 15H, $PhCH_2$), 5.02 (d, 1H, J = 11.8 Hz, CH_2Ph), 4.95 (d, 1H, J = 11.2 Hz, CH_2Ph), 4.86 (d, 1H, J = 10.9 Hz, CH_2Ph), 4.83 (d, 1H, J = 12.3 Hz, CH_2Ph), 4.68 (d, 1H, J = 12.0 Hz, CH_2Ph), 4.65 (d, 1H, J = 3.5 Hz, H-1), 4.20 (dd, 1H, J = 17.3, 1.1 Hz, H-6), 4.06 (app t, 1H, J = 9.6 Hz, H-3), 4.02 (ddd, 1H, J = 9.6, 3.3, 1.1 Hz, H-5), 3.82 (d, 3H, J_{H-P} = 10.5 Hz, CH_3OP), 3.77 (d, 3H, J_{H-P} = 10.6 Hz, CH_3OP), 3.71 (app dt, 1H, J = 9.6, 1.1, H-4), 3.51 (dd, 1H, J = 9.6, 3.5 Hz, H-2), 3.49 (s, 3H, CH_3O); ^{13}C NMR (125 MHz, $CDCl_3$): δ 134.7, 134.3, 134.1, 124.5(6), 124.5(2), 124.4(7), 124.1, 124.0(3), 124.0(2), 123.9, 123.7, 94.6 (C-1), 77.9 (C-3), 75.7 (C-2), 72.3 (d, J = 10.3$_{C-P}$ Hz, C-4), 71.8 (CH_2Ph), 71.1 (CH_2Ph), 69.5 (CH_2Ph), 65.7 (C-5), 62.0 (d, J_{C-P} = 162.4 Hz, C-6), 51.8 (CH_3O), 49.6 (d, J_{C-P} = 6.4, Hz, CH_3OP), 48.6 (d, J_{C-P} = 7.2 Hz, CH_3OP); ^{31}P NMR (160 MHz, $CDCl_3$): δ 24.78.

4.2.5. Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-*E*/*Z*-(diethoxyphosphoryl-1′-bromomethylene)-α-D-glucopyranoside (3)

Tetraethyl methylenediphosphonate (TEMDP) (640 μL, 2.58 mmol, 2.05 equiv.) was added dropwise to a stirring solution of 60% NaH in mineral oil (103 mg, 2.58 mmol, 2.05 equiv) in nhydrous THF (12.5 mL) under N_2 at 0 °C. After 30 min *N*-bromosuccinimide (471 mg, 2.65 mmol, 2.1 equiv) was added in one portion. After stirring at rt for 13 h, the reaction was concentrated in vacuo to remove the THF and re-dissolved in CH_2Cl_2. The organic layer was subsequently washed with a saturated NH_4Cl solution and brine, dried over Na_2SO_4, and concentrated in vacuo to yield a faint yellow oil (**10**; crude wt: 1.0 g). NMR indicated a 67% conversion to the monobromo product and the ^{31}P NMR chemical shifts matched those reported in the literature.[39] The product was dried on a high vacuum without further purification.

Compound **10** was removed from the high vacuum and dissolved in anhydrous THF (10 mL). The solution was subsequently cooled to 0 °C under N_2 and to it was added 60% NaH in mineral oil (100 mg, 2.52 mmol, 2.0 equiv) with rigorous stirring. After 30 min crude aldehyde **8**, as prepared in the synthesis of **9** (0.583 g, 1.26 mmol, 1.0 equiv), in anhydrous THF (7.5 mL), was added to the solution by cannula. The solution was allowed to warm to rt and stirring was continued under N_2 for 1 h. TLC in 1:2 EtOAc:hexane indicated two spots with R_f values of 0.78 and 0.44 that were later determined to be the *E* and *Z* alkenes, respectively. Column chromatography (1:2 EtOAc–hexane to 1:1 EtOAc–hexane) furnished two products (*E* isomer, 195 mg, 0.290 mmol, 23%; *Z* isomer, 289 mg, 0.429 mmol, 37%) as colorless oils. Data for the *E* isomer: R_f = 0.78 (1:1 EtOAc–hexane); $[\alpha]_D$ +41.90 (*c* 0.21, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.37–7.26 (m, 15H, $PhCH_2$), 6.63 (dd, 1H, J = 38.0, 10.0 Hz, H-6), 5.36 (app t, 1H, J = 10.0 Hz, H-5), 4.97 (d, 1H, J = 10.7 Hz, CH_2Ph), 4.84 (d, 1H, 11.1 Hz, CH_2Ph), 4.79 (d, 2H, J = 11.6 Hz, CH_2Ph), 4.66 (d, 1H, J = 12.2 Hz, CH_2Ph), 4.60 (d, 1H, J = 11.4 Hz, CH_2Ph), 4.53 (d, 1H, J = 3.5 Hz, H-1), 4.17–4.00 (m, 4H, CH_3CH_2OP + H-3), 3.88–3.82 (m, 1H, CH_3CH_2OP), 3.49 (dd, 1H, J = 9.5, 3.5 Hz, H-2), 3.46 (s, 3H, CH_3O), 3.27 (app t, 1H, J = 9.5 Hz, H-4), 1.33 (t, 3H, J = 7.1 Hz, CH_3CH_2OP), 1.16 (t, 3H, J = 7.1 Hz, CH_3 CH_2OP); ^{13}C NMR (125 MHz, $CDCl_3$): δ 149.2 (d, J_{C-P} = 5.0 Hz, C-6), 138.7, 138.2, 138.1, 128.5(5), 128.5(3), 128.4(8), 128.4(5), 128.4(2), 128.3, 128.2, 128.1(6), 128.1(2), 128.0, 127.9, 127.7, 127.6(6), 116.8 (d, J_{C-P} = 194.6 Hz, C-7), 98.4 (C-1), 81.5 (C-3), 81.2 (C-4), 80.0 (C-2), 75.8 (CH_2Ph), 74.8 (CH_2Ph), 73.4 (CH_2Ph), 67.5 (d, J_{C-P} = 2.8 Hz, C-5), 63.3 (d, J = 5.2 Hz, CH_3CH_2OP), 63.1 (d, J = 5.2 Hz, CH_3CH_2OP), 55.6 (CH_3O), 16.1(4) (d, J_{C-P} = 4.6 Hz, CH_3-CH_2OP), 16.1(8) (d, J_{C-P} = 4.9 Hz, CH_3CH_2OP), ^{31}P NMR (202 MHz, $CDCl_3$) δ 6.52. HR ESIMS: *m*/*z* [M+Na$^+$] calcd for: $C_{33}H_{40}O_8PBrNa$: 697.1536. Found: 697.1524. Data for the *Z* isomer: R_f = 0.44 (1:1 EtOAc–hexane); $[\alpha]_D$ +11.29 (*c* 0.54, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37–7.24 (m, 15H, $PhCH_2$), 7.06 (dd, 1H, J = 14.5, 8.6 Hz, H-6), 4.94 (d, 1H, J = 10.8 Hz, CH_2Ph), 4.81 (d, 1H, J = 10.8 Hz, CH_2 Ph), 4.80 (d, 1H, 12.1 Hz, CH_2Ph), 4.77 (d, 2H, J = 10.8 Hz, CH_2Ph), 4.72 (app td, 1H, J = 9.5, 1.8 Hz, H-5), 4.66 (d, 1H, J = 12.1 Hz, CH_2 Ph), 4.58 (d, 1H, J = 11.2 Hz, CH_2Ph), 4.56 (d, 1H, J = 3.5 Hz, H-1), 4.19–3.94 (m, 5H, CH_3CH_2OP + H-3), 3.53 (dd, 1H, J = 9.5, 3.5 Hz, H-2), 3.44–3.43 (m, 4H, CH_3O + H-4), 1.34 (td, 3H, J = 7.1, 0.4 Hz, CH_3CH_2OP), 1.25 (td, 3H, J = 7.1, 0.5 Hz, CH_3CH_2OP); ^{13}C NMR

(125 MHz, $CDCl_3$): δ 145.1 (d, J_{C-P} = 4.7 Hz, C-6), 138.6, 138.1, 137.8, 130.0(1), 129.9(7), 129.7, 128.5, 128.4, 128.3, 128.1, 128.0, 127.9, 127.7(2), 127.7(0), 118.2 (d, J_{C-P} = 202.3 Hz, C-7), 98.4 (C-1), 81.4(4) (C-4), 81.4(2) (C-3), 79.8 (C-2), 76.0 (*C*H_2Ph), 75.2 (*C*H_2-Ph), 73.6 (*C*H_2Ph), 69.3 (d, J_{C-P} = 15.2 Hz, C-5), 63.4 (d, J = 5.7 Hz, CH_3CH_2OP), 63.3 (d, J = 5.7 Hz, CH_3CH_2OP), 55.7 (*C*H_3O), 16.2 (m, 2 × *C*H_3CH_2OP), ^{31}P NMR (160 MHz, $CDCl_3$) δ 8.22. HR ESIMS: *m*/*z* [M+Na$^+$] calcd for: $C_{33}H_{40}O_8PBrNa$: 697.1536. Found: 697.1536.

4.2.6. Methyl 6-deoxy-6-*E*-(diethoxyphosphoryl-1′-bromomethylene)-α-D-glucopyranoside (11)

To the *E* isomer of **3** (34.0 mg, 0.050 mmol, 1.0 equiv) in anhydrous CH_2Cl_2 (2 mL) under N_2 was added anhydrous $FeCl_3$ (74.0 mg, 0.454 mmol, 9.0 equiv) with stirring in one batch at rt. This dark brown heterogeneous solution was stirred for 15 min before it was quenched with H_2O (0.5 mL). Stirring for 15 min resulted in a light brown heterogeneous solution that was filtered through a bed of Celite and eluted with 90:9:1 CH_2Cl_2–MeOH–NEt_3. The solvent was then removed in vacuo and dissolved in MeOH. To this solution Amberlite 120 resin (H^+ form) was added to remove some of the NEt_3·HCl salt. After stirring for 2 h the resin was filtered off and the eluent was purified using column chromatography (9:1 CH_2Cl_2–MeOH) to furnish a light brown oil. This oil was dissolved in MeOH and Amberlite 120 resin (H^+ form) was added to remove any remaining NEt_3·HCl salt. After 2 h the resin was filtered off and the solvent was removed in vacuo to produce a light brown oil of compound **11** (16.2 mg, 0.038 mmol, 76%). R_f = 0.68 (9:1 CH_2Cl_2–MeOH); $[\alpha]_D$ +47.59 (*c* 0.20, CH_3OD); ^{1}H NMR (500 MHz, CD_3OD) δ 6.88 (dd, 1H, J = 39.1, 9.9 Hz, H-6), 5.14 (app t, 1H, J = 9.4 Hz, H-5), 4.64 (d, 1H, J = 3.8 Hz, H-1), 4.23–4.14 (m, 4H, 2 × CH_3CH_2OP), 3.63 (app t, 1H, J = 9.4 Hz, H-3), 3.42 (s, 3H, C*H*$_3$O), 3.40 (dd, 1H, J = 9.4, 3.8 Hz, H-2), 3.15 (app t, 1H, J = 9.4 Hz, H-4), 1.36 (td, 3H, J = 7.1, 0.6 Hz, C*H*$_3$CH$_2$OP), 1.36 (td, 3H, J = 7.1, 0.6 Hz, C*H*$_3$CH$_2$OP); ^{13}C NMR (125 MHz, CD_3OD): δ 150.7 (d, J_{C-P} = 15.2 Hz, C-6), 113.9 (d, J_{C-P} = 199.2 Hz, C-7), 100.2 (C-1), 73.6 (C-4), 73.2 (C-3), 71.9 (C-2), 68.8 (d, J_{C-P} = 2.8 Hz, C-5), 63.5–63.4, (m, 2 × CH_3CH_2OP), 54.6 (*C*H_3O), 15.1 (*C*H_3CH_2OP), 15.0 (*C*H_3CH_2OP); ^{31}P NMR (202 MHz, CD_3OD) δ 7.36. HR ESIMS: *m*/*z* [M+Na$^+$] calcd for: $C_{12}H_{22}O_8PBrNa$: 427.0128. Found: 427.0122.

4.2.7. Methyl 6-deoxy-6-*Z*-(diethoxyphosphoryl-1′-bromomethylene)-α-D-glucopyranoside (12)

Compound **12** was prepared according to the $FeCl_3$ debenzylation conditions used in the synthesis of **11**. The *Z* isomer of compound **3** (32.2 mg, 0.048 mmol, 1.0 equiv) was reacted with anhydrous $FeCl_3$ (70.0 mg, 0.43 mmol, 9.0 equiv) in anhydrous CH_2Cl_2 at rt for 15 min to furnish **12** (16.2 mg, 0.038 mmol, 79%). R_f = 0.53 (9:1 CH_2Cl_2–MeOH); $[\alpha]_D$ +14.27 (*c* 0.36, CH_3OD); ^{1}H NMR (500 MHz, CD_3OD) δ 6.99 (dd, 1H, J = 14.7, 8.4 Hz, H-6), 4.68 (d, 1H, J = 3.8 Hz, H-1), 4.55 (app td, 1H, J = 9.3, 2.0 Hz, H-5), 4.20–4.03 (m, 4H, 2 × CH_3CH_2OP), 3.65 (app t, 1H, J = 9.3 Hz, H-3), 3.47 (s, 3H, C*H*$_3$O), 3.43 (dd, 1H, J = 9.3, 3.8 Hz, H-2), 1.35(1) (t, 3H, J = 7.1 Hz, C*H*$_3$CH$_2$OP), 1.34(6) (t, 3H, J = 7.1 Hz, C*H*$_3$CH$_2$OP); Note: H-4 is hidden beneath the *CD*$_3$OD peak. ^{13}C NMR (125 MHz, CD_3OD): δ 146.1 (d, J_{C-P} = 14.4 Hz, C-6), 116.7 (d, J_{C-P} = 206.2 Hz, C-7), 100.4 (C-1), 73.6 (C-4), 73.2 (C-3), 72.1 (C-2), 69.9 (d, J_{C-P} = 15.0 Hz, C-5), 63.7–63.6, (m, 2 × CH_3CH_2OP), 54.9 (*C*H_3O), 15.1(2) (*C*H_3CH_2OP), 15.0(7) (*C*H_3CH_2OP); ^{31}P NMR (202 MHz, CD_3OD) δ 8.62. HR ESIMS: *m*/*z* [M+Na$^+$] calcd for: $C_{12}H_{22}O_8PBrNa$: 427.0128. Found: 427.0121.

4.2.8. Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-(diethylphosphonomethylene)-α-D-glucopyranoside (13)

The synthesis of compound **13** was adapted from Roach et al.[18] and Vidil et al.[49] Crude aldehyde **8** was prepared as described for compound **9**. To a solution of TEMDP (2.5 mL, 9.95 mmol, 2.5 equiv) in anhydrous THF (30 mL) under an N_2 atmosphere was added NaH (60% in mineral oil, 318 mg, 7.96 mmol, 2.0 equiv) with vigorous stirring at 0 °C. After 30 min, a pre-dissolved solution of aldehyde **8** (1.85 g, 3.98 mmol, 1.0 equiv) in anhydrous THF (20 mL) was added dropwise via syringe over 30 min. After the addition was complete, the reaction was warmed to rt and stirring continued for 10 min. The THF was then removed in vacuo and the crude residue was dissolved in EtOAc (100 mL) and washed with 1 M HCl (60 mL) and brine (60 mL), dried over Na_2SO_4, and concentrated. The residue was purified using column chromatography (3:2 EtOAc–hexane) to provide **13** (1.68 g, 2.83 mmol, 71%) as a pale yellow oil. R_f = 0.34 (3:2 EtOAc–hexane); $[\alpha]_D$ +57.51 (*c* 3.00, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.35–7.26 (m, 15H, *Ph*CH_2), 6.88 (ddd, 1H, J = 22.5, 17.3, 4.3 Hz, H-6), 6.04 (ddd, 1H, J = 20.9, 17.3, 1.7 Hz, H-7), 4.96 (d, 1H, J = 10.9 Hz, C*H*$_2$Ph), 4.83 (d, 1H, 10.6 Hz, C*H*$_2$Ph), 4.82 (d, 1H, J = 11.9 Hz, C*H*$_2$Ph), 4.80 (d, 1H, J = 12.2 Hz, C*H*$_2$Ph), 4.66 (d, 1H, J = 12.2 Hz, C*H*$_2$Ph), 4.61 (d, 1H, J = 3.6 Hz, H-1), 4.57 (d, 1H, J = 10.6 Hz, C*H*$_2$Ph), 4.25–4.21 (m, 1H, H-5), 4.09–4.02 (m, 4H, 2 × CH_3CH_2OP), 4.01 (app t, 1H, J = 9.6 Hz, H-3), 3.51 (dd, 1H, J = 9.6, 3.6 Hz, H-2), 3.36 (s, 3H, C*H*$_3$O), 3.23 (dd, 1H, J = 9.6, 8.9 Hz, H-4), 1.31–1.27 (m, 6H, 2 × C*H*$_3$CH$_2$OP); ^{13}C NMR (125 MHz, $CDCl_3$): δ 145.3 (d, J_{C-P} = 5.9 Hz, C-6), 136.1, 135.6, 135.2, 126.0, 125.9(7), 125.9(3), 125.6, 125.5, 124.4(9), 125.4(5), 125.3(8), 125.2, 115.6 (d, J_{C-P} = 188.4 Hz, C-7), 95.7 (C-1), 79.3 (C-3), 79.2 (C-4), 77.3 (C-2), 73.3 (*C*H_2Ph), 72.9 (*C*H_2Ph), 71.0 (*C*H_2Ph), 67.4 (d, J_{C-P} = 21.1 Hz, C-5), 59.3–59.2, (m, 2 × CH_3 *C*H_2OP), 52.9 (*C*H_3O), 13.9 (*C*H_3CH_2OP), 13.8 (*C*H_3CH_2OP); ^{31}P NMR (202 MHz, $CDCl_3$) δ 17.91. HR ESIMS: *m*/*z* [M+Na$^+$] calcd for $C_{33}H_{41}O_8PNa$: 619.2431. Found: 619.2426.

4.2.9. Methyl 2,3,4-tri-*O*-benzyl-6,7-dideoxy-7-diethylphosphoryl-α-D-*gluco*-heptopyranoside (14)

A solution of **13** (1.293 g, 2.17 mmol, 1.0 equiv) in MeOH (100 mL) was cooled to −78 °C. With stirring, $NiCl_2$·$6H_2O$ (2.579 g, 10.85 mmol, 5.0 equiv) was added followed by $NaBH_4$ (763 mg, 20.17 mmol, 10.0 equiv) in 3 portions every 5 min. Stirring was continued at −78 °C for 15 min before the cooling bath was removed and the reaction was allowed to warm to rt. While warming the reaction, the color changed from a homogeneous green solution to a heterogeneous black mixture. The reaction mixture was then partially concentrated in vacuo to reduce the volume before being diluted with CH_2Cl_2 (200 mL). 1 M HCl (100 mL) was added and the flask was stirred vigorously for 15 min. The mixture was then extracted with CH_2Cl_2 (3 × 100 mL) and the combined organic layers were washed with brine (2 × 100 mL), dried over Na_2SO_4, and concentrated in vacuo to afford a **14** (1.251 g, 2.09 mmol, 97%) as a spectroscopically pure colorless oil. Further purification of the compound could be performed by passage through a silica plug (hexane then EtOAc), which reduced the yield to 85%. R_f = 0.43 (EtOAc); $[\alpha]_D$ +63.11 (*c* 0.50, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.38–7.28 (m, 15H, *Ph*CH_2), 4.98 (d, 1H, J = 10.8 Hz, C*H*$_2$Ph), 4.90 (d, 1H, 10.8 Hz, C*H*$_2$Ph), 4.81 (d, 1H, J = 10.8 Hz, C*H*$_2$Ph), 4.79 (d, 1H, J = 12.2 Hz, C*H*$_2$Ph), 4.66 (d, 1H, J = 12.1 Hz, C*H*$_2$Ph), 4.61 (d, 1H, J = 10.8 Hz, C*H*$_2$Ph), 4.54 (d, 1H, J = 3.6 Hz, H-1), 4.12–4.02 (m, 4H, 2 × CH_3CH_2OP), 3.96 (app t, 1H, J = 9.4 Hz, H-3), 3.57 (app td, 1H, J = 9.2, 2.7 Hz, H-5), 3.50 (dd, 1H, J = 9.4, 3.6 Hz, H-2), 3.35 (s, 3H, C*H*$_3$O), 3.18 (app t, 1H, J = 9.4 Hz, H-4), 2.19–2.09 (m, 1H, H-6a), 2.03–1.92 (m, 1H, H-7a), 1.75–1.57 (m, 2H, H-6b, H-7b), 1.30 (t, 6H, J = 7.1 Hz, 2 × C*H*$_3$CH$_2$OP); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.7, 138.2, 138.1, 128.5, 128.4, 128.1, 128.0(2), 128.0(0), 127.9(6), 127.8, 127.7, 97.9 (C-1), 82.0 (C-3), 81.7 (C-4), 80.1 (C-2), 75.8 (*C*H_2Ph), 75.3 (*C*H_2Ph), 73.4 (*C*H_2Ph), 70.0 (d, J_{C-P} = 17.5 Hz, C-5), 61.6–61.5, (m, 2 × CH_3CH_2OP), 55.1 (*C*H_3O), 24.8 (d, J_{C-P} = 4.1 Hz, C-6), 21.8 (d, J_{C-P} = 142.8 Hz, C-7), 16.5 (d, J_{C-P} = 1.3 Hz, *C*H_3CH_2OP),

16.4 (d, J_{C-P} = 1.5 Hz, CH_3CH_2OP); ^{31}P NMR (160 MHz, $CDCl_3$): δ 32.00. HR ESIMS: m/z [M+Na$^+$] calcd for $C_{33}H_{43}O_8PNa$: 621.2588. Found: 621.2579.

4.2.10. Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-[(diethoxypho sphoryl)bromomethyl]-α-D-glucopyranoside (15)

A solution of *n*-butyl lithium (2.5 M in hexanes, 163 μL, 0.407 mmol, 3.0 equiv) was dissolved in anhydrous THF (1 mL) under N_2, and cooled to –98 °C in a liquid N_2–MeOH bath. To this solution was added **14** (81.1 mg, 0.136 mmol, 1.0 equiv) in anhydrous THF (2 mL), dropwise at a rate of 100 μL/min. The rate of addition was maintained by use of a syringe pump. During the addition of **14** it was essential that the temperature did not rise above −90 °C. After complete addition, stirring was continued at −98 °C for an additional 5 min before the reaction was quenched with bromine (21 μL, 0.407 mmol, 3.0 equiv). The reaction was kept at −98 °C for 5 min longer before H_2O (0.5 mL) was added and the cooling bath was removed. The reaction was partially concentrated in vacuo to remove the majority of the THF before diluting with EtOAc (15 mL) and washing with H_2O (10 mL). The aqueous layer was then extracted with EtOAc (2 × 15 mL) and the combined organic layers were washed with brine (10 mL), dried over Na_2SO_4, and concentrated. Purification using column chromatography (1:2 EtOAc–hexane then 1:1 EtOAc–hexane) produced **15** (32.2 mg, 0.0476 mmol, 35%) as a colorless oil and a ~1:1 mixture of diastereomers that co-eluted on silica gel. R_f = 0.28 (1:1 EtOAc–hexane); $[\alpha]_D$ +35.27 (*c* 2.70, CH_2Cl_2); HR ESIMS: m/z [M+Na$^+$] calcd for: $C_{33}H_{46}O_8PBrNa$: 699.1693. Found: 699.1701. NMR data for the slightly major isomer: ^1H NMR (500 MHz, $CDCl_3$) δ 7.37–7.25 (m, 15H, *Ph*CH_2), 5.02 (d, 1H, *J* = 10.8 Hz, C*H*$_2$Ph), 4.92 (d, 1H, *J* = 11.2 Hz, C*H*$_2$Ph), 4.83 (d, 1H, *J* = 12.1 Hz, C*H*$_2$Ph), 4.82 (d, 1H, *J* = 11.7 Hz, C*H*$_2$Ph), 4.69 (d, 1H, *J* = 12.2 Hz, C*H*$_2$Ph), 4.64 (d, 1H, *J* = 11.2 Hz, C*H*$_2$Ph), 4.57 (d, 1H, *J* = 3.6 Hz, H-1), 4.26–3.92 (m, 7H, 2 × CH_3CH_2OP, H-5, H-3, H-7), 3.52 (dd, 1H, *J* = 9.5, 3.5 Hz, H-2b), 3.48 (s, 2.4H, C*H*$_3$O), 3.25 (app t, 1H, *J* = 9.5 Hz, H-4), 2.37 (dddd, 1H, *J* = 14.7, 10.8, 5.6, 2.2 Hz, H-6a), 2.00 (dddd, 1H, *J* = 14.7, 10.6, 5.1, 2.0 Hz, H-6), 1.39–1.32 (m, 6H, 2 × C*H*$_3$CH$_2$OP); ^{13}C NMR (125 MHz, $CDCl_3$): δ 136.2, 136.1, 135.7, 135.6, 135.5, 126.0, 125.9(3), 125.9(2), 125.8(9), 125.6(3), 125.6(2), 125.5, 125.4(9), 125.4(7), 125.4(1), 125.3, 125.2(8), 125.2(4), 125.1(7), 95.5 (C-1), 79.5 (C-4), 79.4 (C-3), 77.5 (C-2), 73.3 (*C*H$_2$Ph), 72.6 (*C*H$_2$Ph), 70.9 (*C*H$_2$Ph), 64.2 (d, J_{C-P} = 12.3 Hz, C-5), 61.3–61.1 (m, 2 × CH_3CH_2OP), 53.2 (*C*H$_3$O), 35.2 (d, J_{C-P} = 160.3 Hz, C-7), 32.5 (C-6), 13.9(2)-13.8(7) (m, 2 × *C*H$_3$CH$_2$OP); ^{31}P NMR (160 MHz, $CDCl_3$): δ 17.80. NMR data for the slightly minor isomer: 7.37–7.25 (m, 15H, *Ph*CH_2), 5.02 (d, 1H, *J* = 10.8 Hz, C*H*$_2$Ph), 4.98 (d, 1H, *J* = 10.9 Hz, C*H*$_2$Ph), 4.84 (d, 1H, *J* = 9.9 Hz, C*H*$_2$Ph), 4.82 (d, 1H, *J* = 11.0 Hz, C*H*$_2$Ph), 4.69 (d, 1H, *J* = 12.2 Hz, C*H*$_2$Ph), 4.67 (d, 1H, *J* = 12.3 Hz, C*H*$_2$Ph), 4.58 (d, 1H, *J* = 3.6 Hz, H-1), 4.26–3.92 (m, 7H, 2 × CH_3CH_2OP, H-5, H-3, H-7), 3.54 (dd, 1H, *J* = 9.5, 3.6 Hz, H-2), 3.45 (s, 3H, C*H*$_3$O), 3.31 (app t, 1H, *J* = 9.5 Hz, H-4), 2.75 (dddd, 1H, *J* = 15.1, 9.9, 6.2, 4.4 Hz, H-6a), 2.19–2.10 (m, 1H, H-6b), 1.39–1.32 (m, 6H, 2 × C*H*$_3$CH$_2$OP); ^{13}C NMR (125 MHz, $CDCl_3$): δ 136.2, 136.1, 135.7, 135.6, 135.5, 126.0, 125.9(3), 125.9(2), 125.8(9), 125.6(3), 125.6(2), 125.5, 125.4(9), 125.4(7), 125.4(1), 125.3, 125.2(8), 125.2(4), 125.1(7), 95.6 (C-1), 79.9 (C-4), 79.0 (C-3), 77.6 (C-2), 73.3 (*C*H$_2$Ph), 72.6 (*C*H$_2$Ph), 70.9 (*C*H$_2$Ph), 66.1 (d, J_{C-P} = 9.8 Hz, C-5), 61.3–61.1 (m, 2 × CH_3CH_2OP), 53.2 (*C*H$_3$O), 34.8 (d, J_{C-P} = 157.5 Hz, C-7), 34.5 (C-6), 13.9(2)–13.8(7) (m, 2 × *C*H$_3$CH$_2$OP); ^{31}P NMR (160 MHz, $CDCl_3$): δ 17.59.

4.2.11. Methyl 2,3,4-tri-*O*-benzyl-6-deoxy-6-[(diethoxypho sphoryl)dibromomethyl]-α-D-glucopyranoside (16)

A solution of *n*-butyl lithium (2.5 M in hexanes, 373 μL, 0.933 mmol, 3.0 equiv) in anhydrous THF (2 mL) under N_2 was cooled to −98 °C in a liquid N_2–MeOH bath. To this solution was added **14** (186 mg, 0.311 mmol, 1.0 equiv) in anhydrous THF (5 mL), dropwise at a rate of 100 μL/min. The rate of addition was maintained by use of a syringe pump. During the addition of **14** it was essential that the temperature did not rise above −90 °C. After complete addition, stirring was continued at −98 °C for an additional 5 min before the reaction was quenched with bromine (48 μL, 0.933 mmol, 3.0 equiv). The reaction was kept at −98 °C for 5 min longer before H_2O (1 mL) was added and the cooling bath was removed. The reaction was partially concentrated in vacuo to remove the majority of the THF before diluting with EtOAc (30 mL) and washing with H_2O (20 mL). The aqueous layer was then extracted with EtOAc (2 × 30 mL) and the combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo to produce a crude mixture of **15** as a brown oil (200 mg) that was not purified before use in the next step. The oil was dried on a high vacuum for a minimum of 12 h prior to use.

To a solution of anhydrous diisopropylamine (125 μL, 0.887 mmol, 3.0 mmol) in anhydrous THF cooled to 0 °C under an inert atmosphere of N_2 was added *n*-butyl lithium (2.5 M solution in hexanes, 350 μL, 0.887 mmol, 3.0 equiv) dropwise with stirring. Stirring was continued at 0 °C for 15 min before the flask was cooled to −98 °C in a liquid N_2–MeOH bath. To this solution, crude **15** (200 mg, 0.296 mmol, 1.0 equiv) in anhydrous THF (5 mL) was added dropwise at a rate of 100 μL/min. The rate of addition was maintained by use of a syringe pump. Once again the temperature of the bath did not rise above −90 °C. After complete addition, stirring was continued at −98 °C for an additional 5 min before the reaction was quenched with bromine (46 μL, 0.887 mmol, 3.0 equiv). The reaction was kept at −98 °C for 5 min longer before H_2O (1 mL) was added and the cooling bath was removed. The reaction was partially concentrated in vacuo to remove the majority of the THF before being diluted with EtOAc (30 mL) and washed with H_2O (20 mL). The aqueous layer was then extracted with EtOAc (2 × 30 mL) and the combined organic layers were washed with brine (20 mL), dried over Na_2SO_4, and concentrated in vacuo. Purification using column chromatography (1:2 EtOAc–hexane then 1:1 EtOAc–hexane furnished **16** (33.2 mg, 0.0440 mmol, 14% from **11**) as a colorless oil followed by recovered **15** (63.2 mg, 0.0935 mmol, 30% from **11**) also as a colorless oil and as a ~1:1 mixture of diastereomers that co-eluted. R_f = 0.50 (1:1 EtOAc–hexane); $[\alpha]_D$ +25.31 (*c* 1.00, CH_2Cl_2); ^1H NMR (500 MHz, $CDCl_3$): δ 7.38–7.26 (m, 15H, *Ph*CH_2), 5.01 (d, 1H, *J* = 10.7 Hz, C*H*$_2$Ph), 4.93 (d, 1H, 11.0 Hz, C*H*$_2$Ph), 4.82–4.79 (m, 2H, 2 × C*H*$_2$Ph), 4.67 (d, 1H, *J* = 12.2 Hz, C*H*$_2$Ph), 4.66 (d, 1H, *J* = 12.1 Hz, C*H*$_2$Ph), 4.61 (d, 1H, *J* = 3.6 Hz, H-1), 4.58 (d, 1H, *J* = 11.1 Hz, C*H*$_2$Ph), 4.38–4.25 (m, 5H, 2 × CH_3CH_2OP, H-5), 4.07 (app t, 1H, *J* = 9.4 Hz, H-3), 3.57 (s, 3H, C*H*$_3$O), 3.52 (dd, 1H, *J* = 9.4, 3.6 Hz, H-2), 3.21 (app t, 1H, *J* = 9.4 Hz, H-4), 3.06 (dd, 1H, *J* = 15.2, 6.1 Hz, H-6a), 2.52 (ddd, 1H, 15.2, 8.9, 5.0 Hz, H-6b), 1.35–1.31 (m, 6H, 2 × C*H*$_3$CH$_2$OP); ^{13}C NMR (125 MHz, $CDCl_3$): δ 138.6, 138.2, 137.9, 128.5, 128.4(8), 128.3(8), 128.1, 128.0(6), 128.0(0), 127.9, 127.8, 127.7, 98.2 (C-1), 81.9 (C-3), 80.3 (C-4), 79.8 (C-2), 75.8 (*C*H$_2$Ph), 75.2 (*C*H$_2$Ph), 73.4 (*C*H$_2$Ph), 69.2 (d, J_{C-P} = 10.8 Hz, C-5), 65.9 (d, J_{C-P} = 7.2 Hz, CH_3CH_2OP), 65.8 (d, J_{C-P} = 7.5 Hz, CH_3CH_2OP), 56.7 (*C*H$_3$O), 55.9 (d, J_{C-P} = 170.4 Hz, C-7), 44.5 (C-6), 16.5 (*C*H$_3$CH$_2$OP), 16.4 (*C*H$_3$CH$_2$OP); ^{31}P NMR (202 MHz, $CDCl_3$) δ 11.63. HR ESIMS: m/z [M+Na$^+$] calcd for: $C_{33}H_{41}O_8PBr_2Na$: 777.0798. Found: 777.0803.

4.2.12. Methyl 6-deoxy-6-[(diethoxyphosphoryl)bromomethyl]-α-D-glucopyranoside (17)

Compound **17** was prepared according to the $FeCl_3$ debenzylation conditions used in the synthesis of **11**. Compound **15** (65 mg, 0.0961 mmol, 1.0 equiv) was reacted with anhydrous $FeCl_3$ (140 mg, 0.865 mmol, 9.0 equiv) in anhydrous CH_2Cl_2 at rt for

15 min to furnish **17** (32 mg, 0.0788 mmol, 82%) as a light brown oil. R_f = 0.32 (9:1 CH_2Cl_2–MeOH); $[\alpha]_D$ +66.65 (*c* 0.39, CH_3OH); HR ESIMS: *m*/*z* [M+Na^+] calcd for: $C_{12}H_{24}O_8PBrNa$: 429.0284. Found: 429.0273. NMR data for the slightly major isomer: ^{1}H NMR (500 MHz, CD_3OD): δ 4.62 (d, 1H, *J* = 3.9 Hz, H-1), 4.25–4.18 (m, 5H, H-7, 2 × CH_3CH_2OP), 3.83–3.76 (m, 1H, H-5), 3.61–3.50 (m, 1H, H-3), 3.42 (s, CH_3O), 3.36 (dd, 1H, *J* = 5.1, 3.9 Hz, H-2), 3.12–3.06 (m, 1H, H-4), 2.39 (dddd, 1H, *J* = 14.8, 10.9, 5.6, 2.1 Hz, H-6a), 2.12–1.99 (m, 1H, H-6b), 1.36–1.33 (m, 6H, 2 × CH_3CH_2OP); ^{13}C NMR (125 MHz, CD_3OD): δ 101.5 (C-1), 76.6 (C-4), 74.9 (C-3), 73.6 (C-2), 70.5 (d, J_{C-P} = 9.5 Hz, C-5), 65.5–65.4 (m, CH_3CH_2OP), 65.3–65.2 (m, CH_3CH_2OP), 56.1 (CH_3O), 38.6 (C-6), 38.5 (d, J_{C-P} = 161.6 Hz, C-7), 16.8 (m, CH_3CH_2OP), 16.7 (m, CH_3CH_2OP); ^{31}P NMR (202 MHz, CD_3OD): δ 20.93. NMR data for the slightly minor isomer: ^{1}H NMR (500 MHz, CD_3OD): δ 4.65 (d, 1H, *J* = 3.9 Hz, H-1), 4.28 (m, 1H, H-7a), 4.25–4.18 (m, 4H, 2 × CH_3CH_2OP), 3.83–3.76 (m, 1H, H-5), 3.61–3.50 (m, 1H, H-3), 3.46 (s, CH_3O), 3.38 (dd, 1H, *J* = 5.0, 3.9 Hz, H-2), 3.12–3.06 (m, 1H, H-4), 2.68 (dddd, 1H, *J* = 15.0, 9.9, 6.6, 4.6 Hz, H-6a), 2.12–1.99 (m, 1H, H-6b), 1.36–1.33 (m, 6H, 2 × CH_3CH_2OP); ^{13}C NMR (125 MHz, CD_3OD): δ 101.4 (C-1), 75.6 (C-4), 75.0 (C-3), 73.7 (C-2), 68.9 (d, J_{C-P} = 13.7 Hz, C-5), 65.5–65.4 (m, CH_3CH_2OP), 65.3–65.2 (m, CH_3CH_2OP), 56.2 (CH_3O), 37.9 (d, J_{C-P} = 158.8 Hz, C-7), 36.3 (C-6), 16.8 (m, CH_3CH_2OP), 16.7 (m, CH_3CH_2OP); ^{31}P NMR (202 MHz, CD_3OD): δ 20.92.

4.2.13. Methyl 6-deoxy-6-[(diethoxyphosphoryl)dibromo methyl]-α-D-glucopyranoside (18)

Compound **18** was prepared according to the $FeCl_3$ debenzylation conditions used in the synthesis of **11**. Compound **16** (19.0 mg, 0.0252 mmol, 1.0 equiv) was reacted with anhydrous $FeCl_3$ (37.0 mg, 0.227 mmol, 9.0 equiv) in anhydrous CH_2Cl_2 at rt for 15 min to furnish **18** (8.2 mg, 0.0162 mmol, 64%) as a light brown oil. R_f = 0.35 (9:1 CH_2Cl_2–MeOH); $[\alpha]_D$ +27.32 (*c* 0.32, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ 4.65 (d, 1H, *J* = 3.8 Hz, H-1) 4.16–4.05 (m, 4H, 2 × CH_3CH_2OP), 4.16 (app t, 1H, *J* = 9.0 Hz, H-5), 3.63 (app t, 9.4 Hz, H-3), 3.56 (s, 3H, CH_3O), 3.38 (dd, 1H, *J* = 9.4, 3.8 Hz, H-2), 3.14 (ddd, 1H, *J* = 15.4, 6.4, 1.0 Hz, H-6a), 3.04 (app t, 1H, *J* = 9.4 Hz, H-4), 2.52 (ddd, 1H, *J* = 15.4, 8.4, 6.0 Hz, H-6b), 1.40–1.36 (m, 6H, 2 × CH_3CH_2OP); ^{13}C NMR (125 MHz, CD_3OD): δ 100.1 (C-1), 73.6 (C-4), 73.4 (C-3), 72.0 (C-2), 69.9 (d, J_{C-P} = 10.3, C-5), 66.0 (d, J_{C-P} = 7.7 Hz, CH_3CH_2OP), 66.71 (d, J_{C-P} = 7.5 Hz, CH_3CH_2OP), 55.7 (CH_3O), 54.7 (d, J_{C-P} = 173.0 Hz, C-7), 44.7 (C-6), 15.3(3) (CH_3CH_2OP), 15.2(9) (CH_3CH_2OP); ^{31}P NMR (202 MHz, CD_3OD): δ 11.71. HR ESIMS: *m*/*z* [M+Na^+] calcd for: $C_{12}H_{23}O_8PBr_2Na$: 506.9390. Found: 506.9385.

4.2.14. Methyl 6-deoxy-6-(diethoxyphosphorylmethylene)-α-D-glucopyranoside (19)

Compound **19** was prepared according to the $FeCl_3$ debenzylation conditions used in the synthesis of **11**. Compound **13** (37.0 mg, 0.062 mmol, 1.0 equiv) was reacted with anhydrous $FeCl_3$ (90.5 mg, 0.56 mmol, 9.0 equiv) in anhydrous CH_2Cl_2 at rt for 15 min to furnish **19** (16.6 mg, 0.051 mmol, 82%) as a light brown oil. R_f = 0.43 (9:1 CH_2Cl_2–MeOH); $[\alpha]_D$ +85.16 (*c* 0.58, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD) δ 6.97 (ddd, 1H, *J* = 22.0, 17.2, 3.8 Hz, H-6), 6.06 (ddd, 1H, *J* = 21.9, 17.2, 2.0 Hz, H-7), 4.76 (d, 1H, *J* = 3.8 Hz, H-1), 4.16–4.06 (m, 5H, 2 × CH_3CH_2OP, H-5), 3.65 (app t, 1H, *J* = 9.4 Hz, H-3), 3.42 (dd, 1H, *J* = 9.4, 3.8 Hz, H-2), 3.41 (s, 3H, CH_3O), 3.09 (app t, 1H, *J* = 9.4 Hz, H-4), 1.33 (t, 6H, *J* = 7.1 Hz, CH_3CH_2OP); ^{13}C NMR (125 MHz, CD_3OD): δ 149.8 (d, J_{C-P} = 6.2 Hz, C-6), 115.7 (d, J_{C-P} = 188.9 Hz, C-7), 100.2 (C-1), 74.0 (d, J_{C-P} = 1.5 Hz, C-4), 73.7 (C-3), 71.9 (C-2), 70.5 (d, J_{C-P} = 20.9 Hz, C-5), 62.1–62.0, (m, 2 × CH_3CH_2OP), 54.5 (CH_3O), 15.2(2) (CH_3CH_2OP), 15.1(8) (CH_3CH_2OP); ^{31}P NMR (202 MHz, CD_3OD) δ 19.20.

4.2.15. Methyl 6-deoxy-6-[monobromophosphorylmethyl]-α-D-glucopyranoside, bis-lithium salt (1)

To a solution of **17** (27.2 mg, 0.0670 mmol, 1.0 equiv) in anhydrous CH_3CN under a N_2 environment was added trimethylsilyl bromide (88 μL, 0.660 mmol, 10.0 equiv) dropwise at 0 °C with rigorous stirring. After 5 min of stirring, anhydrous NEt_3 (65 μL, 0.804 mmol, 10.5 equiv.) was added dropwise and the reaction was allowed to slowly warm to rt. After stirring for 12 h the reaction was concentrated in vacuo to leave a dark red oil. The residue was then washed with H_2O–MeOH (1:9) (3 × 2.5 mL) which was re-concentrated in vacuo after each dissolution. The residue was then dissolved in H_2O and the pH was increased to 7.0 using triethylamine. To this solution Amberlite 120 resin (Li^+ form) was added and stirring continued for 5 h. After 5 h the resin was filtered off and the H_2O was removed via lyophilization to yield a pale yellow, amorphous solid. Et_2O was added to the crude solid to create a suspension which was stirred vigorously as a minimum volume of 100% EtOH was carefully added dropwise to dissolve the excess LiBr salt. The suspension was filtered and the remaining solid was dissolved in H_2O. Removal of the H_2O via lyophilization furnished **1** as a colorless foam (73:27 bis-lithium salt–lithium/$HNEt_3^+$ salt mixture: 15.3 mg, 0.0382 mmol, 57%). $[\alpha]_D$ +66.69 (*c* 0.34, H_2O); Data for the slightly major isomer: ^{1}H NMR (500 MHz, D_2O): δ 4.79 (d, 1H, *J* = 3.8 Hz, H-1), 4.05–3.95 (m, 1H, H-7), 3.92–3.82 (m, 1H, H-5), 3.64–3.54 (m, 2H, H-3 + H-2), 3.49 (s, 3H, CH_3O), 3.28 (app t, 1H, *J* = 9.4 Hz, H-4), 2.34–2.26 (m, 1H, H-6a), 2.14–2.03 (m, 1H, H-6b); ^{13}C NMR (125 MHz, D_2O): δ 99.2 (C-1), 74.3 (C-4), 73.1 (C-3), 71.4 (C-2), 68.8 (d, J_{C-P} = 12.6 Hz, C-5), 55.6 (CH_3O), 43.3 (d, J_{C-P} = 143.1 Hz, C-7), 35.2 (C-6); ^{31}P NMR (160 MHz, D_2O) δ) δ 17.18. Data for the slightly minor isomer: ^{1}H NMR (500 MHz, D_2O): δ 4.79 (d, 1H, *J* = 3.8 Hz, H-1), 4.05–3.95 (m, 1H, H-7), 3.92–3.82 (m, 1H, H-5), 3.64–3.54 (m, 2H, H-3 + H-2), 3.46 (s, 3H, CH_3O), 3.32 (app t, 1H, *J* = 9.4 Hz, H-4), 2.73–2.66 (m, 1H, H-6a), 2.14–2.03 (m, 1H, H-6b); ^{13}C NMR (125 MHz, D_2O): δ 99.3 (C-1), 74.3 (C-4), 73.2 (C-3), 71.3 (C-2), 70.7 (d, J_{C-P} = 12.4 Hz, C-5), 55.6 (CH_3O), 43.8 (d, J_{C-P} = 142.5 Hz, C-7), 36.9 (C-6); ^{31}P NMR (160 MHz, D_2O) δ 17.02. HR ESIMS: *m*/*z* [M−H^-] calcd for: $C_8H_{15}O_8PBrNa$: 348.9693. Found: 348.9692.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carres.2013.08.003.

References

1. Nordlie, R. C.; Foster, J. D.; Lange, A. J. *Annu. Rev. Nutr.* **1999**, *19*, 379–406.
2. Charkoudian, L. K.; Farrell, B. P.; Khosla, C. *Med. Chem. Commun.* **2012**, *3*, 926–931.
3. Ashmore, J.; Hastings, A. B.; Nesbett, F. B. *Proc. Natl. Acad. Sci. U.S.A.* **1954**, *40*, 673–678.
4. Kim-Muller, J. Y.; Accili, D. *Science* **2011**, *331*, 1529–1531.
5. Ghosh, A.; Shieh, J. J.; Pan, C. J.; Sun, M. S.; Chou, J. Y. *J. Biol. Chem.* **2002**, *277*, 32837–32842.
6. Pan, C. J.; Lei, K. J.; Annabi, B.; Hemrika, W.; Chou, J. Y. *J. Biol. Chem.* **1998**, *273*, 6144–6148.
7. Engel, R. *Chem. Rev.* **1977**, *77*, 349–367.
8. Berkowitz, D. B.; Bose, M. *J. Fluorine Chem.* **2001**, *112*, 13–33.
9. Romanenko, V. D.; Kukhar, V. P. *Chem. Rev.* **2006**, *106*, 3868–3935.

10. Panigrahi, K.; Eggen, M.; Maeng, J. H.; Shen, Q. R.; Berkowitz, D. B. *Chem. Biol.* **2009**, *16*, 928–936.
11. Smith-Griffin, B.; Burger, A. *J. Am. Chem. Soc.* **1956**, *78*, 2336–2338.
12. Nifant'ev, E. E.; Gudkova, I. P.; Kochetkov, N. K. *Zh. Obshch. Khim.* **1970**, *40*, 460–463.
13. Lemarechal, P.; Froussios, C.; Level, M.; Azerad, R. *Carbohydr. Res.* **1981**, *94*, 1–10.
14. Padyukova, N. S.; Afsar, S.; Dixon, H. B. F.; Karpeiskii, M. Y. *Bioorg. Khim.* **1995**, *21*, 382–385.
15. Huang, Z.; Kakinuma, K.; Eguchi, T. *Bioorg. Chem.* **2005**, *33*, 82–89.
16. Hirayama, T.; Kudo, F.; Huang, Z.; Eguchi, T. *Bioorg. Med. Chem.* **2007**, *15*, 418–423.
17. Adams, P. R.; Harrison, R.; Inch, T. D.; Rich, P. *Biochem. J.* **1976**, *155*, 1–4.
18. Roach, D. J. W.; Harrison, R.; Hall, C. R.; Inch, T. D. *Biochem. J.* **1981**, *197*, 735–737.
19. Benyoseph, O.; Sparkes, M. J.; Dixon, H. B. F. *Anal. Biochem.* **1993**, *210*, 195–198.
20. Berkowitz, D. B.; Bhuniya, D.; Peris, G. *Tetrahedron Lett.* **1999**, *40*, 1869–1872.
21. Berkowitz, D. B.; Bose, M.; Pfannenstiel, T. J.; Doukov, T. *J. Org. Chem.* **2000**, *65*, 4498–4508.
22. Berkowitz, D. B.; Bose, M.; Asher, N. G. *Org. Lett.* **2001**, *3*, 2009–2012.
23. Berkowitz, D. B.; Eggen, M.; Shen, Q.; Sloss, D. G. *J. Org. Chem.* **1993**, *58*, 6174–6176.
24. Berkowitz, D. B.; Sloss, D. G. *J. Org. Chem.* **1995**, *60*, 7047–7050.
25. Taylor, W. P.; Zhang, Z. Y.; Widlanski, T. S. *Bioorg. Med. Chem.* **1996**, *4*, 1515–1520.
26. Kumar, S.; Zhou, B.; Liang, F. B.; Wang, W. Q.; Huang, Z. H.; Zhang, Z. Y. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 7943–7948.
27. Tulsi, N. S.; Downey, A. M.; Cairo, C. W. *Bioorg. Med. Chem.* **2010**, *18*, 8679–8686.
28. Jiang, G. W.; Xu, Y.; Fujiwara, Y.; Tsukahara, T.; Tsukahara, R.; Gajewiak, J.; Tigyi, G.; Prestwich, G. D. *ChemMedChem* **2007**, *2*, 679–690.
29. Zhang, H. L.; Xu, X. Y.; Gajewiak, J.; Tsukahara, R.; Fujiwara, Y.; Liu, J. X.; Fells, J. I.; Perygin, D.; Parrill, A. L.; Tigyi, G.; Prestwich, G. D. *Cancer Res.* **2009**, *69*, 5441–5449.
30. Gupte, R.; Patil, R.; Liu, J. X.; Wang, Y. H.; Lee, S. C.; Fujiwara, Y.; Fells, J.; Bolen, A. L.; Emmons-Thompson, K.; Yates, C. R.; Siddam, A.; Panupinthu, N.; Pham, T. C. T.; Baker, D. L.; Parrill, A. L.; Mills, G. B.; Tigyi, G.; Miller, D. D. *ChemMedChem* **2011**, *6*, 922–935.
31. Jiang, G. W.; Madan, D.; Prestwich, G. D. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5098–5101.
32. Thompson, R. C.; Blout, E. R. *Biochemistry* **1973**, *12*, 44–47.
33. Jones, J. B.; Hysert, D. W. *Can. J. Chem.* **1971**, *49*, 325–332.
34. Roach, P. J.; Depaoli-Roach, A. A.; Hurley, T. D.; Tagliabracci, V. S. *Biochem. J.* **2012**, *441*, 763–787.
35. Julina, R.; Vasella, A. *Helv. Chim. Acta* **1985**, *68*, 819–830.
36. Tatsuta, K.; Fujimoto, K.; Kinoshita, M.; Umezawa, S. *Carbohydr. Res.* **1977**, *54*, 85–104.
37. Pudovik, A. N.; Konovalova, I. V. *Synthesis* **1979**, 81–96.
38. Bernotas, R. C.; Pezzone, M. A.; Ganem, B. *Carbohydr. Res.* **1987**, *167*, 305–311.
39. Iorga, B.; Savignac, P. J. *Organomet. Chem.* **2001**, *624*, 203–207.
40. Kobayashi, Y.; William, A. D. *Org. Lett.* **2002**, *4*, 4241–4244.
41. Serafimova, I. M.; Pufall, M. A.; Krishnan, S.; Duda, K.; Cohen, M. S.; Maglathlin, R. L.; McFarland, J. M.; Miller, R. M.; Frödin, M.; Taunton, J. *Nat. Chem. Biol.* **2012**, *8*, 471–476.
42. Park, M. H.; Takeda, R.; Nakanishi, K. *Tetrahedron Lett.* **1987**, *28*, 3823–3824.
43. Rodebaugh, R.; Debenham, J. S.; FraserReid, B. *Tetrahedron Lett.* **1996**, *37*, 5477–5478.
44. Khurana, J. M.; Gogia, A. *Org. Prep. Proced. Int.* **1997**, *29*, 1–32.
45. Molander, G. A.; Quirmbach, M. S.; Silva, L. F.; Spencer, K. C.; Balsells, J. *Org. Lett.* **2001**, *3*, 2257–2260.
46. Cantat, T.; Ricard, L.; Le Floch, P.; Mezailles, N. *Organometallics* **2006**, *25*, 4965–4976.
47. Kitamura, M.; Tokunaga, M.; Noyori, R. *J. Am. Chem. Soc.* **1995**, *117*, 2931–2932.
48. Polozov, A. M.; Cremer, S. E. *J. Organomet. Chem.* **2002**, *646*, 153–160.
49. Vidil, C.; Morere, A.; Garcia, M.; Barragan, V.; Hamdaoui, B.; Rochefort, H.; Montero, J. L. *Eur. J. Org. Chem.* **1999**, 447–450.
50. Ko, K. S.; Zea, C. J.; Pohl, N. L. *J. Am. Chem. Soc.* **2004**, *126*, 13188–13189.
51. Taussky, H. H.; Shorr, E. *J. Biol. Chem.* **1953**, *202*, 675–685.
52. Hers, H. G. *Annu. Rev. Biochem.* **1976**, *45*, 167–189.
53. Garland, R. C.; Cori, C. F.; Chang, H. W. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 3805–3809.
54. Foster, J. D.; Bode, A. M.; Nordlie, R. C. *FASEB J.* **1993**, *7*, A848.
55. Foster, J. D.; Bode, A. M.; Nordlie, R. C. *BBA-Protein Struct. M.* **1994**, *1208*, 222–228.
56. Morrison, J. F. *Trends Biochem. Sci.* **1982**, *7*, 102–105.
57. Davison, A. N. *Biochem. J.* **1957**, *67*, 316–322.
58. Madsen, P.; Westergaard, N. *Expert Opin. Ther. Pat.* **2001**, *11*, 1429–1441.